Cryo-EM structure of rotavirus B NSP2 reveals its unique tertiary architecture

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Supplemental Figure S1. **RVB NSP2 cryo-EM data processing.** (A) Representative micrograph. (B) Representative two-dimensional classes after several rounds of two-dimensional classification. (C) Threedimensional reconstruction pipeline.



Supplemental Figure S2. **NSP2 cryo-EM map.** (**A**) Four-fold symmetry and (**B**) two-fold symmetry views of RVB NSP2 (blue) with an additional density that may correspond to the RNA oligonucleotide (yellow).

Supplemental Table S1. Data collection and processing

Microscope	Titan Krios G3i
Camera	Gatan K3
Energy filter	Gatan BioQuantum with 20 eV slit
Voltage (kV)	300
Magnification	105 000x
Electron exposure $(e/Å^2)$	40
Defocus range (µm)	-2.5, -2.0, -1.5
Pixel size (Å)	0.86
Initial particle images (no.)	2 705 762
Final particle images (no.)	22 131
Symmetry imposed	D4
Resolution at 0.143 FSC (masked, Å)	3.83
Map sharpening B factor $(Å^2)$	-201.7
EMBD accession code	EMD-17323

Supplemental Table S2. Refinement and validation statistics

PDB accession code	8P00
Number of non-hydrogen atoms	16992
Protein residues	2376
Bonds (RMSD): Length (Å)	0.002
Bonds (RMSD): Angles (°)	0.581
Validation: Rotamer outliers (%)	0.06
Ramachandran plot: Outliers / Allowed / Favored (%)	0.00 / 4.58 / 95.42