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1 **CD177, a specific marker of neutrophil activation, is associated with COVID-19**
2 **severity and death**

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41

42 **Abstract**

43 The identification of COVID-19 patients with high-risk of severe disease is a challenge in
44 routine care. We performed cell phenotypic, serum and RNA-seq gene expression analyses
45 in severe hospitalized patients (n = 61). Relative to healthy donors, results showed
46 abnormalities of 27 cell populations and an elevation of 42 cytokines, neutrophil chemo-
47 attractants, and inflammatory components in patients. Supervised and unsupervised
48 analyses revealed a high abundance of *CD177*, a specific neutrophil activation marker,
49 contributing to the clustering of severe patients. Gene abundance correlated with high serum
50 levels of CD177 in severe patients. Higher levels were confirmed in a second cohort and in
51 ICU than non-ICU patients ($P < 0.001$). Longitudinal measurements discriminated between
52 patients with the worst prognosis, leading to death, and those who recovered ($P = 0.01$).
53 These results highlight neutrophil activation as a hallmark of severe disease and CD177
54 assessment as a reliable prognostic marker for routine care.

55

56 **Introduction**

57 The Coronavirus Disease 2019 (COVID-19) pandemic is caused by a newly described highly
58 pathogenic beta coronavirus, Severe Acute Respiratory Syndrome coronavirus 2 (SARS-
59 CoV-2) (Coronaviridae Study Group of the International Committee on Taxonomy of, 2020;
60 Phelan et al., 2020). COVID-19 consists of a spectrum of clinical symptoms that range from
61 mild upper respiratory tract disease in most cases to severe disease that affects
62 approximately 15% of patients requiring hospitalization (Wu and McGoogan, 2020), some of
63 whom require intensive care because of severe lower respiratory tract illness, acute
64 respiratory distress syndrome (ARDS), and extra-pulmonary manifestations, leading to multi-
65 organ failure and death. Several recent studies have provided important clues about the
66 pathophysiology of COVID-19 (Blanco-Melo et al., 2020; Chen et al., 2020a; Kuri-Cervantes
67 et al., 2020; Mehta et al., 2020; Qin et al., 2020). Most compared the immune and
68 inflammatory status of patients at different stages of the disease (Hadjadj et al., 2020;
69 Mathew et al., 2020; Wilk et al., 2020). Thus, several important biomarkers associated with
70 specific phases of the evolution of COVID-19 have thus far been identified (Ponti et al., 2020;
71 Silvin et al., 2020). Inflammation, cytokine storms, and other dysregulated immune
72 responses have been shown to be associated with severe disease pathogenesis (Lucas et
73 al., 2020; Ong et al., 2020). Severe COVID-19 patients are characterized by elevated
74 numbers of monocytes and neutrophils and lymphopenia (Giamarellos-Bourboulis et al.,
75 2020; Huang et al., 2020; Mathew et al., 2020; Zhou et al., 2020), and a high neutrophil to
76 lymphocyte ratio predicts in-hospital mortality of critically-ill patients (Fu et al., 2020). High
77 levels of pro-inflammatory cytokines, including IL-6, IL-1 β , TNF, MCP-1, IP-10, and G-CSF, in
78 the plasma (Chen et al., 2020a; Chen et al., 2020b; Giamarellos-Bourboulis et al., 2020;
79 Mathew et al., 2020; Zhou et al., 2020) and a possible defect in type I interferon activity have
80 been reported in patients with severe COVID-19 (Arunachalam et al., 2020; Hadjadj et al.,
81 2020; Ong et al., 2020). However, these responses are dynamic, changing rapidly during the
82 clinical course of the disease, which may explain the high variability of the immunological

83 spectrum described (Arunachalam et al., 2020; Bouadma et al., 2020; Lucas et al., 2020).

84 This makes it difficult to deduce a unique profile of the pathophysiology of this infection,
85 which is still undetermined. Furthermore, the high amplitude of the signals generated by the
86 inflammation associated with the disease may hide other pathways that are involved.

87 From a clinical standpoint, clinicians face the daily challenge of predicting worsening patients
88 due to the peculiar clinical course of severe COVID-19, characterized by a sudden
89 deterioration of the clinical condition 7 to 8 days after the onset of symptoms. Determination
90 of the onset of the pathological process once infection has been established in a patient with
91 a severe stage of infection is highly imprecise because of the possible pauci- or
92 asymptomatic phase of the infection, as well as the low specificity of self-limited “flu” illness.

93 We used a systems immunology approach to identify host factors that were significantly
94 associated with the time to illness onset, severity of the disease (ICU or transfer to ICU), and
95 mortality of COVID-19 patients enrolled in the multicentric French COVID cohort
96 (Yazdanpanah, 2020). In addition to the depletion of T cells and mobilization of B cells,
97 neutrophil activation, and severe inflammation, we show upregulation of *CD177* gene
98 expression and protein levels in the blood of COVID-19 patients in both the COVID-19 cohort
99 and a “confirmatory” cohort, i.e., Swiss cohort, relative to healthy subjects. CD177, a
100 neutrophil activation marker, characterized critically ill patients and marked disease
101 progression and death. Our finding highlights the major role of neutrophil activation through
102 CD177 over-expression in the critical clinical transition point in the trajectory of COVID-19
103 patients.

104

105

106 **Results**

107 **Overview of the phenotype, cytokine, and inflammatory profiles of COVID-19 patients**

108 Patient characteristics from the French COVID cohort enrolled in this analysis are shown in
109 Table 1. All patients from this cohort were stratified as severe according to criteria of the
110 French COVID cohort (clinicaltrials.gov NCT04262921) (Yazdanpanah, 2020), with 53 (87%)
111 hospitalized in an ICU (either initially or after clinical worsening or death) and eight not. The
112 median age was 60 years ([interquartile range (IQR)], [50-69]) and 80% were male. Sampling
113 for immunological analyses was performed within three days of entry and after a median of
114 11 days [7-14] after symptoms onset. We first assessed leukocyte profiles by flow cytometry
115 using frozen peripheral blood mononuclear cells (PBMCs) from 50 COVID-19 patients (with
116 available PBMC samples) and 18 healthy donors (HDs) (14 or 15 HDs were used as controls
117 per immune-cell subset).

118 We analyzed 52 immune cell populations (gating strategies are shown in Figure S1), of
119 which 23 showed significant differences (Wilcoxon test adjusted for multiple comparisons)
120 between the COVID-19 patients and HDs. We not only confirmed previously reported
121 abnormalities but also revealed new immunological features of COVID-19 patients (Figure
122 S2). The COVID-19 patients showed a significant reduction in the frequency of total CD3⁺ T
123 cells and CD8⁺ T cells relative to HDs, as previously reported (De Biasi et al., 2020; Xu et al.,
124 2020), that expressed an activated phenotype (CD38⁺HLA-DR⁺) (Figure 1A). The COVID-19
125 patients also showed lower frequencies of resting memory B cells contrasting with higher
126 frequencies of activated memory B cells and exhausted B cells (Figure 1B). As previously
127 reported (Bouadma et al., 2020; Mathew et al., 2020), the proportion of plasmablasts was
128 markedly higher in COVID-19 patients (median [Q1-Q3]: 10.85% [3-23]) than HDs (0.76%
129 [0.4-0.8]) ($P < 0.001$). Total NK-cell frequencies, more precisely those of the CD56^{bright} and
130 CD56^{dim}CD57⁻ NK-cell subpopulations, were lower than in HDs ($P = 0.017$, $P < 0.001$, and P
131 $= 0.004$, respectively) (Figure 1C), while a higher proportion of these NK cells, as well as
132 NKT cells, were cycling, expressing Ki67 antigen (CD56^{bright}: 22% [13-30], CD56^{dim}CD57⁻:

133 16.8% [11.5-27], and NKT: 10% [5.6-18.2]) ($P = 0.003$, $P = 0.004$, and $P = 0.001$ compared
134 to HD) (Figure 1C). In addition, COVID 19-patients showed significantly smaller classical
135 ($CD14^+CD16^-$), intermediate ($CD14^+CD16^+$), and non-classical ($CD14^-CD16^+$) monocyte
136 subpopulations than HDs ($P = 0.013$, $P = 0.017$, $P < 0.001$, respectively) (Figure 1D).
137 Interestingly, COVID-19 patients tended to exhibit a higher frequency of $\gamma\delta$ T cells than HDs
138 (median 10.4% [7.5-16.1] vs 7.3% [6-10] in HDs; $P = 0.068$) (Figure 1E), with a significant
139 proportion of $\gamma\delta$ T cells showing higher expression of the activation marker CD16 ($P = 0.01$)
140 and lower expression of the inhibitory receptor NKG2A ($P < 0.001$) than HDs (Figure 1E).
141 Finally, we observed markedly smaller frequencies of dendritic cells (DCs) for all populations
142 studied (pre-DC, plasmacytoid DC (pDC), and conventional DC (cDC1 and cDC2) in COVID-
143 19 patients than in HDs ($P < 0.001$, for all comparisons) (Figure 1F). Neutrophils count were
144 available in 44 patients and the concentration was more elevated in COVID19 patients
145 belonging to group 2 and group 3 compared to group 1 (8.109/L vs 3.109/L, $p < 0.03$). It was
146 also more elevated in patients hospitalized in ICU (8.109/L vs 2.109/L, $p < 0.009$).

147 We then evaluated the levels of 71 serum cytokines, chemokines, and inflammatory factors
148 in 33 COVID-19 patients and 5 HDs. Forty-four analytes differed significantly (Wilcoxon test
149 adjusted for multiple comparisons) between the COVID-19 patients and HDs (shown in the
150 heatmap in Figure 2 and detailed in Figure S3). The levels of 42 factors were higher, among
151 them, pro-inflammatory factors (IL-1a, IL-6, IL-18, tumor necrosis factor- α and β (TNF- α ,
152 TNF- β), IL-1ra, ST2/IL-1R4, the acute phase protein lipopolysaccharide binding protein LBP,
153 IFN- α 2); Th1 pathway factors (IL-12 (p70), IFN- γ , IP-10, IL-2Ra); Th2/regulatory cytokines
154 (IL-4, IL-10, IL-13); IL-17, which also promotes granulocyte-colony stimulating factor (G-
155 CSF)-mediated granulopoiesis and recruits neutrophils to inflammatory sites; T-cell
156 proliferation and activation factors (IL-7, IL-15); growth factors (SCF, SCGF-b, HGF, b-FGF,
157 b-NGF); and a significant number of cytokines and chemokines involved in macrophage and
158 neutrophil activation and chemotaxis (RANTES (CCL5), MIP-1a and b (CCL3 and CCL4),
159 MCP-1 (CCL2), MCP-3 (CCL7), M-CSF, MIF, Gro-a (CXCL1), monokine inducible by γ

160 interferon MIG/CXCL9, IL-8, IL-9). Interestingly, we found higher levels of midkine, a marker
161 usually not detectable in the serum, which enhances the recruitment and migration of
162 inflammatory cells and contributes to tissue damage (Cai et al., 2020). In parallel, Granzyme
163 B and IL-21 levels were significantly lower in COVID-19 patients than HDs ($P = 0.007$ and P
164 $= 0.004$, respectively) (Figure S3).

165 **Whole blood gene expression profiles show a specific signature for COVID-19 patients**

166 The comparison of gene abundance in whole blood between COVID-19 patients ($n = 44$) and
167 HDs ($n = 10$) showed 4,079 differentially expressed genes (DEG) with an absolute fold
168 change ≥ 1.5 , including 1,904 that were upregulated and 2,175 that were downregulated
169 (Figure 3A). The main pathways associated with the DEG correspond to the immune
170 response, including neutrophil and interferon signaling, T and B cell receptor responses,
171 metabolism, protein synthesis, and regulators of the eIF2 and mammalian target of
172 rapamycin (mTOR) signaling pathways (Figure 3A). Although several of these pathways
173 involved multiple cell types, analysis of the neutrophil pathway showed higher abundance of
174 genes mainly related to neutrophil activation, their interaction with endothelial cells, and
175 migration (Figure 3B). Among the most highly expressed genes, this signature included
176 *CD177*, a specific marker of neutrophil adhesion to the endothelium and transmigration
177 (Bayat et al., 2010), *HP* (Haptoglobin), a marker of granulocyte differentiation and released
178 by neutrophils in response to activation (Theilgaard-Monch, 2006), *VNN1* (hematopoietic cell
179 trafficking), *GPR84* (neutrophil chemotaxis), *MMP9* (neutrophil activation and migration), and
180 *S100A8* and *S100A12* (neutrophil recruitment, chemotaxis, and migration). The *S100A12*
181 protein is produced predominantly by neutrophils and is involved in inflammation and the
182 upregulation of vascular endothelial cell adhesion molecules (Roth et al., 2003) (Figure 3B).

183 In parallel, we observed a higher abundance of several interferon stimulating genes (ISG)
184 (*IFI27*, *IFITM3*, *IFITM1*, *IFITM2*, *IFI6*, *IRF7*, *IRF4*) (Figure 3C) and cytokines and cytokine
185 receptors (*IL-1R*, *IL-18R1*, *IL-18RAP*, *IL-4R*, *IL-17R*, *IL-10*) (Figure 3D). Consistent with
186 profound T-cell lymphopenia, the expression of several families of T-cell Receptor (TCRA)

187 genes was lower (Figure 3E). We observed severe dysregulation of T-cell function that
188 involved inhibition of serine/threonine kinase PKC θ signaling (z-score = -4.46), as well as the
189 inducible T-cell co-stimulator/ICOSL axis (z-score = -4.5) (Figure S4). In contrast to the
190 results for T cells, the peripheral expansion of memory B cells and plasmablasts was
191 associated with broad expansion of the B-Cell Receptor (BCR) (Figure 3F and Table S2).

192 We also observed genes belonging to several crucial pathways and biological processes that
193 had not been previously reported to characterize COVID-19 patients to be underrepresented.
194 These included eIF2 signaling, with many downregulated genes, such as ribosomal proteins
195 (RP) and eukaryotic translation initiation factors (EIFs) (Figure S4A), common targets of the
196 integrated stress response (ISR), including antiviral defense (Levin and London, 1978;
197 Pakos-Zebrucka et al., 2016). In addition, we also found genes involved in signaling through
198 mTOR (Figure S4B), a member of the phosphatidylinositol 3-kinase-related kinase family of
199 protein kinases. Prediction analysis using Ingenuity pathways showed both lower eIF2 (z-
200 score = - 6.8) and mTOR (z-score = -2.2) signaling in COVID-19 patients than HDs.

201 **Unsupervised whole blood gene expression profiles reveal distinct features of COVID-** 202 **19 patients.**

203 Unsupervised classification of 44 COVID-19 patients and 10 HDs identified three distinct
204 groups of COVID-19 patients: 10 in group 1, 16 in group 2, and 18 in group 3 (Figure 4).
205 Detailed patient characteristics according to group are presented in Table S1. Among a large
206 set of clinical and biological characteristics, the analysis showed the differential clustering to
207 not be explained by the severity of the disease. Indeed, the median Sequential Organ Failure
208 Assessment (SOFA) score and Simplified Acute Physiology Score (SAPS2), which include a
209 large number of physiological variables (Le Gall, 1993; Vincent et al., 1998) and evaluate the
210 clinical severity of the disease (a high score is associated with a worse prognosis) of the
211 COVID-19 patients, were 6 [4-7] and 36 [28-53], respectively, with no significant differences
212 between groups. Nevertheless, we observed a significant difference from symptoms onset to
213 the admission, which ranged from 7 [6 –11] days for patients in group 1 to 11 [10-14] and 13

214 [9-14] days for patients in groups 2 and 3, respectively ($P = 0.04$, Kruskal-Wallis test). Finally,
215 group 1 which was the closest to HDs in terms of gene profile, consisted of patients in the
216 early days of the disease (Table S1).

217 Analysis of the genes contributing to the differences between groups confirmed and
218 extended the findings described above (Figure 3 and Figure S4). Several pathways were
219 highly represented in sectors of the heatmap defined according to gene abundance across
220 patient groups. For example, 97% of the genes making up the BCR and 65% of those
221 involved in neutrophil responses were represented among the genes showing a greater
222 abundance in COVID-19 groups 2 and 3 than group 1 and HDs (Figure 4). Other pathways,
223 such as those for interferon (64%), TCR (100%), iCOS-iCOS-L (88%), mTOR (81%), and
224 eIF2 signaling (92%) were also highly represented. The interferon signaling genes, such as
225 *IFI44L*, *IFIT2*, and *IRF8*, a regulator of type I Interferon (α , β), were significantly more
226 abundant at earlier stages (in patients from group 2) and tended to be less abundant in group
227 3, at more advanced stages of the disease. Finally, the abundance of genes belonging to T-
228 cell pathways (TCR, iCOS-iCOSL signaling) or mTOR and eIF2 signaling was lower in group
229 3, that is to say, those who were analyzed after a longer time from symptom onset to the
230 admission. The findings described above highlight the heterogeneity of COVID-19 patients.

231 **Integrative analysis of all biomarkers reveals the major contribution of CD177 in the** 232 **clinical outcome of COVID-19 patients.**

233 We performed an integrative analysis using all available data to disentangle the relative
234 contribution of the various markers at the scale of every patient. We thus pooled the data for
235 29,302 genes from whole blood RNA-seq, cell phenotypes (52 types), and cytokines (71
236 analytes) using the recently described MOFA approach (Argelaguet et al., 2019), which is a
237 statistical framework for dimension reduction adapted to the multi-omics context. The data
238 are reduced to components that are linear combinations of variables explaining inter-patient
239 variability across the three biological measurement modalities. The first component, that we
240 called our integrative score, discriminated between the three groups of COVID-19 patients

241 (initially defined by hierarchical classification based on gene abundance only) and HDs
242 (Figure 5A), although it only explained a portion of the variability within each of the three
243 types of markers (14% of gene expression, 14% of cell phenotypes, and 5% of cytokines).
244 Figures 5 B-D showed the contribution of each type of markers to the integrative score. The
245 main contributors for the cell phenotype were the significantly lower frequency of cDC2 and T
246 cells and, marginally, the higher number of plasmablasts and CD16⁺ γδ T cells in COVID-19
247 patients (Figure 5B). The contribution of soluble factors was marked by higher levels of
248 soluble CD163 (sCD163), a marker of polarized M2 macrophages involved in tissue repair
249 (Zhi et al., 2017), in more advanced COVID-19 groups (Figure 5C). Indeed, CD163 gene
250 expression was also significantly higher in COVID-19 patients than HDs (log₂ fold change =
251 +1.55; FDR = 4.79 10⁻²). sCD163 has also been reported to be a marker of disease severity
252 in critically ill patients with various inflammatory or infectious conditions (Buechler et al.,
253 2013). Interestingly, the genes that contribute the most to the synthesis of this factor were
254 part of the neutrophil module (*CD177*, *ARG1*, *MMP9*) (Figure 5D). Integrated analysis also
255 revealed higher expression of proprotein convertase subtilisin/kexin type 9 (*PCSK9*). High
256 plasma PCSK9 protein levels highly correlate with the development and aggravation of
257 subsequent multiple organ failure during sepsis (Boyd et al., 2016; Dwivedi et al., 2016). Of
258 note, high PCSK9 levels have been recently associated with severe Dengue infection (Gan
259 et al., 2020). Finally, the increasing abundance of CD177 gene expression according to
260 group was again clearly apparent (Figure S4). An additional cell-type specific significance
261 analysis (csSAM) has been performed to check the robustness of the CD177 differential
262 expression according to the cell-type frequencies (Shen-Orr et al., 2010). We found that
263 CD177 differential expression between COVID-19 and HD patients was not fully explained by
264 population variations. Indeed, it remained significant after deconvolution in several
265 leucocytes subpopulations (notably FDR of 0.04 within T-cells and 0.03 within Monocytes).

266

267 **Serum CD177 protein levels are associated with the clinical outcome of COVID-19**
268 **patients.**

269 Given the contribution of the neutrophil activation pathway in the clustering of COVID-19
270 patients, we sought neutrophil-activation features that could act as possible reliable markers
271 of disease evolution. We focused on CD177 because: i) it is a neutrophil-specific marker
272 representative of neutrophil activation, ii) it was the most highly differentially expressed gene
273 in patients, and iii) the protein can be measured in the serum, making its use as a marker
274 clinically applicable. Thus, we used an ELISA to quantify CD177 in the serum of 203 COVID-
275 19 patients (115 patients from the French cohort and 88 patients from the Swiss COVID-19
276 cohort that we used as “a confirmatory” cohort, patient characteristics are described in Table
277 2), 21% of whom the measurements were repeated (from 2 to 10 measurements per
278 individual). First, we confirmed the significantly higher median serum protein level in the
279 global cohort of COVID-19 patients (4.5; [2.2-7.4]) relative to that of 16 HDs (2.2 [0.9-4.2]) (P
280 = 0.015, Wilcoxon test) (Figure 6A). Second, we found a robust agreement between CD177
281 gene expression measured by RNA-seq and CD177 protein levels measured by ELISA
282 (intraclass correlation coefficient 0.88), (Figure 6B).

283 Then, we examined the association of clinical characteristics and outcomes with serum
284 CD177 concentration at the time of admission. The serum CD177 concentration was
285 positively associated with the time from symptom onset to the admission ($r=0.22$, $P = 0.0026$)
286 (Figure 6C) and was higher for patients hospitalized in an ICU (6.0 ng/ml [3.5-9.4] vs 3.3
287 ng/ml [1.5-5.6], $P < 0.001$) (Figure 6D). The association between serum CD177 levels and
288 hospitalization in an ICU was independent of the usual risk factors, such as age, sex, chronic
289 cardiac or pulmonary diseases, or diabetes (multivariable logistic regression, adjusted odds
290 ratio 1.14 per unit increase, $P < 0.001$). We observed a trend towards a positive association
291 with the SOFA and SAPS2 risk scores that was not statistically significant ($P = 0.17$ and $P =$
292 0.074, respectively) (Figure S6A and B). CD177 levels were not associated with other
293 conditions that contribute to a high risk of severe disease, such as diabetes ($P = 0.632$),

294 chronic cardiac disease ($P = 0.833$), chronic pulmonary disease ($P = 0.478$), or age of the
295 patient ($P = 0.83$).

296 We then examined the dynamics of the CD177 concentration in 172 COVID-19 patients, with
297 longitudinal serum samples, using all available measurements (Figure 6E). At the first
298 measurement, the average concentration of CD177 was not significantly different between
299 the patients who died and those who recovered (5.93 vs 5.06, $P = 0.26$, Wald test). When
300 looking at the change of CD177 concentration over time, it appears clearly that the
301 concentration was decreasing in those who recovered (-0.22 ng/mL/day, 95% CI -0.307 ; $-$
302 0.139) whereas it was stable in those who died later on ($+0.10$ ng/mL/day, 95% CI -0.014 ;
303 $+0.192$). These results show that the stability of CD177 protein levels in severe COVID-19
304 patients during the course of the disease is a hallmark of a worse prognosis, leading to
305 death.

306 **Discussion**

307 Here, we investigated factors that influence the clinical outcomes of severe COVID-19
308 patients involved in a multicentric French cohort combining standardized whole-blood RNA-
309 Seq analyses, in-depth phenotypic analysis of immune cells, and measurements of a large
310 panel of serum analytes. An integrated and global overview of host markers revealed several
311 pathways associated with the course of COVID-19 disease, with a prominent role for
312 neutrophil activation. This signature included CD177, a specific neutrophil marker of
313 activation, adhesion to the endothelium, and transmigration. The correlation between *CD177*
314 gene abundance and serum protein levels in the blood of COVID-19 patients underscores
315 the importance of this marker, making the measurement of CD177 protein levels a reliable
316 approach that is largely accessible in routine care. We also demonstrated that the dynamics
317 of serum CD177 levels is strongly associated with the severity of COVID-19 disease, ICU
318 hospitalization, and survival in an additional cohort of patients. During follow-up, the CD177
319 protein levels decrease in patients who are recovering while staying high in those who died

320 CD177 is a glycosylphosphatidylinositol (GPI)-anchored protein expressed by a variable
321 proportion of human neutrophils. It plays a key role in the regulation of neutrophils by
322 modulating their migration and activation. For example, the CD177 molecule has been
323 identified as the most dysregulated parameter in purified neutrophils from septic shock
324 patients (Demaret et al., 2016) and in severe influenza (Tang et al., 2019). Clinically,
325 neutrophil chemotaxis, infiltration of endothelial cells, and extravasation into alveolar spaces
326 have been described in lung autopsies from deceased COVID-19 patients (Fu et al., 2020).
327 Elevated *CD177* mRNA expression has also been described for patients with acute
328 Kawasaki Disease (KD) (Huang et al., 2019) and resistant to IV Ig therapy (Jing et al., 2020;
329 Ko et al., 2019), a syndrome that has been described as a possible complication of SARS-
330 CoV-2 infection in children (Toubiana et al., 2020; Viner and Whittaker, 2020). Our results
331 are also consistent with those obtained using animal models, suggesting an important role
332 for neutrophil activation in the severity of infection with respiratory viruses through their

333 migration towards infected lungs, and in humans infected with influenza (Brandes et al.,
334 2013; Narasaraju et al., 2011; Zaas et al., 2009) .

335 The neutrophil activation signature is a specific feature of the homing of activated neutrophils
336 toward infected lung tissue in acute lung injury (Juss et al., 2016), followed by the initiation of
337 aggressive responses and the release of neutrophil extracellular traps (NETs), leading to an
338 oxidative burst and the initiation of thrombus formation (Darbousset et al., 2012). Previous
339 studies have reported elevated levels of circulating NETs in COVID-19 disease (Barnes et
340 al., 2020a). Consistent with this finding and extending these data, we showed the differential
341 expression of NET-related genes (Brandes et al., 2013; Narasaraju et al., 2011; Tang et al.,
342 2019) (*S100A8*, *S100A9*, and *S100A12*), confirming the recently described elevated
343 expression of calprotectin (heterodimer of *S100A8* and *A9*) in severe COVID-19 patients
344 (Silvin et al., 2020). The association of neutrophil activation signature with COVID-19 severity
345 has also been described recently with *CD177* gene being one of the most differentially
346 expressed gene in advanced disease (Aschenbrenner et al., 2021; Schulte-Schrepping et al.,
347 2020). Likely, we believe that our data extended significantly these recent observations
348 showing that *CD177* is increased both at the level of coding RNA and at the protein level.
349 Moreover, we show also that *CD177* is not only a marker of severity but also of death as
350 revealed by the longitudinal analysis which was confirmed in a second cohort.

351 Although, it is difficult to formally conclude whether *CD177* is a causal factor of disease
352 progression or a consequence of the severity of the disease, our data strongly show that
353 *CD177* is a valid hallmark of the physiopathology of COVID-19. This observation suggests
354 that the activation of neutrophils, triggered by the infection, is a fundamental element of the
355 innate response. However, persistent activation of this pathway may constitute, along with
356 other factors (e.g., “cytokine storm”), fatal harm possibly associated with the critical turning
357 point in the clinical trajectory of patients during the second week of the infection.

358 Neutrophil activation was associated with significant changes in the level of gene expression
359 of several pathways, some concordantly associated with disturbances in immune-cell

360 populations and cytokine and inflammatory profiles. We reveal a complex picture of the
361 activation of innate immunity, assessed by significant changes in the expression of several
362 genes involved in interferon signaling and the response to stress and the production of
363 inflammatory/activation markers, with a balance between pro-inflammatory signals
364 (increased expression of IL-1R1, IL-18R1 and its accessory chain IL-18 RAP) and anti-
365 inflammatory cytokines or regulators (increased expression of IL-10, IL-4R, IL-27, IL-1RN)
366 (Kim et al., 2005; Migliorini et al., 2020). The frequency of $\gamma\delta$ T cells, a subpopulation of
367 CD3⁺ T cells that were first described in the lung (Augustin et al., 1989) and that play critical
368 roles in anti-viral immune responses, tissue healing, and epithelial cell maintenance (Cheng
369 and Hu, 2017), was elevated and they expressed an activation marker (CD16) and low levels
370 of the inhibitory receptor NKG2A, suggesting possible killing capacity. In accordance with our
371 observation that eIF2 signaling is significantly inhibited in COVID-19 patients, recent studies
372 have shown that coronaviruses encode ISR antagonists, which act as competitive inhibitors
373 of eIF2 signaling (Rabouw et al., 2016; Rabouw et al., 2020). Similarly, the inhibition of
374 mTOR signaling that we found in COVID-19 patients is consistent with the impaired mTOR
375 signaling reported in blood myeloid dendritic cells of COVID-19 patients (Arunachalam et al.,
376 2020). Interestingly, we observed a markedly smaller proportion of all DC (pre-DC, pDC,
377 cDC1 and cDC2) and monocyte cell populations, including classical, intermediate, and non-
378 classical subpopulations, in COVID-19 patients than in HDs. Based on these observations, it
379 can be hypothesized that the impairment in IFN- α production observed in severe COVID-19
380 patients may be the result of both a decrease in the number of pDCs, which are natural IFN-
381 producing cells (Ali et al., 2019), and inhibition of mTOR signaling, a regulator of IFN- α
382 production, in these cells (Kaur et al., 2012).

383 We confirmed the previously reported expansion of B-cell populations (Arunachalam et al.,
384 2020; Bouadma et al., 2020) in COVID-19 patients and our results showed that the anti-
385 SARS-CoV-2 B cell repertoire is commonly mobilized. The marked upregulation of IGV gene
386 families included the *IgHV1-24* family, described to be specific for COVID-19 (Brouwer et al.,

387 2020). The expanded *VH4-39* family was also recently reported to be the most highly
388 represented in S-specific SARS-CoV-2 sequences (Brouwer et al., 2020). We also found
389 enrichment of *VH3-33*, previously described in a set of clonally related anti-SARS-CoV-2
390 receptor-binding domain antibodies (Barnes et al., 2020b).

391 Globally, these results show that the defense against SARS-CoV-2 following pathogen
392 recognition triggers a fine-tuned program that not only includes the production of antiviral
393 (Interferon signaling) and pro-inflammatory cytokines but also signals the cessation of the
394 response and a strong disturbance of adaptive immunity.

395 The same pathways (immune and stress responses through eIF2 signaling, neutrophil and
396 Interferon signaling, T- and B-cell receptor responses, and mTOR pathways) contributed to
397 the ability to discriminate between three groups of severe COVID-19 patients in an
398 unsupervised analysis. One limitation of our study was that we did not repeat the RNA-seq
399 analyses in these specific groups of patients. Nonetheless, it is noteworthy that these groups
400 differed significantly by the time from disease onset. These findings provide clues in our
401 understanding of the wide range of profiles previously described for COVID-19 by showing
402 that these patterns may be mainly related to time-dependent changes in the blood during the
403 course of the infection (Laing et al., 2020; Ong et al., 2020). For example, the observed lower
404 abundance of IFN signaling genes in the last group of COVID-19 patients may be due to
405 decreased abundance in more advanced disease and/or patients who constitutively present
406 a lower abundance of ISG, as described in previous studies (Bastard et al., 2020).

407 Several months after the emergence of this new disease, treatment options for patients with
408 severe disease requiring hospitalization are still limited to corticosteroids, which has emerged
409 as the treatment of choice for critically ill patients (Prescott and Rice, 2020; Sterne et al.,
410 2020). However, interventions that can be administered early during the course of infection to
411 prevent disease progression and longer-term complications are urgently needed. A major
412 obstacle for the design of “adapted “therapies to the various stages of disease evolution is a
413 lack of markers associated with sudden worsening of the disease of patients with moderate

414 to severe disease and markers to predict improvement. Our results show that the
415 measurement of CD177 during the course of the disease may be helpful in following the
416 response to treatment and revision of the prognosis. In addition, they suggest that therapies
417 aiming to control neutrophil activation and chemotaxis should be considered for the treatment
418 of hospitalized patients.

419 **Limitations of the study**

420 Healthy donors were collected from the French Blood Donors Organisation (Etablissement
421 Français du sang (EFS)) before the COVID-19 outbreak. Age to donate blood is limited
422 between 18 and 65 years (with the necessary medical agreement beyond 60 years). Due to
423 the age of hospitalized COVID-19 patients (median: 61 y.o), it was not possible to have age-
424 matched HD. So we could not exclude that differences observed between HD and COVID-19
425 patients could be associated to the age difference. Samples were not available for the entire
426 cohort of 61 patients for every experimentation but clinical characteristics were not different
427 between included and excluded patients for each assay, that is cell phenotype (all $p > 0.97$),
428 seric markers (all $p > 0.58$) and gene expression (all $p > 0.15$). Finally, although we have
429 identified a set of gene characterizing activation of a neutrophil pathway in patients, as well
430 as an association with increased serum CD177, a marker highly specific of neutrophils, we
431 did not study the phenotype of blood neutrophils from patients included in our cohorts for
432 practical reasons.

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444 interpretation, or writing of the manuscript.

445 **Author Contributions**

446 YL and AW conceived and designed the study. MC, JFT, YY, LB, DB, CLa, GP, and MP
447 participated in sample and clinical data collection. EF, MDe, MS, CLe, and PT performed the
448 experiments. MD, MG, BH, and CLe analyzed the data. YL, RT, AW, HH, MS, BJH, and CL
449 analyzed and interpreted the data. YL, RT, AW, and HH drafted the first version and wrote
450 the final version of the manuscript. All authors approved the final version.

451 **Conflict of interest statement**

452 None of the authors has any conflict of interest to declare.

453

454

455 **Figure legends**

456 **Figure 1. Frequency of immune-cell subsets between HD (n = 18) and COVID-19**
457 **patients (n = 50).** **A** Frequency of total CD3 T cells, CD4 and CD8 T-cell subsets, and
458 activated CD38⁺HLADR⁺ CD8 T cells. **B** Frequency of B-cell subsets (CD21⁺CD27⁺: resting
459 memory, CD21⁻CD27⁺: activated memory, CD21⁻CD27⁻: exhausted) and plasmablasts
460 (CD38⁺⁺CD27⁺) gated on CD19⁺ B cells. **C** Frequency of NK-cell subsets (gated on CD3⁻
461 CD14⁻) CD56^{Bright}: CD56⁺⁺CD16⁺, CD56^{dim}: CD56⁺CD16⁺⁺CD57^{+/-}, differentiated Ki67⁺ NK
462 cells (gated on CD56^{Bright} or CD56^{dim}CD57⁻ NK cells) and differentiated Ki67⁺ NKT cells
463 (gated on CD3⁺CD56⁺ cells). **D** Monocyte subsets (gated on CD3⁻CD56⁻) (classical
464 monocytes: CD14⁺CD16⁻, intermediate monocytes: CD16⁺CD14⁺, non-classical monocytes:
465 CD14⁻CD16⁺). **E** Frequency of $\gamma\delta$ T cells (gated on CD3⁺ T cells) and CD16 and NKG2A
466 expression (gated on $\gamma\delta$ CD3 T cells). **F** Frequency of DC subsets (gated on HLADR⁺Lin⁻)
467 (pDC: CD45RA⁺CD33⁻CD123⁺, pre-DC: CD123⁺CD45RA⁺, cDC1: CD33⁺CD123⁻
468 CD141⁺CD1c^{low}, cDC2: CD33⁺CD123⁻CD14⁺CD1c⁺) detected by flow cytometry in PBMCs
469 from n = 50 COVID19 patients and n = 18 HDs. The differences between the two groups
470 were evaluated using Wilcoxon rank sum statistical tests. The lower and upper boundaries of
471 the box represent the 25% and 75% percentiles, the whiskers extend to the most extreme
472 data point that is no more than 1.5 times the interquartile range away from the box. Median
473 values (horizontal line in the boxplot) are shown. See also Figure S1 and S2

474 **Figure 2. Heatmap of analyte abundance in serum.** The colors represent standardized
475 expression values centered around the mean, with variance equal to 1. HD: healthy donors
476 (n = 5), COVID: COVID19 patients (n = 33). Each column represents a subject. Each line
477 represents an analyte. See also Figure S3

478 **Figure 3. Whole blood gene expression in COVID-19 patients and HDs.** **A.** Volcano plot
479 showing differentially expressed genes (DEG) according to the log2 fold change (log2 FC)
480 and Benjamini-Hocberg False Discovery Rate (FDR) with thresholds at absolute log2 FC \geq
481 log2(1.5) and FDR \leq 0.05. **B** Main top DEG related to neutrophils. **C** and **D** Main DEG related

482 to *IFN* and interleukin responses, respectively. **E** Main *TCRV* T-cell repertoire DEG **F** Main
483 B-cell *IGHV* repertoire DEG. Red symbols represent overabundant genes in COVID-19
484 relative to HD, green symbols represent underabundant genes. See also Figure S4 and
485 Table S2

486 **Figure 4. Heatmap of standardized gene expression.** The colors represent standardized
487 expression values centered around 0, with variance equal to 1. Each column represents a
488 subject. This heatmap was built by unsupervised hierarchical clustering of log2-counts-per-
489 million RNA-seq transcriptomic data from whole blood (29,302 genes) and subjects (n = 54)
490 using the Euclidean distance and Ward's method. Seven blocks are highlighted according to
491 the features of gene expression across the groups of individuals. Enrichment (number and %
492 of genes of a given pathway selected in the block) of pathways of interest are shown for each
493 block. See also Table S1

494 **Figure 5. Integrative analysis.** Integrative analysis of the data of RNA-seq (29,302 genes)
495 from 44 COVID-19 patients, cell phenotype (52 types) from 45 COVID-19 patients, and
496 serum analytes (71 analytes) from 33 COVID-19 patients using a sparse principal component
497 analysis approach, MOFA v2. **A** Integrative score according to the patient groups defined by
498 the hierarchical clustering of the RNA-seq data. Top 10 marker contributions (according to
499 the weight from -1 to 1) of the cell phenotypes (**B**), serum analytes (**C**), and RNA-seq (**D**).
500 The integrative score corresponds to the first factor of the analysis and allows the ordering of
501 individuals along an axis centered at 0. Individuals with an opposite sign for the factor
502 therefore have opposite characteristics. See also Figure S5

503 **Figure 6: Distribution of the CD177 marker and association with clinical outcomes of**
504 **COVID-19 patients. A** Measurement of CD177 (ng/ml). HD: Healthy donors (n = 16),
505 COVID-19 patients (n = 203). The difference between the two groups was evaluated using
506 Wilcoxon rank sum statistical tests. The median values (horizontal line in the boxplot) are
507 shown. The lower and upper boundaries of the box represent the 25% and 75% percentiles.
508 **B** Correlation between normalized *CD177* values of gene expression measured by RNA-seq

509 and CD177 protein by ELISA (ng/ml) from 36 COVID-19 patients. The blue line represents
510 the linear regression line and the grey area the 95% prediction confidence interval. **C**
511 Association between CD177 serum concentration and time from symptom onset to the
512 admission (n = 192). This association was tested using Spearman correlation tests. The blue
513 line represents the linear regression line and the grey area the 95% confidence interval. **D**
514 Measurement of CD177 serum concentration in patients hospitalized in an intensive care unit
515 (ICU) or not (n = 196). Wilcoxon rank tests were used. The median values (horizontal line in
516 the boxplot) are shown. The lower and upper boundaries of the box represent the 25% and
517 75% percentiles. **E.** Change of CD177 concentration over time according to the occurrence
518 of death for 172 COVID-19 patients and a total of 248 measurements. Predictions were
519 calculated using a mixed effect models for longitudinal data. See also Figure S6
520
521

522 **STAR Methods**

523 **Resource availability**

524 ***Lead contact***

525 Further information and requests for resources should be directed to and will be fulfilled by
526 the lead contact, Yves Lévy (yves.levy@aphp.fr).

527 ***Materials availability***

528 No materials were newly generated for this paper.

529 ***Data and code availability***

530 RNA sequencing data that support the findings of this study have been deposited in Gene
531 Expression Omnibus (GEO) repository with the accession codes GSE171110. Further
532 information and requests for resources and reagents should be directed to and will be fulfilled
533 by the Lead Contact: Yves Lévy (yves.levy@aphp.fr)

534 **Experimental model and subject details**

535 ***Subjects***

536 We enrolled a subgroup of COVID19 patients of the prospective French COVID cohort in this
537 immunological study which is part of the cohort main objectives. Median age of COVID19
538 patients was 60 years [50-69], 80% were male. Ethics approval was given on February 5th
539 by the French Ethics Committee CPP-Ile-de-France VI (ID RCB: 2020-A00256-33). Eligible
540 patients were those who were hospitalized with virologically confirmed COVID-19. Briefly,
541 nasopharyngeal swabs were performed on the day of inclusion for SARS-CoV-2 testing
542 according to WHO or French National Health Agency guidelines. Viral loads were quantified
543 by real-time semi-quantitative reverse transcriptase polymerase chain reactions (RT-PCR)
544 using either the Charité WHO protocol (testing the E gene and RdRp) or the Pasteur institute
545 assay (testing the E gene and two other RdRp targets, IP2 and IP4). The study was
546 conducted with the understanding and the consent of each participant or its surrogate
547 covering the sampling, storage, and use of biological samples. The time from symptom onset
548 to the admission has been retrospectively collected by the interview of patients enrolled in

549 the national “French Covid-19 cohort”. The Swiss cohort was approved by the ethical
550 commission (CER-VD; Swiss ethics protocol ID: 2020-00620) and all subjects provided
551 written informed consent. Blood from healthy donors was collected from the French Blood
552 Donors Organization (Etablissement Français du sang (EFS)) before the COVID-19
553 outbreak. HD characteristics are shown in Table S3.

554 **Method details**

555 ***Quantification of serum analytes***

556 In total, 71 analytes were quantified in heat-inactivated serum samples by multiplex magnetic
557 bead assays or ELISA. Serum samples from five healthy donors were also assayed as
558 controls. The following kits were used according to the manufacturers’ recommendations:
559 LXSAHM-2 kits for CD163,ST2,CD14 and LBP (R&D Systems); the LXSAHM-19 kit for IL-21,
560 IL-23, IL-31, EGF, Flt-3 Ligand, Granzyme B, Granzyme A, IL-25, PD-L1/B7-H1, TGF- α ,
561 Aggrecan, 4-1BB/CD137, Fas, FasL,CCL-28, Chemerin, sCD40L, CXCL14, and Midkine
562 (R&D Systems); and the 48-Plex Bio-Plex Pro Human Cytokine screening kit for IL-1 β , IL-
563 1 α , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8 / CXCL8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A /
564 CTLA8, Basic FGF (FGF-2), Eotaxin / CCL11, G-CSF, GM-CSF, IFN- γ , IP-10/CXCL10,
565 MCP-1 /CCL2, MIP-1 α / CCL3, MIP-1 β / CCL4, PDGF-BB (PDGF-AB/BB), RANTES/CCL5,
566 TNF- α , VEGF (VEGF-A), IL-1a, IL-2Ra (IL-2R), IL-3, IL-12 (p40), IL-16, IL-18, CTACK /
567 CCL27, GRO-a /CXCL1 (GRO), HGF, IFN- α 2, LIF, MCP-3 / CCL7, M-CSF, MIF,
568 MIG/CXCL9, b-NGF, SCF, SCGF-b ,SDF-1 α ,TNF-b/LTA, and TRAIL (Bio-Rad). The data
569 were acquired using a Bio-Plex 200 system. Extrapolated concentrations were used and the
570 out-of-range values were entered at the highest or lowest extrapolated concentration. Values
571 were standardized for each cytokine across all displayed samples (centered around the
572 observed mean, with variance equal to 1). CD177 quantification was performed on non-
573 inactivated serum samples (diluted 1:2 or 1:10) using a Human CD177 ELISA Kit
574 (ThermoFisher Scientific), according to the manufacturer’s instructions.

575 ***Cell phenotyping***

576 Immune-cell phenotyping was performed using an LSR Fortessa 4-laser (488, 640, 561, and
577 405 nm) flow cytometer (BD Biosciences) and Diva software version 6.2. FlowJo software
578 version 9.9.6 (Tree Star Inc.) was used for data analysis. CD4⁺ and CD8⁺ T cells were
579 analyzed for CD45RA and CCR7 expression to identify the naive, memory, and effector cell
580 subsets for co-expression of activation (HLA-DR and CD38) and exhaustion/senescence
581 (CD57 and PD1) markers. CD19⁺ B-cell subsets were analyzed for the markers CD21 and
582 CD27. ASC (plasmablasts) were identified as CD19⁺ cells expressing CD38 and CD27. We
583 used CD16, CD56, and CD57 to identify NK-cell subsets. $\gamma\delta$ T cells were identified using an
584 anti-TCR $\gamma\delta$ antibody. HLA-DR, CD33, CD45RA, CD123, CD141, and CD1c were used to
585 identify dendritic cell (DC) subsets, as previously described (See et al., 2017). Extracellular
586 labelling was performed for all antibodies except for Ki 67 for which an intracellular labelling
587 was performed with the BD cytofix/cytoperm kit (BD Biosciences).

588 ***RNA sequencing***

589 Total RNA was purified from whole blood using the Tempus™ Spin RNA Isolation Kit
590 (ThermoFisher Scientific). RNA was quantified using the Quant-iT RiboGreen RNA Assay Kit
591 (Thermo Fisher Scientific) and quality control performed on a Bioanalyzer (Agilent). Globin
592 mRNA was depleted using GlobinClear Kit (Invitrogen) prior to mRNA library preparation with
593 the TruSeq® Stranded mRNA Kit, according to the Illumina protocol. Libraries were
594 sequenced on an Illumina HiSeq 2500 V4 system. Sequencing quality control was performed
595 using Sequence Analysis Viewer (SAV). FastQ files were generated on the Illumina
596 BaseSpace Sequence Hub. Transcript reads were aligned to the hg18 human reference
597 genome using Salmon v0.8.2 (Patro et al., 2017) and quantified relative to annotation model
598 "hsapiens_gene_ensembl" recovered from the R package biomaRt v2.42.1 (Durinck et al.,
599 2009). Quality control of the alignment was performed via MultiQC v1.4 (Patro et al., 2017).
600 Finally, counts were normalized as counts per million.

601 ***Quantification and statistical analysis***

602 Subgroups of COVID-19 patients were identified from unsupervised hierarchical clustering of
603 log₂-counts-per-million RNA-seq transcriptomics from whole-blood using the Euclidean
604 distance and Ward's method. Differential expression analysis was carried out using dearseq
605 (Gauthier et al., 2019) to contribute to the analysis of genes of which the abundance differed
606 across the three COVID-19 patient subgroups and healthy subjects. Once the groups were
607 defined by hierarchical clustering, the analysis of the genes contributing to each group was
608 performed by selecting genes with an absolute fold-change ≥ 1.5 in the comparison of
609 interest for which the difference in expression between HDs and COVID-19 patients was
610 significant ($P \leq 0.05$) (to avoid so called "double dipping" [<https://arxiv.org/abs/2012.02936>]).
611 Pathway analyses of the genes involved in each comparison was performed using Ingenuity
612 Pathway Analysis (IPA ®, Qiagen, Redwood City, California, Version 57662101, 2020). For
613 canonical pathway analysis, a Z-score ≥ 2 was defined as the threshold for significant
614 activation, whereas a Z-score ≤ -2 was defined as the threshold for significant inhibition.

615 The integrative analysis of the three types of biological data (RNA-Seq, cell phenotypes,
616 serum analytes) was performed using MOFA+ (Argelaguet et al., 2019) (, a sparse Factor
617 Analysis method. It provides latent variables which are linear combination of the most
618 influential factors for explaining inter-patient variability across the three biological
619 measurement modalities. The first component is presented and called integrative score here.
620 The analyses of factors associated with CD177 protein concentration were performed using
621 non parametric Wilcoxon test or Spearman correlation coefficient when appropriate. To look
622 at the independent association of CD177 with ICU, a logistic regression for the prediction of
623 hospitalization in ICU adjusted for age, sex, chronic cardiac disease, chronic pulmonary
624 disease, diabetes was fitted. The analysis of repeated measurements of CD177 over time
625 was done by using a linear mixed effect model adjusted for time from hospitalization and an
626 interaction with survival outcome (death or recovery). The model included a random intercept
627 and a random slope with an unstructured matrix for variance parameters. Predictions of

628 marginal trajectories were performed. All analyses, if not stated otherwise, were performed
 629 using R software version 3.6.3 (R Core Team (2020)). R: A language and environment for
 630 statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL:
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632 **Additional Ressources**

633 A subgroup of COVID19 patients of the prospective French COVID cohort was enrolled in
 634 this study. French COVID cohort was registered at:

635 <https://clinicaltrials.gov/ct2/show/NCT04262921> **Table 1. Patient characteristics of the**

636 **French COVID cohort (n=61)**

	Number of patients	
Demographic characteristics		
Age - Median (IQR) - years	61	60 (50 - 69)
Male sex – No./total No. (%)	61	49/61 (80)
ICU or transfer to ICU or death – No./total No. (%)	61	53/61 (87)
Outcome – No./total No. (%)	61	
Death		21/61 (34)
Discharge alive		40/61 (66)
Median interval from first symptoms on admission (IQR)-days	61	11 (7 - 14)
Comorbidities – No./total No. (%)		
Any	61	14/61 (23)
Chronic cardiac disease	61	9/61 (15)
Hypertension	61	22/61 (36)
Chronic pulmonary disease	61	5/61 (8)

Asthma	61	4/61 (7)
Chronic kidney disease	61	6/61 (10)
Chronic neurological disorder	61	2/61 (3)
Obesity	60	23/60 (38)
Diabetes	61	12/61 (20)
Smoking History – No./total No. (%)		
Smoking	61	5/61 (8)
Laboratory findings on admission - Median (IQR)		
Hemoglobin - g/dL	57	13 (11 - 14)
WBC count - x10 ⁹ /L	57	6 (5 - 9)
Platelet count - x10 ⁹ /L	57	189 (143 - 270)
C-reactive protein (CRP) - mg/L	57	120 (66 - 195)
Blood Urea Nitrogen (urea) - mmol/L	57	7 (5 - 12)
Symptoms on admission - No./total No. (%)		
Fever	59	51/59 (86)
Cough	57	40/57 (70)
Sore throat	56	4/56 (7)
Wheezing	54	6/54 (11)
Myalgia	56	21/56 (38)
Arthralgia	55	9/55 (16)
Fatigue	57	27/57 (47)
Dyspnea	57	46/57 (81)
Headache	57	11/57 (19)
Altered consciousness	56	3/56 (5)
Abdominal pain	53	8/53 (15)
Vomiting / nausea	56	10/56 (18)

Diarrhea	56	11/56 (20)
Clinical characteristics on admission - Median (IQR)		
SOFA score (ICU patients)	34	6 (4 - 8)
SAPS2 (ICU patients)	36	32 (27 - 53)
Heart rate - beats per minute	61	87 (76 - 104)
Respiratory rate - breaths per minute	55	24 (20 - 32)
Systolic blood pressure - mmHg	60	130 (109 - 145)
Diastolic blood pressure - mmHg	60	77 (70 - 87)
Oxygen saturation - percent	61	96 (91 - 98)
Oxygen saturation on – No./total No. (%)	56	
Room air		17/56 (30)
Oxygen therapy		39/56 (70)
Treatments – No./total No. (%)		
Antiviral	60	40/60 (66)
Antibiotic	60	46/60 (77)
Corticosteroids	60	33/60 (55)
Antifungal	60	9/60 (15)
Hydroxychloroquine	59	8/59 (14)

637

638

639 **Table 2. Characteristics of patients involved in the CD177 analysis**

	Number of patients	French cohort (n = 115)	Swiss cohort (n = 88)
Demographic characteristics			
Age - Median (IQR) - years	200	62 (54-72)	63 (57-74)
Male sex - No./total No. (%)	201	82/113 (73)	56/88 (64)
ICU or transfer to ICU or death - No. /total No. (%)	200	61/112 (54)	40/88 (45)
Outcome - No. /total No. (%)	173		
Death		32/107 (30)	8/66 (12)
Discharge alive		75/107 (70)	58/66 (88)
Median interval from first symptoms on admission (IQT)	192	13 (9-18)	12 (9-17)
Comorbidities - No./total No. (%)			
Chronic cardiac disease	197	22/109 (20)	25/88 (28)
Chronic pulmonary disease	197	14/109 (13)	9/88 (10)
Diabetes	197	23/109 (21)	26/88 (30)
Laboratory findings on admission - Median (IQR)			
C-reactive protein (CRP) - mg/L	34	122 (62-196)	
Lactate dehydrogenase (LDH) UI/L	31	466 (337-533)	
Clinical characteristics on admission - Median (IQR)			
Score SOFA	41	4 (2-7)	
Score SAPS2	40	32 (27-49)	

640

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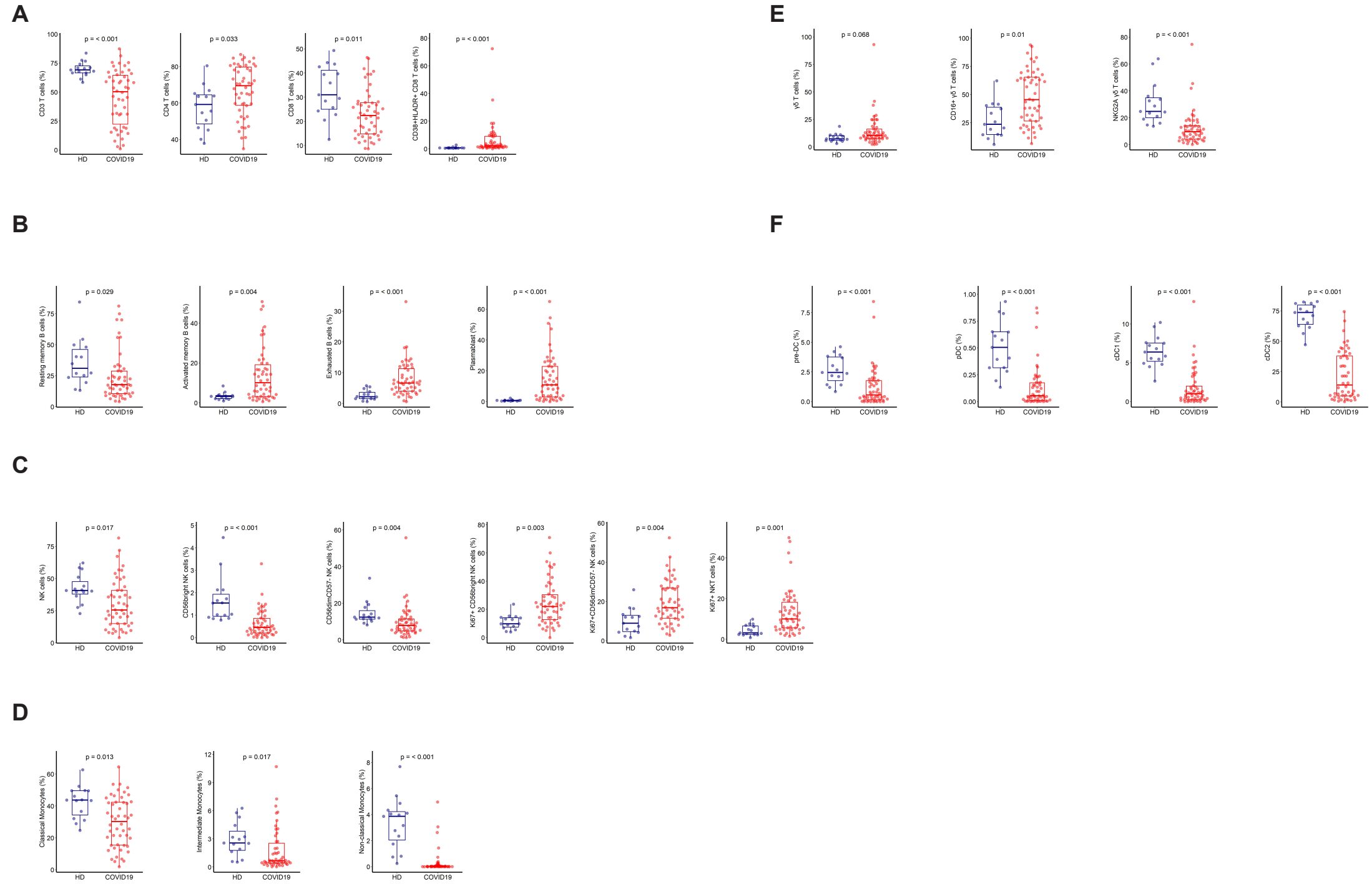
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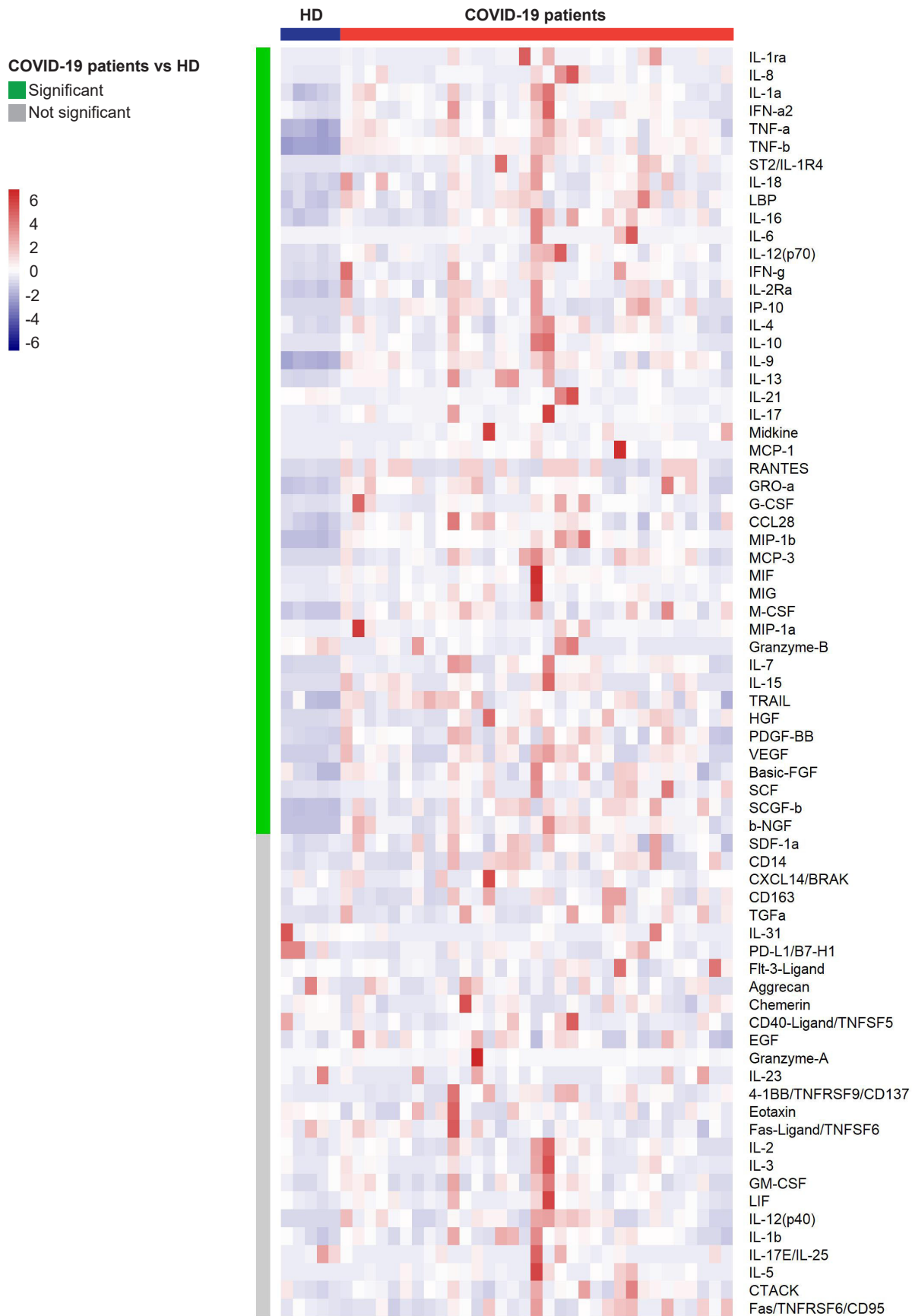
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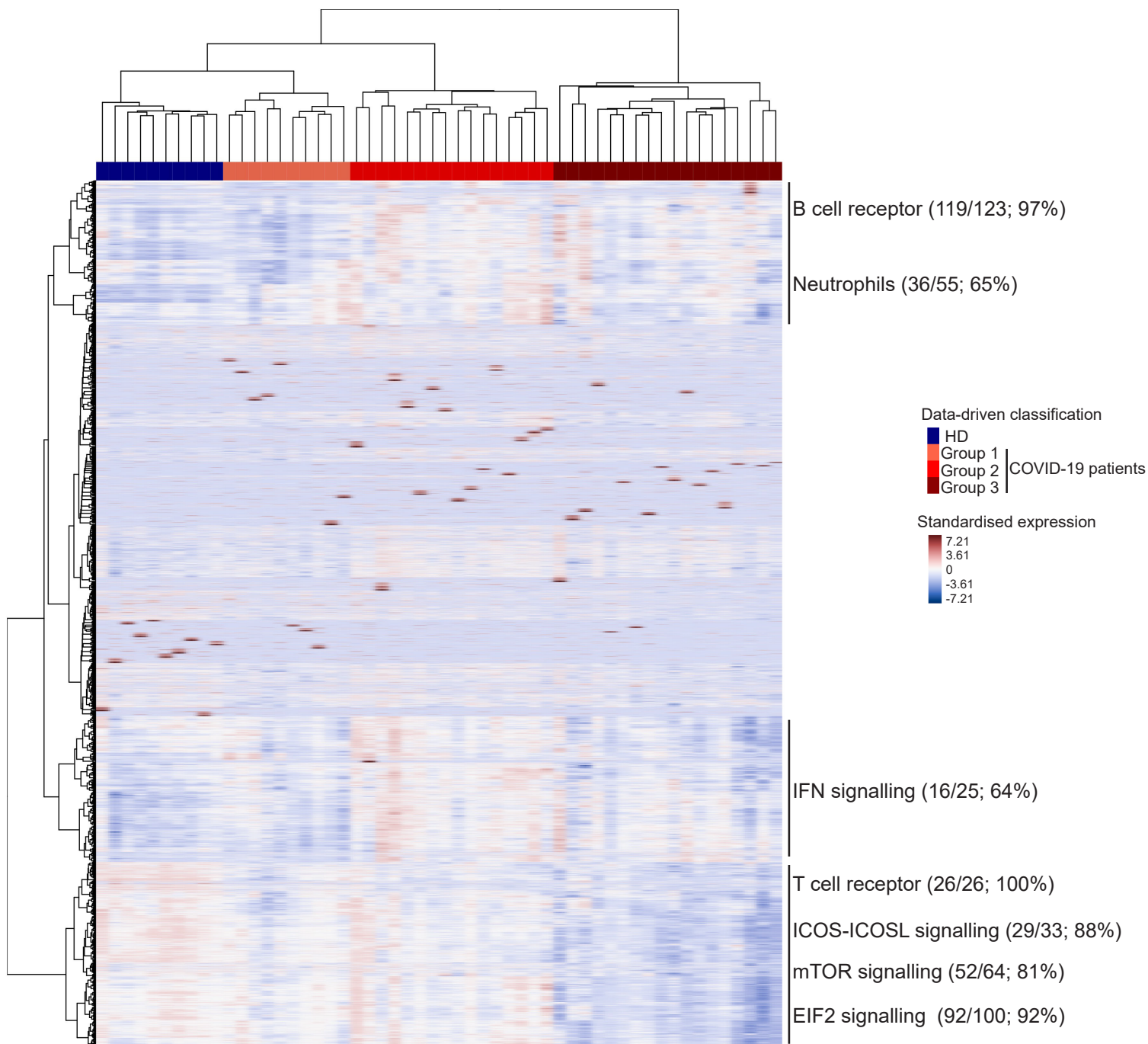
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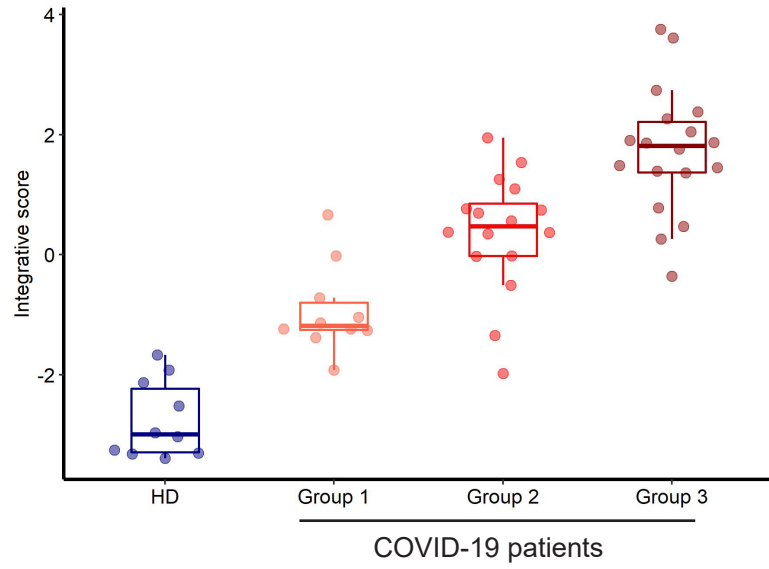
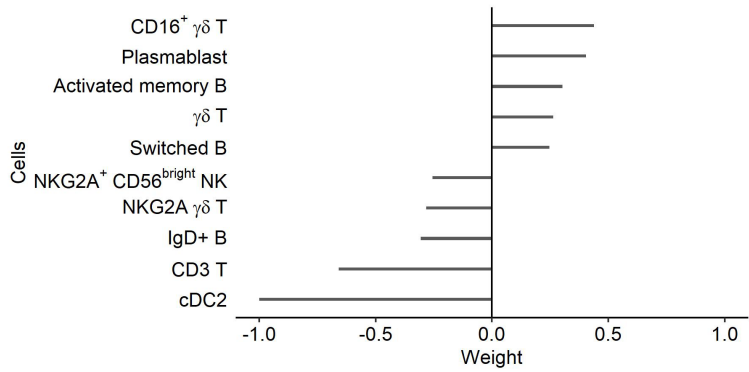
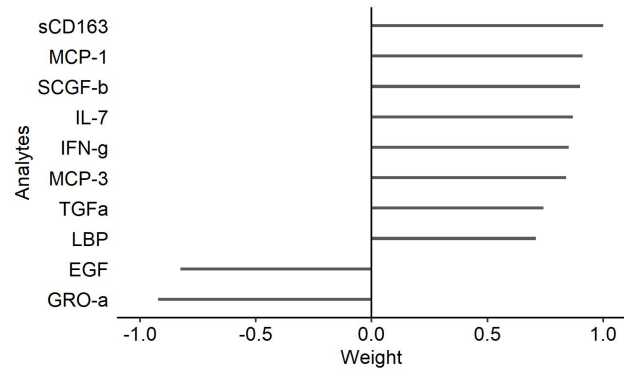
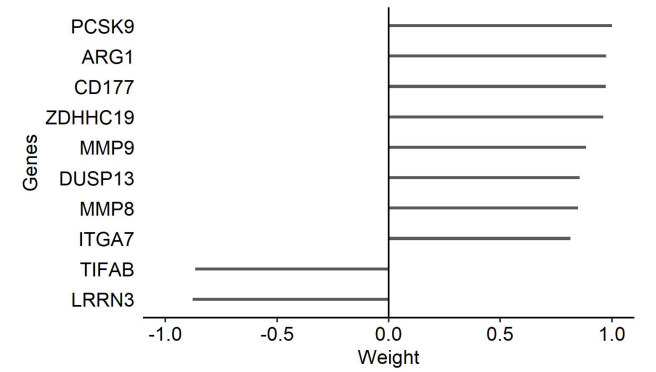
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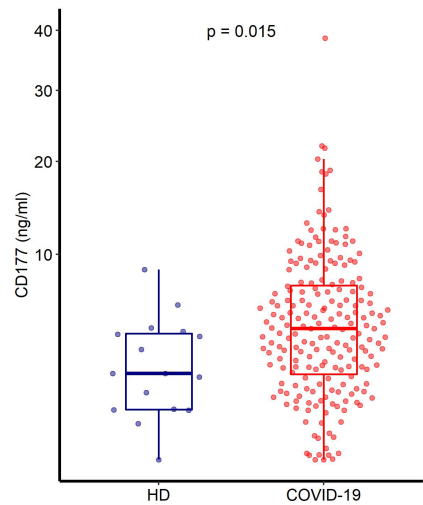
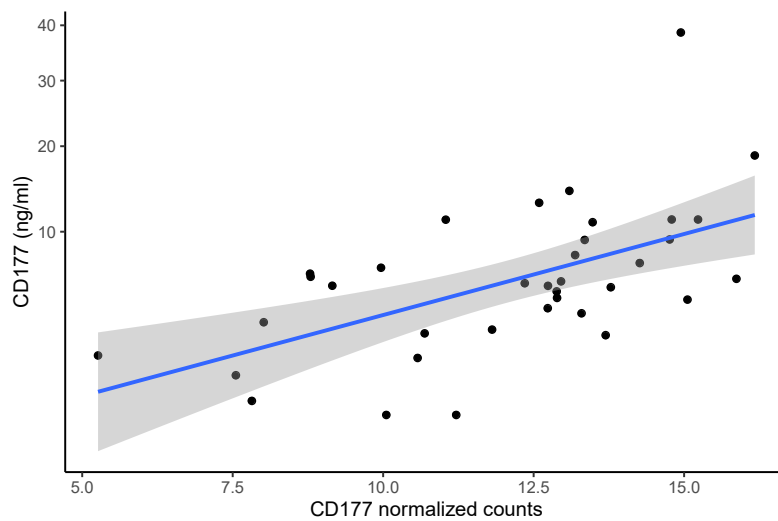
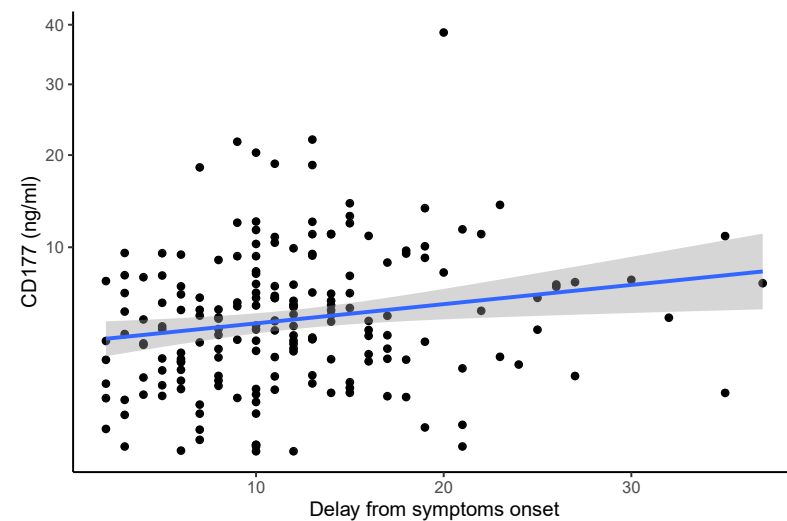
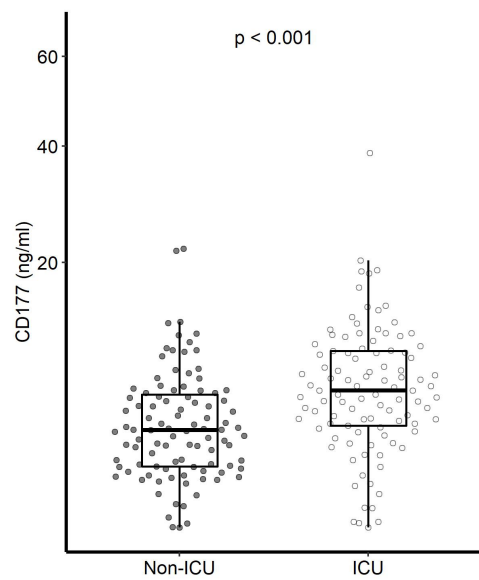
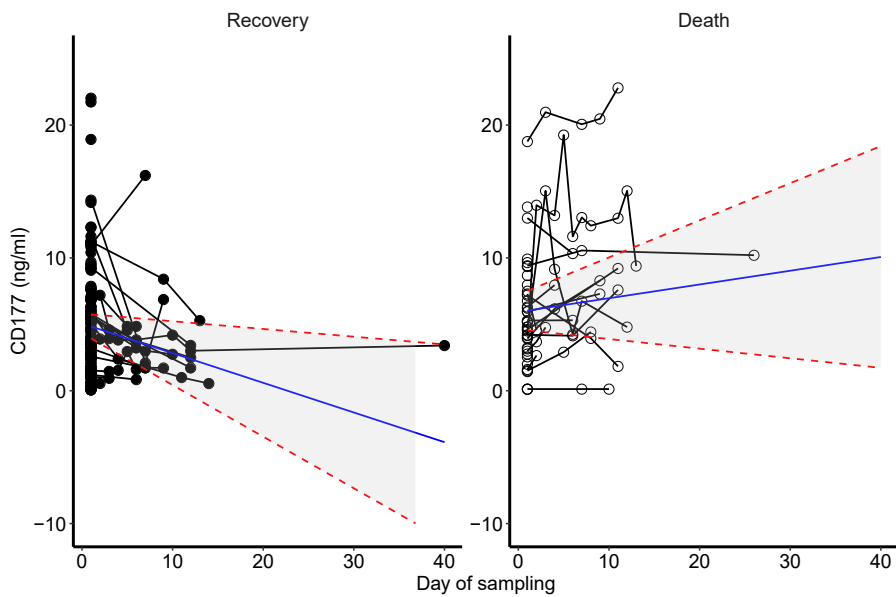
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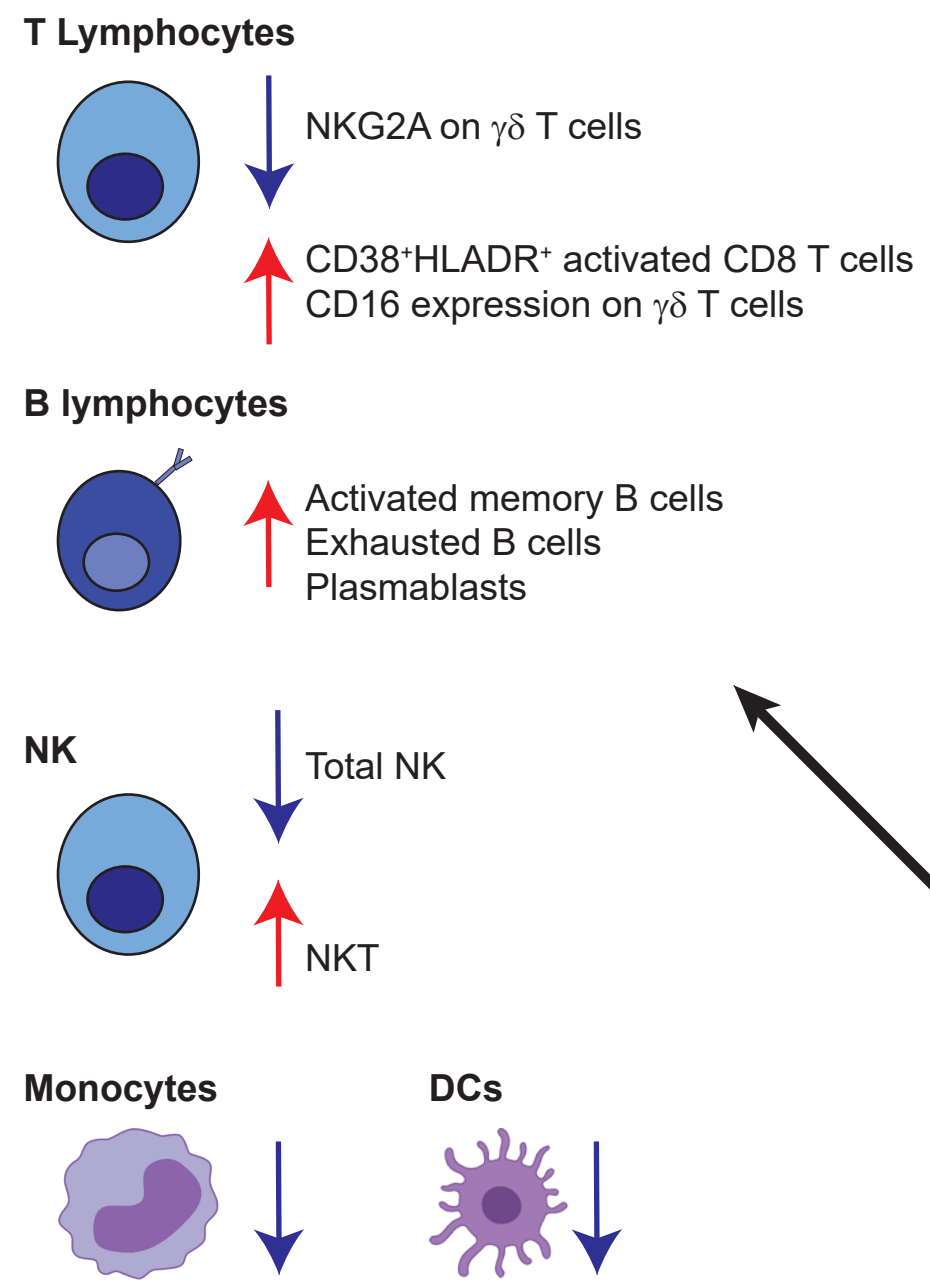




A**B****C****D**

A**B****C****D****E**

Phenotype, cytokine and inflammatory profiles



Whole blood gene expression

