Supplementary Tables and Figures

Supplementary Tables

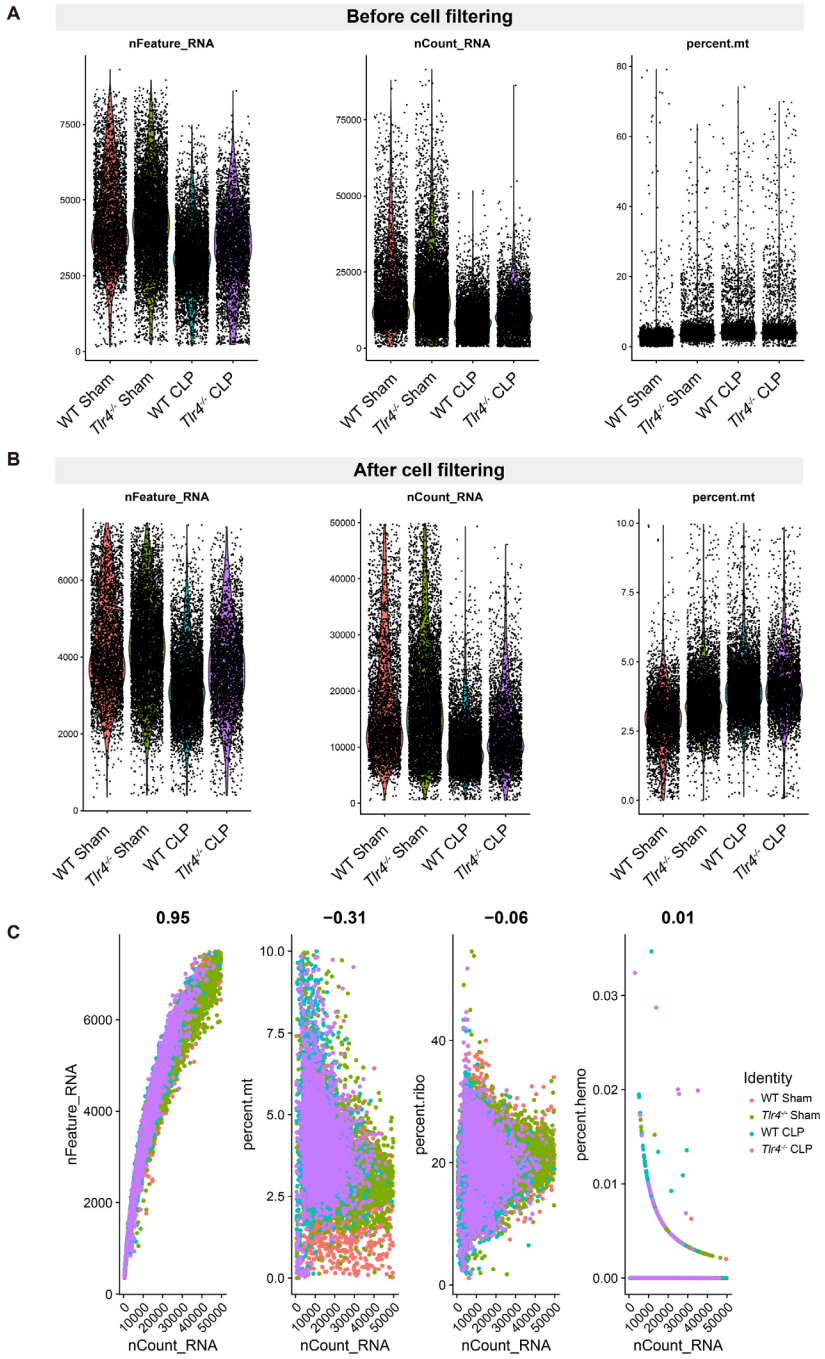
Supplementary Table 1. A list of antibodies used for western blot (WB) and immunofluorescence (IF).

Name of antibody	Cat. No	Company	Mol weight	Dilution (WB/IF)
TLR4	sc-293072	Santa Cruz	100 kDa	1:1000 (WB); 1:100 (IF)
β-actin	#93473	CST	45 kDa	1:1000 (WB)
ABCA1	MA5-16026	ThermoFisher	220 kDa	1:100 (IF)
F4/80	ab300421	Abcam	150 kDa	1:100 (IF)
GAPDH	#5174	CST	37 kDa	1:1000 (WB)
ZO-1	ab276131	Abcam	220 kDa	1:1000 (WB)
Occludin	#68534	CST	65 kDa	1:1000 (WB)

Supplementary Table 2. A list of antibodies used for flow cytometry (FC).

Name of antibody	Cat. No	Company	Conjugate	Dilution (FC)
iNOS	53-5920-82	ThermoFisher	Alexa Fluor™ 488	0.06 μg/test
Arg1	12-3697-82	ThermoFisher	PE	0.5 μg/test
CD206	12-2061-82	ThermoFisher	PE	0.125 μg/test
F4/80	15-4801-82	ThermoFisher	PE-Cyanine5	0.25 μg/test
PDPN	127415	Biolegend	FITC	0.5 μg/test
CD31	160203	Biolegend	PE	0.25 μg/test

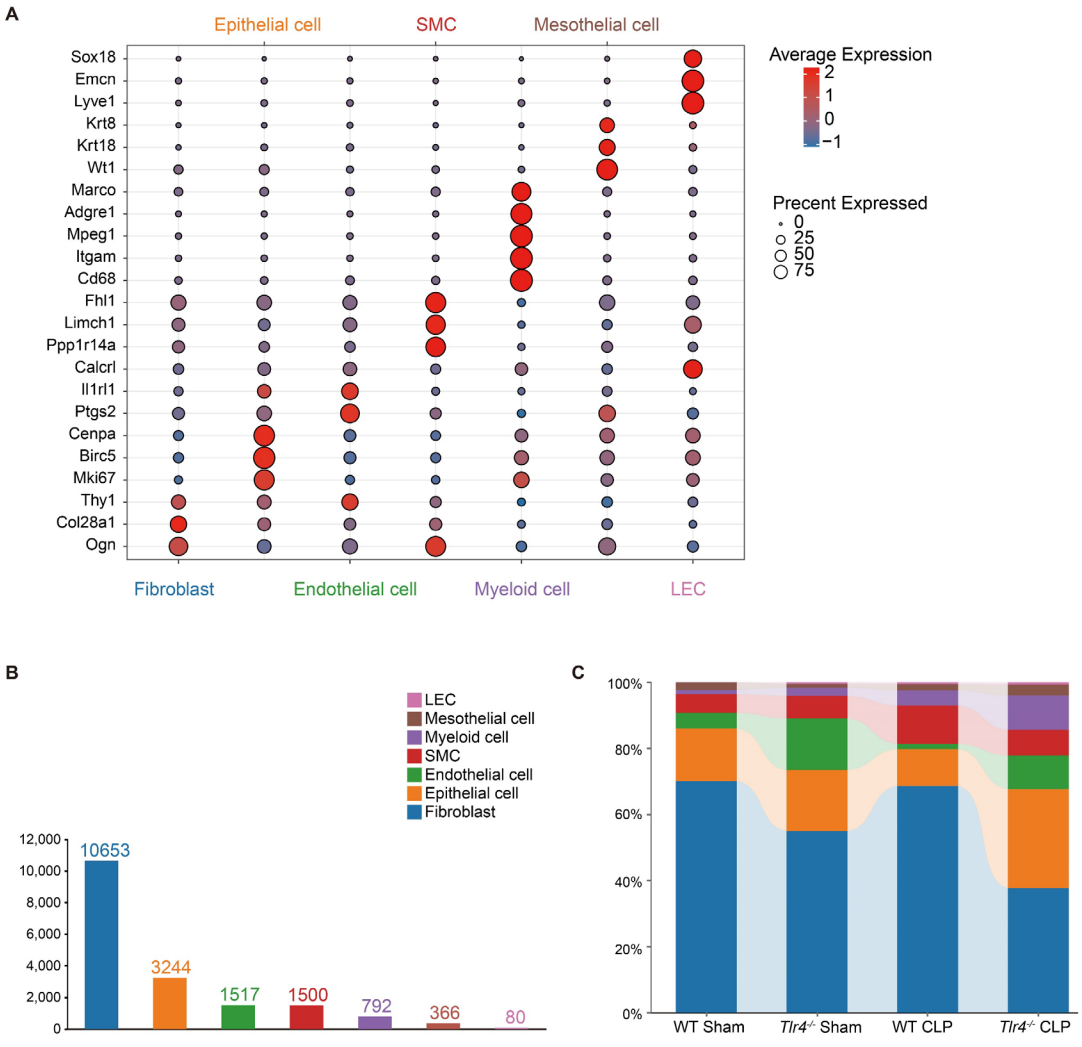
Supplementary Figures and Figure Legends



Supplementary Figure 1. scRNA-seq data sources and quality control.

A. Violin plots showing the distribution of detected features (nFeature-RNA), total RNA counts (nCount-RNA), and the percentage of mitochondrial gene expression (percent.mt) across all groups prior to cell filtering. These plots provide a summary of cell quality metrics before excluding low-quality cells. **B.** Violin plots showing the same metrics (nFeature-RNA, nCount-RNA, and percent.mt) after filtering for cell

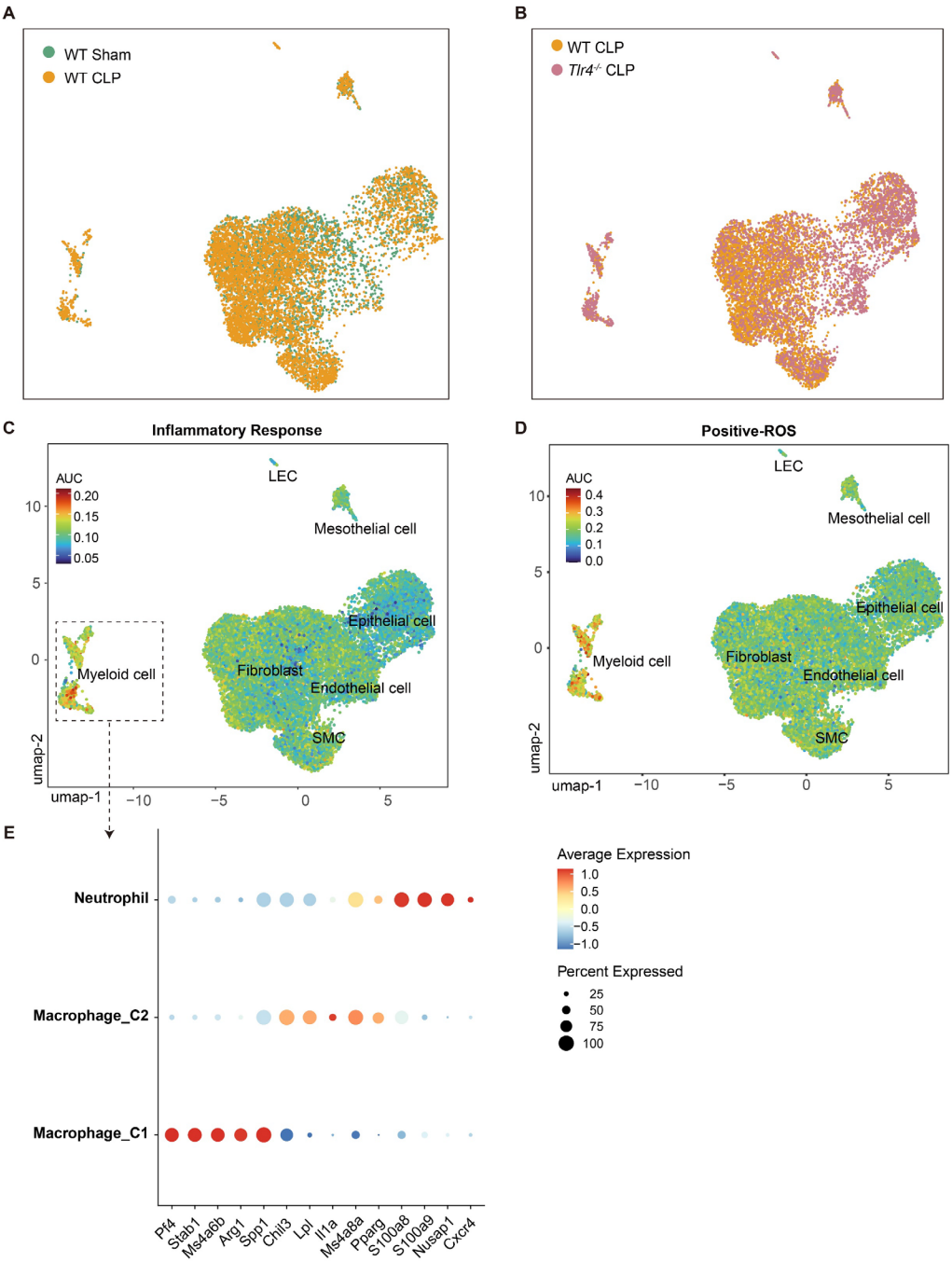
quality. The data shows reduced variability and improved consistency in cell quality, indicating the successful removal of low-quality cells. **C.** Scatter plots demonstrating the correlation between nCount-RNA and other metrics: nFeature-RNA, percent.mt (mitochondrial genes), percent.ribo (ribosomal genes), and percent.hemo (hemoglobin genes). The correlation coefficients are shown above each plot, with a strong positive correlation between nCount-RNA and nFeature-RNA (0.95), while weaker correlations with percent.mt, percent.ribo, and percent.hemo suggest minimal impact of these metrics on total RNA counts.



Supplementary Figure 2. Cell type markers and counts.

A. Dot plot showing cell type markers used to identify distinct cell clusters. Dot size represents the proportion of cells expressing each marker, while color intensity

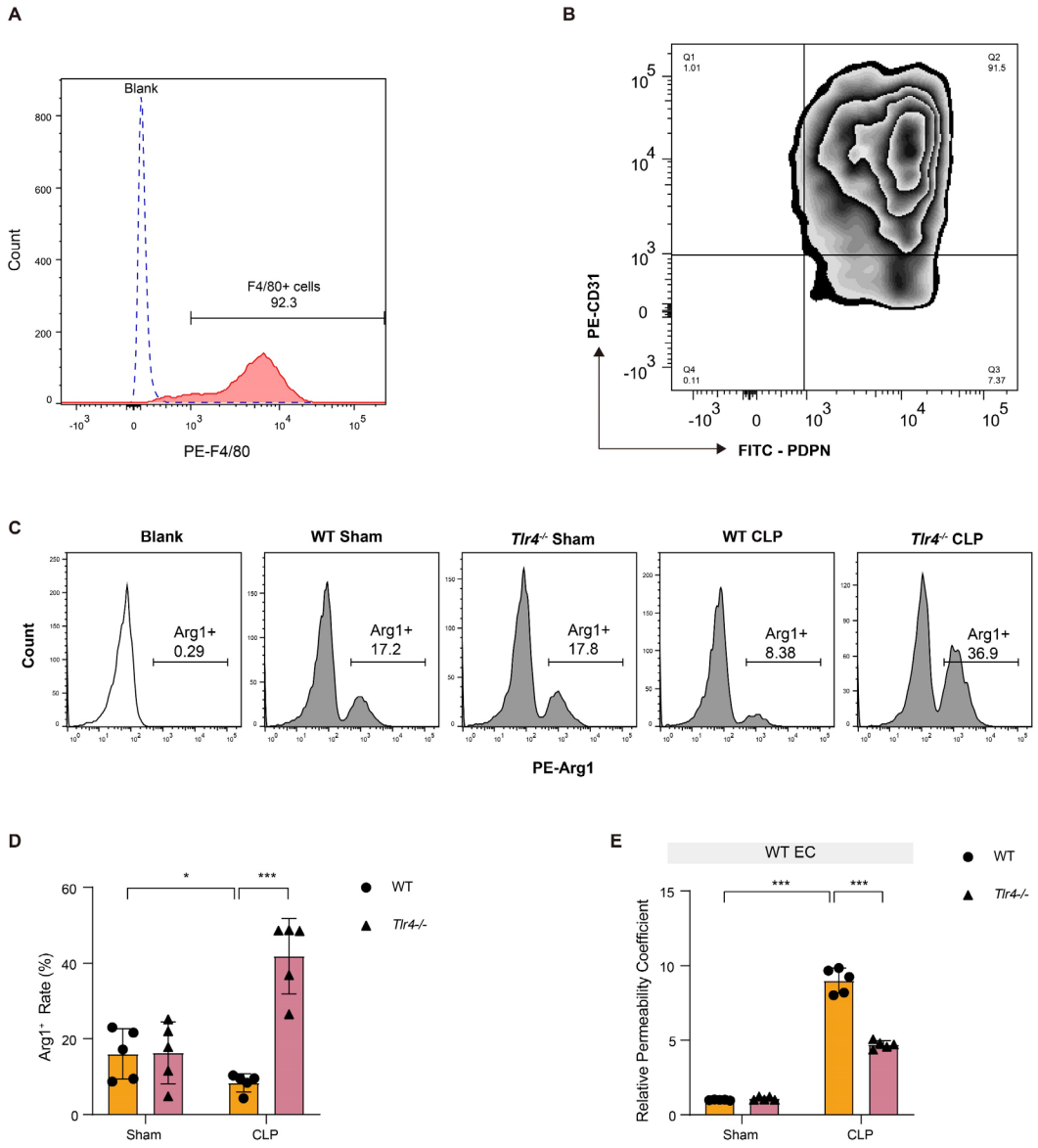
indicates the average expression level. **B.** The bar graph illustrates the absolute count of each cell type. **C.** The stacked bar chart depicts the proportional distribution of cell types within each experimental group.



Supplementary Figure 3. Single-cell transcriptional analysis reveals distinct cellular responses in sepsis-induced ALI.

A. UMAP showing the localization of cells from WT Sham and WT CLP groups. **B.** UMAP showing the localization of cells from *Tlr4*$^{-/-}$ CLP and WT CLP groups. **C.**

UMAP representation of all cell types, with cells colored by the area under the curve (AUC) scores using AUCell to represent the inflammatory response signature (derived from the HALLMARK_INFLAMMATORY_RESPONSE gene set in MSigDB). **D.** UMAP representation of all cell types, showing AUC scores for positive reactive oxygen species (ROS) signature (derived from the relevant gene set in MSigDB). **E.** Dot plot showing marker genes used to identify distinct immune cell subpopulations. The size of each dot represents the percentage of cells expressing the marker gene within each immune cell cluster, while the color intensity indicates the average expression level. Three major immune cell populations were identified: Macrophage_C1, Macrophage_C2, and neutrophils.



Supplementary Figure 4. Validation of cell isolation and characterization of macrophages and ECs.

A. Flow cytometric analysis of F4/80+ cells to assess macrophage purity extracted from BALF. **B.** Flow cytometric analysis using PDPN and CD31 markers to assess the purity of LECs isolated from lung tissue. **C-D.** The percentage of $Arg1^+$ macrophages from the BALF of each mouse group was detected by flow cytometry (n=5). **E.** Assessment of endothelial barrier function by FITC-dextran permeability assay. WT ECs were co-cultured with macrophages isolated from BALF of WT Sham, *Tlr4*$^{-/-}$ Sham, WT CLP, and *Tlr4*$^{-/-}$ CLP mice for 24 hours in a Transwell system. Endothelial permeability was evaluated by adding FITC-dextran (10 kDa, 10 mg/ml)

to the upper chamber for 2 hours. Data are presented as normalized relative permeability coefficient (n=5). Data are represented as mean ± SD, *P < 0.05, ***P < 0.001.

Supplementary Figure 5. The original uncropped blot/gel images of the main figures.