PNAS

Supplementary Information for

Cryo-EM structures of alphavirus conformational intermediates in low pH triggered pre-fusion states

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This PDF file includes:

Figures S1 to S12
Tables S1 to S5

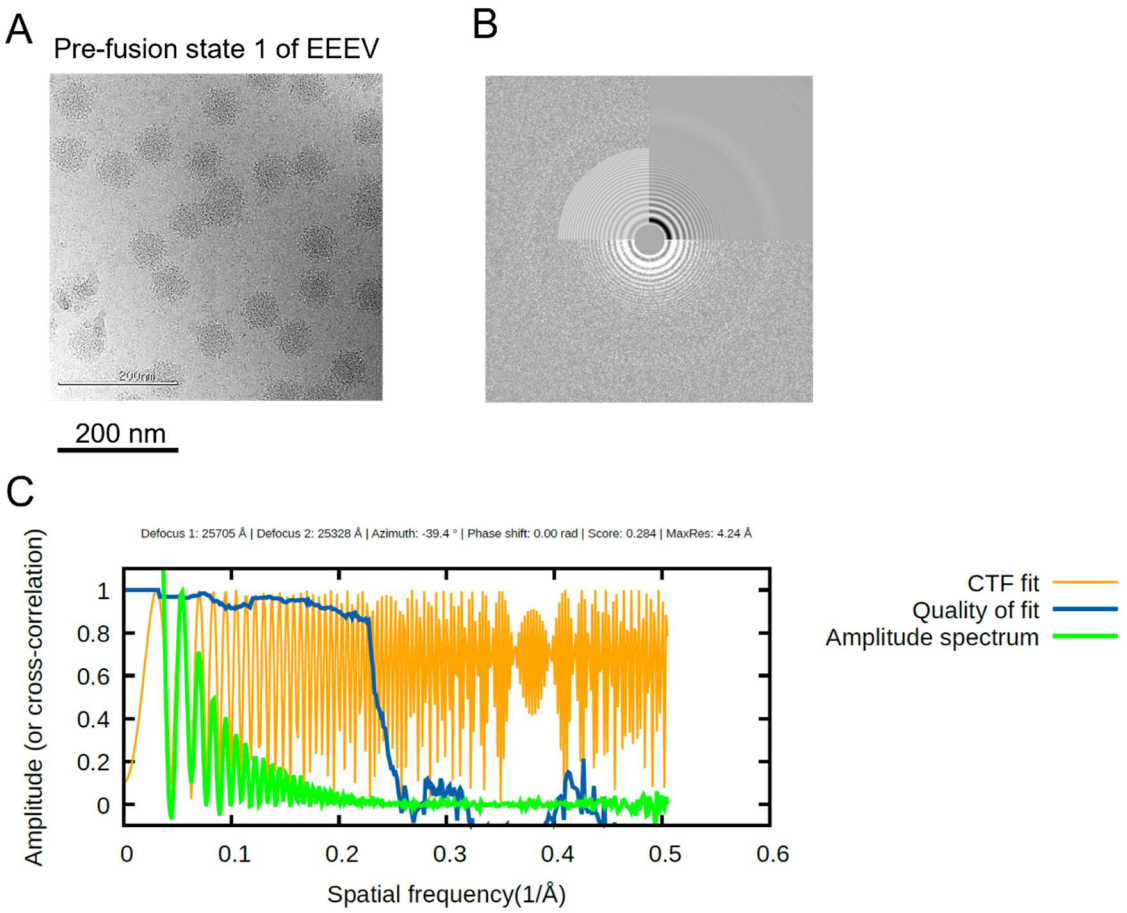

Figure S1. Micrograph of pre-fusion state 1 of EEEV. **(A)** A representative micrograph from the dataset from which pre-fusion state 1 of EEEV was reconstructed. **(B)** The power spectrum of the representative micrograph. **(C)** CTF estimation of the representative micrograph. The results of **(B)** and **(C)** were produced using the CTFFind4 implanted in RELION3.1.

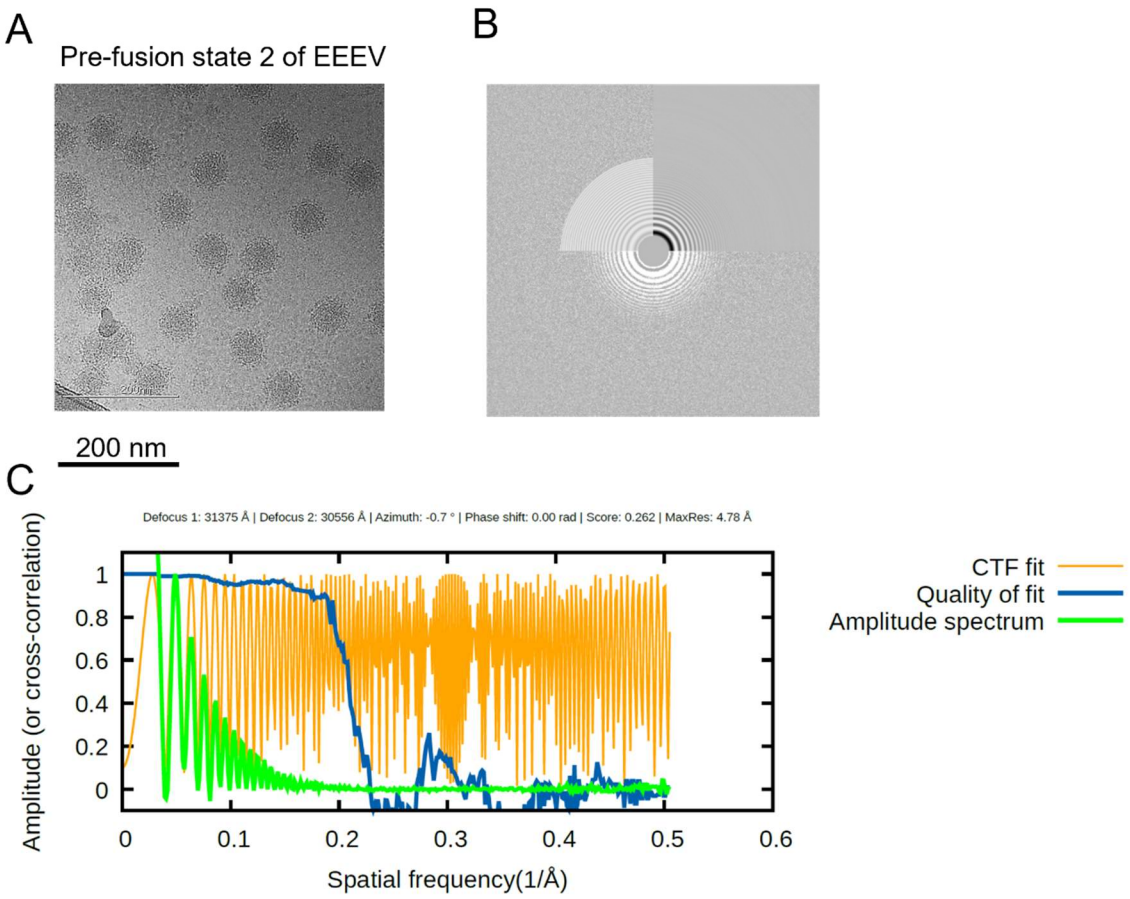

Figure S2. Micrograph of pre-fusion state 2 of EEEV. **(A)** A representative micrograph from the dataset from which pre-fusion state 2 of EEEV was reconstructed. **(B)** The power spectrum of the representative micrograph. **(C)** CTF estimation of the representative micrograph. The results of **(B)** and **(C)** were produced using the CTFFind4 implanted in RELION3.1.

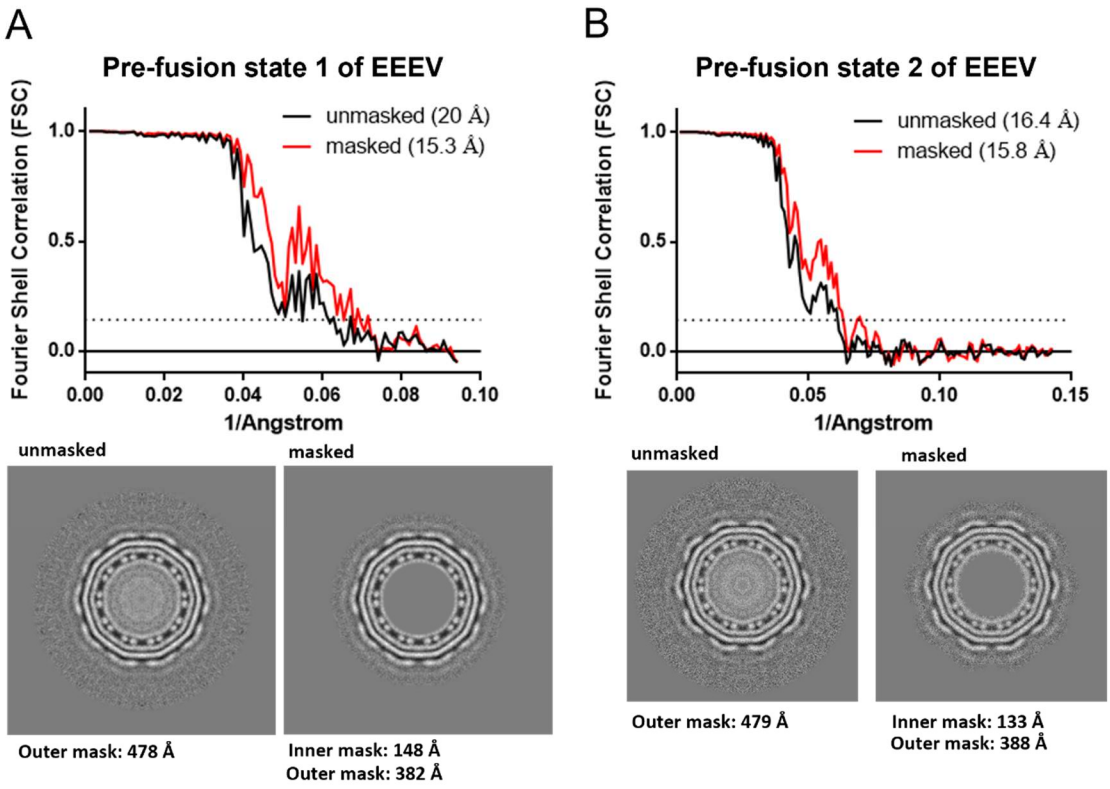

Figure S3. Plots of Fourier-shell correlation (FSC) of the low pH EEEV reconstructions. (A) FSC curve of the two half cryo-EM reconstructed maps of pre-fusion state 1 of EEEV (upper panel). The unmasked FSC was calculated from the half maps with only the outer spherical mask far from the virus density with a radius of 478 Å (lower left panel). The masked FSC was calculated from the half maps with inner mask (radius: 148 Å) to remove the RNA density and outer mask (radius: 382 Å) which is close to the spike density (lower right panel) **(B)** FSC curve of the two half cryo-EM reconstructed maps of pre-fusion state 2 of EEEV (upper panel). The unmasked FSC was calculated from the half maps with only the outer spherical mask far from the virus density with a radius of 479 Å (lower left panel). The masked FSC was calculated from the half maps with inner mask (radius: 133 Å) to remove the RNA density and outer mask (radius: 388 Å) which is close to the spike density (lower right panel)**.** Dashed lines in (**A**) and (**B**) indicate the FSC cutoff value of 0.143.

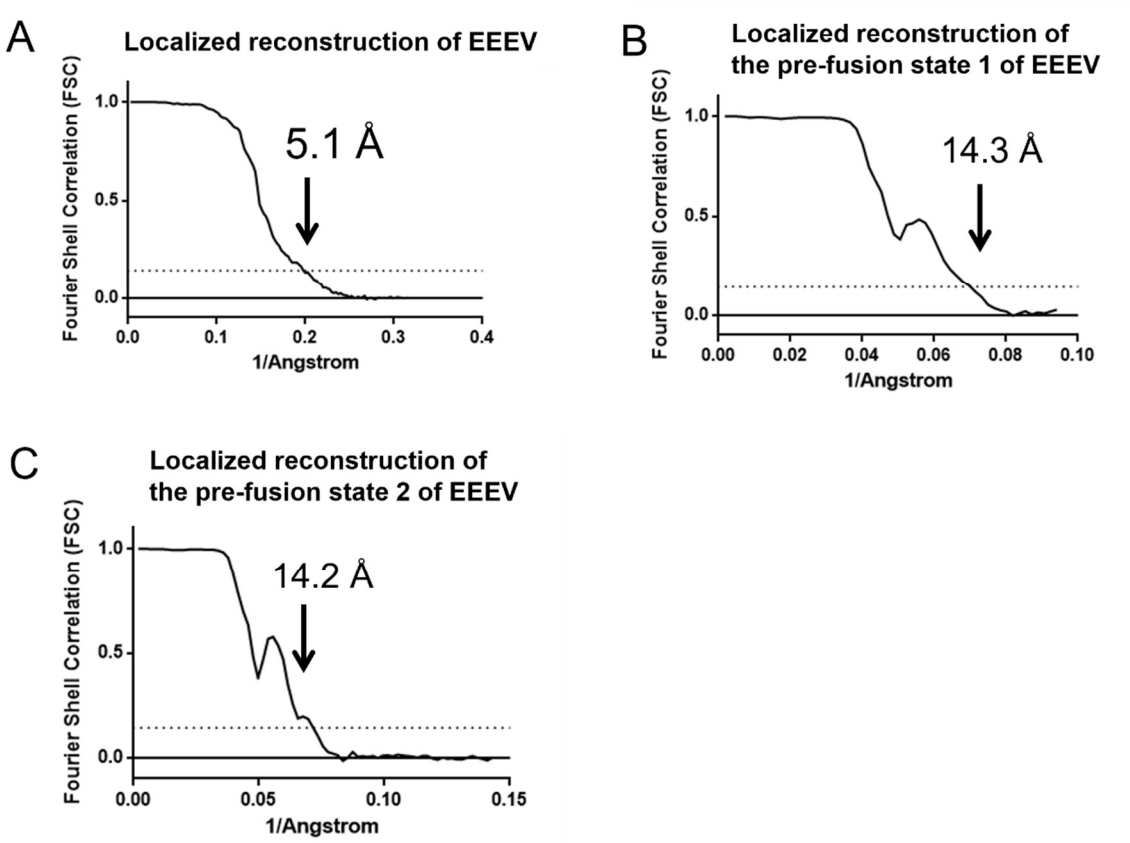

Figure S4. Fourier shell correlation plots of cryo-EM localized reconstructions of (A) native, **(B)** pre-fusion state 1, and **(C)** pre-fusion state 2 of EEEV**.** The FSC values of two half maps in each of the reconstructions were obtained using the EMAN2 software (e2proc3d.py). Dashed lines correspond to a FSC value of 0.143 and the resolution for each reconstruction is marked by an arrow.

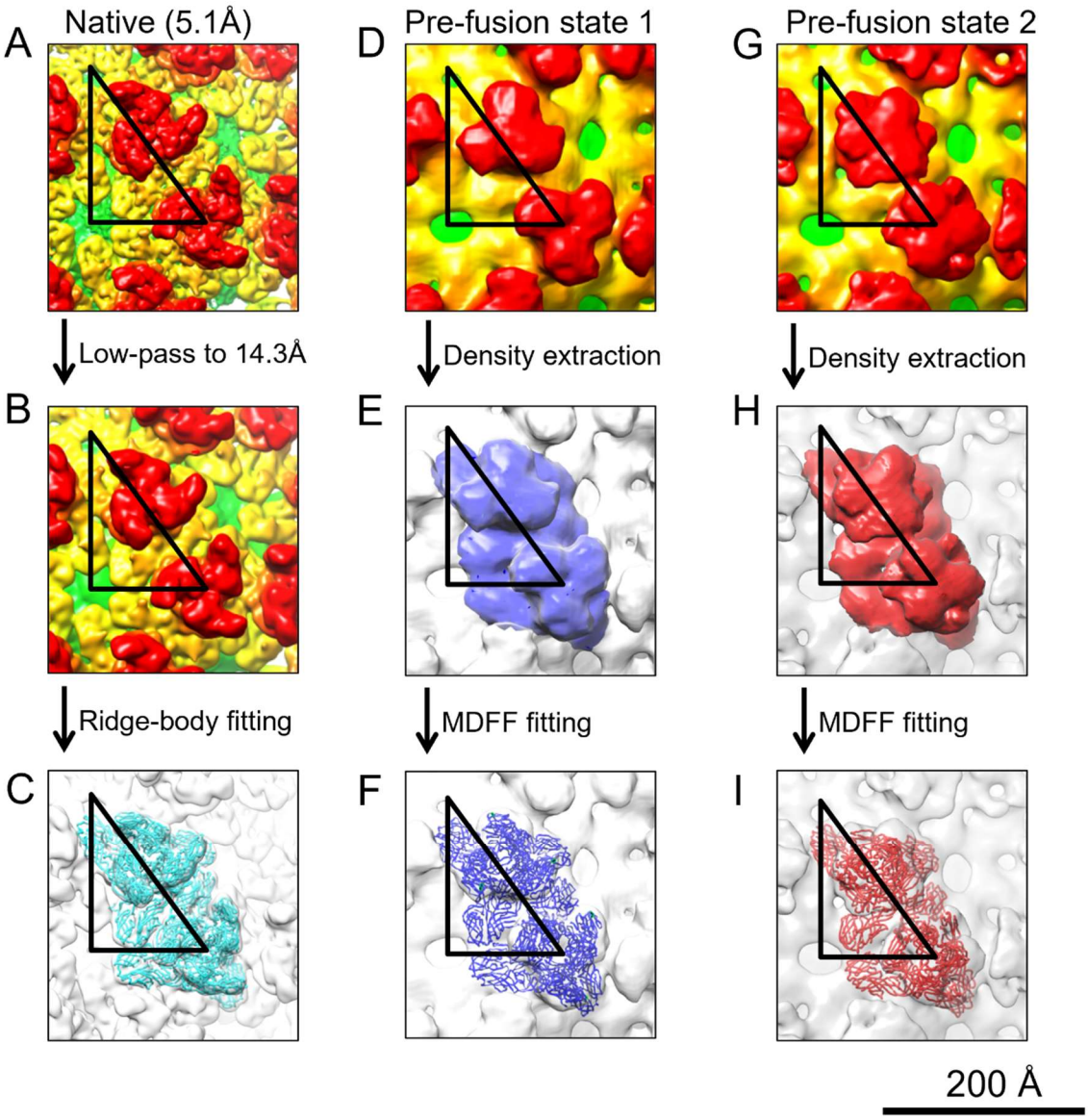

Figure S5. Fitting E1-E2 glycoproteins into the native and two low pH EEEV cryo-EM reconstructions. (A) Localized-reconstruction map of native EEEV. **(B)** Low-pass filtered map at a resolution of ~14.3 Å derived from **(A). (C)** Superposition of the rigid-body fitted E1-E2 model (cyan) with the native EEEV map (white) from **(B)**. **(D)** Localized-reconstruction map of pre-fusion state 1 of EEEV. **(E)** Superposition of the q3-i3 segmented map (blue) with the cryo-EM map (white) from **(D). (F)** Superposition of the MDFF fitted E1-E2 model with the cryo-EM map (white) from **(D). (G)** Localized-reconstruction map of pre-fusion state 2 of EEEV **(H)** Superposition of the q3-i3 segmented map with the cryo-EM (white) map from **(G). (I)** Superposition of the MDFF fitted E1-E2 model with the cryo-EM map (white) from **(G).**

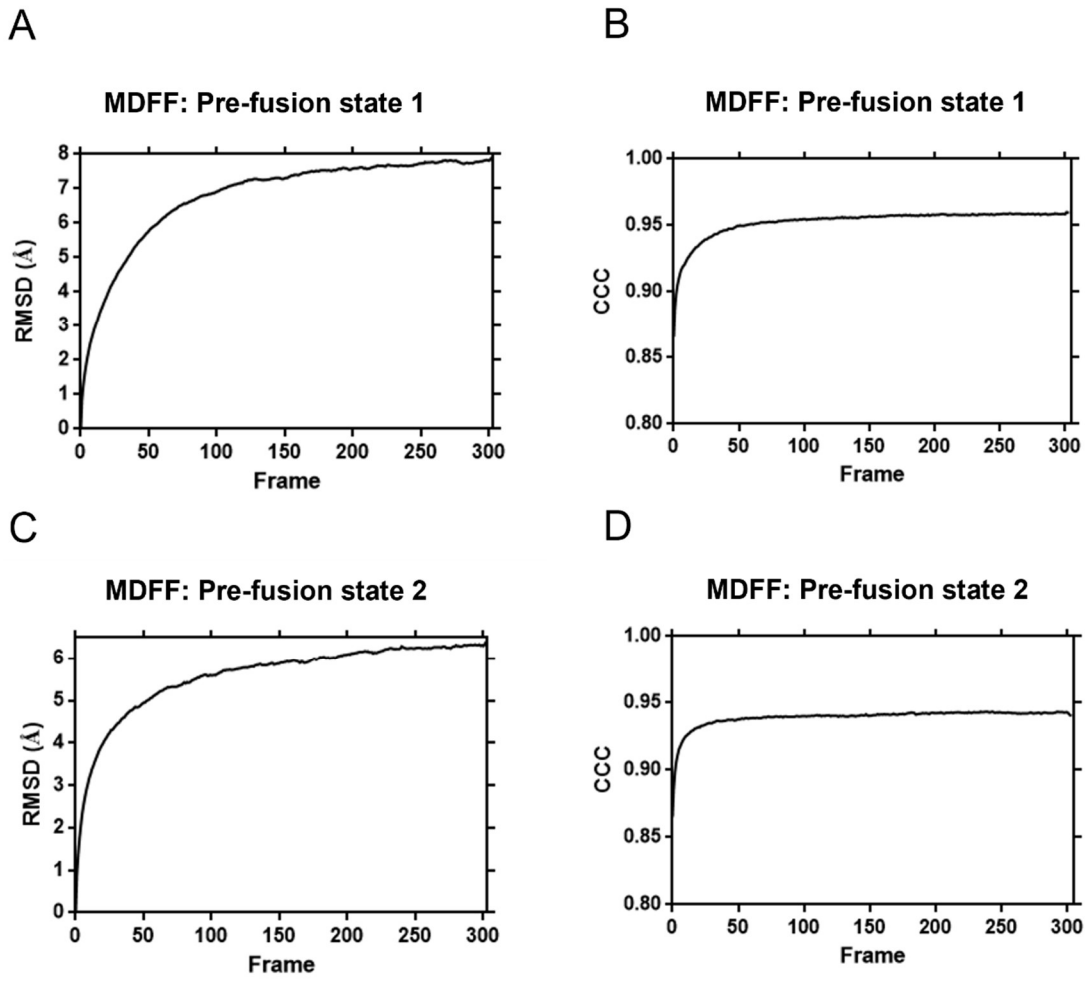

Figure S6. Root-mean-square deviation (RMSD) and cross-correlation coefficient (CCC) plots from molecular dynamics flexible fitting (MDFF). (A) and **(B)** RMSD and CCC plots for evaluating the convergence of model fitting into the segmented cryo-EM map of pre-fusion state 1 of EEEV. **(C)** and **(D)** RMSD and CCC plots for evaluating the convergence of model fitting into the segmented cryo-EM map of pre-fusion state 2 of EEEV.

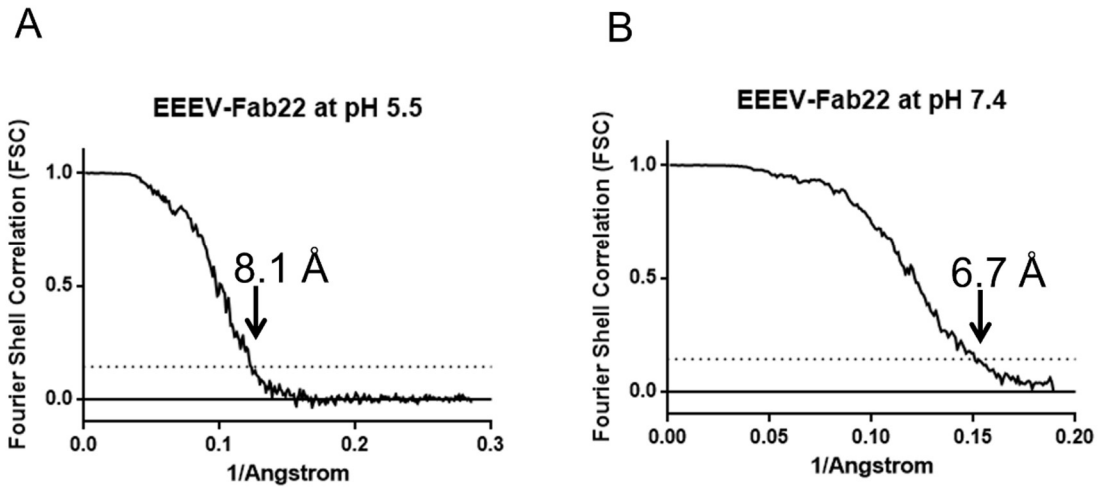

Figure S7. Fourier shell correlation plots of the cryo-EM reconstructions of Fab22 bound to EEEV at pH 5.5 and pH 7.4. (A) FSC plot of the two half cryo-EM reconstructed maps of EEEV-Fab22 at pH 5.5. **(B)** FSC plot of the two half cryo-EM reconstructed maps of EEEV-Fab22 at neutral pH. Dashed lines in (**A**) and (**B**) indicate the FSC coefficient value of 0.143.

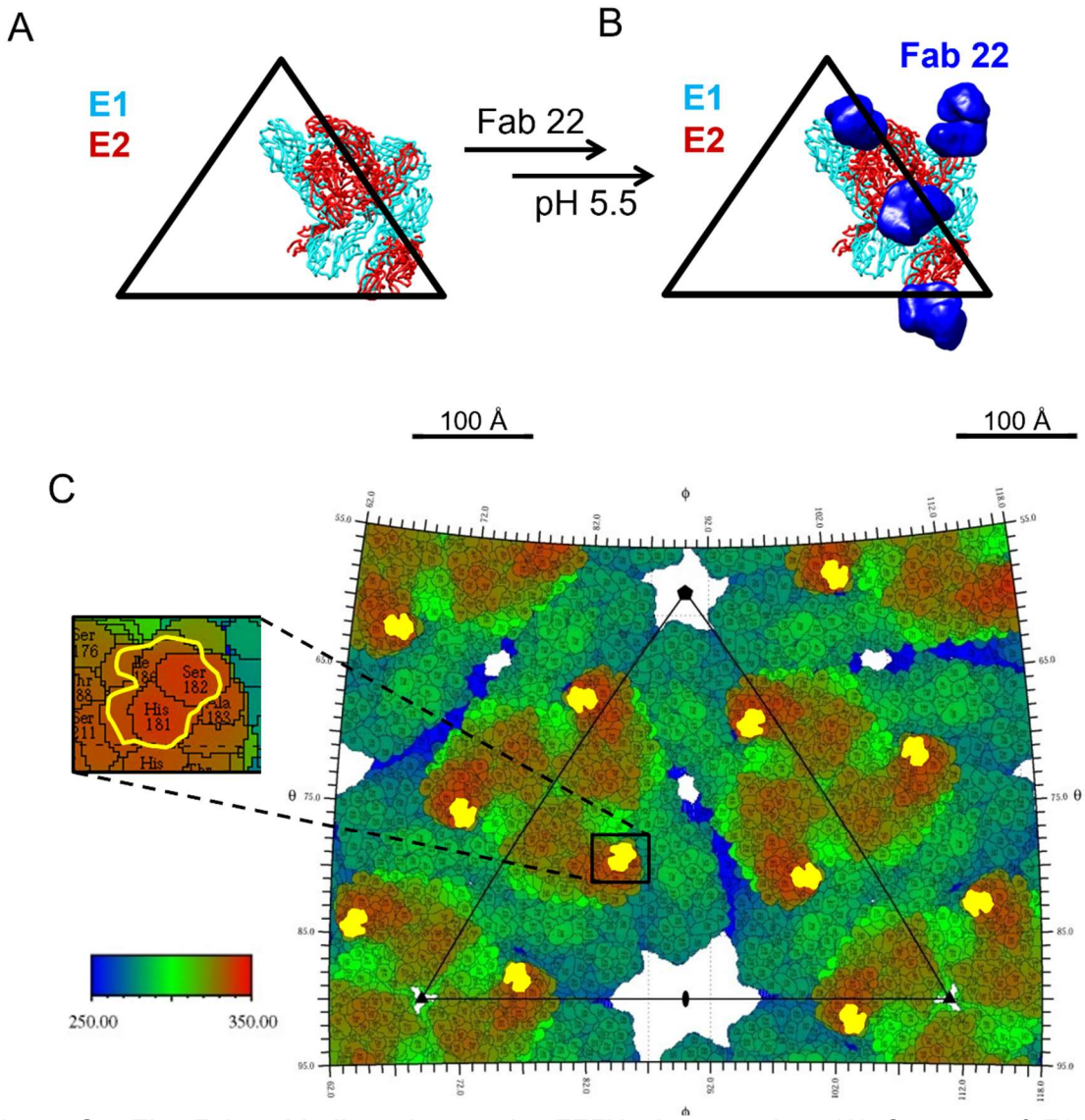

Figure S8. The Fab22 binding site on the EEEV glycoproteins. (A) Structure of E1-E2 glycoproteins of EEEV. E1 (cyan) and E2 (red) glycoproteins were rigid-body fitted into the low pH EEEV-Fab22 cryo-EM map**. (B)** Superimposition of Fab22 densities (blue) with the E1-E2 glycoproteins. **(C)** Roadmap of the EEEV glycoproteins. The EEEV-22 epitope containing I180, H181 and S182 on the E2-B domain is indicated in yellow.

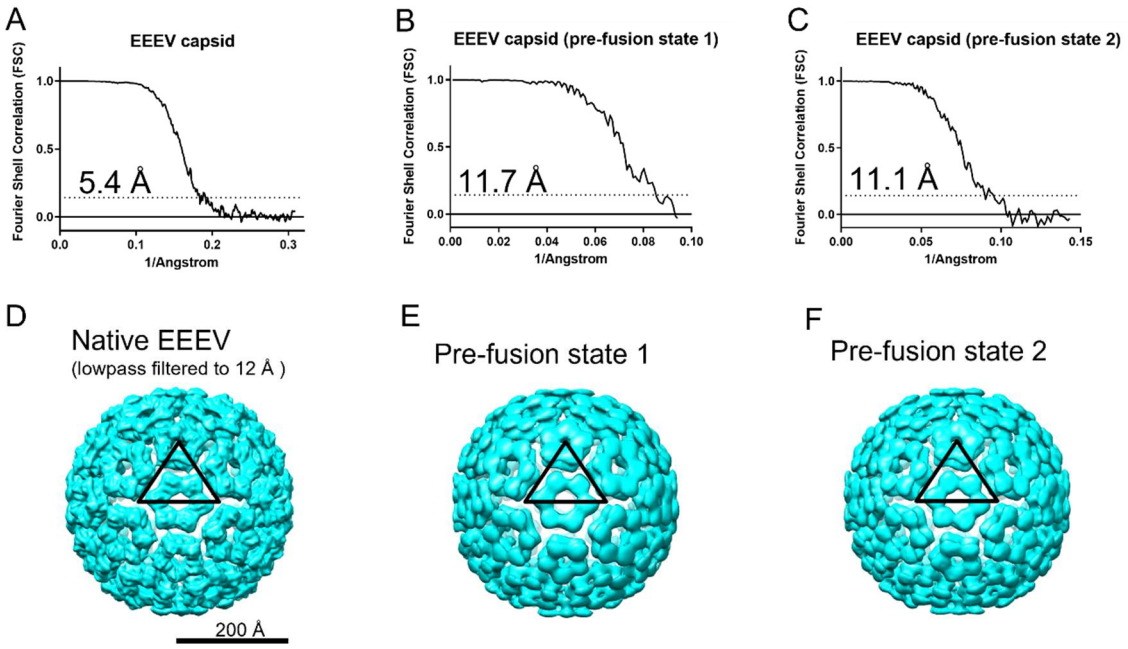

Figure S9. EEEV capsid cores in the native and two pre-fusion states of EEEV. (A) Resolution estimation of the capsid core in native EEEV. **(B)** Resolution estimation of the capsid core in pre-fusion state 1 of EEEV. **(C)** Resolution estimation of the capsid core in pre-fusion state 2 of EEEV. In **(A)**, **(B)** and **(C)**, the capsid densities from the two half maps were used to calculate FSC and resolution was estimated based on the Fourier shell correlation cutoff of 0.143 (dashed lines). **(D)** Lowpass filtered (12 Å) capsid core structure of native EEEV derived from the structure in panel **(A)**. **(E)** Capsid core structure of pre-fusion state 1 of EEEV. **(F)** Capsid core structure of pre-fusion state 2 of EEEV.

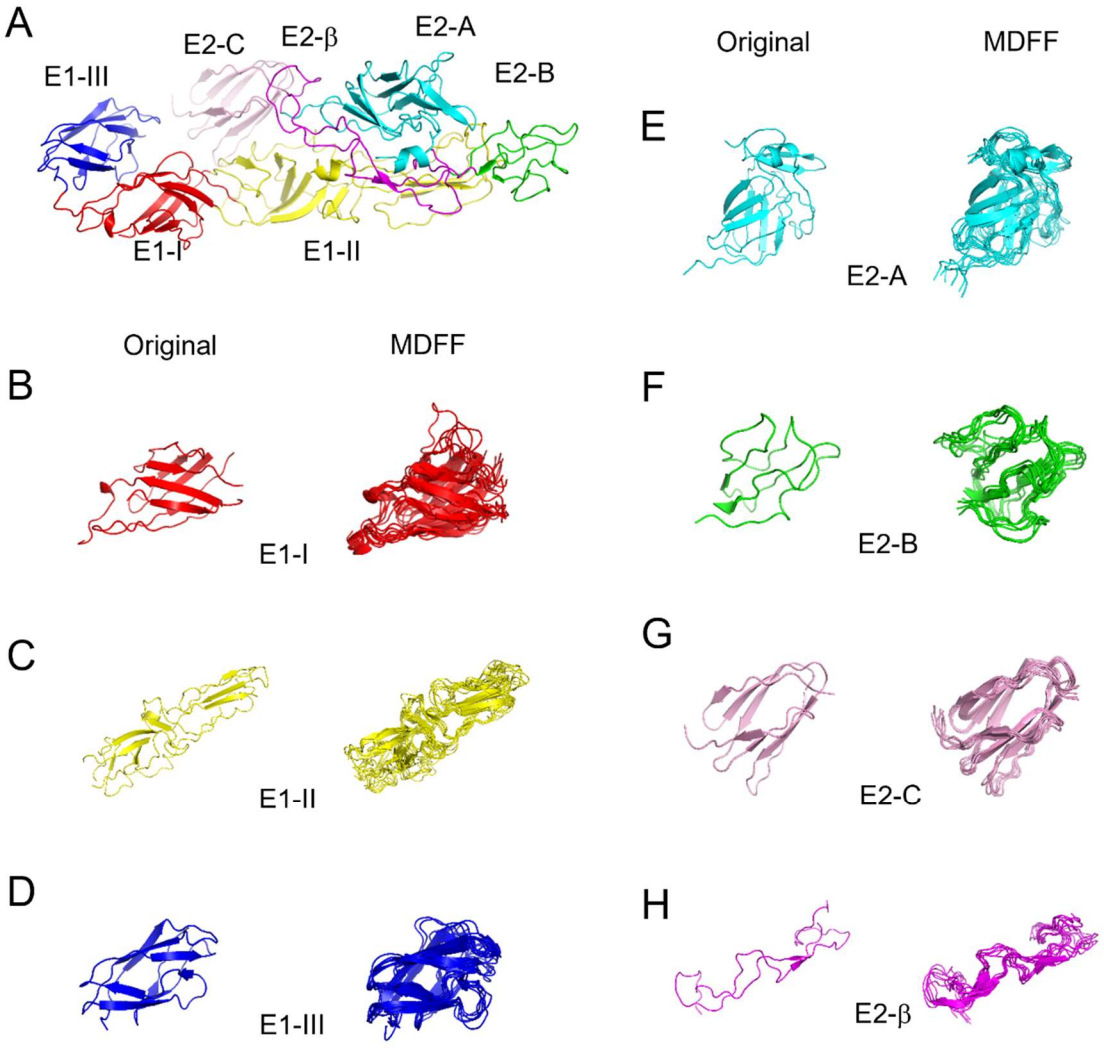

Figure S10. Domain alignments of E1-E2 glycoproteins in pre-fusion state 1 of EEEV to those in native EEEV (6MX4). (A) The cartoon diagram of E1-E2 glycoproteins from native EEEV (6MX4). Each of the domains was colored and labeled, as also shown in **Fig 3A**. **(B)-(H)** Domain alignment. The left panel in **(B)-(H)** shows the domain from native EEEV. The right panel **in (B)-(H)** shows the domain structures from the six E1E2 glycoproteins (q3 and i3 spikes) in pre-fusion state 1 of EEEV. The domain structures in each of the right panels were aligned to the structures in each of the left panels in **(B)-(H)**.

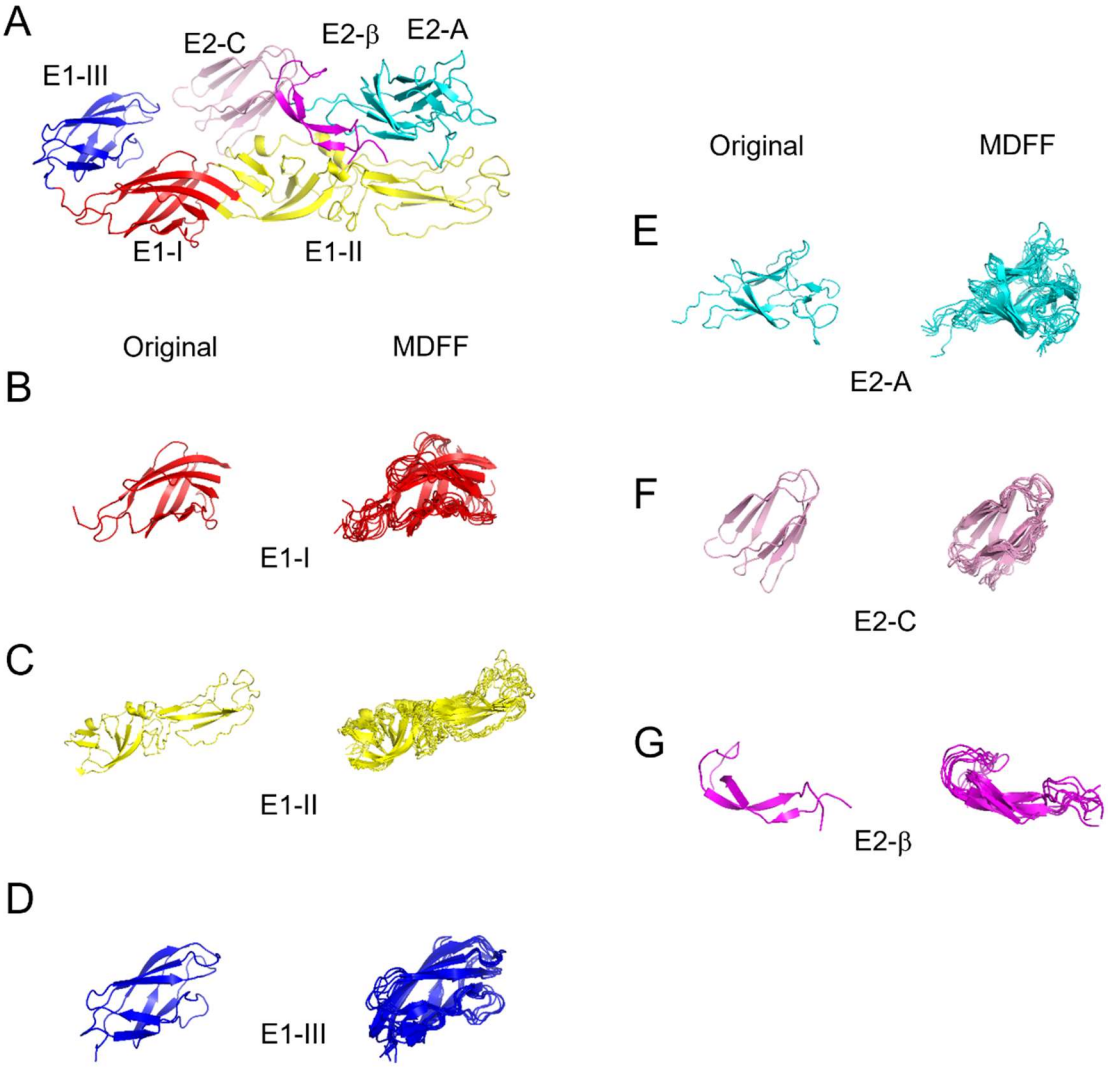

Figure S11. Domain alignments of E1-E2 glycoproteins in pre-fusion state 2 of EEEV to those from a simulated EEEV structure. (A) The cartoon diagram of the E1-E2 glycoproteins simulated from the crystal structure of SINV E1 and E2 glycoproteins at low pH (3MUU). Each of the domains was colored and labeled, as also shown in **Fig 3A.** The simulated EEEV structure does not contain E2-B and parts of E2-β, which were not shown in the low pH SINV E1-E2 crystal structure (3MUU). **(B)-(G)** Domain alignment. The left panel in **(B)-(G)** shows the domain from the simulated EEEV structure. The right panel **in (B)-(G)** shows the domain structures from the six E1E2 glycoproteins (q3 and i3 spikes) in pre-fusion state 2 of EEEV. The domain structures in each of the right panels were aligned to the structures in each of the left panels in **(B)-(G)**.

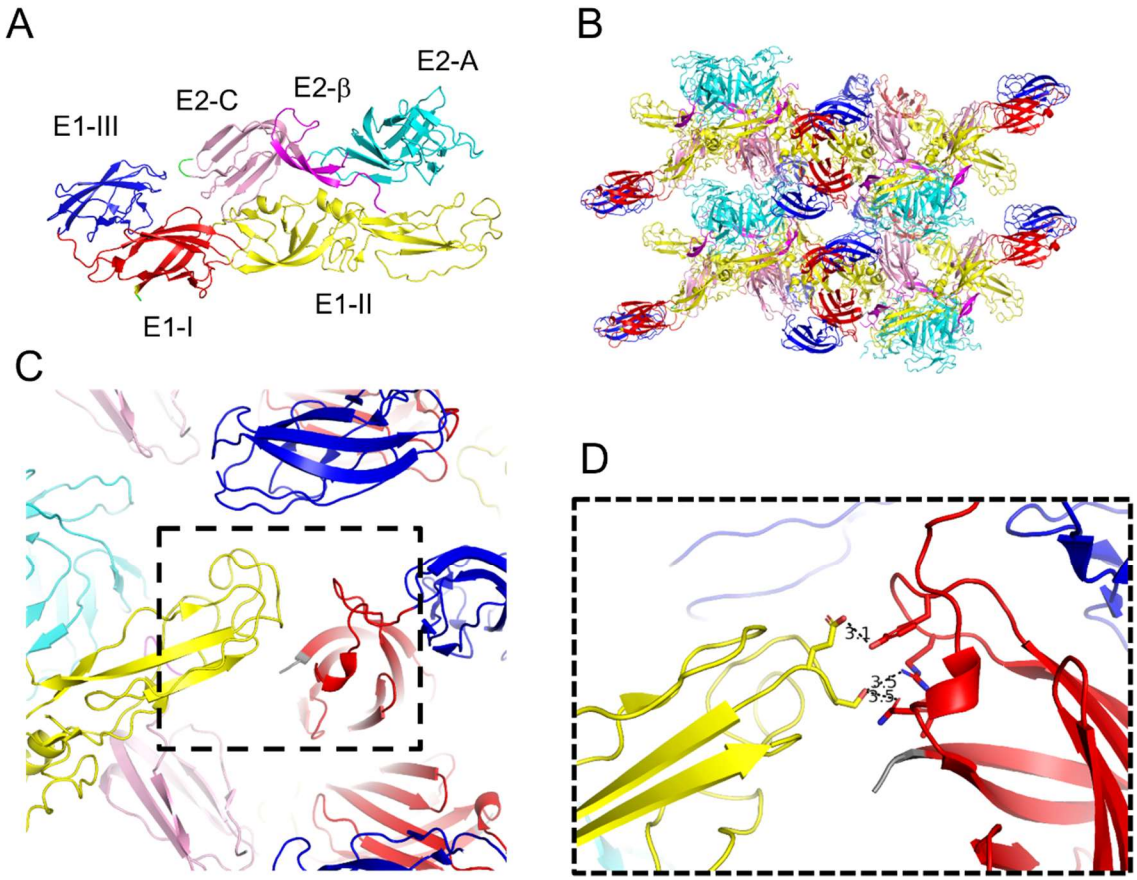

Figure S12. Crystal packing of Sindbis virus (SINV) E1E2 heterodimers (PDB:3MUU). (A) SINV E1E2 heterodimer. The domains of E1 and E2 are indicated and colored accordingly. **(B)** E1E2 heterodimers packed in two adjacent unit cells. Each unit cell has six copies of E1E2 heterodimers. **(C)** The E1-II domain (yellow) is packed and surrounded by neighboring E1-I (red) and E1-III (blue) from different heterodimers. **(D)** Polar contacts between E1-II (yellow) and the adjacent E1-I domain (red).

Table S1. Rotation of E2-B with the rigid-fit method

*Rigid-fit (individual E1E2)	Rotation angle (°)
i3-1	8.29
i3-2	6.09
i3-3	8.41
q3-1	8.32
q3-2	6.20
q3-3	11.55
mean ±SD (q3+i3)	8.14 ± 1.99

*Individual E1E2 molecules were fitted into the pre-fusion state 1 cryo-EM map

Table S2. Model Fitting information

	Pre-fusion state 1	Pre-fusion state 2
Rigid-fit (q3 & i3 spikes)		
Map-model correlation[1]	0.5354	0.9064
Clash score[2]	8	16
Rigid-fit (individual E1E2)		
Map-model correlation[1]	0.5869	0.9033
Clash score[2]	18	61
MDFF		
Map-model correlation[1]	0.791	0.9688
Clash score[2]	4	3

[1] Map-model correlation was calculated using "Fit in map" plugin in Chimera. [2] Clash score was estimated using the validation tool in the wwPDB server.

Table S3. Structural alignment of E1 and E2 domains*

RMSD ± SD (Å)		**Pre-fusion state 1**	**Pre-fusion state 2**
E1			
	E1-I	2.46 ± 0.42	1.52 ± 0.12
	E1-II	3.02 ± 0.51	2.10 ± 0.44
	E1-III	1.81 ± 0.54	1.63 ± 0.21
E2			
	E2-A	2.12 ± 0.44	2.86 ± 0.55
	E2-B	2.37 ± 0.56	----
	E2-C	1.25 ± 0.13	1.56 ± 0.18
	E2-b	2.88 ± 0.39	2.55 ± 0.52

*After fitting the structure into the localize-reconstructed maps (Fig 2E and 2F, Supplementary Fig 4) using MDFF, each of the domain structures were extracted from the E1-E2 glycoproteins in q3 and i3 spikes and aligned to the reference structures using all atoms.

Table S4. Distance between EEEV E2 and CHIKV E3 domains

	E3 (CHIKV) coordinate (x,y,z)	Distance of E3--E2 (native EEEV) (Å)	Distance of E3--E2 (Prefusion state 1 of EEEV) (Å)	Distance of E3--E2 (Prefusion state 2 of EEEV) (Å)	Distance of native EEEV E2 and Prefusion state 1 of EEEV E2 (Å)	Distance of native EEEV E2 and Prefusion state 2 of EEEV E2 (Å)
i3-1	(81.29, 24.11, 307.47)	28.5	18.2	20.0	13.6	42.4
i3-2	(109.20, -41.82, 295.98)	28.5	17.7	12.9	14.9	37.6
i3-3	(148.35, 17.28, 280.72)	28.5	14.1	23.2	17.4	46.7
q3-1	(93.14, 70.41, 296.86)	28.5	12.6	17.9	18.6	44.7
q3-2	(58.14, 132.81, 285.00)	28.5	10.9	18.3	19.1	42.2
q3-3	(22.03, 75.22, 310.25)	28.5	9.7	13.1	22.0	39.3
Average		28.5	13.9	17.6	17.6	42.1

* The CHIKV structure containing E3 (PDB: 3N41) were aligned to q3 and i3 spikes of EEEV. Each center of mass of E3 at q3 and i3 spikes was obtained in chimera. The distances of E2 and E3 were calculated based on the coordinates of E2 in native EEEV and the pre-fusion states (1 and 2) of EEEV (Table 2).

Table S5 Estimation of E3 percentage overlap

	CHIKV E3 density volume	CHIKV E3 density volume overlapped with EEEV prefusion state 1	CHIKV E3 density volume overlapped with EEEV prefusion state 2
i3_1	8143	2108	1828
i3_2	8124	3252	2156
i3_3	8037	2853	1402
q3_1	8058	3148	2873
q3_2	8143	2950	805
q3_3	8086	3280	1504
average	8101	2862	1813
E3 percentage overlap (%)		35	22

* The CHIKV structure containing E3 (PDB: 3N41) were aligned to q3 and i3 spikes of EEEV and a simulated CHIKV density map at 14Å resolution was generated using the molmap command in chimera. The E3 density outside the EEEV prefusion state density were manually erased. Each of the density volume values was measured in chimera.