Figure S1

A)

AhRPE cultures express Claudin-3 and Claudin-19. Cell extracts from ahRPE and fhRPE were immunoblotted 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 15μM CFTRinh-172 decreased $J_v$ from baseline levels by 2.2μl 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 5\( \mu \)M of CFTR-inhibitor 172 basally and 100\( \mu \)M Bumetanide apically decreased \( J_v \) 3.71\( \mu \)l 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 100µM 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.

<table>
<thead>
<tr>
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<th>Epinephrine (10nM)</th>
<th>ATP (100µM)</th>
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<tbody>
<tr>
<td></td>
<td>nhRPE (n=1)</td>
<td>ahRPE cultures (n=5)</td>
</tr>
<tr>
<td>$\Delta$TEP (mV)</td>
<td>2.4*</td>
<td>0.33 ± 0.04</td>
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<tr>
<td>$\Delta R_T$ (Ω·cm²)</td>
<td>8*</td>
<td>17.9 ± 4.3</td>
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