

# Immediate myeloid depot for SARS-CoV-2 in the human lung

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**Biological Sciences - Article**

**Keywords:**

**Posted Date:** May 17th, 2022

**DOI:** <https://doi.org/10.21203/rs.3.rs-1639631/v1>

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## Immediate myeloid depot for SARS-CoV-2 in the human lung

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1 **Abstract**

2 In the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic<sup>1</sup>,  
3 considerable focus has been placed on a model of viral entry into host epithelial populations,  
4 with a separate focus upon the responding immune system dysfunction that exacerbates or  
5 causes disease. We developed a precision-cut lung slice model<sup>2,3</sup> to investigate very early  
6 host-viral pathogenesis and found that SARS-CoV-2 had a rapid and specific tropism for  
7 myeloid populations in the human lung. Infection of alveolar macrophages was partially  
8 dependent upon their expression of ACE2, and the infections were productive for amplifying  
9 virus, both findings which were in contrast with their neutralization of another pandemic virus,  
10 Influenza A virus (IAV). Compared to IAV, SARS-CoV-2 was extremely poor at inducing  
11 interferon-stimulated genes in infected myeloid cells, providing a window of opportunity for  
12 modest titers to amplify within these cells. Endotracheal aspirate samples from humans with  
13 the acute respiratory distress syndrome (ARDS) from COVID-19 confirmed the lung slice  
14 findings, revealing a persistent myeloid depot. In the early phase of SARS-CoV-2 infection,  
15 myeloid cells may provide a safe harbor for the virus with minimal immune stimulatory cues  
16 being generated, resulting in effective viral colonization and quenching of the immune system.

17 The SARS-CoV-2 pandemic has led to over 6 million deaths worldwide. Respiratory viruses  
18 like SARS-CoV-2 are known to infect and replicate in airway epithelial cells, inducing lung  
19 injury with often fatal outcomes<sup>1</sup>. The access to large numbers of human samples has given  
20 the opportunity to extensively study immune responses to COVID-19<sup>4-6</sup>. Unfortunately, once  
21 patients are hospitalized, the host-pathogen responses have been in progress for days or  
22 weeks, creating a challenging problem for understanding the very early host responses to  
23 SARS-CoV-2 infection in humans. While animal models are useful in this regard, they do not  
24 fully recapitulate human complexity, including appropriate expression of relevant ligands.  
25 Here, we used precision-cut lung slices (PCLS) obtained from human lungs to study early host-  
26 pathogen responses in a system replete with the full repertoire of lung stromal and immune  
27 cells.

28

29 We have previously developed a model of PCLS in the mouse lung<sup>2,3</sup> that we have now applied  
30 to human lungs donated for research (Supplementary Table 1). A lung lobe was inflated using  
31 2% low melting point agarose and 300  $\mu$ m PCLSs were produced for tissue culture and direct  
32 infection with SARS-CoV-2 (USA-WA1/2020; MOI 0.1 – 1) for up to 72 hours (Fig. 1a). Imaging  
33 of the infected PCLS (Fig. 1b-c; non-infected in Extended Data Fig. 1) revealed spike staining  
34 in epithelial cells (EpCAM<sup>+</sup>) that were also ACE2 positive (Fig. 1b). PCLSs were enzymatically  
35 digested for multicolor flow cytometry analysis (Fig. 1a). To further investigate infection, cells  
36 were stained for dsRNA as well as spike (Fig. 1d-e). Epithelial cells (CD45<sup>-</sup> EpCAM<sup>+</sup> CD31<sup>-</sup>)  
37 displayed both dsRNA and spike signal after 72h of SARS-CoV-2 infection (Fig. 1d and  
38 Extended Data Fig. 2a). At 72h post-infection, 4 to 10% of epithelial cells were spike<sup>+</sup> and 2 to  
39 6% dsRNA<sup>+</sup>. Both imaging and flow cytometry showed that SARS-CoV-2 was able to induce  
40 epithelial infection in a small-scale human lung model.

41

42 Using both imaging and flow cytometry, we also observed spike and dsRNA signal in lung  
43 immune cells (Fig. 1c and e), the former prevalent from the earliest 48-hour timepoint. Spike  
44 was colocalized to CD45<sup>+</sup> ACE2<sup>+</sup> cells (Fig. 1c; non-infected in Extended Data Fig. 1b) and

45 similarly dsRNA was found in these cells (Extended Data Fig. 2b), supporting that concept that  
46 immune cells may either be infected by SARS-CoV-2 or phagocytose the virus. Flow cytometry  
47 allowed us to further characterize spike<sup>+</sup> and dsRNA<sup>+</sup> immune cells (Fig. 1e). We observed  
48 significant dsRNA and spike signal in lung myeloid cells (CD45<sup>+</sup> CD3<sup>-</sup> CD19<sup>-</sup> HLA-DR<sup>+</sup> CD14<sup>+</sup>  
49 cells, including interstitial macrophages, monocytes and monocyte-derived dendritic cells<sup>7</sup>) at  
50 48-72h post-infection. These data support that immune cells, particularly myeloid cells, have  
51 profound and early interactions with SARS-CoV-2, potentially leading to a major role in shaping  
52 the immune response.

53  
54 To further characterize the transcriptional influence of SARS-CoV-2 infection upon specific cell  
55 populations, during the first days of exposure, we applied single-cell RNA sequencing (scRNA-  
56 seq) analysis to cells obtained at various timepoints after PCLS infection (Fig. 2). Hierarchical  
57 analysis identified clusters representing highly heterogenous lung complexity, including eight  
58 populations of non-immune cells, lymphocytes (T cells, B cells, NK), and four populations of  
59 myeloid cells (Fig. 2a and Extended Data Fig. 3a)<sup>8</sup>. To appreciate unique changes in lung  
60 composition and gene expression induced by SARS-CoV-2, we compared lung slices infected  
61 by either SARS-CoV-2 or Influenza A virus (IAV) (Fig. 2b). In response to IAV, the most  
62 profound change was a reduction in lung fibroblast and epithelial cell proportions, consistent  
63 with previous reports<sup>9</sup>. SARS-CoV-2, in contrast, produced no significant trends in lung non-  
64 immune cell populations, compared to controls. IAV was similarly destructive in the immune  
65 populations, producing a notable overall decrease in cells clustered in “Myeloid 1” (Extended  
66 Data Fig. 3a). Conversely, SARS-CoV-2 infection actually increased the myeloid fraction over  
67 time, increasing by almost 50% relative to controls at 72h (Fig. 2b).

68  
69 Aligning the scRNA-seq data on the two viral genomes revealed that the main targets for IAV  
70 infection were epithelial cells and fibroblasts (Fig. 2c), consistent with the observed loss of  
71 these populations in PCLSs (Fig. 2b). In addition, IAV reads were also sporadically distributed  
72 in several other populations including immune cells. This may be attributable to the IAV entry

73 receptor (sialic acid) being widely distributed on the surfaces of many cell types or perhaps to  
74 phagocytosis. SARS-CoV-2 reads by comparison were distinctly enriched in myeloid cells,  
75 even at the earliest 24h timepoint. Only a few reads were identified in non-immune cells (Fig.  
76 2c). To identify the subpopulation of myeloid cells targeted by SARS-CoV-2, the four myeloid  
77 clusters from Fig. 2a were combined and re-clustered, resulting in the definition of ten myeloid  
78 populations that included neutrophils, dendritic cells (DCs) and multiple subpopulations of  
79 monocytes and macrophages (Fig. 2d and Extended Data Fig. 3b). IAV reads remained  
80 sporadic across all 10 clusters (Fig. 2e). Conversely, SARS-CoV-2 tropism among these  
81 clusters was more selective with alveolar macrophages (AMs), IGSF21<sup>+</sup> DCs, and monocytes  
82 accounting for the majority of significant viral reads (Fig. 2e). To account for the variable  
83 frequency of each population in Fig. 2e, we analyzed the SARS-CoV-2 read frequency and  
84 plotted this over time. This demonstrated that viral reads rose in unison in both myeloid and  
85 epithelial cell populations from 24 to 48h and then decreased in the myeloid populations at 72h  
86 (Fig. 2f). This could be the result of resolution of the infection in the PCLS, or an exhaustion of  
87 host cells targeted by the virus.

88

89 To investigate how SARS-CoV-2 affects human lung myeloid cells, we focused on the  
90 dominant AMs, which are also anatomically within the airspaces and so directly exposed to  
91 virus<sup>10</sup>. A bronchoalveolar lavage (BAL) in the donor lungs (Fig. 3a) produced a sample  
92 enriched in AMs, defined here as CD45<sup>+</sup> CD169<sup>+</sup> HLA-DR<sup>+</sup> (Extended Data Fig. 4a, d-e)<sup>11</sup> and  
93 comprising notably few epithelial cells (<1% of live cells) (Extended Data Fig. 4b-c). AMs were  
94 analyzed for ACE2 expression (Fig. 3b), and we observed variability amongst lung donors with  
95 on average ~10-20% of ACE2<sup>+</sup> AMs, but with one donor at ~80% (Fig. 3c). We incubated the  
96 cell population with SARS-CoV-2 and then analyzed cells by flow cytometry (Fig. 3a, d-e;  
97 Extended Data Fig. 4a, f-g). After 48h with SARS-CoV-2 at MOI 0.1, we detected spike in 1-  
98 10% of the AMs (Fig. 3d, Extended Data Fig. 4a). Somewhat surprisingly, an MOI of 1 did not  
99 significantly increase spike<sup>+</sup> AM percentage compared to MOI 0.1 (Fig. 3d), suggesting that  
100 either cells were somehow protected at higher titers—perhaps due to increase antiviral sensing

101 and subsequent ISGs—or that a plateau was reached in the number of cells that were capable  
102 of being infected. At 48h, viability of AMs was high in both MOI groups, an effect that suggests  
103 the virus was not inducing AM cell death (Extended Data Fig. 4f) contrary to observations in  
104 blood monocytes from COVID-19 subjects<sup>12</sup>. However, of the spike<sup>+</sup> AMs, the majority were  
105 ACE2<sup>+</sup> (Extended Data Fig. 4g) pointing to a specific but not obligate role for ACE2 in licensing  
106 AM viral entry. In support of this, when we used an ACE2 blocking antibody<sup>13</sup> incubated with  
107 AMs 2h prior to SARS-CoV-2, we always observed a significant decrease in spike<sup>+</sup> AMs (Fig.  
108 3e) but this was rarely complete, even despite using saturating concentrations of the ACE2  
109 antibody. Taken together, these data indicate that SARS-CoV-2 entry into AMs was  
110 significantly mediated by ACE2 expression and did not require epithelial cells as an  
111 intermediate host, since our BAL preparation lacked these cells.

112

113 Macrophage infection by viruses such as IAV has long been described as abortive, but several  
114 studies have shown that the IAV H5N1 strain is capable of virus production in macrophages<sup>14</sup>.  
115 To study the ability of AMs that were exposed to virus to produce and release new viruses,  
116 SARS-CoV-2 and IAV (fluorescent strain, PR8-venus) were incubated with BAL cells (MOI 0.1  
117 or 1) as above and the resulting titer in the media was then re-assessed. As a control, the  
118 same quantities of viruses were incubated only with media. After 48h of incubation, cell-free  
119 supernatant was then collected from the control (media) and BAL groups and used to infect  
120 Vero E6 (SARS-CoV-2) or MDCK cells (IAV). After 24h, the infection of these readout cells  
121 was assessed by spike staining and flow cytometry (Fig. 3f-g, Extended Data Fig. 5a-b). By  
122 quantifying Vero E6 cells that are positive for spike (SARS-CoV-2) or MDCK cells positive for  
123 Venus (IAV), we could quantify the infective load of virus present in cell-free supernatant after  
124 incubation with either BAL cells or cell-free media (Fig. 3a). For SARS-CoV-2, incubating the  
125 virus with BAL cells amplified virus at both MOI 0.1 and 1 compared to incubating with media  
126 alone (Fig. 3f, fold-change in Extended Data Fig. 5a). We observed the opposite effect with  
127 IAV, which decreased the amount of virus present at MOI 0.1 (Fig. 3g, fold-change in  
128 Extended Data Fig. 5b).



129 SARS-CoV-2 has been continuously evolving, resulting in the emergence of several variants  
130 of concern (VOC), the most pathogenic of which has been the delta variant, which we directly  
131 compared to ancestral USA-WA1/2020 in our system. As previously done, BAL cells were  
132 infected with SARS-CoV-2 viruses for 48h. To determine SARS-CoV-2 viral production by BAL  
133 cells, we used a plaque assay on BAL supernatant (Fig. 3h). BAL cells increased viral titer for  
134 both ancestral and delta (Fig. 3h, plaque assay in Extended Data Fig. 5c, fold-change in  
135 Extended Data Fig. 5d) confirming productive infection of BAL cells by these variants.

136

137 We next sorted AMs from BAL (gated as CD3<sup>-</sup> CD19<sup>-</sup> EpCAM<sup>-</sup> CD45<sup>+</sup> HLA-DR<sup>+</sup> CD169<sup>+</sup>) before  
138 infection. After 48h infection with SARS-CoV-2 variants (ancestral and delta) at MOI 0.1 or 1,  
139 cell-free supernatant was harvested and used for the plaque assay (Fig 3i). Consistent with  
140 results from BAL incubations, AM incubation with SARS-CoV-2 induced an increase of viral  
141 titer at both MOI 0.1 and 1 (Fig. 3j, plaque assay in Extended Data Fig. 5e, fold-change in  
142 Extended Data Fig. 5f). Anti-viral responses (ISG induction) were increased in both variants  
143 with increasing MOI most significantly with ancestral (Extended Data Fig. 5g). Taken together,  
144 our results show that incubation of AMs with SARS-CoV-2, but not IAV, leads to a productive  
145 infection of these cells and viral propagation without cell death.

146

147 To compare our findings in the isolated human lung model to clinical COVID-19 cases,  
148 endotracheal aspirates (ETA) were sampled from seven intubated subjects with the acute  
149 respiratory distress syndrome (ARDS) from COVID-19 (Supplementary Table 2) and analyzed  
150 by scRNA-seq (Fig. 4a). Clustering showed that the cellular population was predominantly  
151 composed of myeloid cells (Fig. 4a, far right), which contained macrophages, neutrophils, and  
152 some DCs (Fig. 4c). Analysis of SARS-CoV-2 normalized expression revealed that SARS-  
153 CoV-2 reads localized mainly to macrophages, but in some cases were also found in  
154 neutrophils and T cells (Fig. 4b), similar to prior results<sup>15</sup>. The ETA samples were obtained at  
155 different times after intubation, but the timing did not correlate with the quantity or SARS-CoV-  
156 2 reads (Extended Data Fig. 6). In fact, one subject was sampled at 40 days after intubation

157 but still had detectable viral reads in macrophages, which may point to a long-lasting depot  
158 effect. As in the PCLS model, several macrophage subpopulations (Extended Data Fig. 7)  
159 were found to have SARS-CoV-2 reads with almost 25% of AMs being virus positive (Fig. 4d).

160

161 Finally, we analyzed differential gene expression in infected versus uninfected AMs obtained  
162 from COVID-19 ETA samples. Multiple interferon-stimulated genes (ISGs) were increased in  
163 infected compared to non-infected AMs in ETA samples (Fig. 4e), consistent with their being  
164 exposed to viruses more profoundly than neighboring cells. To ask whether ISG expression  
165 was a prominent feature of early infection, we returned to the PCLS system and compared  
166 uninfected, IAV, and SARS-CoV-2 exposure. We indeed found that AMs exposed to SARS-  
167 CoV-2 had upregulated ISGs. However, this induction was typically ~10-fold less in SARS-  
168 CoV-2, compared to IAV infection (Fig. 4f).

169

170 AMs are the sentinel immune cells in lung alveoli and relied upon to efficiently engulf and  
171 neutralize pathogens<sup>16</sup>. Here, we show that these cells are targeted by two of the most  
172 significant SARS-CoV-2 variants, leading to productive infection, viral propagation, and yet  
173 blunted interferon responses compared to IAV. These results suggest a depot effect where  
174 protective defenses are hijacked to facilitate viral production. AMs are migratory in the  
175 alveolar spaces<sup>16</sup> and SARS-CoV-2-infected AMs could potentially spread infection to  
176 uninvolved areas of the lung leading to catastrophic viral loading of the lung, including as  
177 discussed in the accompanying manuscript, the loading of interstitial macrophage populations.  
178 We also found multiple macrophage subpopulations with SARS-CoV-2 viral reads, and it is  
179 certainly possible that these non-AM cells are also capable of productive infections. As ACE2  
180 is an interferon-stimulated gene<sup>17</sup>, initial infection may be self-propagating, since our results  
181 indicated a critical dependency on ACE2 expression, although other viral entry mechanisms  
182 are potentially involved. As one recent example, in later stages of COVID-19 infection, and  
183 when antibodies have been generated, Fc gamma receptors appear to lead to monocyte  
184 infection<sup>12</sup>. In our model, the lack of antibodies likely rules out a role of Fc receptors in viral

185 entry into AMs. It is intriguing to consider the long-lasting effects of this macrophage depot,  
186 which could be linked to non-resolving critical illness and long-term complications of COVID-  
187 19.

188

189 In summary, by examining the very early immune events in the human lung after SARS-CoV-  
190 2 infection, we discovered a specific tropism for lung myeloid populations and evidence of  
191 productive infection by AMs that has broad implications in unraveling the pathogenesis of  
192 severe SARS-CoV-2 infections in humans.

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265 **Methods**

266 **Precision Cut Lung Slices (PCLS).** Human donor lungs were obtained from Donor Network  
267 West. Lung lobes were inflated using 2% low-melting point agarose and incubated at 4°C<sup>2,3</sup>.  
268 After agarose consolidation, 1 cm<sup>3</sup> lung tissue was placed on the precision compresstome VF-  
269 200 (Precisionary Instruments Inc.) for slicing. 300 µm slices were obtained and cultured in  
270 DMEM (UCSF Media Production), 1% penicillin/streptomycin (UCSF Media Production), and  
271 10% fetal bovine serum (FBS) (Corning) in a 24-well plate.

272

273 **SARS-CoV-2 infections.** Vero E6 and Vero-TMPRSS2 cells (gift from Dr. Melanie Ott) were  
274 cultured in DMEM supplemented with 10% FBS, penicillin/streptomycin, and L-glutamine  
275 (Corning) in a humidified incubator at 37°C and 5% CO<sub>2</sub>. SARS-CoV-2 virus (USA-WA1/2020  
276 strain) was provided by Dr. Melanie Ott and propagated in Vero E6 or Vero-TMPRSS2 cells.  
277 SARS-CoV-2 B.1.617.2 (delta) variant was acquired from the California Department of Public  
278 Health, cultured in Vero-TMPRSS2 cells. For propagation, the Vero or Vero-TMPRSS2 cells  
279 were infected with the SARS-CoV-2 virus, incubated at 37°C, 5% CO<sub>2</sub>, and at 72h the  
280 supernatant was collected. The virus was aliquoted and stored at -80°C. All work was done  
281 under Biosafety Level 3 (BSL-3) conditions. Viral titer was quantified using a plaque assay in  
282 Vero cells<sup>18</sup>. Briefly, 10-fold dilutions of the virus stock were added to Vero cells in a 12-well  
283 plate for 1 hour, after which an overlay of 1.2% Avicel RC-581 in DMEM was added. The cells  
284 were incubated at 37°C, 5% CO<sub>2</sub> for 96 hours. The cells were fixed with 10% formalin, stained  
285 with crystal violet, and washed with water. The plaques were counted to determine the titer of  
286 the virus stock.

287

288 **Influenza A virus (IAV) infections.** As a virus of reference, we used influenza A/Puerto  
289 Rico/8/34 (PR8, H1N1) virus labelled with Venus to infect lung slices and BAL cells. PR8-  
290 Venus IAV was a gift from Yoshihiro Kawaoka (University of Wisconsin-Madison). The virus  
291 was produced in pathogen-free fertilized chicken eggs (Charles River) as published<sup>19</sup>. In brief,  
292 eggs were kept in an egg turner for 10 days. PR8-Venus IAV was injected into the allantoic

293 cavity. Infected chicken embryos were incubated with the virus for 48 hours. Allantoic fluid was  
294 harvested, filtered, and snap-frozen in liquid nitrogen. Titers were determined with a  
295 hemagglutination assay. The work was done under BSL-2 conditions.

296

297 **Immunofluorescence imaging.** After infection, PCLS were fixed in 4% PFA for at least 30  
298 min. After saturation in PBS with 1% BSA, slices were stained with anti-CD45 (HI30, 304056,  
299 BioLegend), anti-spike (40150-R007, Sino Biological), anti-ACE2 (bs-1004R, BIOSS) anti-  
300 dsRNA (J2, 10010, Scicons) or anti-EpCAM-AF488 (9C4, 324210, BioLegend), for 1 hour in  
301 PBS with 1% BSA media at room temperature. PCLS were washed in PBS, counterstained  
302 with DAPI and attached to plastic coverslips using Vetbond (3M). Confocal imaging was  
303 performed using a Nikon A1R laser scanning confocal microscope with NIS-Elements software  
304 and a 16X LWD water dipping objective. Images were taken at more than 25 µm deep inside  
305 the slices to avoid the cutting artifact. 50 – 100 µm-thick images with a z-step of 1.5 µm were  
306 taken and analyzed using Imaris (Bitplane).

307

308 **Flow cytometry.** At selected time points, PCLS were dissociated using DNase (4µg/ml) and  
309 Collagenase IV (200U/ml) at 37°C for 30min. Cells were filtered and stain for viability and  
310 surface markers (Supplementary Table 3). After fixation and permeabilization (BD  
311 cytofix/Cytoperm), cells were stained for spike and dsRNA (Supplementary Table 3). Data  
312 were collected using the BD LSRII Cytometer and analyzed using FlowJo version 10 (BD  
313 Biosciences).

314

315 **Bronchoalveolar lavage.** A bronchoalveolar lavage (BAL) was done in a human lung lobe  
316 using ice-cold PBS. BAL cells were filtered and red blood cells lysed. BAL cells were plated  
317 in 24 well plates at  $5 \times 10^5$  cells per well in DMEM, 1% PS, 10% FBS containing 50 ng/ml of  
318 rhM-CSF (300-25, Peprotech). For selected experiments, BAL cells were treated for 2h before  
319 infection with an ACE2 blocking antibody at 10 µg/ml (AF933, R&D systems). SARS-CoV-2  
320 was added to the cells at MOI 0.1 or 1. After 48h of infection, cells were recovered and stained



321 for viability and surface markers (Supplementary Table 4). After fixation and permeabilization,  
322 cells were stained for intracellular spike expression. In selected experiments, AMs (live,  
323 EpCAM-, CD3-, CD19-, CD45+, HLA-DR+, CD169+) were flow-sorted prior to infection with  
324 SARS-CoV-2 using FACSAria Fusion (BD Biosciences).

325

326 **Virus replication assay.** BAL cells were infected for 48h either with SARS-CoV-2 or IAV-  
327 Venus at MOI 0.1 or 1. At the end of the incubation, cell-free supernatant was recovered. This  
328 solution was used as inoculum for Vero E6 cells (SARS-CoV-2) or MDCK cells (IAV-Venus,  
329 gift from Michael A. Matthay). After 24 hours of incubation, cells were recovered and infection  
330 was assessed by flow cytometry.

331

332 **Single-cell RNA-sequencing.** PCLS were dissociated as described above and dead cells  
333 were removed using Miltenyi Dead Cell Removal Kit (130-090-101, Miltenyi Biotec). For the  
334 multiplexing purpose, cells were then labeled with lipid modified oligonucleotides (LMO) and  
335 barcode oligos using the Multi-seq technique<sup>20</sup>. Cells were counted and the targeted cell  
336 number for loading was 8000 cells per sample. 10X encapsulation and library construction  
337 were done using Chromium Next GEM Single Cell 3' Reagent Kits v3.1 per the manufacturer's  
338 instruction. Multi-seq library preparation was done as previously described<sup>20</sup>. Libraries were  
339 mixed at an approximate 10:1 molar ratio of gene expression to LMO barcodes for sequencing.  
340 The sequencing was done on the Illumina NovaSeq 6000 using 10X Genomics recommended  
341 sequencing parameters.

342

343 **Data pre-processing of 10x Genomics Chromium scRNA-seq data.** Sequencer-generated  
344 bcl data (Gene expression and Lipid Hashtag) was demultiplexed into individual fastq libraries  
345 using the mkfastq command on the Cellranger 3.0.2 suite of tools  
346 (<https://support.10xgenomics.com>). Feature-barcode matrices for all samples were generated  
347 using the Cellranger count command. Briefly, raw gene-expression fastqs were aligned to the  
348 GRCh38 reference genome annotated with Ensembl v85, and Lipid Hashtag fastqs were

349 processed to count the incidences of each expected index per cell. Feature-barcode matrices  
350 were read into Seurat 4.0.1<sup>21</sup> and poorly-captured genes (in < 3 cells) were dropped from the  
351 analyses. Matrices were further filtered to remove events with greater than 20% mitochondrial  
352 content, events with greater than 50% ribosomal content, or events with fewer than 100 total  
353 genes.

354

355 **Data quality control and normalization.** The gene expression count matrices were  
356 normalized, and variance stabilized using negative binomial regression using the scTransform  
357 algorithm<sup>22</sup> in the Seurat package. Cellular mitochondrial content, ribosomal content, and cell  
358 cycle state were regressed out of the data at this stage to prevent any confounding signal. The  
359 normalized matrices were transformed into a lower subspace using Principal Component  
360 Analysis (PCA) and 50 PCs per samples were used to generated Uniform Manifold  
361 Approximation and Projection (UMAP) visualizations. Cell clustering via expression was  
362 conducted using the Louvain algorithm. Cluster identities were assigned using Gene scores  
363 generated via the Seurat AddModuleScore function on a list of gene sets obtained from the  
364 Human Lung Cell Atlas.

365

366 **Demultiplexing of pooled single-cell libraries.** In Lipid hashtagged libraries, the raw lipid  
367 tag counts were normalized using the Centered Log Ratio method (CLR) where HTO counts  
368 are divided by the geometric mean for that HTO across all cells and then log normalized. The  
369 resulting matrix was demultiplexes into donor samples using the Seurat HTODemux function<sup>22</sup>  
370 using default parameters.

371

372 **Data integration and batch correction.** scTransformed count matrices from all samples were  
373 integrated together using the in-built integration method provided by Seurat. The following  
374 commands were run in order, to integrate datasets `SelectIntegrationFeatures` to identify  
375 shared “anchor” features for integration, `PrepSCTIntegration` to subset objects based on  
376 identified anchor features, `FindIntegrationAnchors` to identify anchor points between datasets

377 based on the anchor features, and finally `IntegrateData` to actually integrate the datasets  
378 based on the computed anchors. Downstream processing (PCA, UMAP, clustering and cluster  
379 assignment) was conducted similarly to the individual libraries.

380 **Endotracheal aspirates samples (ETA).** Endotracheal aspirate (ETA) samples were  
381 prospectively collected from seven adults requiring mechanical ventilation for the acute  
382 respiratory distress syndrome (ARDS) from COVID-19 as part of the COVID Multiphenotyping  
383 for Effective Therapies (COMET) study, as previously described<sup>23</sup>. Human participants were  
384 enrolled at two tertiary care hospitals in San Francisco, CA (UCSF Medical Center, Zuckerberg  
385 San Francisco General Hospital) under research protocol 20-30497 approved by the University  
386 of California San Francisco Institutional Review Board. ETA samples were collected within 1-  
387 40 days post-intubation for scRNA-sequencing. See Supplementary Table 2 for details.

388 **Statistical analyses.** Statistical analysis was performed using GraphPad Prism v7.0e. For  
389 PCLS experiments (Fig. 1), significance was assessed using ANOVA with Sidak's multiple  
390 comparison test. For Fig. 3 and Extended Data Fig. 5 (BAL cells and AM experiments), each  
391 dot represents the mean of 2 to 3 replicates from a single donor. Donors are color-coded  
392 across experiments. Paired multiple group comparisons (Fig. 3d and Extended Data Fig. 5g)  
393 were analyzed by one-way ANOVA with Dunnett's multiple comparisons. Ratio paired t-test  
394 was used to compare two paired groups (ACE2 blockade, virus propagation assay, plaque  
395 assay).

396 **Acknowledgements.** This work was supported by NIH funding as follows: NIAID-sponsored  
397 Immunophenotyping Assessment in a COVID-19 Cohort  
398 (IMPACC) Network [NIH/NIAID U19 AI1077439], R35 HL140026 (C.S.C.), 3P01AI091580-  
399 09S1 (JPR), R01 AI052116 (M.F.K.), R01 AI160167 (M.R.L.), R35 HL161241 (M.R.L.), P30  
400 DK063720 (UCSF Flow Cytometry CoLab), and Shared Instrument Grant 1S10OD021822-01  
401 (UCSF Flow Cytometry CoLab). We also acknowledge funding from COVID-19 Fast Grants  
402 (M.O. and M.R.L.), UCSF Clinical and Translational Science Institute (CTSI), and the Bakar  
403 UCSF ImmunoX Initiative. M.O. thanks the Rodenberry foundation, Pam and Ed Taft,  
404 the Innovative Genomics Institute, and the Gladstone Institutes for their support.

405

406 **Author contributions.**

407 All authors contributed to manuscript preparation. Conceptualization: MM, RY, MFK, MRL.  
408 Experimentation: MM, RY, LR, CRS, LH, JPR. Human samples: The UCSF COMET  
409 Consortium, CL, PGW, DJE, CMH, CSC, MAM, CRL. Data analysis: MM, RY, AAR, RTD,  
410 KHH, MFK, MRL. Reagents: AS, MO. Supervision: MFK, MRL. Manuscript writing: MM, RY,  
411 AAR, MFK, MRL.

412

413 **Competing interests.** The authors declare no competing interests.

**Supplementary Table 1. Human lung donor demographics and experimental assignments.**

Donor	Gender	Race/ Ethnicity	Age	Cause of death	Donation	Comorbidities	Smoking (>20PY)	Experiment Type
1	M	White	63	Anoxia	DBD	Hypertension, Pulmonary nodules	yes	BAL
2	F	White	49	Anoxia	DCD	Asthma, COPD, Sepsis pneumonia	yes	PCLS flow, PCLS imaging
3	F	White	37	Anoxia	DBD	None	no	PCLS flow, PCLS imaging
4	M	White	51	Stroke	DBD	Diabetes, CAD, Hypertension	no	PCLS flow, PCLS imaging
5	M	White	47	Stroke	DCD	None	no	PCLS flow, PCLS imaging
6	M	Asian/White	32	Head Trauma	DBD	Diabetes	no	PCLS flow
7	F	Hispanic	44	Stroke	DBD	None	no	PCLS flow, PCLS imaging
8	M	Asian	50	Stroke	DCD	Asthma, Hypertension	no	PCLS flow
9	M	White	46	Suicide	DCD	None	yes	PCLS flow
10	F	Asian	56	Stroke	DCD	Hypertension	no	PCLS imaging
11	F	White	57	Stroke	DBD	None	no	PCLS flow, PCLS imaging
12	M	Hispanic	27	Head Trauma	DBD	Asthma	no	PCLS SCS
13	M	White	43	Anoxia	DCD	None	no	BAL, PCLS flow, PCLS imaging
14	F	Hispanic	44	Stroke	DBD	None	no	PCLS imaging, PCLS SCS
15	M	White	43	Anoxia	DCD	None	no	PCLS flow
16	M	Hispanic	57	Stroke	DBD	None	no	BAL
17	M	White	50	Head Trauma	DCD	Hypertension	no	BAL
18	M	White	31	Head Trauma	DCD	None	no	BAL
19	M	Hispanic	50	Stroke	DBD	Hypertension	no	BAL
20	F	White	62	Head Trauma	DBD	Cancer	no	BAL
21	M	White	27	Anoxia	DBD	None	no	BAL
22	F	Hispanic	39	Head trauma	DBD	None	no	BAL
23	F	White	58	Anoxia	DCD	Diabetes, Hypertension	no	BAL
24	F	White	62	Anoxia	DCD	COPD, Hypertension, Hypersensitivity Lung Disease, and diabetes	yes	BAL
25	M	White	32	Anoxia	DCD	Asthma	no	BAL
26	M	Hispanic	25	Anoxia	DCD	None	no	BAL
27	M	Hispanic	33	Head trauma	DBD	Hypertension	no	BAL
28	M	White	33	Anoxia	DBD	Diabetes	yes	BAL
29	M	White	46	Stroke	DBD	Asthma	no	BAL

DBD: donation after brainstem death

COPD: chronic obstructive pulmonary disease

BAL: bronchoalveolar lavage

PCLS Imaging: precision cut lung slice immunofluorescence imaging

DCD: donation after cardiac death

CAD: coronary artery disease

PCLS flow: precision cut lung slice flow cytometry

SCS: single-cell RNA sequencing

**Supplementary Table 2. Demographic and clinical information for COVID-19 ETA samples.**

Subject	Age	Gender	Ethnicity	ARDS (Y/N) Berlin definition	ETA sampling after intubation (days)	P/F ratio at time of ETT sampling	ICU LOS (days)	Hospital LOS (days)	Death (Y/N)
1 (1)	34	F	White	Yes	0	180	12	14	No
2 (365)	55	M	White	Yes	40	110	35	60	No
3 (414)	58	M	Other / Multiple Races	Yes	1, 3	165	19	27	No
4 (415)	62	F	Black / African American	Yes	2	124	14	21	No
5 (419)	68	M	Other / Multiple Races	Yes	7	175	26	50	No
6 (476)	59	M	White	Yes	12	160	44	78	No
7 (389)	66	F	White	Yes	2	144	39	55	No

ARDS: acute respiratory distress syndrome

ETA: endotracheal tube aspirate

P/F ratio: PaO<sub>2</sub>/FiO<sub>2</sub>

ICU LOS: intensive care unit length of stay

Hospital LOS: hospital length of stay

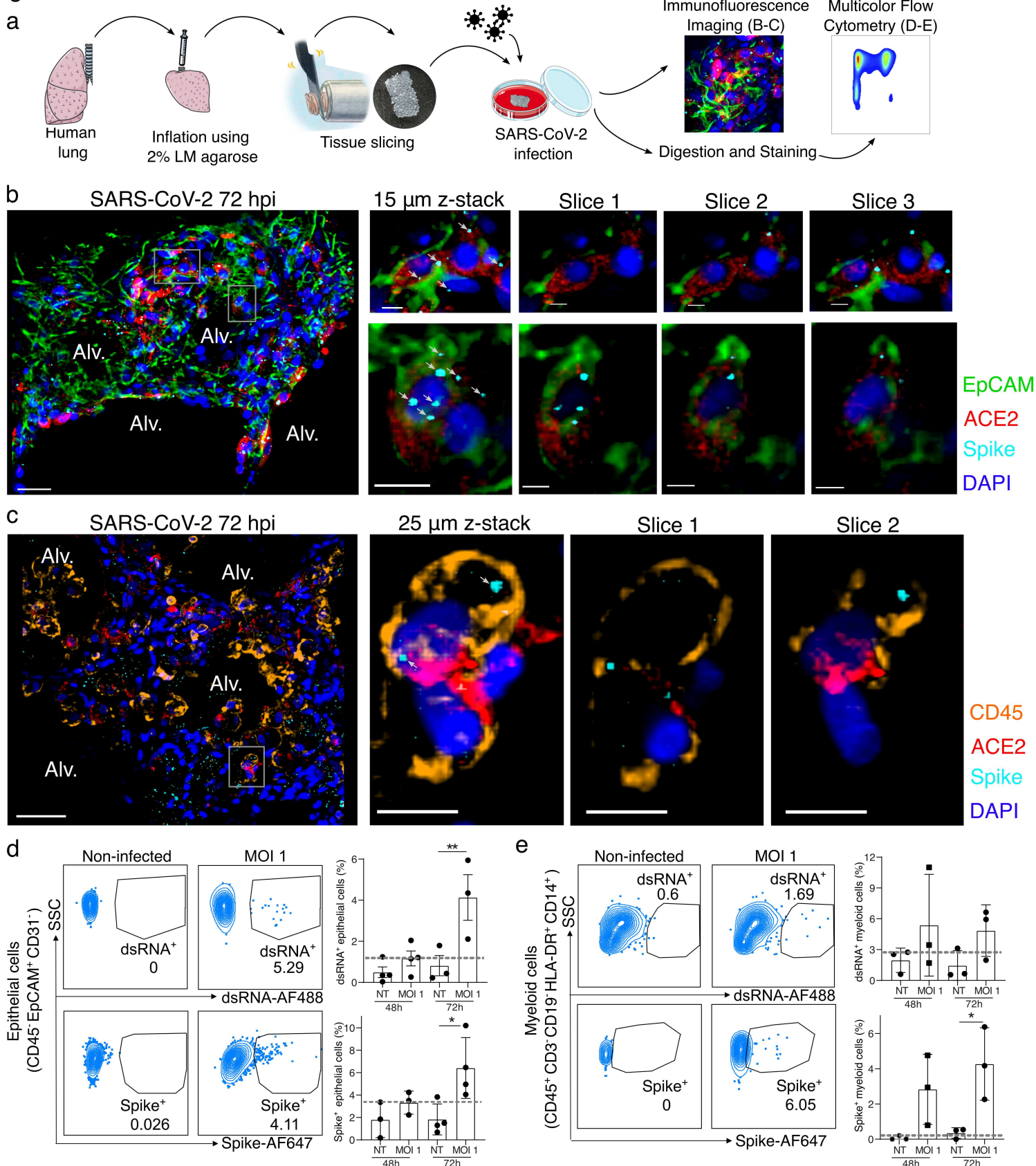
**Supplementary Table 3. Flow cytometry panel for PCLS experiments.**

<b>Staining</b>	<b>Antibody (clone)</b>	<b>Lot number</b>	<b>Dye</b>	<b>Catalog number (Supplier)</b>
<b>Surface Staining</b>	CD169	1621398	AF594	FAB5197T (R&D Systems)
	ACE2	BJ07067522	PE	bs-1004R (BIOSS)
	EpCAM	B250368	BV650	324226 (BioLegend)
	CD31 (WM59)	8232937	BV605	562855 (BD Biosciences)
	CD45 (HI30)	B333796	BV421	304032 (BioLegend)
	CD14 (M5E2)	B275828	BV711	301838 (BioLegend)
	CD3 (SK7)	0314461	BB700	566575 (BD Biosciences)
	CD19 (SJ25C1)	1069967	BB700	566396 (BD Biosciences)
	Zombie (viability)	B331984	NIR	77184 (BioLegend)
	HLA-DR (G46-6)	1266420	BUV395	564040 (BD Biosciences)
<b>Intracellular Staining</b>	Spike	1619059	AF647	FAB105805R (R&D Systems)
	dsRNA (J2)	J2-2007	AF488	10010 (Scicons)

**Supplementary Table 4. Flow cytometry panel for BAL experiments.**

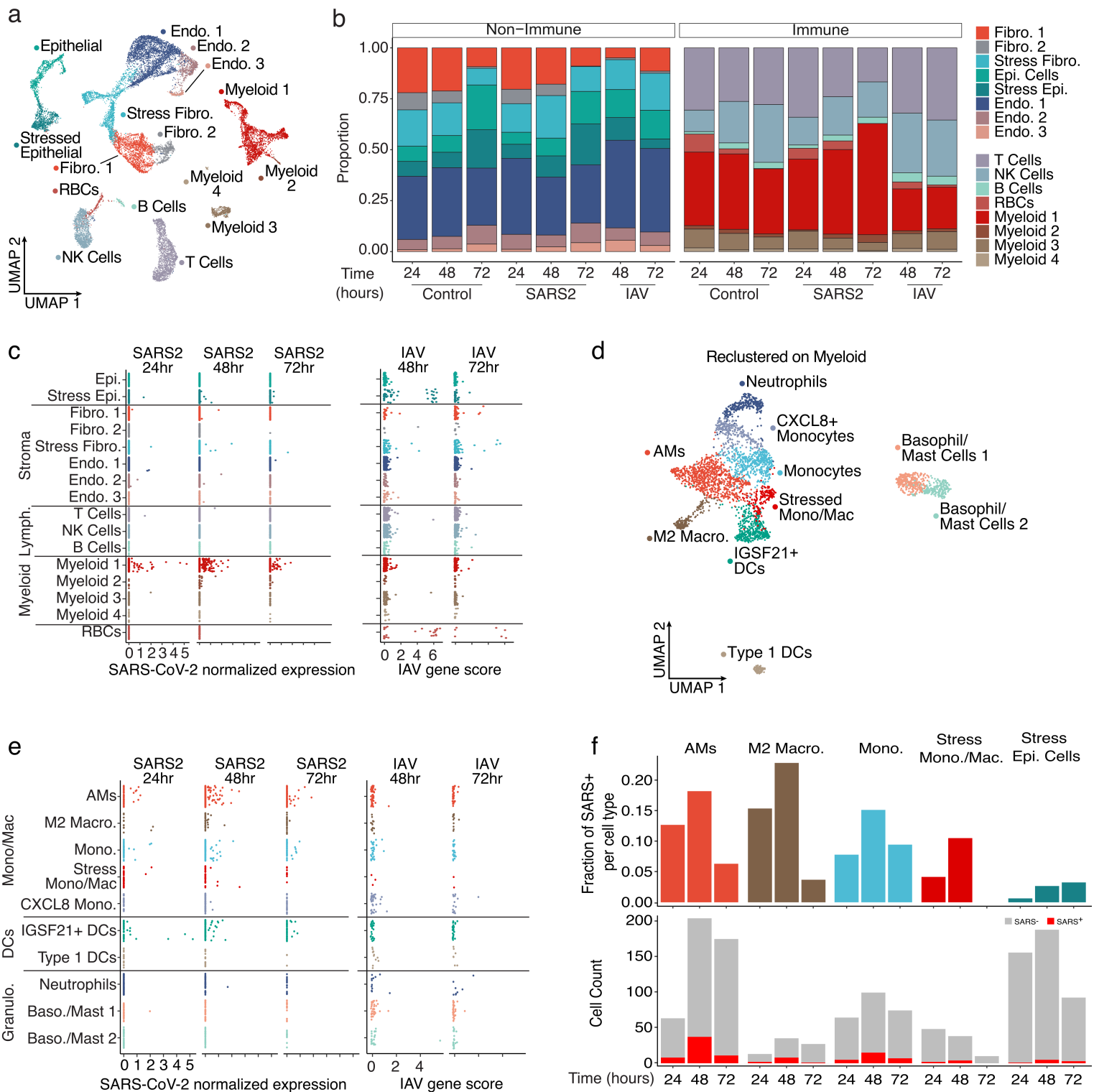
<b>Staining</b>	<b>Antibody</b>	<b>Lot number</b>	<b>Dye</b>	<b>Reference</b>
<b>Surface Staining</b>	CD169	1621398	AF594	FAB5197T (R&D Systems)
	ACE2	BJ07067522	PE	bs-1004R (BIOSS)
	CD15 (W6D3)	1089776	BV786	741013 (BD Biosciences)
	CD16 (3G8)	B321940	BV605	302040 (BioLegend)
	CD45 (HI30)	B333796	BV421	304032 (BioLegend)
	CD14 (M5E2)	B275828	BV711	301838 (BioLegend)
	CD3 (SK7)	0314461	BB700	566575 (BD Biosciences)
	CD19 (SJ25C1)	1069967	BB700	566396 (BD Biosciences)
	Zombie (viability)	B331984	NIR	77184 (BioLegend)
	HLA-DR (G46-6)	1266420	BUV395	564040 (BD Biosciences)
<b>Intracellular Staining</b>	Spike	1619059	AF647	FAB105805R (R&D Systems)
	IFITM3 (EPR5242)	GR3416716-1	AF488	Ab198559 (Abcam)



**Figure 1**

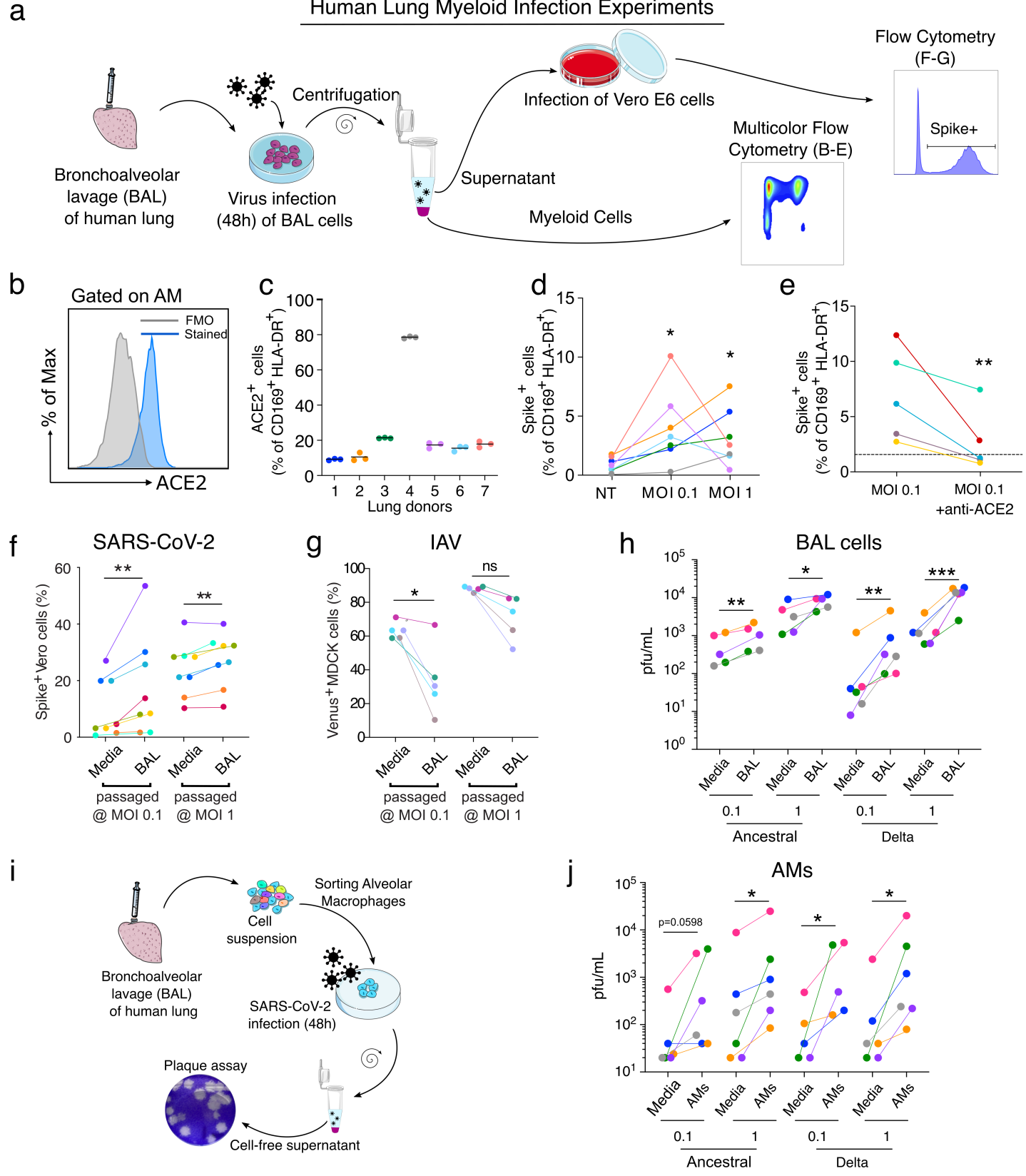
**Figure 1. SARS-CoV-2 infects both epithelial and immune cells in human PCLSs.** (a) Schematic diagram of the experimental design. Human lung lobes were inflated with 2% low-melting point agarose and sectioned into 300  $\mu$ m precision cut lung slices (PCLS), which were cultured in 24-well plates and infected with SARS-CoV-2 for 48 or 72h. After incubation, PCLS were either fixed and stained for confocal imaging (b, c) or dissociated and stained for flow cytometry (d, e). (b, c) PCLS were infected with SARS-CoV-2 for 72h at MOI 0.1 or 1 and used for confocal imaging. Alveolar spaces (Alv.) are indicated in the large image (scale bar = 50  $\mu$ m). Zoom area is marked by the white rectangle. For each zoomed area, 15 or 25  $\mu$ m z-stacks appears on the side with single x-y sections (scale bar = 10  $\mu$ m). (b) PCLS were stained for DAPI (dark blue), EpCAM (green), ACE2 (red) and spike (light blue). (c) PCLS were stained for DAPI (dark blue), CD45 (orange), ACE2 (red) and spike (light blue). (d, e) PCLS were infected at MOI 1 for 48 and 72h. PCLS were dissociated and cell suspension was stained for flow cytometry analysis (n=3-4). Infection was assessed by intracellular spike and dsRNA staining in (d) epithelial and (e) myeloid cells. Grey dashed lines indicate detection limit of assays. Data are mean  $\pm$  SEM. Each dot represents the average percentage of dsRNA<sup>+</sup> or spike<sup>+</sup> myeloid cells of 2 to 3 individual lung slices from one donor. \*p<0.05, \*\*p<0.01.

**Figure 2**



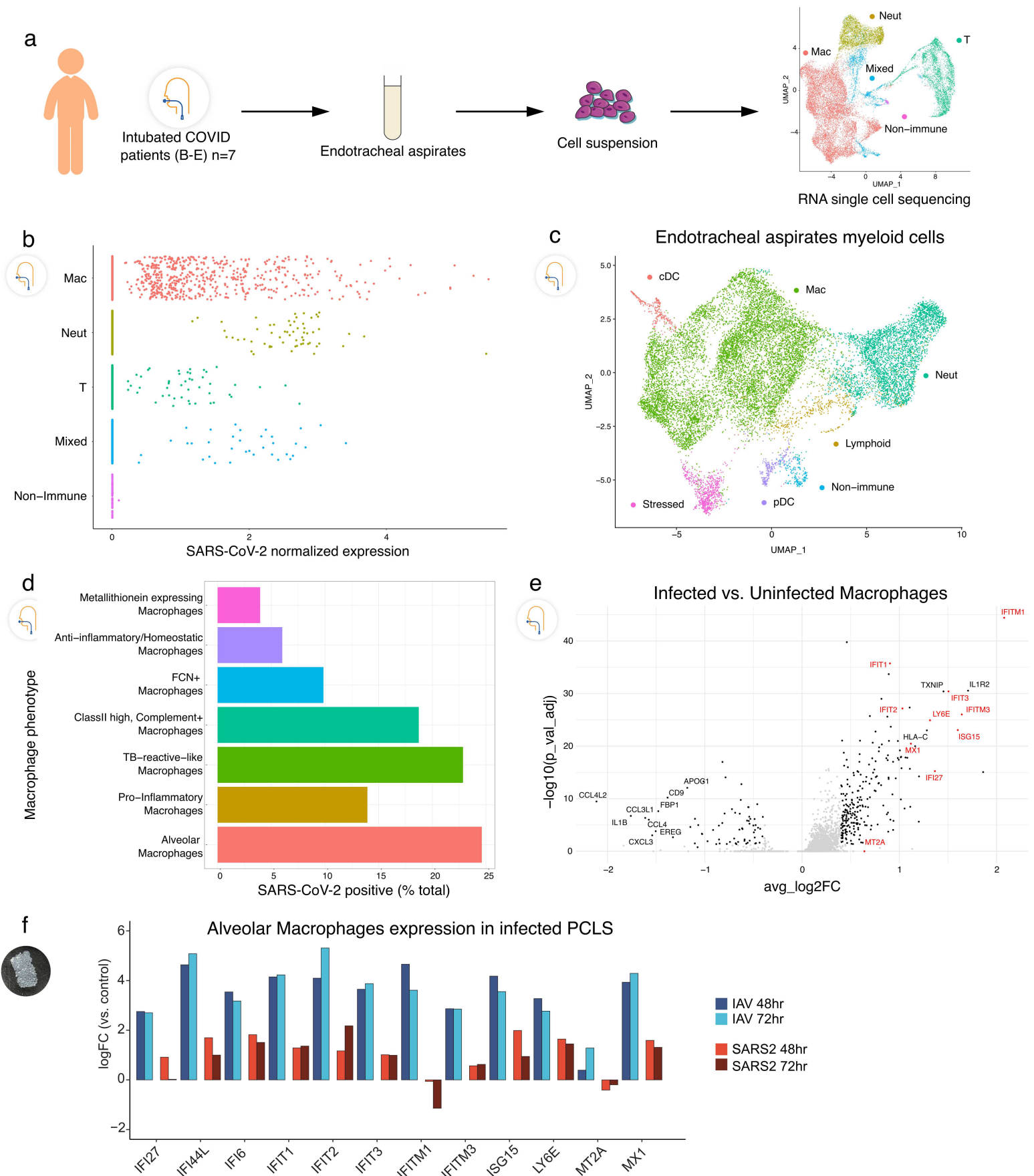
**Figure 2. SARS-CoV-2 displays tropism for myeloid cells compared to IAV.** (a) A Uniform Manifold Approximation and Projection (UMAP) visualization of cells from control, SARS-CoV-2, and IAV-infected PCLS collected at distinct times. (b) Relative quantification of cell types from the different experimental conditions, stratified by timepoint. (c) Scatterplots describing the library-normalized SARS-CoV-2 expression across various cell types in SARS-CoV-2-infected PCLS (left) or IAV gene score in IAV-infected PCLS (right). (d) A UMAP of finely annotated myeloid cell types in the dataset. (e) Distribution of infected myeloid cells similar to (c). (f) The fraction (top) and numbers of SARS-CoV-2-positive cells (bottom) at different timepoints.

Figure 3



**Figure 3. SARS-CoV-2 infection of alveolar macrophages is ACE2-dependent and amplifies viral titer.** (a) Bronchoalveolar lavage (BAL) of human lungs yields cells that were infected with SARS-CoV-2 for 48h. Infected cells were analyzed by multicolor flow cytometry. Supernatant of infected BAL cells was used to infect Vero E6 cells. 24h after infection, infected cells were quantified by flow cytometry. (b) ACE2 protein expression was assessed on alveolar macrophages (AMs: CD169<sup>+</sup> HLA-DR<sup>+</sup> cells) by flow cytometry. (c) Proportion of ACE2<sup>+</sup> AMs was measured (n=7). (d) AM infection was measured by flow cytometry using spike staining (each color represents a human lung donor, n=7). (e) ACE2 blocking antibody was added to BAL cells before infection (MOI 0.1). Cells were analyzed by flow cytometry (n=5). (f) BAL cells were infected with SARS-CoV-2 (BAL passaged). As a control, SARS-CoV-2 was incubated in culture media alone (Media). Cell-free supernatant was used to infect Vero E6 cells. At 24h after infection, Vero E6 cells were stained for intracellular spike expression and analyzed using flow cytometry (n=8). (g) Similarly, IAV-Venus was used to infect BAL cells or incubated with media. Cell-free supernatant was used to infect MDCK cells. At 24h after infection, Venus expressing MDCKs percentage was measured by flow cytometry (n=5). (h) Plaque assay was used to further assess viral titer in supernatant of infected BAL cells (n=5-6). (i) AMs were sorted from BAL samples. (j) Following 48h of SARS-CoV-2 infection (Ancestral, delta), viral titer was determined by plaque assay (n=5-6). ns=not significant, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

Figure 4



**Figure 4. Tropism of SARS-CoV-2 for myeloid cells in endotracheal aspirates from COVID-19 subjects with ARDS.** (a) Endotracheal aspirates were collected from SARS-CoV-2 infected subjects with ARDS and subjected to scRNA-seq. UMAP at far right shows landmark populations. (b) Distribution of per-cell normalized SARS-CoV-2 expression in landmark cell types. (c) UMAP projection of myeloid subtypes in endotracheal aspirates. (d) Fraction of SARS-CoV-2 positive cells per myeloid cell type (e) A volcano plot of SARS-Cov-2 positive AMs vs uninfected AMs. Interferon stimulated genes (ISGs) are highlighted in red. (f) Log2 Fold Change of select ISGs in the PCLS experiment in IAV- and SARS-CoV-2-infected cells at 48 and 72h vs control.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AllExtendedDataFigures1.pdf](#)