## High Pathogenicity of Nipah Virus from *Pteropus lylei* Fruit Bats, Cambodia

## Appendix

Appendix Table. Genetic pairwise comparisons of NiV nonstructural proteins V, W, and C between NiV/Cambodia and other NiV isolates\*

	Nonstructural NiV proteins, % nucleotide identity/% homology of deduced amino						
	acid						
NiV, GenBank accession no.	V	W	С				
Nipah/Malaysia/2000/human, NC_002728.1	97.2/95.7	97.3/95.8	98.6/98.2				
Nipah/Malaysia/2010/Pvampyrus, FN869553.1	97.1/95.5	96.6/94.0	99.4/100				
Nipah/Bangladesh/2004/human, AY988601.1	92.4/89.4	92.5/89.6	97.6/97.0				
Nipah/Bangladesh/2008/human, JN808863.1	91.8/88.5	91.9/88.7	97.4/97.0				
Nipah/India/2007/human, FJ513078.1	92.1/88.9	92.2/89.1	97.6/97.0				
Nipah/India/2018/human, MH396625.1	91.7/88.0	91.8/88.0	97.2/96.4				

\* NiV, Nipah virus.

†The nucleotide identity and deduced amino acid homology were calculated using p-distance method.



**Appendix Figure 1.** Maximum-likelihood phylogenetic trees based on genes coding for *Henipavirus*. A) Phosphoprotein, B) Matrix protein, C) Fusion protein, D) Attachment glycoprotein, and E) Polymerase. The Cambodia 2003 Nipah virus isolate CSUR381 (GenBank MK801755, red triangle) clusters with NiV-Malaysia isolates. Bootstrap statistical support is shown on branch nodes. Hasegawa-Kishino-Yano model and Tamura-3-parameter were calculated as the best DNA model to conduct the analysis for these genes. The phylogenetic trees are drawn to scale and scale bar represents branch lengths measured in the number of substitutions per site.

- -	10	20	30	40	50	60 7	0 80	90	100 110
MK801755 Nipah/Cambodia/2003/Pteropuslylei AY029767.1 Nipah/Malaysia/2001/human AY988601 1 Nipah/Bangladesh/2004/human	MPAESKKVRFE	NTTSDKGKIPS	VV	DIRKINEGLLI	SKILSAFNTVIAL	LGSIVIIVMNIMII	CNYTRSTENGAV	IKDALQGIQQQIKG	SLADKIGTEIGPKVSLI
MK801755 Nipah/Cambodia/2003/Pteropuslylei AY029767.1 Nipah/Malaysia/2001/human AY988601.1_Nipah/Bangladesh/2004/human	110 1 VSLIDTSSTIT	20 13 IPANIGLLGSK	0 14 ISQSTASINE	0 150 NVNEKCKFTLF	160 PLKIHECNISCPN	170 PLPFREYRPCTEGV	180 190 SNLVGLPNNICLO	200 2KTSNGILKPKLIS	210 220 SYTLPVVGCSGTCITDP
						ĸ			
MR801755 Nipah/Cambodia/2003/Pteropuslylei AY029767.1 Nipah/Malaysia/2001/human AY988601.1_Nipah/Bangladesh/2004/human	GTCITDPLLAN	DEGYFAYSHLE	RIGSCSRGVS	RCKIIGVGEVI	DRGDEVPSLEMTN	VWTPPNPNTVYHCs	AVYNNEFYYVLC:	AVSTVGDPILNSTY	WSGSLMMTRLAVKPKS
			K			s			N
	330	340	350	360	370 380	390	400	410 420	430 <b>•</b>
MK801755 Nipah/Cambodia/2003/Pteropuslylei AY029767.1 Nipah/Malaysia/2001/human AY988601.1_Nipah/Bangladesh/2004/human	.GF.	LRSIEKGRYDK	VMPYGPSGIR	QGDTLYFPAVG	FLVRTEFRYNDSN	CPITKCQYSKPENC	RLSMGIRPNSHY	/LRSGLLKYNLSDG	ENPRIVFIEISDORLS
	440	450	460	470	480 49	0 500	510	520 53	0 540
MK801755 Nipah/Cambodia/2003/Pteropuslylei AY029767.1 Nipah/Malaysia/2001/human AY988601.1_Nipah/Bangladesh/2004/human	RLSIGSPSKIY	DSLGQPVFYQA	SESWDTMIKE	GDVGTVNPLVV	SWRDNTVISRPGC NN N	SCCPRENTCPEICW	EGVYNDAFLIDR	INWISAGVFLDSNG	TAENPVFTVFKDNEIL
MK801755 Nipah/Cambodia/2003/Pteropuslylei AY029767.1 Nipah/Malaysia/2001/human AY988601.1_Nipah/Bangladesh/2004/human	530 ISNCTAENPVE	540 TVFKDNEILYR	550 ACLASEDTNA	560 5 QKTITNCFLLK	70 580 NRIWCISLVEIYD	590 TGDNVIRPKLFAVK	600 IPECCT*- *		

**Appendix Figure 2.** Amino acid multiple alignment of the attachment glycoprotein (G) of three Nipah virus (NiV) isolates used in the study. Specific amino acid changes were found in NiV/Cambodia G sequence (V24 $\rightarrow$ I, R248 $\rightarrow$ K, G327 $\rightarrow$ D, I408 $\rightarrow$ V, N478 $\rightarrow$ S). The glycosylation site in G attachment protein (N529/Q530/T531) is conserved among the three tested isolates (marked in red). Polymorphism at Ephrin B2 and B3 potential binding sites was not observed (W504, Q530, T531, A532, and N557, E505, E533, respectively).



**Appendix Figure 3.** Amino acid multiple alignment of the fusion protein (F) of three Nipah virus (NiV) isolates used in the study. Specific NiV/C amino acid changes were found in the beginning of F2 subunit ( $I4 \rightarrow V$ ,  $I15 \rightarrow M$ ). Fusion protein cleavage region (N104-R109, marked in red) and fusion peptide (L110-V133, marked in blue) are preserved among all analyzed NiV isolates. Predicted N-terminal and C-

terminal heptad repeat regions (HRN and HRC) are marked in green and orange respectively. V159 $\rightarrow$ I mutation was observed in HRN region, while no variability was found within the HRC.