

Supplementary Information

Neutralizing antibodies against Chikungunya virus and structural elucidation of their mechanism of action

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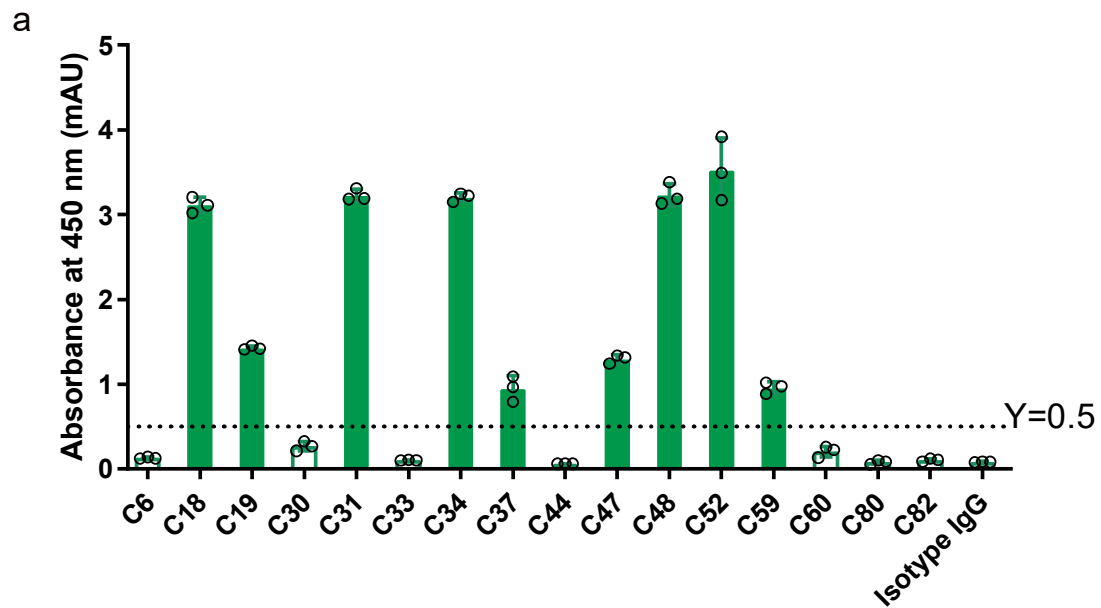
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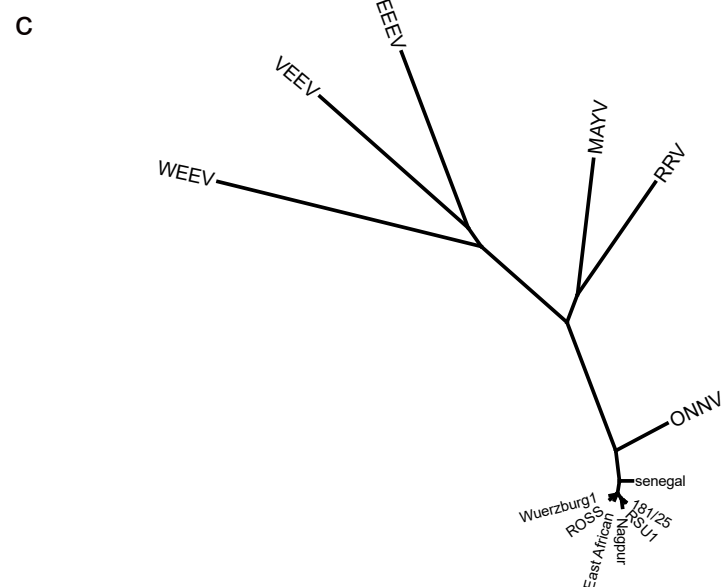
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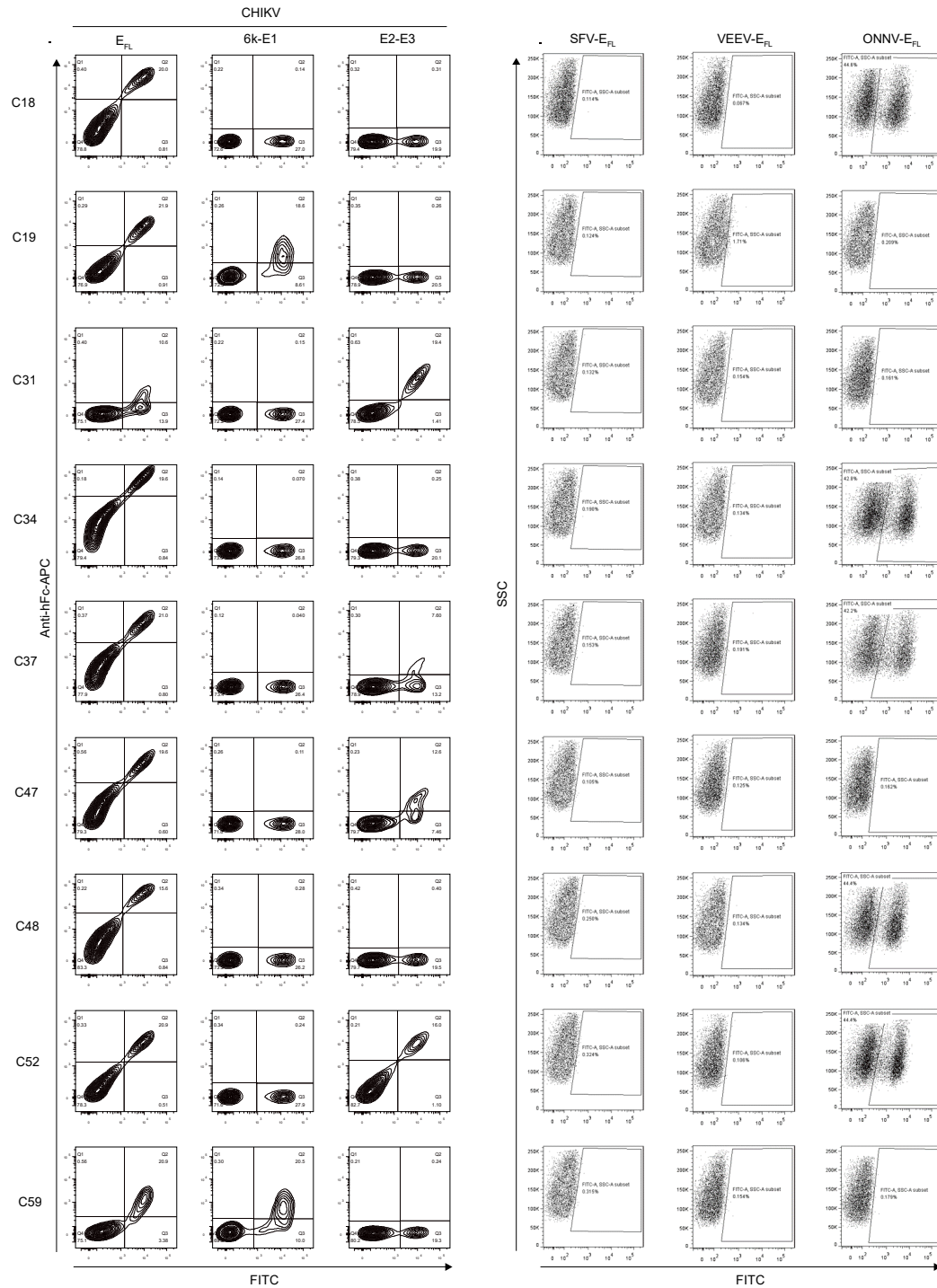
b

	Heavy Chain				Light Chain			SHM(%)
	V-H allele	D-H allele	J-H allele	CDR3-H(aa)	V-L allele	J-L allele	CDR3-L(aa)	
C18	3-23*04	6-25*01	5*02	CAKVTAAGWFDPW	4-1*01	5*01	CQYYSTPQITF	7,4
C19	44-4*02	3-22*01	4*02	CARAVGNYYDISGYFIDYW	1-5*03	1*01	CQYNTYRTF	5,3
C31	1-24*01	3-9*01	6*02	CATRYYDLLTGRIYYGM DVW	1-12*01	4*01	CQQANSFPLTF	4,5
C34	3-9*01	5-12*01	6*02	CAKDMGYSGNDDYYG MDVW	1-39*01	1*01	CQQSYRTLTRTF	0,5
C37	4-39*02	1-1*01	4*02	CASRQNWNDGYFDYW	3-11*01	5*01	CQQRGNWPPITF	6,3
C47	4-59*01	3-22*01	3*02	CARGDMVYYDSSTYAFDIW	2-28*01	2*01	CMQALQTPYAF	7,3
C48	3-48*03	3-10*01	4*02	CARRKGLGTFDYW	4-1*01	2*02	CQYYTTPCTF	3,4
C52	1-69*01	1-26*01	4*02	CARDIRAYSGRYSFPDYW	1-5*01	1*01	CQYNSYSRTF	7,3
C59	4-39*02	3-22*01	5*02	CAREVTMVVVAW	3-15*01	4*01	CQYDNLPLTF	2,4

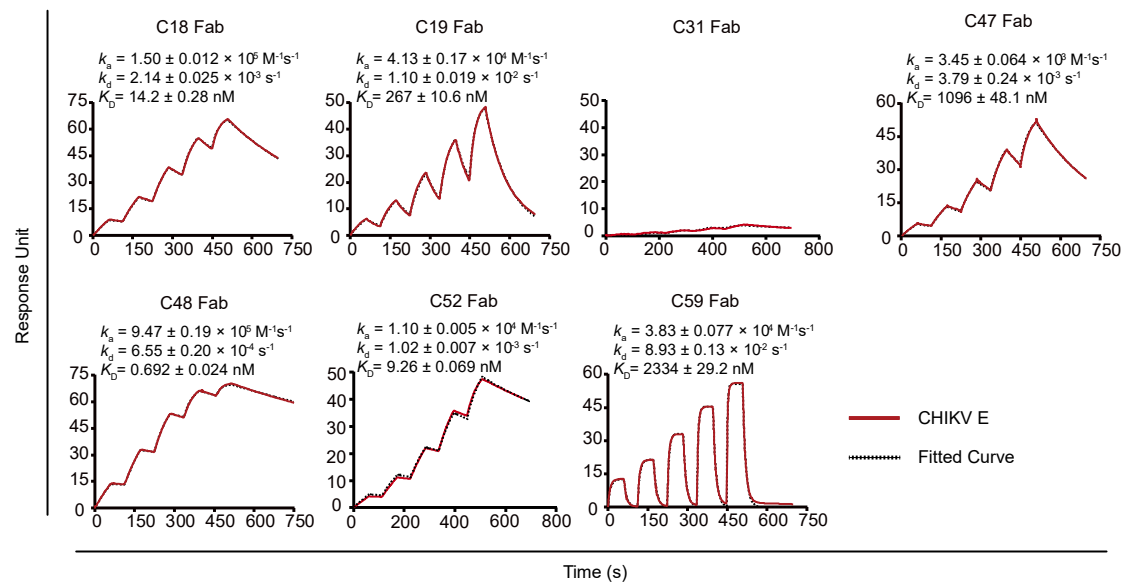


Supplementary Fig. 1 Characterization of isolated mAbs. (a) Binding of isolated

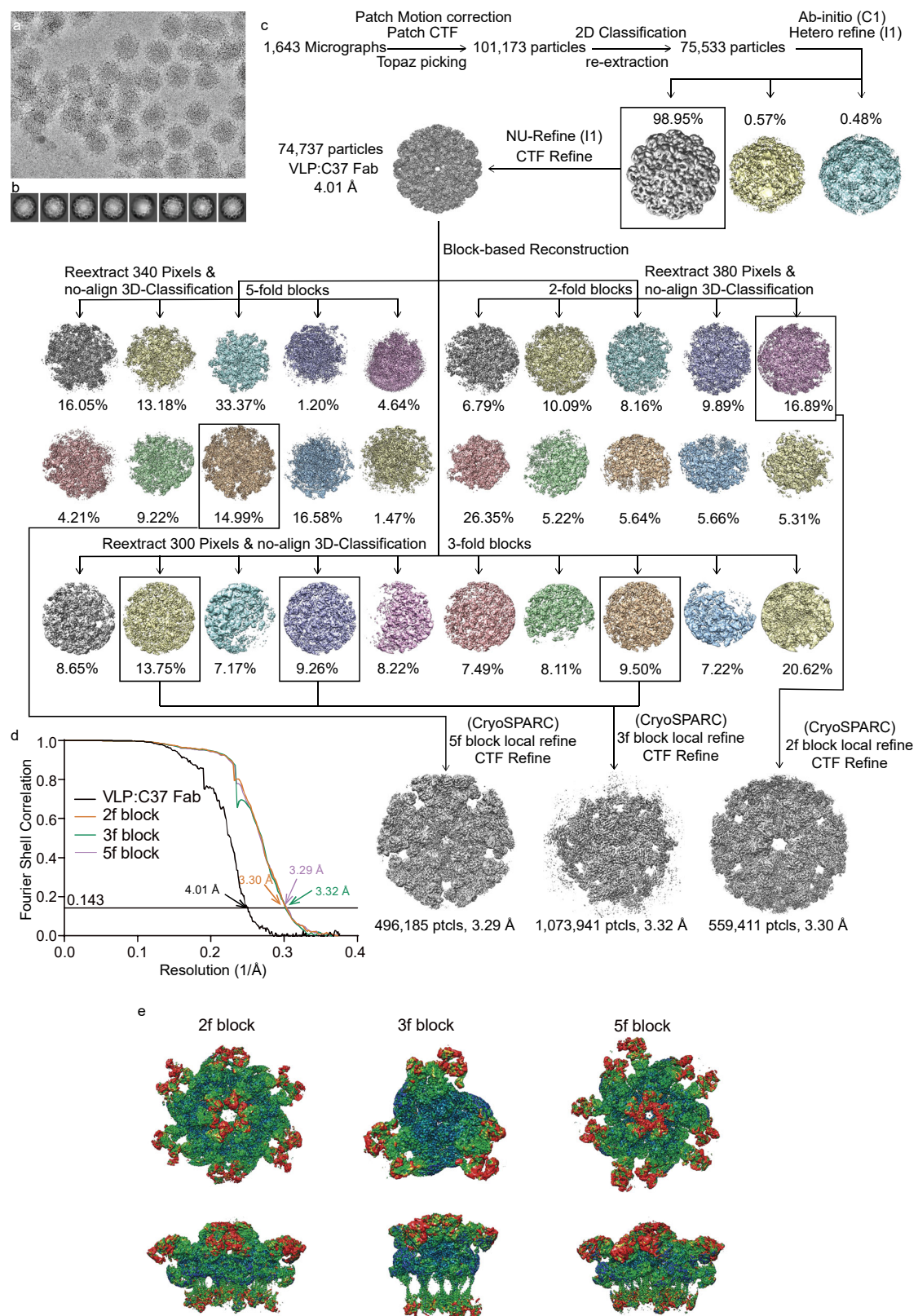
mAbs to the full-length E protein examined by ELISA. ELISA plates were coated with CHIKV E protein overnight. After blocking with 5% defatted milk, cell supernatants were added to the plates. mAbs with OD450 fluorescence higher than 2.1 times the Mock are represented as solid columns, while mAbs with OD450 fluorescence lower than 2.1 times the Mock are represented as outlined columns. Results are presented as mean $\pm$ s.d. from three independent experiments. (b) V-(D)-J rearrangement summary for the isolated mAbs. The top matches for *Homo sapiens* germline genes corresponding to the V, D, and J segments of the heavy chains and the V and J segments of the light chains are listed. Sequences of the CDR3 regions in the heavy and light chains are provided. The total numbers of somatic hypermutations (SHMs) in the heavy and light chains are also listed. (c) genetic relationship of CHIKV to other representative alphaviruses. The C-E3-E2-E1 amino acid sequences of ONNV (OQ238859.1), RRV (MK028844.2), MAYV (PP505832.1), VEEV (U34999.1), WEEV (PP747354.1), EEEV (MK028842.1), and various strains of CHIKV: Senegal (AY726732.1), Wuerzburg1 (EU037962.1), Nagpur (AY424803.1), EastAfrican (OQ567725.1), 181/25 (W473668.1), ROSS (AF490259.3), and RSU1 (HM045797.1) were aligned with ClustalOmega and a phylogenetic tree was produced with Geneious Prime.



Supplementary Fig. 2 Flow cytometry results for binding tests of sorted mAbs. Binding tests of sorted mAbs to full-length E protein, p62-E1, and E2 of CHIKV, as well as full-length E proteins of BFV, VEEV, and ONNV. This is related to Fig 1a.

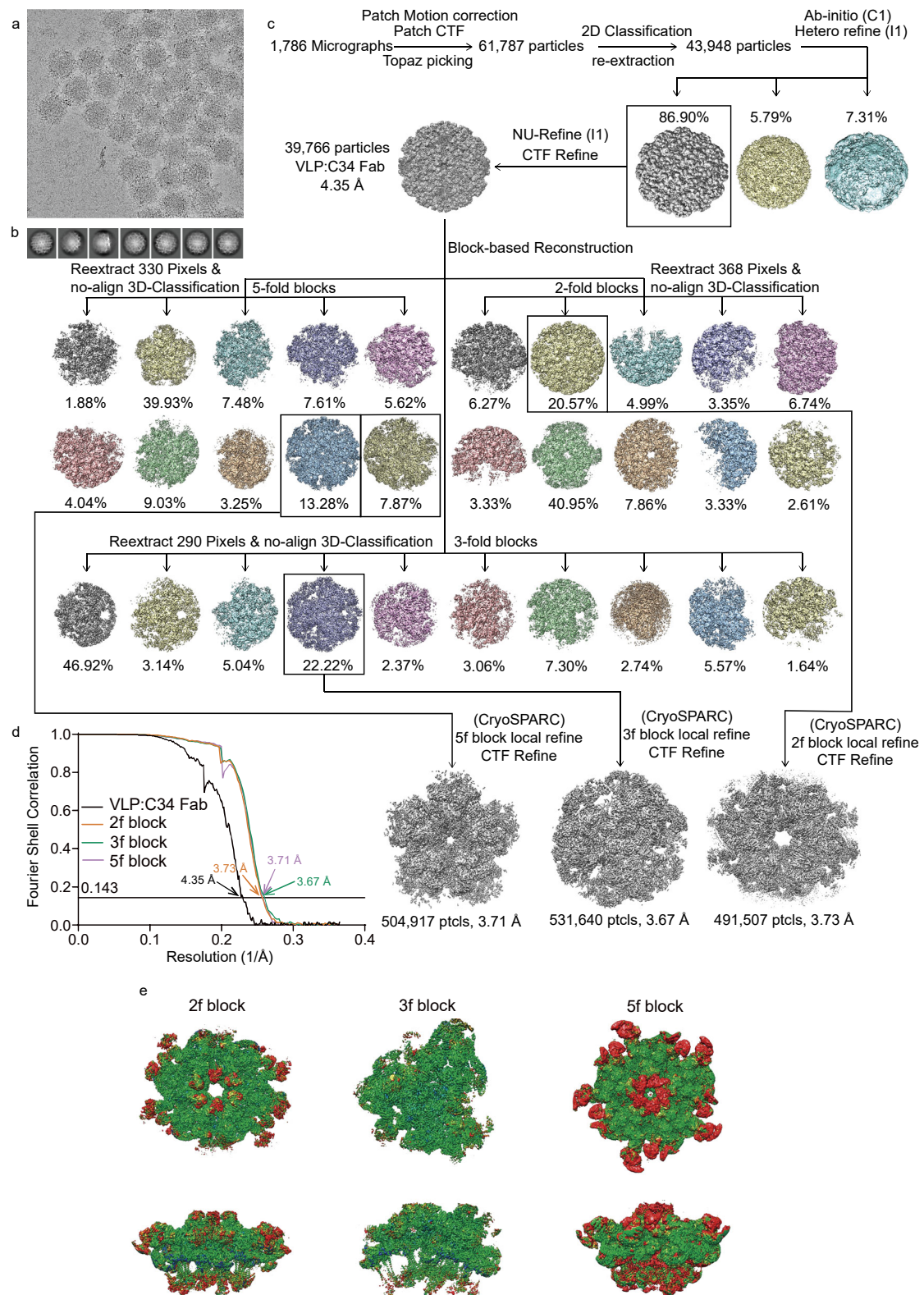


Supplementary Fig. 3 SPR assay characterizing the specific binding between indicated Fab forms of mAbs and full-length E protein. The black lines represent the fitting curves. The calculated KD values for each mAb binding to the E protein are listed above the curves. Data are representative of three independent experiments. This is related to Fig 1c.



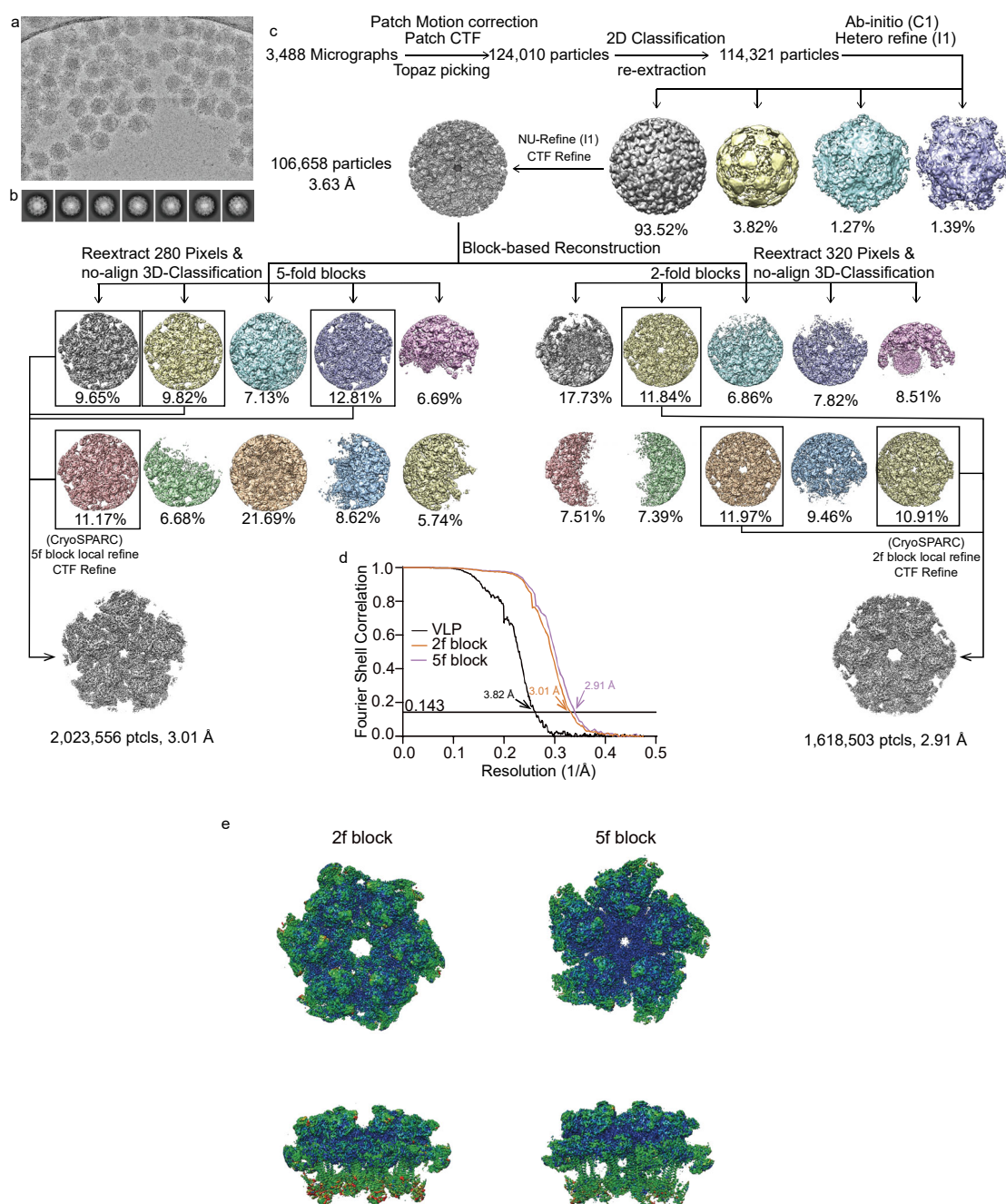
Supplementary Fig. 4 Reconstruction of VLP:C37 Fab complex structure. Related to Fig 3. (a) A representative cryo-EM micrograph showing particles of the complex. (b) averaged images of selected classes of particles from two-dimensional classification. (c) Workflow of the cryo-EM data processing and reconstruction procedure. (d) Gold standard FSCs of the reconstructions calculated with the 5f, 3f and 2f blocks,

respectively. (e) local resolution maps of the 2f, 3f and 5f blocks.

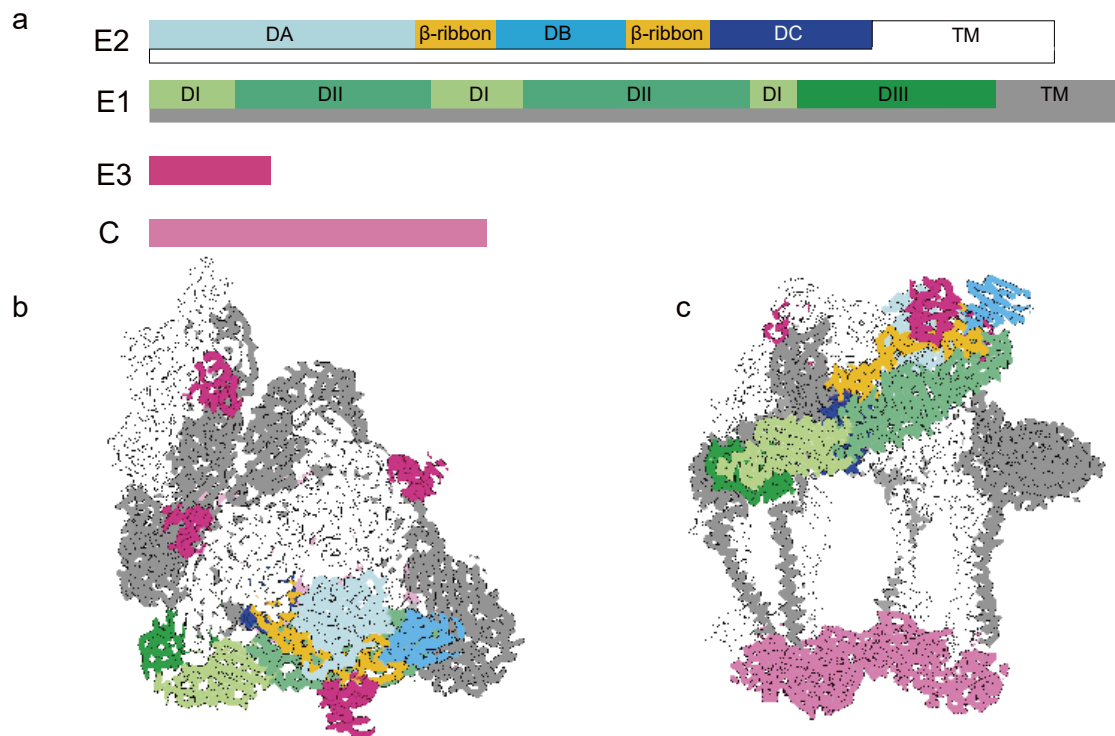


Supplementary Fig. 5 Reconstruction of VLP:C34 Fab complex structure. Related to Fig 3. (a) A representative cryo-EM micrograph showing particles of the complex. (b) averaged images of selected classes of particles from two-dimensional classification.

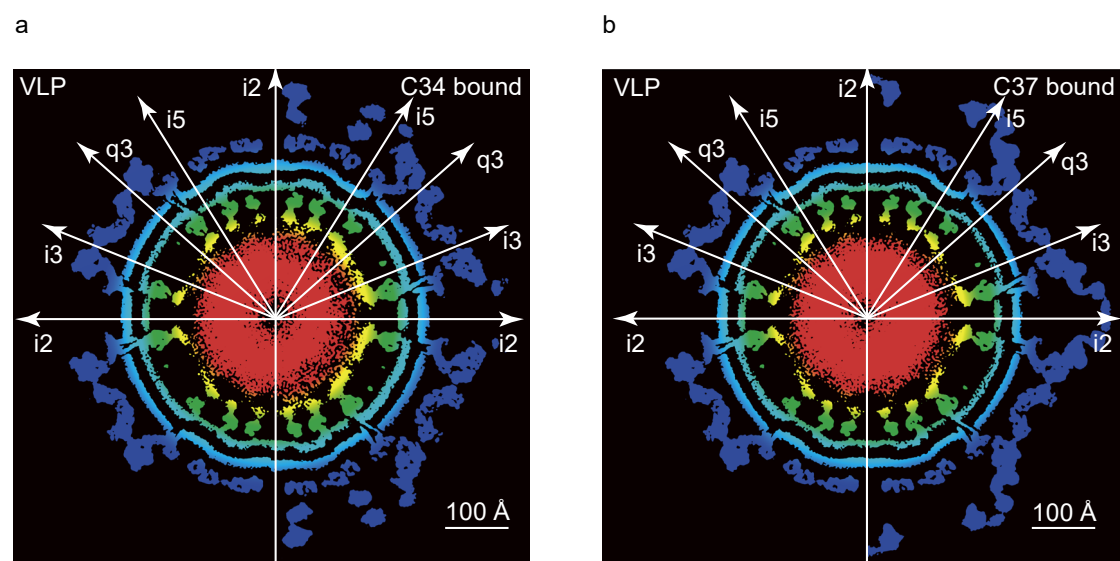
(c) Workflow of the cryo-EM data processing and reconstruction procedure. (d) Gold standard FSCs of the reconstructions calculated with the 5f, 3f and 2f blocks, respectively. (e) local resolution maps of the 2f, 3f and 5f blocks.



Supplementary Fig. 6 Reconstruction of VLP structure. Related to Fig 3. (a) A representative cryo-EM micrograph showing particles of the complex. (b) averaged images of selected classes of particles from two-dimensional classification. (c) Workflow of the cryo-EM data processing and reconstruction procedure. (d) local resolution maps of the 2f and 5f blocks.

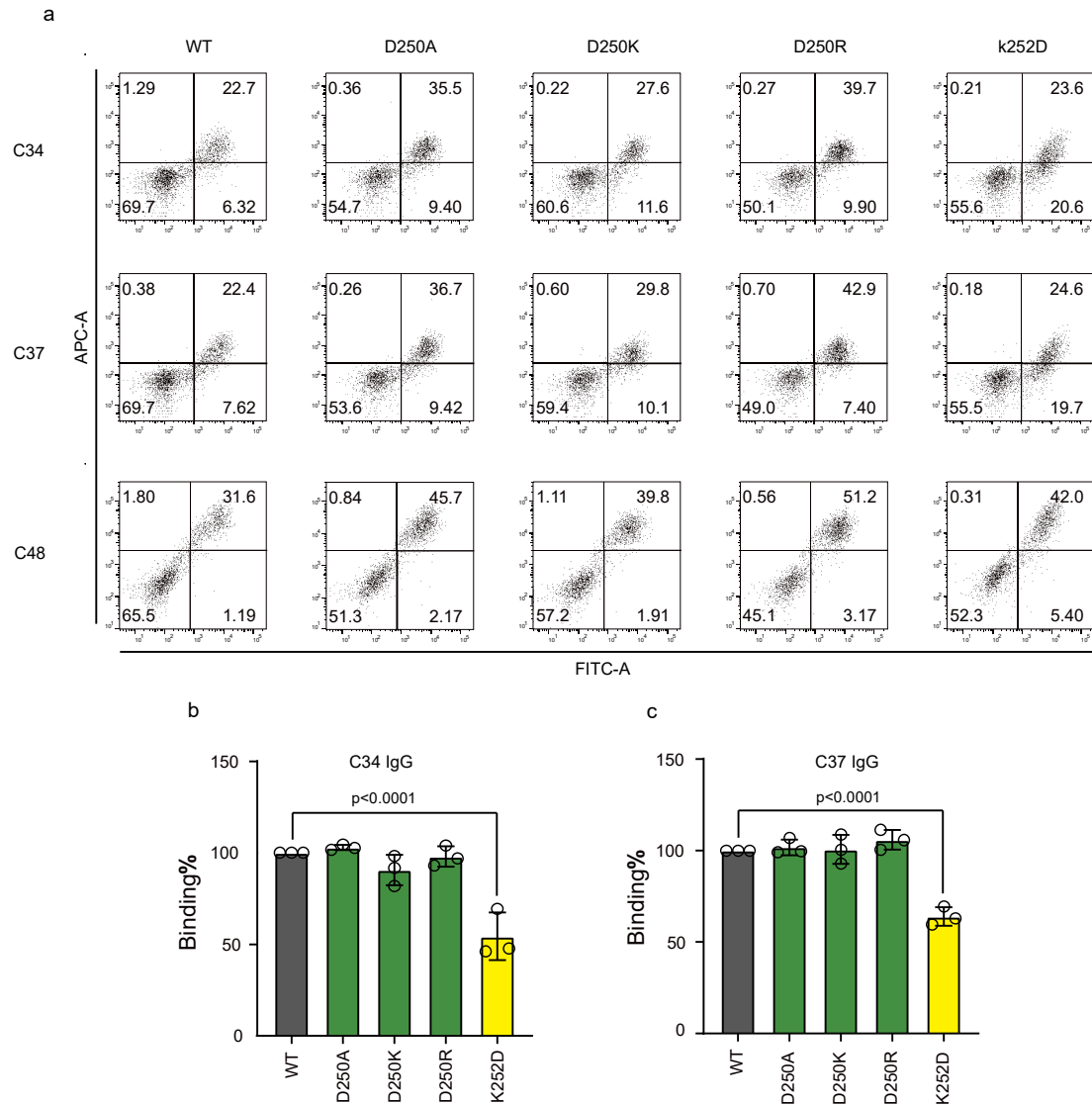


Supplementary Fig. 7 Structural arrangement of CHIKV spike. (A) Domain architecture of proteins consisting the CHIKV spike. (B) and (C) Surface representation of CHIKV spike protein colored according to domains in (A). The density of an asymmetric unit covering four E1-E2-E3-C complex of infectious CHIKV virion is shown. The density is postprocessed with EMReady 2.1.

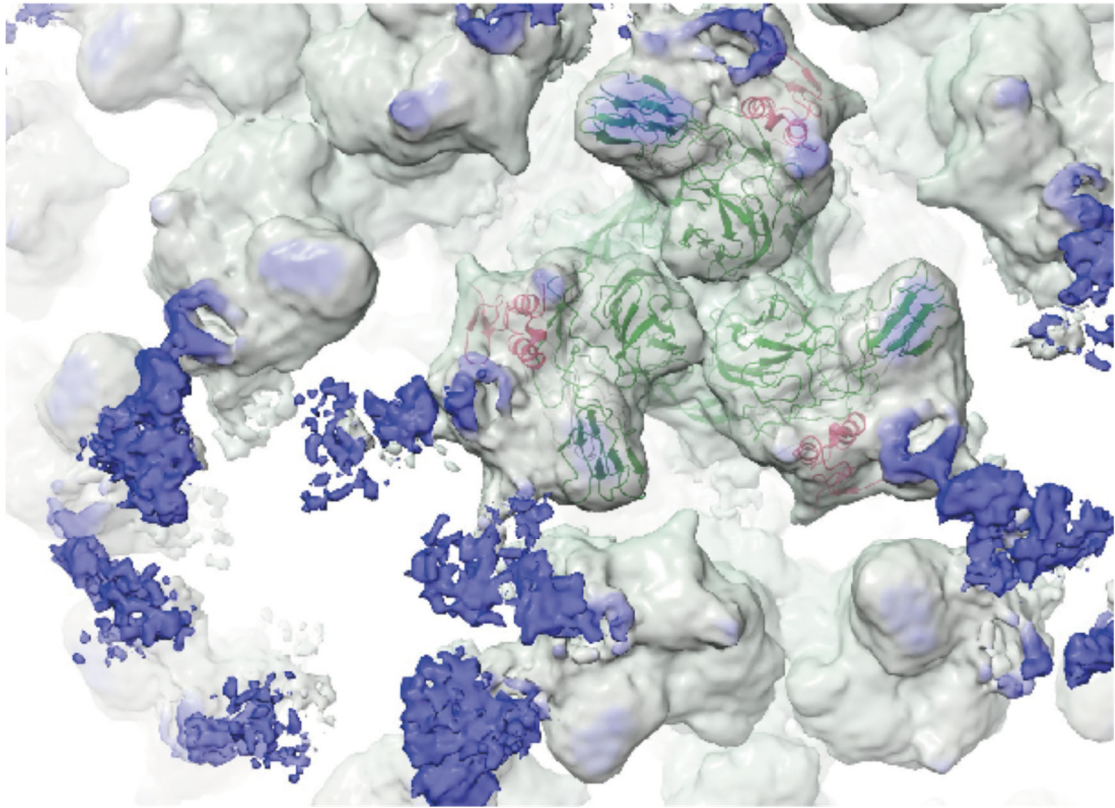


Supplementary Fig. 8 Diameter of VLP remain unchanged upon C34 or C37 binding. Two-dimensional equatorial slice of CHIKV VLP before (left) and after (right) C34 (A) or C37 (B)

binding. The overall diameters of VLP and distribution of spike proteins remain unchanged upon antibody bound. The icosahedral symmetry axes are plotted by white arrows.

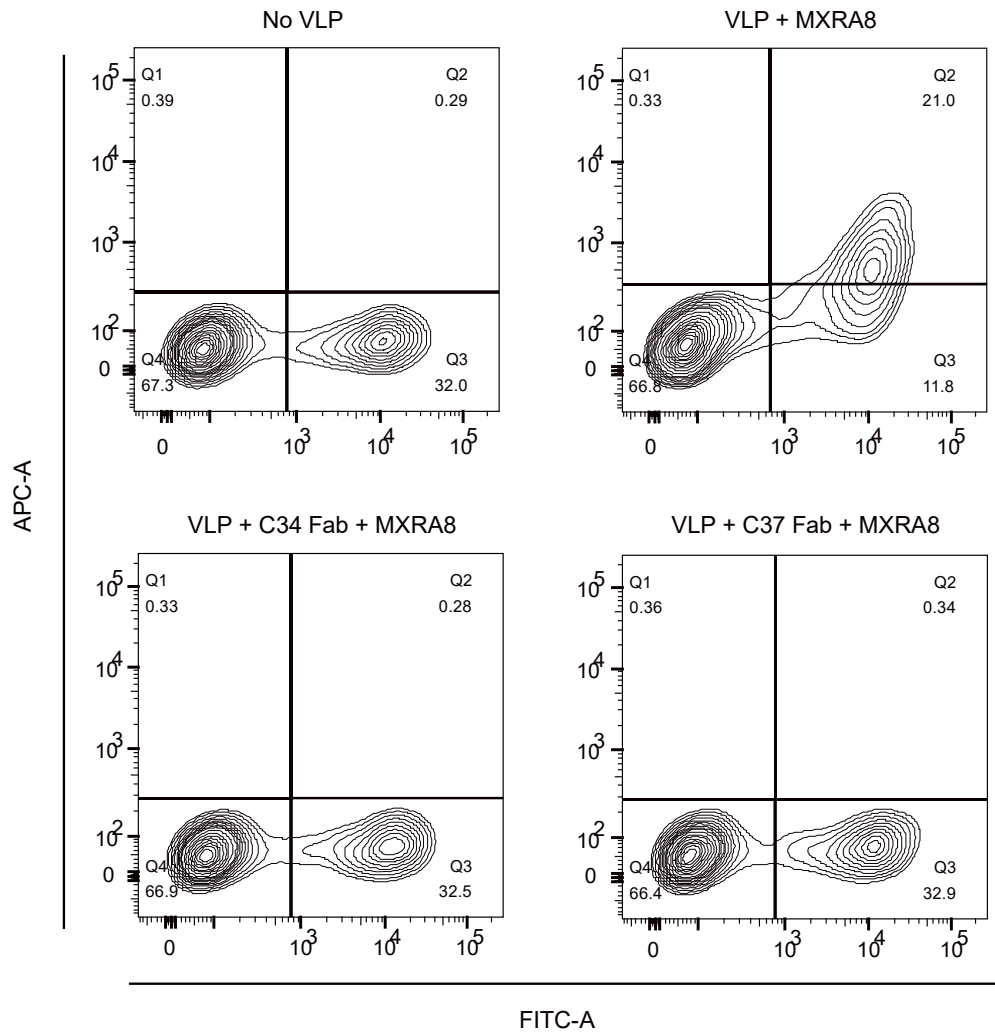


Supplementary Fig. 9 Mutagenesis analysis of the binding epitopes. (a) mutations introduced into CHIKV E2 ectodomain, and binding by each antibody was determined by flow cytometry. (b) Relative binding change to C34 IgG of indicated mutations were plotted. (c) Relative binding change to C37 IgG of indicated mutations were plotted. Each graph shows mean plus SD from three independent experiments.

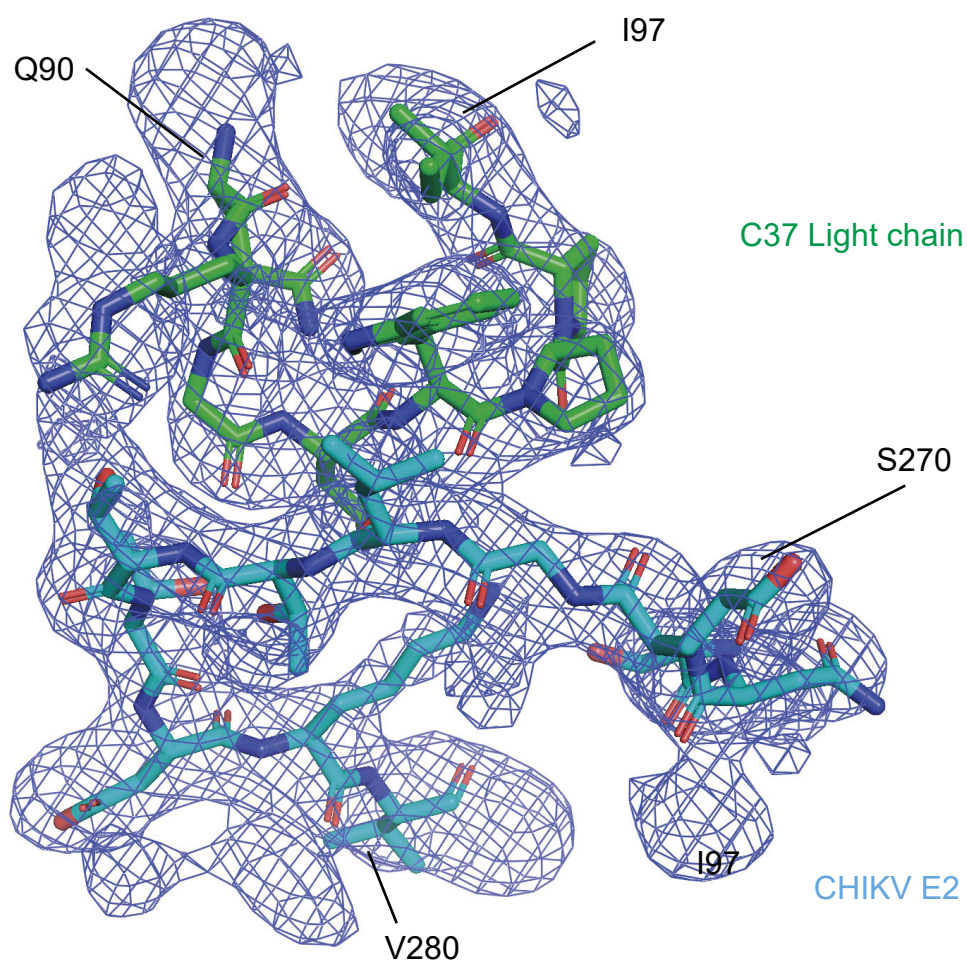


Supplementary Fig. 10 Density of C34 Fab around each spike. Related to Fig 4.

Cryo-EM structure of the infectious virus particle incubated with the C34 antibody. The reconstructed density map shows clear densities for E3 (red) and E2 (green). The C34 Fab density (dark blue) appears discontinuous and exhibits low occupancy.



Supplementary Fig. 11. C34 and C37 block binding of Mxra8 to VLP confirmed by flow cytometry. Flow cytometry analysis of VLPs incubated with C34 or C37 antibodies, followed by staining of HEK293T cells transiently transfected with Mxra8-GFP expression plasmids. Cells were subsequently stained with APC-labeled anti-human IgG-Fc. The figures show the corresponding flow cytometry results for the staining protocol.



Supplementary Fig. 12 Crystal structure of C37-CHIKV E complex. Fo-Fc simulated annealing omit electron density map for interacting surface between CHIKV E2 and C37 light chain. The map is countered at 1.5σ and superimposed with the structure model in stick representation. C37 and CHIKV E2 are colored green and cyan respectively.

Suppl. Table 1. Cryo-EM data collection, refinement and validation statistics

	VLP:C34 Fab (EMDB-60997, 60999, 61002) (PDB 9IYI)			VLP:C37 Fab (EMDB-61025, 61026, 61030) (PDB 9IZ9)			VLP alone (EMDB-60970, 60972) (PDB 9IXI)		Infectious virion (EMDB-61168, 61169) (PDB 9J6D)		C34-E1-E2 (EMDB-60966) (PDB 9IXA)	
Data collection and processing												
Magnification	105,000 ×			105,000 ×			130,000 ×		105,000 ×		105,000 ×	
Voltage (kV)	300			300			300		300		300	
Electron exposure (e−/Å²)	60			60			50		50		50	
Defocus range (μm)	-1.8 to -2.2			-1.5 to -2.0			-1.5 to -2.0		-1.8 to -2.2		-1.5 to -2.2	
Pixel size (Å)	1.34			1.32			1.04		1.32		1.32	
Symmetry imposed	Icosahedral (I1)			Icosahedral (I1)			Icosahedral (I1)		Icosahedral (I1)		C1	
Initial particle images (no.)	61,787 4.35			101,173 4.01			36,959 3.83		85,089 3.63		1,074,703 3.05	
Map Resolution (Å)												
Refined blocks	2-fold	3-fold	5-fold	2-fold	3-fold	5-fold	2-fold	5-fold	2-fold	5-fold	n/a	
Final particle images (no.)	491,507	531,640	504,917	559,411	1,073,941	496,185	240,393	248,526	1,618,503	2,023,556	229,912	
Map resolution (Å)	3.73	3.67	3.71	3.30	3.32	3.29	3.01	2.91	3.11	3.01	3.05	
FSC threshold	0.143			0.143			0.143		0.143		0.143	
Map resolution range (Å)	6.82-2.92	6.64-2.92	6.79-2.92	6.68-2.91	6.80-2.91	6.78-2.91	6.66-2.25	6.34-2.27	5.74-2.91	5.51-2.96	-	
Map sharpening <i>B</i> factor	-108.3	-116.0	-109.7	-71.1	-107.7	-67.8	-60.6	-59.2	-94.3	-95.9	-94.1	

(Å²)

Refinement					
Initial model used (PDB code)	8FCG	8FCG	8FCG	8FCG, 6JO8	6JO8
Model resolution (Å)	3.73	3.30	3.01	3.11	3.05
Model composition					
Non-hydrogen atoms	44589	45102	31117	32801	8909
Protein residues	5774	5787	4044	4254	1158
Ligands	56	53	-		1
<i>B</i> factors (Å ²)					
Protein	136.55	127.07	111.43	116.34	59.19
Ligand	116.82	148.67			87.83
R.m.s. deviations					
Bond lengths (Å)	0.006	0.004	0.010	0.010	0.010
Bond angles (°)	0.977	0.770	1.014	0.985	0.967
Validation					
MolProbity score	1.98	1.75	0.83	0.75	0.86
Clashscore	15.43	11.24	1.17	0.82	1.31
Poor rotamers (%)	0.71	0.30	0.38	0.03	0.10
Ramachandran plot					
Favored (%)	95.80	96.90	98.11	98.53	98.26
Allowed (%)	1.06	3.06	1.62	1.3	1.74
Disallowed (%)	0.14	0.03	0.27	0.17	0.00

Suppl. Table 2. Statistics of Crystallographic Analysis

E1-E2:C37 Fab (PDB 9IW2)	
Data collection and processing	
Wavelength (Å)	0.97853
Space group	P2 ₁ 2 ₁ 2 ₁
Cell dimensions	
a, b, c (Å)	57.087, 118.995, 228.048
α, β, γ (°)	90, 90, 90
Resolution (Å)	40.34-3.20 (3.31-3.20)
CC _{1/2}	0.966
CC*	0.991
Completeness (%)	91.18 (64.87)
Unique reflections	24163
Refinement	
Resolution (Å)	40.34-3.20
No. of reflections	24163 (1693)
R _{work} /R _{free}	0.2154/0.2726
No. of atoms	
Protein	8260
Ligand/ion	0
Waters	0
B factors (Å ²)	
Protein	67.29
Ligand/ion	-
Waters	-
Root-mean-square deviation	
Bond lengths (Å)	0.004
Bond angles (°)	0.83
Ramachandran plot (%)	
Favored	93.97
Allowed	6.03
Outliers	0