1	Structures of the mumps virus polymerase complex via cryo-
2	electron microscopy
3	
4 5	Tianhao Li ^{1,2,3,4,8} , Mingdong Liu ^{1,2,3,8} , Zhanxi Gu ^{5,6} , Xin Su ^{1,2,3,7} , Yunhui Liu ^{1,2,3} , Jinzhong Lin ⁷ , Yu Zhang ⁶ , Qing-Tao Shen ^{1,2,3,4*}
6	
7	Affiliations:
8 9	¹ School of Life Sciences, Department of Chemical Biology, Southern University of Science and Technology, Shenzhen 518055, China.
10 11	² Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China.
12 13	³ Institute for Biological Electron Microscopy, Southern University of Science and Technology, Shenzhen, 518055, China.
14	⁴ School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China.
15	⁵ University of Chinese Academy of Sciences, Beijing 100049, China.
16 17	⁶ Key Laboratory of Synthetic Biology, Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China.
18 19	⁷ State Key Laboratory of Genetic Engineering, School of Life Sciences, Zhongshan Hospital, Fudan University, Shanghai, 200438, China.
20	⁸ These authors contributed equally: Tianhao Li, Mingdong Liu
21	*Correspondence to shenqt@sustech.edu.cn (QT.S.)
22	
23	Supplementary Materials:
24	Supplementary Fig.1 to Fig.11



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



39

40 Supplementary Fig. 3 FSC curves of MuV L–P complex. a–d, FSC curves of MuV L_{integral}–P.

⁴¹ e,f, FSC curves of MuV L_{body}–P.



Leu₃₄₆ PIV-5 P1 PIV-5 P2 PIV-5 P3 PIV-5 P4 (247–284, "parallel") (247–290, 346–392, "parallel") (247–275, "parallel") (247–298, "parallel")

43

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).





Supplementary Fig. 5 Priming loops and intrusion loops of the structurally resolved nsNSV
L. a, The apo-L of MuV and PIV-5 in the family *Paramyxoviridae*. b, The apo-L of HRSV and
HMPV in the family *Pneumoviridae*. c, The apo-L of VSIV and RABV in the family

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





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



Supplementary Fig. 7 Diagram of the MuV N-P-L interaction map. Red lines: new
interactions identified in this work. Black solid lines: interactions proposed by previous studies.

69 Black dashed lines: potential interactions.





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.

MuV_L			10		مععد	α1 Q	مععد	د	α2 η1	α3	. معمومومو
MuV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	.MDPI MDP .MEVHDFETI MLDPGEVYDI	H MAGL MAGS INGNS LNEST DEFNDFN DPIDPIE MAT	NEILLPH REILLPH ANVYLTI VNVYLPI EDDYATH LE2 QHTQYPI	VHLNSP VHLNSP SYLKGV SYLKGV EFLNPD AEPRGTP ARLSSP	IVRYKLF IVKHKLY ISFSECN ISFSETN ERMTYLN IVPNILR IVLDQCD	Y ALGS. AIGS. HAD NSD LVTRA	YILHGQI YILLGNI YIFNGPS CLLKRPS YNL YNL CGLYSSS	PNDLEI	2DDLGPLA DDLGPLH LDDLGPLH	50 NQNWKAIRAE NQNWNQIAHE N N	ESQVHARLK ESNLAQRLV DYTNLI DNTAKV PQLR
MuV_L MuV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	a4 2000202000 79 QIRVELIAR NVRNFLITH SRQNPLIEH . NSPLIED . NSPLIED . NCKLPKH	η2 ΩΩΩΩ IPSLR IPDLR M.NLK V.RL.K V.RL.K PARLMLE IYRL.K	80 WTRSQR KGHWQE KLNITQS NAVNSKN KFNSLP WLKTGN YDVTVTP	SLISKYH MKISDY. KFLSDVP		β1 90 IAILI VNVII EPTYF EPVNM IP.SM RP.YF LPIDF	WPR WPR QSLLMTY QHEIMKN WD TVPILLE	KSMTSS V S TDNCS (ALS)	Q III SEQIATIN HSCELT KNWDGVL SRSFRVLK SNGFCPVE	η3 PIL PLI .LLKKIIRRA .LLKQFLRS EMLTSCQANP DYFKKVDLGS PRCQQFLDEI	QQ 100 QAY PDF IEISDVKVY KNISTLKLN ISTS LKVGGM IKY
MuV_L MuV_L PIV-5_L HRSV_L HNPV_L VSIV_L RABV_L EBOV_L	TT QQ 1100 DIRQSMQLP KINDQLPPLL AILINKLGLK MICDWLQLK QMHKWMGSW TMQDALFLK	QQQQQQQQ IVWEKLT KNWDKLV .EKDKIK ST LMSDNHD LYGAHSE .YYL	α5 <u>000</u> 120 QST KESS SNNGQDF ASQG SNRS KNVG	. Q C EDNSVIT . Y . R	Q QQQQ I NLISDGL SVINAGT TIIKDDI	α6 00000 30 ERVVI SQCIC LSAVK 	HISNQLI NLSYGLI DNQSHLF	η4 2 222 CGKPNLH CGRGNLH KADKN	150 TTRSRAGQ TTRSRELS HST	2 160 DTKDYSIPST GDRRDIDL KQKDTIKTTL SD.DTSI VDKEAEITFD VDKEAEITFD AQEDCVDDHF	α7 QCCCO 17 Q RELSQTW KTVVAAW LKKLMCSMQ LSFIDVE VVETFIRGW VVETFIRGW QEKILSSIQ
MuV_L MuV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	QQ FNNE HDSD FIPS GNKP GNRGLRIPPP GN	IEYI EGVLSCL	U WSC WL WL KKERWTI ERVDYD1 EF	SVKTW. SVKTW. SIJHW. IHW. SNW. DSFK.ILL NAFGRYL. LHQ.	AYLCQKF ANTYSSY	LDLHK LFFHV	α8 LM FN FS LTLI LNA /ITLYMNA MF	. IKYR IKFQI . IKFQI . LYTKI . NWYNI AVSEVEI ALDWDEI . FWYD]	RQLITNQ RQLITNQ INILTVQ NNILTQY NKLILEF LNLARTF KTILALW AILTRRG	.2 200 .KTGE .TDHN RSNEVKNHG .RREEVIRTG KGKVRRSSHG .KDLTSVDIG .RLNRGNSRS	LTDLV DSDLI FTLI SILC TNIC KDLV TWFVHDDLI
MuV_L MuV_L PIV-5_L HRSV_L HNPV_L VSIV_L RABV_L EBOV_L	β2 IIVDTRST TYIENREGI DNQTLSGFQ . RSLGKLV RIRVPSLGP KFKDQIWGL DILGYGDYV	CIITP IIITP FIL FIV LIV FWKIPIS	LLPLNT	QGIPHAA	22 NQYGCI SSYGCI MDWYQTS	β4 ELVAL ELVAL VY SEG TKD VFKEA	230 YSSEHKZ FFNTENH HKELKE SNKSKF WAYFKKI WAYFKKI VQGHTHJ	β5 LTYLTI LTYMTI VSFFT DILMO SNCLFDI VSVST2	20000000 240 FENVLMVT FOIVLMVT NOFLITWK NOFLITWK NFLLMVK NFLLMVK ADVLIMCK	009 250 DMLEGRLNVS DMYEGRHNIL DISLSRLNVC DVMLSRFNAN DVIIGRMQTV DLFLSRFNSL DLITCRFNTT	20020 269 SICTASH SLCTVST LITWISN FCIWYSN LSMVCRIDN MVLLSPPEP LISKIAE
MuV_L MuV_L PIV-5_L HRSV_L HNPV_L VSIV_L RABV_L EBOV_L	20000 270 YLSPLKKRI YLNPLKKRI CLNTLNKSL SLNENQEGL LF RY VEDPVCS	GLRCGFN GLRSNLQ SEQD SDDL DYPN	α10 ΕνΓ ΤΥΙ NVILTQI GMLTNKI IFSLLN ISQLCQI FKIVSMI	28 LTLVDD LLSVDN FLYGDC YETVDY YRIGDK YIAGDQ YQSGDY	η QQQQQQQ Q LALLMGD LAFQIGD ILKLFHN MLSLCCN VLSMCGN LLSILGS	5 2 9 0 2 9 0 KVYGI AVYNI EGFYI EGFYI FSYDI SGYEV DGYKI	α11 2000000 IALLESE IKEVEGE VKEFEGE IKMVEPJ IKILEPJ IKFLEPJ	VYAQLO VYAQLO VYAQLO MSLI IMSLI CNLKLI VVNSLV CLAKIO	2 MSDP NITE RITE KLARESR VQRAEKFR 2LCSK	2000 310 .VIDIKGTFY .IPELRGOFH EDQFRKRFY .HAQFSTRFR PLVPQFPHFE PLIHSLGDFP .YTERKGRFL	α12 QCCCQCOQ 320 GFTCNETLD AFVCSEILD NSMLNNITD NTLLNGLTD NTLLNGLTD NHIKTSVDE VFIKDKVSQ TQMHLAVNH
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MuV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	1290 TPLPTSANI TPLPTSANI FPQYLSVNY LPRFMSVNE .FKRTGSP .FKRTGSP TPSHYSGNI	1300 THRLDDGT THRLDDGT THRLTVSS THRLSVSS THRFSTSR THRFSTSR THRFKSAR THRFKSAR	1310 TQLKFTPASSY TTLKFTPASSY RPCEFPASTP RPMEFPASV.P MSHGGFASQST YSEGGYSSVCP SPHSFMANR.M	β2 1320 AFSSFVH] AFSSFTH] AYRTINYHFD] AYRTINYHFD] AL.IRLMATI NLL.SHISVS] SNSATRLIVS]	2 TT 130, 12 SNDEQTLEIDDQVTI SNDEQTLTINDKTAL SPINRILTEKYGDE SPINQALSERFGNE DTMRDLGDQN DTMSDLTQDGKN NTLGEFSGGGQSAR	031 2000000000000000 3011YOVMITGLALI SNIIYOVMITGLALI 101VFONAISCGISIM INLVFONAISCGISIM 10LVFONAISCGISIM 10LVFONAISCGISIM 10FDFLGATLLYAQITT 19DFMFOPLMLYAQITT 19NIIFONVINYAVALF	2000 T 1360 ETWNNP ETWNNP SVVEQFTNVC SVVEQLTGRS TVQR DIKFRNTEAT
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MuV_L	<u>0000000000</u> 1430	<u>000000000</u> 1440 1	α53 00000000000 450 1460	1470	α54 202000 200 1480 1490	α55 2020.20200 1500
MuV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	IEDIAYQTRIGGLD LESLSFQAQLGNID ISDYFHNTYI RENYFHGNNL.IE: GSWGQEIKQI. .VPHFQRLPDI. DNPFFQGKQLNIIE!	2IPLLEKIPLLA AVDMTGKLTLLS LSTNLAG SLSAALAC YPLEA RLRPG D.D.LIRLPHLS	HLTAKQMVNSTTGLDEJ QFTARQIINALTGLDEJ HWILILQLM HWCGLLTQ NWKNLAPAEQ DFESSGREK GWELAKTIM.Q	ATSIMNDAVVQA SVSLTNDAIVAS KDSKGIFEK CIENNIFKKI SIISDSNNSSTDI	DYTSNWISECCYTYIDS DYVSNWISECMYTKDDE DWGEGYITDHMFINLKV WGDGFISDHAFMDFKI SYQV SHHI PISSGETRSFTI	SVFV.YSGWA ELFM.YCGWE 7.FFNAYKTY 1.FLCVFKTK GRCIG GSAQG HELTYPKIG
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MuV_L	η17 <u>000</u> 1570 SLDYTE	α59 200000000000 1580	0000000 1590 1600 VIINTSOCIDVELVUD	<u>0000</u> 1610 Sesoltisdevij	α60 20202020202020 1620 NIVARKISIJATIWAN	1630 VNVDDK
HRV_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	SLDYIR IYLIDK SYILLR GDRGLSDAARLFLR	ASVDAILWGCKR ASVDAILWGCKR HSFKL GFKN LSVSPPFLSL LDNHPSLYIM TSISSFLTFVKE	VINVLSNGGDLELVVT WF. LKRLNVAEFTV. WF. IEQLRSAELHE. TRSGP. LREPS WIINRGTIV.	SEDSLILSDRSMI	NLIARKITLISIIHHN. CPWVVN IPWVVN IROELETIPHKIE LRGEIFSIPQKIE PLWIVY	CGLELPK NID.Y.HPTH NAE.G.DLVE CTSYPTS CAAYPTT YPLEGQNPTP
MuV_L	η18 α6 ΤΤ <u>000000000</u> 1640 1651	1 2020202 2 1660		α62 <u>00000000</u> 1670	<u>ورن</u> 1680 1690	
MUV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	IKGGSPEDEKCGALI IKGSPDEKCFALI MKAIL IKSIK NRDMGVIVRI MKEGNRSIL VN	FLRKVVNSGLS FLRKVVNSGLS TYIDLVRMGLIN TYLQLIEQSLFL YYFKYQCR YLQHVLRYERE FLHQIVELLVH	1	LEKINIRAMIIEI ENLSNFMYNVENI HIKNKHKF RSHYSQLWLFS. SPENDWLWIFS. HPHDNLVY	PRLAAFSNLFYLSKK. .NDEFYTSNLFYINYNF DEFYTSNLFYINYNF DVLSIDFI.G.PF DFRSAKMTYLS.LI .TCKSTASNFFH <mark>AS</mark> LAY	SSDNTHLLTK XTDMAHALTR SSISTTLLQI ITYQSHLLLQ WWRSRHRNSN
	<i>4</i> 63		af	<i></i>		
MuV_L MuV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	HIRIANSELE.NNY LIRKKLM.LYKPELSGKDKNEL RVERNLSKSMRDNL RKDLT.RNS	NKLYHPTPETLE CD RELA RQLSSTGSST	Q000000 00.000 1700 LLNAIRDIES.GOVI NILANPIKSNDK.KTL NALITPI NLSSLLRSGEGWEDI .SLMRQVLGGHGEDTLI NNSDGHIKRSQE.QTT	U00000 1710 LIASYYNSF AVTSYYESL NDYCIGKNVD VKFFTKD SSDDNIQRLLKD RDPHDGTER.	1720 SYLEPIIMESKIFN SYDSLKLTPHVPG SIMLPLLSNKKLIKSSA SSPMVNLTQVID ILCPEIR SL.RRTRWVDQEVR SLVLQMSHEIKRTI	AMIRTNY HAC HAA TIPQENTHQG
MuV L			β24		η19 • • • • • •	
MuV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	1730 ISSSE: SKQDLYN PTTQLD RTMTGD PSFQSFLSDSACGT	1740 SASLTEFD DDSLCTND LFPMVVID (FPKITFE GIPKDNNKDMS (SPNKKVSRKVG ANPKLNFD	1750 FILNLELSDA.NLE. FILNLESDA.NLE. RILDHSGNTA.KSNQ RLKNYDTS. VPPWGRESRGTITIP CSEWVCSAQQVAVSTS: RSRHNVKSQD.H.NS	1760 XYSIP.SLLMTA XYPIP.NSPEDD LYTTTSHQISLV VYYTTTP.YPKKGI VYYTTTP.YPK ANPAPVS.ELD ASKREGI	1770 ENMDNPFQPPLHHULF SNFHNFKLNAPSHHIL HNSTSLYCMLPWHHIN KLTRNYMILLPWQHVNF MLEMPPRIQNPLISGIF TRALSKRFQNPLISGIF HQIISHRLVLPFFILS	RP RF RF RI V ZGTRQLTSSN
MuV_L			α65 <u>000000000000000000000000000000000000</u>	200 B00 1810	325 α66 <u>00000000</u> 1820	β26
MuV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	ESQTQDEISKYLRQ	NFVFS NFVFS NFVFS	LGLSSTSWYKTISVI LGLSSTAWYKGISCC STGCKISIEYILKD.L STGCKVSIKTCIGKLM GQLPTGAHYKIRSII VQWATGAHYKLKPII FTGIV <u>SSM</u> HYKLDEVI	NYLERLKLPQGD RYLERLKLPQGD KIKDPNC KDLNPKV HGMGTHYRD DDLNVFPSL WEIENFKSAV	HLYLAEGSGASMSTIET HLYIAEGSGASMTIIEY LAFIGEGAGNLLLRTVV LYFIGEGAGNWMARTAC FLSCEDGSGGMTAALLF CLVVGDGSGGISRAVLN TLAEGEGAGALLLLIQKY	TF.LPGETIW YL.FPGRKIY VELHPDIRYI CE.YPDIKFV R.ENVHSRGI M.MFPDAKLV YQVKTLF

Figure continued on the next page

MuV_L	η20 200 1840	TT	η21 <u>200</u> 1860	α67 <u>0000000</u> 1870	TT 1880	α68 <u>2000</u> 1890	α69 <u>000000000</u> 1900
MuV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	YNSLFNSGEN YNSLFSSGD YRSLKDCNDE YRSLKDCLDE FNSLLELSGS FNSLLEVNDI FNTLATESS	IPPQI IPPQI ISLPI IHYPI VMI MAS ESEIVSGMTTPI	NFAPLPTQFIE NYAPMPTQFIE EFLRLYNGI EYQRVIGEI GASPEPP GTHPLPP MLLPVMSKFHNI	SVPYRLIQAGT SVPYKLWQAHTD HSRIID LSRIID SALET SAIMR DQIEII	AGNGIVQSFYPI QYPEIFEDFIPI YG. LGGDK.SRCVNG RGGNDIVSRVIDI LN.	WNGNSDITDIS WNGNAAMTDIG ENLTIPATDAT EGLSMETTDAT ETCWEYPSDIC .NSASQITDIT	TKTSVEYIIHK. MTACVEFIINR. NNIHWSY.LHI. QKTHWDLY.LHI. DPRTWDYFLRLK NLATWKYFQSVQ NPTWFKD.QRA.
MuV_L MuV_L PIV-5_L HRSV_L HMFV_L VSIV_L RABV_L EBOV_L	TT 1910 .VGADTCALV .VGPRTCSLV .KFAEPISLE .VSKDALLI AGLGLQIDLE .RLPRQVEV	1920 HVDLESSASIN VCDASLSVIVW VCDASLSVIVW LCDAEFKDRDD VMDWEVRDSST ICDASVTDIAS TMDASTTENIN	0000000 1930 SMIERAQVHALI QCISRPIINAI SKIIIEWSKHVI FKMVILWRKHVI LKIETNVRNYVI NRITLUNGPA SKUYEAVHKLI	270 <u>β</u> 1940 LITVTVLKP TTATTVLCF RCKYCSSVMK. USCRICTTYGT. HRILDE LSIDG LHH.VDPSVLK.	29 β30 1950 GGLHILKA HGVILKY CM.HIVKYHAQC DLYFAKYHAKC QGVIYKTYG PLYVFKTYG AVVKVFLSDTE	QOOOC 1960 SWEPFNRFS SWLPFTRFS DIDFKLDN CNVKLPFF TYICESEK TMLVNPNY	α71 1970 SFLLTVLWQFFST ITTLKTYVCL ITTLKTYVCL VRSVATFIMQ NAVTILGPMFKT KAIQHLSRAFPS NDNLAPFFAT
MuV_L MuV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	331 I RILRSSYSI IRILRSSYSI ITVLRSTYSI GSI GSI GSI VDLVQTEFSS VDLVQTEFSS VTGFITQVTS GYLIKPITSS	β32 1990 PPNNHEVYIIATI PANHEVYIIATI LKGSPYILLT SSQTSPYMVCKG SSTSSELYLRFS ARSSEWILCLTN	AVDP AVDP ANNF LKKLIDEPNP RGKFFRDAEYL FLST	DWSSINESWKNI ISSTLREMSLVI	0 2010 .TTSSFTTALNF .AFQTVSQATGM IGP .YAFQSSEQEFAP FNCSSPKSEMQF TRKMPE	72 2020 2020 AMTLTDQGF. ANIFPVFNVVC HNSLPCHGEIC AKKVSTYTLI ARSLNYQDLVF QNHLSCKQVII	QQQQQQQ 2039
MuV_L MuV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	073 2020.0000 2040 WRKR.VEQGH IMPKKADKES YAAKKLDNKS VNIETMI NEMITTI WLSHLT	2000000000000 2050 IIQDCIDKVISE IDANIKS DIEANCKS QIFGVPTGVSHI IDSDVESFLVHF QYADCDL	20000 2060 CCVRQYLADNN ENALFNSSDNE L L L MVDDLELQRGT H	α74 ÎILQ IILK IPFL LSGL LTISLFYMATI LSKVAIIIAIMI LSYI	2070 	α75 2080 STRKWLDLPDY NARNLIDIPV ITKKGINTALS INKKELDRQRF RVGPIPVPPS SLEKVLYHRYN	2000000000 2099 255FNELQSEMAR VATFIEFEQLICT 5
MuV_L MuV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	α76 2100 LITIHLK NUTTHLK NVGIAITGIS HFNICCST.N SVTQHLA	200000000 2110 EVIEILKGQASI EIIDIRSGTQI GDILS SFWLSLMEKDIPI MYLSTALGDVPS HLRAEIRELTNI	2120 HDTLL.FT YESLL.LT. YSIAG.RNEV VATVG.GSKI YQQCLAVIQQS FARLHDLYNRP YNQQR.QSRT(200000 2130 SYNVGPLGKIN PYNLGLLGKIS SSNKL.INNRAS FIRWEAVSVKG TYYFRKQVIRG 2TYHF.IRTAKG	20000202 2140 TILRLIV TIVRLIT NILK GYKQKWSTRGDG NVYLSWSWSNDT RITKLVN	7 2ERILMY ERILNH ERILNH SUPKRVACNSS DYLKFFLIVQF	2250 2150 TVRNWCILPT WFNHVL WFNHVL SLAPIGNWIRSLE SLSLSHWIRLIY LKHNGTWQAEFK
MuV_L MuV_L PIV-5_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	α78 <u>000000000</u> 2160 QTRLTLRQSI SLRMIVKQDI NFR NSP KIVKTTR KLP	B33 a79 20 → 2020 2170 21 ELGEFRLRDVIT EFGIFRITSILN	α80 200000 80 219 PMEILKLSPNRI STELNYNHI KGELNYNHI LNPFNI LNPFNI LVGSII ELISVCNRI	081 200000 2200 XYEKSALNQSTF FEA FEA KDL KDL	n22 β34 2210 2210 NHLMGETSDILI RKLIEGDCNIDI FNQLCRTVD SREVERHLH IRDCNCEE	QQQQ NRAYQ TYPYLSELNS TYPNMIKLIDN NHLKW RYNRW RFLVQTLYLHF	082 2220 KRIWKA KQIWKA SLTINELKKLIKI JLGNAEIKKLIKI JLGNAEIKKLIK SNLRRN ITLEDIRSR MQDSEVKLIERL
MuV_L MuV_L HRSV_L HMPV_L VSIV_L RABV_L EBOV_L	222020 IGCVI LGCVV TGSLL TGYML TGYML SSLLD TGLISLFPDO	224 230 224 YCFGLLTPDVE YCFDPMDQRES YNFHNE WINRRISKEDE YS SLYRFD.	GSERIDVD.ND 	2260 IPDYDIHGDII SIDRGIDGEEI 			

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,
- strain SAD-B19, Uniport ID: P16289; EBOV, Uniport ID: X5H5B6.



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



Rhabdoviridae

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.