Supplementary Materials for

Combating the SARS-CoV-2 Omicron (BA.1) and BA.2 with potent bispecific antibodies engineered from non-Omicron neutralizing antibodies

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Table S1. Mutations in spike proteins of Omicron, BA.1.1, BA.2 and BA.3.

| Spkie | Mutation | BA.1 | BA.1.1 | BA.2 | BA.3 |
|-------|----------|------|--------|------|------|
| NTD   | T19I     |      |        |      |      |
|       | A24-26   |      |        |      |      |
|       | A27S     |      |        |      |      |
|       | A67V     |      |        |      |      |
|       | Δ69-70   |      |        |      |      |
|       | T95I     |      |        |      |      |
|       | G142D    |      |        |      |      |
|       | A143-145 |      |        |      |      |
|       | A21I     |      |        |      |      |
|       | L212I    |      |        |      |      |
|       | V213G    |      |        |      |      |
|       | ins214EPE|      |        |      |      |
|       | G339D    |      |        |      |      |
|       | R346K    |      |        |      |      |
|       | S371L/F  | L    | L      | F    | F    |
|       | S373P    |      |        |      |      |
|       | S375F    |      |        |      |      |
|       | T376A    |      |        |      |      |
|       | D405N    |      |        |      |      |
|       | R408S    |      |        |      |      |
|       | K417N    |      |        |      |      |
|       | N440K    |      |        |      |      |
|       | G446S    |      |        |      |      |
|       | S477N    |      |        |      |      |
|       | T478K    |      |        |      |      |
|       | E484A    |      |        |      |      |
|       | Q493R    |      |        |      |      |
|       | G496S    |      |        |      |      |
|       | Q498R    |      |        |      |      |
|       | N501Y    |      |        |      |      |
|       | Y505H    |      |        |      |      |
| SD1   | T547K    |      |        |      |      |
| SD2   | D614G    |      |        |      |      |
|       | H655Y    |      |        |      |      |
|       | N679K    |      |        |      |      |
|       | P681H    |      |        |      |      |
| FP    | N764K    |      |        |      |      |
|       | D796Y    |      |        |      |      |
|       | N856K    |      |        |      |      |
| HR1   | Q954H    |      |        |      |      |
|       | N969K    |      |        |      |      |
|       | L981F    |      |        |      |      |
| Donor ID | mAb ID  | VH          | CDRH3 sequence | VL      | CDRL3 sequence |
|----------|---------|-------------|----------------|---------|----------------|
| Donor 1  | GW01    | IGHV3-43    | ARDRSYSGFDVFNYEYGMVD | IGLV1-44 | AAWDDSLNWV     |
| Donor 1  | 4L12    | IGHV3-66    | ARDLITYGMDV       | IGKV1-9 | QQLNSYPFLLT    |
| Donor 2  | 16L9    | IGHV3-53    | ARGEIQPYHHGMVD    | IGLV2-8 | SSYAGSSNDV     |

Table S2. The germline and CDRH3 sequences of GW01, 4L12, and 16L9.
Table S3. Cryo-EM data collection and refinement statistics of SARS-CoV-2 complexed with IgG FD01.

| Data collection and processing | State 1 | State 2 | State 3 | State 4 | State 5 | State 6 | Local refine NRF |
|-------------------------------|---------|---------|---------|---------|---------|---------|-----------------|
| Magnification                 | 81,000  |         |         |         |         |         |                 |
| Voltage (kV)                  | 300     |         |         |         |         |         |                 |
| Electron exposure (e⁻/Å²)     | 58      |         |         |         |         |         |                 |
| Defocus range (μm)            | -1.2 to -2.5 |       |         |         |         |         |                 |
| Pixel size (Å)                | 1.064   |         |         |         |         |         |                 |
| Initial particles (no.)       | 1,003,956 |       |         |         |         |         |                 |
| Symmetry imposed              | C1      |         |         |         |         |         |                 |
| Final particles (no.)         | 194,026 | 62,040  | 74,415  | 141,577 | 39,294  | 71,568  | 249,122         |
| Map resolution (Å)            | 3.47    | 3.70    | 3.91    | 3.47    | 3.87    | 6.11    | 3.51            |

| Refinement                    |         |         |         |         |         |         |                 |
| R.m.s. deviations             |         |         |         |         |         |         |                 |
| Bond lengths (Å)              | 0.003   | 0.003   | 0.003   | 0.003   | 0.003   | 0.002   | 0.002           |
| Bond angles (°)               | 0.539   | 0.506   | 0.499   | 0.557   | 0.527   | 0.437   | 0.524           |
| Validation                    |         |         |         |         |         |         |                 |
| MolProbity score              | 2.52    | 2.48    | 2.46    | 2.50    | 2.53    | 2.41    | 2.88            |
| Clashscore                    | 9.18    | 8.06    | 8.38    | 8.44    | 8.14    | 7.67    | 10.97           |
| Rotamer outlier (%)           | 5.14    | 5.34    | 5.30    | 5.39    | 5.97    | 5.33    | 9.21            |
| Ramachandran plot             |         |         |         |         |         |         |                 |
| Favored (%)                   | 91.75   | 92.00   | 92.74   | 91.84   | 91.67   | 93.20   | 88.62           |
| Allowed (%)                   | 7.95    | 7.90    | 7.14    | 8.13    | 8.17    | 6.80    | 11.38           |
| Disallowed (%)                | 0.30    | 0.11    | 0.13    | 0.03    | 0.16    | 0.00    | 0.00            |

| EMDB                          | 32655   | 32656   | 32657   | 32659   | 32660   | 32661   | 32654           |
| PDB                           | 7WOQ    | 7WOR    | 7WOS    | 7WOU    | 7WOV    | 7WOW    | 7WOP            |
Fig. S1. Binding affinities of GW01, 16L9, 4L12, REGN10987, and ten bispecific antibodies to SARS-CoV-2 RBD-his, Omicron trimer-his and Omicron RBD-his measured by bilayer interferometry. Antibodies were immobilized on anti-human IgG (AHC) biosensors and then tested for their binding abilities to the target proteins.
Fig. S2. Cryo-EM data collection and processing of FD01 bound SARS-CoV-2 Omicron S. (a) Representative electron micrograph and 2D classification results of FD01 bound SARS-CoV-2 S. (b) The reconstruction map of the complex structures at
six states. (c) Gold-standard Fourier shell correlation curves generated in RELION for structures of six states. The 0.143 cut-off is indicated by a horizontal dashed line. (d) The local-refined map of the NRF region. (e) Gold-standard Fourier shell correlation curves generated in cryoSPARC for local-refined map. (f) Density maps of residues around the interface.
Fig. S3. Data processing flowchart of FD01 bound SARS-CoV-2 Omicron S trimer. Particles number above cyan line is used for particle counting statistics.
Fig. S4. Data processing flowchart of local refinement of RBD-FD01.

Select all 3D-classes of OS-trimer with good RBD and FD01 density
De-duplicate

217,667 particles (1.064 Å/pixel)

Auto-refine with reference aligned C3 symmetry

Expand particles with C3 symmetry
653,001 particles

Subtract with one NRF mask
No-alignment 3D class

249,122 particles

Export to cryoSPARC
Local refinement

3.51 Å
Fig. S5. IgG FD01 crosslinks Omicron S trimers to form trimer dimer conformation. (a) SDS-PAGE of the FD01 in IgG and Fab form; (b) Negative stain images of Omicron S trimer-FD01 Fab and Omicron S trimer-FD01 IgG, showing that only IgG FD01 can induce the formation of trimer dimer.
Fig. S6. Sequence alignment of SARS-CoV-2 WT and all VOCs RBDs. Conserved amino acids are highlighted as red. Residues involved in 16L9 or GW01 are marked with triangles in green or magenta, respectively. Residues involved in both 16L9 and GW01 binding are marked with triangles in blue.
Fig. S7. Five representative bispecific antibodies block RBD binding to ACE2.

Binding of ACE2 to the SARS-CoV-2 RBD in competition with bispecific antibodies (red), S309 (blue), control IgG1 (green), and IgG1+ACE2 (black).
Fig. S8. Hypothesis of binding features when Omicron S trimer meets with mAbs of 16L9 or GW01.
Supplementary Video: Conformation transitions of Omicron S trimer and the representative bispecific antibody GW01-16L9 (FD01) in six states.