*NPPC mRNA Expression Analysis*

For tissue CNP gene NPPC mRNA analysis, total RNA from 100mg canine cortex or medulla (n=4 each group) were collected using Trizol method. Total RNA from human primary glomerular microvascular endothelial cells (HGMEC, Cell Systems, Kirkland, MA) and human primary renal proximal tubular epithelial cells (HRPTC) were collected with RNeasy kits from Qiagen. RT-PCR was performed with SYBR method. GAPDH gene was used for normalization for NPPC analysis. NPPC (QT00211946, Qiagen, Germantown, MD) and GAPDH (Hs99999905_m1, Thermofisher Scientific, Waltham, MA) primers were used.

Human Renal Fibroblasts Proliferation by Cell Counting

Human renal fibroblasts (HRF, Cell Biologics, Chicago, IL) were counted before being seeded into 48-well plates at a density of 0.1×10^5 cells/well, with complete medium and then incubated at 37 °C, 5% CO_2 overnight. The culture medium was replaced with fresh medium containing PBS or with CNP concentration of 10^{-8}, 10^{-7} M and then cultured for 72 hours. Fresh CNP was added at 24 and 48 hours. At 72 hours, HRF were trypsinized, stained with 0.2% trypan blue and then counted using a Cedex XS Analyzer (Roche, Indianapolis, IN).
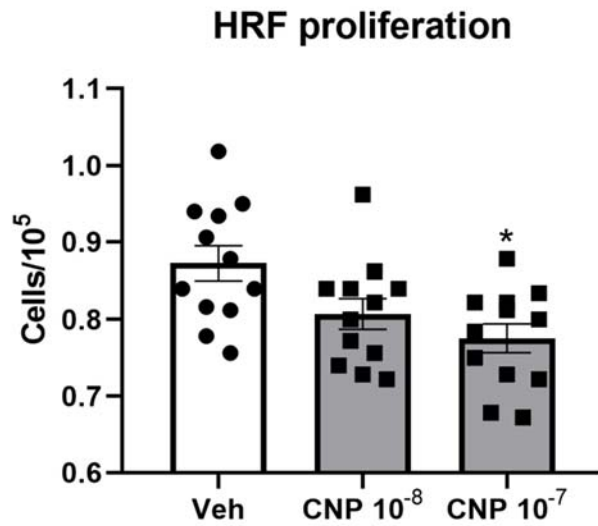
Supplemental Figure IA

NPPC gene

Supplemental Figure IB

NPPC gene
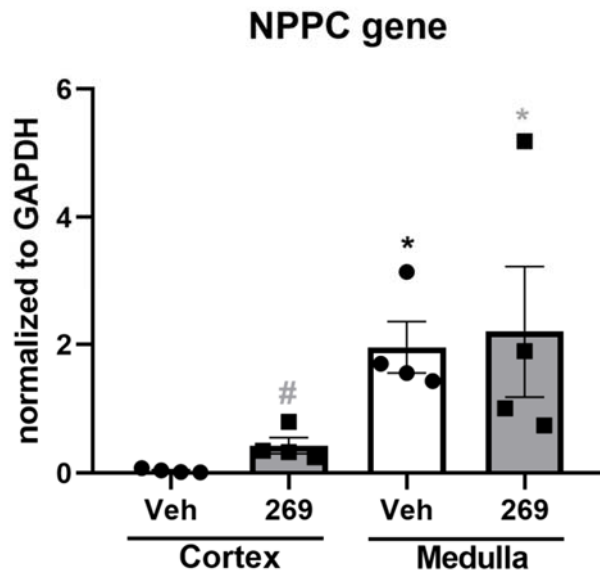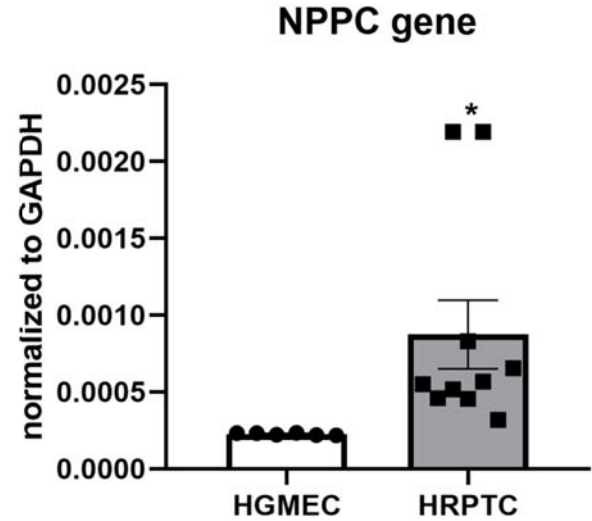
Supplemental Figure IA. Renal CNP gene (NPPC) mRNA by RT-PCR, normalized to GAPDH in CRRL269 or vehicle infusion group. n=4 Veh and n=4 CRRL269, # $p<0.05$ vs Veh with unpaired t-test, * $p<0.05$ vs cortex with paired t-test. **Supplemental Figure IB**. In vitro CNP gene expression was higher in HPRTC than HGMEC. CNP gene (NPPC) mRNA expression by RT-PCR in human glomerular microvascular endothelial cells (HGMEC) and human renal proximal tubular epithelial cells (HPRTC). n=6 HGMEC, n=10 HRPTC, * $p<0.05$ vs HGMEC using unpaired t-test

Supplemental Figure IC. Human renal fibroblasts (HRF) proliferation was reduced by CNP by classical cell counting. HRF were treated with PBS or two concentrations 10^{-8}, 10^{-7} M of CNP every 24 hours and cell numbers were counted at 72 hours. n=12 wells each group, * $p < 0.05$ vs vehicle using unpaired t-test.