Expanded View Figures

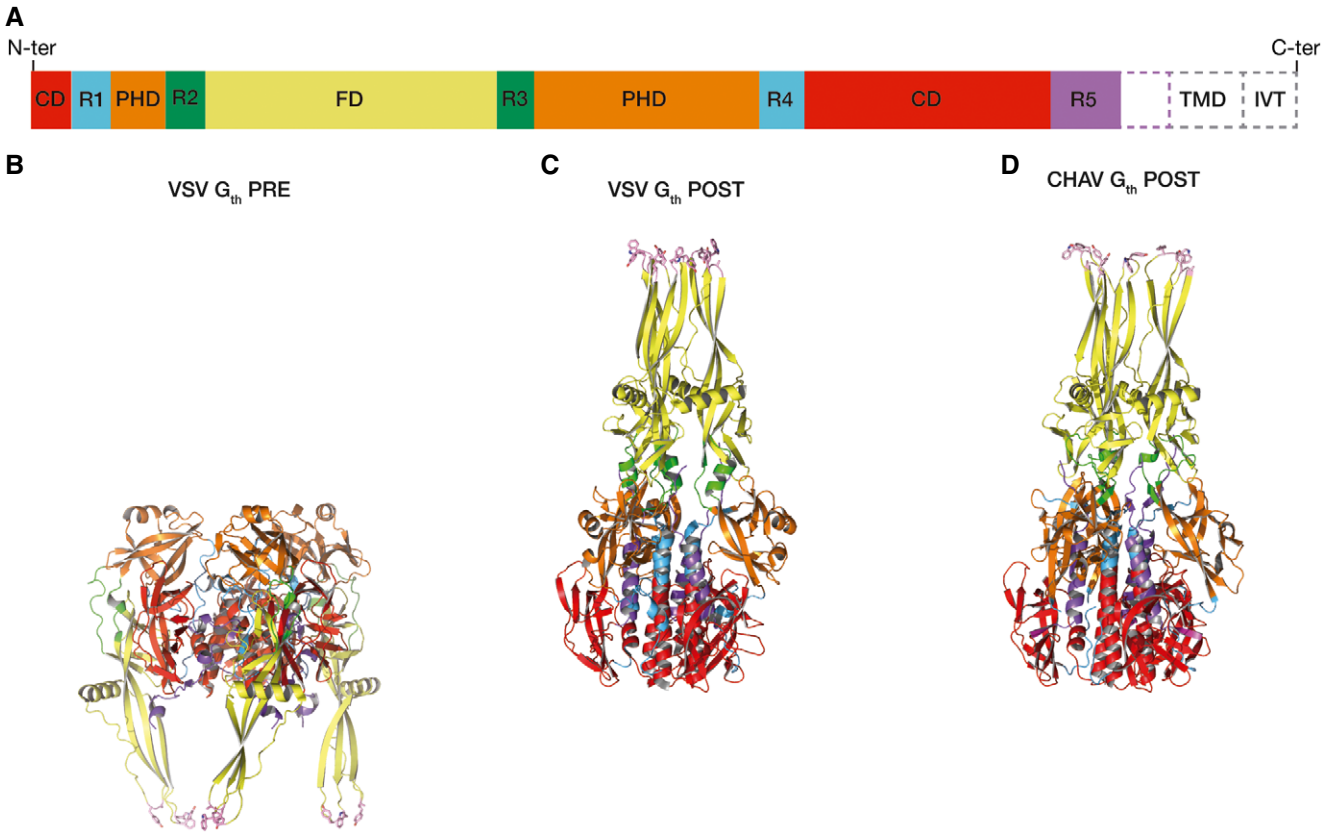

Figure EV1. Structure of the pre- and post-fusion states of vesiculovirus G ectodomain.

A Domain organization of vesiculovirus G ectodomain polypeptide chain. TMD: Transmembrane domain; IVT: Intraviral tail.
B Ribbon diagram of VSV G_{th} in its trimeric pre-fusion conformation.
C Ribbon diagram of VSV G_{th} in its trimeric post-fusion conformation.
D Ribbon diagram of CHAV G_{th} in its trimeric post-fusion conformation.

Data information: Domains and segments are colored as indicated in Table 1. Hydrophobic residues of the fusion loops are in pink sticks. FD and the C-terminal part of the ectodomain are flipped around the CD (which has the same orientation in B, C, and D) during the structural transition.

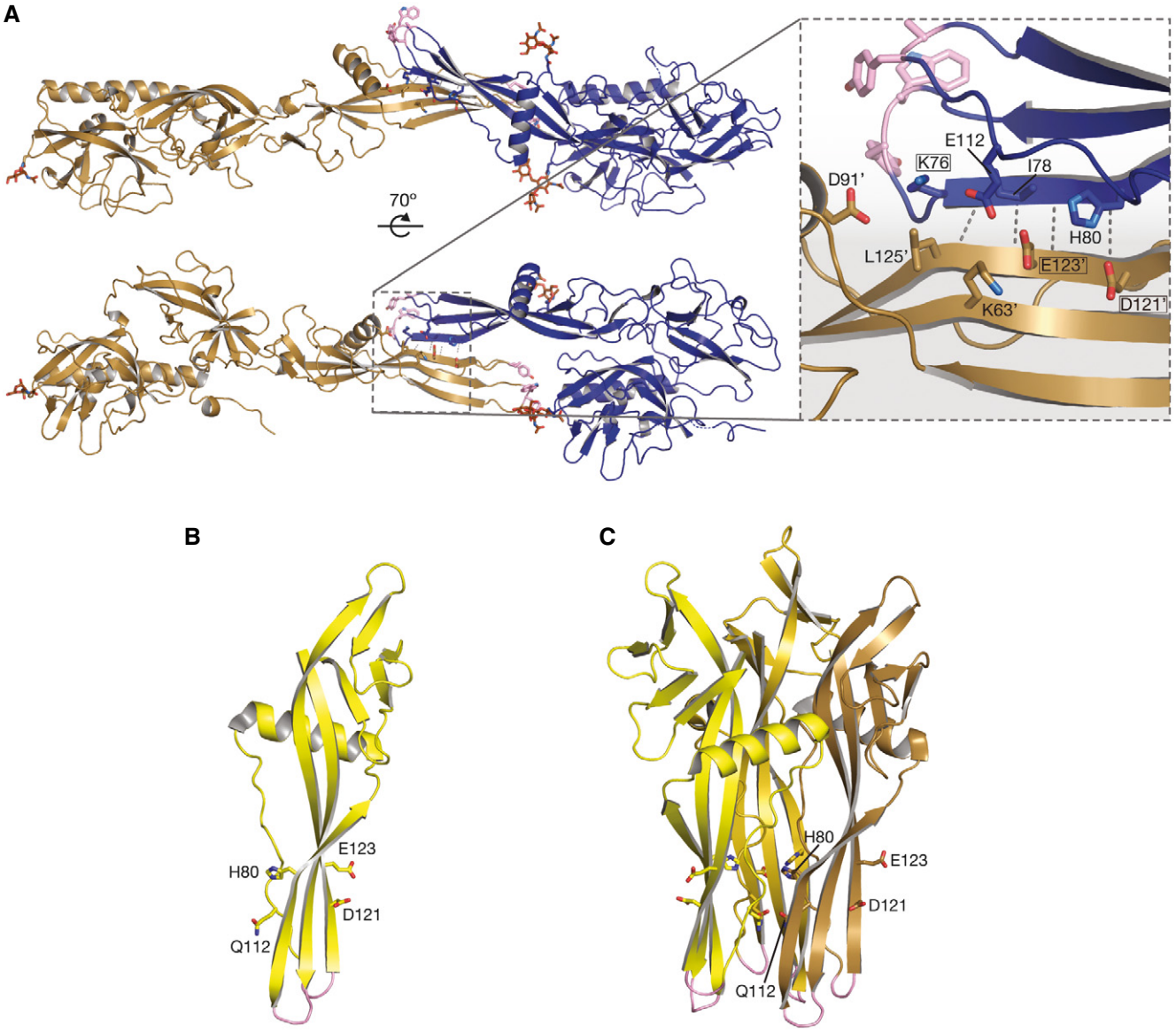

Figure EV2. An antiparallel contact between the EI and LI fusion domains.

A Views of the antiparallel contact between the EI and LI fusion domains in the tetrameric crystal structure. CHAV G_{th} LI is in brown, and CHAV G_{th} EI is in blue. Hydrophobic residues in the fusion loops are in pink sticks. Glycan chains are also represented in a sticks model. The inset shows a zoom on one of the intermolecular EI/LI beta sheets. Sites of mutations previously reported to affect the fusion properties are boxed. Side chains that cluster together (including H80, E112, D121, E123) in the interface on the sheet's face opposite the EI's fusion loops are shown as sticks and labeled, with for LI residues.

B, C Localization of residues H80, E112, D121, E123 in the fusion domain in the pre-fusion (B) and post-fusion (C) conformations of VSV G.

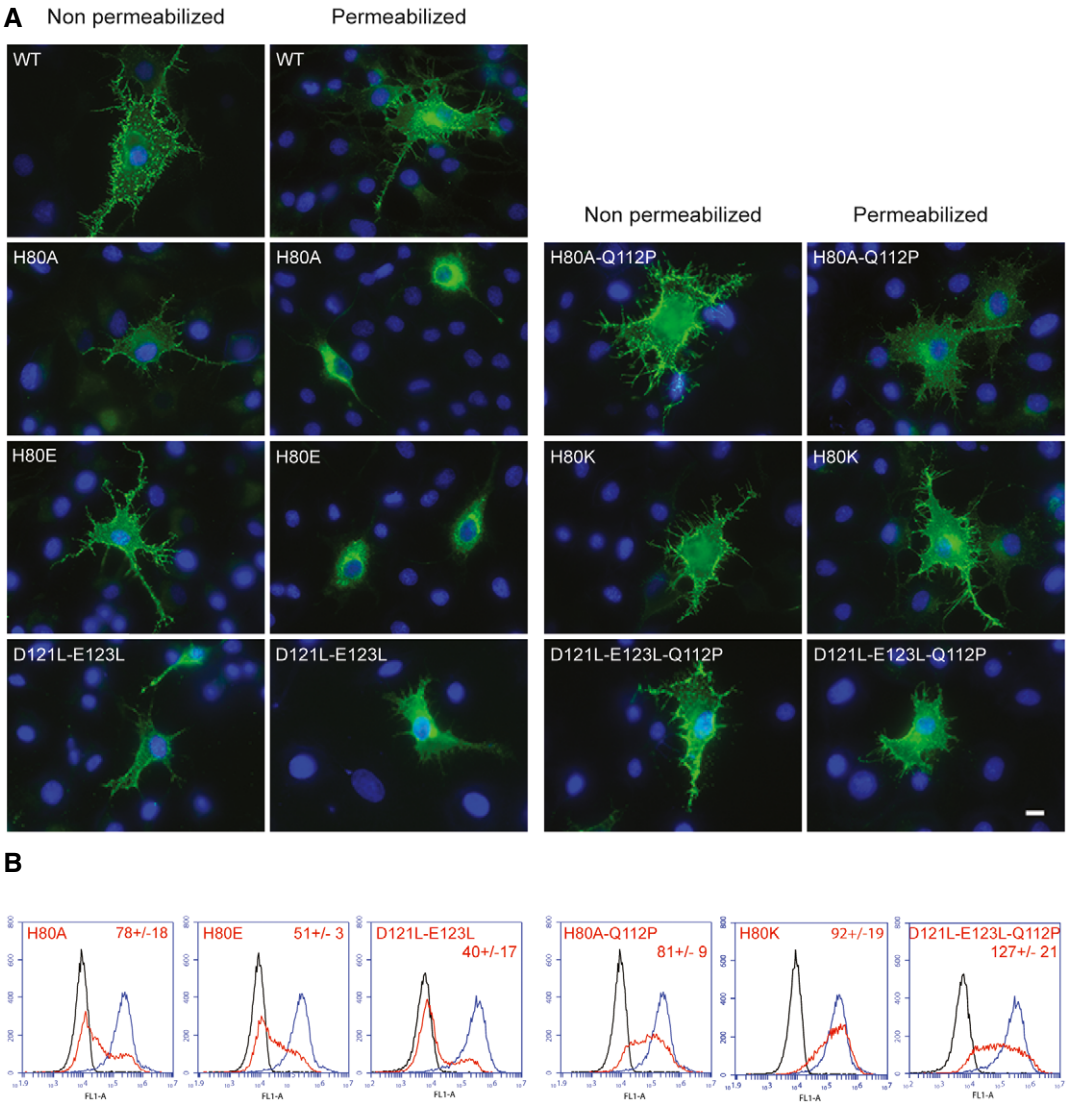

Figure EV3. Effect of mutations of residues H80, D121, and E123 on VSV G transport and surface expression in mammalian cells.

A Expression and subcellular localization of G proteins (WT and mutants) in BSR cells. Epifluorescence images of transfected cells. At 24 h post-transfection, cells were permeabilized (or not) and fixed and G was detected by immunostaining with a monoclonal antibody to VSV G (KeraFAST, 8G5F11), which was subsequently detected by using a goat anti-mouse IgG secondary antibody conjugated to Alexa Fluor 488. Nuclei were stained with DAPI. All the images are at the same scale, scale bar (bottom right): 10 μm.

B Surface expression of G in HEK cells. The surface G protein expression was detected with a monoclonal antibody to VSV G (KeraFAST, 8G5F11) and a goat anti-mouse IgG secondary antibody conjugated to Alexa Fluor 488. A total of 10,000 cells were counted by flow cytometry. In each frame, the black curve corresponds to the fluorescence of untransfected cells, the blue (resp. red) one corresponds to the fluorescence of cells transfected by pCAGGS plasmids encoding WT G (resp. indicated mutant). Experiments were performed three times, and the data were used to determine the average level of surface expression for positive cells (indicated for each mutant ± SD). Although the percentage of cells expressing G at their surface is lower for the mutants, the average levels of surface expression for positive cells are similar to wild type (varying from 40 to 127% of wild type).