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Supplemental information

Alterations in T and B cell function persist in convalescent COVID-19 patients

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Supplemental Table 1. Clinical characteristics of acute COVID-19 patients, Related to Figures 1-4**.** Data are median (IQR)[m] , where[m] is the number of missing data points, n (%) or n/N (%), where N is the total number with available data. PE, pulmonary embolism, AKI, Acute kidney injury. [a] Admission observations.

Acute Patients

	Overall (58)	**Mild (22)**	**Moderate (23)**	**Severe (13)**
Age	58.5 (49.5-71)	58 (45-71)	58 (50-66.5)	67 (55-74)
Gender				
Male	36/58 (62.1%)	13/22 (59.1%)	13/23 (56.5%)	10/13 (77%)
Female	22/58 (37.9%)	9/22 (40.9%)	10/23 (43.5%)	3/13 (23%)
BMI	28.5 (25.2-30.6)[14]	27.8 (23.2-33.1)[8]	29 (26.8-30.3)[3]	27.6 (25.4-30.2)[3]
Day of admission recruited	2 (2-3)	3 (2-4.75)	2 (2-3)	2 (2-3)
Medical History				
Diabetes	13/58 (22.41%)	6/22 (27.27%)	2/23 (8.7%)	5/13 (38.46%)
Ischaemic Heart Disease	8/58 (13.79%)	3/22 (13.64%)	2/23 (8.7%)	3/13 (23.08%)
Hypertension	20/58 (34.48%)	6/22 (27.27%)	9/23 (39.13%)	5/13 (38.46%)
COPD	11/58 (18.97%)	5/22 (22.73%)	4/23 (17.39%)	2/13 (15.38%)
Asthma	9/58 (15.52%)	4/22 (18.18%)	4/23 (17.39%)	1/13 (7.69%)
Malignancy	5/58 (8.6%)	0/22 (0%)	2/23 (8.7%)	3/13 (23%)
Differential counts on admission (x 10^9/L)				
Total white cell count	7.1 (6-10)[9]	6.85 (5.55-8.42)[4]	7.3 (6.3-10)[2]	7.25 (6.55-10.02)[3]
Lymphocytes	1 (0.82-1.5)[9]	1.2 (0.92-1.77)[4]	0.9 (0.8-1.5)[2]	0.9 (0.82-0.98)[3]
Neutrophils	5.3 (4.4-8)[9]	5.15 (3.8-7.05)[4]	5.5 (4.7-7.7)[2]	6.25 (4.82-8.62)[3]
Monocytes	0.4 (0.2-0.6)[9]	0.25 (0.2-0.73)[4]	0.5 (0.4-0.7)[2]	0.35 (0.3-0.57)[3]
Other inpatient investigations				
Positive SARS CoV2 PCR	49/58 (84%)	18/22 (81%)	20/23 (87%)	11/13 (85%)
Highest CRP (mg/L)	144 (83.9-226)	99 (35-176)	131 (86.5-195.6)	256 (198-283)
Chest Imaging				
Bilateral opacification	49/56 (87.5%)	14/20 (70%)	22/23 (95.7%)	13/13 (100%)
Unilateral opacification	3/56 (5.4%)	2/20 (10%)	1/23 (4.3%)	0/13 (0%)
Clear	4/56 (7.1%)	4/20 (20%)	0/23 (0%)	0/13 (0%)
Outcome				
Length of stay (days)	6 (4-11.3)[2]	5.5 (3.3-9.2)	6 (4-10)[2]	10 (6-13)
Mortality	9/58 (15.5%)	1/22 (4.5%)	0/23 (0%)	8/13 (61.5%)

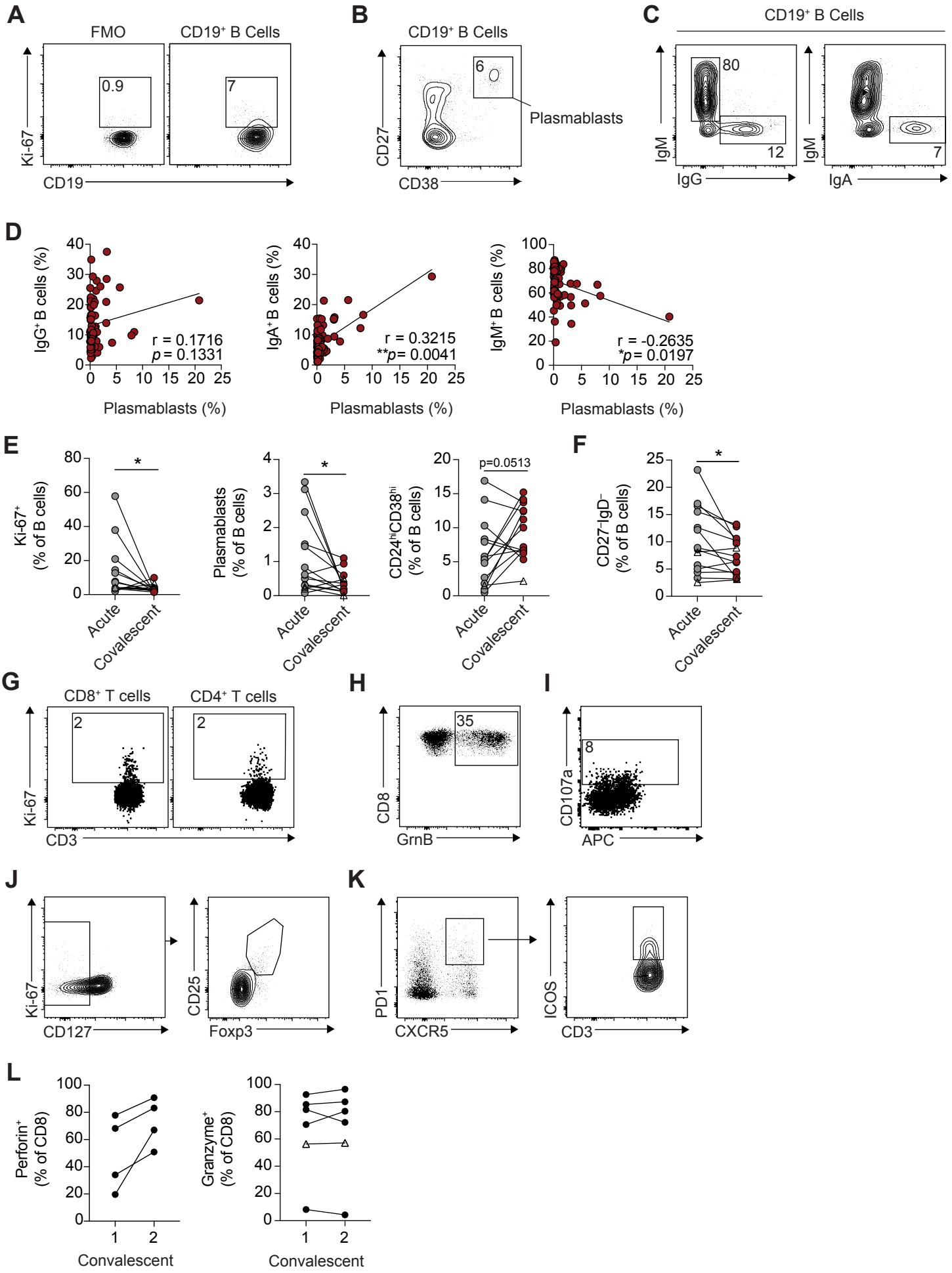
Supplemental Table 2. Clinical characteristics of convalescent COVID-19 patients Related to Figures 1-4. Data are median (IQR)[m] , where[m] is the number of missing data points, n (%) or n/N (%), where N is the total number with available data. PE, pulmonary embolism, AKI, Acute kidney injury. [a] Admission observations.

Convalescent Patients

	Overall (83)	Mild (15)	Moderate (27)	Severe (41)
Age (years)	60 (51 - 66.5)	51 (45 - 59.54)	60 (51.5 - 71)	60 (55.63 - 66)
Gender				
Male	51/83 (61.4%)	10/15 (66.7%)	14/27 (51.9%)	27/41 (65.9%)
Female	32/83 (38.6%)	5/15 (33.3%)	13/27 (48.1%)	14/41 (34.1%)
BMI	31 (28.7 - 34.7)[31]	34.8 (31.9 - 38.4)[5]	30.9 (28.8 - 34.6)[13]	29.6 (28.5 - 33.1)[13]
Medical History				
Diabetes	21/83 (25.3%)	4/15 (26.67%)	9/27 (33.33%)	8/41 (19.51%)
Ischaemic heart disease	17/83 (20.48%)	2/15 (13.33%)	12/27 (44.44%)	3/41 (7.32%)
Hypertension	32/83 (38.55%)	3/15 (20%)	13/27 (48.15%)	16/41 (39.02%)
COPD	14/83 (16.87%)	3/15 (20%)	9/27 (33.33%)	2/41 (4.88%)
Asthma	30/83 (36.14%)	7/15 (46.67%)	9/27 (33.33%)	14/41 (34.15%)
Malignancy	5/83 (6.02%)	0/15 (0%)	3/27 (11.1%)	2/41 (4.9%)
ACUTE ADMISSION				
Differential blood counts on acute admission ($x10^9$/L)				
Total white cell count	7.3 (5.77 - 10.1) [11]	6.1 (4.8 - 7.5) [2]	6.65 (5.63 - 9.55) [7]	8.7 (6.8 - 11.35)[2]
Lymphocytes	0.89 (0.7 - 1.21) [11]	1.1 (0.86 - 1.63) [2]	0.98 (0.73 - 1.42) [7]	0.8 (0.62 - 0.95)[2]
Neutrophils	5.99 (4.13 - 8.75) [11]	4.04 (3.7 - 5.7) [2]	4.81 (4.04 - 7.68) [7]	7.03 (5.34 - 10.2)[2]
Monocytes	0.4 (0.27 - 0.56) [11]	0.27 (0.23 - 0.55) [2]	0.48 (0.3 - 0.6) [7]	0.4 (0.3 - 0.52)[2]
Other inpatient investigations				
Positive SARS CoV2 test	78/83 (94%)	15/15 (100%)	25/27 (92.6%)	38/41 (92.7%)
Highest CRP (mg/L)	135 (66 - 245.5)[3]	50 (26.5 - 88.5)	113 (70 - 151.5)[1]	256 (131 - 330)[2]
Chest imaging				
Bilateral opacification	59/64 (92.1%)	7/9 (77.8%)	21/23 (91.3%)	31/32 (96.9%)
Unilateral opacification	4/64 (6.3%)	1/9 (11.1%)	2/23 (8.7%)	1/32 (3.1%)
Clear	1/64 (1.6%)	1/9 (11.1%)	0/23 (0%)	0/32 (0%)
Outcome				
Length of stay (days)	11 (5.5 – 21.5)	2 (1 - 5.5)	7 (4.5 - 13)	20 (13 - 39)
CONVALESCENCE				
Admission to follow up (days)	158 (116.5 - 184.5)	174 (140.5 - 185)	140 (105 - 171.5)	155 (126 - 192)
Discharge to follow up (days)	131 (90 - 178)	169 (138.5 - 181.5)	125 (95.5 - 165)	117 (87 - 180)
Symptoms and investigations at follow up				
Dyspnoea	38/82 (46.34%)	6/15 (40%)	16/27 (59.26%)	16/40 (40%)
Resolved CXR	55/83 (66.3%)	12/15 (80%)	19/27 (70.4%)	24/41 (58.5%)
Persistent CXR features	28/83 (33.7%)	3/15 (20%)	8/27 (29.6%)	17/41 (41.5%)

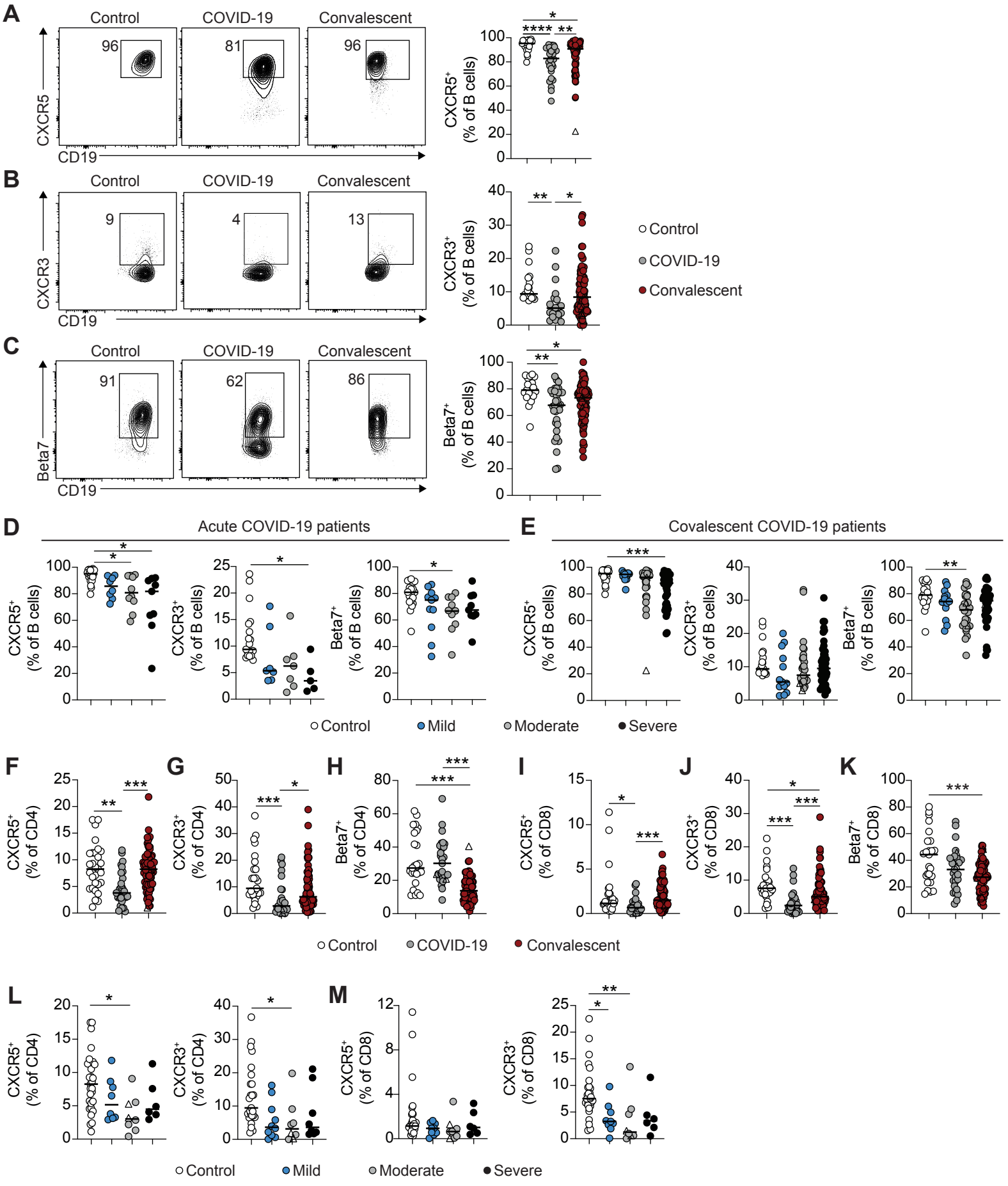
Supplemental Table 3. Patient categorisation Information Related to Figures 1-4**.** Criteria for patient stratification. NIV, non-invasive ventilation; CPAP, continuous positive airway pressure; ICU, intensive care.

Severity Score	**Criteria**
Mild	- <3l or 28% supplemental oxygen required to maintain oxygen saturations. - Managed in a ward based environment.
Moderate	- Breathless - <10l or <60% supplemental oxygen required to maintain oxygen saturations. - Managed in a ward based environment. - Chronic NIV or CPAP (home use) or acute NIV for COPD.
Severe	Any of: - >10l or 60% supplemental oxygen required to maintain oxygen saturations. - Use of acute NIV (not for COPD) - Managed in ICU/invasive ventilation.



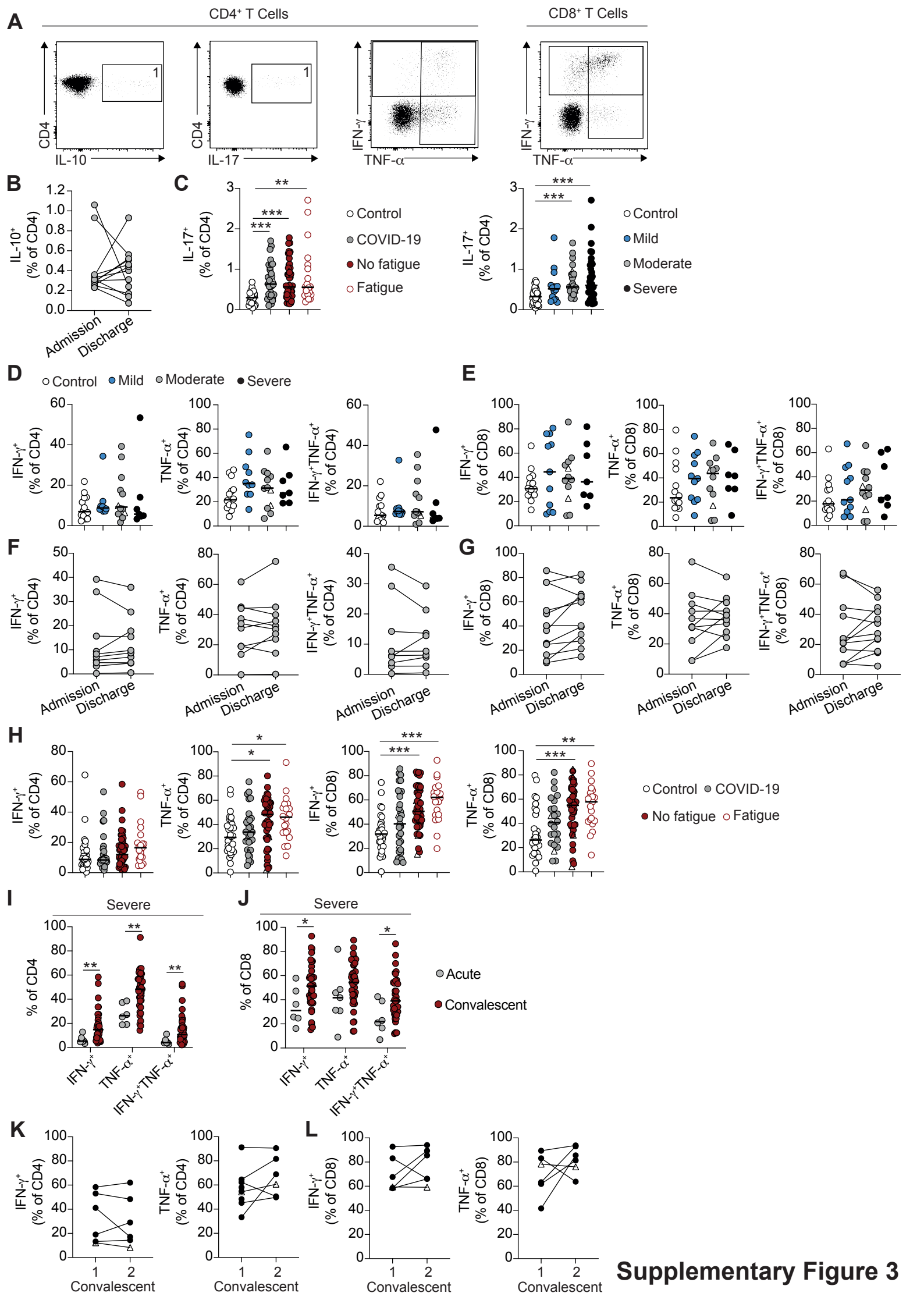
Supplementary Figure 1

Supplemental Figure 1. T and B cell subsets in hospitalized COVID-19 patients, Related to Figures 1 and 2. **(A)** Representative FACS plots showing Ki-67 staining on $CD19^+$ B cells. **(B)** Representative FACS plots showing the gating strategy for $CD27^{hi}CD38^{hi}$ plasmablasts. **(C)** Representative FACS plots gated on $CD19^+$ B cells staining positive for IgG, IgM or IgA.**(D)** Graphs showing correlation between plasmablasts and IgG^+ (left), IgA^+ (middle) or IgM^+ (right) B cell frequencies in convalescent COVID-19 patients (n=78) . **(E,F)** Graphs track frequencies of **(E)** $Ki67^+$ B cells, plasmablasts, transitional ($CD24^{hi}CD38^{hi}$) B cells, and **(F)** $CD27^-IgD^-$ B cells in the same COVID-19 patient at acute (grey circles) and convalescent (maroon circles) time-points (n=14). (**G**) Representative FACS plots showing Ki-67 staining on $CD4^+$ and $CD8^+$ T cells. **(H,I)** Representative FACS plots showing $CD8^+$ T cells staining positive for **(H)** GranzymeB and **(I)** CD107a. **(J)** Representative FACS plots gated on $CD3^+CD4^+CD127^{lo/neg}$ T cells staining positive for CD25 and foxp3. **(K)** Representative FACS plots showing gating to identify Tfh cells ($CD3^+CD4^+$PD- 1^+CXCR5^+ $ICOS^+$). **(L)** Graphs track frequencies of $CD8^+$ T cells which are $perforin^+$ and $granzymeB^+$ in the same COVID-19 patient at convalescent time-points pre (1) and post (2) 6 months since hospital discharge (n=4-6). In all graphs, triangles represent SARS-CoV-2 PCR-negative patients.



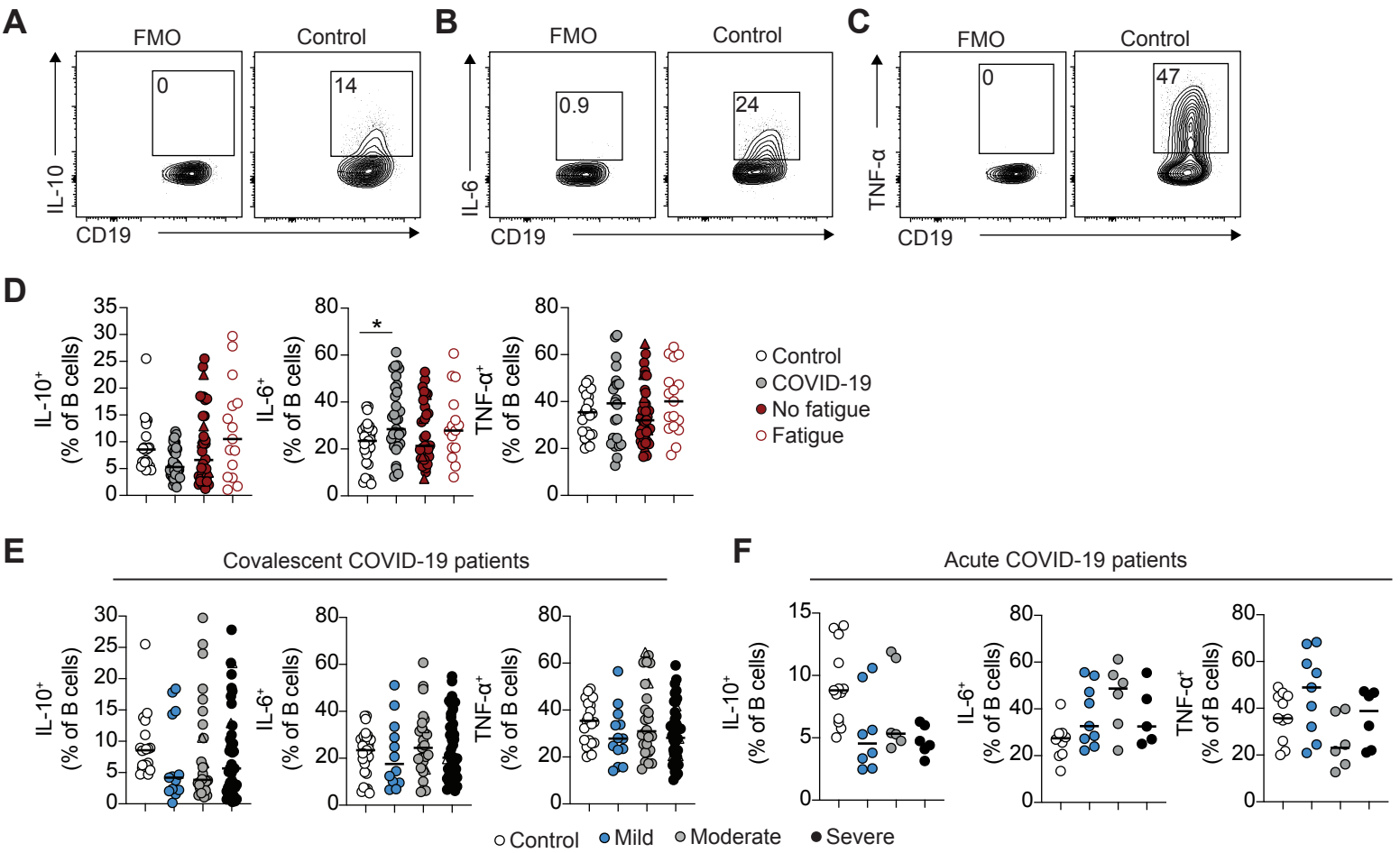
Supplementary Figure 2

Supplemental Figure 2. Altered expression of migratory markers in acute but not convalescent COVID-19 patients, Related to Figures 1 and 2. **(A-C)** Representative flow cytometry plots and graphs showing frequencies of B cells expressing **(A)** CXCR5, **(B)** CXCR3 and **(C)** β7 in healthy individuals (n=19-21), acute (n=21-34) and convalescent (n=81) COVID-19 patients. **(D)** Graphs showing frequencies of B cells expressing CXCR3, CXCR5 and β7 in healthy individuals (n=19) and acute COVID-19 patients with mild (n=7-11), moderate (n=7-10) and severe (n=5-9) disease. **(E)** Graphs showing frequencies of B cells staining positive for CXCR3, CXCR5 and β7 in healthy individuals (n=19-21) and convalescent COVID-19 patients which initially presented with mild (n=13), moderate (n=26) and severe (n=41) disease. **(F-H)** Graphs showing frequencies of $CD4^+$ cells positive for **(F)** CXCR5, **(G)** CXCR3 and **(H)** β7 in healthy individuals (n=25-29), acute (n=26-27) and convalescent (n=80-83) COVID-19 patients. **(I-K)** Graphs showing frequencies of $CD8^+$ cells positive for **(I)** CXCR5, **(J)** CXCR3 and **(K)** β7 in healthy individuals (n=25-29), acute (n=27-28) and convalescent (n=80-81) COVID-19 patients. **(L,M)** Graphs showing frequencies of **(L)** $CD4^+$ and **(M)** $CD8^+$ T cells staining positive for CXCR3 and CXCR5 in healthy individuals (n=25-29) and acute COVID-19 patients with mild (n=8-10), moderate (n=8) and severe (n=6-8) disease. Graphs show individual patient data, with the bar representing median values. In all graphs, triangles represent SARS-CoV-2 PCR-negative patients. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, one-way ANOVA with Holm-Sidak post-hoc testing (A-E, K and L (CXCR5)) or Kruskal-Wallis test with Dunn's post-hoc testing (F-J, L(CXCR3), M) for multiple comparisons.



Supplementary Figure 3

Supplemental Figure 3. Long-lasting changes in type-I cytokine production T cells in convalescent COVID-19 patients, Related to Figure 3. **(A)** Representative FACS plots showing example staining for cytokine$^+$ CD4$^+$ and CD8$^+$ T cells. **(B)** Graph shows frequency of IL-10$^+$CD4$^+$ T cells following 3 hour stimulation with PMA and ionomycin in acute COVID-19 patients at the first and last time points of hospitalization. **(C)** Graph show frequencies of IL-17$^+$CD4$^+$ T cells following 3 hour stimulation with PMA and ionomycin in healthy individuals (n=22-30), acute COVID-19 patients (n=31) and convalescent COVID-19 patients stratified by fatigue reporting (not reporting (n=50) and reporting (n=20) enhanced fatigue) and mild (n=14), moderate (n=27) and severe (n=36) acute disease severity. **(D, E)** Graphs show frequencies of (**D**) CD4$^+$ and (**E**) CD8$^+$ T cells which stain positive for IFNγ and TNFα following 3 hour stimulation with PMA and ionomycin in healthy individuals (n=14) and acute COVID-19 patients with mild (n=10-11), moderate (n=12) and severe (n=7) disease. (**F, G**) Graphs show frequencies of **(F)** CD4$^+$ and **(G)** CD8$^+$ T cells that stain positive for IFNγ and TNFα following 3 hour stimulation with PMA and ionomycin in acute COVID-19 patients at the first and last time points of hospitalization. **(H)** Graphs show frequencies of CD4$^+$ and CD8$^+$ T cells which stain positive for IFNγ and TNFα following 3 hour stimulation with PMA and ionomycin in healthy individuals (n=28-30)**,** acute COVID-19 patients (n=24-33) and convalescent COVID-19 patients not reporting (n=44-49) and reporting (n=20-21) enhanced fatigue. **(I, J)** Graphs show frequencies of CD4$^+$ and CD8$^+$ T cells which stain positive for IFNγ and TNFα in all severe COVID-19 patients at acute (grey) and convalescent (maroon) time-points. **(K, L)** Graphs track frequencies of CD4$^+$ and CD8$^+$ T cells which stain positive for IFNγ and TNFα following 3 hour stimulation with PMA and ionomycin in the same COVID-19 patient at convalescent time-points pre (1) and post (2) 6 months since hospital discharge (n=4-6). Graphs show individual patient data, with the bar representing median values. In all graphs, triangles represent SARS-CoV-2 PCR-negative patients. *p<0.05, **p<0.01, ***p<0.001, one-way ANOVA with Kruskal-Wallis test with Dunn's post-hoc testing for multiple comparisons (except for graphs showing CD4$^+$TNFα^+ and CD8$^+$TNFα^+ T cells in D and E where One-way ANOVA with Holm-Sidak post-hoc test was employed).



Supplementary Figure 4

Supplemental Figure 4. Cytokine production by B cells from acute and convalescent COVID-19 patients, Related to Figure 3. **(A-C)** Representative flow cytometry plots showing gating strategy of $CD19^+$ B cells positive for IL-10, IL-6 and TNFα following 48 hour stimulation with CpGB. **(D)** Graphs show frequencies of $CD19^+$ B cells staining positive IL-10, IL-6 and TNFα following 48 hour stimulation with CpGC in healthy individuals (n=22-27), acute COVID-19 patients (n=22-37) and convalescent COVID-19 patients not reporting (n=36-38) and reporting (n=15-17) enhanced fatigue. **(E)** Graphs show frequencies $CD19^+$ B cells staining positive for IL-10, IL-6 and TNFα in healthy individuals (n=22-23), and convalescent COVID-19 patients which initially presented with mild (n=12-13), moderate (n=25-26) and severe (n=41) disease. **(F)** Graphs show frequencies $CD19^+$ B cells staining positive for IL-10, IL-6 and TNFα in healthy individuals (n=11-13), and acute COVID-19 patients with mild (n=8-9), moderate (n=6) and severe (n=6) disease. Graphs show individual patient data, with the bar representing median values. *$p<0.05$, one-way ANOVA with Kruskal-Wallis test with Dunn's post-hoc testing for multiple comparisons.