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Supplementary Information for

High resolution asymmetric structure of a Fab-virus complex reveals overlap with the receptor binding site

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Movies S1 to S2

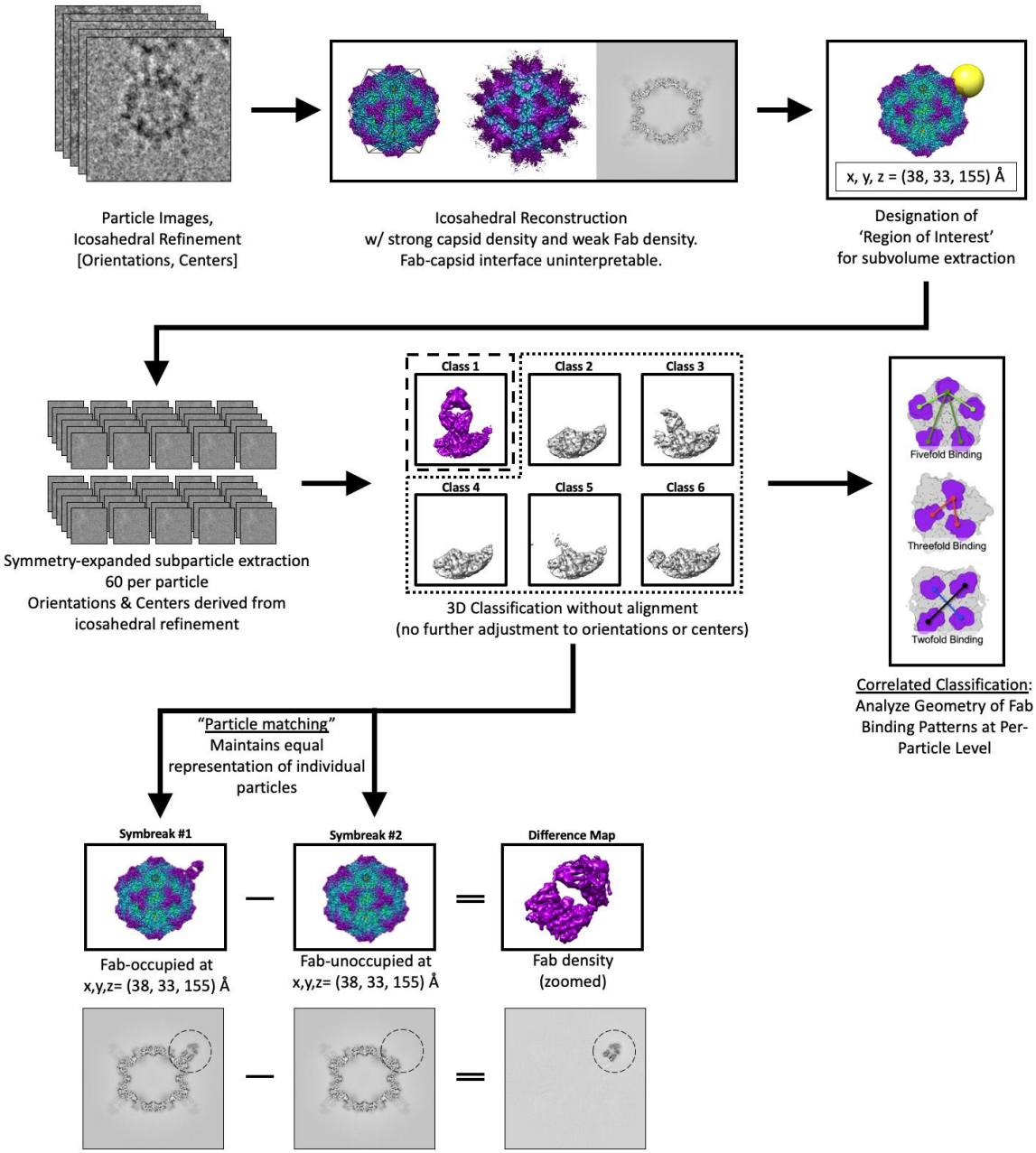

Fig. S1. Flow chart of the reconstruction and classification process.

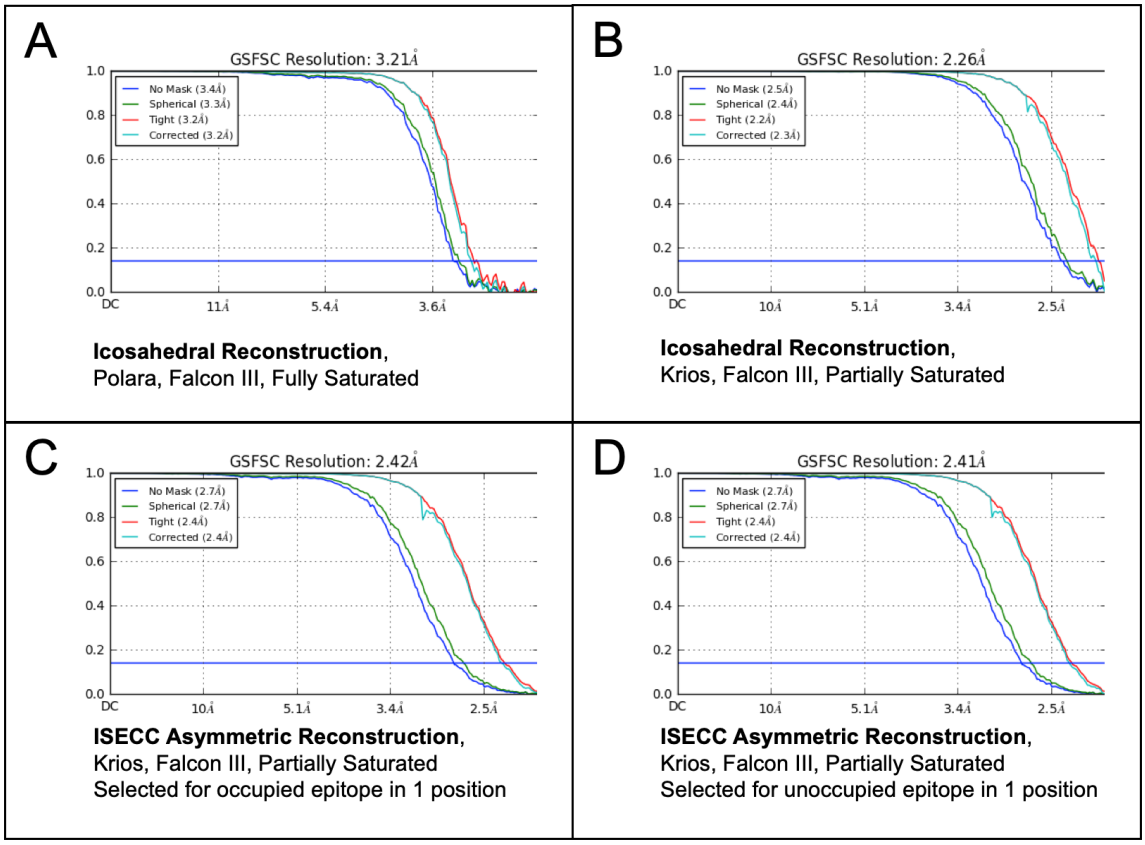

Fig. S2. Global FSC curves. (A, B) Different instrumentation and sample preparations allowed the partially saturated dataset to achieve higher resolution than the fully saturated dataset (2.3Å vs 3.2Å). (C,D) Asymmetric symmetry-break operations sacrificed some of this resolution to resolve the capsid witha selected site either in the presence or absence of Fab (both 2.4Å global resolution). Local resolution at the selected epitope is shown in Fig. 4.

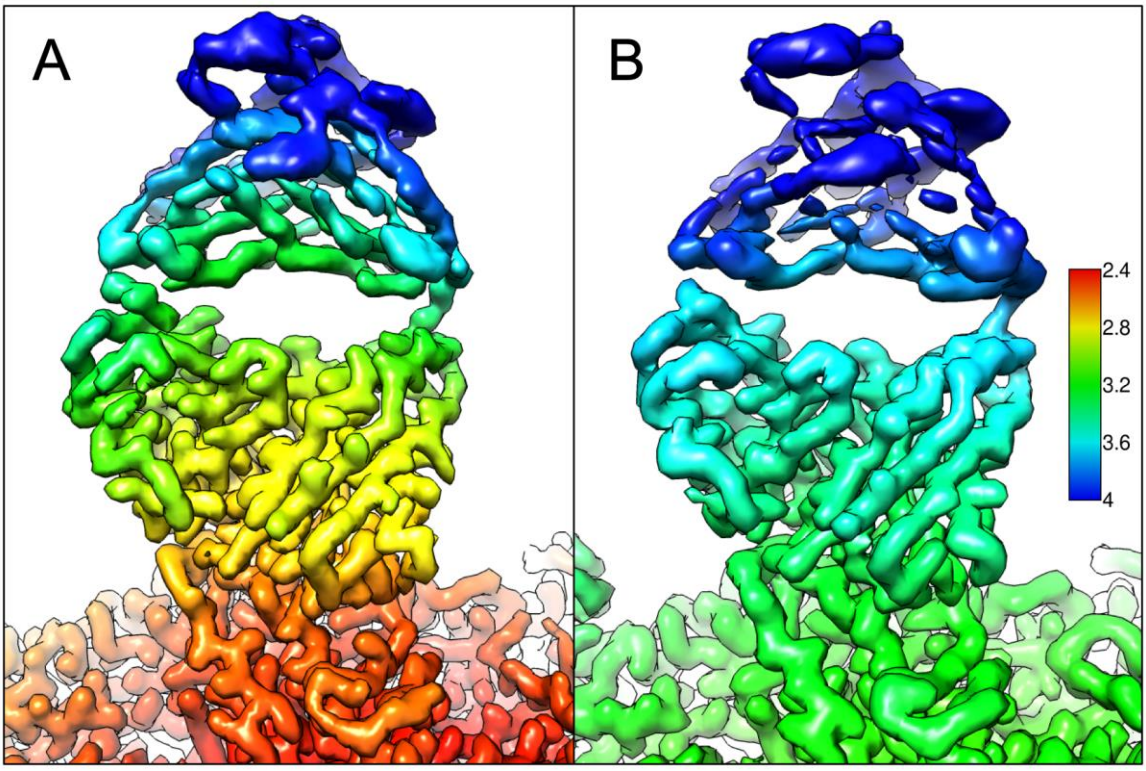

Fig. S3. Comparison of whole Fab structures. Fab-density of the asymmetric low-Fab map colored by local resolution (A, left) was consistently better at the Fab-capsid interface than that of the full-fab icosahedrally averaged map (B, right).

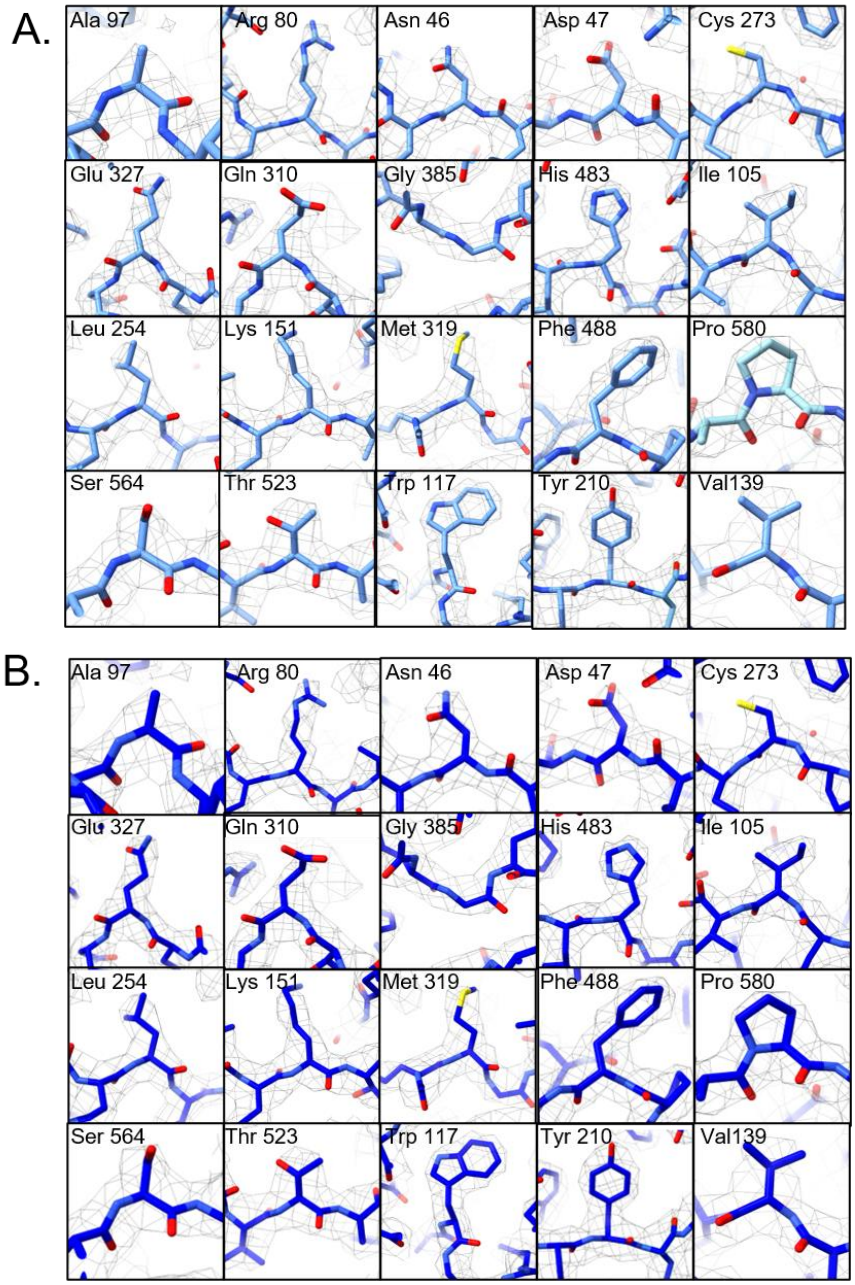

Fig. S4. Illustration of side chain densities to highlight map quality. Representative areas are shown for A) Fab unbound (light blue) and B) Fab bound (dark blue) capsid maps at 2.4Å resolution**.**

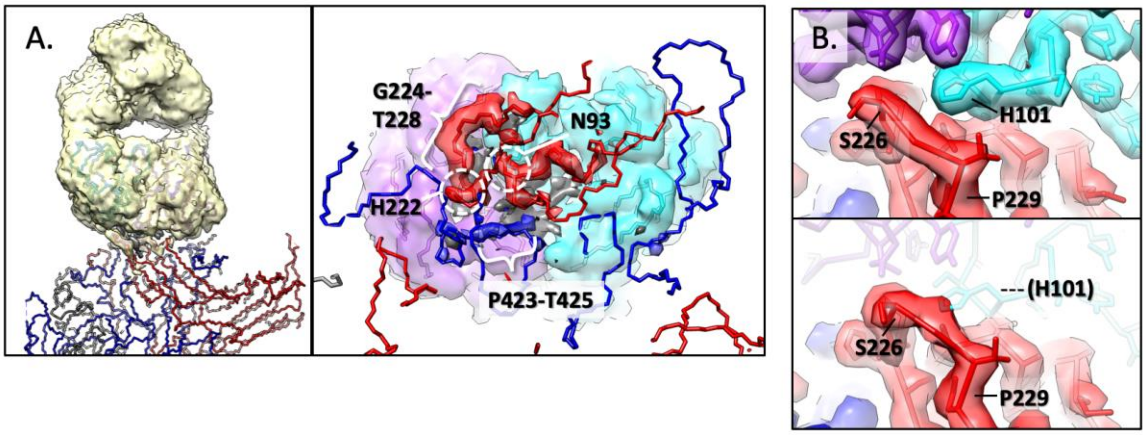

Fig. S5. Positive and negative difference mapping in the Fab-virus interface.
(A, Left) Difference map between occupied and unoccupied asymmetric maps (Figure 1) shows all positive density (taupe) most of which corresponds to Fab14. Two symmetry related copies of the VP2 capsid protein (red and blue wire) comprise the binding site. (Right) The same difference map is rotated 90° with the virus slabbed to show the interface from the virus surface looking outward visualizing positive difference density of the Fab heavy (cyan) and light chains (purple). This zoomed view illustrates additional positive difference density (red and blue) corresponding to the virus loops. The positive difference density corresponds to capsid residues 88-94, 222, 224-228, and 423-425 (colored by nearest chain, denoted by white brackets and circles). Negative difference density (dark grey) can be seen adjacent to the positive difference density, suggesting loop movement. (B) Zoomed image of the virus (red and blue) and Fab (purple and cyan) interface in the region of the 225 loop and H101. The VP2 228 loop is displaced by a maximum of 1.9Å upon interaction with Fab heavy chain residue H101 (top vs bottom). This same view is provided as a morph-map in S.Movie 2.

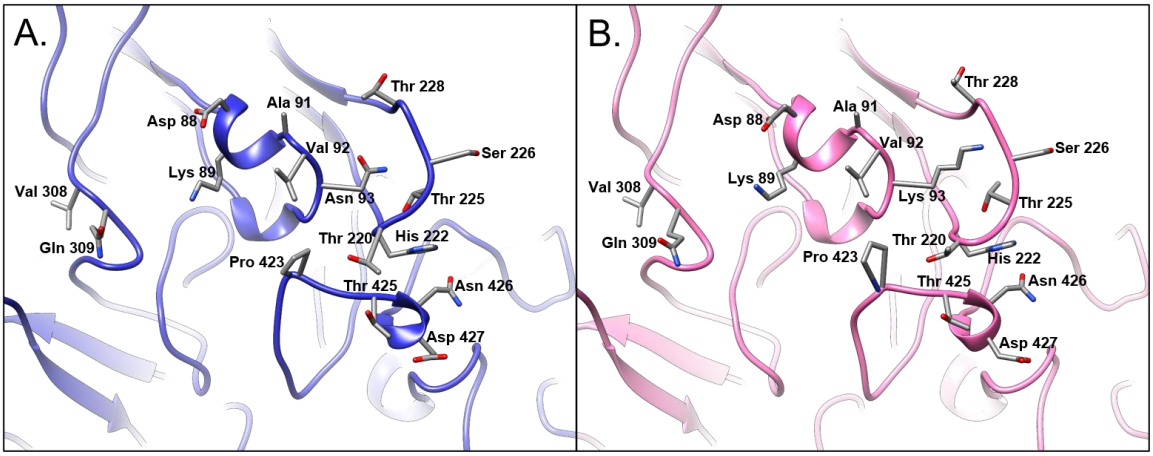

Fig. S6. Comparison of CPV and FPV at Fab14 antibody binding site. A) Fab14 antibody binding site on CPV crystal structure colored in blue, binding site residues colored by element (PDB: 2CAS). B) Fab 14 antibody binding site on FPV crystal structure colored in pink, binding site residues colored by element (PDB: 1C8F). Both oriented as in Fig. 2 A,B right for direct comparison to cryo EM structures.

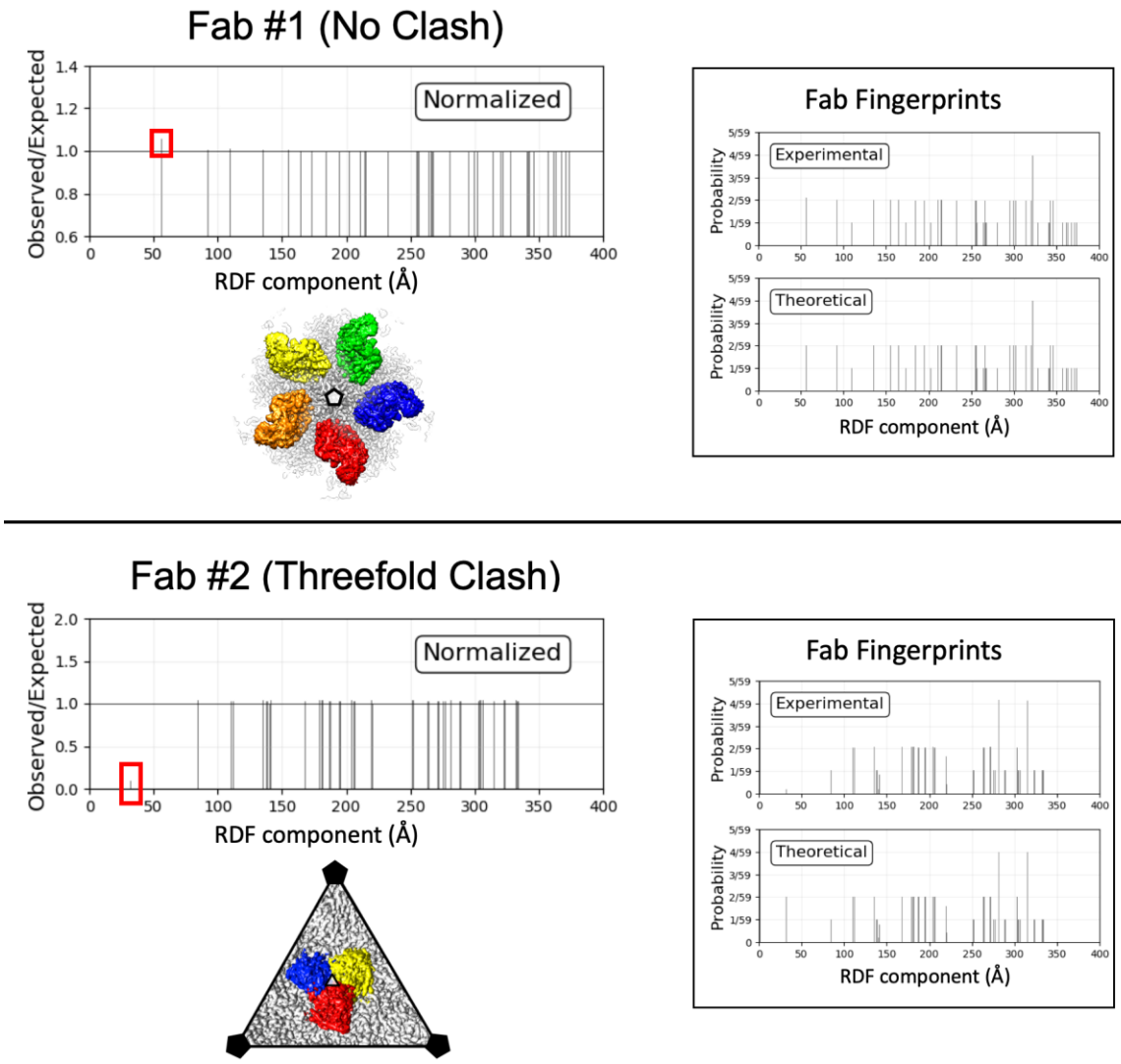

Fig. S7. Further validation of RDFs for clash analysis. Two undersaturated enterovirus-Fab complexes are shown to demonstrate the impact of Fab binding geometry on cooperativity via RDF analysis. Fab #1 features no symmetry clash, with the closest interaction being the fivefold-relationship (top panel, Fab shown in red, orange, yellow, green, blue). Fab #2 features strong symmetry clash across the threefold symmetry axis (bottom panel, Fab shown in red, blue, yellow). The behavior of Fab #1 largely matched CPV-Fab 14, with a slight excess of the most proximal RDF component. Consistent with steric clash, the behavior of Fab #2 matched that of CPV-FabE.
Enterovirus-Fab structures and corresponding biological analysis will be published separately. They are shown as further validation of Fab fingerprints in assessing binding cooperativity.

Table S1. Cryo-EM statistics

Fab Occupancy	Symmetry	Microscope /Detector	No. of micrographs	Defocus range (u.m.)	No. of particles selected	No. of particles used	Resolution (Å)
Full-Fab 60 Fab/capsid	I2	FEI Polara, Falcon 3EC	21,075	0.5 – 4.2	142,618	95,353	3.2
Low-Fab 10 Fab/capsid	I2	Titan Krios, Falcon 3EC	2,265	0.7 – 4.9	316,808	162,627	2.3
	C1/ Symmetry -mismatch	Titan Krios, Falcon 3EC	2,265	0.7 – 4.9	316,808	162,627 * 10.2 orientations/particle	2.4

Table S2. Sequence of the Fab and ScFv

Light Chain

DIVMTQSHKFMSTSVGDRVSITCKASQDVNTALAWYQQIPGQSPKLLIYSASNRYTGVPDRFTASGSGTDFTFTISSVQAEDLALYYCQQHYTTPWTFGGGTKLEIKRA---

L1 **L2** **L3**

Heavy Chain

GTELVKPGASAGVKLSCKASGYTFTNYDMNWVRQRPEQGLEWIGWIFPGDGSTRYNEKFKGKATLTTDKSSSTAYQLNRLTSEDSAVYFCARRGSHGSYSFAYWGQGTLVTVS---

H1 **H2** **H3**

Heavy and light chain sequences reported in one letter code with the chain loops underlined and designated. Yellow highlighted residues were identified as contacts with the virus surface in the 2.4 Å map.

Table S3. Refinement and Validation Statistics

(a) Asymmetric Refinement: Fab-occupied epitope

	VP2	Fab 14
Refinement		
Model composition	Capsid asu	Fab
Non-hydrogen atoms	4,238	1,766
Protein residues	532	227
Model-to-map fit		
CC_mask	0.8121	0.7989
R.m.s. deviations		
Bond lengths (Å)	0.0054	0.0047
Bond angles (°)	1.01	1.02
Validation		
MolProbity score	1.57	2.74
Clashscore	3.74	9.82
Rotamer outliers (%)	2.58	8.47
Ramachandran plot		
Favored (%)	97.53	91.03
Outliers (%)	0.19	0

(b) Asymmetric Refinement: Fab-unoccupied epitope

	VP2
Refinement	
Model composition	Capsid asu
Non-hydrogen atoms	4,238
Protein residues	532
Model-to-map fit	
CC_mask	0.8111
R.m.s. deviations	
Bond lengths (Å)	0.0108
Bond angles (°)	0.75
Validation	
MolProbity score	2.14
Clashscore	4.71
Rotamer outliers (%)	6.67
Ramachandran plot	
Favored (%)	96.01
Outliers (%)	0

(c) Icosahedral Refinement: Low-Fab vs. Full-Fab data

	$VP2^{Low\text{-}Fab}$	$VP2^{Full\text{-}Fab}$	$Fab\ 14^{Full\text{-}Fab}$
Refinement			
Model composition	Capsid asu		Fab
Non-hydrogen atoms	4,238		1,766
Protein residues	532		227
Model-to-map fit			
CC_mask	0.7831	0.8545	0.7162
R.m.s. deviations			
Bond lengths (Å)	0.0050	0.010	0.008
Bond angles (°)	0.99	1.25	1.17
Validation			
MolProbity score	1.72	1.81	1.96
Clashscore	4.35	5.53	6.93
Rotamer outliers (%)	3.44	0.21	0.53
Ramachandran plot			
Favored (%)	97.53	91.21	88.79
Outliers (%)	0	0.37	0

All statistics generated via phenix.molprobity on phenix/1.14

Movie S1 (separate file). Occupied-Unoccupied epitope morph map. Morphing between the two particle-matched asymmetric maps suggests subtle hinging of the 228 loop on VP2. Key residues in the Fab 14 binding footprint are labeled. The Fab density was segmented away to provide this top-down view of the epitope. For side view, density is colored as in S.Fig. 4.

Movie S2 (separate file). Occupied-Unoccupied epitope morph map with Fab. Side view of morphing between the two particle-matched asymmetric maps suggests subtle hinging of the 228 loop on VP2., density is colored as in S.Fig. 4.