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2 **Single-cell transcriptomic atlas of individuals receiving inactivated COVID-19**

3 **vaccines reveals distinct immunological responses between vaccine and natural**

4 **SARS-CoV-2 infection**

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39

Abstract

41 To control the ongoing COVID-19 pandemic, CoronaVac (Sinovac), an inactivated

42 vaccine, has been granted emergency use authorization by many countries. However,

43 the underlying mechanisms of the inactivated COVID-19 vaccine-induced immune

44 response remain unclear, and little is known about its features compared to SARS-

45 CoV-2 infection. Here, we implemented single-cell RNA sequencing (scRNA-seq) to

46 profile longitudinally collected PBMCs (peripheral blood mononuclear cells) in six

47 individuals immunized with CoronaVac and compared these to the profiles of

48 COVID-19 infected patients from a Single Cell Consortium. Both inactivated

49 vaccines and SARS-CoV-2 infection drove changes in immune cell type proportions,

50 caused B cell activation and differentiation, and induced the expression of genes

51 associated with antibody production in the plasma. The inactivated vaccine and

52 SARS-COV-2 infection also caused alterations in peripheral immune activity such as

53 interferon response, inflammatory cytokine expression, innate immune cell apoptosis

54 and migration, effector T cell exhaustion and cytotoxicity, however, the magnitude of

55 change was greater in COVID-19 patients, especially those with severe disease, than

56 in immunized individuals. Further analyses revealed a distinct peripheral immune cell

57 phenotype associated with CoronaVac immunization (HLA class II upregulation and

58 *IL21R* upregulation in naïve B cells) versus SARS-CoV-2 infection (HLA class II

59 downregulation and *IL21R* downregulation in naïve B cells severe disease). There

60 were also differences in the expression of important genes associated with

61 proinflammatory cytokines and thrombosis. In conclusion, this study provides a

62 single-cell atlas of the systemic immune response to CoronaVac immunization and

63 reveals distinct immune responses between inactivated vaccines and SARS-CoV-2

64 infection.

65 **Keywords:** Inactivated vaccine, CoronaVac, COVID-19, SARS-CoV-2, Single-cell

66 sequencing, Immunological responses

Introduction

COVID-19 (coronavirus disease 2019), caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), is an unprecedented threat to global public health and has rapidly spread throughout the world (Cevik et al., 2020). COVID-19 has led to high mortality and morbidity worldwide, and as of Aug 24, 2021, 213,752,662 laboratory-confirmed cases of SARS-CoV-2 infection have been reported, resulting in 4,459,381 deaths (WHO COVID-19 Dashboard). As there are no effective drugs available at this time against COVID-19, safe and effective COVID-19 vaccines are urgently required to control the pandemic and reduce the global burden of SARS-CoV-2 (Mehra et al., 2020).

Various candidate vaccines including inactivated viral vaccines, live attenuated vaccines, nucleic acid vaccines, viral-vectored vaccines, and protein or peptide subunit vaccines are being rapidly developed, tested, and granted approval for emergency use (Amanat and Krammer, 2020). Each vaccine has advantages and disadvantages and these have been reviewed elsewhere (Dong et al., 2020; Poland et al., 2020). Among these candidate vaccines, the inactivated COVID-19 vaccines are among one of the most widely used and well developed vaccines due to their ease of production and scale-up, and relatively low cost. They are produced by growing SARS-CoV-2 in cell culture (e.g., Vero cells), followed by chemical inactivation of the virus (Krammer, 2020). Inactivated vaccines present the whole SARS-COV-2 virus for immune recognition, thus the immune responses are likely to target not only the unique protein (e.g., S protein) of the virus but also matrix, nucleoprotein and envelope (Krammer, 2020). Moreover, the inactivated vaccines exhibit stable expression of conformation-dependent antigenic epitopes, and also offer advantages in a variety of different populations (e.g., those with degrees of immune senescence) (Iversen and Bavari, 2021b).

CoronaVac (initially known as PiCoVacc) from Sinovac, is a leading Chinese COVID-19 vaccine and was devised with β-propiolactone as an inactivating agent and formulated with aluminum hydroxide as an adjuvant (Gao et al., 2020). The

96   inactivated CoronaVac vaccine is a whole-virus preparation administered in a two-

97   dose regimen (at day 0 and day>21), and the immunogenicity, safety and tolerability

98   have been assessed in different populations, including children and adolescent aged 3-

99   17 years old (Han et al., 2021), adults aged 18-59 (Zhang et al., 2021), and adults

100   aged 60 years and older (Wu et al., 2021). Within the scope of combating the SARS-

101   CoV-2 pandemic, CoronaVac has been granted an emergency use authorization by

102   Chinese authorities in July, 2020 (Poland et al., 2020), and a host of others countries

103   such as Turkey, Chile, Brazil, Indonesia, etc. (Bayram et al.; Fortner and Schumacher,

104   2021; Muena et al., 2021; Shervani et al., 2020).

105   Currently, the knowledge about the immunity generated by COVID-19 vaccines

106   (including the inactivated vaccines) are limited with researchers understanding less

107   about this than about immunity to natural SARS-CoV-2 infection. Although clinical

108   trial data have demonstrated that the current COVID-19 vaccines approved (including

109   CoronaVac) can elicit immunity with a high degree of safety, efficacy and tolerability,

110   much remains to be learned concerning the genetic drivers of COVID-19 vaccine-

111   elicited humoral and/or cellular immunity, defining detailed targets of the immune

112   response at the epitope level, and characterizing the B-cell receptor and T-cell

113   receptor repertoire induced by COVID-19 vaccines (Poland et al., 2020). Inactivated

114   vaccines (such as CoronaVac) have been shown to keep the immunogenicity of the

115   SARS-CoV-2 virus, and can elicit an immune response, however whether there is a

116   distinct immune response landscape between natural SARS-CoV-2 infection and the

117   inactivated COVID-19 vaccine remains unclear.

118   Here, we implemented scRNA-seq (single-cell RNA sequencing) to obtain a

119   comprehensive and unbiased visualization of PBMCs (Peripheral blood mononuclear

120   cells) from healthy adults immunized with the inactivated COVID-19 vaccine,

121   CoronaVac, at 3 pivotal time points, including day 0 (before vaccination), day 21 after

122   the first dose and day 14 after the second dose (**Figure 1A**). This study provides a

123   high-resolution transcriptomic landscape of PBMCs during the immune response to

124   CoronaVac immunization, which will foster a better understanding of the protective

125   immune response generated by inactivated COVID-19 vaccines. Then, we compared

126   this data to the reported profiles from a Single Cell Consortium for COVID-19 (Ren

127   et al., 2021), revealing the distinct immune features between natural SARS-CoV-2

128   infection and the inactivated COVID-19 vaccine.

129

130   **Results**

131   **Single-cell transcription profiling of PBMCs**

132   To identify features of the immunological hallmarks from individuals receiving

133   inactivated COVID-19 vaccines (CoronaVac), the droplet-based scRNA-seq (10 X

134   Genomics) was conducted for studying the transcriptomic profiles of PBMCs, which

135   were longitudinally collected from six individuals at three pivotal time points (**Fig**

136   **1A**). Single-cell B cell receptor (BCR) and T cell receptor (TCR) sequencing were

137   also performed for each sample. According to the time points, these samples were

138   classified into three conditions: no injection (NJ, day=0, PMBCs were collected

139   before vaccination), first injection (FJ, Day=21, PBMCs were collected at day 21 after

140   the first dose) and second injection (SJ, Day=35 PBMCs were collected at day 14

141   after second dose). The associated metadata of the six individuals enrolled and the

142   three conditions are detailed in **Supplementary Table S1**. After the single-cell

143   analysis pipeline (Refer to Methods), we obtained ~0.895 billion unique transcripts

144   belonging to 178268 cells from the PBMCs of vaccinated individuals. Among these

145   cells, 60783 cells (34.1%) were from the NJ conditions, 47451 cells (26.6%) were

146   from the FJ condition, and 70034 cells (39.3%) from the SJ condition. Next, we

147   integrated all high-quality cells into an unbatched and comparable dataset, which was

148   subjected to principal component analysis after correction for read depth and

149   mitochondrial read counts (**Fig S1A-B**)

150   To reveal immune cell populations in individuals administered with inactivated

151   COVID-19 vaccines, the graph-based clustering of UMAP (uniform manifold

152   approximation and projection) was performed. According to the expression of

153   canonical cell-type markers, we identified 10 major cell types (**Fig 1B (left), C-D**;

154 **S1A-B**): B cells (CD79A$^+$CD79B$^+$MS4A$^+$), plasma cells (XBP1$^+$MZB1$^+$), γδ T cells

155 (TRDV1$^+$TRDV9$^+$), natural killer (NK) cells (CD11b$^+$NKG7$^+$KLRD1$^+$NKG2A$^+$),

156 CD4$^+$ T cells (CD3D$^+$CD3E$^+$CD40LG$^+$), CD8$^+$ T cells (CD3D$^+$CD3E$^+$CD8A$^+$CD8B$^+$),

157 mucosal-associated invariant T (MAIT) cells (CD3D$^+$CD3E$^+$SLC4A10$^+$), monocytes

158 (CST3$^+$LYZ$^+$CD68+), dendritic cells (CST3$^+$LYZ$^+$CD163$^+$) and megakaryocytes

159 (CST3$^+$LYZ$^+$PPBP$^+$). At the more granular level, we identified 27 different cell

160 subtypes (**Fig 1B (right)**, **S1A-B**). Likewise, we also successfully identified 10 major

161 cell types (**Fig 1E** (**Left**)) and 27 cell subtypes (**Fig 1E** (**Right**)) for the PBMCs

162 samples reported by a Single Cell Consortium for COVID-19 (Ren et al., 2021) (**Fig**

163 **S1C-F**). As such, the composition of cell subpopulations in peripheral blood from

164 individuals with COVID-19 vaccine and COVID-19 patients were clearly defined

165 (**Fig 1B**, **E**; **S1G**).

166

167 **Differences in major cell type compositions across conditions**

168 We firstly uncovered the differences in cell composition (10 major cell types) across

169 two conditions (FJ and SJ) and then compared that with NJ. According to scRNA-seq

170 data (**Fig 2A-D**), we calculated the relative percentage of 10 major cell types in the

171 PBMCs of each participant at 3 conditions. The relative abundance of CD4+ T cells,

172 CD8+ T cells, γδ T cells, NK cells and MAIT cells at FJ and SJ conditions remained

173 similar when compared with the NJ condition (**Fig 2D**). The relative percentage of B

174 cells appeared to increase in FJ and SJ conditions in comparison with NJ condition,

175 implying that the change in B cells may be related with the humoral immune response

176 after vaccination (**Fig 2D**). The proportions of dendritic cells (DCs), monocytes

177 (Mono) and megakaryocytes (Mega) increased after vaccination (**Fig 2D**). Increased

178 DCs may be involved in antigen presentation to stimulate the immune response to

179 CoronaVac, while increased Mono and Mega may be involved with the potential

180 inflammatory response after vaccination. Of note, the percentage of plasma cells did

181 not significantly increase in the FJ and SJ conditions (**Fig 2D**). This may be due to

182 plasma cells requiring a strong level of continuous antigen stimulation, or that the

183   plasma cell level had decreased or was restored when the PBMC samples were

184   collected at the FJ (at day 21 after first dose) and SJ (at day 14 after first dose)

185   conditions.

186   Next, we compared the cell composition between vaccine and natural SARS-

187   CoV-2 infection. Patients with COVID-19 (n=64) were classified into four conditions:

188   control (Cont; n=15), mild (mild; n=12), severe (seve; n=4) and convalescent (conv;

189   n=33) (**Fig 2E-G, Fig S2A**). After SARS-CoV-2 infection, the proportion of innate

190   immune cells, including NK cells, $\gamma\delta$ T cells, MAIT cells and DCs, decreased with

191   disease severity (**Fig 2E-G**). This trend was different to what was observed with

192   vaccines (**Fig 2D**, **G**; **S2B-D**). Similar with vaccination, the relative abundance of

193   monocytes and megakaryocytes in COVID-19 patients increased with disease severity,

194   and the relative percentage of these cells later declined in Conv conditions (**Fig 2G**).

195   Unlike the inactivated COVID-19 vaccine, a decrease in $CD4^+$ T and $CD8^+$ T cells

196   were observed in COVID-19 infected patients, and this was related with disease

197   severity (**Fig 2D**, **G**; **S2D**). A slight increase in B and plasma cell levels were

198   observed after SARS-CoV-2 mild infection, whereas a massive increase in plasma

199   cells was observed in the Seve condition (**Fig G**; **S2D**). These data suggested that

200   both vaccination with CoranaVac and natural SARS-CoV-2 infection can cause

201   changes in the cell compositions of PBMC.

202

203   **Features of B cell subsets across samples**

204   To reveal the dynamic changes in different B cell subtypes in immunized (**Figure 3A-**

205   **E, Fig S3A**) and infected individuals (**Fig 3F-G**, **S3B-E**), we classified B cells into 6

206   subsets according to the distribution and expression of classical subtype markers. We

207   successfully identified one naïve B subcluster ($MS4A1^+IGHD^+$), one memory B

208   subcluster ($MS4A1^+CD27^+$), one germinal center B subcluster ($MS4A1^+NEIL1^+$), one

209   intermediated transition memory B subcluster (Intermediate memory B,

210    $MS4A1^+IGHD^+CD27^+$), one plasma B subcluster (MZB^+CD38^+) and one

211   proliferating plasma B subsets ($MZB^+CD38^+MKI67+$).

7

212   The general patterns of B/plasma cells were compared across conditions. The

213   relative percentages of an active state B subtype (germinal center B) significantly

214   increased after vaccination, suggesting that B cells may be activated after vaccination

215   (**Fig 3E**), Other B cell subsets, including naïve B cells, memory B cells and

216   intermediate memory B cells, remained similar across the three vaccination conditions

217   (NJ, FJ and SJ) (**Fig 3E**), suggesting that the inactivated COVID-19 vaccine has a

218   relatively low impact on the composition of these B cell subsets. Likewise, SARS-

219   CoV-2 infection also had relatively low impact on the composition of these B cell

220   subsets, with only the memory B subtype decreased in severe COVID-19 patients

221   (**Fig 3G**). Increased plasma and dividing plasma cells were observed in COVID-19

222   patients with the percentage of plasma cells in severe COVID-19 patients reaching 15%

223   while mild COVID-19 patients only reached 3% (**Fig 3G**) (Ren et al., 2021),

224   suggesting that antibody production may be stronger in severe COVID-19 patients,

225   reminiscent of previous findings that higher antibody titers are related with worse

226   clinical outcomes (Long et al., 2020; Okba et al., 2020). Although the levels of plasma

227   cells and dividing plasma cells did not significantly increase after vaccination, the

228   levels of neutralizing antibodies (anti-S-RDB-specific antibody) did significantly

229   increase after the second injection for all 6 individuals (**Fig 3H**, **Fig S3G**, **Table S2**).

230   Interestingly, the levels of neutralizing antibody did not significantly increase after the

231   first dose of CoronaVac (**Fig 3H**, **Fig S3G**, **Table S2**) and significant production of

232   neutralizing antibody was only obtained after the second dose. This implies that two

233   doses of CoronaVac are required for efficient seroconversion.

234   After vaccination, the plasma cells in PBMCs had highly expressed genes which

235   encode the constant regions of immunoglobulin G1 (Ig G1), IgG2, IgA1 or IgA2. This

236   correlates with their function in secreting antigen-specific antibodies and implies that

237   the serum of immunized individuals may have had high titers of SARS-CoV-2-

238   specific antibodies (**Fig 3H-I, S3G**). These findings were also observed in COVID-19

239   patients (**Fig 3J**), and is consistent with previous studies showing high titers of

240   antigen-specific antibodies in the serum of COVID-19 patients (Ni et al., 2020; Ren et

241   al., 2021). Similar to natural SARS-CoV-2 infection, genes encoding IgA1 and IgA2

242   were activated by CoronaVac, and this finding has also been observed in influenza

243   vaccines (Neu et al., 2019).

244

245   **Transcriptomic changes in B cells after vaccination and SARS-CoV-2 infection**

246   To investigate differential transcriptomic changes in B/plasma cells after vaccination,

247   we compared the expression profiles of B/plasma cells in FJ or SJ conditions with the

248   NJ condition. As expected, genes involved in B cell activation, adaptive immune

249   response, response to interferon, and antigen processing and presentation were

250   specifically enriched in B cells after vaccination (**Fig 4A**). This suggests that the B

251   cells were responding to the inactivated COVID-19 vaccine. For SARS-CoV-2

252   infection, genes involved in defense response to virus and interferon signaling

253   pathways were the most highly upregulated in the B cell subset (**Fig S4A**). Notably,

254   we observed that genes associated with the "IFN response" were enriched in both

255   post-vaccination samples and COVID-19 patients (**Fig 4A** and **Fig S4A**).

256   Next, we further examined the expression of important genes (e.g., *PRDM1*, T-

257   bet) that are involved in B/plasma-cell-activation-related processes after vaccination

258   and SARS-CoV-2 infection. Two GO pathways (GO:0002312 and GO:0042113) that

259   were related to the activation of B cells were significantly enriched after vaccination

260   (**Fig 4B**). Several genes (e.g., *PTPRC*, *HMCES* and *SWAP70*) involved in the B cell

261   activation GO pathways (GO:0002312 and GO:0042113), were highly upregulated in

262   the naïve B cell subtype, implying activation of naïve B cells after vaccination (**Fig**

263   **4C**). In contrast, these three genes (*PTPRC*, *HMCES* and *SWAP70*) were down-

264   regulated in severe COVID-19 patients, suggesting that activation of naïve B cells in

265   these individuals may be impaired (**Fig S4B**). Naïve B cells in vaccinated samples

266   also highly expressed *IL4R* and *IL21R* (**Fig 4D**), but these genes were downregulated

267   in activated and memory B cells (**Fig 4E**). This indicates that naïve B subsets were

268   more responsive than activated and memory B cells to IL-4 (interleukin 4) and IL-21,

269   which regulate class switching to IgG, including IgG1, IgG3 or IgG4 (Horns et al.,

270   2020; Pène et al., 2004). Similar findings for *IL4R* and *IL21R* expression were also

271   observed in SARS-CoV-2 mild infection, while *IL4R* and *IL21R* were significantly

272   downregulated in severe COVID-19 patients (**Fig S4C-D**).

273   Six transcription factors (TBX21, ZEB2, TFEC, ZBTB32 and YBX3) associated

274   with the activation of memory B cells were highly expressed in intermediate transition

275   memory B cell (also referred to as activated memory B cells) compared to memory B

276   cells (**Fig 4F** and **Fig S4E**) (Horns et al., 2020). TBX21 (also known as T-bet) has

277   been hypothesized to play a key regulatory role in activation and is required for class

278   switching to IgG2 (Wang et al., 2012). This transcription factor showed higher

279   expression in activated memory B cells than memory B cells (**Fig 4F** and **Fig S4E**). A

280   triad of transcription factors, including PRDM1, XBP1 and IRF4, also had increased

281   expression in activated B cells (which encompasses: germinal center B cell,

282   intermediate transition memory B cell, dividing plasma and plasma) from COVID-19

283   patients and immunized individuals than non-activated B cell (including naïve B cell

284   and memory B cell) (**Fig 4G** and **Fig S4F**). These transcription factors are associated

285   with B-cell-differentiation-related pathways and are required for activating the ASC

286   (antibody-secreting cell) program (Shi et al., 2015). PRDM1 plays a core role in

287   determining and shaping the secretory arm of B cell differentiation and in promoting

288   Ig synthesis. XBP1 is a positively acting transcription factor of the CREB-ATF family

289   that is highly express in plasma cells and is important for increasing protein synthesis

290   in plasma cells (Shaffer et al., 2004). IRF4 is crucial for regulating Ig class-switch

291   recombination, and a previous study has found that sustained and increased

292   concentration of this transcription factor promotes the generation of plasma cells

293   (Ochiai et al., 2013). Finally, three B cell-promoting transcription factors: BACH2,

294   BCL6 and PAX5, showed increased expression in B cells after vaccination (**Fig 4H**)

295   while in severe COVID-19 patients (**Fig S4G**), these transcription factors decreased.

296   BACH2, BCL6 and PAX5 play a key role in determining the fate of B cells during

297   differentiation (Shi et al., 2015).

298   Interestingly in COVID-19 infected samples, expression of important chemokine

299   receptors such as CXCR5 were significantly decreased, especially for severe samples

300   (**Fig S4H**). However, this loss of chemokine receptors was not observed in

301   immunized samples (**Fig 4I**). Decreased chemokine receptors can impair germinal

302   center reactions and ultimately cause dysregulated humoral immunity responses

303   (Mathew et al., 2020; Okada et al., 2002; Reimer et al., 2017). Our data also observed

304   significant upregulation of HLA class II genes after immunization (**Fig 4J**), implying

305   that there is an enhancement of immune cell crosstalk between the adaptive immune

306   cell classes. However in COVID-19 patients, several HLA class II genes were

307   significantly downregulated, especially for severe COVID-19 patients (**Fig S4I**). This

308   suggests a dysregulation of immune cell crosstalk between the adaptive immune cell

309   classes during infection. Together, these data define the transcriptional hallmarks of

310   CoronaVac-induced B cell activation and clonal expansion and revealed underlying

311   differences in the B cell transcriptome between vaccine-induced immunity and SARS-

312   CoV-2 infection.

313

314   **V(D)J gene usage and clonal expansion in B cells after vaccination and SARS-**

315   **CoV-2 infection**

316   BCR information was detected in all B/plasma subsets and in >80% of cells while

317   clonal expansion (clonal size >10) was observed in memory B cells, intermediate

318   transition memory B cells, dividing plasma cells, and plasma cells (**Fig 5A**, **Fig S5A-**

319   **B**). The largest proportion of BCR in B cells was the IGHM subtype and the largest

320   proportion of BCR in plasma cells were IGHA1 and IGHG1 (**Fig 5B**). After

321   vaccination, the percentage of IGHM significantly increased in B cells (**Fig 5C, Fig**

322   **S5A**) while the percentage of IGHA1 decreased in plasma cells (**Fig 5C, S5C, S5D**).

323   The light chain type, IGK and IGL, did not change in B and plasma cells (**Fig 5D**).

324   We compared the clonal expansion of B cell subtypes under different conditions

325   in the vaccine cohort (**Fig 5E**). BCR clonal expansion of plasma cells increased from

326   NJ to FJ to SJ, suggesting that two-doses of CoronaVac induced plasma proliferation

327   of specific BCR clonotypes. In COVID-19 patients, increased clonal expansion in

328 CD8+ T was also observed in severe COVID-19 patients (**Fig 5E**), in agreement with

329 Zhang et al. (Zhang et al., 2020).

330   BCR diversity, as measured by alpha diversity, showed no change after

331 vaccination but was significantly decreased in severe COVID-19 infections (**Fig 5H**).

332 The length distribution of the CDR3 region was similar for all conditions except

333 COVID-19 severe condition (**Fig5I**). These results suggest that vaccine induced

334 immune protection in very gentle way.

335   The usage of IGH V(D)J genes across vaccination and infection conditions were

336 compared (**Fig 5F**). The combinations of the 6 IGHJ and >40 IGHV genes

337 demonstrated that CoronaVac induced many changes in the IGH V(D)J genes (**Fig 5F**

338 left panel). The V(D)J pair pattern were significantly altered after two doses of

339 CoronaVac. For example, the most prevalent pair in NJ was IGHJ2/IGHV3-23 which

340 shifted to IGHJ2/IGHV4-59 and IGHJ1/IGHV3-23 following vaccination. The

341 percentage of IGHJ1/IGHV3-43 and IGHJ1/IGHV3-15 also increased after

342 vaccination. In addition, we also analyzed the usage of IGK/L V(D)J genes (**Fig 5F**

343 right panel) and observed that the V(D)J pair pattern were also altered after two doses

344 of CoronaVac. For example, IGKJ5/IGKV3-11, IGLJ1/IGKV2-14 and IGLJ7/IGKV1-

345 51 were all increased after vaccination. Interestingly, IGLJ1/IGKV2-14 and

346 IGLJ7/IGKV1-51 were also increased in COVID-19 Mild and Conv conditions

347 compared to Cont. These results suggest a similarity in B-cell protective responses

348 between vaccine and Mild/Conv conditions.

349

350 **Characterization of innate immune cells**

351 To investigate vaccine (**Fig 6A-D**, **S6A**) and infection-driven (**Fig 6F**, **S6B-E**)

352 changes in innate immune cells, the distribution and expression of classical subtype

353 markers were used to classify innate cell types. We identified 6 innate cell types

354 including NK cells, $\gamma\delta$ T cells, MAIT cells, DC cells, monocytes and megakaryocytes

355 (**Fig 6A-B, 6F, S6A-E**). The dendritic cells were further classified into 2 subtypes

356 including pDCs, (plasmacytoid dendritic cells) and mDC (monocyte-derived dendritic

357   cells), while monocytes were classified into 3 subtypes including CD16$^+$ monocytes,

358   CD14$^+$ monocytes and CD14$^+$CD16$^+$ monocytes (**Fig 6A-B, 6F, S6A-E**).

359   To obtain further insights into the features of innate cells, we examined the

360   distribution of each subset across three conditions (NJ, FJ and SJ). The proportions of

361   γδ T cells, NK cells, MAIT cells, CD16+ Mono, CD14+CD16+ Mono were similar

362   across the three conditions (NJ, FJ and SJ) (**Fig 6E**, **6H**, **S6F**), while the relative

363   percentages of γδ T cells, NK cells and MAIT cells decreased after SARS-CoV-2

364   infection, especially in severe patients (**Fig 6G**, **6H**, **S6F**). The innate cell subsets

365   mDC, pDC, megakaryocytes and CD14$^+$ monocytes increased after vaccination (**Fig**

366   **6E**, **6H**, **S6F**). For COVID-19 patients, the percentages of mDC and pDC

367   significantly decreased in severe patients but were restored in convalescent samples

368   (**Fig 6G**, **6H**, **S6F**). In contrast, the proportions of megakaryocytes and

369   CD14$^+$monocytes were significantly increased in severe patients and were also

370   increased in convalescent samples (**Fig 6G**, **6H**). These findings of changes to innate

371   cell subsets in COVID-19 patients are consistent with previous reports and flow-

372   cytometry-based results (Jouan et al., 2020; Wilk et al., 2020). These data also

373   indicate that both vaccines and SARS-CoV-2 infection alter components of the innate

374   cells in PBMCs and reveals distinct differences in innate cells between vaccine-

375   induced immunity and natural SARS-CoV-2 infection.

376

377   **Transcriptomic changes in innate immune cells after vaccination and SARS-**

378   **CoV-2 infection**

379   Next, we investigated the transcriptomic changes in innate immune cells after

380   vaccination. GO (Gene Ontology) analyses were conducted to obtain functional

381   insights into innate cell subtypes between FJ/SJ conditions with NJ condition (**Fig**

382   **7A**). Genes associated with the adaptive immune response (such as "T cell activation",

383   "immune response-activating signal transduction" and "antigen processing and

384   presentation") were enriched after vaccination (**Fig 7A**), while for COVID-19

385   infection, other pathways (such as "response to virus" and "defense response to virus")

386   were enriched (**Fig S7A**). Genes associated with "Response to IFN signaling" were

387   enriched in both vaccination and SARS-CoV-2 infection (**Fig 7A-B**, **S7B-7C**). INF

388   response is an essential pathway for innate cells to respond to viral infections, and our

389   data indicates that CoronaVac successfully induces the INF response in innate cells

390   (**Fig 7B**, **S7B**). The "INF response" of innate immune cells was stronger in SARS-

391   CoV-2 infection (especially for severe COVID-19 patients) compared to vaccination

392   (**Fig 7B**). We observed that four innate immune cell types (monocytes, $\gamma\delta$ T, MAIT

393   and NK cells) exhibited significantly upregulated IFN after vaccination (**Figure S7B**),

394   while all 6 innate cell types had higher IFN in COVID-19 patients (**Fig S7C**).

395   Our data showed that levels of cellular apoptosis and migration were

396   significantly upregulated in innate cells at the bulk level after vaccination and SARS-

397   CoV-2 infection (**Fig 7C, S7D-E**). The expression of HLA-II genes at FJ and SJ

398   conditions was higher compared to NJ, suggesting the enhancement of crosstalk

399   across cells after vaccination (**Fig 7E**). However, some HLA-II genes (e.g., *HLA-*

400   *DRB5*, *HLA-DPB1* and *HLA-DPA1*) were downregulated after SARS-CoV-2 infection,

401   especially in severe COVID-19 patients (**Fig 7F**), implying possible impairment of

402   crosstalk across cells. Genes which encoded HLA class I molecules (such as HLA-A,

403   HLA-B and HLA-E) were upregulated in innate cells from immunized samples

404   relative to NJ condition (**Fig S8**). Similar results were also seen in COVID-19 patients

405   (**Fig S9**). The underlying mechanism and effect of changes in HLA-I molecules

406   requires further investigation. In addition, we also investigated the expression of

407   several critical genes related with platelet aggregation (P2RX1, P2RY1 and TBXA2R)

408   in megakaryocytes (Sangkuhl et al., 2011). P2RX1, P2RY1 and TBXA2R were not

409   significantly upregulated after immunization which suggests a low risk of thrombosis

410   following CoronaVac immunization (**Fig 7G**). P2RX1, P2RY1 and TBXA2R were

411   significantly upregulated in mild COVID-19 patients (**Fig 7G**), which may imply a

412   higher risk of thrombosis for mild COVID-19 patients than severe COVID-19 patients.

413   We further analyzed monocytes, as previous reports suggested that this cell

414   subset appeared to be the source of inflammation in COVID-19 patients (Ren et al.,

415　2021). We evaluated the expression of genes reported to encode inflammatory

416　cytokines (**Table S3**) (Ren et al., 2021; Wilk et al., 2020). We found elevated

417　expression of inflammatory genes in COVID-19 patients compared to healthy controls

418　at the bulk level, indicating that peripheral monocytes are potential contributors to the

419　inflammatory cytokine storm observed in COVID-19 patients. However, severe

420　COVID-19 patients did not show higher expression of inflammatory cytokines

421　compared to mild patients (**Fig I**). We also identified increased expression of

422　inflammatory response genes in vaccinated individuals (FJ and NJ conditions)

423　compared to NJ condition at the bulk level, especially after the second dose (**Fig 7J**).

424　Of note, expression of inflammatory response genes in vaccinated individuals was

425　much lower than COVID-19 patients (**Fig K**). This implies that post-vaccination may

426　not cause an increase in inflammatory cytokines in peripheral blood or causes a lower

427　increase in inflammatory cytokines. To validate this result, we investigated the levels

428　of 11 cytokines (including pro-inflammatory cytokines: TNF-α, IL-1B and IL-6) in the

429　sera of the 18 samples using a bead-based flow-cytometry assay on the BD

430　LSRFortessa X-20 platform. No obvious post-vaccination elevation in most cytokines

431　were observed (**Fig S10**), which further suggests that post-vaccination does not lead

432　to significant increases in inflammatory cytokines in peripheral blood. Interestingly,

433　CD14+monocytes contributed to the highest proportion of cell composition (**Fig 7L**)

434　and inflammatory scores (**Fig 7M**) after vaccination or SARS-CoV-2 infection,

435　suggesting that CD14+ monocytes may be the major source of inflammation (Ren et

436　al., 2021; Zhou et al., 2020).

437

438　**Features of T cell subsets in individuals after vaccination and SARS-CoV-2**

439　**infection**

440　To investigate changes in individual T cell subclusters, the T cells from PBMCs of

441　vaccinated individuals across three conditions (NJ, FJ and SJ) (**Fig 8A-B**, **S11A**) and

442　in COVID-19 patients (**Fig S11B-D**) were subclustered into 12 subtypes according to

443　the distribution and expression of classical T cell markers. These include 7 CD4$^+$T

444   cell subtypes (CD3D$^+$ CD3E$^+$ CD40LG) and 5 CD8$^+$T cell subtypes (CD3D$^+$ CD3E$^+$

445   CD8A$^+$CD8B$^+$).

446   For CD4$^+$T cells, we defined one naïve CD4$^+$ T cell subset (CCR7+SELL+), one

447   memory CD4$^+$ T cell subset (S100A4$^+$GPR183$^+$), one effector memory CD4$^+$ T cell

448   subset (S100A4$^+$GPR183$^+$GZMA$^+$), one regulatory CD4$^+$ T cell subset (T$_{reg}$;

449   FOXP3$^+$IL2RA$^+$), one follicular T helper (Tfh) cell subset (CXCR5$^+$, ICOS+,

450   SLAMF1+) and two effector CD4$^+$ T cell subsets (CD4$^+$ effector-GZMK and CD4$^+$

451   effector-GNLY). Notably, the CD4+ effector-GNLY cell subtype was characterised

452   with high expression of genes related with cytotoxicity, such as *GNLY*, *GZMB*, *NKG7*

453   and *KLRD1*, whereas the CD4$^+$ effector-GZMK cell subtype displayed high

454   expression of *GZMK* and low expression of other cytotoxic genes (**Fig 8A-B**, **S11A,**

455   **Table S5**). The CD4+ effector-GNLY subtype also had highly expressed TBX-21 (T-

456   bet), suggesting that this subcluster were Th-1 (Type 1 helper)-like cells (**Table S5**).

457   For CD8$^+$ T cells, we defined one naïve CD8$^+$ T cell subset (CCR7$^+$SELL$^+$), one

458   cycling CD8+ T cell subset (MKI67+), one effector memory CD8$^+$ T cell subset

459   (S100A4$^+$GPR183$^+$GZMA$^+$), two effector CD8$^+$ T cell subsets (CD8$^+$ effector-GNLY

460   and CD8$^+$ effector-GZMK) (**Fig 8A-B**, **S11A, Table S5**).

461   To gain further insights into the characteristics of the T cell subclusters, we

462   examined the distribution of each subtype across three vaccine timepoints (**Fig 8C-E**)

463   and compared these profiles with data collected from COVID-19 patients (**Fig 8F**,

464   **S11E-G**). Three T cell subsets were significantly altered after vaccination (**Fig 8E**) in

465   comparison to NJ with CD4+ effector-GNLY and Tfh cell subsets decreasing while

466   Treg cells increased. For SARS-CoV-2 infection, the relative percentage of naïve

467   CD8+ T cells significantly decreased in severe COVID-19 patients whereas no

468   significant changes were observed in naïve CD4+ T cell subsets (**Fig 8F**). The

469   proportion of CD4+ effector memory decreased in COVID-19 patients compared to

470   controls (**Fig 8F**) and in the convalescence stage, the CD4+ effector memory cells

471   remained low and were not restored to the levels observed in the controls. In contrast,

472   two cytotoxic subsets, including CD4+ effector-GNLY and CD4+ effector-GZMK,

473   were present in higher percentages for convalescence patients (**Fig 8F**). Of particular

474   note, the cycling CD8+ subset was almost absent in controls but were highly enriched

475   in COVID-19 patients, especially for severe patients (**Fig 8F**). Besides naïve CD8+,

476   CD4+ effector-GNLY, CD4+ effector-GZMK, CD4+ effector memory and cycling

477   CD8+ subsets, others T cell subsets were not significantly altered (**Fig 8F**, **S11F-G**).

478

479   **Transcriptomic changes in T cells after vaccine and SARS-CoV-2 infection**

480   We investigated transcriptomic changes in the T cell subsets of vaccinated participants

481   and identified differences between vaccine and natural infection induced responses.

482   GO analyses found that genes associated with "T cell activation", "antigen processing

483   and presentation" and "response to interferon" were enriched in T cell subsets after

484   vaccination, implying an ongoing adaptive immune response to vaccination (**Fig 9A**).

485   For SARS-CoV-2 infection, "Interferon signaling pathway", "response to virus" and

486   "defense response to virus" were specifically enriched in T cell subsets, suggesting an

487   ongoing response against the virus (**Fig S12A**). IFN response is essential to the

488   immune response triggered by vaccines or viral infections and consistently, we found

489   that T cell subsets exhibited significant upregulation of IFN after vaccination and

490   SARS-CoV-2 infection (**Fig 9B**, **S12B**). Four activated state T cell subsets, including

491   CD4+ effector memory, CD4+ effector-GZMK, CD8+ effector-GNLY and CD8+

492   effector memory, showed significantly upregulated IFN after vaccination, whereas in

493   SARS-CoV-2 infection, all activated state T cell subsets had IFN significantly

494   upregulated (**Fig 9C**, **S12B**). COVID-19 patients had stronger expression of IFN than

495   vaccinated individuals (**Fig 9D**).

496   We then evaluated the cytotoxicity scores of different effector T cell subsets after

497   vaccination. Our data showed that effector T cell subsets had lower cytotoxicity

498   scores after vaccination than NJ condition at the bulk level (**Fig 9E**), and only two

499   effector T cell subsets (CD8+ effector-GNLY and CD8+ effector memory) showed

500   higher cytotoxicity scores after vaccination (**Fig 9F**). In contrast, the effector T cell

501   subsets exhibited higher cytotoxicity scores in COVID-19 patients than controls at the

502   bulk level, and all effector T cell subsets showed higher cytotoxicity scores in

503   COVID-19 patients (**Fig S12C**). COVID-19 patients also exhibited higher

504   cytotoxicity scores than vaccinated individuals (**Fig 9G**). Interestingly, we did not

505   observe significant elevation in exhaustion scores for effector T cell subsets after

506   vaccination at the bulk level (**Fig 9H**) however only CD4+ effector-GNLY and CD8+

507   effector-GZMK showed an increase in exhaustion scores after vaccination (**Fig 9I**).

508   For SARS-CoV-2 infection, effector T cell subsets did show higher exhaustion scores

509   at the bulk level (**Fig S12D**). All effector T cell clusters in mild and severe COVID-19

510   patients had higher exhaustion scores than controls, with severe patients having the

511   highest exhausted score (**Fig 9J, S12D**). COVID-19 infection did not displayed a

512   stronger exhaustion score in comparison to vaccinated individuals (**Fig 9J**).

513   We also investigated the apoptosis and migration scores of different T cell subsets.

514   Our data indicated that both vaccination and SARS-CoV-2 infection showed a high

515   level of apoptosis and migration in T cell subsets at the bulk level (**Fig 9K-P**, **S12E-**

516   **F**), however stronger apoptosis and migration of T cell subsets were observed in

517   COVID-19 patients, especially in the severe group (**Fig 9K-P**, **S12E-F**). These results

518   suggest that significant activation of cell apoptosis and migration pathways in the

519   PBMCs of severe disease may be associated with lymphopenia, consistent with

520   previous studies (Chen et al., 2020; Tan et al., 2020).

521   In addition, we observed that the expression of CD2AP significantly upregulated on

522   activated CD4+ T cells after vaccination and SARS-CoV-2 infection (**Fig 9Q**).

523   CD2AP, as an adaptor protein in CD4+ T cell, can modulate the differentiation of Tfh

524   cells and promote protective antibody responses in viral infection (Raju et al., 2018).

525   The expression of CD258 (TNFSF14) also significantly upregulated on activated

526   CD4+ T cells (**Fig 9R**) and cytotoxic CD8+ T cells (**Fig 9T**) after vaccination and

527   SARS-CoV-2 infection. TNFSF14 serves as a key component for T cell recruitment to

528   tissues from peripheral blood as well as promote T cell activation. The expression of

529   KDM5A significantly elevated in cytotoxic CD8+ T cells after vaccination and

530   SARS-CoV-2 infection (**Fig 9S**). KDM5A encodes a demethylase-H3K4me3, which

18

531 is needed for T cell activation. These data suggested that increased activation of T

532 cells in vaccinated individuals and COVID-19 patients may contribute to defense

533 against the vaccine and SARS-CoV-2 virus.

534

535 **V(D)J gene usage and clonal expansion in T cells after vaccination and SARS-**

536 **CoV-2 infection**

537 TCR information was detected in all subsets and in ~60% of T cells. Clonal expansion

538 of CD8+ effector T cells was larger (clonal size >100) than CD8+ cycling T cells and

539 CD4+ T cells (**Fig10A-B**). >70% of T cells in the vaccine cohort and 53%~61% of T

540 cells in COVID-19 cohorts had unique TCR clonotypes (**FigS13A-B**). A negative

541 correlation between clone size and clonotype number was observed, consistent with

542 previous report (Zhang et al., 2020) and suggests that large clonal expansion is a rare

543 event (**Fig 10C**). Next, we compared the expression of T cell receptor β-chain

544 constant domains 1 and 2 (TRBC1 and TRBC2). The percentage of TRBC1 increased

545 after vaccination, while in COVID-19 patients, the percentage of TRBC1 was highest

546 in severe disease and lowest in controls (**Fig 10D, H, S13C**). This suggests that

547 immune activation by vaccination and infection are able to modulate T cell receptor β-

548 chain ratios.

549 We compared T cell clonal expansion after vaccination and observed a

550 significant decrease in clonal expansion of CD8+ T cells, especially CD8+T effector-

551 GNLY cells, from NJ to FJ to SJ. This suggests that two-doses of CoronaVac induced

552 immunogenic proliferation leading to many new unique TCR clonotypes (**Fig 10E**,

553 **S13D**). For COVID-19 infection, decreased CD8+ T cell clonal expansion was also

554 observed in infected patients compared to Cont and Conv (**Fig 10E**), in agreement

555 with previous report (Tanriover et al., 2021). Clonal expansion in CD4+ T cells was

556 also decreased from NJ to SJ but was higher in severe than controls, suggesting that

557 CD4+ T cells may play different roles in vaccine and infection immune responses

558 (**Fig 10E**).

559 The length distribution of the CDR3 region were similar for all conditions

560 (**Fig10G**). COVID-19 infected patients tended to have lower TCR diversity while

561 vaccinated patients had higher diversity (**Fig 10I**) which may suggest a protective role

562 for TCR diversity. The usage of TRB V(D)J genes across vaccine and infection

563 conditions were compared (**Fig 10F**). The combination of the most prevalent 11 TRBJ

564 and 22 TRBV genes indicates that vaccination induced greater diversity in TRB

565 V(D)J genes (**Fig10F** top panel). Mild COVID-19 infection also induced greater

566 diversity however no new V(D)J pair patterns were observed. In contrast, severe

567 COVID-19 infection induced new prominent V(D)J pairs, including TRBJ1-6/TRBV9,

568 TRBJ1-4/TRBV27 and TRBJ1-5/TRBV4-1. The change in pattern for TRAJ and

569 TRAV pairs showed similar trends to TRB V(D)J (**Fig10F** bottom panel). In addition,

570 the CDR3 peptides sequences showed large individual differences however we did not

571 observe any interesting patterns across conditions (**Fig S14**).

572

573 **Discussion**

574 As an emerging virus, SARS-CoV-2 is highly pathogenic and is responsible for the

575 COVID-19 pandemic. Currently, there are no effective drugs or optimal treatments for

576 SARS-CoV-2 infection, thus considerable effort has been put into developing safe and

577 effective vaccines against COVID-19. Inactivated SARS-CoV-2-based vaccines are

578 one of the most-widely used COVID-19 vaccines due to its low cost, ease of scale-up

579 and production (Iversen and Bavari, 2021a). CoronaVac is an inactivated COVID-19

580 vaccine candidate which has had its safety and potency validated in both animal

581 models and clinical trials (Tanriover et al., 2021; Zhang et al., 2021). However,

582 current knowledge of the host immune response to the inactivated COVID-19 vaccine

583 is still limited, making it difficult to inform and improve the design of new

584 generations of COVID-19 vaccines. In addition, little is known about how this

585 immune response compares to natural SARS-CoV-2 infection.

586 In this report, we performed single-cell RNA sequencing in PBMCs of six

587 individuals immunized with CoronaVac and compared these to the single-cell profiles

588 in COVID-19 patients. Overall, both inactivated SARS-CoV-2 vaccine and natural

20

589   SARS-CoV-2 infection affect the composition of peripheral immune cells, with

590   greater changes observed in COVID-19 patients, especially for severe disease. After

591   CoronaVac injection, the inactivated SARS-CoV-2 virus initially encounters antigen

592   presenting cells (APCs) in the innate immune system which then triggers the adaptive

593   immune response. Our data confirmed that the proportion of DCs, one of the main

594   APCs, are significantly elevated after vaccination (**Fig 2**), thus suggesting that

595   CoronaVac is able to successfully initiate the adaptive immune response. However in

596   severe COVID-19 patients, the relative percentage of DCs significantly decreased,

597   implying a possible subversion of the adaptive immune response in severe disease.

598   Monocytes are the major source of inflammatory cytokines in SARS-CoV-2 infection

599   (Ren et al., 2021; Zhou et al., 2020), and were elevated after immunization with

600   CoronaVac and SARS-CoV-2 infection (**Fig S2**). However, the level of monocytes

601   was significantly greater in COVID-19 patients (especially for severe disease)

602   compared to immunization. This implies that CoronaVac immunization causes a

603   weaker inflammatory response than natural SARS-CoV-2 infection. The relative

604   abundance of γδ T, MAIT, NK, CD4+ effector memory, and naïve CD8+ T cells

605   decreased with disease severity while the proportion of cycling CD8+ T cells

606   increased with disease severity, suggesting that these subsets may be associated with

607   disease severity. We did not observe significant changes to these subsets after

608   immunization with CoronaVac (**Fig 2, 6, 9, S2, S6** and **S11**).

609   IFNs are produced during viral infection and has an antiviral role. However

610   when produced excessively, IFNs can cause immunopathological damages.

611   Interestingly, 'IFN response' was enriched by GO analysis in different cell subclusters

612   after vaccination in our study (**Fig 4, 7 and 9**). Compared to pre-vaccination samples,

613   the response to IFN-α (type I IFN) pathway was also significantly elevated in most

614   PBMC cell types in post-vaccination samples. Together, this suggests CoronaVac

615   induces IFNs as part of the antiviral response. Similarly, 'IFN response' was also

616   enriched by GO analysis after SARS-CoV-2 infection (**Fig S4, S7** and **S12**). The

617   expression level of IFN-α was also significantly upregulated in COVID-19 patients

618   (**Fig S4, S7** and **S12**), suggesting an antiviral role triggered by IFNs. Notably,

619   COVID-19 patients showed higher response to IFN-α than immunized individuals

620   with the strongest response to IFN-α found in severe disease (**Fig 7**, **9**).This indicates

621   that the stronger response to IFNs in COVID-19 patients may be involved in immune

622   pathology (e.g., lung injury) (Liu et al., 2020).

623   In this report, we observed broad immune activation after immunization with

624   CoronaVac. First, the levels of neutralizing antibodies (anti-S-RDB-specific antibody)

625   significantly increased after the second injection (**Fig 3**, **S3**, **Table S2**). Second, B cell

626   activation in PBMCs post-vaccination is supported by our data and evidenced by: (i)

627   enrichment of genes involving in 'B cell activation', 'adaptive immune response' and

628   'antigen processing and presentation'; (ii) significant upregulation of two important B

629   cell activation pathways (GO:0002312 and GO:0042113); (iii) the activation of naïve

630   B and memory B cell subsets; (iv) significant expression of key genes (e.g., *PRDM1*,

631   *XBP1*, *IFR4*, *PAX5*, *IL4R* and *IL21R*). Third, the activation of innate immune cells

632   was observed in our study as genes associated with 'antigen processing and

633   presentation', 'IFNs response', 'T cell activation' and 'immune response-activating

634   signal transduction' were enriched by GO analysis (**Fig 7**). Fourth, the activation of T

635   cell subsets was also supported by our data as genes involved in 'T cell activation', 'T

636   cell mediated immunity', 'antigen processing and presentation', and 'IFNs response'

637   were also enriched by GO analysis (**Fig 9**). Similarly, broad immune activation was

638   also observed after SARS-CoV-2 infection (**Fig S4, S7, S12**). However, we observed

639   significant downregulation of some HLA class II genes in B, T and innate cells in

640   COVID-19 patients, especially for severe disease, implying a dysregulation in

641   crosstalk between adaptive immune cell classes. Furthermore, some key chemokine

642   receptor genes (e.g., CXCR5) were also significantly downregulated in the PBMCs of

643   severe COVID-19 patients (**Fig S4**), which may impair germinal center reaction,

644   resulting in a dysregulated humoral immune response (Mathew et al., 2020).

645   We analyzed the apoptosis, migration, cytotoxicity and exhaustion scores in

646   different immune cell subsets from immunized individuals and compared their

647 expression levels with COVID-19 patients. Overall, innate immune and T cell subsets

648 showed higher apoptosis and migration scores after immunization with CoranaVac or

649 SARS-CoV-2 infection (**Fig 7, 9, Fig S7** and **S12**). However, compared to post-

650 vaccination, SARS-CoV-2 infection exhibited higher apoptosis and migration scores,

651 with severe disease having the highest score. This suggests that severe patients likely

652 had increased lymphocyte apoptosis and migration which may be associated with

653 lymphopenia, a clinical predictor for severe COVID-19 disease (Tan et al., 2020). At

654 the bulk level, post-vaccination effector T cell subsets did not display higher

655 exhaustion scores compared to pre-vaccination samples. However for COVID-19

656 patients, all samples had higher exhaustion scores compared to controls, and those

657 with severe disease displayed the highest exhaustion scores. It is possible that the high

658 exhaustion status of effector T cell subsets may be associated with functional

659 impairment (Zheng et al., 2020). Interestingly, the effector T cell subset from post-

660 vaccination showed lower cytotoxicity scores at the bulk level than those of pre-

661 vaccination. In contrast, the cytotoxicity scores of the effector T cell subset from

662 COVID-19 patients were higher than the controls with severe disease having the

663 highest cytotoxic score. Similarly, we observed that the cytotoxicity scores in each

664 subset of effector T cells were also significant elevated after SARS-CoV-2 infection.

665 Although increased expression of pro-inflammatory cytokine genes was

666 observed in monocytes post-vaccination, this upregulation may not be adequate to

667 cause a significant increase in systemic levels of pro-inflammatory cytokines. This is

668 supported by our immunoassay results which showed no obvious increase in several

669 key pro-inflammatory cytokines (e.g., IL-6 and TNF) post-vaccination (**Fig S10**). In

670 addition, the expression level of pro-inflammatory cytokine genes after vaccination

671 was significantly lower than SARS-CoV-2 infection. These evidences suggest that

672 CoronaVac may not contribute to acute inflammation or the cytokine storm commonly

673 observed in severe COVID-19 patients (Zhou et al., 2020). Among the monocyte

674 subsets, CD14+ monocytes were identified as the major contributor to inflammation

675 for both immunization and SARS-CoV-2 infection. This is evidenced by: (i) a large

676 increase in CD14+ monocytes observed after vaccination and SARS-CoV-2 infection

677 (**Fig 3 and Fig S3**) (Zhou et al., 2020) and (ii) CD14+ monocytes was the largest

678 contributor to the inflammatory scores (**Fig 7**). In addition, due to rising concerns

679 about the bloodclotting side effect observed in several vaccine types, we also

680 examined several key genes (e.g., *2RX1*, *P2RY1* and *TBXA2R*) involved in platelet

681 aggregation in megakaryocytes (Sangkuhl et al., 2011).   Unlike SARS-CoV-2

682 infection, our data demonstrated that the expression level of platelet aggregation-

683 associated genes were not significantly upregulated after immunization with

684 CoronaVac. This may explain the fewer number of thrombus-related adverse events

685 reported CoronaVac.

686

687 **Methods**

688 **Volunteer cohort**

689 Three male and three female healthy adults (n = 6) were admitted at the Sanya People's Hospital

690 and enrolled in the study. Peripheral blood samples were collected at 3 key timepoints (Fig. 1A):

691 pre-vaccine baseline (timepoint 1, day 0), 3 weeks following the first dose (timepoint 2, day 21),

692 which was also the same day they received the second dose, and finally, 2 weeks following the

693 second dose (timepoint 3, day 35). All six volunteers had matching samples at 3 time points. This

694 study design allowed us to investigate the kinetics of the immune responses following both

695 primary and secondary immunizations.

696 **Sample collection**

697 **Supplementary Table 1** summarizes the characteristics of individuals assessed in each assay. Full

698 cohort information is described in figure 1. The fresh blood samples for each timepoint

699 immediately underwent peripheral blood mononuclear cells (PBMCs) isolation using standard

700 density gradient centrifugation. PBMCs are typically employed to assess immune-regulatory

701 effects at the single-cell level. PBMCs were isolated using HISTOPAQUE-1077 (Sigma-Aldrich,

702 10771) solution according to the manufacturer's instructions. Briefly, 3 mL of fresh peripheral

703 blood was collected in EDTA anticoagulant tubes and subsequently layered onto HISTOPAQUE-

704 1077. After centrifugation, PBMCs remained at the plasma-HISTOPAQUE-1077 interface and

705   were carefully transferred to a new tube. Erythrocytes were removed using Red Blood Cell Lysis

706   Solution (10×) (Miltenyi, 130-094-183) and washed twice with 1× PBS (Gibco, 10010023). The

707   cell pellets were re-suspended in sorting buffer (PBS supplemented with 2% fetal bovine serum

708   (FBS, Gibco, 10099141)). Cell viability of PBMCs were assessed using the Countstar cell

709   viability detection kit and showed greater than 90% viability. PBMCs were then used in

710   immunological analysis and cell encapsulation. The 10x Genomics single-cell transcriptome

711   platform was used to generate the 5' gene expression profiles, T cell receptor (TCR) and B cell

712   receptor (BCR) data. This approach employs a commercial emulsion-based microfluidic platform

713   (Chromium 10x) that enables the generation of amplified cDNA used for both the preparation of

714   single cell RNA-seq libraries and TCR/BCR target enrichment and sequencing.

715   **Single cell RNA library preparation and sequencing**

716   Cell suspensions were barcoded through the 10x Chromium Single Cell platform using Chromium

717   Single Cell 5' Library, Gel Bead and Multiplex Kit, and Chip Kit (10x Genomics). Single-cell

718   RNA libraries were prepared using the Chromium Single Cell 5' Kit v2 (10x Genomics; PN-

719   1000263), Chromium Single Cell V(D)J Reagent kits (10x Genomics, PN-1000252(TCR), PN-

720   1000253(BCR)) according to the manufacturer's instructions. Each sequencing library was

721   generated with a unique sample index. The libraries were sequenced on the Illumina Novaseq6000

722   sequencer with a paired-end 150-bp (PE150) reading strategy. With the provided sample sheet, the

723   CellRanger (v.5.0.0) *mkfastq* command was used to demultiplex the flow cells' raw base call files

724   into fastq files.

725

726   **SARS-CoV-2-specific IgM/IgG ELISA and plasma cytokine detection**

727   The S-specific IgG/IgM and plasma cytokine detection were detected according to our previous

728   study (Wang et al., 2021).

729

730   **Quantification and Statistical analysis**

731   **Single-cell RNA-seq and data processing**

732   The human reference (v.GRCh38-3.0.0) was downloaded from the 10x Genomics official website

733   in Mar-2021. Raw and filtered gene expression matrices were generated for each sample using the

734   kallisto/bustools (kb v0.24.4) pipeline coupled with human GRCh38. The *kb count* command was

735   called to generate single cell feature counts for each sample by specifying the library name in the

736   argument. Then the filtered feature, barcode and matrix files were analyzed in python (v3.8.10)

737   using the anndata (ad) (v0.7.6) and scanpy (sc) (v1.7.2) packages. Data files of all 18 samples and

738   the largest dataset of Chinese PBMC COVID-19 infection (64 fresh PBMC samples from Cell

739   2021 Zhangzemin) were merged together by the ad.concat function. Cells and genes were filtered

740   by the sc.pp.filter_cells and sc.pp.filter_genes function for further analyses. First, genes expressed

741   at a proportion >0.1% of the cells were selected. Second, to minimize technical artifacts from low-

742   quality cells and potential doublets, cells meeting the following criteria were filtered out: (1) <

743   1000 or > 25000 unique molecular identifiers (UMIs, representing unique mRNA transcripts); (2)

744   <500 or > 5000 genes; or (3) > 10% UMIs derived from the mitochondrial genes. The scanpy's

745   external module Scrublet (Wolock et al., 2019) was called using the sc.external.pp.scrublet

746   function to identify potential doublets using default parameters. An automatically set threshold

747   labelled 299 cells with a doubletScore > 0.25 as "predicted_doublets" and were filtered out. After

748   quality control, a total of 585860 cells remained. The violin distribution and scatter plot for

749   computed quality measures including gene counts per cell, UMI counts per cell and mitochondrial

750   gene percentage are shown in Supplementary Fig 1. The gene expression matrix were normalized

751   by library size to 10,000 reads per cell by sc.pp.normalize_total function, so that all cells were

752   comparable in UMI counts. Next, the normalized counts were natural log transformed ($X = \log(X$

753    $+ 1)$) by sc.pp.log1p function. The log transformed expression values were used for downstream

754   analysis.

755   **Batch effect correction and dataset integration**

756   Gene features with high cell-to-cell variation in the data were prioritized using the

757   sc.pp.highly_variable_genes function (supplemental Fig 1). Briefly, the informative highly-

758   variable genes (HVGs) were selected within each sample separately and merged to select the

759   consensus set of 1,500 top-HVGs. All ribosomal and mitochondrial genes were removed from

760   HVGs as described (Cell 2021 Zhangzemin). The HVGs subset matrix extracted from the full

761   expression matrix was used for downstream integration steps. Then each gene was scaled to unit

762   variance and zero mean and clipped when values exceeded 10.

763   Integration of different datasets was conducted in the order of dimension reduction by principal

764   component analysis (PCA), batch effect correction using Harmony algorithm (Korunsky et al.

765   (2019), Fast, sensitive and accurate integration of single-cell data with Harmony) and

766   unsupervised clustering using Louvain algorithm (Traag et al., 2019). Specifically, the main axes

767   of variation was identified using the sc.tl.pca function with parameter svd_solver='arpack'.

768   Dimensionality of the datasets was reduced to 50 PCA components and fed into

769   sc.external.pp.harmony_integrate function implemented in the python package harmonypy. The

770   parameter theta was set as 2.5 for sample for technical covariate correction. Nearest neighbor

771   graph of cells was built using the sc.pp.neighbors function with batch-corrected matrix.

772   **Cell clustering and annotations**

773   Unsupervised clustering of cells was then computed by sc.tl.louvain at different resolutions using

774   the neighborhood relations of cells. Cluster-specific signature genes were identified using the

775   sc.tl.rank_genes_groups function. Cluster annotation was done manually by matching canonical

776   cell marker genes with Cluster-specific signature genes.

777   Clustering analysis of cell types consisted of two rounds. The first round (Louvain resolution = 1.2)

778   was performed on all cells and identified 10 major cell types (**Fig 1, S1**). The second round (with

779   Louvain resolution ranging from 0.3 to 1.8) was performed on CD4+/CD8+ T cells, B cells,

780   monocyte and DC cells separately to subdivide each cell type into sub-clusters. These sub-clusters

781   represented distinct immune cell lineages within each major cell type. Each sub-cluster was

782   manually analyzed by domain experts and considered as distinctive enough when they had at least

783   one highly expressed signature gene compared to other cells. The complete list of canonical

784   marker genes and cluster-specific highly expressed signatures are provided in Fig 3, 6, 8, S3, S6

785   and S11.

786   **Cell state score of cell subtypes**

787   After cluster annotation were completed, several gene sets from important immune processes were

788   used to compare overall activation level or physiological activity of cell clusters. Gene sets related

789   to cytokine storm and immune exhaustion were collected from previous literature (Nature Immun

790   2020, Cell 2021 zhangzemin) and gene sets about Response To Interferon Alpha (GO:0035455),

791   Response To Interferon Beta (GO:0035456), Acute Inflammatory Response (GO:0002526),

792   Apoptotic Signaling Pathway (GO:0097190), Leukocyte Migration (GO:0050900) were collected

793   from the MsigDB database. Cell state scores were calculated using the sc tl.score_genes function

794   available in Scanpy.

795   The cell scores of the cell were defined as the average expression of the genes from the

796   predefined gene set with respect to reference genes. Comparison of the cell state score of one

797   condition versus another condition was statistically assessed using the Mann-Whitney rank test

798   (two-tail, p-value < 0.01, adjusted using the Benjamini–Hochberg method).

799   **TCR and BCR V(D)J immune repertoire sequencing and analysis**

800   From one aliquot of the gene expression 5′ libraries, full-length TCR/BCR V(D)J segments were

801   enriched from transcriptome cDNA via PCR amplification using the Chromium Single-Cell V(D)J

802   Enrichment kit according to the manufacturer's protocol. Similar to the gene expression pipeline,

803   immune repertoire preprocessing was performed using Cell Ranger (v.6.0.0) *vdj* command with

804   human vdj reference vGRCh38-alts-ensembl-5.0.0. This pipeline includes demultiplexing by

805   index and barcodes, TCR/BCR V(D)J sequence discovery and TCR/BCR clonotype assignment to

806   each cell. V(D)J immune repertoire was analyzed by the python-toolkit, Scirpy. In brief, the

807   productive chains of each cell were identified and connected with the cell's barcode information.

808   Each unique TRA(s)-TRB(s) pair was defined as a TCR clonotype and each unique IGH(s)-

809   IGK/IGL(s) pair was defined as a BCR clonotype. If one clonotype was present in at least two

810   cells, cells harboring this clonotype were considered to be clonal and the number of cells with

811   such pairs indicated the degree of clonality of the clonotype. Only cells with at least one

812   productive clonotype was used in the following analysis. The TCR/BCR downstream analysis

813   were similar for the most part. TCR/BCR data table of cells loaded by Scirpy was matched

814   together with gene expression profiles already prepared by Scanpy in the AnnData data structure.

815   Clonotypes were then clustered based on the similarity of their CDR3 amino acid sequences.

816   TCR/BCR diversity metric, containing clonotype frequency and clonotype composition, was

817   obtained using scirpy.pl.alpha_diversity function based on 'normalized_shannon_entropy'.

818   **Statistics**

819   The statistical analysis, visualization and method details described in this study were performed in

820   python and R and are provided with the results of the main text, in the figure legends or in the

821   above Methods sections. In all figures with significance marks, the following convention for

822   symbols indicating statistical significance were used:

823   ns: $p > 0.05$

824   *: $p <= 0.05$

825   **: $p <= 0.01$

826   ***: $p <= 0.001$

827   ****: $p <= 0.0001$

828

829   **Code availability**

830   Experimental protocols and the data analysis pipeline used in our work follow the 10X Genomics

831   and Seurat official websites. The analysis steps, functions and parameters used are described in

832   detail in the Methods section. Custom scripts for analyzing data are available upon reasonable

833   request. The software and algorithms used in this report are presented in Table S8.

839 **Ethical approval:** This study was approved by the Ethics Committee of the Sanya People's Hospital
840 (SYPH-2021-26).

841 **Transparency declaration:** The lead author and guarantor affirm that the manuscript is an honest,
842 accurate, and transparent account of the study being reported; that no important aspects of the study
843 have been omitted; and that any discrepancies from the study as planned and registered have been
844 explained.

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985

986 **Figure legends**
987 **Fig 1**. **Study design and overall results of single-cell transcriptomic profiling of PBMCs**
988 **isolated from vaccine recipients without COVID-19 infection.**
989 **A**, A schematic diagram of the overall study design. The PBMCs from six recipients, 3 male and 3
990 female adults, and across three conditions were subjected to scRNA-seq gene expression profiling,
991 TCR and BCR profiling analyses. The data set was integrated with a published COVID-19
992 scRNA-seq data set comprised of 64 fresh PBMC samples.
993 **B**. Cell populations identified and 2-D visualization. The UMAP projection of 180k single cell
994 transcriptomes from NJ (n = 6), FJ (n = 6) and SJ (n = 6) samples, showing the presence of 10
995 major clusters and 27 smaller clusters with their respective colors. Each dot corresponds to a
996 single cell, colored according to the annotated major cell type (left panel) or subcelltype (right
997 panel). **C**. Canonical single cell RNA markers were used to label major clusters by cell identity as
998 expression level on the UMAP plot. Cells are colored according to log transformed and
999 normalized expression levels of twelve genes (CD3D, CD8A, CD40LG, etc.).
1000 **D**. Expression distribution of cell identity specific RNA markers of vaccine cohort samples. The
1001 rows represent 10 cell clusters labeled with different colors and the columns represent log
1002 transformed gene expression of the RNAs. The distribution of a gene in a cluster is shown as one
1003 small violin plot.
1004 **E**. Similar to **Fig 1B**, Cell populations identified and 2-D visualization of 410k single cell
1005 transcriptomes from Cont (Control), Conv (Convalescence), Mild (Mild), and Seve (Severe)
1006 samples from Ren et al..
1007
1008 **Fig 2. Comparison of cell composition across sample groups.**
1009 **A.** UMAP projection of the single cells from NJ, FJ and SJ conditions. Each dot corresponds to a
1010 single cell, colored by its major cell type.
1011 **B.** Average proportion of each major cell type derived from NJ, FJ and SJ groups.
1012 **C.** Proportion of each major cell type derived from each NJ, FJ and SJ individual sample.
1013 **D.** The box plot shows cell compositions of NJ, FJ and SJ conditions at a single sample level.
1014 Condition preference of each cluster compared side by side. y axis, percentage of cell types. Boxes
1015 are colored by conditions. Each plot panel represents one cell type.
1016 **E.** Average proportion of each major cell type derived from Cont, Conv, Mild, and Seve conditions
1017 from the COVID-19 infection cohort.
1018 **F.** Proportion of each major cell type derived from individual samples of Cont, Conv, Mild, and
1019 Seve groups from the COVID-19 infection cohort
1020 **G.** The box plot shows cell compositions of Cont, Conv, Mild, and Seve conditions from the
1021 COVID-19 infection cohort at a single sample level.
1022 In 2D and 2G, all pairwise differences with $P < 0.05$ using two-sided unpaired Mann–Whitney U-
1023 test are marked to show significance levels.
1024
1025 **Fig 3. Characterization of B cell composition differences in individuals across vaccination**
1026 **and infection conditions**
1027 **A.** UMAP projection of all B cells from NJ, FJ and SJ conditions. Each dot corresponds to a single
1028 cell, colored by its cell subtype.
1029 **B.** Expression levels of canonical B cell RNA markers were used to identify and label major cell

35

1030   clusters on the UMAP plot. Cells are colored according to log transformed and normalized
1031   expression levels of eight genes. Cells are from NJ, FJ and SJ conditions

1032   **C.** Average proportion of each B cell subtype derived from NJ, FJ and SJ groups.

1033   **D.** Proportion of each B cell subtype derived from NJ, FJ and SJ individual samples.

1034   **E.** The box plot shows the composition of B cells before (NJ) and after vaccination (FJ, SJ) at a
1035   single sample level.

1036   **F.** UMAP projection of all B cells from Cont, Conv, Mild and Seve conditions. Each dot
1037   corresponds to a single cell, colored by its cell subtype.

1038   **G.** Proportion of each B cell subtype derived from Cont, Conv, Mild and Seve individual samples.

1039   **H.** Antibody levels of IgM and IgG at NJ, FJ and SJ conditions in serum.

1040   **I.** The composition of Ig classes in the vaccine cohort identified by BCR single cell sequencing.

1041   **J.** The composition of Ig classes in the COVID-19 infected cohort identified by BCR single cell
1042   sequencing.

1043   All pairwise differences with P < 0.05 using two-sided unpaired Mann–Whitney U-test are marked
1044   to show significance levels.

1045

1046   **Fig 4. Characterization of gene expression differences in B cells in individuals across**
1047   **vaccination and infection conditions.**

1048   **A.** GO enrichment analysis of DEGs identified by comparing before- and after- vaccination
1049   conditions. DEGs refer to genes with Benjamini–Hochberg adjusted P value (two-sided unpaired
1050   Mann–Whitney U-test) ≤0.01 and average log2 fold change ≥1 in both FJ/NJ and SJ/NJ
1051   comparisons.

1052   **B.** Violin plots of B cell expression activities in three vaccine conditions. Cells are grouped and
1053   colored by conditions. Y axis represents the normalized expression score of gene sets related to
1054   B_CELL_ACTIVATION_INVOLVED_IN_IMMUNE_RESPONSE   (GO:0002312)   and
1055   B_CELL_ACTIVATION (GO:0042113)

1056   **C.** Dot plots of the gene expression level of naïve B cells in three vaccine conditions. Rows
1057   represent conditions; columns represent genes. Dots are colored by mean expression levels in each
1058   condition.

1059   **D.** (Left) Dot plots of *IL4R* and *IL21R* expression level of naïve B cells between pre-vaccination
1060   and post-vaccination. Rows represent conditions; columns represent genes. Dots are colored by
1061   mean expression levels in each condition. (Right) Violin plots show the expression level of the
1062   two genes in before- and after- vaccination conditions.

1063   **E.** Violin plots of the expression level of *IL4R* and *IL21R* genes in naïve B cells, activated B cells
1064   and memory B cells.

1065   **F.** Dot plots of gene expression level of memory B and intermediate transition memory B cells in
1066   vaccine cohort. Rows represent genes (*TBX21*, *ZEB2*, *TFEC*, *ZBTB32*, *YBX3*); columns represent
1067   B cell subtypes. Dots are colored by mean expression levels in each group.

1068   **G.** *PRDM1*, *XBP1*, *IRF4* gene expression level of activated B cells and other B cells in the vaccine
1069   cohort. Dot plots (Left) and violin plots (Right) are used for visualization.

1070   **H.** *PAX5*, *BCL6* and *BACH2* gene expression level of B cells in before- and after-vaccination
1071   conditions. Dot plots (Left) and violin plots (Right) are used for visualization.

1072   **I.** *CXCR5*, *CXCR4* and *CCR6* gene expression level of B cells in before- and after-vaccination
1073   conditions. Dot plots (Left) and violin plots (Right) are used for visualization.

1074   **J.** Gene expression level of HLA-II genes in B cells in before- and after-vaccination conditions.
1075   Dot plots (Left) show individual genes and violin plots (Right) show normalized average
1076   expression of the HLA-II gene set.
1077   **K.** Gene expression level of HLA-II genes in B cells in Cont, Conv, Mild and Seve conditions.
1078   Dot plots (Left) show individual genes and violin plots (Right) show normalized average
1079   expression of the HLA-II gene set.
1080   All pairwise differences with $P < 0.05$ using two-sided unpaired Mann–Whitney U-test are marked
1081   to show significance levels.
1082

1083   **Fig 5. Changes in BCR clones and selective usage of V(D)J genes.**
1084   **A.** UMAP projection of B cells derived from PBMCs. Cells are colored by conditions (Panel 1), B
1085   cell subtypes (Panel 2), if BCR detection was successful (Panel 3) and clone-type expansion size
1086   (panel 4)
1087   **B.** Pie graph showing the distribution of IGHA, IGHD, IGHG and IGHM in B cells and plasma
1088   cells.
1089   **C.** Box plot showing the percentages IGHA, IGHD, IGHG and IGHM in B cells and plasma cells
1090   under each condition.
1091   **D.** Pie graph showing the distribution of light chain IGK and IGL in B cells and plasma cells
1092   under each condition.
1093   **E.** Stacked bar plots showing the clone state of each B cell subtype in each condition.
1094   **F.** Heat maps showing differential IGH/K/L rearrangement. Prevalent IGHV-IGHJ combination
1095   pairs (left) and IGKV-IGKJ combination pairs (right) are compared across conditions. Usage
1096   percentage are sum normalized by column.
1097   **H.** Box plot showing the alpha diversity of clonotypes in each PBMC sample. Data points are
1098   colored by condition.
1099   **I.** Density curve plots showing the distribution shift of IGK/L and IGH chain CDR3 region length
1100   in BCR clone types for each condition.
1101

1102   **Fig 6. Characterization of innate cell composition differences in individuals across**
1103   **vaccination and infection conditions.**
1104   **A.** UMAP projection of all innate cells from NJ, FJ and SJ conditions. Each dot corresponds to a
1105   single cell, colored by its cell subtype.
1106   **B.** Expression levels of canonical innate cell RNA markers were used to identify and label major
1107   cell clusters on the UMAP plot. Cells are colored according to log transformed and normalized
1108   expression levels of eight genes. Cells are from NJ, FJ and SJ conditions.
1109   **C.** Average proportion of each innate cell subtype derived from NJ, FJ and SJ groups.
1110   **D.** Proportion of each innate cell subtype derived from NJ, FJ and SJ individual samples.
1111   **E.** The box plot shows the composition of innate cells in NJ, FJ and SJ conditions at a single
1112   sample level.
1113   **F.** UMAP projection of all innate cells from Cont, Conv, Mild, and Seve conditions. Each dot
1114   corresponds to a single cell, colored by its cell subtype.
1115   **G.** Proportion of each innate cell subtype derived from Cont, Conv, Mild and Seve individual
1116   samples.
1117   **H.** Proportion of each innate cell subtype derived from ContNJ(NJ), Vacc(FJ+SJ), Conv, MiSe

1118   (Mild and Seve) individual samples.

1119   All pairwise differences with P < 0.05 using two-sided unpaired Mann–Whitney U-test are marked

1120   to show significance levels.

1121

1122   **Fig 7. Characterization of gene expression differences in innate immune cells from vaccine**

1123   **and COVID-19 infected cohort samples.**

1124   **A**, GO enrichment analysis of DEGs identified by comparing the before and after vaccination

1125   conditions. DEGs refer to genes with Benjamini–Hochberg adjusted P value (two-sided unpaired

1126   Mann–Whitney U-test) ≤0.01 and average log2 fold change ≥1 in both

1127   FJ/NJ and SJ/NJ comparisons.

1128   **B**, **C** and **D**, Expression activity of IFN-alpha, apoptosis and migration pathways in innate

1129   immune cells of NJ, FJ, SJ, Cont, Conv, Mild and Seve conditions shown as violin plots and

1130   colored by sample conditions.

1131   **E**, Heatmap dot plot of HLA-II gene expression in innate immune cells of NJ, FJ, and SJ

1132   conditions

1133   **F**, Heatmap dot plot of HLA-II gene expression in innate immune cells of Cont, Conv, Mild and

1134   Seve conditions

1135   **G**, Violin plot showing normalized expression levels of *P2RX1*, *TBXA2R* and *P2RY1* in

1136   megakaryocytes (Mega) from NJ and Vaccine (FJ+SJ) conditions

1137   **H**, Violin plot of normalized expression of *P2RX1*, *TBXA2R* and *P2RY1* in megakaryocytes (Mega)

1138   from Cont, Mild and Seve conditions

1139   **I**, Expression activity of inflammatory pathways in monocytes from NJ, FJ and SJ conditions

1140   shown as box plots. Boxes are colored by sample conditions.

1141   **J**, Expression activity of inflammatory pathways in monocytes from Cont, Conv, Mild and Seve

1142   conditions shown as box plots. Boxes are colored by sample conditions.

1143   **K**, Expression activity of inflammatory pathways in monocytes from NJ, Vacc, Cont, Conv, Mild

1144   and Seve conditions shown as violin plots. Violins are colored by sample conditions.

1145   **L**, Pie graph of relative percentage for CD14+monocytes, CD16+monocytes,

1146   CD14+CD16+monocytes in the vaccine and COVID-19 cohort.

1147

1148   **M**, Pie graph of inflammatory scores from CD14+monocytes, CD16+monocytes,

1149   CD14+CD16+monocytes in the vaccine and COVID-19 cohort.

1150

1151   **Fig 8. Characterization of T cell composition differences in individuals across vaccination**

1152   **and infection conditions.**

1153   **A**, UMAP projection of all T cells from NJ, FJ and SJ conditions. Each dot corresponds to a single

1154   cell, colored by its cell subtype.

1155   **B**, Expression levels of canonical T cell RNA markers were used to identify and label major cell

1156   clusters on the UMAP plot. Cells are colored according to log transformed and normalized

1157   expression levels of eight genes. Cells are from NJ, FJ and SJ conditions

1158   **C**, Average proportion of each T cell subtype derived from NJ, FJ and SJ groups.

1159   **D**, Proportion of each T cell subtype derived from NJ, FJ and SJ individual samples.

1160   **E**, The box plot shows the composition of T cells from NJ, FJ and SJ conditions at a single sample

1161   level.

1162 **F**, The box plot shows the composition of T cells from Cont, Conv, Mild and Seve conditions at a

1163 single sample level.

1164 All pairwise differences with P < 0.05 using two-sided unpaired Mann–Whitney U-test are marked

1165 to show significance levels.

1166

1167 **Fig 9, Characterization of gene expression differences in activated T cells from vaccine and**

1168 **COVID-19 infected cohort samples.**

1169 **A**, GO enrichment analysis of DEGs identified by comparing the before and after vaccination

1170 conditions. DEGs refer to genes with Benjamini–Hochberg adjusted P value (two-sided unpaired

1171 Mann–Whitney U-test) ≤0.01 and average log2 fold change ≥1 in both

1172 FJ/NJ and SJ/NJ comparisons.

1173 **B** and **C**, Expression activity of IFN-alpha pathways in activated T cells (**B**) and subtypes (**C**) of

1174 NJ and Vacc (FJ and SJ) conditions shown as box plots and are colored by sample conditions.

1175 **D**, Expression activity of IFN-alpha in activated T cells of NJ, FJ, SJ, Cont, Conv, Mild and Seve

1176 conditions shown as violin plots and colored by sample conditions.

1177 **E** and **F**, Expression activity of cytotoxicity pathways in activated T cells (**E**) and subtypes (**F**) of

1178 NJ and Vacc (FJ and SJ) conditions shown as box plots and are colored by sample conditions.

1179 **G**, Expression activity of cytotoxicity pathways in activated T cells of NJ, FJ, SJ, Cont, Conv,

1180 Mild and Seve conditions shown as violin plots and colored by sample conditions.

1181 **H** and **I**, Expression activity of exhaustion genes in activated T cells (**H**) and subtypes (**I**) of NJ

1182 and Vacc (FJ and SJ) conditions shown as box plots and are colored by sample conditions.

1183 **J**, Expression activity of exhaustion genes in activated T cells of NJ, FJ, SJ, Cont, Conv, Mild and

1184 Seve conditions shown as violin plots and colored by sample conditions.

1185 **K** and **L**, Expression activity of apoptosis pathways in T cells (**K**) and subtypes (**L**) of NJ and

1186 Vacc (FJ and SJ) conditions shown as box plots and are colored by sample conditions.

1187 **M**, Expression activity of apoptosis pathways in activated T cells of NJ, FJ, SJ, Cont, Conv, Mild

1188 and Seve conditions shown as violin plots and colored by sample conditions.

1189 **N** and **O**, Expression activity of migration pathways in activated T cells (**N**) and subtypes (**O**) of

1190 NJ and Vacc (FJ and SJ) conditions shown as box plots and are colored by sample conditions.

1191 **P**, Expression activity of migration pathways in activated T cells of NJ, FJ, SJ, Cont, Conv, Mild

1192 and Seve conditions shown as violin plots and colored by sample conditions.

1193 **Q** and **R**, Gene expression level of CD2AP (**Q**) and TNFSF14 (**R**) in activated CD4+T cells in NJ,

1194 FJ, SJ, Cont, Conv, Mild and Seve conditions. Violin plots showed normalized average expression

1195 of CD2AP (**Q**) and TNFSF14 (**R**).

1196 **S** and **T**, Gene expression level of KDM5A (**S**) and TNFSF14 (**T**) in cytotoxic CD8+T cells in NJ,

1197 FJ, SJ, Cont, Conv, Mild and Seve conditions. Violin plots showed normalized average expression

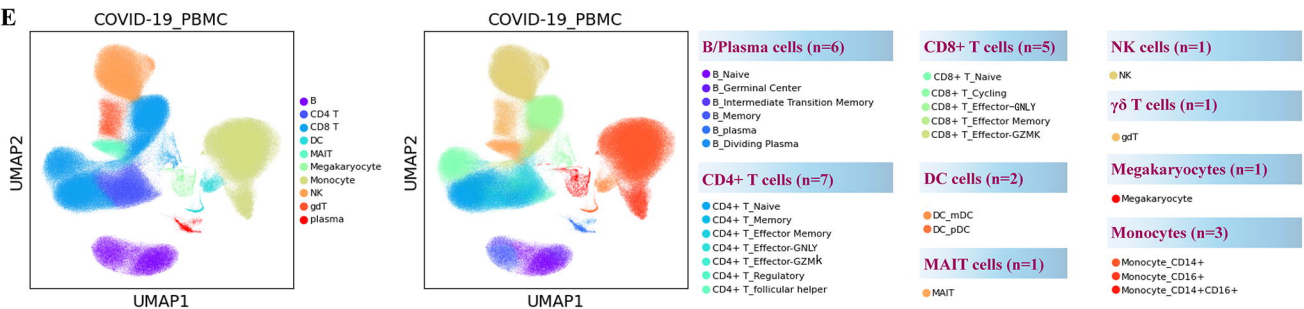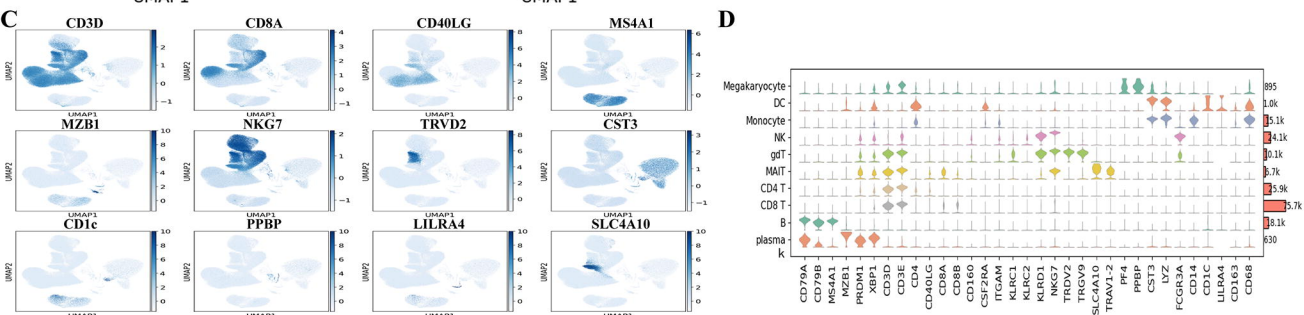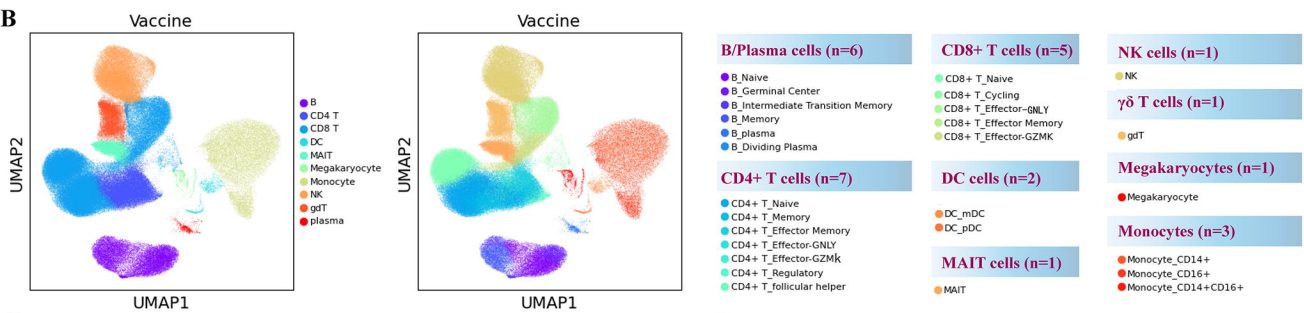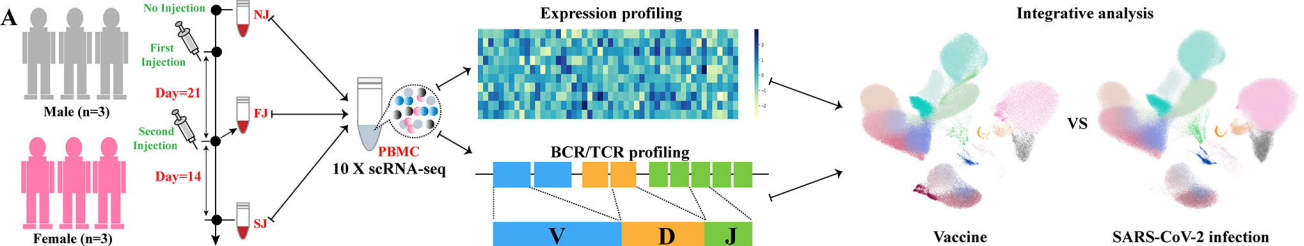1198 of KDM5A (**S**) and TNFSF14 (**T**).

1199 All pairwise differences with P < 0.05 using two-sided unpaired Mann–Whitney U-test are marked
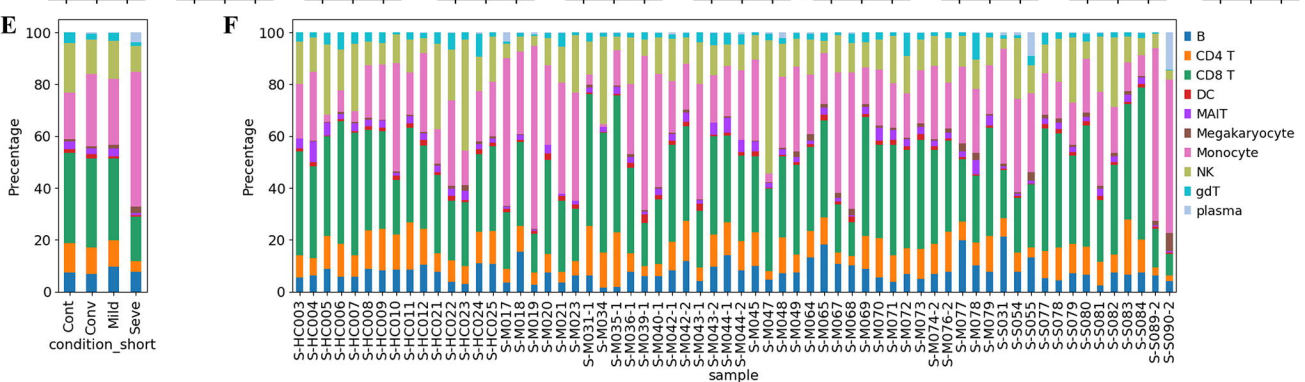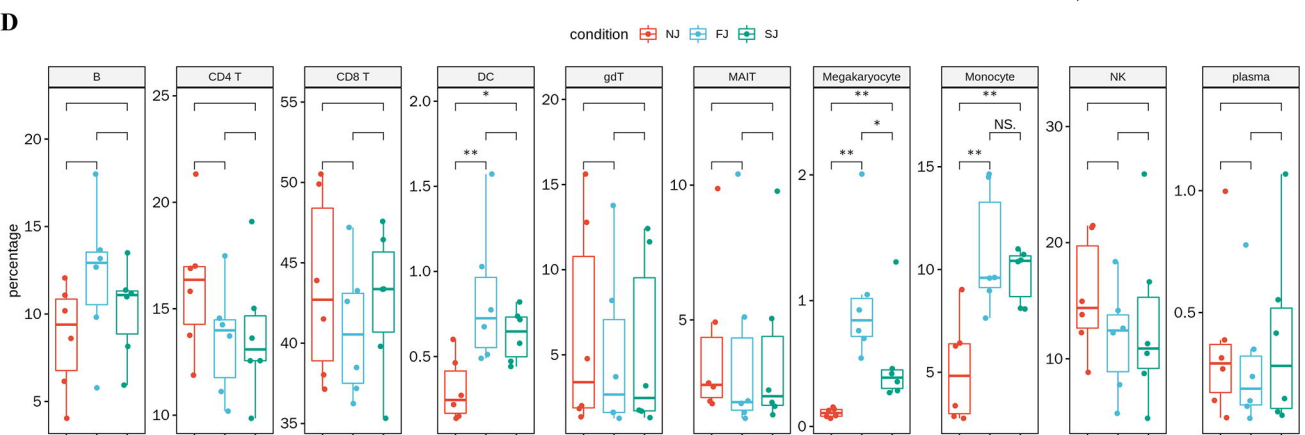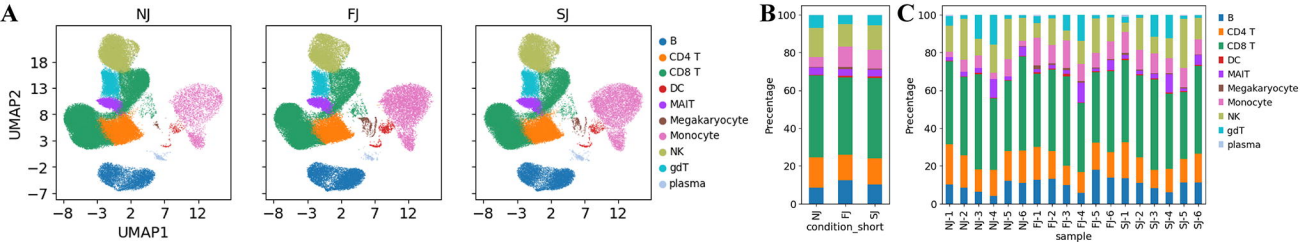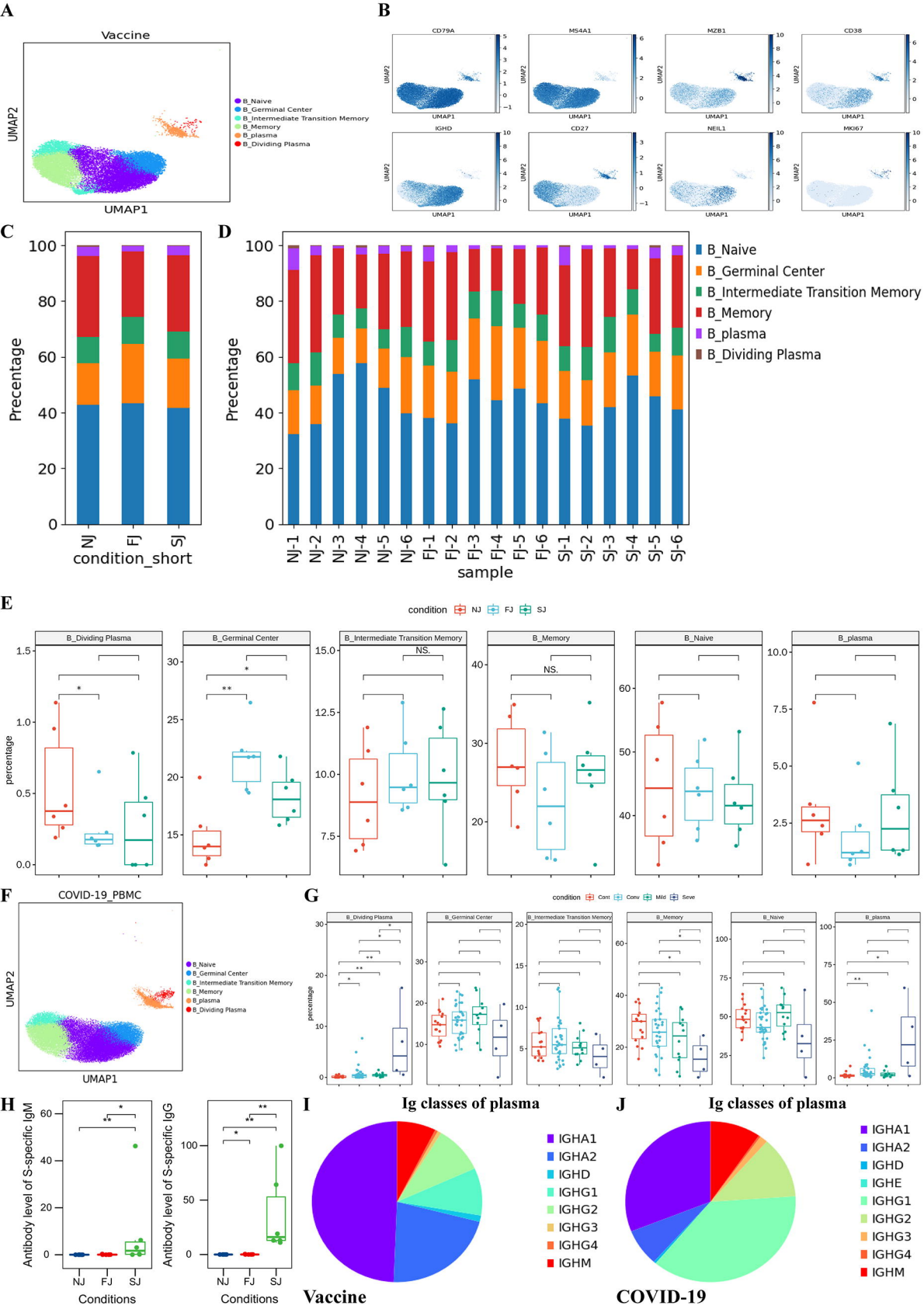
1200 to show significance levels.

1201

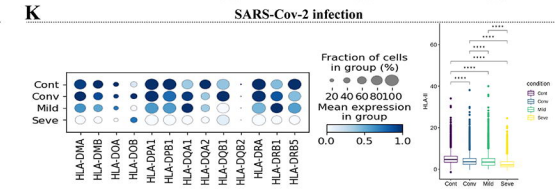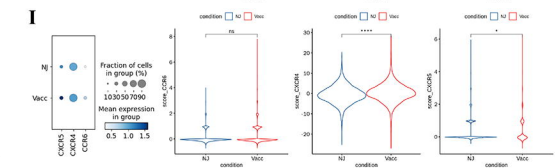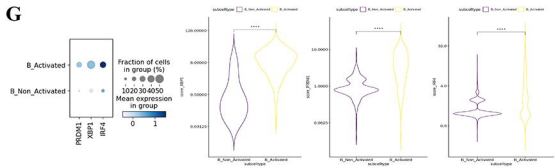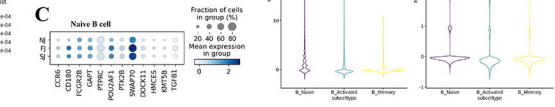1202 **Fig 10. Changes in TCR clones and selective usage of V(D)J genes.**

1203 **A**, UMAP projection of T cells derived from PBMCs. Cells are colored by conditions (Panel 1), T

1204 cell subtypes (Panel 2), if TCR detection was successful (Panel 3) and clonotype expansion size

1205 (panel 4).

1206   **B**, Stacked bar plot shows the TCR detection success rate for each T cell subtype.

1207   **C**, Histogram shows the negative correlation between the number of T cell clones and the number

1208   of cells per clonotype. Y axis is log10 scaled.

1209   **D**, Pie graph showing the distribution of TRBC1 and TRBC2 in T cells under each condition.

1210   **E**, Stacked bar plots showing the clone state of each T cell subtype in each condition.

1211   **F**, Heat maps showing differential TRBV-J and TRAV-J rearrangement. Prevalent TRBV-J
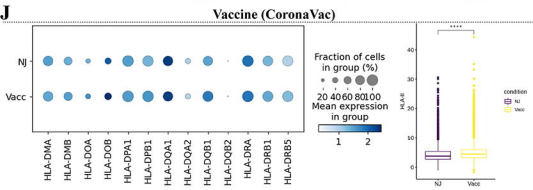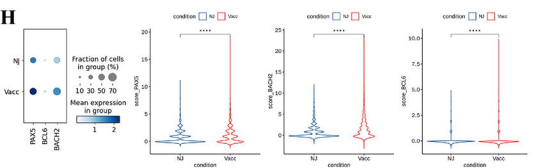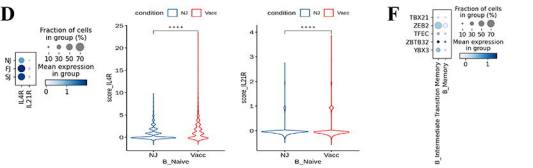
1212   combination pairs (top) and TRAV-J combination pairs (bottom) are compared across conditions.

1213   Usage percentage are sum normalized by column.

1214   **G**, Density curve plots showing the distribution shift of TRA and TRB chain CDR3 region length

1215   in TCR clone types from each condition.

1216   **H**, Box plot showing TRBC1 and TRBC2 percentages in NJ, FJ, SJ, Cont, Conv, Mild and Seve

1217   conditions

1218   **I**, Box plot showing the alpha diversity of TCR clonotypes in each PBMC sample. Data points are

1219   colored by condition.

A

No Injection — NJ
First Injection — Day=21
Second Injection — Day=14
— SJ

Male (n=3)
Female (n=3)
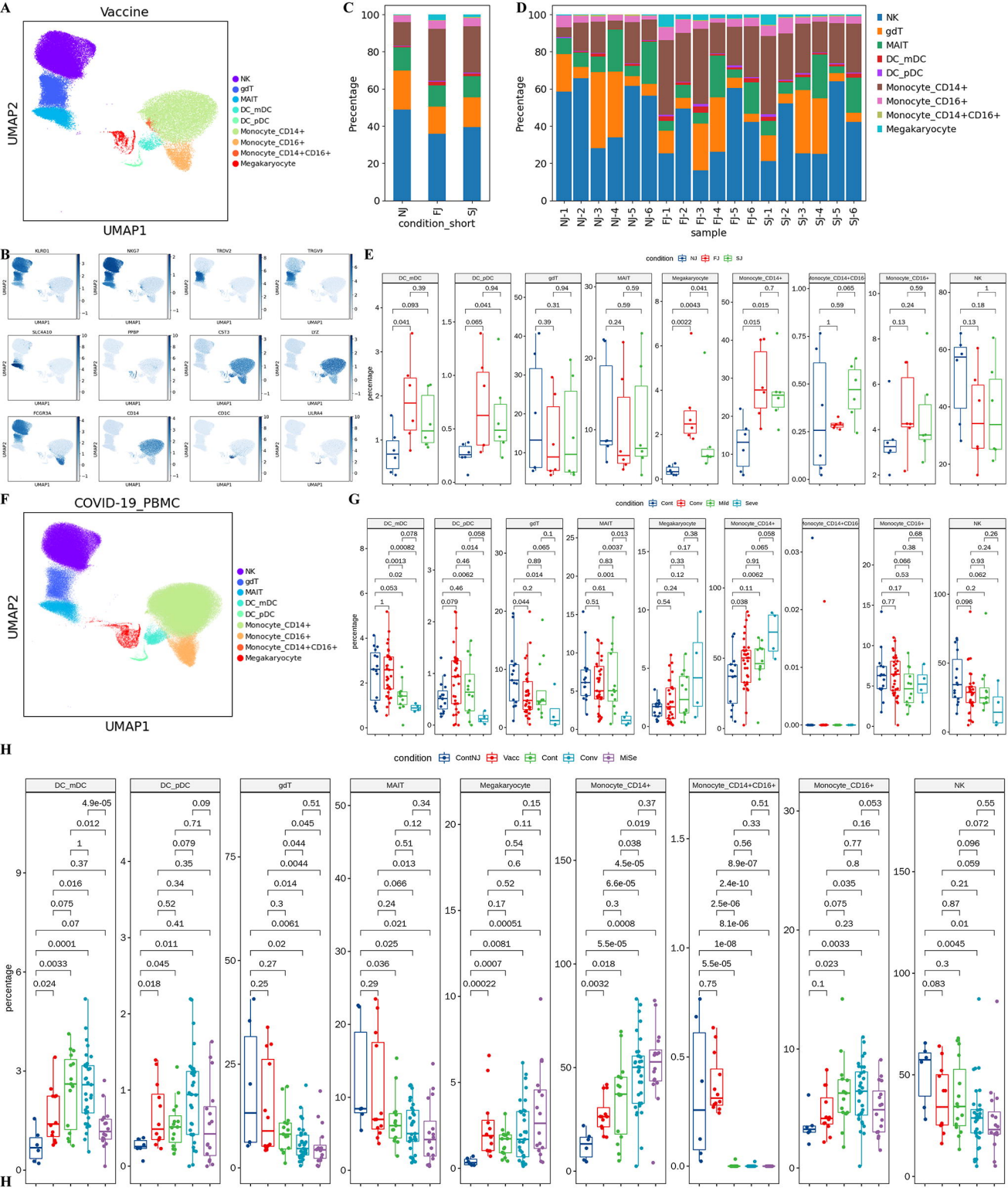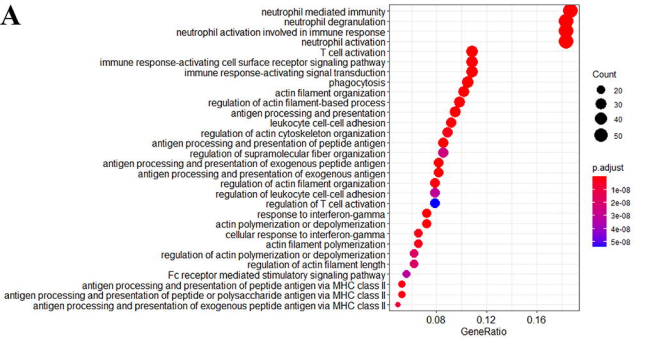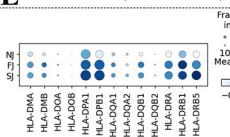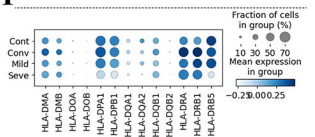
10 X scRNA-seq
PBMC

Expression profiling

BCR/TCR profiling
V D J

Integrative analysis

Vaccine VS SARS-CoV-2 infection

B

Vaccine

UMAP2 / UMAP1

Vaccine

UMAP2 / UMAP1

- B
- CD4 T
- CD8 T
- DC
- MAIT
- Megakaryocyte
- Monocyte
- NK
- gdT
- plasma

B/Plasma cells (n=6)
- B_Naive
- B_Germinal Center
- B_Intermediate Transition Memory
- B_Memory
- B_plasma
- B_Dividing Plasma

CD4+ T cells (n=7)
- CD4+ T_Naive
- CD4+ T_Memory
- CD4+ T_Effector Memory
- CD4+ T_Effector-GNLY
- CD4+ T_Effector-GZMK
- CD4+ T_Regulatory
- CD4+ T_follicular helper

CD8+ T cells (n=5)
- CD8+ T_Naive
- CD8+ T_Cycling
- CD8+ T_Effector-GNLY
- CD8+ T_Effector Memory
- CD8+ T_Effector-GZMK

DC cells (n=2)
- DC_mDC
- DC_pDC

MAIT cells (n=1)
- MAIT

NK cells (n=1)
- NK

γδ T cells (n=1)
- gdT

Megakaryocytes (n=1)
- Megakaryocyte

Monocytes (n=3)
- Monocyte_CD14+
- Monocyte_CD16+
- Monocyte_CD14+CD16+

C

CD3D, CD8A, CD40LG, MS4A1, MZB1, NKG7, TRVD2, CST3, CD1c, PPBP, LILRA4, SLC4A10

D

Megakaryocyte, DC, Monocyte, NK, gdT, MAIT, CD4 T, CD8 T, B, plasma k

895, 1.0k, 5.1k, 4.1k, 10.1k, 3.7k, 25.9k, 5.7k, 8.1k, 630

CD79A, CD79B, MS4A1, MZB1, PRDM1, XBP1, CD3D, CD3E, CD4, CD40LG, CD8A, CD8B, CD160, CSF2RA, ITGAM, KLRC1, KLRC2, KLRD1, NKG7, TRDV2, TRGV9, SLC4A10, TRAV1-2, PF4, PPBP, CST3, FCGR3A, CD14, LYZ, CD1C, LILRA4, CD163, CD68

E

COVID-19_PBMC

UMAP2 / UMAP1

COVID-19_PBMC

UMAP2 / UMAP1

- B
- CD4 T
- CD8 T
- DC
- MAIT
- Megakaryocyte
- Monocyte
- NK
- gdT
- plasma

B/Plasma cells (n=6)
- B_Naive
- B_Germinal Center
- B_Intermediate Transition Memory
- B_Memory
- B_plasma
- B_Dividing Plasma

CD4+ T cells (n=7)
- CD4+ T_Naive
- CD4+ T_Memory
- CD4+ T_Effector Memory
- CD4+ T_Effector-GNLY
- CD4+ T_Effector-GZMK
- CD4+ T_Regulatory
- CD4+ T_follicular helper

CD8+ T cells (n=5)
- CD8+ T_Naive
- CD8+ T_Cycling
- CD8+ T_Effector-GNLY
- CD8+ T_Effector Memory
- CD8+ T_Effector-GZMK

DC cells (n=2)
- DC_mDC
- DC_pDC

MAIT cells (n=1)
- MAIT

NK cells (n=1)
- NK

γδ T cells (n=1)
- gdT

Megakaryocytes (n=1)
- Megakaryocyte

Monocytes (n=3)
- Monocyte_CD14+
- Monocyte_CD16+
- Monocyte_CD14+CD16+

A

condition_short
- NJ
- FJ
- SJ
- Cont
- Conv
- Mild
- Seve

subcelltype
- B_Naive
- B_Germinal Center
- B_Intermediate Transition Memory
- B_Memory
- B_plasma
- B_Dividing Plasma

receptor_type
- BCR

clone_id_size

B

B cells
- IGHA1
- IGHA2
- IGHD
- IGHE
- IGHG1
- IGHG2
- IGHG3
- IGHG4
- IGHM

Plasma
- IGHA1
- IGHA2
- IGHD
- IGHE
- IGHG1
- IGHG2
- IGHG3
- IGHG4
- IGHM

C

B cells — condition NJ FJ SJ

Plasma — condition NJ FJ SJ

D

B cells

NJ 58% 42% | FJ 59% 41% | SJ 60% 40% | Cont 58% 42% | Conv 60% 40% | Mild 58% 42% | Seve 61% 39%

IGH+IGK / IGH+IGL

Plasma

NJ 54% 46% | FJ 61% 39% | SJ 54% 46% | Cont 55% 45% | Conv 57% 43% | Mild 64% 36% | Seve 52% 48%

IGH+IGK / IGH+IGL

E

NJ · FJ · SJ · Cont · Conv · Mild · Seve

clone
- 1
- 2
- 3
- >= 4

B_Naive, B_Germinal Center, B_Intermediate Transition Memory, B_Memory, B_plasma, B_Dividing Plasma

H

condition
- NJ
- FJ
- SJ
- Cont
- Conv
- Mild
- Seve

alpha_diversity_clone_id

I

Seve, Mild, Conv, Cont, SJ, FJ, NJ

IGK/L cdr3 aa length   IGH cdr3 aa length

F

A

condition_short | subcelltype | receptor_type | clone_id_size

- NJ
- FJ
- SJ
- Cont
- Conv
- Mild
- Seve

subcelltype:
- CD4+ T_Naive
- CD4+ T_Memory
- CD4+ T_Effector Memory
- CD4+ T_Effector-GNLY
- CD4+ T_Effector-GZMA
- CD4+ T_Regulatory
- CD4+ T_follicular helper
- CD8+ T_Naive
- CD8+ T_Cycling
- CD8+ T_Effector
- CD8+ T_Effector Memory
- CD8+ T_Effector-GZMK

receptor_type:
- TCR
- no IR

B With TCR: True / False

C

D
TRBC1 / TRBC2

NJ 32% / 68%
FJ 34% / 66%
SJ 34% / 66%
Cont 33% / 67%
Conv 35% / 65%
Mild 34% / 66%
Seve 41% / 59%

E clone expansion: Unique, 1-5, 6-20, 21-50, >50

F

G TRA cdr3 aa length / TRB cdr3 aa length

H condition: NJ, FJ, SJ, Cont, Conv, Mild, Seve — TRBC1 / TRBC2

I condition: NJ, FJ, SJ, Cont, Conv, Mild, Seve