

1 **Single-cell analysis of severe COVID-19 patients reveals a**  
2 **monocyte-driven inflammatory storm attenuated by Tocilizumab**

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39 **ABSTRACT**

40 Despite the current devastation of the COVID-19 pandemic, several recent studies  
41 have suggested that the immunosuppressive drug Tocilizumab can powerfully treating  
42 inflammatory responses that occur in this disease. Here, by employing single-cell  
43 analysis of the immune cell composition of severe-stage COVID-19 patients and these  
44 same patients in post Tocilizumab-treatment remission, we have identified a  
45 monocyte subpopulation specific to severe disease that contributes to inflammatory  
46 storms in COVID-19 patients. Although Tocilizumab treatment attenuated the strong  
47 inflammatory immune response, we found that immune cells including plasma B cells  
48 and CD8<sup>+</sup> T cells still exhibited an intense humoral and cell-mediated anti-virus  
49 immune response in COVID-19 patients after Tocilizumab treatment. Thus, in  
50 addition to providing a rich, very high-resolution data resource about the immune cell  
51 distribution at multiple stages of the COVID-19 disease, our work both helps explain  
52 Tocilizumab's powerful therapeutic effects and defines a large number of potential  
53 new drug targets related to inflammatory storms.

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60 **Keywords** : Coronavirus disease 2019 (COVID-19); Severe acute respiratory  
61 syndrome coronavirus 2 (SARS-CoV-2); Tocilizumab; Single-cell RNA sequencing  
62 (scRNA-seq); Inflammatory storm; Monocyte

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## 64 **Introduction**

65 As of May 1, 2020, the WHO has reported 224,172 deaths out of 3,175,207  
66 confirmed cases for infection by severe acute respiratory syndrome coronavirus 2  
67 (SARS-CoV-2), and these numbers are still growing rapidly<sup>1</sup>. Approximately 14% of  
68 patients with COVID-19 experienced severe disease, and 5% were critically ill,  
69 among which there was a 49% fatality rate<sup>2</sup>; it has been speculated that this high  
70 mortality is related to abnormal immune system activation<sup>3, 4, 5</sup>. Hence, there is an  
71 urgent need for researchers to understand how the immune system responds to  
72 SARS-CoV-2 viral infection at the severe stage, which may highlight potential  
73 effective treatment strategies.

74 Studies have shown that the inflammatory storm caused by excessive immune  
75 responses was strongly associated with mortality in COVID-19<sup>6, 7</sup>. Plasma  
76 concentrations of a series of inflammatory cytokines, such as  
77 granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-6<sup>4</sup>,  
78 tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), IL-2, 7, 10, and granulocyte colony-stimulating  
79 factor (G-CSF)<sup>8</sup> were increased after SARS-CoV-2 infections. Further investigation  
80 demonstrated that peripheral inflammatory monocytes and pathogenic T cells may  
81 incite cytokine storm in severe COVID-19 patients<sup>4, 6</sup>. Tocilizumab, an  
82 immunosuppressive drug that targets IL-6 receptors, has been used to treat severe  
83 COVID-19 patients<sup>9, 10</sup>, as it is effective for treating severe and even life-threatening  
84 cytokine-release syndrome<sup>11, 12</sup>. After receiving Tocilizumab, the body temperature of  
85 the patients returned to normal after 24 hours, and Tocilizumab was shown to  
86 significantly decrease the concentration of oxygen inhalation by COVID-19 patients  
87 by the 5<sup>th</sup> day of treatment<sup>13</sup>. Despite the apparent efficacy of Tocilizumab for treating  
88 severe COVID-19 patients, the lack of single-cell level analyses has prevented any  
89 deepening of our understanding about how Tocilizumab impacts the typical  
90 COVID-19 induced activation of an inflammatory storm.

91 In the present study, we profiled the single-cell transcriptomes of 13,239  
92 peripheral blood mononuclear cells (PBMCs) isolated at the severe and remission

93 disease stages of two severe COVID-19 patients treated with Tocilizumab. We  
94 identified a severe-stage-specific monocyte subpopulation that clearly contributes to  
95 the patients' inflammatory storms. Comparison between the severe and remission  
96 disease stages at the single-cell level revealed that Tocilizumab treatment weakens the  
97 excessively activated inflammatory immune response and also showed that immune  
98 cells, including plasma B cells and CD8<sup>+</sup>T cells, still exhibit boosted humoral and  
99 cell-mediated anti-virus immune responses in post-treatment COVID-19 patients. Our  
100 study thus provides a rich, high-resolution data set about the immune context at  
101 multiple stages of COVID-19, and helps to explain how a promising candidate drug  
102 both alters immune cell populations and reduces patient mortality.

103

## 104 **Results**

### 105 **An atlas of peripheral immune cells in severe COVID-19 patients**

106 We obtained 5 peripheral blood samples from 2 severe COVID-19 patients at 3  
107 time points including the severe and remission stages during Tocilizumab treatment  
108 (Fig. 1a). Specifically, we collected blood samples at day 1— within 12 hours of  
109 Tocilizumab administration—and at day 5 for both patients; note that we also  
110 obtained a blood sample from patient P2 on day 7 of Tocilizumab treatment because  
111 P2 still had a positive result for a SARS-Cov-2 nucleic acid test of a throat swab  
112 specimen on day 5. At day 1, the patients both had a decreased number of  
113 lymphocytes compared to healthy reference interval, as well as increased percentages  
114 of neutrophils and elevated concentrations of C-reaction protein and increased  
115 expression of IL-6 (Supplementary Table 1). Since the clinical symptoms of most of  
116 the severe COVID-19 patients, including both patients in this study, were remarkably  
117 improved by 5 days of Tocilizumab treatment<sup>13</sup> (Supplementary Table 1), we defined  
118 the blood draws from day 5 as the “remission stage”. For patient P2, we took another  
119 blood draw at day 7, when his nucleic acid test turned negative (Fig. 1a). It is worth  
120 noting that patient P1 was discharged on day 8, and patient P2 on day10, and these

121 discharges were both at 3 days after a nucleic acid test of a throat swab specimen was  
122 negative.

123 We isolated the PBMCs from the COVID-19 patients' blood samples and  
124 subjected them to single-cell mRNA sequencing (scRNA-seq) using the 10X platform  
125 (Fig. 1a). After rigorous quality control definition (Supplementary Fig. 1a-d,  
126 Supplementary Table 2), low quality cells were filtered; we also removed cell  
127 doublets using Scrublet<sup>14</sup>. Correlation of the gene expression for the samples from  
128 either patient emphasized the excellent reproducibility between the technical and  
129 biological replicates of our dataset (Supplementary Fig. 1e-f). After quality control  
130 (QC) and doublet removal, our dataset comprised a total of 13,239 high-quality  
131 transcriptomes for single PBMCs.

132 Due to the similarities between the single-cell transcriptomes of most of the  
133 identified cell subsets at the severe and remission disease stages, we initially  
134 combined the samples from both patients from day 1 as the "severe stage" and  
135 combined the samples from day 5 (and day 7 for P2) as the "remission stage"; note  
136 that we also conducted separate analyses for each patient, which yielded similar data  
137 trends (Supplementary Fig. 2a, b). In total, the combined analyses of all the single-cell  
138 transcriptomes for the COVID-19 patients included 4,344 cells from the severe  
139 disease stage and 8,895 were from the remission disease stage.

140 To investigate heterogeneity among the PBMCs for the COVID-19 patients  
141 compared to healthy controls, we applied Seurat<sup>15</sup> (version 3.1.4) to integrate our  
142 COVID-19 single-cell transcriptomes with the published single-cell profiles of  
143 healthy PBMCs from the 10X official website<sup>16</sup>, enabling an analysis with a total of  
144 68,190 cells (See **Methods**). We then normalized and clustered the gene expression  
145 matrix; this identified 18 unique cell subsets, which were visualized via uniform  
146 manifold approximation and projection (UMAP) (Fig. 1b-d). Cell lineages, including  
147 monocytes, CD4<sup>+</sup> and CD8<sup>+</sup> T,  $\gamma\delta$ T, natural killer (NK), B, plasma B and myeloid  
148 dendritic cells (mDC), plasmacytoid dendritic cells (pDC), platelets, and CD34<sup>+</sup>

149 progenitor cells were identified based on the expression of known marker genes (Fig.  
150 1e). This analysis represents a delineation of the landscape of circulating immune  
151 cells for severe COVID-19 patients.

152 We also used another integration method, Harmony<sup>17</sup>, to help assess the accuracy  
153 of the cell clustering results from Seurat<sup>15</sup> (version 3.1.4) and again visualized the  
154 results in UMAP (Supplementary Fig. 3a). We found strong correlations for the  
155 identified cell subsets and the detected gene expression patterns between the cell  
156 clusters with the two integration methods (Supplementary Fig. 3b, c), supporting the  
157 robustness of our cell clustering results.

158 We next explored the distribution of immune cells from the severe and remission  
159 stage COVID-19 patients, as well as in healthy control individuals (Supplementary  
160 Fig. 4a). We observed that a number of subpopulations, such as pDCs (cluster 15),  
161 mDCs (cluster 10), and most monocytes (clusters 2 and 13) were present in remission  
162 stage COVID-19 patients and in healthy controls but not in severe COVID-19 patients  
163 (Supplementary Fig. 4b), indicating that Tocilizumab treatment gradually restores a  
164 normal distribution of these cell types in circulating blood. Some cell subsets such as  
165 NK cells (cluster 7) and CD4<sup>+</sup> T cells (cluster 1 and 4) were quite heterogeneous  
166 between the two COVID-19 patients, so we did not examine these cell types further.  
167 These analyses revealed the conspicuous presence of four cell populations that were  
168 uniquely present in the COVID-19 patients (albeit to differencing extents in the severe  
169 vs. remission disease stages), including a monocyte subpopulation (cluster 9), plasma  
170 B cells (cluster 11), effector CD8<sup>+</sup> T cells (cluster 6), and proliferative MKI67<sup>+</sup>CD8<sup>+</sup>  
171 T cells (cluster 12) patients (Supplementary Fig. 4c). Given our study's aim of  
172 characterizing the COVID-19-specific and Tocilizumab-sensitive immune cell  
173 populations of COVID-19 patients, the majority of our subsequent detailed analyses  
174 focused on these four cell populations.

175

176 **A monocyte subpopulation contributes the inflammatory storm in severe stage**

## 177 COVID-19 patients

178 Monocytes have been reported to play a vital role in CAR-T induced  
179 cytokine-release syndrome<sup>18</sup> and in SARS-CoV-2 infection triggered inflammatory  
180 storms<sup>4</sup>, so we explored the features and functions of the aforementioned monocyte  
181 subpopulation that we detected in our single-cell analysis of the two COVID-19  
182 patients. We detected 1,677 monocytes in patients, with 916 from the severe disease  
183 stage and 761 from the remission stage; we examined these alongside the data for  
184 9,517 monocytes from health controls. The UMAP plot displayed two main clouds of  
185 monocytes that were clearly segregated (Fig. 2a). One monocyte subpopulation  
186 (cluster 9) consisted of 98.3% of all monocytes at severe stage, while this ratio was  
187 only 12.1% at remission stage and 0% in healthy controls (Fig. 2b), so we initially  
188 assessed these severe-stage-specific monocytes.

189 Transcriptional differences among monocytes subtypes was detected based on a  
190 pairwise comparison of the gene expression in the severe and remission stages and in  
191 respective comparisons against healthy control individuals. A large number of  
192 differentially expressed genes (DEGs) with reported inflammation-related functions  
193 were observed in the severe-stage-specific monocytes, including the previously  
194 reported cytokine-storm-related genes such as *TNF*<sup>8</sup>, *IL10*<sup>8</sup>, *CCL3*<sup>8</sup>, and *IL6*<sup>4</sup>;  
195 inflammatory related chemokine genes *CCL4*, *CCL20*, *CXCL2*, *CXCL3*, *CCL3L1*,  
196 *CCL4L2*, *CXCL8* and *CXCL9*; and inflammasome activation associated genes *NLRP3*  
197 and *IL1B* in the severe-stage-specific monocytes (Fig. 2c, fold change > 2,  $P < 10^{-3}$ ,  
198 Wilcoxon rank-sum test; Fig. 2d; and Supplementary Table 3). Collectively, the large  
199 number of DEGs with reported inflammation-related functions support the idea that  
200 the severe-stage-specific monocyte subpopulation we detected in our single-cell  
201 COVID-19 patient data may strongly support development of inflammatory responses  
202 in severe COVID-19 patients.

203 A GO analysis indicated enrichment of genes with annotations related to  
204 “regulation of acute inflammatory response” , “regulation of leukocyte activation”,

205 “cell chemotaxis” and “cellular response to chemokine” in severe-stage COVID-19  
206 patients compared to remission-stage patients and healthy controls (Fig. 2e, f,  $P <$   
207  $10^{-117}$ , Wilcoxon rank-sum test; Supplementary Fig. 5; and Supplementary Table 4, 5),  
208 suggesting that the inflammatory storm caused by this monocyte subpopulation is  
209 suppressed by Tocilizumab treatment.

210 Next, we explored transcription factors (TFs) in monocytes which may be  
211 involved in the promoting of the inflammatory storm. We used SCENIC<sup>19</sup> and  
212 predicted 9 TFs that may regulate genes that were up-regulated in  
213 severe-stage-specific monocytes (Fig. 2g). We then constructed a gene regulatory  
214 network among the SCENIC predicted TFs and a set of inflammation-relevant genes  
215 that were collected from the literatures<sup>20, 21</sup>. We found that 3 of the SCENIC  
216 predicted TFs, namely *ATF3*, *NFIL3*, and *HIVEP2*, may have the capacity to regulate  
217 the detected inflammation-relevant genes (Supplementary Fig. 6). Additionally, we  
218 found that the expression of *ATF3*, *NFIL3*, and *HIVEP2* and their motif enrichment  
219 which was predicted by the expressing of their potential target genes were enhanced  
220 in the severe-stage-specific monocyte subpopulation (Fig. 2h), further supporting that  
221 these 3 TFs may regulate the observed inflammatory storm in monocytes.

222 Recent studies have shown that over 20% of the severe COVID-19 patients had  
223 symptoms of severe septic shock, which affects several organ systems and contributes  
224 to liver injury<sup>22</sup>, acute kidney failure<sup>23</sup>, and abnormal heart damage<sup>24</sup>. We therefore  
225 checked whether this severe-stage-specific monocyte subpopulation is unique to  
226 COVID-19. We downloaded scRNA-seq datasets from patients with sepsis at a mild  
227 stage (Int-URO) and patients with sepsis at a severe stage (ICU-SEP), as well as  
228 critically ill patients without sepsis (ICU-NoSEP) and healthy controls (Control)<sup>25</sup>.  
229 We then integrated these data sets with our COVID-19 patients’ single-cell data using  
230 Seurat<sup>15</sup> (version 3.1.4), which revealed a total of 10 monocyte cell clusters  
231 (Supplementary Fig. 7a, b). Interestingly, the cells from the severe stage COVID-19  
232 patients clearly overlapped with only one of the integrated monocyte clusters (cluster



233 VI) (Supplementary Fig. 7c), suggesting that the severe-stage-specific monocyte  
234 population might be unique to COVID-19.

235

### 236 **A monocyte-centric cytokine/receptor interaction network in severe-stage** 237 **COVID-19 patients**

238 Given that monocytes in the severe stage may be involved in the regulation of a  
239 variety of immune cell types, we used the accumulated ligand/receptor interaction  
240 database<sup>26</sup> CellPhoneDB ([www.cellphonedb.org](http://www.cellphonedb.org)) to identify alterations of molecular  
241 interactions between monocytes and all of the immune cell subsets we identified in  
242 our single-cell analysis (Supplementary Table 6). We found 15 cytokine/receptors  
243 pairs whose interactions were significantly boosted in severe-stage COVID-19  
244 patients as compared to remission stage patients and healthy controls (Fig. 3a). It is  
245 notable that the expression of multiple inflammatory-storm-related  
246 cytokines/receptors was significantly increased in severe stage COVID-19 patients  
247 (Fig. 3b), which seems plausible that monocytes may have a substantially increased  
248 propensity for interaction with other immune cells in blood vessels. Our comparison  
249 between severe stage and remission stage patients also suggested obvious attenuation  
250 of increased cytokine/receptor interaction activity among the immune cells of  
251 remission COVID-19 patients (Fig. 3b). While clearly preliminary, our data support a  
252 role for Tocilizumab in reducing monocyte receptor compositions that have been  
253 previously implicated in the induction of inflammatory storms.

254 Consistent with a previous reported that the inflammatory monocytes released  
255 IL-6 play an vital role in inciting inflammatory storm in severe COVID-19 patients<sup>4</sup>,  
256 we found monocytes were predicted to communicate with CD4<sup>+</sup> T cells and plasma B  
257 cells at severe stage COVID-19 patients through the cytokine/receptor pairs of  
258 IL-6/IL-6R. We also detected that the severe-stage-specific monocytes featured  
259 elevated expression of other cytokine/receptor pairs that may contribute to a broad  
260 spectrum of immune cell communications, such as TNF- $\alpha$  and its receptors, through

261 which monocytes may interact with CD4<sup>+</sup> T, CD8<sup>+</sup> T and B cells. Similarly, the  
262 severe-stage monocytes had elevated levels of IL-1 $\beta$  and its receptor, suggesting  
263 potentially functional interaction of these monocytes with CD8<sup>+</sup> T cells. Chemokines  
264 such as CCL4L2, CCL3, and CCL4 and their respective receptors were also found to  
265 be enriched in severe stage monocytes, indicating the potential of targeting these  
266 cytokines and/or their receptors as possible drug targets for treating severe-stage  
267 COVID-19 patients. Indeed, it is notable that inhibitors targeting some of these  
268 cytokine/receptor pairs are currently undergoing anti-COVID-19 clinical trials in  
269 multiple places around the world (Supplementary Table 7). Collectively, these  
270 findings help illustrate the possible molecular basis of cell-cell interactions at the  
271 peripheral blood of COVID-19 patients, leading to a better understanding of the  
272 mechanisms of inflammatory storm of the disease.

273

#### 274 **Boosted humoral and cell-mediated immunity in severe COVID-19 patients**

275 Studies of avian H7N9 disease have revealed that viral infection can elicit robust,  
276 multi-factorial immune responses<sup>27, 28</sup>, and a very recent study reported effective  
277 immune responses from a non-severe COVID-19 patient<sup>29</sup>. However, it is not clear  
278 whether the anti-virus immune responses are affected by Tocilizumab treatment. We  
279 assessed the anti-virus immune responses—both humoral and cell-mediated immune  
280 responses—of severe-stage COVID-19 patients as compared with both remission  
281 stage patients and healthy controls. As expected for un-infected controls, there were  
282 hardly any plasma B cells in healthy individuals (Fig. 4a). In contrast, there were  
283 many plasma B cells in both the severe and remission stage COVID-19 patients (Fig.  
284 4a, b), suggesting that SARS-CoV-2 infection may elicit the anti-virus humoral  
285 immune responses, which are not affected by the Tocilizumab treatment.

286 CD8<sup>+</sup> T cells are function in cell-mediated immunity against viral infections by  
287 killing infected cells and secreting proinflammatory cytokines<sup>30</sup>. Our single-cell  
288 analysis detected a total of 13,602 CD8<sup>+</sup> T cells. Clustering of these cells revealed 3

289 subtypes: naïve CD8<sup>+</sup> T cells (cluster 3), effector CD8<sup>+</sup> T cells (cluster 6), and a  
290 subset of CD8<sup>+</sup> T cells with obvious expression of known proliferation markers  
291 (cluster 12) (Fig. 4c, d). The CD8<sup>+</sup> T cells of the severe patients were primarily of the  
292 effector CD8<sup>+</sup> T cell cluster (Fig. 4c, d). We then conducted pairwise comparisons to  
293 identify DEGs in the effector CD8<sup>+</sup> T cells among the severe and remission stage  
294 patients and in healthy controls (Fig. 4e, Supplementary Table 8). A GO analysis  
295 indicated that DEGs in severe stage effector CD8<sup>+</sup> T cells exhibited enrichment for  
296 “positive regulation of cell activation” (Fig. 4f,  $P < 10^{-10}$ ; hypergeometric test;  
297 Supplementary Table 9). Conversely, DEGs of the CD8<sup>+</sup> T cells from severe and  
298 remission stage COVID-19 patients (i.e., vs. healthy controls) were enriched for  
299 functional annotations relating to “cell chemotaxis” and “regulation of cell killing”  
300 (Fig. 4g,  $P < 10^{-6}$ ; hypergeometric test; Supplementary Table 9). We also detected  
301 significant elevated expression of the 306 and 94 genes involved in these GO terms  
302 (Fig. 4h, i,  $P < 10^{-32}$ , Wilcoxon rank-sum test; Supplementary Table 10). Together,  
303 these results indicate that SARS-CoV-2 infection elicits robust adaptive immune  
304 responses and suggest that Tocilizumab treatment further promotes such responses.

305 To gather additional empirical support from COVID-19 patients, we downloaded  
306 the bulk RNA-seq data of PBMCs from 3 severe COVID-19 patients and 3 healthy  
307 controls<sup>31</sup>, and applied AutoGeneS<sup>32</sup> to deconvolute the composition of cell clusters  
308 based on the signature genes identified in our single-cell analysis. Our results  
309 indicated that there were significantly more severe-stage-specific monocytes (cluster  
310 9), plasma B cells (cluster 11), and proliferating CD8<sup>+</sup> T cells (cluster 12) in severe  
311 COVID-19 patients compared with healthy controls (Supplementary Fig. 8a-c,  $P <$   
312 0.05, Student’s t-test), findings consistent with our main conclusions.

313

## 314 Discussion

315 The immune system exerts essential functions in fighting off viral infections<sup>33</sup>,  
316 <sup>34</sup>. Recent studies have indicated that monocytes can exacerbate and even be a

317 primary factor in the mortality of COVID-19 by contributing to inflammatory storms<sup>4</sup>.  
318 In the present study, we used single-cell mRNA sequencing and discovered a specific  
319 monocyte subpopulation that may lead to the inflammatory storm in severe-stage  
320 COVID-19 patients. By analyzing the monocyte-centric cytokine/receptor  
321 complements and predicting interaction networks, we uncovered a  
322 severe-stage-specific landscape of peripheral immune cell communication which may  
323 drive the inflammatory storm in COVID-19 patients. Our identification of this  
324 monocyte subpopulation and these cytokine-storm-related cytokine/receptor provides  
325 mechanistic insights about the immunopathogenesis of COVID-19 and suggests the  
326 potential of these cytokine/receptor molecules as candidate drug targets for treating  
327 the disease.

328 There have long been questions about whether treatment with the  
329 immunosuppressive agent Tocilizumab may affect the body's antiviral responses<sup>35, 36</sup>.  
330 Our single-cell profiles illustrated a sustained humoral and cell-mediated anti-virus  
331 immune response in severe and remission stage COVID-19 patients. For example,  
332 Tocilizumab treatment of severe-stage COVID-19 patients retained a high proportion  
333 of plasma B cells with antibody-secreting functions and we found that the cytotoxicity  
334 and cytokine production of effector CD8<sup>+</sup> T cells remained stable upon Tocilizumab  
335 treatment.

336 Our work represents a collaborative clinical/basic effort that does provide an  
337 unprecedented empirical window for studying single-cell resolution profiles from  
338 severe COVID-19 patients. Deconvolution analysis of published bulk RNA-seq data<sup>31</sup>  
339 from 3 additional severe COVID-19 patients and healthy controls helps support our  
340 conclusions on the enrichment of severe-stage-specific monocytes and plasma B cells  
341 in severe-stage COVID-19 patients. We further integrated additional single-cell  
342 datasets from sepsis patients and found the severe-stage-specific monocytes we  
343 observed are unique to severe COVID-19. Based on the incorporation of diverse  
344 additional data, our study and empirical data provide actionable insights that will help

345 the multiple research communities who are still fighting against the virus, including  
346 clinical physicians, drug developers, and basic scientists.

347

## 348 **Methods**

349

### 350 **Human samples**

351 Peripheral blood samples were obtained from two severe COVID-19 patients. The  
352 patient severity was defined by the "Diagnosis and Treatment of COVID-19 (Trial  
353 Version 6)" which was released by The General Office of the National Health  
354 Commission and the Office of the National Administration of Traditional Chinese  
355 Medicine. Patient P1 was defined as a severe patient for his peripheral capillary  
356 oxygen saturation (SPO<sub>2</sub>) <93% without nasal catheter for oxygen. Patient P2 was  
357 defined as critical ill for respiratory failure, multiple organ dysfunction (MOD) and  
358 SPO<sub>2</sub> <93 without nasal catheter for oxygen. Two peripheral blood samples were  
359 obtained for patient P1 on day 1 and day 5, and three peripheral blood samples were  
360 obtained for patient P2 on day 1, day 5 and day 7. For both patients, peripheral blood  
361 samples of day 1 were collected within 12 hours of Tocilizumab administration, when  
362 the patients were still at severe stage. Our decision to obtain blood draws from the  
363 two patients at day 5 were guided by information from the authors of the recent study  
364 published in *PNAS*<sup>13</sup>, which guided our decision to consider day 5 of Tocilizumab  
365 treatment as a "remission stage". For patient P2, we observed that his SARS-CoV-2  
366 nucleic acid test of a throat swab specimen was still positive at day 5, so we took  
367 another blood draw at day 7 for P2, at point by which a throat swab specimen nucleic  
368 acid test was negative. All samples were collected from the First Affiliated Hospital of  
369 University of Science and Technology of China. Before blood draws, informed  
370 consent was obtained from each patient. Ethical approvals were obtained from the  
371 ethics committee of the First Affiliated Hospital of the University of Science and  
372 Technology of China (No. 2020-XG(H)-020).

373

374 **Cell Isolation**

375 We collected 2ml peripheral blood each time from the COVID-19 patients. Peripheral  
376 blood mononuclear cells (PBMC) were freshly isolated from the whole blood by  
377 using a density gradient centrifugation using Ficoll-Paque and cryopreserved for  
378 subsequent generation of single-cell RNA library.

379

380 **Single-cell RNA-seq**

381 We generated single-cell transcriptome library following the instructions of single-cell  
382 3' solution v2 reagent kit (10x Genomics). Briefly, after thawing, washing and  
383 counting cells, we loaded the cell suspensions onto a chromium single-cell chip along  
384 with partitioning oil, reverse transcription (RT) reagents, and a collection of gel beads  
385 that contain 3,500,000 unique 10X Barcodes. After generation of single-cell gel  
386 bead-in-emulsions (GEMs), RT was performed using a C1000 Touch™ Thermal  
387 Cycler (Bio-Rad). The amplified cDNA was purified with SPRIselect beads  
388 (Beckman Coulter). Single-cell libraries were then constructed following  
389 fragmentation, end repair, polyA-tailing, adaptor ligation, and size selection based on  
390 the manufacturer's standard parameters. Each sequencing library was generated with  
391 unique sample index. Libraries were sequenced on the Illumina NovaSeq 6000  
392 system.

393

394 **Single-cell RNA-seq data processing**

395 The raw sequencing data of patients and health donors were processed using Cell  
396 Ranger (version 3.1.0) against the GRCh38 human reference genome with default  
397 parameters, and data from different patients and disease stages were combined by the  
398 Cell Ranger 'aggr' function. We are uploading the scRNA-seq data of PBMCs from  
399 the 2 severe COVID-19 patients to the Genome Sequence Archive at BIG Data Center  
400 and the accession number will be available upon request. We also used the  
401 scRNA-seq data of PBMCs from 2 healthy donors, which can be downloaded from

402 the 10X genomics official website. Firstly, we filtered low quality cells using Seurat<sup>15</sup>  
403 (version 3.1.4). For cells from COVID-19 patients (P1 and P2), we retained cells with  
404 detected gene numbers between 500 and 6,000 and mitochondrial unique molecular  
405 identifiers (UMIs) less than 10%. For cells from healthy donors, we retained cells  
406 with detected gene numbers between 300 and 5,000 and mitochondrial UMIs less than  
407 10%. Subsequently we adopted Scrublet<sup>14</sup> (version 0.2.1) to eliminate doublets in the  
408 PBMCs from the COVID-19 patients and healthy donors. We used the default  
409 parameters for Scrublet (i.e. “min\_gene\_variability\_pctl=85, n\_prin\_comps=30,  
410 threshold=0.25”) and detected 50 doublets from the patients and 997 doublets from  
411 the healthy donors. After removing the doublets, we normalized gene counts for each  
412 cell using the “NormalizeData” function of Seurat with default parameters.

413 In downstream data processing, we used canonical correlation analysis and the  
414 top 40 canonical components to find the “anchor” cells between patients and healthy  
415 controls. We then used the “IntegrateData” function in Seurat to integrate cells from  
416 COVID-19 patients and healthy controls. We clustered all the cells based on the  
417 integrated gene expression matrix using Seurat with parameter “resolution=0.3” and  
418 generated 20 clusters. To display cells in a 2-dimensional space, we ran the principal  
419 component analysis on the integrated dataset and adopted the first 50 principal  
420 components (PCs) for the uniform manifold approximation and projection (UMAP)  
421 analysis.

422 In the integration of cells from COVID-19 and sepsis patients using Seurat, we  
423 applied the same functions and parameters as described above. We adopted Seurat  
424 to cluster the integrated gene expression matrix (with resolution = 0.3) and identified  
425 monocyte clusters based on the expression of known marker genes *CD14* and *CD68*.  
426 We then extracted all the monocytes from the integrated dataset and re-clustered them.  
427 Finally, we generated 10 cell clusters.

428

429 **Integration of cells from patients and healthy controls by Harmony**

430 To verify the reliability of the integration results obtained using Seurat (version 3.1.4),  
431 we also applied Harmony<sup>17</sup> to integrate the PBMCs from COVID-19 patients and  
432 healthy controls. We used the same gene expression matrix and applied the same  
433 parameters as Seurat, and adopted the first 50 PCs to perform the data integration by  
434 calling the “RunHarmony” function in Harmony. We then used the same clustering  
435 algorithm as Seurat to cluster cells and generated 23 clusters (“resolution=0.5”) based  
436 on the integration results obtained from Harmony. Jaccard index was applied to gauge  
437 the similarity between the cell clusters, with cell integration processed by Seurat or  
438 by Harmony. The Jaccard similarity between each pair of Seurat cluster (cluster *i*) and  
439 Harmony cluster (cluster *j*) were defined as follows

$$\text{Jaccard similarity} = \frac{(\text{cells in cluster } i) \cap (\text{cells in cluster } j)}{(\text{cells in cluster } i) \cup (\text{cells in cluster } j)}$$

440

#### 441 **Differential expression analysis**

442 To search for the differentially expressed genes (DEGs), we first assign the negative  
443 elements in the integrated expression matrix to zero. We used Wilcoxon rank-sum test  
444 to search for the DEGs between each pair of the 3 stages of cells (i.e. severe stage,  
445 remission stage and healthy control). We applied multiple thresholds to screen for  
446 DEGs, including mean fold change >2, *P* value <0.001, and were detected in >10% of  
447 cells in at least one stage.

448 We defined stage A specific-DEGs as the intersections between the DEGs in stage  
449 A versus stage B and the DEGs in stage A versus stage C. We defined stage A and B  
450 common-DEGs as the intersections of the DEGs in stage A versus stage C and the  
451 DEGs in the stage B versus stage C, minus the DEGs between stage A and B. In this  
452 way, we obtained the specific-DEGs for each stage, and the common-DEGs for each  
453 pair of the 3 stages. We then uploaded these DEG groups to the Metascape<sup>37</sup> website  
454 (<https://metascape.org/gp/index.html#/main/step1>), and used the default parameters to  
455 perform Gene Ontology (GO) analysis for each stage.

456



#### 457 **Motif enrichment and regulatory network**

458 We adopted SCENIC<sup>19</sup> (version 1.1.2) and RcisTarget database to build the gene  
459 regulatory network of CD14<sup>+</sup> monocytes. Since the number of CD14<sup>+</sup> monocytes  
460 from healthy control (N = 9,618) was more than those from the severe and remission  
461 stages (N = 1,607), to balance their contributions in the motif analysis, we randomly  
462 sampled 2,000 CD14<sup>+</sup> monocytes from the healthy control for calculation. We  
463 selected 13,344 genes that were detected in at least 100 monocytes or included in the  
464 DEGs of the 3 stages as the input features for SCENIC. With default parameters,  
465 SCENIC generated the enrichment scores of 427 motifs. We used the student's t-test  
466 to calculate the *P* values of these motifs between severe stage and healthy control, and  
467 selected severe-specific enriched motifs with fold change >1.5 and *P* value < 10<sup>-100</sup>.

468 We then applied the enrichment scores of the severe-specific enriched motifs and  
469 the expression of their targeted genes to Cytoscape<sup>38</sup> to construct a connection map  
470 for the gene regulatory network, as shown in Supplementary Fig. 6. The thickness of  
471 line connecting TFs and target genes represented the weight of regulatory link  
472 predicted by SCENIC.

473

#### 474 **Cytokine/receptor interaction analysis**

475 To identify potential cellular communications between monocytes and other cell types  
476 (CD4<sup>+</sup> T, CD8<sup>+</sup> T, B, plasma B and NK cells), we applied the CellphoneDB<sup>26</sup>  
477 algorithm to the scRNA-seq profiles from the the severe and remission stages, and in  
478 healthy control individuals. CellphoneDB evaluated the impact of a ligand/receptor  
479 interactions based on the ligand expression in one cell type and its corresponding  
480 receptor expression in another cell type. We focused on the enriched cytokine/receptor  
481 interactions in severe-stage COVID-19 patients, and selected the cytokine/receptor  
482 interactions with more significant (*P* value < 0.05) cell-cell interaction pairs in the  
483 severe stage than that in the remission and healthy stages. We also included  
484 cytokine/receptor pairs which were highly expressed in severe stage.

485

#### 486 **Deconvolution of cell clusters from bulk RNA-seq data**

487 We applied AutoGeneS<sup>32</sup> to deconvolute the composition of cell clusters based on the  
488 signature genes identified in our single-cell analysis. Specifically, we first obtained a  
489 gene-by-cluster expression matrix from our normalized single-cell profile, where the  
490 matrix elements were the average expression of each gene in each cell cluster. We  
491 then defined the top 5000 most variable genes between cell cluster and the 2000  
492 DEGs used for cell clustering as the “VarGenes”, and extracted the  
493 VarGenes-by-cluster expression matrix as the feature gene expression profile for  
494 AutoGeneS. We set the input parameters as “model = 'nusvr', ngen = 1000, seed = 0,  
495 nfeatures = 1500” to deconvolute the cell composition in AutoGeneS.

496

#### 497 **Statistical analysis**

498 The two-tailed Wilcoxon rank-sum test (also called the Mann-Whitney U test) was  
499 used to search for the DEGs and to compare the expression differences of a gene set  
500 of interest between two conditions. In CellphoneDB, a permutation test was used to  
501 evaluate the significance of a cytokine/receptor pair. Metascape utilizes the  
502 hypergeometric test and Benjamini-Hochberg *P* value correction algorithm to identify  
503 the ontology terms that contain a statistically greater number of genes in common  
504 than expected. We used the Student’s t-test to evaluate the significance of the  
505 expression differences of the TFs (and their target genes) between samples from  
506 severe stage patients and healthy controls.

507

#### 508 **Data Availability**

509 The scRNA-seq data of PBMCs from the 2 severe COVID-19 patients can be  
510 obtained from the Genome Sequence Archive (GSA) at BIG Data Center and the  
511 accession number is CRA002509. We also used published datasets as controls or  
512 comparable data, including (1) the scRNA-seq data of PBMCs from 2 healthy donors

513 downloaded from the 10X genomics official website  
514 [https://support.10xgenomics.com/single-cell-gene-expression/datasets/3.1.0/5k\\_pbmc](https://support.10xgenomics.com/single-cell-gene-expression/datasets/3.1.0/5k_pbmc)  
515 [\\_NGSC3\\_aggr](#), (2) the scRNA-seq data of PBMCs from 22 sepsis patients and 19  
516 related controls<sup>25</sup> that is available on Institute Single Cell Portal  
517 ([https://singlecell.broadinstitute.org/single\\_cell](https://singlecell.broadinstitute.org/single_cell)) with accession number SCP548, (3)  
518 the bulk RNA-seq data of PBMCs from 3 COVID-19 patients and 3 related controls<sup>31</sup>  
519 downloaded from the GSA at BIG Data Center with accession number CRA002390.

520

### 521 **Code Availability**

522 Analysis scripts are accessible from github:  
523 <https://github.com/QuKunLab/COVID-19>.

524

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658

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667

## 668 **Author Contributions**

669 K.Q. conceived and supervised the project; K.Q., C.G. and J.L. designed the  
670 experiments; C.G. and J.L. performed the experiments and conducted all the sample  
671 preparation for next-generation sequencing with the help from H.M. and T.C.; B.L.  
672 performed the data analysis with the help from P.C., Q.Y., L.Z., L.J., C.J., Q.L., D.Z.,  
673 W.Z., Y.L., K.L., X.G. and J.F.; T.C., X.W., L.L., J.W. and X.M. provided  
674 COVID-19 blood samples and clinical information; J.W. contributed to the revision of  
675 the manuscript; K.Q., C.G., J.L. and B.L. wrote the manuscript with the help of B.F.,  
676 H.W. and all the other authors.

677

### 678 **Competing interests**

679 Jingwen Fang is the chief executive officer of HanGen Biotech.

680

### 681 **Figure Legends**

682

#### 683 **Figure 1 | An atlas of peripheral immune cells in severe COVID-19 patients. a,**

684 Flowchart depicting the overall design of the study. Blood draws from patient P1 were  
685 performed at 2 time points (day 1 and day 5), and from P2 at 3 time points (day 1, day  
686 5 and day 7). P1 at day 1 and P2 at day 1 and day 5 were positive for the nucleic acid  
687 test of a throat swab specimen. P1 at day 5 and P2 at day 7 were negative for the  
688 nucleic acid test of a throat swab specimen. Patients at day 1 were at the severe stage,  
689 were in the remission stage at day 5 (P1 and P2); the day 7 blood draw for P2 (still  
690 remission stage) was based on a positive nucleic acid test at day 5. Note that samples  
691 on day 1 were collected within 12 hours of Tocilizumab treatment. **b-d**, UMAP  
692 representations of integrated single-cell transcriptomes of 69,237 PBMCs, with  
693 13,239 cells from our COVID-19 patients and 55,998 were from 10X official  
694 website<sup>16</sup>. Cells are color-coded by clusters (**b**), disease state (**c**), and sample origin  
695 (**d**). Dotted circles represented cell types with > 5% proportion of PBMCs in (**b**), and  
696 clusters significantly enriched in patients versus controls in (**c**, **d**). Mono, monocyte;  
697 NK, natural killer cells; mDC, myeloid dendritic cells; pDC, plasmacytoid dendritic

698 cells. **e**, Violin plots of selected marker genes (upper row) for multiple cell  
699 subpopulations. The left column presents the cell subtypes as identified based on  
700 combinations of marker genes.

701

702 **Figure 2 | A unique monocyte subpopulation contributes to the inflammatory**

703 **storm in severe-stage COVID-19 patients. a**, UMAP plot showing 3 clusters of

704 CD14<sup>+</sup> monocytes and 1 cluster of CD16<sup>+</sup> monocyte. Cells are color-coded by

705 clusters. **b**, Bar plot of the proportion of monocytes in cluster 9 at the severe and

706 remission stages, and in healthy control individuals. **c**, Heatmap of differentially

707 expressed genes (DEGs) in monocytes from pairwise comparison between the severe

708 stage patients, remission stage patients, and healthy control individuals. **d**, UMAP

709 plots showing the expression of selected cytokines in all monocyte clusters. **e,f**, Box

710 plot of the average expression of genes involved in the signaling pathway "

711 Regulation of acute inflammatory response " and "Cell chemotaxis" in monocytes

712 from the severe and remission stages, and in healthy control individuals. Center line,

713 median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range;

714 points, outliers; \*\*\*\* represents  $P$  value  $< 10^{-100}$ , Wilcoxon rank-sum test. **g**, Heatmap

715 of the area under the curve (AUC) scores of expression regulation by transcription

716 factors (TFs), as estimated using SCENIC. Shown are the top-ranked TFs having the

717 highest difference in expression regulation estimates in monocytes from severe-stage

718 COVID-19 patients. **h**, UMAP plots showing the expression of the *ATF3*, *NFIL3*, and

719 *HIVEP2* genes in monocytes (top) and the AUC of the estimated regulon activity of

720 the corresponding TFs, predicting the degree of expression regulation of their target

721 genes (bottom).

722

723 **Figure 3 | The monocyte-centric molecular interactions of peripheral immune**

724 **cells in severe-stage COVID-19 patients. a**, Dot plot of predicted interactions

725 between monocytes and the indicated immune cell types in the severe and remission



726 stages, and in healthy control individuals. *P* values were measured by circle sizes,  
727 scale on right (permutation test). The means of the average expression level of  
728 interacting molecule 1 in cluster 1 and interacting molecule 2 in cluster 2 are  
729 indicated by color. Assays were carried out at the mRNA level, but are extrapolated to  
730 protein interactions. **b**, Summary illustration depicting the potential cytokine/receptor  
731 interactions between monocytes and other types of peripheral immune cells in the  
732 severe and remission stages, and in healthy control individuals. Bolder lines indicate  
733 predicted enriched cytokine/receptor interactions between monocytes and other  
734 immune cell types.

735

736 **Figure 4 | Enhanced humoral and cell-mediated immunity in severe COVID-19**  
737 **patients. a**, UMAP representations of B and plasma B cell clusters from the severe  
738 and remission stages, and in healthy control individuals. **b**, Bar plot of the proportions  
739 of plasma B cells in the B cell lineage from the severe and remission stages, and in  
740 healthy control individuals. **c**, UMAP representations of CD8<sup>+</sup> T cell subtypes (left)  
741 and the distribution of cells from the severe and remission stages, and in healthy  
742 control individuals in each subtype (right). **d**, Dot plot of the expression of the *CCR7*,  
743 *PRDM1*, and *MKI67* genes in all CD8<sup>+</sup> T cell subtypes. **e**, Heatmap of differentially  
744 expressed genes in effector CD8<sup>+</sup> T cells from pairwise comparisons between the  
745 severe stage patients, remission stage patients, and healthy control individuals. **f, g**,  
746 Bar plots of GO terms enriched in effector CD8<sup>+</sup> T cells from the severe stage (**f**) or  
747 the severe and remission stages (**g**). **h, i**, Box plots of the average expression of genes  
748 “cell chemotaxis” and “regulation of cell killing” in the effector CD8<sup>+</sup> T cells from  
749 severe stage, remission stage, and healthy controls. Center line, median; box limits,  
750 upper and lower quartiles; whiskers, 1.5x interquartile range; points, outliers; \*\*\*\*  
751 represents *P* value < 10<sup>-30</sup>. Wilcoxon rank-sum test.

752

753 **Supplementary Figure Legends and Supplementary Tables**

754

755 **Supplementary Figure 1 | Quality control of single-cell data for PBMC samples**  
756 **from severe COVID-19 patients. a**, Summary of captured cells, median genes per  
757 cell, median UMIs per cell, and the number of cells that passed quality control (QC)  
758 in distinct batches of single-cell data from severe COVID-19 patients. **b-d**, Box plots  
759 showing the gene number (**b**), UMI number (**c**), and percentage of mitochondrial  
760 RNA (**d**) in distinct batches of single-cell data from severe COVID-19 patients. **e, f**,  
761 Aggregated scRNA-seq one-to-one reproducibility plots for technical replicates (**e**)  
762 and biological replicates (**f**). The correlation (**R**) represents the Pearson correlation  
763 across all genes. Box-whisker plot; the lower whisker is the lowest value greater than  
764 the 25% quantile minus 1.5 times the interquartile range (IQR), the lower hinge is the  
765 25% quantile, the middle is the median, the upper hinge is the 75% quantile and the  
766 upper whisker is the largest value less than the 75% quantile plus 1.5 times the IQR.

767

768 **Supplementary Figure 2 | Single-cell transcriptomes of PBMCs from patient P1**  
769 **or P2 at each time point. a**, UMAP plot showing single-cell transcriptomes from  
770 patient P1 and P2 at day 1. **b**, UMAP plot showing single-cell transcriptomes from  
771 patient P1 at day 5 and P2 at day 5 and day 7.

772

773 **Supplementary Figure 3 | Single-cell profiling of peripheral immune cells in**  
774 **severe COVID-19 integrated with healthy controls using Harmony. a**, UMAP  
775 representations of single-cell transcriptomes of 69,237 PBMCs integrated by  
776 Harmony. Cells are color-coded by clusters and disease state (see legend for key).  
777 Mono, monocyte; NK, natural killer cells; mDC, myeloid dendritic cells; pDC,  
778 plasmacytoid dendritic cells. **b**, Violin plots of selected marker genes (upper row) for  
779 multiple cell subpopulations. The left column presents the cell subtypes as identified  
780 based on combinations of marker genes. **c**, Jaccard similarities between the cell  
781 clusters with the integration processed by Seurat (version 3.1.4) and with the

782 integration processed by Harmony.

783

784 **Supplementary Figure 4 | The composition of cell clusters identified in the**  
785 **integrated single-cell transcriptomes of PBMCs from the severe and remission**  
786 **stages, and in healthy control individuals. a**, Bar chart showing the percentage of  
787 cell clusters in the severe and remission stages, and in healthy controls. **b**, Pie chart  
788 showing the proportion of cells from each disease state in selected cell clusters  
789 (cluster 2, 13, 10, 15), which were present in remission-stage patients and in healthy  
790 controls, but not in severe-stage patients. **c**, Pie chart showing the proportion of cells  
791 from each disease state in selected cell clusters (cluster 9, 6, 12, 11), which were  
792 present in severe and remission stages but not in healthy controls.

793

794 **Supplementary Figure 5 | GO terms enriched among DEGs highly expressed in**  
795 **monocyte at the severe stage or at severe and remission stages. a, b**, Bar plots of  
796 enriched GO terms of genes highly expressed in monocytes at the severe stage (**a**) or  
797 at the severe and remission stages (**b**).

798

799 **Supplementary Figure 6 | Severe-stage-specific monocyte regulatory network**  
800 **predicted by SCENIC**. Transcription factors are shown as rectangles; their target  
801 genes are shown as circles. Student's t-test.

802

803 **Supplementary Figure 7 | Integrated single-cell transcriptome analysis from**  
804 **patients with sepsis and our COVID-19 patients. a, b**, UMAP representations of  
805 integrated single-cell transcriptomes from patients with sepsis at mild stage (Int-URO,  
806  $n = 7$ )<sup>25</sup>, patients with sepsis at severe stage (ICU-SEP,  $n = 8$ )<sup>25</sup>, critically ill patients  
807 without sepsis (ICU-NoSEP,  $n = 7$ )<sup>25</sup>, healthy controls from outside our study  
808 (Control,  $n = 19$ )<sup>25</sup>, and our COVID-19 patients (Severe COVID-19 and remission  
809 COVID-19). Cells are color-coded by clusters (**a**), disease states (**b**). **c**, Bar chart

810 showing the proportion of cell clusters in (a) in each disease state.

811

812 **Supplementary Figure 8 | The composition of cell clusters identified in our**

813 **single-cell analysis in a bulk RNA-seq from three severe COVID-19 patients and**

814 **healthy controls. a,** Bar chart showing an estimation of the composition of each cell

815 cluster of PBMCs deconvoluted from bulk RNA-seq data from three COVID-19

816 patients and healthy controls<sup>31</sup>. **b, c,** Bar chart showing the percentage of

817 severe-stage-specific monocytes (cluster 9, **b**) and plasma B cells (cluster 11, **c**) in

818 COVID-19 patients and healthy controls, deconvoluted from bulk RNA-seq. Student's

819 t-test.

820

821 **Supplementary Table 1 | Baseline characteristics and laboratory findings for the**

822 **two COVID-19 patients in this study.**

823 **Supplementary Table 2 | Sequencing data quality.**

824 **Supplementary Table 3 | DEGs of different disease stages of the monocytes.**

825 **Supplementary Table 4 | GO terms enriched among DEGs in different disease**

826 **stages of the monocytes.**

827 **Supplementary Table 5 | Sets of genes entailed in the enriched GO terms from**

828 **Figure 2e and 2f.**

829 **Supplementary Table 6 | Interactions of cytokines and receptors in different**

830 **disease stages, predicted using CellphoneDB.**

831 **Supplementary Table 7 | Drugs targeting cytokines or cytokine receptors.**

832 **Supplementary Table 8 | DEGs of different disease stages of effector CD8<sup>+</sup> T**

833 **cells.**

834 **Supplementary Table 9 | GO terms enriched among DEGs in different disease**

835 **stages of the effector CD8<sup>+</sup> T cells.**

836 **Supplementary Table 10 | Set of genes entailed in the enriched GO terms from**

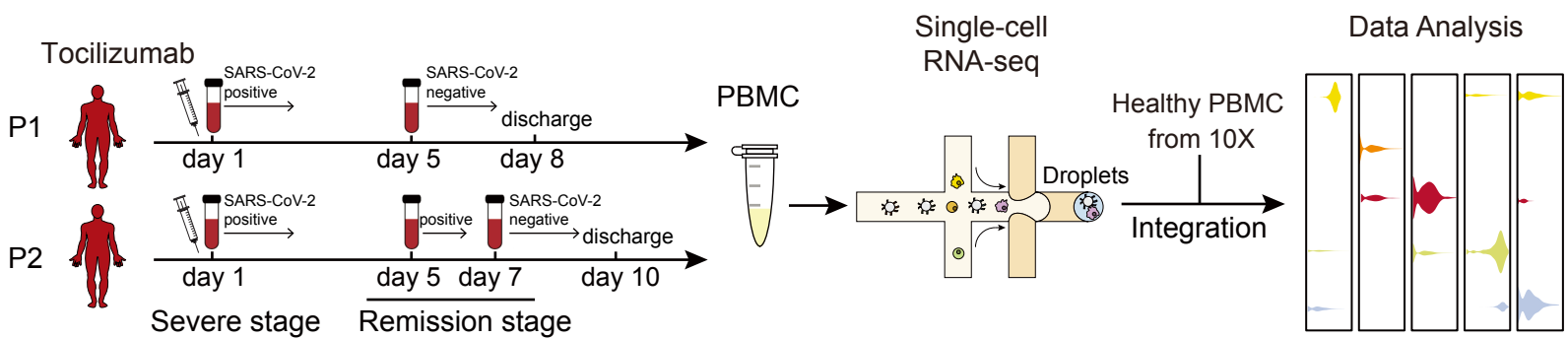
837 **Figure 4h and 4i.**

838

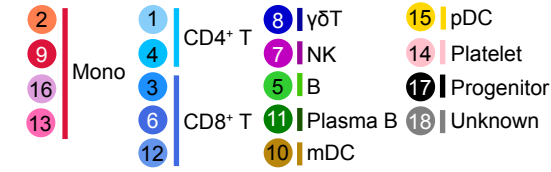
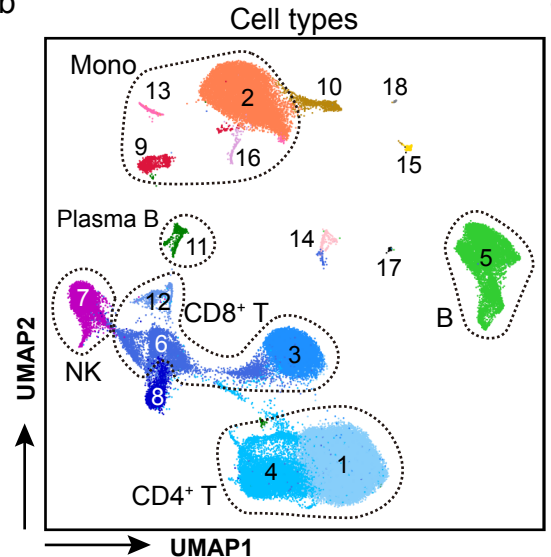
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Figure 1

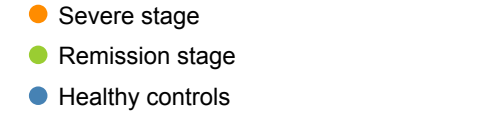
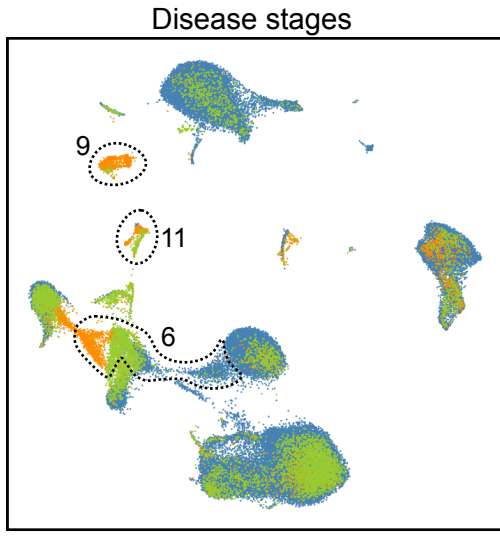
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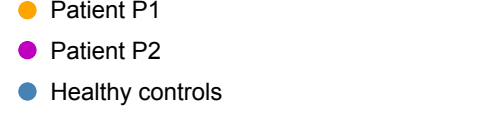
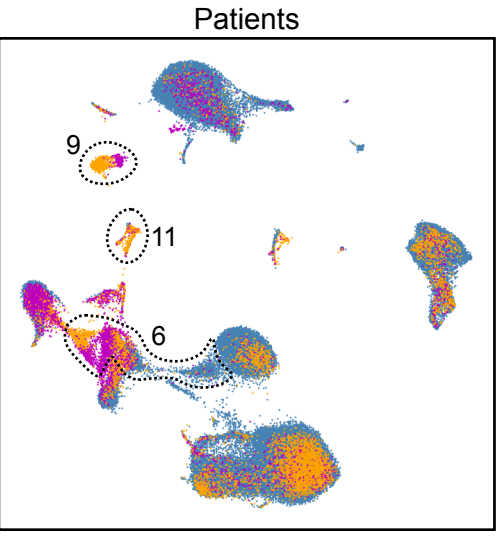
b



c



d



e

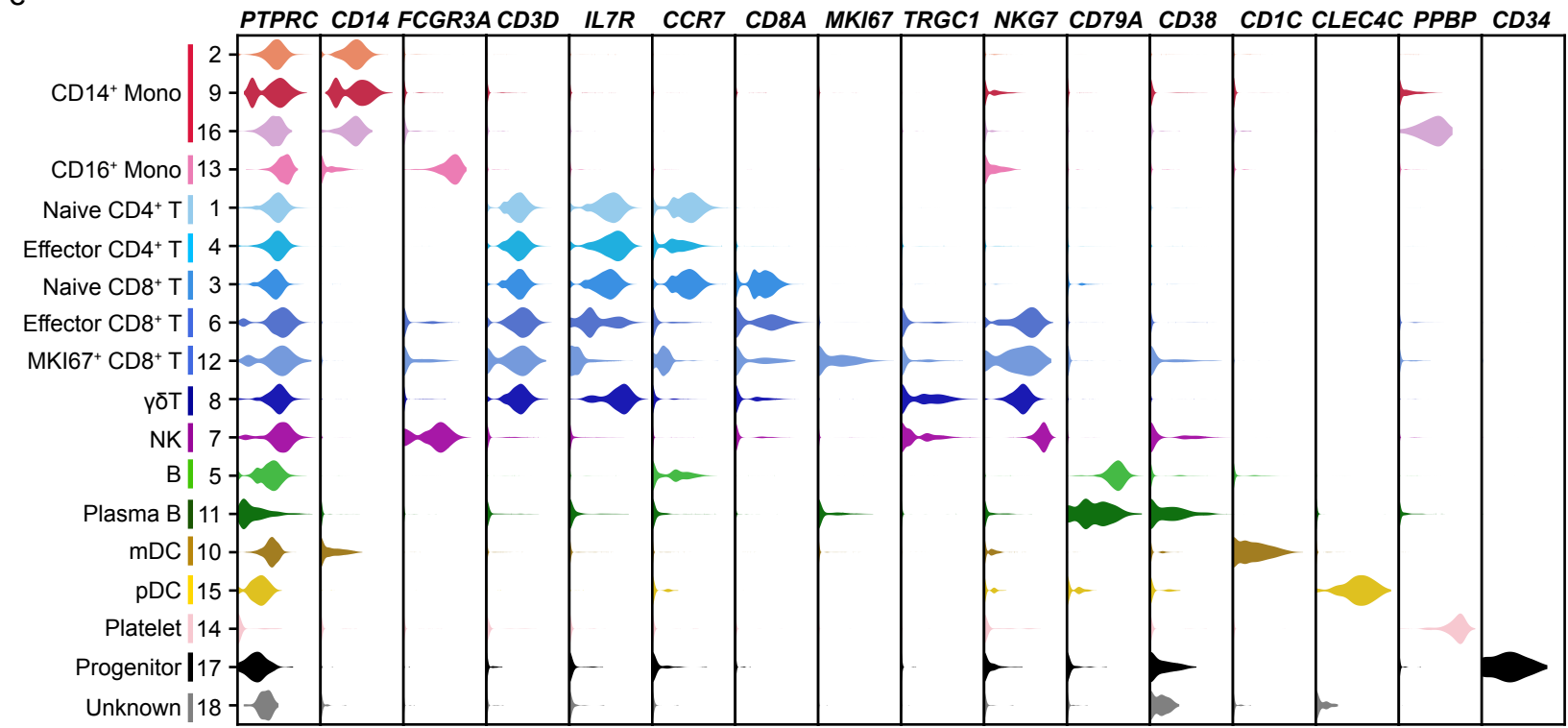


Figure2

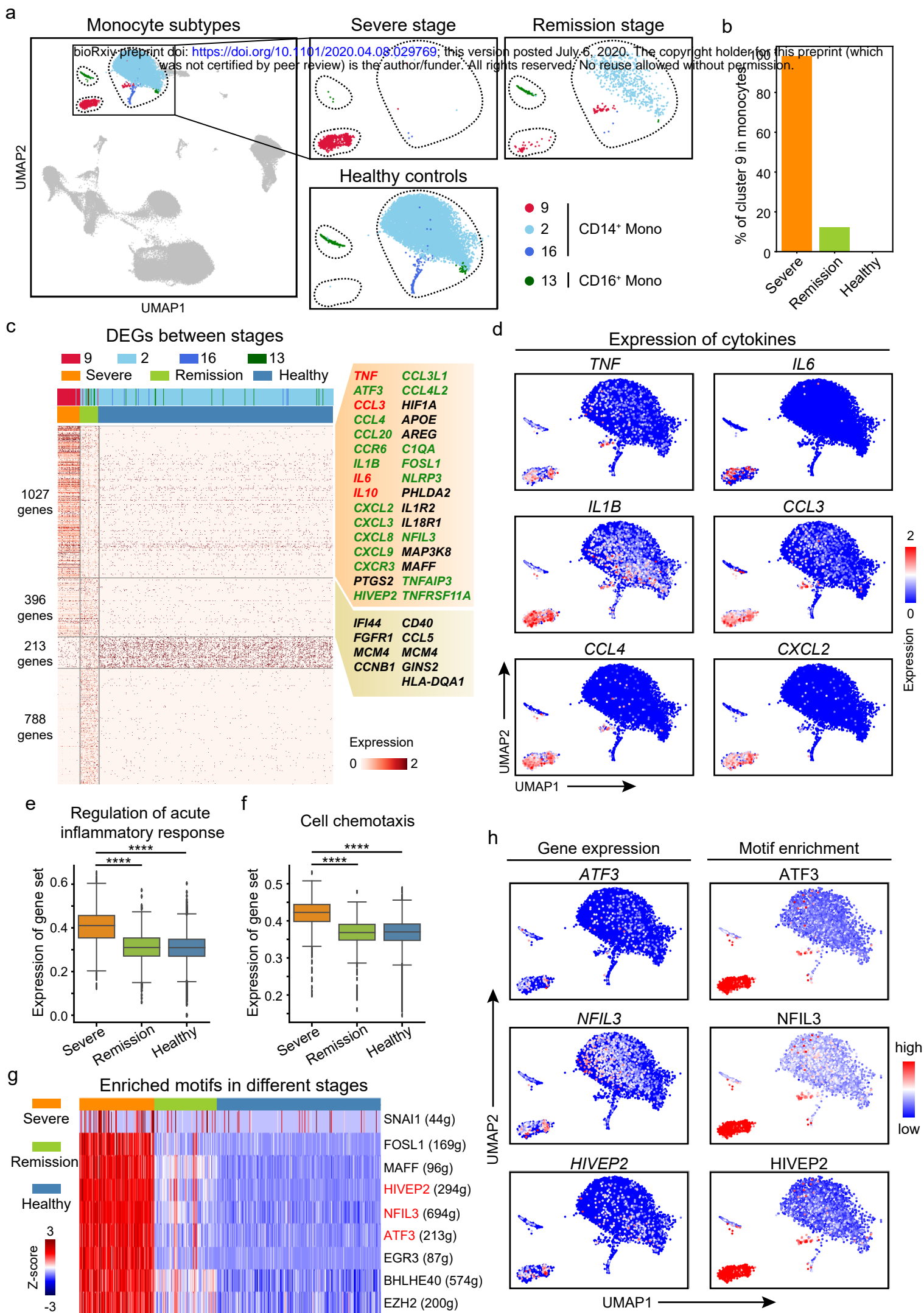
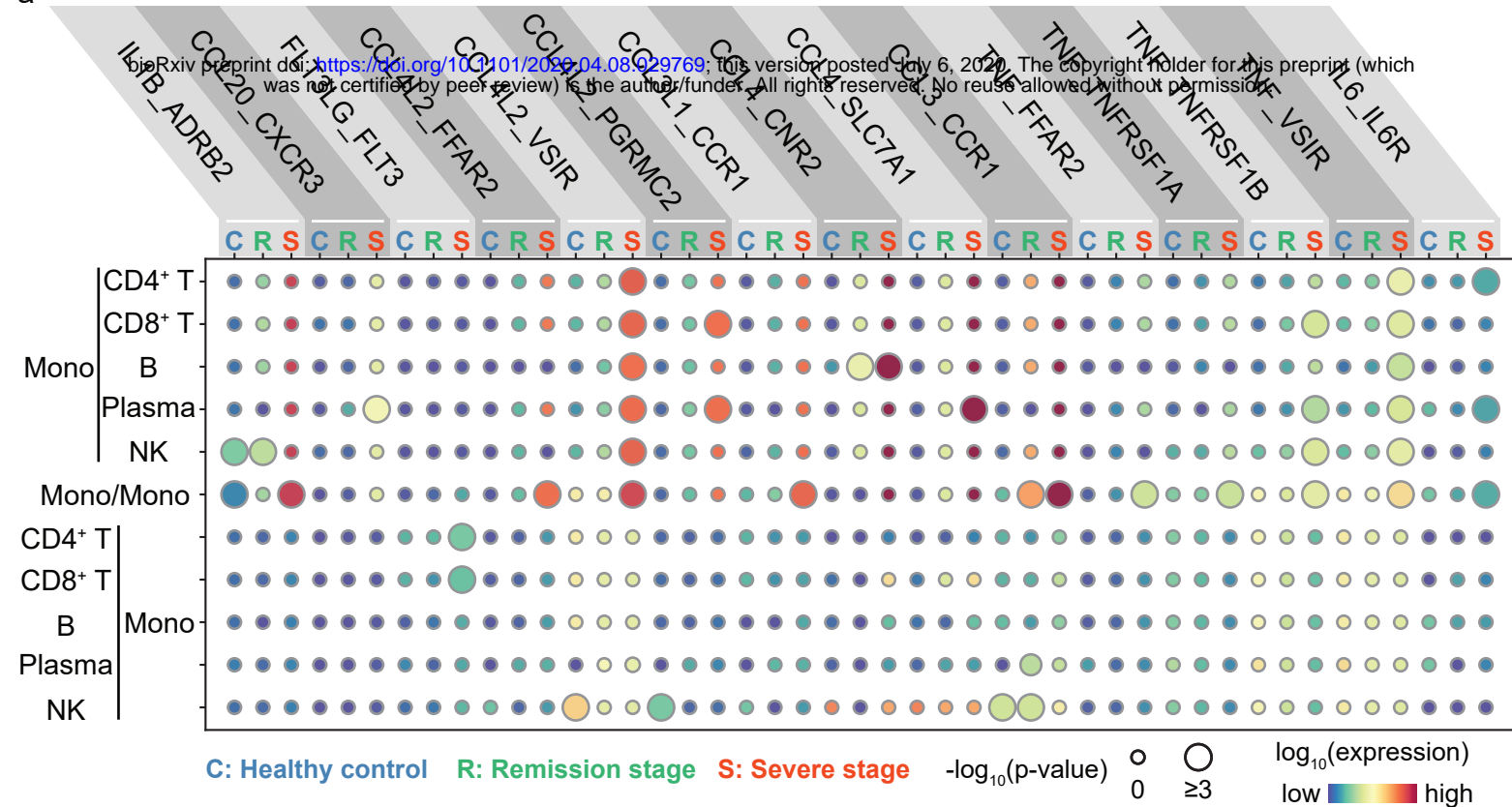


Figure 3

a



b

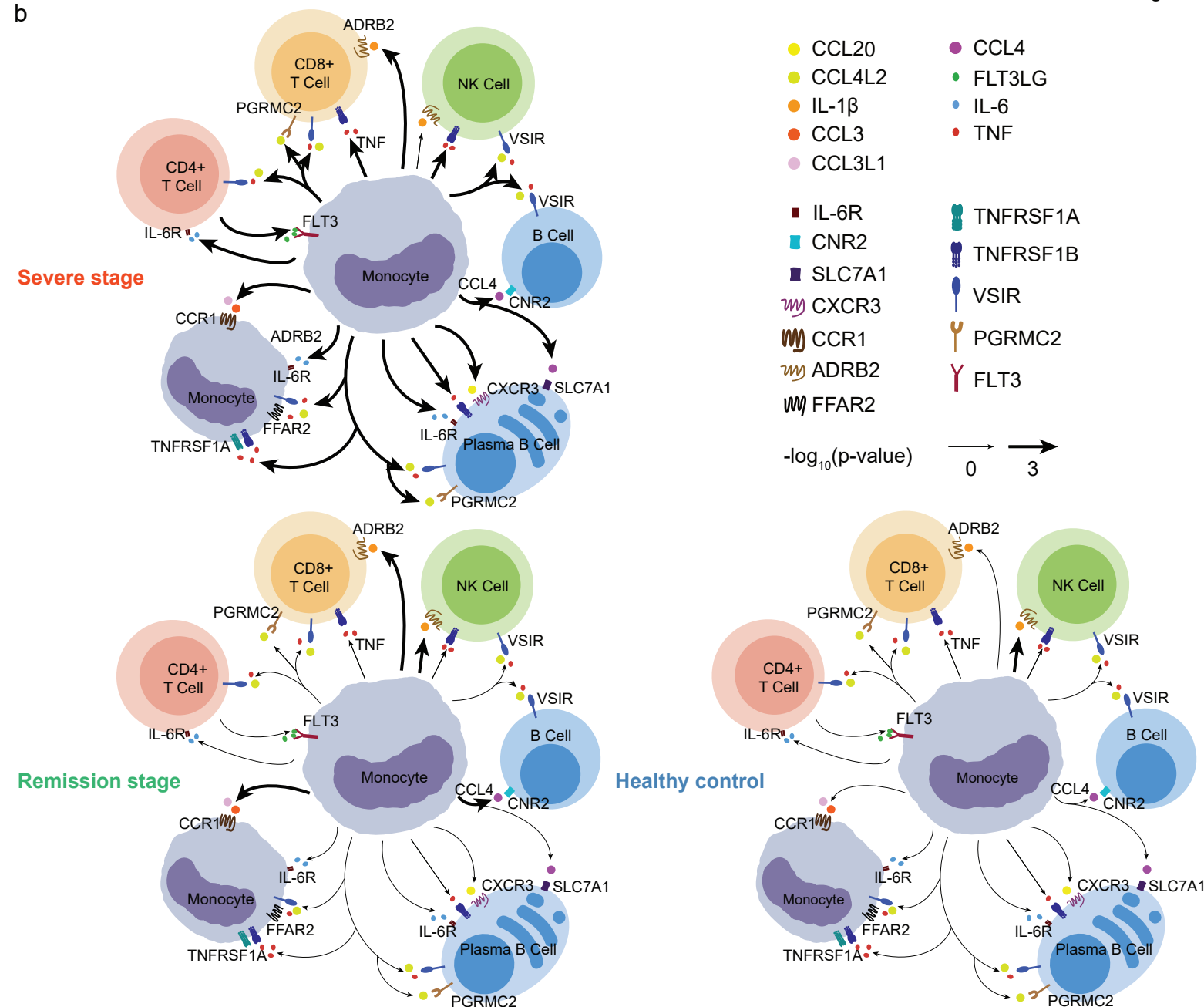
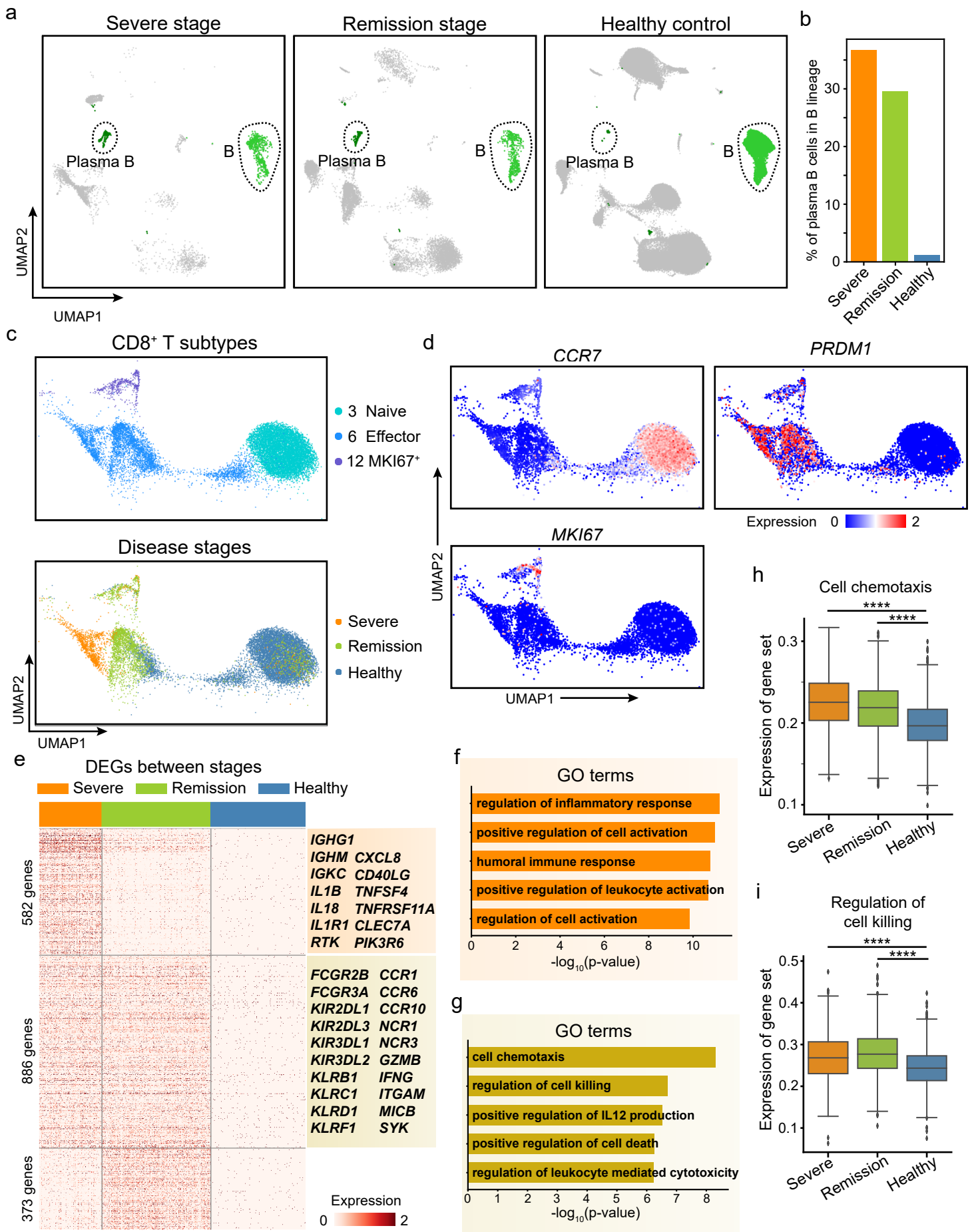




Figure 4

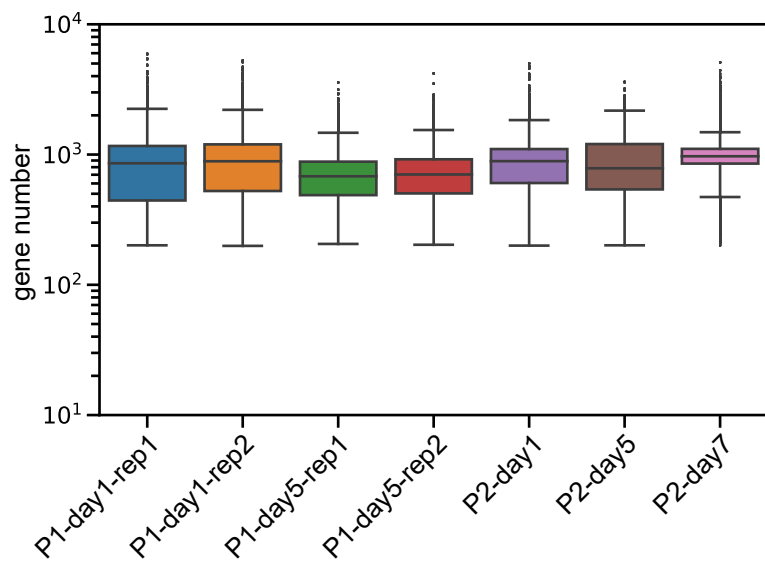


# Supplementary Fig. 1

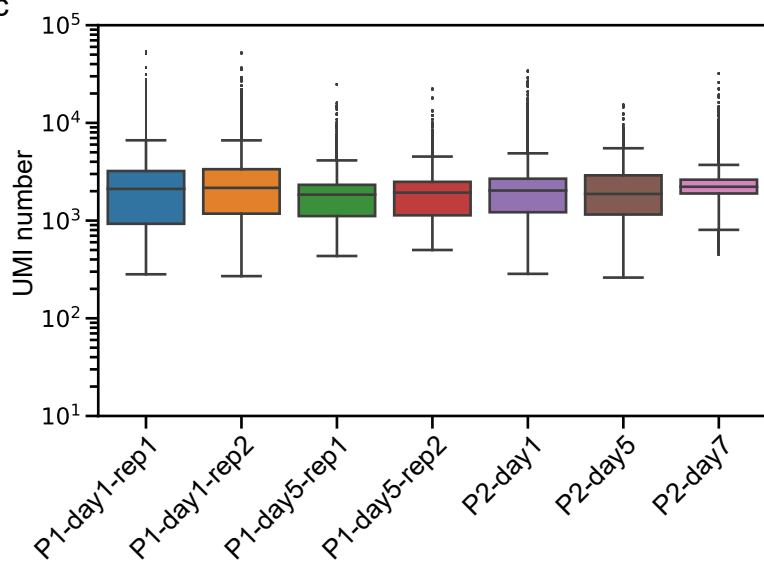
a

Batch name	Captured cells	Median genes per cell	Median UMIs per cell	Cells passed QC
P1-day1-rep1	2591	840	2068	1511
P1-day1-rep2	2081	862	2100	1257
P1-day5-rep1	3129	682	1850	2160
P1-day5-rep2	3851	691	1895	2699
P2-day1	2280	914	2067	1576
P2-day5	1488	810	1909	1027
P2-day7	3332	977	2238	3009

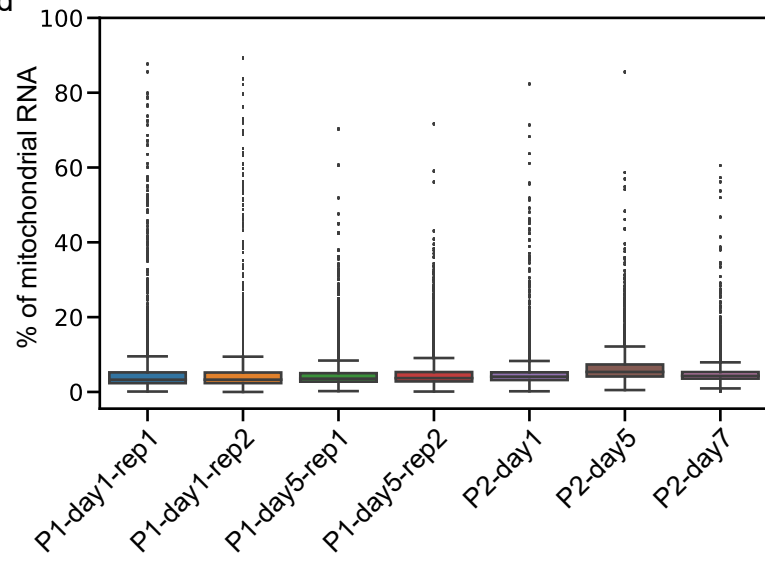
b



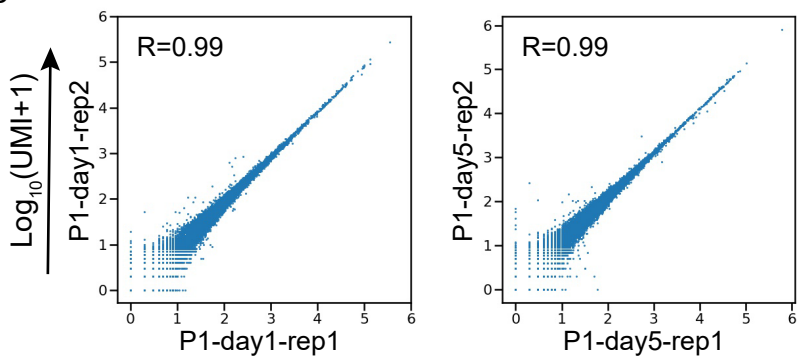
c



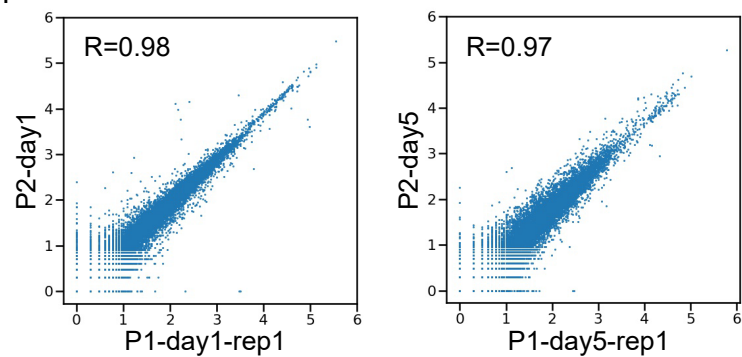
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e

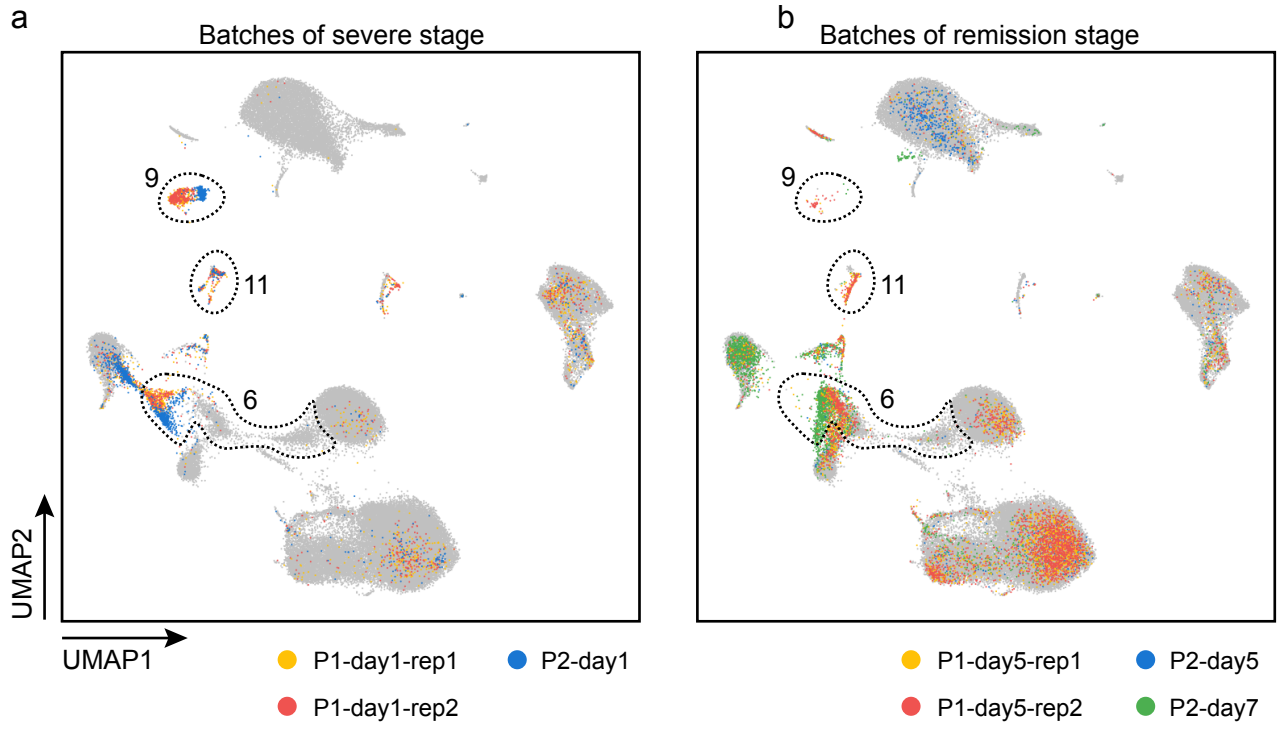


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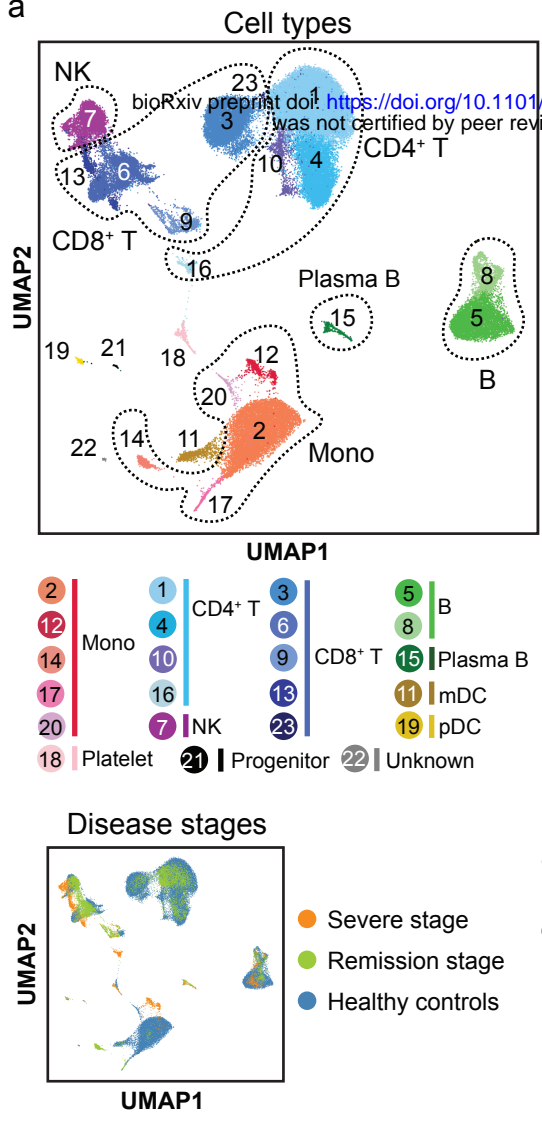
$\text{Log}_{10}(\text{UMI}+1)$

Supplementary Fig. 2

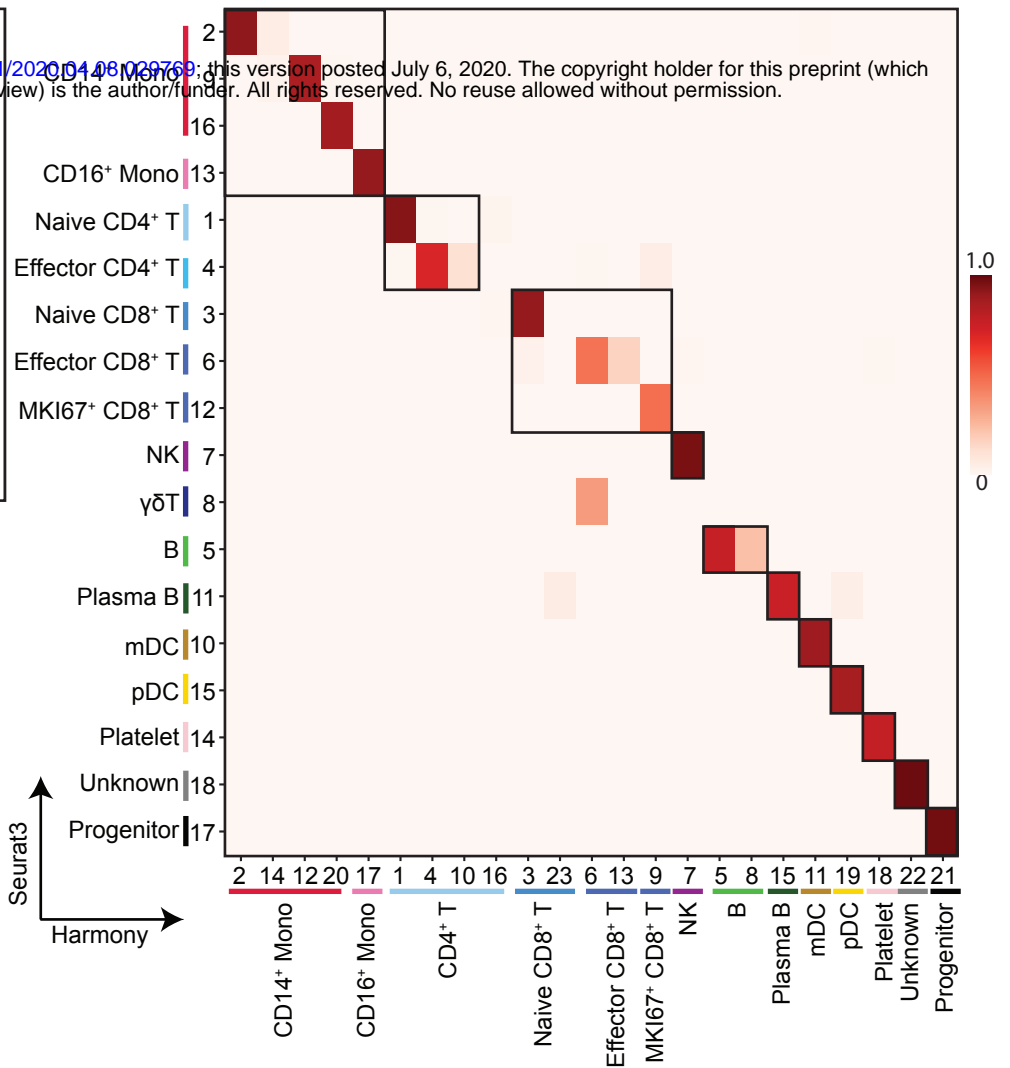


Supplementary Fig. 3

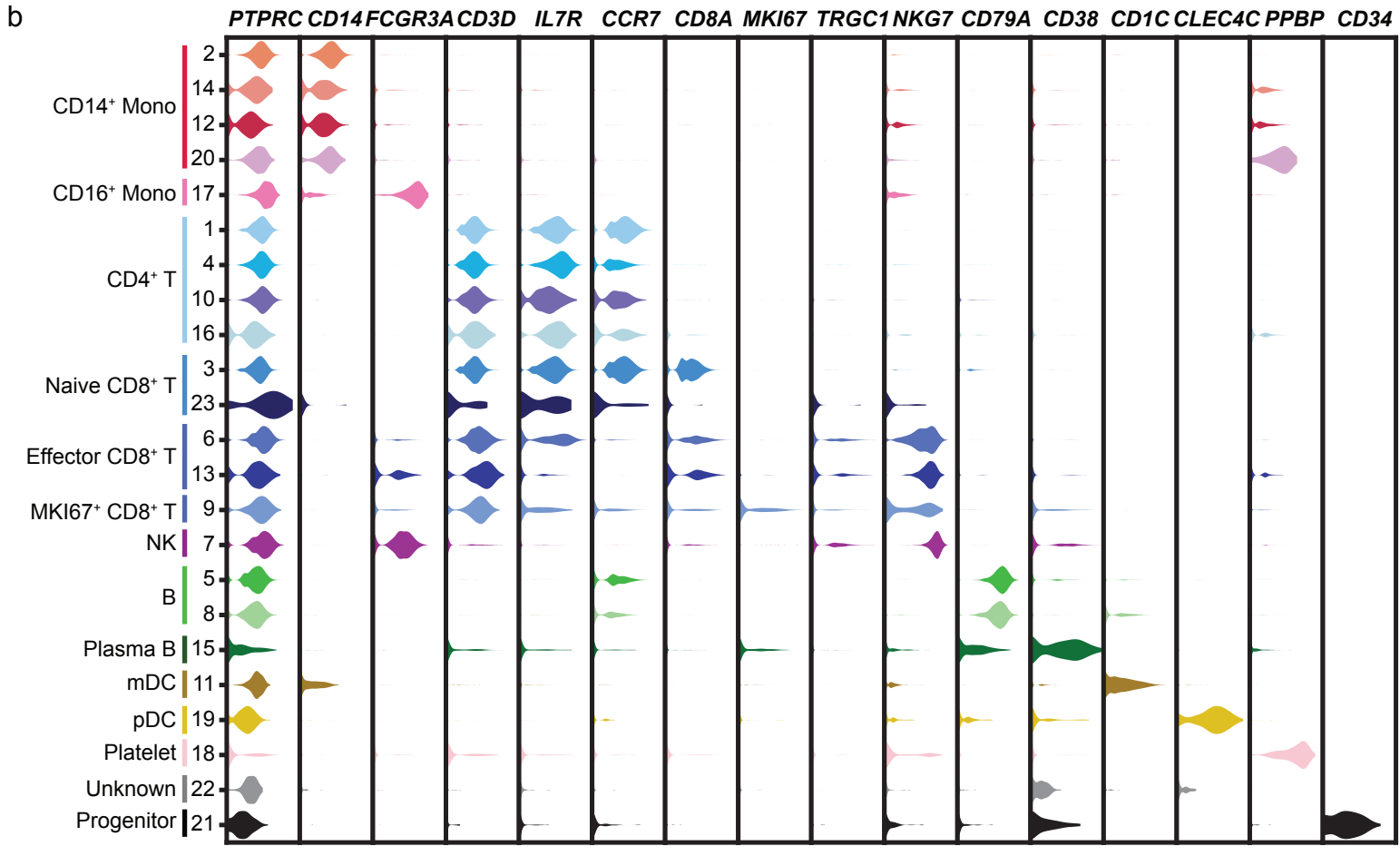
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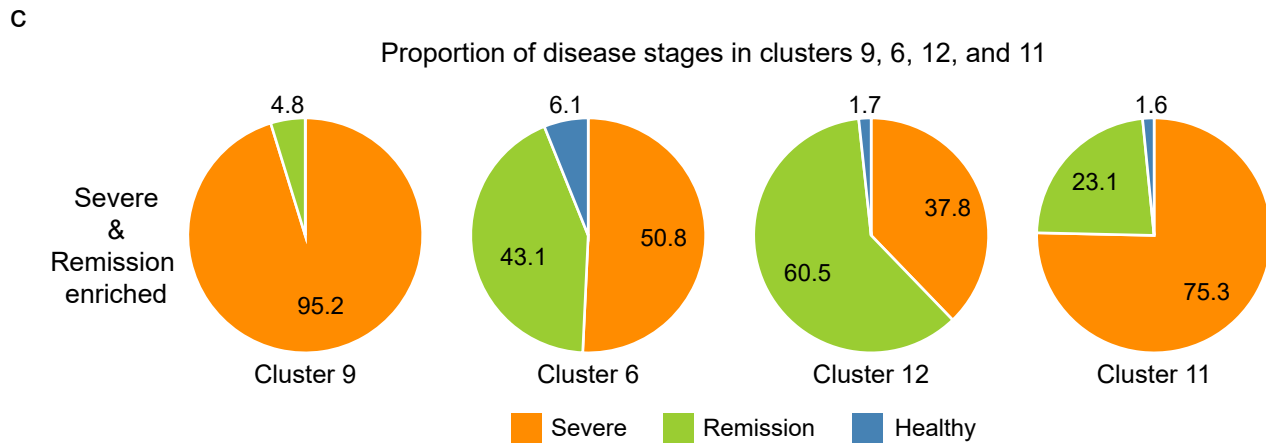
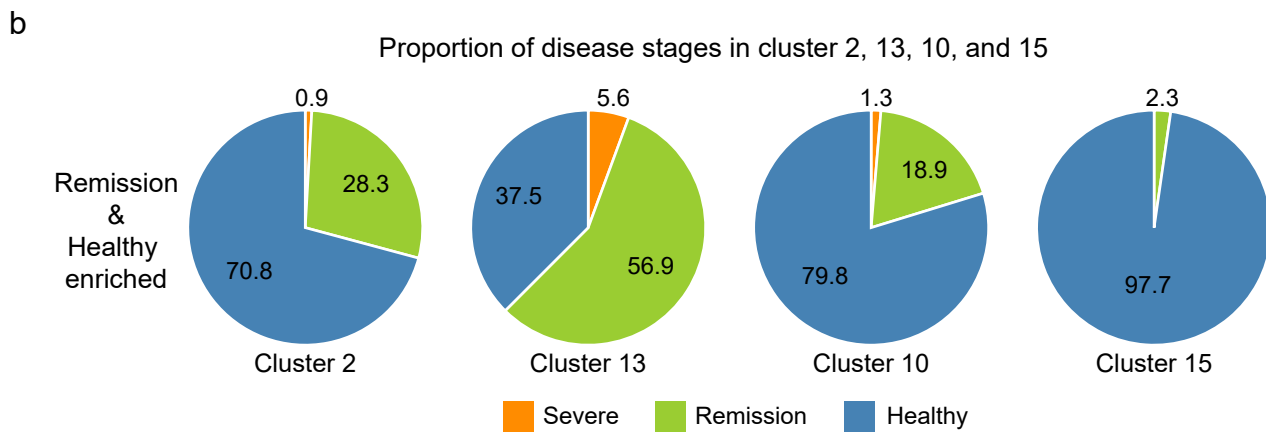
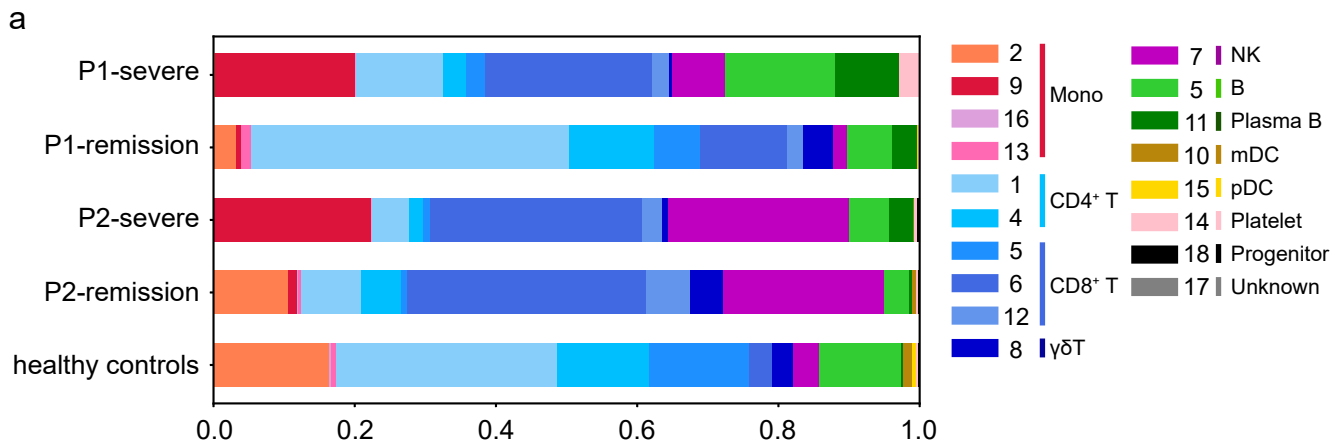
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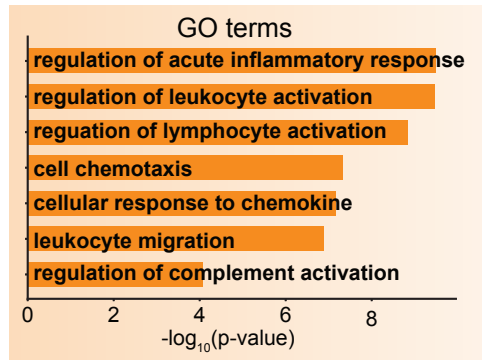


Supplementary Fig. 4

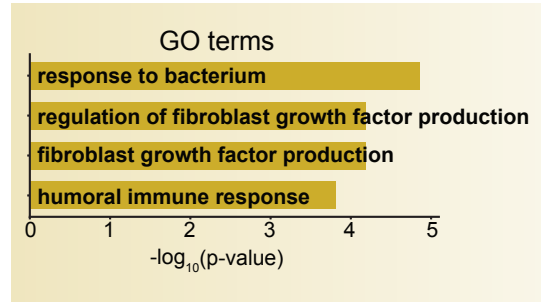


## Supplementary Fig. 5

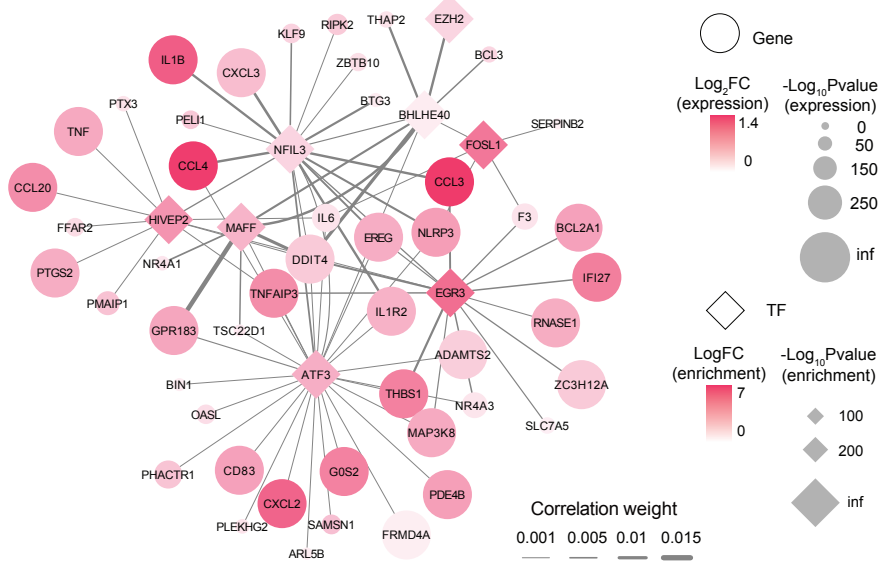
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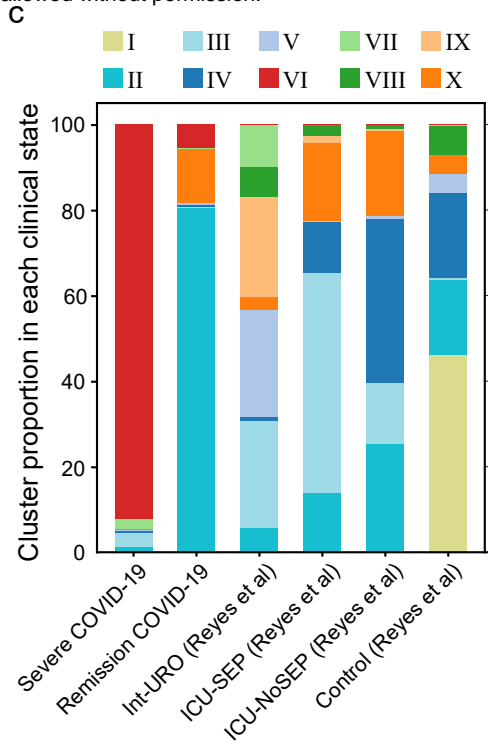
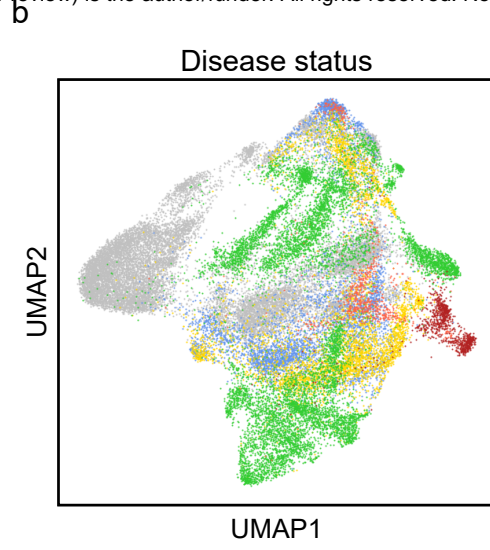
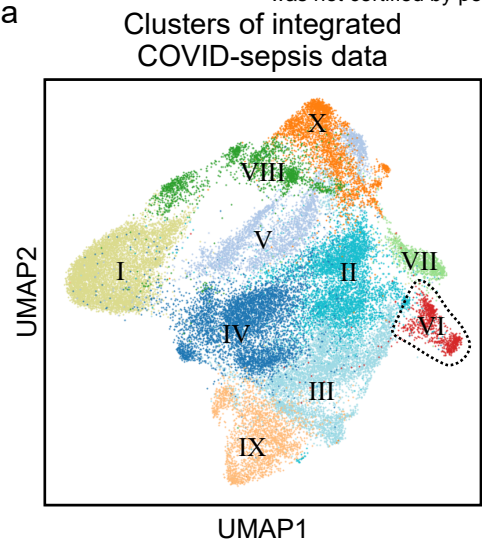


b



## Supplementary Fig. 6





● I ● III ● V ● VII ● IX  
● II ● IV ● VI ● VIII ● X

● Severe COVID-19 ● ICU-SEP (Reyes et al)  
● Remission COVID-19 ● ICU-NoSEP (Reyes et al)  
● Int-URO (Reyes et al) ● Control (Reyes et al)



## Supplementary Fig. 8

