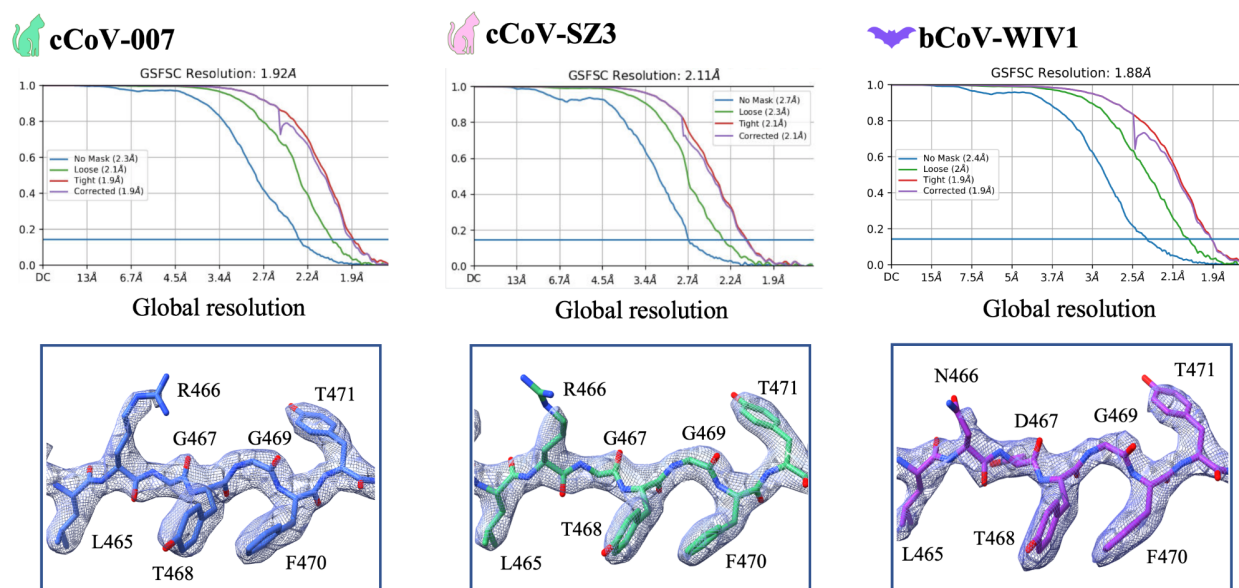
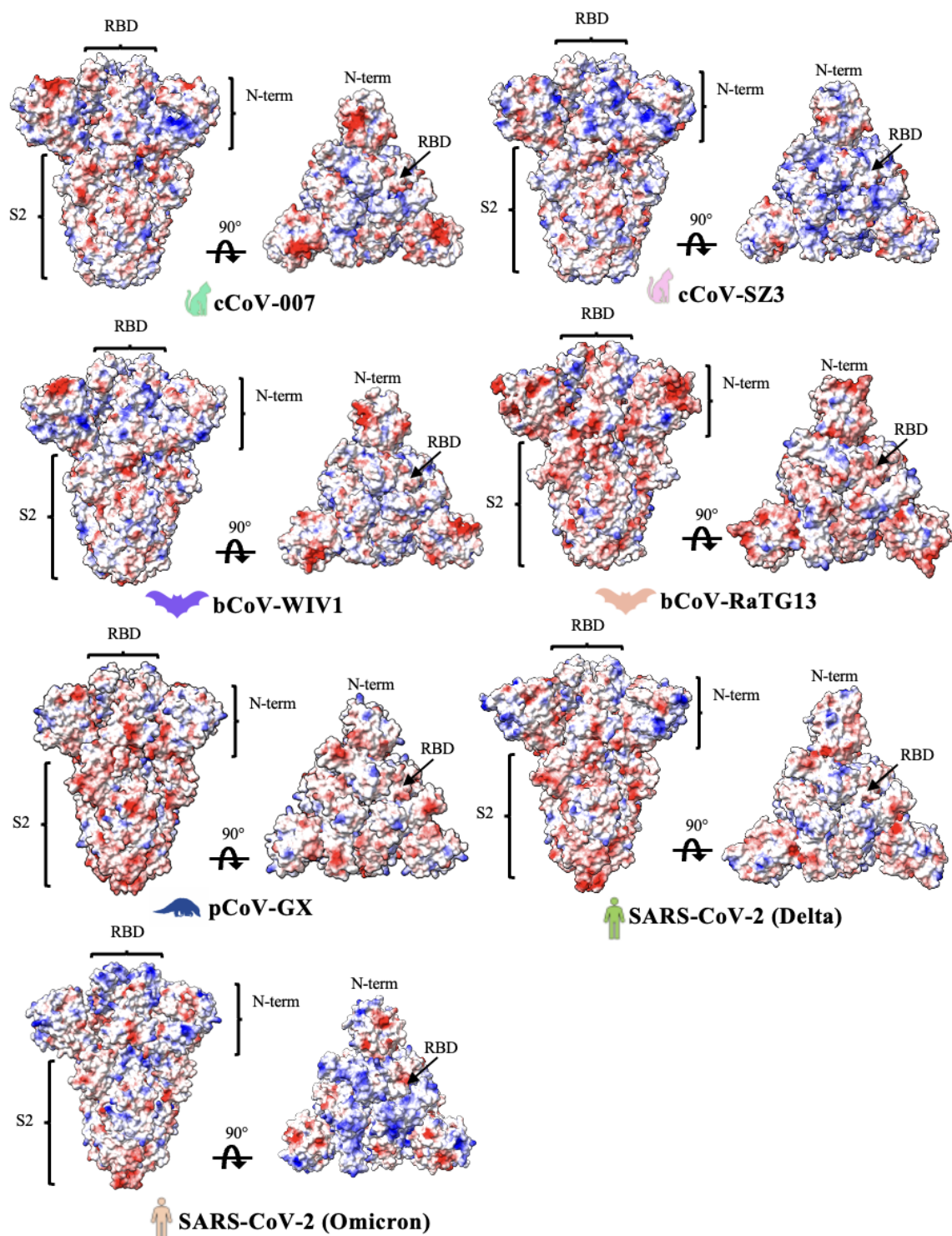


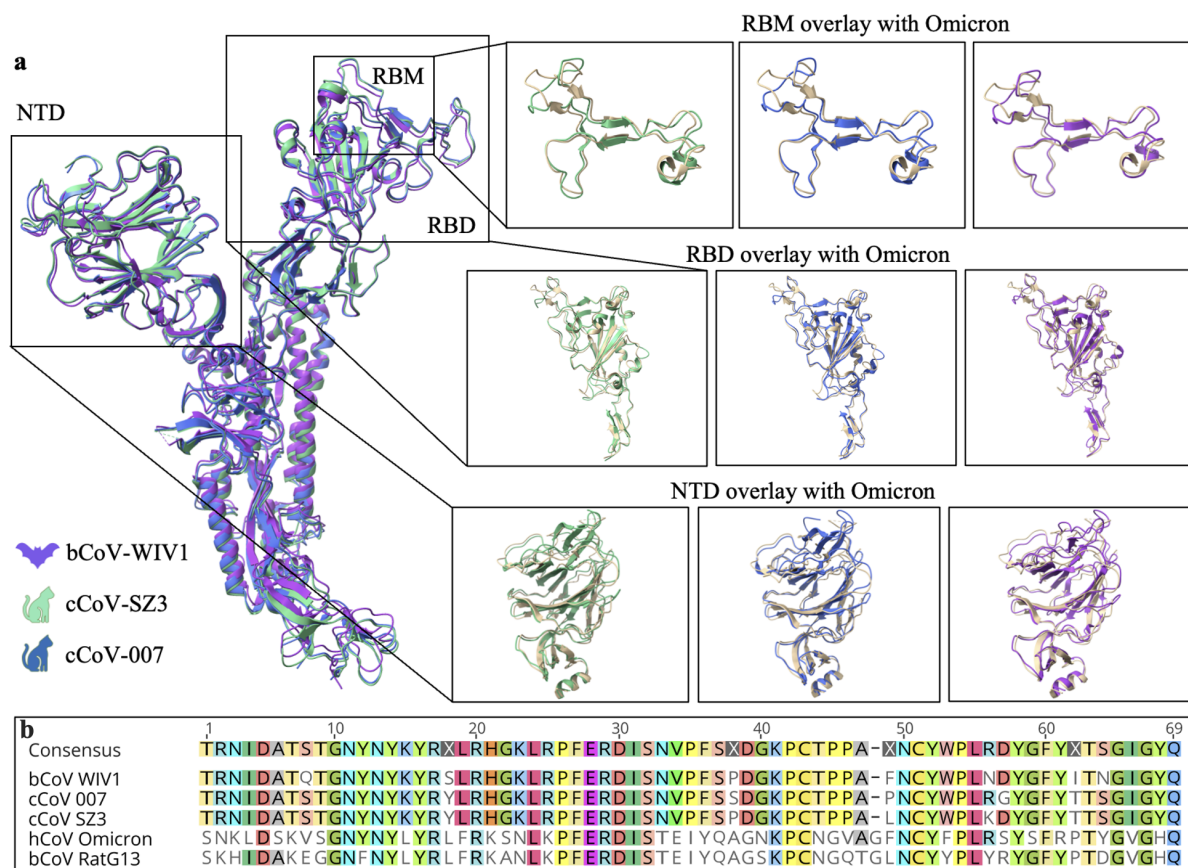
## Supplementary Materials



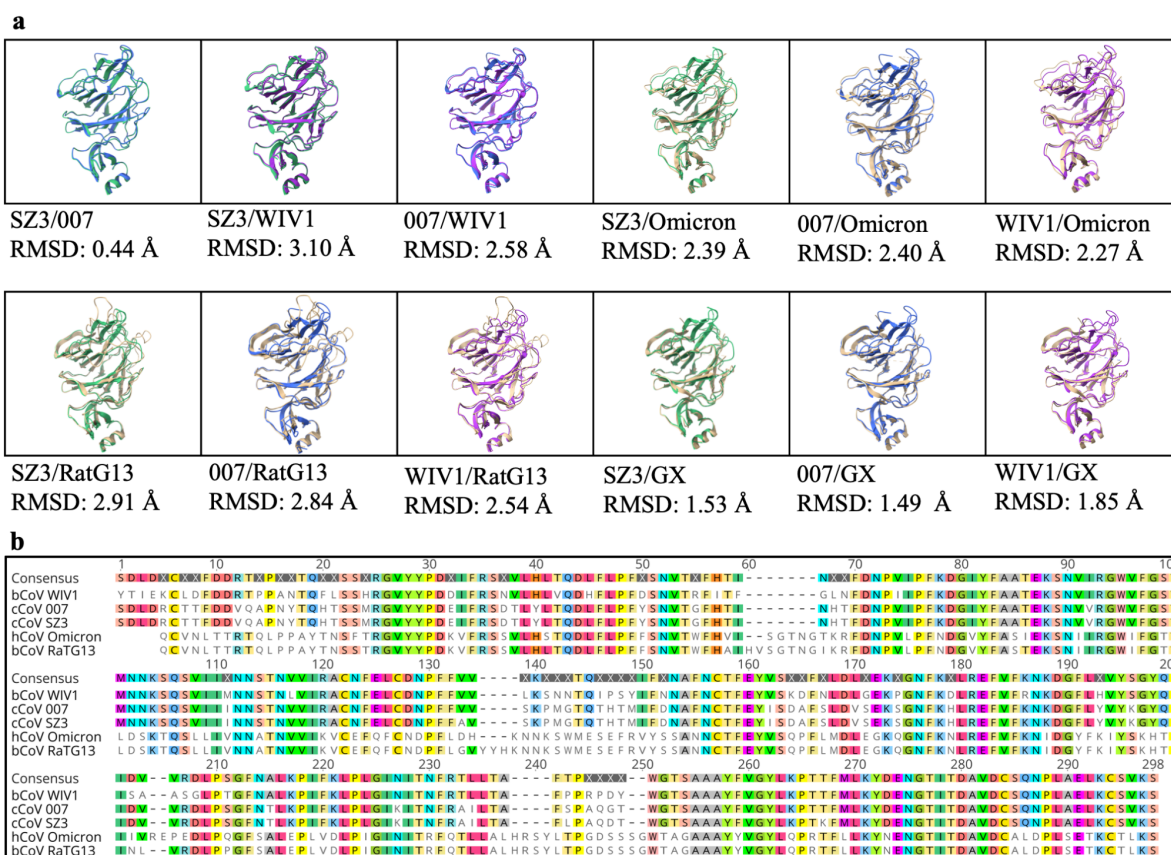
**Supplementary figure 1. Comparison of reconstruction resolutions and map quality between SARSr-CoV glycoproteins.** (Top) Gold-standard Fourier shell correlation plots are computed within cryoSPARC from two independent half-maps and report a global resolution for each reconstruction. (Bottom) Amino acid residues contained within the receptor binding motif loop (L465-T471) are presented within the electron density, set to a standard deviation threshold of 0.25 in all panels.



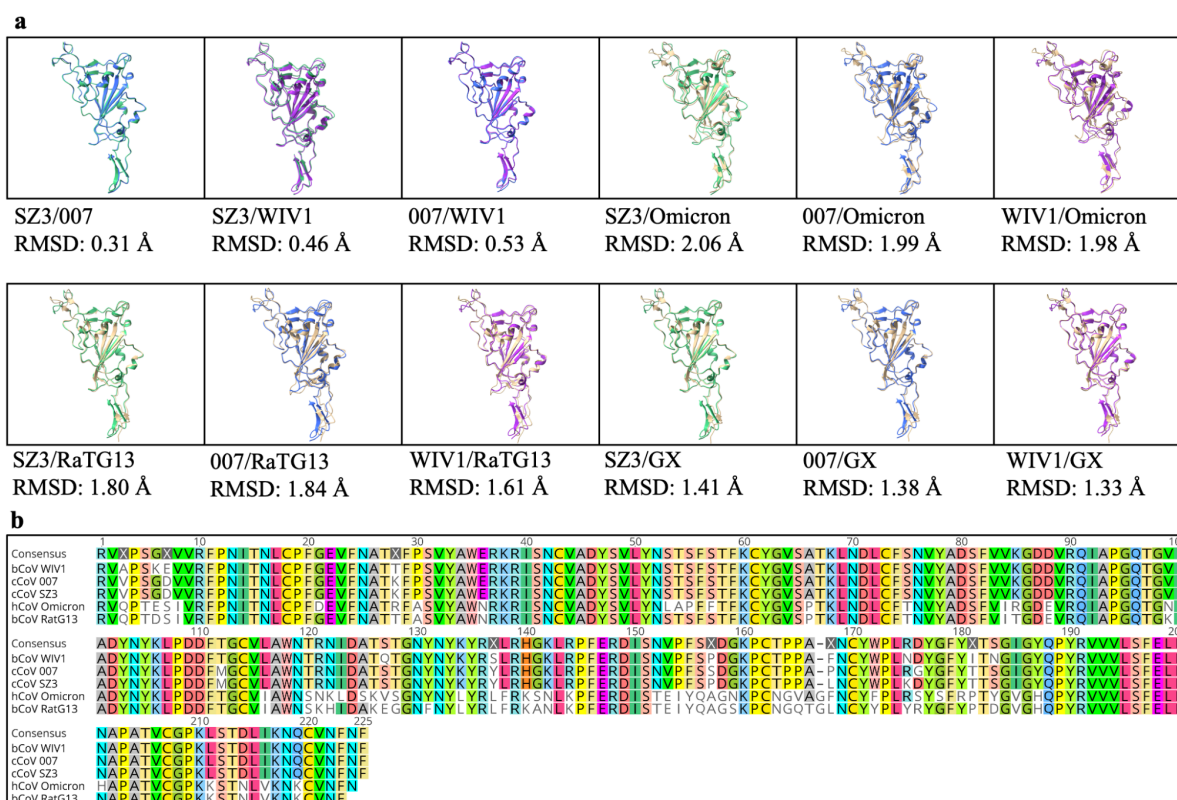
**Supplementary figure 2. Coulombic electrostatic potential of SARSr-CoV glycoproteins.** Molecular surface representation of side and top views of SARSr-CoV spike proteins are coloured by Coulombic electrostatic potential calculated in UCSF ChimeraX according to Coulombs law:  $\phi = \sum [q_i / (\epsilon d_i)]$ . Negative potential is coloured blue and positive potential is coloured red. The SARS-CoV-2 Wuhan-Hu-1 (PDB: 7E7B) and Omicron spikes (PDB: 7EWK), as well as SARSr-CoV spikes pCoV-GX (PDB: 7BBH) and bCoV-RaTG13 spike (PDB: 7CN4) were downloaded from the Protein Data Bank.



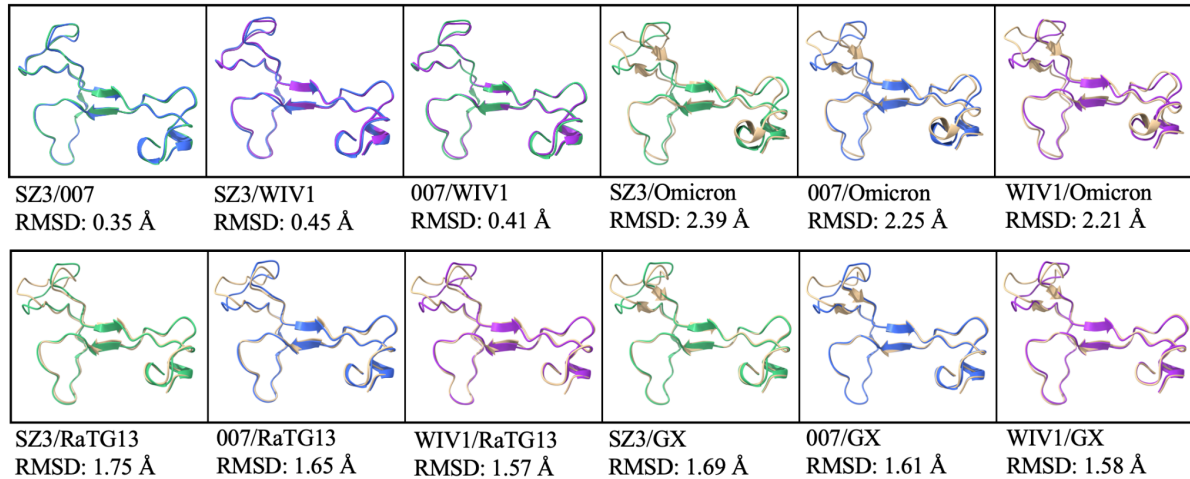
**Supplementary figure 3. Structural and sequence based comparison of S1 domains between SARSr-CoV spike glycoprotein and Omicron.** **a**, Alignment of glycoprotein monomer cCoV SZ3 (green), cCoV 007 (blue) and bCoV WIV1 (purple), followed by alignments of the receptor binding motif (RBM), receptor binding domain (RBD) and the N-Terminal domain (NTD) of novel glycoprotein structures against omicron (PDB 7WK2). **b**, Geneious global sequence alignment of RBM from 6 spike glycoproteins, bCoV-WIV1, cCoV-SZ3, cCoV-007, human (h) CoV Omicron, bCoV RaTG13 and pangolin (p) CoV GX (PDB: 7BBH).



**Supplementary figure 4. Structural and sequence based comparison of N-terminal domain between SARSr-CoV spike glycoproteins. a**, Alignment and RMSD calculation of N-terminal domains (NTD) from civet (c) CoV-SZ3 shown in green, cCoV-007 shown in blue, bat (b) CoV-WIV1 shown in purple. Human (h) Omicron (PDB: 7WK2), bCoV-RaTG13 (PDB: 7CN4), pangolin (p) CoV-GX (PDB: 7BBH) all shown in tan. RMSD values calculated using unpruned atom pairs **b**, GENEIOUS global sequence alignment of NTDs from 6 spike glycoproteins, bCoV-WIV1, cCoV-SZ3, cCoV-007, hCoV Omicron, bCoV RaTG13 and pCoV GX (PDB: 7BBH).

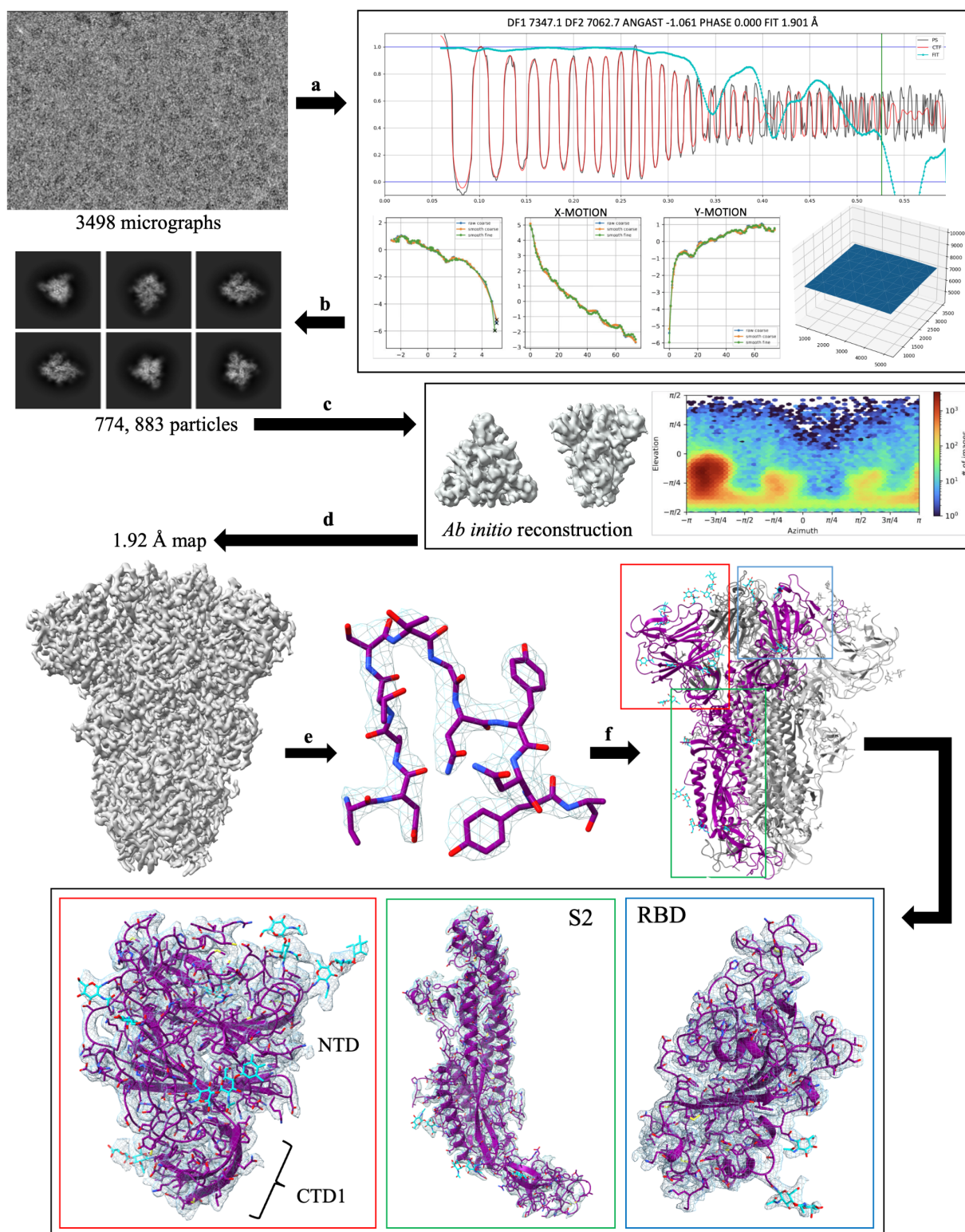


**Supplementary figure 5. Structural and sequence based comparison of receptor binding domain between SARSr-CoV spike glycoproteins. a**, Alignment and RMSD calculation of receptor binding domain (RBD) from civet (c) CoV-SZ3 shown in green, cCoV-007 shown in blue, bat (b) CoV-WIV1 shown in purple. Human (h) Omicron (PDB: 7WK2), bCoV-RaTG13 (PDB: 7CN4), pangolin (p) CoV-GX (PDB: 7BBH) all shown in tan. RMSD values calculated using unpruned atom pairs **b**, Geneious global sequence alignment of RBDs from 6 spike glycoproteins, bCoV-WIV1, cCoV-SZ3, cCoV-007, hCoV Omicron, bCoV RaTG13 and pCoV GX (PDB: 7BBH).

**a****b**

|              |   |    |    |    |    |    |    |    |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|--------------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|              | 1 | 10 | 20 | 30 | 40 | 50 | 60 | 69 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Consensus    | T | R  | N  | I  | D  | A  | T  | S  | T | G | N | Y | N | K | Y | R | X | L | R | H | G | K | L | R | P | F | E | R | D | I | S | N | V | P | F | S | X | D | G | K | P | C | T | P | P | A | - | X | N | C | Y | W | P | L | R | D | Y | G | F | Y | X | T | S | G | I | G | Y | Q |
| bCoV WIV1    | T | R  | N  | I  | D  | A  | T  | Q  | T | G | N | Y | N | K | Y | R | S | L | R | H | G | K | L | R | P | F | E | R | D | I | S | N | V | P | F | S | P | D | G | K | P | C | T | P | P | A | - | F | N | C | Y | W | P | L | R | D | Y | G | F | Y | I | T | N | G | I | G | Y | Q |
| cCoV 007     | T | R  | N  | I  | D  | A  | T  | S  | T | G | N | Y | N | K | Y | R | Y | L | R | H | G | K | L | R | P | F | E | R | D | I | S | N | V | P | F | S | S | D | G | K | P | C | T | P | P | A | - | P | N | C | Y | W | P | L | R | G | Y | G | F | Y | T | T | S | G | I | G | Y | Q |
| cCoV SZ3     | T | R  | N  | I  | D  | A  | T  | S  | T | G | N | Y | N | K | Y | R | Y | L | R | H | G | K | L | R | P | F | E | R | D | I | S | N | V | P | F | S | P | D | G | K | P | C | T | P | P | A | - | L | N | C | Y | W | P | L | R | D | Y | G | F | Y | T | T | S | G | I | G | Y | Q |
| hCoV Omicron | S | N  | K  | L  | D  | S  | K  | V  | S | G | N | Y | N | L | Y | R | L | F | R | K | S | N | L | K | P | F | E | R | D | I | S | T | E | I | Y | Q | A | G | N | K | P | C | N | G | V | A | G | F | N | C | Y | F | P | L | R | S | Y | S | E | R | P | T | Y | G | V | G | H | Q |
| bCoV RatG13  | S | K  | H  | I  | D  | A  | K  | E  | G | G | N | F | N | Y | L | Y | R | L | F | R | K | A | N | L | K | P | F | E | R | D | I | S | T | E | I | Y | Q | A | G | S | K | P | C | N | G | T | G | L | N | C | Y | F | P | L | R | Y | G | F | Y | P | T | D | G | V | G | H | Q |   |

**Supplementary figure 6. Structural and sequence based comparison of receptor binding motif between SARSr-CoV spike glycoproteins. a**, Alignment and RMSD calculation of receptor binding motif (RBM) from civet (c) CoV-SZ3 shown in green, cCoV-007 shown in blue, bat (b) CoV-WIV1 shown in purple. Human (h) Omicron (PDB: 7WK2), bCoV-RaTG13 (PDB: 7CN4), pangolin (p) CoV-GX (PDB: 7BBH) all shown in tan. RMSD values calculated using unpruned atom pairs **b**, Geneious global sequence alignment of RBMs from 6 spike glycoproteins, bCoV-WIV1, cCoV-SZ3, cCoV-007, hCoV Omicron, bCoV RaTG13 and pCoV GX (PDB: 7BBH).



**Supplementary figure 7. Image processing and reconstruction of cCoV-007 glycoprotein.** (a) Data processing included patch-based motion correction and patch-based CTF correction. (b) Particles were picked by automated blob picking, extracted and classed to produce templates used in template-based particle picking. Following iterative 2D classing, 774,883 particles were selected for use in downstream reconstruction jobs. (c) *Ab initio* reconstruction produced an initial 3D model. (d) The initial model was refined in iterative homogeneous and non-uniform refinement jobs using the particle stack from (b) to produce a final model extending to a global atomic resolution of 1.92 Å. (e) An initial model was built using a reference model (PDB: 7E7B) and fit into the electron density using ISOLDE. (f) Refinement was performed using Stand-Alone Coot and Phenix.

**Supplementary Table 1. Cryo-EM data collection, refinement and validation statistics for SARSs-CoV glycoproteins.**

| Data collection and processing            | cCoV-007         | cCoV-SZ3         | bCoV-WIV1        |
|-------------------------------------------|------------------|------------------|------------------|
| Magnification                             | ×105,000         | ×105,000         | ×130,000         |
| Detector                                  | K3 (gatan)       | K3 (gatan)       | K3 (gatan)       |
| Voltage (kV)                              | 300              | 300              | 300              |
| Electron exposure (e /Å <sup>2</sup> )    | 59               | 59               | 67               |
| Spherical aberration (mm)                 | 2.7              | 2.7              | 2.7              |
| Defocus range (μm)                        | -0.6 – -2        | -0.6 – -2        | -0.6 – -2        |
| Pixel size (Å/pix)                        | 0.84             | 0.84             | 0.65             |
| Frames                                    | 75               | 75               | 95               |
| Symmetry imposed                          | C3               | C3               | C3               |
| Initial particle images (no.)             | >5,000,000       | >3,000,000       | >5,000,000       |
| Final particle images (no.)               | ~775,000         | ~800,000         | ~800,000         |
| Map resolution (Å)                        | 1.92             | 2.11             | 1.88             |
| <b>Refinement</b>                         |                  |                  |                  |
| Initial model used (PDB code)             | <i>ab initio</i> | <i>ab initio</i> | <i>ab initio</i> |
| Model resolution (Å)                      | N/A              | N/A              | N/A              |
| Map sharpening B factor (Å <sup>2</sup> ) | -46              | -53              | -41              |
| Bond lengths (Å)                          | 0.004            | 0.021            | 0.020            |
| Bond angles (°)                           | 0.662            | 2.23             | 2.187            |
| Protein residues                          | 3264             | 3261             | 3273             |
| <b>Validation</b>                         |                  |                  |                  |
| MolProbity score                          | 1.47             | 1.59             | 1.76             |
| EMRinger score                            | 5.76             | 5.76             | 4.77             |
| Clashscore                                | 3.49             | 2.79             | 4.92             |
| Poor rotamers (%)                         | 0 (0)            | 1.6              | 1.4              |
| Ramachandran plot                         |                  |                  |                  |
| Favored (%)                               | 94.63            | 94.63            | 94.22            |
| Allowed (%)                               | 2.19             | 5.37             | 5.71             |
| Disallowed (%)                            | 0.9              | 0                | 0.06             |
| Rama-Z (Ramachandran plot Z-score, RMSD)  |                  |                  |                  |
| whole (N = 3240)                          | 0.06 (0.14)      | -3.08 (0.12)     | -2.66 (0.12)     |
| helix (N = 675)                           | 2.06             | -2.21 (0.14)     | -2.49 (0.13)     |
| sheet (N = 726)                           | (0.20)           | -2.60 (0.18)     | -1.45 (0.22)     |
| loop (N = 1839)                           | 0.30 (0.19)      | -1.78 (0.12)     | -1.60 (0.12)     |
|                                           | -0.86 (0.13)     |                  |                  |
| C-beta outliers (%)                       | 0.13             | 0.3              | 0.07             |

