**Cell Reports Medicine, Volume *2***

## Supplemental information

# Spatial mapping of SARS-CoV-2 and H1N1 lung injury identifies differential transcriptional signatures

**Camilla Margaroli, Paul Benson, Nirmal S. Sharma, Matthew C. Madison, Sarah W. Robison, Nitin Arora, Kathy Ton, Yan Liang, Liang Zhang, Rakesh P. Patel, and Amit Gaggar**

# Supplementary Materials for

## Spatial mapping of SARS-CoV-2 and H1N1 Influenza Lung Injury Identifies Differential Transcriptional Signatures

C. Margaroli, P. Benson, N.S Sharma, M.C. Madison, S.W. Robison, N. Arora, K. Ton, Y. Liang, L. Zhang, R.P. Patel, A. Gaggar

Correspondence to: agaggar@uabmc.edu

**This PDF file includes:**

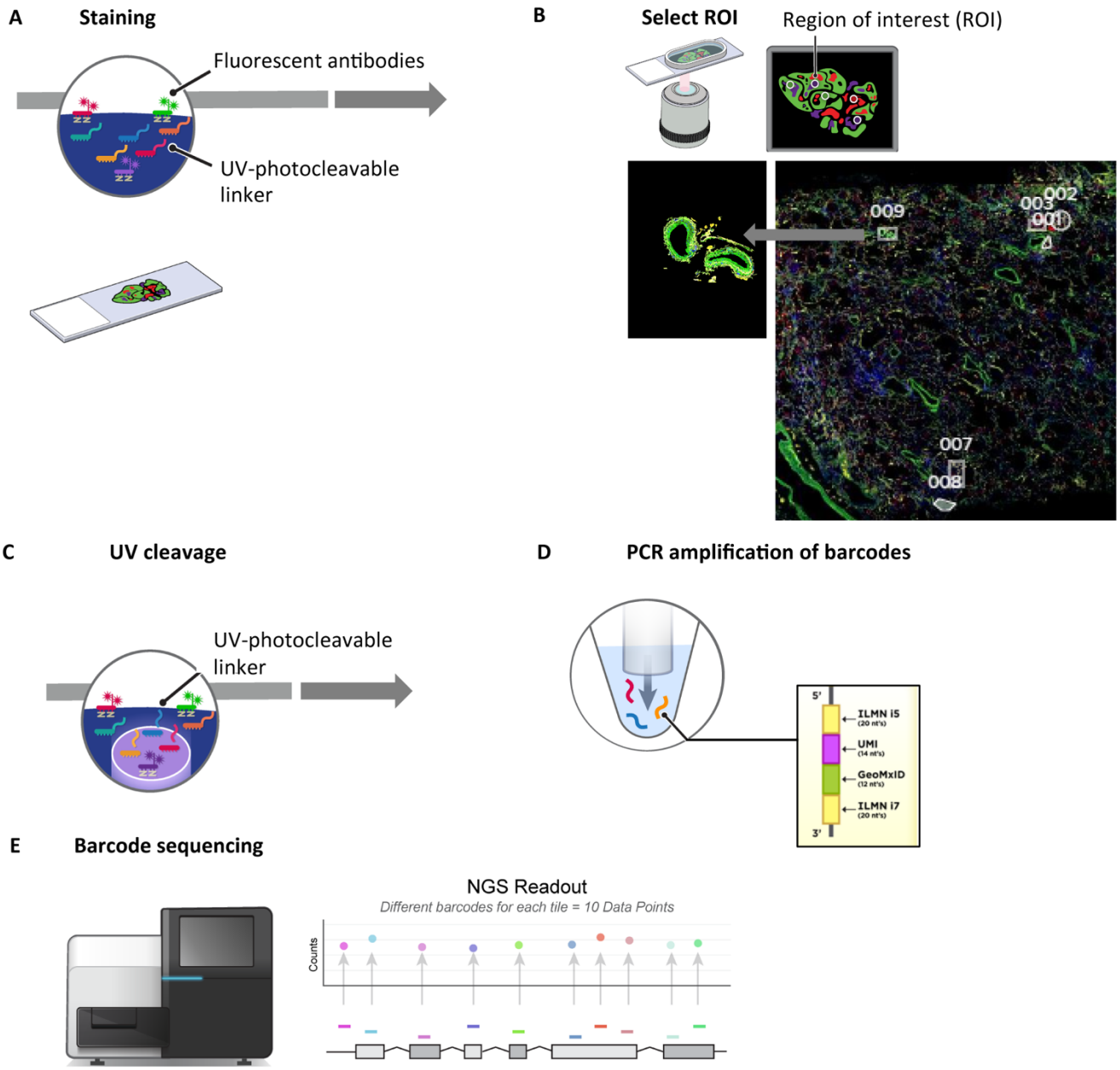


**Fig. S1 related to STAR Methods. Nanostring workflow. (A)** The tissue is processed and stained using a standard ISH protocol with both fluorescent labeled morphology markers and sequence specific RNA probes. **(B)** Morphology markers are used for Region of Interest (ROI) and Area of Interest (AOI) selection. **(C)** After ROI/AOI selection, UV light is shone on the ROI/AOI decoupling the barcodes from the RNA probes only from the selected area or cells of interest. Five probes per mRNA target are used and are coupled to molecular barcodes and a unique barcode identifies a specific gene target. The UMI identifies a specific molecule and accounts for amplification bias from PCR. **(D)** RNA probes for the ROI/AOI are aspirated and deposited into a unique well of a 96 well plate. The PCR step adds dual indexing barcodes (Index1 and Index2) to identify AOIs and adds Illumina flow cell adapter regions (P5 and P7) to enable sequencing. **(E)** The barcodes are sequenced.

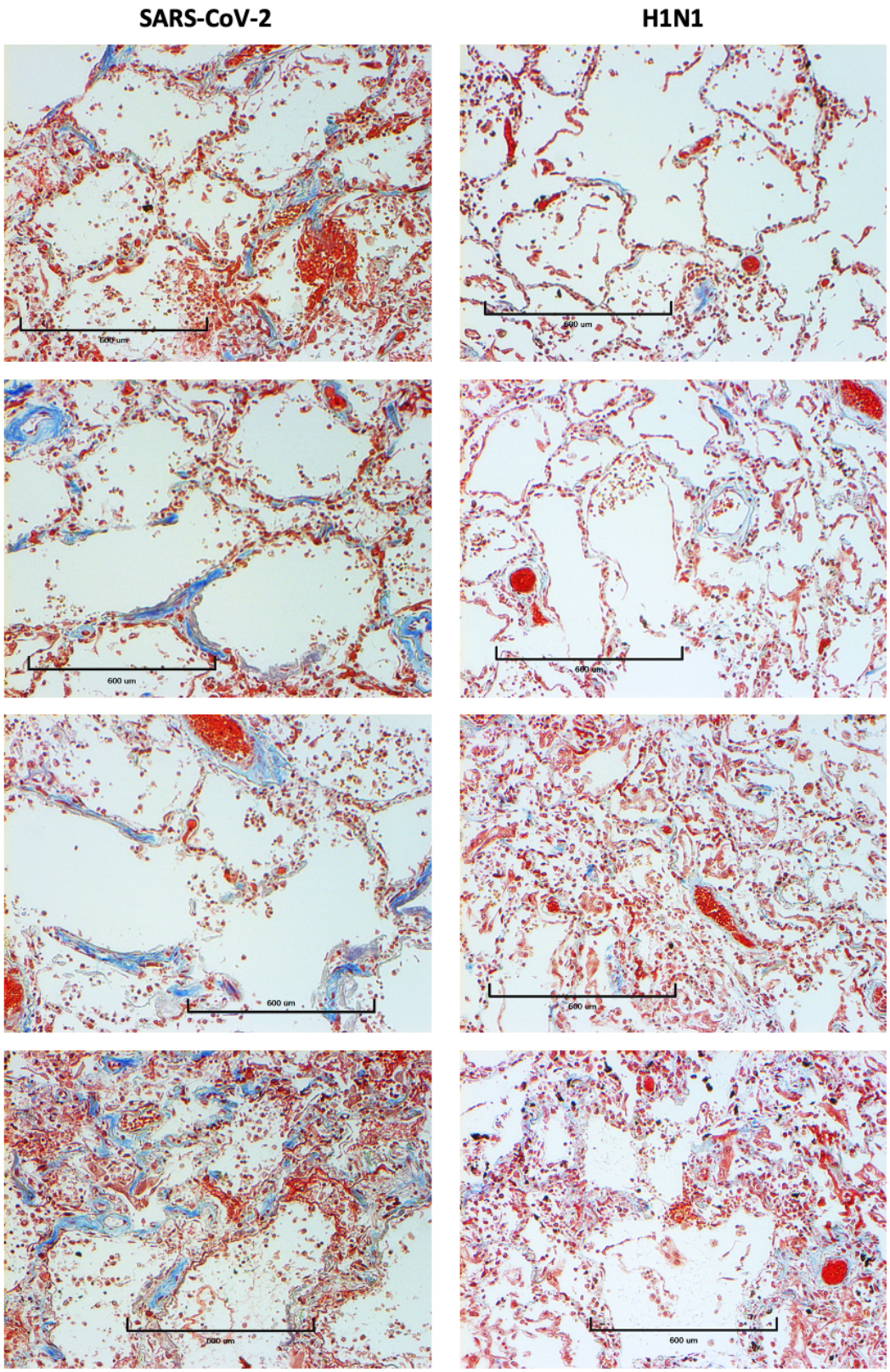


**Fig. S2 related to Figure 1.** Representative areas of collagen deposition in H1N1 and SARS-CoV-2 lung tissue. Collagen staining (blue) was performed by Masson's trichrome staining on paraffin embedded lung tissues. Scale bar 600$\mu$m. SARS-CoV-2 infected patients (n=3), H1N1 (n=3).

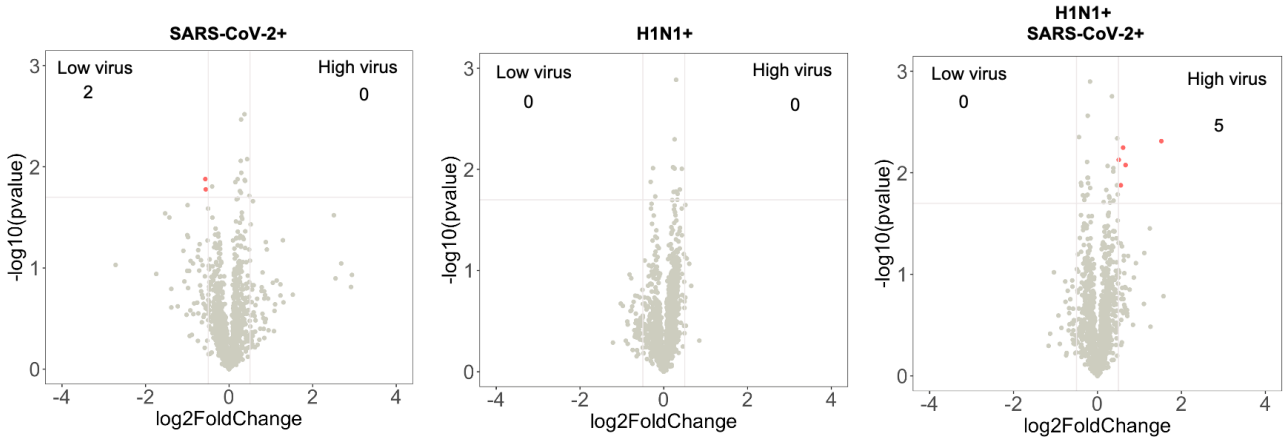


**Fig. S3 related to Figure 1. Viral load does not influence gene expression.** Differential gene expression comparing areas of high and low viral presence determined by immunofluorescence in patients infected by SARS-CoV-2, H1N1 or both viruses, showed no differential modulation of gene expression. P-value threshold for differential gene expression were set at p=0.02 and log2 fold change of 0.5. Biological replicates were analyzed for SARS-CoV-2 infected patients (n=3) and H1N1 (n=3), technical replicates were used for SARS-CoV-2/H1N1 (high n=2, low n=2).

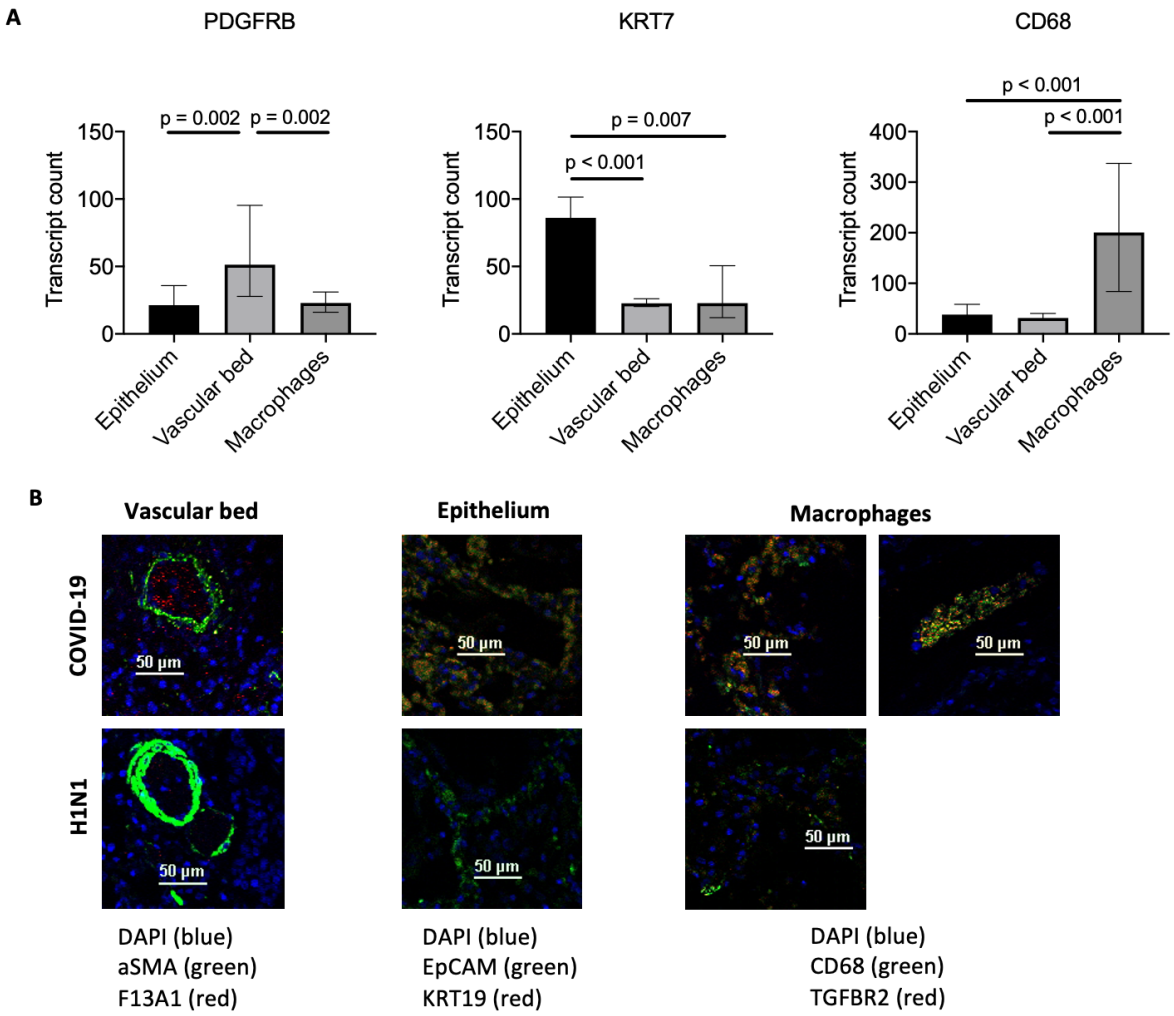


**Fig. S4 related to Figures 2, 3, and 4. Gene and protein expression in cell populations selected by immunofluorescence.** Specific cell populations for transcriptional profiling were selected using immunofluorescence staining. **(A)** Validation of cellular purity was performed by gene expression in the selected samples, for vascular bed (PDGFRB), alveolar epithelium (KRT7), and macrophages (CD68). Replicates for expression include SARS-CoV-2 infected patients (n=3), H1N1 (n=3), and SARS-CoV-2/H1N1 (n=1). **(B)** Validation of colocalization for high expressed genes in SARS-CoV-2 tissue for epithelium, vascular bed and macrophages. Scale bars= 50$\mu$m. Results are shown as median and interquartile range. Data were analyzed using Mann-Whitney test.

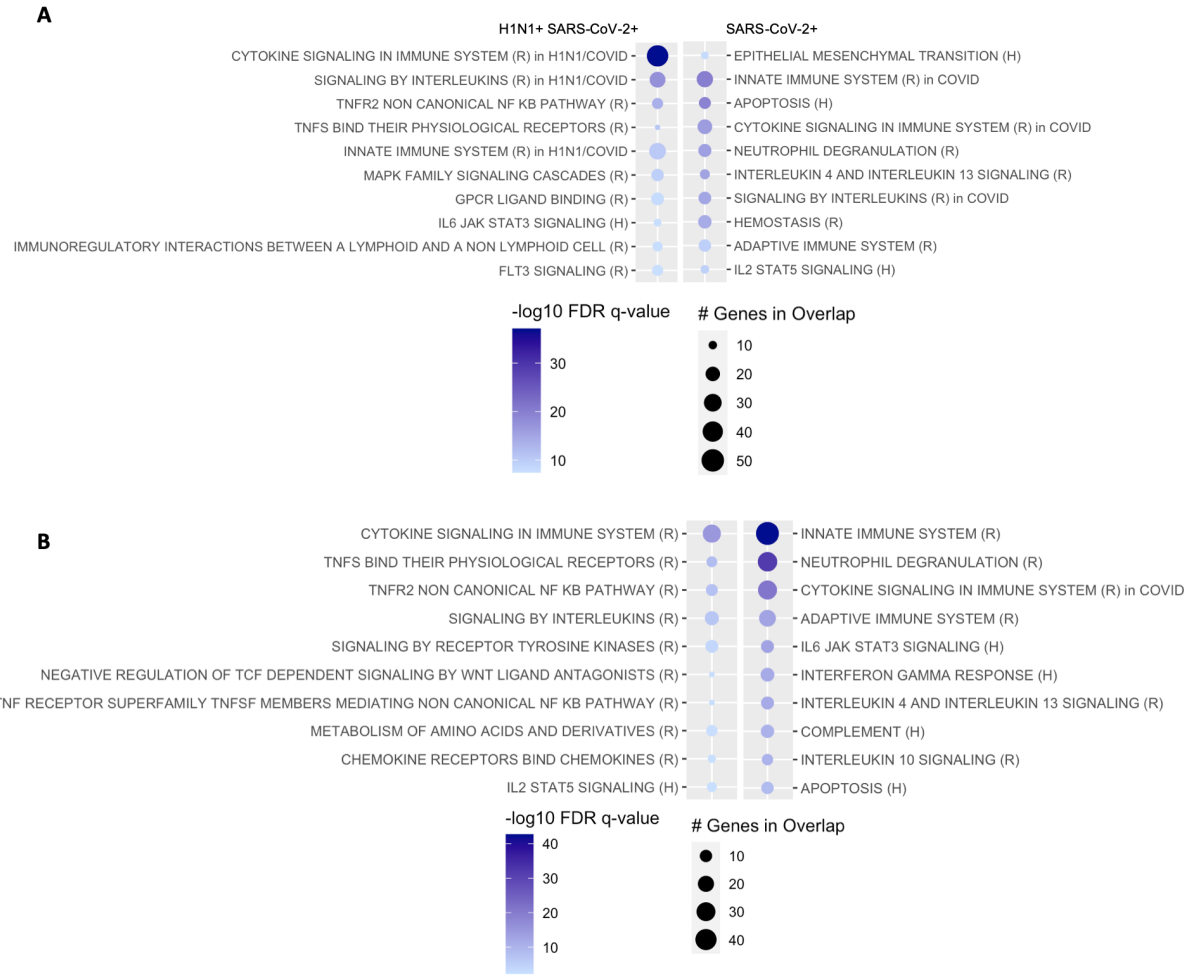


**Fig. S5 related to Figures 3 and 4. SARS-CoV-2/H1N1 infected patient mimic H1N1 only gene expression.** Geneset enrichment analysis using reactome (R) and hallmark (H) datasets for up-regulated or down-regulated genes in SARS-CoV-2 infected patients compared to SARS-CoV-2/H1N1 sample set for the epithelium **(A)** (technical replicates n= 12 for SARS-CoV-2 and n=5 SARS-CoV-2/H1N1), and macrophages **(B)** (technical replicates n= 9 for SARS-CoV-2 and n=3 SARS-CoV-2/H1N1). Differential gene expression was defined as p-value of 0.02 and log2 fold change of 0.5. SARS-CoV-2 infected patients (n=3) and SARS-CoV-2/H1N1 (n=1).

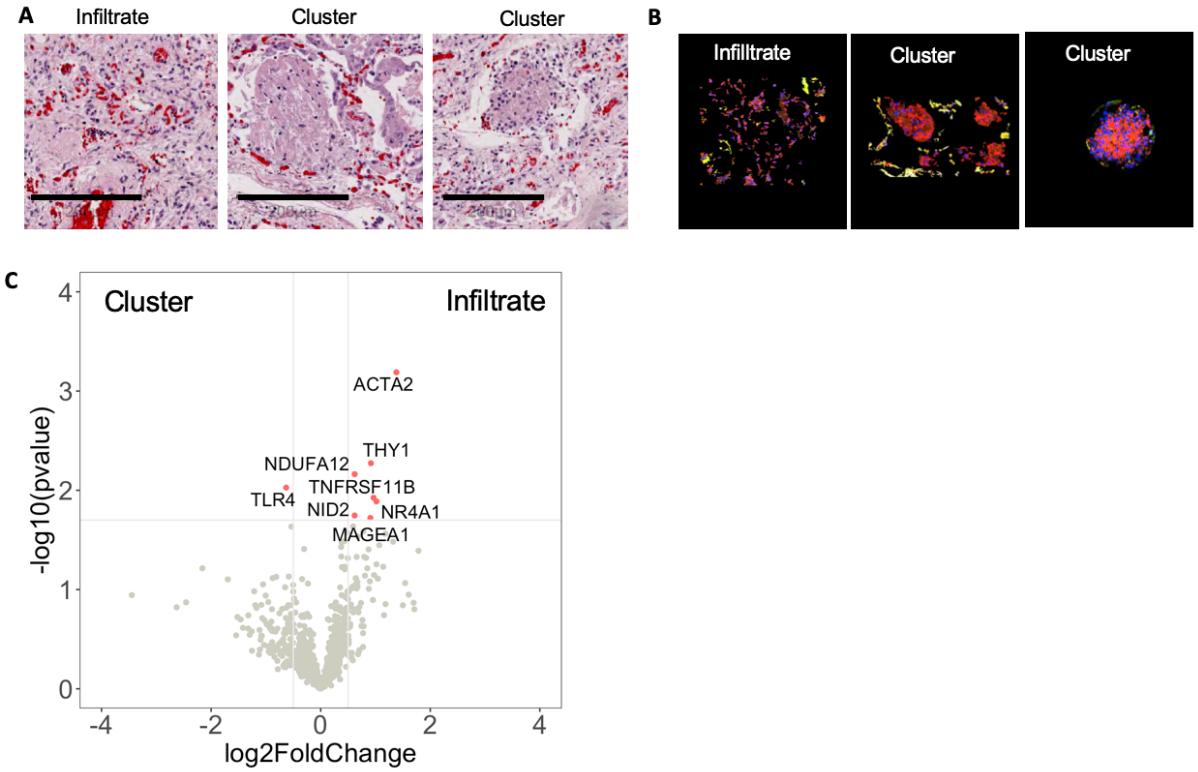


**Fig. S6 related to Figure 4. Analysis of clustering macrophage transcriptional phenotype in SARS-CoV-2 patients.** **(A)** Histological analysis of tissues sections stained by H&E revealed presence of clustering macrophages (scale bars = 200$\mu$m), which was confirmed by **(B)** CD68 staining (Blue = nuclei, Red = CD68, Yellow = EpCAM). Differential gene expression analysis of clustering vs infiltrating macrophages in SARS-CoV-2 infected patients showed only 8 differentially regulated genes. Differential gene expression was defined as p-value of 0.02 and log2 fold change of 0.5. SARS-CoV-2 infected patients (n=2). Technical replicates n= 4 for infiltrate and n=4 for cluster.

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 |
|---|---|---|---|---|---|---|---|
| **VIRAL TESTING** | | | | | | | |
| SARS-CoV-2 | Positive | Positive | Positive | Negative | Negative | Negative | Positive |
| Influenza- H1N1 | Negative | Negative | Negative | Positive | Positive | Positive | Positive |
| **DEMOGRAPHICS** | | | | | | | |
| Age in years | 86 | 69 | 75 | 57 | 50 | 63 | 58 |
| Race | C | AA | C | C | C | AA | AA |
| Gender | Male | Male | Male | Female | Female | Male | Female |
| **CO-MORBIDITIES** | | | | | | | |
| Diabetes Mellitus | None | Yes | None | None | None | None | None |
| CV Disease | None | None | None | None | HFpEF | None | None |
| Chronic Lung Disease | Asthma | None | None | None | COPD | None | CTD-ILD, COPD |
| Chronic Kidney Disease | None | None | None | None | None | None | None |
| **INTERVENTIONS** | | | | | | | |
| Antiviral (days) | None | None | Remdesivir (2) | Oseltamivir (7) | Oseltamivir (7) | Oseltamivir (10) | Oseltamivir (10) |
| Steroids (days) | MP (3) | HC (16) | Dex (1) | HC (3) | MP (2) | Pred (3) | HC (6) |
| Epoprostenol (days) | None | None | EPO 2d | None | EPO (16) | NO, EPO (3) | EPO (4) |
| Mechanical Ventilation | 100% FiO2 via NRB | 5.7cc/kg Tv, 12 PEEP, 60% FiO2 | 5.3cc/kg Tv, 16 PEEP, 60%FiO2 | 6cc/kg Tv, 16 PEEP, 100% FiO2 | 5.3cc/kg Tv, 18 PEEP, 100% FiO2 | 5.1cc/kg Tv, 15 PEEP, 90% FiO2 | 5.9cc/kg Tv, 10 PEEP, 100% FiO2 |
| Proning (days) | None | Yes (9) | Yes (3) | None | Yes (5) | Yes (4) | None |
| ECMO (days) | None | None | None | VA (5) | None | None | None |
| **ICU ADMISSION** | | | | | | | |
| BMI ($kg/m^2$) | 22.6 | 28 | 32.3 | 30 | 68.9 | 27.5 | 31 |
| WBC ($x10^3/cmm$) | 14.8 | 12.7 | 8.14 | 0.9 | 6.35 | 11.92 | 11.6 |
| % PMN | 81% | 90% | 88% | 4% | 87% | 86% | 84% |
| % Lymphocytes | 6% | 6% | 7% | 72% | 7% | 5% | 9% |
| Troponin I (ng/L) | <0.3 | 88 | 17 | 38 | 128 | n/a | 1761 |
| BNP (pg/mL) | n/a | 78 | 536 | 445 | 581 | n/a | 299 |
| AST (U/L) | 34 | 106 | 68 | 15 | 41 | 32 | 43 |
| Creatinine (mg/dL) | 1.9 | 1.6 | 1.1 | 0.9 | 1.5 | 1.4 | 1.0 |
| D-Dimer (ng/mL) | n/a | >20,000 | 921 | 2,978 | n/a | n/a | 12,301 |
| PT (sec) | 16.2 | 14.6 | 13.4 | 15.1 | 16.5 | 13.1 | 13.2 |
| LDH (U/L) | n/a | 777 | n/a | 198 | n/a | n/a | 472 |
| **PHYSIOLOGY** | | | | | | | |
| Chest Radiograph (# of quadrants [1-4]) | 4 | 2 | 4 | 2 | 4 | 4 | 4 |
| PaO2:FiO2 ratio | 61 | 120 | 173 | 64 | 47 | 132 | 113 |
| APACHE II Score<br>(a) admission<br>(b) 48h before death | <br>(a) 20<br>(b) 24 | <br>(a) 26<br>(b) 35 | <br>(a) 20<br>(b) 43 | <br>(a) 17<br>(b) 24 | <br>(a) 13<br>(b) 37 | <br>(a) 20<br>(b) 38 | <br>(a) 14<br>(b) 17 |
| SOFA Score<br>(a) admission<br>(b) 48h before death | <br>(a) 5<br>(b) 6 | <br>(a) 12<br>(b) 18 | <br>(a) 10<br>(b) 17 | <br>(a) 10<br>(b) 17 | <br>(a) 11<br>(b) 14 | <br>(a) 7<br>(b) 15 | <br>(a) 7<br>(b) 7 |
| **30-DAY** | | | | | | | |
| ICU Days | 5 | 16 | 4 | 6 | 19 | 11 | 10 |
| Ventilator Days | 0 | 16 | 4 | 6 | 17 | 8 | 8 |
| New Organ Failure | 3 | 5 | 3 | 5 | 2 | 3 | 3 |

**Table S1 related to STAR methods. Summary of patient demographics.** Abbreviations: C: Caucasian; AA: African American; CV: cardiovascular; HFpEF: heart failure preserved ejection fraction; CTD-ILD: connective tissue disease associated interstitial lung disease; COPD: chronic obstructive lung disease; MP: Methylprednisone; HC: Hydrocortisone; TV: tidal volume, PEEP: positive end-expiratory pressure, FIO2: fraction of inspired oxygen, ECMO: extracorporeal membrane oxygenation, V-A: venous-arterial; BMI: body mass index; WBC: white blood cells; BNP: brain natriuretic

peptide; AST: aspartate aminotransferase; PT: prothrombin time; LDH: lactic acid dehydrogenase; PaO2: partial pressure arterial oxygen; APACHE: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; ICU: intensive care unit.

**COVID-19 added gene set**

| | |
|---|---|
| S | Spike Protein |
| ORF1ab | ORF1ab |
| ORF1ab_REV | Negative Strand ORF1ab |
| ACE2 | angiotensin I converting enzyme 2 |
| ABCA3 | ATP binding cassette subfamily A member 3 |
| AQP5 | aquaporin 5 |
| DHX58 | DEXH (Asp-Glu-X-His) box polypeptide 58 |
| FURIN | furin (paired basic amino acid cleaving enzyme) |
| HAS2 | hyaluronan synthase 2 |
| HOPX | HOP homeobox |
| IFNLR1 | interferon lambda receptor 1 |
| IL10RB | interleukin 10 receptor subunit beta |
| MUC13 | mucin 13, cell surface associated |
| MUC2 | mucin 2, oligomeric mucus/gel-forming |
| MUC5AC | mucin 5AC, oligomeric mucus/gel-forming |
| MUC5B | mucin 5B, oligomeric mucus/gel-forming |
| NAPSA | napsin A aspartic peptidase |
| PGC | progastricsin (pepsinogen C) |
| SCGB1A1 | secretoglobin family 1A member 1 |
| SFTPA1 | surfactant protein A1 |
| SFTPB | surfactant protein B |
| SFTPC | surfactant protein C |
| SFTPD | surfactant protein D |
| TP63 | tumor protein p63 |
| CC2D1B | coiled-coil and C2 domain containing 1B |
| SF3A3 | splicing factor 3a subunit 3 |

**Table S2 related to STAR methods. GeoMx spatial profiling SARS-CoV-2 gene set.**