Supplementary Information

A 3.4-Å cryo-EM structure of the human coronavirus spike trimer computationally derived from vitrified NL63 virus particles

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Fig. S1. Single-particle cryo-EM data processing of HCoV-NL63 coronavirus spike glycoprotein. 
(a). Workflow of the data processing. (b) Gold standard FSC plot for the final 3D reconstruction. 
(c) Euler angle distribution of the particle images of computationally extracted spikes, calculated in cryoSPARC. (d) Cross-correlation coefficient between the 3D reconstructions with (green) and without (grey) C3 symmetry applied.
**CoV2 Domain A' to NL63 Domain 0**

Aligned length=159, RMSD=3.48Å, Identity (#identical/#aligned)=7.5%

(TM)

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**CoV2 Domain A to NL63 Domain B**

Aligned length=159, RMSD=3.48Å, Identity (#identical/#aligned)=7.5%

(TM)

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**CoV2 Domain B' to NL63 Domain 0**

Aligned length=159, RMSD=3.48Å, Identity (#identical/#aligned)=7.5%

(TM)

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**CoV2 Domain C' to NL63 Domain C**

Aligned length=160, RMSD=3.48Å, Identity (#identical/#aligned)=7.5%

(TM)

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**CoV2 Domain D' to NL63 Domain D**

Aligned length=160, RMSD=3.48Å, Identity (#identical/#aligned)=7.5%

(TM)

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Fig. S2. Structure-based alignment of domains of spike protein from HCoV-NL63 and SARS-CoV-2. The method of TM-align was used, yielding good structural alignments illustrated in Fig. S2. Aligned sequences along with RMSD, percent identity, and TM-score are shown for all domain pairs.
Table S1. Cryo-EM data collection, refinement, and validation statistics.

| Data collection and processing |   |
|-------------------------------|--|
| Magnification                | 64k |
| Voltage (kV)                 | 300 |
| Electron exposure (e⁻/Å²)    | 48  |
| Defocus range (μm)           | -0.4 to -3.6 |
| Pixel size (Å)               | 1.4 |
| Symmetry imposed             | C3  |
| Initial particle images (no.)| 944,822 |
| Final particle images (no.)  | 82,030 |
| Map resolution (Å)           | 3.39 |
| FSC threshold                | 0.143 |
| Map resolution range (Å)     | 2.7-7.0 |

| Refinement                  |   |
|-------------------------------|--|
| Initial model used (PDB code)| 5SZS |
| Map sharpening $B$ factor (Å²)| -90 |
| Model composition            |   |
| Non-hydrogen atoms           | 30,612 |
| Protein residues             | 3,576 |
| Ligands                      | 222 |
| $B$ factors (Å²)             |   |
| Protein                      | 94.84 |
| Ligand                       | 141.21 |
| R.M.S. deviations            |   |
| Bond lengths (Å)             | 0.009 |
| Bond angles (°)              | 1.30 |
| Validation                   |   |
| MolProbity score             | 1.44 |
| Clashscore                   | 1.88 |
| Poor rotamers (%)            | 0  |
| Ramachandran plot            |   |
| Favored (%)                  | 92.06 |
| Allowed (%)                  | 7.86 |
| Disallowed (%)               | 0.08 |
Table S2. Comparison of the HCoV-NL63 S glycans in different studies.

| Amino acid residue site | Sequon | MS identified (Walls et al) | Cryo-EM observed (Walls et al) | Cryo-EM observed (this study) |
|------------------------|--------|-----------------------------|------------------------------|------------------------------|
| 24                     | NLSM   | ND                          | ND                           | 24                           |
| 35                     | NSST   | 35                          | 35                           | 35                           |
| 52                     | NQST   | 52                          | 52                           | 52                           |
| 98                     | NASV   | 98                          | 98                           | 98                           |
| 111                    | NTTF   | ND                          | ND                           | ND                           |
| 119                    | NASS   | ND                          | ND                           | ND                           |
| 155                    | NVTR   | 155                         | 155                          | 155                          |
| 178                    | NYSC   | ND                          | ND                           | ND                           |
| 187                    | NATV   | 187                         | 187                          | 187                          |
| 193                    | NVTT   | 193                         | 193                          | 193                          |
| 203                    | NYTV   | ND                          | ND                           | 203                          |
| 240                    | NGST   | 240                         | 240                          | 240                          |
| 276                    | NATG   | 276                         | 276                          | 276                          |
| 301                    | NFSA   | 301                         | 301                          | 301                          |
| 330                    | NSSS   | ND                          | ND                           | 330                          |
| 354                    | NSTI   | ND                          | 354                          | 354                          |
| 358                    | NTTH   | 358                         | 358                          | 358                          |
| 403                    | NVTT   | 403                         | 403                          | 403                          |
| 426                    | NVSA   | ND                          | 426                          | 426                          |
| 486                    | NFTA   | 486                         | 486                          | 486                          |
| 506                    | NISL   | 506                         | 506                          | 506                          |
| 512                    | NTSV   | 512                         | 512                          | 512                          |
| 626                    | NCTK   | 626                         | 626                          | 626                          |
| 645                    | NQSL   | 645                         | 645                          | 645                          |
| 666                    | NVST   | 666                         | 666                          | 666                          |
| 699                    | NERS   | 699                         | 699                          | 699                          |
| 723                    | NCTT   | ND                          | 723                          | 723                          |
| 749                    | NSSD   | ND                          | 749                          | 749                          |
| 762                    | NLSI   | ND                          | 762                          | 762                          |
| 768                    | NWTT   | 768                         | 768                          | 768                          |
| 844                    | NVTS   | 844                         | 844                          | 844                          |
| 852                    | NLSS   | 852                         | 852                          | 852                          |
| 1111                   | NGTH   | 1111                        | 1111                         | 1111                         |
| 1196                   | NVTF   | 1196                        | 1196                         | 1196                         |
| 1201                   | NISR   | 1201                        | 1201                         | 1201                         |
| 1218                   | NKTL   | 1218                        | 1218                         | 1218                         |
| 1242                   | NLTY   | 1242                        | ND                           | ND                           |
| 1247                   | NLSS   | ND                          | ND                           | ND                           |
| 1277                   | NSTY   | ND                          | ND                           | ND                           |
| TOTAL # of sites       | 39     | 28                          | 31                           | 33                           |