

## SUPPLEMENTARY FIGURES

**Supplementary Table S1.** Primer sequences used for the construction of WNV nonstructural (NS) gene plasmids.

WNV gene constructs	Primer	Nucleotide Position*	Primer Sequence (5' → 3')
NS1	forward	2398 – 2418	accaccatg <sup>a</sup> ATAGGTCCATAGCTCTCACG
	reverse	3502 – 3525	AGCATTCACCTTGTGACTGCACGAG
NS2B	forward	4219 – 4234	accaccatg <sup>a</sup> GGATGGCCCCGCAACTG
	reverse	4686 – 4611	TCTCTTTGTGTATTGGAGAGTTATCC
NS3	forward	4612 – 4631	accaccatg <sup>a</sup> GGAGGCGTGTGTGGGACAC
	reverse	6444 – 6468	GACGTTTTCCCGAGGCGAAGTCCTTG
NS4B-NS5	forward <sup>b</sup>	6865 – 6882	accaccatg <sup>a</sup> <i>gct</i> <sup>c</sup> CTAGCCGTGTTCTCCTGATT
	reverse	10370 – 10395	GCAGTACTGTGTCCTCAACCAAAGTTG
NS5	forward	7681 – 7698	accaccatg <sup>a</sup> GTGGGGCAAAGGACGC
	reverse	10370 – 10395	GCAGTACTGTGTCCTCAACCAAAGTTG

<sup>a</sup>Kozak sequence is lowercase, and the viral sequences are in uppercase. <sup>b</sup>NS4B forward primer includes 17-aa signal sequence at the -COOH end of NS4A. <sup>c</sup>Codon filling nucleotides are lowercase and italicized. \*GenBank Accession No. DQ211652.

**Supplementary Table S2.** Antibodies and dilutions used for immunofluorescence and western blot staining.

Protein	Primary Ab	Catalog Number	IF	WB	Secondary Antibody	Catalog Number	IF	WB
Dilutions				Dilutions				
WNV NS5	rabbit pAb	GTX131961 <sup>a</sup>		1:3,000	anti-rabbit IRDye 800CW	926-32211 <sup>b</sup>		1:10,000
WNV NS3	rabbit pAb	GTX131955 <sup>a</sup>		1:3,000	anti-rabbit IRDye 800CW	926-32211 <sup>b</sup>		1:10,000
WNV NS2B	rabbit pAb	GTX132060 <sup>a</sup>		1:3,000	anti-rabbit IRDye 800CW	926-32211 <sup>b</sup>		1:10,000
JEV NS4B	rabbit pAb	GTX125865 <sup>a</sup>		1:4,000	anti-rabbit IRDye 800CW	926-32211 <sup>b</sup>		1:10,000
			1:100		anti-rabbit IgG Pacific Blue	P10994 <sup>b</sup>	1:500	
Calnexin	rabbit pAb	C4731 <sup>c</sup>	1:100		anti-rabbit IgG AF 555	A21428 <sup>d</sup>	1:1,000	
				1:2,000	anti-rabbit IRDye 800CW	926-32211 <sup>b</sup>		1:10,000
Giantin	rabbit pAb	ab80864 <sup>e</sup>	1:100		anti-rabbit IgG AF 555	A21428 <sup>d</sup>	1:1,000	
Tubulin β	rabbit pAb	RB9249 <sup>d</sup>	1:100		anti-rabbit IgG AF 555	A21428 <sup>d</sup>	1:1,000	
	mouse mAb	sc-5274 <sup>f</sup>		1:1,000	anti-mouse IRDye 680RD	926-68170 <sup>b</sup>		1:10,000
IκB kinase subunit ε (IKKε)	rabbit mAb	2905S <sup>g</sup>	1:100		anti-rabbit IgG AF 555	A21428 <sup>d</sup>	1:1,000	
Green fluorescent protein (GFP)	rabbit pAb	G10362 <sup>d</sup>	1:100		anti-rabbit IgG AF 488	A11008 <sup>d</sup>	1:1,000	
				1:4,000	anti-rabbit IRDye 800CW	926-32211 <sup>b</sup>		1:10,000
V5/His epitope	mouse mAb	R960-25 <sup>c</sup>	1:100		anti-mouse IgG Pacific Blue	P31582 <sup>b</sup>	1:500	
				1:2,000	anti-mouse IRDye 680RD	926-68170 <sup>b</sup>		1:10,000

<sup>a</sup>Genetex, Irvine, CA, USA; <sup>b</sup>Li-Cor Biosciences, Lincoln, NE, USA; <sup>c</sup>Millipore Sigma, Burlington, MA, USA; <sup>d</sup>ThermoFisher Scientific, Waltham, MA, USA; <sup>e</sup>Abcam, Cambridge, MA, USA; <sup>f</sup>Santa Cruz Biotechnology, Dallas, TX, USA; <sup>g</sup>Cell Signaling Technology, Danvers, MA, USA; pAb, polyclonal antibody; mAb, monoclonal antibody; IF, immunofluorescence; WB, Western blot; AF, Alexa Fluor.