**Supporting Information to accompany**

# Cryo-EM structure of eastern equine encephalitis virus in complex with heparan sulfate analogues

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## The SI Appendix includes:

Figures S1 through S6

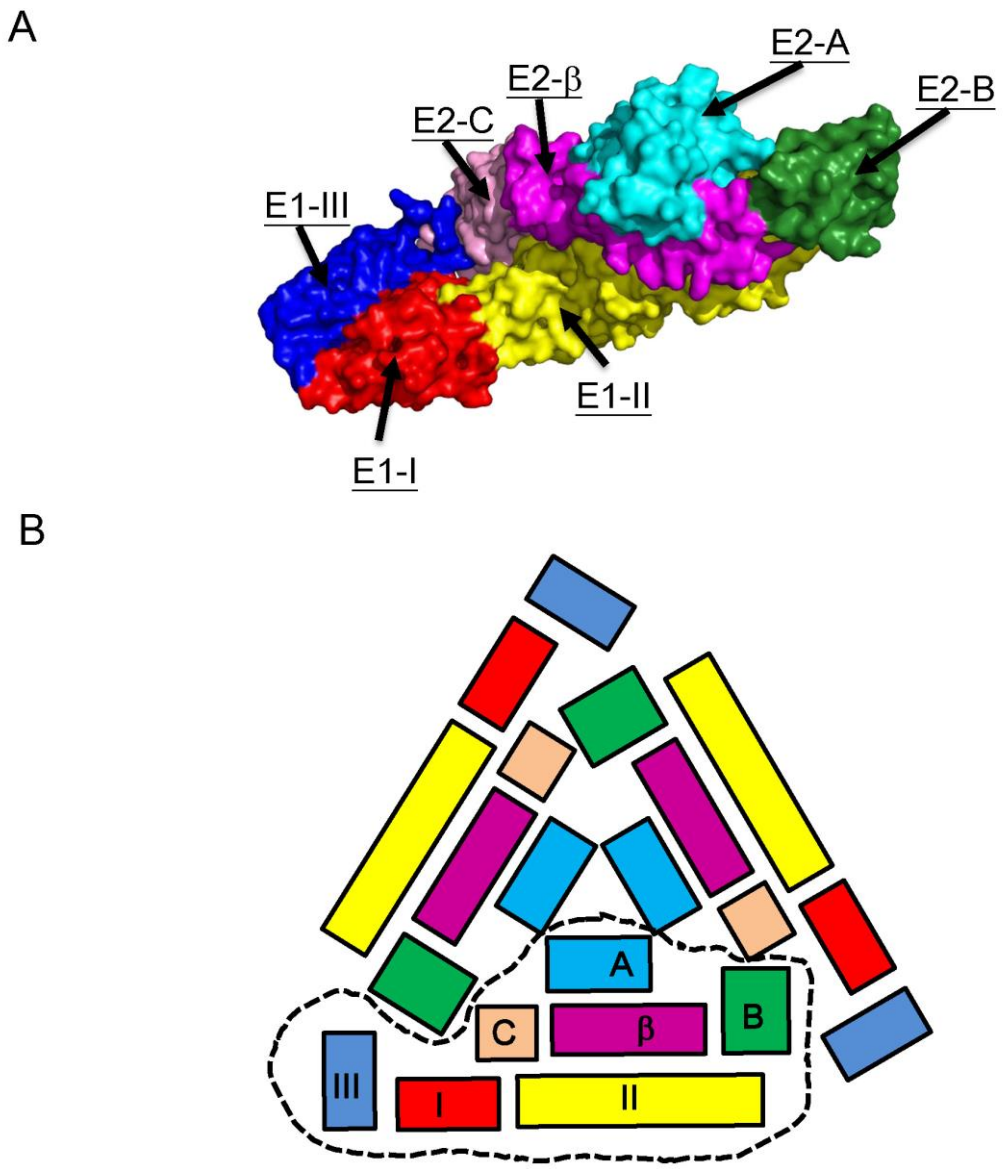


**Fig S1. Domains of E1and E2 glycoproteins. (A)** Surface representation of the domains in an E1-E2 heterodimer. E1: Domain I (red), Domain II (yellow) and Domain III (blue). E2: Domain A (cyan), β-ribbon connector (magenta), Domain B (green) and Domain C (pink). **(B)** Cartoon illustration of the domains in an E1-E2 in a trimeric spike. One E1-E2 heterodimer is circled by dashed lines and labeled and colored the same as in (A).

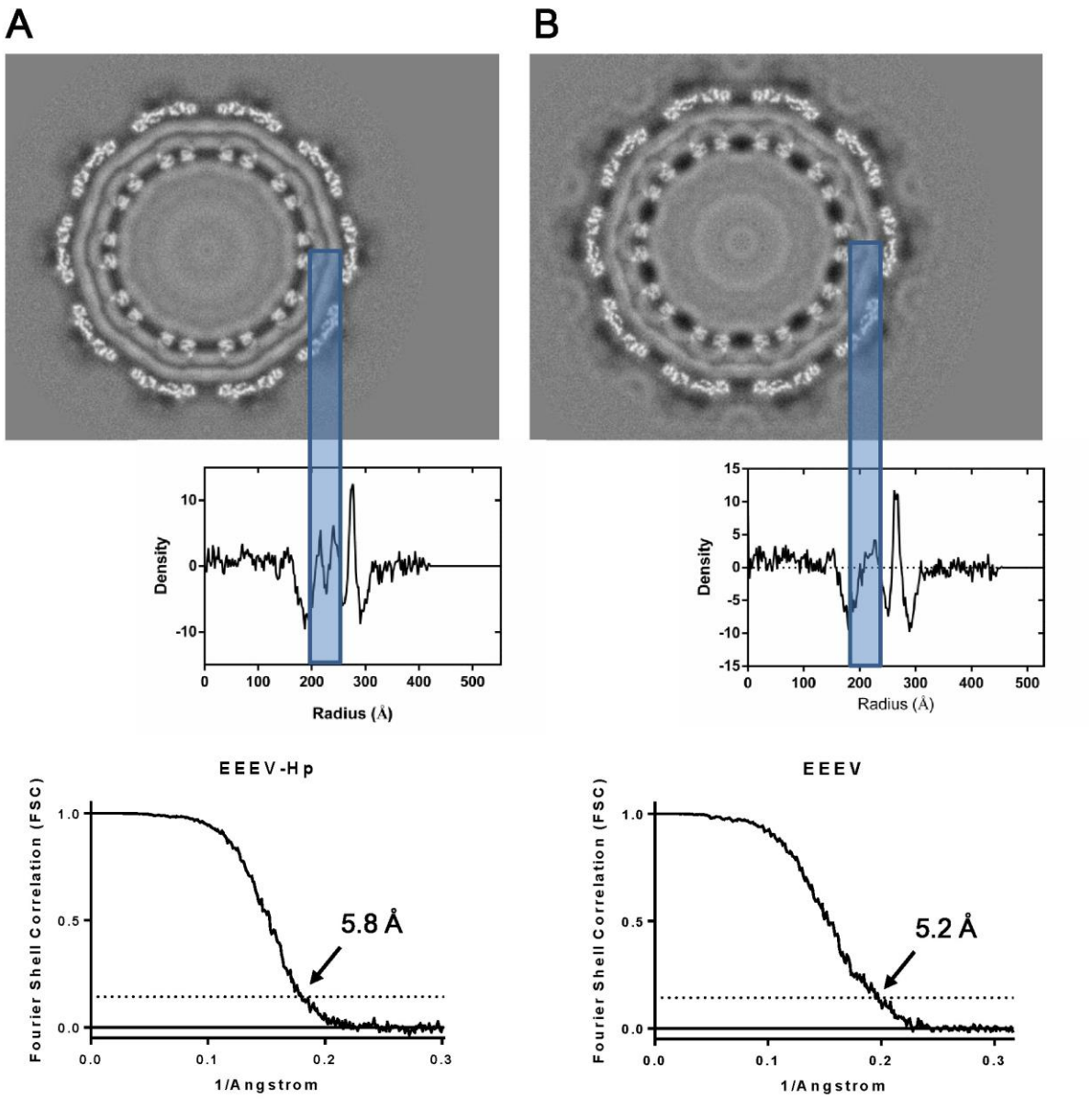


**Fig. S2. Comparison of the EEEV-heparin (Hp) and EEEV cryo-EM reconstructions. (A)** The central section of the EEEV-Hp cryo-EM map and its radial density distribution. The membrane portions in this map and the corresponding radial density plot are indicated by a blue rectangle. Fourier shell correlation between two half maps of the EEEV-Hp reconstruction is shown at the bottom (Dashed lines: FSC=0.143). **(B)** The central section of the native EEEV cryo-EM map and its radial density distribution along the x-axis. The membrane portions in the map and the corresponding density plot are again indicated by a blue rectangle. Fourier shell correlation between two half maps of the EEEV reconstruction is shown at the bottom (Dashed lines: FSC=0.143).

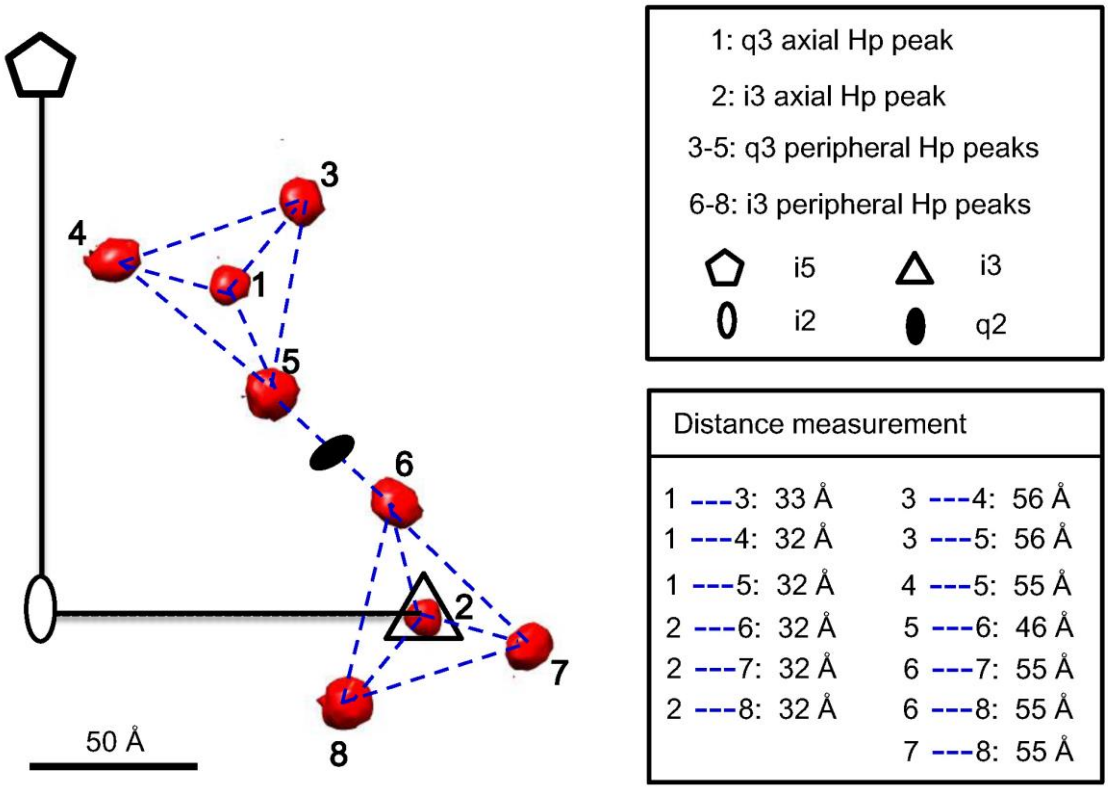


**Fig. S3. Heparin density distribution on EEEV.** Distance measurements between the various q3 and i3 axial and peripheral HS densities are provided.

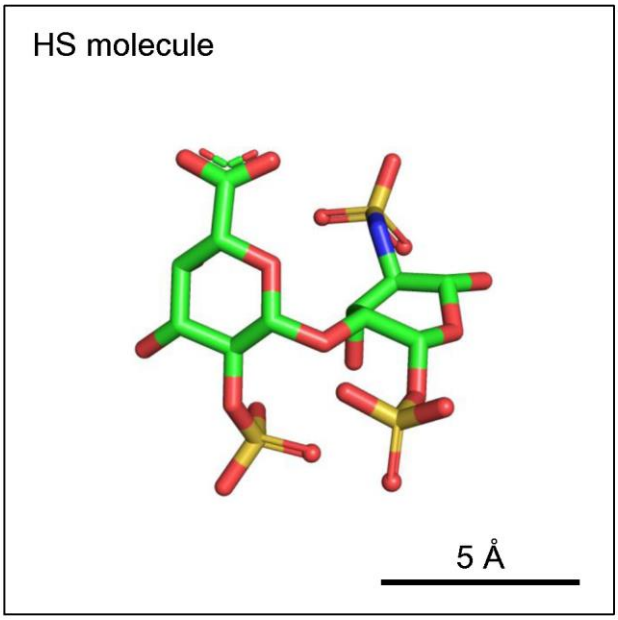


**Fig. S4. A model of the HS disaccharide molecule, GlcA(2S)-GlcNAc(6S), built using the PRODRG server.** Carbon is illustrated in green, oxygen in red, sulfur in gold, and nitrogen in blue.

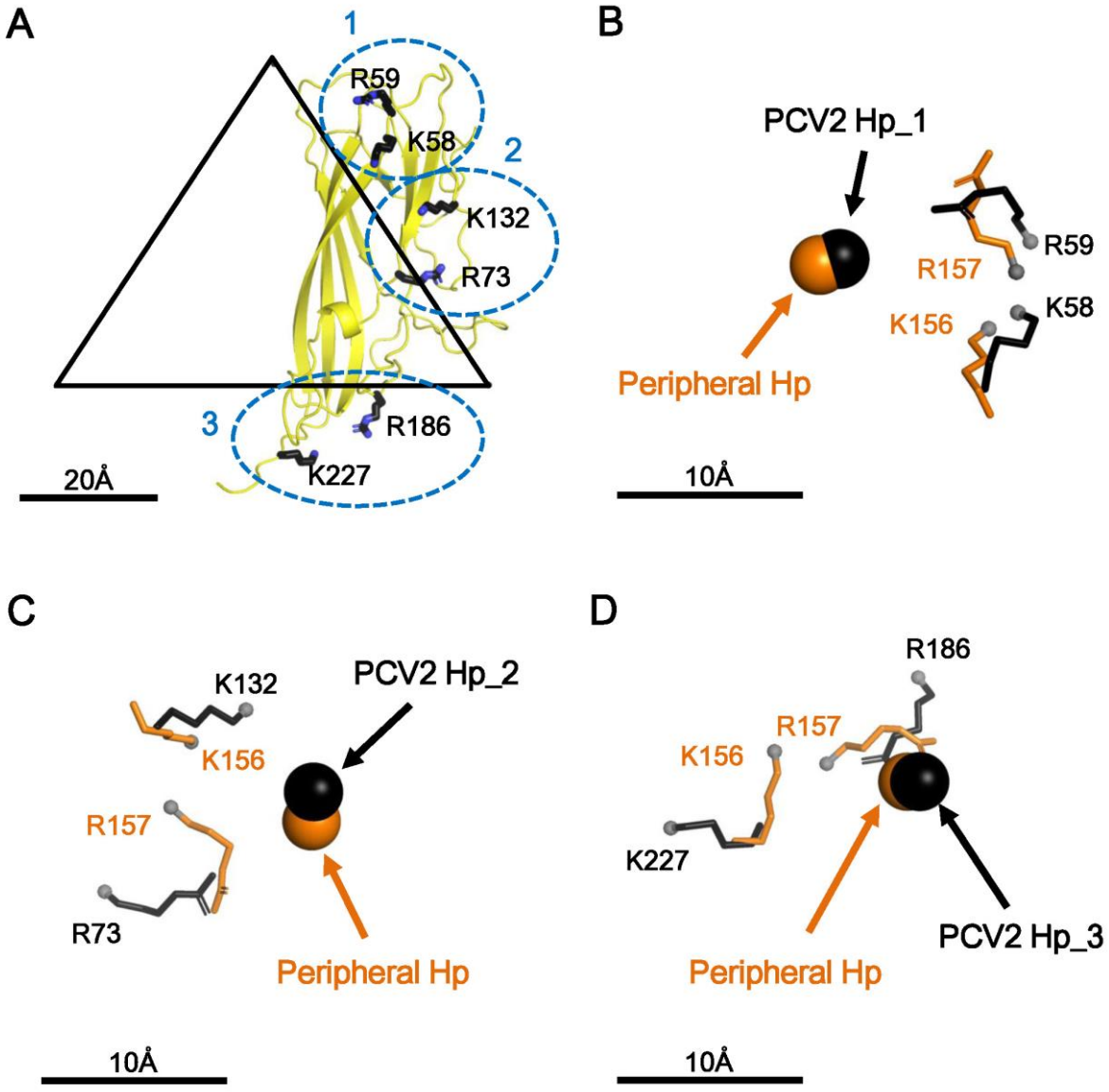


**Fig. S5. Structural alignment of the EEEV peripheral HS site and the porcine circovirus 2 (PCV2) HS binding sites. (A)** The structure of one PCV2 capsid protein in an asymmetric unit (black triangle). The side-chains of positively charged residues in three HS binding sites, 1: R73-K132, 2: R186-K227 and 3: K58-R59, are surrounded by blue dashed lines. **(B)** Structural alignment of the EEEV peripheral site and the PCV2 site 1. **(C)** Structural alignment of the EEEV peripheral site and the PCV2 site 2. **(D)** Structural alignment of the EEEV peripheral site and the PCV2 site 3. In (B), (C), and (D), the centers of modeled PCV2 HS molecules are shown as black spheres and the center of the peripheral heparin densities are shown as orange spheres. The residues in the EEEV peripheral site are labeled and colored in orange; the residues from the PCV2 sites are shown and labeled in black. The Cα atoms of residues are represented as grey spheres.

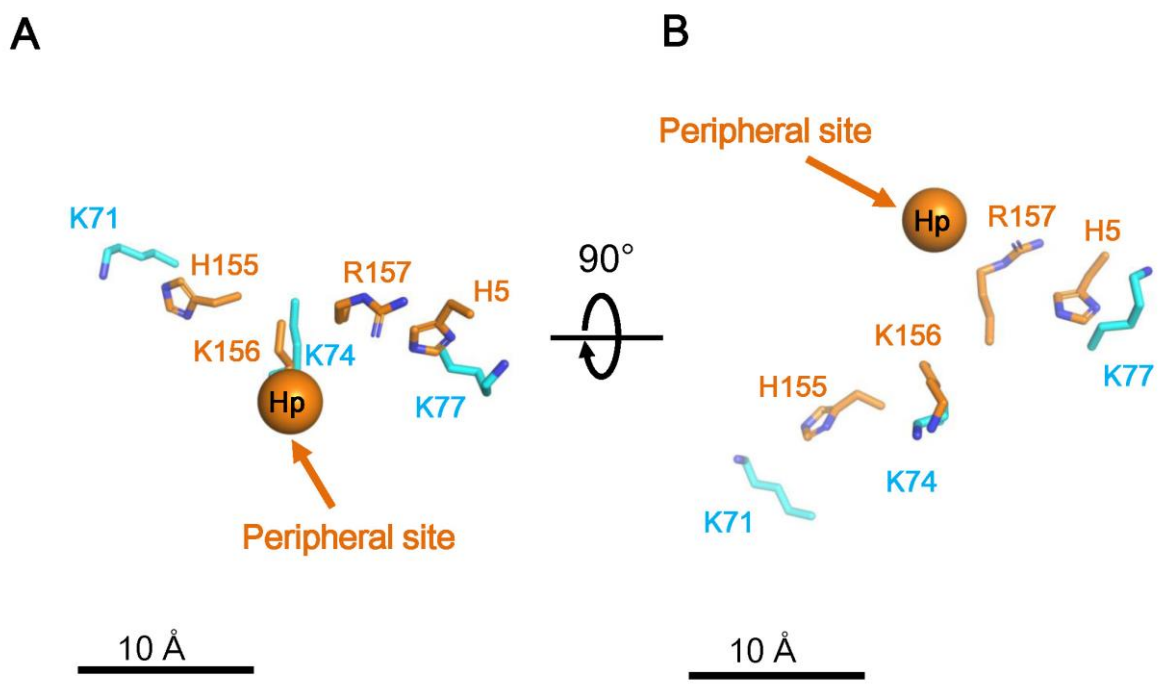


**Fig. S6. Superposition of the peripheral site residues with the 71KXX74KXXK77 sequence.** In both left and right panels, the E2 residues from the peripheral sites are labeled in orange. The three lysine residues in the 71KXX74KXXK77 sequence of E2 are labeled in cyan. The center of the peripheral heparin density is shown as an orange sphere.