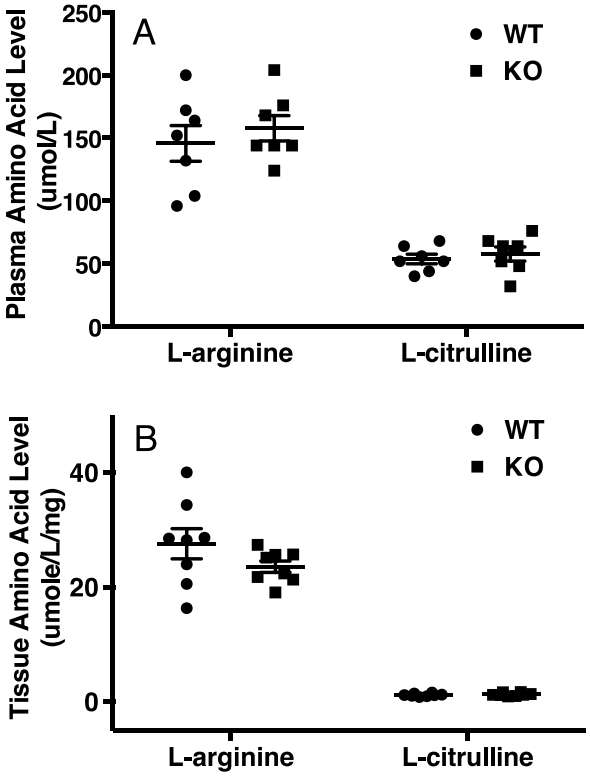
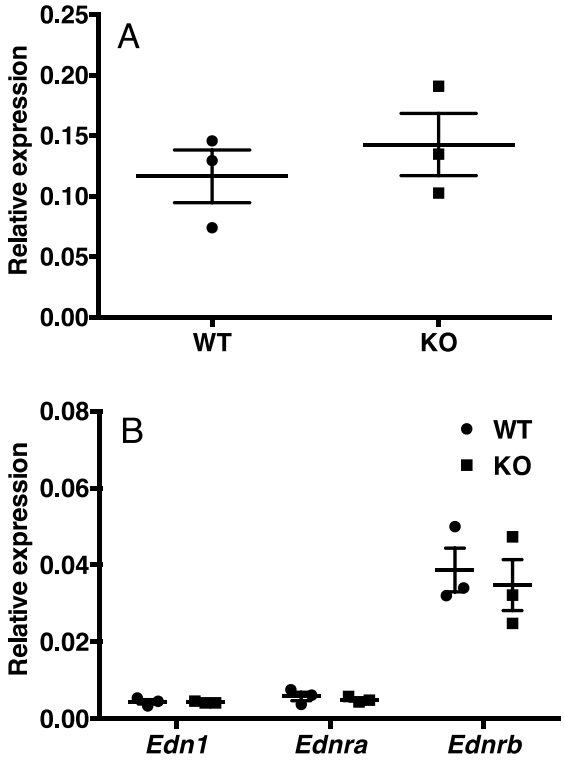
SUPPLEMENTAL DATA

Supplemental Figure 1.



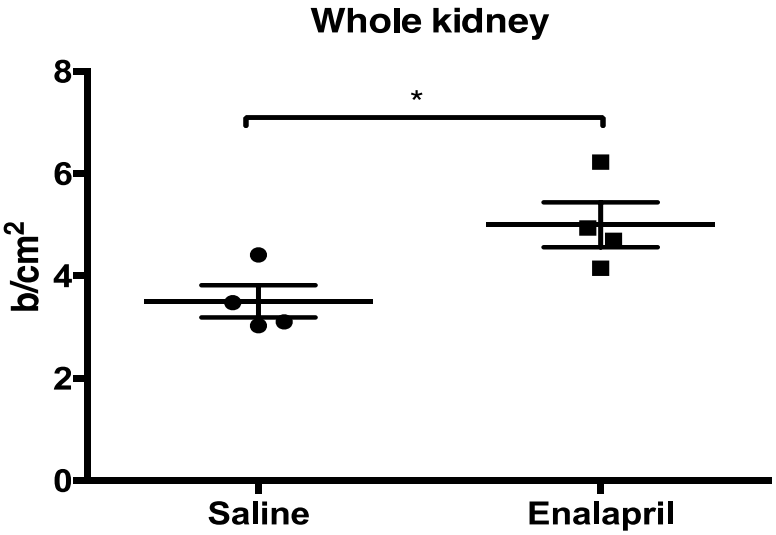
Plasma and tissue levels of L-arginine and L-citrulline are not different between WT (n = 8) and KO (n=8) mice. Plasma L-arginine (umol/L): WT 145.7 ± 37.4, KO 157.7 ± 26.7. Plasma L-citrulline (umol/L): WT- 53.7 ± 10.0, KO 57.7 ± 14.8. (B) Tissue L-arginine (umol/L/mg): WT 27.6 ± 7.5, KO 23.6 ± 2.82. Tissue L-citrulline (umol/L/mg): WT 1.23 ± 0.26, KO 1.32 ± 0.27. p = NS by t-test.

Supplemental Figure 2.



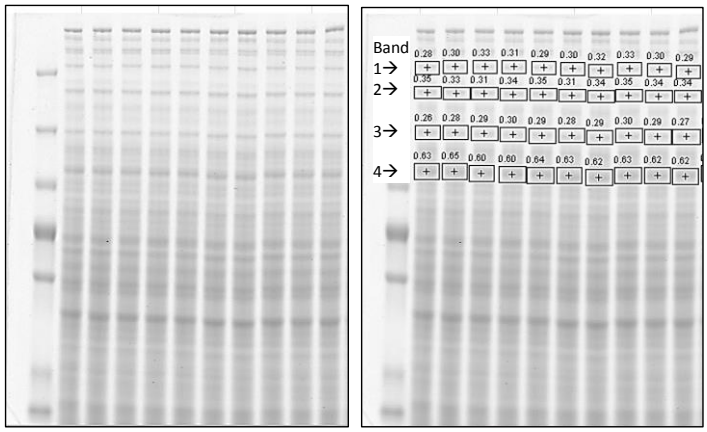
Renal mRNA expression of AT_{1A} receptor (*Agtr1a*), endothelin (ET-1, *Edn1*), ET_A (*Ednra*) and ET_B receptors (*Ednrb*), normalized to *Hprt*, are not different between WT (n = 3) and KO (n=3) mice. p = NS. (A) Renal mRNA expression of *Agtr1a*. (B) Renal mRNA expression of *Edn1*, *Ednra* and *Ednrb*. p = NS, by t-test.

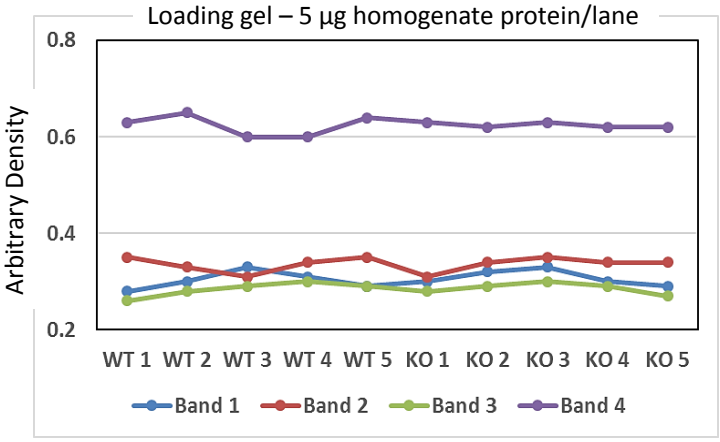
Supplemental Figure 3.



Contrast enhanced ultrasound detects a significant 38% increase in whole kidney renal blood flow in enalapril treated mice (n = 4, average b/cm^2 = 5.0 ± 0.88) compared to saline treated mice (n = 4, b/cm^2 = 3.6 ± 0.63), *p = 0.03. All mice were wild-type males between 12 – 16 weeks of age. Enalapril was administered by intraperitoneal injection (IP) at a dose of 5 mg/kg body weight in volume of 10 ul/gram body weight. Saline was administered IP at 10 ul/gram body weight. Renal blood flow (see Methods section) was determined 2 hours after administration of enalapril or saline.

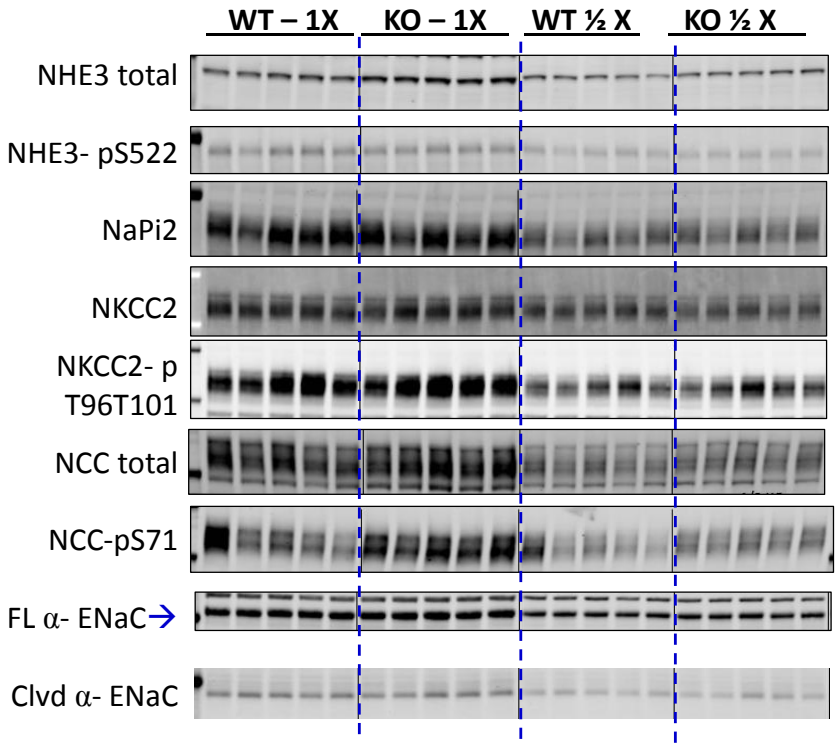
Supplemental Figure 4





Direct protein staining of the gel was implemented to demonstrate equivalent protein loading per lane. Top left: Coomassie stained SDS-PAGE gel (not blot) of renal cortex homogenate samples loaded at 5 µg/lane. Top right: image indicates 4 unidentified protein bands arbitrarily chosen for quantitation by the Licor Odyssey system. Below: summary display of the band densities. Sample to sample variation was less than 5%, viewed as acceptable; normalizing was unnecessary.

Supplemental Figure 5.



Linearity of the detection system. Illustration of the layout of the quantitative immunodetection: Samples loaded at 1X and ½ X on each gel, placed on one piece of blot matrix for same antibody incubations and quantitation. Shown are the molecular weight lanes and baseline samples. It is evident that loading ½ reduced the signal proportionately (verified by quantitation). Amounts loaded per lane: except for NHE3-p analyzed with 5 µg (1X) and 2.5 µg (½ X), all transporters were analyzed at 40 µg (1X) and 20 µg (½ X) per lane. 1X amounts shown in the main manuscript.

SUPPLEMENTAL METHODS

Ion exchange chromatography for plasma and tissue amino acid analysis. The assay was run in the Medical Laboratories of the University of Virginia, Biochemical Genetics Division, using the Amino Acid Analyzer (Agilent 1260 Infinity Quaternary LC) system dedicated for amino acid analysis in physiological fluids. Deproteinized physiological fluids are applied to a resin bed contained in a high pressure metal column where amino acids are separated depending on their dissociation characteristics which are influenced by pH, ionic concentration of eluents and temperature. Eluted amino acids, except for proline and hydroxyproline, then react with fluorescent reagents (o-phthalaldehyde and thiofluor), post column, to yield fluorescence proportional to the amino acid concentration present. The fluorescence (excitation 330nm; emission 465 nm) is measured by the fluorometer and processed by software version C.01.03 of Agilent OpenLAB CDS ChemStation Edition.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR). Gene expression differences between collectrin KO and WT were examined using qRT-PCR. Briefly, whole kidneys from mice aged 10-12 weeks of both genotypes (n = 3 of each) were harvested and snap frozen in liquid nitrogen. Samples were stored at -80°C for later processing. Total mRNA was isolated using the RNeasy Mini Kit (Qiagen). Each sample was additionally treated with DNase (RNase-Free DNase Set, Qiagen) during the isolation. Quantity and quality of isolated total RNA was measured using the Synergy/HT microplate

reader (BioTek). Complementary DNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad), and qRT-PCR was performed using iQ SYBR Green Supermix (Bio-Rad) based on manufacturer's instruction. The primer sequences of each gene are listed below.

	Forward primer	Reverse primer
Agtr1a	AATGAGCACGCTCTCCTACC	GGTCGTGAGCCATTTAGTCC
Edn1	GCCACAGACCAGGCAGTTAG	CGAAAAGATGCCTTGATGCTATT
Ednra	CCCAAAACCTCCCAAGTCTCTC	TGGAAATGACATGCGCGGTAT
Ednrb	AGCTGGTGCCCTTCATACAG	GGGGCTTTCCTTTGTAGTCC
Hprt	CAAACTTTGCTTTCCCTGGT	CAAGGGCATATCCAACAACA