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Supplementary Information for

WNK4 kinase is a physiological intracellular chloride sensor

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This PDF file includes:

Figs. S1 to S7

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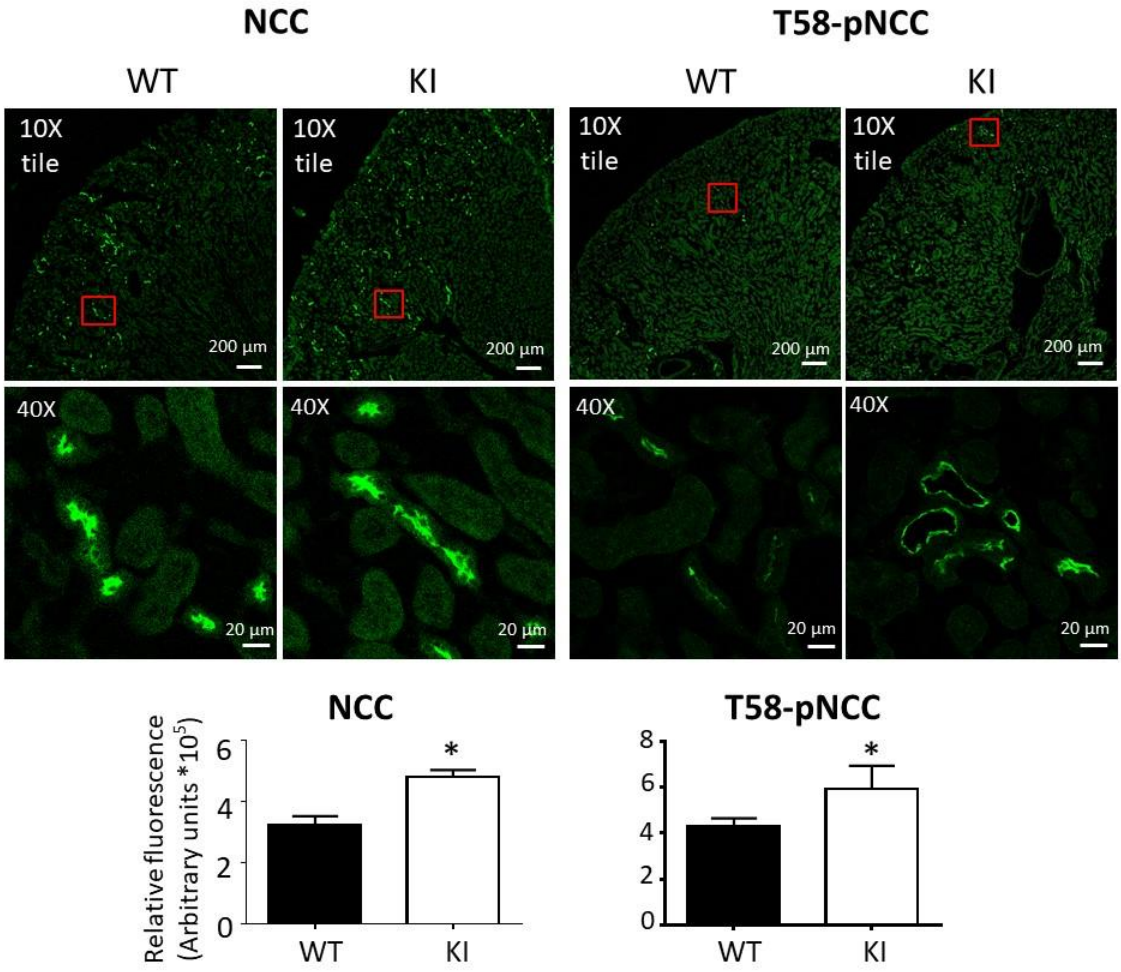

Fig. S1. Immunofluorescence staining of NCC and phospho-NCC in WNK4 knockin mice.

The low-powered view (10X tile) of the kidney slice of wild-type (WT) or knockin (KI) mice was shown, and the fluorescence intensities of NCC and T58 phospho-NCC were quantified using ImageJ. High-power view (40X) magnified the red square area in the low-powered view and displayed the subcellular localization of NCC in DCT. *$p < 0.05$ versus WT group using Student's t-test. The following antibodies: anti-NCC antibody (AB3553)(1:100 dilution; Millipore), anti-NCC phospho-Thr-58 antibody (1:100 dilution), anti-Rabbit (A-21206)(1:200 dilution; Invitrogen) were used.

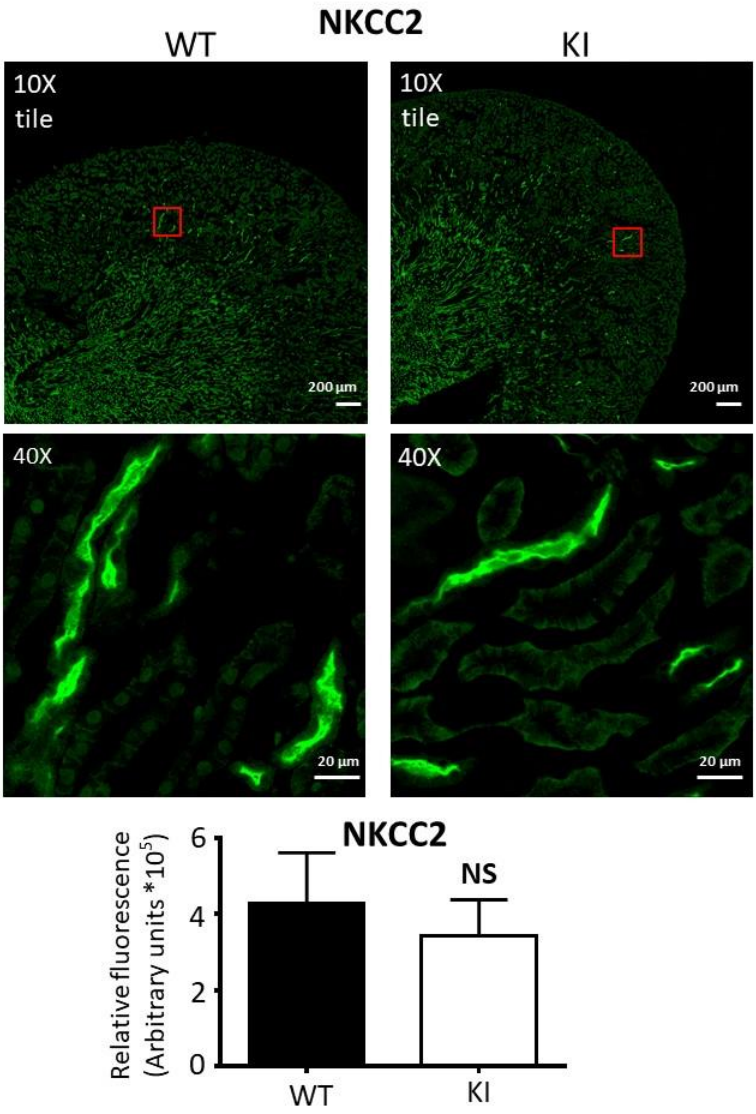

Fig. S2. Immunofluorescence staining of NKCC2 in WNK4 knockin mice.

The NKCC2 abundance (10X, tile) and distribution (40X) in the kidneys of wild-type (WT) or WNK4 knockin (KI) mice were shown. NS: statistically not significant versus WT group using Student's t-test. The following antibodies: anti-NKCC2 (AB2281)(1:100 dilution; Millipore), anti-Rabbit (A-21206)(1:200 dilution; Invitrogen) were used.

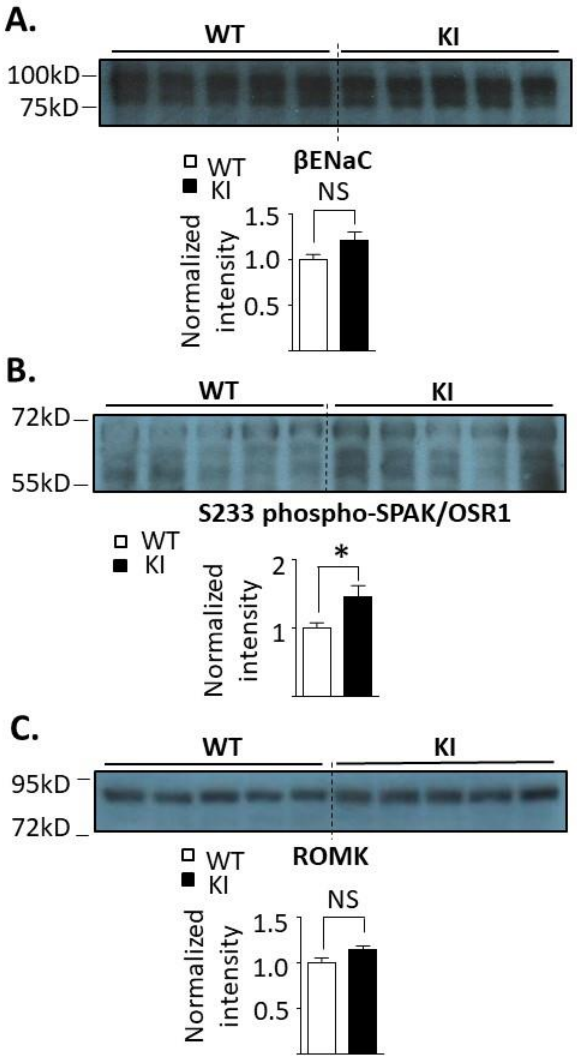

Fig. S3. βENaC, S233 phospho-SPAK/OSR1, and ROMK in WNK4 knockin mice.

The western blot analyses of (A) βENaC, (B) S233 phospho-SPAK/OSR1, and (C) ROMK in whole-kidney lysates of wild-type (WT) or WNK4 knockin (KI) mice were shown. The protein level was normalized to actin and reported relatively to WT controls. Results are presented as mean±SEM, and data were analyzed by unpaired t-test. *$p < 0.05$, NS: statistically not significant versus WT group.

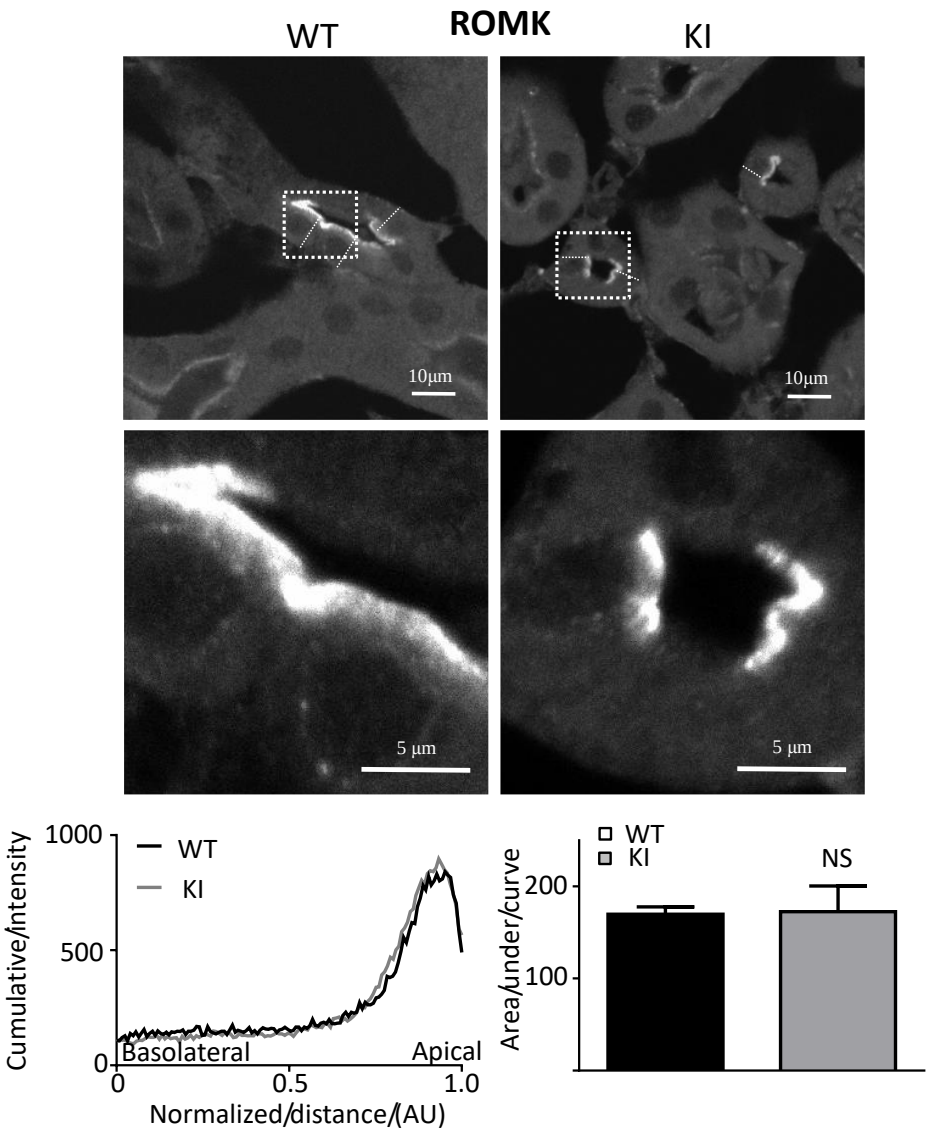

Fig. S4. Analysis of immunofluorescence signal intensity of ROMK across cortical collecting duct of WNK4 knockin mice.

Top two panels are the representative immunofluorescence staining of ROMK in kidneys of wild-type (WT) or WNK4 knockin (KI) mice. The lower panels are magnified views of the indicated region (white dashed box) in the upper panels. The bottom left panel depicts the cumulative intensity profiles for the segments (white dashed lines) in the top panels. The length of each segment was normalized to arbitrary units (AU), with 0 being the basolateral side of the cell, and 1 the apical side. The bottom right panel measured the area under curve of the bottom left panel. The experiments were repeated three times with similar results. The following antibodies: anti-ROMK antibody (sc-393189)(1:50 dilution; Santa Cruz Biotechnology), anti-Mouse (GTX213111-04)(1:200 dilution; GeneTex) were used.

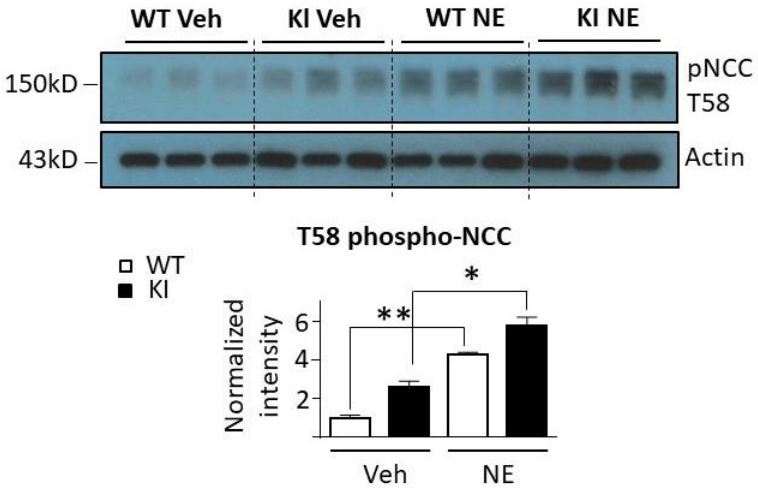

Fig. S5. Norepinephrine further stimulates NCC phosphorylation in WNK4 knockin mice.

Thirty minutes after norepinephrine (NE, 1250 ng/g) or vehicle (Veh) intraperitoneal injection, the kidney lysates of wild-type (WT) and WNK4 knockin (KI) mice were analyzed by Western blot for T58 phospho-NCC. Results are presented as mean±SEM, and data were analyzed by unpaired t-test. *$p < 0.05$, **$p < 0.01$ between indicated groups using Student's t-test.

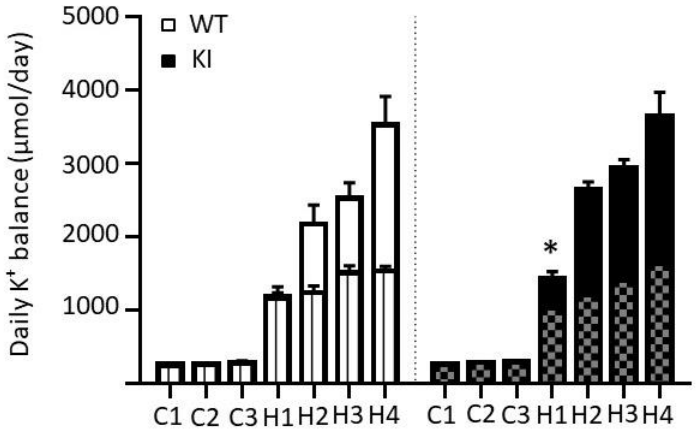

Fig. S6. Daily potassium balance in mice on a 4-day K^+-rich diet.

The daily urinary K^+ excretion (WT: white bar; KI: black bar) and dietary K^+ intake (WT: area with the vertical line; KI: checkered area) are superimposed. The difference between urinary K^+ excretion and dietary K^+ intake indicates daily K^+ balance. *$p < 0.05$ versus WT group on the same day using Student's t-test.

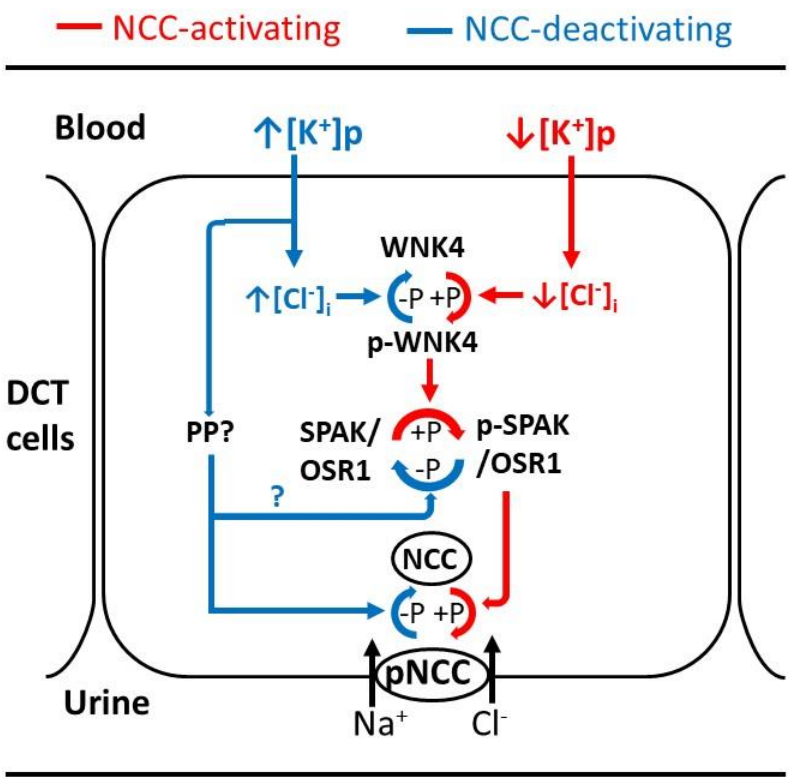

Fig. S7. Mechanisms of plasma K^+ mediated NCC regulations.

NCC-activating (red line) and NCC-deactivating (blue line) signaling coexist in DCT cells in response to the alteration of plasma K^+ concentration ($[K^+]p$). Low $[K^+]p$ activates NCC mainly through Cl^--sensing stimulation of WNK4. In contrast, high $[K^+]p$ deactivates NCC through Cl^--sensing inhibition of WNK4 and WNK4-independent pathways. (+P: phosphorylation; -P: dephosphorylation; PP: protein phosphatase).