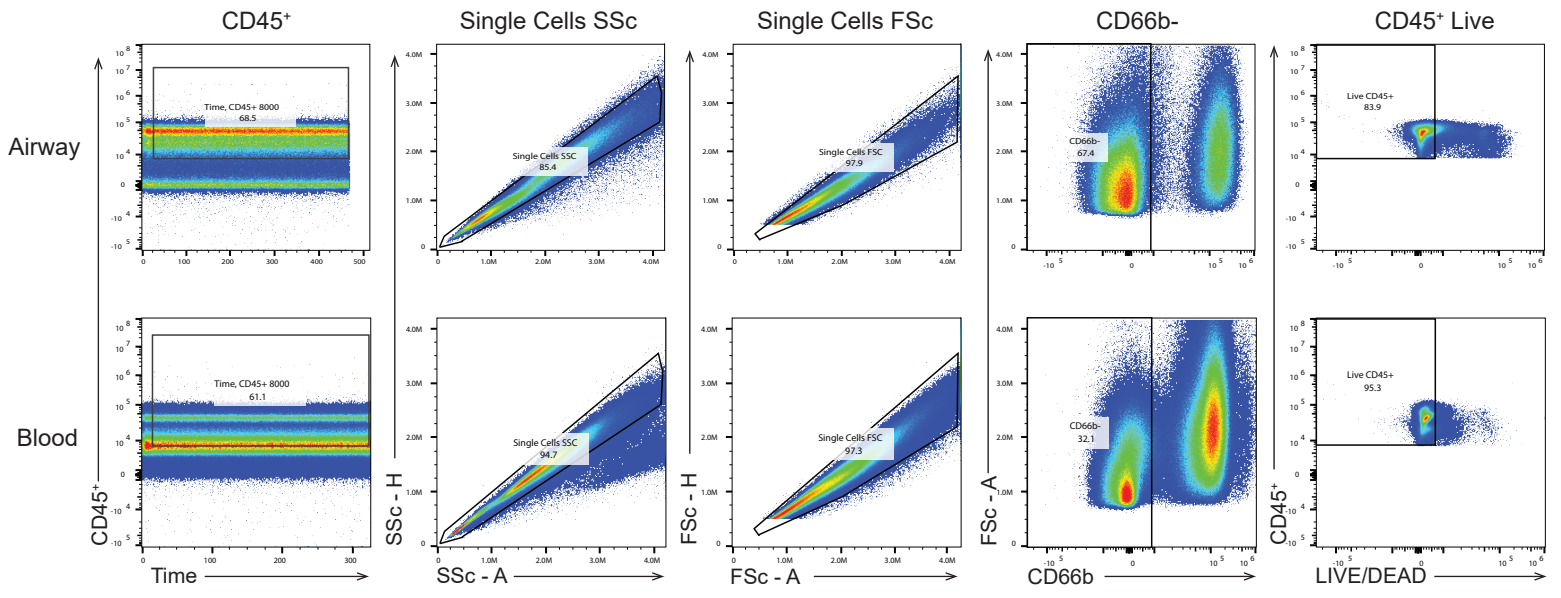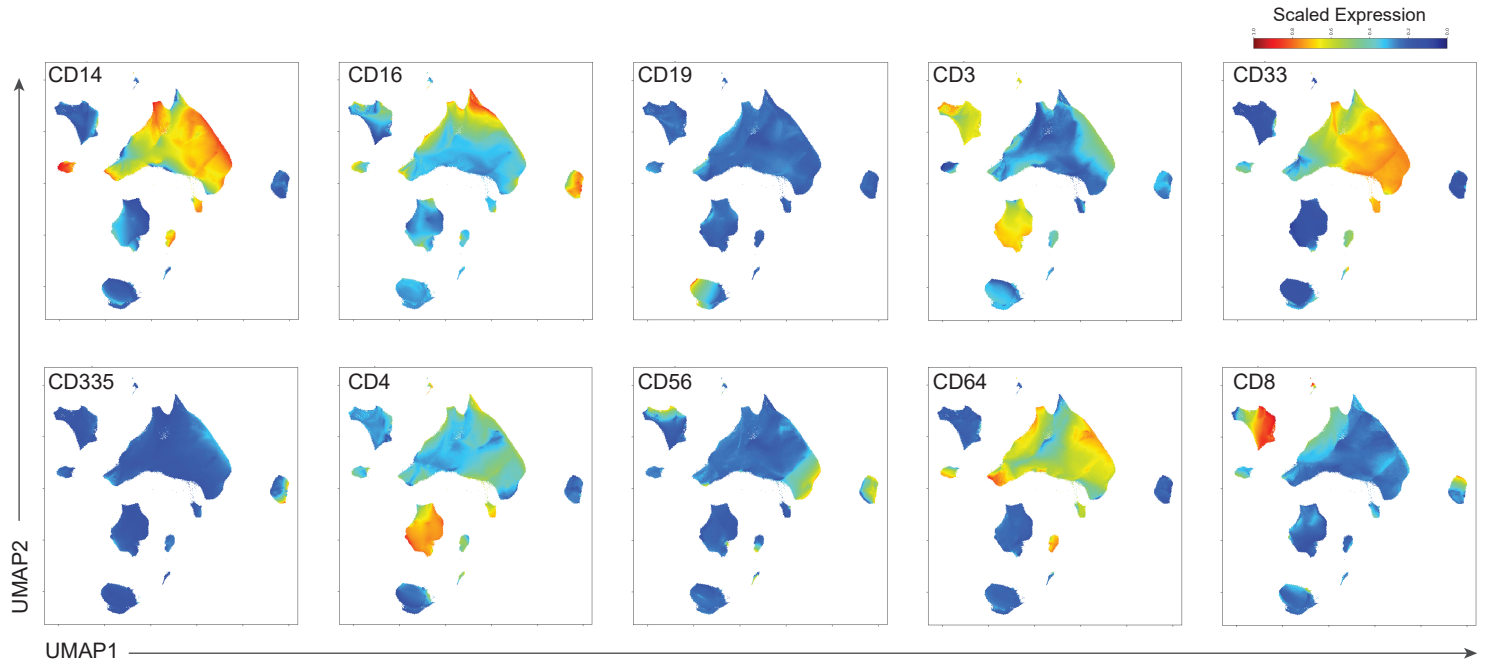**Supplemental information**

Longitudinal profiling of respiratory and systemic

immune responses reveals myeloid

cell-driven lung inflammation in severe COVID-19

Peter A. Szabo, Pranay Dogra, Joshua I. Gray, Steven B. Wells, Thomas J. Connors, Stuart P. Weisberg, Izabela Krupska, Rei Matsumoto, Maya M.L. Poon, Emma Idzikowski, Sinead E. Morris, Chloé Pasin, Andrew J. Yates, Amy Ku, Michael Chait, Julia Davis-Porada, Xinzheng V. Guo, Jing Zhou, Matthew Steinle, Sean Mackay, Anjali Saqi, Matthew R. Baldwin, Peter A. Sims, and Donna L. Farber

A

Airway

Blood

CD45+

Single Cells SSc

Single Cells FSc

CD66b-

CD45+ Live

Time, CD45+ 8000
68.5

Single Cells SSC
85.4

Single Cells FSC
97.9

CD66b-
67.4

Live CD45+
83.9

Time, CD45+ 8000
61.1

Single Cells SSC
94.7

Single Cells FSC
97.3

CD66b-
32.1

Live CD45+
95.3

CD45+

Time

SSc - H

SSc - A

FSc - H

FSc - A

FSc - A

CD66b

CD45+

LIVE/DEAD

B

Scaled Expression

CD14

CD16

CD19

CD3

CD33

CD335

CD4

CD56

CD64

CD8

UMAP2

UMAP1

Figure S1 related to Figure 1. Gating strategy for flow cytometry analysis and expression of lineage defining markers. (A) FACS plots denoting the gating strategy used for analysis of myeloid and lymphocyte populations by flow cytometry from complex populations in airway samples, with complementary gating for blood cells. Total cells were initially gated on CD45$^+$ cells versus time to eliminate non-hematopoietic cells and debris; doublets were excluded followed by exclusion of neutrophils (FSC-A^{hi} CD66b$^+$). Finally, dead cells were excluded by gating on CD45$^+$ and LIVE/DEAD blue. The resulting populations contained the full complement of mononuclear immune cells used for downstream analysis. (B) UMAP embedding displaying expression of lineage-defining markers for the major immune cell subsets in combined airway and blood samples from 15 COVID-19 patients.
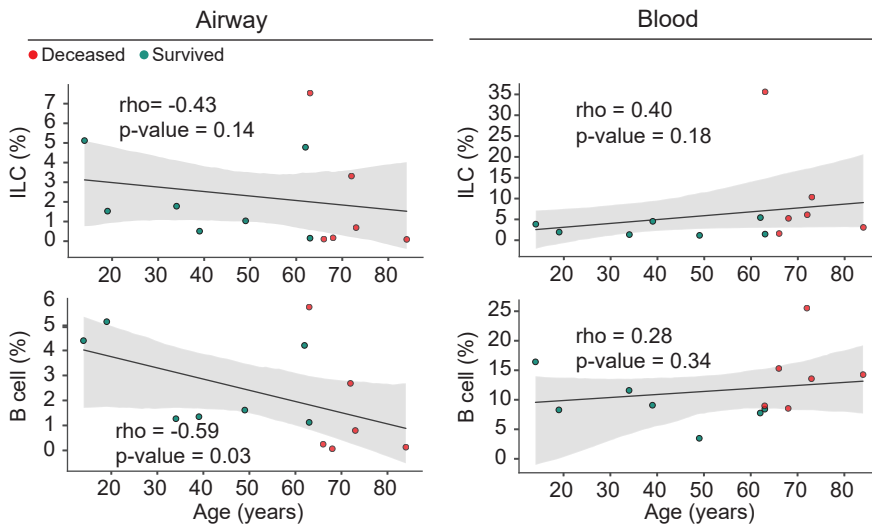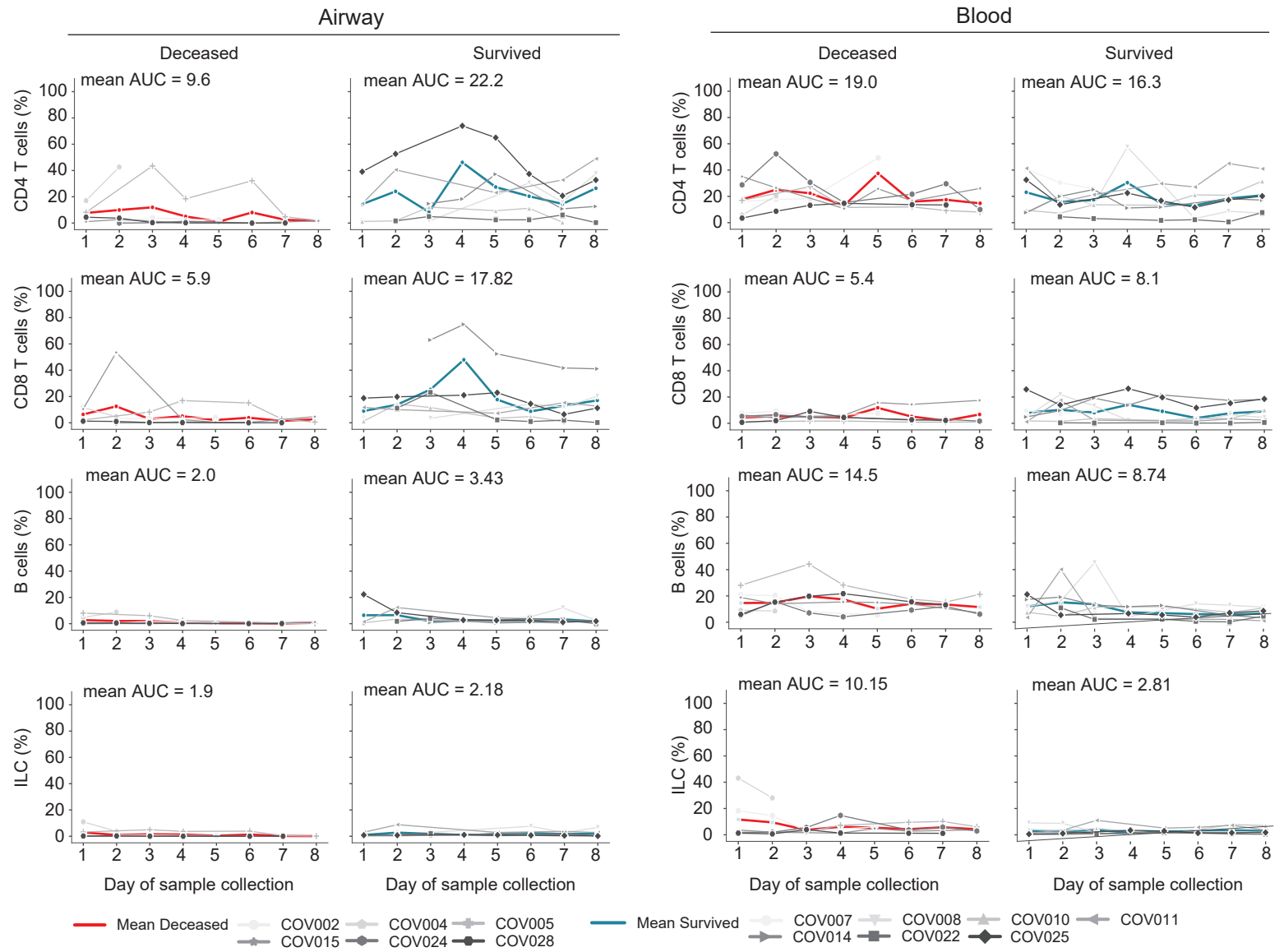
Figure S2 related to Figure 2. Major immune cell lineages over time in COVID-19 patients.

(A) Correlation of B cell and ILC frequency in the airways (left) and blood (right) with age. Each dot is representative of the mean immune cell frequency for each donor from all time points, and color denotes whether each patient survived (blue) or died (red). Statistical significance was calculated by Spearman correlation (showing rho). (B) Frequencies of CD4$^+$ T cells, CD8$^+$ T cells, B cells and ILCs in the airway (left) and blood (right) for each patient over time, separated by deceased and survived outcome. Solid red and blue lines show mean cell lineage frequency for deceased and survived patients respectively. Area-under-the-curve (AUC) was calculated for each patient, normalized for number of sampling days, and is shown as mean for each cell lineage and outcome.
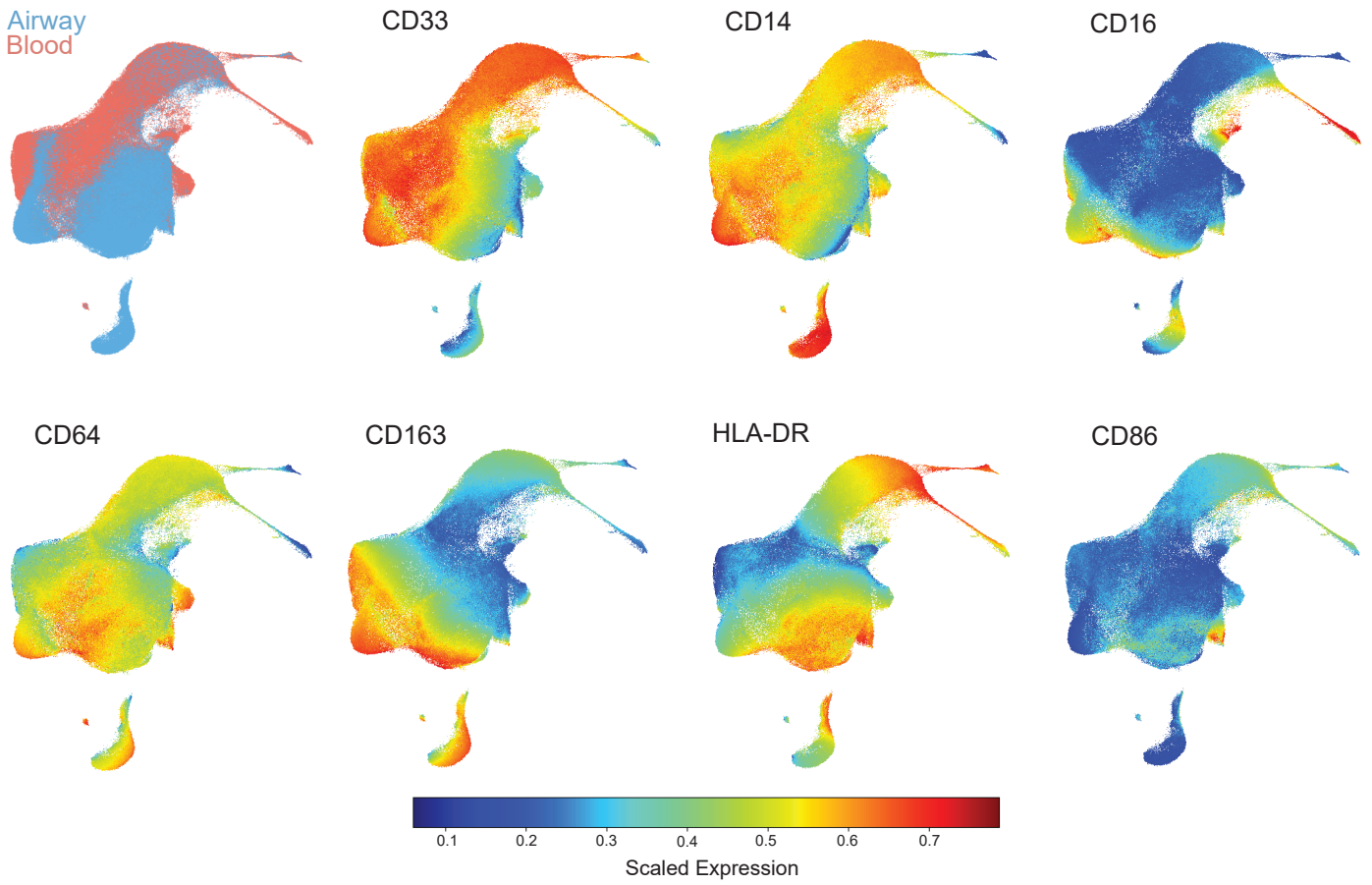
A
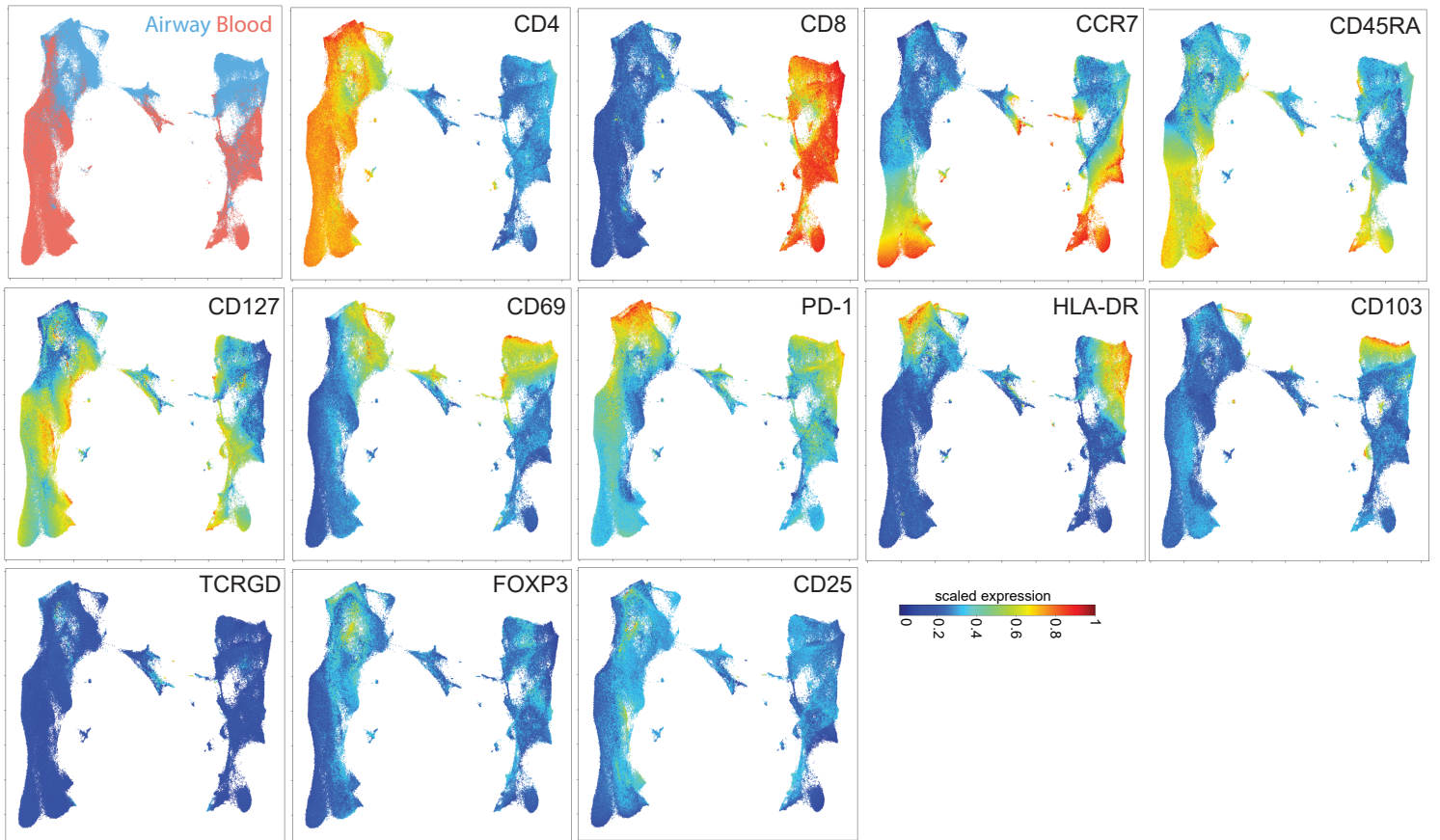
B
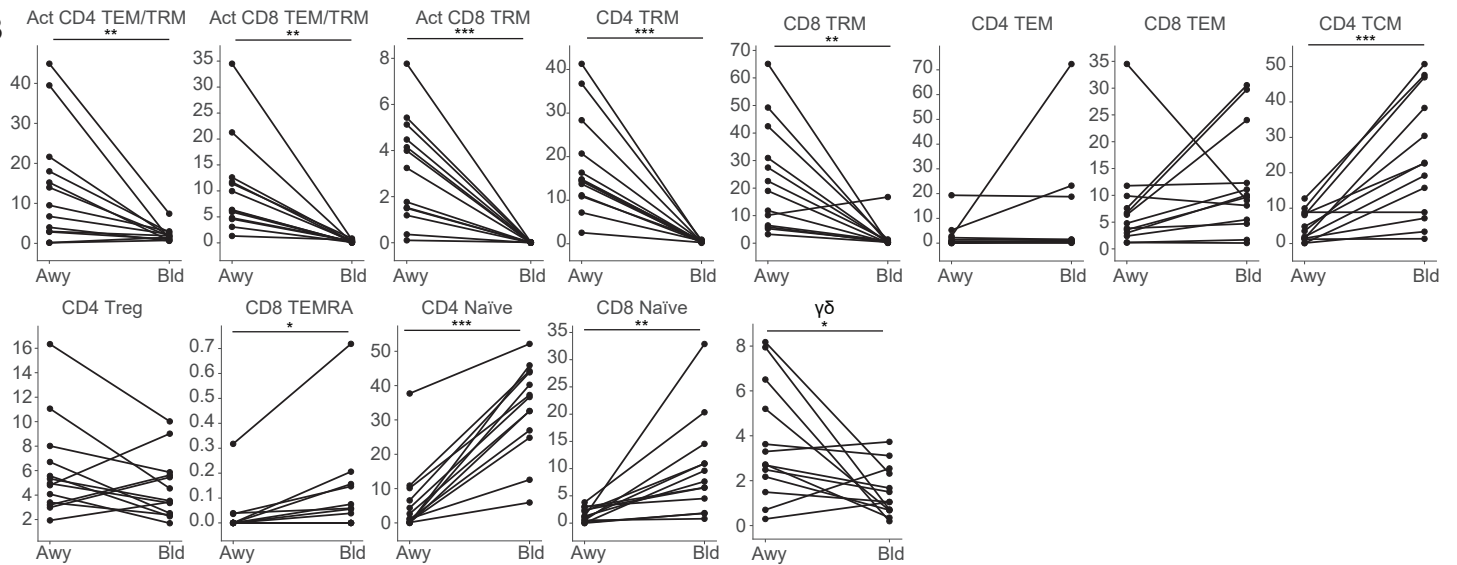
Figure S3 related to Figure 3. T cell marker expression in the airways and blood of COVID-19 patients. (A) UMAP embeddings indicating site of origin or scaled expression of indicated markers by flow cytometry in merged blood and airway samples from COVID-19 patients. (B) Paired quantitation of defined T cell subsets in blood and airway samples from COVID-19 patients. Statistical significance was calculated using a paired T-test and indicated by ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$.
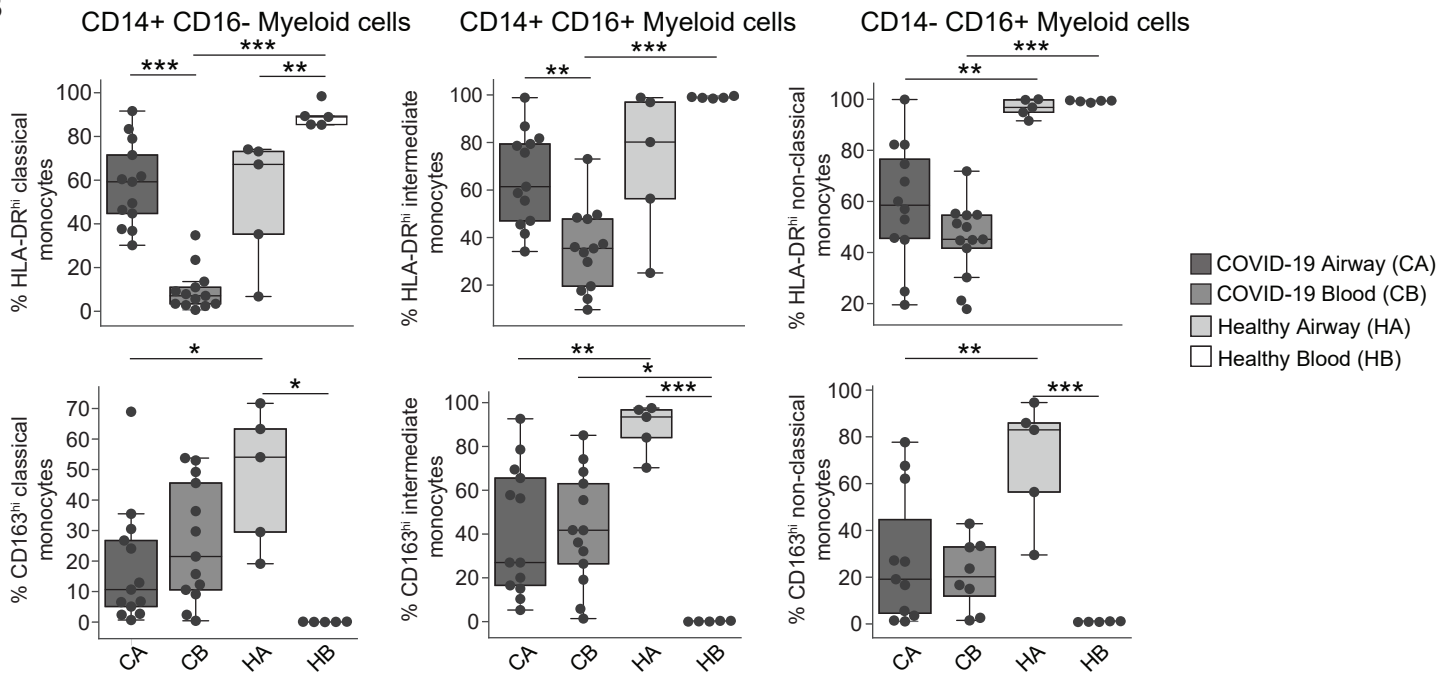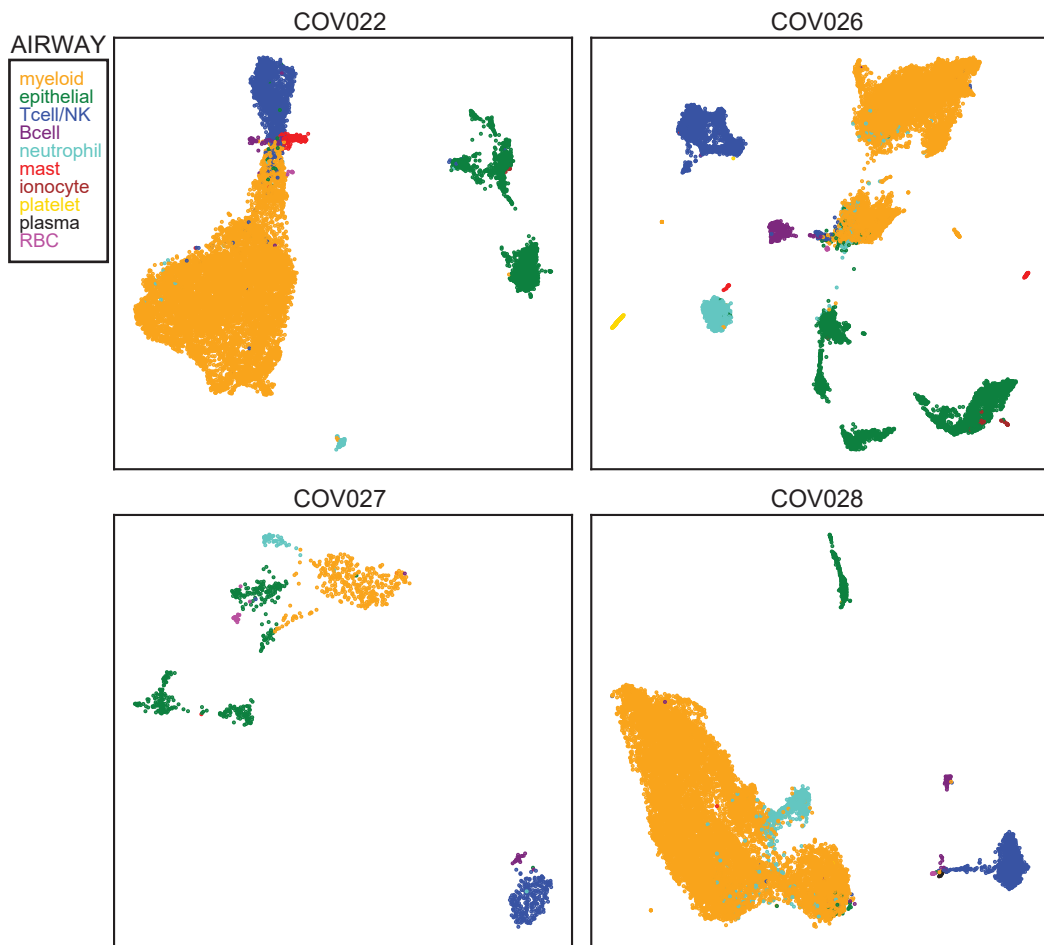
Figure S4

Figure S4 related to Figure 4. Myeloid cell marker expression in blood and airways of COVID-19 patients and healthy controls. (A) UMAP embeddings indicate site of origin for total myeloid cells in the airway (light blue) and blood (light red) of COVID-19 patients and healthy controls (top left) and scaled expression of indicated myeloid cell markers. (B) Boxplots indicate percent expression of HLA-DR (*top row*) and CD163 (*bottom row*) for the COVID-19 myeloid cell dataset gated according to classical nomenclature with $CD14^{hi}$ $CD16^{lo}$ designated as classical monocytes, $CD14^{hi}$ $CD16^{hi}$ as intermediate monocytes and $CD14^{lo}$ $CD16^{hi}$ and non-classical monocytes. Statistical significance was calculated using a one-way ANOVA followed by a Tukey HSD and indicated by ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$.

A

AIRWAY
myeloid
epithelial
Tcell/NK
Bcell
neutrophil
mast
ionocyte
platelet
plasma
RBC

COV022

COV026

COV027

COV028

B

BLOOD
myeloid
Tcell/NK
Bcell
platelet
plasma/plasmablast
RBC
dendritic
neutrophil

COV022

COV026

COV027

COV028
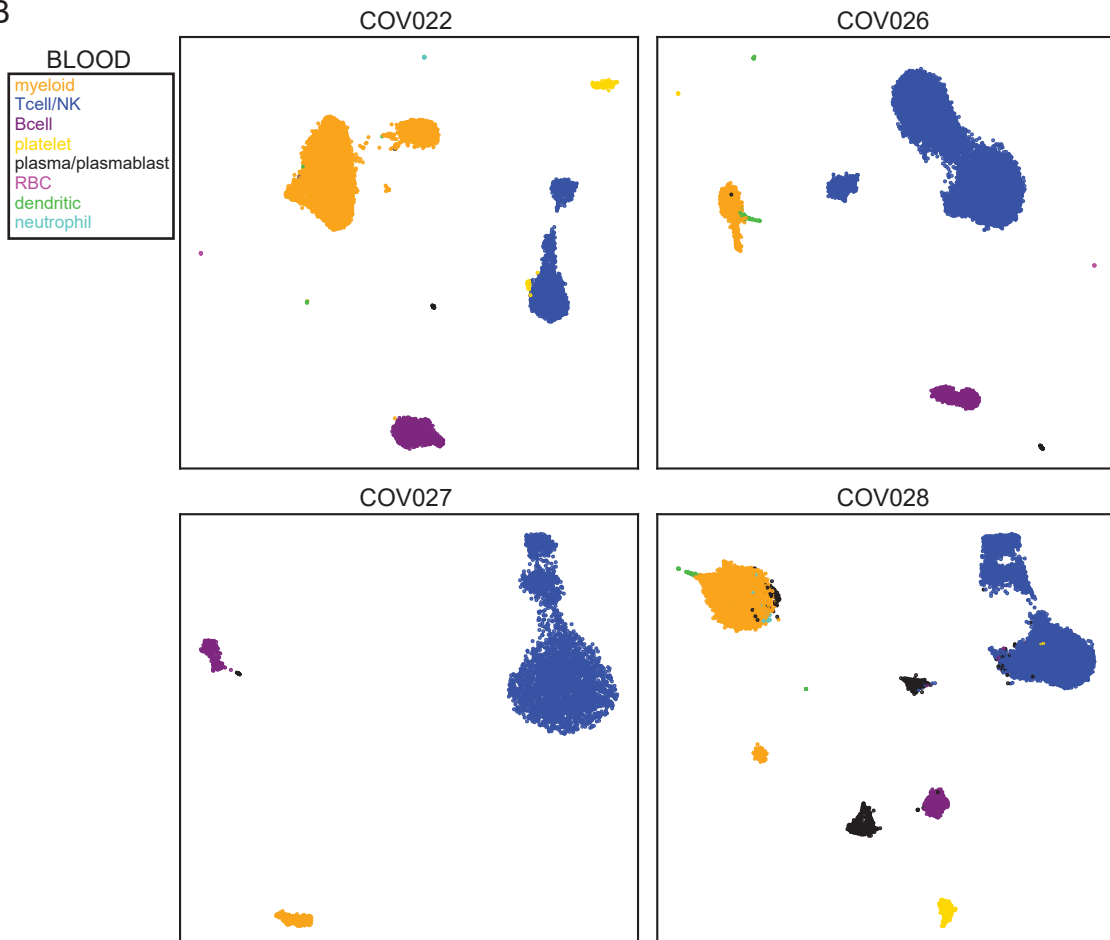
Figure S5 related to Figure 5. UMAPs of major immune lineages by scRNA-seq.

(A) UMAP of scRNA-seq data displaying all cells in airway samples from all timepoints for each patient. (B) UMAP of scRNA-seq data displaying all cells present in blood samples from all timepoints for each patient.
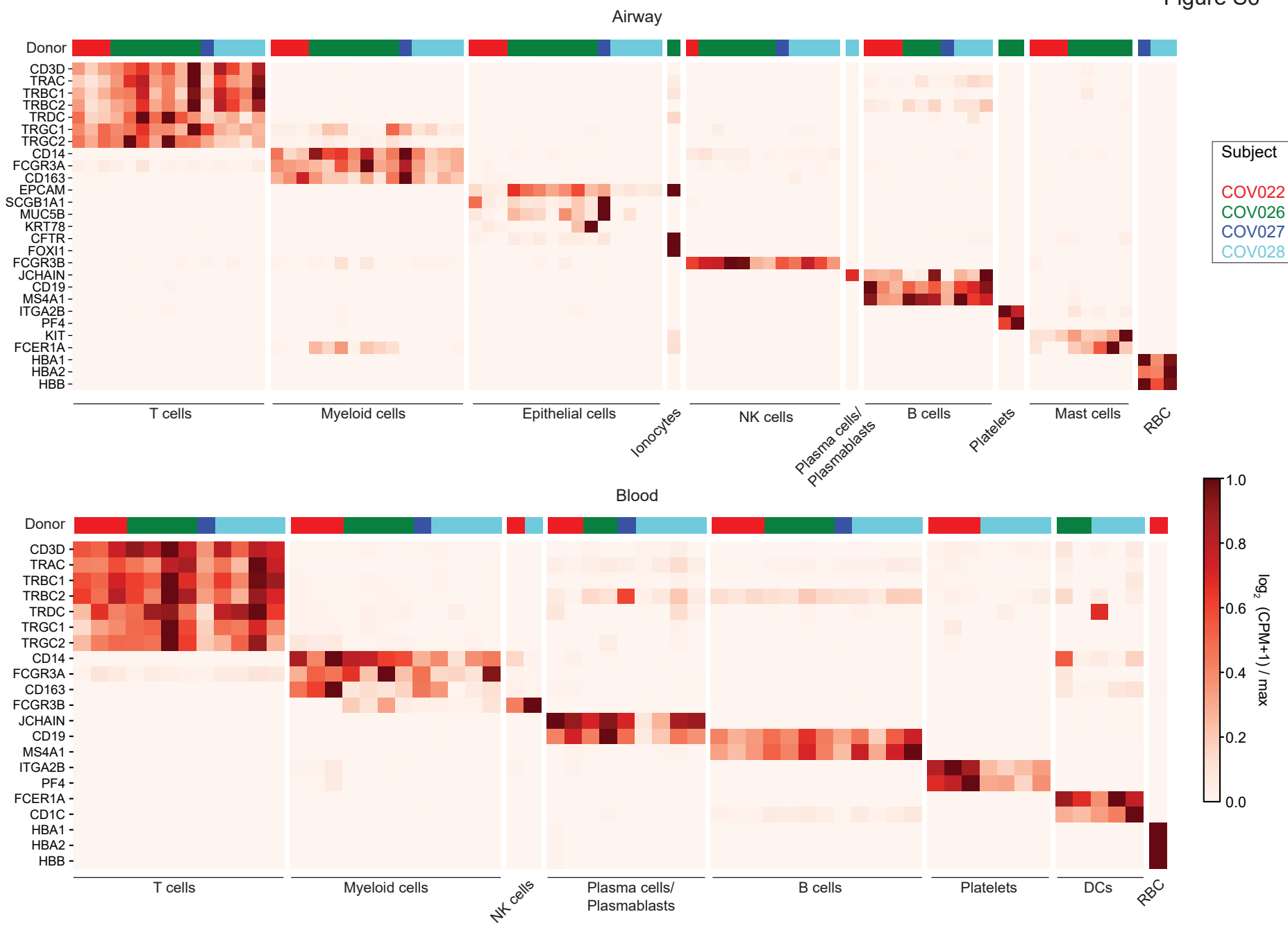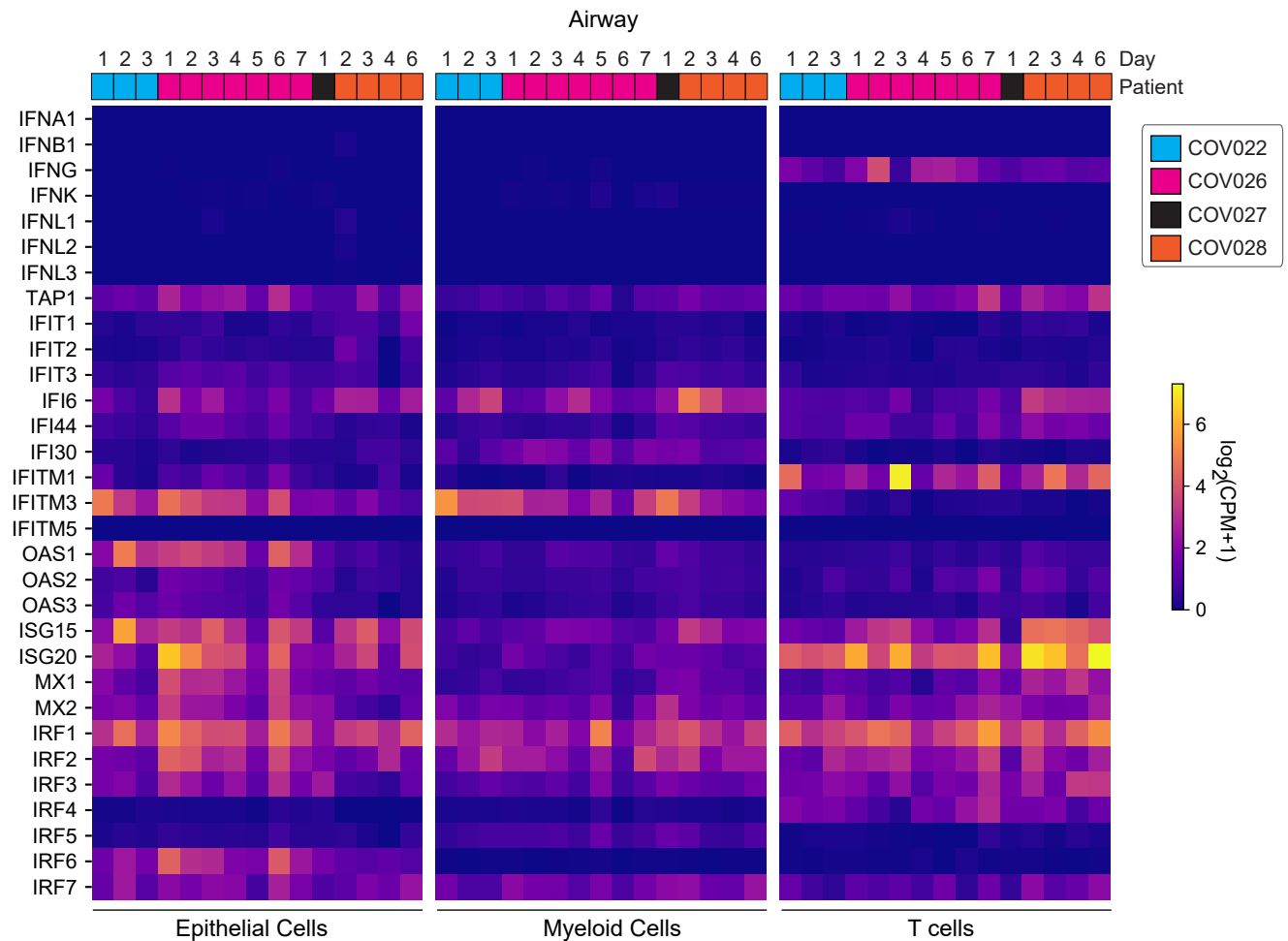
Figure S6

Figure S6 related to Figure 5. scRNA-seq analysis identifies specific features of immune cell types in airway and blood of COVID-19 patients. (A) Heatmap of scRNA-seq data displaying gene expression of lineage defining markers for both the airway and blood across the four patients. Heatmap data are colored by $\log_2$(CPM+1)/max values for each sample. (B) Heatmap showing $\log_2$(CPM+1) expression of *IFN* and *IFN*-related genes by the indicated airway cell types as determined by scRNA-seq for each patient sample, indicated by color.
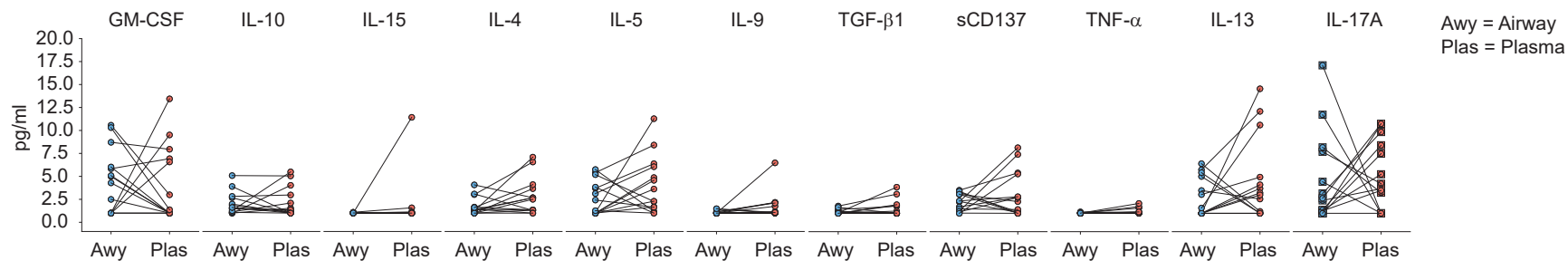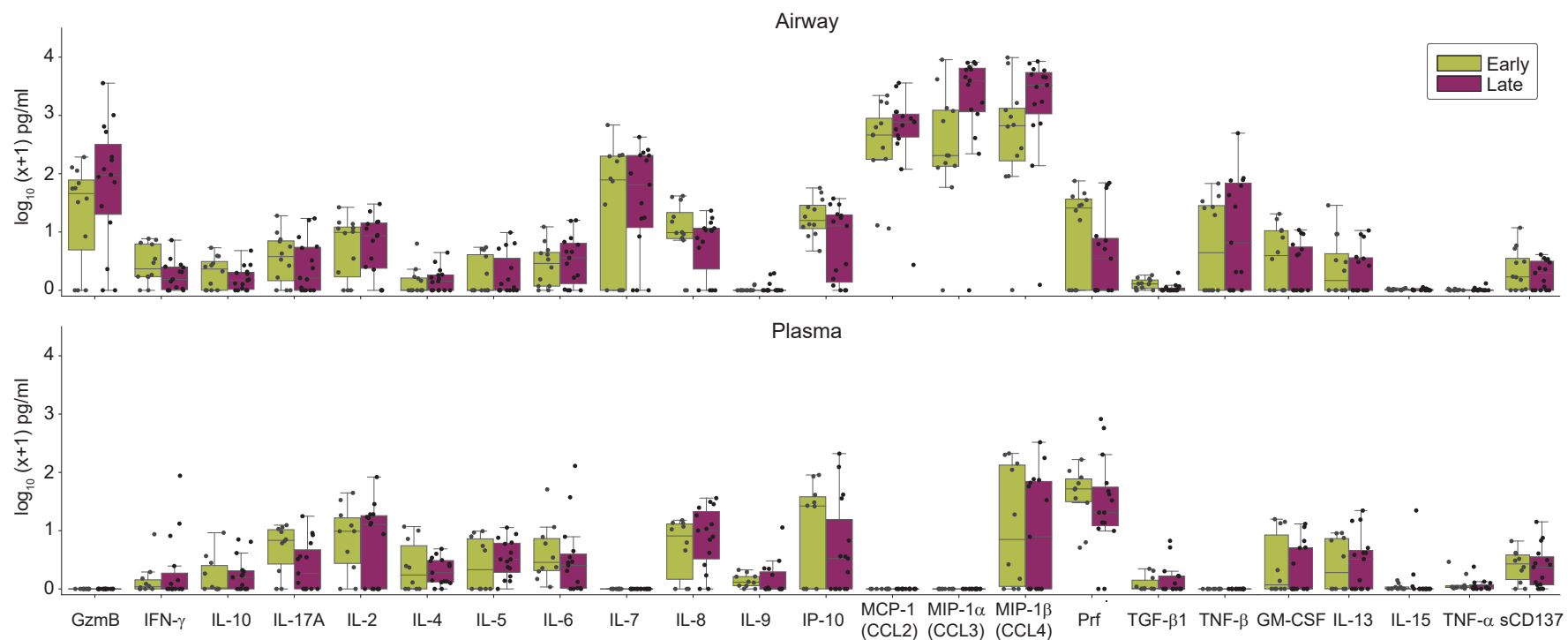
Figure S7

Figure S7 related to Figure 6. Airway is the major site for production of inflammatory cytokines and chemokines in COVID-19 patients. (A) Pairwise comparison of cytokine levels averaged across both timepoints in airway wash and blood plasma samples collected from 15 patients. A p-value of < 0.05 was considered significant. For figures, p-value < 0.05 = *, p-value < 0.01 = ** and p-value < 0.001 = **. (B) Box plots showing $\log_{10}(X+1)$ normalized cytokine expression profiles in the airway wash (top) and blood plasma (bottom) samples for an early and late time point collected from 15 patients. Each dot represents an individual data point.

Table S1. Related to Figures 1-6. Clinical information for COVID-19 patients in this study.

	Deceased (n=8)	Survived (n=7)	*P value*
Clinical Characteristics			
Age, years median (range)	72.5 (63-84)	39 (14- 63)	0.0005
Sex, male, n (%)	5 (62.5%)	4 (57.1%)	
Body Mass Index, kg/m2, median (IQR)	31.1 (29.3-35.0)	36.3 (30.5-39.4)	0.6200
SOFA, median (IQR)[a]	11.5 (10.3-14.8)	12 (10.5-13.5)	0.9826
Acute Respiratory Distress Syndrome, n (%)	8 (100%)	7 (100%)	
SARS-CoV-2 PCR Positive	8 (100%)	7 (100%)	
Days Post Symptom Onset, median (IQR)[b]	13.5 (8.8-16.5)	12 (9-13)	0.5523
Hospitalization, Days, median (range)	14.5 (1-36)	43 (19-88)	0.0140
Race or Ethnic Group, n (%)[c]			
Hispanic or Latino	2 (25%)	2 (28.6%)	
Black or African American	0 (0%)	3 (42.9%)	
White	2 (25%)	2 (28.6%)	
Other or Unknown	6 (75%)	2 (28.6%)	
Co-Morbidities, n (%)			
Hypertension	6 (75%)	1 (14.3%)	
Diabetes	3 (37.5%)	2 (28.6%)	
Chronic Cardiac Disease	0 (0%)	0 (0%)	
Current or Former Smoker	3 (37.5%)	1 (14.3%)	
Chronic Obstructive Pulmonary Disease	1 (12.5%)	1 (14.3%)	
Chronic Neurologic Diseases and Dementia	1 (12.5%)	0 (0%)	
Asthma	1 (12.5%)	1 (14.3%)	
Laboratory Values, Median (IQR)[a]			
Absolute Neutrophil Count, x10(3)/μL	17.2 (12.7-23.2)	15.1 (11.7-28.5)	0.8333
Absolute Lymphocyte Count, x10(3)/μL	0.95 (0.74-1.27)	0.64 (0.52-1.05)	0.5237
Absolute Monocyte Count, x10(3)/μL	0.86 (0.63-1.26)	0.77 (0.52-0.87)	0.5556
D-Dimer, μg/mL[d]	7.5 (3.3-14.3)	10.2 (2.8-17.9)	0.9860
Ferritin, ng/mL	1250 (636-2580)	1767 (806-3929)	0.6943
High Sensitivity C-reactive Protein, mg/L[d]	75 (38-157)	94 (24-199)	0.4557
Interleukin-6, pg/mL[d]	121.7 (86.9-315)	64.5 (13.9-226.1)	0.1732
Lactate Dehydrogenase, U/L	646 (614-1129)	598 (287-1052)	0.4452
PaO_2/FiO_2 ratio	122 (83-153)	168 (119-180)	0.3969
Treatment, n (%)[e]			
Convalescent Plasma	0 (0%)	4 (57.1%)	0.0125
Intravenous Immunoglobulin	0 (0%)	1 (14.3%)	0.2685
Monoclonal Antibody Therapy[f]	3 (37.5%)	2 (28.6%)	0.7144
Remdesivir	5 (62.5%)	2 (28.6%)	0.1888
Steroids[g]	8 (100%)	5 (71%)	0.2000
Abbreviations: SOFA - Sequential Organ Failure Assessment Score, PaO2 – arterial partial pressure of oxygen, FiO2 – fraction of inspired oxygen			
a First available value within 48 hours of study enrollment			
b Days from onset of respiratory symptoms to study enrollment			
c Subjects included in all categories for which they identified			
d Values above upper limits of test entered as; D-Dimer (20mg/mL), C-reactive Protein (200mg/L), Interleukin-6 (315pg/mL)			
e All samples obtained during or post-treatment. Treatments initiated after study completion include: Hydrocortisone (n=3 Survived), Remdesivir (n=2, Survived)			
f Tocilizumab (n=2 Deceased, n=2 Survived), enrolled in double-blind randomized controlled Sarilumab trial (n=1 Deceased)			
g Hydrocortisone or Methylpredisone			
All statistical testing done with 2 tailed Mann-Whitney Test, Chi-squared Test, or Fischer's Exact test.			

Table S2. Related to Figure 1. Number of paired airway and blood samples obtained from individual COVID-19 patients and assays performed with the samples.

Study ID	Age/Sex	Outcome	#paired samples	Flow Cytometry	Cytokines	scRNAseq
COV002	73M	Deceased	4	X	X	
COV004	63M	Deceased	2	X		
COV005	72M	Deceased	7	X	X	
COV007	63F	Survived	4	X	X	
COV008	14F	Survived	10	X	X	
COV010	39M	Survived	6	X	X	
COV011	62M	Survived	7	X	X	
COV014	34F	Survived	7	X	X	
COV015	84M	Deceased	7	X	X	
COV022	49M	Survived	7	X	X	X
COV024	68M	Deceased	7	X	X	
COV025	19M	Survived	8	X	X	
COV026	74F	Deceased	7		X	X
COV027	82F	Deceased	1			X
COV028	66F	Deceased	6	X	X	X

Table S3. Related to Figures 1, 5-7. Summary of sample details for scRNA-seq analysis.

Sample Name	Patient ID	Days Post-Intubation	Sample Source	Number of Cells
COV022A1	COV022	1	Airway Wash	2,489
COV022A2	COV022	2	Airway Wash	9,852
COV022A3	COV022	3	Airway Wash	2,487
COV022B1	COV022	1	Fresh PBMCs	4,603
COV022B2	COV022	2	Fresh PBMCs	11,574
COV022B3	COV022	3	Fresh PBMCs	9,337
COV026A1	COV026	1	Airway Wash	4,651
COV026A2	COV026	3	Airway Wash	5,834
COV026A3	COV026	4	Airway Wash	4,147
COV026A4	COV026	5	Airway Wash	1,324
COV026A5	COV026	6	Airway Wash	2,834
COV026A6	COV026	7	Airway Wash	810
COV026A7	COV026	8	Airway Wash	2,065
COV026B2	COV026	3	Frozen PBMCs	5,608
COV026B3	COV026	4	Frozen PBMCs	5,080
COV026B6	COV026	7	Frozen PBMCs	4,846
COV026B7	COV026	8	Frozen PBMCs	4,755
COV027A1	COV027	1	Airway Wash	1,074
COV027B1	COV027	1	Frozen PBMCs	4,232
COV028A2	COV028	2	Airway Wash	7,025
COV028A3	COV028	3	Airway Wash	3,434
COV028A4	COV028	4	Airway Wash	2,598
COV028A6	COV028	7	Airway Wash	4,594
COV028B2	COV028	2	Fresh PBMCs	7,097
COV028B3	COV028	3	Fresh PBMCs	4,792
COV028B4	COV028	4	Fresh PBMCs	5,696
COV028B6	COV028	7	Fresh PBMCs	3,726

Table S4. Related to Figure 1. PCA loadings of markers for PC1 and PC2 for plots in Figure 1B.

Marker	PC1	PC2
CD3	0.077054	0.006976
CD27	0.058595	-0.02115
CD4	0.046202	-0.00891
CD8	0.038877	0.026317
CD45RA	0.038415	-0.05869
CD127	0.03697	0.009139
CD69	0.029454	0.037681
CD28	0.027359	0.001804
CCR7	0.025786	-0.01655
KLRG1	0.015328	0.016481
PD_1	0.007538	0.033281
CD57	0.007306	-0.00865
CXCR5	0.004977	0.009335
CD103	0.003101	0.002303
CD19	0.000995	0.015749
FOXP3	-0.00317	0.028677
CD335	-0.00361	0.002101
CD25	-0.00422	0.008298
CD86	-0.00468	0.015079
CD56	-0.00506	-0.01521
CD66B	-0.00632	0.019469
CD16	-0.01384	-0.00482
TCRGD	-0.01789	0.018678
CD95	-0.02823	0.005061
HLA_DR	-0.03622	0.074729
CD163	-0.08021	-0.03492
CD64	-0.08127	0.019918
CD11C	-0.08342	0.031825
CD14	-0.10606	-0.01016
CD33	-0.12646	-0.0358

Table S7. Related to Figures 1, 3, 4, 7. Deceased donor information for control airway and blood samples and COVID-19 autopsy samples.

Donor Number	Status	Sex	Age	Flow/IF[a]
D328	Control	M	52	IF
D370	Control	M	52	IF
D372	Control	M	68	IF
D481	Control	M	29	Flow
D484	Control	M	59	Flow
D488	Control	M	55	Flow
D489	Control	F	67	Flow
D490	Control	M	26	Flow
IMG001	COVID-19	M	73	IF
IMG002	COVID-19	F	93	IF
IMG003	COVID-19	M	71	IF
IMG004	COVID-19	F	81	IF
IMG005	COVID-19	M	63	IF

[a]Flow, flow cytometry; IF, Immunofluorescence imaging