

Supplementary Figure 1 | **Crystal packing of GII.4 HOV VLPs. a** The asymmetric unit of GII.4 HOV VLP crystal contains 15 copies of A (yellow), B (blue), and C (pink) chains. For clarity, only C subunits are labeled. The five-fold and three-fold axes are indicated by a pentagon and a triangle, respectively. b Four asymmetric units assemble into a VLP particle that consists of 180 copies of VP1. The icosahedron cage is shown with black lines. The symbol of each asymmetric units is shown as a blue sphere. **c** The crystal packing of VLPs shows the C subunits interacting with the neighboring VLP particles colored in gold, green, purple, and blue.



Supplementary Figure 2 | a Fourier shell correlation (FSC) curve indicates the cryo-EM structure's resolution. The resolution estimation of the reconstructions was based on the 0.143 criteria for the comparison between two half-data sets. b-c Representative section cryo-EM map of VLP. The density map is shown in gray mesh with the fitted atomic model of VP1. The residues are shown as stick models and labeled. d Local resolution of the cryo-EM map.



Supplementary Figure 3 | **Modeling of GII.4 HOV VLP in the raised conformation. a-b** GII.4 HOV VP1 fitted into the 'rising' and 'raised' conformations in the T=3 states. **c-d** Structural comparison of GII.4 HOV AB and CC dimers in both states. The P domain axis was determined by mass weighing using Chimera. The ration angles and the distance between the 'resting' and 'raised' states are calculated using Chimera.



Supplementary Figure 4 | Density map of GII.4 Minerva (T = 4) VLP. Density at the VP1 dimeric interface around the residue H460 is shown in level 0.1 (**a**) and 0.14 (**b**), respectively (PDB ID: 60UU; EMD-20206). The positions of H460 and Q463 are highlighted by blue and red arrows, respectively. No density between H460 residues was observed.



Supplementary Figure 5 | The 3D variability analysis using CryoSPARC. The reaction coordinate plots for the VLP alone (a) and in the presence of EDTA (b). The axes 0, 1, and 2, correspond to the three different components (eigenvectors) that were solved using 3DVA. The upper panel shows coordinates for 0 vs 1, and the bottom panel for 1 vs 2.

	+	
	432 - NLPCLLPQEY ISHLASEQAPTVGEAALL	HYVDPDTGENLGEFKAYPDGFLTCVPNGASSGPQQLPINGVFVFVSWVSRFYQLKPVGTASSAKGRLGLRR-
GI.4/1-544	445 NLVPCLLPQEYITHFISEQAPIQGEAALL	HYVDPDTNENLGEFKLYPGGYLTCVPNSSST <mark>GP</mark> QQLPLDGVFVFASWVSRFYQLKPVGTAGPARGRLGVRR-
GI.6/1-540	441 LRVPCLIPOEFITHFCNEQAPIAGEAALL	HYVDPDTGRNLGEFKLYPDGFMTCVPNSISSGPOTLPINGVFVFVSWVSRFYQLKPVGTASAAR-RLGLRRI
GI.5/1-543 GI 2/1 546	446 NEVPCLEPUEFVIFFVSEQAPSMGEAALV	HTYDPDTNENLGEFILTPEGFILCVPNG IGPUQLPLNGV-VFASWVSRFTQLKPVGTASSARGRLGVRR-
GI.3/1-544	445 MRIPCTLPOEYVAHEVNEQAPTRGEAALL	HYVDPDTHENLGEFKMYPEGFMTCVPNSSGSGPOTLPINGVFTEVSWVSRFYOLKPVGTAGPAR-RLGIRRS
GI.7/1-539	441 AQIPCTLPQEFVSHFVEQQAPIRGEAALL	H YVD PD TH RNL GEF KL YPDGF I TC VPNT - GGGPQNL PTNGVF VFS SWVSR YYQL K PVGT AGPAR - RL GVRR V
GI.9/1-550	451 AAIPCLLPQEFVAHFVNEQAPIRGEAALL	HYIDPDTHENLGEFKIYPEGFVTCVPNVGGTGPQSLPTNGVFVFVSWVSRYYQLKPVGTAGQAR-RLGFRRV
GI.8/1-543	445 PKVPCTL POEFVSHFVNEQAPTRGDAALL	HYVDPDTHENLGEFKMYPEGYMTCVPNAGG-GPOTLPINGVFVFISWVSRYVQLKPVGTAGAAR-RLGLRRS
GNA1.1/1-543 GIII 1/1-519	445 NETPOLLPUEWVGHFMGEQVPAQGDAALL	NTYNPED TSRULAEFALTFEGFLICSFGONSFGALVLPINGVFVFVSWVSAFTULRFVGIANSGANTAFAN
GIII.2/1-522	424 NPCPCLLPQEWITHFVSERAALQSDVALL	NYVNPNTGRVLFECKLYANGFLTVNLGASDOATLPVDGIFKFVSWVSFYYOLRPVGNASVGRRLPRLDGF
GIII.3/1-521	423 NPLPCLLPLEWINHFVAERPAKOSDVALL	NYINPLIGRVLFECKLYSAGFLAVNLGSQTEVTLPVDGIFKFVSWVSYYYQLRPVGTVSVGRRLPRLDGH
GVII.1/1-528	433 LAVQCLLPSEWITHFYSEAAPIQGEAMLL	RYVOPDLGRILFEAKLYKEGFVTVAGGTNPVTFPIDGTFVAVSWVSRNFVLSPMGSGOGRRAALAE
GII.3/1-548	455 GVLDCLVPQEWVQHFYQESAPAQTQVALV	RYVNPDTGRVLFEAKLHKLGFMTTAKNGDSPTTVPPNGYFRFESWVNPFYTLAPMGTGNGRRKTG
GII 1/1-535	442 GALDOLL POEWLOHEYOESAPSPTDVAL I	BY THE DIG VILLE ALL HOOFLIVARY - GSEP VVPPNGTEND SWINGFYSLAPMGTGNGREEVO
GII.2/1-542	449 PAIDCLL POEWVOHFYOEAAPSMSEVALV	RYINPDTGRALFEAKLHRAGFMTVSSN TSAPVVVPANGYFRFDSWVNOFYSLAPMGTGNGRRRVQ
GII.5/1-540	447 PAIDCLVPQEWVQHFYQESAPSLGDVALV	RYVNPDTGRVLFEAKLHKGGFLTVSST <u>S</u> TGPVVVPANGYFRFDSWVNQFYSLAPMGTGNGRRRFQ
GII.25/1-538	448 PAIDCLLPQEWIQHFYQESAPSOTDVAL -	LNPDTGRVLFEAKLHROGYITIAKSGDGPIVVPPNGYFRFDSWVNOFYSLAPMGNGNGRRRVG
GII.16/1-540 GIV 1/1-556	447 PF IDCL VPQEWIGHFYQE SAPAQS SVALL	MYNPD GRILFEALHREGFI VSS - ENRY I VPPNGTRFDSWVNGTSLAPMGI GNGRRRMU
GII.14/1-536	443 GAIDCLL POEWIEHFYGEAAPSOSDIALV	REINPDTGRVLLEAKLHKOGELTVAASGDHPIVMPTNGYFREEAWVNPFYTLAPVGTGSGRRRIG
GII.11/1-547	454 GWLDCLL POEWVOHFFOE SATSOSDVALV	RYVNPTTGRVLFEAKLHKOGFLTVAAS - GSYPLVVPADGYFRFESWVNQFYTLAPMGNGSGRRRAR
GII.8/1-537	444 GAIDCLLPQEWVAHFYQEAATAQTDVALI	RFVNPDTGRVLFEGKLHKQGFITISNSGDHPIVMPANGYFRFEAWVKOFYSLAPVGTGSGRRRVQ
GVI.1/1-582	487 KDIRCLLPNEYVQHLYDTQAPSLSDVALV	RYVNRDTGRVLFEAKLHRDGFMTVNASSTTVLPVDGYFRFDSWVNOFYALSPVGNASGRRGRSROAN-
GII.13/1-542	449 AFTDELL PUEWINHFTGEAAPSGADVALT	
GVI.2/1-580	485 RRISCLLPNEFVQHFYDLQAPSOSDVALL	RYVHPESGRVLFECKLYRDGFLTVNGASLAEFPVNGYFRFDSWVNOFFTLSPVGTGSGRSGRSGRSAD-
GII.21/1-537	444 IEIDCLIPQEWVSHFYQESAPSQSDVALV	RYVNPDTGRTIFEAKLHROGFITIAATGSNPVVVPPNGYFRFDSWVNOFYALAPMGTGNGRRRIQ
GIV.NA1/1-554	459 QKIHCLLPQQFITHFFDSQAPALAEAALL	RYVHPDSSRVLFETKLYREGFMVVSAP T - GREDEPLDGYFREDSWVNSFYVLSPVGSGQGRRGRSKVV
GII.22/1-541	448 PAIDCLL POEWIGHFYGE SAPSOT SVALI	RYVNPDTGRVLFEGKLHROGFITIAKS GDGPIVVPPNGYFRFDSWVNOFYSLAPMGNGNGRRRIG
GIL27/1-543 GIV 2/1-578	483 OK HOLL PNEELTHLEDLOAPALAEAALL	
GII.23/1-543	450 PFIDCLVPQEWVAHFYQESAPAQTDVALI	RYVNPETGRVLFEAKLHROGFITVAKS - GDSPINVPANGYFRFDSWVNOFYSLAPMGTGNGRRRIG
GII.19/1-548	455 GWLDCLLPQEWIQHFYQD SAAAQSDVALV	RYINPATGRVLFEAKLHROGFLTVSAAGPHPVVAPADGYFRFESWVNOFYTLAPMGNGSGRRRVY
GII.18/1-557	464 SVLDCLLPQEWIQHFYQESFAPQSDIALL	RYVNPDTGRVLFEAKLHROGYITVAGAGTSPVAVPPNGYFRFESWVSQFYTLAPMGSGTGRRRNQ
GII.12/1-535	442 PVIDCLL POEWIGHLYGE SAPSOSDVALI	RFINPDTGRVLFEGKLHRSGYIVANT - GSRFIVPANGYFRFDSWVNOFYSLAPMGTGNGRRRV0
GII 9/1-537	444 GENDOLL POFEVOHEYOFAATARSEVALL	REVNEDTGRAN FESKI HKOGFMUSA
GIX.1/1-555	459 GEIDSLLPQEWITHFYAEQAPTQGDAALL	RYYNPDTGRVLFECKLHREGFITINYT GSNALAVPVNGVFRFEGWVNKFYTLTPMGNGNGRRGRRREL
GII.24/1-542	449 SHIDCLVPQEWIQHFYQESAPSQTDVALI	RYVNPETGRVLFEAKLHRQGYITVARSGSSPINVPANGYFRFDSWVNQFYSLAPMGTGNGRRRIQ
GNA2.1/1-555	459 WYIDCLLPNEYVSHFYSLAAPRRGDLALV	RYINPESNEVLFEAKLHPEGFFTISGA - NEGPLVFPTNGYFRFDSWVSRFYTLSPVGSGSGRSGRTKAH -
GII.4/1-539 GII.6/1-550	446 MNLDCLLPQEWVLHFYQEAAPAQSDVALL	REVINED TO RELEASE TO CALLER SOTT VAN OF VDL VLPPNGTFRED SWANDET TLAPMONGTORK AL
GII.7/1-540	447 GYMDCL VPQEWVQHFYQEAATAQSEVAL I	REINPDTGRVLFEAKLHKOGFLTVAHT - GDNPLVMPPNGYFRFEAWVNOFYSLAPVGTGNGRBBVG
GII.NA2/1-542	449 TAIDCLVPQEWVQHFYQESAPAQSDVALL	RYVNPETGRVLFEAKLHROGFLTVARSGESPINVPANGYFRFDSWVNOFYSLAPMGTGNGRRRFQ
GII.20/1-534	442 TN IDCL I PQEWVQHFYQEGAPARTDVALL	REINPDIGEVLEECKLHKHGEITVAYS - GNHDLVMPPNGYEREESWVNQEHTLAPMGIGRAEEIQ
GII.26/1-542	449 STIDCLVPQEWIQHFYQESAPSQTDVALI	YVNPELGRVLFEAKLHNOGFITVAKT - GDSPINVPANGYFEFDSWVNSFYSLAPMGTGNGRRRIG
GV 2/1-588	494 OPIDCAL POEFVTHLATHGYTLOSDALLL	RYRNIR TGOLICEAKI YAEGELALSYS. GTGPLOL PTDGVFFV SWORLYOLAPVGTSTAGESLGS.
GX.1/1-540	448 ATLDCAL PAEWI TWFL VHNF VPQGDAALL	RYRNRGTGQILFECKLYKEGFLAVAGV - S-TRTVFPTSGVFEFVSWVPVFYQLTPVGSSGARKEQR
GV.1/1-541	447 EAIDCAL POEFVSWFASNAFTVOSEALLL	RYRNTLTGQLLFECKLYNEGYIALSYSGSGPLTFPTDGIFEVVSWVPRLYQLASVGSLATGRMLKQ

Supplementary Figure 6 | **Sequence alignment of HuNoV VP1 from 49 genotypes.** Multiple sequence alignment was carried using ClustalW and visualized by Jalview. The conservation of Histidine residue in all the genotypes, except GX.1 and GV.1, shown by the blue arrow at the top. The sequences we selected based on structure availability in Fig. 8 are indicated by red arrows.

Genotype	Strain	Expression	VP2	Structure	Resolution	Symmetry	P dimer	PDB or EMDB	Reference
GI.1	Norwalk	Insect cells	+	Crystal	3.4 Å	T = 3	Resting	PDB: 1IHM	Prasad (1999)
GI.1	Norwalk	Tobacco leaves	-	Cryo-EM	2.9 Å	T = 3	Resting	PDB: 6OUT	Jung (2019)
								EMD-20199/EMD-20205	
GI.7	Houston	Insect cells	+	Cryo-EM	2.9 Å	T = 3	Resting	PDB: 60U9	Jung (2019)
								EMD-20197/EMD-20198	
GII.2	SMV	Tobacco leaves	-	Cryo-EM	3.1 Å	T = 3	Resting	PDB: 60TF	Jung (2019)
								EMD-20195	
GII.2	SMV	Tobacco leaves	-	Cryo-EM	2.7 Å	T = 1	Resting	PDB: 6OUC	Jung (2019)
								EMD-20201/EMD-20202	
GII 4	Minerva	Tobacco leaves	-	Cryo-EM	4.1 Å	T = 4	Raised	PDB: 60UU	Jung (2019)
								EMD-20206	
GII.4	NSW-2012	Insect cells	-	Cryo-EM	7.3 Å	T = 4	Raised	EMD-4550	Devant (2019)
GII.4	CHDC-1974	Insect cells	-	Cryo-EM	6.1 Å	T = 4	Raised	EMD-4549	Devant (2019)
GII.4	GII.4c	HEK-293T	-	Cryo-EM	4.5 Å	T = 4	Raised	EMD-10758	Devant (2021)
GII.4	GII.4c	Insect cells	-	Cryo-EM	4.2 Å	T = 4	Raised	EMD-10755	Devant (2021)
GII.4	GII.4c	Insect cells	-	Cryo-EM	8.0 Å	T = 3	Raised	EMD-10756	Devant (2021)
GII.4	GII.4c	Insect cells	-	Cryo-EM	4.2 Å	T = 1	Raised	EMD-10757	Devant (2021)
GII.10	Vietnam 026	Insect cells	-	Cryo-EM	10 Å	T = 3	Raised	EMD-5374	Hansman (2012)
GII.17	Kawasaki 2014	Insect cells	-	Cryo-EM	5.8 Å	T = 3	Raised	EMD-10759	Devant (2021)
GII.4	Houston	Insect cells	+	Crystal	3.0 Å	T = 3	Resting	PDB: 7K6V	This study
GII.4	Houston	Insect cells	+	Cryo-EM	3.8 Å	T = 3	Resting	PDB 7MRY EMD-23960	This study

Supplementary Table 1 | Human norovirus VLP structures.

PDB ID	7K6V				
Wavelength (Å)	0.9795				
Resolution range (Å)	29.99 - 3.0 (3.1 - 3.0)				
Space group	I 2 2 2				
Unit cell					
a, b, c (Å)	420.2, 446.6 464.0				
α, β, γ (°)	90, 90, 90				
Unique reflections	819642 (61796)				
Multiplicity					
Completeness (%)	95.67 (72.59)				
Mean I/sigma(I)	7.0/1.0				
Wilson B-factor	67.97				
R-merge (%)	13.6 (83.5)				
R-work (%)	20.71 (31.13)				
R-free (%)	24.18 (36.21)				
Number of non-hydrogen atoms	176522				
macromolecules	176443				
ligands	76				
solvent	3				
Protein residues	22902				
R.M.S. deviations					
Bond lengths (Å)	0.004				
Bond angles (°)	0.77				
Ramachandran					
Favored (%)	96.72				
Allowed (%)	3.28				
Disallowed (%)	0				
Average B-factor	67.79				
macromolecules	67.78				
ligands	80.77				
solvent	41.45				

Supplementary Table 2 | X-ray crystallography data collection and refinement statistics.

Statistics for the highest-resolution shell are shown in parentheses.

Supplementary Table 3 | Cryo-EM data collection, reconstruction, and model refinement

parameters.

	HOV VLP					
	(EMDB-23960)					
	(PDB 7MRY)					
Data collection and						
processing						
Magnification	30,000					
Voltage (kV)	300					
Electron exposure $(e - / Å^2)$	55.96					
Defocus range (µm)	0.6 to 2.6					
Pixel size (Å)	1.24					
Symmetry imposed	I2					
Map resolution (Å)						
FSC threshold	0.143					
Map resolution (Å)	3.8					
Refinement						
Initial model used (PDB code)	7K6V					
Model resolution (Å)	3.8					
FSC threshold	0.143					
Map sharpening <i>B</i> factor (Å ²)	-210.9					
Model composition						
Non-hydrogen atoms	11329					
Protein residues	1460					
Ligands	0					
<i>B</i> factors (Å ²)						
Protein	72.34					
Ligand	0					
R.m.s. deviations						
Bond lengths (Å)	0.002					
Bond angles (°)	0.529					
Validation						
MolProbity score	1.45					
Clashscore	5.47					
Poor rotamers (%)	0					
Ramachandran plot						
Favored (%)	97.11					
Allowed (%)	2.89					
Disallowed (%)	0					