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Supplemental Information

Single-Cell Sequencing of Peripheral Mononuclear Cells Reveals Distinct Immune Response Landscapes of COVID-19 and Influenza Patients

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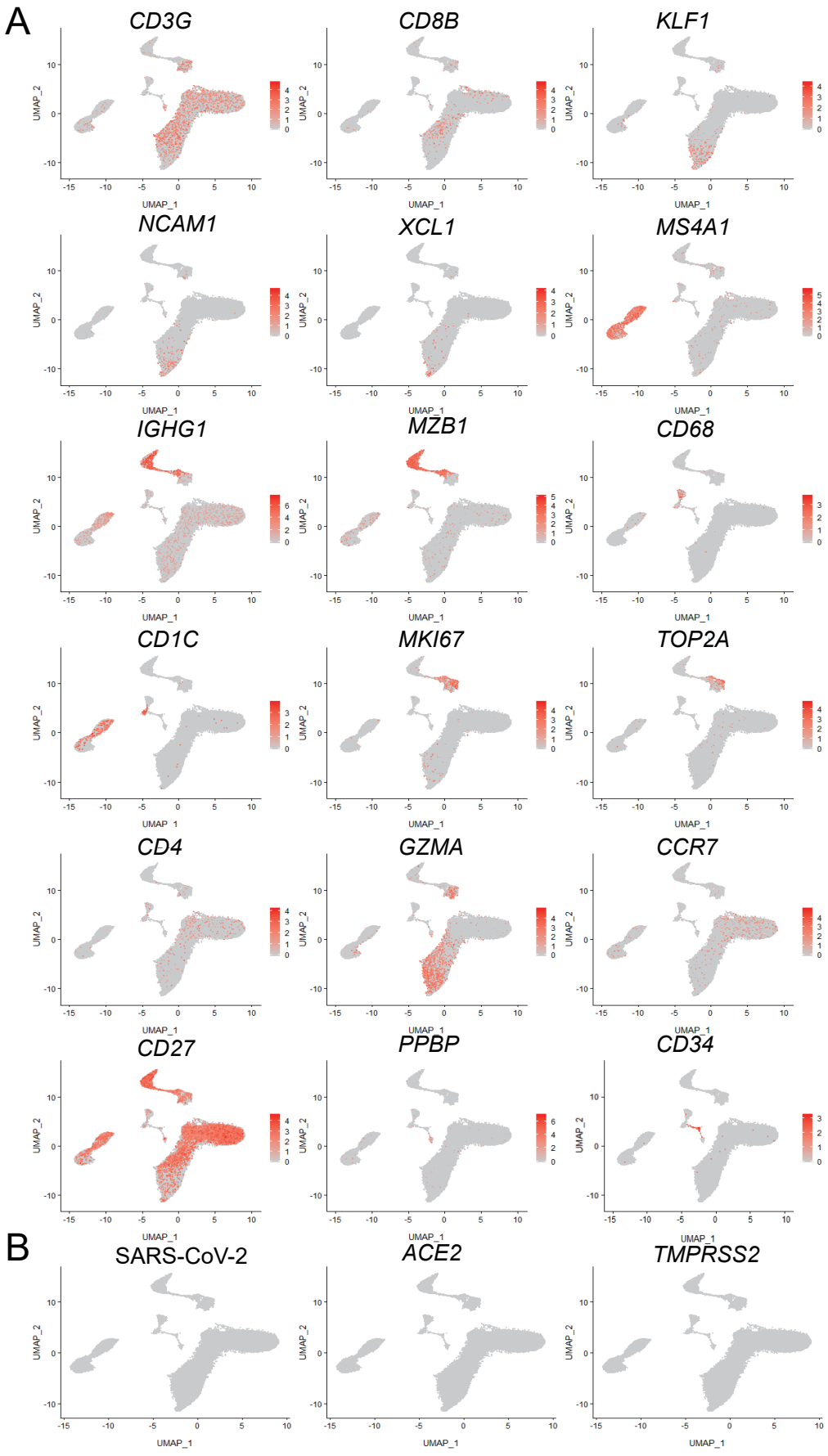

Figure S1. Single-cell RNA-seq analysis of immune cells from COVID-19 patients, Related to Figure 1.
(A) The clustering result of 46,022 cells from 10 donors, split by samples. Each point represents one single cell, colored according to cell type.
(B) The expression level of cell typing genes by UMAP. Cells are colored by expression level.

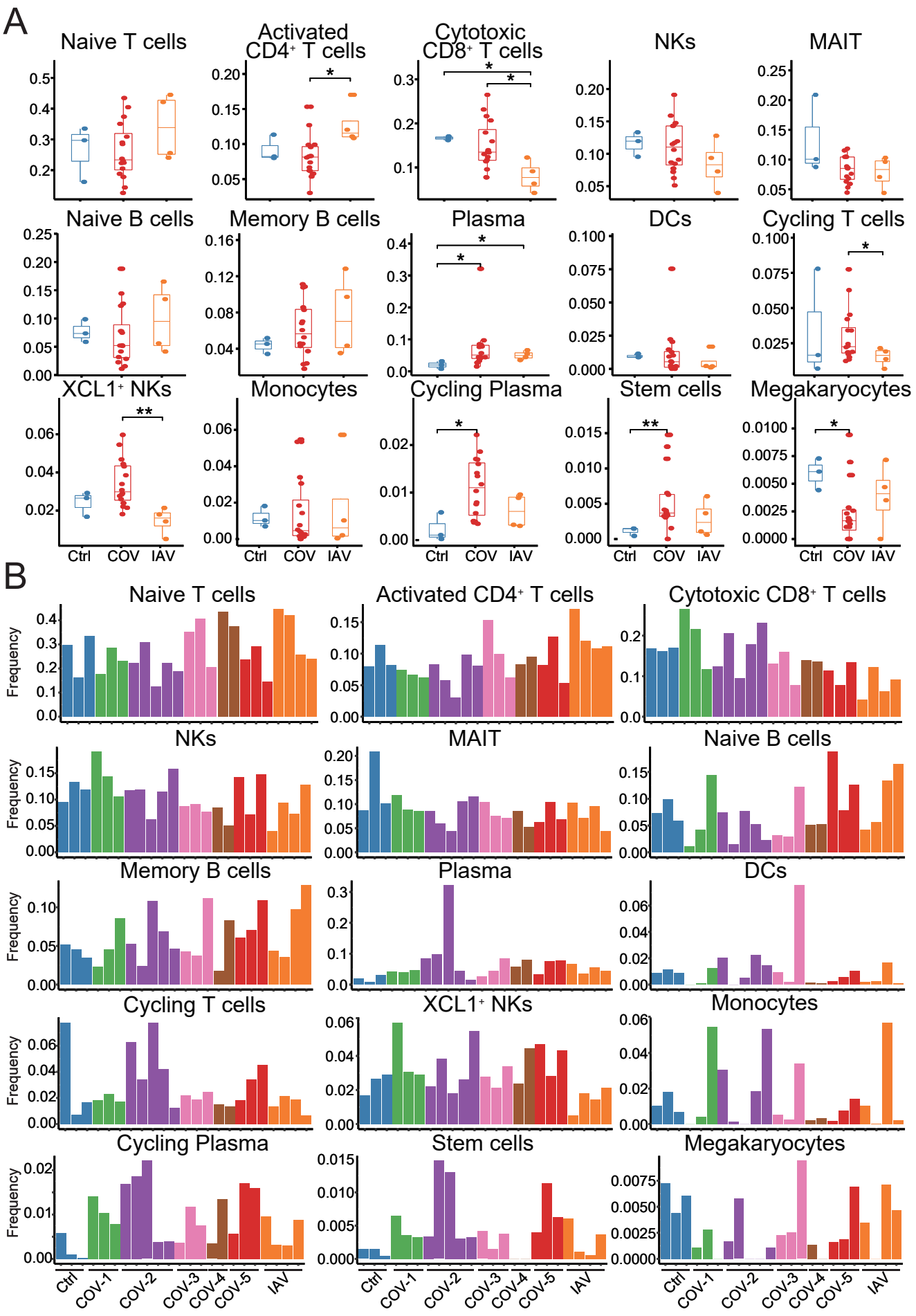

Figure S2. Dynamic changes of immune cells reveal immune response in COVID-19 patients, Related to Figure 2.
(A) The difference of the proportion of each cell populations among samples from healthy donors (Ctrl) (n=3), IVA patients (IVA) (n=4) and COVID-19 patients (COV) (n=16). Student's t test was applied to test the significance of the difference. * p<0.05, ** p<0.01, *** p<0.001.
(B) The dynamic change of each cell population among samples from Controls (n=3), IVA patients (n=4) and COVID-19 patients (n=16) at different time points.

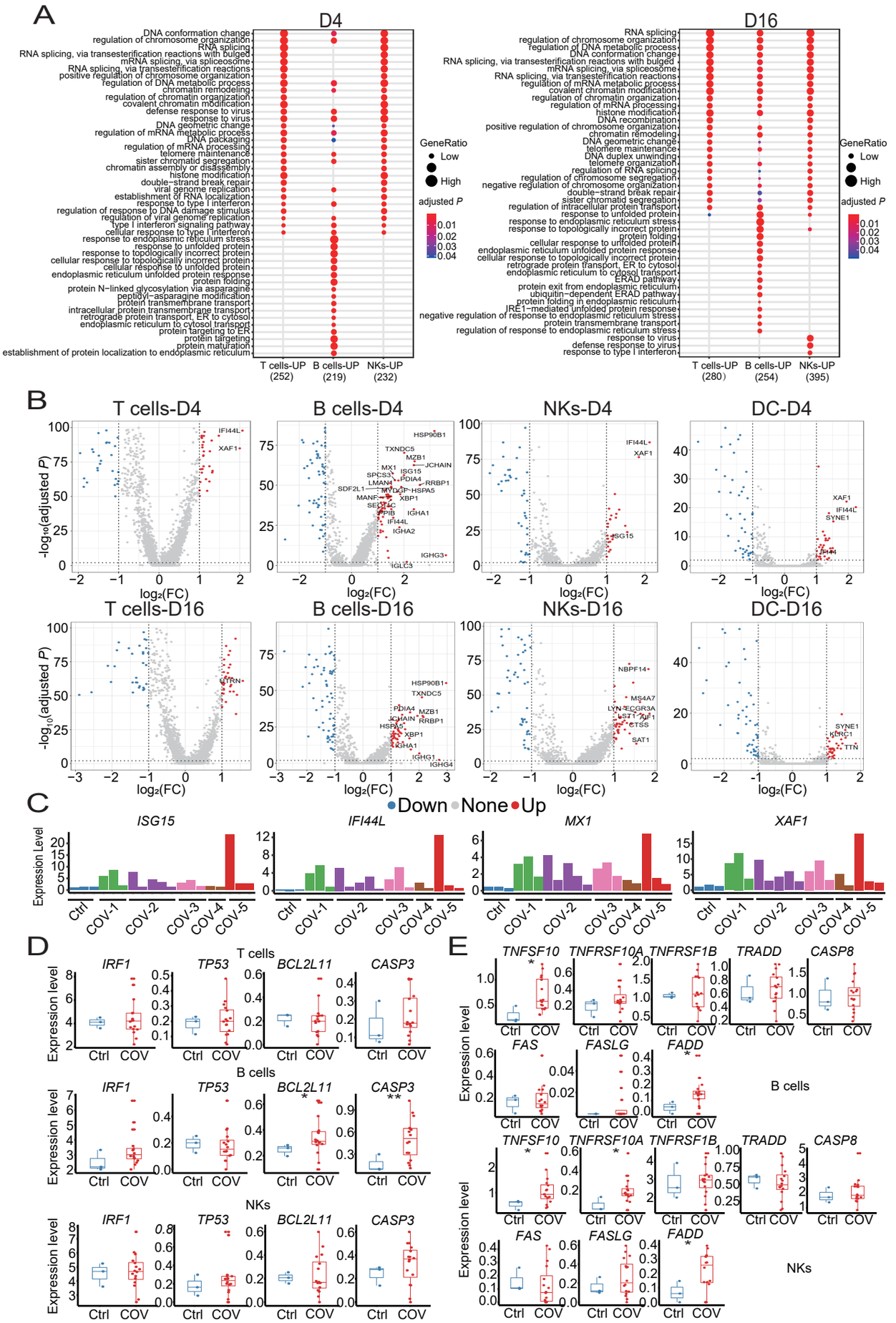

Figure S3. Enrichment of biological processes in D4 and D16 COVID-19 patients, and expression of apoptosis-associated genes, Related to Figure 3.

(A) The top 20 enriched biological process by Gene Ontology analysis in Day4 and Day16 samples from COVID-19 patients, compared to healthy controls in different cell populations. Dot color indicates the statistical significance of the enrichment (P-value) and dot size represents gene ratio annotated to each term.

(B) The differential expressed genes in Day4 and Day16 samples comparing COVID-19 patients to healthy donors in different cell subsets. Red dots represent genes up-regulated in COVID-19 patients (adjusted P-value < 0.01 and FC >= 2) while blue dots represent downregulated genes in COVID-19 patients (adjusted P-value < 0.01 and FC <= 0.5). Genes with $\log_2$(FC) >=1.5 were labeled by gene symbols.

(C) Dynamic changes in *ISG15*, *IFI44L*, *MX1*, and *XAF1* gene expression in healthy controls and at different time-points in COVID-19 patients.

(D) The expression level of XAF1-related genes in COVID-19 patients (COV) (n=16) and healthy donors(Ctrl) (n=3) in T, B and NK cells.

(E) The expression level of apoptosis-associated genes in COVID-19 patients (COV) (n=16) and healthy donors (Ctrl) (n=3) in B and NK cells. Student's t test was applied to test the significance of the difference. * p<0.05, **p<0.01, ***p<0.001.

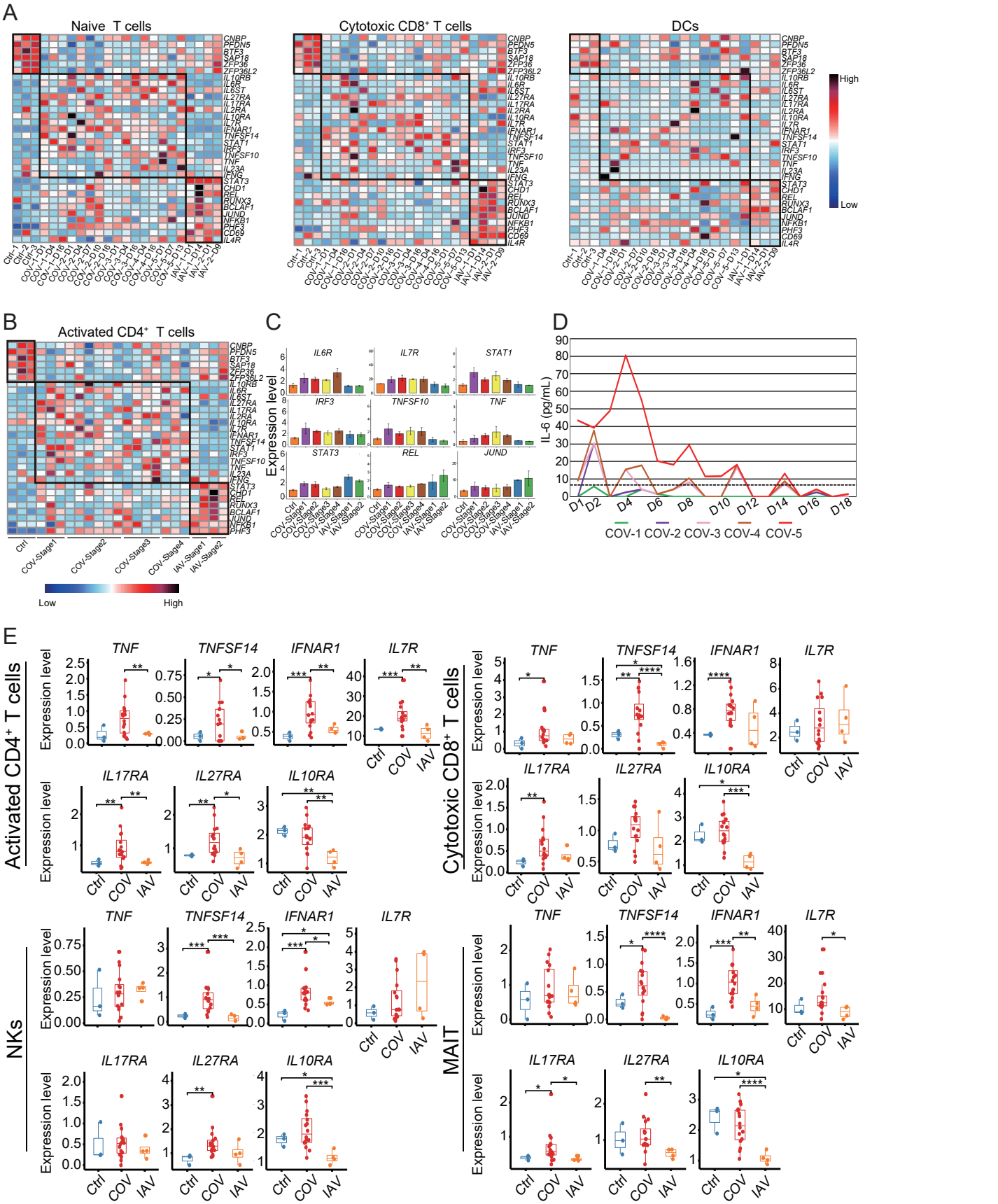

Figure S4. Analysis of cytokines, cytokine receptors and transcription factors, Related to Figure 4.

(A) The relative expression level (Z-score) of key cytokines, cytokine receptors and transcription factors among COVID-19 patients, IVA patients and healthy controls in naive T cells, cytotoxic CD8⁺ T cells, NKs, DCs, respectively.

(B) The relative expression level (Z-score) of key cytokines, cytokine receptors and transcription factors among COVID-19 patients, IVA patients and healthy controls in CD4⁺ T cells. Samples were ordered by disease progression stage.

(C) Bar plots showing the expression levels of 9 genes in different disease progression stage of COVID-19 patients and IAV patients. Data are represented as mean ±SEM.

(D) The concentration of IL-6 in blood in 5 COVID-19 patients at multiple time-points. D1 corresponds to the first day when PBMCs were collected for scRNA-seq analysis. The dotted line indicates the upper limit of the normal range of IL-6 concentration.

(E) The expression level of key cytokines and cytokine receptors in activated CD4⁺ T cells, cytotoxic CD8⁺ T cells, NKs, and MAITs. Each dot represents the average expression level of a gene in a cell population in one sample. Student's t test was applied to test the significance of the difference. * p<0.05, **p<0.01, ***p<0.001.