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## Supplemental Information

# Structure and immune recognition of the porcine epidemic diarrhea virus spike protein

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**Supplemental Information**
Structure and immune recognition of the porcine epidemic diarrhea virus spike protein

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| | |
|---|---|
| EMDB | 21391 |
| **Data and reconstruction statistics** | |
| Microscope | Talos Actica |
| Voltage (kV) | 200 |
| Detector | Gatan K2 Summit |
| Dose rate ($e^-$/pix/sec) | 5.67 |
| Exposure (s) | 12.8 |
| Dose ($e^-/Å^2$) | 54.9 |
| Frames | 64 |
| Defocus Range (μm) | 0.6-1.4 |
| Initial Particles | 19,436 |
| Final Particles | 19,436 |
| B-factor ($Å^2$) | -70 |
| Resolution (Å) | 3.5 |
| | |
| **Coordinate model refinement** | |
| PDB | 6VV5 |
| Residues | 3,396 |
| RMSD Bonds (Å) | 0.017 |
| RMSD Angles (°) | 1.579 |
| Ramachandran | |
| Favored (%) | 98.3 |
| Allowed (%) | 1.6 |
| Outliers (%) | 0.1 |
| Rotamer Outliers (%) | 1.3 |
| Clash score | 4.8 |
| Molprobity score | 1.32 |
| EM Ringer Score | 3.3 |

**Table S1. Data collection and refinement of PEDV spike by cryo-electron microscopy, Related to STAR Methods.**

| Sample | PEDV S 1-1322 | PEDV S 1-1322 | PEDV S 1-1322 | PEDV S 1-1322, N264D | PEDV S 1-1322 | PEDV S 1-33, 231-1322 |
|---|---|---|---|---|---|---|
| Expression System | 293F | 293F+Kif | 293S | 293F | Sf9 | Sf9 |
| EMDB | 21393 | 21394 | 21395 | 21396 | 21392 | 21397 |
| Pixel size | 2.05 | 2.05 | 2.05 | 2.05 | 2.05 | 4.1 |
| Particles | 29910 | 39819 | 11268 | 10698 | 12896 | 18070 |
| Resolution | 12 | 10 | 13 | 12 | 13 | 23 |

**Table S2. Data collection for PEDV spikes by negative-stain electron microscopy, Related to STAR Methods.** All data was collected on a Technai G2 Spirit operating at 120 kV with a Teitz 4K × 4K camera and collecting a total dose of 25 $e^-/Å^2$.

| Sample | Negative control | Sample 1, side-binding | Sample 1, apex-binding | Sample 2, side-binding | Sample 2, apex-binding | Sample 3, side-binding | Sample 3, apex-binding |
|---|---|---|---|---|---|---|---|
| Expression System | Sf9, Sus Scrofa | Sf9, Sus Scrofa | Sf9, Sus Scrofa | Sf9, Sus Scrofa | Sf9, Sus Scrofa | Sf9, Sus Scrofa | Sf9, Sus Scrofa |
| EMDB | 21398 | 21399 | 21400 | 21401 | 21402 | 21403 | 21404 |
| Pixel size | 4.1 | 4.1 | 4.1 | 4.1 | 4.1 | 4.1 | 4.1 |
| Particles | 44484 | 4552 | 753 | 6504 | 5903 | 36978 | 15570 |
| Resolution | 15 | 22 | 28 | 23 | 23 | 15 | 17 |

**Table S3. Data collection for PEDV spikes and porcine $F_{ab}$ by negative-stain electron microscopy, Related to STAR Methods.** All data was collected on a Technai G2 Spirit operating at 120 kV with a Teitz 4K × 4K camera and collecting a total dose of 25 $e^-/Å^2$.

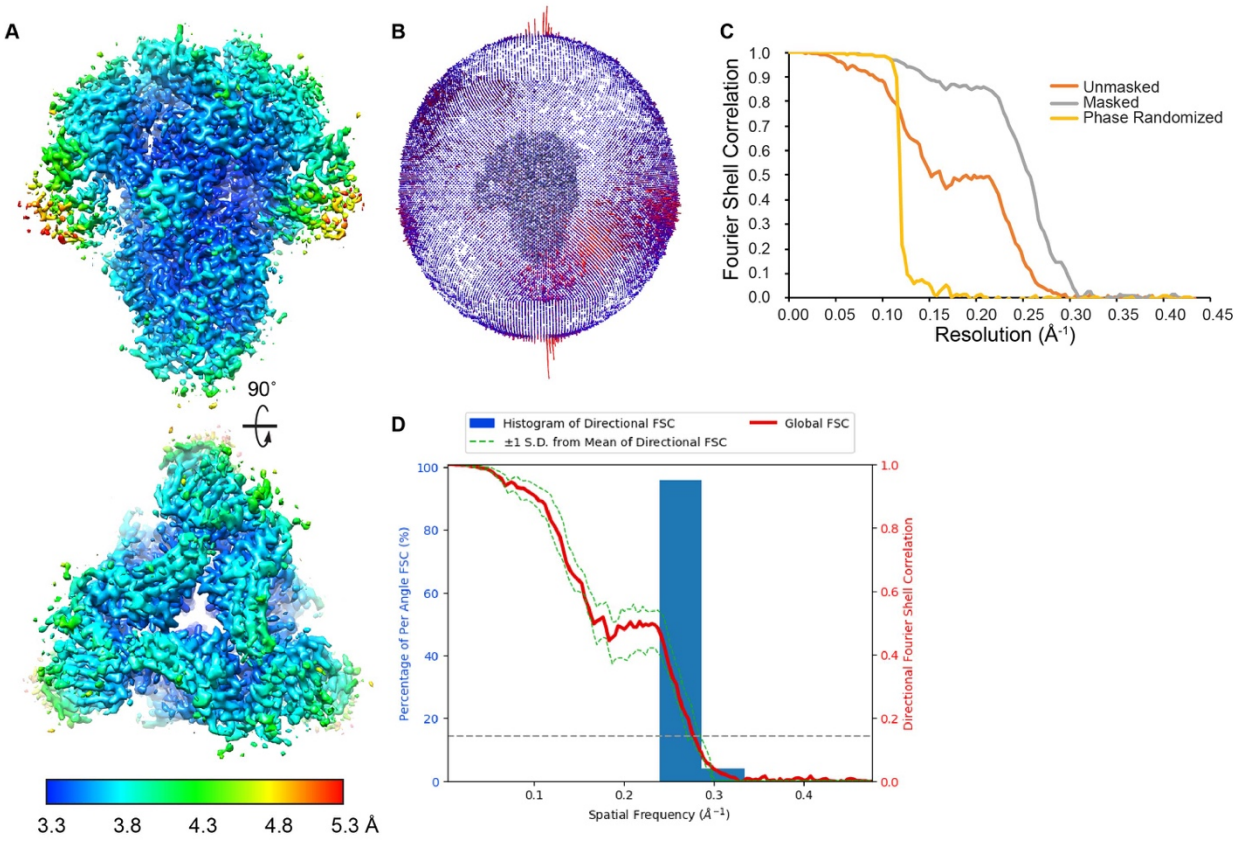


**Figure S1. Validation of cryo-electron microscopy reconstruction, Related to Figure 1.** A) Local resolution of the reconstruction calculated with RELION-3.0 (Zivanov et al., 2018). B) Plot of angular distribution. C) Fourier shell correlation curves for unmasked, masked and phase randomized correlations. D) Plot of the directional Fourier shell correlation. The sphericity of the reconstruction is 0.69.

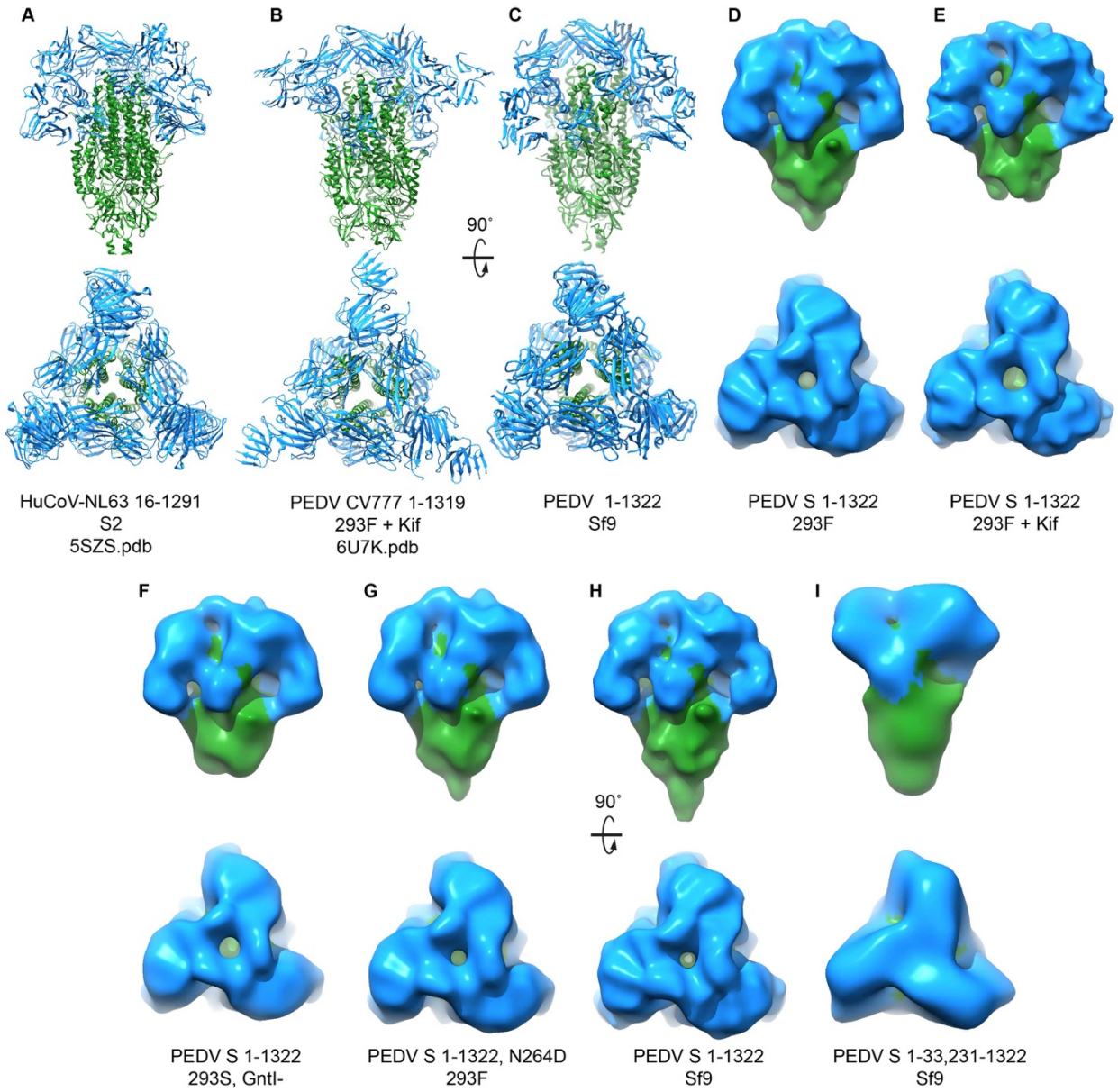


**Figure S2. Conformations of Alphacoronavirus spike variants, Relate to Figure 1.** A) The HuCoV-NL63 amino acids 16-1291 spike cryoEM structure expressed from Drosophila melanogaster S2 cells. B) The previously published PEDV spike cryoEM structure, strain CV777, amino acids 1-1319 expressed in human 293F cells with kifunensine. C) The PEDV spike cryoEM structure, amino acids 1-1322 expressed in Sf9 cells. D) PEDV spike was expressed in 293F cells, adding complex glycans. E) PEDV spike was expressed in 293F cells in the presence of kifunensine adding high-mannose glycans. F) PEDV spike was expressed in 293S GntI- cells adding high-mannose glycans. G) PEDV spike carrying a knockout of the glycan at Asn264 was expressed in 293F cells. H) PEDV spike was expressed in Sf9 insect cells, adding short high-mannose glycans. I) PEDV spike was truncated to remove the first N-terminal domain, domain 0 (amino acids 34-230) and expressed in Sf9 insect cells. With the exception of (B), all PEDV spike structures are of strain USA/Colorado/2013.

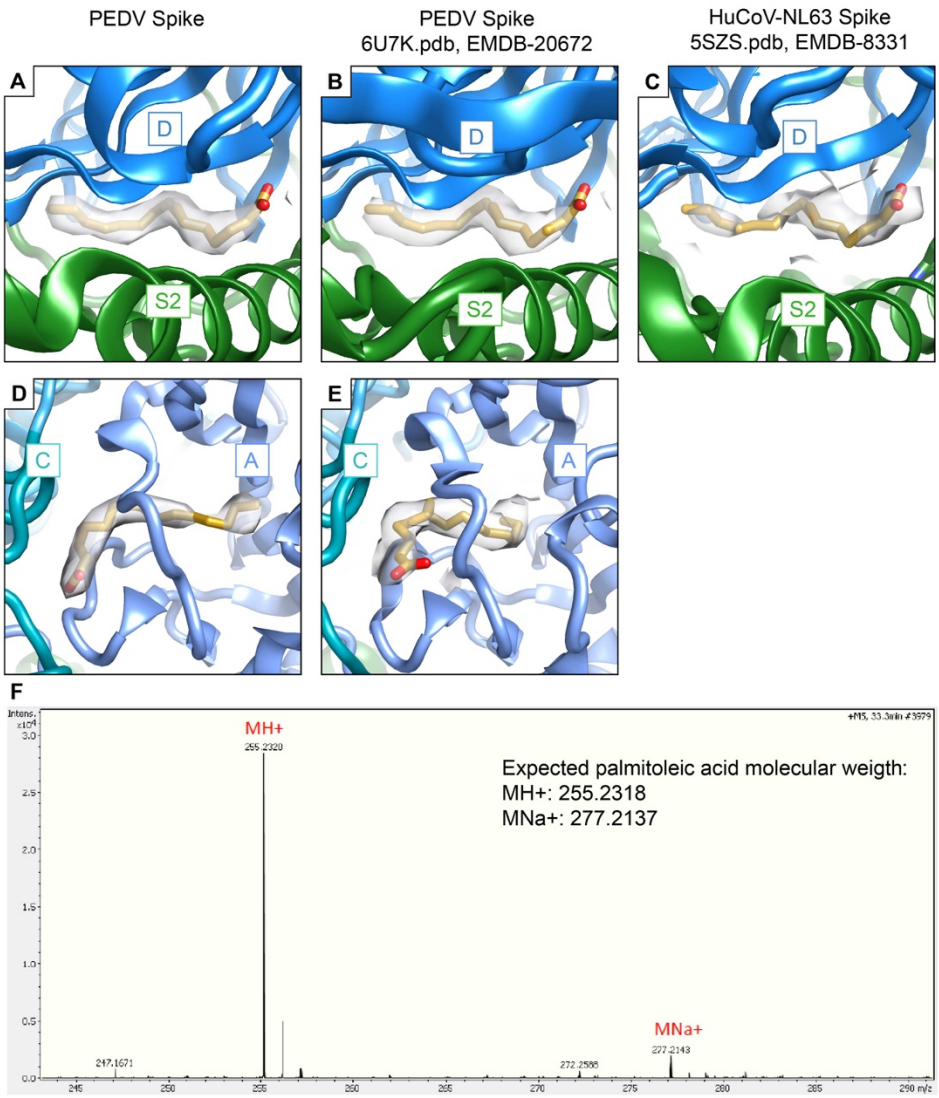


**Figure S3. Alphacoronavirus spike proteins bind to palmitoleic acid, Related to Figure 3.** Comparison of the palmitoleic acid binding site in density maps for A) the PEDV spike structure determined here, B) the previously determined PEDV spike structure (Wrapp and McLellan, 2019) and C) the previously determined HuCoV-NL63 spike structure (Walls et al., 2016b). The coordinates of palmitoleic acid built in the PEDV spike structure presented here were superposed onto the other spike structures based on neighboring protein regions. Comparison of density in the second palmitoleic acid binding sites located within PEDV S1 domain A in D) the spike structure presented here as well as E) the previously determined PEDV spike structure (Wrapp and McLellan, 2019). Note that the palmitoleic acid density appears to adopt an alternate conformation in the previously determined PEDV spike structure. The coordinates of palmitoleic acid were real space refined to reflect this altered conformation. The altered fatty acid conformation may be due to differences in the conformation of this region of the protein (see main text).

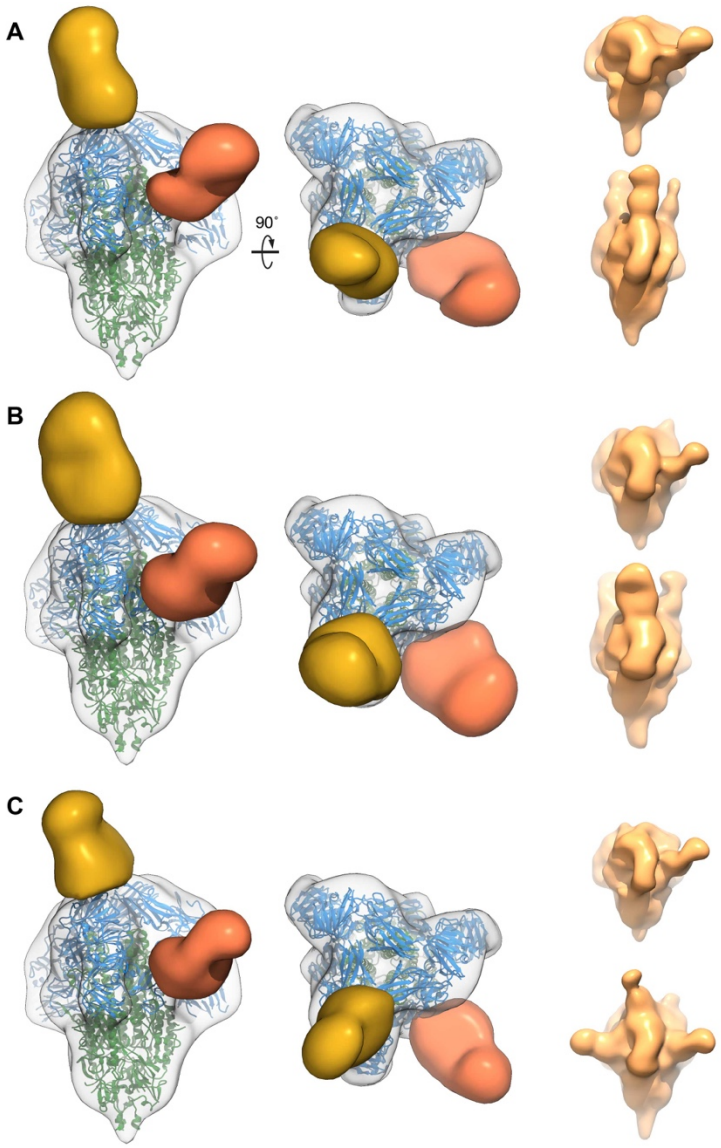


**Figure S4. Polyclonal antibody analysis of pigs experimentally infected with PEDV, Related to Figure 4.** The polyclonal antibody sera from pigs experimentally infected with PEDV was bound to PEDV spikes and analyzed by negative-stain electron microscopy using 3D classification and C1 symmetry. Antibody Fabs recognizing unique epitopes were isolated from the reconstructions by segmentation and positioned onto a negative stain reconstruction of PEDV spike (EMDB-21392) with the cryoEM coordinate model (6VV5.pdb) docked in. Antibody Fabs from three individual pigs (A, B and C) confirm the recognition of the same two antibody epitopes on PEDV spikes. For each sera sample, representative non-segmented maps are shown at right.