

## Supplementary Tables

**Table S1, related to Figure 1. Plasmids used in this study.**

AP-1 core	Hs AP1S3/His-TEVsite-Hs AP1B1 (1-584)/Mm AP1G (1-595)-TEVsite-GST/Mm AP1M1 in pST44 vector
AP-1 $\Delta\beta$ Arf	AP1B1_I85D/V88D in AP-1 core/pST44 construct
AP-1 $\Delta\gamma$ Arf	AP1G_L68D/L71E/L102E in AP-1 core/pST44 construct
Arf1	His-TEVsite-Hs Arf1 (17-181)-Q71L in pHis2 vector
Tetherin-Nef	His-TEVSite-Hs Tetherin (1-21)- GSDEASEGSG-NL4-3 Nef in pHis2 vector
Tetherin-Nef_LL/AA	His-TEVSite-Hs Tetherin (1-21)- GSDEASEGSG-NL4-3 Nef-L164A/L165A in pHis2 vector
Tetherin-MRCA Nef	His-TEVSite-Hs Tetherin (1-21)- GSDEASEGSG-O-MRCA Nef in LIC 1B vector
MHC-Nef	His-TEVSite-Hs MHC-I (338-365)-NL4-3 Nef in pHis2 vector
Nef	His-TEVSite-NL4-3 Nef in pHis2 vector
His-MBP-CK1	His-MBP-TEVsite-Rat CK1 $\delta$ (1-317) in LIC 2C-T vector
HIV-1 $\Delta$ Vpu $\Delta$ Nef	NL4-3 HIV-1 provirus with deletion of nt3-nt146 in vpu and aa 1,4 and 5 mutated to stop in nef in pBR vector
O-MRCA Nef	OMRCA Nef with C-terminal AU1-tag in pCG IRES-eGFP vector
NL4-3 Nef	NL4-3 Nef with C-terminal AU1-tag in pCG IRES-eGFP vector
O-MRCA Nef C174S	OMRCA Nef C174S with C-terminal AU1-tag in pCG IRES-eGFP vector
NL4-3 Nef S169A	NL4-3 Nef S169A with C-terminal AU1-tag in pCG IRES-eGFP vector
Vpu	vpu with C-terminal AU1-tag in pCG IRES-eGFP vector
Empty vector control	nef with stop codon at position 2,3 and 4 in pCG vector
Tetherin	human tetherin in pCG vector

**Table S2, related to Figure 1. EM and model statistics.**

Map	Wild type Monomer	Wild type Trimer monomeric	Wild type Trimer	Wild type Trimer (stable subset)	Mutant L2A Dimer monomeric	Mutant L2A Dimer
<b>Data collection</b>						
Microscope	FEI Titan Krios	FEI Titan Krios	FEI Titan Krios	FEI Titan Krios	FEI Titan Krios	FEI Titan Krios
Voltage (kV)	300	300	300	300	300	300
Detector	Gatan K2	Gatan K2	Gatan K2	Gatan K2	Gatan K2	Gatan K2
Recording mode	Super	Super	Super	Super	Super	Super
Magnification	53,350	53,350	53,350	53,350	53,350	53,350
Movie micrograph pixel size (Å)	1.067	1.067	1.067	1.067	1.067	1.067
Dose rate (e <sup>-</sup> /Å <sup>2</sup> /sec)	6.57	6.57	6.57	6.57	7.09	7.09
Number of frames per movie micrograph	38	38	38	38	28	28
Frame exposure time (ms)	250	250	250	250	200	200
Movie exposure time (ms)	9,500	9,500	9,500	9,500	5,600	5,600
Total dose (e <sup>-</sup> /Å <sup>2</sup> )	62.42	62.42	62.42	62.42	39.7	39.7
Defocus range (um)	-0.75 to -2.00	-0.75 to -2.00	-0.75 to -2.00	-0.75 to -2.00	-0.75 to -2.50	-0.75 to -2.50
<b>EM data processing</b>						
EMDB code	7455	7457	7458	7563	7453	7454
Complex ordered molecular weight (kDa)	233	233	698	698	466	233
Number of movies/micrographs	2,200	2,200	2,200	2,200	1,989	1,989
Box size (px)	384	224	384	384	256	384
Particle number (total)	252,212	252,212	252,212	252,212	399,032	399,032
Particle number (post 2D)	209,816	209,816	209,816	209,816	162,509	162,509
Particle number (post 3D)	53,123	61,929	61,929	11,108	85,804	42,902
Particle number (used in final map)	53,123	53,841	61,929	11,108	85,592	42,902
Refinement angular accuracy (°)	2.61	2.28	2.70	1.66	1.43	1.12
Refinement offset accuracy (Å)	1.34	0.99	1.90	1.22	0.94	0.54
Symmetry	C1	C1	C1	C1	C1	C1
Map resolution (FSC 0.143)	3.90	3.73	4.27	6.83	4.27	6.72
Measured map B-factor (Å <sup>2</sup> )	-93.6	-136.6	-124.6	-277.2	-151.2	-398.7
CryoEF score	0.77	0.76	0.78	0.70	0.46	0.47
CryoEF worst/best PSF resolution (Å)	4.39/2.29	4.58/2.45	4.39/2.45	5.20/2.45	7.18/2.18	6.96/2.18
3DFSC sphericity	0.97	0.99	0.96	0.98	0.77	0.97
<b>Structure Building and Validation</b>						
PDB ID	6DFF	6CM9		6CRI	6D83	6D84
Number of atoms in deposited model	16,594	17,683		53,049	16,588	33,176
MolProbity clashscore, all atoms	4.64	5.85		11.73	6.08	8.05
MolProbity score	1.59	1.81		1.94	1.69	1.76
EMRinger score	2.07	2.28		-	1.15	-
Refinement program	Phenix	Phenix		Phenix	Phenix	Phenix
RMSD from ideal						
Bond length (Å)	0.01	0.01		0.01	0.01	0.01
Bond angles (°)	1.17	1.32		1.23	1.19	1.16
Ramachandran plot						
Favored (%)	94.9	92.0		94.8	95.0	95.4
Allowed (%)	5.1	8.0		5.2	5.0	4.6
Outliers (%)	0.0	0.0		0.0	0.0	0.0
Favored rotamers (%)	100.0	99.7		99.9	100.0	99.9
Average atomic	84.6	29.3		425.2	89.2	381.7
FSC (0.5) model-vs-map	3.99	3.85		8.04	4.35	8.93
CC model-vs-map (masked)	0.81	0.76		0.79	0.76	0.69

