Supplemental information

Integrated immune dynamics define correlates of COVID-19 severity and antibody responses

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Figure S1. Violin plots of proportions and activation of innate immune cells by FlowSOM analysis in acute COVID-19 (n=55), and convalescent COVID-19 (n=53) samples. Comparisons were performed using a Wilcoxon rank-sum test (equivalent to the Mann-Whitney test) with the wilcox.test function in R. Statistics displayed across scatter graphs were uncorrected. Mean and SEM are shown. Related to Figure 1.
Figure S2. Violin plots of proportions and activation of adaptive immune cells by FlowSOM analysis in acute COVID-19 (n=55), and convalescent COVID-19 (n=53) samples. Comparisons were performed using a Wilcoxon rank-sum test (equivalent to the Mann-Whitney test) with the `wilcox.test` function in R. Statistics displayed across scatter graphs were uncorrected. Mean and SEM are shown. Related to Figure 1.
Figure S3. After analysis using TrackSOM (Figure 1), the dataset was analysed with UMAP. Cells belonging to each of the four TrackSOM clusters labelled in Figure 1 are highlighted in the UMAP plot to confirm their identity. Cells from the B cell cluster 'MM' are contained within CD19 expressing cells, cells from the CD4 T cell cluster 'GG' are contained largely within the CD3^+CD4^+ expressing cells, and cells from the two monocyte clusters 'LL' and 'FF' are contained within the CD14 expressing cells. Related to Figure 1.
**Figure S4.** Cytokine responses in COVID-19 plasma samples. (A) Cytokine levels in healthy (n=32), acute COVID-19 (n=74), and convalescent COVID-19 (n=65) plasma samples. Statistical significance was determined by Kruskal-Wallis test with Dunn’s correction for multiple comparisons. (B) Cytokine levels over time after disease onset (n=119). Related to Figure 1.
Figure S5. Gating strategies of FACS data for immunophenotyping of whole blood. (A) Analysis of innate and adaptive immune cells. (B) Analysis of adaptive B cell and T cell subsets. (C) Analysis of cytotoxicity profiles of CD8+ and CD4+ T cells. Related to Figure 2.
Figure S6. Innate and adaptive immune cells in COVID-19 blood samples based on manual gating strategy. (A) Volcano plots of differential immune profiles, based on manual gating strategy, of acute versus convalescent COVID-19 samples and healthy controls. (B) Proportions and cell numbers of innate and adaptive immune
populations between healthy (n=32), acute COVID-19 (n=60), and convalescent COVID-19 (n=58) samples. (C) Proportions of CD27/CD45RA subsets of γδ T cells between healthy (n=32), acute COVID-19 (n=60), and convalescent COVID-19 (n=58). (D) Cell numbers of the parent lymphocyte and monocyte populations. (E) Proportions and numbers of ASCs and activated cTfh cell subsets between healthy (n=49), acute COVID-19 (n=60), and convalescent COVID-19 (n=58). (F) Proportions and numbers of activated CD8+ and CD4+ T cells between healthy (n=32), acute COVID-19 (n=60), and convalescent COVID-19 (n=58). For CD38+CXCR3+CD8+ T cells, CD38+PD-1+CD8+ T cells, CD38+ICOS+CXCR3+CD4+ T cells and CD38+ICOS+CXCR3+CD4+ T cells, n=11 for the healthy group for which data were available. (G) Proportions of CD27/CD45RA subsets of CD8+ and CD4+ T cells between healthy (n=32), acute COVID-19 (n=60), and convalescent COVID-19 (n=58).

(B, D, E, F) Statistical significance was determined by Kruskal-Wallis test with Dunn’s correction for multiple comparisons, median and IQR are shown. (C, G) Statistical significance was determined by 2-way ANOVA with Tukey’s correction for multiple comparisons, mean and SD are shown. Related to Figure 2.
Figure S7. Granzyme and perforin profiling of CD8+ and CD4+ T cells. (A) Representative histograms and (B) proportion of granzyme/perforin-expressing CD38+HLA-DR+ CD8+ T cells in healthy, acute and convalescent COVID-19 samples. (C) Representative histograms and (D) proportion of granzyme/perforin-expressing CD38+HLA-DR+ CD4+ T cells in healthy (n=20), acute (n=57) and convalescent (n=58) COVID-19 samples. (b, d) Statistical significance was determined by Kruskal-Wallis test with Dunn’s correction for multiple comparisons. Related to Figure 2.
FlowSOM dataset – granzymes

Figure S8. Violin plots of granzyme and perforin expression in subsets of CD8⁺ and CD4⁺ T cells by FlowSOM analysis in for healthy (n=16), acute COVID-19 (n=51), and convalescent COVID-19 (n=51) samples. Comparisons were performed using a Wilcoxon rank-sum test (equivalent to the Mann-Whitney test) with the \texttt{wilcox.test} function in R. Statistics displayed across scatter graphs were uncorrected. Mean and SEM are shown. Related to Figure 2.
Figure S9. RBD ELISA curves for patients with multiple timepoints for IgG, IgM and IgA. Horizontal orange lines within each graph represent the cut-off used to determine the end-point titre while vertical blue lines represent the seropositivity cut-off value for each isotype (antibody titre > mean + 2xSD of healthy individuals). Related to Figure 3.
Figure S10. Avidity antibody responses in COVID-19 plasma samples. (A) Linear regression analysis of the numbers of acute ASCs, PD-1^ICOS^ cTfh1 or PD-1^ICOS^ cTfh2/17 cells and paired convalescent RBD-specific titres for each isotype, n=14. Avidity antibody responses in COVID-19 plasma samples. (B) Avidity analysis for IgG and IgM RBD-specific antibodies in paired samples. The percent of antibody binding after treatment with 6M Urea compared to without treatment is shown. For IgG, data from 1:31.6 and 1:316 plasma dilutions are shown and for IgM, 1:31.6 and 1:100 plasma dilutions are shown. Samples 1 and 2 were collected between 7-70 days apart. Statistical significance was assessed with a Wilcoxon matched-pairs signed rank test, n=13. (C) Avidity analysis for IgG and IgM RBD-specific analysis in all samples assessed, grouped by days post onset. Statistical significance was assessed with a Kruskal-Wallis test with Dunn’s correction for multiple comparisons between week 1 (n=12), week 2 (n=22), and week 3+ (n=32). Related to Figure 4.
Figure S11. Innate and adaptive immune cells in healthy controls (n=32), non-COVID-19 ILI (n=13), acute ward (n=36) and ICU (n=36) COVID-19 blood samples. (A) Absolute numbers of activated lymphocytes and CD14+ and CD16+ monocytes in ward and ICU samples. (B) Absolute cell numbers of the parent lymphocyte and monocyte populations in ward and ICU samples. (C) Cytokine levels in ward and ICU samples. (D) Absolute numbers of activated cTfh1 and ASC populations in ward and ICU samples. Statistical significance was determined by Kruskal-Wallis test with Dunn’s correction for multiple comparisons. Related to Figure 5.
Figure S12. Proportions of immune cell populations as determined by FlowSOM analysis between healthy (n=21), acute ward (n=32), acute ICU (n=18) and convalescent (n=54) samples. Related to Figure 5.
**Figure S13.** Correlation analysis of cytokines and immune cell populations defined by FlowSOM. Heatmap summarising the correlations between cytokine levels and immune cell populations in acute samples as determined by FlowSOM analysis. Statistical significance was defined as FDR-corrected p-value q<0.1. Related to Figure 6.
Figure S14. Heatmap summarising all the healthy, acute and convalescent COVID-19 patients and sample time points. (A) Contains all patient timepoints and all immune features, and (b) contains only immune features that were present in each of the healthy, acute, and convalescent groups. Statistics displayed for each column in (B) represent comparisons using a Wilcoxon rank-sum test (equivalent to the Mann-Whitney test) with the
wilcox.test function in R. Significant (p<0.05) for both uncorrected and FDR-corrected p-values are indicated in blue and red respectively. Related to Figure 6.
## Supplementary Table

### Supplementary Table 1: Patient summary, related to Figure 1A.

|                     | COVID-19 Symptomatic /hospitalized cases | COVID-19 Recovered cases | Other ILI Symptomatic /hospitalized cases | Healthy |
|---------------------|------------------------------------------|--------------------------|-------------------------------------------|---------|
| Sample size         | 33                                       | 52                       | 66                                        |         |
| Age, median (range) | 54 (21-78)                               | 59 (18-76)               | 62 (22-87)                                | 51 (12-94) |
| Female (%)          | 19 (68%)                                 | 23 (44%)                 | 4 (36%)                                   | 40 (61%) |
| Total days in hospital, median (range) | 8 (0-103) | 7 (1-29) | 3 (1-33) | N/A |
| Days from disease onset to admission, median (range) | 5 (0-22) | 4 (0-14) | 2 (1-14) | N/A |
| Days from disease onset to discharge, median (range) | 15 (3-111) | 15 (5-32) | 8 (3-34) | N/A |
| Oxygen status       |                                          |                          |                                           |         |
| Invasive ventilation | 8                                        | 0                        | 0                                         | N/A     |
| Non-invasive support required | 7 | 1 | 1 | N/A |
| Acute samples - location during disease |                     |                          |                                           |         |
| Intensive care unit required (ICU) | 12 | N/A | 1 | N/A |
| Ward                | 20                                       | N/A                      | 10                                        | N/A     |
| Outpatient          | 1                                        | N/A                      | N/A                                       |         |
| Convalescent samples - location during disease |                     |                          |                                           |         |
| Ward                | 12                                       | 9                        | 9                                         | N/A     |
| Home                | 1                                        | 43                       | 0                                         | N/A     |
| Days from disease onset to convalescent sampling, median (range) | 40 (20-80) | 40 (28-102) | 37 (36-63) | N/A |

N/A, not applicable

### Supplementary Table 2: Patient demographics and clinical data for COVID-19 patients, related to Figure 1A.

| Patient | Age | Gender | Days from ILI to | Hospital | Discharge | Days in hospital | Location during disease | Oxygen |
|---------|-----|--------|------------------|----------|-----------|------------------|-------------------------|--------|
| 1-073   | 63  | F      | 4                | 39       |           | 4                | Ward                    | No     |
| 1-074   | 25  | F      | 2                | 6        |           | 0                | Ward                    | No     |
| 1-080   | 72  | F      | 7                | 11       | 38        | 7                | Ward                    | Yes - Supplemental oxygen |
| 1-082   | 54  | F      | 8                | 52       | 7         | 12               | Ward                    | No     |
| 1-087   | 49  | M      | 10               | 14       | 39        | 9                | 22                      | Ward    |
| 1-088   | 58  | M      | 15               | 23       | 36        | 43               | 111                     | 103 ICU |
| 1-089   | 38  | M      | 6                | 12       | 41        | 5                | 14                      | Ward    |
| 1-090   | 73  | F      | 7                | 10       | 44        | 2                | 12                      | Ward    |
| 2-026   | 47  | F      | 7                | 8        | 9         | 20               | 5                       | 11 Ward |
| 2-027   | 48  | M      | 3                | 46       |           | 2                | 3                       | Ward    |
| 2-028   | 71  | F      | 1                | 2        |           | 0                | 3                       | Ward    |
| 2-029   | 43  | F      | 6                |           |           | 5                | 7                       | Ward    |
| 2-030   | 66  | M      | 23               |           |           | 17               | 24                      | Ward    |
| 2-031   | 61  | F      | 11               | 12       |           | 10               | 15                      | Ward    |
| 2-032   | 41  | F      | 33               | 35       | 37        | 39               | 22                      | 39 ICU  |
| 2-033   | 29  | F      |                   | 31       | 78        |                 |                         | Home    |
| 2-034   | 57  | M      | 9                | 11       | 13        | 17               | 21                      | 8 22 ICU | Yes - Invasive ventilation |
| 2-035   | 54  | F      | 3                | 5        | 7        | 9                | 13                      | 0 14 Ward | No |
| 2-036   | 37  | M      | 10               |           |           | 10               | 10                      | 0 Ward    |
| 2-037   | 48  | M      | 6                | 8        | 10        | 13               | 5                       | 18 13 ICU | Yes - High-flow nasal cannula O2 therapy |
| 3-001   | 56  | F      | 18               |           |           | 13               | 19                      | 6 Ward    |
| 3-002   | 39  | M      | 8                | 9        |           | 71               |                         | 6 14 Ward  |
| 3-003   | 61  | M      | 9                | 11       | 17        | 30               | 45                      | 8 60 52 ICU | Yes - Invasive ventilation |
| 4-001   | 62  | M      | 5                |           |           | 53               |                         | 4 9 5 Ward | No |
| 4-002   | 54  | M      | 5                | 10       |           | 34               | 80                      | 5 9 4 Ward | No |
| 4-003   | 45  | M      | 5                | 7        |           | 39               |                         | 4 10 6 Ward | No |
| 5-001   | 21  | F      | 8                |           |           | 28               |                         | Home No |
| 5-002   | 55  | F      |                   | 32        |           |                 |                         | Home No |
| 5-003   | 62  | M      |                   | 10       | 12        | 2                |                         | 10 12 Ward | No |
| 5-004   | 70  | M      |                   | 30        | 0         | 7                |                         | 0 7 7 Ward  | Yes - Nasal cannula |
| 5-006   | 65  | F      |                   | 38        |           |                 |                         | Home No |
| 5-007   | 69  | M      |                   | 35        |           |                 |                         | Home No |
| Patient ID | Gender | Age | Admission Date | Admission Site | Acuity | Mechanical Ventilation |
|------------|--------|-----|----------------|----------------|--------|------------------------|
| 5-008      | F      | 62  | 41             | Home           | No     | No                     |
| 5-009      | M      | 75  | 42             | Home           | No     | No                     |
| 5-010      | F      | 54  | 39             | Home           | No     | No                     |
| 5-011      | M      | 55  | 39             | Home           | No     | No                     |
| 5-012      | M      | 63  | 36             | Home           | No     | No                     |
| 5-013      | F      | 54  | 36             | Home           | No     | No                     |
| 5-014      | M      | 59  | 39             | Home           | No     | No                     |
| 5-015      | F      | 61  | 37             | Home           | No     | No                     |
| 5-016      | M      | 76  | 36             | Home           | No     | No                     |
| 5-017      | F      | 52  | 37             | Home           | No     | No                     |
| 5-018      | F      | 22  | 42             | Home           | No     | No                     |
| 5-019      | M      | 54  | 39             | Home           | No     | No                     |
| 5-020      | F      | 66  | 40             | Home           | No     | No                     |
| 5-022      | F      | 58  | 27             | Home           | No     | No                     |
| 5-023      | M      | 60  | 27             | Home           | No     | No                     |
| 5-024      | F      | 61  | 41             | Home           | No     | No                     |
| 5-025      | M      | 60  | 41             | Home           | No     | No                     |
| 5-026      | M      | 65  | 41             | Home           | No     | No                     |
| 5-028      | F      | 57  | 33             | Home           | No     | No                     |
| 5-029      | M      | 18  | 33             | Home           | No     | No                     |
| 5-030      | F      | 49  | 33             | Home           | No     | No                     |
| 5-031      | M      | 64  | 42             | Home           | No     | No                     |
| 5-032      | M      | 66  | 40             | Home           | No     | No                     |
| 5-033      | M      | 66  | 35             | Home           | No     | No                     |
| 5-034      | M      | 65  | 46             | Home           | No     | No                     |
| 5-035      | M      | 49  | 31             | Home           | No     | No                     |
| 5-036      | M      | 55  | 33             | Home           | No     | No                     |
| 5-037      | F      | 50  | 33             | Home           | No     | No                     |
| 5-038      | M      | 64  | 40             | Home           | No     | No                     |
| 5-039      | M      | 58  | 26             | Home           | No     | No                     |
| 5-040      | M      | 61  | 47             | Home           | No     | No                     |
| 5-041      | M      | 58  | 39             | Home           | No     | No                     |
| 5-042      | F      | 59  | 46             | Home           | No     | No                     |
| 5-043      | M      | 56  | 36             | Home           | No     | No                     |
| 5-044      | F      | 22  | 29             | Home           | No     | No                     |
| 5-045      | M      | 49  | 44             | Home           | No     | No                     |
| 5-046      | M      | 52  | 41             | Home           | No     | No                     |
| 5-047      | F      | 56  | 35             | Home           | No     | No                     |
| 6-002      | F      | 62  | 47             | Ward           | No     | No                     |
| 6-003      | M      | 54  | 46             | Ward           | No     | No                     |
| 6-004      | F      | 55  | 46             | Ward           | No     | No                     |
| 7-121      | M      | 71  | 75             | Ward           | No     | No                     |
| 7-123      | F      | 32  | 45             | Home           | No     | No                     |
| 7-124      | M      | 74  | 90             | Home           | No     | No                     |
| 7-125      | F      | 62  | 88             | Home           | No     | No                     |
| 7-126      | F      | 57  | 61             | Home           | No     | No                     |
| 8-013      | F      | 69  | 37             | ICU            | Yes    | Endotracheal tube      |
| 8-022      | M      | 70  | 34             | ICU            | Yes    | Nasal prong            |
| 8-036      | F      | 52  | 26             | ICU            | Yes    | High-flow nasal prong  |
| 8-037      | F      | 59  | 36             | ICU            | Yes    | Endotracheal tube      |
| 8-043      | F      | 78  | 15             | ICU            | Yes    | Endotracheal tube      |
| 8-068      | F      | 41  | 2              | ICU            | Yes    | Endotracheal tube      |
| 8-069      | F      | 73  | 0              | ICU            | Yes    | Endotracheal tube      |

N/A, not applicable; A, acute visit; C, convalescent visit
**Supplementary Table 3:** Binned groupings for TrackSOM algorithm, related to Figure 1.

| Bin | Days post disease onset | Number of patients | Number of samples |
|-----|-------------------------|--------------------|-------------------|
| 1   | 1-4                     | 5                  | 6                 |
| 2   | 5-8                     | 12                 | 15                |
| 3   | 9-12                    | 11                 | 14                |
| 4   | 13,14,15,17             | 6                  | 7                 |
| 5   | 18,21,23,30             | 6                  | 6                 |
| 6   | 31,33,34,35             | 3                  | 4                 |
| 7   | 36-39                   | 6                  | 7                 |
| 8   | 41,42,43,45             | 4                  | 4                 |
| 9   | 46,47,52,53             | 6                  | 6                 |
| 10  | 71,75,80,101,102        | 6                  | 6                 |