

Supplementary Materials for
Cryo-EM structures of the Crimean-Congo Haemorrhagic
Fever Virus RNA Dependent RNA Polymerase

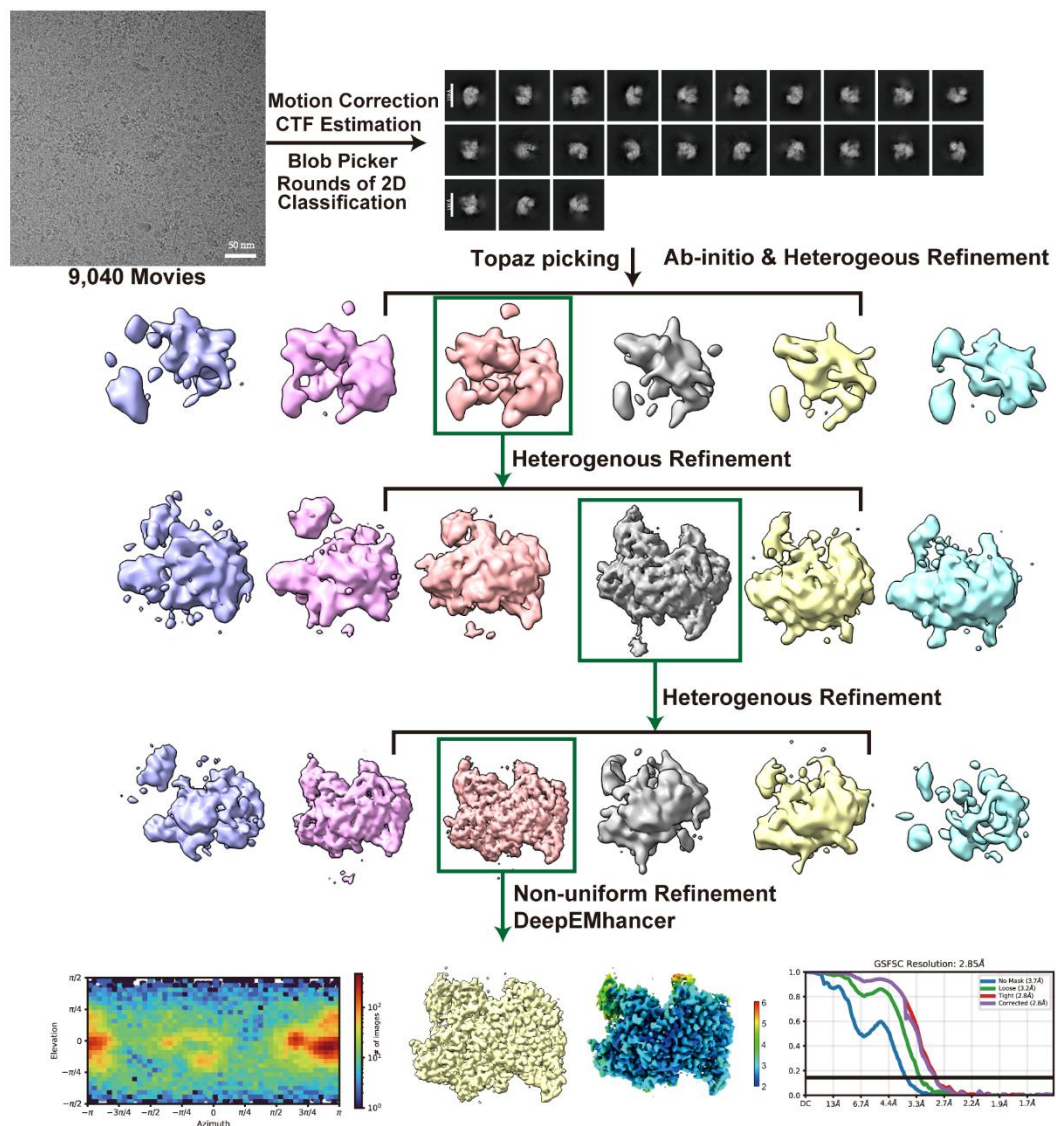
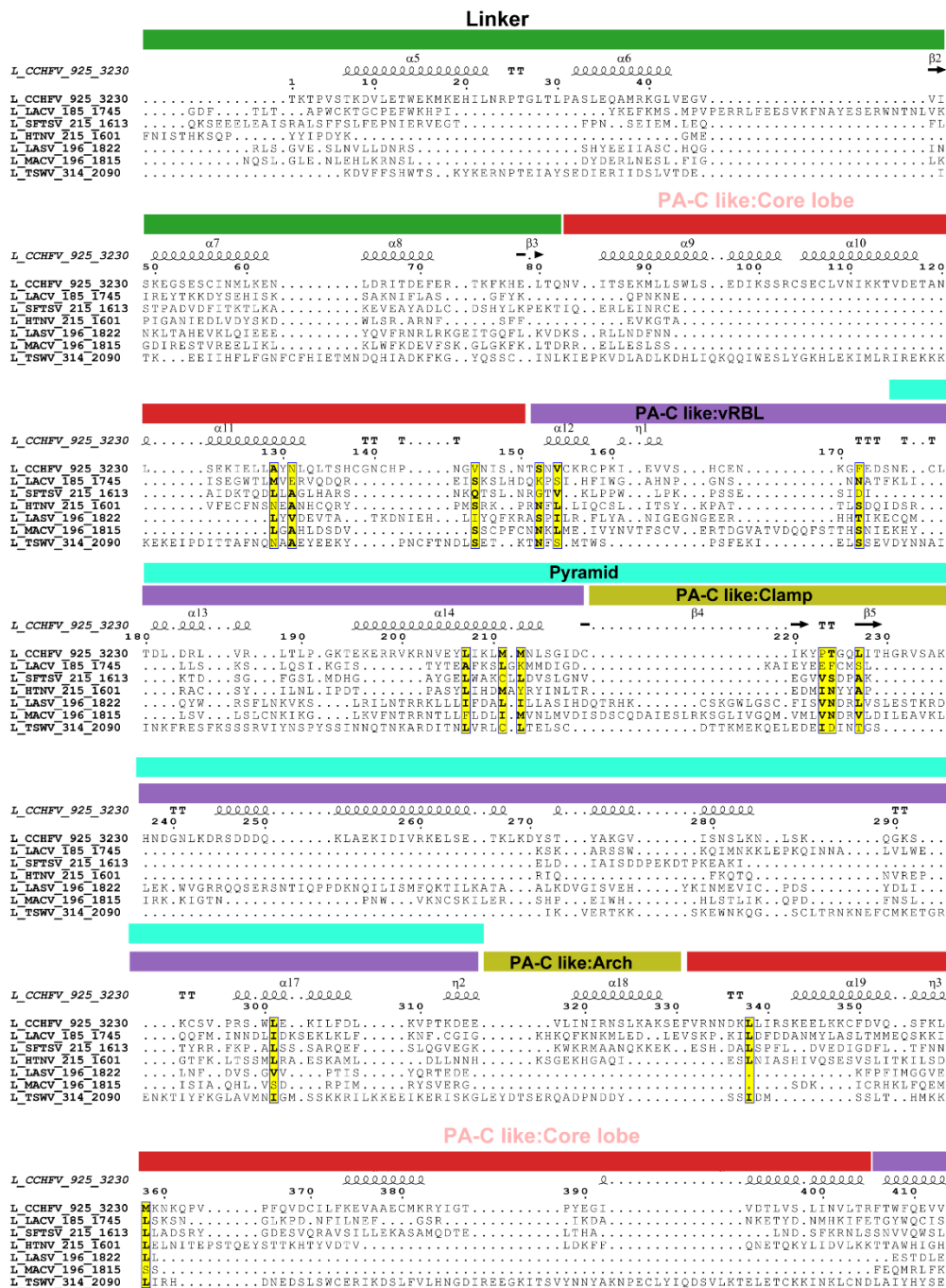


Figure S1 Data processing workflow of cryo-EM data analysis of apo structure of the CCHFV L Core.



PA-C like:vRBL

α22 420 TT 430 β6 440 β7 450 TT 460 β8 TT

L_CCHFV_925_3230
L_LACV_185_1745
L_SFTSV_215_1613
L_BTNV_215_1601
L_LASV_196_1822
L_MACV_196_1815
L_TSWV_314_2090

β9 470 α23 480 490 500 η4 510 α24 520 530

L_CCHFV_925_3230
L_LACV_185_1745
L_SFTSV_215_1613
L_BTNV_215_1601
L_LASV_196_1822
L_MACV_196_1815
L_TSWV_314_2090

α25 540 η5 550 α26 560 α27 570 α28 580 η6 590 600 610

L_CCHFV_925_3230
L_LACV_185_1745
L_SFTSV_215_1613
L_BTNV_215_1601
L_LASV_196_1822
L_MACV_196_1815
L_TSWV_314_2090

PA-C like:Core lobe Motif G

β10 620 α29 630 α30 640 α31 650 660 670

L_CCHFV_925_3230
L_LACV_185_1745
L_SFTSV_215_1613
L_BTNV_215_1601
L_LASV_196_1822
L_MACV_196_1815
L_TSWV_314_2090

New motif K

α32 680 η7 690 α33 700 α34 710 720 730 740 750

L_CCHFV_925_3230
L_LACV_185_1745
L_SFTSV_215_1613
L_BTNV_215_1601
L_LASV_196_1822
L_MACV_196_1815
L_TSWV_314_2090

New motif J Fingers

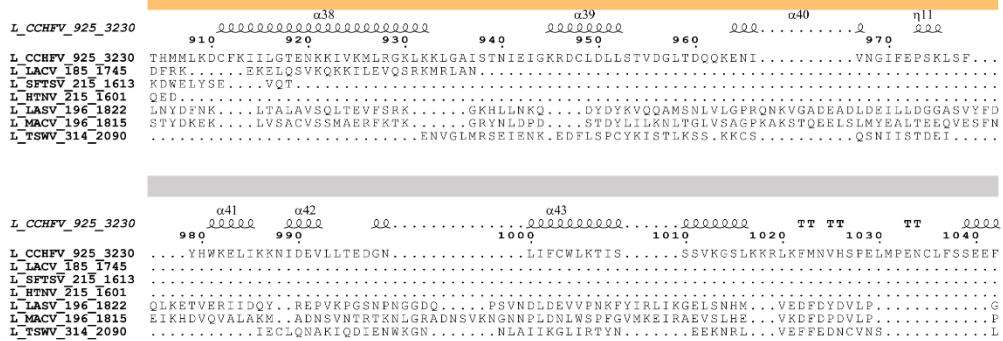
β11 760 770 780 790 800 810 820 830 840

L_CCHFV_925_3230
L_LACV_185_1745
L_SFTSV_215_1613
L_BTNV_215_1601
L_LASV_196_1822
L_MACV_196_1815
L_TSWV_314_2090

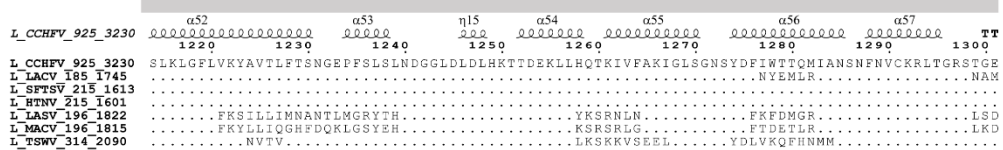
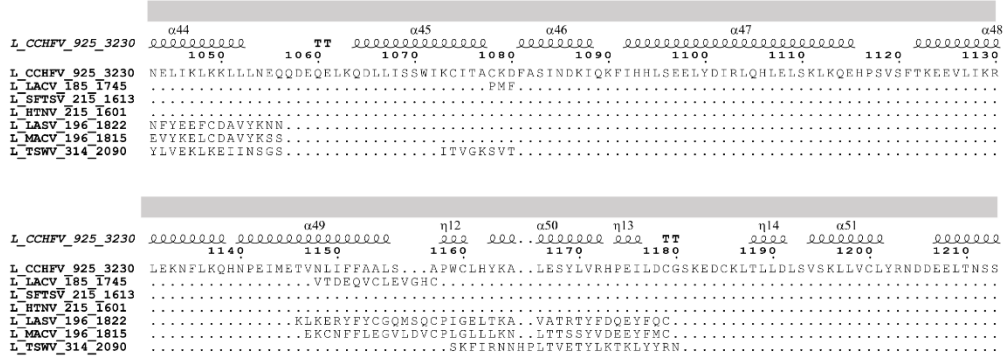
α35 840 α36 850 η8 860 α37 870 η9 880 α38 890 β12 900

L_CCHFV_925_3230
L_LACV_185_1745
L_SFTSV_215_1613
L_BTNV_215_1601
L_LASV_196_1822
L_MACV_196_1815
L_TSWV_314_2090

α -ribbon like

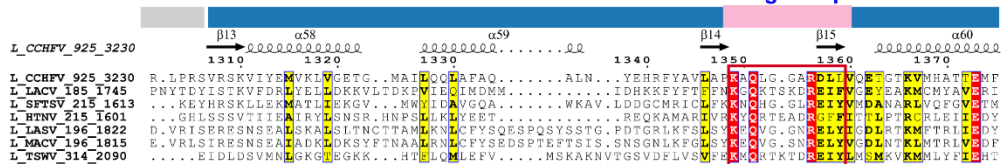


Larger pendant Insertion



Fingers

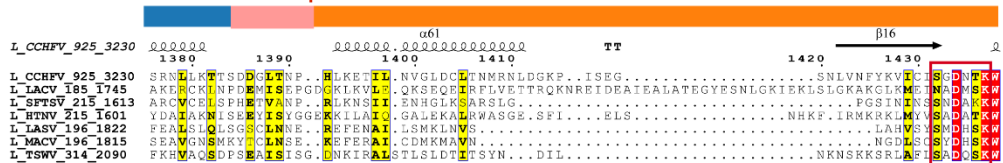
Fingers-tips



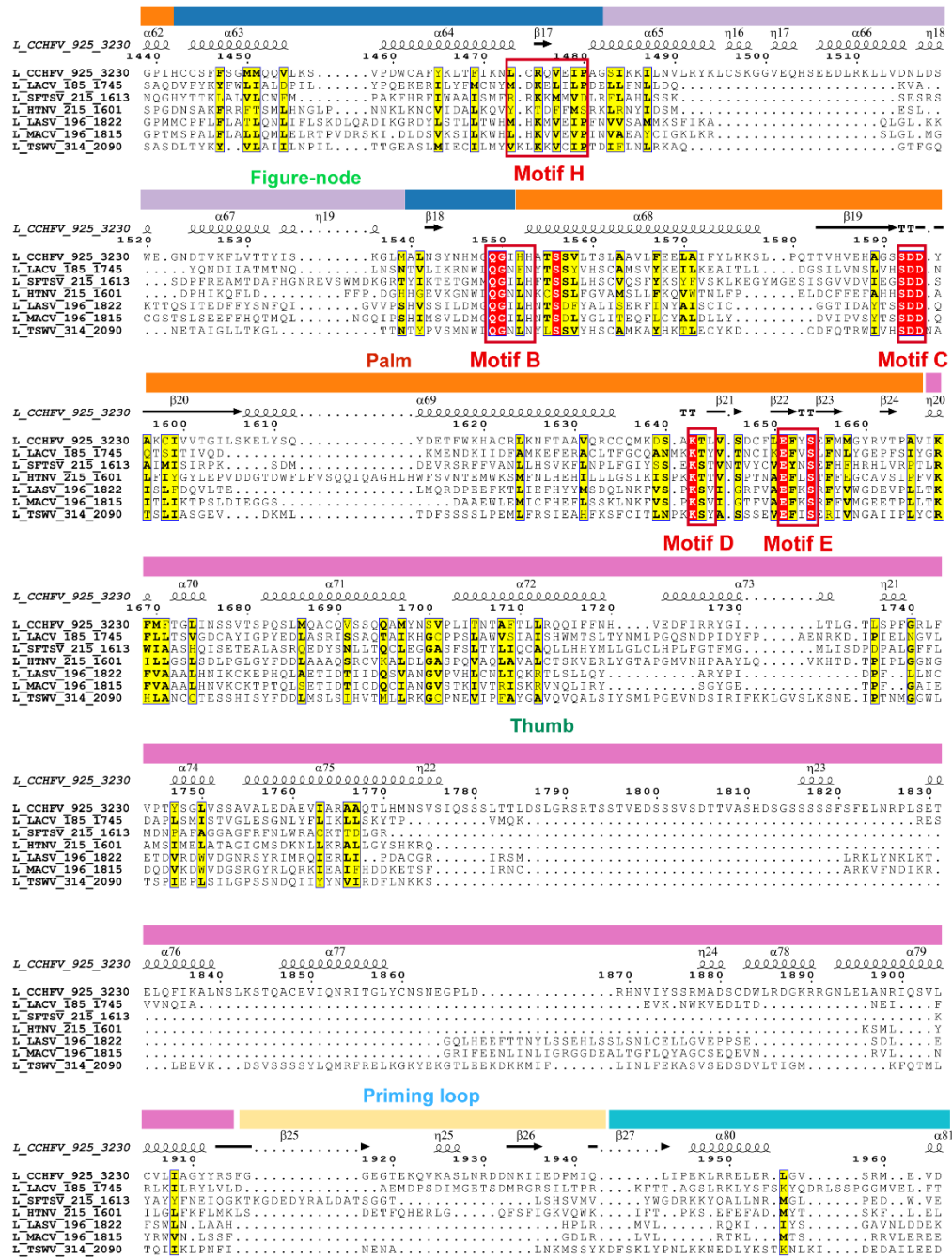
PR Loop

Palm

Motif F



Motif A



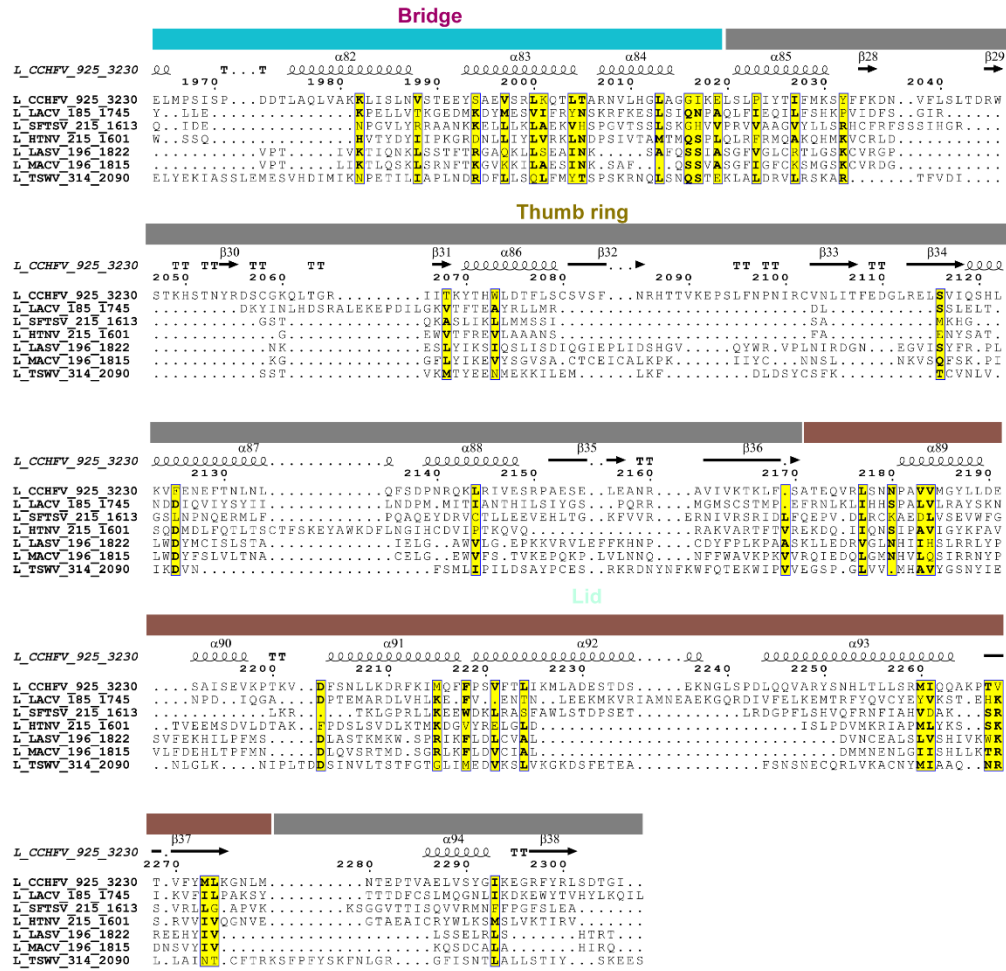
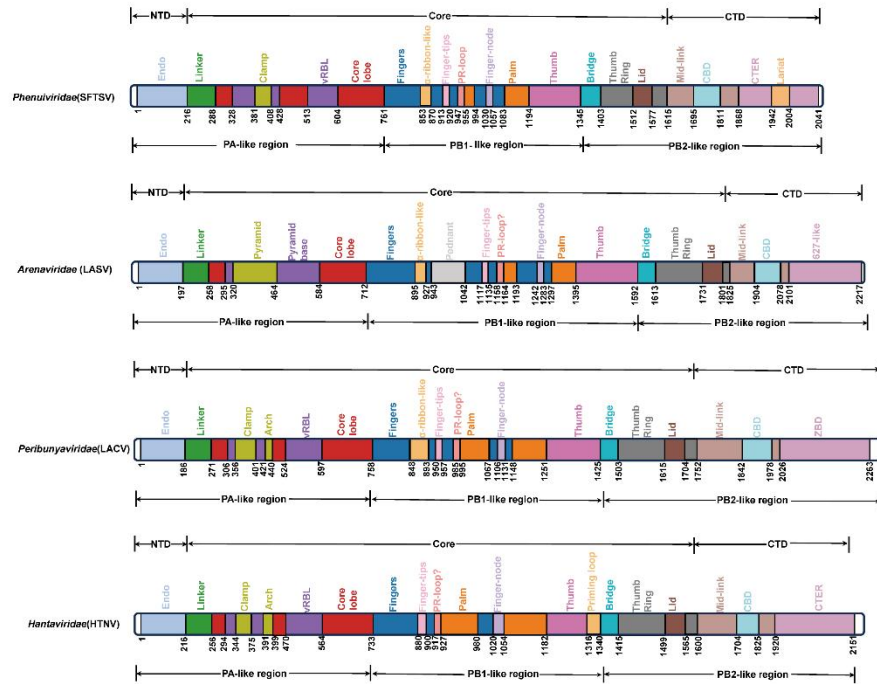


Figure S2 Structure-based sequence alignment of the polymerase core of CCHFV L with representative sNSVs.

Structure-based sequence alignment of the core region from CCHFV, LACV, SFTSV, HTNV, LASV, MACV and TSWV L proteins. Conserved polymerase motifs A-H are outlined with red boxes, and strictly or highly conserved residues are shaded in yellow. The secondary-structure elements of CCHFV L corresponding to the alignment are shown above the sequences. The domain organization of CCHFV L is indicated by colored bars and follows the domain definition presented in Fig. 1a.

a



b

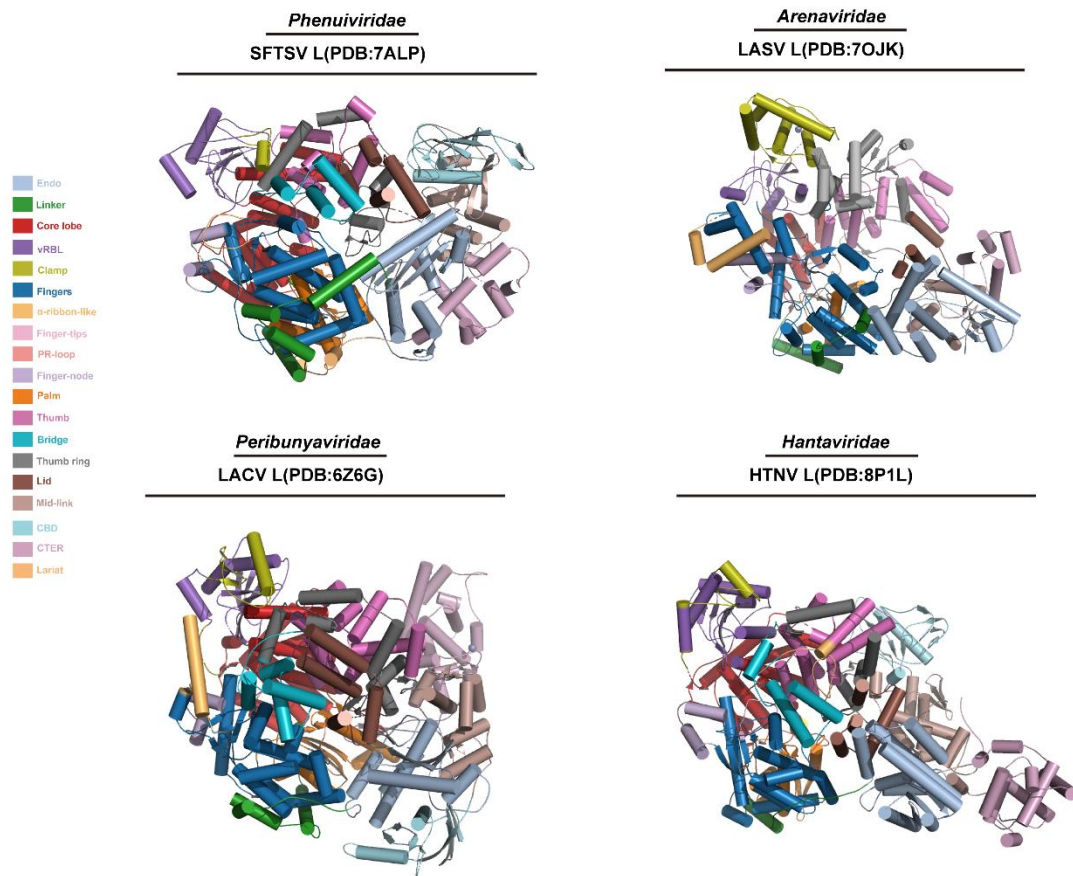


Figure S3 Comparison of domain organization and core architectures of representative *Bunyavirales* polymerases.

a. Schematic domain organization of L proteins from four representative *Bunyavirales* members: SFTSV (*Phenuiviridae*), LASV (*Arenaviridae*), LACV (*Peribunyaviridae*) and

HTNV (*Hantaviridae*). The PA-like, PB1-like (core) and PB2-like regions are indicated below each bar. Individual domains and structural elements are color-coded as in Fig. 1a, with residue boundaries labeled beneath the diagrams.

- b. Ribbon representations of the L of SFTSV (PDB: 7ALP), LASV (PDB: 7OJK), LACV (PDB: 6Z6G) and HTNV (PDB: 8P1L), shown in the same color scheme as in panel (A).

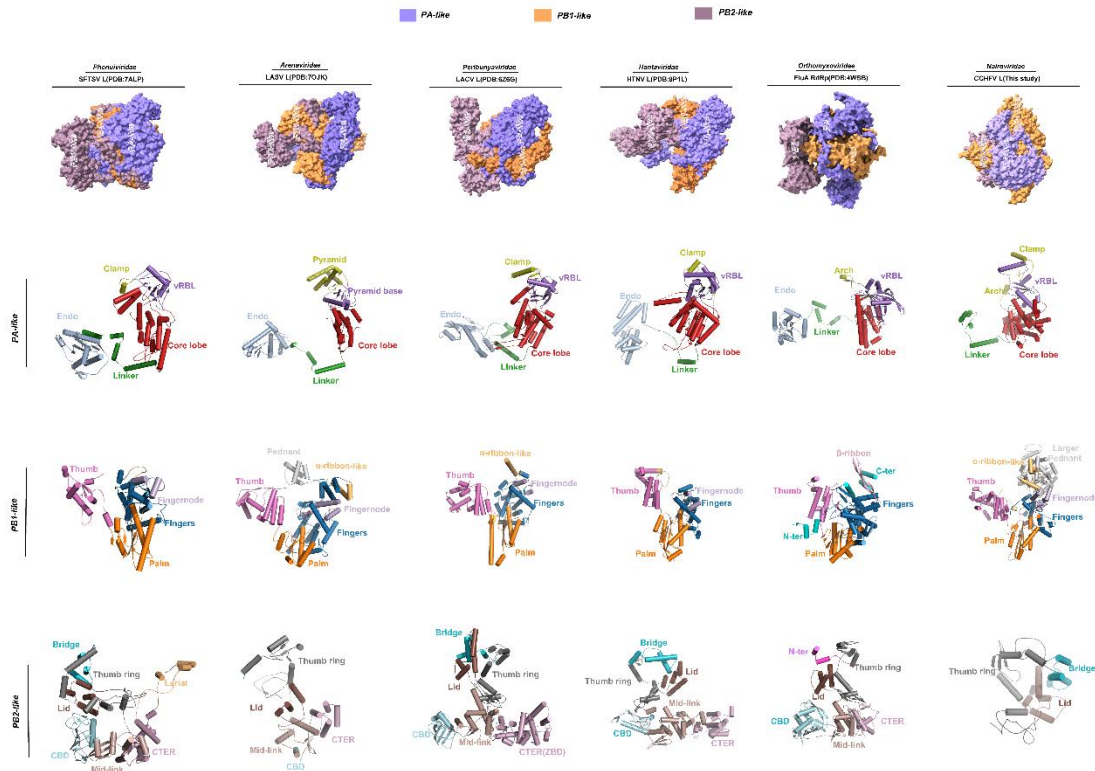


Figure S4 Comparative architecture of PA-like, PB1-like, and PB2-like regions in representative Bunyavirales polymerases and influenza A polymerase.

Upper: Surface and cartoon representations of L proteins from five *Bunyavirales* families: SFTSV (*Phenuiviridae*, PDB: 7ALP), LASV (*Arenaviridae*, PDB: 7OJK), LACV (*Peribunyaviridae*, PDB: 6Z6G) and HTNV (*Hantaviridae*, PDB: 8P1L) and CCHFV (*Nairoviridae*, this study), together with the influenza A virus polymerase (FluA, PDB: 4WSB) shown for reference. Lower: Cartoon representations highlighting the domain organization of each polymerase. The structures are partitioned into PA-like, PB1-like, and PB2-like regions, corresponding to the influenza polymerase subunits, to facilitate comparison of domain architecture across Bunyavirales polymerases.

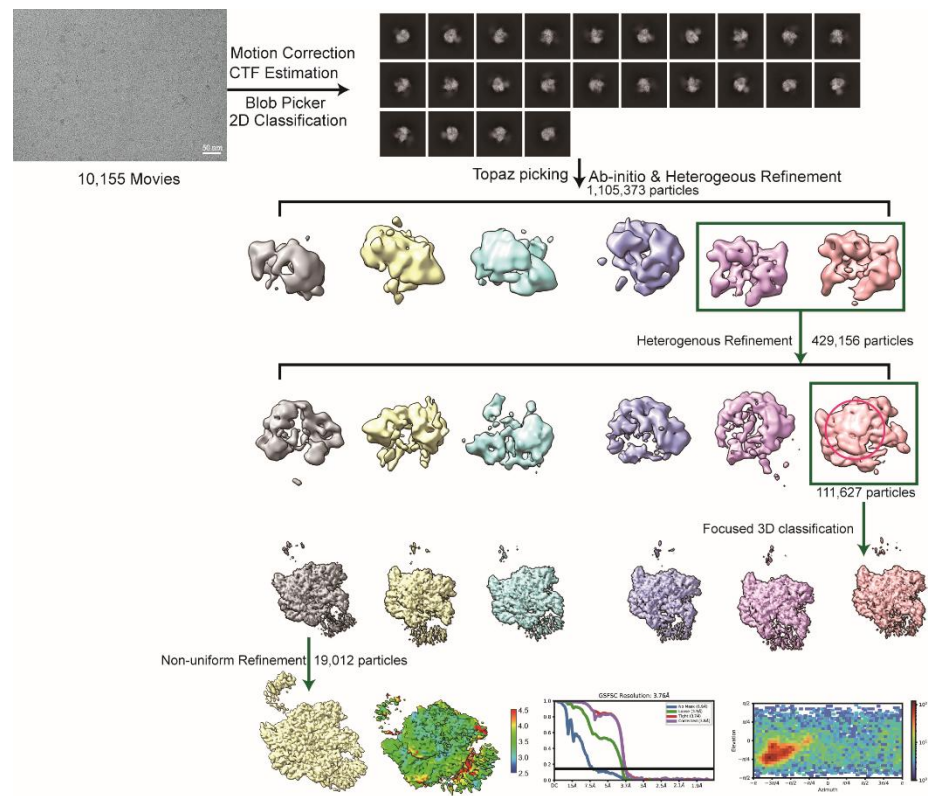


Figure S5 Data processing workflow of cryo-EM data analysis of 5' hook vRNA bound CCHFV L.

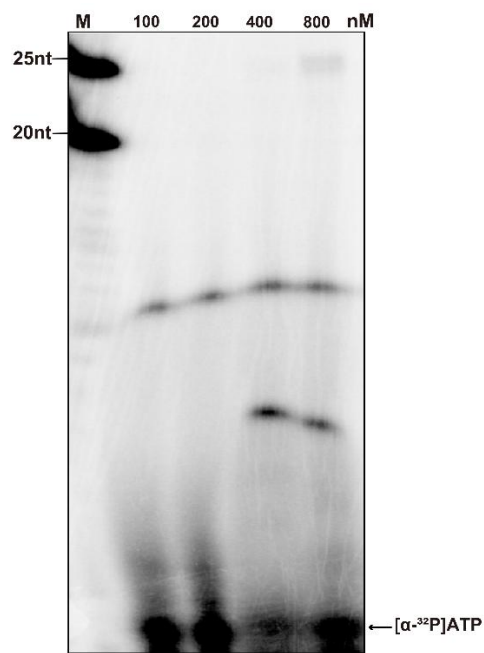


Figure S6 In vitro RNA synthesis activity of the core domain of the CCHFV L protein.

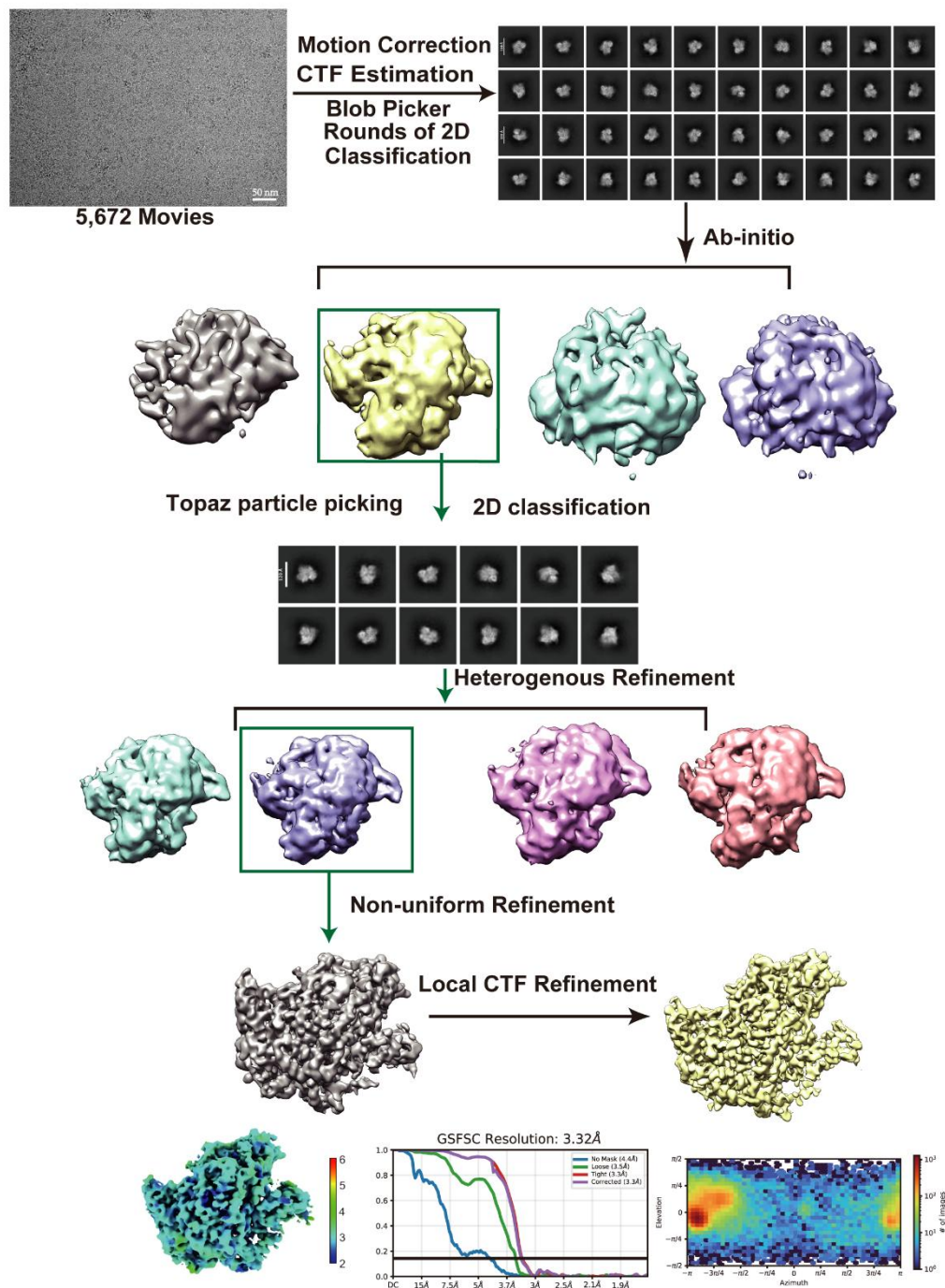


Figure S7 Data processing workflow of cryo-EM data analysis of 5' hook vRNA bound CCHFV L Core.

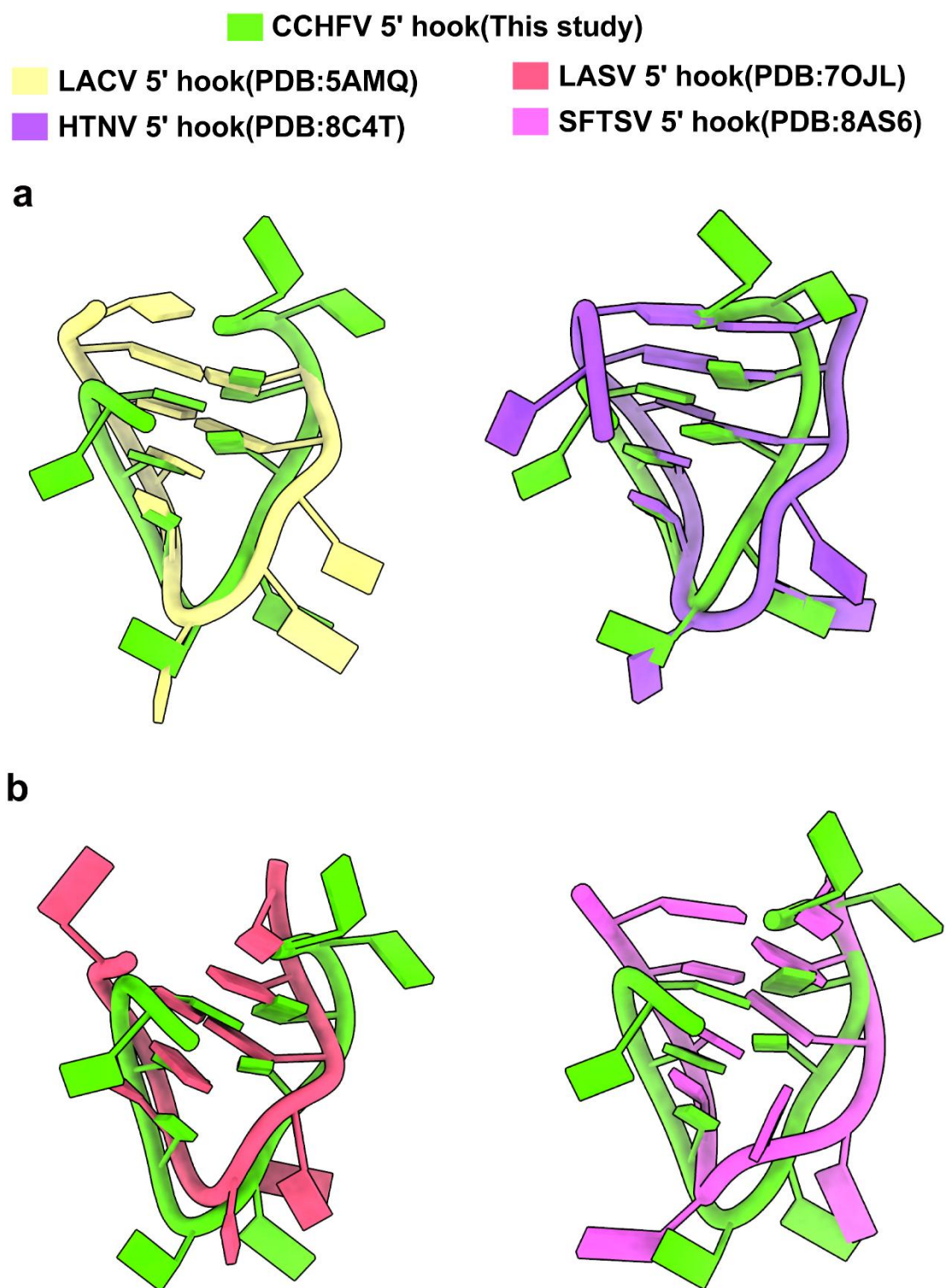


Figure S8 Comparison of 5' vRNA hook structures from sNSVs.

- a.** Structural superposition of the CCHFV 5' hook with the LASV (left) and SFTSV (right) hooks.
- b.** Structural superposition of the CCHFV 5' hook with the LACV (left) and HTNV (right) hooks.

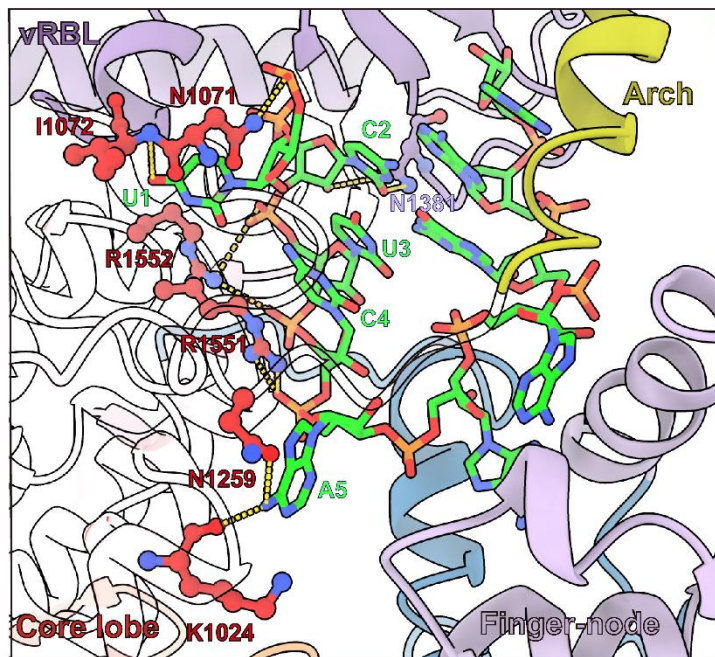


Figure S9 Detailed interactions between the CCHFV L polymerase and the first five nucleotides of the 5' RNA hook.

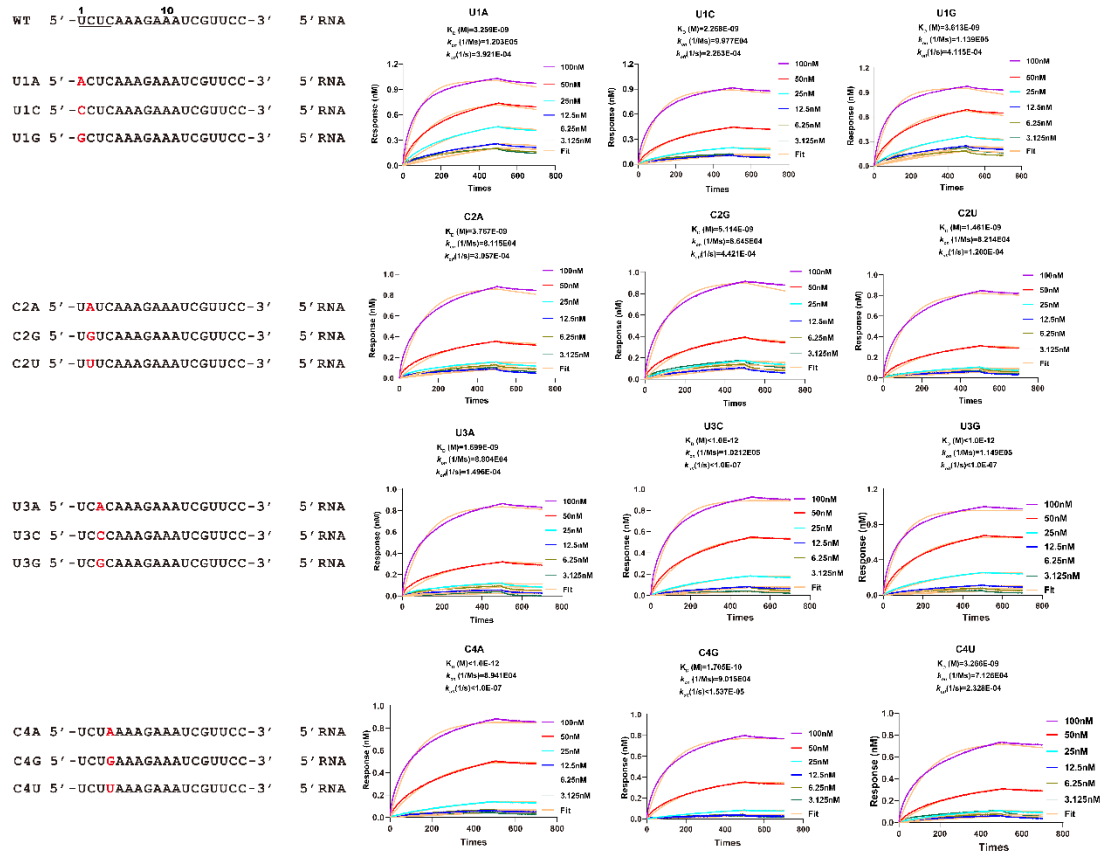


Figure S10 BLI analysis of CCHFV L binding to mutant 5' RNA constructs.

BLI analysis for wild-type and mutant 5' RNA constructs carrying single-nucleotide substitutions within the first four nucleotides. Mutated residues are highlighted in red. Most substitutions had little or modest effects on binding, whereas selected mutations enhanced binding affinity.

CCHEV 1 10 20 30 40 50 60 70 80 90
CCHEV MDFTFNDITQVLAGQVYVNPFNISDYFEIVROPCDGNCFYHSIAETIPNKTDSYHNEHLTCLANRKYVCEPEAKHLIGSLSDLRKMSDN
NSDV MDFTNSTPDEIVVQGTANPFPQVTDYFEIVROPCDGNCFYHSIAETFPVNDNDSPRLVKQHLCLANRKYVCEPEAKGLGSLERKLVAVAMCDN
DUGV MDFTDSLINRVVDROYINPFCVSDYFEIVROPCDGNCFYHSIAETFPDVKTSPSRKVKRHLCLAAEVYVDTEPEAVGTGISKDEYIKVAMKDN
HAZV MDFTBGTINDSVSDIOEVSNPFTITDYFEIVROPCDGNCFYHSIAETIYPNRSDHAYRLVKNEHRAAEKYVPTPEAAATCMRLDEYLDOTALRDN

CCHEV 100 110 120 130 140 150 160 170 180 190
CCHEV EWGSELEASMLAKSMQFTIITIVVAASDPFAAINTFCQDVFTFVNLHSCQTHFDALRLITPEADTR...ETTLVDKVIITVDQLTSSSSDLDLQ
NSDV EWGSELEASMLAKHLDTIVIVIEGSRVAAAVKFCQGVVAGAINLHHCYNHFDALRLITVDDSOVSR...QPRDIETRIEIVESEVLSDREETFF
DUGV EWGSELEASMLAKHLDTIITLVVNSTEQVTAATKFCQGRVSTALNLMHVGRTHFDALRLITVQLNNQPDQRNRLDIADRIAASAEVYVRSISIEDNLQ
HAZV EWGSELEASMLAKSRHLGFTTVVILVLDGSRNVVCAATFCRGSGLKTAHLHLSGTHFDALRLITAEEDPQQ...ETMTLVEKMLVEFRFTITTEGEECLQ

CCHEV 200 210 220 230 240 250 260 270 280
CCHEV YEDIALALATSAEPYRRSSLEDEVTLSSKQAEMLRQKNSLSKLVNKKQNIPIRVGRVLDCLNCKLQVEISADTILIRPESKRTIGEVMLRQLQGH
NSDV EEDLLNFATTEIVEE...KLT.R.QDKKQVDEMQRRALIGKIIKKGENIPIRVGRVLDCLNCKLQVEISADTILIRPESKRTIGEVMLRQLQGH
DUGV EDEFFDYAREDEISE...DVS.APGGSRATELKKKAILLNKTVKGENIPIRVGRVLDCLNCKLQVEISADTILIRPESKRTIGEVMLRQLQGH
HAZV EEEELLDSPTATAAP...E.E.EPSISEPRLRSQAILHLRLVRHGENIPIRVGRVLDCLNCKLQVEISADTILIRPESKRTIGEVMLRQLQGH

CCHEV 290 300 310 320 330 340 350 360 370 380
CCHEV KLILTRDKQIKQDFSRHKLIVYTRDLIDHLDVGGLLRSAPFCPTGERHMLQLHSEMDLIDICTVVLGVMLSFFLYGSNNRKKKRFITNCLLSTLSGKKV
NSDV KLILTRDKQIKQDYARSKLIVYTRDLIDHLDVGGLLRSAPFCPTGERHMLQLHSEMDLIDICTVVLGVMLSFFLYGSNNRKKKRFITNCLLSTLSGKKV
DUGV KLILTRDRHIMDYARSKLIVYTRDLIDHLDVGGLLRSAPFCPTGERHMLQLHSEMDLIDICTVVLGVMLSFFLYGSNNRKKKRFITNCLLSTLSGKKV
HAZV KLILTRDKQIKQDFSRHKLIVYTRDLIDHLDVGGLLRSAPFCPTGERHMLQLHSEMDLIDICTVVLGVMLSFFLYGSNNRKKKRFITNCLLSTLSGKKV

CCHEV 390 400 410 420 430 440 450 460 470 480
CCHEV YKVVLGNLGNELLYKAPRKALAVCSAIDFCQKINKLQNCFRITISDYSLLALRNDFDCLSVQDYNGMIECMSKIDNDVDFNHRITADLNQITSLRLIT
NSDV FKALGKLTGTTLYRSPRNALSHVCQTTLYGKMMQLQSYISVMSPISSLALRNDFDCLSVQDYNGMIECMSKIDNDVDFNHRITADLNQITSLRLIT
DUGV FKALGKLTGQMLYRTPKRAVSVSQELLYGKMLKVXNNLEGMGPISSLALRNDFDCLSVQDYNGMIECMSKIDNDVDFNHRITADLNQITSLRLIT
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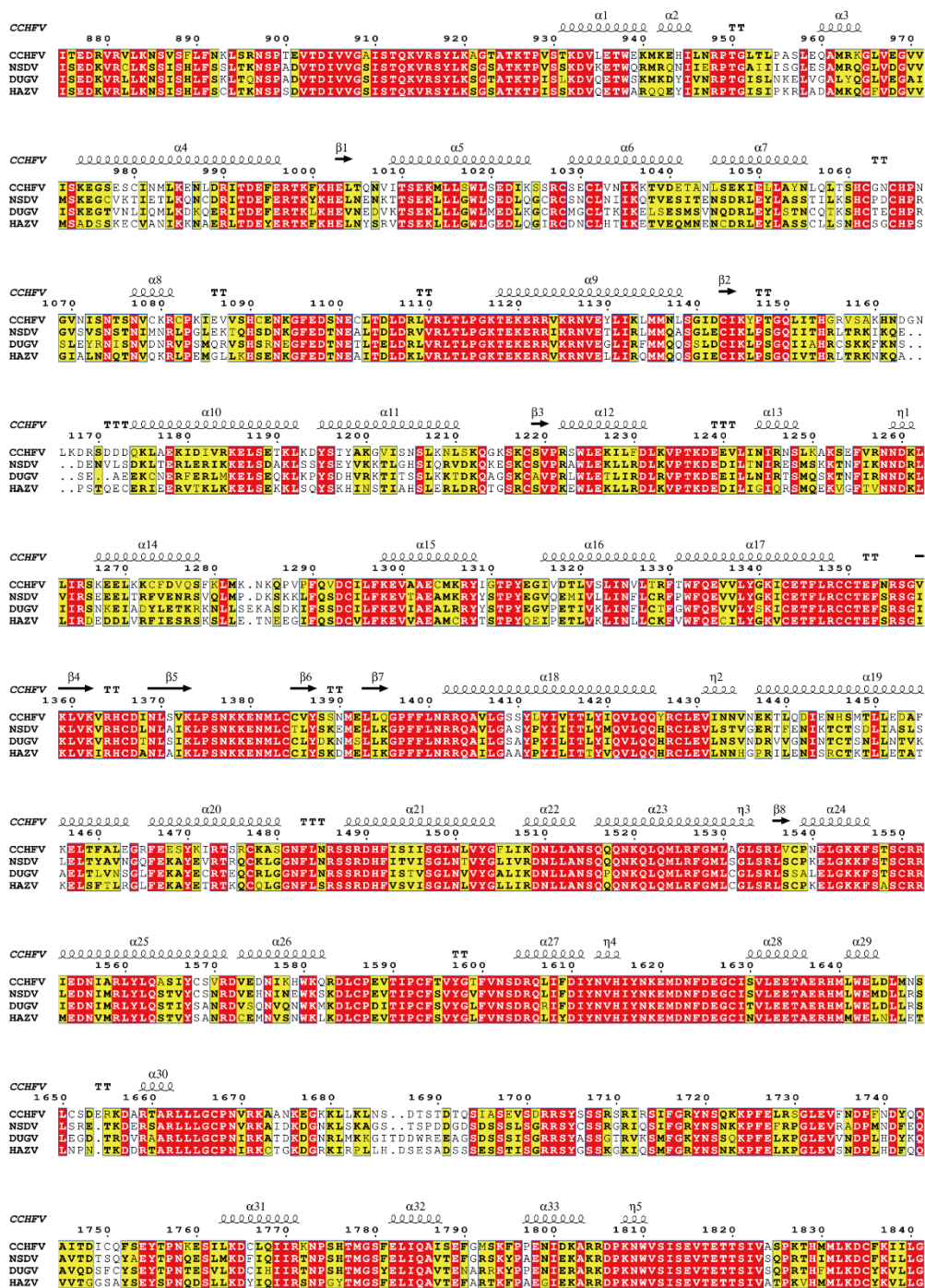
CCHEV 490 500 510 520 530 540 550 560 570
CCHEV LRKKEKDTDLKQWFSGGDLSRRSTNVANAEFTISEFPKKKDKIMKFTSTSGKASSACITGNVLSYAHNLYLSKSSLNMTSSEDISQLLIEIKRMYAL
NSDV LRKEGRADBLKNWYKEEEDTKRSLSRVQNASSEFLISDYFKKKDKIMKFTSTSGKASSACITGNVLSYAHNLYLSKSSLNMTSSEDISQLLIEIKRMYAL
DUGV LOKSQDVNDLKLWFKEEVLTKRSSQSVGNASEFLINDYFKKKDKIMKFTSTSGKASSACITGNVLSYAHNLYLSKSSLNMTSSEDISQLLIEIKRMYAL
HAZV LAGKRNKPDVLWYKKEEDHRAKLRDITAGAOEHLISDFPRKKDKIMKFTSTSGKASSACITGNVLSYAHNLYLSKSSLNMTSSEDISQLLIEIKRMYAL

CCHEV 580 590 600 610 620 630 640 650 660 670
CCHEV QGDSEVPPIALICDGIQGNKQLFISLPPCARCEQVLFDIRNSPSSHAWKHALRLKGTAYEGMFAKQVGNQYIPEDIKPSLTMLIQTLPFKFE
NSDV QGEQSIEPIALICDKLEQFRKLFKELPPECSELEQQLFDIRNSGSSHAWKHALRLKGTAYEGMFAKQVGNQYIPEDIKPSLTMLIQTLPFKFE
DUGV QGDLSEVPPIALICDKLEQFRKLFKELPPECSELEQQLFDIRNSPSSHAWKHALRLKGTAYEGMFAKQVGNQYIPEDIKPSLTMLIQTLPFKFE
HAZV QGDQSVPIALICDRLDSFRRLCRELPADCAEQVLFDIRNSPSSHAWKHALRLKGTAYEGMFAKQVGNQYIPEDIKPSLTMLIQTLPFKFE

CCHEV 680 690 700 710 720 730 740 750 760 770
CCHEV NPLDRTQLHPEFRDLTPDFSLTQKVHKKRNQIPSVENVOESISDADLPSVDAVPVTERKKMFPLPETPVDEVHSIERIMENDTRIMHGGKPSATKKDE
NSDV NPLDRTQLHPEFRDLTPDFALTQKVYFKKNKIVGIGNQNTQVVIDSSLEGSVDAVPVVEKKMFPLPETPVDEVHSIQIRIKSRDRKVENDKRKEEEDG
DUGV NPLDRTQLHPEFRDLTPDYALTQKIFPFRNTIPRTENROAIDVSLGSVDAVPVVEKKMFPLPETPVDEANSISRVMNIDKEKREESMOKKLEHDR
HAZV NPLDRTQLHPEFRDLTPDYALTQKVYFKKNQIVEMVSHQESIGELDPSVDAHPVEKKMFPLPETPVGEVYSIQSIKNIDQKIDRCEDDSKASA.

CCHEV
CCHEV DLT.....EQ
NSDV KAPASES.....M.EDQKGGSVQDD....KPAPITKK.....AMQEESEERAGESKSNLESRGADKL...QPVLAS
DUGV QAEANRLKSAGLSASKAEQEVCSAQDRKEEKERTTEPAGKQQRTEDLVIEGNQDEGSDPQKKVDEKTVPGESKQSKSSSSSSTNQMSQKVVDV
HAZV .L.....

CCHEV 780 790 800 810 820 830 840 850 860 870
CCHEV DSQQNATEHSSSISAFKDYGBRGIVGCHHMRSEEDQLETRQLLLVVGCFQTDLDGKIRTDKKWKDILRLLELDGIRCSFVACADCSSTPDRNWH
NSDV TGPR...GMAQSATRGPLIYSDRILIDENSTELTEEELEKROILLVVGQYQTDVVGKINTDKKKWKDILRLLEMDIKCSFVACADCSSTPADNWH
DUGV PSVEDSSDAQAGDFPDYGYFKRIIVMDESGLVTEPAQLEKROILLVVGQYQTDVVGKINTDKKKWKDILRLLEMDIKCSFVACADCSSTPSNNWH
HAZV ..EE...GKASDSAGEQLDINSRIIIBKNNIEISDEEPELEKROILLVVGQYQTDVVGKINTDKKKWKDILRLLEMDIKCSFVACADCSSTPSDSWH



CCHFV $\eta 6$ $\alpha 34$ $\alpha 35$ $\eta 7$ $\eta 8$ $\alpha 36$ $\alpha 37$ $\alpha 38$

CCHFV TENKKIVKMLRGLKLLGALISNDIICRRDCLDLNTVVGSLSECKKNIVNGIFEPKSLSPFYHWKDLTKKDLVSVLLTDGNGVFCWLKTTSSVVG

NSDV TENKKIVKMLRGLKLLGALISNDIICRRDCLDLNTVVGSLSECKKNIVNGIFEPKSLSPFYHWKDLTKKDLVSVLLTDGNGVFCWLKTTSSVVG

DUGV TENKKIVKMLRGLKLLGALISNDIICRRDCLDLNTVVGSLSECKKNIVNGIFEPKSLSPFYHWKDLTKKDLVSVLLTDGNGVFCWLKTTSSVVG

HAZV TENKKIVKMLRGLKLLGALISNDIICRRDCLDLNTVVGSLSECKKNIVNGIFEPKSLSPFYHWKDLTKKDLVSVLLTDGNGVFCWLKTTSSVVG

CCHFV $\alpha 39$ $\alpha 40$ $\alpha 41$ $\alpha 42$

CCHFV SLKKKLPFNNVHSPETLMPNCFPSSEPFNFKLKLINLQDDECEIKKQDLFSSWKKCTTACKDFASITKTKFTHHEEELVDYDLOHLELSK

NSDV ALKKELPFNNNGGVLSLNSGPFSEDPFELNLVKKELTGNKSLDRRLNTELTLASWFKCVYKPKREGASIVQSGLEALAKAMAGELYEILQHLBLTR

DUGV SLKKDLPFNNVHSPETLMPNCFPSSEPFNFKLKLINLQDDECEIKKQDLFSSWKKCTTACKDFASITKTKFTHHEEELVDYDLOHLELSK

HAZV RLKKDLPFNNNGGVLSLNSGPFSEDPFELNLVKKELTGNKSLDRRLNTELTLASWFKCVYKPKREGASIVQSGLEALAKAMAGELYEILQHLBLTR

Larger pendant Insertion

CCHFV $\alpha 43$ $\alpha 44$ $\eta 9$ $\alpha 45$ $\eta 10$ $\alpha 46$ $\alpha 47$

CCHFV LKQHPDVSFTKEEVVLKRLERKFNFKHNDLMTVNLIPFAALSSVIVRHPEILDGSGSKCKLTLTDSVQSKLVLYRN...D

NSDV MKKNDPVSFTKEEVVLKRLERKFNFKHNDLMTVNLIPFAALSSVIVRHPEILDGSGSKCKLTLTDSVQSKLVLYRN...D

DUGV LKQHPDVSFTKEEVVLKRLERKFNFKHNDLMTVNLIPFAALSSVIVRHPEILDGSGSKCKLTLTDSVQSKLVLYRN...D

HAZV LKQHPDVSFTKEEVVLKRLERKFNFKHNDLMTVNLIPFAALSSVIVRHPEILDGSGSKCKLTLTDSVQSKLVLYRN...D

CCHFV $\alpha 48$ $\alpha 49$ $\alpha 50$ $\alpha 51$ $\alpha 52$ $\alpha 53$

CCHFV DLELTNSSSLLKGLFHVYAVTLFTANGEPFSLSLDGGGLDLDHHTTDEKLLQTKVVFARGLSCNSYDFIHTQMANSNFNVCKRITGRSTGER

NSDV DLDSEVLNEMKVRFAVYVTLFTANGEPFSLSLDGGGLDLDHHTTDEKLLQTKVVFARGLSCNSYDFIHTQMANSNFNVCKRITGRSTGER

DUGV DLELTNSSSLLKGLFHVYAVTLFTANGEPFSLSLDGGGLDLDHHTTDEKLLQTKVVFARGLSCNSYDFIHTQMANSNFNVCKRITGRSTGER

HAZV DLDSEVLNEMKVRFAVYVTLFTANGEPFSLSLDGGGLDLDHHTTDEKLLQTKVVFARGLSCNSYDFIHTQMANSNFNVCKRITGRSTGER

CCHFV $\alpha 54$ $\alpha 55$ $\beta 9$ $\alpha 56$

CCHFV LPRSVRSKVIYEMVKLVGETGMAILQQLARQAALNYDHRFYAVLAPKAQLGGRDLLVQETGTKVHATTEMFSRNLLKTTSDGGLTNPHLKETILN

NSDV LPRSVRSKVIYEMVKLVGETGMAILQQLARQAALNYDHRFYAVLAPKAQLGGRDLLVQETGTKVHATTEMFSRNLLKTTSDGGLTNPHLKETILN

DUGV LPRSVRSKVIYEMVKLVGETGMAILQQLARQAALNYDHRFYAVLAPKAQLGGRDLLVQETGTKVHATTEMFSRNLLKTTSDGGLTNPHLKETILN

HAZV LPRSVRSKVIYEMVKLVGETGMAILQQLARQAALNYDHRFYAVLAPKAQLGGRDLLVQETGTKVHATTEMFSRNLLKTTSDGGLTNPHLKETILN

CCHFV $\alpha 57$ $\alpha 58$ $\alpha 59$ $\alpha 60$

CCHFV VGLDCLTNMRLNDGKPISECSNLDVNFYKVVICISGDNTKWGPICCSFFSGMMQQLKDVDPDWCSFYKLTFFKNLCROVEIPASITKRIINVLRFKLS

NSDV VGLDALSTMRLNDGKPISECSNLDVNFYKVVICISGDNTKWGPICCSFFSGMMQQLKDVDPDWCSFYKLTFFKNLCROVEIPASITKRIINVLRFKLS

DUGV VGLDCLTNMRLNDGKPISECSNLDVNFYKVVICISGDNTKWGPICCSFFSGMMQQLKDVDPDWCSFYKLTFFKNLCROVEIPASITKRIINVLRFKLS

HAZV VGLDALSTMRLNDGKPISECSNLDVNFYKVVICISGDNTKWGPICCSFFSGMMQQLKDVDPDWCSFYKLTFFKNLCROVEIPASITKRIINVLRFKLS

CCHFV $\alpha 61$ $\alpha 62$ $\eta 11$ $\alpha 63$ $\beta 12$

CCHFV KGGVESEEDLRLKLVVDNLDSWGNLVKFLITTYISKGIMANSYNHMGQGIHATSSVLTSLAAVLFEELAIFFYKRRPQTLTVNVHAGSSD

NSDV KGGVESEEDLRLKLVVDNLDSWGNLVKFLITTYISKGIMANSYNHMGQGIHATSSVLTSLAAVLFEELAIFFYKRRPQTLTVNVHAGSSD

DUGV KGGVESEEDLRLKLVVDNLDSWGNLVKFLITTYISKGIMANSYNHMGQGIHATSSVLTSLAAVLFEELAIFFYKRRPQTLTVNVHAGSSD

HAZV KGGVESEEDLRLKLVVDNLDSWGNLVKFLITTYISKGIMANSYNHMGQGIHATSSVLTSLAAVLFEELAIFFYKRRPQTLTVNVHAGSSD

CCHFV $\alpha 64$ $\alpha 65$ $\alpha 66$ $\alpha 67$

CCHFV DYAKCIVVGLSLKELYSCVDFTFWKHAACKLNFTAAVORCCQMKDSAKTLVDCDFLEFYSEFMMGNRVTPAVIKFIFTGLINSSVTSPOSLQACH

NSDV DYAKCIVVGLSLKELYSCVDFTFWKHAACKLNFTAAVORCCQMKDSAKTLVDCDFLEFYSEFMMGNRVTPAVIKFIFTGLINSSVTSPOSLQACH

DUGV DYAKCIVVGLSLKELYSCVDFTFWKHAACKLNFTAAVORCCQMKDSAKTLVDCDFLEFYSEFMMGNRVTPAVIKFIFTGLINSSVTSPOSLQACH

HAZV DYAKCIVVGLSLKELYSCVDFTFWKHAACKLNFTAAVORCCQMKDSAKTLVDCDFLEFYSEFMMGNRVTPAVIKFIFTGLINSSVTSPOSLQACH

CCHFV $\alpha 68$ $\alpha 69$ $\alpha 70$ $\alpha 71$

CCHFV VSSQQAAMNSVPLTNTAFTLLRQQVFVHVEDFRRYGLITLGLTSGFGRLEVPYISGLVSSVALEDSVLTARAAQTTHMNSVSIQSSSLTTLDS

NSDV VSSQQAAMNSVPLTNTAFTLLRQQVFVHVEDFRRYGLITLGLTSGFGRLEVPYISGLVSSVALEDSVLTARAAQTTHMNSVSIQSSSLTTLDS

DUGV VSSQQAAMNSVPLTNTAFTLLRQQVFVHVEDFRRYGLITLGLTSGFGRLEVPYISGLVSSVALEDSVLTARAAQTTHMNSVSIQSSSLTTLDS

HAZV VSSQQAAMNSVPLTNTAFTLLRQQVFVHVEDFRRYGLITLGLTSGFGRLEVPYISGLVSSVALEDSVLTARAAQTTHMNSVSIQSSSLTTLDS

CCHFV $\alpha 72$ $\alpha 73$ $\alpha 74$

CCHFV LGRSRSTSTVSDSSVDFTTVASGDSGSSSSSFTFELNRPLETELOLITNALNSLSTQACEVIONRITGLYCNSSNECPDLRHHNVYSSRMADSCDW

NSDV LKDDTSAAKARDDESSEIETTISTESGSSSSSFTFELNRPLETELOLITNALNSLSTQACEVIONRITGLYCNSSNECPDLRHHNVYSSRMADSCDW

DUGV LGRSRSTSTVSDSSVDFTTVASGDSGSSSSSFTFELNRPLETELOLITNALNSLSTQACEVIONRITGLYCNSSNECPDLRHHNVYSSRMADSCDW

HAZV LKDDTSAAKARDDESSEIETTISTESGSSSSSFTFELNRPLETELOLITNALNSLSTQACEVIONRITGLYCNSSNECPDLRHHNVYSSRMADSCDW

α75 α76 β17 η14 β18 α77 TT
CCHFV 2810 2820 2830 2840 2850 2860 2870 2880 2890 2900
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NSDV RDQKRRGLDANIOSVIVTACGYRSFGSGTEKQVKAQLNRDDNRVIEDPMIQLPEKLRRELERLGVSRMEVDLMPSTPDDTIALQVAK
DUGV RDQKRRGLDANIOSVIVTACGYRSFGSGTEKQVKAQLNRDDNRVIEDPMIQLPEKLRRELERLGVSRMEVDLMPSTPDDTIALQVAK
HAZV RDQKRRGLDANIOSVIVTACGYRSFGSGTEKQVKAQLNRDDNRVIEDPMIQLPEKLRRELERLGVSRMEVDLMPSTPDDTIALQVAK

α78 α79 η15 α80 β19 TT TT TT β20 α81
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CCHFV KLISLNVSTEEYSAEVSRKQTLTARNVLHGLAGGIKELSLPIYITFLKSYFFKDNVFLSDDRWSTKHSNRYRDSCKLTCRIITKRYTHWLDTFI
NSDV KLISLNVSTEEYSAEVSRKQTLTARNVLHGLAGGIKELSLPIYITFLKSYFFKDNVFLSDDRWSTKHSNRYRDSCKRLTGRVITKRYTHWLDTFI
DUGV KLISLNVSTEEYSAEVSRKQTLTARNVLHGLAGGIKELSLPIYITFLKSYFFKDNVFLSDDRWSTKHSNRYRDSCKRLTGRVITKRYTHWLDTFI
HAZV KLISLNVSTEEYSAEVSRKQTLTARNVLHGLAGGIKELSLPIYITFLKSYFFKDNVFLSDDRWSTKHSNRYRDSCKRLTGRVITKRYTHWLDTFI

β21 TT TT β22 β23 α82 α83 β24 TT
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CCHFV SCSVSFNRRHTVKKRSLFNPNCRCVNLITEDGLRRLSVQSHLVFENFTNLNLQFSDNRQKIKIVESRPPSELEANKRAVIVKSKLFAVEV
NSDV NCTVSNIRNQEIKNSLFNPNCRCVNLITEDGLRRLSVQSHLVFENFTNLNLQFSDNRQKIKIVESRPPSELEANKRAVIVKSKLFAVEV
DUGV SCVVSANRSQEIKNSLFNPNCRCVNLITEDGLRRLSVQSHLVFENFTNLNLQFSDNRQKIKIVESRPPSELEANKRAVIVKSKLFAVEV
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α84 α85 η16 α86 β25
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TTT
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NSDV MLKGNLNNTEPTVVALVSVCIKEGRFLRLSDGLDASTYSVKYWKILHCISAIGLPLSPKADSSLLSFLNKRNVNMDIRASDCPLSNHASTITSEF
DUGV MLKGNLNNTEPTVVALVSVCIKEGRFLRLSDGLDASTYSVKYWKILHCISAIGLPLSPKADSSLLSFLNKRNVNMDIRASDCPLSNHASTITSEF
HAZV MLKGNLNNTEPTVVALVSVCIKEGRFLRLSDGLDASTYSVKYWKILHCISAIGLPLSPKADSSLLSFLNKRNVNMDIRASDCPLSNHASTITSEF

CCHFV 3300 3310 3320 3330 3340 3350 3360 3370 3380 3390
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DUGV DGOVIANLASELSSVRKDSERDGLTDLDIYVNSPELLKKKPYLGTCKPNTWGDSSNRSGKFTYSSRSGEAIGIFVAGKLIHILTSDESLALCEVE
HAZV DGOVIANLASELSSVRKDSERDGLTDLDIYVNSPELLKKKPYLGTCKPNTWGDSSNRSGKFTYSSRSGEAIGIFVAGKLIHILTSDESLALCEVE

CCHFV 3400 3410 3420 3430 3440 3450 3460 3470 3480
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DUGV ROVSWLNNRRRTDITKEQHQFLLFLPQSHSELOKHKDGSALSWIPDGNPNRLDKFVPLKGLAVVKIKKOILTVKKQVVFDAESEPRLOWHGCG
HAZV ROVSWLNNRRRTDITKEQHQFLLFLPQSHSELOKHKDGSALSWIPDGNPNRLDKFVPLKGLAVVKIKKOILTVKKQVVFDAESEPRLOWHGCG

CCHFV 3490 3500 3510 3520 3530 3540 3550 3560 3570 3580
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NSDV SIVYDECEOTTYHENLLKQKLVDCSTDRKKLLDQSVFSDSKVVLRSIKFKTELLLSLLHLCFLKHAQSDAIMEVESKSLHKKFKKGGVQR
DUGV SIVYDECEOTTYHENLLKQKLVDCSTDRKKLLDQSVFSDSKVVLRSIKFKTELLLSLLHLCFLKHAQSDAIMEVESKSLHKKFKKGGVQR
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CCHFV 3590 3600 3610 3620 3630 3640 3650 3660 3670 3680
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NSDV NTEVLLREKMNKVVKDNLEQMEESIEFCNNNTKIVSENPLDLSQWSEVQNYIEDIGFNNVLVNIIDRNIVKSELVWRKTLTDNVSTITKDVRL
DUGV NTEVLLREKMNKVVKDNLEQMEESIEFCNNNTKIVSENPLDLSQWSEVQNYIEDIGFNNVLVNIIDRNIVKSELVWRKTLTDNVSTITKDVRL
HAZV NTEVLLREKMNKVVKDNLEQMEESIEFCNNNTKIVSENPLDLSQWSEVQNYIEDIGFNNVLVNIIDRNIVKSELVWRKTLTDNVSTITKDVRL

CCHFV 3690 3700 3710 3720 3730 3740 3750 3760 3770
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DUGV VSVYSTETIPKFLFLFLYDVLNMLISQCKAVKELINSTGLSDLELESLLTICAFYQSECSKRRDGPGRCSFALLSLVHEDWCKTGKNTLVRRNNE
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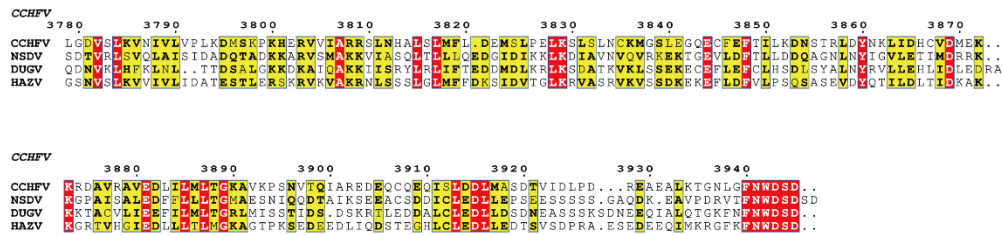


Figure S11 Structure-based sequence alignment of the CCHFV L protein with representative orthonairoviruses.

Structure-based sequence alignment of the L proteins from CCHFV, NSDV, DUGV, and HAZV. Conserved residues are outlined with red boxes, whereas strictly or highly conserved residues are highlighted in yellow. The secondary-structure elements of the CCHFV L protein corresponding to the aligned regions are shown above the sequences. CCHFV, Crimean–Congo hemorrhagic fever virus; NSDV, Nairobi sheep disease virus; DUGV, Dugbe virus; HAZV, Hazara virus.

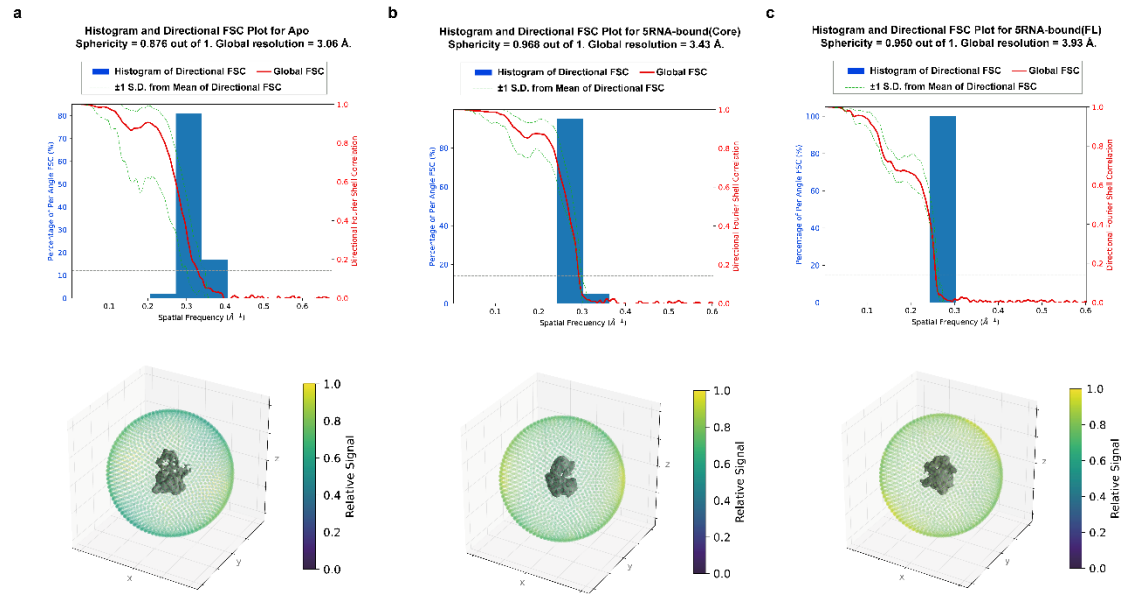


Figure S12 3D FSC analysis of cryo-EM reconstructions.

a-c: Upper: Directional FSC plots and histograms for the apo, 5'RNA-bound core, and 5'RNA-bound full-length reconstructions. Red lines indicate global FSC, and green dashed lines represent ± 1 standard deviation of directional FSC. The sphericity values (0.876, 0.968, and 0.950) indicate largely isotropic reconstructions. Lower: Directional signal distributions mapped onto a sphere show broadly distributed signal across orientations, supporting sufficient angular sampling.

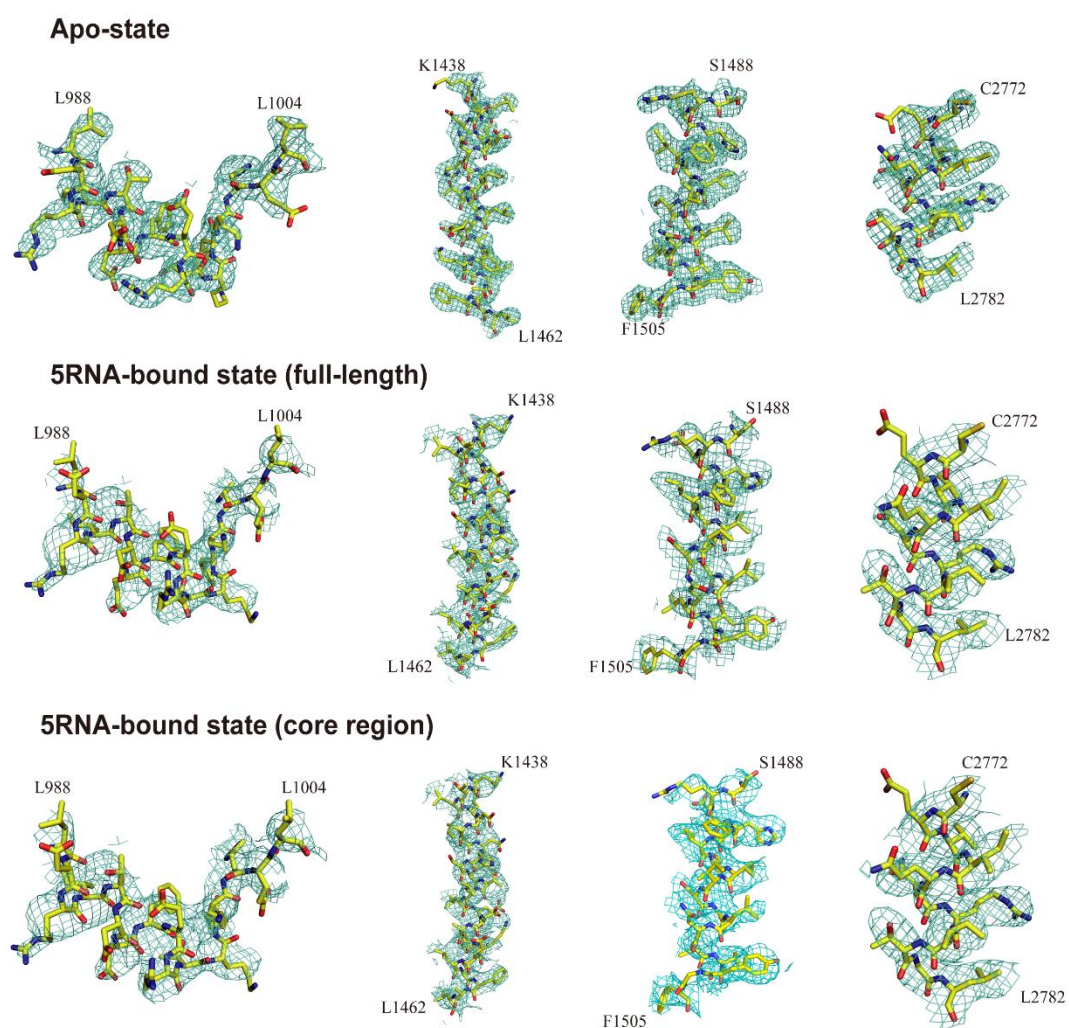


Figure S13 Representative EM densities for the different structures. The local map density for representative protein regions are indicated.

Supplementary Table S1 Cryo-EM data collection, refinement and validation statistics.

	Apo	5RNA-bound (FL)	5RNA-bound (Core)
Data collection and processing			
Magnification	165,000	105,000	105,000
Voltage (kV)	300	300	300
Election exposure (e ⁻ /Å ²)	60	60	60
Defocus range (μm)	-1.5 to -2.0	-1.5 to -2.0	-1.5 to -2.0
Pixel size (Å)	0.74	0.83	0.83
Micrographs (no.)	9,040	11,010	5,672
Symmetry imposed	C1	C1	C1
Detector	Falcon4	K3	K3
Initial particle images (no.)	1,317,709	1,802,531	1,195,460
Final particle images (no.)	55,537	19,012	69,639
Map resolution (Å)	2.85	3.76	3.32
FSC threshold	0.143	0.143	0.143
Refinement			
Initial model used	Alphafold	Alphafold	Alphafold
Model resolution (Å)	1.8	3.6	2.1
FSC threshold	0.143	0.143	0.143
Map sharpening <i>B</i> factor (Å ²)	-74.3	-64.3	-94.1
Map Correlation Coefficient	0.69	0.59	0.72
Model composition			
Non-hydrogen atoms	12090	17243	11649
Protein residues	1511	2128	1431
Nucleotide	0	10	10
Ligands	0	0	0
Water	0	0	0
<i>B</i> factors (Å ²)			
Protein	149.78	248.89	83.25
Nucleotide	N/A	144.93	97.08
R.m.s deviations			
Bond lengths (Å)	0.004	0.004	0.004
Bond angles (°)	0.827	0.822	0.954
Validation			
MolProbity score	2.16	2.30	2.79
Clash score	10.77	20.23	18.73
Poor rotamers (%)	2.45	0.20	3.88
Ramachandran plot			

Disallowed (%)	0	0.8	0.5
Allowed (%)	4.52	7.58	10.63
Favored (%)	95.48	91.62	88.87

Supplementary Table S2

Synthesized genes in this study.

Gene names	genes sequence
<i>His-CCHFV L-twin strepII</i>	> ATGCATCATCACCATCACCATGGTACCATGGATTTCCTGAGAAACCTGGACT GGACCCAAGTGATCGCCGGACAATACGTGACCAACCCTAGATTCAACATT CAGACTACTTTGAGATCGTGCGCCAGCCTGGAGATGGGAATTGCTTCTACC ACTCTATCGCTGAGCTGACAATGCCGAACAAGACGGATCACAGCTACCATA ACATCAAGCACCTGACAGAGTTGGCCGCTAGAAAATACTACCAGGAAGAA CCTGAAGCCAAGCTGATTGGCCTGAGCCTAGAGGACTACCTGAAGAGGAT GTTGTCTGGACAATGAGTGGGGCAGCACCCCTGGAGGCCAGCATGTTGGCAA AGGAGATGGGCATCACCATCATCATCTGGACCGTGGCCGCCTCTGACGAGG TGGAAGCCGGAATCAAGTTCGGCGACGGCGACGTGTTCAACGCCGTGAAC CTGCTGCACAGCGGCCAGACACACTTTGATGCTCTGCGGATACTGCCTCAG TTCGAAGCCGACACAAGAGAGACCCTTAGCCTGGTGGACAAGGTGATCAC AGTGGACCAACTTACATCATCCTCTTCCGACGAACTGCAGGACTACGAGGA TCTGGCCCTGGCCCTGACCAGCGCTGAGGAACCTTACCGGAGATCTAGCCT GGACGAAGTGACCCTGTGAAAAAGCAGGCTGAAATGCTGAGGCAGAAG GCCAGCCAACCTATCGAAACTGGTCAACAAGTCTCAGAACATCCCTACCAGA GTGGGCAGGGTACTGGACTGCATGTTCAACTGCAAGCTGTGCGTGGAAATC AGTGCTGACACACTGATCCTGAGACCCGAATCCAAGGAAAGAATCGGAGA AGTGATGAGCCTCCGACAGCTCGGCCACAAGCTGCTGACCAGAGACAAGC AGATCAAGCAGGAGTTCTCCAGAATGAAGCTGTACGTGACAAAGGACCTG TTGGACCACCTCGACGTGGGCGGTCTGCTCAGAGCCGCATTCCCCGGCACA GGCATCGAGAGACACATGCAGCTGCTGCACAGCGAAATGATCCTGGACATC TGCACCGTTAGCCTGGGCGTGATGCTCAGCACCTTCCTGTACGGCTCCAAC AACAAGAACAAGAAGAAGTTTATCACGAACTGCCTCCTGAGACCGCCCT GAGTGGAAAAAAGGTGTACAAGGTGCTGGGCAACCTGGGAAACGAACTG CTGTACAAGGCCCTAGAAAGGCTCTTGCCACTGTGTGCAGCGCCCTGTTC GGCAAGCAGATTAACAAGCTGCAGAACTGTTTCAGAACCATCAGCCCTGT GAGCCTGCTGGCCCTGAGAAACCTGGACTTTGATTGCCTGTCTGTGCAGGA CTACAACGGCATGATCGAGAACATGAGCAAACTCGATAATACCGACGTGGA GTTCAACCATAGAGAGATCGCAGATCTCAACCAGTTAACTAGCCGGCTTAT CACCCTGAGAAAGGAGAAAGACACGGACCTGCTGAAACAATGGTTCTCGG AAGGCGACCTGAGTCGGCGGTCTACCAGAAACGTGGCCAACGCCGAAGA GTTTATCATCAGCGAGTTCTTCAAGAAGAAGGACATCATGAAATTCATTTCG ACAAGCGGAAAGGCCATGAGCGCCGTAAGATTGGCAACGTGCTGTCTTAT GCCCACAACCTGTACCTGAGCAAATCTAGCCTGAACATGACAAGCGAAGA CATCAGCCAGCTGCTTATCGAGATCAAGCGGCTATACGCTCTGCAGGAGGA

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	GCGGGACGGCAAGCGGAGAGGCAACCTGGAGCTGGCCAACAGAATCCAG TCTGTGCTGTGCGTGCTAATAGCTGGATACTACAGATCTTTCGGCGGCGAGG GCACAGAAAAGCAGGTGAAGGCCAGTCTGAATCGAGATGACAACAAGATC ATCGAAGATCCTATGATTGAGCTGATCCCAGAGAAGCTGAGAAGGGAAGT GAACGGCTGGGAGTGAGCAGAATGGAGGTGGACGAGCTGATGCCTAGCAT CTCTCCTGACGACACCCTCGCTCAACTGGTTGCCAAAAAGCTGATTAGCCT GAATGTGTCTACCGAAGAGTACTCTGCCGAGGTGAGCCGGCTGAAACAGA CCCTGACAGCCAGAAAACGTGTTGCACGGCCTTGCTGGAGGCATCAAGGAG CTGTCCCTGCCTATCTACACCATTTTCATGAAATCTTACTTCTTTAAGGATAA CGTGTTCCTGAGCCTGACAGACAGATGGTCCACCAAGCATTCTACCAACTA CAGAGACTCCTGCGGCAAAACAGCTGACCGGAAGAATCATCACCAAGTACA CCCACTGGCTGGACACCTTCTTATCCTGCTCAGTTTCATTCAACCGGCACAC CACCGTGAAAGAGCCTAGCCTGTTTAAACCCTAACATCCGGTGCGTGAACCT GATAACATTTGAAGATGGCCTGAGAGAACTAAGTGTGATTGAGAGCCACCT CAAGGTCTTCGAGAACGAGTTCACAAACCTTAACCTGCAGTTTTAGACCC TAACAGGCAGAAACTGCGCATTGTGGAAAGCCGGCCTGCAGAAAGCGAAC TTGAAGCGAACAGAGCTGTTATCGTTAAGACAAAGCTGTTCTCTGCCACAG AGCAGGTTAGACTGAGCAACAACCCCGCCGTAGTGATGGGCTACCTGTTGG ACGAGAGCGCCATCAGCGAGGTGAAGCCCACCAAGGTGGACTTTAGCAAT CTGCTGAAGGACCGGTTTAAAGATCATGCAATTCTTCCCTAGCGTGTTACCC TTATCAAAATGCTGGCCGACGAGTCCACCGACTCTGAAAAGAATGGCCTCT CCCCCGACCTTCAGCAGGTGGCCAGATACAGCAATCACCTGACCCTGCTCT CCAGAATGATCCAGCAGGCCAAGCCTACGGTGACAGTCTTCTATATGCTGA AAGGAAACCTGATGAACACCGAACCCACTGTGGCTGAGCTGGTGAGCTAC GGCATCAAGGAGGGCAGATTCTACAGATTGAGCGATACAGGTATCGACGCC AGCACCTACAGCGTGAAATACTGGAAGATCCTGCATTGCATCAGCGCCATT GGATGCCTGCCCCTGAGCCAGGCCGACAAGAGCAGCCTGCTGATGTCTTTT CTTAATTGGCGGGTGAACATGGACATCAGAGCTAGCGACTGTCTCTGTCC AACCACGAGGCCTCTATACTGTCAGAGTTTGATGGACAGGTATCGCTAAC ATCTTGCGGAGCGAACTGAGCTCAGTGAAGCGGGACAGCGAAAGAGAAG GCCTGACCGACCTGCTGGACTACTTGAACCTACCTACAGAACTGCTGAAGA AAAAGCCCTACCTGGGGACAACCTGCAAGTTTAATACCTGGGGAGATTCCA ACAGAAGCGGCAAGTTCACATACTCCTCAAGAAGCGGCGAGTCTATCGGG ATCTTTATTGCCGGCAAGCTGCACATCCACCTGTCTAGCGAGTCTATCGCCC TCCTGTGTGAAACCGAACGGCAGGTACTGAGCTGGATGAGCAAGAGACGG ACCGAGGTGATCACAAAGGAACAGCACCAGCTGTTCCCTGAGCCTGCTCCC TCAGTCCCACGAGTGCCTGCAGAAGCACAAAGATGGCTCTGCCCTGAGCG TGATCCCCGACGGCAGCAATCCTAGACTGCTGAAGTTTCGTGCCTCTGAAGA AAGGCCTCGCTGTGGTGAAGATCAAGAAGCAAATCCTGACAGTGAAGAAG CAGGTGGTGTTCGACGCCGAGTCTGAGCCTAGACTGCAGTGGGGCCACGG CTGCCTGAGCATCGTGTACGACGAGACCGACACCCAGACCACTTACCACG AGAACCTGCTGAAGGTGAAGCAGCTGGTGGATTGCAGCACAGATAGAAAG AAGCTGCTGCCTCAGAGCGTGTTCTCCGACAGCAAGGTGGTGTGAGCCG GATCAAATTCAAGACTGAGCTGCTGCTGAACAGCCTGACGCTGCTGCACTG
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	CTTCCTGAAACACGCCCCCTCTGATGCTATAATGGAAGTGGAAGTAAGTC GAGCCTGCTGCATAAGTTCCTGAAGCTGGGCGGCGTGAGACAGAGAAACA CCGAAGTTCTGCTCCGGGAGAAAATGAACAAGGTGGTGATCAAGGACAAT CTGGAACAGGGCATGGAGGAGGAGATCGAGTTTTGTAACTTGACTAA AACCGTGAGCGAAAACCTCTGCCTCTGTCTTGTGGTCTGAGGTGCAGAA CTACATCGAGGACATCGGATTCAATAACGTGCTGGTGAACATTGATAGAAA CACTGTGAAATCCGAGCTCCTGTGGAAATTCACGCTCGACACAAATGTCAG CACCACCAGCACCATCAAAGACGTGCGTACCTGGTTTCTTACGTGAGCAC AGAGACAATCCCCAAGTTCCTGCTGGCCTTCCTGCTTTATGAGGAAGTGCT CATGAACCTGATCTCTCAGTGTAAGGCCGTGAAGGAGCTTATTAACAGCAC AGGCCTGTCCGACTTGAACTGGAGAGCCTGCTGACCCTGTGTGCTTTTTTA CTTCCAGTCAGAATGTTCCAAGCGGGACGGTCCTAGATGCAGCTTCGCTGC CTTGCTGTCCCTGGTGCACGAGGACTGGCAGAAAATCGGCAAGAACATCC TCGTGCGAGCCAACAACGAGCTGGGCGACGTGTCCTTGAAGGTGAACATC GTTTTGGTGCCCCTGAAGGATATGAGCAAACCTAAGCACGAGAGAGTGGT GATCGCTAGAAGAAGCCTGAACCACGCTCTGTCACTAATGTTCTGGACGA GATGTCTCTTCTGAGCTGAAGAGCCTCAGTCTGAACTGCAAGATGGGCAG CCTGGAAGGCCAGGAGTGCTTCGAGTTCACCATCCTGAAGGACAATTCTAC CAGACTGGACTATAACAAGCTGATCGACCACTGTGTGGACATGGAAAAGA AGAGAGATGCCGTACGCGCCGTCGAGGACCTGATCCTGATGCTAACCGGCA AGGCCGTGAAGCCCAGCAATGTGACCCAGATCGCCAGAGAGGATGAGCAG TGCCAGGAGCAGATCAGCCTTGATGACCTGATGGCCTCTGACACCGTGATC GACCTGCCAGATAGAGAAGCCGAGGCCCTAAAGACAGGGAACCTGGGCTT CAACTGGGACAGCGATTGGTCGACCCCTCAGTTCGAGAAGGGCGGCGGCT CTGGCGGGGGCAGCGGCGGCAGCGCCTGGAGCCACCCCCAGTTTGAAAAG TGA
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Supplementary Table S3

Oligonucleotides used in this work.

Oligonucleotides	Sequence (5'-3' direction)	Description
5'-vRNA	UCUCAAGAAAUCGUUCC	For cryo-EM sample preparation, the 5' end was phosphorylated. For BLI assays, the 5' end was biotinylated.
5'-vRNA-U1A	ACUCAAGAAAUCGUUCC	5'-Biotin
5'-vRNA-U1G	GCUCAAGAAAUCGUUCC	5'-Biotin
5'-vRNA-U1C	CCUCAAGAAAUCGUUCC	5'-Biotin
5'-vRNA-C2G	UGUCAAGAAAUCGUUCC	5'-Biotin
5'-vRNA-C2A	UAUCAAGAAAUCGUUCC	5'-Biotin
5'-vRNA-C2U	UUUCAAGAAAUCGUUCC	5'-Biotin
5'-vRNA-U3A	UCACAAAGAAAUCGUUCC	5'-Biotin
5'-vRNA-U3G	UCGCAAAGAAAUCGUUCC	5' '-Biotin

5'-vRNA-U3C	UCCCAAAGAAAUCGUUCC	5'-Biotin
5'-vRNA-C4G	UCUGAAAGAAAUCGUUCC	5'-Biotin
5'-vRNA-C4A	UCUAAAAGAAAUCGUUCC	5'-Biotin
5'-vRNA-C4U	UCUAAAAGAAAUCGUUCC	5'-Biotin
5'-vRNA	UCUCAAGAUAUAGCUGCGCGC	For ³² P-labelled radionucleotide extension assay
3'-vRNA	GCGCGCUGAUUCUUUGCUA	For ³² P-labelled radionucleotide extension assay