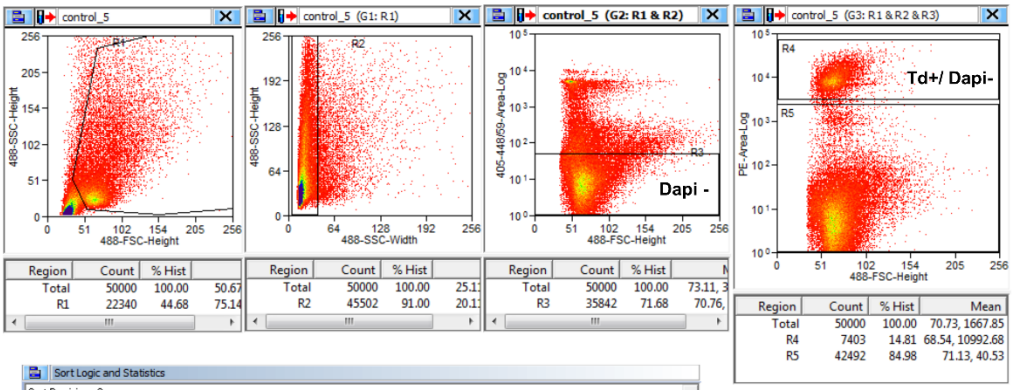
SUPPLEMENTAL MATERIAL

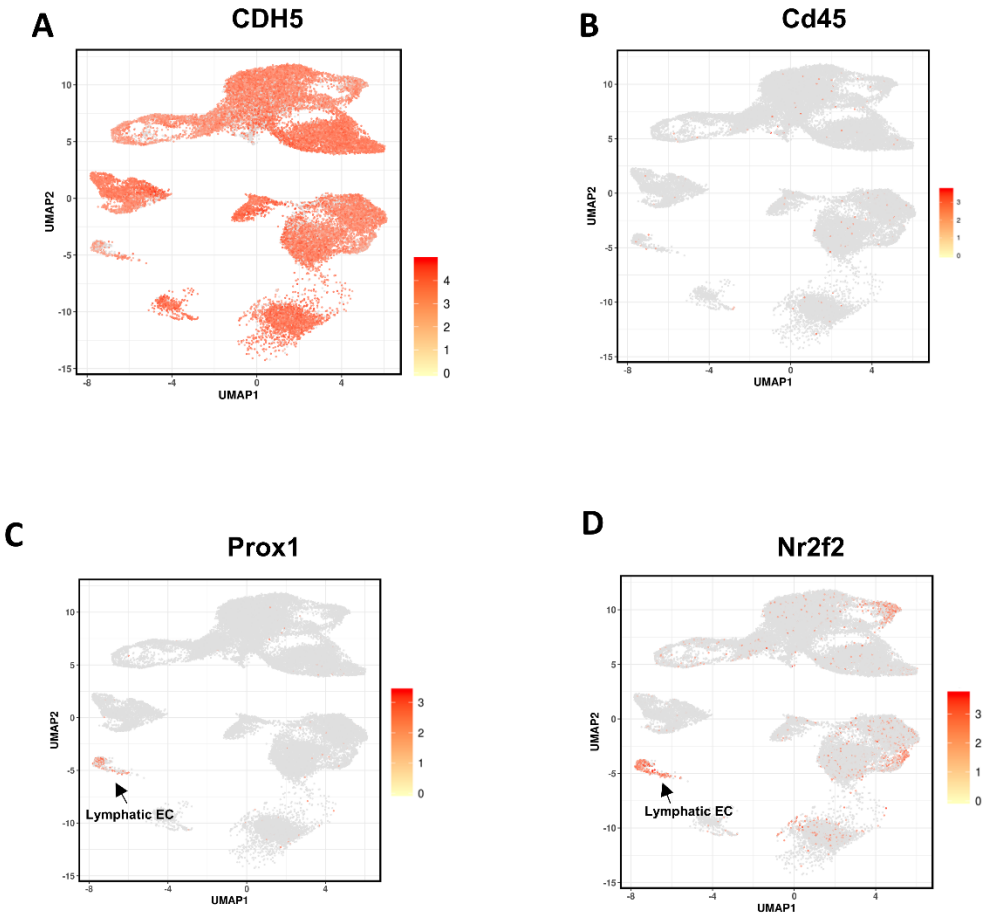


Sort Logic and Statistics

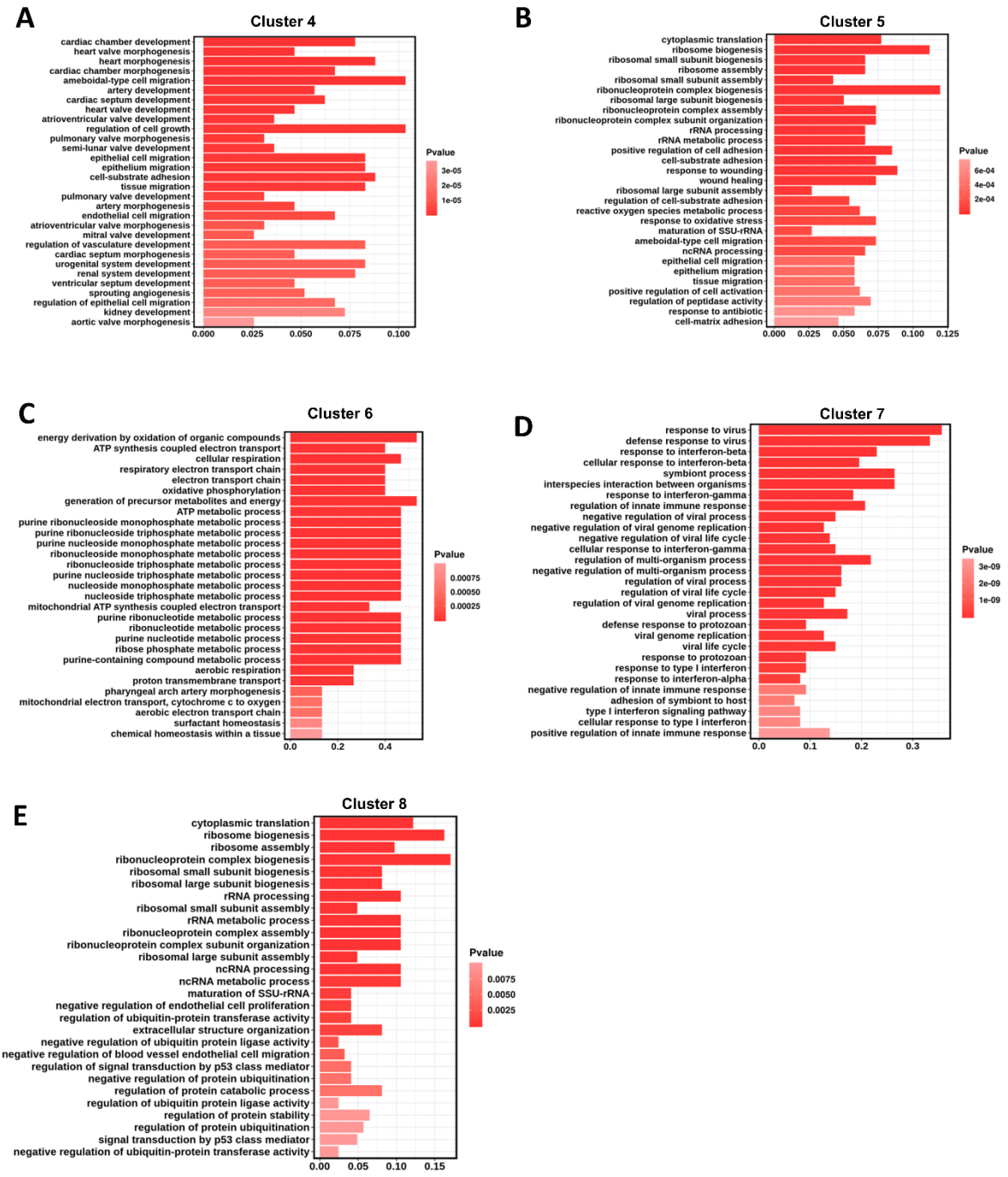
Sort Decisions 3

	Left 2	Left 1	Right 2	Right 3
Logic	R1 & R2 & R3 & R5		R1 & R2 & R3 & R4	
Limit	None	None	None	None
Abort Mode	Single	Purify	Single	Purify
Drop Envelope	0.5	1-2	0.5	1-2
Abort Stream	Waste	Waste	Waste	Waste
Sort Count	774412	0	139354	0
Sort Rate	934	0	168	0
Abort Count	2180266	0	374817	0
Abort Rate	2630	0	452	0
% Total	6.73	0.00	1.21	0.00
% Efficiency	26.21	N/A	27.10	N/A
Sigma Sort	1972136	0	357163	0
Sigma Abort	5633759	0	973062	0

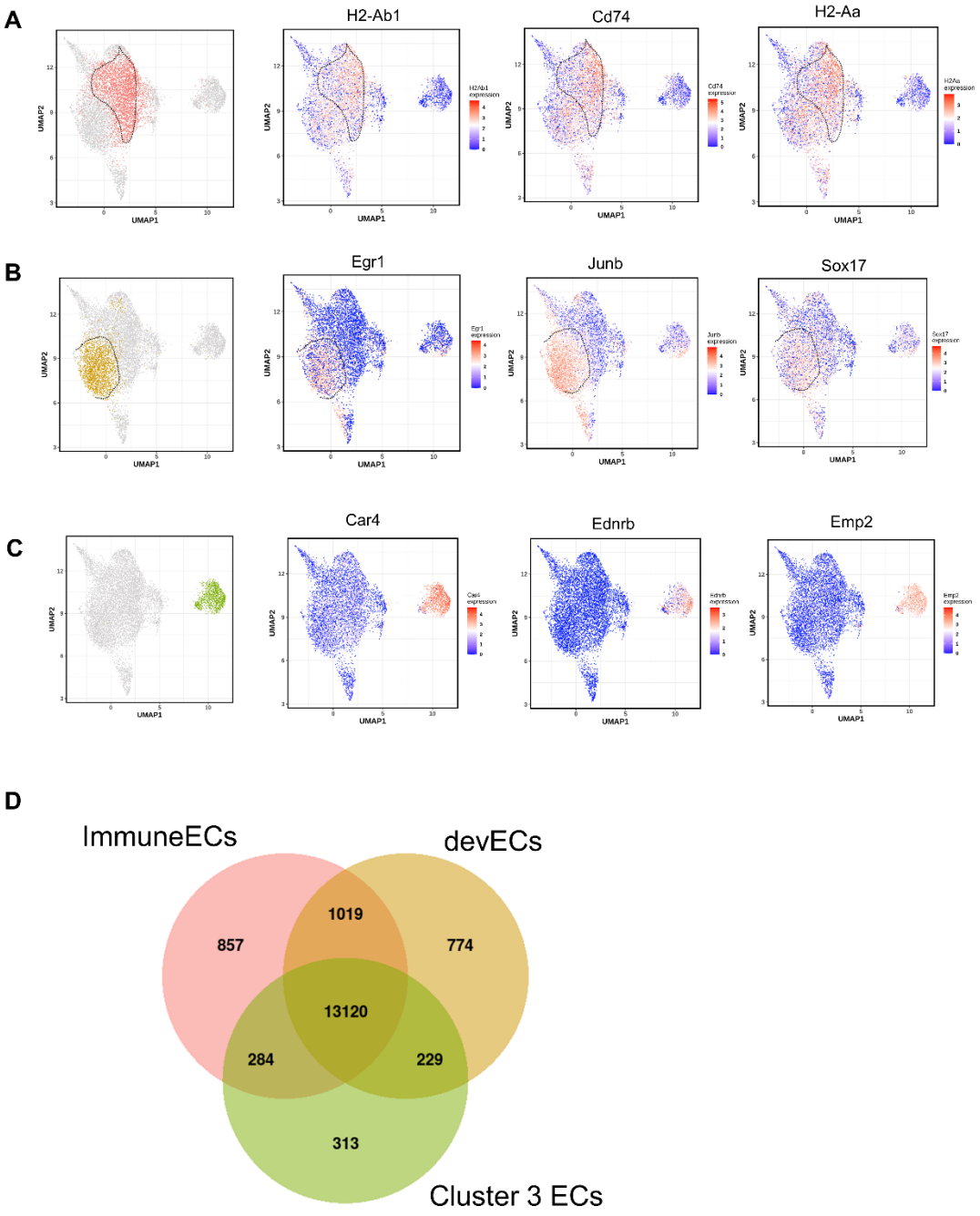
Supplemental Figure 1. The gating condition for sorting tdTOMATO (Td)+ /DAPI– cells in Fluorescence-activated cell sorting (FACS).



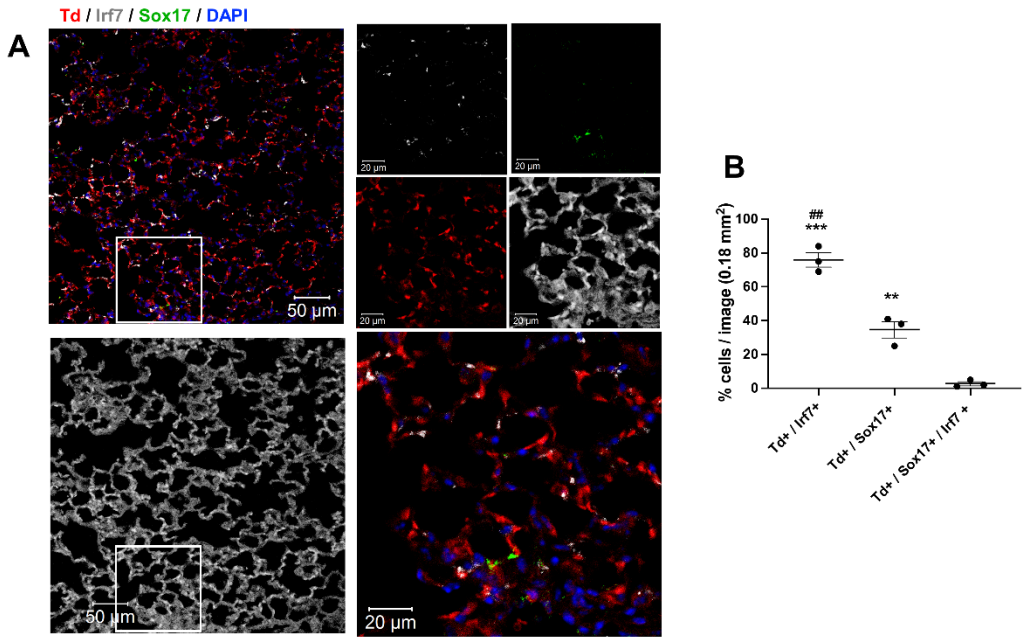
Supplemental Figure 2. The UMAP of gene expression levels in lung endothelial cells of all time points. (**A**) *Cdh5*, (**B**)*Cd45*, (**C**) *Prox1* and (**D**) *Nr2f2*. Each dot represents an individual cell. The color bars show gene expression level in $\log_2$ scale. Light red to red color represents low to high expression level.



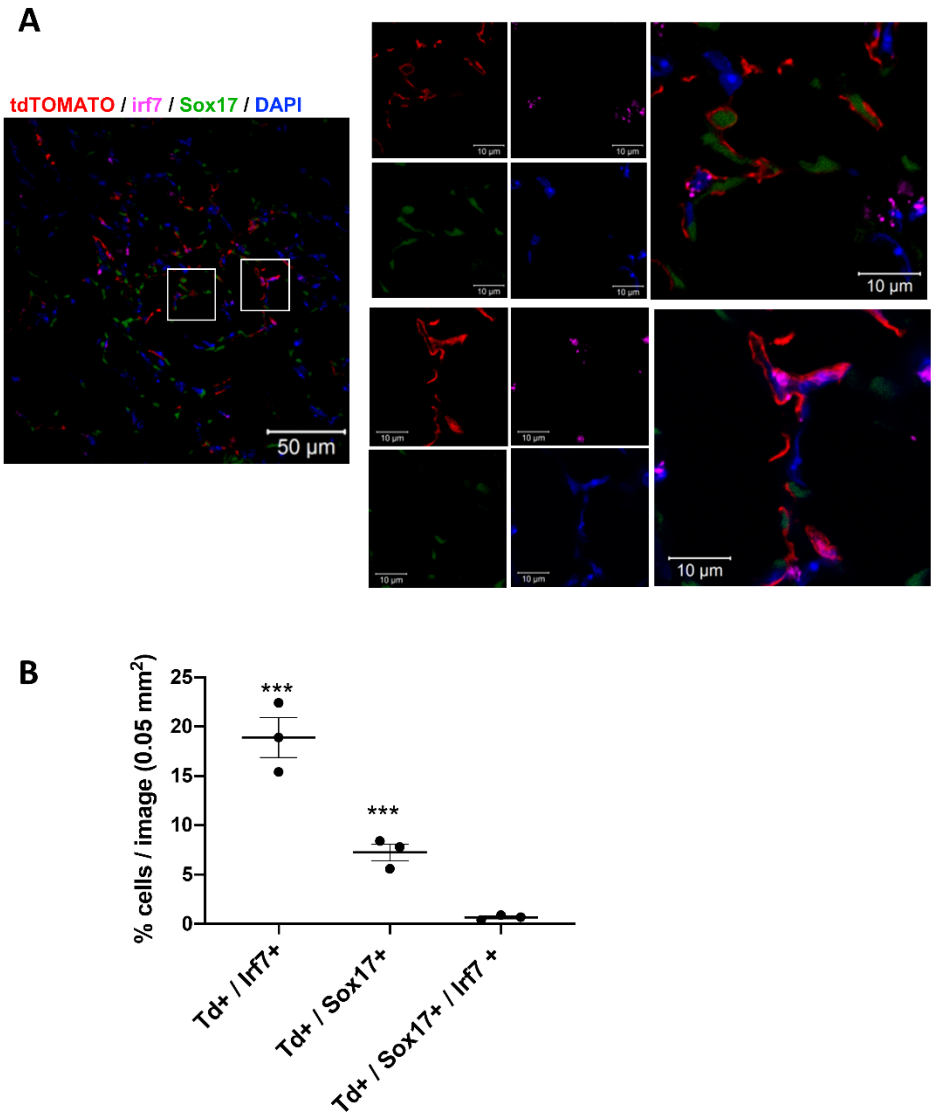
Supplemental Figure 3. The biological process GO terms for cluster4 (**A**), 5 (**B**), 6 (**C**), 7 (**D**), and 8 (**E**) at baseline with P values indicated as the color of the bars. Light red to red represents low to high significance.



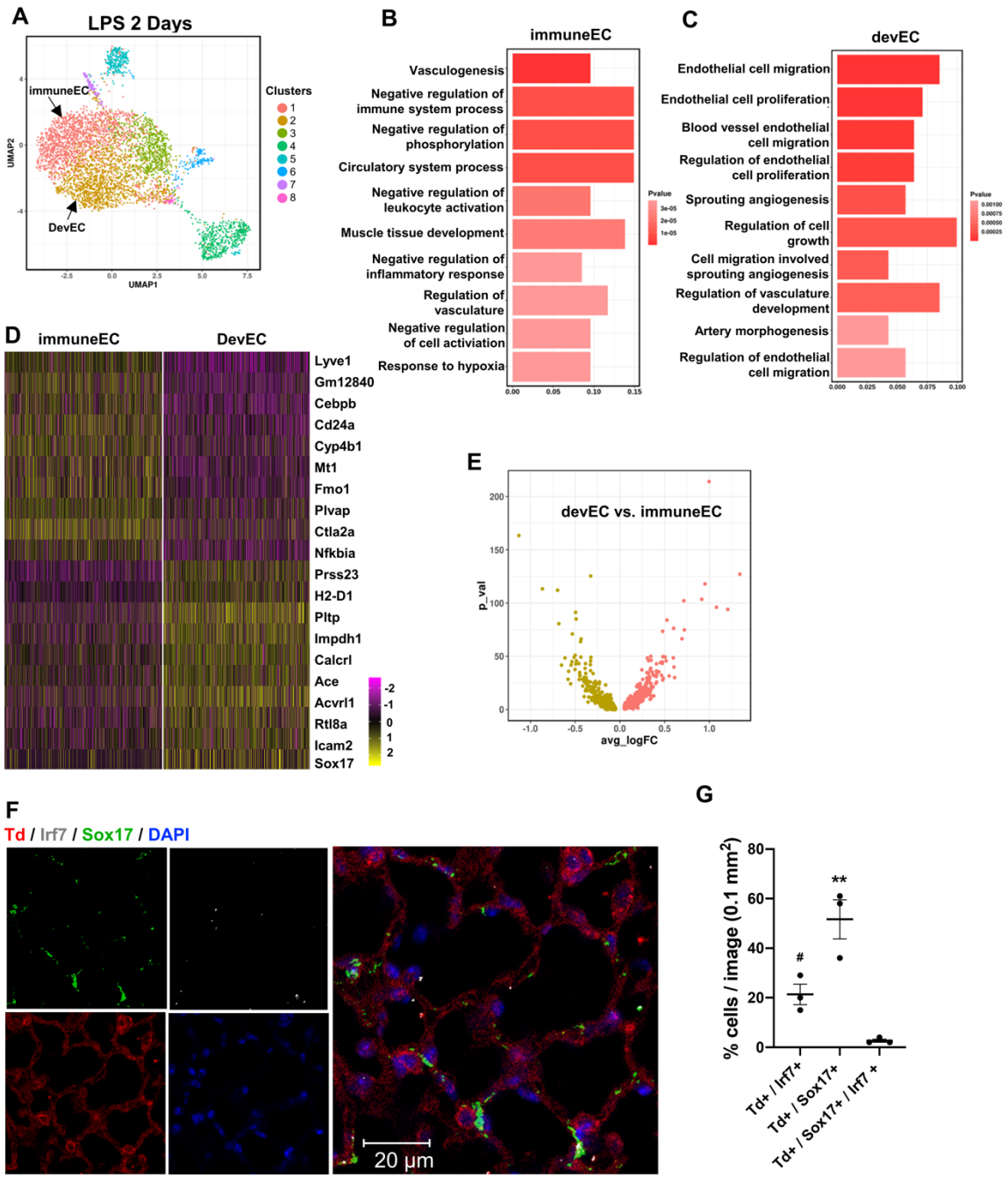
Supplemental Figure 4. (**A-C**) UMAPs to visualize immuneEC (**A**), devEC (**B**), and cluster 3 ECs (**C**), and the expression levels of their representative marker genes. The color bars in UMAPs indicate gene expression levels in $\log_2$ scale. (**D**) Venn diagram of shared and differentially expressed genes among three major subpopulations at baseline – immuneECs, devECs and cluster 3 ECs (Car4 high EC population).



Supplemental Figure 5. Confocal images (A) and quantification (B) of immunofluorescence of mouse lung paraffin embedded tissues following LPS treatment for 6 hours. The lung structures are shown in A by enhancing autofluorescence of lung tissues. Lung ECs expressed tdTomato (Td) in tamoxifen-treated tdTomato$^{fl/fl}$:Cdh5-CreERT(+) mice. Irf7 and Sox17 proteins are identified by immunofluorescence as grey and green respectively. Nuclei were stained by DAPI. The small region is zoomed in as shown on the right. Scale bars are 50μm on the left and 20μm on the right. The statistical analysis of numbers of Td+/Irf7+, Td+/Sox17+, and Td+/Sox17+/Irf7+ cells in lung sections of mice were shown in B. Data are shown as means ± SE from three independent mice. ***: P<0.001 and **:P<0.01 compared to Td+/Sox17+/Irf7+ cells, ##: P<0.01 compared to Td+/Sox17+ by one-way ANOVA.

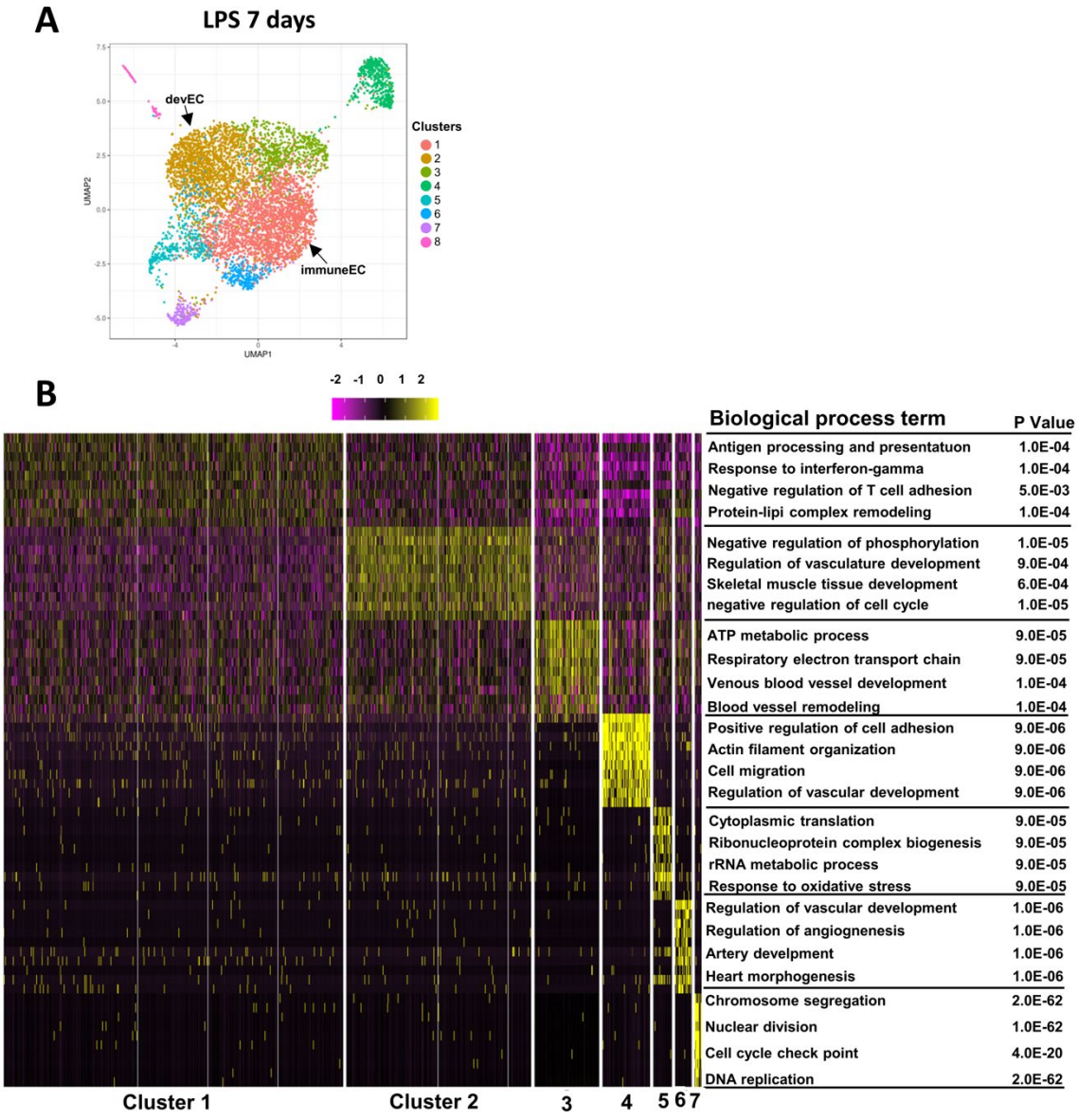


Supplemental Figure 6. (A) Confocal images of RNA FISH of mouse lung sections following LPS treatment for 24 hours. The lung ECs expressed tdTOMATO (Td). The RNAs of Irf7 and Sox17 were probed and labeled with magenta and green respectively. Nuclei were stained by DAPI. The small regions in square are shown at higher magnification on the right. Scale bar is 50μm on the left and 10μm on the right. (B) The statistical analysis of numbers of Td+/Irf7+, Td+/Sox17+, and Td+/Sox17+/Irf7+ cells in lung sections of mice following LPS treatment for 24 hours. Data are shown as means ± SE from three independent mice with each dot presenting one mouse. ***: P<0.001 compared to Td+/Sox17+/Irf7+ cells, by one-way ANOVA.

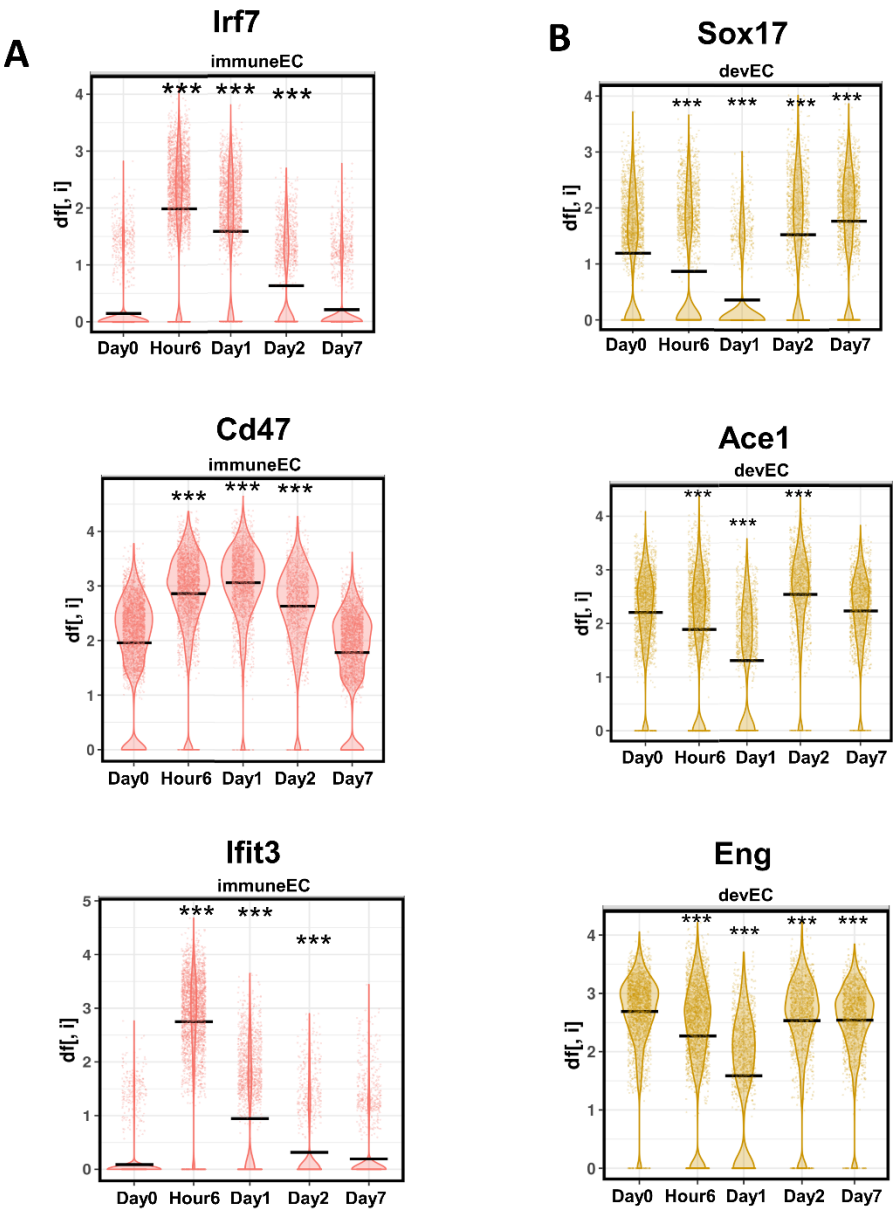


Supplemental Figure 7. Analysis of inflammatory and developmental lung EC subpopulations of lung ECs during the resolution phase (LPS treatment 2 days). (**A**) The UMAP of 4,608 individual lung ECs isolated at 2 days following systemic LPS injury. Different colors indicate different clusters with immuneEC and devEC labeled. (**B-C**) The GO terms of immuneEC (**B**) and devEC (**C**) indicate the enriched biological processes in each cluster. P values are indicated on the left in a color scale. (**D**) The heatmap of most differentially expressed genes of immuneEC and devEC subpopulations with the color bar showing gene expression levels on a $\log_2$ scale. (**E**) The volcano plot of immuneEC and devEC with differentially expressed genes between devEC and immuneEC. The genes were selected by $\log_2$ fold change $p< 0.05$. The x-axis is the average expression log fold change. y axis is the log p-value. (**F-G**) Confocal images

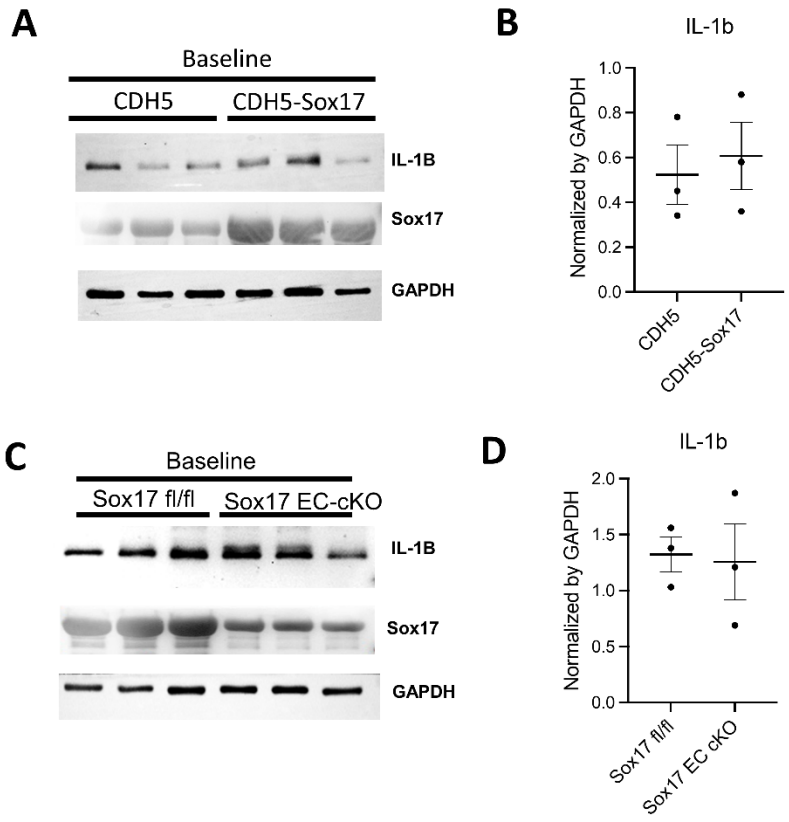
(**F**) and quantification (**G**) of protein immunostaining in mouse lung sections following LPS treatment for 2 days. Irf7 and Sox17 proteins are identified by immunofluorescence as grey and green respectively. Scale bars are 20μm. The quantification of Td+/Irf7+, Td+/Sox17+, and Td+/Sox17+/Irf7+ cells in lung sections of mice is shown in **I**. Data are shown as mean ± SE from three independent mice. **: $P<0.01$ compared to Td+/Sox17+/Irf7+ cells, #: $P<0.05$ compared to Td+/Sox17+ by one-way ANOVA.



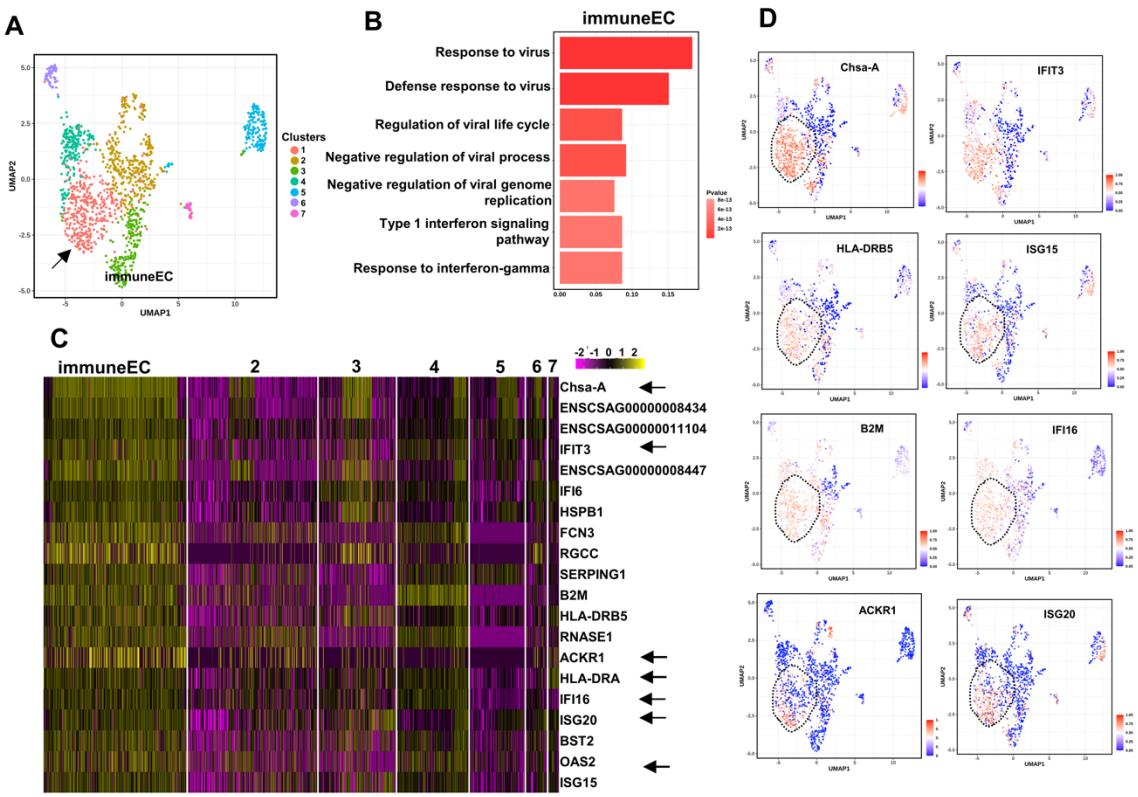
Supplemental Figure 8. (**A**) The UMAP of 6,171 individual lung endothelial cells following LPS treatment 7 days clustered by unbiased Seurat (v2.3) R package with different colors presenting different clusters. The immuneEC (cluster 1) and devEC (cluster 2) were indicated. (**B**) The heatmap of most differentiated genes of lung endothelial cells following LPS treatment 7 days among cluster 1 to 7 with representative biological process GO terms (Biological Process terms) and P value indicated in left. The color bars indicate gene expression level in $\log_2$ scale. Purple to yellow color represents low to high expression level.



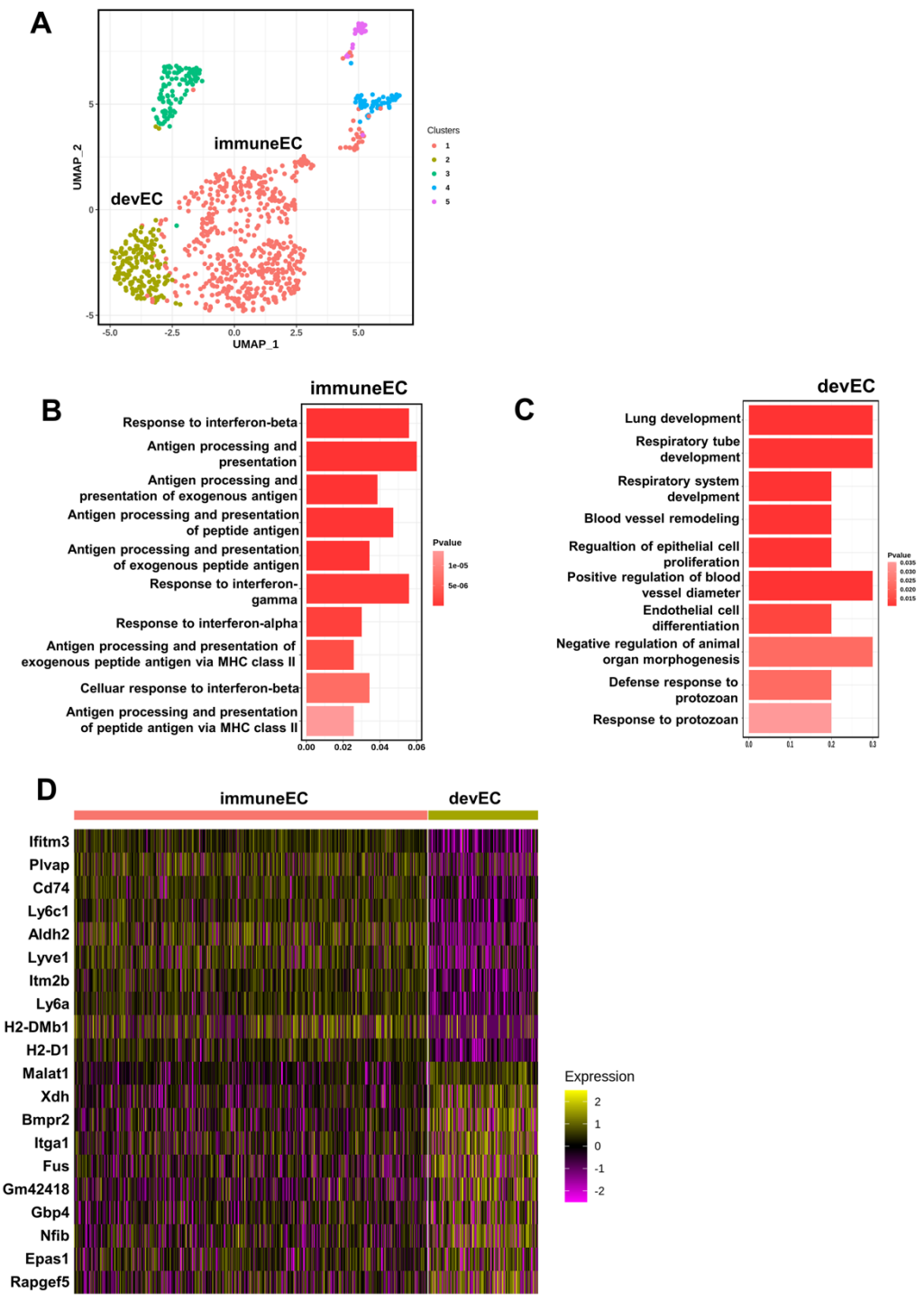
Supplemental Figure 9. The violin plots of representative genes in immuneEC (**A**) and devEC (**B**).X-axis is LPS injury at different time points. Y-axis is the relative expression level of genes of interest. ***: P<0.001 compared to control by one-way ANOVA.



Supplemental Figure 10. (**A**) Western blots of fresh isolated lung ECs from C57BL/6J mice with liposome delivery of CDH5 vector or CDH5-Sox17 for 3 days. N=3 for each group. (**B**) The quantification of IL-1β protein level normalized by GAPDH. No significance between two groups was found by umpired t-test. (**C**) Western blots of fresh isolated lung ECs from Sox17 fl/fl mice or Sox17 EC cKO mice. N=3 for each group. (**D**) The quantification of IL-1β protein level normalized by GAPDH. No significance between two groups was found by umpired t-test.



Supplemental Figure 11. **Analysis of lung ECs obtained from non-human primates infected with SARS-CoV-2 at day 3.** African green monkeys were inoculated with 2.6X10^6 $TCID_{50}$ (50% tissue culture infectious dose) live SARS-CoV-2 (nCoV-WA1-2020 (MN985325.1). The scRNA-seq was performed using harvested lung ECs from 4 non-human primates at day 3 following SARS-CoV-2 infection. (**A**) UMAP of 3,253 individual lung EC with different colors indicate distinct clusters. immuneEC: immune EC subpopulation. devEC. (**B**) The GO terms of cluster 1 - immuneEC indicates the enriched biological processes in immune and inflammatory feature. P values are indicated on the left in a color scale. X-axis represents the percentage of differentially expressed genes in each GO term. (**C**) The heatmap of most differentially expressed genes of monkey lung EC in cluster 1 (immuneEC) compared to cluster 2 – 7. The color bars indicate gene expression level in log_2 scale. Arrows highlight genes shown in **D**. (**D**) The UMAP of visualization of the expression levels of the representative marker genes in immuneEC. The color bars in UMAP indicate gene expression level in log_2 scale.



Supplemental Figure 12. Analysis of inflammatory and developmental lung EC subpopulations of lung ECs at day 7 post H1N1-inoculation. (**A**) The UMAP of 923 individual lung ECs isolated at day 7 post H1N1-inoculation. Different colors indicate different clusters with immuneEC and devEC labeled. (**B-C**) The GO terms of immuneEC (**B**) and devEC (**C**) indicate the enriched biological processes in each cluster. P values are indicated on the left in a color scale. (**D**) The heatmap of most differentially expressed genes of immuneEC and devEC subpopulations with the color bar showing gene expression levels on a $\log_2$ scale.

Supplemental Table 1

General information of scRNA-seq of lung endothelial cells at different time points

	baseline	LPS 6 hours	LPS 24 hours	LPS 2 days	LPS 3 days	LPS 7 days
Cell number	8,191	6,527	5,158	4,608	5,318	6,171
Median gene number per cell	1,568	1,147	1,252	1,770	1,897.5	1,872
Total gene number	17,035	16,409	16,069	16,501	16,664	16,966
UMI* number per cell	3,003	2,273	2,544.5	3,788	4,289	3,694
Total UMI* number	29,013,732	16,689,633	15,099,705	19,572,952	27,346,395	25,398,294
Cell number of inflamEC	3460	2759	2278	1531	-	3083
Median gene number of inflamEC	1,502	1,090	1,229.5	1,758	-	1,884
Total gene number of inflamEC	15,280	14,730	14,649	14,638	-	15,499
Cell number of devEC	2206	2601	1561	1424	-	1683
Median gene number of devEC	1,567.5	1,154	1,242	1,723.5	-	1,903
Total gene number of devEC	15,142	14,861	14,059	14,650	-	15,234
Cell number of devEC	-	-	-	-	635	-
Median gene number of proEC	-	-	-	-	2,951	-
Total gene number of proEC	-	-	-	-	14,265	-