

**Supplementary Materials:** The following supporting information can be downloaded at [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1), Figures S1-S8; Tables S1-S3.

Figure S1: Vulnerability of CFTR-KD epithelium to PAO1 strains with attenuated virulence at 6h and 16h post-infection

Figure S2: mRNA expression level of junctional proteins in CFTR-CTL and CFTR-KD epithelia

Figure S3: Expression of junctional proteins in CFTR-CTL and CFTR-KD monolayers

Figure S4: Restoration of ZO-1 expression and localization in apically rehydrated CFTR-KD-epithelium

Figure S5: Protection of CFTR-KD epithelium to  $\Delta$ *fliC* and  $\Delta$ *lasR* strains by apical rehydration

Figure S6: Protection of CFTR-KD epithelium to  $\Delta$ *fliC* strain by previous apical rehydration

Figure S7: Contribution of the secretome to PAO1 growth

Figure S8: Immune gene profile of CFTR-CTL and CFTR-KD epithelia subjected to apical liquid manipulations

Table S1: List of products

Table S2: List of primer pairs used for qPCR

Table S3: List of immunology genes evaluated in uninfected and  $\Delta$ *lasR*-infected CFTR-CTL and CFTR-KD epithelia after apical liquid manipulations

**Author Contributions:** Conceptualization of the study, M.C. and J.L.S.; conceptualization of the infection experiments, A.L., D.L. and T.K.; methodology, investigation and formal analysis for infection experiments, J.L.S., A.L. and D.L.; methodology, investigation and formal analysis for all other experiments, J.L.S.; investigation for experiments on epithelial cells, M.B.; formal analysis, D.L., C.v.D. and T.K.; writing – original draft preparation, J.L.S. and M.C.; supervision, project administration and funding acquisition, M.C.. All authors reviewed the manuscript.

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**Institutional Review Board Statement:** “Not applicable” for studies not involving humans or animals.

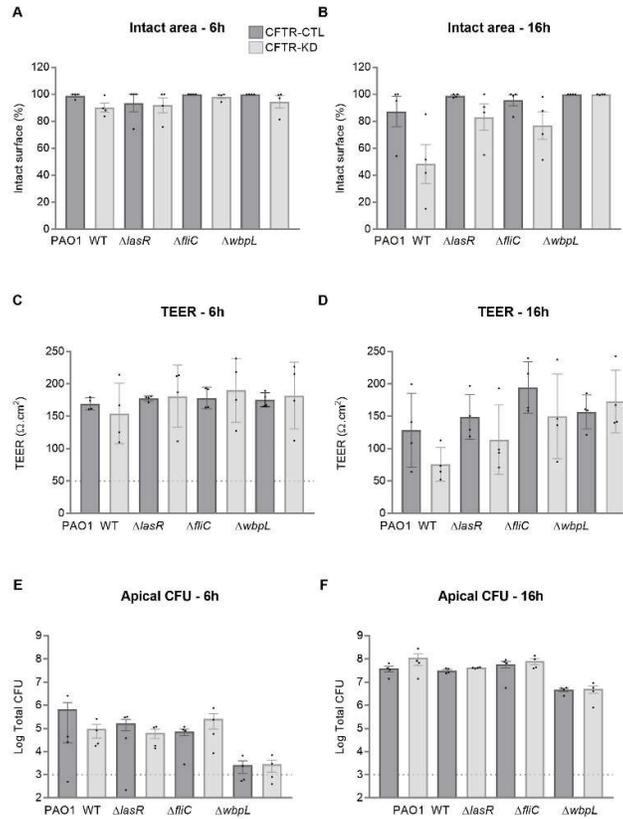
**Data Availability Statement:** The dataset for this article can be found at the following DOI: 10.26037/yareta:ckvglc7mgbdjml5k6nllgtlhe. It will be preserved for 10 years.

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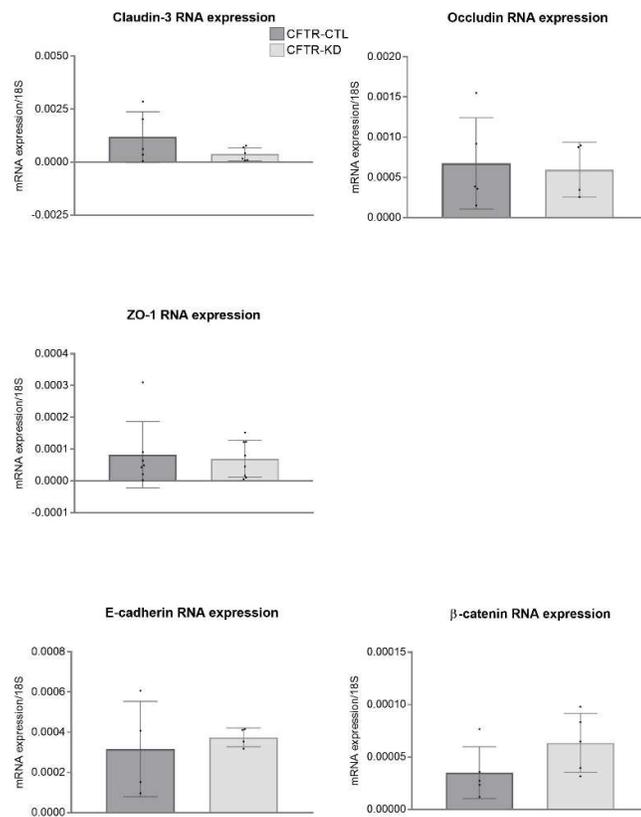
## Appendix

Supplementary data

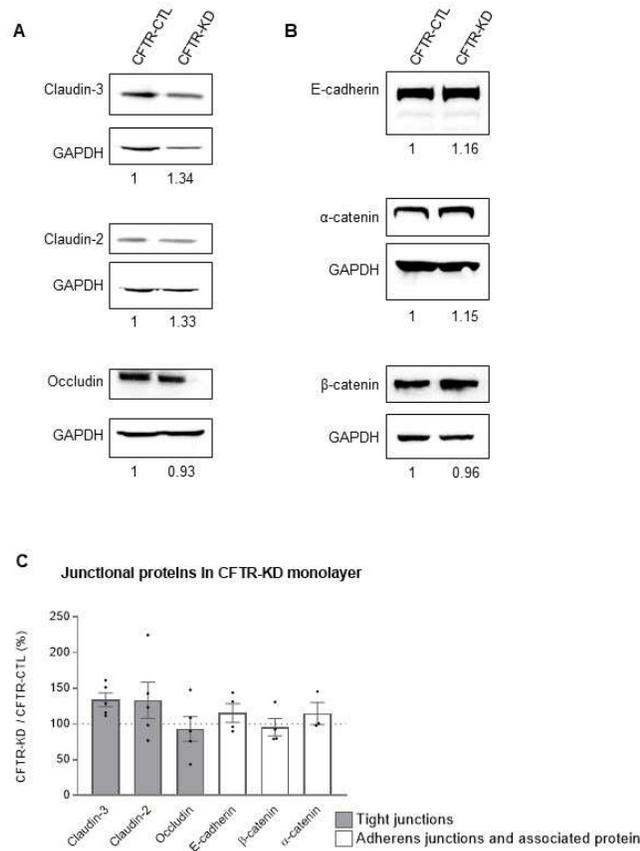
**Figure S1.** Vulnerability of CFTR-KD epithelium to PAO1 strains with attenuated virulence at 6h and 16h post-infection

**Figure S1.** CFTR-CTL (dark gray) and CFTR-KD (light gray) epithelium were apically infected with  $10^3$  CFU of the wild type PAO1 strain PAO1 mutants ( $\Delta lasR$ ,  $\Delta fliC$ ,  $\Delta wbpL$ ) for 6h (A, C, E) and 16h (B, D, F). The intact epithelial surface (A, B), TEER value (C, D) and apical CFUs (E, F) were determined. (A, B) The intact epithelial surface was expressed as % of the initial area measured in uninfected conditions (6h:  $N=4$ ,  $n \geq 1$ ; 16h:  $N=4$ ,  $n=1$ ). (C, D) TEER values, which were measured in duplicate for each insert, were expressed in  $\Omega \cdot cm^2$ . The dotted line indicates the average TEER values of empty Transwell filters (6h:  $N=4$ ,  $n \geq 2$ ; 16h:  $N=4$ ,  $n=2$ ). (D, E) The dotted line represents the initial amount of inoculated bacteria (6h:  $N=4$ ,  $n \geq 2$ ; 16h:  $N=4$ ,  $n=2$ ). \* indicates the degree of significance, two-way Anova tests.

**Figure S2.** mRNA expression level of junctional proteins in CFTR-CTL and CFTR-KD epithelia

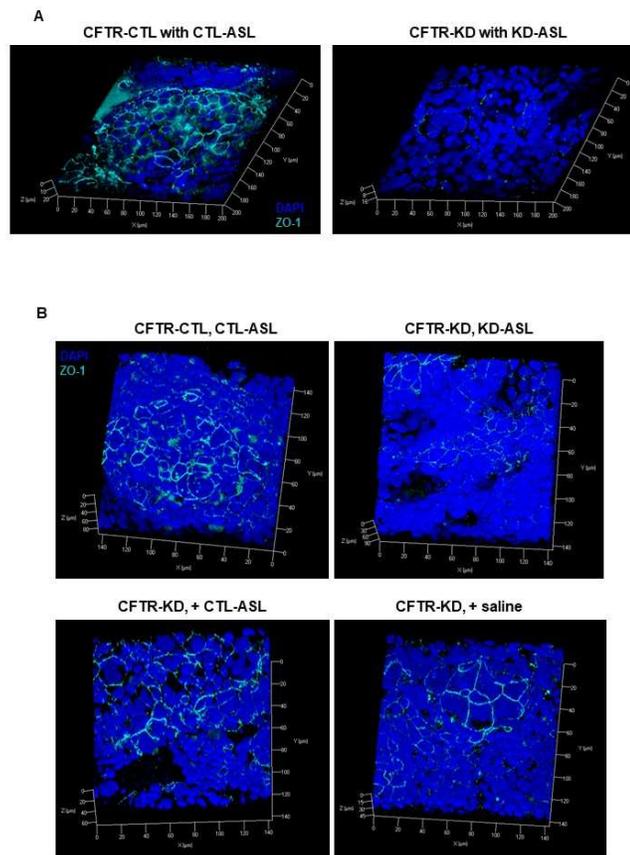


**Figure S2.** Quantification of claudin-3 ( $N \geq 5$ ,  $n=3$ ), occludin ( $N \geq 4$ ,  $n=3$ ), ZO-1 ( $N \geq 7$ ,  $n=3$ ), Ecadherin ( $N=4$ ,  $n=3$ ) and  $\beta$ -catenin ( $N=5$ ,  $n=3$ ) mRNA level expression in CFTR-CTL (dark gray) and CFTR-KD (light gray) epithelia relative to 18S control RNA level. No difference was detected between CFTR-CTL and CFTR-KD filters, paired Student tests.

**Figure S3.** Expression of junctional proteins in CFTR-CTL and CFTR-KD monolayers.

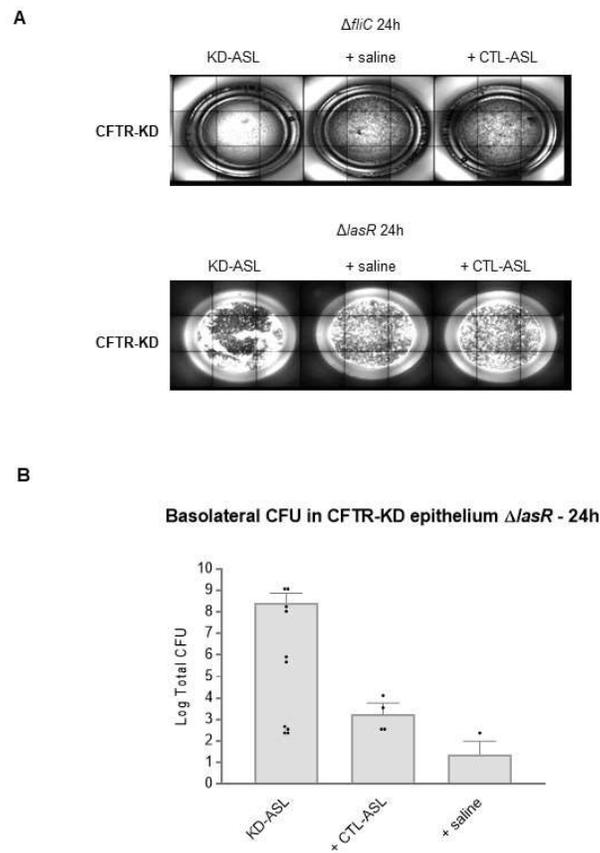
**Figure S3.** Representative western blots for tight junction (A) and adherens junction (B) proteins. The relative expression level is indicated below each lane. (C) Quantification of claudin-3 ( $N=5$ ,  $n=1$ ), claudin-2 ( $N=5$ ,  $n=1$ ), occludin ( $N=5$ ,  $n=1$ ), E-cadherin ( $N=4$ ,  $n=1$ ),  $\beta$ -catenin ( $N=4$ ,  $n=1$ ) and  $\alpha$ -catenin ( $N=3$ ,  $n=1$ ) expression in CFTR-KD epithelia relative to their expression in CFTR-CTL cells (dotted line). No difference was detected between CFTR-CTL and CFTR-KD monolayers, paired Student tests.

**Figure S4.** Restoration of ZO-1 expression and localization in apically rehydrated CFTR-KD-epithelium



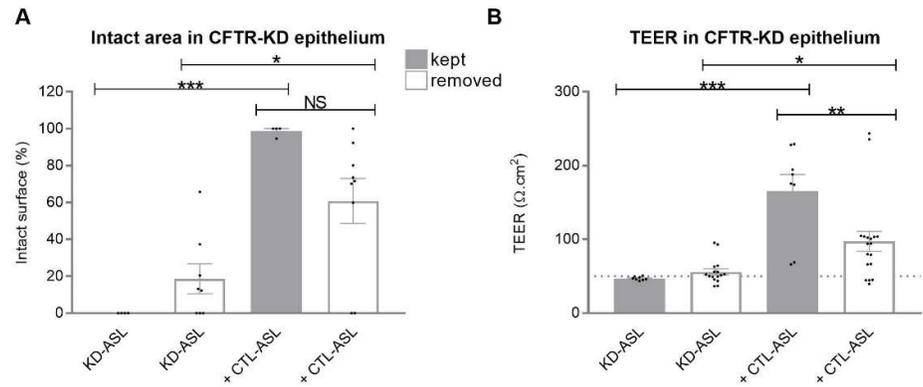
**Figure S4.** ZO-1 expression and localization were evaluated by immunofluorescence. (A) Representative images from z-stacks confocal acquisitions of immunofluorescence for ZO-1 detection on CFTR-CTL (left panel) and CFTR-KD (right panel) Transwell inserts ( $N=8$ ). DAPI: dark blue staining; ZO-1: light blue staining. (B) Representative images from z-stacks confocal acquisitions of CFTR-CTL and CFTR-KD epithelial immunostained for ZO-1. Apical transfer of CTL-ASL or saline for 24-48h restored the cell-cell expression of ZO-1 in CFTR-KD cultures ( $N=3$ ). DAPI: dark blue staining; ZO-1: light blue staining.

**Figure S5.** Protection of CFTR-KD epithelium to  $\Delta fliC$  and  $\Delta lasR$  strains by apical rehydration

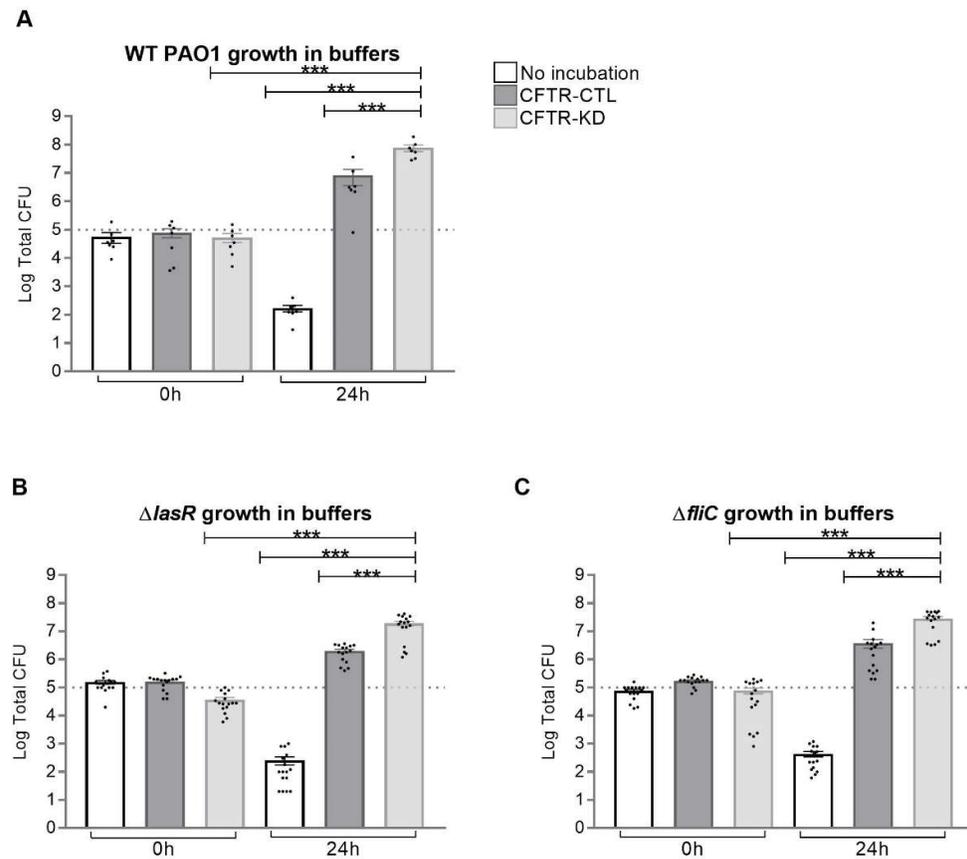


**Figure S5.** (A) Representative images from CFTR-KD epithelium infected with  $\Delta fliC$  and  $\Delta lasR$  for 24h. (B) Basolateral CFU ( $N=6$ ,  $n \geq 1$ ) determined after infection with  $\Delta lasR$  for 24h before and after ASL rehydration with CTL-ASL or saline. Points with a value of zero do not appear in logarithmic scale.

**Figure S6.** Protection of CFTR-KD epithelium to  $\Delta$ *fliC* strain by previous apical rehydration

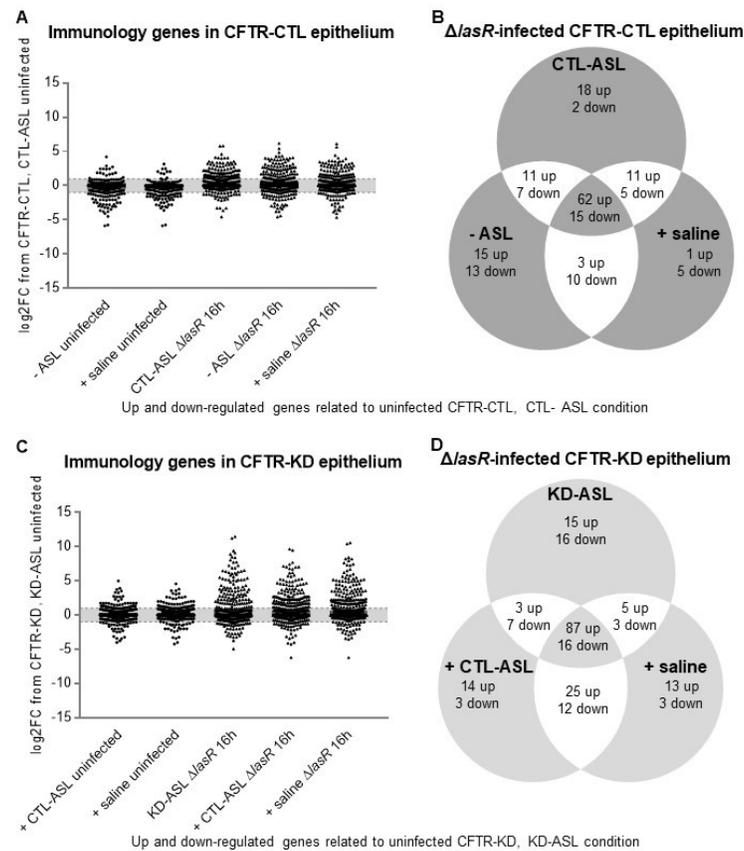


**Figure S6.** The KD-ASL were rehydrated with CTL-ASL for 48h, which were kept (filled bars) or removed (open bars) from the apical surface before infection with  $10^3$  CFU of  $\Delta$ *fliC* for 24h. The intact epithelial surface (A) and TEER values were determined. (A) The intact epithelial surface was expressed as % of the initial area measured in uninfected conditions (kept:  $N=4$ ,  $n=1$ ; removed:  $N=5$ ,  $n \geq 1$ ). (B) TEER values ( $\Omega \cdot \text{cm}^2$ ) were measured in duplicate for each insert. The dotted line indicates the average TEER values of empty Transwell filters (kept:  $N=4$ ,  $n=2$ ; removed:  $N=5$ ,  $n \geq 2$ ). \*, \*\*, \*\*\* indicate the degree of significance, two-way Anova tests.

**Figure S7.** Contribution of the secretome to PAO1 growth

**Figure S7.** ASLs were removed from CFTR-CTL and CFTR-KD cultures and replaced with 95 $\mu$ L of physiological saline for 4h. The conditioned salines with secretomes from CFTR-CTL (dark gray) and CFTR-KD (light gray) cultures were collected and infected with 10<sup>5</sup> CFU of WT PAO1 ( $N=7$ ,  $n \geq 2$ ) (A),  $\Delta lasR$  ( $N=4$ ,  $n=4$ ) (B) or  $\Delta fliC$  ( $N=4$ ,  $n=4$ ) (C) for 24h. CFU were counted in conditioned and in control saline at time 0 and 24h after infection. The dotted lines in A, B and C represent the initial amount of inoculated bacteria. \*\*\* indicate the degree of significance, two-way Anova tests.

**Figure S8.** Immune gene profile of CFTR-CTL and CFTR-KD epithelia subjected to apical liquid manipulations



**Figure S8.** (A) Dot plots showing distribution of immune gene expression in CFTR-CTL cultures after removal of the CTL-ASL, replacement of CTL-ASL with physiological saline, infection for 16h with  $10^3$  CFU of  $\Delta$ lasR, infection with  $\Delta$ lasR in the absence of CTL-ASL or infection with  $\Delta$ lasR while CTL-ASL was replaced with physiological saline. (B) Venn diagrams showing distribution of uniquely and commonly upregulated (blank) and downregulated (dark gray) gene counts in  $\Delta$ lasR-infected CFTR-CTL cultures subjected to ASL manipulation. (C) Dot plots showing distribution of immune gene expression in CFTR-KD cultures after addition of the CTL-ASL, addition of physiological saline, infection for 16h with  $10^3$  CFU of  $\Delta$ lasR, infection with  $\Delta$ lasR while CTL-ASL was added to the KD-ASL or infection with  $\Delta$ lasR while saline was added to the KD-ASL. (D) Venn diagrams showing distribution of uniquely and commonly upregulated (blank) and downregulated (light gray) gene counts in  $\Delta$ lasR-infected CFTR-KD cultures subjected to ASL manipulation. Gene expression was normalized to the initial uninfected CFTR-CTL (A, B) or CFTR-KD (C, D) conditions. A two-fold change was considered significant.

**Table S1:** List of products

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Calu-3 cell line	ATCC	HTB-55™
<b>Antibodies</b>		
Claudin-3	Abcam	ab15102
Occludin	Invitrogen	71-1500
Claudin-2	Abcam	ab53032
GAPDH	Merck	MAB374
$\beta$ -actin	Sigma	A1978
$\alpha$ 1-catenin	Abcam	ab51032
ZO-1	Invitrogen	61-7300
$\beta$ -catenin	Cell Signaling	8480S
E-cadherin	Cell Signaling	3195S
Goat anti-Rabbit HRP	Sigma	Cat# A8275, RRID:AB_258382
Goat anti-Mouse HRP	Sigma	Cat# A5278, RRID:AB_258232
Alexa Fluor 647 goat anti-rabbit (H+L)	ThermoFisher	Cat# A-21245, RRID:AB_2535813
<b>Other reagents</b>		
MEM <i>GlutaMAX</i>	Gibco®	41090-28
Fetal Bovine Serum (FBS)	Gibco®	10270-106
<i>Non-essential amino acids 100X</i>	Gibco®	11140-035
<i>HEPES 1M</i>	Gibco®	15630-056
<i>Sodium pyruvate 100X</i>	Gibco®	11360-039
Penicillin/Streptomycin/Fungizone®	BioConcept	4-02F00-H
Nonidet-P40	AppliChem	A1694
complete™ <i>Protease Inhibitor</i> Cocktail	Roche	04693124001
Pierce BCA protein assay kit	ThermoFisher	23228
SDS-PAGE	Bio-Rad	161-0301
Porablot NCP nitrocellulose membrane	Macherey-Nagel	741280
BSA	Sigma	A7906
PBS	Gibco®	14190-094
Tween	PanReac Applichem	A4974
HRP substrate Immobilon™ Western	Millipore	WBKLS0500
Trypsin-EDTA 10X	Gibco®	15400-054
Paraformaldehyde (PFA)	Sigma	158127
Triton 100X	Sigma	T8787
DAPI	AppliChem	A4099
<i>Amiloride</i>	Millipore	129876
<i>Forskolin</i>	Sigma	F6886
<i>IBMX</i>	Sigma	I5879
GlyH-101	Merck	219671
Bumetanide	Sigma	B3023
MgATP	Sigma	A9187
RNeasy kit	Qiagen	74106
nCounter inflammation panel Human v2	NanoString®	XT_PGX_HuV2_Inflammation_CS0
QuantiTect Reverse Transcription Kit	Qiagen	205311
PowerUp™ SYBR™ Green Master Mix	Appliedbiosystems	A2574

**Table S2:** List of primer pairs used for qPCR

Gene	Forward	Reverse
Claudin-3	CTGCTCTGCTGCTCGTGTCC	TTAGACGTAGTCCTTGCGGTCGTAG
Occludin	CACACAGGACGTGCCTTCAC	GAGTATGCCATGGGACTGTCAA
ZO-1	CAGCCGGTCACGATCTCCT	TCCGGAGACTGCCATTGC
E-cadherin	GGTTATTCCTCCCATCAGCT	CTTGGCTGAGGATGGTGTA
$\beta$ -catenin	TCGCCAGGATGATCCCAGC	GCCCATCCATGAGGTCCTG
18S	GTAACCCGTTGAACCCATT	CCATCCAATCGGTAGTAGCG