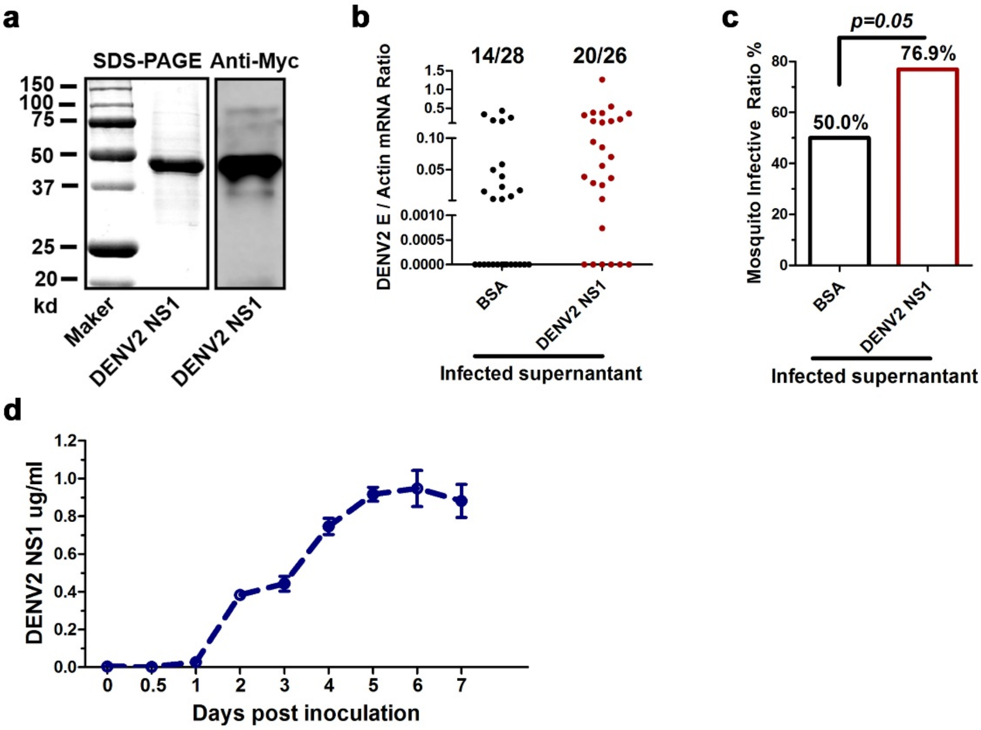
nature microbiology
SUPPLEMENTARY INFORMATION
ARTICLE NUMBER: 16087 | DOI: 10.1038/NMICROBIOL.2016.87

Flavivirus NS1 protein in infected host sera enhances viral acquisition by mosquitoes

Jianying Liu, Yang Liu, Kaixiao Nie, Senyan Du, Jingjun Qiu, Xiaojing Pang,

Penghua Wang, Gong Cheng



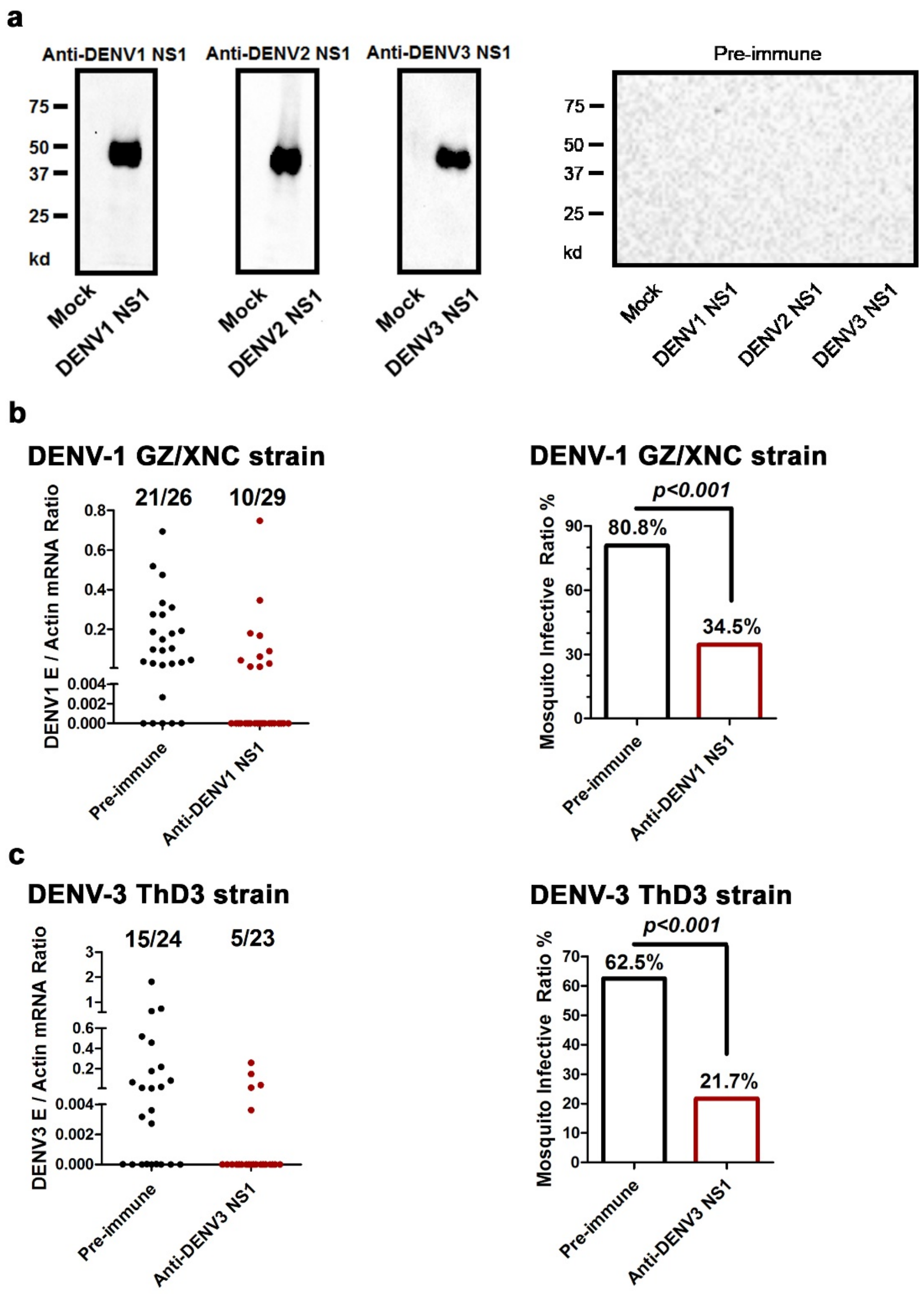
Supplementary Figure 1. Recombinant DENV2 sNS1 increased DENV2 acquisition by *A. aegypti*, and measuring concentration of sNS1 in the supernatant of DENV2-infected Vero cells.

(**a**) Purification of DENV2 sNS1 protein from a *Drosophila* S2 expression system. The DENV2 *NS1* gene was cloned into a pMT/BiP/N-myc-His-A DNA vector. Recombinant DENV2 NS1 was expressed and purified on a Cobalt-His column (Left panel). Protein expression was evaluated using an anti-myc mAb (Right panel).

(**b,c**) Recombinant DENV2 sNS1 increased DENV2 acquisition by *A. aegypti*. Recombinant DENV2 sNS1 protein was expressed and purified using the *Drosophila* S2 expression system. Following this, 10 µg of purified sNS1 was incubated with fresh human blood (500 µl) and DENV2 in the supernatant of Vero cells (500 µl) to feed *A. aegypti* via an *in vitro* membrane blood meal. A concentration of $1x10^6$ pfu/ml DENV2 was used for mosquito oral infection. Mosquitoes fed an equivalent quantity of BSA served as negative controls. Mosquito infectivity was determined by TaqMan qPCR at 8 days post blood meal. (**b**) The number of infected mosquitoes relative to total mosquitoes is shown at the top of each column. Each dot represents a mosquito. (**c**) The data are represented as the percentage of mosquito infection. Differences in mosquito infective ratios were compared using Fisher's exact test.

(**d**) Concentration of the DENV2 sNS1 was detected in the supernatant of DENV2-infected Vero cells by ELISA. The values in the graph represent the mean ± SEM.

(**a-d**) The DENV2 NGC strain was used to infect Vero cells. The experiments were biologically reproduced at least 3 times.

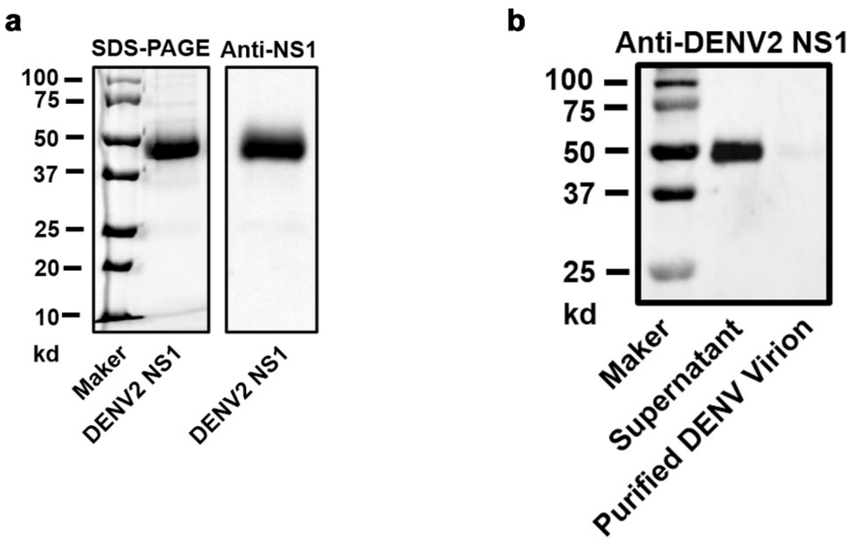


Supplementary Figure 2. Immunoblockade of sNS1 in infected supernatant reduced DENV acquisition by *A. aegypti*.

(**a**) Generation of murine polyclonal antibodies against the NS1s of different DENV serotypes. The *NS1* genes of three DENV serotypes were cloned into a pET-28a (+) expression vector and expressed in *E. coli* BL21 DE3. The recombinant proteins, expressed in inclusion bodies, were dissolved in 8 M urea and purified using a Cobalt-His column for antibody generation. The antibodies were validated by immunostaining with S2-expressed correlated NS1 proteins.

(**b,c**) Immunoblockade of DENV1 NS1 (**b**) and DENV3 NS1 (**c**) in infected Vero supernatant reduced DENV acquisition. An aliquot of 10 µl antisera against DENV1 NS1 (GZ/XNC strain) or DENV3 NS1 (ThD3 strain) was mixed with supernatant from corresponding DENV-infected Vero cells (500 µl) and with fresh human blood (500 µl) for *in vitro* membrane feeding by *A. aegypti*. Both 1×10^6 pfu/ml DENV1 and 1×10^6 pfu/ml DENV3 were used for mosquito oral infection. An equivalent quantity of pre-immune serum served as a mock control. Mosquito infectivity was determined by TaqMan qPCR at 8 days post blood meal. The number of infected mosquitoes relative to total mosquitoes is shown at the top of each column (Left panel). Each dot represents a mosquito. The data are represented as the percentage of mosquito infection. Differences in mosquito infective ratios were compared using Fisher's exact test (Right panel).

(**a-c**)The experiments were biologically repeated 3 times with similar results.

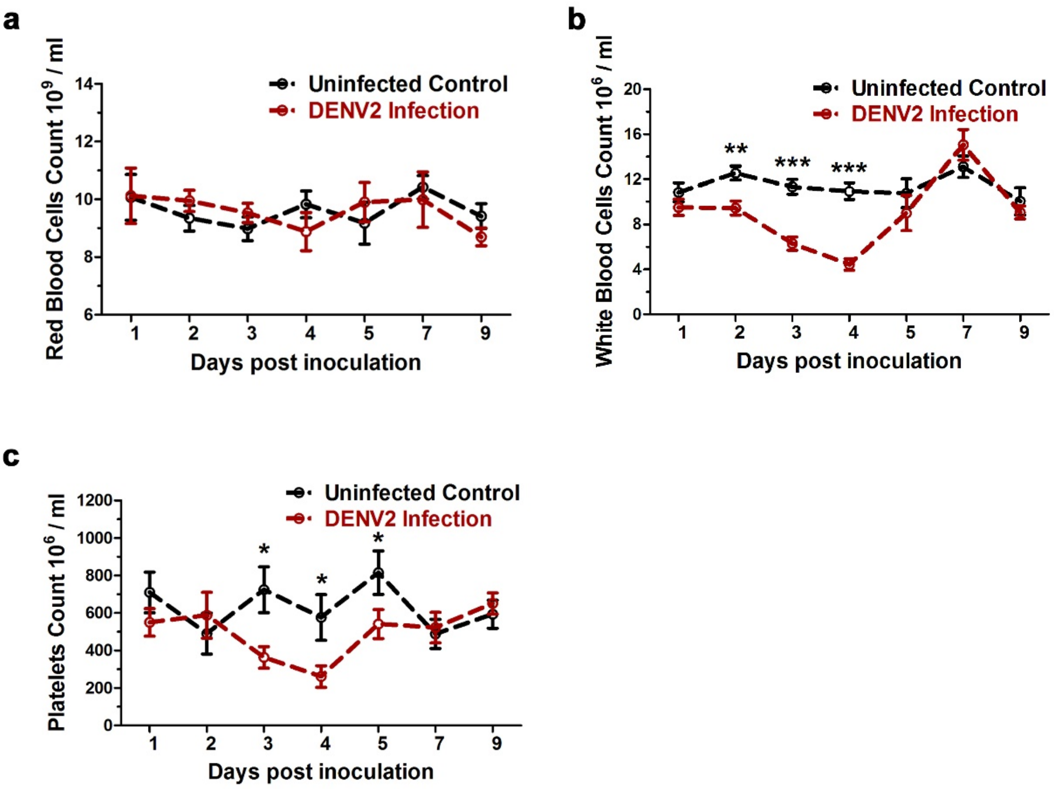


Supplementary Figure 3. Purification of infectious DENV2 particles and native sNS1 protein from supernatant of DENV2-infected Vero cells.

(**a**) Purification of native DENV2 sNS1. Purified polyclonal antibodies were coupled to cyanogen bromide-activated Sepharose 4B beads for affinity purification. Native sNS1 was isolated from the supernatant of DENV2-infected Vero cells at 5 days post infection. The quality of the purified native DENV2 sNS1 was assessed via SDS-PAGE and western blotting.

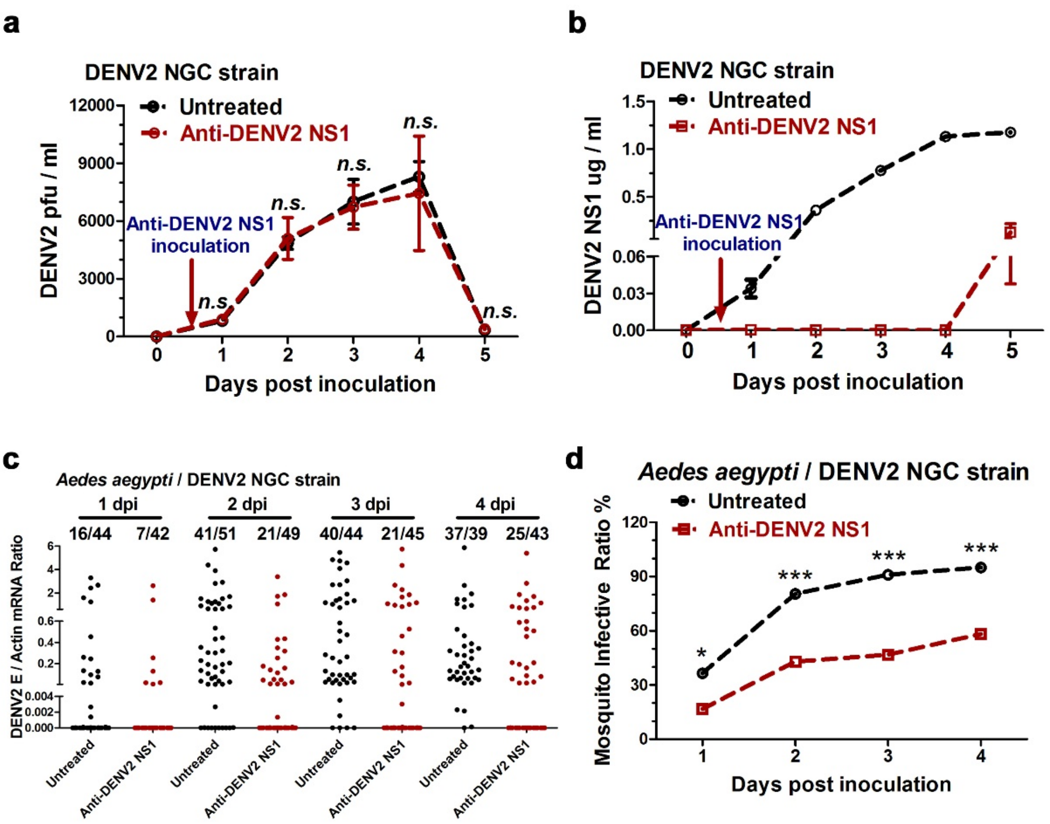
(**b**) Removal of sNS1 from purified DENV2 virions. Vero cells were infected with DENV2 (NGC strain) for 5 days, and supernatant from the infected cells was subsequently collected to isolate infectious particles via high-speed centrifugation. Removal of the NS1 protein from the purified DENV2 particles was determined by western blotting using an anti-DENV2 NS1 antibody.

(**a,b**) The experiments were biologically repeated 3 times with similar results.



Supplementary Figure 4. DENV2 infection produces leukopenia and thrombocytopenia in AG6 mice.

(**a-c**) Blood from DENV2 infected and uninfected AG6 mice was daily collected for counting red blood cells (**a**), white blood cells (**b**), and platelets (**c**) using a hemocytometer. The DENV2 43 strain was used for animal infections. The data are representative of at least five AG6 mice. The values in the graph represent the mean ± SEM. A non-parametric Mann-Whitney test was used to determine significant differences. "*", "**" and "***" represent $p<0.05$, $p<0.01$, and $p<0.001$, respectively. The experiment was biologically reproduced at least 3 times.

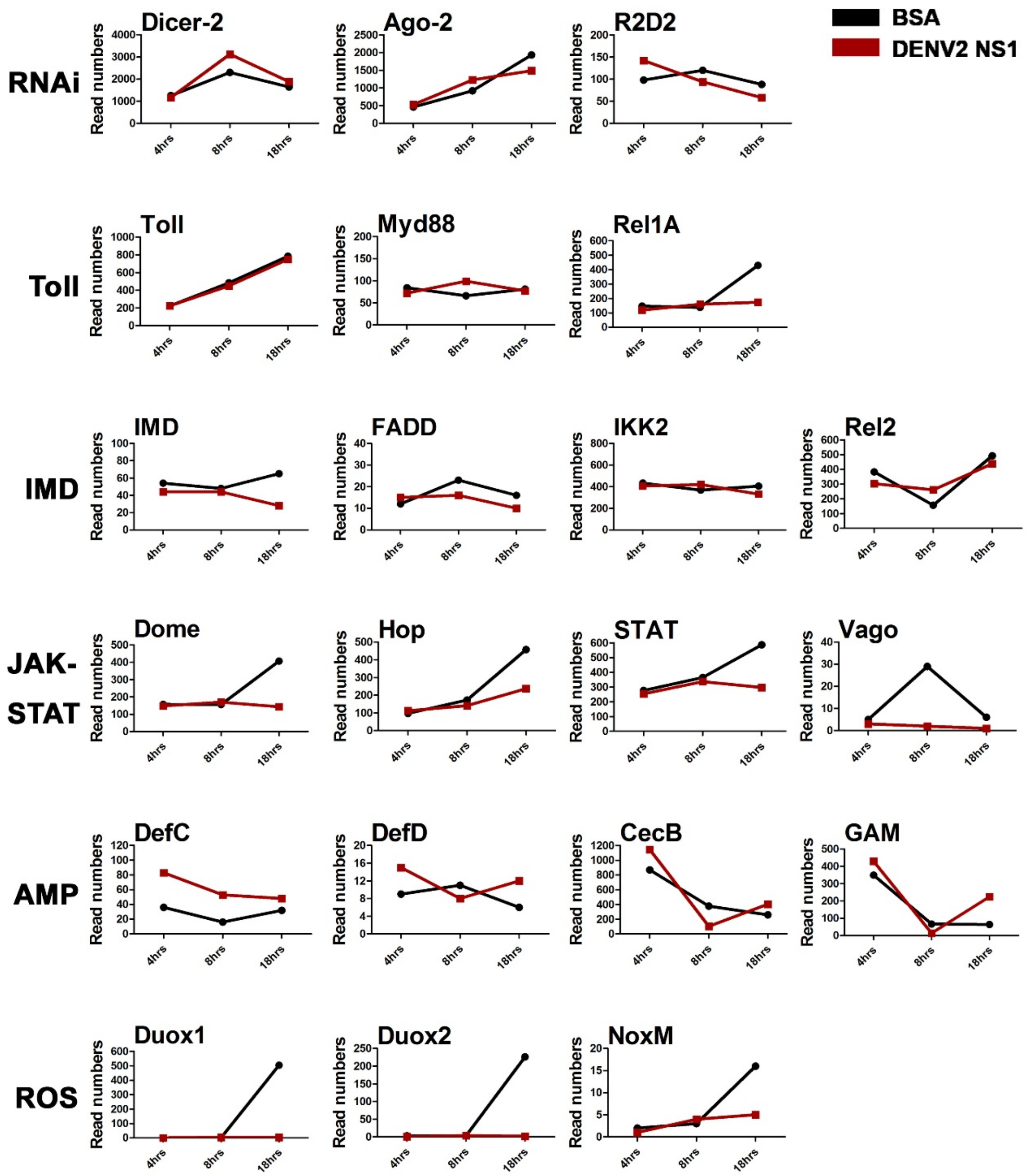


Supplementary Figure 5. Immunoblockade of sNS1 in the infected AG6 mice prevents acquisition of the DENV2 NGC strain by *A. aegypti*.

(**a,b**) Infection of AG6 mice with DENV2 NGC. AG6 mice were immunized and infected with the DENV2 NGC strain following the same procedure as shown in **Fig.2a**. (**a**) Detection of DENV2 viremia in the blood of infected AG6 mice. Blood was collected from the tail veins of infected mice from 0 to 5 days post infection. The presence of infectious viral particles in the blood plasma was assessed using a plaque assay. (**b**) Measurement of DENV2 sNS1 concentration. Mouse sera were used to measure the amounts of DENV2 sNS1 from 0 to 5 days post infection by ELISA. (**a,b**) The data are representative of at least five infected AG6 mice. The values in the graph represent the mean ± SEM. A non-parametric Mann-Whitney test was used to determine significant differences.

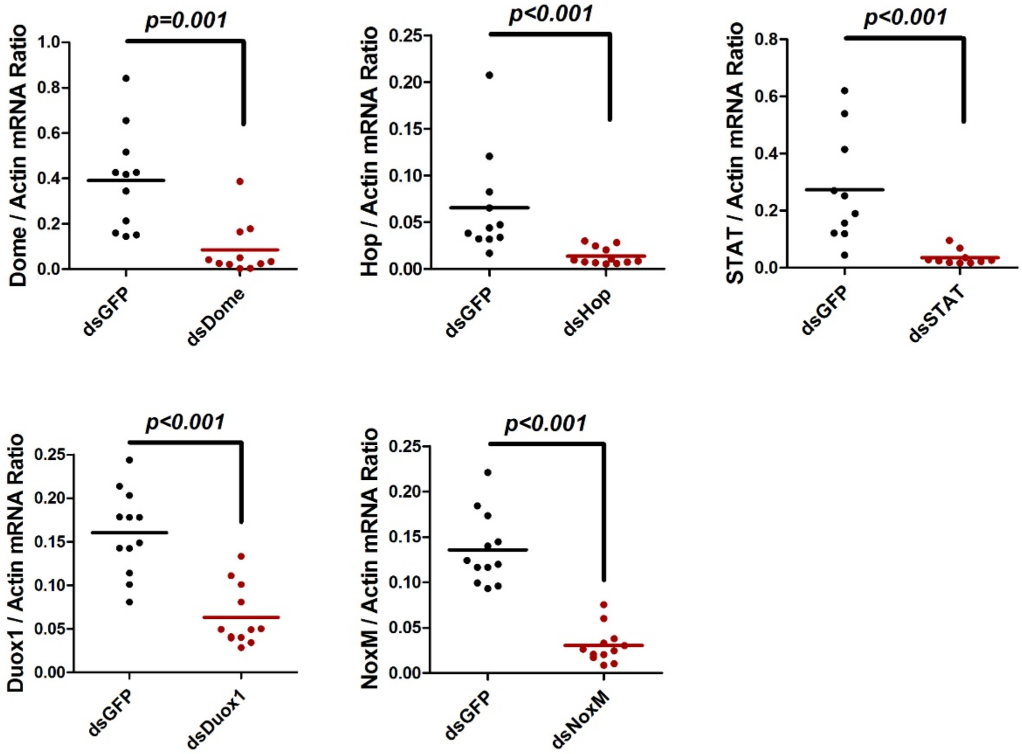
(**c,d**) Immunoblockade of sNS1 prevents acquisition of the DENV2 NGC strain by *A. aegypti*. The fed mosquitoes were reared for an additional 8 days for DENV detection by TaqMan qPCR. (**c**) The number of infected mosquitoes relative to total mosquitoes is shown at the top of each column. Each dot represents a mosquito. (**d**) The data are represented as the percentage of mosquito infection. Differences in mosquito infective ratios were compared using Fisher's exact test. "*", "**" and "***" represent $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

(**a-d**) The experiments were biologically reproduced at least 3 times.

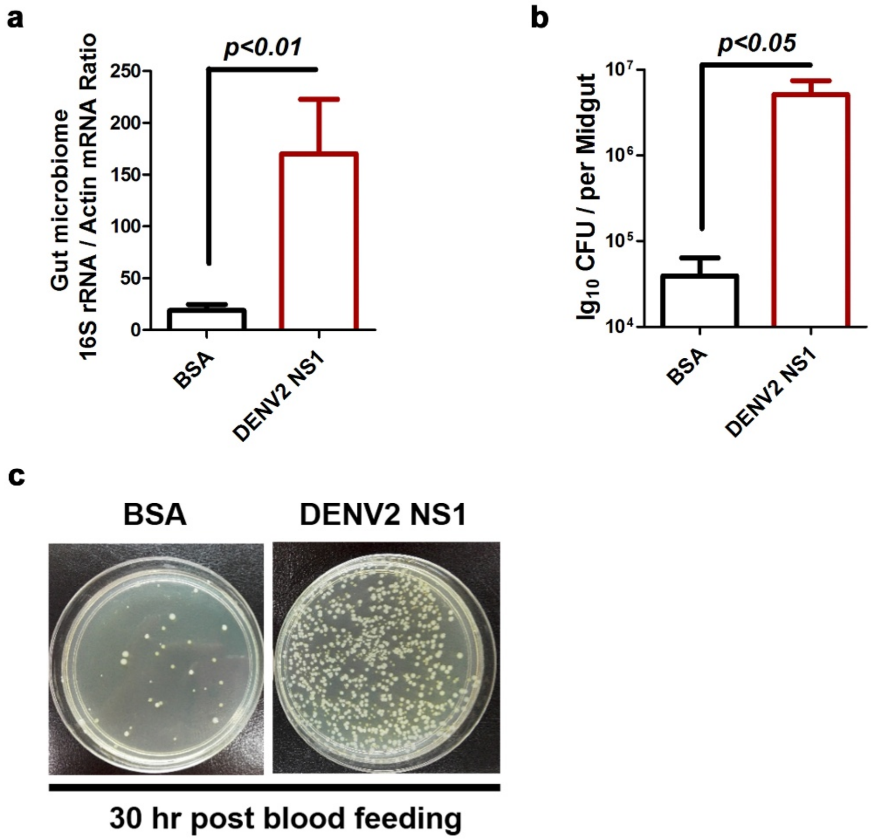


Supplementary Figure 6. Regulation of immune-related genes in the midguts of mosquitoes feeding DENV2 sNS1.

Read numbers of immune-related genes were extracted from the RNA-Seq data in Supplementary Table 1. Gene regulation is presented based on categorized immune pathways and factors.



Supplementary Figure 7. dsRNA-mediated knockdown of immune genes in *A. aegypti*. Genes encoding proteins in the JAK-STAT pathway and ROS system, which were suppressed in the mosquito midgut following feeding with DENV2 sNS1, were silenced by dsRNA inoculation in *A. aegypti*. *GFP* dsRNA served as a mock control. The expression levels of these immune-related genes were determined by qPCR and normalized against *A. aegypti actin* (*AAEL011197*). The qPCR primers are shown in **Supplementary Table 2**. The data are representative of at least three independent trials. The values in the graph represent the mean ± SEM. A non-parametric Mann-Whitney test was performed to determine significant differences.

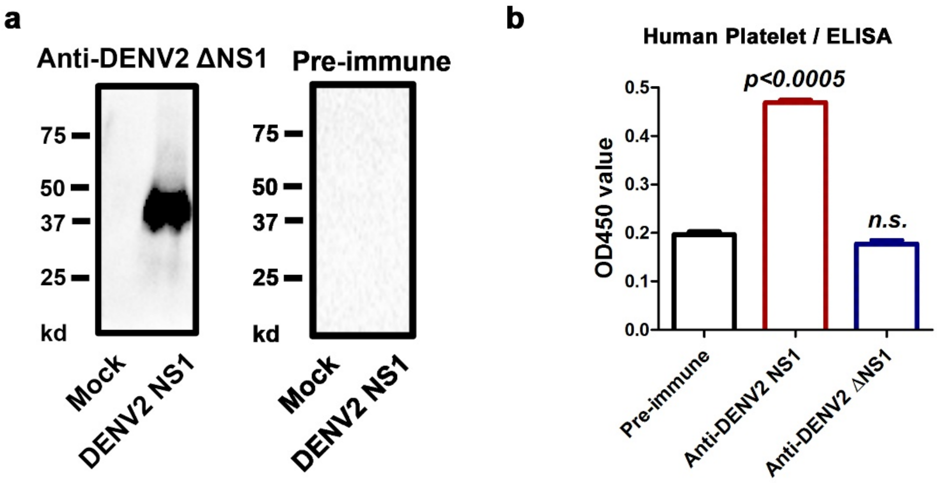


Supplementary Figure 8. Feeding DENV2 sNS1 increased the burden of commensal bacteria in the mosquito midgut.

(**a,b**) The midguts of mosquitoes fed either 10 µg/ml DENV2 sNS1 or BSA were separately dissected at 30 hr post blood feeding. The burden of commensal bacteria was determined by qPCR either using a pair of universal bacterial primers (**a**) or a colony-forming units (CFU) assay (**b**). The data are representative of at least three independent trials. The values in the graph represent the mean ± SEM. A non-parametric Mann-Whitney test was used to determine significant differences.

(**c**) Culturable commensal bacteria were counted on LB agar plates. Lysates of mosquito midguts were diluted 1000 times in PBS for counting in 90 mm petri dishes.

(**a-c**) The data are representative of at least three independent trials.

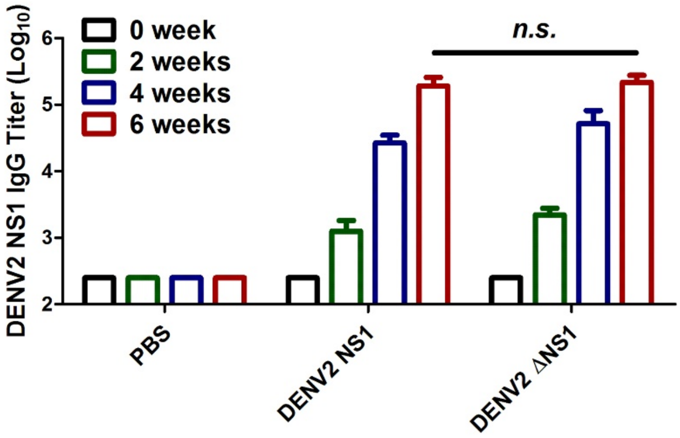


Supplementary Figure 9. An antibody generated against DENV2 ΔNS1 does not cross-react with human primary platelet cells.

(**a**) DENV2 ΔNS1 remains antigenic activity. Murine antibodies generated against DENV2 ΔNS1 can efficiently recognize S2-expressed, full-length DENV2 NS1 protein during western blotting. Supernatant from empty vector-transfected S2 cells was used as a mock control.

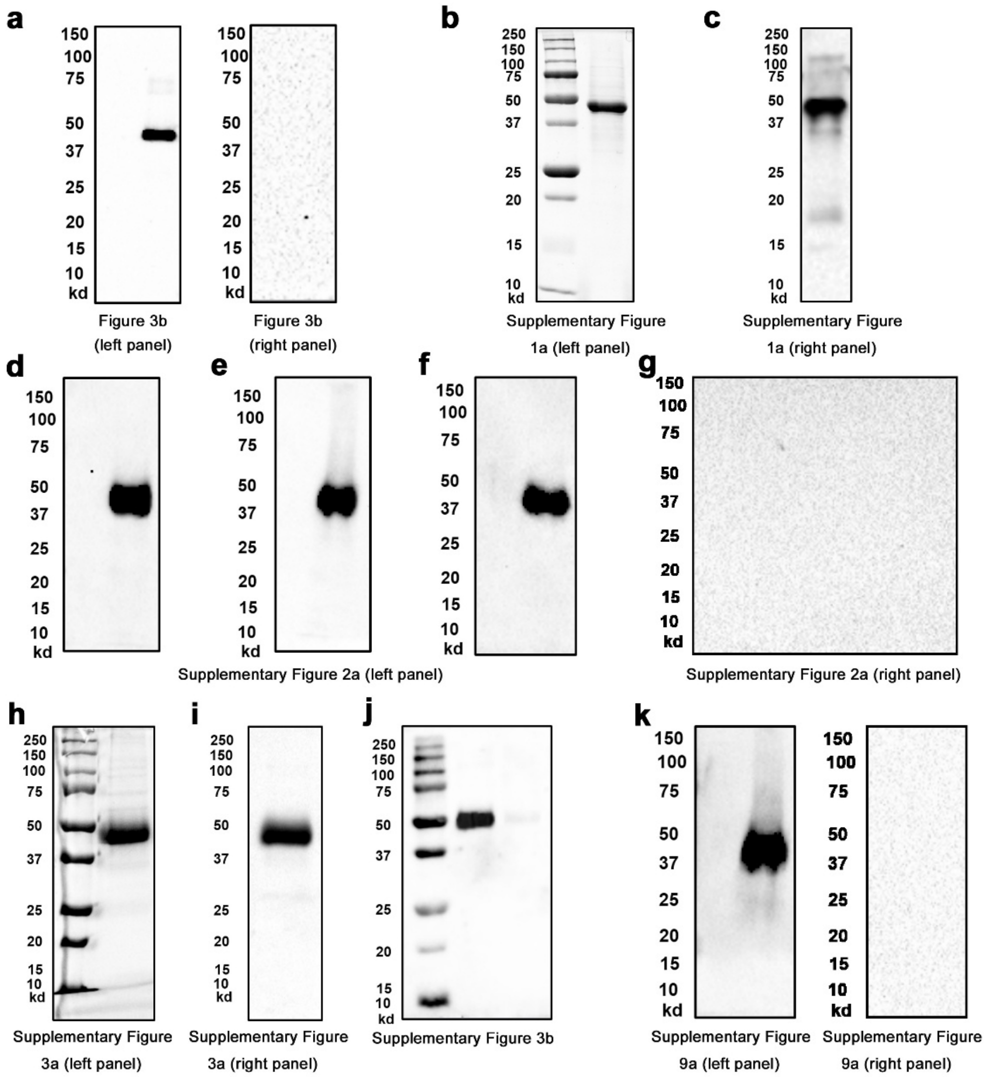
(**b**) Attachment of antibodies to DENV2 full-length NS1 or DENV2 ΔNS1 in human primary platelet cells. Purified antibodies against DENV2 full-length NS1 or ΔNS1 were incubated with human platelets. A pre-immune antibody served as a mock control. Antibody attachment to human platelets was analyzed by ELISA. The values in the graph represent the mean ± SEM. A non-parametric Mann-Whitney test was used for significant analysis. The *p* values were adjusted using Bonferroni correction to account for multiple comparisons. Differences were considered significant if $p<0.025$.

(**a,b**)The experiments were biologically repeated 3 times with similar results.



Supplementary Figure 10. Titration of DENV2 NS1-specific antibodies in immunized AG6 mice.

Immunized and control mice were bled in the 0th, 2nd, 4th and 6th week after the first immunization. NS1-specific antibodies were titrated by ELISA using purified recombinant DENV2 NS1 protein. The titer was determined at least 5 immunized AG6 mice. The values in the graph represent the mean ± SEM. A non-parametric Mann-Whitney test was used to determine significant differences. The experiment was biologically repeated 3 times with similar result.



Supplementary Figure 11. Full-length blots from Figure 3 and Supplementary Figure 1, 2, 3, 9.

(**a**) Production of murine polyclonal antibodies against JEV NS1. (**b,c**) Purification of DENV2 sNS1 protein from a *Drosophila* S2 expression system. (**d-g**) Generation of murine polyclonal antibodies against the NS1s of different DENV serotypes. (**h,i**) Purification of native DENV2 sNS1. (**j**) Removal of sNS1 from purified DENV2 virions. (**k**) Generation of murine polyclonal antibodies against DENV 2 ΔNS1.

Supplementary Table 1. Regulation of immune genes in the midgut of DENV2 NS1 fed *A. aegypti*.

Gene ID	Gene Name	Function Group	Log_2 Ratio		
			4 hr	**8 hr**	**18 hr**
AAEL001612	Dicer-1	RNA interference	0.11	0.38	-1.94
AAEL006794	Dicer-2	RNA interference	-0.14	0.51	0.41
AAEL012410	Ago-1	RNA interference	-0.37	0.34	-1.26
AAEL017251	Ago-2	RNA interference	0.16	0.49	-0.16
AAEL011753	R2D2	RNA interference	0.50	-0.28	-0.39
AAEL008592	Drosha	RNA interference	0.20	0.17	-1.21
AAEL008687	Loq	RNA interference	-0.40	0.59	-0.70
AAEL000709	CACT	Toll pathway	0.31	0.00	-0.21
AAEL007768	MYD	Toll pathway	0.20	0.24	0.44
AAEL006571	Pelle	Toll pathway	0.55	-0.45	-0.73
AAEL007696	Rel1A	Toll pathway	-0.34	0.28	-1.10
AAEL015018	Toll	Toll pathway	-0.02	-0.03	0.15
AAEL009551	Toll11	Toll pathway	0.29	-1.93	-0.46
AAEL007613	Toll1A	Toll pathway	-2.36	-0.71	-3.19
AAEL017523	Toll4	Toll pathway	0.55	-1.15	-1.68
AAEL007619	Toll5A	Toll pathway	-0.56	-0.35	-0.59
AAEL000057	Toll5B	Toll pathway	-0.04	0.74	0.40
AAEL002583	Toll7	Toll pathway	-0.09	-0.19	0.54
AAEL013441	Toll9A	Toll pathway	-0.14	-0.08	0.42
AAEL011734	Toll9B	Toll pathway	-0.40	0.22	0.16
AAEL011363	Traf6	Toll pathway	-0.02	0.46	0.38
AAEL007642	Tube	Toll pathway	-1.04	0.09	-0.71
AAEL006212		Toll pathway	N/A	-0.11	0.09
AAEL009474	PGRPS1	Toll pathway	2.46	-0.56	0.09
AAEL017056	PGRPS4	Toll pathway	0.97	0.76	0.01
AAEL009176	GNBPB3	Toll pathway	3.44	-4.36	0.39
AAEL009178	GNBPB4	Toll pathway	-2.36	2.17	-0.79
AAEL007897	Spz4	Toll pathway	-5.56	-1.07	-0.52
AAEL001929	Spz5	Toll pathway	-3.46	-0.15	0.80
AAEL013434		Toll pathway	-1.26	1.49	-0.11
AAEL007624	Rel2	IMD pathway	-0.37	0.81	0.05
AAEL001932	FADD	IMD pathway	0.29	-0.45	-0.46
AAEL003245	IKK1	IMD pathway	-0.13	-0.49	-0.17
AAEL012510	IKK2	IMD pathway	-0.13	0.26	-0.08
AAEL010083	IMD	IMD pathway	-0.33	-0.05	-1.00
AAEL012380	PGRPLA	IMD pathway	-0.04	-0.21	0.13
AAEL010171	PGRPLB	IMD pathway	1.01	0.16	0.02
AAEL014640	PGRPLC	IMD pathway	-0.38	0.05	0.62
AAEL013112	PGRPLE	IMD pathway	0.42	-0.76	-0.14
AAEL012471	Dome	JAK-STAT pathway	-0.12	0.20	-1.30

AAEL009692	STAT	JAK-STAT pathway	-0.16	-0.04	-0.77
AAEL012553	Hop	JAK-STAT pathway	0.17	-0.22	-0.74
AAEL000200	Vago	JAK-STAT pathway	0.97	-8.65	-5.19
AAEL000165	Vago	JAK-STAT pathway	-2.04	-2.74	-2.11
AAEL018117	Vir-1	JAK-STAT pathway	0.47	0.17	-0.12
AAEL007563	Duox1	ROS	N/A	2.07	-6.76
AAEL007562	Duox2	ROS	-1.62	0.49	-6.61
AAEL010179	NoxM	ROS	-1.04	0.49	-1.46
AAEL004223	CecB	Anti-microbial peptide	0.36	-1.82	0.86
AAEL017211	CecH	Anti-microbial peptide	0.46	-1.98	1.66
AAEL003841	DefA	Anti-microbial peptide	0.97	2.07	-0.71
AAEL003832	DefC	Anti-microbial peptide	1.17	1.80	0.80
AAEL003857	DefD	Anti-microbial peptide	0.70	-0.39	1.21
AAEL004522	GAM	Anti-microbial peptide	0.26	-2.19	2.02
AAEL015308	SCRAL1	Scavenger Receptor	-3.21	3.77	-6.47
AAEL009192	SCRASP1	Scavenger Receptor	5.03	-5.87	-6.18
AAEL008370	SCRB17	Scavenger Receptor	0.04	0.56	-0.25
AAEL000234	SCRB7	Scavenger Receptor	0.00	0.15	0.22
AAEL000227	SCRB8	Scavenger Receptor	-0.05	0.21	0.10
AAEL000256	SCRB9	Scavenger Receptor	0.97	-0.08	-0.11
AAEL009420	SCRBQ1	Scavenger Receptor	-0.33	0.13	0.12
AAEL009423	SCRBQ2	Scavenger Receptor	-0.11	0.22	-0.05
AAEL009432	SCRBQ3	Scavenger Receptor	-0.04	-0.13	0.22
AAEL001914		Scavenger Receptor	-0.07	0.61	0.22
AAEL000087	TEP22	Thioester-containing protein	-0.04	-2.25	-0.56
AAEL001163	TEP23	Thioester-containing protein	0.00	0.48	0.41
AAEL001794	TEP20	Thioester-containing protein	-0.04	-4.88	-2.68
AAEL012267	TEP13	Thioester-containing protein	-0.15	0.31	-0.72
AAEL014078	SRPN	Serine protease inhibitor	-0.04	-2.10	-2.31
AAEL007765	SRPN10	Serine protease inhibitor	-0.39	0.13	-0.01
AAEL003686	SRPN11	Serine protease inhibitor	-1.04	-3.47	-3.94
AAEL002720	SRPN20	Serine protease inhibitor	0.48	-2.58	1.02
AAEL005665	SRPN3	Serine protease inhibitor	0.48	-1.39	-3.06
AAEL013936	SRPN4	Serine protease inhibitor	0.92	-2.92	-2.59
AAEL014141	SRPN5	Serine protease inhibitor	0.97	0.23	-3.15
AAEL010769	SRPN6	Serine protease inhibitor	-0.04	-5.92	-1.18
AAEL011777	SRPN8	Serine protease inhibitor	-1.04	-2.15	-3.08
AAEL008364	SRPN9	Serine protease inhibitor	0.42	0.22	-4.05
AAEL000074	CLIPB1	Clip-Domain Serine Protease	-0.62	-4.25	-1.16
AAEL003243	CLIPB13A	Clip-Domain Serine Protease	0.97	-6.07	-5.16
AAEL014349	CLIPB15	Clip-Domain Serine Protease	1.72	0.50	-0.03
AAEL008668	CLIPB22	Clip-Domain Serine Protease	1.84	-0.46	0.68
AAEL007993	CLIPB27	Clip-Domain Serine Protease	-0.34	-0.20	1.80
AAEL006161	CLIPB31	Clip-Domain Serine Protease	0.82	-0.57	0.35

AAEL000099	CLIPB33	Clip-Domain Serine Protease	-0.04	-4.18	0.80
AAEL000028	CLIPB34	Clip-Domain Serine Protease	0.55	-2.99	-3.01
AAEL006168	CLIPB42	Clip-Domain Serine Protease	1.19	-1.39	0.37
AAEL005093	CLIPB46	Clip-Domain Serine Protease	-2.84	0.07	-0.15
AAEL005064	CLIPB5	Clip-Domain Serine Protease	1.29	-2.74	-3.51
AAEL000038	CLIPB6	Clip-Domain Serine Protease	0.21	-0.87	-1.88
AAEL003625	CLIPB8	Clip-Domain Serine Protease	3.29	-2.10	-0.96
AAEL011991	CLIPC1	Clip-Domain Serine Protease	-5.05	-0.93	-2.49
AAEL012712	CLIPC13	Clip-Domain Serine Protease	-0.94	0.33	-5.58
AAEL012713	CLIPC16	Clip-Domain Serine Protease	1.77	-2.51	-0.98
AAEL007593	CLIPC2	Clip-Domain Serine Protease	0.55	-1.51	-4.28
AAEL002124	CLIPD6	Clip-Domain Serine Protease	-0.67	-2.15	-2.96
AAEL015439	CLIPD7	Clip-Domain Serine Protease	-0.69	0.80	-0.16
AAEL009726		Clip-Domain Serine Protease	-0.33	-2.79	0.56
AAEL009722		Clip-Domain Serine Protease	-1.04	-0.93	-0.14

Supplementary Table 2. Primers and probes for gene cloning and qPCR

Primers for cloning into pET28a(+)	**Upper primer**	**Lower primer**	
DENV1 NS1 (His tag in both N and C-terminal)	GGGCTTCCATATGGACTCGGGATGTGTA	ATAAGAATGCGGCCGCTGCAGAGACCAATGA	
DENV2 NS1 (His tag in both N and C-terminal)	GGCATTCCATATGGATAGTGGTTGCGTTG	TAATTCCTCGAGGGCTGTGACCAAGGA	
DENV3 NS1 (His tag in both N and C-terminal)	TACTCTAGCTAGCGACATGGGGTGTGTC	GATTCCGCTCGAGCGCTGAGACTAAAGA	
JEV NS1 (His tag in both N and C-terminal)	TATCTAGCTAGCGACACTGGATGTGCC	TAGCCCGCTCGAGTTCACCATTGAAAGC	
Primers for cloning into pMT/BiP/Myc-His/A	**Upper primer**	**Lower primer**	
DENV1 NS1 (Myc tag in N-terminal)	TATTCCCCCGGGGACTCGGGATGTGTA	ATTTGCGGCCGCGTGCAGAGACCAATGA	
DENV2 NS1 (Myc tag in N-terminal)	TCTCGGGGTACCTGATAGTGGTTGCGTT	TATTACGCTCGAGAAGGCTGTGACCAAGGAG	
DENV3 NS1 (Myc tag in N-terminal)	ATAATAATGCGGCCGCGACATGGGGTGTGTC	TACTCCCCGCGGCGCTGAGACTAAAGA	
JEV NS1 (Myc tag in N-terminal)	TATCGGGGTACCTGACACTGGATGTGCC	TATCCGCTCGAGTTTTCACCATTGAAAGC	
The primers for double-strand RNA synthesis	**Upper primer**	**Lower primer**	
dsRNA GFP	TAATACGACTCACTATAGGGGTGAGCAAGGGCGAGGAG	TAATACGACTCACTATAGGGCATGATATAGACGTTGTGGCTGTT	
dsRNA Dome	TAATACGACTCACTATAGGGCCATCTCCACCACGAAACTT	TAATACGACTCACTATAGGGCCGGTGGTTGCCATATAATC	
dsRNA Hop	TAATACGACTCACTATAGGGGTAAACCCCATCTGTCCTC	TAATACGACTCACTATAGGGTGTTGCATTTCCCCGGCAT	
dsRNA STAT	TAATACGACTCACTATAGGGTGGAATACCAAGGACCAAAT	TAATACGACTCACTATAGGGTGCCCAACTGCAAAGCTTGA	
dsRNA Duox1	TAATACGACTCACTATAGGGGGCTGAACACACAGATCTTAG	TAATACGACTCACTATAGGGGAGTGCGCAAACGCGAATAT	
dsRNA Noxm	TAATACGACTCACTATAGGGGTGGGTGTTATCCATAGCATAG	TAATACGACTCACTATAGGGGTTCCTCACTGCAAATAGCC	
The primers for SYBR Green RT-QPCR	**Upper primer**	**Lower primer**	
SYBR Dicer-2	CGCTCGGCTTTGGTGAAT	TGCCGACTCTGCCAGGAT	
SYBR Ago-2	CCTCGTGCGCAACGTAATG	TTGATCCGGGTGTTTCTTGTT	
SYBR R2D2	GAAAGGGCTGAGCGATATTGA	CCCGCACTTCGGTCACTTTA	
SYBR Toll	TTGGATGGAAACGAGATATCAGAA	CTTCCTGAATCTCGGTCAACTTG	
SYBR Myd88	CGTGATTGGCGAGGGTTGTTTC	ATCCGCTCCAATGCTCGTTTCC	
SYBR Rel1A	TGGTGGTGGTGTCCTGCGTAAC	CTGCCTGGCGTGACCGTATCC	
SYBR IMD	ATCCCGACATCTGGGATATG	GGGTTGACTTTGTCGTCGTT	
SYBR IKK2	GCGGCCAGTGTCATCATG	AGAGGCATCGAGGAATTCATCA	
SYBR FADD	AGTATCGAGCAACGTTAGAGG	AAGGTGCTCCAATTGCGAC	
SYBR Rel2	TGGACATGCTCGATCTTTTCAA	CGACGACCTCTACCAGATTGG	
SYBR Dome	AAACGGTGGCAAAATGAACT	CATACAGCCGGCTTTCTTCT	
SYBR Hop	GCTGGTAGTAATGCTTCGAGTGAGT	GCCGGTGCTGTAATGACTAGAA	
SYBR STAT	CACACAAAAAGGACGAAGCA	TCCAGTTCCCCTAAAGCTCA	
SYBR Vago	CAGTAGCATTTGCCGGTCAGA	CGATGTTGGATCGTAGCACTTC	
SYBR DefC	CTTTGTTTGATGAACTTCCGGAG	GAACCCACTCAGCAGATCGC	
SYBR DefD	GGCGTTGGTGATAGTGCTTG	CACACCTTCTTGGAGTTGCAG	
SYBR CecB	GCTGAAGAAGCTGGGAAAAAAG	CTTCCCAGTCCCTTGATGCC	
SYBR GAM	GGTGTTTGTTTATGCCAAAACC	CGATGTAGCATTCGGTGATG	
SYBR Duox1	CACGTGGTGGAATTCGAATG	GCCATGCCCATCAGGAACT	
SYBR Duox2	CGTGGCGGAGTATTTCAGT	GCAAATTCATCAGCAACCAC	
SYBR Noxm	ATATCCGCAGGCCAGTACGT	CTGTAAACGGGTGCCACTCA	
SYBR 16S rRNA for commensal bacteria	TCCTACGGGAGGCAGCAGT	GGACTACCAGGGTATCTAATCCTGTT	
SYBR *Aedes albopictus Actin*	CGGAAGAGCACCCAGTTCTC	TGTGTCATCTTCTCGCGGTTAG	
The primers for Taqman RT-QPCR	**Upper primer**	**Lower primer**	**Probe (for Taqman QPCR)**
DENV1 Envelope gene	GCTGACATGGCTAGGATTAAATTCA	GGTACAGTGTAACCATGCCAACTG	FAM-CGCGTCCCTTTCGATGACGTGC-TAMRA
DENV2 Envelope gene	CATTCCAAGTGAGAATCTCTTTGTCA	CAGATCTCTGATGAATAACCAACG	FAM-ATGCTGAAACGCGAGAGAAACCGC-TAMRA
DENV3 Envelope gene	GGGAAAACCGTCTATCAATA	CGCCATAACCAATTTCATTGG	FAM-CACAGTTGGCGAAGAGATTCTCAAGAGGA-TAMRA
JEV Envelope gene	CTGGTCCATAGGGAGTGGTTTC	CTCCACGCTGTGCTCGAA	FAM-TGACCTCGCTCTCCCCTGGACG-TAMRA
Aedes aegypti Actin	GAACACCCAGTCCTGCTGACA	TGCGTCATCTTCTCACGGTTAG	FAM-AGGCCCCGCTCAACCCGAAG-TAMRA
Mouse Actin	AGCCATGTACGTAGCCATCCA	TCTCCGGAGTCCATCACAATG	FAM-TGTCCCTGTATGCCTCTGGTCGTACCAC-TAMRA