

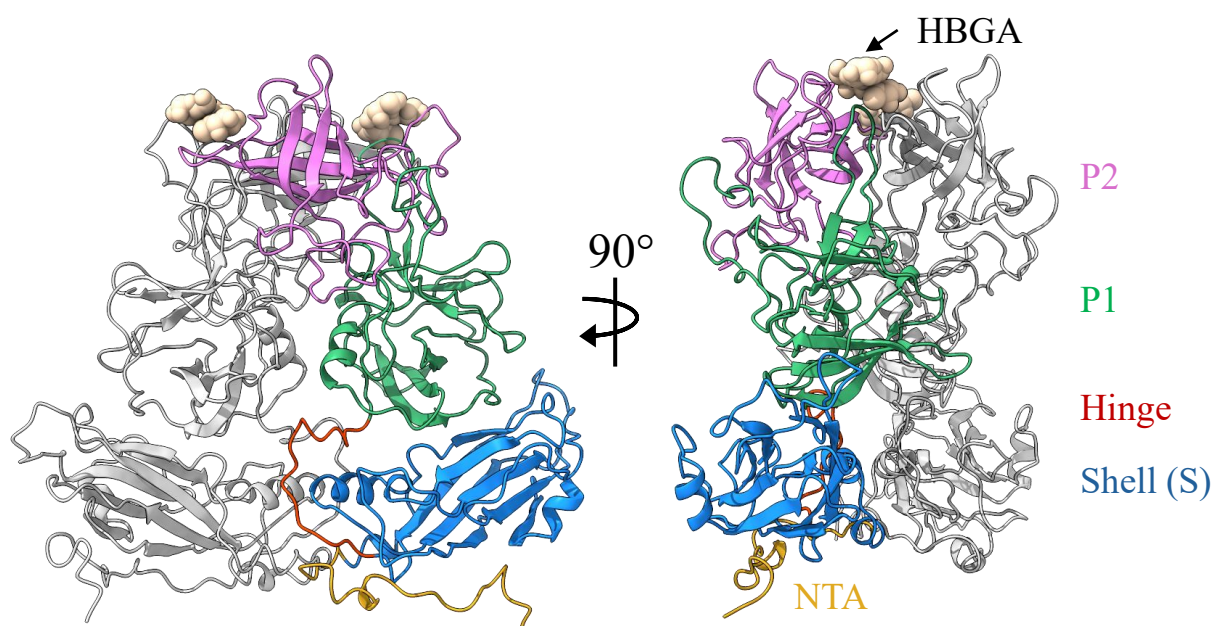


Figure S1: Domain organization of HuNoV VP1. HuNoV VP1 dimer (PDB: 7MRY) showing one monomer colored in grey and the other according to domain organization: N-terminal arm (gold), shell (blue), hinge (red), P1 subregion (green) and P2 subregion (violet). HBGA polysaccharide ligands (PDB: 5J35) are shown as tan spheres.

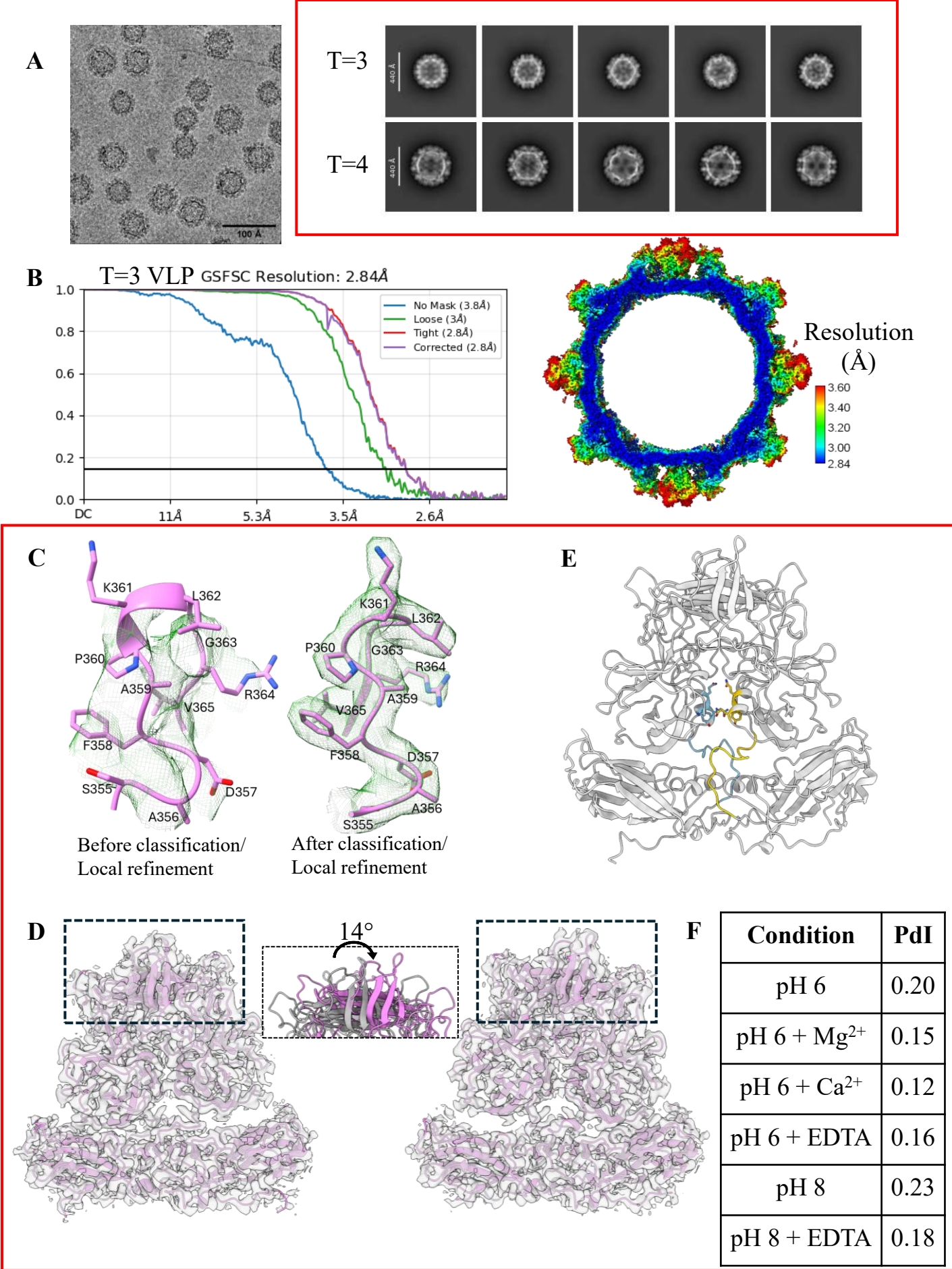


Figure S2, related to Figure 1: Cryo-EM of GII.4 Sydney, T=3 VLPs. **A (Left)** Representative view from a micrograph containing a mixed GII.4 Sydney VLP population used for particle picking. Scale bar = 100 Å. **(Right)** 2D classes show a clear distinction between T=3 and T=4 particles. **B (Left)** Gold-Standard Fourier Shell Correlation (GSFSC) plot with cut-off at 0.143 indicated by horizontal line, shows maximum resolution of the T=3 VLP map. **(Right)** Cross-section of T=3 VLP colored by local resolution. **C** Improvement of density fitting on focused refinement of C-C dimer on the extremities of P-domain. **D** Two distinct conformations of C-C dimers identified by 3D classification are shown inside the cryo-EM density. **Inset:** Superimposition of the C-C dimers via the shell highlights a 14° tilt about the 2-fold axis between the P-domains. **E** A-B dimer (grey) from the T=3 VLP, with hinges and metal ion pocket shown as sticks identified in GII.4 HOV colored in blue and gold. **F** VLP homogeneity under different conditions measured by DLS. Polydispersity indices (PdIs) show little variation and near monodisperse VLP samples (PdI < 0.2).

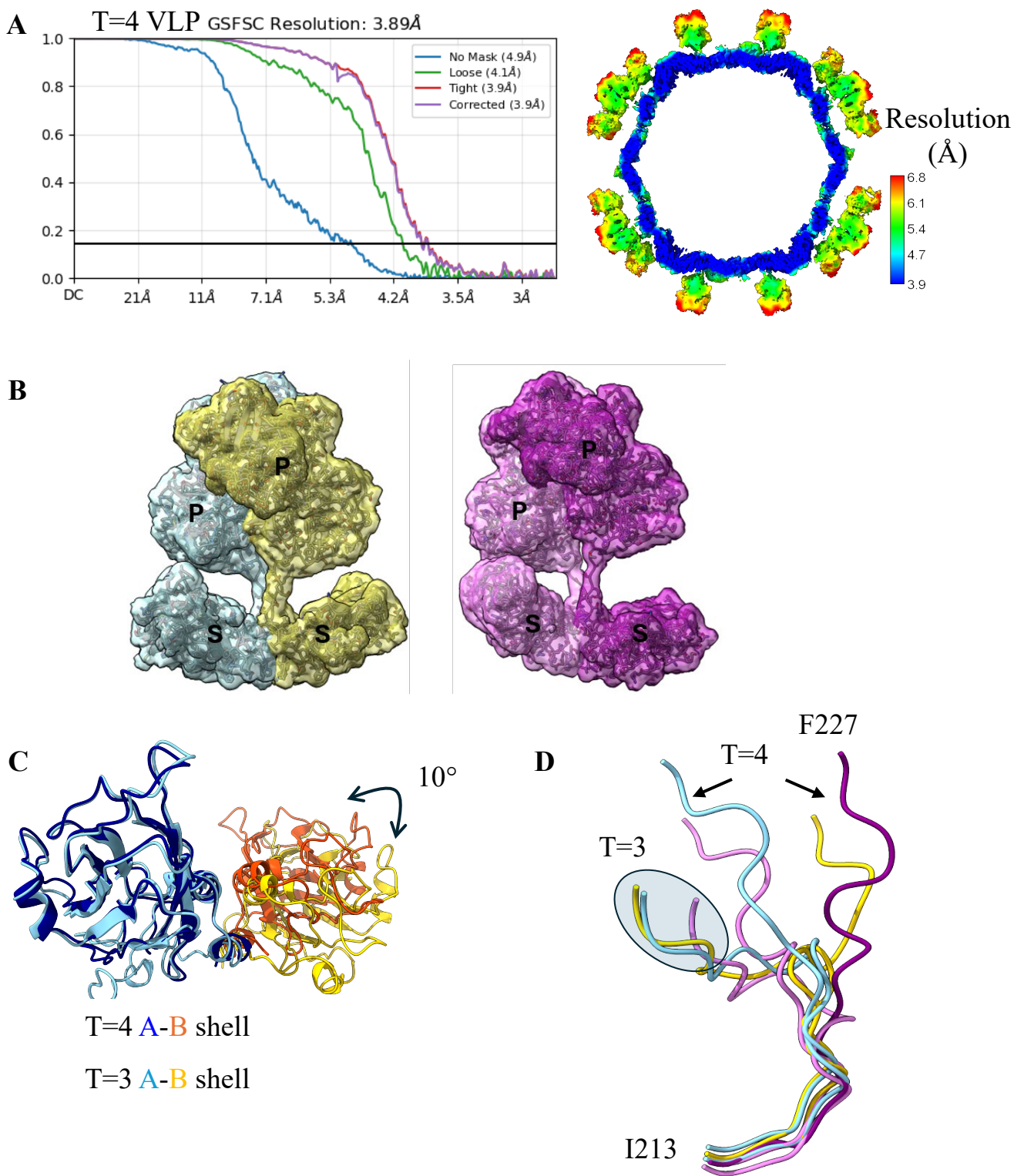


Figure S3, related to Figure 1: Cryo-EM of GII.4 Sydney, T=4 VLPs. A GSFSC plot shows the maximum resolution of the T=4 VLP map. **(Right)** Cross-section of T=4 VLP colored by local resolution. **B** Placement of chains in the A/B (left) and C/D (right) dimers in one asymmetric unit of the T=4 lattice inside the cryo-EM density, following the same color scheme as in Fig. 1F. **C** Superimposition of A-B shell domains from T=3 (light colors) and T=4 (dark colors) shows a larger angle between the domains in T=4 particles. **D** Conformational variations in the hinge region (residues 213-227) in T=3 (indicated by an oval) and T=4 VLP structures based on the superposition of the S domains.

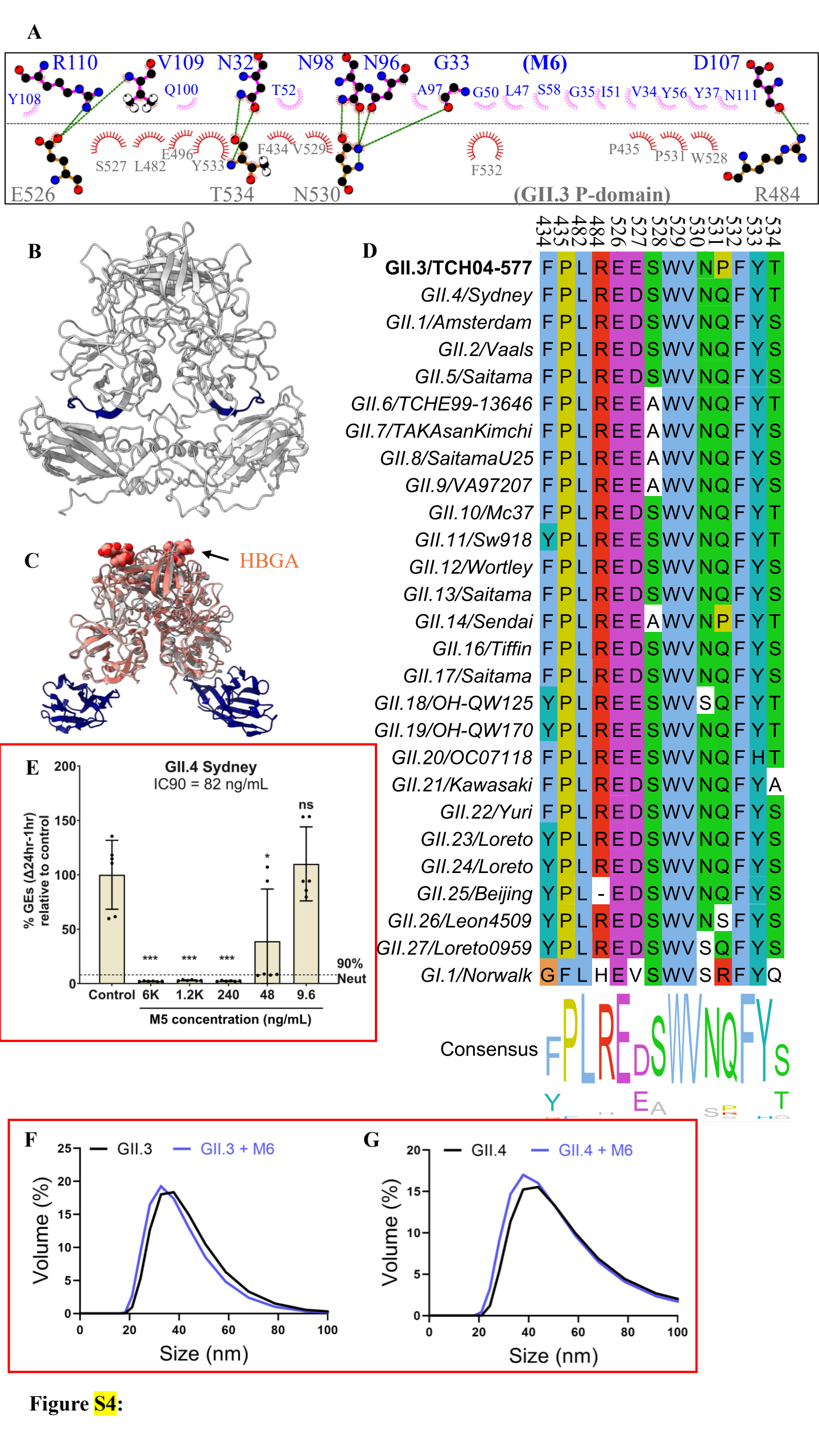


Figure S4:

Figure S4, related to Figure 2: Detailed view of linear M6 epitope and its conservation across GI/GII genotypes. **A** LigPLOT+ diagram of the interaction network between the P-dimer and M6. Green dashed lines indicate H-bonds and spoked half-circles represent hydrophobic interactions. **B** VP1 dimer structure (grey) with the linear M6 epitope in dark blue highlighting the inaccessibility in the resting capsid conformation due to steric clashes. **C** Superimposition of the M6-bound P-dimer structure onto the crystal structure of a GII.3 P-dimer (salmon red) bound to HBGA ligands shown as spheres (PDB: 6IS5). **D** Multiple sequence alignment of VP1 from strains belonging to genotypes GII.1-GII.27 and prototypical GI genotype (GI.1) highlights the broad conservation of the M6 epitope identified in the crystal structure. The reference sequence (GII.3) is in bold and residue numbers are indicated at the top. Alignment was performed with Clustal Omega with default parameters and visualized with Jalview v2.11.3.0, following the Clustal color palette. **E** Neutralization assays measuring GII.4 Sydney viral replication inside HIEs in the presence of different concentrations of M5 nanobody. The assay measures fold changes in genome equivalents (GEs) at 24 hr post infection relative to an untreated control. The dashed line represents the fold change in GEs expected at 90% neutralization by M5. Error bars denote the standard deviation, and data bars represent the mean of n= 6 wells. Significance was determined using two-tailed Student's *t* test and is shown by *** = $p < 0.001$, * = $p < 0.05$ and ns = $p > 0.05$. **F, G** Raw DLS data showing the change in distribution of GII.3 (**F**) and GII.4 Sydney (**G**) VLPs in response to M6 addition. Graphs represent the average of three rounds of measurements.

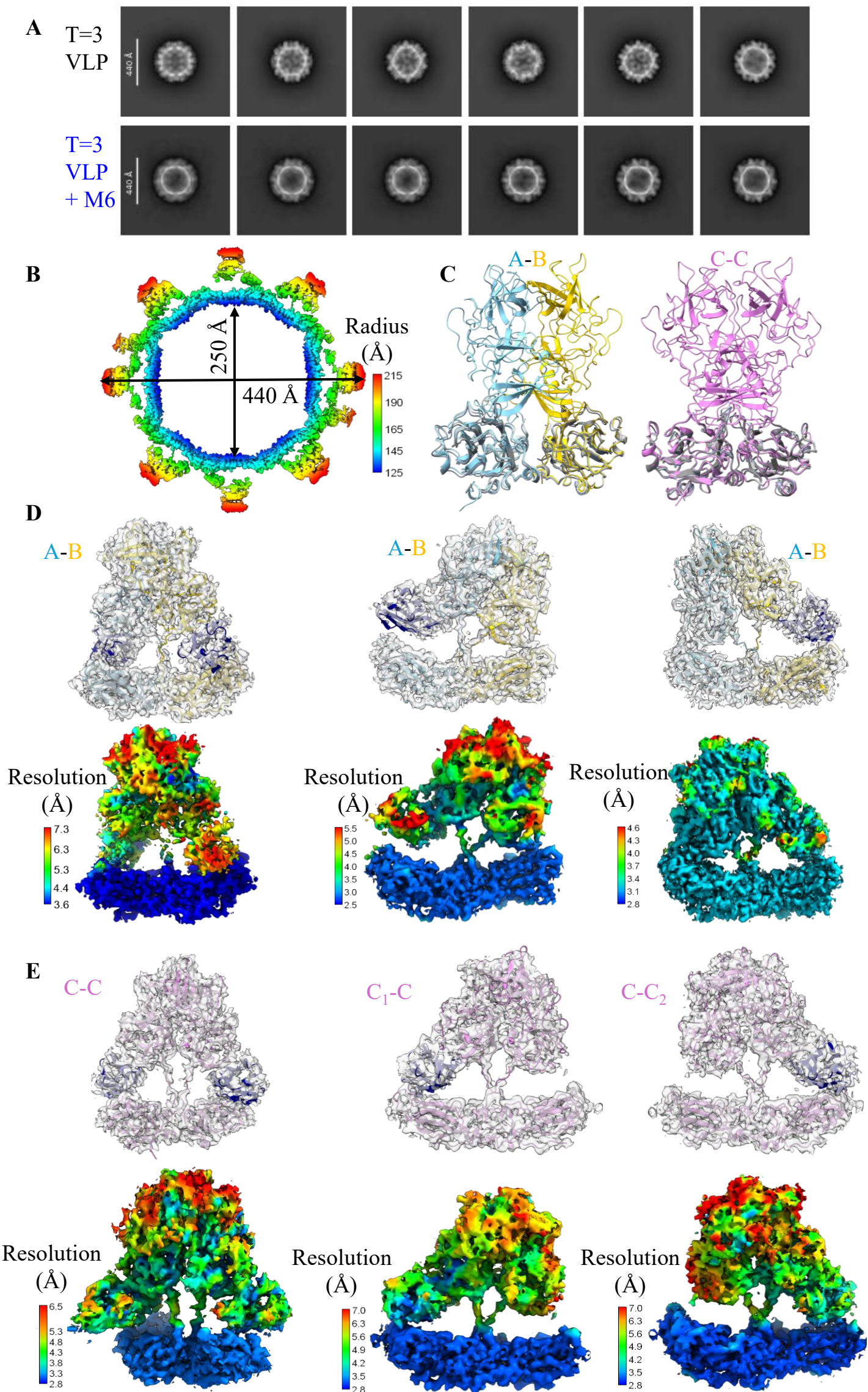


Figure S5:

Figure S5, related to Figures 3-4: Cryo-EM of M6-bound T=3 VLPs. **A** 2D classes of GII.4 Sydney VLPs in T=3 conformation untreated (**top**) or incubated with M6 (**bottom**). **B** Cross-section of **M6-bound** T=3 VLP cryo-EM map resulting from homogeneous refinement with strict icosahedral symmetry imposed, colored by radius and with VLP dimensions marked. **C** Superimposition of A-B and C-C shell domains from M6-bound structures (all conformations, grey) onto native T=3 A-B and C-C dimers (shown in color). **D** The three distinct conformations of M6-bound A-B dimers from 3D classification and focused refinement, shown inside the transparent cryo-EM density map (**top**) along with the maps colored by resolution range (**bottom**). **E** The three distinct conformations of M6-bound C-C dimers from 3D classification and focused refinement, shown inside the transparent cryo-EM density map (**top**) along with the maps colored by resolution range (**bottom**).

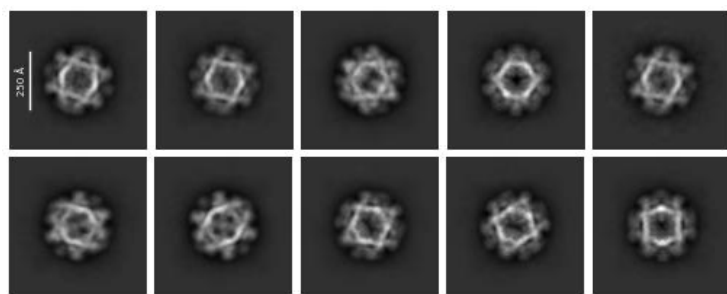
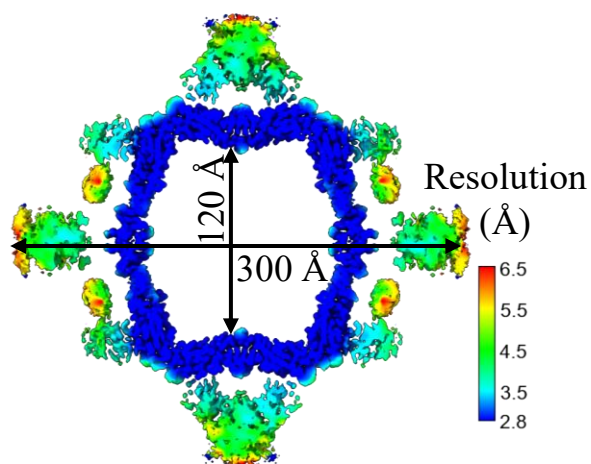
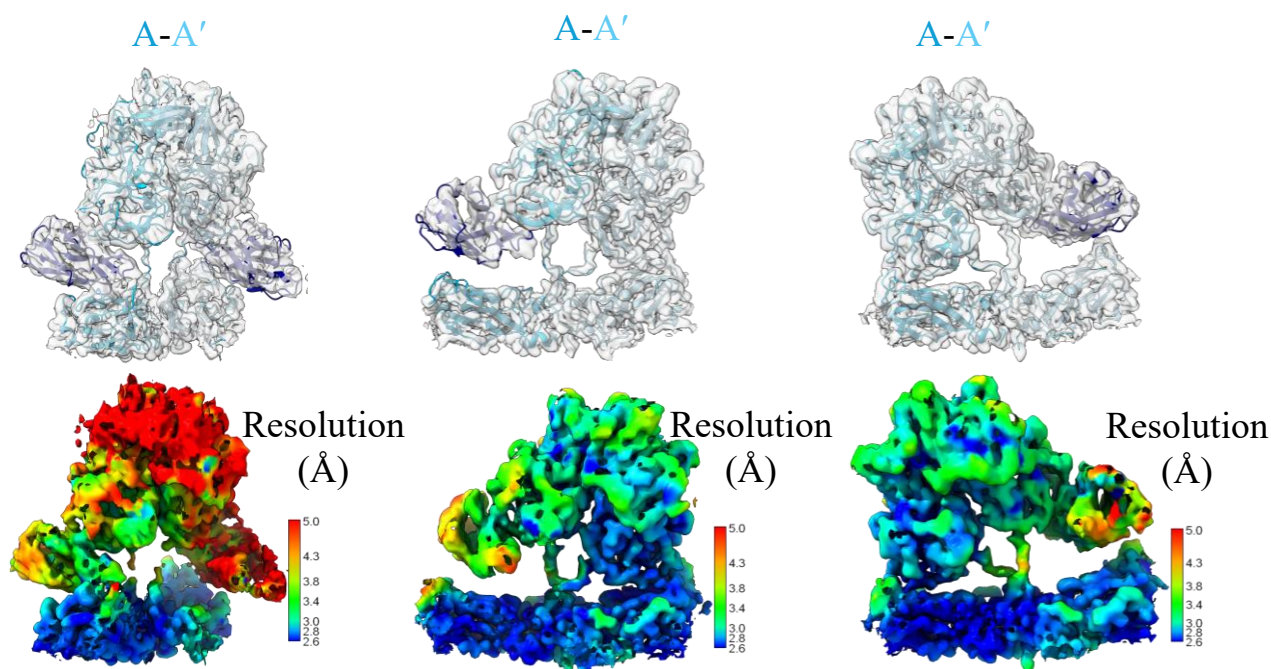
A**B****C**

Figure S6, related to Figure 5: Cryo-EM of T=1 particles assembled from broken T=4 VLPs after M6 binding. **A** 2D classes of T=1 particles picked from micrographs of M6-treated GII.4 Sydney VLPs. **B** Cross-section of a T=1 VLP map generated by homogeneous refinement with strict I symmetry imposed. The map is colored by local resolution and includes the VLP dimensions. **C** Three distinct A-A' dimer conformations with one or two M6 bound shown inside the cryo-EM map (transparent density) to highlight the map completeness (**top**) and with the map colored by local resolution (**bottom**).

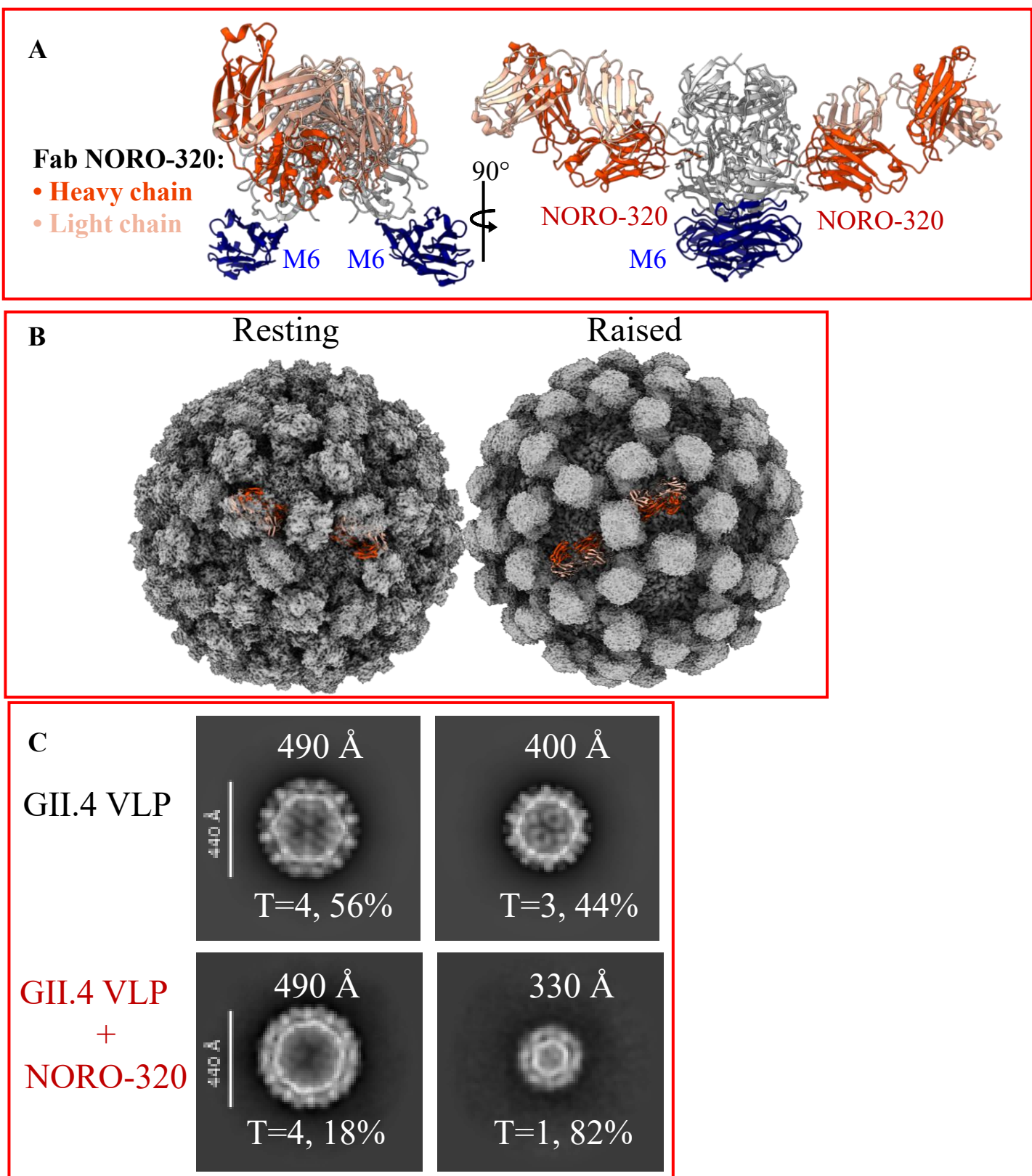


Figure S7, related to Figure 7: Comparison of P-domain epitopes between M6 nanobody and human Fab NORO-320. **A** Crystal structures of NORO-320 (PDB: 7JIE) and M6 superimposed onto the same P dimer highlighting distinct hidden epitopes occluded by either the shell (M6) or adjacent P-domains (NORO-320). **B** Modelling of NORO-320 Fab (PDB: 7JIE) binding to a T=3 A-B dimer in the resting (**left**) or raised (**right**) capsid conformations, generated using the T=3 maps from resting (**Fig. 1A**) or raised (EMD-10756) states. **C** Representative 2D classes of GII.4 Sydney VLPs without treatment (**top**) or after incubation with NORO-320 (**bottom**). VLP diameters and T numbers are indicated for each class, **along with relative abundance**.

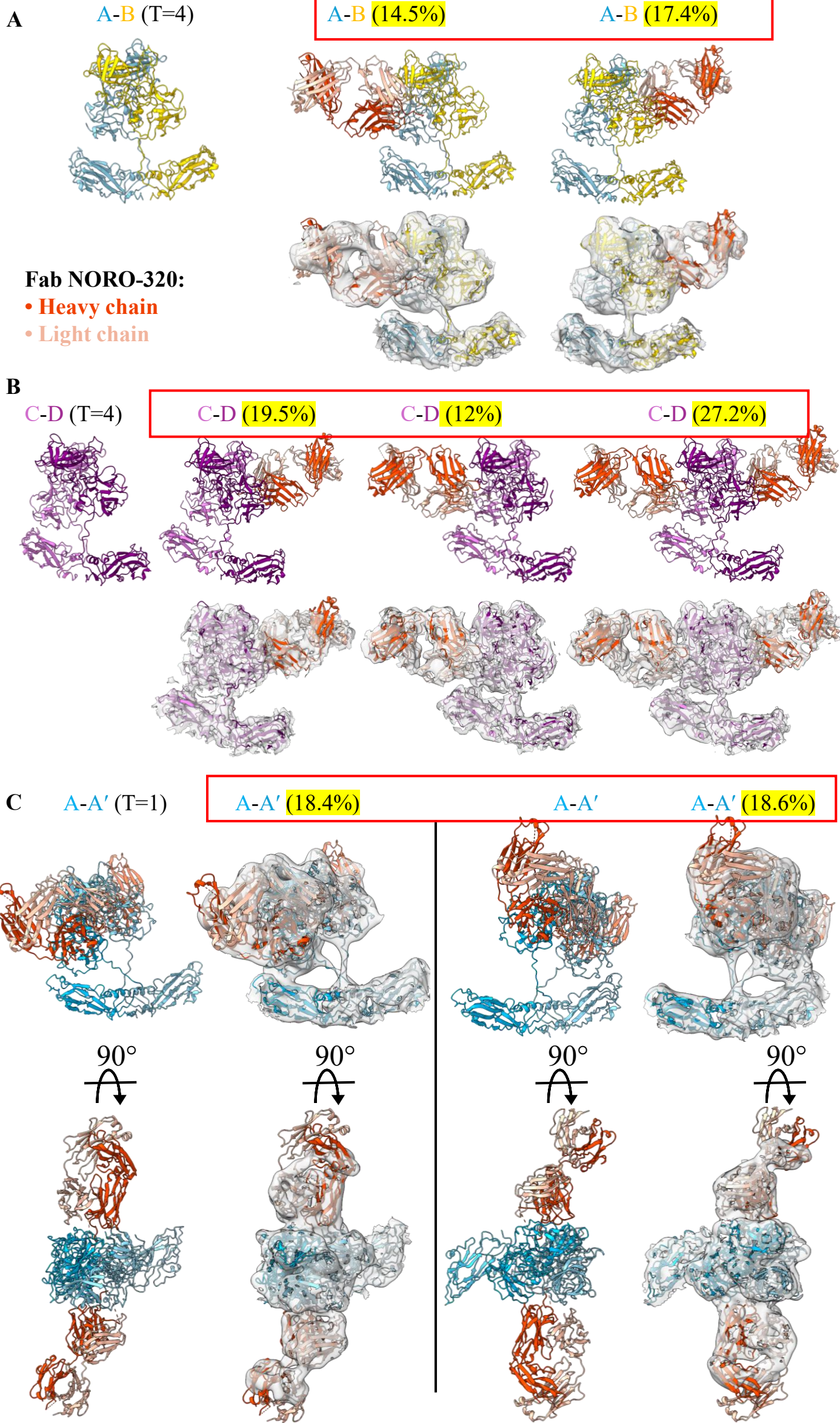


Figure S8:

Figure S8, related to Figure 7: VP1 dimers from T=4 and T=1 VLPs bound to one or two NORO-320. **A** A-B dimer from an untreated T=4 particle as reference (**left**, also **Fig. 1E**) and with NORO-320 binding from the 5-fold axis (**middle**) or the quasi 6-fold axis (**right**). Bottom views show the protein chains inside the cryo-EM density (transparent). **B** C-D dimer from an untreated T=4 particle as reference (**left**, also **Fig. 1E**) and with NORO-320 binding from only one quasi 6-fold axis (**middle two**) or from both quasi 6-fold axes (**right**). Bottom views show the protein chains inside the cryo-EM density (transparent). **C** Two distinct A-A dimer conformations (T=1) with P-dimer twisted towards one S-domain or another to accommodate two NORO-320 binding simultaneously. Righthand views show the protein chains inside the cryo-EM density (transparent).

Metal
binding
pocket

	211	Hinge										219	230	459	460	463							
2012-Sydney-GenBank-AFV08795.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2023-Milwaukee0499-GenBank-PQ207934.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2022-SanFrancisco-SA-GenBank-WKD84244.1/1-541	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2022-Maricopa0179-GenBank-XEJ39712.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2021-LosAngeles0043-GenBank-XER78455.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2021-Wichita0031-GenBank-XEO39786.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2020-Huron0309-GenBank-XEJ39052.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2019-Sydney-GenBank:UEA14085.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2015-Sydney-Y1677-GenBank:_UEA14085.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2014-WI_SP-0251_121714_WI_/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	F	Q
2012-Woonona-GenBank-AFV08792.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2012-OH-GenBank-BAV10400.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2011-NewOrleans-GenBank-ANP93425.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2009New_Orleans1805-GenBank-ADD10375.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2008-Orange-GenBank-ACX31889.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2008-Shimane2-GenBank-BAJ13975.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	Q	H	Q
2007-Apeldoorn317-GenBank-BAG55289.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	Y	Q
2007-Pune_India-GenBank-ACL31360.2/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	Q	H	Q
2007-Osaka-GenBank-BAG30923.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	Q	H	Q
2006-Minerva-GenBank-ABW74543.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	Q	H	Q
2006-DenHaag89-GenBank-ABL74395.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	Q	H	Q
2006-Yerseke38-GenBank-ABL74391.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	H	Q
2005-Sakai-GenBank-BAE98194.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	Q	H	Q
2004-Hunter504D-GenBank-AAZ31396.2/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	S	V	P	Q	H	Q
2002-HoV-GenBank-ABY27560.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	L	H	Q
2002-FarmingtonHills-GenBank-AAR97663.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	Q	H	Q
2000-Henry-GenBank-ACL27298.1/1-540	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	Q	H	Q
1996-US95-96-GenBank-AAL13025.1/1-539	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	Q	H	Q
1995-Miami-GenBank-AAL12983.1/1-539	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	Q	H	Q
1994-Richmond-GenBank-ABW74532.1/1-539	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	M	H	Q
1993-Bristol-GenBank-CAA54134.1/1-539	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	L	H	Q
1974-CHDC5191-GenBank:-ACT76139.1/1-539	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	L	H	Q
1974-CHDC2094-GenBank:-ACT76142.1/1-539	D	F	I	F	L	V	P	P	T	V	E	S	R	T	K	P	F	T	V	P	S	H	Q

Figure S9, related to Discussion: Sequence alignment of pre- and post-2007 GII.4 VP1 sequences in the hinge and metal ion binding pocket as observed in the GII.4 HOV structure (PDB: 7MRY).