
Figures and figure supplements

A putative ATPase mediates RNA transcription and capping in a dsRNA virus

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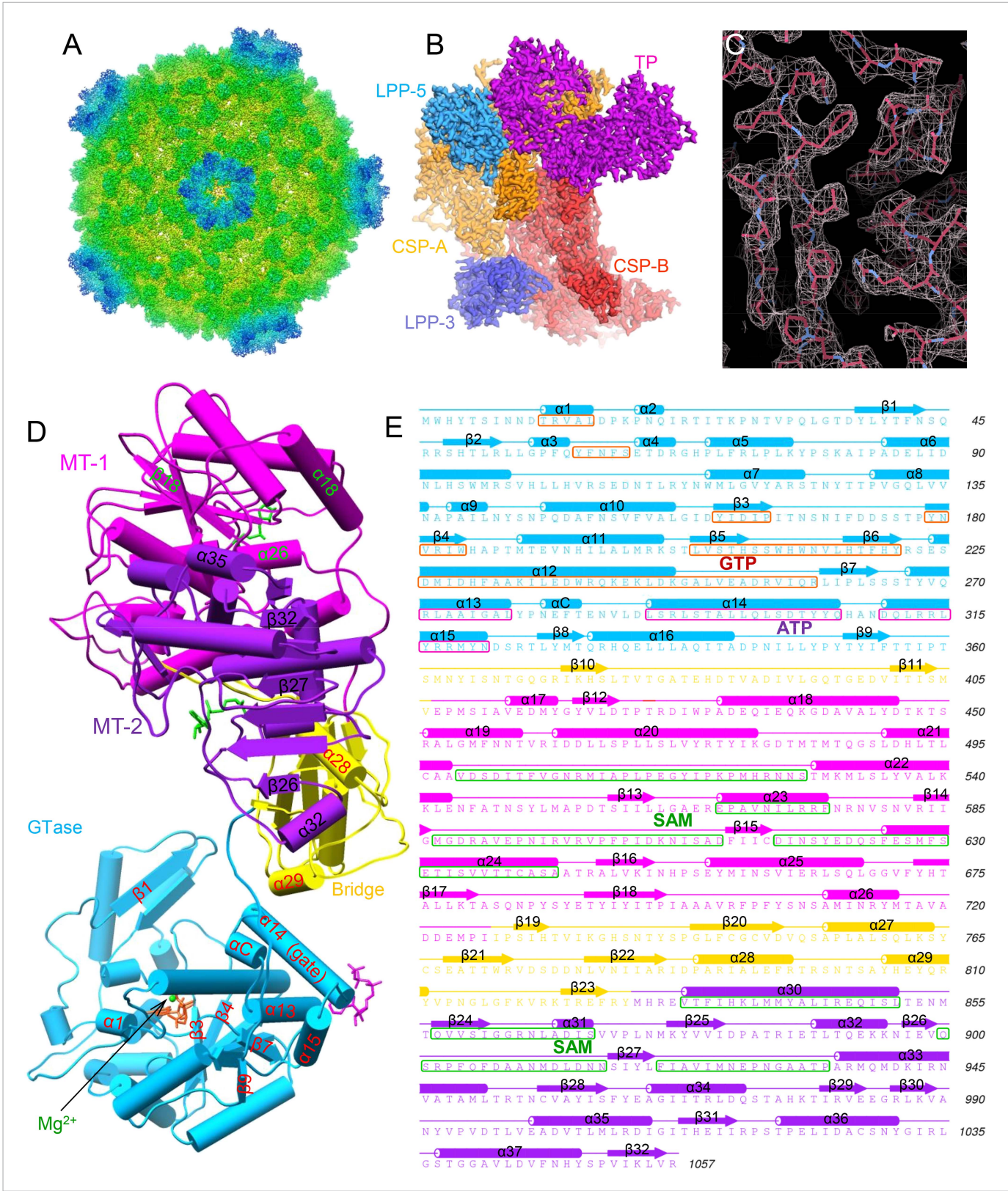


Figure 1. Structural overviews of cytoplasmic polyhedrosis virus (CPV) bound with different ligands involved in regulation and capping for viral RNA transcription. (A) Radially colored G-CPV reconstruction at 2.9 Å resolution as viewed along a fivefold axis. (B) Density map of an asymmetric unit of G-CPV is colored by protein subunit. (C) Density map (mesh) and atomic model (stick) of a selected region from CSP-A of G-CPV, showing characteristic side Figure 1. continued on next page

Figure 1. Continued

chains. (D) Structures of turret protein (TP) and ligands in t-CPV. TP is colored by domain. The Mg^{2+} and GTP in the guanylyltransferase (GTase) site are in green and orange, respectively; ATP in the putative ATPase site is in magenta; the two S-adenosyl-L-methionines (SAMs) in MT-1 and MT-2 are in green. (E) Schematic illustration of t-CPV TP structure. Secondary elements involved in hydrogen bonding or stacking interactions with GTP and ATP are highlighted in orange red and magenta, respectively. Secondary elements involved in interactions with SAM are highlighted in green.

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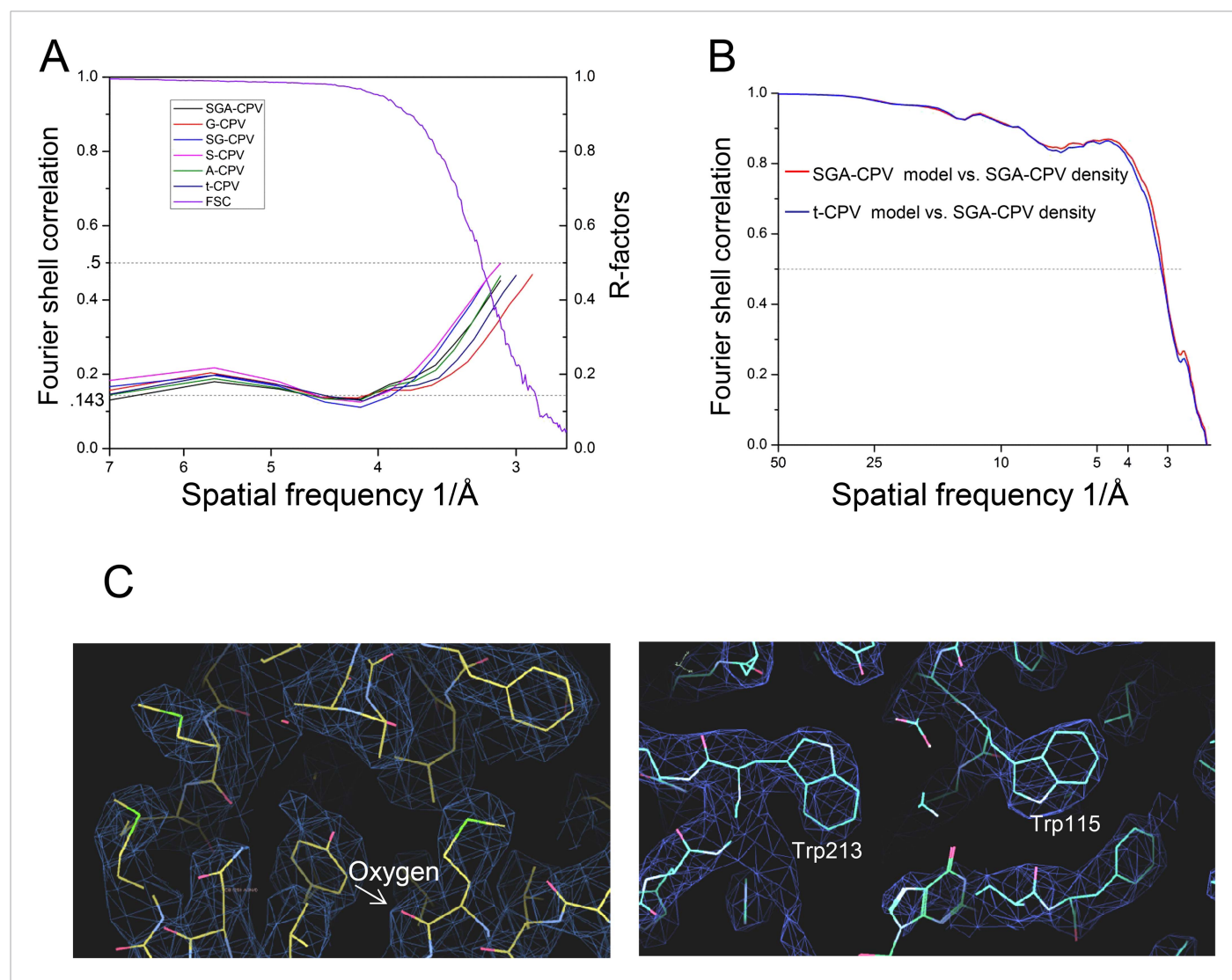


Figure 1—figure supplement 1. Resolution assessment of CPV particle reconstructions. (A) R-factors of the six different CPV particles and Fourier shell correlation coefficient (FSC) of G-CPV. (B) FSC curve between the SGA-CPV map and the SGA-CPV model (red line) and that between the SGA-CPV map and the t-CPV model (blue line). (C) Density maps (mesh) and atomic models (stick) of a selected region from LPP-5 (left) and TP (right) of G-CPV at 2.9 Å resolution, showing characteristic side chains and main chain carbonyl oxygen.

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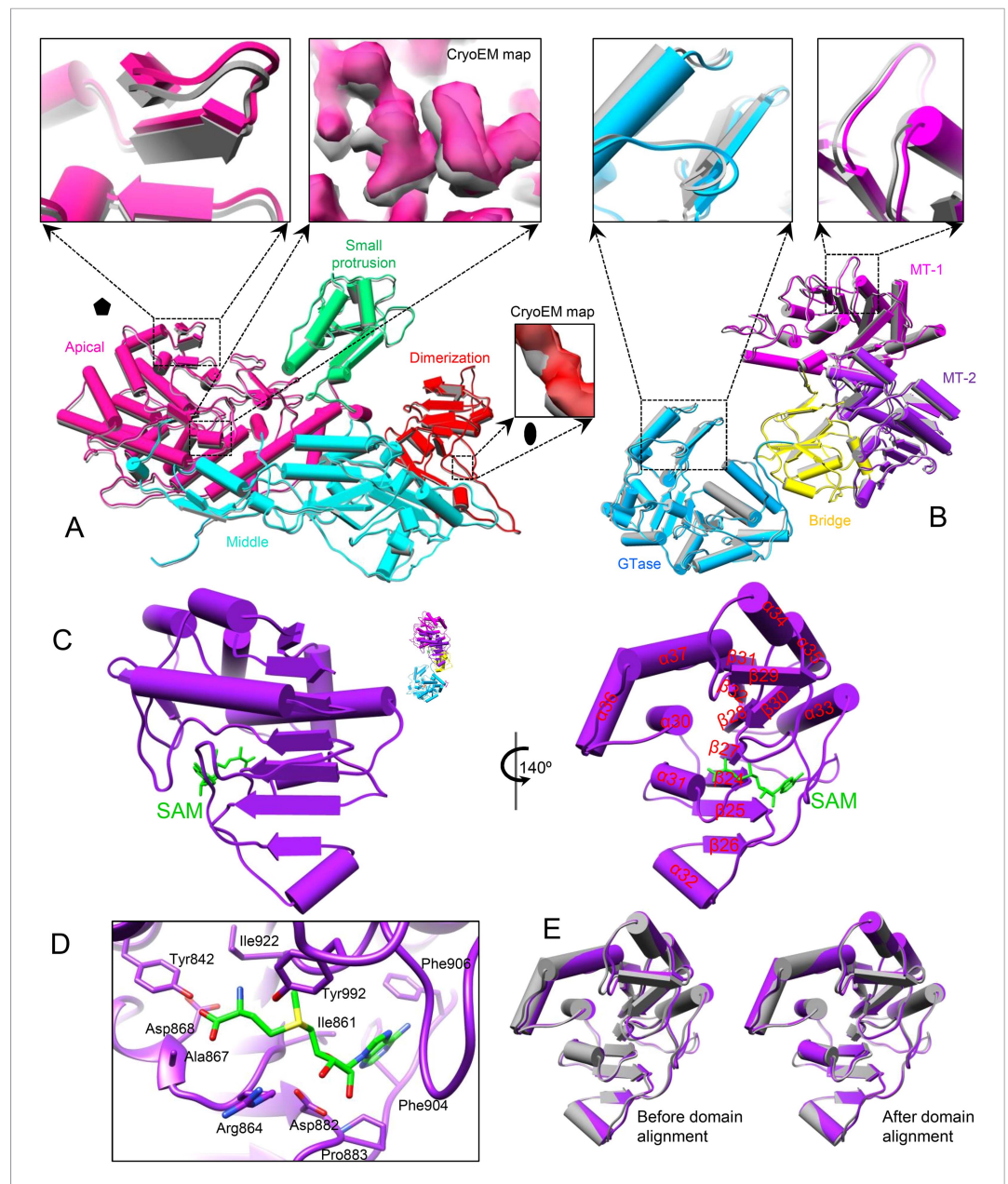


Figure 2. SAM alone binds to MT-2 of TP and triggers global movement of all capsid proteins. **(A)** Superimposition of CSP-A between unliganded CPV (gray) and S-CPV (colored by domain). Insets: zoom-in views of the boxed regions. The twofold and fivefold axes are indicated by a pentagon and an oval, respectively. **(B)** Superimposition of TP between unliganded CPV (gray) and S-CPV (colored by domain as in **Figure 1D**). Insets: zoom-in views of the boxed regions from GTase and MT-1 domains, respectively. **(C)** Structure of MT-2 (purple) and SAM (green). Left, view as the guide map (inset). Right, view rotated as indicated. **(D)** Active site of MT-2. SAM is colored by element: carbon in green, nitrogen in blue, oxygen in red, and sulfur in yellow. Side chains of those amino acids interacting with SAM are shown. **(E)** Superimposition of MT-2 between unliganded CPV (gray) and S-CPV (purple) before (left) and after (right) domain alignment using C α positions.

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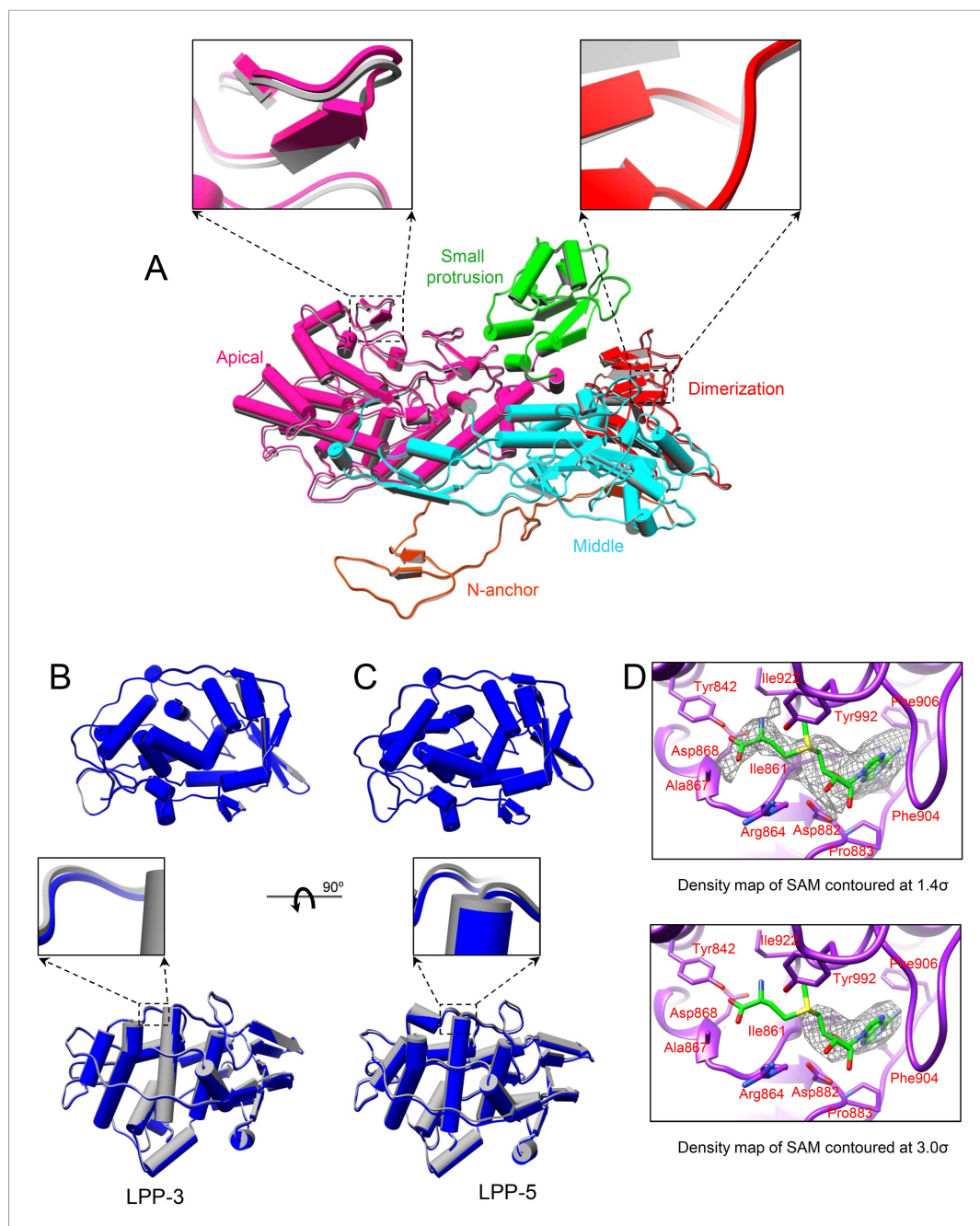


Figure 2—figure supplement 1. Global movement of viral capsid proteins caused by SAM bound to the externally located MT-2. **(A)** Superimposition of CSP-B between unliganded CPV (gray) and S-CPV (colored by domain). Left inset, zoom-in view of a boxed region from apical domain. Right inset, zoom-in view of a boxed region from dimerization domain. **(B)** Superimposition of LPP-3 between unliganded CPV (gray) and S-CPV (blue). Upper: viewed from outside. Lower: view rotated as indicated. Inset: zoom-in view of the boxed region. **(C)** Superimposition of LPP-5 between unliganded CPV (gray) and S-CPV (blue). Upper: viewed from outside. Lower: view rotated as indicated. Inset: zoom-in view of the boxed region. **(D)** Structure of MT-2 active site and the bound SAM in S-CPV. MT-2 is in purple. SAM is colored as in **Figure 2D**. Side chains of amino acids involved in interactions with SAM are shown. Density map of bound SAM is contoured at 1.4σ (upper) and 3.0σ (lower) above the means, respectively.

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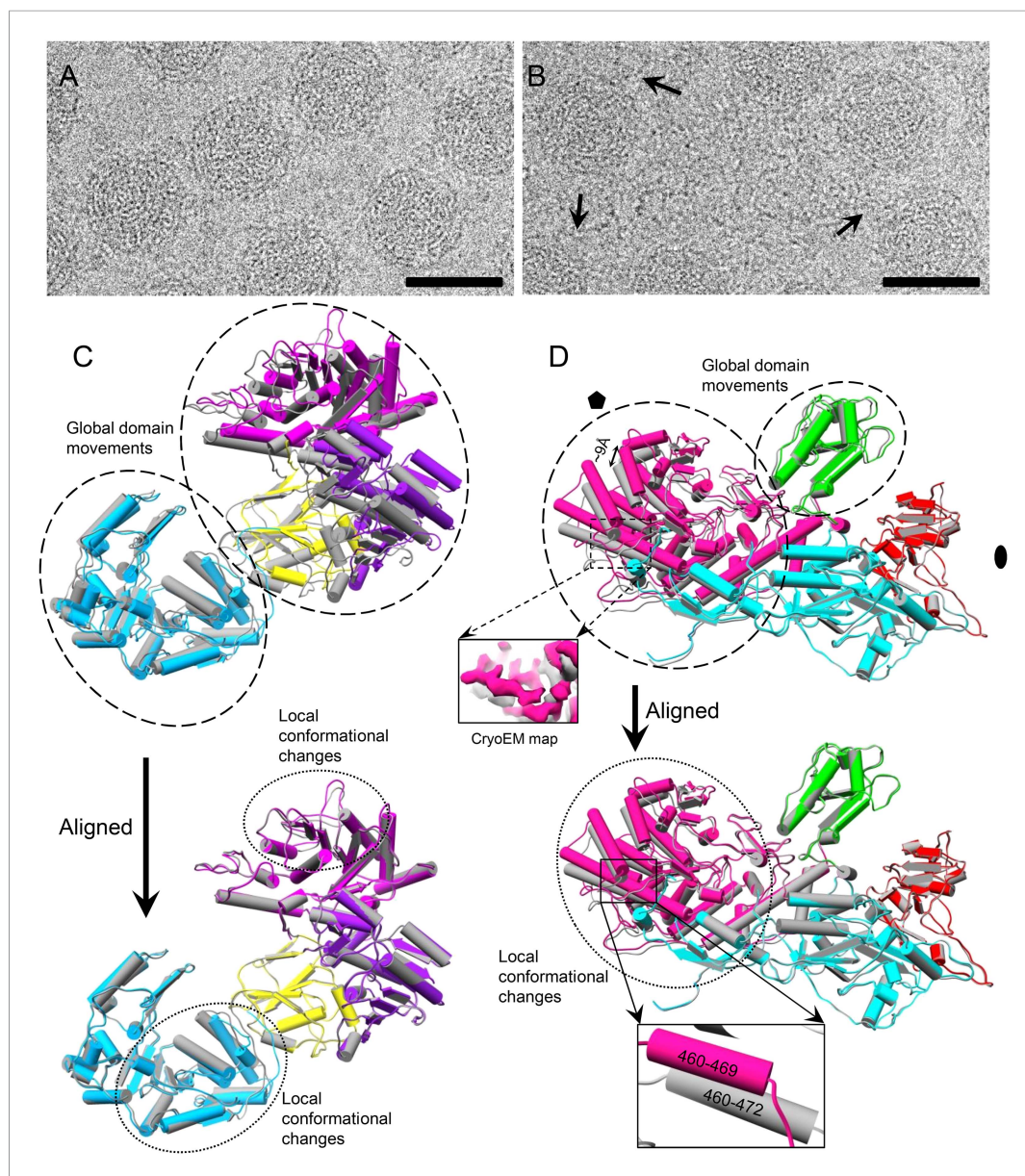


Figure 3. Comparison of S-CPV and t-CPV reveals global protein movements and local conformational changes. (A, B) Cryo-electron microscopy (cryoEM) images of S-CPV and t-CPV. Unlike that of S-CPV (A), the cryoEM image of t-CPV (B) shows characteristic string-like densities emanating from virus particles (arrows). Scale bars, 50 nm. (C) Superimposition of TP between S-CPV (gray) and t-CPV (colored by domain as in **Figure 1D**). Upper, domains that show global movements are indicated by dashed ellipses. Lower, GTase domain of t-CPV was aligned to that of S-CPV using C α positions for residues in small sub-domain. Each of other three domains in t-CPV was aligned to its counterpart in S-CPV using C α positions for residues in each domain. Regions that undergo local conformational changes are indicated by dotted ellipses. (D) Superimposition of CSP-A between S-CPV (gray) and t-CPV (colored as in **Figure 2A**). Upper, domains that show global movements are indicated by dashed ellipses. Inset, density maps of S-CPV (gray) and t-CPV (pink) from the boxed region. Lower, molecules were aligned using C α positions for residues in small protrusion, middle and dimerization domains. Region that undergoes local conformational change is indicated by dotted ellipse. Part (470–472) of a helix (residues 460–472) in S-CPV becomes a loop in t-CPV (inset). DOI: [10.7554/eLife.07901.011](https://doi.org/10.7554/eLife.07901.011)

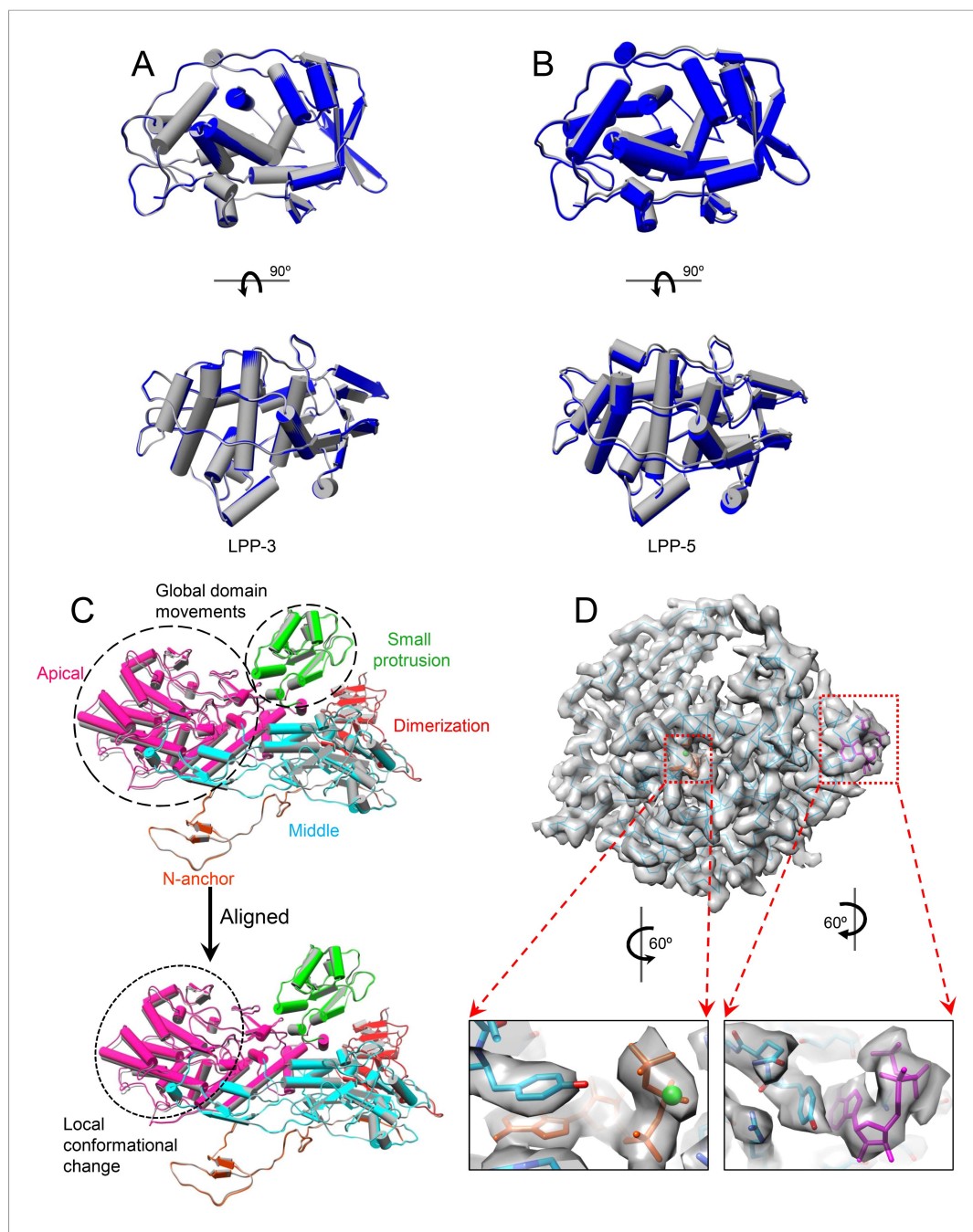


Figure 3—figure supplement 1. Global movements and local conformational changes of capsid proteins observed in t-CPV. (A) Superimposition of LPP-3 between S-CPV (gray) and t-CPV (blue). Upper: viewed from outside. Lower, view rotated as indicated. (B) Superimposition of LPP-5 between S-CPV (gray) and t-CPV (blue). Upper: viewed from outside. Lower: view rotated as indicated. (C) Superimposition of CSP-B between S-CPV (gray) and t-CPV (colored by domain). Upper: domains that show global movements are indicated by dashed ellipses. Lower, the CSP-B molecules were aligned using Ca positions for residues in small protrusion, middle and dimerization domains. Region that undergoes local conformational change is indicated by dotted ellipse. (D) Structure of GTase domain with bound ligands in t-CPV. Density map and atomic model of GTase domain are in transparent gray and sky blue, respectively. Density map is contoured at 3σ above the means. GTP and ATP models are in orange red and magenta, respectively. Left inset, zoom-in view rotated from the boxed region showing the GTP density in the GTase site. Right inset, zoom-in view rotated from the boxed region showing the density of a ligand bound to the large sub-domain of GTase domain.

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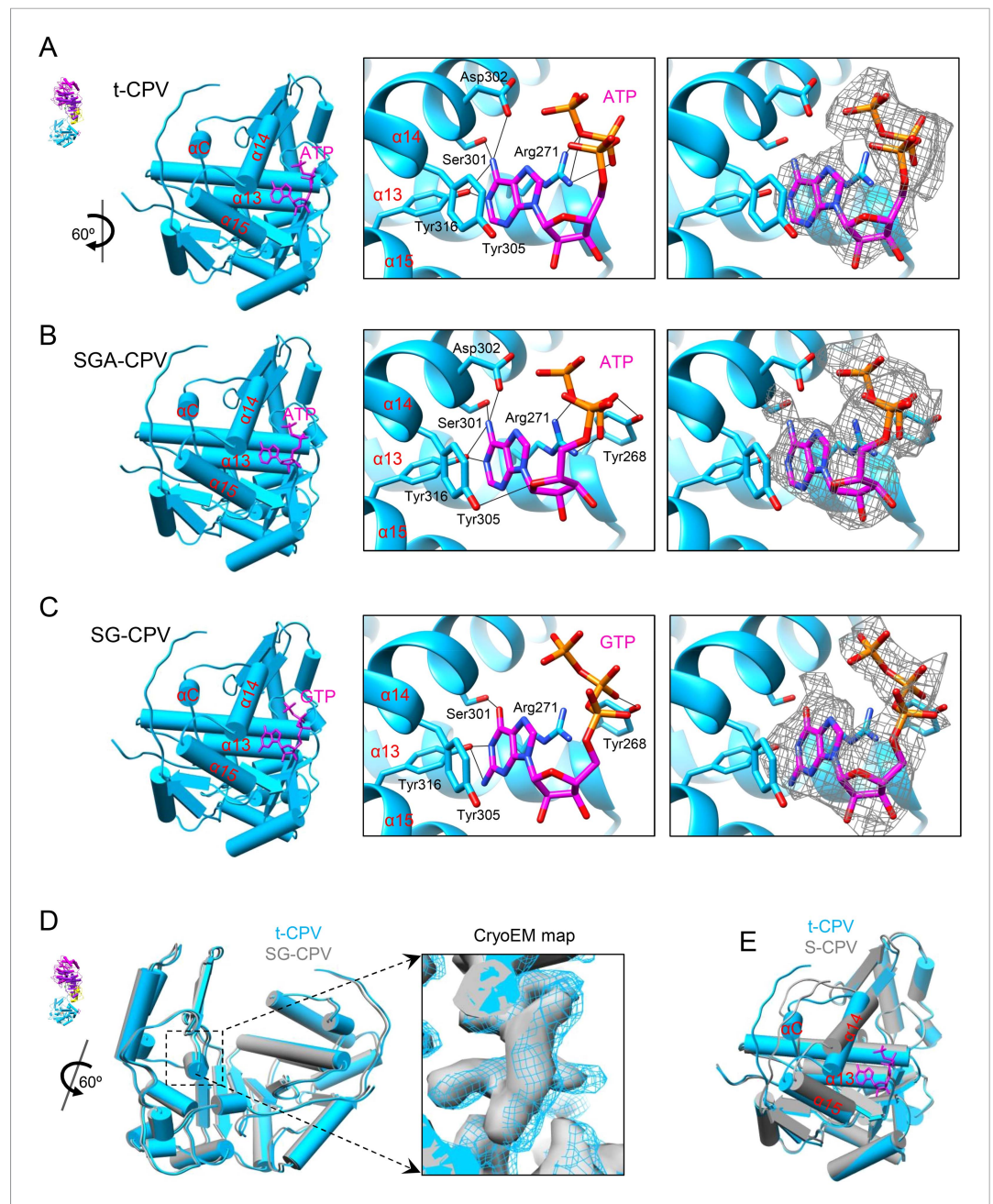


Figure 4. Discovery of the viral ATP-binding site. **(A)** Structure of GTase domain and ATP in t-CPV. Left, view rotated from the guide map (inset) as indicated. GTase domain is in sky blue. ATP is in magenta. Middle, zoom-in view of the putative ATP-binding site. ATP is colored by element: carbon atoms are magenta, nitrogen atoms are blue, and oxygen atoms are red. The hydrogen bonds are indicated by black lines. Side chains of Tyr305 and Arg271 form pi-pi and cation-pi interactions with the adenine ring of ATP, respectively. Right, same view as the middle. The density map of bound ATP (gray mesh) is contoured at 3σ above the means. **(B)** Structure of GTase domain and ATP in SGA-CPV. Molecules are viewed and colored as in **A**. **(C)** Structure of GTase domain and GTP in SG-CPV. Molecules are viewed and colored as in **A**. GTP is colored analogously. The density map of bound GTP (gray mesh) is contoured at 1.4σ above the means. **(D)** Superimposition of GTase domain between SG-CPV (gray) and t-CPV (sky blue). Inset: zoom-in view of the boxed region. Density maps from t-CPV (sky blue) and SG-CPV (gray) are contoured at 3.0σ above the means. **(E)** Superimposition of the large sub-domain of GTase domain between S-CPV (gray) and t-CPV (sky blue). Molecules were aligned using Ca positions for residues in small sub-domain. The bound ATP of t-CPV is in magenta.

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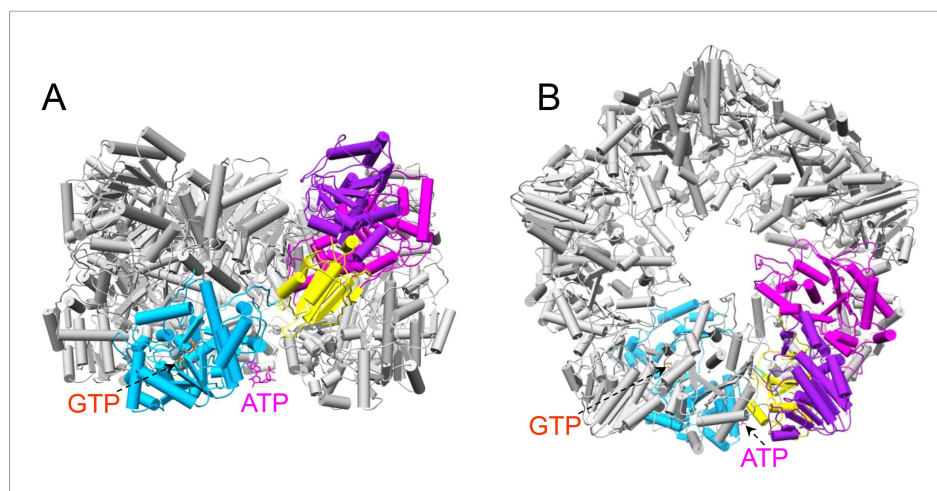


Figure 4—figure supplement 1. The pentameric turret complex of t-CPV. (A, B) The turret viewed from the side and top, respectively. Four monomers are in gray. One monomer is colored by domain as in **Figure 1D**. The GTP and ATP ligands bound to the GTase domain (sky blue) are in orange red and magenta, respectively.

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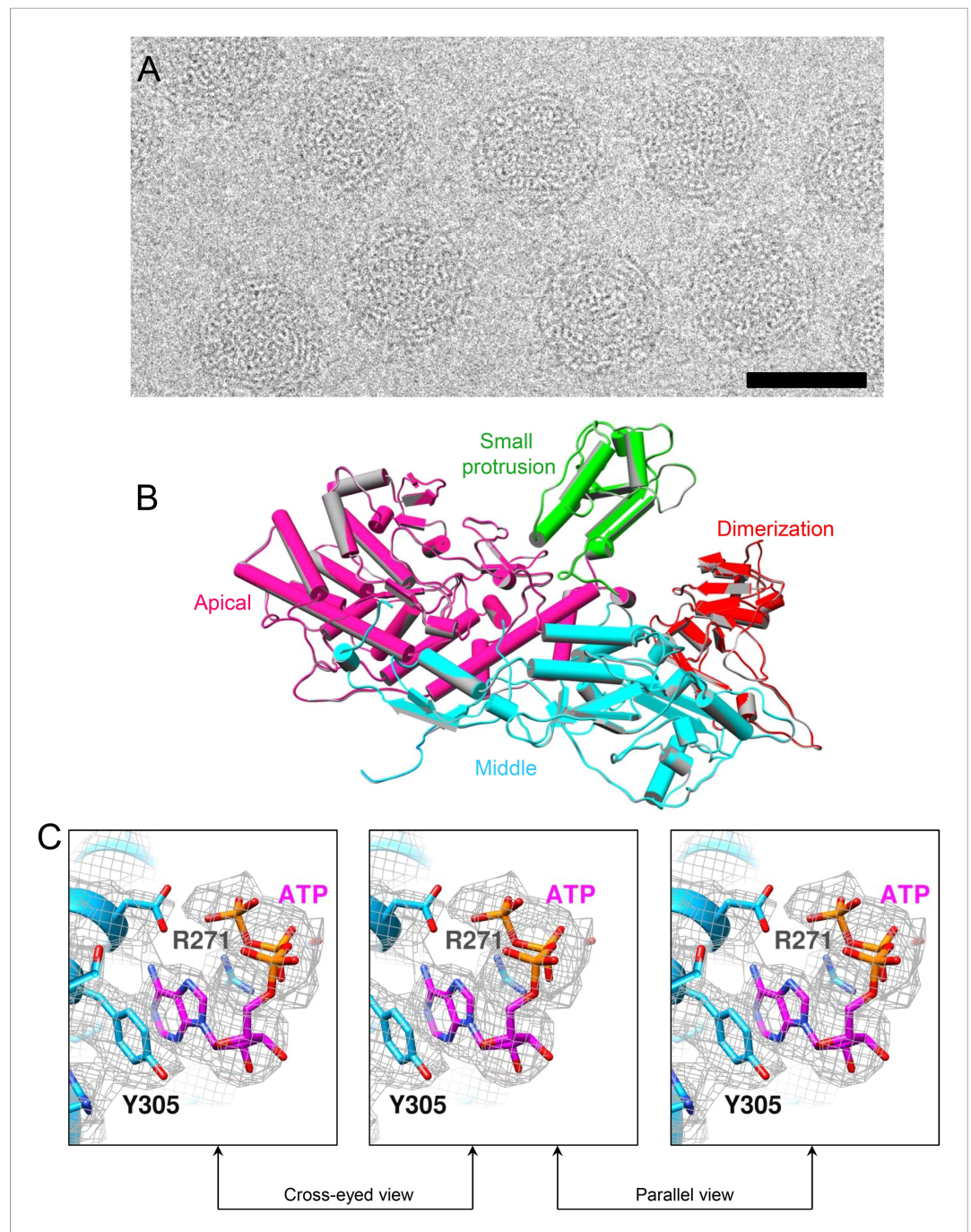


Figure 4—figure supplement 2. CryoEM of SGA-CPV. (A) CryoEM image of SGA-CPV. Scale bar, 50 nm. (B) Superimposition of CSP-A between SGA-CPV (gray) and t-CPV (colored by domain). (C) Stereo view of ATP-binding site and ATP in SGA-CPV. GTase domain is in sky blue. ATP is colored by element as in **Figure 4B**. The density map (gray mesh) of protein and bound ATP is contoured at 3σ above the means.
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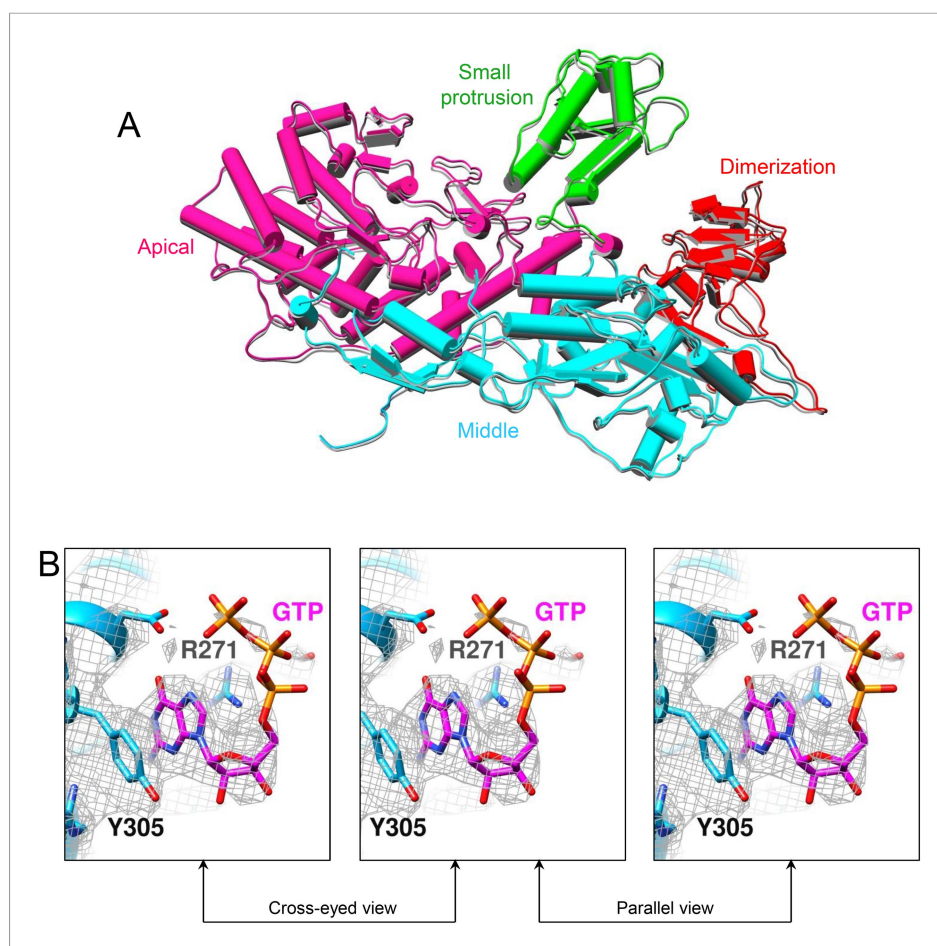


Figure 4—figure supplement 3. CryoEM of SG-CPV. (A) Superimposition of CSP-A between SG-CPV (gray) and t-CPV (colored by domain). (B) Stereo view of ATP-binding site and GTP in SG-CPV. GTase domain is in sky blue. GTP is colored by element as in **Figure 4C**. The density map (gray mesh) of protein and bound GTP is contoured at 3σ above the means.

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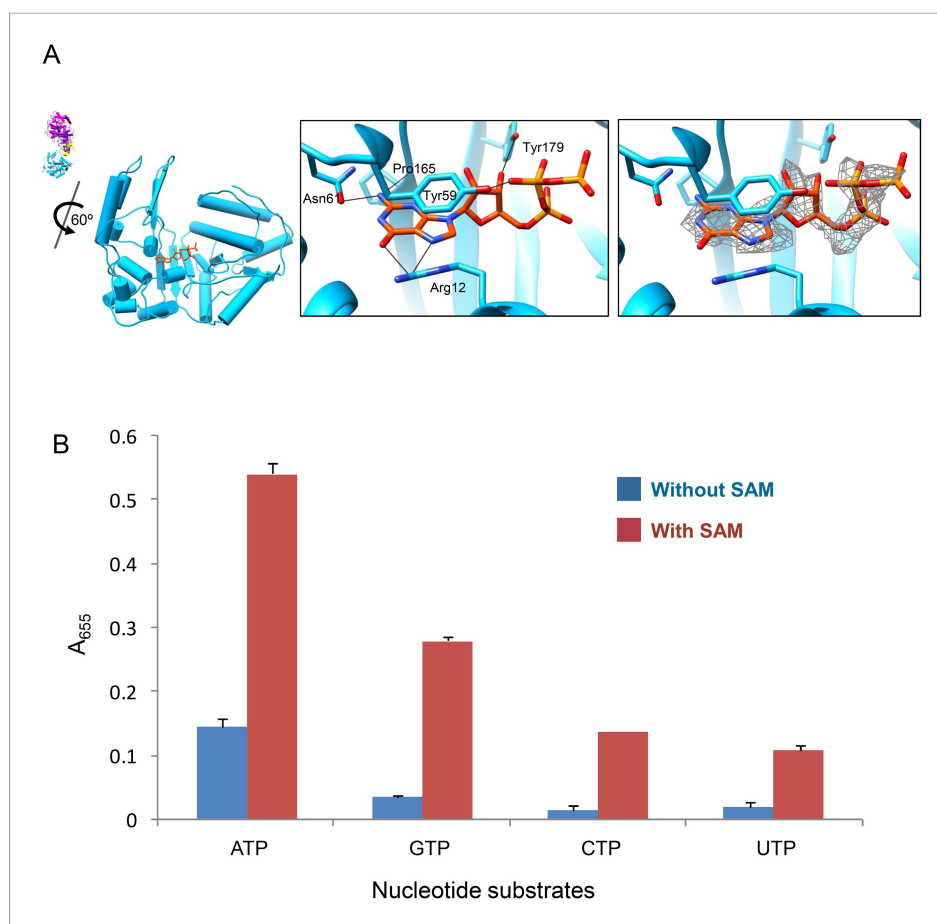


Figure 5. ATP binding and hydrolysis by the viral ATPase is SAM-dependent. **(A)** Structure of GTase domain and GTP in G-CPV. Left, view rotated from the guide map (inset) as indicated. GTase domain is in sky blue. GTP is in orange red. Middle, active site of GTase. GTP is colored by element: carbon atoms are orange red, nitrogen atoms are blue, and oxygen atoms are red. The hydrogen bonds are indicated by black lines. Side chain of Tyr59 also forms pi-pi stacking interaction with the guanylyl ring of GTP. Right, same view as the middle. The density map of bound GTP (gray mesh) is contoured at 3σ above the means. **(B)** Nucleotide substrates specificity by CPV nucleoside triphosphatase. Values are means derived from duplicate experiments. Standard deviations are indicated by error bar.

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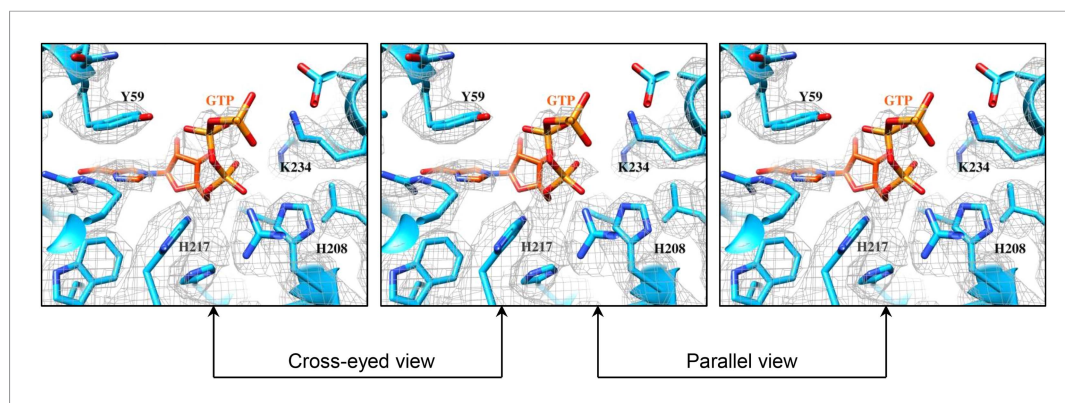


Figure 5—figure supplement 1. Stereo view of GTPase site and GTP in G-CPV. GTase domain is in sky blue. GTP is colored by element as in **Figure 5A**. The density map (gray mesh) of protein and bound GTP is contoured at 3σ above the means.

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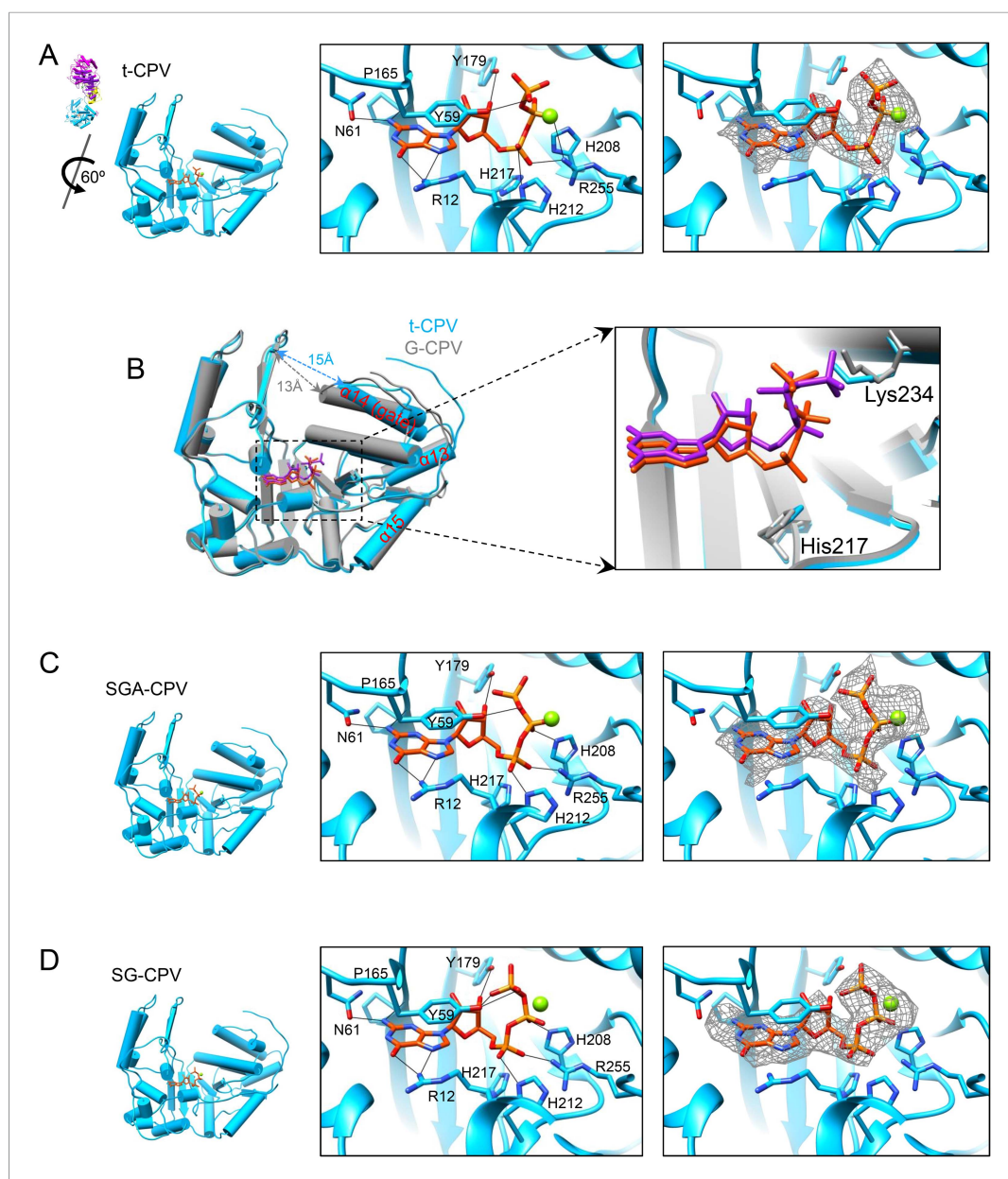


Figure 6. The catalytic activity of viral GTase is regulated by the viral ATPase through allosteric effect. **(A)** Structure of GTase domain and the bound Mg^{2+} -GTP in t-CPV. Left, view rotated from the guide map (inset) as indicated. GTase domain is in sky blue. GTP is in orange red. Mg^{2+} is in green. Middle, active site of GTase with bound Mg^{2+} -GTP. GTP is colored by element as in **Figure 5A**. The hydrogen bonds are indicated by black lines. Side chains of the two conserved His208 and His217 are shown. Right, same view as the middle. The density map of bound GTP (gray mesh) is contoured at 3σ above the means. **(B)** Superimposition of GTase domain between G-CPV (gray) and t-CPV (sky blue). Molecules were aligned using Ca positions for residues in small sub-domain. GTPs bound to the GTase sites of G-CPV and t-CPV are in purple and orange red, respectively. Inset, zoom-in view of the boxed region. **(C)** Structure of GTase domain and the bound Mg^{2+} -GTP in SGA-CPV. Molecules and Mg^{2+} are viewed and colored as in **A**. Side chain of the conserved His217 is shown. **(D)** Structure of GTase domain and the bound Mg^{2+} -GTP in SG-CPV. Molecules and Mg^{2+} are shown as in **A**. Side chain of the conserved His217 is shown.

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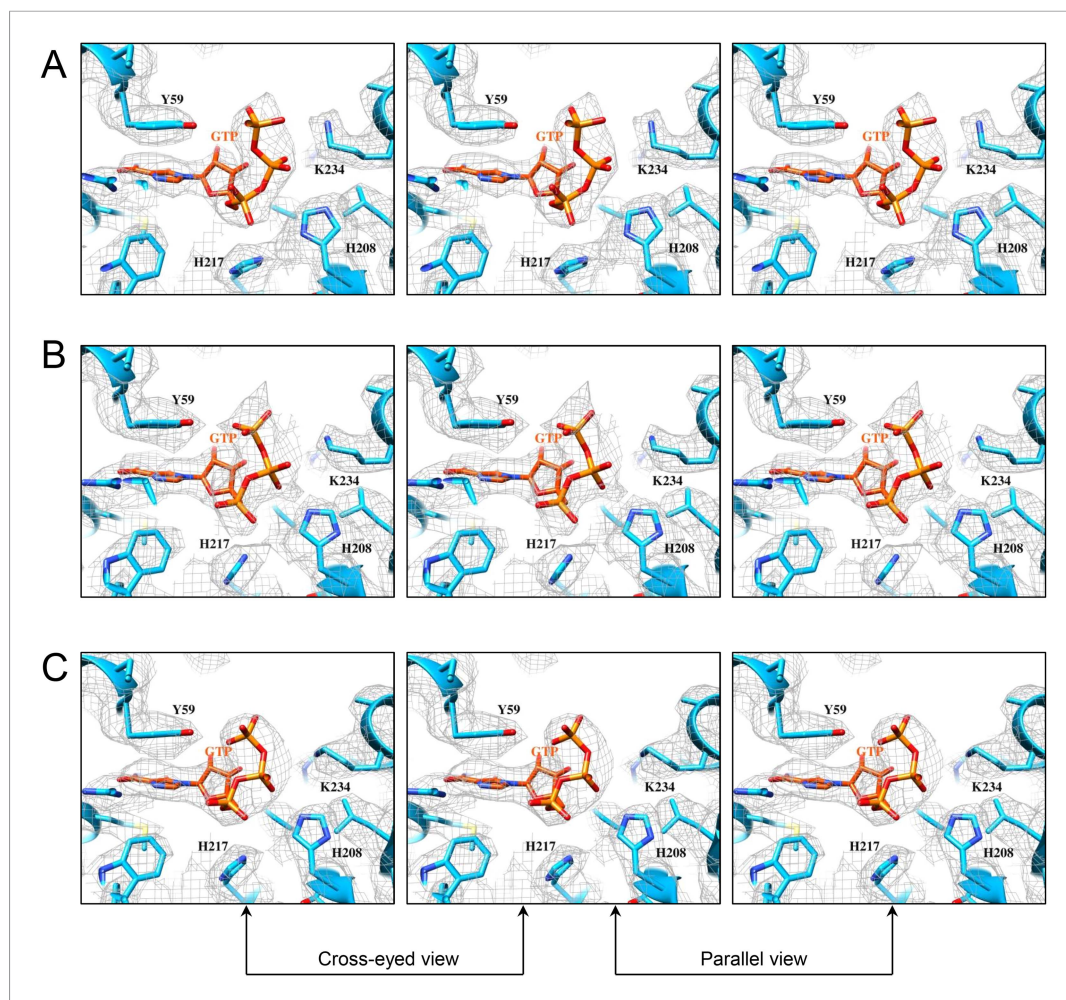


Figure 6—figure supplement 1. Structures of GTase sites and bound GTPs. **(A)** Stereo view of GTase site and GTP in t-CPV. Molecules and density map are colored and displayed as in **Figure 6A**. **(B)** Stereo view of GTase site and bound GTP in SGA-CPV. Molecules and density map are colored and displayed as in **A**. **(C)** Structure of GTase site and bound GTP in SG-CPV. Molecules and density map are colored and displayed as in **A**.

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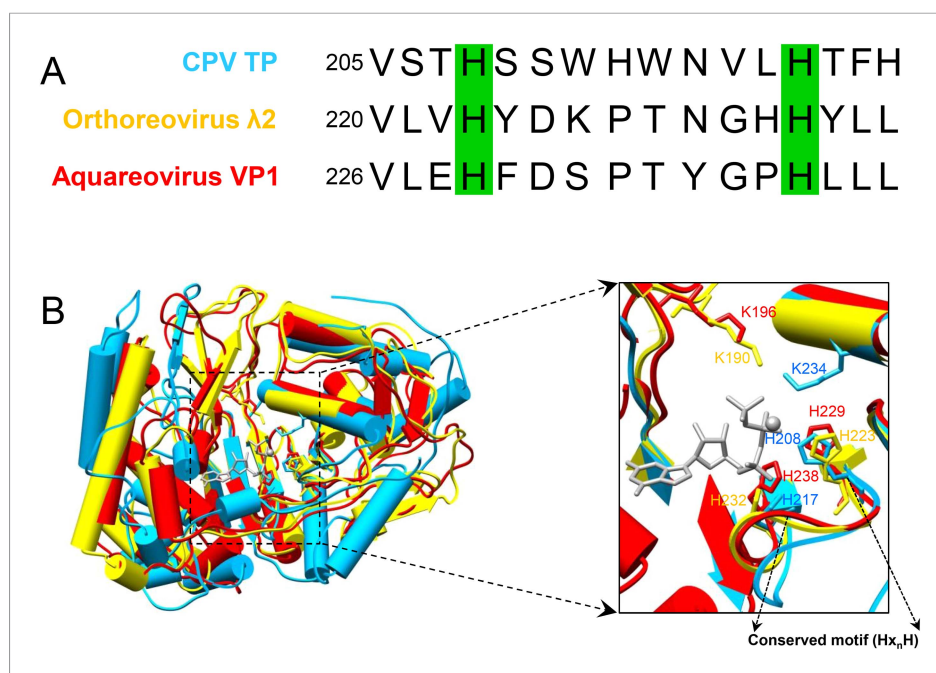


Figure 6—figure supplement 2. The conserved His217 is the catalytic amino acid for guanylation of GTase in CPV. **(A)** Sequence alignment showing the conserved histidines of GTase domains among 3 different members in *Reoviridae* family. The conserved histidines are highlighted by green boxes. **(B)** Superimposition of GTase domains of CPV TP, orthoreovirus λ 2, and aquareovirus VP1. Molecules were aligned using C α positions for residues in domains. The GTase domains of CPV, orthoreovirus, and aquareovirus are in sky blue, yellow, and red, respectively. The GTP and Mg²⁺ bound to CPV GTase site are colored in gray. Inset: zoom-in view of the boxed region. Side chains of K234, H208, and H217 of CPV, K190, H223, and H232 of orthoreovirus and K196, H229, and H238 of aquareovirus are shown.

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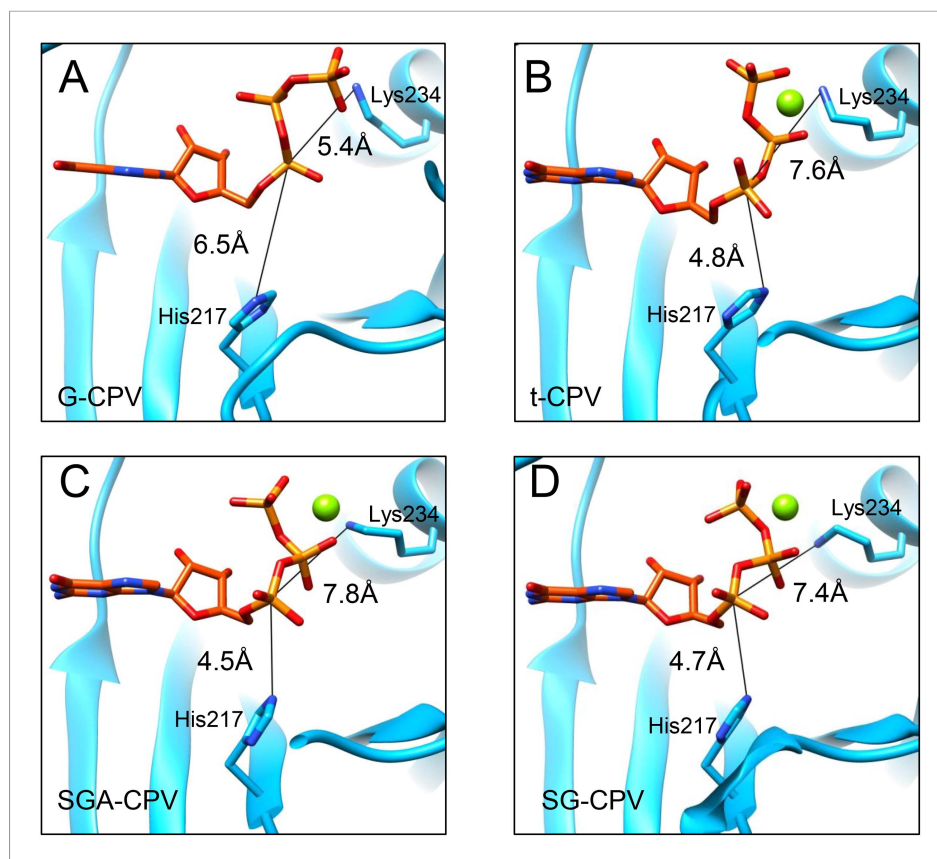


Figure 7. The α -phosphorus of GTP bound to the GTase site moves towards His217 and away from Lys234 accompanying the activation of GTase. **(A)** The distance between the Ne2 of His217 and the α -phosphorus of GTP in G-CPV is ~ 6.5 Å. The distance between the Ne of Lys234 and the α -phosphorus is ~ 5.4 Å. Molecules and Mg^{2+} are colored as in **Figure 5B**. **(B)** The distance between Ne2 of His217 and the α -phosphorus of GTP in t-CPV is ~ 4.8 Å. The distance between the Ne of Lys234 and the α -phosphorus is ~ 7.6 Å. **(C)** The distance between Ne2 of His217 and the α -phosphorus of GTP in SGA-CPV is ~ 4.5 Å. The distance between the Ne of Lys234 and the α -phosphorus is ~ 7.8 Å. **(D)** The distance between Ne2 of His217 and the α -phosphorus of GTP in SG-CPV is ~ 4.7 Å. The distance between the Ne of Lys234 and the α -phosphorus is ~ 7.4 Å.

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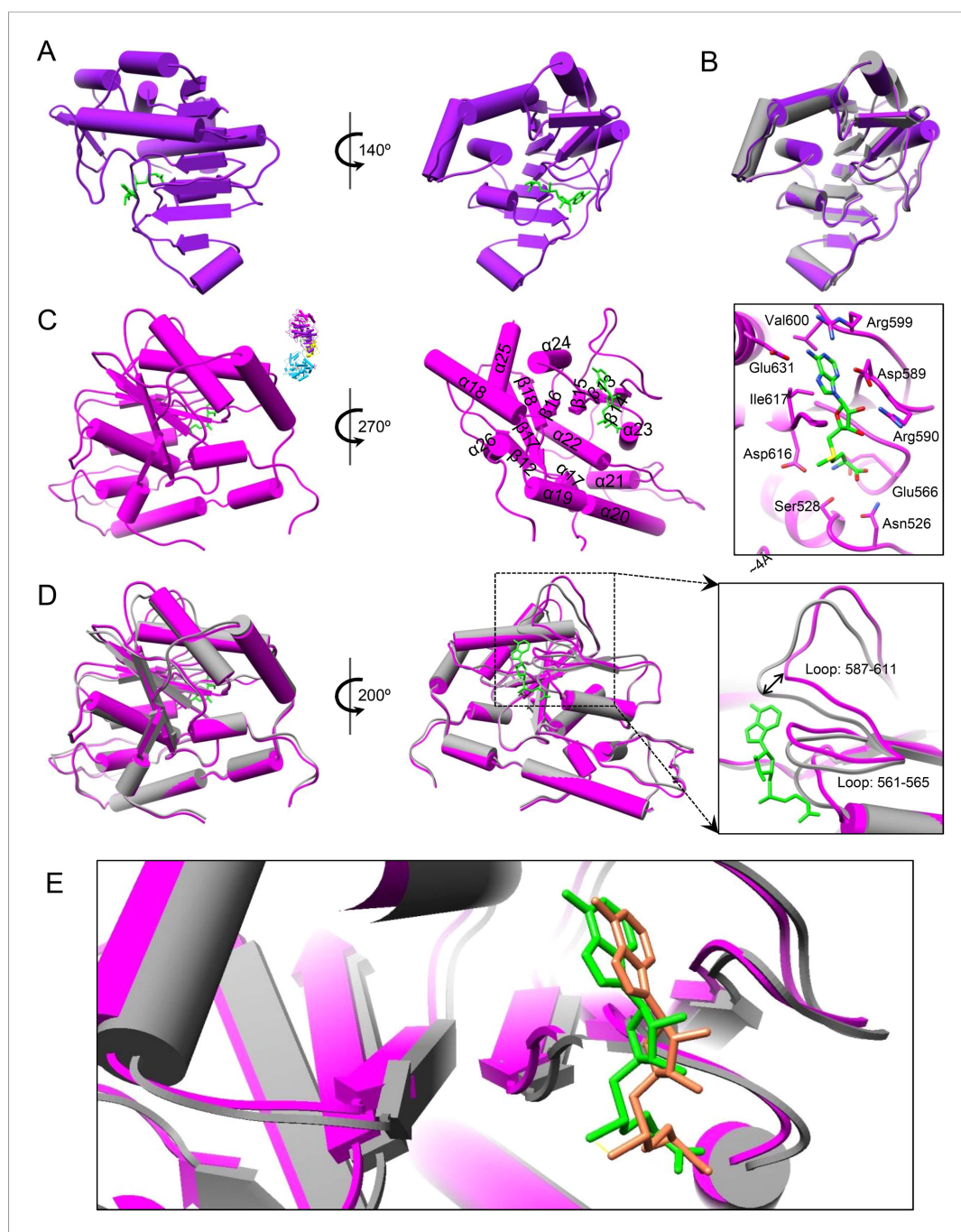


Figure 8. The catalytic activity of MT-1 is also regulated by the viral ATPase through allosteric effect. **(A)** Structure of MT-2 domain and the bound SAM in t-CPV. MT-2 domain is in purple. SAM is in green. Left, viewed as in **Figure 2C**. Right, view rotated as indicated. **(B)** Superimposition of MT-2 between S-CPV (gray) and t-CPV (purple). Molecules were aligned using Ca positions for residues in t-CPV. **(C)** Structure of MT-1 domain and the bound SAM in t-CPV. MT-1 domain is in magenta. SAM is in green. Left, view as the guide map (inset). Middle, view rotated as indicated. Right, active site of MT-1 domain. SAM is colored as in **Figure 2D**. Side chains of amino acids involved in interactions with SAM are shown. **(D)** Superimposition of MT-1 between S-CPV (gray) and t-CPV (magenta). Molecules were aligned using Ca positions for residues in MT-1 domain. The bound SAM of t-CPV is in green. Left, viewed as the guide map in **C**. Right, view rotated as indicated. Inset: zoom-in view of MT-1 active site. **(E)** Superimposition of MT-1 active site between SG-CPV (gray) and t-CPV (magenta). The SAM molecules bound to the active sites of SG-CPV and t-CPV are colored in coral and green, respectively.

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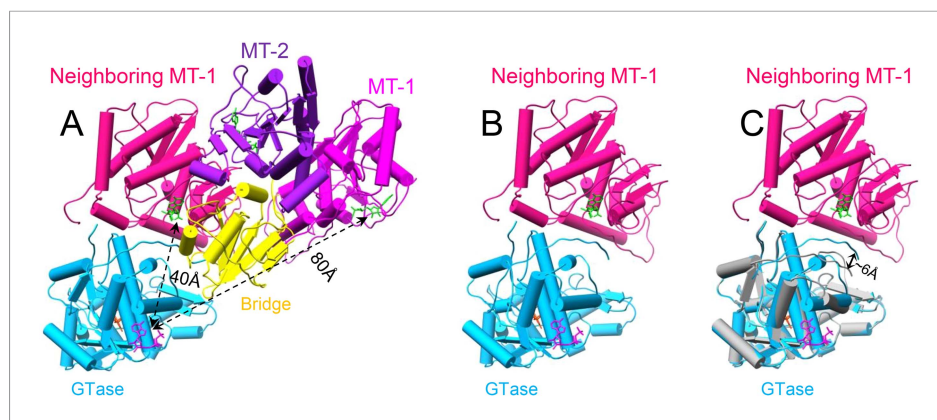


Figure 8—figure supplement 1. The putative viral ATPase regulates the methyl transfer activity of MT-1. **(A)** One TP monomer (domain colored) and its neighboring MT-1 domain (red) of t-CPV. SAM, GTP, and ATP are in green, orange red, and magenta, respectively. **(B)** One GTase domain (sky blue) and one MT-1 domain (red) from its neighboring TP molecule. **(C)** One GTase domain (sky blue) and one MT-1 domain (red) from its neighboring TP molecule in t-CPV and one GTase domain of S-CPV (gray). The GTase domain of S-CPV was aligned into the GTase domain of t-CPV using Ca positions for residues in domain.

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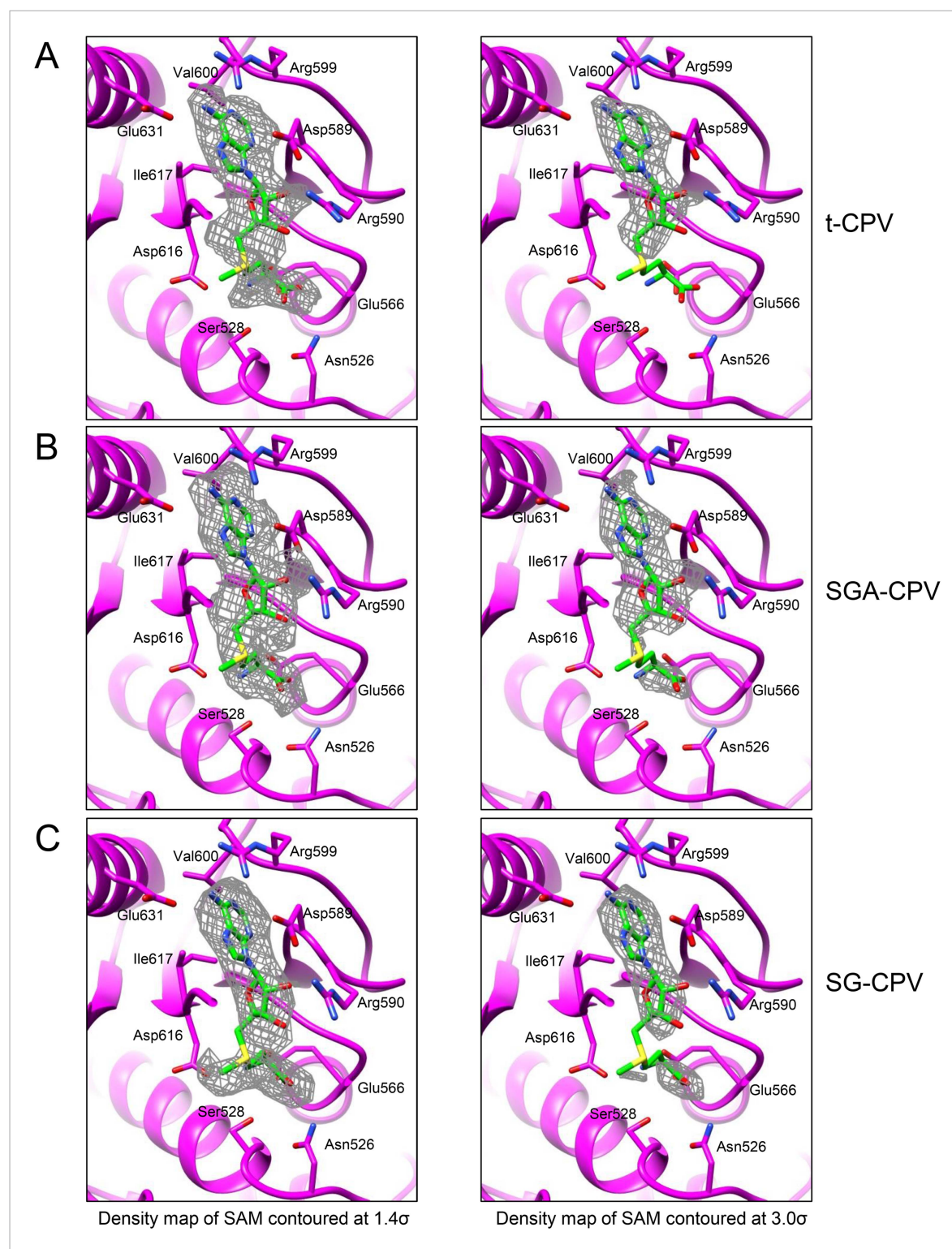


Figure 8—figure supplement 2. Structures of MT-1 active sites and SAMs. **(A)** Structure of MT-1 active site and SAM in t-CPV. MT-1 domain is in magenta. SAM is colored by element as in **Figure 2D**. Side chains of amino acids interacting with SAM are shown. Left: density map (gray mesh) of SAM is contoured at 1.4σ above the means. Right: density map of SAM is contoured at 3.0σ above the means. **(B)** Structure of MT-1 active site and SAM in SGA-CPV. Models and density map are colored and contoured as in **A**. **(C)** The structure of MT-1 active site and SAM in SG-CPV. Models and density map are colored and contoured as in **A**.

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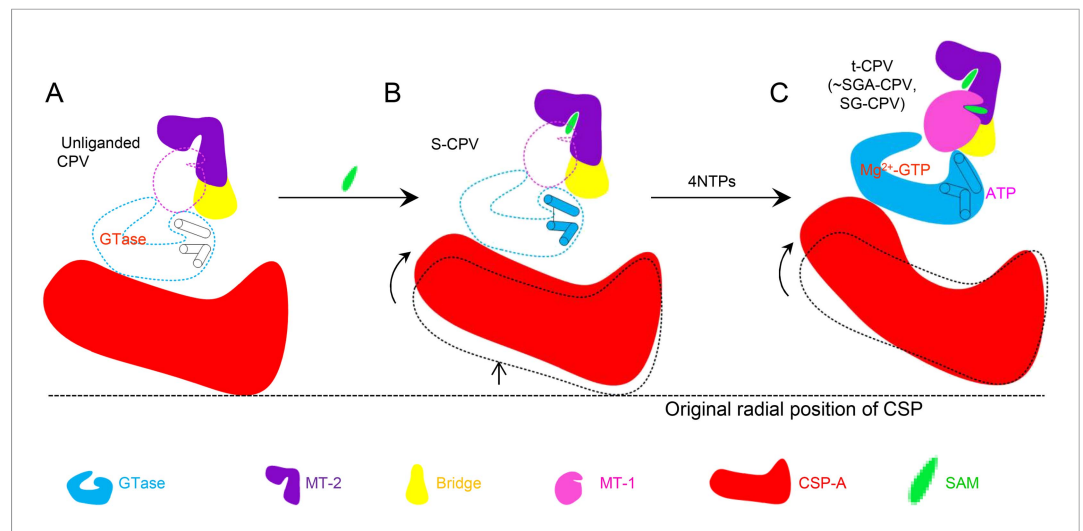


Figure 9. Schematic illustration of the putative viral ATPase-mediated activation of mRNA transcription and capping. In this illustration, the active open states of enzymes are shown in filled colors and the inactive closed states of enzymes are shown in dotted color lines. **(A)** CSP-A (red) and GTase, bridge and MT-2 domains from the same TP molecule, and a neighboring MT-1 (colored as in **Figure 1D**) of unliganded CPV. The inactive ATPase site is indicated by three empty cylinders. **(B)** CSP-A and GTase, bridge and MT-2 domains from the same TP molecule, and a neighboring MT-1 of S-CPV. SAM alone can only bind to MT-2 domain (purple) to cause conformational change and activate the putative viral ATPase. The activated ATPase site is indicated by three colored cylinders. **(C)** CSP-A and GTase, bridge and MT-2 domains from the same molecule, and a neighboring MT-1 of t-CPV.

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