Supporting Information

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SI Methods

Urine and Blood Analysis. Freshly collected urine samples obtained from *Kir5.1*$^{+/+}$ and *Kir5.1*$^{-/-}$ mice in the metabolic cages were analyzed for protein content and creatinine, Na^+, K^+, Cl^-, Mg^{2+}, Ca^{2+}, and PO_4^{2-} concentrations by using a Konelab 20I analyzer (Thermo Fisher Scientific). The urinary ammonium concentration was determined in 1:60 diluted samples according the colorimetric protocol of Berthelot (1), and by absorbance measurements at 623 nm. Urinary pH was measured by using a small-diameter glass pH microelectrode and a 691 pH meter (Metrohm). Urinary aldosterone was measured by a Coat-A-Count aldosterone competitive RIA kit (Diagnostic Products), and values were converted into concentrations according the manufacturer's instructions.

Venous blood from the retroorbital plexus of conscious *Kir5.1*$^{+/+}$ and *Kir5.1*$^{-/-}$ mice was analyzed for pH, hematocrit, gas contents, and concentrations of Na^+, Ca^{2+} (calculated at pH 7.4), and Cl^- by a ABL77 blood gas analyzer (Radiometer). Plasma creatinine, osmolality, and K^+ were measured from blood samples taken from the tail artery or at the time the animals were killed. Blood K^+ was measured by using the ABL77 analyzer, and plasma creatinine levels were measured by a Dionex BX-500 high-pressure liquid ion chromatograph (Dionex) according to a previously described method (2) and converted into concentrations by using an external standard curve. Urine and plasma osmolalities were measured by an Autocal 13DR freezing point osmometer (Hermann Roebling) after 1:10 or 1:20 dilution of the samples as appropriate. Whole kidney filtration rates, fractional excretions of ions, blood and urine anion gaps, and blood bicarbonates were calculated from the values measured.

At the end of the experimental period, the animals were killed after anesthetizing by the peritoneal injection of ketamine and xylazine (0.1 and 0.01 mg/g body weight, respectively). Blood was collected on heparin, and the kidneys were removed.

Immunoblot Analysis. *Kir5.1*$^{+/+}$ and *Kir5.1*$^{-/-}$ mice were anesthetized by peritoneal injection of ketamine and xylazine (0.1 and 0.01 mg/g body weight, respectively) and killed by cervical dislocation. The kidneys were rapidly removed and washed in ice-cold buffer, and the medullary tissue was discarded. Membrane fraction preparation and immunoblotting procedures for comparing two sets of samples of renal cortical membranes with regard to the relative abundance of specific proteins were as described (3, 4). Coomassie-stained polyacrylamide gels were used to check that equal quantities of protein were loaded for each series. The dilutions of primary antibodies were as follows: anti-NKCC2, 1:10,000; anti–α-ENaC, 1:10000; anti-NCC, 1:30,000; and anti-NHE3 1:5,000. Densitometric values for *Kir5.1*$^{-/-}$ mice were normalized to the mean for *Kir5.1*$^{+/+}$ mice, which was defined as 100%, and results were expressed as means ± SEM.

Arterial Blood Pressure. Systolic blood pressure was measured in conscious mice by using a BP-2000 blood pressure analysis tail cuff system (Visitech Systems). Variability in testing conditions was minimized by measuring all mice of a given strain at the same time. For each mouse, 10 measurements were repeated over a period of 3 d for acclimatization purposes, and data were discarded. Test measurements were performed over the next 2 d. Systolic blood pressure values lower than 80 mm Hg on individual test measurements, which were probably attributable to a pulse-detection failure, were discarded. The systolic blood pressure from the remaining individual test measurements were averaged, and taken as the blood pressure for each mouse.

1. Berthelot MPE (1859) Violet d'aniline. *Repert Chim Appl* 1:284.
2. Dunn SR, Qi Z, Bottinger EP, Breyer MD, Sharma K (2004) Utility of endogenous creatinine clearance as a measure of renal function in mice. *Kidney Int* 65:1959–1967.
3. Quentin F, et al. (2004) The Cl^-/HCO_3^- exchanger pendrin in the rat kidney is regulated in response to chronic alterations in chloride balance. *Am J Physiol Renal Physiol* 287: F1179–F1188.
4. Quentin F, et al. (2004) Regulation of the Cl^-/HCO_3^- exchanger AE2 in rat thick ascending limb of Henle's loop in response to changes in acid-base and sodium balance. *J Am Soc Nephrol* 15:2988–2997.

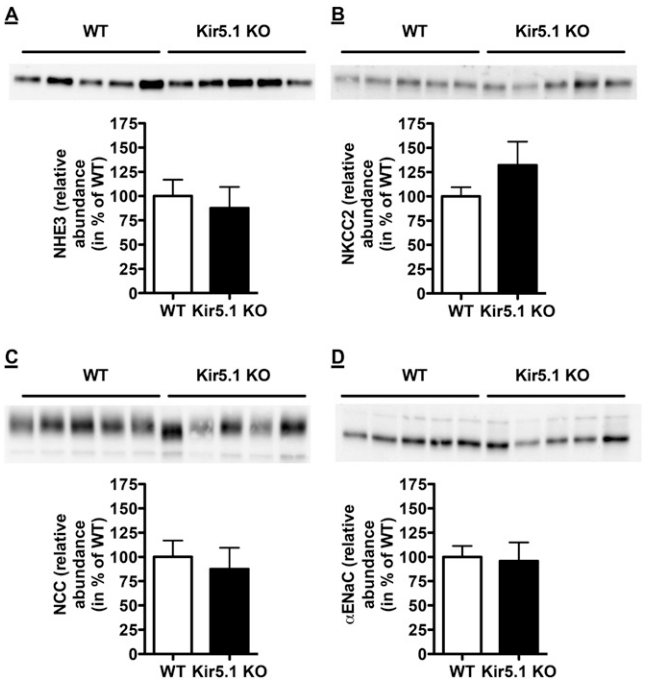

Fig. S1. Semiquantitative immunoblotting of membrane fractions from cortex dissected from *Kir5.1*$^{+/+}$ and *Kir5.1*$^{-/-}$ mice kidneys. Immunoblots of samples from *Kir5.1*$^{+/+}$ ($n = 5$) and *Kir5.1*$^{-/-}$ ($n = 5$) mice incubated with (*A*) anti-Na/H exchanger (NHE3) antibody showing a distinct band at approximately 87 kDa, with (*B*) anti-NaK2Cl cotransporter (NKCC2) showing a distinct band at 160 to 170 kDa, with (*C*) anti-NCC showing a distinct band at approximately 130 kDa, and with (*D*) anti-αENaC antibody showing a distinct band at approximately 100 kDa. Densitometric analysis showed that the abundance (means ± SEM) of NHE3, NKCC2, NCC, and αENaC in *Kir5.1*$^{-/-}$ mice (filled bars) were not different compared with *Kir5.1*$^{+/+}$ mice (open bars).

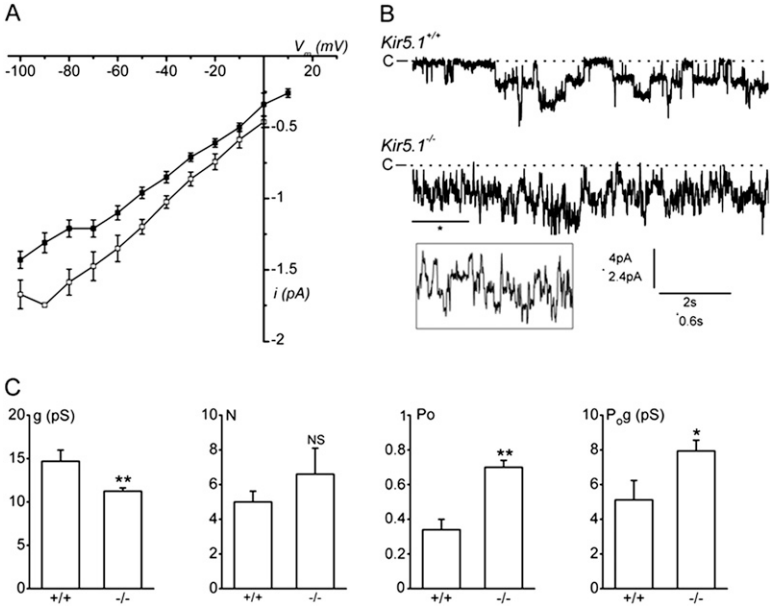

Fig. S2. K^+ currents in the basolateral membrane of DCT tubules with 15 mM K^+ in the pipette. (*A*) Current (*i*)–voltage (V_m) relationships for K^+ channels recorded in the DCTs of *Kir5.1*$^{+/+}$ (□) and *Kir5.1*$^{-/-}$ (■) mice in the cell-attached configuration. Tubules were bathed with physiological saline solution, and the pipette contained 15 mM KCl. Values are means of seven and 10 patches from *Kir5.1*$^{+/+}$ mice and *Kir5.1*$^{-/-}$ mice, respectively. Error bars represent SEM when larger than symbols. (*B*) Current traces recorded in the cell-attached mode at a clamped potential of −60 mV. "C" indicates the closed current level. The expanded inset trace corresponds to the segment of recordings in *Kir5.1*$^{-/-}$ mice indicated by the asterisk. (*C*) Diagrams for the single-channel conductances (*g*), the number of channels per patch (N), the P_o, and the averaged unit conductance (P_o**g*) for the two groups of mice. Values are means of the numbers patches in *Kir5.1*$^{+/+}$ and *Kir5.1*$^{-/-}$ mice given in *A*. Error bars represent SEM; $^{**}P < 0.01$ and $^{*}P < 0.05$; NS, no significant difference.

Table S1. Effects of furosemide on urine parameters of *Kir5.1*$^{+/+}$ and *Kir5.1*$^{-/-}$ mice

	Kir5.1$^{+/+}$		Kir5.1$^{-/-}$	
Parameter	−Furosemide	+Furosemide	−Furosemide	+Furosemide
Urinary volume, μL/g BW/h	2.9 ± 0.3	26.7 ± 2.6*	3.9 ± 0.2	23.8 ± 2.7*
Osmolality, mOsm./kg H_2O	1555 ± 123	317 ± 49*	1120 ± 128	285 ± 48*
pH	5.37 ± 0.06	5.17 ± 0.04	5.34 ± 0.03	5.08 ± 0.06
Mg^{2+}, mmol/mmol creatinine	2.3 ± 1.5	5.2 ± 0.4*	3.3 ± 0.3	6.1 ± 0.3*
K^+, mmol/mmol creatinine	12.5 ± 1	30.8 ± 2.1*	16.8 ± 2.4	38.5 ± 3.5*

Parameters were determined before and after a 6-h treatment with furosemide. Values are means ± SEM, for 12 *Kir5.1*$^{+/+}$ and 12 *Kir5.1*$^{-/-}$ mice. BW, body weight.
*$P < 0.05$, Wilcoxon signed-rank test.

Table S2. Short-term effects of HCTZ on urine parameters of *Kir5.1*$^{+/+}$ and *Kir5.1*$^{-/-}$ mice

	Kir5.1$^{+/+}$		*Kir5.1*$^{-/-}$	
Parameter	−HCTZ	+HCTZ	−HCTZ	+HCTZ
Urinary volume, μL/g BW/6 h	9.2 ± 1.2	35.3 ± 6.1	15.6 ± 2	56 ± 10
Osmolality, mOsm/kg H_2O	2965 ± 111	2562 ± 207*	1775 ± 147	1417 ± 152*
pH	5.39 ± 0.06	5.48 ± 0.02	5.38 ± 0.05	5.51 ± 0.09
K^+, mmol/mmol creatinine	6.8 ± 1.6	22.3 ± 3.4*	12.5 ± 2.1	35.2 ± 4.2*
Cl^-, mmol/mmol creatinine	99.7 ± 11.6	198.3 ± 22*	104.6 ± 21.3	304 ± 42*
Mg^{2+}, mmol/mmol creatinine	2.9 ± 0.4	5.1 ± 0.7*	4.6 ± 0.6	7 ± 0.3*

Parameters were determined before and after a 4-d treatment with HCTZ. Values are means ± SEM, for 8 *Kir5.1*$^{+/+}$ and 7 *Kir5.1*$^{-/-}$ mice. BW, body weight.
*$P < 0.05$, Wilcoxon signed-rank test.