

Supplementary information

Renal Production, Uptake, and Handling of Circulating α Klotho

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Running title: Kidney Handling of α Klotho

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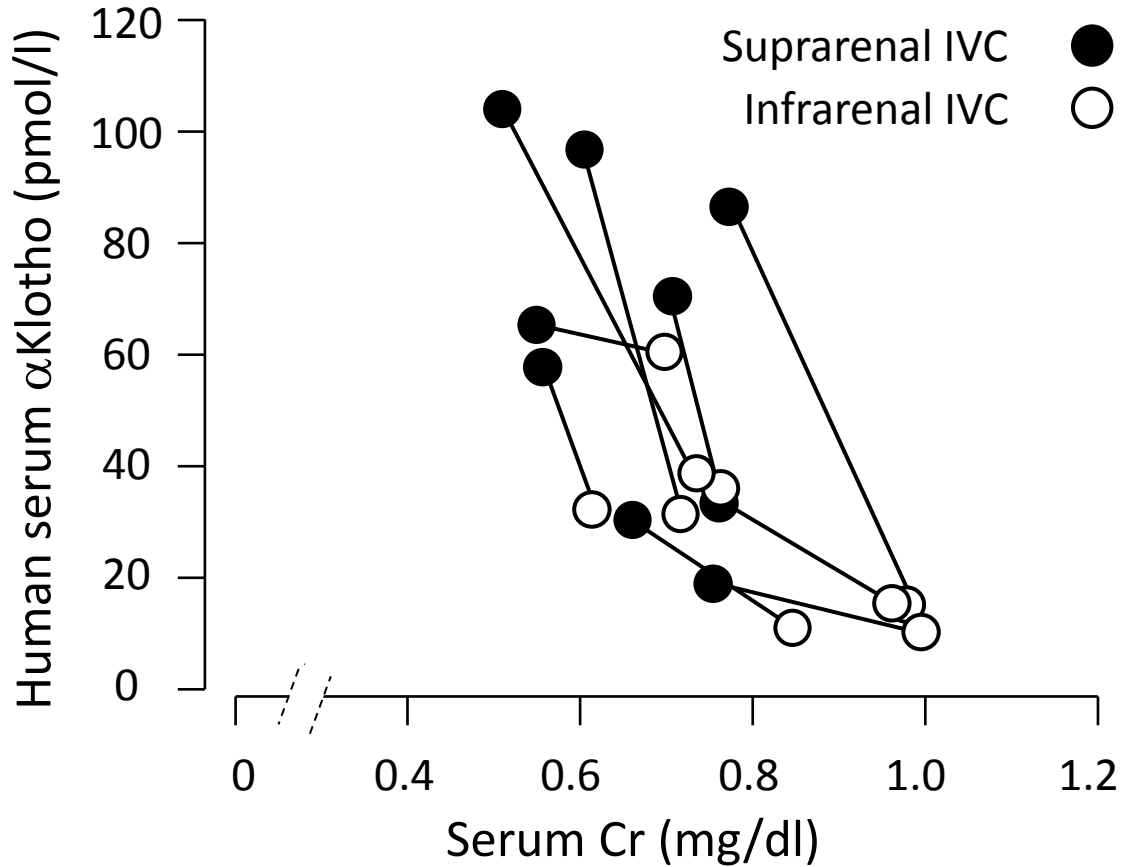
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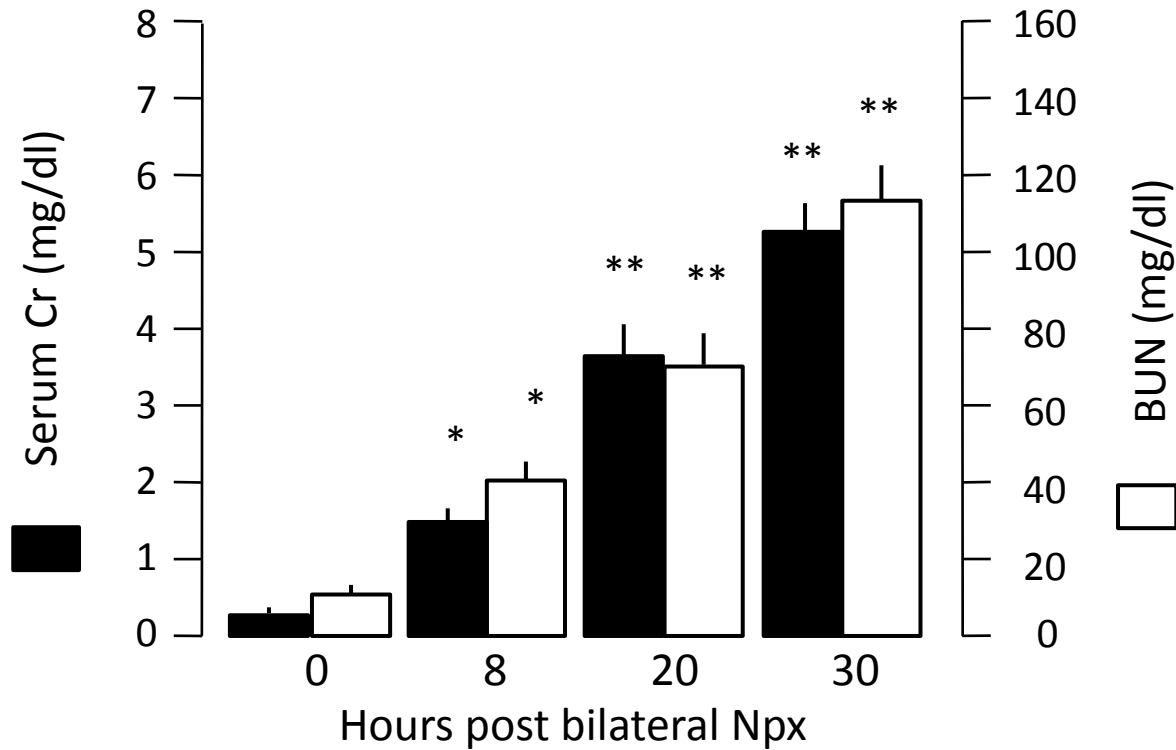
Supplementary Table 1: Demography of humans underwent right heart catheterization

Case	Age (yr)	Sex	Race	Ethnicity	Reason for Catheterization	Co-morbidity	Medications	Serum Pi mg/dl (mM)	Serum Ca mg/dl (mM)
1	60	F	B	NH	CHF	HTN	Nitrate, α/β blocker, ACEI, NSAID	3.0 (0.96)	8.6 (2.2)
2	46	F	W	H	Mitral stenosis	Pulmonary congestion	NSAID	2.9 (0.94)	9.0 (2.3)
3	45	F	B	NH	Aortic stenosis, CHF	CHD, HTN	Estrogen, ACEI, iron, Ca and Vitamin D	2.0 (0.64)	8.6 (2.2)
4	50	F	W	H	Cardiomyopathy	CAD, DM	α/β blocker, ACEI, Insulin, Statin, NSAID,	4.0 (1.3)	8.2 (2.1)
5	43	M	B	NH	CHF	HTN, ashma	Spironolactone, ACEI, β agonist inhaler	4.1 (1.3)	9.7 (2.4)
6	56	F	B	NH	PAH	Lupus	Prednisone	3.5 (1.1)	9.2 (2.3)
7	53	F	B	NH	PAH	HTN, ashma, hypercholesterolemia	α blocker, ACEI, Tricor, NSAID, β agonist inhaler	3.8 (1.2)	8.0 (2.0)
8	43	F	W	NH	PAH			3.8 (1.2)	8.0 (2.0)
9	45	F	W	NH	PAH			4.9 (1.6)	8.8 (2.2)

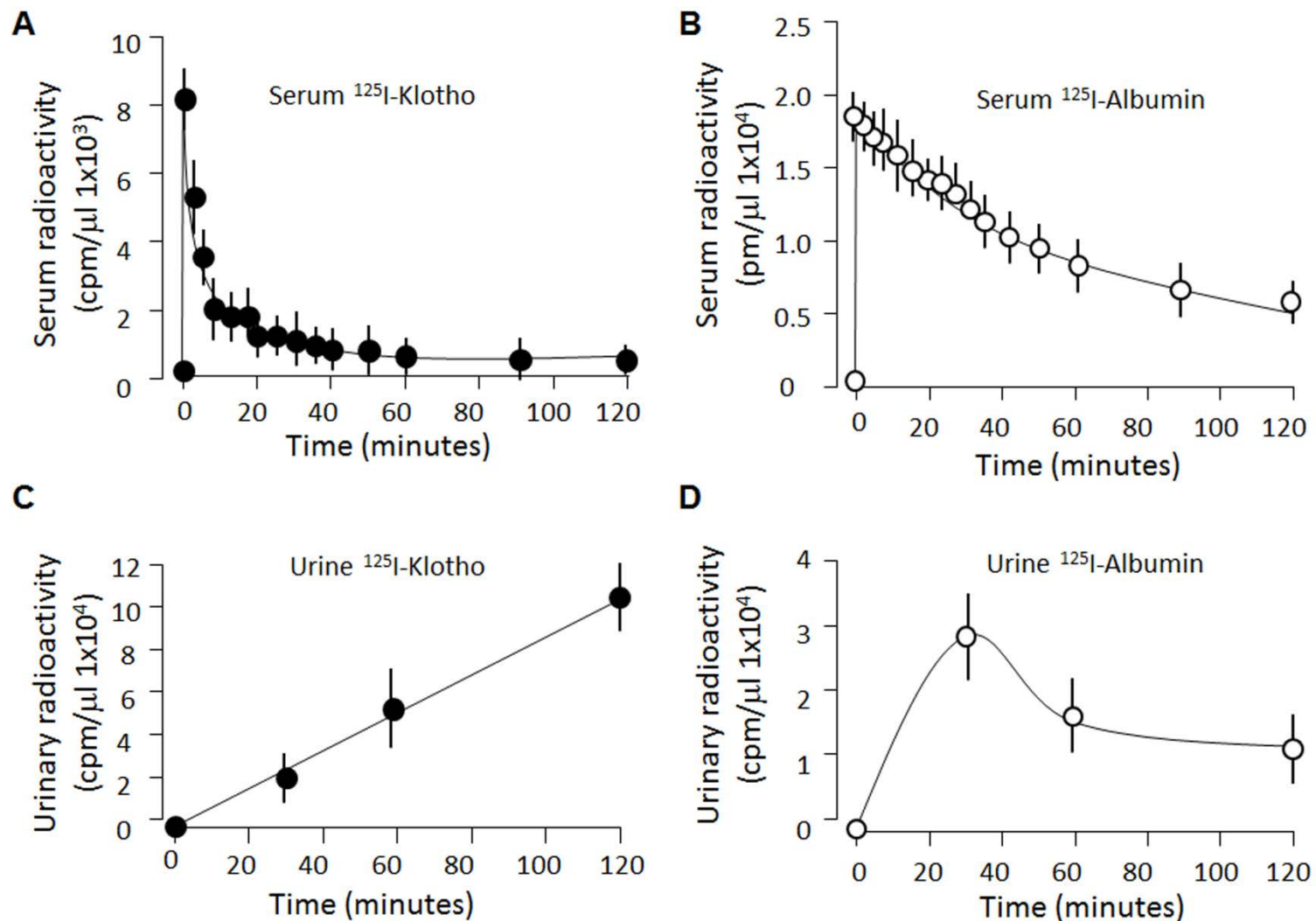
ACEI: angiotensin converting enzyme inhibitor; Ca: serum calcium; CAD: coronary artery disease, CHD: congenic heart defect/CHF: Congestion heart failure; DM: diabetes mellitus; H: Hispanic; HTN: hypertension; NH: non-hispanic; NSAID: non-steroidal anti-inflammatory drug; Pi: serum phosphate; PAH: Pulomary arterial hypertension; yr: year



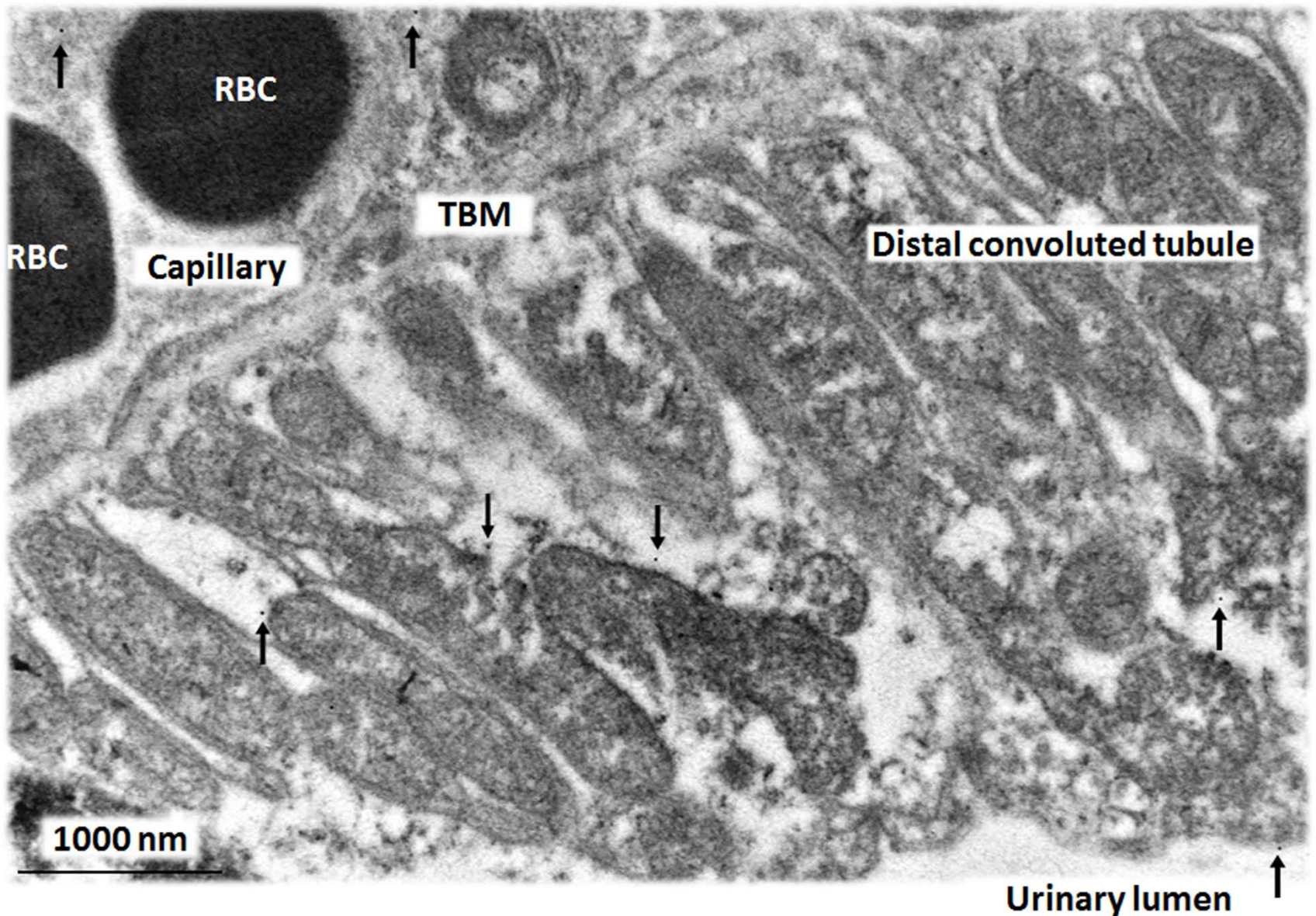
Supplementary Figure 1. Serum αKlotho and serum creatinine in the same samples from human suprarenal and infrarenal inferior vena cava (IVC).
The lines join samples from the same subject.



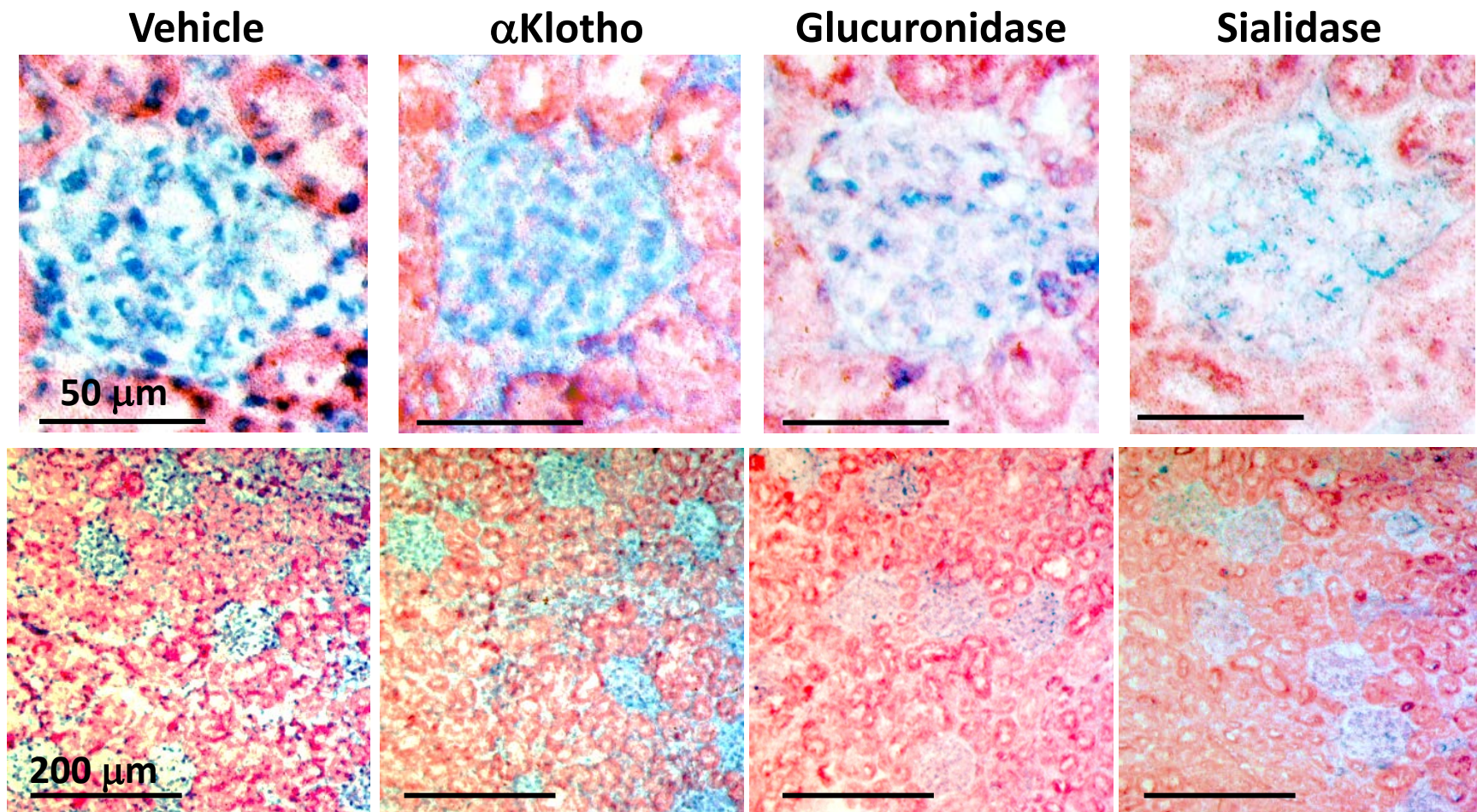
Supplementary Figure 2. Serum creatinine and BUN in anephric rats. Normal SD rats underwent bilateral nephrectomy and blood was drawn at the specified time points post-nephrectomy. Summary of serum creatinine (Cr) and blood urea nitrogen (BUN) from 3 independent experiments. Data is expressed as means \pm SD. Statistical significance was assessed by ANOVA followed by Student-Newman-Keuls test, and significant differences were accepted when $P < 0.05^*$; $P < 0.01^{**}$ vs. 0 hour. BUN: Blood urea nitrogen; Cr: Creatinine.



Supplementary Figure 3. Blood and urine ^{125}I -labeled αKlotho and albumin clearance in *WT* mice. (A and B) ^{125}I -labeled αKlotho was intravenously injected into normal rats and blood and urine were collected at specific time point up to 2 hours post injection. (A) Radioactivity of ^{125}I - αKlotho in serum was quantified and plotted against time. (B) Radioactivity of ^{125}I - αKlotho in urine was quantified and plotted against time. (C and D) ^{125}I -labeled albumin was intravenously injected into normal rats and blood and urine were collected at specific time point up to 2 hours post injection. (C) Radioactivity of ^{125}I -albumin in serum was quantified and plotted against time. (D) Radioactivity of ^{125}I -albumin in urine was quantified and plotted against time. Circles and error bars represent mean \pm SD.



Supplementary Figure 4. Representative electron micrograph of exogenous aKlotho in renal distal tubule. aKlotho protein was intraperitoneally injected into homozygous aKlotho-deficient mice and the kidneys were harvested for immunogold electron microscopy. Arrows indicate gold particle (labelled aKlotho protein) in capillary lumen, cytosol and apical membrane of distal tubule. RBC: red blood cells; TBM: tubular basement membrane. Scale bar = 1000 nm.



Supplementary Figure 5. α Klotho did not reduce glomerular negative charge. The negative charge in glomeruli was evaluated by the Alcian blue stain^{1,2} with Eosin as contrast stain. α Klotho protein was added onto kidney cryosections and its effect was compared with that of glucuronidase (Sigma-Aldrich, St. Louis, MO) and sialidase (Sigma-Aldrich, St. Louis, MO). PBS was used as vehicle for control. **Upper panel:** representative photos at high magnification (Scale bar= 50 μ m); and **bottom panel:** representative photos with low magnification (Scale bar= 200 μ m).

Supplementary method for Alcian blue staining.

The negative charge in glomeruli were evaluated by the Alcian blue stain and fast red nuclear stain^{1,2}.

1. Kanwar YS.Carone FA Reversible changes of tubular cell and basement membrane in drug-induced renal cystic disease. *Kidney Int* 1984; 26(1):35-43.
2. Rollason TP.Brewer DB A study of glomerular basement membrane anionic sites and glomerular visceral epithelial cell coat in protein overload proteinuria in the rat. *J Pathol* 1984; 142(4):301-316.