**SI APPENDIX**

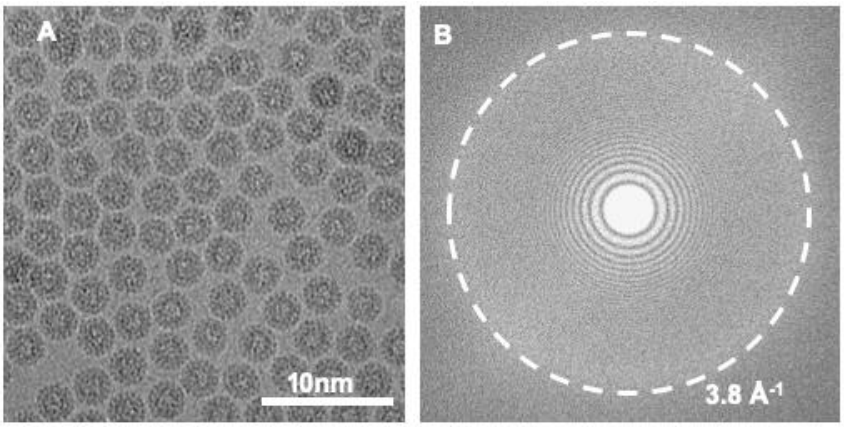


**Figure S1. (A)** Representative cryoEM micrograph of BMV3+4. Scale bar is 10 nm. **(B)** Fourier transform power spectrum of the micrograph pictured in (A). The outer ring is the diffraction ring associated with $H_2O$, at a resolution of 3.8 Å.

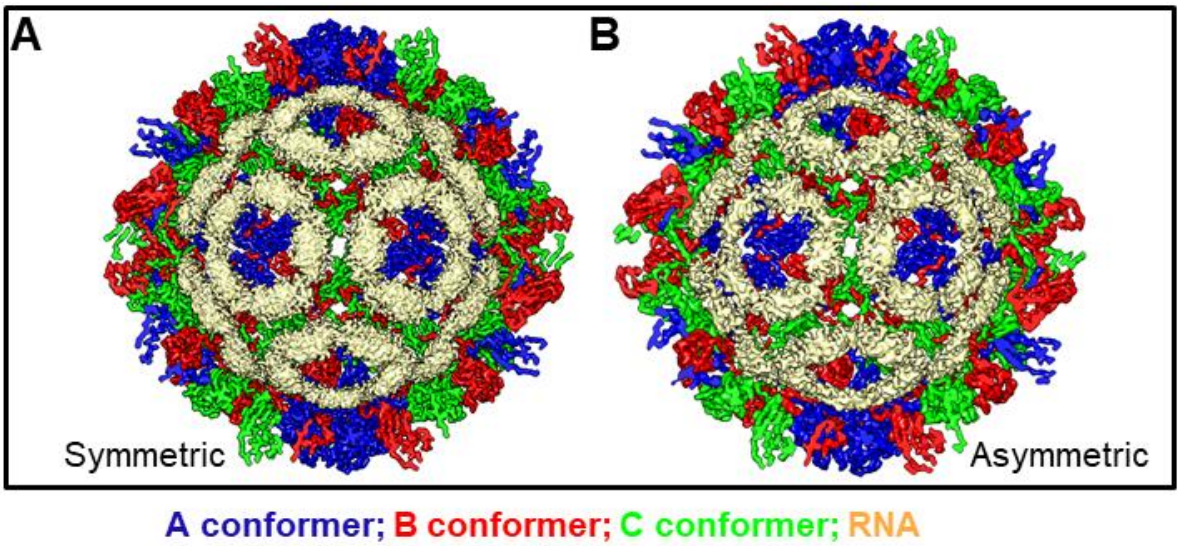


**Figure S2. (A,B)** Interior view of the 3.1 Å symmetric (A) and 3.9 Å asymmetric (B) reconstructions showing that they are essentially identical.

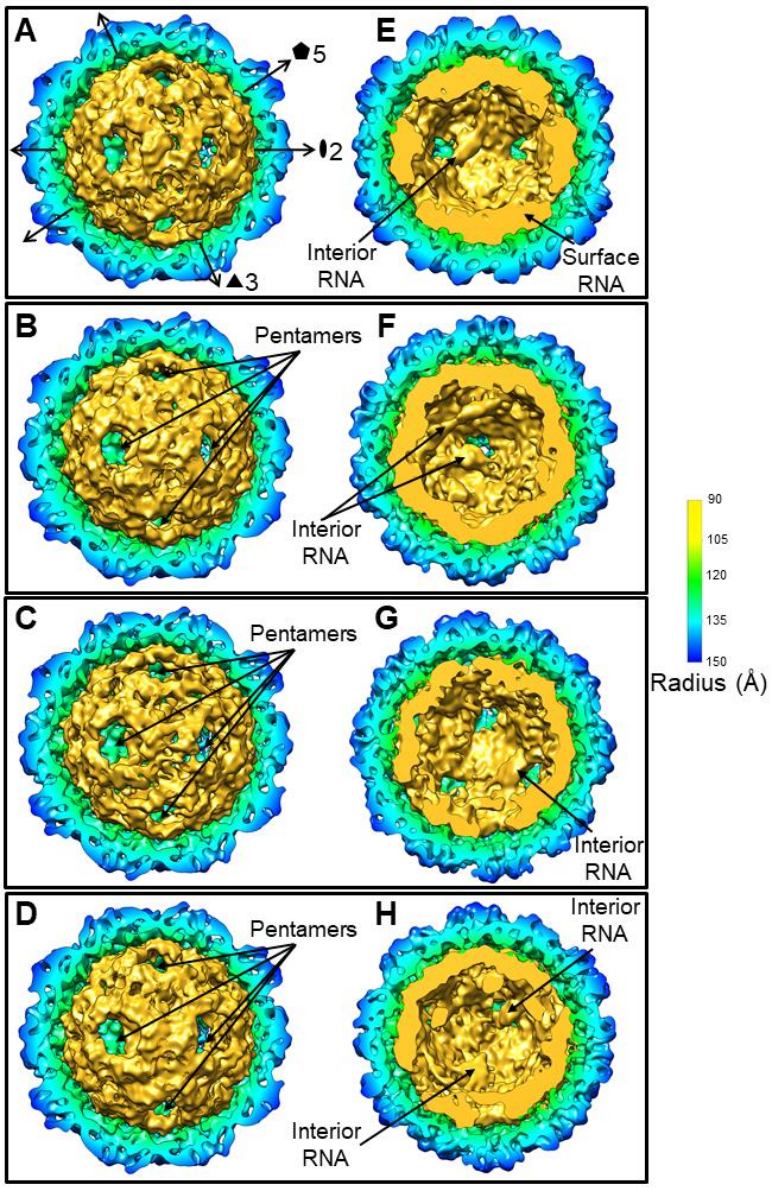


**Figure S3.** Capsid-subtracted reconstruction shows an ensemble of RNA structures. (**A**-**H**) Interior radially-colored view of four representative structures from capsid-subtracted reconstructions showing the back half of the capsid protein shell, and either the entire (**A**-**D**) or the back half (**E**-**H**) of the RNA genome colored orange. For guidance the capsid symmetry axes are shown on the RNA genome in (**A**) and the location of the pentameric capsomers is indicated in (**B**). While all of the RNA structures involve most of the RNA as a disordered ring near the surface (Surface RNA) of the hexameric capsomers, each structure is unique and several have some RNA near the center of the particle (Interior RNA).

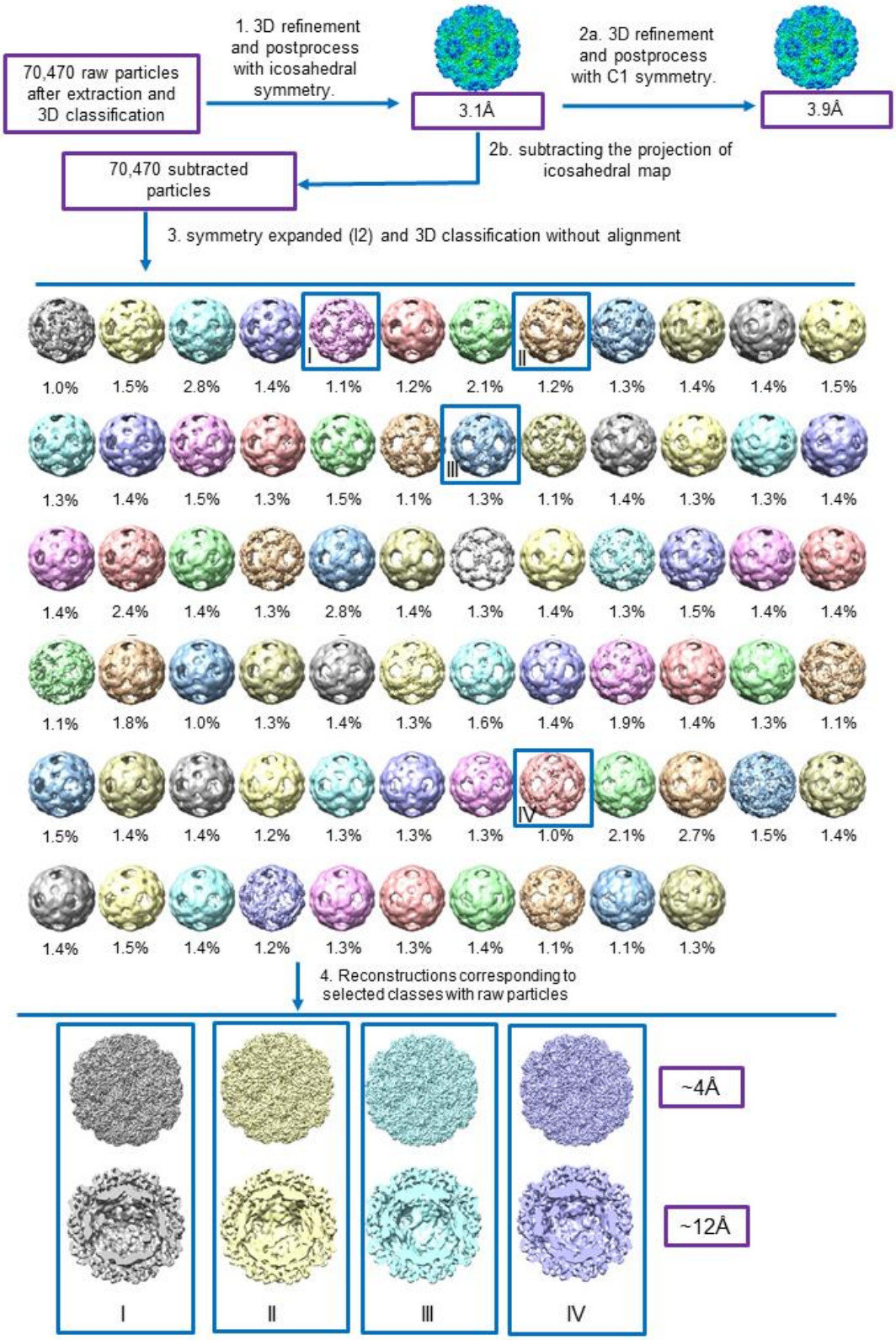


**Figure S4.** Workflow for reconstructions of the whole virus particles, including virions by symmetric (I2) reconstruction, virions by asymmetric (C1) reconstruction, and capsid-subtracted core particles by asymmetric reconstruction.

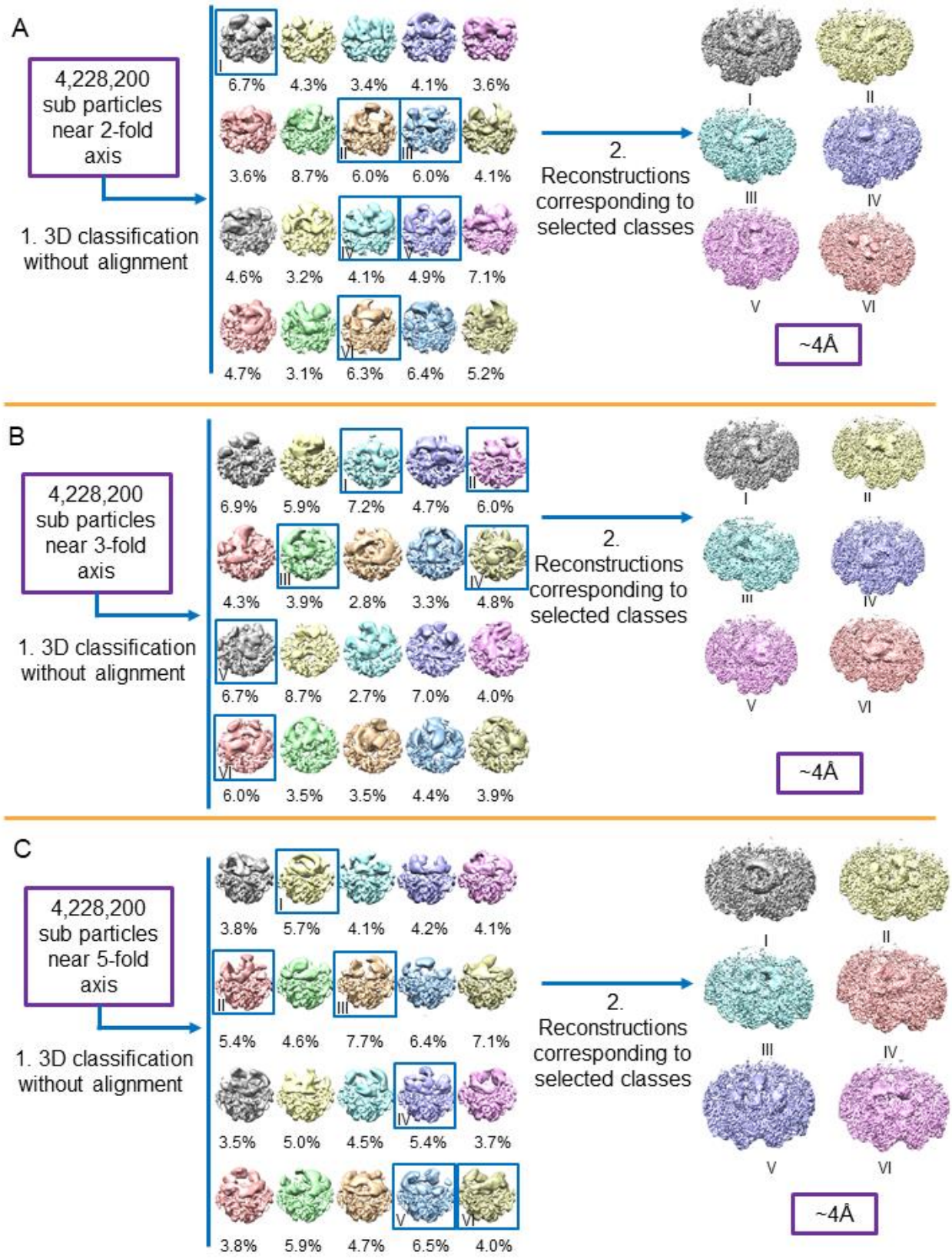


**Figure S5.** Workflow for sub-particle reconstructions for sub-particles extracted around the 2-fold symmetry axes (A), 3-fold symmetry axes (B) and 5-fold symmetry axes (C).

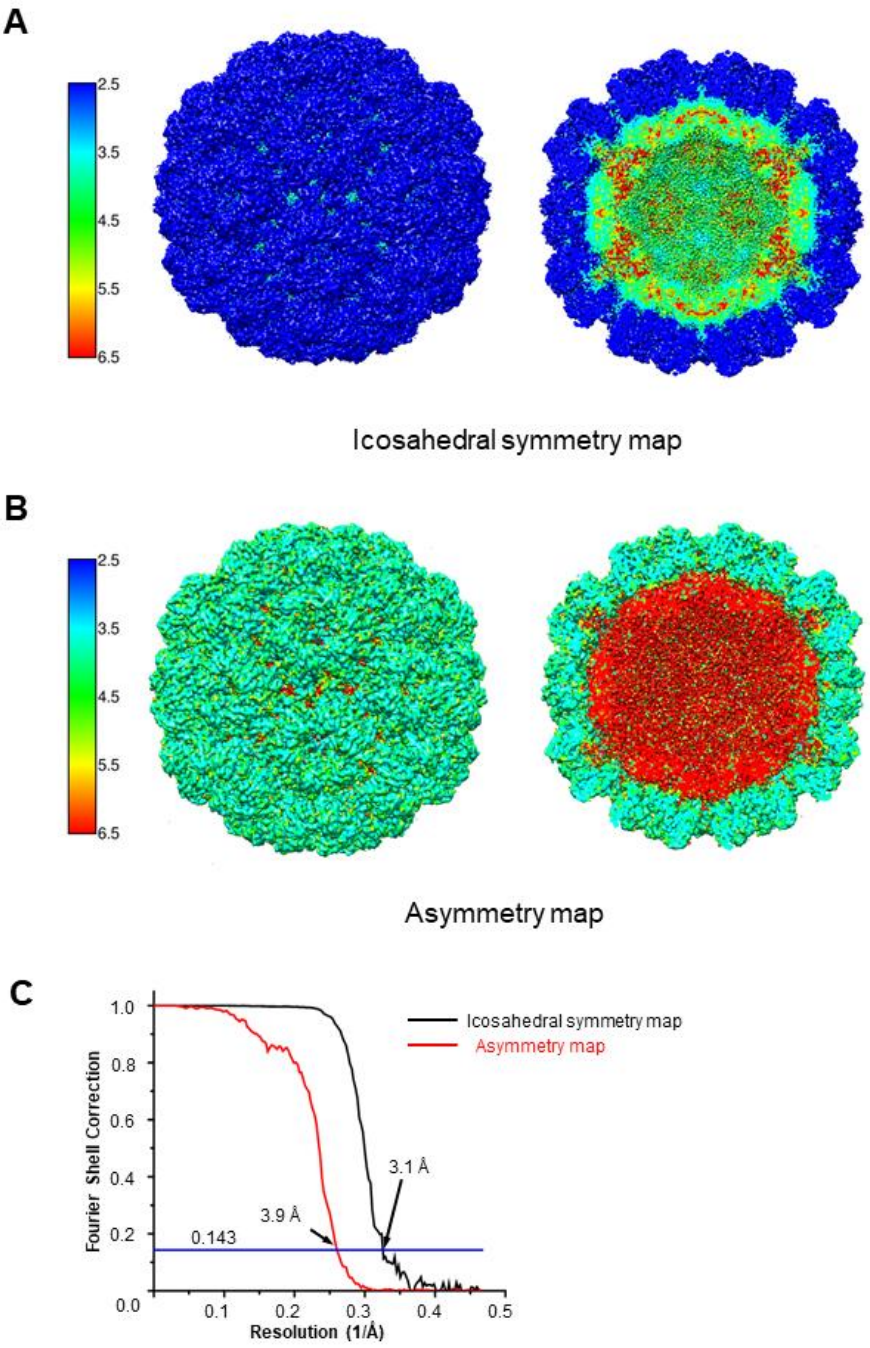


**Figure S6.** Resolution assessments. **(A,B)** Local resolutions of the icosahedral (I2) symmetric **(A)** and C1 asymmetric **(B)** reconstructions as determined by *ResMap* (66). Unit in the color bars is angstrom (Å). **(C)** Global resolution evaluation based on "gold standard" Fourier shell correlation (FSC) of both the symmetric reconstruction (3.1 Å) and the asymmetric reconstruction (3.9 Å). Note that the structures show high-resolution features of amino acid side chains that support *de novo* atomic modeling.

# Supplementary table 1: Cryo-EM data collection, processing, model refinement and validation statistics

| | Icosahedral symmetry reconstruction (EMD-21260; PDB: 6VOC) | C1 symmetry reconstruction (EMD-21261) | RNA-focused C1 symmetry reconstructions (class I: EMD-21279 class II: EMD-21280 class III: EMD-21281 class IV: EMD-21282) | 2-fold sub-particle reconstructions (class I: EMD-21283 class II: EMD-21284 class III: EMD-21285 class IV: EMD-21286 class V: EMD-21287 class VI: EMD-21288) | 3-fold sub-particle reconstructions (class I: EMD-21289 class II: EMD-21290 class III: EMD-21291 class IV: EMD-21292 class V: EMD-21293 class VI: EMD-21294) | 5-fold sub-particle reconstructions (class I: EMD-21295 class II: EMD-21296 class III: EMD-21297 class IV: EMD-21298 class V: EMD-21299 class VI: EMD-21300) |
|---|---|---|---|---|---|---|
| **Data collection and processing** | | | | | | |
| Magnification | | | 130,000× | | | |
| Voltage (kV) | | | 300kV | | | |
| Electron exposure ($e^{-}$/Å$^2$) | | | 48 | | | |
| Defocus range (μm) | | | 0.8-3 | | | |
| Pixel size (Å) | | | 1.07 | | | |
| Symmetry imposed | I2 | | C1 | | | |
| Used particle images (no.) | 70,470 | 70,470 | class I: 47,676 class II: 48,699 class III: 53,652 class IV: 41,934 | class I: 286,153 class II: 251,607 class III: 252,697 class IV: 173,336 class V: 208,131 class VI: 267,320 | class I: 307,099 class II: 251,553 class III: 163.362 class IV: 200,647 class V: 283,819 class VI: 253,402 | class I: 243,444 class II: 226,088 class III: 327,402 class IV: 226,414 class V: 274,177 class VI: 168,464 |
| Map resolution (Å) | 3.1 | 3.9 | 4.0 | | | |
| Map sharpening *B* (Å$^2$) | -80 | -80 | | | | |
| FSC threshold | 0.143 | 0.143 | | | | |
| Map resolution range (Å) | 2.9-30 | 3.6-30 | 4.0-30 | | | |
| **Refinement** | | | | | | |
| Initial model used | 3J7L | | | | | |
| Model resolution (Å) | 3.0 | | | | | |
| FSC threshold | 0.5 | | | | | |
| Model resolution range (Å) | 2.6-3.2 | | | | | |
| Model composition | | | | | | |
| Nonhydrogen atoms | 3,570 | | | | | |
| Protein residues | 476 | | | | | |
| Ligands | 0 | | | | | |
| *B* factors (Å$^2$) | | | | | | |
| Protein | 90.32 | | | | | |
| Ligand | | | | | | |
| R.m.s. deviations | | | | | | |
| Bond lengths (Å) | 0.006 | | | | | |
| Bond angles (°) | 0.891 | | | | | |
| Validation | | | | | | |
| MolProbity score | 1.10 | | | | | |
| Clashscore | 3.05 | | | | | |
| Poor rotamers (%) | 0.00 | | | | | |
| Ramachandran plot | | | | | | |
| Favored (%) | 98.09 | | | | | |
| Allowed (%) | 1.91 | | | | | |
| Disallowed (%) | 0 | | | | | |

**Legends for Supplementary Movies**

**Movie 1.** The video begins with an exterior view of the 3.1 Å symmetric density map colored radially. After one rotation, most of the capsid is colored grey except for a single asymmetric unit composed of the A, B and C conformers which are colored yellow, blue and purple, respectively. We then zoom in on the density map of the asymmetric unit, and rotate it 180° while zooming in on the C conformer. The density map rocks vertically once, and then the atomic model is fit into the now translucent density. The video slowly zooms out and reverts back to the original radially colored capsid exterior. After some rotation, the front-half of the capsid density is hidden showing the internal RNA genome (orange). The map rocks horizontally and then vertically.

**Movie 2.** The movie begins with a radially colored exterior view of a representative class generated by capsid subtraction and asymmetric reconstruction. After some rotation, the front-half of the capsid density is hidden showing the internal RNA genome (orange). The map then rocks horizontally one time. The capsid is then completely hidden, and three more representative 3D classes are shown (only the RNA). The four maps then rock horizontally one time.

**Movie 3.** Exterior view of a representative map generated by 3-fold sub-particle reconstruction. The map has been colored by CP conformer, with the A, B and C conformers colored blue, red and green, respectively. The map rocks horizontally once, and then is rotated 180° to show the RNA genome (orange). Then most of the capsid is colored gray, leaving only the last 10 amino acids of each CP colored by conformer, and the structure rocks horizontally once. The map is then rotated 90°, and half of the density is slowly removed to

show the interior of the density map. This view highlights that the N-termini of the B and C conformers are interacting with the RNA. We then zoom out and remove the capsid density entirely, leaving behind the RNA. 5 more representative classes are then shown (only the RNA), and these maps rock horizontally and then vertically.

**Movie 4.** Exterior view of a representative map generated by 5-fold sub-particle reconstruction. The map has been colored by CP conformer, with the A, B and C conformers colored blue, red and green, respectively. The map rocks horizontally once, and then is rotated 180° to show the RNA genome (orange). Then most of the capsid is colored gray, leaving only the last 10 amino acids of each CP colored by conformer, and the structure rocks horizontally once. The map is then rotated 90°, and half of the density is slowly removed to show the interior of the density map. This view highlights that the N-termini of the A conformers are far away from the RNA. We then zoom out and remove the capsid density entirely, leaving behind the RNA. 5 more representative classes are then shown (only the RNA), and these maps rock horizontally and then vertically.

**Movie 5.** Exterior view of a representative map generated by 2-fold sub-particle reconstruction. The map has been colored by CP conformer, with the A, B and C conformers colored blue, red and green, respectively. The map rocks horizontally once, and then is rotated 180° to show the RNA genome (orange). Then most of the capsid is colored gray, leaving only the last 10 amino acids of each CP colored by conformer, and the structure rocks horizontally once. The map is then rotated 90°, and half of the density is slowly removed to show the interior of the density map. This view highlights that the N-termini of the B and C conformers are interacting with the RNA, while the N-termini of the A conformers are far

away from the RNA. We then zoom out and remove the capsid density entirely, leaving behind the RNA. 5 more representative classes are then shown (only the RNA), and these maps rock horizontally and then vertically.