

1 **Lasting alterations in monocyte and dendritic cell subsets in individuals after**  
2 **hospitalization for COVID-19**

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19 Running title: Long-term myeloid cell alterations following COVID-19

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24 **ABSTRACT**

25 After more than two years the COVID-19 pandemic continues to burden healthcare systems and  
26 economies worldwide, and it is evident that long-term effects of the disease can persist for months  
27 post-recovery in some individuals. The activity of myeloid cells such as monocytes and dendritic cells  
28 (DC) is essential for correct mobilization of the innate and adaptive responses to a pathogen. Impaired  
29 levels and responses of monocytes and DC to SARS-CoV-2 is likely to be a driving force behind the  
30 immune dysregulation that characterizes severe COVID-19. Here, we followed, for 6-7 months, a  
31 cohort of COVID-19 patients hospitalized during the early waves of the pandemic. The levels and  
32 phenotypes of circulating monocyte and DC subsets were assessed to determine both the early and  
33 long-term effects of the SARS-CoV-2 infection. We found increased monocyte levels that persisted for  
34 6-7 months, mostly attributed to elevated levels of classical monocytes. While most DC subsets  
35 recovered from an initial decrease, we found elevated levels of cDC2/cDC3 at the 6-7 month timepoint.  
36 Analysis of functional markers on monocytes and DC revealed sustained reduction in PD-L1 expression  
37 but increased CD86 expression across almost all cell types examined. Finally, viral load and CRP  
38 correlated to the appearance of circulating antibodies and levels of circulating DC and monocyte  
39 subsets, respectively. By elucidating some of the long-term effects that SARS-CoV-2 infection has on  
40 these key innate myeloid cells, we have shed more light on how the immune landscape remains  
41 affected in the months following severe COVID-19.

42

## 43 INTRODUCTION

44 Since coronavirus disease 2019 (COVID-19) was declared a pandemic on March 11 2020, great  
45 progress has been made in combating the disease. To date, more than ten vaccines have been  
46 developed and granted emergency use listing by the World Health Organization <sup>1</sup>. As of July  
47 2022, the mortality from the disease stands at 6.3 million deaths, with 550 million cumulative  
48 cases worldwide <sup>2</sup>, although with different standards of testing worldwide, the true human  
49 cost of the pandemic is likely to be much higher. The evolution of new variants indicate that  
50 the pandemic is still far from over.

51 The innate and adaptive immune responses during severe acute respiratory syndrome  
52 coronavirus 2 (SARS-CoV-2) infection have been extensively studied. Different pathways,  
53 markers, and factors of interest for drug targets or vaccine development have been identified  
54 and provide a better understanding of the impact of COVID-19 on the human host. While the  
55 various T cell responses in COVID-19 have been delineated <sup>3, 4</sup> there is still much unknown  
56 about other immune cells and their role in the disease.

57 The myeloid cell compartment consists of a variety of innate immune cell populations  
58 including dendritic cells (DC) and monocytes <sup>5</sup>. Monocytes can be divided into different  
59 subsets based on their expression levels of CD14 and CD16 <sup>6</sup>. Classical monocytes (cMo,  
60 CD14<sup>+</sup>CD16<sup>-</sup>) are highly phagocytic and can upregulate proteins associated with anti-bacterial  
61 activity, while non-classical monocytes (ncMo, CD14<sup>-/low</sup>CD16<sup>+</sup>) have a less inflammatory  
62 phenotype and are associated with wound healing <sup>7</sup>. Intermediate monocytes (iMo,  
63 CD14<sup>+</sup>CD16<sup>+</sup>) are not completely understood but display a capacity for both antigen  
64 presentation and inflammatory responses <sup>8,9</sup>. In addition to monocytes, there are also myeloid

65 derived suppressor cells (MDSC) that are strongly immunosuppressive, which increase in  
66 numbers in settings such as chronic infections and cancers <sup>10</sup>.

67 The pathogenic role of monocytes in respiratory viral infection has been demonstrated in  
68 mouse models of influenza A infection, where inflammatory monocytes drive the virus-  
69 induced damage to the lung <sup>11</sup>. Alterations to the monocyte compartment occur during SARS-  
70 CoV-2 infection, with most findings showing strong inflammatory monocyte responses in  
71 severe disease <sup>12, 13</sup>, whereas the opposite is found for mild disease <sup>14</sup>. Indeed, it has been  
72 shown that classical monocytes are drivers of the cytokine storm that frequently proves fatal  
73 in SARS-CoV-2 infection <sup>15</sup>.

74 The DC serve as a bridge between the innate and adaptive responses and are essential for the  
75 development of an adaptive response <sup>16</sup>. Like monocytes, DC can be classified into different  
76 subsets with the ability to sense pathogens, produce cytokines and activate T cell responses  
77 <sup>17</sup>. Plasmacytoid DC (pDC) are defined by surface expression of CD303 (BDCA-2) and are the  
78 main type I interferon (IFN) producers <sup>18, 19</sup>. Conventional DC (cDC) are further divided into  
79 different subsets, with cDC1 having a high capacity to cross-present antigens and activate T  
80 helper (Th)1 responses <sup>17, 20</sup>, while cDC2 activate a wider range of Th responses <sup>17</sup>. Recent work  
81 has divided cDC2 further, based on their CD5 expression, into a CD5<sup>high</sup> population mainly  
82 promoting Th1 responses, and a CD5<sup>low</sup> population promoting Th2, Th17, Th22, and T  
83 regulatory cell responses <sup>21</sup>. In addition, within the traditionally defined cDC2 population the  
84 most newly characterized populations are the two cDC3 populations <sup>17</sup>.

85 Functionality of monocyte and DC subsets can be defined by their expression of surface  
86 markers such as HLA-DR, CD86, CCR7 and PD-L1. In COVID-19 patients there is evidence of  
87 lower HLA-DR expression on DC subtypes, but not monocytes, indicating an impaired ability

88 to activate T cells. Increased PD-L1 expression is also indicative of an impairment of the  
89 effector function of DC <sup>22</sup>.

90 Studies of mild and severe COVID-19 patients have demonstrated an early overall decrease in  
91 circulating DC populations, particularly the pDC <sup>23, 24</sup>. Furthermore, in severe disease the  
92 different DC subsets are impaired in their ability to sense pathogens, present antigens, and  
93 stimulate T cell responses <sup>25-27</sup>. The importance of a functional DC compartment is illustrated  
94 by the correlation between the activation of early and effective T cell responses, and a positive  
95 clinical outcome in SARS-CoV-2 infection <sup>28,29</sup>. So far, there have been few longitudinal studies  
96 investigating the effects of COVID-19 on myeloid cell subsets during the disease and after  
97 recovery <sup>30</sup>, and more data is needed to provide better insight into the long-term effects of  
98 COVID-19.

99 Here, we have characterized the effects that severe COVID-19 exerts on circulating DC,  
100 monocytes, and MDSC, in patients that needed hospitalization, using spectral flow cytometry.  
101 Furthermore, the effects of several clinical markers on levels of myeloid cell subsets were  
102 assessed.

103 In accordance with previous studies, all circulating DC subsets were decreased at inclusion,  
104 during acute infection. While pDC and cDC1 subsequently returned to levels comparable to  
105 healthy controls, the cDC2 and cDC3 combined subset was significantly elevated at the 6-7  
106 month time point. We further found the frequencies of different monocyte subsets to be  
107 altered. An initial increase of cMo and decrease of iMo and ncMo was observed, and this  
108 profile was maintained long-term for cMo and iMo in comparison to healthy controls.  
109 Additionally, the MDSC compartment remained elevated, confirming a long-term  
110 immunosuppressive environment post-COVID-19. In addition, there was an alteration of

111 immunomodulatory markers, such as HLA-DR, CD86 and PD-L1, in all cell subsets. Together,  
112 our findings highlight sustained long-term alterations in monocyte and DC subsets after  
113 COVID-19, that could be linked to initial elevated circulating C-reactive protein (CRP) levels.

114

## 115 MATERIALS AND METHODS

### 116 Clinical data and study design

117 This study was approved by the Swedish Ethical Review Authority (Ethics No. 2020-02580).  
118 The hospitalized COVID-19 patients were included at the Clinic of Infectious Diseases and the  
119 Intensive Care Unit (ICU) at the Vrinnevi Hospital, Norrköping, Sweden. Furthermore, healthy  
120 COVID-19 negative controls were enrolled among the staff at the same hospital. For this study  
121 we used longitudinal samples from hospitalized COVID-19 patients (N=21; age range 32-83  
122 years) and samples from healthy controls (N=16; age range 23-80 years). Sample collection  
123 from COVID-19 patients was performed at four timepoints throughout the study: at enrolment  
124 when the COVID-19 patients were admitted to the Department of Infectious Diseases and  
125 after 2 weeks, 6 weeks, and 6-7 months post-enrolment. Both male and female participants  
126 were included, and all individuals had provided written informed consent prior to enrolment.  
127 This study was carried out in accordance with the International Conference on Harmonization  
128 Guidelines and the Declaration of Helsinki. Clinical data is described in **Table 1**.  
129 Data regarding the clinical symptoms related to COVID-19 and general health status were  
130 collected from all participants included in the study. The information is summarized in **Table**  
131 **1**. The severity of the disease was determined in the individuals hospitalized for COVID-19 as  
132 per the criteria defined by the National Institutes of Health <sup>31</sup>, i.e., estimated with regards to  
133 maximum oxygen needed, and highest level of care provided. COVID-19 severity was classified  
134 as: mild (admitted to pandemic department, no oxygen supplementation), moderate  
135 (admitted to pandemic department, oxygen supplementation <5L/min), severe (admitted to  
136 pandemic department or intermediate care unit, oxygen need ≥5L/min, supplemented by high

137 flow nasal oxygen therapy (HFNOT) and continuous positive airway pressure therapy (CPAP)  
138 and critical (intensive care unit, with or without mechanical ventilation).

### 139 **Sample collection and processing**

140 Blood samples were collected from study subjects in EDTA-treated Vacuette® tubes (Griener  
141 Bio-one GmbH, Kremsmünster, Austria) and peripheral blood mononuclear cells (PBMCs)  
142 were isolated by density gradient centrifugation (Ficoll-Paque, GE Healthcare, ThermoFisher).  
143 The PBMCs were washed and cryopreserved in freezing medium (8% DMSO in FBS) at -150°C  
144 until use. Sera were isolated from whole blood samples that had been collected in 3.5 ml  
145 Vacuette® tubes (Griener Bio-one GmbH, Kremsmünster, Austria), by centrifugation at 1000 g  
146 for 10 minutes at room temperature. Sera were frozen and stored at -80°C until testing.

### 147 **Spectral Flow cytometry**

148 Cryopreserved PBMCs were thawed and washed in RPMI before resuspension in buffer (0.2%  
149 FBS in PBS) at concentration of  $1 \times 10^6$  cells/ml in FACS tubes (Falcon® Brand, VWR). Before  
150 addition of the cocktail of 21 monoclonal antibodies, cells were blocked with a cocktail of FcγR  
151 (1/15 dilution, Miltenyi), Novablock (Phitonex), and stained with live/dead violet viability dye  
152 (**Supplementary Table 1 for the antibodies and spectral flow reagents**), for 20 mins at 4°C.  
153 After washing, 30 µl of antibody cocktail was added to the cells, and the mix incubated for 30  
154 minutes at 4°C. The stained PBMCs were washed and resuspended in 200 µl buffer and  
155 samples acquired using a Cytex Aurora (USA) spectral flow cytometer. Data was processed  
156 using OMIQ (California, USA).

157

158



159 **SARS-CoV-2 neutralizing antibodies**

160 To measure neutralizing antibodies, against the SARS-CoV-2, we utilized the TECO<sup>®</sup> SARS-CoV-  
161 2 Neutralization Antibody Assay (TECOmedical AG, Sissach, Switzerland). The assay was  
162 performed according to the protocol provided by the manufacturer. Briefly, serum samples  
163 were diluted in sample buffer (1:10, 1:30, and 1:90) and incubated at 37°C for 30 minutes in a  
164 96-well plate coated with ACE2 (provided in the kit). The plate was washed 3 times with  
165 diluted wash buffer (provided in the kit) and incubated with (S)-RBD–horseradish peroxidase  
166 conjugate for 15 minutes at 37°C. Finally, stop solution (provided in the kit) was added and  
167 optical density measured within 5 minutes at 450 nm (SpectraMax iD3 Molecular Devices,  
168 USA). The inhibition rate was calculated, and a positive value with the cutoff set at  $\geq 20\%$ .

169 **Data analysis and statistics**

170 Data analysis and statistical calculations were performed with GraphPad Prism v9 (GraphPad  
171 Software, CA, USA). Differences among the study groups were analyzed with either unpaired,  
172 parametric T test with Welch’s correction, or Brown-Forsythe and Welch ANOVA tests, with  
173 no correction for multiple comparisons. In addition, bivariate analysis with Spearman’s  
174 correlation coefficient was performed. All differences with p values of  $< 0.05$  were considered  
175 statistically significant.

176

## 177 **RESULTS**

### 178 **Study outline and clinical parameters**

179 Patients suffering from COVID-19 and admitted to a hospital in Norrköping, Sweden were  
180 recruited into our study from May 2020 and throughout 2021, during and after the first and  
181 second waves of the pandemic in Sweden. Healthy controls who were confirmed SARS-CoV-2  
182 negative by PCR and antibody tests, and had no prior history of COVID-19, were also recruited  
183 from within the hospital staff. Detailed clinical data were recorded for all individuals within  
184 the hospitalized cohort (**Table 1**). Whole blood, serum, and nasal swabs were collected at  
185 inclusion, and patients were followed up for further sample collection at 2 weeks, 6 weeks,  
186 and 6-7 months post-inclusion (**Figure 1A**). For this study, we have utilized longitudinal  
187 samples from 21 hospitalized COVID-19 patients and 16 healthy controls. Disease severity was  
188 defined according to the NIH guidelines, which are based largely on supplemental oxygen  
189 requirements<sup>31</sup>. An initial assessment, at study inclusion, was made of an array of clinical  
190 parameters (**Figure 1B-C**), showing a significant decrease in lymphocytes, basophils,  
191 eosinophils, and neutrophils in the COVID-19 patients. The CRP and lactate dehydrogenase  
192 (LDH) levels were both significantly elevated in patients compared to healthy controls. All  
193 patients developed neutralizing SARS-CoV-2-specific antibodies that, in most individuals,  
194 lasted until the end of the study, even if the levels of neutralizing antibodies were significantly  
195 decreased at the 6-7 months timepoint compared to 2 and 6 weeks (**Figure 1D**). These findings  
196 are in agreement with previous publications<sup>32-36</sup>. We performed correlation analysis of clinical  
197 parameters and found that CRP correlated with viral load at inclusion (**Supplementary Figure**  
198 **1A**). Additional correlation analysis of clinical parameters and antibody levels revealed that  
199 anti-spike IgG levels and neutralizing antibody titers were negatively correlated with viral load

200 at inclusion (**Supplementary Figure 1B**). The connection between viral load and both spike  
201 and nucleocapsid antibody levels has been shown previously<sup>37, 38</sup>. Furthermore, there was  
202 negative correlation of neutralizing antibody titers and anti-spike IgG levels to CRP at inclusion  
203 (**Supplementary Figure 1C**). Following spectral flow data acquisition, lineage exclusion was  
204 performed to remove T cells, B cells, NK cells and basophils (**Figure 1E**), and remaining cells  
205 were defined as myeloid and used for further analysis of monocyte and DC populations. Major  
206 immune cell types from within PBMCs, and their changing distributions over time were  
207 visualized following dimensionality reduction analysis (**Figure 1F**).

208

### 209 **Increased levels of monocytes in the mononuclear myeloid compartment**

210 The proportion of total HLA-DR<sup>+</sup> myeloid cells did not change significantly over the 6-7 months  
211 of the study (**Figure 2A**). Within the myeloid compartment in COVID-19 patients, monocytes  
212 were defined as CD88<sup>+</sup> cells, with CD88 being exclusively expressed on blood monocytes<sup>39</sup>.  
213 Monocytes increased significantly during the first 2 weeks after inclusion and reached a  
214 plateau that was significantly higher at 6-7 months post COVID-19 compared to controls  
215 (**Figure 2B**). The proportion of CD14<sup>+</sup>HLA-DR<sup>-</sup> MDSC was raised compared to healthy controls  
216 at inclusion and at 2 weeks and 6-7 months follow up, but surprisingly, there was a significant  
217 dip at the 6 week time point (**Figure 2C**). Compared to the healthy controls there was a shift  
218 towards the non-monocytes, i.e., CD88<sup>-</sup> cells, in the myeloid compartment following COVID-19  
219 (**Figure 2D**). Together our results show that there is a sustained shift in the balance of cell  
220 types within the myeloid compartment (**Figure 2D**), despite the total level of myeloid cells  
221 remaining the same (**Figure 2A**).

222

### 223 **Long term changes in classical and intermediate monocytes post COVID-19.**

224 The CD14<sup>+</sup>CD16<sup>-</sup> cMo, CD14<sup>+</sup>CD16<sup>+</sup> iMo and CD14<sup>-/lo</sup>CD16<sup>+</sup> ncMo were examined to compare  
225 COVID-19 patients to healthy controls (**Figure 3**). The CD14<sup>+</sup> and CD16<sup>+</sup> cell levels within the  
226 myeloid compartment were initially low and increased over the 6-7 months (**Figure 3A**). The  
227 proportion of cMo at inclusion was significantly higher than in healthy controls and remained  
228 significantly elevated even after 6-7 months (**Figure 3B**). The iMo were reduced for the entire  
229 duration of the study (**Figure 3C**). A drastic reduction in ncMo at inclusion was observed in  
230 patients, but had already returned by 2 weeks to a level comparable to controls (**Figure 3C**).  
231 Together these data revealed that there was a sustained effect on the proportion of cMo and  
232 iMo in COVID-19 patients, while the change in ncMo was restored within 2 weeks.

233

#### 234 **Circulating conventional DC subsets and plasmacytoid DC levels recovered after initial** 235 **depletion**

236 We assessed the different circulating DC subsets (**Figure 4A-B**) and found decreased levels of  
237 all DC subsets in the patients at study inclusion. The proportion of pDC were significantly  
238 reduced in COVID-19 patients at inclusion and that these cells started to recover already at  
239 two weeks (**Figure 4A**). The pDC fraction amongst the CD88<sup>-</sup> myeloid cell compartment clearly  
240 increased until it reached a similar level to healthy controls (**Figure 4C**). The level had  
241 increased significantly by 2 weeks but did not fully return to normal until 6 weeks (**Figures 4A-**  
242 **C-D**). Next, we explored the different cDC subsets in COVID-19 patients. Among the cDC,  
243 CD141<sup>+</sup> cDC1 constitute a very small fraction<sup>17</sup>. This DC subset (**Figure 4A**) was significantly  
244 reduced at inclusion but throughout the 6-7 months studied, returned to a level comparable  
245 to controls (**Figure 5A**). Here, we analyzed cDC2 and cDC3 as one combined population due to  
246 the contrasting methods used by different groups to further define distinct cDC  
247 subpopulations within classical cDC2. While the proportion of the cumulative cDC2 and cDC3

248 subset was significantly reduced at inclusion, this recovered over the study period and even  
249 significantly surpassed the levels found in healthy controls and at 6-7 months (**Figure 5B**).  
250 Following this, cDC2 and cDC3 were further divided based on their expression of CD5 and  
251 CD14, respectively. Interestingly, neither the CD5<sup>+</sup> cDC2 nor the CD5<sup>-</sup> cDC2 were significantly  
252 altered at any of the study timepoints (**Figure 5C**). There was a significant increase in CD14<sup>+</sup>  
253 cDC3 at the 2 week and 6-7 month time points, but no change in the CD14<sup>-</sup> cDC3 (**Figure 5D**).  
254 Together, we found that both pDC and cDC1 recovered from initial reductions during the  
255 study. Additionally, there were major alterations over time in the combined cDC2 and cDC3  
256 population, though the proportions of the subpopulations within these DC subsets were  
257 unaffected.

258

### 259 **Prolonged decrease in PD-L1 and elevated CD86 and HLA-DR expression levels on monocyte** 260 **subsets**

261 The expression levels of functional surface markers were examined on monocyte subsets and  
262 MDSC (**Figure 6A, Supplementary Figure 2**). The clearest pattern was the decreased PD-L1  
263 expression, which was observed at all time points on the monocyte subsets and the MDSC  
264 (**Figure 6A-B, Supplementary Figure 2**). In addition, there were increased CD86 and HLA-DR  
265 levels from the 2 week timepoint onwards on the monocyte subsets (**Figure 6A-C-D**). The iMo  
266 were selected to represent the PD-L1 expression pattern seen across all monocytic cell types,  
267 which was significantly reduced at all time points compared to healthy controls (**Figure 6B**). A  
268 detailed look at CD86 expression levels revealed a significant increased expression from 2  
269 weeks onwards in iMo and ncMo, from 6 weeks onwards in MDSC, but not until 6 months in  
270 cMo (**Figure 6C**). HLA-DR expression was initially reduced in cMo before reverting to levels  
271 found in healthy controls at the 2 week time point. At the same time, there were increased

272 HLA-DR levels from the 2 week time point onwards in iMo. HLA-DR expression on ncMo was  
273 similar at all time points (**Figure 6D**).

274

275 **Prolonged decrease in PD-L1 expression on all DC subsets and elevated CD86 levels on**  
276 **conventional DC subsets**

277 The DC subsets were assessed for the expression levels of functional surface markers (**Figure**  
278 **7A-B, Supplementary Figure 3**). There was a clear reduction in CD83 at 6 months and a  
279 decrease in PD-L1 at all time points, across all DC subsets (**Figure 7A**). The pDC and cDC3 were  
280 found to have decreased expression of CCR7 at the 6-7 month time point (**Figure 7A,**  
281 **Supplementary Figure 3**). PD-L1 was significantly decreased during the entire study across all  
282 DC subtypes studied (**Figure 7A, Supplementary Figure 3**). CD86 expression on pDC was  
283 significantly increased at inclusion but returned, by 2 weeks, to a comparable level to controls  
284 and remained so for the remainder of the 6-7 months post-COVID-19. On cDC1, CD86 was  
285 increased at both inclusion and the 6-7 month time point, but not in the intervening period.  
286 In the cumulative cDC2 and cDC3 population, CD86 expression increased to a significantly  
287 higher level than controls at 6 weeks and 6-7 months (**Figure 7D**). Only cumulative cDC2 and  
288 cDC3 showed any significant changes to HLA-DR expression, with a significant increase over  
289 time, until the expression from 6 weeks onwards showed no significant difference to healthy  
290 controls (**Figure 7E**). Taken together these data show that COVID-19 elicited lasting alterations  
291 in PD-L1 and CD86 expression levels on DC subsets.

292

293 **CRP at inclusion correlated with alterations in monocyte and DC subsets in COVID-19**  
294 **patients**

295 To determine if the initial inflammatory response to the SARS-CoV-2 infection affected the  
296 myeloid cell compartment in the COVID-19 patients, we assessed correlations between clinical  
297 parameters and myeloid cell populations. We found that the proportion of iMo at inclusion  
298 positively correlated with CRP (**Figure 8A**), and negative correlations were found with CRP and  
299 all DC subtypes, both at 2 and 6 weeks post-inclusion (**Figure 8B**).

## 300 **DISCUSSION**

301 To address the lack of knowledge concerning myeloid cells in COVID-19 we have investigated  
302 the effects that SARS-CoV-2 infection exerts, both initially and long-term, on monocyte and  
303 DC subsets. Decreased frequencies of all DC subsets were found during acute COVID-19 with  
304 the levels subsequently returning to normal for all except the cDC2 and cDC3 combined  
305 subset, which still had higher levels at 6-7 months follow up. There were major alterations in  
306 monocyte subsets, with elevated levels of cMo that were still present at the long-term follow  
307 up. Notably, MDSC levels were still elevated at the 6-7 month timepoint. Additionally, our  
308 phenotypic assessment of functional markers showed elevated levels of CD86 on the  
309 monocyte subsets and MDSC at the final time point. On all cells HLA-DR expression was either  
310 unaffected or showed an initial decrease followed by recovery, except for iMo that had  
311 elevated HLA-DR from the second week and throughout the study period. Regarding CD86,  
312 the level at acute infection was elevated, especially on pDC, and high levels were sustained  
313 long term on cDC1 and cDC2/cDC3. HLA-DR expression was unaffected or low at inclusion on  
314 DC subsets and stayed low on the cDC2 and cDC3 combined subset. A lower expression of  
315 HLA-DR could be indicative of an initial suppressed functionality of amongst these DC.

316 Here we present a well-characterized, representative cohort, with clinical features commonly  
317 observed in hospitalized COVID-19 patients, as highlighted by marked lymphopenia, elevated  
318 serum CRP and LDH, and changes in the polymorphonuclear leukocytes, all in accordance with  
319 current knowledge <sup>32-35, 40</sup>. Previous studies have illustrated a reduction of many of the  
320 myeloid cell subsets in COVID-19 <sup>23, 24, 27, 41</sup>. Monocyte frequencies have been found to be  
321 lower during acute COVID-19 compared to patients in convalescence <sup>42</sup>. In line with this, the  
322 levels of overall monocytes in the patients in our study were significantly decreased at  
323 inclusion, when compared to the later time points. The three subpopulations of monocytes



324 are distinguished primarily by their expression of CD14 and CD16, but also by other cell surface  
325 markers, including CD36 and CCR2 for cMo, CCR5 and CD74 for iMo, and CXCR4 and CX3CR1  
326 for nCMo. These markers aid in the wide range of functions of monocytes, including their  
327 antiviral activity <sup>43</sup>. Our observed sustained increase in cMo in COVID-19 patients in  
328 comparison to healthy controls is consistent with previously published data <sup>23</sup>. Key roles of  
329 cMo include tissue repair and anti-apoptotic functions <sup>44, 45</sup>, and the elevated levels of these  
330 cells at 6-7 months could be part of the host resolving long-term effects of the SARS-CoV-2  
331 infection. We saw an initial drastic drop in the level of ncMo, which recovered in  
332 convalescence, consistent with other reports <sup>23, 46</sup>. Different patterns are seen in severe  
333 compared to mild COVID-19 in the levels of circulating iMo, with decreased levels in severe  
334 disease when measured during active disease <sup>24</sup>. We found decreased levels of these cells  
335 compared to healthy controls and that they failed to reach the levels of the healthy controls  
336 before the 6-7 month follow up, indicating a long-lasting effect. In some COVID-19 patients  
337 with post-acute sequelae, i.e., long COVID, levels of ncMo and iMo were increased even 15  
338 months post SARS-CoV-2 infection <sup>41</sup>. From acute infection to convalescence, there was a  
339 decrease in cMo and an increase in ncMo <sup>47</sup>. The decreased ncMo in severe COVID-19 patients  
340 during acute disease has been suggested to occur due to the recruitment of CD16<sup>+</sup> myeloid  
341 cells to inflamed lung tissue during infection <sup>24, 48, 49</sup>. In mouse models of acute lung injury and  
342 in acute respiratory distress syndrome, as seen in severe COVID-19, circulating monocytes  
343 have been shown to play a pivotal role in driving inflammation <sup>50</sup>. Another factor affecting the  
344 levels of monocyte subsets could be virus-mediated cell death during COVID-19, since they all  
345 express the SARS-CoV-2 receptor, ACE2 <sup>42</sup>, and there is evidence of viral antigens present  
346 within ncMo for over a year after initial infection <sup>41</sup>. In addition, CD16<sup>+</sup> monocytes can also be  
347 infected in an ACE2-independent manner, leading to inflammatory cell death <sup>51</sup>.

348 Recent studies, through single-cell resolution methods, point to the expansion of suppressive  
349 myeloid cells in the blood as a hallmark of severe COVID-19 <sup>14, 23</sup>, as confirmed by our data.  
350 These cells (MDSC) are a heterogeneous population of myeloid cells spanning granulocytes,  
351 macrophages and DC and these cells exist during normal conditions at diverse frequencies at  
352 different tissue <sup>52</sup>. The levels of MDSC have been found to correlate to impaired T cell  
353 responses <sup>53, 54</sup>. This sustained elevation suggests a long-lasting suppressive state, which we  
354 have found to be reflected in the suppressive T cell phenotypes defined in our COVID-19  
355 cohort <sup>55</sup>.

356 In line with other studies, we found that COVID-19 had a major impact on circulating DC  
357 subpopulations <sup>23</sup>, with major reductions during active disease for pDC <sup>14</sup>, cDC1 <sup>24</sup>, and  
358 combined cDC2/cDC3 <sup>56</sup>. Of note, the low level of pDC was independent of the study  
359 participants' age and sex, which is in line with previous findings <sup>57</sup>. Indeed, the reason for the  
360 reduction seen in pDC and cDC1 at inclusion could be due to several factors. A redirection of  
361 circulating pDC and cDC1 to lymphoid tissue has been well documented <sup>25, 58</sup> and cDC2  
362 subtypes have been shown to be recruited into the lung tissue, though this is not seen for pDC  
363 and cDC1 <sup>24, 59</sup>. The myeloid DC populations, cDC1 and cDC2/cDC3 returned to normal levels  
364 within 2 weeks. Of note, there were long-lasting effects, with higher levels of combined  
365 cDC2/cDC3 compared to healthy controls. A lasting effect with higher levels of HLA-DR<sup>+</sup>CD11c<sup>+</sup>  
366 DC has been noted in individuals needing hospitalization, whereas normal levels of CD141<sup>+</sup>  
367 DC, i.e., cDC1 were observed <sup>30</sup>. Regarding pDC, we found these cells to be affected for a longer  
368 time, and to not fully return to the level found in healthy controls until the 6 week time point.  
369 Conversely, at 7 months in the study by Pérez-Gómez <sup>30</sup> the pDC levels were still not restored  
370 to normal.

371 Increased levels of CD163<sup>+</sup>CD14<sup>+</sup> DC3 could play a role in COVID-19 mediated inflammation <sup>56</sup>.  
372 In addition, the elevated levels of these cells could indicate an ongoing long-term systemic  
373 inflammation due to the damage inflicted by COVID-19 on the host. In addition to relocation  
374 from circulation, the observed decreases in DC might depend on the killing of immune cells by  
375 direct or indirect viral effects. Contrary to monocytes, circulating DC lack expression of ACE2.  
376 However, other DC receptors such as CD147 and DC-SIGN can facilitate SARS-CoV-2 binding  
377 and entry <sup>60,61</sup> and this might enable their infection. This interaction has been shown to induce  
378 pro-apoptotic p53 pathways in pDC <sup>25</sup>. Almost all DC functions are affected during COVID-19  
379 <sup>25</sup> with probable consequences for the progression and outcome of the illness. Initial type I  
380 and III IFN responses are essential to the resolution of viral infection and are found to be  
381 impaired in severe COVID-19 <sup>62</sup>. One possible explanation for this may be the reduction of  
382 pDC, which are a major source of type I IFN. Given the importance of DC for the initial  
383 activation of T cell responses, their depletion or tissue relocation could hamper the  
384 subsequent T cell response <sup>63</sup>. Additionally, the long-lasting alteration in the DC compartment  
385 could explain the sustained T cell dysfunction seen following COVID-19 <sup>55,64</sup>.

386 In different diseases such as cancer and severe infections, the phenotype and functionality of  
387 circulating monocyte and DC subsets are altered <sup>65,66</sup>. Our phenotypic assessment for  
388 functional markers affecting cellular activation and suppression showed little to no decrease  
389 in CD86 expression during acute disease, on monocyte subsets and MDSC. This was followed  
390 by elevated CD86 levels on all these cells at the long-term follow up. Previous studies have  
391 shown that monocyte subsets, and especially iMo, have decreased CD86 during acute  
392 infection <sup>67,68</sup>. HLA-DR expression on monocyte subsets had a similar initial decrease as found  
393 for CD86, with a recovery to levels in healthy controls. The exception to this was iMo, that had  
394 elevated HLA-DR from the second week and throughout the study period. The decreased HLA-

395 DR levels in acute infection for cMo, ncMo are in accordance with previous studies <sup>14, 46, 67, 69,</sup>  
396 <sup>70</sup>. The iMo have been found to have downregulated HLA-DR during severe acute COVID-19 <sup>14,</sup>  
397 <sup>46, 69</sup>, which differs to our observation of no effect on HLA-DR compared to healthy control at  
398 inclusion. For HLA-DR a more pronounced decrease has been connected to severe disease <sup>69</sup>.  
399 In severe COVID-19, HLA-DR has previously been found to be reduced across all circulating DC  
400 besides cDC1 <sup>23</sup>. In our study HLA-DR was only significantly altered on cDC2/3 where it was  
401 reduced at early time points before returning to levels found in controls, which is in agreement  
402 with Marongiu et al. who showed a reduction of HLA-DR in cDC2 and cDC3 subsets <sup>71</sup>. The  
403 observed initial low expression of HLA-DR on cDC1, cDC2/cDC3, cMo, and ncMo could be a  
404 sign of immunosuppression in these cells) <sup>26, 69, 72, 73</sup>. Interestingly, the elevated levels on iMo  
405 found after a few weeks and throughout the study period could be an indication of an ongoing  
406 inflammatory environment due to residual effects of the disease <sup>8, 9</sup>.

407 Regarding the costimulatory molecule CD86 on DC, we found an increased level of expression  
408 on pDC and cDC1 at inclusion but not cDC2/cDC3. The elevated CD86 levels on pDC have been  
409 documented during acute SARS-CoV-2 infection <sup>57</sup>. However, other studies found no effect on  
410 pDC, nor did they find decreased CD86 expression on HLA-DR<sup>+</sup>CD11c<sup>+</sup> DC <sup>27, 70</sup>, or on other  
411 myeloid DCs subsets in acute disease <sup>7, 56</sup>. The expression of CD86 returned to levels  
412 comparable to healthy controls quickly for pDC but remained elevated on cDC1 and cDC2 and  
413 cDC3 subsets at 6-7 months. This contrasts with a previously published study which showed  
414 long-term reduction in CD86 expression on pDC and HLA-DR<sup>+</sup>CD11c<sup>+</sup> DC subsets in hospitalized  
415 COVID-19 patients <sup>30</sup>. Elevated CD86 expression was found on ki67<sup>+</sup> cDC2 and cDC3 subsets,  
416 i.e., a similar pattern as we have seen for these DC subsets, highlighting that the level seems  
417 to be connected to the time that they have been in circulation <sup>56</sup>. CD86 is found to be elevated

418 in cancer and chronic infections such as HIV-1, and the increased CD86 we see could be  
419 another indicator of ongoing, low-level systemic inflammation from lung repair following  
420 COVID-19<sup>74</sup>. The differences between our and other results could be due to the method of  
421 defining DC subsets, with many studies assessing CD14<sup>-</sup>HLA-DR<sup>+</sup>CD11c<sup>+</sup> DC and not the  
422 different cDC subsets we have used in our study.

423 In our study we observed a consistently lower PD-L1 surface expression across pDC and cDC  
424 subsets, in keeping with gene and protein expression levels in hospitalized patients during  
425 acute disease<sup>30, 75, 76</sup>. This decrease of PD-L1 has not been seen in other studies, rather an  
426 increased surface expression of PD-L1 has been seen when examining the bulk monocytes<sup>70,</sup>  
427 <sup>77</sup>, HLA-DR<sup>+</sup>CD11c<sup>+</sup> DC, and pDC<sup>57, 70</sup>. Interestingly, on cDC1 and cDC2 recently entering  
428 circulation (ki67<sup>+</sup> cells), the levels of PD-L1 were decreased in COVID-19 patients compared to  
429 controls<sup>56</sup>. In our data this reduced expression of PD-L1 persisted for the entire duration of  
430 the study, as was seen also in a previous study at 7 months post-COVID-19<sup>30</sup>. Furthermore,  
431 PD-L1 expression was reduced on monocyte subsets when compared to the healthy controls  
432 throughout the 6-7 month period of our study. The loss of PD-L1 might be due to shedding of  
433 soluble PD-L1, which is found to be elevated in the serum of COVID-19 patients<sup>75</sup>. Of note,  
434 the severity of disease seems to correlate to the level of soluble PD-L1 in circulation<sup>75</sup>. The  
435 lasting reduction that we observe in PD-L1 expression across all myeloid cell subsets requires  
436 further study to explore if it plays any role in COVID-19 pathogenesis.

437 Concerning the migratory receptor CCR7, there was little change in the DC and monocyte  
438 subsets during acute disease compared to healthy controls, while long-term effects included  
439 decreased CCR7 on cMo, pDC and the two cDC3 populations. This decrease is in accordance  
440 with previous findings for cDC at 7 months<sup>30</sup>, but not for pDC, which had long-term increased  
441 CCR7<sup>30</sup>, though this study measured the percentage of positive cells whereas we measured

442 level of expression. CD83 expression has been explored at gene level in DC in COVID-19 <sup>14, 78</sup>,  
443 however, not much is known regarding surface protein expression. We did not find any major  
444 alterations in the surface expression of CD83 across DC, which concurs with Venet et al. <sup>76</sup>.  
445 Overall, we identified marked alterations to the expression of surface markers across myeloid  
446 cell types following COVID-19. The long-term changes to the surface expression of these  
447 proteins on monocytes and DC may be due to epigenetic changes resulting from severe  
448 COVID-19 <sup>79</sup>, possibly altering progenitors in the bone marrow.

449 COVID-19 severity has been linked to an array of clinical parameters such as the level of  
450 soluble urokinase plasminogen activator receptor (suPAR), CRP, and viral load <sup>80-83</sup>. In this  
451 study, the viral load correlated positively with the CRP levels and negatively with anti-spike  
452 IgG and neutralizing SARS-CoV-2 antibody levels, at inclusion. In addition, the COVID-19  
453 patients with high CRP levels had lower levels of anti-spike IgG and neutralizing antibodies at  
454 inclusion. This negative association between CRP and antibody levels early on during SARS-  
455 CoV-2 infection has, to our knowledge, not yet been made. It has previously been shown that  
456 higher levels of specific antibodies during convalescence correlated with initial higher CRP  
457 levels <sup>84</sup>, though we did not see this. During acute disease, the levels of pDC, cDC2, and  
458 CD163<sup>+</sup>CD14<sup>-</sup> cDC3 were negatively correlated to CRP levels <sup>56, 69</sup>, which we confirm in our  
459 study to be the case for all circulating DC subsets, i.e., a faster DC recovery could be predicted  
460 by lower initial levels of CRP. Conversely, during mild disease a positive correlation of pDC  
461 proportions with CRP has been shown <sup>30</sup>. The levels of all monocyte subsets were previously  
462 found to negatively correlate to CRP in acute disease <sup>85</sup>, while we found a positive correlation  
463 for iMo levels in our study. The failure to induce a strong type I IFN response in COVID-19 leads  
464 to a prolonged high viral load and induces a highly inflammatory environment that includes

465 raised CRP <sup>86</sup>. This in turn might have long-term effects on monocyte and DC subsets, which  
466 our data strongly supports.

467 A potential limitation of our study is the imperfect age and sex matching of the controls to the  
468 patients. This is of particular importance for some DC subsets such as pDC, which are  
469 decreased in older (>40 years) healthy individuals, whereas there are no significant effects on  
470 cDC <sup>87, 88</sup>. Given that the levels of pDC returned to the levels of healthy controls we do not  
471 believe this is an issue. While biological sex does not seem to play a role for the levels of MDSC  
472 in general, their levels could be influenced during disease. A study of MDSC in mild to severe  
473 COVID-19 found higher levels of monocytic MDSC in males than in females <sup>89</sup>. The increase we  
474 found of MDSC in blood from hospitalized COVID-19 patients did not correlate with biological  
475 sex or age, however, our cohort was relatively small and did not contain cases of mild disease,  
476 so a larger cohort may be needed to observe these associations. The MDSC levels increase  
477 with age and highly elevated levels are seen in severe infections and cancers <sup>90-96</sup>. For instance,  
478 there are increased conventional CD33<sup>+</sup>HLA-DR<sup>-</sup>CD45<sup>+</sup> MDSC in older adults compared to  
479 younger adults. A general problem, not specific for our study, when comparing data from  
480 different COVID-19 studies is the definition of disease severity, that diverges depending on  
481 country. We defined severity according to the NIH guidelines, which are based largely on  
482 supplemental oxygen requirements <sup>31</sup>, as opposed to the WHO scale <sup>97</sup>.

483 In conclusion, given the long-lasting changes in the monocyte, DC and MDSC compartments,  
484 as seen in the altered frequencies of cell populations and expression levels of various surface  
485 markers, it is evident that COVID-19 imprints the development and fate of these cells. Further  
486 studies will be required to determine for exactly how long these alterations persist after  
487 severe COVID-19, and if they affect the type and quality of immune responses elicited against  
488 future infections.

## 489 **Authorship contribution**

490 F.R.H., M.G., C.S., J.N., and H.W. conducted experiments. F.R.H., M.G., C.S., J.N., H.W., S.N.,  
491 and M.L. analyzed the data. F.R.H., M.G., and M.L were involved in the writing of the initial  
492 manuscript, and F.R.H., M.G., C.S., J.N., A.N., A.J.H., M.H., J.S., S.N., and M.L. helped in the  
493 revision of the manuscript. M.L. designed the experiments. A.N., A.J.H., M.H., J.S., S.N., and  
494 M.L. were involved in establishing the cohort study. All authors read and approved the final  
495 manuscript.

496

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513

514 **Competing interest/conflict**

515 The authors declare no competing interest/conflict.

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772

773 **Figure legends**

774 **Figure 1. Study outline and experimental design.**

775 PBMCs and serum were collected from 21 COVID-19 patients and 16 healthy donors over a 6-  
776 7 month period. (A) Design of COVID cohort study illustrating timepoints for sample collection  
777 and experimental outputs, created with BioRender.com. Initial clinical assessments of (B)  
778 blood cell counts, (C) plasma C-reactive protein (CRP) and lactate dehydrogenase (LDH), and  
779 (D) neutralizing SARS-CoV-2 antibody titers. (E) Representative gating strategy to demonstrate  
780 live/dead gating and lineage exclusion, to leave myeloid cell populations for further analysis.  
781 (F) tSNE plots showing distinct clustering of all live cells, with 17 patients overlaid, and 16  
782 controls overlaid. Data is represented as mean with 95% CI, with significance of \* $p \leq 0.05$ ,  
783 \*\*\*\* $p \leq 0.0001$ , determined using Brown-Forsythe and Welch ANOVA tests. Inc = Study  
784 inclusion, 2W = 2 weeks, 6W = 6 weeks, 6M = 6-7 months, HC = healthy control. Mo =  
785 monocytes; cMo = classical iMo = intermediate, ncMo = non-classical, MDSC = myeloid-  
786 derived suppressor cells, DC = dendritic cells; pDC = plasmacytoid, cDC1/2/3 = conventional.

787

788 **Figure 2. Dysregulation in the frequency of myeloid compartments persists for 6 months in**  
789 **hospitalized COVID-19 patients.**

790 PBMCs obtained from 21 COVID-19 patients and 16 healthy donors over a 6-7 month period  
791 were stained with monoclonal antibodies and assessed by flow cytometry to identify the  
792 myeloid cell subsets. Percentage of the cells comprising (A) the total HLA-DR<sup>+</sup>Lin<sup>-</sup> myeloid  
793 compartment and (B) CD88<sup>+</sup> monocytes. (C) Representative gating and percentage of  
794 CD14<sup>+</sup>HLA-DR-Lin<sup>-</sup> MDSC. (D) Ratio of CD88<sup>+</sup> monocytes to CD88<sup>-</sup> myeloid cells. Data is  
795 represented as mean with 95% CI, with significance of \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ ,



796 \*\*\*\* $p \leq 0.0001$ , determined using Brown-Forsythe and Welch ANOVA tests. Inc = Study  
797 inclusion, 2W = 2 weeks, 6W = 6 weeks, 6M = 6-7 months, HC = healthy control.

798

799 **Figure 3. Classical and intermediate monocyte cells subsets remain altered in COVID-19**  
800 **patients at 6 months.**

801 PBMCs were collected from COVID-19 patients that required hospitalization (N=21) and  
802 healthy controls (N=16) over a 6-7-month period. Cells were stained with antibodies for flow  
803 cytometry to define the monocyte subsets. (A) tSNE plots displaying myeloid cell clusters,  
804 colored according to their intensity of CD14 and CD16 expression. (B) Representative plots  
805 illustrating how monocyte subsets were defined. (C) Frequencies of the CD14<sup>+</sup>CD16<sup>-</sup> cMo,  
806 CD14<sup>+</sup>CD16<sup>+</sup> iMo, and CD14<sup>-</sup>CD16<sup>-</sup> ncMo. Data is represented as mean with 95% CI, with  
807 significance of \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , \*\*\*\* $p \leq 0.0001$ , determined using Brown-  
808 Forsythe and Welch ANOVA tests. Inc = Study inclusion, 2W = 2 weeks, 6W = 6 weeks, 6M = 6-  
809 7 months, HC = healthy control.

810

811 **Figure 4. Plasmacytoid DCs in COVID-19 patients are severely depleted in blood during**  
812 **COVID-19.**

813 PBMCs collected from hospitalized COVID-19 patients (N = 21) and healthy controls (N = 16),  
814 were stained for flow cytometry to observe the effect of SARS-CoV-2 on the overall DC  
815 compartment. (A) tSNE plots illustrating the changing distribution of DC within CD88<sup>-</sup> myeloid  
816 cells. (B) Gating strategy for DC subsets. (C) Ratio of CD303<sup>-</sup> cells to pDC during COVID-19. (D)  
817 Percentages CD303<sup>+</sup> pDC. Data are presented as mean with 95% CI, with significance of \* $p \leq$

818 0.05, \*\*\* $p \leq 0.001$ , \*\*\*\*  $p \leq 0.0001$ , determined using Brown-Forsythe and Welch ANOVA  
819 tests. Inc = Study inclusion, 2W = 2 weeks, 6W = 6 weeks, 6M = 6-7 months, HC = healthy  
820 control.

821

822 **Figure 5. Initial depletion of classical DC populations during COVID-19.**

823 PBMCs from hospitalized COVID-19 patients (N = 21) and healthy donors (N = 16), were stained  
824 for flow cytometry to observe changes in the composition of the cDC compartment during  
825 COVID-19. Proportions of (A) CADM1<sup>+</sup>CD141<sup>+</sup> cDC1, (B) CD11c<sup>+</sup>FCεR1a<sup>+</sup> cDC2 and cDC3  
826 combined, (C) CD5<sup>+</sup>CD163<sup>-</sup> cDC2 and CD5<sup>-</sup>CD163<sup>-</sup> cDC2, and (D) CD5<sup>-</sup>CD14<sup>+</sup>CD163<sup>+</sup> cDC3 and  
827 CD5<sup>-</sup>CD14<sup>+</sup>CD163<sup>+</sup> cDC3 are shown. Data is presented as mean with 95% CI, with significance  
828 of \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , \*\*\*\* $p \leq 0.0001$ , determined using Brown-Forsythe and  
829 Welch ANOVA tests. Inc = Study inclusion, 2W = 2 weeks, 6W = 6 weeks, 6M = 6-7 months, HC  
830 = healthy control.

831

832 **Figure 6. Long-term changes in the phenotype of blood monocyte subsets and myeloid  
833 derived suppressor cells in COVID-19 patients.**

834 PBMCs were collected from hospitalized COVID-19 patients (N=21) and healthy controls (N =  
835 16) over a 6-7 month period. (A) Heatmaps showing the mean fluorescence intensity (MFI) of  
836 CCR7, PD-L1, CD86, and HLA-DR on monocyte subsets and MDSC, from all donors. (B)  
837 Representative graph of PD-L1 on iMo. MFI of (C) CD86 and (D) HLA-DR on monocyte subsets  
838 and MDSC. Data is represented as mean with 95% CI, with significance of \* $p \leq 0.05$ , \*\* $p \leq 0.01$ ,

839 \*\*\* $p \leq 0.001$ , \*\*\*\* $p \leq 0.0001$ ., determined using Brown-Forsythe and Welch ANOVA tests. Inc  
840 = Study inclusion, 2W = 2 weeks, 6W = 6 weeks, 6M = 6-7 months, HC = healthy control.

841

842 **Figure 7. Altered phenotype of circulating dendritic cell subsets in COVID-19 patients.**

843 PBMCs collected from hospitalized COVID-19 patients (N = 21) and healthy controls (N = 16)  
844 over a 6-7 months period, were assessed for phenotypical changes. (A) Heatmaps showing the  
845 mean fluorescence intensity (MFI) of CD83, CCR7, PD-L1, CD86, and HLA-DR on different DC  
846 subsets. (B) tSNE plots showing CD88<sup>-</sup> myeloid cells, colored according to intensity of PD-L1,  
847 CD86, and HLA-DR expression. (C) Representative graph of PD-L1 on pDC. MFI of (D) CD86 and  
848 (E) HLA-DR on DC subsets. Data is represented as mean with 95% CI, with significance of  
849 \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , \*\*\*\* $p \leq 0.0001$ ., determined using Brown-Forsythe and  
850 Welch ANOVA tests. Inc = Study inclusion, 2W = 2 weeks, 6W = 6 weeks, 6M = 6-7 months, HC  
851 = healthy control.

852 **Figure 8. CRP levels correlated negatively with various monocyte and DC subsets.**

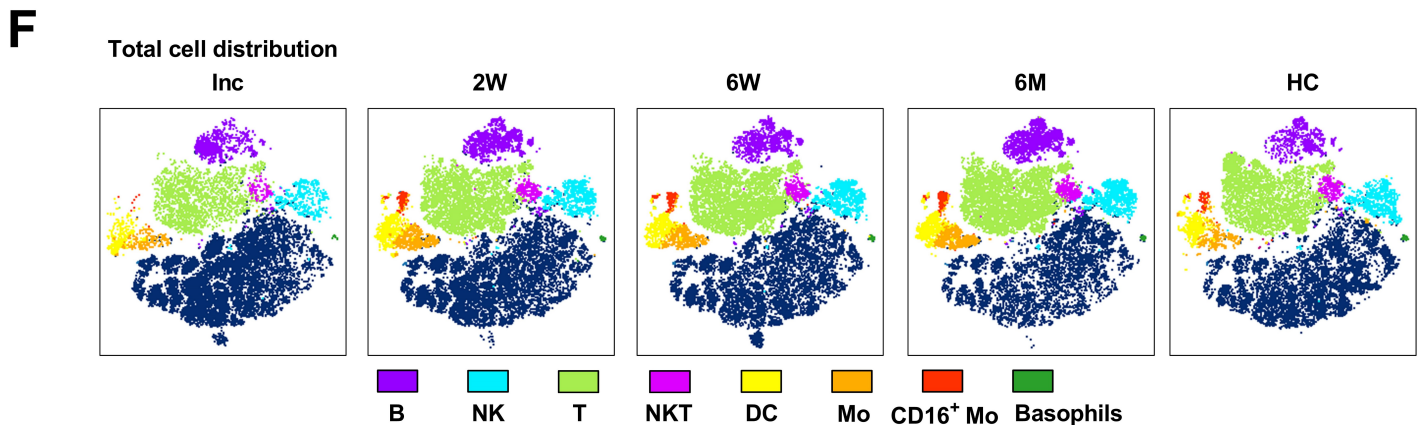
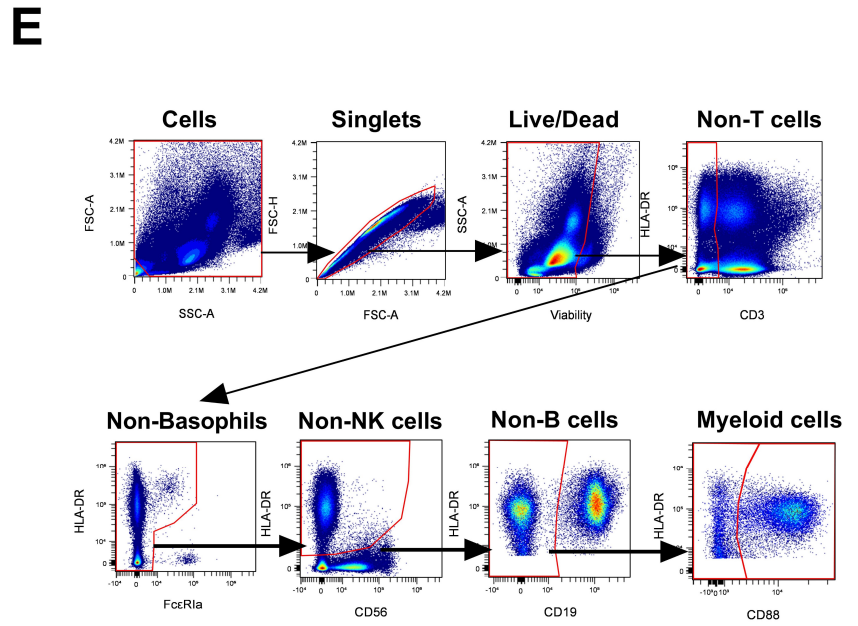
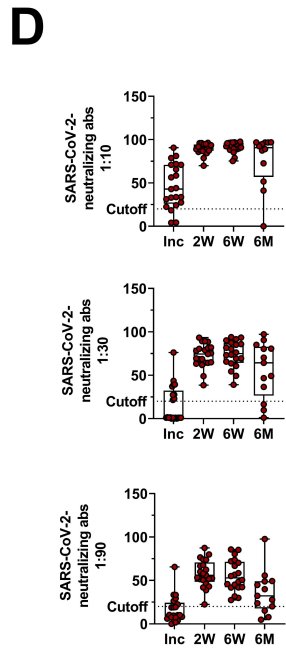
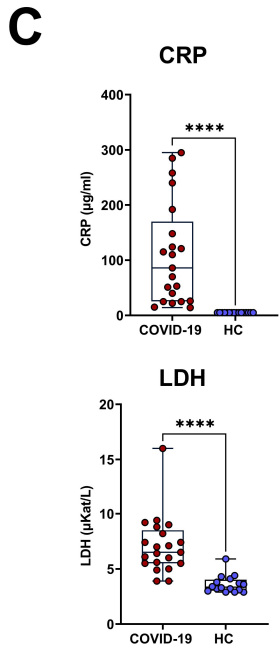
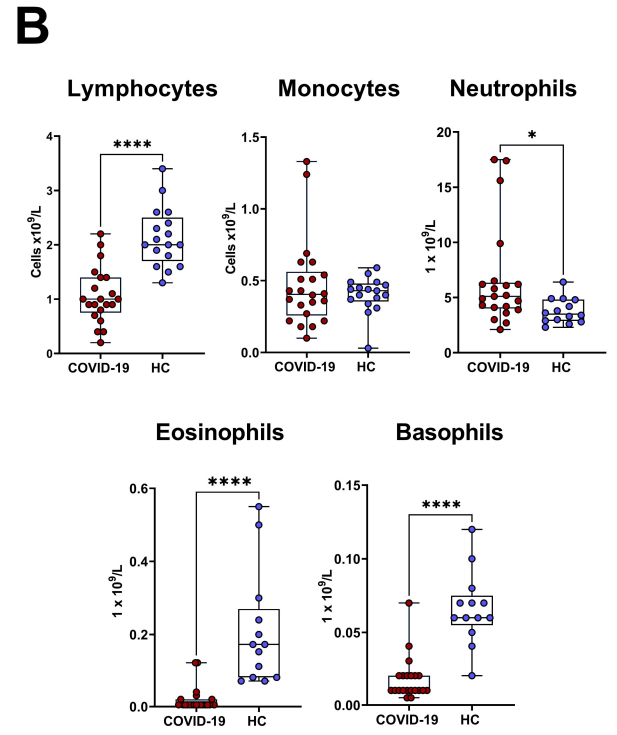
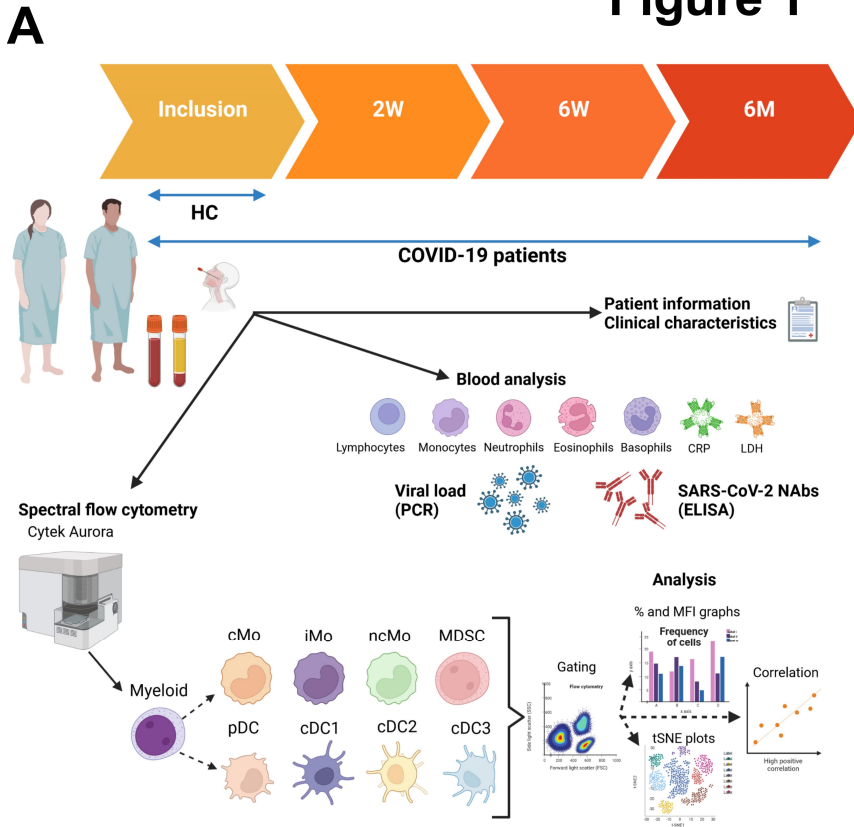
853 Bivariate analysis with Spearman's correlation coefficient was performed using an array of  
854 clinical parameters and myeloid cell types. Significant correlations shown for (A) iMo at  
855 inclusion and (B) circulating DC subsets at 2 and 6 weeks post-inclusion, all against CRP. The p  
856 value and Spearman's correlation coefficient (R) are shown for each analysis. N = 21. Inc =  
857 Study inclusion, 2W = 2 weeks, 6W = 6 weeks, 6M = 6-7 months, HC = healthy control.

858 **Table 1. Clinical and-demographical characteristics of hospitalized COVID-19 patients.**

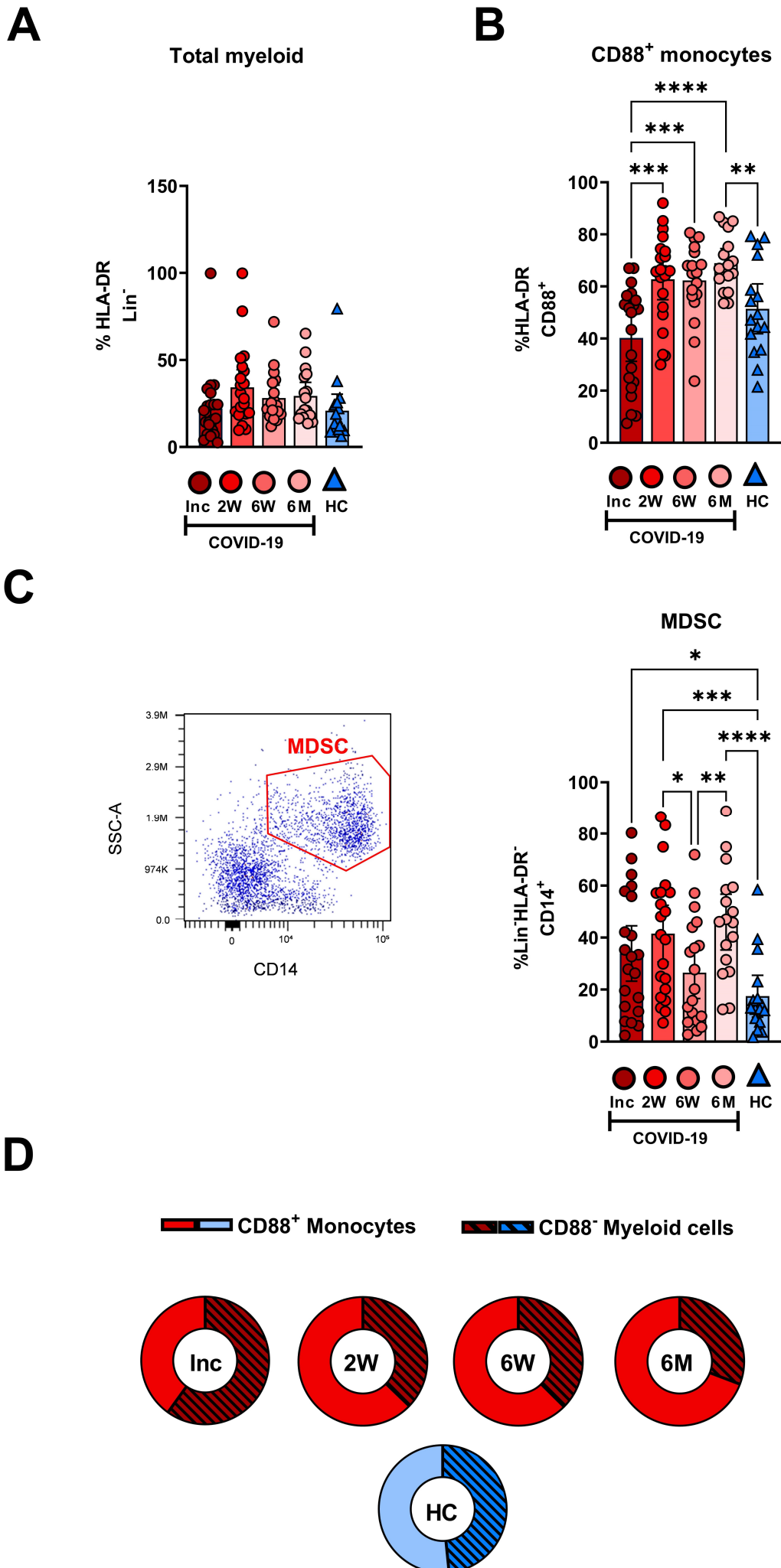
Variable	Clinical data	Reference range
Number of patients	21	
Age, median (range)	56 (32–83)	
Body mass index, median (range)	30.7 (23.7–45.2)	
Biological sex, % (N)	38.1 F/61.9 M (8F/13M)	
Days in hospital, median (range)	9 (4–34)	
ICU/pandemic Ward %, (N)	14.3/85.7 (3/18)	
Days with symptoms before inclusion, median (range)	11 (5–30)	
Spike IgG antibody positive at inclusion, % (N)	76.2 (16)	
Nucleocapsid IgG antibody positive at inclusion, % (N)	76.2 (16)	
Viral load at inclusion, median (range)(copies/ml)	3589 (1071–111 x 10 <sup>6</sup> )	
Antiviral treatment, % (N)	42.9 (9)	
Corticosteroid treatment, % (N)	76.2 (16)	
No/oxygen/HFNOT:CPAP <sup>1</sup> /mechanical ventilation, % (N)	4.8/38.1/52.3/4.8 (1/8/11/1)	
Cardiovascular disease, % (N)	52.4 (11)	
Pulmonary disease, % (N)	28.6 (6)	
Diabetes mellitus, % (N)	19 (4)	
Two of the underlying conditions, % (N)	19 (4)	
Three of the underlying conditions, % (N)	9.5 (2)	
Disease score: moderate/severe, % (N)	85.7 (18)/14.3 (3)	
Smoker/snus, % (N)	19 (4)	
Previous history of smoking/snus, % (N)	47.6 (10)	
Leukocytes (x 10 <sup>9</sup> /L), median (range)	6.6 (3.5–20.4)	3.5–8.8
Thrombocytes (x 10 <sup>9</sup> /L), median (range)	275 (151–476)	150–400
Lymphocytes (x 10 <sup>9</sup> /L), median (range)	0.9 (0.2–2.8)	1.1–4.8
Monocytes (x 10 <sup>9</sup> /L), median (range)	0.4 (0.1–1.33)	0.1–1
Lactate dehydrogenase (μKat/L), median (range)	7.4 (3.9–16)	>70 years <3.5, <70 years < 4.3
C-reactive protein (mg/L), median (range)	86 (14–295)	0–10

859 <sup>1</sup>High flow nasal oxygen therapy (HFNOT), continuous positive airway pressure therapy (CPAP).  
 860 Diabetes mellitus, cardiovascular, and chronic pulmonary diseases defined by the individuals being medicated for these  
 861 conditions.

## Figure 1

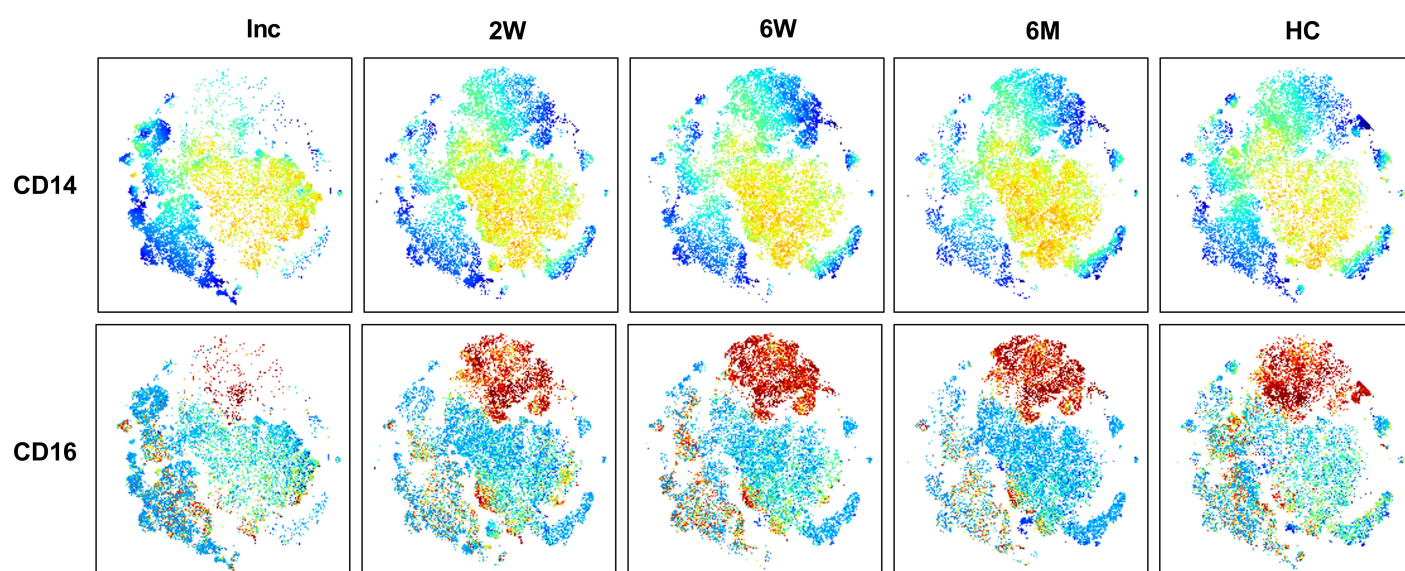


## Figure 2

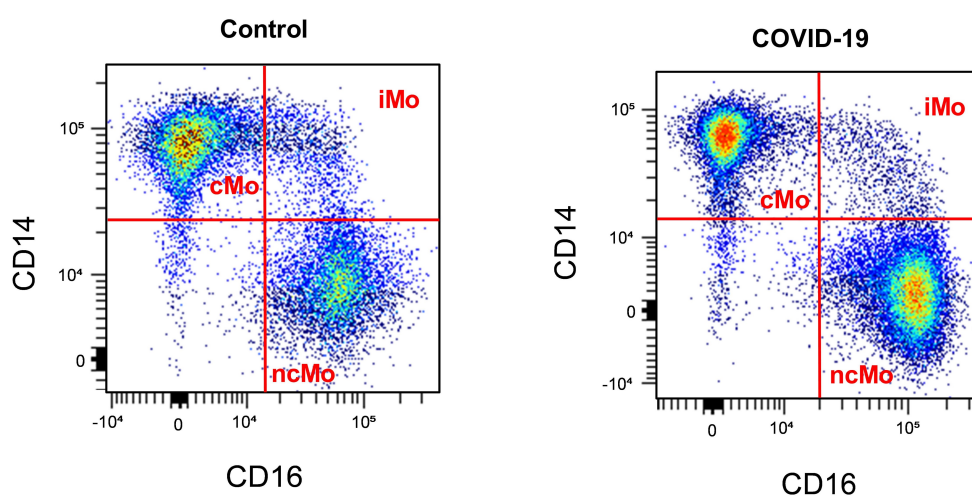


## Figure 3

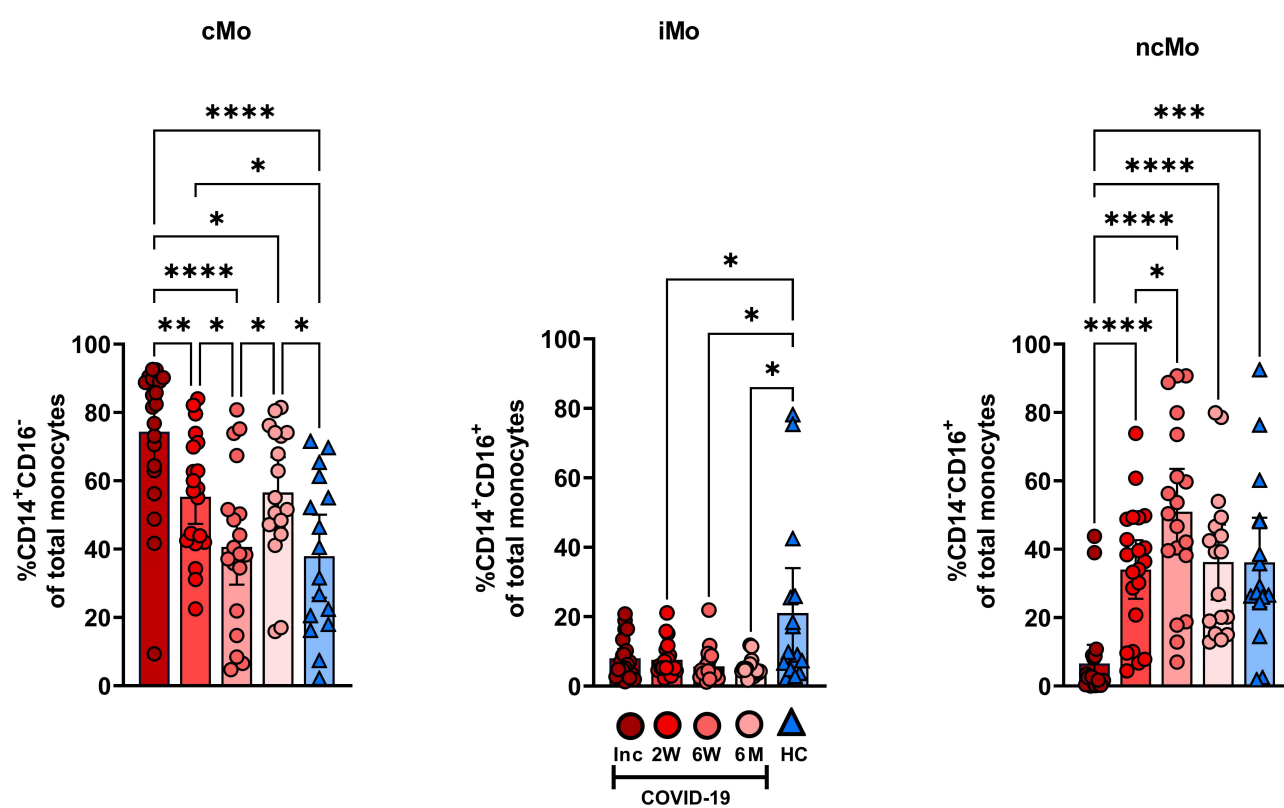
**A**



**B**



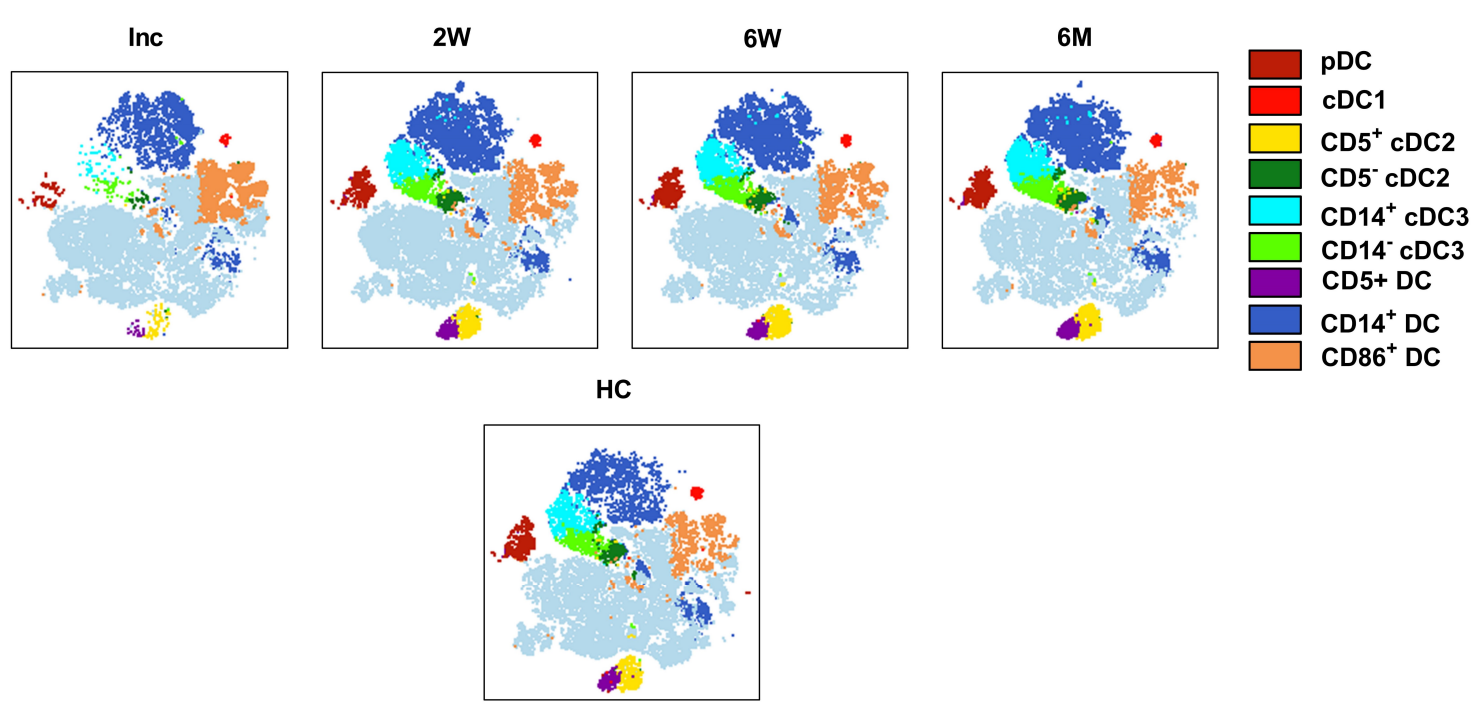
**C**



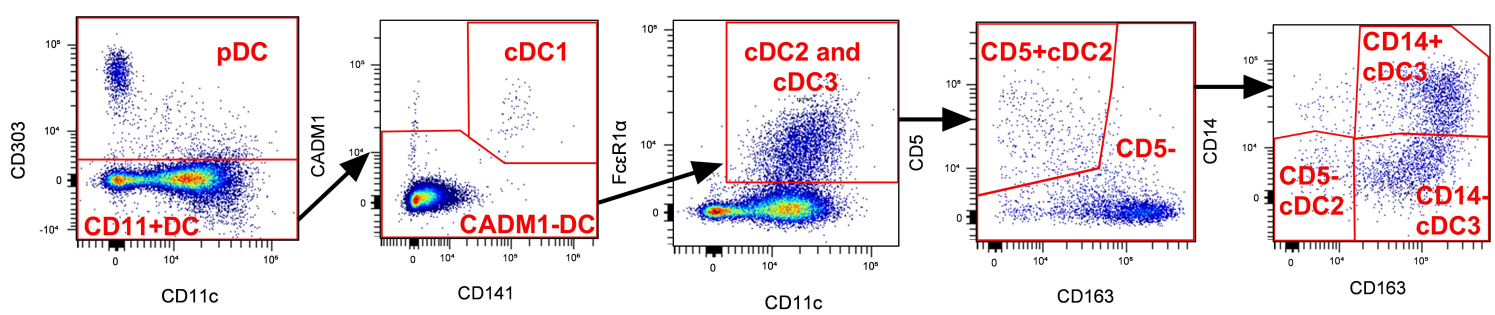
# Figure 4

**A**

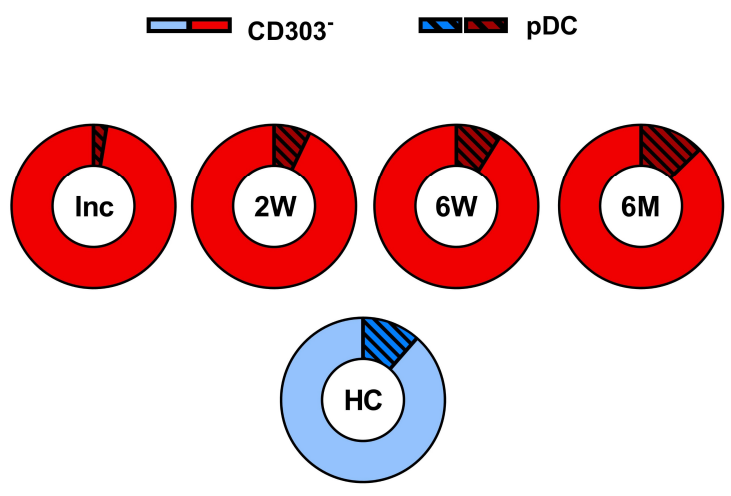
## DC clusters



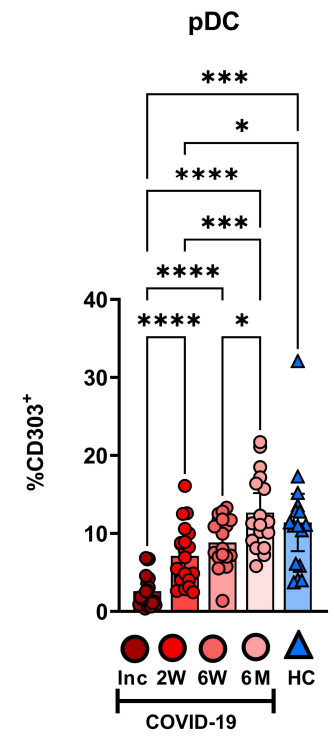
**B**



**C**

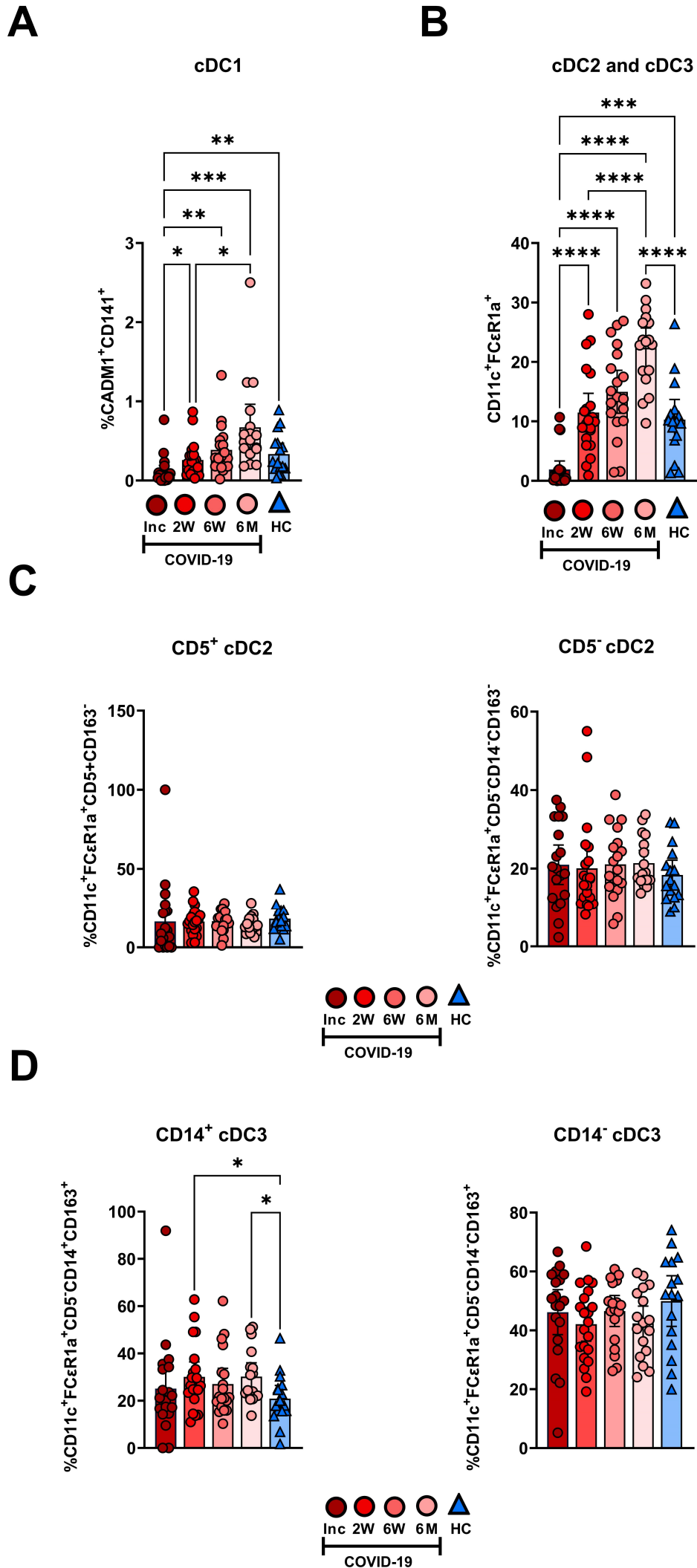


**D**

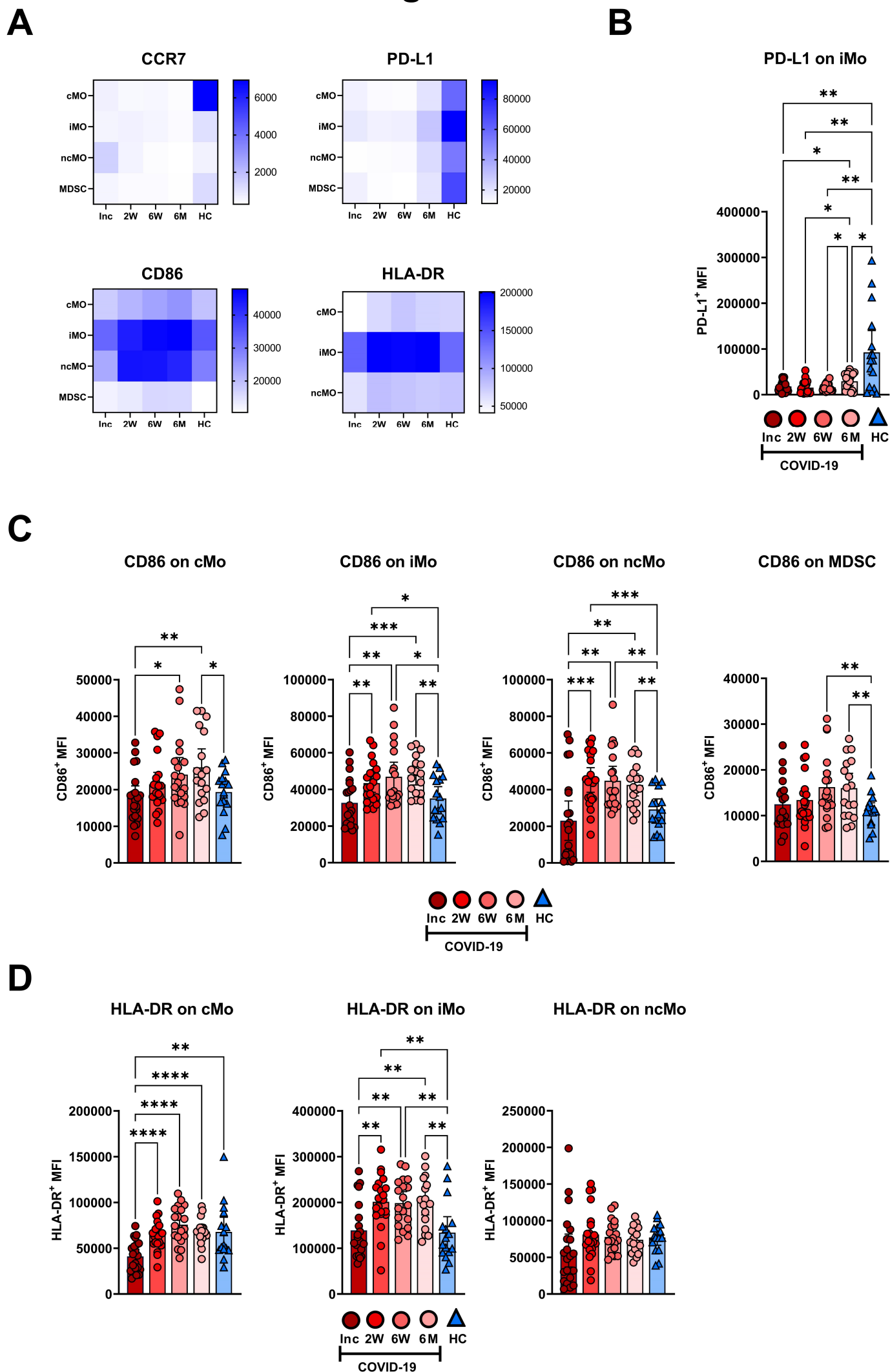




# Figure 5

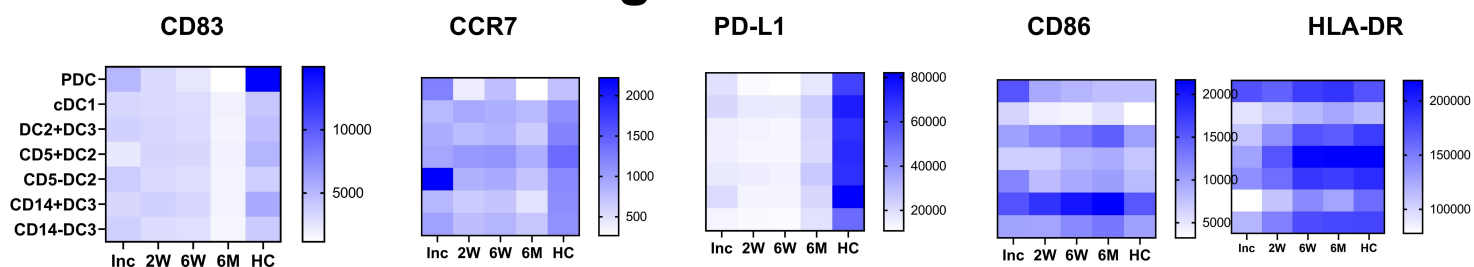


# Figure 6

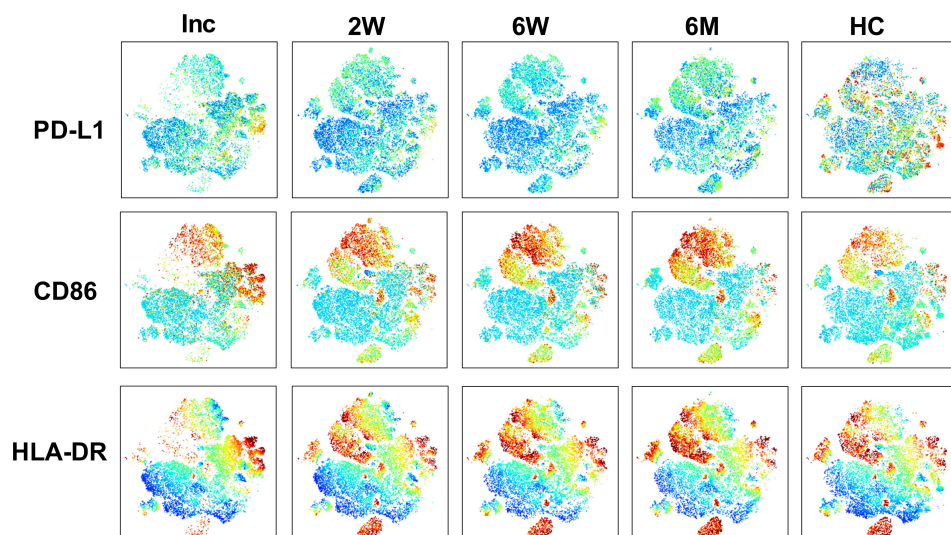


# Figure 7

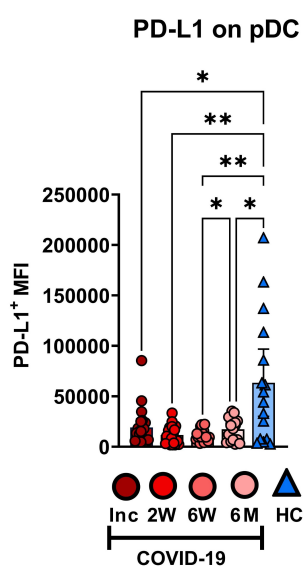
**A**



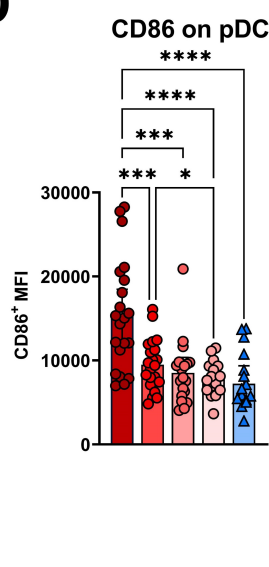
**B**



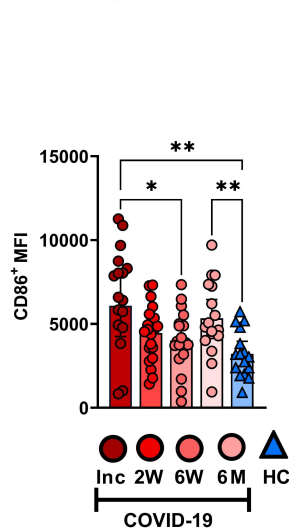
**C**



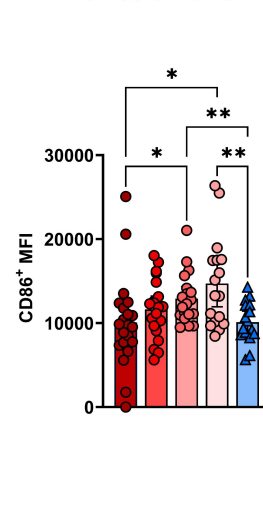
**D**



**CD86 on cDC1**

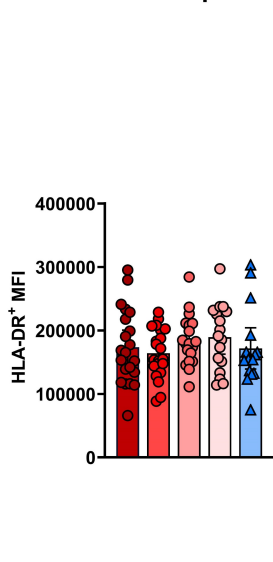


**CD86 on cDC2 and cDC3**

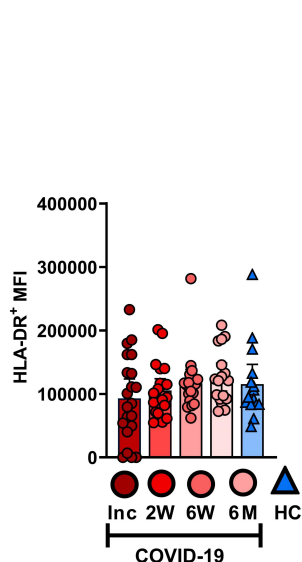


**E**

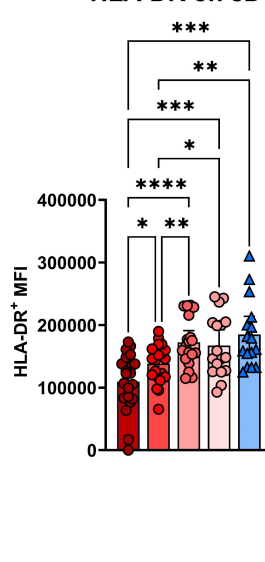
**HLA-DR on pDC**



**HLA-DR on cDC1**

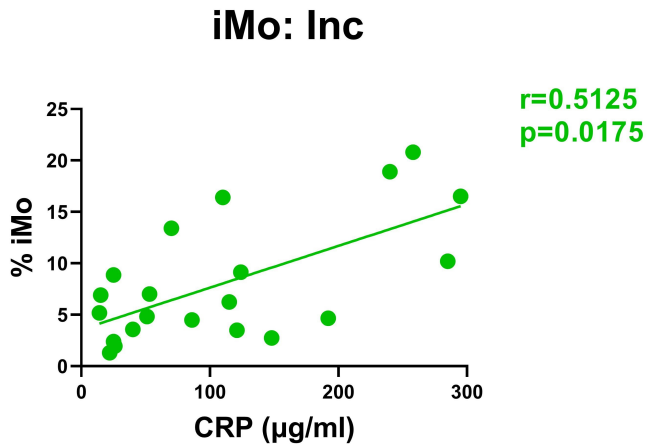


**HLA-DR on cDC2 and cDC3**



## Figure 8

**A**



**B**

