

Supplementary Materials for

**Early non-neutralizing, afucosylated antibody responses are associated with  
COVID-19 severity**

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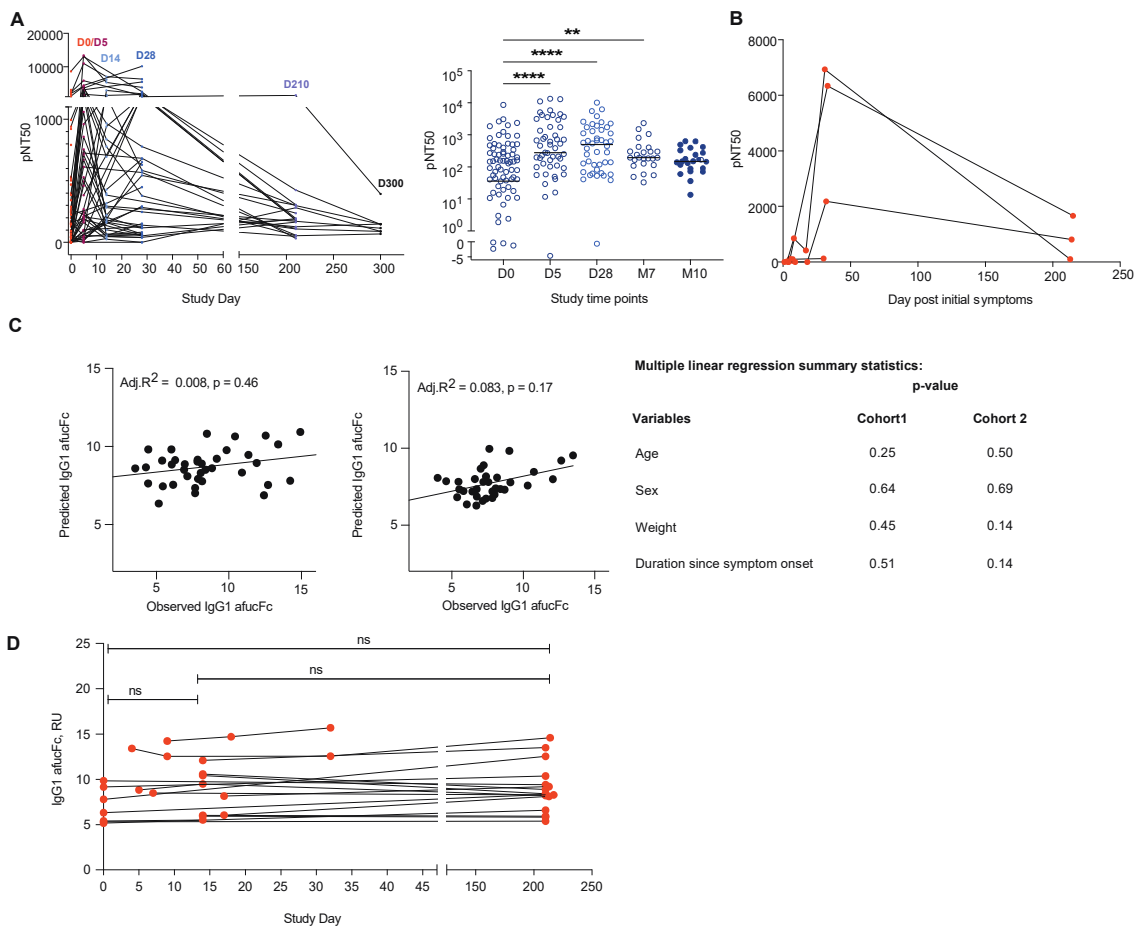
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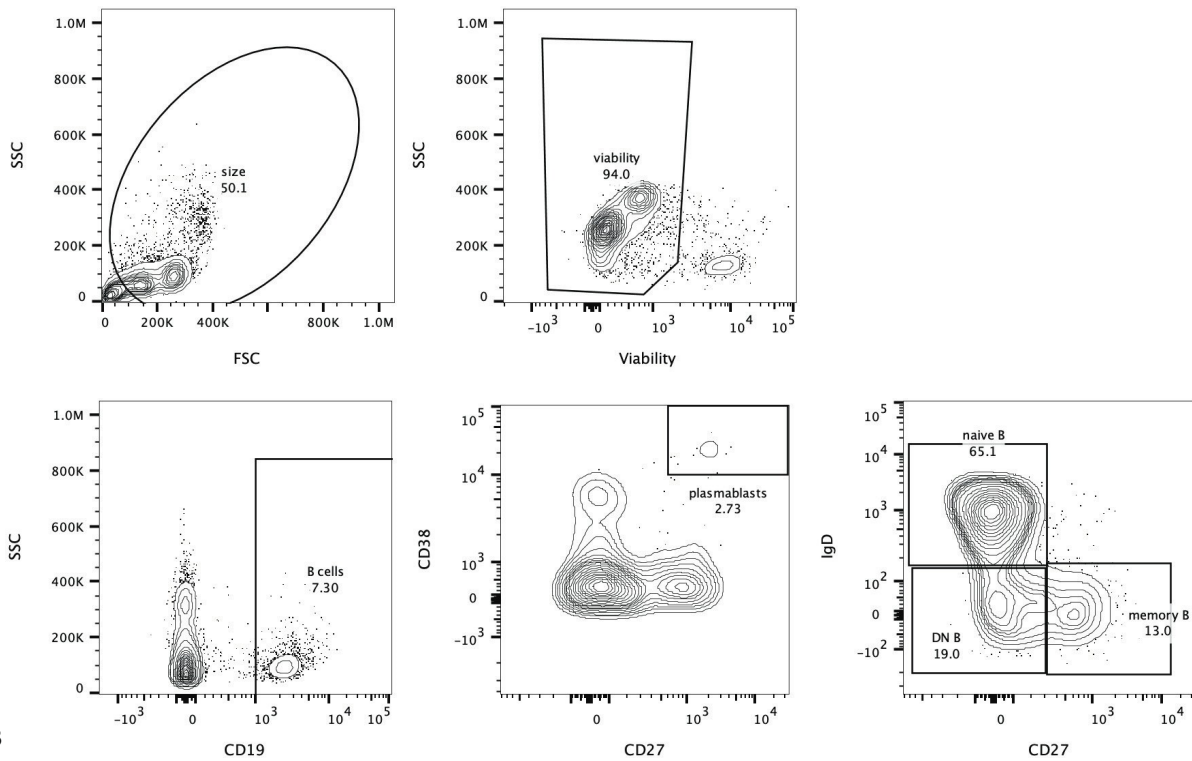
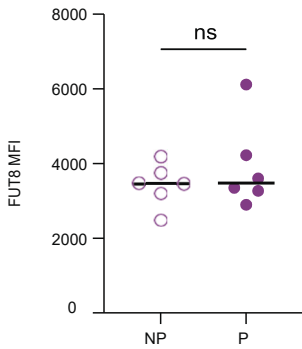
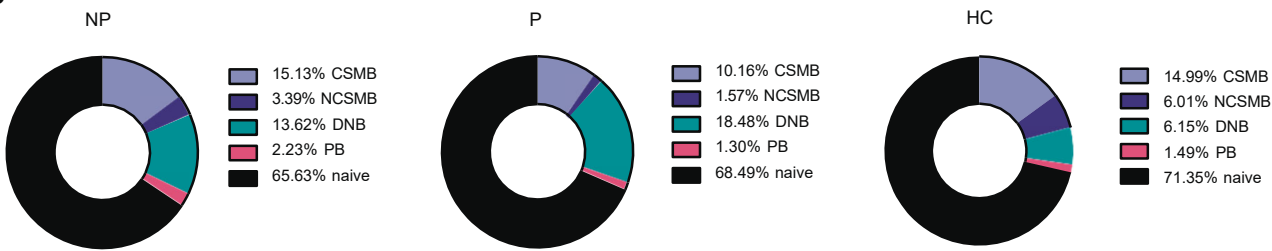
Figs. S1 to S5  
Tables S1 to S4

**Other Supplementary Material for this manuscript includes the following:**

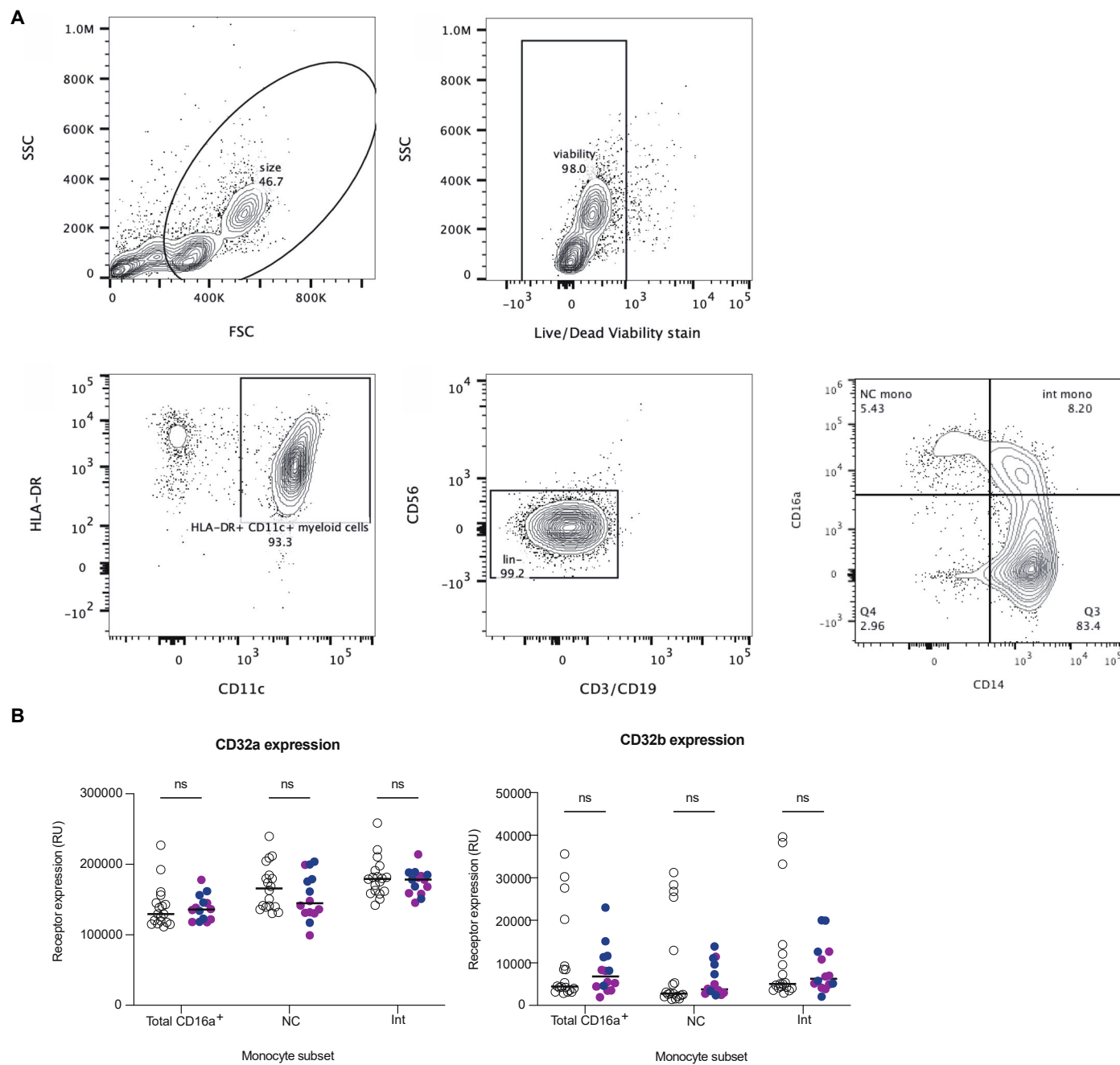
Data file S1  
MDAR Reproducibility Checklist



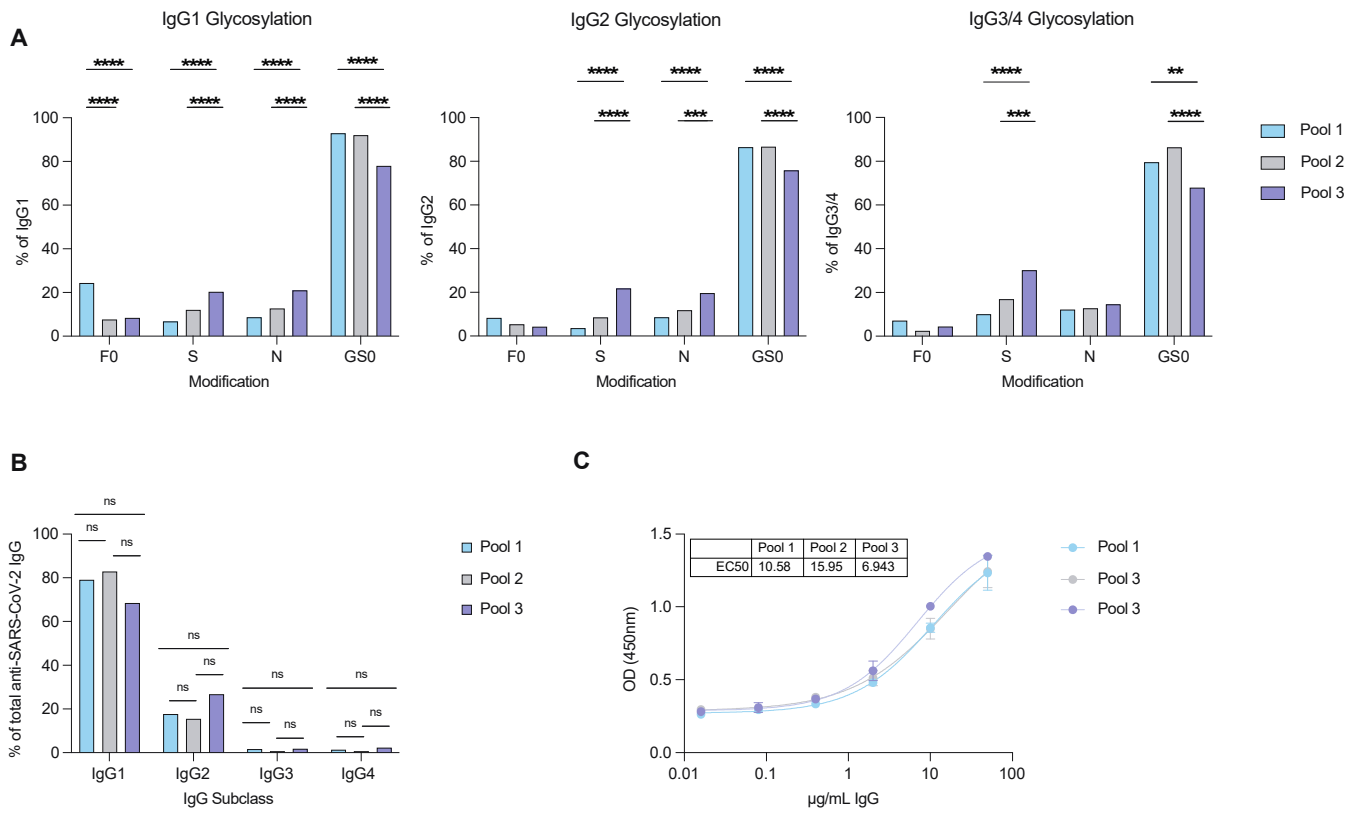
**Fig. S1. Neutralizing titers and abundance of IgG1 Fc afucosylation in COVID-19 outpatients over time. (A)** Half-maximal SARS-CoV-2 pseudovirus neutralizing titers (pNT50) are shown for each study time point. Left panel: graphed to match format of Figure 1A. Right panel: plotted to clearly show the median pNT50 at each study time point. Horizontal bars indicate median. **(B)** The kinetics of neutralizing antibody response are shown over time in progressors in Cohort 1. **(C)** Multivariate regression analysis was used to test the contribution of age, sex, weight, or duration since COVID-19 onset on abundance of afucosylated anti-RBD IgG1 in two cohorts **(D)** Abundance of SARS-CoV-2 specific afucosylated IgG1 (afucFc) is shown for Cohort 1 COVID-19 outpatients over time (n=18). RU, relative units. P values in (A) and (D) were calculated using Kruskal Wallis test with Dunn's correction for multiple comparisons. \*\*P < 0.01; \*\*\*\*P < 0.0001; ns, not significant.

**A****B****C**

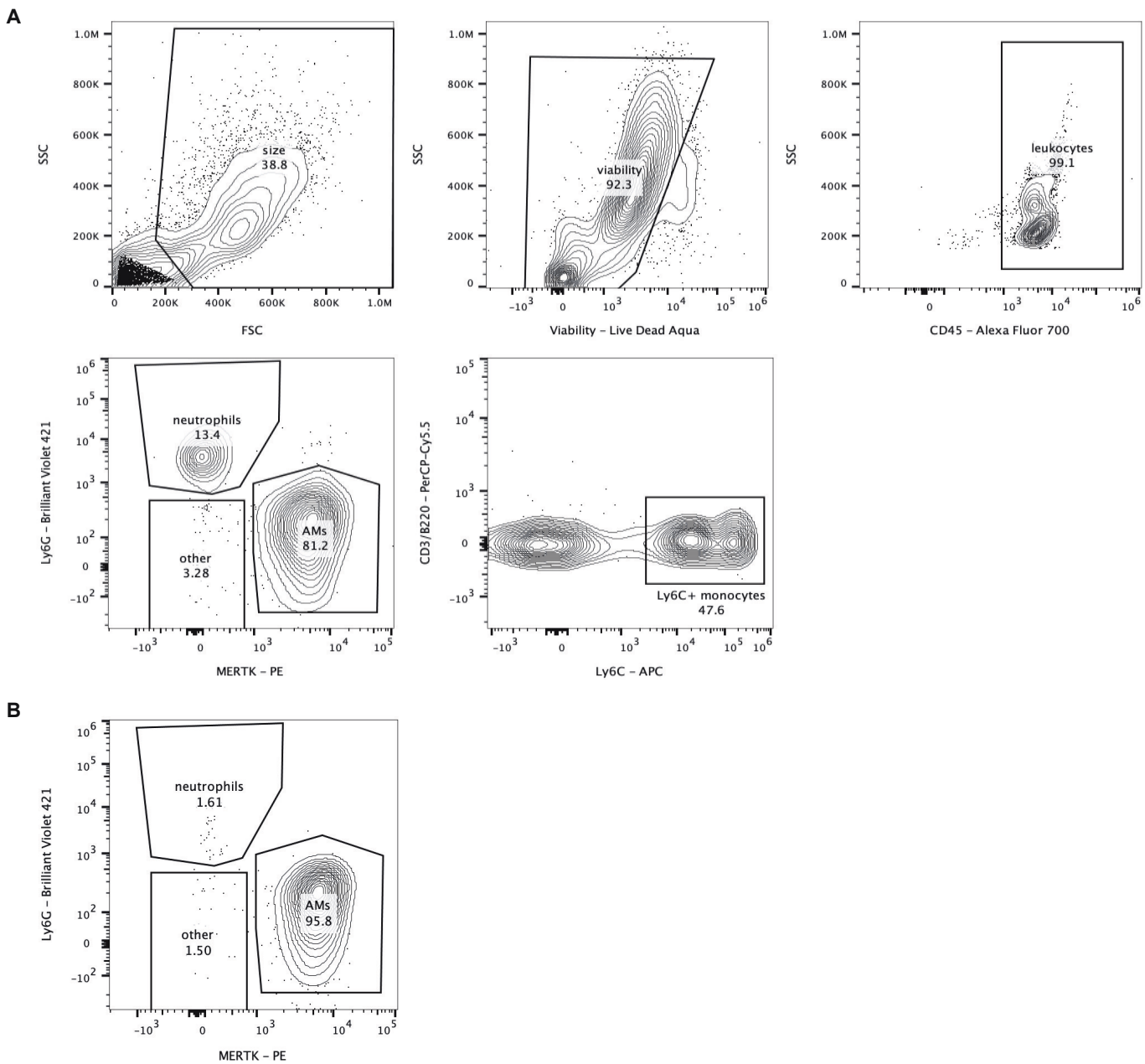
**Fig. S2. Characterization of B cell subsets in COVID-19 outpatients.** (A) The gating strategy for B cell subsets is shown. Total CD19<sup>+</sup> B cells were assessed from within viable peripheral blood mononuclear cells (PBMCs). Plasmablasts were further defined as CD19<sup>+</sup> CD27<sup>+</sup> CD38<sup>++</sup>. Memory B cells were defined as CD19<sup>+</sup> CD27<sup>+</sup> IgD<sup>-</sup>, double negative (DN) B cells were CD19<sup>+</sup> CD27<sup>-</sup> IgD<sup>-</sup>, and naïve B cells were CD19<sup>+</sup> CD27<sup>-</sup> IgD<sup>+</sup>. FSC, forward scatter; SSC, side scatter. (B) FUT8 expression is shown within total viable PBMCs in progressors (P; n = 6) and sex-matched non-progressors (NP; n = 6). MFI, median fluorescence intensity. Horizontal bars indicate median. (C) Distribution of B cell subsets (class switched memory B cells (CSMB), non-class switched memory B cells (NCSMB), CD27<sup>-</sup> IgD<sup>-</sup> double negative B cells (DNB), plasmablasts (PB), and naïve B cells in progressors (P; n = 6), non-progressors (NP, n = 6), and healthy controls (HC, n = 4) are shown. P values in (B) was calculated using unpaired Student's t test; ns, not significant.



**Fig. S3. Characterization of monocyte subsets in COVID-19 outpatients. (A)** The gating strategy for day 0 monocyte subsets and Fc $\gamma$ R expression is shown. Bulk myeloid cells were defined as viable CD3<sup>-</sup> CD19<sup>-</sup> CD56<sup>-</sup> CD11c<sup>+</sup> HLA-DR<sup>+</sup> cells, while CD16a<sup>+</sup> monocytes within this population were additionally positive for CD16a. Within CD16a<sup>+</sup> monocytes, non-classical (NC) monocytes were CD16a<sup>+</sup> CD14<sup>-</sup> while intermediate (Int) monocytes were CD16a<sup>+</sup> CD14<sup>+</sup>. **(B)** Fc $\gamma$ R (CD32a and CD32b) expression on myeloid cell subsets is shown. Horizontal bars indicate median. P values were determined by unpaired Student's test. ns, not significant.



**Fig. S4. Characterization of polyclonal IgG pools. (A)** Glycan composition (F0-afucosylation, GS0-Galactosylation, S-Sialylation and N-Bisection) of IgG1, IgG2 and IgG3/4 was measured for individually purified patient IgG comprising polyclonal pool 1 (F0, F0>20%), pool 2 (F, F0<10%), and pool 3 (Vax). **(B)** IgG subclass distribution is shown for the individually purified patient IgG comprising polyclonal pool 1, pool 2, and pool 3. **(C)** SARS-CoV-2 full length spike binding to polyclonal pool 1, pool 2, and pool 3 is shown; OD, optical density. Data in (C) are presented as mean  $\pm$  SD; EC50, half maximal binding concentration. P values in (A and B) were calculated using two-way ANOVA with Sidak's correction. \*\*P < 0.01; \*\*\*P < 0.001; \*\*\*\*P < 0.0001; ns, not significant.



**Fig. S5. Gating strategy for immune cell infiltrates in mouse BAL. (A)** Neutrophils were defined as viable Ly6G<sup>+</sup> CD11b<sup>+</sup> CD3<sup>-</sup> B220<sup>-</sup> leukocytes. Monocytes were defined as viable CD11b<sup>+</sup> Ly6G<sup>-</sup> MERTK<sup>-</sup> MHC IA/IE<sup>-</sup> CD3<sup>-</sup> B220<sup>-</sup> or Ly6C<sup>+</sup> CD11b<sup>+</sup> Ly6G<sup>-</sup> MERTK<sup>-</sup> MHC IA/IE<sup>-</sup> CD3<sup>-</sup> B220<sup>-</sup> leukocytes. **(B)** Alveolar macrophages were magnetically sorted based on positive expression of MERTK. This sorting method consistently resulted in >90% purity, as determined by flow cytometry. PE, phycoerythrin; APC, allophycocyanin.

## Tables

**Table S1. Demographics of the COVID-19 infection cohorts**

| <b>Stanford Lambda cohort (Cohort 1)</b>                       | <b>Non-progressor (n=102)</b> | <b>Missing values</b> | <b>Progressor (n=8)</b> | <b>Missing values</b> | <b>p value</b> |
|--|-------------------------------|-----------------------|-------------------------|-----------------------|----------------|
| Age, years (median, 95%CI)                                     | 36 (32.8-38.2)                | 0                     | 45 (34.4-55.7)          | 0                     | ns             |
| Sex Female   | 45 (44.1%)                    | 0                     | 3 (38%)                 | 0                     | ns             |
| Race/Ethnicity   |                               | 0                     |                         | 0                     |                |
| Latinx   | 65 (63.7%)                    |                       | 5 (62.5%)               |                       |                |
| White  | 29 (28.4%)                    |                       | 2 (25%)                 |                       |                |
| Asian  | 7 (6.9%)                      |                       | 0 (0%)                  |                       |                |
| Others   | 1(1%)                         |                       | 1 (12.5%)               |                       |                |
| Weight (lbs) (median, 95% CI)                                  | 175 (168.1-181.9)             | 1                     | 198.5 (148.8-248.2)     | 0                     | ns             |
| Body mass index (BMI) (median, 95% CI)                         | 27.2 (26.5-28.9)              | 3                     | 28.8 (23.9-33.7)        | 0                     | ns             |
| <25  | 25 (25.3%)                    |                       | 2 (25%)                 |                       |                |
| 25-30  | 38 (38.4%)                    |                       | 3 (37.5%)               |                       |                |
| >30  | 36 (36.4%)                    |                       | 3 (37.5%)               |                       |                |
| Median days from symptoms onset to study enrollment            | 4                             | 17                    | 3.5                     | 0                     | ns             |
| Mean days from symptoms onset to study enrollment              | 5                             | 17                    | 3.6                     | 0                     | ns             |
| Median days from randomization to disease resolution           | 9                             | 0                     | 16                      | 4                     | ns             |
| <b>Stanford Favipiravir Cohort (Cohort 2)</b>                  | <b>Non-progressor (n=67)</b>  | <b>Missing values</b> | <b>Progressor (n=7)</b> | <b>Missing values</b> | <b>p value</b> |
| Age, years (median, 95%CI)                                     | 40 (36.9-43.1)                | 0                     | 52 (45.9-58)            | 0                     | **             |
| Sex Female   | 35 (52.2%)                    | 0                     | 3 (42.9%)               | 0                     | ns             |
| Race/Ethnicity   |                               | 4                     |                         | 0                     |                |
| Latinx   | 26 (38.8%)                    |                       | 5 (71.4%)               |                       |                |
| White  | 27 (40.2%)                    |                       | 1 (14.3%)               |                       |                |
| Asian  | 7 (10.4%)                     |                       | 0 (0%)                  |                       |                |
| Others   | 3(4.5%)                       |                       | 1 (14.3%)               |                       |                |
| Weight (lbs) (median, 95% CI)                                  | 180 (170.4-189.6)             | 0                     | 190 (146.5-233.4)       | 0                     | ns             |
| Body mass index (BMI) (median, 95% CI)                         | 28.3 (26.8-29.7)              | 0                     | 30.8 (24.7-37)          | 0                     | ns             |
| <25  | 20 (29.9%)                    |                       | 2 (28.6%)               |                       |                |
| 25-30  | 21 (31.3%)                    |                       | 1 (14.3%)               |                       |                |
| >30  | 26(38.8%)                     |                       | 4 (57.1%)               |                       |                |
| Median days from symptoms onset to study enrollment            | 5                             | 3                     | 5                       | 0                     | ns             |
| Mean days from symptoms onset to study enrollment              | 5.7                           | 3                     | 6.4                     | 0                     | ns             |
| Median days from randomization to disease resolution           | 11                            | 0                     | 16                      | 0                     | ns             |
| <b>Mount Sinai Cohort</b>                                      | <b>Hospitalized (n=52)</b>    |                       |                         |                       |                |
| Age, years (median, 95%CI)                                     | 65(60.6-69.4)                 |                       |                         |                       |                |
| Sex Female   | 21(40.4%)                     |                       |                         |                       |                |
| Race/Ethnicity   |                               |                       |                         |                       |                |
| Latinx   | 13 (25%)                      |                       |                         |                       |                |
| White  | 7 (13.5%)                     |                       |                         |                       |                |
| Asian  | 3 (5.8%)                      |                       |                         |                       |                |
| Others   | 29 (55.8%)                    |                       |                         |                       |                |
| Weight (lbs) (median, 95% CI)                                  | N/A                           |                       |                         |                       |                |
| Body mass index (BMI) (median, 95% CI)                         | 26.79(24.6-29.01)             |                       |                         |                       |                |
| <25  | 15(28.8%)                     |                       |                         |                       |                |
| 25-30  | 21(40.4%)                     |                       |                         |                       |                |
| >30  | 16(30.8%)                     |                       |                         |                       |                |
| Median days of sample time point post hospitalization (95% CI) | 4(2.87-5.13)                  |                       |                         |                       |                |

P values were calculated by comparing features between the progressors and non- progressors using unpaired t-tests with Welch's correction for categorical variables or Fisher's t-test for continuous variables. CI, confidence intervals; lbs, pounds; N/A not available; ns, not significant



**Table S2. Description of progressors from Cohort 1**

| <b>Age</b> | <b>Sex</b> | <b>Days of symptoms prior to enrollment</b> | <b>Days to Hospitalization/ED visit post assessment of mild COVID-19</b>                                      |
|------------|------------|---|---|
| 25         | Female     | 3   | 0; progressive symptoms leading to hospitalization within 24-hours.   |
| 58         | Male       | 4   | 5; progressively worsening respiratory symptoms. Referred to ED by study-associated physician on study day 5. |
| 44         | Female     | 3   | 1; progressively worsening respiratory symptoms.  |
| 34         | Male       | 2   | 13; worsening symptoms.   |
| 47         | Male       | 5   | 3; two ED visits within first 3 days for worsening respiratory symptoms.                                      |
| 59         | Female     | 7   | 0; progressive symptoms leading to hospitalization within 24-hours.   |
| 28         | Male       | 4   | 0; progressive symptoms leading to hospitalization within 24-hours.   |
| 46         | Male       | 1   | 2; progressive symptoms leading to hospitalization.   |

**Table S3. Description of progressors from Cohort 2**

| <b>Age</b> | <b>Sex</b> | <b>Days of symptoms prior to enrollment</b> | <b>Days to Hospitalization/ED visit post assessment of mild COVID-19</b>                                     |
|------------|------------|---|--|
| 44         | Female     | 8   | 8; worsening respiratory symptoms  |
| 54         | Male       | 2   | 1; sent by study team to ED for worsening respiratory symptoms and leg swelling, resulted in hospitalization |
| 57         | Male       | 4   | 2; sent by study team to ED for worsening respiratory symptoms; hospitalized                                 |
| 46         | Male       | 5   | 3; worsening symptoms and new rash   |
| 63         | Female     | 10  | 5; sent by study team to ED for worsening symptoms   |
| 52         | Male       | 4   | 10; sent by study team to ED for worsening respiratory symptoms; hospitalized                                |
| 50         | Female     | 8   | 3; sent by study team to ED for worsening respiratory symptoms; hospitalized                                 |

**Table S4: Demographics of the Stanford Adult Vaccination Cohort**

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| <b>Characteristics</b>     |               |
|----------------------------|---------------|
| Age, years (median, 95%CI) | 36(28.3-43.7) |
| Sex Female                 | 11(64.7%)     |
| Race/Ethnicity             |               |
| Latinx                     | 0(0.0%)       |
| White                      | 7(41.2%)      |
| Asian                      | 8(47.1%)      |
| Others                     | 2(11.8%)      |

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