Adverse mechanical ventilation and pneumococcal pneumonia induce immune and mitochondrial dysfunctions mitigated by mesenchymal stem cells in rabbits.

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Supplemental digital content 2

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Supplementary Figures

Figure S1

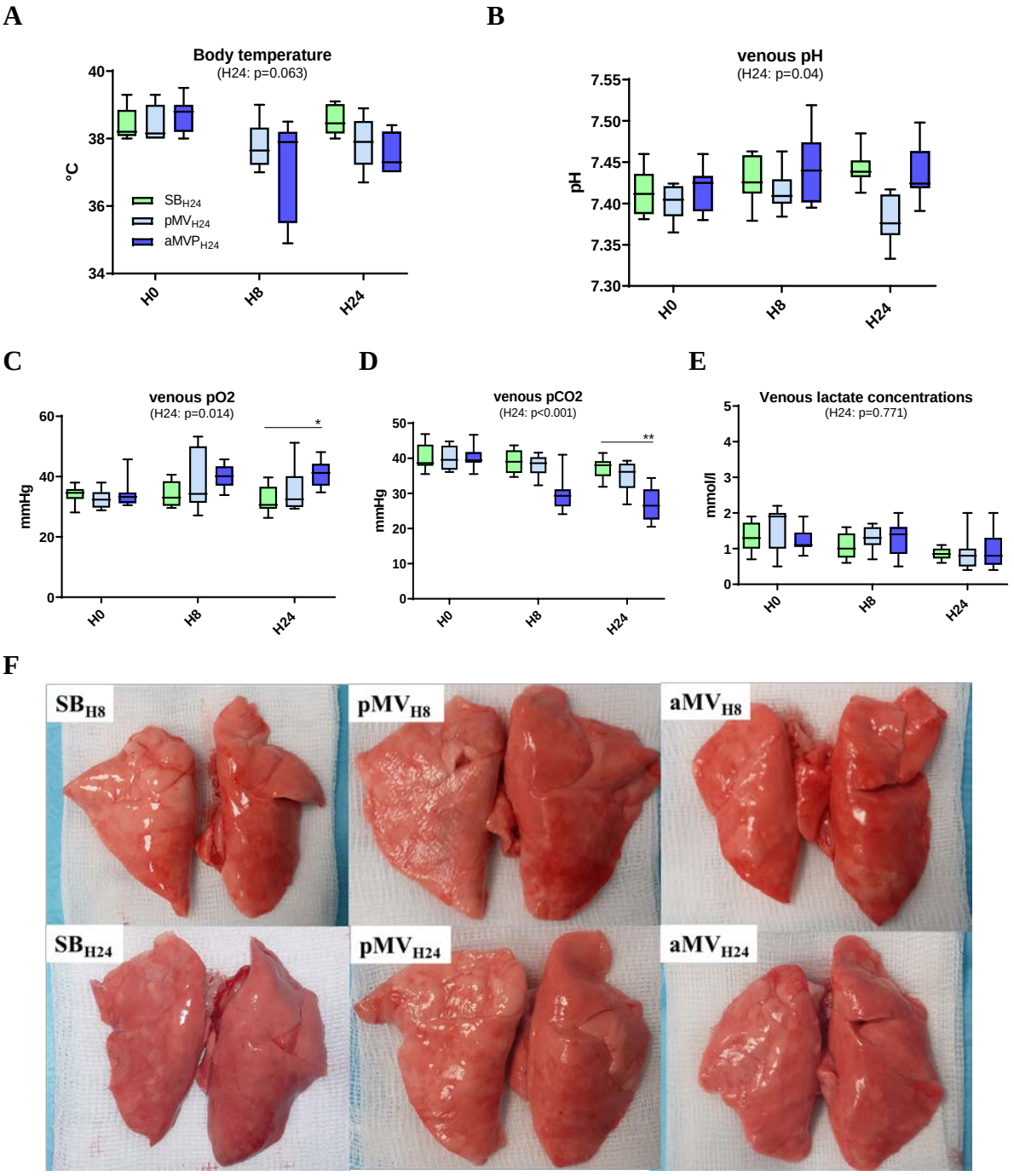


Figure S1. Safety of either protective or adverse mechanical ventilation protocols in rabbits.
(A) Central temperature was measured at baseline (H0), H8 and H24 (not measured at H8 in animals in spontaneous breathing). Blood samples were obtained at H0, H8 and H24 to measure venous (B) pH; (C) venous oxygen partial pressure (PvO2); (D) partial pressure of carbon dioxide (PvCO2); and (E) lactate concentrations. (F) Lung pictures.
Data are expressed as box-and-whisker diagrams (n=7 per group, excepted for pMV_{H8} n=6)). The Kruskal-Wallis test was performed (*p*-value reported for each time point in the figure), and the Dunn's post-hoc correction for multiple comparisons was used when appropriate: *, $p < 0.05$;**, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Figure S2

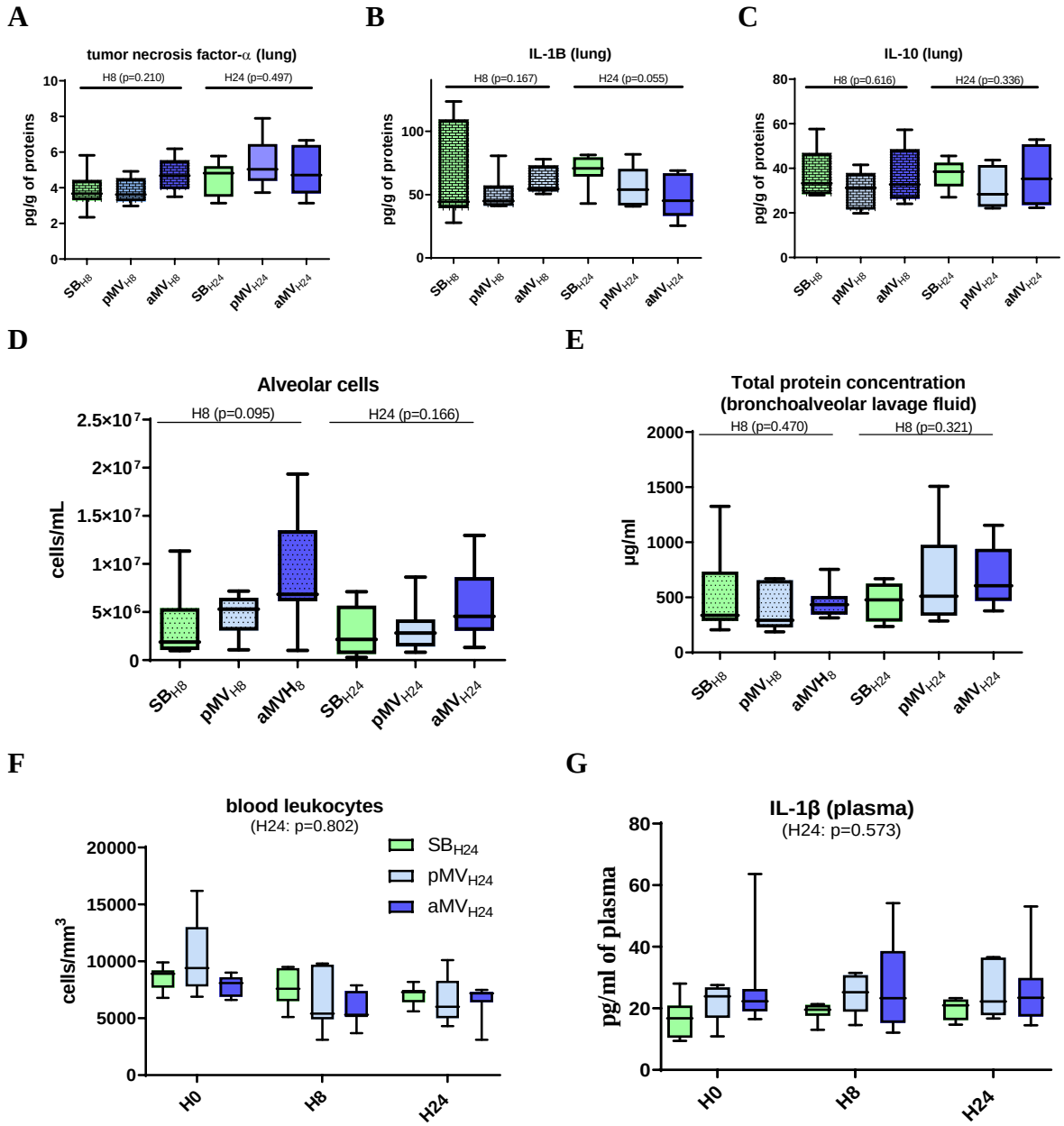


Figure S2. Lung inflammation and immune response during either protective or adverse mechanical ventilation.

Lung concentrations of (A) tumor necrosis factor-α, (B) IL-1β, and (C) Interleukin-10 and alveolar (D) cell count and (E) protein concentration were measured in rabbits submitted to protective or adverse mechanical ventilation or kept in spontaneous breathing at 8 or 24 hours. Blood samples were obtained at baseline (H0), H8 and H24 to measure: (F) leukocytes count and (G) plasma concentrations of IL-1β.

Data are expressed as box-and-whisker diagrams (n=7/group, excepted for pMV_{H8} n=6). The Kruskal-Wallis test was performed (*p*-value reported for each time point in the figure), and the Dunn's post-hoc correction for multiple comparisons was used when appropriate: *, p < .05;**, p < .01; ***, p < .001; ****, p < .0001.

Figure S3

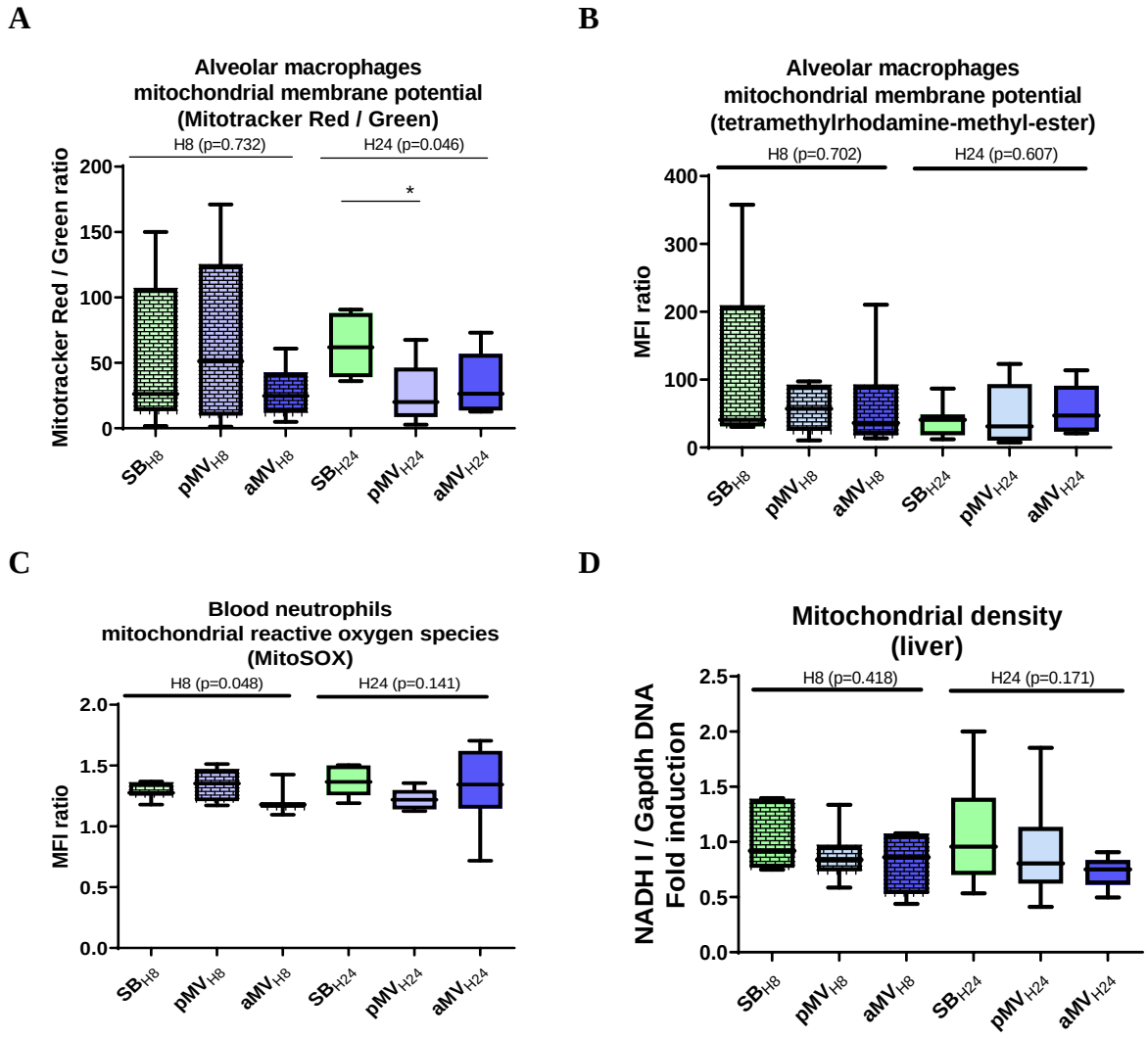


Figure S3. Mitochondrial derangements in the alveolar and systemic compartment during either protective or adverse mechanical ventilation.

The mitochondrial membrane potential of alveolar macrophages was measured by flow cytometry and using (A) Mitotracker Red/Green ratio or (B) tetramethylrhodamine-methyl-ester. Blood samples were obtained at baseline (H0), H8 and H24 to measure: (C) mitochondrial reactive oxygen species production of blood neutrophils by flow cytometry and using the MitoSOX probe. (D) Mitochondrial DNA levels were measured in the liver tissue (as a reflection of mitochondrial density). Data are expressed as box-and-whisker diagrams (n=7 per group, excepted for pMV_{H8} n=6). The Kruskal-Wallis test was performed (*p*-value reported for each time point in the figure), and the Dunn's post-hoc correction for multiple comparisons was used when appropriate: *, $p < 0.05$;**, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Figure S4

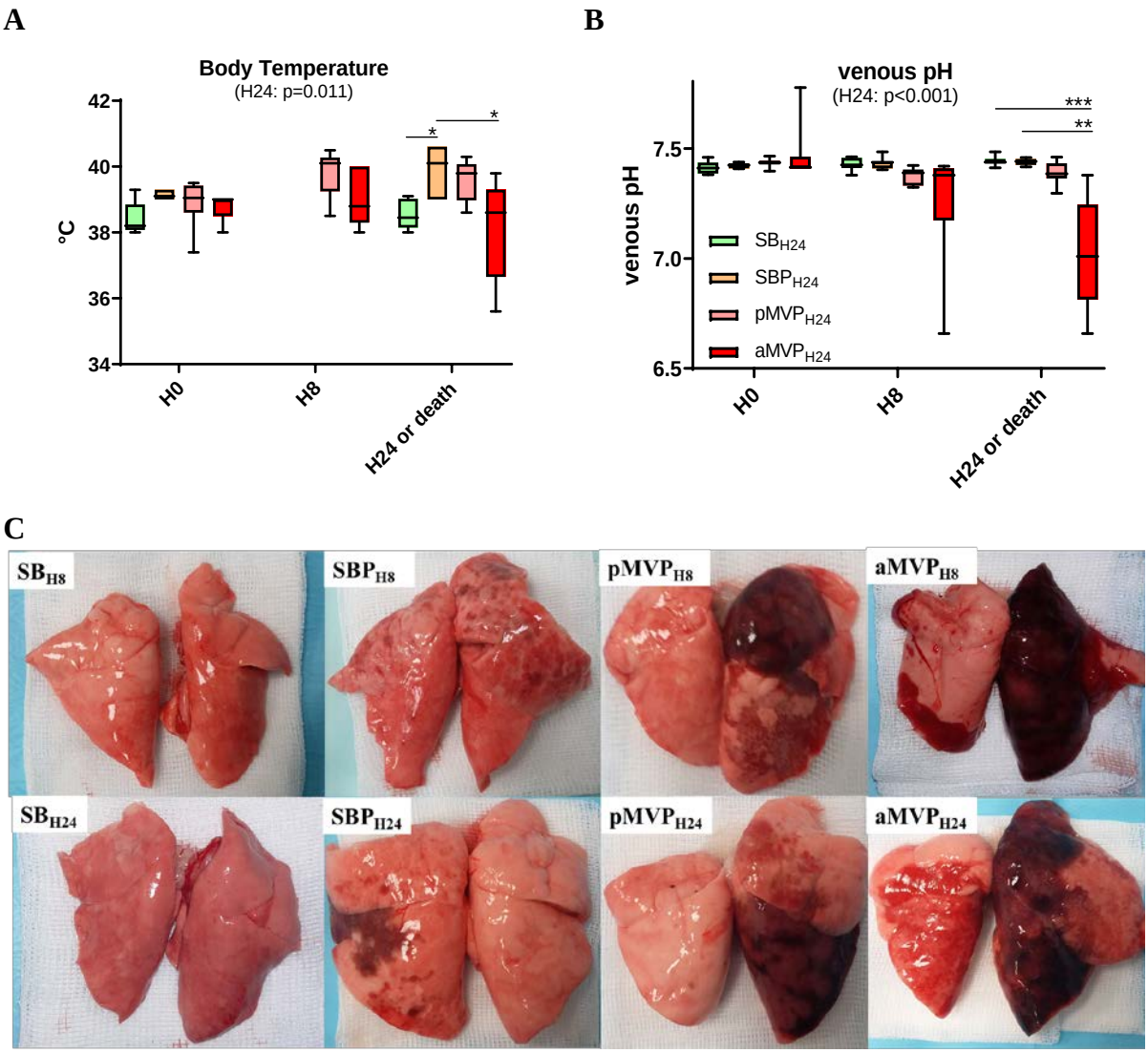


Figure S4. In the setting of pneumococcal pneumonia, adverse mechanical ventilation led to physiological derangements and extensive pneumonia.

(A) Body temperature (not measured at H8 in non-ventilated groups (SB and SBP)); (B) venous pH; (C) lung pictures. Data are expressed as box-and-whisker diagrams (n=7/group). The Kruskal-Wallis test was performed (*p*-value reported for each time point in the figure), and the Dunn's post-hoc correction for multiple comparisons was used when appropriate: *, $p < 0.05$;**, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Figure S5

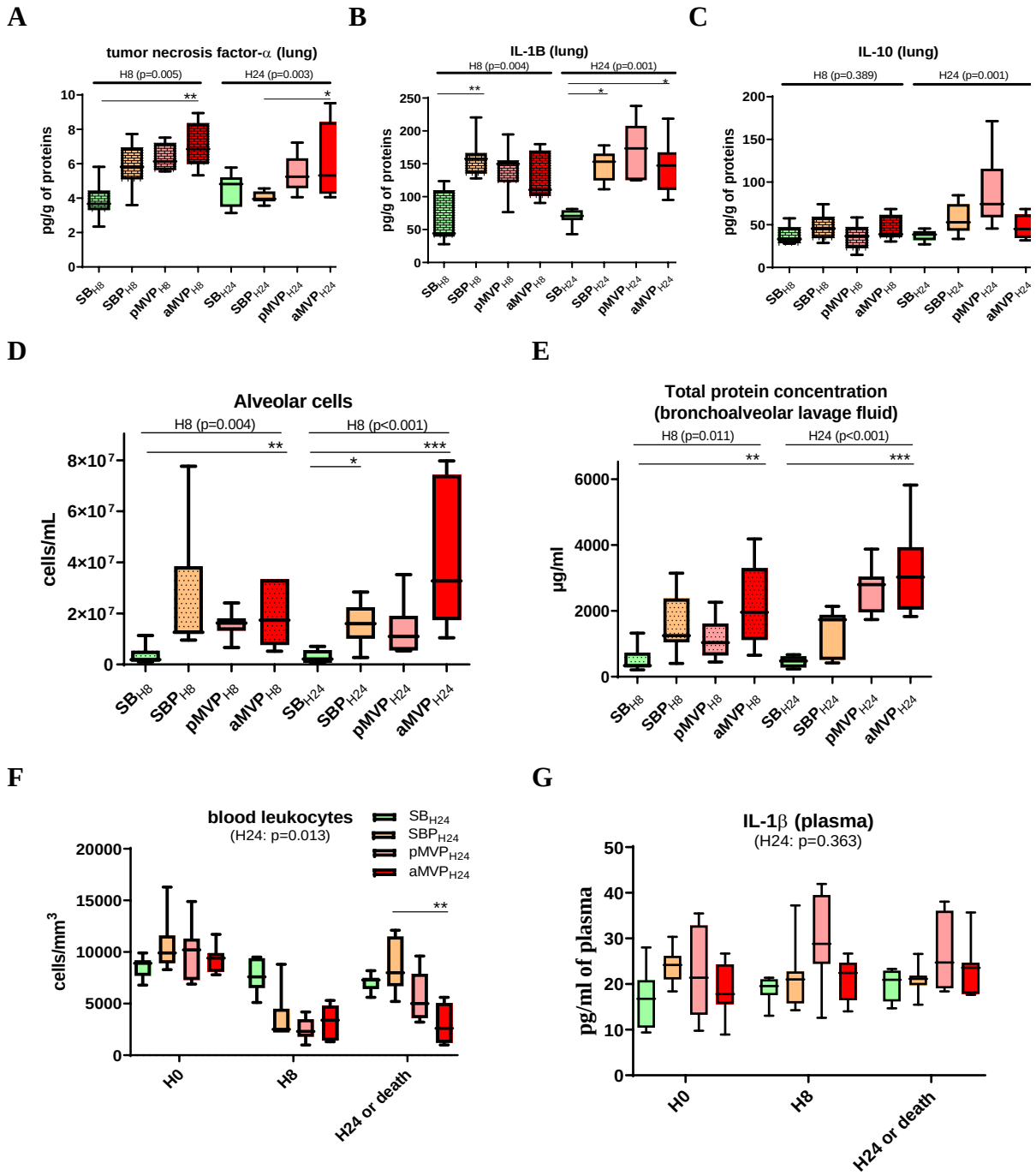


Figure S5. In the setting of pneumococcal pneumonia, adverse mechanical ventilation led to immune derangements in both the lung and the systemic compartments.

Lung concentrations of (A) IL-1β, (B) tumor necrosis factor-α, and (C) IL-10, and alveolar (D) cell count and (E) protein concentration. Blood samples were obtained at baseline (H0), H8 and H24 (or just prior to death if appropriate) to measure: (F) blood leukocyte count, and (G) IL-1β plasma concentrations. Data are expressed as box-and-whisker diagrams (n=7/group). The Kruskal-Wallis test was performed (*p*-value reported for each time point in the figure), and the Dunn's post-hoc correction for multiple comparisons was used when appropriate: *, $p < 0.05$;**, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Figure S6

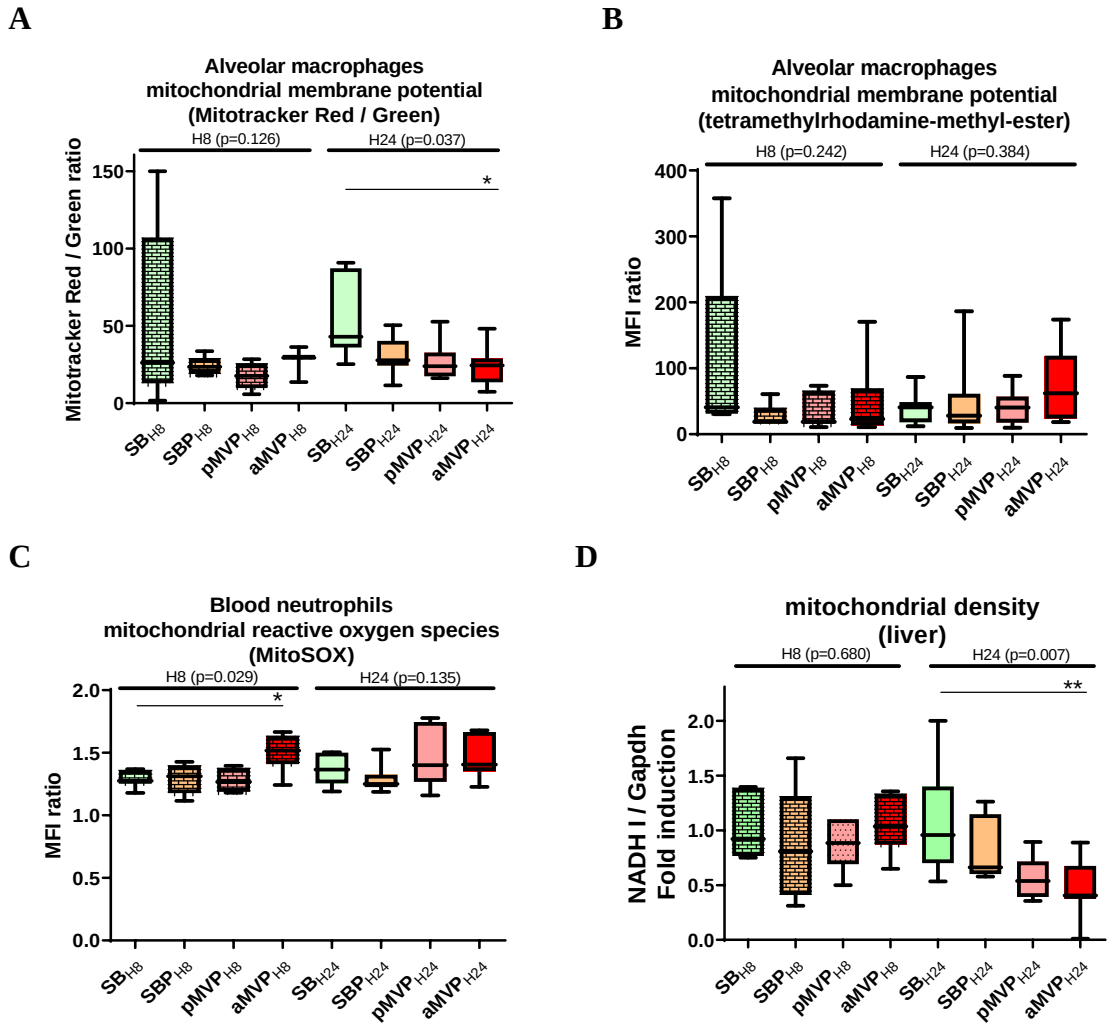


Figure S6. In the setting of pneumococcal pneumonia, adverse mechanical ventilation led to mitochondrial derangements in the lung and systemic compartments.

Flow cytometry was used to measure mitochondrial membrane potential of alveolar macrophages with (A) the Mitotracker Red/Green ratio and (B) the tetramethylrhodamine-methyl-ester probe. (C) The MitoSOX probe was used to measure mitochondrial reactive oxygen species production of blood neutrophils. (D) Mitochondrial DNA (NADH-I) levels were measured in the liver tissue (as a reflection of mitochondrial density) by quantitative polymerase chain reaction. Data are expressed as box-and-whisker diagrams (n=7/group). The Kruskal-Wallis test was performed (*p*-value reported for each time point in the figure), and the Dunn's post-hoc correction for multiple comparisons was used when appropriate: *, $p < 0.05$;**, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Figure S7

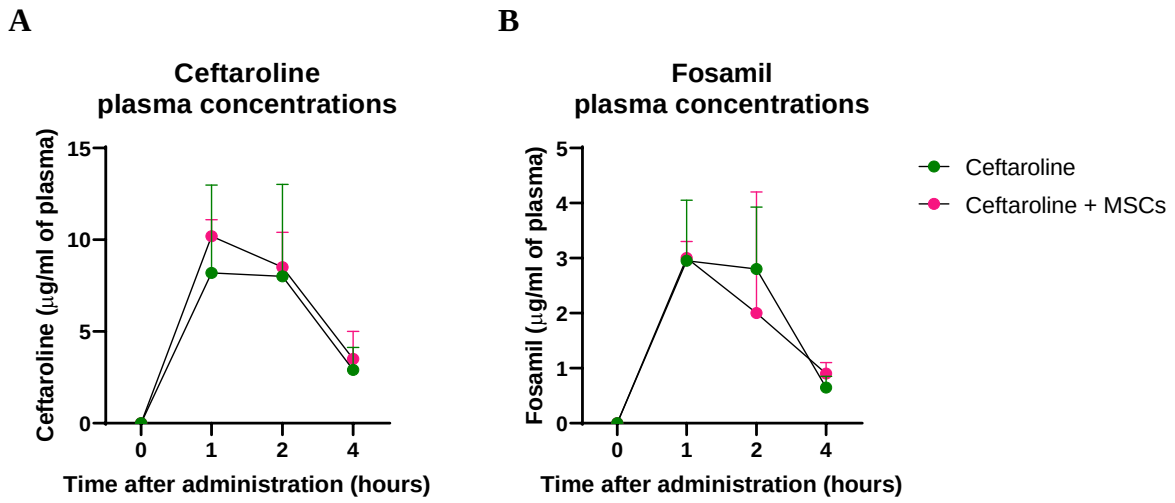


Figure S7. Plasma concentrations of ceftaroline and fosamil.

In the two groups treated with ceftaroline (ceftaroline (n=6) and ceftaroline + MSCs groups; (n=7)), blood sample were taken at different time points: before the intramuscular administration of 20mg/kg ceftaroline-fosamil and then 1, 2 and 4 hours after the infusion. Plasma concentrations of ceftaroline and fosamil were measured by liquid chromatography with tandem mass spectrometry.

Figure S8

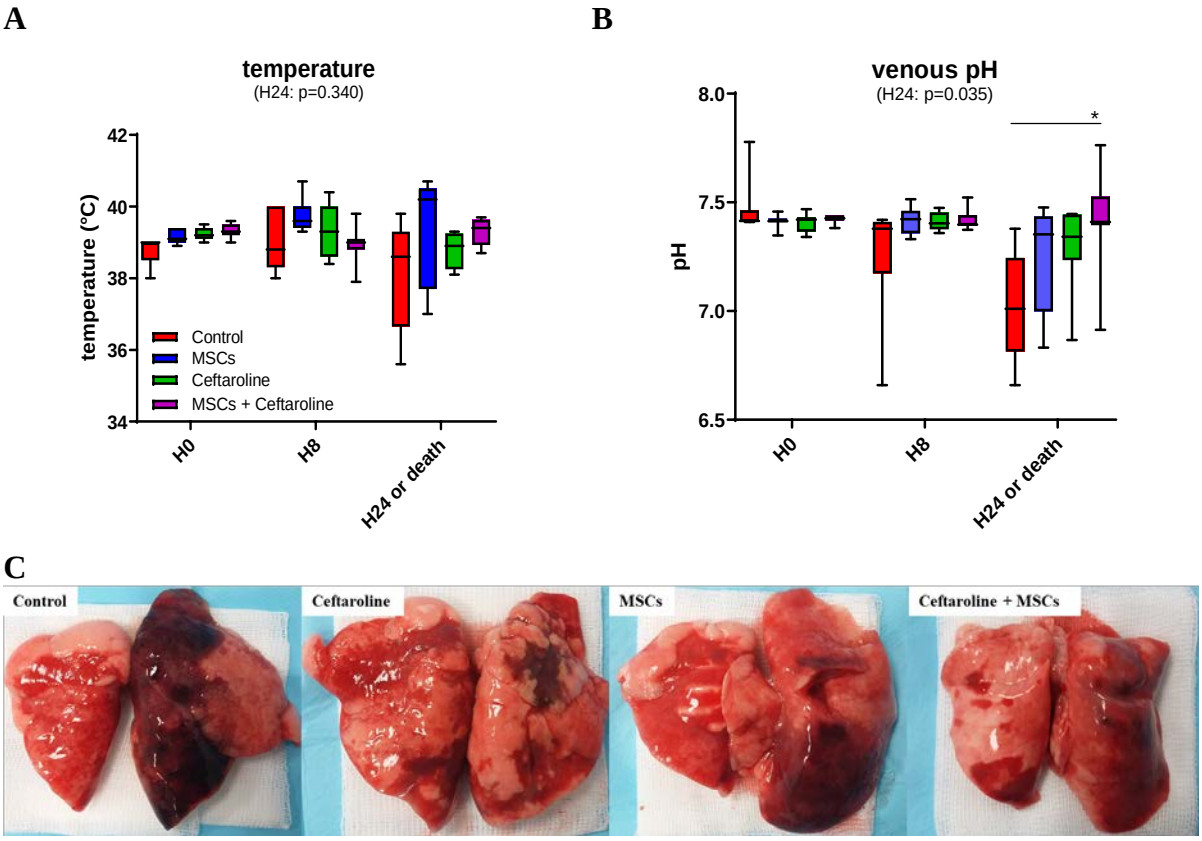


Figure S8. Mesenchymal stem cells contributed to the improvement of the physiological response to pneumococcal pneumonia and the reduction of pneumonia extension in rabbits submitted to adverse mechanical ventilation.

(A) body temperature; (B) venous pH; (C) lung pictures. The Kruskal-Wallis test was performed (*p*-value reported for each time point in the figure), and the Dunn's post-hoc correction for multiple comparisons was used when appropriate: *, $p < 0.05$;**, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Figure S9

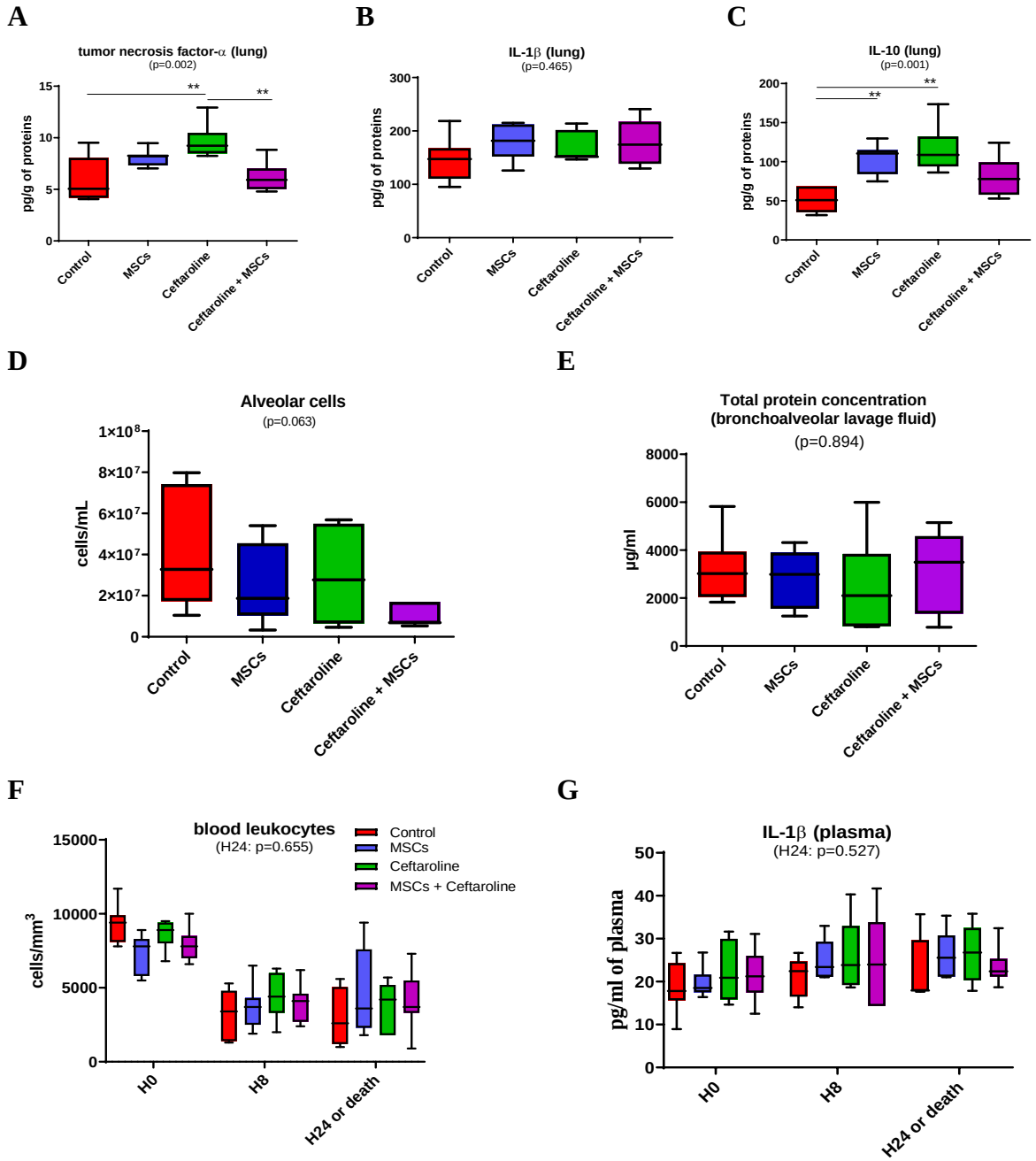


Figure S9. Mesenchymal stem cells contributed to the improvement of the immune response to pneumococcal pneumonia in rabbits submitted to adverse mechanical ventilation.

Lung concentrations of (A) tumor necrosis factor-α (B) IL-1β, (C) IL-10, and alveolar (D) cell count and (E) total protein concentration. Blood samples were obtained at baseline (H0), H8 and H24 (or just prior death if occurred) to measure: (F) leukocyte count, and (G) IL-1β plasma concentrations. The Kruskal-Wallis test was performed (*p*-value reported for each time point in the figure), and the Dunn's post-hoc correction for multiple comparisons was used when appropriate: *, $p < 0.05$;**, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Figure S10

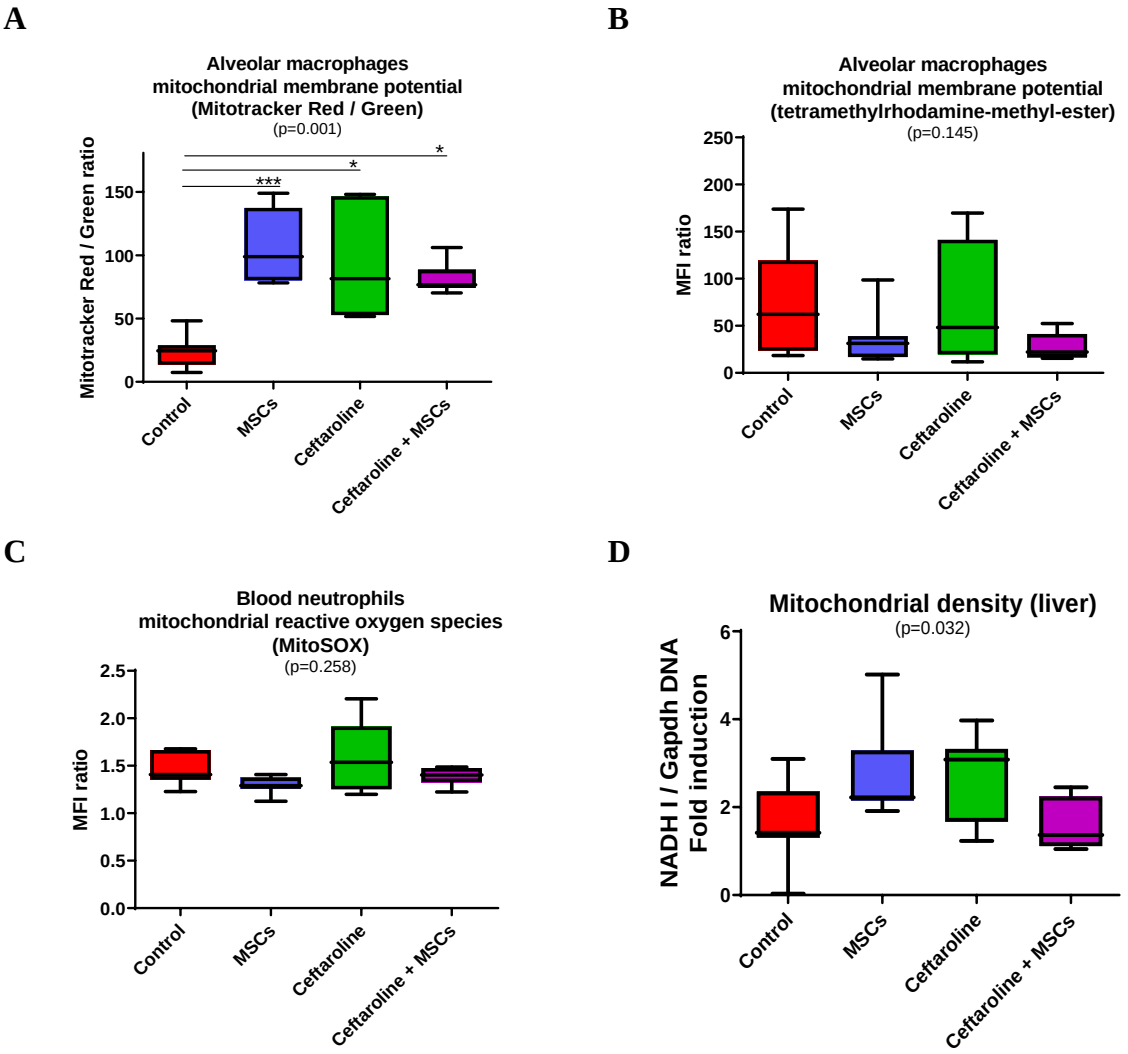


Figure S10 : Mesenchymal stem cells contributed to the resolution of mitochondrial derangements, in rabbits submitted to adverse mechanical ventilation.

Mitochondrial derangements were measured within the alveolar and systemic compartments. The mitochondrial membrane potential of alveolar macrophages was measured by flow cytometry and using (A) Mitotracker Red/Green or (B) tetramethylrhodamine-methyl-ester. (C) Mitochondrial reactive oxygen species production of blood neutrophils was measured by flow cytometry and using the MitoSOX probe. (D) Mitochondrial DNA levels were measured in the liver tissue (as a reflection of mitochondrial density). Data are expressed as box-and-whisker diagrams (n=7/group). The Kruskal-Wallis test was performed (*p*-value reported for each time point in the figure), and the Dunn's post-hoc correction for multiple comparisons was used when appropriate: *, $p < 0.05$;**, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Figure S11

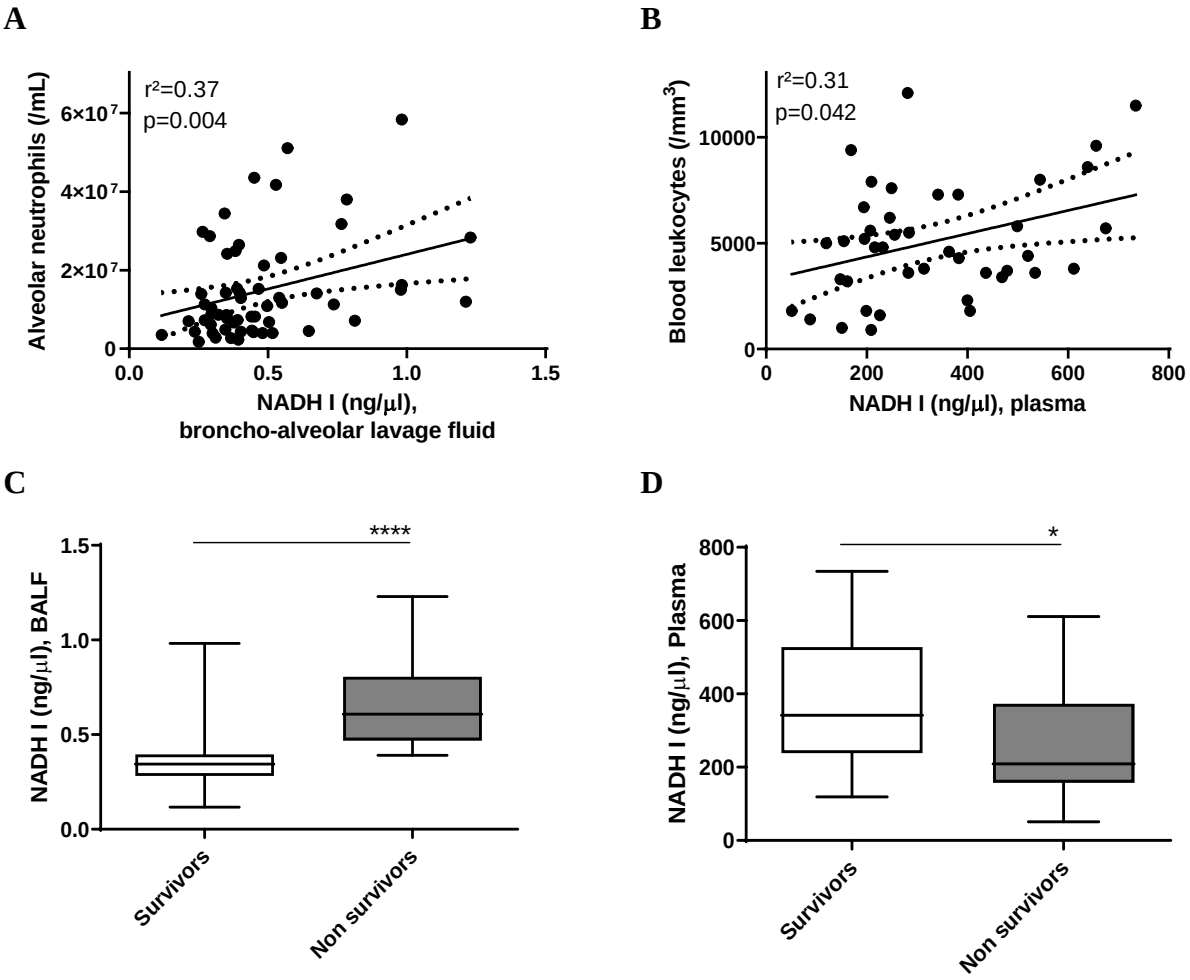
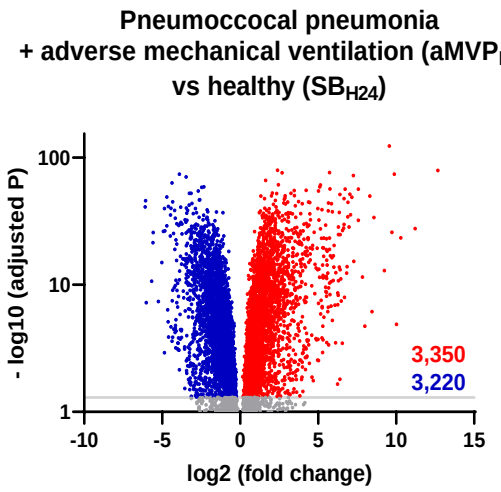


Figure S11. Cell-free extracellular mitochondrial DNA concentrations and blood leukocyte counts are closely related in both bronchoalveolar lavage fluids and plasma.

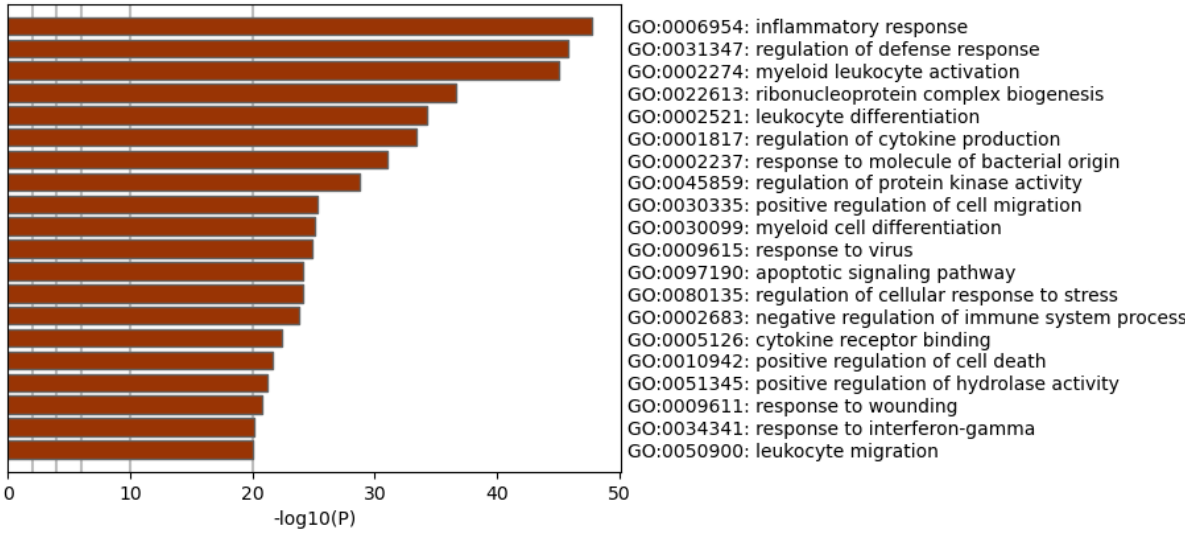
All rabbits infected with *Streptococcus pneumoniae* and assessed at H24 (or before if death occurred earlier) were considered for the analysis, regardless of mechanical ventilation or treatment. First, we observed a significant correlation (A) between mitochondrial DNA concentrations in bronchoalveolar lavage fluids measured by quantitative polymerase chain reaction, and alveolar neutrophils count, and (B) between plasma mitochondrial DNA concentrations and blood leukocyte counts. Then, we investigated the prognostic value of bronchoalveolar lavage fluids and plasma mitochondrial DNA concentrations and found (C) a significant association between mitochondrial DNA alveolar concentrations and mortality (p=0.004), and (D) an inverse association between plasma mitochondrial DNA concentrations and mortality (p=0.042).

Figure S12

A **RNA-seq volcano plot**



B **Up-regulation**



C **Down-regulation**

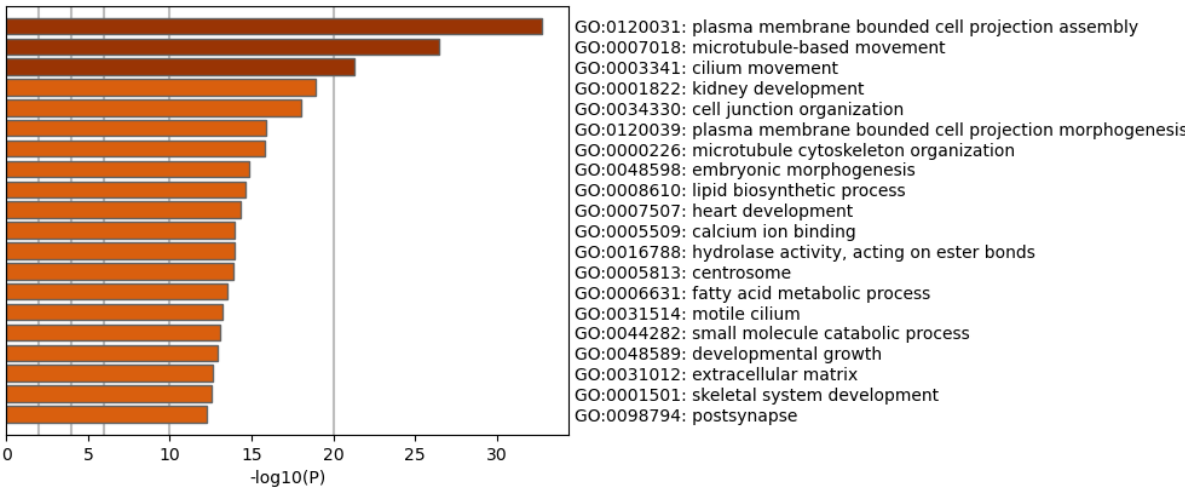


Figure S12. Transcriptomic responses in the lungs of animals submitted to the two adverse conditions (*Streptococcus pneumoniae* infection and adverse mechanical ventilation) as compared to healthy rabbits.

Pulmonary gene expression was analyzed in the lung of rabbits using transcriptomics analysis. Animals submitted to *Streptococcus pneumoniae* pneumonia + adverse mechanical ventilation ($aMVP_{H24}$) were compared to uninfected control rabbits (SB_{H24}) (A-C). Volcano plot (integrating adjusted *p*-values and fold expression [log_2 fold change] depicting the global alteration in gene expression. The horizontal line indicates False Discovery Rate adjusted $p < 0.05$. Red dots denote over-expressed genes; blue dots indicate under-expressed genes (A). Gene Ontology term analysis of upregulated (B) or down-regulated (C) genes according to Metascape analysis.