

Structures of the mumps virus polymerase complex via cryo-electron microscopy

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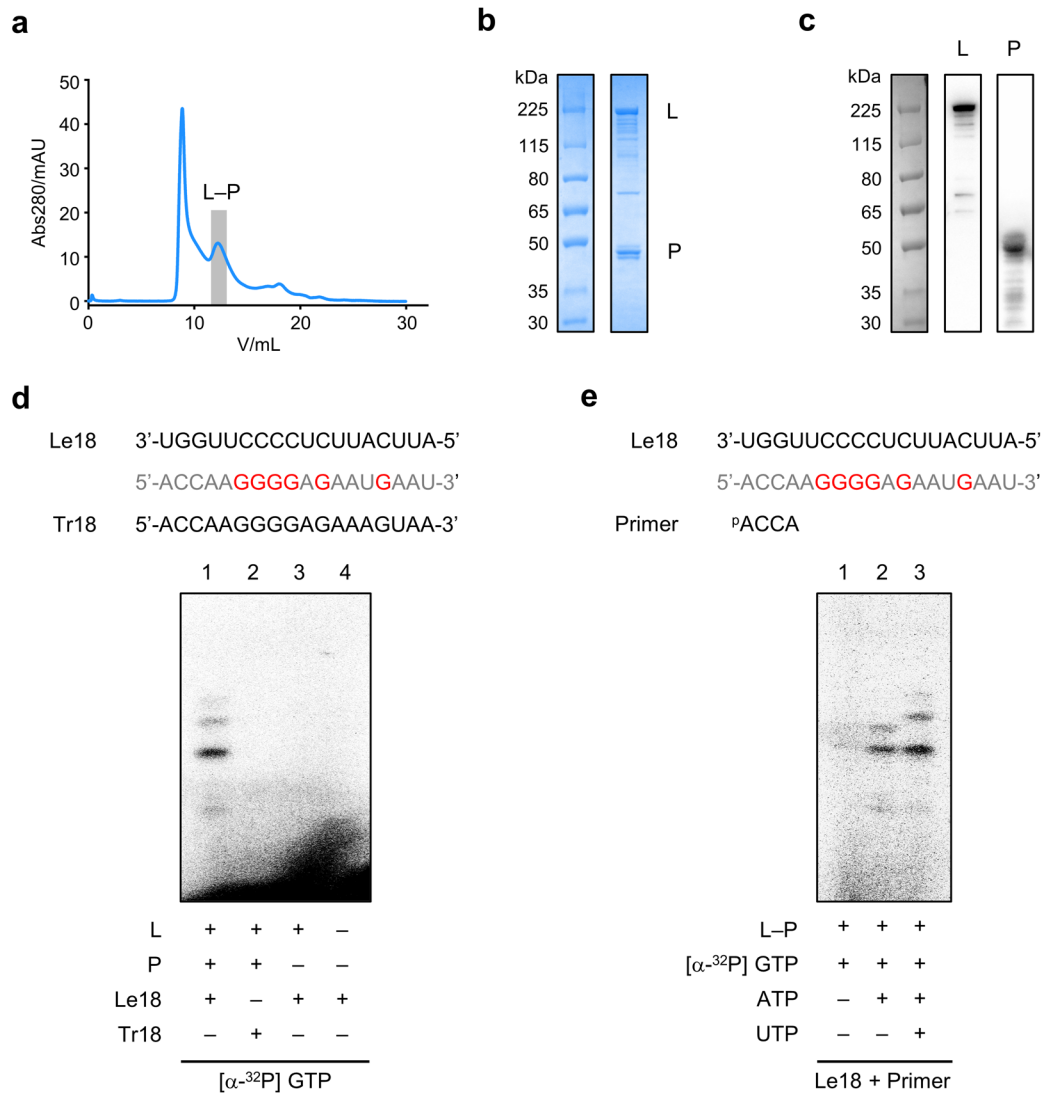
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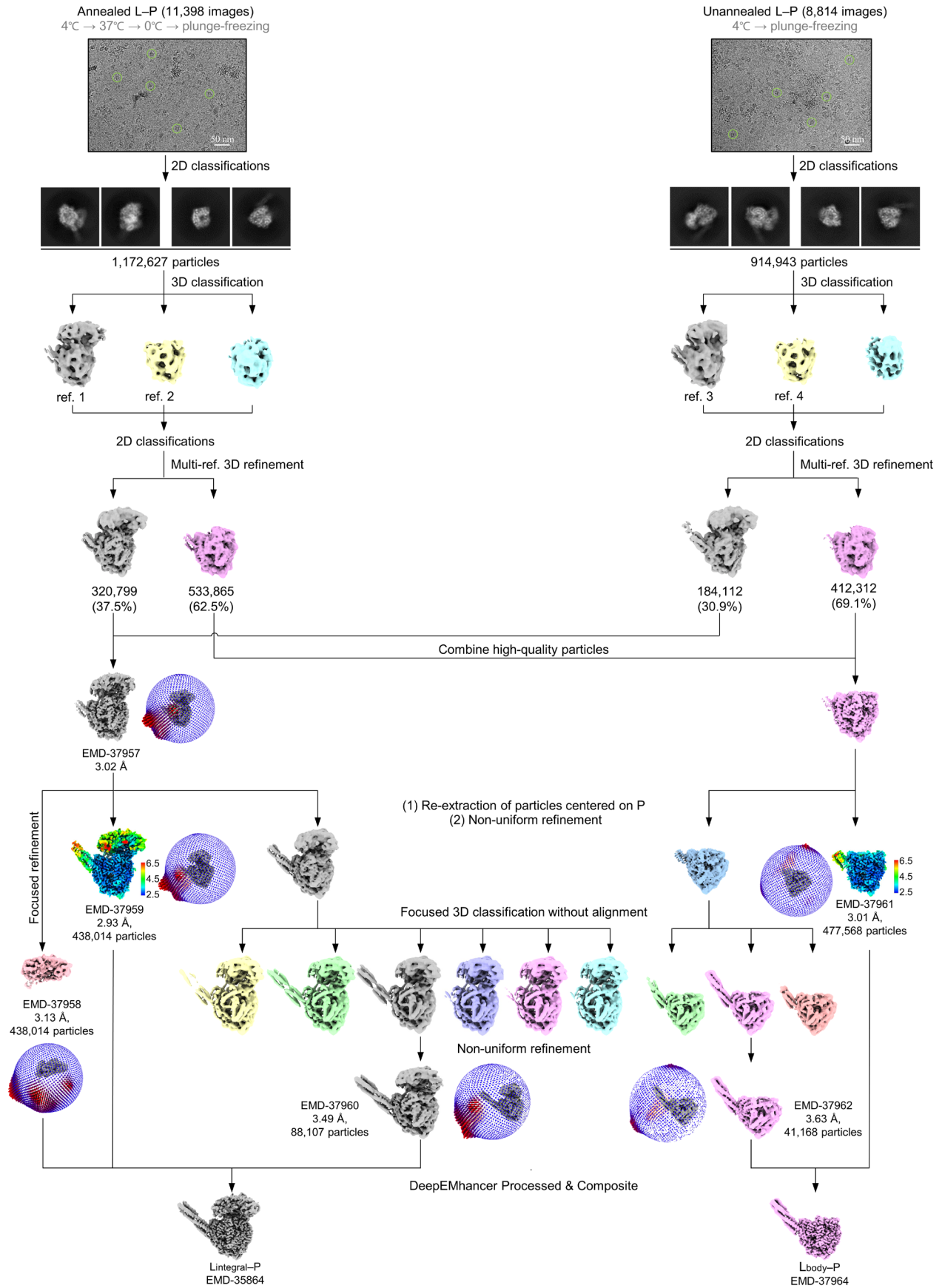
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Supplementary Materials:

Supplementary Fig.1 to Fig.11

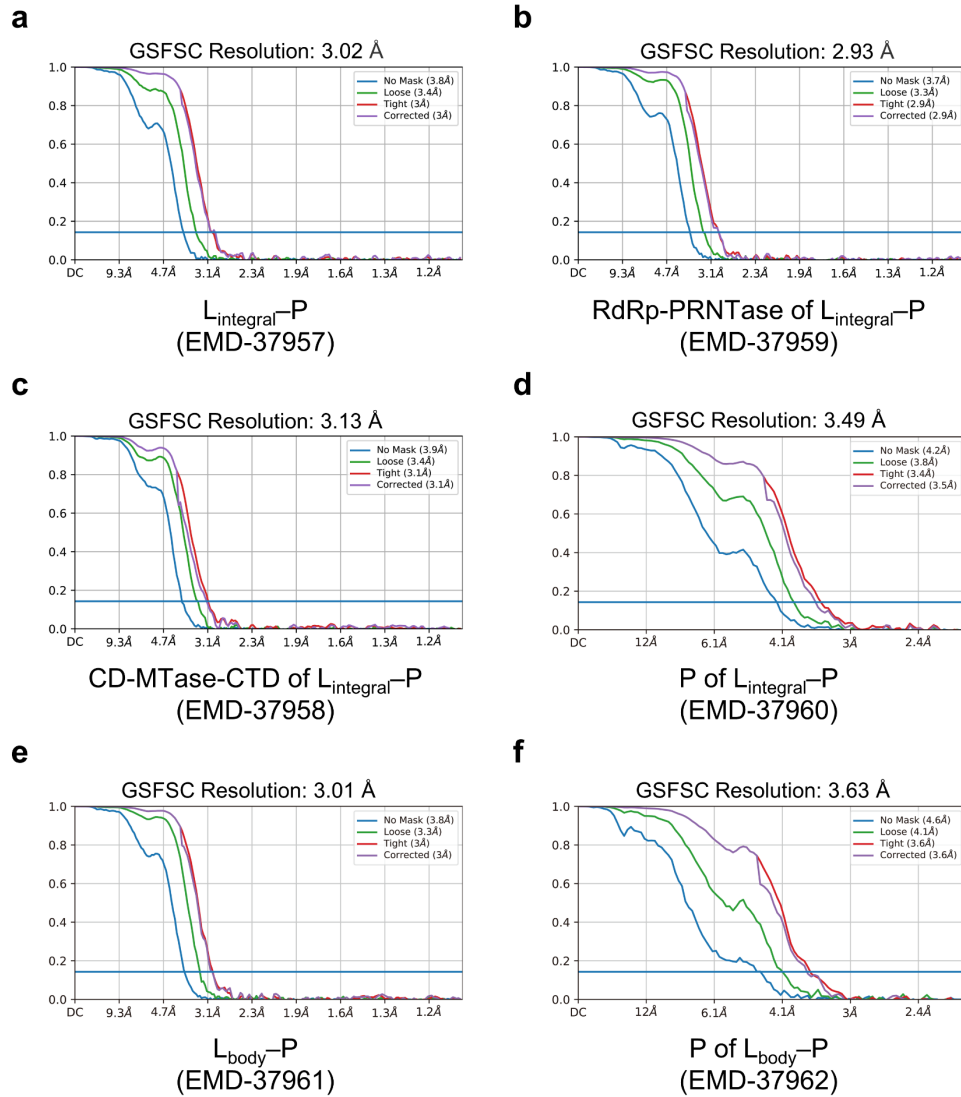


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 26 **Supplementary Fig. 1 Purification and verification of MuV L-P complex.** **a**, Size-exclusive
 27 chromatogram of MuV L-P. The grey rectangle indicates the fractions collected for cryo-EM
 28 sample preparation and verifications in **b-e**. **b,c**, The SDS-PAGE gel and western blotting profiles
 29 of purified MuV L-P complex. **d**, The *de novo* RNA synthesis assay of MuV L-P complex. The
 30 first 18 nucleotides of the leader sequence (Le18) are used as a template. The control is the 18
 31 nucleotides at the 5' end of the genome (Tr18). **e**, The primer-extension assay of MuV L-P
 32 complex. A 4-nt primer was incubated with the Le18 template before initiating the reaction. Source
 33 data in **a-e** are provided as a Source Data file.



35 **Supplementary Fig. 2 Flowchart of cryo-EM data processing on MuV L–P complex.** The
36 angular distribution of the particles for the locally refined maps is shown. Local resolution maps
37 for MuV L_{integral}–P (EMD-37959) and L_{body}–P (EMD-37961) are shown.

38

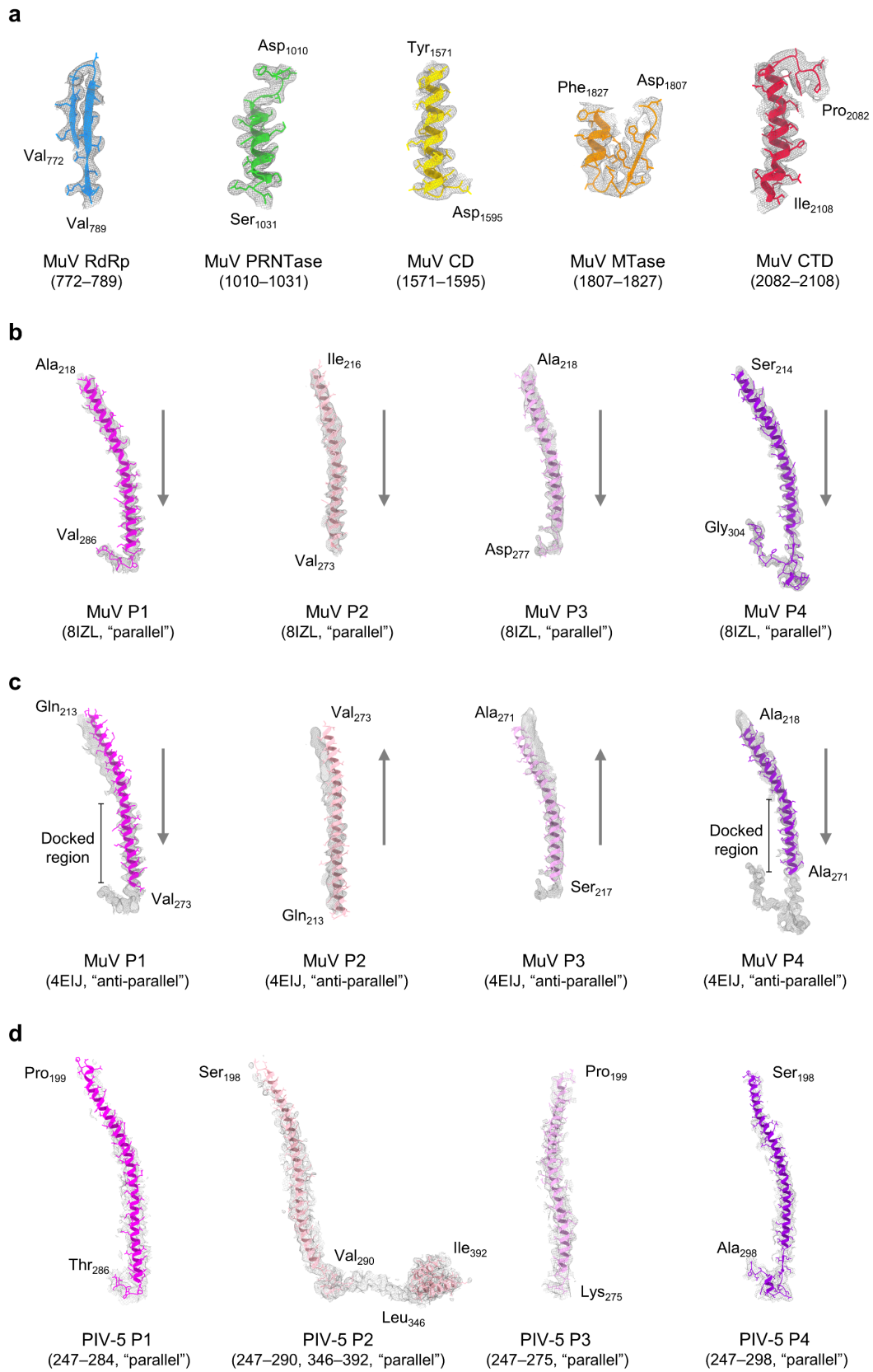


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40 **Supplementary Fig. 3 FSC curves of MuV L-P complex. a-d, FSC curves of MuV $L_{\text{integral}}\text{-P}$.**

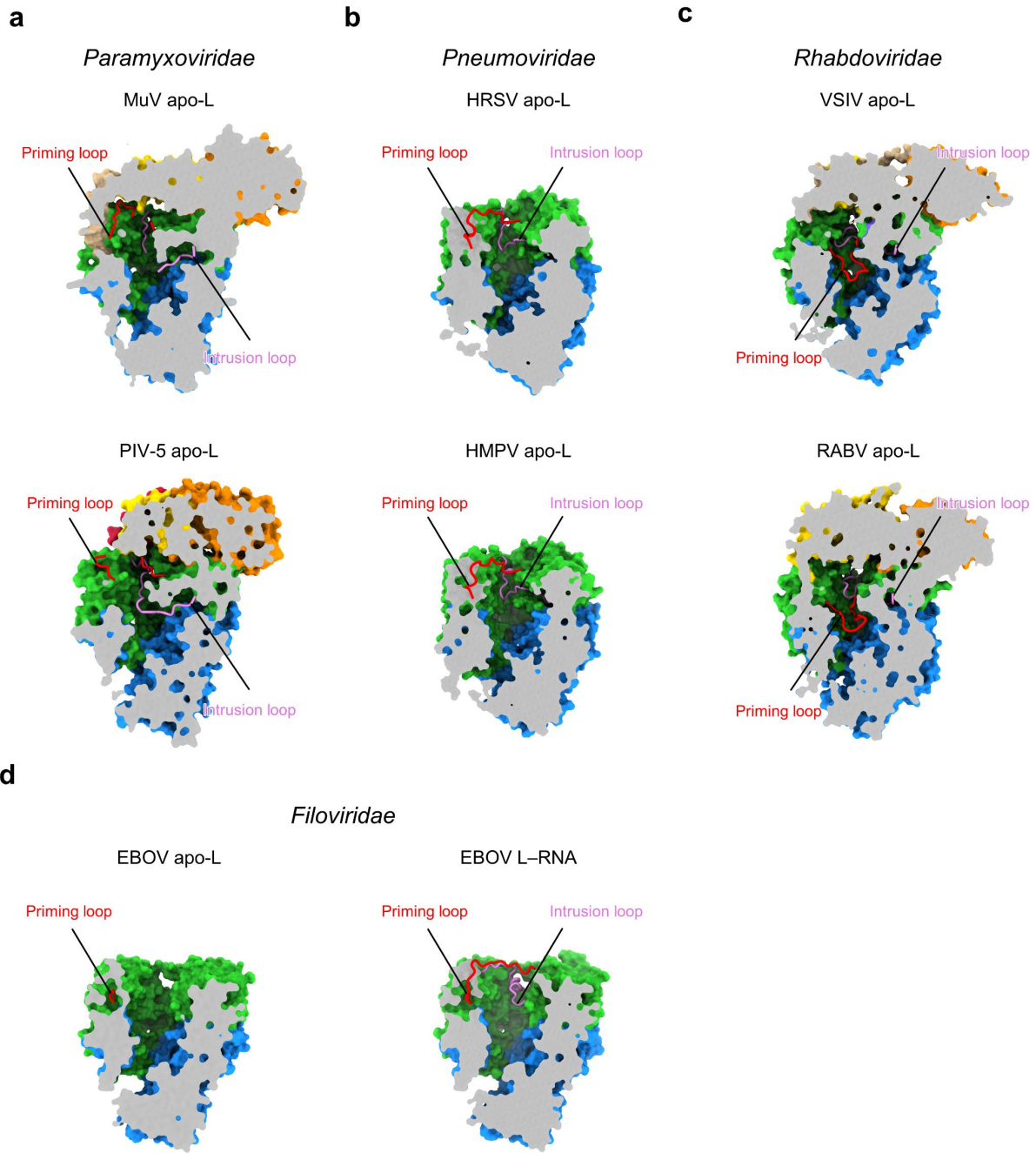
41 **e,f, FSC curves of MuV $L_{\text{body}}\text{-P}$.**

42



44 **Supplementary Fig. 4 Representative model fittings into L and P of MuV and PIV-5. a,**
45 **Representative fragments of MuV L. b,** Representative fragments of four parallel MuV P
46 molecules (PDB ID 8IZL). Arrow describes the helical direction of each P molecule. **c,**
47 Representative fragments of four antiparallel MuV P molecules (PDB ID 4EIJ). Arrow describes
48 the helical direction of each P molecule. **d,** Representative fragments of PIV-5 P (EMD-21095)
49 and the respective modified atomic models after comparing MuV P with the original model (PDB
50 ID 6V85).

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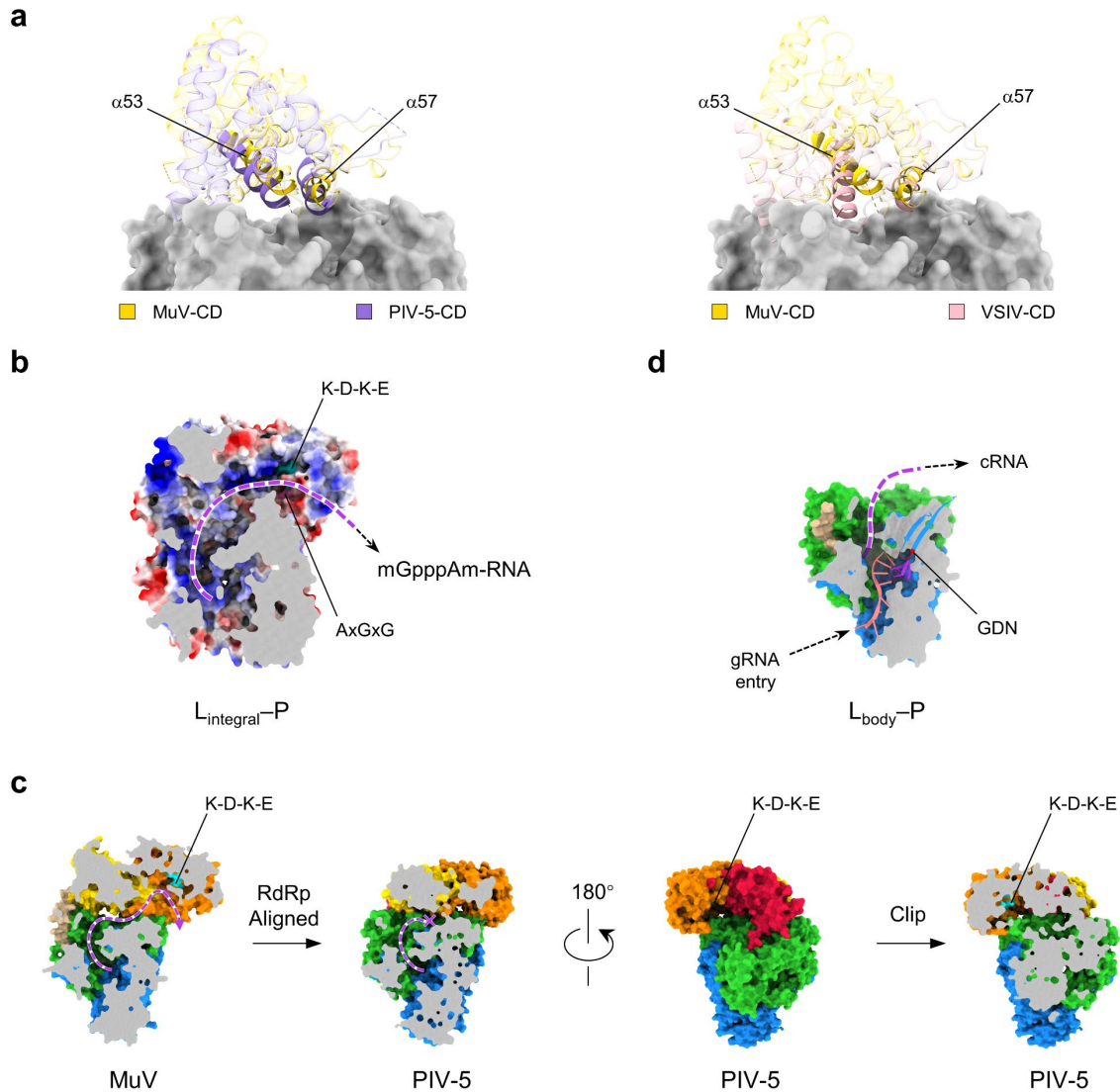
52

53 **Supplementary Fig. 5 Priming loops and intrusion loops of the structurally resolved nsNSV**

54 **L. a,** The apo-L of MuV and PIV-5 in the family *Paramyxoviridae*. **b,** The apo-L of HRSV and

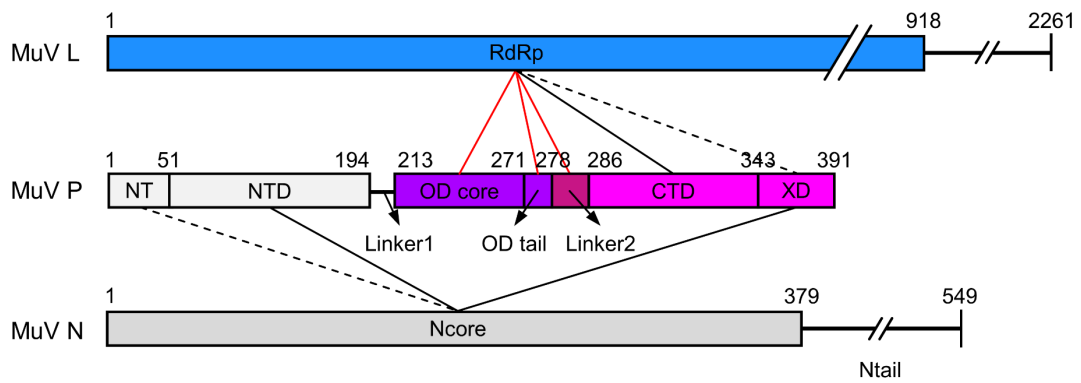
55 HMPV in the family *Pneumoviridae*. **c,** The apo-L of VSIV and RABV in the family

- 56 *Rhabdoviridae*. **d**, The apo-L and RNA-binding L of EBOV in the family *Filoviridae*. Priming
- 57 loop, red; Intrusion loop, violet.



58

59 **Supplementary Fig. 6 RNA tunnel display.** **a**, Comparison of MuV, PIV-5, and VSIV L CD
 60 positioning. Their RdRp and PRNTase are structurally aligned and displayed as grey surfaces. CD
 61 of these three viruses are displayed as semi-transparent ribbons. Helices $\alpha 53$ and $\alpha 57$ are
 62 highlighted. **b**, Electrostatic surface of MuV L. The purple dashed line indicates the positively
 63 charged tunnel for mRNA maturation. The K-D-K-E and AxGxG motifs are colored in cyan and
 64 hot pink, respectively. **c**, The K-D-K-E motif of PIV-5 L is not inside the RNA tunnel. **d**, Exposed
 65 RNA cavity of MuV $L_{\text{body}}-P$.

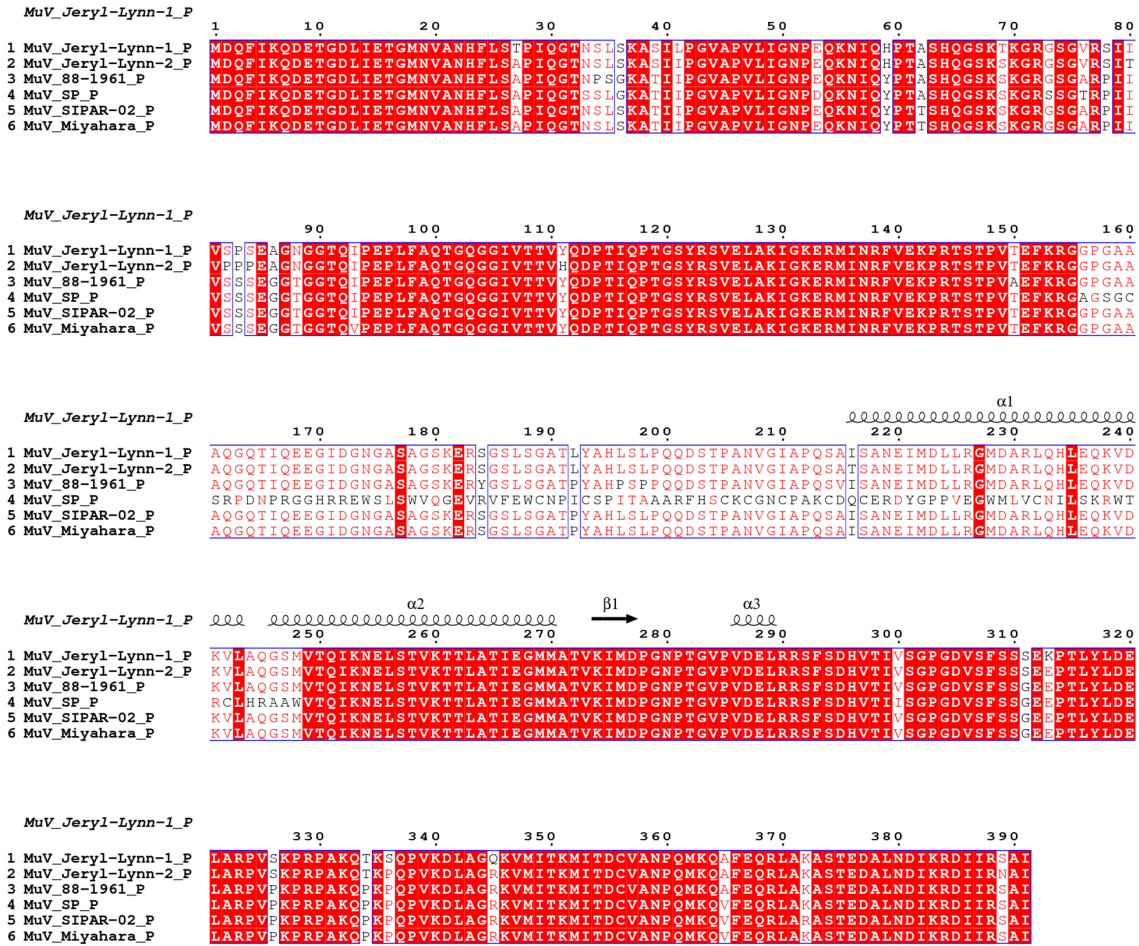


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67 **Supplementary Fig. 7 Diagram of the MuV N-P-L interaction map.** Red lines: new

68 interactions identified in this work. Black solid lines: interactions proposed by previous studies.

69 Black dashed lines: potential interactions.



70

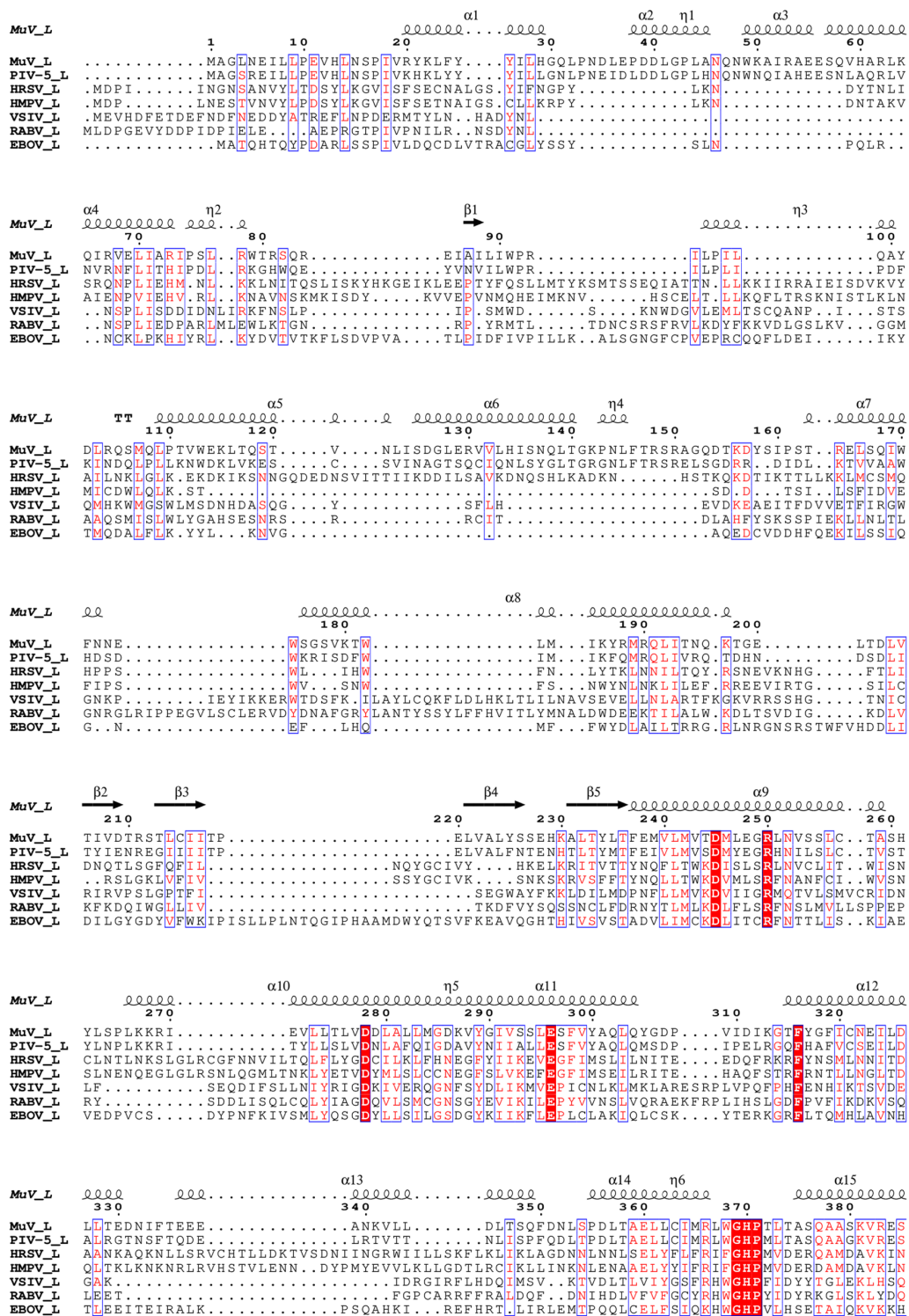
71 **Supplementary Fig. 8 Sequence alignment of P from six MuV strains.** Jeryl-Lynn-1, Uniport

72 ID: Q9J4L6; Jeryl-Lynn-2, Uniport ID: Q8QV71; 88-1961, Uniport ID: Q8QY72; SP, Uniport ID:

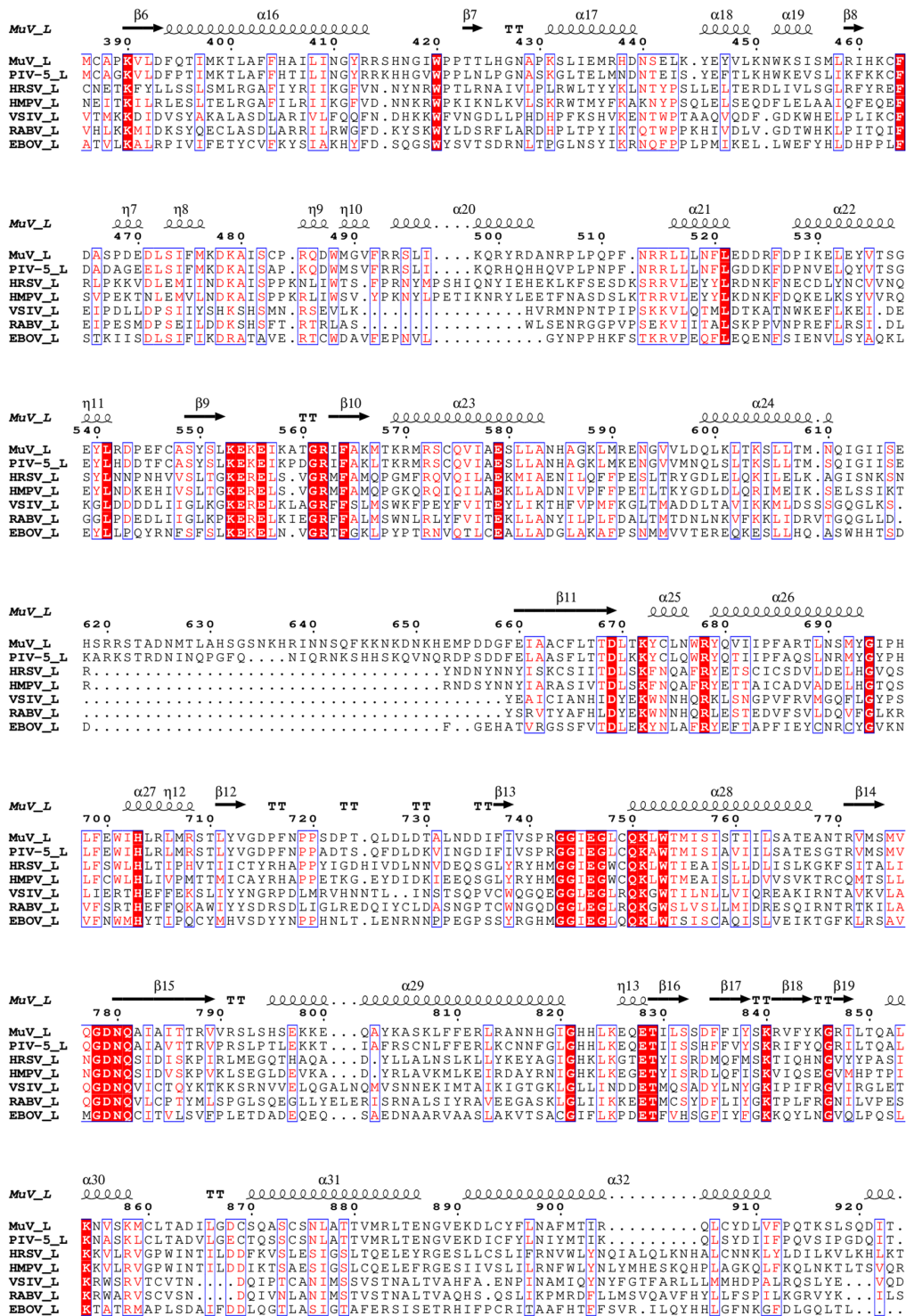
73 B5TE95; SIPAR-02, Uniport ID: Q910S3; Miyahara, Uniport ID: P30928.

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α_{33} α_{34} η_{14} η_{15} α_{35} α_{36}
 MuV_L 930 940 950 960 970 980 990
 MuV_L . NAYLNHPF I L I S R L C L L P S Q I G G L N P L S C S R L F N R N I G D P L V S A T A D V K R L I K A G C L D I W V L Y N
 PIV-5_L . L E Y I N N P H V S R L A L L P S Q I G G L N V L S C S R L F N R N I G D P V V S A V A D L K R L I K S G C M D Y W I L Y N
 HRSV_L F F N I D N I D T A L T L Y M N L P M L F G G D P N L L Y R S F Y R R T P D F L T E A I V H S V F L I S Y T N H D L K D K L Q D L S D D R L N K F L T C I I
 HMPV_L F F E I K R E N E V V D L W M N I P M O F G G D P V V F Y R S F Y R R T P D F L T E A I S H V D I L I K I S A N T K N E T K V S F F K A L L
 VSIV_L K I P G L H S S T F K Y A M L Y L D P S L G I G V S G M S L S R F L I R A P F D P V T E S L S S W R F I H V H A R S E H L K E M S A V F G N P E I A K F
 RABV_L A E . G E S F L L A M S R I I Y L D P S L G I G S G M S L G R F H I R Q F S D P V S E G L S F W R E I W L S S Q E S N I H A L C Q E A G N P D L G E R
 EBOV_L . . G R P L D F G T I S L A L A V P Q V L G G L S F L N P E K C E Y R N L G D P V T I S G L F Q L K T Y L R M I E M D D L F L P L .

α_{37} α_{38} α_{39}
 MuV_L 990 1000 1010 1020 1030 1040 1050 1060 1070
 MuV_L I L G R R P G K G K W S T L A A D E V T L N I D Y L V P S T F F L K K H A Q Y T L M E R S V N P M L R G V F S E N A A E E E E E L A Q Y I L L D R E V V M P
 PIV-5_L L L N G K P G N G S W A T L A A D E V S I N I E Y Q Y P P T A L K R H T Q Q A L M E L S T M L R G I F S D N A Q A E E N N L R M L L D R E V I F P
 HRSV_L T F D K N P . N A E F V T L M R D P Q A L G S E R O A K I T S E I N R L A V T E V L S T A P N K I F S K S A Q H Y T T E I D L N D I M O N I E T P Y P
 HMPV_L S I E K N E . R A T I T T L M R D P A V G S E R O A K V T S I N R T A V T S I L S L S P N Q L F S D S A I H Y S R N E E E V G I I A E N I T P V P
 VSIV_L R . I T H I D K L V E D P T S L N I A M G M S P A N L K T E V K K C L I E . S R Q T I R N Q V I K D A T I Y L Y H E E D R L R S F L W S I N P L F P
 RABV_L T . L E S T R L L E D P T L N I R G G A S P T I L L K D A I R K A L Y D . E V D K V E N S E F R E A I L L S K T H R D N F I L F L I S V E P L F P
 EBOV_L . I A I N P G N C T A I D F V L N P S G L N V P G S Q D L T S F L R Q I V R R T I T L S A K N K L I N T L F H A S A D F E D E M V C K W L S S T P V M S

α_{40} α_{41} α_{42} α_{43} α_{44} η_{16}
 MuV_L 1070 1080 1090 1100 1110 1120 1130 1140
 MuV_L R V A H V I L A Q S S C G R R K O I Q G Y L D S T R T I I R Y S L E V R P L S A K K L N T V I E Y N L L Y L S Y N L E T I E K P N I V Q P . . F L N A I N
 PIV-5_L R V A H I I I E Q T S V G R R K O I Q G Y L D S T R S I M R K S L E I K P L S N R K L N E I L D Y N I N Y L A Y N L A L L K N A I E P P T . . Y L K A M T
 HRSV_L H G L R V V Y E S L P F Y K A E K I V N L I S G T K S I T N I L E K T S A I D L T D I D R A T E M M R K N I T L L I R I L P L D C N R D K R . . E I L S M E
 HMPV_L H G L R V L Y E S L P F H K A E K V V N M I S G T K S I T N L L E Q T S A I N G E D I D R A V S M M L E N L G L S R I L S V V V D S E I . . P I K S N G
 VSIV_L R F L S E F K S G T F L G V P D G L I S L P Q N S R T I R N S F K K K Y H R E L D D L I V R S E I V S S L T H L G K L H L R R G S C K
 RABV_L R F L S E L F S S S F L G I P E S I I G L I Q N S R T I R R Q R F R K S L S K T L E E S F Y N S E I H G I S R M Q T P Q R V G G
 EBOV_L R F A A D I F S R T P S G K R L Q I L G Y L E G T R T L L A S K I I N N N T E T P V L D R L R K I T L Q R W S L W F S Y L D H C . . D N I L A . . E . . A L T

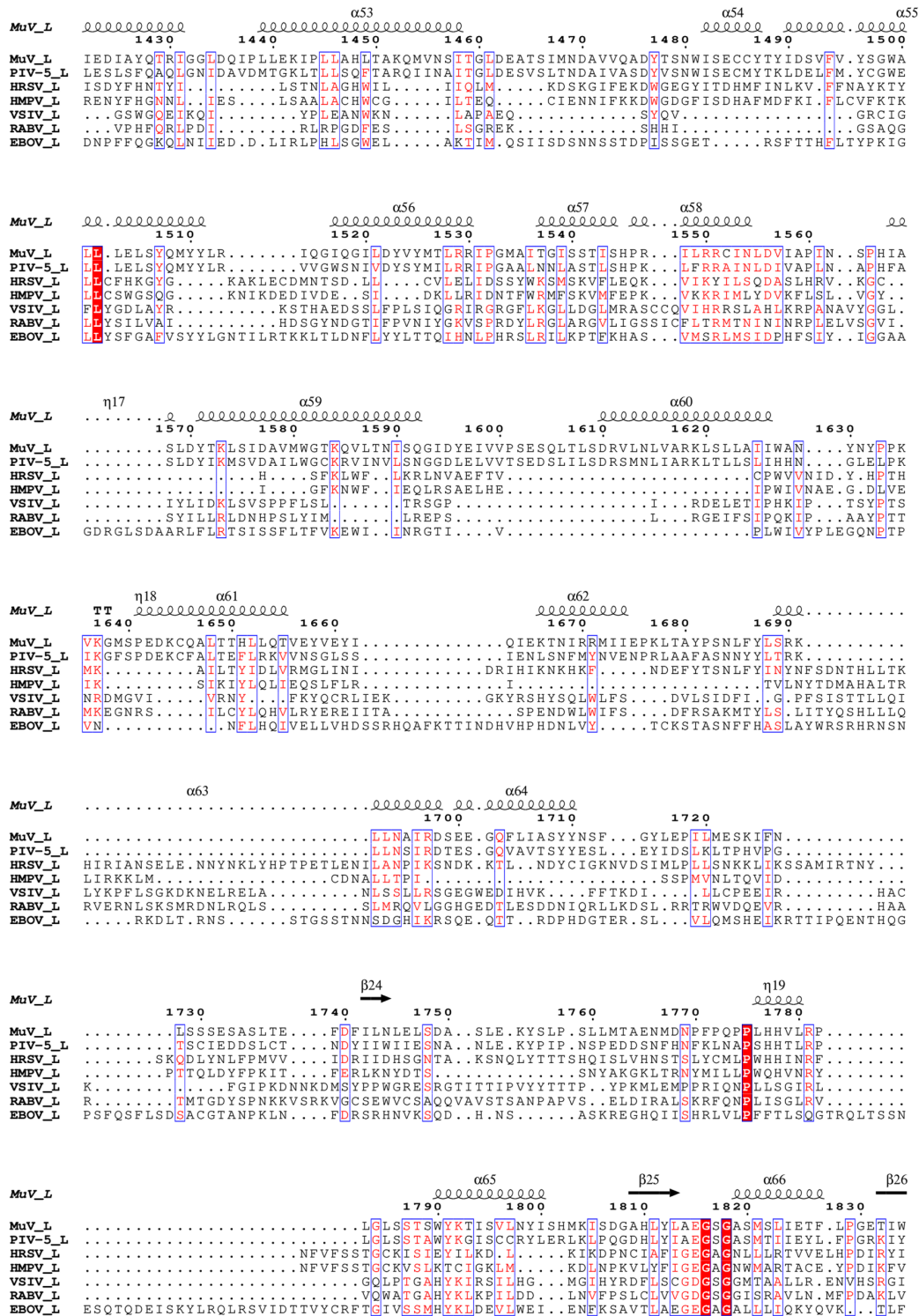
α_{45} β_{20} α_{47} β_{21}
 MuV_L 1140 1150 1160 1170 1180 1190 1200 1210
 MuV_L V D I C S I D I A R S L R K I S W A T L L N G R P I E G L E T P D F I E L V H G C L I I G S D E C E H C S S G D D K F T W F F L P K G I R L D D D
 PIV-5_L L E T C S I D I A R N L R K I S W A P L L G R N L E G L E T P D F I E T A G A L I V G S G Y C E Q C A A G D N R F T W F L P S G I E I G D D
 HRSV_L N L S . I T E L S K V R E R S W L S N I V G V T S P S I M T D I K Y T S T I S S G I I I E K Y N V N S
 HMPV_L R L I . C C Q I S R P L R E S W N M E I V G V T S P S I M T C M D V I Y A T S S H L K S I I I E K F S T D R
 VSIV_L M W T C S A T H A D L T R Y S W R T V I G T T V P H P L E M L G . P Q H R K E T C A P C N T S . G F N V V S H C D G I H D V
 RABV_L V W P C S S E R A D L L R E S W G R K V V G T I V P H P S E M L G . L L P K S S I S C T C G A T G G N P R V S V S V L P S F D Q S
 EBOV_L Q I T C T V D L A Q L R E Y S W A H I L P G R P L I G A T L E C M I E Q K V V W L K P Y E Q C P Q C S N A K Q P G G K P F V S V A V K H I I S A W P N A S

α_{48} α_{49} α_{50}
 MuV_L 1220 1230 1240 1250 1260 1270 1280
 MuV_L . P A S N P I R V P Y I G S K T D E R R V A S M A Y I R G A . S V S L R S A T L A G V Y I I A F G D T E E S W Q D A Y E L A S T R V N L T L E Q T Q S L
 PIV-5_L . L R N P I R V P Y I G S R T D E R R V A S M A Y I R G A . S S S L R A V I R L A G V Y I I A F G D T L E N W I D A L D L S H T R V N I T L E Q L Q S L
 HRSV_L L T R N G E R G T K P P W G S S T Q E K K T M P V Y N R Q V L . T K K Q D Q I D L L A K L D W V Y A S I D N K D E F M E E L S I G T L G I Y E K A K K L
 HMPV_L T T R G O R G P K S P W G S S T Q E K K L V P V Y N R Q I L . S K Q Q R E Q L E A I G K M R W V Y K G T P G L R R L L N K I C L G S L G I S Y K C V K P L
 VSIV_L F S S R G L P A Y L G S K T S E S T S I L Q P W E R E S K V P L I K R A T R L R D A I S W F E P D S K L A M T I L S N I S L T G B E W T K R Q H G
 RABV_L F S S R G L K G Y L G S S T S M S T O L F H A W E K V T N V H V V R K A L S L K E S I N W F I T R D S N L A Q A L I R N I M S L T G P D F L E E A P V
 EBOV_L R I S W T I G D G I P Y I G S R T E D K I G O P A I K P K C P . S A A L R E A I E L A S R L T W I T Q G S S N S D L L I K P F L E A R V N L S V Q E L L Q M

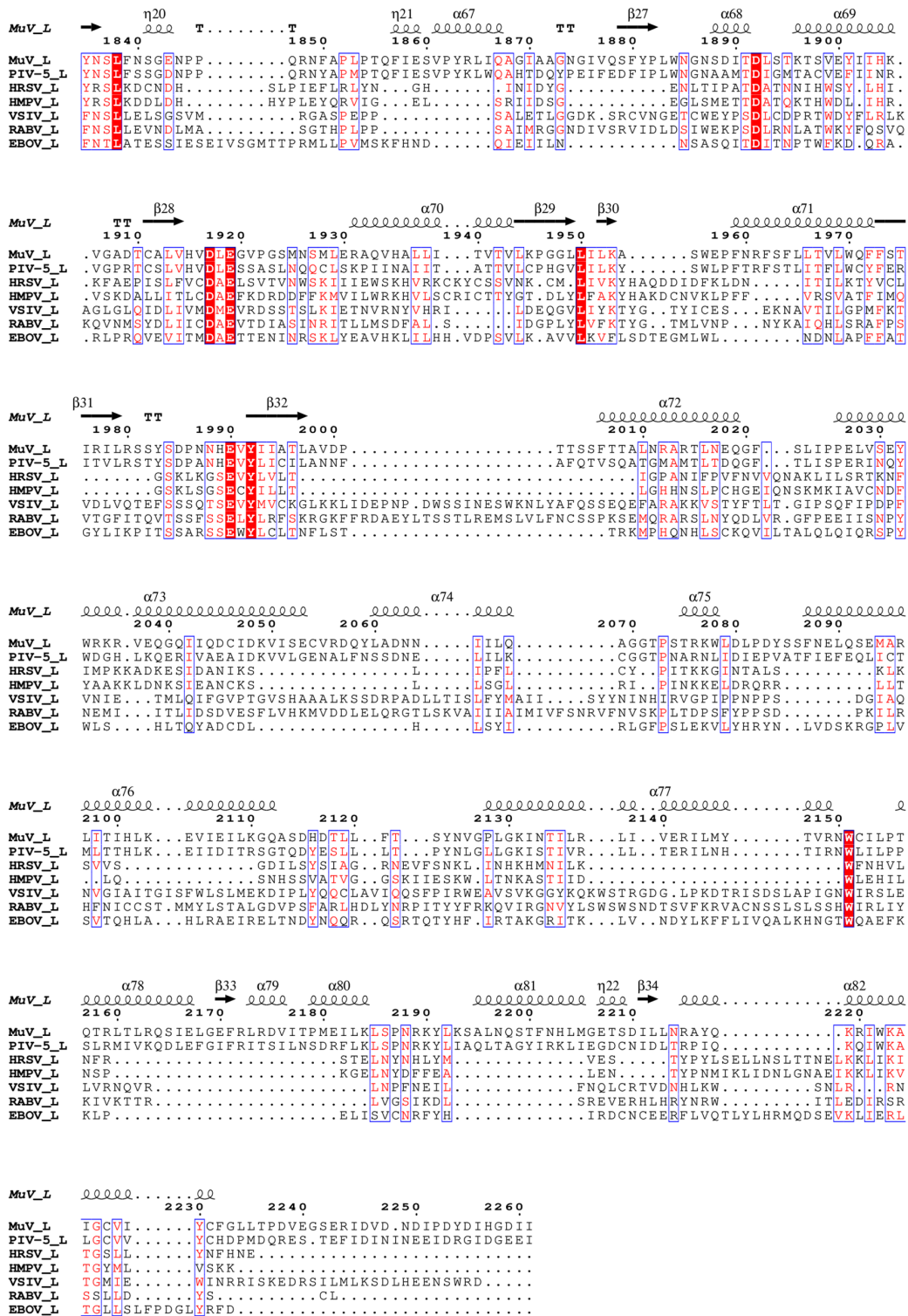
β_{22} α_{51}
 MuV_L 1290 1300 1310 1320 1330 1340 1350 1360
 MuV_L T P L P T S A N L V H R L D D G T T Q L K F T P A S S Y A F S S F V H I S N D C Q I L E I D D Q V T D S N L I Y Q V M I T G L A L I E T W N N P
 PIV-5_L T P L P T S A N L T H R L D D G T T T L K F T P A S S Y T F S S F T H I S N D E Q Y L T I N D K T A D S N I Y Q L M I T G L I L E T W N N P
 HRSV_L F P Q Y L S V N Y L H R L T V S S R P C E F P A S I . P A Y R T N Y H F D T S P I N R I L T E K Y G D E I D I V E N C I S F G L S I M S V V E Q F T N C R
 HMPV_L L P R F M S V N Y L H R L S V S S R P M E F P A S V . P A Y R T N Y H F D T S P I N Q A L S E R F G N E D I N L V E O N A I S C G L S I M S V V E Q L T G R S
 VSIV_L F K R T G S A L H R F S T R M S H G G F A S Q S T A A L . T R L M A T T D T M R D L G . D O N F D F L Q A T L L Y A Q I T T T V A R
 RABV_L F K R T G S A L H R F S A R Y S E G G Y S V C P N L L . S H I S V S T D T M S D L T Q D G K N Y D F M P O P L M L Y A Q T W T S E L V Q R
 EBOV_L T S B Y S G N I V H R Y N D Q Y S P H S E M A N R . M S N S A T R L I V S T N T L G E F S G G G Q S A R D S N I T R O N V I N Y A V A L P D I K F R N T E A T

β_{23} α_{52}
 MuV_L 1370 1380 1390 1400 1410 1420
 MuV_L P I N F S V Y E T T L H L H L T G S S C I R P V E S C V V N P P L L P V P L I N V Q M N K F V Y D P E P L S L E M E K
 PIV-5_L P I N R T F E E S T L H L H L T G A S C V R P V D S C I L S E A L T V K P H I T V P Y S N K F V F D E D P L S E Y E T A K
 HRSV_L P N R I I L P K L N E I H L M K . P P I F T G D V I H K L K Q V I . Q K Q H M F L P D K I S L T . Q Y V E L F L S N K T L K S G S H V N S N L I A H K
 HMPV_L P K Q V L I P Q L N E I D I M P . P P V F Q G K F N Y K L V D K I T . S D Q H I F S P D K I D M L . T L G K M L M P T I K G Q K T D Q F L N K
 VSIV_L G W I T S C T D Y H I A C K S C L R P I E E I T L D S S M D Y T P D V S H V L K T W R N G E
 RABV_L T R L R D T F H W H L R C N R C V R I D D V T L E T S Q I F E F D V S K R I S R M V S G A
 EBOV_L D I Q Y R A H L H L . T K C T R N V P A Q Y I T Y T S L L D D L T . R Y R E N E L I Y D N N P L R G G L N C N I S P

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83 Figure continued on the next page

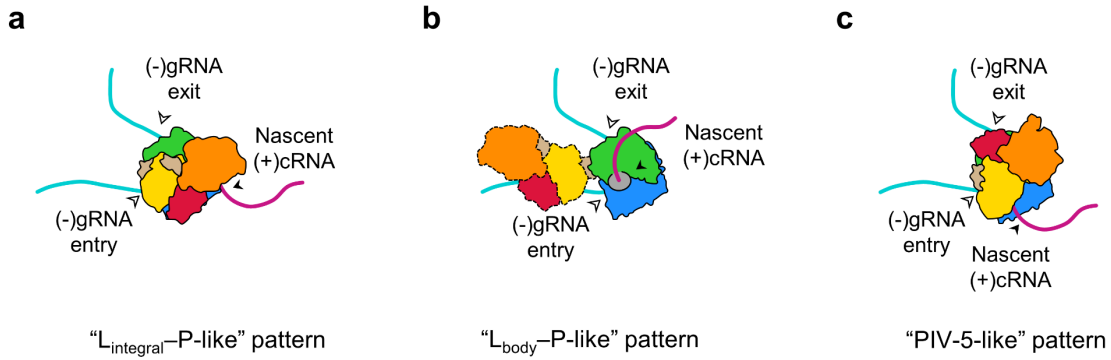


84

85 **Supplementary Fig. 9 Sequence alignment of nsNSV L. MuV, strain Jeryl-Lynn-1, Uniport ID:**

86 **Q9J4L0; PIV-5, strain W3, Uniport ID: Q88434; HRSV, strain A2, Uniport ID: P28887; HMPV,**

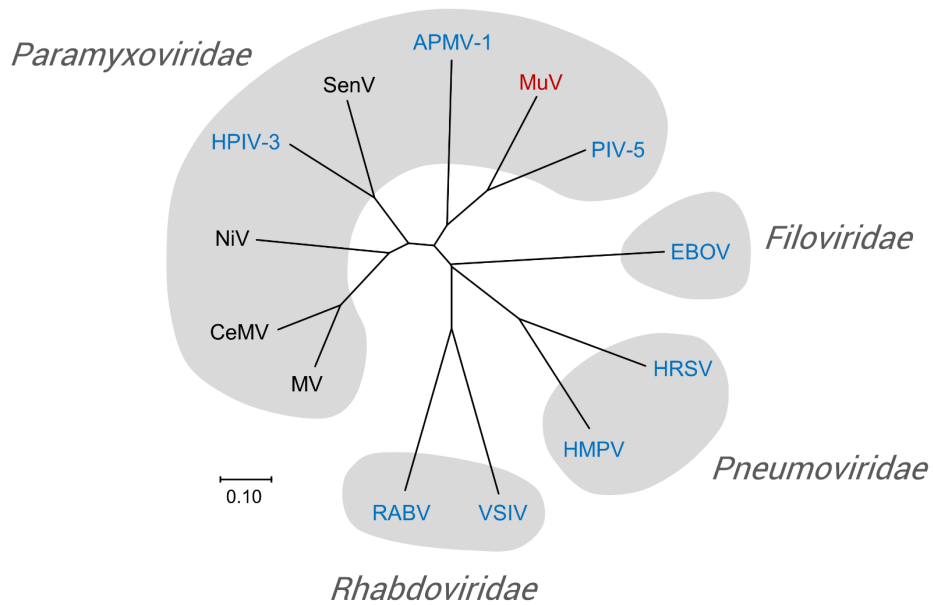
- 87 strain CAN97-83, Uniport ID: Q6WB93; VSIV, strain San-Juan, Uniport ID: P03523; RABV,
- 88 strain SAD-B19, Uniport ID: P16289; EBOV, Uniport ID: X5H5B6.



89

90 **Supplementary Fig. 10 Proposed models for genome replication of nsNSVs.** In the replication
 91 mode, the conformation of CD-MTase-CTD determines the exit of the complementary RNA
 92 (cRNA). **a**, L adopts the “L_{integral}-P-like” pattern as in MuV and VSIV. “L_{integral}-P-like” structures
 93 may bypass the MTase domain for genome replication. **b**, L adopts the “L_{body}-P-like” pattern as
 94 in MuV, HRSV, HMPV, and EBOV. The appendage (CD-MTase-CTD) is not fixed on the top of
 95 RdRp and PRNTase. The cRNA comes out directly from the central RNA cavity. **c**, L adopts the
 96 “PIV-5-like” pattern. The cRNA comes out from the hole fenced by RdRp and CD.

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98

99 **Supplementary Fig. 11 Phylogenetic tree based on L sequences in nsNSVs.** Structurally

100 resolved polymerase complexes are labeled in blue and red. MV: measles virus; CeMV: Cetacean

101 morbillivirus; NiV: Nipah virus; HPIV-3: human parainfluenza virus 3; SenV: Sendai virus;

102 APMV-1: Newcastle disease virus; MuV: mumps virus; PIV-5: parainfluenza virus 5; EBOV:

103 Ebola virus; HRSV: human respiratory syncytial virus; HMPV: human metapneumovirus; VSIV:

104 vesicular stomatitis Indiana virus.

105