Supplementary data.

Immunocytochemistry

Confluent $mCCD_{cl1}$ cells grown in 3D culture on Transwell filter inserts were fixed with 4% paraformaldehyde, permeabilized and blocked with 2% BSA + 0.1% Triton X in PBS for 15 min before incubation with primary antibodies (1:100; $K_{ir}4.1$ # A9826 Abclonal, $K_{ir}5.1$ #APR-123 Alomone, N^+/K^+-ATPase #sc-21712 Santa Cruz) in a 2% BSA-PBS solution overnight at 4^0 C. After washing, cells were probed with Alexa labeled secondary antibodies (1:500) then incubated with Hoescht nuclear dye (100 µg ml^{-1}) before mounting on slides with Fluormount. Cells were imaged on a Nikon A1 at 60x with Elements software with 1µm z-steps, and final maximum projection images were processed using ImageJ.

Supplementary Figure 1. The expression of $K_{ir}5.1$, $K_{ir}4.1$, and Na^+/K^+-ATPase in polarized $mCCD_{cl1}$ cells. Confocal images of $mCCD_{cl1}$ cells labeled with Hoechst nuclear dye and antibodies for $K_{ir}4.1$, $K_{ir}5.1$, and N^+/K^+-ATPase confirm the expression of these proteins in cultured $mCCD_{cl1}$ cells. Scale bars are 20 µm.

Supplementary Figure 2. Effect of $K_{ir}1.1$ (ROMK) potassium channel inhibitor VU591 on short-circuit current (I_{eq}) in polarized epithelial cortical collecting principal $mCCD_{cl1}$ cells. Apical application of 1 µM VU591 did not produce significant changes in amiloride-sensitive I_{eq} measured at different time points (n=5 per point).

Supplementary Figure 3. Effect of amitriptyline (a) and VU992 (b) in CHO cells overexpressing ENaC. Examples of ENaC single-channel activity and response to amitriptyline (n = 5) and VU992 (n = 6). Conformational closed (c) and open states are indicated by solid and dashed lines, correspondingly. Summary graphs demonstrating single-channel activity (NP_o) (right panel).