

Pediatric Long COVID Is Characterized by Myeloid CCR6 Suppression and Immune Dysregulation – Supplemental information

Supplemental Table 1: 37-color panel used for spectral cytometry.

| Marker | Fluorochrome | Clone |
|------------------|---------------------|--------------|
| CD19 | Spark NIR 685 | HIB19 |
| HLADR | BV510 | L243 |
| IgD | BV480 | IA6-2 |
| IgM | BV570 | MHM-88 |
| IgA | FITC | REA1014 |
| CD27 | APC | M-T271 |
| CD21 | V405 | B-ly4 |
| CD38 | APC/Fire 810 | HIT2 |
| CD24 | BUV615 | ML5 |
| CD11c | BUV805 | B-ly6 |
| CCR7 | BV421 | G043H7 |
| CCR6 | BV711 | G034E3 |
| CXCR5 | BV750 | RF8B2 |
| CXCR3 | PE/Cy7 | G025H6 |
| CD1c | Alexa Fluor 647 | L161 |
| PD-1 | BV785 | EH12.2H7 |
| CD95 | PE/Cy5 | DX2 |
| CD25 | PE-Alexa Fluor 700 | 3G10 |
| CD127 | APC-R700 | HIL-7R-M21 |
| CD3 | PerCP | OKT3 (SKT7) |
| TCR V α 2 | PerCP-Vio | REA771 |
| CD14 | Spark Blue 550 | 63D3 |
| CD4 | cFluor 568 | SK3 |
| CD8 | Pacific Orange | 3B5 |
| CD45RA | BUV395 | 5H9 |
| CD28 | BV650 | CD28.2 |
| CD57 | BV605 | C10-1 |
| CCR5 | BUV563 | 2D7 |
| CD56 | BUV737 | NCAM16.2 |
| CD16 | BUV496 | 3G8 |
| NKG2C | BUV661 | 134591 |
| NKG2A | PE-Vio615 | REA110 |
| NKp46 | APC/Fire 750 | 9E2 |
| CD123 | Super Bright 436 | 6H6 |
| CD11b | BB515 | ICRF44 |
| CD169 | BB700 | 7-239 |
| CD137L | PE | 5F4 |

Supplemental Table 2: Cell count, marker annotation and myeloid cell population for 17 clusters

| Cluster | Cell count | Marker | | | | | | | | Myeloid cell population 1 | Myeloid cell population 2 |
|---------|------------|--------|------|-------|-------|------|-------|-------|------|---------------------------|---------------------------|
| | | CD14 | CD16 | CD11c | CD123 | CD1c | CD11b | HLADR | CD95 | | |
| 1 | 342,575 | + | + | + | - | - | + | + | + | Monocytes | Intermediate monocytes |
| 2 | 7,583,745 | + | - | + | - | - | + | + | + | Monocytes | Classical monocytes |
| 3 | 192,759 | - | + | + | - | - | - | + | + | Monocytes | Non-classical monocytes |
| 4 | 8,905 | - | + | - | - | - | low | low | + | Monocytes | Non-classical monocytes |
| 5 | 33,642 | - | + | - | - | - | low | - | + | Monocytes | Non-classical monocytes |
| 6 | 452,565 | - | - | + | - | + | - | + | + | Dendritic cells | cDCs |
| 7 | 68,514 | + | - | + | - | + | -/low | + | + | Dendritic cells | cDCs |
| 8 | 273,434 | + | - | + | - | - | low | + | + | Monocytes | Classical monocytes |
| 9 | 69,908 | - | - | - | - | - | - | low | + | Dendritic cells | CD123- CD11c- DCs |
| 10 | 74,789 | + | - | low | - | - | low | low | + | Monocytes | Classical monocytes |
| 11 | 11,384 | + | - | + | + | - | + | + | + | Monocytes | Classical monocytes |
| 12 | 1,441,128 | + | - | + | - | - | + | low | + | Monocytes | Classical monocytes |
| 13 | 649,848 | + | - | + | - | - | + | - | + | Monocytes | Classical monocytes |
| 14 | 653,258 | - | - | - | + | - | - | + | - | Dendritic cells | pDCs |
| 15 | 72,292 | - | - | + | - | - | - | + | - | Dendritic cells | cDCs |
| 16 | 354,045 | - | - | - | - | - | - | + | - | Dendritic cells | CD123- CD11c- DCs |
| 17 | 40,095 | + | - | - | - | - | + | + | -/+ | Monocytes | Classical monocytes |

Supplemental Table 3: Cell count, marker annotation and NK-cell population for 33 clusters

| Cluster | Cell count | Marker | | | | | | | | NK-cell population |
|---------|------------|--------|------|------|-------|-------|-------|------|-------|-----------------------|
| | | CD56 | CD16 | CD57 | NKG2C | NKG2A | NKp46 | CCR5 | CXCR3 | |
| 1 | 2,306,493 | dim | + | - | - | + | - | - | - | CD56dim |
| 2 | 46,356 | - | + | - | - | - | - | - | + | CD56- |
| 3 | 56,548 | dim | + | - | - | - | - | - | + | CD56dim |
| 4 | 393,957 | - | + | - | - | - | - | - | - | CD56- |
| 5 | 3,284,317 | dim | + | - | - | - | - | - | - | CD56dim |
| 6 | 18,331 | dim | + | - | - | - | - | + | + | CD56dim |
| 7 | 350,004 | dim | + | - | - | + | - | - | + | CD56dim |
| 8 | 33,772 | dim | - | - | - | + | - | - | + | CD56dim |
| 9 | 99,504 | dim | - | - | - | + | + | - | + | CD56dim |
| 10 | 187,417 | dim | + | - | - | + | + | - | + | CD56dim |
| 11 | 25,002 | - | + | - | - | - | + | - | low | CD56- |
| 12 | 150,315 | dim | + | - | - | - | + | - | + | CD56dim |
| 13 | 379,804 | dim | + | + | - | - | - | - | - | CD56dim |
| 14 | 85,325 | dim | + | - | - | + | + | + | + | CD56dim |
| 15 | 211,698 | bright | + | - | - | + | + | - | + | CD56bright |
| 16 | 45,591 | bright | - | - | - | - | + | - | + | CD56bright |
| 17 | 492,009 | bright | - | - | - | + | + | - | + | CD56bright |
| 18 | 42,988 | bright | + | - | + | - | + | - | + | CD56bright and NKG2C+ |
| 19 | 181,583 | dim | + | + | - | + | - | - | - | CD56dim |
| 20 | 273,266 | dim | + | + | + | - | - | - | - | CD56dim and NKG2C+ |
| 21 | 23,394 | dim | - | - | - | + | - | + | + | CD56dim |
| 22 | 17,574 | dim | - | - | - | + | + | + | + | CD56dim |
| 23 | 222,765 | dim | - | - | - | - | - | - | - | CD56dim |
| 24 | 17,905 | bright | - | - | + | - | + | - | + | CD56bright and NKG2C+ |

| | | | | | | | | | | |
|----|---------|--------|---|---|---|---|---|-----|-----|-----------------------|
| 25 | 84,213 | bright | - | - | + | + | + | - | + | CD56bright and NKG2C+ |
| 26 | 46,549 | bright | - | - | - | + | - | - | low | CD56bright |
| 27 | 73,682 | bright | - | - | - | + | + | - | low | CD56bright |
| 28 | 327,552 | dim | + | - | + | - | - | - | - | CD56dim and NKG2C+ |
| 29 | 26,106 | dim | - | - | + | - | - | - | + | CD56dim and NKG2C+ |
| 30 | 6,280 | - | + | - | - | - | - | low | + | CD56- |
| 31 | 44,625 | dim | - | - | - | - | - | - | + | CD56dim |
| 32 | 42,584 | dim | - | - | - | - | - | + | low | CD56dim |
| 33 | 66,657 | dim | + | - | + | - | - | - | - | CD56dim and NKG2C+ |

Supplemental Table 4: Cell count, marker annotation and T-cell population for 37 clusters

| Cluster | Cell count | Marker | | | | | | | | | | | | T cell population 1 | T cell population 2 |
|---------|------------|--------|-----|-----|--------|------|------|------|------|-------|-------|------|-------|---------------------|------------------------------------|
| | | TCRVd2 | CD4 | CD8 | CD45RA | CCR7 | CD95 | CD27 | CD28 | CCR6 | CXCR3 | CD25 | CD127 | | |
| 1 | 2,504,123 | - | - | - | -/+ | -/+ | -/+ | -/+ | -/+ | -/+ | -/+ | - | -/+ | DN T cells | - |
| 2 | 16,264,042 | - | + | - | + | + | - | + | + | - | - | - | + | CD4 T cells | Tn CD4 |
| 3 | 1,109,850 | - | + | - | + | + | - | + | + | - | - | + | - | CD4 T cells | Treg |
| 4 | 7,785,873 | - | - | + | + | + | - | + | + | - | -/+ | - | + | CD8 T cells | Tn CD8 |
| 5 | 1,427,263 | - | - | + | - | - | + | + | + | -/+ | - | + | -/+ | CD8 T cells | Ttm CD8 |
| 6 | 667,707 | - | - | + | + | + | + | + | + | - | + | - | + | CD8 T cells | Tscm CD4 |
| 7 | 496,750 | - | - | + | + | - | + | + | - | - | + | -/+ | + | CD8 T cells | Temra CD8 |
| 8 | 111,744 | - | + | - | + | + | + | + | + | -/+ | + | -/+ | -/+ | CD4 T cells | Tscm CD4 |
| 9 | 245,538 | - | + | - | + | + | - | + | + | - | + | - | + | CD4 T cells | Tn CD4 |
| 10 | 53.276 | - | + | - | + | - | + | + | + | -/+ | + | -/+ | + | CD4 T cells | Temra CD4 |
| 11 | 21.127 | - | + | - | + | + | + | + | + | - | - | - | - | CD4 T cells | Tscm CD4 |
| 12 | 1.931.523 | - | - | + | - | - | + | + | + | + | -/+ | - | + | CD8 T cells | Ttm CD8 |
| 13 | 1.554.760 | - | + | - | - | + | + | + | + | - | - | -/+ | + | CD4 T cells | Tcm CD4 |
| 14 | 238.059 | - | + | - | - | - | + | + | + | - | - | -/+ | + | CD4 T cells | Ttm CD4 |
| 15 | 685.024 | - | - | + | + | - | -/+ | + | - | - | -/+ | - | - | CD8 T cells | Temra CD8 |
| 16 | 307,529 | - | - | + | - | + | + | + | + | low/+ | + | + | + | CD8 T cells | Tcm CD8 |
| 17 | 1,611,587 | - | - | + | + | - | + | - | - | - | -/+ | - | - | CD8 T cells | Temra CD8 |
| 18 | 490,180 | - | - | + | - | - | + | + | - | - | -/+ | - | - | CD8 T cells | Tem CD8 |
| 19 | 632,504 | - | - | + | - | - | + | - | - | - | -/+ | - | - | CD8 T cells | CD8+, CD45RA-, CCR7-, CD27-, CD28- |
| 20 | 2,278,361 | - | + | - | - | + | + | + | + | - | + | -/+ | + | CD4 T cells | Tcm CD4 |
| 21 | 949,601 | - | + | - | - | - | + | + | + | - | + | -/+ | + | CD4 T cells | Ttm CD4 |
| 22 | 10,791 | - | + | + | - | -/+ | + | + | + | - | - | -/+ | + | DP T cells | - |
| 23 | 70,577 | - | - | + | - | + | + | + | + | - | - | -/+ | + | CD8 T cells | Tcm CD8 |

| | | | | | | | | | | | | | | | |
|----|-----------|---|---|---|-----|-----|---|---|-----|-------|-----|-----|---|------------------------|------------------------------------|
| 24 | 25,965 | - | - | + | - | - | + | + | + | - | - | -/+ | + | CD8 T cells | Ttm CD8 |
| 25 | 84,7667 | - | + | - | - | -/+ | + | + | + | -/+ | -/+ | + | - | CD4 T cells | Treg |
| 26 | 307,928 | - | + | - | - | - | + | - | - | - | -/+ | - | - | CD4 T cells | CD4+, CD45RA-, CCR7-, CD27-, CD28- |
| 27 | 177,860 | - | - | + | - | - | + | - | + | + | -/+ | - | + | CD8 T cells | CD8+, CD45RA-, CCR7-, CD27-, CD28+ |
| 28 | 1,265,483 | - | + | - | - | - | + | - | + | - | -/+ | -/+ | + | CD4 T cells | Tem CD4 |
| 29 | 1,155,959 | - | + | - | - | + | + | + | + | + | + | + | + | CD4 T cells | Tcm CD4 |
| 30 | 522,811 | - | + | - | - | - | + | + | + | + | + | + | + | CD4 T cells | Ttm CD4 |
| 31 | 1,176,983 | - | + | - | - | + | + | + | + | + | - | + | + | CD4 T cells | Tcm CD4 |
| 32 | 169,533 | - | + | - | - | - | + | + | + | + | - | + | + | CD4 T cells | Ttm CD4 |
| 33 | 406,018 | - | + | - | - | - | + | - | + | + | + | + | + | CD4 T cells | Tem CD4 |
| 34 | 362,257 | - | + | - | - | - | + | - | + | + | - | + | + | CD4 T cells | Tem CD4 |
| 35 | 333,288 | + | - | + | -/+ | - | - | + | -/+ | - | + | low | + | $\gamma\delta$ T cells | - |
| 36 | 3,042,351 | + | - | - | -/+ | - | - | + | -/+ | - | + | - | + | $\gamma\delta$ T cells | - |
| 37 | 1,196,647 | + | - | - | - | - | + | + | + | low/+ | + | low | + | $\gamma\delta$ T cells | - |

Supplemental Table 5: Cell count, marker annotation and B-cell population for 31 clusters

| Cluster | Cell count | Marker | | | | | | | | | | | | B cell population |
|---------|------------|--------|-----|-----|------|------|------|------|-------|------|------|-------|-------|-------------------------|
| | | IgD | IgM | IgA | CD27 | CD21 | CD38 | CD24 | CD11c | CCR7 | CCR6 | CXCR5 | CXCR3 | |
| 1 | 113,849 | - | - | + | + | + | low | + | - | + | + | + | - | Switched memory B cells |
| 2 | 41,077 | - | - | + | + | + | low | low | -/+ | - | + | + | - | Switched memory B cells |
| 3 | 30,013 | - | -/+ | - | + | - | high | - | - | - | - | - | - | Plasmablasts |
| 4 | 27,104 | - | - | + | + | - | high | - | - | - | - | - | - | Plasmablasts |
| 5 | 29,758 | - | - | - | - | - | - | - | + | - | + | - | + | Switched memory B cells |
| 6 | 22,410 | - | - | -/+ | low | - | high | - | - | - | - | - | - | Plasmablasts |
| 7 | 3,089,971 | + | + | - | - | + | low | low | - | + | + | + | - | Naïve B cells |
| 8 | 19,772 | + | - | - | - | - | - | - | -/+ | - | + | - | + | IgD-only B cells |
| 9 | 50,271 | + | + | - | - | - | + | + | - | - | low | + | - | Transitional B cells |
| 10 | 175,072 | + | + | - | - | + | high | high | - | - | low | -/+ | - | Transitional B cells |
| 11 | 38,086 | - | - | + | + | + | low | + | - | + | + | + | + | Switched memory B cells |
| 12 | 69,857 | - | - | - | + | + | - | low | - | - | + | + | - | Switched memory B cells |
| 13 | 138,813 | - | - | - | + | + | low | + | - | + | + | + | + | Switched memory B cells |
| 14 | 52,186 | + | - | - | - | + | - | - | - | - | - | + | - | IgD-only B cells |
| 15 | 21,621 | - | - | - | - | - | - | - | - | - | - | low | - | Switched memory B cells |
| 16 | 28,476 | - | - | - | + | -/+ | low | low | -/+ | - | + | + | + | Switched memory B cells |
| 17 | 55,557 | - | - | - | + | + | - | high | - | - | + | + | - | Switched memory B cells |
| 18 | 206,392 | - | - | - | low | + | low | + | - | + | + | + | - | Switched memory B cells |
| 19 | 13,268 | - | -/+ | - | + | + | + | - | low | - | + | low | - | Switched memory B cells |
| 20 | 586,535 | + | + | - | - | + | + | + | - | + | + | + | - | Transitional B cells |
| 21 | 18,601 | - | + | - | + | + | - | high | - | - | + | + | + | IgM-only B cells |
| 22 | 20,245 | - | + | - | + | + | low | high | - | + | + | + | - | IgM-only B cells |
| 23 | 81,751 | + | + | - | + | + | - | high | - | - | + | + | - | MZ-like B cells |
| 24 | 338,391 | + | + | - | low | + | - | + | - | + | + | + | - | Naïve B cells |

| | | | | | | | | | | | | | | |
|----|--------|---|---|---|-----|-----|-----|------|-----|---|---|-----|-----|----------------------|
| 25 | 25,560 | + | + | - | + | + | - | high | low | - | + | + | + | MZ-like B cells |
| 26 | 37,847 | + | + | - | low | + | - | + | - | + | + | + | + | Naïve B cells |
| 27 | 96,888 | + | + | - | - | + | low | low | - | + | + | + | + | Naïve B cells |
| 28 | 5,427 | + | - | - | + | + | - | -/+ | -/+ | - | + | + | -/+ | IgD-only B cells |
| 29 | 14,453 | + | + | - | - | low | - | - | -/+ | - | + | low | + | Naïve B cells |
| 30 | 20,324 | + | + | - | - | + | - | + | - | - | + | + | + | Naïve B cells |
| 31 | 14,056 | + | + | - | - | + | + | + | - | - | + | + | + | Transitional B cells |

Supplemental Table 6: Frequency and annotation of different immune cell populations in CYP with and without LC.

| Cell type | Annotation | LC cohort | Control cohort | LC cohort | Control cohort |
|---|---|--------------------------------|--------------------------------|---------------------------|---------------------------|
| | | % of total PBMCs (median, IQR) | % of total PBMCs (median, IQR) | % of parent (median, IQR) | % of parent (median, IQR) |
| Monocytes | (SSC ⁻ A/FSC ⁻ A) CD14 ^{+/-} , CD16 ^{-/+} | 10.15 (7.61, 12.6) | 11.51 (10.00, 16.74) | - | - |
| Classical monocytes | CD14 ⁺ , CD16 ⁻ | - | - | 94.71 (93.30, 96.23) | 95.05 (93.46, 96.70) |
| Intermediate monocytes | CD14 ⁺ , CD16 ⁺ | - | - | 2.91 (1.95, 3.98) | 3.02 (1.96, 4.45) |
| Non-classical monocytes | CD14 ⁻ , CD16 ⁺ | - | - | 2.18 (1.40, 2.85) | 1.50 (0.65, 2.26) |
| Dendritic Cells | CD3 ⁻ , CD19 ⁻ , HLADR ⁺ , CD14 ^{-/+} , CD16 ⁻ | | 1.65 (1.45, 1.81) | - | - |
| cDCs | CD14 ^{-/+} , CD16 ⁻ , CD11c ⁺ , CD123 ⁻ | - | - | 38.62 (32.32, 46.16) | 34.46 (25.68, 42.62) |
| pDCs | CD14 ⁻ , CD16 ⁻ , CD11c ⁻ , CD123 ⁺ | - | - | 34.17 (27.26, 42.80) | 40.42 (35.87, 47.16) |
| CD11c ⁻ , CD123 ⁻ DCs | CD14 ⁻ , CD16 ⁻ , CD11c ⁻ , CD123 ⁻ | - | - | 19.84 (15.11, 24.86) | 16.43 (13.41, 22.26) |
| NK cells | CD3 ⁻ , CD19 ⁻ , HLADR ⁻ , CD14 ⁻ | 9.29 (7.16, 13.19) | 9.76 (5.81, 12.68) | - | - |
| Early NK cells | CD56 ^{bright} | - | - | 10.23 (6.97, 14.92) | 12.52 (8.46, 15.00) |
| Mature NK cells | CD56 ^{dim} | - | - | 84.52 (78.21, 88.24) | 82.85 (78.95, 84.69) |
| Exhausted NK cells | CD56 ⁻ | - | - | 4.09 (2.67, 5.85) | 3.95 (2.33, 5.73) |
| NKG2C ⁺ memory NK cells | NKG2C ⁺ | - | - | 3.35 (1.41, 10.95) | 1.98 (1.07, 5.68) |
| CD4 T cells | CD14 ⁻ , CD3 ⁺ , TCRVd2 ⁻ , CD4 ⁺ , CD8 ⁻ | 31.57 (26.73, 35.67) | 25.74 (21.08, 34.33) | - | - |
| CD4 Tn | CD45RA ⁺ , CCR7 ⁺ , CD95 ⁻ | - | - | 57.07 (48.38, 64.21) | 53.24 (42.01, 57.95) |
| CD4 Tscm | CD45RA ⁺ , CCR7 ⁺ , CD95 ⁺ | - | - | 0.30 (0.22,0.43) | 0.31 (0.24,0.42) |
| CD4 Tcm | CD45RA ⁻ , CCR7 ⁺ | - | - | 20.80 (15.61, 25.48) | 23.90 (15.61, 25.48) |
| CD4 Ttm | CD45RA ⁻ , CCR7 ⁻ , CD27 ⁺ , CD28 ⁺ | - | - | 5.69 (4.05, 7.94) | 6.06 (4.43, 9.77) |
| CD4 Tem | CD45RA ⁻ , CCR7 ⁻ , CD27 ⁻ , CD28 ⁺ | - | - | 6.42 (4.05, 7.94) | 6.06 (4.43, 9.77) |
| CD45RA ⁻ , CCR7 ⁻ , CD27 ⁻ , CD28 ⁻ | CD45RA ⁻ , CCR7 ⁻ , CD27 ⁻ , CD28 ⁻ | - | - | 0.11 (0.03, 0.94) | 0.11 (0.06, 1.17) |
| CD4 Temra | CD45RA ⁺ , CCR7 ⁻ | - | - | 0.06 (0.04, 0.11) | 0.07 (0.04, 0.10) |
| CD4 Treg | CD25 ⁺ , CD127 ⁻ | - | - | 6.77 (5.55, 8.04) | 6.30 (5.22, 7.26) |

| | | | | | |
|---|---|----------------------|----------------------|----------------------|----------------------|
| CD8 T cells | CD14 ⁻ , CD3 ⁺ , TCRVd2 ⁻ , CD4 ⁻ , CD8 ⁺ | 17.35 (14.80, 19.36) | 19.01 (16.43, 21.81) | - | - |
| CD8 Tn | CD45RA ⁺ , CCR7 ⁺ , CD95 ⁻ | - | - | 48.35 (38.29, 60.20) | 42.96 (31.70, 54.98) |
| CD8 Tscm | CD45RA ⁺ , CCR7 ⁺ , CD95 ⁺ | - | - | 3.63 (2.56, 4.77) | 4.75 (3.36, 5.62) |
| CD8 Tcm | CD45RA ⁻ , CCR7 ⁺ | - | - | 2.03 (1.12, 3.46) | 1.22 (0.65, 1.59) |
| CD45RA ⁻ , CCR7 ⁻ , CD27 ⁻ , CD28 ⁺ | CD45RA ⁻ , CCR7 ⁻ , CD27 ⁻ , CD28 ⁺ | - | - | 0.69 (0.44, 1.44) | 0.61 (0.46, 1.88) |
| CD8 Ttm | CD45RA ⁻ , CCR7 ⁻ , CD27 ⁺ , CD28 ⁺ | - | - | 20.31 (15.06, 25.88) | 19.01 (14.08, 26.81) |
| CD8 Tem | CD45RA ⁻ , CCR7 ⁻ , CD27 ⁺ , CD28 ⁻ | - | - | 2.59 (1.68, 4.16) | 2.80 (1.69, 4.03) |
| CD45RA ⁻ , CCR7 ⁻ , CD27 ⁻ , CD28 ⁻ | CD45RA ⁻ , CCR7 ⁻ , CD27 ⁻ , CD28 ⁻ | - | - | 2.58 (1.28, 5.44) | 3.09 (0.90, 8.47) |
| CD8 Temra | CD45RA ⁺ , CCR7 ⁻ | - | - | 13.51 (6.22, 21.14) | 14.51 (11.06, 28.55) |
| γδ T cells | CD14 ⁻ , CD3 ⁺ , TCRVd2 ⁺ | 3.78 (2.38, 5.87) | 4.00 (2.28, 6.91) | - | - |
| DN T cells | CD14 ⁻ , CD3 ⁺ , TCRVd2 ⁻ , CD4 ⁻ , CD8 ⁻ | 2.46 (1.98, 3.15) | 2.78 (2.41, 3.84) | - | - |
| DP T cells | CD14 ⁻ , CD3 ⁺ , TCRVd2 ⁻ , CD4 ⁺ , CD8 ⁺ | 0.007 (0.004, 0.014) | 0.006 (0.004, 0.007) | - | - |
| B cells | CD3 ⁻ , CD19 ⁺ , CD14 ⁻ | 5.91 (4.73, 7.52) | 8.13 (4.67, 9.52) | - | - |
| Transitional B cell | IgD ⁺ , IgM ⁺ , CD27 ⁻ , CD38 ^{+/hi} , CD24 ^{+/hi} | - | - | 12.85 (9.42, 18.04) | 16.46 (12.09, 21.62) |
| Naïve B cell | IgD ⁺ , IgM ⁺ , CD27 ⁻ , CD38 ^{-/lo} | - | - | 65.77 (58.83, 70.72) | 69.97 (61.01, 73,85) |
| MZ-like B cell | IgD ⁺ , IgM ⁺ , CD27 ⁺ , CD21 ⁺ | - | - | 1.32 (0.76, 1.98) | 0.63 (0.48, 1.49) |
| IgM-only B cell | IgM ⁺ , IgD ⁻ , CD27 ⁺ | - | - | 0.64 (0.41, 1.04) | 0.55 (0.39, 0.66) |
| IgD-only B cell | IgD ⁺ , IgM ⁻ | - | - | 1.56 (1.15, 2.65) | 1.59 (0.91, 2.31) |
| Switched memory B cells | IgD ⁻ , IgM ⁻ , CD38 ^{-/+} | - | - | 13.19 (9.90, 18.32) | 10.77 (9.15, 12.94) |
| Plasmablasts | IgD ⁻ , IgM ⁻ , CD38 ^{hi} | - | - | 1.05 (0.66, 1.80) | 0.91 (0.70, 1.80) |

Supplemental Table 7: Coefficients and p-values of linear regression models

| | Coefficients | | | | LRT p values | | | |
|-----------------------|--------------|----------|-------------------------|------------|--------------|-------|----------|----------|
| | Age | Sex=Male | Exposure= Two exposures | Group=PASC | Age | Sex | Exposure | Group |
| S2 IgG | 0.024 | -0.091 | 0.711 | 0.061 | 0.235 | 0.418 | 6.12E-07 | 0.669 |
| RBD IgG | 0.014 | 0.269 | 1.109 | -0.743 | 0.562 | 0.066 | 4.41E-10 | 8.99E-05 |
| S2 IgA | 0.039 | -0.001 | 0.318 | -0.061 | 0.038 | 0.925 | 0.011 | 0.652 |
| RBD IgA | 0.013 | 0.013 | 0.728 | -0.310 | 0.549 | 0.915 | 2.01E-06 | 0.046 |
| Neutralization | 0.039 | 0.204 | 0.568 | -0.330 | 0.192 | 0.112 | 1.47E-04 | 0.048 |

Supplemental Table 8: SARS-CoV-2 antigen and RNA persistence in CYP with and without long COVID

| ID | Cohort | Spike (fg/mL) | Nucelocapsid (fg/mL) | 3'UTR SARS-CoV-2 (copies/mL) | N SARS-CoV-2 (copies/mL) | Ng SARS-CoV-2 (copies/mL) | Nsg SARS-CoV-2 (copies/mL) |
|----|------------|---------------|----------------------|------------------------------|--------------------------|---------------------------|----------------------------|
| 1 | Long COVID | - | - | - | - | - | - |
| 2 | Long COVID | - | 842 | - | - | - | - |
| 3 | Long COVID | - | - | - | - | - | - |
| 4 | Long COVID | - | - | - | - | - | - |
| 6 | Long COVID | - | - | - | - | - | - |
| 7 | Long COVID | - | - | - | - | - | - |
| 8 | Long COVID | - | - | - | - | - | - |
| 9 | Long COVID | - | - | - | - | - | - |
| 10 | Long COVID | - | - | - | - | - | - |
| 12 | Long COVID | - | - | - | - | - | - |
| 14 | Long COVID | - | - | - | - | - | - |
| 15 | Long COVID | - | 11,944 | - | - | - | - |
| 17 | Long COVID | - | - | - | - | - | - |
| 19 | Long COVID | - | - | - | - | - | - |
| 20 | Long COVID | - | - | - | - | - | - |
| 21 | Long COVID | - | - | - | - | - | - |
| 23 | Long COVID | - | - | - | - | - | - |
| 24 | Long COVID | - | - | - | - | - | - |
| 25 | Long COVID | - | - | - | - | - | - |
| 26 | Long COVID | - | - | - | - | - | - |
| 27 | Long COVID | 260 | - | - | - | - | - |
| 28 | Long COVID | 262 | - | - | - | - | - |
| 29 | Long COVID | - | - | - | - | - | - |
| 30 | Long COVID | - | - | - | - | - | - |
| 31 | Long COVID | - | - | - | - | - | - |

| | | | | | | | |
|----|------------|-----|-----|----|---|---|----|
| 33 | Long COVID | - | - | - | - | - | - |
| 34 | Long COVID | - | - | - | - | - | - |
| 35 | Long COVID | - | - | - | - | - | - |
| 36 | Long COVID | - | - | - | - | - | - |
| 37 | Long COVID | - | - | - | - | - | - |
| 38 | Long COVID | 358 | - | - | - | - | - |
| 39 | Long COVID | - | - | - | - | - | - |
| 40 | Long COVID | 267 | - | - | - | - | - |
| 42 | Long COVID | - | - | - | - | - | - |
| 43 | Long COVID | - | - | - | - | - | - |
| 44 | Long COVID | - | - | - | - | - | - |
| 45 | Long COVID | - | - | - | - | - | - |
| 46 | Long COVID | - | - | - | - | - | - |
| 47 | Long COVID | - | - | - | - | - | - |
| 48 | Long COVID | - | - | 14 | - | - | - |
| 49 | Long COVID | - | - | - | - | - | - |
| 50 | Long COVID | - | - | - | - | - | - |
| 51 | Long COVID | - | - | - | - | - | 14 |
| 52 | Long COVID | - | - | - | - | - | - |
| 53 | Long COVID | - | - | - | - | - | - |
| 55 | Long COVID | - | 527 | - | - | - | - |
| 56 | Long COVID | - | - | - | - | - | - |
| 57 | Long COVID | - | - | - | - | - | - |
| 58 | Long COVID | - | - | - | - | - | - |
| 59 | Long COVID | - | - | - | - | - | - |
| 60 | Long COVID | - | - | - | - | - | - |
| 61 | Long COVID | - | - | - | - | - | - |
| 62 | Long COVID | - | 529 | - | - | - | - |

| | | | | | | | |
|----|------------|-----|-------|---|----|---|---|
| 63 | Long COVID | - | - | - | - | - | - |
| 65 | Long COVID | - | - | - | - | - | - |
| 67 | Long COVID | - | - | - | - | - | - |
| 68 | Long COVID | 250 | 9,324 | - | - | - | - |
| 69 | Long COVID | - | - | - | - | - | - |
| 70 | Long COVID | - | - | - | - | - | - |
| 71 | Long COVID | - | - | - | - | - | - |
| 73 | Long COVID | - | - | - | - | - | - |
| 74 | Long COVID | - | 8,555 | - | - | - | - |
| 76 | Long COVID | - | - | - | - | - | - |
| 77 | Long COVID | - | - | - | 13 | - | - |
| 78 | Long COVID | - | - | - | - | - | - |
| 79 | Long COVID | - | - | - | - | - | - |
| 80 | Long COVID | - | - | - | - | - | - |
| 81 | Long COVID | - | - | - | - | - | - |
| 82 | Long COVID | - | - | - | - | - | - |
| 83 | Long COVID | - | - | - | - | - | - |
| 84 | Long COVID | - | - | - | - | - | - |
| 85 | Long COVID | - | - | - | - | - | - |
| 86 | Long COVID | - | 869 | - | - | - | - |
| 87 | Long COVID | - | - | - | - | - | - |
| 88 | Long COVID | - | - | - | - | - | - |
| 89 | Long COVID | - | - | - | - | - | - |
| 90 | Long COVID | - | - | - | - | - | - |
| 91 | Long COVID | - | - | - | - | - | - |
| 92 | Long COVID | - | - | - | - | - | - |
| 93 | Long COVID | - | - | - | - | - | - |
| 94 | Long COVID | - | - | - | - | - | - |

| | | | | | | | |
|-----|------------|-----|-----|---|----|---|----|
| 96 | Long COVID | - | 659 | - | - | - | - |
| 98 | Long COVID | - | - | - | - | - | - |
| 99 | Long COVID | - | - | - | - | - | - |
| 100 | Long COVID | - | - | - | - | - | - |
| 101 | Long COVID | - | - | - | - | - | - |
| 102 | Long COVID | - | - | - | - | - | - |
| 103 | Long COVID | - | 671 | - | - | - | - |
| 104 | Long COVID | - | - | - | 16 | - | - |
| 105 | Long COVID | - | - | - | - | - | - |
| 106 | Long COVID | - | - | - | - | - | - |
| 107 | Long COVID | 267 | - | - | - | - | 13 |
| 108 | Long COVID | - | - | - | - | - | - |
| 109 | Long COVID | - | - | - | - | - | - |
| 110 | Long COVID | - | - | - | - | - | - |
| 111 | Long COVID | - | - | - | - | - | - |
| 112 | Long COVID | - | - | - | - | - | - |
| 114 | Long COVID | - | - | - | - | - | - |
| 115 | Long COVID | - | - | - | - | - | - |
| C1 | Controls | - | - | - | - | - | - |
| C2 | Controls | - | - | - | - | - | - |
| C3 | Controls | - | - | - | - | - | - |
| C4 | Controls | - | - | - | - | - | - |
| C5 | Controls | - | - | - | - | - | - |
| C7 | Controls | - | - | - | - | - | 25 |
| C8 | Controls | - | - | - | - | - | - |
| C9 | Controls | - | - | - | - | - | - |
| C10 | Controls | - | - | - | - | - | - |
| C11 | Controls | - | - | - | - | - | - |

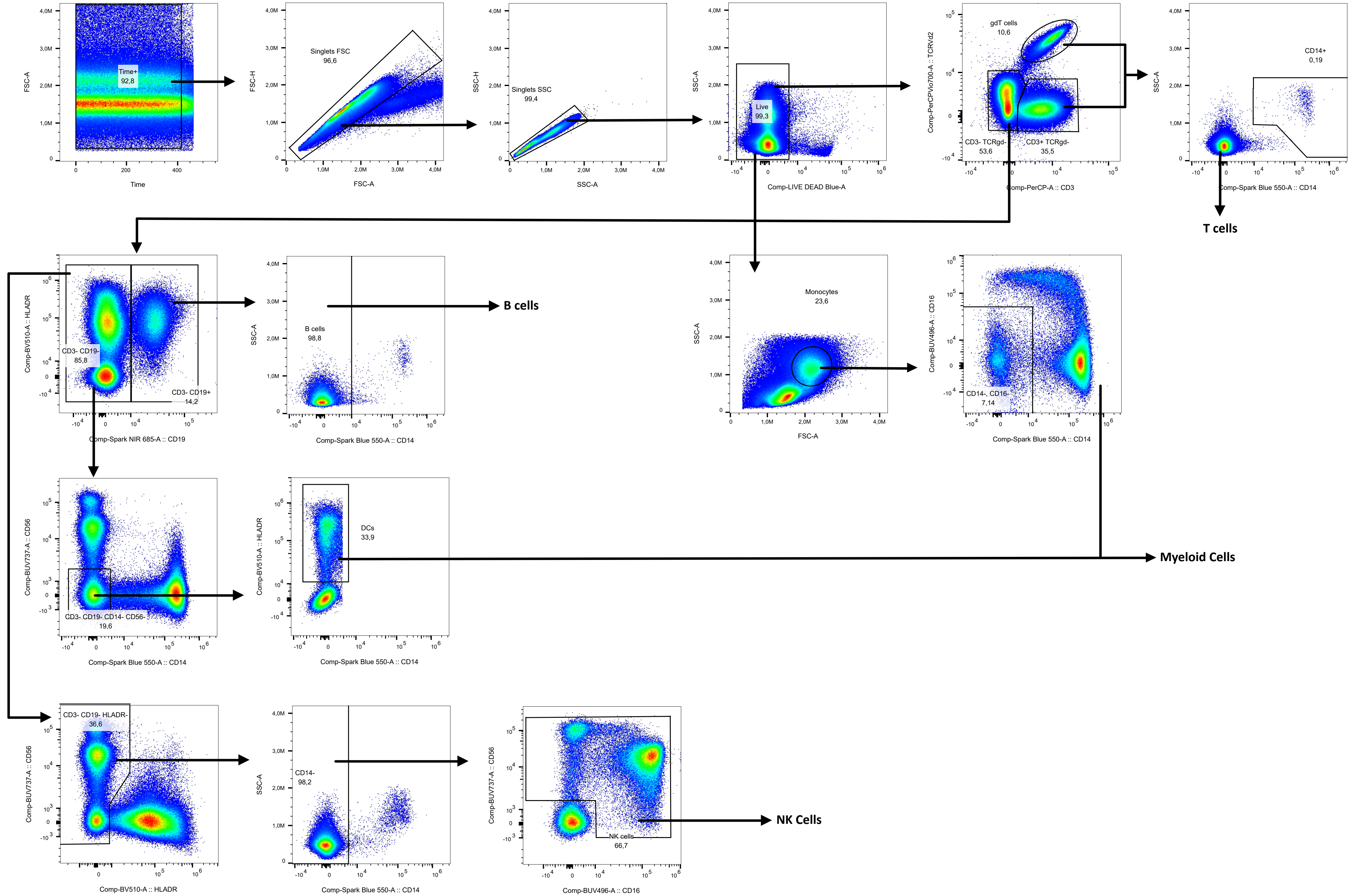
| | | | | | | | | |
|-----|----------|---|---|---|---|---|---|----|
| C13 | Controls | - | - | - | - | - | - | - |
| C14 | Controls | - | - | - | - | - | - | - |
| C16 | Controls | - | - | - | - | - | - | - |
| C17 | Controls | - | - | - | - | - | - | 28 |
| C18 | Controls | - | - | - | - | - | - | - |
| C19 | Controls | - | - | - | - | - | - | - |
| C20 | Controls | - | - | - | - | - | - | - |
| C21 | Controls | - | - | - | - | - | - | - |

Supplemental Table 9: Spearman correlations of time since last antigen exposure

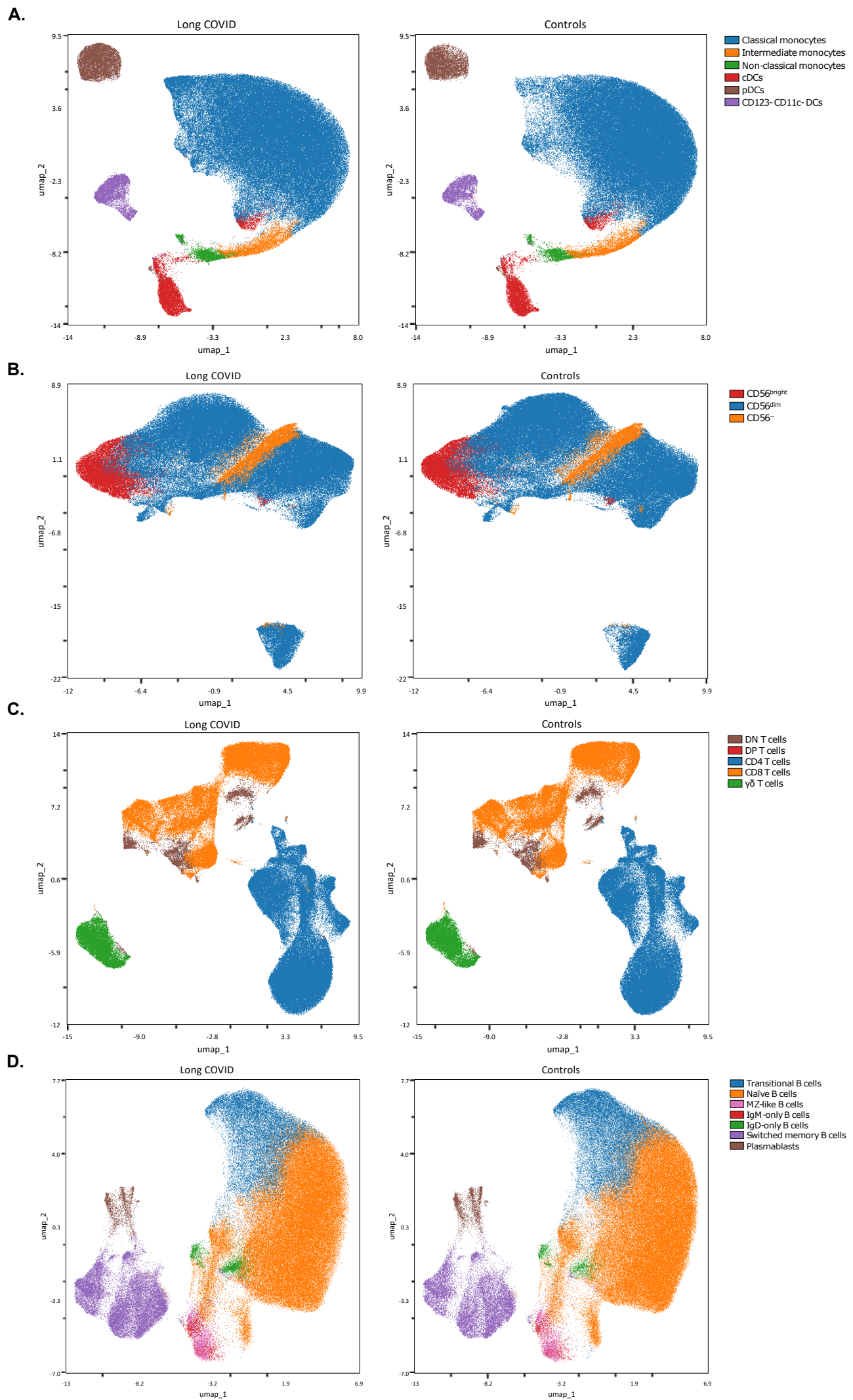
| Group | Variable | Rho | P.value |
|--------------|---------------------------------|------------|----------------|
| Long COVID | CCR6.MFI.CD56bright.NK.cells | 0.267 | 0.008 |
| Long COVID | CD8.MFI.CD56-.NK.cells | -0.256 | 0.011 |
| Long COVID | CXCR5.MFI.cDCs | -0.139 | 0.171 |
| Controls | CCR6.MFI.Intermediate.monocytes | -0.368 | 0.178 |
| Controls | CXCR5.MFI.Tem.CD4 | -0.396 | 0.182 |
| Controls | CD8.MFI.CD56..NK.cells | -0.243 | 0.382 |
| Long COVID | CCR6.MFI.Classical.monocytes | -0.082 | 0.420 |
| Long COVID | CCR6.MFI.Intermediate.monocytes | -0.082 | 0.421 |
| Long COVID | CCR6.MFI.Monocytes | -0.076 | 0.458 |
| Long COVID | CD1c.MFI.IgM.only.B.cells | -0.076 | 0.467 |
| Long COVID | CD1c.MFI.MZ.like.B.cells | -0.070 | 0.503 |
| Long COVID | CXCR5.MFI.Tem.CD4 | 0.028 | 0.788 |
| Controls | CXCR5.MFI.cDCs | -0.061 | 0.832 |
| Controls | CD1c.MFI.MZ.like.B.cells | -0.036 | 0.903 |
| Controls | CCR6.MFI.CD56bright.NK.cells | -0.036 | 0.903 |
| Controls | CD1c.MFI.IgM.only.B.cells | -0.021 | 0.944 |
| Controls | CCR6.MFI.Classical.monocytes | -0.018 | 0.954 |
| Controls | CCR6.MFI.Monocytes | -0.018 | 0.954 |

Supplemental Table 10: Mann-Whitney Test comparing the significant results between infected and uninfected (vaccinated) controls

| Variable | p.value | p.adj |
|-----------------------------|----------------|--------------|
| Cluster.19.B.cells | 0.013 | 0.451 |
| Cluster.15.B.cells | 0.028 | 0.451 |
| CCR6.CD56bright.NK.cells | 0.040 | 0.451 |
| CD4.Tcm | 0.112 | 0.587 |
| CD8.tcm | 0.190 | 0.886 |
| Cluster.6.NK.cells | 0.254 | 1.000 |
| CD4.T.cells | 0.364 | 1.000 |
| CD8.Tscm | 0.364 | 1.000 |
| IgD.only.B.cells | 0.371 | 1.000 |
| Non.classical.monocytes | 0.371 | 1.000 |
| Switched.memory.B.cells | 0.440 | 1.000 |
| CD8.CD56..NK.cells | 0.440 | 1.000 |
| cDCs | 0.513 | 1.000 |
| Cluster.3.T.cells | 0.518 | 1.000 |
| Cluster.25.T.cells | 0.518 | 1.000 |
| pDCs | 0.594 | 1.000 |
| Cluster.23.B.cells | 0.679 | 1.000 |
| Cluster.25.B.cells | 0.679 | 1.000 |
| Cluster.25.NK.cells | 0.679 | 1.000 |
| CD1c.MZ.like | 0.679 | 1.000 |
| Cluster.16.T.cells | 0.699 | 1.000 |
| Cluster.23.T.cells | 0.699 | 1.000 |
| Cluster.17.B.cells | 0.768 | 1.000 |
| Cluster.15.NK.cells | 0.768 | 1.000 |
| CXCR5.cDCs | 0.768 | 1.000 |
| Treg | 0.797 | 1.000 |
| Cluster.7.M.cells | 0.859 | 1.000 |
| Cluster.11.M.cells | 0.859 | 1.000 |
| Cluster.26.NK.cells | 0.859 | 1.000 |
| CD1c.IgM.only | 0.859 | 1.000 |
| CXCR5.Tem.CD4 | 0.898 | 1.000 |
| Monocytes | 0.953 | 1.000 |
| DCs | 0.953 | 1.000 |
| CCR6.Intermediate.monocytes | 0.953 | 1.000 |
| CCR6.Classical.monocytes | 1.000 | 1.000 |
| CCR6.Total.monocytes | 1.000 | 1.000 |

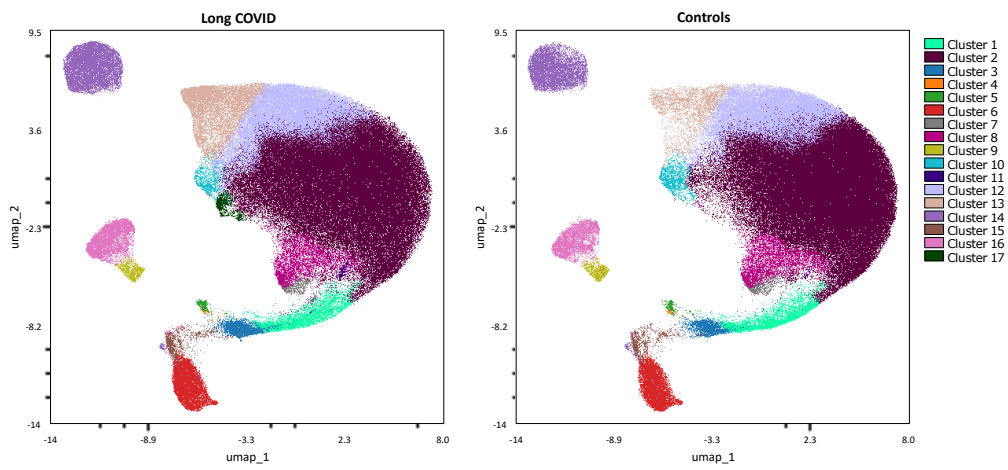


Supplemental Figure 1: Manual gating strategy

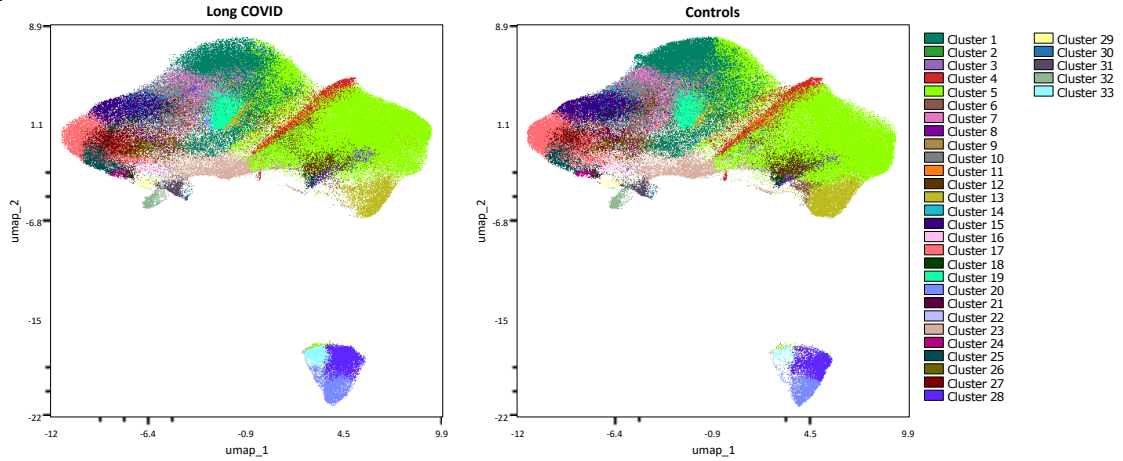


Supplemental Figure 2: UMAP representing all cell populations analyzed across the different major immune cell types. (A) Uniform manifold approximation and projection for dimension reduction (UMAP) representing the 6 major myeloid cell populations in which the 17 clusters analyzed using Flow-SOM were grouped depending on specific marker expression. **(B)** UMAP representing the 4 major NK-cell populations into which the 33 clusters analyzed using Flow-SOM were grouped depending on specific marker expression. **(C)** UMAP representing the 5 major T-cell populations into which the 37 clusters analyzed using Flow-SOM were grouped depending on specific marker expression. **(D)** UMAP representing the 6 major B-cell populations in which the 31 clusters analyzed using Flow-SOM were grouped depending on specific marker expression.

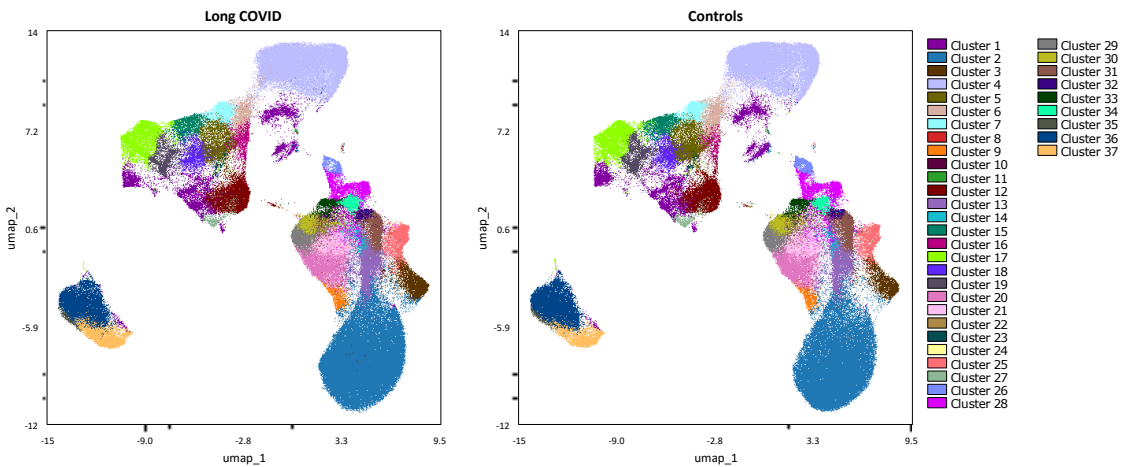
A. Myeloid cells



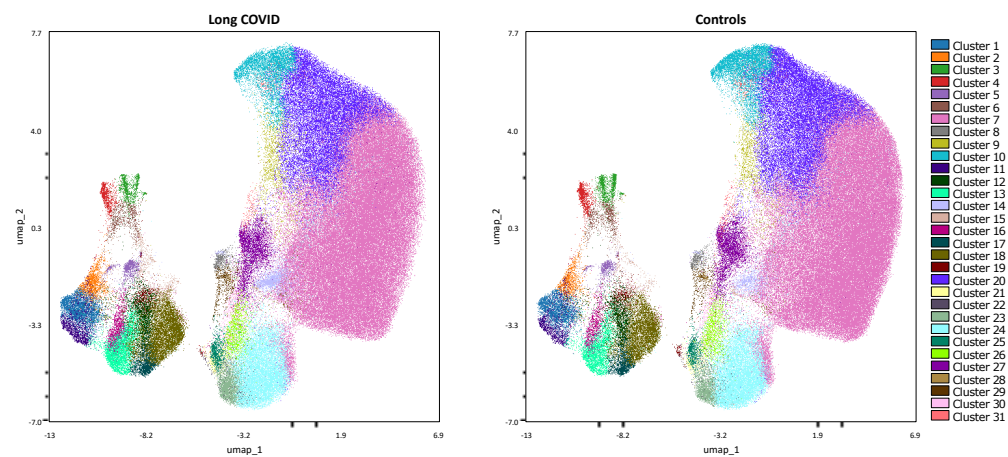
B. NK cells



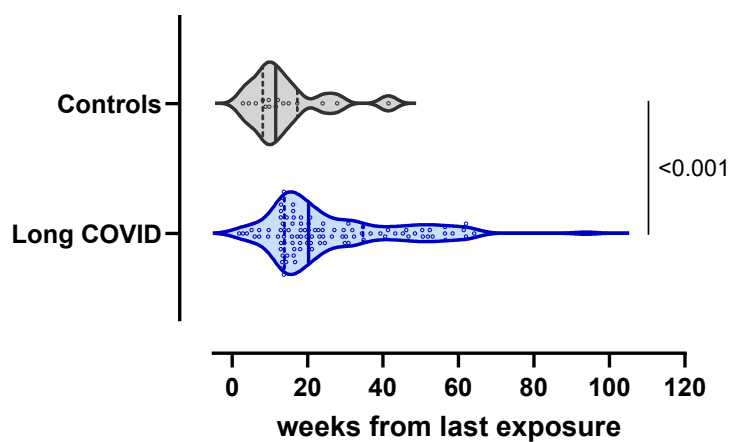
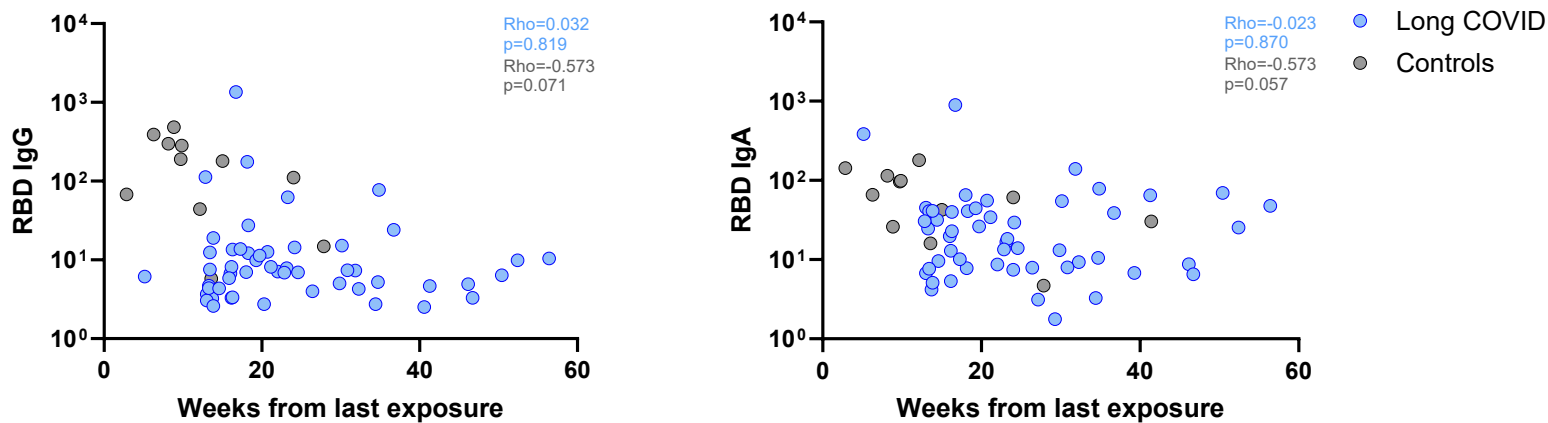
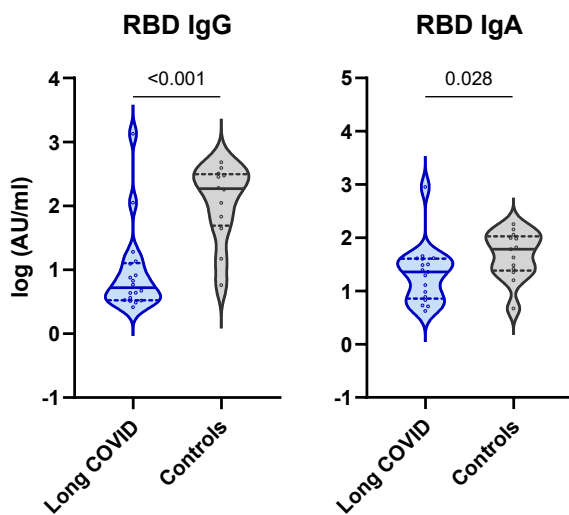
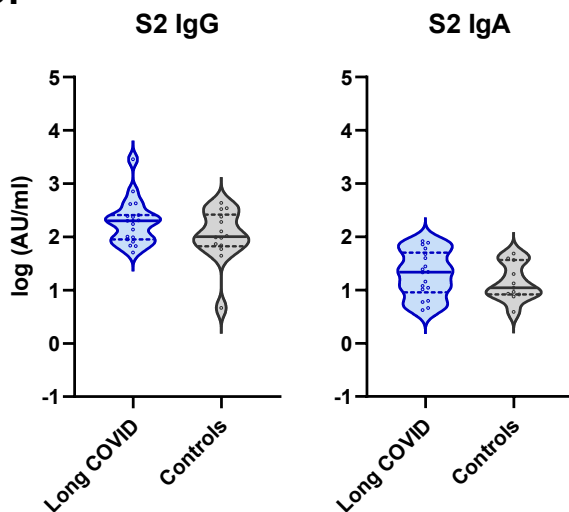
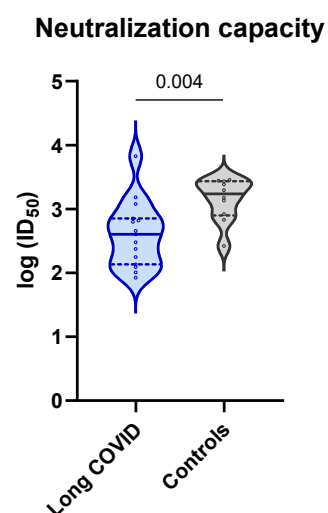
C. T cells



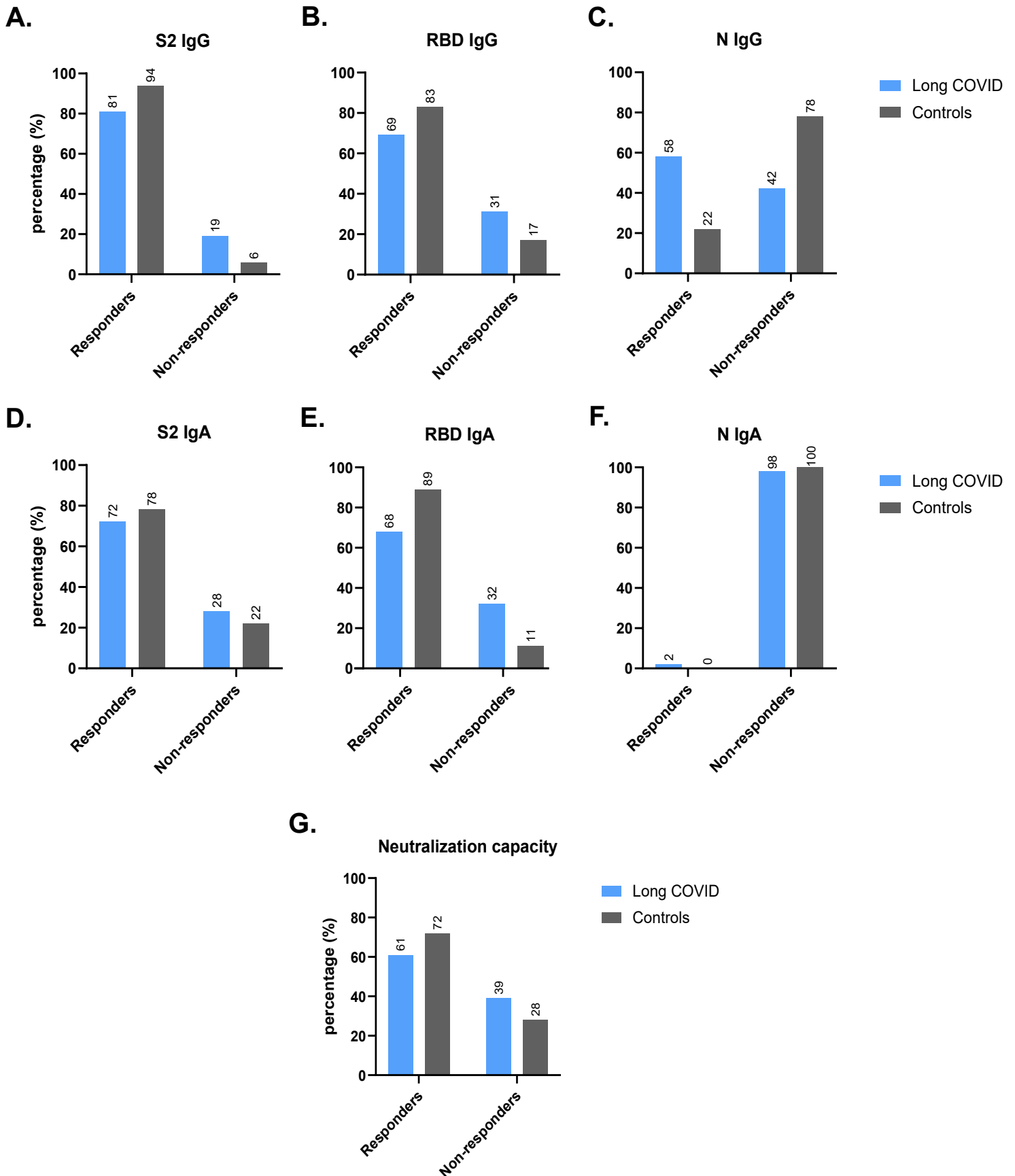
D. B cells



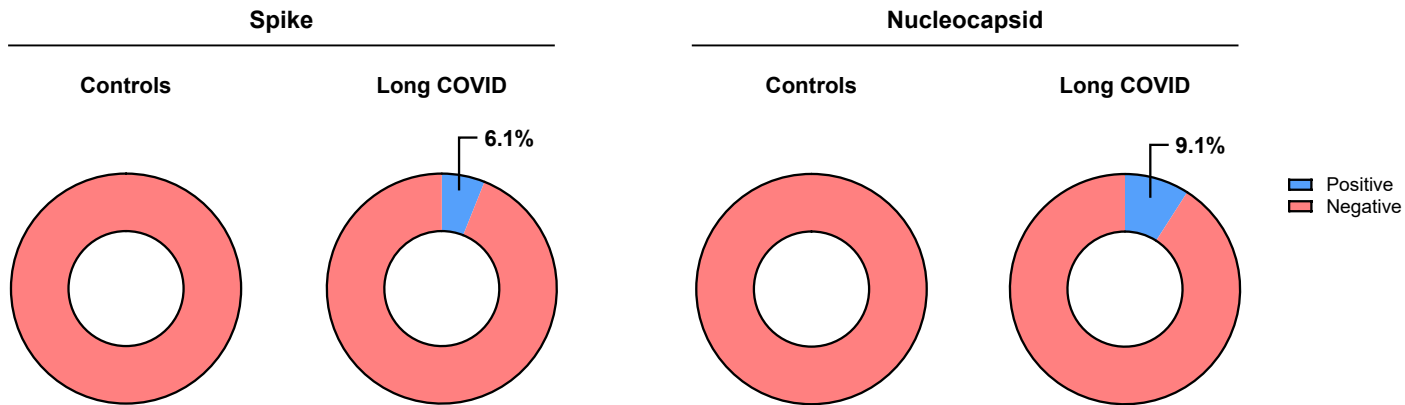
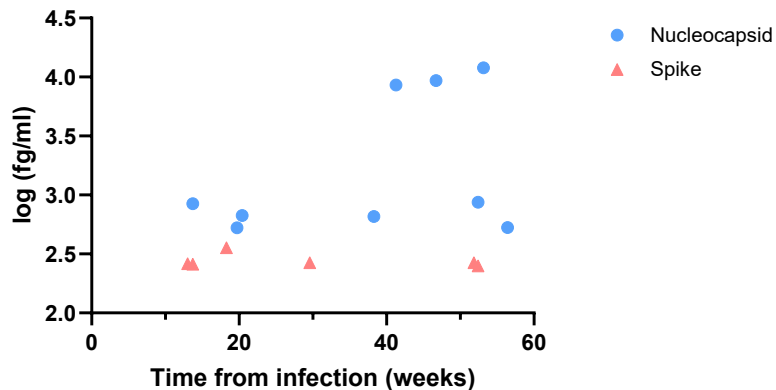
Supplemental Figure 3: UMAP representing all clusters analyzed across the different cell populations. (A) Uniform manifold approximation and projection for dimension reduction (UMAP) representing the 17 clusters analyzed in the myeloid cell population using Flow-SOM and grouped depending on specific marker expression. **(B)** UMAP representing the 33 clusters analyzed in the NK-cell population using Flow-SOM and grouped depending on specific marker expression. **(C)** UMAP representing the 37 clusters analyzed in the T-cell population using Flow-SOM and grouped depending on specific marker expression. **(D)** UMAP representing the 31 clusters analyzed in the B-cell population using Flow-SOM and grouped depending on specific marker expression.

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

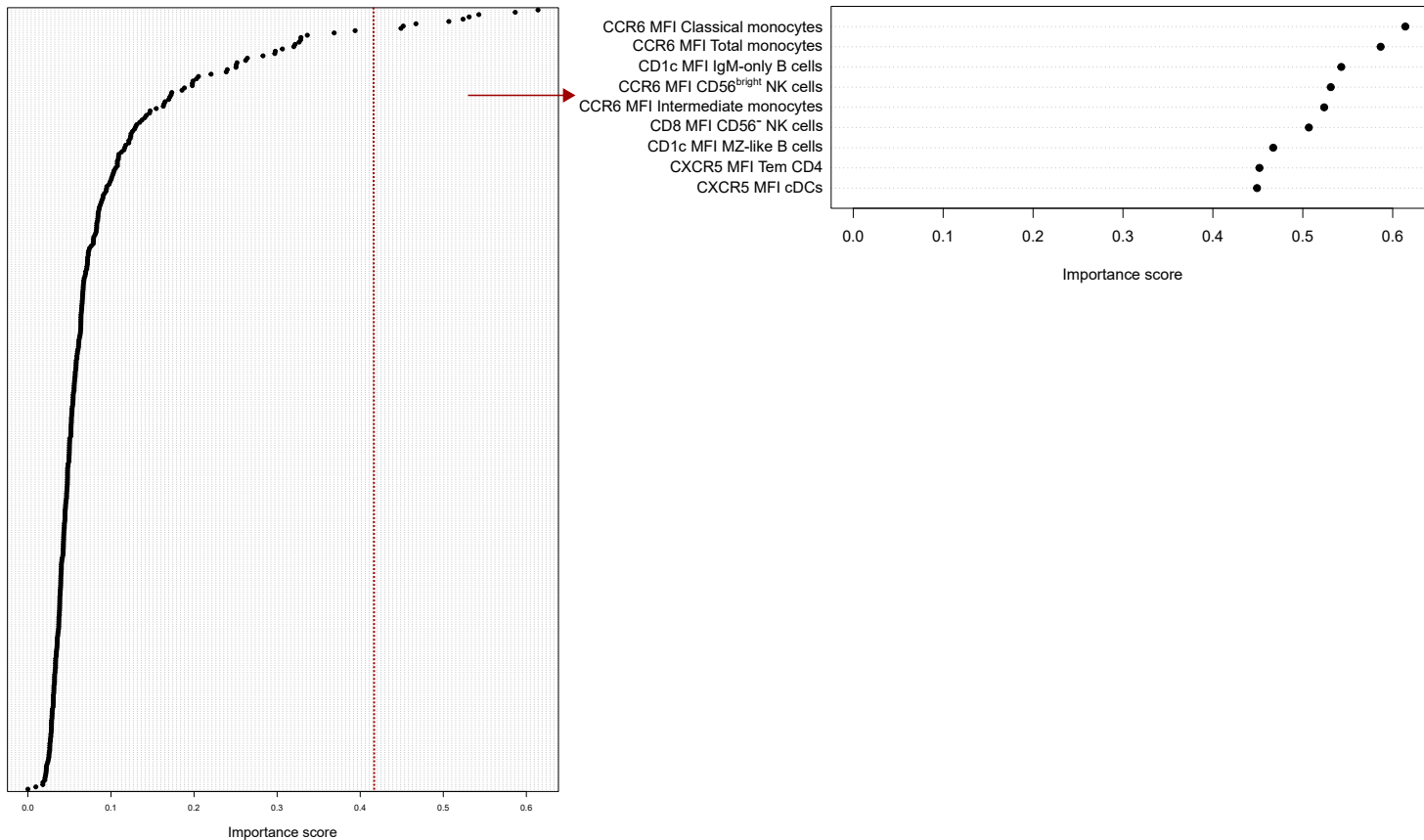
Supplemental Figure 4: Effect of time on antibody levels among CYP with and without LC. (A) Time to sample collection from the last exposure (weeks) in the LC and control cohort (median weeks [IQR]; LC: 20.3 [13.9-34.7]; controls: 11.6 [8.1-17.3]) ($p < 0.001$). (B) Correlations of anti-RBD IgG (left) and anti-RBD IgA (right) in the LC and control cohorts (all $p = n.s.$) restricted to one-exposure samples. (C) Specific anti-RBD IgG (left) ($p < 0.001$) and IgA (right) ($p = 0.028$) antibody levels of responders. (D) Specific anti-S2 IgG (left) ($p = n.s.$) and IgA (right) ($p = n.s.$) antibody levels of responders. (E) Specific SARS-CoV-2 neutralizing capacity of responders in half-maximal inhibitory dilution (ID₅₀) among the LC and control cohort ($p = 0.004$). Analyses of antibody and neutralization levels were restricted to one-exposure samples with no differences in time from the last exposure (median weeks [IQR]; LC: 13.9 [13.4-16.1]; controls: 11.0 [8.3-21.8]). Each dot represents an individual, and median and IQR values are indicated. p-values according to the Mann-Whitney test. Significant p-values < 0.05 .



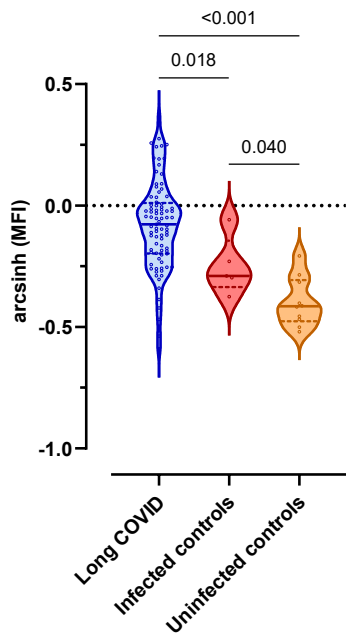
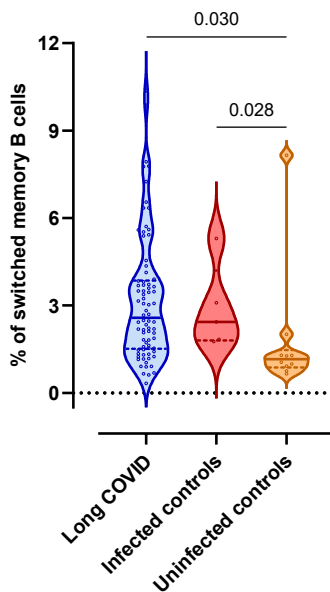
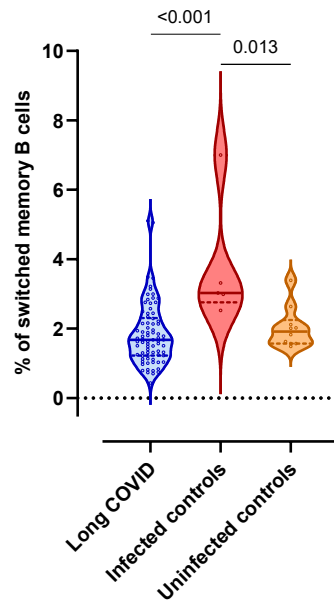
Supplemental Figure 5: Prevalence of responders and non-responders among CYP with and without LC. (A) Prevalence of anti-S2 IgG–specific responders and non-responders (%) among the LC and control cohorts. **(B)** Prevalence of anti-RBD IgG–specific responders and non-responders (%) among the LC and control cohorts. **(C)** Prevalence of Specific anti-N IgG responders and non-responders (%) among the LC and control cohorts. **(D)** Prevalence of Specific anti-S2 IgA responders and non-responders (%) among the LC and control cohorts. **(E)** Prevalence of specific anti-RBD IgA responders and non-responders (%) among the LC and control cohorts. **(F)** Prevalence of Specific anti-N IgA responders and non-responders (%) among the LC and control cohorts. **(G)** Prevalence of SARS-CoV-2–specific neutralizing capacity responders and non-responders (%) among the LC and control cohorts. LC n=99; controls n=18 in all analyses.

A.**B.**

Supplemental Figure 6: SARS-CoV-2 antigen persistence in CYP with and without long COVID. (A) Percentage of antigen positivity of individuals with and without long COVID (right Spike, left Nucleocapsid). **(B)** Concentration of Spike (triangle, red) and Nucleocapsid (circle, blue) of positive samples in log(fg/ml) versus time from infection in weeks.



Supplemental Figure 7: Factor importance in random forest analysis. Relative variable importance of the factors included in the random forest analysis.

A.**CCR6 MFI CD56^{bright} NK cells****B.****Cluster 15**(switched memory B cells IgA⁺, CD21⁻, CD27⁻)**C.****Cluster 19**(switched memory B cells CD27⁺, CD21⁺, CD24⁻)

Supplemental Figure 8: Significant differences between infected and uninfected (vaccinated) controls. (A) Mean fluorescence intensity (MFI) of CCR6 in CD56^{bright} NK cells. **(B)** Frequency of Cluster 15 with respect to total switched memory B cells. **(C)** Frequency of Cluster 19 with respect to total switched memory B cells. Mann-Whitney test, p-values < 0.05 were considered statistically significant.