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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 $\beta$ , 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<sup>+</sup> T  
123 cells and CD8<sup>+</sup> T cells relative to HDs, as previously reported (De Biasi et al., 2020; Xu et al.,  
124 2020), that expressed an activated phenotype (CD38<sup>+</sup>HLA-DR<sup>+</sup>) (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<sup>bright</sup> and  
130 CD56<sup>dim</sup>CD57<sup>-</sup> 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<sup>bright</sup>: 22% [13-30], CD56<sup>dim</sup>CD57<sup>-</sup>:

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- $\alpha$  and  $\beta$  (TNF- $\alpha$ ,  
152 TNF- $\beta$ ), IL-1ra, ST2/IL-1R4, the acute phase protein lipopolysaccharide binding protein LBP,  
153 IFN- $\alpha$ 2); Th1 pathway factors (IL-12 (p70), IFN- $\gamma$ , 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  $\gamma$

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  $\geq 1.5$ , 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 $\theta$  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 ( $\alpha$ ,  $\beta$ ), 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<sup>+</sup> 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 (log<sub>2</sub> fold change =  
251 +1.55; FDR = 4.79 10<sup>-2</sup>). 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<sup>+</sup> 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- $\alpha$  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- $\alpha$   
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<sup>+</sup>HLADR<sup>+</sup> CD8 T cells. **B** Frequency of B-cell subsets (CD21<sup>+</sup>CD27<sup>+</sup>: resting  
459 memory, CD21<sup>-</sup>CD27<sup>+</sup>: activated memory, CD21<sup>-</sup>CD27<sup>-</sup>: exhausted) and plasmablasts  
460 (CD38<sup>++</sup>CD27<sup>+</sup>) gated on CD19<sup>+</sup> B cells. **C** Frequency of NK-cell subsets (gated on CD3<sup>-</sup>  
461 CD14<sup>-</sup>) CD56<sup>Bright</sup>: CD56<sup>++</sup>CD16<sup>+</sup>, CD56<sup>dim</sup>: CD56<sup>+</sup>CD16<sup>++</sup>CD57<sup>+/-</sup>, differentiated Ki67<sup>+</sup> NK  
462 cells (gated on CD56<sup>Bright</sup> or CD56<sup>dim</sup>CD57<sup>-</sup> NK cells) and differentiated Ki67<sup>+</sup> NKT cells  
463 (gated on CD3<sup>+</sup>CD56<sup>+</sup> cells). **D** Monocyte subsets (gated on CD3<sup>-</sup>CD56<sup>-</sup>) (classical  
464 monocytes: CD14<sup>+</sup>CD16<sup>-</sup>, intermediate monocytes: CD16<sup>+</sup>CD14<sup>+</sup>, non-classical monocytes:  
465 CD14<sup>-</sup>CD16<sup>+</sup>). **E** Frequency of  $\gamma\delta$  T cells (gated on CD3<sup>+</sup> T cells) and CD16 and NKG2A  
466 expression (gated on  $\gamma\delta$  CD3 T cells). **F** Frequency of DC subsets (gated on HLADR<sup>+</sup>Lin<sup>-</sup>)  
467 (pDC: CD45RA<sup>+</sup>CD33<sup>-</sup>CD123<sup>+</sup>, pre-DC: CD123<sup>+</sup>CD45RA<sup>+</sup>, cDC1: CD33<sup>+</sup>CD123<sup>-</sup>  
468 CD141<sup>+</sup>CD1c<sup>low</sup>, cDC2: CD33<sup>+</sup>CD123<sup>-</sup>CD14<sup>+</sup>CD1c<sup>+</sup>) 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- $\alpha$ ,  
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 $\beta$ , IL-  
563 1 $\alpha$ , 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- $\gamma$ , IP-10/CXCL10,  
565 MCP-1 /CCL2, MIP-1 $\alpha$  / CCL3, MIP-1 $\beta$  / CCL4, PDGF-BB (PDGF-AB/BB), RANTES/CCL5,  
566 TNF- $\alpha$ , 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- $\alpha$ 2, LIF, MCP-3 / CCL7, M-CSF, MIF,  
568 MIG/CXCL9, b-NGF, SCF, SCGF-b ,SDF-1 $\alpha$  ,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<sup>+</sup> and CD8<sup>+</sup> 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<sup>+</sup> B-cell subsets were analyzed for the markers CD21 and  
582 CD27. ASC (plasmablasts) were identified as CD19<sup>+</sup> 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 log<sub>2</sub>-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  $\geq 1.5$  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  $\geq 2$  was defined as the threshold for significant  
614 activation, whereas a Z-score  $\leq -2$  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:  
 631 <https://www.R-project.org/>

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)**

	<b>Number of patients</b>	
<b>Demographic characteristics</b>		
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)
<b>Median interval from first symptoms on admission (IQR)-days</b>	61	11 (7 - 14)
<b>Comorbidities – No./total No. (%)</b>		
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)
<b>Smoking History – No./total No. (%)</b>		
Smoking	61	5/61 (8)
<b>Laboratory findings on admission - Median (IQR)</b>		
Hemoglobin - g/dL	57	13 (11 - 14)
WBC count - x10 <sup>9</sup> /L	57	6 (5 - 9)
Platelet count - x10 <sup>9</sup> /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)
<b>Symptoms on admission - No./total No. (%)</b>		
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)
<b>Clinical characteristics on admission - Median (IQR)</b>		
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)
<b>Treatments – No./total No. (%)</b>		
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)

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

	<b>Number of patients</b>	<b>French cohort (n = 115)</b>	<b>Swiss cohort (n = 88)</b>
<b>Demographic characteristics</b>			
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)
<b>Median interval from first symptoms on admission (IQT)</b>	192	13 (9-18)	12 (9-17)
<b>Comorbidities - No./total No. (%)</b>			
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)
<b>Laboratory findings on admission - Median (IQR)</b>			
C-reactive protein (CRP) - mg/L	34	122 (62-196)	
Lactate dehydrogenase (LDH) UI/L	31	466 (337-533)	
<b>Clinical characteristics on admission - Median (IQR)</b>			
Score SOFA	41	4 (2-7)	
Score SAPS2	40	32 (27-49)	

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641 **References**

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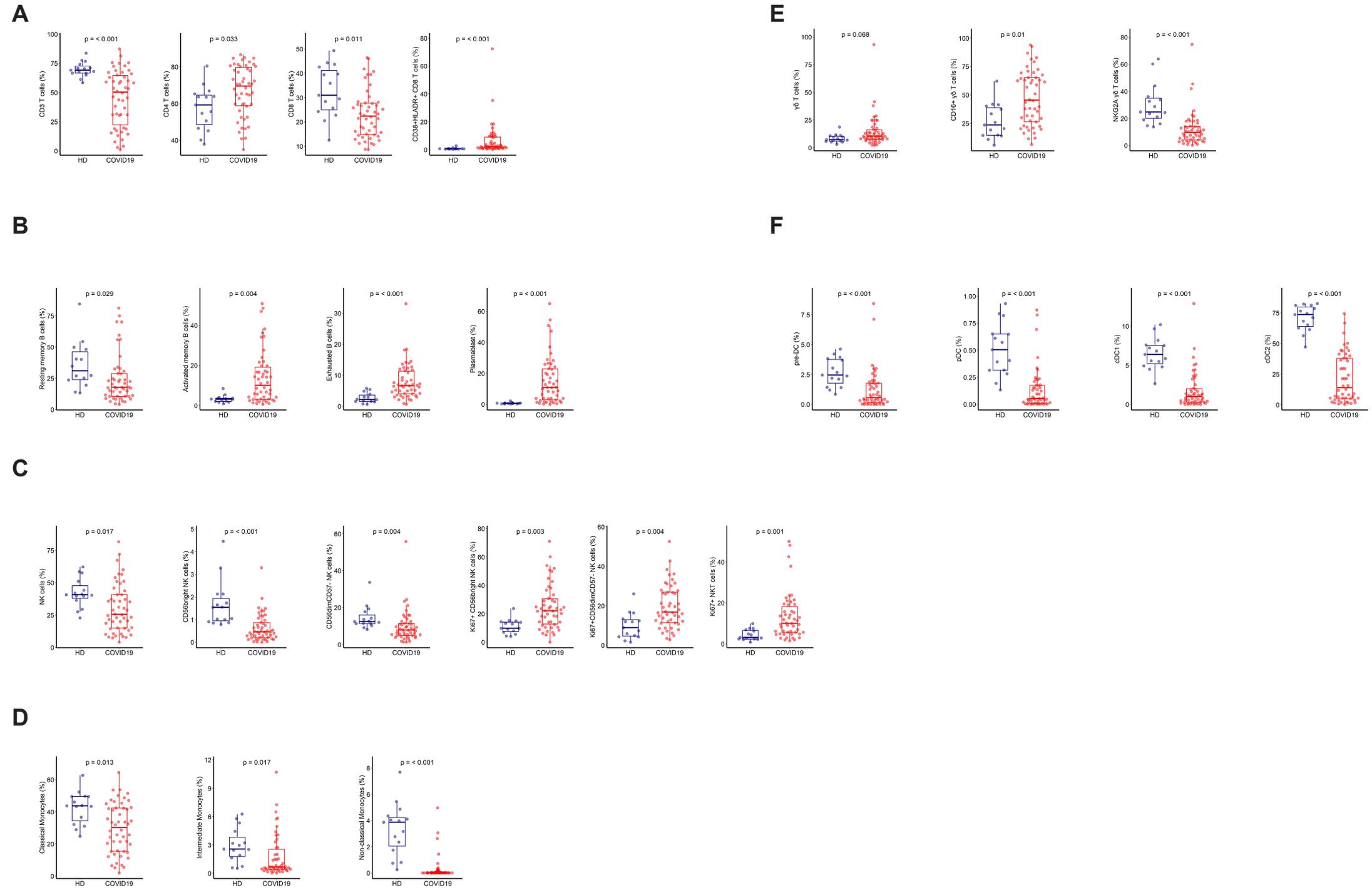
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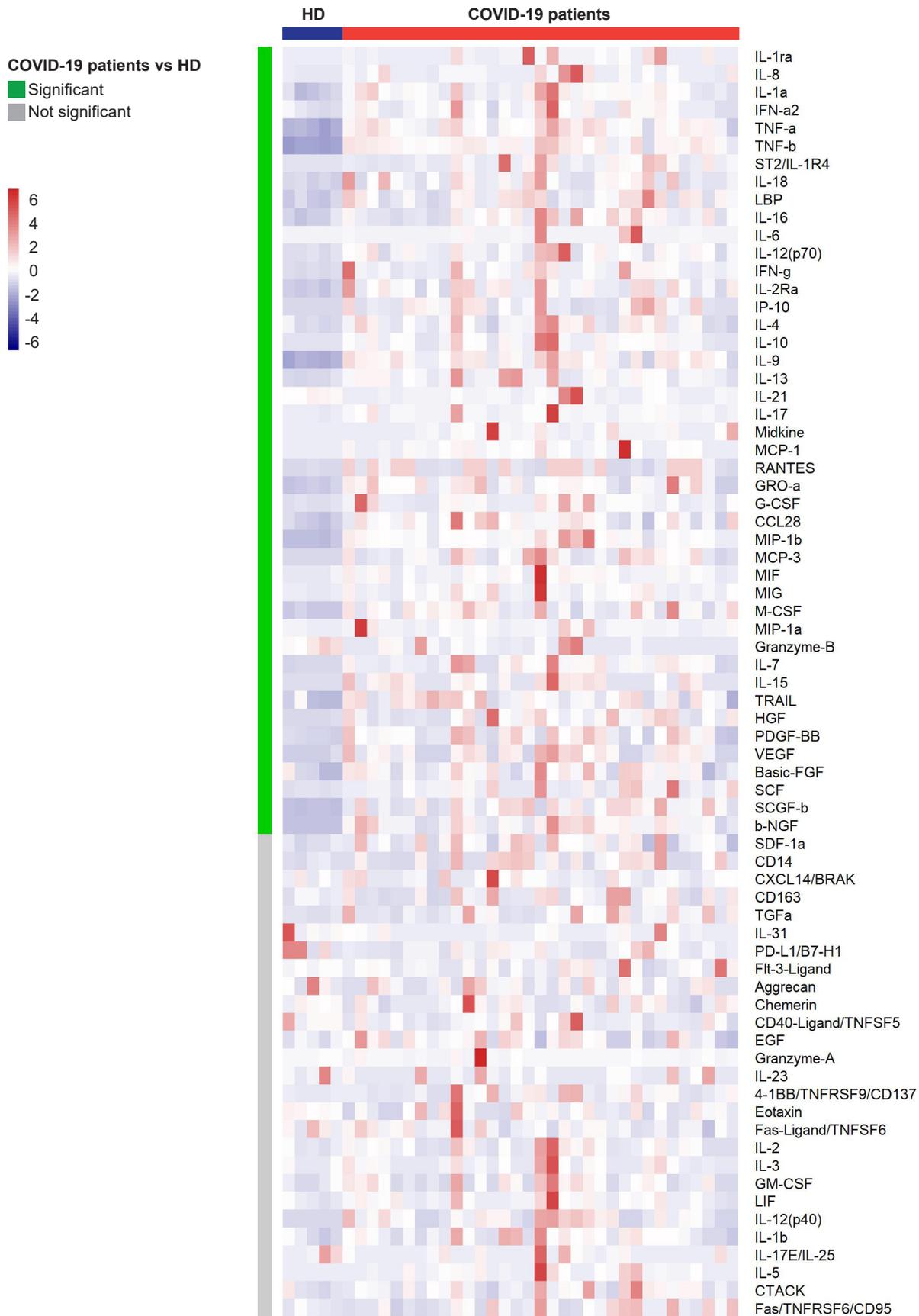
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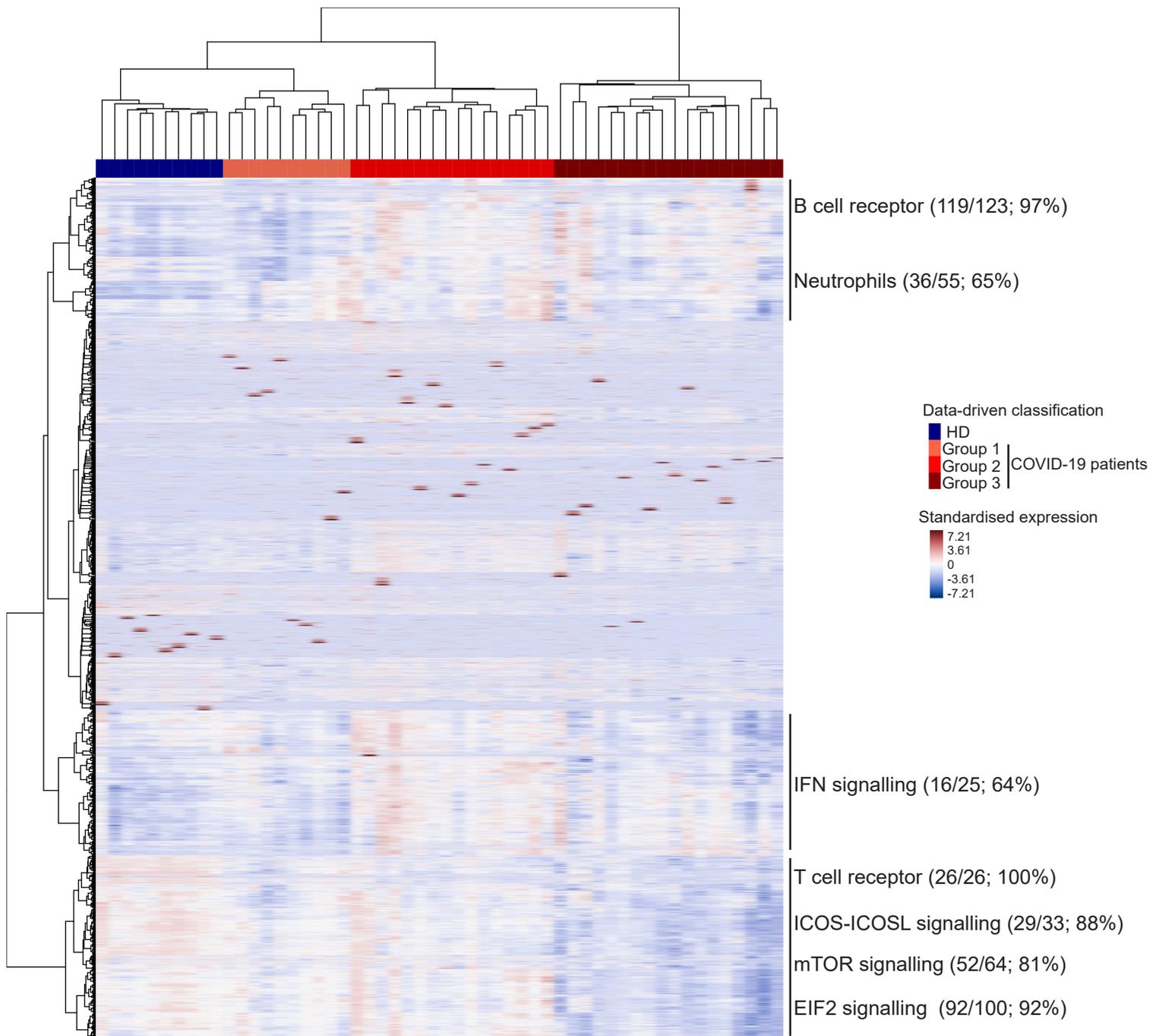
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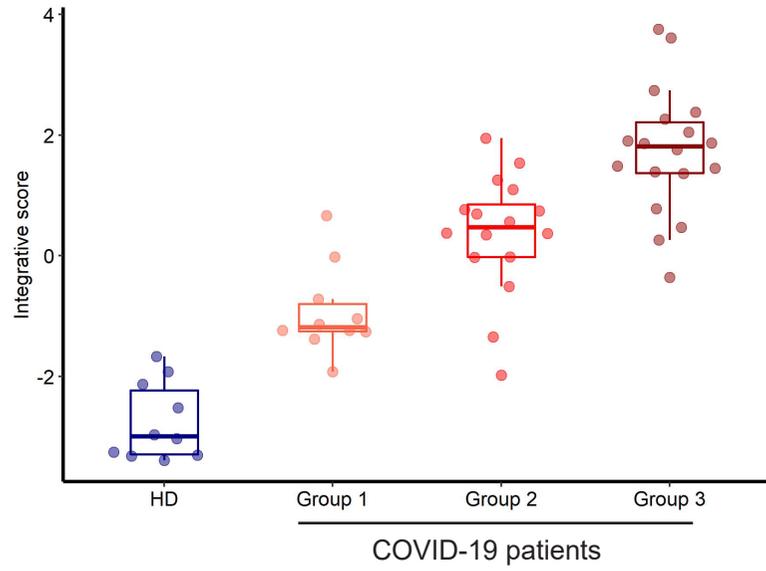
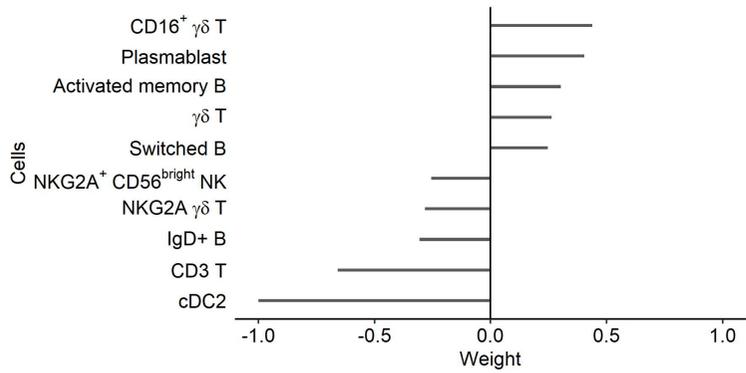
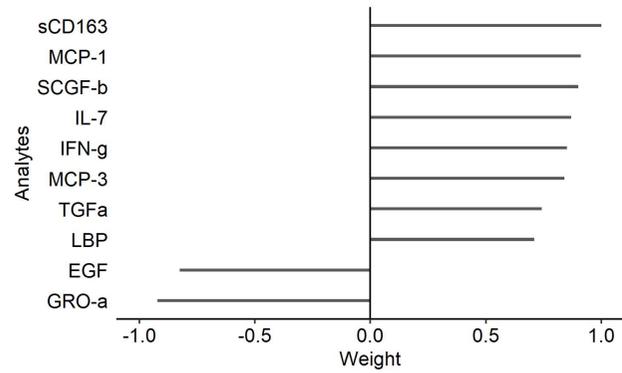
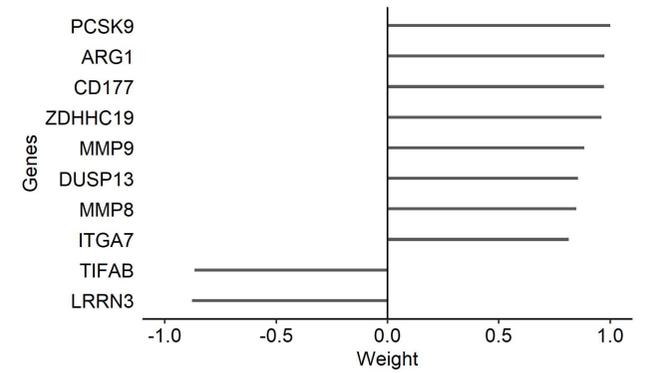
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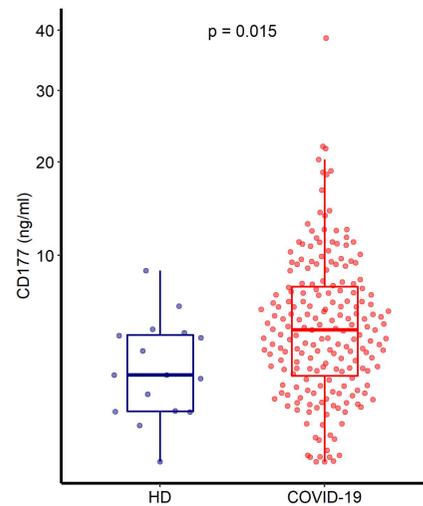
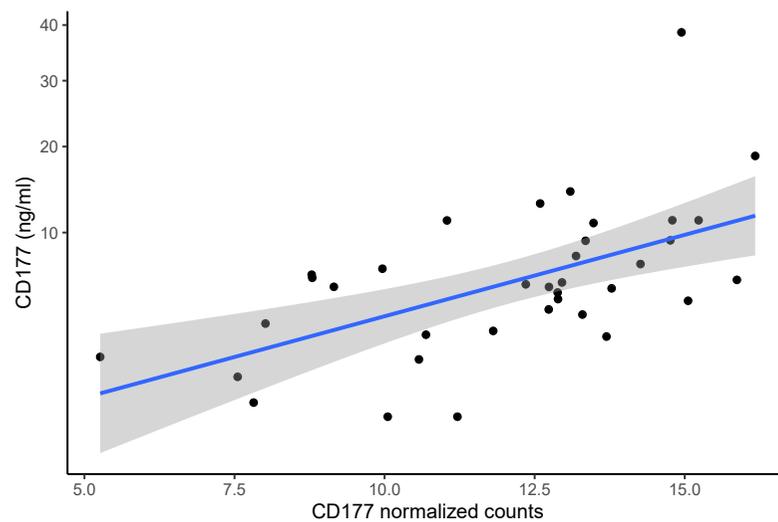
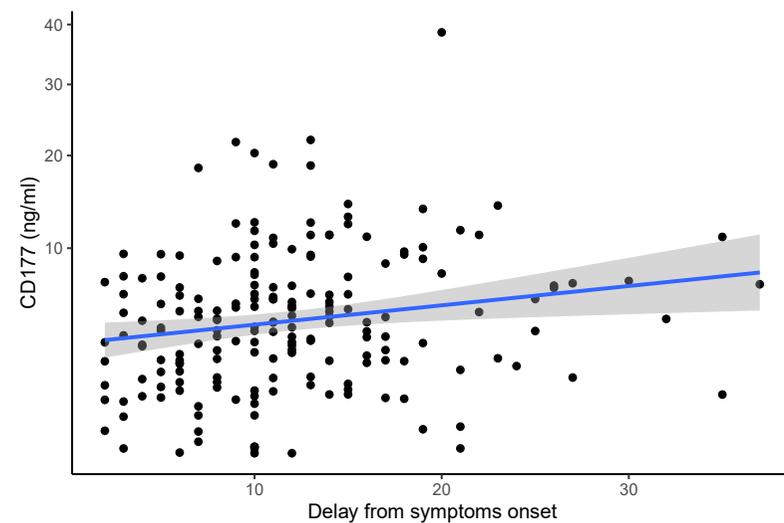
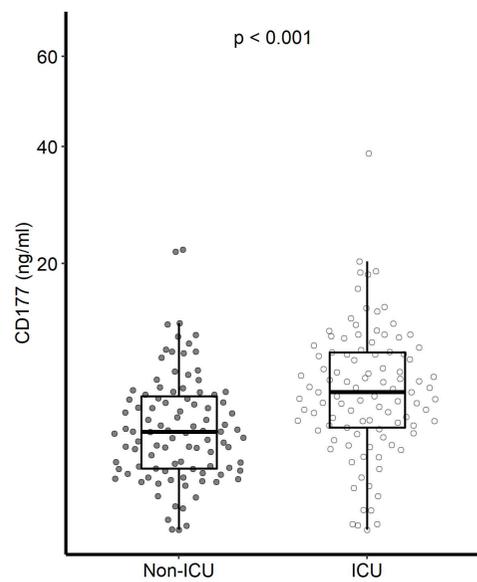
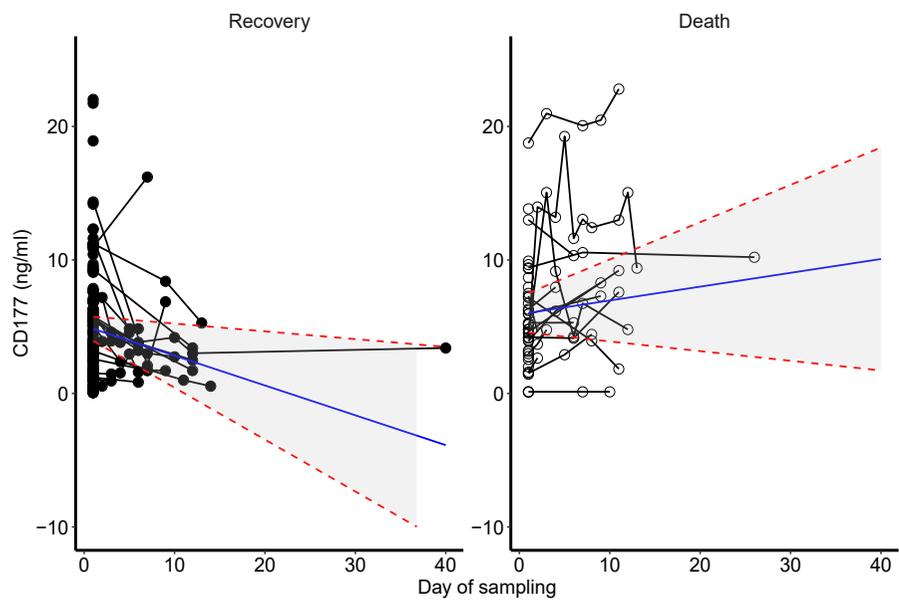




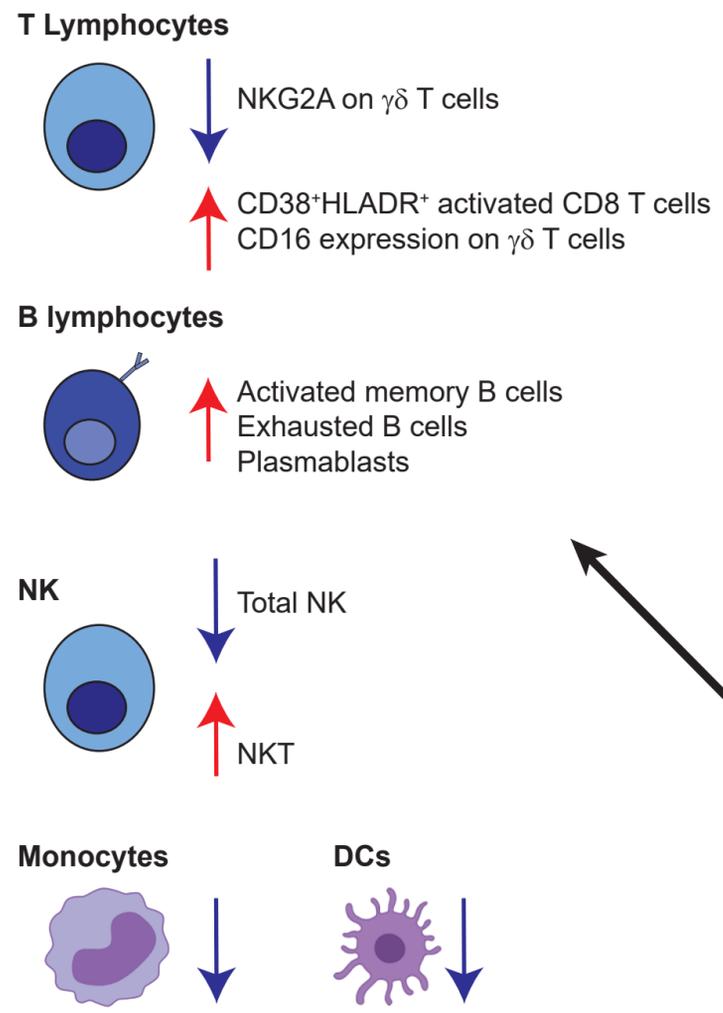




**A****B****C****D**

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

# Phenotype, cytokine and inflammatory profiles



# Whole blood gene expression

