nature microbiology

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41564-018-0328-y

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Characterization of a filovirus (Měnglà virus) from *Rousettus* bats in China

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Running title: Filovirus in *Rousettus* bats in China

Key Words: filovirus, *Rousettus,* bats, NPC1, cell tropism, zoonosis

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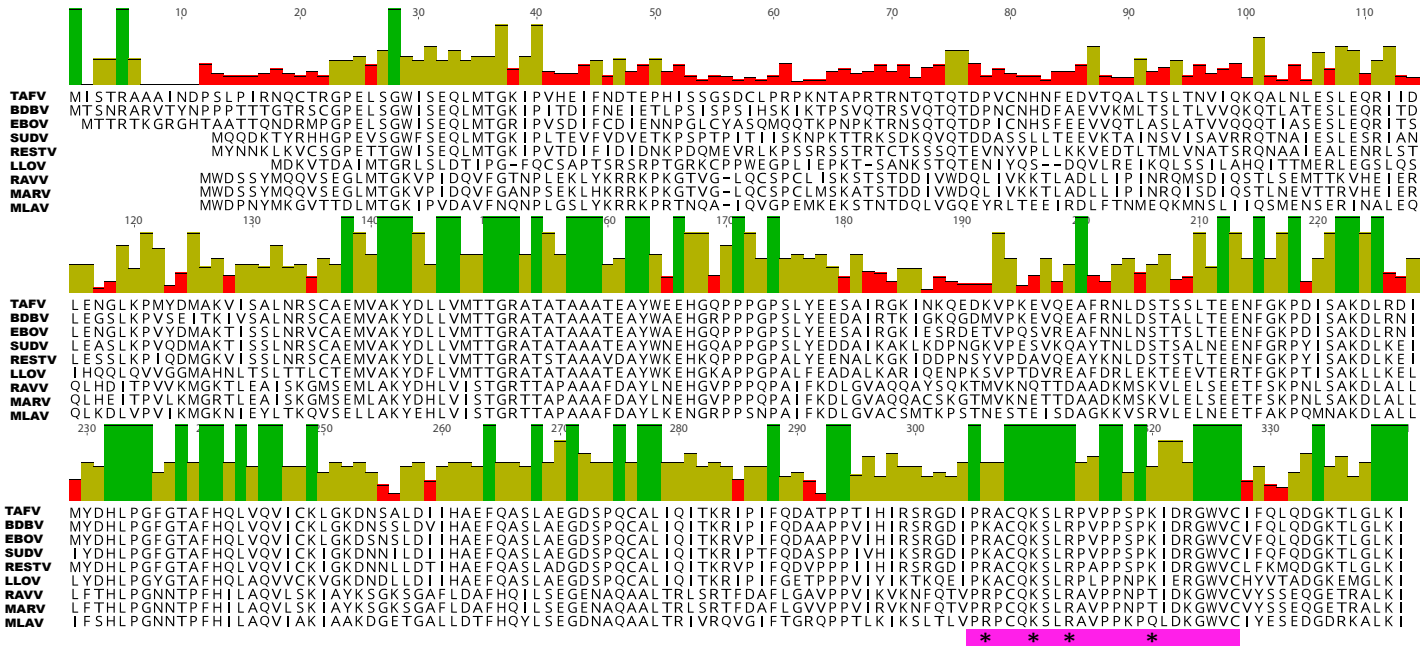
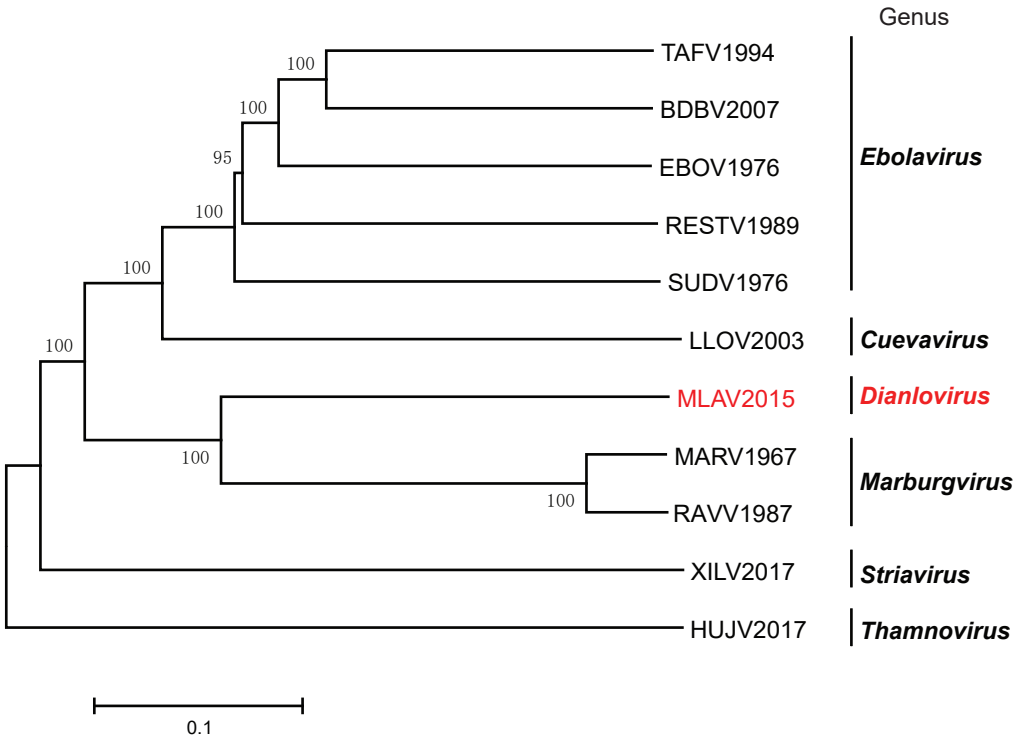
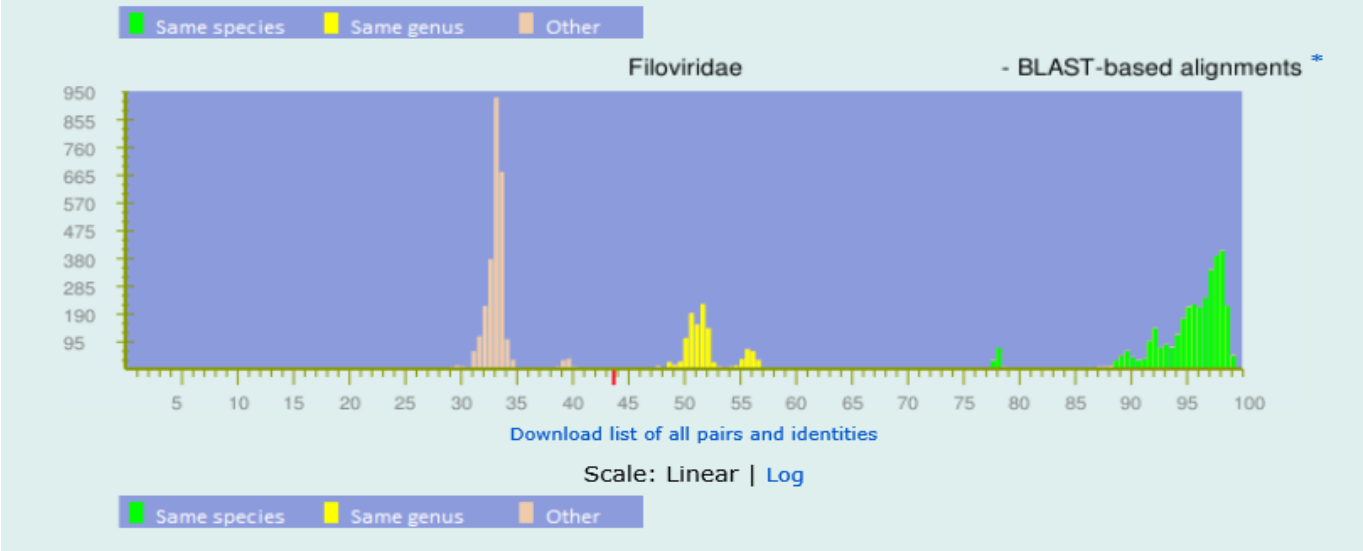


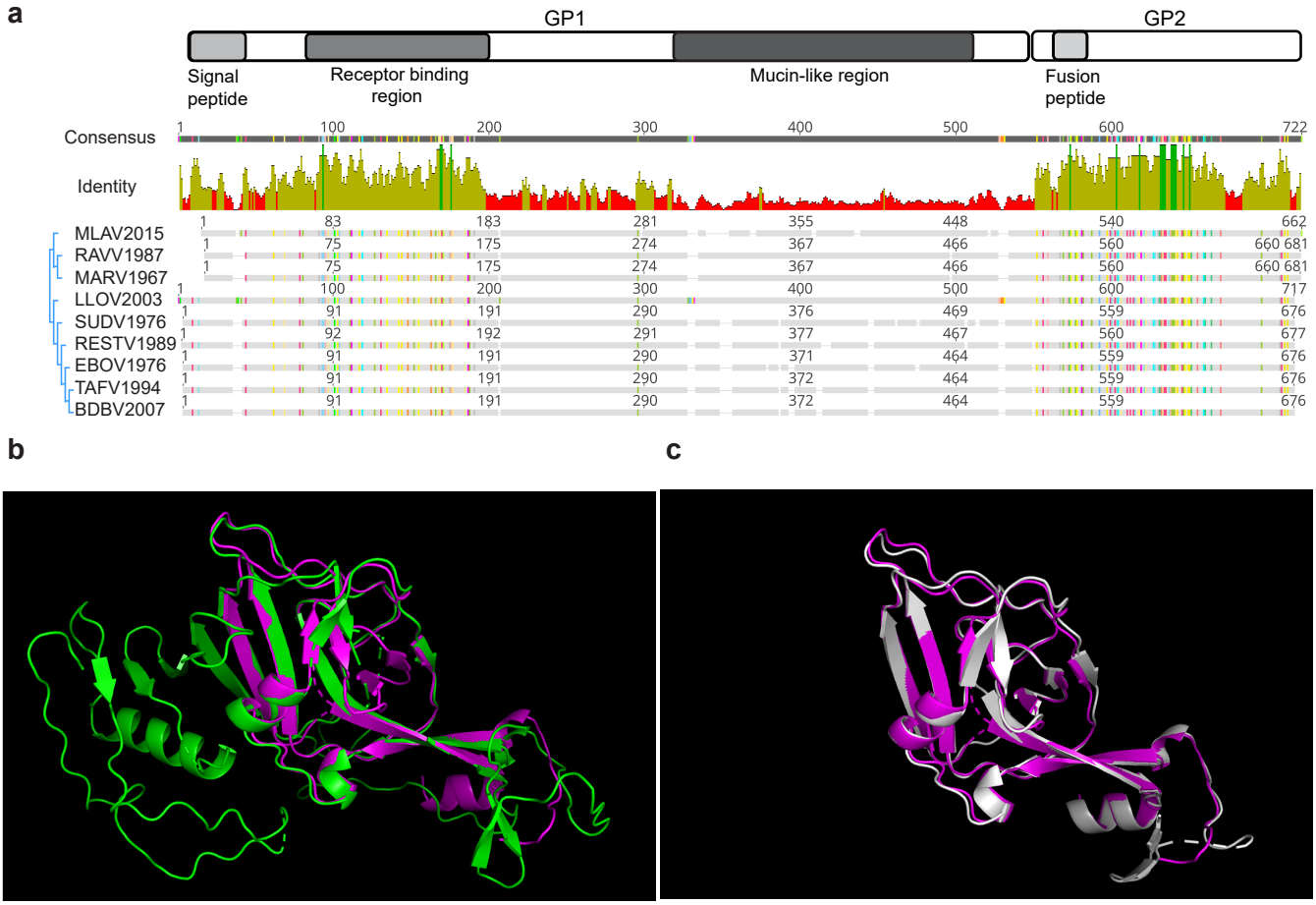
Supplementary Figure 1| Comparation of amino acid sequence of VP35 of filoviruses. Alignment results of VP35 using ClustalW in Geneious 10.0.2 (Biomatters, New Zealand). The identity (sliding window size of 1) lower than 29% is shown in red, 30%-99% in yellow, and 100% in green. Purple filled box indicate the basic amino acid motif for interferon antagonist, black asterisk indicate the conserved sites. GenBank accession numbers: NC014372 for TAFV; NC014373 for BDBV; NC002549 for EBOV; FJ968794 for SUDV; NC004161 for RESTV; NC016144 for LLOV; Z29337 for MARV; DQ447649 for RAVV.



Supplementary Figure 2| Phylogenetic tree of filoviruses based on genomic sequences. The sequence alignment was performed using ClustalW in Geneious software 10.0.2. The phylogenetic tree was built using MEGA7 using the Neighbor-Joining method with p-distance model. The bootstrap value is 1000, and the scale bar represents the units of the number of base differences per site. The position of MALV is highlighted in red. GenBank accession numbers: NC014372 for TAFV1994; NC014373 for BDBV2007; NC002549 for EBOV1976; FJ968794 for SUDV1976; NC004161 for RESTV1989; NC016144 for LLOV2003; Z29337 for MARV1967; DQ447649 for RAVV1987; MG599980 for XILV2017; MG599981 for HUJV2017.

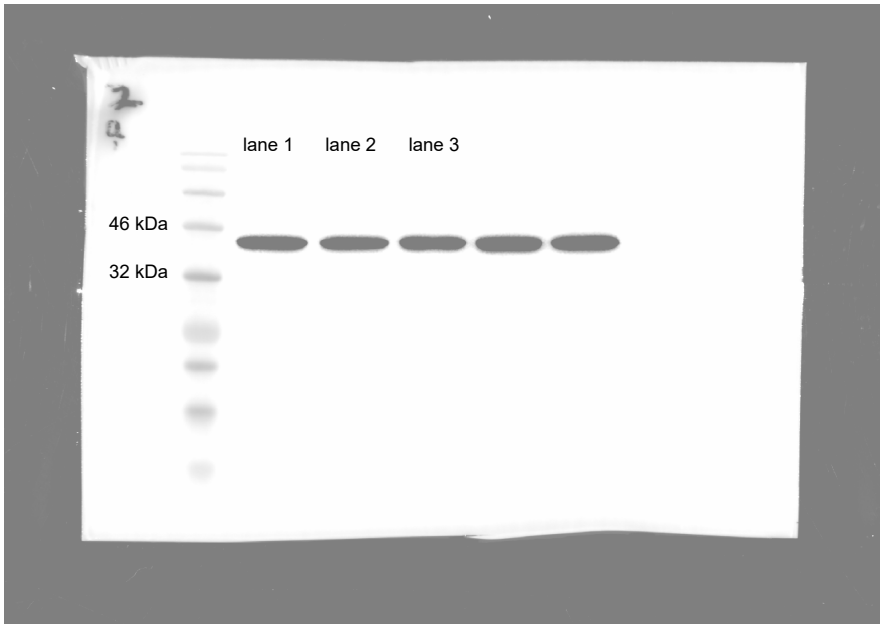


Supplementary Figure 3| PASC analysis results of coding-complete genome sequence of MLAV. Pairwise Sequence Comparison (PASC) analysis was conducted using the online tool from the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi). Shown here is a screenshot of the results obtained for MALV. The red vertical line on identity ruler represents MLAV shared 43.86% similarity with Marburgvirus (JN408064) from the BLAST-based alignments. Brown bars represent genome pairs assigned to (three) different genera; yellow bars represent genome pairs assigned to (seven) separate species; and green bars represent genome pairs assigned to the same species. BLAST: Basic Local Alignment.



Supplementary Figure 4| MLAV GP analysis based on predicted sequence and structure. a, Alignment results of GPs using ClustalW. The identity (sliding window size of 3) lower than 29% is shown in red, 30%-99% in yellow, and 100% in green. The GP functional regions are indicated on top of the alignment. **b,** The predicted structure of receptor binding region for MLAV GP in comparison with that of EBOV GP (NP_066246). The pink and green color represents MLAV and EBOV GP, respectively. **c,** The predicted structure of receptor binding region for MLAV GP in comparison with that of MARV GP (CAA82539). The pink and gray color represents MLAV and MARV GP, respectively. Structure prediction was conducted using CPHmodels3.2 (http://www.cbs.dtu.dk/services/CPHmodels/) and visualized using PyMOL 2.2.0 (https://pymol.org/2/).

a



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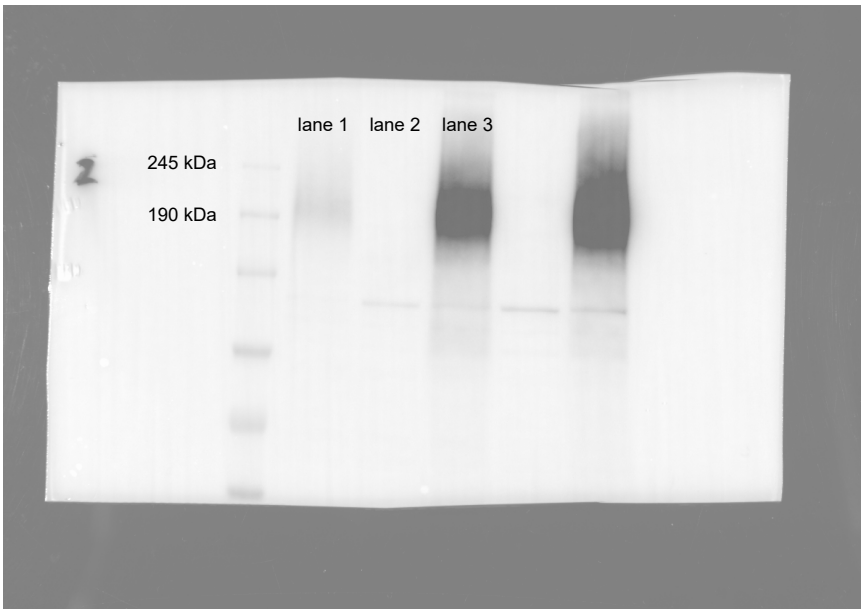

Supplementary Figure 5| Original blots of Figure 2b. a, Western blot analysis of β-actin. **b**, Western blot analysis of NPC1. The size of molecular weight markers are shown. Lane 1, normal HEK293; lane 2, HEK293△NPC1; lane 3, HEK293△NPC1 with stably expressed hNPC1.

Supplementary Table 1. Genome features of MLAV and gene comparison with other filoviruses

MLAV		Percentage (%) sequence identity (nt/aa)							
Gene	**ORF nt/aa**	**TAFV**	**BDBV**	**EBOV**	**SUDV**	**RESTV**	**LLOV**	**RAVV**	**MARV**
Coding-complete genome	18300/na	40/na	41/na	41/na	41/na	40/na	39/na	54/na	54/na
NP	2094/697	45/35	45/ 35	45/ 36	45/35	44/34	43 /33	60/56	61/56
VP35	990/329	50/35	47/34	48/34	47/34	48/33	47/32	61/57	59/55
VP40	915/304	41/25	41/25	40/26	39/26	40/26	38/27	60/59	59/58
GP	1989/662	39/24	39/24	39/23	38/25	38/25	35/22	53/39	53/39
VP30	882/293	45/37	47/37	48/38	45/34	46/38	45/33	56/51	57/57
VP24	762/253	40/28	42/28	39/28	40/27	43/28	40/25	58/57	56/52
L	6831/2276	51/45	51/45	50 /46	50 /45	50/45	49/44	60/56	60/56

MLAV, Měngl à virus, KX371887; TAFV, Ta ïforest virus, NC014372; BDBV, Bundibugyo virus, NC014373; EBOV, Ebola virus, NC002549; SUDV, Sudan virus, FJ968794; RESTV, Reston virus, NC004161; LLOV, Lloviu virus, NC016144; MARV, Marburg virus, Z29337; RAVV, Ravn virus, DQ447649

Supplementary Table 2. Cell tropism of MLAV compared with EBOV and MARV.

Cell line	Host	Origin of cell line	Pseudotype susceptibility*			
			MLAV-GP	EBOV-GP	MARV-GP	VSV-GP
A549	Human	*Homo sapiens* lung	+	++	+	++
HeLa		*H. sapiens* cervix	+	++	+	+
HEK293		*H.sapiens* embryonic kidney	+++	+++	+++	+++
RlKi	Bat**	*Rousettus leschenaultii* kidney	+++	+++	+++	+++
MdKi		*Myotis davidii* kidney	+	+	+	+
EsKi		*Eonycteris spelaea* kidney	+	+	+	+
EsLu		*E. spelaea* lung	+	+	+	+
EsIn		*E. spelaea* intestine	++	++	+	++
VeroE6	Monkey	*Chlorocebus aethiops* kidney	+++	+++	+++	+++
LLC-MK2		*Macaca mulatta* kidney	++	+++	+++	+++
MDCK	Dog	*Canis familiaris* kidney	++	+++	++	+
BHK21	Hamster	*Mesocricetus auratus* kidney	+++	+++	+++	+++

* Efficiency of transduction is defined by GFP positive cell ratio; +, <5%; ++, between 5% and 10%; +++, >10% (3 independent experiments were repeated for all the cell lines). VSV-GP is included as a baseline control for comparison of relative susceptibility/efficiency of different cell lines using the VSV system.

**All bat cell lines are primary cell line.