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Supplemental information

SARS-CoV-2 Nsp16 activation mechanism and a cryptic pocket with pan-coronavirus antiviral potential

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Figure S1.

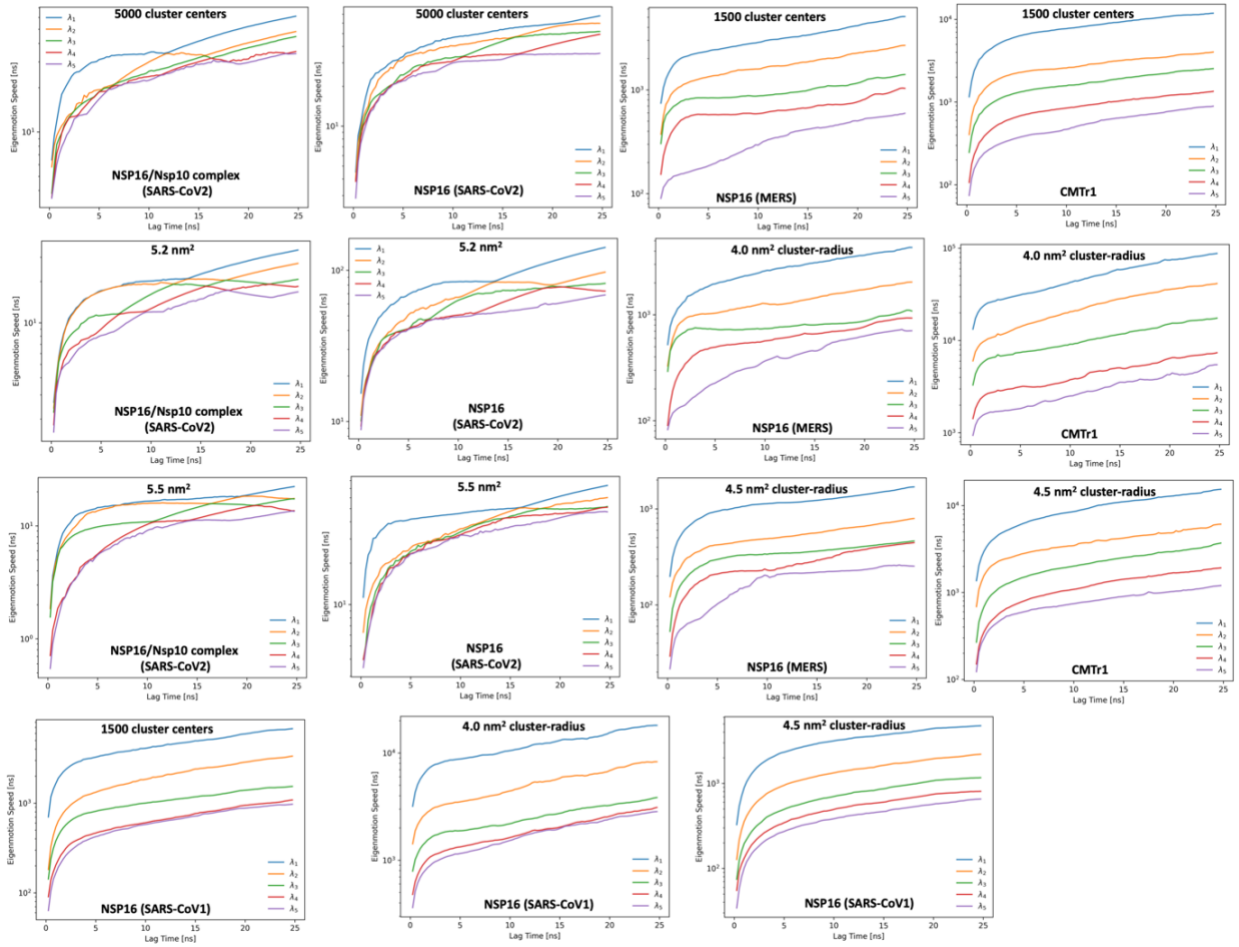

Figure S1. Implied timescales plotted as a function of the lag time for MSMs for Nsp16/Nsp10 complex (SARS-CoV2), Nsp16 homologs (SARS-CoV2, SARS-CoV1, & MERS) and human CMTr1 for different clustering cut-offs. 5 ns lag-time was selected to build the final MSMs used in this study.

Figure S2.

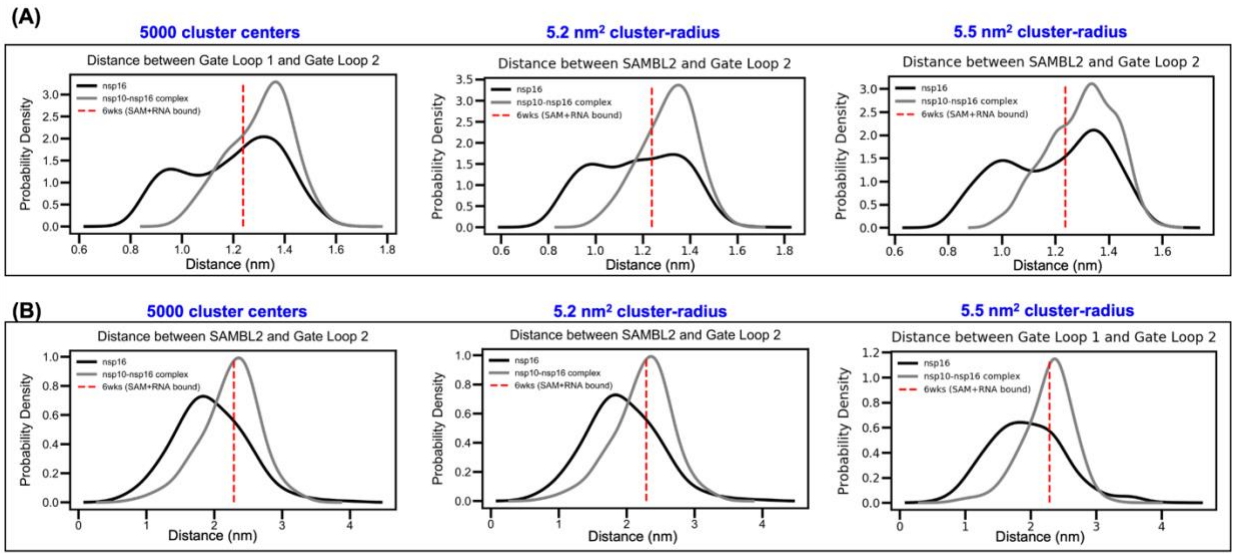

Figure S2. (A) Probability-weighted distance distribution between RNA-binding gate loops 1 and 2 comparing monomeric Nsp16 (black) to the Nsp10-Nsp16 complex (gray) are shown for three different clustering cut-offs. (B) Probability-weighted distance distribution between SAM-binding loop 2 and gate loop 2, comparing monomeric Nsp16 (black) to the Nsp10-Nsp16 complex (gray) are shown for three different clustering cut-offs. The distance for a SAM and RNA bound crystal structure is also plotted (red dotted line) in all figures.

Figure S3.

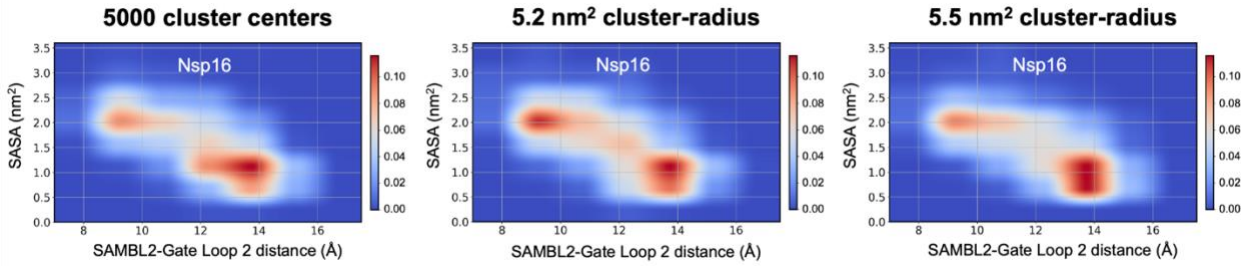

Figure S3. Equilibrium probability weighted 2D histograms of solvent-accessible surface area (SASA) of the cryptic pocket residues and the distance between SAMBL2 and gate loop 2 in Nsp16, derived from MSMs built with three different clustering cut-offs (5000 cluster centers, 5.2 nm^2 cluster radius and 5.5 nm^2 cluster radius)

Figure S4.

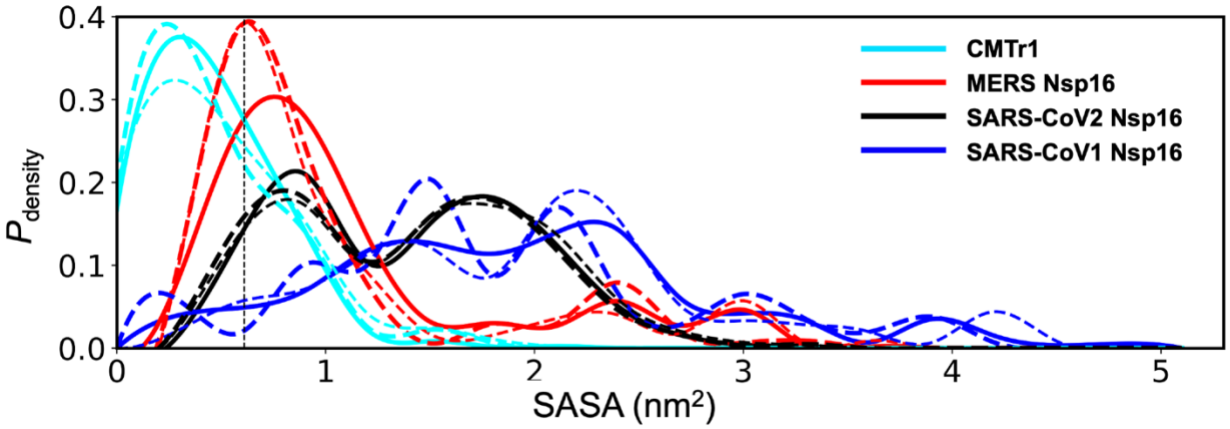

Figure S4. Equilibrium probability-weighted distribution of the solvent exposure of pocket forming residues for SARS-CoV2 (black), SARS-CoV1 (blue), MERS (red) and CMTr1 (cyan). Solid lines show the distributions derived from MSM built on 5000 clusters (for SARS-CoV2 Nsp16) and 1500 clusters (for other homologs). Thick dashed lines show the distributions derived from MSM built on clustering with 5.5 nm^2 (for SARS-CoV2 Nsp16) and 4.5 nm^2 clusters (for other homologs). Thin dashed lines show the distributions derived from MSM built on clustering with 5.2 nm^2 (for SARS-CoV2 Nsp16) and 4.0 nm^2 clusters (for other homologs). Black dotted line depicts SASA of pocket residues in the crystal structure of Nsp16/Nsp10 complex (PDB: 6wks).

Figure S5.

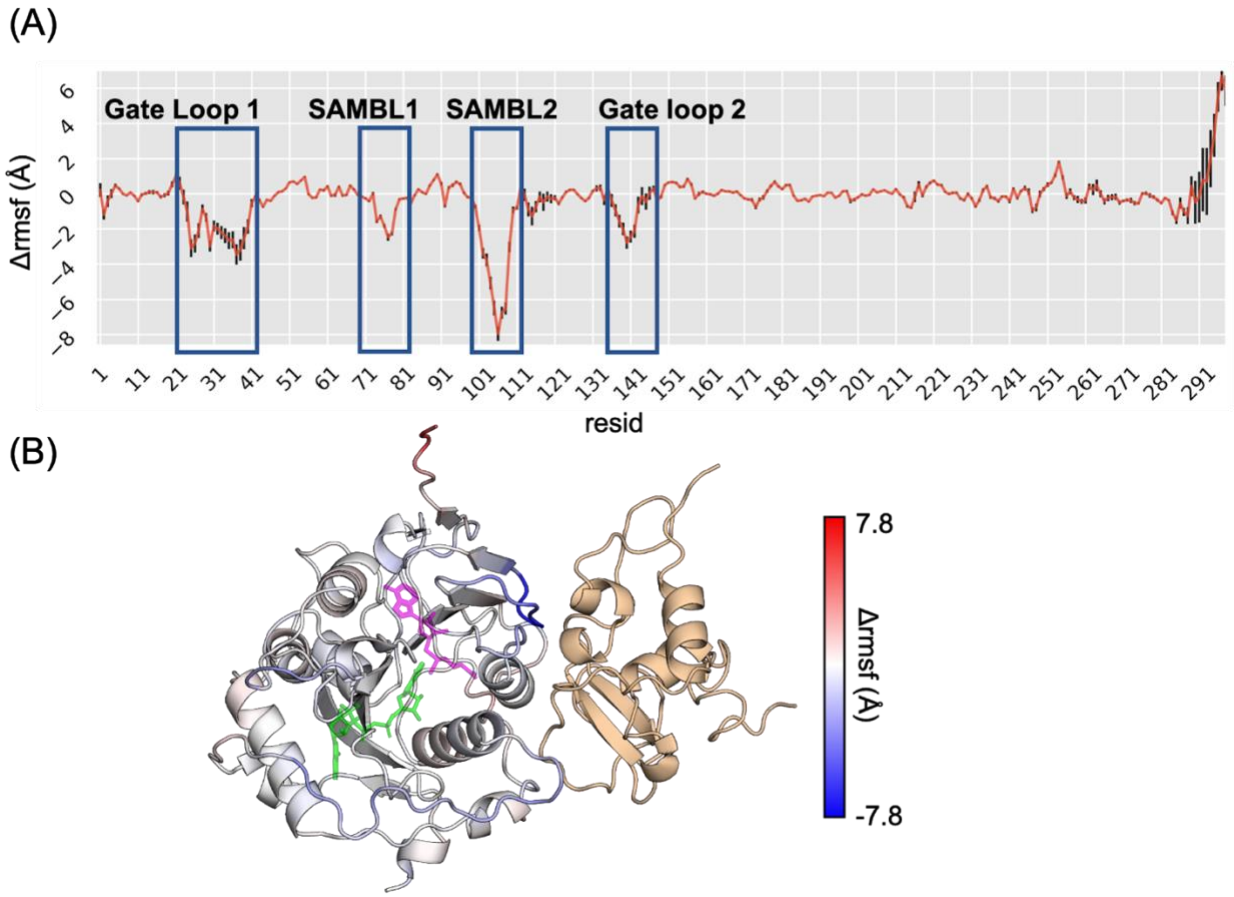

Figure S5. Change in root mean square fluctuation (rmsf) of Nsp16 upon Nsp10 association. (A) Probability weighted Δrmsf of Nsp16' residues upon Nsp10 binding is plotted. Negative values represent a decrease in rmsf upon Nsp10 binding. RNA binding loops (gate loop 1 and 2) and SAM binding loops (SAMBL1 and 2) are highlighted by the blue colored boxes. (B) Probability weighted Δrmsf of Nsp16 is mapped on its structure, with negative values shown in blue and positive values in red.

Figure S6.

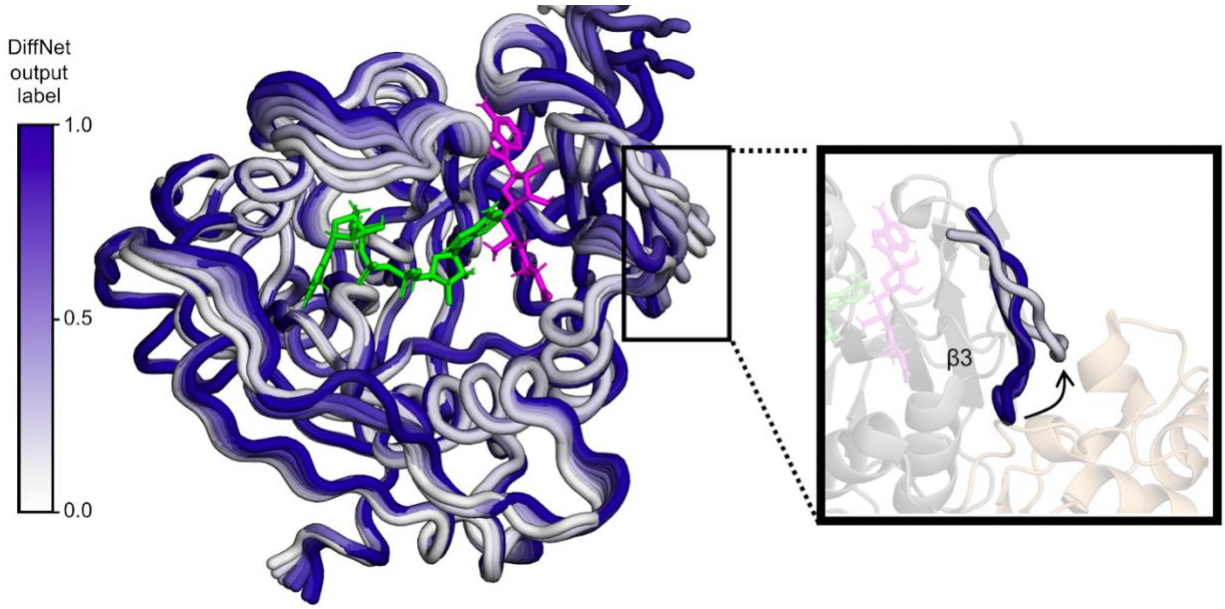

Figure S6. DiffNets predict that β4 peels away from β3 in Nsp16 inactive structural states. (Left) Structural states changing from inactive to active (white to purple) as predicted by the DiffNet. (Right) The loop connecting β3 and β4 peels away from β3 into solution in predicted inactive states.

Figure S7.

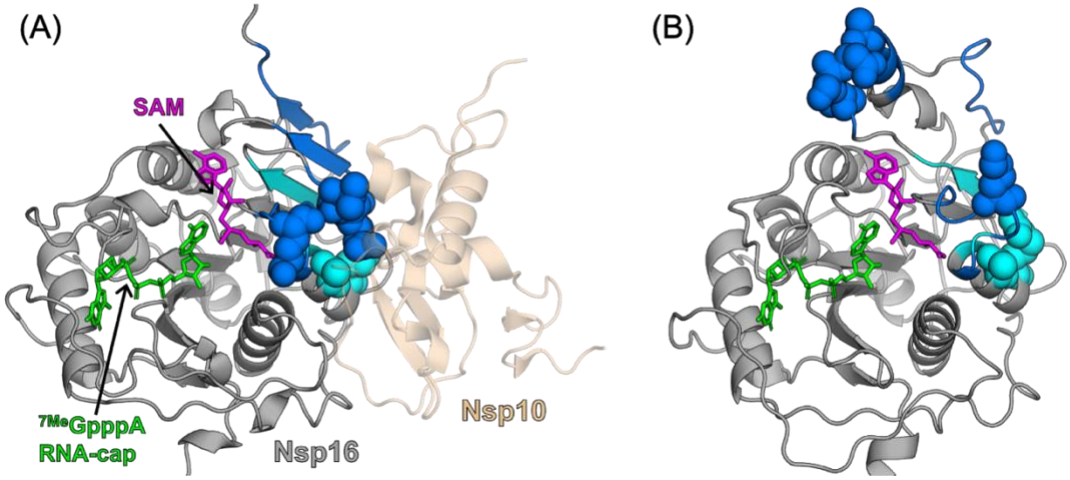

Figure S7. Displacement of Nsp10 binding residues by cryptic pocket opening. (A) Structure of Nsp16 in cryptic pocket closed state is shown in grey. Cryptic pocket forming residues and the residues undergoing opening motion are shown in cyan and blue, respectively. Cryptic pocket residues that contact Nsp10 are depicted in spheres. (B) Opening motion of the cryptic pocket shows the displacement of Nsp10 binding residues.

Figure S8.

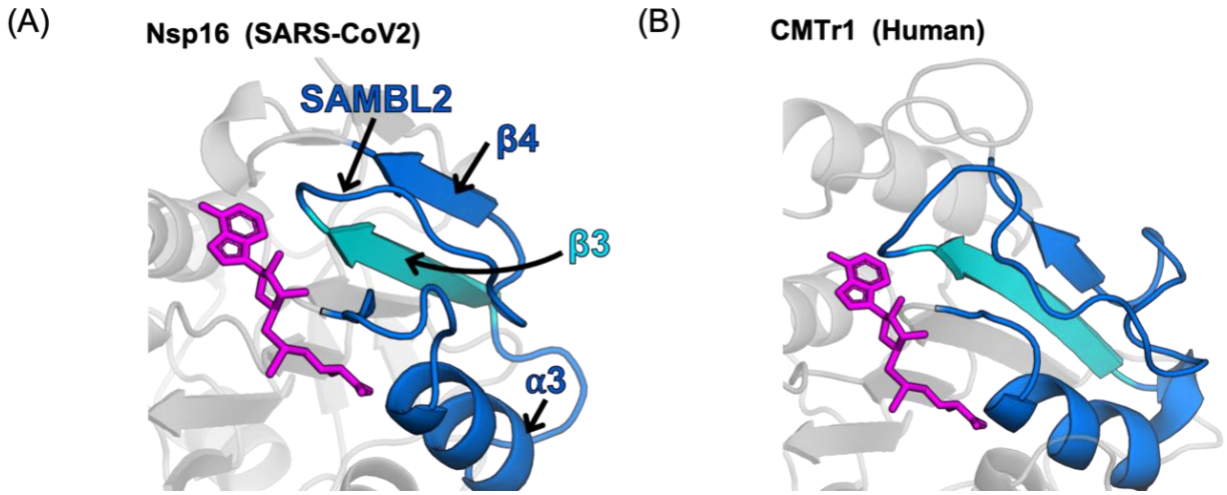

Figure S8. Structural comparison of β3-β4 cryptic pocket in SARS-CoV2 Nsp16 and human CMTr1. (A) β3 and residues lining the cryptic pocket in SARS-CoV2 are shown in cyan and blue, respectively. (B) Regions of human CMTr1, structurally equivalent to β3 and the pocket lining regions are depicted in cyan and blue, respectively.

Table S1. Timescales for transitioning between the pocket closed and open states in Nsp16 homologs.

Nsp16 homolog	Transition time (microseconds) 'Closed' → 'Open'	Transition time (microseconds) 'Open' → 'Closed'
SARS-CoV2	77.0	81.4
SARS-CoV1	26.0	13.5
MERS	19.5	9.9

Figure S9.

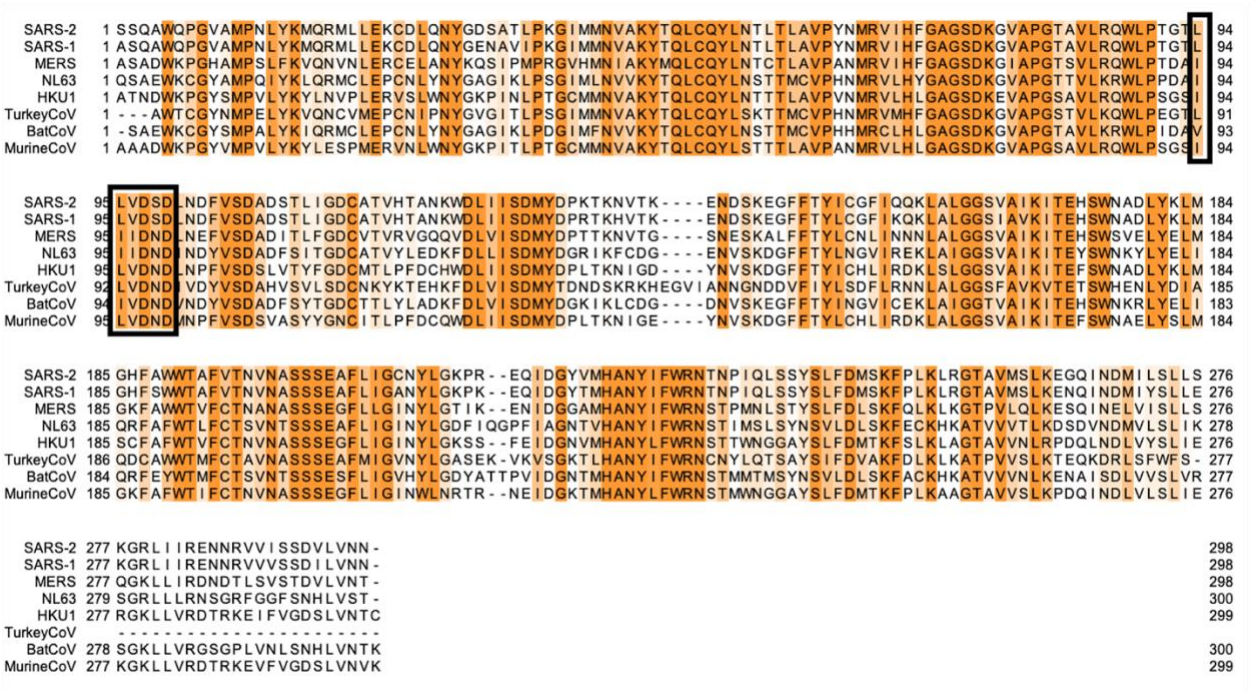

Figure S9. Multiple sequence alignment of Nsp16 homologs from coronaviruses. The color ranges from white to orange for the sequence conservation score ranging from 0 to 10, where 10 denotes 100% sequence identity. Residues of β3 are enclosed in the black box. Uniprot ids of the sequences used for the alignment are given in the Methods section.