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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40 **ABSTRACT**

41

42 Coronavirus disease 2019 (COVID-19) has caused more than 40,000 deaths

43 worldwide[1]. Approximately 14% of patients with COVID-19 experienced severe

44 disease and 5% were critically ill[2]. Studies have shown that dysregulation of the

45 COVID-19 patients' immune system may lead to inflammatory storm and cause severe

46 illness and even death[3,4]. Tocilizumab treatment targeting interleukin 6 receptor has

47 shown inspiring clinical results of severe COVID-19 patients[5]. However, the immune

48 network with Tocilizumab treatment at single cell resolution has not been uncovered.

49 Here, we profiled the single-cell transcriptomes of 13,289 peripheral blood

50 mononuclear cells isolated at three longitudinal stages from two severe COVID-19

51 patients treated with Tocilizumab. We identified a severe stage-specific monocyte

52 subpopulation and these cells centric immune cell interaction network connected by the

53 inflammatory cytokines and their receptors. The over-activated inflammatory immune

54 response was attenuated after Tocilizumab treatment, yet immune cells including

55 plasma B cells and $CD8^+$ T cells still exhibited an intense humoral and cell-mediated

56 anti-virus immune response in recovered COVID-19 patients. These results provided

57 critical insights into the immunopathogenesis of severe COVID-19 and revealed

58 fundamentals of effectiveness in Tocilizumab treatment.

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Main

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

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

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

123   in patients versus control.

124   Monocytes were reported to play a vital role in CAR-T induced cytokine-release

125   syndrome[14] and SARS-CoV-2 infection caused inflammatory storm[4], therefore we

126   explored the features and functions of the monocyte from COVID-19 patients. We

127   detected 1,737 monocytes in patients, 927 from severe stage and 810 from recovery

128   stage, and integrated 9,787 monocytes from health control. The UMAP plot displayed

129   two main clouds of monocytes that were clearly segregated (Fig 2a). One monocyte

130   subpopulation (cluster 9) was almost exclusively consisted of cells from severe stage

131   and others (cluster 2, 13, 14, 17) were dominated by cells from the recovery and healthy

132   stages (Fig 2b), suggesting a severe-stage specific monocyte subpopulation.

133   We then investigated the expressions of several selected inflammatory cytokines

134   and observed that these genes were all significantly enriched in severe stage-specific

135   monocytes (Fig. 2c, $P < 0.001$, Wilcoxon rank-sum test). To further explore the

136   transcriptional differences among the monocytes' subtypes, we performed a pairwise

137   comparison of the gene expressions in severe, recovery and healthy stages. We obtained

138   2,335 differentially expressed genes (DEGs) enriched in each stage, within which

139   reported cytokine storm related genes, such as *TNF*[9], *IL10*[9], *CCL3*[9] and *IL6*[4] were found

140   significantly higher expressed in severe stage-specific monocytes (Fig 2d,

141   Supplementary Table 3). In addition, we also discovered a large number of significant

142   and inflammatory related genes that were less reported (Fig 2d, fold change > 2, $P <$

143   10^{-3}), including chemokine genes *CCL4*, *CCL20*, *CXCL2*, *CXCL8* and *CXCL9*,

144   inflammasome activation associated genes *NLRP3* and *IL1B*, and complement pathway

145   genes *C1QA*, *C1QB* and *C1QC* (Extended Data Fig.2a-c, Supplementary Table 4).

146   These results indicated that this monocyte subpopulation may contribute to the

147   inflammatory storm in severe COVID-19 patients.

148   We also observed that genes involved in "acute inflammatory response" and

149   "leukocyte chemotaxis" were significantly decreased at recovery and healthy stage

150   (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; $P < 10^-$

207 [5]). We further detected elevated expression of the 108 and 449 genes involved in these

208 GO terms (Fig. 4h, i, Supplementary Table 10). Together, these results demonstrated

209 the critical evidence that robust adaptive immune responses against SARS-CoV-2

210 infection exist in severe stage and remain after Tocilizumab treatment.

211 The immune system is crucial to fight off viral infection[20,21]. Recent studies have

212 illustrated that monocytes may be the main cause of exacerbation and even death of

213 COVID-19 patients through inflammatory storms[4]. In this study, we discovered a

214 specific monocyte subpopulation that may lead to the inflammatory storm in patients

215 at severe stage through single-cell mRNA sequencing. By analyzing the monocyte-

216 centric ligand/receptor interactions, we revealed a severe stage-specific landscape of

217 peripheral immune cell communication that may drive the inflammatory storm in

218 COVID-19 patients. With that we obtained a list of cytokine storm relevant

219 ligand/receptors that can serve as candidate drug targets to treat the disease, and

220 provided mechanistic insights of the immunopathogenesis of COVID-19.

221 There are always questions about whether Tocilizumab treatment may affect the

222 antiviral effect of the body[22,23]. Our single cell profiles illustrated a sustained humoral

223 and cell-mediated anti-virus immune response of COVID-19 patients at both severe and

224 recovery stage. For example, the proportion of plasma B cells with antibody-secreting

225 function were keeping at high levels and the cytotoxicity and cytokine production of

226 effector CD8$^+$ T cells were also remained stable in severe COVID-19 patients after

227 Tocilizumab treatment.

228 The distributions of NK cells were significantly different in the two patients

229 (Extended Data Fig.1b), and the analysis of gene expression differences did not enrich

230 significant biological functions, therefore we did not discuss them in depth. The normal

231 functions of other cell types, such as γδT cells and DCs, were almost lost under severe

232 conditions, and the contribution of these cells to the progression of the disease requires

233 further investigation.

234

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

264   contain 3,500,000 unique 10X Barcodes. After generation of single-cell gel bead-in-

265   emulsions (GEMs), RT was performed using a C1000 Touch^{TM} Thermal Cycler (Bio-

266   Rad). The amplified cDNA was purified with SPRIselect beads (Beckman Coulter).

267   Single-cell libraries were then constructed following fragmentation, end repair, polyA-

268   tailing, adaptor ligation, and size selection based on the manufacturer's standard

269   parameters. Each sequencing library was generated with unique sample index. Libraries

270   were sequenced on the Illumina NovaSeq 6000 system.

271

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

273   The raw sequencing data of patients and health donors were processed using Cell

274   Ranger (version 3.1.0) against the GRCh38 human reference genome with default

275   parameters, and data from different patients and disease stages were combined by the

276   Cell Ranger 'aggr' function. We are uploading the scRNA-seq data of PBMCs from the

277   2 severe COVID-19 patients to the Genome Sequence Archive at BIG Data Center and

278   the accession number will be available upon request. We also used the scRNA-seq data

279   of PBMCs from 2 healthy donors, which can be downloaded from the 10X genomics

280   official website. Firstly, we filtered low quality cells using Seurat[13] (version 3.1.4). For

281   cells from COVID-19 patients (PZ and PW), we retained cells with detected gene

282   numbers between 500 and 6,000 and mitochondrial UMIs less than 10%. For cells from

283   healthy donors, we retained cells with detected gene numbers between 300 and 5,000

284   and mitochondrial UMIs less than 10%. Subsequently we normalized gene counts for

285   each cell using the "NormalizeData" function of Seurat with default parameters.

286   In downstream data processing, we used canonical correlation analysis (CCA) and

287   the top 40 canonical components to find the "anchor" cells between patients and healthy

288   controls. We then used the "IntegrateData" function in Seurat to integrate all the cells.

289   We clustered cells based on the integrated dataset using Seurat with parameter

290   "resolution=0.3" and generated 20 clusters. To display cells in a 2-dimensional space,

291   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

320 values of these motifs between severe stage and healthy control, and selected severe-

321 specific enriched motifs with fold change >1.5 and P value < 10^{-100}.

322 We then applied the enrichment scores of the severe-specific enriched motifs and

323 the expressions of their targeted genes to Cytoscape[25] to construct a connection map for

324 the gene regulatory network, as shown in Extended Data Fig. 2b. The thickness of line

325 connecting TFs and target genes represented the weight of regulatory link predicted by

326 SCENIC.

327

328 **Ligand/receptor interaction analysis**

329 To identify potential cellular communications between monocytes and other cell types

330 (CD4$^+$ T, CD8$^+$ T, B, plasma B and NK cells), we applied the CellphoneDB[16] algorithm

331 to the scRNA-seq profiles from the severe, recovery and healthy stages. CellphoneDB

332 evaluated the impact of a ligand/receptor interactions based on the ligand expression in

333 one cell type and its corresponding receptor expression in another cell type. To identify

334 the enriched ligand/receptor interactions in patients at severe stage, we selected the

335 ligand/receptor interactions with more significant (P value < 0.05) cell-cell interaction

336 pairs in the severe stage than that in the recovery and healthy stages. We also included

337 ligand/receptor pairs which were highly expressed in severe stage.

338

339 **Data Availability**

340 We are uploading the scRNA-seq data of PBMCs from the 2 severe COVID-19 patients

341 to the Genome Sequence Archive at BIG Data Center and the accession number will be

342 available upon request. We also used the scRNA-seq data of PBMCs from 2 healthy

343 donors, which can be downloaded from the 10X genomics official website.

344

352

353   **Author Contributions**

354   K.Q. conceived and supervised the project; K.Q., C.G. and J.L. designed the

355   experiments; C.G. and J.L. performed the experiments and conducted all the sample

356   preparation for NGS with the help from H.M. and T.C.; B.L. performed the data

357   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.,

358   X.G. and J.F; T.C., X.W., L.L. and X.M. provided COVID-19 blood samples and

359   clinical information. K.Q., C.G., J.L. and B.L. wrote the manuscript with the help of

360   B.F., H.W. and all the other authors.

361

362   **Competing interests**

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

364

365   **Figure Legends**

366

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

368   Flowchart depicting the overall design of the study. Blood draws from patient PZ were

369   performed at 2 time points (Day1 and Day5), and PW at 3 time points (Day1, Day5,

370   and Day7). Patients at Day 1 were at severe stage and Day 5 and Day 7 were at recovery

371   stages. Samples were collected within 12 hours of Tocilizumab was given at Day 1. **b-**

372   **d**, UMAP representations of single-cell transcriptomes of 13,289 PBMCs. Cells are

373   color-coded by clusters **(b)**, disease stages **(c)**, and the corresponding patient or healthy

374   control **(d)**. Dotted circles represented cell types with > 5% proportion of PBMCs in **(b)**

375   and clusters significantly enriched in patients versus control in **(c, d)**. Mono, monocyte;

376   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

433 and recovery stages **(b)**, and healthy controls **(c)**. **d,** Severe stage specific monocyte

434 regulatory network predicted by SCENIC. Transcription factors were shown in

435 rectangles and their target genes in circles.

436

437 **Supplementary Table 1 | Baseline characteristcs and laboratory findings of**

438 **COVID-19 patients in this study.**

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

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

441 **Supplementary Table 4 | GOterms of DEGs of CD14 monocytes.**

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

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

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

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

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

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

448

449 **Reference:**

450

451 1   WHO. Coronavirus disease 2019 (COVID-19) Situation Report - 72.   (2020).

452 2   Wu, Z. & McGoogan, J. M. Characteristics of and Important Lessons From the
453   Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a
454   Report of 72314 Cases From the Chinese Center for Disease Control and
455   Prevention. *JAMA*, doi:10.1001/jama.2020.2648 (2020).

456 3   Mehta, P. *et al.* COVID-19: consider cytokine storm syndromes and
457   immunosuppression. *Lancet* **395**, 1033-1034, doi:10.1016/S0140-
458   6736(20)30628-0 (2020).

459 4   Zhou, Y. *et al.* Pathogenic T cells and inflammatory monocytes incite
460   inflammatory storm in severe COVID-19 patients. *National Science Review*,
461   doi:10.1093/nsr/nwaa041 (2020).

462 5   Xu, X., Han, Mingfeng, Li, Tiantian, Sun, Wei, Wang, Dongsheng, Fu, Binqing,
463   Zhou, Yonggang, Zheng, Xiaohu, Yang, Yun, Li, Xiuyong, Zhang, Xiaohua, Pan,
464   Aijun, Wei, Haiming. Effective Treatment of Severe COVID-19 Patients with
465   Tocilizumab. *[ChinaXiv:202003.00026]* (2020).

466 6   Zumla, A., Hui, D. S., Azhar, E. I., Memish, Z. A. & Maeurer, M. Reducing

467   mortality from 2019-nCoV: host-directed therapies should be an option. *Lancet*
468   **395**, e35-e36, doi:10.1016/S0140-6736(20)30305-6 (2020).

469   7   Chaofu Wang, J. X., Lei Zhao et al. Aveolar Macrophage Activation and
470   Cytokine Storm in the Pathogenesis of Severe COVID-19. *PREPRINT (Version*
471   *1) available at Research Square [+*https://doi.org/10.21203/rs.3.rs-19346/v1*+]*
472   (2020).

473   8   Li, G. *et al.* Coronavirus infections and immune responses. *J Med Virol* **92**, 424-
474   432, doi:10.1002/jmv.25685 (2020).

475   9   Huang, C. *et al.* Clinical features of patients infected with 2019 novel
476   coronavirus in Wuhan, China. *Lancet* **395**, 497-506, doi:10.1016/S0140-
477   6736(20)30183-5 (2020).

478   10   Kotch, C., Barrett, D. & Teachey, D. T. Tocilizumab for the treatment of
479   chimeric antigen receptor T cell-induced cytokine release syndrome. *Expert Rev*
480   *Clin Immunol* **15**, 813-822, doi:10.1080/1744666X.2019.1629904 (2019).

481   11   Le, R. Q. *et al.* FDA Approval Summary: Tocilizumab for Treatment of
482   Chimeric Antigen Receptor T Cell-Induced Severe or Life-Threatening
483   Cytokine Release Syndrome. *Oncologist* **23**, 943-947,
484   doi:10.1634/theoncologist.2018-0028 (2018).

485   12   Zheng, G. X. *et al.* Massively parallel digital transcriptional profiling of single
486   cells. *Nat Commun* **8**, 14049, doi:10.1038/ncomms14049 (2017).

487   13   Butler, A., Hoffman, P., Smibert, P., Papalexi, E. & Satija, R. Integrating single-
488   cell transcriptomic data across different conditions, technologies, and species.
489   *Nat Biotechnol* **36**, 411-420, doi:10.1038/nbt.4096 (2018).

490   14   Norelli, M. *et al.* Monocyte-derived IL-1 and IL-6 are differentially required for
491   cytokine-release syndrome and neurotoxicity due to CAR T cells. *Nat Med* **24**,
492   739-748, doi:10.1038/s41591-018-0036-4 (2018).

493   15   Aibar, S. *et al.* SCENIC: single-cell regulatory network inference and clustering.
494   *Nat Methods* **14**, 1083-1086, doi:10.1038/nmeth.4463 (2017).

495   16   Vento-Tormo, R. *et al.* Single-cell reconstruction of the early maternal-fetal
496   interface in humans. *Nature* **563**, 347-353, doi:10.1038/s41586-018-0698-6
497   (2018).

498   17   Guan, W. *et al.* Clinical Correlations of Transcriptional Profile in Patients
499   Infected With Avian Influenza H7N9 Virus. *J Infect Dis* **218**, 1238-1248,
500   doi:10.1093/infdis/jiy317 (2018).

501   18   Wang, Z. *et al.* Recovery from severe H7N9 disease is associated with diverse
502   response mechanisms dominated by CD8(+) T cells. *Nat Commun* **6**, 6833,
503   doi:10.1038/ncomms7833 (2015).

504   19   Thevarajan, I. *et al.* Breadth of concomitant immune responses prior to patient
505   recovery: a case report of non-severe COVID-19. *Nature Medicine*,
506   doi:10.1038/s41591-020-0819-2 (2020).

507   20   Braciale, T. J., Sun, J. & Kim, T. S. Regulating the adaptive immune response
508   to respiratory virus infection. *Nat Rev Immunol* **12**, 295-305,

509 doi:10.1038/nri3166 (2012).

510 21 Rouse, B. T. & Sehrawat, S. Immunity and immunopathology to viruses: what
511 decides the outcome? *Nat Rev Immunol* **10**, 514-526, doi:10.1038/nri2802
512 (2010).

513 22 Ahn, S. S. *et al.* Safety of Tocilizumab in Rheumatoid Arthritis Patients with
514 Resolved Hepatitis B Virus Infection: Data from Real-World Experience. *Yonsei*
515 *Med J* **59**, 452-456, doi:10.3349/ymj.2018.59.3.452 (2018).

516 23 Bersanelli, M. Controversies about COVID-19 and anticancer treatment with
517 immune checkpoint inhibitors. *Immunotherapy*, doi:10.2217/imt-2020-0067
518 (2020).

519 24 Zhou, Y. *et al.* Metascape provides a biologist-oriented resource for the analysis
520 of systems-level datasets. *Nat Commun* **10**, 1523, doi:10.1038/s41467-019-
521 09234-6 (2019).

522 25 Cline, M. S. *et al.* Integration of biological networks and gene expression data
523 using Cytoscape. *Nat Protoc* **2**, 2366-2382, doi:10.1038/nprot.2007.324 (2007).

Figure1

a Monocyte subtypes | Severe stage | Recovery stage | Healthy control

UMAP2 / UMAP1

Cluster
- 9
- 2
- 13
- 17

CD14⁺ Mono

- 14

CD16⁺ Mono

b % of cluster 9 in monocytes — 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 — Severe, Recovery, Healthy
**** ****

f Leukocyte chemotaxis

Average gene expression — 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

Severe stage   Recovery stage   Healthy control

Plasma B   Plasma B   Plasma B

B   B   B

UMAP2

UMAP1

b

% of plasma B cells in B lineage

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

CD83	TNFRSF18
IGHG1	MAP3K8
IGHM	CLEC7A
IGKC	PIK3R6
IL1B	
IL18	

893 genes

FCGR2B	NCR1
FCGR3A	NCR3
KIR2DL1	FASLG
KIR2DL4	GZMB
KIR3DL1	PRF1
KIR3DL2	TNF
KLRB1	PIK3R3
KLRC1	
KLRD1	
KLRF1	

322 genes

Expression

low   high

f

GO terms

regulation of leukocyte activation

regulation of cell activation

positive regulation of leukocyte activation

regulation of lymphocyte activation

-log$_{10}$(p-value)

g

GO terms

leukocyte mediated cytotoxicity

positive regulation of cytokine production

regulation of leukocyte mediated cytotoxicity

regulation of cytokine production

-log$_{10}$(p-value)

h

Leukocyte mediated cytotoxicity

****

****

Average gene expression

Severe   Recovery   Healthy

i

Positive regulation of cytokine production

****

****

Average gene expression

Severe   Recovery   Healthy