Tocilizumab treatment in severe COVID-19 patients attenuates the inflammatory storm incited by monocyte centric immune interactions revealed by single-cell analysis

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ABSTRACT

Coronavirus disease 2019 (COVID-19) has caused more than 40,000 deaths worldwide[1]. Approximately 14% of patients with COVID-19 experienced severe disease and 5% were critically ill[2]. Studies have shown that dysregulation of the COVID-19 patients' immune system may lead to inflammatory storm and cause severe illness and even death[3,4]. Tocilizumab treatment targeting interleukin 6 receptor has shown inspiring clinical results of severe COVID-19 patients[5]. However, the immune network with Tocilizumab treatment at single cell resolution has not been uncovered. Here, we profiled the single-cell transcriptomes of 13,289 peripheral blood mononuclear cells isolated at three longitudinal stages from two severe COVID-19 patients treated with Tocilizumab. We identified a severe stage-specific monocyte subpopulation and these cells centric immune cell interaction network connected by the inflammatory cytokines and their receptors. The over-activated inflammatory immune response was attenuated after Tocilizumab treatment, yet immune cells including plasma B cells and $CD8^+$ T cells still exhibited an intense humoral and cell-mediated anti-virus immune response in recovered COVID-19 patients. These results provided critical insights into the immunopathogenesis of severe COVID-19 and revealed fundamentals of effectiveness in Tocilizumab treatment.

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

66 **Main**

67 As of Apr 1, 2020, WHO reported 40,598 deaths out of 823,626 confirmed cases
68 infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and these
69 numbers are still growing rapidly[1]. Approximately 14% of patients with COVID-19
70 experienced severe disease, and 5% were critically ill suffered from 49% fatality rate[2],
71 which may be caused by patients' abnormal immune system activation[3,4,6]. Hence, there
72 is an urgent need for researchers to understand how the immune system respond to the
73 viral infections at severe stage and thereby provide effective treatment strategies.

74 Studies have shown that the inflammatory storm caused by excessive immune
75 responses was associated with the crucial cause for mortality in COVID-19[7,8]. Plasma
76 concentrations of a series of inflammatory cytokines, such as granulocyte-macrophage
77 colony-stimulating factor (GM-CSF), interleukin (IL)-6[4], tumor necrosis factor α
78 (TNF-α), IL-2, 7, 10 and granulocyte colony-stimulating factor (G-CSF)[9] were
79 increased after SARS-CoV-2 infections. Further investigation demonstrated peripheral
80 inflammatory monocytes and pathogenic T cells may incite cytokine storm in severe
81 COVID-19 patients[4,7]. To calming inflammatory storm, Tocilizumab, which targeting
82 IL-6 receptors and has proved its effectiveness in the treatment of cytokine release
83 syndrome that is severe or life-threatening[10,11], was used in the treatment of severe
84 COVID-19. After receiving Tocilizumab, the body temperature of the patients returned
85 to normal after 24 hours. The concentration of oxygen inhalation was significantly
86 decreased on the 5th day[5]. However, the immune network arousing the inflammatory
87 storm in severe or recovery stage during Tocilizumab therapy at single cell level has
88 not been uncovered.

89 Here, we profiled the peripheral immune cells of COVID-19 patients using single-
90 cell transcriptome sequencing. We obtained 5 peripheral blood samples from 2 severe
91 COVID-19 patients at 3 consecutive time-points from the severe to recovery stages
92 during Tocilizumab treatment (Fig. 1a). Specifically, the blood samples at severe stage
93 were collected within 12 hours of Tocilizumab was given. The blood samples at
94 recovery stage were collected at the 5th and 7th day after Tocilizumab treatment. The

95 patients at severe stage had decreased number of lymphocytes, increased percentage of
96 neutrophil and concentrations of C-reaction protein, and increased expression of IL-6
97 (Supplementary Table 1). Peripheral blood mononuclear cells (PBMCs) were isolated
98 and subjected to single-cell mRNA sequencing (scRNA-seq) using the 10X platform
99 (Fig. 1a, Supplementary Table 2). After filtering low quality cells, we retained a total
100 of 13,289 single transcriptomes of PBMCs. Of these, 4,364 cells were from severe stage
101 and 8,925 were from patients at recovery stage.

102 To investigate the heterogeneity and differences of PBMCs between COVID-19
103 patients and healthy controls, we integrated our COVID-19 single cell transcriptomes
104 with the published single-cell profiles of healthy PBMC from the 10X official website[12]
105 and obtained a total of 69,237 cells (See **Methods**) (Fig. 1b-d). We then applied Seurat[13]
106 to normalize and cluster the gene expression matrix, and identified 20 unique cell
107 subsets, which were visualized via uniform manifold approximation and projection
108 (UMAP) (Fig. 1b-d). Cell lineages, including monocytes, $CD4^+$ and $CD8^+$ T, γδT, NK,
109 B, plasma B and myeloid dendritic cells (mDC), plasmacytoid dendritic cells (pDC),
110 platelet and $CD34^+$ progenitor cells were identified based on the expression of known
111 marker genes (Fig. 1e, Extended Data Fig.1a). With that, we delineated the landscape
112 of circulating immune cells in severe COVID-19 patients.

113 We next explored the distribution of immune cells from the severe, recovery and
114 healthy stage in each cell subpopulation (Fig. 1f, Extended Data Fig.1b). We observed
115 that a monocyte subpopulation (cluster 9) existed only in patients at severe stage.
116 Plasma B cells (cluster 11), effector $CD8^+$ T (cluster 6), proliferative $MKI67^+CD8^+$ T
117 cells (cluster 12) and NK cells (cluster 7) were significantly enriched in patients versus
118 control. However, a number of subpopulations, such as γδ T cells (cluster 8), pDCs
119 (cluster 15) and mDCs (cluster 10 and 19), most monocytes (cluster 2, 13 and 14)
120 existed only in patients at recovery stage and healthy controls, indicating that these cell
121 types gradually become normal after the treatment. No significant differences were
122 observed in $CD4^+$ T (cluster 1 and 4), naïve $CD8^+$ T (cluster 3) and B cells (cluster 5)

in patients versus control.

Monocytes were reported to play a vital role in CAR-T induced cytokine-release syndrome[14] and SARS-CoV-2 infection caused inflammatory storm[4], therefore we explored the features and functions of the monocyte from COVID-19 patients. We detected 1,737 monocytes in patients, 927 from severe stage and 810 from recovery stage, and integrated 9,787 monocytes from health control. The UMAP plot displayed two main clouds of monocytes that were clearly segregated (Fig 2a). One monocyte subpopulation (cluster 9) was almost exclusively consisted of cells from severe stage and others (cluster 2, 13, 14, 17) were dominated by cells from the recovery and healthy stages (Fig 2b), suggesting a severe-stage specific monocyte subpopulation.

We then investigated the expressions of several selected inflammatory cytokines and observed that these genes were all significantly enriched in severe stage-specific monocytes (Fig. 2c, $P < 0.001$, Wilcoxon rank-sum test). To further explore the transcriptional differences among the monocytes' subtypes, we performed a pairwise comparison of the gene expressions in severe, recovery and healthy stages. We obtained 2,335 differentially expressed genes (DEGs) enriched in each stage, within which reported cytokine storm related genes, such as *TNF*[9], *IL10*[9], *CCL3*[9] and *IL6*[4] were found significantly higher expressed in severe stage-specific monocytes (Fig 2d, Supplementary Table 3). In addition, we also discovered a large number of significant and inflammatory related genes that were less reported (Fig 2d, fold change > 2, $P < 10^{-3}$), including chemokine genes *CCL4*, *CCL20*, *CXCL2*, *CXCL8* and *CXCL9*, inflammasome activation associated genes *NLRP3* and *IL1B*, and complement pathway genes *C1QA*, *C1QB* and *C1QC* (Extended Data Fig.2a-c, Supplementary Table 4). These results indicated that this monocyte subpopulation may contribute to the inflammatory storm in severe COVID-19 patients.

We also observed that genes involved in "acute inflammatory response" and "leukocyte chemotaxis" were significantly decreased at recovery and healthy stage (Fig.2e, f, Supplementary Table 5), suggesting that the inflammatory storm caused by

151 this monocyte subpopulation was suppressed after the Tocilizumab treatment.

152 Next, we explored the transcription factors (TFs) that may be involved in the
153 regulation of inflammatory storm in monocytes. We used SCENIC[15] and predicted 15
154 TFs that may regulate genes which were enriched in severe stage-specific monocyte
155 (Fig 2g). By constructing a gene regulatory network, we found 3 of them, namely ETS2,
156 NFIL3 and PHLDA2 were regulating the cytokine storm relevant genes (Extended Data
157 Fig.2d). In addition, we found the expressions of *ETS2*, *NFIL3* and *PHLDA2* and their
158 target genes were enhanced in severe-specific monocyte subpopulation (Fig 2h),
159 suggesting these 3 TFs may regulate the inflammatory storm in monocytes.

160 Given that monocytes in the severe stage may be involved in the regulation of a
161 variety of immune cell types, we used the accumulated ligand/receptor interaction
162 database[16] CellPhoneDB (www.cellphonedb.org) to identify alterations of molecular
163 interactions between monocytes and all the immune cell subsets (Supplementary Table
164 6). We found 15 pairs of cytokines and their receptors whose interaction were
165 significantly altered in severe versus recovery and healthy stages (Fig 3a). Consistent
166 with previous study[4], we found monocytes interacted with $CD4^+$ T cells and plasma B
167 cells in patients at severe stage through the ligand/receptor pairs of IL-6/IL-6R. This
168 interaction, together with many other cytokine storm relevant cell-cell communications
169 were then extensively attenuated after the treatment of Tocilizumab (Fig 3b), suggesting
170 that this drug may functioning by effectively blocking the inflammatory storm in severe
171 COVID-19 patients.

172 In addition, we also discovered many other ligand/receptor pairs involved in a
173 broader spectrum of immune cell communications enriched at the severe stage. For
174 example, TNF-α and its receptors, which connected monocytes with many other
175 immune cells. Others like IL-1β and its receptor, which connected monocytes with
176 $CD8^+$ T cells. Chemokines, such as CCL4L2, CCL3 and CCL4 and their receptors were
177 also found enriched at severe stage. These cytokines and their receptors may serve as
178 potential drug targets to treat COVID-19 patients at severe stage, and some of their

179 inhibitors are undergoing clinical trials in multiple places around the world
180 (Supplementary Table 7). Collectively, these findings illustrated the molecular basis of
181 cell-cell interactions at the peripheral blood of COVID-19 patients, leading to a better
182 understanding of the mechanisms of inflammatory storm of the disease.

183 Robust multi-factorial immune responses can be elicited against viral infection,
184 such as avian H7N9 disease[17,18]. A recent report has found effective immune responses
185 from a non-severe COVID-19 patient[19]. However, it is not clear whether the anti-virus
186 immune response would be affected after Tocilizumab treatment. Therefore, the anti-
187 virus immune responses from the humoral and cell-mediated immunity in COVID-19
188 patients at severe stage were compared with recovery stage and healthy controls. As
189 expected, we found plasma B cells were barely existing in healthy controls (Fig. 4a).
190 By contrast, significantly higher proportion of plasma B cells was exclusively increased
191 in both severe and recovery stages (Fig. 4a, b), suggesting powerful anti-virus humoral
192 immune responses during Tocilizumab treatment.

193 $CD8^+$ T cells are a critical component of cell-mediated immunity against viral
194 infections by killing infected cells and secreting proinflammatory cytokines. To identify
195 the anti-virus immune responses from the cell-mediated immunity during Tocilizumab
196 treatment, we detected 12,121 $CD8^+$ T cells from our analysis. Clustering these cells
197 revealed 3 subtypes: naïve $CD8^+$ T cells (cluster 3), effector $CD8^+$ T cells (cluster 6)
198 and a subset of $CD8^+$ T cells with proliferation characteristics (cluster 12) (Fig. 4c, d).
199 Among them, the cells from the severe patients were mainly distributed in the effector
200 $CD8^+$ T cell cluster (Fig. 4c, d). We then conducted pairwise comparisons to identify
201 DEGs of $CD8^+$ T cells between the severe, recovery and healthy stages (Fig. 4e,
202 Supplementary Table 8). We found that genes enriched in severe stage showed
203 "regulation of cell activation" signatures (Fig. 4f, Supplementary Table 9; $P < 10^{-10}$).
204 Meanwhile, genes involved in "leukocyte mediated cytotoxicity" and "positive
205 regulation of cytokine production" were highly enriched in $CD8^+$ T cells from COVID-
206 19 patients at both severe and recovery stage (Fig. 4g, Supplementary Table 9; $P < 10^{-}$

[5]). We further detected elevated expression of the 108 and 449 genes involved in these GO terms (Fig. 4h, i, Supplementary Table 10). Together, these results demonstrated the critical evidence that robust adaptive immune responses against SARS-CoV-2 infection exist in severe stage and remain after Tocilizumab treatment.

The immune system is crucial to fight off viral infection[20,21]. Recent studies have illustrated that monocytes may be the main cause of exacerbation and even death of COVID-19 patients through inflammatory storms[4]. In this study, we discovered a specific monocyte subpopulation that may lead to the inflammatory storm in patients at severe stage through single-cell mRNA sequencing. By analyzing the monocyte-centric ligand/receptor interactions, we revealed a severe stage-specific landscape of peripheral immune cell communication that may drive the inflammatory storm in COVID-19 patients. With that we obtained a list of cytokine storm relevant ligand/receptors that can serve as candidate drug targets to treat the disease, and provided mechanistic insights of the immunopathogenesis of COVID-19.

There are always questions about whether Tocilizumab treatment may affect the antiviral effect of the body[22,23]. Our single cell profiles illustrated a sustained humoral and cell-mediated anti-virus immune response of COVID-19 patients at both severe and recovery stage. For example, the proportion of plasma B cells with antibody-secreting function were keeping at high levels and the cytotoxicity and cytokine production of effector $CD8^+$ T cells were also remained stable in severe COVID-19 patients after Tocilizumab treatment.

The distributions of NK cells were significantly different in the two patients (Extended Data Fig.1b), and the analysis of gene expression differences did not enrich significant biological functions, therefore we did not discuss them in depth. The normal functions of other cell types, such as γδT cells and DCs, were almost lost under severe conditions, and the contribution of these cells to the progression of the disease requires further investigation.

Methods

Human samples

Peripheral blood samples were obtained from two severe COVID-19 patients. The patient severity was defined by the "Diagnosis and Treatment of COVID-19 (Trial Version 6)" which was released by The General Office of the National Health Commission and the Office of the National Administration of Traditional Chinese Medicine. Patient PZ was defined as a severe patient for his peripheral capillary oxygen saturation (SPO2) <93%. Patient PZ provided 2 blood samples at severe stage (Day 1) and recovery stage (Day 5). Patient PW was defined as critical ill for respiratory failure, multiple organ dysfunction (MOD) and SPO2 <93% under high flow oxygen (50 L/min, FIO2 50%). Patient PW provided 3 blood samples at severe stage (Day 1) and recovery stage (Day 5 and Day 7). All samples were collected from the First Affiliated Hospital of University of Science and Technology of China. Before blood draw, informed consent was obtained from each patient. Ethical approvals were obtained from the ethics committee of the First Affiliated Hospital of the University of Science and Technology of China (No. 2020-XG(H)-020).

Cell Isolation

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

Single-cell RNA-seq

We generated single-cell transcriptome library following the instructions of single-cell 3' solution v2 reagent kit (10x Genomics). Briefly, after thawing, washing and counting cells, we loaded the cell suspensions onto a chromium single-cell chip along with partitioning oil, reverse transcription (RT) reagents, and a collection of gel beads that

contain 3,500,000 unique 10X Barcodes. After generation of single-cell gel bead-in-emulsions (GEMs), RT was performed using a C1000 Touch^{TM} Thermal Cycler (Bio-Rad). The amplified cDNA was purified with SPRIselect beads (Beckman Coulter). Single-cell libraries were then constructed following fragmentation, end repair, polyA-tailing, adaptor ligation, and size selection based on the manufacturer's standard parameters. Each sequencing library was generated with unique sample index. Libraries were sequenced on the Illumina NovaSeq 6000 system.

Single-cell RNA-seq data processing

The raw sequencing data of patients and health donors were processed using Cell Ranger (version 3.1.0) against the GRCh38 human reference genome with default parameters, and data from different patients and disease stages were combined by the Cell Ranger 'aggr' function. We are uploading the scRNA-seq data of PBMCs from the 2 severe COVID-19 patients to the Genome Sequence Archive at BIG Data Center and the accession number will be available upon request. We also used the scRNA-seq data of PBMCs from 2 healthy donors, which can be downloaded from the 10X genomics official website. Firstly, we filtered low quality cells using Seurat[13] (version 3.1.4). For cells from COVID-19 patients (PZ and PW), we retained cells with detected gene numbers between 500 and 6,000 and mitochondrial UMIs less than 10%. For cells from healthy donors, we retained cells with detected gene numbers between 300 and 5,000 and mitochondrial UMIs less than 10%. Subsequently we normalized gene counts for each cell using the "NormalizeData" function of Seurat with default parameters.

In downstream data processing, we used canonical correlation analysis (CCA) and the top 40 canonical components to find the "anchor" cells between patients and healthy controls. We then used the "IntegrateData" function in Seurat to integrate all the cells. We clustered cells based on the integrated dataset using Seurat with parameter "resolution=0.3" and generated 20 clusters. To display cells in a 2-dimensional space, we ran the principal component analysis (PCA) on the integrated dataset and adopted

292 the first 50 principal components for the uniform manifold approximation and
293 projection (UMAP) analysis.
294
295 **Differential expression analysis**
296 To search for the differentially expressed genes (DEGs), we first assign the negative
297 elements in the integrated expression matrix to zero. We used Wilcoxon rank-sum test
298 to search for the DEGs between each pair of the 3 stages of cells (i.e. severe stage,
299 recovery stage and healthy control). We applied multiple thresholds to screen for DEGs,
300 including mean fold-change >2, *P* value <0.001, and were detected in >10% of cells in
301 at least one stage.
302 We defined stage A specific-DEGs as the intersections between the DEGs in stage
303 A versus stage B and the DEGs in stage A versus stage C. We defined stage A and B
304 common-DEGs as the intersections of the DEGs in stage A versus stage C and the
305 DEGs in the stage B versus stage C, minus the DEGs between stage A and B. In this
306 way, we obtained the specific-DEGs for each stage, and the common-DEGs for each
307 pair of the 3 stages. We then uploaded these DEG groups to the Metascape[24] website
308 (https://metascape.org/gp/index.html#/main/step1), and used the default parameters to
309 perform Gene Ontology (GO) analysis for each stage.
310
311 **Motif enrichment and regulatory network**
312 We adopted SCENIC[15] (version 1.1.2) and RcisTarget database to build the gene
313 regulatory network of $CD14^+$ monocytes. Since the number of $CD14^+$ monocytes from
314 healthy control (N = 9,618) was more than those from the severe and recovery stages
315 (N = 1,607), to balance their contributions in the motif analysis, we randomly sampled
316 2,000 $CD14^+$ monocytes from the healthy control for calculation. We selected 13,344
317 genes that were detected in at least 100 monocytes or included in the DEGs of the 3
318 stages as the input features for SCENIC. With default parameters, SCENIC generated
319 the enrichment scores of 427 motifs. We used the student's t-test to calculate the *P*

values of these motifs between severe stage and healthy control, and selected severe-specific enriched motifs with fold change >1.5 and *P* value < 10^{-100}.

We then applied the enrichment scores of the severe-specific enriched motifs and the expressions of their targeted genes to Cytoscape[25] to construct a connection map for the gene regulatory network, as shown in Extended Data Fig. 2b. The thickness of line connecting TFs and target genes represented the weight of regulatory link predicted by SCENIC.

Ligand/receptor interaction analysis

To identify potential cellular communications between monocytes and other cell types ($CD4^+$ T, $CD8^+$ T, B, plasma B and NK cells), we applied the CellphoneDB[16] algorithm to the scRNA-seq profiles from the severe, recovery and healthy stages. CellphoneDB evaluated the impact of a ligand/receptor interactions based on the ligand expression in one cell type and its corresponding receptor expression in another cell type. To identify the enriched ligand/receptor interactions in patients at severe stage, we selected the ligand/receptor interactions with more significant (*P* value < 0.05) cell-cell interaction pairs in the severe stage than that in the recovery and healthy stages. We also included ligand/receptor pairs which were highly expressed in severe stage.

Data Availability

We are uploading the scRNA-seq data of PBMCs from the 2 severe COVID-19 patients to the Genome Sequence Archive at BIG Data Center and the accession number will be available upon request. We also used the scRNA-seq data of PBMCs from 2 healthy donors, which can be downloaded from the 10X genomics official website.

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Author Contributions

K.Q. conceived and supervised the project; K.Q., C.G. and J.L. designed the experiments; C.G. and J.L. performed the experiments and conducted all the sample preparation for NGS with the help from H.M. and T.C.; B.L. performed the data analysis with the help from P.C., Q.Y., L.Z., L.J., C.J., Q.L., D.Z., W.Z., Y.L., K.L., X.G. and J.F; T.C., X.W., L.L. and X.M. provided COVID-19 blood samples and clinical information. K.Q., C.G., J.L. and B.L. wrote the manuscript with the help of B.F., H.W. and all the other authors.

Competing interests

Jingwen Fang is the chief executive officer of HanGen Biotech.

Figure Legends

Figure 1 | An atlas of peripheral immune cells in severe COVID-19 patients. a, Flowchart depicting the overall design of the study. Blood draws from patient PZ were performed at 2 time points (Day1 and Day5), and PW at 3 time points (Day1, Day5, and Day7). Patients at Day 1 were at severe stage and Day 5 and Day 7 were at recovery stages. Samples were collected within 12 hours of Tocilizumab was given at Day 1. **b-d**, UMAP representations of single-cell transcriptomes of 13,289 PBMCs. Cells are color-coded by clusters **(b)**, disease stages **(c)**, and the corresponding patient or healthy control **(d)**. Dotted circles represented cell types with > 5% proportion of PBMCs in **(b)** and clusters significantly enriched in patients versus control in **(c, d)**. Mono, monocyte; NK, natural killer cells; mDC, myeloid dendritic cells; pDC, plasmacytoid dendritic

377 cells. **e**, Violin plots of selected marker genes (upper row) for multiple cell
378 subpopulations. The left column are cell subtypes identified by combination of marker
379 genes. **f**, Bar chart showing the proportion of immune cells from the severe, recovery
380 and healthy stage in each cell subpopulation.
381
382 **Figure 2 | A unique monocyte subpopulation contributes to the inflammatory**
383 **storm in COVID-19 patients at severe stage. a**, UMAP plot showing 4 clusters of
384 $CD14^+$ monocytes and 1 cluster of $CD16^+$ monocyte. Cells are color-coded by clusters.
385 **b**, Bar plot of the proportion of monocytes in cluster 9 at the severe, recovery and
386 healthy stages. **c**, UMAP plots showing the expressions of selected cytokines in all
387 monocytes clusters. **d**, Heatmap of differentially expressed genes (DEGs) in monocytes
388 from pairwise comparison between severe, recovery and healthy stages. **e,f,** Box plot
389 of the average expressions of genes involved in the signaling pathway "Acute
390 inflammatory response" and "Leukocyte chemotaxis" in monocytes from severe,
391 recovery and healthy stages. Center line, median; box limits, upper and lower quartiles;
392 whiskers, 1.5x interquartile range; points, outliers; **** represents P value < 10^{-100},
393 student's t-test. **g**, Heatmap of the area under the curve (AUC) scores of expression
394 regulation by transcription factors (TFs) estimated using SCENIC. Shown are the top
395 differential TFs. **h**, UMAP plots showing the expressions of genes *ETS2*, *NFIL3* and
396 *PHLDA2* in monocytes (top) and the AUC of the estimated regulon activity of the
397 corresponding TFs, indicating the degree of expression regulation of their target genes
398 (bottom).
399
400 **Figure 3 | The monocyte-centric molecular interactions of peripheral immune cells**
401 **in COVID-19 patients at severe stage. a**, Dot plot of predicted interactions between
402 monocytes and indicated immune cell types in the severe, recovery and healthy stages.
403 P values were measured by circle sizes. The expression levels of the interacted genes
404 were indicated by colors, scales on the right. **b**, Summary illustration depicting the

405 cytokine/receptor interactions between monocytes and other types of peripheral
406 immune cells in severe, recovery and healthy stages. Bolder lines indicated predicted
407 enriched ligand/receptor interactions between monocytes and other immune cell types.
408
409 **Figure 4 | Enhanced humoral and cell-mediated immunity in severe COVID-19**
410 **patients. a**, UMAP representations of B and plasma B cell clusters from the severe,
411 recovery and healthy stages. **b**, Bar plot of the proportions of plasma B cells in B cell
412 lineage from severe, recovery and healthy stages. **c**, UMAP representations of $CD8^+$ T
413 cell subtypes (left) and the distribution of cells from severe, recovery and healthy stages
414 in each subtype (right). **d**, Dot plot of the expression of *CCR7, PRDM1* and *MKI67* in
415 all $CD8^+$ T cell subtypes. **e**, Heatmap of differentially expressed genes in effector $CD8^+$
416 T cells from pairwise comparison between the severe, recovery and healthy stages. **f, g,**
417 Bar plots of GO terms enriched in effector $CD8^+$ T cells from the severe stage **(f)** or
418 severe and recovery stages **(g)**. **h, i,** Box plots of the average expressions of genes
419 involved in the signaling pathway "Leukocyte mediated cytotoxicity" and "Positive
420 regulation of cytokine production" in the effector $CD8^+$ T cells from severe stage,
421 recovery stage and healthy controls. Center line, median; box limits, upper and lower
422 quartiles; whiskers, 1.5x interquartile range; points, outliers; **** represents P value <
423 10^{-30}.
424
425 **Extended Data Figure Legends and Supplementary Tables**
426
427 **Extended Data Figure 1 | Identification of single-cell subpopulations. a**, UMAP
428 plots showing the expressions of selected marker genes in all identified cells. **b**, Bar
429 chart showing the percentage of cell subpopulations in different clinical stages of
430 patients and healthy controls.
431 **Extended Data Figure 2 | Features of monocyte subpopulations. a-c,** Bar plots of
432 enriched GO terms of genes highly expressed in monocytes at severe stage **(a)**, severe

and recovery stages **(b)**, and healthy controls **(c)**. **d,** Severe stage specific monocyte regulatory network predicted by SCENIC. Transcription factors were shown in rectangles and their target genes in circles.

Supplementary Table 1 | Baseline characteristcs and laboratory findings of COVID-19 patients in this study.

Supplementary Table 2 | Sequencing data quality.

Supplementary Table 3 | DEGs of different stages of CD14 monocytes.

Supplementary Table 4 | GOterms of DEGs of CD14 monocytes.

Supplementary Table 5 | Gene sets of GO terms in Figure 2e and 2f.

Supplementary Table 6 | Interactions of cytokines and receptors in different stages.

Supplementary Table 7 | Drugs for targeting cytokines or their receptors.

Supplementary Table 8 | DEGs of different stages of effector CD8 T cells.

Supplementary Table 9 | GOterms of DEGs of effector CD8 T cells.

Supplementary Table 10 | Gene sets of GO terms in Figure 4h and 4i.

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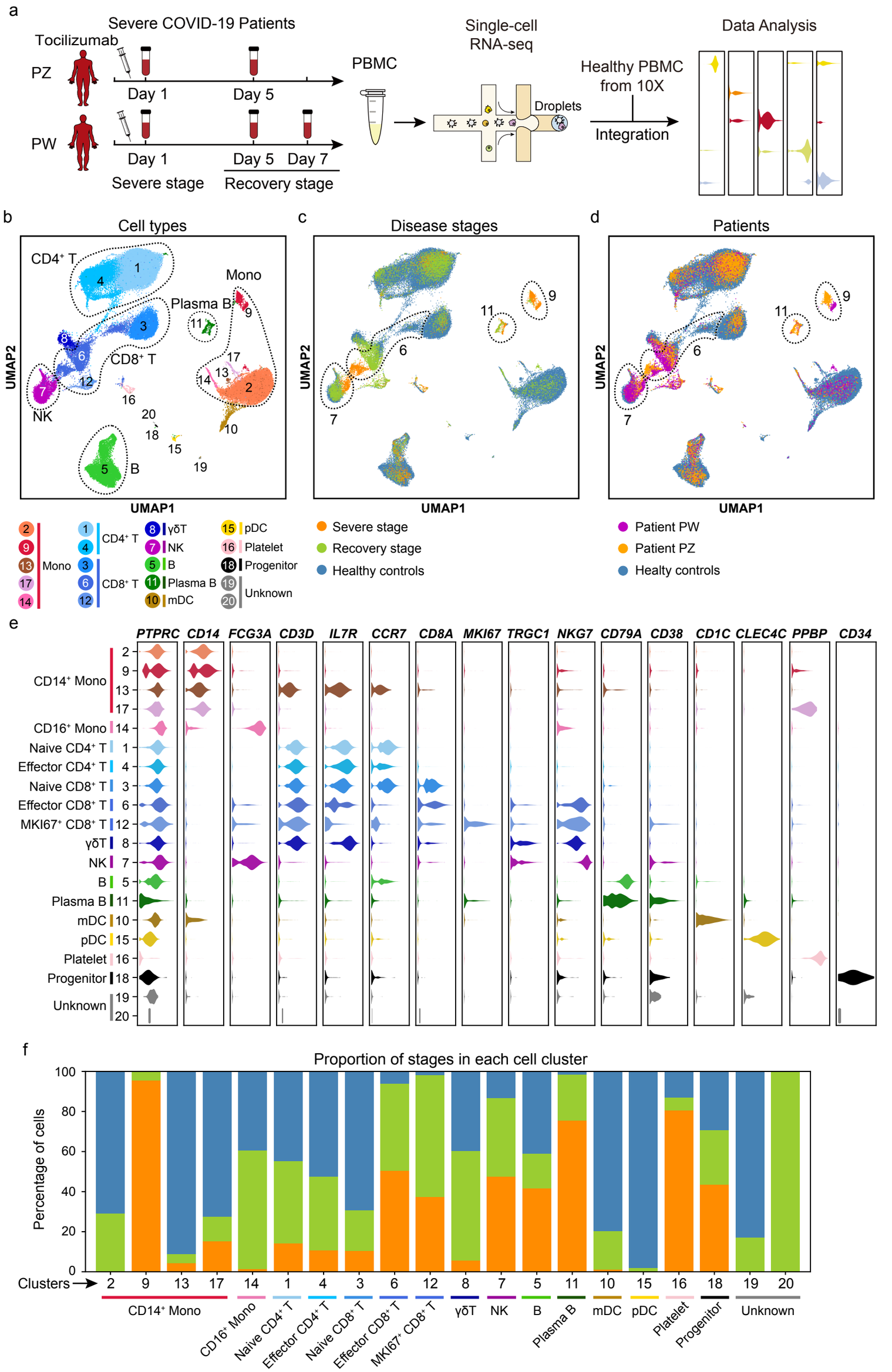
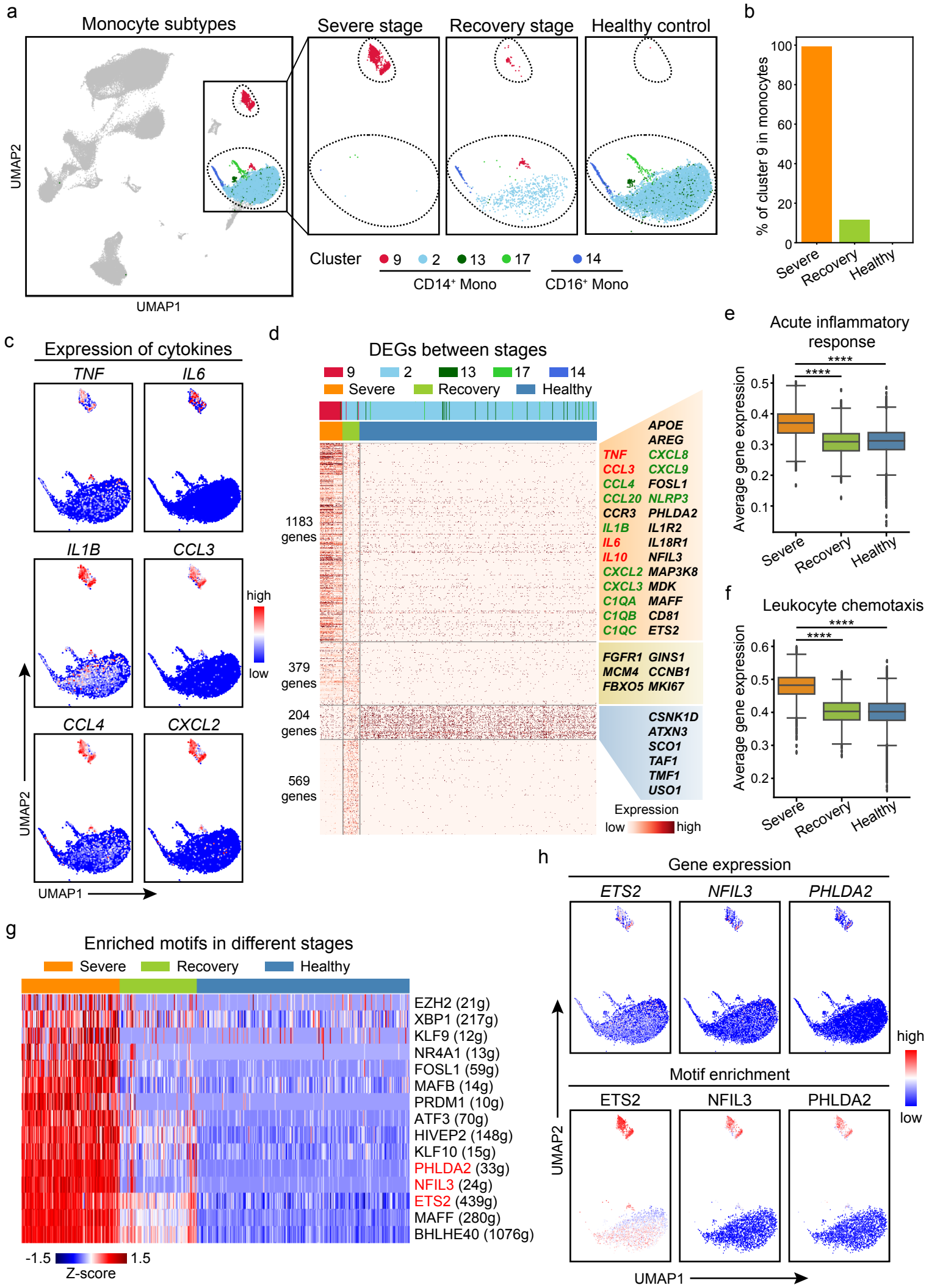
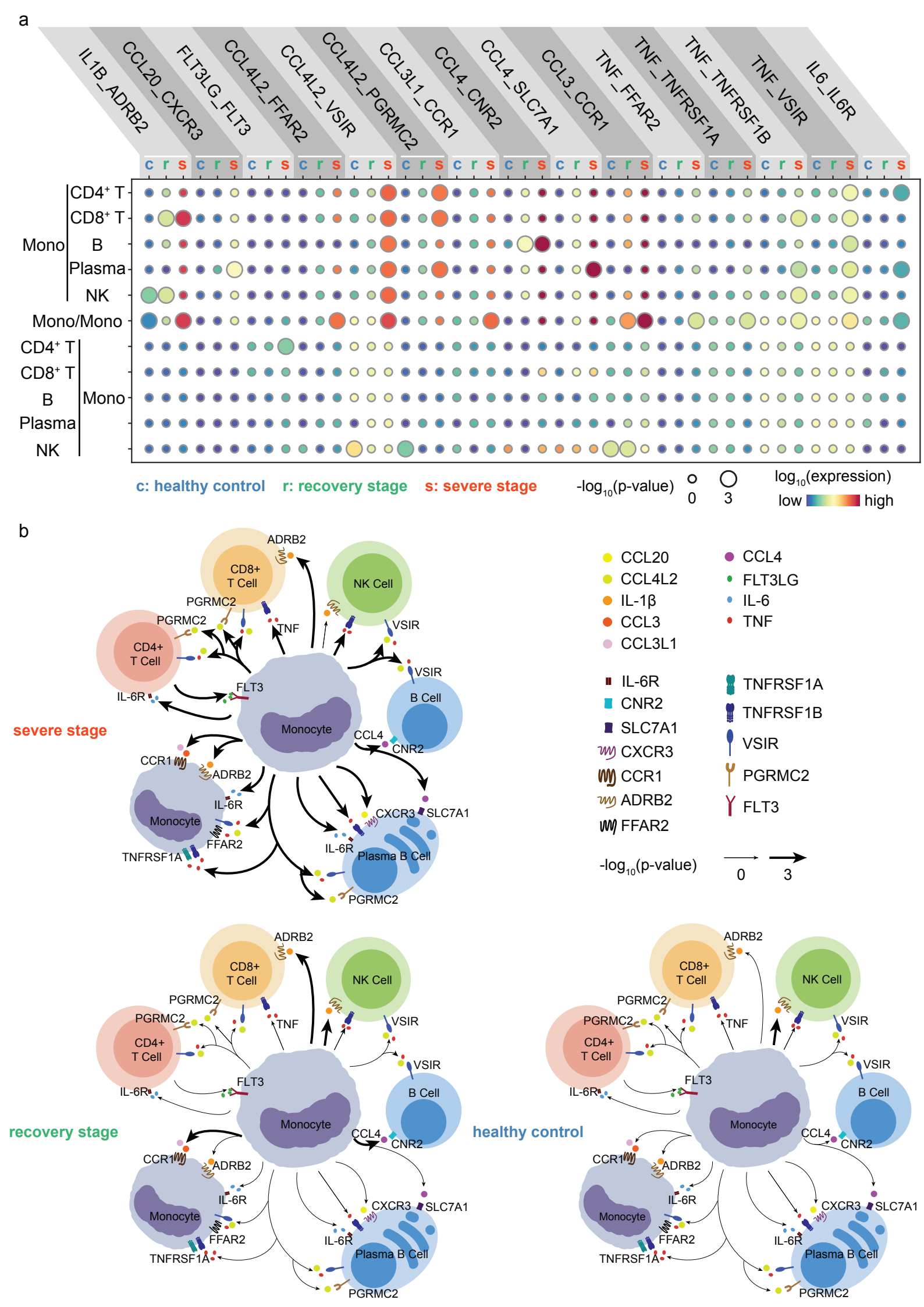
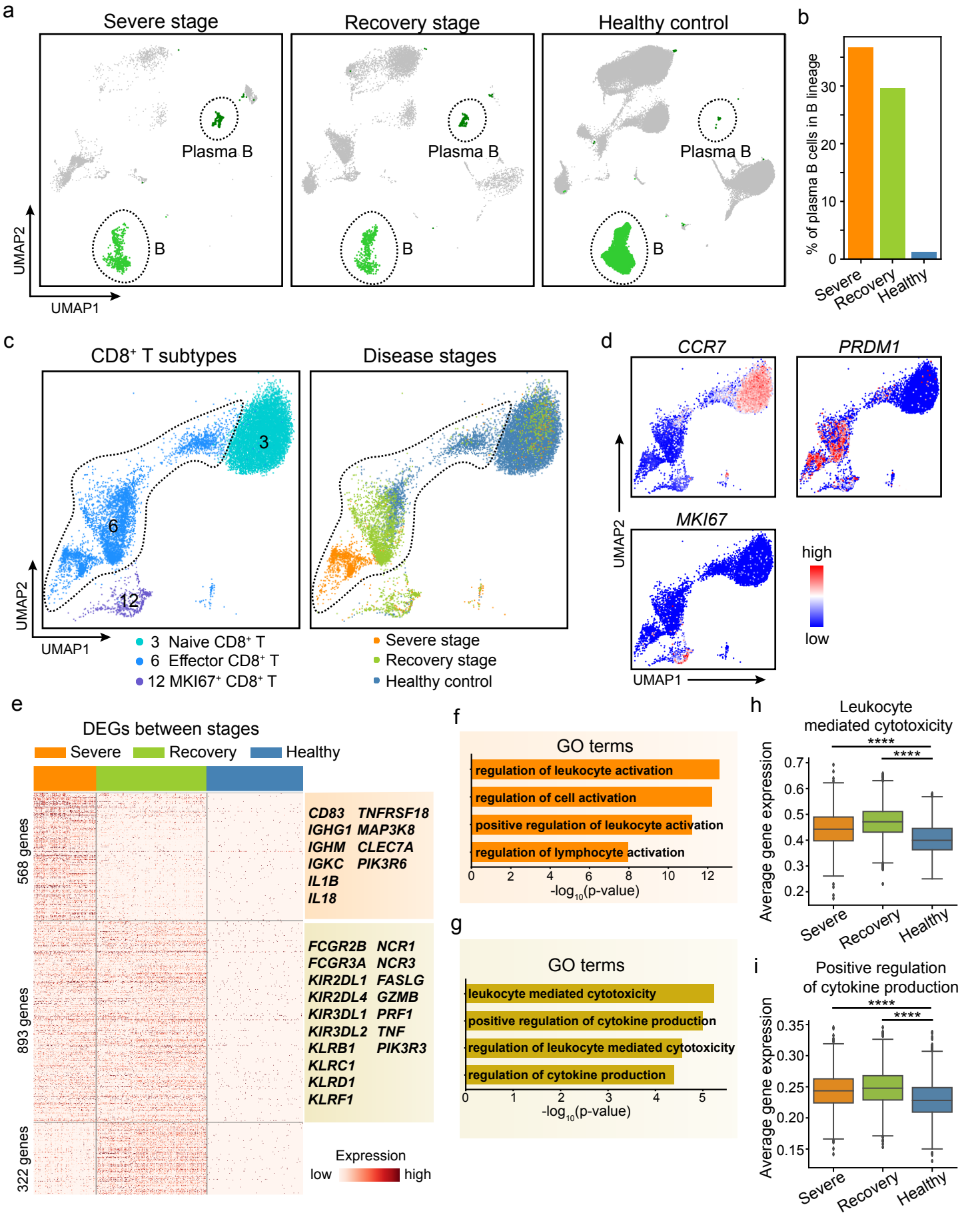
Figure1


a
Severe COVID-19 Patients
Tocilizumab
PZ
PW
Day 1
Day 5
Day 1
Day 5
Day 7
Severe stage
Recovery stage
PBMC
Single-cell RNA-seq
Droplets
Healthy PBMC from 10X
Integration
Data Analysis
b
Cell types
CD4⁺ T
Mono
Plasma B
CD8⁺ T
NK
B
UMAP2
UMAP1
c
Disease stages
d
Patients
Severe stage
Recovery stage
Healthy controls
Patient PW
Patient PZ
Healty controls
CD4⁺ T
CD8⁺ T
Mono
γδT
NK
B
Plasma B
mDC
pDC
Platelet
Progenitor
Unknown
e
PTPRC
CD14
FCG3A
CD3D
IL7R
CCR7
CD8A
MKI67
TRGC1
NKG7
CD79A
CD38
CD1C
CLEC4C
PPBP
CD34
CD14⁺ Mono
CD16⁺ Mono
Naive CD4⁺ T
Effector CD4⁺ T
Naive CD8⁺ T
Effector CD8⁺ T
MKI67⁺ CD8⁺ T
γδT
NK
B
Plasma B
mDC
pDC
Platelet
Progenitor
Unknown
f
Proportion of stages in each cell cluster
Percentage of cells
Clusters
CD14⁺ Mono
CD16⁺ Mono
Naive CD4⁺ T
Effector CD4⁺ T
Naive CD8⁺ T
Effector CD8⁺ T
MKI67⁺ CD8⁺ T
γδT
NK
B
Plasma B
mDC
pDC
Platelet
Progenitor
Unknown


a
Monocyte subtypes
Severe stage
Recovery stage
Healthy control
UMAP2
UMAP1
Cluster
9
2
13
17
14
CD14⁺ Mono
CD16⁺ Mono
b
% of cluster 9 in monocytes
100
80
60
40
20
0
Severe
Recovery
Healthy
c
Expression of cytokines
TNF
IL6
IL1B
CCL3
CCL4
CXCL2
high
low
UMAP2
UMAP1
d
DEGs between stages
9
2
13
17
14
Severe
Recovery
Healthy
1183 genes
379 genes
204 genes
569 genes
APOE
AREG
TNF
CXCL8
CCL3
CXCL9
CCL4
FOSL1
CCL20
NLRP3
CCR3
PHLDA2
IL1B
IL1R2
IL6
IL18R1
IL10
NFIL3
CXCL2
MAP3K8
CXCL3
MDK
C1QA
MAFF
C1QB
CD81
C1QC
ETS2
FGFR1
GINS1
MCM4
CCNB1
FBXO5
MKI67
CSNK1D
ATXN3
SCO1
TAF1
TMF1
USO1
Expression
low
high
e
Acute inflammatory response
****
****
Average gene expression
0.5
0.4
0.3
0.2
0.1
Severe
Recovery
Healthy
f
Leukocyte chemotaxis
****
****
Average gene expression
0.6
0.5
0.4
0.3
0.2
Severe
Recovery
Healthy
g
Enriched motifs in different stages
Severe
Recovery
Healthy
EZH2 (21g)
XBP1 (217g)
KLF9 (12g)
NR4A1 (13g)
FOSL1 (59g)
MAFB (14g)
PRDM1 (10g)
ATF3 (70g)
HIVEP2 (148g)
KLF10 (15g)
PHLDA2 (33g)
NFIL3 (24g)
ETS2 (439g)
MAFF (280g)
BHLHE40 (1076g)
-1.5
1.5
Z-score
h
Gene expression
ETS2
NFIL3
PHLDA2
high
low
Motif enrichment
ETS2
NFIL3
PHLDA2
UMAP2
UMAP1


a
IL1B_ADRB2
CCL20_CXCR3
FLT3LG_FLT3
CCL4L2_FFAR2
CCL4L2_VSIR
CCL4L2_PGRMC2
CCL3L1_CCR1
CCL4_CNR2
CCL4_SLC7A1
CCL3_CCR1
TNF_FFAR2
TNF_TNFRSF1A
TNF_TNFRSF1B
TNF_VSIR
IL6_IL6R
c r s
Mono
CD4+ T
CD8+ T
B
Plasma
NK
Mono/Mono
c: healthy control
r: recovery stage
s: severe stage
-log10(p-value)
0
3
log10(expression)
low
high
b
severe stage
recovery stage
healthy control
CD8+ T Cell
NK Cell
CD4+ T Cell
B Cell
Monocyte
Plasma B Cell
ADRB2
PGRMC2
TNF
VSIR
FLT3
IL-6R
CCL4
CNR2
CCR1
CXCR3
SLC7A1
FFAR2
TNFRSF1A
CCL20
CCL4L2
IL-1β
CCL3
CCL3L1
CCL4
FLT3LG
IL-6
TNF
IL-6R
CNR2
SLC7A1
CXCR3
CCR1
ADRB2
FFAR2
TNFRSF1A
TNFRSF1B
VSIR
PGRMC2
FLT3
-log10(p-value)
0
3


a
Severe stage
Recovery stage
Healthy control
Plasma B
B
UMAP2
UMAP1
b
% of plasma B cells in B lineage
0
10
20
30
Severe
Recovery
Healthy
c
CD8⁺ T subtypes
Disease stages
3
6
12
UMAP2
UMAP1
3 Naive CD8⁺ T
6 Effector CD8⁺ T
12 MKI67⁺ CD8⁺ T
Severe stage
Recovery stage
Healthy control
d
CCR7
PRDM1
MKI67
UMAP2
UMAP1
high
low
e
DEGs between stages
Severe
Recovery
Healthy
568 genes
893 genes
322 genes
CD83 TNFRSF18
IGHG1 MAP3K8
IGHM CLEC7A
IGKC PIK3R6
IL1B
IL18
FCGR2B NCR1
FCGR3A NCR3
KIR2DL1 FASLG
KIR2DL4 GZMB
KIR3DL1 PRF1
KIR3DL2 TNF
KLRB1 PIK3R3
KLRC1
KLRD1
KLRF1
Expression
low
high
f
GO terms
regulation of leukocyte activation
regulation of cell activation
positive regulation of leukocyte activation
regulation of lymphocyte activation
0
2
4
6
8
10
12
-log10(p-value)
g
GO terms
leukocyte mediated cytotoxicity
positive regulation of cytokine production
regulation of leukocyte mediated cytotoxicity
regulation of cytokine production
0
1
2
3
4
5
-log10(p-value)
h
Leukocyte mediated cytotoxicity
****
****
Average gene expression
0.2
0.3
0.4
0.5
0.6
0.7
Severe
Recovery
Healthy
i
Positive regulation of cytokine production
****
****
Average gene expression
0.15
0.20
0.25
0.30
0.35
Severe
Recovery
Healthy