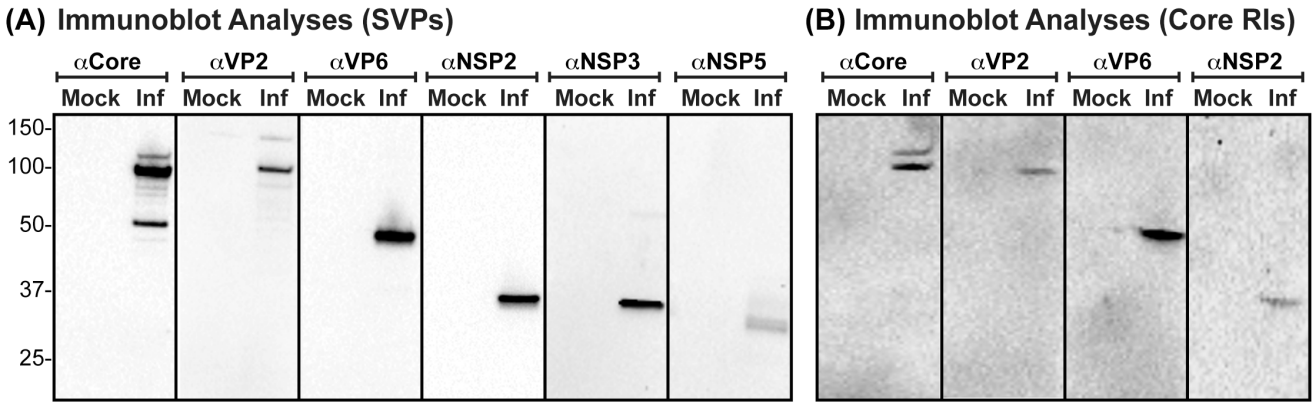

Fig. S1. Immunoblot analyses. To detect viral proteins in the SVP (A) or gel-purified core RI (B) preparations derived from mock-infected (Mock) or RV-infected (Inf) cells, proteins were resolved by SDS-PAGE in 4-20% gradient gels and transferred to nitrocellulose membranes for immunoblot analyses. All guinea pig primary antibodies (listed at top of respective blots) were provided by Dr. John T. Patton (National Institutes of Health) and were used at a 1:1000 dilution. Secondary anti-guinea pig horse radish peroxidase-conjugated antibody (KPL) was used at 1:10,000. Enhanced chemiluminesense reagent (Pierce) was used for detection according to the manufacturer's instructions. Molecular weight markers (kDas) are listed to the left of the blots.

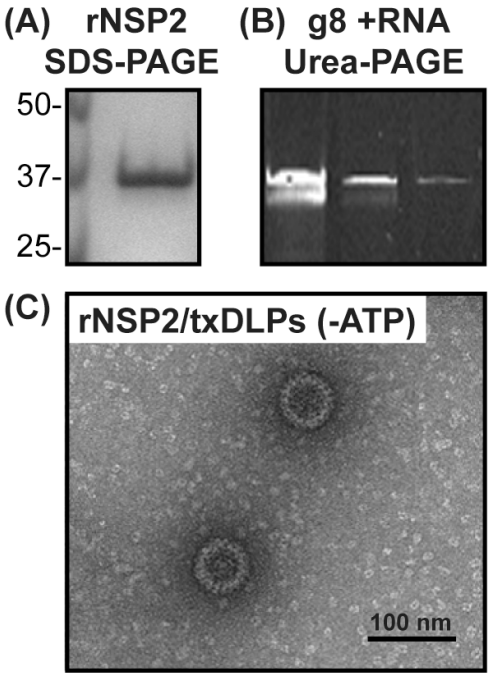

Fig. S2. Quality control of rNSP2, g8 +RNA, and control transcription reaction. (A) Purified rNSP2 was resolved by SDS-PAGE and visualized following Gel Code Blue (Pierce) staining. Molecular weight markers (kDas) are listed to the left of the gel. (B) In vitro transcribed gene (g8) +RNA was resolved in a urea-PAGE gel and visualized following ethidium bromide staining. Serial dilutions of the +RNA are shown. (C) EM image of negatively-stained control transcription reaction (lacking ATP) mixed with rNSP2. No darkly-stained strings could be detected.

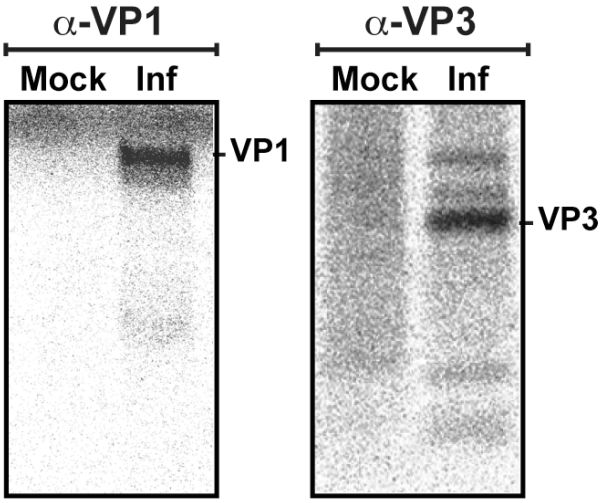

Fig. S3. Specificity of antibodies used for immunoaffinity capture EM. Proteins in mock-infected (Mock) or RV-infected (Inf) cells were labeled using [^{35}S]-methionine/cysteine. Lysates were then subjected to immunoprecipitation using either αVP1 or αVP3 (1:250 dilution) bound to protein A-sepharose beads. Proteins were resolved by SDS-PAGE and visualized using a phosphorimager.

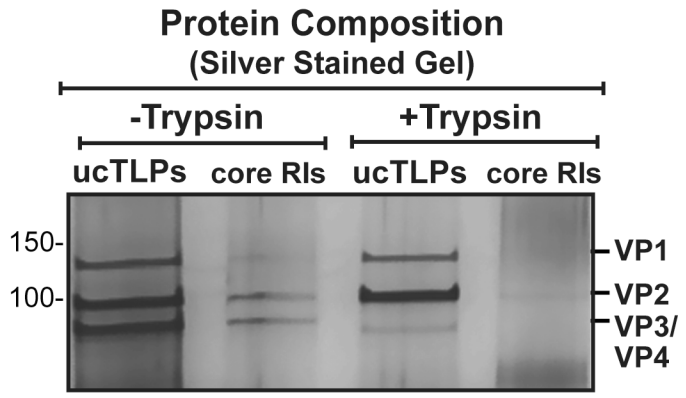

Fig. S4. Trypsin proteolysis of TLPs and core RIs. Control virion particles in which VP4 is uncleaved (unTLPs) were purified using cesium chloride gradients. Approximately 500 ng of either ucTLPs or core RIs were either mock-treated (-Trypsin) or treated with 1 μg of porcine pancreatic trypsin (+Trypsin) for 1 hour at 37°C. Proteins were then resolved by SDS-PAGE in a 4-20% gradient gel and visualized by silver staining. Molecular weight markers (kDas) are listed to the left of the blots. The expected migration of viral proteins VP1 (125 kDa), VP2 (102 kDa), VP3 (98kDa), and VP4 (86 kDa) is indicated to the right of the gel.