SI Appendix:

Asymmetric cryo-EM structure of the canonical Allolevivirus Qβ reveals a single maturation protein and the genomic ssRNA *in situ*

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Movie Legends

Movie S1. Asymmetric structure of Qβ. Coat proteins are in salmon (conformer A), green (conformer B) and blue (conformer C), respectively. A_2 is in hot pink. RNA is in yellow and low-pass filtered to 10Å resolution.

Movie S2. Symmetry breaking in the coat proteins around A_2.

Movie S3. The deviations of the coat protein dimers in the asymmetric structure from their locations in the icosahedral structure.

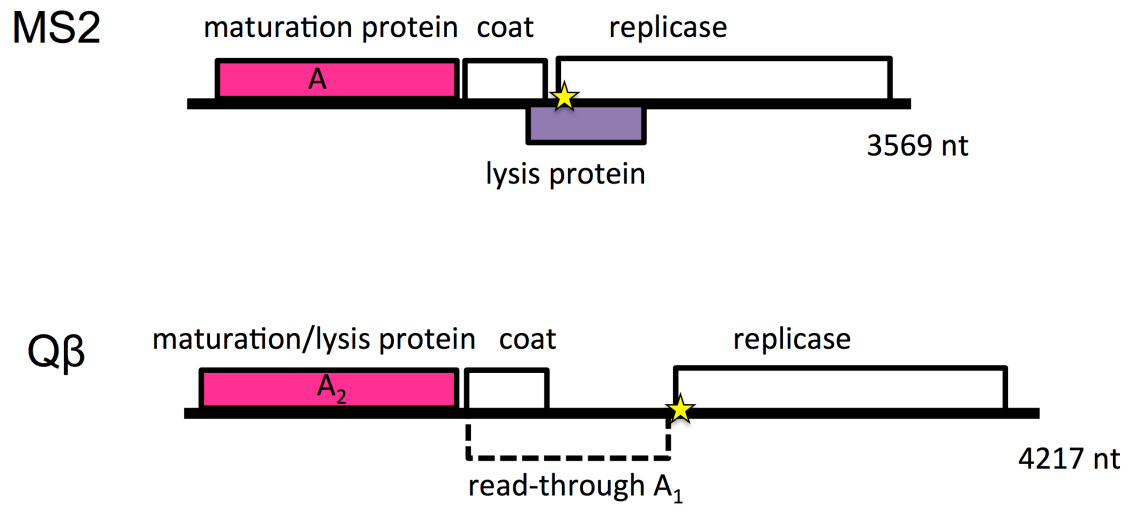

Figure S1. Genomic maps of *Leviviridae* MS2 (top; Levivirus) and Qβ (bottom; Allolevivirus). The maturation proteins are highlighted in hot pink. The lysis protein of MS2 is highlighted in purple. A_1 of Qβ is labeled as dashed box. Yellow stars indicate the operators near the start of their respective replicase genes.

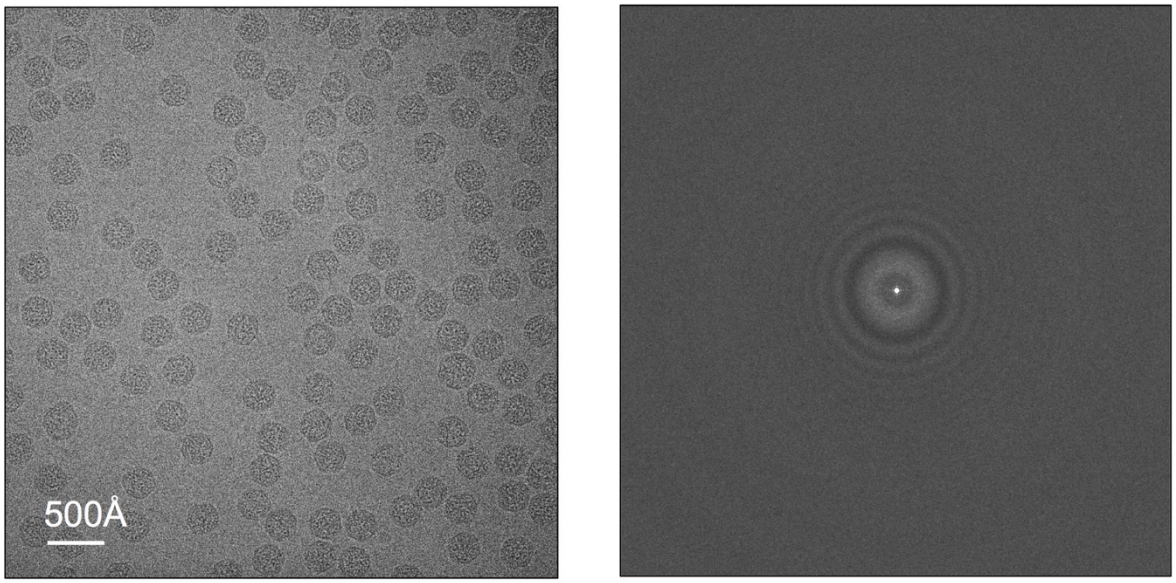

Figure S2. Drift-corrected micrograph of Qβ (left) and its power spectrum (right).

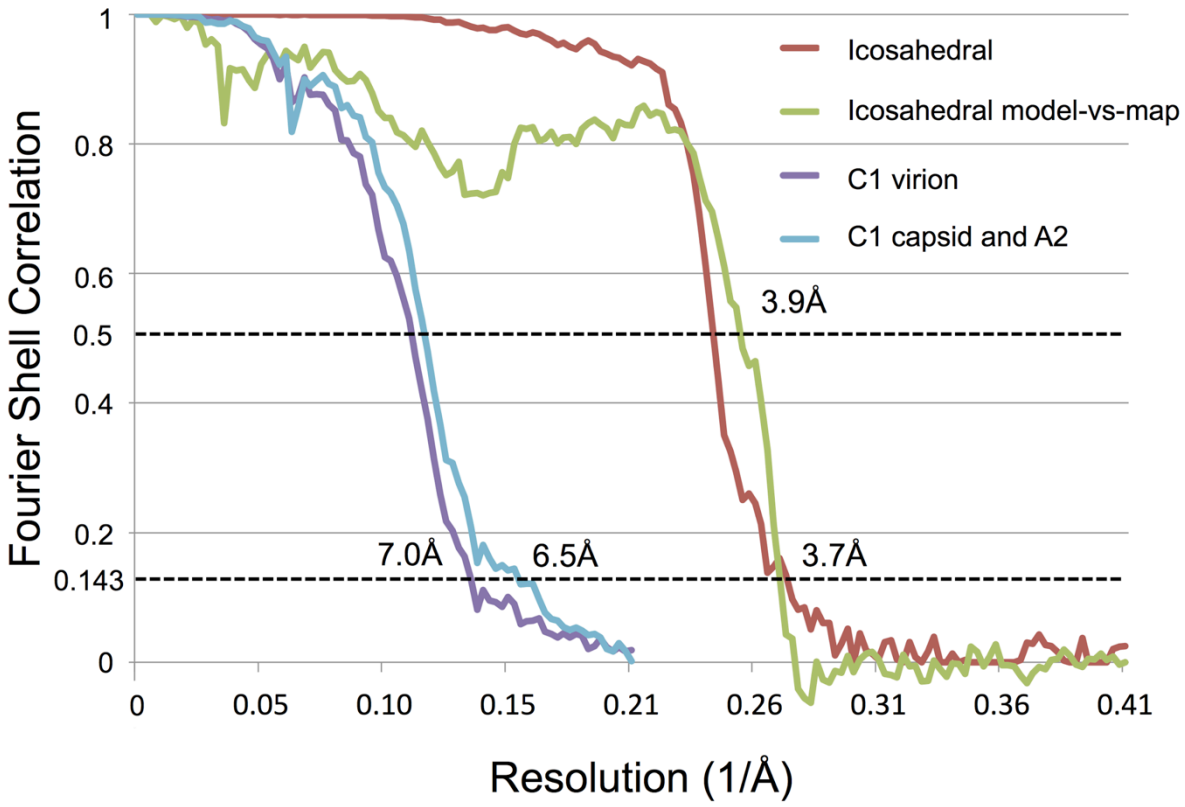

Figure S3. Gold-standard Fourier shell correlation (FSC) for the cryo-EM reconstructions of Qβ with and without symmetry and between the icosahedral map and the refined PDB model.

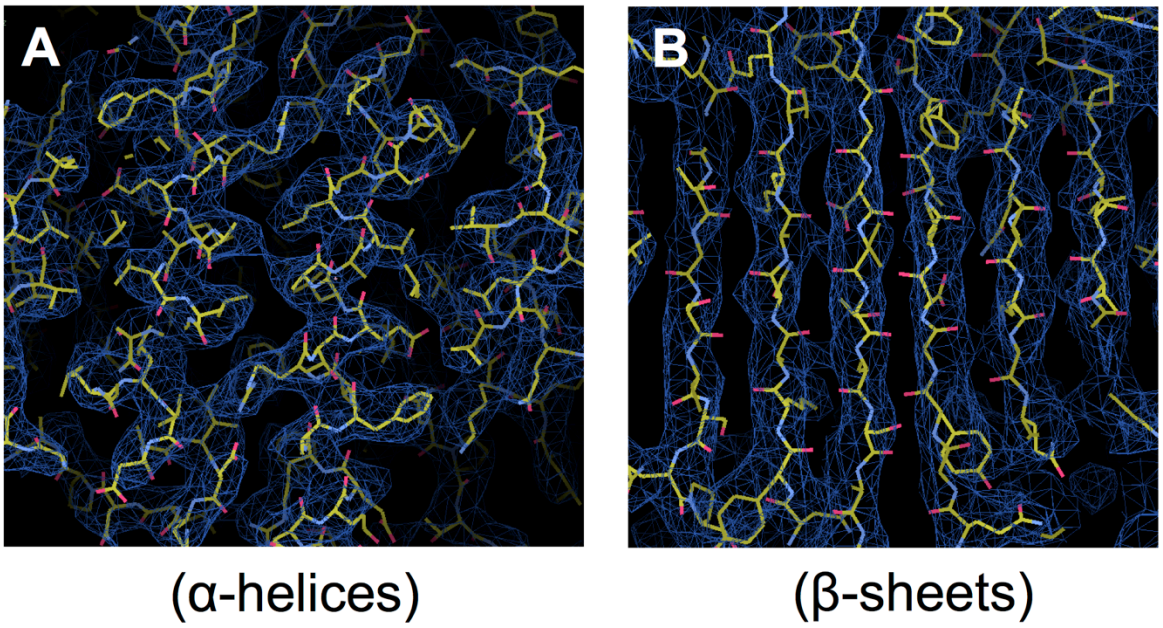

Figure S4. Fitting of the crystal structure (PDB ID 1QBE) into our icosahedral cryo-EM density map at 3.7Å resolution.

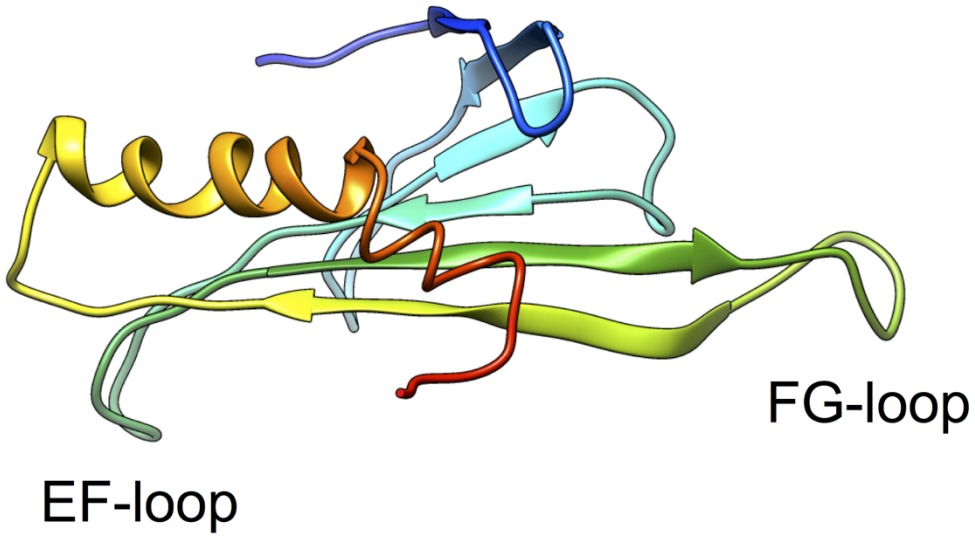

Figure S5. PDB model of Qβ coat protein showing the location of EF-loop and FG-loop. The protein backbone is colored blue to red from N-terminus to C-terminus.

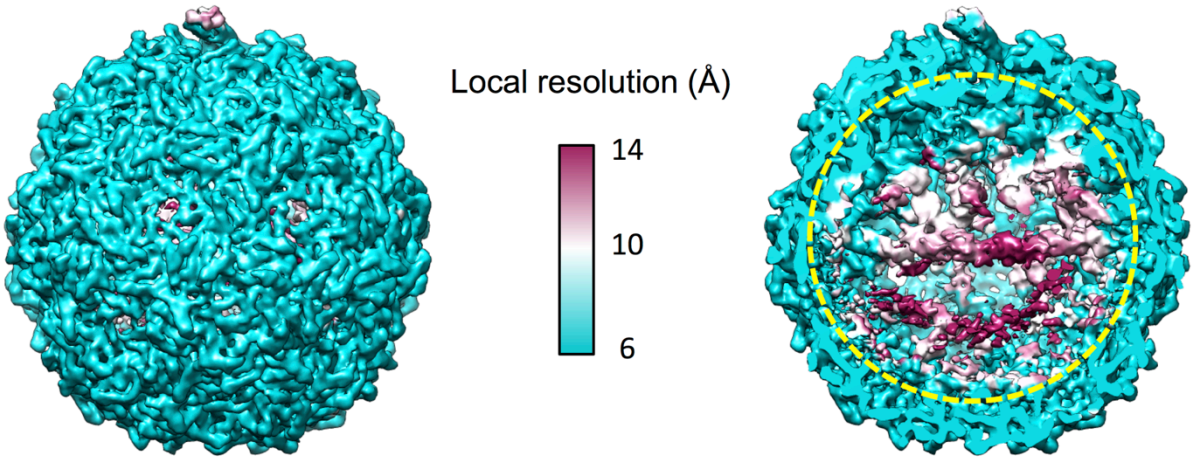

Figure S6. Local resolution of the asymmetric reconstruction of Qβ from outside (left) and cutaway (right) views. The yellow dashed circle encloses the gRNA densities.

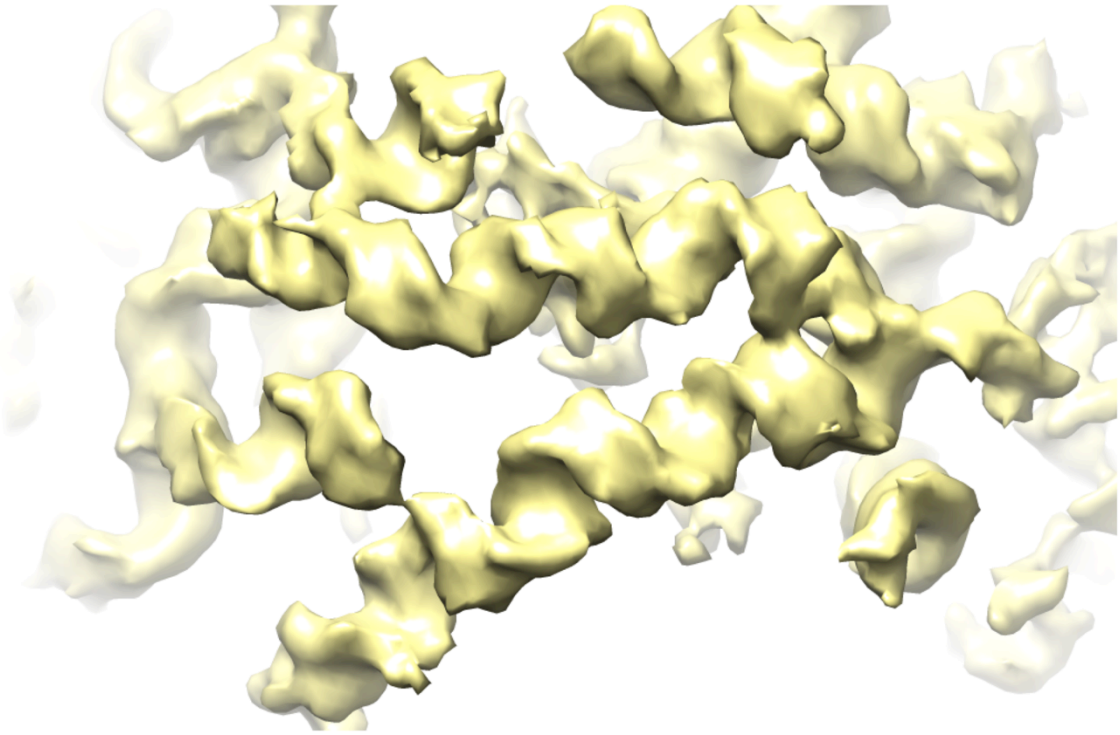

Figure S7. Segmented density map of genomic RNA showing major and minor grooves.

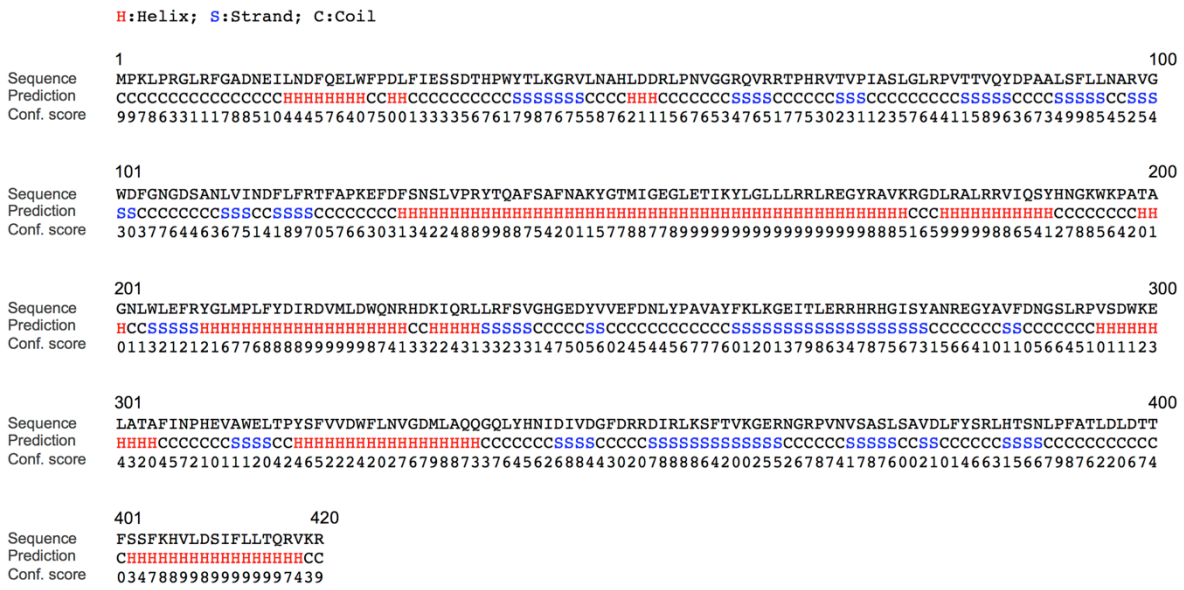

Figure S8. Secondary structure prediction for the A_2 protein from I-TASSER, with the first line as peptide sequence, second line as predicted secondary structures and the third line as the confidence score of the predicted secondary structures. The larger the confidence score, the higher the probability of the predicted secondary structures.

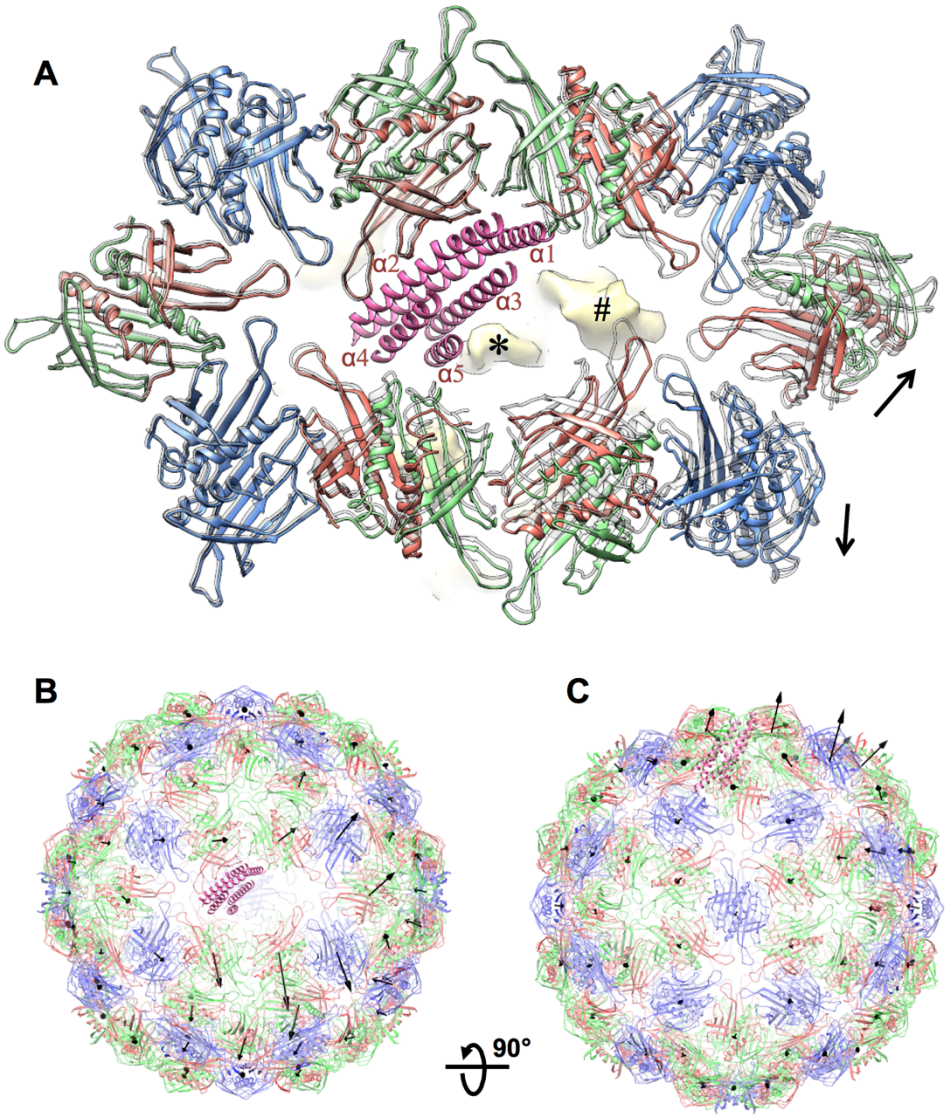

Figure S9. Deviation of the coat protein dimers' position in the asymmetric structure from their positions in the icosahedral reconstruction. (A) Zoom-in top view of the capsid proteins in the asymmetric structures (salmon, green and blue ribbon models) overlaid with their positions in the icosahedral structure (grey ribbon models). The α-region of A_2 is shown as a five-helix bundle (α1-5, hot pink ribbon models). Two RNA stem-loops (yellow density) are labeled with the star and pound symbols, respectively. Black arrows label the disruption of the interface between two dimers. (B) Zoom-out top view of asymmetric Qβ capsid with the black arrows indicating the direction and amount of deviation for each coat protein dimer from its position in the icosahedral structure. The length of each arrow equals 10 times the deviation between the corresponding dimer's centers-of-mass in the icosahedral and asymmetric capsids. (C) The side view with viewing angle rotated 90° from the top view.

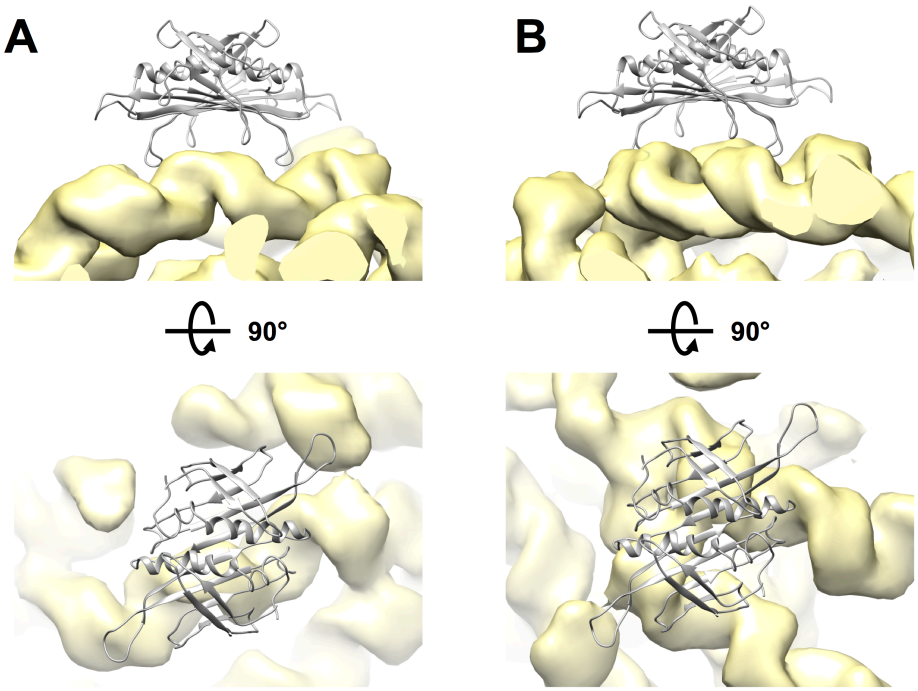

Figure S10. Examples of coat protein dimers (grey ribbon models) interacting with gRNA (yellow densities), which do not have an operator-like hairpin fold. (A) Side view and top view of one coat protein dimer interacting with the middle of an RNA helix. (B) Side view and top view of one coat protein dimer interacting with an RNA junction.

Table S1. Molprobity model statistics for the coat proteins built from the icosahedral map.

All-atom Clashscore	10.42
Poor rotamers	0.00%
Favored rotamers	98.06%
Ramachandran outliers	0.00%
Ramachandran favored	83.08%
MolProbity score	2.23
Cβ deviations >0.25Å	0.00%
Bad bonds:	0.00%
Bad angles:	0.07%