Online Data Supplement

Inhibition of pendrin by a small molecule reduces lipopolysaccharide-induced acute lung injury

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Supplemental Materials

Materials & Methods

All animal experiments were conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Transgenic Experimental animals

Pendrin knock-out (KO) mice used in the experiment were provided by JY Choi of Yonsei University (Supplementary Table S1). Transgenic NF-κB reporter/SPC-Cre-ER<sup>T2</sup> mice were used for IVIS in this study. Briefly, NF-κB reporter mice contain a ROSA26 lox-STOP-lox-cassette inserted between a promoter and the NF-κB-luciferase-dTomato gene (Supplementary Figure S1). Normally, the stop gene is located between loxP and loxP, and NF-κB-luciferase-dTomato is not expressed. The surfactant protein C (SPC)-Cre-ER<sup>T2</sup> mice were bred with ROSA26R mice to obtain NF-κB reporter/SPC-Cre-ER<sup>T2</sup> mice. Cre-ER<sup>T2</sup> recombinase activity in these transgenic mice was induced by tamoxifen [1,2]. These NF-κB reporter/SPC-Cre-ER<sup>T2</sup> mice express NF-κB activity in the alveolar epithelium either through dTomato fluorescence or luciferase in the presence of tamoxifen. Increased fluorescence in IVIS image means increased NF-κB activity in the alveolar epithelium. Tamoxifen (Sigma, USA) was dissolved in a 10:1 sunflower seed oil/ethanol mixture (10 mg/mL). Each 4-week-old mouse was injected intraperitoneally with 100 mL of tamoxifen/day for 5 consecutive days. One week after the last injection, mice were used for IVIS or intravital imaging. Transgenic NF-κB reporter and SPC-Cre-ER<sup>T2</sup> mice were provided by KT Nam and BC Cho of Yonsei University (Table S1).
Isolation of bronchoalveolar lavage

All mice were euthanized by a lethal overdose of ketamine and xylazine. BALF was obtained by tracheal cannula using 1 mL sterile saline. The BALF was centrifuged (4°C, 3000 rpm, 10 min) and the supernatant was stored at −80°C for further analysis. The cell pellet was reconstituted in 100 μL PBS and used for cell counts and cytospin samples. Total cell numbers in each sample were determined using a hemocytometer (Marienfield) according to the manufacturer’s protocol. A 90 μL aliquot of each sample was transferred into the slide chambers, which were then inserted into a cytospin with the slide facing outward. Slides were centrifuged at 800 rpm for 5 min, then removed from the cytocentrifuge and dried prior to staining. Cytospins were prepared with a cytocentrifuge (Shandon Cytospin 4 cytocentrifuge, Thermo Scientific, Waltham, MA, USA) and stained with Diff-Quik Stain Set (Dade Behring, Newark, DE, USA) to assess inflammation. The protein concentrations of the BAL supernatant were determined using a BCA assay (Thermo Fisher Scientific). Two microliters of each sample and 198 μL of working reagent were pipetted into a microplate well and mixed thoroughly on a plate shaker for 30 s. After incubation for 30 min at 37°C, the plate was cooled and the absorbance read at 562 nm in a spectrophotometer.

Lung tissue harvest and histological examination

The right lung was isolated and stored at −80°C prior to protein extraction after flushing the pulmonary vasculature with saline under low pressure. The left lung was inflated via a tracheotomy with low-melting point agarose (4%) in PBS at 25 cm H₂O pressure until the pleural margins sharpened. The lungs were then excised and fixed overnight in 10% formaldehyde in PBS and embedded in paraffin for 5 μm sectioning. Left lung sections were stained with H & E and subjectively evaluated by light microscopy. Histopathology was reviewed in a blinded manner by two qualified investigators (EH Lee and MS Park). Five easily identifiable pathological processes were scored using the weighted scale presented in the official ATS workshop report [3]. Lung sections
were processed for immunohistochemistry using anti-rabbit SLC26A4 (ab98091, abcam) antibody.

Measurement of Cl⁻/SCN⁻ exchange

The human alveolar epithelial cells (hAEC) purchased from Science Cell (Catalog #3200), which consisted of alveolar type I and type II epithelial cells, lined more than 99% of the internal surface area of the lung. The hAEC from Science Cell research Laboratories were isolated from human lung tissues, cryopreserved at P0, and delivered frozen. The hAEC transient transfected human pendrin (PDS) and YFP-F46L/H148Q/I152L were plated in 96-well plate at a density of $2 \times 10^4$ cells per well and incubated for 48 h. Each well of the 96-well plate was washed two times with 200 µL of PBS, and it was filled with 100 µL of PBS. To measure the effect of YS-01 on hPDS-mediated Cl⁻/SCN⁻ exchange activity, cells were pre-treated with YS-01. After 10 min of incubation at 37°C, the 96-well plate was placed on the stage of an inverted fluorescence microscope (Nikon, Tokyo, Japan) equipped with a cooled charge-coupled device camera (Zyla sCMOS), image acquisition and analysis software (Meta Imaging Series 7.7). Each well was assayed individually for hPDS-mediated SCN⁻ influx by recording YFP fluorescence continuously (2 s per point) for 4 s (baseline). Then, 100 µL of 140 mM SCN⁻ solution was added at 4 s and then YFP fluorescence was recorded for 14 s. To investigate the effect of long-term treatment of YS-01 in LPS exposed hAEC, we treated the hAEC with LPS (10 µg/ml or 20 µg/ml) for 24 hours with or without YS-01 (20 µM/ml).

Transepithelial SCN⁻ transport

Human nasal epithelial (HNE) cells were plated on transwell permeable supports and cultured at the air-liquid interface for 14 days. Complete differentiated HNE cells were incubated with LPS (10 µg/ml) or DMSO (control) for 24 hours with or without YS-01 (20 µM/ml). The basolateral side was treated with 1 mL of PBS containing 10 mM glucose and 5 µCi of $^{35}$CN (total concentration of SCN⁻:
86 μM). The apical side of fluid was collected and placed in scintillation vials for the evaluation of radioactivity.

Real-time RT-PCR analysis

Total messenger RNA (mRNA) was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed using random hexamer primers, an oligo (dT) primer, and SuperScript® III Reverse Transcriptase (Invitrogen). Quantitative real-time PCRs were performed using the StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and Thunderbird SYBR qPCR mix (Toyobo, Osaka, Japan). The thermal cycling conditions included an initial step of 95°C for 5 min followed by 40 cycles of 95°C for 10 s, 55°C for 20 s, and 72°C for 10 s in a 96-well reaction plate. The primer sequences are described in Table E3.

ELISA

Macrophage inflammatory protein (MIP-2), interleukin-1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α) levels in lung lysates were measured using ELISA kits (Millipore) according to the manufacturer’s directions. Human BALF was centrifuged (10 min; 1500 g) and the supernatant was cryopreserved at −80°C until use. Pendrin levels of human BALF were measured using human SLC26A4 ELISA kits (MBS764789, Mybiosource) according to the manufacturer’s directions.

Western blotting

Lung tissues were harvested and lysed in homogenization buffer (PRO-PREP™ Extraction solution, iNtRON Biotechnology). The samples were centrifuged at 13000 g for 30 min at 4°C. Supernatant
protein concentration was determined by BCA assay (Thermo Fisher Scientific). Equal amounts of 
protein were separated by SDS/PAGE and transferred to a nitrocellulose membrane. Membranes were 
blocked with 5% skim milk in TBS-T (TBS (170-6435, Bio-Rad Laboratories) and 1% Tween-20 
(170-6531, Bio-Rad Laboratories) for 1 h at room temperature. Membranes were then incubated 
overnight with primary antibody diluted in 5% skim milk in TBS-T at 4°C. After washing with TBS-
T, the blots were incubated with horseradish peroxidase-conjugated secondary antibodies and 5% 
skim milk in TBS-T for 1 h at room temperature, then developed using a Super- Signal West Pico 
chemiluminescence detection kit (Pierce). The antibodies used in the present study included rabbit 
SLC26A4 (ab98091, abcam), mouse phospho-IκB (9246, Cell Signaling Technology), mouse IκB 
(4814, Cell Signaling Technology), and rabbit α-tubulin (PA5-16891, Cell Signaling Technology). 
Western blot quantification was conducted using ImageJ (Image Processing and Analysis in Java; 
NIH, USA) software.

IVIS (in vivo optical imaging)

Imaging of live animals and organs was performed using an IVIS kinetic imaging system (Caliper 
Life Sciences, Preston Brook Runcorn, UK). The IVIS system consisted of a cooled charge-coupled 
device camera mounted onto a light-tight specimen chamber. The fluorescent excitation light was 
provided by a halogen lamp in combination with appropriate excitation filters. Emission filters were 
placed in front of the camera aperture to allow recording of specific wavelengths of light, depending 
on the emission spectra of the FP examined. Fluorescence imaging was obtained with an excitation 
wavelength of 554 nm and emission wavelength of 581 nm (dTomato). Mice were divided into three 
groups for IVIS imaging, (DMSO + PBS, DMSO + LPS, and YS-01 (10 mg/kg, i.p) + LPS), and ex 
vivo lungs were aseptically removed 6 h after LPS treatment. When organs were imaged, they were 
placed as flat as possible to allow full and consistent light penetration in order to minimize potential 
variation in the measurements due to differing tissue thicknesses. Fluorescence was quantified using
the region of interest tool in Living Image software (version 3.2, Caliper Life Sciences).
Figure S1. NF-κB reporter mouse
### SUPPLEMENTARY TABLE LEGENDS

**Table S1. Mouse genotyping PCR Primers**

| Mouse genotyping primers            | Sequence                                      |
|-------------------------------------|-----------------------------------------------|
| **Pendrin KO mouse**                |                                               |
| pPNTloxp (22)                       | GGG TGC GGA GAA AGA GGT AAT G                 |
| exon 8 (25)                         | GCA TTG TAG TTC TTT TCC AAG TTG G             |
| exon 7 (18)                         | TGC CGA TTT CAT CGC TGG                       |
| **NF-κB reporter mouse**            |                                               |
| ROSA                                |                                               |
| ROSA I (forward-common)             | AAA GTC GCT CTG AGT TGT TAT                   |
| ROSA-2 (reverse-WT)                 | GGA GCG GGA GAA ATG GAT ATG                   |
| ROSA (reverse-mut)                  | GGC GGG CCA TTT ACC GTA AG                    |
| Insert                              |                                               |
| NFxB_For                            | GAT CCC CAT CAA GCT GAT CCG G                 |
| NFxB_Rev                            | GCT GCG AAA TGC CCA TAC TG                    |
| **SPC-CreER**                       |                                               |
| Forward                             | GTC GAT GCA ACG AGT GAT GA                    |
| Reverse                             | TCA GCT ACA CCA GAG AC                       |
| Final diagnosis     | Age | Sex | BMI  | Pneumonia/ Sepsis | ARDS | P/F ratio | Cause of Pneumonia | Time from intubation to BAL (days) | Hospital stay (days) | Mortalities | BALF analysis |
|---------------------|-----|-----|------|-------------------|------|-----------|-------------------|-----------------------------------|---------------------|-------------|---------------|
|                     |     |     |      |                   |      |           |                   |                                   |                     |             |               |
| 1 Benign nodule     | 67  | M   | 23.3 | 0                 | 0    | 0         |                   | 3                                 | 0                   | 0           |               |
| 2 Ewing sarcoma     | 52  | M   | 21.1 | 0                 | 0    | 0         |                   | 2                                 | 0                   | 0           |               |
| 3 Benign nodule     | 69  | M   | 25.1 | 0                 | 0    | 0         |                   | 3                                 | 0                   | 0           |               |
| 4 Lung cancer       | 74  | M   | 18.0 | 0                 | 0    | 0         |                   | 3                                 | 0                   | 0           |               |
| 5 Benign nodule     | 79  | M   | 26.8 | 0                 | 0    | 0         |                   | 2                                 | 0                   | 0           |               |
| 6 Lung cancer       | 73  | M   | 35.4 | 0                 | 0    | 0         |                   | 2                                 | 0                   | 0           |               |
| 7 Benign nodule     | 44  | F   | 19.1 | 0                 | 0    | 0         |                   | 2                                 | 0                   | 0           |               |
| 8 Benign nodule     | 67  | M   | 23.1 | 0                 | 0    | 0         |                   | 2                                 | 0                   | 0           |               |
| 9 Benign nodule     | 61  | M   | 21.8 | 0                 | 0    | 0         |                   | 2                                 | 0                   | 0           |               |
| 10 Lung cancer      | 62  | M   | 20.4 | 0                 | 0    | 0         |                   | 2                                 | 0                   | 0           |               |
| 11 Mild bronchitis  | 64  | M   | 26.1 | 0                 | 0    | 0         |                   | 1                                 | 0                   | 0           |               |
| 12 Benign nodule    | 73  | M   | 24.9 | 0                 | 0    | 0         |                   | 2                                 | 0                   | 0           |               |
| 13 Benign nodule    | 81  | M   | 24.2 | 0                 | 0    | 0         |                   | 2                                 | 0                   | 0           |               |
| 14 Lung cancer      | 51  | F   | 19.5 | 0                 | 0    | 0         |                   | 2                                 | 0                   | 0           |               |
| 15 Focal inflammation | 58  | F   | 22.4 | 0                 | 0    | 0         |                   | 3                                 | 0                   | 0           |               |
| 16 Lung cancer      | 55  | M   | 24.9 | 0                 | 0    | 0         |                   | 2                                 | 0                   | 0           |               |
| 17 Lung cancer      | 74  | F   | 26.8 | 0                 | 0    | 0         |                   | 19                                | 0                   | 0           |               |
| 18 Benign nodule    | 60  | M   | 25.3 | 0                 | 0    | 0         |                   | 2                                 | 0                   | 0           |               |
| 19 Benign nodule    | 55  | M   | 28.7 | 0                 | 0    | 0         |                   | 3                                 | 0                   | 0           |               |
| 20 Lung cancer      | 72  | F   | 27.6 | 0                 | 0    | 0         |                   | 2                                 | 0                   | 0           |               |
| 21 Benign nodule    | 57  | M   | 35.1 | 0                 | 0    | 0         |                   | 4                                 | 0                   | 0           |               |
| No. | Diagnosis                | Age | Gender | Vital Signs | Others | Microbiology                                      | Imaging | Pathology | Other Comments |
|-----|--------------------------|-----|--------|-------------|--------|--------------------------------------------------|---------|-----------|----------------|
| 22  | Benign nodule 58 M       | 21.1| M      | 0 0         |        |                                                  |         |           |                |
| 23  | Benign nodule 55 M       | 23.9| M      | 0 0         |        |                                                  |         |           |                |
| 24  | Benign nodule 58 M       | 27.3| M      | 0 0         |        |                                                  |         |           |                |
| 25  | Lung cancer 77 M         | 20.6| M      | 0 0         |        |                                                  |         |           |                |
| 26  | Pneumonia 65 M           | 26.1| M      | 1 1 133     |        | Pseudomonas aeruginosa                            | 11 29   | 1 0      | 190 35 35 20  |
| 27  | Pneumonia, AGC 76 M      | 23.8| M      | 1 1 111     |        | Pseudomonas aeruginosa                            | 8 94 0 0 | 0 0      | 1500 78 7 13  |
| 28  | Pneumonia, pancreatic cancer 63 M | 23.5| M      | 1 1 100     |        | Pseudomonas aeruginosa                            | 3 39 1 0 | 0 0      | 1400 86 5 8  |
| 29  | Aspiration pneumonia 60 M | 16.9| M      | 1 1 146     |        | Acinetobacter baumannii                           | 6 125 1 0 | 0 0      | 175 30 51 19  |
| 30  | Pneumonia 69 F           | 22.8| F      | 1 1 136     |        | Pseudomonas aeruginosa                            | 1 44 1 0 | 0 0      | 550 89 11 0  |
| 31  | Pneumonia, AML 50 M      | 22.2| M      | 1 1 240     |        |                                                  | 1 31 1 0 | 0 0      | 97 90 6 4  |
| 32  | Pneumonia, thyroid cancer 76 F | 28.1| F      | 1 1 150     |        | Acinetobacter baumannii                           | 2 34 1 0 | 0 0      | 284 95 1 2  |
| 33  | Pneumonia 67 M           | 17.9| M      | 1 1 130     |        | Pseudomonas aeruginosa                            | 4 169 0 0 | 0 0      | 283 92 8 0  |
| 34  | Pneumonia 59 M           | 20.0| M      | 1 1 160     |        | Acinetobacter baumannii                           | 9 39 1 0 | 0 0      | 100 90 6 4  |
| 35  | Pneumonia 75 M           | 18.7| M      | 1 1 140     |        | Acinetobacter baumannii                           | 9 164 0 0 | 0 0      | 6800 100 0 0  |
| 36  | Pneumonia 51 M           | 23.0| M      | 1 1 166     |        | Pseudomonas aeruginosa                            | 2 29 0 0 | 0 0      | 813 77 21 2  |
| 37  | Pneumonia 72 M           | 24.3| M      | 1 1 132     |        | Enterobacter cloacae                              | 4 26 1 1 | 1 0      | 190 63 5 32  |
|   | Diagnosis                        | Age | Gender | Temperature | WBC | Characteristic | Pathogen               | Count | CRP | ESR | M | LCR | Count | Platelet | CRP | ESR | M | LCR | Count |
|---|----------------------------------|-----|--------|-------------|-----|---------------|-------------------------|-------|-----|-----|---|-----|-------|----------|-----|-----|---|-----|-------|
|38 | Pneumonia                        | 73  | M      | 19.2        | 1   | 1             | Acinetobacter baumannii | 3     | 40  | 1   | 0 | 210 | 76    | 13       | 10  |
|39 | Pneumonia                        | 69  | M      | 18.9        | 1   | 1             | Acinetobacter baumannii | 15    | 48  | 1   | 0 | 2400| 69    | 25       | 6   |
|40 | Pneumonia                        | 74  | M      | 19.7        | 1   | 1             | Acinetobacter baumannii | 6     | 90  | 0   | 0 | 650 | 44    | 3        | 53  |
|41 | Pneumonia                        | 61  | M      | 15.2        | 1   | 1             | Pseudomonas aeruginosa   | 6     | 83  | 0   | 0 | 337 | 94    | 1        | 3   |
|42 | Pneumonia                        | 86  | M      | 27.0        | 1   | 1             | Klebsiella pneumoniae   | 2     | 28  | 0   | 0 | 740 | 78    | 5        | 17  |
|43 | Pneumonia, Lymphoma              | 78  | M      | 25.4        | 1   | 1             | MRSA                    | 1     | 37  | 1   | 0 | 1400| 97    | 7        | 1   |
|44 | Pneumonia                        | 84  | F      | 27.3        | 1   | 1             | Acinetobacter baumannii | 6     | 32  | 1   | 0 | 285 | 77    | 17       | 6   |
|45 | Pneumonia                        | 65  | M      | 22.3        | 1   | 1             | Klebsiella pneumoniae   | 28    | 73  | 0   | 0 | 358 | 90    | 7        | 3   |
|46 | Aspiration pneumonia              | 60  | M      | 25.3        | 1   | 1             | MRSA                    | 2     | 46  | 0   | 0 | 300 | 58    | 39       | 3   |
|47 | Pneumonia, Esophageal cancer      | 67  | F      | 24.1        | 1   | 1             | Acinetobacter baumannii | 3     | 31  | 1   | 0 | 660 | 23    | 6        | 32  |
|48 | Necrotizing pneumonia, Sigmoid colon cancer | 69  | M      | 23.3        | 1   | 1             | Klebsiella pneumoniae   | 1     | 2   | 1   | 1 | 850 | 98    | 0        | 3   |
|49 | Pneumonia, Multiple myeloma       | 75  | M      | 25.7        | 1   | 1             | VRE                     | 1     | 40  | 1   | 0 | 158 | 87    | 1        | 12  |
|   | Pneumonia,   | 62 | M  | 23.1 | 1  | 1  | 250 | Klebsiella pneumoniae | 2  | 62 | 1  | 0  | 208 | 0  | 28 | 72 |
|---|--------------|----|----|------|----|----|-----|-----------------------|----|----|----|----|-----|----|----|----|
| 51| Pneumonia,   | 76 | F  | 22.0 | 1  | 1  | 112 | Acinetobacter baumannii | 6  | 15 | 1  | 1  | 850 | 94 | 1  | 5  |
| 52| Pneumonia, AML| 37 | M  | 21.0 | 1  | 1  | 160 | Pseudomonas aeruginosa | 0  | 12 | 1  | 1  | 300 | 43 | 21 | 27 |
| 53| Pneumonia    | 77 | M  | 24.9 | 1  | 1  | 150 | Acinetobacter baumannii | 1  | 31 | 1  | 0  | 2100 | 75 | 8  | 17 |
| 54| Pneumonia    | 80 | M  | 20.2 | 1  | 1  | 240 | —                      | 0  | 8  | 1  | 1  | 26  | 60 | 15 | 10 |
| 55| Pneumonia    | 72 | M  | 24.2 | 1  | 1  | 106 | Pseudomonas aeruginosa | 0  | 49 | 1  | 0  | 700 | 23 | 45 | 23 |
| 56| Pneumonia    | 76 | F  | 27.0 | 1  | 1  | 100 | Pseudomonas aeruginosa | 0  | 18 | 1  | 1  | 110 | 41 | 29 | 27 |
| 57| Pneumonia    | 59 | F  | 18.1 | 1  | 1  | 220 | Enterobacter cloacae | 0  | 36 | 1  | 0  | 1991 | 95 | 2  | 3  |
| 58| Pneumonia    | 62 | M  | 24.4 | 1  | 1  | 93  | Klebsiella pneumoniae | 0  | 61 | 0  | 0  | 290 | 76 | 7  | 15 |
| 59| Pneumonia    | 26 | F  | 19.8 | 1  | 1  | 117 | —                      | 0  | 26 | 1  | 1  | 120 | 21 | 53 | 23 |
| 60| Pneumonia    | 45 | M  | 24.1 | 1  | 1  | 237 | Acinetobacter baumannii | 0  | 13 | 1  | 1  | 142 | 84 | 8  | 8  |
| 61| Pneumonia    | 74 | M  | 24.3 | 1  | 1  | 180 | Pseudomonas aeruginosa | 1  | 9  | 1  | 1  | 550 | 80 | 8  | 12 |
| No. | Diagnosis                        | Sex | Age | Comorbidities | Cause of Death | SIRS | P/F | ARDS | BAL | Septic | PMN | Lympho | Mono | MRSA | VRE | Unatable |
|-----|----------------------------------|-----|-----|---------------|----------------|------|-----|------|-----|--------|-----|--------|------|------|-----|----------|
| 62  | Pneumonia, Diffuse large B-cell lymphoma | F   | 25.9 | 1 1 85        | Acinetobacter baumannii | 0    | 52  | 0    | 0   | 80     | 44  | 47     | 6    |       |     |          |
| 63  | Pneumonia, Lung cancer           | M   | 20.9 | 1 1 190       | Pseudomonas aeruginosa  | 0    | 15  | 1    | 1   | 510    | 56  | 14     | 19   |       |     |          |
| 64  | Pneumonia, Esophageal cancer     | M   | 21.2 | 1 1 242       | —                | 0    | 18  | 0    | 0   | 694    | 40  | 10     | 41   |       |     |          |
| 65  | Aspiration pneumonia             | M   | 22.4 | 1 1 260       | Pseudomonas aeruginosa | 0    | 29  | 0    | 0   | Unatable |     |        |      |       |     |          |
| 66  | Pneumonia                        | M   | 22.4 | 1 1 102       | Acinetobacter baumannii | 13   | 80  | 1    | 0   | Unatable |     |        |      |       |     |          |

0=no, 1=yes, BMI=body mass index, ARDS=acute respiratory distress syndrome, P/F=P_{\text{O}_2}/F_{\text{I}_O_2}, BAL=bronchoalveolar lavage, PMN=polymorphonuclear leukocyte, Lympho=lymphocyte, Mono=monocyte, MRSA=Methicillin-resistant Staphylococcus aureus, VRE=vancomycin-resistant Enterococcus.

Sepsis was identified according to the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference guidelines [4]. Sepsis is defined as SIRS and a known or suspected infection. ARDS was defined according to the Berlin Definition [5]. All ARDS patients were endotracheally intubated and mechanically ventilated using a low tidal volume ventilation strategy according to ARDSNet protocol [6].
Table S3. Primer sequences used for quantitative RT-PCR.

| Gene            | Primer sequence                  | PCR product size |
|-----------------|----------------------------------|------------------|
| Human Pendrin   | 5′-TTC CCA AAG TGC CAA TCC ATA G-3′  
|                 | 5′-CCG CAG TGA TCT CAC TCC AAC-3′   | 83 bp            |
| Human Duox2     | 5′-ACG CAG CTC TGT GTC AAA GGT-3′  
|                 | 5′-TGA TGA ACG AGA CTC GAC AGC-3′   | 90 bp            |
| Human β-actin   | 5′-GCA AAG ACC TGT ACG CCA ACA C-3′ 
|                 | 5′-ATC TCC TTC TGC ATC CTG TC-3′    | 82 bp            |
| Mouse Pendrin   | 5′-CAT CTG CAG AAC CAG GTC AA-3′    
|                 | 5′-GCA TTC ATC TCT GCC TCC AT-3’    | 94 bp            |
| Mouse β-actin   | 5′-TGT TAC CAA CTG GGA CGA CA-3′    
|                 | 5′-GGG GTG TTG AAG GTC TCA AA-3’    | 165 bp           |
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