

Supplementary Material

Supplementary Table 1. Antibodies and flow cytometry reagents used for flow cytometry analysis.

| Antibody/reagent | Manufacturer | Catalog number |
|--------------------------------------|---------------------|-----------------------|
| BUV496 anti-human CD19 | BD Horizon | 564656 |
| APC/Cy7 anti-human CD27 | BioLegend | 302816 |
| PerCP/Cyanine5.5 anti-human IgD | BioLegend | 348208 |
| Brilliant Violet 421 anti-human CD24 | BioLegend | 31122 |
| Brilliant Violet 650 anti-human CD38 | BioLegend | 356620 |
| Alexa Fluor 700 anti-human CD21 | BioLegend | 354918 |
| PE/Dazzle 594 anti-human CD11c | BioLegend | 301642 |
| Zombie Green fixable viability kit | BioLegend | 423112 |

Supplementary Table 2. B cell subsets definitions and phenotypes.

| Parental population | Subset definitions and phenotype |
|---|--|
| Transitional 1/2 | Transitional CD21^{neg/lo} (Tr CD21^{neg/lo}) CD19 ^{pos} CD27 ^{neg} CD38 ^{hi} CD24 ^{hi} CD21 ^{neg/lo} |
| | Transitional CD21^{pos} (Tr CD21^{pos}) CD19 ^{pos} CD27 ^{neg} CD38 ^{hi} CD24 ^{lo} CD21 ^{pos} |
| Naive | Resting Naive (resN) CD19 ^{pos} CD27 ^{neg} IgD ^{pos} CD38 ^{pos} CD24 ^{neg} CD11c ^{neg} |
| | Activated Naive (actN) CD19 ^{pos} CD27 ^{neg} IgD ^{pos} CD38 ^{neg} CD24 ^{neg} CD11c ^{pos} |
| Antibody-Secreting Cells | Plasmablasts (ASC) CD19 ^{pos} CD27 ^{hi} CD38 ^{hi} |
| Classic Memory | Unswitched Classical Memory (USwM) CD19 ^{pos} CD27 ^{pos} IgD ^{pos} |
| | Switched Classical Memory (SwM) CD19 ^{pos} CD27 ^{pos} IgD ^{neg} |
| Non-classic Memory (CD27 ^{neg}) | Non-Classic CD27^{neg} IgD^{neg} Memory (M CD27^{neg} IgD^{neg}) CD19 ^{pos} CD38 ^{neg/lo} CD24 ^{pos} CD27 ^{neg} IgD ^{neg} |
| | Non-Classic CD27^{neg} IgD^{pos} Memory (M CD27^{neg} IgD^{pos}) CD19 ^{pos} CD38 ^{neg/lo} CD24 ^{pos} CD27 ^{neg} IgD ^{pos} |
| Double Negative (CD27 ^{neg} IgD ^{neg}) | Double Negative 1 (DN1) CD19 ^{pos} CD27 ^{neg} IgD ^{neg} CD38 ^{neg/pos} CD24 ^{neg} CD21 ^{pos} CD11c ^{neg} |
| | Double Negative 2 (DN2) CD19 ^{pos} CD27 ^{neg} IgD ^{neg} CD38 ^{neg} CD24 ^{neg} CD21 ^{neg} CD11c ^{pos} |
| | Double Negative 3 (DN3) CD19 ^{pos} CD27 ^{neg} IgD ^{neg} CD38 ^{neg/pos} CD24 ^{neg} CD21 ^{neg} CD11c ^{neg} |
| | Double Negative 4 (DN4) CD19 ^{pos} CD27 ^{neg} IgD ^{neg} CD38 ^{neg} CD24 ^{neg} CD21 ^{pos} CD11c ^{pos} |

Supplementary Table 3. B cell subsets absolute counts.

| B Cell Subset | Control | Mild/Moderate | Severe | Critical | P value (Mild/Moderate vs Severe) | P value (Mild/Moderate vs Critical) |
|----------------------------------|---------------------------|----------------------------|----------------------------|----------------------------|--|--|
| CD19+ B cells count/mL | 58,310 (36,971-68,804) | 99,810 (54,599-166,600) | 73,041 (39,747-108,953) | 67,147 (29,062-101,090) | ns | ns |
| Memory B cells count/mL | 20,925 (19,214-25,679) | 23,126 (12,956-37,155) | 14,258 (6,412-18,904) | 17,593 (6,379-24,273) | 0.018 | 0.048 |
| USwM B cells count/mL | 2,867 (1,630-3,375) | 2,667 (1,821-4,522) | 1,030 (510-1,848) | 1,316 (606-2,136) | 0.001 | 0.007 |
| SwM B cells count/mL | 18,425 (16,125-22,080) | 20,328 (10,240-32,953) | 11,353 (5,689-16,401) | 15,847 (5,655-21,351) | 0.02 | ns |
| ASC count/mL | 334 (162-437) | 1,276 (669-1,725) | 1,127 (922-2,781) | 909 (296-3,082) | ns | ns |
| T1/T2 B cells count/mL | 697 (544-976) | 7,497 (2,065-9,608) | 1,674 (602-5,098) | 1,433 (473-976) | 0.006 | 0.006 |
| TrCD21neg/lo B cells count/mL | 137 (71-297) | 2,626 (549-3,503) | 810 (304-1,616) | 695 (106-3,043) | ns | 0.02 |
| TrCD21pos B cells count/mL | 571 (461-803) | 4,376 (1,312-6,120) | 304 (38-1,785) | 358 (272-1,981) | 0.0002 | 0.002 |
| Non-Classic Memory count/mL | 8,945 (3,013-12,604) | 11,578 (9,270-18,929) | 12,579 (5,685-16,615) | 9,912 (2,678-16,499) | ns | ns |
| M CD27neg IgDneg count/mL | 4,321 (1,956-7,114) | 7,691 (6,068-12,274) | 7,597 (3,839-1,678) | 6,556 (1,496-9,592) | ns | ns |
| M CD27neg IgDpos count/mL | 2,641 (1,054-4,603) | 3,838 (2,977-9,169) | 3,064 (1,399-5,664) | 3,008 (1,338-5,565) | ns | ns |
| DN B cells | 7,706 | 14,439 | 10,240 | 15,919 | ns | ns |

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|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|-------|-------|
| count/mL | (4,436-10,009) | (7,964-25,352) | (5,605-25,317) | (4,334-19,937) | | |
| DN1 B cells count/mL | 3,818 (3,235-5,219) | 6,414 (3,752-11,897) | 3,605 (1,595-5,127) | 3,586 (500-6,751) | 0.008 | 0.009 |
| DN2 B cells count/mL | 30 (14-245) | 46 (24-98) | 82 (24-477) | 24 (0-46) | ns | ns |
| DN3 B cells count/mL | 2,862 (1,122-5,580) | 5,044 (2,992-13,778) | 5,962 (2,924-20,086) | 10,446 (2,558-14,506) | ns | ns |
| DN4 B cells count/mL | 4 (2-10) | 0 (0-18) | 0 (0-14) | 0 (0-12) | ns | ns |
| Naive B cells count/mL | 12,131 (7,814-22,430) | 20,113 (7,649-56,431) | 16,885 (7,053-30,327) | 13,380 (4,928-28,841) | ns | ns |
| resN B cells count/mL | 12,066 (7,755-22,361) | 17,567 (7,649-56,268) | 16,761 (7,035-30,111) | 13,279 (4,902-28,748) | ns | ns |
| actN B cells count/mL | 62 (32-71) | 82 (41-333) | 128 (57-260) | 90 (9-152) | ns | ns |

Data presented are the median (IQR). P values comparing mild/moderate and severe or critical cases were obtained by a Kruskal-Wallis non-parametric ANOVA and Dunn's uncorrected test. IQR, interquartile range.

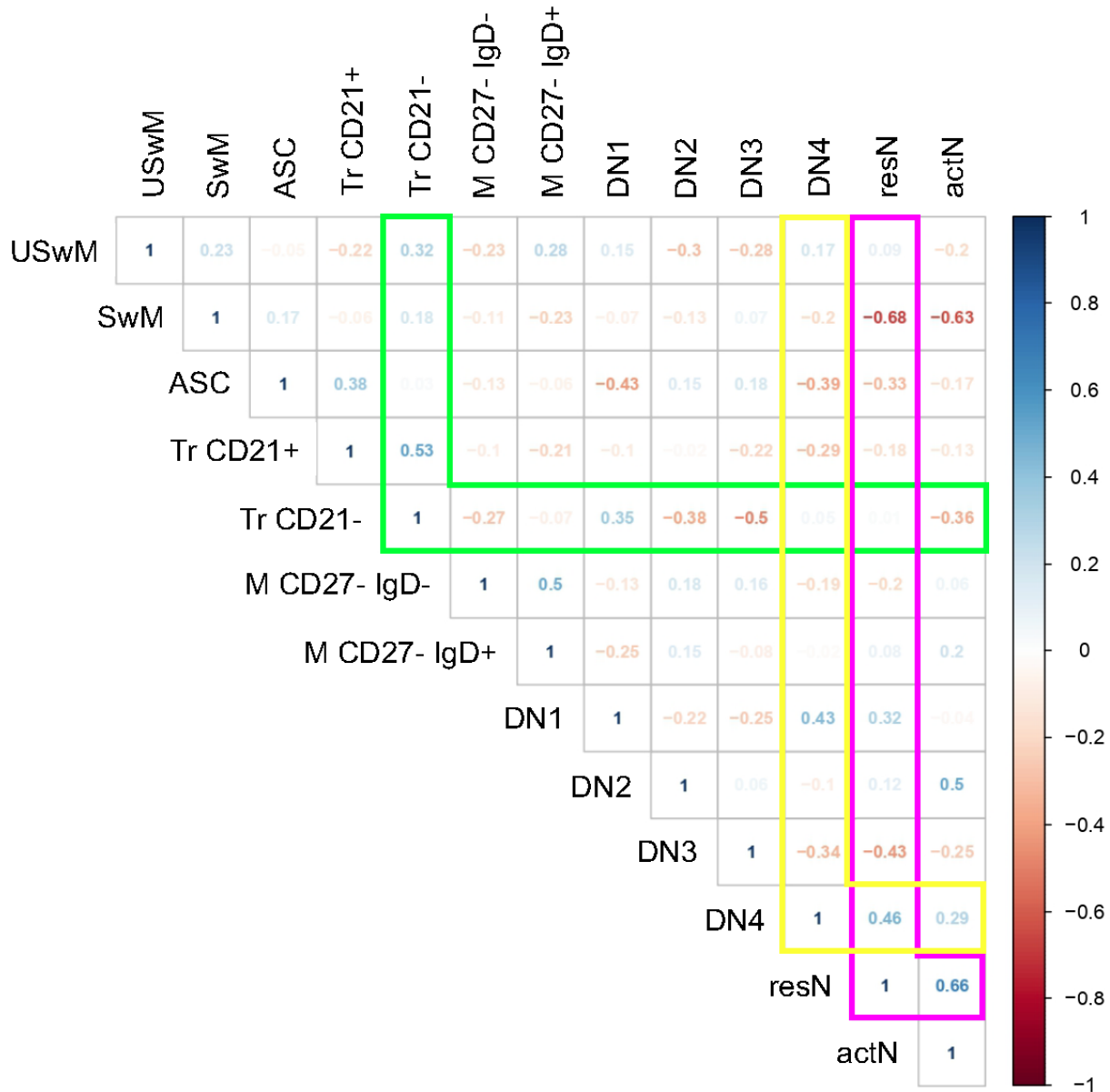
Supplementary Table 4. B cell subsets frequency comparisons in age-matched COVID-19 patients vs. healthy subjects.

| B cell subset | p-value (Healthy subjects vs COVID-19 patients) | Test |
|----------------------------|--|-------------|
| T1/T2 B cells (%) | 0.0030** | U |
| Tr CD21 neg/lo B cells (%) | 0.0009*** | U |
| Tr CD21 pos B cells (%) | 0.0057** | U |
| ASC (%) | 0.0019** | U |
| Memory B cells (%) | 0.0493* | t |
| USwM B cells (%) | 0.0102* | U |
| SwM B cells (%) | 0.0859 ⁺ | t |
| DN1 B cells (%) | 0.0617 ⁺ | U |
| DN2 B cells (%) | ns | U |
| DN3 B cells (%) | 0.0285* | U |
| actN B cells (%) | ns | U |

*** p < 0.001; ** p < 0.01; * p < 0.05; ⁺ p < 0.1

P-values by results of U-Mann-Whitney or t-test for the comparison of the B cell subset frequencies of between healthy controls (n=7) and COVID-19 patients (n=11). All subjects in this analysis are 32-41 years old.

Supplementary Figure 1.



Supplementary Figure 1. Correlation matrix comparing B cell subsets from COVID-19 patients.

This matrix comparing all parental-derived B cell subsets, showing the Spearman's correlation coefficient values according to the color scale on the right side; the color intensity of numbers denotes correlation strength. Enclosed values in green, yellow, and magenta indicate a high number of moderately-strong correlations among T CD21⁻, DN4, and resN, thus making them susceptible to exclusion from hierarchical clustering analysis.