Supporting Information

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SI Methods

Western Blot Analysis. One day after transfection, CHO cells (KCNJ10) or HEK293 cells (KCNJ16) were washed twice with Ringer's solution, scraped into ice-cold RIPA buffer [0.1% SDS, 1% Igepal, and 0.5% sodium deoxycholate, with protease inhibitor mixture in PBS (pH 7.4)], and homogenized using a 27-gauge needle. After incubation for 20 min on ice, cellular debris was removed by centrifugation at 10,000 × *g* at 4 °C for 1 min. The protein concentration of the supernatant was determined by a Bradford-based protein assay (Bio-Rad). Equal amounts of proteins were mixed with reducing Laemmli sample buffer (Bio-Rad) and separated by electrophoresis through SDS (80 g/l)/PAGE. After transfer to a PVDF membrane, the blot was rinsed with PBS and blocked with nonfat milk (50 g/L) and 0.1% Tween in PBS for 1 h. Membranes were incubated overnight with the primary antibodies (rabbit–anti-KCNJ10 at 1:800 dilution; Alomone Labs or rabbit–anti-KCNJ16 at 1:1,000 dilution; custom-made by Davids) in Tween/PBS with BSA (15 g/L) and 0.02% NaN_3. They were then washed (15 g/L) and incubated with HRP-conjugated secondary antibody (1:7,500 dilution) for 1 h. After an additional washing step, images were developed with Western Blotting Luminol Reagent (Santa Cruz) and visualized on medical X-ray film (Fotochemische Werke GmbH).

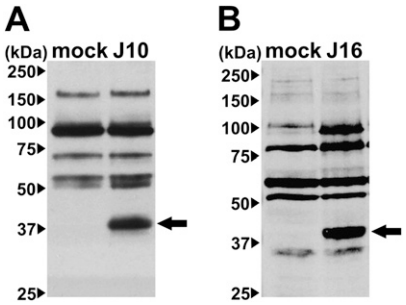

Fig. S1. Western blotting of KCNJ10 and KCNJ16. To test the specificity of the antibody used in this study, Western blot experiments were performed on transfected cells. The antibodies labeled a 40-kDa band in KCNJ10-expressing cells (expected size of 42.45 kDa) (*A*) and a 42-kDa band in KCNJ16-expressing cells (expected size of 47.96 kDa) (*B*), respectively. Additionally, bands of higher molecular weight were labeled with both antibodies. They were considered to be nonspecific because mock-transfected cells also showed these bands.

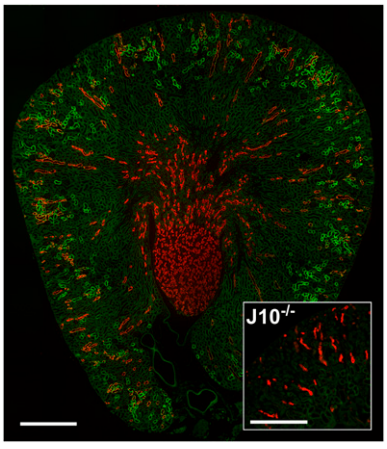

Fig. S2. KCNJ10 in mouse kidney. As a control for the KCNJ10 antibody staining, kidneys from neonatal KO mice were stained. In contrast to sections of WT littermates, KO tissue was devoid of specific staining. KCNJ10 (green) was stained in adult WT mouse kidney. Aquaporin-2 (red) was used to label connecting tubules and collecting ducts. (Inset) Section of a 1-d-old KCNJ10 KO mouse that was negative for KCNJ10 staining. (Scale bar: WT kidney, 1 mm; KCNJ10 KO kidney, 100 μm.)