Supplemental Data Table of Contents.

Supplementary Data.

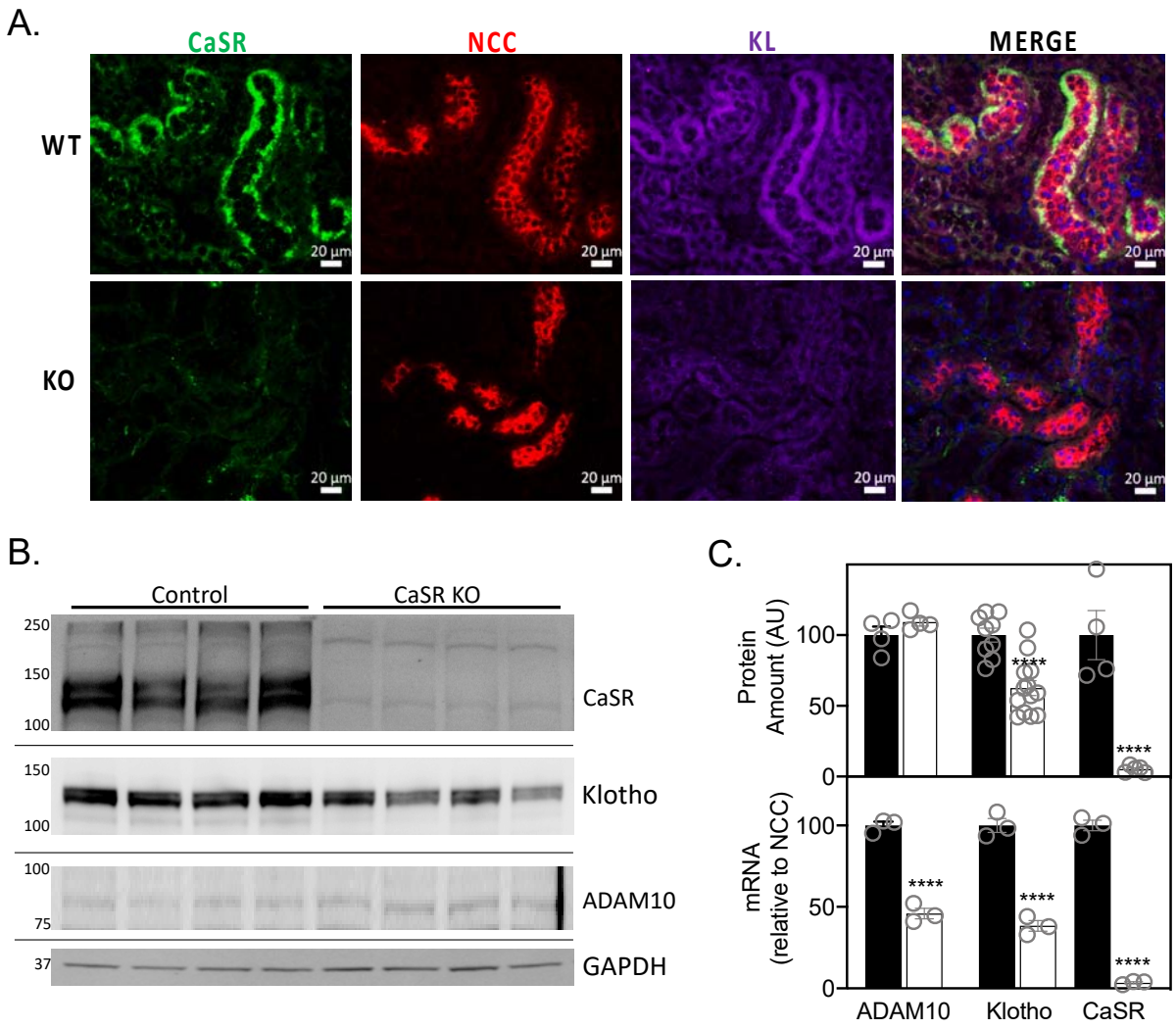

Figure S1. Expression of the CaSR and Klotho in the DCT and absence of CaSR expression in TS-CaSR$^{-/-}$ mice. A) Immunofluorescent images of CaSR$^{fl/fl}$ (WT) and TS-CaSR$^{-/-}$ (KO) kidneys. Perfusion-fixed mouse kidney cortex sections were stained with antibodies recognizing the CaSR (green), NCC (red) and Klotho (magenta). CaSR staining seen in a baso-lateral distribution that overlaps with Klotho in the sections from the CaSR$^{fl/fl}$ mice is associated with NCC staining in the same segments in an apical distribution. Klotho staining is present more widely than the DCT. In sections from the TS-CaSR$^{-/-}$ mice, CaSR staining is not seen in cells that stain for NCC, and Klotho is present in a diffuse distribution with less intensity at the membrane. Scale bar = 20 µm. **B)** Immunoblot of mouse renal cortical homogenates for CaSR, Klotho, ADAM10 and GAPDH, from four separate CaSR$^{fl/fl}$ control mice and TS-CaSR$^{-/-}$ KO mice, as indicated. C) Quantification of kidney tissue extract immunoblots (upper panel) and total mRNA qRT-PCR (lower panel) for indicated genes in control (black bars), and CaSR KO (white bars). Scattered circles each represent an individual mouse; ****P<0.0001 by ANOVA (GraphPad Prism).

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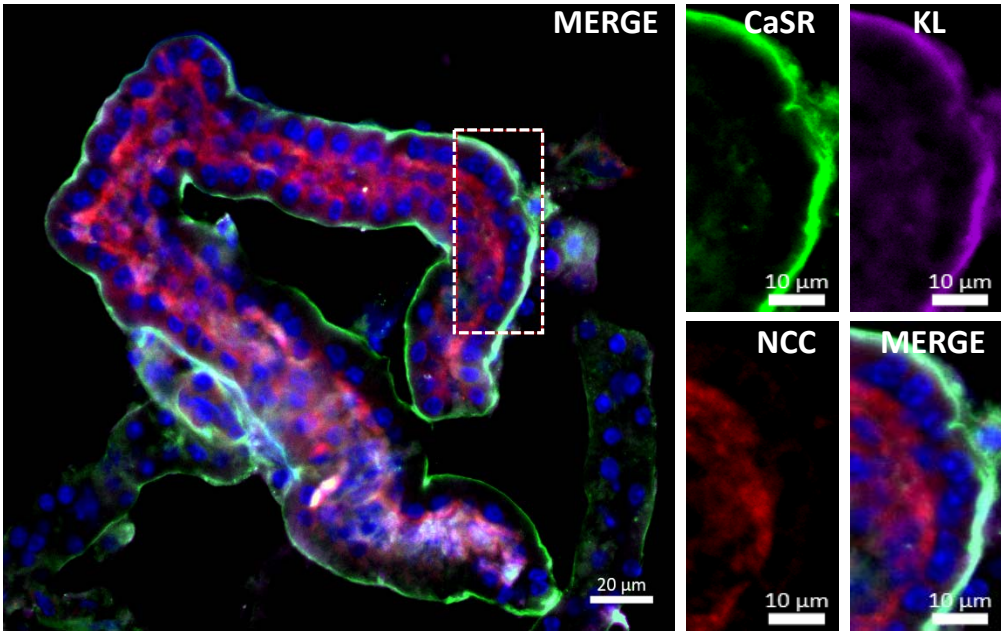

Figure S2. Expression of the CaSR and Klotho in isolated mouse DCT. Mouse tubules were freshly isolated and adhered to poly-L-lysine coated microscope slides. DCT was identified from NCC co-staining. A representative DCT is shown with enlarged inset that shows co-localization of the CaSR with Klotho.

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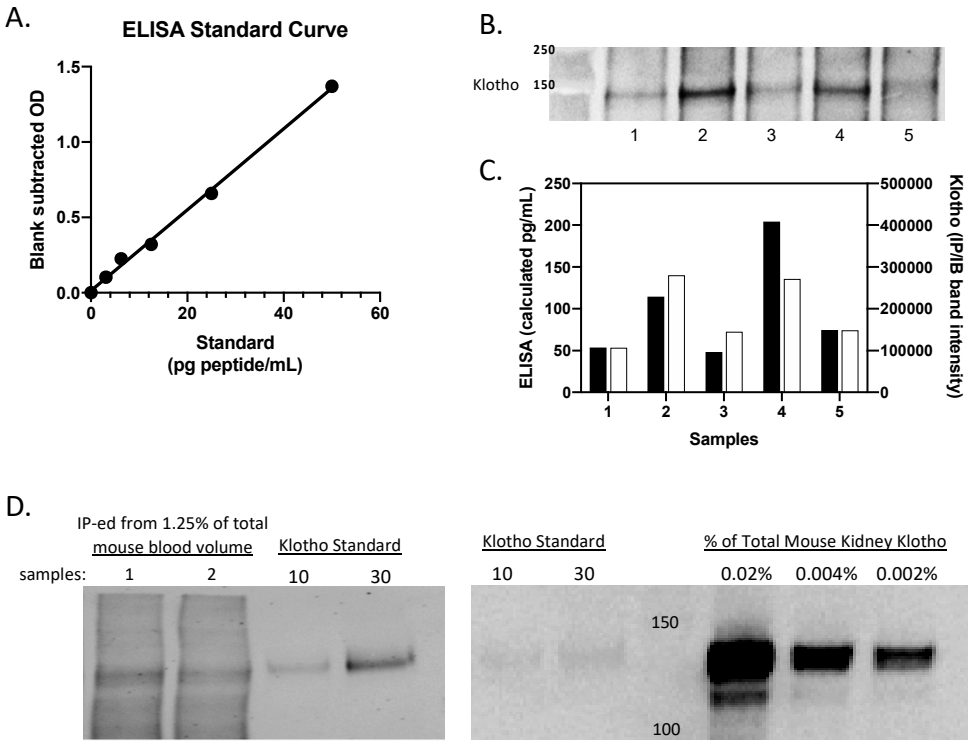

Figure S3. Comparison of Klotho IP/IB and commercially available ELISA. Measurement of serum Klotho amounts are routinely conducted by immunoprecipitation/immunoblot (IP/IB) procedures, through the O'Brien Core at UTSW. To circumvent limitations imposed by blood volume requirements of IP/IB, we used a commercially available Klotho ELISA (Cloud-Clone), which requires less mouse serum per well. This assay was used in several reviewed publications. The general trend of measured Klotho amounts in samples of mouse serum was compared using the two methods. **A)** Standard curve of Klotho peptide for the ELISA used to calculate serum Klotho levels in C (left). **B)** Immunoblot of serum Klotho that was immunoprecipitated from 5 different WT control samples. **C)** Amount of Klotho in the same serum from the 5 control samples, diluted 20x and measured by ELISA (black bars), compared to quantified bands from IP/IB (white bars) shown in panel B. **D)** Comparison of Klotho immunoprecipitated from 20 μL of mouse serum (gel band calculated to represent 1.25% of total mouse blood volume), with mouse kidney extract. The band intensity relative to standard signal on same membrane was used to estimate approximate percentage of total circulating sKlotho relative to Klotho in total kidney extract. The total amount in circulation, assuming 2 mL blood volume is 0.016% of all Klotho detectable in 290 mg of kidney tissue.

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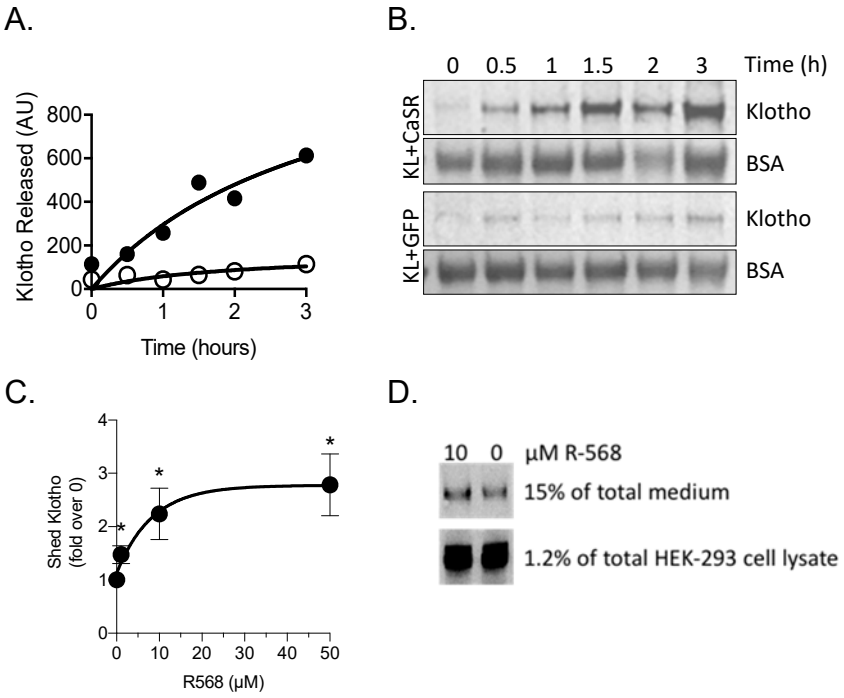

Figure S4. CaSR-mediated increase in shed Klotho from transiently transfected HEK-293 cells. **A)** High extracellular calcium (4mM) induced shed Klotho from HEK-293 cells co-transfected with Klotho and CaSR (filled) or GST (receptor-negative) control and Klotho (open). **B)** Immunoblot of shed Klotho with Ponceau-stained loading control BSA from the same membrane. **C)** R-568 concentration dependent shed Klotho from HEK-293 cells co-transfected with Klotho and CaSR at 1 hour. Average ± S.D., N=3. *P<0.05 by ANOVA comparison to untreated control. **D)** Representative immunoblot of shed Klotho and matched HEK-293 cell lysate after incubation for 1 hour in 10μM R-568. The amount of shed Klotho is <0.005% of total expressed Klotho.

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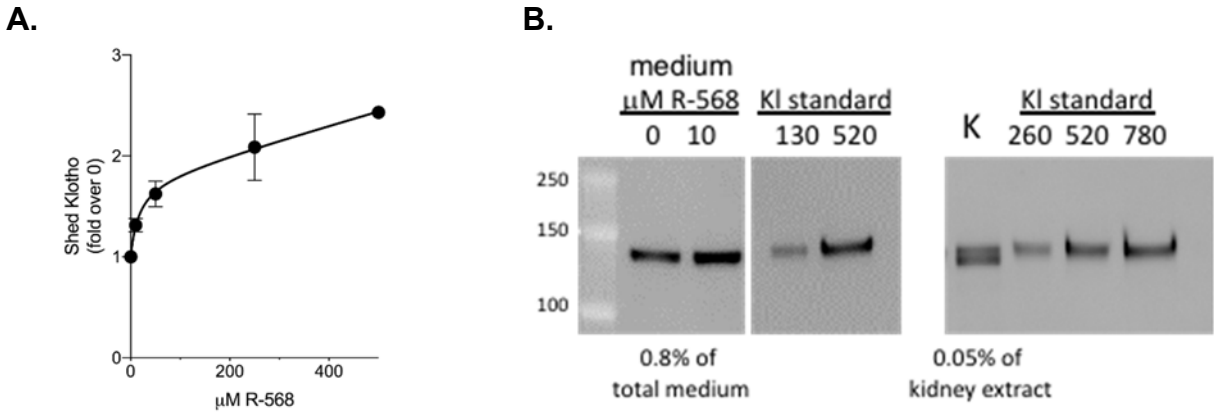

Figure S5. R-568 dose response in minced kidney. A) R-568 concentration dependent shed Klotho from minced kidney at 1 hour. Average ± S.E., N=3. **B)** Representative immunoblots: (Left) Shed Klotho after incubation for 1 hour in 20x tissue weight adjusted volume of 10μM R-568 in DMEM/F12. Purified Klotho standard was run on the same membrane. (Right) Kidney cortex extracted in 20x tissue weight adjusted volume of RIPA buffer, "K", with the same purified Klotho standard. After normalization to the Klotho standard, shed Klotho signal in the sample treated with R-568 is 6.48% of total calculated available Klotho. Compared to control sample, the amount activated by 10μM R-568 is 2.72% of total available Klotho in equivalent weight adjusted kidney extract.

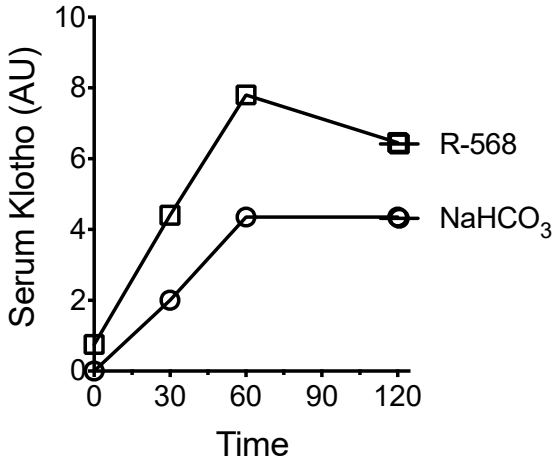

Figure S6. Time-course for in vivo response to R-568 and NaHCO3. Representative time course of 0.4 mg/kg R-568 (square, N=1) or 20 mEq/kg body weight of $NaHCO_3$ (circle, N=1) induced increase in serum Klotho *in vivo*, measured by ELISA from the same mouse, at different times pre- and post-treatment.