Expanded View Figures

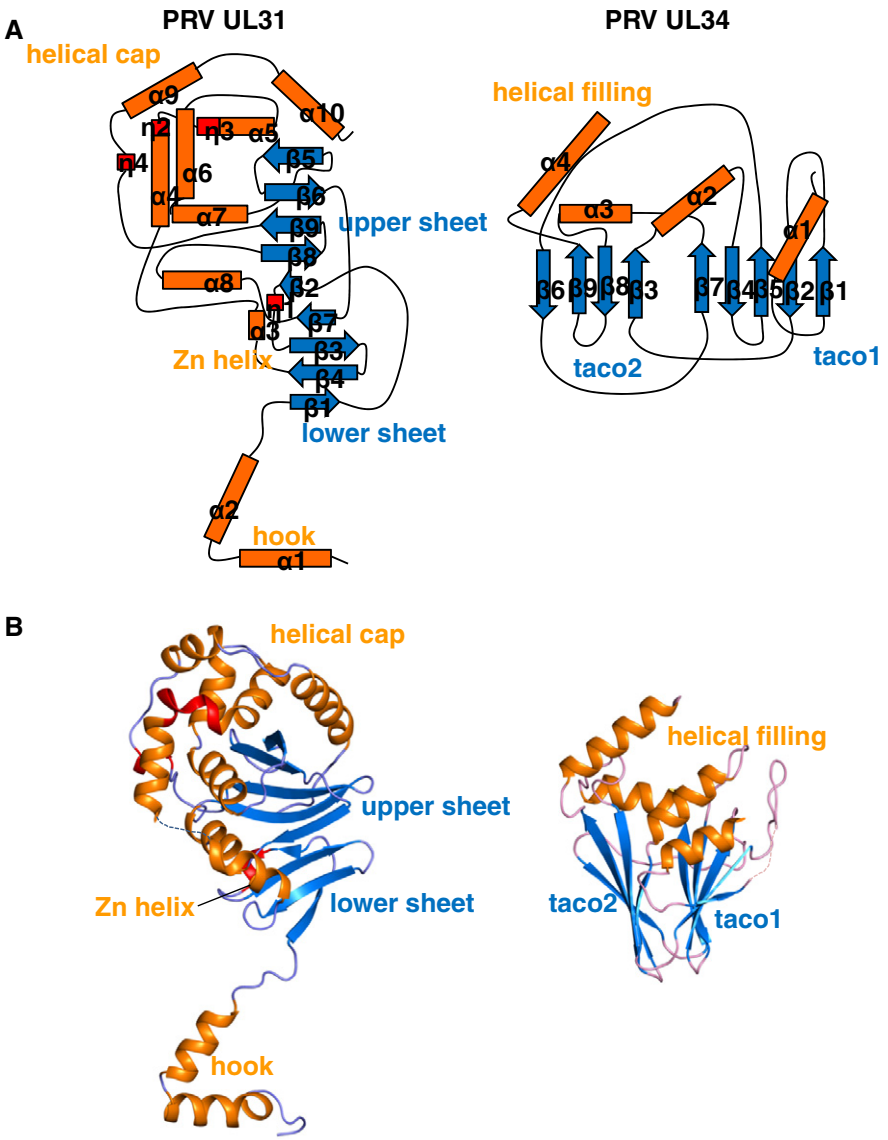

Figure EV1. Secondary structure assignment in PRV UL31 and UL34.

A Topology diagram for the PRV UL31 and UL34.

B PRV UL31 and UL34 structures are shown separately.

Data information: Colors are as in Fig 2C.

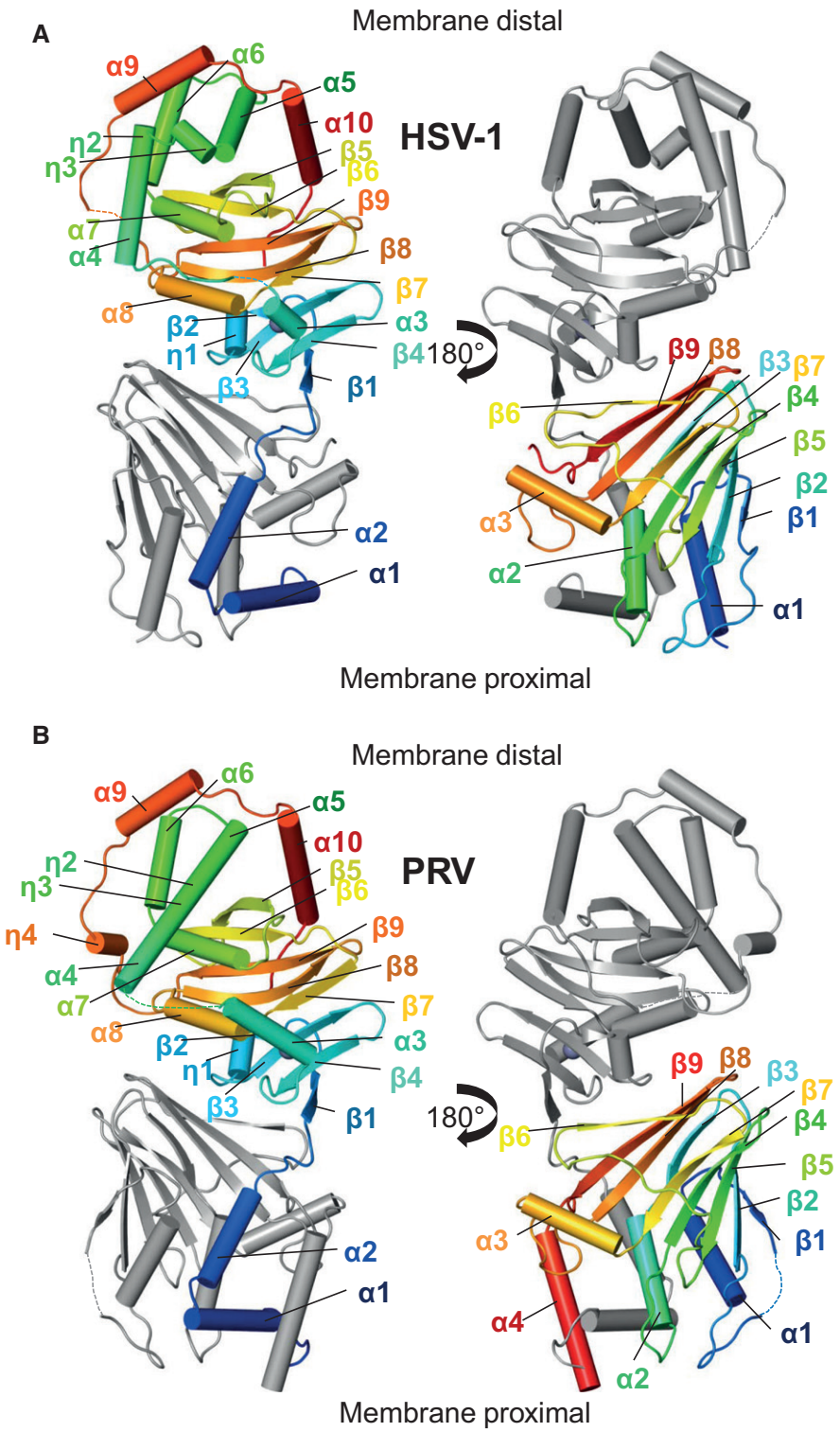

Figure EV2. Crystal structures of HSV-1 and PRV UL31 and UL34.

A, B Crystal structures of HSV-1 (A) and PRV (B) UL31 and UL34, colored according to Fig 2A and B. The top of the NEC in the shown orientation represents the membrane-distal end, whereas the bottom of the NEC represents the membrane-proximal end.

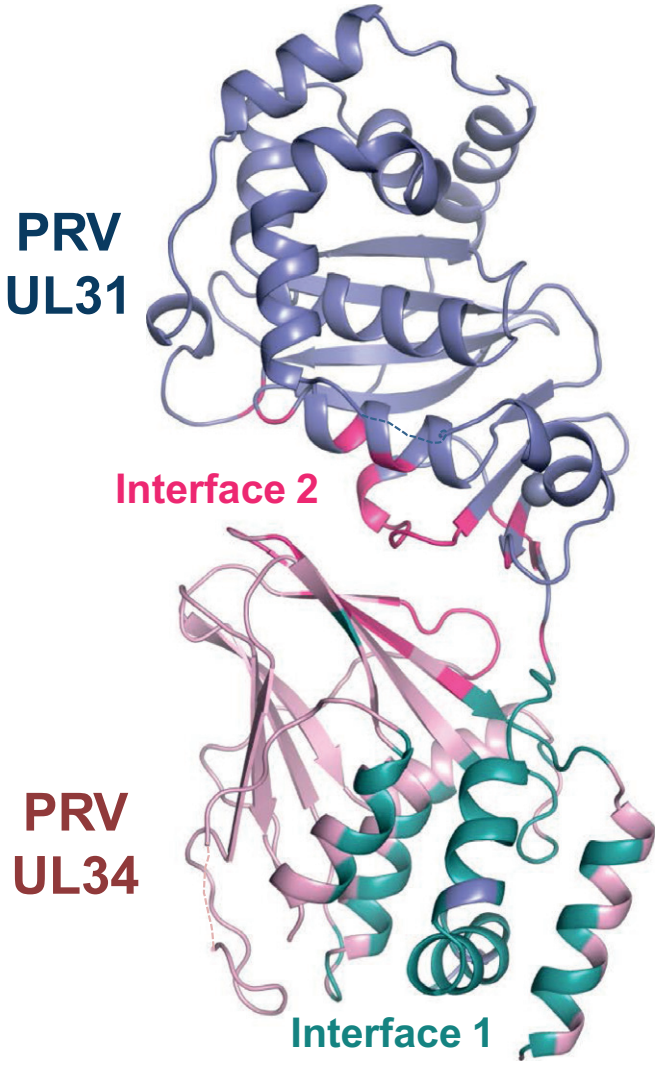

Figure EV3. UL31 binds to UL34 using two distinct interfaces.
PRV UL31 is colored in light blue and PRV UL34 in light pink. Residues involved in interface 1 are shown in deep teal, and residues involved in interface 2 are shown in hot pink.

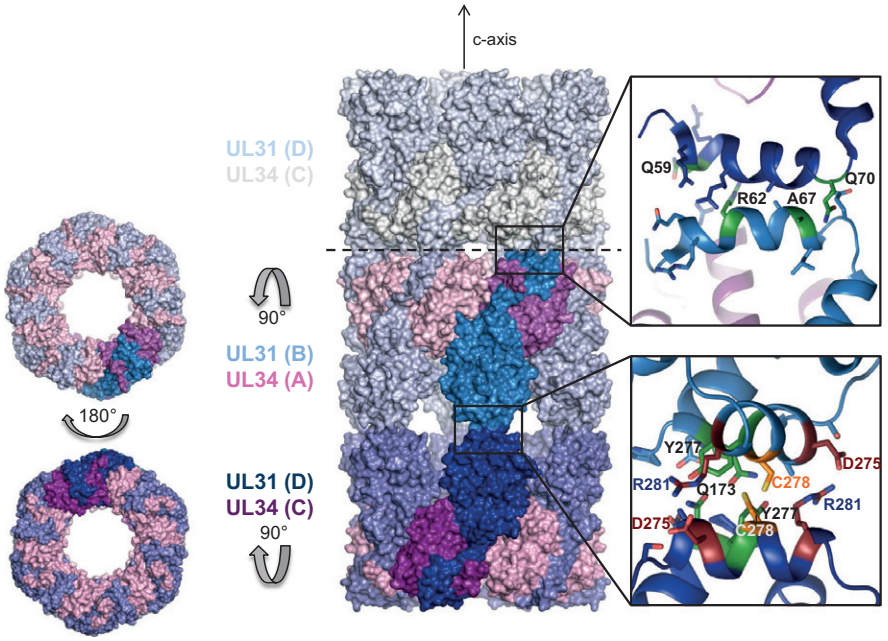

Figure EV4. Crystal packing for HSV-1 NEC.
UL31 molecules are colored in shades of blue and UL34 molecules in shades of pink. The two different hexagonal lattices are stacked on top of each other, but because the individual NEC molecules are tilted toward the crystallographic c-axis, the two different lattices can be accommodated. One asymmetric unit is shown in bold colors, visualizing that the only contacts mediating the NCS interface are provided by residues from helices α6 and α9 in UL31. The interactions are shown in detail next to the interface. The proposed disulfide bond is shown in yellow, hydrogen bonds in green, and salt bridges in firebrick. Other residues that are involved in hydrophobic interactions are not labeled, but the side chains are shown. The tail-to-tail interactions link the next layer (gray) to the lattice. These interactions are mediated by helix α1 from UL31. A detailed view is shown on the top right. Again, hydrogen bonds are colored green and the side chains of other residues involved in the interaction are shown.

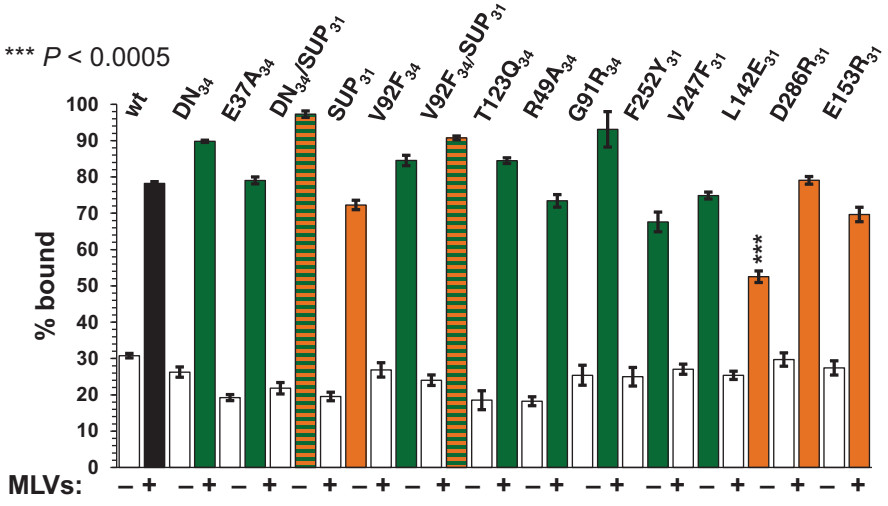

Figure EV5. Cosedimentation assay with NEC mutants and multilamellar vesicles (MLVs).
Most mutants bind to membranes comparable to WT NEC220, except for $L142E_{31}$, which shows reduced binding. The reported values represent averages of the results of three individual experiments. Error bars represent the standard errors of measurement. The statistical analysis used is the Student's *t*-test. ****P*-value < 0.005. The asterisks above $L142E_{31}$ represent the significance compared to WT. All other samples do not show significant changes in membrane binding compared to WT. Coloring corresponds to Fig 6C, with hexamer mutants in green and inter-hexamer mutants in orange.