(12) STANDARD PATENT (11) Application No. **AU 2015339786 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Treatment of cardiovascular disease using ActRII ligand traps

(51) International Patent Classification(s)
A61P 9/00 (2006.01) ***A61K 38/00*** (2006.01)

(21) Application No: **2015339786** (22) Date of Filing: **2015.10.08**

(87) WIPO No: **WO16/069234**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/062,021	**2014.10.09**	**US**
62/167,052	**2015.05.27**	**US**
62/170,015	**2015.06.02**	**US**
62/078,321	**2014.11.11**	**US**
62/103,515	**2015.01.14**	**US**

(43) Publication Date: **2016.05.06**
(44) Accepted Journal Date: **2021.03.04**

(71) Applicant(s)
Celgene Corporation;Washington University

(72) Inventor(s)
Hruska, Keith;Fang, Yifu;Smith, William;Chen, Nianhang

(74) Agent / Attorney
FB Rice Pty Ltd, L 23 44 Market St, Sydney, NSW, 2000, AU

(56) Related Art
WO 2014071158 A1
WO 2006012627 A2
FANG et al., "Early chronic kidney disease-mineral bone disorder stimulates vascular calcification.", Kidney International, (2014-01), vol. 85, no. 1, pages 142 - 150

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 May 2016 (06.05.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/069234 A1

(51) International Patent Classification:
A61P 9/00 (2006.01) *A61K 38/00* (2006.01)

(21) International Application Number:
PCT/US2015/054674

(22) International Filing Date:
8 October 2015 (08.10.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/062,021	9 October 2014 (09.10.2014)	US
62/078,321	11 November 2014 (11.11.2014)	US
62/103,515	14 January 2015 (14.01.2015)	US
62/167,052	27 May 2015 (27.05.2015)	US
62/170,015	2 June 2015 (02.06.2015)	US

(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).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

[Continued on next page]

(54) Title: TREATMENT OF CARDIOVASCULAR DISEASE USING ACTRII LIGAND TRAPS

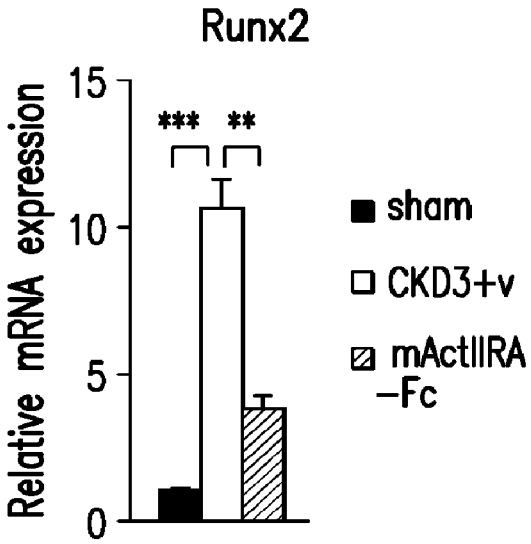


FIG. 4A

(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.

WO 2016/069234 A1

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

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.

2015339786 10 Feb 2021

[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.

[0007a] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each claim of this application.

[0007b] Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

6. SUMMARY

[0007c] Provided herein is a method of treating and/or preventing a 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 been selected based on:

(a) elevated levels of snail homolog 1 (Snai1) as compared to levels of Snai1 in a reference population;

(b) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population;

(c) elevated levels of collagen type 1 alpha 1 (col1a1) as compared to levels of col1a1 in a reference population;

(d) elevated levels of Osterix as compared to levels of Osterix in a reference population;

(e) decreased levels of alpha smooth muscle actin (alpha-SMA) as compared to levels of alpha-SMA in a reference population;

(f) decreased levels of myocardin (MYOCD) as compared to levels of MYOCD in a reference population;

2015339786 10 Feb 2021

(g) decreased levels of smooth muscle protein 22-alpha (Sm22-alpha) as compared to levels of Sm22-alpha in a reference population

(h) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population;

(i) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or

(j) decreased levels of Axin2 as compared to levels of Axin 2 in a reference population,

wherein the reference population:

(i) consists of healthy people;

(ii) consists of people of the same age, weight, and/or gender as the subject;

(iii) consists of people without cardiovascular disease;

(iv) consists of people without vascular calcification;

(v) consists of people without renal disease;

(vi) consists of people without chronic kidney disease;

(vii) consists of people without pathologically elevated levels of arterial stiffness; or

(viii) consists of people without left ventricular hypertrophy, and

wherein the disease is:

(a) vascular calcification;

(b) cardiovascular disease associated with and/or resulting from vascular calcification;

(c) arterial stiffness;

(d) cardiovascular disease associated with and/or resulting from arterial stiffness;

(e) left ventricular hypertrophy (LVH);

(f) cardiovascular disease associated with and/or resulting from left ventricular hypertrophy;

(g) cardiovascular disease associated with and/or resulting from renal disease; or

(h) bone resorption associated with chronic kidney disease-mineral and bone disease (CKD-MBD), and

wherein the ActRII signaling inhibitor is a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) at least 90% identical to SEQ ID NO:2;

(b) at least 95% identical to SEQ ID NO:2;

(c) at least 98% identical to SEQ ID NO:2;

(d) SEQ ID NO:2;

(e) at least 90% identical to SEQ ID NO:3;

(f) at least 95% identical to SEQ ID NO:3;

(g) at least 98% identical to SEQ ID NO:3;

(h) SEQ ID NO:3;

(i) at least 90% identical to SEQ ID NO:6;

(j) at least 95% identical to SEQ ID NO:6;

(k) at least 98% identical to SEQ ID NO:6;

(l) SEQ ID NO:6;

(m) at least 90% identical to SEQ ID NO:7;

(n) at least 95% identical to SEQ ID NO:7;

(o) at least 98% identical to SEQ ID NO:7;

(p) SEQ ID NO:7;

(q) at least 90% identical to SEQ ID NO:12;

(r) at least 95% identical to SEQ ID NO:12;

(s) at least 98% identical to SEQ ID NO:12;

(t) SEQ ID NO:12;

(u) at least 90% identical to SEQ ID NO:17;

(v) at least 95% identical to SEQ ID NO:17;

(w) at least 98% identical to SEQ ID NO:17;

(x) SEQ ID NO:17;

(y) at least 90% identical to SEQ ID NO:20;

(z) at least 95% identical to SEQ ID NO:20;

(aa) at least 98% identical to SEQ ID NO:20;

(bb) SEQ ID NO:20;

(cc) at least 90% identical to SEQ ID NO:21;

(dd) at least 95% identical to SEQ ID NO:21;

(ee) at least 98% identical to SEQ ID NO:21;

(ff) SEQ ID NO:21;

(gg) at least 90% identical to SEQ ID NO:25;

(hh) at least 95% identical to SEQ ID NO:25;

(ii) at least 98% identical to SEQ ID NO:25; and

(jj) SEQ ID NO:25.

2015339786 10 Feb 2021

[0007d] Provided herein is a method of treating and/or preventing a disease in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an activin receptor type II (ActRII) signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1, Osterix, alpha-SMA, MYOCD, 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, col1a1, Osterix, alpha-SMA, MYOCD, 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 treating and/or preventing the disease,

and wherein the disease is:

(a) vascular calcification;

(b) cardiovascular disease associated with and/or resulting from vascular calcification;

(c) arterial stiffness;

(d) cardiovascular disease associated with and/or resulting from arterial stiffness;

(e) left ventricular hypertrophy (LVH);

(f) cardiovascular disease associated with and/or resulting from left ventricular hypertrophy;

(g) cardiovascular disease associated with and/or resulting from renal disease; or

(h) bone resorption associated with chronic kidney disease-mineral and bone disease (CKD-MBD), and

wherein the ActRII signaling inhibitor is a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) at least 90% identical to SEQ ID NO:2;

(b) at least 95% identical to SEQ ID NO:2;

(c) at least 98% identical to SEQ ID NO:2;

(d) SEQ ID NO:2;

(e) at least 90% identical to SEQ ID NO:3;

(f) at least 95% identical to SEQ ID NO:3;

(g) at least 98% identical to SEQ ID NO:3;
(h) SEQ ID NO:3;
(i) at least 90% identical to SEQ ID NO:6;
(j) at least 95% identical to SEQ ID NO:6;
(k) at least 98% identical to SEQ ID NO:6;
(l) SEQ ID NO:6;
(m) at least 90% identical to SEQ ID NO:7;
(n) at least 95% identical to SEQ ID NO:7;
(o) at least 98% identical to SEQ ID NO:7;
(p) SEQ ID NO:7;
(q) at least 90% identical to SEQ ID NO:12;
(r) at least 95% identical to SEQ ID NO:12;
(s) at least 98% identical to SEQ ID NO:12;
(t) SEQ ID NO:12;
(u) at least 90% identical to SEQ ID NO:17;
(v) at least 95% identical to SEQ ID NO:17;
(w) at least 98% identical to SEQ ID NO:17;
(x) SEQ ID NO:17;
(y) at least 90% identical to SEQ ID NO:20;
(z) at least 95% identical to SEQ ID NO:20;
(aa) at least 98% identical to SEQ ID NO:20;
(bb) SEQ ID NO:20;
(cc) at least 90% identical to SEQ ID NO:21;
(dd) at least 95% identical to SEQ ID NO:21;
(ee) at least 98% identical to SEQ ID NO:21;
(ff) SEQ ID NO:21;
(gg) at least 90% identical to SEQ ID NO:25;
(hh) at least 95% identical to SEQ ID NO:25;
(ii) at least 98% identical to SEQ ID NO:25; and
(jj) SEQ ID NO:25.

[0007e] Provided herein is the use of an activin receptor type II (ActRII) signaling inhibitor in the manufacture of a medicament for treating and/or preventing a disease in a subject, wherein the subject has been selected based on:

2015339786 10 Feb 2021

(a) elevated levels of snail homolog 1 (Snai1) as compared to levels of Snai1 in a reference population;

(b) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population;

(c) elevated levels of collagen type 1 alpha 1 (col1a1) as compared to levels of col1a1 in a reference population;

(d) elevated levels of Osterix as compared to levels of Osterix in a reference population;

(e) decreased levels of alpha smooth muscle actin (alpha-SMA) as compared to levels of alpha-SMA in a reference population;

(f) decreased levels of myocardin (MYOCD) as compared to levels of MYOCD in a reference population;

(g) decreased levels of smooth muscle protein 22-alpha (Sm22-alpha) as compared to levels of Sm22-alpha in a reference population;

(h) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population;

(i) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or

(j) decreased levels of Axin2 as compared to levels of Axin2 in a reference population,

wherein the reference population:

(i) consists of healthy people;

(ii) consists of people of the same age, weight, and/or gender as the subject;

(iii) consists of people without cardiovascular disease;

(iv) consists of people without vascular calcification;

(v) consists of people without renal disease;

(vi) consists of people without chronic kidney disease;

(vii) consists of people without pathologically elevated levels of arterial stiffness; or

(viii) consists of people without left ventricular hypertrophy, and

wherein the disease is:

(a) vascular calcification;

(b) cardiovascular disease associated with and/or resulting from vascular calcification;

(c) arterial stiffness;

2015339786 10 Feb 2021

(d) cardiovascular disease associated with and/or resulting from arterial stiffness;

(e) left ventricular hypertrophy (LVH);

(f) cardiovascular disease associated with and/or resulting from left ventricular hypertrophy;

(g) cardiovascular disease associated with and/or resulting from renal disease; or

(h) bone resorption associated with chronic kidney disease-mineral and bone disease (CKD-MBD); and

wherein the ActRII signaling inhibitor is a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) at least 90% identical to SEQ ID NO:2;

(b) at least 95% identical to SEQ ID NO:2;

(c) at least 98% identical to SEQ ID NO:2;

(d) SEQ ID NO:2;

(e) at least 90% identical to SEQ ID NO:3;

(f) at least 95% identical to SEQ ID NO:3;

(g) at least 98% identical to SEQ ID NO:3;

(h) SEQ ID NO:3;

(i) at least 90% identical to SEQ ID NO:6;

(j) at least 95% identical to SEQ ID NO:6;

(k) at least 98% identical to SEQ ID NO:6;

(l) SEQ ID NO:6;

(m) at least 90% identical to SEQ ID NO:7;

(n) at least 95% identical to SEQ ID NO:7;

(o) at least 98% identical to SEQ ID NO:7;

(p) SEQ ID NO:7;

(q) at least 90% identical to SEQ ID NO:12;

(r) at least 95% identical to SEQ ID NO:12;

(s) at least 98% identical to SEQ ID NO:12;

(t) SEQ ID NO:12;

(u) at least 90% identical to SEQ ID NO:17;

(v) at least 95% identical to SEQ ID NO:17;

(w) at least 98% identical to SEQ ID NO:17;

(x) SEQ ID NO:17;

(y) at least 90% identical to SEQ ID NO:20;

(z) at least 95% identical to SEQ ID NO:20;

(aa) at least 98% identical to SEQ ID NO:20;

(bb) SEQ ID NO:20;

(cc) at least 90% identical to SEQ ID NO:21;

(dd) at least 95% identical to SEQ ID NO:21;

(ee) at least 98% identical to SEQ ID NO:21;

(ff) SEQ ID NO:21;

(gg) at least 90% identical to SEQ ID NO:25;

(hh) at least 95% identical to SEQ ID NO:25;

(ii) at least 98% identical to SEQ ID NO:25; and

(jj) SEQ ID NO:25.

[0007f] Provided herein is the use of an activin receptor type II (ActRII) signaling inhibitor in the manufacture of a medicament for treating and/or preventing a disease in a subject, wherein the medicament is formulated for administration at an initial dose, wherein a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1, Osterix, alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha is measured in the subject, wherein a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1, Osterix, alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha is measured in the subject after a period of time, wherein the medicament is formulated for administration at an adjusted dose based on the detected change between the first measurement and the second measurement and wherein the disease is:

(a) vascular calcification;

(b) cardiovascular disease associated with and/or resulting from vascular calcification;

(c) arterial stiffness;

(d) cardiovascular disease associated with and/or resulting from arterial stiffness;

(e) left ventricular hypertrophy (LVH);

(f) cardiovascular disease associated with and/or resulting from left ventricular hypertrophy;

(g) cardiovascular disease associated with and/or resulting from renal disease; or

(h) bone resorption associated with chronic kidney disease-mineral and bone disease (CKD-MBD), and

wherein the ActRII signaling inhibitor is a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) at least 90% identical to SEQ ID NO:2;
(b) at least 95% identical to SEQ ID NO:2;
(c) at least 98% identical to SEQ ID NO:2;
(d) SEQ ID NO:2;
(e) at least 90% identical to SEQ ID NO:3;
(f) at least 95% identical to SEQ ID NO:3;
(g) at least 98% identical to SEQ ID NO:3;
(h) SEQ ID NO:3;
(i) at least 90% identical to SEQ ID NO:6;
(j) at least 95% identical to SEQ ID NO:6;
(k) at least 98% identical to SEQ ID NO:6;
(l) SEQ ID NO:6;
(m) at least 90% identical to SEQ ID NO:7;
(n) at least 95% identical to SEQ ID NO:7;
(o) at least 98% identical to SEQ ID NO:7;
(p) SEQ ID NO:7;
(q) at least 90% identical to SEQ ID NO:12;
(r) at least 95% identical to SEQ ID NO:12;
(s) at least 98% identical to SEQ ID NO:12;
(t) SEQ ID NO:12;
(u) at least 90% identical to SEQ ID NO:17;
(v) at least 95% identical to SEQ ID NO:17;
(w) at least 98% identical to SEQ ID NO:17;
(x) SEQ ID NO:17;
(y) at least 90% identical to SEQ ID NO:20;
(z) at least 95% identical to SEQ ID NO:20;
(aa) at least 98% identical to SEQ ID NO:20;
(bb) SEQ ID NO:20;
(cc) at least 90% identical to SEQ ID NO:21;
(dd) at least 95% identical to SEQ ID NO:21;
(ee) at least 98% identical to SEQ ID NO:21;
(ff) SEQ ID NO:21;
(gg) at least 90% identical to SEQ ID NO:25;
(hh) at least 95% identical to SEQ ID NO:25;

2015339786 10 Feb 2021

(ii) at least 98% identical to SEQ ID NO:25; and

(jj) SEQ ID NO:25.

[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 **Error! Reference source not found.** 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 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.

[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 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, 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, Snail, 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, Snail, 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, Snail, 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, Snail, 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, Snail, 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, Snail, 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, Snail, 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, Snail, 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/dl)	8.3 ± 1.8	8.9 ± 0.9	9.4 ± 0.8	8.8 ± 0.3
Phosphorus (mg/dl)	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 CCCATGGTGCCGGGGGGAGACCGGTCTCCTTCC 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 TTGTTTTTCTTTCTTTCTTTTTTTTTTTTTTTCACT 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

SEQ ID NO:	Description	Sequence
		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 ATCAATCTTTTCTCAAAAAAAAAAAAAAAAAA
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 TCCCCGAAGTCCAACTGCTGTCCTCACCAAGCCC 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
		GGCTGGGAGTTGGAAAAAGCTAAAATTTCAGA ACAGTCTCTGTAAGGCTCTCTGTGGCTCCAGTT CACCATTTTATATTGTTGCATGCTGTAGAAAGG AGCTATTGCTGTTGTTTTGTTTTTTTATTTAAAT CACTAAGGCACTGTTTTTATCTTTTGTAAAAAA AAAAAAAAAGTTGTTCACTGTGCACTTATAGAA AAAATAATCAAAAATGTTGGGATTTTAGAAGCT CTCTTTTTGATAAACCAAAGATTTAGAAGTCAT TCCATTGTTAACTTGTAAAAATGTGTGAACACA GAGAGTTTTTGGTGATTGCTACTCTGAAAGCTG CCAGATCTTATTCTGGGGGTGGGATGTGGAGGA ATACACATACACACACAAACATACATGTATGTA TAATAGATATATACATATGTGTATATTATATCT GTGTGTGCATGTATCTCCAAAAGCGGCGTTACA GAGTTCTACACCAAAAGCCTTTAACCCTTAATC TGCTGTGAATGATACCTGGCCTTTCTCACTATG AATTTCTGATTAACCAACCAGACTACACGTTGC CTCTCTGTGTATGACTAACGGCTCCAACCCGAT GACTCACAGCTACTTGCTTATCGTGAACAAGCT CATCTTGGCAATGAATATGGATGTGAAAAGACA GAACAGCTTCACCATTAGTAGCTGGAAATGGTA TCACAGTCTCTTATAGAGGAATATGAAAGGAAC AAGAAAATCATTTTACATTCCTTTTATCTGTATT GTGCTTTAAAAGATCCACATGGTAAATTTTTTA TTTTGCTTTTATGTCAGTCATCAGAACCAAAAA AATCCAGAAGAAAAAATTGCCAGTGTTTCCTTT GAAGATGAAGCTACTGGGGAAGAAAACCTTAT TAATACACTCCACACATTTGTTCATTCCTCAGCT GTTGGTGTTTTCTTGGGGTCTTGACAAAGCTTGC TGGTCAGTGCACTTTTCAGGTGTCACGTTTTGCT GTTTGTATGTTTTTTCTTCCCCTTACTTCCTTTGG AAAACAAACTCACACAGTGCCCCTACTCTGAGA CCTGGGACTGAGTGTTAATTATTTTTTCCTTGGG TATTTCTATCTGAGAGACTAGACCTAGTTAGGA GGCCTCTGTACTTCTCCAGATTGTACCTTTTTAT GGGGATCTTTGAGGCTATGACCCAGGACTGATA GATATGCCTTACGGAAGACAAAAGATAAAATG GTTCCTATATCCTAATGCAAACCAACACAGTTA AAAGAGCAGATCTCTGGATAACTGCTCTCAACC TGCTTCTACAGTCTCCACAAACCGCATTCACCC TCTCTCTTCATAGCTCAGACATGAAATTTGAGG GAGAAAACTGGAGATAATTGGGAGAAAATTGA TGAAGTTGGCTGCTTCCAGTAGATCAGATAATC CATGAATTTGTCTCCCATTGAGAATTTTATTTTA AATTCTTTTAAACTCTTCGTTGTGTCTTTTGTGA

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

SEQ ID NO:	Description	Sequence
		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
		ACTTCAGTCTCTTCCTTGGAGGCCGAGGCCTAT GCTGCCTTCCCAGGCTTGGGCCAAGTGCCCAAG CAGCTGGCCCAGCTCTCTGAGGCCAAGGATCTC CAGGCTCGAAAGGCCTTCAACTGCAAATACTGC AACAAGGAATACCTCAGCCTGGGTGCCCTCAAG ATGCACATCCGAAGCCACACGCTGCCCTGCGTC TGCGGAACCTGCGGGAAGGCCTTCTCTAGGCCC TGGCTGCTACAAGGCCATGTCCGGACCCACACT GGCGAGAAGCCCTTCTCCTGTCCCCACTGCAGC CGTGCCTTCGCTGACCGCTCCAACCTGCGGGCC CACCTCCAGACCCACTCAGATGTCAAGAAGTAC CAGTGCCAGGCGTGTGCTCGGACCTTCTCCCGA ATGTCCCTGCTCCACAAGCACCAAGAGTCCGGC TGCTCAGGATGTCCCCGCTGACCCTCGAGGCTC CCTCTTCCTCTCCATACCTGCCCCTGCCTGACAG CCTTCCCCAGCTCCAGCAGGAAGGACCCCACAT CCTTCTCACTGCCATGGAATTCCCTCCTGAGTGC CCCACTTCTGGCCACATCAGCCCCACAGGACTT TGATGAAGACCATTTTCTGGTTCTGTGTCCTCTG CCTGGGCTCTGGAAGAGGCCTTCCCATGGCCAT TTCTGTGGAGGGAGGGCAGCTGGCCCCCAGCCC TGGGGGATTCCTGAGCTGGCCTGTCTGCGTGGG TTTTTGTATCCAGAGCTGTTTGGATACAGCTGCT TTGAGCTACAGGACAAAGGCTGACAGACTCACT GGGAAGCTCCCACCCCACTCAGGGGACCCCACT CCCCTCACACACACCCCCCCCACAAGGAACCCTC AGGCCACCCTCCACGAGGTGTGACTAACTATGC AATAATCCACCCCCAGGTGCAGCCCCAGGGCCT GCGGAGGCGGTGGCAGACTAGAGTCTGAGATG CCCCGAGCCCAGGCAGCTATTTCAGCCTCCTGT TTGGTGGGGTGGCACCTGTTTCCCGGGCAATTT AACAATGTCTGAAAAGGGACTGTGAGTAATGG CTGTCACTTGTCGGGGGCCCAAGTGGGGTGCTC TGGTCTGACCGATGTGTCTCCCAGAACTATTCT GGGGGCCCGACAGGTGGGCCTGGGAGGAAGAT GTTTACATTTTTAAAGGTACACTGGTATTTATAT TTCAAACATTTTGTATCAAGGAAACGTTTTGTA TAGTTATATGTACAGTTTATTGATATTCAATAA AGCAGTTAATTTATATATTAAAAAAAAAAAAA AAAAA
75	Dkk1 mRNA	GCAGAGCTCTGTGCTCCCTGCAGTCAGGACTCT GGGACCGCAGGGGGCTCCCGGACCCTGACTCTG CAGCCGAACCGGCACGGTTTCGTGGGGACCCA GGCTTGCAAAGTGACGGTCATTTTCTCTTTCTTT CTCCCTCTTGAGTCCTTCTGAGATGATGGCTCTG

SEQ ID NO:	Description	Sequence
		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

SEQ ID NO:	Description	Sequence
		ACAGTGTCTAAATATAAGACAATATTGATCAGC TCTAGAATAACTTTAAAGAAAGACGTGTTCTGC ATTGATAAACTCAAATGATCATGGCAGAATGAG AGTGAATCTTACATTACTACTTTCAAAAATAGT TTCCAATAAATTAATAATACCTAAAAAAAAAAA
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

SEQ ID NO:	Description	Sequence
		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
		AGACTGGCAACCTCAAGAAGGCCCTGCTCCTCC AGGGCTCCAACGAGATCGAGATCCGCGCCGAG GGCAACAGCCGCTTCACCTACAGCGTCACTGTC GATGGCTGCACGAGTCACACCGGAGCCTGGGG CAAGACAGTGATTGAATACAAAACCACCAAGA CCTCCCGCCTGCCCATCATCGATGTGGCCCCCT TGGACGTTGGTGCCCCAGACCAGGAATTCGGCT TCGACGTTGGCCCTGTCTGCTTCCTGTAAACTCC CTCCATCCCAACCTGGCTCCCTCCCACCCAACC AACTTTCCCCCCAACCCGGAAACAGACAAGCA ACCCAAACTGAACCCCCTCAAAAGCCAAAAAA TGGGAGACAATTTCACATGGACTTTGGAAAATA TTTTTTTCCTTTGCATTCATCTCTCAAACTTAGTT TTTATCTTTGACCAACCGAACATGACCAAAAAC CAAAAGTGCATTCAACCTTACCAAAAAAAAAA AAAAAAAAAGAATAAATAAATAACTTTTTAAA AAAGGAAGCTTGGTCCACTTGCTTGAAGACCCA TGCGGGGGTAAGTCCCTTTCTGCCCGTTGGGCT TATGAAACCCCAATGCTGCCCTTTCTGCTCCTTT CTCCACACCCCCCTTGGGGCCTCCCCTCCACTC CTTCCCAAATCTGTCTCCCCAGAAGACACAGGA AACAATGTATTGTCTGCCCAGCAATCAAAGGCA ATGCTCAAACACCCAAGTGGCCCCCACCCTCAG CCCGCTCCTGCCCGCCCAGCACCCCCAGGCCCT GGGGGACCTGGGGTTCTCAGACTGCCAAAGAA GCCTTGCCATCTGGCGCTCCCATGGCTCTTGCA ACATCTCCCCTTCGTTTTTGAGGGGGTCATGCC GGGGGAGCCACCAGCCCCTCACTGGGTTCGGA GGAGAGTCAGGAAGGGCCACGACAAAGCAGAA ACATCGGATTTGGGGAACGCGTGTCAATCCCTT GTGCCGCAGGGCTGGGCGGGAGAGACTGTTCT GTTCCTTGTGTAACTGTGTTGCTGAAAGACTAC CTCGTTCTTGTCTTGATGTGTCACCGGGGCAACT GCCTGGGGGCGGGGATGGGGGCAGGGTGGAAG CGGCTCCCCATTTTATACCAAAGGTGCTACATC TATGTGATGGGTGGGGTGGGGAGGGAATCACT GGTGCTATAGAAATTGAGATGCCCCCCCAGGCC AGCAAATGTTCCTTTTTGTTCAAAGTCTATTTTT ATTCCTTGATATTTTTCTTTTTTTTTTTTTTTTT GTGGATGGGGACTTGTGAATTTTTCTAAAGGTG CTATTTAACATGGGAGGAGAGCGTGTGCGGCTC CAGCCCAGCCCGCTGCTCACTTTCCACCCTCTCT CCACCTGCCTCTGGCTTCTCAGGCCTCTGCTCTC CGACCTCTCTCCTCTGAAACCCTCCTCCACAGC TGCAGCCCATCCTCCCGGCTCCCTCCTAGTCTGT

SEQ ID NO:	Description	Sequence
		CCTGCGTCCTCTGTCCCCGGGTTTCAGAGACAA CTTCCCAAAGCACAAAGCAGTTTTTCCCCCTAG GGGTGGGAGGAAGCAAAAGACTCTGTACCTAT TTTGTATGTGTATAATAATTTGAGATGTTTTTAA TTATTTTGATTGCTGGAATAAAGCATGTGGAAA TGACCCAAACATAA
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 TATTTTATGCCTTTTTTATTACAAGTCTTGTACTC AATGACTTTTGTCATGACATTTTGTTCTACTTAT ACTGTAAATTATGCATTATAAAGAGTTCATTTA AGGAAAATTACTTGGTACAATAATTATTGTAAT TAAGAGATGTAGCCTTTATTAAAATTTTATATTT

SEQ ID NO:	Description	Sequence
		TTCAAAAAAAAAAA
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.

2015339786 10 Feb 2021

WHAT IS CLAIMED:

1. A method of treating and/or preventing a 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 been selected based on:

(a) elevated levels of snail homolog 1 (Snai1) as compared to levels of Snai1 in a reference population;

(b) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population;

(c) elevated levels of collagen type 1 alpha 1 (col1a1) as compared to levels of col1a1 in a reference population;

(d) elevated levels of Osterix as compared to levels of Osterix in a reference population;

(e) decreased levels of alpha smooth muscle actin (alpha-SMA) as compared to levels of alpha-SMA in a reference population;

(f) decreased levels of myocardin (MYOCD) as compared to levels of MYOCD in a reference population;

(g) decreased levels of smooth muscle protein 22-alpha (Sm22-alpha) as compared to levels of Sm22-alpha in a reference population

(h) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population;

(i) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or

(j) decreased levels of Axin2 as compared to levels of Axin 2 in a reference population,

wherein the reference population:

(i) consists of healthy people;

(ii) consists of people of the same age, weight, and/or gender as the subject;

(iii) consists of people without cardiovascular disease;

(iv) consists of people without vascular calcification;

(v) consists of people without renal disease;

(vi) consists of people without chronic kidney disease;

(vii) consists of people without pathologically elevated levels of arterial stiffness; or

(viii) consists of people without left ventricular hypertrophy, and

wherein the disease is:

(a) vascular calcification;

(b) cardiovascular disease associated with and/or resulting from vascular calcification;

2015339786 10 Feb 2021

(c) arterial stiffness;

(d) cardiovascular disease associated with and/or resulting from arterial stiffness;

(e) left ventricular hypertrophy (LVH);

(f) cardiovascular disease associated with and/or resulting from left ventricular hypertrophy;

(g) cardiovascular disease associated with and/or resulting from renal disease; or

(h) bone resorption associated with chronic kidney disease-mineral and bone disease (CKD-MBD), and

wherein the ActRII signaling inhibitor is a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) at least 90% identical to SEQ ID NO:2;

(b) at least 95% identical to SEQ ID NO:2;

(c) at least 98% identical to SEQ ID NO:2;

(d) SEQ ID NO:2;

(e) at least 90% identical to SEQ ID NO:3;

(f) at least 95% identical to SEQ ID NO:3;

(g) at least 98% identical to SEQ ID NO:3;

(h) SEQ ID NO:3;

(i) at least 90% identical to SEQ ID NO:6;

(j) at least 95% identical to SEQ ID NO:6;

(k) at least 98% identical to SEQ ID NO:6;

(l) SEQ ID NO:6;

(m) at least 90% identical to SEQ ID NO:7;

(n) at least 95% identical to SEQ ID NO:7;

(o) at least 98% identical to SEQ ID NO:7;

(p) SEQ ID NO:7;

(q) at least 90% identical to SEQ ID NO:12;

(r) at least 95% identical to SEQ ID NO:12;

(s) at least 98% identical to SEQ ID NO:12;

(t) SEQ ID NO:12;

(u) at least 90% identical to SEQ ID NO:17;

(v) at least 95% identical to SEQ ID NO:17;

(w) at least 98% identical to SEQ ID NO:17;

(x) SEQ ID NO:17;

(y) at least 90% identical to SEQ ID NO:20;

2015339786 10 Feb 2021

(z) at least 95% identical to SEQ ID NO:20;

(aa) at least 98% identical to SEQ ID NO:20;

(bb) SEQ ID NO:20;

(cc) at least 90% identical to SEQ ID NO:21;

(dd) at least 95% identical to SEQ ID NO:21;

(ee) at least 98% identical to SEQ ID NO:21;

(ff) SEQ ID NO:21;

(gg) at least 90% identical to SEQ ID NO:25;

(hh) at least 95% identical to SEQ ID NO:25;

(ii) at least 98% identical to SEQ ID NO:25; and

(jj) SEQ ID NO:25.

2. The method of claim 1, wherein the pharmaceutically effective dose of the ActRII signaling inhibitor is:

(a) 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;

(b) administered *via* injection; and/or

(c) administered:

(i) once every 14 days;

(ii) once every 21 days;

(iii) once every 28 days;

(iv) once every 42 days; or

(v) continuously and/or indefinitely.

3. The method of claim 1 or claim 2, wherein the pharmaceutical composition is used such that the ActRII signaling inhibitor is:

(a) administered in an amount of about 1.0 mg/kg;

(b) administered via injection; and/or

(c) administered once every 21 days.

4. The method of any one of claims 1-3, wherein the elevated levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1 and/or Osterix, are:

2015339786 10 Feb 2021

(a) 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, col1a1 and/or Osterix, respectively, in the reference population; or

(b) 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, col1a1 and/or Osterix in the top 10%, top 5%, top 4%, top 3%, top 2%, or top 1% in the reference population.

5. The method of any one of claims 1-3, wherein the decreased levels of alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha are:

(a) about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the levels of alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha, respectively, in the reference population; or

(b) equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the levels of alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population.

6. A method of treating and/or preventing a disease in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an activin receptor type II (ActRII) signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1, Osterix, alpha-SMA, MYOCD, 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, col1a1, Osterix, alpha-SMA, MYOCD, 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 treating and/or preventing the disease, and wherein the disease is:

(a) vascular calcification;

(b) cardiovascular disease associated with and/or resulting from vascular calcification;

(c) arterial stiffness;

(d) cardiovascular disease associated with and/or resulting from arterial stiffness;

(e) left ventricular hypertrophy (LVH);

2015339786 10 Feb 2021

(f) cardiovascular disease associated with and/or resulting from left ventricular hypertrophy;

(g) cardiovascular disease associated with and/or resulting from renal disease; or

(h) bone resorption associated with chronic kidney disease-mineral and bone disease (CKD-MBD), and

wherein the ActRII signaling inhibitor is a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) at least 90% identical to SEQ ID NO:2;

(b) at least 95% identical to SEQ ID NO:2;

(c) at least 98% identical to SEQ ID NO:2;

(d) SEQ ID NO:2;

(e) at least 90% identical to SEQ ID NO:3;

(f) at least 95% identical to SEQ ID NO:3;

(g) at least 98% identical to SEQ ID NO:3;

(h) SEQ ID NO:3;

(i) at least 90% identical to SEQ ID NO:6;

(j) at least 95% identical to SEQ ID NO:6;

(k) at least 98% identical to SEQ ID NO:6;

(l) SEQ ID NO:6;

(m) at least 90% identical to SEQ ID NO:7;

(n) at least 95% identical to SEQ ID NO:7;

(o) at least 98% identical to SEQ ID NO:7;

(p) SEQ ID NO:7;

(q) at least 90% identical to SEQ ID NO:12;

(r) at least 95% identical to SEQ ID NO:12;

(s) at least 98% identical to SEQ ID NO:12;

(t) SEQ ID NO:12;

(u) at least 90% identical to SEQ ID NO:17;

(v) at least 95% identical to SEQ ID NO:17;

(w) at least 98% identical to SEQ ID NO:17;

(x) SEQ ID NO:17;

(y) at least 90% identical to SEQ ID NO:20;

(z) at least 95% identical to SEQ ID NO:20;

(aa) at least 98% identical to SEQ ID NO:20;

(bb) SEQ ID NO:20;

2015339786 10 Feb 2021

(cc) at least 90% identical to SEQ ID NO:21;

(dd) at least 95% identical to SEQ ID NO:21;

(ee) at least 98% identical to SEQ ID NO:21;

(ff) SEQ ID NO:21;

(gg) at least 90% identical to SEQ ID NO:25;

(hh) at least 95% identical to SEQ ID NO:25;

(ii) at least 98% identical to SEQ ID NO:25; and

(jj) SEQ ID NO:25.

7. The method of claim 6, wherein the initial dose of the ActRII signaling inhibitor is:

(a) 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

(b) administered *via* injection; and/or

(c) administered:

(i) once every 14 days;

(ii) once every 21 days;

(iii) once every 28 days; or

(iv) once every 42 days.

8. The method of claim 6 or claim 7, wherein the initial dose of the ActRII signaling inhibitor is:

(a) about 1.0 mg/kg;

(b) administered via injection; and/or

(c) administered once every 21 days.

9. The method of any one of claims 6-8, wherein the adjusted dose of the ActRII signaling inhibitor is greater than the initial dose if:

(a) the level of Snai1 is elevated as compared to the level of Snai1 in a reference population;

(b) the level of phosphosmad2 is elevated as compared to the level of phosphosmad2 in a reference population;

2015339786 10 Feb 2021

(c) the level of col1a1 is elevated as compared to the level of col1a1 in a reference population;

(d) the level of Osterix is elevated as compared to the level of Osterix in a reference population;

(e) the level of alpha-SMA is decreased as compared to the level of alpha-SMA in a reference population;

(f) the level of MYOCD is decreased as compared to the level of MYOCD in a reference population;

(g) the level of Sm22-alpha is decreased as compared to the level of Sm22-alpha in a reference population;

(h) the level of phosphosmad3 is elevated as compared to the level of phosphosmad3 in a reference population;

(i) the level of urinary protein is elevated as compared to the level of urinary protein in a reference population; and/or

(j) the level of Axin2 is decreased compared to the level of Axin2 in a reference population, and wherein the reference population

(i) consists of healthy people;

(ii) consists of people of the same age, weight, and/or gender as the subject;

(iii) consists of people without cardiovascular disease;

(iv) consists of people without vascular calcification;

(v) consists of people without renal disease;

(vi) consists of people without chronic kidney disease;

(vii) consists of people without pathologically elevated levels of arterial stiffness; or

(viii) consists of people without left ventricular hypertrophy.

10. The method of claim 9, wherein the adjusted dose is:

(a) 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

(b) administered more frequently than the initial dose; and/or

(c) administered every 5, 10, 15, 20, 25, 28, 30, 35, or 40 days.

11. The method of any one of claims 6-8, wherein the adjusted dose of the ActRII signaling inhibitor is less than the initial dose if:

2015339786 10 Feb 2021

(a) the level of Snai1 is decreased as compared to the level of Snai1 in a reference population;

(b) the level of phosphosmad2 is decreased as compared to the level of phosphosmad2 in a reference population;

(c) the level of col1a1 is decreased as compared to the level of col1a1 in a reference population;

(d) the level of Osterix is decreased as compared to the level of Osterix in a reference population;

(e) the level of alpha-SMA is elevated as compared to the level of alpha-SMA in a reference population;

(f) the level of MYOCD is elevated as compared to the level of MYOCD in a reference population;

(g) the level of Sm22-alpha is elevated as compared to the level of Sm22-alpha in a reference population;

(h) the level of phosphosmad3 is decreased as compared to the level of phosphosmad3 in a reference population;

(i) the level of urinary protein is decreased as compared to the level of urinary protein in a reference population; and/or

(j) the level of Axin2 is elevated as compared to the level of Axin2 in a reference population, and

wherein the reference population:

(i) consists of healthy people;

(ii) consists of people of the same age, weight, and/or gender as the subject;

(iii) consists of people without cardiovascular disease;

(iv) consists of people without vascular calcification;

(v) consists of people without renal disease;

(vi) consists of people without chronic kidney disease;

(vii) consists of people without pathologically elevated levels of arterial stiffness; or

(viii) consists of people without left ventricular hypertrophy.

12. The method of claim 11, wherein the adjusted dose is:

(a) 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

2015339786 10 Feb 2021

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;

(b) administered less frequently than the initial dose;

(c) administered every 30, 35, 40, 42, 50, 60, 70, 80, or 90 days; and/or

(d) administered continuously and/or indefinitely.

13. The method of any one of claims 6-12, wherein:

(a) the first measurement is taken prior to the commencement of the treatment;

(b) 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; or

(c) 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.

14. The method of claim 9, wherein

(a) the elevated levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1 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, col1a1 and/or Osterix, respectively, in the reference population;

(b) the decreased levels of alpha-SMA, MYOCD, 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 alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha, respectively, in a reference population;

(c) the elevated levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1 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, col1a1 and/or Osterix in the top 10%, top 5%, top 4%, top 3%, top 2%, or top 1% in the reference population; and/or

(d) the decreased levels of alpha-SMA, MYOCD, 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 alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population.

15. The method of claim 11, wherein

2015339786 10 Feb 2021

(a) the elevated levels of alpha-SMA, MYOCD, 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 alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha, respectively, in the reference population;

(b) the decreased levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1 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, col1a1 and/or Osterix, respectively, in a reference population;

(c) the elevated levels of alpha-SMA, MYOCD, 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 alpha-SMA, MYOCD, 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

(d) the decreased levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1 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, col1a1 and/or Osterix in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population.

16. The method of any one of claims 1-15, wherein the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1, Osterix, alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha is the protein level of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1, Osterix, alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha, respectively, preferably wherein the protein level is determined by enzyme-linked immunosorbent assay (ELISA),

more preferably, wherein the ELISA is performed with

(a) Snai1-specific antibody sc-393172 (Santa Cruz) to determine Snai1 levels;

(b) Phosphosmad2-specific antibody sc-101801 (Santa Cruz) to determine phosphosmad2 levels;

(c) Phosphosmad3-specific antibody sc-130218 (Santa Cruz) to determine phosphosmad3 levels;

(d) Col1a1-specific antibody sc-8784 (Santa Cruz) to determine col1a1 levels;

(e) Osterix-specific antibody SC-22538 (Santa Cruz) to determine Osterix levels;

(f) Alpha-SMA-specific antibody SC-53142 (Santa Cruz) to determine alpha-SMA levels;

(g) MYOCD-specific antibody SC-21561 (Santa Cruz) to determine MYOCD levels; and/or

2015339786 10 Feb 2021

(h) Sm22-alpha-specific antibody SC-271719 (Santa Cruz) to determine Sm22-alpha levels.

17. The method of any one claims 1-15, wherein the level of Snai1, col1a1, Osterix, alpha-SMA, MYOCD and/or Sm22-alpha is the mRNA level of Snai1, col1a1, Osterix, alpha-SMA, MYOCD and/or Sm22-alpha, respectively, preferably wherein the mRNA level is determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR),

more preferably, wherein the qRT-PCR is performed with

(a) Col1a1-specific primers (SEQ ID NOS: 82 and 83) to determine col1a1 levels;

(b) Snai1-specific primers (SEQ ID NOS:78 and 79) to determine Snai1 levels;

(c) Osterix-specific primers (SEQ ID NOS: 52 and 53) to determine Osterix levels; and/or

(d) Sm22-alpha-specific primers (SEQ ID NOS: 56 and 57) to determine Sm22-alpha levels.

18. The method of any one of claims 1-17, wherein:

(a) the levels are in a tissue, preferably wherein the tissue is aorta, serum, bone marrow, liver, or spleen;

(b) the subject is human; and/or

(c) the reference population consists of 1, 5, 10, 25, 50, 75, 100, 200, 250, 300, 400, 500, or 1000 individuals.

19. The method of any one of claims 1-18, wherein the ActRII signaling inhibitor is:

(a) a polypeptide comprising the amino acid sequence of SEQ ID NO:7, or

(b) a humanized fusion-protein consisting of the extracellular domain of ActRIIA and the human IgG1 Fc domain.

20. The method of any one of claims 1-18, wherein the ActRII signaling inhibitor is a polypeptide comprising: (i) a fragment of the extracellular domain of ActRIIB, wherein the fragment consists of the sequence of amino acid 25 to 131 of SEQ ID NO:28 and wherein the fragment carries the L79D amino acid substitution; (ii) a linker; and (iii) an Fc of an IgG.

21. Use of an activin receptor type II (ActRII) signaling inhibitor in the manufacture of a medicament for treating and/or preventing a disease in a subject, wherein the subject has been selected based on:

2015339786 10 Feb 2021

(a) elevated levels of snail homolog 1 (Snai1) as compared to levels of Snai1 in a reference population;

(b) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population;

(c) elevated levels of collagen type 1 alpha 1 (col1a1) as compared to levels of col1a1 in a reference population;

(d) elevated levels of Osterix as compared to levels of Osterix in a reference population;

(e) decreased levels of alpha smooth muscle actin (alpha-SMA) as compared to levels of alpha-SMA in a reference population;

(f) decreased levels of myocardin (MYOCD) as compared to levels of MYOCD in a reference population;

(g) decreased levels of smooth muscle protein 22-alpha (Sm22-alpha) as compared to levels of Sm22-alpha in a reference population;

(h) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population;

(i) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or

(j) decreased levels of Axin2 as compared to levels of Axin2 in a reference population,

wherein the reference population:

(i) consists of healthy people;

(ii) consists of people of the same age, weight, and/or gender as the subject;

(iii) consists of people without cardiovascular disease;

(iv) consists of people without vascular calcification;

(v) consists of people without renal disease;

(vi) consists of people without chronic kidney disease;

(vii) consists of people without pathologically elevated levels of arterial stiffness; or

(viii) consists of people without left ventricular hypertrophy, and

wherein the disease is:

(a) vascular calcification;

(b) cardiovascular disease associated with and/or resulting from vascular calcification;

(c) arterial stiffness;

(d) cardiovascular disease associated with and/or resulting from arterial stiffness;

(e) left ventricular hypertrophy (LVH);

(f) cardiovascular disease associated with and/or resulting from left ventricular hypertrophy;

2015339786 10 Feb 2021

(g) cardiovascular disease associated with and/or resulting from renal disease; or

(h) bone resorption associated with chronic kidney disease-mineral and bone disease (CKD-MBD); and

wherein the ActRII signaling inhibitor is a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) at least 90% identical to SEQ ID NO:2;

(b) at least 95% identical to SEQ ID NO:2;

(c) at least 98% identical to SEQ ID NO:2;

(d) SEQ ID NO:2;

(e) at least 90% identical to SEQ ID NO:3;

(f) at least 95% identical to SEQ ID NO:3;

(g) at least 98% identical to SEQ ID NO:3;

(h) SEQ ID NO:3;

(i) at least 90% identical to SEQ ID NO:6;

(j) at least 95% identical to SEQ ID NO:6;

(k) at least 98% identical to SEQ ID NO:6;

(l) SEQ ID NO:6;

(m) at least 90% identical to SEQ ID NO:7;

(n) at least 95% identical to SEQ ID NO:7;

(o) at least 98% identical to SEQ ID NO:7;

(p) SEQ ID NO:7;

(q) at least 90% identical to SEQ ID NO:12;

(r) at least 95% identical to SEQ ID NO:12;

(s) at least 98% identical to SEQ ID NO:12;

(t) SEQ ID NO:12;

(u) at least 90% identical to SEQ ID NO:17;

(v) at least 95% identical to SEQ ID NO:17;

(w) at least 98% identical to SEQ ID NO:17;

(x) SEQ ID NO:17;

(y) at least 90% identical to SEQ ID NO:20;

(z) at least 95% identical to SEQ ID NO:20;

(aa) at least 98% identical to SEQ ID NO:20;

(bb) SEQ ID NO:20;

(cc) at least 90% identical to SEQ ID NO:21;

(dd) at least 95% identical to SEQ ID NO:21;

2015339786 10 Feb 2021

(ee) at least 98% identical to SEQ ID NO:21;

(ff) SEQ ID NO:21;

(gg) at least 90% identical to SEQ ID NO:25;

(hh) at least 95% identical to SEQ ID NO:25;

(ii) at least 98% identical to SEQ ID NO:25; and

(jj) SEQ ID NO:25.

22. The use according to claim 21, wherein the pharmaceutically effective dose of the ActRII signaling inhibitor is:

(a) 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;

(b) administered *via* injection; and/or

(c) administered:

(i) once every 14 days;

(ii) once every 21 days;

(iii) once every 28 days;

(iv) once every 42 days; or

(v) continuously and/or indefinitely.

23. The use according to claim 21 or claim 22, wherein the initial dose of the ActRII signaling inhibitor is:

(a) about 1.0 mg/kg;

(b) administered via injection; and/or

(c) administered once every 21 days.

24. The use according to any one of claims 21-23, wherein the elevated levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1 and/or Osterix are:

(a) 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, col1a1 and/or Osterix, respectively, in the reference population; or

(b) 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

2015339786 10 Feb 2021

protein, col1a1 and/or Osterix in the top 10%, top 5%, top 4%, top 3%, top 2%, or top 1% in the reference population.

25. The use according to any one of claims 21-23, wherein the decreased levels of alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha are:

(a) about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the levels of alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha, respectively, in the reference population; or

(b) equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the levels of alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population.

26. Use of an activin receptor type II (ActRII) signaling inhibitor in the manufacture of a medicament for treating and/or preventing a disease in a subject, wherein the medicament is formulated for administration at an initial dose, wherein a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1, Osterix, alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha is measured in the subject, wherein a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1, Osterix, alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha is measured in the subject after a period of time, wherein the medicament is formulated for administration at an adjusted dose based on the detected change between the first measurement and the second measurement and wherein the disease is:

(a) vascular calcification;

(b) cardiovascular disease associated with and/or resulting from vascular calcification;

(c) arterial stiffness;

(d) cardiovascular disease associated with and/or resulting from arterial stiffness;

(e) left ventricular hypertrophy (LVH);

(f) cardiovascular disease associated with and/or resulting from left ventricular hypertrophy;

(g) cardiovascular disease associated with and/or resulting from renal disease; or

(h) bone resorption associated with chronic kidney disease-mineral and bone disease (CKD-MBD), and

wherein the ActRII signaling inhibitor is a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) at least 90% identical to SEQ ID NO:2;

(b) at least 95% identical to SEQ ID NO:2;

2015339786 10 Feb 2021

(c) at least 98% identical to SEQ ID NO:2;

(d) SEQ ID NO:2;

(e) at least 90% identical to SEQ ID NO:3;

(f) at least 95% identical to SEQ ID NO:3;

(g) at least 98% identical to SEQ ID NO:3;

(h) SEQ ID NO:3;

(i) at least 90% identical to SEQ ID NO:6;

(j) at least 95% identical to SEQ ID NO:6;

(k) at least 98% identical to SEQ ID NO:6;

(l) SEQ ID NO:6;

(m) at least 90% identical to SEQ ID NO:7;

(n) at least 95% identical to SEQ ID NO:7;

(o) at least 98% identical to SEQ ID NO:7;

(p) SEQ ID NO:7;

(q) at least 90% identical to SEQ ID NO:12;

(r) at least 95% identical to SEQ ID NO:12;

(s) at least 98% identical to SEQ ID NO:12;

(t) SEQ ID NO:12;

(u) at least 90% identical to SEQ ID NO:17;

(v) at least 95% identical to SEQ ID NO:17;

(w) at least 98% identical to SEQ ID NO:17;

(x) SEQ ID NO:17;

(y) at least 90% identical to SEQ ID NO:20;

(z) at least 95% identical to SEQ ID NO:20;

(aa) at least 98% identical to SEQ ID NO:20;

(bb) SEQ ID NO:20;

(cc) at least 90% identical to SEQ ID NO:21;

(dd) at least 95% identical to SEQ ID NO:21;

(ee) at least 98% identical to SEQ ID NO:21;

(ff) SEQ ID NO:21;

(gg) at least 90% identical to SEQ ID NO:25;

(hh) at least 95% identical to SEQ ID NO:25;

(ii) at least 98% identical to SEQ ID NO:25; and

(jj) SEQ ID NO:25.

2015339786 10 Feb 2021

27. The use of claim 26, wherein the initial dose of the ActRII signaling inhibitor is:

(a) 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;

(b) administered *via* injection; and/or

(c) administered:

(i) once every 14 days;

(ii) once every 21 days,

(iii) once every 28 days; or

(iv) once every 42 days.

28. The use according to claim 26 or claim 27, wherein the initial dose of the ActRII signaling inhibitor is:

(a) about 1.0 mg/kg;

(b) administered via injection; and/or

(c) administered once every 21 days.

29. The use of any one of claims 26-28, wherein the adjusted dose of the ActRII signaling inhibitor is greater than the initial dose if:

(a) the level of Snai1 is elevated as compared to the level of Snai1 in a reference population;

(b) the level of phosphosmad2 is elevated as compared to the level of phosphosmad2 in a reference population;

(c) the level of col1a1 is elevated as compared to the level of col1a1 in a reference population;

(d) the level of Osterix is elevated as compared to the level of Osterix in a reference population;

(e) the level of alpha-SMA is decreased as compared to the level of alpha-SMA in a reference population;

(f) the level of MYOCD is decreased as compared to the level of MYOCD in a reference population;

(g) the level of Sm22-alpha is decreased as compared to the level of Sm22-alpha in a reference population;

2015339786 10 Feb 2021

(h) the level of phosphosmad3 is elevated as compared to the level of phosphosmad3 in a reference population;

(i) the level of urinary protein is elevated as compared to the level of urinary protein in a reference population;

and/or

(j) the level of Axin2 is decreased compared to the level of Axin2 in a reference population, and

wherein the reference population:

(i) consists of healthy people;

(ii) consists of people of the same age, weight, and/or gender as the subject;

(iii) consists of people without cardiovascular disease;

(iv) consists of people without vascular calcification;

(v) consists of people without renal disease;

(vi) consists of people without chronic kidney disease;

(vii) consists of people without pathologically elevated levels of arterial stiffness; or

(viii) consists of people without left ventricular hypertrophy.

30. The use of claim 29, wherein the adjusted dose is:

(a) 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;

(b) administered more frequently than the initial dose; and/or

(c) administered every 5, 10, 15, 20, 25, 28, 30, 35, or 40 days.

31. The use of any one of claims 27-29, wherein the adjusted dose of the ActRII signaling inhibitor is less than the initial dose if:

(a) the level of Snai1 is decreased as compared to the level of Snai1 in a reference population;

(b) the level of phosphosmad2 is decreased as compared to the level of phosphosmad2 in a reference population;

(c) the level of col1a1 is decreased as compared to the level of col1a1 in a reference population;

2015339786 10 Feb 2021

(d) the level of Osterix is decreased as compared to the level of Osterix in a reference population;

(e) the level of alpha-SMA is elevated as compared to the level of alpha-SMA in a reference population;

(f) the level of MYOCD is elevated as compared to the level of MYOCD in a reference population;

(g) the level of Sm22-alpha is elevated as compared to the level of Sm22-alpha in a reference population;

(h) the level of phosphosmad3 is decreased as compared to the level of phosphosmad3 in a reference population;

(i) the level of urinary protein is decreased as compared to the level of urinary protein in a reference population

and/or

(j) the level of Axin2 is elevated as compared to the level of Axin2 in a reference population, and

wherein the reference population:

(i) consists of healthy people;

(ii) consists of people of the same age, weight, and/or gender as the subject;

(iii) consists of people without cardiovascular disease;

(iv) consists of people without vascular calcification;

(v) consists of people without renal disease;

(vi) consists of people without chronic kidney disease;

(vii) consists of people without pathologically elevated levels of arterial stiffness; or

(viii) consists of people without left ventricular hypertrophy.

32. The use of claim 31, wherein the adjusted dose is:

(a) 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;

(b) administered less frequently than the initial dose;

(c) administered every 30, 35, 40, 42, 50, 60, 70, 80, or 90 days; and/or

(d) administered continuously and/or indefinitely.

33. The use of any one of claims 26-32, wherein:

2015339786 10 Feb 2021

(a) the first measurement is taken prior to the commencement of the treatment;

(b) 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; or

(c) 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.

34. The use of claim 29, wherein:

(a) the elevated levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1 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, col1a1 and/or Osterix, respectively, in the reference population;

(b) the decreased levels of, alpha-SMA, MYOCD, 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 alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha, respectively, in a reference population;

(c) the elevated levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1 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, col1a1 and/or Osterix in the top 10%, top 5%, top 4%, top 3%, top 2%, or top 1% in the reference population; and/or

(d) the decreased levels of alpha-SMA, MYOCD, 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 alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population.

35. The use of claim 31, wherein

(a) the elevated levels of alpha-SMA, MYOCD, 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 alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha, respectively, in the reference population;

(b) the decreased levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1 and/or Osterix are about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80% 90%, or 100%

2015339786 10 Feb 2021

less than the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1 and/or Osterix, respectively, in a reference population;

(c) the elevated levels of alpha-SMA, MYOCD, 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 alpha-SMA, MYOCD, 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

(d) the decreased levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1 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, col1a1 and/or Osterix in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population.

36. The use of any one of claims 21-35 wherein the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1, Osterix, alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha is the protein level of Snai1, phosphosmad2, phosphosmad3, urinary protein, col1a1, Osterix, alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha, respectively,

preferably wherein the protein level is determined by enzyme-linked immunosorbent assay (ELISA),

more preferably, wherein the ELISA is performed with

(a) Snai1-specific antibody sc-393172 (Santa Cruz) to determine Snai1 levels;

(b) Phosphosmad2-specific antibody sc-101801 (Santa Cruz) to determine phosphosmad2 levels;

(c) Phosphosmad3-specific antibody sc-130218 (Santa Cruz) to determine phosphosmad3 levels;

(d) Col1a1-specific antibody sc-8784 (Santa Cruz) to determine col1a1 levels;

(e) Osterix-specific antibody SC-22538 (Santa Cruz) to determine Osterix levels;

(f) Alpha-SMA-specific antibody SC-53142 (Santa Cruz) to determine alpha-SMA levels;

(g) MYOCD-specific antibody SC-21561 (Santa Cruz) to determine MYOCD levels; and/or

(h) Sm22-alpha-specific antibody SC-271719 (Santa Cruz) to determine Sm22-alpha levels.

37. The use of any one of claims 19-31, wherein the level of Snai1, col1a1, , Osterix, alpha-SMA, MYOCD and/or Sm22-alpha is the mRNA level of Snai1, col1a1, Osterix, alpha-SMA, MYOCD and/or Sm22-alpha, respectively,

2015339786 10 Feb 2021

preferably wherein the mRNA level is determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR),

more preferably, wherein the qRT-PCR is performed with

(a) Col1a1-specific primers (SEQ ID NOS: 82 and 83) to determine col1a1 levels;

(b) Snai1-specific primers (SEQ ID NOS:78 and 79) to determine Snai1 levels;

(c) Osterix-specific primers (SEQ ID NOS: 52 and 53) to determine Osterix levels; and/or

(d) Sm22-alpha-specific primers (SEQ ID NOS: 56 and 57) to determine Sm22-alpha levels.

38. The use of any one of claims 21-37, wherein:

(a) the levels are in a tissue, preferably wherein the tissue is aorta, serum, bone marrow, liver, or spleen;

(b) the subject is human; and/or

(c) the reference population consists of 1, 5, 10, 25, 50, 75, 100, 200, 250, 300, 400, 500, or 1000 individuals.

39. The use of any one of claims 21-38, wherein the ActRII signaling inhibitor is:

(a) a polypeptide comprising the amino acid sequence of SEQ ID NO:7, or

(b) a humanized fusion-protein consisting of the extracellular domain of ActRIIA and the human IgG1 Fc domain.

40. The use of any one of claims 21-38, wherein the ActRII signaling inhibitor is a polypeptide comprising: (i) a fragment of the extracellular domain of ActRIIB, wherein the fragment consists of the sequence of amino acid 25 to 131 of SEQ ID NO:28 and wherein the fragment carries the L79D amino acid substitution; (ii) a linker; and (iii) an Fc of an IgG.

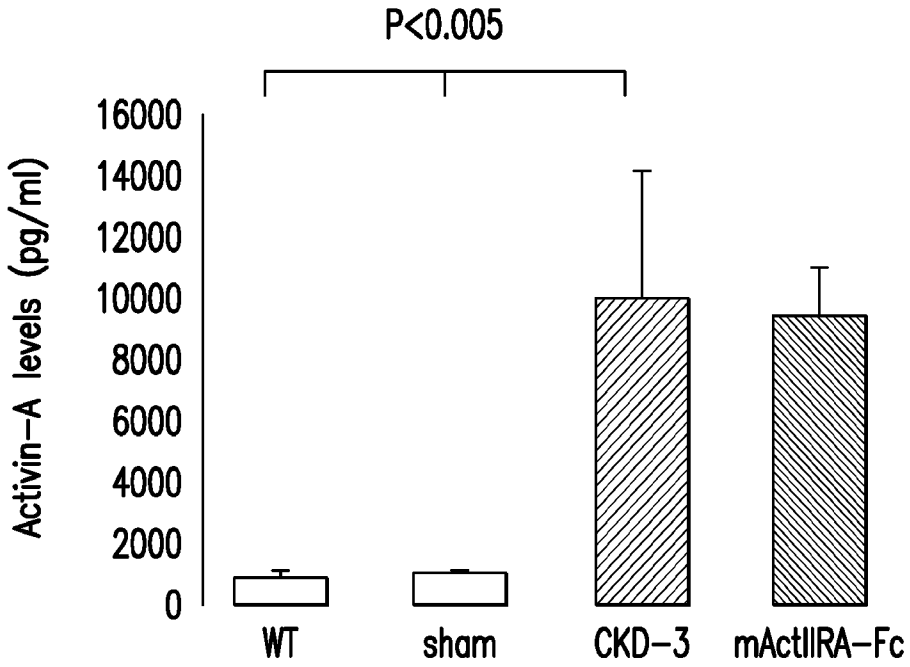

P<0.005
Activin-A levels (pg/ml)
16000
14000
12000
10000
8000
6000
4000
2000
0
WT
sham
CKD-3
mActIIRA-Fc

FIG. 1A

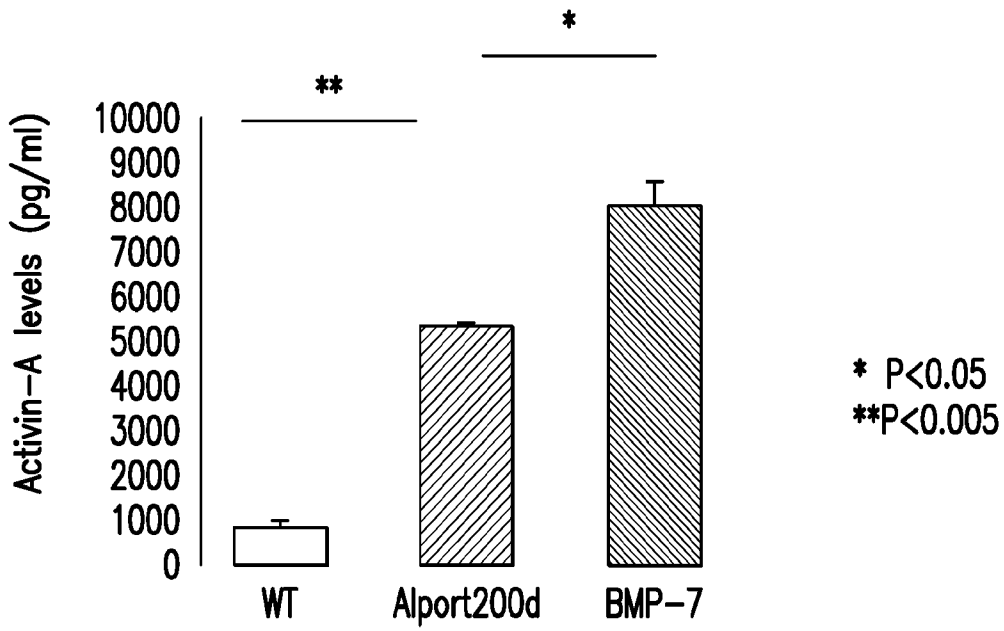

*
**
Activin-A levels (pg/ml)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
WT
Alport200d
BMP-7
* P<0.05
**P<0.005

FIG. 1B

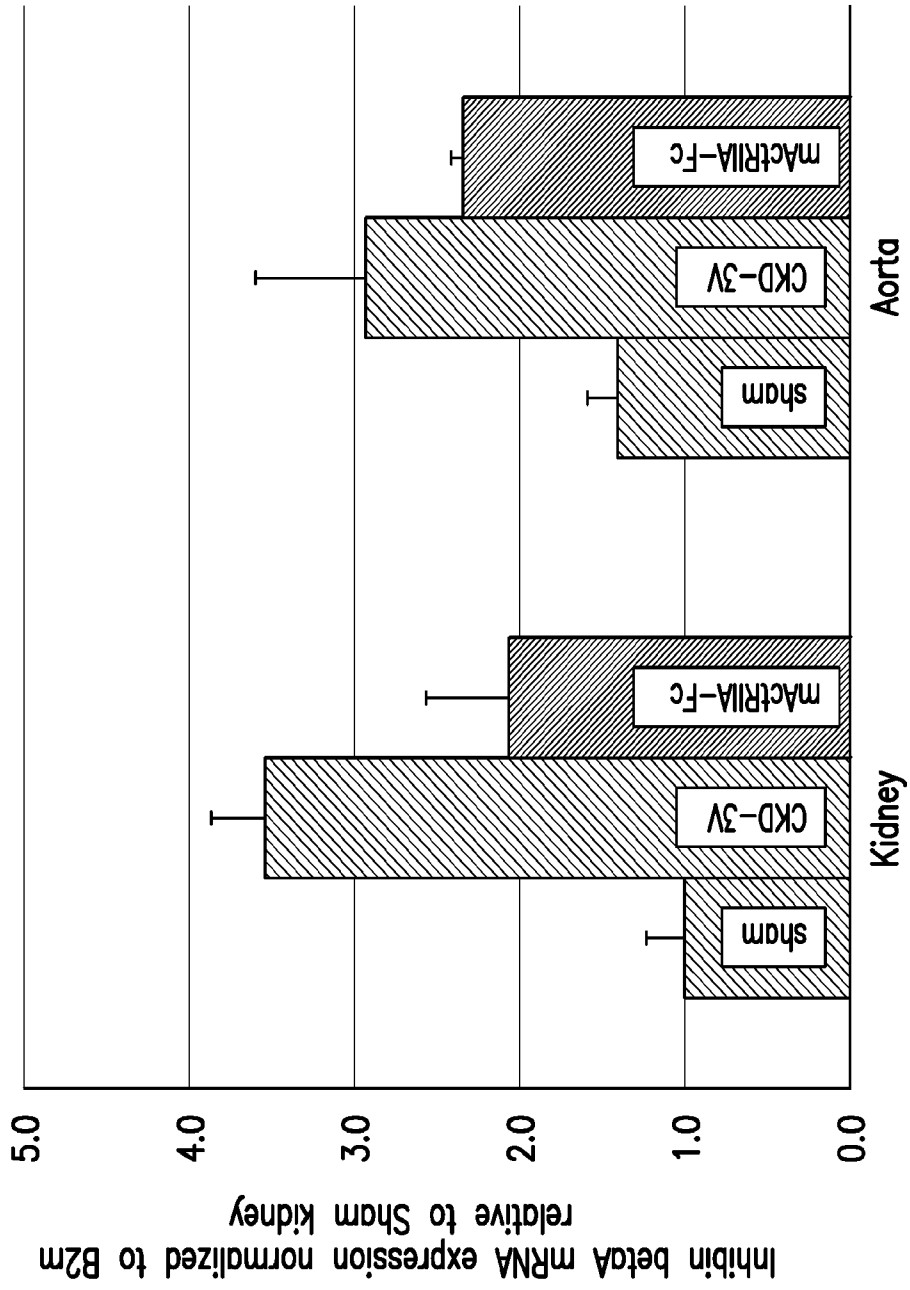

Inhibin betaA mRNA expression normalized to B2m
relative to Sham kidney
5.0
4.0
3.0
2.0
1.0
0.0
Kidney
Aorta
sham
CKD-3V
mActRIIA-Fc
sham
CKD-3V
mActRIIA-Fc

FIG. 2

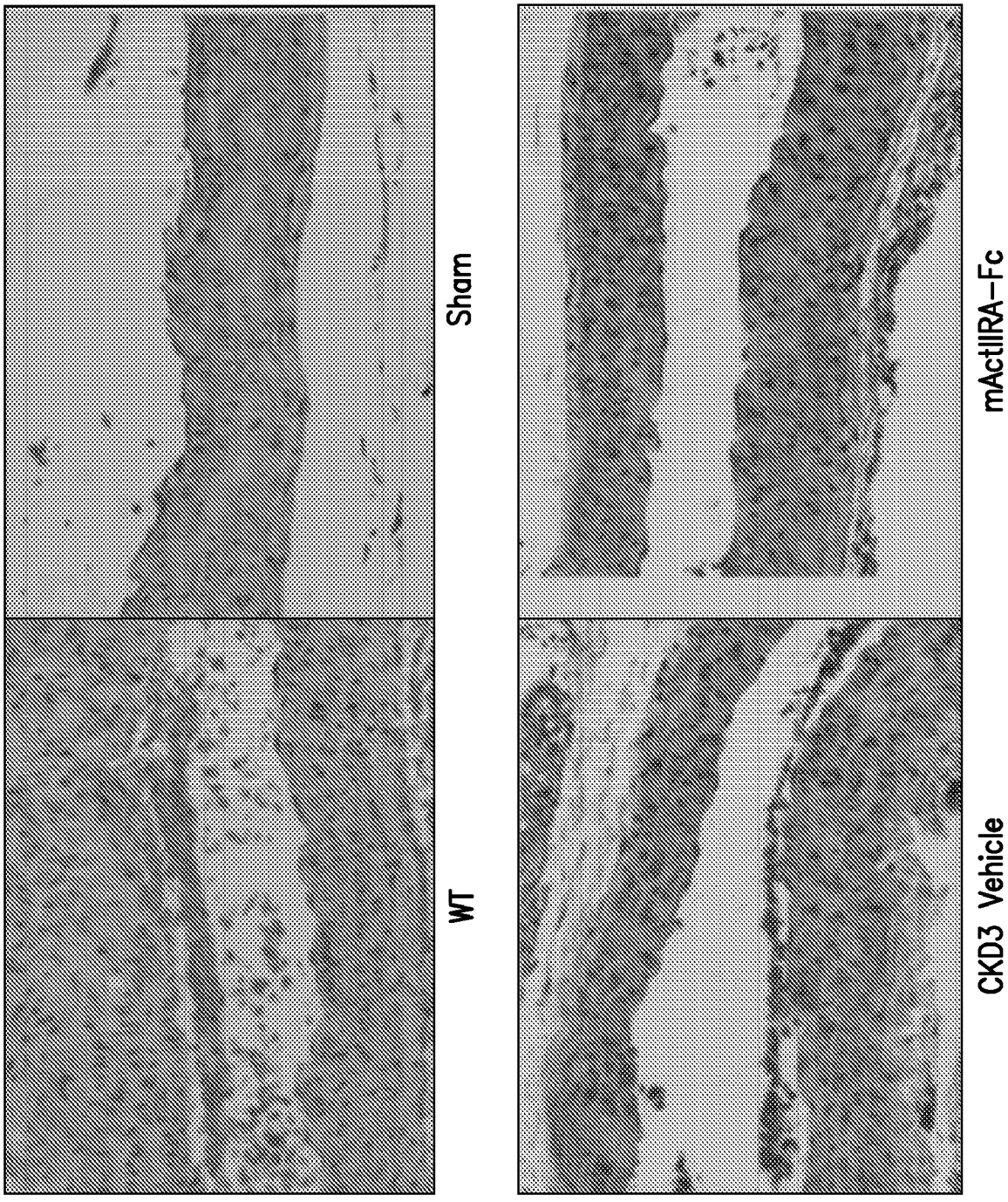


FIG. 3A

FIG. 3B

FIG. 3C

FIG. 3D

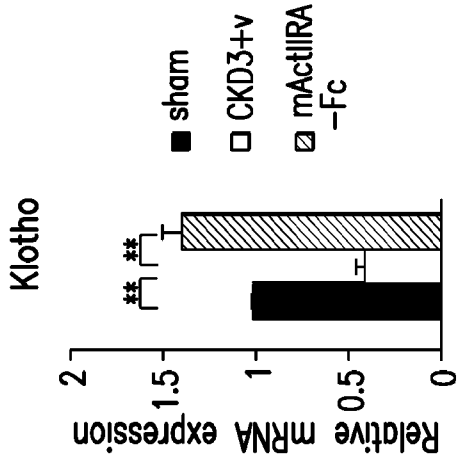


FIG. 4C

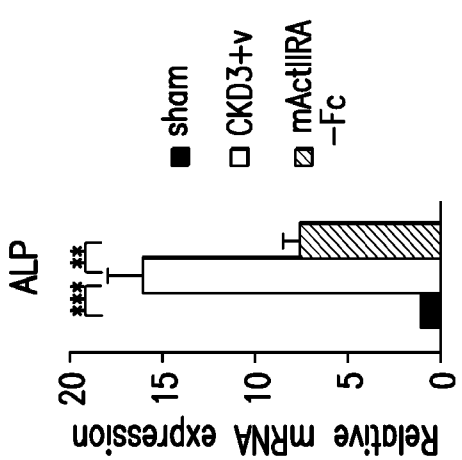


FIG. 4B

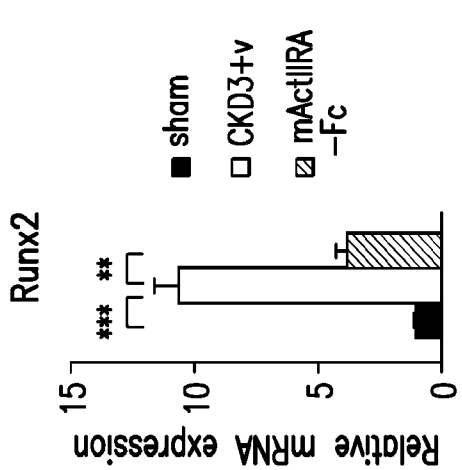


FIG. 4A

*p<0.05
**p<0.01
***p<0.005

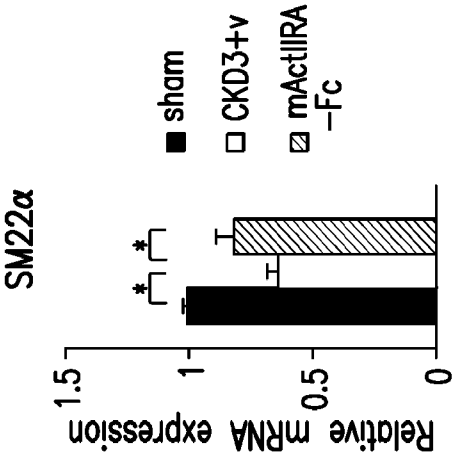


FIG. 4E

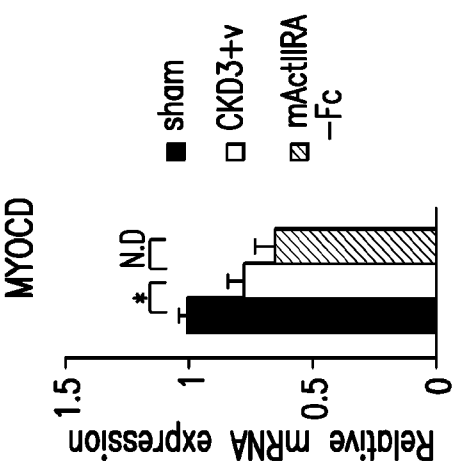


FIG. 4D

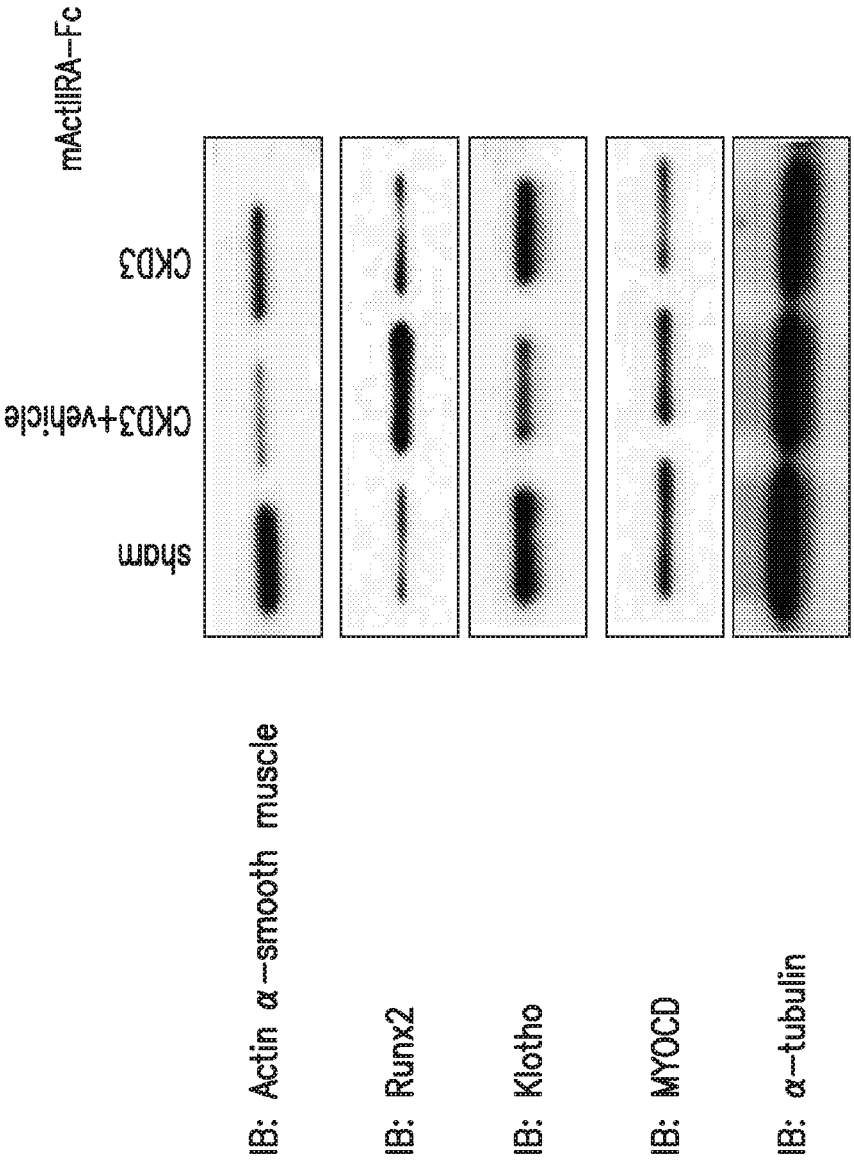
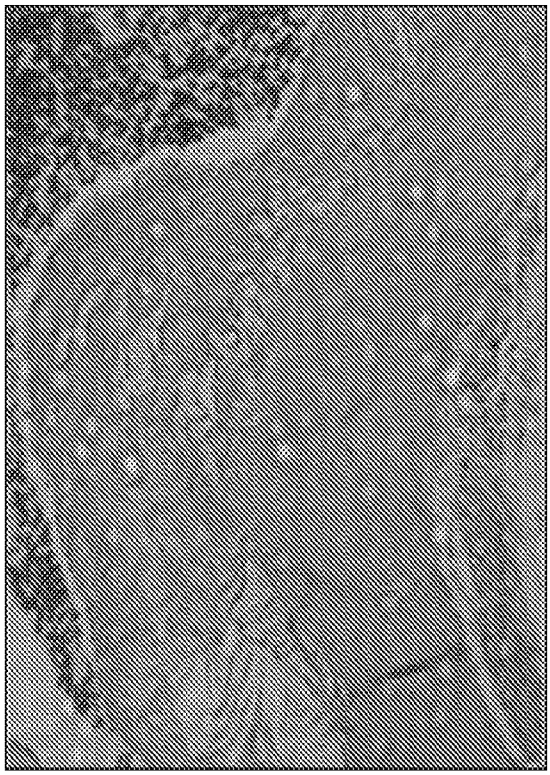

mActIIRA-Fc
sham
CKD3+vehicle
CKD3
IB: Actin α-smooth muscle
IB: Runx2
IB: Klotho
IB: MYOCD
IB: α-tubulin

FIG. 4F



CKD-3 mActIIRA-Fc Rx

FIG. 5B

CKD-3 veh. Rx

FIG. 5A

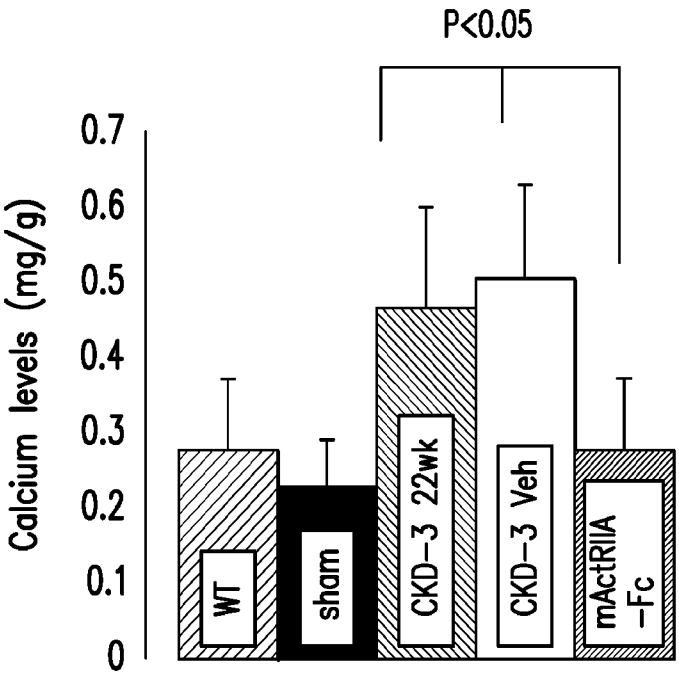

P<0.05
Calcium levels (mg/g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
WT
sham
CKD-3 22wk
CKD-3 Veh
mActRIIA -Fc

FIG. 6A

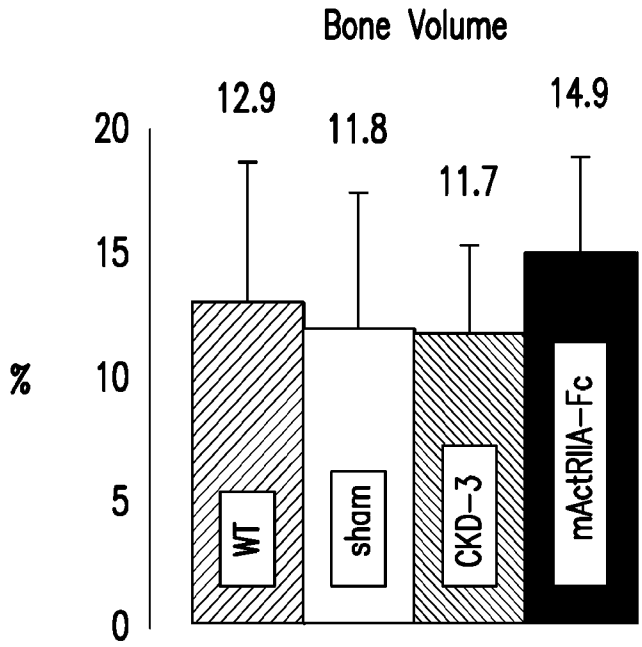
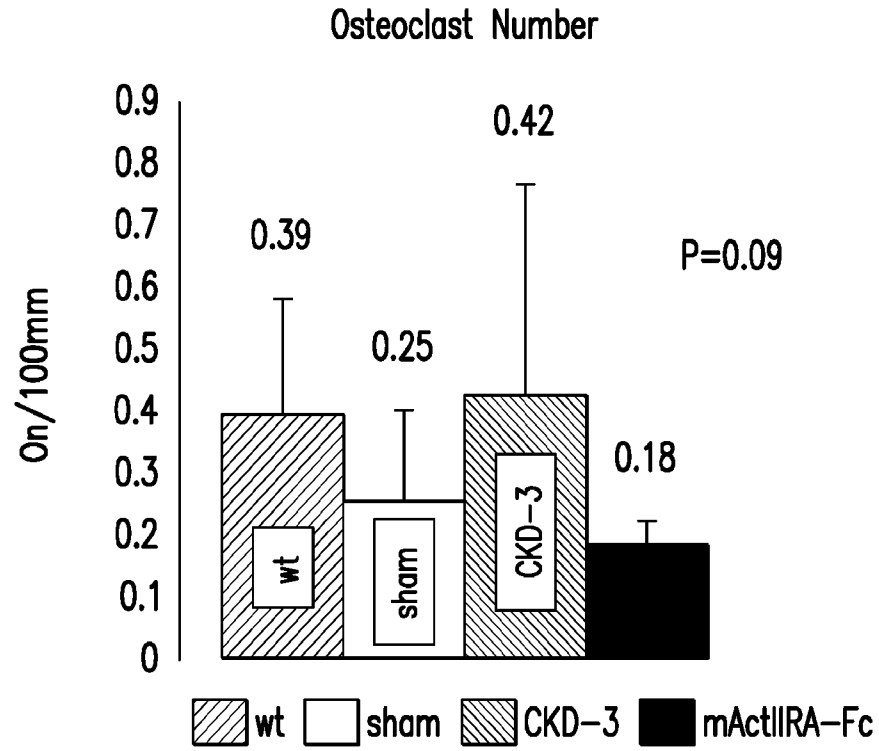

Bone Volume
12.9
11.8
11.7
14.9
20
15
10
5
0
%
WT
sham
CKD-3
mActRIIA-Fc

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

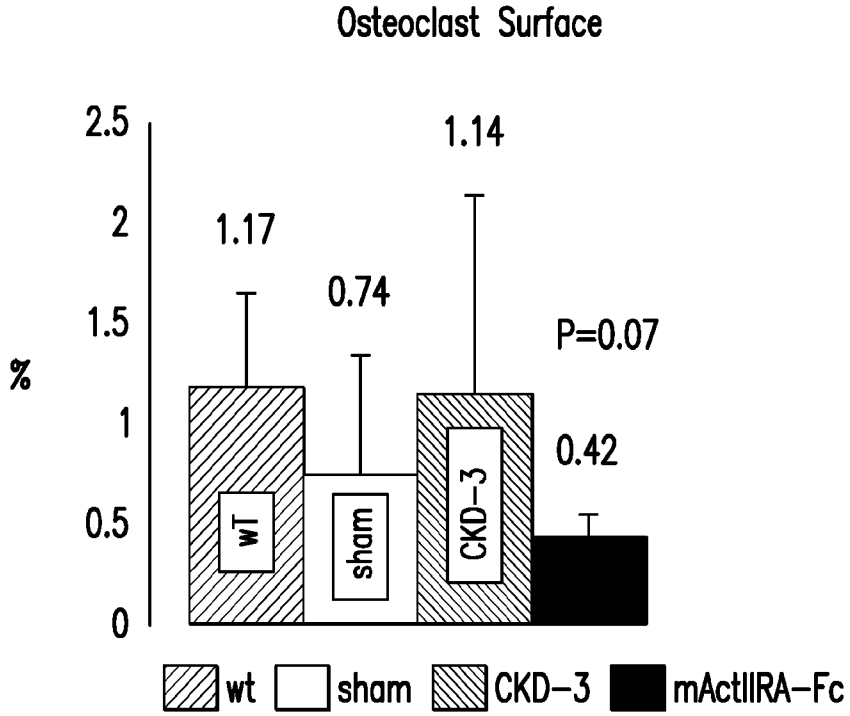

Osteoclast Surface
%
2.5
2
1.5
1
0.5
0
1.17
0.74
1.14
0.42
P=0.07
wT
sham
CKD-3
wt
sham
CKD-3
mActIIRA-Fc

FIG. 6D

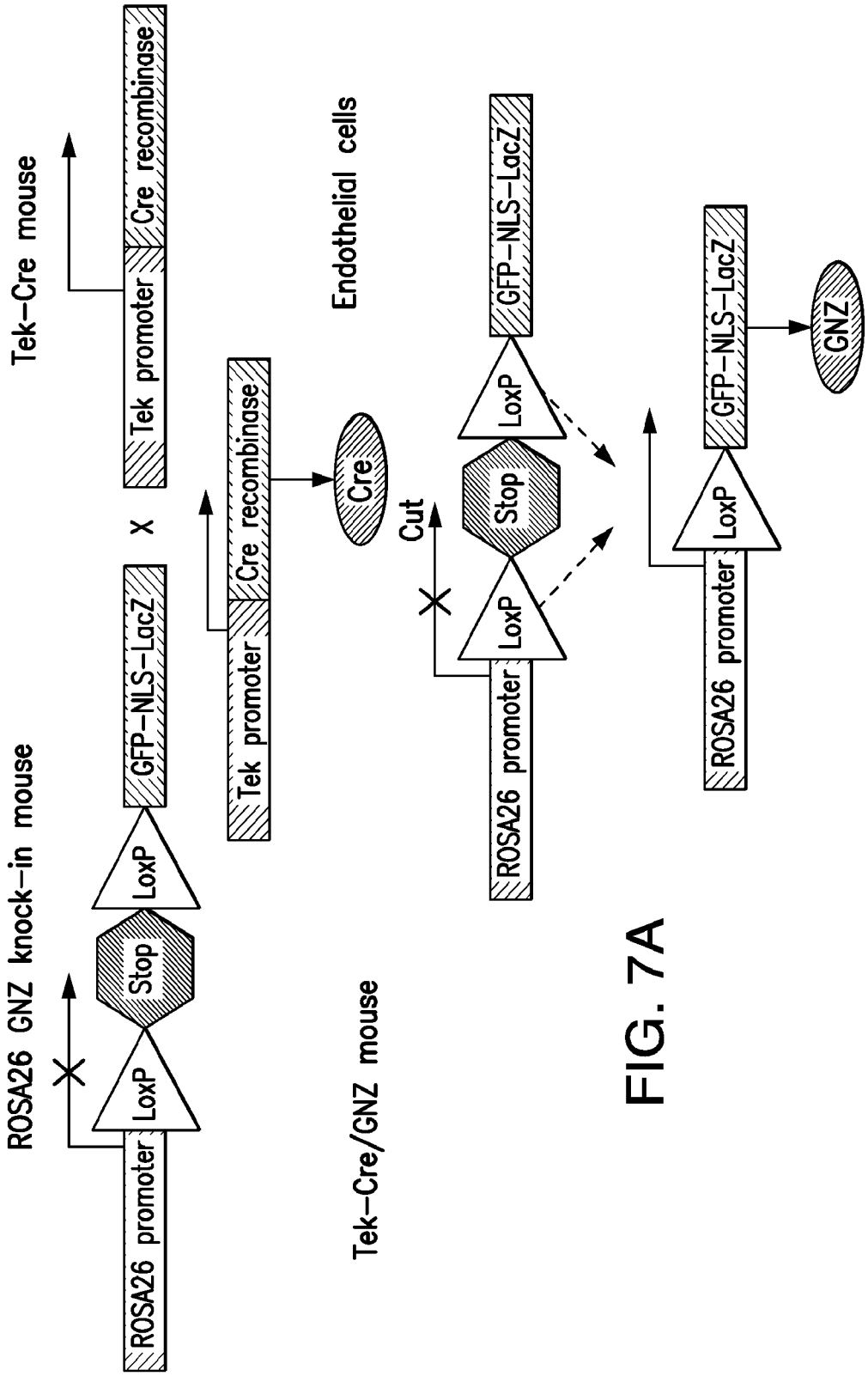

Tek–Cre mouse
Cre recombinase
Tek promoter
X
ROSA26 GNZ knock–in mouse
GFP–NLS–LacZ
LoxP
Stop
LoxP
ROSA26 promoter
Tek–Cre/GNZ mouse
Cre recombinase
Tek promoter
Cre
Endothelial cells
Cut
GFP–NLS–LacZ
LoxP
Stop
LoxP
ROSA26 promoter
GFP–NLS–LacZ
LoxP
ROSA26 promoter
GNZ

FIG. 7A

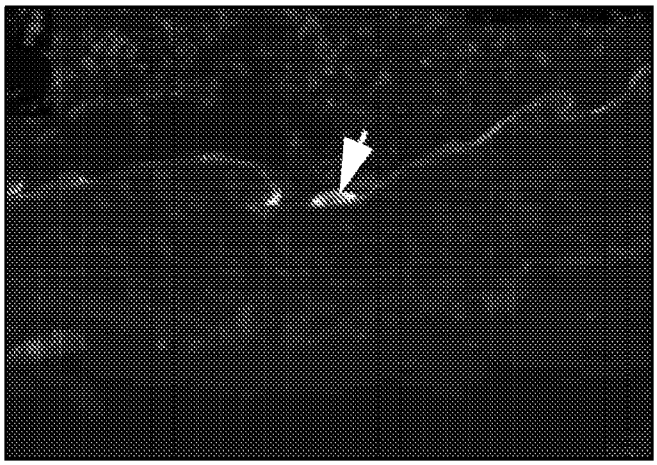


FIG. 7B

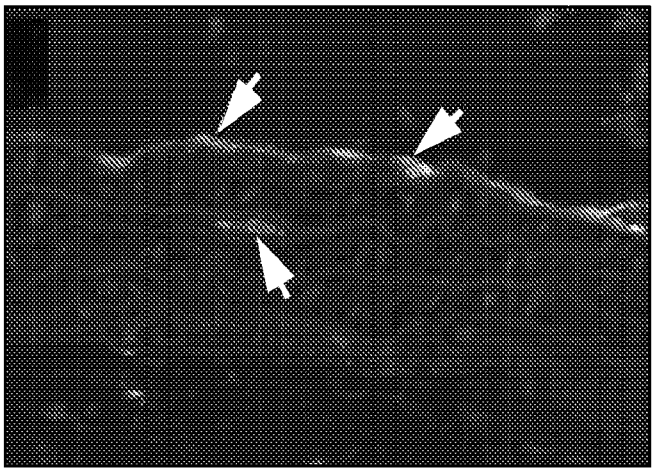


FIG. 7C

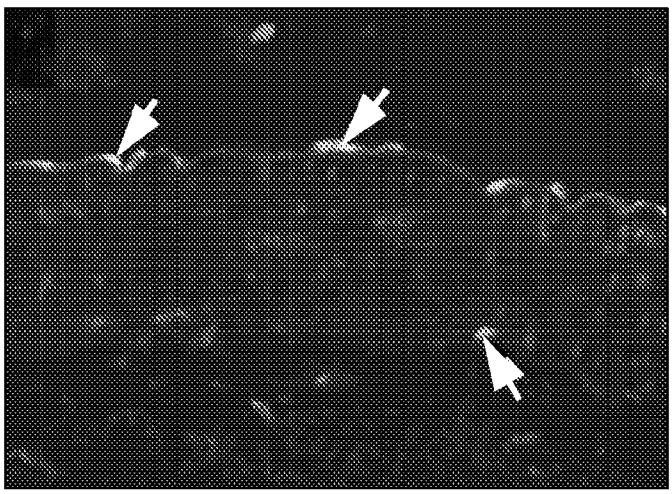


FIG. 7D

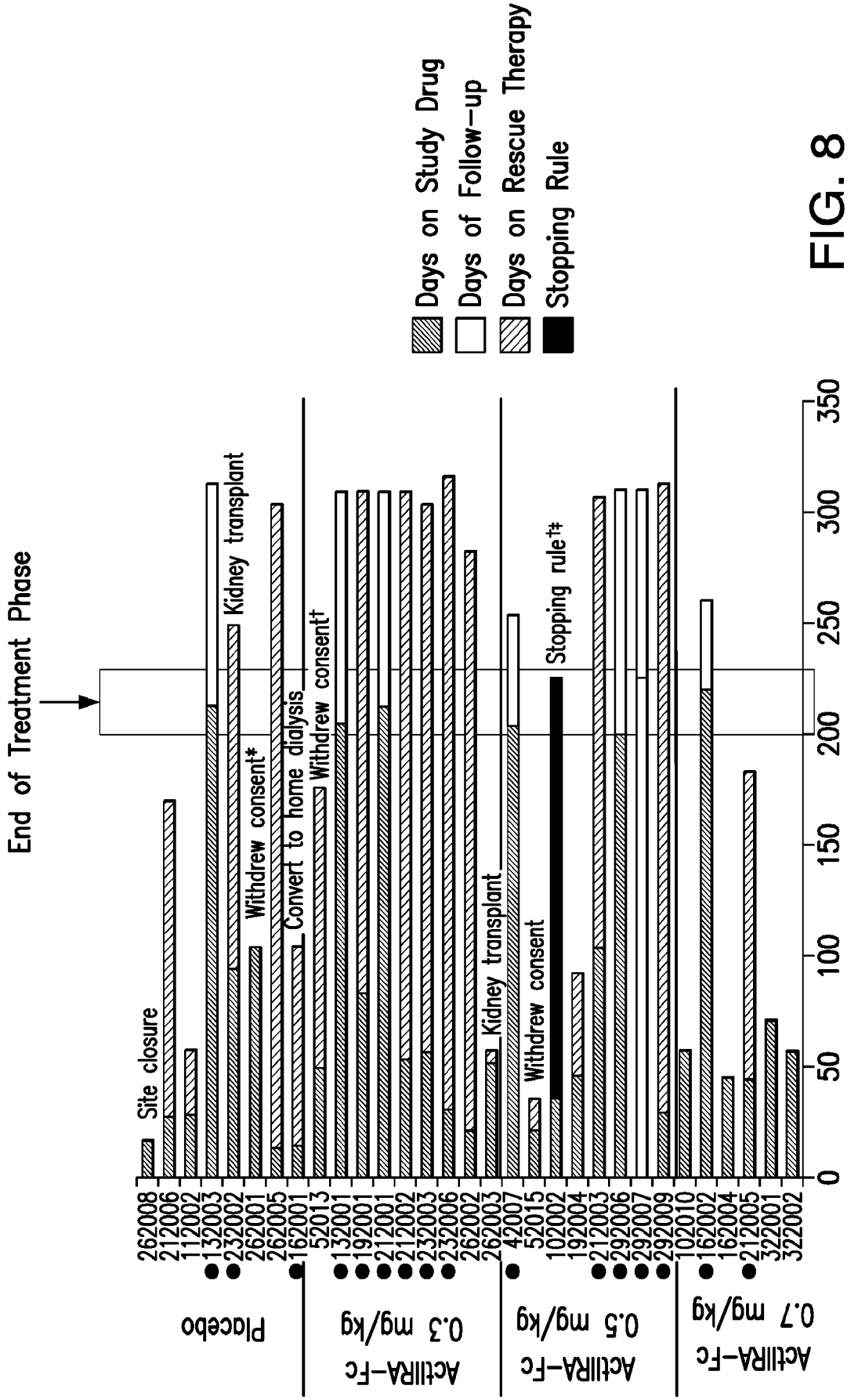

Days on Study Drug
Days of Follow-up
Days on Rescue Therapy
Stopping Rule
End of Treatment Phase
Site closure
Withdrew consent*
Kidney transplant
Convert to home dialysis
Withdrew consent†
Kidney transplant
Withdrew consent
Stopping rule†‡
Placebo
0.3 mg/kg
0.5 mg/kg
0.7 mg/kg
ActIIRA-Fc
ActIIRA-Fc
ActIIRA-Fc
262008
212006
112002
132003
232002
262001
262005
162001
52013
132001
192001
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

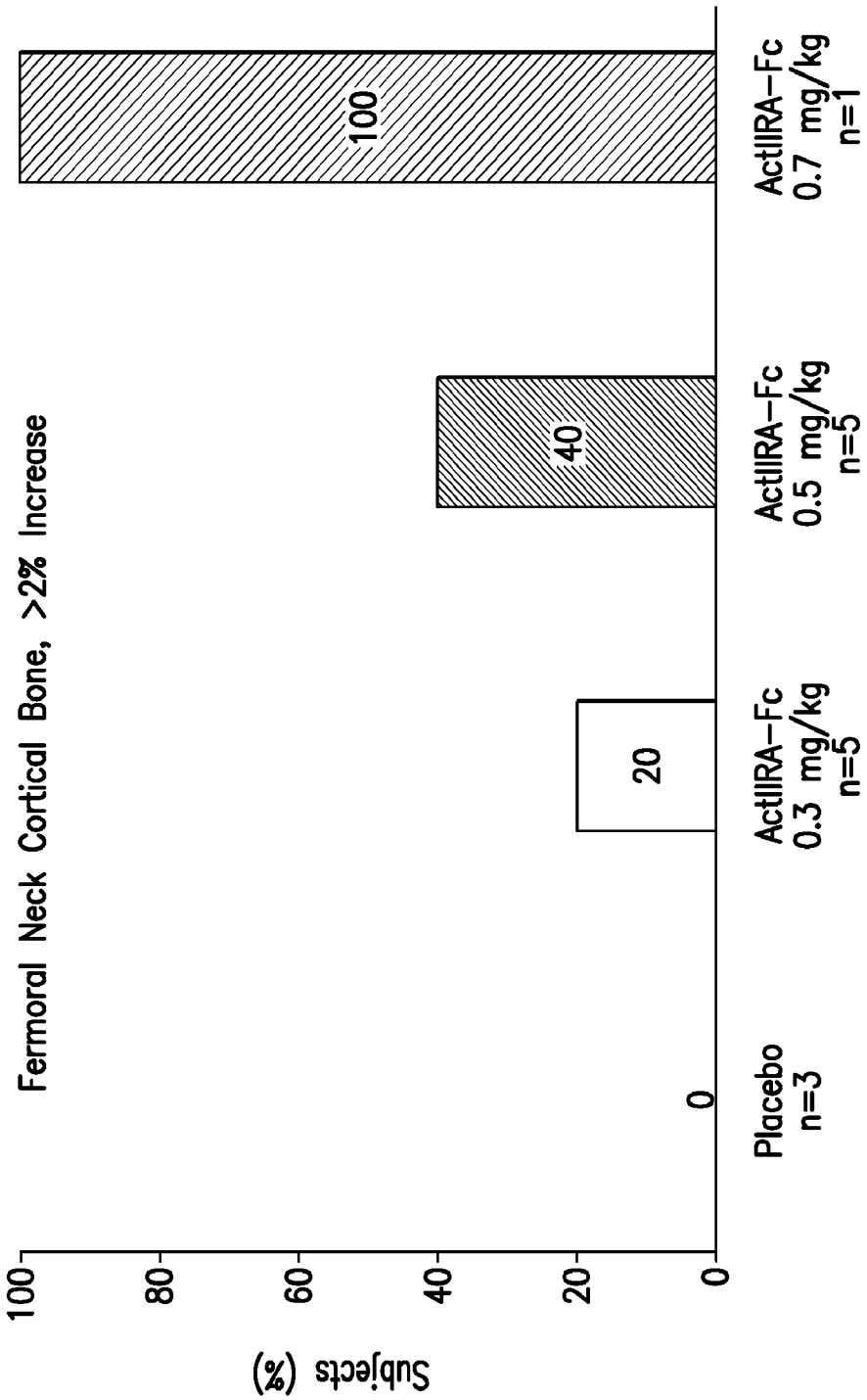

Femoral Neck Cortical Bone, >2% Increase
Subjects (%)
100
80
60
40
20
0
0
20
40
100
Placebo n=3
ActIIRA-Fc 0.3 mg/kg n=5
ActIIRA-Fc 0.5 mg/kg n=5
ActIIRA-Fc 0.7 mg/kg n=1

FIG. 9A

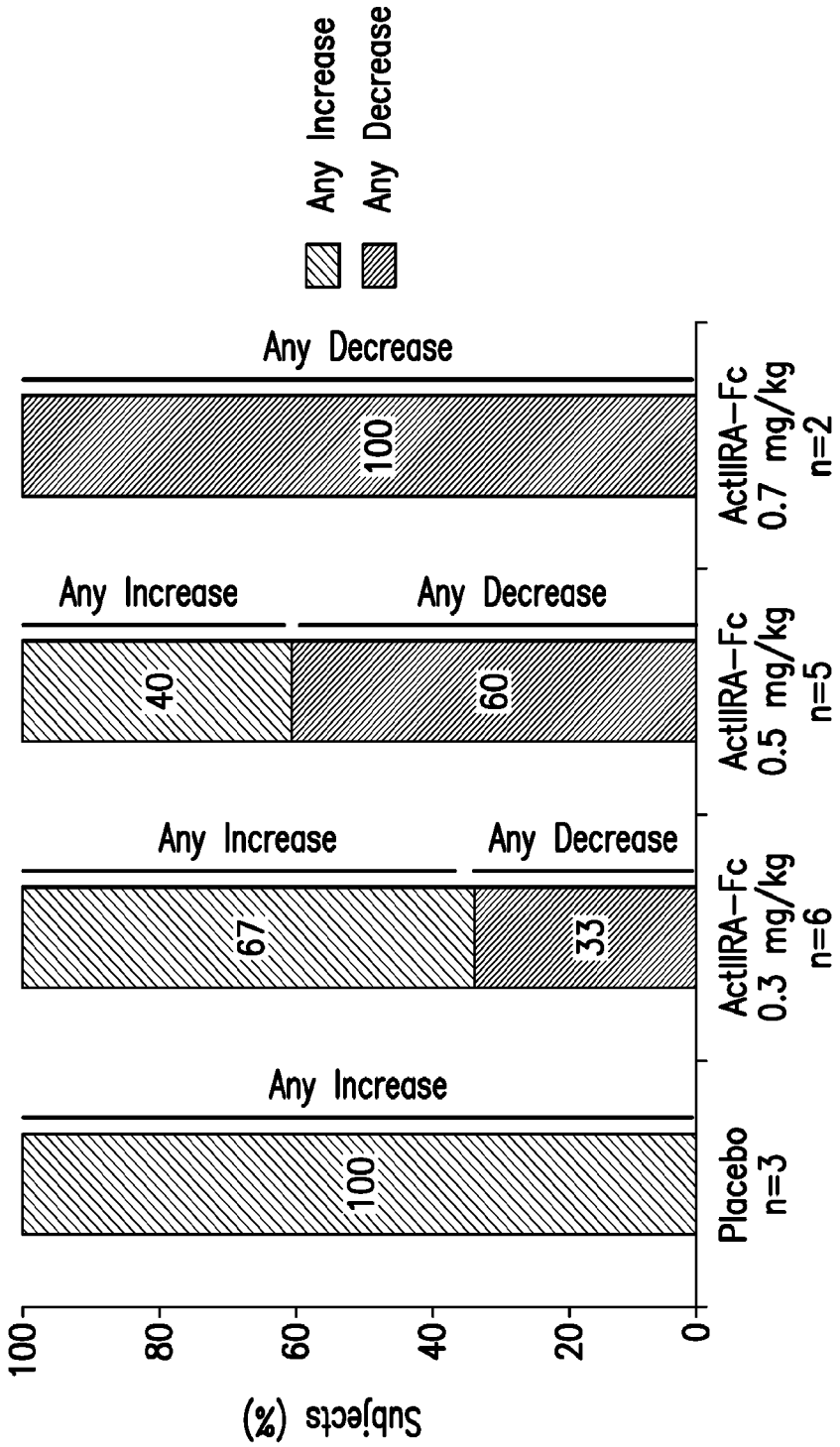

Any Increase
Any Decrease
Any Decrease
100
ActIIRA-Fc
0.7 mg/kg
n=2
Any Increase
Any Decrease
40
60
ActIIRA-Fc
0.5 mg/kg
n=5
Any Increase
Any Decrease
67
33
ActIIRA-Fc
0.3 mg/kg
n=6
Any Increase
100
Placebo
n=3
100
80
60
40
20
0
Subjects (%)

FIG. 9B

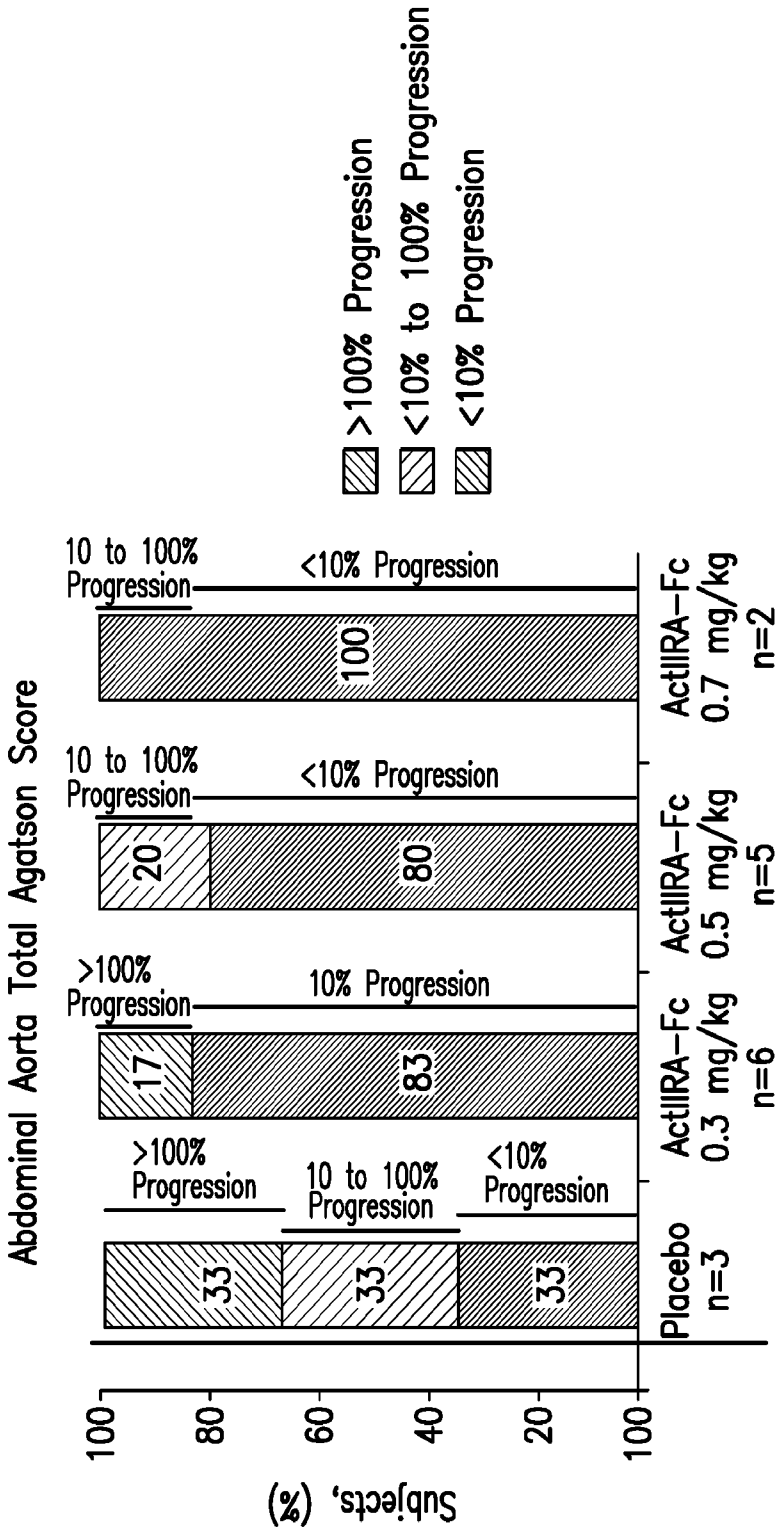

>100% Progression
<10% to 100% Progression
<10% Progression
Abdominal Aorta Total Agatson Score
Subjects, (%)
100
80
60
40
20
100
10 to 100% Progression
<10% Progression
100
ActIIRA-Fc 0.7 mg/kg n=2
10 to 100% Progression
<10% Progression
20
80
ActIIRA-Fc 0.5 mg/kg n=5
>100% Progression
10% Progression
17
83
ActIIRA-Fc 0.3 mg/kg n=6
>100% Progression
10 to 100% Progression
<10% Progression
33
33
33
Placebo n=3

FIG. 10A

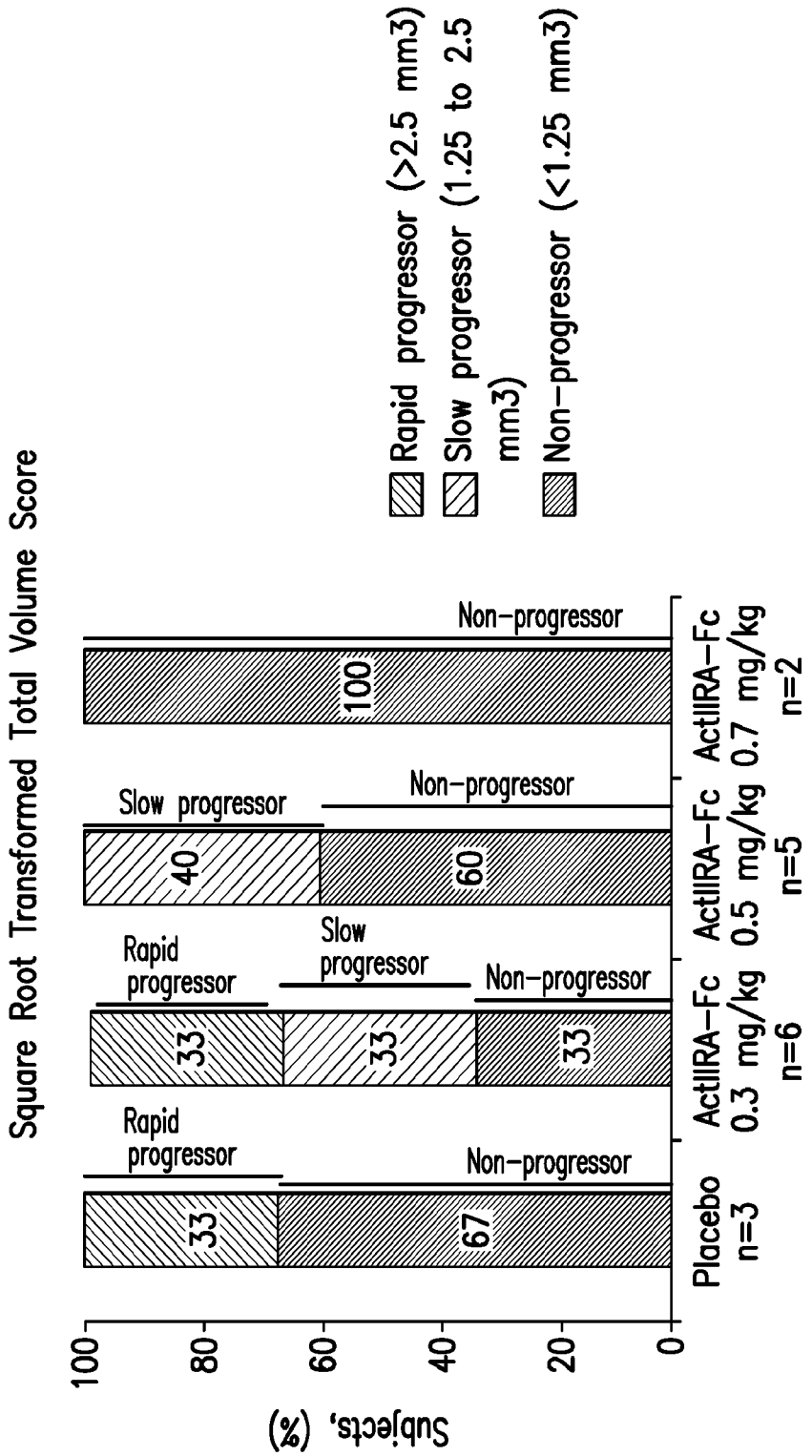
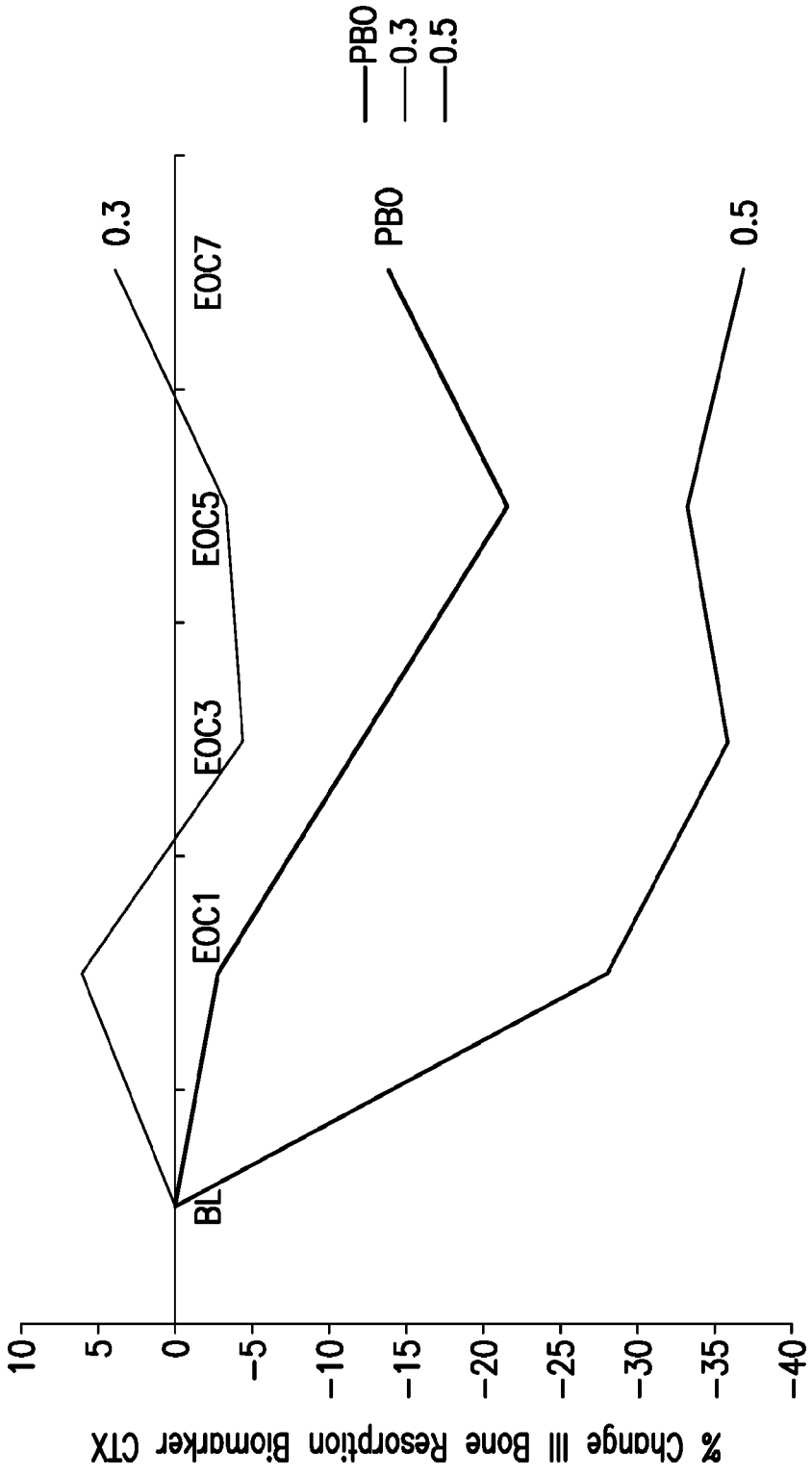

Square Root Transformed Total Volume Score
Rapid progressor (>2.5 mm3)
Slow progressor (1.25 to 2.5 mm3)
Non-progressor (<1.25 mm3)
Placebo n=3
ActIIRA-Fc 0.3 mg/kg n=6
ActIIRA-Fc 0.5 mg/kg n=5
ActIIRA-Fc 0.7 mg/kg n=2
Rapid progressor
Non-progressor
33
67
Rapid progressor
Slow progressor
Non-progressor
33
33
33
Slow progressor
Non-progressor
40
60
Non-progressor
100
Subjects, (%)
100
80
60
40
20
0

FIG. 10B



EOC=end of dose cycle.

FIG. 11

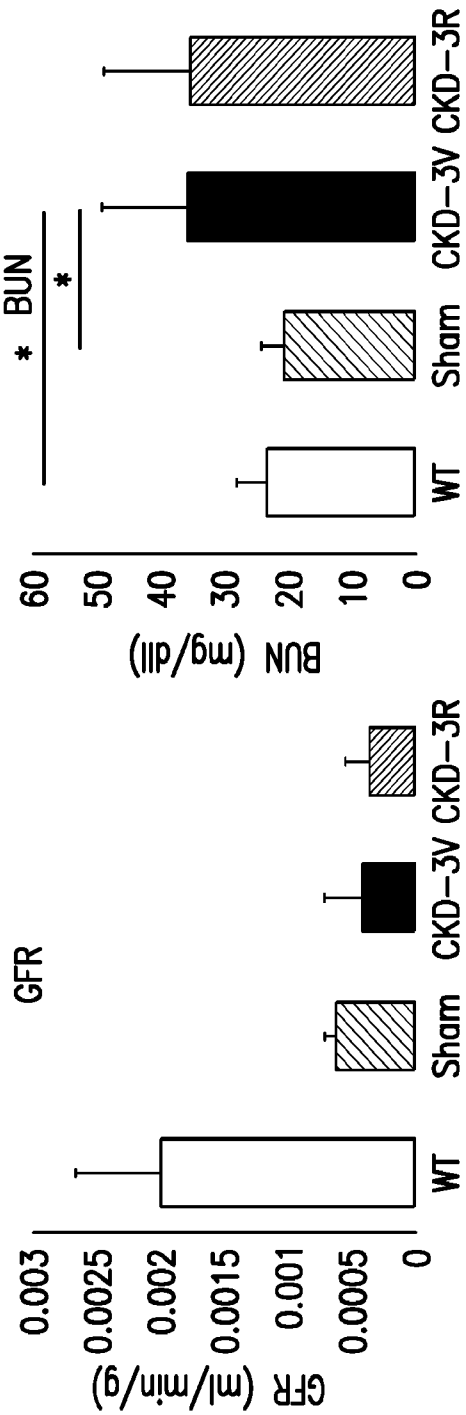


FIG. 12A

FIG. 12B

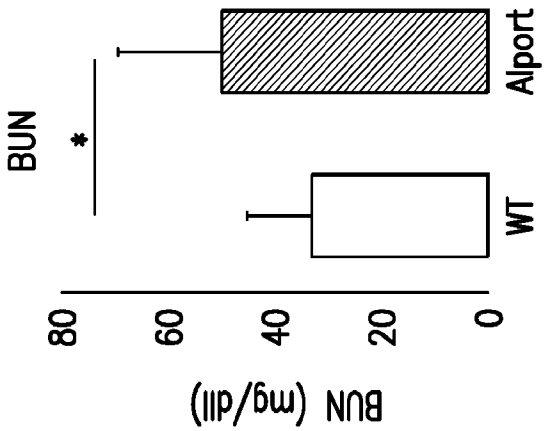


FIG. 12D

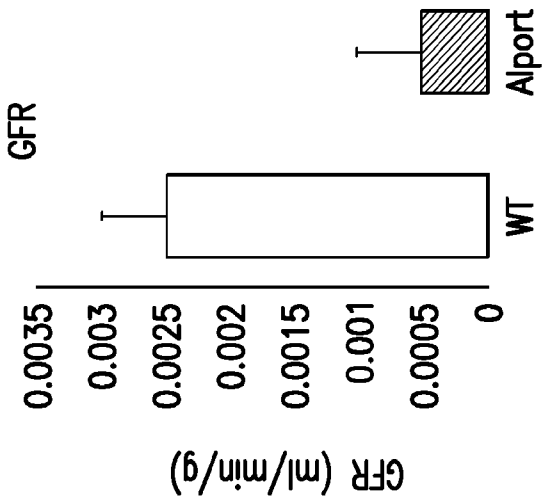


FIG. 12C

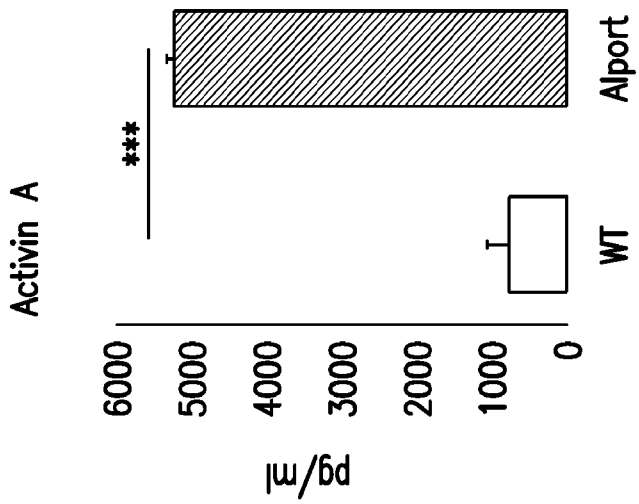


FIG. 13B

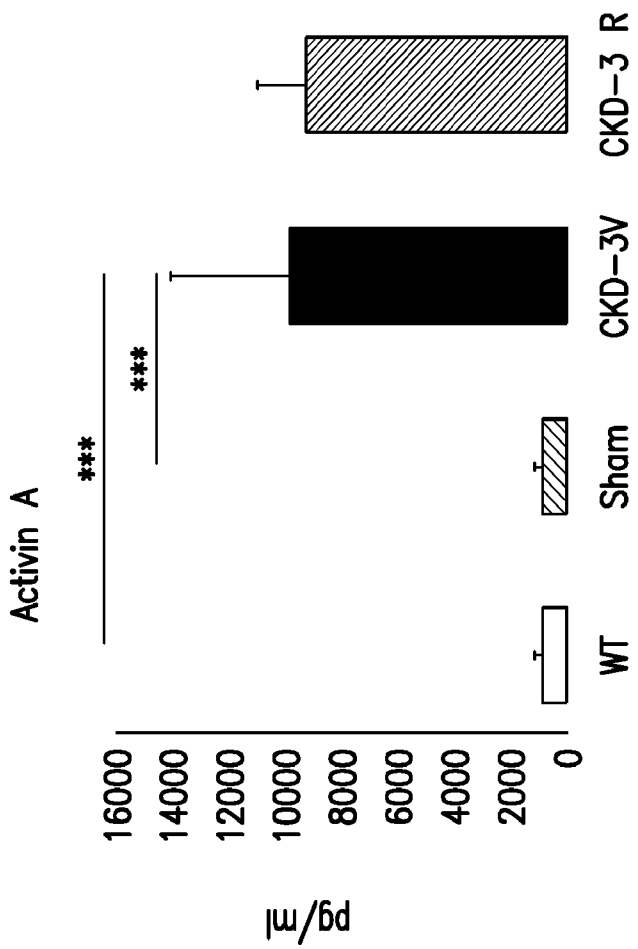


FIG. 13A

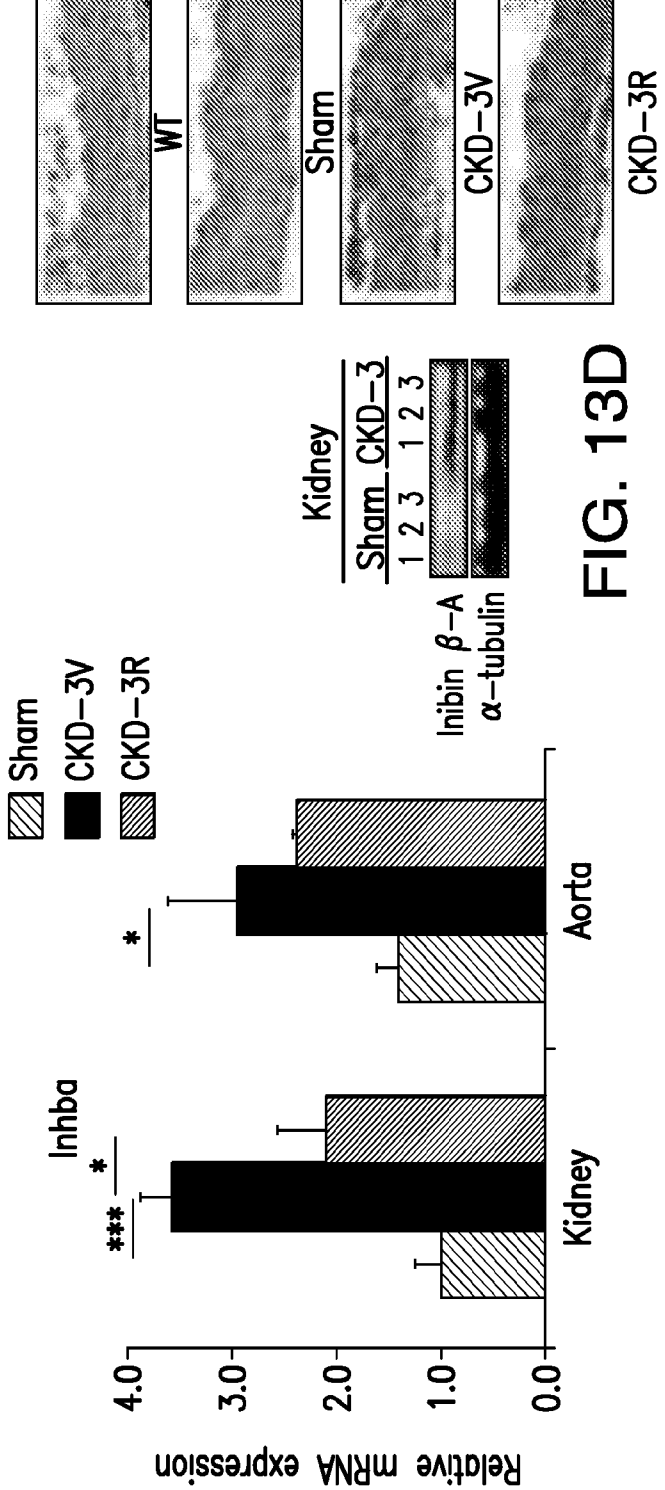


FIG. 13C

FIG. 13D

FIG. 13E

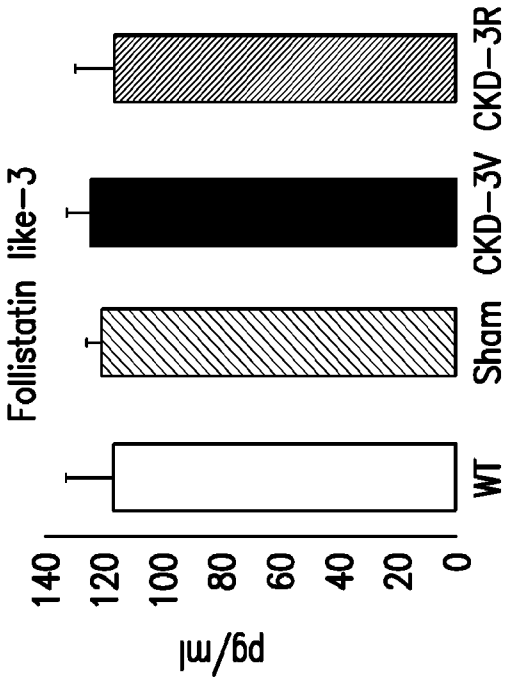


FIG. 13G

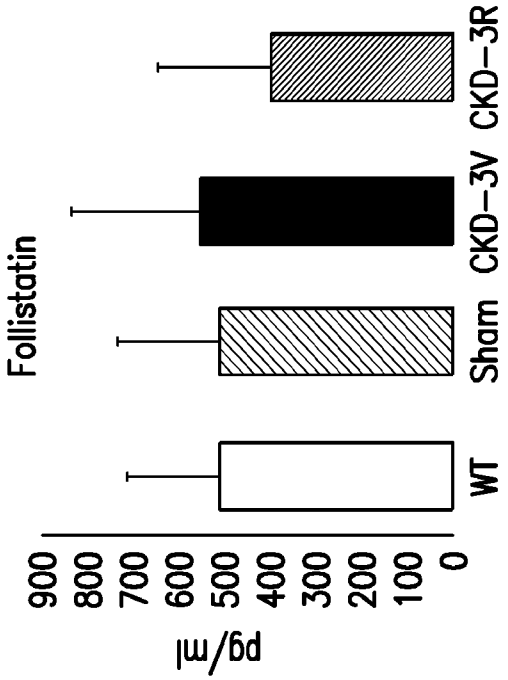


FIG. 13F

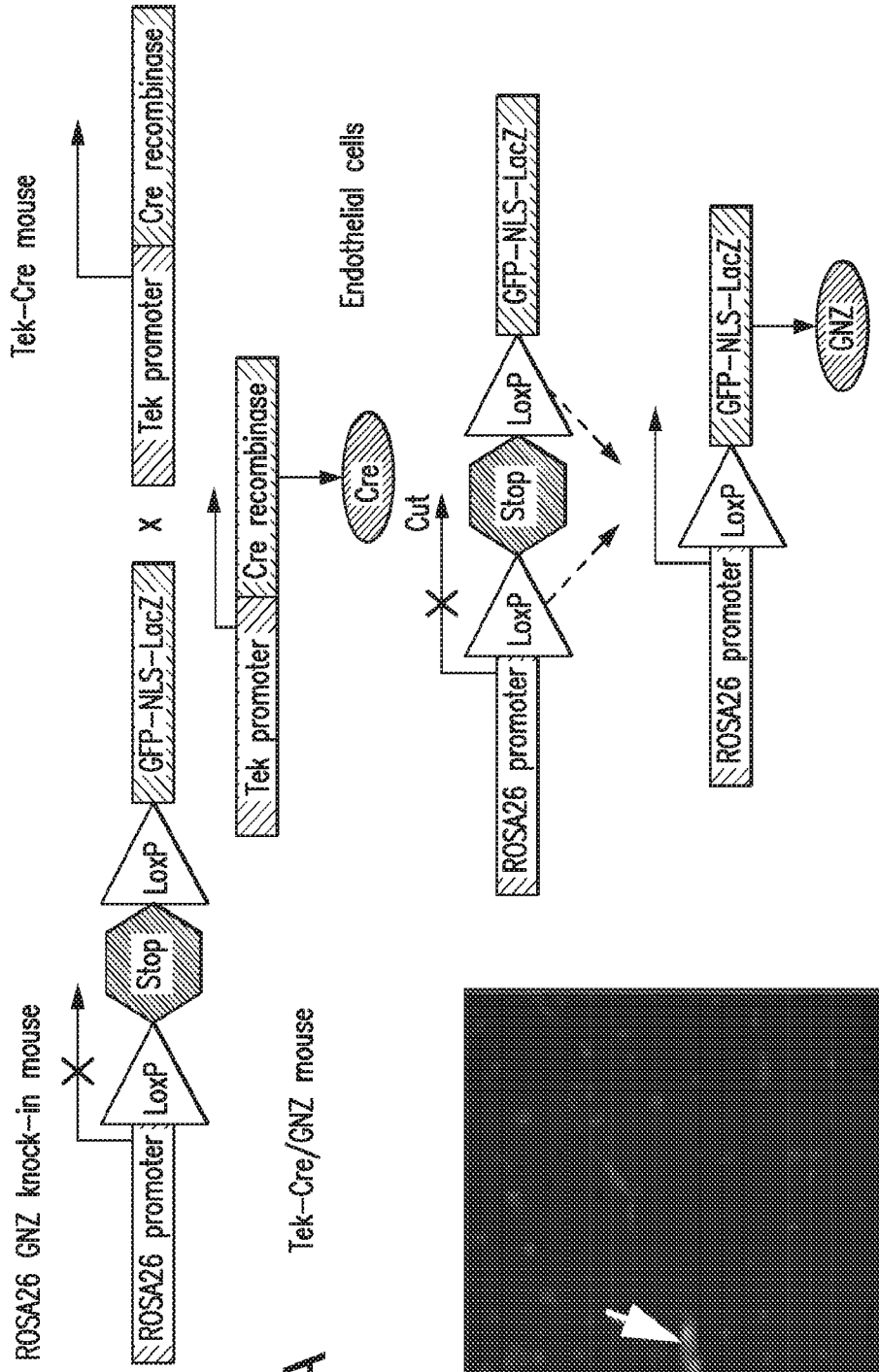


FIG. 14A Tek-Cre/GNZ mouse

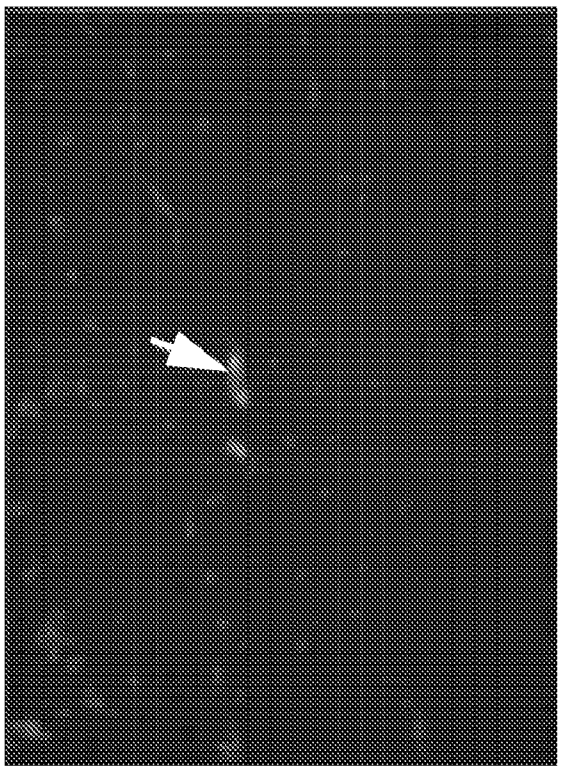


FIG. 14B

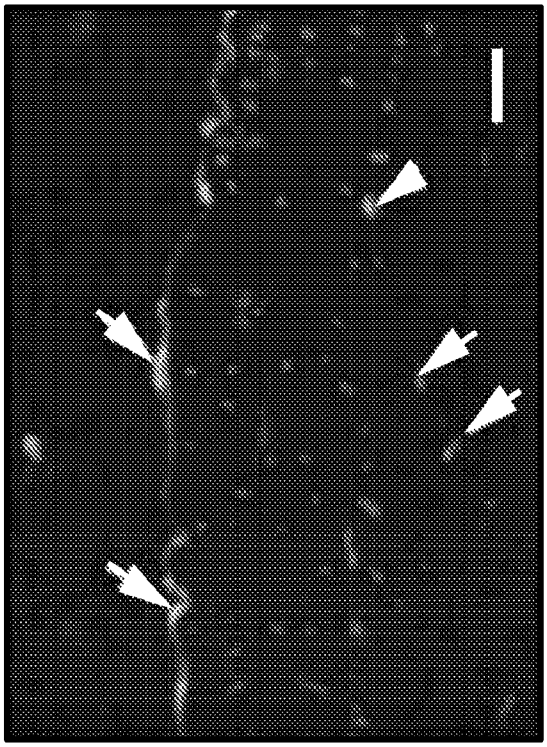


FIG. 14D

FIG. 14C

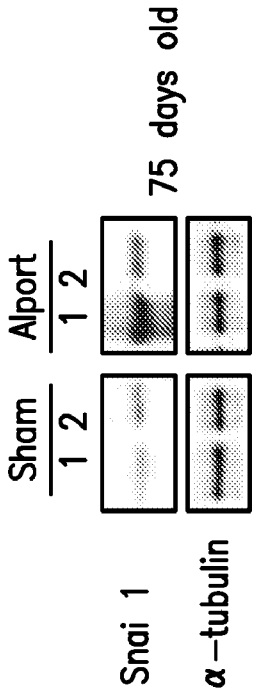


FIG. 14E

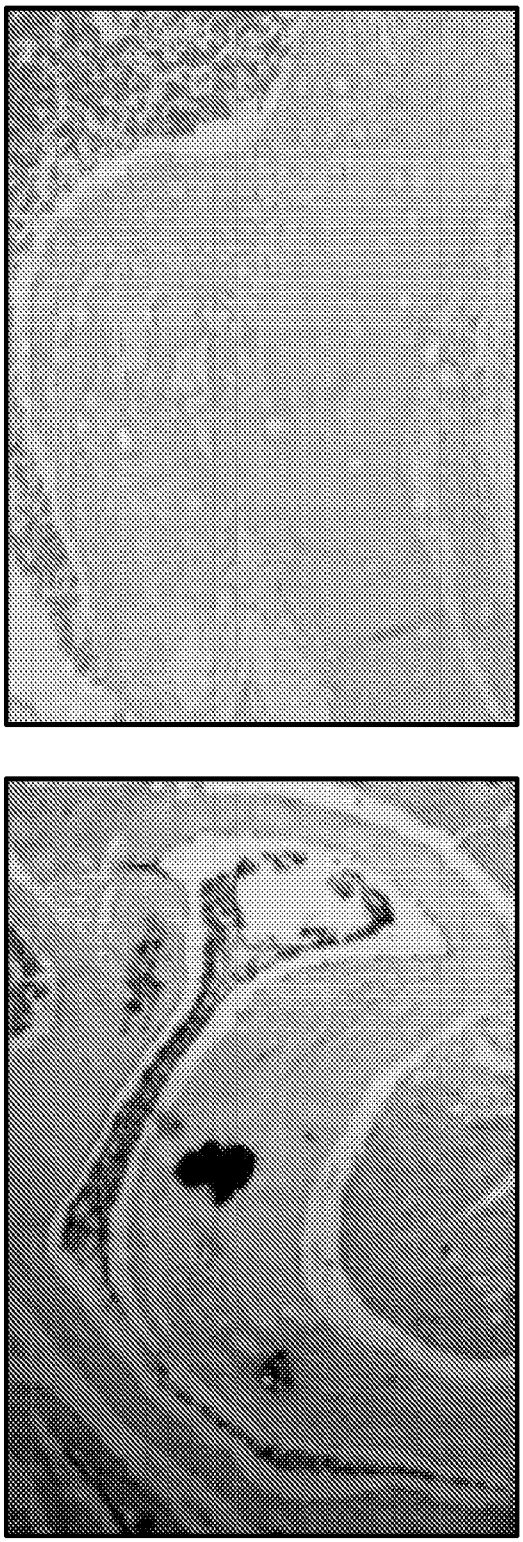


FIG. 15A

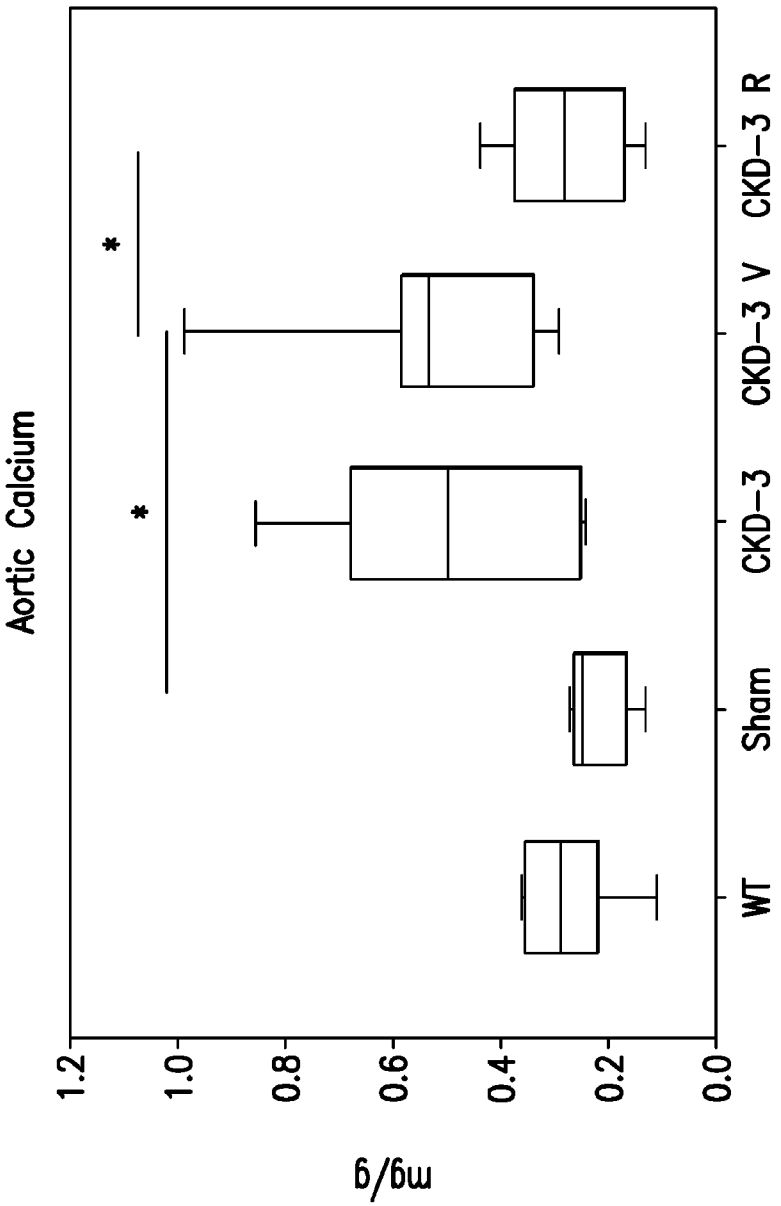

Aortic Calcium
mg/g
1.2
1.0
0.8
0.6
0.4
0.2
0.0
WT
Sham
CKD-3
CKD-3 V
CKD-3 R
*
*

FIG. 15B

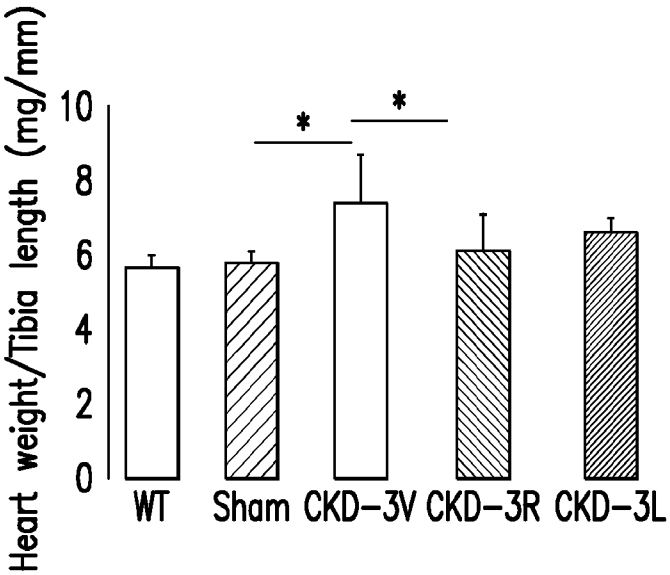


FIG. 16A

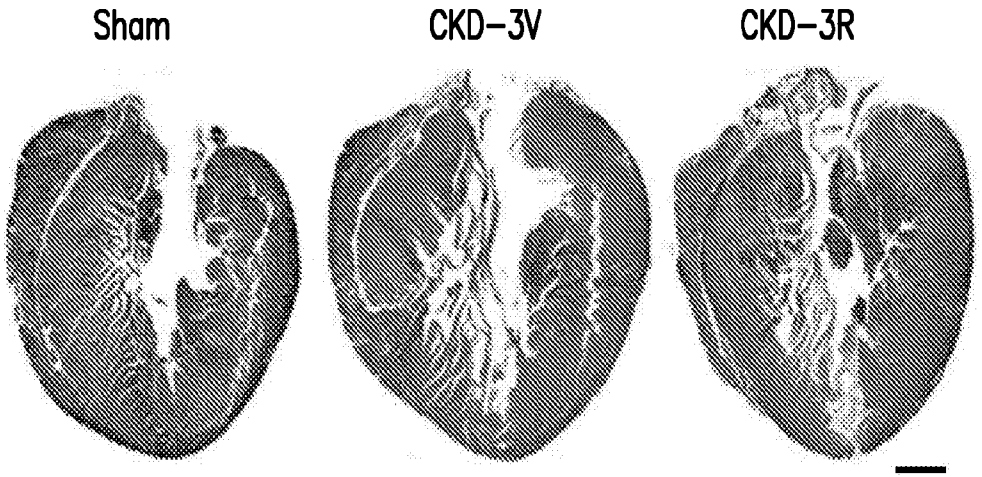


FIG. 16B

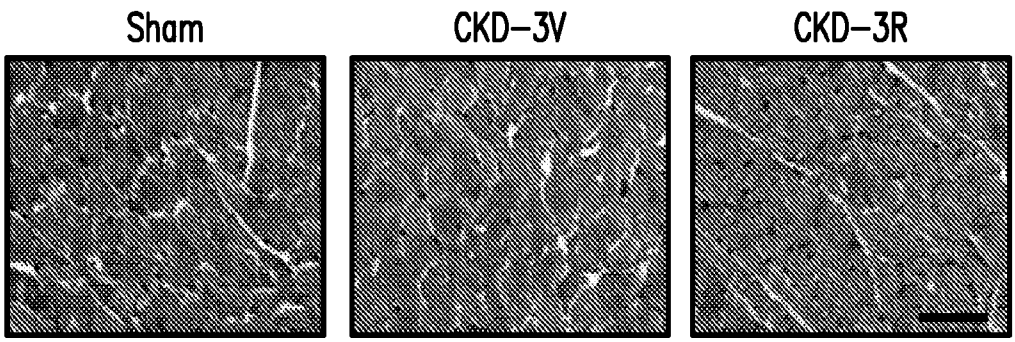


FIG. 16C

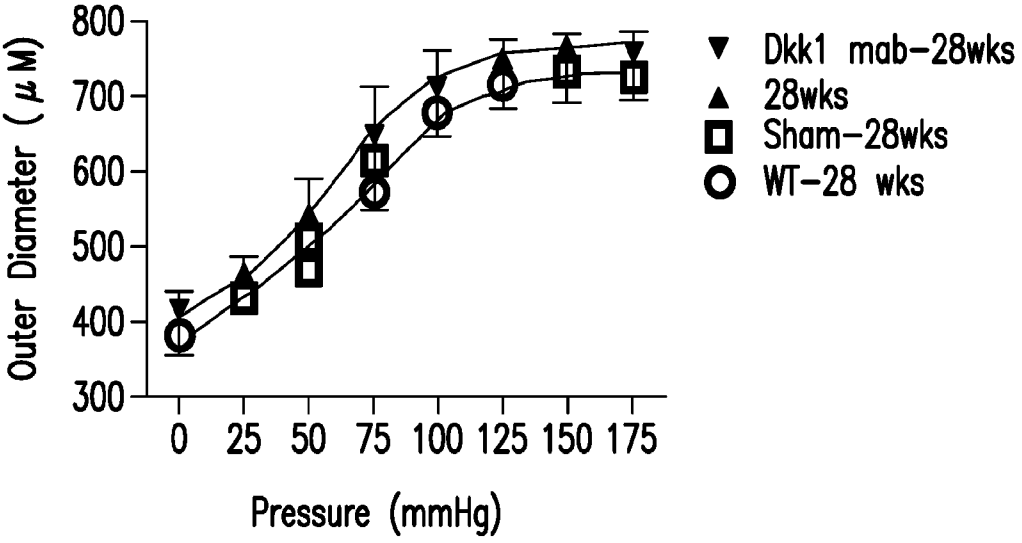


FIG. 17A

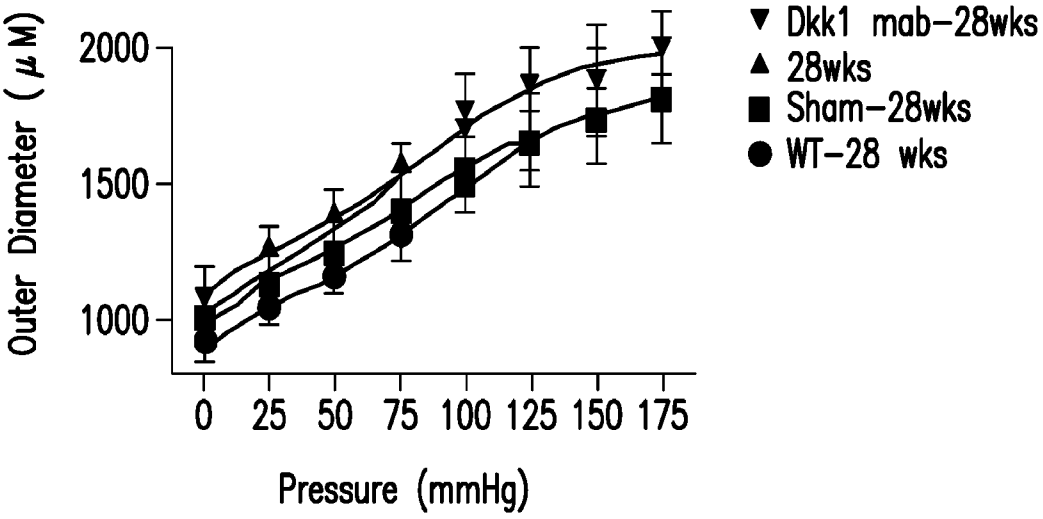


FIG. 17B

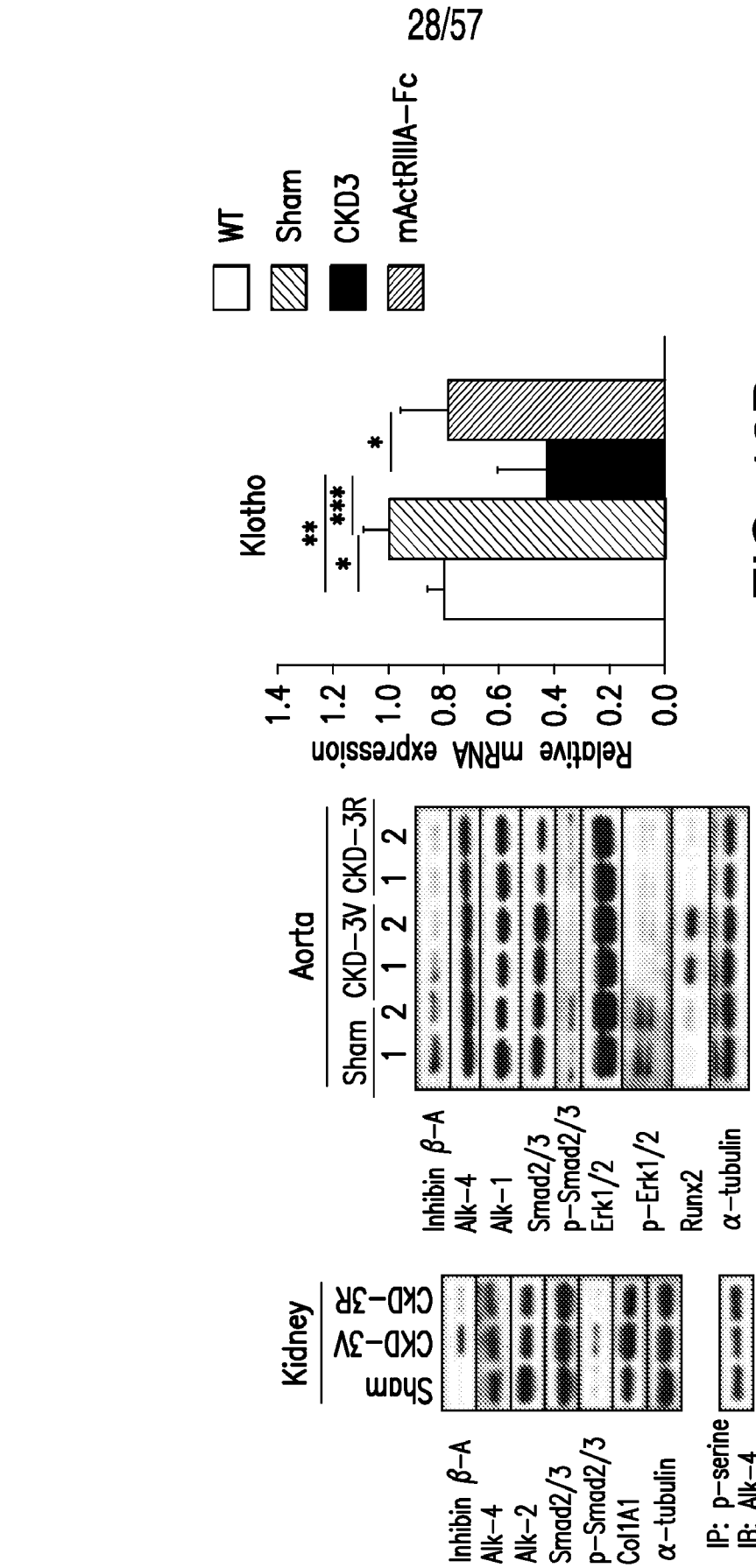


FIG. 18A

FIG. 18B

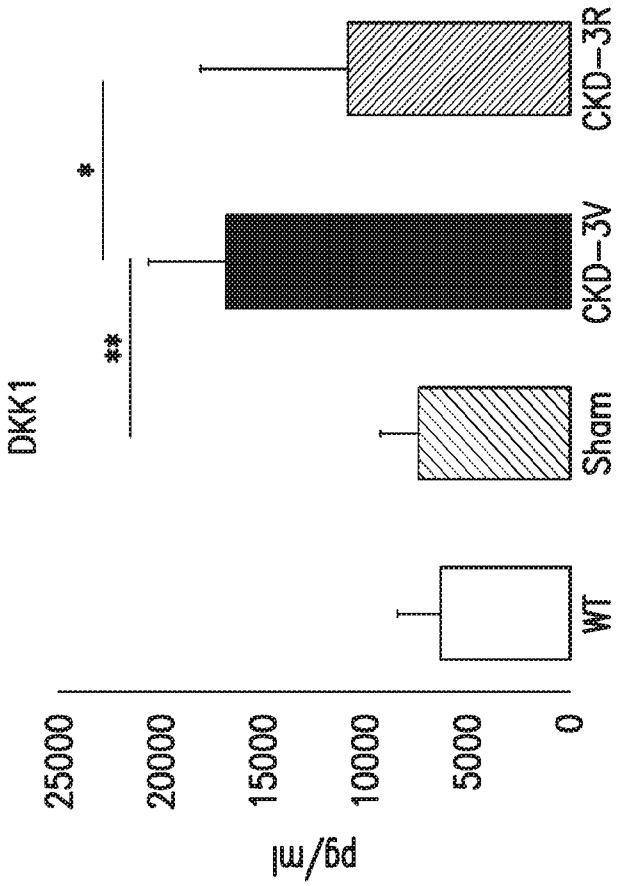

DKK1
pg/ml
25000
20000
15000
10000
5000
0
WT
Sham
CKD-3V
CKD-3R
*
**
Kidney
Aorta
Sham
CKD-3V
CKD-3R
DKK1
α-tubulin
DKK1
α-tubulin

FIG. 18C

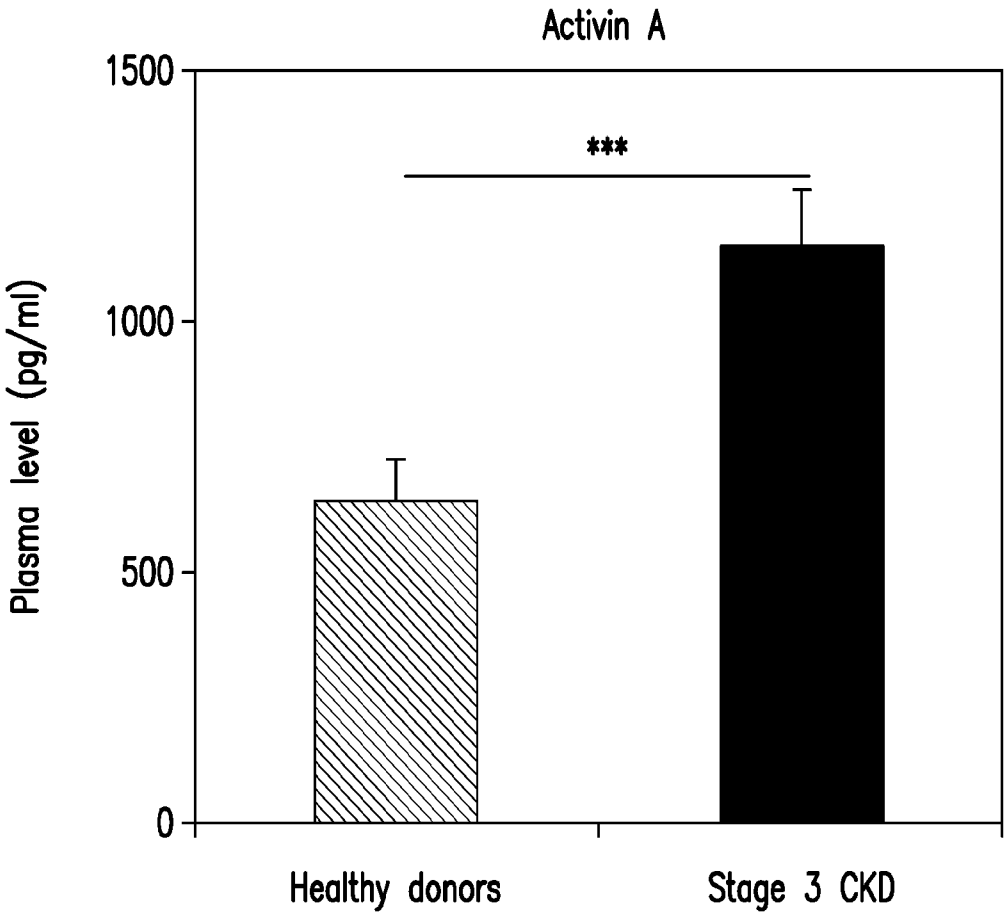

Activin A
1500
1000
500
0
***
Plasma level (pg/ml)
Healthy donors
Stage 3 CKD

FIG. 19

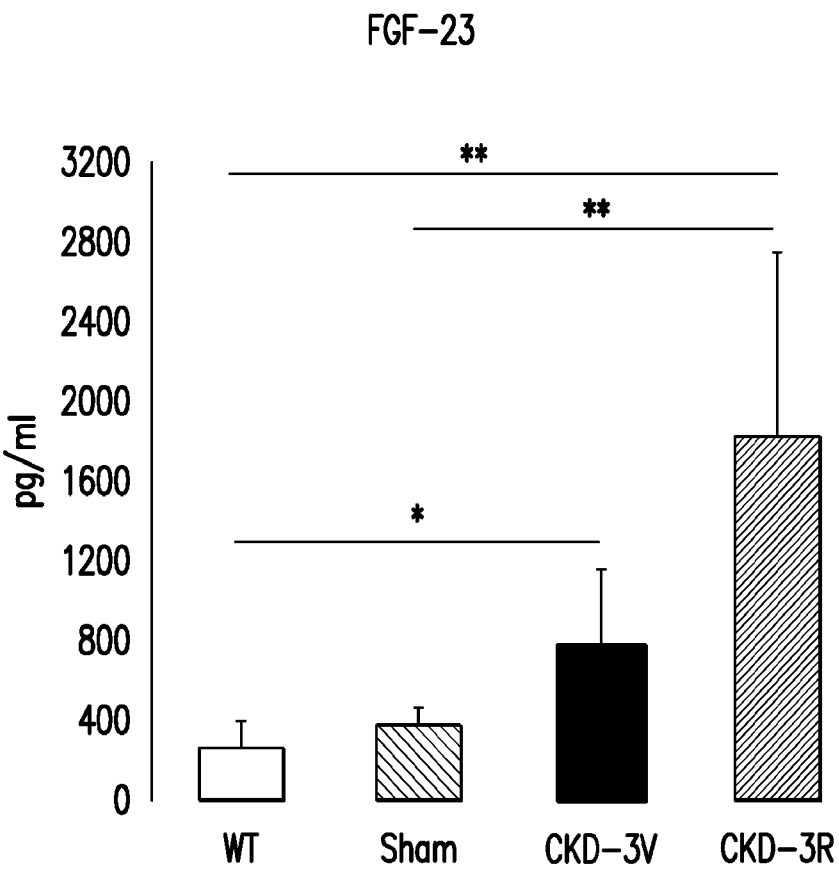

FGF-23
3200
2800
2400
2000
1600
1200
800
400
0
pg/ml
**
**
*
WT
Sham
CKD-3V
CKD-3R

FIG. 20

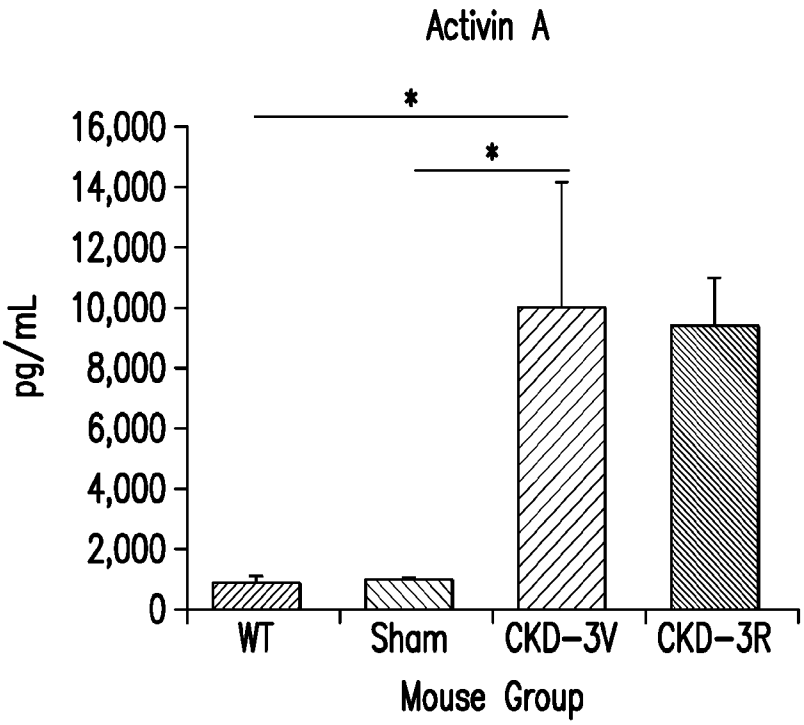


FIG. 21A

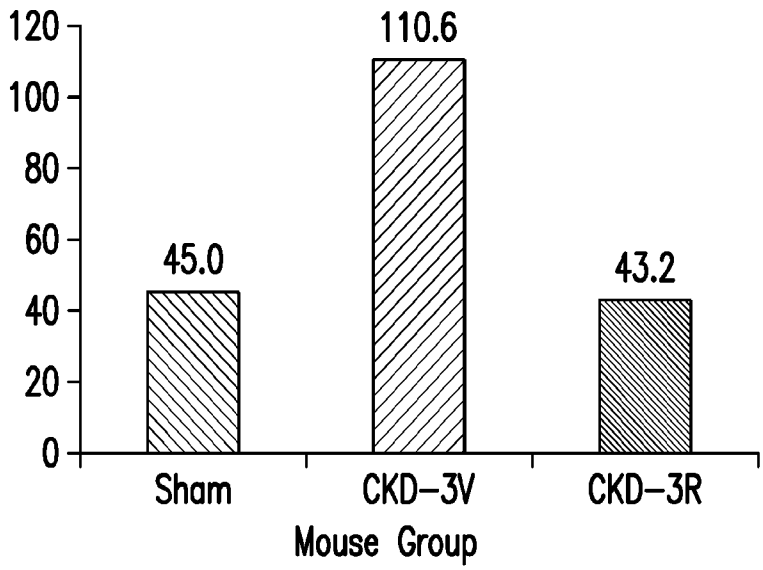


FIG. 21B

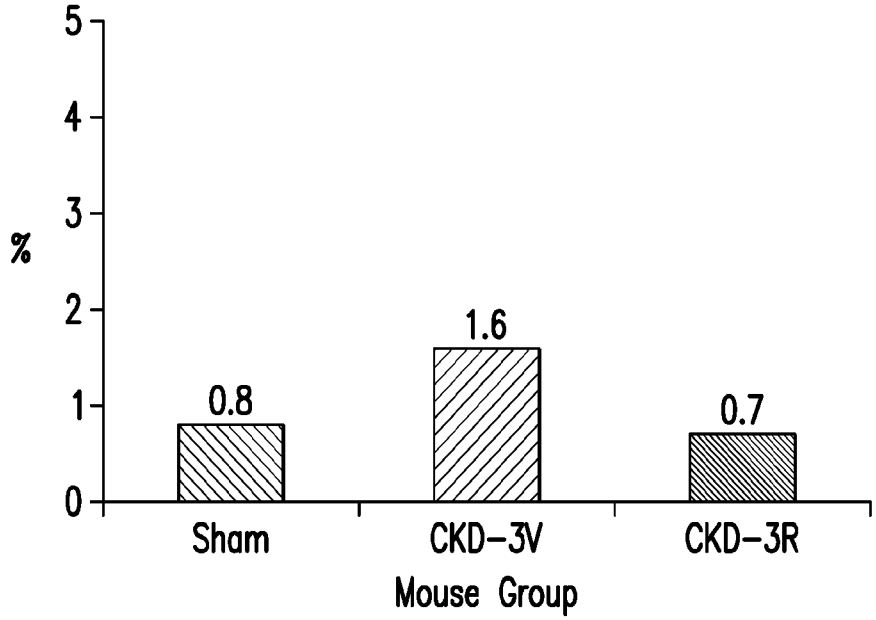


FIG. 21C

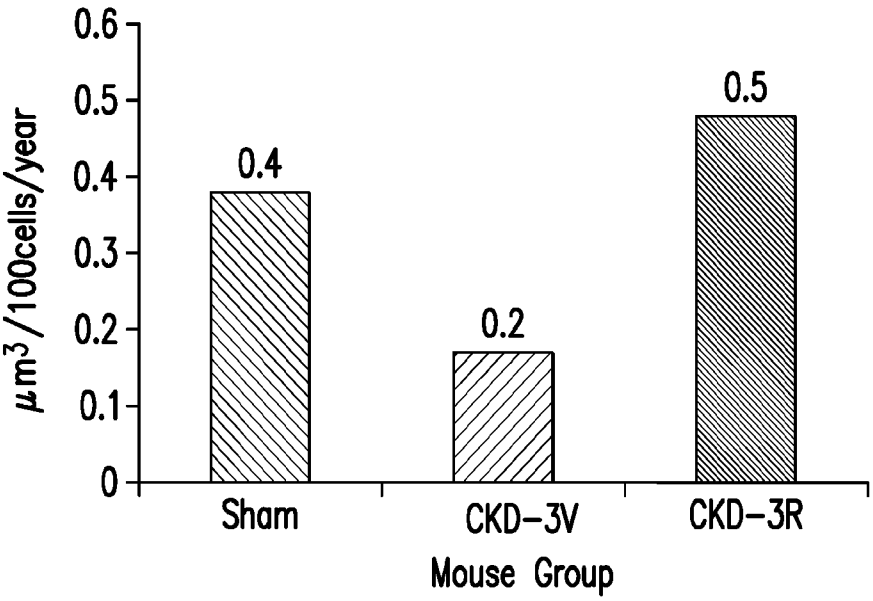
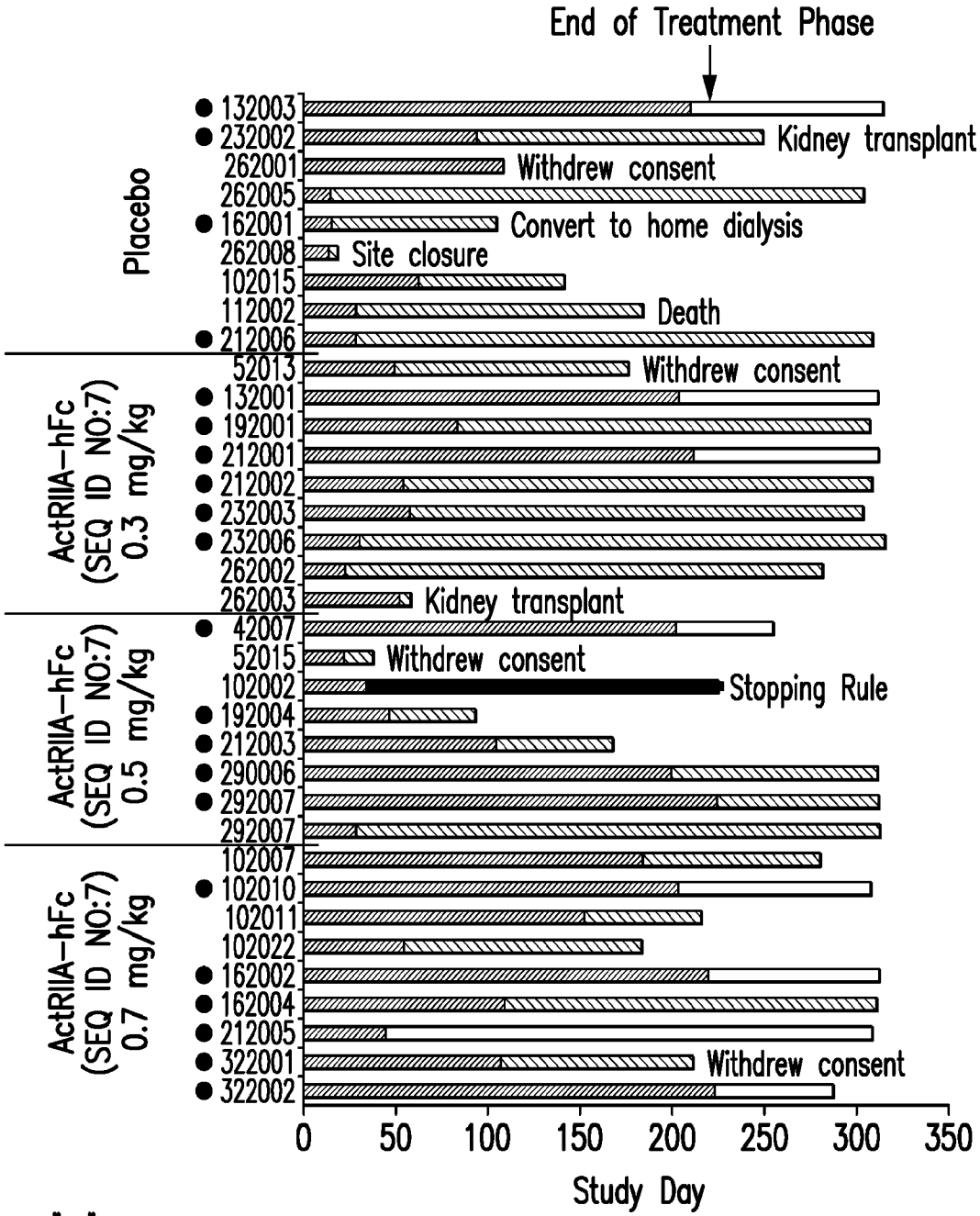


FIG. 21D



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.

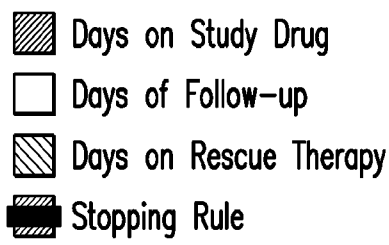


FIG. 22A

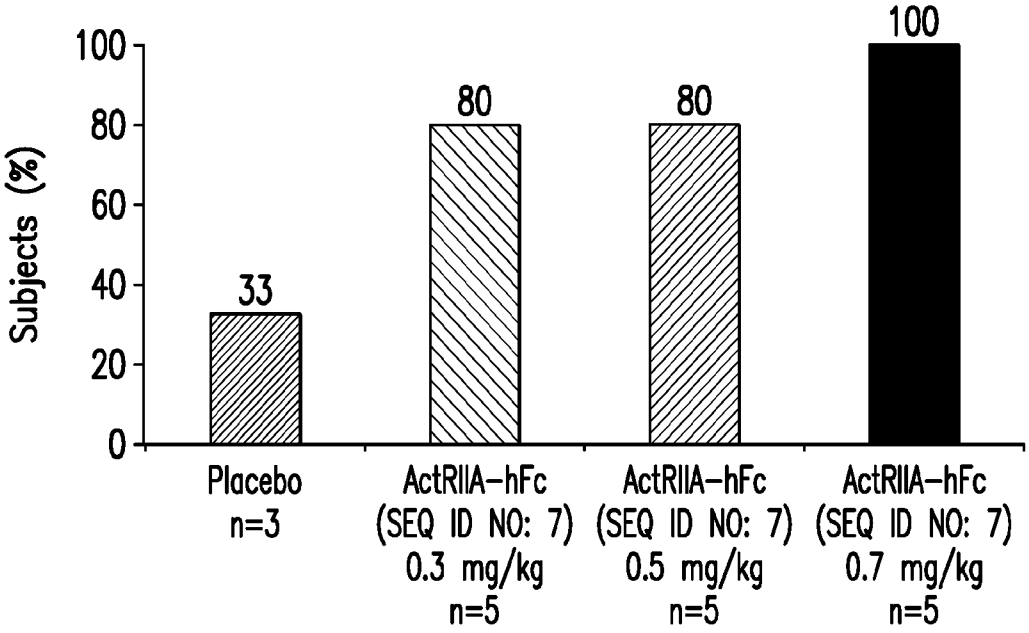


FIG. 22B

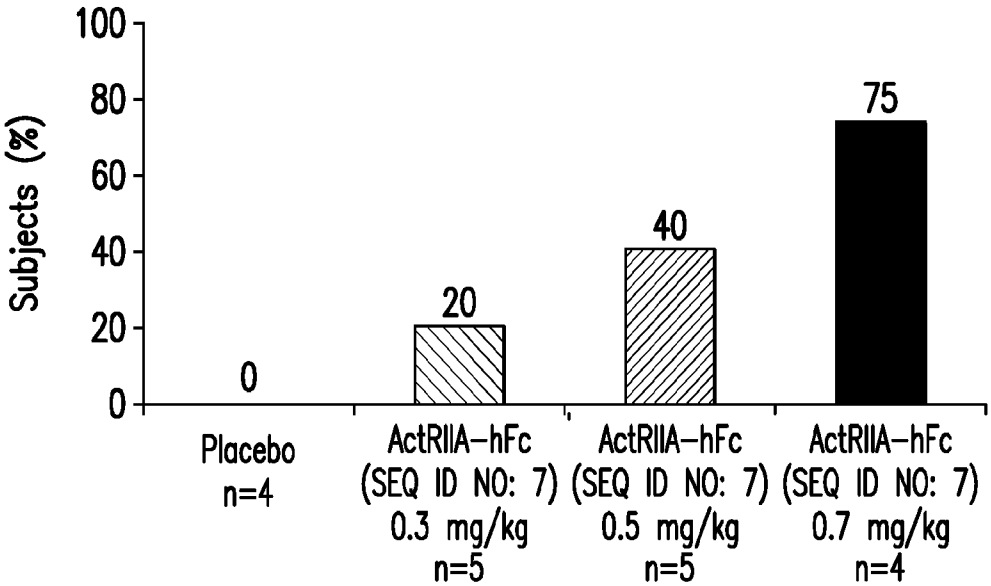


FIG. 22C

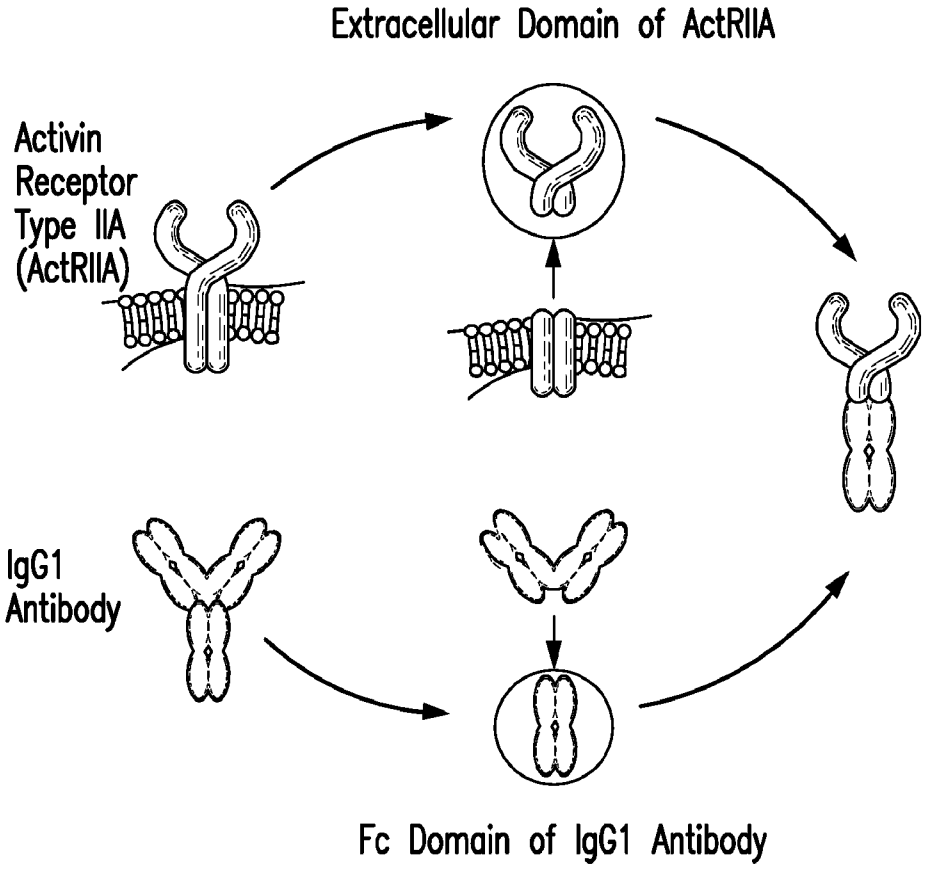


FIG. 23A

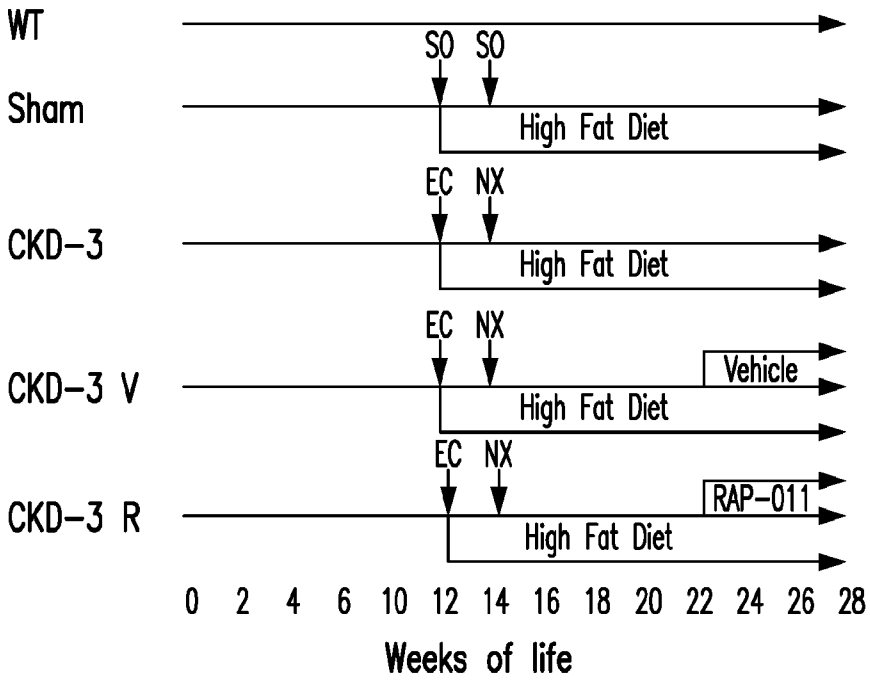


FIG. 23B

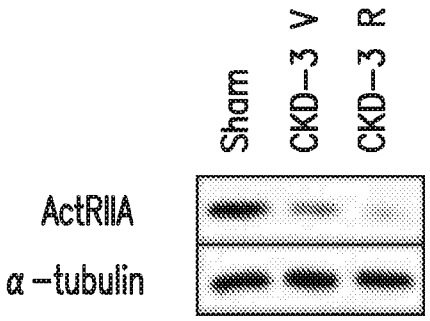


FIG. 24A

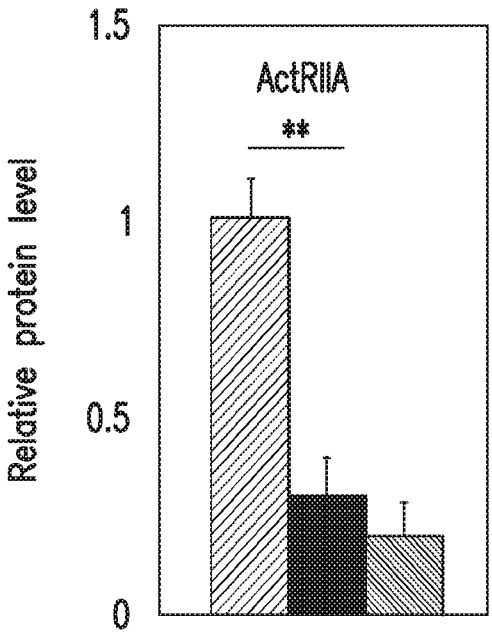


FIG. 24B

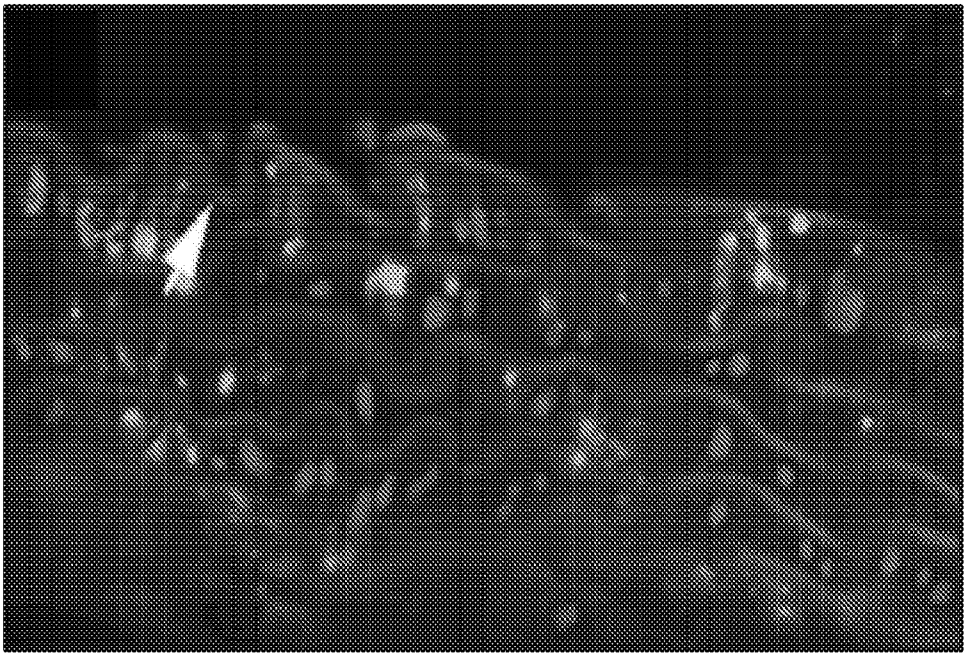


FIG. 24C

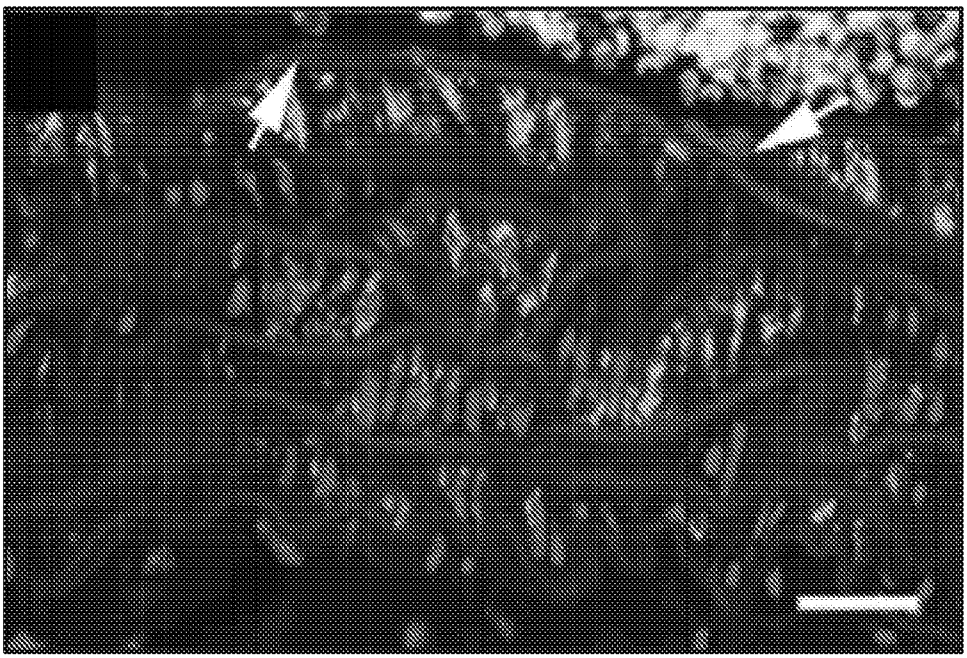


FIG. 24D

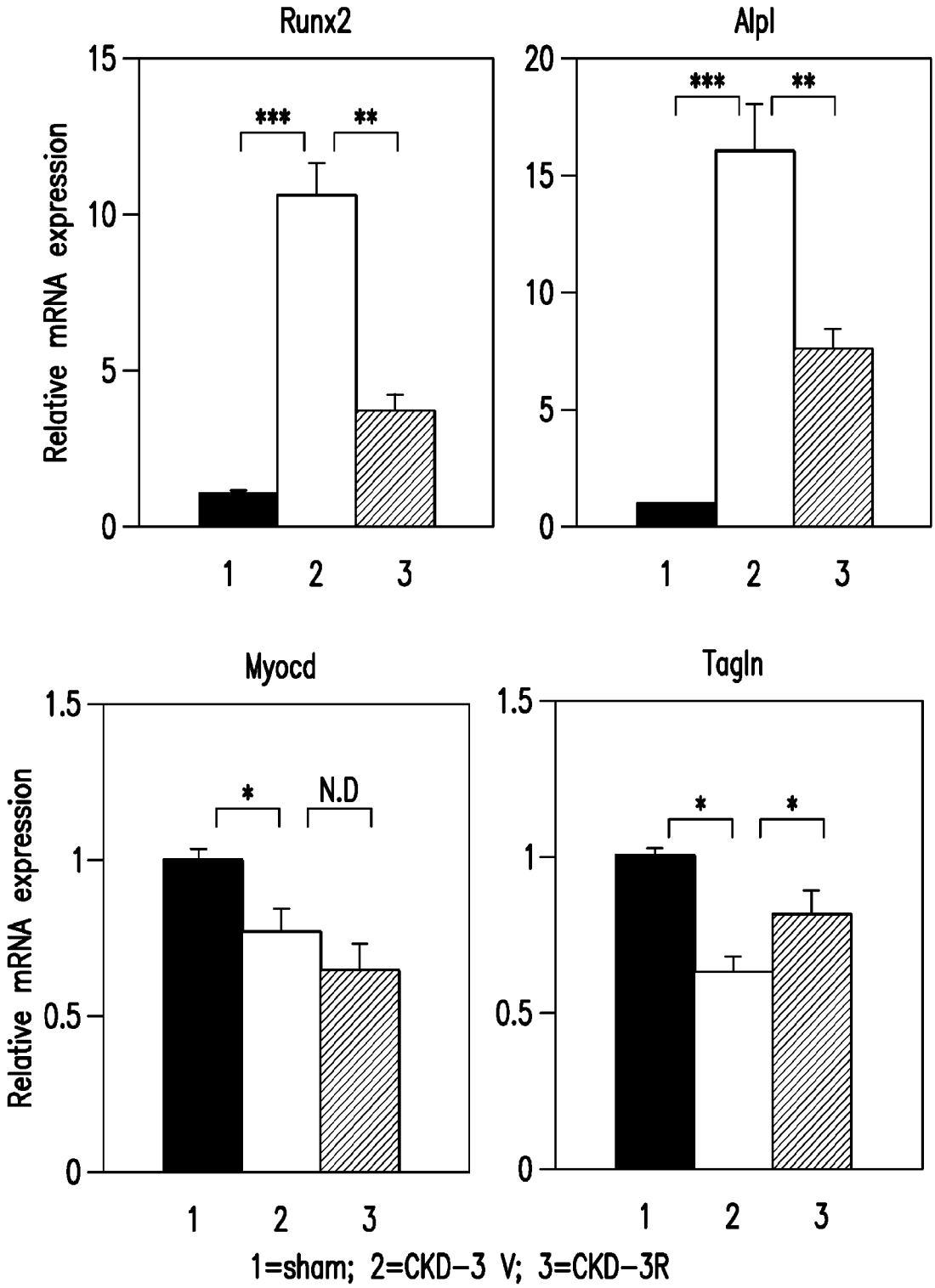

Runx2
Alpl
Myocd
Tagln
Relative mRNA expression
Relative mRNA expression
***
**
*
N.D
1=sham; 2=CKD-3 V; 3=CKD-3R

FIG. 25A

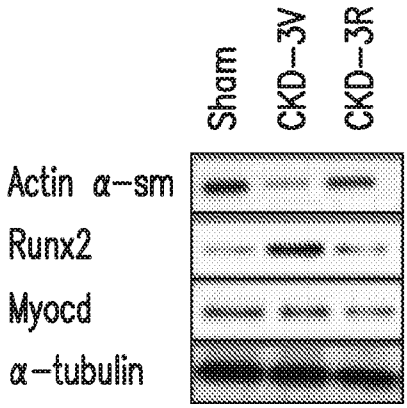


FIG. 25B

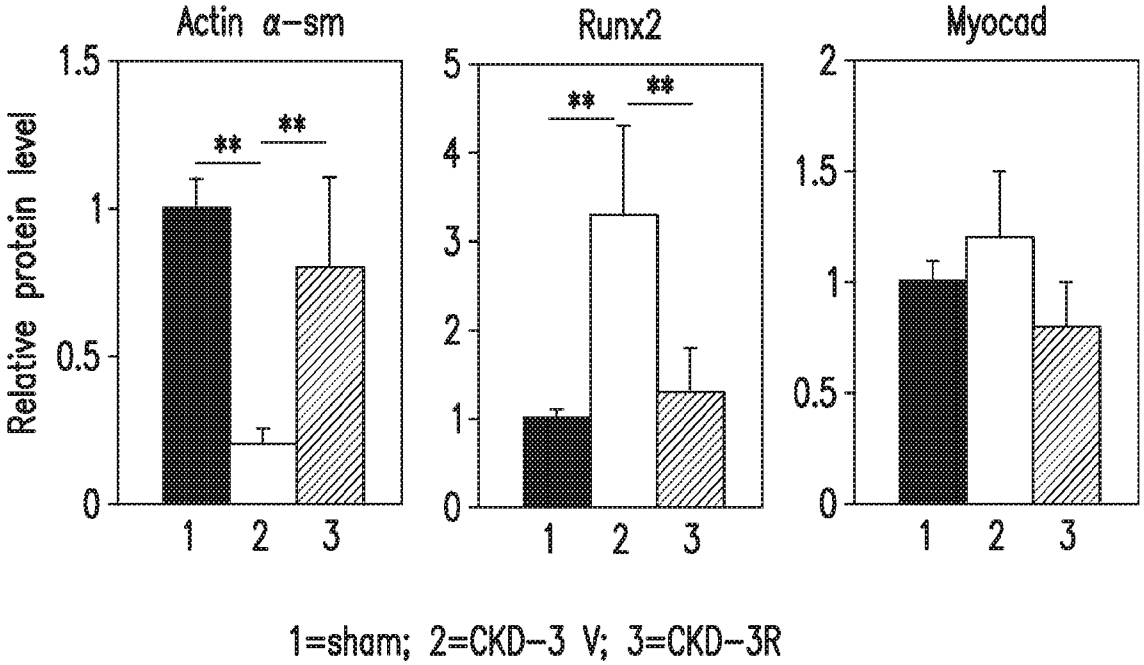
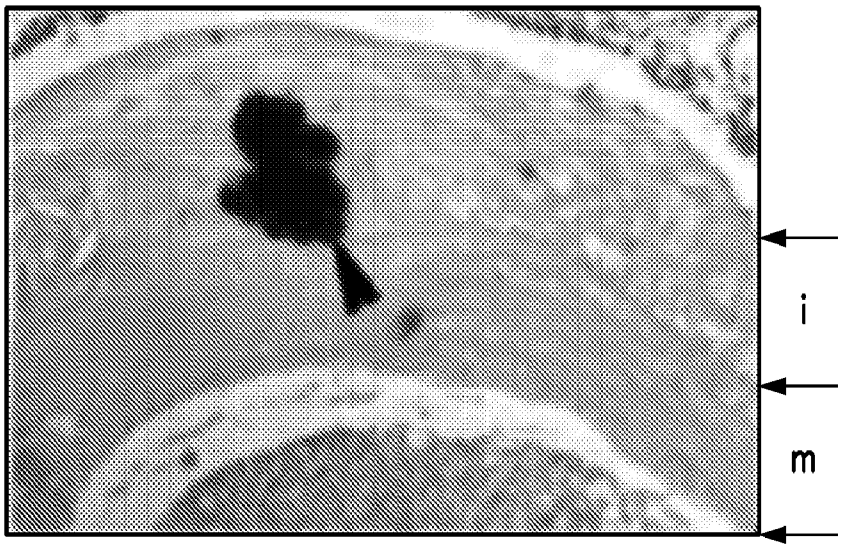
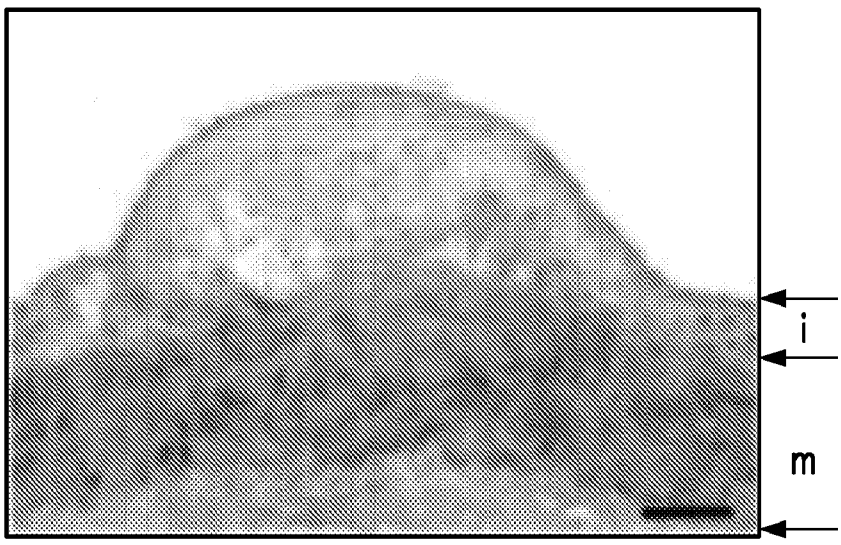


FIG. 25C



CKD-3 V

FIG. 26A



CKD-3 RAP-011

FIG. 26B

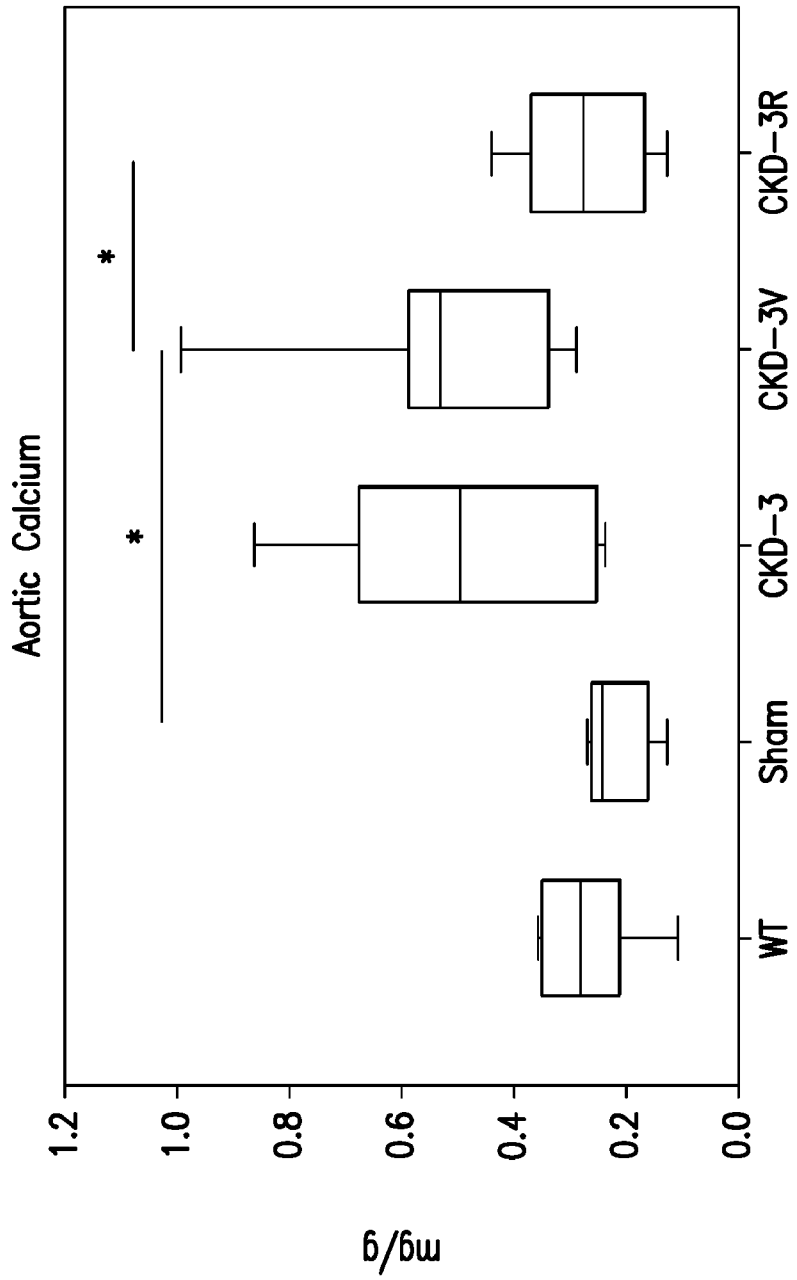

Aortic Calcium
mg/g
1.2
1.0
0.8
0.6
0.4
0.2
0.0
WT
Sham
CKD-3
CKD-3V
CKD-3R
*
*

FIG. 26C

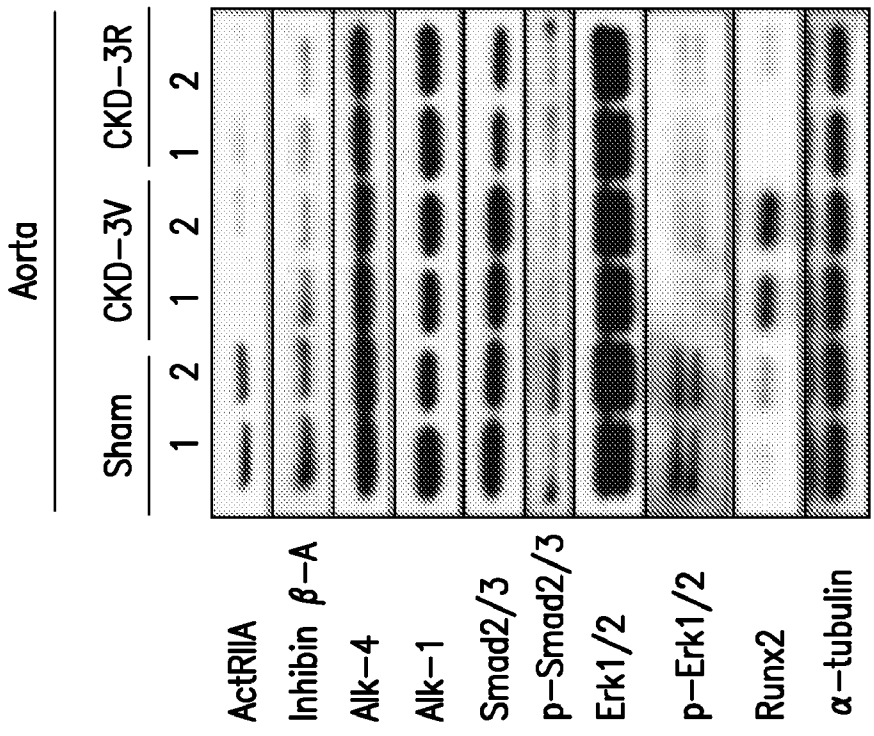


FIG. 27A

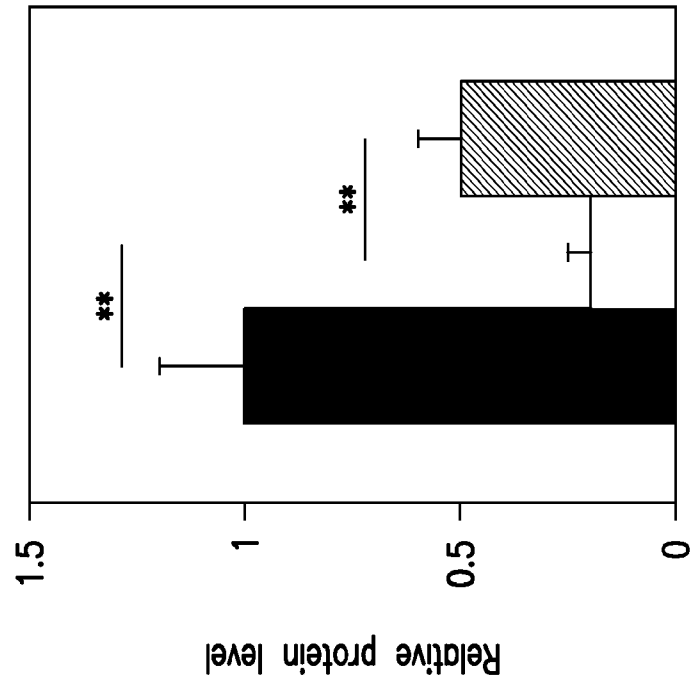


FIG. 27B

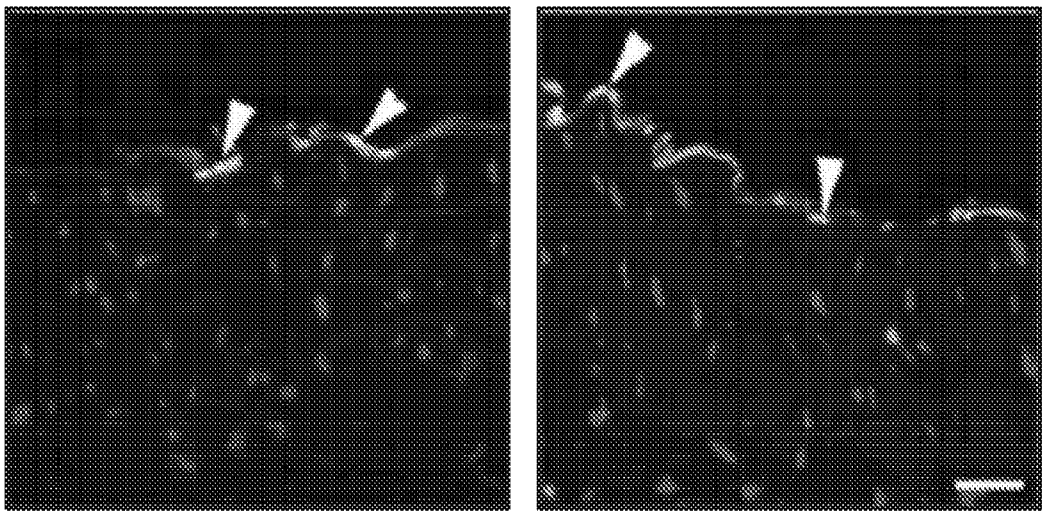


FIG. 28A

FIG. 28B

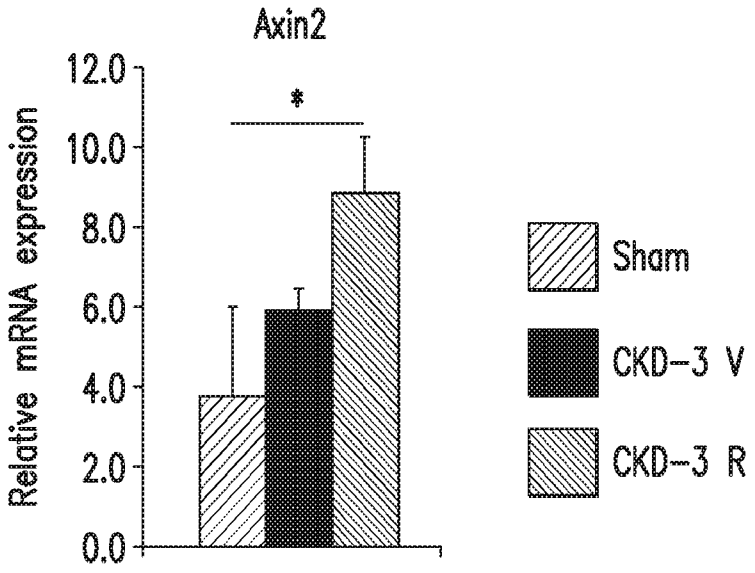


FIG. 28C

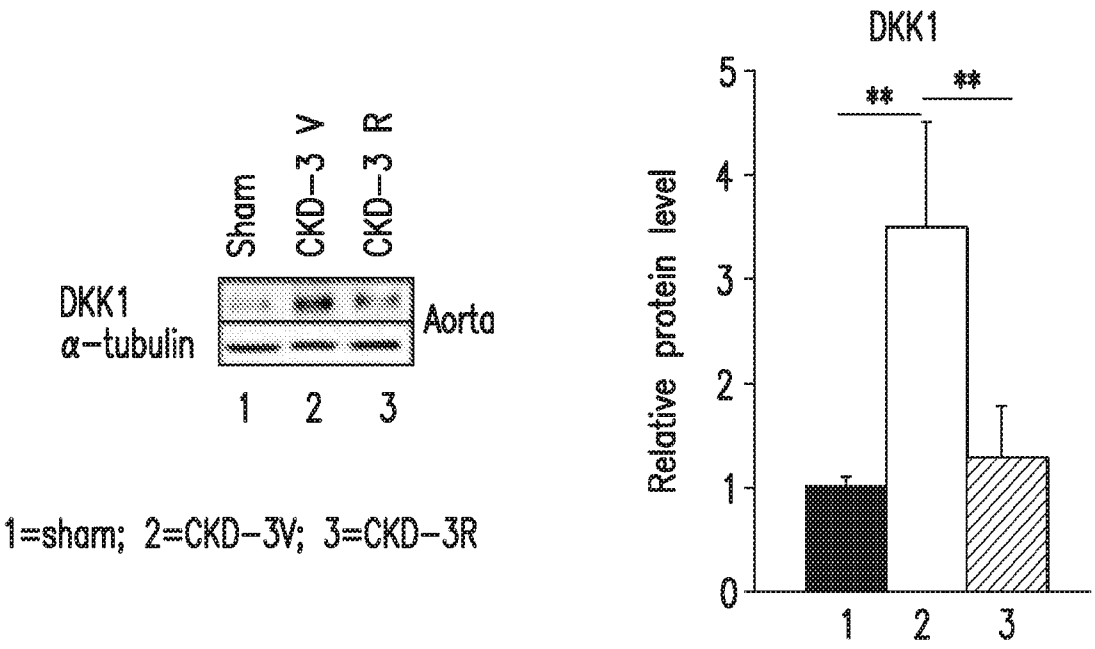


FIG. 28D

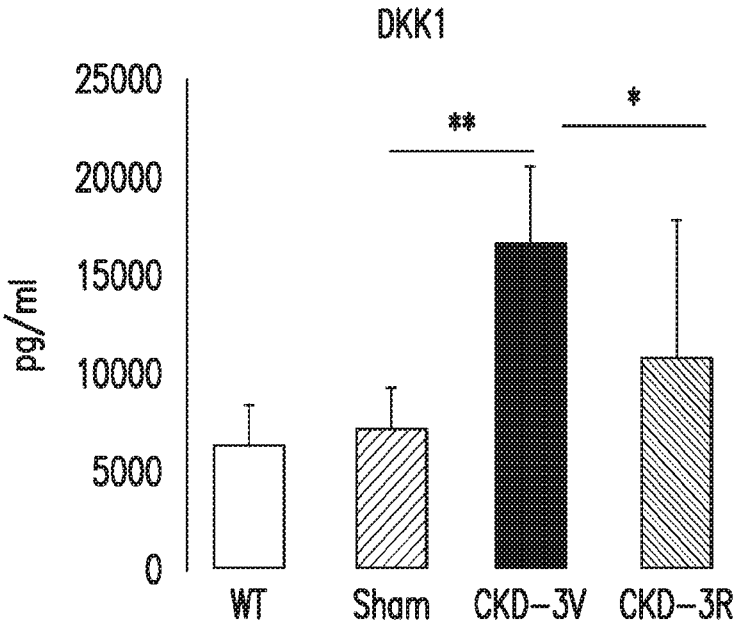


FIG. 28E

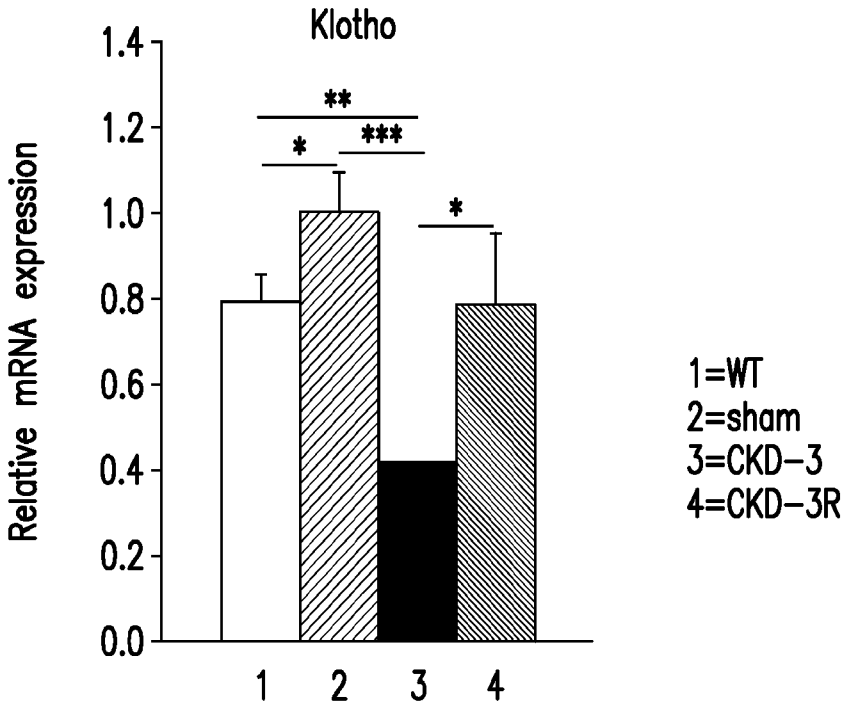


FIG. 29A

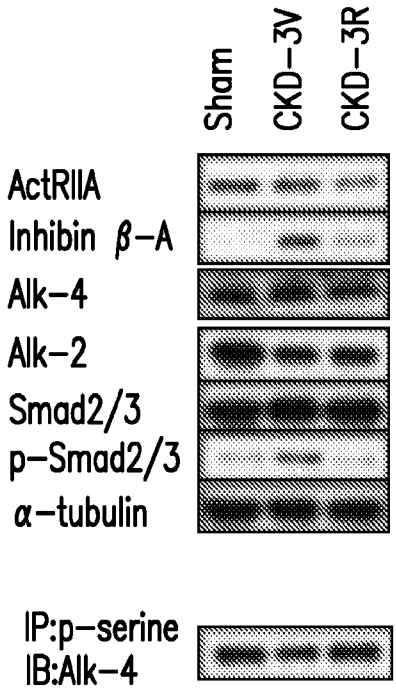


FIG. 29B

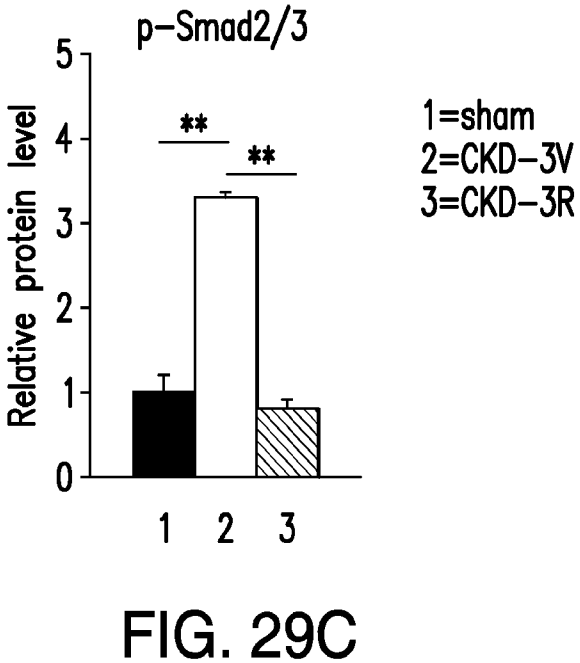


FIG. 29C

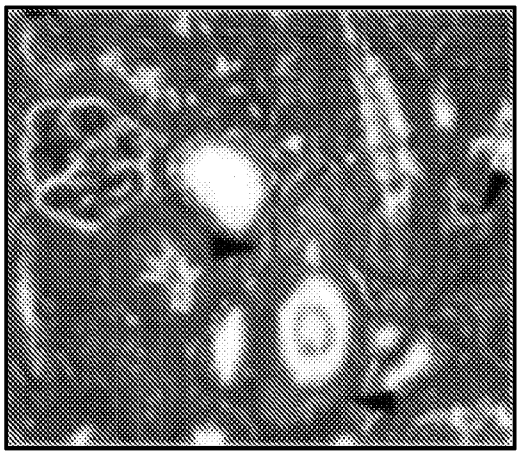


FIG. 30A

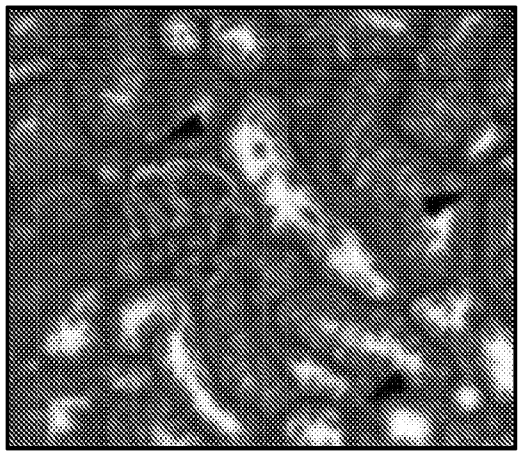


FIG. 30B

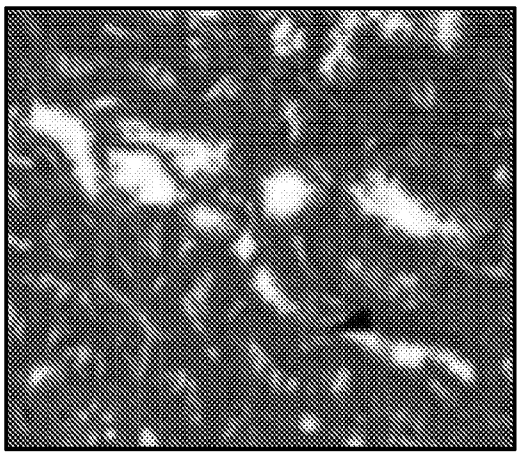


FIG. 30C

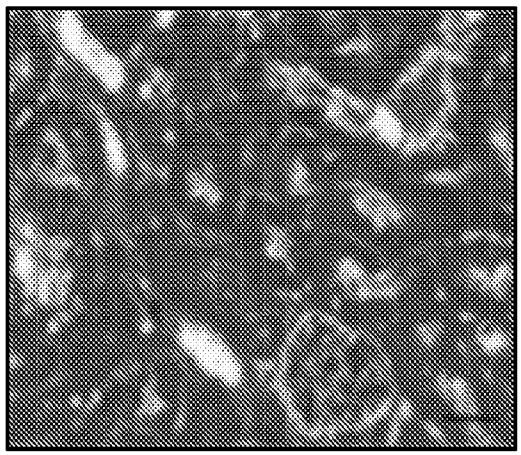


FIG. 30D

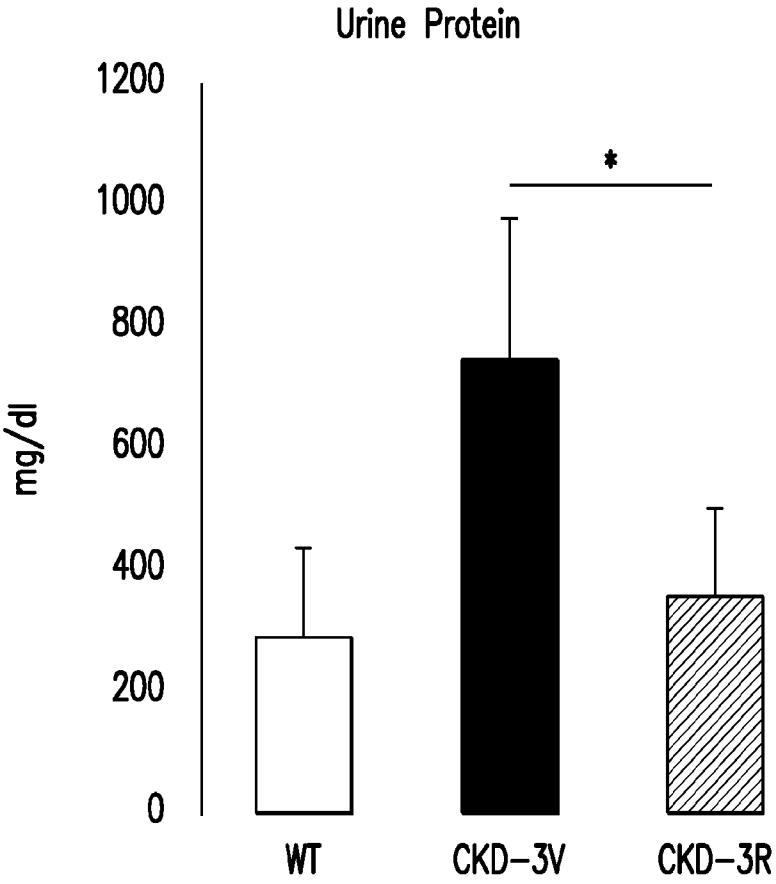


FIG. 30E

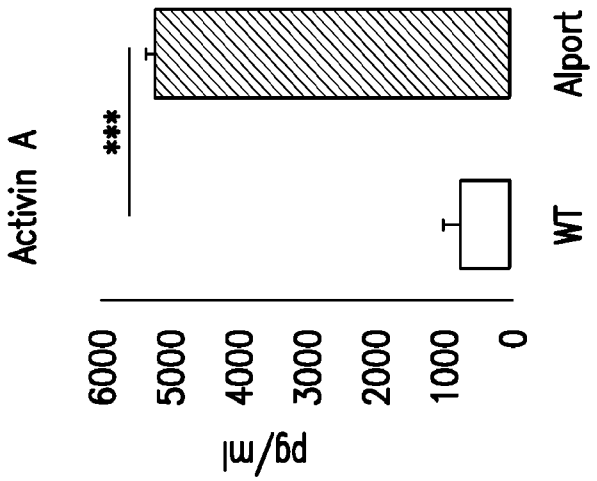


FIG. 31B

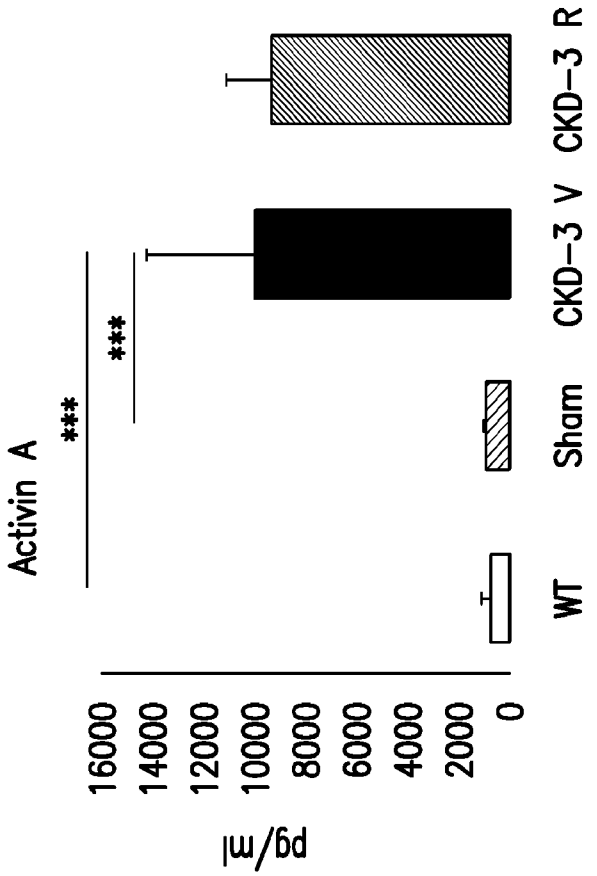


FIG. 31A

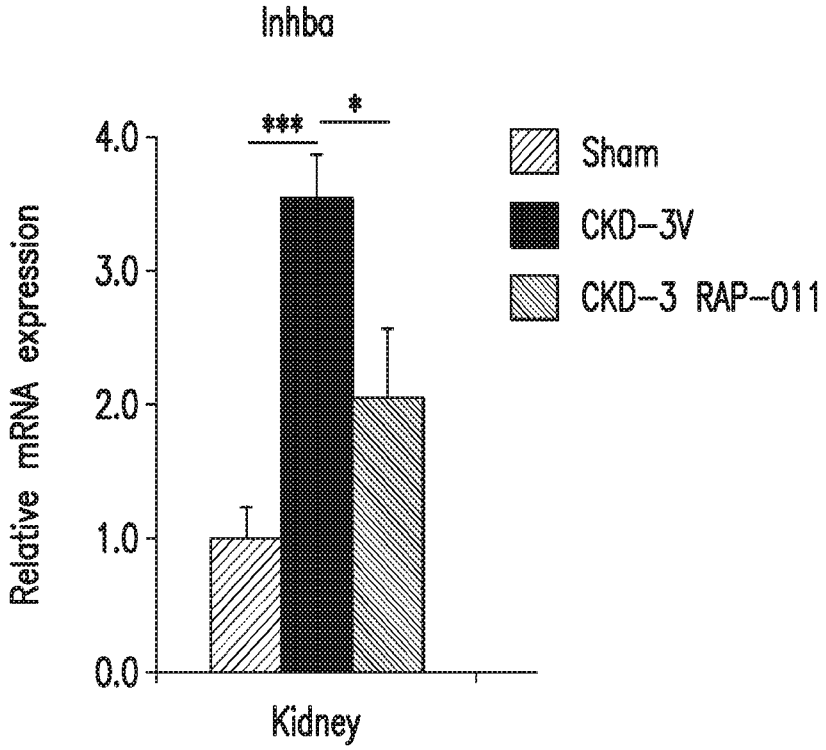


FIG. 31C

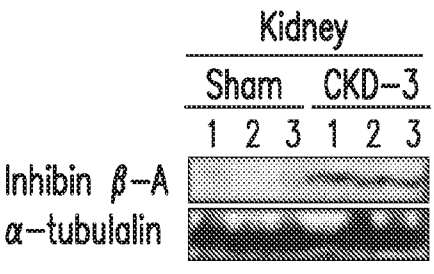


FIG. 31D

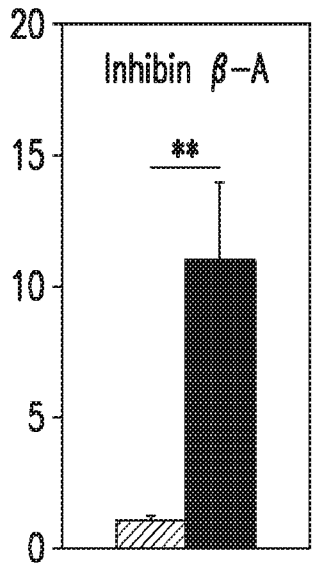


FIG. 31E

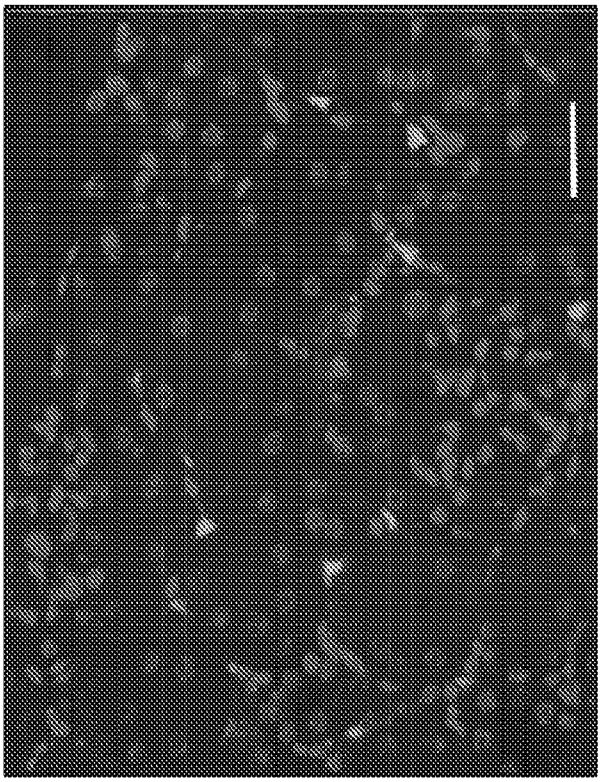


FIG. 32B

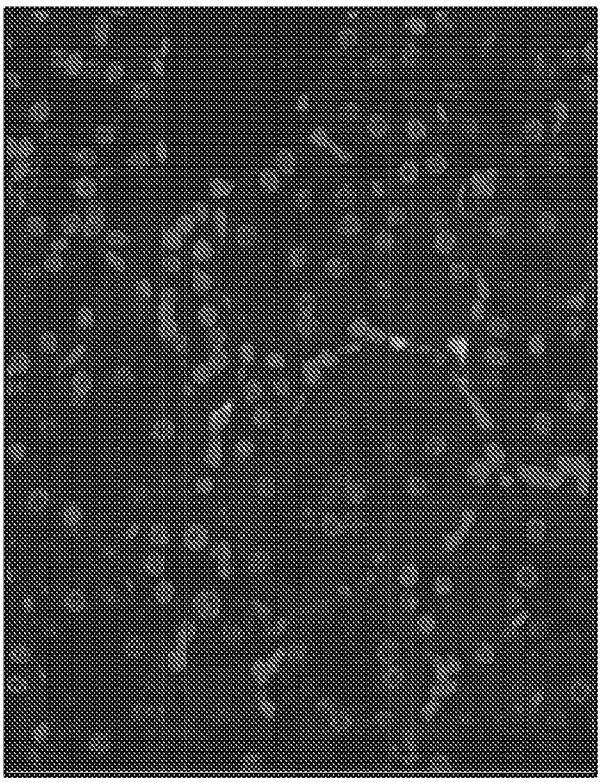


FIG. 32A

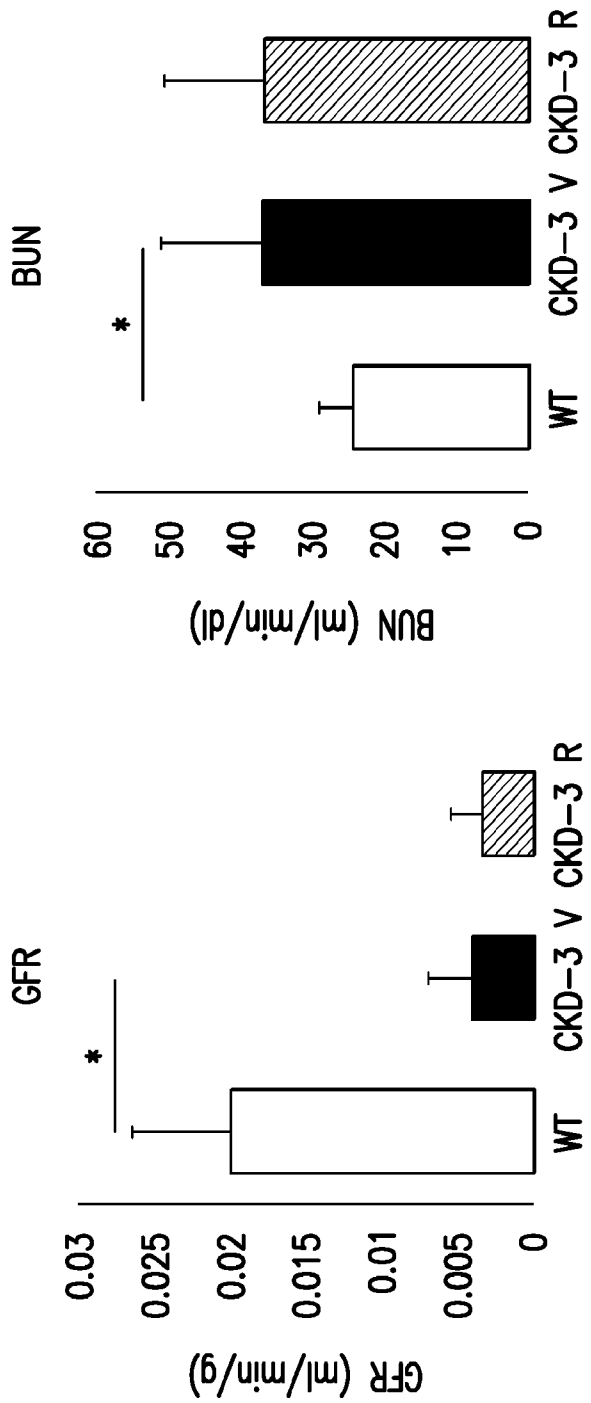

BUN
BUN (ml/min/dl)
60
50
40
30
20
10
0
*
WT
CKD-3 V
CKD-3 R
GFR
GFR (ml/min/g)
0.03
0.025
0.02
0.015
0.01
0.005
0
*
WT
CKD-3 V
CKD-3 R

FIG. 33A

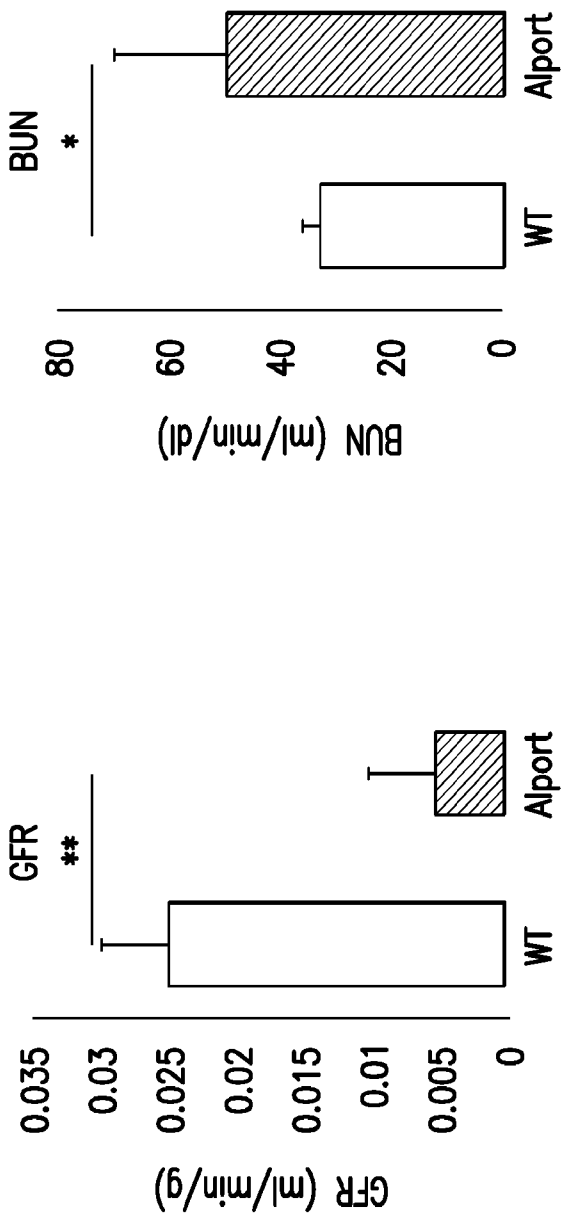

BUN (ml/min/dl)
80
60
40
20
0
BUN
*
WT
Alport
GFR (ml/min/g)
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
GFR
**
WT
Alport

FIG. 33B

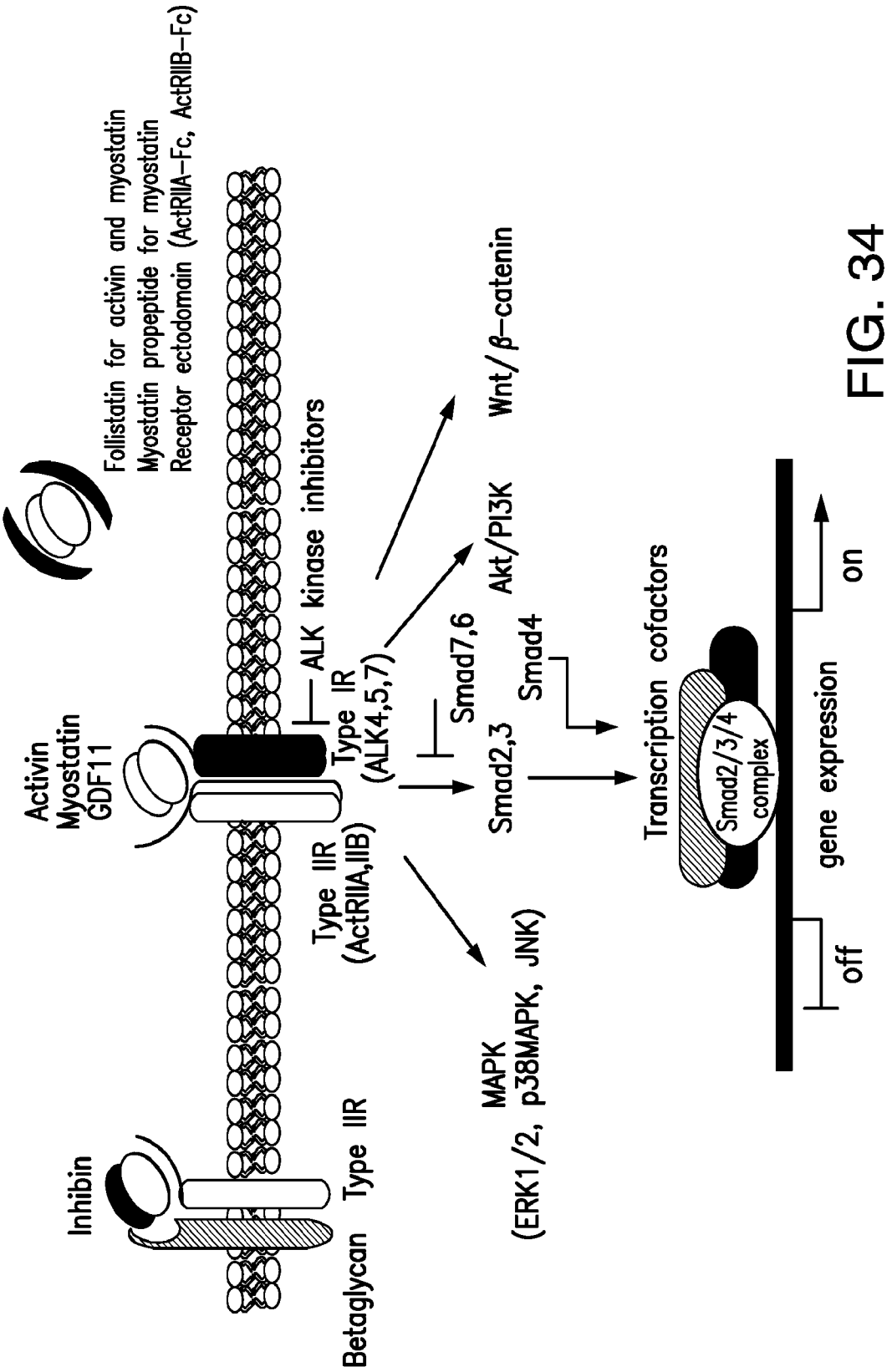

Follistatin for activin and myostatin
Myostatin propeptide for myostatin
Receptor ectodomain (ActRIIA-Fc, ActRIIB-Fc)
ALK kinase inhibitors
Activin
Myostatin
GDF11
Type IR
(ALK4,5,7)
Type IIR
(ActRIIA,IIB)
Inhibin
Betaglycan
Type IIR
Smad7,6
Smad2,3
Smad4
Akt/PI3K
Wnt/β-catenin
MAPK
(ERK1/2, p38MAPK, JNK)
Transcription cofactors
Smad2/3/4
complex
gene expression
on
off

FIG. 34

FIG. 35B

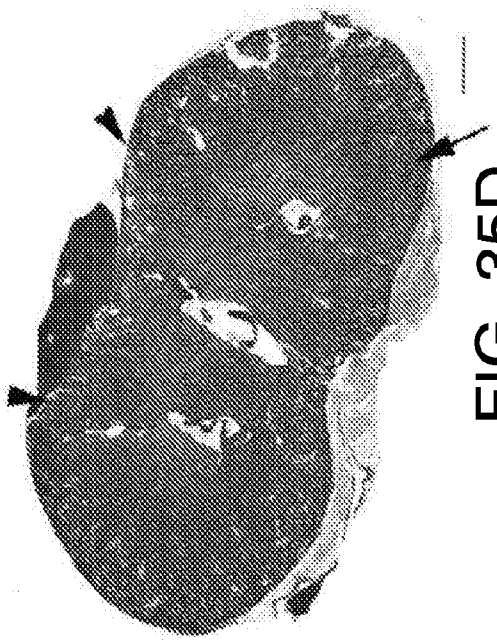


FIG. 35D

FIG. 35A

FIG. 35C

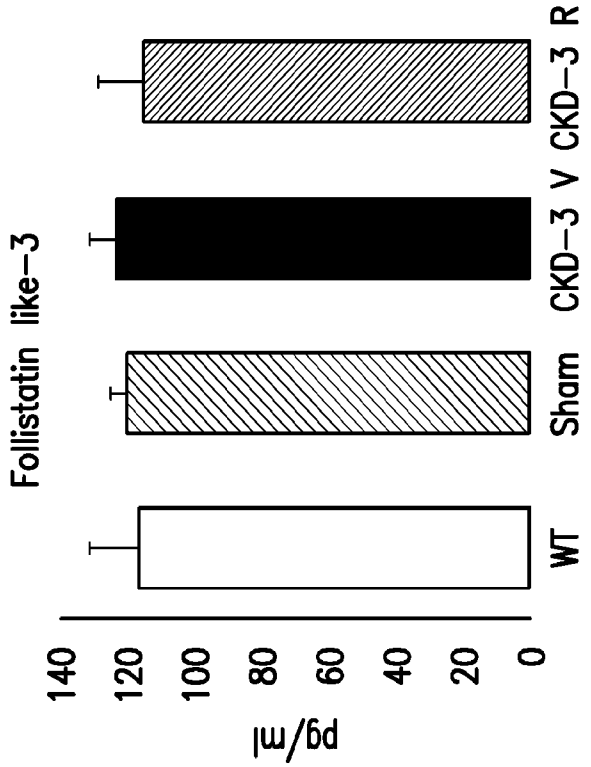


FIG. 36B

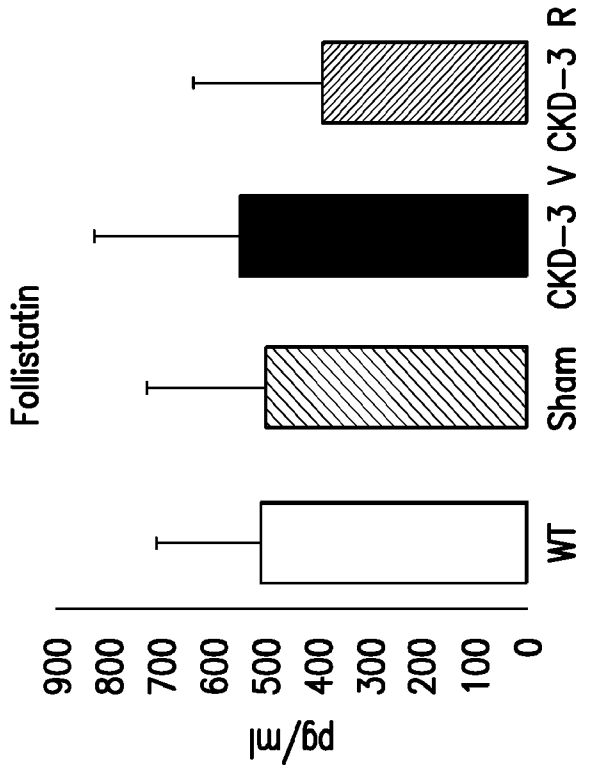


FIG. 36A

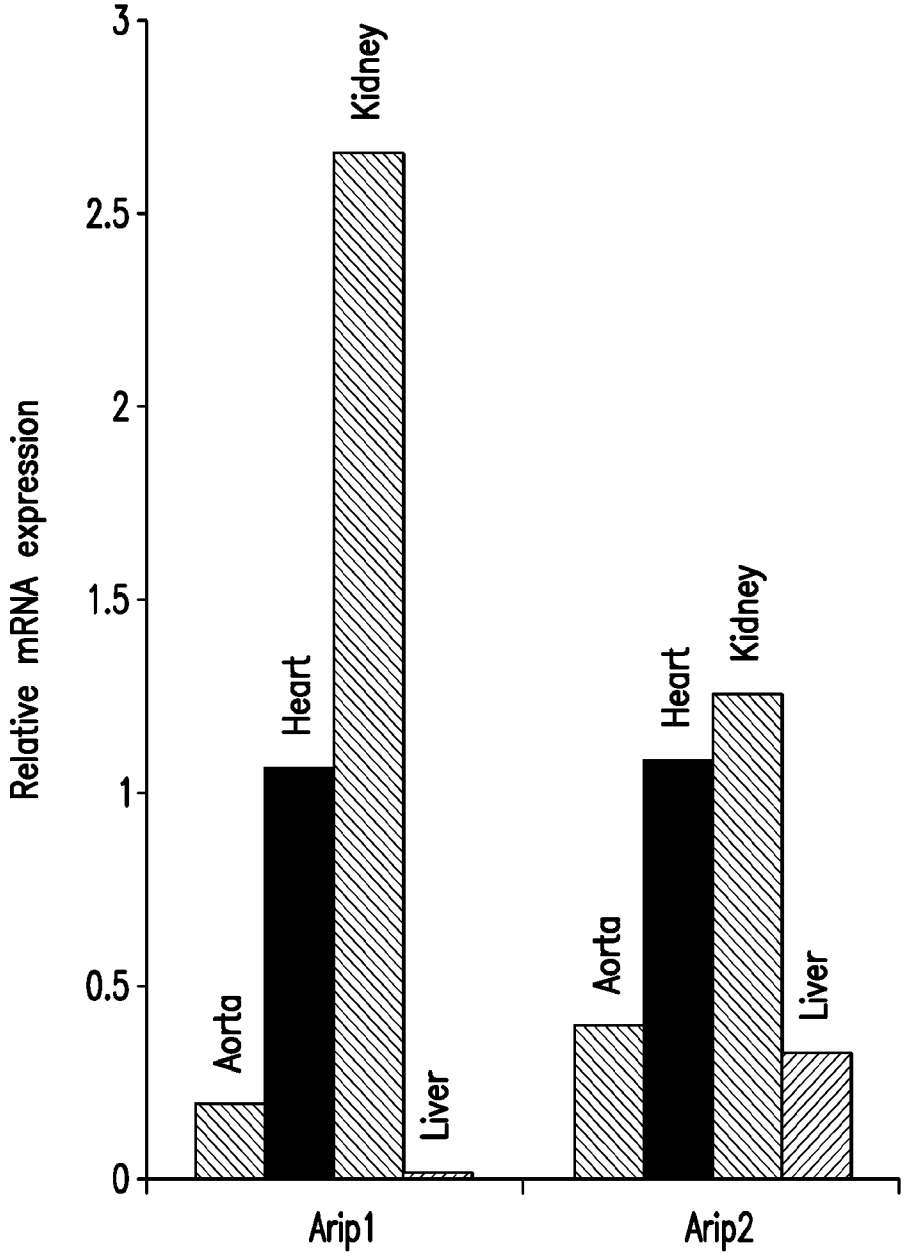

Relative mRNA expression
3
2.5
2
1.5
1
0.5
0
Aorta
Heart
Kidney
Liver
Arip1
Aorta
Heart
Kidney
Liver
Arip2

FIG. 37

20153397786 28 Nov 2017

SEQUENCE LISTING

<110> Celgene Corporation
Washington University

<120> TREATMENT OF CARDIOVASCULAR DISEASE USING ACTRII LIGAND TRAPS

<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> Homo sapiens

<220>
<223> human ActRIIA precursor polypeptide

<400> 1

Met Gly Ala Ala Ala Lys Leu Ala Phe Ala Val Phe Leu Ile Ser Cys
1 5 10 15

Ser Ser Gly Ala Ile Leu Gly Arg Ser Glu Thr Gln Glu Cys Leu Phe
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

2015339786 28 Nov 2017

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

2015339786 28 Nov 2017

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> Homo sapiens

<220>
<223> human ActRIIA soluble (extracellular), processed polypeptide sequence

<400> 2

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

2015339786 28 Nov 2017

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> Artificial Sequence

<220>
<223> human ActRIIA soluble (extracellular), processed polypeptide sequence with the C-terminal 15 amino acids deleted

<400> 3

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
100

<210> 4
<211> 1542
<212> DNA
<213> Homo sapiens

<220>
<223> nucleic acid sequence encoding human ActRIIA precursor protein

<400> 4
atgggagctg ctgcaaagtt ggcgtttgcc gtctttctta tctcctgttc ttcaggtgct 60

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

tattttgtt gctgtgaggg caatatgtgt aatgaaaagt tttcttattt tccagagatg 360

2015339786 28 Nov 2017

gaagtcacac agcccacttc aaatccagtt acacctaagc caccctatta caacatcctg 420

ctctatt cct tggtgccact tatgttaatt gcggggattg tcatttgtgc attttgggtg 480

tacaggcatc acaagatggc ctaccctcct gtacttgttc caactcaaga cccaggacca 540

cccccacctt ctccattact agggttgaaa ccactgcagt tattagaagt gaaagcaagg 600

ggaagatttg gttgtgtgct g 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 catttgctgac 1020

tttgggttgg ccttaaaatt tgaggctggc aagtctgcag gcgataccca tggacaggtt 1080

ggtacccgga ggtacatggc tccagaggta ttagagggtg ctataaactt cgaaagggat 1140

gcattttttga 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 ttgacttttcc tcccaaagaa tctagtctat ga 1542

<210> 5
<211> 345
<212> DNA
<213> Homo sapiens

<220>
<223> nucleic acid sequence encoding a human ActRIIA soluble (extracellular) polypeptide

<400> 5
atacttggta gatcagaaac tcaggagtgt cttttcttta atgctaattg ggaaaaagac 60

agaaccaatc aaactggtgt tgaaccgtgt tatggtgaca aagataaacg gcggcattgt 120

tttgctacct ggaagaatat ttctggttcc attgaaatag tgaaacaagg ttgttggctg 180

gatgatatca actgctatga caggactgat tgtgtagaaa aaaaagacag ccctgaagta 240

tattttttgtt gctgtgaggg caatatgtgt aatgaaaagt tttcttatttt tccagagatg 300

gaagtcacac agcccacttc aaatccagtt acacctaagc caccc 345

<210> 6
<211> 228
<212> PRT

2015339786 28 Nov 2017

<213> Artificial Sequence

<220>
<223> fusion protein comprising a soluble extracellular domain of ActRIIA fused to an Fc domain

<220>
<221> misc_feature
<222> (44)..(44)
<223> Xaa = Asp or Ala

<220>
<221> misc_feature
<222> (102)..(102)
<223> Xaa = Lys or Ala

<220>
<221> misc_feature
<222> (215)..(215)
<223> Xaa = Asn or Ala

<400> 6

```
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
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
```

28 Nov 2017

2015339786

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
210 215 220

Ser Pro Gly Lys
225

<210> 7
<211> 344
<212> PRT
<213> Artificial Sequence

<220>
<223> Extracellular domain of human ActRIIA fused to a human Fc domain

<400> 7

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 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

2015339786 28 Nov 2017

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> Artificial Sequence

<220>
<223> Leader sequence of Honey bee mellitin (HBML)

<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> Artificial Sequence

2015339786 28 Nov 2017

<220>
<223> Leader sequence of Tissue Plasminogen Activator (TPA)

<400> 9

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
20

<210> 10
<211> 20
<212> PRT
<213> Unknown

<220>
<223> Native ActRIIA leader

<400> 10

Met Gly Ala Ala Ala Lys Leu Ala Phe Ala Val Phe Leu Ile Ser Cys
1 5 10 15

Ser Ser Gly Ala
20

<210> 11
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> ActRIIA-hFc and mActRIIA-Fc N-terminal sequence

<400> 11

Ile Leu Gly Arg Ser Glu Thr Gln Glu
1 5

<210> 12
<211> 329
<212> PRT
<213> Artificial Sequence

<220>
<223> ActRIIA-Fc Protein with deletion of the C-terminal 15 amino acids of the extracellular domain of ActRIIA

<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

28 Nov 2017

2015339786

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

2015339786 28 Nov 2017

Lys Ser Leu Ser Leu Ser Pro Gly Lys
325

<210> 13
<211> 369
<212> PRT
<213> Artificial Sequence

<220>
<223> Unprocessed ActRIIA-hFc with TPA leader sequence

<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

28 Nov 2017 2015339786

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> Artificial Sequence

<220>
<223> Nucleic acid sequence encoding Unprocessed ActRIIA-hFc with TPA leader sequence

<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

2015339786 28 Nov 2017

agtcttcctc ttcccccaa 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> Artificial Sequence

<220>
<223> 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

<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

28 Nov 2017 2015339786

<213> Homo sapiens

<220>
<223> human ActRIIB precursor protein sequence (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
```

28 Nov 2017 2015339786

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

2015339786 28 Nov 2017

<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 19-134 of SEQ ID NO:16)

<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> Artificial Sequence

<220>
<223> human ActRIIB soluble (extracellular), processed polypeptide sequence with the C-terminal 15 amino acids deleted (amino acids 19-119 of SEQ ID NO:16)

<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

28 Nov 2017 2015339786

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> Homo sapiens

<220>
<223> nucleic acid sequence encoding a human ActRIIB(A64) precursor protein

<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 gccaccccg acagccccca ccctgctcac ggtgctggcc 420

tactcactgc tgcccatcgg gggcctttcc ctcatcgtcc tgctggcctt ttggatgtac 480

cggcatcgca agccccctca cggtcatgtg gacatccatg aggaccctgg gcctccacca 540

ccatccctc tggtgggcct gaagccactg cagctgctgg agatcaaggc tcgggggcgc 600

tttggctgtg tctggaaggc ccagctcatg aatgacttg tagctgtcaa gatcttccca 660

ctccaggaca agcagtcgtg gcagagtgaa cgggagatct tcagcacacc tggcatgaag 720

cacgagaacc tgctacagtt catgctgcc gagaagcgag gctccaacct cgaagtagag 780

ctgtggctca tcacggcctt ccatgacaag ggctccctca cggatttacct caaggggaac 840

atcatcacat ggaacgaact gtgtcatgta gcagagacga tgtcacgagg cctctcatac 900

ctgcatgagg atgtgccctg gtgccgtggc gagggccaca agccgtctat tgcccacagg 960

gacttttaaaa 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

cacccttcgt tggaggagct gcaggaggtg gtggtgcaca agaagatgag gcccaccatt 1320

2015339786 28 Nov 2017

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> Artificial Sequence

<220>
<223> fusion protein comprising a soluble extracellular domain of ActRIIB (A64; SEQ ID NO:17) fused to an Fc domain

<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

28 Nov 2017

2015339786

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> Artificial Sequence

<220>
<223> 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

<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

28 Nov 2017

2015339786

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

2015339786 28 Nov 2017

<210> 22
<211> 105
<212> PRT
<213> Artificial Sequence

<220>
<223> 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

<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> Artificial Sequence

<220>
<223> 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

<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

2015339786 28 Nov 2017

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> Artificial Sequence

<220>
<223> 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

<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> Artificial Sequence

<220>
<223> 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

<400> 25

Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1 5 10 15

28 Nov 2017

2015339786

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

28 Nov 2017
2015339786

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> Artificial Sequence

<220>
<223> human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 20-134 of SEQ ID NO: 16)

<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> Artificial Sequence

<220>
<223> human ActRIIB soluble (extracellular), processed polypeptide sequence with the C-terminal 15 amino acids deleted (amino acids 20-119 of SEQ ID NO: 16)

<400> 27

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn

28 Nov 2017 2015339786

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> Homo sapiens

<220>
<223> human ActRIIB precursor protein sequence (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

28 Nov 2017
2015339786

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

2015339786 28 Nov 2017

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> Artificial Sequence

<220>
<223> human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 19-134 of SEQ ID NO: 28)

<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

2015339786 28 Nov 2017

Thr Ala Pro Thr
115

<210> 30
<211> 101
<212> PRT
<213> Artificial Sequence

<220>
<223> human ActRIIB soluble (extracellular), processed polypeptide sequence with the C-terminal 15 amino acids deleted (amino acids 19-119 of SEQ ID NO:28)

<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> Artificial Sequence

<220>
<223> human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 20-134 of SEQ ID NO:28)

<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

28 Nov 2017 2015339786

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> Artificial Sequence

<220>
<223> human ActRIIB soluble (extracellular), processed polypeptide sequence with the C-terminal 15 amino acids deleted (amino acids 20-119 of SEQ ID NO: 28)

<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> Artificial Sequence

<220>
<223> 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

28 Nov 2017
2015339786

(amino acids 25-131 of SEQ ID NO:16) and with an L79D mutation

<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> Artificial Sequence

<220>
<223> 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:16) and with an L79D mutation and with TPA leader sequence

<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

28 Nov 2017 2015339786

```
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
```

28 Nov 2017

2015339786

<210> 35
<211> 335
<212> PRT
<213> Artificial Sequence

<220>
<223> 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

<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

2015339786 28 Nov 2017

```
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>  Artificial Sequence

<220>
<223>  human ActRIIB soluble (extracellular), processed polypeptide
       sequence (amino acids 20-134 of SEQ ID NO: 28) with L79D mutation

<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
```

2015339786 28 Nov 2017

Ala Pro Thr
115

<210> 37
<211> 115
<212> PRT
<213> Artificial Sequence

<220>
<223> human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 20-134 of SEQ ID NO:16) with L79D mutation

<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> Artificial Sequence

<220>
<223> 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

<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

28 Nov 2017

2015339786

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

28 Nov 2017

2015339786

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> Artificial Sequence

<220>
<223> human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 20-134 of SEQ ID NO:16) with L79D mutation fused to an Fc domain

<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

28 Nov 2017
2015339786

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> Artificial Sequence

<220>
<223> 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

<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

28 Nov 2017

2015339786

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

28 Nov 2017

2015339786

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> Artificial Sequence

<220>
<223> 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

<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

28 Nov 2017

2015339786

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> Artificial Sequence

<220>
<223> human ActRIIB soluble (extracellular), processed polypeptide sequence having a variant C-terminal sequence (disclosed in WO2007/053775)

<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

28 Nov 2017 2015339786

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> Artificial Sequence

<220>
<223> human ActRIIB soluble (extracellular), processed polypeptide sequence having a variant C-terminal sequence (disclosed in WO2007/053775) having an L79D mutation

<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

2015339786 28 Nov 2017

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> Artificial Sequence

<220>
<223> human ActRIIB soluble (extracellular), processed polypeptide sequence having a variant C-terminal sequence (disclosed in WO2007/053775) having an L79D mutation fused to an Fc domain with a TGGG linker

<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

28 Nov 2017 2015339786

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> Artificial Sequence

<220>
<223> Nucleic Acid Sequence Encoding SEQ ID NO:24

<400> 45
atggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttcgtt 60

tcgcccggcg ccgccgaaac ccgcgaatgt atttattaca atgctaattg ggaactcgaa 120

cggacgaacc aatccgggct cgaacggtgt gaggggggaac aggataaacg cctccattgc 180

tatgcgtcgt ggaggaactc ctccgggacg attgaactgg tcaagaaagg gtgctgggac 240

gacgatttca attgttatga ccgccaggaa tgtgtcgcga ccgaagagaa tccgcaggtc 300

2015339786 28 Nov 2017

tatttctgtt gttgcgaggg gaatttctgt aatgaacggt ttacccacct ccccgaagcc 360

ggcgggcccg aggtgaccta tgaaccccg 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 ctttcctctat 960

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1020

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc cccgggtaaa 1080

tga 1083

<210> 46
<211> 344
<212> PRT
<213> Artificial Sequence

<220>
<223> fusion protein comprising a soluble extracellular domain of ActRIIB (R64; SEQ ID NO:29) fused to an Fc domain

<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

28 Nov 2017

2015339786

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> Artificial Sequence

<220>
<223> fusion protein comprising a soluble extracellular domain of ActRIIB (R64) with the C-terminal 15 amino acids deleted (SEQ ID

28 Nov 2017

2015339786

NO:30) fused to an Fc domain

<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
```

28 Nov 2017
2015339786

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> Artificial Sequence

<220>
<223> Runx2 qRT-PCR primer 1

<400> 48
ccgcacgaca accgcaccat 20

<210> 49
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Runx2 qRT-PCR primer 2

<400> 49
cgctccggcc cacaaatctc 20

<210> 50
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Alp qRT-PCR primer 1

<400> 50
acgtggctaa gaatgtcatc 20

<210> 51
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Alp qRT-PCR primer 2

<400> 51
ctggtaggcg atgtcctta 19

28 Nov 2017

2015339786

<210> 52
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> osterix qRT-PCR primer 1

<400> 52
tagtggtttg gggtttgttt accgc 25

<210> 53
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> osterix qRT-PCR primer 2

<400> 53
aaccaaataa ctcttattcc ctaagt 26

<210> 54
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Klotho qRT-PCR primer 1

<400> 54
gctctcaaag cccacatact g 21

<210> 55
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Klotho qRT-PCR primer 1

<400> 55
gcagcataac gatagaggcc 20

<210> 56
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Sm22-alpha qRT-PCR primer 1

<400> 56
gttccagact gttgacctct tt 22

<210> 57
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Sm22-alpha qRT-PCR primer 2

28 Nov 2017
2015339786

<400> 57
ctgcgctttc ttcataaacc 20

<210> 58
<211> 5553
<212> DNA
<213> Homo sapiens

<220>
<223> Human 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 ctttttgggat ccgagcacca gccggcgctt cagccccccc 300
tccagcagcc tgcagcccgg caaaatgagc gacgtgagcc cggtggtggc tgcgcaacag 360
cagcagcaac agcagcagca gcaacagcag cagcagcagc agcaacagca gcagcagcag 420
caggaggcgg cggcggcggc tgcggcggcg gcggcggctg cggcggcggc agctgcagtg 480
ccccggttgc ggccgcccca cgacaaccgc accatggtgg agatcatcgc cgaccacccg 540
gccgaactcg tccgcaccga cagccccaac ttcctgtgct cggtgctgcc ctcgcactgg 600
cgctgcaaca agaccctgcc cgtggcctac aaggtggtag ccctcggaga ggtaccagat 660
gggactgtgg ttactgtcat ggcgggtaac gatgaaaatt attctgctga gctccggaat 720
gcctctgctg ttatgaaaaa ccaagtagca aggttcaacg atctgagatt tgtgggccgg 780
agtggacgag gcaagagttt cacctgacc ataaccgtct tcacaaatcc tccccaagta 840
gctacctatc acagagcaat taaagttaca gtagatggac ctcgggaacc cagaaggcac 900
agacagaagc ttgatgactc taaacctagt ttgttctctg accgcctcag tgattttaggg 960
cgcattcctc atcccagtat gagagtaggt gtcccgcctc agaaacccacg 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
cgaatgcact atccagccac ctttactttac accccgccag tcacctcagg catgtccctc 1440
ggtatgtccg ccaccactca ctaccacacc tacctgccac caccctaccc cggctcttcc 1500
caaagccaga gtggaccctt ccagaccagc agcactccat atctctacta tggcacttcg 1560
tcaggatcct atcagtttcc catggtgccg gggggagacc ggtctcctcc cagaatgctt 1620
ccgccatgca ccaccacctc gaatggcagc acgctattaa atccaaatct gcctaaccag 1680

2015339786 28 Nov 2017

aat gat ggt g t t gacgct ga t ggaagccac agcagt t ccc caact gt t t t gaat t ct agt 1740
ggcagaat gg at gaat ct gt t t ggcgacca t at t gaaat t cct cagcagt ggcccagt gg 1800
t at ct ggggg ccacat ccca cacgt at caa t at at acat a t at agagaga gt gcat at at 1860
at gt at at cg at t agct at c t acaaagt gc ct at t t t t t a gaagat t t t t cat t cact ca 1920
ct cagt cat g at ct t gcagc cat aagaggg t agat at t ga gaagcagaag gct caagaga 1980
gacaat t gca at cgagct t c agat t gt t t a ct at t t aaga t gt act t t t a caaaggaaca 2040
aagaagggaa aaggt at t t t t gt t t t t gt t gt t t ggt ct g t t at cat caa t aacct gt t c 2100
at at gccaat t cagagaggt ggact ccagg t t caggaggg agaagagcaa agccgct t cc 2160
t ct ct gt gct t t gaaact t c acaccct cac ggt ggcagct gt gt at ggac cagt gccct c 2220
cgcagacagc t cacaaaacc agt t gaggt g cact aaaggg acat gaggt a gaat ggat gc 2280
t t ccat caca gt accat cat t cagaat aac t ct t ccaat t t ct gct t t ca gacat gct gc 2340
aggt cct cat ct gaact gt t gggt t cgt t t t t t t t t t t t t cct gct ccaagaaagt 2400
gact t caaaa at aact gat c aggat agat t at t t t at t t t act t t t t aac act cct t ct c 2460
ccct t t t ccc act gaaccaa aaagaaat cc cat ccct aaa acct gcct t c t cct t t t at g 2520
caaaact gaa aat ggcaat a cat t at t at a gccat aat gg t at agat agt gat t gcgt t t 2580
ggct at gt gt t gt t t t ct t t t t t t t t aaat t at gaat at g t gt aaaat ct gaggt aact t 2640
gct aacgt ga at ggt cat at aact t t aaag at at at t t at aat t at t t aa t gacat t t gg 2700
accct t gaaa cat t t ct t ag t gt at t gat a t gt t gact t c ggt ct ct aaa agt gct ct t t 2760
at t aaat aac aaat t t ct t c agt ggt ct ag agccat at ct gaaat at t gc t aagcaat t t 2820
cagt t cat cc aggcacaat g t gat t t t aaa aaat act t cc at ct ccaaat at t t t agat a 2880
t agat t gt t t t t gt gat gt a t gaaggaaat gt t at gt t t a gt t ct t t cag at ct t t gaat 2940
gcct ct aaca cagct t t gcc t t ct aaagcg gt aat t aggg at t t aaaaaa caacct t t ag 3000
ccct t t at ca gcat gaaat g ct ggagt gat gt ggt t t t ct aat t t ct t t g gggt aat t at 3060
gact ct t gt c at at t aaaaa gacaagcaca agt aaat cat t gaact acag aaaaat gt t c 3120
t gt ggt t t ca t agt t aagca aaact ct aaa t cgccaggct t cat agcaaa gacat agt ca 3180
gct aaaagcc gcacat gt gg at agagggt t caat t at gag acacct agt a caggagagca 3240
aaat t gcacc agagat t ct t aaccaaccag cct t accaaa caacacaaca ggggaacccc 3300
aat ct gcct t acccaaggcc ccact ggcag ct t t ccacag aat t t gcat t t agaggagca 3360
gaat gacat c act gt cct t t gggagt aggt cct ct gaaaa ggcagcaggt t ccagcaggt 3420
agct gagct g agaggacat a t ggcccacgg ggacct acag acagcct t t g acat t t gt at 3480
t t ct t acaat ggagggccaa ggagggcaag gggct gt gga gt t t ggt gt c t act agt gt g 3540
t at gaat t t g agct agagt c ct t ct gt ggc at gcact t t g accact cct g gcagt cacat 3600
ggcagat t t c caagt gcaaa t cct t aat cc aaacaaggat cat ct aat ga caccaccagg 3660
ccaat ccct g ct ct cct ccc cgaaaagt ca gggt ccct t c at t ggaat cc t ccacccacc 3720

2015339786 28 Nov 2017

caagcagaat ttagcagaga tttgcctt ca aaccctaacg gccccctt gt tctctggtcc 3780
ttctcaaacc cacctttgta ggccacccag cattgcagga cagcgtgtgg ggcagctgga 3840
cctgtgcttc ctgcctggga gtctcccttg gaattcatcc tgactcctt c taataaaaat 3900
ggatgggaaa gcaaaacact ttgcctt ct a aaggccgtat accaagtatg ctt agataaa 3960
taagccactt ttctattact taagtaagaa ggaagtagt a attgatacta tttattgttt 4020
gtgtgtggta gcttgaagca caccactgtc cattt attt g taagtgtaaa atatgtgtgt 4080
ttgtttcagc agcacttaaa aaagccagtg tctggttaca catttcaatt ttaattaatt 4140
gacataaaaa tgctaccgcc agtgccagct gcatcctatt taatt aaaaa ggtactatat 4200
ttgtacatta tttttt aatg ttaaaagggc ttttttaagt ttacagtaca catac cgagt 4260
gacttt aggg atgctttgt gttgaaatgt tactatagtg gctgcaggca gcaacccaga 4320
aacactttag aagctttttt tcctt gggaa aaattcaagc acttctt ccc tccaccctca 4380
ctccaaccac cccaatgggg gtaattcaca tttcttagaa caaattctgc cctttttt gg 4440
tctagggatt aaaatttgt ttttctttct ttctttttt t tttttttca ctgaaccctt 4500
aatttgcact gggtcatgtg tttgattt gt gatttcaaga ccaaagcaaa gtcttactac 4560
tactgtggaa ccatgtacta gttcctggga attaaaatag cgtggttctc tttgtagcac 4620
aaacatt gct ggaatttgca gtctttt caa tgcagccaca ttttt atcca tttcagttgt 4680
ctcacaaatt ttaacccata tcagagttcc agaacaggta ccacagcttt ggttttagat 4740
tagtggaata acattcagcc cagaactgag aaactcaaca gattaactat cgttt gctct 4800
ttagacggtc tcactgcctc tcacttgcca gagccctttc aaaatgagca gagaagtcca 4860
caccattagg gaccatctgt gataaatt ca gaagggagga gatgtgtgta cagctttaag 4920
gatttccct ca attccgagga aagggactgg cccagaatcc aggttaatac atggaaacac 4980
gaagcatt ag caaaagtaat aatt at acct atggtatttg aaagaacaat aataaaagac 5040
actt ctt cca aaccttgaat ttgttgtttt tagaaaacga atgcatttaa aaatatttc 5100
tatgtgagaa ttttttagat gtgtgtttac ttcatgttta caaataactg tttgctttt t 5160
aatgcagtac tttgaaatat atcagccaaa accataactt acaataatt t ctt aggtatt 5220
ctgaataaaa ttccatttct tttggatatg ctttaccatt cttaggttt c tgtggaacaa 5280
aaatatttgt agcatttttgt gtaaatacaa gctttcattt tttatttttt c caatt gctat 5340
tgcccaagaa ttgctttcca tgcacatatt gtaaaaatt c cgctttgtgc cacaggtcat 5400
gatttgtggat gagtttactc ttaacttcaa agggactatt tgtattgtat gttgcaactg 5460
taaatt gaat tatttggcat ttttctcatg attgtaatat taatttgaag tttgaatt ta 5520
atttt caata aaatggctt t tttggttttg tta 5553

<210> 59
<211> 3173
<212> DNA
<213> Homo sapiens

28 Nov 2017 2015339786

<220>
<223> Human Alp mRNA

<400> 59
ccgggcctca ctcgggcccc gcggccgcct ttataaggcg gcgggggtgg tggcccgggc 60
cgcgttgcgc tcccgccact ccgcgcccgc tatcctggct ccgtgctccc acgcgcttgt 120
gcctggacgg acccctcgcca gtgctctgcg caggattgga acatcagtta acatctgacc 180
actgccagcc cacccccttcc cacccacgtc gattgcatct ctgggctcca gggataaagc 240
aggtcttggg gtgcaccatg attttcaccat tcttagtact ggccatttggc acctgcctta 300
ctaactcctt agtgccagag aaagagaaag accccaagta ctggcgagac caagcgcaag 360
agacactgaa atatgccctg gagcttcaga agctcaacac caacgtggct aagaatgtca 420
tcatgttcct gggagatggg atgggtgtct ccacagtgac ggctgcccgc atcctcaagg 480
gtcagctcca ccacaaccct ggggaggaga ccaggctgga gatggacaag ttcccttcg 540
tggccctctc caagacgtac aacaccaatg cccaggtccc tgacagcgcc ggcaccgcca 600
ccgcctacct gtgtggggtg aaggccaatg agggcaccgt gggggtaagc gcagccactg 660
agcgttcccg gtgcaacacc acccagggga acgaggtcac ctccatcctg cgctgggcca 720
aggacgctgg gaaatctgtg ggcattgtga ccaccacgag agtgaaccat gccacccca 780
gcgccgccta cgcccactcg gctgaccggg actggtactc agacaacgag atgcccctg 840
aggcctgag ccagggctgt aaggacatcg cctaccagct catgcatcac atcagggaca 900
ttgacgtgat catgggggt ggccggaaat acatgtaccc caagaataaa actgatgtgg 960
agtatgagag tgacgagaaa gccaggggca cgaggctgga cggcctggac ctcgttgaca 1020
cctggaagag ctttcaaaccg agatacaagc actcccactt catctggaac cgcacggaac 1080
tcctgaccct tgacccccac aatgtggact acctattggg tctcttcgag ccaggggaca 1140
tgcagtacga gctgaacagg aacaacgtga cggacccgtc actctccgag atggtggtgg 1200
tggccatcca gatcctgcgg aagaacccca aaggcttctt tctgctggtg gaaggaggca 1260
gaattgacca cgggcaccat gaaggaaaag ccaagcaggc cctgcatgag gcggtggaga 1320
tggaccgggc catcgggcag gcaggcagct tgacctcctc ggaagacact ctgaccgtgg 1380
tcactgcgga ccatttcccac gtcttcacat ttggtggata caccccccgt ggcaactcta 1440
tctttggtct ggcccccat gctgagtgaca cagacaagaa gccctttcact gccatcctgt 1500
atggcaatgg gcctggctac aaggtggtgg gcggtgaacg agagaatgtc tccatggtgg 1560
actatgctca caacaactac caggcgcagt ctgctgtgcc cctgcgccac gagacccacg 1620
gcggggagga cgtggccgtc ttctccaagg gccccatggc gcacctgctg cacggcgtcc 1680
acgagcagaa ctacgtcccc cacgtgatgg cgtatgcagc ctgcatcggg gccaacctcg 1740
gccactgtgc tcctgccagc tcggcaggca gcctttgctgc aggccccctg ctgctcgcgc 1800
tggccctcta ccccctgagc gtcctgttct gagggcccag ggcccgggca cccacaagcc 1860
cgtgacagat gccaacttcc cacacggcag ccccccccct caaggggcagg gaggtggggg 1920

28 Nov 2017 2015339786

cctcctcagc ctctgcaact gcaagaaagg ggacccaaga aaccaaagtc tgccgcccac 1980

ctcgctcccc tctggaatct tccccaaggg ccaaacccac ttctggcctc agcctttgc 2040

tccctccccg ctgcccttg gccaacaggg tagatttctc ttgggcaggc agagagtaca 2100

gactgcagac attctcaaag cctcttattt ttctagcgaa cgtatttctc cagacccaga 2160

ggccctgaag cctccgtgga acattctgga tctgaccctc ccagtctcat ctcctgaccc 2220

tcccactccc atctccttac ctctggaacc ccccaggccc tacaatgctc atgtccctgt 2280

ccccaggccc agccctcctt caggggagtt gaggtctttc tcctcaggac aaggcctgc 2340

tcactcactc actccaagac caccagggtc ccaggaagcc ggtgcctggg tggccatcct 2400

acccagcgtg gcccaggccg ggaagagcca cctggcaggg ctcacactcc tgggctctga 2460

acacacacgc cagctcctct ctgaagcgac tctcctgttt ggaacggcaa aaaaaaatt t 2520

ttttttctct ttttggtggt ggttaaaagg gaacacaaaa catttaaata aaacttcca 2580

aatattccg aggacaaaaa aaaaaa 2606

<210> 60
<211> 3173
<212> DNA
<213> Homo sapiens

<220>
<223> Human Osterix mRNA

<400> 60
cggcgggcgg cagcagccta ggcagcagca gtagcagaag cagcagccgc cgagcagcag 60

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 ccccctggcca tgctgacggc agcgtgcagc 360

aaatttggtg gctctagccc tctgcgggac tcaacaactc tgggcaaagc aggcacaaag 420

aagccgtact ctgtgggcag tgacctttca gcctccaaaa ccatggggga tgcttatcca 480

gccccctta caagcactaa tgggctcctt tcacctgcag gcagtcctcc agcacccacc 540

tcaggctatg ctaatgatta ccctccctt tcccactcat tccctgggcc cacaggcacc 600

caggaccctg ggctactagt gcccaagggg cacagctctt ctgactgtct gcccagtgtc 660

tacacctctc tggacatgac acacccctat ggctcctggt acaaggcagg catccatgca 720

ggcatttcac caggcccagg caacactcct actccatggt gggatatgca ccctggaggc 780

aactggctag gtggtgggca gggccagggt gatgggctgc aagggacact gcccacaggt 840

ccagctcagc ctccactgaa ccccagctg cccacctacc catctgactt tgctcccctt 900

aatccagccc cctacccagc tccccacctc ttgcaaccag ggccccagca tgtcttgccc 960

caagatgtct atаaacccaa ggcagtggga aatagtgggc agctagaagg gagtggtgga 1020

2015339786 28 Nov 2017

gccaaacccc cacggggt gc aagcact ggg ggt agt ggt g gat at ggggg cagt ggggca 1080

gggcgct cct cct gcgact g ccct aat t gc caggagct ag agcggct ggg agcagcagcg 1140

gct gggct gc ggaagaagcc cat ccacagc t gccacat cc ct ggct gcgg caaggt gt at 1200

ggcaaggct t cgcacct gaa ggcccact t g cgct ggcaca caggcgagag gccct t cgt c 1260

t gcaact ggc t ct t ct gcgg caagaggt t c act cgt t cgg at gagct gga gcgt cat gt g 1320

cgcact caca cccgggagaa gaagt t cacc t gcct gct ct gct ccaagcg ct t t acccga 1380

agcgaccacc t gagcaaaca ccagcgcacc cat ggagaac caggcccggg t cccct ccc 1440

agt ggcccca aggagct ggg ggagggccgc agcacggggg aagaggaggc cagt cagacg 1500

ccccgacct t ct gcct cgcc agcaacccca gagaaagccc ct ggaggcag ccct gagcag 1560

agcaact t gc t ggagat ct g agccgggt gg aaggt ct ccc accccagggc t gccct gaca 1620

gt ct ct ct t g gct ct ct aga ccact gct t g ccaat cact c t ct t t acccc at gcat gcca 1680

t cct t cgggg ct ct ct ccct ct gt ct ccct cct ggccat t ct gggct t gg gt at ct cct t 1740

gcat gcct cc t cagct cacc t t ct ct ct t c accat gagac t ggct t t cca caaact ct ca 1800

t ct caggccc t cccct t gt g cct gat acct gcact ccggc t t cct agact ct ggccct gc 1860

cacaccaaca cact t t ct at t t gggct ccc aacact at t t ct ccat ct ca ct cct t gaca 1920

t gt acccct t t ct gct t ct c aagct t at t t cct gct gt cc ct cagcct cc aggct t cagt 1980

ct t cccaact t ct t acacca t t gct t t cca t t ct ccagaa ct ct t t t t t c ct t t t t acaa 2040

acacaat gat aat gat aat t t at t gccccc t ggt ggcct c t t cat caggg gt at t ggggt 2100

t agt gacct g gccagagggt gccaagaggg gggcagacca gt ggggat ct gat cccaaag 2160

at ggggt gac cccagggt ca gggaggct gc ccccaggcct gt at at t t aa cccct at gt a 2220

ccaggagt aa t gaat agt aa t aat t ct at t t at gt aagt t at gat gacgg gt caggt aga 2280

gt gagct ggg gagggaagt g gat ccat t t c t gct aaggaa at t ct agt ca aat gcat ct c 2340

t gt at agaca aaat gt t agt ggagaagat c t t gt t aat ag aat gt ct at c at cagaat ct 2400

cagt t gat ag ggt t t ct ct t gt aat gaagt ct ct acaaat t gggt t agct acat ct ct gc 2460

t aaacagt t g at ggggt at c t ct t gat t ag ggggat ccct aat at cccca gccccagcca 2520

gaagct gt ga aacct caagt cct at ggagg ggagaaggac t ggaat gt ac cccat ct ccc 2580

t t gact gcag agcaggt t cc t ccact gccc cacccct t ag acaccat gac cccat caggt 2640

t aat cccct g t t gccat ggt t at ggagagc t t gcagct gc cat ct t agat gt gct ct t t g 2700

gggaagccca t ct aacagga ggacat t ggt t t gggggt gc acct cct gaa gaat gggt gg 2760

ggaaggct t t ct ct aggat c agat t caaat aagt at gt at t gagt gcct a ct ct gt gcaa 2820

ggcact at gc t agat ct ggt gcct agaagc cct gagaaag aact t aaaga gct aggagga 2880

cagaggcccc caagct gat c t ggt ggt gca t ccacgcacc cccaccct gg gact t t ggat 2940

gct cccat ct ccacct ccag t gact t t t aa agccgct t cg t gcct t t cct gt aacgt t gg 3000

at cct cct t t t ct gt cccct gct gt ct caa ggccccaagt t aaagggt t a aagccgct gg 3060

28 Nov 2017
2015339786

agcttgggga gagaacattg tggaatggaa gggatcatgc cctttgtgga gtctttttt 3120

tttaatttaa taaataaaag ttggatttga aaaaaaaaaa aaaaaaaaaa aaa 3173

<210> 61
<211> 5003
<212> DNA
<213> Homo sapiens

<220>
<223> Human Klotho mRNA

<400> 61
cgcgcagcat gcccgccagc gccccgccgc gccgcccgcg gccgccgccg ccgtcgctgt 60

cgctgctgct ggtgctgctg ggcctgggcg gccgccgcct gcgtgcggag ccgggcgacg 120

gcgcgcagac ctgggcccgt gtctcgcggc ctcctgcccc cgaggccgcg ggcctcttcc 180

agggcacctt ccccgacggc ttcctctggg ccgtgggcag cgccgcctac cagaccgagg 240

gcggctggca gcagcacggc aagggtgcgt ccatctggga cacgttcacc caccaccccc 300

tggcaccccc gggagactcc cggaacgcca gtctgccgtt gggcgccccg tcgccgctgc 360

agcccgccac cggggacgta gccagcgaca gctacaacaa cgtcttccgc gacacggagg 420

cgctgcgcga gctcggggtc actcactacc gctttctcca tctcgtgggcg cgagtgctcc 480

ccaatggcag cgcgggcgtc cccaaccgcg aggggctgcg ctactaccgg cgcctgctgg 540

agcggctgcg ggagctgggc gtgcagcccg tggtcaccct gtaccactgg gacctgcccc 600

agcgcctgca ggacgcctac ggcggctggg ccaaccgcgc cctggccgac cacttcaggg 660

attacgcgga gctctgcttc cgccacttcg gcggtcaggt caagtactgg atcaccatcg 720

acaaccccta cgtggtggcc tggcacggct acgccaccgg gcgcctggcc cccggcatcc 780

ggggcagccc gcggctcggg tacctggtgg cgcacaacct cctcctggct catgccaaag 840

tctggcatct ctacaatact tctttccgtc ccactcaggg aggtcaggtg tccattgccc 900

taagctctca ctggatcaat cctcgaagaa tgaccgacca cagcatcaaa gaatgtcaaa 960

aatctctgga ctttgtacta ggttggttg ccaaacccgt attattgat ggtgactatc 1020

ccgagagcat gaagaataac ctttcatcta ttctgcctga ttttactgaa tctgagaaaa 1080

agttcatcaa aggaactgct gacttttttg ctctttgctt tggacccacc ttgagttttc 1140

aacttttgga ccctcacatg aagttccgcc aattggaatc tcccaacctg aggcaactgc 1200

tttcctggat tgaccttgaa tttaaccatc ctcaaatatt tattgtggaa aatggctggt 1260

ttgtctcagg gaccaccaag agagatgatg ccaaatatat gtattacctc aaaaagttca 1320

tcatggaaac cttaaaagcc atcaagctgg atggggtgga tgtcatcggg tataccgcat 1380

ggtccctcat ggatggttac gagtggcaca gaggttacag catcaggcgt ggactcttct 1440

atgttgactt tctaagccag gacaagatgt tgttgccaaa gtcttcagcc ttgttctacc 1500

aaaagctgat agagaaaaat ggcttccctc ctttacctga aaatcagccc ctagaaggga 1560

cattttccct gtgacttttgct tggggagttg ttgacaactta catcaagtta gataccactc 1620

28 Nov 2017 2015339786

t gt ct cagt t t accgacct g aat gt t t acc t gt gggat gt ccaccacagt aaaaggct t a 1680
t t aaagt gga t ggggt t gt g accaagaaga ggaaat cct a ct gt gt t gac t t t gct gcca 1740
t ccagcccca gat cgct t t a ct ccaggaaa t gcacgt t ac acat t t t cgc t t ct ccct gg 1800
act gggccct gat t ct ccct ct gggt aacc agt cccaggt gaaccacacc at cct gcagt 1860
act at cgct g cat ggccagc gagct t gt cc gt gt caacat caccccagt g gt ggccct gt 1920
ggcagcct at ggccccgaac caaggact gc cgcgcct cct ggccaggcag ggcgcct ggg 1980
agaacccct a cact gccct g gcct t t gcag agt at gcccg act gt gct t t caagagct cg 2040
gccat cacgt caagct t t gg at aacgat ga at gagccgt a t acaaggaat at gacat aca 2100
gt gct ggcca caacct t ct g aaggcccat g ccct ggct t g gcat gt gt ac aat gaaaagt 2160
t t aggcat gc t cagaat ggg aaaat at cca t agcct t gca ggct gat t gg at agaacct g 2220
cct gccct t t ct cccaaaag gacaaagagg t ggccgagag agt t t t ggaa t t t gacat t g 2280
gct ggct ggc t gagcccat t t t cggct ct g gagat t at cc at gggt gat g agggact ggc 2340
t gaaccaaag aaacaat t t t ct t ct t cct t at t t cact ga agat gaaaaa aagct aat cc 2400
agggt acct t t gact t t t t g gct t t aagcc at t at accac cat cct t gt a gact cagaaa 2460
aagaagat cc aat aaaat ac aat gat t acc t agaagt gca agaaat gacc gacat cacgt 2520
ggct caact c ccccagt cag gt ggcggt ag t gccct gggg gt t gcgcaaa gt gct gaact 2580
ggct gaagt t caagt acgga gacct cccca t gt acat aat at ccaacgga at cgat gacg 2640
ggct gcat gc t gaggacgac cagct gaggg t gt at t at at gcagaat t ac at aaacgaag 2700
ct ct caaagc ccacat act g gat ggt at ca at ct t t gcgg at act t t gct t at t cgt t t a 2760
acgaccgcac agct ccgagg t t t ggcct ct at cgt t at gc t gcagat cag t t t gagccca 2820
aggcat ccat gaaacat t ac aggaaaat t a t t gacagcaa t ggt t t cccg ggcccagaaa 2880
ct ct ggaaag at t t t gt cca gaagaat t ca ccgt gt gt ac t gagt gcagt t t t t t t caca 2940
cccgaaagt c t t t act ggct t t cat agct t t t ct at t t t t t gct t ct at t at t t ct ct ct 3000
ccct t at at t t t act act cg aagaaaggca gaagaagt t a caaat agt t c t gaacat t t t 3060
t ct at t cat t cat t t t gaaa t aat t at gca gacacat cag ct gt t aacca t t t gcacct c 3120
t aagt gt t gt gaaact gt aa at t t cat aca t t t gact t ct agaaaacat t t t t gt ggct t 3180
at gacagagg t t t t gaaat g ggcat aggt g at cgt aaaat at t gaat aat gcgaat agt g 3240
cct gaat t t g t t ct ct t t t t gggt gat t aa aaaact gaca ggcact at aa t t t ct gt aac 3300
acact aacaa aagcat gaaa aat aggaacc acaccaat gc aacat t t gt g cagaaat t t g 3360
aat gacaaga t t aggaat at t t t ct t ct gc acccact t ct aaat t t aat g t t t t t ct gga 3420
agt agt aat t gcaagagt t c gaat agaaag t t at gt acca agt aaccat t t ct cagct gc 3480
cat aat aat g cct agt ggct t cccct ct gt caaat ct agt t t cct at gga aaagaagat g 3540
gcagat acag gagagacgac agagggt cct aggct ggaat gt t cct t t cg aaagcaat gc 3600
t t ct at caaa t act agt at t aat t t at gt a t ct ggt t aat gacat act t g gagagcaaat 3660

2015339786 28 Nov 2017

tatggaaatg tgtatttat atgattttg aggtcctgtc taaaccctgt gtccctgagg 3720

gatctgtctc actggcatct tgttgagggc cttgcacata ggaaacttt gataagtatc 3780

tgcggaaaaa caaacatgaa tcctgtgata ttgggctctt caggaagcat aaagcaattg 3840

tgaaatacag tataccgcag tggctctagg tggaggaaag gaggaaaaag tgcttattat 3900

gtgcaacatt atgattaatc tgattataca ccatttttga gcagatcttg gaatgaatga 3960

catgacctt ccctagagaa taaggatgaa ataatcactc attctatgaa cagtgacact 4020

actttctatt ctttagctgt actgtaattt ctttgagttg atagttttac aaatttcttaa 4080

taggttcaaa agcaatctgg tctgaataac actggatttg tttctgtgat ctctgaggtc 4140

tatttttatgt ttttgctgct acttctgtgg aagtagcttt gaacttagtt tacttttgaac 4200

tttcacgctg aaacatgcta gtgatatcta gaaagggcta attaggtctc atccttttaat 4260

gcccctt aaa taagtcttgc tgatttt cag acagggaagt ctctctatta cactggagct 4320

gttttataga taagtcaata ttgtatcagg caagataaac caatgtcata acaggcattg 4380

ccaacctcac tgacacaggg tcatagtgta taataataata ctgtactata taatatatca 4440

tctttagagg tatgatttttt tcatgaaaga taagctttg gtaatattca ttttaaagt g 4500

gacttattaa aattggatgc tagagaatca agttttatttt atgtatatat ttttctgatt 4560

ataagagtaa tatatgttca ttgtaaaaat ttttaaaaca cagaaactat atgcaaagaa 4620

aaaataaaaa ttatctatAA tctcagaacc cagaaatagc cactattaac atttcctacg 4680

tatttttatt tacatagatc atattgtata tagttagtat ctttattaat ttttattatg 4740

aaacttttcct ttgtcattat tagtcttcaa aagcatgatt tttaatagtt gttgagtatt 4800

ccaccacagg aatgtatcac aacttaaccg ttcccgtttg ttagactagt ttcttattaa 4860

tgttgatgaa tgttgttttaa aaataatttt gttgctacat ttacttttaat ttcctttgact 4920

gtaaagagaa gtaatttttgc tcctttgataa agtattatat taataataaa tctgcctgca 4980

acttttttgcc ttctttcata atc 5003

<210> 62
<211> 1574
<212> DNA
<213> Homo sapiens

<220>
<223> Human Sm22-alpha mRNA

<400> 62
tcaccacggc ggcagccctt taaacccctc acccagccag cgccccatcc tgtctgtccg 60

aacccagaca caagtcttca ctccttcctg cgagccctga ggaagccttg tgagtgcatt 120

ggctggggct tggagggaag ttgggctgga gctggacagg agcagtgggt gcatttcagg 180

caggctctcc tgaggtccca ggcgccagct ccagctccct ggctagggaa acccaccctc 240

tcagtcagca tgggggccca agctccaggc agggtgggct ggatcactag cgtcctggat 300

ctctctcaga ctgggcagcc ccgggctcat tgaaatgccc cggatgactt ggctagtgca 360

2015339786 28 Nov 2017

gaggaattga tggaaaccac cggggtgaga gggaggctcc ccatctcagc cagccacatc 420
cacaaggtgt gtgtaagggt gcaggcgccg gccggttagg ccaaggctct actgtctgtt 480
gcccctccag gagaacttcc aaggagcttt ccccagacat ggccaacaag ggtccttcct 540
atggcatgag ccgcgaagtg cagtccaaaa tcgagaagaa gtatgacgag gagctggagg 600
agcggctggt ggagtggatc atagtgcagt gtggccctga tgtgggccgc ccagaccgtg 660
ggcgcttggg cttccaggtc tggctgaaga atggcgtgat tctgagcaag ctggtgaaca 720
gcctgtaccc tgatggctcc aagccggtga aggtgcccga gaacccaccc tccatggtct 780
tcaagcagat ggagcaggtg gctcagttcc tgaaggcggc tgaggactat ggggtcatca 840
agactgacat gttccagact gttgacctct ttgaaggcaa agacatggca gcagtgcaga 900
ggaccctgat ggctttgggc agcttggcag tgaccaagaa tgatgggcac taccgtggag 960
atcccaactg gtttatgaag aaagcgcagg agcataagag ggaattcaca gagagccagc 1020
tgcaggaggg aaagcatgtc attggcctcc agatgggcag caacagaggg gcctcccagg 1080
ccggcatgac aggctacgga cgacctcggc agatcatcag ttagagcgga gagggctagc 1140
cctgagcccg gccctccccc agctccttgg ctgcagccat cccgcttagc ctgcctcacc 1200
cacacccgtg tggtacctcc agccctggcc aagcttttgag gctctgtcac tgagcaatgg 1260
taactgcacc tgggcagctc ctccctgtgc ccccagcctc agcccaactt cttacccgaa 1320
agcatcactg cctggccccc tccctcccgg ctgcccccat cacctctact gtcctccccc 1380
tgggctaagc aggggagaag cgggctgggg gtagcctgga tgtgggccaa gtccactgtc 1440
ctccttggcg gcaaaagccc attgaagaag aaccagccca gcctgccccc tatcttgtcc 1500
tggaatattt ttggggttgg aactcaaaaa aaaaaaaaaa aaatcaatct tttctcaaaa 1560
aaaaaaaaaa aaaa 1574

<210> 63
<211> 521
<212> PRT
<213> Homo sapiens

<220>
<223> Human Runx2 protein

<400> 63

Met Ala Ser Asn Ser Leu Phe Ser Thr Val Thr Pro Cys Gln Gln Asn
1 5 10 15

Phe Phe Trp Asp Pro Ser Thr Ser Arg Arg Phe Ser Pro Pro Ser Ser
20 25 30

Ser Leu Gln Pro Gly Lys Met Ser Asp Val Ser Pro Val Val Ala Ala
35 40 45

Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
50 55 60

2015339786 28 Nov 2017

Gln Gln Gln Gln Gln Gln Gln Glu Ala Ala Ala Ala Ala Ala Ala Ala
65 70 75 80

Ala Ala Ala Ala Ala Ala Ala Ala Ala Val Pro Arg Leu Arg Pro Pro
85 90 95

His Asp Asn Arg Thr Met Val Glu Ile Ile Ala Asp His Pro Ala Glu
100 105 110

Leu Val Arg Thr Asp Ser Pro Asn Phe Leu Cys Ser Val Leu Pro Ser
115 120 125

His Trp Arg Cys Asn Lys Thr Leu Pro Val Ala Phe Lys Val Val Ala
130 135 140

Leu Gly Glu Val Pro Asp Gly Thr Val Val Thr Val Met Ala Gly Asn
145 150 155 160

Asp Glu Asn Tyr Ser Ala Glu Leu Arg Asn Ala Ser Ala Val Met Lys
165 170 175

Asn Gln Val Ala Arg Phe Asn Asp Leu Arg Phe Val Gly Arg Ser Gly
180 185 190

Arg Gly Lys Ser Phe Thr Leu Thr Ile Thr Val Phe Thr Asn Pro Pro
195 200 205

Gln Val Ala Thr Tyr His Arg Ala Ile Lys Val Thr Val Asp Gly Pro
210 215 220

Arg Glu Pro Arg Arg His Arg Gln Lys Leu Asp Asp Ser Lys Pro Ser
225 230 235 240

Leu Phe Ser Asp Arg Leu Ser Asp Leu Gly Arg Ile Pro His Pro Ser
245 250 255

Met Arg Val Gly Val Pro Pro Gln Asn Pro Arg Pro Ser Leu Asn Ser
260 265 270

Ala Pro Ser Pro Phe Asn Pro Gln Gly Gln Ser Gln Ile Thr Asp Pro
275 280 285

Arg Gln Ala Gln Ser Ser Pro Pro Trp Ser Tyr Asp Gln Ser Tyr Pro
290 295 300

Ser Tyr Leu Ser Gln Met Thr Ser Pro Ser Ile His Ser Thr Thr Pro
305 310 315 320

Leu Ser Ser Thr Arg Gly Thr Gly Leu Pro Ala Ile Thr Asp Val Pro
325 330 335

2015339786 28 Nov 2017

Arg Arg Ile Ser Asp Asp Asp Thr Ala Thr Ser Asp Phe Cys Leu Trp
340 345 350

Pro Ser Thr Leu Ser Lys Lys Ser Gln Ala Gly Ala Ser Glu Leu Gly
355 360 365

Pro Phe Ser Asp Pro Arg Gln Phe Pro Ser Ile Ser Ser Leu Thr Glu
370 375 380

Ser Arg Phe Ser Asn Pro Arg Met His Tyr Pro Ala Thr Phe Thr Tyr
385 390 395 400

Thr Pro Pro Val Thr Ser Gly Met Ser Leu Gly Met Ser Ala Thr Thr
405 410 415

His Tyr His Thr Tyr Leu Pro Pro Pro Tyr Pro Gly Ser Ser Gln Ser
420 425 430

Gln Ser Gly Pro Phe Gln Thr Ser Ser Thr Pro Tyr Leu Tyr Tyr Gly
435 440 445

Thr Ser Ser Gly Ser Tyr Gln Phe Pro Met Val Pro Gly Gly Asp Arg
450 455 460

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> Homo sapiens

<220>
<223> Human Alp protein

<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

28 Nov 2017

2015339786

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

28 Nov 2017 2015339786

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> Homo sapiens

<220>
<223> Human Osterix protein

<400> 65

Met Ala Ser Ser Leu Leu Glu Glu Glu Val His Tyr Gly Ser Ser Pro
1 5 10 15

2015339786 28 Nov 2017

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

2015339786 28 Nov 2017

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> Homo sapiens

<220>
<223> Human Klotho protein

<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

28 Nov 2017

2015339786

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

28 Nov 2017

2015339786

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

28 Nov 2017
2015339786

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

28 Nov 2017 2015339786

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> Homo sapiens

<220>
<223> Human Sm22-alpha protein

<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

2015339786 28 Nov 2017

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> Homo sapiens

<220>
<223> Human alpha-SMA protein

<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

28 Nov 2017
2015339786

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> Homo sapiens

2015339786 28 Nov 2017

<220>
<223> Human MYOCD protein

<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

2015339786 28 Nov 2017

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

2015339786 28 Nov 2017

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

2015339786 28 Nov 2017

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> Homo sapiens

<220>
<223> Human alpha-SMA mRNA

<400> 70
ctctccccgc ccccgcgggg cggcgcgcac tcacccaccc gcgccggagc ggacctttgg 60

cttggcttgt cagggcttgt ccaggagttc cgctcctctc tccaaccggg gtccccctcc 120

agcgacccta aagcttccca gacttccgct tcaattcctg tccgcacccc acgcccacct 180

caacgtggag cgcagtggtc tccgaggagc gccggagctg ccccgcctgc ccagcggggt 240

cagcacttcg catcaaggcc caagaaaagc aagtcctcca gcgttctgag cacccgggcc 300

20153397 86 28 Nov 2017

tgagggaagg tcctaacagc ccccgggagc agtctccaa cgcctcccgc agcagcccgc 360

cgctcccagg tgcccgcgtg cgccgctgcc gccgcaatcc cgcacgcgtc ccgcgcccgc 420

cccacttt gc ctatccccgg gactaagacg ggaatcctgt gaagcagctc cagctatgtg 480

tgaagaagag gacagcactg cctt ggtgtg tgacaatggc tctgggctct gtaaggccgg 540

cttt gctggg gacgatgctc ccagggctgt tttcccatcc attgtgggac gtcccagaca 600

tcaggggtg atggtgggaa tgggacaaaa agacagctac gtgggtgacg aagcacagag 660

caaaagagga atcctgaccc tgaagtaccc gatagaacat ggcatcatca ccaactggga 720

cgacatggaa aagatctggc accactcttt ctacaatgag cttcgtgttg cccctgaaga 780

gcatcccacc ctgctcacgg aggcacccct gaaccccaag gccaaccggg agaaaatgac 840

tcaaatt atg tttgagactt tcaatgtccc agccatgtat gtggctatcc aggcggtgct 900

gtctctctat gcctctggac gcacaactgg catcgtgctg gactctggag atggtgtcac 960

ccacaatgtc cccatctatg agggctatgc cttgccccat gccatcatgc gtctggatct 1020

ggctggccga gatctcactg actacctcat gaagatcctg actgagcgtg gctattcctt 1080

cgttactact gctgagcgtg agattgtccg ggacatcaag gagaaactgt gttatgtagc 1140

tctggacttt gaaaatgaga tggccactgc cgcatcctca tcctccctgg agaagagtta 1200

cgagttgcct gatgggcaag tgatcaccat cggaaatgaa cgttttccgct gcccagagac 1260

cctgttccag ccatccttca tcgggatgga gtctgctggc atccatgaaa ccacctacaa 1320

cagcatcatg aagtgtgata ttgacatcag gaaggacctc tatgctaaca atgtcctatc 1380

agggggcacc actatgtacc ctggcattgc cgaccgaatg cagaaggaga tcacggccct 1440

agcacccagc accatgaaga tcaagatcat tgcccctccg gagcgcaaat actctgtctg 1500

gatcggtggc tccatcctgg cctctctgtc cacctttccag cagatgtgga tcagcaaaca 1560

ggaatacgat gaagccgggc cttccattgt ccaccgcaaa tgcttctaaa acacttttcct 1620

gctcctctct gtctctagca cacaactgtg aatgtcctgt ggaattatgc cttcagttct 1680

tttccaaatc attcctagcc aaagctctga ctcgttacct atgtgttttt taataaatct 1740

gaaataggct actggtaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaa 1798

<210> 71
<211> 8580
<212> DNA
<213> Homo sapiens

<220>
<223> Human MYOCD mRNA

<400> 71
aatcgccggc agcctatgac atcagacagg aacgcctggg atgccgcgct gctcctggcc 60

aacctccgag gaggaggagg gtcccgccgg ctaagagtta attagccccg cacggcgagg 120

ggggaggcgc cagttttctg gggacactgg ctgccactgt actcctaccc aggggagctc 180

acggagagtt ggatgaattc ttgggttgta gctgcggtca gctgggctcc cgggagcctg 240

28 Nov 2017 2015339786

t t gct ggt gg agaacagggg gcgcct ggcc aagggaccag cggct t gct g agact caaca 300
t gacact cct ggggt ct gag cat t cct t gc t gat t aggag caagt t caga t cagt t t t ac 360
agt t aagact t caacaaaga aggacccagg aacaact ggc t aaccaaggc at aat accac 420
cact gaaacg t ccagct gaa t t ccat gagc aaagaaaaca t t t ggat agt gacaaggct a 480
aaaat t ccct gaagcgcaaa gccagaaaca ggt gcaacag t gccgact t g gt t aat at gc 540
acat act cca agct t ccact gcagagaggt ccat t ccaac t gct cagat g aagct gaaaa 600
gagcccgact cgccgat gat ct caat gaaa aaat t gct ct acgaccaggg ccact ggagc 660
t ggt ggaaaa aaacat t ct t cct gt ggat t ct gct gt gaa agaggccat a aaaggt aacc 720
aggt gagt t t ct ccaaat cc acggat gct t t t gcct t t ga agaggacagc agcagcgat g 780
ggct t t ct cc ggat cagact cgaagt gaag acccccaaaa ct cagcggga t ccccgccag 840
acgct aaagc ct cagat acc cct t cgacag gt t ct ct ggg gacaaaccag gat ct t gct t 900
ct ggct caga aaat gacaga aat gact cag cct cacagcc cagccaccag t cagat gcgg 960
ggaagcaggg gct t ggcccc cccagcaccc ccat agccgt gcat gct gct gt aaagt cca 1020
aat cct t ggg t gacagt aag aaccgccaca aaaagcccaa ggaccccaag ccaaaggt ga 1080
agaagct t aa at at caccag t acat t cccc cagaccagaa ggcagagaag t cccct ccac 1140
ct at ggact c agcct acgct cggct gct cc agcaacagca gct gt t cct g cagct ccaaa 1200
t cct cagcca gcagcagcag cagcagcaac accgat t cag ct acct aggg at gcaccaag 1260
ct cagct t aa ggaaccaaat gaacagat gg t cagaaat cc aaact ct t ct t caacgccac 1320
t gagcaat ac cccct t gt ct cct gt caaaa acagt t t t t c t ggacaaact ggt gt ct ct t 1380
ct t t caaacc aggcccact c ccacct aacc t ggat gat ct gaaggt ct ct gaat t aagac 1440
aacagct t cg aat t cggggc t t gcct gt gt caggcaccaa aacggct ct c at ggaccggc 1500
t t cgaccct t ccaggact gc t ct ggcaacc cagt gccgaa ct t t ggggat at aacgact g 1560
t cact t t t cc t gt cacaccc aacacgct gc ccaat t acca gt ct t cct ct t ct accagt g 1620
ccct gt ccaa cggct t ct ac cact t t ggca gcaccagct c cagccccccg at ct ccccag 1680
cct cct ct ga cct gt cagt c gct gggt ccc t gccggacac ct t caat gat gcct cccct 1740
cct t cggcct gcacccgt cc ccagt ccacg t gt gcacgga ggaaagt ct c at gagcagcc 1800
t gaat ggggg ct ct gt t cct t ct gagct gg at gggct gga ct ccgagaag gacaagat gc 1860
t ggt ggagaa gcagaaggt g at caat gaac t cacct ggaa act ccagcaa gagcagaggc 1920
aggt ggagga gct gaggat g cagct t caga agcagaaaag gaat aact gt t cagagaaga 1980
agccgct gcc t t t cct ggct gcct ccat ca agcaggaaga ggct gt ct cc agct gt cct t 2040
t t gcat ccca agt acct gt g aaaagacaaa gcagcagct c agagt gt cac ccaccggct t 2100
gt gaagct gc t caact ccag cct ct t ggaa at gct cat t g t gt ggagt cc t cagat caaa 2160
ccaat gt act t t ct t ccaca t t t ct cagcc cccagt gt t c ccct cagcat t caccgct gg 2220
gggct gt gaa aagcccacag cacat cagt t t gccccccat c acccaacaac cct cact t t c 2280

20153397 86 28 Nov 2017

t gccct cat c ct ccggggcc cagggagaag ggcacagggt ct cct cgccc at cagcagcc 2340
aggt gt gcac t gcacagaac t caggagcac acgat ggcca t cct ccaagc t t ct ct cccc 2400
at t ct t ccag cct ccacccg ccct t ct ct g gagcccaagc agacagcagt cat ggt gccg 2460
ggggaaaccc t t gt cccaaa agcccat gt g t acagcaaaa gat ggct ggt t t acact ct t 2520
ct gat aaggt ggggccaaag t t t t caat t c cat ccccaac t t t t t ct aag t caagt t cag 2580
caat t t caga ggt aacacag cct ccat cct at gaagat gc cgt aaagcag caaat gaccc 2640
ggagt cagca gat ggat gaa ct cct ggacg t gct t at t ga aagcggagaa at gccagcag 2700
acgct agaga ggat cact ca t gt ct t caaa aagt cccaaa gat acccaga t ct t cccgaa 2760
gt ccaact gc t gt cct cacc aagccct cgg ct t cct t t ga acaagcct ct t caggcagcc 2820
agat cccct t t gat ccct at gccaccgaca gt gat gagca t ct t gaagt c t t at t aaat t 2880
cccagagccc cct aggaaag at gagt gat g t caccct t ct aaaaat t ggg agcgaagagc 2940
ct cact t t ga t gggat aat g gat ggat t ct ct gggaaggc t gcagaagac ct ct t caat g 3000
cacat gagat ct t gccaggc cccct ct ct c caat gcagac acagt t t t ca ccct ct t ct g 3060
t ggacagcaa t gggct gcag t t aagct t ca ct gaat ct cc ct gggaaacc at ggagt ggc 3120
t ggacct cac t ccgccaaat t ccacaccag gct t t agcgc cct caccacc agcagcccca 3180
gcat ct t caa cat cgat t t c ct ggat gt ca ct gat ct caa t t t gaat t ct t ccat ggacc 3240
t t cact t gca gcagt ggt ag aat gcccaat gcaccagt gc t at ggaagac caat ggagt t 3300
ccat ggggga aagcacacag ccat acat ac t t t act gt cc aaaaacagaa gaagaagaag 3360
agaat t aaaa agaagcaat g at t t ct gt gc caat gaacaa gaacaaaagt cat t t t t aga 3420
aat acat at a ct gt aat at t t accaacagt cagt aact gt t aat gat t t c aacaat gcat 3480
t aaaagaat g t gct t t ct ca gat t aaggat gccaaaaaag at at t t cact gcct t t t caa 3540
agaccagt at at t t t ct agc ccat aat t t t t ct caggcat t gt t ggggca t aagct caca 3600
ct gt aagct t t t ct cat gaa t t cact agac at aacgt gga aggaaaacgt agt ct t t t gg 3660
gagt acaggg aagccagccc ct caaagct t at ggaagaca t acct gcaat ggaagct gt t 3720
gcccaat gt c t ccat t act a t ct t t caaaa gagaagccag acccagct t c agat caaaag 3780
t t ct t gagac agaggaacaa aaccaat cga t t t ccaggga agct aat caa ct ct ct t t t c 3840
cct ct accac aaaact gccc t gct ggagt g gt t ct gaacc t gt acccagg act cgat gt g 3900
gt cact aat a acaat t aacc t gaact gagt ccacagaact ccact cggaa ct t t ct t ct t 3960
t t t t aact ag t ggcccaat c at t cccacca t ct ct gt gct gat aagt acg t gt cct agat 4020
gagaaccct g aagaat gcag acct t ct t cc cccgaaggag at gccacaag ct ct ccaaca 4080
cagcccct t t agt t ccaaa gact agagat gaccacat t g gt agaagt at at ct cgaggc 4140
acaggaaggg agccccacca gggat aat t c agacaggact agagaat aac at cat t t cac 4200
at accct ggg at aaacaccc t gggt t cct a t agaaggact at t act t at g ggagt ccaac 4260
t t ct cct t t t gt t t t gt t at t at cagt t t a t ct t t ct ccc act ccact t t t cct t caagg 4320

28 Nov 2017 2015339786

t accaat cct t t cct gt t cc t cgt t t ggcc at ct t t ct t t t t ct gcct cc acat t gggag 4380
gggaggact t ct cagt t ct a acaagct gcc at act cct aa gaaagccat t t t t gaaaaat 4440
t t aacaat cc aggt t ct t ct ggagaact ca t t ct ccacac gcacagt t t g ct gcaaaagg 4500
aagt t gcaag aat t t ct t ga ggaagaaact ggt gact t gg t ccat cagt c acgaagt t ct 4560
t t ct at t ct c gt t t agt t t t caagaaat t a t t ggt t t gt g t t gct ct ggg gaaat t ggaa 4620
at cat t acat t gt aaagaca aat at ggat g at at t t acaa gagagaat t t cagat ct ggg 4680
t t t t t gaaag aaaacagaat t gcgcat t ga aaacgat gga aggaaaaaga caat ggt ct a 4740
at gt gcat t c ct cat t acct ct cgt ggct t t ggct gggag t t ggaaaaag ct aaaat t t c 4800
agaacagt ct ct gt aaggct ct ct gt ggct ccagt t cacc at t t t at at t gt t gcat gct 4860
gt agaaagga gct at t gct g t t gt t t t gt t t t t t t at t t a aat cact aag gcact gt t t t 4920
t at ct t t t gt aaaaaaaaaa aaaaagt t gt t cact gt gca ct t at agaaa aaat aat caa 4980
aaat gt t ggg at t t t agaag ct ct ct t t t t gat aaaccaa agat t t agaa gt cat t ccat 5040
t gt t aact t g t aaaaat gt g t gaacacaga gagt t t t t gg t gat t gct ac t ct gaaagct 5100
gccagat ct t at t ct ggggg t gggat gt gg aggaat acac at acacacac aaacat acat 5160
gt at gt at aa t agat at at a cat at gt gt a t at t at at ct gt gt gt gcat gt at ct ccaa 5220
aagcggcgt t acagagt t ct acaccaaaag cct t t aaccc t t aat ct gct gt gaat gat a 5280
cct ggcct t t ct cact at ga at t t ct gat t aaccaaccag act acacgt t gcct ct ct gt 5340
gt at gact aa cggct ccaac ccgat gact c acagct act t gct t at cgt g aacaagct ca 5400
t ct t ggcaat gaat at ggat gt gaaaagac agaacagct t caccat t agt agct ggaaat 5460
ggt at cacag t ct ct t at ag aggaat at ga aaggaacaag aaaat cat t t t acat t cct t 5520
t t at ct gt at t gt gct t t aa aagat ccaca t ggt aaat t t t t t at t t t gc t t t t at gt ca 5580
gt cat cagaa ccaaaaaaat ccagaagaaa aaat t gccag t gt t t cct t t gaagat gaag 5640
ct act gggga agaaaacct t at t aat acac t ccacacat t t gt t cat t cc t cagct gt t g 5700
gt gt t t t ct t ggggt ct t ga caaagct t gc t ggt cagt gc act t t t cagg t gt cacgt t t 5760
t gct gt t t gt at gt t t t t t c t t cccct t ac t t cct t t gga aaacaaact c acacagt gcc 5820
cct act ct ga gacct gggac t gagt gt t aa t t at t t t t t c ct t gggt at t t ct at ct gag 5880
agact agacc t agt t aggag gcct ct gt ac t t ct ccagat t gt acct t t t t at ggggat c 5940
t t t gaggct a t gacccagga ct gat agat a t gcct t acgg aagacaaaag at aaaat ggt 6000
t cct at at cc t aat gcaaac caacacagt t aaaagagcag at ct ct ggat aact gct ct c 6060
aacct gct t c t acagt ct cc acaaaccgca t t caccct ct ct ct t cat ag ct cagacat g 6120
aaat t t gagg gagaaaact g gagat aat t g ggagaaaat t gat gaagt t g gct gct t cca 6180
gt agat caga t aat ccat ga at t t gt ct cc cat t gagaat t t t at t t t aa at t ct t t t aa 6240
act ct t cgt t gt gt ct t t t g t gat gacaaa t caggcat ga ct aaaagat g t acagagact 6300
t acgaagat g gt cacat t ca agt t ccct aa t gct ct t aga acct gaagat gaccat gt gt 6360

2015339786 28 Nov 2017

agt t t t ct t a agacct ct ga acccccat gg t gat gaagac t t gaagacat t t gcagct at 6420
ct gct gcagt ct ggt agat t cat act t at c t aaagaagt c aaaaaat t t a t t cgt gcaag 6480
t gct t gcagg aagccagt gc t t at t agt ag t gaccct gct t ct at caacg t t at t gagac 6540
aacacat at t ct at t ct aag ggagaaagag ggaggaagag agggagggag ggaggaagaa 6600
gagggaggga gcgaggaagg aagat aggag at gggt aggg gggt aaagag aaaaggaggg 6660
agaagggaag gaaggaaaga agagaggaaa gaaaggaggg aaggaaaaaa gggccaaact 6720
t t ct gat ct a t gaact t ct c agt t cagct g t cacat t at g agaagt aaat cagaat t t t t 6780
t t aaggagaa gt cat t ct t a gcact acaat aat t gt acca gt aat t gagg aaaccaagac 6840
aat ct t cacc t gaat aat ag agggt ct gag aact gt cagc ct t t t gccat t caaaaacat 6900
t t at gt ccaa cct gaaaaaa aagcat caat aaaacct at c ccaagcat t c aaaat agt cc 6960
t t t ccaaat g t t at t t at t t t aaagt caat cagct ct t t t agaaacagat t ct ggt ct gg 7020
ct gaaaact c ccacaacaaa t t t act cat c cagt ggct aa t at t t aat gc ccaccat ggg 7080
cagagcacac aaat ct t cag at aaacaat a ct gaat t gat t cagcacaga t t gt t cagat 7140
t t gaat gacc agggagt t gt at t t gcacat gcaagaacac t aagaat ct c ccagt cct ca 7200
aact agaaac ct t t cagct a cgat gaaaaa aaaaaagggg t ct t cat t t t t ccaagaggg 7260
ggt ggaggt g gggat cact t t t t agct aaa agct at ct ct cact t caaaa t t ct t gt ct t 7320
t t t ct t t gt g gacaaacacc agt agt ct at cact t ggaga t ct t t t aat a t ct cccat ca 7380
t t t aaaacat ccacgagagt t t gaagat t t gt gt t gat t g ccagat acag aagcccct t g 7440
aaaat aagga aagggt ggag gaagcat t t t t gt gt cct at ccct act t at cgt agcagct 7500
ct at agacaa aagggacact t act ggt gag cct ct ggccc t t aaaagaaa at cat ct aag 7560
aat at gaagg caat t t gat t t ccccccaca gccct cagct gcct t cct ca cagaaggaag 7620
t t cccaaaat t gct ggt aca cagt t t gcaa t caaat at ca gat at gagaa aacct gt agt 7680
gaagagt ct g ggt t ct t ggt t t t ct cat aa at ccaat at a aat t t gt agg t t ggt t cagg 7740
gt caaaat t g ccagt gct t t at t agacaga t gat act gat agacacacag agcccaggt c 7800
ct ggaacaag acaat cct gt agt gccaaga t ct ggt cagt t gcgt t aagg agct gggt t t 7860
gat t ct agag t ccaggt t t a t agagaaacc ct ggct agat t gagcct acc cat ggggaga 7920
cgat t t caag acaggat gag at ct gggaag aat t t t gt t g t cat ct gcca gggaaat t at 7980
cacaggact c at t gaat gca at aacat gt g agt aagt t cc ct t t t gat t c t gggaat cag 8040
cgat t t t ccc t gt ggat t aa gacaaaccaa cgccagaagg t ct cct gt gc t t at t t t aac 8100
cat ct gct cc cat cgt gaac cct ggagcat gcat t t cct a gaagt ggt t t cat agct cct 8160
gt gt gt t cat ggaaaagggg agt at aat ga t ggggat gct ggaagct t t t t t aat gt t t t 8220
ccaaaggaaa ggaacccaca ct gct cccca gagt t cct t t ccaat ggccc t gcagt aaga 8280
acggaggaca at gt at t gct gggt gct t aa aat cct ccct cagt gaagca caaagagaca 8340
ct t t gt aaag aaaaaaagag caagcat agg t t ct ct gt gg gacct t gt gg agt ggt gt t t 8400

2015339786 28 Nov 2017

tcacgttggt ctctttggct caattgagca taatcagaaa gaaatgtggg ttattgggaa 8460

gagacaaaaa gcagtggcta aaataccaaa gttggcatgt gttctttttt aaaaaaaaaa 8520

aaaaatgcat atatttttaa ataaaatgtt tattttaaaa agaaaaaaaa aaaaaaaaaa 8580

<210> 72
<211> 524
<212> PRT
<213> Homo sapiens

<220>
<223> Human BSAP protein

<400> 72

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

2015339786 28 Nov 2017

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

2015339786 28 Nov 2017

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> 73
<211> 2606
<212> DNA
<213> Homo sapiens

<220>
<223> Human BSAP mRNA

<400> 73
ccgggcctca ctcgggcccc gcggccgcct ttataaggcg gcgggggtgg tggcccgggc 60

cgcgttgcgc tcccgccact ccgcgcccgc tatcctggct ccgtgctccc acgcgcttgt 120

gcctggacgg acccctcgcca gtgctctgcg caggattgga acatcagtta acatctgacc 180

actgccagcc caccccctcc cacccacgtc gattgcatct ctgggctcca gggataaagc 240

aggtcttggg gtgcaccatg attttcacca ttcttagtact ggccattggc acctgcctta 300

ctaactcctt agtgccagag aaagagaaag accccaagta ctggcgagac caagcgcaag 360

agacactgaa atatgccctg gagcttcaga agctcaacac caacgtggct aagaatgtca 420

tcatgttcct gggagatggg atgggtgtct ccacagtgac ggctgcccgc atcctcaagg 480

gtcagctcca ccacaaccct ggggaggaga ccaggctgga gatggacaag ttcccttcg 540

tggccctctc caagacgtac aacaccaatg cccaggtccc tgacagcgcc ggcaccgcca 600

ccgcctacct gtgtggggtg aaggccaatg agggcaccgt gggggtaagc gcagccactg 660

agcgttcccg gtgcaacacc acccagggga acgaggtcac ctccatcctg cgctgggcca 720

aggacgctgg gaaatctgtg ggcattgtga ccaccacgag agtgaaccat gccacccca 780

gcgccgccta cgcccactcg gctgaccggg actggtactc agacaacgag atgcccctg 840

aggccttgag ccagggctgt aaggacatcg cctaccagct catgcatcaac atcagggaca 900

ttgacgtgat catgggggt ggccggaaat acatgtaccc caagaataaa actgatgtgg 960

agtatgagag tgacgagaaa gccaggggca cgaggctgga cggcctggac ctcgttgaca 1020

cctggaagag cttcaaaccg agatacaagc actcccactt catctggaac cgcacggaac 1080

tcctgaccct tgacccccac aatgtggact acctattggg tctcttcgag ccaggggaca 1140

tgcagtacga gctgaacagg aacaacgtga cggacccgtc actctccgag atggtggtgg 1200

tggccatcca gatcctgcgg aagaacccca aaggcttctt cttgctggtg gaaggaggca 1260

gaattgacca cgggcaccat gaaggaaaag ccaagcaggc cctgcatgag gcggtggaga 1320

tggaccgggc catcgggcag gcaggcagct tgacctcctc ggaagacact ctgaccgtgg 1380

20153397 86 28 Nov 2017

tcactgcgga ccattcccac gtcttcacat ttggtggata caccccccgt ggcaactcta 1440

tctttggtct ggcccccatg ctgagtgaca cagacaagaa gccctt cact gccatcctgt 1500

atggcaatgg gcctggctac aaggtggtgg gcggtgaacg agagaatgtc tccatggtgg 1560

actatgctca caacaactac caggcgcagt ctgctgtgcc cctgcgccac gagacccacg 1620

gcggggagga cgtggccgtc tt ctccaagg gccccatggc gcacctgctg cacggcgtcc 1680

acgagcagaa ctacgtcccc cacgtgatgg cgtatgcagc ctgcatcggg gccaacctcg 1740

gccactgtgc tcctgccagc tcggcaggca gcctt gctgc aggcccctg ctgctcgcgc 1800

tggccctcta ccccctgagc gtcctgttct gagggcccag ggcccgggca cccacaagcc 1860

cgtgacagat gccaacttcc cacacggcag ccccccccct caaggggcagg gaggtggggg 1920

cctcctcagc ctctgcaact gcaagaaagg ggacccaaga aaccaaagtc tgccgcccac 1980

ctcgctcccc tctggaatct tccccaaggg ccaaacccac ttctggcctc cagcctttgc 2040

tccctccccg ctgccctttg gccaacaggg tagatttctc ttgggcaggc agagagtaca 2100

gactgcagac attctcaaag cctcttattt ttctagcgaa cgtatttctc cagacccaga 2160

ggccctgaag cctccgtgga acattctgga tctgaccctc ccagtctcat ctcctgaccc 2220

tcccactccc atctccttac ctctggaacc ccccaggccc tacaatgctc atgtccctgt 2280

ccccaggccc agccctcctt caggggagtt gaggtctttc tcctcaggac aaggcctt gc 2340

tcactcactc actccaagac caccagggtc ccaggaagcc ggtgcctggg tggccatcct 2400

acccagcgtg gcccaggccg ggaagagcca cctggcaggg ctcacactcc tgggctctga 2460

acacacacgc cagctcctct ctgaagcgac tctcctgttt ggaacggcaa aaaaaaatt t 2520

ttttttctct ttttggtggt ggttaaaagg gaacacaaaa catttaaata aaacttt cca 2580

aatattt ccg aggacaaaaa aaaaaa 2606

<210> 74
<211> 1722
<212> DNA
<213> Artificial Sequence

<220>
<223> Snai1 mRNA

<400> 74
attcattgcg ccgcggcacg gcctagcgag tggttcttct gcgctactgc tgcgcgaatc 60

ggcgacccca gtgcctcgac cactatgccg cgctctttcc tcgtcaggaa gccctccgac 120

cccaatcgga agcctaacta cagcgagctg caggactcta atccagagtt tacctt ccag 180

cagccctacg accaggccca cctgctggca gccatcccac ctccggagat cctcaacccc 240

accgcctcgc tgccaatgct catctgggac tctgtcctgg cgccccaagc ccagccaatt 300

gcctgggcct ccctt cggct ccaggagagt cccagggtgg cagagctgac ctccctgtca 360

gatgaggaca gtgggaaagg ctcccagccc cccagcccac cctcaccggc tcctt cgtcc 420

ttctcctcta cttcagtctc ttccttggag gccgaggcct atgctgcctt cccaggcttg 480

2015339786 28 Nov 2017

ggccaagtgc ccaagcagct ggcccagctc tctgaggcca aggatctcca ggctcgaaag 540
gcctt caact gcaaatactg caacaaggaa tacctcagcc tgggtgccct caagatgcac 600
atccgaagcc acacgctgcc ctgcgtctgc ggaacctgcg ggaaggcctt ctctaggccc 660
tggctgctac aaggccatgt ccggacccac actggcgaga agcccttctc ctgtccccac 720
tgcagccgtg cctt cgctga ccgctccaac ctgcgggccc acctccagac ccactcagat 780
gtcaagaagt accagtgcca ggcgtgtgct cggacctt ct cccgaatgtc cctgctccac 840
aagcaccaag agtccggctg ctcaggatgt ccccgctgac cctcgaggct ccctcttcct 900
ctccatacct gcccctgcct gacagcctt c cccagctcca gcaggaagga ccccacatcc 960
tt ctcactgc catggaatt c cctcctgagt gccccacttc tggccacatc agccccacag 1020
gactttgatg aagaccattt tctggttctg tgtcctctgc ctgggctctg gaagaggcct 1080
tcccatggcc attttctgtgg agggagggca gctggccccc agccctgggg gattcctgag 1140
ctggcctgtc tgcgtgggtt tttgtatcca gagctgttg gatacagctg ctttgagcta 1200
caggacaaag gctgacagac tcactgggaa gctcccaccc cactcagggg accccactcc 1260
cctcacacac acccccccac aaggaaccct caggccaccc tccacgaggt gtgactaact 1320
atgcaataat ccacccccag gtgcagcccc agggcctgcg gaggcggtgg cagactagag 1380
tctgagatgc cccgagccca ggcagctatt tcagcctcct gttttggtggg gtggcacctg 1440
tttcccgggc aatttaacaa tgtctgaaaa gggactgtga gtaatggctg tcacttgtcg 1500
ggggcccaag tggggtgctc tggtctgacc gatgtgtctc ccagaactat tctgggggcc 1560
cgacaggtgg gcctgggagg aagatgttta catttttaaa ggtacactgg tatttatatt 1620
tcaaacattt tgtatcaagg aaacgttttg tatagttata tgtacagttt attgatattc 1680
aataaagcag ttaatttata tatttaaaaaa aaaaaaaaaa aa 1722

<210> 75
<211> 1815
<212> DNA
<213> Artificial Sequence

<220>
<223> Dkk1 mRNA

<400> 75
gcagagctct gtgctccctg cagtcaggac tctgggaccg caggggctc ccggaccctg 60
actctgcagc cgaaccggca cggttt cgtg gggacccagg cttgcaaagt gacggtcatt 120
ttctctttct ttctccctct tgagtcctt c tgagatgatg gctctgggcg cagcgggagc 180
tacccgggtc tttgtcgcga tggtagcggc ggctctcggc ggccaccctc tgctgggagt 240
gagcgccacc ttgaactcgg ttctcaatt c caacgctatc aagaacctgc ccccaccgct 300
gggcggcgct gcggggcacc caggctctgc agtcagcgcc gcgccgggaa tcctgtaccc 360
gggcgggaat aagtaccaga ccattgacaa ctaccagccg tacccgtgcg cagaggacga 420
ggagtgcggc actgatgagt actgcgctag tcccacccgc ggaggggacg caggcgtgca 480

28 Nov 2017 2015339786

aatctgtctc gcctgcagga agcgccgaaa acgctgcatg cgtcacgcta tgtgctgccc 540
cgggaattac tgcaaaaatg gaatatgtgt gtcttctgat caaaatcatt tccgaggaga 600
aattgaggaa accatcactg aaagcttttgg taatgatcat agcaccttgg atgggtattc 660
cagaagaacc acctttgtctt caaaaatgta tcacaccaaa ggacaagaag gttctgttttg 720
tctccggtca tcagactgtg cctcaggatt gtgttgtgct agacacttct ggtccaagat 780
ctgtaaacct gtcctgaaag aaggtcaagt gtgtaccaag cataggagaa aaggctctca 840
tggactagaa atattccagc gttgttactg tggagaaggt ctgtcttgcc ggatacagaa 900
agatcaccat caagccagta attcttctag gcttcacact tgtcagagac actaaaccag 960
ctatccaaat gcagtgaact cctttatat aatagatgct atgaaaacct tttatgacct 1020
tcatcaactc aatcctaagg atatacaagt tctgtggttt cagttaagca ttccaataac 1080
acctttccaaa aacctggagt gtaagagctt tgttttctttta tggaactccc ctgtgattgc 1140
agtaaattac tgtattgtaa attctcagtg tggcacttac ctgtaaatgc aatgaaactt 1200
ttaatttatttt ttctaaaggt gctgcactgc ctatttttcc tcttgttatg taaatttttg 1260
tacacattga ttgttatctt gactgacaaa tattctatat tgaactgaag taaatcattt 1320
cagcttatag ttcttaaaag cataacccctt tacccccatttt aatttctagag tctagaacgc 1380
aaggatctct tggaatgaca aatgataggt acctaaaatg taacatgaaa atactagctt 1440
attttctgaa atgtactatc ttaatgcttta aatttatatttt ccctttaggc tgtgatagtt 1500
tttgaaataa aatttaacat ttaatatcat gaaatgttat aagtagacat acatttttggg 1560
attgtgatct tagaggttttg tgtgtgtgta cgtatgtgtg tgttctacaa gaacggaagt 1620
gtgatatgtt taaagatgat cagagaaaag acagtgtcta aatatagac aatattgatc 1680
agctctagaa taacttttaaa gaaagacgtg ttctgcattg atgaaactcaa atgatcatgg 1740
cagaatgaga gtgaatcttta catttactact ttcaaaaata gttttccaata aatttaataat 1800
acctaaaaaa aaaaa 1815

<210> 76
<211> 5927
<212> DNA
<213> Artificial Sequence

<220>
<223> Col1a1 mRNA

<400> 76
tcgtcggagc agacgggagt ttctcctcgg ggtcggagca ggaggcacgc ggagtgtgag 60
gccacgcatg agcggacgct aacccccttcc ccagccacaa agagtctaca tgtctagggt 120
ctagacatgt tcagcttttgt ggacctccgg ctcctgctcc tcttagcggc caccgccctc 180
ctgacgcacg gccaagagga aggccaagtc gagggccaag acgaagacat cccaccaatc 240
acctgcgtac agaacggcct caggtaccat gaccgagacg tgtggaaacc cgagccctgc 300
cggatctgcg tctgcgacaa cggcaaggtg ttgtgcgatg acgtgatctg tgacgagacc 360

28 Nov 2017 2015339786

aagaact gcc ccggcgccga agt ccccgag ggcgagt gct gt cccgt ct g ccccgacggc 420
t cagagt cac ccaccgacca agaaaccacc ggcgt cgagg gacccaaggg agacact ggc 480
ccccgaggcc caaggggacc cgcaggcccc cct ggccgag at ggcat ccc t ggacagcct 540
ggact t cccg gacccccccgg acccccccgga cct cccggac cccct ggcct cggaggaaac 600
t t t gct cccc agct gt ct t a t ggct at gat gagaaat caa ccggaggaat t t ccgt gcct 660
ggccccat gg gt ccct ct gg t cct cgt ggt ct ccct ggcc cccct ggt gc acct ggt ccc 720
caaggct t cc aaggt ccccc t ggt gagcct ggcgagcct g gagct t cagg t cccat gggt 780
ccccgaggt c ccccaggt cc ccct ggaaag aat ggagat g at ggggaagc t ggaaaacct 840
ggt cgt cct g gt gagcgt gg gcct cct ggg cct cagggt g ct cgaggat t gcccggaaca 900
gct ggcct cc ct ggaat gaa gggacacaga ggt t t cagt g gt t t ggat gg t gccaaggga 960
gat gct ggt c ct gct ggt cc t aagggt gag cct ggcagcc ct ggt gaaaa t ggagct cct 1020
ggt cagat gg gcccccgt gg cct gcct ggt gagagaggt c gccct ggagc ccct ggccct 1080
gct ggt gct c gt ggaaat ga t ggt gct act ggt gct gccg ggcccct gg t cccaccggc 1140
cccgct ggt c ct cct ggct t ccct ggt gct gt t ggt gct a agggt gaagc t ggt ccccaa 1200
gggccccgag gct ct gaagg t ccccagggt gt gcgt ggt g agcct ggccc ccct ggccct 1260
gct ggt gct g ct ggccct gc t ggaaaccct ggt gct gat g gacagcct gg t gct aaaggt 1320
gccaat ggt g ct cct ggt at t gct ggt gct cct ggct t cc ct ggt gcccg aggcccct ct 1380
ggaccccagg gccccggcgg ccct cct ggt cccaagggt a acagcggt ga acct ggt gct 1440
cct ggcagca aaggagacac t ggt gct aag ggagagcct g gccct gt t gg t gt t caagga 1500
cccct ggcc ct gct ggaga ggaaggaaag cgaggagct c gaggt gaacc cggacccact 1560
ggcct gcccg gacccct gg cgagcgt ggt ggacct ggt a gccgt ggt t t ccct ggcgca 1620
gat ggt gt t g ct ggt cccaa gggt cccgct ggt gaacgt g gt t ct cct gg ccct gct ggc 1680
cccaaaggat ct cct ggt ga agct ggt cgt cccggt gaag ct ggt ct gcc t ggt gccaag 1740
ggt ct gact g gaagccct gg cagccct ggt cct gat ggca aaact ggccc ccct ggt ccc 1800
gccggt caag at ggt cgccc cggacccca ggcccacct g gt gcccgt gg t caggct ggt 1860
gt gat gggat t ccct ggacc t aaaggt gct gct ggagagc ccggcaaggc t ggagagcga 1920
ggt gt t cccg gacccct gg cgct gt cggt cct gct ggca aagat ggaga ggct ggagct 1980
cagggacccc ct ggccct gc t ggt cccgct ggcgagagag gt gaacaagg ccct gct ggc 2040
t cccccggat t ccagggt ct ccct ggt cct gct ggt cct c caggt gaagc aggcaaacct 2100
ggt gaacagg gt gt t cct gg agacct t ggc gcccct ggcc cct ct ggagc aagaggcgag 2160
agaggt t t cc ct ggcgagcg t ggt gt gcaa ggt cccct g gt cct gct gg t ccccgaggg 2220
gccaacggt g ct cccggcaa cgat ggt gct aagggt gat g ct ggt gcccc t ggagct ccc 2280
ggt agccagg gcgcccct gg cct t caggga at gcct ggt g aacgt ggt gc agct ggt ct t 2340
ccagggcct a agggt gacag aggt gat gct ggt cccaaag gt gct gat gg ct ct cct ggc 2400

28 Nov 2017 2015339786

aaagat ggcg t ccgt ggt ct gact ggcccc at t ggt cct c ct ggccct gc t ggt gcccct 2460
ggt gacaagg gt gaaagt gg t cccagcggc cct gct ggt c ccact ggagc t cgt ggt gcc 2520
cccggagacc gt ggt gagcc t ggt cccccc ggccct gct g gct t t gct gg ccccct ggt 2580
gct gacggcc aacct ggt gc t aaaggcgaa cct ggt gat g ct ggt gct aa aggcgat gct 2640
ggt cccct g gccct gccgg acccgct gga ccccct ggcc ccat t ggt aa t gt t ggt gct 2700
cct ggagcca aaggt gct cg cggcagcgct ggt cccct g gt gct act gg t t t ccct ggt 2760
gct gct ggcc gagt cggt cc t cct ggcccc t ct ggaaat g ct ggaccccc t ggccct cct 2820
ggt cct gct g gcaaagaagg cggcaaaggt ccccgt ggt g agact ggccc t gct ggacgt 2880
cct ggt gaag t t ggt ccccc t ggt cccct ggccct gct g gcgagaaagg at cccct ggt 2940
gct gat ggt c ct gct ggt gc t cct ggt act cccgggcct c aaggt at t gc t ggacagcgt 3000
ggt gt ggt cg gcct gcct gg t cagagagga gagagaggct t ccct ggt ct t cct ggcccc 3060
t ct ggt gaac ct ggcaaaca aggt ccct ct ggagcaagt g gt gaacgt gg t ccccct ggt 3120
cccat gggcc cccct ggat t ggct ggaccc cct ggt gaat ct ggacgt ga gggggct cct 3180
ggt gccgaag gt t cccct gg acgagacggt t ct cct ggcg ccaagggt ga ccgt ggt gag 3240
accggccccg ct ggaccccc t ggt gct cct ggt gct cct g gt gcccct gg ccccgt t ggc 3300
cct gct ggca agagt ggt ga t cgt ggt gag act ggt cct g ct ggt cccgc cggt cct gt c 3360
ggccct gt t g gcgcccgt gg ccccgccgga ccccaaggcc cccgt ggt ga caagggt gag 3420
acaggcgaac agggcgacag aggcat aaag ggt caccgt g gct t ct ct gg cct ccagggt 3480
cccct ggcc ct cct ggct c t cct ggt gaa caaggt ccct ct ggagcct c t ggt cct gct 3540
ggt ccccgag gt cccct gg ct ct gct ggt gct cct ggca aagat ggact caacggt ct c 3600
cct ggcccca t t gggccccc t ggt cct cgc ggt cgcact g gt gat gct gg t cct gt t ggt 3660
ccccccggcc ct cct ggacc t cct ggt ccc cct ggt cct c ccagcgct gg t t t cgact t c 3720
agct t cct gc cccagccacc t caagagaag gct cacgat g gt ggccgct a ct accgggct 3780
gat gat gcca at gt ggt t cg t gaccgt gac ct cgaggt gg acaccaccct caagagcct g 3840
agccagcaga t cgagaacat ccggagccca gagggcagcc gcaagaaccc cgcccgcacc 3900
t gccgt gacc t caagat gt g ccact ct gac t ggaagagt g gagagt act g gat t gacccc 3960
aaccaaggct gcaacct gga t gccat caaa gt ct t ct gca acat ggagac t ggt gagacc 4020
t gcgt gt acc ccact cagcc cagt gt ggcc cagaagaact ggt acat cag caagaacccc 4080
aaggacaaga ggcat gt ct g gt t cggcgag agcat gaccg at ggat t cca gt t cgagt at 4140
ggcggccagg gct ccgaccc t gccgat gt g gccat ccagc t gacct t cct gcgcct gat g 4200
t ccaccgagg cct cccagaa cat cacct ac cact gcaaga acagcgt ggc ct acat ggac 4260
cagcagact g gcaacct caa gaaggccct g ct cct ccagg gct ccaacga gat cgagat c 4320
cgcgccgagg gcaacagccg ct t cacct ac agcgt cact g t cgat ggct g cacgagt cac 4380
accggagcct ggggcaagac agt gat t gaa t acaaaacca ccaagacct c ccgcct gccc 4440

28 Nov 2017 2015339786

atcatcgatg tggccccctt ggacgttggt gccccagacc aggaattcgg cttcgacgtt 4500

ggccctgtct gcttcctgta aactccctcc atcccaacct ggctccctcc cacccaacca 4560

actttccccc caacccggaa acagacaagc aacccaaact gaaccccctc aaaagccaaa 4620

aaatgggaga caatttcaca tggacttttgg aaaatattt tttcctttgc attcatctct 4680

caaacttagt ttttatcttt gaccaaccga acatgaccaa aaaccaaaag tgcattcaac 4740

cttaccaaaa aaaaaaaaaa aaaaagaata aataaataac ttttttaaaaa aggaagcttg 4800

gtccacttgc ttgaagaccc atgcgggggt aagtcccttt ctgcccgttg ggcttatgaa 4860

accccaatgc tgccctttct gctccttttct ccacaccccc cttggggcct cccctccact 4920

cctttcccaaa tctgtctccc cagaagacac aggaaacaat gtattgtctg cccagcaatc 4980

aaaggcaatg ctcaaacacc caagtggccc ccaccctcag cccgctcctg cccgcccagc 5040

acccccaggc cctgggggac ctggggttct cagactgcca aagaagcctt gccatctggc 5100

gctcccatgg ctcttgcaac atctcccctt cgttttgag ggggtcatgc cgggggagcc 5160

accagcccct cactgggttc ggaggagagt caggaagggc cacgacaaag cagaaacatc 5220

ggatttgggg aacgcgtgtc aatccctgt gccgcagggc tgggcgggag agactgttct 5280

gttccttgtg taactgtgtt gctgaaagac tacctcgttc ttgtcttgat gtgtcaccgg 5340

ggcaactgcc tgggggcggg gatgggggca gggtggaagc ggctccccat tttataccaa 5400

aggtgctaca tctatgtgat gggtggggtg gggagggaat cactggtgct atagaaattg 5460

agatgccccc ccaggccagc aaatgttcct ttttgttcaa agtctatttt tattccttga 5520

tatttttctt ttttttttttt ttttttgtg gatggggact tgtgaatttt tctaaaggtg 5580

ctatttaaca tgggaggaga gcgtgtgcgg ctccagccca gcccgctgct cacttttccac 5640

cctctctcca cctgcctctg gcttctcagg cctctgctct ccgacctctc tcctctgaaa 5700

ccctcctcca cagctgcagc ccatcctccc ggctccctcc tagtctgtcc tgcgtcctct 5760

gtccccgggt ttcagagaca acttcccaaa gcacaaagca gttttcccc ctaggggtgg 5820

gaggaagcaa aagactctgt acctatttg tatgtgtata ataattgag atgttttaa 5880

ttatttgat tgctggaata aagcatgtgg aaatgaccca aacataa 5927

<210> 77
<211> 1203
<212> DNA
<213> Artificial Sequence

<220>
<223> Activin A mRNA

<400> 77
atgccttgc tttggctgag aggatttctg ttggcaagtt gctggattat agtgaggagt 60

tccccaccc caggatccga ggggcacagc gcggcccccg actgtccgtc ctgtgcgctg 120

gccgccctcc caaaggatgt acccaactct cagccagaga tggtggaggc cgtcaagaag 180

cacatttaa acatgctgca cttgaagaag agacccgatg tcacccagcc ggtacccaag 240

2015339786 28 Nov 2017

gcggcgcttc tgaacgcgat cagaaagctt catgtgggca aagtcgggga gaacgggtat 300

gtggagatag aggatgacat tggaaggagg gcagaaatga atgaacttat ggagcagacc 360

tcggagatca tcacgtttgc cgagtcaggt tggtgctggc attggcaggg ggtggggagg 420

ggtggggggt gggagggtaa aatatatttc tttgacagtc ccaggaggaa ctctctttcc 480

cttcagctgg aaactgcctg ggaaggttat tagttattag gtgatggtag cggactagcc 540

gacggagggc aggcagggga gggggagagg actttacaga aaaggaattc tcggtcgagc 600

tctgcctgga gatgactggc ttacacttac taaacccagc gggtcacaca gagaggaagc 660

tcggggccaa tgttgagctg gaaggcagac tgtgaggggc tgccttgccc tgcctgtgaa 720

acagatctga gcagccggag gaaagccgcg gcattttcgg gtgctagggg agcagaggag 780

gcttccggac cccatccaag ttttttattga gggtagaggg gtgaatgtac caggattgga 840

gtggaatggc acagatgaag tcactctctt aaaacaaacc ttcccctttа aaagtccaat 900

ctggggccac attggagaag cagggcatat ttatgagtga cagtcatttt tacctttaga 960

aaatgtctat aagtgcacag gcaccacatt caagacaggg aagagctact ttgggggaca 1020

gttgtcattg aaccagcagt tacttttggg acactgactt ttgctctctg aaagaaaaaa 1080

aaataaataa aacaaccagt tttgttcttt ctaaagttac taagagctct ctgccaagga 1140

acgaaccttg acaaagtact ctcagatact acgctgaagt cactcaatct taagaggaag 1200

aag 1203

<210> 78
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Snai1 primer 1

<400> 78
tcggaagcct aactacagcg a 21

<210> 79
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Snai1 primer 2

<400> 79
agatgagcat tggcagcgag 20

<210> 80
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Dkk1 primer 1

28 Nov 2017 2015339786

<400> 80
ccttgaactc ggttctcaat tcc 23

<210> 81
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Dkk1 primer 2

<400> 81
caatggtctg gtacttattc ccg 23

<210> 82
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Col1a1 primer 1

<400> 82
gagggccaag acgaagacat c 21

<210> 83
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Col1a1 primer 2

<400> 83
cagatcacgt catcgcacaa c 21

<210> 84
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Activin primer 1

<400> 84
tcatcacgtt tgccgagtca 20

<210> 85
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Activin primer 2

<400> 85
gctagtccgc taccatcacc 20

<210> 86
<211> 4241
<212> DNA
<213> Homo sapiens

28 Nov 2017 2015339786

<220>
<223> Human Axin2 mRNA

<400> 86
actggctccg cgagcctggc ccgggggagt cggctggagc cggctgcgct ttgataaggt 60
cctggcaact cagtaacagc ccgagagccg ggaaataaaa ataacccctc agagcgatgg 120
attttcgggc cgcccggcgg ccgaggcgcc cgccgaaggc cctgctgtaa aagagaggag 180
gttcagatga gcccctgctg acttgagaga gacagagaga ccacgccgat tgctgagagg 240
aactggaaga agaaaaatcc ccagactcag tgggaagagc tccctcacca tgagtagcgc 300
tatgttggtg acttgcctcc cggaccccag cagcagcttc cgtgaggatg ccccgcggcc 360
cccagtgcca ggggaagaag gggagacccc accgtgtcag ccaggggtgg gcaagggcca 420
ggtcaccaaa cccatgcctg tctcttccaa caccaggcgg aacgaagatg ggttggggga 480
gccggagggg cgggcatctc cggattcccc tctgacccgg tggaccaagt cctcacactc 540
cttattgggc gatcaagacg gtgcttacct gttccgaact ttcctggaga gggagaaatg 600
cgtggatacc ttagacttct ggttttgcct gcaatggatc aggcagatga acctgaagga 660
taccaaaact ttacgagtag ccaaagcgat ctacaaaagg tacattgaga acaacagcat 720
tgtctccaag cagctgaagc ctgccaccaa gacctacata agagatggca tcaagaagca 780
gcagattgat tccatcatgt ttgaccaggc gcagaccgag atccagtcgg tgatggagga 840
aaatgcctac cagatgtttt tgacttctga tatatacctc gaatatgtga ggagtggggg 900
agaaaacaca gcttacatga gtaatggggg actcgggagc ctaaaggtcg tgtgtggcta 960
tctccccacc ttgaatgaag aagaggagtg gacttgtgcc gacttcaagt gcaaacttct c 1020
gccaaccgtg gttggcttgt ccagcaaaac tctgagggcc acggcgagtg tgaggtccac 1080
ggaaactgtt gacagtggat acaggtcctt caagaggagc gatcctgtta atcctatca 1140
cataggttct ggctatgtct ttgcaccagc caccagcgcc aacgacagtg agatatccag 1200
tgatgcgctg acggatgatt ccatgtccat gacggacagc agtgtagatg gaattcctcc 1260
ttatcgtgtg ggcagtaaga aacagctcca gagagaaatg catcgcagtg tgaaggccaa 1320
tggccaagtg tctctacctc atttcccgag aacccaccgc ctgcccaagg agatgacccc 1380
cgtggaaccc gccacctttg cagctgagct gatctcgagg ctggaaaagc tgaagctgga 1440
gttggagagc cgccacagcc tggaggagcg cctgcagcag atccgagagg atgaagagag 1500
agagggctcc gagctcacac tcaattcgcg ggagggggcg cccacgcagc acccctctcc 1560
cctactgccc tccggcagct acgaggaaga cccgcagacg atactggacg atcacctgtc 1620
cagggtcctc aagacccctg gctgccagtc tccaggcgta ggccgctata gccccccgct c 1680
ccgctccccg gaccaccacc accaccacca ttcgcagtac cactccctgc tcccgcccgg 1740
tggcaagctg cctcccgcgg ccgcctcgcc gggcgcctgc cccctcctcg ggggcaaagg 1800
cttttgtgacc aagcagacga cgaagcatgt ccaccaccac tacatccacc accatgccgt 1860
ccccaagacc aaggaggaga tcgaggcgga ggccacgcag cgggtgcact gcttctgccc 1920

28 Nov 2017 2015339786

t gggggcagc gagt at t act gct act cgaa at gcaaaagc cact ccaagg ct ccggaaac 1980
cat gcccagc gagcagt t t g gcggcagcag aggcagt acc t t gcccaaac gcaat gggaa 2040
aggcacggag ccgggcct gg ccct gcccgc cagggaagga ggggcccccg gcggagct gg 2100
ggccct gcag ct t ccccggg aggaaggaga caggt cgcag gat gt ct ggc agt ggat gct 2160
ggagagt gag cggcagagca agcccaagcc ccat agt gcc caaagcacaa aaaaggcct a 2220
cccct t ggag t ct gcccgct cgt ct ccagg cgaacgagcc agccggcacc at ct gt gggg 2280
gggcaacagc gggcaccccc gcaccacccc ccgt gcccac ct gt t caccc aggaccct gc 2340
gat gcct ccc ct gaccccac ccaacacgct ggct cagct g gaggaggcct gt cgcaggct 2400
agct gaggt g t cgaagcccc caaagcagcg gt gct gt gt g gccagt cagc agagggacag 2460
gaat cat t cg gccact gt t c agacgggagc cacaccct t c t ccaat ccaa gcct ggct cc 2520
agaagat cac aaagagccaa agaaact ggc aggt gt ccac gcgct ccagg ccagt gagt t 2580
ggt t gt cact t act t t t t ct gt ggggaaga aat t ccat ac cggaggat gc t gaaggct ca 2640
gagct t gacc ct gggccact t t aaagagca gct cagcaaa aagggaaat t at aggt at t a 2700
ct t caaaaaa gcaagcgat g agt t t gcct g t ggagcggt g t t t gaggaga t ct gggagga 2760
t gagacggt g ct cccgat gt at gaaggccg gat t ct gggc aaagt ggagc ggat cgat t g 2820
agccct gggg t ct ggct t t g gt gaact gt t ggagcccgaa gct ct t gt ga act gt ct t gg 2880
ct gt gagcaa ct gcgacaaa acat t t t gaa ggaaaat t aa accaat gaag aagacaaagt 2940
ct aaggaaga at cggccagt gggcct t cgg gagggcgggg ggaggt t gat t t t cat gat t 3000
cat gagct gg gt act gact g agat aagaaa agcct gaact at t t at t aaa aacat gacca 3060
ct ct t ggct a t t gaagat gc t gcct gt at t t gagagact g ccat acat aa t at at gact t 3120
cct agggat c t gaaat ccat aaact aagag aaact gt gt a t agct t acct gaacaggaat 3180
cct t act gat at t t at agaa cagt t gat t t ccccat ccc cagt t t at gg at at gct gct 3240
t t aaact t gg aaggggggaga caggaagt t t t aat t gt t ct gact aaact t aggagt t gag 3300
ct aggagt gc gt t cat ggt t t ct t cact aa cagaggaat t at gct t t gca ct acgt ccct 3360
ccaagt gaag acagact gt t t t agacagac t t t t t aaaat ggt gccct ac cat t gacaca 3420
t gcagaaat t ggt gcgt t t t gt t t t t t t t t t cct at gct gct ct gt t t t gt ct t aaagg 3480
t ct t gagggt t gaccat gt t gcgt cat cat caacat t t t g ggggt t gt gt t ggat gggat 3540
gat ct gt t gc agagggagag gcagggaacc ct gct cct t c gggccccagg t t gat cct gt 3600
gact gaggct cccct cat g t agcct cccc aggcccaggg ccct gaggcc t gct agaat c 3660
act gccgct g t gct t t cgt g gaaat gacag t t cct t gt t t t t t t t gt t t c t gt t t t t gt t 3720
t t acat t agt cat t ggacca cagccat t ca ggaact accc cct gccccac aaagaaat ga 3780
acagt t gt ag ggagacccag cagcacct t t cct ccacaca cct t cat t t t gat gt t cggg 3840
t t t t t gt gt t aagt t aat ct gt acat t ct g t t t gccat t g t t act t gt ac t at acat ct g 3900
t at at agt gt acggcaaaag agt at t aat c cact at ct ct agt gct t gac t t t aaat cag 3960

28 Nov 2017

2015339786

tacagtacct gtacctgcac ggtcacccgc tccgtgtgtc gccctatatt gagggctcaa 4020

gctttccctt gtttttttgaa aggggtttat gtataaatat attttatgcc ttttttattac 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> Homo sapiens

<220>
<223> Human Axin2 amino acid sequence

<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

2015339786 28 Nov 2017

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

2015339786 28 Nov 2017

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

2015339786 28 Nov 2017

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