Figure S1

A) AhRPE cultures express Claudin-3 and Claudin-19. Cell extracts from ahRPE and fhRPE were immunoblotted using using antibodies directed against claudin-3 and claudin-19. Actin was included as a loading control. Claudin-19 was readily detected, but Claudin-3 required a much longer exposure. Claudins-1, 2, and 10 were found in trace amounts in hfRPE were not detected in ahRPE (data not shown).

B) Gene expression of junction-related proteins comparing ahRPE to fhRPE. cDNA from 3 independent cultures of ahRPE and a culture of fhRPE was compared by quantitative PCR.

Figure S1 Tight junction associated genes expression were similar between fhRPE and ahRPE. A) AhRPE cultures express Claudin-3 and Claudin-19. Cell extracts from ahRPE and fhRPE were immunoblotted using using antibodies directed against claudin-3 and claudin-19. Actin was included as a loading control. Claudin-19 was readily detected, but Claudin-3 required a much longer exposure. Claudins-1, 2, and 10 were found in trace amounts in hfRPE were not detected in ahRPE (data not shown). B) Gene expression of junction-related proteins comparing ahRPE to fhRPE. cDNA from 3 independent cultures of ahRPE and a culture of fhRPE was compared by quantitative PCR.
The data for fhRPE were comparable to published data for the claudins {Peng, 2011 #1798}. Because gap, adherens, and tight junctions form an integrated signaling complex, data was normalized to house-keeping genes and then to claudin-19 and plotted in $\log_{10}$ scale. cDNA with a cycle # $[C(t)] > 31$ were at the limits of detection under these conditions. Error bars indicate the SE for ahRPE.
Figure S2. Basal application of CFTR inhibitor decreases fluid transport in ahRPE. $J_v$ was plotted as a function of time in the top trace and net fluid absorption (apical to basal bath) is indicated by positive values; TEP (line) and $R_T$ (dotted line) are plotted as function of time in the lower traces. Addition of 15$\mu$M CFTRinh-172 decreased $J_v$ from baseline levels by 2.2 $\mu$L cm$^{-2}$ hr$^{-1}$. N=1.
Figure S3. Apical application of Bumetanide decreased ahRPE fluid transport. $J_v$ was plotted as a function of time in the top trace and net fluid absorption (apical to basal bath) is indicated by positive values; TEP (line) and $R_T$ (dotted line) are plotted as function of time in the lower traces. Apical application of 200$\mu$M ATP mildly increased $J_v$. Afterwards, apical application of 100$\mu$M of Bumetanide decreased $J_v$ by 3.65 $\mu$l cm$^{-2}$ hr$^{-1}$. N=2.
Figure S4. Co-administration of Bumetanide and CFTR-inhibitor 172 decreased fluid transport.

$J_v$ was plotted as a function of time in the top trace and net fluid absorption (apical to basal bath) is indicated by positive values; TEP (line) and $R_T$ (dotted line) are plotted as function of time in the lower traces. The combined application of 5μM of CFTR-inhibitor 172 basally and 100μM Bumetanide apically decreased $J_v$ 3.71 μl·cm⁻²·hr⁻¹. N=1.
Table S1. Responses to Epinephrine and ATP by nhRPE, ahRPE and fhRPE are compared.

Changes in TEP and $R_T$ were compared after application of 10nM of Epinephrine (right) and 100$\mu$M ATP. The largest changes by epinephrine were observed in nhRPE, followed by fhRPE and lastly ahRPE. There is no published data on the response of nhRPE to ATP. *Data from Quinn and Miller 1992. Measurements reported are mean ± standard error of the mean.