**Supplemental Digital Content 1**

**Materials and Methods**

**Experimental Protocol**

Protocols were approved by the Subcommittee on Research Animal Care and the Institutional Animal Care and Use Committee of the Massachusetts General Hospital (Boston, Massachusetts), and followed National Institutes of Health and ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

All experiments lasted for approximately 12h, starting in the early morning. After premedication, twelve female sheep (weight 18.2±2.3 kg) were anesthetized, paralyzed, intubated, and mechanically ventilated. Anesthesia was maintained with a continuous infusion of propofol (5 mg·kg-1·hour-1), xylazine (50 µg·kg-1·hour-1) and fentanyl (10 μg·kg-1·hour-1). Paralysis was established with a rocuronium bolus at induction (0.5 mg/kg) and subsequent continuous infusion (0.5 mg·kg-1·hour-1). For monitoring and collection of blood samples, we percutaneously cannulated a femoral artery and introduced a pulmonary artery catheter via the jugular vein using sterile techniques. As a surrogate for pleural pressure, esophageal pressure was measured by an esophageal balloon placed in the lower third of the esophagus. A stepwise lung recruitment maneuver was performed with PEEP steps of 2-3 cmH2O for 30-60s up to a plateau pressure of 30-32 cmH2O. After a period of stabilization, baseline physiological variables, arterial and central venous blood samples were acquired. After a period of stabilization, baseline physiological variables, arterial and central venous blood samples were acquired.

To achieve lung atelectasis, animals were placed in the right lateral recumbent position and lateral thoracotomy was performed in the fifth intercostal space. Left lung isolation was produced with a bronchial blocker, while the right lung was mechanically ventilated for eight hours. Aiming to increase normally aerated lung compartments, tidal volume (VT)=10 ml/kg was used throughout the whole experiment as previous studies1,2. Additional ventilation settings were as follows: volume control mode; positive end-expiratory pressure (PEEP)=2 cmH2O; inspired oxygen fraction (FIO2)=0.3; inspiratory-to-expiratory ratio=1:2; and initial respiratory rate=25 breaths/min and adjusted to maintain the arterial carbon dioxide partial pressure between 32 and 45 mmHg (normocapnia). FIO2 was increased if needed to maintain oxygenation (oxygen saturation > 88%). If normal blood gases were not obtained with FIO2=1, then PEEP was increased in steps of 1 cmH2O. A new set of physiological measurements were collected following stabilization of cardiopulmonary variables (Atelectasis-0h).

Then, animals were divided in two groups: lipopolysaccharide (LPS)-unexposed (n=6, 18.1±2.4 kg) or LPS-exposed (n=6, 18.3±2.4 kg) groups. Each animal was studied in a separate day, and group allocation consisted of alternate sequences of 3 experiments for each group, starting from LPS exposure. In LPS exposure group, an intravenous infusion of LPS (10 ng·kg-1·min-1, Escherichia coli O55:B5; List Biologic Laboratories Inc., USA) was started for 30 minutes and continued (5 ng·kg-1·min-1) for the whole experiment. In order to ensure cardiovascular stability while maintaining continuity of the extrapulmonary inflammatory stimulus, an algorithm was followed to manage hypotension with lactated Ringer's solution and reduction in endotoxin infusion (Figure S13)3. Respiratory mechanics and hemodynamic parameters were continuously monitored. At the end of the study, physiologic data were collected, and animals were euthanized under deep general anesthesia (Atelectasis-8h).

**Image acquisition**

High resolution computed tomography and positron emission tomography imaging were performed for assessment of regional aeration, strain, and inflammation. After six hours of one-lung collapse and mechanical ventilation, computed tomography images (tube current of 7 mA and voltage of 140 kVp) were acquired during end-inspiratory and end-expiratory breath holds for lung aeration and strain analysis. An additional mean lung volume computed tomography image for positron emission tomography attenuation correction and delineation of regions-of-interest was acquired during tidal breathing (2 min). Reconstructed computed tomography images consisted of a matrix of 512 × 512 × 82 voxels of 0.49 × 0.49 × 2.5 mm3 each4.

The acquisition protocol for dynamic positron emission tomography imaging of 18F-FDG has been previously described3,5. Briefly, starting 30 s before a fast injection (30 s) of 18F-FDG (~2 mCi) in the central venous port of a Swan-Ganz catheter, sequential positron emission tomography frames were acquired for a total of 90 min. Blood samples (1 ml) were collected from the pulmonary artery at 3.0, 5.5, 7.5, 9.5, 25, 37, and 42.5 min and centrifuged. Plasma tracer concentration was measured in a well counter cross calibrated with the positron emission tomography camera and corrected for radioactivity decay from time of injection for posterior calibration of the image derived input function6.

**Image analysis**

Computed Tomography: Lung aeration and strain analyses have been described in detail previously4.

*Selection of Regions of Interest for Analysis*

Two regions of interest were studied: the atelectatic left lung and a homogeneously aerated region of the ventilated lung. The delineation of both regions was manually performed by one of two investigators with knowledge of the sheep lung anatomy using a computed tomography image acquired at mean lung volume just before the positron emission tomography acquisition. In the ventilated lung, the homogeneously aerated region was defined as contiguous voxels with gas fraction ≥0.4 in the upper two thirds of the lung (approximately the right upper or middle lobes).

*Aeration*

Briefly, lung aeration was quantified as voxel gas fraction (Fgas) = voxel Hounsfield units/-1000 with air =-1000 Hounsfield units and tissue =0 Hounsfield units. Aeration is reported as the mean voxel aeration within the mask defining the aerated or atelectatic regions in each animal.

*Strain*

Strain analysis was performed at the voxel level using image registration to calculate the Jacobian of the transformation that mapped each end-inspiratory image to the corresponding end-expiratory image7–10. Before registration, images were rescaled to convert Hounsfield units inside the parenchyma from their maximum (=tissue density) to zero and from their minimum (=air) to one. Each dimension was then cropped to the limits of the end-inspiratory mask and padded with a margin of 50 voxels at each side. Registration was implemented with diffeomorphic transforms and B-Spline regularization in a multistage approach (increasing image resolution and decreasing B-Spline knots distance), using Advanced Normalization Tools 2.1.0 (ANTs)11. The cost function used cross-correlation inside a radius of four voxels and the B-spline knots were initialized with 26 mm, and halved in each of the subsequent three steps. The registration framework used was previously validated using landmarks4. The volumetric strain of the aerated and atelectatic regions were computed as the median volumetric strain of the voxels within the corresponding regional mask.

Positron Emission Tomography:

Lung 18F-FDG kinetics was analyzed with a four-compartment model12. 18F-FDG is transported into cells by the same mechanism as glucose. Once in the cell, 18F-FDG is phosphorylated by hexokinase to 18F-FDG-6-phosphate, which stays trapped in the cell. During acute lung injury, neutrophils are highly activated and the pulmonary 18F-FDG signal is predominantly determined by their number and state of activation13. Therefore, the 18F-FDG uptake rate (Ki) has been used as a measure of pulmonary regional neutrophilic inflammation3,5. Ki is expressed as Ki=Fe × k3, where Fe is the fractional distribution volume of 18F-FDG available for phosphorylation and k3 the phosphorylation rate. The utilized 4-compartment model includes an additional compartment to the classic Sokoloff model of vascular, tissue and metabolized 18F-FDG pools12,14. This additional compartment describes the volume of distribution of 18F-FDG not immediately available for phosphorylation (Fee), interpreted as indicative of lung edema15. Additionally, the model fitting provides an estimation of the fractional blood volume within a region-of-interest. With this, values of Ki, Fe and Fee were normalized for lung tissue by dividing those variables by (1-gas fraction-fractional blood volume) to yield Kis, Fes and Fees.

**Purification of total RNA**

Immediately following collection, lung tissues were immersed in RNA*later*TM solution (Invitrogen) and stored at -80°C. Total RNA from lung tissues was extracted using PAXgene tissue RNA kit (Qiagen, Germany) according to the manufacturer's instruction. Concentration and purity was determined with Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and integrity of RNA was assessed using the RNA 6000 Nano Kit and a Bioanalyzer (both Agilent Technologies, USA). RNA integrity numbers typically vary between 5 and 10 depending on species and tissue. If RNA integrity numbers were below that threshold, samples were not used.

**RNA-Sequencing**

Gene expressions were quantified by RNA-sequencing in an Illumina HiSeq2500 providing 20-30 million single-end reads per sample. The raw reads were processed using an RNA-seq pipeline implemented in the bcbio-nextgen project (<https://bcbio-nextgen.readthedocs.org/en/latest/>) and examined for quality issues using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) to ensure library generation and sequencing were suitable for further analysis. The RNA-seq pipeline provided two matrixes of counts, one generated by featureCounts16 from reads aligned by STAR17 (sheep genome build Oar\_v31), and other from quasi-alignments using Salmon18. The first matrix provided extended quality metrics for quality control and clustering of per sample gene counts, while the Salmon counts have been shown to be more accurate for differential expression analysis19,20.

**Gene set enrichment analysis**

Differential gene expression was assessed with DESeq2 Bioconductor package21. Statistical significance was calculated by the Wald test, which fits the count data with the negative binomial model distribution. Differentially expressed genes were defined by an absolute fold change > 1.5 and an adjusted P value (Padj, Benjamini-Hochberg) < 0.05. Fold change values were estimated using fold-shrinkage as implemented in the DESeq2 package. To link differentially expressed genes to known biological functions, functional analysis of enriched biological process gene ontology was performed with a cut-off-free gene set enrichment analysis using clusterProfiler22 (1000 permutations and gene set sizes between 100 and 500) and p-value weighted fold change calculations from DESeq2. Functional redundancy in enriched gene ontology terms was reduced by semantic similarity using REVIGO23. The quality control, differential expression and functional analyses on the count data were performed in R, based on templates from the bcbioRNASeq package24. Network based on gene set enrichment analysis was generated and visualized by using EnrichmentMap (version 3.1.0)25 and WordCloud (version 3.1.2)26 in Cytoscape (http://www.cytoscape.org). In the resulting networks, the color and size of nodes indicate the corresponding FDR-adjusted enrichment P values (Q values)25. Overlaps of genes between gene sets are indicated by edges in figures. Significantly enriched gene sets (Padj<0.05) were also visualized in either GraphPad Prism software v.7.0 (GraphPad Software, USA) or Rstudio (R version 3.4.4). Cells were colored according to normalized enrichment scores, the primary statistic for examining gene set enrichment results. Enrichment *P* values were plotted in log10 scale. The interferon-stimulated genes (ISGs) included in INTERFEROME database (http://www.interferome.org) were further analyzed based on the most significantly enriched immune-related processes in atelectatic lung tissue during LPS exposure.

**Transcription-factor prediction analysis**

We used transcription factor prediction analysis in iRegulon (version 1.3) in combination with the provided 10K position weight matrices (PWMs) motif collection27 to predict potential transcription regulators involved in the observed gene expression patterns. Differentially expressed genes between atelectatic and aerated lung regions in the absence or presence of LPS exposure were used as input. To identify only highly enriched transcription factor predictions, we filtered the results by an enrichment score of 3 and only reported transcription factors with a maximum false discovery rate (FDR-adjusted P value) of less than 0.001. Resulting transcriptional regulators were grouped into clusters with high motif similarity using the STAMP algorithm with iRegulon standard parameters. The transcriptional regulators in their respective clusters were ordered by decreasing fold change and their gene expression was visualized as log2FC.

**Wet/dry weight ratio**

Used to estimate lung water content in samples from the atelectatic and aerated lungs. Samples were weighted before and after drying for 72 hours in an oven at 80°C. Wet/dry ratios were calculated as the average of the ratios of three samples from each region.

**Validation of RNA-seq by Real-time PCR**

For real-time polymerase chain reaction (PCR) validation, total RNA was isolated from frozen lung tissue samples using a RNeasy Mini Kit (Qiagen) according to the manufacturer’s standard protocol. The concentration and purity of the isolated RNA were assessed using a NanoDrop 2000 spectrophotometer. Complementary DNA was generated by ProtoScript® First Strand cDNA Synthesis Kit (New England BioLabs® Inc., USA). TaqMan reverse transcription PCR (using TaqMan™ Gene Expression Master Mix (Thermo Fisher Scientific, USA) was performed in an ABI Prism 7300 Sequence Real-time PCR System (Applied Biosystems, USA) using universal thermal cycling parameters. Gene expression was analyzed using the comparative threshold cycle (ΔΔCt) method with the housekeeping gene β-actin as the endogenous control.

**Immunofluorescent Staining and Analysis**

YAP staining was performed on 5 µm paraffin sections from aerated and atelectatic regions of LPS treated and untreated lungs. Sections were dewaxed and antigen retrieval was performed using a standard microwave procedure in a citrate-based buffer (Vector Laboratories, H-3300). Blocking was done for 1 hour in 5% donkey serum in TBS-T. Samples were incubated in primary antibody (Cell Signaling Technology, YAP [D8H1X] XP) diluted 1:100 in blocking solution at 4˚C overnight. After washing with TBS-T samples were incubated in secondary antibody (Jackson ImmunoResearch, 711-606-152) diluted 1:500 in blocking solution for 1 hour, washed in TBS-T, and mounted with Prolong Gold (Life Technologies, P36930). Images were acquired using a Zeiss AxioObserver D1 equipped with a X-Cite 120LED System. For each sample, five images were randomly taken by an investigator blinded to lung region and treatment. The pixel intensity of YAP in the nucleus was quantified using CellProfiler by masking on nuclei after subtracting the autofluorescence contributed by red blood cells. The values of YAP nuclear intensity based on these five images for each sample were then averaged to yield a single value for each animal and condition.

**Histological examination and myeloperoxidase measurement**

After sample collection for gene expression analysis, the lungs were excised and filled with trump fixative to 27 cm H2O. After fixation, tissue blocks from atelectatic (left) lung, and normal aeration in the ventilated (right) lung based on computed tomography images were sampled and analyzed. Paraffin sections (5 um) were examined by two investigators blinded to the lung region using stereologic point-counting technique at a magnification of ×400 across random, noncoincident microscopic fields. Lung histology was assessed by using a score based on 4 measures of lung injury: capillary congestion, alveolar hemorrhage, alveolar wall thickness, and infiltration of neutrophils28. Each measure was examined semi-quantitatively according to a five-point scale of damage: 0 = minimal, 1 = mild, 2 = moderate, 3 = severe, and 4 = maximal. Neutrophils were assessed by visual characterization based on size, color, cellular and nuclear morphology29. Neutrophil infiltration was scaled in the alveolar space or vessel wall per high power field (HPF) as 0 = 0 cells/HPF, 1 < 5 cells/HPF, 2 = 5-10 cells/HPF, 3 = 10-15 cells/HPF, 4 >15 cells/HPF. An average lung histopathology score was calculated for each section by summarizing the values from the aforementioned criteria.

Myeloperoxidase staining measurement was performed to assess extravasation of neutrophils. All IHC was performed on the Leica Bond III automated staining platform. Antibody myeloperoxidase from Dako, catalogue # A0398, Polyclonal, was run at 1:1000 dilution using the Leica Biosystems Refine Detection Kit with EDTA antigen retrieval. A semi-quantitative score was used to evaluate the density of myeloperoxidase staining within the field of representative area in each lung regions30. Briefly, 10 random fields (400 × magnification) were evaluated and a score ranging from 0-3 (where 0 corresponds to no staining, 1 corresponds to mild, 2 corresponds to moderate and 3 corresponds to extensive staining) was given to each field, and then an average obtained.

**Immunoblotting**

To detect total NFKB and STAT1 relative to β-actin, we obtained whole lysates by lysing lung tissues in RIPA buffer containing protease and phosphatase inhibitors. SDS-PAGE and western blotting were carried out. The following primary antibodies were used for sheep tissues: NF-κB p65 (Cell Signaling Technology, 4764, Leiden, Netherlands), STAT1 (Cell Signaling Technology, 14994, Leiden, Netherlands), and β-actin (Cell Signaling Technology, 4970, Leiden, Netherlands). Appropriate anti-rabbit horseradish-peroxidase-conjugated secondary antibody (Cell Signaling Technology, 93702, Leiden, Netherlands) were obtained from Cell Signaling. Protein bands were visualized with the enhanced chemiluminescence system and quantified by densitometric analysis with the ChemiDoc MP System with Image Lab Software v.4.0 (all from Bio-Rad Laboratories, CA, USA).

**Enzyme-linked immunosorbent assay (ELISA)**

Regional lung tissues were harvested from atelectatic and normally aerated regions. Protein levels in lung tissue were measured in duplicate using the commercial ELISA kits for CXCL8 (MyBioSource, San Diego, CA, USA), CXCL10 (Lifespan Biosciences, Inc., Seattle, WA, USA), CCL5 (RayBiotech, Inc., Norcross, GA, USA), THBS1 (MyBioSource, San Diego, CA, USA) and SERPINE1 (Lifespan Biosciences, Inc., Seattle, WA, USA), according to the manufacturer's instructions. Protein levels in the lung were normalized to total protein concentrations in the tissue homogenate. The absorbance was determined at 450 nm using a microplate reader.

**Statistical Analysis**

No statistical power calculation was conducted prior to the study and sample size was determined based on a previous study indicating six biological replicates as a general guideline for RNA-seq experiments31. Outliers for cardiopulmonary physiology and imaging variables were assessed by visual inspection, with no data deletion. No samples for genomic analysis were excluded after quality control. Samples were examined for technical failure via various metrics, including mapping percentage, gene detection (how many genes had at least one read mapped to them), genomic mapping context (i.e. intronic, exonic or intergenic) and ribosomal RNA mapping. No technical failures were detected. PCA analysis of transcriptomic data also failed to identify any outliers.

Data are presented as mean ± SD if normally distributed and median and interquartile interval (25 to 75%) otherwise. Global cardiopulmonary variables were compared using repeated-measures ANOVA with post hoc Tukey test for normally distributed data and Kruskal–Wallis test otherwise. The effects of ventilation (atelectatic vs aerated) and systemic LPS in the parameters of 18F-FDG kinetics were assessed by two-way ANOVA for repeated measurements. The interaction between conditions was included when significant. Multiple comparisons were corrected by Tukey method. The statistical significance of mRNA expression, densitometric analyses of protein levels was calculated by a paired, two-tailed Student's t-test. The Pearson correlation between wet/dry ratios and the standardized tissue volume of distribution of 18F-FDG not immediately available for phosphorylation (Fees) was computed from measurements in atelectatic and normally aerated lung samples from each one of the studied animals (n=12). Tests were two tailed and performed in R (R version 3.4.4). Significance was considered at P<0.05 and only corrected values were reported.

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**Additional Tables**

**Table S1. Cardiopulmonary variables in one-lung atelectasis and mechanical ventilation.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Lipopolysaccharide(-) | | | Lipopolysaccharide(+) | | | P-value | | |
|  | Baseline | Atelectasis-0h | Atelectasis-8h | Baseline | Atelectasis-0h | Atelectasis-8h | Group | Time | Group×Time |
| **Respiratory**  **variables** |  |  |  |  |  |  |  |  |  |
| VT, ml/kg | 9.7±0.3 | 9.6±0.3 | 10.0±0.7 | 9.5±0.2 | 9.4±0.1 | 9.3±0.4 | 0.038 | 0.031 | 0.056 |
| PEEP, cm H2O | 2±0 | 2±0 | 2±0 | 2±0 | 2±0 | 5±3 | 0.083 | 0.079 | 0.029 |
| RR, min-1 | 29±4 | 28±4 | 28±4 | 26±4 | 26±4 | 25±3 | 0.342 | 0.163 | 0.870 |
| PMAX, cm H2O | 19.7±5.9 | 23.9±2.7 | 32.5±12.0 | 16.3±1.6 | 24.7±3.8 | 38.8±8.4 | 0.577 | <0.001 | 0.140 |
| Pplateau, cm H2O | 15.7±2.7 | 22.5±2.9 | 28.6±10.6 | 14.5±1.4 | 22.5±4.1 | 32.8±6.1 | 0.602 | <0.001 | 0.404 |
| Cdyn, ml/cm H2O | 14±3 | 9±2 | 8±3 | 15±4 | 9±1 | 6±2 | 0.791 | <0.001 | 0.241 |
| Driving Pressure, cm H2O | 13.6±2.6 | 20.3±2.8 | 28.4±11.0 | 12.0±1.3 | 21.0±4.0 | 30.3±7.4 | 0.834 | <0.001 | 0.654 |
| Pesophageal, cm H2O | 5.7±2.5 | 3.6±1.6 | 2.6±0.5 | 5.5±1.5 | 2.6±1.3 | 4.9±3.0 | 0.690 | 0.009 | 0.130 |
| pH | 7.37±0.05 | 7.41±0.08 | 7.34±0.05 | 7.44±0.05 | 7.42±0.06 | 7.11±0.26 | 0.370 | <0.001 | 0.006 |
| PaCO2, mm Hg | 42 ±5 | 40±6 | 44±12 | 36±4 | 37±6 | 52±11 | 0.913 | 0.004 | 0.039 |
| PaO2, mm Hg | 145±25 | 88±13 | 79±14 | 176±50 | 76±12 | 78±31 | 0.507 | <0.001 | 0.032 |
| FiO2 | 0.50±0.00 | 0.50±0.00 | 0.55±0.12 | 0.50±0.00 | 0.50±0.00 | 1.00±0.00 | <0.001 | <0.001 | <0.001 |
| PaO2/FiO2, mm Hg | 299±46 | 175±24 | 146±42 | 351±91 | 152±21 | 81±30 | 0.871 | <0.001 | 0.008 |
| PvO2, mm Hg | 47±5 | 44±3 | 42±5 | 49±8 | 42±5 | 43±17 | 0.912 | 0.215 | 0.878 |
| Bicarbonate, mM | 23.40±1.65 | 24.56±2.82 | 22.65±3.28 | 24.30±2.95 | 23.63±2.95 | 16.45±5.88 | 0.251 | <0.001 | 0.006 |
| ETCO2, mm Hg | 38±4 | 34±5 | 34±5 | 26±2 | 32±3 | 32±3 | 0.257 | 0.002 | 0.962 |
| **Cardiovascular variables** |  |  |  |  |  |  |  |  |  |
| HR, beats/min | 123±25 | 113±14 | 112±6 | 114±27 | 115±19 | 137±52 | 0.585 | 0.479 | 0.182 |
| BP, mm Hg | 93±11 | 88±16 | 85±10 | 89±10 | 99±12 | 64±18 | 0.353 | 0.004 | 0.018 |
| MPAP, mm Hg | 19±3 | 22±2 | 22±3 | 16±3 | 23±2 | 39±6 | 0.007 | <0.001 | <0.001 |
| PCWP, mm Hg | 9±3 | 9±2 | 11±4 | 9±2 | 7±2 | 18±6 | 0.177 | <0.001 | 0.005 |
| CO, L/min | 3.1±0.7 | 3.3±0.8 | 3.5±0.9 | 3.0±0.7 | 3.2±0.4 | 2.1±0.8 | 0.124 | 0.230 | 0.030 |
| Temperature, oF | 101.1±1.1 | 101.5±1.5 | 102.6±0.7 | 101.2±1.1 | 100.1±1.7 | 99.8±3.0 | 0.113 | 0.553 | 0.004 |

Cardiopulmonary function in mechanically ventilated sheep after anesthesia induction (baseline, two lung ventilation), shortly after induction of one-lung atelectasis (Atelectasis-0h, one lung ventilation) and after 8h of atelectasis (Atelectasis-8h) under lipopolysaccharide (LPS)-unexposed (-) and LPS-exposed (+) conditions.

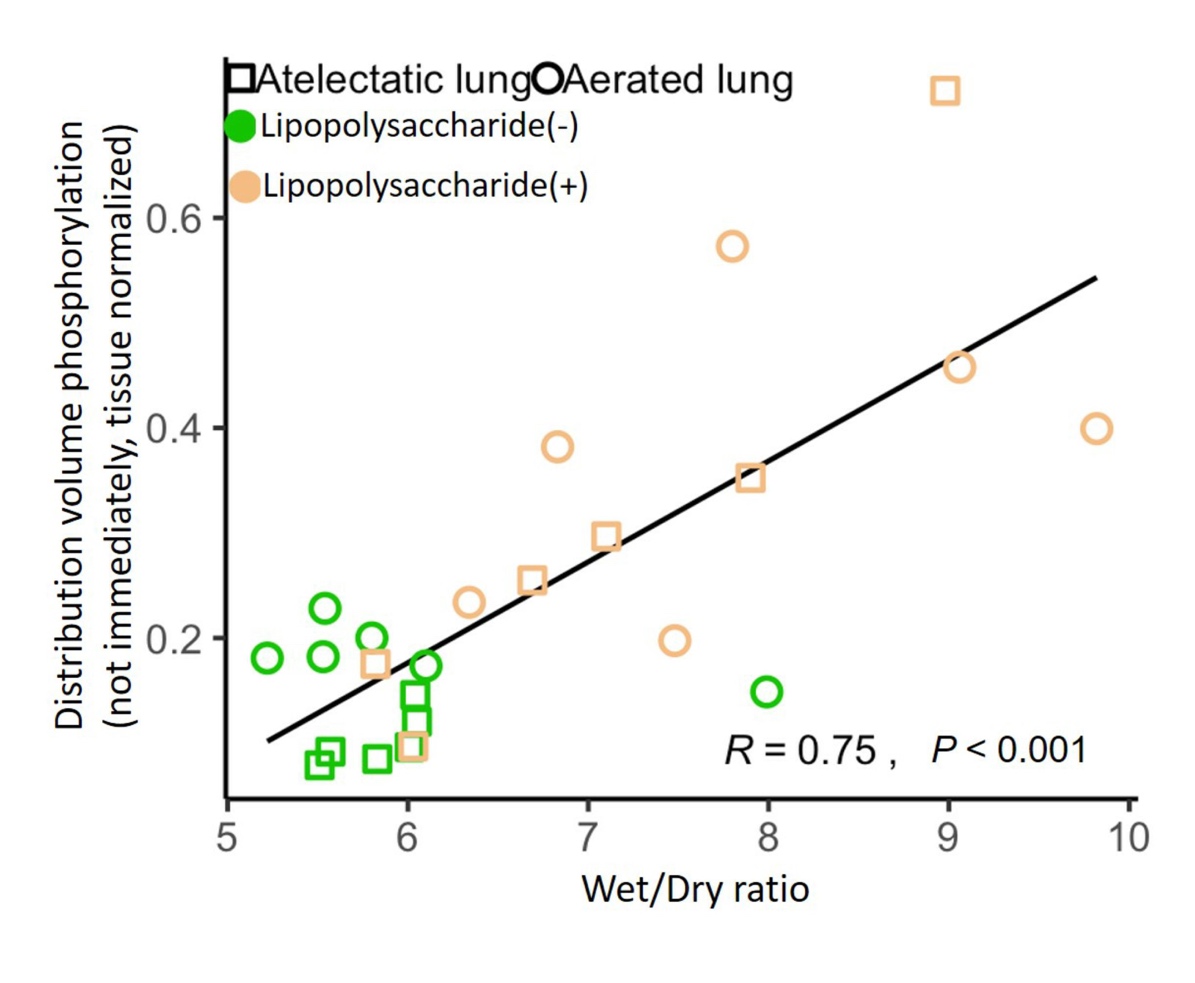
VT = tidal volume; PEEP = positive end-expiratory pressure; RR = respiratory rate; PMAX = peak airway pressure; Pplateau = plateau airway pressure; Cdyn = dynamic compliance; Pesophageal = esophageal pressure; PaCO2 = arterial carbon dioxide tension; PaO2 = arterial oxygen tension; FIO2 = inspired oxygen fraction; PaO2/FIO2 = ratio of PaO2 to inspired oxygen fraction (FIO2); PvO2 = mixed venous oxygen tension; ETCO2 = end-tidal carbon dioxide; HR = heart rate; BP = blood pressure; MPAP = mean pulmonary arterial pressure; PCWP = pulmonary capillary wedge pressure; CO = cardiac output. The P-values corresponding to each of the analyzed effects (LPS exposure (Group), time point (Time), and the interaction term (Group×Time)) are indicated in the table.

**Table S2. Histological lung injury assessment.**

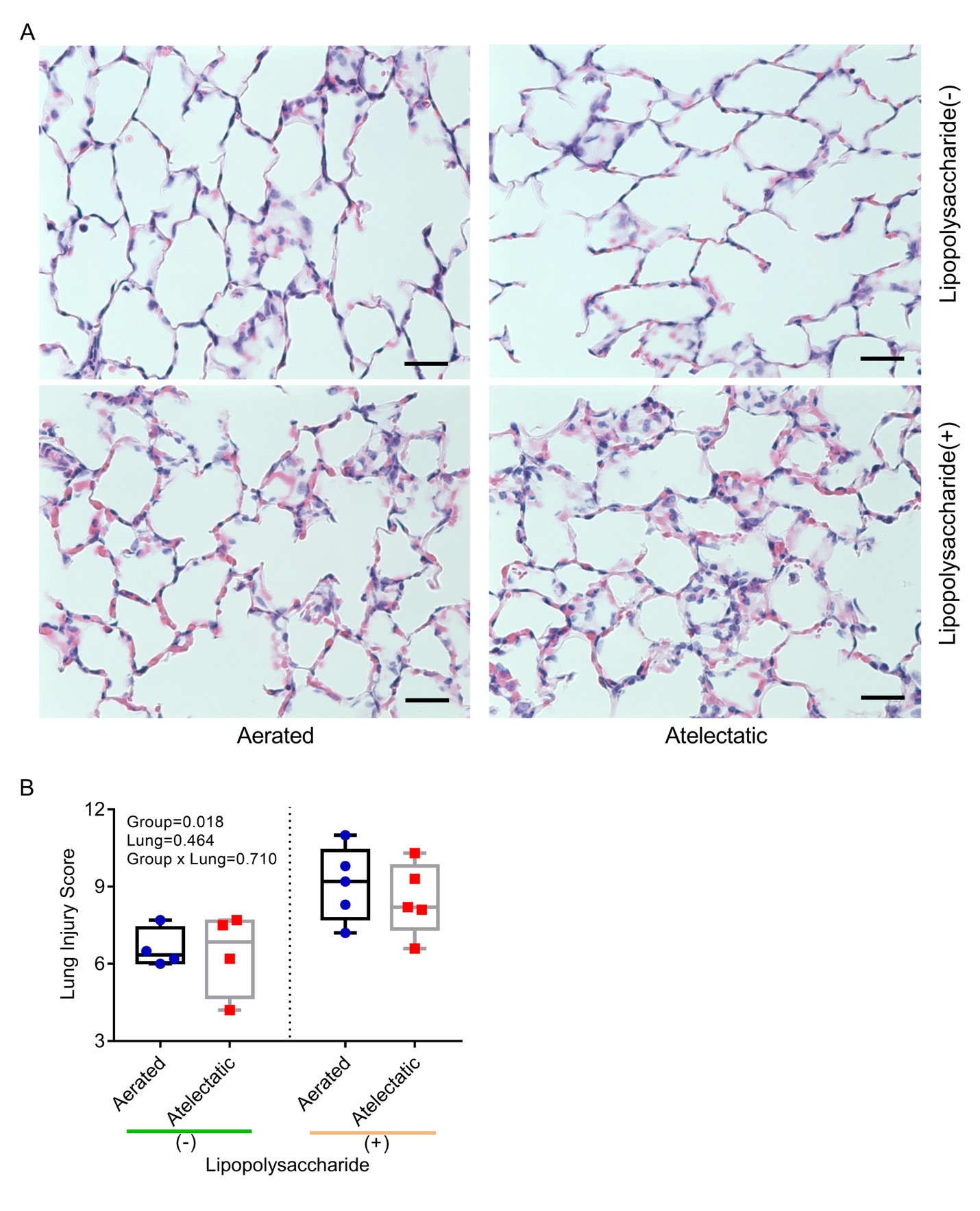
Histological lung injury in atelectatic and aerated lung regions was assessed in the absence or the presence of systemic lipopolysaccharide (LPS) exposure. The scoring system was based on 4 parameters of lung injury: capillary congestion, alveolar hemorrhage, alveolar wall thickness, and neutrophils infiltration. LPS exposure increased total lung injury scores in both atelectatic and aerated regions (P=0.018), with severe capillary congestion (P=0.017) and infiltration of neutrophils (P=0.046). The P-values corresponding to analyzed effects (LPS exposure (Group), lung regions (Lung), and their interaction (Group×Lung)) are indicated in the table.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Lipopolysaccharide(-) | | Lipopolysaccharide(+) | | P-value | | |
|  | Aerated | Atelectatic | Aerated | Atelectatic | Group | Lung | Group×Lung |
| Capillary congestion | 1.5±0.2 | 1.9±0.6 | 3.1±0.7 | 2.9±0.9 | 0.017 | 0.703 | 0.103 |
| Alveolar hemorrhage | 1.1±0.6 | 1.1±0.5 | 1.4±1.1 | 1.4±0.2 | 0.528 | 0.966 | 0.702 |
| Alveolar wall thickness | 3.5±0.1 | 2.9±0.7 | 3.7±0.3 | 3.6±0.4 | 0.085 | 0.086 | 0.317 |
| Neutrophils infiltration | 0.5±0.1 | 0.4±0.1 | 0.9±0.3 | 0.7±0.3 | 0.046 | 0.145 | 0.521 |
| Total | 6.6±0.7 | 6.4±1.6 | 9.1±1.4 | 8.5±1.4 | 0.018 | 0.464 | 0.710 |

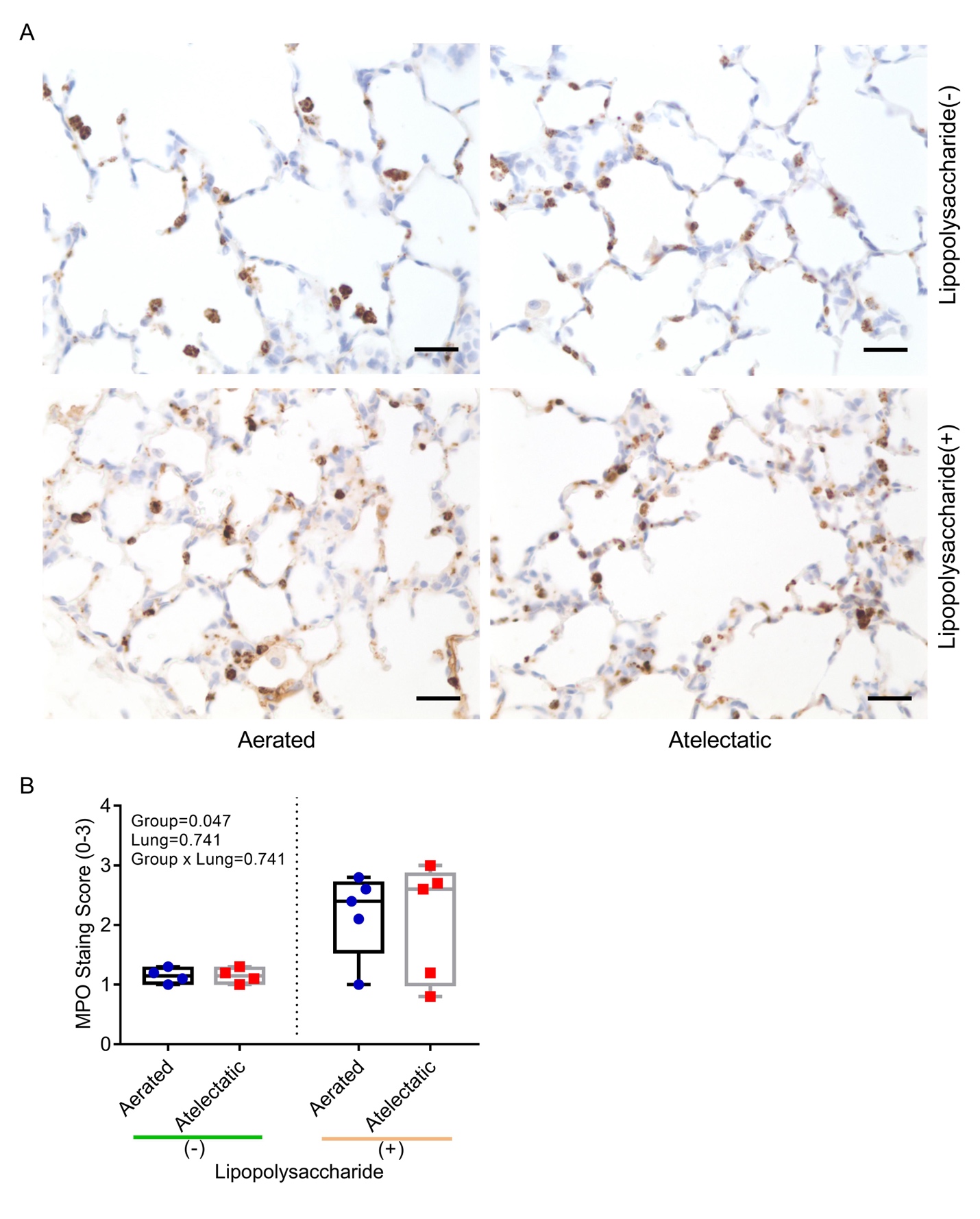
**Additional Figures**



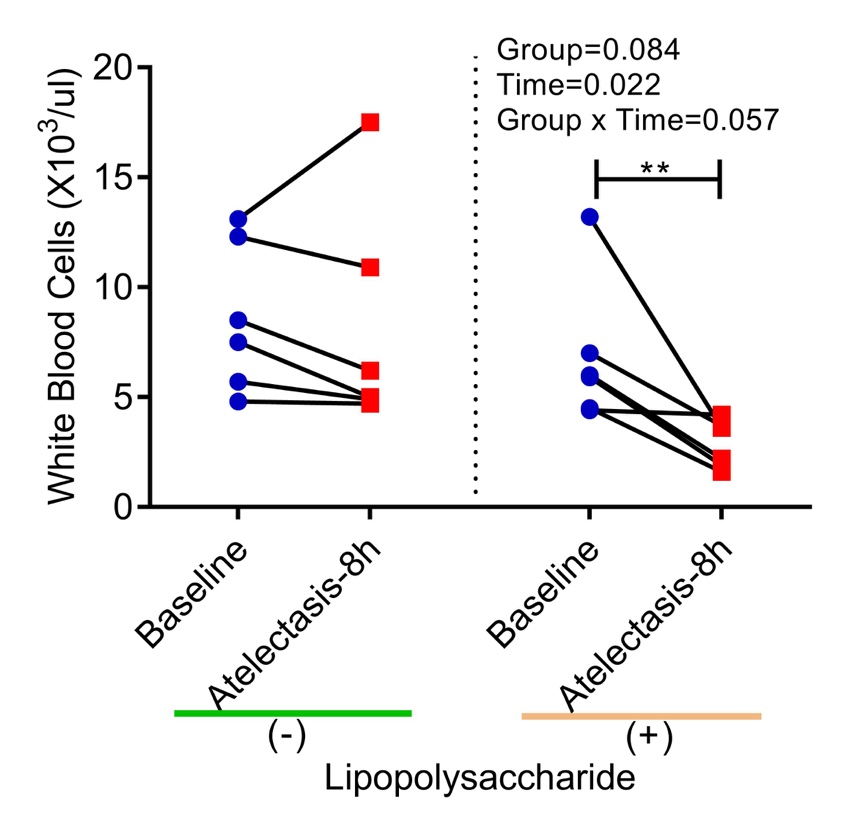
**Figure S1. Wet/Dry ratios correlate with the volume of distribution of lung tissue 2-deoxy-2-[18F] fluoro-D-glucose (18F-FDG) not immediately available for phosphorylation (Fees).** Wet/Dry ratios correlated directly with the *in vivo* lung edema parameter Fees derived from the 18F-FDG kinetics (R=0.75, P<0.001). Each point represents samples from atelectatic and aerated lung from studied animals exposed or not to systemic lipopolysaccharide.



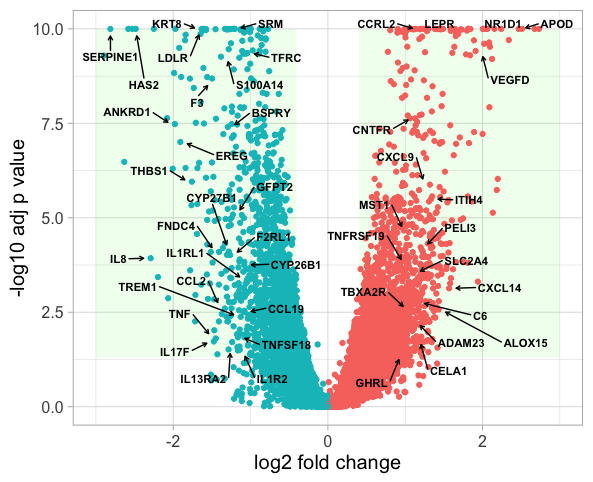
**Figure S2. Histological injury in aerated and atelectatic lung regions according to lipopolysaccharide (LPS) exposure.** (A) Worsened lung injury included severe capillary congestion and neutrophil infiltration (in high-power fields, magnification, ×400). Scale bars represent 50 µm. (B) Histological assessment showed that LPS exposure increased the lung injury scores (P=0.018; *n*=4 animals in LPS(-), *n*=5 animals in LPS(+)). The P-values corresponding to analyzed effects (LPS exposure (Group), lung region (Lung), and their interaction (Group × Lung)) are indicated in the figure.



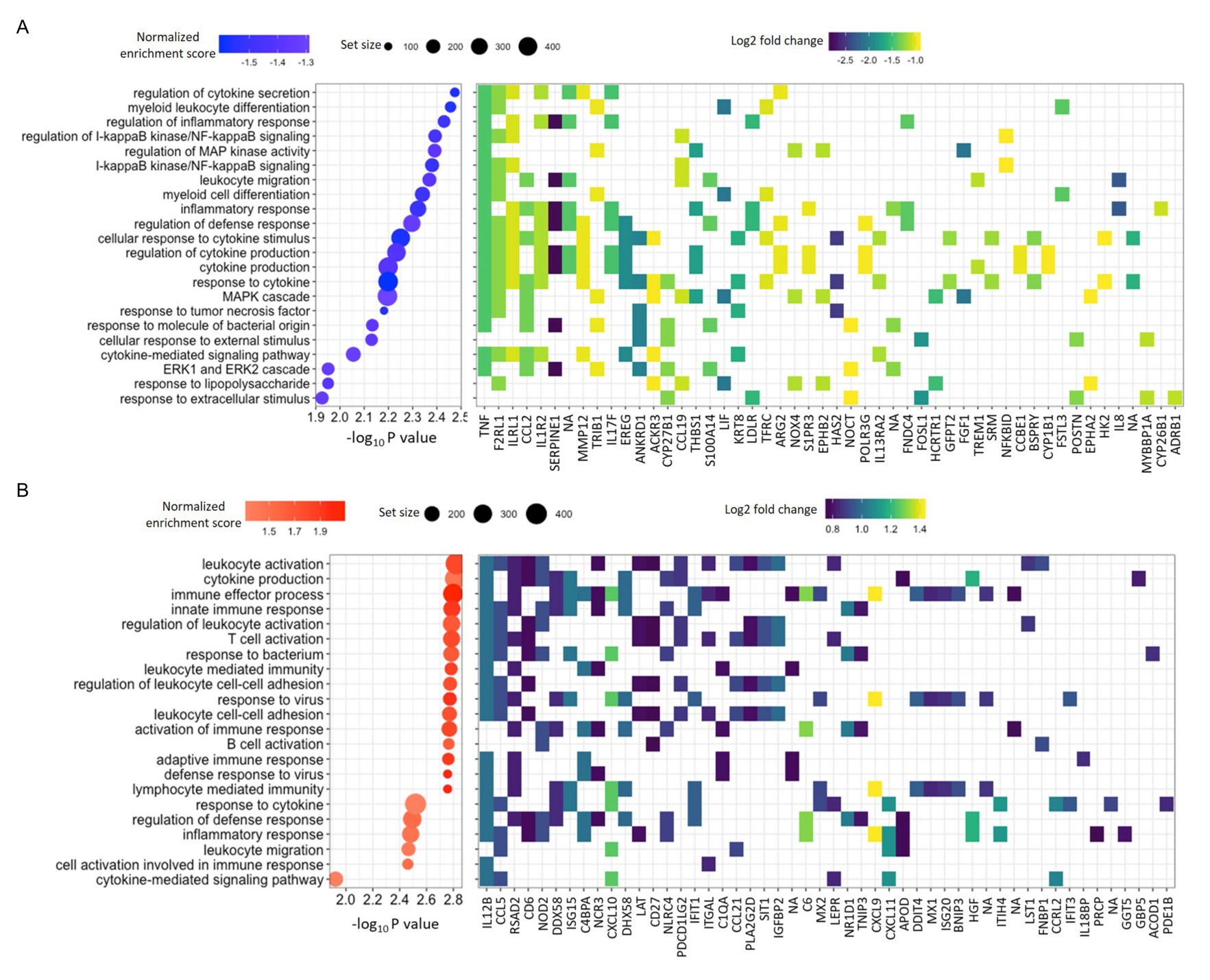
**Figure S3. Myeloperoxidase staining in aerated and atelectatic lung regions according to lipopolysaccharide (LPS) exposure.** (A) The representative images (in high-power fields, magnification, ×400) showing increased neutrophils in atelectatic and aerated regions after LPS exposure. Scale bars represent 50 µm. (B) The quantitative scores of myeloperoxidase (from 0 = no staining to 3 = maximal staining) showed that exposure to LPS increased the number of neutrophils in both atelectatic and aerated regions (P=0.047; *n*=4 animals in LPS(-), *n*=5 animals in LPS(+)). The number of neutrophils in atelectatic regions was comparable to that in aerated regions, independent of LPS exposure (P=0.741). The P-values corresponding to analyzed effects (LPS exposure (Group), lung region (Lung), and their interaction (Group × Lung)) are indicated.



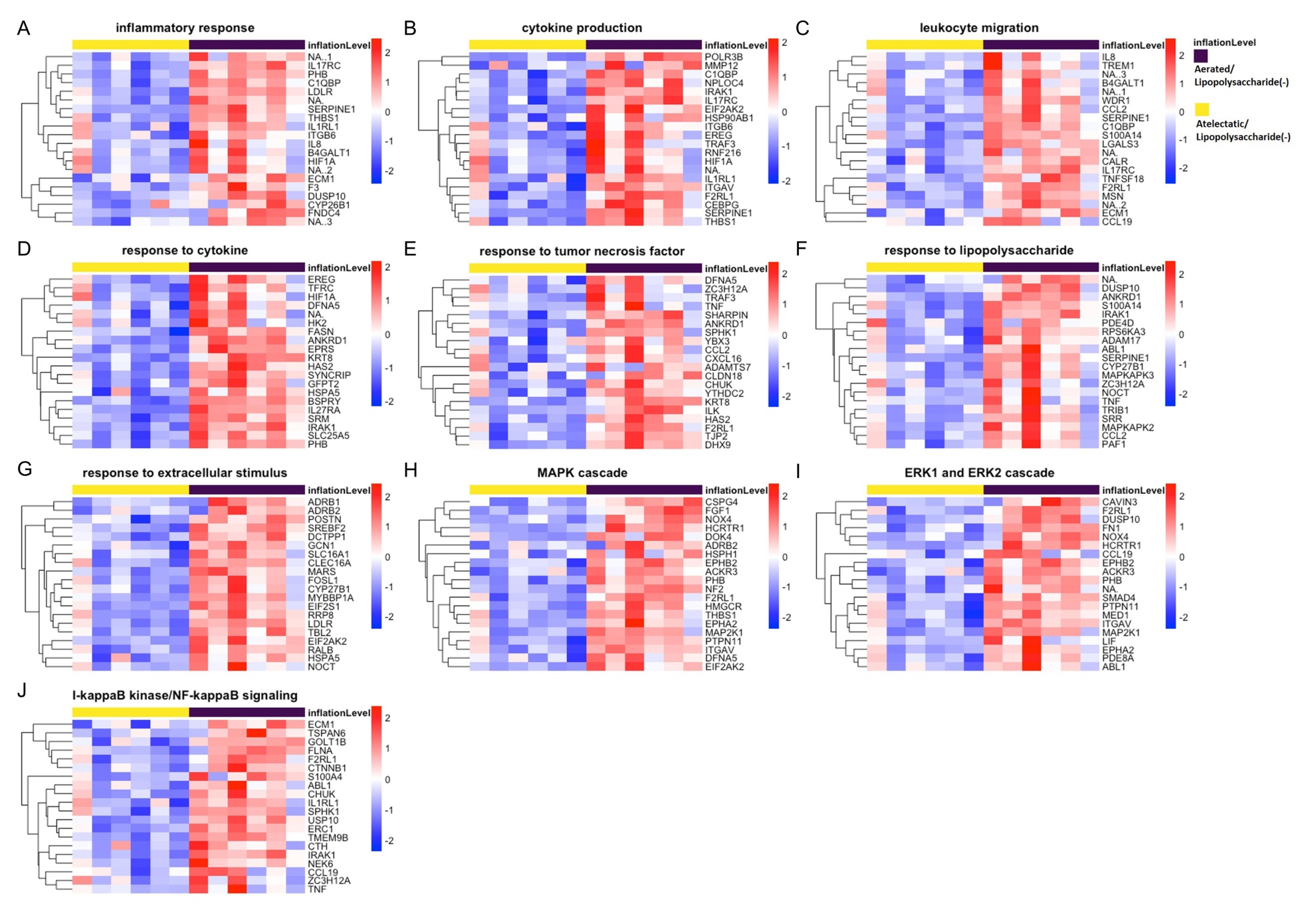
**Figure S4. Peripheral white blood cells at baseline and 8 hours later of lung atelectasis with or without lipopolysaccharide (LPS) exposure.** Peripheral white blood cells were decreased at the end of 8 hours lung atelectasis. LPS exposure dramatically decreased peripheral white blood cells after 8 hours of lung atelectasis when compared with baseline blood samples (P=0.022). The P-values corresponding to analyzed effects (LPS exposure (Group), time point (Time), and their interaction (Group × Time) are indicated. \*\* *P* < 0.01.



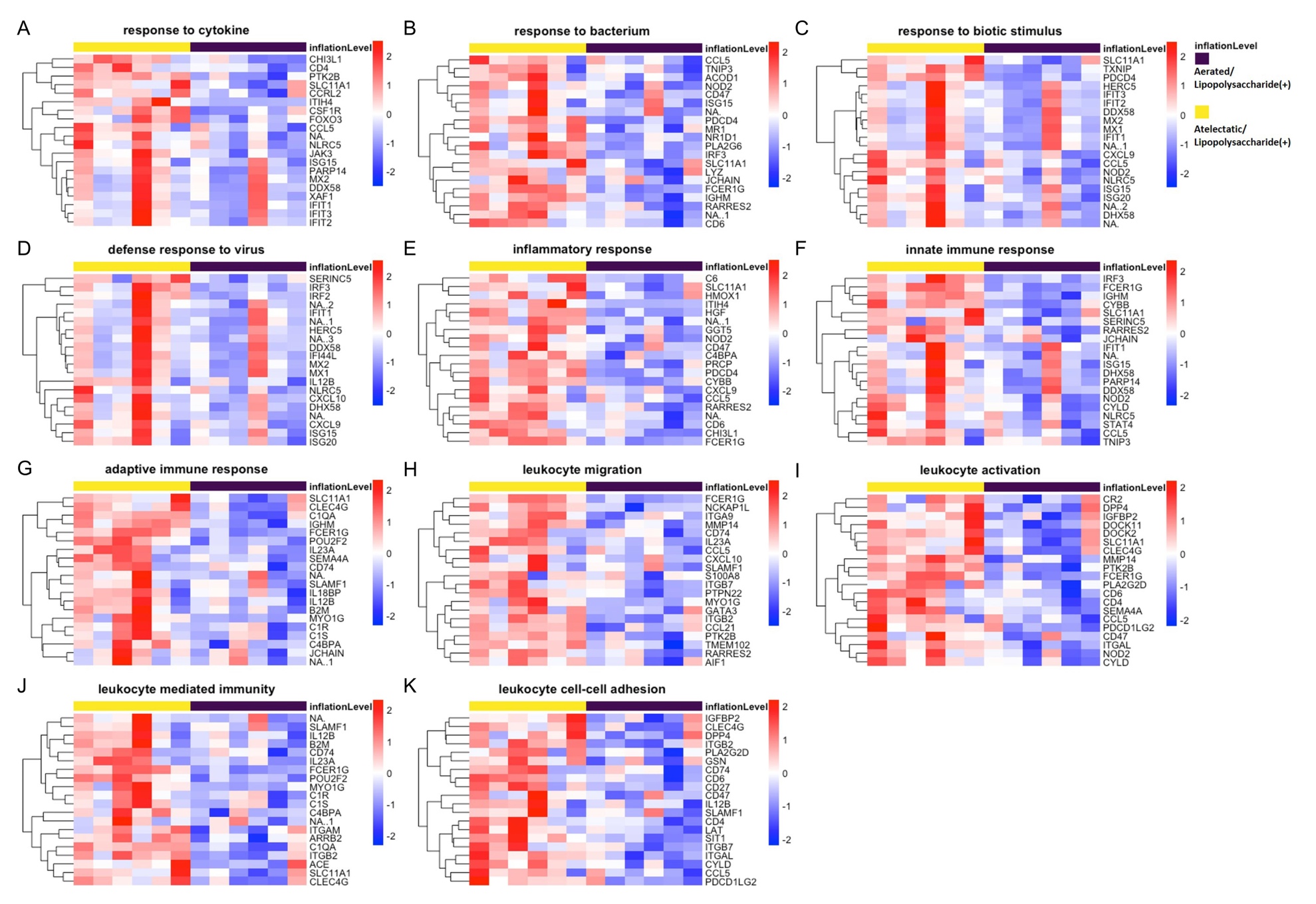
**Figure S5. Volcano plot visualization of differential gene expression in atelectatic vs aerated lung in the absence of lipopolysaccharide exposure.** Differentially expressed gene are defined by fold change>1.5 and adjusted P<0.05. The x-axis shows the fold-change in gene expression between (paired) atelectatic and aerated lung, and the y-axis shows the statistical significance of the differences. Colors represent different genes: red for genes upregulated in atelectasis and blue for genes downregulated in atelectasis. Top 50 differentially expressed genes are labeled with gene names. Each dot represents a specific gene transcript.

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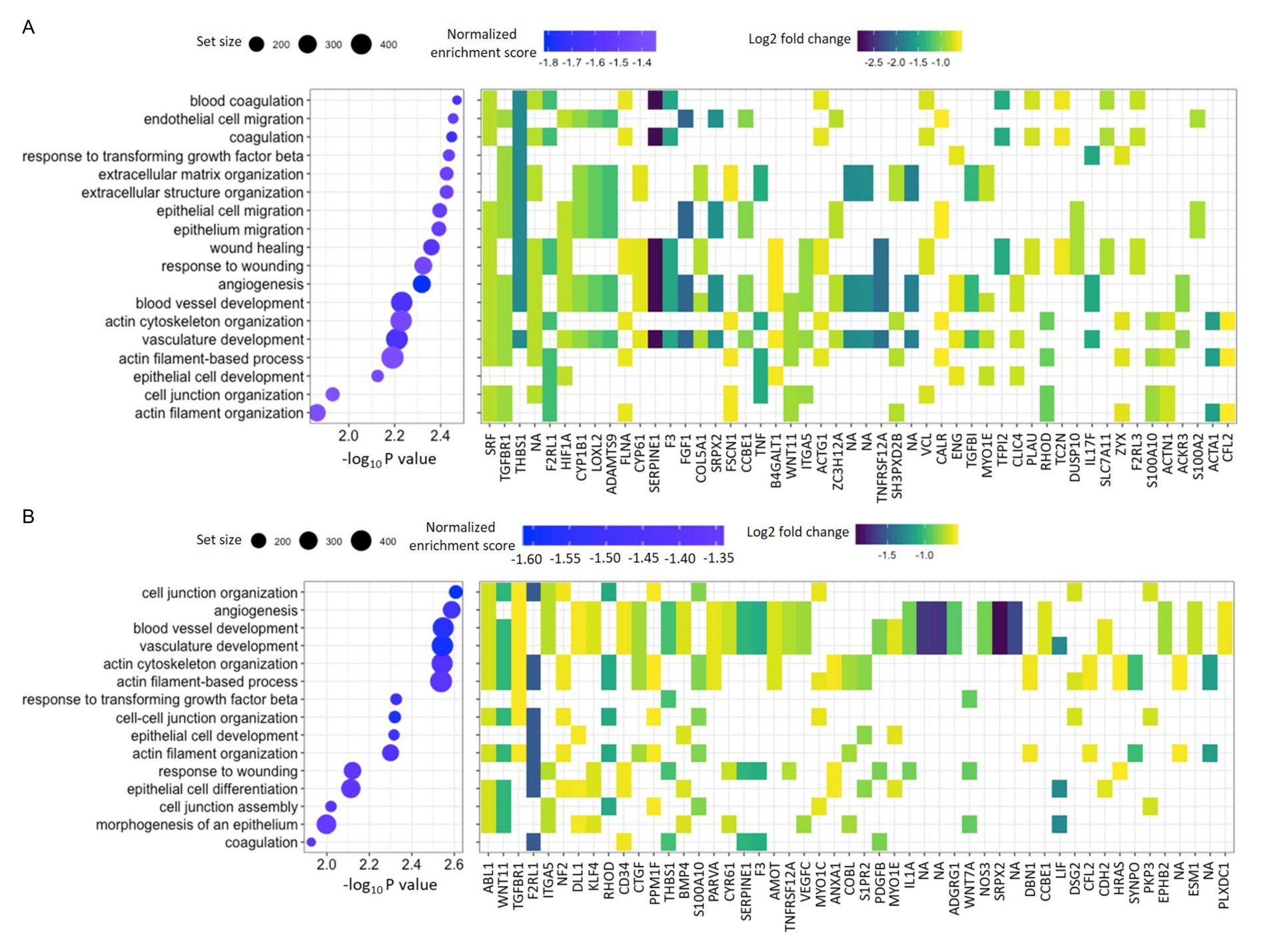
**Figure S6. Transcriptomic changes associated with immune** **function** **enriched in atelectasis in the absence or presence of lipopolysaccharide (LPS) exposure.** (A) Panel of negatively enriched gene sets relevant to immune response in the absence of LPS exposure and excluding redundant gene sets, along with genes mostly involved in these biological processes. For the gene sets, node color represents normalized enrichment score and node size represents set size. For the gene list, color is log-fold change with dark blue as stronger downregulation in atelectasis vs aerated regions. NA represents RNA sequences without a known symbol for sheep. (B) Panel of positively enriched gene sets relevant to pulmonary immunity and excluding redundant ontologies in the condition of LPS exposure, along with genes mostly involved in these biological processes. For the gene sets, node color represents normalized enrichment score and node size represents set size. For the gene list, color is log-fold change with light yellow as stronger upregulation in atelectasis vs aerated regions. NA represents RNA sequences without a known symbol for sheep.

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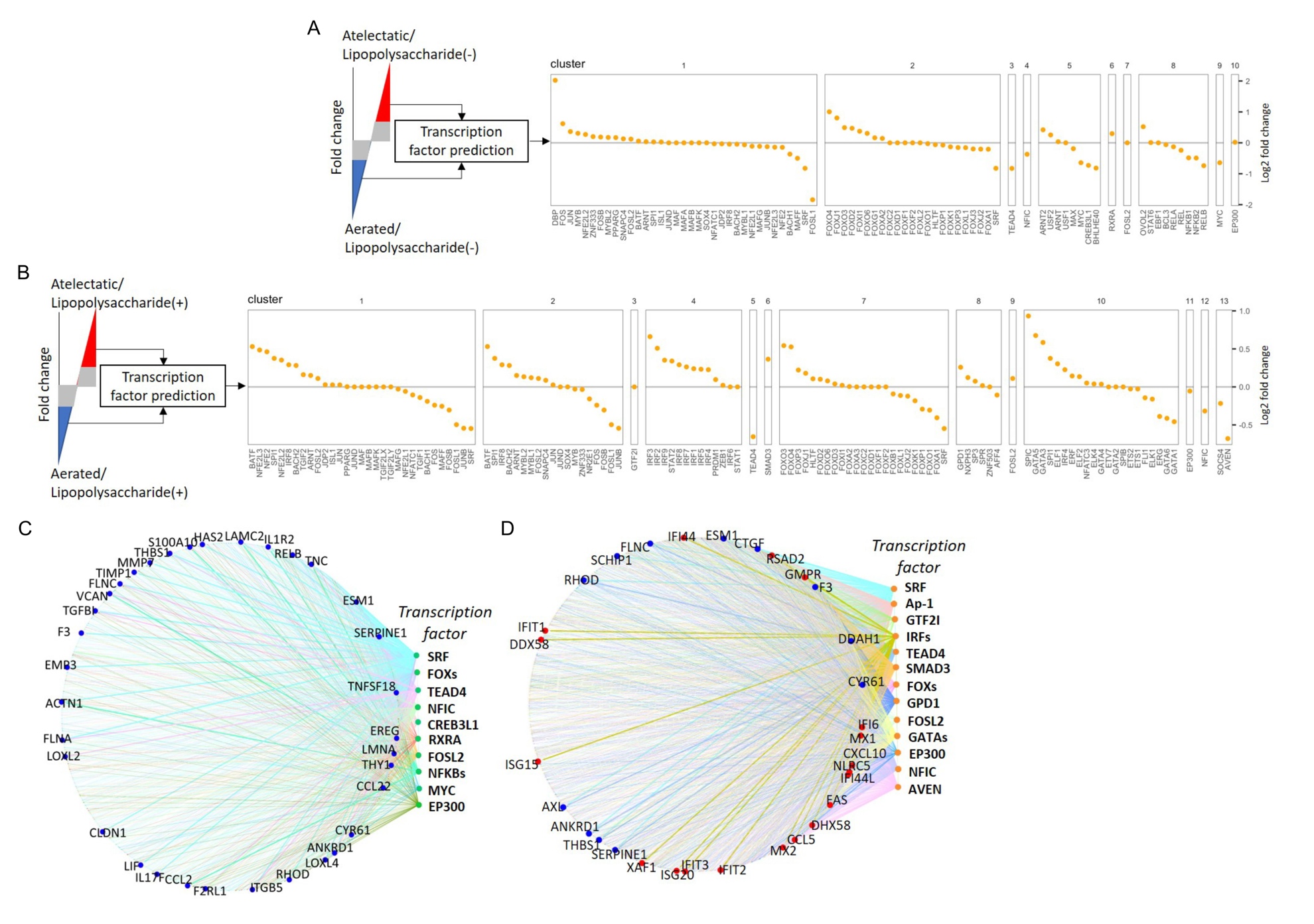
**Figure S7. Atelectasis dysregulated immune response in the absence of lipopolysaccharide (LPS).** (A-C) Heat map showing gene sets involved in immunity system, such as inflammatory response (A), cytokine production (B), as well as leukocyte migration (C), were downregulated in atelectatic regions as compared to aerated regions in the absence of LPS. (D-G) Heat map showing gene sets associated with cellular stress responses, including response to cytokine (D), response to TNF (E), response to lipopolysaccharide (F) and response to extracellular stimulus (G), were downregulated in atelectatic regions compared to aerated regions in the absence of LPS. (H-J) Heat map showing gene sets associated with immune-associated signaling pathways, such as MAPK cascade (H), ERK1 and ERK2 cascade (I), as well as NF-κB signaling (J), were downregulated in LPS-unexposed atelectatic regions relative to aerated regions.

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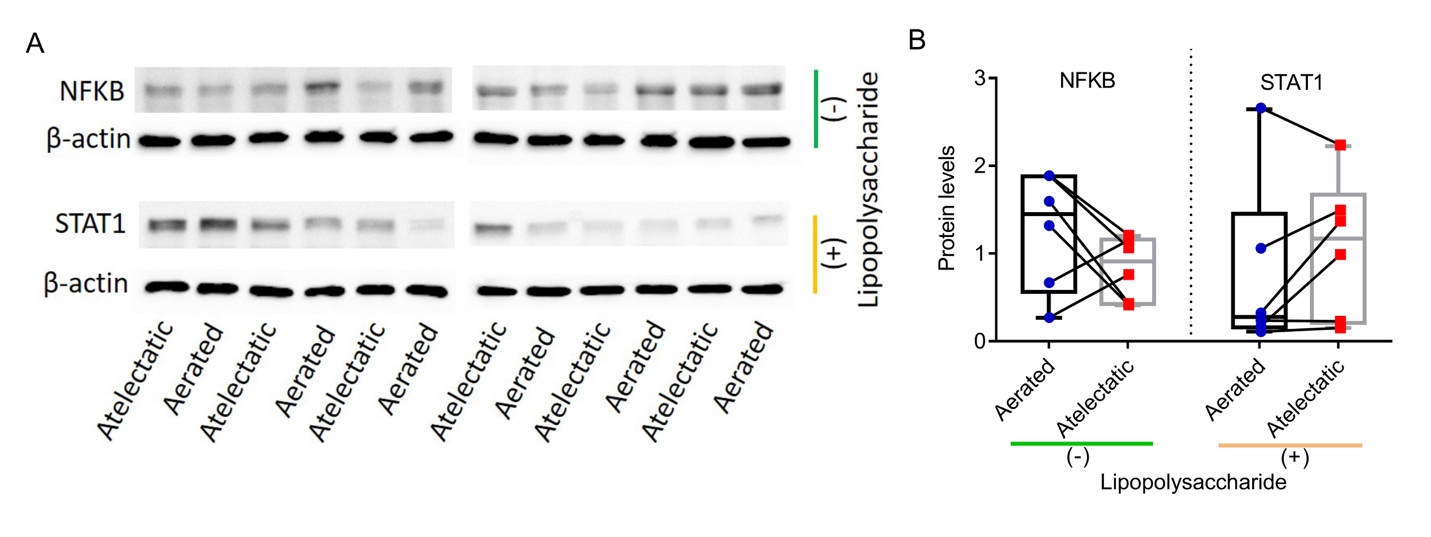
**Figure S8. Atelectasis was associated with positively enriched immune-related processes during lipopolysaccharide (LPS) exposure.** (A-C) Heat map showing gene sets associated with stress responses to cytokine (A), to bacterium (B) and to biotic stimulus (C) were upregulated in atelectatic vs aerated lung tissue during LPS exposure. (D-G) Heat map showing gene sets associated with immune responses, such as defense response to virus (D), inflammatory response (E) as well as innate (F) and adaptive (G) immune response, were upregulated in LPS-exposed atelectatic vs aerated lung tissue. (H-K) Heat map showing gene sets associated with leukocyte-related processes, such as leukocyte migration (H) and activation (I), leukocyte-mediated immunity (J) and leukocyte cell-cell adhesion (K), were upregulated in atelectatic vs aerated lung tissue during LPS exposure.

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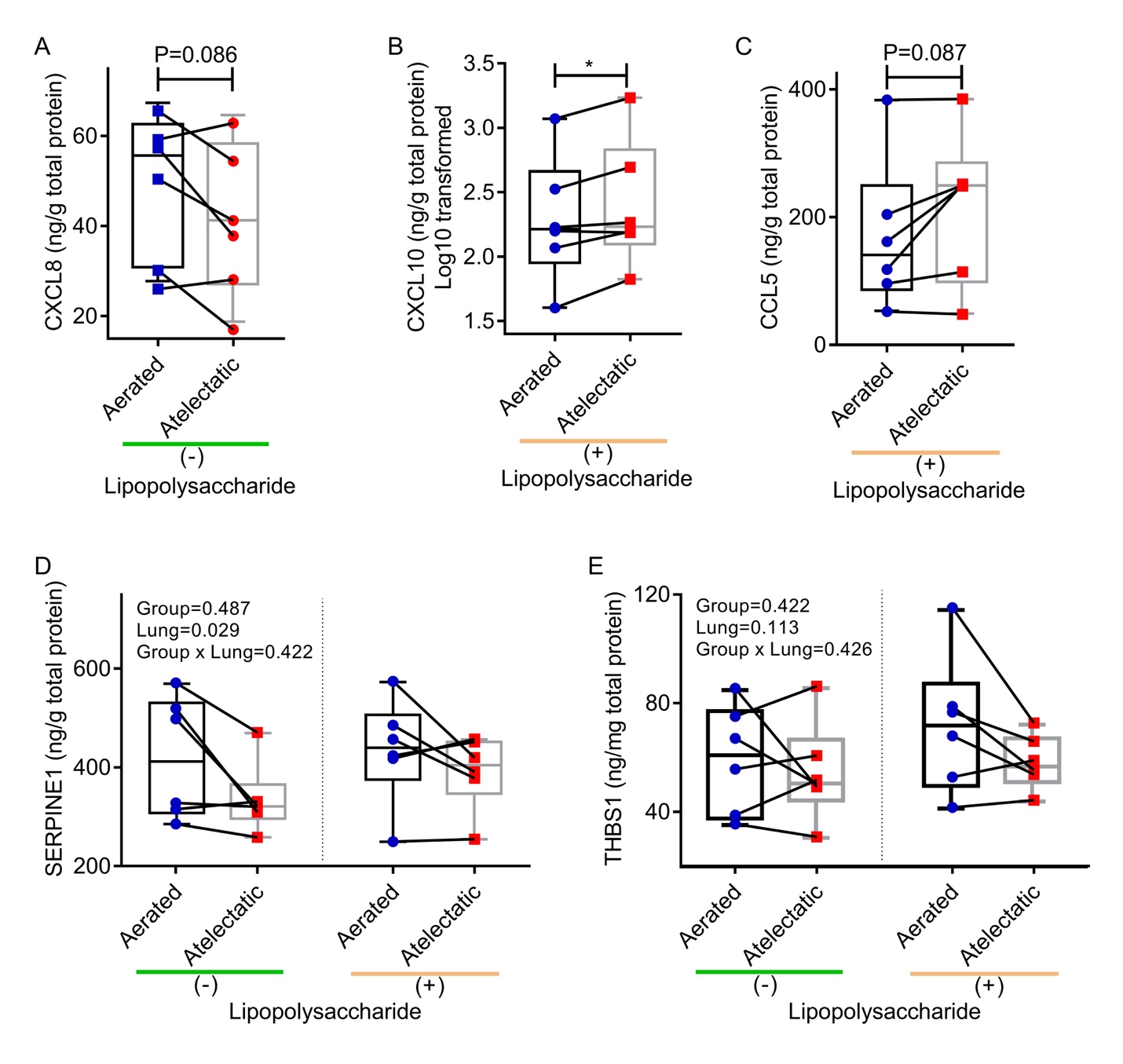
**Figure S9. Transcriptomic changes in barrier function** **enriched in atelectasis in the presence or absence of lipopolysaccharide (LPS) exposure.** Panel of negatively enriched gene sets in the absence (A) or the presence (B) of LPS exposure relevant to barrier function identified by gene set enrichment analysis and excluding redundant gene sets, along with genes mostly involved in these biological processes. For the gene sets, node color represents normalized enrichment score and node size represents set size. For the gene list, color is log-fold change with dark blue as stronger downregulation in atelectasis. NA represents RNA sequences without a known symbol for sheep.

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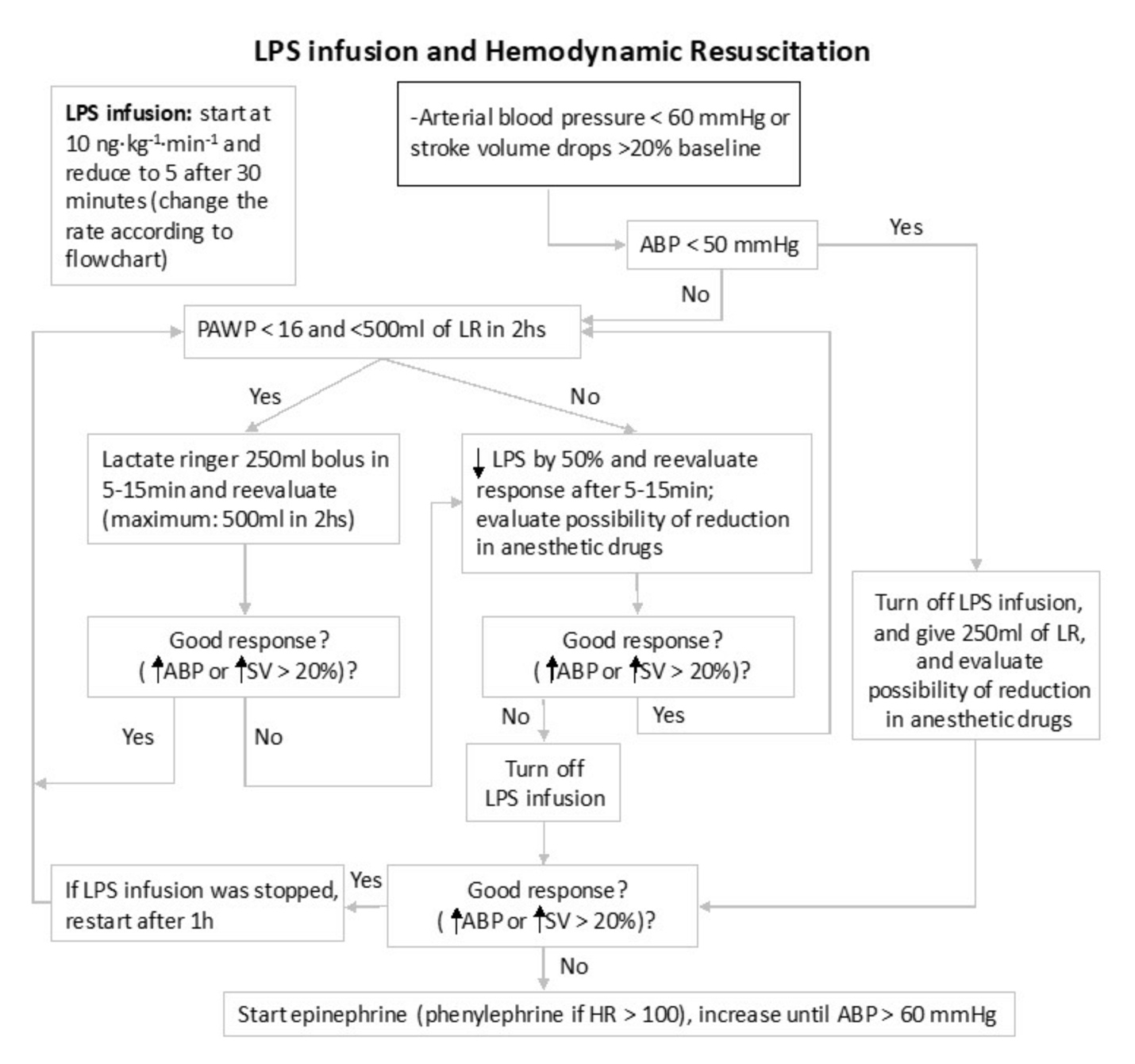
**Figure S10. Transcription factor analysis in atelectasis in the presence or absence of lipopolysaccharide (LPS) exposure.** (A, B) Transcription factor prediction analysis based on differentially expressed genes of atelectatic lung tissue in the absence (A) or the presence (B) of LPS exposure as compared to the corresponding aerated lung tissue. The transcription factors in their respective clusters were ordered by decreasing fold change and their gene expression was visualized as log2 fold change. (C, D) Gene targets involved in immune and barrier function in transcription factor prediction analysis in the absence (C) or the presence (D) of LPS exposure as compared to the corresponding aerated lung tissue. Predicted targets based on positive log2 fold change are depicted by red nodes, and those based on negative log2 fold change by blue nodes.

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**Figure S11. Transcription factor validations in atelectasis in the presence or absence of lipopolysaccharide (LPS) exposure.** Western blot analysis (A) and quantification (B) showing the protein levels of transcription factors NFKB p65 in the absence of LPS and STAT1 in the presence of LPS condition, in both atelectatic and aerated lung tissues. Representative results from six animals are shown.



**Figure S12. Quantification of protein levels in atelectatic and aerated lung tissues related to the observed transcriptomic findings.** (A) Protein levels of NF-κB-related factor CXCL8 by enzyme-linked immunosorbent assay (ELISA) tended to be lower in atelectatic regions than those in aerated regions in the absence of lipopolysaccharide (LPS) exposure (P=0.086, n=6 animals). (B and C) With LPS exposure, the protein levels of interferon-stimulated genes (ISGs) such as CXCL10 (P=0.022) and CCL5 (P=0.087) increased in atelectatic regions vs aerated regions (n=6 animals), consistent with the magnified transcriptomic response. (D and E) Protein levels of Yes-associated protein (YAP)-responsive genes including SERPINE1 (P=0.029) and THBS1 (P=0.113) were found low expression in atelectasis when compared to aerated regions, independent of LPS exposure (n=6 animals). The P-values corresponding to each of the analyzed effects (LPS exposure (Group), lung region (Lung), and the interaction term (Group × Lung) are indicated. \* P<0.05.



**Figure S13. Algorithm for hemodynamic management and adjustment of lipopolysaccharide infusion rate.**