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Supplemental information

Evolution of long-term vaccine-induced and hybrid immunity in healthcare workers after different COVID-19 vaccine regimens

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SUPPLEMENTARY INFORMATION

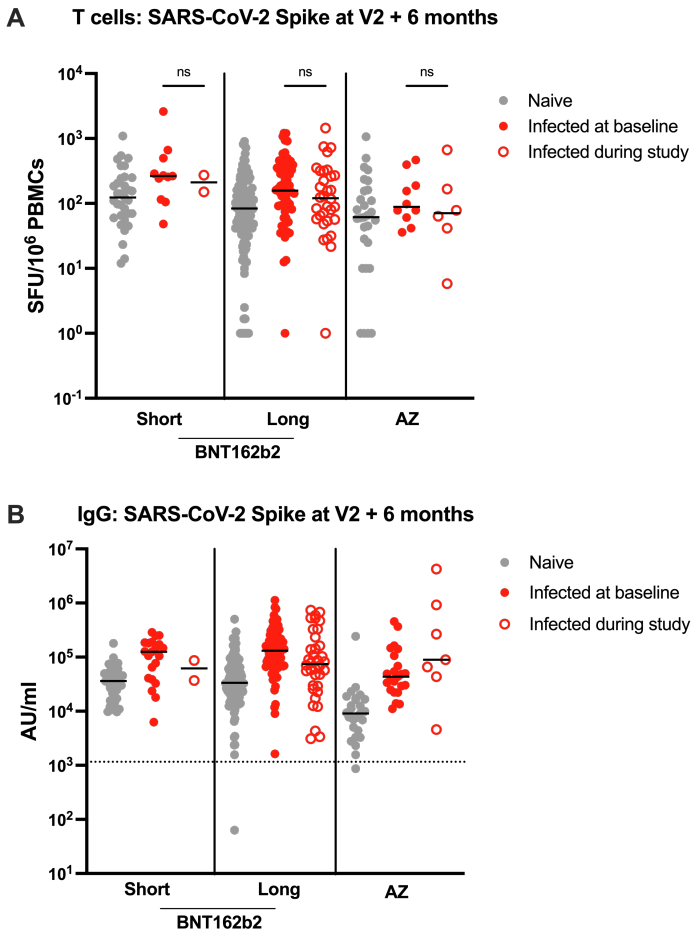

Figure S1. Comparison of T cell and IgG responses in those previously infected at baseline, infected during study, or infected at any time, at six months post second vaccine (related to main figure 1).

(A) Comparison of IFNy ELISpot responses to S (ancestral strain) from cryopreserved PBMCs in short naïve (n=33), infected during study (n=2), previously infected at baseline (n=11) individuals; long naïve (n=116), infected during study (n=32), previously infected at baseline (n=62) individuals; AZ naïve (n=29), infected during study (n=6), previously infected at baseline (n=10) individuals. (B) Effect of vaccine regime and infection status on SARS-CoV-2 S-specific IgG responses in short naïve (n=38), infected during study (n=2), previously infected at baseline (n=21); long naïve (n=132), infected during study (n=36), previously infected at baseline (n=96); AZ naïve (n=27), infected during study (n=7), previously infected at baseline (n=23). Grey circles = naïve; solid red circles = previous infection at baseline; open red circles = infected during study. ELISpot values are expressed as SFU/10^6 PBMCs, with values displayed responses to peptide pools representing S1 and S2 units of S (ancestral strain). IgG responses were measured in serum 6 months after the second dose using multiplexed MSD immunoassays and are shown in arbitrary units (AU)/mL. Horizontal bars represent the median. Vaccine regimens and vaccine status was compared using the Kruskal-Wallis test and Dunn's multiple comparisons correction, with 2-tailed p-values shown above linking lines where significant ($p \leq 0.05$). ns = not significant.

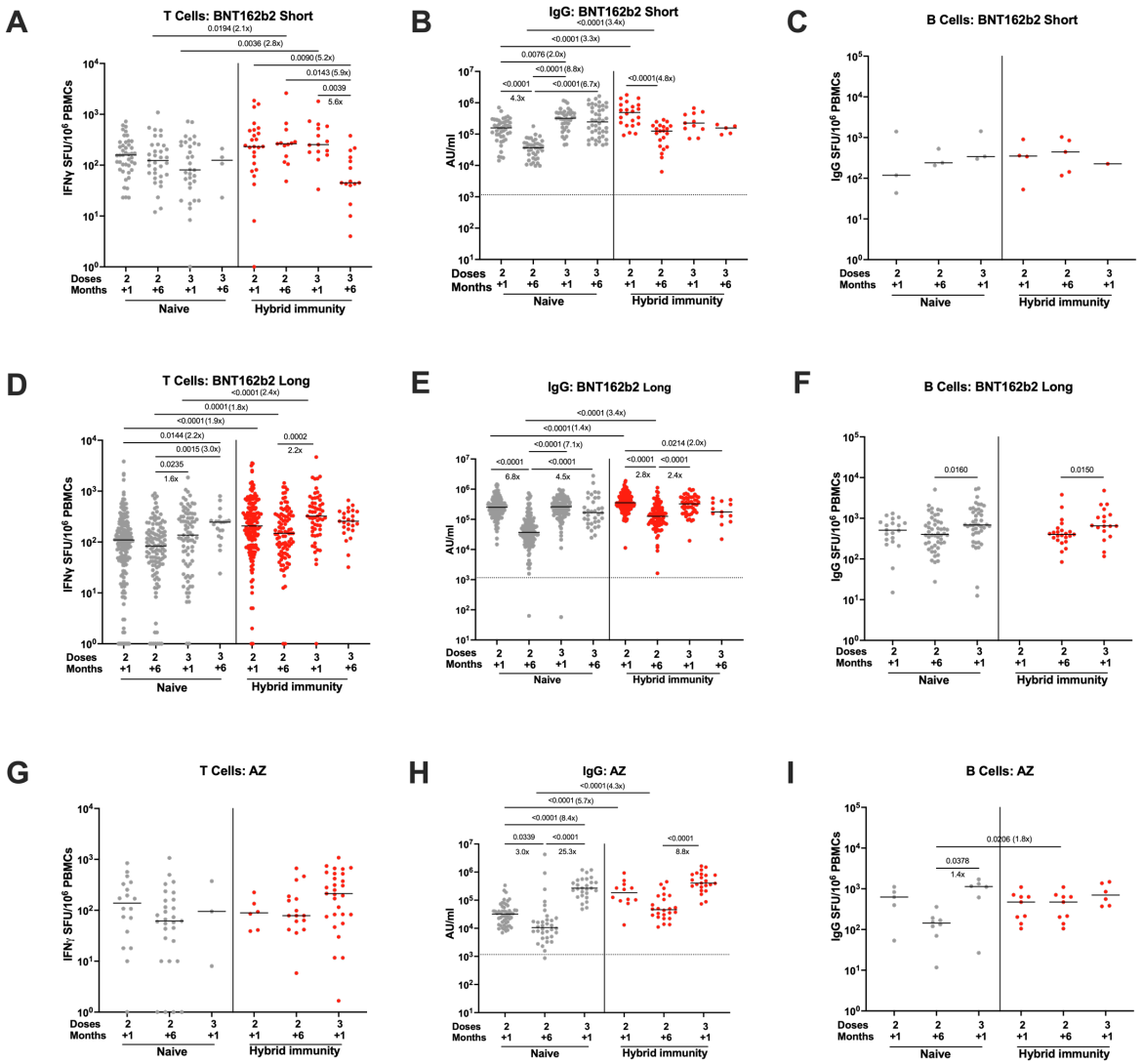

Figure S2. Time course of T cell, binding IgG and B cell responses 1 and 6 months after 2 doses of BNT162b2 (short or long interval) or AZD1222 vaccine (related to main figure 1). Responses are shown one and 6 months after 2 doses and following a third dose of BNT162b2 for (A) T cell responses to SARS-CoV-2 spike by IFNγ ELISpot assay after BNT162b2 (Pfizer-BioNTech) delivered with a short dosing interval ("Short", 3-5 weeks, n=11-44 naïve, n=10-24 hybrid immunity), (B) IgG responses to SARS-CoV-2 spike by MesoScale Discovery (MSD) assay after BNT162b2 Short (n=24-59 naïve, n=8-24 hybrid immunity) and (C) B cell responses to SARS-CoV-2 spike by B cell Elispot assay after BNT162b2 Short (n=6-13 naïve, n=1-4 hybrid immunity). Responses are shown 1 and 6 months after 2 doses and following a third dose of BNT162b2 for (D) T cell responses to spike after a long interval ("Long", 6-17 weeks, n=49-189 naïve, n=31-156 hybrid immunity), (E) IgG responses to spike by MSD assay after BNT162b2 Long (n=123-178 naïve, 78-203 hybrid immunity) and (F) B cell responses to spike after BNT162b2 Long (n=12-47 naïve, n=22-39 hybrid immunity). Responses are shown 1 and 6 months after 2 doses and following a third dose of BNT162b2 for (G) T cell responses to spike after AZD1222 (AstraZeneca) vaccine ("AZ", n=18-26 naïve, 6-26 hybrid immunity), (H) IgG responses to spike by MSD assay after AZD1222 (n=28-54 naïve, n=16-44 hybrid immunity) and (I) B cell responses to spike after AZD1222 (n=5-8 naïve, n=7-10 hybrid immunity).

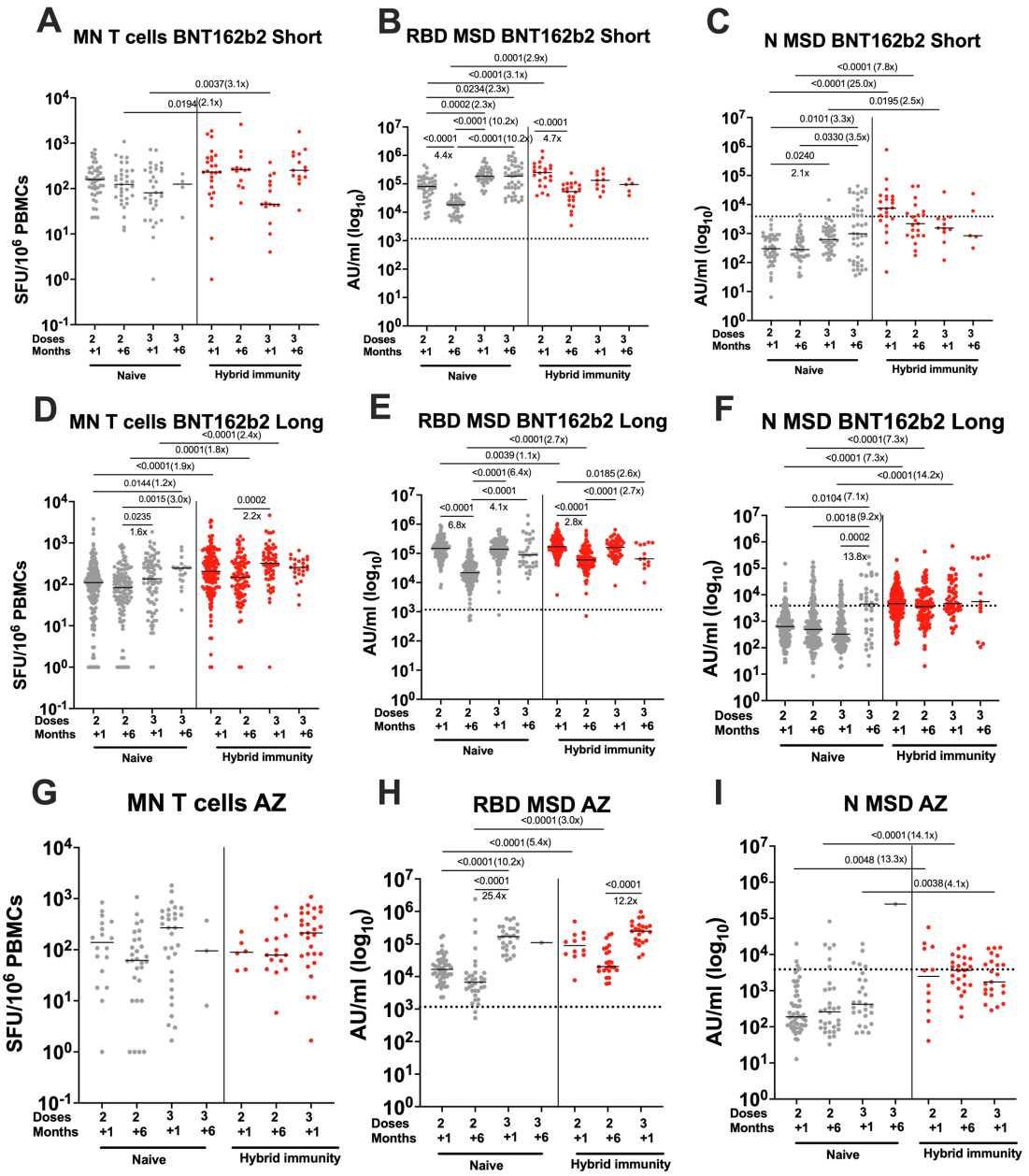

Figure S3. T cell and IgG Antibody responses to membrane protein, nucleocapsid protein and receptor binding domain (related to main figure 1).

T cell and IgG antibody responses to membrane (M) and nucleocapsid (N) protein in participants receiving a primary course of BNT162b2 short dosing interval. (A) IFNy ELISpot responses in PBMCs, (B) IgG against receptor binding domain (RBD) and (B) IgG against nucleocapsid (N). T cell and IgG antibody responses to membrane (M) and nucleocapsid (N) protein in participants receiving a primary course of BNT162b2 long dosing interval. (D) IFNy ELISpot responses in PBMCs, (E) IgG against receptor binding domain (RBD) and (F) IgG against nucleocapsid (N). T cell and IgG antibody responses to membrane (M) and nucleocapsid (N) protein in participants receiving a primary course of AstraZeneca. (G) IFNy ELISpot responses in PBMCs, (H) IgG against receptor binding domain (RBD) and (I) IgG against nucleocapsid (N). Grey circles = naïve individuals, red circles = hybrid immunity. Bars represent the median. Comparisons are with the Kruskal-

Wallis nonparametric test and Dunn’s multiple comparisons correction, with 2-tailed p values shown above linking lines for significant differences with $p \leq 0.05$.

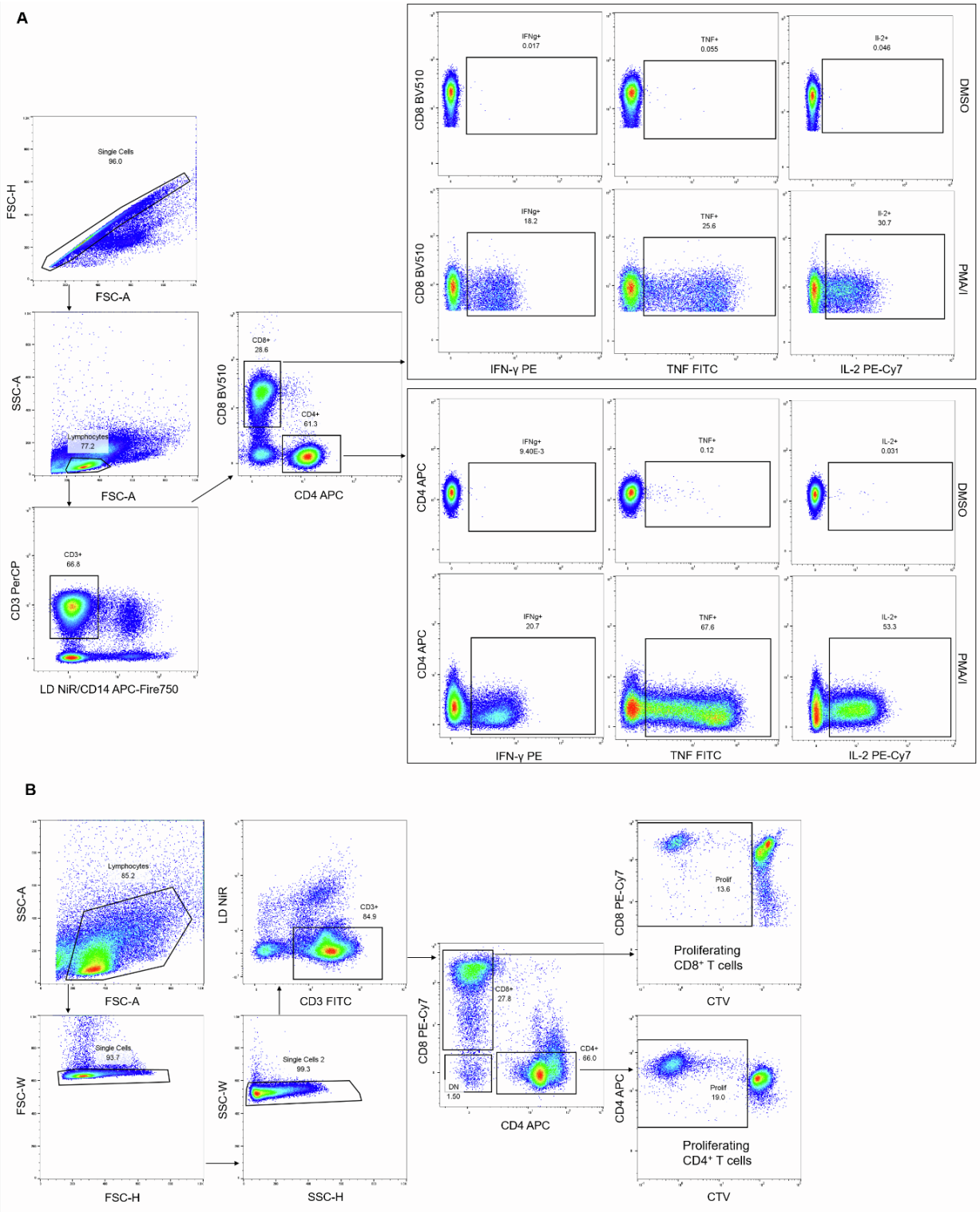

Figure S4. Gating strategy for T cell cytokine secretion (ICS) and proliferation (related to main figures 2 and 3 and STAR methods).
(A) For ICS assays single cells were gated using forward scatter (FSC)- area (A) and FSC-height (H) followed by a lymphocyte gate using FSC-A and side scatter (SSC)- A. Live $CD3^+$ T cells were gated based on exclusion of dead cells (LD-NiR) and monocytes (CD14 APC Fire-750) as well as positivity for CD3 PerCP. T cell subsets were identified based on staining for CD4 APC and CD8 BV510 respectively and expression of cytokines (IFNγ,

TNF, IL-2) was then identified in the $CD4^+CD8^-$ gate as well as the $CD8^+CD4^-$ gate. Representative gating is shown for the DMSO negative control and the PMA/Ionomycin positive control. In the case of PMA/Ionomycin the $CD4^+$ gate was extended all the way to the $CD4^-$ population due to downregulation of expression upon treatment (not shown in the figure). (B) For proliferation assays, lymphocytes were gated FSC-A and SSC-A parameters, followed by two subsequent single cell gates on FSC-H and width (W) as well as SSC-H and W to exclude doublets. From there live T cells were gated (LD-NiR low $CD3^+$) and T cell subsets were identified ($CD4^+CD8^-$ and $CD8^+CD4^-$) using $CD4^+$ APC and $CD8^+$ PE-Cy7. Within the $CD4^+$ and the $CD8^+$ T cell gate proliferating cells were identified by gating on cells with reduced CTV (CellTrace™ Violet) fluorescence intensity.

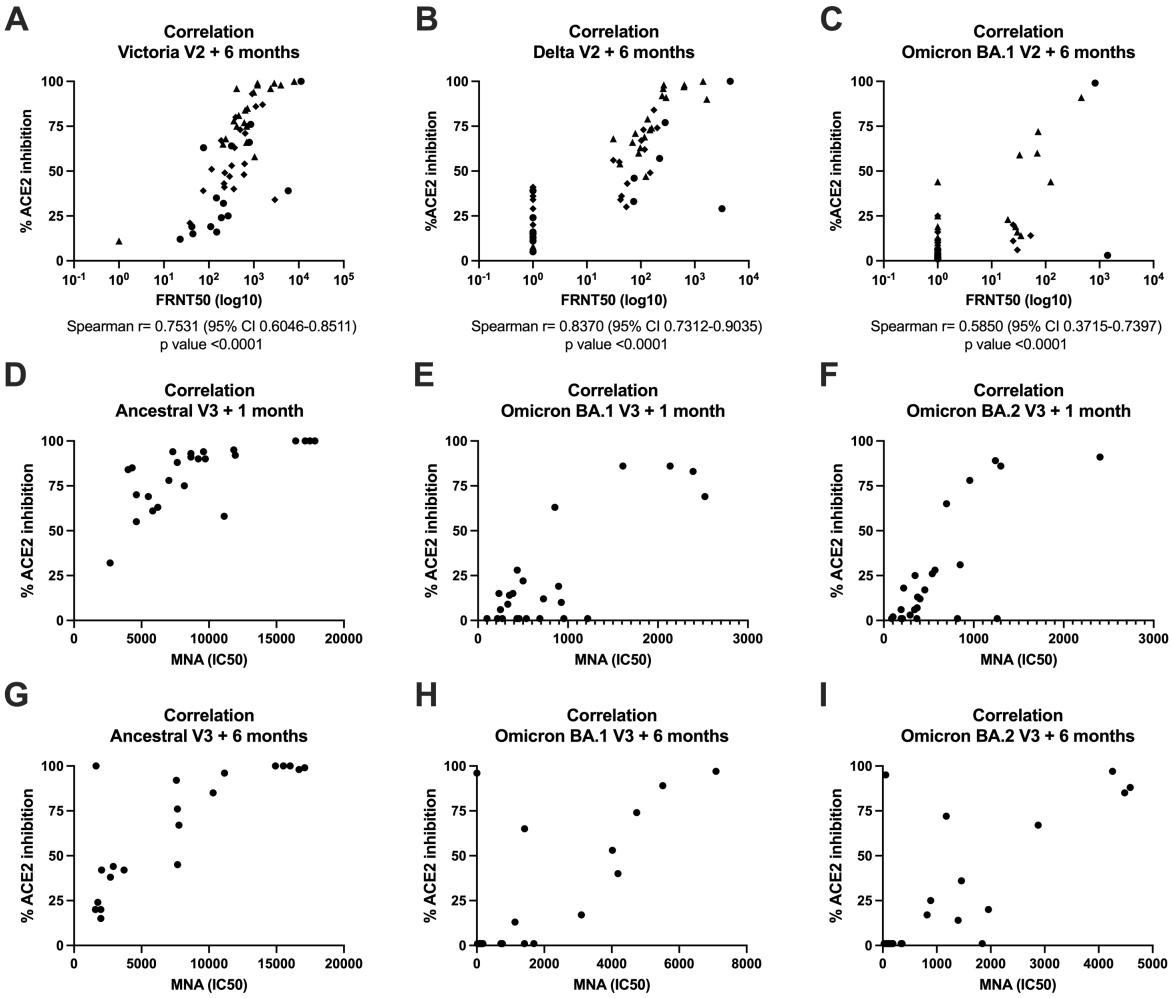

Figure S5. Correlation between ACE2 inhibition and neutralising antibodies (related to main figure 4).

Correlation between the percentage of ACE2 inhibition and neutralisation titres against (A) Victoria, (B) Delta (B.1.617.2) and (C) Omicron BA.1 (B.1.1.529 BA.1), expressed as Focus Reduction Neutralization Assay 50% ($FRNT_{50}$), determined in infection-naïve participants after receiving two doses of BNT162b2 (Pfizer-BioNTech) vaccine delivered in a short ("Short", 3-5 weeks, n=20) or long ("Long", 6-14 weeks, n=20) dosing interval, or two doses of AZD1222 (AstraZeneca) vaccine ("AZ", n=15) 6 months after the second dose. Correlation between the percentage of ACE2 inhibition and neutralisation titres against (D) Ancestral, (E) Omicron BA.1 and (F) Omicron BA.2 expressed as half maximal inhibitory concentration (IC50), determined in infection-naïve participants one month after third vaccine dose. Correlation between the percentage of ACE2 inhibition and neutralisation titres against (G) Ancestral, (H) Omicron BA.1 and (I) Omicron BA.2 expressed as IC50, determined in infection-naïve participants 6 months after third vaccine dose. Pairwise correlations were assessed using Spearman's rank-order correlation. Rhombus = Pfizer short, triangle= Pfizer Long, circle=AZ.

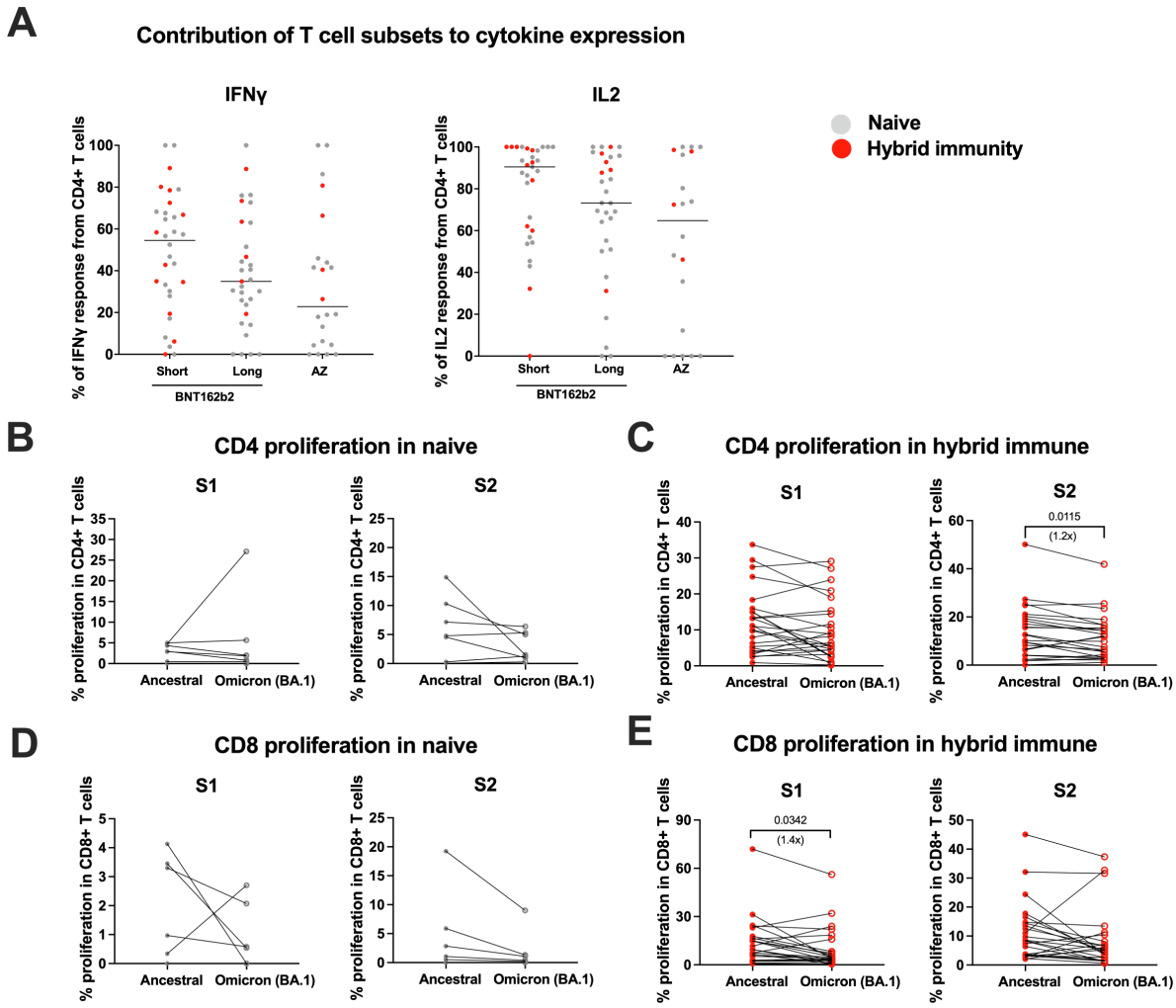

Figure S6. T cell cytokine responses and proliferation to the omicron (BA.1) variant 6 months after the primary vaccine course with BNT162b2 or AZD1222 (related to main figure 6).

(A) Combined data from naïve (grey) and hybrid immunity (red) participants shows cytokine responses (IFN-γ and IL-2) in CD4+ T cells at 6 months post second dose of either BNT162b2 (short and long dosing interval) or AZD1222.

Proliferative responses of $CD4^+$ and $CD8^+$ T cells to SARS-CoV2 spike (S1+S2) from the ancestral strain were compared to the omicron BA.1 variant in a subset of (B, D) naïve (n=9) and (C, E) hybrid immunity (hybrid immunity, n=27) participants from all three vaccine regimens 6 months after the second dose. Individual data points are presented, and paired values are connected with a line. Paired testing was performed using Wilcoxon signed rank test and 2-tailed p values for significant differences ($p \leq 0.05$) are displayed. Closed circles = ancestral spike, open circles = omicron BA.1 spike, Grey = naïve individuals, red= individuals with hybrid immunity.

Supplementary Tables - regression analysis related to Figure 1

Effects of age, sex, SARS-CoV-2 infection and vaccine regimen on binding antibody and T cell responses against SARS-CoV-2 spike protein

All analyses were conducted after log10 transformation of the data.

Reference groups are: BNT162b2 long interval, SARS-CoV-2 unexposed (naive), Female sex

Supplementary Table 1 - Antibody response

Effect of age, sex, SARS-CoV-2 infection and vaccine regimen on IgG MSD binding one month post third (booster) mRNA vaccine dose.

All participants:

```
##
## Call:
## glm(formula = MSD_Spike_log ~ Group + Sex + Age + Exposure, data = Data_antibody)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -3.5670  -0.1714   0.0439   0.2548   0.6932
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)           5.635162   0.105456  53.436   <2e-16 ***
## GroupPFIZER LONG     -0.111552   0.066192  -1.685   0.0931 .
## GroupPFIZER SHORT    -0.036692   0.082460  -0.445   0.6567
## SexM                 -0.014505   0.055182  -0.263   0.7929
## Age                  -0.003842   0.002079  -1.848   0.0658 .
## ExposureConvalescent  0.130881   0.055727   2.349   0.0196 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 0.1636672)
##
##     Null deviance: 45.974  on 274  degrees of freedom
## Residual deviance: 44.026  on 269  degrees of freedom
## AIC: 290.62
##
## Number of Fisher Scoring iterations: 2
```

BNT162b2 short interval group.

```
##
## Call:
## glm(formula = MSD_Spike_log ~ Age + Sex + Exposure, data = Pfizer_short)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -0.82245  -0.19484   0.04456   0.21637   0.62484
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)           5.368752   0.286966  18.709   <2e-16 ***
## Age                   0.002358   0.005965   0.395    0.694
## SexM                 -0.030752   0.099487  -0.309    0.759
## ExposureConvalescent -0.136321   0.133891  -1.018    0.314
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 0.1182208)
##
##     Null deviance: 5.8044  on 51  degrees of freedom
## Residual deviance: 5.6746  on 48  degrees of freedom
## AIC: 42.377
##
## Number of Fisher Scoring iterations: 2
```

BNT162b2 long interval group.

```
##
## Call:
## glm(formula = MSD_Spike_log ~ Age + Sex + Exposure, data = Pfizer_long)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -3.5206  -0.1581   0.0399   0.2384   0.7742
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)           5.632926   0.128801  43.733   <2e-16 ***
## Age                  -0.006836   0.002836  -2.410   0.0170 *
## SexM                  0.071247   0.077723   0.917   0.3606
## ExposureConvalescent  0.144525   0.071920   2.010   0.0461 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 0.1819026)
##
##     Null deviance: 32.412  on 172  degrees of freedom
## Residual deviance: 30.742  on 169  degrees of freedom
## AIC: 202.06
##
## Number of Fisher Scoring iterations: 2
```

AZD1222 group.

```
##
## Call:
## glm(formula = MSD_Spike_log ~ Age + Sex + Exposure, data = Astrazeneca)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -0.81362  -0.17685   0.02944   0.23610   0.57438
##
## Coefficients:
##                        Estimate Std. Error t value Pr(>|t|)
## (Intercept)           5.5367729  0.1553644  35.637   <2e-16 ***
## Age                  -0.0005614  0.0035145  -0.160   0.8738
## SexM                 -0.1977244  0.1114208  -1.775   0.0826 .
## ExposureConvalescent  0.1761229  0.1159844   1.519   0.1357
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 0.1310527)
##
##     Null deviance: 7.0066  on 49  degrees of freedom
## Residual deviance: 6.0284  on 46  degrees of freedom
## AIC: 46.117
##
## Number of Fisher Scoring iterations: 2
```

Supplementary Table 2 - T cell response

Effect of age, sex, SARS-CoV-2 infection and vaccine regimen on the T cell response one month post third (booster) mRNA vaccine dose.

All participants

```
##
## Call:
## glm(formula = log_Spike ~ Group + Sex + Age + Exposure, data = Data_ELISpot)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -2.22573  -0.35072   0.09392   0.44558   1.15404
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)           1.987710   0.156052  12.738  < 2e-16 ***
## GroupPFIZER SHORT    -0.103426   0.105601  -0.979  0.32836
## GroupAZ              -0.090612   0.095477  -0.949  0.34355
## SexM                 -0.241905   0.091264  -2.651  0.00857 **
## Age                   0.004959   0.003317   1.495  0.13631
## ExposureConvalescent  0.275695   0.087091   3.166  0.00175 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## (Dispersion parameter for gaussian family taken to be 0.3799997)
##
##     Null deviance: 102.01  on 245  degrees of freedom
## Residual deviance:  91.20  on 240  degrees of freedom
##   (1 observation deleted due to missingness)
## AIC: 468.02
##
## Number of Fisher Scoring iterations: 2
```

BNT162b2 short interval group.

```
##
## Call:
## glm(formula = log_Spike ~ Age + Sex + Exposure, data = Pfizer_short_T)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -1.77111  -0.29550   0.00585   0.32323   1.05414
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)           1.939336   0.403439   4.807  1.9e-05 ***
## Age                   0.002752   0.008533   0.322   0.7487
## SexM                 -0.267279   0.169757  -1.574   0.1227
## ExposureConvalescent  0.465206   0.188587   2.467   0.0177 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 0.3016591)
##
##     Null deviance: 16.836  on 46  degrees of freedom
## Residual deviance: 12.971  on 43  degrees of freedom
##   (1 observation deleted due to missingness)
## AIC: 82.872
##
## Number of Fisher Scoring iterations: 2
```

BNT162b2 long interval group.

```
##
## Call:
## glm(formula = log_Spike ~ Age + Sex + Exposure, data = Pfizer_long_T)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -2.14673  -0.37439   0.05956   0.40233   1.14516
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)           1.761305   0.203070   8.673 1.23e-14 ***
## Age                   0.008030   0.004414   1.819 0.071151 .
## SexM                 -0.034019   0.122910  -0.277 0.782375
```

```
## ExposureConvalescent  0.418604   0.110259   3.797 0.000221 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 0.3445951)
##
##     Null deviance: 53.260  on 137  degrees of freedom
## Residual deviance: 46.176  on 134  degrees of freedom
## AIC: 250.54
##
## Number of Fisher Scoring iterations: 2
```

AZD1222 group.

```
##
## Call:
## glm(formula = log_Spike ~ Age + Sex + Exposure, data = Astrazeneca_T)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -1.7305  -0.3509   0.1424   0.4367   1.2522
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)           2.258726   0.280138   8.063  5.4e-11 ***
## Age                   0.002383   0.006221   0.383  0.70308
## SexM                 -0.598650   0.197288  -3.034  0.00363 **
## ExposureConvalescent -0.109155   0.190335  -0.573  0.56857
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 0.4656948)
##
##     Null deviance: 31.042  on 60  degrees of freedom
## Residual deviance: 26.545  on 57  degrees of freedom
## AIC: 132.36
##
## Number of Fisher Scoring iterations: 2
```