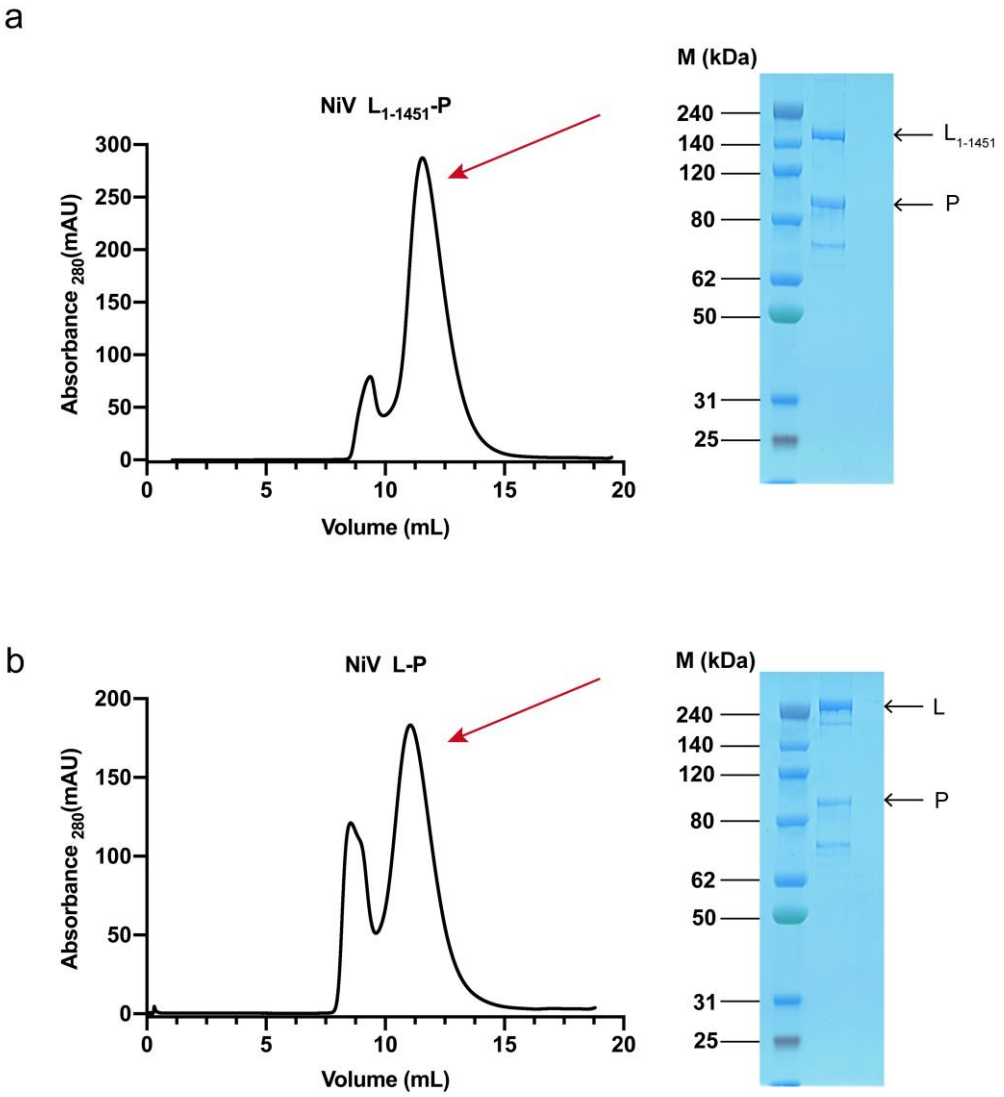
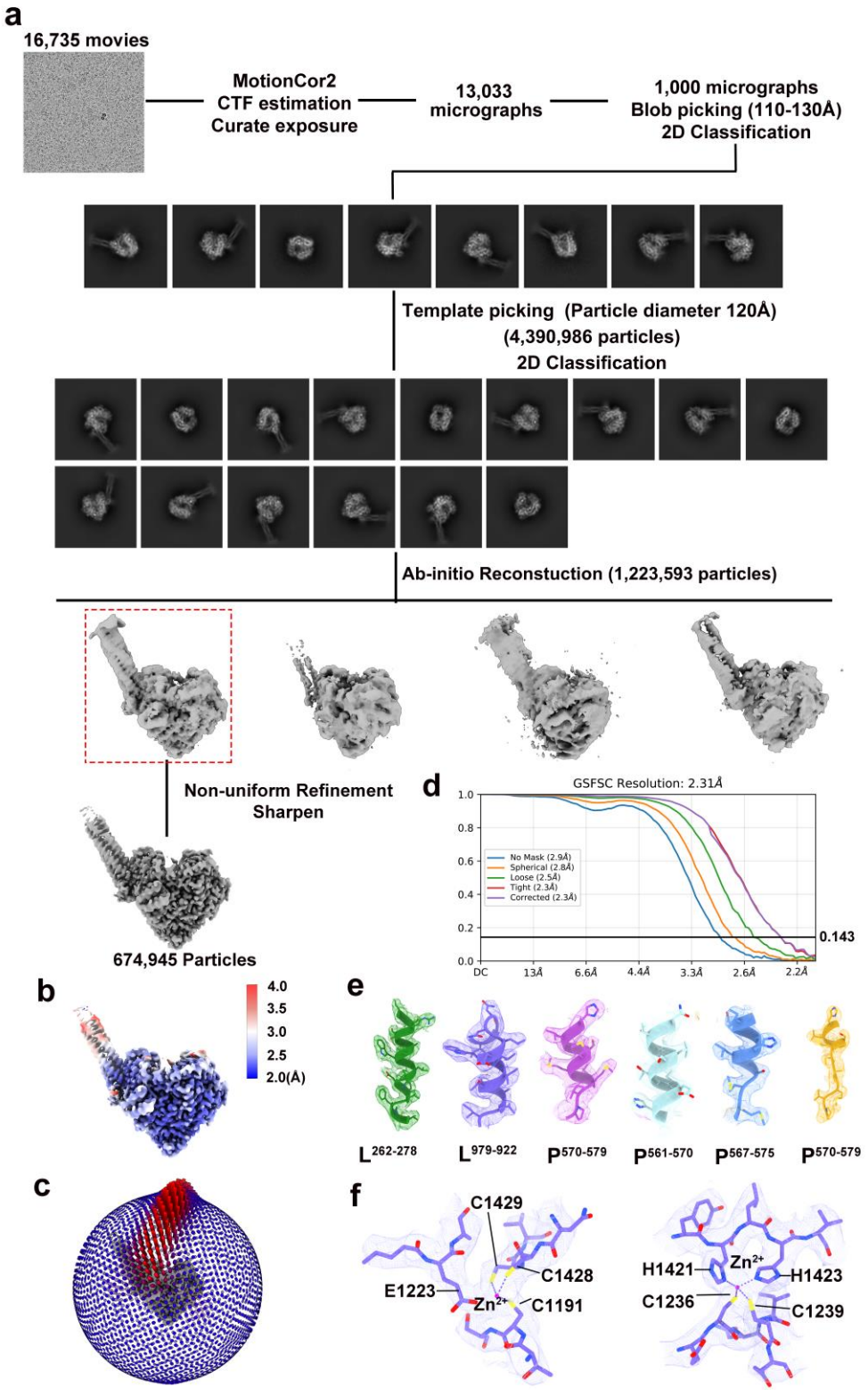


Figure S1 | Purification of the truncated (L_{1-1451}-P) and full-length (L-P) NiV polymerase complexes.

a. Size-exclusion chromatography and Coomassie-stained SDS-PAGE analysis of purified NiV L_{1-1451}-P complex. The molecular weights of the L_{1-1451} protein and P protein are about 170 kDa and 80 kDa, respectively. **b.** Size-exclusion chromatography and Coomassie-stained SDS-PAGE

analysis of purified NiV L-P complex. The molecular sizes of the L protein and P protein are about 250 kDa and 80 kDa, respectively.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(4,390,986 particles)
2D Classification
Ab-initio Reconstuction (1,223,593 particles)
Non-uniform Refinement
Sharpen
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3.5
3.0
2.5
2.0(Å)
c
d
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Tight (2.3Å)
Corrected (2.3Å)
0.143
DC
13Å
6.6Å
4.4Å
3.3Å
2.6Å
2.2Å
e
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P570-579
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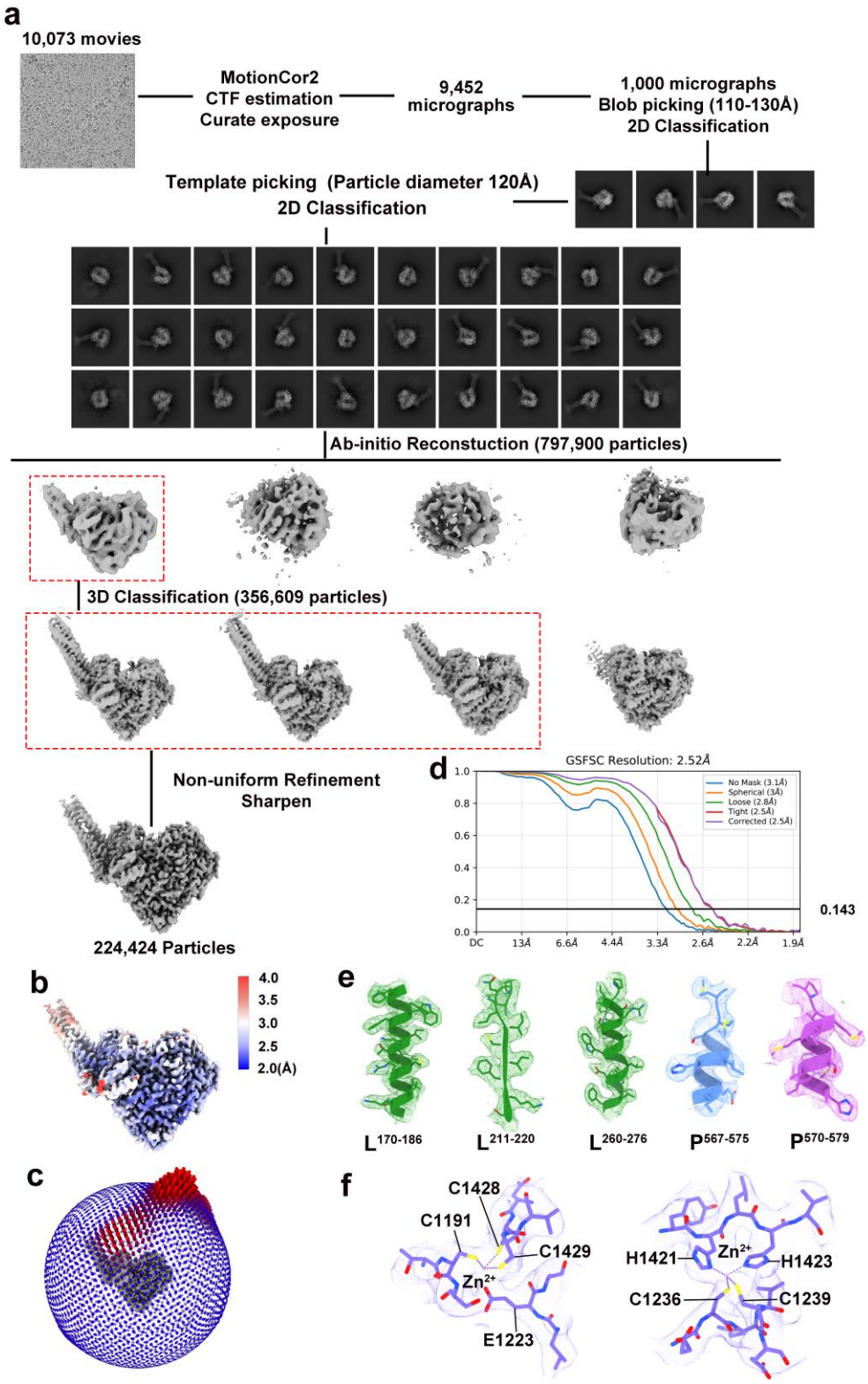
P570-579
f
C1429
C1428
E1223
Zn2+
C1191
H1421
Zn2+
H1423
C1236
C1239

Figure S2 | Cryo-EM data processing and analysis of the NiV $L_{1\text{-}1451}$-P complex.

a. Cryo-EM image processing flowchart for the NiV $L_{1\text{-}1451}$-P complex sample. **b.** A local resolution map for the NiV $L_{1\text{-}1451}$-P complex. **c.** Angular distribution of the particles used for the reconstruction of the NiV $L_{1\text{-}1451}$-P complex structure**. d.** Fourier shell correlation curves of the reconstructed map for the NiV $L_{1\text{-}1451}$-P complex. Overall resolution of the structure was assessed by the gold-standard FSC 0.143 cut-off criteria. **e.** Representative densities from the structure of the NiV $L_{1\text{-}1451}$-P complex. **f.** Cryo-EM densities for the two zinc-binding sites within the PRNTase domain of NiV.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2D Classification
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224,424 Particles
d
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4.0
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2.5
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f
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C1429
Zn2+
E1223
H1421
Zn2+
H1423
C1236
C1239

Figure S3 | Cryo-EM data processing and analysis of the NiV L-P complex.

a. Cryo-EM image processing flowchart for the NiV L-P complex sample. **b.** A local resolution map for the NiV L-P complex. **c.** Angular distribution of the particles used for the reconstruction of the NiV L-P complex structure**. d.** Fourier shell correlation curves of the reconstructed map for the NiV L-P complex. Overall resolution of the structure was assessed by the gold-standard FSC 0.143 cut-off criteria. **e.** Representative densities from the structure of the NiV L-P complex. **f.** Cryo-EM densities for the two zinc-binding sites within the PRNTase domain of NiV.

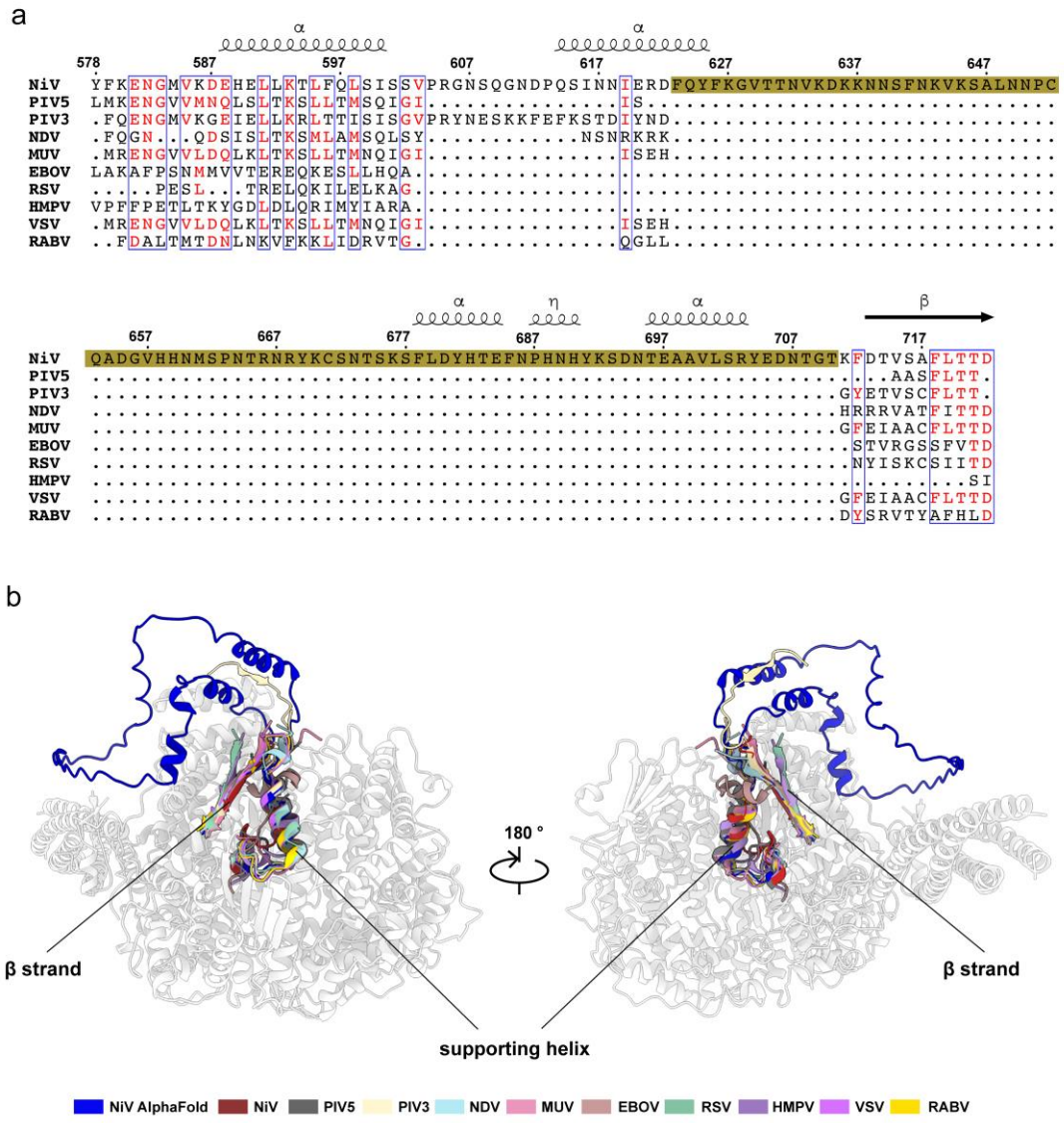


Figure S4 | The RdRp domain of NiV L protein contains a long insertion sequence compared to other NNS RNA virus polymerases.

a. Multiple sequence alignment of polymerase L protein sequences, the long insertion sequence is highlighted in brown. L protein sequences used are NiV (nipah virus), PIV5 (Parainfluenza virus 5), PIV3 (Parainfluenza virus type 3), NDV (Newcastle disease virus), MuV (Mumps virus), EBOV (Ebola virus), RSV (respiratory syncytial virus), HMPV (Human

metapneumovirus), VSV (Vesicular stomatitis virus) and RABV (Rabies virus). **b.** Structural superposition of NiV polymerase complex structure (gary) with an AlphaFold2 predicted long insertion sequence structure (blue) and experimental polymerase strcutures of *Mononegavirales* (colored) indicates that the long insertion sequence (highlighted in brown) form a large loop. This loop is potentially flexible, situated between the supporting helix and a β-sheet in the NiV polymerase.

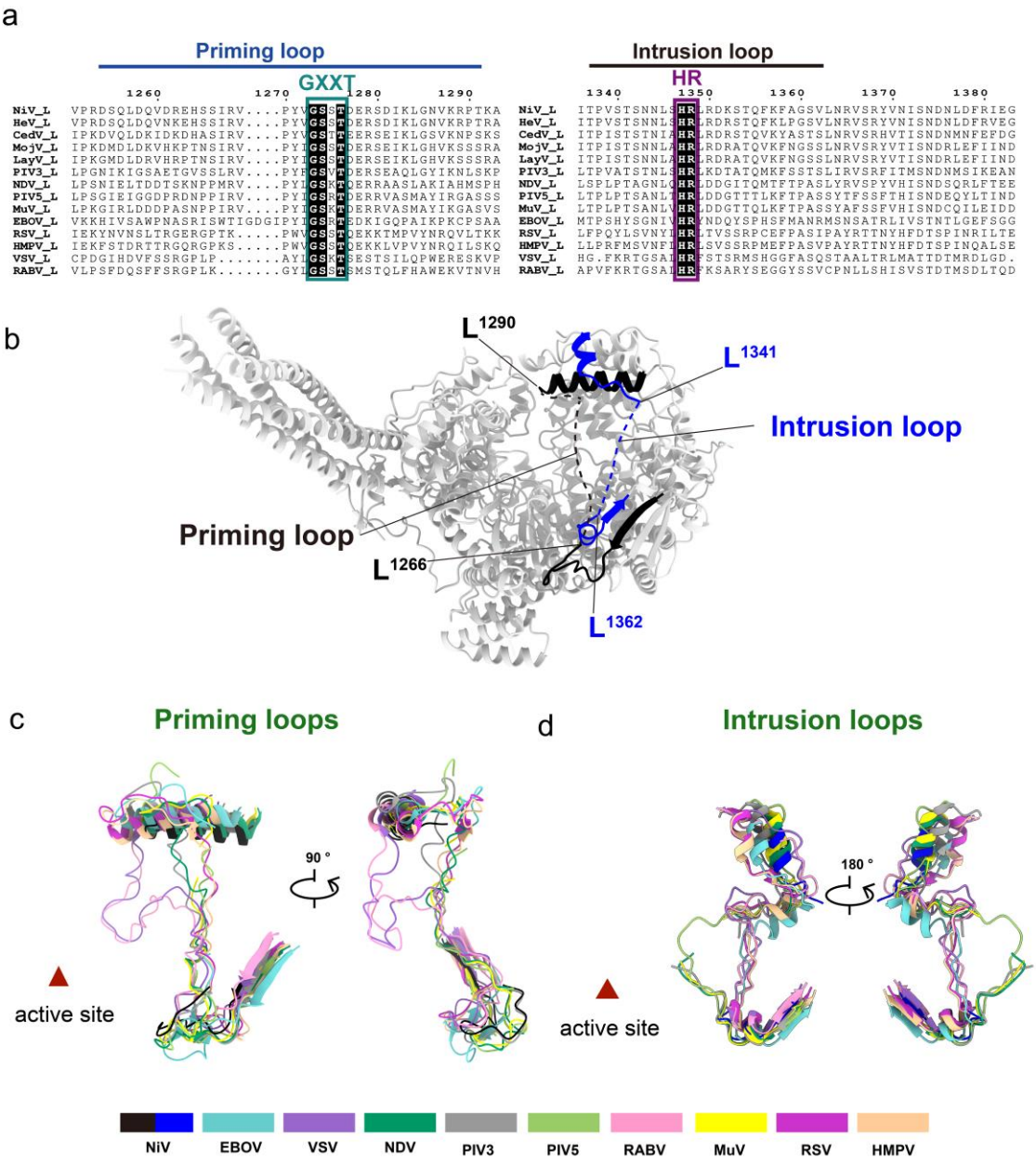


Figure S5 | Structural features of the priming and intrusion loops in NiV polymerase compared to those of other NNS RNA viruses.

a. Multiple sequence alignment of the GXXT and HR motifs in different *Mononegavirales*. The highly conserved GXXT and HR motifs among *Mononegavirales* are highlighted in colored boxes. **b.** The locations of the priming loop and the intrusion loop in the structure of the NiV L-P complex

structure. **c-d.** Comparison of priming (**c**) and intrusion (**d**) loops in the NiV polymerase with those of other *Mononegavirales* shows that the backbone trajectories of residues at both ends of the NiV priming and intrusion loops align well with the conformations observed for HMPV, NDV, and PIV5 polymerases. Polymerase structures used are EBOV (PDB: 8JSM), VSV (PDB: 5A22), NDV (PDB: 7YOU), PIV3 (PDB: 8KDC), PIV5 (PDB: 6V85), RABV (PDB: 6UEB), MuV (PDB: 8IZL), RSV (PDB: 8SNX) and HMPV (PDB: 6U5O). The location of the polymerase catalytic site is indicated by a red triangle.

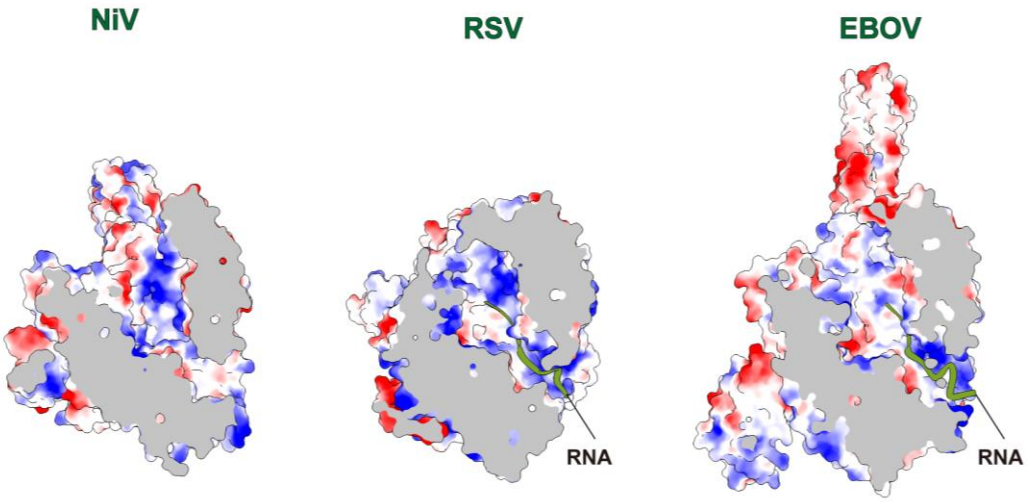


Figure S6 | Polymerase cavity electrostatic potentials compared among NiV, RSV and EBOV.

Cut views to show the electrostatic potentials of NiV, RSV and EBOV polymerase cavities. Bound template RNAs (green) are included for the RSV (PDB: 8SNX) and EBOV (PDB: 8JSM) strcutures. Blue and red colors indicate positive and negative surface charges.

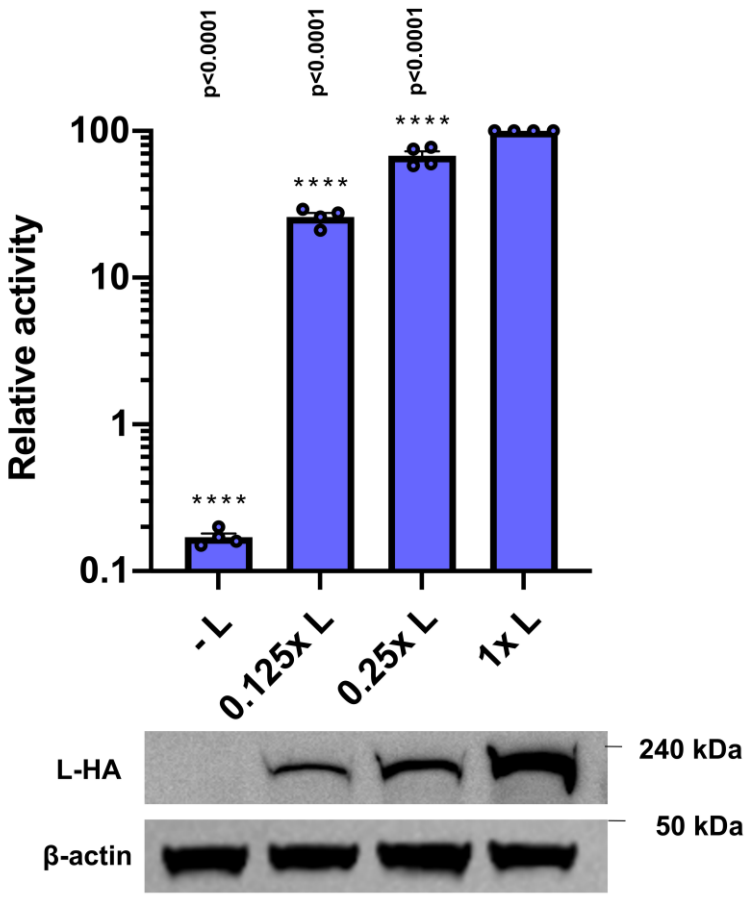


Figure S7 | L protein expression titration experiment.

1×L, 0.25×L, 0.125×L and -L lanes represent protein expression levels in mini-replicon experiments, where 1μg, 0.25 μg, 0.125 μg and 0 μg of L protein expression plasmid were transfected. Corresponding mini-replicon activities are reported as mean ± SEM from four independent experiments (n = 4). All statistics used one-way ANOVA Dunnett's multiple comparisons test. (ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

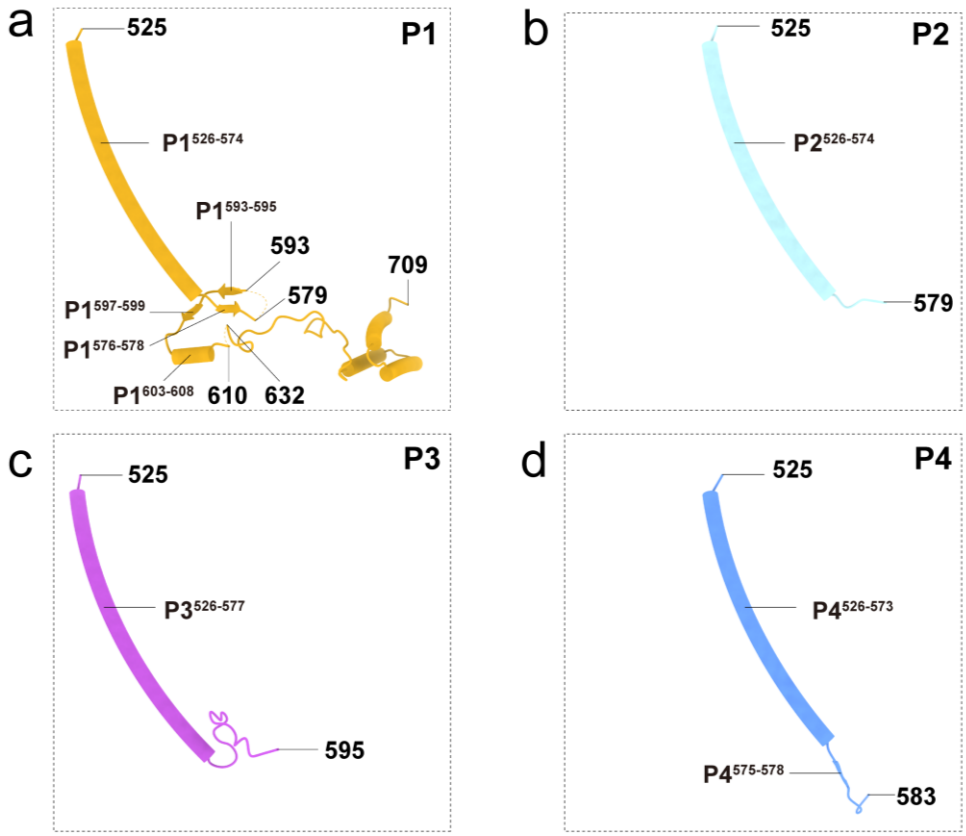


Figure S8 | Structures of P protein monomers within the P protein bundle within the NiV L_{1-1451}-P complex.

a-d. Strcutures of the four P protein monomer – P1 (**a**), P2 (**b**), P3 (**c**) and P4 (**d**) within the L protein bound P protein bundle. Secondary structural elements and their starting and end residues are shown.

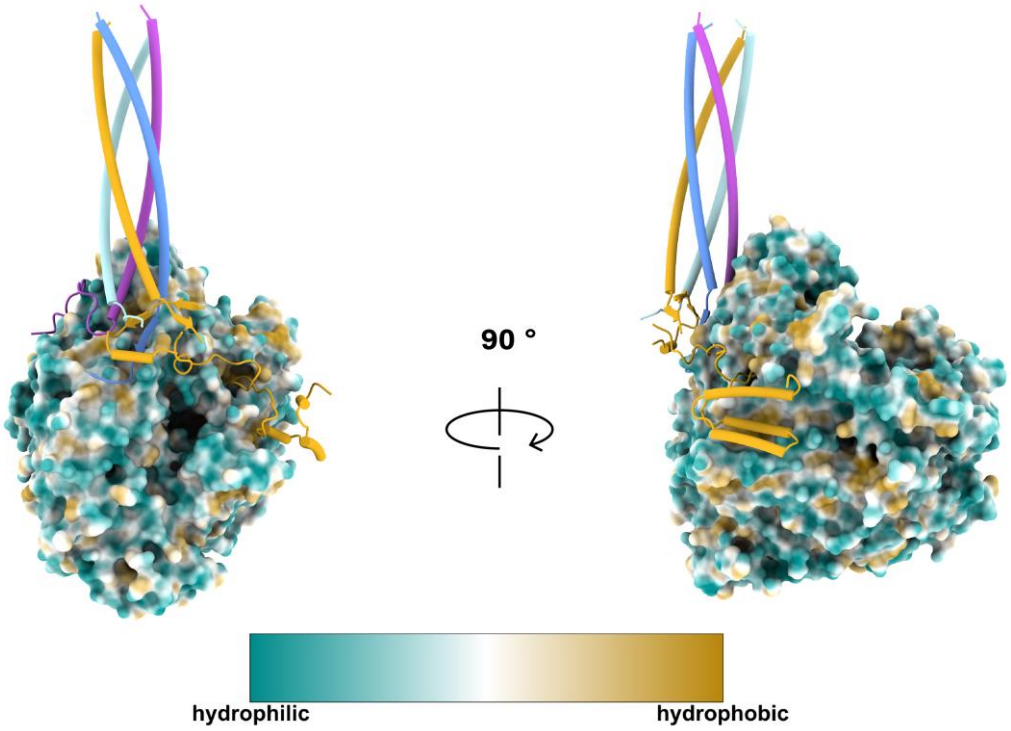


Figure S9 | Binding of the P protein bundle on the L protein surface.

The surface of the L protein is coloured according to the hydrophobicity of its surface residues. The P1, P2, P3 and P4 monomers of the P protein bundle are colored yellow, cyan, magenta and blue, respectively.

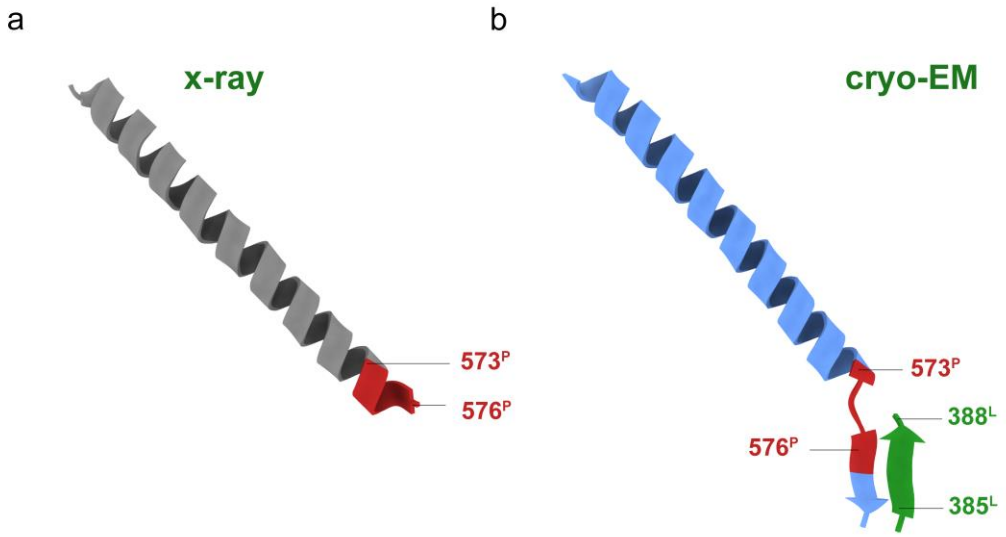
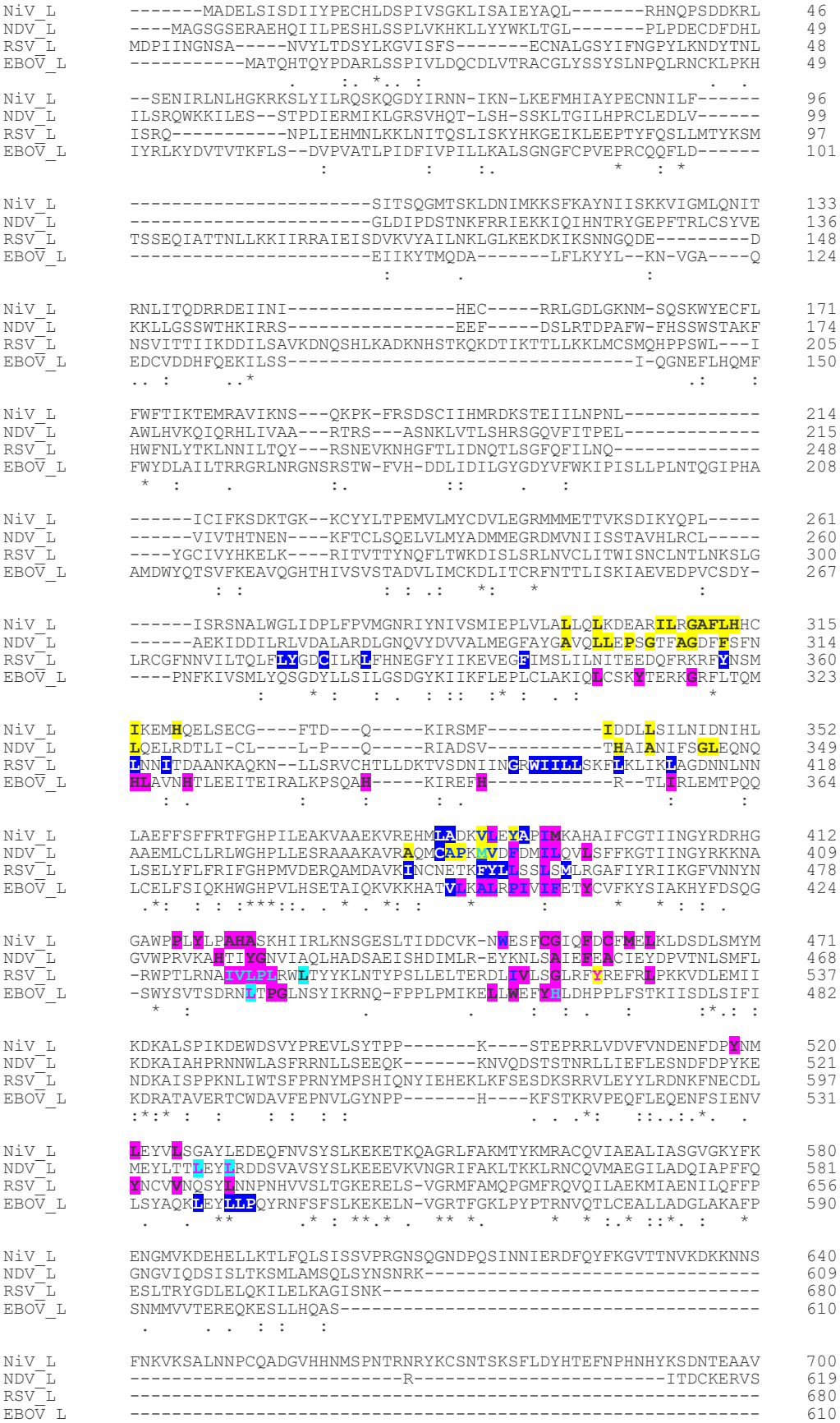


Figure S10 | Conformational change of the NiV P protein upon L protein binding.

a. An α-helix extracted from the X-ray structure of the NiV P protein tetramer. Residues 573-576 at the C-terminal end of the helix is shown in red. **b.** The structure of P4 within the P protein tetramer in the cryo-EM structure. Upon L binding, the residues ($P^{573\text{-}576}$, red) refold to transform into a loop and part of a β-strand to bind $L^{385\text{-}388}$, forming an antiparallel sheet.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RSV_L MDPIINGNSA-----NVYLTDSYLKGVISFS-------ECNALGSYIFNGPYLKNDYTNL 48
EBOV_L -----------MATQHTQYPDARLSSPIVLDQCDLVTRACGLYSSYSLNPQLRNCKLPKH 49
NiV_L --SENIRLNLHGKRKSLYILRQSKQGDYIRNN-IKN-LKEFMHIAYPECNNILF------ 96
NDV_L ILSRQWKKILES--STPDIERMIKLGRSVHQT-LSH-SSKLTGILHPRCLEDLV------ 99
RSV_L ISRQ-----------NPLIEHMNLKKLNITQSLISKYHKGEIKLEEPTYFQSLLMTYKSM 97
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EBOV_L ----------EIIKYTMQDA-------LFLKYYL--KN-VGA----Q 124
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NDV_L KKLLGSSWTHKIRRS----------EEF-----DSLRTDPAFW-FHSSWSTAKF 174
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RSV_L -RWPTLRNALVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMII 537
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EBOV_L SNMMVVTEREQKESLLHQAS---------- 610
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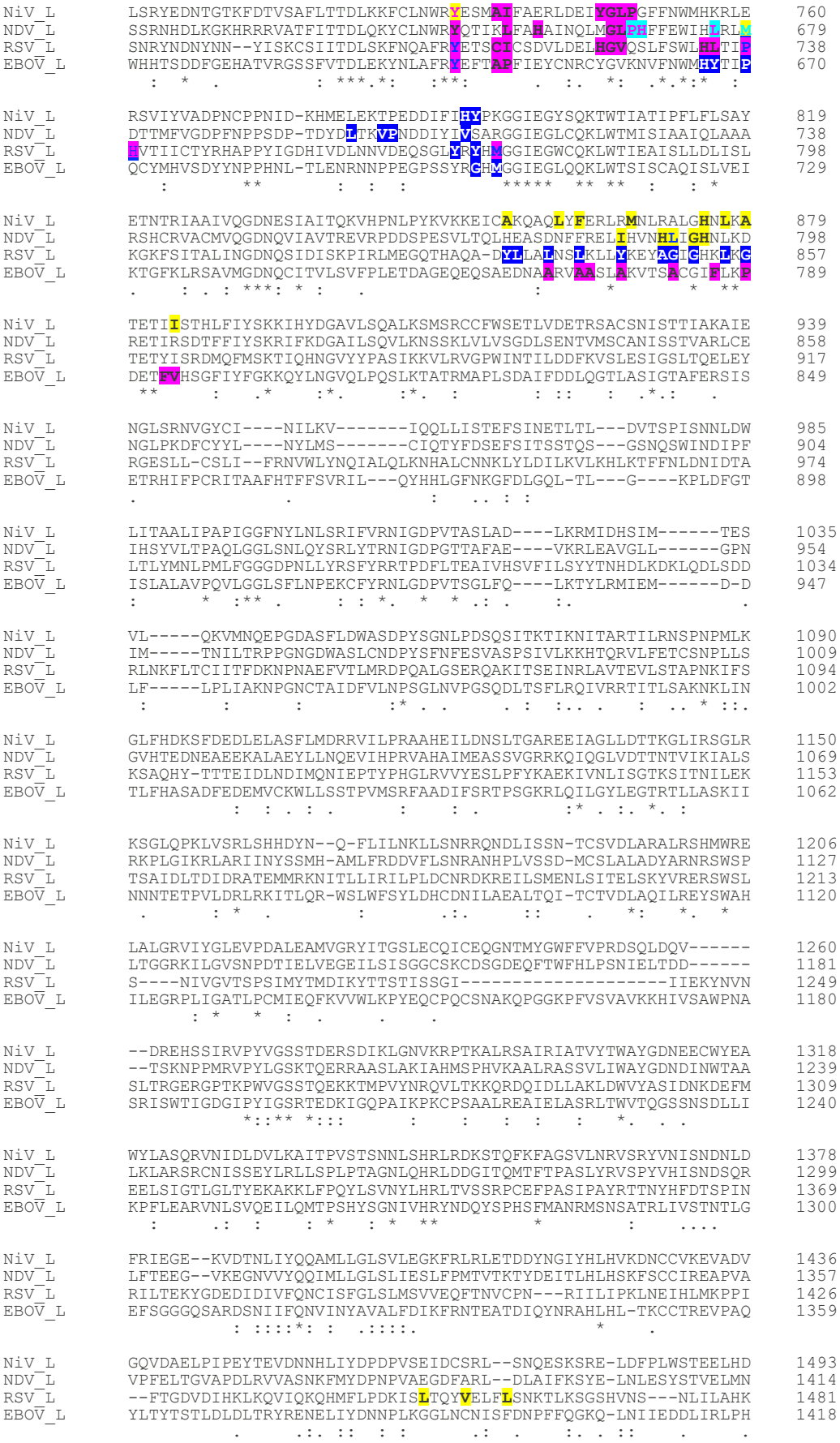
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EBOV_L      L---------SGWELAK-TIMQ-SIISDSNNSST---DPISSGETRSFTTHFLTYPKIGL    1464
            :          .   .   :    : . .:           .  ..  :.

NiV_L       ALYLGQSISIKWAFEIHHRRPRGRHTMVDLL--SDLVSN---TSKHTYKVLSNALSHPRV    1597
NDV_L       FEYAALEVLLDCSYQLYYLRVRGLNNVVLYM--SDLYKN---MPGILLSNIAATISHPII    1518
RSV_L       --FFNAYKTYL----LCFHKGYGKAKLECDMNTSDLLCVLELIDSSYWKSMSKVFLEQKV    1587
EBOV_L      LYSFGAFVSYYLGNTILRTKKLTLDNFLYYL--TTQIHN---LPHRSLRILKPTFKHASV    1519
                        :   :     ..   :  :              :  .: .  :

NiV_L       FKRFVNCGLLLP------TQGPYLHQQDFEKLSQNLLVTSYMIYLMNWCDFKK--SPFLI    1649
NDV_L       HSRLHTVGLISH------DGSHQLADTDFIELSAKLLVSCTRRVVSGLYAGNK--YDLLF    1570
RSV_L       IKYILSQDASLH--------------------------------RVKGC-----HSFKLWF    1611
EBOV_L      MSRLMSIDPHFSIYIGGAAGDRGL--SDAARLFLRTSISSFLTFVKEWIINRGTIVPLWI    1577
             . : . .                                      :           : :

NiV_L       AEQDE----TVISLREDII-------TSKHLCVIIDLYANHHKPPWIIDLNPQ--EKICV    1696
NDV_L       PSVLD----DNLNEKMLQL-------ISRLCCLYTVLFATTREIPKIRGLPAE--EKCAM    1617
RSV_L       LKRLN--------------------------------VAEFTVCPWVVNIDYHPTHMKAI    1639
EBOV_L      VYPLEGQNPTPVNNFLHQIVELLVHDSSRHQAFKTTIND--H-------VHPH-------    1621
                :                                            :  .

NiV_L       LRDFISKSRHVD--TSS--RSWNTSDLDFVIFYASLTYLRRGIIKQLR------------    1740
NDV_L       LTEYLLSDAVRPLLSPE--QVDSITSPSIVTFPANLYYMSRKSLNLIR------------    1663
RSV_L       LT-YIDLVRM-GLINIDRIHIKNKHKFNDEFYTSNLFYINYNFSDNTH-LLTKHIRIANS    1696
EBOV_L      ---------------DN--LVYTCKSTASNFFHASLAYWRSRHRNSNRKDLTRNSSTG-S    1663
                          .     .  .     : :.* *      .  :

NiV_L       -----------------IRQVTEVIDT------T----------TMLRDNIIVENPPIK-    1766
NDV_L       -----------------EREDRDSILA------L----------MFPQEPLFEFPLVQD    1689
RSV_L       ELENNYNKLYHPTPETLENILANPIKSNDKKTLNDYCIGKNVDSIMLPLLS--NKKLIK-    1753
EBOV_L      STNNSDGHIK----RSQEQTTRDPHDGTERSLVL--QMSHEIKRTTIPQENTHQGPSFQ-    1716
                          .   :                        :     :   .:

NiV_L       ----------------TGVLDIRGCIIYNLEE--------ILSMNTKSASK---------    1793
NDV_L       IGARVKDQLTMKPAAFLHELDLSAPARYDAYT--------LE---QAR-SD---------    1728
RSV_L       -------------SSAM-IRTNYSKQDLYNLFPMVVIDR--IIDHSGNTAKS---NQ----    1791
EBOV_L      -----------------SFLSDSACGTANPKLNFDRSRHNVKSQDHNSASKREGHQIISH    1759
                           .      :          :       ..

NiV_L       -------------KIFNL----NSRPSVEN------------------HKYRRIGLNSSS    1818
NDV_L       -------------CALAD----MGEDQLVR------------------YLFRGVGTASSS    1753
RSV_L       --------------LYTT---TSHQISLVHNSTSLYCMLPWHHINRFNFVFSSTGCKISI    1834
EBOV_L      RLVLPFFTLSQGTRQLTSSNESQTQDEISKYLRQLRSVI------DTTVYCRFTGIVSSM    1813
                             . .: .                          *   *

NiV_L       CYKALNLSPLIQRYLPSGAQRLFIGEGSGSMMLLYQSTLGQSISFYN-SGIDGDYIPGQR    1877
NDV_L       WYKASHLLSVPEIRCARHGNSLYLAEGSGAIMSLLELHIPHETIYYN-TLFSNEMNPPQR    1812
RSV_L       EYILKDL------KIKDPNCIAFIGEGAGNLLLRTVVELHPDIRYIYRSLKDCNDH----    1884
EBOV_L      HYKLDEVLWEIEN--FKSAVTLAEGEGAGALLL--IQKYQVKTLFFN-TLATESSIESEI    1868
             *   .:               .**:* ::         .  :   :    .

NiV_L       ELK--------LFPSEYSIAEEDPSLTGKLKGLVVPLFNGRPETTWIGNLD---------    1920
NDV_L       HFG--------PTPTQFLNSVVYRNLQAEVP--CKDGFVQEFRTLWRENTEE--SDLTSD    1860
RSV_L       -----------SLPIEFL-----------------RLYNGHINIDYGENLTIPATDATNN    1916
EBOV_L      VSGMTTPRMLLPVMSKFHNDQIEIILNNSA-----SQITDITNPTWFKDQRA--------    1915
                        ::                          .  :  :

NiV_L       -SYEYIINRTAGRSIGLVHSDMESGIDKNVEEILVEHSHLISI-AINVMMEDGLLVSKIA    1978
NDV_L       KAVGYITSVVPYRSVSLLHCDIEIPPGSNQSLLDQLATNLSLI-AMHSVREGGVVIVKIL    1919
RSV_L       IHWSYL-HIKFAEPISLFVCDAELSVTVNWSKIIIEWSKHVRKCKYCSSVNKCMLIVKYH    1975
EBOV_L      ---------RLPRQVEVITMDAETTENINRSKLYEAVHKLILH-HVDPSVLK-AVVLKVF    1964
                     . : :.  * *     * . :    :              :: *

NiV_L       YTPGFPISRLFNMYRSYFGLVLVCFPVYSNPDSTEVYLLCLQKTV------KTIVPPQK-    2031
NDV_L       YSMGYYFHLLVNLFTPCSVKGYVLSNGYACRGDMECYVVFVMGYLGGPTFVNEVVRMAKT    1979
RSV_L       AQDDID--FKLDNIT--ILKTYVCLG--SKLKGSEVYLVLTIGPANIFPV-FNVVQNAKL    2028
EBOV_L      LSDTEGMLWLNDNLAPFFATGYLIKPITSSARSSEWYL-CLTNFLSTTRK----MPHQNH    2019
                        :          :    :   . * *:                :   :

NiV_L       -VLEHSNLHDEVNDQGITSVIFKIKNSQSKQFHDDLK---------KYYQIDQPFFV-PT    2080
NDV_L       LIQRHGTLLAKSDETALMA-LF---TSQKQRVDNILS-------------SPLPRLA-KL    2021
RSV_L       ILSRTKNFI-------MP------KKADKESIDANIK-------------SLIPFLCYPI    2062
EBOV_L      LS---------CKQVILTALQLQIQR--SPYWLSHLTQYADCDLHLSYIRLGFPSLE-KV    2067
                           :          .     :.              * :

NiV_L       KITSDEQVLLQAGLKLNGPEILKS---------EISYDIGSDINTLRDTIIIMLNEAMNY    2131
NDV_L       LRRNIDTALIEAGGQPVRPFCAESLVNTLSDITQTTQVIASHIDTVIRSVIYMEAE----    2077
RSV_L       TKKGINTALSKLKSVVSGDILSYSIAGRN-EVFS-----NKLINHKHMNILKWFNHVLNF    2116
EBOV_L      LYHRYNLVDSK-----RGPLVS---------V-------TQHLAHLRAEIRELTND-YNQ    2105
                 : .  :                              . :     :     .
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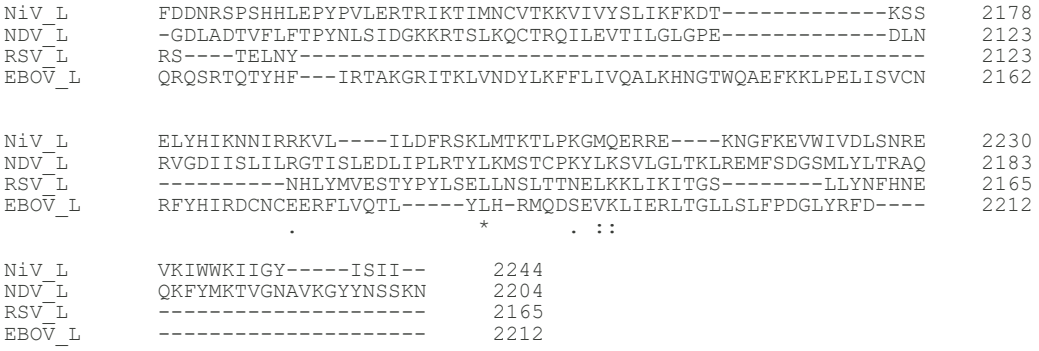


Figure S11 | Aligment of NiV, NDV, RSV, and EBOV L protein sequences.

Hydrophobic or aromatic residues contacted by P1 (yellow), P2 (cyan), P3 (magenta) and P4 (blue) monomers of the P tetramer are shade according to the P monomer they contacted with. Residues contacted by two P monomers are indicated by both shades and colored letters. Residues contacted by three P monomers are indicated by shades, colored letters, and underlines.

Total BSA = 3537 Å^2
>NiV_P1 (BSA = 1743 Å^2)
MDKLELVNDGLNIIDFIQKNQKEIQKTYGRSSIQQPSIKDQTKAWEDFLQCTSGESEQVEGG
MSKDDGDVERRNLEDLSSTSPTDGTIGKRVSNTRDWAEGSDDIQLDPVVTDVVYHDHGGECT
GYGFTSSPERGWSDYTSGANNGNVCLVSDAKMLSYAPEIAVSKEDRETDLVHLENKLSTTGL
NPTAVPFTLRNLSDPAKDSPVIAEHYYGLGVKEQNVGPQTSRNVNLDSIKLYTSDDEEADQL
EFEDEFAGSSSEVIVGISPEDEEPSSVGGKPNESIGRTIEGQSIRDNLQAKDNKSTDVPGAG
PKDSAVKEEPPQKRLPMLAEEFECSGSEDPIIRELLKENSLINCQQGKDAQPPYHWSIERSI
SPDKTEIVNGAVQTADRQRPGTPMPKSRGIPIKKGTDAKYPSAGTENVPGSKSGATRHVRGS
PPYQEGKSVNAENVQLNASTAVKETDKSEVNPVDDNDSLDDKYIMPSDDFSNTFFPHDTDRL
NYHADHLGDYDLETLCEESVLMGVINSIKLINLDMRLNHIEEQVKEIPKIINKLESIDRVLA
KTNTALSTIEGHLVSMMIMIPGKGKGERKGKNNPELKPVIGRDILEQQSLFSFDNVKNFRDG
SLTNEPYGAAVQLREDLILPELNFEETNASQFVPMADDSSRDVIKTLIRTHIKDRELRSELI
GYLNKAENDEEIQEIANTVNDIIDGNI
>NiV_P2
MDKLELVNDGLNIIDFIQKNQKEIQKTYGRSSIQQPSIKDQTKAWEDFLQCTSGESEQVEGG
MSKDDGDVERRNLEDLSSTSPTDGTIGKRVSNTRDWAEGSDDIQLDPVVTDVVYHDHGGECT
GYGFTSSPERGWSDYTSGANNGNVCLVSDAKMLSYAPEIAVSKEDRETDLVHLENKLSTTGL
NPTAVPFTLRNLSDPAKDSPVIAEHYYGLGVKEQNVGPQTSRNVNLDSIKLYTSDDEEADQL
EFEDEFAGSSSEVIVGISPEDEEPSSVGGKPNESIGRTIEGQSIRDNLQAKDNKSTDVPGAG
PKDSAVKEEPPQKRLPMLAEEFECSGSEDPIIRELLKENSLINCQQGKDAQPPYHWSIERSI
SPDKTEIVNGAVQTADRQRPGTPMPKSRGIPIKKGTDAKYPSAGTENVPGSKSGATRHVRGS
PPYQEGKSVNAENVQLNASTAVKETDKSEVNPVDDNDSLDDKYIMPSDDFSNTFFPHDTDRL
NYHADHLGDYDLETLCEESVLMGVINSIKLINLDMRLNHIEEQVKEIPKIINKLESIDRVLA
KTNTALSTIEGHLVSMMIMIPGKGKGERKGKNNPELKPVIGRDILEQQSLFSFDNVKNFRDG
SLTNEPYGAAVQLREDLILPELNFEETNASQFVPMADDSSRDVIKTLIRTHIKDRELRSELI
GYLNKAENDEEIQEIANTVNDIIDGNI
>NiV_P3 (BSA = 1176 Å^2)
MDKLELVNDGLNIIDFIQKNQKEIQKTYGRSSIQQPSIKDQTKAWEDFLQCTSGESEQVEGG
MSKDDGDVERRNLEDLSSTSPTDGTIGKRVSNTRDWAEGSDDIQLDPVVTDVVYHDHGGECT
GYGFTSSPERGWSDYTSGANNGNVCLVSDAKMLSYAPEIAVSKEDRETDLVHLENKLSTTGL
NPTAVPFTLRNLSDPAKDSPVIAEHYYGLGVKEQNVGPQTSRNVNLDSIKLYTSDDEEADQL
EFEDEFAGSSSEVIVGISPEDEEPSSVGGKPNESIGRTIEGQSIRDNLQAKDNKSTDVPGAG
PKDSAVKEEPPQKRLPMLAEEFECSGSEDPIIRELLKENSLINCQQGKDAQPPYHWSIERSI
SPDKTEIVNGAVQTADRQRPGTPMPKSRGIPIKKGTDAKYPSAGTENVPGSKSGATRHVRGS
PPYQEGKSVNAENVQLNASTAVKETDKSEVNPVDDNDSLDDKYIMPSDDFSNTFFPHDTDRL
NYHADHLGDYDLETLCEESVLMGVINSIKLINLDMRLNHIEEQVKEIPKIINKLESIDRVLA
KTNTALSTIEGHLVSMMIMIPGKGKGERKGKNNPELKPVIGRDILEQQSLFSFDNVKNFRDG
SLTNEPYGAAVQLREDLILPELNFEETNASQFVPMADDSSRDVIKTLIRTHIKDRELRSELI
GYLNKAENDEEIQEIANTVNDIIDGNI
>NiV_P4 (BSA = 617 Å^2)
MDKLELVNDGLNIIDFIQKNQKEIQKTYGRSSIQQPSIKDQTKAWEDFLQCTSGESEQVEGG
MSKDDGDVERRNLEDLSSTSPTDGTIGKRVSNTRDWAEGSDDIQLDPVVTDVVYHDHGGECT
GYGFTSSPERGWSDYTSGANNGNVCLVSDAKMLSYAPEIAVSKEDRETDLVHLENKLSTTGL
NPTAVPFTLRNLSDPAKDSPVIAEHYYGLGVKEQNVGPQTSRNVNLDSIKLYTSDDEEADQL
EFEDEFAGSSSEVIVGISPEDEEPSSVGGKPNESIGRTIEGQSIRDNLQAKDNKSTDVPGAG
PKDSAVKEEPPQKRLPMLAEEFECSGSEDPIIRELLKENSLINCQQGKDAQPPYHWSIERSI
SPDKTEIVNGAVQTADRQRPGTPMPKSRGIPIKKGTDAKYPSAGTENVPGSKSGATRHVRGS
PPYQEGKSVNAENVQLNASTAVKETDKSEVNPVDDNDSLDDKYIMPSDDFSNTFFPHDTDRL
NYHADHLGDYDLETLCEESVLMGVINSIKLINLDMRLNHIEEQVKEIPKIINKLESIDRVLA
KTNTALSTIEGHLVSMMIMIPGKGKGERKGKNNPELKPVIGRDILEQQSLFSFDNVKNFRDG
SLTNEPYGAAVQLREDLILPELNFEETNASQFVPMADDSSRDVIKTLIRTHIKDRELRSELI
GYLNKAENDEEIQEIANTVNDIIDGNI

Total BSA = 3575 Å^2
>NDV_P1 (BSA = 1303 Å^2)
MATFTDAEIDELFETSGTVIDSIITAQGKPVETVGRSAIPQGKTKALSLAWEKHGNTNTPAA
QESAGEQDQHGQNQASNSNRATPEEGPHSSQAQAATQPQEDANESQLKTGASSSLLSMLDKL
SNKSSNAKKGPPQSPPQQALHSKGSPAVEQTQHGANQGRAQQETGHQAAPSPGPPGTGVNIA
FPGQRGVSPQSVGATQPAPQSGQNQGSTPASADHVQPPVDFVQAMMSMMEAISQRVSKIDYQ
LDLVLKQTSSIPTMRSEIQQLKTSVAVMEANLGMMKILDPGCANVSSLSDLRAVAKSHPVLI
AGPGDPSPYVTQGGEIALNKLSQPVPHPSDLIKHATSGGPDIGIERDTVRALILSRPMHPSS
SSKLLSKLDSAGSVEEIRKIKRLALNG
>NDV_P2 (BSA = 366 Å^2)
MATFTDAEIDELFETSGTVIDSIITAQGKPVETVGRSAIPQGKTKALSLAWEKHGNTNTPAA
QESAGEQDQHGQNQASNSNRATPEEGPHSSQAQAATQPQEDANESQLKTGASSSLLSMLDKL
SNKSSNAKKGPPQSPPQQALHSKGSPAVEQTQHGANQGRAQQETGHQAAPSPGPPGTGVNIA
FPGQRGVSPQSVGATQPAPQSGQNQGSTPASADHVQPPVDFVQAMMSMMEAISQRVSKIDYQ
LDLVLKQTSSIPTMRSEIQQLKTSVAVMEANLGMMKILDPGCANVSSLSDLRAVAKSHPVLI
AGPGDPSPYVTQGGEIALNKLSQPVPHPSDLIKHATSGGPDIGIERDTVRALILSRPMHPSS
SSKLLSKLDSAGSVEEIRKIKRLALNG
>NDV_P3 (BSA = 983 Å^2)
MATFTDAEIDELFETSGTVIDSIITAQGKPVETVGRSAIPQGKTKALSLAWEKHGNTNTPAA
QESAGEQDQHGQNQASNSNRATPEEGPHSSQAQAATQPQEDANESQLKTGASSSLLSMLDKL
SNKSSNAKKGPPQSPPQQALHSKGSPAVEQTQHGANQGRAQQETGHQAAPSPGPPGTGVNIA
FPGQRGVSPQSVGATQPAPQSGQNQGSTPASADHVQPPVDFVQAMMSMMEAISQRVSKIDYQ
LDLVLKQTSSIPTMRSEIQQLKTSVAVMEANLGMMKILDPGCANVSSLSDLRAVAKSHPVLI
AGPGDPSPYVTQGGEIALNKLSQPVPHPSDLIKHATSGGPDIGIERDTVRALILSRPMHPSS
SSKLLSKLDSAGSVEEIRKIKRLALNG
>NDV_P4 (BSA = 923 Å^2)
MATFTDAEIDELFETSGTVIDSIITAQGKPVETVGRSAIPQGKTKALSLAWEKHGNTNTPAA
QESAGEQDQHGQNQASNSNRATPEEGPHSSQAQAATQPQEDANESQLKTGASSSLLSMLDKL
SNKSSNAKKGPPQSPPQQALHSKGSPAVEQTQHGANQGRAQQETGHQAAPSPGPPGTGVNIA
FPGQRGVSPQSVGATQPAPQSGQNQGSTPASADHVQPPVDFVQAMMSMMEAISQRVSKIDYQ
LDLVLKQTSSIPTMRSEIQQLKTSVAVMEANLGMMKILDPGCANVSSLSDLRAVAKSHPVLI
AGPGDPSPYVTQGGEIALNKLSQPVPHPSDLIKHATSGGPDIGIERDTVRALILSRPMHPSS
SSKLLSKLDSAGSVEEIRKIKRLALNG

Total BSA = 4583 Å^2
>RSV_P1 (BSA = 427 Å^2)
MEKFAPEFHGEDANNRATKFLESIKGKFTSPKDPKKKDSIISVNSIDIEVTKESPITSNSTI
INPTNETDDTAGNKPNYQRKPLVSFKEDPTPSDNPFSKLYKETIETFDNNEEESSYSYEEIN
DQTNDNITARLDRIDEKLSEILGMLHTLVVASAGPTSARDGIRDAMIGLREEMIEKIRTEAL
MTNDRLEAMARLRNEESEKMAKDTSDEVSLNPTSEKLNNLLEGNDSDNDLSLEDFKGENKYF
QG
>RSV_P2 (BSA = 187 Å^2)
MEKFAPEFHGEDANNRATKFLESIKGKFTSPKDPKKKDSIISVNSIDIEVTKESPITSNSTI
INPTNETDDTAGNKPNYQRKPLVSFKEDPTPSDNPFSKLYKETIETFDNNEEESSYSYEEIN
DQTNDNITARLDRIDEKLSEILGMLHTLVVASAGPTSARDGIRDAMIGLREEMIEKIRTEAL
MTNDRLEAMARLRNEESEKMAKDTSDEVSLNPTSEKLNNLLEGNDSDNDLSLEDFKGENKYF
QG
>RSV_P3 (BSA = 1526 Å^2)
MEKFAPEFHGEDANNRATKFLESIKGKFTSPKDPKKKDSIISVNSIDIEVTKESPITSNSTI
INPTNETDDTAGNKPNYQRKPLVSFKEDPTPSDNPFSKLYKETIETFDNNEEESSYSYEEIN
DQTNDNITARLDRIDEKLSEILGMLHTLVVASAGPTSARDGIRDAMIGLREEMIEKIRTEAL
MTNDRLEAMARLRNEESEKMAKDTSDEVSLNPTSEKLNNLLEGNDSDNDLSLEDFKGENKYF
QG
>RSV_P4 (BSA = 2433 Å^2)
MEKFAPEFHGEDANNRATKFLESIKGKFTSPKDPKKKDSIISVNSIDIEVTKESPITSNSTI
INPTNETDDTAGNKPNYQRKPLVSFKEDPTPSDNPFSKLYKETIETFDNNEEESSYSYEEIN
DQTNDNITARLDRIDEKLSEILGMLHTLVVASAGPTSARDGIRDAMIGLREEMIEKIRTEAL
MTNDRLEAMARLRNEESEKMAKDTSDEVSLNPTSEKLNNLLEGNDSDNDLSLEDFKGENKYF
QG

Total BSA = 3348 Å^2
>EBOV_P1
MTTRTKGRGHTAATTQNDRMPGPELSGWISEQLMTGRIPVSDIFCDIENNPGLCYASQMQQT
KPNPKTRNSQTQTDPICNHSFEEVVQTLASLATVVQQQTIASESLEQRITSLENGLKPVYDM
AKTISSLNRVCAEMVAKYDLLVMTTGRATATAAATEAYWAEHGQPPPGPSLYEESAIRGKIE
SRDETVPQSVREAFNNLNSTTSLTEENFGKPDISAKDLRNIMYDHLPGFGTAFHQLVQVICK
LGKDSNSLDIIHAEFQASLAEGDSPQCALIQITKRVPIFQDAAPPVIHIRSRGDIPRACQKS
LRPVPPSPKIDRGWVCVFQLQDGKTLGLKI
>EBOV_P2 (BSA = 161 Å^2)
MTTRTKGRGHTAATTQNDRMPGPELSGWISEQLMTGRIPVSDIFCDIENNPGLCYASQMQQT
KPNPKTRNSQTQTDPICNHSFEEVVQTLASLATVVQQQTIASESLEQRITSLENGLKPVYDM
AKTISSLNRVCAEMVAKYDLLVMTTGRATATAAATEAYWAEHGQPPPGPSLYEESAIRGKIE
SRDETVPQSVREAFNNLNSTTSLTEENFGKPDISAKDLRNIMYDHLPGFGTAFHQLVQVICK
LGKDSNSLDIIHAEFQASLAEGDSPQCALIQITKRVPIFQDAAPPVIHIRSRGDIPRACQKS
LRPVPPSPKIDRGWVCVFQLQDGKTLGLKI
>EBOV_P3 (BSA = 2093 Å^2)
MTTRTKGRGHTAATTQNDRMPGPELSGWISEQLMTGRIPVSDIFCDIENNPGLCYASQMQQT
KPNPKTRNSQTQTDPICNHSFEEVVQTLASLATVVQQQTIASESLEQRITSLENGLKPVYDM
AKTISSLNRVCAEMVAKYDLLVMTTGRATATAAATEAYWAEHGQPPPGPSLYEESAIRGKIE
SRDETVPQSVREAFNNLNSTTSLTEENFGKPDISAKDLRNIMYDHLPGFGTAFHQLVQVICK
LGKDSNSLDIIHAEFQASLAEGDSPQCALIQITKRVPIFQDAAPPVIHIRSRGDIPRACQKS
LRPVPPSPKIDRGWVCVFQLQDGKTLGLKI
>EBOV_P4 (BSA = 1094 Å^2)
MTTRTKGRGHTAATTQNDRMPGPELSGWISEQLMTGRIPVSDIFCDIENNPGLCYASQMQQT
KPNPKTRNSQTQTDPICNHSFEEVVQTLASLATVVQQQTIASESLEQRITSLENGLKPVYDM
AKTISSLNRVCAEMVAKYDLLVMTTGRATATAAATEAYWAEHGQPPPGPSLYEESAIRGKIE
SRDETVPQSVREAFNNLNSTTSLTEENFGKPDISAKDLRNIMYDHLPGFGTAFHQLVQVICK
LGKDSNSLDIIHAEFQASLAEGDSPQCALIQITKRVPIFQDAAPPVIHIRSRGDIPRACQKS
LRPVPPSPKIDRGWVCVFQLQDGKTLGLKI

Figure S12 | NiV, NDV, RSV, and EBOV P protein sequences and their L protein interacting residues.

Hydrophobic residues in P1 (yellow), P2 (cyan), P3 (magenta) and P4 (blue) monomers of the P teramers contacting the L proteins are colored.

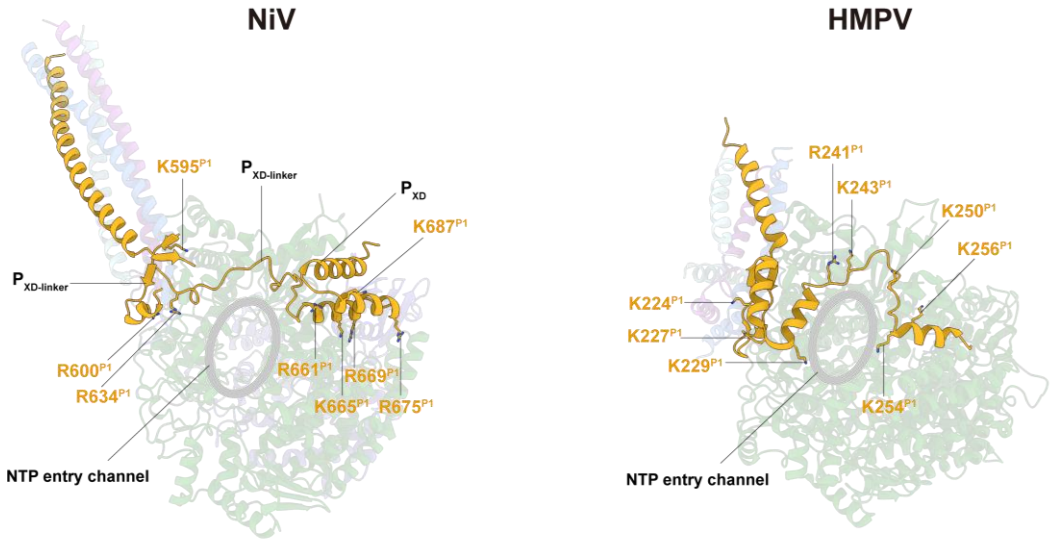


Figure S13 | The NiV $P1_{XD}$ domain and $P1_{XD}$ linker may play a potential role in NTP entry.

For HMPV, basic residues ($K224^{P1}$, $K227^{P1}$, $K229^{P1}$, $R241^{P1}$, $K243^{P1}$, $K250^{P1}$, $K254^{P1}$ and $K256^{P1}$) in the C-terminal region of subunit P1, located around the polymerase NTP entry tunnel, form a positively charged arch that may attract NTPs to the NTP entry tunnel. Similarly, for NiV, we observe several basic amino acids ($K595^{P1}$, $R600^{P1}$, $R634^{P1}$, $R661^{P1}$, $K665^{P1}$, $R669^{P1}$, $R675^{P1}$ and $K687^{P1}$) present in the $P1_{XD}$ domain and $P1_{XD}$ linker.

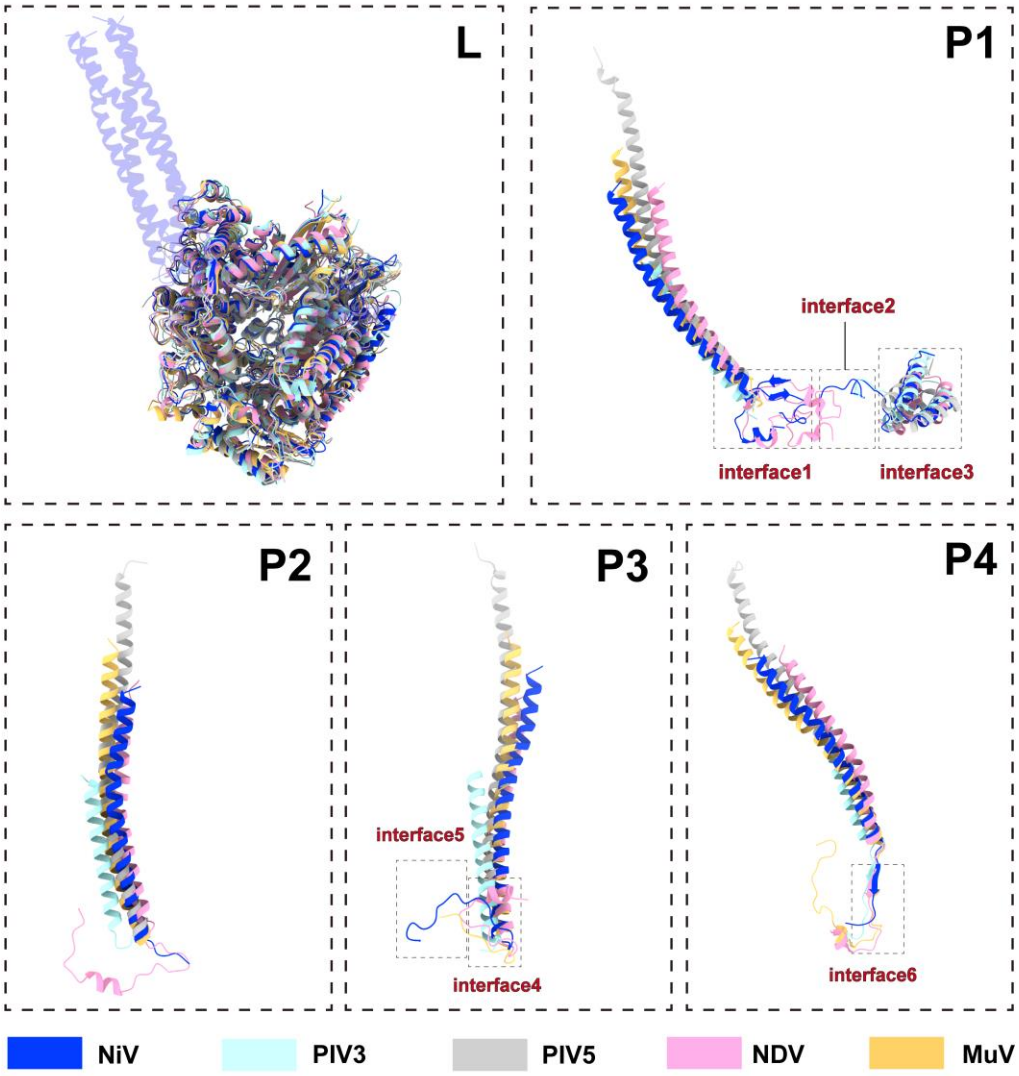


Figure S14 | Comparison of P protein strcutures among paramyxovirus L–P complexes.

Structural superposition of the paramyxoviruses L-P complexes reveals that P (P1, P2, P3 and P4) proteins from different viruses exhibit conformational dynamics on the surface of the L proteins (NiV: blue; PIV3: cyan, 8KDC; PIV5: gray, 6V85; NDV: pink, 7YOV; MuV: yellow, 8IZL).

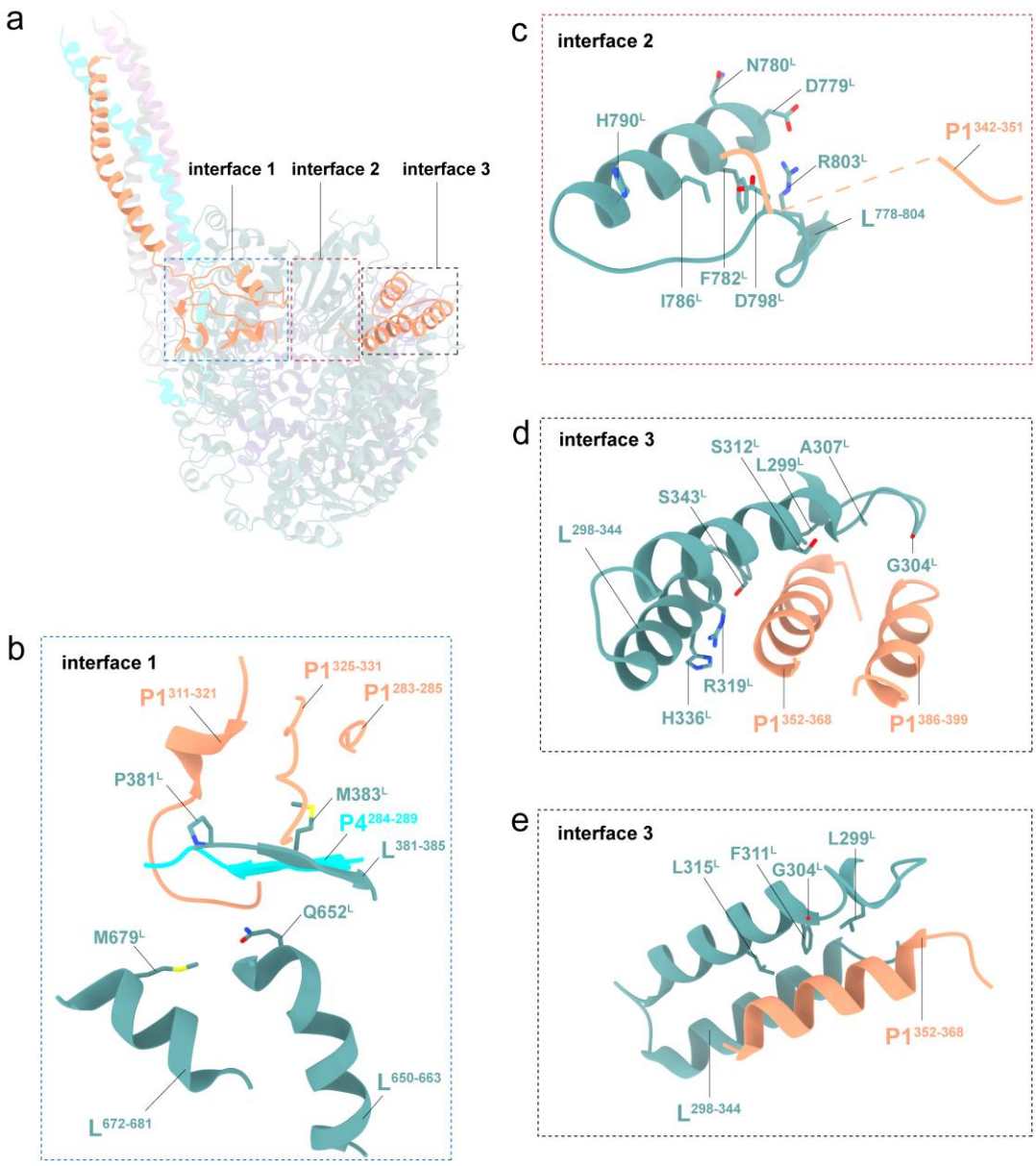


Figure S15 | Structural features of the L-P interactions in interfaces 1-3 of the NDV polymerase complex.

a. The overall structure of the NDV L-P complex (PDB: 7YOV). The locations of the interfaces 1-3, equivalent to those in the NiV L-P complex, are indicated by the dashed boxes. **b-e**. Strcurual details in the NDV L-P interfaces. The L residues in NDV, equivalent to those involved in the NiV

L-P interaction, are shown as sticks and labeled. These residues exhibit substantial variations when compared to those in NiV (see **Fig. 4**).

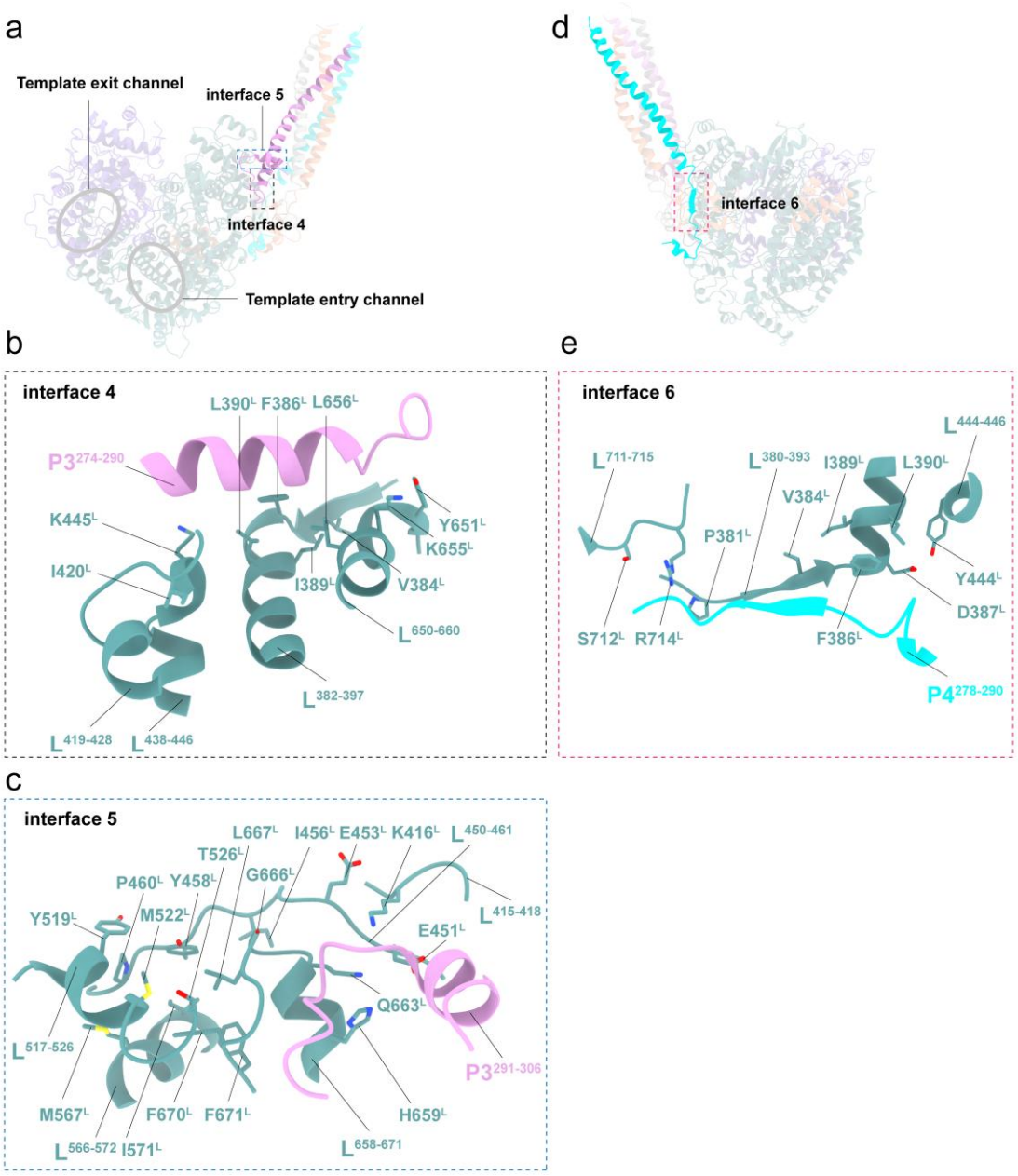


Figure S16 | Structural features of the L-P interactions in interfaces 4-6 of the NDV polymerase complex.

a. The overall structure of the NDV L-P complex (PDB: 7YOV). The locations of the interfaces 4 and 5, equivalent to those in the NiV L-P complex, are indicated by the dashed boxes. **d.** The overall structure of the

NDV L-P complex (PDB: 7YOV) in a different view. The location of the interfaces 6 is indicated by the dashed box. **b-c and e.** Strcurual details in the NDV L-P interfaces. The L residues in NDV, equivalent to those involved in the NiV L-P interaction, are shown as sticks and labeled. These residues exhibit substantial variations when compared to those in NiV (see **Fig. 5**).

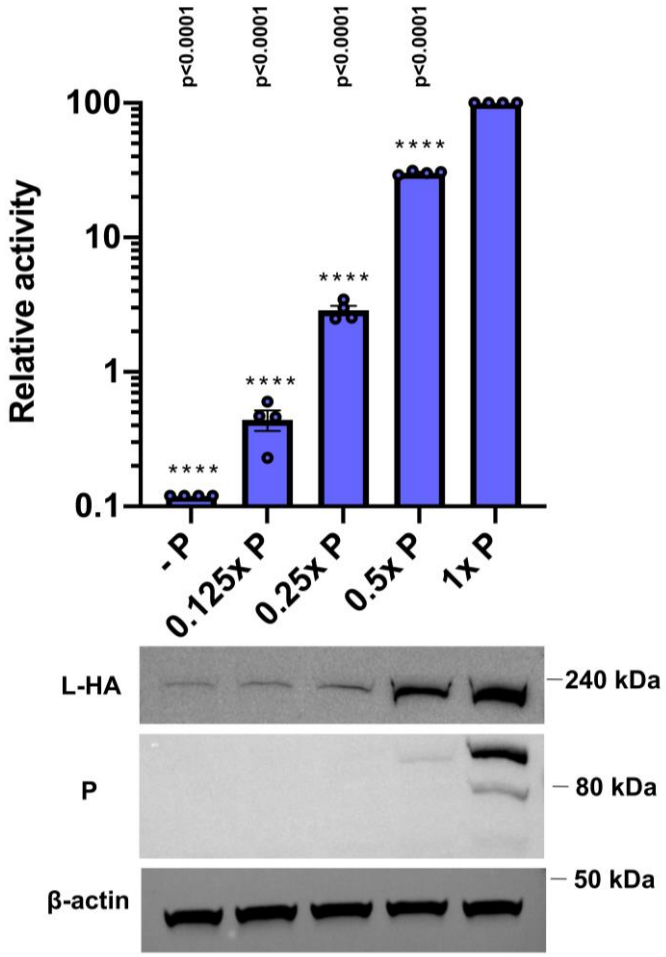
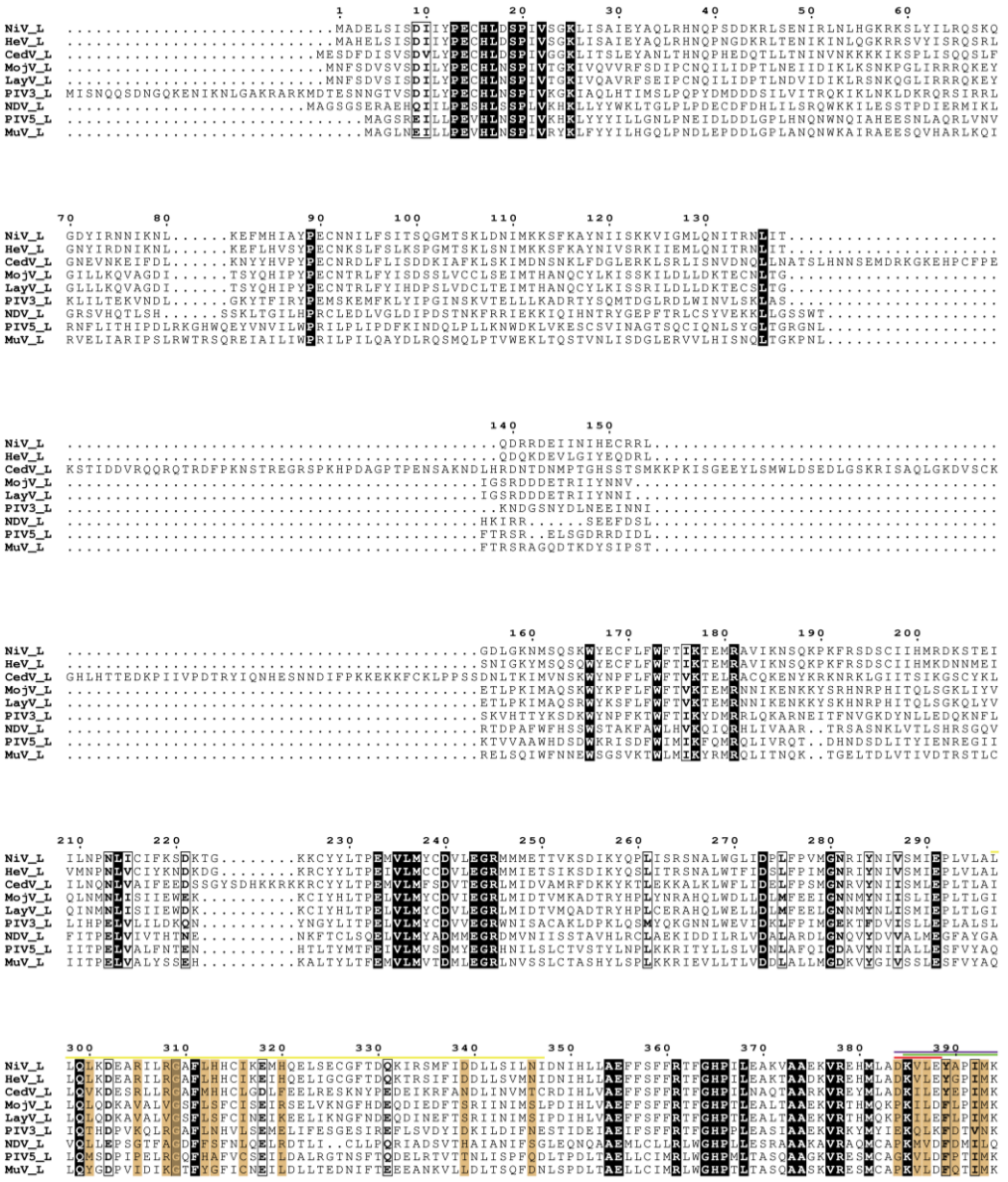
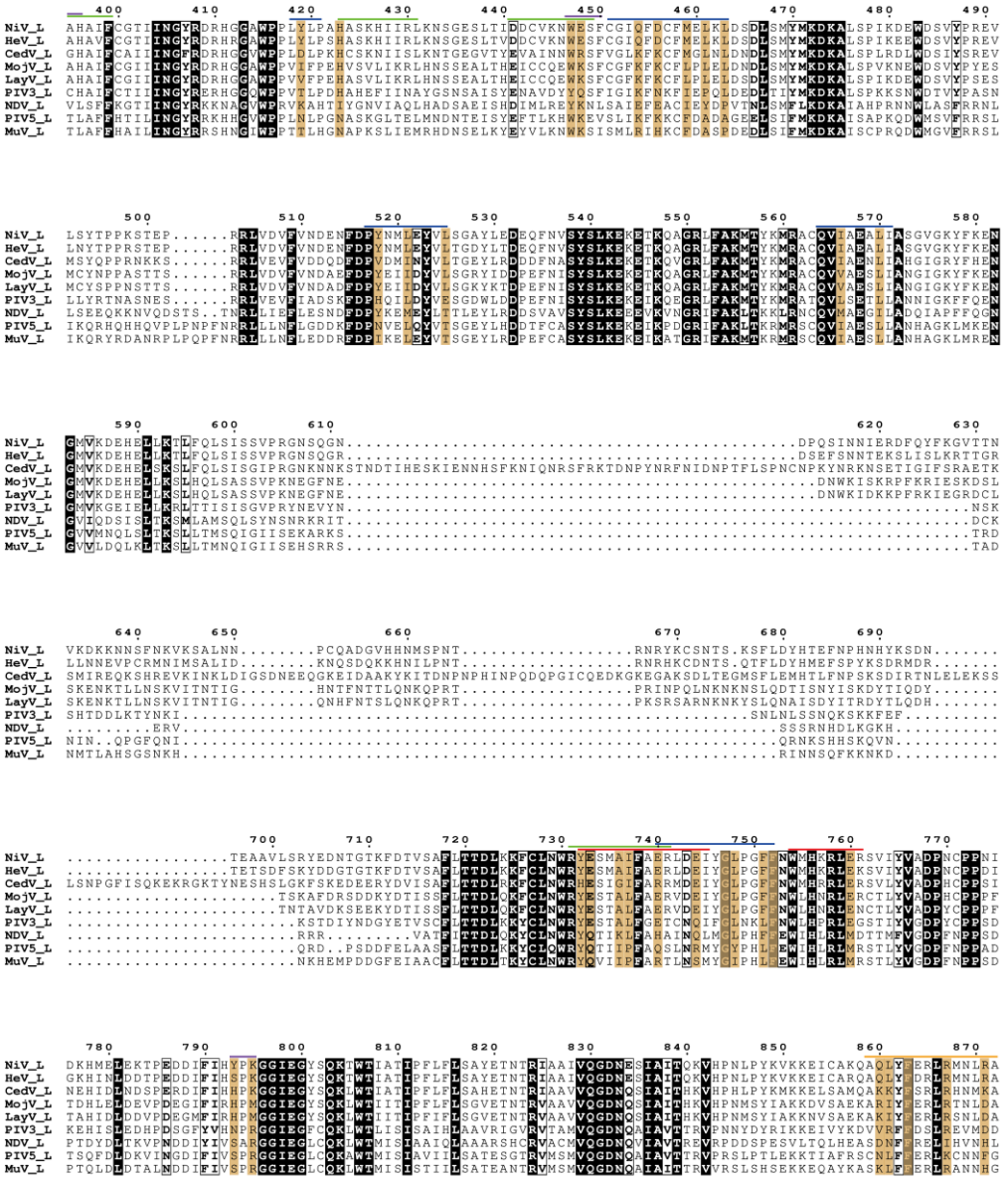


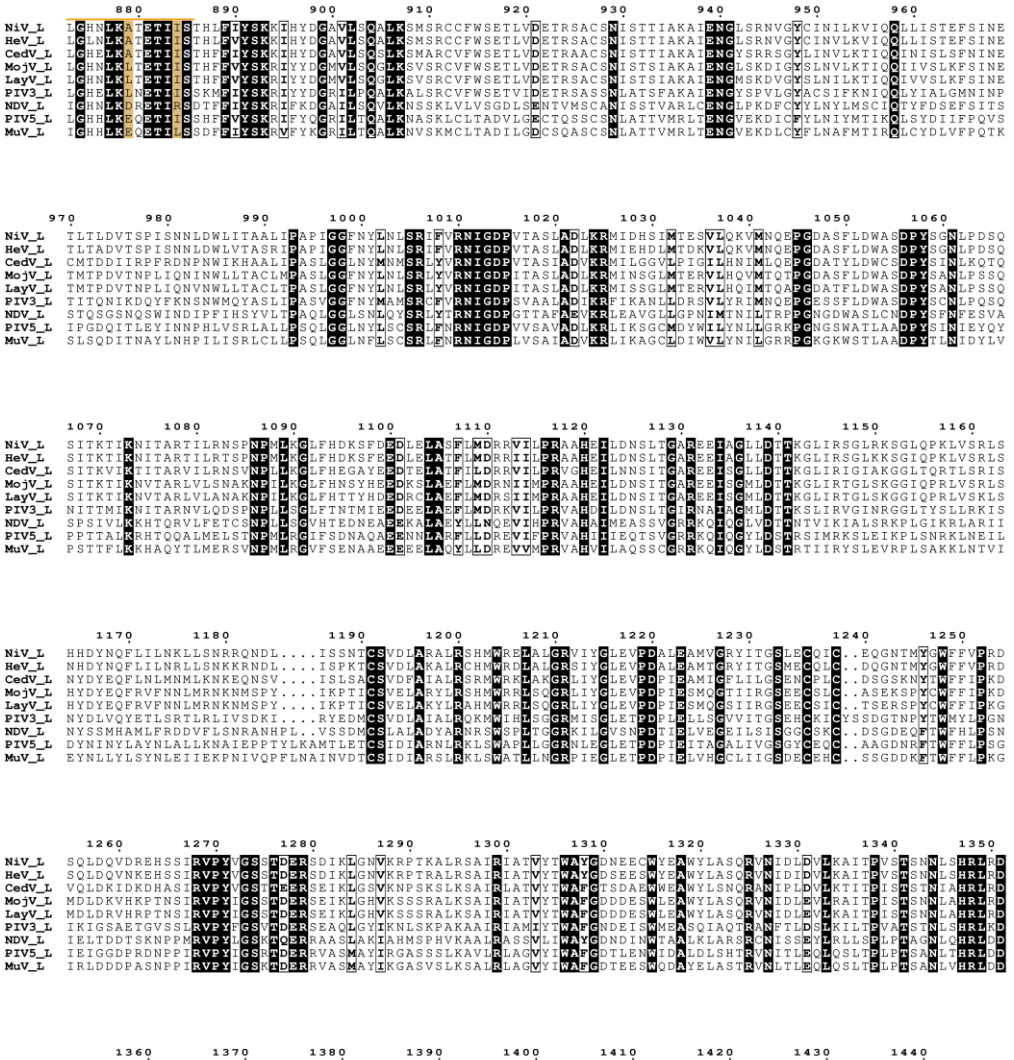
Figure S17 | P protein expression titration experiment showing P protein expression dependent L protein expression and mini-replicon activity.

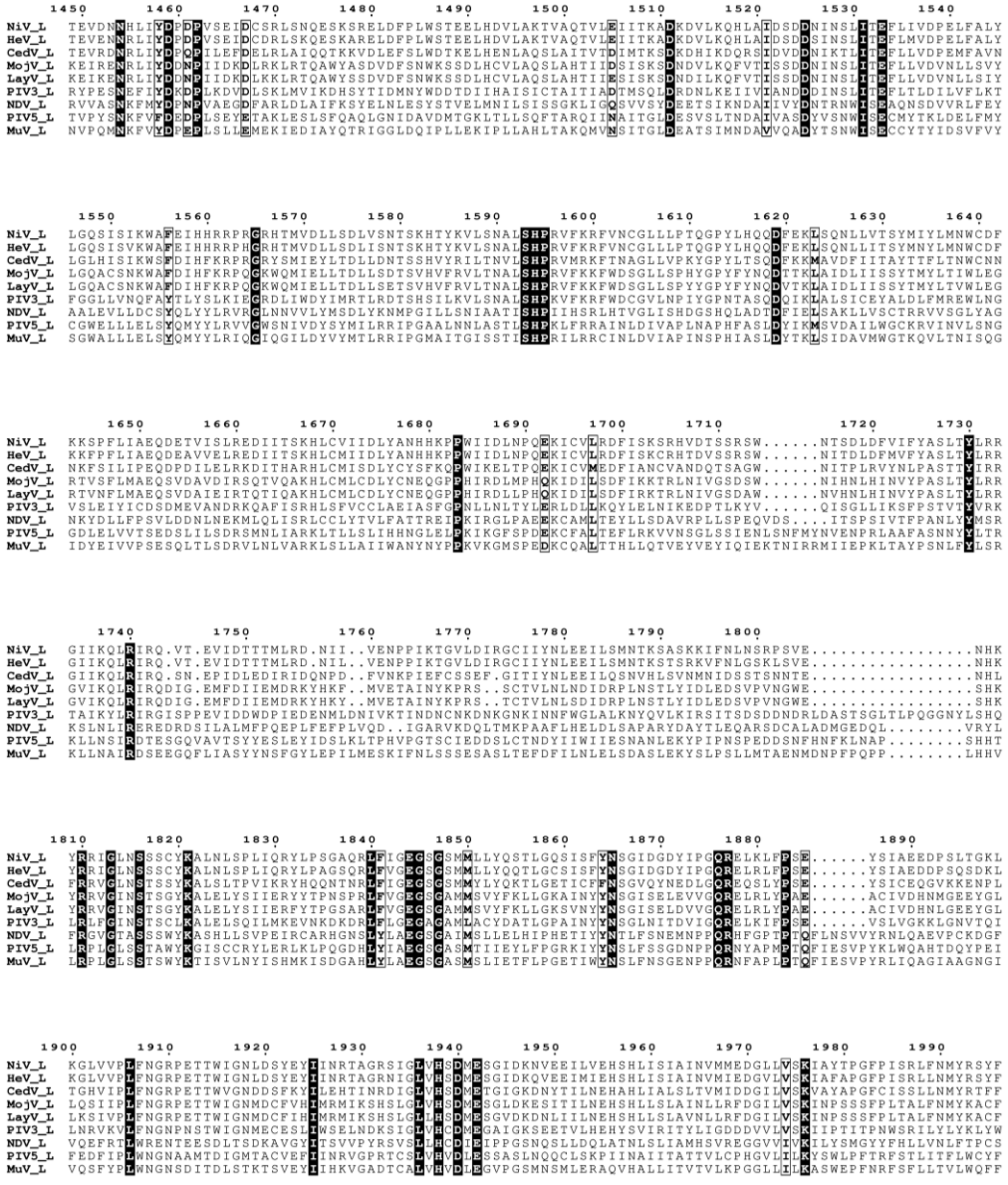
1×P, 0.5×P, 0.25×P, 0.125×P and -P lanes represent protein expression levels in mini-replicon experiments, where 0.5μg, 0.25 μg, 0.125 μg, 0.0625 μg and 0 μg of P protein expression plasmid were transfected. Corresponding mini-replicon activities are reported as mean ± SEM from four independent experiments (n = 4). All statistics used one-way ANOVA

Dunnett's multiple comparisons test. (ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).









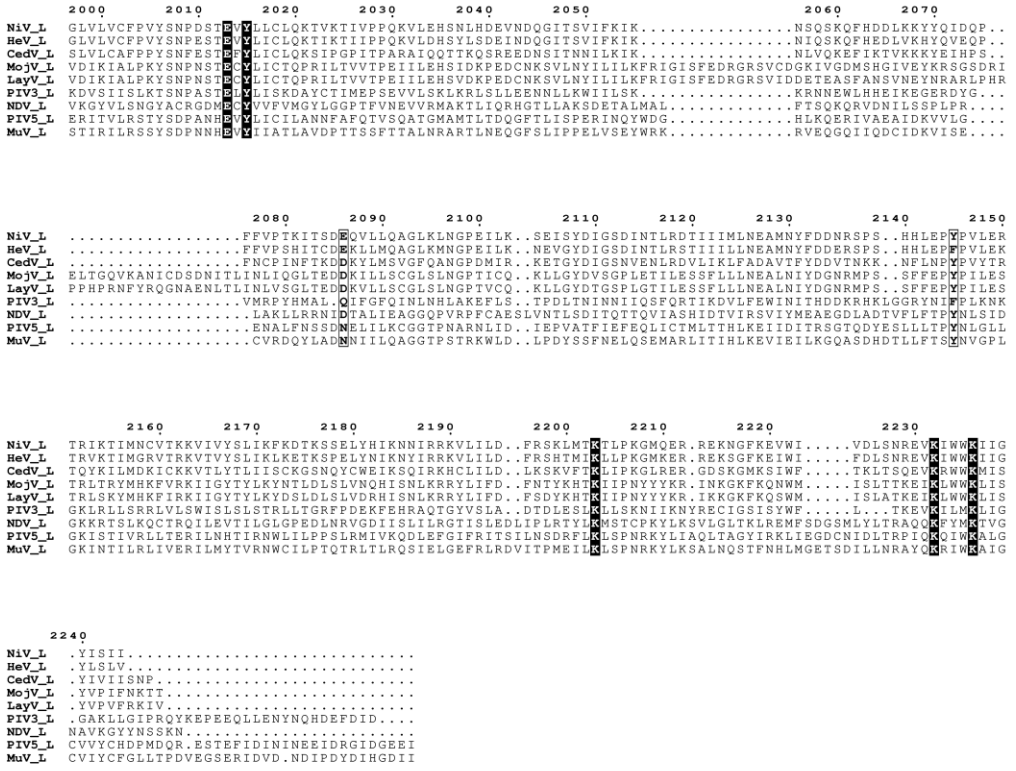
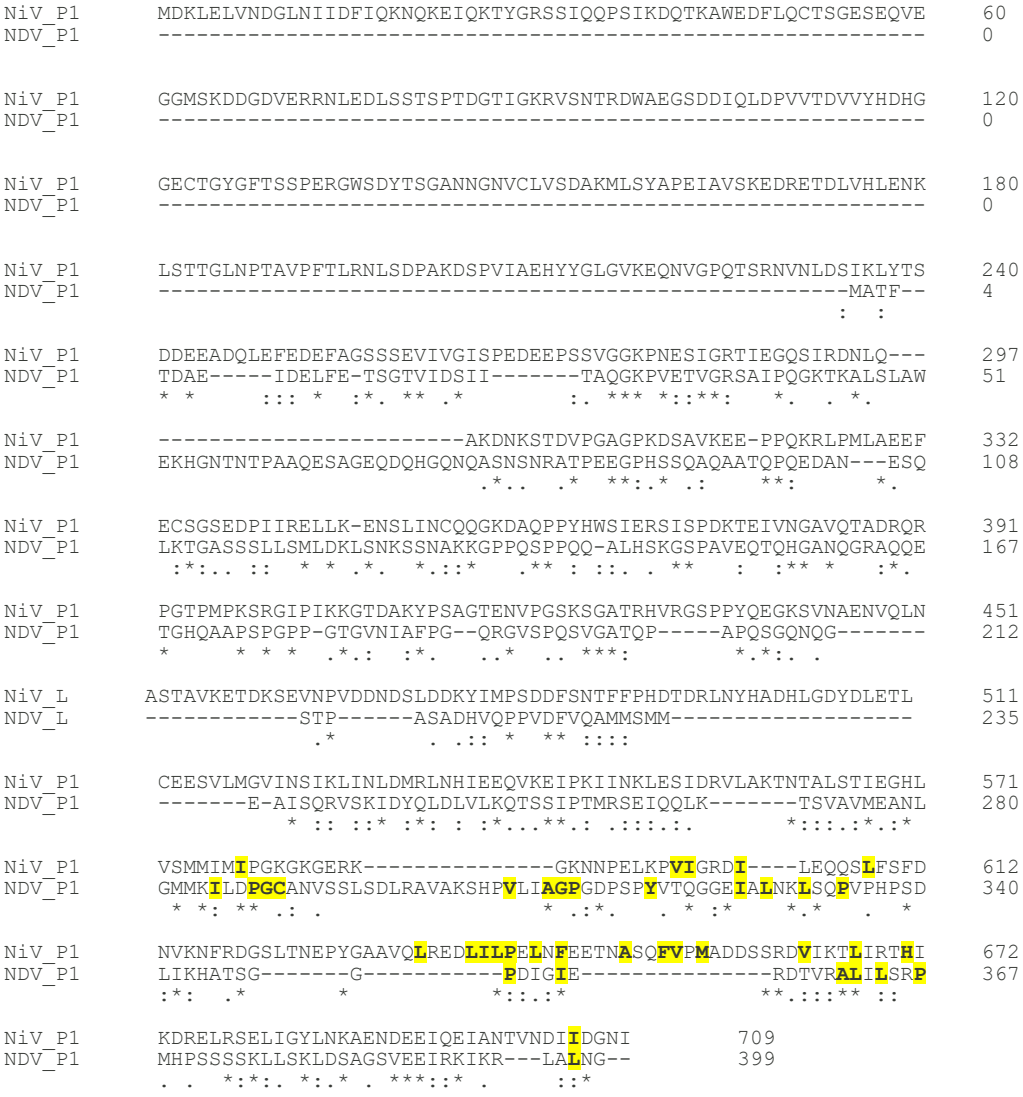


Figure S18 | Multiple sequence alignment of the L proteins from different paramyxovirus.

Residues in the NiV L protein interacting with the P proteins were identified. These residues and their equivalent residues in L proteins of the other paramyxoviruses are highlighted in the brown boxes. The NiV L sequences involved in interfaces 1, 2, 3, 4, 5 and 6 are indicated by red, orange, yellow, green, blue and purple overlines, respectively. Highly conserved amino acid residues are shaded black. L protein sequences used are NiV (Nipah virus), HeV (Hendra virus), CedV (Cedar virus), MojV

(Mojiang virus), LayV (Langya virus), PIV3 (Parainfluenza virus type 3), NDV (Newcastle disease virus), PIV5 (Parainfluenza virus 5), MuV (Mumps virus), EBOV (Ebola virus), RSV (Respiratory syncytial virus), HMPV (Human metapneumovirus), VSV (Vesicular stomatitis virus) and RABV (Rabies virus).


NiV_P1 MDKLELVNDGLNIIDFIQKNQKEIQKTYGRSSIQQPSIKDQTKAWEDFLQCTSGESEQVE 60
NDV_P1 ---------- 0
NiV_P1 GGMSKDDGDVERRNLEDLSSTSPTDGTIGKRVSNTRDWAEGSDDIQLDPVVTDVVYHDHG 120
NDV_P1 ---------- 0
NiV_P1 GECTGYGFTSSPERGWSDYTSGANNGNVCLVSDAKMLSYAPEIAVSKEDRETDLVHLENK 180
NDV_P1 ---------- 0
NiV_P1 LSTTGLNPTAVPFTLRNLSDPAKDSPVIAEHYYGLGVKEQNVGPQTSRNVNLDSIKLYTS 240
NDV_P1 ----------MATF-- 4
NiV_P1 DDEEADQLEFEDEFAGSSSEVIVGISPEDEEPSSVGGKPNESIGRTIEGQSIRDNLQ--- 297
NDV_P1 TDAE-----IDELFE-TSGTVIDSII-------TAQGKPVETVGRSAIPQGKTKALSLAW 51
NiV_P1 ----------AKDNKSTDVPGAGPKDSAVKEE-PPQKRLPMLAEEF 332
NDV_P1 EKHGNTNTPAAQESAGEQDQHGQNQASNSNRATPEEGPHSSQAQAATQPQEDAN---ESQ 108
NiV_P1 ECSGSEDPIIRELLK-ENSLINCQQGKDAQPPYHWSIERSISPDKTEIVNGAVQTADRQR 391
NDV_P1 LKTGASSSLLSMLDKLSNKSSNAKKGPPQSPPQQ-ALHSKGSPAVEQTQHGANQGRAQQE 167
NiV_P1 PGTPMPKSRGIPIKKGTDAKYPSAGTENVPGSKSGATRHVRGSPPYQEGKSVNAENVQLN 451
NDV_P1 TGHQAAPSPGPP-GTGVNIAFPG--QRGVSPQSVGATQP-----APQSGQNQG------- 212
NiV_L ASTAVKETDKSEVNPVDDNDSLDDKYIMPSDDFSNTFFPHDTDRLNYHADHLGDYDLETL 511
NDV_L ----------STP------ASADHVQPPVDFVQAMMSMM---------- 235
NiV_P1 CEESVLMGVINSIKLINLDMRLNHIEEQVKEIPKIINKLESIDRVLAKTNTALSTIEGHL 571
NDV_P1 -------E-AISQRVSKIDYQLDLVLKQTSSIPTMRSEIQQLK-------TSVAVMEANL 280
NiV_P1 VSMMIMIPGKGKGERK---------------GKNNPELKPVIGRDI----LEQQSLFSFD 612
NDV_P1 GMMKILDPGCANVSSLSDLRAVAKSHPVLIAGPGDPSPYVTQGGEIALNKLSQPVPHPSD 340
NiV_P1 NVKNFRDGSLTNEPYGAAVQLREDLILPELNFEETNASQFVPMADDSSRDVIKTLIRTHI 672
NDV_P1 LIKHATSG-------G-----------PDIGIE---------------RDTVRALILSRP 367
NiV_P1 KDRELRSELIGYLNKAENDEEIQEIANTVNDIIDGNI 709
NDV_P1 MHPSSSSKLLSKLDSAGSVEEIRKIKR---LALNG-- 399

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NiV_P2      MDKLELVNDGLNIIDFIQKNQKEIQKTYGRSSIQQPSIKDQTKAWEDFLQCTSGESEQVE    60
NDV_P2      ------------------------------------------------------------    0

NiV_P2      GGMSKDDGDVERRNLEDLSSTSPTDGTIGKRVSNTRDWAEGSDDIQLDPVVTDVVYHDHG    120
NDV_P2      ------------------------------------------------------------    0

NiV_P2      GECTGYGFTSSPERGWSDYTSGANNGNVCLVSDAKMLSYAPEIAVSKEDRETDLVHLENK    180
NDV_P2      ------------------------------------------------------------    0

NiV_P2      LSTTGLNPTAVPFTLRNLSDPAKDSPVIAEHYYGLGVKEQNVGPQTSRNVNLDSIKLYTS    240
NDV_P2      ------------------------------------------------------MATF--    4
                                                                   :  :

NiV_P2      DDEEADQLEFEDEFAGSSSEVIVGISPEDEEPSSVGGKPNESIGRTIEGQSIRDNLQ---    297
NDV_P2      TDAE-----IDELFE-TSGTVIDSII-------TAQGKPVETVGRSAIPQGKTKALSLAW    51
            * *     ::: *  :*. ** .*        :. *** *::**:   *.  . *.

NiV_P2      ------------------------AKDNKSTDVPGAGPKDSAVKEE-PPQKRLPMLAEEF    332
NDV_P2      EKHGNTNTPAAQESAGEQDQHGQNQASNSNRATPEEGPHSSQAQAATQPQEDAN---ESQ    108
                                  .*..  .*  **:.* .:    **:      *.

NiV_P2      ECSGSEDPIIRELLK-ENSLINCQQGKDAQPPYHWSIERSISPDKTEIVNGAVQTADRQR    391
NDV_P2      LKTGASSSLLSMLDKLSNKSSNAKKGPPQSPPQQ-ALHSKGSPAVEQTQHGANQGRAQQE    167
             :*:.. ::  * * .*.  *.::*   .** : ::. . **   :  :** *   :*.

NiV_P2      PGTPMPKSRGIPIKKGTDAKYPSAGTENVPGSKSGATRHVRGSPPYQEGKSVNAENVQLN    451
NDV_P2      TGHQAAPSPGPP-GTGVNIAFPG--QRGVSPQSVGATQP-----APQSGQNQG-------    212
            *     * * *  .*.:  :*.   ..*  .. ***:       *.*:. .

NiV_P2      ASTAVKETDKSEVNPVDDNDSLDDKYIMPSDDFSNTFFPHDTDRLNYHADHLGDYDLETL    511
NDV_P2      ------------STP------ASADHVQPPVDFVQAMMSMM-------------------    235
                        .*       . .:: *  ** ::::

NiV_P2      CEESVLMGVINSIKLINLDMRLNHIEEQVKEIPKIINKLESIDRVLAKTNTALSTIEGHL    571
NDV_P2      -------E-AISQRVSKIDYQLDLVLKQTSSIPTMRSEIQQLK-------TSVAVMEANL    280
                     * :: ::* :*: : :*...**.: .:::.:.       *:::.:*.:*

NiV_P2      VSMMIMIPGKGKGERK---------------GKNNPELKPVIGRDI----LEQQSLFSFD    612
NDV_P2      GMMKILDPGCANVSSLSDLRAVAKSHPVLIAGPGDPSPYVTQGGEIALNKLSQPVPHPSD    340
             * *: ** .: .                  * .:*.   . * :*    *.*   .  *

NiV_P2      NVKNFRDGSLTNEPYGAAVQLREDLILPELNFEETNASQFVPMADDSSRDVIKTLIRTHI    672
NDV_P2      LIKHATSG-------G-----------PDIGIE---------------RDTVRALILSRP    367
            :*:  .*       *           *::.:*               **.:::** ::

NiV_P2      KDRELRSELIGYLNKAENDEEIQEIANTVNDIIDGNI        709
NDV_P2      MHPSSSSKLLSKLDSAGSVEEIRKIKR---LALNG--        399
            . .  *:*:. *:.* . ***::* .     ::*
```

```
NiV_P3      MDKLELVNDGLNIIDFIQKNQKEIQKTYGRSSIQQPSIKDQTKAWEDFLQCTSGESEQVE     60
NDV_P3      ------------------------------------------------------------     0

NiV_P3      GGMSKDDGDVERRNLEDLSSTSPTDGTIGKRVSNTRDWAEGSDDIQLDPVVTDVVYHDHG    120
NDV_P3      ------------------------------------------------------------     0

NiV_P3      GECTGYGFTSSPERGWSDYTSGANNGNVCLVSDAKMLSYAPEIAVSKEDRETDLVHLENK    180
NDV_P3      ------------------------------------------------------------     0

NiV_P3      LSTTGLNPTAVPFTLRNLSDPAKDSPVIAEHYYGLGVKEQNVGPQTSRNVNLDSIKLYTS    240
NDV_P3      ------------------------------------------------------MATF--     4
                                                                     :  :

NiV_P3      DDEEADQLEFEDEFAGSSSEVIVGISPEDEEPSSVGGKPNESIGRTIEGQSIRDNLQ---    297
NDV_P3      TDAE-----IDELFE-TSGTVIDSII-------TAQGKPVETVGRSAIPQGKTKALSLAW     51
            * *     ::: *  :*. ** .*        :. *** *::**:   *.  . *.

NiV_P3      ------------------------AKDNKSTDVPGAGPKDSAVKEE-PPQKRLPMLAEEF    332
NDV_P3      EKHGNTNTPAAQESAGEQDQHGQNQASNSNRATPEEGPHSSQAQAATQPQEDAN---ESQ    108
                                  .*..  .*  **:.* .:    **:      *.

NiV_P3      ECSGSEDPIIRELLK-ENSLINCQQGKDAQPPYHWSIERSISPDKTEIVNGAVQTADRQR    391
NDV_P3      LKTGASSSLLSMLDKLSNKSSNAKKGPPQSPPQQ-ALHSKGSPAVEQTQHGANQGRAQQE    167
             :*:.. ::  * * .*.  *.::*   .** : ::. . **   :  :** *   :*.

NiV_P3      PGTPMPKSRGIPIKKGTDAKYPSAGTENVPGSKSGATRHVRGSPPYQEGKSVNAENVQLN    451
NDV_P3      TGHQAAPSPGPP-GTGVNIAFPG--QRGVSPQSVGATQP-----APQSGQNQG-------    212
            *     * * *  .*.:  :*.   ..*  .. ***:        *.*:. .

NiV_P3      ASTAVKETDKSEVNPVDDNDSLDDKYIMPSDDFSNTFFPHDTDRLNYHADHLGDYDLETL    511
NDV_P3      ------------STP------ASADHVQPPVDFVQAMMSMM-------------------    235
                        .*       . .:: *  ** ::::

NiV_P3      CEESVLMGVINSIKLINLDMRLNHIEEQVKEIPKIINKLESIDRVLAKTNTALSTIECHL    571
NDV_P3      -------E-AISQRVSKIDYQLDLVLKQTSSIPTMRSEIQQLK-------TSVAVMEANL    280
                     * :: ::* :*: : :*...**.: .:::.:.       *:::.:*.:*

NiV_P3      VSMMIMIPCKGKGERK---------------CKNNPELKPVIGRDI----LEQQSLFSFD    612
NDV_P3      GMMKTLDPGCANVSSLSDLRAVAKSHPVLIAGPGDPSPYVTQGGEIALNKLSQPVPHPSD    340
             * *: ** .: .                  * .:*.   . * :*    *.*   .  *

NiV_P3      NVKNFRDGSLTNEPYGAAVQLREDLILPELNFEETNASQFVPMADDSSRDVIKTLIRTHI    672
NDV_P3      LIKHATSG-------G-----------PDIGIE---------------RDTVRALILSRP    367
            :*:  .*       *           *::.:*               **.:::** ::

NiV_P3      KDRELRSELIGYLNKAENDEEIQEIANTVNDIIDGNI        709
NDV_P3      MHPSSSSKLLSKLDSAGSVEEIRKIKR---LALNG--        399
            . .  *:*:. *:.* . ***::* .     ::*
```

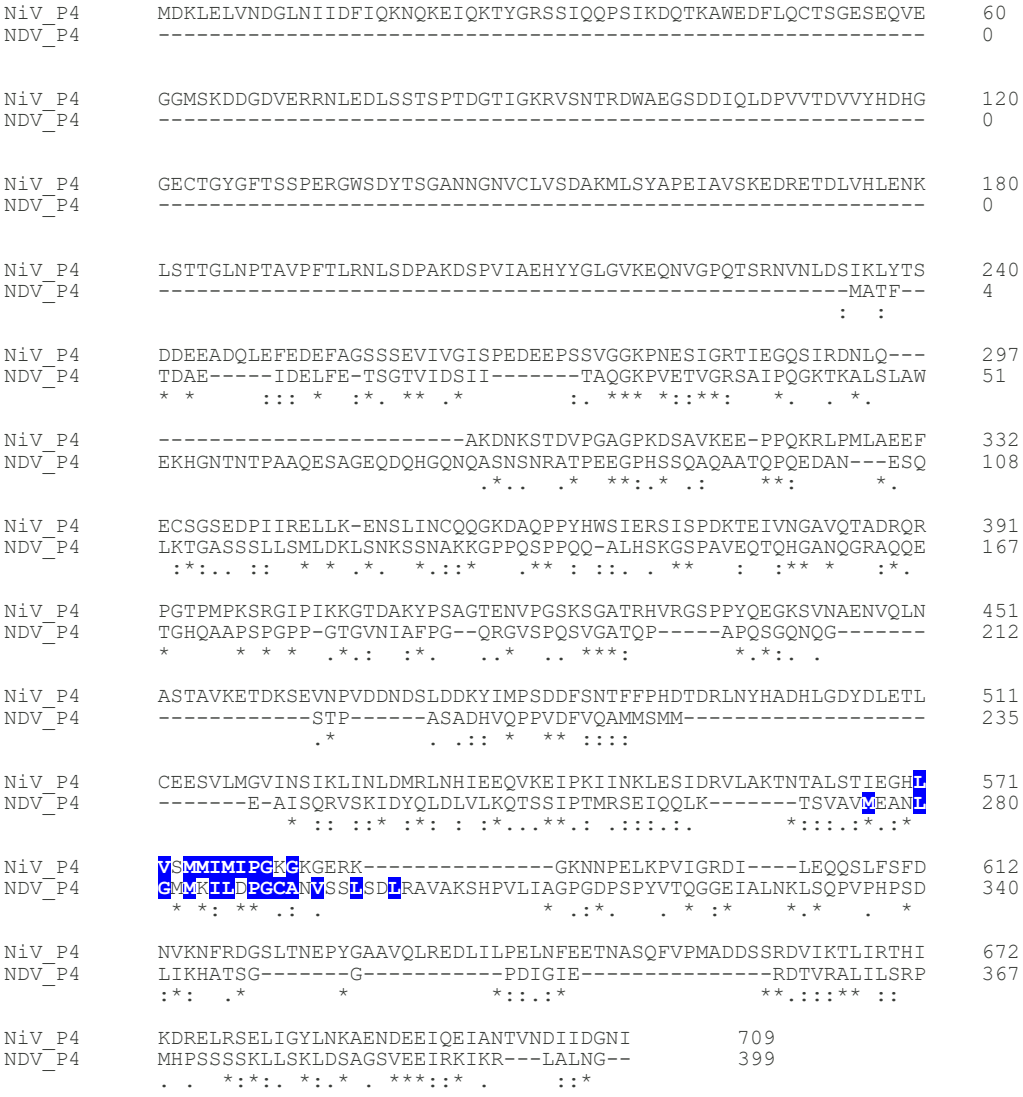

Figure S19 | Hydrophobic residues in P1 (yellow), P2 (cyan), P3 (magenta) and P4 (blue) monomers contacting the L proteins are compared between NiV and NDV.

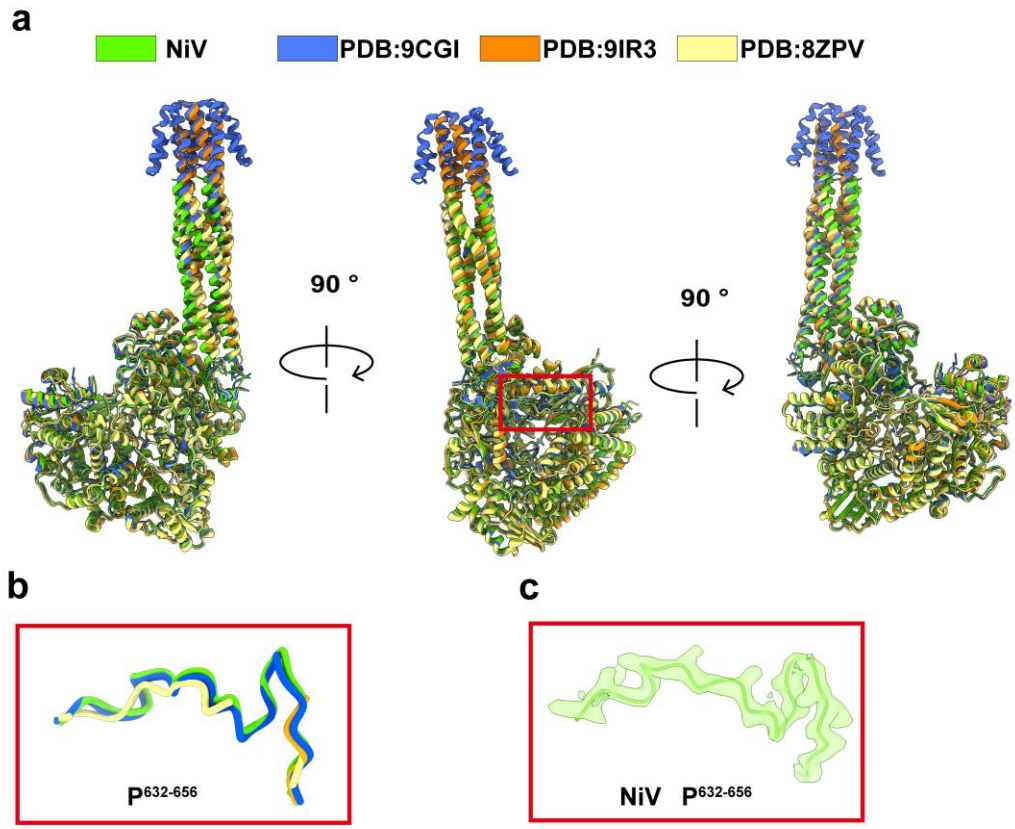

Figure S20 | A comparison of the reported NiV L-P complex structures. **a.** Three different views of our NiV $L_{1\text{-}1451}$-P complex structure superposed onto the released NiV L-P complex structures (9CGI, 9IR3 and 8ZPV). **b.** Close-up view of the modelled structures for the residues $P^{632\text{-}656}$. **c.** The cryo-EM density for the residues $P^{632\text{-}656}$ in the NiV $L_{1\text{-}1451}$-P complex structure.

Supplementary Table 1. Cryo-EM data collection, refinement and validation statistics.

	NiV $L_{1\text{-}1451}$-P complex	NiV L-P complex
Data collection and processing		
Magnification	165000	165000
Voltage (kV)	300	300
Electron exposure (e^{-}/Å^2)	50	50
Defocus range (μm)	0.6-2.4	0.6-2.4
Pixel size (Å)	0.73	0.73
Movies (no.)	16735	10073
Initial particle images (no.)	4390986	7475406
Symmetry imposed	*C1*	*C1*
Final particle images (no.)	674945	224424
Map resolution (Å)	2.31	2.52
FSC threshold	0.143	0.143
Map resolution range (Å)	2.30-37.23	2.15-38.17
Refinement		
Initial model used	PDB 6EB8	PDB 9IV9
Model resolution (Å)	2.56	2.87
FSC threshold	0.5	0.5
Map sharpening *B* factor (Å^2)	-88	-89
Model composition		
Non-hydrogen atoms	13048	12944
Protein residues	1624	1611
Ligands	2	2
B factors (Å^2)		
Protein	104.83	101.08
Ligand	146.19	124.50
R.m.s. deviations		
Bond lengths (Å)	0.004	0.004
Bond angles (°)	0.798	0.669
Validation		
MolProbity score	1.34	1.34
Clash score	3.69	3.45
Poor rotamers (%)	0	0
Ramachandran plot		
Favored (%)	96.94	96.73
Allowed (%)	3.06	3.27
Disallowed (%)	0	0

Supplementary Table 2. Interactions between L and P proteins.

Interface	Molecule	Sites	L	Interaction Pattern
Interface1	P1	I576^{P1} I578^{P1} L608^{P1} L633^{P1} L637^{P1} I638^{P1}	V386^{L}	Hydrophobic interactions
		R634^{P1}	D384^{L}	long-range electrostatic interaction
		R600^{P1}	E733^{L} E760^{L}	salt bridges
	P4	M575^{P4} M577^{P4} P579^{P4}	V386^{L}	Hydrophobic interactions
Interface2	P1	L639^{P1}#	R871^{L}	hydrogen bond
		P640^{P1}#	R867^{L}	hydrogen bond
		Q651^{P1}	Q860^{L}	hydrogen bond
		L642^{P1} F644^{P1}	L861^{L} F863^{L} A879^{L} I884^{L}	Hydrophobic interactions
Interface3	P1	D658^{P1}# D662^{P1}	H320^{L}	hydrogen bond salt bridge
		S660^{P1}	H313^{L}	hydrogen bond
		K665^{P1} R669^{P1}	D339^{L}	salt bridges
		T670^{P1}	N346^{L}	hydrogen bond
		H671^{P1}	L300^{L}#	hydrogen bond
		N702^{P1} D706^{P1}	R308^{L}	hydrogen bond salt bridge
		D706^{P1} D703^{P1}	R305^{L}	salt bridges
		F652^{P1} V663^{P1} L667^{P1}	L300^{L} G309^{L} L312^{L} I316^{L}	Hydrophobic interactions
interface4	P3	S565^{P3}	H423^{L}	hydrogen bond
		H570^{P3}	Y389^{L} E448^{L}	hydrogen bond salt bridge
		I576^{P3} M577^{P3} P579^{P3}	L387^{L} I392^{L} M393^{L} Y732^{L} A736^{L} I737^{L}	Hydrophobic interactions
Interface5	P3	K583^{P3}	E740^{L} E744^{L}	salt bridges
		E585^{P3}#	Q454^{L}	hydrogen bond
		K587^{P3}	Y419^{L}	cation-π interaction
		K589^{P3}#	D456^{L}	salt bridge
		N591^{P3}	M459^{L}# Y746^{L}#	hydrogen bonds

		$E593^{P3}$	$L461^{L}$# $Y518^{L}$	hydrogen bonds
		$L594^{P3}$	$L461^{L}$ $L463^{L}$ $Y518^{L}$ $L521^{L}$ $L525^{L}$ $I566^{L}$ $L570^{L}$ $L748^{L}$ $F751^{L}$ $F752^{L}$	Hydrophobic interactions
Interface6	P4	$G580^{P4}$#	$D384^{L}$	hydrogen bond
		$G582^{P4}$#	$K795^{L}$	hydrogen bond
		$K583^{P4}$	$Y793^{L}$	cation-π interaction
		$V572^{P4}$ $M574^{P4}$ $I576^{P4}$ $I578^{P4}$	$L387^{L}$ $Y389^{L}$ $A390^{L}$ $I392^{L}$ $M393^{L}$ $W447^{L}$	Hydrophobic interactions

The "#" symbol indicates the formation of hydrogen bonds with nitrogen or oxygen in the main chain.