

1 **Longitudinal omics in Syrian hamsters integrated with human data unravel complexity**  
2 **of moderate immune responses to SARS-CoV-2**

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## 52 **Highlights**

- 53 • Our hamster data allow for comparison and alignments with patient data
- 54 • Only few pulmonary epithelial cells are infected and show a weak response
- 55 • We provide kinetics and origin of SARS-CoV-2-induced mediator expression,  
56 including expression programs in endothelial cell subtypes
- 57 • Neutrophil recruitment is minor and resolved before peak inflammation
- 58 • Monocyte-derived macrophages and endothelial cell subtypes strongly activate  
59 pro-inflammatory and T cell attracting chemokine expression
- 60 • Cytotoxic effector CD8<sup>+</sup> and CD4<sup>+</sup> T cells are recruited and their arrival  
61 coincides with viral clearance and symptom cessation

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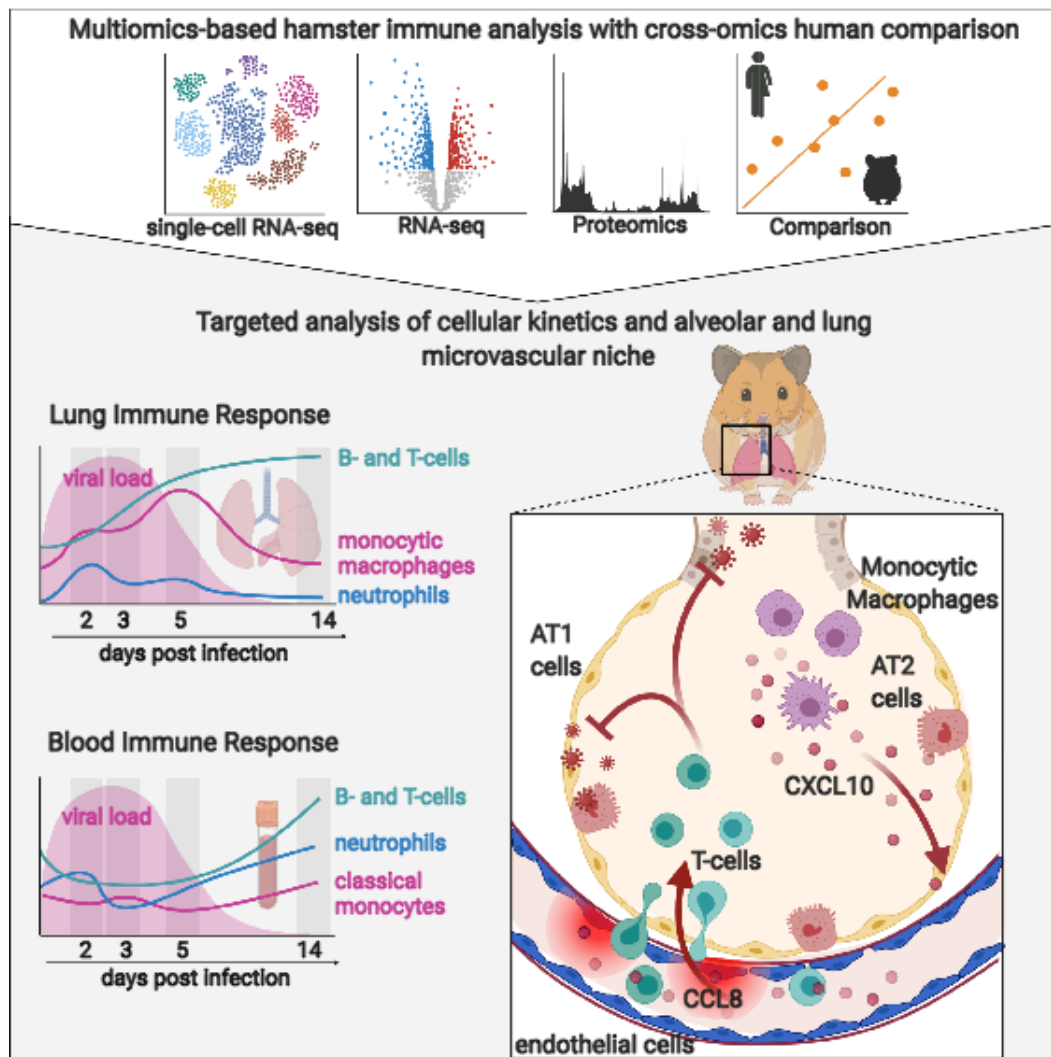
## 63 **In brief**

64 Using omics tools with the SARS-CoV-2 infected Syrian hamster as model for COVID-  
65 19, along with published datasets from COVID-19 patients, Nouailles et al. present a  
66 detailed longitudinal analysis of systemic and pulmonary quantitative and qualitative

67 immune responses in a moderate disease setting. Targeted analysis of the alveolar  
68 and microvascular niche revealed a dominant role for monocyte-derived macrophages  
69 and endothelial cells regarding early anti-viral genes as well as pro-inflammatory and  
70 T cell recruiting chemokine expression. Recruitment of cytotoxic effector T cells  
71 coincided with viral clearance. This combined response was favorable for a moderate  
72 and self-limited disease course.

73

## 74 Graphical abstract



75

76

## 77 Summary

78 In COVID-19, the immune response largely determines disease severity and is key to  
79 therapeutic strategies. Cellular mechanisms contributing to inflammatory lung injury  
80 and tissue repair in SARS-CoV-2 infection, particularly endothelial cell involvement,  
81 remain ill-defined. We performed detailed spatiotemporal analyses of cellular and  
82 molecular processes in SARS-CoV-2 infected Syrian hamsters. Comparison of

83 hamster single-cell sequencing and proteomics with data sets from COVID-19 patients  
84 demonstrated inter-species concordance of cellular and molecular host-pathogen  
85 interactions. In depth vascular and pulmonary compartment analyses (i) supported the  
86 hypothesis that monocyte-derived macrophages dominate inflammation, (ii) revealed  
87 endothelial inflammation status and T-cell attraction, and (iii) showed that CD4<sup>+</sup> and  
88 CD8<sup>+</sup> cytotoxic T-cell responses precede viral elimination. Using the Syrian hamster  
89 model of self-limited moderate COVID-19, we defined the specific roles of endothelial  
90 and epithelial cells, among other myeloid and non-myeloid lung cell subtypes, for  
91 determining the disease course.

92

93 **Keywords:** coronavirus disease 2019; COVID-19; Syrian hamster; SARS-CoV-2;  
94 inflammatory monocytes; macrophages; cytotoxic T cells; endothelial cells

95

## 96 **Introduction**

97 The enduring severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)  
98 pandemic has emphasized the urgent need for experimental models to rapidly identify  
99 pathomechanisms and therapeutic targets of Corona Virus Disease 2019 (COVID-19).  
100 COVID-19 causes a wide range of disease manifestations, spanning from  
101 asymptomatic infections to acute respiratory distress syndrome (ARDS) and fatal multi-  
102 organ dysfunction (Guan et al., 2020). Disease severity is influenced by age, sex and  
103 specific comorbidities, making it evident that host-specific factors influence the course  
104 of the disease and require further investigation. While blood of COVID-19 patients is  
105 accessible to detailed longitudinal investigation, irrespective of disease severity, and  
106 bronchoalveolar lavage (BAL) can be safely performed in intubated patients,  
107 pulmonary tissue responses remain inaccessible in mild and moderate COVID-19  
108 courses, since lung tissue is only available upon autopsy from patients with fatal  
109 disease. Hence, experimental models of COVID-19 are needed, which reflect the  
110 complexity of human responses to SARS-CoV-2 infections, including the  
111 spatiotemporal dynamics of airway and alveolar infection, local pulmonary immune  
112 responses, the activation of systemic inflammatory, complement and coagulation  
113 cascades, the impairment of endothelial barrier function, and also mechanisms of  
114 resilience, resolution and repair.

115 The hamster family (Cricetinae) is of particular interest for experimental modelling of  
116 COVID-19, as we and others have observed that animals without genetic modifications

117 can be infected with SARS-CoV-2 and develop phenotypes ranging from mild to lethal  
118 COVID-19, depending on age and species (Bertzbach et al., 2020; Imai et al., 2020;  
119 Osterrieder et al., 2020; Sia et al., 2020; Trimpert et al., 2020). Notably, immune cell  
120 influx into the lungs, bronchointerstitial pneumonia and diffuse alveolar damage in  
121 hamsters bear resemblance to COVID-19 in human patients (Gruber et al., 2020;  
122 Kreye et al., 2020a; Lee et al., 2020).

123 Since its initial description as animal model for SARS-CoV (Roberts et al., 2005), the  
124 Syrian hamster has been used to study different aspects of SARS-CoV and Middle  
125 East Respiratory Syndrome (MERS) coronavirus infection (Gong and Bao, 2018;  
126 Gretebeck and Subbarao, 2015; Subbarao and Roberts, 2006). Consequently, it now  
127 serves as a versatile non-transgenic rodent model to study SARS-CoV-2 infection and  
128 therapeutic interventions such as antiviral treatments, immunomodulatory therapies  
129 and vaccines (Imai et al., 2020; Kreye et al., 2020b; Tostanoski et al., 2020). The  
130 disease observed in hamster species primarily affects the lower respiratory tract, which  
131 more closely resembles the common courses of human disease as opposed to  
132 clinically severely affected transgenic mice, in many of which infection of the central  
133 nervous system (CNS) is the predominant manifestation of the disease (Jiang et al.,  
134 2020; Winkler et al., 2020).

135 Despite the advantages of hamster models for investigating COVID-19 pathogenesis,  
136 unavailability of molecular tools and reagents for hamsters limits investigations of  
137 immuno-pathomechanisms, leaving unanswered how closely SARS-CoV-2 evoked  
138 disease in hamsters models human COVID-19. We therefore in-depth evaluated  
139 SARS-CoV-2-infected Syrian hamsters (*Mesocricetus auratus*) elucidating the innate  
140 and adaptive steps of immunity and pathogenesis by pairing single-cell RNA  
141 sequencing (scRNA-Seq) data from lung cells and white blood cells (WBC),  
142 histopathology and quantitative proteomics analysis of lungs and blood following nasal  
143 SARS-CoV-2-infection of *Mesocricetus auratus*. We compared our findings with own  
144 data from scRNA-Seq and proteomics analyses from human biosamples of COVID-19  
145 patients. This enabled in-depth investigations on central COVID-19 pathomechanisms  
146 in compartments inaccessible in humans, particularly in moderate disease.

147

## 148 **Results**

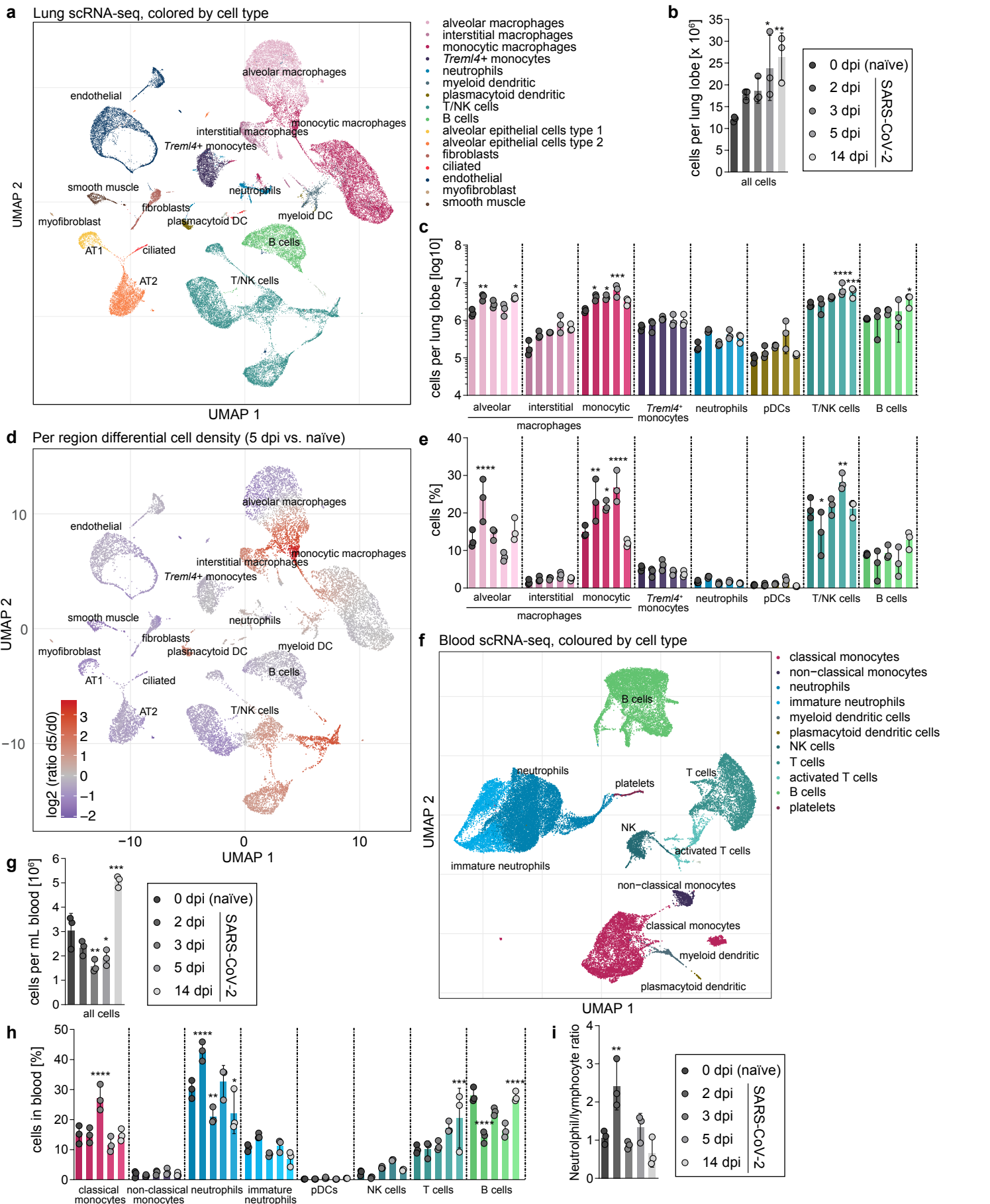
### 149 **SARS-CoV-2 induces self-resolving moderate pneumonia and robust pulmonary** 150 **immune cell recruitment in Syrian hamsters**

151 After infection with SARS-CoV-2 (Figure S1), clinical disease manifested in Syrian  
152 hamsters with moderate transient weight loss analogous to previous reports ((Imai et  
153 al., 2020; Osterrieder et al., 2020; Sia et al., 2020), Figure S2a). High viral loads were  
154 detected in both the upper and lower respiratory tracts at 2, 3 and 5 days post infection  
155 (dpi). At 14 dpi, only minimal viral RNA load remained, and no replication competent  
156 virus was detected in the respiratory tract (Figure S2b – d). Lack of viral RNA in the  
157 blood of all animals suggested absence of systemic infection (data not shown).

158 Similar to previous observations (Gruber et al., 2020; Osterrieder et al., 2020),  
159 histopathology identified necrosuppurative bronchitis and bronchointerstitial  
160 pneumonia at 2 and 3 dpi, characterized by intrabronchial and intraalveolar infiltration  
161 by neutrophils and macrophages as well as severe, diffuse alveolar damage. Numbers  
162 and density of infiltrating immune cells, hyperplasia of bronchial and alveolar epithelial  
163 cells as well as alveolar and interstitial edema and endothelialitis peaked at 5 dpi. By  
164 14 dpi, cellular influx into alveolar spaces was largely resolved, with fewer neutrophils,  
165 macrophages and lymphocytes observed within the interalveolar septa, while marked  
166 hyperplasia of alveolar epithelial cells remained (Figure S2e – n). Again, consistent  
167 with previous reports (Gruber et al., 2020; Osterrieder et al., 2020) no evidence of  
168 thrombotic events were observed.

169 To obtain a higher resolution of the tissue response, we performed scRNA-Seq at time  
170 points spanning early infection, peak inflammation and the resolution phase of viral  
171 pneumonia. Cell type clusters detected in lungs corresponding to leukocyte-subset-  
172 signatures included alveolar, interstitial, and monocytic macrophages, *Trem14*<sup>+</sup>-  
173 monocytes, neutrophils, dendritic cells, B, T, and natural killer (NK) cells. We further  
174 identified typical resident cell types, including alveolar epithelial cells type 1 (AT1) and  
175 2 (AT2), ciliated epithelial, endothelial, and smooth muscle cells, and fibroblasts  
176 (Figure 1a, S3a). By integrating scRNA-Seq-derived cell frequencies with manually  
177 counted cell numbers over time, we mapped the dynamics of infection-induced  
178 pulmonary leukocyte recruitment compared to uninfected animals (Figure 1b, c).  
179 Neutrophils were the first immune cells recruited to the lungs (peak at 2 dpi), closely  
180 followed by an influx of monocyte-derived macrophages (peak at 5 dpi). NK and T  
181 lymphocyte recruitment to lungs was first detected at 5 dpi and peaked at day 14

## Figure 1



**Figure 1: Single-cell dynamics in lungs and blood of SARS-CoV-2 infected Syrian hamsters**

(a), UMAP plot of identified cell populations in Syrian hamster lungs. (b), Cell count of isolated cells per lung lobe over time (2, 3, 5 and 14 dpi) and control group (naïve, “d0”). (c), Count of hematopoietic cells per lung lobe in naïve hamsters and over time pi. (d), Changes in cellular density of lung cells in UMAP projection. Coloration indicates  $\log_2$  fold change between control group and 5 dpi. (e), Percentage of hematopoietic cells per lung lobe in naïve hamsters and over time pi. (f), UMAP plot of identified cell populations in blood samples. (g), Cell count of isolated cells per mL blood in naïve hamsters and over time pi. (h), Percentage of identified cell populations in blood samples over time pi and naïve animals. (i), Neutrophil–lymphocyte ratio in blood samples over time pi and naïve animals. (a, d and f), Clusters defined by Louvain clustering,  $n = 3$  per time point. (b, c, e, g, h and i), Data display means  $\pm$  SD.  $n = 3$  per time point. Ordinary one-way ANOVA, Sidak’s multiple comparisons test versus corresponding 0 dpi (naïve). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$

182 (Figure 1c). The peak of lung inflammation on 5 dpi (Figure S2l) coincided with the  
183 highest proportion of inflammatory macrophages (monocytic macrophage cluster) and  
184 proliferating cytotoxic cells (T/ NK cell cluster) among lung cells (Figure S3b).

185 Notably, despite pronounced neutrophilic bronchitis (Figure S2g), the overall neutrophil  
186 frequencies remained low. In line with histopathology, the peak of neutrophil  
187 recruitment was at 2 dpi, when neutrophil proportions presented ~3 % of all isolated  
188 lung cells (Figure 1e). In contrast, monocytic macrophages population at day 5 peaked  
189 at ~25 % of all lung cells (Figure 1d, e, S3b). Relative numbers of pulmonary tissue  
190 cell subsets fluctuated mildly during infection, declining proportionally as inflammatory  
191 cell influx rose at 2, 3 and 5 dpi (Figure S3c, d). At 14 dpi, increased numbers of AT2  
192 pneumocytes matched histopathology observations of epithelial hyperplasia, indicating  
193 tissue repair (Figure S2i, k, S3c).

194 Analogously, we analyzed scRNA-Seq data from WBC populations, to study systemic  
195 responses evoked by pulmonary SARS-CoV-2 infection. Detected cell populations  
196 included neutrophils, monocytes, dendritic, NK, B and T cells and various  
197 subpopulations thereof (Figure 1f, S3e). Infected hamsters displayed significant  
198 leukopenia at 3 and 5 dpi, probably reflecting pulmonary leukocyte recruitment. By 14  
199 dpi this trend was inverted and peripheral blood leukocyte numbers were significantly  
200 higher than in naïve animals (Figure 1g, S3f). Increased proportions of neutrophils at  
201 2 dpi suggested inflammation-induced granulopoiesis, and increasing proportions of T  
202 cells at 14 dpi indicated their clonal expansion (Figure 1h, S3b). Notably, calculated  
203 neutrophil–lymphocyte ratios only transiently increased at 2 dpi to a minor extend,  
204 matching observations in humans with non-severe as opposed to severe COVID-19  
205 (Lagunas-Rangel, 2020; Liu et al., 2020) (Figure 1i). Overall scRNA-Seq cell profiling  
206 substantially extended histopathology analysis in hamsters, by defining kinetics of  
207 innate and adaptive immune cell trafficking to lungs in greater detail.

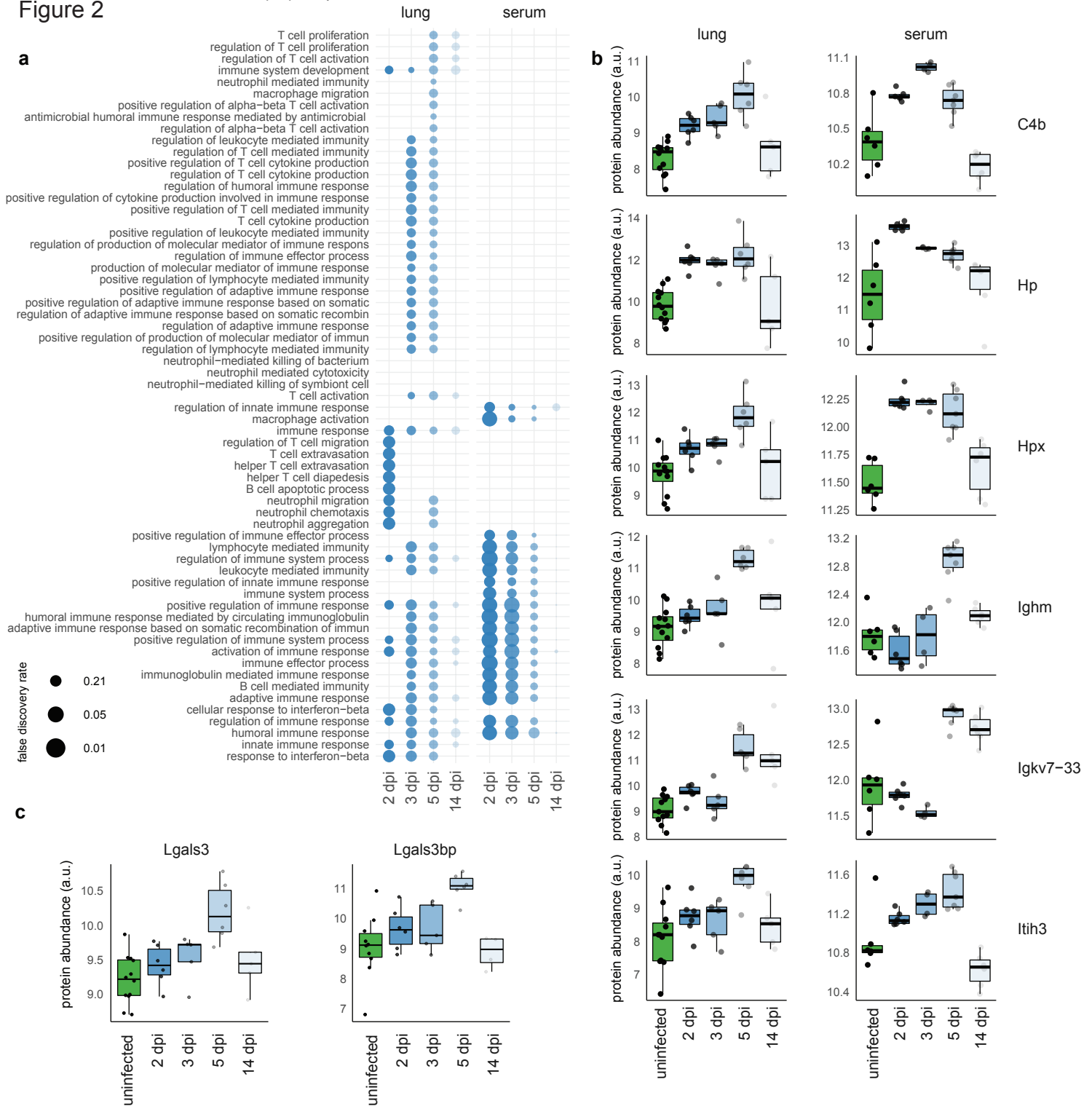
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### 209 **Bulk transcriptomics, proteomics and single-cell RNA sequencing reveal** 210 **activation of anti-SARS-CoV-2 pro-inflammatory immunity in hamsters**

211 After evaluating immune cell dynamics in blood and lungs, we performed bulk RNA-  
212 sequencing of lungs and blood and matched proteomics of lungs and serum to gain  
213 insights into qualitative changes into transcriptome and proteome alterations of SARS-  
214 CoV-2-induced immune responses.



## Figure 2



### Figure 2: Proteomics analysis recapitulates transcriptomics and human COVID-19 patient data

(a), Temporal evolution of gene ontology/biological process terms connected with immune system response in lung tissue (left part) and in serum fluid (right part), for the indicated time points compared to samples from uninfected animals. Enriched terms were filtered for terms mentioning “immune”, “interferon”, “neutrophil”, “T cell” and “B cell”. Size of dots correspond to the inverse of the false discovery rate. (b), Expression values for differentially regulated proteins in hamster serum (control 3 dpi, p value < 0.01) and lung (control 5 dpi, p value < 0.01) that correlate with disease severity in human plasma. Controls from different days are plotted together. The thick line represents median of all values, lower and upper hinges correspond to medians of lower and upper half of set, respectively. Whiskers extend to a maximum of 1.5 times distance between first and third quartile. All values are shown. (c) Expression values for the differentially expressed proteins Lgals3 and Lgals3bp (only detected in lung samples).

215 Gene ontology (GO) enrichment analysis of the most differentially expressed genes in  
216 lungs and blood at 2, 3, 5 and 14 dpi versus naïve hamsters provided expected GO  
217 terms such as *defense response to virus*, *innate immune response*, and *cell activation*  
218 (Figure S4a, b). Pulmonary gene sets related to *type 1 interferon (IFN) signaling*  
219 correlated with presence of viral RNA, thus vanished by 14 dpi. Similarly, pulmonary  
220 gene sets related to *response to interferon-gamma (Ifng)* were highest at 5 dpi (Figure  
221 S4c). In blood, *type 1 IFN signaling* and *response to IFN- $\gamma$*  gene sets were highest at  
222 2 and 3 dpi (Figure S4d), perhaps reflecting recruitment of specific cells from blood to  
223 lungs. Overall, bulk RNA Seq identified an anti-viral immune response that was  
224 effectively resolved when the virus was cleared.

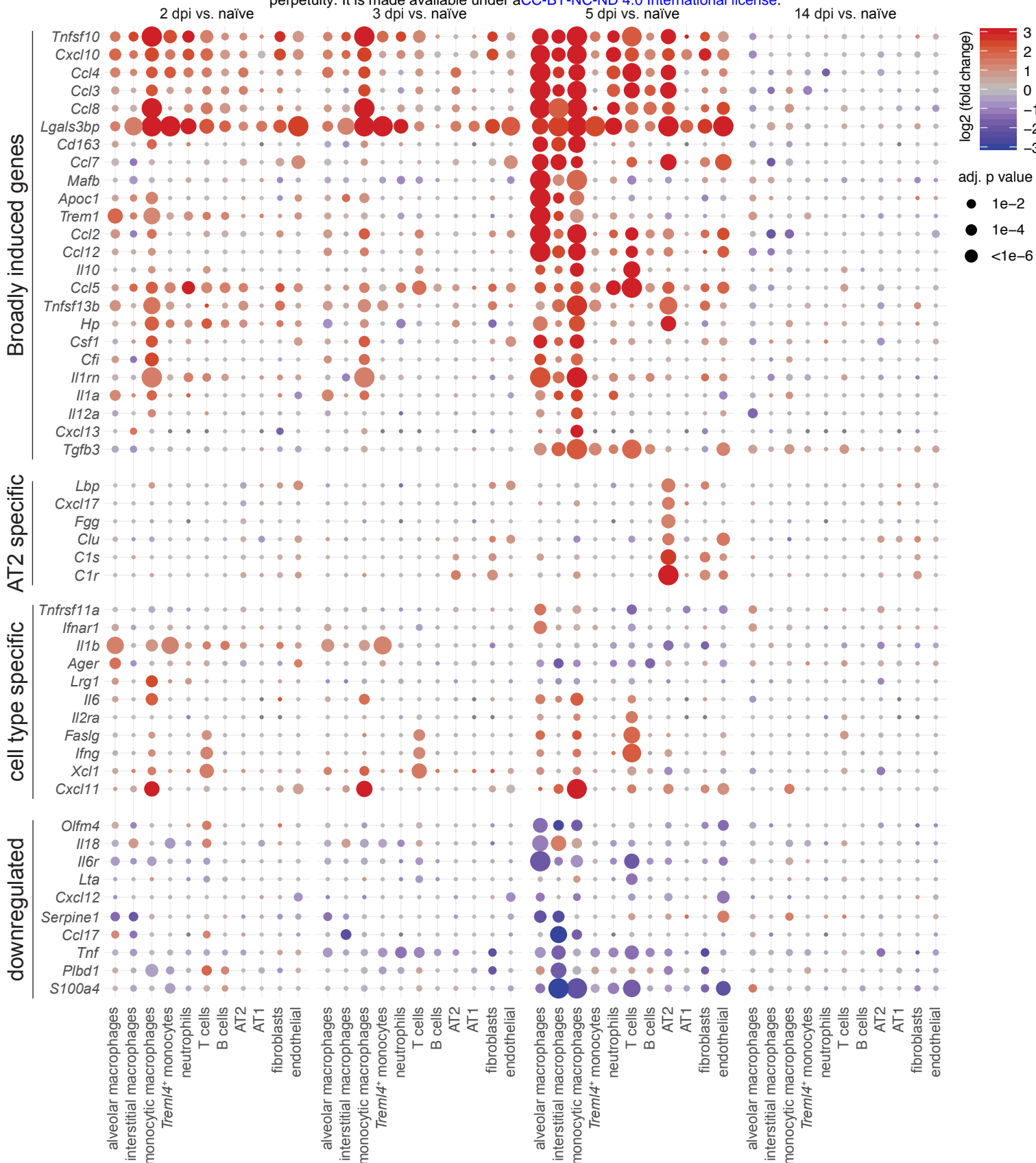
225 Proteome host response was in line with sequencing data, serum proteins peaked at  
226 3 dpi and lung proteins at 5 dpi (Figure S4e). Agreement between bulk RNA  
227 sequencing and proteomics was highly consistent in lungs at 5 dpi ( $r = 0.9$ ) (Figure  
228 S4f). Functional terms connected with immune response, such as innate and adaptive  
229 immunity, activation of complement system, humoral immune response and regulation  
230 of immune system processes were the most enriched in both lung and serum (Figure  
231 2a). In lungs, the response to interferon-beta peaked at 3 dpi and stayed high until 5  
232 dpi (Figure 2a). Most processes were resolved by 14 dpi.

233 We next aimed at comparing our data to published datasets from COVID-19 patients.  
234 In hamster serum, a total of 37 differentially expressed proteins were identified ( $\alpha =$   
235 0.01, providing FDR below 6 %), 17 compared to control and 31 proteins compared to  
236 14 dpi when most effects are resolved. 20 of 31 proteins have been reported in human  
237 COVID-19 studies, 7 (Actg1, Apoa1, Apoc1, Gsn, Hp, Itih3, Lbp) of which correlate  
238 with disease severity (Messner et al., 2020b), all showing the same direction and a  
239 response at WHO 4 with the maximum amplitude at WHO grade 7 (Figure S4g).

240 In hamster lungs, at peak response (5 dpi) we identified 150 differentially expressed  
241 proteins. 13 differentially expressed proteins have been reported to be regulated in  
242 human plasma (Demichev et al., 2020b) with 9 showing the same trend (Table S2 for  
243 protein lists). Of these, 6 proteins (C4b, Hp, Hpx, Ighm, Igkv7-33, Itih3) are also  
244 changed in hamster serum (Figure 2b). Although a comparison to moderate disease  
245 in human lung tissue is not possible, 22 proteins are reported to be regulated in human  
246 BAL fluid of critically ill patients (Zeng et al., 2020). The few proteins showing opposite  
247 regulation to COVID-19 patients were confirmed by bulk sequencing. Five out of 7  
248 conflicting responses, namely C4b, Hpx, Rbp4, Cfd, and Agt in hamster serum

Figure 3

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**Figure 3: Induction of inflammatory mediators are strongest and earliest in myeloid cells**

Dotplots of differentially expressed cytokines and inflammatory mediators in lungs. Shown are genes that display a significant absolute log<sub>2</sub>-transformed fold change of at least one in at least one comparison, and are grouped into four categories indicated on the left. Coloration and point size indicate log<sub>2</sub>-transformed fold changes and P values, respectively, of genes at 2 dpi relative to control groups (naïve). Adjusted P values were calculated by DEseq2 using Benjamini-Hochberg corrections of Wald test p values. Genes are ordered by unsupervised clustering, cell type as in Figure 1.

249 compared to human plasma as well as 8 out of 12 for hamster lung tissue compared  
250 to human BAL were confirmed.

251 Next, we refined our transcriptome analyses with the higher resolution of scRNA-Seq  
252 and related identified bulk GO terms to cell types, concentrating on inflammatory  
253 mediators in lungs and blood (Figure 3). Indeed, various pro-inflammatory chemokines  
254 were expressed by lung cells and showed distinct cellular and temporal expression  
255 patterns. Classical pro-inflammatory cytokines, e.g. *Il1a* and *Il1b* transcripts were  
256 elevated only early in infection at 2 and 3 dpi in alveolar and monocytic macrophages,  
257 and alveolar macrophages and *Triggering receptor expressed on myeloid cells-like 4*  
258 (*Trem14*)<sup>+</sup> monocytes, respectively. By 5 dpi, AT2 cells showed a unique range of  
259 upregulated inflammatory mediators such as *Cxcl17*, *Lipopolysaccharide Binding*  
260 *Protein (Lbp)*, *fibrinogen gamma gene (Fgg)*, and *clusterin (Clu)*. Within the analysis in  
261 Figure 3 *galectin 3-binding protein (Lgals3bp)* gene stood out as being upregulated in  
262 many different cell types from 2 dpi to 5 dpi. Notably, we likewise measured increased  
263 levels of the Lgals3bp protein in lungs (Figure 2c), which was shown to be regulated  
264 also in plasma of COVID-19 patients and correlated with severity (Messner et al.,  
265 2020b).

266 Taken together, we identified clear changes in transcriptome, proteome and pro-  
267 inflammatory signatures on single-cell level in response to SARS-CoV-2 infection,  
268 displaying a highly active immune response that to large extents were described in  
269 COVID-19 patients underlying the validity of our model.

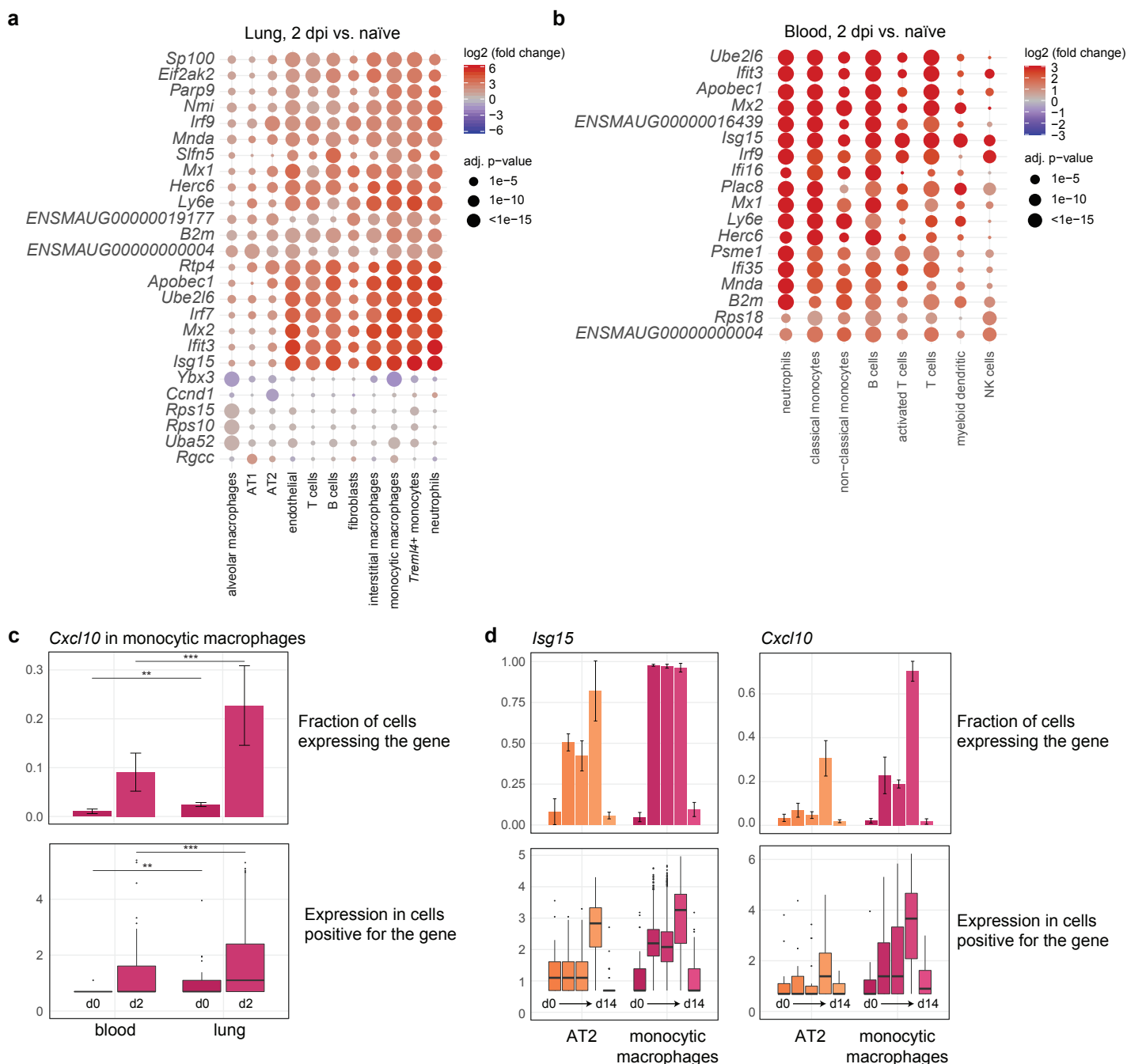
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### 271 **Migratory myeloid cells dominate pulmonary transcriptional response to** 272 **SARS-CoV-2 infection in Syrian hamsters and COVID-19 patients**

273 To pinpoint the individual role of the identified cells in anti-SARS-CoV-2 immunity, we  
274 analyzed the 15 - 20 most differently expressed genes in each cell subset. In the early  
275 stage of infection at 2 dpi, robust, local transcriptome changes were observed primarily  
276 in lung monocytic and interstitial macrophages, neutrophils and endothelial cells,  
277 whereas AT1 and AT2 epithelial cells and alveolar macrophages showed comparably  
278 little change in mRNA expression (Figure 4a).

279 A common set of anti-viral effector genes was found upregulated in many cell types  
280 (Schoggins, 2019). These include e.g. *interferon-stimulated gene 15 (Isg15)*, *MX*  
281 *dynamin like GTPase (Mx)1*, *Mx2*, *Interferon-induced protein with tetratricopeptide*  
282 *repeats 3 (Ifit3)*, and *Sp100*, as well as transcription factors, such as *Interferon*

Figure 4



**Figure 4: Transcriptional response to SARS-CoV-2 infection is strong in myeloid and moderate in epithelial cells** (a, b), Dotplot of differentially expressed genes in lungs (a) and blood (b). Shown are genes that are in at least one cell type among the top 4 most changing genes as ranked by adjusted p value. Example: top 4 most changing genes in alveolar macrophages are *Ybx3*, *Rps15*, *Rps10*, and *Uba52*. Coloration and point size indicate  $\log_2$ -transformed fold changes and p values, respectively, of genes at 2 dpi relative to control groups (naïve). Adjusted p values were calculated by DEseq2 using Benjamini-Hochberg corrections of Wald test p values. Cell types and genes are ordered by unsupervised clustering. (c), Top, fraction of monocytic macrophages expressing *Cxcl10* in blood and lungs from naïve animals and 2 dpi. Bottom, boxplots of *Cxcl10* gene expression values of cells positive for *Cxcl10* expression. Significance levels were calculated using generalized linear mixed-effects models for bar plot and a Wilcoxon rank sum test on all cells (i.e. not only the ones expressing the gene) for boxplots. \* means p value 0.05-0.01, \*\* means p value 0.01-0.0001, \*\*\* means p value smaller 0.0001. See materials and methods for details. (d), As in (c), but only in lungs, all time points and both alveolar epithelial cells type 2 (AT2) and monocytic macrophages. Displayed are values for *Isg15* (left) and *Cxcl10* (right). For all bar plots, data display means  $\pm$  SD,  $n = 3$  per time point. For all boxplots, lower and upper hinges correspond to first and third quartiles. Whiskers extend to a maximum of 1.5 times the distance between first and third quartile. Outliers beyond are marked by single dots.

283 *regulatory factor (Irf) 7 and Irf9* (Figure 4a, Figure S5a). Blood transcriptome analysis  
284 recapitulated this early transcriptional activity at 2 dpi (Figure 4b, Figure S5b), but  
285 declined by 5 dpi, whereas the signature persisted in the lungs until 14 dpi (Figure S5a,  
286 b). The genes that differed most between classical blood monocytes and their  
287 counterparts in the lungs encoded chemokines and activation markers, including *CXC*  
288 *chemokine ligand 10 (Cxcl10)*, *Slamf9*, *Il18bp*, *Ifitm2*, *Ccl8*, *Ccl4* and *Ccl5* (Figure 4c,  
289 Figure S5c), indicating that activation and acquisition of effector function occurred in  
290 the lungs.

291 Although AT2 cells are a main target of SARS-CoV-2 in lungs (Hou et al., 2020), they  
292 displayed weaker and later transcriptional changes upon infection compared to  
293 monocytic macrophages (Figure 4a, d, S5a, d).

294 Notably, at 14 dpi differential transcriptional responses related to defense resolved in  
295 blood and lung cells. Instead, we observed upregulation of cell cycle proliferation  
296 genes in AT2 cells including *Marker of Proliferation Ki-67 (Mki67)*, *Ubiquitin-*  
297 *conjugating enzyme E2 C (Ube2c)*, *Aurora B kinase (Aurkb)*, and *Stathmin (Stmn1)*  
298 (Figure S5a). This transcriptome profile indicated initiation of a repair program by AT2  
299 cells, proliferating to replace damaged AT1 cells (Olajuyin et al., 2019), and matching  
300 the increase in AT2 numbers and alveolar epithelial hyperplasia observed earlier  
301 (Figure S2, S3). Finally, we put our hamster lung scRNA-Seq data in context with BAL  
302 scRNA-Seq data from patients with moderate-to-severe COVID-19 (Liao et al., 2020)  
303 and healthy controls (Morse et al., 2019). As in the hamster data, we observed stronger  
304 transcriptional responses in macrophages compared to epithelial cells (Figure S5e).  
305 Furthermore, the upregulated gene program containing e. g. *CXCL10*, *CCL2* or *CCL8*  
306 was substantially overlapping (Figure S5f).

307 To test whether hamster tissue responses are representative of infected human  
308 epithelial cells, we next referred to our scRNA-Seq dataset derived from  
309 nasopharyngeal swabs of 19 COVID-19 patients and 5 healthy controls (Chua et al.,  
310 2020). Here again, human and hamster epithelial cells derived from infected individuals  
311 and animals, respectively, showed a similar, moderate induction of most inflammatory  
312 mediators (Figure S6a, b). As notable difference, the strong induction of neutrophil-  
313 recruiting chemokines targeting CXC chemokine receptor (CXCR) 2, such as *CXCL1*,  
314 *CXCL3*, *CXCL6* and *CXCL8*, were found only in human basal and secretory cells with  
315 severe COVID-19 but were absent in moderately ill Syrian hamsters (Figure S6a,  
316 Figure 3). Aside from the epithelial inductions of neutrophil-attractant transcripts unique

317 to severe COVID-19, SARS-CoV-2 infected hamsters and patients displayed strikingly  
318 similar pro-inflammatory immune profiles specifically in migratory myeloid cells.

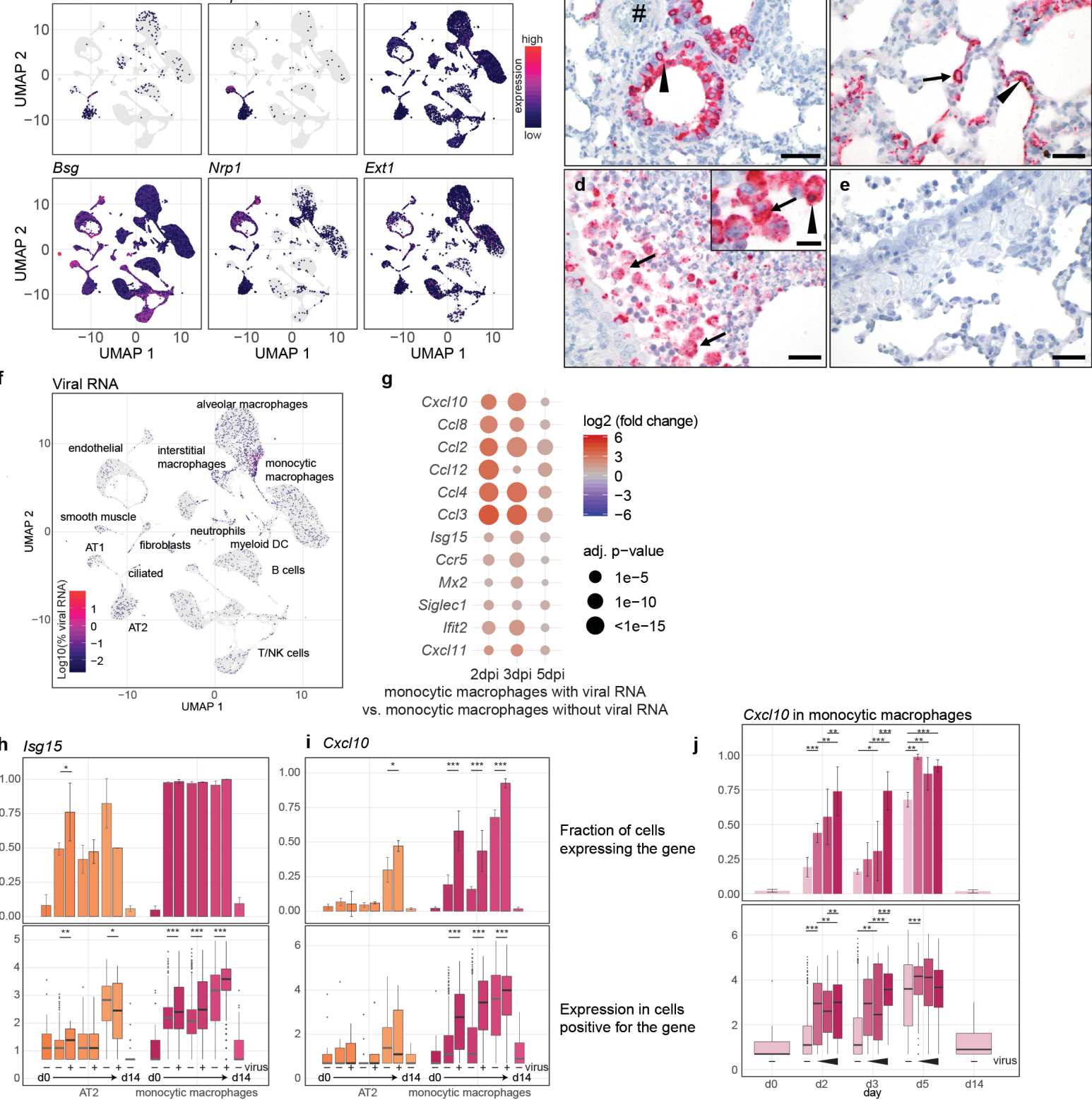
319

### 320 **Early activation of TLR/NF- $\kappa$ B dependent transcription of pro-inflammatory** 321 **cytokines in monocytic macrophages by SARS-CoV-2 infection**

322 Next, we asked whether the observed cellular transcriptional response to SARS-CoV-  
323 2 infection was directly or indirectly influenced by the presence of virus in each cell  
324 type. First, we determined the fractions of cells expressing SARS-CoV-2 entry  
325 receptors, *Angiotensin-converting enzyme 2 (Ace2)* and *transmembrane serine*  
326 *protease 2 (Tmprss2)*, putative alternative receptors, *Basigin (Bsg)* and *Furin*, and  
327 cofactors, such as neuropilins (*Nrp1*), and heparan sulfate (*exostosin-1, Ext1*)  
328 (Cantuti-Castelvetri et al., 2020; Daly et al., 2020; Davies et al., 2020) in hamster lungs  
329 (Figure 5a, Table S3). Ciliated epithelial cells most frequently expressed *Ace2* (~4 –  
330 22 %), as did a smaller proportion of AT2 cells (~3 – 5 %) (Table S3). By *in situ*-  
331 hybridization, we visualized SARS-CoV-2 RNA in bronchial epithelial cells (Figure 5b),  
332 and alveolar epithelial cells morphologically consistent with both type I (Figure 5c,  
333 arrowhead) and type II (Figure 5c, arrow) cells, whereas endothelial cells were  
334 consistently devoid of virus (Figure 5b, hash). Importantly, viral RNA was detected in  
335 high numbers of intrabronchial and intraalveolar macrophages (Figure 5d, arrows) at  
336 early time points. A fraction of macrophages contained high loads of virus without cell  
337 debris, pointing towards uptake of cell-free virus (Figure 5d, inset). For comparison, a  
338 section of alveoli from uninfected hamsters is shown (Figure 5e).

339 In accordance with *in situ*-hybridization data, scRNA-Seq data revealed that most viral  
340 RNA content was found in monocytic macrophages, and not in epithelial cells (Figure  
341 5f, Table S3). For epithelial and endothelial cells, frequencies of virus-positive cells  
342 were highest at 3 dpi, declining by 5 dpi to become absent at 14 dpi, indicating removal  
343 of virus-containing cells (Table S3). In contrast, alveolar macrophages showed highest  
344 viral loads at 5 dpi (~25 %), paralleling decline of virus-positive tissue cells, thus  
345 pointing towards potential increase in phagocytic activities (Table S3). To further  
346 investigate how cell-specific gene expression is modulated by the cell-associated viral  
347 RNA, we tested the correlation between gene expression and viral load in monocytic  
348 macrophages.

349 We first compared gene expression levels in monocytic macrophages that did  
350 (vRNA(+)), or did not (vRNA(-)) contain viral RNA (Figure 5g, S7a). This revealed a set



**Figure 5: Virus RNA in monocytic macrophages leads to dose-dependent activation of pro-inflammatory cytokines by TLR signaling**

(a), Feature plots of entry factor expression in UMAP projection. Coloration indicates expression values of indicated genes. (b-e), Detection of viral RNA by in situ-hybridization. Labeled are supposed endothelium (b, hash), bronchial epithelial cells (b, arrowhead), AT1 (c, arrowhead) and AT2 (c, arrow). (d, inset), macrophages containing viral RNA and cell debris (arrowhead), and an example of high levels of viral RNA without cell debris in the inset (arrow). For (b-e), red signals viral RNA and blue hemalaun counterstain. Time points: (b-c) from 2 dpi, (d) from 3 dpi, (e) from naïve. Bars: (b, d, e) = 50  $\mu$ m, (c) = 100  $\mu$ m, Inset in (c) = 20  $\mu$ m. (f), Cells in the UMAP projection are colored by amount of viral RNA (log<sub>10</sub> transformed percentage of viral RNA per cell), along with overview of identified cell types in lungs. (g), Dotplot of cytokine expression in monocytic macrophages containing viral RNA compared to those without viral RNA. Coloration and point size indicate log<sub>2</sub> fold change and P value for each time point 2, 3 and 5 dpi. (h, i), Box- and barplots of *Isg15* (h) and *Cxcl10* (i) gene expression in AT2 and monocytic macrophages along, comparing cells containing viral RNA to those without for 2, 3, and 5 dpi. Barplot shows percentage of cells positive for respective gene, with mean  $\pm$  SD, n = 3. Boxplots show gene expression levels in cells positive for respective gene. (j), Box- and barplots of *Cxcl10* in monocytic macrophages and fraction of *Cxcl10* positive cells for each time point pi and naïve animals, with cells grouped by increasing virus levels for 2, 3, and 5 dpi. (e and f), for barplots, data display means  $\pm$  SD. Significance levels calculated using generalized linear mixed-effects models for barplot and a wilcoxon rank sum test on all cells (i.e. not only the ones expressing the gene) for boxplots. \* means p value 0.05-0.01, \*\* means p value 0.01-0.0001, \*\*\* means p value smaller 0.0001.



351 of genes that were present at much higher levels in vRNA(+) monocytic macrophages  
352 at the earlier time points (2 dpi, 3 dpi), but not at 5 dpi (Figure S7a). Gene ontology  
353 and KEGG pathway analysis showed that this gene set is enriched for Toll-like receptor  
354 (TLR) signaling (Figure S7b). Specifically, this gene set contained a range of pro-  
355 inflammatory cytokines such as *Cxcl10* or *Ccl2* (Figure 5g), which are activated by the  
356 NF- $\kappa$ B pathway downstream of TLRs (Liu et al., 2017). On the other side, expression  
357 levels of NF- $\kappa$ B independent ISGs such as *Isg15* or *Mx2*, induced by interferons or  
358 cytosolic RNA sensors (Schneider et al., 2014), were only slightly more elevated in  
359 vRNA(+) compared to vRNA(-) monocytic macrophages (Figure 5g).

360 We therefore investigated expression of *Isg15* and *Cxcl10* in monocytic macrophages  
361 in more detail, and in comparison to AT2 cells (Figure 5h, i). In AT2 cells, *Isg15*  
362 expression was induced in about 50 percent of the cells at 2 dpi, with the gene being  
363 present (Figure 5h, upper panel) and expressed at somewhat higher levels in vRNA(+)  
364 cells. In monocytic macrophages however, *Isg15* was expressed in all cells, and at  
365 slightly elevated levels in vRNA(+) cells. In comparison, *Cxcl10* was barely present in  
366 AT2 cells at 2 dpi and 3 dpi (Figure 5i). On the other hand, monocytic macrophages  
367 start to express *Cxcl10* already at 2 dpi, and much stronger so the vRNA(+) cells  
368 (Figure 5i). At 5 dpi, the difference between vRNA(-) and vRNA(+) monocytic  
369 macrophages becomes smaller, but is still significant (Figure 5i).

370 We further analyzed the dose-dependency of this transcriptional response to virus  
371 uptake in monocytic macrophages. Cells were binned in three groups of equal size  
372 with increasing content of viral RNA (Figure 5j). We found that at earlier time points (2  
373 and 3 dpi), but not a 5 dpi, cells with higher amounts of viral RNA-signal, also  
374 expressed more *Cxcl10* (Figure 5j). Overall, this indicates that viral RNA in monocytic  
375 macrophages induces early, strong activation of dose-dependent virus sensing,  
376 leading to a substantial increase of NF- $\kappa$ B-regulated pro-inflammatory chemokines.

377 At 5 dpi, broad inflammation likely masks direct viral RNA-triggered responses by  
378 activating the expression of pro-inflammatory genes in vRNA(+) and vRNA(-) cells  
379 equally. In contrast, AT2 cells, supposed primary site of viral replication, show by far  
380 less activation of both NF- $\kappa$ B dependent and independent transcriptional responses as  
381 compared to monocytic macrophages.

382

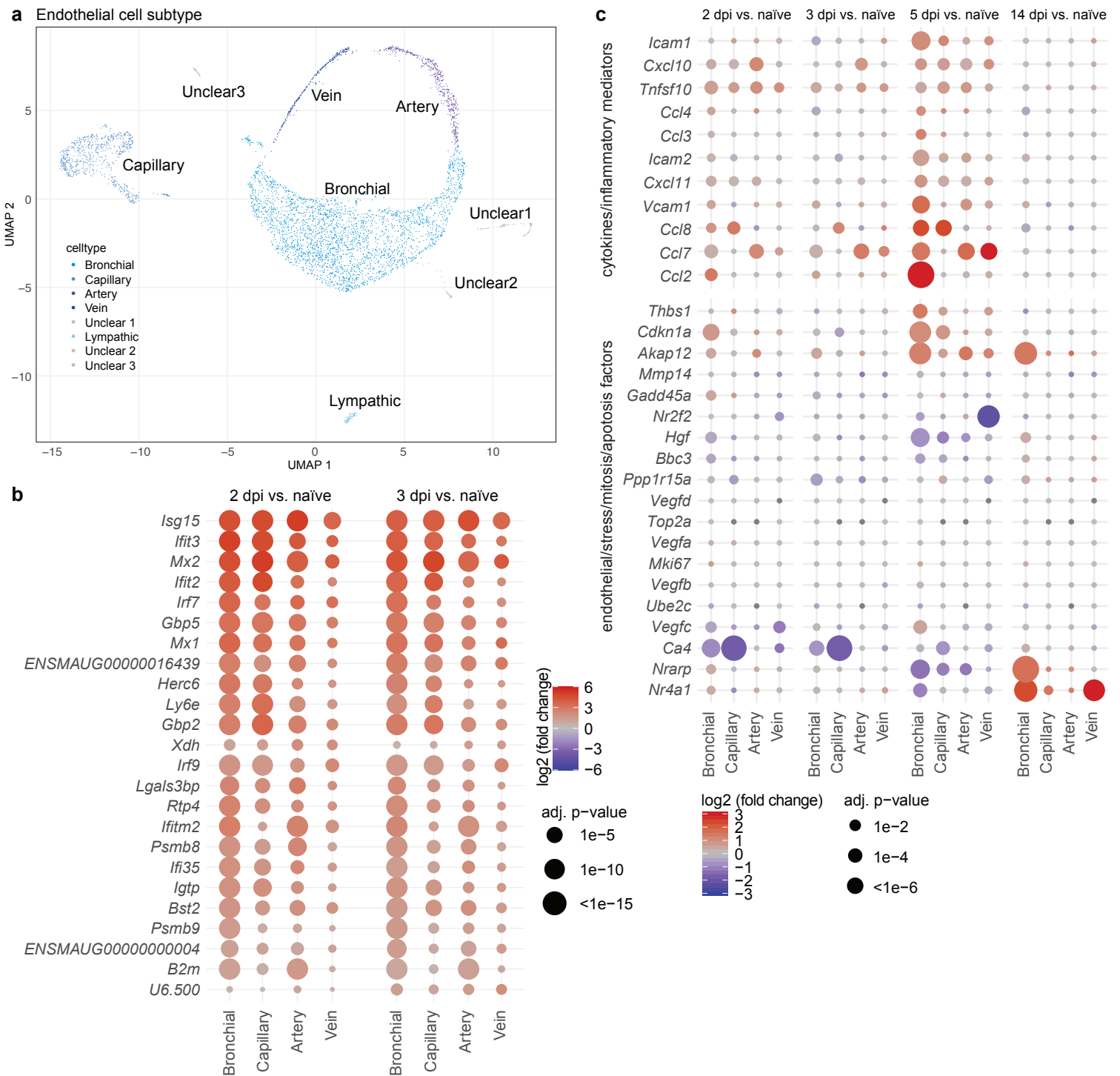
### 383 **Endothelial cells participate in anti-viral and pro-inflammatory responses**

384 Having observed vast similarities of human and Syrian hamster immune responses in  
385 moderate SARS-CoV-2 infection on transcriptomic and proteomic levels, we next  
386 turned our attention to dissection of the molecular mechanisms in lung tissue  
387 compartments that have so far not been assessed longitudinally in moderate COVID-  
388 19 patients, since invasive tissue sampling is hardly possible.

389 Endothelial cells likely participate in COVID-19 pathogenesis (Ackermann et al., 2020;  
390 Michalick et al., 2020), but little is known about the dynamics of their response to  
391 inflammation *in vivo*. Subclustering of cells of endothelial origin identified endothelial  
392 cells of lymphatic and bronchial vasculature, pulmonary arteries, pulmonary capillaries  
393 and pulmonary veins with unique features (Figure 6a, Figure S8a, b). Interestingly,  
394 bronchial endothelial cells, pulmonary artery and capillary endothelial cells all  
395 displayed strong and early anti-viral gene expression profiles at 2 dpi (Figure 6b).  
396 Pulmonary arterial endothelial cells responded most rapidly to infection, with high  
397 expression of *Cxcl10*, *Tnfsf10*, and *Ccl7* by 2 dpi (Figure 6c). Responses of bronchial  
398 vasculature, pulmonary capillary and pulmonary vein endothelial cells were similar but  
399 delayed, peaking at 5 dpi. In addition to the distinct temporal dynamics of endothelial  
400 activation in different tissues, we observed a spatial regulation of expression of  
401 monocyte and effector T cells attractants. Pulmonary artery and vein endothelial cells  
402 preferentially transcribed *Ccl7*, a chemoattractant binding multiple CC receptors,  
403 including CCR1, CCR2, CCR3, CCR5, and CCR10 (Ford et al., 2018). Pulmonary  
404 capillary endothelial cells, however, preferentially expressed the pleiotropic *Ccl8*,  
405 binding at least CCR2, CCR3 and CCR5 (Błaszczuk et al., 2000), while bronchial  
406 vasculature endothelial cells were characterized by *Ccl2* (Figure 6c). ICAM-1 and  
407 VCAM-1 upregulation, expressed by endothelial cells following inflammatory stimuli to  
408 allow for leukocyte transmigration (Schnoor et al., 2015), was highest in bronchial  
409 endothelial cells and pulmonary artery cells at 5 dpi, corresponding with the influx of T  
410 cells revealed by scRNA-Seq data (Figure 6c). Overall lung endothelial cells shared  
411 an anti-viral gene profile but revealed distinct patterns of chemokines targeting  
412 primarily monocytes and Th1 cells. Unlike cells of epithelial origin, endothelial cells  
413 failed to show evidence of proliferation and cell cycle activity that could have indicated  
414 their participation in tissue repair processes during the study period (*DNA*  
415 *topoisomerase 2-alpha* (*Top2a*), *Mki67*, *Ube2c*) (Figure 6c).

416

Figure 6



**Figure 6: Endothelial cells show subtype and time specific activation of cytokines**

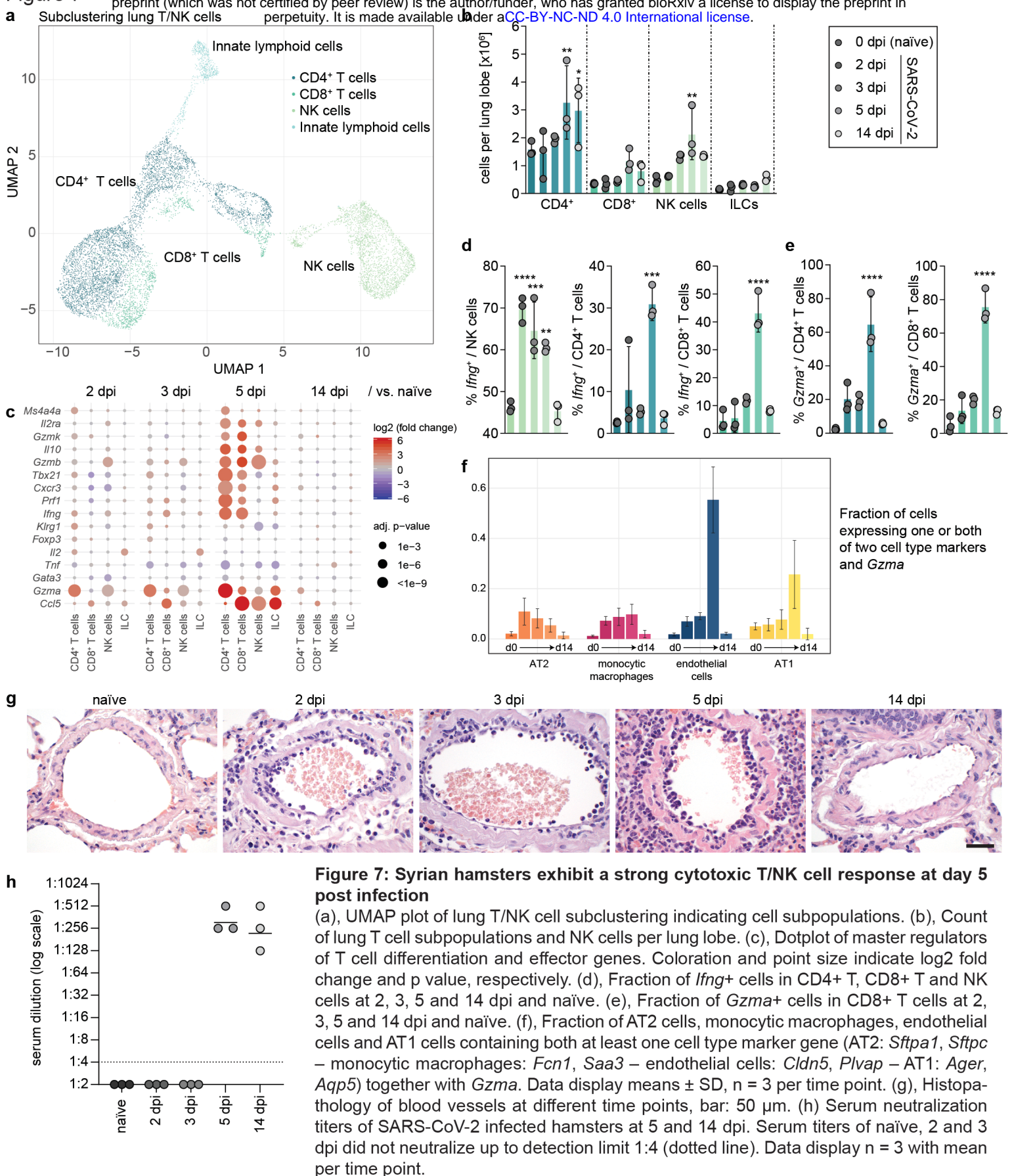
(a), UMAP plot of lung endothelial cell subpopulations. (b), Dotplot of differentially expressed genes in lung endothelial cell subpopulations over time. Shown are genes that are in at least one cell type among the top 10 most changing genes as ranked by adjusted p value. (c), Dotplot of differentially expressed genes from two curated sets as indicated in lung endothelial cell subpopulations over time. For both (b) and (c), coloration and point size indicate log<sub>2</sub>-transformed fold changes and P values, respectively, of genes at 2 dpi relative to control groups (naïve). Adjusted p values were calculated by DEseq2 using Benjamini-Hochberg corrections of Wald test p values.

## 417 **Type 1 effector T cells are efficiently recruited to lungs in SARS-CoV2 infection**

418 Our initial cellular analysis of scRNA-Seq data from lung samples had grouped T and  
419 NK cells in one set of connected clusters, and we had observed their significant  
420 increase in lungs at 5 and 14 dpi (Figure 1). We hypothesized that cytotoxic immunity  
421 might be linked to viral clearance observed at 5 dpi, and elimination by 14 dpi (Figure  
422 S2). Therefore, we subclustered NK and T cells to identify 4 subpopulations based on  
423 *Cd3e*, *Cd4*, *Cd8a* and *Natural Killer Cell Granule Protein 7 (Nkg7)* gene expression  
424 (Figure 7a, S9a), CD4<sup>+</sup> T cells (*Cd3e*<sup>+</sup>*Cd4*<sup>+</sup>), CD8<sup>+</sup> T cells (*Cd3e*<sup>+</sup>*Cd8a*<sup>+</sup>), NK cells  
425 (*Cd3e*<sup>-</sup>*Nkg7*<sup>+</sup>) and innate lymphoid-(ILC) like cells (*Cd3e*<sup>-</sup>*Cd4*<sup>-</sup>*Cd8a*<sup>-</sup>*Nkg7*<sup>-</sup>). CD4<sup>+</sup> T,  
426 CD8<sup>+</sup> T and NK cell numbers increased with infection time and peaked at 5 dpi (Figure  
427 7b). SARS-CoV-2 infection initiated type 1 immunity and cytotoxic effector  
428 mechanisms in lungs (Figure 7c, S9b). The fraction of NK cells expressing interferon  
429 gamma (*Ifng*) increased significantly at 2 dpi and peaked at 3 dpi (Figure 7d, left). In  
430 contrast, *Ifng*<sup>+</sup> effector T cells (*Ifng*<sup>+</sup> CD4<sup>+</sup> and CD8<sup>+</sup> T cells) peaked at 5 dpi (Figure  
431 7d, right). By 14 dpi, both *Ifng*<sup>+</sup> NK and T cell responses had declined to naïve levels  
432 (Figure 7d).

433 NK cells and CD8<sup>+</sup> T cells expressed high levels of cytotoxic genes, but upregulation  
434 of *Gzma* was highest in CD4<sup>+</sup> T cells (Figure 7c, S9b). Cytotoxic effector function of T  
435 cells was evident, 60 % of all CD4<sup>+</sup> T and 70 % of all CD8<sup>+</sup> T expressed *Gzma* at 5 dpi  
436 (Figure 7e). Furthermore, we detected “cells” carrying both a specific cell type marker  
437 (for AT1 / AT2 / endothelial cells, or monocytic macrophages), and simultaneously the  
438 cytotoxic cell marker *Gzma*. These possible doublets were absent in naïve animals  
439 and appeared particularly and reproducibly for endothelial and AT1 cells at the peak  
440 of effector T cell recruitment 5 dpi (Figure 7f), indicating possibly either killer-target  
441 interaction or more likely T cell transmigration. The latter notion was supported by the  
442 strong immigration of lymphocytes in the endothelium observed in histopathology  
443 (Figure 7g).

444 Induction of high-affinity neutralizing antibodies is the primary aim of most vaccination  
445 strategies against viruses. In the here described primary infection with SARS-CoV-2,  
446 neutralizing antibodies were evident by 5 dpi and declined mildly until 14 dpi (Figure  
447 7h). Matching course of mu chain protein levels measured by proteomics (Ighm, Figure  
448 2b). Along with cytotoxic CD8<sup>+</sup> T cells, we found pronounced recruitment of CD4<sup>+</sup> Th1  
449 cells expressing *Ifng*, neutralizing antibodies reached relevant numbers only by 14 dpi



**Figure 7: Syrian hamsters exhibit a strong cytotoxic T/NK cell response at day 5 post infection**

(a), UMAP plot of lung T/NK cell subclustering indicating cell subpopulations. (b), Count of lung T cell subpopulations and NK cells per lung lobe. (c), Dotplot of master regulators of T cell differentiation and effector genes. Coloration and point size indicate log<sub>2</sub> fold change and p value, respectively. (d), Fraction of *Irfg*<sup>+</sup> cells in CD4<sup>+</sup> T, CD8<sup>+</sup> T and NK cells at 2, 3, 5 and 14 dpi and naïve. (e), Fraction of *Gzma*<sup>+</sup> cells in CD8<sup>+</sup> T cells at 2, 3, 5 and 14 dpi and naïve. (f), Fraction of AT2 cells, monocyte macrophages, endothelial cells and AT1 cells containing both at least one cell type marker gene (AT2: *Sftpa1*, *Sftpc* – monocyte macrophages: *Fcn1*, *Saa3* – endothelial cells: *Cldn5*, *Plvap* – AT1: *Ager*, *Aqp5*) together with *Gzma*. Data display means ± SD, n = 3 per time point. (g), Histopathology of blood vessels at different time points, bar: 50 μm. (h) Serum neutralization titers of SARS-CoV-2 infected hamsters at 5 and 14 dpi. Serum titers of naïve, 2 and 3 dpi did not neutralize up to detection limit 1:4 (dotted line). Data display n = 3 with mean per time point.

450 – thus, the peak of T cells corresponded with a vanishing virus responses, indicating  
451 successful cellular effector programs.

452

## 453 **Discussion**

454 Detailed understanding of COVID-19 pathophysiology is imperative for the  
455 development of therapies to reduce numbers of patients developing lung injury.  
456 Notably, recent patient-centered research on COVID-19 was compromised by three  
457 blind spots: (i) Biomaterial is usually sampled after hospital admission, therefore the  
458 early phase of infection and host response has rarely been investigated. (ii) the BAL  
459 procedure to access the alveolar compartment is too dangerous for non-intubated  
460 patients with COVID-19 pneumonia, so that alveolar host responses can hardly be  
461 investigated in mild and moderate COVID-19. (iii) Lung tissue can exclusively be  
462 harvested after death in COVID-19 patients, precluding from investigations of non-  
463 myeloid alveolar or endothelial host responses in early disease, and enabling for  
464 analysis of later disease stadium only in case of fatal outcome. Thus, we performed in-  
465 depth analysis of the full course of moderate, self-limited disease that develops in  
466 SARS-CoV-2 infected Syrian hamsters. In this model, lungs are fully accessible,  
467 providing for detailed analysis of myeloid and non-myeloid compartments including  
468 vascular endothelium. Yet, while the immune response is qualitatively similar between  
469 human and hamsters in central aspects, the viral dose applied in experimental  
470 infections is likely to be higher than in natural aerosol infection. Consequently, infection  
471 kinetics, viral decay rate and immune responses are accelerated (Johansen et al.,  
472 2020; Khoury et al., 2020). In line, we observed the pulmonary peak of viral load,  
473 inflammation and cellular response between 3 and 5 dpi, whereas at 14 dpi the  
474 infection was resolved and mechanisms of tissue regeneration were induced. This  
475 course of disease confirms observations in other animal models including non-human  
476 primates.

477 Syrian hamsters developed rapid, but moderate, neutrophil recruitment predominantly  
478 to bronchi and lung parenchyma, which resolved by 3 dpi. We also observed little of  
479 the typical neutrophil-dependent alveolar damage. Although single-cell RNA-  
480 sequencing analysis likely underestimates the numbers of this fragile cell type (10x  
481 Genomics, 2020), these findings suggest a minor role for neutrophils. Nonetheless, the  
482 neutrophil response is noteworthy, as many other respiratory viruses, e.g. Influenza A  
483 Virus and MERS Virus, initiate little or no neutrophil trafficking in rodents (Dietert et al.,

484 2017). In fact, blood N/L ratios in COVID-19 patients were reported as markers for  
485 disease severity, and neutrophil extracellular traps as well as reactive oxygen species  
486 are suspected to contribute to adverse vascular events (Fu et al., 2020; Middleton et  
487 al., 2020). Confirming our classification of hamsters as a moderate disease model, N/L  
488 ratios were only mildly elevated at 2 dpi, and gene signatures of dysfunctional  
489 immunosuppressive neutrophils were observed in severe COVID-19 patients (Schulte-  
490 Schrepping et al., 2020), but not in Syrian hamsters.

491 In line with COVID-19 patient reports, alveolar macrophage numbers did not decrease  
492 in moderate disease settings (Liao et al., 2020), and monocytic macrophages were the  
493 largest cell population recruited to the lungs, with notable recruitment from 2 dpi on,  
494 and peak presence at 5 dpi (Chua et al., 2020; Schulte-Schrepping et al., 2020).  
495 Monocyte trafficking to lungs was initiated by local expression of CCR2 ligands, and  
496 endogenous chemokine expression served as a feedforward loop. Consequently,  
497 macrophages were the predominant inflammatory cell type in alveolar spaces as  
498 identified by histopathology. Moreover, macrophages presented the earliest and  
499 strongest transcriptional response to the infection, primarily responding to intracellular  
500 viral RNA with pro-inflammatory cytokines such as CXCL10, CCL2 and others.  
501 Notably, the monocytic macrophage pro-inflammatory expression profile was rather  
502 productive than dysregulated as it tended more towards effector T cell recruiting  
503 chemokines targeting CXCR3 and CCR5, and less towards pro-inflammatory cytokine  
504 expression. This matches observations on moderate versus severe courses of COVID-  
505 19 in patients and stands in contrast to findings in animal models with severe disease  
506 progression such as K18-hACE2 mice (Liao et al., 2020; Winkler et al., 2020). It seems  
507 unlikely that intracellular viral RNA found in macrophages is the result of active  
508 infection of these cells, as they have been demonstrated to be largely resistant to  
509 infection *ex vivo* (Hönzke et al., 2020) (Hönzke, personal communications). scRNA-  
510 Seq data obtained from African green monkeys infected with SARS-CoV-2 likewise did  
511 not support virus replication in macrophages (Speranza et al., 2020). Instead, virus  
512 uptake may derive from complement receptor and Fc receptor-mediated phagocytosis  
513 of complement and antibody-labeled virus, as suggested by neutralizing antibody titers  
514 at 5 dpi (Van Strijp et al., 1989). Further, we observed early increase in complement  
515 factors and IgM mu chain by proteomics analysis in lung tissue. Taken together, the  
516 Syrian hamster model endorses the hypothesis that monocyte-derived macrophages  
517 are a primary source of the strong pro-inflammatory response observed in COVID-19,

518 yet highlights that their presence not necessarily results in fatal outcome (Merad and  
519 Martin, 2020).

520 Presumably, SARS-CoV-2 primarily infects AT2 cells (Hou et al., 2020). Of note, only  
521 few of them were infected and they reacted with only marginal transcriptional  
522 responses, which is probably explained by the recent observation in human lungs that  
523 less than 10% of AT2 cells express the crucial entry receptor ACE2 (Hönzke et al.,  
524 2020). Moreover, coronaviruses are endowed with a multitude of mechanisms that  
525 block immunological cascades downstream of interferon signaling and cytoplasmic  
526 RNA sensing (V'Kovski et al., 2020). Only a small subset of *in vitro* highly infected cells  
527 express pro-inflammatory genes (Fiege et al., 2020; Wyler et al., 2020). Thus, despite  
528 being the primary target for viral replication, epithelial cells were not accountable for  
529 early systemic propagation of anti-viral or pro-inflammatory signatures.

530 Endothelial barrier dysfunction, resulting from endothelial cell stress or death, evokes  
531 lung edema and thus contributes to lung failure in severe COVID-19 (Ackermann et  
532 al., 2020; Escher et al., 2020; Marchetti, 2020; Thompson et al., 2017). However,  
533 mechanisms driving endothelial barrier failure in COVID-19 are not well understood,  
534 as the endothelial compartment is not accessible in living humans suffering from  
535 COVID-19. Autopsy studies reported presence of viral particles in human endothelial  
536 cells (Ackermann et al., 2020), but infection of endothelial cells by SARS-CoV has  
537 been questioned (To and Lo, 2004). In SARS-CoV-2 infected non-human primates viral  
538 infection of endothelial cells was not observed (Speranza et al., 2020). Here, we found  
539 that endothelial cells showed a rapid and strong induction of anti-viral response genes,  
540 but considering the absence of histopathological evidence for intraendothelial virus,  
541 we speculate that virus-positive endothelial cells found by scRNA-Seq were not  
542 infected, but were rather an artifact originating from contact with ambient virus or its  
543 RNA (Gruber et al., 2020; Osterrieder et al., 2020). Concomitant cellular and molecular  
544 inflammatory responses in blood suggested that systemic responses were additive to  
545 direct, local endothelial cell activation. Notably, this observation is in line with our  
546 recent findings that virus-free plasma of COVID-19 patients induced significant  
547 endothelial gap formation and loss of junctional VE-cadherin in human endothelial  
548 monolayers and lung tissue (Michalick et al., 2020). Endothelialitis, as observed in  
549 autopsies of deceased COVID-19 patients, was also found in infected hamsters  
550 (Gruber et al., 2020; Osterrieder et al., 2020) and corresponded to their transcriptional  
551 pro-inflammatory chemokine responses (Ackermann et al., 2020). Histopathological



552 evidence of pronounced lymphocyte trafficking via capillary endothelial cells also  
553 correlated with endothelial *Cc18* expression.

554 Lymphocyte recruitment in response to CXCR3 and CCR5 targeting chemokines  
555 resulted in the presence of CD4<sup>+</sup> and CD8<sup>+</sup> T cells with cytotoxic expression pattern in  
556 lungs starting at 3 dpi. Most importantly, viral clearance coincided with appearance of  
557 effector T and NK cells stressing their relevance for resolution of SARS-CoV-2 infection  
558 and highlighting their importance in vaccination strategies. Studies from other  
559 coronaviruses suggest that type 1 immunity is the primary mechanism controlling the  
560 infection (Janice Oh et al., 2012; Shin et al., 2019). In severe COVID-19, blood CD4<sup>+</sup>  
561 T, CD8<sup>+</sup> T and NK cells expressed markers of exhaustion (Varchetta et al., 2020), a  
562 finding not mirrored in moderately sick Syrian hamsters. In contrast, T and NK  
563 responses were effective and self-resolving. This matches observations of broad T cell  
564 antigen-specificity in the majority of resolved cases independent of mild or severe  
565 infection (Peng et al., 2020). In our study, we found SARS-CoV-2 neutralizing  
566 antibodies at 5 dpi, likely of IgM type, as early appearance and corresponding elevated  
567 Ighm protein levels suggested. Seroconversion in COVID-19 patients occurred 7 to 14  
568 days post diagnosis with IgG titers appearing at later time points (Gudbjartsson et al.,  
569 2020; Ni et al., 2020).

570 At 14 dpi, infectious virus was no longer detected in hamster lungs, and most  
571 transcriptional activity had returned to basal levels. Upregulation of mitotic markers in  
572 AT2 cells may reflect regeneration mechanisms after clearing the infection.

573 The pulmonary capillary microvascular niche in lungs supports alveolar epithelial repair  
574 mechanisms following injury, e.g. by secretion of MMP14, VEGF, thrombospondin-1  
575 (THBS1) (Mammoto and Mammoto, 2019). Analysis of pulmonary endothelial cell  
576 subclusters revealed that bronchial, pulmonary capillary and pulmonary vein  
577 endothelial cells showed increased expression of *Thbs1* at 5, but not 14 dpi. Similarly,  
578 no increase in *Mmp14* or *Vegf* expression was detected at 14 dpi. In murine influenza  
579 characterized by lung injury and pronounced alveolar damage, Niethamer et al.  
580 described a pulmonary population of proliferating endothelial cells at 14 dpi (Niethamer  
581 et al., 2020) that was absent in SARS-CoV-2 infected Syrian hamsters, indicating that  
582 the alveolar endothelial and epithelial damage remained moderate in our model. Most  
583 notably, lung endothelial cells showed an anti-fibrotic gene signature at 14 dpi (*Nr4a1*  
584 (Palumbo-Zerr et al., 2015), *Akap12* (Lee et al., 2018), *Nrarp* (downstream of Notch-

585 signaling) (Cao et al., 2016), indicating regeneration rather than repair of lung tissue,  
586 thereby matching histopathology findings.

587 Our study has several limitations, including limited disease spectrum by restriction to  
588 moderate disease, lack of detailed B cell response analysis beyond 14 dpi, and need  
589 for improvement of gene annotation in the current version hamster genomes.  
590 Furthermore, it does not recapitulate the various covariates and preexisting conditions  
591 that can affect disease outcome. Clearly, we are only at the beginning of matching the  
592 course of disease in hamsters and humans in greater detail.

593 Nevertheless, we already provide clear evidence that Syrian hamsters recapitulate the  
594 course of moderate human SARS-CoV-2 infection. Hamsters displayed nearly  
595 prototypic antiviral immune responses starting with rapid, yet self-restricted  
596 neutrophilic response, along with a fast and strong monocytic innate immune response  
597 following activation after virus uptake, augmenting local anti-viral responses and pro-  
598 inflammatory CC chemokine production recruiting a potent type 1 T cell response that  
599 probably contributed to elimination of pulmonary residing virus via cytotoxic effector  
600 mechanisms. Neutralizing antibodies of IgM type aided in preventing viral spread and  
601 fostered cellular virus uptake. Viral infection and inflammatory response in and by lung  
602 epithelium is not predominant. Upon successful elimination of virus, alveolar epithelial  
603 repair mechanisms started, along with endothelial suppression of fibrotic programs,  
604 thus enabling pulmonary regeneration in convalesced hamsters.

605 Hence, Syrian hamsters represent a highly suitable model to study pathophysiology of  
606 moderate COVID-19, virus-directed and immunomodulatory therapies, and potentially  
607 vaccines. SARS-CoV-2 infected Syrian hamsters mount immune responses consistent  
608 with COVID-19 patients and enable for detailed investigations on the kinetics and role  
609 of specific cell populations, highlighting the dominant contribution of monocytic  
610 macrophages, endothelial cells and T cells to inflammatory responses and resolution  
611 of SARS-CoV-2 infection.

612

### 613 **Data and code availability**

614 Raw and processed data is available at the NCBI gene expression omnibus, entry  
615 GSE162208. The code used for data analysis is available at github.com,  
616 <https://github.com/Berlin-Hamster-Single-Cell-Consortium>. Further supplementary  
617 data is available at <http://www.mdc-berlin.de/singlecell-SARSCoV2>.

618

619 **Author Contributions:** Conceptualization, G.N., E.W., J.T and M.W.; Methodology,  
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666 interpretation of data, in the writing of the manuscript, or in the decision to publish the  
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668

669 **Figure legends:**

670 **Figure 1: Single-cell dynamics in lungs and blood of SARS-CoV-2 infected**  
671 **Syrian hamsters**

672 (a), UMAP plot of identified cell populations in Syrian hamster lungs. (b), Cell count of  
673 isolated cells per lung lobe over time (2, 3, 5 and 14 dpi) and control group (naïve,  
674 “d0”). (c), Count of hematopoietic cells per lung lobe in naïve hamsters and over time  
675 pi. (d), Changes in cellular density of lung cells in UMAP projection. Coloration  
676 indicates log<sub>2</sub> fold change between control group and 5 dpi. (e), Percentage of  
677 hematopoietic cells per lung lobe in naïve hamsters and over time pi. (f), UMAP plot of  
678 identified cell populations in blood samples. (g), Cell count of isolated cells per mL  
679 blood in naïve hamsters and over time pi. (h), Percentage of identified cell populations  
680 in blood samples over time pi and naïve animals. (i), Neutrophil–lymphocyte ratio in  
681 blood samples over time pi and naïve animals. (a, d and f), Clusters defined by Louvain  
682 clustering, n = 3 per time point. (b, c, e, g, h and i), Data display means ± SD. n = 3  
683 per time point. Ordinary one-way ANOVA, Sidak’s multiple comparisons test versus  
684 corresponding 0 dpi (naïve). \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$

685

686 **Figure 2: Proteomics analysis recapitulates transcriptomics and human**  
687 **COVID-19 patient data**

688 (a), Temporal evolution of gene ontology/biological process terms connected with  
689 immune system response in lung tissue (left part) and in serum fluid (right part), for the  
690 indicated time points compared to samples from uninfected animals. Enriched terms  
691 were filtered for terms mentioning “immune”, “interferon”, “neutrophil”, “T cell” and “B  
692 cell”. Size of dots correspond to the inverse of the false discovery rate. (b), Expression  
693 values for differentially regulated proteins in hamster serum (control 3 dpi,  $p$  value <  
694 0.01) and lung (control 5 dpi,  $p$  value < 0.01) that correlate with disease severity in  
695 human plasma. Controls from different days are plotted together. The thick line  
696 represents median of all values, lower and upper hinges correspond to medians of  
697 lower and upper half of set, respectively. Whiskers extend to a maximum of 1.5 times  
698 distance between first and third quartile. All values are shown. (c) Expression values  
699 for the differentially expressed proteins *Lgals3* and *Lgals3bp* (only detected in lung  
700 samples).

701

702 **Figure 3: Induction of inflammatory mediators are strongest and earliest in**  
703 **myeloid cells**

704 Dotplots of differentially expressed cytokines and inflammatory mediators in lungs.  
705 Shown are genes that display a significant absolute  $\log_2$ -transformed fold change of  
706 at least one in at least one comparison, and are grouped into four categories indicated  
707 on the left. Coloration and point size indicate  $\log_2$ -transformed fold changes and  $P$   
708 values, respectively, of genes at 2 dpi relative to control groups (naïve). Adjusted  $P$   
709 values were calculated by DEseq2 using Benjamini-Hochberg corrections of Wald test  
710  $p$  values. Genes are ordered by unsupervised clustering, cell type as in Figure 1.

711

712 **Figure 4: Transcriptional response to SARS-CoV-2 infection is strong in**  
713 **myeloid and moderate in epithelial cells**

714 (a, b), Dotplot of differentially expressed genes in lungs (a) and blood (b). Shown are  
715 genes that are in at least one cell type among the top 4 most changing genes as ranked  
716 by adjusted  $p$  value. Example: top 4 most changing genes in alveolar macrophages  
717 are *Ybx3*, *Rps15*, *Rps10*, and *Uba52*. Coloration and point size indicate  $\log_2$ -  
718 transformed fold changes and  $p$  values, respectively, of genes at 2 dpi relative to  
719 control groups (naïve). Adjusted  $p$  values were calculated by DEseq2 using Benjamini-

720 Hochberg corrections of Wald test  $p$  values. Cell types and genes are ordered by  
721 unsupervised clustering. (c), Top, fraction of monocytic macrophages expressing  
722 *Cxcl10* in blood and lungs from naïve animals and 2 dpi. Bottom, boxplots of *Cxcl10*  
723 gene expression values of cells positive for *Cxcl10* expression. Significance levels  
724 were calculated using generalized linear mixed-effects models for bar plot and a  
725 Wilcoxon rank sum test on all cells (i.e. not only the ones expressing the gene) for  
726 boxplots. \* means  $p$  value 0.05-0.01, \*\* means  $p$  value 0.01-0.0001, \*\*\* means  $p$  value  
727 smaller 0.0001. See materials and methods for details. (d), As in (c), but only in lungs,  
728 all time points and both alveolar epithelial cells type 2 (AT2) and monocytic  
729 macrophages. Displayed are values for *Isg15* (left) and *Cxcl10* (right). For all bar plots,  
730 data display means  $\pm$  SD,  $n = 3$  per time point. For all boxplots, lower and upper hinges  
731 correspond to first and third quartiles. Whiskers extend to a maximum of 1.5 times the  
732 distance between first and third quartile. Outliers beyond are marked by single dots.

733

734 **Figure 5: Virus RNA in monocytic macrophages leads to dose-dependent**  
735 **activation of pro-inflammatory cytokines by TLR signaling**

736 (a), Feature plots of entry factor expression in UMAP projection. Coloration indicates  
737 expression values of indicated genes. (b-e), Detection of viral RNA by in situ-  
738 hybridization. Labeled are supposed endothelium (b, hash), bronchial epithelial cells  
739 (b, arrowhead), AT1 (c, arrowhead) and AT2 (c, arrow). (d, inset), macrophages  
740 containing viral RNA and cell debris (arrowhead), and an example of high levels of viral  
741 RNA without cell debris in the inset (arrow). For (b-e), red signals viral RNA and blue  
742 hemalaun counterstain. Time points: (b-c) from 2 dpi, (d) from 3 dpi, (e) from naïve.  
743 Bars: (b, d, e) = 50  $\mu\text{m}$ , (c) = 100  $\mu\text{m}$ , Inset in (c) = 20  $\mu\text{m}$ . (f), Cells in the UMAP  
744 projection are colored by amount of viral RNA (log10 transformed percentage of viral  
745 RNA per cell), along with overview of identified cell types in lungs. (g), Dotplot of  
746 cytokine expression in monocytic macrophages containing viral RNA compared to  
747 those without viral RNA. Coloration and point size indicate log2 fold change and  $P$   
748 value for each time point 2, 3 and 5 dpi. (h, i), Box- and barplots of *Isg15* (h) and *Cxcl10*  
749 (i) gene expression in AT2 and monocytic macrophages along, comparing cells  
750 containing viral RNA to those without for 2, 3, and 5 dpi. Barplot shows percentage of  
751 cells positive for respective gene, with mean  $\pm$ SD,  $n = 3$ . Boxplots show gene  
752 expression levels in cells positive for respective gene. (j), Box- and barplots of *Cxcl10*  
753 in monocytic macrophages and fraction of *Cxcl10* positive cells for each time point pi

754 and naïve animals, with cells grouped by increasing virus levels for 2, 3, and 5 dpi. (e  
755 and f), for barplots, data display means  $\pm$  SD. Significance levels calculated using  
756 generalized linear mixed-effects models for barplot and a wilcoxon rank sum test on  
757 all cells (i.e. not only the ones expressing the gene) for boxplots. \* means  $p$  value 0.05-  
758 0.01, \*\* means  $p$  value 0.01-0.0001, \*\*\* means  $p$  value smaller 0.0001. See materials  
759 and methods for details.

760

## 761 **Figure 6: Endothelial cells show subtype and time specific activation of**

### 762 **cytokines**

763 (a), UMAP plot of lung endothelial cell subpopulations. (b), Dotplot of differentially  
764 expressed genes in lung endothelial cell subpopulations over time. Shown are genes  
765 that are in at least one cell type among the top 10 most changing genes as ranked by  
766 adjusted  $p$  value. (c), Dotplot of differentially expressed genes from two curated sets  
767 as indicated in lung endothelial cell subpopulations over time pi. For both (b) and (c),  
768 coloration and point size indicate  $\log_2$ -transformed fold changes and  $P$  values,  
769 respectively, of genes at 2 dpi relative to control groups (naïve). Adjusted  $P$  values  
770 were calculated by DEseq2 using Benjamini-Hochberg corrections of Wald test  $P$   
771 values.

772

## 773 **Figure 7: Syrian hamsters exhibit a strong cytotoxic T/NK cell response at day**

### 774 **5 post infection**

775 (a), UMAP plot of lung T/NK cell subclustering indicating cell subpopulations. (b),  
776 Count of lung T cell subpopulations and NK cells per lung lobe. (c), Dotplot of master  
777 regulators of T cell differentiation and effector genes. Coloration and point size indicate  
778  $\log_2$  fold change and  $P$  value, respectively. (d), Fraction of *Ifng*<sup>+</sup> cells in CD4<sup>+</sup> T, CD8<sup>+</sup>  
779 T and NK cells at 2, 3, 5 and 14 dpi and naïve. (e), Fraction of *Gzma*<sup>+</sup> cells in CD8<sup>+</sup> T  
780 cells at 2, 3, 5 and 14 dpi and naïve. (f), Fraction of AT2 cells, monocyte macrophages,  
781 endothelial cells and AT1 cells containing both at least one cell type marker gene (AT2:  
782 *Sftpa1*, *Sftpc* – monocyte macrophages: *Fcn1*, *Saa3* – endothelial cells: *Cldn5*, *Plvap*  
783 – AT1: *Ager*, *Aqp5*) together with *Gzma*. Data display means  $\pm$  SD,  $n = 3$  per time  
784 point. (g), Histopathology of blood vessels at different time points, bar: 50  $\mu\text{m}$ . (h)  
785 Serum neutralization titers of SARS-CoV-2 infected hamsters at 5 and 14 dpi. Serum  
786 titers of naïve, 2 and 3 dpi did not neutralize up to detection limit 1:4 (dotted line). Data  
787 display  $n = 3$  with mean per time point.

## 788 **Methods**

### 789 **Ethics statement, animal husbandry and study design**

790 All experiments involving animals were approved by institutional and governmental  
791 authorities (Freie Universität Berlin and Landesamt für Gesundheit und Soziales Berlin,  
792 Germany, approval number 0086/20) and performed in accordance with relevant  
793 national and international regulations. Female and male Syrian hamsters  
794 (*Mesocricetus auratus*; breed RjHan:AURA, Janvier Labs, Saint-Berthevin, France)  
795 were housed in biosafety level 3 (BSL-3) conditions in individually ventilated cages  
796 with enrichment (IVCs; Tecniplast, Buguggiate, Italy; Carfil, Oud-Tunrhout, Belgium).  
797 Food and water was provided *ad libidum*. Daily cage temperature and relative humidity  
798 measurements ranged from 22–24 °C and 40–55 %, respectively. Animals were  
799 acclimatized for a minimum of 7 days prior to infection.

800

### 801 **Virus Stocks**

802 SARS-CoV-2 isolate (BetaCoV/Germany/BavPat1/2020) [32235945] was kindly  
803 provided by Daniela Niemeyer and Christian Drosten, Charité Berlin, Germany. Virus  
804 stocks were propagated under BSL-3 conditions in Vero E6 cells (ATCC CRL-1586) in  
805 minimal essential medium (MEM; PAN Biotech, Aidenbach, Germany) supplemented  
806 with 10 % fetal bovine serum (PAN Biotech), 100 IU/mL penicillin G and 100 g/mL  
807 streptomycin (Carl Roth, Karlsruhe, Germany). All hamsters described here received  
808 virus from the same batch.

809

### 810 **Animal infection**

811 At 10–12 weeks of age hamsters were intranasally infected with  $1 \times 10^5$  pfu SARS-  
812 CoV-2 under anesthesia as previously described (Osterrieder et al., 2020). Clinical  
813 signs and weight were monitored daily. Animals with >15 % body weight loss over 48  
814 h were euthanized in accordance with the animal use protocol. Naïve (n = 3) and  
815 infected hamsters were randomly selected for analyses at time points 2, 3, 5 or 14  
816 days post infection (dpi) (n = 3, each). Exsanguination and euthanasia under  
817 anesthesia were performed as previously described (Osterrieder et al., 2020). 1 mL  
818 peripheral blood was collected in EDTA-coated syringes. The left lung lobe was  
819 collected for histopathology, the right caudal lobe for single-cell analysis, the right  
820 cranial lobe for virological assessments and the right medial for bulk RNA as well as



821 proteomics analysis. Experimental design and analysis are summarized in the  
822 supplemental material (Figure S1).

823

#### 824 **Viral burden assessment in lungs and tracheal swabs**

825 *Virus titers* were determined by serial dilutions of lung homogenates (50 mg) plated on  
826 Vero E6 cells. Virus adsorption was allowed for 2 h, medium was then removed and  
827 cells were overlaid with MEM supplemented with 5 % FBS, 100 IU/mL penicillin G and  
828 100 g/mL streptomycin and 1.25 % Avicel (Sigma-Aldrich, St. Louis, MO) and  
829 incubated for 48 h. Cells fixed in 4 % formalin were stained with 0.75 % crystal violet  
830 in aqueous solution and plaques were counted.

831 For *RNA extractions and quantitative RT-PCR*, RNA from oropharyngeal swabs, 25  
832 mg lung tissue and 25  $\mu$ L whole blood was isolated with the innuPREP Virus DNA/RNA  
833 Kit (Analytic Jena) according to the manufacturer's instructions. One-step RT qPCR  
834 reaction with the NEB Luna Universal Probe One-Step RT-qPCR (New England  
835 Biolabs) and the 2019-nCoV RT-qPCR primers and probe (E\_Sarbeco) [31992387]  
836 quantified viral RNA according to the manufacturer's instructions and normalization of  
837 viral RNA to cellular *Rpl18* occurred as previously described [32698441]. Primers and  
838 probes are listed in the Supplemental Material (Table S1), standard curves for  
839 quantification were generated as previously described [32698441]. Viral RNA copies  
840 were calculated per  $1 \times 10^5$  hamster *Rpl18* transcripts.

841

#### 842 **Measurement of neutralizing antibodies titer**

843 Serum neutralization tests were performed by two fold serial dilutions (1:4 to 1:512) of  
844 complement inactivated (56°C, 2 h) hamster serum plated on sub-confluent  
845 monolayers of Vero E6 cells in 96 well plates. 50 pfu SARS-CoV-2 were added per  
846 well and incubated for 72 h at 37°C, fixed with 10 % formalin for 24 h and stained with  
847 crystal violet (0.75% aqueous solution). Serum neutralization was considered effective  
848 in wells that did not shows any cytopathic effect, the highest effective dilution was  
849 counted.

850

#### 851 **Histopathology and *in situ*-hybridization of SARS-CoV-2 RNA**

852 For histopathology and *in situ*-hybridization (ISH), lungs were processed as described  
853 (29361238). Left lung lobes were immersion-fixed in 10 % formalin, pH 7.0, for 48 h,  
854 embedded in paraffin, and cut into 2  $\mu$ m sections. Hematoxylin and eosin (HE) staining

855 and *in situ*-hybridizations were performed as described [32817952] using the  
856 ViewRNA™ ISH Tissue Assay Kit (Invitrogen by Thermo Fisher Scientific, Darmstadt,  
857 Germany) following the manufacturer's instructions with minor adjustments. SARS-  
858 CoV-2 RNA was localized with probes detecting N gene sequences (NCBI database  
859 NC\_045512.2, nucleotides 28,274 to 9533, assay ID: VPNKRHM). An irrelevant probe  
860 for the detection of streptococcal pneumolysin was used to control for sequence-  
861 specific binding (Bertzbach et al., 2020). Amplifier and label probe hybridizations were  
862 performed following the manufacturer's instructions using fast red as chromogen with  
863 hemalaun counterstain. Tissues were histopathologically evaluated by board-certified  
864 veterinary pathologists (KD, ADG) in a blinded fashion following standardized  
865 recommendations (Gruber et al., 2020), including pneumonia-specific scoring  
866 parameters (Dietert et al., 2017) as previously described for SARS-CoV-2 infection in  
867 hamsters (Osterrieder et al., 2020).

868

#### 869 **Single Cell Isolation from Whole Blood**

870 Protocols were adapted for BSL-3 facility regulations. For isolation of cells from whole  
871 blood, 250 µL blood were lysed in red blood cell lysis buffer (BioLegend), washed and  
872 centrifuged according to the manufacturer's instructions. Resulting RBC-free pellets  
873 were resuspended in low-BSA buffer (1× PBS, 0.04 % BSA), filtered with 40 µm FloMi  
874 filters (Merck) suitable for volumes below 500 µL and counted microscopically by  
875 hemocytometer in trypan blue.

876

#### 877 **Single Cell Isolation from Hamster Lungs**

878 Protocols were adapted for BSL-3 facility regulations. For isolation of single cells *lobus*  
879 *caudalis* of the right lung was removed and placed in storage medium (1× PBS, 0.5 %  
880 BSA) until further processing. Storage and isolation media contained 2 µg/mL  
881 ActinomycinD. Tissues and cells were centrifuged at 350 x *g* for 6 minutes at 4 °C.  
882 Lung lobes were mechanically disassociated with tweezers for 2 minutes in enzymatic  
883 digestion medium containing 3.4 mg/mL Collagenase Cls II (Merck) and 1 mg/mL  
884 DNase I (PanReac AppliChem) in 2 mL Dispase medium (Corning) per lung lobe  
885 followed by 30 minutes incubation at 37 °C and 5 % CO<sub>2</sub>. After repeated pipetting of  
886 digested lung tissue with 5 mL pipettes, cell suspensions were pressed through 70 µm  
887 cell strainers with syringe plungers. Red blood cells were lysed (BioLegend), washed  
888 with an excess of PBS/BSA and resuspended in low-BSA buffer (1× PBS, 0.04 %

889 BSA), and filtered with 40  $\mu$ m low-volume FloMi filters (Merck). Cells were counted with  
890 trypan blue.

891

## 892 **Single Cell RNA Sequencing**

893 *Barcoding, cDNA Library generation and sequencing*; Filtered cells were adjusted to a  
894 final concentration of  $\sim$ 1,000 cells /  $\mu$ L in 1 $\times$  PBS with 0.04% BSA and subjected to  
895 partitioning into Gel-Beads-in-Emulsions (GEMs) with the aim of recovering a  
896 maximum of 6,000 single cells per hamster and organ by following the instructions for  
897 the Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1 provided by  
898 the manufacturer (10x Genomics). The procedure entailed tagging polyadenylated  
899 mRNA from individual cells with unique 16-bp 10x barcodes and 10-bp Unique  
900 Molecular Identifiers (UMI). Following instructions, reverse transcribed full-length and  
901 barcoded cDNA were bulk-amplified. Enzymatic fragmentation and size selection  
902 followed, resulting in double-stranded cDNA amplicons optimized for library  
903 construction. Adaptor ligation and sample index PCR introduce all reads, sequences  
904 and sample index into the cDNA library necessary for Illumina bridge amplification and  
905 sequencing.

906 Single-cell libraries were quantified using Qubit (ThermoFisher) and quality-controlled  
907 using the Bioanalyzer system (Agilent). Sequencing was performed on a Novaseq  
908 6000 device (Illumina), with SP or S1 flow cells (read1: 28 nucleotides, read2: 64  
909 nucleotides).

910

## 911 **Bulk RNA sequencing**

912 *For lung RNA Bulk Sequencing*, the *lobus medialis* of the right lung lobe was removed  
913 carefully and stored in RNA Later Solution for a maximum of 24 hours at 4  $^{\circ}$ C  
914 (Thermofisher). Lung Tissue was homogenized using the TissueLyser II system  
915 (Qiagen) and the homogenate stored in Trizol reagent (Zymo research). For *WBC RNA*  
916 *Bulk Sequencing*, white blood cells were isolated as described for scSeq followed by  
917 lysis in Trizol reagent. RNA extractions were performed according to the Direct-zol  
918 RNA Miniprep protocol (Zymoresearch). Bulk RNA sequencing libraries constructed  
919 using the Nebnext Ultra II Directional RNA Library Prep Kit (New England Biolabs),  
920 and sequenced on a Nextseq 500 device with read length 76.

921

## 922 **Proteomics**

### 923 *Sample preprocessing*

924 Approximately 5 mg homogenized lung tissue and 5 µl of hamster serum were added  
925 to the lysis and inactivation buffer (RIPA) and boiled for 10 min at 95 °C before storage  
926 at -80 °C. Samples were thawed on ice and 20 µl of the extract were transferred to a  
927 96 well plate. The volume was adjusted to 50 µl with water and 25 µl of 50 U  
928 benzonase, 50 mM ABC, 2 mM MgCl<sub>2</sub> added before incubation for 30 min at 37 °C.  
929 The lysates were processed on a Biomek i7 workstation using the SP3 protocol as  
930 previously described with single-step reduction and alkylation (Muller et al., 2020). In  
931 brief, 20 µl reduction and alkylation buffer (40 mM TCEP, 160 mM CAA, 200mM ABC)  
932 were added and the samples were incubated for 5 min at 95 °C. To bind the proteins,  
933 200 µg paramagnetic beads (1:1 ratio of hydrophilic/hydrophobic beads) were added  
934 and proteins precipitated with 50 % ACN. The samples were washed 3 times with EtOH  
935 and once with ACN, before reconstitution in 35 µl 100 mM ABC, addition of 20 µl trypsin  
936 (1 µg) ON digestion at 37 °C. The reaction was stopped by addition of formic acid to a  
937 final concentration of 0.1 %. Samples were then transferred to a new plate and used  
938 for LC-MS/MS analysis without additional conditioning or clean-up.

939 For proteomics, we combine conventional approaches with latest generation  
940 acquisition methods, (i.e. Scanning SWATH) (Messner et al., 2020a) coupled with high  
941 flow chromatographic separation. This combination combines the strengths of the  
942 depth of conventional proteomics, with the strength of very high measurement  
943 precision at high throughput achieved with the high-flowrate LC proteomic methods,  
944 which overcome limits in the clinical application of proteomics.

### 945 *Liquid Chromatography–Mass Spectrometry Analysis (LC–MS)*

946 *High-throughput analysis of serum and lung tissue;* Peptide separation has been  
947 accomplished in a 5-minute water to acetonitrile gradient on an Agilent Infinity II HPLC  
948 coupled to a Sciex Triple TOF 6600 mass spectrometer (IonDrive TurboV Source)  
949 operating in ScanningSWATH mode with minor changes in the liquid chromatography  
950 method (Messner et al., 2020a). As follows: 5 µg of peptides were loaded and resolved  
951 in a linear gradient from 1-35 % buffer B in 4.5 min before increasing to 40 % B in 0.5  
952 min and washing for 0.2 min with 80 % buffer B before equilibration for 2.2 min with  
953 initial conditions (buffer A: 0.1 % formic acid, buffer B: 100 % ACN, 0.1 % formic acid).

954 *Computational proteomics;* For *Mesocricetus auratus* serum samples, a project  
955 specific library was generated by gas-phase fractionation (GPF), whereas the lung

956 tissue library was constructed using standard settings in library free mode with DIA-  
957 NN (version 1.7.12) (Demichev et al., 2020a). Proteins were annotated using the *M.*  
958 *auratus* genome sequence generated in this work. The libraries were automatically  
959 refined based on the project dataset at 0.01 global q-value (using the “Generate  
960 spectral library” option in DIA-NN) as previously described (Messner et al., 2020b).  
961 The output was filtered at 0.01 false discovery rate (FDR) at the peptide level. For GPF,  
962 6 single 1 µg injections of pooled serum samples were analyzed by online nanoflow  
963 liquid chromatography tandem mass spectrometry on a Ultimate3000 Thermo  
964 Scientific Q Exactive Orbitrap, LC-MS instrument (Thermo Fisher Scientific, Waltham,  
965 USA). The peptides were concentrated for 3 min on a trap column (PepMap C18, 5  
966 mm x 300 µm x 5 µm, 100Å, Thermo Fisher Scientific) with a buffer containing 2:98  
967 (v/v) acetonitrile/water containing 0.1% (v/v) trifluoroacetic acid at a flow rate of 20  
968 µl/min. They were separated by a 250 mm LC column (Acclaim PepMap C18, 2 µm;  
969 100 Å; 75µm, Thermo Fisher Scientific). The mobile phase (A) was 0.1 % (v/v) formic  
970 acid in water, and (B) 80 % acetonitrile in 0.1 % (v/v) formic acid. In 155 min total  
971 acquisition time gradient B increased in 90 min to 25 %, and in 30 min to 40 % with a  
972 flow rate of 300 nl/ min. The MS instrument was operated in the data independent  
973 mode as followed: the Orbitrap worked in centroid mode with 4 m/z DIA spectra (4 m/z  
974 precursor isolation windows at 17,500 resolution, AGC target 1e6, maximum inject time  
975 60 ms, 27 NCE). An overlapping window pattern from narrow mass ranges using  
976 window placements (i.e., 395-505, 495-605,595-705, 695-805,795-805,895-905 m/z)  
977 was set. Two precursor spectra, a wide spectrum (395-505 m/z at 35,000 resolution)  
978 and a narrow spectrum matching the range using an AGC target of 1e6 and a  
979 maximum inject time of 60 ms were interspersed every 25 MS/MS spectra at resolution  
980 of 17,500. Typical mass spectrometric conditions were as follows: spray voltage, 2.1  
981 kV; no sheath and auxiliary gas flow; heated capillary temperature: 275 °C; normalized  
982 HCD collision energy 27 %. As lock mass acted the background ion m/z 445.1200.

983 *Materials Proteomics*; Hydrophobic Sera-Mag magnetic carboxylate modified particles  
984 (44152105050250 Fisher Scientific), hydrophilic Sera-Mag magnetic carboxylate  
985 modified particles (24152105050250 Fisher Scientific), Twintec skirted low bind plates  
986 (0030129512 Eppendorf), TCEP (646547 Sigma Aldrich), SDS (A7249.1000  
987 Applichem), CAA (22788 Merck/Millipore), ammonium bicarbonat (/871.2 Roth), 100  
988 % ACN (955-212 Fisher Scientific), 80 % ethanol (1.00983.2500 Millipore), 230 µl  
989 Biomek Tips (B85903 Beckmann Coulter), Eppendorf 500 µl deep well plates

990 (30501101 Eppendorf), Waters Acquity UPLC 700  $\mu$ l plates (186005837 Waters  
991 GmbH) Sequencing grade modified Trypsin (V5117 Promega), Pierce Quantitative  
992 Fluorometric Peptide Assay (number 23290), formic acid (85178 Thermo Scientific),  
993 water (1.15333.2500 Merck), protease inhibitor cocktail complete mini (Roche  
994 04693124001), benzonase nuclease (Sigma Aldrich E1014-25KU)  
995 *Data pre-processing*; Four serum samples showed low quality and were removed.  
996 Peptides with excessive missing values (> 30% per group) were excluded from  
997 analysis. Batch correction was applied. The peptide matrix was filtered using factor  
998 “Proteotypic” keeping only peptides belonging to one protein group. To obtain a  
999 quantitative protein data matrix, the log<sub>2</sub>-intensities of peptides belonging to one  
1000 protein group were summarised by “maxLFQ” method (Cox et al., 2014) into protein  
1001 log intensity.  
1002 *Statistical analysis*; Statistical analysis of proteomics data was carried out using  
1003 internally developed R scripts. Linear modelling was based on the R package LIMMA  
1004 (Ritchie et al., 2015). Following model was applied to the sets of lung/serum samples  
1005 (log(p) is log<sub>2</sub> transformed expression of a protein):  $\log(p) \sim 0 + \text{Class}(\text{Day}) + \text{Gender}$   
1006 Here, categorical factor Class(Day) has 8 levels:  
1007 Infected(D02), Infected(D03), Infected(D05), Infected(D14),  
1008 Control(D02), Control(D03), Control(D05), Control(D14)  
1009 Categorical factor Gender has two levels: male, female.  
1010 The following contrasts were evaluated to trace time dependence of response to viral  
1011 infection (Note that Contrast5 addresses the average difference between infected and  
1012 recovered animals and Contrast6 addresses the difference between infected and  
1013 control animals on average):  
1014 Contrast1: Infected(D02) – Control(D02)  
1015 Contrast2: Infected(D03) – Control(D03)  
1016 Contrast3: Infected(D05) – Control(D05)  
1017 Contrast4: Infected(D14) – Control(D14)  
1018 Contrast5:  $[\text{Infected}(\text{D02}) + \text{Infected}(\text{D03}) + \text{Infected}(\text{D05})] / 3 - [\text{Control}(\text{D02}) +$   
1019  $\text{Control}(\text{D03}) + \text{Control}(\text{D05})] / 3 - [\text{Infected}(\text{D14}) - \text{Control}(\text{D14})]$   
1020 Contrast6:  $[\text{Infected}(\text{D02}) + \text{Infected}(\text{D03}) + \text{Infected}(\text{D05})] / 3 - [\text{Control}(\text{D02}) +$   
1021  $\text{Control}(\text{D03}) + \text{Control}(\text{D05})] / 3$   
1022 In serum set of samples there was only one control group at 3 dpi and it was used to  
1023 build contrasts replacing control groups at other dpi’s.

1024 For finding regulated features following criteria were applied:

1025 Significance level alpha was set to guarantee false discovery rate below 10% at the  
1026 response maximum (5 dpi) in both sample types. We found that alpha = 0.01 was  
1027 delivering regulated proteins with Benjamini–Hochberg FDR below 8% in lung tissue  
1028 and below 6% in serum and used it for feature selection.

1029 The log fold change criterion was applied to guarantee that the measured signal is  
1030 above the average noise level. As such we have taken mean residual standard  
1031 deviation of linear model:  $\log_2(T) = \text{mean residual SD of linear modelling}$  ( $T = 1.45$  in  
1032 lung and  $T=1.37$  in serum).

1033 *Functional analysis of proteomics data*; Functional analysis was carried out using  
1034 gprofiler2 R package (Kolberg and Raudvere, 2020). For selecting the most  
1035 (de)regulated GO terms we applied filter:  $2 \leq \text{term size} \leq 200$ . Analyses for each  
1036 Contrast 1 – 6 (Table 1) and then all in parallel were carried out with Benjamini–  
1037 Hochberg FDR threshold 0.2. Organism for search was specified as mauratus –  
1038 Mesocricetus auratus (Syrian hamster). Statistical domain scope was set to custom,  
1039 list of all identified proteins was provided as background.

1040

#### 1041 **Statistical Analyses of clinical data**

1042 Graph-Pad Prism v8 (GraphPad Software Inc., San Diego, CA, USA) software was  
1043 used for statistical analysis of clinical data. The statistical details of all analyzed  
1044 experiments are given in the respective figure legends.

1045

#### 1046 **Annotation of the *M. auratus* genome**

1047 The *M. auratus* genome (MesAur1.0) sequence and annotation (gtf file, version 99)  
1048 was downloaded from Ensembl. We noticed that 3'-UTRs in this annotation were  
1049 frequently too short to capture all transcriptome reads and particularly the 3' end reads  
1050 in single-cell RNA-sequencing, so we extended all 3'-UTRs for coding genes by 1,000  
1051 bp. The *Ifit2* gene was extended by 2,000 bp. For key genes analyzed in this  
1052 manuscript, we verified that this extension did not lead to overlaps with downstream  
1053 genes.

1054 The Ensemble annotation was extended by mapping ENSEMBL gene ids without  
1055 annotated gene names to entrez identifiers and to the homolog associated gene  
1056 names using biomaRt (Durinck et al., 2009). Wherever existing, we extracted the gene  
1057 name from the NCBI's All\_Mammalia.gene\_info (download from

1058 [ftp://ftp.ncbi.nlm.nih.gov/gene/DATA/GENE\\_INFO/Mammalia/](ftp://ftp.ncbi.nlm.nih.gov/gene/DATA/GENE_INFO/Mammalia/)) table, capturing 1067  
1059 gene names. Otherwise we used available homolog associated gene names yielding  
1060 1193 additional entries.

1061

### 1062 **Analysis of bulk RNA-sequencing data**

1063 Reads were aligned to the genome using hisat2 (Kim et al., 2019) and quantified using  
1064 quasR (Gaidatzis et al., 2015). We then performed gene set enrichment analysis with  
1065 tmod (Zyla et al., 2019) and Hallmark, Reactome and GO BP gene sets from MSigDB  
1066 v7.0 (Liberzon et al., 2015), ranking genes by the product of the sign of the log2 fold  
1067 change and log10 adjusted p-value and converting hamster gene names to human  
1068 using the biomaRt mouse-to-human mapping.

1069

### 1070 **Analysis of single-cell RNA-sequencing data**

1071 Data analysis was done in R (R Core Team, 2019), using Seurat (Butler et al., 2018)  
1072 and packages from tidyverse (Wickham et al., 2019) and glmer (Bates et al., 2015). All  
1073 used code with annotation is available through Github at [https://github.com/Berlin-](https://github.com/Berlin-Hamster-Single-Cell-Consortium)  
1074 [Hamster-Single-Cell-Consortium](https://github.com/Berlin-Hamster-Single-Cell-Consortium).

1075 Raw single-cell sequencing data was processed using CellRanger 3.1.0 (10x  
1076 Genomics) with standard parameters, based on a combined MesAur1.0/SARS-CoV-2  
1077 (GenBank entry MN908947) reference. Raw feature barcode matrices from the  
1078 CellRanger output were read into Seurat using the Read10X function and a Seurat  
1079 object created using the CreateSeuratObject function. Cells with more than 7 %  
1080 mitochondrial reads, based on the percentage feature expression of the mitochondrial  
1081 genes *Cox1*, *Cytb*, *Nd1*, *Nd2*, *Nd4*, *Nd5*, *Nd6* were excluded (reads from other  
1082 mitochondrial genes were not detected in the data). Furthermore, cells with less than  
1083 1000 (lung) or 500 (blood) detected genes were also excluded from downstream  
1084 analysis. Sample sets (all lung, or all blood, or blood/lung combined from the individual  
1085 time points) were then integrated using the SCTransform workflow, as illustrated on  
1086 the Seurat website (Stuart et al., 2019). Briefly, the Seurat object was split by the  
1087 hamster that the data points originated from and separately transformed using  
1088 SCTransform to normalise and scale the data. To prevent batch specific/animal  
1089 specific effects from obscuring results, these split objects were integrated using the  
1090 SelectIntegrationFeatures, PrepSCTIntegration, FindIntegrationAnchors and  
1091 IntegrateData functions in succession. PCA and UMAP dimensional reduction



1092 analyses respectively were performed on the integrated object, using 30 dimensions  
1093 for the UMAP as the SCT workflow reportedly shows more robust results with higher  
1094 dimensionality. Cells were subjected to Louvain clustering using the FindNeighbours  
1095 and FindClusters (with a resolution parameter of 0.8 for lung samples and 0.5 for the  
1096 blood samples) functions.

1097 To annotate cell types in the lung scRNAseq data, we used Seurat's TransferData  
1098 workflow (Stuart et al., 2019) and two different reference data sets: Tabula Muris  
1099 (Tabula Muris Consortium, 2018) and the Human Lung Cell Atlas (Travaglini et al.,  
1100 2020). Integration with mouse data was performed using matching gene names  
1101 between mouse and hamster, while gene names in the human data were converted to  
1102 mouse using biomaRt. We then used the predicted cell type of the majority of cells in  
1103 each cluster to guide cluster annotation, also taking into account cell marker genes  
1104 published in literature and cell marker databases (Franzen et al., 2019; Zhang et al.,  
1105 2019). The following populations were confirmed: Alveolar macrophages (*Siglec<sup>f</sup>*,  
1106 *Marco<sup>+</sup>*) (Han et al., 2018), interstitial macrophages (*C1qb<sup>+</sup>*) (Angelidis et al., 2019),  
1107 monocytic macrophages (*Ccr2<sup>+</sup>*, *Ccr5<sup>+</sup>*, *Arg1<sup>+</sup>*) (Lechner et al., 2017; Shaheen et al.,  
1108 2019), *Trem14<sup>+</sup>*-monocytes (*Trem14<sup>+</sup>*) (Briseno et al., 2016), neutrophils (*S100a8<sup>+</sup>*,  
1109 *Cxcr2<sup>+</sup>*, *Camp<sup>+</sup>*) (Schulte-Schrepping et al., 2020; Zhao et al., 2019), myeloid dendritic  
1110 cells (mDC) (*Flt3<sup>+</sup>*, *H2-Ab1<sup>hi</sup>*, *Irf8<sup>lo</sup>*, *Tcf4<sup>lo</sup>*) and plasmacytoid dendritic cells (pDC) (*Flt3<sup>+</sup>*,  
1111 *H2-Ab1<sup>hi</sup>*, *Irf8<sup>hi</sup>*, *Tcf4<sup>hi</sup>*) (Dutertre et al., 2019; Monaghan et al., 2019; Rodrigues et al.,  
1112 2018; Tabula Muris Consortium, 2018), T / NK / cells (*Cd3e<sup>+</sup>*, *Cd4<sup>+</sup>* or *Cd8a<sup>+</sup>*, *Gzma<sup>+</sup>*,  
1113 *Nkg7<sup>+</sup>*) (Raredon et al., 2019; Smith et al., 2020; Tabula Muris Consortium, 2018; Zhao  
1114 et al., 2019; Zhu et al., 2009), B cells (*Cd79b<sup>+</sup>*, *Ms4a1<sup>+</sup>*) (Cohen et al., 2018; Schulte-  
1115 Schrepping et al., 2020), alveolar epithelial cells type 1 (*Rtn2<sup>+</sup>*) (Angelidis et al., 2019),  
1116 endothelial cells (*Pecam1<sup>+</sup>*) (Han et al., 2018), ciliated epithelial cells (*Foxj1<sup>+</sup>*)  
1117 [30318149], alveolar epithelial cells type 2 (*Lamp3<sup>+</sup>*) (Cohen et al., 2018), smooth  
1118 muscle cells (*Tagln<sup>+</sup>*, *Acta2<sup>+</sup>*) (Angelidis et al., 2019; Tawfik et al., 2014), fibroblasts  
1119 (*Dcn<sup>+</sup>*) (Angelidis et al., 2019) as well as myofibroblasts (*Dcn<sup>+</sup>*, *Tagln<sup>hi</sup>*, *Acta2<sup>hi</sup>*).

1120 Analysis of single-cell sequencing data for blood samples was similar to that of lung  
1121 samples with minor exceptions. Following initial clustering, cluster marker genes were  
1122 identified using the FindAllMarkers function and clusters expressing high levels of  
1123 erythrocyte marker genes *Snca*, *Fam46c* and *Alas2* were identified as containing  
1124 erythrocyte contamination (Barbour et al., 2008; Chiabrando et al., 2014; Durussel et  
1125 al., 2016). Cells in these clusters, as well as a cluster of most likely dead cells, marked

1126 by the expression of mitochondrial genes, were removed and the data was re-  
1127 integrated using the workflow described above. Cell type annotations were assigned  
1128 to the identified cluster using the follow marker genes: Classical (inflammatory)  
1129 monocytes (*Ccr2*<sup>+</sup>, *Cx3cr1*<sup>lo</sup>, *Adgre1*<sup>+</sup>); non-classical (residential) monocytes (*Ccr2*<sup>-</sup>,  
1130 *Cx3cr1*<sup>hi</sup>, *Adgre1*<sup>+</sup>) (Burgess et al., 2019; Cochain et al., 2018; Ronning et al., 2019);  
1131 mature neutrophils (*Cxcr2*<sup>+</sup>, *S100a8*<sup>+</sup>, *Camp*<sup>lo</sup>, *Retn*<sup>lo</sup>, *Ltf*<sup>lo</sup>) and immature neutrophils  
1132 (*Cxcr2*<sup>+</sup>, *S100a8*<sup>+</sup>, *Camp*<sup>hi</sup>, *Retn*<sup>hi</sup>, *Ltf*<sup>hi</sup>) (Kim et al., 2017; Schulte-Schrepping et al.,  
1133 2020; Xie et al., 2020; Zhao et al., 2019); myeloid dendritic cells (*Flt3*<sup>+</sup>, *H2-Ab1*<sup>hi</sup>, *Irf8*<sup>lo</sup>,  
1134 *Tcf4*<sup>lo</sup>) and plasmacytoid dendritic cells (*Flt3*<sup>+</sup>, *H2-Ab1*<sup>hi</sup>, *Irf8*<sup>hi</sup>, *Tcf4*<sup>hi</sup>) (Dutertre et al.,  
1135 2019; Monaghan et al., 2019; Rodrigues et al., 2018; Tabula Muris Consortium, 2018);  
1136 T cells (*Cd3e*<sup>+</sup>, *Cd4*<sup>+</sup> or *Cd8a*<sup>+</sup>) and activated T cells (*Cd3e*<sup>+</sup>, *Cd4*<sup>+</sup> and/or *Cd8a*<sup>+</sup>,  
1137 *Gzma*<sup>+</sup>) (Raredon et al., 2019; Tabula Muris Consortium, 2018; Zhao et al., 2019; Zhu  
1138 et al., 2009), natural killer (NK) cells (*Cd3e*<sup>-</sup>, *Nkg7*<sup>+</sup>) (Smith et al., 2020; Zhao et al.,  
1139 2019); B cells (*Cd79a*<sup>+</sup>, *Ms4a1*<sup>+</sup>) (Cohen et al., 2018; Schulte-Schrepping et al., 2020;  
1140 Zuccolo et al., 2013) and platelets (*Gng11*<sup>+</sup>, *Ppbp*<sup>+</sup>) (El-Gedaily et al., 2004; Schoggins,  
1141 2019). While cluster 17 showed no expression of *Ccr2*, the levels of *Adgre1* and *Cd14*  
1142 were considerable and it was considered as comprising classical monocytes for the  
1143 purposes of this study (Sampath et al., 2018).

1144 Previously published scRNAseq data of bronchoalveolar lavages originating from  
1145 COVID-19 patients data from Liao et al. (Liao et al., 2020) was processed using the  
1146 same (Seurat) workflow in R. We kept cells with less than 10 % mitochondrial reads,  
1147 less than 50000 UMIs and less than 6000 genes and used IntegrateData to combine  
1148 different samples. We then again used the Human Lung Cell Atlas reference and  
1149 TransferData to annotate clusters.

1150 Differential cell density was calculated as in (Praktiknjo et al., 2020) by plotting the log2  
1151 ratio of two separate 2D kernel density estimators interpolated on the UMAP  
1152 coordinates of each cell.

1153

1154

1155

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