

Supplementary Materials for

Lung transplantation for patients with severe COVID-19

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Figure S2. Single cell RNA sequencing identifies similarities between end-stage pulmonary fibrosis and organizing pneumonia resulting from COVID-19.

Table S1. Description of the specimens used in this study.

Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/scitranslmed.abe4282/DC1)

Table S2. Marker genes for epithelial cell, macrophage and mesenchymal cell clusters.

Table S3. Differentially expressed genes in COVID-19 and IPF lung samples for each epithelial cell cluster.

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Movie S1. Three-dimensional matrix imaging of COVID-19 and non-COVID-19 lung tissue.

SUPPLEMENTARY MATERIALS

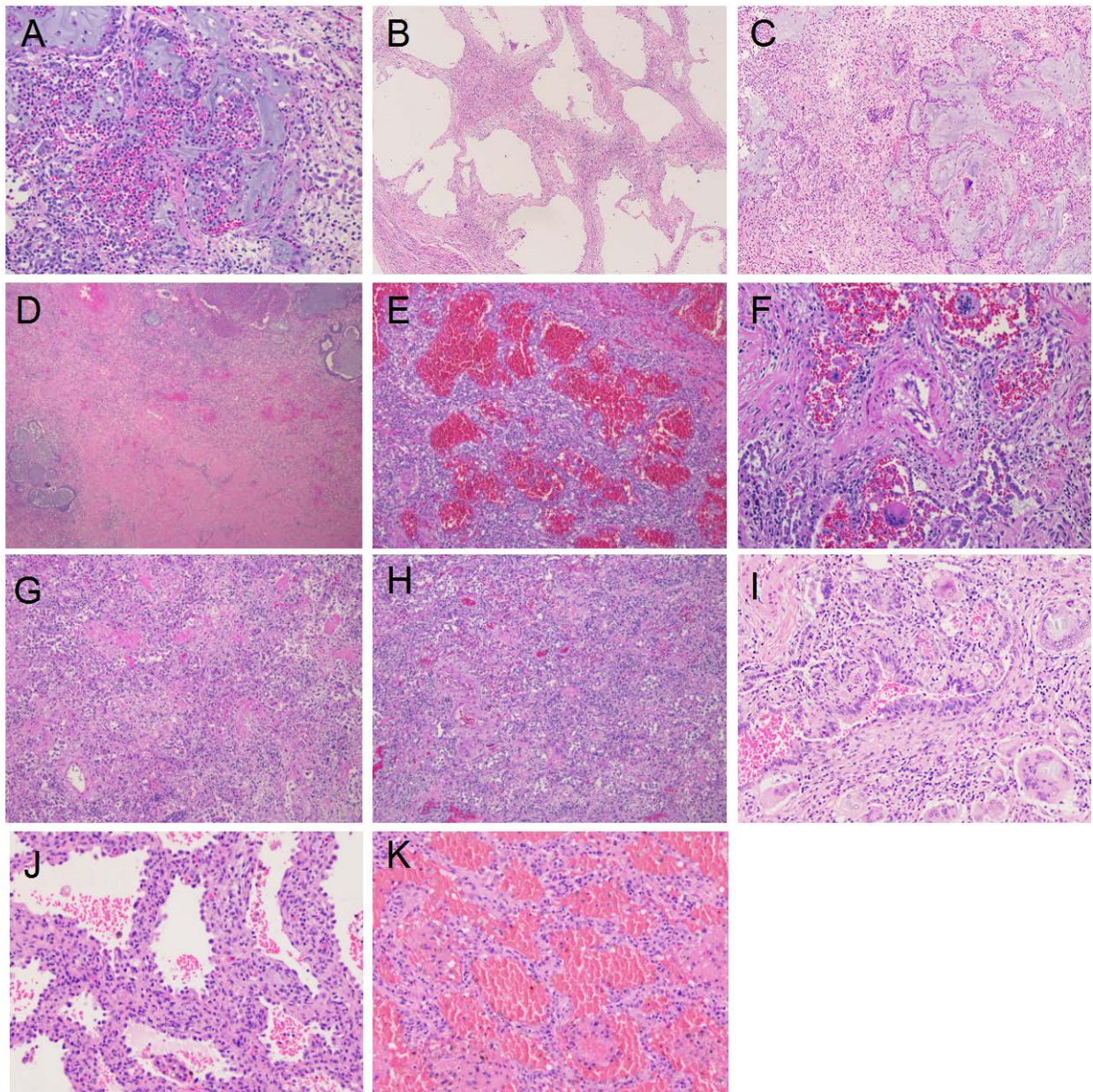


Figure S1. Common histological features of warm lung autopsies. **A.** Bronchiolitis (PMB2, 200x). **B.** Cystic change. Acute and chronic organizing pneumonia (PMB2,40x). **C.** Organizing DAD/pneumonia, obliteration of the normal architecture. Dense fibrosis (PMB2, 40x). **D.** Neutrophilic abscess formation. Dense fibrosis. Bronchiolitis (PMB2, 40x). **E.** Cystic airspaces with hemorrhage. Acute and chronic interstitial pneumonia (PMB1, 100x). **F.** DAD with type II pneumocyte hyperplasia. Multinucleate giant cells.

Alveolar hemorrhage. Organizing pneumonia with dense fibrosis (PMB1, 200x). **G.** Organizing DAD/pneumonia. Dense collagen whorls with numerous fibroblasts. Chronic inflammation (PMB1, 100x). **H.** Organizing DAD/pneumonia. Obliteration of the normal architecture. Airspaces are filled with type II pneumocytes, macrophages, fibrin membranes, red blood cells and fibroblasts. Dense collagen is present (PMB1, 100x). **I.** Multinucleated giant cell with foreign body (200x). **J.** Alveolar septal expansion by reactive pneumocytes and chronic inflammatory cells (200x). **K.** Intra alveolar fibrin and hemorrhage (200x).

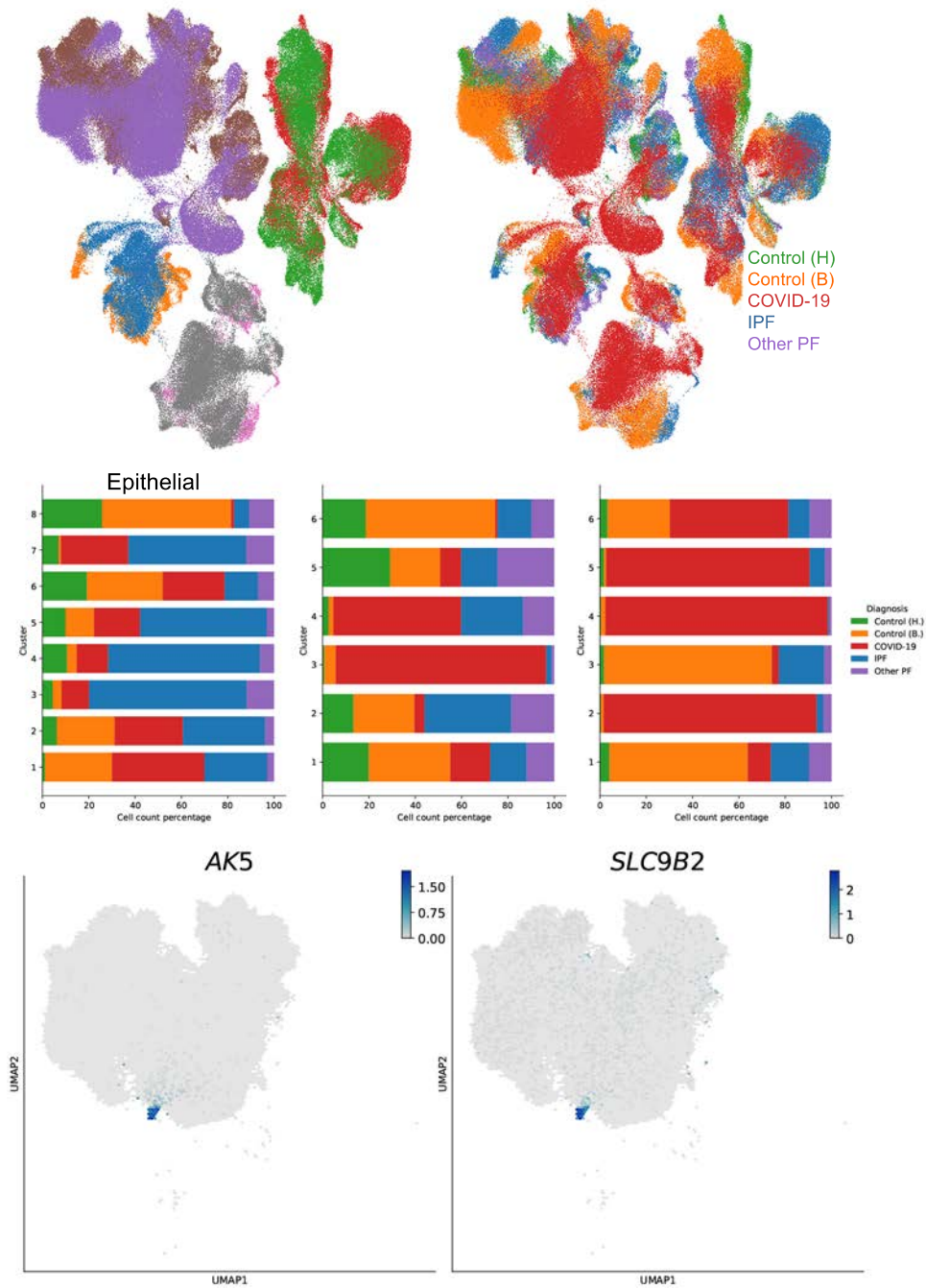


Figure S2. Single cell RNA sequencing identifies similarities between end-stage pulmonary fibrosis and organizing pneumonia resulting from COVID-19. A. UMAP plot showing integrative analysis of single cell transcriptomes from control and subjects with pulmonary fibrosis (Haberman et al., 2020) and three patients with COVID-19 and

two donor lungs using scArches. Main cell lineages are shown: epithelial, immune, mesenchymal and endothelial, color-coded by the dataset. H. indicates cells from Haberman et al., dataset, B. indicates cells from our dataset. **B.** UMAP plot showing cluster composition according to the diagnosis. All non-IPF samples from Habermann et al., were collapsed into “Other PF” category. **C–E.** Stacked barplots demonstrating relative abundance of cells per each cluster in the specific lineage: epithelial (C.), macrophages (D.), and mesenchymal (E.). **F.** UMAP plot showing expression of AK5 and SLC9B2 in a subset of macrophages from cluster 4.

Patient	Abbreviation	Age	Sex	Comorbidity	Gross pathology & Histology	RNA-Scope for SARS-CoV-2	Tissue Clearing	Single Cell RNA-Seq
COVID-19 transplant Case 1	Case 1	28	F	Neuromyelitis optica	Yes	Yes	Yes	Yes
COVID-19 transplant Case 2	Case 2	62	M	Hypertension	Yes	Yes	No	No
COVID-19 transplant Case	Case 3	43	M	Diabetes Mellitus Type 2	Yes	No	No	No
Post-mortem biopsy 1	PMB1	54	M	Hyperlipidemia	Yes	No	Yes	Yes
Post-mortem biopsy 2	PMB2	57	F	None	Yes	No	Yes	Yes
Idiopathic pulmonary fibrosis patient 1	IPF1	NA	NA	NA	No	No	Yes	No
Idiopathic pulmonary fibrosis patient 2	IPF2	NA	NA	NA	No	No	Yes	No
Donor 1	Donor1	52	M	Stroke	No	No	No	Yes
Donor 2	Donor2	25	F	Head trauma	No	No	No	Yes
Normal Control	PE control	43	M	Pulmonary Embolism	No	Yes	Yes	No
COVID-19 no ICU treatment	Palliative COVID-19	81	F	Cirrhosis	No	Yes	No	No

Table S1. Description of the specimens used in this study.

*N/A consent does not allow sharing of patient demographics.

Table S2. Marker genes for epithelial cell, macrophage and mesenchymal cell clusters.

Marker genes for each cluster of Epithelial cells (Figure 4A), Macrophages (Figure 4C) and Mesenchymal cells (Figure 4E). Marker genes were computed with scanpy package using Wilcoxon rank-sum test, and top 100 markers were selected.

Table S3. Differentially expressed genes in COVID-19 and IPF lung samples for each epithelial cell cluster. Differentially expressed genes between cells from COVID-19 and IPF samples for each cluster of Epithelial cells (Figure 4A). Only clusters with at

least 100 cells for each condition were kept. Wilcoxon rank-sum test. Only genes that were expressed in more than 5% of cells in both conditions are shown.

Table S4. Differentially expressed genes in COVID-19 and IPF lung samples for each macrophage cluster. Differentially expressed genes between cells from COVID-19 and IPF samples for each cluster of Macrophages (Figure 4C). Only clusters with at least 100 cells for each condition were kept. Wilcoxon rank-sum test. Only genes that were expressed in more than 5% of cells in both conditions are shown.

Table S5. Differentially expressed genes in COVID-19 and IPF lung samples for each mesenchymal cell cluster. Differentially expressed genes between cells from COVID-19 and IPF samples for each cluster of Mesenchymal cells (Figure 4E). Only clusters with at least 100 cells for each condition were kept. Wilcoxon rank-sum test. Only genes that were expressed in more than 5% of cells in both conditions are shown.

Video S1. Three-dimensional matrix imaging of COVID-19 and non-COVID-19 lung tissue. A 360° view of SHIELD cleared lung tissue allowing visualization of the collagen structure and matrix (cyan). The video shows images of different cleared lung samples in the following order: normal lung (CONTROL), explanted lung of a COVID-19 patient who underwent a bilateral lung transplant (EXPLANT), warm autopsy lung biopsies from two patients who died of severe COVID-19 (PMB1, PMB2), and explanted lung from a patient with end-stage idiopathic pulmonary fibrosis (IPF) who underwent a bilateral lung transplant.