Supplemental information

The antibody response to SARS-CoV-2

Beta underscores the antigenic
distance to other variants

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**Figure S1. Binding / neutralisation comparisons of Beta-43 and Beta-49/50.** (A) Binding of Beta-43 to plate immobilized Wuhan, Alpha, Beta and Gamma NTDs was determined by ELISA, mAb 407 is a cross reactive anti-NTD mAb. (B) Cross reactivity of Beta 49, 50 with SARS-CoV-1 S trimer measured by ELISA and compared with mAb S309. (C) SARS-CoV pseudovirus neutralisation curves for mAb S309, Beta-49, 50, 53. (D) Neutralization curves of Beta using mAb S309, Beta-49, 50, 53. The data points for S309 have been previously published (Dejnirattisai et al., 2021b). Related to Figure 2.
Figure S2. Cluster analysis (see Methods) of the pairwise BLI competition experiment. The points are projected onto two dimensions with the minimal information loss. There is a reasonably continuous distribution which essentially runs from one flank up across the shoulder, neck, shoulder to the opposite flank in the torso analogy. Antibody names are abbreviated, from Beta-n to simply n. Related to Figure 3.
Figure S3. Gold-standard FSC curves for global and locally refined S-Fab complexes. Local refined maps are colored in cyan, orange and light grey for RBD, Fab and the rest respectively, and fitted RBD/Fab models in burgundy (with black outline). Map/model pairs are 180° rotated with respect to one another. Related to Figure 4.
Figure S4. Epitopes of antibodies Beta-49 and 50. (A) The binding mode of Beta-49 compared to Beta-53 and S309. The crystal structure of Beta-49/Beta-RBD is shown with the RBD as grey surface with the three mutation sites highlighted in magenta, the VhVl as ribbons and N343 glycans as sticks (left panel). The middle panel compares the binding mode of Beta-49 to Beta-53 (cyan, PDB ID 7PS2), and the right panel to S309 (blue, PDB ID 7BEP). (B) Sequences of the RBD region for SARS-CoV-2 and SARS-CoV-1. The conserved major epitope is marked in cyan, N and C mark the ends of the natural sequence in the soluble RBD construct. Secondary structure in SARS-CoV-2 is marked above and sequence numbers are given for SARS-CoV-2. Drawn with ESPript (Gouet et al 1999). Related to Figure 6.
Figure S5. NTD conformational changes. Comparison of the crystal structures of Beta-43 bound Beta-NTD and that of the original virus strain. (A) Comparing the two Beta-NTD/Beta-43 complexes in the crystal asymmetric unit by overlapping the Beta-NTD. The Beta-NTD, HC and LC are coloured green, red and blue in one complex, and pale green, salmon and light blue in the second. Mutation sites are shown as magenta spheres. The Cα of residue 241 is in orange, after which there is a three-residue deletion in the Beta variant. (B) Overlap of the Beta-NTD (green) with the biliverdin bound NTD (Grey, PDB ID 7B62). The boxed area shows the large differences between the two structures. (C), (D) Comparisons of Beta-NTD/Beta-43 complex (coloured as in (A)) with NTD/N11 Fab complex (C) (grey, salmon and light blue for NTD, HC and LC respectively. PDB ID 7E7X) and NTD/S2M28 Fab complex (D) (colour scheme is as for (C). PDB ID 7LY3). Related to Figure 7.
Table S1A. IC50 titres of 28 Beta SARS-CoV-2-specific mAbs against live virus strains Victoria, B.1.1.7, B.1.351, P.1, B.1.1.7+E484K, B.1.525 and 1.617.2. The data are shown as mean±s.e.m. of 2 independent experiments. Related to Figure 2.

| mAbs        | IC50 (µg/ml) |
|-------------|--------------|
|             | Victoria | Alpha | Beta | Gamma | Alpha+E484K | B.1.525 | Delta |
| β06         | >10      | 0.024±0.002 | 0.008±0.002 | 0.015±0.003 | 0.034±0.003 | >10     | >10   |
| β10         | >10      | 0.064±0.042 | 0.015±0.000 | 0.025±0.011 | 0.322±0.071 | >10     | >10   |
| β20         | >10      | >10      | 0.005±0.011 | 0.345±0.122 | >10      | >10     | >10   |
| β22         | >10      | 6.58±2.988 | 0.025±0.004 | 0.030±0.007 | 3.672±1.538 | >10     | >10   |
| β23         | >10      | 0.009±0.001 | 0.011±0.001 | 0.002±0.000 | 0.019±0.002 | >10     | >10   |
| β24         | >10      | 0.007±0.001 | 0.002±0.001 | 0.005±0.001 | 0.011±0.003 | >10     | >10   |
| β26         | 2.74±0.208 | >10      | 0.012±0.003 | 0.016±0.000 | 0.030±0.009 | 0.025±0.001 | >10   |
| β27         | 0.018±0.002 | 0.018±0.000 | 0.009±0.000 | 0.006±0.002 | 0.026±0.002 | 0.025±0.004 | 0.021±0.004 |
| β29         | >10      | 1.372±0.016 | 0.027±0.003 | 0.023±0.009 | 0.149±0.065 | >10     | >10   |
| β30         | 2.643±0.88 | 0.004±0.001 | 0.003±0.001 | 0.004±0.000 | 0.007±0.002 | 0.966±0.239 | 0.350±0.035 |
| β32         | 0.248±0.003 | 0.119±0.044 | 0.053±0.025 | 0.027±0.014 | 0.173±0.097 | 0.257±0.171 | 0.267±0.068 |
| β33         | 2.016±0.051 | 0.234±0.013 | 0.017±0.003 | 0.007±0.001 | 0.042±0.014 | 1.359±0.406 | 0.334±0.005 |
| β34         | 8.241±1.067 | 0.146±0.336 | 0.032±0.010 | 0.092±0.003 | 0.072±0.002 | 0.387±0.034 | >10   |
| β38         | >10      | >10      | 0.011±0.003 | 0.043±0.025 | 0.017±0.010 | 0.021±0.006 | >10   |
| β40         | 0.075±0.005 | 0.001±0.000 | 0.001±0.000 | 0.001±0.000 | 0.001±0.001 | 0.118±0.045 | 0.107±0.031 |
| β43         | >10      | 0.048±0.024 | >10      | >10     | >10      | >10     | >10   |
| β44         | 0.007±0.002 | 0.028±0.008 | 0.015±0.008 | 0.071±0.026 | 0.035±0.005 | 0.019±0.001 | >10   |
| β45         | >10      | >10      | 0.018±0.003 | 0.015±0.006 | 0.068±0.010 | 0.027±0.005 | >10   |
| β47         | 0.006±0.001 | 0.008±0.003 | 0.003±0.001 | 0.004±0.002 | 0.018±0.003 | 0.010±0.007 | 0.005±0.000 |
| β48         | 0.034±0.011 | 0.020±0.008 | 0.009±0.001 | 0.011±0.001 | 0.011±0.001 | 0.087±0.016 | 0.042±0.016 |
| β49         | 0.009±0.000 | 0.011±0.001 | 0.007±0.000 | 0.019±0.003 | 0.014±0.002 | 0.008±0.002 | 0.008±0.003 |
| β50         | 0.011±0.000 | 0.014±0.006 | 0.007±0.001 | 0.015±0.005 | 0.010±0.002 | 0.018±0.001 | 0.019±0.005 |
| β51         | 0.119±0.008 | 0.242±0.024 | 0.005±0.000 | 0.019±0.001 | 0.008±0.003 | 0.005±0.001 | >10   |
| β53         | 0.005±0.000 | 0.032±0.009 | 0.004±0.000 | 0.017±0.001 | 0.023±0.009 | 0.008±0.001 | 0.007±0.000 |
| β54         | 0.232±0.092 | 0.002±0.001 | 0.001±0.000 | 0.002±0.001 | 0.002±0.000 | 0.834±0.247 | 0.409±0.071 |
| β55         | 0.108±0.060 | 0.028±0.004 | 0.010±0.003 | 0.022±0.001 | 0.014±0.003 | 0.075±0.016 | 0.076±0.020 |
| β56         | 0.046±0.013 | 0.001±0.000 | 0.001±0.000 | 0.002±0.000 | 0.002±0.000 | 0.034±0.014 | 0.022±0.002 |

Table S1B. Immunoglobulin heavy and light chain gene families of 28 Beta SARS-CoV-2-specific human mAbs. Related to Figure 3.
Table S2. X-ray data collection and structure refinement statistics. Related to Figure 4.

| Structure | RBD/Beta-6/ Fab45 * | RBD/Beta-22 * | RBD/Beta- 24 | RBD/Beta- 27 | RBD/Beta-29/Beta-53 * | Beta-32 Fab | RBD/Beta-40 | RBD/Beta-47 * | RBD/Beta-44/Beta-54 | RBD/Beta-49/FI3A | RBD/Beta-50-beta-54 | NTD/Beta-43 |
|-----------|---------------------|----------------|--------------|--------------|------------------------|------------|------------|-------------|------------------|----------------|-----------------|-------------|
| PDB ID    | 7PRY               | 7PRZ           | 7PS0         | 7PS1         | 7PS2                   | 7PS3       | 7PS4       | 7PS7        | 7PS5             | 7PS6           | 7Q0G            | 7Q0H        | 7Q0I        |
| Data collection |              |                |              |              |                        |            |            |             |                  |                |                 |             |
| Space group | P2₁             | R32            | P2₁          | C2           | P2₁                    | P2₁        | P2₁        | P2₁         | P2₁             | P2₁            | P2₁             | P2₁         |
| Cell dimensions |                |                |              |              |                        |            |            |             |                  |                |                 |             |
| a, b, c (Å) | 86.4, 166.8, 120.2 | 199.4, 199.4, 202.4 | 81.8, 110.8, 85.4 | 193.4, 85.7, 57.4 | 213.4, 213.4, 135.0 | 60.6, 66.8, 135.0 | 55.1, 118.5, 109.3 | 41.2, 139.9, 116.4 | 78.1, 114.4, 169.0 | 129.4, 112.6, 99.9 | 89.3, 106.5, 215.6 | 102.2, 42.5, 138.4 | 117.8, 67.0, 143.2 |
| α, β, γ (°) | 90, 108.5, 90     | 90, 90, 120    | 90, 90, 120  | 90, 90, 120  | 90, 90, 90             | 90, 90, 90 | 90, 90, 90 | 90, 90, 90  | 90, 90, 90  | 90, 90, 90  | 90, 90, 90  | 90, 90, 90  |
| Resolution (Å) | 74–3.10          | (3.15–3.10)   | 87–3.20      | (2.35–3.20)  | 67–2.92                | (2.97–2.92)| 50–2.99    | (3.04–2.99) | 68–1.70         | (1.73–1.70) | 59–1.94         | (1.97–1.94) | 60–3.90       | (3.96–3.90) | 65–2.66      | (2.30–2.26) |
| R_{free} | 0.246 (---)       | 0.472 (---)    | 0.181 (---)  | 0.181 (---)  | 0.293 (---)            | 0.293 (---)| 0.056 (---)| 0.056 (---) | 0.059 (---)    | 0.059 (---) | 0.059 (---)    | 0.059 (---) | 0.059 (---)  | 0.059 (---) | 0.059 (---)  |

| Refinement |                |                |              |              |                        |            |            |             |                  |                |                 |             |
| Resolution (Å) | 58–3.10          | 87–3.20      | 65–2.92      | 57–2.40      | 48–2.99                | 48–1.70    | 55–1.94    | 57–3.90    | 57–3.14         | 50–2.26       | 108–1.82        | 89–3.65     | 143–2.39     |
| No. reflections | 55715/2711       | 24332/1326   | 30862/1496   | 32810/1642   | 38058/2011             | 57383/5002 | 59822/5034 | 11260/568  | 25227/1307      | 63593/3455   | 89133/4708      | 12841/705  | 58316/2931   |
| R_{free} / R_{free} | 0.245/0.290      | 0.190/0.223  | 0.222/0.271  | 0.190/0.216  | 0.234/0.267            | 0.171/0.188| 0.199/0.231| 0.233/0.284| 0.226/0.274      | 0.212/0.239  | 0.192/0.224      | 0.255/0.303| 0.202/0.242   |
| No. atoms | 16222            | 4954          | 9487         | 4767         | 8293                   | 3387      | 9625       | 9460        | 4876            | 8032          | 8068            | 8142        | 11158        |
| Protein   | 76               | 137           | 58           | 227          | 138                    | 418       | 528        | ---         | 131             | 129           | 513             | 60          | 446          |
| Ligand/ion/water |                |                |              |              |                        |            |            |             |                  |                |                 |             |
| B factors (Å²) |                |                |              |              |                        |            |            |             |                  |                |                 |             |
| Protein   | 107              | 130           | 75           | 55           | 146                    | 38        | 67         | 172         | 128             | 80            | 47              | 126         | 52           |
| Ligand/ion/water |                |                |              |              |                        |            |            |             |                  |                |                 |             |
| Bond lengths (Å) | 0.003            | 0.003         | 0.002        | 0.002        | 0.003                  | 0.004     | 0.006      | 0.008       | 0.003            | 0.003         | 0.008            | 0.003       | 0.003        |
| Bond angles (°) | 0.5              | 0.6           | 0.5          | 0.5          | 0.6                    | 0.7       | 0.8        | 0.8         | 0.5             | 0.6           | 1.0             | 0.6         | 0.6          |

* In Beta-47/RBD, the RBD most distant from the epitope (close to the N- and C-terminal regions) is not well ordered. In Beta-29/Beta-53/RBD and Beta-6/Fab-45/RBD the constant domains of both Fabs are less well ordered.

bSA022 glycosylated at N35 of the light chain, and SA047 at N102 of the heavy chain.

cValues in parentheses are for highest-resolution shell.
Table 53. Sequences for heavy chain CDRs for IgVH4-30, 4-39, 3-30 and 3-33 gene families and for other structures determined in this paper. Related to Figure 5.

| IgVH4-30 | CDR-H1    | CDR-H2    | CDR-H3          |
|----------|-----------|-----------|-----------------|
| Beta-24  | CSVSDGISSSSDYYWSW | EWIGIYYT-GSTYY | YYCARLVVPS-----PKGSWFDPWG |
| Beta-6   | CTVSGGISISSSHYSYWG | EWIGSIYYS-ESAYY | YYCARVTEPR-----WSACYFDYWG |
| Beta-10# | CTVSGGISISSSSYYWG | EWIGNYYS-GSTYY | YYCARERSAP-----LAGNWFDPWG |
| Beta-23# | CTVSGGISISSSSSYWG | EWIGSNYS-GSTYY | YYCARWIWEFGE-----PAGGDFYWG |
| Beta-40  | CTVSGGISISSSSYYWG | EWIGSNYS-GSAYY | FLYCARHASSAP-----PGDNWFDPWG |
| Beta-54  | CTVFGSGITSSNHYWVW | EWIGSMYYS-GSTAY | YYCARQIGPKRPS-----QVADWFDPWG |
| Beta-55# | CTVSGGISISSSRYWGW | EWIGTFYYS-GITYY | YYCARPRPFPDYDYN-SGALLFDIWG |

| IgVH3-30 | CDR-H1    | CDR-H2    | CDR-H3          |
|----------|-----------|-----------|-----------------|
| Beta-22  | CAASG--FTFSNYGIHW | EWVAVISYDGSHKYY | YYCAKDSAAIP-----YYYGMDVWG |
| Beta-29  | CAASG--FTFSNYGMHR | EWVALISYEESNYY | YYCAKDQGPATVMTAIRGMDVWG |

| IgVH3-33 | CDR-H1    | CDR-H2    | CDR-H3          |
|----------|-----------|-----------|-----------------|
| Beta-20# | CAASG--FPFSNYGMHW | EWVAVIYWDGNSKYY | YYCAKDGYTA-----HYYYYMDVWG |

| IgVH1-69 | CDR-H1    | CDR-H2    | CDR-H3          |
|----------|-----------|-----------|-----------------|
| Beta-49  | CKASG--GTFSSSVISW | EWMMGIIPFLFSAANY | YYCAKV--------SQWALILFWG |
| Beta-50  | CKASR--GTFNTRYFTW | EWMMGIIPFFTADY | YYCSRL--------SQWDLPLMGW |

Others with known structure

| Beta-26  | CTVSG--ASISNYWYW | EWVGYIYTYGSTNH | YYCARAYCSGG-----SCFDTFIDWG |
| Beta-27  | CAASG--LTVRSNYMNW | EWVSLIYS-GSTFY | YYCARD--------LVVYMDVWG |
| Beta-32  | CKASG--YTFTGYMHW | EWGWINPSGSGTNY | YYCAVRGADHDYD-----SSDNWFDPWG |
| Beta-38  | CKGSG--YSFTNYWG | EWGMIIPDSSGTRY | YYCARGVSGATGG--YYDYMDVWG |
| Beta-43  | CAASG--FTFSSYGMHW | EWVAVIYWDGSSNFY | YYCARRSJCSTGF--FGYYYGLDVWG |
| Beta-44  | CKAASG--YFTFTYGISW | EWGWIISYPYNTHY | YYCARRDG--------LLGWFDPWG |
| Beta-47  | CKAASG--FTFITSQVQ | EWGWIAVGGSGTNY | YYCAAPHNCRT--------SCDGFIDW |
| Beta-53  | CKGSG--HNPSYWISW | EWGGRIDPSDSYTNY | YYCARHVLALTHL-----YPDWYG |
Table S4 Cryo-EM data collection, refinement and validation statistics of spike/Fab complexes
Related to Figure 4
* Rigid body refinement only. Numbers in square brackets refer to local refinement results.

| Microscope, Detector | mAb-222 (EMD-13868, PDB 7Q9G) | Beta-6 (EMD-13875, PDB 7Q9I) | Beta-26 (EMD-13871, PDB 7Q9J) | Beta-32 (EMD-13872, PDB 7Q9K) | Beta-43 (EMD-13870, PDB 7Q9l) | Beta-44 (EMD-13874, PDB 7Q9E) | Beta-49 (EMD-13868, PDB 7Q9F) | Beta-53 (EMD-13873, PDB 7Q9M) |
|----------------------|---------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| **Voltage** | 300 |
| **Dose(e^-Å²)** | 50.2 | 40.3 | 40.3 | 40.3 | 40.0 | 45 | 38.4 | 38.4 | 38.2 |
| **Focus range, increment, um** | -0.8 to -2.6 (-0.3) |
| **Nominal magnification kx** | 165 | 165 | 165 | 165 | 165 | 215 | 165 | 165 | 165 |
| **Pixel size [super res] (Å)** | 0.72 | 0.83 | 0.82 | 0.82 | 0.83 | 0.576 | 0.82 | 0.82 | 0.82 |
| **Symmetry imposed** | C1 | C1 | C1 | C1 | C1 | C1 | C1 | C1 | C1 |
| **Particles in final reconstruction** | 86,742 | 79,744 | 50,878 | 54,370 | 89,350 | 61,603 | 222,689 | 40,869 | 32,899 |
| **Map resolution (Å)** | 3.3 | 4.5 | 4.0 | 4.5 | 4.9 | 3.9 | 2.7 | 3.6 | 3.7 |
| **Map sharp B factor (Å²)** | -83.9 | -162.7 | -109.5 | -150.1 | -209.2 | N/A | -68.4 | -47.6 | -36.1 |

**Refinement**

| Initial model | 7NXA | 7PRY | AlphaFold VHH. RBD from 7NXA. | RBD from 7NXA, 7PS3. | 7Q01 | N/A | 7Q0G | 7Q0H | 7PS2 |
|---------------|------|------|-------------------------------|----------------------|------|-----|------|------|------|
| RBD #up / #fabs | 2 / 3 | 2 / 3 | 3 / 3 | 2 / 2 | 1 (NTD) | 2 / 2 | 0 / 3 | 0 / 3 | 2 / 2 |

**Final model:**

| Protein | 28973 | 30494 | 30308 | 27225 | 30966 | N/A | 34341 | 34264 | 28406 |
|---------|-------|-------|-------|-------|-------|-----|-------|-------|-------|
| Ligands | 3632 | 3807 | 3797 | 3388 | 3881 | 4327 | 4312 | 3613 |
| B factors (Å²) | 52 | 52 | 57 | 60 | 53 | 74 | 64 | 43 |
| Protein | 91.1 | 319.3 | 202.5 | 524.5 | 301.9 | 65.1 | 123.5 | 142.0 |
| Ligand | 77.6 | 267.2 | 175.9 | 489.4 | 283.8 | 76.2 | 124.3 | 124.7 |

**R.m.s. deviations**

| Bond lengths(Å) | 0.003 | 0.004 | 0.004 | 0.004 | 0.003 | N/A | 0.003 | 0.003 | 0.004 |
| Bond angles(°) | 0.455 | 0.635 | 0.529 | 0.849 | 0.538 | 0.479 | 0.463 | 0.524 |

**Validation**

| Resolution at FSC to model 0.5 | 3.4 | 6.4 | 4.2 | 8.1 | 5.4 | N/A | 2.8 | 3.7 | 3.7 |
| CCbox, phenix | 0.82 | 0.81 | 0.83 | 0.82 | 0.76 | 0.84 | 0.88 | 0.84 |
| MolProbity score | 1.37 | 1.91 | 1.57 | 1.58 | 1.47 | 1.25 | 1.49 | 1.39 |
| Clashscore | 3.97 | 8.90 | 6.17 | 5.48 | 4.70 | 3.20 | 4.43 | 4.26 |
| Poor rotamers (%) | 0.67 | 1.20 | 0.64 | 0.44 | 0.80 | 0.48 | 0.45 | 0.75 |

**Ramachandran plot**

| Favored (%) | 96.9 | 94.6 | 96.4 | 95.9 | 96.5 | 97.3 | 96.1 | 96.9 |
| Allowed (%) | 3.10 | 4.98 | 3.56 | 3.73 | 3.45 | 2.70 | 3.93 | 2.92 |
| Disallowed (%) | 0.00 | 0.40 | 0.00 | 0.36 | 0.03 | 0.00 | 0.00 | 0.14 |