Structure *16*

Supplemental Data

Mechanism for Coordinated RNA Packaging and Genome Replication by Rotavirus Polymerase VP1

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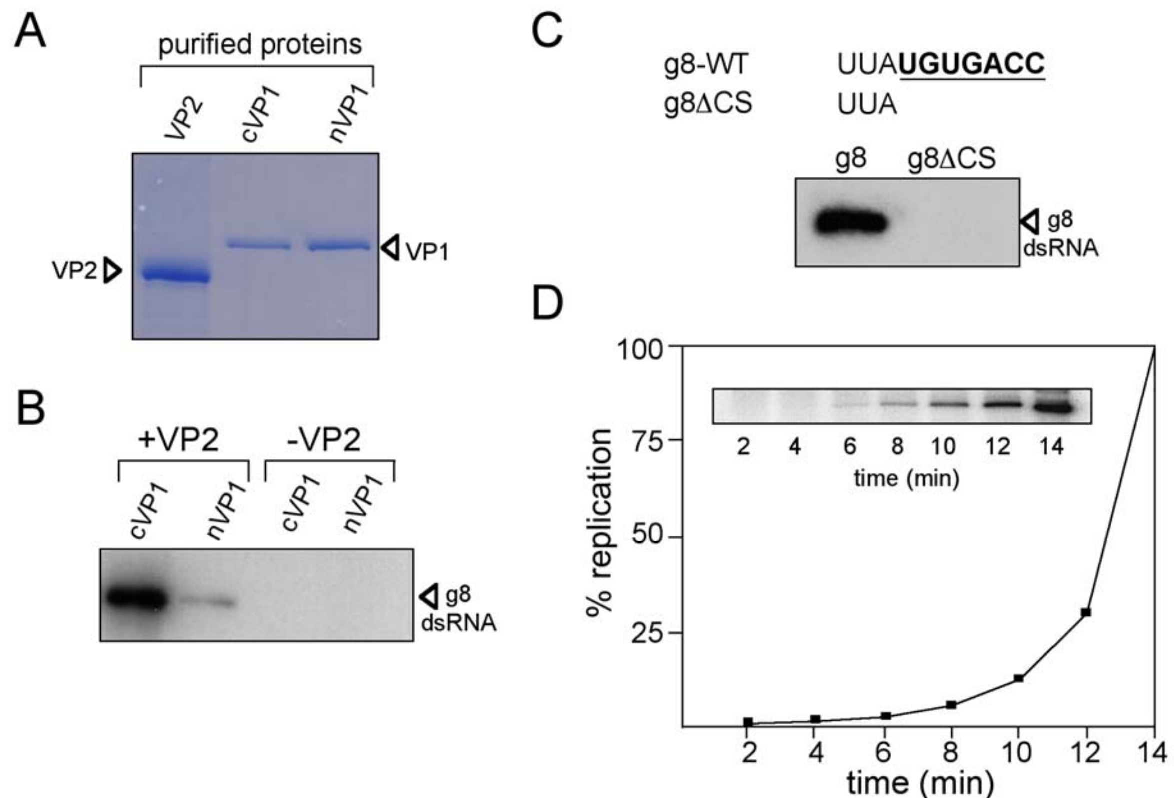

Figure S1. Biochemical Activity of His-Tagged VP1 Proteins
(A) Gel analysis of purified VP1 and VP2. Approximately 4 pmols of recombinant VP1 (cVP1 or nVP1) and 16 pmols of recombinant VP2 were used for all *in vitro* dsRNA synthesis assays.
(B) cVP1 shows robust dsRNA synthesis. Reaction mixtures included cVP1or nVP1, gene 8 +RNA, Mg^{2+}, NTPs, ^{32}P-UTP, with and without VP2 (+VP2 and -VP2, respectively). Radiolabeled dsRNA products were resolved by SDS-PAGE and detected by autoradiography.
(C) cVP1 requires a +RNA containing an intact 3'CS. Wild-type (g8) and mutant gene 8 +RNAs with a deletion of the 3'CS (ΔCS) were prepared by T7 transcription. The 3' terminal sequences are indicated and the ^{32}P-labeled dsRNA products of replicase assays containing cVP1, VP2, and the indicated template were analyzed as described in (B).
(D) Kinetics of cVP1 activity. A reaction mixture containing cVP1, VP2, gene 8 +RNA, and GTP were incubated at 37°C to promote formation of initiation complexes. Afterwards, 1.25 mM each of ATP and CTP, 0.125 mM UTP, and ^{32}P-UTP were added to allow elongation. Aliquots taken at the indicated times of the elongation period were analyzed by SDS-PAGE and autoradiography (inset). The dsRNA product was first

detected in reaction mixtures after 6 min of incubation, indicating an elongation rate of ~180 nucleotides per min.

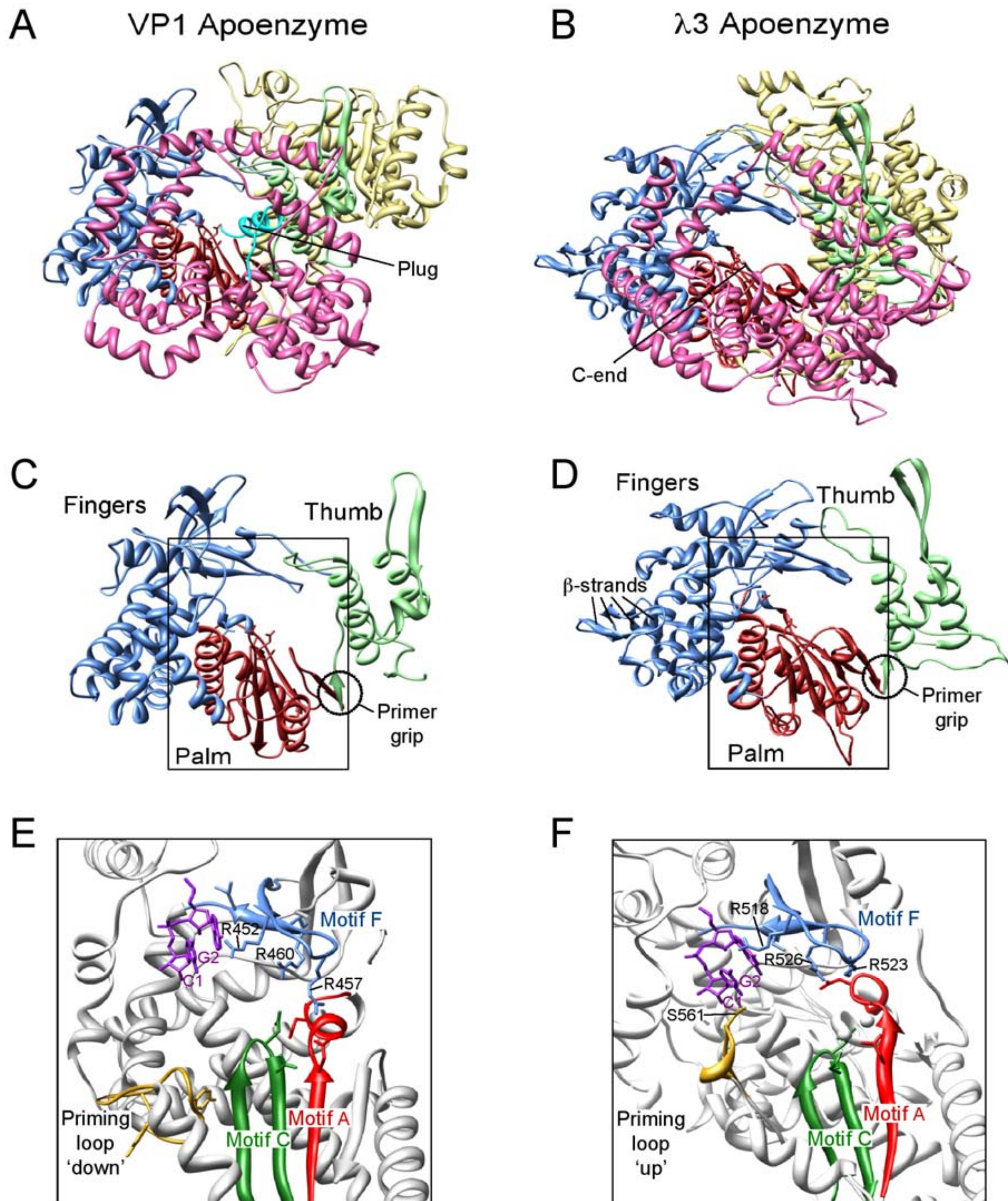

Figure S2. Comparison of the VP1 and λ3 Structures

(A) Ribbon diagram of the rotavirus VP1 apoenzyme.

(B) Ribbon diagram of the reovirus λ3 apoenzyme. The N- and C-terminal (bracelet) domains are in yellow and pink, respectively; the VP1 C-terminal plug is in cyan. The subdomains of the polymerase domain are in light blue (fingers), red (palm), and green (thumb) and the primer grip and β-strands are labeled.

(C and D) This domain is shown alone for each enzyme (C) VP1 and (D) λ3.

(E and F) The boxed region is magnified in panels (E) VP1 and (F) λ3 to show the arrangement of the catalytic sites. Incoming nucleotides are purple, motif-F is light blue, the priming loop is gold, and motifs A, B, and C are colored red, yellow, and green, respectively. These motifs are also depicted in Figure S3.

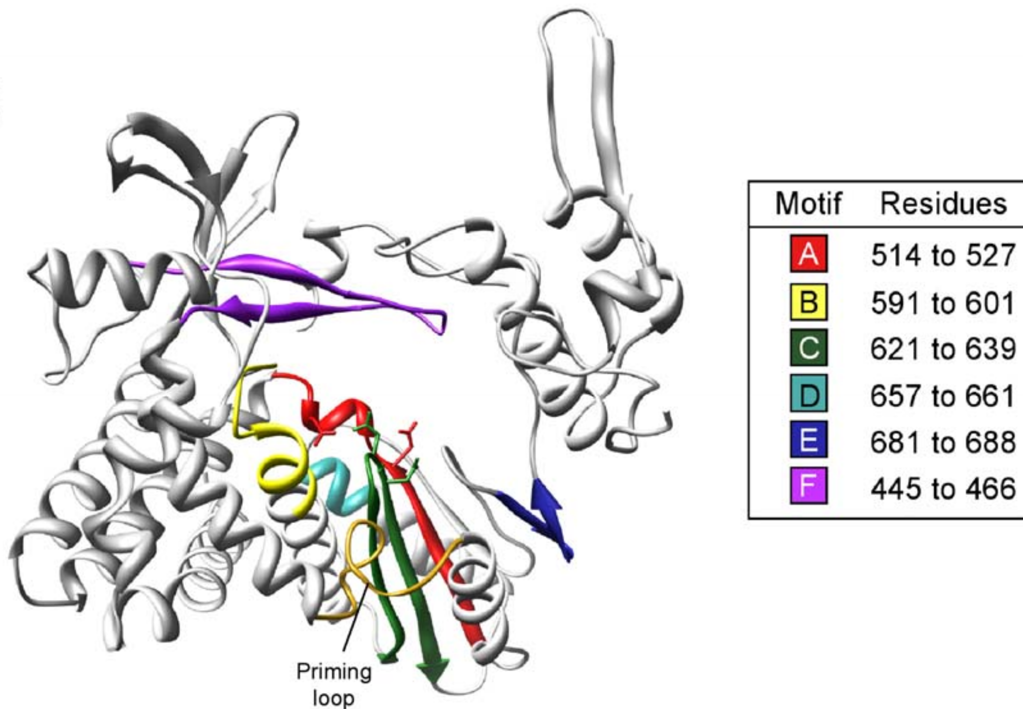

Figure S3. VP1 Secondary Structures and Motifs

(A) The amino acid sequence of VP1 is shown with secondary structural elements indicated as rods (helices) or arrows (strands) above the corresponding residues. The N- and C-terminal (bracelet) domains are in yellow and pink, respectively; the VP1 C-terminal plug is in cyan. The subdomains of the polymerase domain are in light blue (fingers), red (palm), and green (thumb).

(B) Structural motifs of the polymerase domain are colored according to the legend on the right of the ribbon diagram. The priming loop (gold) is labeled.

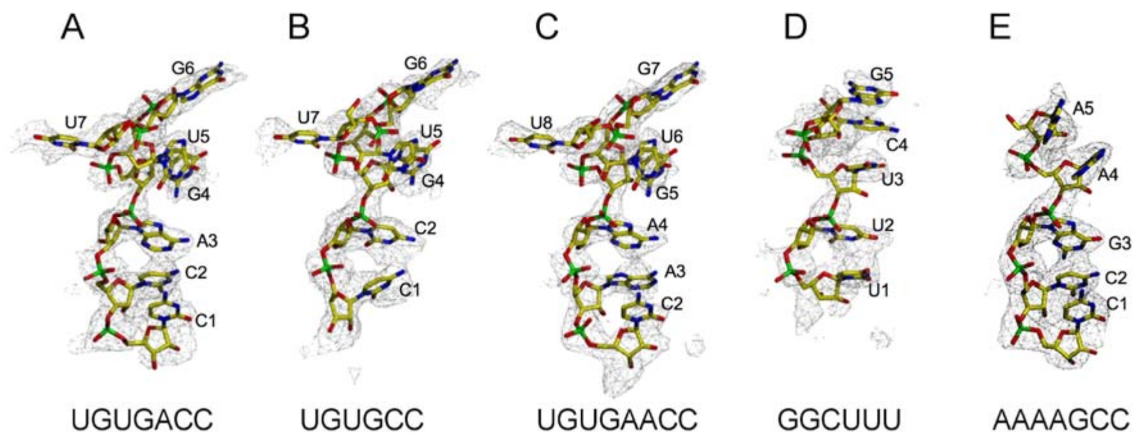

Figure S4. RNA Oligonucleotide Densities
Conformations and corresponding difference electron density is shown for five of the oligonucleotides used in soaking experiments: (A) +RNA 3'CS, (B) A3-deletion 3'CS, (C) A-insertion 3'CS, (D) a biologically irrelevant rotavirus sequence, and (E) the -RNA 3' terminus.