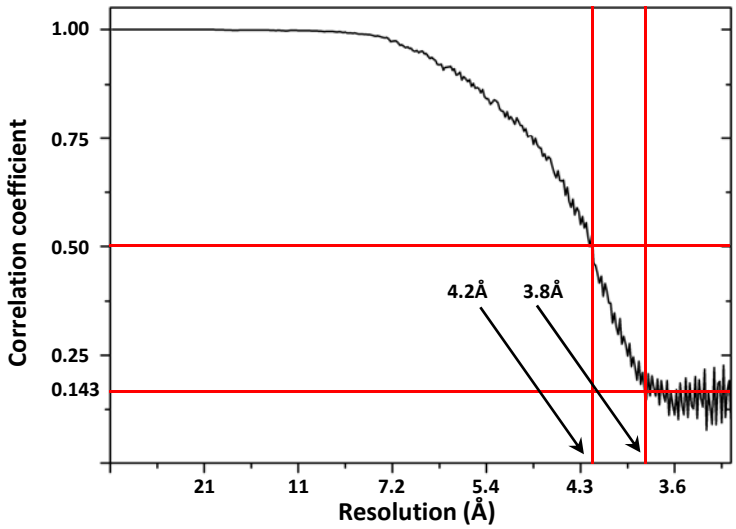

Figure S1 Fourier shell correlation curve (FSC) of the transcribing CPV capsid structure. The estimated resolution is 4.2 Å at FSC=0.5 and 3.8 Å at FSC=0.143.

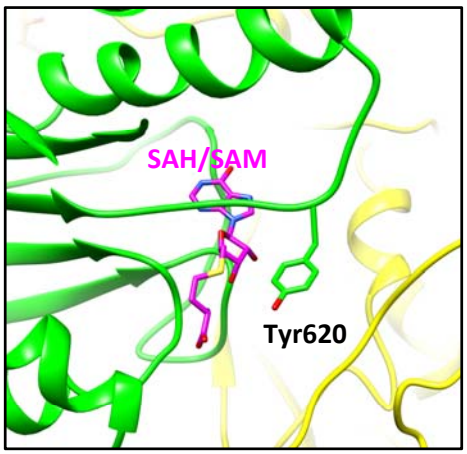

Figure S2 Interaction between SAM/SAH and Tyr620 in the MTase-1 domain (2'-O-MTase).

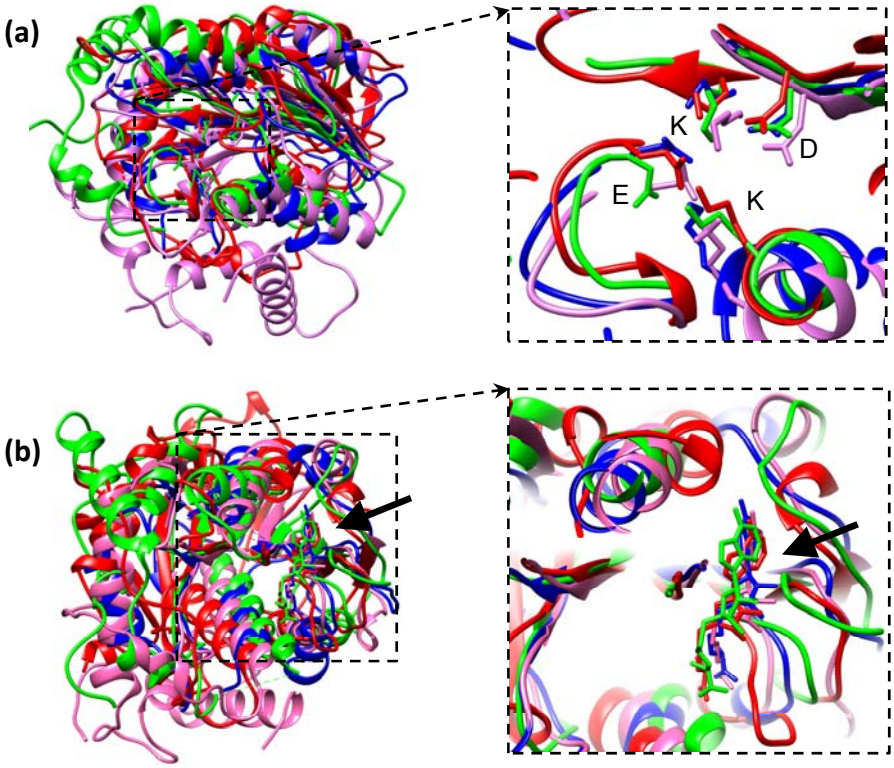

Figure S3 Two views of alignments of 2'-O-Mtases of cypovirus (green), bluetongue virus (blue), SARS virus [1] (red), and vaccinia virus [2] (pink) are shown. (a) These side chains of the catalytic tetrad KDKE closely overlap. (b) Their SAH molecules are closely overlapped (pointed by a black arrowhead).

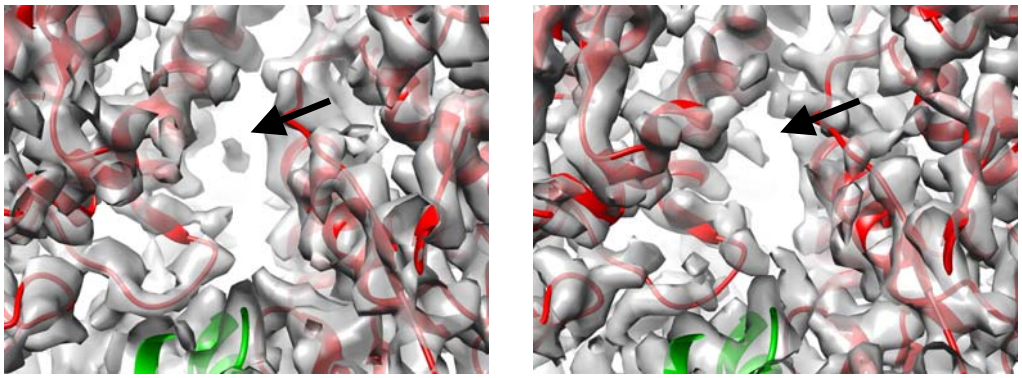

Figure S4 The density feature (arrowed), attributed to a partially resolved RNA in the peripentonal channels of the density map of 4.1-Å resolution (left) [3], is also visible at nearly the same location of the density map of 3.8-Å (right). The atomic models (ribbon) superimposed on the density maps are two conformers of capsid shell protein VP1A (red) and VP1B (green).

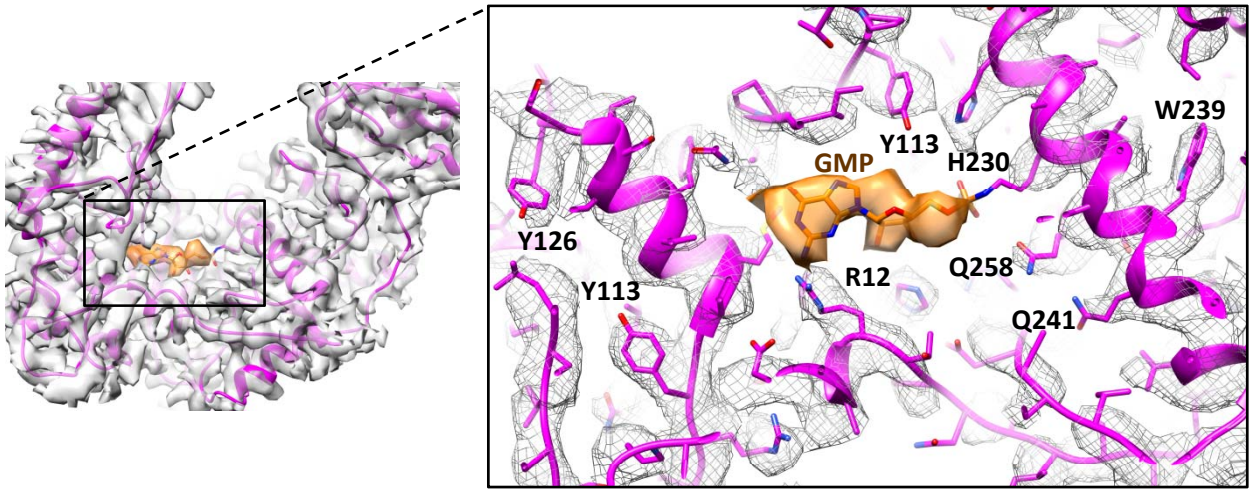

Figure S5 The density map (transparent) of GMP in the VP3 GTase domain with its atomic model superimposed. The GMP moiety is in orange.

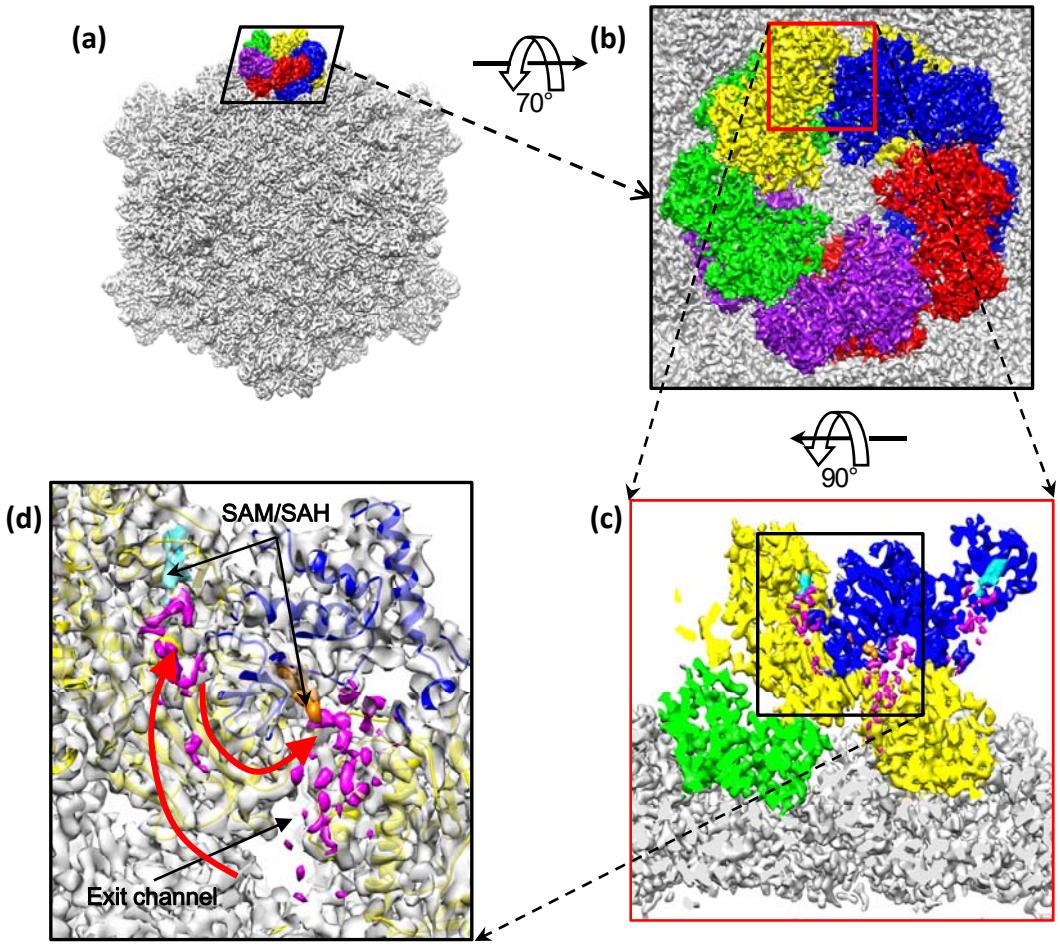

Figure S6 VP3 in the capsid. (a) a VP3 pentamer in the capsid. (b) Zoom-in view of the VP3 pentamer. (c) and (d) Two zoom-in views of a 7-N-MTase and a 2'-O-MTase in the VP3 pentamer. The RNA channel-1 and -2 are labeled with red arrows, and the exit channel are indicated by a black arrow. The SAM/SAH of 7-N-MTase is in cyan and the SAM/SAH of 2'-O-MTase is in orange. The fragmented density features of RNA are in magenta.

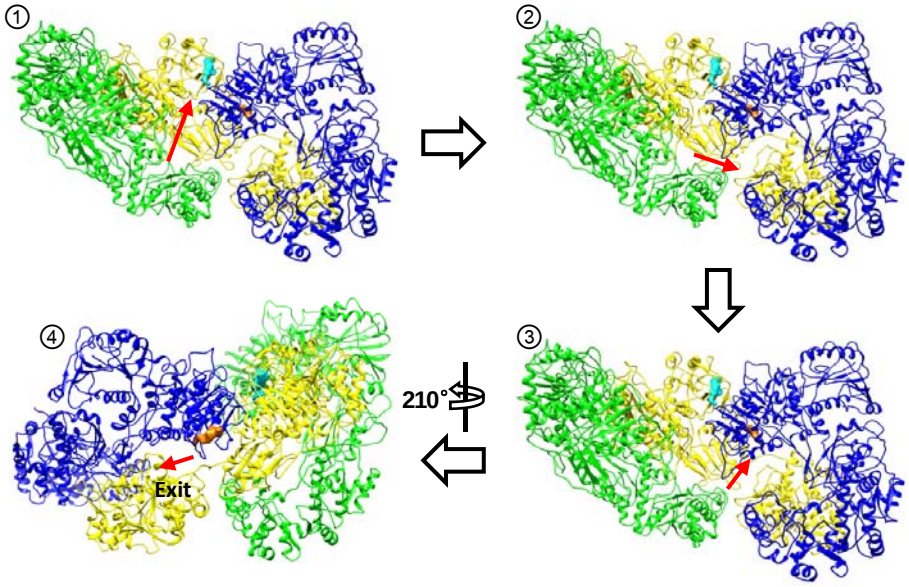

Figure S7 The RNA pathway inside the turret. The orientations and color scheme of VP3 monomers and SAM/SAH molecules are identical to those in Fig. 3a. The red arrowheads represent the directions and traces of RNA pathway. Step 1: After the guanylyltransfer reaction in the GTase domain of the green VP3 monomer, RNA reaches the active site of the 7-N-MTase domain of the yellow VP3 monomer through channel-1. Step 2 and 3: RNA withdraws from the 7-N-MTase domain through channel-1 and approaches the active site of the adjacent 2'-O-MTase domain of the blue VP3 monomer. Step 4: After the 7-N and 2'-O methylations, RNA is released from the turret into the cytoplasm through the third channel, which is formed by the 2'-O-MTase domain of the blue VP3 monomer and the GTase domain of the yellow VP3 monomer.

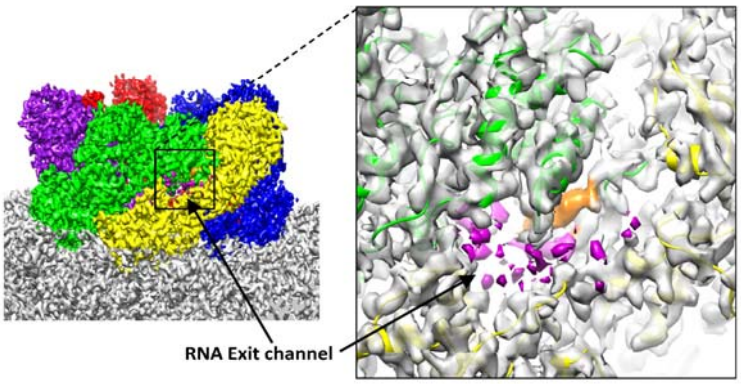

Figure S8 Some fragmented density features attributable to RNA can be observed at the channel outside.

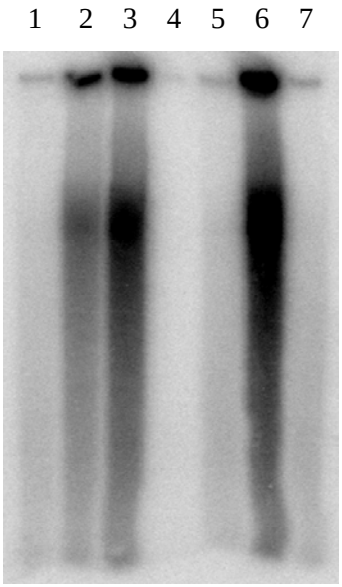

Figure S9 Polyacrylamide gel electrophoresis analysis of transcription reaction mixtures either containning different concentrations of GTP or incubated for different lengths of time. Lane 2, 3, and 6: these transcription reaction mixtures consisting of 70 mM Tris-Ac (pH 8), 10 mM MgAc2, 100 mM NaAc, 4 mM ATP, 2 mM CTP, 2 mM GTP, 2 mM UTP, 20 μCi [α-^{32}P] UTP (specific activity 3,000 Ci/mM), 1 mM SAM, 1 U/μL RNase inhibitor and purified CPV suspension were incubated at 31 ℃ for 1 h, 3 h, and 6 h, respectively. Lane 1, 5, and 7: transcription reaction mixtures in which the concentration of GTP was decreased to 0.04 mM were incubated at 31 ° C for 1 h, 3 h, and 6 h, respectively. Lane 4: transcription reaction mixture in the absence of GTP.

Movie S1 Atomic model of the turret formed by five VP3 monomers in red, purple, green, yellow, and blue, respectively. The two VP3 monomers in red and purple were for an inside view of the turret. The color scheme in the movie is the same as that in Fig. 3A. The first enzymatic domain that the nascent RNA reaches is the GTase domain of monomer C (green). A channel (denoted as channel-1), which is formed by a bridge domain and a brace domain of monomer A (yellow), and a brace domain of monomer B (blue), guides the RNA to the active site of 7-N-MTase of monomer A (yellow). Then, the RNA withdraws from the 7-N-MTase domain of monomer A (yellow) through channel-1 and approaches the active site of the adjacent 2'-O-MTase domain of monomer B (blue) through the channel-2, which is formed by bridge domain of the monomer A (yellow), GTase domain and 2'-O-MTase domain of the monomer B (blue).

References

[1] Decroly E, Debarnot C, Ferron F, Bouvet M, Coutard B, Imbert I, et al. Crystal structure and functional analysis of the SARS-coronavirus RNA cap 2'-O-methyltransferase nsp10/nsp16 complex. Plos Pathog 2011;7:e1002059.

[2] Hodel AE, Gershon PD, Shi X, Quiocho FA. The 1.85 A structure of vaccinia protein VP39: a bifunctional enzyme that participates in the modification of both mRNA ends. Cell 1996;85:247-56.

[3] Yang C, Ji G, Liu H, Zhang K, Liu G, Sun F, et al. Cryo-EM structure of a transcribing cypovirus. Proc Natl Acad Sci USA 2012;109:6118-23.