

Supplementary Data

Supplementary Table 1. **Synthetic RNA oligonucleotides used in different assays.** This table lists all the RNA oligonucleotides used in this study. The sequence, length in nucleotides (nt) and the specific identifier used to label the RNA in the experimental descriptions are provided. All RNAs were chemically synthesized by Biomers with the exception of the primer RNA, which was chemically synthesized by ChemGenes and then manually capped using the ScriptCap kit (see Materials and Methods).

	Length [nt]	Identifier	Sequence		
5' RNA	20	L 5' (1-20)	5' HO-	ACACAGAGACGCCCAGAUGA	-OH 3'
3' RNA	20	L 3'-mid AAA (1-20)	3' HO-	UGUGUAAAUGGCGGGUCUAG	-OH 5'
	26	L 3'-6A (1-26)	3' HO-	UGUGUUUCUGGCGGGUCUAGAAAAAA	-OH 5'
Primers	24	Primer A-PP (1-24)	PP-	AAAACGCAACCAACACACACACAC	-OH 3'
	32	Primer B-PP (1-32)	PP-	AAAACGCAACCAACACACACACACACACAC	-OH 3'
	25 (incl. cap)	Primer A-CAP (1-25)	5' Cap ⁰ -	AAAACGCAACCAACACACACACAC	-OH 3'
	33 (incl. cap)	Primer B-CAP (1-33)	5' Cap ⁰ -	AAAACGCAACCAACACACACACACACACAC	-OH 3'
	17 (incl. cap)	Primer C-CAP (1-17)	5' Cap ⁰ -	AAAACGCAACCAACAC	-OH 3'

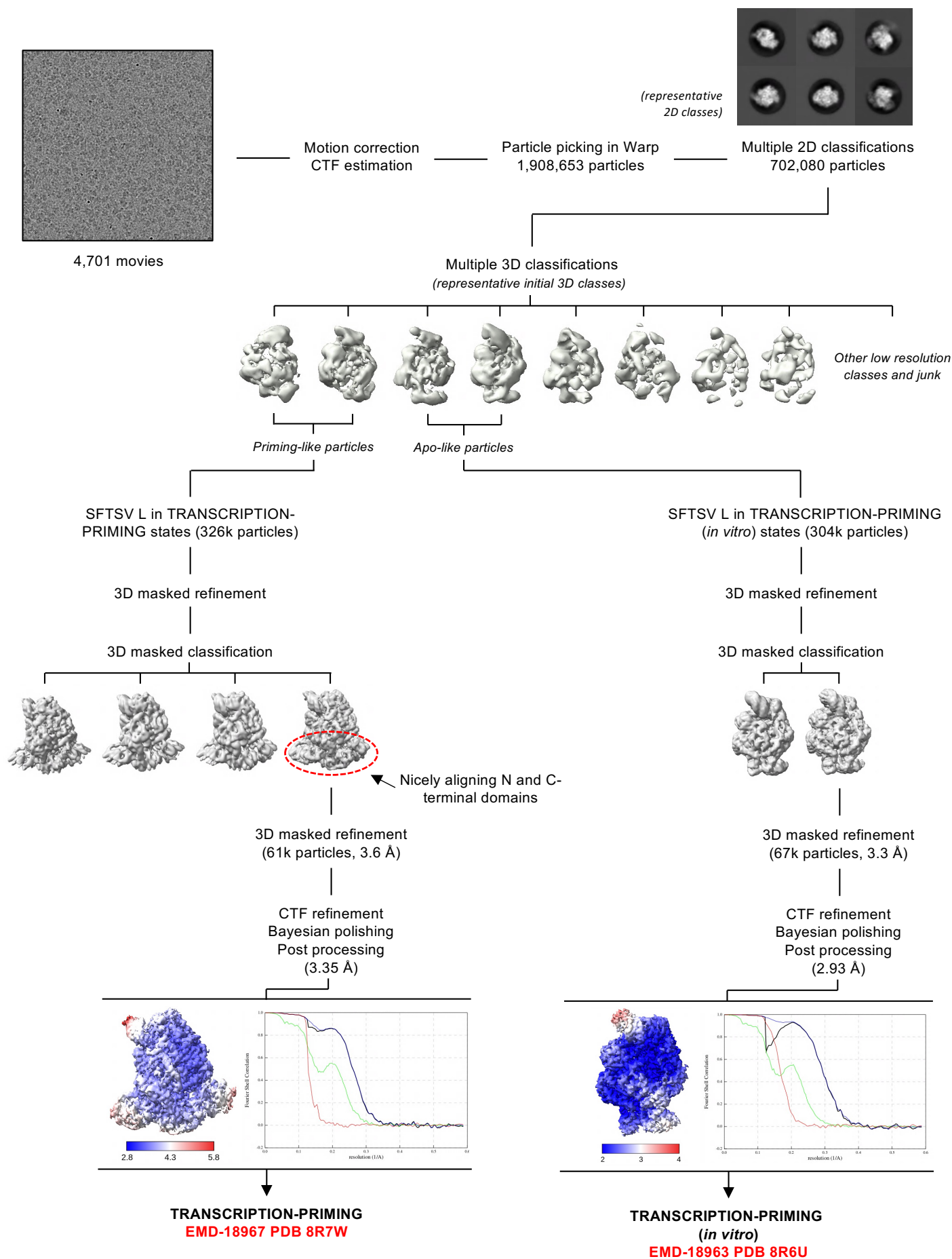
Supplementary Table 2. **Cryo-EM data collection, processing, refinement, and validation statistics.** This table provides the parameters and statistics for the data collection, processing, refinement, and structure validation of the cryo-EM structures described. Refinement statistics were generated using the Phenix package.

	TRANSCRIPTION-PRIMING	TRANSCRIPTION-EARLY-ELONGATION	TRANSCRIPTION-PRIMING (<i>in vitro</i>)
PDB Code	8R6W	8R7Y	8R6U
EMDB Code	EMD-18967	EMD-18969	EMD-18963
Data collection and processing			
Microscope	Titan Krios G3	Titan Krios G3	Titan Krios G3
Voltage (kV)	300	300	300
Camera	Gatan K3 BioQuantum	Gatan K3 BioQuantum	Gatan K3 BioQuantum
Magnification	105,000	130,000	105,000
Nominal negative defocus range (μm)	0.8-2.6	0.8 – 2.4	0.8-2.6
Exposure time (s)	2.5	1.45	2.5
Electron dose ($\text{e}^-/\text{\AA}^2$)	50	50	50
Frames collected (no.)	50	50	50
Frames processed (no.)	50	50	50
Pixel size ($\text{\AA}$)	0.85	0.67	0.85
Micrographs (no.)	4,701 (DATASET 1)	27,095 (DATASET 2)	4,701 (DATASET 1)
Total particle images	1,908,653 (DATASET 1)	2,998,852 (DATASET 2)	1,908,653 (DATASET 1)
Refinement			
Final particle subset (no.)	61,698	264,083	67,479
Map resolution ($\text{\AA}$), 0.143 FSC	3.35	3.40	2.98
Map sharpening <i>B</i> factor ($\text{\AA}^2$)	-43	-60	-20

Map vs. model cross-correlation CC-MASK	0.81	0.85	0.87
Model composition			
Non-hydrogen atoms	16,539	16,665	13,408
Protein residues	1,974	1,991	1,566
Nucleotide residues	39	36	43
Other	1 ADM	1 MG, 1 ADM, 1 2KH	1 MG
Mean B factors (Å)			
Protein	93.63	136.57	67.75
Nucleotide	147.24	183.25	117.26
Ligand	138.12	146.48	29.14
R.m.s. deviations			
Bond lengths (Å)	0.003	0.003	0.007
Bond angles (°)	0.741	0.665	0.728
Vanlilation			
MolProbity score	2.59	2.03	2.30
All-atom clashscore	6.53	5.59	4.89
Poor rotamers (%)	9.45	3.38	6.46
Ramachandran plot (%)			
Favoured	91.98	95.34	93.38
Allowed	7.87	4.66	6.62
Outliers	0.15	0.00	0.00

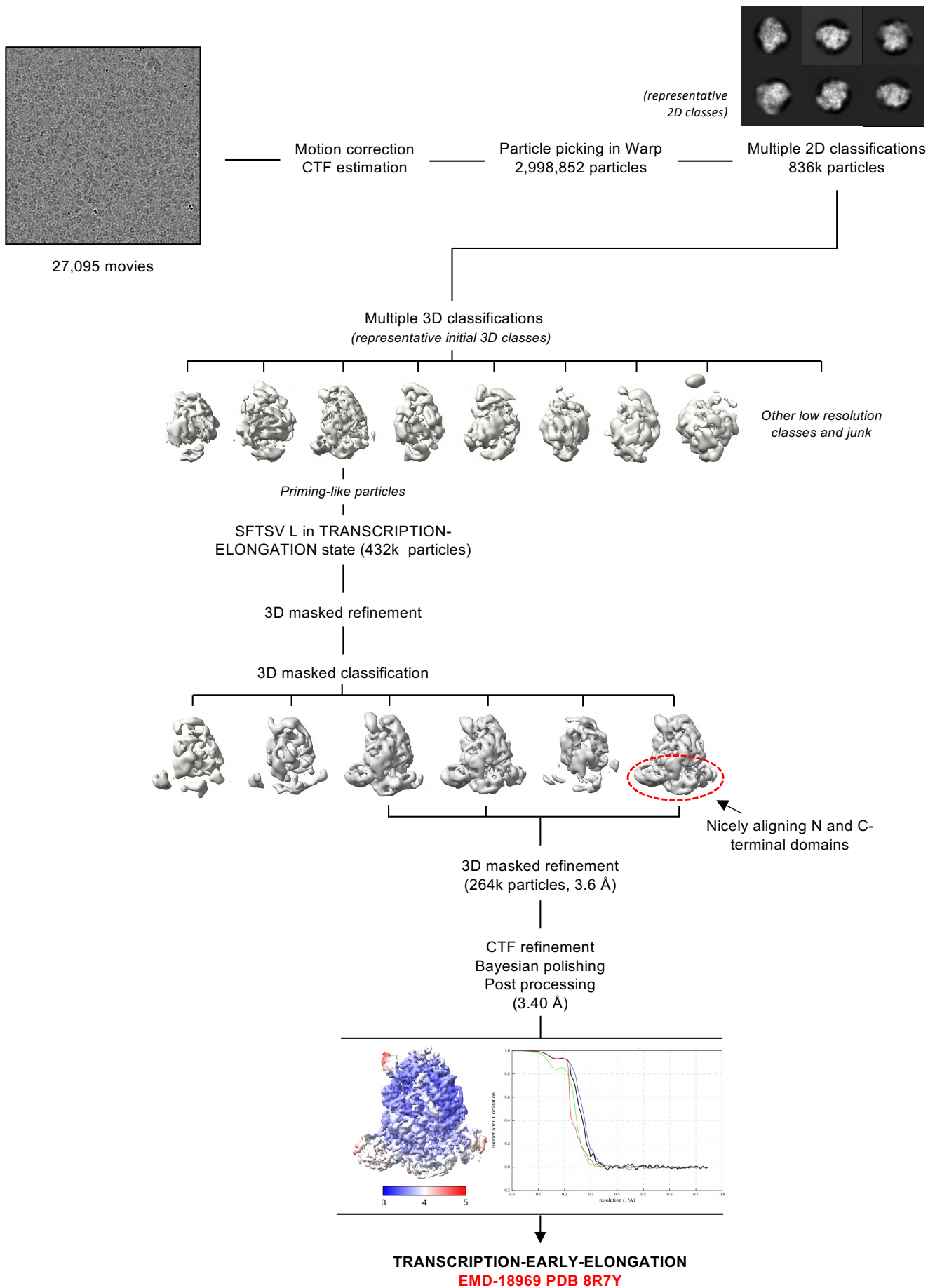
Supplementary Table 3. **Raw data from RVFV mini replicon assays.** For each mutant, the calculated Ren-Luc activity (as a % compared to the WT) from 3 biological replicates (as indicated in the table), the calculated average and standard deviation is provided.

RVFV Residue	Replicate 1	Replicate 2	Replicate 3	Average	Standard Deviation
RNA-interacting residues					
K1680A	11.65	32.32	20.02	21.33	10.40
S1719A	114.89	147.45	131.45	131.26	16.28
V1726G	111.49	78.92	146.70	112.37	33.90
R1841A	42.88	105.57	60.21	69.55	32.37
S1861A	70.93	65.76	118.90	85.20	29.30
T1863A	101.63	157.96	176.30	145.30	38.91
S1231A	24.98	35.71	42.49	34.39	8.83
N1846A	16.08	19.38	19.18	18.21	1.85
H1858A	0.03	0.03	5.92	1.99	3.40
R1200A	0.81	0.86	4.16	1.94	1.92
D1497A	59.93	69.73	62.07	63.91	5.15
R1501A	0.02	0.05	0.03	0.03	0.02
E1565A	16.74	10.44	18.11	15.10	4.09
S1865A	125.96	155.97	163.30	148.41	19.78
T1563A	3.56	3.28	3.58	3.47	0.17
V656G	109.92	154.89	107.90	124.24	21.69
W1205G	0.02	0.04	0.02	0.03	0.01
Blocking motif residues					
H1852A	29.80	39.27	24.15	26.98	4.00
Q1855A	102.60	102.41	89.00	98.00	7.80



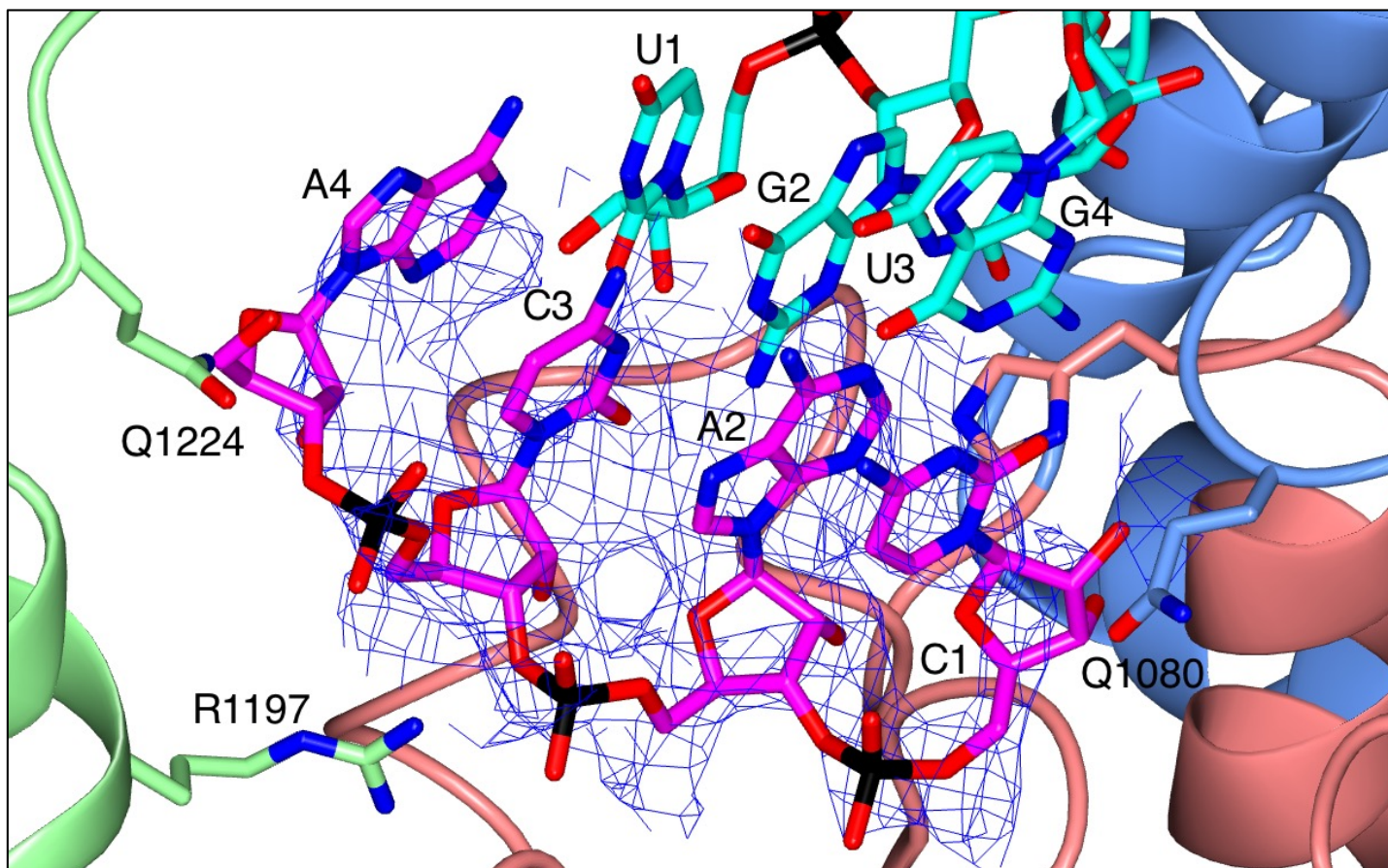
Supplementary Figure 1

Supplementary Figure 1. **Image processing strategy to obtain TRANSCRIPTION-PRIMING and TRANSCRIPTION-PRIMING (*in vitro*) structures.** Schematic representation of the image processing strategy used with the data collected on a Titan Krios G3 equipped with a Gatan K3 BioQuantum detector to obtain the **TRANSCRIPTION-PRIMING** and **TRANSCRIPTION-PRIMING (*in vitro*)** structures. Representative micrographs, 2D class averages, and 3D class averages are displayed. All processing steps, including 2D classification, 3D classification, 3D refinement, and post-processing were completed in RELION with the exception of the **TRANSCRIPTION-PRIMING** structure, the map for which was further processed in LocScale in CCP-EM. Local resolution EM maps are coloured according to the resolution key shown. Fourier shell correlation curves (FSC) curves are also displayed.



Supplementary Figure 2

Supplementary Figure 2. **Image processing strategy to obtain TRANSCRIPTION-EARLY-ELONGATION structure.** Schematic representation of the image processing strategy used with the data collected on a Titan Krios G3 equipped with a Gatan K3 BioQuantum detector to obtain the **TRANSCRIPTION-EARLY-ELONGATION** structure. Representative micrographs, 2D class averages, and 3D class averages are displayed. All processing steps, including 2D classification, 3D classification, 3D refinement, and post-processing were completed in RELION. Local resolution EM maps are coloured according to the resolution key shown. Fourier shell correlation curves (FSC) curves are also displayed.



Supplementary Figure 3

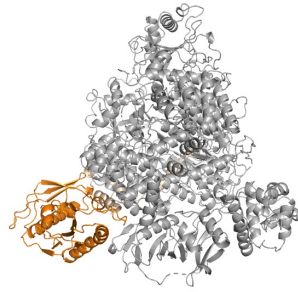
Supplementary Figure 3. **Zoomed-in view of the TRANSCRIPTION-PRIMING structure active site.** A zoomed-in view of the active site in the **TRANSCRIPTION-EARLY-ELONGATION** structure is shown with the respective map overlaid. Residues from the SFTSV L protein are labelled and coloured according to the assigned domain (blue for fingers domain, light green for the thumb and thumb ring domains, and coral for the palm domain). RNAs are shown as sticks and coloured either yellow (5' RNA) or magenta (primer RNA). The overlaid map is clipped to the primer RNA.

FRONT VIEW

SIDE VIEW

REAR VIEW

**TRANSCRIPTION
PRIMING**
(PDB: 8R6W)



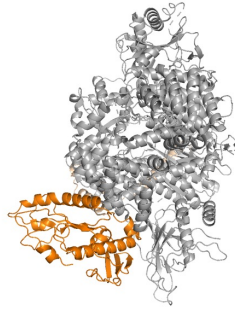
90°



90°



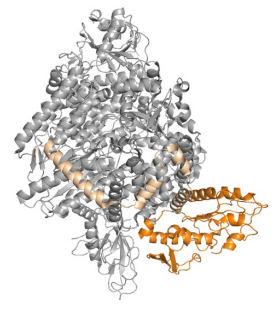
**EARLY-
ELONGATION-
ENDO**
(PDB: 8ASB)



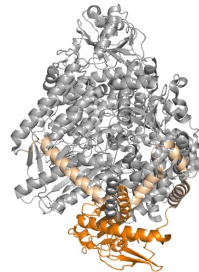
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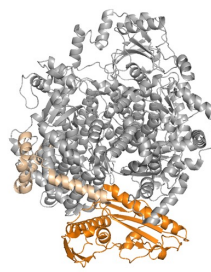
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**EARLY-
ELONGATION**
(PDB: 8AS7)



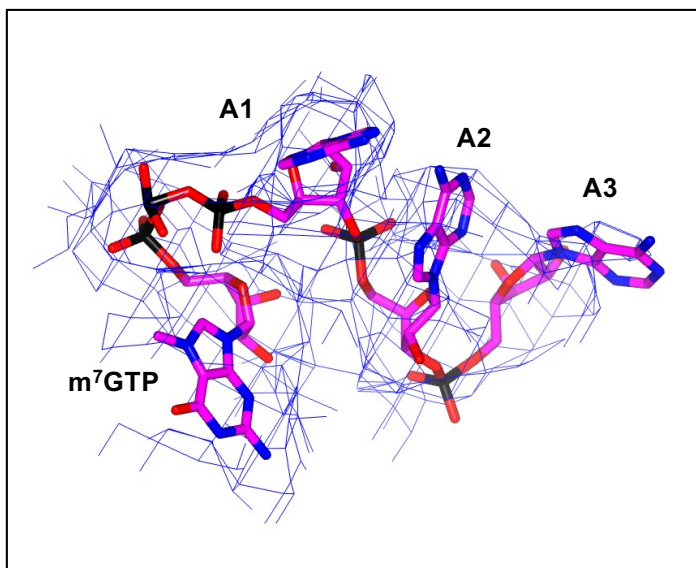
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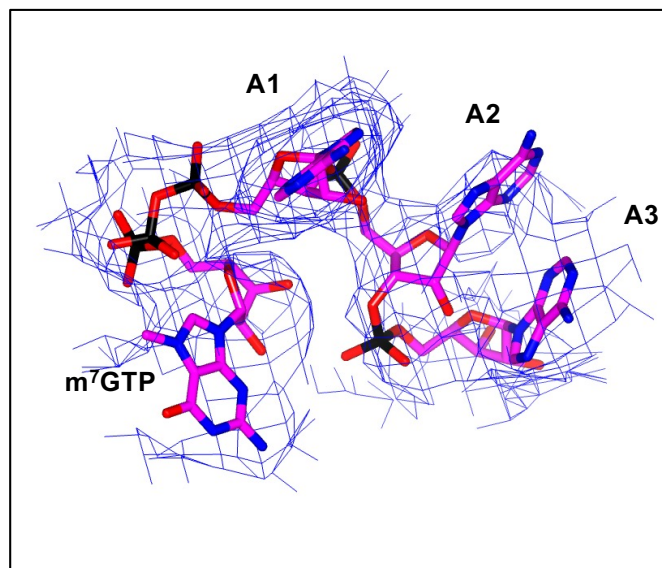
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Supplementary Figure 4. **Hyper-flexibility of the SFTSV L protein endonuclease domain.** The SFTSV L protein TRANSCRIPTION-PRIMING, EARLY-ELONGATION (PDB: 8AS7), and EARLY-ELONGATION-ENDO (PDB: 8ASB) structures are shown having been superposed by a least-squares fit of main-chain RNA-dependent RNA polymerase residues. For clarity, the majority of the L protein is coloured in light grey with only the endonuclease and endonuclease linker coloured orange and wheat, respectively. Bound RNA in each structure is not shown. Three views are shown for each structure to enable comparison.

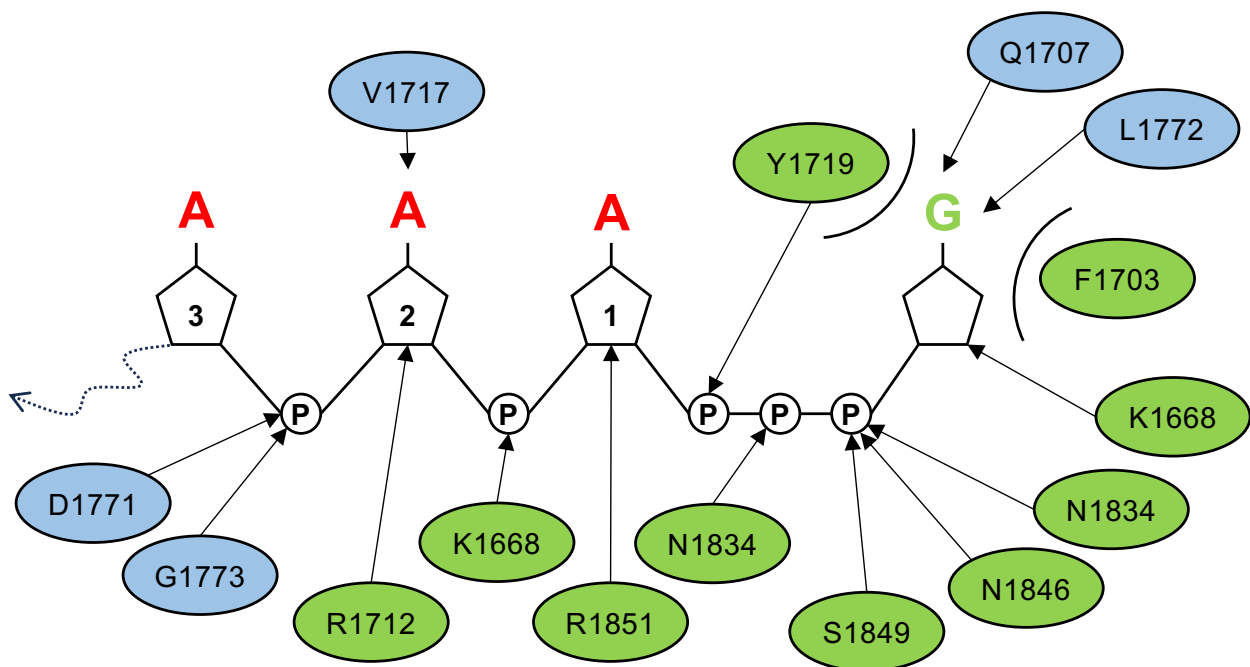


Capped RNA in TRANSCRIPTION-PRIMING structure

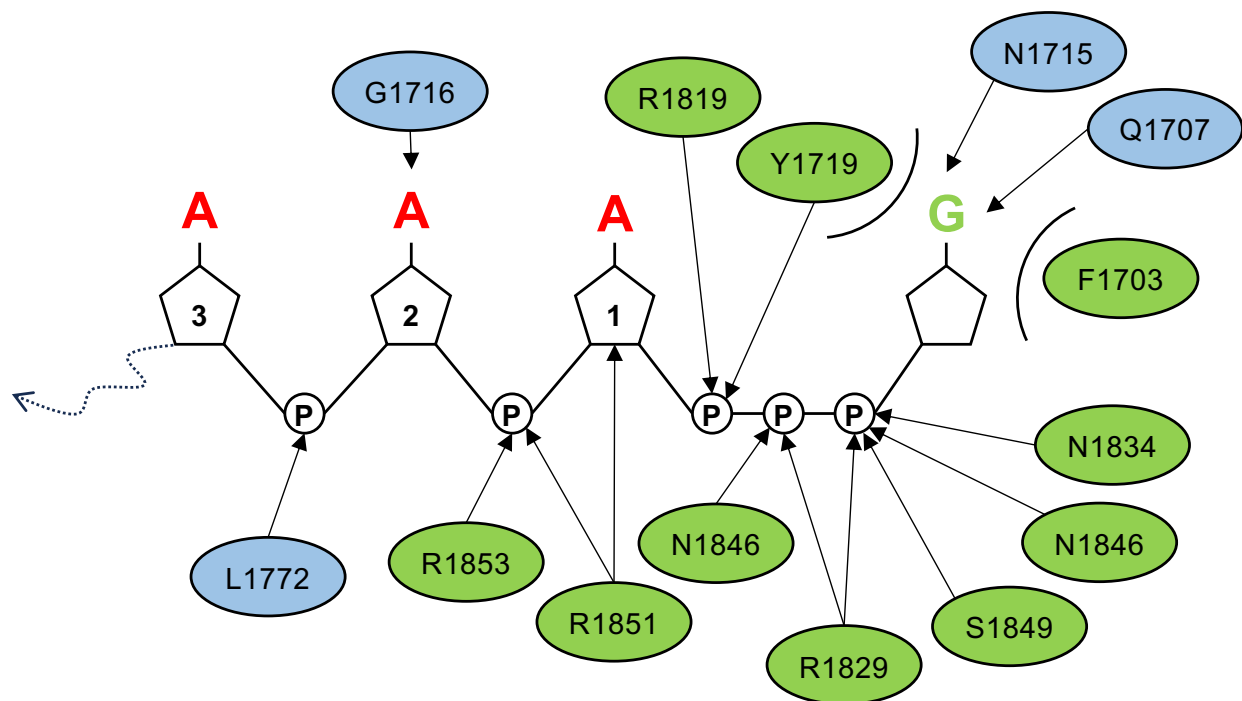


Capped RNA in TRANSCRIPTION-EARLY-ELONGATION structure

Supplementary Figure 5. **Quality of the map for the bound capped RNA.** Zoomed-in views of the capped RNA (shown as sticks in magenta) in the **TRANSCRIPTION-PRIMING** and **TRANSCRIPTION-EARLY-ELONGATION** structures are shown with the relevant map overlaid. In both cases, the map is clipped to the capped RNA.

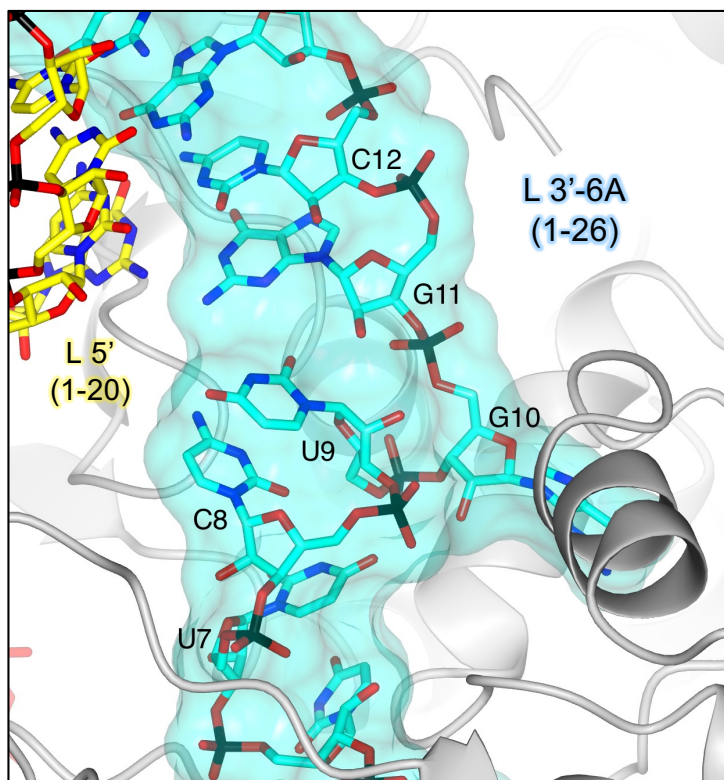


Capped RNA interactions in TRANSCRIPTION-PRIMING structure

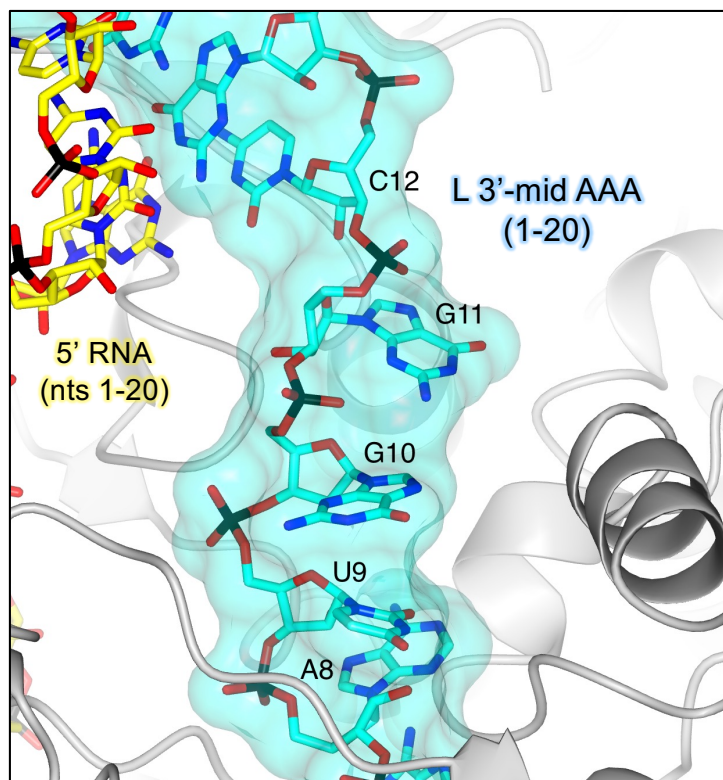


Capped RNA interactions in TRANSCRIPTION-EARLY-ELONGATION structure

Supplementary Figure 6. **Capped RNA interaction map.** A schematic overview of all interactions between SFTSV L protein residues and bound capped RNA is shown. Mainchain interactors are coloured blue, whereas sidechain interactors are coloured green.

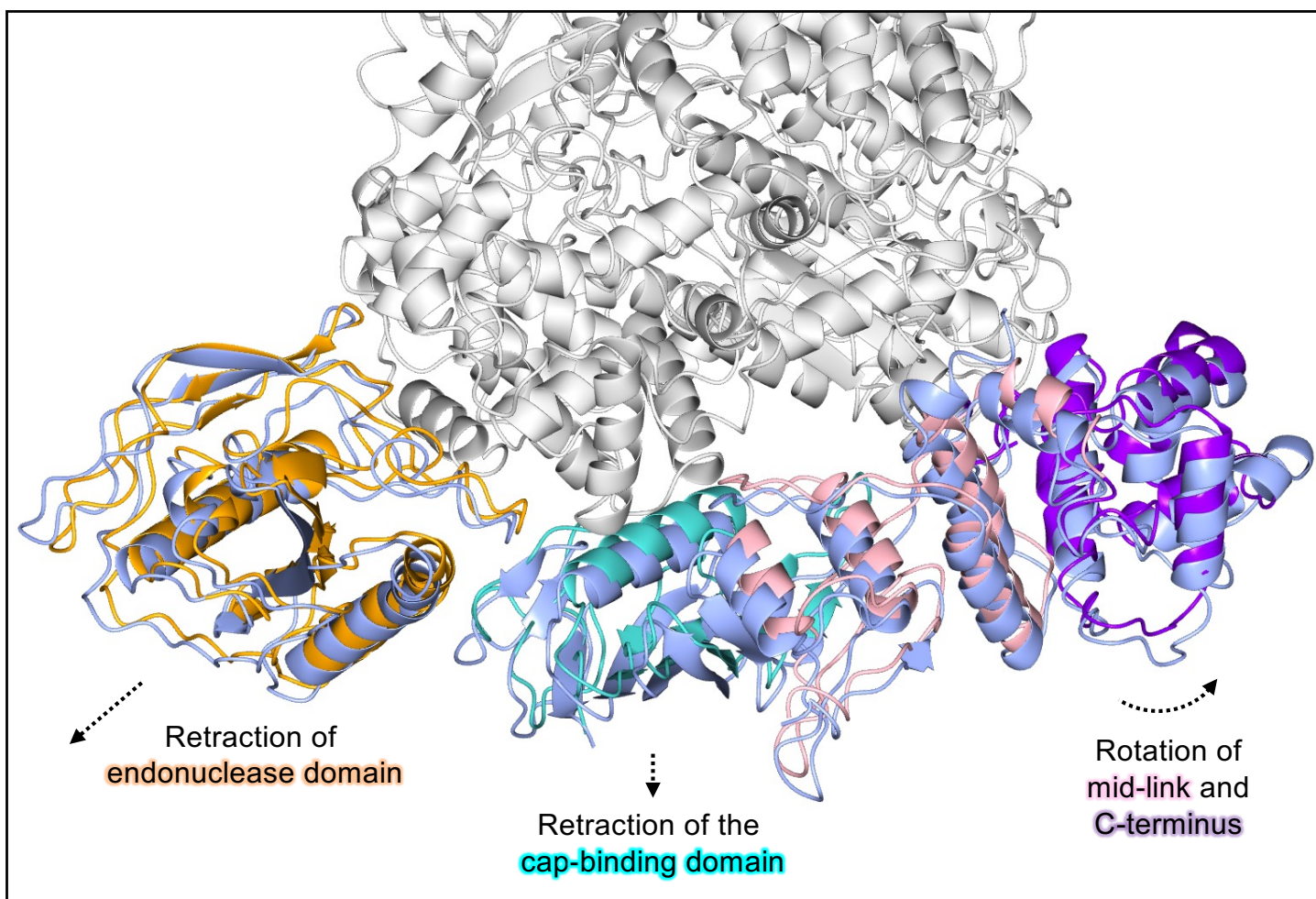


Template RNA in TRANSCRIPTION-PRIMING structure



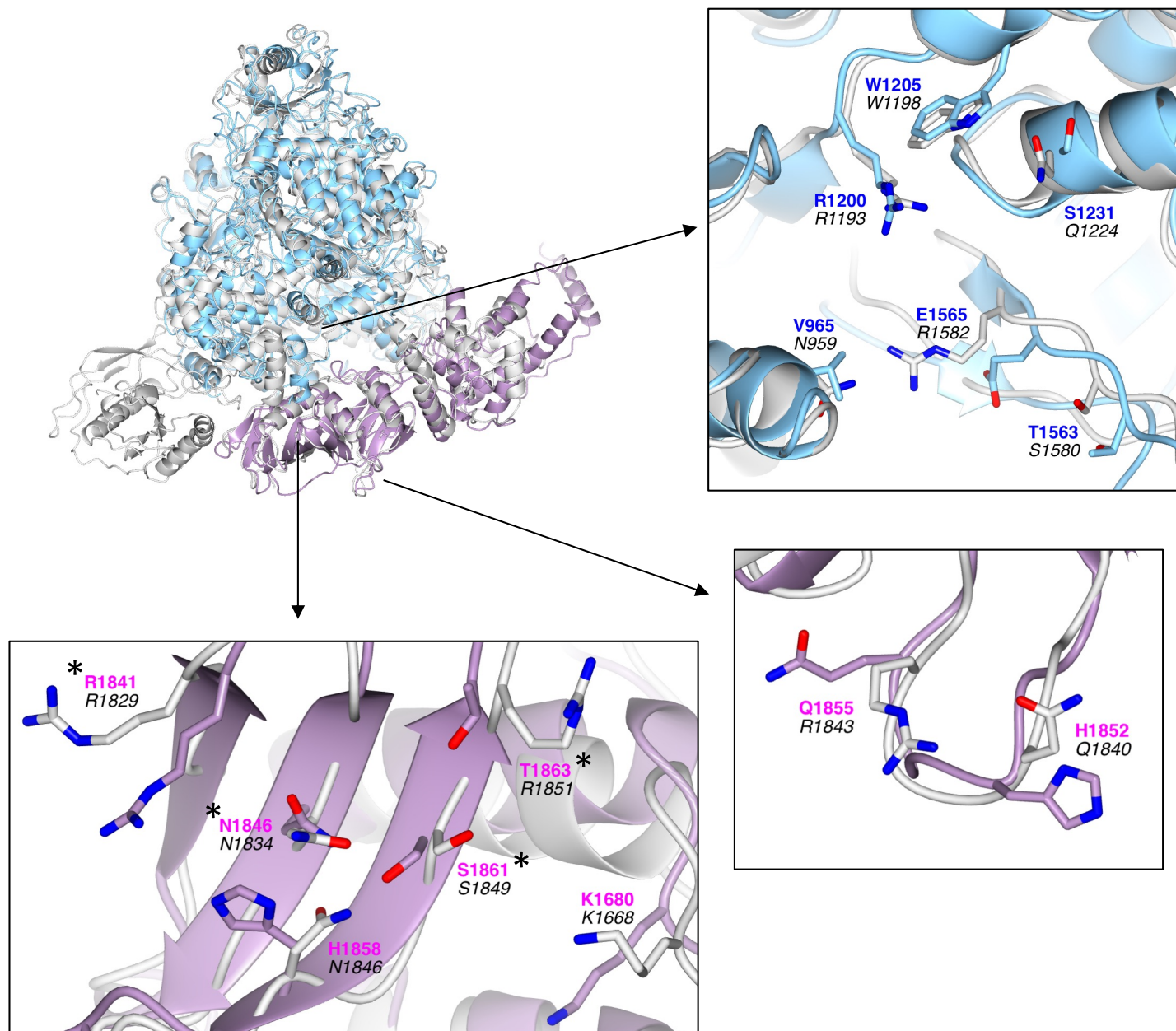
Template RNA in TRANSCRIPTION-EARLY-ELONGATION structure

Supplementary Figure 7. **Status of the 3' RNA between the distal duplex and the template entry channel.** A zoomed-in view of the SFTSV L protein TRANSCRIPTION-PRIMING (left) and TRANSCRIPTION-EARLY-ELONGATION (right) are shown. RNA in both structures is shown as sticks with surface overlaid (30% transparency) and coloured either yellow (5' RNA) or cyan (3' RNA).



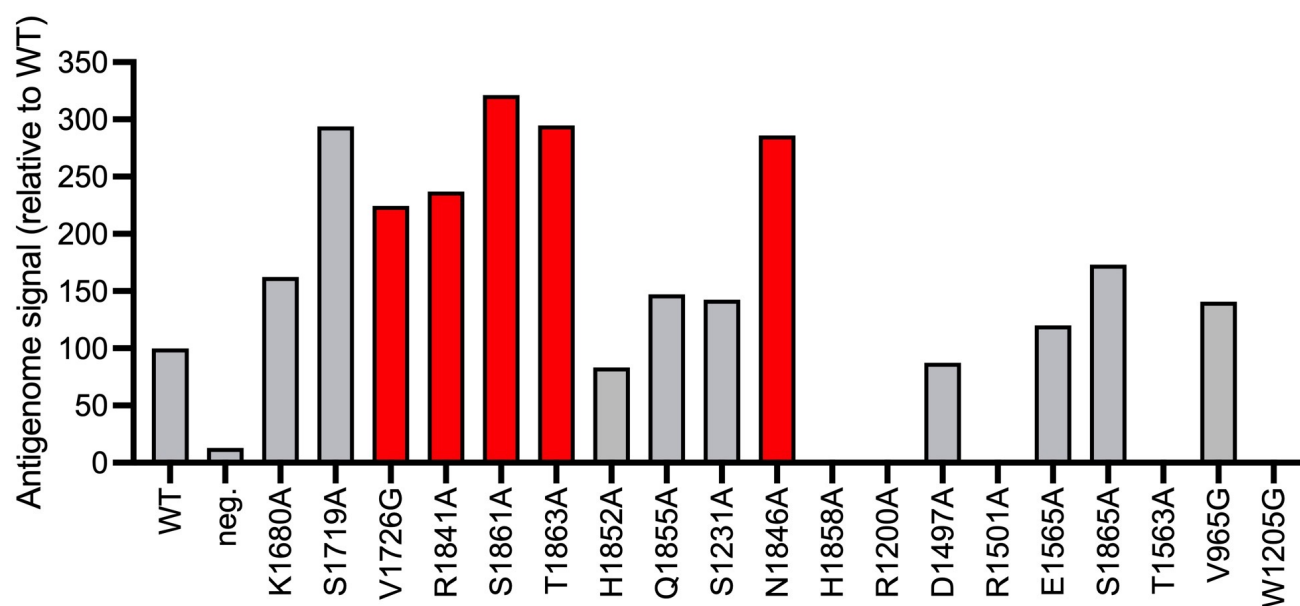
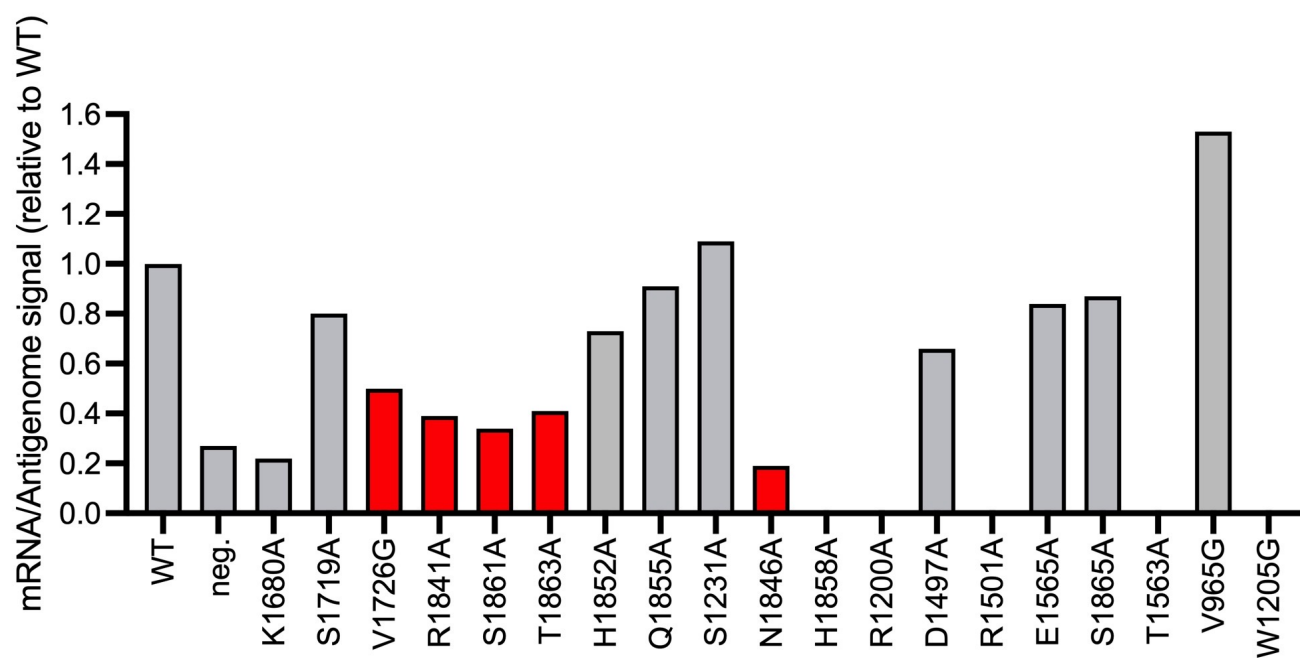
Supplementary Figure 8

Supplementary Figure 8. **Conformational changes as the SFTSV L protein moves from transcription priming to a transcription-specific early elongation state.** The SFTSV L protein **TRANSCRIPTION-PRIMING** and **TRANSCRIPTION-EARLY-ELONGATION** structures are shown. Key L protein domains in the TRANSCRIPTION-PRIMING structure are coloured canonically, i.e.: endonuclease (orange), cap-binding domain (cyan), mid-link domain (light pink), and extreme C-terminus (purple). For comparison, the same domains in the **TRANSCRIPTION-EARLY-ELONGATION** are each coloured ice blue. Dashed arrows indicate the direction of movement for each domain.



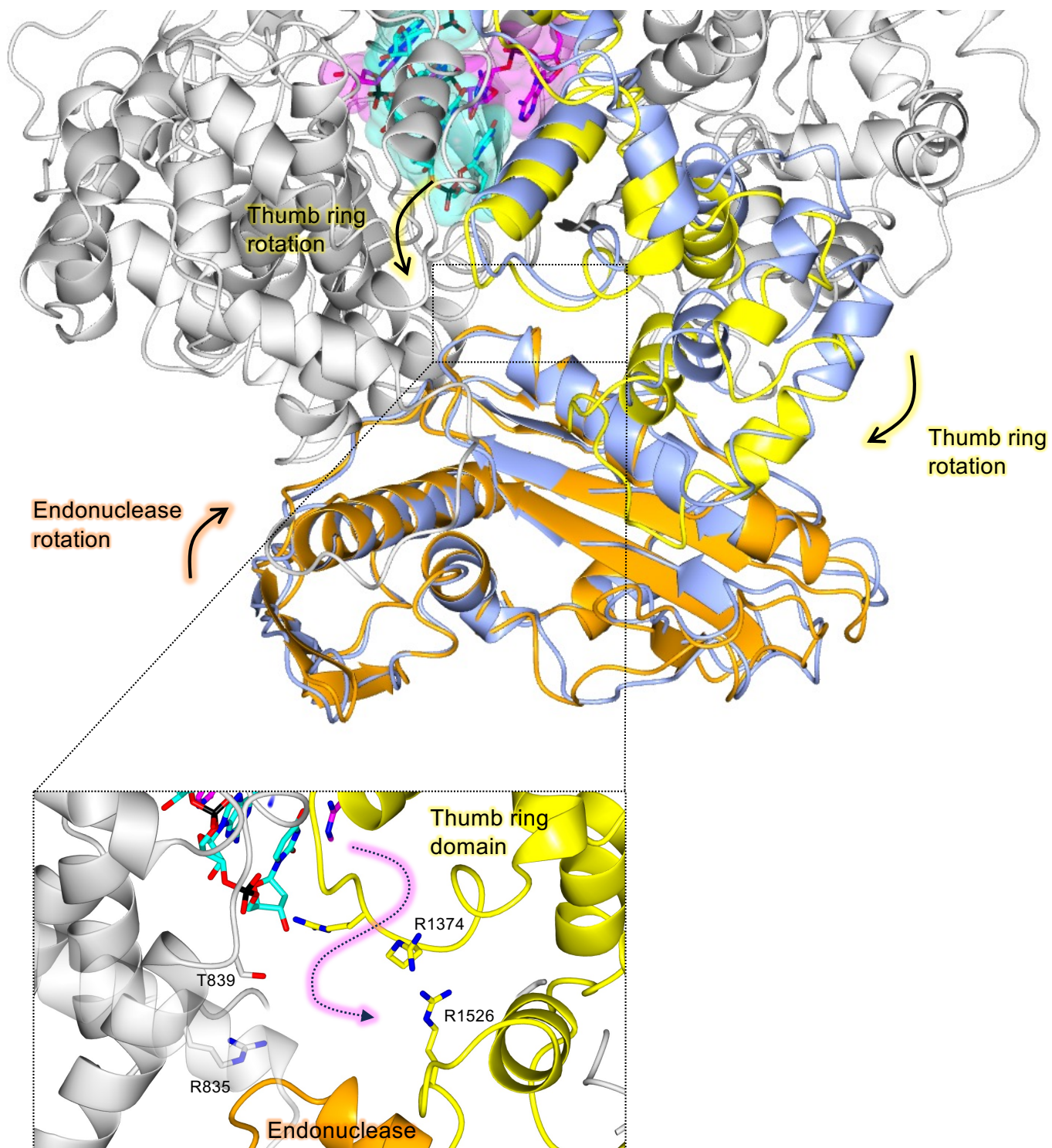
Supplementary Figure 9

Supplementary Figure 9. **Structural superposition of the RVFV L protein onto the SFTSV L protein.** The SFTSV L protein (TRANSCRIPTION-PRIMING) is shown with the RVFV L protein (PDB: 7EEI, backbone coloured light blue). The RVFV L protein C-terminus (residues 1649-2149, predicted using an AlphaFold2 version implemented on the DESY MAXWELL computing cluster) is also shown (backbone coloured lilac). RVFV L has been superposed onto the SFTSV L superposed by SSM in CCP4mg. Sidechains of key amino acids mutated in the cell-based mini-replicon are shown individually with close-up views. Residues from the RVFV L protein are labelled and coloured according to their source (i.e. light blue for the experimentally determined 7EEI structure, or lilac for AlphaFold2 prediction) with the corresponding SFTSV L protein residue labelled in black. For clarity, not all residues mutated in the cell-based mini-replicon are shown but 14 of the 19 in total are shown. Mutants with an asterisk beside them show a transcription-specific reduction in our mini-replicon assay.



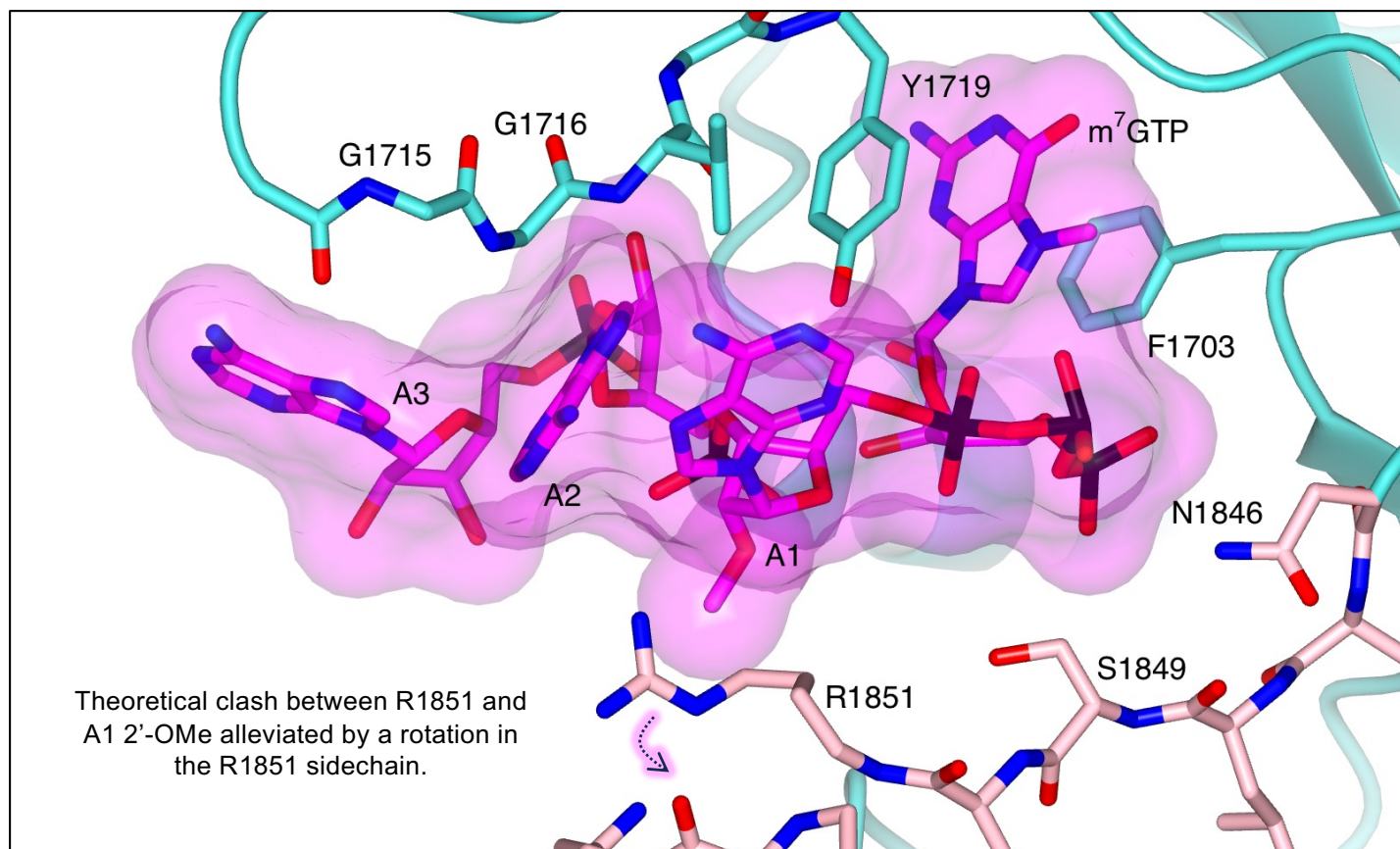
Supplementary Figure 10

Supplementary Figure 10. **Northern blot quantification.** (Left) RNA signals on northern blots for selected mutants were quantified using ImageJ/FIJI and the mRNA-to- antigenome signal ratio was calculated. (Right) Antigenome signals for selected mutants in northern blots were quantified via intensity profiles using ImageJ/FIJI. For both analyses, the wild-type ratio was set at 100% for each experiment allowing the comparison of independent experiments. Note, wherever no discernible signal can be detected on the Northern blot for the purposes of statistical analyses this is recorded as *nil*. Mutants with a transcription-specific reduction are indicated in red. Data plotted in Prism.



Supplementary Figure 11

Supplementary Figure 11. **Insights into cap-independent primer-elongation.** The SFTSV L protein **TRANSCRIPTION-PRIMING (*in vitro*)** structure is shown with the published apo L protein structure (PDB: 6Y6K) superposed by SSM in CCP4mg. Parts in grey are similar in both structures and, where this is true, only the **TRANSCRIPTION-PRIMING (*in vitro*)** structure is depicted. Domains of the **TRANSCRIPTION-PRIMING (*in vitro*)** structure that are shown to either rotate or remodel to enable primer entry and base pairing with the 3' RNA are coloured (orange for the endonuclease and yellow for the thumb ring domain). The corresponding apo structure domains are shown in ice blue. RNAs are shown as sticks with surface overlaid (30% transparency) and coloured either cyan (3' RNA) or magenta (primer RNA). Movements to the endonuclease and thumb ring domains precipitated by the progression to late elongation are indicated. Additionally, a zoomed-in view of the proposed product exit channel through which the primer has likely entered the RdRp core is shown. The path of the primer RNA, which is coloured magenta, is indicated by a dashed arrow.



Supplementary Figure 12

Supplementary Figure 12. **Exploring the effect of cap1 methylation.** The cap-binding domain is shown with bound capped RNA. The cap-binding domain is coloured cyan, whereas the neighbouring mid-link domain is shown in light pink. The bound capped RNA is shown as sticks with surface overlaid (30% transparency). The primer RNA has been modified by the substitution of A1 with 2'-O-methyladenosine (PDB: A2M). Sidechains of key interacting amino acids, coloured according to the protein domain, are shown as sticks and labelled accordingly. The addition of the methyl group at the O2' position would clash with the current placement of the R1851 sidechain, however, as indicated, the R1851 sidechain. could rotate and in doing so create sufficient space for cap1 RNA.