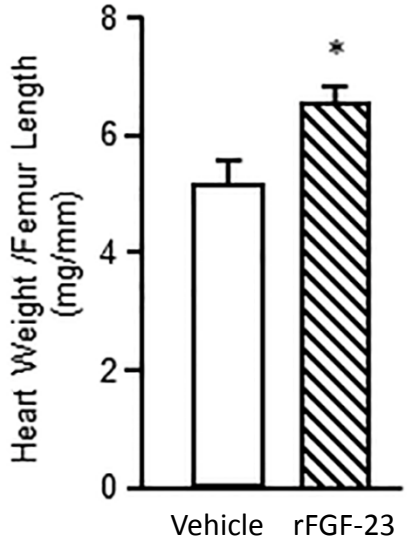
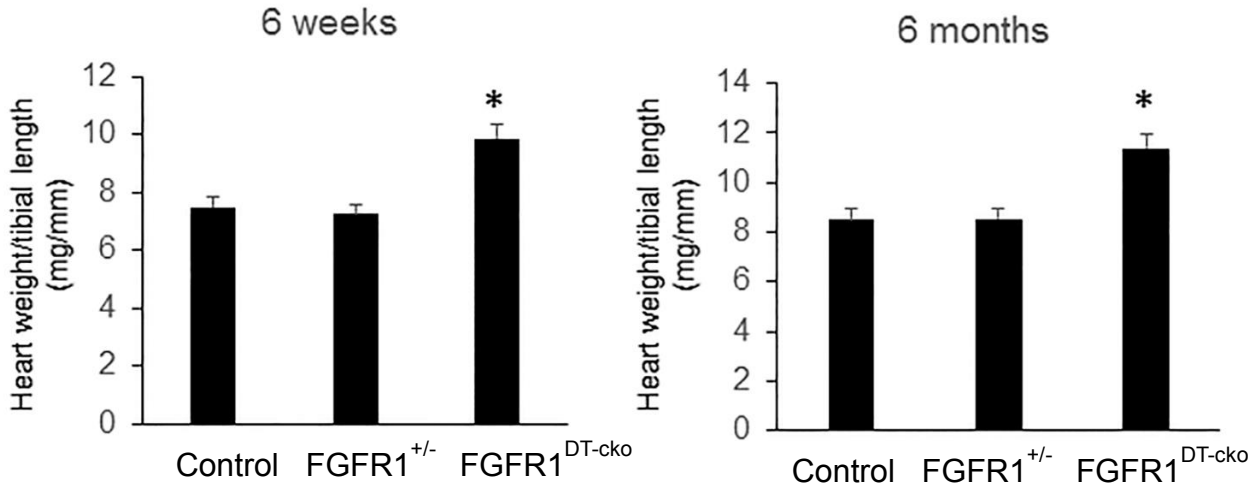
Supplemental data: S1



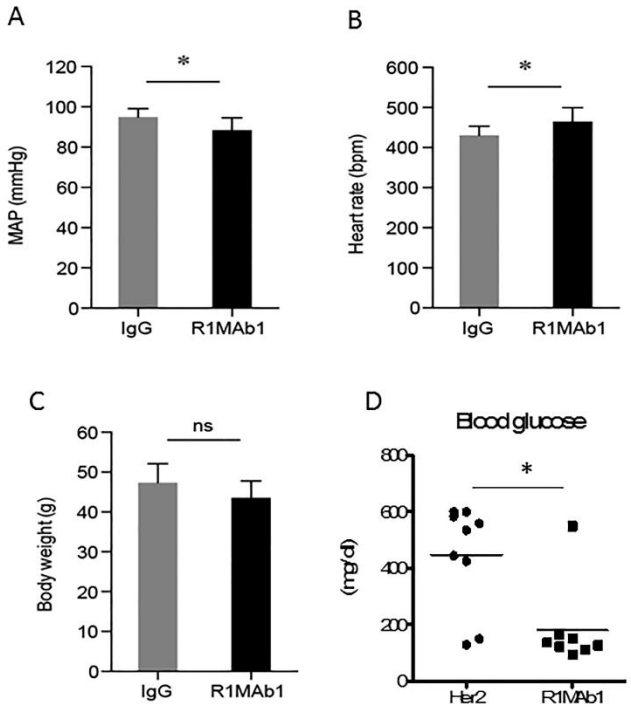
S1. FGF-23 induced cardiac hypertrophy. The seven to eight weeks old wild-type mice were injected IP 50 ng/g rFGF23 (from Amgen) twice daily for 5 days. Heart hypertrophy is expressed as the ratio of heart weight (mg) to femur length (mm). * Significant difference from vehicle control group and FGF23 stimulated group at $p<0.05$ (n=5).

Supplemental data: S2



S2. Deletion of FGFR1 in distal tubule of kidney results in LVH in *FGFR1*$^{DT\ cKO}$ mice. (**A**) *FGFR1*$^{DT\ cKO}$ mice showed increased ratio of heart weight to tibia length in 6-week-old and 6-month-old mice (n=5-8/group). (**p* < 0.05 vs controls by Student's *t* test).

Supplemental data: S3



S3. Activation of FGFR1 reduces blood pressure in *db/db* mice. Effect of R1MAb1 administration on mean arterial pressure (A), heart rate (B), body weight (C), and blood glucose (D) of hypertensive db/db diabetic mice that received single i.p. injection of anti-FGFR1 agonistic mAb R1MAb1 or control IgG. Each measurement was taken on day 6 after the mAb injection. Note that R1Mab1 administration reduced arterial pressure and increased heart rate without significantly reducing body weight at (n=9/group, *$p < 0.05$ vs controls by Student's *t* test). *$p < 0.05$ vs controls by Student's *t* test. All values are shown as mean ± S.D.

Supplemental data: Table 1. Nucleotide primers for qRT-PCR

FGFR1	Forward	AACCTCTAACCGCAGAAC
	Reverse	GAGATCCACTTCCACAG
α-Klotho	Forward	TGATGTCGTCCAACACGTAGGCTT
	Reverse	GCAAAGTGCTCAACTGGCTAAGGT
s-Klotho	Forward	AGATGTGGCCAGCGATAGTTA
	Reverse	ACTTGACCTGACCACCGAAGT
TRPC6	Forward	CGCTGCCACCGTATGG
	Reverse	CCGCCGGTGAGTCAGT
Ace2	Forward	CTTCTCTTCTCAGTGCCCAACCCA
	Reverse	CCCGTGCGCCAAGATCCCAT
Renin	Forward	GAGGCCTTCCTTGACCAATC
	Reverse	TGTGAATCCCACAAGCAAGG
Angiotensinogen	Forward	CACCCCTGCTACAGTCCATTG
	Reverse	GTCTGTACTGACCCCCTCCAG
NCC	Forward	CTTCGGCCACTGGCATTCTG
	Reverse	GATGGCAAGGTAGGAGATGG
NKCC2	Forward	GGCTTGATCTTTGCTTTTGC
	Reverse	CCATCATTGAATCGCTCTCC
GAPDH	Forward	TATGTCGTGGAGTCTACTGG
	Reverse	AGTGATGGCATGGACTGTGG

COMPLETE METHODS

Study approval: All animal research was conducted according to guidelines provided by the National Institutes of Health and the Institute of Laboratory Animal Resources, National Research Council. The University of Tennessee Health Science Center's Animal Care and Use Committee or the Institutional Animal Care and Use Committee at Genentech reviewed and approved all animal studies (Protocol number: 15–128.0 and #10-1033).

Animals breeding and genotyping

CMV-Cre mice were originally purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and maintained in C57BL/6J background. Female *db/db* mice on BKS background were also purchased from the Jackson Laboratory (Bar Harbor, ME, USA). The floxed *Fgfr1* mice (*Fgfr1* $^{flox/flox}$) were obtained from Dr. Chuxia Deng at National Institute of Diabetes and Digestive and Kidney Diseases and maintained in C57BL/6J background for at least six generations. *Ksp-Cre* (40) mice were used to delete the floxed *Fgfr1* in kidney as described previously and maintained in C57BL/6J background for at least five generations. All mice were maintained on a standard diet (7912, Harlan Teklad, Madison, WI, USA) (20). First, we crossed *Fgfr1*$^{flox/+}$ to *CMV*-Cre to obtain a germline-specific deletion of *Fgfr1* (*Fgfr1*$^{null/+}$). The *Fgfr1*$^{null/+}$ mice were crossed to *Ksp*-Cre mice to obtain heterozygous *Ksp*-Cre;*Fgfr1*$^{null/+}$ mice. Then, *Fgfr1*$^{flox/flox}$ females were crossed to *Ksp*-Cre;*Fgfr1*$^{null/+}$ males to obtain the kidney-specific deletion of *Fgfr1* in distal tubule of these mice. For the entire study, samples were collected from 3-month-old *Fgfr1*$^{flox/+}$ (control equivalent) control, conditional *Ksp*-Cre;*Fgfr1*$^{null/flox}$ (*Fgfr1*$^{DT\text{-}cKO}$)-null mice, respectively. Tail clips were collected to genotype the mice. REDExtract-N-Amp Tissue PCR Kit (Sigma-Aldrich, St. Louis, MO, USA) was used for DNA extraction and PCR amplification. Mice were genotyped for the *Fgfr1*^{flox} allele using forward primer 5′-CTG GTA TCC TGT GCC TAT C-3′ and reverse primer 5′-CCA ATC TGA TCC CAA GAC CAC-3′ (325 bp product for the Fgfr1+ control allele, 400 bp

product for the Fgfr1flox floxed allele), and for the Fgfr1null allele using forward primer 5′-GTA TTG CTG GCC CAC TGT TC-3′ and reverse primer 5′-CCA ATC TGA TCC CAA GAC CAC-3′ (300 bp product for the *Fgfr1*^{null} null allele). Mice were euthanized by exposing overdose of isoflurane followed by cervical dislocation. For recombinant FGF-23 (rFGF-23) intervention study, mice were injected IP 50 ng/g rFGF23 (from Amgen) twice daily for 1 or 5 days with vehicle (PBS) or rFGF-23 (from Amgen). Power calculations to determine minimal sample size were performed as previously described (67).

Echocardiography and blood pressure measurement

Non-invasive ultrasound examination of the cardiovascular system was performed using a Vevo 2100 Ultrasound System (VisualSonics Inc, Toronto, Ontario, Canada) following standard procedures. Mice were lightly anesthetized with isoflurane at a constant volume and anesthetic time throughout the procedure. Hair was removed from the anterior chest with a combination of shaving and chemical hair remover under anesthesia, and the animals were placed on a warming pad in a left lateral decubitus position. Ultrasound coupling gel was applied to the chest. Examination of cardiac structure and function under these near-physiologic conditions was obtained with hand-held manipulation of the ultrasound transducer (Sonos 4500). Care was taken to maintain adequate contact while avoiding excessive pressure on the chest. The parasternal long-axis and short-axis views under two-dimensional, M-mode, and Doppler ultrasound examination was performed from multiple views. After completion of the imaging studies, mice were kept warm and allowed to recover until returned to their cage. Digitally acquired images were analyzed to compute left ventricular mass (LVM), LV wall thickness, and ejection fractions ratio using Vevo 2100 analysis software. Blood pressure was measured using mice-tail cuff technique (CODA, Kent Scientific). Mice were maintained in still and unperturbed position throughout the measurement period. Mice were conditioned to the restraint and the warming chamber for 10-20 min/day for 3 days before measurements. The computerized blood pressure

monitor was set for desired sensitivity, number of cycles, the maximum tail-cuff inflation pressure, the rate of deflation and the interval between.

FGFR1 activating antibody, sKI, and FGF-23 intervention

Recombinant anti-FGFR1 R1MAb1 antibody and isotype control Trastuzumab (anti-human Her2) were produced in CHO cells and purified to homogeneity in PBS. Trastuzumab does not react with mouse Her2. At baseline, female *db/db* mice were randomized on the basis of body weight and blood glucose levels. On day 0, mice received 3 mg/kg i.p. injection of R1MAb1 or the control Trastuzumab. On day 6, all animals receive a carotid artery cannula as follows. The ventral and dorsal neck areas were shaved and prepped with betadine and alcohol. A small skin incision was made over the carotid artery. Using blunt dissection techniques, the intended vessel was freed from surrounding tissue and two sutures were threaded under the artery. The cranial suture was tied, the vessel was nicked, catheter was inserted, and the distal suture was used to secure the catheter. All animals remained under anesthesia for 20 minutes heated to 37°C during blood pressure measurement using Biopac blood pressure collection equipment. For studies using WT, *FGFR1*$^{DT\text{-}cKO}$, or Hyp mice, mice (4-week-old) received 3 mg/kg i.p. injection of R1MAb1 or the control IgG on day 0. On day 28, blood pressure was measured using mice-tail cuff technique (CODA, Kent Scientific). For studies using Hyp (4-week-old) received 0.01 mg/kg i.p. injection of sKI (R&D systems) or PBS on day 0 for 4 consecutive days [58], and then blood pressure was measured. For recombinant FGF-23 (rFGF-23) administration, mice were injected IP 50 ng/g rFGF23 (from Amgen) twice daily for 1 or 5 days with vehicle (PBS) or rFGF-23 (from Amgen).

Serum and urine biochemistry

Blood samples were collected by retro-orbital bleeding at the endpoint. Urine samples were collected overnight (from 6:00 pm to 6:00 am) in mice housed in metabolic cages. Calcium was measured using a Calcium CPC Liquicolor Kit (Stanbio Laboratories, Boerne, TX, USA) and phosphorus was measured using the Phosphorus Liqui-UV procedure (Stanbio Laboratories). Sodium was measured using a Sanbio Soudium kit. Serum aldosterone was determined using an Aldosterone ELISA kit from Abcam.

Immunohistochemistry and morphometric of mouse heart.

After fixation in 10% formalin neutral buffered solution immediately after sacrifice, heart and kidney were embedded in paraffin. Samples were rehydrated in decreasing ethanol solutions, then immersed in phosphate buffered saline for 10 min. Slides were blocked for 1 hour in the blocking buffer (SuperBlock T20, Thermo Scientific). Primary antibodies (Klotho antibody # AF1819, 1:100 from R&D Laboratory, Minneapolis, MN; FGFR1 antibody #9740, FGFR4 antibody # 8562, 1:200 from Cell Signaling Technology; TRPC6 antibody # ACC-017, 1:50 and TRPV5 antibody # ACC-035 1:100 from Alomone Labs, Jerusalem, Israel; ACE2 antibody # ab15348, 1:200 from Abcam; NKCC2 antibody # AB3562P, 1:200 from EMD Millipore Corporation, CA, USA) were applied to slides and incubated for overnight at 4°C. For immunofluorescence staining, the secondary antibody was Alexa Fluor 568 # A11011, 1:1000, or 488 # A11008, 1:1000 dye, respectively (Life Technologies). Controls were performed by omitting primary antibodies. DAPI was used for counterstaining of the kidney sections. To visualize cellular borders, fixed tissue was stained with wheat germ agglutinin (WGA) conjugated to Alexa Fluor555 (# W32464, Invitrogen) at 1 mg/ml in PBS containing 10 mm sodium azide. Immunofluorescence images were taken on a Nikon Eclipse Ti-S inverted microscope system. An ImageScope software was used to quantify cross-sectional area of 30 cells at 4 fields along the mid-chamber free wall based on WGA-positive staining. To examine myocardial fibrosis, short-axis cardiac sections from mice were stained with

picrosirius red solution (Sigma-Aldrich) for 1 hour. Images (10-12) were taken and collagen was detected by picrosirius-red-positive area.

Kidney RNA isolation and real-time reverse transcriptase (RT)-qPCR

Total RNA was isolated from whole kidney and heart of mouse at 3 months of age using an RNeasy Mini Kit (Qiagen, Germany). For quantitative real-time RT-PCR, 1.0 μg total RNA isolated from kidney or heart of two genotypes mice was reverse transcribed using an iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA) by following the manufacturer's instructions. PCR reactions contained 1 μl of cDNA (equivalent to 50 ng of total RNA), 300 nM each primers, and 1× iQ SYBR Green supermix (Bio-Rad, Hercules, CA, USA) in a total of 25 μl reaction volume performed with CFX96 Real-Time PCR Detection Systems (Bio-Rad). Primers used for quantitative real-time PCR of genes including FGFR1, α-Klotho, s-Klotho, TRPC6, Ace2, renin, angiotensinogen, NCC, NKCC2, and GAPDH are listed in Table 2. Primers used for quantitative real-time PCR of genes including α-MHC, β-MHC, ANP, BNP, fibronectin, col1α2, col5α1, trimp1 were described previously (68). We also collected kidneys from control and *Fgfr1*$^{DT\text{-}cKO}$ mice injected with vehicle (PBS) or rFGF-23 (100 ng/g/mouse, Sigma) for analyzing expression of Ace2 and Klotho. Relative expression values were evaluated with the $2^{-\Delta\Delta}$Ct or $2^{-\Delta}$Ct method using GAPDH as housekeeping gene.

Western blot analysis

Tissue (~10 mg) was transferred into T-PER tissue protein extraction reagent (Thermo Scientific, Rockford, IL) and 1x protease inhibitor cocktail with 1mM phenylmethylsulfonyl fluoride (PMSF)(Cell Signaling, Danvers, MA, USA). After three 30-second sonication, samples were centrifuged at 13,000x *g* for 10 minute and protein contents in the supernatants were quantified and samples were stored at -80°C until use. For electrophoresis, samples were prepared by

mixing 3x SDS loading buffer (Cell signaling) with 1x DTT. About 50 µg of protein were loaded onto NuPAGE 4–12% Bis-Tris Gel (Invitrogen, Carlsbad, CA, USA). Proteins were separated at 120 V for 60 minutes and transferred to nitrocellulose membrane (Invitrogen, Carlsbad, CA). Membranes were blocked with Superblock blocking buffer in TBST (Thermo Scientific, Rockford, IL, USA) for 60 minutes and then incubated with primary antibodies Primary antibodies (FGFR1 antibody #9740, TRPC6 antibody # ACC-017, 1:1000 and TRPV5 antibody # ACC-035 1:1000 from Alomone Labs, Jerusalem, Israel; ACE2 antibody # ab15348, 1:1000 from Abcam; NKCC2 antibody # AB3562P, 1:1000 from EMD Millipore Corporation, CA, USA; β-actin antibody # sc-1615, 1:1000, Santa Cruz Biotechnology) with gentle agitation overnight at 4°C. After 3 washes with TBST (15 min once and 2x 5 min), membrane was incubated with secondary antibody in Superblock blocking buffer at room temperature for 1 hour. Membrane was then washed 4 times (15 min and 3x 5 min) and subjected to ECL (Thermo Scientific) and analyzed with the FOTO/Analyst Luminary/FX imaging workstation (FOTODYNE INCORPORATED, Hartland, WI, USA). Western bolt using β-actin antibody (Santa Cruz Biotechnology, Dallas, TX) was used as internal control of protein loadings. The intensity of bands was quantified using Image J software (http://rsb.info.nih.gov/ij/).

Statistics

Statistical analyses were performed using 2-tailed Student's *t* test. All values are expressed as means ± SD. All statistical tests are performed with an alpha of 0.05 as the significance threshold. All computations were performed using GraphPad Prism5 (GraphPad Software Inc. La Jolla, CA, USA).