Supplemental information

Impact of SARS-CoV-2 variants on the total CD4+ and CD8+ T cell reactivity in infected or vaccinated individuals

Alison Tarke, John Sidney, Nils Methot, Esther Dawen Yu, Yun Zhang, Jennifer M. Dan, Benjamin Goodwin, Paul Rubiro, Aaron Sutherland, Eric Wang, April Frazier, Sydney I. Ramirez, Stephen A. Rawlings, Davey M. Smith, Ricardo da Silva Antunes, Bjoern Peters, Richard H. Scheuermann, Daniela Weiskopf, Shane Crotty, Alba Grifoni, and Alessandro Sette
Fig. S1. SARS-COV-2 serology, DMSO controls, and ancestral Spike MP responses for all the cohorts analyzed in this study, related to Figures 1, 2 and 3 and Table 1.

(A) Spike RBD serology in COVID-19 convalescents (n=28, filled circles) and COVID-19 vaccinees (Pfizer/BioNTech BNT162b2 (n=14, open triangles) and Moderna COVID-19 vaccines (n=15, open circles)). Unexposed donors (n=23, not plotted) were seronegative or collected prior to the emergence of SARS-CoV-2. (B-C) AIM+ CD4+ (B) or CD8+ (C) T cells stimulated with DMSO or the ancestral S MP at 1ug/mL. (D) IFNγ SFC per million PBMC stimulated with DMSO or the ancestral S MP at 1ug/mL. (E-F) AIM+ CD4+ (E) or CD8+ (F) T cells stimulated with the ancestral S MP at 1ug/mL. Dotted black lines indicate the threshold of positivity calculated based on median +2 SD of the DMSO controls (>80). Data is plotted after background subtraction and SI>2. (G) IFNγ SFC per million PBMC stimulated with the ancestral S MP at 1ug/mL. Data is plotted after background subtraction, SI>2, and p value <0.05 by Poisson or T test.
Fig. S2. Fold-change analyses of convalescent COVID-19 and unexposed donor responses to variant MPs, Related to Figures 1 and 2.

(A-G) Convalescent COVID-19 (n = 28) and (H-M) unexposed (n = 23) donors were stimulated with SARS-CoV-2 MPs corresponding to the ancestral reference strain (black) and the B.1.1.7 (grey), B.1.351 (red), P.1 (orange) and CAL.20C (light blue) SARS-CoV-2 variants. (A-B) Fold-change of AIM⁺ CD4⁺ (A) and CD8⁺ (B) T cells with S MPs at 1μg/mL. (C) Fold-change of total IFNγ SFC/10⁶ PBMC with S MPs at 1μg/mL. (D-E) Fold-change of AIM⁺ CD4⁺ (D) and CD8⁺ (E) T cells with Spike MPs at 0.1 and 0.01 μg/mL. (F-G) Fold-change of the sum of AIM⁺ CD4⁺ (F) and CD8⁺ (G) T cells for all SARS-CoV-2 antigens. (H-I) Percentages of AIM⁺ (OX40⁺CD137⁺) CD4⁺ T cells (H) and AIM⁺ (CD69⁺CD137⁺) CD8⁺ T cells (I) for the total reactivity. (J-K) Percentages of AIM⁺ (OX40⁺CD137⁺) CD4⁺ T cells (J) and AIM⁺ (CD69⁺CD137⁺) CD8⁺ T cells (K) for each MP. Bars represent the geometric mean. (L-M) Fold-change of the sum of AIM⁺ CD4⁺ (L) and CD8⁺ (M) T cells for all SARS-CoV-2 antigens in unexposed donors. p values listed at the top of graphs correspond to the discrepancy and were calculated by one sample Wilcoxon Signed Rank test compared to the lower bound fold change threshold of 0.47 for CD4 AIM⁺, 0.55 for CD8 AIM⁺, and 0.6 for IFNγ SFC/10⁶ PBMC.
Figure S3. Fold-change analyses of COVID-19 vaccinees tested with the variant MPs, related to Figure 3. PBMCs of Pfizer/BioNTech BNT162b2 (n=14, triangles) and Moderna COVID-19 vaccines (n=15, circles) were stimulated with the Spike MPs corresponding to the ancestral reference strain (black) and the B.1.1.7 (grey), B.1.351 (red), P.1 (orange) and CAL.20C (light blue) SARS-CoV-2 variants. (A-B) Fold-change of AIM⁺ CD4⁺ (A) and CD8⁺ (B) T cells with Spike MPs at 1 µg/mL. (C) Fold-change of total IFNγ SFC/10⁶ PBMC with Spike MPs at 1 µg/mL. (D-E) Fold-change of AIM⁺ CD4⁺ (D) and CD8⁺ (E) T cells with Spike MPs at 0.1 and 0.01 µg/mL. p values listed at the top of graph correspond to the discrepancy and were calculated by one sample Wilcoxon Signed Rank test compared to the lower bound fold change threshold of 0.47 for CD4 AIM⁺, 0.55 for CD8 AIM⁺, and 0.6 for IFNγ SFC/10⁶ PBMC.
Figure S4. Memory phenotype of COVID-19 convalescent and vaccinated donors, related to Figures 1, 2, and 3.
(A-B) The memory phenotype of COVID-19 convalescent donors (n = 28) was analyzed to determine the makeup of naïve (CD45RA⁺CCR7⁺), central memory (CD45RA⁻CCR7⁺), effector memory (CD45RA⁻CCR7⁻), and terminally differentiated effector memory (CD45RA⁺CCR7⁻) in the bulk and SARS-CoV-2 AIM⁺ populations for CD4⁺ (A) and CD8⁺ (B) T cells. (C-D) The memory phenotype was also analyzed for the COVID-19 vaccinees (n = 29) for CD4⁺ (C) and CD8⁺ (D) T cells. The violin plots represent the bulk CD4⁺ or CD8⁺ T cell populations (white) or the subsets of AIM⁺ T cells positive for the ancestral reference strain (black) and the B.1.1.7 (grey), B.1.351 (red), P.1 (orange) and CAL.20C (light blue) SARS-CoV-2 variants. p values were calculated comparing the bulk population to the AIM⁺ populations separately for each variant by two-tailed Wilcoxon test.
Figure S5. Gating strategy for AIM assay and memory phenotyping, related to Figures 1, 2, and 3.
Representative graphs illustrating the gating strategy used in the flow cytometry AIM assays in order to define antigen-specific CD4+ (outlined in blue) and CD8+ (outlined in red) T cells by the expression of OX40+CD137+ and CD69+CD137+, respectively. The memory compartment was analyzed by gating naïve (CD45RA+CCR7+), central memory (CD45RA−CCR7+), effector memory (CD45RA−CCR7+), and terminally differentiated effector memory (CD45RA−CCR7−) cells on the bulk CD4+ and CD8+ T cells as well as the AIM+ subsets, overlaid in blue and red, respectively. These graphs depict one of the COVID-19 convalescent donors from this study tested with each of the VOCs S MP and are representative of the gating strategy utilized with all donors tested.
| Protein | Amino acid position | Ancestral (Wu) | B.1.1.7 (UK) | B.1.351 (SA) | P.1. (BR) | CAL.20C (CA) |
|---------|---------------------|----------------|--------------|--------------|-----------|--------------|
| S       | 13                  | S              |              | F            | F         |              |
| S       | 20                  | T              |              |              | N         |              |
| S       | 26                  | P              |              |              | S         |              |
| S       | 69                  | H              | Del          |              |           |              |
| S       | 70                  | V              | Del          |              |           |              |
| S       | 80                  | D              | A            |              |           |              |
| S       | 138                 | D              | Y            |              |           |              |
| S       | 145                 | Y              | Del          |              |           |              |
| S       | 152                 | W              | C            |              |           |              |
| S       | 190                 | R              | S            |              |           |              |
| S       | 215                 | D              | G/H          |              |           |              |
| S       | 241                 | L              | Del          |              |           |              |
| S       | 242                 | L              | Del          |              |           |              |
| S       | 243                 | A              | Del          |              |           |              |
| S       | 417                 | K              | N            | T            |           |              |
| S       | 452                 | L              |              |              | R         |              |
| S       | 484                 | E              | K            | K            |           |              |
| S       | 501                 | N              | Y            | Y            | Y         |              |
| S       | 570                 | A              | D            |              |           |              |
| S       | 614                 | D              | G            | G            | G         | G            |
| S       | 655                 | H              | Y            |              |           |              |
| S       | 681                 | P              | H            |              |           |              |
| S       | 701                 | A              | V            |              |           |              |
| S       | 716                 | T              | I            |              |           |              |
| S       | 938                 | L              |              | F            |           |              |
| S       | 982                 | S              | A            |              |           |              |
| S       | 1027                | T              |              | I            |           |              |
| S       | 1118                | D              | H            |              |           |              |
| S       | 1176                | V              | F            |              |           |              |
| S       | 1191                | K              |              |              |           | N            |
| M       | 162                 | K              |              | N            |           |              |
| N       | 3                   | D              | L            |              |           |              |
| N       | 13                  | P              | S            |              |           |              |
| N       | 32                  | R              | H            |              |           |              |
| N       | 80                  | P              |              | R            |           |              |
| N       | 203                 | R              | K            | K            |           |              |
| N       | 204                 | G              | R            | R            |           |              |
| N       | 205                 | T              | I            | I            |           |              |
| N       | 212                 | G              |              | C            |           |              |
| N       | 234                 | M              |              | I            |           |              |
| N       | 235                 | S              | F            |              |           |              |
| E       | 71                  | P              |              | L            |           |              |
| ORF3a   | 57                  | Q              | H            | H            |           |              |
| ORF3a   | 131                 | W              | L            |              |           |              |
| ORF3a   | 171                 | S              | L            |              |           |              |
| ORF3a   | 253                 | S              | P            |              |           |              |
| ORF7a   | 93                  | V              | F            |              |           |              |
| Protein | 27 | 92 | ORF8 | 121 | 109 | 85 | 339 | 366 | 427 | 563 | 183 | 186 | 370 | 778 | 837 | 890 | 926 | 977 | 1180 | 1412 | 1778 | 395 | 90 | 193 | 106 | 107 | 108 | 125 | 135 | 149 | 167 | 65 | 105 | 323 | 53 | 209 | 260 | 341 | 588 | 177 | 326 | 328 | 91 |   |
|---------|----|----|------|-----|-----|----|-----|-----|-----|-----|-----|-----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|----|----|-----|-----|-----|-----|-----|----|-----|-----|-----|-----|-----|-----|----|-----|-----|-----|
|         |    |    |      |     |     |    |     |     |     |     |     |     |    |    |     |     |     |     |     |     |     |     |    |    |     |     |     |     |     |    |    |     |     |     |     |     |    |    |     |     |    |    |
| Protein | Aminoacid position (Start) | Aminoacid position (End) | SARS-CoV-2 strain | Sequence |
|---------|---------------------------|--------------------------|-------------------|----------|
| nsp3    | 173                       | 187                      | B.1.1.7           | QDGEDNQTITRPT |
| nsp3    | 178                       | 192                      | B.1.1.7           | DNIQEEITPTEVQF |
| nsp3    | 183                       | 197                      | B.1.1.7           | RHIQDTEVPQGQMLD |
| nsp3    | 878                       | 892                      | B.1.1.7           | QDAYYRARAGEADNFI |
| nsp3    | 883                       | 897                      | B.1.1.7           | RARAEADNFCALII |
| nsp3    | 888                       | 902                      | B.1.1.7           | EADNFCAALIAYCN |
| nsp3    | 1398                      | 1412                     | B.1.1.7           | NYLKLPSNFKLIDE |
| nsp3    | 1403                      | 1417                     | B.1.1.7           | PNFKLNIHITWFL |
| nsp3    | 1408                      | 1422                     | B.1.1.7           | LNIHTWFLILLSVCL |
| nsp6    | 92                        | 106                      | B.1.1.7           | MRMJMTWLMVDVTSILK |
| nsp6    | 97                        | 111                      | B.1.1.7           | WLDMDVTDLSKLKDCV |
| nsp6    | 102                       | 116                      | B.1.1.7           | DTSLLKLDCCVYMAASA |
| nsp6    | 107                       | 121                      | B.1.1.7           | KLKDCKMVASAVLVE |
| nsp12   | 309                       | 323                      | B.1.1.7           | HCANCNFNYLFSYFVPL |
| nsp12   | 314                       | 328                      | B.1.1.7           | NVLPSYTVFPLTSGF |
| nsp12   | 319                       | 333                      | B.1.1.7           | TVNFPLTSGFPLYRKO |
| nsp12   | 66                        | 80                       | B.1.1.7           | MLGMPGNQGNAPAHL |
| nsp12   | 191                       | 205                      | B.1.1.7           | RNSRNSPFGYK |-
| Donor   | MP 1    | MP 2    | MP 3    | MP 4    | MP 5    | MP 6    | MP 7    | MP 8    | MP 9    | MP 10   | MP 11   |
|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|         | MP 1    | MP 2    | MP 3    | MP 4    | MP 5    | MP 6    | MP 7    | MP 8    | MP 9    | MP 10   | MP 11   |
|         | 0.381   | 0.2116  | 0.125   | 0.1312  | 0.1412  |         |         |         |         |         |         |
| 4801    | 0.231   | 0.1616  | 0.171   | 0.0626  |         |         |         |         |         |         |         |
| 4837    | 0.2255  | 0.2745  | 0.1555  | 0.0575  |         |         |         |         |         |         |         |
| 4842    | 0.1478  | 0.1443  | 0.16674 |         |         |         |         |         |         |         |         |
| 4851    | 0.13846 | 0.039   |         |         |         |         |         |         |         |         |         |
| 4863    | 0.53843 | 0.71211 |         |         |         |         |         |         |         |         |         |
| 4866    | 0.17948 | 0.106   |         |         |         |         |         |         |         |         |         |
| 4867    | 0.36489 | 0.335   | 0.064   | 0.22567 |         |         |         |         |         |         |         |
| 4868    | 1.81486 | 1.1895  | 0.40857 |         |         |         |         |         |         |         |         |
| 4878    | 0.16885 | 0.0455  | 0.158   |         |         |         |         |         |         |         |         |
| 4882    | 0.12956 | 0.6415  | 0.104   |         |         |         |         |         |         |         |         |
| 4963    | 0.324   | 0.5052  | 0.29933 |         |         |         |         |         |         |         |         |
| 4972    | 0.318   | 0.5625  |         |         |         |         |         |         |         |         |         |
| 4995    | 0.27203 | 0.2833  |         |         |         |         |         |         |         |         |         |
| 4973    | 0.10415 | 0.1414  | 0.08613 |         |         |         |         |         |         |         |         |
| 5329    | 0.047   | 0.082   |         |         |         |         |         |         |         |         |         |
| 5328    | 0.5267  | 0.7097  |         |         |         |         |         |         |         |         |         |
| 5476    | 0.022   | 0.125   |         |         |         |         |         |         |         |         |         |
| 5475    | 0.067   | 0.091   |         |         |         |         |         |         |         |         |         |
| 5502    | 0.035   |         |         |         |         |         |         |         |         |         |         |
| 5530    | 0.057   | 0.059   |         |         |         |         |         |         |         |         |         |
| 5520    | 0.055   | 0.005   |         |         |         |         |         |         |         |         |         |
| 5521    | 0.024   | 0.041   |         |         |         |         |         |         |         |         |         |
| 5451    | 0.032   | 0.023   |         |         |         |         |         |         |         |         |         |

**Table S3.** Related to Figures 1, 2, and 3. Technical replicates for the AIM and FluoroSPOT assays testing multiple donors in repeated assays with the same SARS-CoV-2 MPs.
| Origin | Ancestral reference sequence | Protein Start | Mutation | Mutated sequence | HLA restriction (IC_{50} nM) | WT (IC_{50} nM) | Mutant (IC_{50} nM) | Fold difference | Effect |
|--------|-----------------------------|----------------|----------|------------------|-------------------------------|----------------|-------------------|----------------|--------|
| CAL.20C | LPLVSSVC | S8 | | S13I | LPLVSSVC | B*05:01 | 402 | 272 | 0.68 | Neutral |
| CAL.20C | YYYHNNKSW | S144 | W152C | | SCMESEFRV | A*29:02 | 49 | 980 | 20 | Decrease |
| CAL.20C | KVGGNNYL | S444 | L452R | KVGGNNYL | A*29:02 | 101 | 505 | 5.0 | Decrease |
| CAL.20C | VGGNNYN | S445 | L452R | VGGNNYN | A*29:02 | 94 | 519 | 5.5 | Decrease |
| CAL.20C | UNNYLRLF | S448 | L452R | UNNYLRLF | A*29:02 | 21 | 108 | 5.1 | Decrease |
| CAL.20C | YQQVNCET | S612 | D164G | YQQVNCET | A*20:06 | 18 | 57 | 3.2 | Decrease |
| CAL.20C | QSAKII | ORF3a | | Q57H | HAISGTNGTK | A*68:01 | 55 | 44 | 0.8 | Neutral |
| CAL.20C | WFFSNYLKR | nsp4 | | S395T | WFFSNYLKR | A*31:01 | 70 | 98 | 1.4 | Neutral |

a) Mutation noted as ancestral residue-position-variant residue. Del refers to deletion of the corresponding residue.

b) For deletion mutants, the peptide sequence shown represents the variant encompassing the same region that has the highest predicted binding affinity for the corresponding restricting allele.

c) Indicates predicted IC_{50} for the corresponding reported restricting allele. Predictions were performed using the NetMHCpan BA 4.1 algorithm, hosted by the IEDB.

d) Increase/decrease in affinity defined by a two-fold difference in predicted IC_{50} nM.