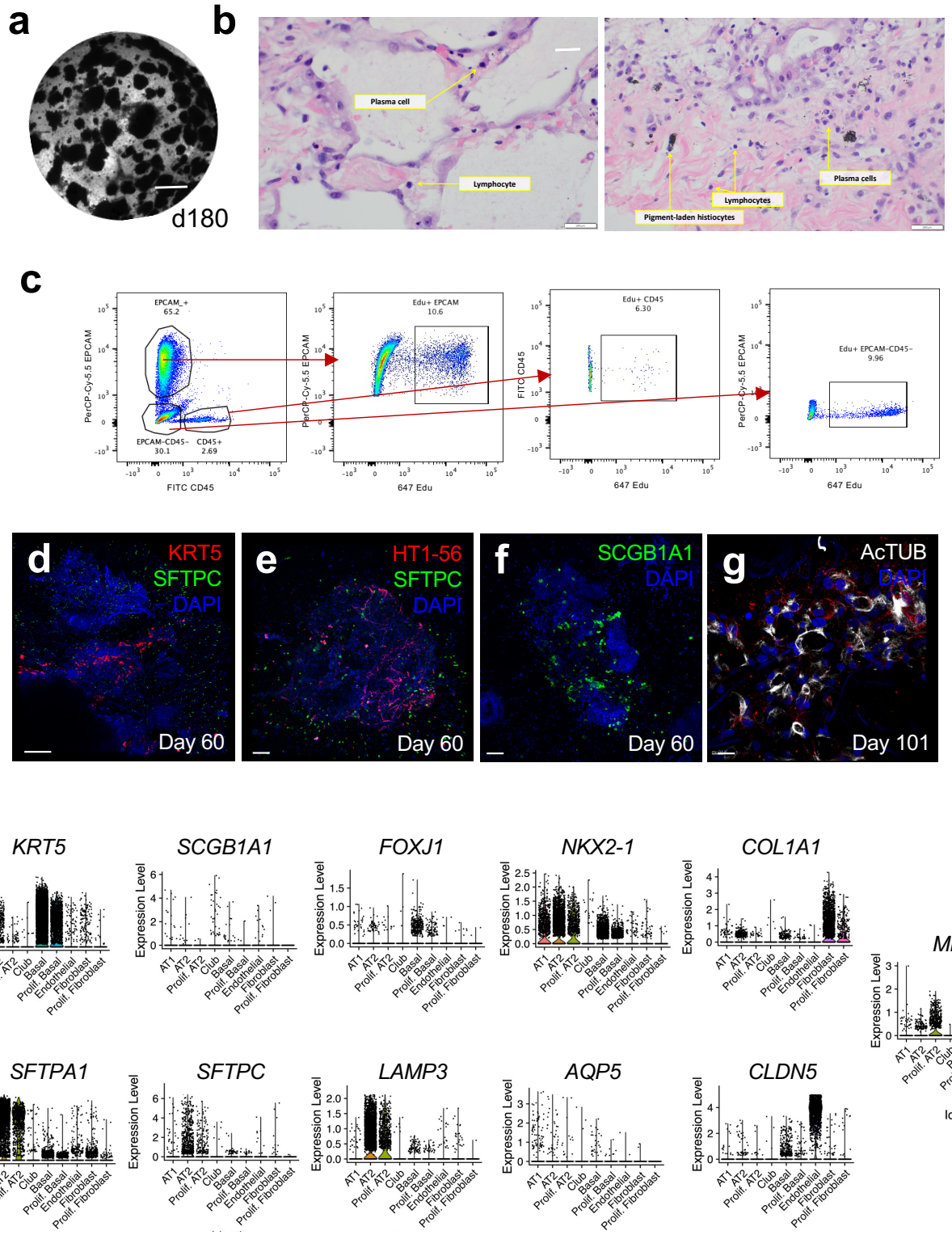
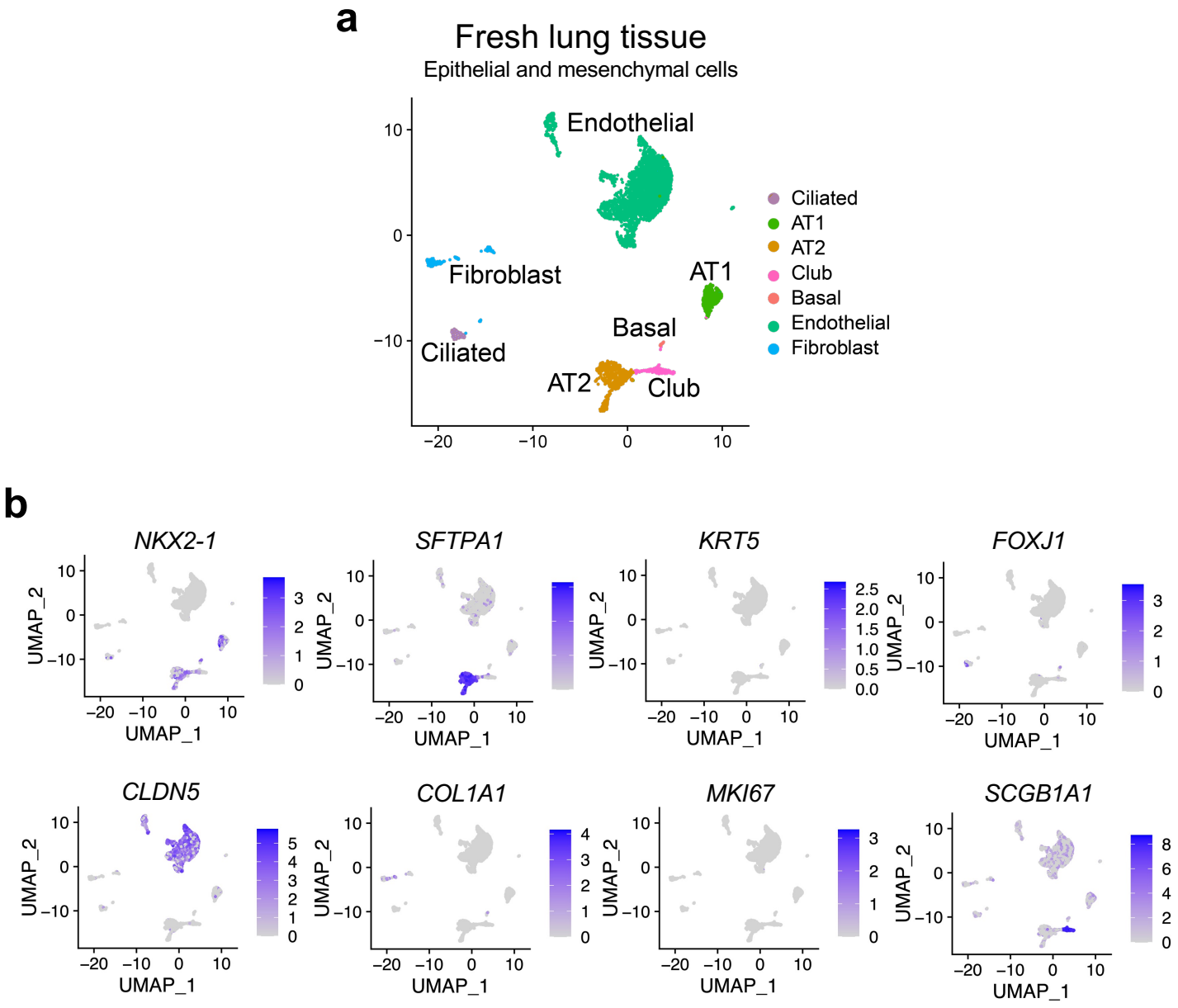
Extended Data Fig. 1



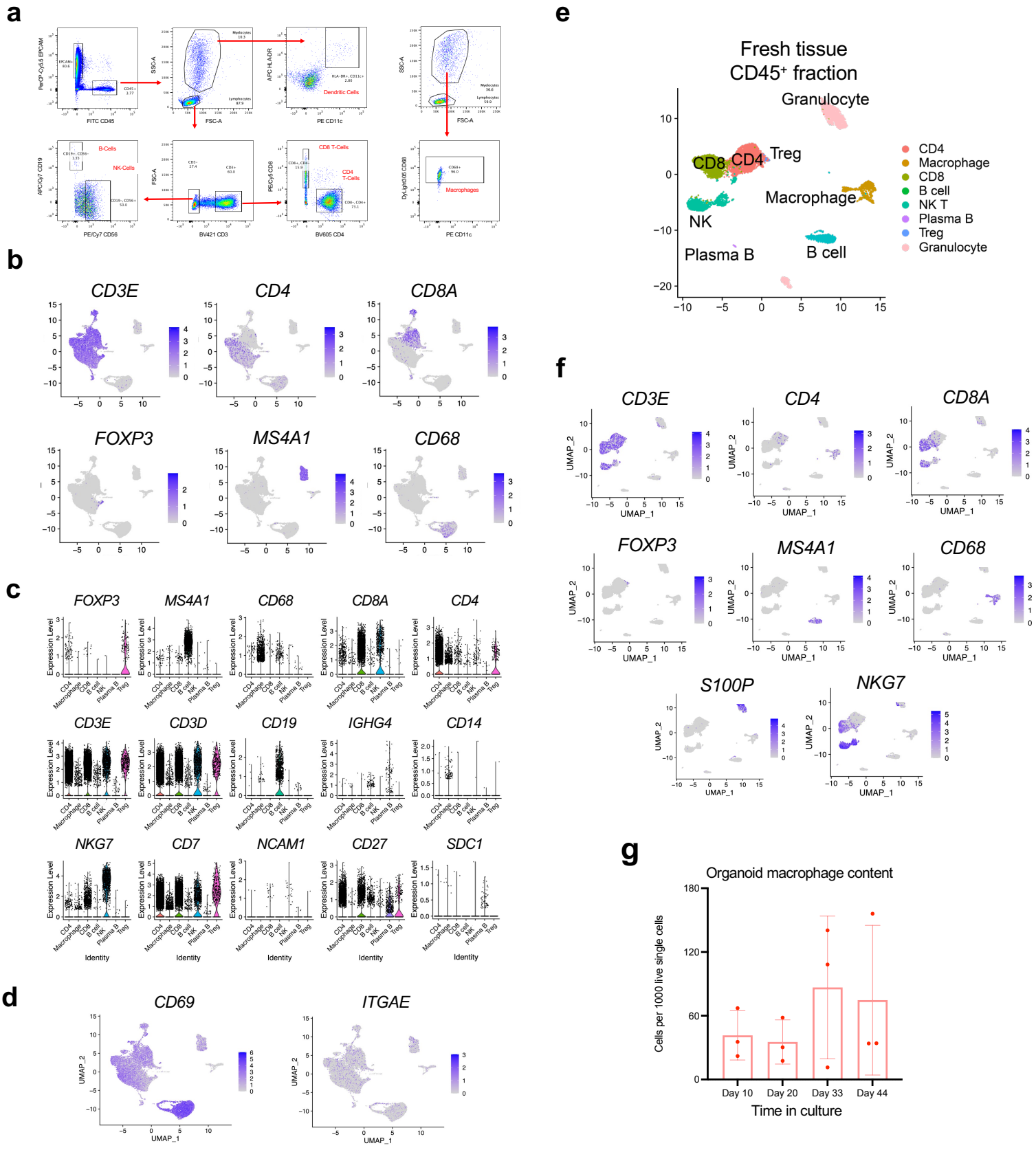
Extended Data Fig. 1. Characterization of epithelial cells in lung ALI organoids. a, Brightfield, lung ALI organoid culture, day 180, scale bar, 2 mm. **b,** H&E of lung ALI organoid, day 8, with plasma cells, lymphocytes, and pigment-laden histiocytes (yellow arrows). Scale bar, 1000 μm. **c,** Flow cytometry plots of EdU^{+} subsets of organoid epithelial, immune, and EPCAM^{-}CD45^{-} cells. **d-g,** IF images of lung ALI organoid basal, AT2, AT1, club, and ciliated cells at extended time points. **d,** KRT5 (red), SFTPC (green), DAPI (blue). Scale bar, 300 μm. **e,** HT1-56 (red), SFTPC (green), DAPI (blue). Scale bar, 100 μm. **f,** SCGB1A1 (green), DAPI (blue). Scale bar, 100 μm. **g,** Acetylated tubulin (white), DAPI (blue). Scale bar, 10 μm. **h,** Violin plots of selected genes from day 12 organoid scRNA-seq, CD45^{-} cells, data are integrated from 3 patients.

Extended Data Fig. 2



Extended Data Fig. 2. scRNA-seq data from fresh lung tissue from one individual used as comparator to cognate organoids in Fig. 1m-o. a, UMAP plot of CD45^{-} cells from fresh lung tissue. **b,** Feature plots of genes used to phenotype clusters from fresh tissue.

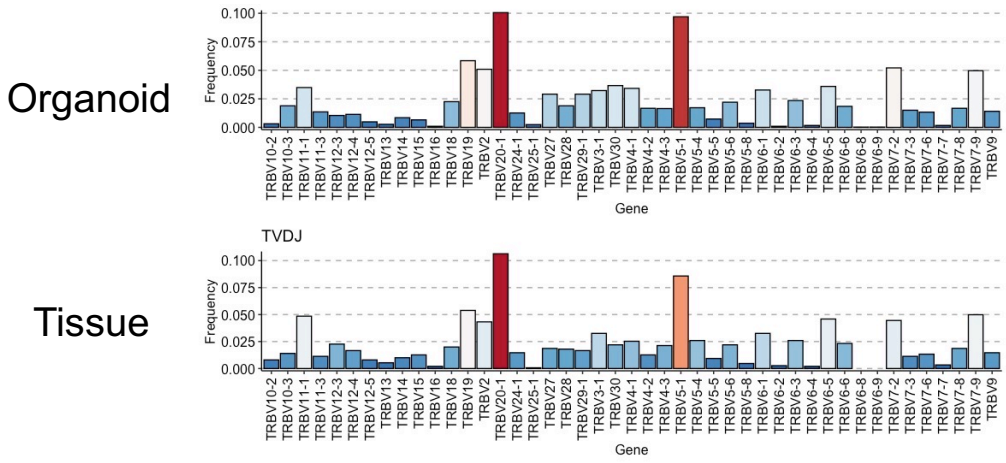
Extended Data Fig. 3



Extended Data Fig. 3. Characterization of immune cells from lung ALI organoids. a, Flow cytometry gating strategy of immune cells from day 12 organoid. **b-c,** scRNA-seq feature plots (b) and violin plots (c) of transcripts used for cell-type assignments in Fig. 2c scRNA-seq. **d,** scRNA-seq feature plots of tissue residency markers *CD69* and *ITGAE (CD103)* from organoid UMAP in Fig. 2c. **e-f,** scRNA-seq of immune cells from fresh tissue from a single individual used as a comparator to Fig. 2d. **e,** UMAP plot showing CD45⁺ cell types from fresh lung tissue (corresponding ALI organoid culture is in Fig. 2c). **f,** Feature plots showing various genes used to phenotype clusters from (e). **g,** Time course of macrophage persistence in ALI lung organoids without cytokine supplementation from Fig. 2g. The y-axis represents the number of cells per 1000 live single cells.

Extended Data Fig. 4

a



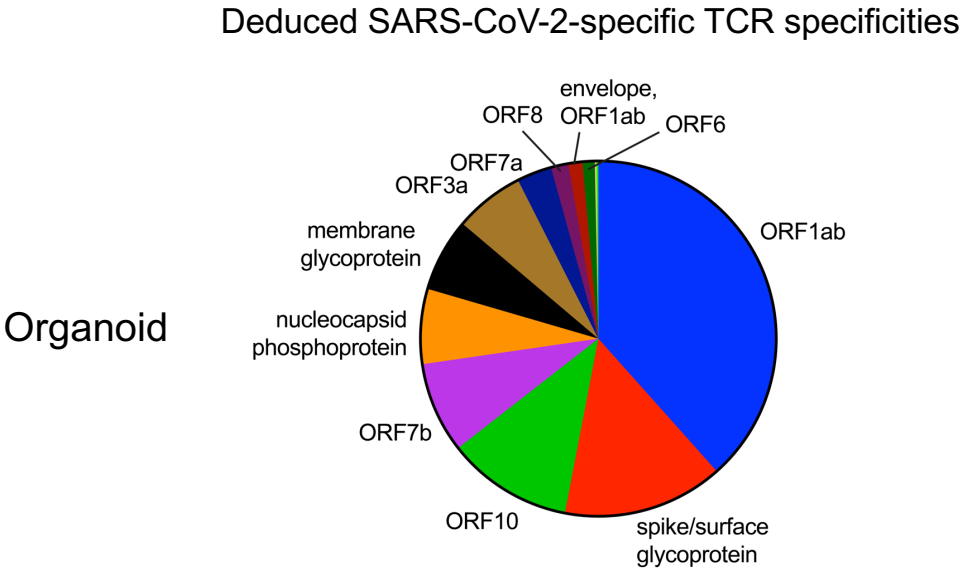
b

SARS-CoV-2	CMV	EBV	Influ. A	YFV	DENV 3/4	DENV 1	HCV	HIV-1
200[a]	74[b]	20[b]	14[b]	6[b]	2[b]	1[b]	1[b]	1[b]

[a] ImmuneCODE database containing 1153,618 known SARS-CoV-2-specific TCR sequences (doi: 10.21203/rs.3.rs-51964/v1)

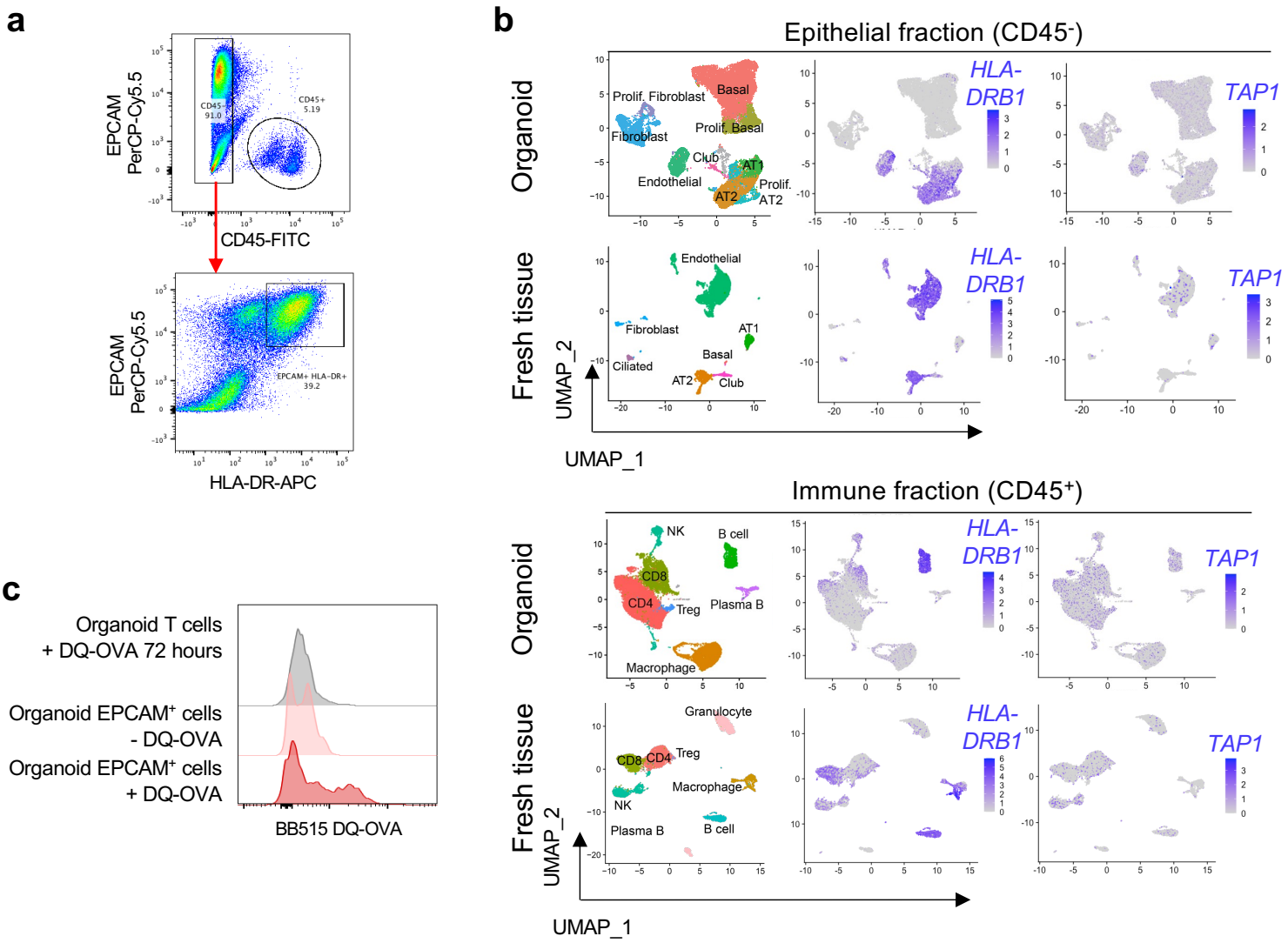
[b] VDJDB curated database containing 39,302 TCR sequences of known antigen specificity (doi: 10.1093/nar/gkx760)

c



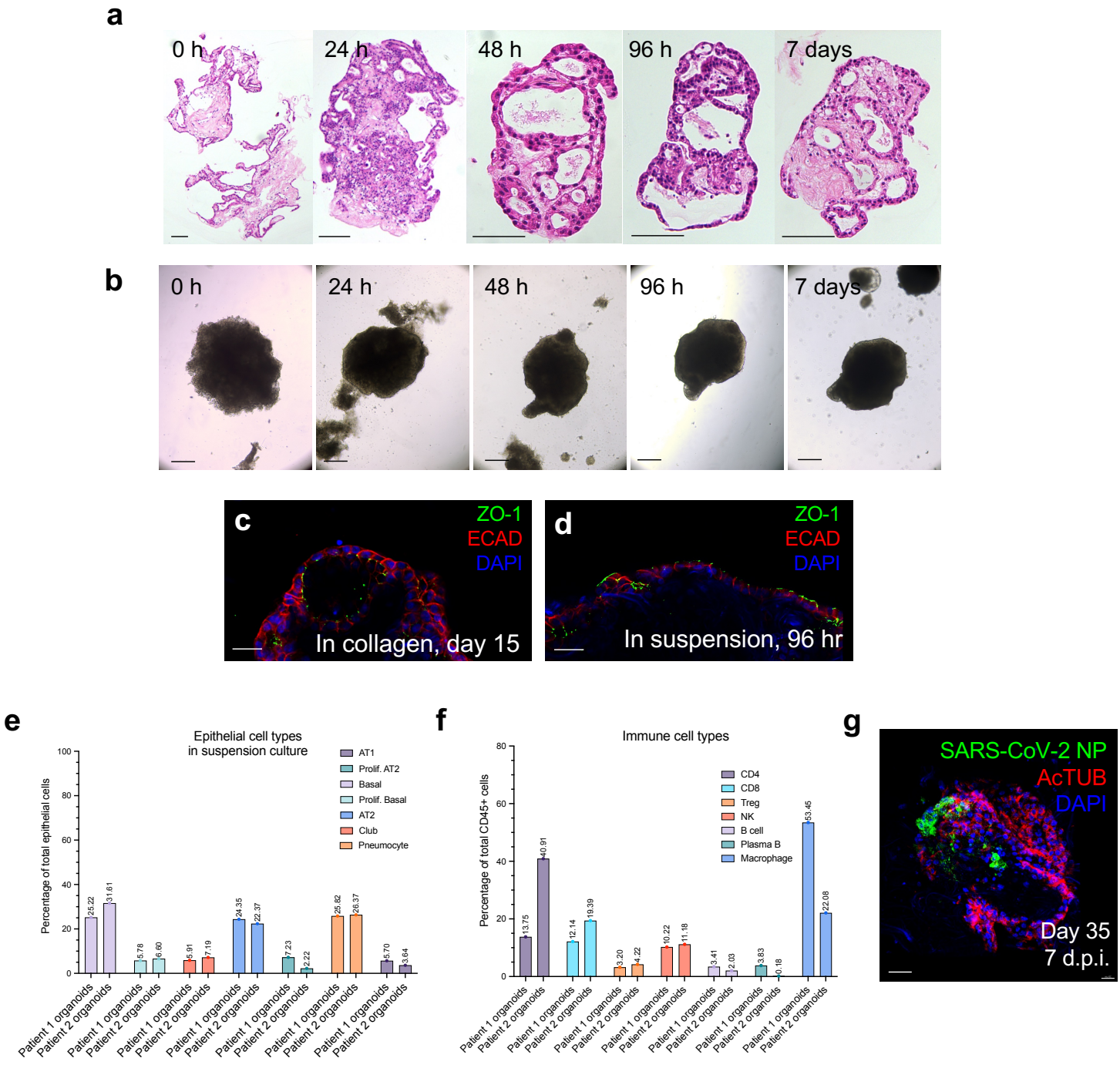
Extended Data Fig. 4. T cell repertoire analysis of lung organoid culture. a, T cell receptor (TCR) V gene usage frequencies comparing fresh tissue and matched day 12 organoid culture from a single individual. **b,** Number of shared TCR clonotypes (identical CDR3 amino acid sequences) between lung organoid culture and two public TCR databases of known viral antigen specificities from (a). **c,** Pie chart of frequencies of deduced SARS-CoV-2 epitope specificities in the lung organoid culture as identified in the reference ImmuneCODE database in panel (b).

Extended Data Fig. 5



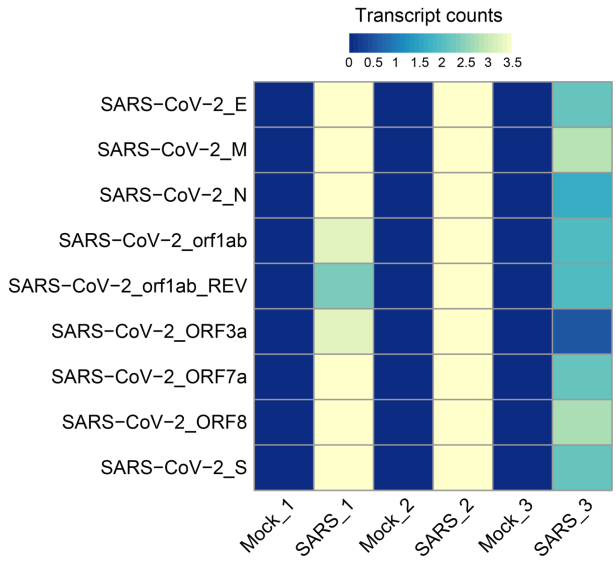
Extended Data Fig 5. Epithelial cells in ALI lung organoids act as antigen-presenting cells. a, Flow cytometry plot showing epithelial expression of surface HLA-DR in day 12 ALI lung organoids. **b,** scRNA-seq feature plots of *HLA-DRB1* and *TAP1* in both day 12 lung ALI organoids (3 individuals for $CD45^-$ and 2 individuals for $CD45^+$) and corresponding fresh tissue (one individual). **c,** DQ-OVA presentation on $EPCAM^+$ cells from organoids, pre-gated on live, single cells. DQ-OVA (20 µg/mL) or PBS control were added to lung ALI organoid cultures for 72 hours. Organoid $CD45^+CD3^+$ T cells and $EPCAM^+$ epithelium were assayed for DQ-OVA uptake by flow cytometry.

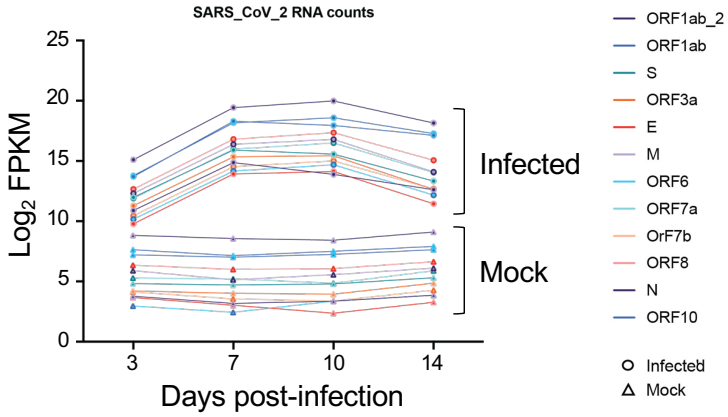
Extended Data Fig. 6

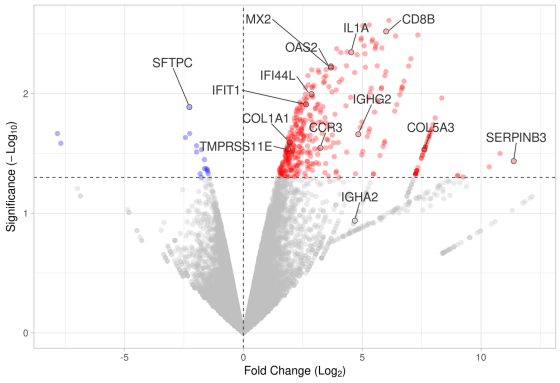


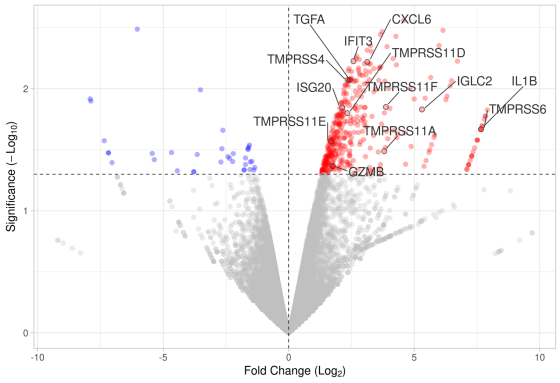
Extended Data Fig. 6. Characterization of suspension lung organoid culture. a, Time course H&E of suspension culture of ALI lung organoids after removal from collagen. Scale bar, 500 μm. **b,** Serial brightfield microscopy of a single lung ALI organoid after removal from collagen. Scale bar, 500 μm. **c-d,** IF of apical tight-junction marker ZO-1 on organoids continuously in collagen (c) or in suspension (d) for 4 days. Both panels are cumulative culture day 15. Scale bar, 50 μm. **e-f,** Quantification of CD45$^-$ epithelial (e) and CD45$^+$ immune (f) cell types from Fig 3h. **g,** IF image of infected AcTUB$^+$ ciliated cells from day 35 organoid, 7 days post-infection. SARS-CoV-2 NP (green), AcTUB (red), DAPI (blue). Scale bar, 100 μm.

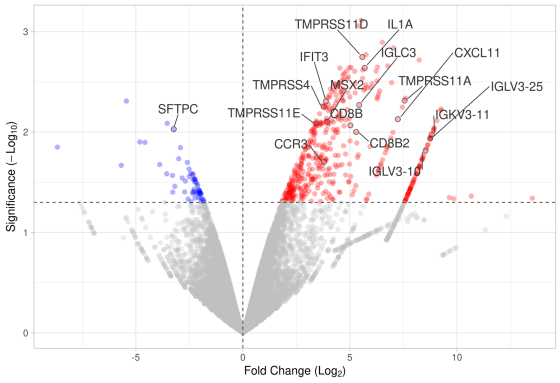
Extended Data Fig. 7

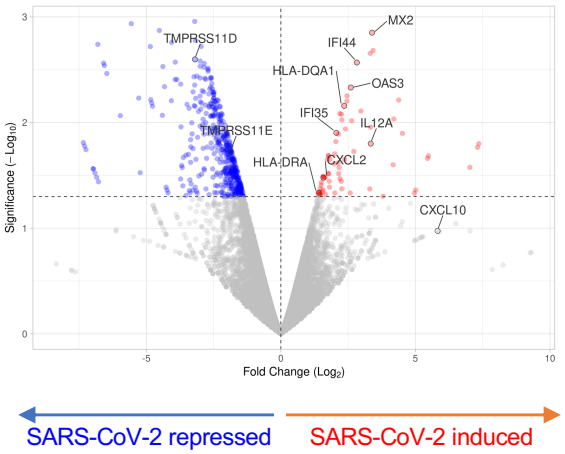






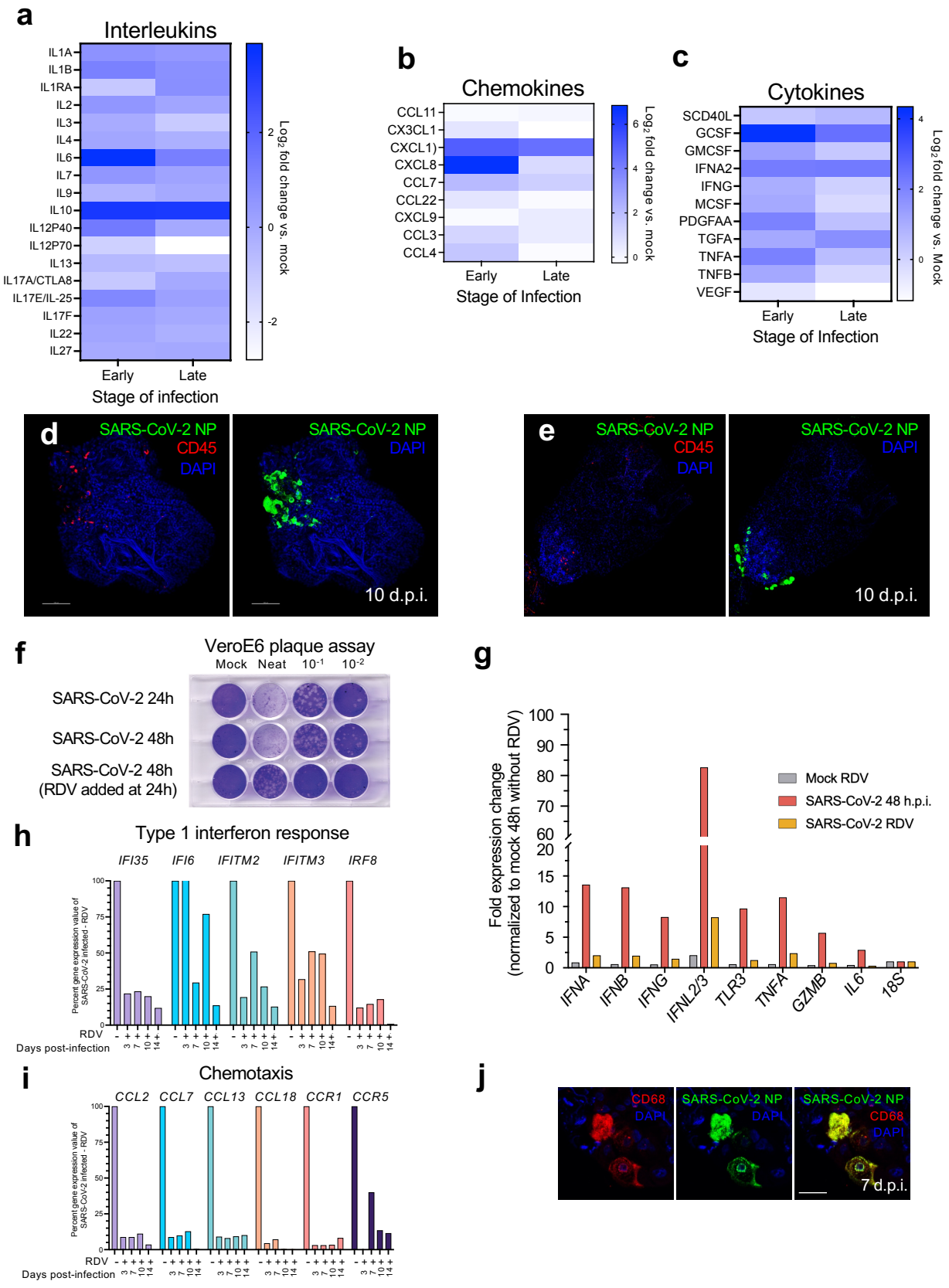






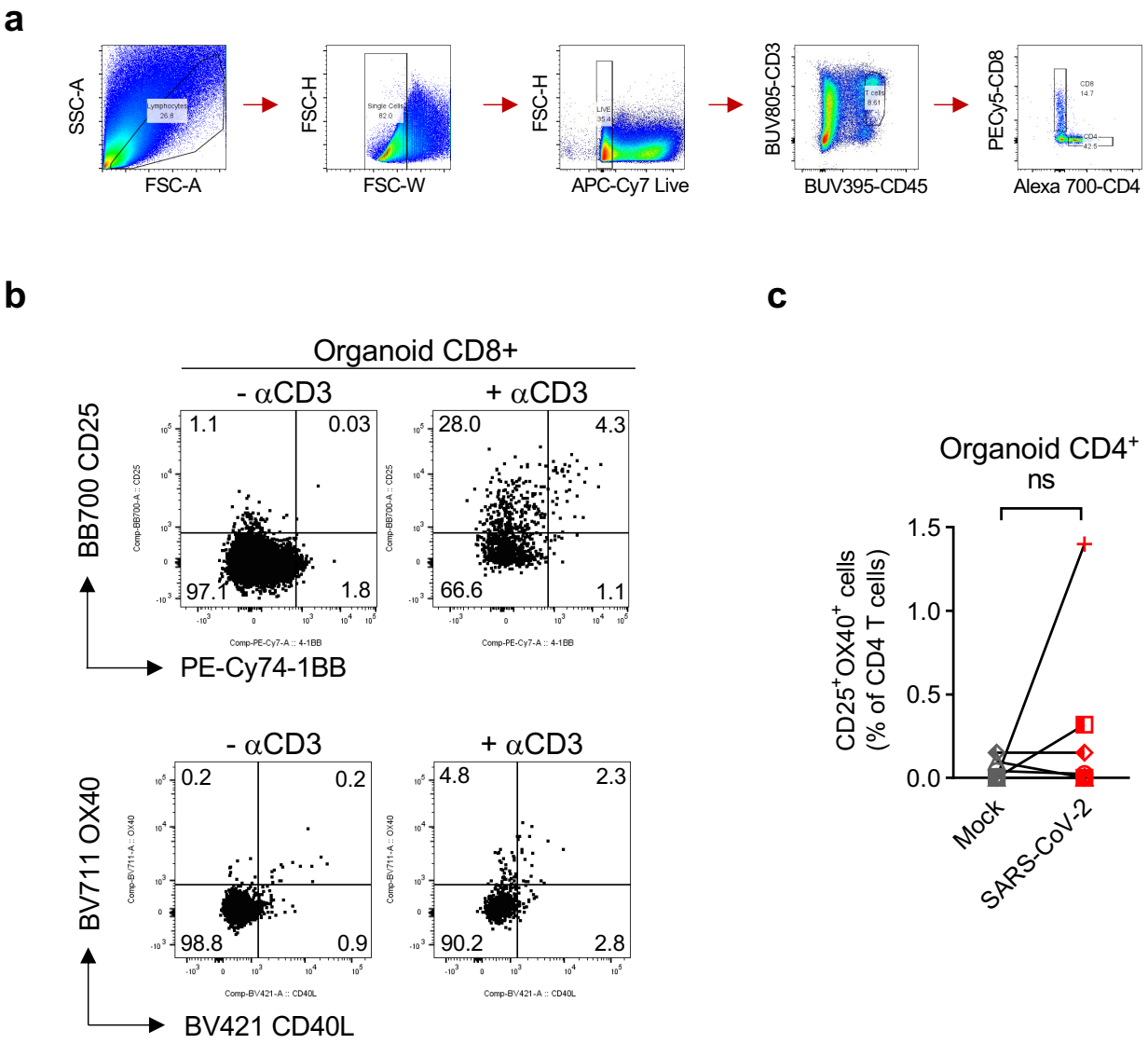
Extended Data Fig. 7. Long-term (3-14 day) SARS-CoV-2 infection of lung ALI organoids in suspension. a, Heatmap of SARS-CoV-2 transcripts detected by Nanostring nCounter in purified lung organoid epithelial cells from three patients, after 7 days of SARS-CoV-2 infection. Color intensity is $\log_2$ normalized transcript counts of the SARS-CoV-2 ORFs. **b,** Bulk RNA-sequencing of SARS-CoV-2 ORFs in lung ALI organoids, 3-14 days post-infection, y-axis is $\log_2$ FPKM. **c,** Volcano plots of bulk RNA sequencing depicting genes that are downregulated (blue) or induced (red) 3-14 days post-infection with SARS-CoV-2 or mock control. Data represent organoids from a single patient. Y-axis is $-\log_{10}$(p value), x-axis is $\log_2$(fold change normalized to mock).

Extended Data Fig. 8



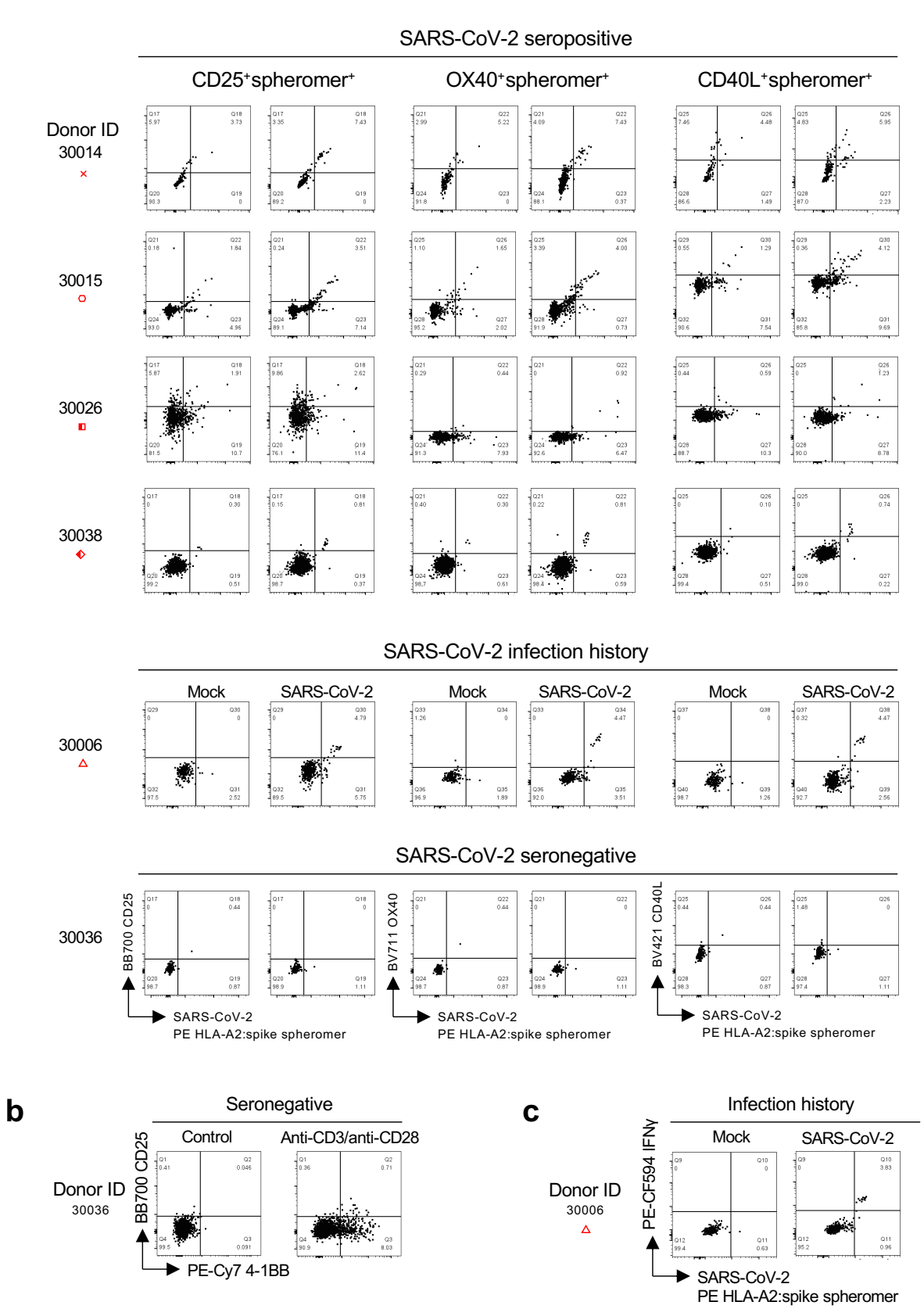
Extended Data Fig. 8. Lung ALI organoids mount innate immune responses to SARS-CoV-2 infection. a-c, Luminex analysis of supernatant from infected suspension organoids from early (3 days) or late (10 days) infection, second biological replicate of Fig 5d, total day 7-17 in culture. **d-e,** Further examples of CD45^{+} cell clustering around infected cells, IF with and without CD45, 10 d.p.i, SARS-CoV-2 NP (green), CD45 (red), DAPI (blue), scale bar, 100 µm. **f,** Vero-E6 cell plaque assay performed with supernatants from SARS-CoV-2-infected suspension lung organoids at 24 and 48 hr post-infection, with or without 10 µM remdesivir (RDV). **g,** qRT-PCR of cytokine expression upon SARS-CoV-2 infection. **h-i,** Bulk RNA sequencing of genes related to interferon signaling (h) and chemotaxis (i) from 3-14 days post infection, -/+ 10 µM RDV. Y-axis represents percentage of gene expression value compared to SARS-CoV-2 infected, no RDV condition. **j,** Representative IF imaging of infected macrophage from organoid 7 days post-infection, SARS-CoV-2 NP (green), CD68 (red), DAPI (blue), scale bar, 30 µm.

Extended Data Fig. 9



Extended Data Fig 9. Organoid T cell activation in response to SARS-CoV-2 infection. a, Gating strategy for AIM assay and spheromer staining in Fig. 6. **b,** Flow cytometry plots showing induction of 4-1BB, CD25, OX40 and CD40L on organoid CD8⁺ T cells with or without organoid anti-CD3/anti-CD28 treatment for 4 days. **c,** Flow cytometry analysis of induction of CD25 and OX40 in organoid CD4⁺ T cells after organoid mock or SARS-CoV-2 infection for 6 days, Wilcoxon matched pairs-test.

Extended Data Fig. 10



Extended Data Fig. 10. Organoid T cell activation in response to SARS-CoV-2 infection. a, Flow cytometry plots showing induction of CD25, OX40 and CD40L on SARS-CoV-2 HLA-A2 spheromer$^+$ organoid CD8 T cells after organoid mock or SARS-CoV-2 infection for 6 days. **b,** Flow cytometry plots showing induction of 4-1BB and CD25 on organoid CD8$^+$ T cells from the SARS-CoV-2 seronegative donor in response to organoid treatment with anti-CD3/anti-CD28 antibody. **c,** Flow cytometry plots showing induction of IFNγ on SARS-CoV-2 HLA-A2 spheromer$^+$ organoid CD8$^+$ T cells after organoid mock or SARS-CoV-2 infection for 6 days.