Methods:Production of animal models:

The animal protocols were approved by the Washington University animal studies committee. 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 #TD.88137, Harlan Laboratories, Indianapolis, IN) 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. A two-step procedure was utilized to create chronic kidney disease as described previously.{Davies, 2003 #6191;Davies, 2005 #8503} 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 increased compared to recent studies of milder reductions in GFR{Fang, 2014 #12382;Fang, 2014 #12452} to produce moderate (CKD) renal injury that was confirmed by inulin clearances at age 26 weeks.{Agapova, 2016 #13749} Control animals received sham operations in which the appropriate kidney was exposed and mobilized but not treated in any other way. Four groups of mice were used in this study (**supplemental Fig. 1**). 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 CKD receiving subcutaneous injections of vehicle (phosphate buffered saline) twice a week beginning at 22 weeks until euthanasia at 28 weeks (CKD V). The fourth group was *ldlr-/-* mice with CKD receiving subcutaneous injections of RAP-011 (Celgene, Summit, NJ), 10mg/kg twice a week beginning at 22 weeks until euthanasia at 28 weeks (CKD R). The dose used was previously shown in PK/PD studies to be an efficacious dose for stimulation of bone formation.{Lotinun, 2010 #12616}

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.

Inulin clearances:

Inulin clearances were performed at 26 wks, 2 weeks before euthanasia at 28 wks, according to manufacturer instructions (BioPal Inc., Worcester, MA).

Blood tests:

Serum was analyzed on the day of blood draw for blood urea nitrogen (BUN), calcium, and phosphate by standard autoanalyzer laboratory methods performed by our animal facility. Plasma PTH levels were determined by ELISA (ALPCO Diagnostic, Salem NH), and FGF23 levels were determined by a mouse C-terminal kit (Immunotopics, San Clemente, CA) 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.

Histomorphometry:

Bone formation rate/bone surface was determined at time of sacrifice. All mice received intraperitoneal tetracycline (5 mg/kg) 7 and 2 days before being sacrificed. Both femurs were dissected at the time of sacrifice and placed in 70% ethanol. All bone samples were dehydrated, and embedded in methylmethacrylate as previously described.{Malluche, 1986 #12352} Serial sections of 4- and 7-μm thicknesses were cut with a Microm microtome (model HM360, C. Zeiss, Thornwood, NY). Sections were stained with the modified Masson-Goldner trichrome stain.{Goldner, #12353} Unstained sections were prepared for phase contrast and fluorescent light microscopy. Parameters of bone structure, formation, and resorption were evaluated at standardized sites in cancellous bone using the semiautomatic method (Osteoplan II, Kontron, Munich, Germany) at a magnification of 200x.{Malluche, #12354;Manaka, #12355} The

histomorphometric parameters comply with the guidelines of the nomenclature committee of the American Society of Bone and Mineral Research.{Parfitt, 1987 #5048} Osteoplan II software has been programmed to transfer data automatically to statistical software (SPSS for Windows; Chicago Ill).

Micro-computed tomography (micro CT) analysis:

Femurs were fixed in 10% neutral buffered formalin overnight and stored in 70% ethanol at 4 C until analysis. Bones were scanned in 1.5% agarose in 15 mm sample holder using μCT 40 scanner (Scanco Medical, Bassersdorf, Switzerland) with the following parameters: 55 kVp energy, 145μA current, 16 μm resolution, 16 μm voxel size, 200-ms integration time. Images were reconstructed and analyzed using Scanco software. Auto-contouring scripts were used to contour trabecular and cortical regions of interest. 1.6 mm of distal femur metaphysis proximal of growth plate and 0.8 mm of mid-diaphysis were analyzed for trabecular and cortical bones respectively. Femurs from four groups of C57BL/6J mice were used for micro CT analysis. One femur from 8 to 13 mice per group were scanned and analyzed.

Western blotting:

Macrophage colony-stimulating factor (M-CSF)-dependent bone marrow macrophages (M-BMMs) were prepared from the femurs and tibias of 12-week-old C57BL/6J male mice (Jackson Laboratories) and cultured with 50 ng/ml M-CSF (Peprotech) and 100 ng/ml receptor activator of nuclear factor kappa-B ligand (RANKL) (Peprotech) with or without 100 ng/ml activin A (Peprotech) for four days. The cells were harvested by day 0 (M-BMMs + M-CSF alone) and day 4 (osteoclasts) using the RIPA buffer (Thermo Scientific) according to the manufacturer's instructions to prepare the whole-cell lysates. The proteins were separated on a 8-12 % SDS-PAGE and electrotransformed to PVDF membrane (Millipore). Immunoblots were performed by using antibodies against ActRIIA (1:200 Santa Cruz), NFATc1 (nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1) (1:1000 Santa Cruz), cathepsin K (1:1000 Cell

Signaling), integrin β_3 (1:1000 Cell Signaling), or α-tubulin (1:1000 Santa Cruz). Blots were developed by chemiluminescence (Thermo Scientific).

Chromatin immunoprecipitation assays:

The cells were treated with RANKL (100 ng/ml) with or without activin A (100 ng/ml) for 24 h. ChIP assay was performed with the simpleChIP® plus enzymatic chromatin IP kit (Cell Signaling) according to the manufacturer's suggestions using antibodies against histone H3 (1:50 Cell Signaling), normal rabbit IgG (1:100 Cell Signaling), p-c-Fos (1:50 Cell Signaling) or p-Smad2 (1:50 Cell signaling). The purified DNA was analyzed by PCR using primers that detect sequences containing the mouse NFATc1 promoter (forward: 5-CCGGGACGCCCATGCAATCTGTTAGTAATT-3, and reverse: 5-GCGGGTGCCCTGAGAAAGCTACTCTCCCTT-3). All primers were synthesized by Integrated DNA Technologies.

Statistics:

Statistical analysis was performed using Student's unpaired t test (micro CT and ELISAs) or one-Way ANOVA for histomorphometry. and the differences between groups were considered significant at $p<0.05$. All data are expressed as mean ± SE, unless other specified in the figure legend. Differences between groups were assessed post hoc using Fisher LSD method and considered significant at $p <0.05$. The differences between groups in the mechanical bending data were assessed by posthoc Tukey tests. Data for all groups represent an "n" of 7-15, and described in detail in the supplementary materials.