Implicating Gene and Cell Networks Responsible for Differential COVID-19 Host Responses via an Interactive Single Cell Web Portal

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Summary

Numerous studies have provided single-cell transcriptome profiles of host responses to SARS-CoV-2 infection. Critically lacking however is a datamine that allows users to compare and explore cell profiles to gain insights and develop new hypotheses. To accomplish this, we harmonized datasets from COVID-19 and other control condition blood, bronchoalveolar lavage, and tissue samples, and derived a compendium of gene signature modules per cell type, subtype, clinical condition, and compartment. We demonstrate approaches to probe these via a new interactive web portal (http://toppcell.cchmc.org/ COVID-19). As examples, we develop three hypotheses: (1) a multicellular signaling cascade among alternatively differentiated monocyte-derived macrophages whose tasks include T cell recruitment and activation; (2) novel platelet subtypes with drastically modulated expression of genes responsible for adhesion, coagulation and thrombosis; and (3) a multilineage cell activator network able to drive extrafollicular B maturation via an ensemble of genes strongly associated with risk for developing post-viral autoimmunity.

Keywords

COVID-19, SARS-CoV-2, single-cell RNA-seq, host-pathogen cell atlas, interactive datamining, bronchoalveolar lavage, systems biology, antiviral host defense, platelets, inflammatory thrombosis, autoimmune disorder.

Introduction

COVID-19 clinical outcomes are variable. The poorer outcomes due to this infection are highly associated with immunological and inflammatory responses to SARS-Cov-2 infection (Shi et al., 2020; Tay et al., 2020) and many recent single cell expression profiling studies have characterized patterns of immunoinflammatory responses among individuals, mostly during acute infection phases. Different studies have revealed a spectrum of responses that range from lymphopenia (Cao, 2020; Wang et al., 2020), cytokine storms (Mehta et al., 2020; Pedersen and Ho, 2020), differential interferon responses (Blanco-Melo et al., 2020; Hadjadj et al., 2020) and emergency myelopoiesis (Schulte-Schrepping et al., 2020; Silvin et al., 2020). However, a variety of obstacles limit the ability of the research and medical communities to explore and compare these studies to pursue additional questions and gain additional insights that could improve our understanding of cell type specific responses to SARS-Cov-2 infection and their impact on clinical outcome.

Whereas many studies have focused on the peripheral blood mononuclear cells (PBMC) (Arunachalam et al., 2020; Guo et al., 2020; Lee et al., 2020; Schulte-Schrepping et al., 2020; Wilk et al., 2020a) due to ease of procurement, other studies have profiled airway locations via bronchoalveolar lavage (BAL) (Grant et al., 2020; Liao et al., 2020), nasopharyngeal swabs, and bronchial brushes (Chua et al., 2020). Additional sampling sites that could also be infected or affected have also been approached in autopsy-derived materials from the central nervous system (Heming et al., 2021; Yang et al., 2020), and other sites (Delorey et al., 2021). Moreover, as major COVID-19 consortiums working on the collection and integration of each of their individual studies and interpreting important features of these individual datasets as downloadable datasets or browsable versions, such as single cell portal (https://singlecell.broadinstitute.org/single_cell/covid19) and COVID-19 Cell Atlas (https://www.covid19cellatlas.org/), using these data beyond markers, cell types, and individual signatures is either not possible or not accomplishable across-datasets. Thus, a well-organized and systematic study of immune cells across tissues for in-depth

biological explorations is an unmet need for a deeper understanding of the underlying basis of the breadth of COVID-19 host defense and pathobiology.

Here we harmonized and analyzed eight high quality publicly available single-cell RNA-seq datasets from COVID-19 and immunologically-related studies that in total covered more than 480,000 cells isolated from peripheral blood, bronchial alveolar lavage and lung parenchyma samples, and assembled an integrated COVID-19 atlas (https://toppcell.cchmc.org/). We established a framework for deriving, characterizing, and establishing reference gene expression signatures from these harmonized datasets using modular and hierarchical approaches based on signatures per class, subclass, and signaling/activation and clinical status per each sample group. Leveraging these gene expression signature modules, we demonstrate datamining approaches that allow for the identification of a series of fundamental disease processes: (1) an intercellular monocytic activation cascade capable of mediating the emergence of hyperinflammatory monocyte-derived alveolar macrophages in severe COVID-19 patients; (2) the generation of several alternatively differentiated platelet subtypes with dramatically different expression of sets of genes associated with critical platelet tasks capable of altering vascular and tissue responses to infectious agents; and (3) a multilineage and multi cell type cooperative signaling network with the potential to drive extrafollicular B maturation at a lesion site, but do so with high risk for the development of B cell-associated immunity. Additionally, immune hallmarks of COVID-19 patients were compared with other immune-mediated diseases using single-cell data from patients with influenza, sepsis, or multiple sclerosis. Consistent and varied compositional and gene patterns were identified across these implicating striking COVID-19 effects in some individuals.

Results

Creating the First COVID-19 Signature Atlas Using ToppCell Portal

To have a comprehensive coverage of cells, we collated single-cell data of COVID-19 patients from eight public datasets, which in total contains 231,800 PBMCs, 101,800

118 BAL cells and 146,361 lung parenchyma cells from donors: 43 healthy; 22 mild; 42
119 severe; and 2 convalescent patients (**Figure 1A, Table S1**).
120
121 In order to assemble an integrated atlas of human cell responses to COVID-19, we
122 sought to harmonize metadata encompassing clinical information, sampling
123 compartments, and cell and gene expression module designations. Doing so provides a
124 rich framework for detecting perturbations of cell repertoire and differentiative state
125 adaptations. We first integrated single cell RNA-seq data in Seurat (Stuart et al., 2019)
126 and annotated cell types using canonical markers (**Table S2**). Further annotations of B
127 cell and T cell subtypes were completed using the reference-based labeling tool
128 Azimuth (Hao et al., 2020). Sub-clustering was applied for some cell types, such as
129 neutrophils and platelets, to interrogate finer resolutions of disease-specific sub-
130 populations (**Figure 1B**). Using the ToppCell toolkit (https://toppcell.cchmc.org/), we
131 created over 3,000 hierarchical gene modules of the most significant differentially
132 expressed genes (DEGs) for all cell classes and sub-clusters across compartments and
133 disease severity (**Table S1**). These modules were then used to infer cell-cell
134 interactions as well as upregulated pathways, which were further combined for
135 functional comparative analysis in a specific cell manner in ToppCluster (Kaimal et al.,
136 2010) (**Figure 1B**), such as sub-clusters of platelets. Integration of ToppCluster output
137 of cells from multiple compartments and disease conditions built pathogenic maps,
138 highlighted by the coagulation map of COVID-19 (**Figure S12**). In addition, perturbation
139 of cell abundance was evaluated either in one cell population, or in multiple populations
140 across diseases. Taken together, we investigated cell abundance changes, severity-
141 associated signatures, mechanisms of COVID-19 specific symptoms and unique
142 features of COVID-19 as an immune-mediated disease (**Figure 1B**).
143
144 **Dynamic Changes and Balance of COVID-19 Immune Repository in Blood and**
145 **Lung**
146 After the aforementioned cell annotation procedure, we identified 28 and 24 distinct cell
147 types in PBMC and BAL respectively (**Figure 2A, 2C; Table S2**). Shifts of Uniform
148 Manifold Approximation and Projection (UMAP) of cell type distributions were observed

149 in both compartments of mild and severe patients (**Figure 2A, 2C, S1A and 3A**). In
150 PBMC, conventional dendritic cells (cDC), plasmacytoid dendritic cells (pDC) and non-
151 classical monocytes displayed a prominent reduction in severe patients (**Figure 2B,**
152 **S1C**), consistent with prior reports (He et al., 2020; Laing et al., 2020; Wilk et al.,
153 2020a). In contrast, severe patients demonstrated dramatic expansion of neutrophils,
154 especially immature stages (**Figure S1C, S2**). Integration with evoked pathways in the
155 following analysis implicated that neutrophil expansion was likely the consequence of
156 emergency myelopoiesis (Wilk et al., 2020b). Additionally, a general down-regulation of
157 T cell and NK cell was observed, consistent with lymphopenia reported in clinical
158 practices (Pedersen and Ho, 2020; Terpos et al., 2020) (**Figure S1C, S2**). However, the
159 trend of T cell subtypes varies across studies and individuals, apart from proliferative T
160 cells which have a dramatic increase in mild and severe patients (**Figure S2**). Notably,
161 plasmablasts substantially increased in COVID-19 patients, and especially so in severe
162 patients, suggesting upregulated antibody production (De Biasi et al., 2020) (**Figure 2B**
163 **and S1C**). Expansion of platelets is another significant change observed in severe
164 patients, possibly leading to immunothrombosis in the lung, which could be closely
165 associated with the severity of the disease (Middleton et al., 2020; Nicolai et al., 2020)
166 (**Figure 2B and S1C**).
167
168 In samples obtained from patients' lungs, we observed the depletion of FABP4^{high}
169 tissue-resident alveolar macrophages (TRAM) and dramatic expansion of FCN1^{high}
170 monocyte-derived alveolar macrophages (MoAM) in severe patients (**Figure 2C, 2D**
171 **and S3D**). Mild patients exhibited a moderate reduction of tissue-resident
172 macrophages, but no evidence of aggregation of monocyte-derived macrophages
173 (**Figure 2C, 2D, S3A and S3D**). Dynamic changes of these two subtypes suggest
174 increased tissue chemoattraction (Merad and Martin, 2020) and potential damage of
175 patients' lungs (McGonagle et al., 2020). In addition, neutrophils were only identified in
176 severe patients in the integrated BAL data (**Figure 2C and S3A**), which might be
177 related with neutrophil extracellular traps (NETs) in the lung (Barnes et al., 2020).
178 However, more samples are required to draw a solid conclusion. We also noted
179 conventional dendritic cells decreased in the severe patients, which is consistent with

180 the trend of the counterpart in PBMC data. Opposite to the change in PBMC, an
181 expansion of plasmacytoid dendritic cells is observed in both mild and severe patients
182 (**Figure 2D**). Other cell types, including T cell and NK cell in the BAL, also have
183 converse changes of their counterparts in PBMC, which could be attracted by lung
184 macrophages or epithelial cells after infection or damages (Chua et al., 2020) (**Figure**
185 **2D and Figure S3D**). These changes were consistently observed in lung parenchyma
186 samples from severe COVID-19 patients (**Figure S4**). With cells well-annotated in the
187 integrated COVID-19 atlas, we drew a global heatmap for cells in both blood and lung
188 using ToppCell gene modules (top 50 DEG in each module) of all identified cell classes.
189 While there was conservation of gene patterns involved in healthy donors and severe
190 COVID-19 patients, there were substantial differences most notably in myeloid cells
191 (**Figure 2E**). Such hierarchically ordered ToppCell gene modules were broadly used in
192 visualization, large-scale comparisons and fine-resolution investigations in the following
193 analyses.
194
195 **Myeloid Cell Atlas: Functionally Distinct Neutrophils at Different Levels of**
196 **Maturation and Derailed Macrophages in the Lung**
197 Dysregulated myeloid cells have been reported as an important marker of severe
198 COVID-19 patients (Schulte-Schrepping et al., 2020; Silvin et al., 2020). In order to gain
199 a deeper and comprehensive understanding of these cells, we applied the sub-
200 clustering strategy on the integrated data of key cell types, such as neutrophils and
201 macrophages, and then generated gene modules for comparative functional analysis
202 and interactome inference. We successfully identified 5 neutrophil sub-clusters after the
203 integration of PBMC and BAL data, including 3 FCGR3B+ mature sub-clusters and 2
204 FCGR3B- immature sub-clusters (**Figure 3A and S5B; Table S3**). They're mainly from
205 severe patients and their gene modules were generated and subjected to comparative
206 functional enrichment using ToppCell and ToppCluster (**Figure 3C, 3D and S5A**). We
207 identified proliferative neutrophils (referred to as pro-neutrophils and Neu4) and
208 $MMP8^{high}$ precursor immature neutrophils (referred to as pre-neutrophils and Neu2)
209 (**Figure 3A and S5B**) consistent with prior studies (Schulte-Schrepping et al., 2020).
210 While immune response genes and pathways were barely activated in the immature

211 neutrophils, they displayed upregulation of granule formation pathways and NETosis-
212 associated proteins, including ELANE, DEFA4 and MPO, especially in Neu4 (Schulte-
213 Schrepping et al., 2020; Wilk et al., 2020b) (**Figure 3C, S5B and S5C**). Upregulated
214 myeloid leukocyte mediated immunity in Neu2 suggests involvement of this cell type in
215 anti-viral function (**Figure S5D**). Yet, the absence of cytokine and interferon response
216 pathways suggests the lack of mature immune responses (**Figure 3D**). Notably,
217 compared to mature neutrophils (Neu0 and Neu1) in the blood, the extravasated
218 hyperinflammatory sub-cluster (Neu3) from BAL of severe patients shows
219 extraordinarily high expression of interferon-stimulated genes, as well as prominent
220 upregulation of productions and responses to cytokines and interferons (**Figure 3C, 3D,**
221 **S5B and S5D**).
222
223 MoAM and TRAM were two main macrophage types in the BAL (**Figure 2C**); both are
224 known to have distinct roles in immune responses in the lung (Liao et al., 2020). As
225 described above, five sub-clusters among the expanded COVID-19 patient-specific
226 MoAM (**Figure 3B; Table S3**) were found, where the loss of HLA class II genes and
227 elevation of interferon-stimulated genes (ISGs) were consistently observed (**Figure 3F**
228 **and S6A**). Relative to MoAM3,4, MoAM1,2,5 displayed an upregulation of interferon
229 responses and cytokine production (**Figure 3D and S6B; Table S3**), indicating their
230 pro-inflammatory characteristics. Notably, MoAM5 shows dramatic upregulation of IL-6
231 secretion and cytokine receptor binding activities (**Figure S7A-D**). However, cells in this
232 sub-cluster were mainly from one severe patient (**Figure S3C**). We still need more data
233 to fully understand such dramatic upregulation of IL-6 secretion in some severe
234 patients. Similar to MoAM, we also identified two distinct groups of TRAM in BAL
235 (**Figure 3B and S6B**), including quiescent TRAM (TRAM1 and TRAM2) and activated
236 TRAM (TRAM3). The quiescent group was mainly from healthy donors with enriched
237 pathways of ATP metabolism (**Figure 3D**), while the activated group from mild and
238 severe patients displays upregulation of ISGs and cytokine signaling pathways (**Figure**
239 **S6B; Table S3**). However, the magnitude of activation and inflammatory responses in
240 TRAM3 is smaller than MoAM1,2,5. Not surprisingly, stronger antigen processing and
241 presentation activities were observed in TRAM3 relative to MoAM1,2,5 (**Figure 3D and**

242 **S6B; Table S3**). Collectively, we concluded that tissue-resident macrophages were
243 greatly depleted in severe patients as the front-line innate immune responders in the
244 lung. Pro-inflammatory monocyte-derived macrophages infiltrate into the lung, leading
245 to the cytokine storm and damage of the lung. Large amounts of infiltration of MoAM
246 were not observed in mild COVID-19 patients, probably due to the controlled infection,
247 which could explain milder lung damages in those patients.
248
249 To develop an understanding of the interaction network in the lung microenvironment of
250 severe COVID-19 patients, we focused on signaling ligands, receptors and pathways
251 using ToppCell and CellChat (**Figure 3E, S8A, S8B**). Notably, basal cells, MoAMs,
252 neutrophils and T cells all contributed to the cytokine, chemokine and interleukin
253 signaling networks. Strikingly, severe patient specific MoAM2 shows the broadest
254 upregulation of signaling ligands, including CCL2, CCL3, CCL7, CCL8, CXCL9, CXCL9,
255 CXCL10, CXCL11, IL6, IL15 and IL27, suggesting its role as a signaling network hub
256 that is distinct from the other major signaling ligand-expressing cells of BAL such as
257 epithelial and other myeloid cell types such as TRAM3 and proliferating myeloid cells
258 (**Figure S8A**). Among the MoAM2 top signaling molecules, attractants CXCL8, CXCL9
259 and CXCL10 are known to target CXCR3 on T cells, suggesting their role is to stimulate
260 migration of T cells to the epithelial interface and into BAL fluid (**Figure 3E**) (Liao et al.,
261 2020). In addition, many of MoAM2's ligands have the potential to cause autocrine
262 signaling activation via IL6-IL6R, IL1RN-ILR2, CCL7-CCR1, CCL2-CCR1 and CCL4-
263 CCR1, indicating its active roles in self-stimulation and development, which further
264 amplify the attraction and migration of T cells and other immune cells. Notably, CCR1
265 was also expressed in activated TRAM3, but with a lower level. Although IL6 expression
266 level is relatively low compared to other ligands in BAL data, substantial expression of
267 IL6R was observed in MoAMs. The CCL and CXCL signaling pathways of neutrophils
268 are less strong than MoAMs (**Figure S8B**), but they displayed high expression levels of
269 CXCR1 and CXCR2, which binds with a large number of the chemokines from MoAM
270 and epithelial cells (**Figure 3E**). In addition, neutrophils exhibit an extraordinarily high
271 level of IL1B, which could potentially in turn activate macrophages (**Figure S8A and**
272 **S8B**). TRAM3 also displayed a unique pattern of signaling molecules, with a substantial

273 level of CCL23 which could potentially attract MoAM by the interaction with CCR1.
274 Secretion of CXCL3 and CXCL5 in TRAM3 towards CXCR2 could be a potential
275 chemoattraction pathway for neutrophils. In turn, neutrophils could activate TRAM3 by
276 secreting IL1B, which binds with IL1RAP. Additionally, CD4+ T cells could also activate
277 TRAM3 by IL10-IL10RB interaction (**Figure 3E, S8A and S8B**).
278
279 In addition to neutrophils and macrophages, the upregulation of ISGs was observed in
280 classical monocytes of both mild and severe patients (cMono3, cMono4), while the
281 reduction of the MHC class II cell surface receptor HLA-DR genes was only observed in
282 severe patients (cMono4) (**Figure S9**). In cDCs, polarization of interleukin secretion was
283 observed in mild-patient and severe-patient specific clusters (**Figure S10F**).
284 Collectively, dynamic changes of marker genes, transcriptional profiles, signaling
285 molecules and biological activities reveal the heterogeneity of myeloid cell sub-clusters
286 across disease severity (**Figure S11C**). Pro-inflammatory gene expression was found in
287 all major myeloid cell types, including cMono4, DC1, DC9 in PBMC and Neu3, DC10,
288 MoAM1, MoAM2, MoAM5 in BAL of COVID-19 patients. The reduction of MHC class II
289 (HLA-II) genes is a common feature of classical monocytes and macrophages in
290 COVID-19 patients and implies impaired capacity to activate T cell adaptive immunity.
291
292 **COVID-19 Coagulation and Immunothrombosis Map**
293 Individuals severely affected during acute phase COVID-19 infection, and in particular
294 those with significantly elevated risk of death, frequently demonstrate striking
295 dysregulation of coagulation and thrombosis characterized by hypercoagulability and
296 microvascular thromboses (endothelial aggregations of platelets and fibrin) and highly
297 elevated D-dimer levels. Yet, COVID-19 does not lead to wide scale consumption of
298 fibrinogen and clotting factors (Iba et al., 2020a; Levi et al., 2020; Middleton et al., 2020;
299 Nicolai et al., 2020; Rapkiewicz et al., 2020). At present, we lack a molecular or cellular
300 explanation of the underlying basis of this pathobiology (Aid et al., 2020; Middleton et
301 al., 2020). To evaluate candidate effectors of this pathobiology, we used a list of genes
302 associated with abnormal thrombosis from mouse and human gene mutation
303 phenotypes and identified parenchymal lung sample endothelial cells and platelets in

304 PBMC as cell types highly enriched with respect to genes responsible for the regulation
305 of hemostasis (**Figure S12**). Because platelet counts were greatly elevated in severe
306 versus mild individuals, we further examined platelet gene expression signatures and
307 cell type differentiation and identified six distinct platelet sub-clusters shared across all
308 datasets after data integration (**Figure 4A and 4B**). Severe-patient-specific PLT0 is an
309 interesting sub-cluster with elevated integrin genes, including ITGA2B, ITGB1, ITGB3,
310 ITGB5, as well as thrombosis-related genes, such as SELP, HPSE, ANO6 and PF4V1.
311 Antibodies against the latter are associated with thrombosis including adverse reactions
312 to recent COVID-19 vaccine ChAdOx1 nCoV-19 (Schultz et al., 2021). In addition,
313 upregulated pathways of hemostasis, wound healing and blood coagulation were also
314 observed in PLT0 (**Figure S13A; Table S4**). Importantly, PLT2 is an inflammatory sub-
315 cluster with an upregulation of ISGs and interferon signaling pathways, while PLT4 is
316 highlighted by upregulated post-transcriptional RNA splicing activities (**Figure S13A**
317 **and S13C**).
318
319 Severity-associated gene patterns were also identified by selecting coagulation-
320 associated genes modules (**Figure 4C; Table S4**), indicating distinct coagulation
321 activities across platelets. Apart from pan-platelet genes, we found dramatic
322 upregulation of genes involved in platelet activation, fibrinogen binding and blood
323 coagulation in platelets of severe COVID-19, including procoagulant heparanase
324 (HPSE) (Osterholm et al., 2013), Anoctamin-6 (ANO6) (Swieringa et al., 2018), and
325 selectin P (SELP) (Sparkenbaugh and Pawlinski, 2013) (**Figure 4C and 4D**).
326 Heparanase is an endoglycosidase that cleaves heparan sulfate constituents, a major
327 component of anti-coagulation glycocalyx on the surface of vascular endothelium
328 (Edovitsky et al., 2006; Iba et al., 2020b). Upregulated heparanase was related to
329 upregulation of cell-matrix adhesion and coagulation (**Figure 4D**). Thrombotic vascular
330 damages could be caused by the degradation function of heparinase enriched in
331 platelets of severe patients. Elevation of ANO6 is known to trigger phospholipid
332 scrambling in platelets, resulting in phosphatidylserine exposure which is essential for
333 activation of the clotting system (Heemskerk et al., 2013). In addition, other upregulated
334 genes involved in coagulation-associated activities were also observed, including

335 wound healing, fibrinolysis, platelet aggregation and activation (**Figure 4D**), which likely
336 collectively contribute to the clotting issue of severe COVID-19 patients.
337
338 **Emergence of Developing Plasmablasts and B Cell Association with**
339 **Autoimmunity**
340 Autoimmune disorders in COVID-19 patients such as immune thrombocytopaenic
341 purpura (ITP) is now recognized as a known disease complication (Ehrenfeld et al.,
342 2020; Fujii et al., 2020; Gruber et al., 2020; Rodríguez et al., 2020; Zhou et al., 2020).
343 However, little is known about the molecular and cellular mechanism behind it. To
344 examine this further, we integrated B cells and plasmablasts from both PBMC and BAL
345 and conducted systematic analysis (**Figure 5A, 5B, S14A and S14B**). Several COVID-
346 19 specific sub-clusters were identified in B cells, such as ISG^{high} activated B cells
347 (cluster 7) (**Figure 5A**). Importantly, activated B cells showed dramatic upregulation of
348 interferon signaling pathways and cytokine productions (**Figure S15A**), indicating its
349 anti-virus characteristics. Notably, plasmablasts were mainly observed in severe
350 COVID-19 patients, where a group of proliferative cells was identified and labeled as
351 developing plasmablasts (**Figure 5B**). In contrast, non-dividing plasmablasts displayed
352 upregulation of immunoglobulin genes (IGHA1, IGHA2, IGKC), B cell markers (CD79A)
353 (Mason et al., 1995), interleukin receptors (IL2RG) and type II HLA complex (HLA-DOB)
354 (**Figure 5C; Table S5**). In addition, non-dividing plasmablasts showed unique isotypes
355 of immunoglobulin (Ig) in sub-regions of UMAP, whereas developing plasmablasts
356 displayed obscure Ig types (**Figure S14E and S14F**). Antibody production activities
357 were upregulated in non-dividing plasmablasts based on gene enrichment analysis
358 (**Figure S15A; Table S5**). Collectively, we inferred that non-dividing plasmablasts had
359 definite immunoglobulin isotypes and were actively involved in immune responses
360 towards COVID infection, while developing plasmablasts were less mature but highly
361 proliferative to replenish the repertoire of plasma cells.
362
363 Since there are few clues of gene associations of autoimmunity in COVID-19, we
364 brought up a hypothesis-driven, prior knowledge-based approach to discover and
365 prioritize genes for the specific phenotype (**Figure 5D**). First, gene modules of B cells

366 and other cells in severe patients were collected and subjected to ToppGene for
367 enrichment analysis. Then we queried autoimmunity-associated terms in the enriched
368 output and identified associated genes. After that, we retrieved interaction pairs using
369 ToppCluster and CellChat database (Jin et al., 2020). In the end, we identified genes
370 that are not only involved in autoimmunity, but have a mediator role in the immune
371 signaling network. Using this approach, we observed several candidate pairs of genes,
372 including TNFSF13B-TNSRSF13, IL10-IL10RA, IL21-IL21RA, IL6-IL6R, CXCL13-
373 CXCR5, CXCL12-CXCR4, CCL21-CCR7, CCL19-CCR7 and CCL20-CCR6 in severe
374 patients, which were enriched for autoimmune diseases, such as autoimmune thyroid
375 diseases, lupus nephritis, autoimmune encephalomyelitis (Aust et al., 2004; Hirota et
376 al., 2007; Kuwabara et al., 2009; Lee et al., 2010; Steinmetz et al., 2008). Candidate
377 cytokine and chemokine ligand genes were expressed in various cell types in PBMC
378 and BAL, including IL21 and CXCL13 from exhausted T cells of BAL, CXCL12 from
379 mesenchymal cells, IL6 and CCL21 from endothelial cells, CCL19 from cDC and
380 CCL20, TNFSF13B, and TNFSF13 from lung macrophages (**Figure 5E; Table S5**).
381 These interaction pairs have been linked with auto-immunity (Klimatcheva et al., 2015;
382 Wong et al., 2010). In addition, we analyzed single-cell studies (Arazi et al., 2019;
383 Zhang et al., 2019) of rheumatoid arthritis and lupus nephritis patients and found that
384 high expression levels of the candidate receptors in B cells and ligands in other cells
385 were also observed, such as CXCL13 in helper T cells and CXCR5 in B cells in both
386 studies (**Figure S15C, S15D**). However, more evidence is still required to infer the
387 association between these interactions and autoimmunity in COVID-19 patients.
388 Supported by the evidence above, we drew a network for potential mediator interactions
389 of B cells and their associations with autoimmune disorders, where linkages with
390 diseases, such as rheumatoid arthritis, systemic lupus erythematosus, were highlighted,
391 as well as linkages with mouse phenotypes, such as abnormal immune tolerance and
392 increased susceptibility to autoimmune disorder (**Figure 5F**). As a caveat, although
393 using prior knowledge to prioritize gene and cell-associated functions and interactions
394 may introduce biases, such approaches also have the potential to highlight key
395 similarities and differences between different disease causes and clinical responses and
396 improve our understanding of the molecular and cellular mechanisms at work.

397

398 **Functional Map and Immune Cell Interplay Landscape in COVID-19**

399 As above, where highly significant enrichments of unique functions and pathways could
400 be identified in the subtypes of multiple cell classes, such as neutrophils, platelets and B
401 cells, we sought to get a more holistic understanding of COVID-19 specific cell class
402 and subclass-level signatures, including T cell subtypes (**Figure S16 and S17**), we built
403 an integrative functional map of all cell types in three compartments across multiple
404 disease conditions using a highly integrated gene module set (**Figure 6A; Table S6**). All
405 enriched functional associations in ToppCluster for gene modules of cell types and sub-
406 clusters were depicted. They were grouped by disease conditions and compartments to
407 show heterogeneity of cellular functions in different circumstances.

408

409 In the heatmap (**Figure 6A**), most enrichments were consistently observed across cells
410 of healthy donors and COVID-19 patients. However, some unique patterns were also
411 identified. For example, T cells and NK cells in healthy donors show enrichments of
412 mitochondrial transport and ATP metabolic process, while activated T cells in mild
413 patients show upregulation of type I interferon production and cytokine signaling.
414 Enrichments of macrophage differentiation and neutrophil migration regulation were
415 uniquely found in MoAM1 in severe patients (**Figure 6A**). The function map provides a
416 high-level approach to investigate functional variations of cells across disease
417 conditions and compartments. The predicted interplay of immune cells across multiple
418 compartments and disease conditions is displayed in **Figure 6B**. Cell proportion
419 changes, sub-cluster specific signatures and cell-cell interaction are also depicted.

420

421 **Similarity and Heterogeneity Between COVID-19 and Other Immune-mediated**
422 **Diseases**

423 To further analyze COVID-19 specific immune signatures, we compared immune cells
424 from COVID-19 patients with cells in other immune-mediated diseases, including severe
425 influenza (Lee et al., 2020), sepsis (Reyes et al., 2020) and multiple sclerosis (Schafflick
426 et al., 2020). 404,125 cells were included after the integration of PBMC single-cell
427 datasets (**Figure 7A, S18; Table S7**). Dynamic changes of cell abundance were

428 compared in diseases versus healthy donors. Similar to COVID-19 patients, severe
429 influenza patients also exhibited the reduction of non-classical monocytes, pDC, cDC
430 and CD4+ TCM, but the effect of the former two types was smaller in magnitude (**Figure**
431 **7B**). However, the reduction of non-classical monocytes is more significant in severe
432 COVID-19 patients than severe influenza or mild COVID-19 patients (**Figure 7B**).
433 Notably, NK cell reduction is associated with COVID-19 severity, whereas T cell
434 depletion is a more dramatic perturbation in severe influenza. Within these
435 comparisons, the expansion of plasmablasts is consistently observed, whereas the
436 accumulation of platelets is unique to SARS-CoV-2 and in particular, to severe COVID-
437 19 clinical status (**Figure 7B**).
438
439 In addition to dynamic changes of cell ratios, we also investigated the regulation of
440 immune mediator genes across various diseases (**Figure 7C; Table S7**). IL-6 is an
441 important factor of cytokine storms in COVID-19 (Zhao, 2020). As shown in the
442 heatmap, naive B cells are the main sources of IL-6 in COVID-19 patients while CD14+
443 monocytes show the highest expression levels in severe influenza patients (**Figure 7C**).
444 Specific ligands, including CXCL2, CXCL3, CCL20 were upregulated in both severe
445 COVID-19 patients and severe influenza patients. CCR4 and IL2RA is uniquely high in
446 CD4+ T cells of COVID-19 patients. Interestingly, most PBMC myeloid cell types
447 displayed upregulated levels of interferon-stimulated genes in both COVID-19 and
448 influenza, especially in COVID-19, where highest levels of ISGs in CD14+ Monocytes,
449 cDC and pDC were observed.
450
451

452 # Discussion

453 In this work, we have constructed an innovative immune signature atlas of the blood
454 and lung of COVID-19 patients using the integrated single cell RNA-sequencing data
455 and Topp-toolkit. By virtue of systemic analysis of large sample size from multiple
456 sampling sites, consistent immunopathology-associated changes of cell abundance and
457 transcriptional profiles were observed in the circulating and lung immune repertoire of
458 COVID-19 patients. The established single cell atlas and the provided public portal
459 (https://toppcell.cchmc.org/) enables the query of candidate molecules and pathways in
460 each of these processes.
461
462 Leveraging this approach, we identified three major candidate mechanisms capable of
463 driving COVID-19 severity: (1) a cascade-like network of proinflammatory autocrine and
464 paracrine ligand receptor interactions among subtypes of differentiating mononuclear,
465 lymphoid, as well as other cell types; (2) the production of emergency platelets whose
466 gene expression signatures implicate significantly elevated potential for adhesion,
467 thrombosis, attenuated fibrinolysis, and potential to enhance the release of heparin-
468 bound cytokines as well as further influence the activation of neutrophils causing further
469 inflammatory cell recruitment and neutrophil netosis; and (3) the extrafollicular activation
470 of naive and immature B cells via a multilineage network that includes monocytic
471 subtypes and exhausted T cells of cytokines and interleukins with the potential to
472 generate local antigen specific response to virus infected targets and collateral
473 autoimmunity. More details will be discussed below.
474

475 We identified dramatically expanded macrophages which were marked by the loss of
476 HLA class II genes and upregulation of interferon-stimulated genes. It implicates a key
477 role for these activated macrophages involved in signaling network and less so in
478 activation of adaptive T cell immunity. Among them, MoAM2 displayed
479 hyperinflammatory responses and extraordinary high levels of signaling molecules,
480 which are involved in both autocrine (e.g. IL-6, CCL2, CCL4 and CCL8) and paracrine
481 (e.g. CXCL2, CXCL9, CXCL10 and CXCL11) signaling pathways. The former pathway

482 contributed to the self-stimulation and development, which amplified the paracrine
483 pathway for T cell and neutrophil chemoattraction. The latter two cell types in turn
484 activated MoAMs with cytokines genes (CCL5, IL10 of T cells and IL1B of neutrophils,
485 respectively). Based on the intercellular and multifactor complexity of the signaling
486 cascade we have outlined, to effectively control a malignant inflammatory cascade, it
487 may be essential to consider simultaneously targeting multiple nodes of this network of
488 cytokines and interleukins. In addition, HLA-DR^{low} monocytes, likely reflecting
489 dysfunctional cells, were observed in severe infection. This, along with evidence of
490 emergency myelopoiesis with immature circulating neutrophils into the circulation was
491 detected in severe COVID-19. These neutrophils had transcriptional programs
492 suggestive of dysfunction and immunosuppression not seen in patients with mild
493 COVID-19. As such, we have presented evidence for the contribution of defective
494 monocyte activation and dysregulated myelopoiesis to severe COVID.

495

496 Platelet expansion is uniquely observed in COVID-19 versus other immune-mediated
497 diseases.
498 Strikingly, these activated platelets were highlighted with abnormal thrombosis and
499 upregulated heparanase, a procoagulant endoglycosidase that cleaves anti-coagulation
500 heparan sulfate constituents on endothelial cells and potentially causes thrombotic
501 vascular damages. Additionally, heparanase-cleaved heparan sulphate (HS) fragments
502 were capable of stimulating the release of pro-inflammatory cytokines, such as IL1B,
503 IL6, IL8, IL10 and TNF through the TLR-4 pathway in PBMC (Goodall et al., 2014),
504 further contributing to the hyperinflammatory environment in COVID-19 patients. Since
505 heparanase is recognized as a hallmark in tumor progression and metastasis
506 (Jayatilleke and Hulett, 2020), we hypothesize COVID-19 infection could be associated
507 with higher occurrence of lung tumor metastasis. However, more data is required to
508 support it. Pro-neutrophil secreted proteins (e.g. ELANE, DEF4) of neutrophil
509 extracellular trap (NET), which have been reported to be associated with higher risk of
510 morbid thrombotic events (Zuo et al., 2021). Approaches to combatting NETs could a
511 potential anticoagulation treatment (Thålin et al., 2019).

512

513 We propose a signaling network which potentially shapes the differentiation of B cells
514 towards the formation of autoantibodies. Proliferation and activation of inflammatory
515 myeloid cells and the formation of exhausted CD4+ T helper around an area of direct or
516 indirect viral tissue injury leads to the production of a set of interleukins and cytokines
517 known to have both direct cell activating and maturing effects on naïve and immature B
518 cells. Previous report had revealed the exaggerated extrafollicular B cell response,
519 which is part of a mechanism that stimulates somatic mutation and maturation of B cells
520 to produce plasma cells with specificity for antigens present in the vicinity of tissue
521 damage sites (Farris and Guthridge, 2020). In the absence of macrophages or dendritic
522 cells to restrict self vs non-self, the presence of IL-10, IL-21, CXCL13 CXC10, IL-6 and
523 others acting on receptors present in naïve and immature B cells leads to the selection
524 and maturation of self-reactive maturation of B cells clones with formation of
525 autoantibodies. Many of these COVID-19-activated genes (e.g. CXCL13, CCL19,
526 CCL20, TNFRSF13) are known to be genetically associated with rheumatoid arthritis,
527 lupus, and risk of developing autoimmune disease in humans and mouse models. The
528 development of different patterns of autoimmunity may be the main hallmark of “Long
529 Haul” Covid disease and could explain why some individuals develop different
530 autoantibodies and suffer different forms of clinical consequences depending on which
531 antigens drive the B-cell maturation. Thus, an additional prediction that could be made
532 based on these findings and our network model is that among individuals treated with
533 corticosteroids at the time these auto-immunogenic processes are activated, there
534 should be a protective effect and lower likelihood of developing post acute sequela of
535 Covid.

536

537 Consistent and varied compositional changes and gene patterns of immune cells were
538 identified in COVID-19, influenza and sepsis. Expansion of plasmablasts, as well as the
539 reduction of non-classical monocytes, are more significant changes in severe COVID-19
540 patients, while the depletion of T cells is more dramatic in severe influenza patients. The
541 accumulation is a unique immune hallmark of COVID-19 within the selected diseases,
542 which contributes to the coagulation abnormalities and thrombosis, a key cause of

543 fatality in COVID-19 patients. Different signaling gene patterns were identified across
544 immune-mediated diseases, with CCR4 only highly expressed in CD4+ T cells of
545 COVID-19 patients, which might be related with extravasation of these cells (Spoerl et
546 al., 2021). Upregulated interferon-stimulated genes of myeloid cells in PBMC revealed
547 the inflammatory environment of COVID-19.
548
549 Collectively, using the COVID-19 single cell atlas data exploration environment, we
550 have illustrated is that researchers are now enabled to systematically explore, learn,
551 and formulate new hypotheses within and between compartments, cell types, and
552 biological processes, and provided access to these reprocessed datasets through a
553 suite of explorative and evaluative tools. Moreover, we have shown different hypotheses
554 can be developed and explored using the approaches that we have outlined and the
555 database that we have provided. Certainly additional critical information will also be
556 obtained using approaches that include in situ spatial, temporal data as well as those of
557 viral products and viral and inflammatory-process affected complexes. Next steps for
558 improving its ability to be mined more deeply will be based on additional statistical
559 methods that extend the current ToppCell / ToppGene Suite based on fuzzy measure
560 similarity, Page-Rank, and cell-cell signaling approaches.
561
562 There are several limitations in our study. Different studies used various standards of
563 COVID-19 severity definition. To generalize conclusions, we simplified disease
564 conditions into several universal groups. Prospectively, a standardized definition of
565 disease stages will assist to the accuracy of future studies. Additionally, the timing of
566 sample collection was not considered as a variable in this study, rather disease stages
567 were used to consolidate data across samples. W lack follow-up data of patients with
568 sequela, which will be helpful for understanding the long-haul effects of the disease.
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Author Contributions

Conceptualization, K.J. and B.A.; Methodology, K.J., B.A., E.B., A.M. and J.Y.W; Investigation, K.J. and B.A.; Writing – Original Draft, K.J.; Writing – Review & Editing, K.J., B.A., M.E.R., A.M., D.A.P.K, S.S.G. and S.B.; Funding Acquisition, B.A.; Resources, K.J.; Data Curation, K.J. and E.B.; Visualization, E.B.; Supervision, B.A..

Declaration of Interests

The authors declare no competing interests.

Resource availability

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Bruce Aronow (bruce.aronow@cchmc.org).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

603 Public single-cell RNA-seq datasets of PBMC in COVID-19 patients are available on
604 NCBI Gene Expression Omnibus and European Genome-phenome Archive, including
605 GSE150728, GSE155673, GSE150861, GSE149689 and EGAS00001004571 (or
606 FastGenomics). BAL single-cell RNA-seq datasets of COVID-19 patients are available
607 on GSE145926 and GSE155249. Lung Parenchyma single-cell RNA-seq data are
608 available on GSE158127.
609 Single-cell RNA-seq data of sepsis patients are available on the Single Cell Portal
610 SCP548 and SCP550. Data of multiple sclerosis patients are available on GSE128266.
611 Data of severe influenza patients are available on GSE149689.
612 Gene modules of all datasets analyzed using ToppCell web portal are available on
613 COVID-19 Atlas in ToppCell, including gene modules from either a single dataset or an
614 integrated dataset. Gene modules from the integration of specific cell types, such as B
615 cells and neutrophils are also listed in ToppCell. More details are listed in Figure1A and
616 Table S1. An interactive interface of integrated PBMC data and subclusters of immune
617 cells will be public on cellxgene.
618 Codes of preprocessing, normalization, clustering and plotting of single-cell datasets will
619 be available on github.
620

621 # Methods

622

623 **Single-cell RNA-seq data source**

624 To have a comprehensive understanding of immune cells in different repertoires, we
625 collected 8 public COVID-19 single-cell RNA-seq datasets of multiple compartments,
626 including peripheral blood mononuclear cells, bronchoalveolar lavage and lung biopsy,
627 which in total covered over 43 healthy donors, 22 mild/moderate, 42 severe and 2
628 convalescent COVID-19 patients. More details can be found in Figure 1A and Table S1.
629 Lung biopsy samples were taken from the explanted lung or post-mortem lungs of
630 COVID-19 patients(Bharat et al., 2020). Various criteria were used in these publications
631 to describe COVID-19 severity. For example, we found asymptomatic, mild, moderate
632 and floor COVID-19 patients under the definition of non-severe COVID-19 patients in
633 our data sources. A recent paper used the WHO score of COVID-19 severity to

634 categorize disease conditions of patients(Wilk et al., 2020b), which is a more
635 standardized and robust approach for the description of disease stages. However, in
636 order to address the issue of missing information for disease stratification and to
637 simplify the comparison, we grouped disease conditions into three groups, including
638 healthy donors, mild COVID-19 patients and severe COVID-19 patients. Convalescent
639 patients were excluded in some of our analysis for simplification. Sequencing data of
640 healthy donors in Guo et al. was excluded since it was not from the same institute(Guo
641 et al., 2020).
642 We also collected PBMC single-cell RNA-seq data from 29 sepsis patients(Reyes et al.,
643 2020) and 4 multiple sclerosis(Schafflick et al., 2020) patients for comparative analysis
644 of immune-mediated diseases (**Figure 1A; Table S1**). Data sources can be found in
645 Data Availability.
646
647 **Data preprocessing and normalization**
648 For datasets with raw UMI counts, we first removed cells with less than 300 detected
649 genes or less than 600 UMI counts. Then cells with more than 15% counts of
650 mitochondrial genes were filtered out. Genes expressed in less than 5 cells were
651 removed. After quality control, we finally harvested 483,765 high-quality cells from 8
652 studies (**Table S1**). We normalized the total UMI counts per gene to 1 million (CPM)
653 and applied $\log_2$(CPM+1) transformation for heatmap visualization and downstream
654 differential gene expression analysis. Steps above were done in Scanpy(Wolf et al.,
655 2018).
656 For some datasets that only provide processed and normalized *h5ad* or *rds* files, we
657 checked their preprocessing procedures in the original publications and confirmed that
658 stringent quality control procedures were used. Most of them used the default
659 normalization approach in the Seurat or Scanpy pipeline. We transferred them to
660 $\log_2$(CPM+1) to make data consistently normalized. We also prepared corresponding
661 raw count files for data integration.
662
663 **Integration of PBMC datasets and BAL datasets using Reciprocal PCA in Seurat**

664 We input raw count files of 5 preprocessed PBMC datasets into Seurat and created a
665 list of Seurat objects. Reciprocal PCA procedure
666 (https://satijalab.org/seurat/v3.2/integration.html#reciprocal-pca) was used for data
667 integration. First, normalization and variable feature detection were applied for each
668 dataset in the list. Then we used *SelectIntegrationFeatures* to select features for
669 downstream integration. Next, we scaled data and ran the principal component analysis
670 with selected features using *ScaleData* and *RunPCA*. Then we found integration
671 anchors and integrated data using *FindIntegrationAchnors* and *IntegrateData*. RPCA
672 was used as the reduction method. After integration, we scaled data and ran PCA on
673 integrated expression values. UMAP was generated using the top 30 reduced
674 dimensions with *RunUMAP*. The same approach was also used in BAL data integration
675 and multi-disease integration. We also used it for the integration of specific cell types
676 across multiple datasets, for example, the integration of neutrophils from PBMC and
677 BAL datasets. Compared with standard workflow and SCTransform
678 (https://satijalab.org/seurat/v3.2/integration.html) in Seurat, we found Reciprocal PCA is
679 much less computation-intensive and time-consuming, making the integration of
680 multiple large single-cell datasets feasible.
681
682 **Cell Annotations using canonical markers after unsupervised clustering**
683 Cell annotations were assigned in each dataset and then mapped to the integrated
684 data. For some datasets without available cell annotations, we first used unsupervised
685 clustering in Scanpy. Detailed steps include (1) detecting top 3,000 highly variable
686 genes using *pp.highly_variable_genes*; (2) scaling each gene to unit variance on highly
687 variable genes using *pp.scale*; (3) running PCA using *arpack* approach in *tl.pca*; (4)
688 finding neighbors using *pp.neighbors*; (5) running leiden clustering with resolution of 1
689 using *tl.leiden* (resolutions were determined swiftly based on the size and complexity of
690 data). More details can be found in the code (point to it). For datasets with available
691 annotations, we checked their validity and corrected wrong annotations. For example,
692 hematopoietic stem and progenitor cells (HSPC) were mistakenly annotated as
693 “SC&Eosinophil” in the original paper(Wilk et al., 2020a) and were corrected in our
694 annotation.

695

696 After unsupervised clustering, well recognized immune cell markers were used to
697 annotate clusters, including CD4+ T cell markers such as TRAC, CD3D, CD3E, CD3G,
698 CD4; CD8+ T cell markers such as CD8A, CD8B, NKG7; NK cell markers such as
699 NKG7, GNLY, KLRD1; B cell markers such as CD19, MS4A1, CD79A; plasmablast
700 markers such as MZB1, XBP1; monocyte markers such as S100A8, S100A9, CST3,
701 CD14; conventional dendritic cell markers such as XCR1, plasmacytoid dendritic cell
702 markers such as TCF4; megakaryocyte/platelet marker PPBP; red blood cell markers
703 HBA1, HBA2; HSPC marker CD34. Exhaustion-associated markers, including PDCD1,
704 HAVCR2, CTLA4 and LAG3 were used to identify exhausted T cells.
705

706 Additionally, other markers were used for annotations of lung-specific cells, including
707 AGER, MSLN for AT1 cells; SFTPC, SFTPB for AT2 cells; SCGB3A2, SCGB1A1 for
708 Club cells; TPPP3, FOXJ1 for Ciliated cells; KRT5 for Basal cells; CFTR for Ionocytes;
709 FABP4, CD68 for tissue-resident macrophages; FCN1 for monocyte-derived
710 macrophages, TPSB2 for Mast cells. More details can be found in Table S2.
711

712 **Cell Annotations using Azimuth**

713 To better annotate T cells in our study, we applied Azimuth
714 (https://satijalab.org/azimuth/), a tool for reference-based single-cell analysis developed
715 in Seurat version 4.0(Hao et al., 2020). High-quality PBMC single-cell data in Azimuth
716 was used as the reference for label projection. After removing annotations with low
717 prediction scores or low mapping scores, we got a collection of well-annotated T cell
718 subtypes, including CD4+ Cytotoxic T cell, CD4+ Naive T cell, CD4+ Central Memory T
719 cell, CD8+ Naive T cell, CD8+ Effector Memory cell, gamma-delta T cell, double-
720 negative T cell. CD4+ Effector Memory T cell and CD8+ Central Memory T cell were
721 found by Azimuth but removed later because of low scores. Apart from annotations of T
722 cell subtypes, we also found CD56-bright NK cell, intermediate B cell and Memory B cell
723 using Azimuth.
724

725 **Sub-clustering for specific cell types**

726 Sub-clustering was used for the discovery of subtypes or distinct stages of a specific
727 cell type. In our work, we applied sub-cluster for various immune cell types, including
728 classical monocytes, neutrophils, conventional dendritic, B cells and platelets. First, all
729 cells in the specific cell type were integrated using the same procedure as PBMC data
730 integration. Then Louvain clustering (resolution = 0.5, except for sub-clustering of
731 classical monocytes where resolution = 0.3) was applied to detect sub-clusters of those
732 cells. Importantly, neutrophils, cDCs and B cells were retrieved from both PBMC and
733 BAL, whereas classical monocytes and platelets were only retrieved from PBMC.
734

735 **Generation of ToppCell gene modules**

736 ToppCell (https://toppcell.cchmc.org/) was designed to parallelly analyze transcriptional
737 profiles of single-cell datasets by organizing differential expressed gene modules in a
738 customized hierarchical order. In our study, we hierarchically annotated cells with
739 multiple layers, including compartments, disease conditions, lineages, cell classes and
740 sub-clusters. All the cells were grouped into specific hierarchical categories. For
741 example, "PBMC_severe COVID-19_myeloid cells_classical-monocytes_cMono1"
742 represents cells belonging to cMono1 (a sub-cluster of classical monocytes) in PBMC of
743 severe COVID-19 patients. With hierarchically ordered cell annotations, we calculated
744 their DEGs in a hierarchical way as well. We defined customized ranges for
745 comparisons and applied t-test based on normalized expression values. More details
746 can be seen on ToppCell website. Usually, the top 200 most differentially genes in each
747 comparison were picked up as the gene modules for the selected cell group, which are
748 the starting point of downstream analysis, including gene enrichment in ToppGene and
749 interaction inference in ToppCluster. All gene modules in our study were curated in
750 COVID-19 Atlas (https://toppcell.cchmc.org/biosystems/go/index3/COVID-19 Atlas) and
751 ImmuneMap (https://toppcell.cchmc.org/biosystems/go/index3/ImmuneMap) on the
752 ToppCell website.
753

754 **Gene Enrichment Analysis using ToppGene**

755 Abundant gene modules were generated with ToppCell. After that, we used ToppGene
756 (https://toppgene.cchmc.org/) for gene enrichment analysis. Genes in each gene

757 module were sent to ToppGene platform as input for enrichment in different domains.
758 GO-Molecular Function, GO-Biological Process and GO-Cellular Component and
759 Mouse Phenotype were usually used for enrichment. P values of enrichment results
760 were adjusted using the Benjamini-Hochberg procedure.
761
762 **Generation of Functional Association Heatmap using ToppCluster**
763 Genes in gene modules of selected cell types or sub-clusters were sent to ToppCluster
764 (https://toppcluster.cchmc.org/). Then multi-group functional enrichment was drawn for
765 input gene modules and -$\log_{10}$(adjusted p-value) was used as the gene enrichment
766 score to represent the strength of association between gene modules and pathways.
767 Scores greater than 10 were trimmed to 10. Pathways from Gene Ontologies, including
768 Molecular Functions, Biological Process and Cellular Component in the option list were
769 used for the enrichment of gene modules in myeloid cells, B cells and platelets. In order
770 to gain a broader knowledge of immunothrombosis-related pathways, “Pathway” and
771 “Mouse Phenotype" in the option list were also selected for enrichment. Morpheus was
772 used for visualization of the heatmap (https://software.broadinstitute.org/morpheus/).
773
774 **Cell Interaction Inference in immunothrombosis activities and cytokine signaling**
775 **pathways**
776 CellChat was used to infer the signaling network in the BAL of severe patients (**Figure**
777 **S8B**). All 3 categories of interactions were used in the database *CellChatDB.human*.
778 Over-expressed ligands or receptors in each cell type were first identified for further
779 identification of over-expressed interaction pairs. Then cytokine, chemokine and IL
780 signaling probability between multiple cell types was inferred using
781 *computeCommunProb* and *computeCommunProbPathway*.
782
783 ToppCell was used to infer interactions in immunothrombosis. We first selected genes
784 related to coagulation or immunothrombosis pathways from subtypes of endothelial
785 cells, platelets, neutrophils, classical monocytes and monocyte-derived macrophages
786 by filtering the output of ToppCluster (**Figure S12A**). Then we used CellChatDB as the
787 knowledge base to find the subset of genes participating in cell-cell interaction, including

genes involved in signaling via secretion, cell-cell contact and extracellular matrix interaction. These genes in each cluster were sent to ToppCluster to infer the interaction network using protein-protein interactions (PPI) between those genes.

Statistics of Cell Proportion Changes in Different Disease Stages

Cell proportion differences between disease groups for specific types and subtypes (**Figure 2, Figure S2-S4**) shown on box plots were measured by Mann-Whitney test (Wilcoxon, paired=False). Significance between two disease conditions were shown on the top.

To investigate the dynamic changes of cell proportions across various immune-mediated diseases, we followed the approach in recent literature (Lee et al., 2020) (**Figure 7B**). For each disease condition, we computed the relative ratio of each cell type in individual disease samples divided by individual healthy samples. Log_2 transformed values were shown in the box plot. Then we calculated relative ratios of each cell type between all sample pairs of healthy donors as a control. To compute the significance, we used a two-sided Kolmogorov-Smirnov (KS) test using relative ratios in diseases and those values in healthy donors.

Generation of Volcano Plots

We first calculated differential expressed genes using *tl.rank_genes_groups* in Scanpy. Adjusted p values and log fold changes in the output were used as the input of volcano plots. R package *EnhancedVolcano (Blighe et al., 2018)* was used to draw figures.

Construction of COVID-19 Functional Enrichment Map

In order to characterize functional properties of cell types and subtypes observed in BAL, PBMC, and lung parenchymal samples from control, mild, and severe COVID-19 patient samples, we used the library of gene expression signatures ("Gene Module Report" from ToppCell) as an input to the ToppCluster enrichment analyzer web server (Kaimal et al 2010). Using categories of *Gene Ontology*, *Human Phenotype*, *Mouse Phenotype*, *Pathway* and *Protein Interaction,* a matrix was constructed using minus log P enrichment values for each celltype gene list and then all cells and enriched features

819 could be clustered and ordered based on their shared or distinct properties that could
820 then be associated with lineage, cell subclass, tissue compartment, and disease state.
821

822 Supplementary Information

823 Table S1. Metadata of data sources and generated resources in this study, relative to
824 Figure 1.
825
826 Table S2. Severity-associated cell distribution in multiple compartments of COVID-19
827 patients, relative to Figure 2.
828
829 Table S3. Gene modules and DEGs of neutrophil and macrophage sub-clusters, relative
830 to Figure 3.
831
832 Table S4. Gene modules and pro-thrombosis genes in platelets of COVID-19 patients,
833 relative to Figure 4.
834
835 Table S5. Developing plasmablast signatures and autoimmunity-associated signatures
836 of COVID-19 B cells, relative to Figure 5.
837
838 Table S6. Enrichment scores of COVID-19 Functional Map, relative to Figure 6.
839
840 Table S7. Dynamic changes of abundances and signatures across immune-mediated
841 diseases, relative to Figure 7.
842
843

844 Reference

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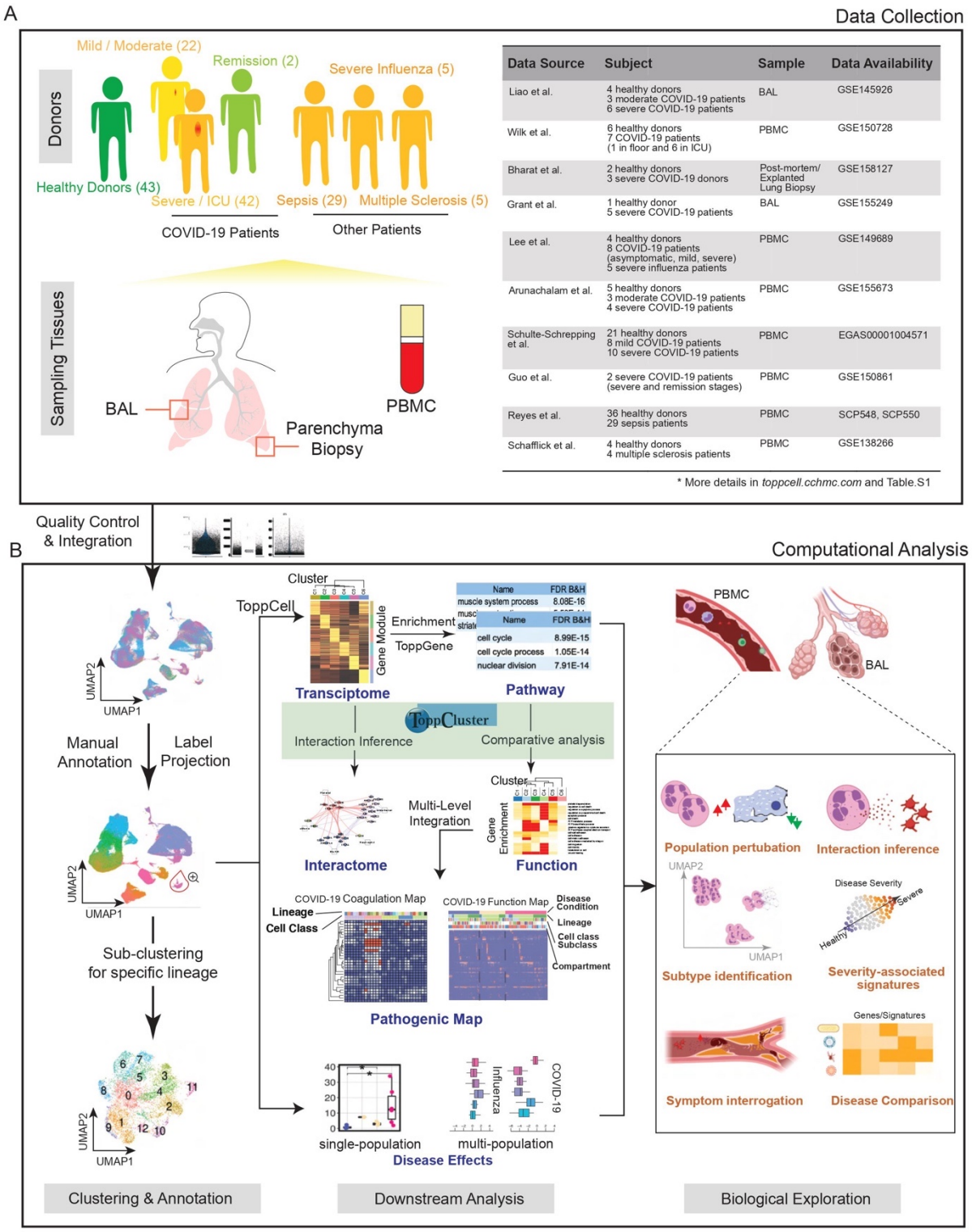
Figures



Data Source	Subject	Sample	Data Availability
Liao et al.	4 healthy donors 3 moderate COVID-19 patients 6 severe COVID-19 patients	BAL	GSE145926
Wilk et al.	6 healthy donors 7 COVID-19 patients (1 in floor and 6 in ICU)	PBMC	GSE150728
Bharat et al.	2 healthy donors 3 severe COVID-19 donors	Post-mortem/ Explanted Lung Biopsy	GSE158127
Grant et al.	1 healthy donor 5 severe COVID-19 patients	BAL	GSE155249
Lee et al.	4 healthy donors 8 COVID-19 patients (asymptomatic, mild, severe) 5 severe influenza patients	PBMC	GSE149689
Arunachalam et al.	5 healthy donors 3 moderate COVID-19 patients 4 severe COVID-19 patients	PBMC	GSE155673
Schulte-Schrepping et al.	21 healthy donors 8 mild COVID-19 patients 10 severe COVID-19 patients	PBMC	EGAS00001004571
Guo et al.	2 severe COVID-19 patients (severe and remission stages)	PBMC	GSE150861
Reyes et al.	36 healthy donors 29 sepsis patients	PBMC	SCP548, SCP550
Schafflick et al.	4 healthy donors 4 multiple sclerosis patients	PBMC	GSE138266

* More details in *toppcell.cchmc.com* and Table.S1

Fig. 1. **Creating a COVID-19 Signature Atlas**. (**A**) Representative aggregation of multiple single-cell RNA-sequencing datasets from COVID-19 and related studies. The present study is derived from a total of 231,800 peripheral blood mononuclear cells (PBMCs), 101,800 bronchoalveolar lavage (BAL) cells and 146,361 lung parenchyma cells from 43 healthy; 22 mild, 42 severe, and 2 convalescent patients. Data was collated from eight public datasets (right). (**B**) Data analysis pipeline of the study using Topp-toolkit. It includes three phases: (1) clustering and annotation; (2) downstream analysis using Topp-toolkit; (3) biological exploration. Output includes the evaluation of abundance of cell populations, cell type (cluster) specific gene modules, functional associations of disease-associated cell classes and clusters, inference of cell-cell interactions, as well as comparative analysis across diseases, including influenza, sepsis and multiple sclerosis. Additional newer datasets not included in this manuscript are present and will continue to be added to ToppCell (http://toppcell.cchmc.org).

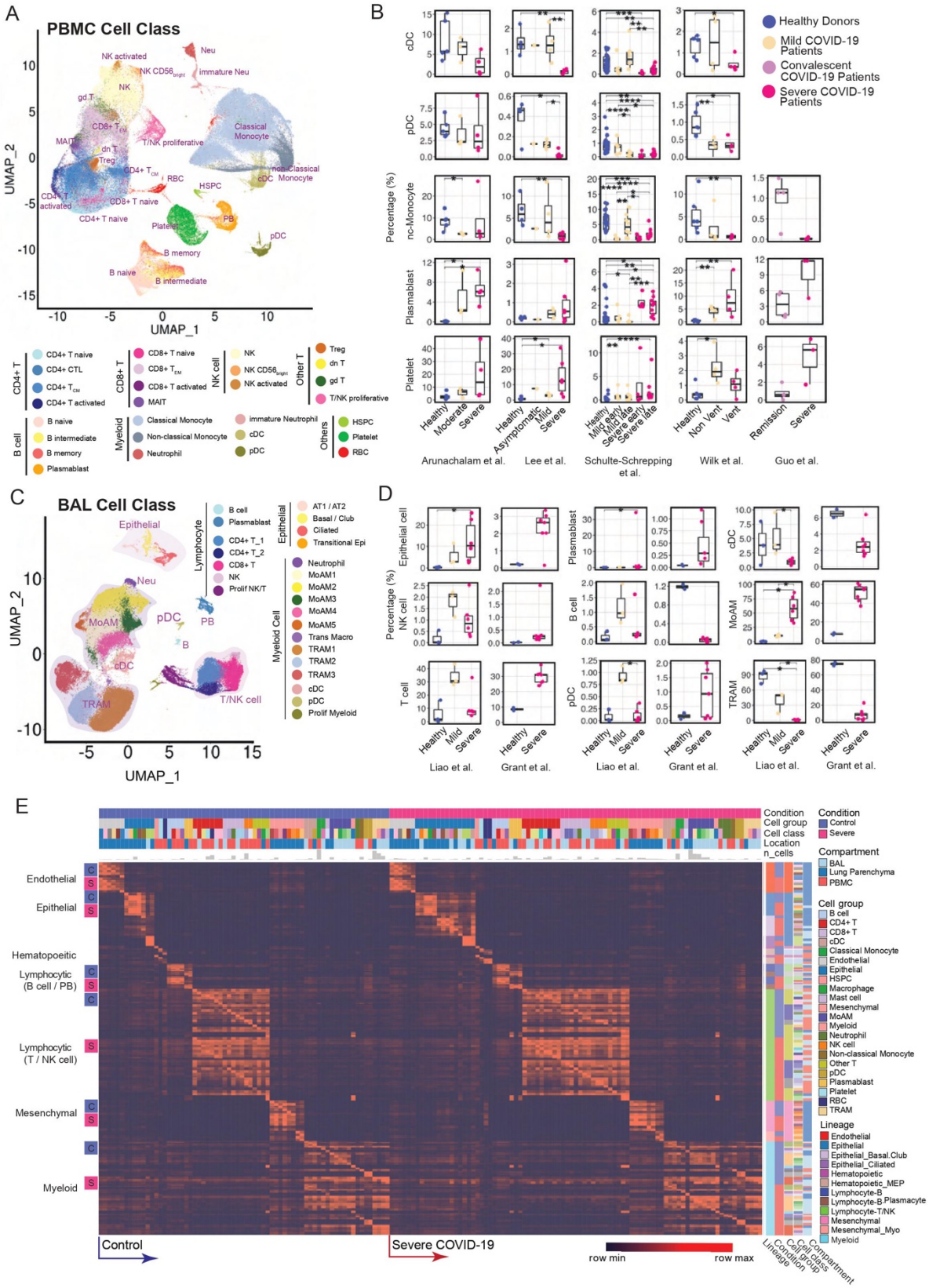

A
PBMC Cell Class
UMAP_1
UMAP_2
B
Healthy Donors
Mild COVID-19 Patients
Convalescent COVID-19 Patients
Severe COVID-19 Patients
Percentage (%)
cDC
pDC
nc-Monocyte
Plasmablast
Platelet
Arunachalam et al.
Lee et al.
Schulte-Schrepping et al.
Wilk et al.
Guo et al.
C
BAL Cell Class
Epithelial
Neu
MoAM
pDC
PB
B
cDC
TRAM
T/NK cell
D
Epithelial cell
Plasmablast
cDC
NK cell
B cell
MoAM
T cell
pDC
TRAM
Liao et al.
Grant et al.
E
Condition
Cell group
Cell class
Location
n_cells
Endothelial
Epithelial
Hematopoietic
Lymphocytic (B cell / PB)
Lymphocytic (T / NK cell)
Mesenchymal
Myeloid
Control
Severe COVID-19
row min
row max
Compartment
BAL
Lung Parenchyma
PBMC
Cell group
Lineage

Fig. 2. Modularized representation of cell type specific gene signatures and dynamic changes of cell abundance. (**A**) Uniform Manifold Approximation and Projection (UMAP) of 28 distinct cell types identified in the integrated peripheral blood mononuclear cell (PBMC) data. (**B**) Comparative analysis of cell abundance effects of COVID-19. Reproducible multi-study data present high impact effects on 5 cell types in PBMC. Percentages of selected cell types in each sample are shown (where Vent: Ventilated patients; Non Vent: Non-ventilated patients). Significance between two conditions was measured by the Mann-Whitney rank sum test (Wilcoxon, paired=False), which was also used in following significance tests of cell abundance changes in this study. *: $p <= 0.05$; **: $p <= 0.01$; ***: $p <= 0.001$; ****: $p <= 0.0001$. (**C**) UMAP of 24 distinct cell types identified in the integrated BAL data. (**D**) Dynamic changes of cell abundances for cell types in two bronchoalveolar lavage (BAL) single-cell datasets. (**E**) ToppCell allows for gene signatures to be hierarchically organized by lineage, cell type, subtype, and disease condition. The global heatmap shows gene modules with top 50 upregulated genes (student t test) for each cell type in a specific disease condition and compartment. Gene modules from control donors and severe COVID-19 patients were included in the figure.

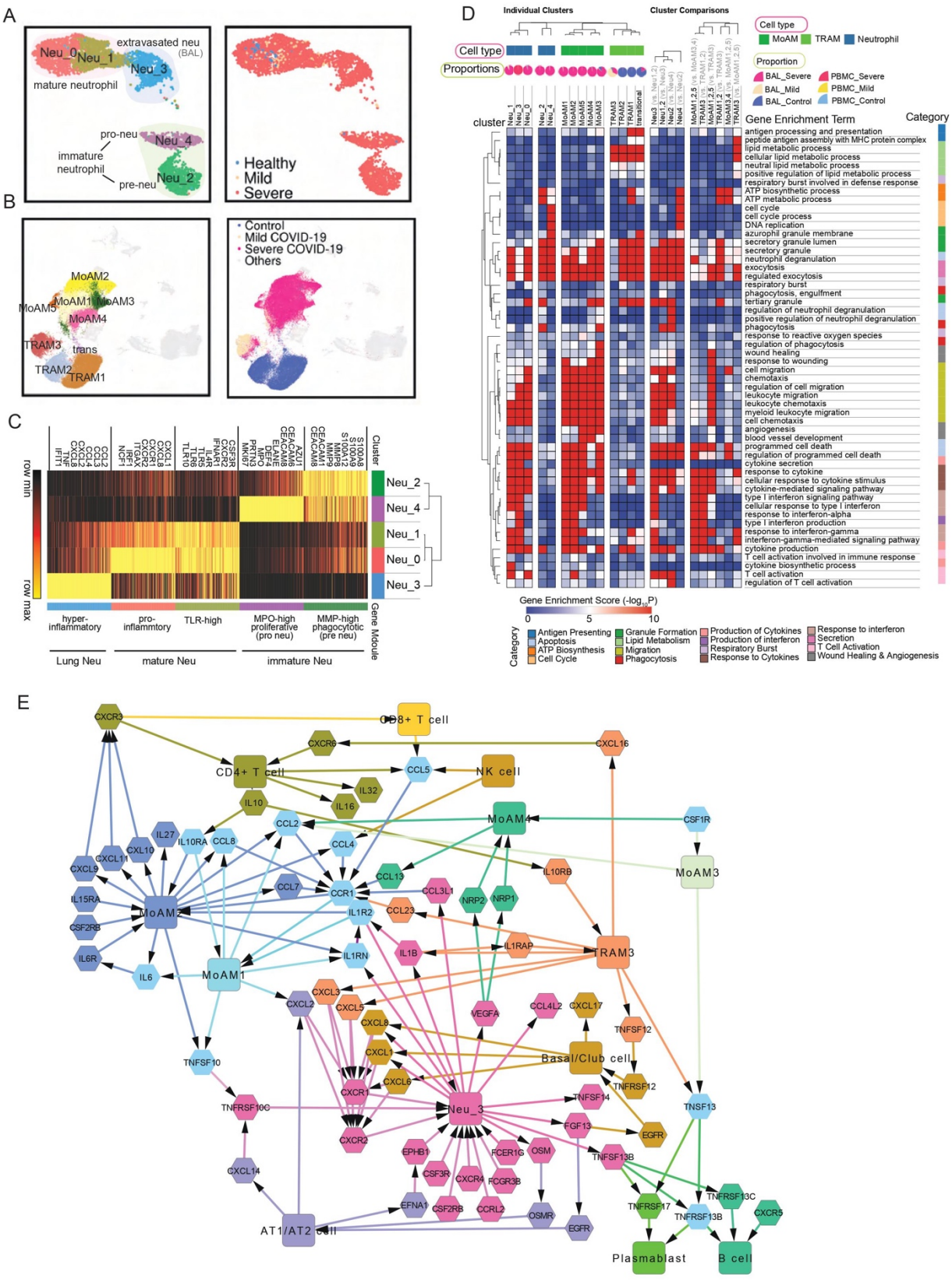

A
Neu_0
Neu_1
extravasated neu
(BAL)
Neu_3
mature neutrophil
pro-neu
Neu_4
immature
neutrophil
pre-neu
Neu_2
Healthy
Mild
Severe
B
MoAM2
MoAM5
MoAM1
MoAM3
MoAM4
TRAM3
trans
TRAM2
TRAM1
Control
Mild COVID-19
Severe COVID-19
Others
C
row min
row max
Cluster
Neu_2
Neu_4
Neu_1
Neu_0
Neu_3
hyper-inflammatory
pro-inflammtory
TLR-high
MPO-high proliferative (pro neu)
MMP-high phagocytotic (pre neu)
Gene Module
Lung Neu
mature Neu
immature Neu
D
Individual Clusters
Cluster Comparisons
Cell type
Proportions
cluster
Cell type
MoAM
TRAM
Neutrophil
Proportion
BAL_Severe
BAL_Mild
BAL_Control
PBMC_Severe
PBMC_Mild
PBMC_Control
Gene Enrichment Term
Category
antigen processing and presentation
peptide antigen assembly with MHC protein complex
lipid metabolic process
cellular lipid metabolic process
neutral lipid metabolic process
positive regulation of lipid metabolic process
respiratory burst involved in defense response
ATP biosynthetic process
ATP metabolic process
cell cycle
cell cycle process
DNA replication
azurophil granule membrane
secretory granule lumen
secretory granule
neutrophil degranulation
exocytosis
regulated exocytosis
respiratory burst
phagocytosis, engulfment
tertiary granule
regulation of neutrophil degranulation
positive regulation of neutrophil degranulation
phagocytosis
response to reactive oxygen species
regulation of phagocytosis
wound healing
response to wounding
cell migration
chemotaxis
regulation of cell migration
leukocyte migration
leukocyte chemotaxis
myeloid leukocyte migration
cell chemotaxis
angiogenesis
blood vessel development
programmed cell death
regulation of programmed cell death
cytokine secretion
response to cytokine
cellular response to cytokine stimulus
cytokine-mediated signaling pathway
type I interferon signaling pathway
cellular response to type I interferon
response to interferon-alpha
type I interferon production
response to interferon-gamma
interferon-gamma-mediated signaling pathway
cytokine production
T cell activation involved in immune response
cytokine biosynthetic process
T cell activation
regulation of T cell activation
Gene Enrichment Score (-log10P)
0
5
10
Category
Antigen Presenting
Apoptosis
ATP Biosynthesis
Cell Cycle
Granule Formation
Lipid Metabolism
Migration
Phagocytosis
Production of Cytokines
Production of interferon
Respiratory Burst
Response to Cytokines
Response to interferon
Secretion
T Cell Activation
Wound Healing & Angiogenesis
E
CD8+ T cell
CD4+ T cell
NK cell
MoAM4
MoAM3
MoAM2
MoAM1
TRAM3
Basal/Club cell
Neu_3
AT1/AT2 cell
Plasmablast
B cell
CXCR3
CXCR6
CXCL16
CCL5
IL32
IL16
IL10
CCL2
CCL4
CSF1R
IL27
IL10RA
CCL8
CXL10
CXCL11
CXCL9
IL15RA
CSF2RB
IL6R
IL6
CCL13
IL10RB
CCL7
CCR1
CCL3L1
NRP2
NRP1
IL1R2
CCL23
IL1RN
IL1B
IL1RAP
CXCL3
CXCL2
CXCL5
CXCL8
CXCL1
CXCL6
CXCR1
CXCR2
VEGFA
CCL4L2
CXCL17
TNFSF12
TNFRSF12
TNFSF14
TNSF13
TNFSF10
TNFRSF10C
FGF13
EGFR
TNFSF13B
EPHB1
CSF3R
CXCR4
FCER1G
OSM
FCGR3B
CXCL14
EFNA1
CSF2RB
CCRL2
OSMR
EGFR
TNFRSF17
TNFRSF13C
TNFRSF13B
CXCR5

Fig. 3. Functional analysis of compartment-specific immature and subtype-differentiated neutrophils and monocytic macrophages in COVID-19 patients. (**A**) Five sub-clusters and three cell groups were identified after the integration of neutrophils in peripheral blood mononuclear cells (PBMC) and bronchoalveolar lavage (BAL) (Left). The distribution of compartments is shown on the right. (**B**) Sub-clusters (Left) and COVID-19 conditions (Right) of monocyte-derived macrophages and tissue-resident macrophages were identified after integration of BAL datasets. (**C**) Heatmap of gene modules from ToppCell with top 200 upregulated genes for each neutrophil sub-cluster. Important neutrophil-associated genes and inferred roles of sub-clusters were shown on two sides. (**D**) Heatmap of associations between subclusters of neutrophils and macrophages and myeloid-cell-associated pathways (Gene Ontology). Gene modules with 200 upregulated genes for sub-clusters were used for enrichment in ToppCluster. Additionally, enrichment of top 200 differentially expressed genes (DEGs) for comparisons in fig. S5D and fig. S6B were appended on the right. Gene enrichment scores, defined as $-\log_{10}$(adjusted p-value), were calculated as the strength of associations. Pie charts showed the proportions of COVID-19 conditions in each cluster. (**E**) Gene interaction network in the BAL of severe patients. Highly expressed ligands and receptors of each cell type were drawn based on fig. S8. Interaction was inferred using both CellChat database and embedded cell interaction database in ToppCell.

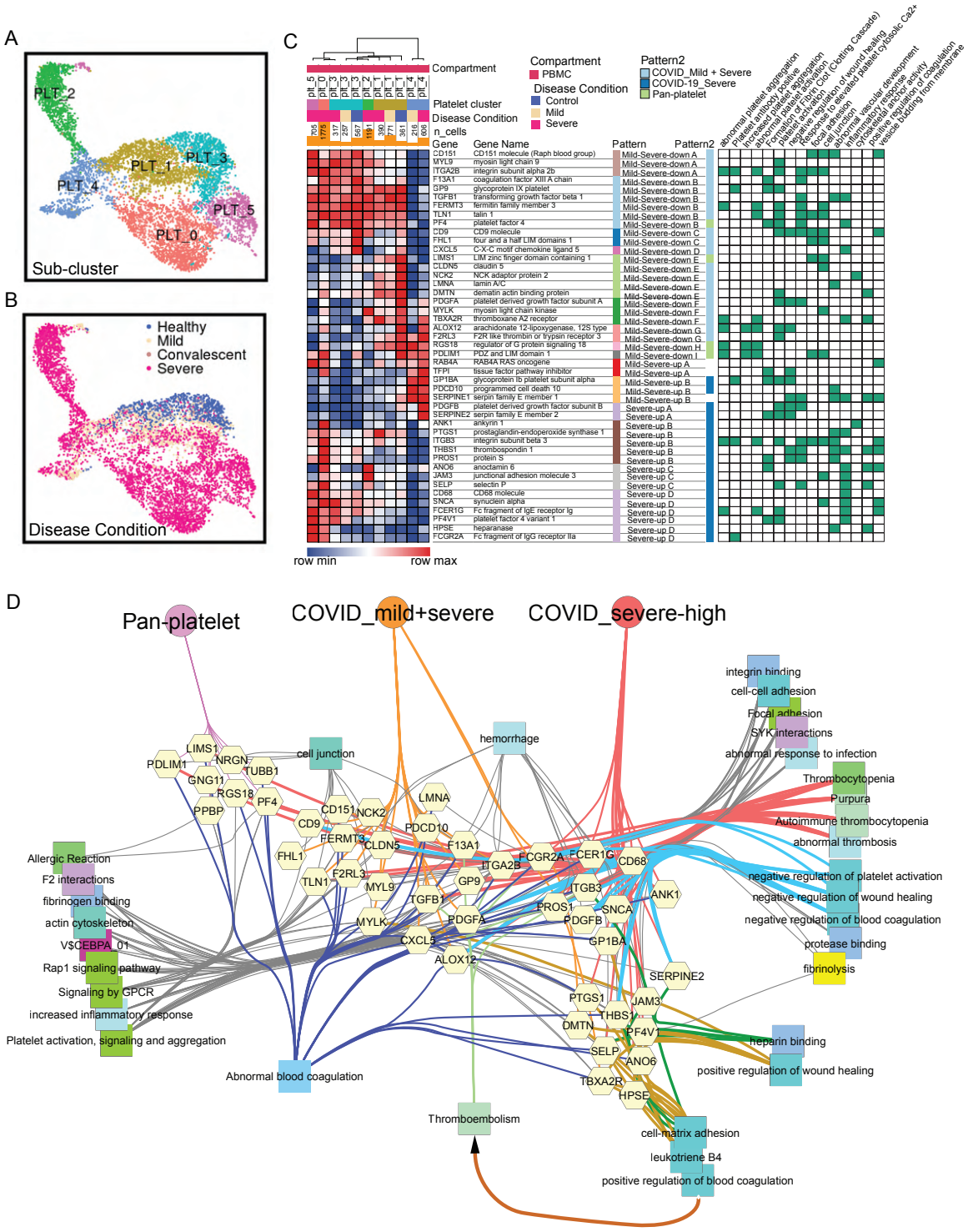

A
PLT_2
PLT_1
PLT_3
PLT_4
PLT_5
PLT_0
Sub-cluster
B
Healthy
Mild
Convalescent
Severe
Disease Condition
C
Compartment
Platelet cluster
Disease Condition
n_cells
Compartment
PBMC
Disease Condition
Control
Mild
Severe
Pattern2
COVID_Mild + Severe
COVID-19_Severe
Pan-platelet
Gene
Gene Name
Pattern
Pattern2
row min
row max
D
Pan-platelet
COVID_mild+severe
COVID_severe-high
integrin binding
cell-cell adhesion
Focal adhesion
SYK interactions
abnormal response to infection
Thrombocytopenia
Purpura
Autoimmune thrombocytopenia
abnormal thrombosis
negative regulation of platelet activation
negative regulation of wound healing
negative regulation of blood coagulation
protease binding
fibrinolysis
heparin binding
positive regulation of wound healing
cell-matrix adhesion
leukotriene B4
positive regulation of blood coagulation
hemorrhage
cell junction
Allergic Reaction
F2 interactions
fibrinogen binding
actin cytoskeleton
V$CEBPA_01
Rap1 signaling pathway
Signaling by GPCR
increased inflammatory response
Platelet activation, signaling and aggregation
Abnormal blood coagulation
Thromboembolism
LIMS1
PDLIM1
NRGN
TUBB1
GNG11
RGS18
PF4
PPBP
CD151
NCK2
CD9
FERMT3
CLDN5
FHL1
TLN1
F2RL3
MYL9
TGFB1
MYLK
CXCL5
ALOX12
LMNA
PDCD10
F13A1
ITGA2B
GP9
PDGFA
FCGR2A
FCER1G
CD68
ITGB3
PROS1
SNCA
PDGFB
GP1BA
ANK1
SERPINE2
PTGS1
JAM3
THBS1
DMTN
PF4V1
SELP
ANO6
TBXA2R
HPSE

Fig. 4. COVID-19 driven reprogramming of platelets leads to drastically altered expression of genes associated with platelet adhesion, activation, coagulation and thrombosis. (**A-B**) Uniform Manifold Approximation and Projections (UMAPs) show distributions of sub-clusters (A) and COVID-19 conditions (B) of platelets after the integration of PBMC datasets. (**C**) Severity-associated coagulation genes were selected and shown on the heatmap, with disease and sub-cluster specific gene patterns identified and labeled. Their functional associations with coagulation pathways were retrieved from ToppGene and shown on the right. (**D**) Functional and phenotypical associations of coagulation-association genes in each gene pattern from (B). Associations were retrieved from ToppGene enrichment. Fibrinolysis is highlighted.

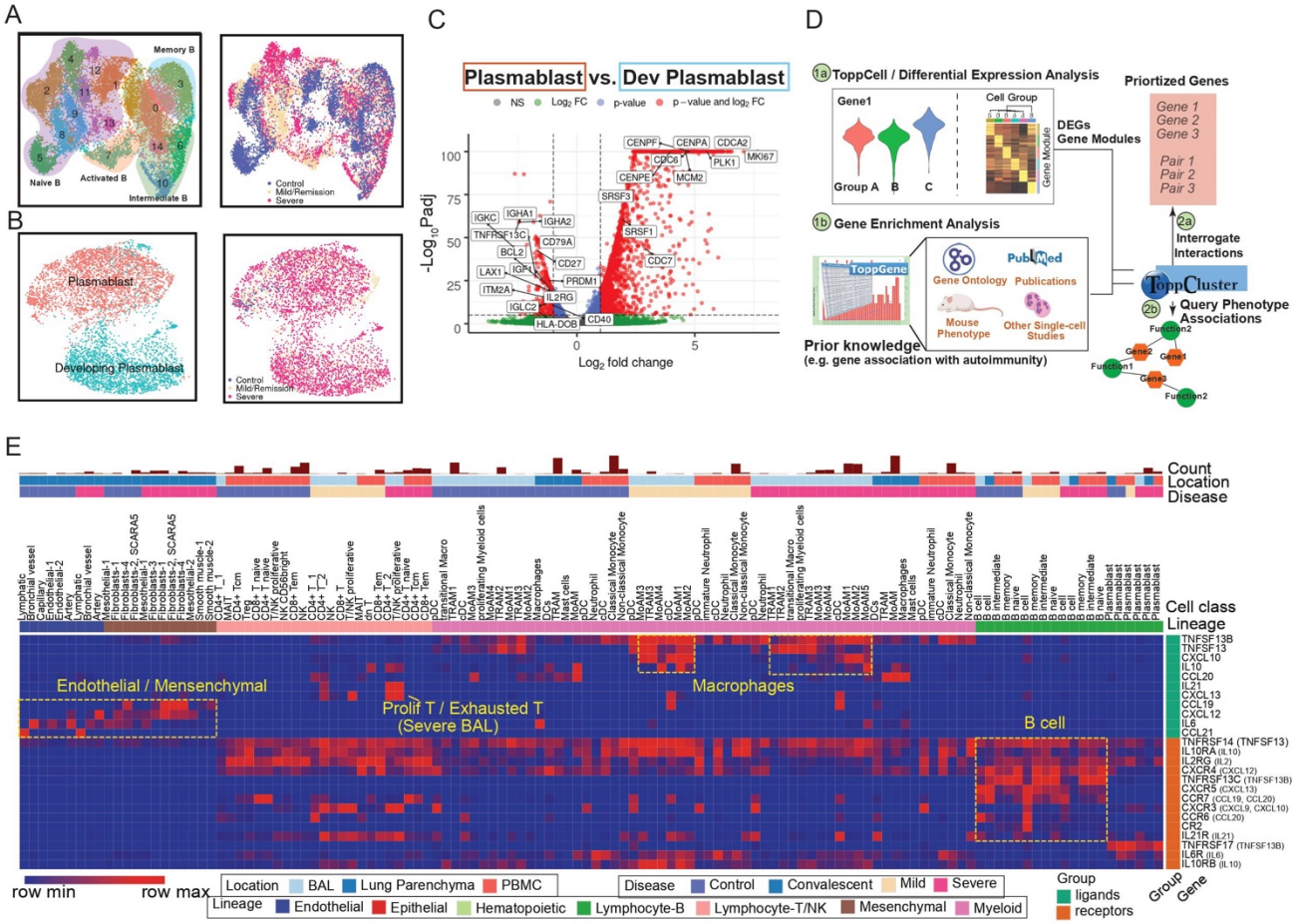
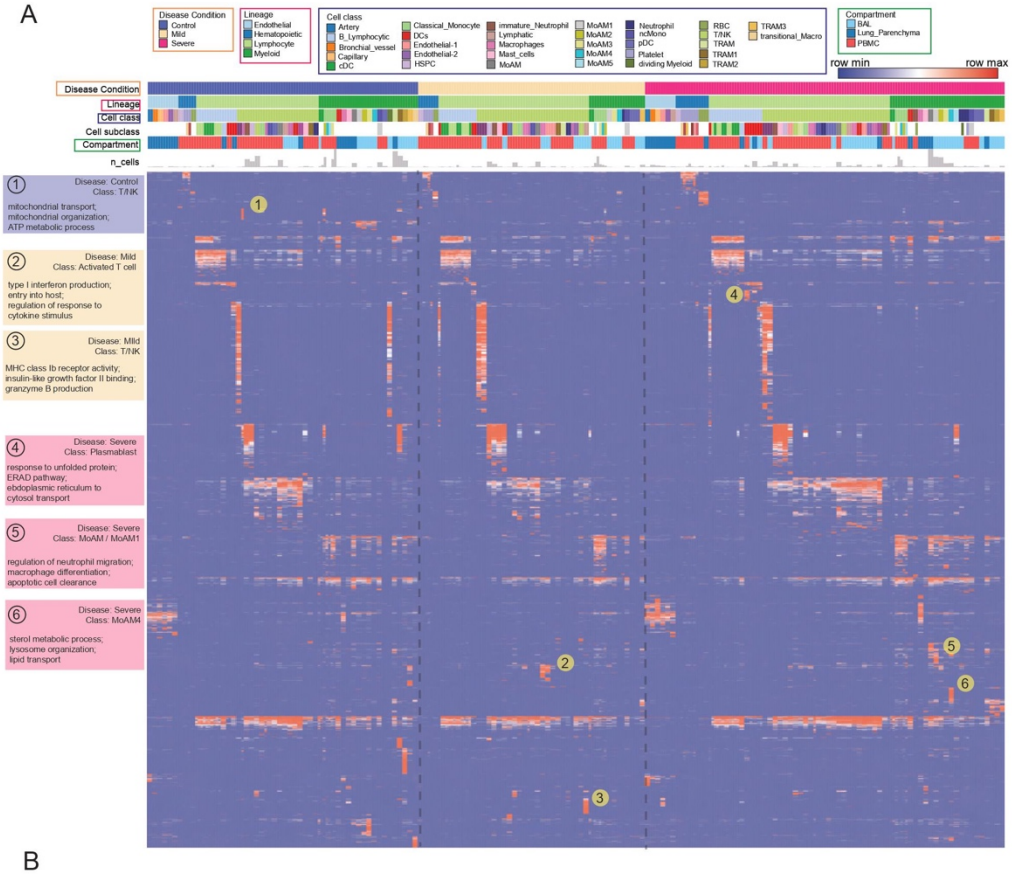

A
Memory B
Naive B
Activated B
Intermediate B
Control
Mild/Remission
Severe
B
Plasmablast
Developing Plasmablast
C
Plasmablast vs. Dev Plasmablast
NS
Log2 FC
p-value
p – value and log2 FC
-Log10 Padj
Log2 fold change
D
1a ToppCell / Differential Expression Analysis
Gene1
Group A
B
C
Cell Group
Gene Module
DEGs
Gene Modules
Priortized Genes
Gene 1
Gene 2
Gene 3
Pair 1
Pair 2
Pair 3
1b Gene Enrichment Analysis
ToppGene
Gene Ontology
Publications
Mouse Phenotype
Other Single-cell Studies
Prior knowledge
(e.g. gene association with autoimmunity)
2a Interrogate Interactions
ToppCluster
2b Query Phenotype Associations
E
Count
Location
Disease
Cell class
Lineage
Endothelial / Mensenchymal
Prolif T / Exhausted T
(Severe BAL)
Macrophages
B cell
row min
row max
Location
BAL
Lung Parenchyma
PBMC
Disease
Control
Convalescent
Mild
Severe
Lineage
Endothelial
Epithelial
Hematopoietic
Lymphocyte-B
Lymphocyte-T/NK
Mesenchymal
Myeloid
Group
ligands
receptors
Group
Gene
F
MP:altered susceptibility to autoimmune disorder
MP: abnormal immune tolerance
CTTTGA_V$LEF1_Q2
receptors
TFBS:ISRE_01
TFBS:ICSBP_Q6
signaling-ligands
NF-kappa B signaling pathway
Immature B cell_mild+severe
Requirements for follicular exclusion and competitive e...
Regulation of CXCR3 and CXCR4 expression during termin...
TACI and BAFF-R mediate isotype switching in B cells
Naive B cell
absent transitional stage B cells
arrested B cell differentiation
decreased mature B cell number
decreased plasma cell number
Plasmablasts
CCR7
CCR6
CXCR5
CXCR4
CXCR3
TNFRSF13C
TNFRSF17
TNFRSF14
IL10RB
IL10RA
IL6R
IL2RG
IL21R
CCL21
CCL19
CCL20
CXCL12
CXCL13
CXCL10
TNFSF13B
TNFSF13
IL10
IL6
IL21
LungParenchym_Lymphatic-Endothelial
LungParenchym_Endothelial-Severe
LungParenchym_MoAM_Severe
LungParenchym_MesenchymFibroblasts
BAL_CD4+ T cell_Exhausted-Severe
BAL_Activated_MoAM_mild+severe
dependence of plasma cells and indepe ...
anti-apoptotic role of BAFF and APRIL
TACI and BCMA stimulation of B cell immune responses.
TFBS:IRF1
Autoimmunity
Rheumatoid Arthritis
Lupus Erythematosus, Systemic
abnormal mature B cell number
decreased B cell number

Fig. 5. Implicating a multi-lineage cell network capable of driving extrafollicular B cell maturation and the emergence of humoral autoimmunity in COVID-19 patients. (**A**) Uniform Manifold Approximation and Projections (UMAPs) of sub-clusters (Left) and COVID-19 conditions (Right) of B cells after integration of peripheral blood mononuclear cells (PBMC) and bronchoalveolar lavage (BAL) datasets. (**B**) UMAPs of subtypes (Left) and COVID-19 conditions (Right) of plasmablasts after integration of PBMC and BAL datasets. (**C**) Volcano plot depicts differentially expressed genes between plasmablasts and developing plasmablasts. Student t-tests were applied and p values were adjusted by the Benjamini-Hochberg procedure. (**D**) Workflow of discovering and prioritizing candidate genes related to a disease-specific phenotype with limited understanding. (**E**) The heatmap shows the normalized expression levels of candidate ligands and receptors for COVID-19 autoimmunity in multiple compartments in healthy donors and COVID-19 patients. Binding ligands of receptor genes were shown in parentheses on the right. Hot spots of expression are highlighted. (**F**) Network analysis of autoimmunity-associated gene expression by COVID-19 cell types. Prior knowledge associated gene associations include GWAS, OMIM, mouse knockout phenotype, and additional recent manuscripts were selected from ToppGene enrichment results of differentially expressed ligands and receptors and shown on the network. Orange arrows present the interaction directions from ligands (green) to receptors (pink) on B cells. Annotations for these genes, including single-cell co-expression (blue), mouse phenotype (light blue), transcription factor binding site (purple) and signaling pathways (green) are shown.

A


Disease Condition
Control
Mild
Severe
Lineage
Endothelial
Hematopoietic
Lymphocyte
Myeloid
Cell class
Artery
B_Lymphocytic
Bronchial_vessel
Capillary
cDC
Classical_Monocyte
DCs
Endothelial-1
Endothelial-2
HSPC
immature_Neutrophil
Lymphatic
Macrophages
Mast_cells
MoAM
MoAM1
MoAM2
MoAM3
MoAM4
MoAM5
Neutrophil
ncMono
pDC
Platelet
dividing Myeloid
RBC
T/NK
TRAM
TRAM1
TRAM2
TRAM3
transitional_Macro
Compartment
BAL
Lung_Parenchyma
PBMC
row min
row max
Disease Condition
Lineage
Cell class
Cell subclass
Compartment
n_cells
1
Disease: Control
Class: T/NK
mitochondrial transport;
mitochondrial organization;
ATP metabolic process
2
Disease: Mild
Class: Activated T cell
type I interferon production;
entry into host;
regulation of response to
cytokine stimulus
3
Disease: Mild
Class: T/NK
MHC class Ib receptor activity;
insulin-like growth factor II binding;
granzyme B production
4
Disease: Severe
Class: Plasmablast
response to unfolded protein;
ERAD pathway;
ebdoplasmic reticulum to
cytosol transport
5
Disease: Severe
Class: MoAM / MoAM1
regulation of neutrophil migration;
macrophage differentiation;
apoptotic cell clearance
6
Disease: Severe
Class: MoAM4
sterol metabolic process;
lysosome organization;
lipid transport

B

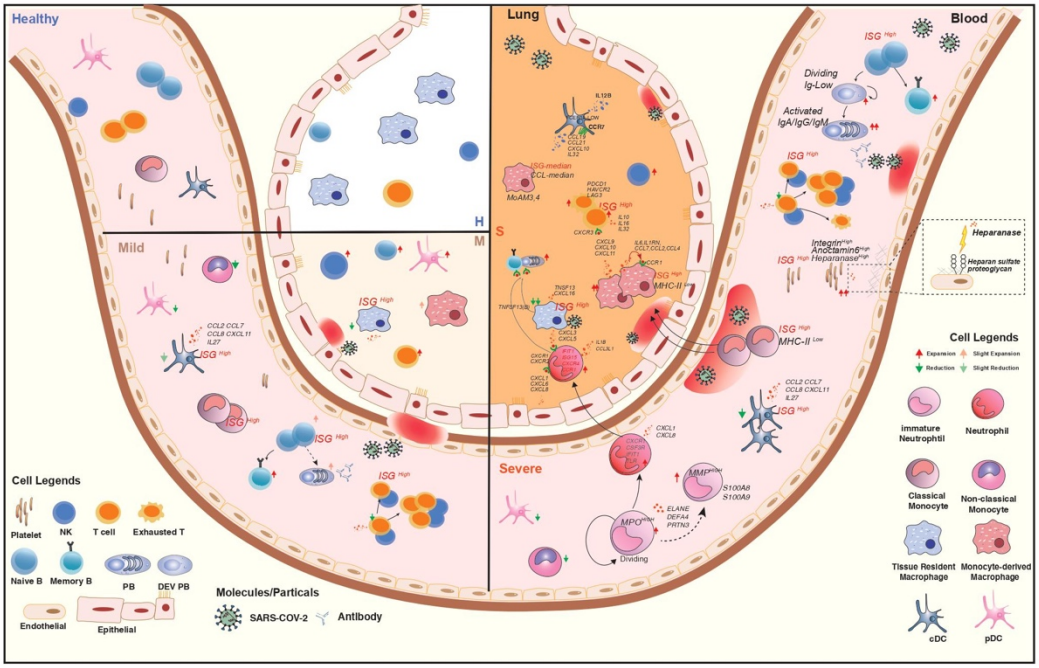

Healthy
Mild
H
M
S
Lung
Severe
Blood
Dividing Ig-Low
Activated IgA/IgG/IgM
Heparanase
Heparan sulfate proteoglycan
Cell Legends
Platelet
NK
T cell
Exhausted T
Naive B
Memory B
PB
DEV PB
Endothelial
Epithelial
Molecules/Particals
SARS-COV-2
Antibody
Cell Legends
Expansion
Slight Expansion
Reduction
Slight Reduction
immature Neutrophil
Neutrophil
Classical Monocyte
Non-classical Monocyte
Tissue Resident Macrophage
Monocyte-derived Macrophage
cDC
pDC

Fig. 6. Comparative analysis of cell type specific gene signatures associated with lineage, class, subclass, compartment, and disease state in the COVID-19 atlas. (**A**) Enrichment scores of gene modules for all cell types across different compartments and COVID-19 conditions were generated by ToppCluster and shown on the heatmap. ToppCluster enriched functions from Gene Ontology, Human Phenotype, Mouse Phenotype, Pathway and Interaction databases were used to generate a feature matrix (cell types by features) and hierarchically clustered. Hot spots of the disease-specific enrichments were highlighted and details were shown on the left. More details can be found in Methods. (**B**) Summarizing predicted functions and interplay of immune cells in COVID-19 blood and lung. Aforementioned key observations in this study were shown in peripheral blood mononuclear cells (PBMC) and bronchoalveolar lavage (BAL) in healthy donors, mild and severe COVID-19 patients, including changes of cell abundance, specific marker genes, upregulated secretion, cell development and cell-cell interactions.

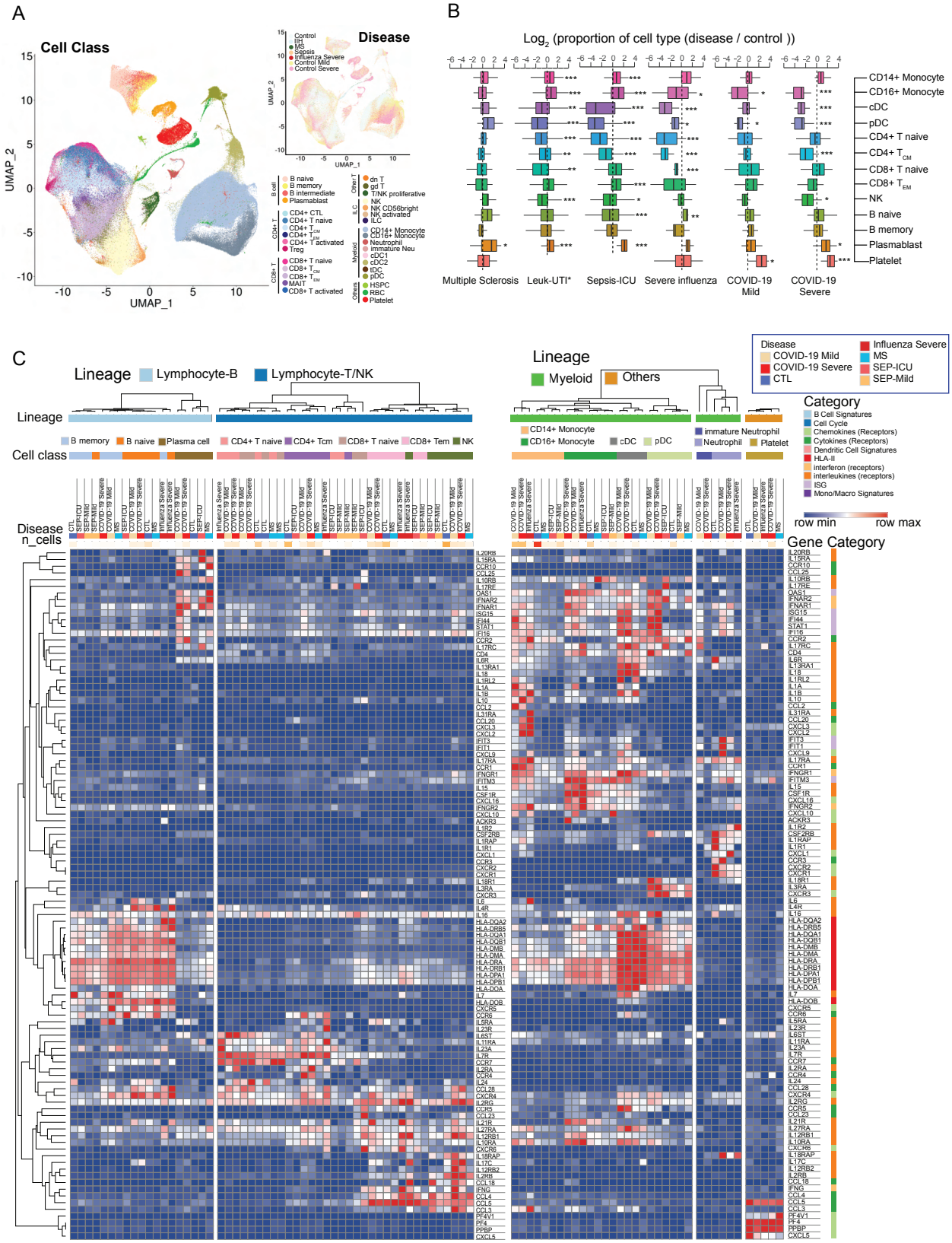

A
Cell Class
UMAP_2
UMAP_1
Disease
Control
ILI
MS
Sepsis
Influenza Severe
Control Mild
Control Severe
B naive
B memory
B intermediate
Plasmablast
CD4+ CTL
CD4+ T naive
CD4+ T activated
Treg
CD8+ T naive
MAIT
CD8+ T activated
dn T
gd T
T/NK proliferative
NK
NK CD56bright
NK activated
ILC
CD14+ Monocyte
CD16+ Monocyte
Neutrophil
immature Neu
cDC1
cDC2
IDC
pDC
HSPC
RBC
Platelet
B
Log2 (proportion of cell type (disease / control))
Multiple Sclerosis
Leuk-UTI*
Sepsis-ICU
Severe influenza
COVID-19 Mild
COVID-19 Severe
CD14+ Monocyte
CD16+ Monocyte
cDC
pDC
CD4+ T naive
CD8+ T naive
NK
B naive
B memory
Plasmablast
Platelet
C
Lineage
Lymphocyte-B
Lymphocyte-T/NK
Cell class
B memory
B naive
Plasma cell
CD4+ T naive
CD4+ Tcm
CD8+ T naive
CD8+ Tem
NK
Myeloid
Others
CD14+ Monocyte
CD16+ Monocyte
cDC
pDC
immature Neutrophil
Neutrophil
Platelet
Disease
COVID-19 Mild
COVID-19 Severe
CTL
Influenza Severe
MS
SEP-ICU
SEP-Mild
Category
B Cell Signatures
Cell Cycle
Chemokines (Receptors)
Cytokines (Receptors)
Dendritic Cell Signatures
HLA-II
interferon (receptors)
interleukines (receptors)
ISG
Mono/Macro Signatures
row min
row max
Disease
n_cells
Gene Category

Fig. 7. Comparative analysis of differentially-expressed immunoregulatory genes between COVID-19 and other immune-mediated diseases. (**A**) Uniform Manifold Approximation and Projection (UMAP) shows the distributions of cell types (Left) and diseases (Top right) after the integration of datasets in multiple studies. MS: multiple sclerosis; IIH: idiopathic intracranial hypertension. IIH patients were recruited as controls in the multiple sclerosis study. (**B**) Dynamic changes of immune cell types in different immune-mediated diseases compared to healthy controls. Log2(ratio) was calculated to show the levels of changes. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$. Statistical models can be found in the Methods. Leuk-UTI: sepsis patients that enrolled into UTI with leukocytosis (blood WBC ≥ 12,000 per mm3) but no organ dysfunction. (**C**) Normalized expression values of key genes involved in immune signaling and responses are shown for cell types across multiple diseases. Lowly expressed genes (maximal average expression level across all cell types in the heatmap is less than 0.5 after Log_2CPM normalization) were removed.

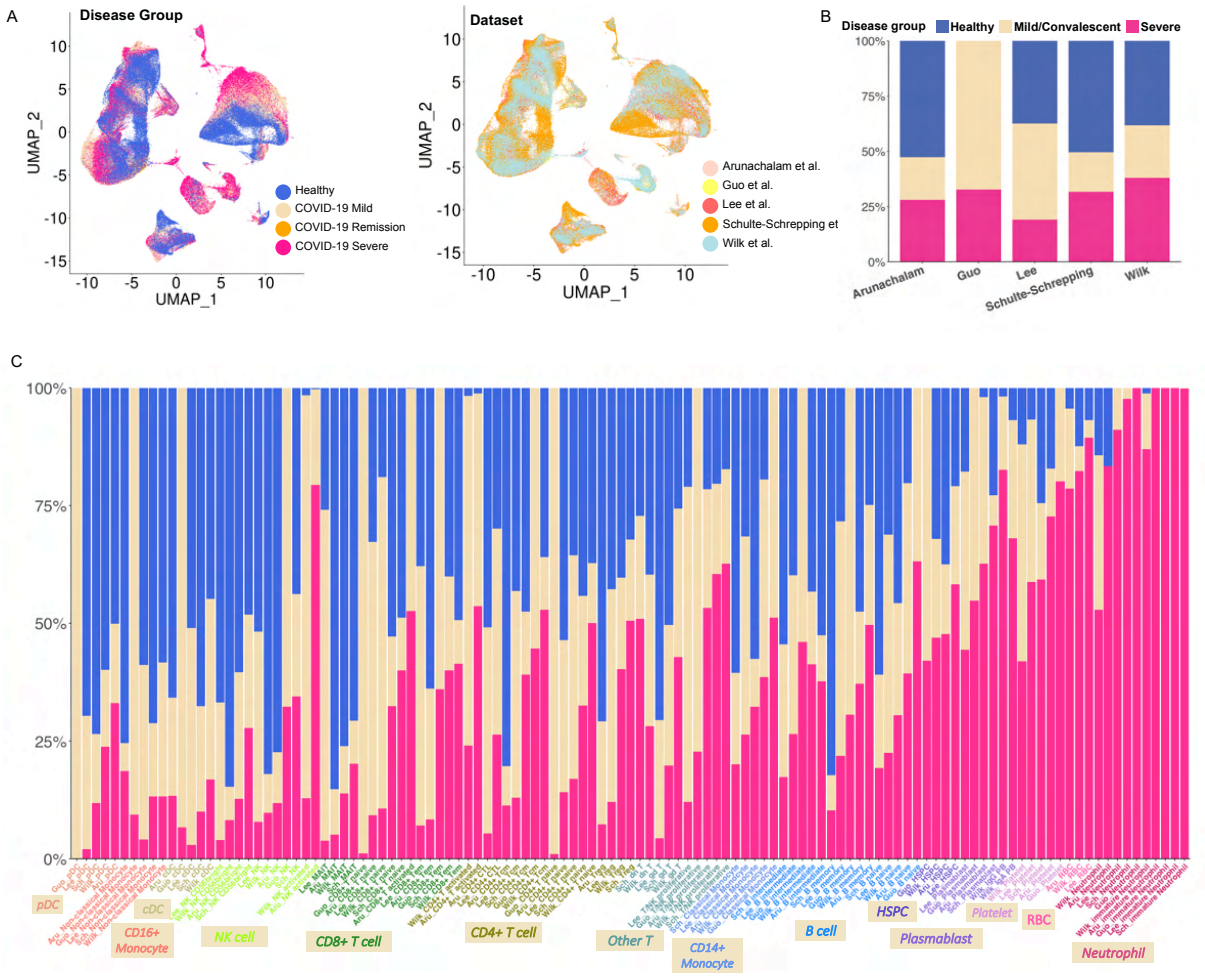


Figure S1. Cell distribution and abundance in the integrated COVID-19 PBMC data, relative to Figure 2. (**A**) Distributions of COVID-19 conditions (Left) and data sources (Right) for the integrated PBMC data are shown on the same UMAP of Figure 2A. (**B**) Bar plot depicts distributions of disease conditions in 5 individual PBMC single-cell datasets. Percentages of 3 disease conditions in each dataset is shown on y axis. (**C**) The integrated bar plot shows percentages of 3 disease conditions in each cell type per dataset. Dataset abbreviations and cell types were concatenated to show disease distributions of specific cell types in the selected datasets. These labels are colored by their cell type designations and ordered by the ascending percentages of COVID-19 conditions.

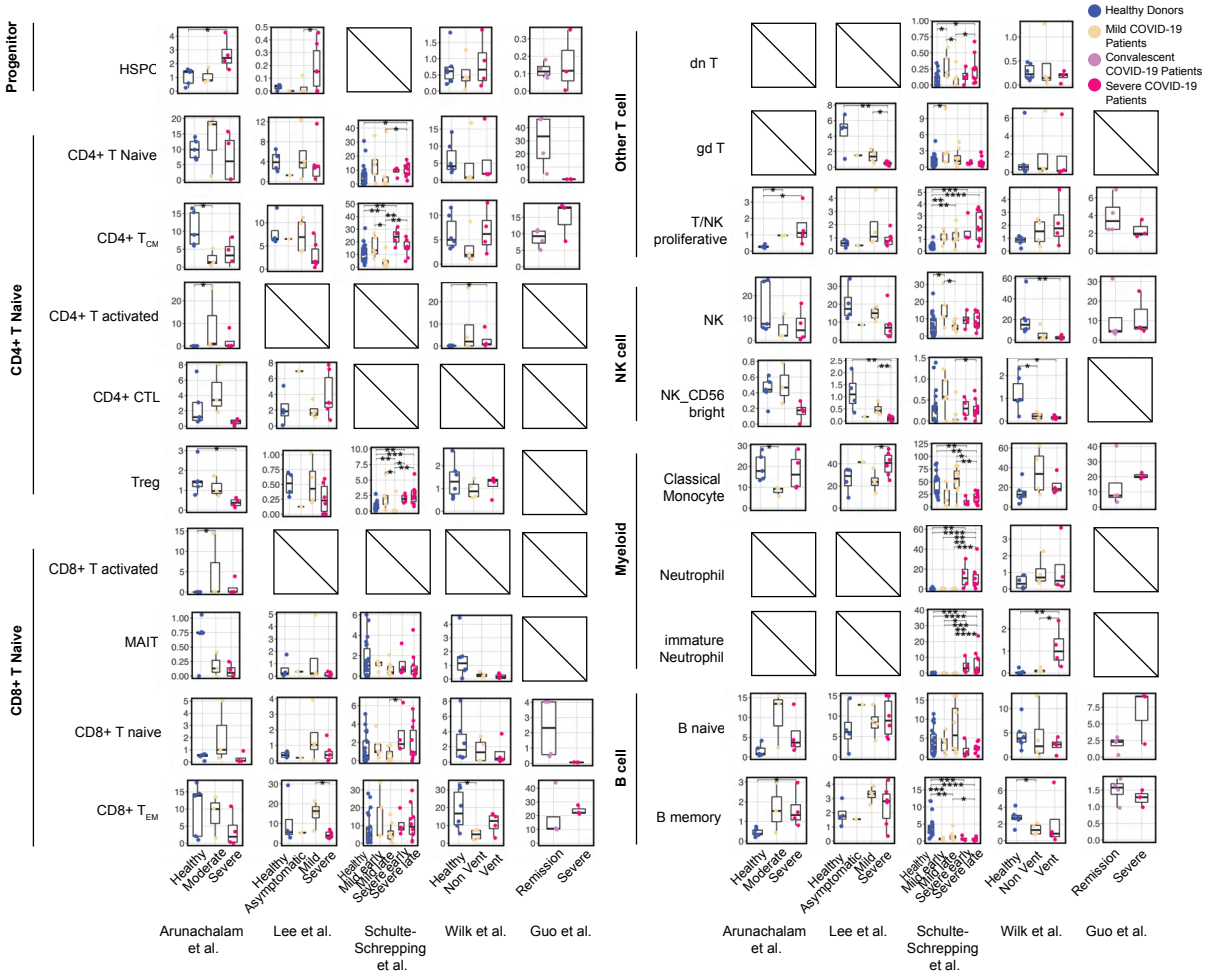


Figure S2. Dynamic changes of cell type abundances in five COVID-19 PBMC datasets, relative to Figure 2. Relative abundances and differences of major cell types in each single cell dataset are shown and compared to controls per each disease condition, per each single-cell dataset. Box plots of all cell types in PBMC are shown except for the 5 highlighted cell types shown in Figure 2B. Statistical methods are the same with Figure 2B.

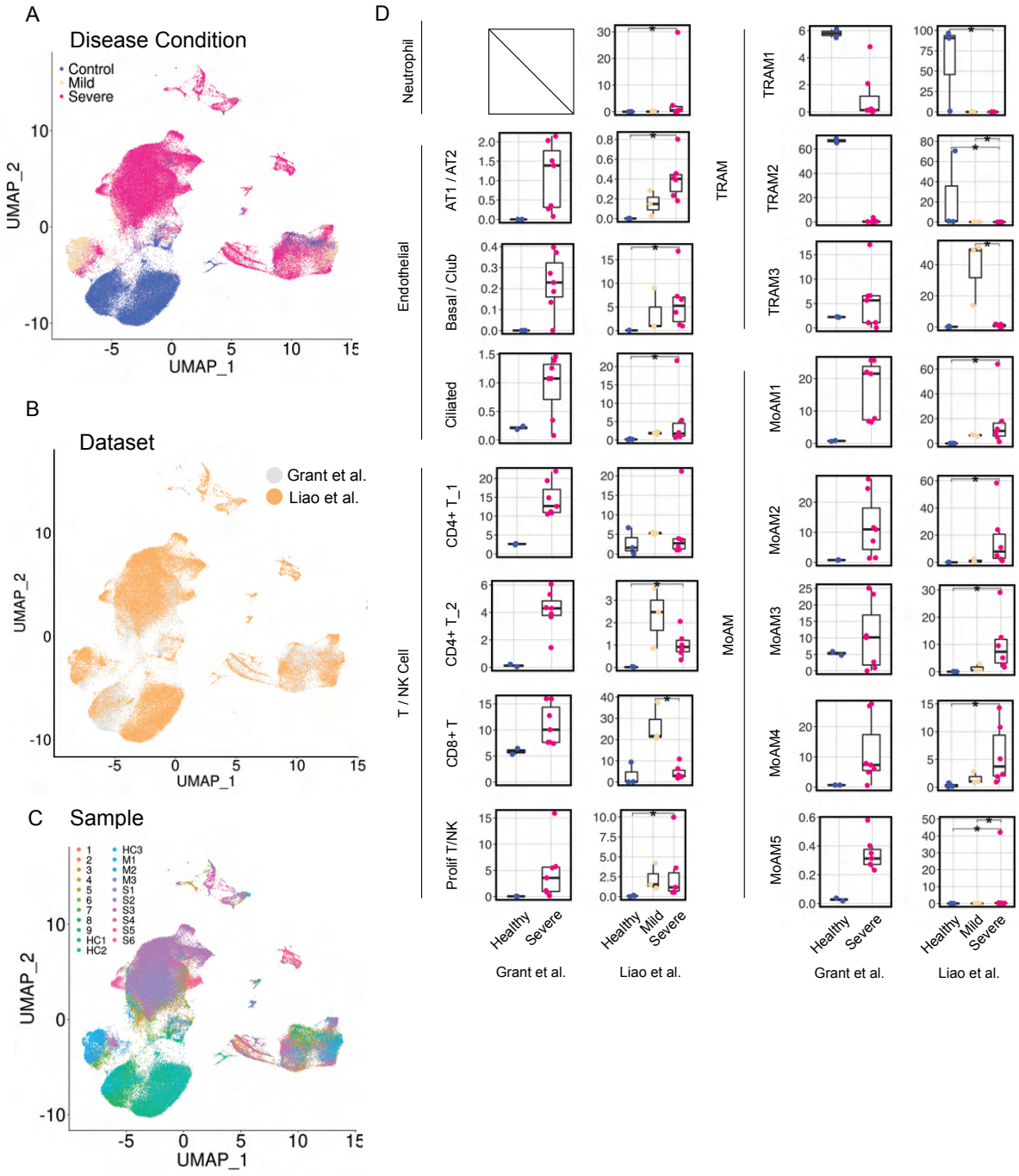


Figure S3. Cell distributions and dynamic changes in the integrated COVID-19 BAL data, relative to Figure 2. (**A-C**) Distributions of disease conditions (A), data sources (B) and samples (C) are shown on the same UMAP of Figure 2C. (**D**) Box plots depict dynamic changes of cell types across COVID-19 conditions in BAL that are not covered in Figure 2D. Statistical methods are the same with Figure 2B.

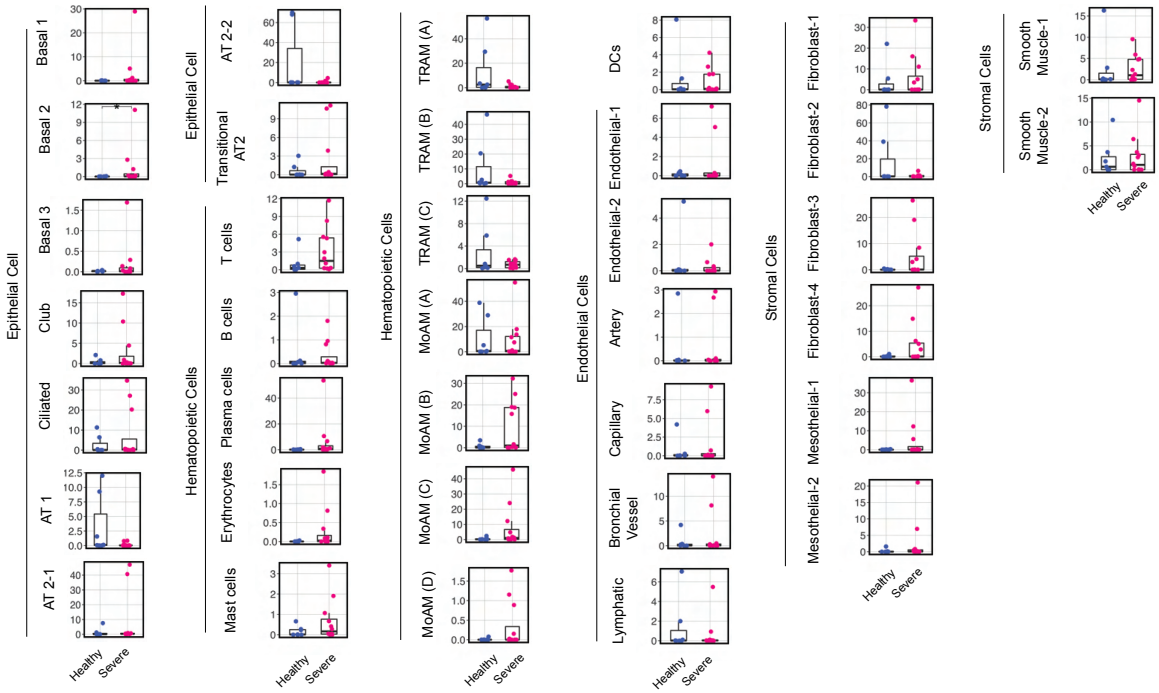


Figure S4. Cell type abundance changes in COVID-19 lung parenchyma dataset, relative to Figure 2. Box plots depict percentages of cell types in control samples and severe COVID-19 samples. We used cell type clusters identified in the original publication but modified cell naming of macrophage subtypes to distinguish monocyte derived macrophage subtypes present in BAL fluid samples. Statistical methods are the same with Figure 2B.

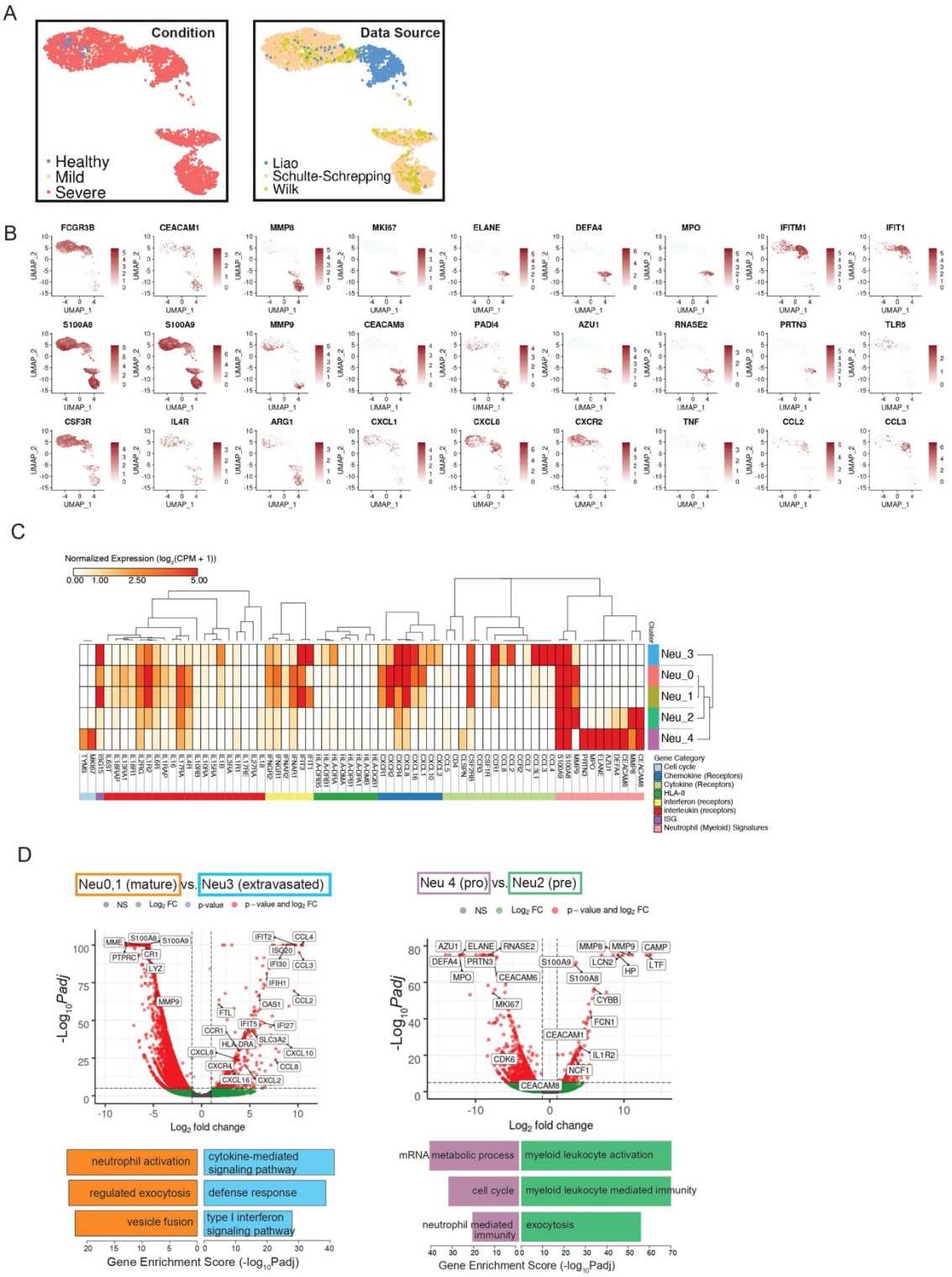


Figure S5. Sub-cluster-specific genes of neutrophils of COVID-19 patients, relative to Figure 3. (**A**) Distribution of disease conditions (Left) and data sources

(Right) for the integrated neutrophil data on the same UMAP of Figure 3A. (**B**) UMAPs of neutrophil sub-cluster-associated genes from Figure 3C. Normalized expression values for each gene were used. (**C**) Normalized expression values of neutrophil-associated genes and other important immune signatures are shown for 5 neutrophil sub-clusters. Lowly expressed genes (genes with maximal average expression level across all neutrophil sub-clusters less than 0.5 after Log_2CPM normalization) were removed from the gene pool of cytokines, chemokines, ISGs, interleukins, interferons, corresponding receptors and MHC-II. (**D**) The volcano plot depicts differentially expressed genes between circulating mature neutrophils (Neu0,1) and extravasated neutrophils (Neu3) (Left); as well as DEGs between pro-neutrophils (Neu4) and pre-neutrophils (Neu2) (Right). Statistical methods are the same with Figure 5C. Representative enriched biological processes (Gene Ontology) are shown in the bottom.

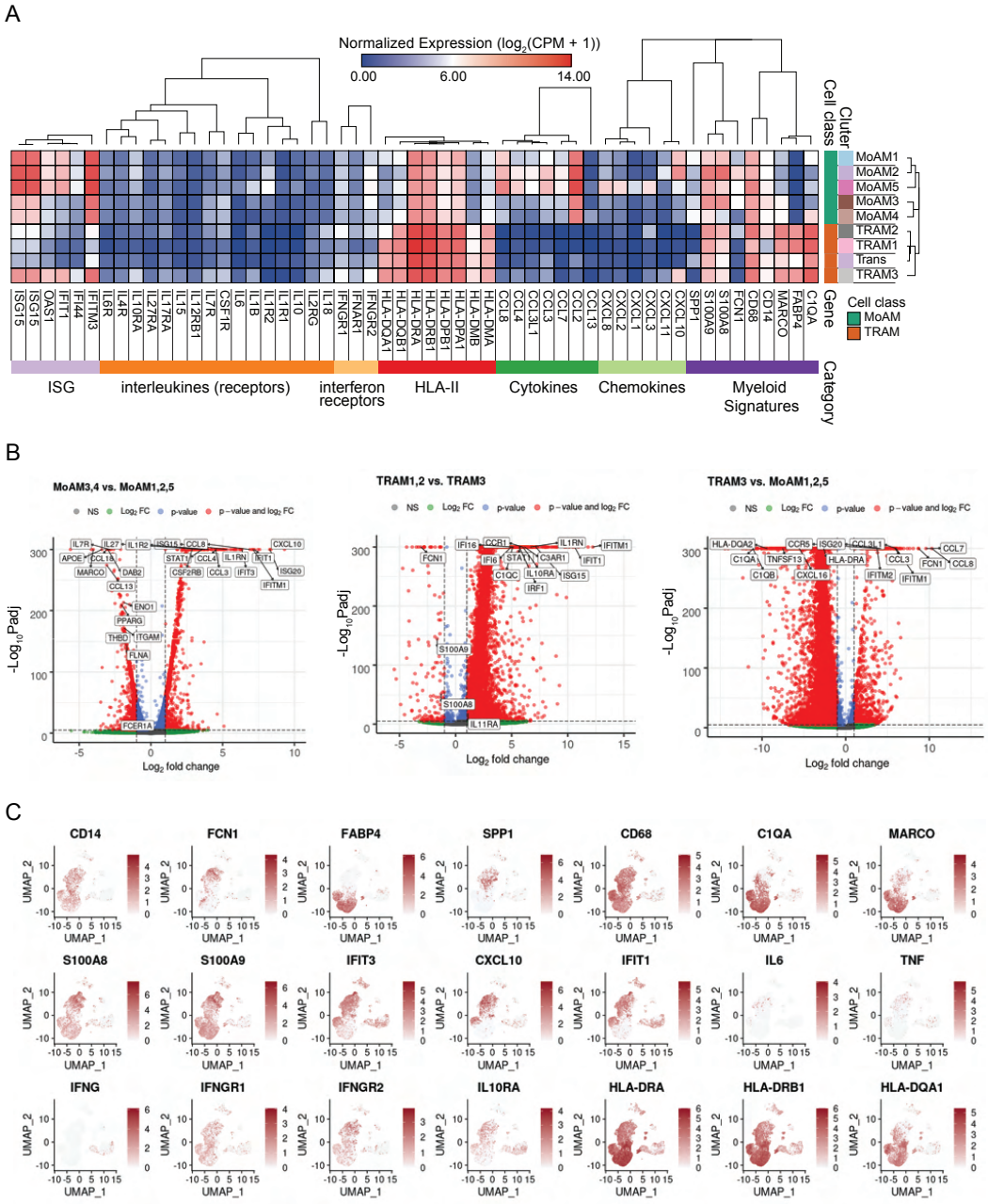


Figure S6. Macrophage-related signatures in the integrated BAL data, relative to Figure 3. (**A**) Normalized expression values of myeloid-cell-associated genes and other important immune signatures are shown for 9 macrophage sub-clusters. Lowly expressed genes (genes with maximal average expression level across all macrophage sub-clusters less than 0.5 after Log_2CPM normalization) were removed from the gene pool of MHC-II, cytokines, chemokines, ISGs, interleukins, interferons and their

receptors. (**B**) Volcano plots were drawn for DEGs of MoAM3,4 versus MoAM1,2,5 (Left) and TRAM 1,2 versus TRAM3 (Middle) and TRAM3 versus MoAM1,2,5 (Right). Statistical methods are the same with Figure 5C. (**C**) Normalized expression values were shown on the same UMAP of Figure 3B for important genes including macrophage signatures, ISGs, interferons, receptors and MHC-II.

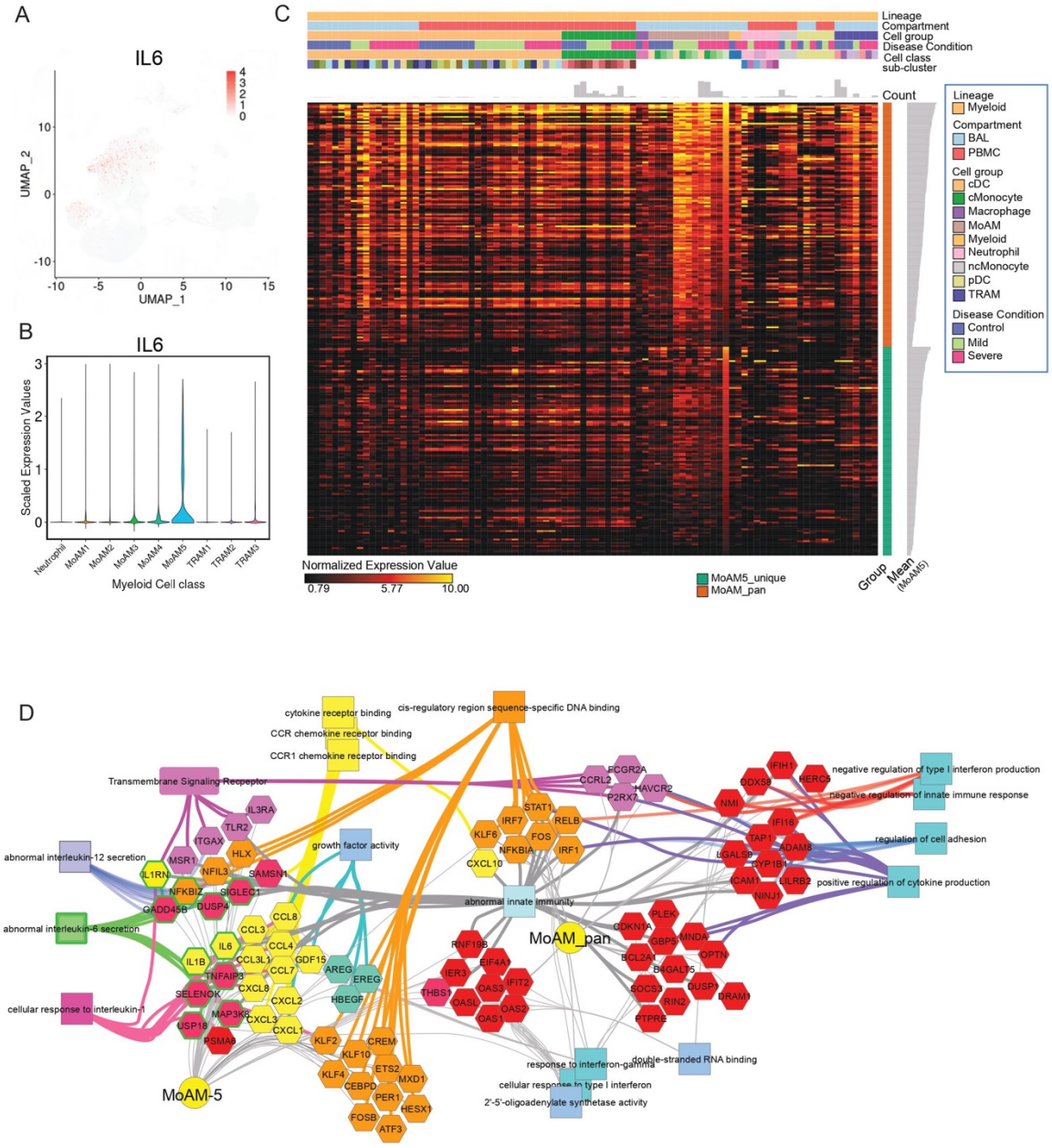


Figure S7. A uniquely-activated monocyte-derived cell type (MoAM5) exhibits a broad signature of cytokines, chemokines, and interleukins including IL6, relative to Figure 3. (**A**) Normalized expression values of IL6 on the same reference UMAP of integrated BAL data as Figure 3B. (**B**) Scale expression levels of IL6 for each macrophage sub-cluster on the violin plot. (**C**) Heatmap of expression levels of pan-MoAM signatures and MoAM5-specifc signatures in all myeloid cells in both PBMC and BAL. (**D**) Network of functional and phenotypic associated pan-MoAM signatures and

MoAM5-specific signatures from (C). Associations were retrieved from ToppGene enrichment results. IL6 is highlighted in the network. As a caveat, the MoAM5 subtype represented a small fraction among the BAL MoAM subtypes and the majority of these cells were observed in a single severely-affected individual.

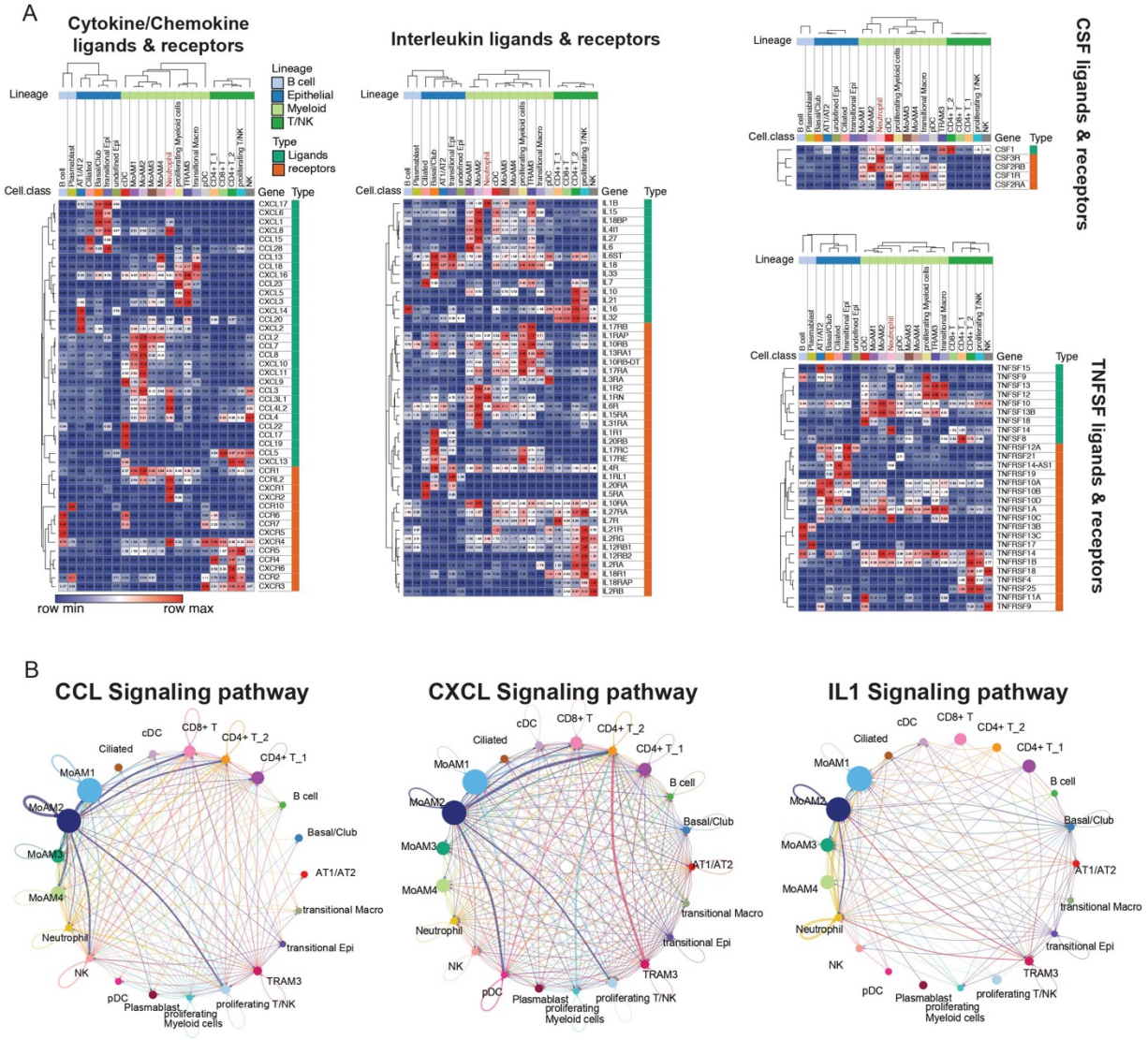


Figure S8. Cell type and cell subtype-specific divisions of cytokine, chemokine, and interleukin signaling pathways in BAL of severe COVID-19 patients, relative to Figure 3. (**A**) Heatmap of expression patterns of ligands and receptors in cytokine, chemokine, interleukin, CSF and TNFSF signaling pathways across cell types of BAL in severe patients. Average normalized expression values were shown and lowly expressed ligands or receptors (maximal normalized expression value for a row in the heatmap < 0.5) were removed. To reduce bias, MoAM5 was removed because cells in the cluster were mainly from one patient. Cell types that have less than 5% cells from severe patients were removed, including TRAM1 and TRAM2. Neutrophils are

highlighted in the heatmap. (**B**) Interaction network of BAL cells in severe patients using CellChat. CCL, CXCL and IL1 signaling pathways were shown. The width of edges represents the strength of interactions and the size of nodes represents the abundance of cell types.

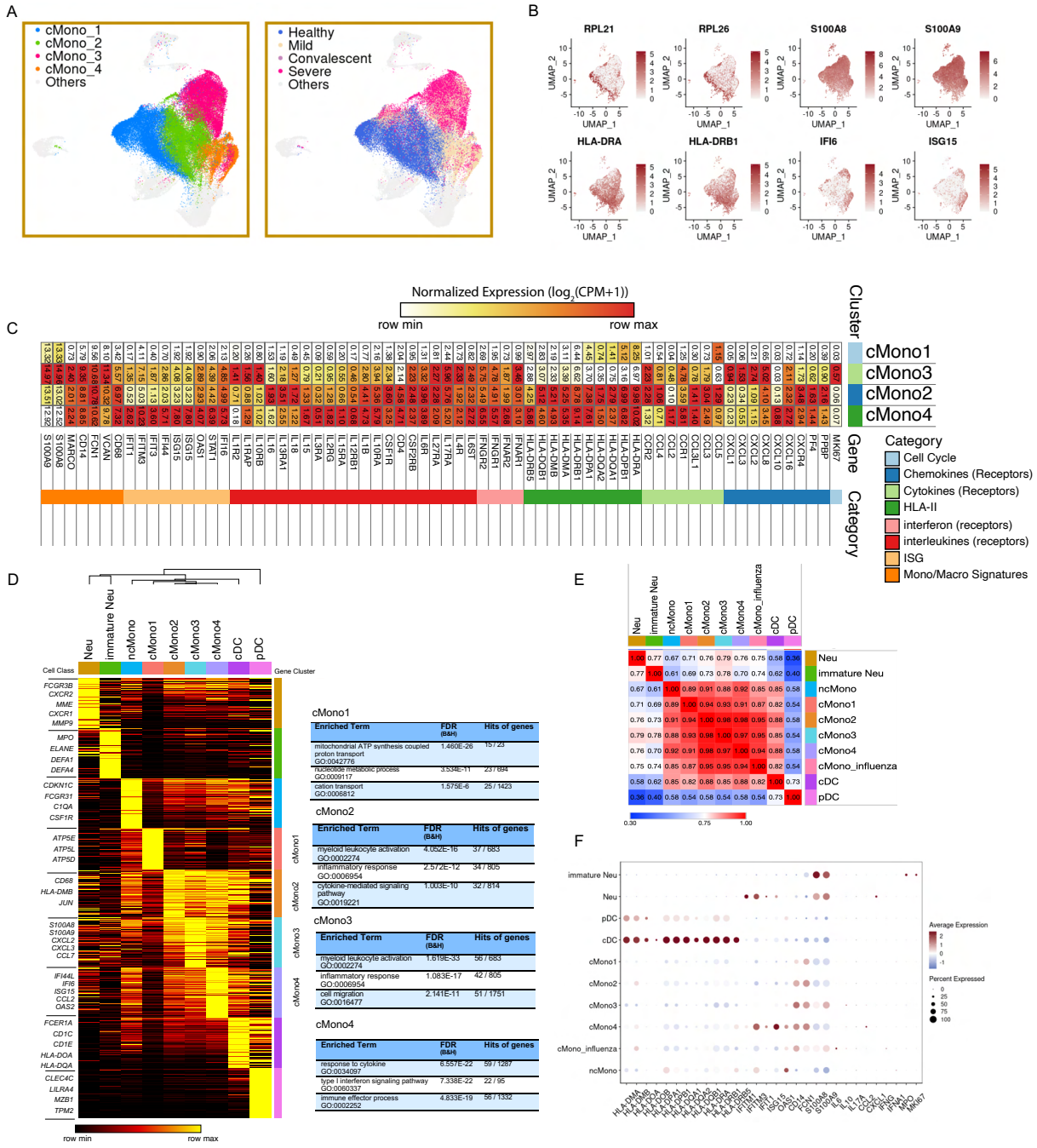


Figure S9. Characteristics of sub-clusters of classical monocytes in the integrated COVID-19 PBMC data, relative to Figure 3. (**A**) UMAPs of 4 sub-clusters (Left) and COVID-19 conditions (Right) of classical monocytes are shown. Grey dots are other myeloid cells in the UMAP of integrated PBMC myeloid data. (**B**) UMAPs of normalized expression values of specific signatures for classical monocyte sub-clusters.

(**C**) Normalized expression values of monocyte-associated genes and other important immune signatures are shown for 4 classical monocyte sub-clusters. (**D**) Gene modules of classical monocyte sub-clusters, as well as other myeloid cell types in the integrated PBMC myeloid data. Representative genes in each module are shown on the left. ToppGene enrichment results for classical monocyte sub-clusters are shown on the right. Columns are clustered using hierarchical clustering. (**E**) Similarity matrix of myeloid cell types using genes in (D). Pearson correlation was used to evaluate similarity. (**F**) Dot plot of MHC-II, ISGs, interleukin genes and cell cycle genes for each myeloid cell type. Scale values were used.

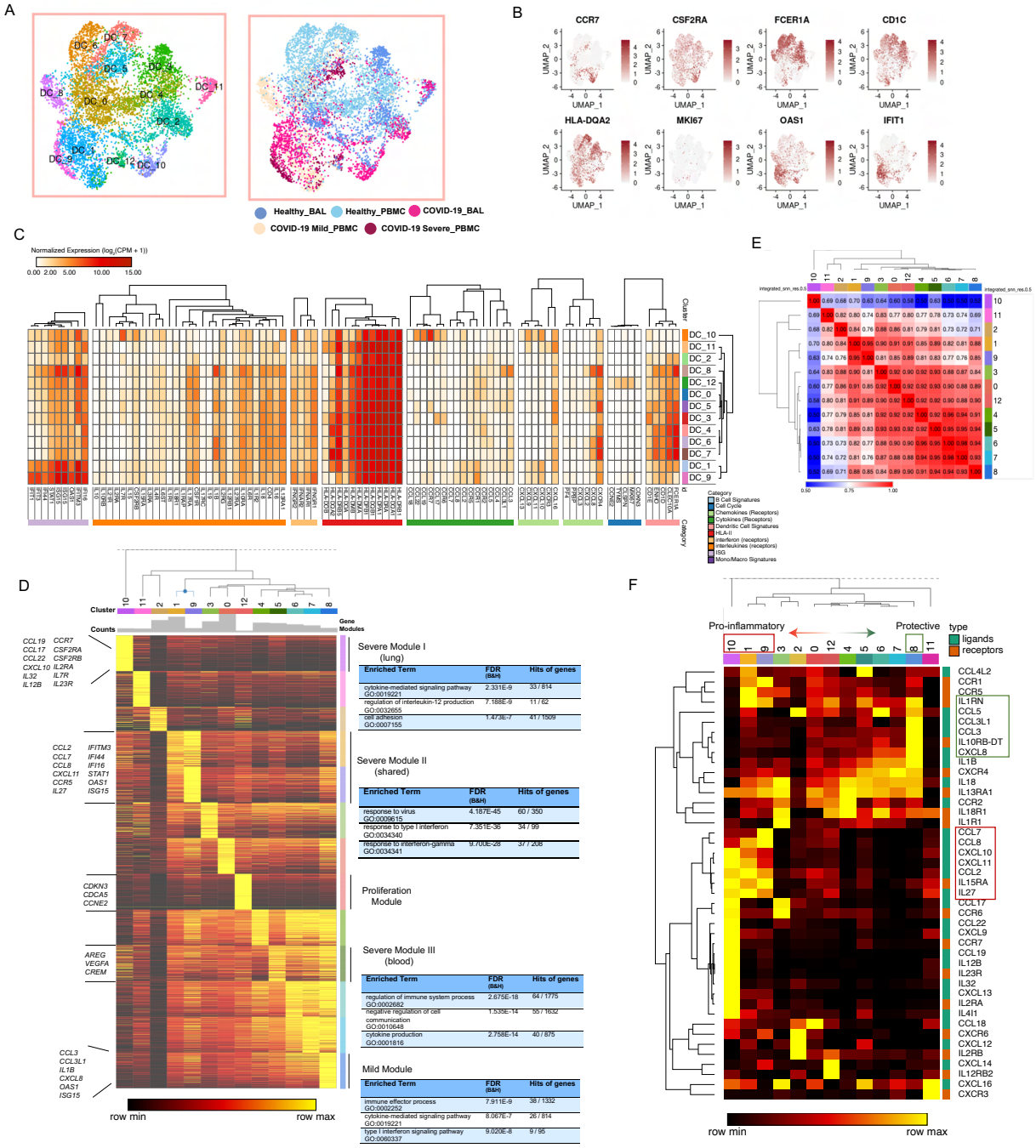


Figure S10. Features of conventional dendritic cell sub-clusters and polarized signaling genes, relative to Figure 3. (**A**) UMAPs of 13 sub-clusters (Left) and sources (Right) of conventional dendritic cells after data integration. (**B**) Normalized expression values of sub-cluster-specific genes on the UMAP. (**C**) Normalized expression values of cDC-associated genes and other important immune signatures are

shown for 13 cDC sub-clusters. (**D**) Gene modules of cDC sub-clusters with 200 most significantly upregulated genes in each module. Representative genes are shown on the left. Gene enrichment results of some modules from ToppGene are shown on the right. (**E**) Similarity matrix of sub-clusters using genes in (D). Pearson correlation was used for similarity scores and hierarchical clustering was applied for rows and columns. (**F**) The heatmap shows the clustering of signaling genes, including cytokines, chemokines, interleukins and their receptors. Red boxes highlight severe patients associated sub-clusters and their upregulated genes. Green boxes highlight mild patients-associated sub-clusters and their upregulated genes.

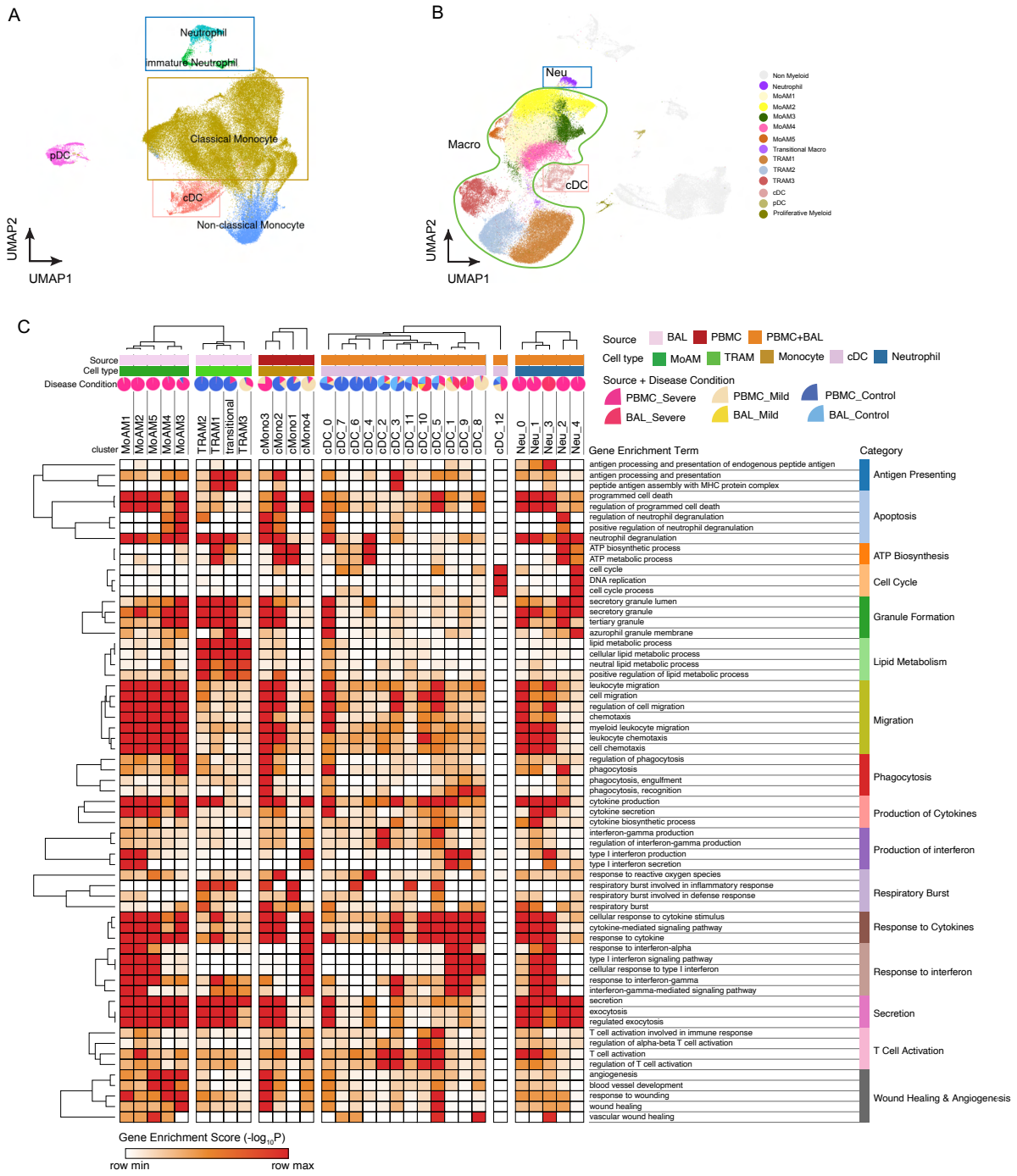


Figure S11. Landscape of myeloid cells in the integrated PBMC and BAL data, relative to Figure 3.

Figure S11. Landscape of myeloid cells in the integrated PBMC and BAL data, relative to Figure 3. (**A-B**) UMAPs of myeloid cells in integrated PBMC (A) and BAL (B)

data. Cell types which were further clustered are highlighted in different colors. (**C**) The heatmap shows associations between subclusters of myeloid cells and myeloid-cell-associated pathways, such as antigen presenting, T cell activation, phagocytosis etc. Gene enrichment scores, defined as $-\log_{10}$(adjusted p value), were calculated as the strength of associations. Pie charts showed the proportions of COVID-19 conditions in each sub-cluster.

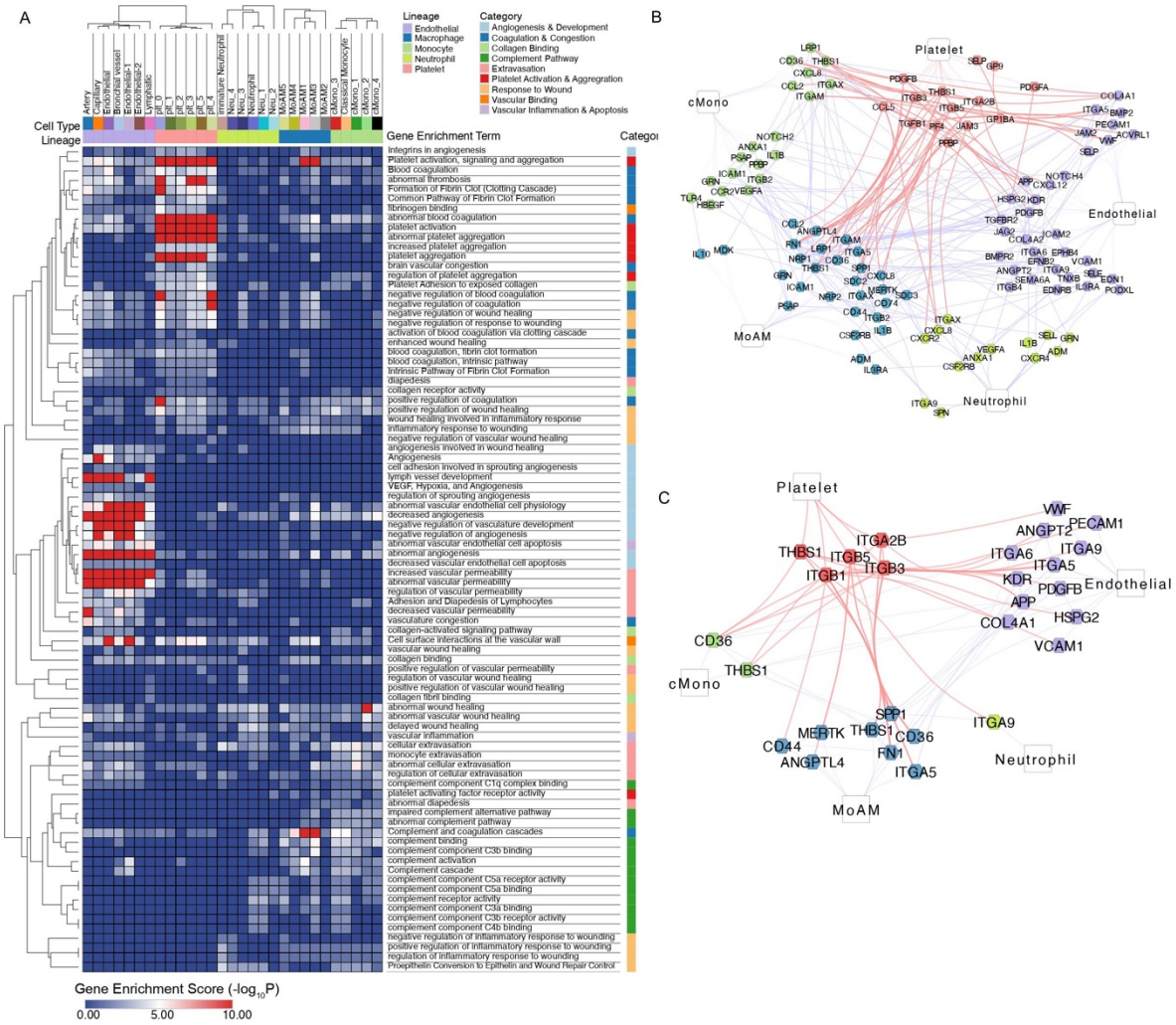


Figure S12. Gene expression signatures of cell types and subtypes activated by COVID-19 are extensively associated with coagulation, hemostasis, and thrombosis-associated pathways, functions, and knockout phenotypes, relative to Figure 4. (**A**) Functional association heatmap of gene signatures from COVID-19 cell types demonstrates differential enrichment for pathways associated with coagulation, vascular permeability, complement, extravasation, platelet activation and aggregation, response to wounding, as shown. Gene modules of cell types and sub-clusters that participate in these pathways were used to calculate enrichment scores. (**B**) Network of upregulated genes in coagulation/thrombosis-associated pathways (A) shows the

potential gene-gene interactions in immunothrombosis of COVID-19 patients. CellChat and ToppCell/ToppGene protein-protein ligand receptor and cell adhesion interaction databases were used to find interaction pairs among upregulated genes. (**C**) A new network derived from (B) shows integrin-associated interactions between platelets and other cells.

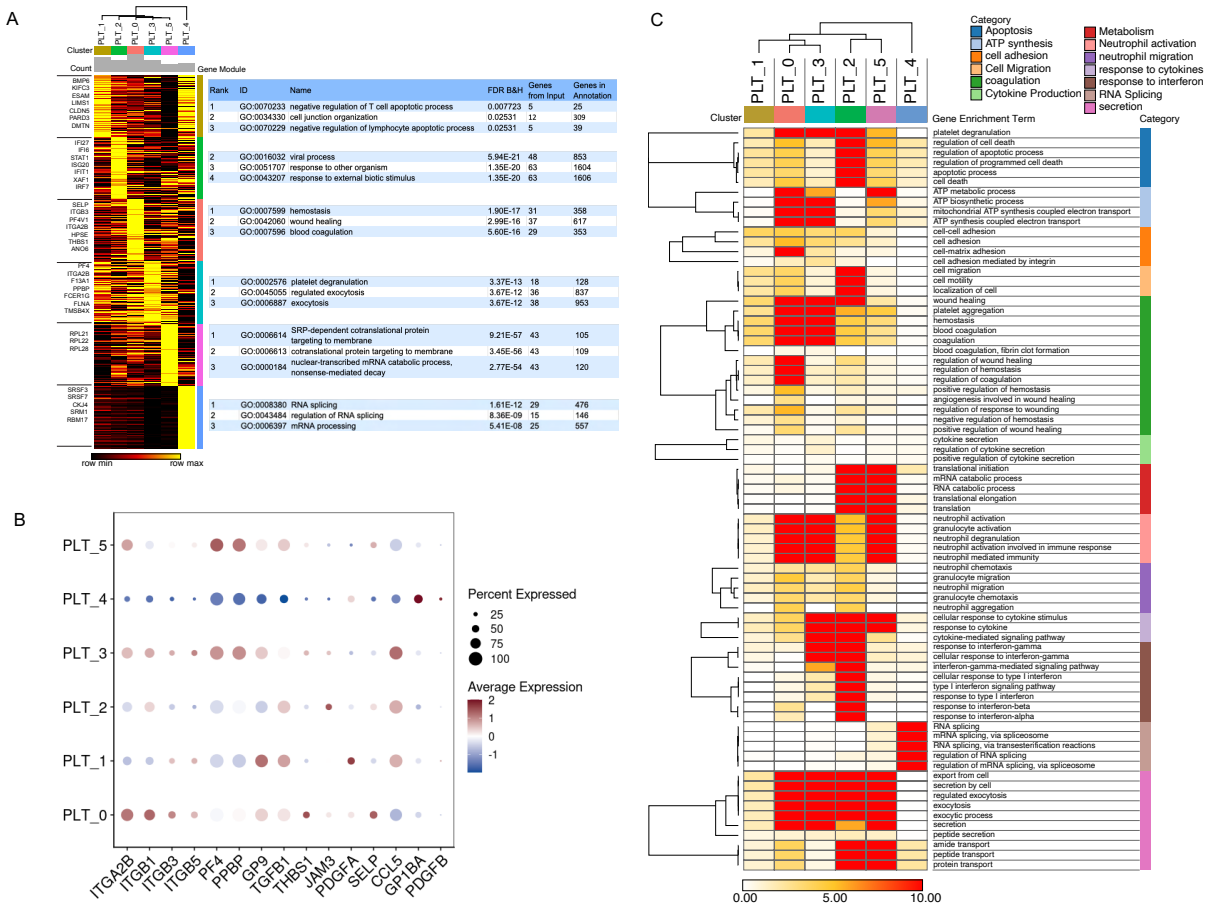


Figure S13. Emergence of platelet subtypes implicating functionally significant alternative roles in hemostasis, coagulation, wound response, and neutrophil recruitment and activation, relative to Figure 4. (**A**) The heatmap shows ToppCell gene modules of 6 platelet sub-clusters in COVID-19 PBMC. Each gene module contains 200 most significant genes for each sub-cluster and important genes are shown on the left. Gene enrichment analysis was conducted using ToppGene and top enrichment results from biological processes (Gene Ontology) are shown on the right. (**B**) Dot plot of integrin and other platelet-associated genes. Scale values are shown on the figure. (**C**) Heatmap of associations between subclusters of platelets and platelet-associated pathways (Gene Ontology). Gene enrichment scores, defined as -$\log_{10}$(adjusted p value), were calculated and shown.

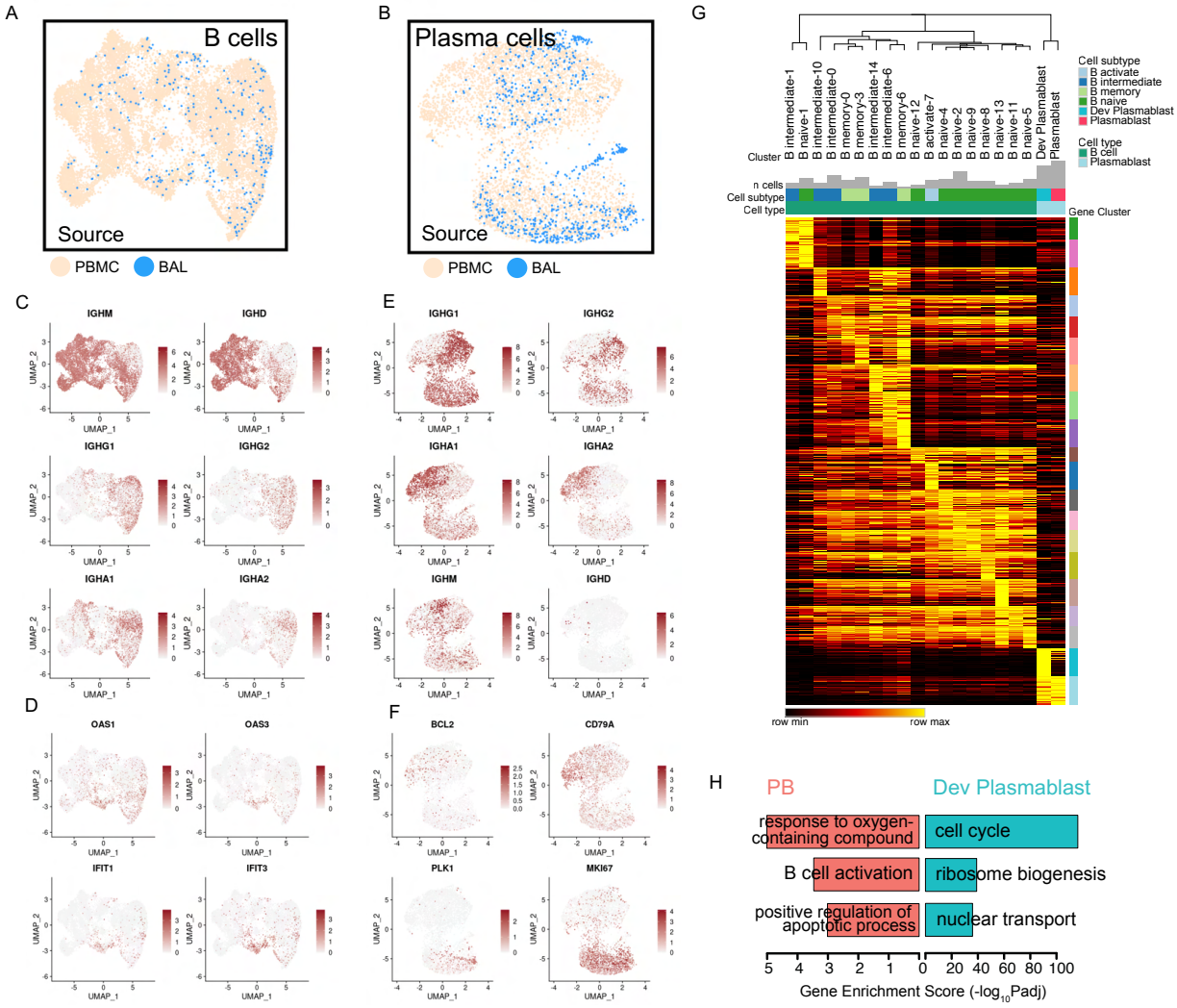


Figure S14. Consistent emergence of a series of early and maturing B cells and plasmablasts in BAL fluid and PBMC across multiple datasets, relative to Figure 5. (**A-B**) UMAPs of B cells (A) and plasmablasts (B) from multiple datasets. (**C-D**) UMAP of normalized expression values of immunoglobulin genes (C) and ISGs (D) for B cells. (**E-F**) UMAP of normalized expression values of immunoglobulin genes (E) and sub-cluster associated genes, such as cell cycle genes and B cell markers (F) for plasmablasts. (**G**) Gene modules of B cell sub-clusters and plasmablast subtypes with 200 most significant genes in each module. Hierarchical clustering was applied for columns. (**H**) Three representative enriched biological processes (Gene Ontology) are shown for these two subtypes using DEGs of plasmablasts in Figure 5C.

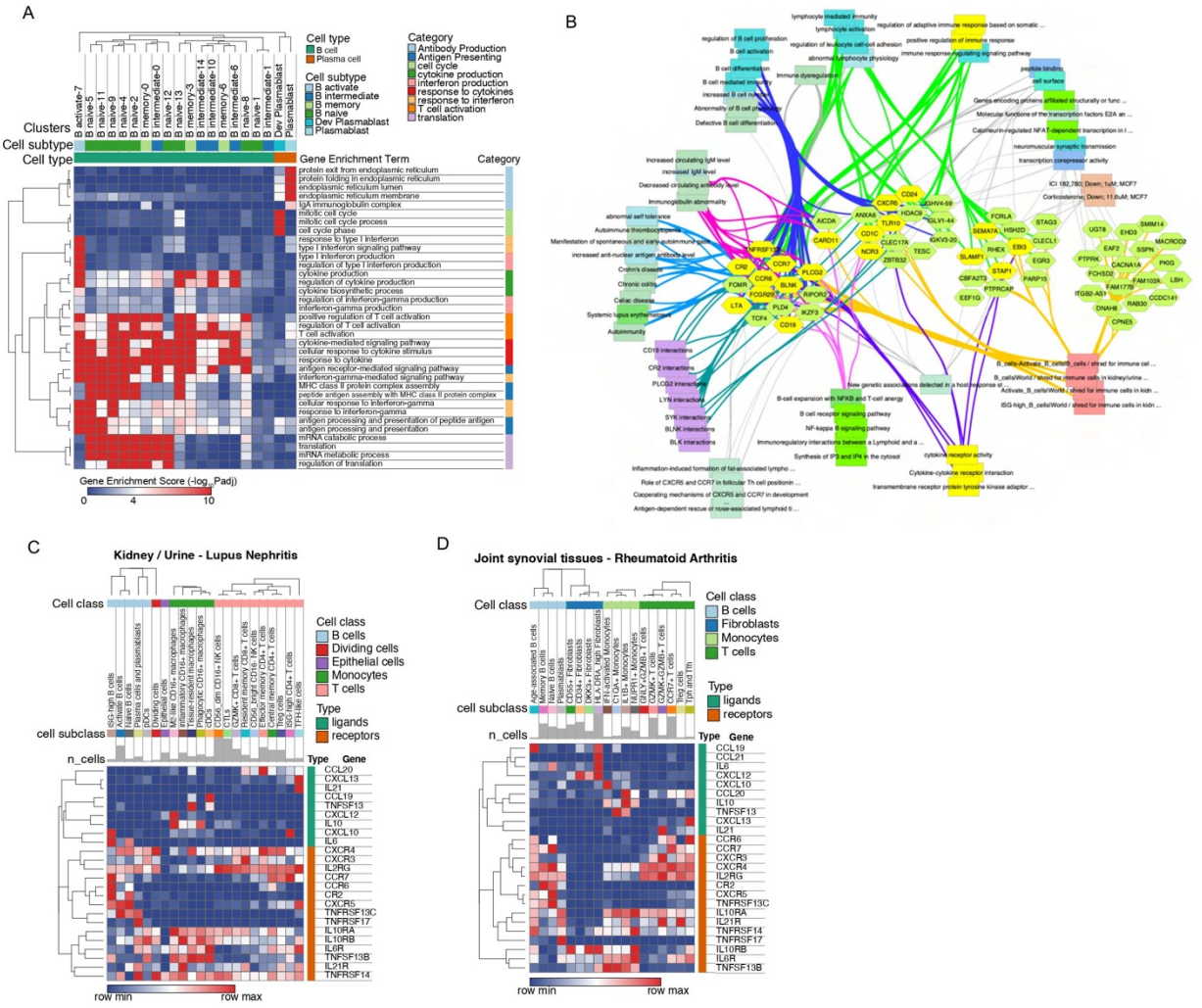


Figure S15. Gene Enrichment analysis of B cell subtypes and autoimmune-associated signatures, relative to Figure 5. (**A**) Heatmap shows gene enrichment scores of B-cell-associated pathways for each B cell sub-cluster and plasmablast subtype. (**B**) Pathway and function association network of upregulated genes in B cells of BAL in mild COVID-19 patients. (**C-D**) Heatmaps show normalized expression levels of autoimmune-associated ligands and receptors (Figure 5E) in lupus nephritis (C) and rheumatoid arthritis (D).

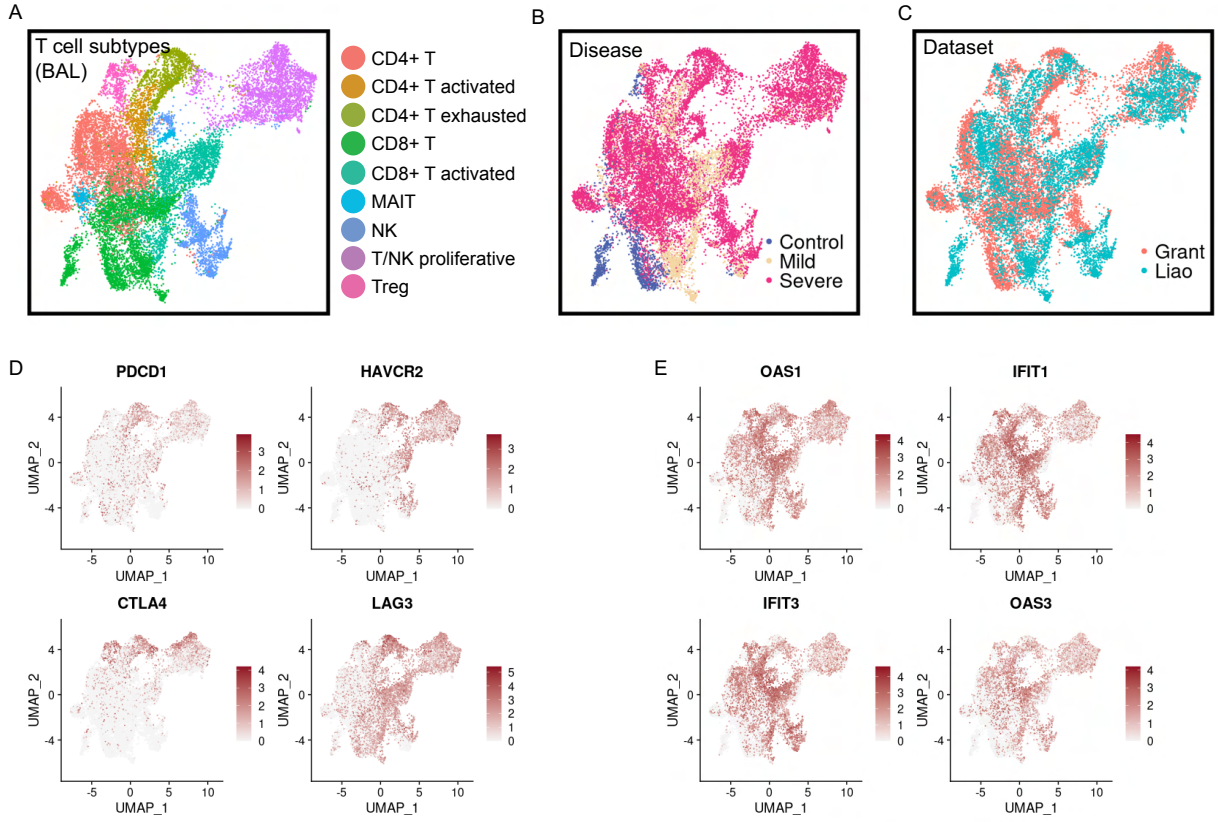


Figure S16. Distinct subtypes of T cells and NK cells in COVID-19 BAL data. (**A-C**) UMAPs of subtypes (A), COVID-19 conditions (B) and data sources (C) of T cells and NK cells in the integrated BAL data. (D-E) UMAPs of normalized expression values of exhausted T cell markers (**D**) and ISGs (**E**).

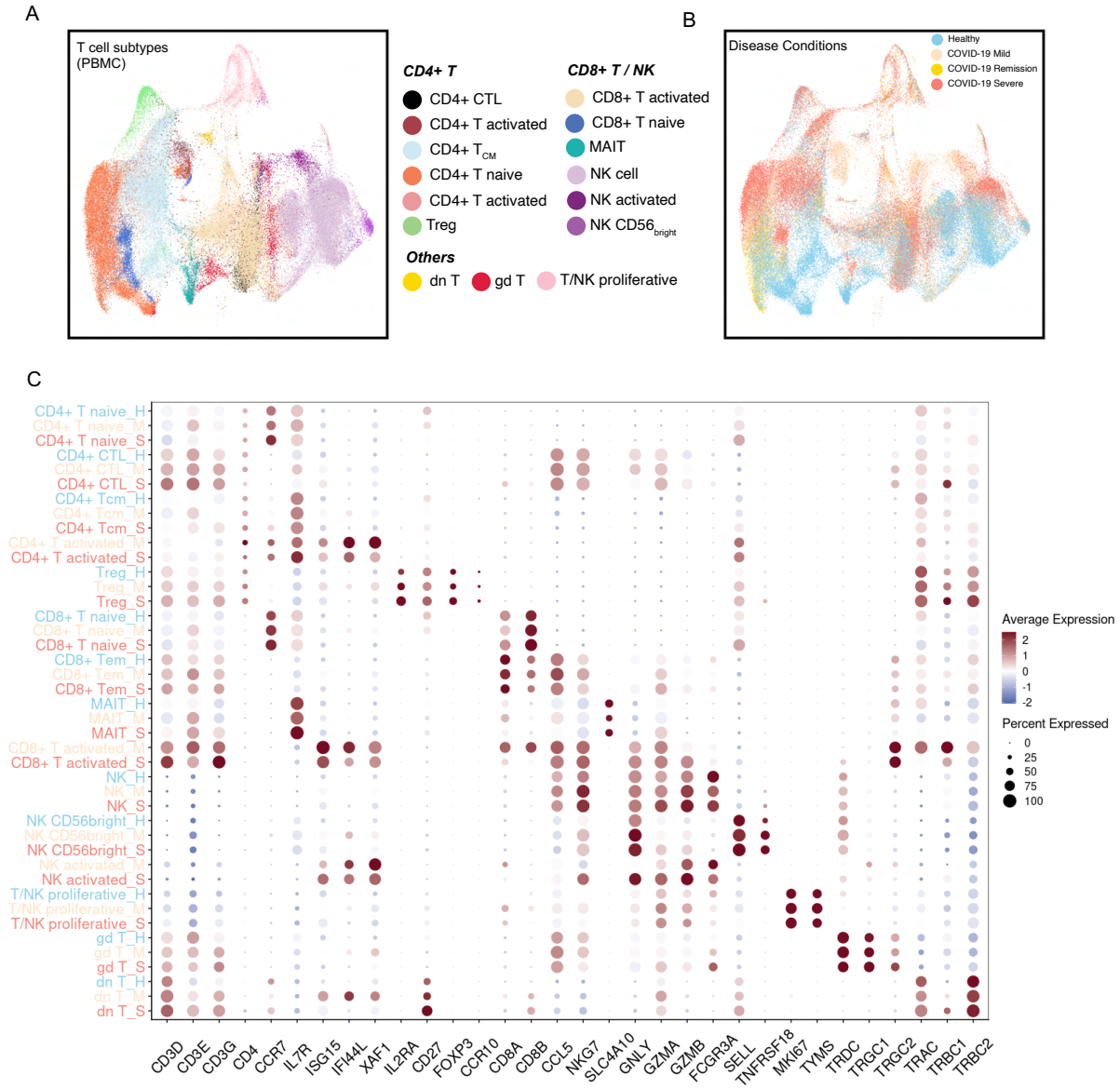


Figure S17. Various T cell and NK cell subtypes in the integrated PBMC data. (**A-B**) UMAPs of T cell and NK cell subtypes (A) and COVID-19 conditions (B) after integration of T cells in 5 PBMC single-cell datasets. (**C**) Dot plot shows T cell and NK cell subtype associated genes for each subtype per disease condition. Labels of cell types of healthy donors, mild patients and severe patients are colored by blue, yellow and red. Scaled expression values are shown using a color scheme.

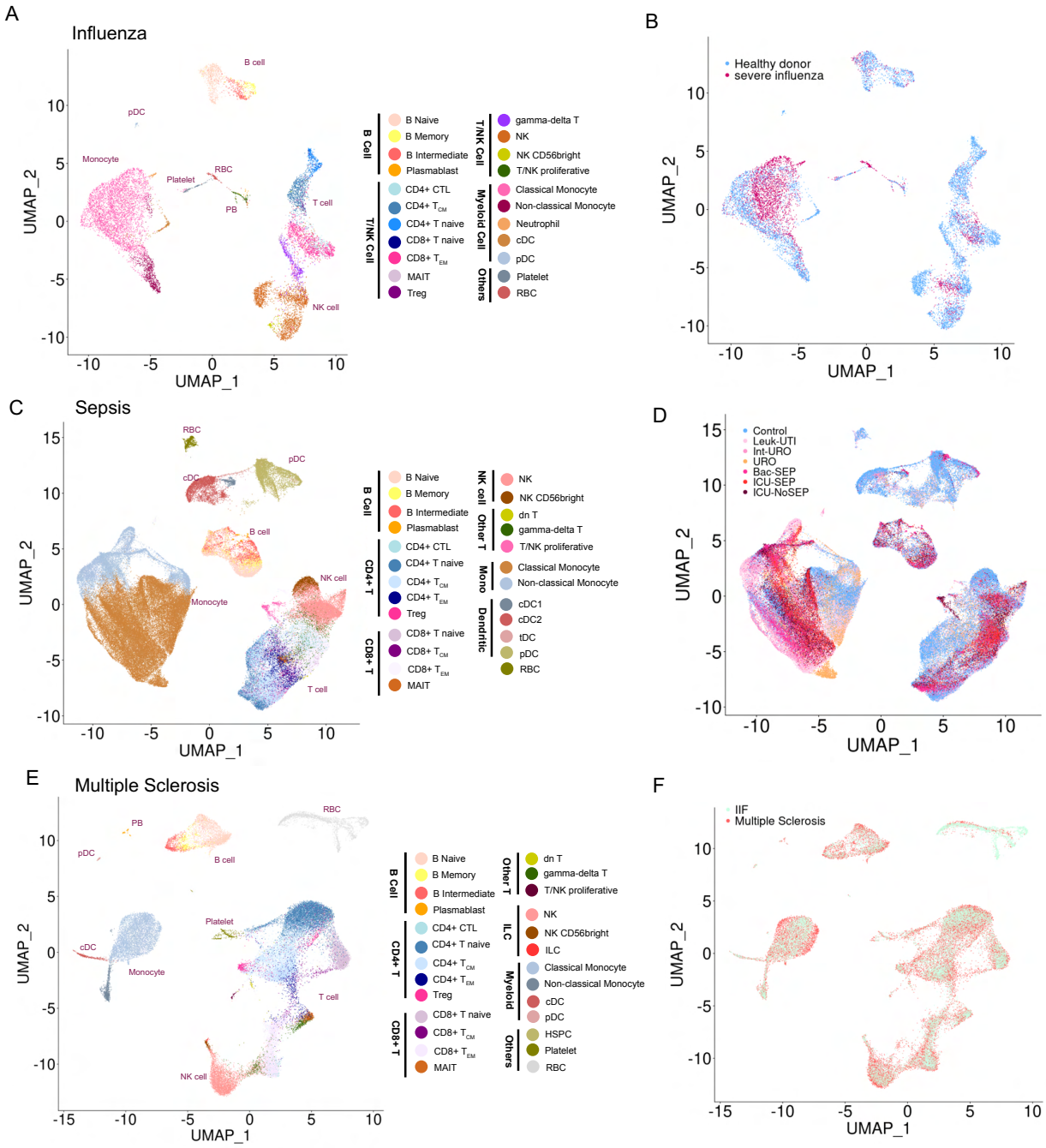


Figure S18. Various cell types in immune-mediated diseases, relative to Figure 7. (**A, C, E**) Distributions of cell types identified in influenza (A), sepsis (C) and multiple sclerosis (E) patients were shown on UMAPs. (**B, D, F**) Distributions of disease conditions in influenza (B), sepsis (D) and multiple sclerosis (F) patients were shown on UMAPs.