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# Supplementary Materials for

## Longitudinal immune profiling reveals key myeloid signatures associated with COVID-19

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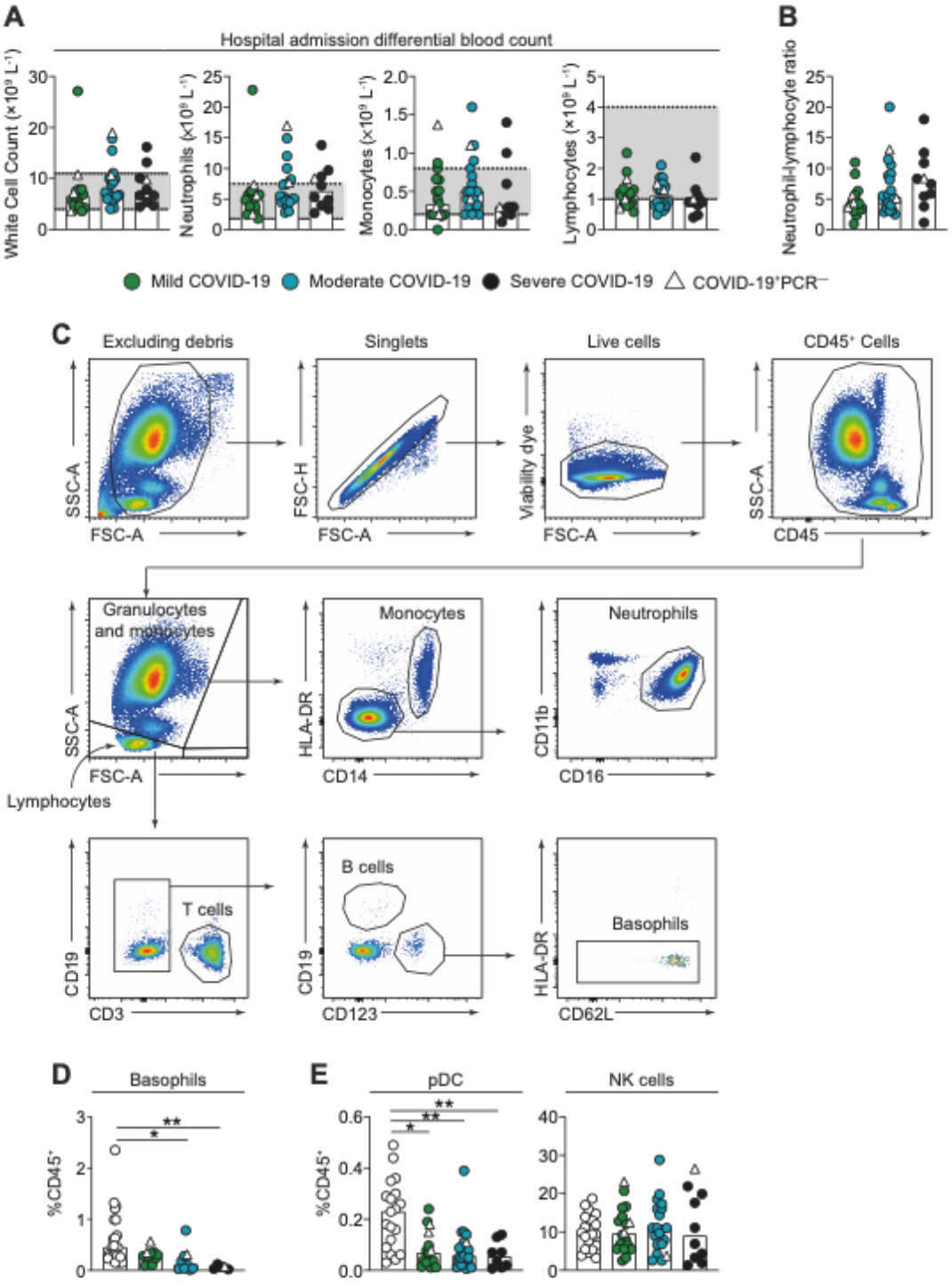
**This PDF file includes:**

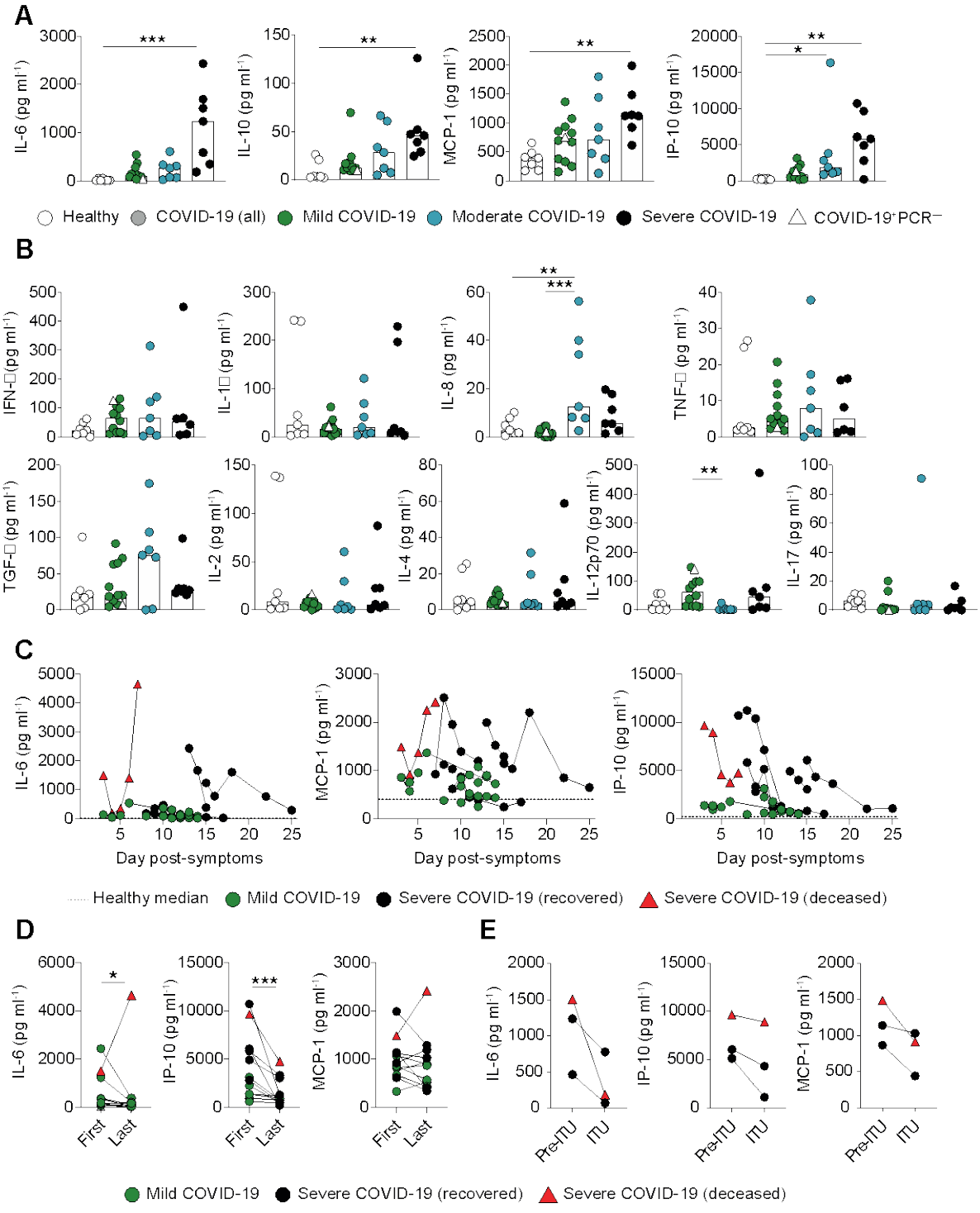


**Other Supplementary Material for this manuscript includes the following:**
(available at immunology.sciencemag.org/cgi/content/full/5/51/eabd6197/DC1)



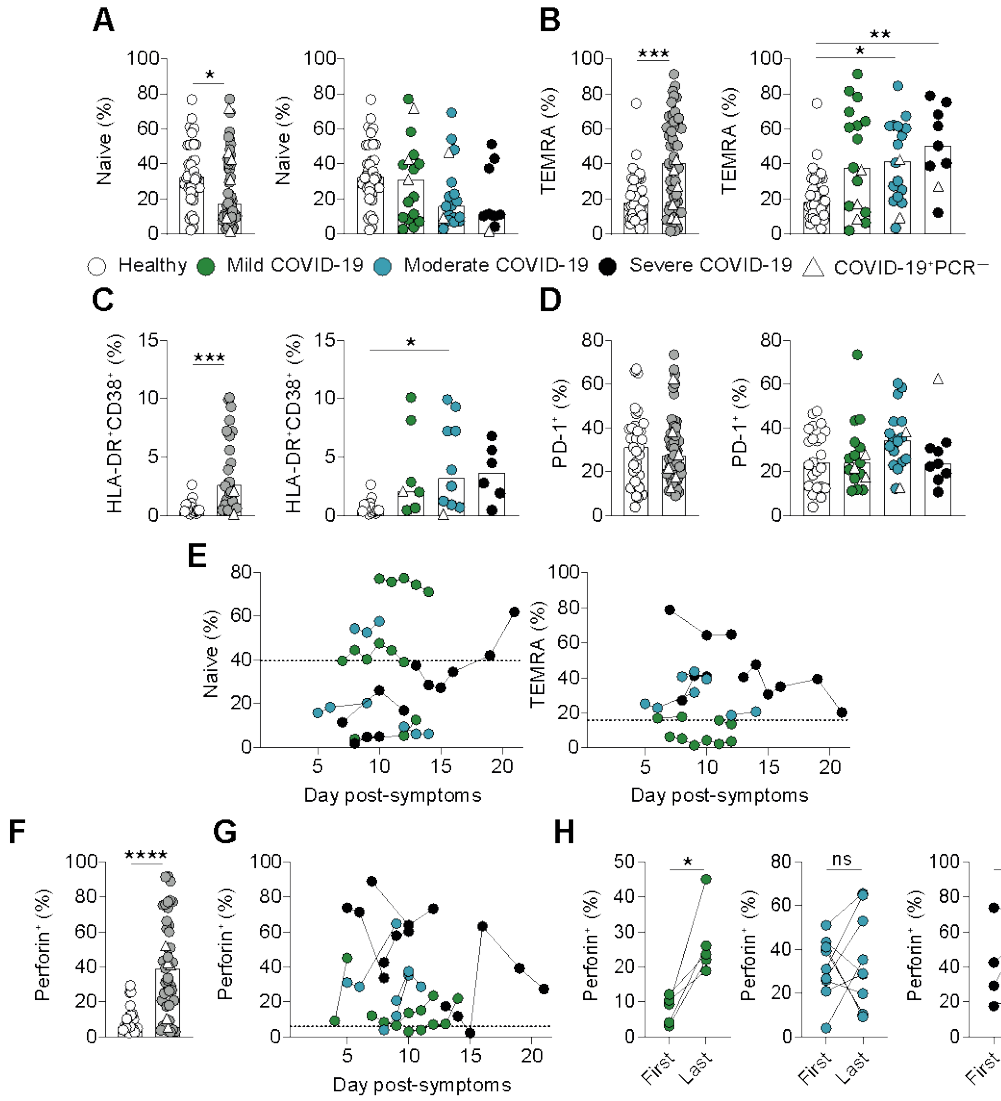

**Supplemental Figure 1**

**Figure S1: Immune cell types in COVID-19 patients. (A)** Hospital assessed white blood cell count (WCC), lymphocyte count, monocyte count, neutrophil count and **(B)** Neutrophil to lymphocyte ratio (NLR) calculated using hospital assessed neutrophil and lymphocyte counts. Grey region represents normal range. Patients stratified into mild (n=17), moderate (n=20) and severe (n=10) disease groups. **(C)** Representative FACS plots outline flow cytometric gating strategy used in analysis of whole blood samples. **(D)** Graph shows frequencies of basophils in whole blood samples in healthy individuals (n=10) and in COVID-19 patients with mild (n=10), moderate (n=9) and severe (n=5) disease. **(E)** Graphs show frequencies of $BDCA2^{+}CD123^{+}$ plasmacytoid DCs and $CD3^{-}$ $CD56^{+}$ NK cells in freshly prepared PBMC from healthy individuals (n=20) and in COVID-19 patients with mild (n=17), moderate (n=18) and severe (n=8) disease. Graphs show individual patient data with the bar representing median values. In all graphs, open triangles represent SARS-CoV-2 PCR negative patients. Kruskal Wallis with Dunn's post-hoc test: S1D; S1E Basophils. One-way ANOVA with Holm-Sidak post-hoc test: S1E NK cells. (*P<0.05, **P<0.01).



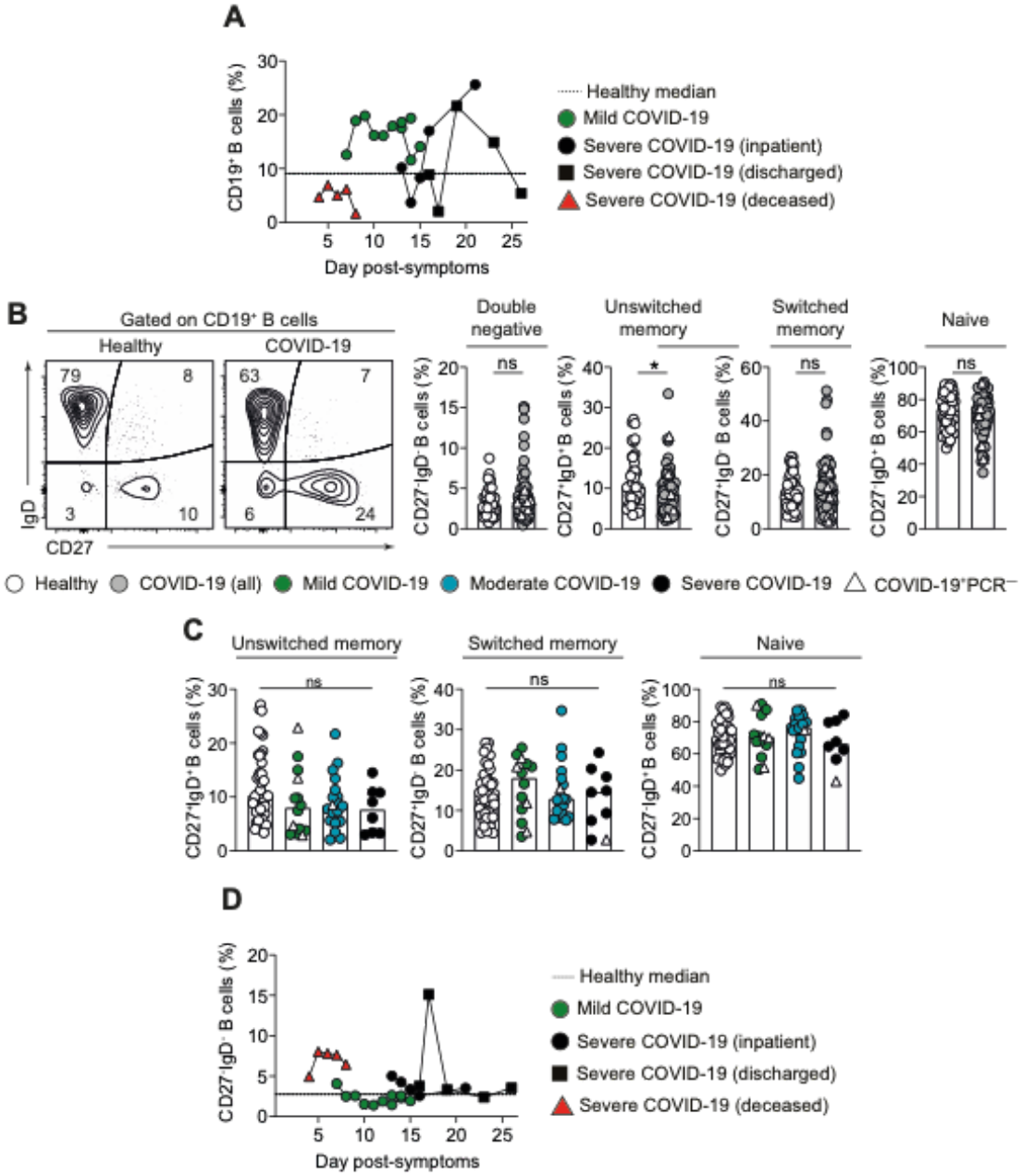

**Supplemental Figure 2**

**Figure S2. Serum cytokines and chemokines in COVID-19 patients. (A,B)** Levels of systemic IL-6, IL-10, MCP-1, IP-10, IFNγ, IL-1β, IL-8, TNF-α, TGF-β, IL-2, IL-4, IL-12p40 and IL-17 were measured in serum from healthy individuals (n=7) and COVID-19 patients with mild (n=12), moderate (n=7) and severe (n=7) disease using LEGENDplex assay. **(C)** Graphs show levels of systemic IL-6, MCP-1 and IP-10 in admitted patients over all measured time points, in mild (green shapes; n=12) and severe (black shapes; n=7) COVID-19 patients, with lines connecting data from the same patient. Deceased patient represented by red triangles. On these graphs x axis values represent the number of days since reported onset of symptoms and the dotted line the median value from healthy individuals. **(D)** Graphs showing serum levels of IL-6, IP-10 and MCP-1 at the first and last time points in mild patients (green circles) and severe patients (black circles). Red triangles represent severe patients that died and are not included in the statistical test. **(E)** Serum levels of IL-6, IP-10 and MCP-1 at last time-point pre-ITU and first time-point after transfer to ITU. Graphs show individual patient data with the bar representing median values. In all graphs, open triangles represent SARS-CoV-2 PCR negative patients. Kruskal Wallis with Dunn's post-hoc test: S2A IL-6, IL-10, IP-10; S2B IFN-γ, IL-1β, TNF-α, IL-2, IL-4, IL-12p70, IL-17. One-way ANOVA with Holm-Sidak post-hoc test: S2A MCP-1; S2B IL-8. Paired *t*-test: S2D MCP-1; S3E. Wilcoxon matched-pairs signed rank test: S2D IL-6 and IL-10. (*P<0.05, **P<0.01, ***P<0.001).



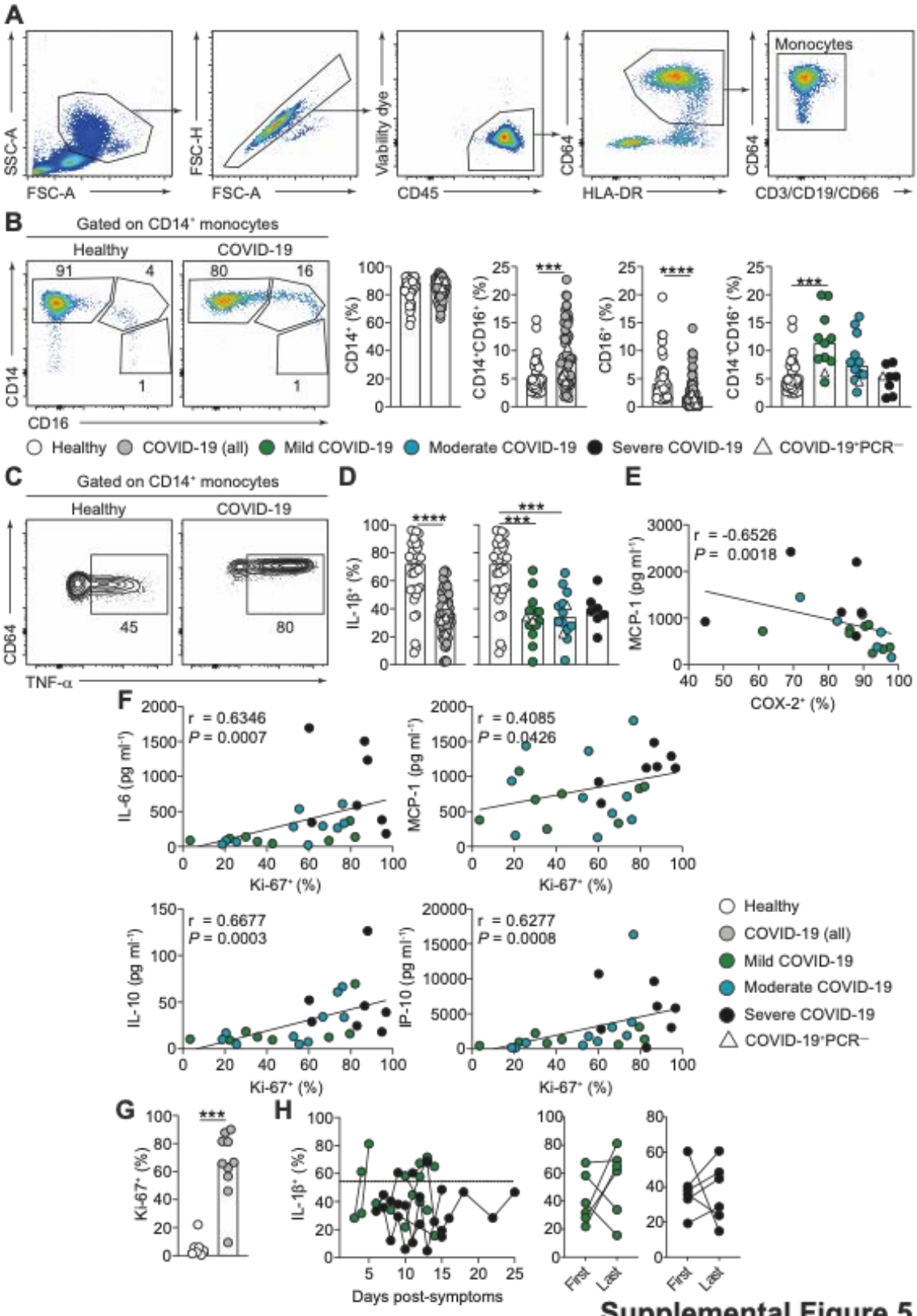

**Supplemental Figure 3**

**Figure S3. T cell subsets in COVID-19 patients. (A-D)** Cumulative data of frequencies of $CD8^+$ T cells which are **(A)** Naïve ($CD45RA^+CCR7^+$), **(B)** TEMRA ($CD45RA^+CCR7^-$), **(C)** $HLA\text{-}DR^+CD38^+$ or **(D)** $PD\text{-}1^+$ in healthy individuals (n=14-36) and COVID-19 patients (n=24-53). **(E)** Graphs show frequencies of $CD8^+$ T cells which are naïve and TEMRA in representative mild, moderate and severe COVID-19 patients at all time points examined. **(F)** Cumulative data of frequencies of $perforin^+$ cells amongst $CD8^+$ T cells in healthy individuals (n=21) and COVID-19 patients (n=43). **(G)** Frequencies of $CD8^+$ T cells which are $perforin^+$ in representative mild, moderate and severe COVID-19 patients at all time points examined. **(H)** Graphs showing frequency of $CD8^+$ T cells which are $perforin^+$ at the first and last time point in (left) mild (n=5) and moderate (n=9) patients and (right) severe (n=4) patients. Graphs show individual patient data with the bar representing median values. In all graphs, open triangles represent SARS-CoV-2 PCR negative patients. Mann-Whitney U test: S3A-D, 3F. Kruskal Wallis with Dunn's post-hoc test: S3A-D. Paired *t*-test: S3H. (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).




**Supplemental Figure 4**

**Figure S4. B cell subsets in COVID-19 patients. (A)** Frequencies of switched memory B cells ($CD27^+IgD^-$) in 3x representative mild and severe COVID-19 patients on the days following symptom onset at all time points examined. Dotted line is the median value from healthy individuals. **(B)** Representative flow cytometry plots and graphs showing frequencies of naïve ($CD27^-IgD^+$), unswitched memory ($CD27^+IgD^+$), switched memory ($CD27^+IgD^-$) and double negative ($CD27^-IgD^-$) B cells in healthy individuals (n=42) and COVID-19 patients (n=73). **(C)** Graphs show frequencies of naïve ($CD27^-IgD^+$), unswitched memory ($CD27^+IgD^+$) and switched memory ($CD27^+IgD^-$) in healthy individuals (n=42) and COVID-19 patients with mild (n=14), moderate (n=19) and severe (n=9) disease. **(D)** Frequencies of double negative B cells in 3x representative mild and severe COVID-19 patients on the days following symptom onset at all time points examined. Dotted line is the median value from healthy individuals. Graphs show individual patient data with the bars representing median values. In all graphs, open triangles represent SARS-CoV-2 PCR negative patients. Mann-Whitney U test; S4B. Kruskal Wallis with Dunn's post-hoc test; S4C. (*P<0.05; ns, not significant).




**Supplemental Figure 5**

**Figure S5. Monocytes in COVID-19 patients. (A)** Representative FACS plots outline flow cytometric gating strategy used for analysis of monocytes in PBMC samples. **(B)** Representative FACS plots of circulating classical ($CD14^+$), intermediate ($CD14^+16^+$) and non-classical ($CD16^+$) monocytes as a proportion of total monocytes in healthy individuals (n=37) and COVID-19 patients (n=60). COVID-19 patients were stratified into mild (n=11), moderate (n=12) and severe (n=8) disease. **(C)** Representative FACS plots demonstrating intracellular TNF-$\alpha$ staining in $CD14^+$ monocytes. **(D)** IL-1β production by $CD14^+$ monocytes following LPS stimulation in healthy individuals (n=34) and COVID-19 patients (n=56). COVID-19 patients were stratified into mild (n=14), moderate (n=14) and severe (n=7) disease. **(E)** Graph showing inverse correlation of serum levels of MCP-1 and frequencies of $COX\text{-}2^+$ $CD14^+$ monocytes in COVID-19 patients. **(F)** Graphs showing correlation of serum levels of IL-6, MCP-1, IL-10 and IP-10 and frequencies of $Ki\text{-}67^+$ $CD14^+$ monocytes in COVID-19 patients. **(G)** Ki-67 expression by unstimulated $CD14^+$ monocytes in healthy individuals (n=7) and COVID-19 patients (n=10). **(H)** (left) Longitudinal graph showing frequencies of $IL\text{-}1\beta^+$ $CD14^+$ monocytes following LPS stimulation in patients over all measured time points, in mild (green, n=7) and severe (black, n=6) COVID-19 patients. X axis represents the days since onset of symptoms and the dotted line is the median value from healthy individuals. (right) Graphs showing frequency of $IL\text{-}1\beta^+$ $CD14^+$ monocytes at the first and last time point in all mild (green circles) and severe (black circles) patients. Graphs show individual patient data with the bar representing median values. In all graphs, open triangles represent SARS-CoV-2 PCR negative patients. Mann-Whitney U test; S5B, S5D, S5G. Kruskal Wallis with Dunn's post-hoc test; S5B, S5D. Spearman ranked coefficient correlation test; S5E, S5F. (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).