Supplemental Material

Supplemental Table 1. Composition of solutions (in mM) in experiments on microperfused single collecting ducts.

	Na-Ringer	30 mM NH_4Cl	140 Na, 0 K	0 Na, 0 K	0 Na, 5 K	calibration pH 6.8	calibration pH 7.8
NaCl	**135**		**140**				
NMDG-Cl		**105**		**140**	**135**		
K_2HPO_4	**2.5**	**2.5**			**2.5**	**2.5**	**2.5**
NH_4Cl		**30**					
$MgSO_4$	**1.2**	**1.2**	**1.2**	**1.2**	**1.2**	**1.2**	**1.2**
L-alanine	**6.0**	**6.0**	**6.0**	**6.0**	**6.0**	**6.0**	**6.0**
HEPES	**5.0**	**5.0**	**5.0**	**5.0**	**5.0**	**25**	**25**
$CaCl_2$	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**
glucose	**5.5**	**5.5**	**5.5**	**5.5**	**5.5**		
lactate	**4.0**	**4.0**	**4.0**	**4.0**	**4.0**		
KCl						**125**	**125**
osmolality	**~290**	**290-300**	**~290**	**~290**	**~290**	**290-300**	**290-300**

Supplemental Table II. Mean urine pH and blood pH measurements from cilium knockout and control mice.

	mean urine pH ± SEM				mean blood pH ± SEM			
	Day 7	Day 14	Day 19-23	Day 28	Day 7	Day 14	Day 19-23	Day 28
cilium knockout mice	5.28±0.05	5.02±0.04	5.05±0.07	5.34±0.11	7.66±0.05	7.51±0.03	7.50±0.04	7.50±0.03
control mice	5.60±0.04	5.73±0.04	6.25±0.1	6.90±0.2	7.60±0.05	7.44±0.03	7.45±0.03	7.32±0.02

Supplemental Figure 1. Calibrating BCECF. I_{495}/I_{440} values were converted to pH_i values using the high-K^+/nigericin technique initially described by Thomas et al. (37), and modified for a one-point calibration as described by Boyarsky et al. (7). Functioning as a K-H exchange carboxylic ionophore, nigericin sets pH_i equal to pH_o if extracellular and intracellular K^+ concentrations are equal. *A*. A full pH-titration curve experiment is shown for a cilium-deficient monolayer initially perfused on both sides with the standard HEPES-buffered solution to obtain a resting pH_i, and then perfused on both sides with 0 Na^+/130 mM K^+ solutions containing 5 μM nigericin and different extracellular pH (pH_o) varying from 5.5 to 8.5. *B*. From panel A-type experiments, steady-state I_{495}/I_{440} values at each pH were normalized to the mean ratio obtained from flanking exposures to the pH-7.0 solution (R_N). As described by Boyarsky et al. (7), the following pH-titration curve was fit to R_N data using a non-linear least-squares method:

$$\frac{I_{490}}{I_{440}} = 1 + b\left[\frac{10^{(pH-pK)}}{1+10^{(pH-pK)}} - \frac{10^{(7-pK)}}{1+10^{(7-pK)}}\right]$$

where *b* and *pK* are 1.611 ± 0.007 (SD) and 7.212 ± 0.005 (SD), respectively, for cilium-competent cells, and 1.302 ± 0.007 (SD) and 7.095 ± 0.006 (SD), respectively for cilium-deficient cells. The best-fit titration curves had lower and upper asymptotes (i.e., R_{min} and R_{max}) of 0.387 and 1.999, respectively, for cilium-competent cells, and 0.420 and 1.722, respectively for cilium-deficient cells. These titration-curve values allowed us to use the one-point calibration approach (7) for other experiments. More specifically, at the end of an experiment, the monolayer was perfused on both sides with the high-K^+/nigericin solution at pH 7.0. All I_{495}/I_{440} values (R_N) of that experiment were normalized to the I_{495}/I_{440} value obtained at pH 7.0, and pH_i was computed using the equation:

$$pH_i = pK + \log\left[\frac{(R_N - R_{\min})}{(R_{\max} - R_N)}\right]$$

Supplemental Figure 2. Measuring intrinsic H^+ buffering power. β_i was computed as $\Delta[NH_4^+]_i/\Delta pH_i$ in experiments where stepwise decreases in the extracellular NH_3/NH_4^+ concentration (e.g., from 20 to 10 to 5 to 2 to 1 to 0 mM) on both sides of the monolayer elicited corresponding decreases in pH_i while both sides of the monolayer were exposed to a Na^+-free solution to minimize acid-base transporter activity (7). β_i vs. the average pH_i before and after each step change in NH_3/NH_4^+ exhibited a pH_i dependence that was best fit with the line β_i= -13.0×pH_i + 105.2 for the cilium-deficient cells, and β_i= -7.1×pH_i + 68.8 for the cilium-competent cells. Although the slopes and y-intercepts were somewhat different for the two lines, β_I values were similar in the pH_i range during pH_i recoveries from acid loads.

Supplemental Figure 3. Apical cariporide inhibition of basolateral Na^+-induced acid extrusion in cilium-competent monolayers. *A*: pH_i recovery from an acid load in the presence and absence of cariporide. pH_i stabilized at the beginning of the experiment with both the apical and basolateral membranes bathed in the standard HEPES-buffered solution (*ab*). The cells were acid loaded by first applying and then removing a Na^+-free solution containing 20 mM NH_4Cl on both the apical and basolateral membranes (*bcde*). Following the acid load, there was little pH_i recovery in the continued absence of external Na^+ (*ef*). As expected, returning apical Na^+ in the continued absence of basolateral Na^+ had little effect on the slow pH_i recovery (*fg*). Returning basolateral Na^+ initiated an increase in pH_i that was slowed by applying apical cariporide (50 μM) ~12 s later (*gh*). Removing cariporide had little effect on pH_i (*hi*). In another experiment, the basolateral Na^+-induced pH_i recovery was considerably faster (*g'h'*) in the absence of cariporide. For clarity, we only show the pH_i vs. time trace immediately prior to returning basolateral Na^+. *B*: pH_i dependence of basolateral Na^+-induced acid extrusion. Total acid extrusion ± cariporide was calculated from the segment-*gh/g'h'* pH_i recoveries in experiments similar to those shown in panel A. n ≥ 3 for each symbol.