

# Supporting information

## **A tick-borne segmented RNA virus contains genome segments derived from unsegmented viral ancestors**

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# Table S1

**Table S1.** Genetic identities between putative JMTV proteins and their homologs from major groups of flaviviruses, including West Nile Virus (WNV), Dengue Virus 1 (DENV1), Tick-borne Encephalitis Virus (TBEV), Apoi virus (APOIV), Cell Fusion Agent (CFA), Tamana bat virus (TABV) and from the cDNA library of *Toxocara canis* (TCLA). Representatives of the more divergent hepatitis C virus (HCV) and pestiviruses (Pesti) are also included.

	<b>TCLA</b>	<b>WNV</b>	<b>DENV1</b>	<b>YFV</b>	<b>TBEV</b>	<b>APOIV</b>	<b>CFAV</b>	<b>TABV</b>	<b>HCV</b>	<b>Pesti</b>
<b>NSP1 (NS5)</b>	0.337*	0.245	0.246	0.234	0.242	0.249	0.252	0.253	0.140	0.176
<b>NSP2 (NS2b-NS3)</b>	0.248	0.215	0.236	0.230	0.229	0.227	0.225	0.209	0.169	0.185
<b>VP1</b>	0.222	–	–	–	–	–	–	–	–	–
<b>VP2</b>	0.194	–	–	–	–	–	–	–	–	–
<b>VP3</b>	0.218	–	–	–	–	–	–	–	–	–

\*The predicted NS5-like protein for TCLA is incomplete compared to JMTV and other members of the *Flaviviridae*. Only partial sequence is involved in similarity comparisons.

# Table S2

**Table S2. Background information on the JMTV positive samples**

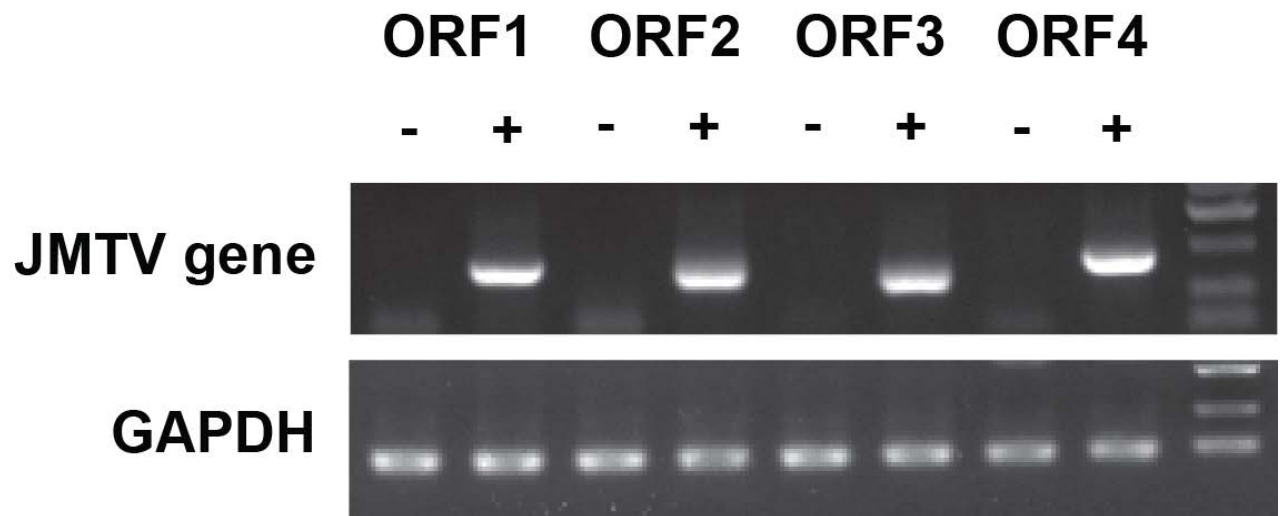
Strain	Host Species	Place of sampling	GenBank Accession	Strain	Host Species	Place of sampling	GenBank Accession
JMTVX01	<i>Haemaphysalis longicornis</i>	Wuhan	KJ001590	JMTV60	<i>Rhipicephalus microplus</i>	Enshi	KJ001615
JMTVX06L	<i>Haemaphysalis campanulata</i>	Wuhan	KJ001591	JMTV62	<i>Rhipicephalus microplus</i>	Enshi	KJ001616
JMTVX08	<i>Haemaphysalis longicornis</i>	Wuhan	KJ001592	JMTV67	<i>Rhipicephalus microplus</i>	Enshi	KJ001617
JMTVX23	<i>Haemaphysalis longicornis</i>	Wuhan	KJ001593	JMTV69A	<i>Rhipicephalus microplus</i>	Enshi	KJ001618
JMTVX27	<i>Rhipicephalus microplus</i>	Wuhan	KJ001594	JMTV69B	<i>Rhipicephalus microplus</i>	Enshi	KJ001619
JMTVX35	<i>Rhipicephalus microplus</i>	Wuhan	KJ001595	JMTV72	<i>Rhipicephalus microplus</i>	Yichang	KJ001620
JMTVX37	<i>Rhipicephalus microplus</i>	Wuhan	KJ001596	JMTV84	<i>Rhipicephalus microplus</i>	Jingmen	KJ001621
JMTVX39H	<i>Haemaphysalis campanulata</i>	Wuhan	KJ001597	JMTV85	<i>Rhipicephalus microplus</i>	Jingmen	KJ001634
JMTVX39L	<i>Haemaphysalis longicornis</i>	Wuhan	KJ001598	JMTV90	<i>Rhipicephalus microplus</i>	Jingmen	KJ001622
JMTVx41	<i>Rhipicephalus microplus</i>	Wuhan	KJ001599	JMTV112	<i>Rhipicephalus microplus</i>	Xiangfan	KJ001623
JMTVX55	<i>Haemaphysalis flava</i>	Wuhan	KJ001600	JMTV118	<i>Rhipicephalus microplus</i>	Xiangfan	KJ001624
JMTVH101	<i>Ixodide sinesis</i>	Wuhan	KJ001630	JMTV123	<i>Rhipicephalus microplus</i>	Xiangfan	KJ001625
JMTV11	<i>Rhipicephalus microplus</i>	Shiyan	KJ001603	JMTV129	<i>Rhipicephalus microplus</i>	Xiangfan	KJ001626
JMTV12	<i>Rhipicephalus microplus</i>	Shiyan	KJ001604	JMTVZY1-7	<i>Haemaphysalis flava</i>	Xiangfan	KJ001601
JMTV16	<i>Rhipicephalus microplus</i>	Shiyan	KJ001605	JMTVZY2-4	<i>Haemaphysalis flava</i>	Xiangfan	KJ001602
JMTV19	<i>Rhipicephalus microplus</i>	Shiyan	KJ001606	JMTV138	<i>Rhipicephalus microplus</i>	Jingmen	KJ001627
JMTV29	<i>Rhipicephalus microplus</i>	Shiyan	KJ001632	JMTV141	<i>Rhipicephalus microplus</i>	Jingmen	KJ001628
JMTV33	<i>Rhipicephalus microplus</i>	Shennongjia	KJ001607	JMTV157	<i>Rhipicephalus microplus</i>	Jingmen	KJ001629
JMTV34	<i>Rhipicephalus microplus</i>	Shennongjia	KJ001608	JMTVWC3	<i>Ixodes granulatus</i>	Wenzhou	KJ001633
JMTV36	<i>Haemaphysalis longicornis</i>	Shennongjia	KJ001631	JMTVningbo	<i>Rhipicephalus sanguineus</i>	ningbo	KJ001589
JMTV39	<i>Rhipicephalus microplus</i>	Shennongjia	KJ001609	JMTVH115-1	<i>Haemaphysalis longicornis</i>	Wuhan	KJ001584
JMTV47	<i>Rhipicephalus microplus</i>	Yichang	KJ001610	JMTVH115-4	<i>Haemaphysalis longicornis</i>	Wuhan	KJ001585
JMTV48	<i>Rhipicephalus microplus</i>	Yichang	KJ001611	JMTVH115-5	<i>Haemaphysalis longicornis</i>	Wuhan	KJ001586
JMTV49	<i>Rhipicephalus microplus</i>	Yichang	KJ001612	JMTVH118-6	<i>Haemaphysalis longicornis</i>	Wuhan	KJ001587
JMTV52	<i>Rhipicephalus microplus</i>	Enshi	KJ001613	JMTVX114-1	<i>Haemaphysalis longicornis</i>	Wuhan	KJ001588
JMTV53	<i>Rhipicephalus microplus</i>	Enshi	KJ001614	JMTVDJ1-1	<i>Armigeres sp</i>	Wuhan	KJ001583

# Table S3

**Table S3. Background information on de-pooled JMTV positive samples**

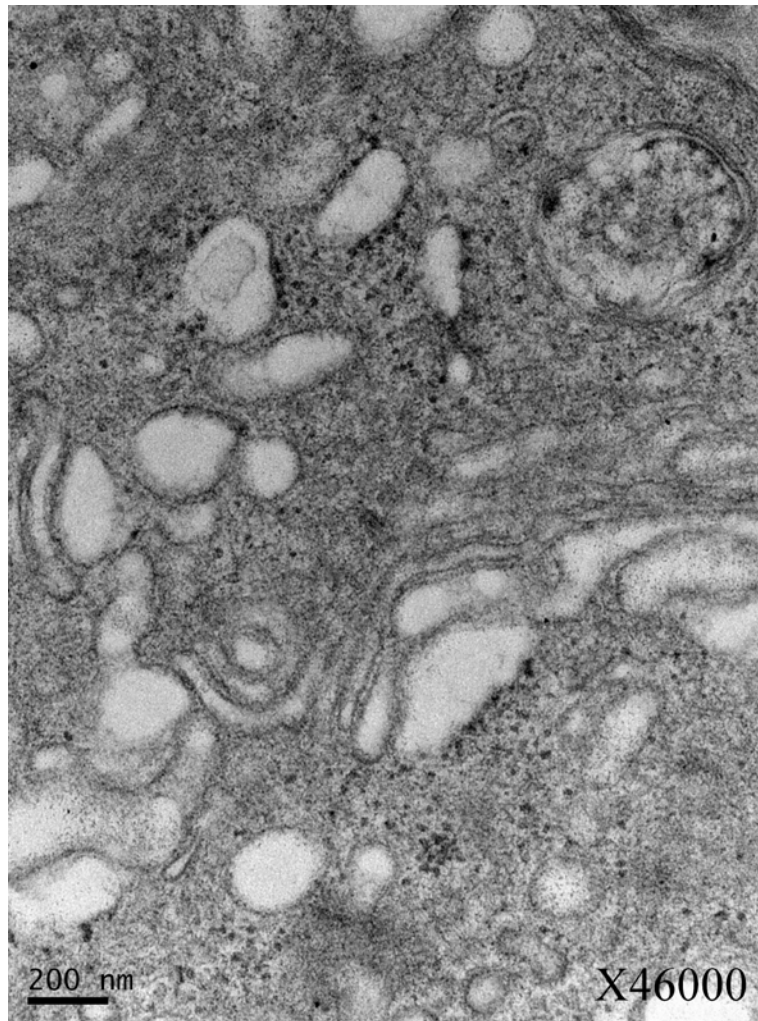
Strain	Host species	Place of Sampling	GenBank Accession			
			Segment 1	Segment 2	Segment 3	Segment 4
YS102-1	<i>Rhipicephalus microplus</i>	Wuhan	KJ001547	KJ001555	KJ001563	KJ001571
YJ3-3	<i>Rhipicephalus microplus</i>	Wenzhou	KJ001548	KJ001556	KJ001564	KJ001572
10	<i>Rhipicephalus microplus</i>	Shiyan	KJ001549	KJ001557	KJ001565	KJ001573
84	<i>Rhipicephalus microplus</i>	Jingmen	KJ001550	KJ001558	KJ001566	KJ001574
85	<i>Rhipicephalus microplus</i>	Jingmen	KJ001551	KJ001559	KJ001567	KJ001575
164	<i>Rhipicephalus microplus</i>	Jingmen	KJ001552	KJ001560	KJ001568	KJ001576
204	<i>Rhipicephalus microplus</i>	Jingzhou	KJ001553	KJ001561	KJ001569	KJ001577
X84-3	<i>Rhipicephalus microplus</i>	Wuhan	KJ001554	KJ001562	KJ001570	KJ001578

# Figure S1



**Figure S1.** Detection of four JMTV segments in infected and uninfected DH82 cells. The GAPDH gene is used as a cellular control.

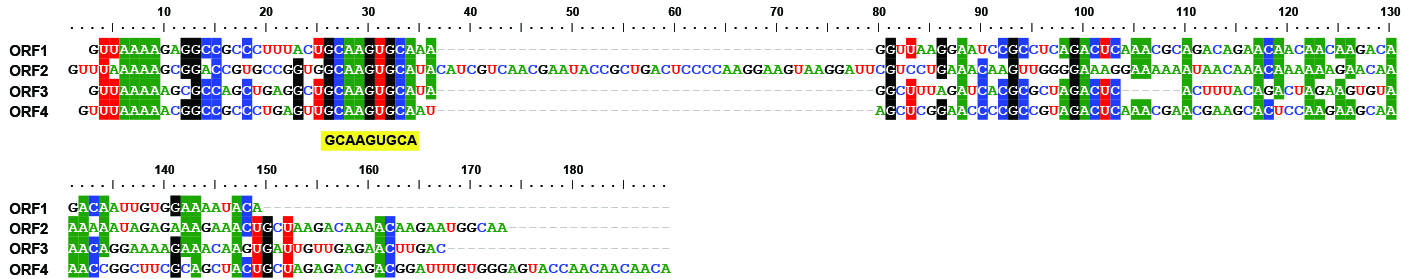
## Figure S2



**Figure S2.** Transmission electron micrograph of uninfected DH82 cells.

# Figure S3

(A)



(B)

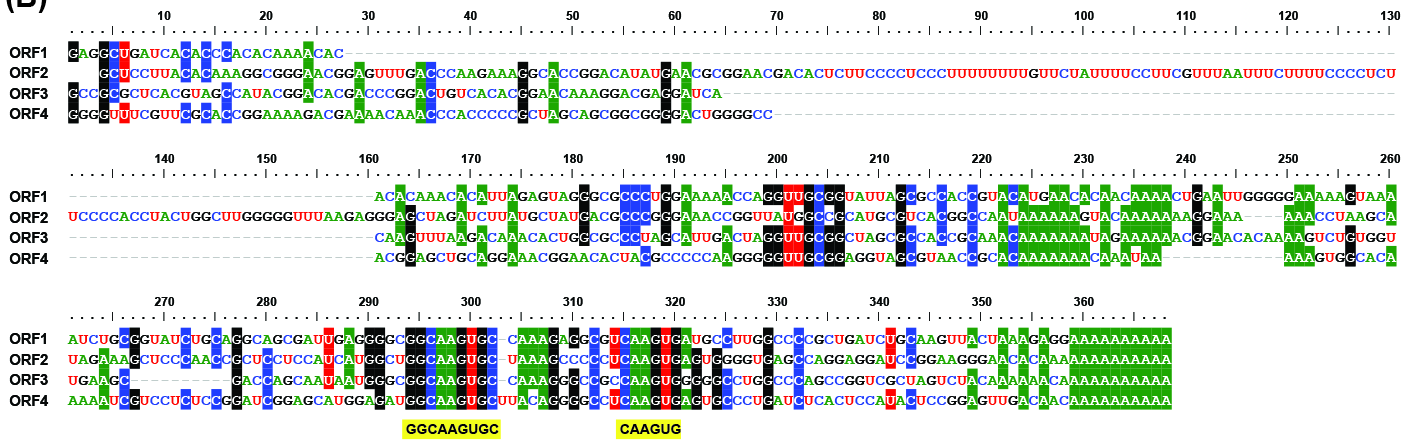


Figure S3. Nucleotide sequence alignment of A) 5' and B) 3' UTRs of the four JMTV segments. The conserved nucleotides are indicated below the sequence and are shaded yellow.

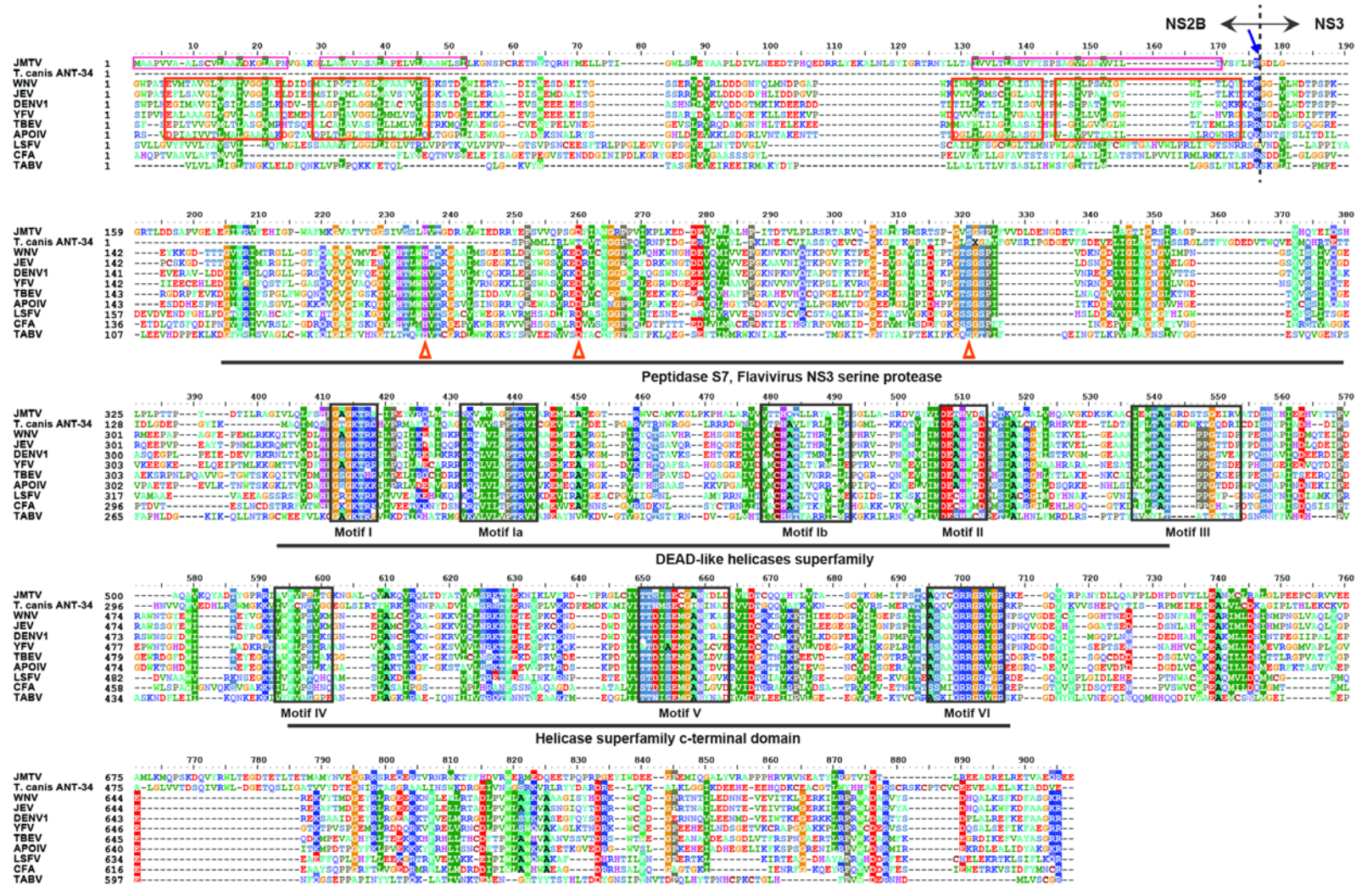
# Figure S4



**Figure S4.** Amino acid alignment of the NS5 proteins of JMTV, TCLA, and representatives from the genus *Flavivirus*. This shows the results of the homology search against the Conserved Domain Database in NCBI (grey lines) and the conserved motifs of the flavivirus methyltransferase and RdRp (grey boxes). Also shown are the predicted transmembrane domains (pink box), signal peptide cleavage site (pink arrows), and serine protease cleavage site (blue arrows) of JMTV.



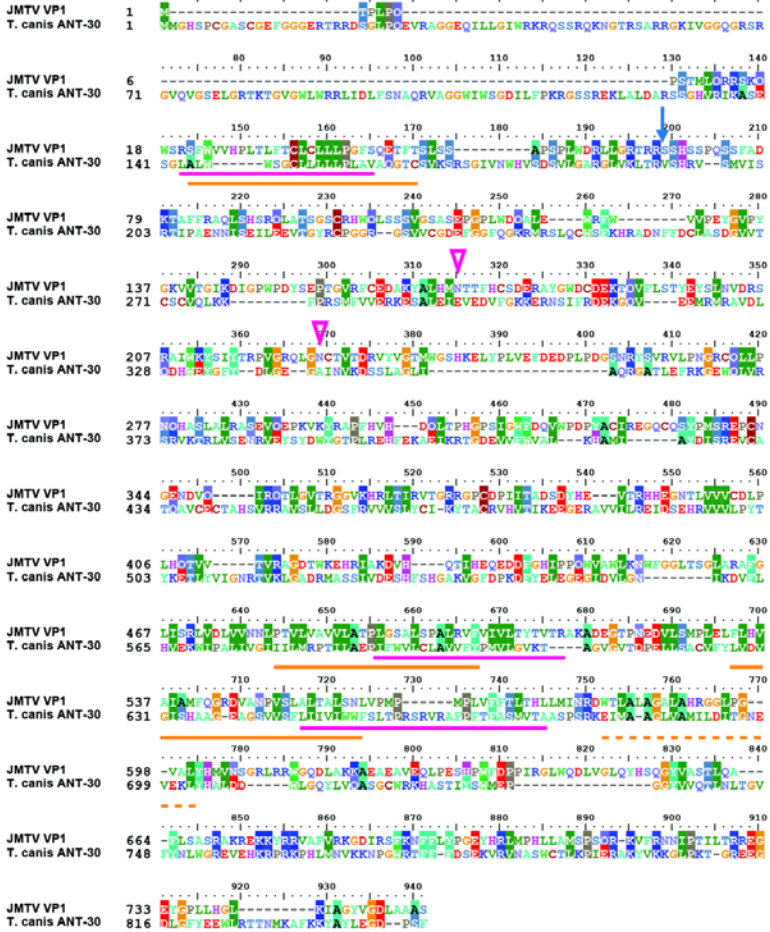
# Figure S5



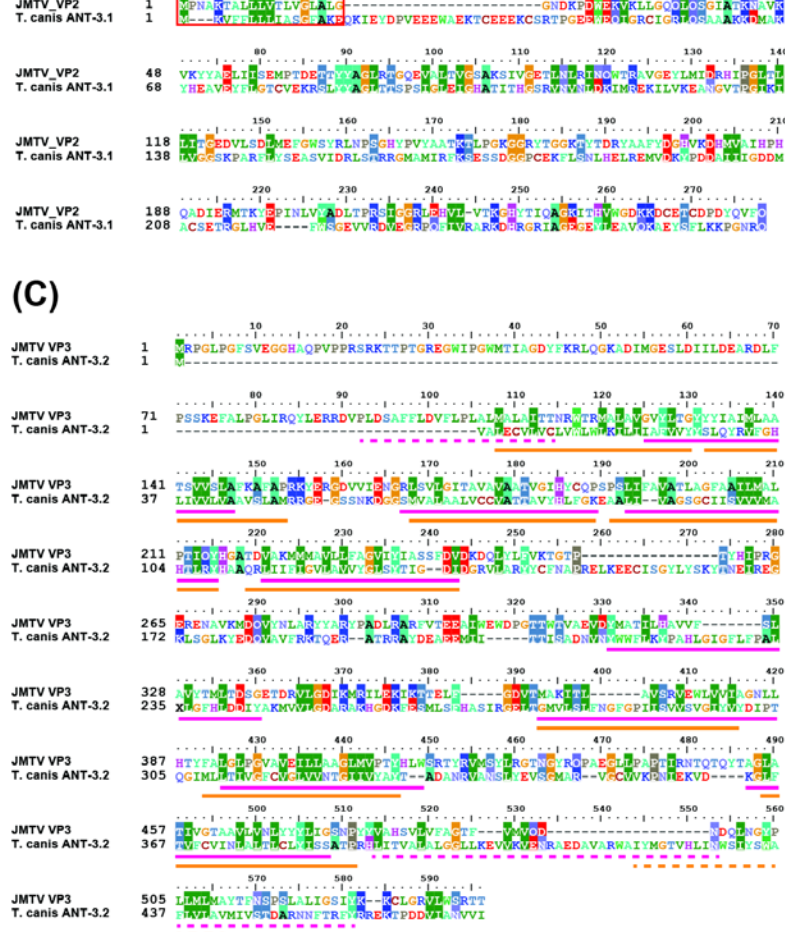
**Figure S5.** Amino acid alignment of the NS2b-NS3 complex of JMTV, TCLA, and representatives from the genus *Flavivirus*. This shows the results of the homology search against the Conserved Domain Database in NCBI (grey lines), the transmembrane domains of the flavivirus NS2B protein (orange boxes), the conserved motifs of the flavivirus helicase (grey boxes), and the positions of the catalytic triad in serine protease (orange triangles). Also shown are the predicated transmembrane domains (pink boxes) and serine protease cleavage site (blue arrow) of JMTV.

# Figure S6

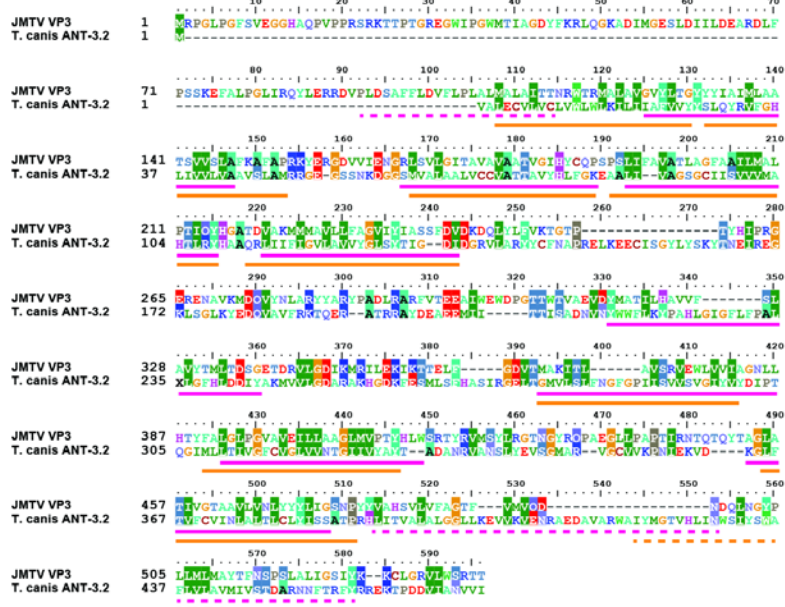
(A)



(B)

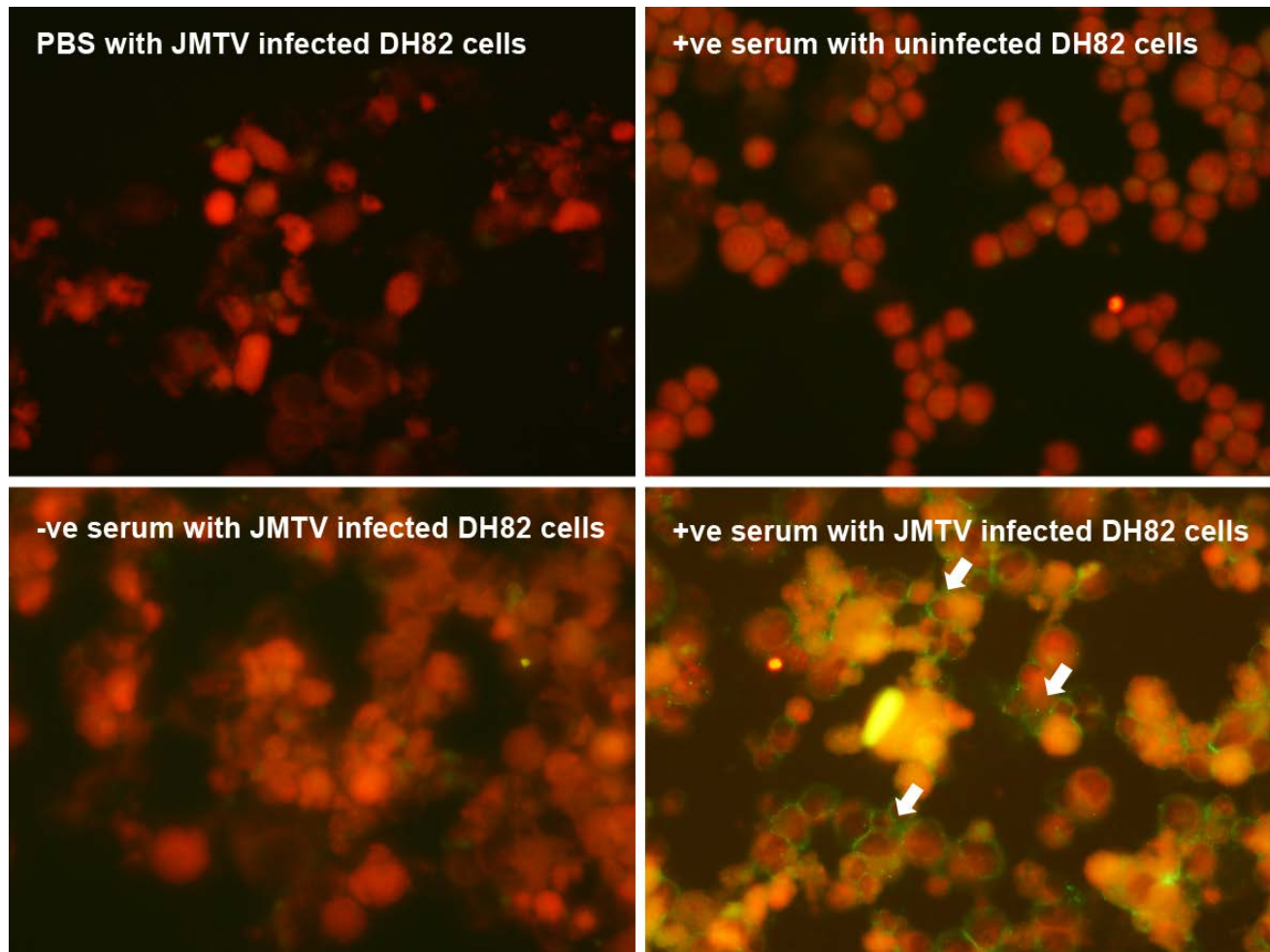


(C)



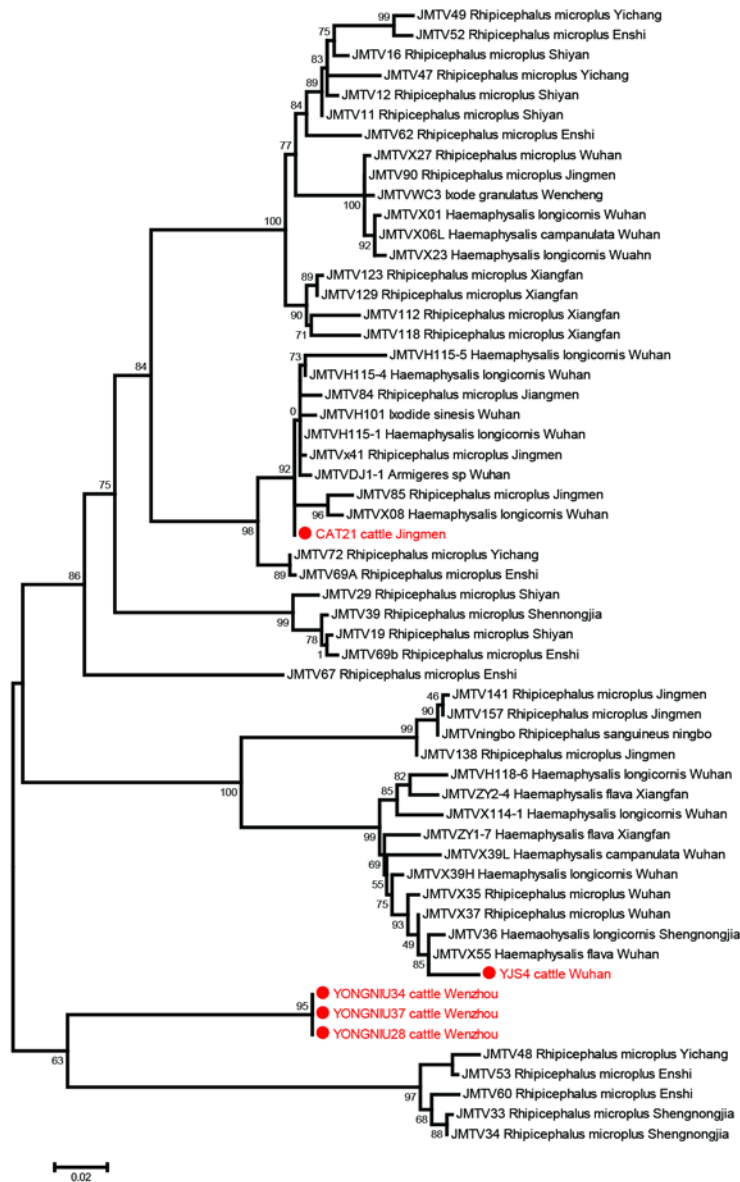
**Figure S6.** Amino acid alignments of the A) VP1, B) VP2 and C) VP3 of JMTV to their corresponding homologs in TCLA, which shows the predicated transmembrane domains (pink lines), serine protease cleavage site (blue arrow), signal peptides (red box), and N-linked glycosylation sites (pink triangles) of JMTV. Also shown are the predicated transmembrane domains (yellow lines), serine protease cleavage site (blue arrow), and signal peptides (red box) of TCLV.

## Figure S7



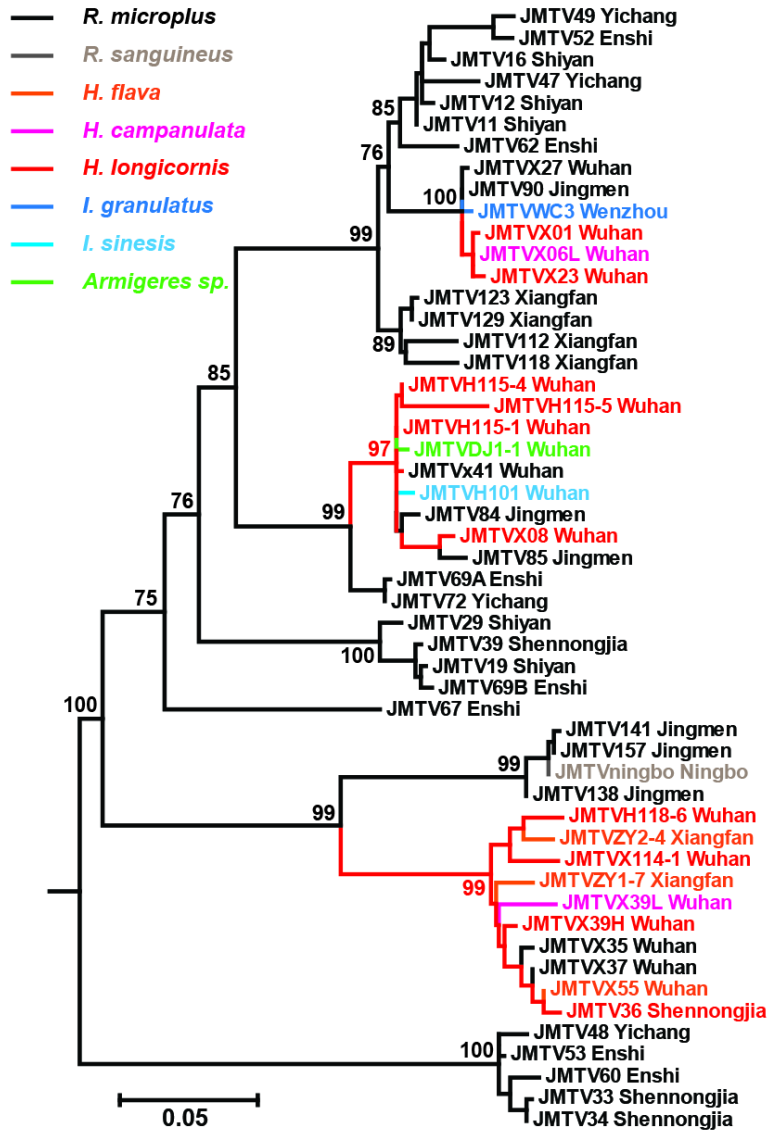
**Figure S7.** Typical serological reactivity of cattle sera with viral antigens in JMTV infected DH82 cells (lower right). The controls are PBS and JMTV negative serum with JMTV infected DH82 cells, and JMTV positive serum with uninfected DH82 cells. The arrows indicate specific staining of cytoplasmatic antigen.

# Figure S8



**Figure S8.** The phylogenetic positions (NSP2 gene) of JMTVs detected from cattle blood. The tree is mid-point rooted and all horizontal branches are drawn to a scale of nucleotide substitutions per site. The cattle blood samples are highlighted in red. The taxon labels contain strain names, host (species), and sample locations.

# Figure S9



**Figure S9.** ML phylogenetic tree of JMTV NSP2 gene sequences sampled from various geographic locations across China. The tree is mid-point rooted and all horizontal branches are drawn to a scale of nucleotide substitutions per site. For clarity, bootstrap values are only shown for internal nodes. The branch and taxon labels are colored based on arthropod species. The taxon labels contain strain names and sample locations for each pool.

**Figure S10**



**Figure S10.** Map of Hubei and Zhejiang provinces showing the locations of sample collection sites.