Supplementary information for

An evolutionarily conserved ubiquitin ligase drives infection and transmission of flaviviruses

Authors

Linjuan Wu, Liming Zhang, Shengyong Feng, Lu Chen, Cai Lin, Gang Wang, Yibin Zhu, Penghua Wang, Gong Cheng*

*Correspondence to: Gong Cheng Ph.D., School of Basic Medical Sciences, Tsinghua University, Beijing, P.R. China, 100084. Phone: (+86)-10-62788494; Fax: (+86)-10-62788494. Email: gongcheng@mail.tsinghua.edu.cn

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Supporting text

Materials and Methods

Cell viability assay

The cell viability was measured by Cell Counting Kit-8 (CCK-8) (C0038, Beyotime) assay. Aag2 cells were transfected with dsRNAs against either *AaHRD1* or *GFP* genes, and A549 cells were transfected with either *HsHRD1* or negative control (NC) siRNAs for 48h. The CCK-8 solution was added and incubated for 1 h, and then the absorbance was measured at 450 nm.

EC50 quantification

C6/36 cells or A549 cells were infected with 0.1 MOI DENV2 and simultaneously incubated with different concentrations of LS-102 for 1 h. The cells were then washed and cultured with fresh medium containing LS-102 for 48 h. Viral yield in cell supernatant samples was quantified using a standard curve. To generate standard curve of DENV2, a segment of DENV2 genome containing the segment for qRT-PCR detection was generated. The PCR products contained the T7 promoter on positive strands, and acted as DNA templates for *in vitro* transcription using the MEGAscript T7 Transcription Kit (AM1334, Ambion) by following the manufacturers' instructions. The number of single-stranded RNA copies was measured as described previously (1). The concentration of LS-102 required to 50% of maximal effect (EC_{50}) was calculated by comparing the values with the DMSO-treated cells in Graphpad Prism 8.0 (GraphPad Software).

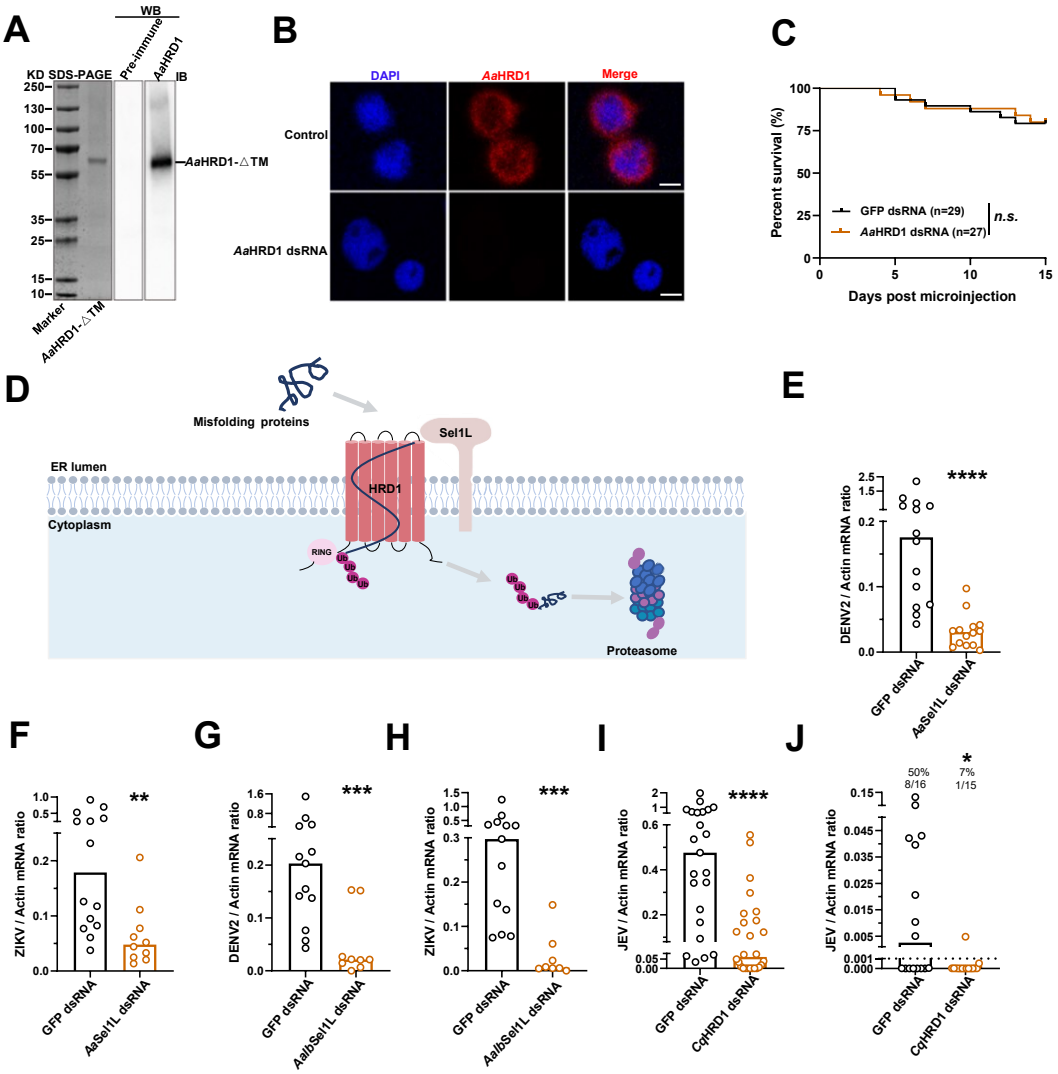

Fig. S1. HRD1s facilitate the infection of flavivirus in mosquitoes, related to Fig.1.

(A) The preparation of anti-*Aa*HRD1 murine polyclonal antibodies. The plasmid encoding *Aa*HRD1-△TM was expressed in the *E. coli* BL21 DE3 strain, and formed the inclusion bodies, followed by purification and dissolving in urea. The purified *Aa*HRD1-△TM protein as an immune antigen to immunize with mice as described in Materials and Methods. The immune antigen was detected by staining with Coomassie blue and analyzed via WB with anti-*Aa*HRD1 murine antibodies. **(B)** The validation specificity of anti-*Aa*HRD1 antibodies via immunofluorescence assay. Aag2 cells were cultured in 24-wells overnight, and then were transfected with or without *Aa*HRD1 dsRNA. After 48 hours transfection, cells were fixed and incubated with anti-*Aa*HRD1 murine polyclonal antibodies overnight and then stained with Alexa Fluor 594-conjugated anti-mouse IgG. The nuclei were stained with DAPI and then analyzed via immunofluorescence assay. Scar bars, 5 μm. **(C)** Silencing *Aa*HRD1 did not

influence the survival of mosquitoes. Female *A. aegypti* mosquitoes were inoculated with dsRNAs against *HRD1* or *GFP* genes via thoracic microinjection. The survival of *A. aegypti* mosquitoes were daily recorded. **(D)** Schematic of the association of HRD1 with Sel1L in the ERAD pathway. **(E, F)** Silencing *Aa*Sel1L inhibited DENV2 and ZIKV infections in *A. aegypti*. *A. aegypti* mosquitoes were inoculated with *AaSel1L* dsRNA for 3 days and then thoracically infected with 10 $M.I.D._{50}$ of DENV2 or ZIKV. Viral loads of DENV2 **(E)** and ZIKV **(F)** were determined via qPCR and normalized against *A. aegypti* actin. **(G, H)** Knockdown of *Aalb*Sel1L impaired DENV2 and ZIKV infections in *A. albopictus*. The *AalbSel1L* dsRNA was microinjected into *A. albopictus* mosquitoes, followed by inoculation with 10 $M.I.D._{50}$ of DENV2 or ZIKV 3 days later. Viral loads of DENV2 **(G)** and ZIKV **(H)** were determined via qPCR and normalized against *A. albopictus* actin. **(I, J)** Silencing *Cq*HRD1 inhibited JEV infection in *C. quinquefasciatus*. *C. quinquefasciatus* mosquitoes were inoculated with dsRNA of *CqHRD1* and microinjected with *GFP* dsRNA served as a negative control. Ten $M.I.D._{50}$ of JEV was inoculated at 3 days post dsRNA microinjection, and viral loads were assessed at 3 days postinfection via SYBR qPCR and normalized against *C. quinquefasciatus* actin **(I)**. *CqHRD1* dsRNA was inoculated into *C. quinquefasciatus* mosquitoes. After 3 days post microinjection, Human blood (50% v/v) and supernatant from JEV-infected Vero cells (50% v/v) were used to feed *C. quinquefasciatus* via an *in vitro* membrane feeding system. The infectivity of JEV were measured at 8 days after a blood meal via qPCR **(J)**. **(C)** The data were analyzed statistically using the log-rank (Mantel–Cox) test. **(E-J)** One dot represents one mosquito, and the median value of the results was shown. Data were analyzed statistically using the Mann–Whitney test **(E-I)**. The number of infected mosquitoes/total number of mosquitoes is shown at the top of each column. The limit of detection for the viral genome/actin ratio is 0.001, indicating with the black dashed line. Differences in the infectivity ratio were compared using Fisher's exact test **(J)**. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, *n.s.*, not significant ($P > 0.05$).

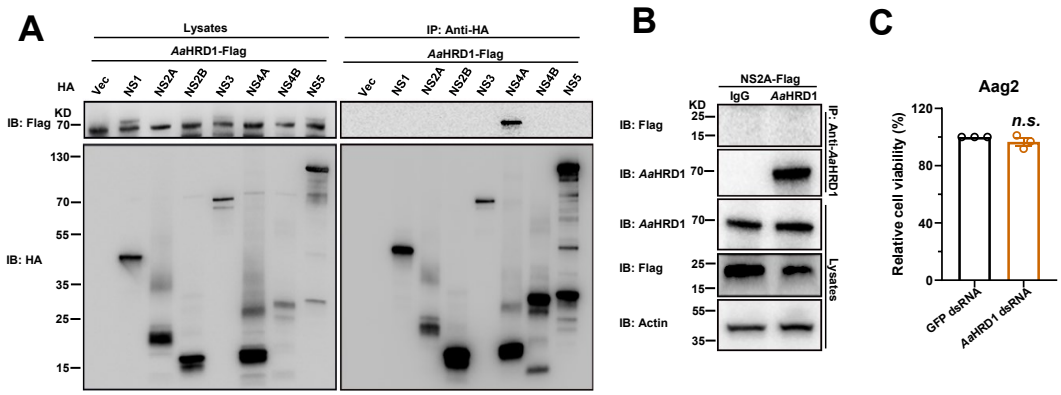

Fig. S2. *Aa*HRD1 interacts with DENV2 NS4A.

(A) The interplay of *Aa*HRD1 with nonstructural proteins of DENV2. Recombinant plasmids encoding codon optimized *Aa*HRD1 and DENV2 nonstructural proteins were cotransfected into HEK293T cells. Cell lysates were subjected to IP with anti-HA antibodies and analyzed via Western blot (WB). **(B)** DENV2 NS2A did not interact with endogenous *Aa*HRD1. Aag2 cells were transfected with plasmid encoding NS2A with the Flag tag. After 48 hours transfection, cell lysates were subjected to IP with anti-*Aa*HRD1 murine polyclonal antibodies and analyzed via WB. **(C)** Silencing *Aa*HRD1 did not influence the cell viability. Aag2 cells were transfected with indicated dsRNAs. After 48 hours transfection, cell viability was measured via CCK-8 Kit. Relative cell viability (%) = (Experiment value-black control value/negative control value-blank control value). **(A-B)** These experiments were repeated three times with similar results. **(C)** Data are presented as the mean±SEM and were analyzed statistically using the unpaired t test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, *n.s.*, not significant (P > 0.05).

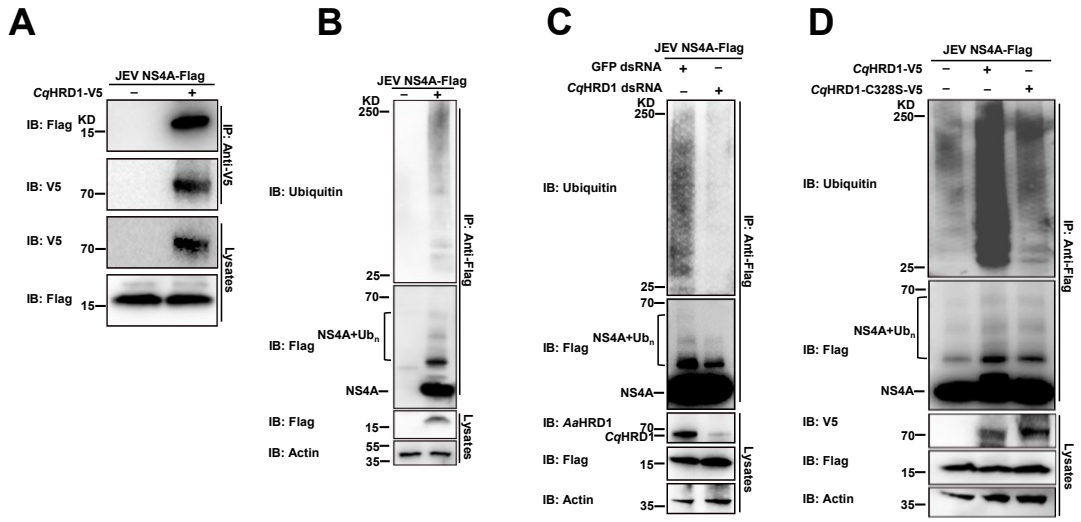

Fig. S3. *Cq*HRD1 interacts and ubiquitylates JEV NS4A.

(A) *Cq*HRD1 interacted with JEV NS4A. Recombinant plasmids expressing *Cq*HRD1 and JEV NS4A were cotransfected into Cxq-1 cells for 48 h. Cell lysates were subjected to IP with anti-V5 antibodies and analyzed via WB. **(B)** JEV NS4A is ubiquitinated in Cxq-1 cells. A plasmid encoding JEV NS4A was transfected into Cxq-1 cells. Cell lysates were subjected to IP with anti-Flag antibodies, and the level of NS4A ubiquitination was measured with anti-Ub. **(C)** Silencing endogenous *Cq*HRD1 impaired the ubiquitination of JEV NS4A. Cxq-1 cells were transfected with *CqHRD1* dsRNA 3 days later, and the cells were transfected with JEV NS4A. The level of NS4A ubiquitination was measured with anti-Ub. **(D)** Overexpression of *Cq*HRD1 enhanced the ubiquitination of JEV NS4A. S2 cells were transfected with the indicated plasmids. IP was performed with anti-Flag antibodies, followed by immunoblotting with anti-Ub to measure the level of NS4A ubiquitination. **(A-D)** These experiments were repeated three times with similar results.

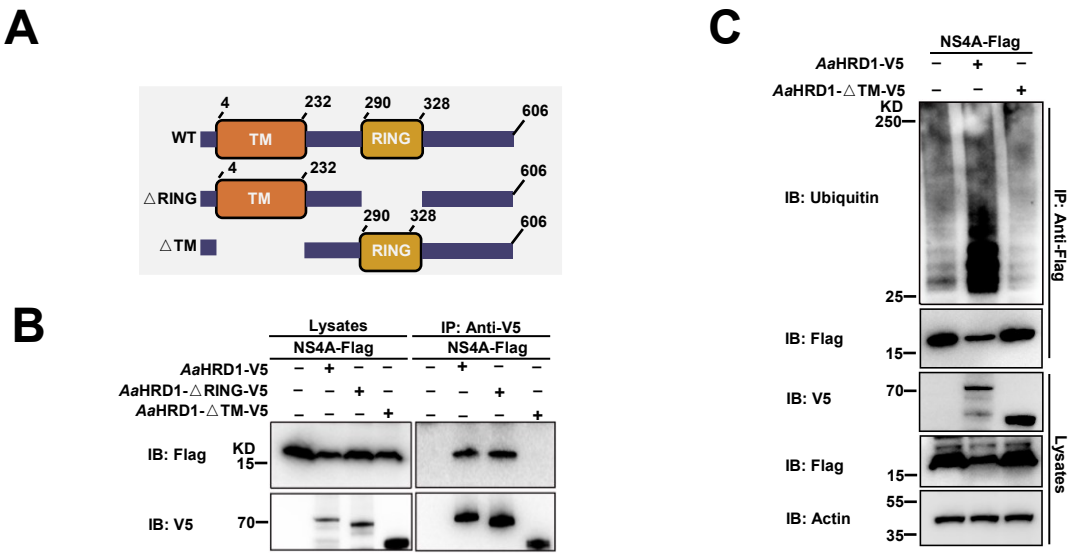

Fig. S4. The transmembrane of *Aa*HRD1 is indispensable for the interaction with NS4A.

(A) Identification of functional domains of *Aa*HRD1 based on its structure. **(B)** The transmembrane domain of *Aa*HRD1 mediated the interaction with NS4A. S2 cells were transfected with the indicated plasmids. IP was conducted with anti-V5 antibodies and analyzed via WB. **(C)** *Aa*HRD1-△TM was unable to ubiquitinate NS4A. S2 cells were transfected with the indicated plasmids. The level of NS4A ubiquitination was determined. **(B-C)** These experiments were repeated three times with similar results.

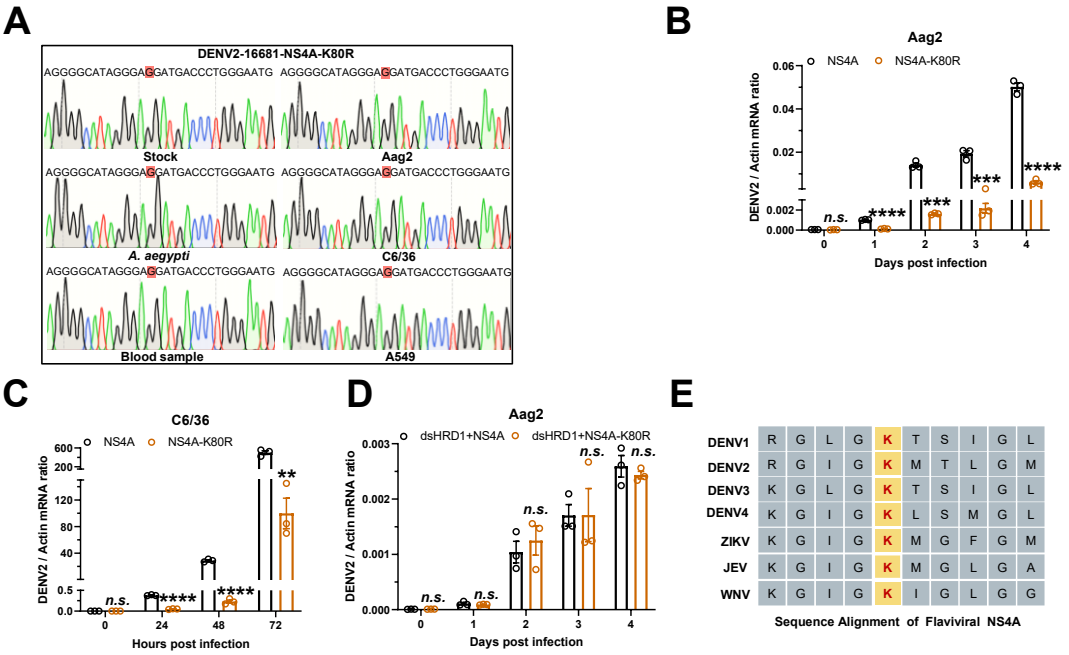

Fig. S5. The replication of DENV2-16681-NS4A-K80R in mosquito cells.

(A) The NS4A-K80R substitution was stable in DENV2-16681-NS4A-K80R. The rescued DENV2-16681-NS4A-K80R mutant stock was passaged 5 times. The rescued mutant stock and the samples of infected cells or animals were sequenced. **(B, C)** Infections of NS4A/NS4A-K80R strains in Aag2 or C6/36 cells. Aag2 or C6/36 cells were infected with NS4A or NS4A-K80R strain at an MOI of 0.1. Viral loads were measured at the indicated time via qPCR. Viral loads were normalized against *A. aegypti actin* (**B**) and *A. albopictus actin* (**C**). **(D)** Infections of NS4A/NS4A-K80R strains in *Aa*HRD1-silenced Aag2 cells. Aag2 cells were transfected with *AaHRD1* dsRNA for 3 days and then infected with NS4A or NS4A-K80R strain at an MOI of 0.1. Viral loads were determined at the indicated time via qPCR. **(E)** Conservative analysis of lysine (K) at the 80th residue of DENV NS4A among flaviviral NS4As. **(B-D)** Data are presented as the mean±SEM and analyzed statistically using the unpaired t test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, *n.s.*, not significant (P > 0.05).

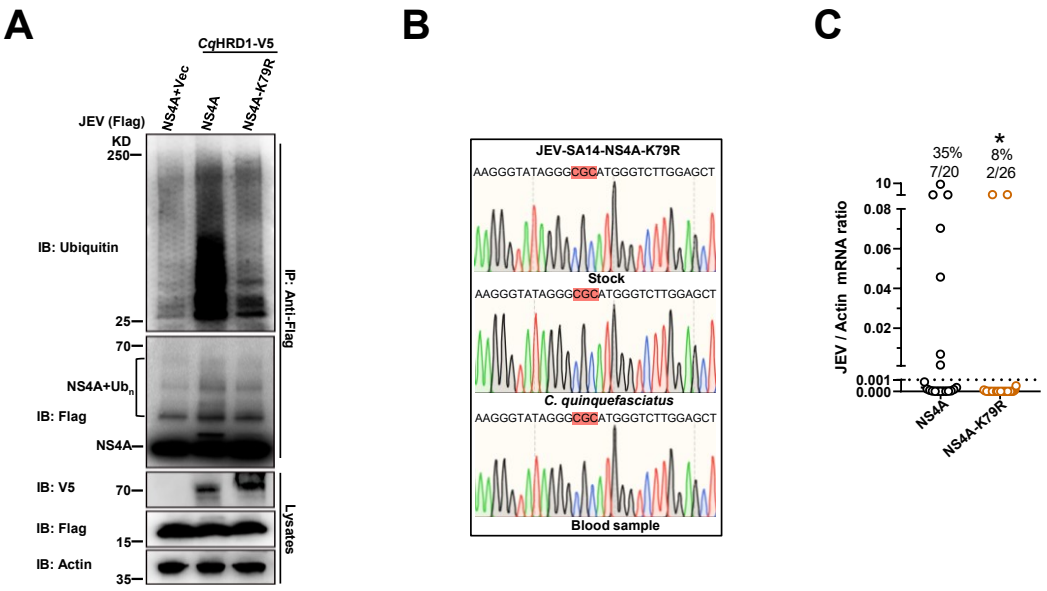

Fig. S6. The K79R substitution of NS4A reduces JEV replication in *C. quinquefasciatus*.

(A) *Cq*HRD1 ubiquitylated the 79th lysine residue of JEV NS4A. S2 cells were transfected with the indicated plasmids. IP was conducted, and then the level of NS4A ubiquitination was measured. **(B)** The NS4A-K79R substitution was stable in JEV-SA14-NS4A-K79R. The rescued mutant stock JEV-SA14-NS4A-K79R was passaged 5 times. The rescued mutant stock and the samples of infected animals were sequenced. **(C)** Infections of NS4A/NS4A-K79R strains in *C. quinquefasciatus*. Either NS4A or NS4A-K79R strain (500 μl) and 500 μl fresh human blood were mixed and then fed on *C. quinquefasciatus* via an *in vitro* blood feeding system. Viral infectivity was determined at 8 days after a blood meal by qPCR. **(A)** The experiment was repeated for three time with similar results. **(C)** One dot represents one mosquito, and the median value of the results was shown. The number of infected mosquitoes/total number of mosquitoes is shown at the top of each column. The limit of detection for the viral genome/actin ratio is 0.001, indicating with the black dashed line. Differences in the infectivity ratio were compared using Fisher's exact test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, *n.s.*, not significant (P > 0.05).

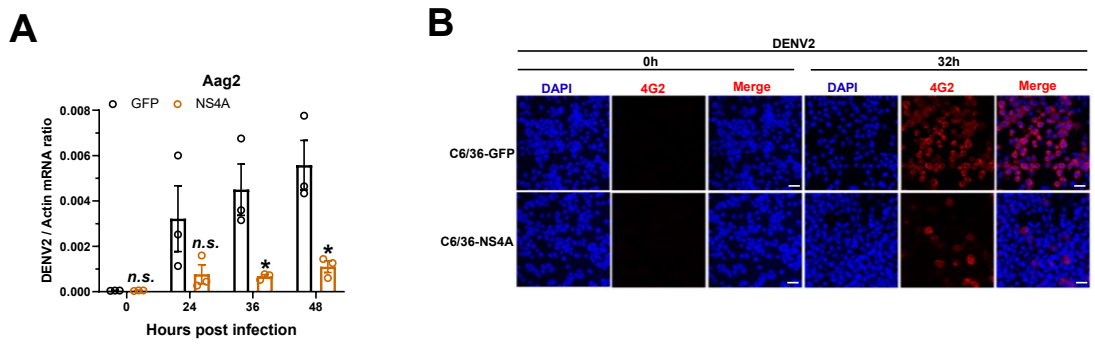

Fig. S7. Overexpression of NS4A inhibits DENV2 replication in mosquito cells.

(A-B) Overexpression of NS4A impaired DENV2 infection. Aag2 cells were transfected with plasmids expressing NS4A or GFP and then incubated with MG132 (10 μM) for 12 h, followed by inoculation with DENV2 at an MOI of 0.01. Viral loads were assessed at the indicated time via qPCR and normalized against *A. aegypti actin* **(A)**. C6/36 cells stably expressing GFP or NS4A were infected DENV2 at 0.01 MOI, and then were harvested, fixed, and then stained with anti-4G2, followed by staining with Alexa Fluor 594-conjugated anti-mouse IgG. Scale bars, 10 μm **(B)**. **(A)** Data are presented as the mean±SEM and analyzed statistically using the unpaired t test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, *n.s.*, not significant (P > 0.05).

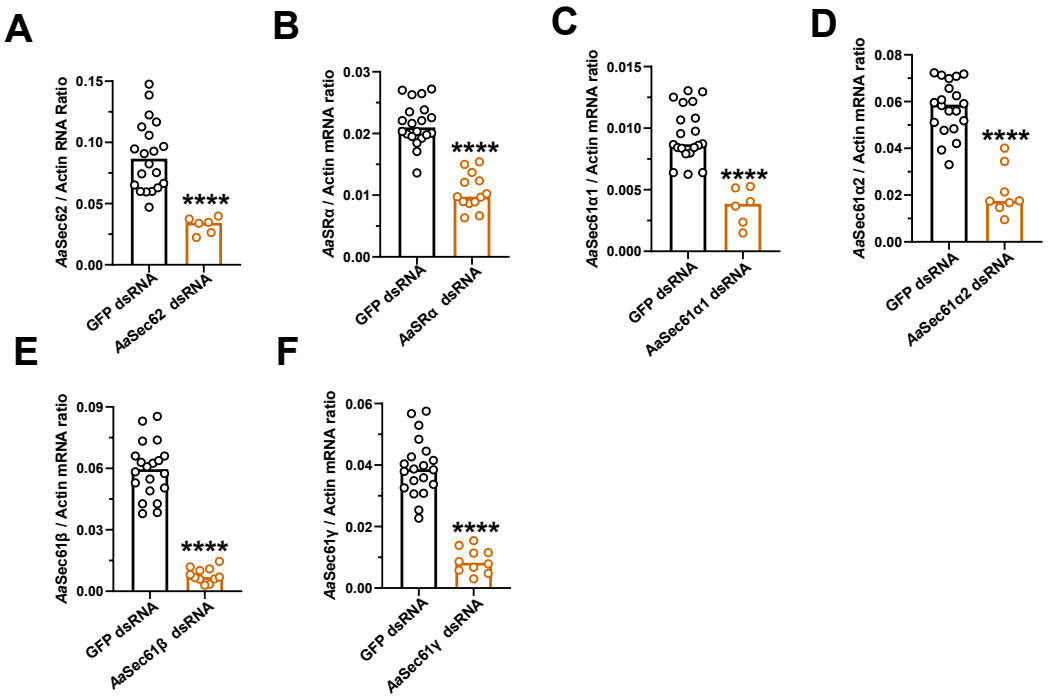

Fig. S8. The determination of silencing efficiency of ER translocation complex. *A. aegypti* were inoculated dsRNAs against of *AaSec62, AaSRα, AaSec61-α/β/γ genes* and microinjected with *GFP* dsRNA served as a negative control. The knockdown efficiency of *AaSec62, AaSRα, AaSec61α1, AaSec61α2, AaSec61β, AaSec61γ* were measured via qPCR. One dot represents one mosquito, and the median value of the results was shown. Data were analyzed statistically using the Mann–Whitney test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, *n.s.*, not significant (P > 0.05).

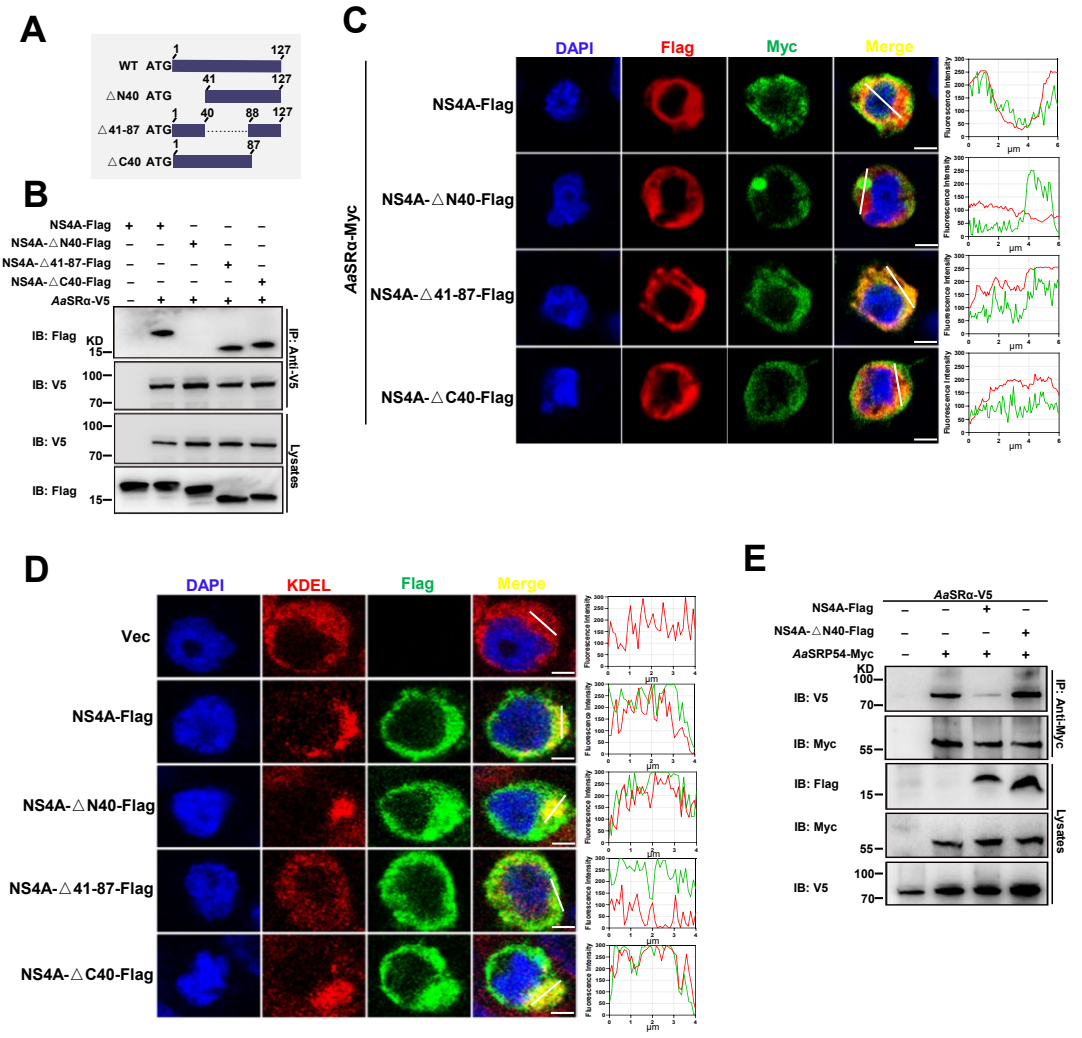

Fig. S9. The N-terminal 40 amino acids of NS4A are indispensable for the interaction with *Aa*SRα.

(A) Schematic of NS4A truncations. **(B)** The N-terminal 40 amino acids of NS4A mediated the interaction with *Aa*SRα. S2 cells were transfected with the indicated plasmids, and cell lysates were subjected to IP with anti-V5 antibodies and analyzed via WB. **(C)** NS4A-△N40 was unable to colocalize with *Aa*SRα. C6/36 cells were transfected with the indicated plasmids and then harvested and fixed. NS4A/truncations and *Aa*SRα were stained with Alexa Fluor 594-conjugated anti-mouse IgG and Alexa Fluor 488-conjugated anti-rabbit IgG, respectively. The nuclei were stained with DAPI and then analyzed via immunofluorescence assay. Scale bars, 3 μm. **(D)** The truncation of 41-87th residues abolished the colocalization of NS4A with ER. C6/36 cells were cultured overnight, and then were transfected with indicated plasmids. After 48 hours transfection, cells were fixed and incubated with anti-Flag and anti-KDEL antibodies

overnight and then stained with Alexa Fluor 488-conjugated anti-rabbit IgG and Alexa Fluor 594-conjugated anti-mouse IgG. The nuclei were stained with DAPI and then analyzed via immunofluorescence assay. Scar bars, 3 μm. **(E)** NS4A-△N40 did not compete with *Aa*SRP54 binding to *Aa*SRα. S2 cells were transfected with the indicated plasmids. IP was performed with anti-Myc antibodies and analyzed via WB. **(B, E)** These experiments were repeated three times with similar results.

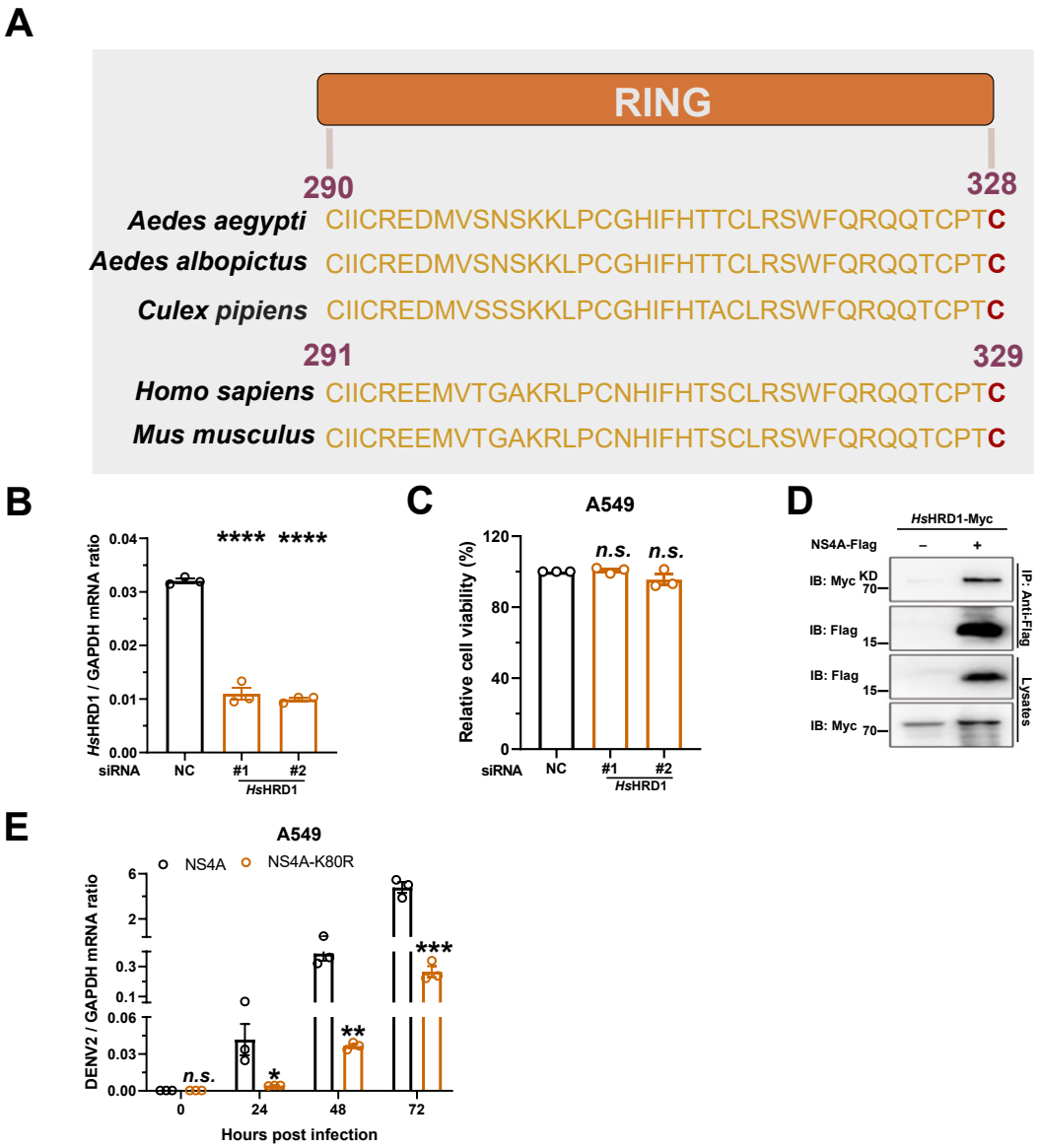

Fig. S10. *Hs*HRD1 ubiquitylates DENV2 NS4A to facilitate flavivirus infection in hosts, related to Fig. 4.

(A) Sequence alignment of HRD1s RING domain from different species. Sequence alignment of HRD1s RING domain from indicated species and catalytic centers are marked in red. **(B)** Identification of *Hs*HRD1 siRNA deficiency via qPCR. A549 cells were transfected with indicated siRNAs for 36 hours. Gene expression was measured via qPCR. **(C)** Silencing *Hs*HRD1 did not influence the cell viability. A549 cells were transfected with indicated siRNAs. After 48 hours transfection, cell viability was measured via CCK-8 Kit. Relative cell viability (%) = (Experiment value-black control value/negative control value-blank control value). **(D)** NS4A interacted with *Hs*HRD1. HEK293T cells were transfected with indicated plasmids. IP

was performed with anti-Flag antibodies and analyzed via WB. **(E)** The K80R substitution in NS4A reduced DENV2 replication in A549 cells. A549 cells were infected with NS4A or NS4A-K80R strain at 0.1 MOI. Viral loads were measured at the indicated time via qPCR. **(D)** The experiment was repeated for three times with similar results. **(B, C, E)** Data are presented as the mean±SEM and analyzed statistically using the unpaired t test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, *n.s.*, not significant (P > 0.05).

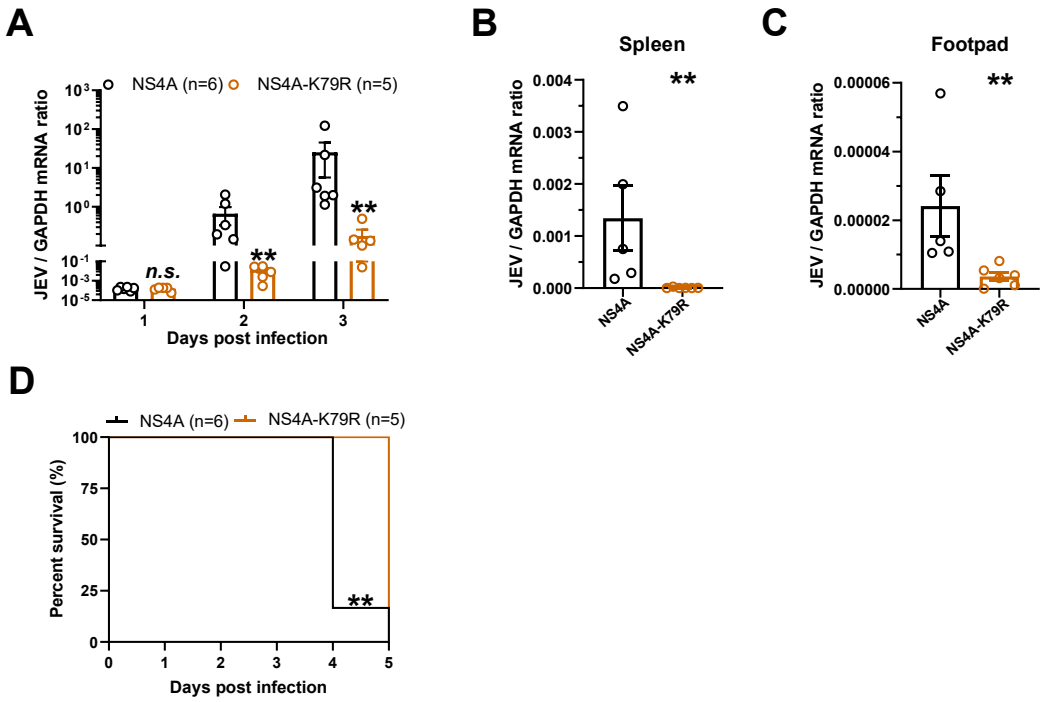

Fig. S11. JEV-SA14-NS4A-K79R reduces the pathogenicity in AG6 mice.

(A-D) The K79R substitution of NS4A reduced JEV replication in AG6 mice. Four-week-old male AG6 mice were infected with 20 f.f.u. of NS4A or NS4A-K79R strain by footpad inoculation. As JEV is highly pathogenic in AG6 mice, blood samples from Days 1 to 3 were collected for viremia assessment via qPCR. The K79R substitution in the NS4A resulted in lower viral burdens in murine blood **(A)**. Infected mice were euthanized at two days postinfection, and viral loads in tissues were determined by qPCR **(B-C)**. The survival rates of the mice were recorded daily **(D)**. **(A, D)** n=6 (NS4A) and n=5 (NS4A-K79R) biological replicates. **(B, C)** n=5 (NS4A) and n=6 (NS4A-K79R) biological replicates. **(A-C)** Data are presented as the mean±SEM and were analyzed statistically using the Mann–Whitney test. **(D)** The data were analyzed statistically using the log-rank (Mantel–Cox) test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, *n.s.*, not significant (P > 0.05).

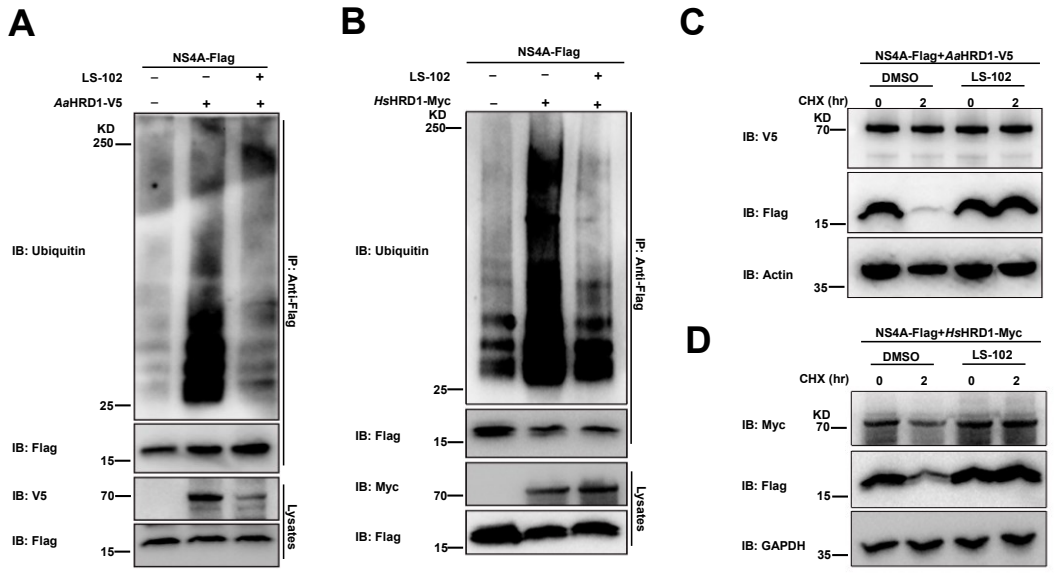

Fig. S12. LS-102 inhibits the ubiquitination and instability of DENV2 NS4A mediated by HRD1s.

(A, B) LS-102 inhibited the ubiquitination of NS4A mediated by *Aa*HRD1 or *Hs*HRD1. S2 cells were transfected with the indicated plasmids and then incubated with LS-102 (20 μM) **(A)**. The indicated plasmids were cotransfected into HEK293T cells. Subsequently, cells were treated with LS-102 (10 μM) **(B)**. The level of NS4A ubiquitination was measured at 48 hours posttransfection by IP with anti-Flag antibodies and then analyzed via WB. **(C, D)** LS-102 offset the instability of NS4A mediated by *Aa*HRD1 or *Hs*HRD1. S2 cells were transfected with NS4A and *Aa*HRD1 and then incubated with LS-102 (20 μM). Subsequently, the cells were treated with CHX (10 μg/ml) for the indicated time. Cells were harvested, and the amount of NS4A was analyzed via WB **(C)**. The indicated plasmids were transfected into HEK293T cells. Cells were incubated with LS-102 (10 μM), followed by treatment with CHX (10 μg/ml) for the indicated time. The expression of NS4A was analyzed via WB **(D)**. **(A-D)** These experiments were repeated three times with similar results.

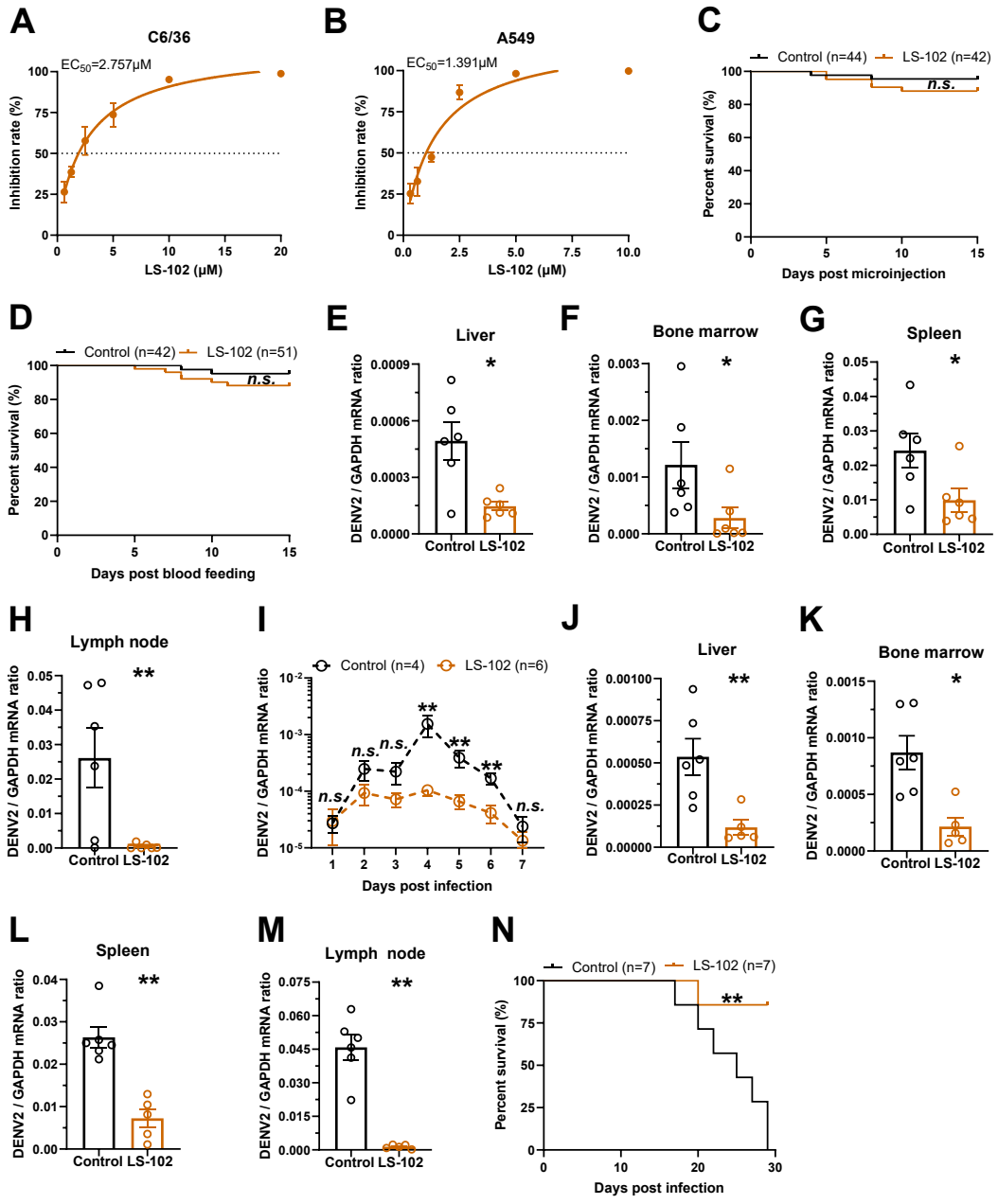

Fig. S13. LS-102 inhibits DENV2 infection in hosts and mosquitoes, related to Fig. 5.

(A, B) Quantification of the EC50 of LS-102 in C6/36 cells and A549 cells. C6/36 or A549 cells were infected with DENV2 at 0.1 MOI and simultaneously treated with the indicated concentration of LS-102 for 48 hours. Viral yield in cell supernatant were quantified by qRT-PCR [% inhibition rate=[1-(viral copies of treatment group/viral RNA copies of control group)] ×100%].The values of the concentration for 50% of maximal effect (EC_{50}) of LS-102 are shown above each plot and indicated with the black dashed line. **(C, D)** The survival rates of mosquitoes were recorded daily after microinjection or oral feeding with LS-102. Per female

mosquito aged 7 days was inoculated with 70 ng LS-102 and then maintained to record survival rates **(C)**. LS-102 mixed with human blood were used to feed mosquitoes via an *in vitro* blood feeding system. The final concentration of LS-102 for feeding was 240 μg ml^{-1}. The survival rates were recorded daily **(D)**. **(E-H)** LS-102 reduced viral loads in AG6 tissues. Four-week-old female AG6 mice were infected with 5×10^4 f.f.u. of the DENV2 16681 strain by footpad inoculation and intraperitoneally inoculated with LS-102 (15 mg/kg) dissolved in corn oil every day postinfection. Infected mice were euthanized at two days postinfection, and viral loads in tissues were measured via qPCR. **(I-N)** The prophylactic effect of LS-102 in DENV2 infection. There-week-old female AG6 mice were intraperitoneally inoculated with LS-102 (15 mg/kg) every day beginning 7 days before infection. After infection with the 5×10^4 f.f.u DENV2 16681 strain, the animals were continuously treated daily with LS-102 (15 mg/kg). Blood samples were collected from infected mice for viremia assessment from Day 1 to Day 7 using qPCR (**I**). Infected mice were euthanized at two days postinfection, and viral loads in the liver (**J**), bone marrow (**K**), spleen (**L**) and lymph nodes (**M**) were measured via qPCR. **(I-M)** Data were combined with two independent experiments. The survival rates of mice were recorded daily (**N**). **(A, B)** Data are presented as the mean±SEM and analyzed statistically using the unpaired t test. **(C, D)** The data were analyzed statistically using the log-rank (Mantel–Cox) test. **(E-H)** n=6 biological replicates. **(I)** n=4 (Control) and n=6 (LS-102) biological replicates. **(J-M)** n=6 (Control) and n=5 (LS-102) biological replicates. **(N)** n=7 biological replicates. **(E-M)** Data are presented as the mean±SEM and were analyzed statistically using the Mann–Whitney test. **(N)** The data were analyzed statistically using the log-rank (Mantel–Cox) test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, *n.s.*, not significant (P > 0.05).

Supplementary Table 1

Table S1. The E3 ligases of *Aedes*. Aegypti

Gene ID	Gene name	Type
AAEL009509	potential E3 ubiquitin-protein ligase ariadne-2	RBR
AAEL012490	E3 ubiquitin-protein ligase RNF19B	RBR
AAEL018318	E3 ubiquitin-protein ligase parkin	RBR
AAEL022272	potential E3 ubiquitin-protein ligase ariadne-1-like	RBR
AAEL023019	potential E3 ubiquitin-protein ligase ariadne-1	RBR
AAEL001205	E3 ubiquitin-protein ligase Su(dx)	HECT
AAEL002306	probable E3 ubiquitin-protein ligase HERC2	HECT
AAEL004790	ubiquitin-protein ligase E3B	HECT
AAEL005774	E3 ubiquitin-protein ligase HECW2	HECT
AAEL005820	probable E3 ubiquitin-protein ligase HERC4	HECT
AAEL005930	E3 ubiquitin-protein ligase Hyd	HECT
AAEL006008	apoptosis-resistant E3 ubiquitin protein ligase 1	HECT
AAEL007705	E3 ubiquitin-protein ligase HECTD1	HECT
AAEL008374	E3 ubiquitin-protein ligase nedd-4	HECT
AAEL010256	E3 ubiquitin-protein ligase SMURF2	HECT
AAEL011541	ubiquitin-protein ligase E3C	HECT
AAEL012500	ubiquitin-protein ligase E3A	HECT
AAEL017357	E3 ubiquitin-protein ligase TRIP12	HECT
AAEL026428	E3 ubiquitin-protein ligase TRIP12-like	HECT
AAEL000424	E3 ubiquitin-protein ligase RFWD2	RING
AAEL000523	RING finger protein 44	RING
AAEL000570	RING-box protein 2	RING
AAEL000590	E3 ubiquitin-protein ligase MYLIP	RING
AAEL000730	E3 ubiquitin-protein ligase Bre1	RING
AAEL000763	E3 ubiquitin-protein ligase SH3RF1	RING
AAEL000776	E3 ubiquitin-protein ligase TRIM37	RING
AAEL001217	tripartite motif-containing protein 45	RING

Table S1. The E3 ligases of *Aedes*. Aegypti

Gene ID	Gene name	Type
AAEL001357	serine/arginine repetitive matrix protein 2	RING
AAEL001410	E3 ubiquitin-protein ligase SHPRH	RING
AAEL001459	protein deltex	RING
AAEL001610	RING finger protein 37	RING
AAEL001765	E3 ubiquitin-protein ligase LRSAM1	RING
AAEL001778	RING finger protein 113A	RING
AAEL001933	uncharacterized protein	RING
AAEL002020	RING finger protein 121	RING
AAEL002078	RING finger protein 141	RING
AAEL002345	E3 ubiquitin-protein ligase listerin	RING
AAEL002642	E3 ubiquitin-protein ligase TRIM9	RING
AAEL002706	E3 ubiquitin-protein ligase RBBP6	RING
AAEL003009	E3 ubiquitin-protein ligase RNF13	RING
AAEL003010	E3 ubiquitin-protein ligase TRIM39	RING
AAEL003104	RING finger protein nhl-1	RING
AAEL003248	probable E3 ubiquitin-protein ligase MGRN1	RING
AAEL003271	E3 ubiquitin-protein ligase FANCL	RING
AAEL003343	RING finger and SPRY domain-containing protein 1	RING
AAEL003466	E3 ubiquitin-protein ligase cullin-4A	RING
AAEL003489	E3 ubiquitin-protein ligase ZNF598	RING
AAEL003680	E3 ubiquitin-protein ligase rnf146	RING
AAEL003787	E3 ubiquitin-protein ligase TRAIP	RING
AAEL004044	E3 ubiquitin-protein ligase highwire	RING
AAEL004356	E3 ubiquitin-protein ligase arkadia-B	RING
AAEL004689	cell growth regulator with RING finger domain protein 1	RING
AAEL004697	E3 ubiquitin-protein ligase HRD1	RING
AAEL004713	E3 ubiquitin-protein ligase TRIM37	RING
AAEL004861	E3 ubiquitin-protein ligase RNF4	RING

Table S1. The E3 ligases of *Aedes*. Aegypti

Gene ID	Gene name	Type
AAEL005040	E3 ubiquitin-protein ligase siah-1	RING
AAEL005267	LON peptidase N-terminal domain and RING finger protein 2	RING
AAEL005288	E3 ubiquitin-protein ligase KCMF1	RING
AAEL005320	E3 ubiquitin-protein ligase MIB2	RING
AAEL005646	RING finger and CHY zinc finger domain-containing protein 1	RING
AAEL005826	E3 ubiquitin-protein ligase	RING
AAEL005874	E3 ubiquitin-protein ligase AMFR	RING
AAEL006550	E3 ubiquitin-protein ligase RNF185	RING
AAEL006589	protein TRC8 homolog	RING
AAEL006839	RING finger protein 3	RING
AAEL006929	E3 ubiquitin-protein ligase cullin-2	RING
AAEL007167	E3 ubiquitin-protein ligase RNF4	RING
AAEL007187	E3 ubiquitin-protein ligase cullin-3	RING
AAEL007227	E3 ubiquitin-protein ligase RNF123	RING
AAEL007353	E3 ubiquitin-protein ligase cullin-5	RING
AAEL007476	probable E3 ubiquitin-protein ligase makorin-1	RING
AAEL007527	tripartite motif-containing protein 2	RING
AAEL007701	putative E3 ubiquitin-protein ligase UBR7	RING
AAEL007797	E3 ubiquitin-protein ligase RNF220	RING
AAEL008184	E3 ubiquitin-protein ligase NRDP1	RING
AAEL008683	E3 ubiquitin-protein ligase Topors	RING
AAEL008854	RING finger protein 10	RING
AAEL009242	E3 ubiquitin-protein ligase RFWD3	RING
AAEL009614	E3 ubiquitin-protein ligase sina	RING
AAEL009739	E3 ubiquitin-protein ligase CBL-B-B	RING
AAEL009874	E3 ubiquitin-protein ligase znrf2	RING
AAEL010211	polycomb protein Pcl	RING
AAEL010831	mitochondrial ubiquitin ligase activator of nfkb 1-A	RING

Table S1. The E3 ligases of *Aedes*. Aegypti

Gene ID	Gene name	Type
AAEL011124	G2/M phase-specific E3 ubiquitin-protein ligase	RING
AAEL011179	ring finger protein 2	RING
AAEL011279	zinc finger protein ubi-d4	RING
AAEL011413	E3 ubiquitin-protein ligase RNF25	RING
AAEL011580	E3 ubiquitin-protein ligase RNF181	RING
AAEL011668	RING finger and transmembrane domain-containing protein 2	RING
AAEL011927	E3 ubiquitin-protein ligase mind-bomb	RING
AAEL012105	zinc finger protein-like 1 homolog	RING
AAEL012209	E3 ubiquitin-protein ligase RING1	RING
AAEL012337	protein goliath	RING
AAEL012428	E3 ubiquitin-protein ligase TRIM37	RING
AAEL012588	E3 ubiquitin-protein ligase CHIP	RING
AAEL012693	E3 ubiquitin-protein ligase TRIM37	RING
AAEL012941	E3 ubiquitin-protein ligase MARCH5	RING
AAEL013402	E3 ubiquitin-protein ligase Mdm2	RING
AAEL013530	E3 ubiquitin-protein ligase cullin-1	RING
AAEL013965	E3 ubiquitin-protein ligase RNF126	RING
AAEL014030	roquin-1	RING
AAEL014545	RING finger protein unkempt	RING
AAEL014744	E3 ubiquitin-protein ligase TRAIP	RING
AAEL017329	B-box type zinc finger protein ncl-1	RING
AAEL020680	probable E3 ubiquitin protein ligase DRIPH	RING
AAEL021579	E3 ubiquitin-protein ligase TRIM37	RING
AAEL023117	E3 ubiquitin-protein ligase MARCH6	RING
AAEL023762	E3 ubiquitin-protein ligase Mdm2-like	RING
AAEL023913	E3 ubiquitin-protein ligase MARCH3-like	RING
AAEL027780	E3 ubiquitin-protein ligase RNF181-like	RING

Supplementary Table 2

Table S2. The Sec61 translocon complex of *Aedes. aegypti*

Gene ID	Gene name
AAEL005856	signal recognition particle receptor subunit alpha homolog (SRα)
AAEL010716	protein transport protein Sec61 subunit alpha (Sec61α1)
AAEL004523	protein transport protein Sec61 subunit alpha (Sec61α2)
AAEL013989	protein transport protein Sec61 subunit beta (Sec61β)
AAEL005471	protein transport protein Sec61 subunit gamma (Sec61γ)
AAEL013226	translocation protein Sec62(Sec62)
AAEL013076	signal recognition particle 54 kDa protein (SRP54)

Supplementary Table 3

Table S3. Primers for dsRNA, siRNA synthesis, sequences for probes and qPCR

Primers for dsRNA synthesis			**Primers for qPCR**	
Gene ID	**Upper primer**	**Lower primer**	**Upper primer（qPCR）**	**Lower primer(qPCR)**
AAEL012490	TAATACGACTCACTATAGGGT CGACCAGTCTGTCGTCTTCG	TAATACGACTCACTATAGGGC GTCCGGCTCGGTGATGTGCT	CGTCATTGATGGCGGCAGTA	CGGCTACTTCCCAGACTAGC
AAEL023019	TAATACGACTCACTATAGGGA CAGTGTCGGCTACTGAGGAA	TAATACGACTCACTATAGGGC CGATGCATAGAGTTTATGCT	ACGAGAGCTTCGATGAGCAC	TCAGCACCTCAAACGGGTAG
AAEL018318	TAATACGACTCACTATAGGGC TGTCTAGACTGCTTCCGCC	TAATACGACTCACTATAGGGG TTCGGTCGCCGTTCGACACT	TTCGCCGGAAAGGAACTCTC	CGGAGATTGGCGATTCCAGT
AAEL022272	TAATACGACTCACTATAGGGG GAACCGCATGGTTCTTCCT	TAATACGACTCACTATAGGGT TCCAAGTACTTCGGGACAGCT T	AATGTAACCGATTGCTTCGC	GATCGTGCCAGTTTTCACCA
AAEL009509	TAATACGACTCACTATAGGGC GGGACTGACTATCACGCTC	TAATACGACTCACTATAGGGG ACTTTCTCGTTGATGCGGGT	CCAAGGTGCTACTCCACGAG	CCGATGGGGCAGCTTTGATA
AAEL005930	TAATACGACTCACTATAGGGG GTTCTCGAACATTAGAAATCG T	TAATACGACTCACTATAGGGA GCCGGATCCGTATTCTTTCT	TCGGATGAACCGCAGGATTT	ATCAACTGGGGTGCGTCTTC
AAEL017357	TAATACGACTCACTATAGGGT TCAGATCCGCACGACTACTA	TAATACGACTCACTATAGGGG CTTTATGGCTACTGCCCGTA	AACCTCCCAAAAGGCAGGAG	TGCTTGCCACTTTCTCTGGT
AAEL012500	TAATACGACTCACTATAGGGC ACCGCCGACCTATCTTTCA	TAATACGACTCACTATAGGGT AGTCGCCGTTCCGAATACAT	GGGCAGTGCTAAGATTCCGT	ACTGCTGCCGCTAAGAGTTT
AAEL007705	TAATACGACTCACTATAGGGA TACCTGCCAACCAAGCTCGA	TAATACGACTCACTATAGGGC ACCAACTTCCACCGAATCGA	GGCCTCCAAAGTCATGAGCA	GGAGGAAACGGTTAAGGGCA
AAEL004790	TAATACGACTCACTATAGGGC TACATGGCGTACTTCCGCA	TAATACGACTCACTATAGGGC GAACCCACGGTGTCGCCTAT	GAAGCCAGGCATGCTTCAAC	GACCGTTCGACAGGTTCCTT
AAEL008374	TAATACGACTCACTATAGGGA AATGGTCTATCCCCGTCGC	TAATACGACTCACTATAGGGC CGCCGCGTTTGGTATAATG	CAGTAACGATCCCAGCGACA	CGATCGATCCAACCGGACAT
AAEL002306	TAATACGACTCACTATAGGGG AGAGTGCCAGCTCGGATAC	TAATACGACTCACTATAGGGT CGTTCAAATGCCCATTCCAC	CGCTGCCAGAAAACGAAACA	TTGGTCGAGTAGTTTCCCGC
AAEL005820	TAATACGACTCACTATAGGGA CGTACTTCTCGCTGTGCAA	TAATACGACTCACTATAGGGA CATGCCATACTTGGGATCCA	ATCGAACTCGATCGGTCAGC	AGCGATCTGAACCACCGTTT

Table S3. Primers for dsRNA, siRNA synthesis, sequences for probes and qPCR

Primers for dsRNA synthesis			**Primers for qPCR**	
Gene ID	**Upper primer**	**Lower primer**	**Upper primer（qPCR）**	**Lower primer(qPCR)**
AAEL005774	TAATACGACTCACTATAGGGT GCTGGAAGATGCCTTCCGCC	TAATACGACTCACTATAGGGC CGATGTCGTTGTCCCGTAT	GAACTTGAAGGGGCTGTTGG	ATTCAGGTGGCTCGTCTTCG
AAEL010256	TAATACGACTCACTATAGGGC CTCAACAGCCAGCTCTCAA	TAATACGACTCACTATAGGGA GTTCGATTGTTGTGATCTA	GTACCTGGGCAAGAACGACA	TCTTTGAGCCGCTGGATTGT
AAEL011541	TAATACGACTCACTATAGGGA TCACTCCAGCTGATGGCCG	TAATACGACTCACTATAGGGC TGTATGCAAAACGGTGGAT	GTCGGACCGCTTGACCATAA	CTGGACAAAACTGCGAACCG
AAEL026428	TAATACGACTCACTATAGGGT TCAGATCCGCACGACTACTA	TAATACGACTCACTATAGGGG CTTTATGGCTACTGCCCGTA	AACCTCCCAAAAGGCAGGAG	TGCTTGCCACTTTCTCTGGT
AAEL006008	TAATACGACTCACTATAGGGC CTCAACAGCCAGCTCTCAA	TAATACGACTCACTATAGGGA GTTCGATTGTTGTGATCTA	GTACCTGGGCAAGAACGACA	TCTTTGAGCCGCTGGATTGT
AAEL001205	TAATACGACTCACTATAGGGT GCAGATCAATCCCGCAAGT	TAATACGACTCACTATAGGGC TCCAAAGGCACCACCTCAT	AGCCAAATCCGTACGTGGAG	GACACAATCACCGTGAAGCG
AAEL008184	TAATACGACTCACTATAGGGT GATTCCCAAGGACGAGTACA	TAATACGACTCACTATAGGGC AATTCTCCATCAGGTCGTCG	AATTTGCTCTCCCGGCTGAA	CTCGCACTCCTCGATATGGG
AAEL012209	TAATACGACTCACTATAGGGC GAATCCTCCATCGGTCAGG	TAATACGACTCACTATAGGGT GCAGAAATTCACCAGACGAT	AGATTGCTGTATCACCCCGC	TGCAGTCGGAACAAAACCGA
AAEL002706	TAATACGACTCACTATAGGGG ATAATGCAGAGCGTTCCGTT	TAATACGACTCACTATAGGGC TCCTCGCAATCTTTTCACTT	AAGCGACTCGGGAAAACGA	GGGGGCATTCTTCAACGGTA
AAEL006550	TAATACGACTCACTATAGGGT CGAGGATGCCACGGAAGACG	TAATACGACTCACTATAGGGT CGCCAAAGTTGAGTGTCGA	CGGCTCCCAGTTTGGAAGAT	AACTGCCCGTATCCGATTCC
AAEL021579	TAATACGACTCACTATAGGGT AACACGAACCGAACGAATGC	TAATACGACTCACTATAGGGC TGTTATTCTCGTTACGATCG	GCAAGCAATCCTTCGTCAGG	CCAGCGGGAAATGCAAAGAT
AAEL002642	TAATACGACTCACTATAGGGT ACGTTCTGGAGCTGGACGAC	TAATACGACTCACTATAGGGC AGCATCTTATCTCGGGCTAC	ATCAGGAGGTGACGAATGCC	GGTGCCAGGGGAAGTTTCAT
AAEL000424	TAATACGACTCACTATAGGGT CCGAAAAGTTCGTGCCACT	TAATACGACTCACTATAGGGG TCTGAGATGGATCGGGTTGC	GAAAGCGAACCGATTCCAGC	TTACGTTGACGTCCGACAGG
AAEL005826	TAATACGACTCACTATAGGGT CATTGTGCGGGGTTCTTCA	TAATACGACTCACTATAGGGA ACGAAGCCGTGGCAGCAGCC	AATGTCCAGGATGTGCCCAG	TCGCTTCCAGCGTGTAGTTT

Table S3. Primers for dsRNA, siRNA synthesis, sequences for probes and qPCR

Primers for dsRNA synthesis			**Primers for qPCR**	
Gene ID	**Upper primer**	**Lower primer**	**Upper primer（qPCR）**	**Lower primer(qPCR)**
AAEL005040	TAATACGACTCACTATAGGGA TTCGAATGCCGATAGCCGT	TAATACGACTCACTATAGGGT CCTGGGACGAGCCGGATATA	CGTGCGTGGACGAAAAGAAC	AGTGCATCCGGATTGCTTGA
AAEL013530	TAATACGACTCACTATAGGGA AGCGTCGATGATTTCCAAAC	TAATACGACTCACTATAGGGG GTATTCTCACTCAACTGTTG	AACGTGAGTGCGAAGAAGGT	TGGTTTCCCCATTACGCTCC
AAEL006929	TAATACGACTCACTATAGGGG GCAAAACGACTCATCCACG	TAATACGACTCACTATAGGGT GGCATAGATGATTCAACCAG	CACCCGATTCCGCCTAATGA	CTGCGATGCACTGCAGTTTT
AAEL007187	TAATACGACTCACTATAGGGA GACCCTGAACCAATCGTGGA	TAATACGACTCACTATAGGGC TCGGTGATACGTGCCTCAAC	TCGTGCGAACACCCAAATCA	ATGATACAATTTTGAAGCGTG ACA
AAEL003466	TAATACGACTCACTATAGGGG GCGGAACTGATAGCCAAGT	TAATACGACTCACTATAGGGC AGCTCTGGGGGCAGTGTGAC	GTACCAAGCCGTGGAGAACA	TTATCGACCGACTCGGCAAG
AAEL007353	TAATACGACTCACTATAGGGA CTACATCCCCGAGGTGGAA	TAATACGACTCACTATAGGGT TGTAACCGTCCGACCAGATT	CATTCGATTCGCAGCTGGTG	CGCGGACGTAGCTTGTAGAT
AAEL001765	TAATACGACTCACTATAGGGG GTCTGGTGGAACAGGTGAG	TAATACGACTCACTATAGGGT CAAGATAGCGGCACGATCC	TGTCTGGCGAAAGAGACACC	ACTCCTTCCGCAGAACCTTG
AAEL005320	TAATACGACTCACTATAGGGA TAACCAGGACGGTGGACCA	TAATACGACTCACTATAGGGT TGGCACAACCCTCATACTG	GTGCGAAATAGGACTTCCG	TCTGGTACCCGACCCGATAA
AAEL003271	TAATACGACTCACTATAGGGA GCACGTGGCGTATCATCAA	TAATACGACTCACTATAGGGC ACGTTCCAACGGATATGGC	AAGCGCGATCATGGCTGTAA	TGTCCGATATGCGCACCATT
AAEL005874	TAATACGACTCACTATAGGGA GCACGTGGCGTATCATCAA	TAATACGACTCACTATAGGGC ACGTTCCAACGGATATGGC	AAGCGCGATCATGGCTGTAA	TGTCCGATATGCGCACCATT
AAEL014744	TAATACGACTCACTATAGGGG TGCTGAACACACCCGATCA	TAATACGACTCACTATAGGG AGCTGTCCAAATCAAGTTCCA G	CCGATATATTCGTGTCGTCGGA	TTTGTTTCGACACTCCGGGC
AAEL003787	TAATACGACTCACTATAGGGA GATGTACCGACTGCAATGTGA	TAATACGACTCACTATAGGGT CCCTCGTTTGATGGTCACG	TGTGTTTCCTTCATCGGTGG	ATTTGCTTCGACACTCCGGG
AAEL000776	TAATACGACTCACTATAGGG ATAGCACCGGTGAAGGAAGC	TAATACGACTCACTATAGGG GTCCATACACCGGACCAAGG	AATTTCTGCGCATTCCGTCG	GATGGGGTGTTCCACGCTTA
AAEL012428	TAATACGACTCACTATAGGG CAGGAGGCCCATCTTTGTCC	TAATACGACTCACTATAGGG TTTCGGTCCATCAGGGTTCC	CAGCAGGAAATGGAGCAAGC	ACAGTGCGGACAAAGATGGG

Table S3. Primers for dsRNA, siRNA synthesis, sequences for probes and qPCR

Primers for dsRNA synthesis			**Primers for qPCR**	
Gene ID	**Upper primer**	**Lower primer**	**Upper primer（qPCR）**	**Lower primer(qPCR)**
AAEL004713	TAATACGACTCACTATAGGGT CGGTCTCTGGCATGTCAAC	TAATACGACTCACTATAGGGC CCAGTGGCTGACAAAATGC	CAGCTTGCCGGCACTTATTC	GCGCAAAAGCAATCCAGACA
AAEL012693	TAATACGACTCACTATAGGGC AGCCGGTCGCAGTTTATTG	TAATACGACTCACTATAGGGC GTCCAGGATGGTCTTCAGG	CAAACTGCAAGACCCGCATC	TTGGACAATTCATGCGCTGC
AAEL003010	TAATACGACTCACTATAGGGT CCATCCTGTTGGAGTGTCG	TAATACGACTCACTATAGGGG ACGCATCTCTGGGCTCATC	GGAGGACTCCAGACTCTCCA	CATTCCCGACAAAAGGGCTG
AAEL001410	TAATACGACTCACTATAGGGA TCGCCCGGAATCATGGAAA	TAATACGACTCACTATAGGGT CCTCTCCGGGGTTGAGAAT	TCTGAGTTTGTTCCTCCGGC	GTCGTTCCAGCTCCTCGAAA
AAEL000763	TAATACGACTCACTATAGGGC AACTGCACAACCGGTCAAG	TAATACGACTCACTATAGGGG CCGCATGTTTTGAGGGAAG	GCAGCAAAATCACCCAAGC	TCTAGCGCGCATTTCCTGCGA T
AAEL013402	TAATACGACTCACTATAGGGA GAGCCAGAAGGATTACCG	TAATACGACTCACTATAGGGT TCCAAACTTTCATGGCGCA	TGCAGCAGTGAAGTAGCAGA	TCTAGCACTCGGTTTGGACT
AAEL004044	TAATACGACTCACTATAGGGC GCCATCGCTGAGTTATCT	TAATACGACTCACTATAGGGC GGAACTCCAGCATCCCTT	CCGAACTGTATCGCTTCCGA	GATGAATCCCGGTACCGTCC
AAEL023117	TAATACGACTCACTATAGGGT CGAGGTATTGCTGTGGCAG	TAATACGACTCACTATAGGGT CGAATGGGCGATCACGTAG	CCACTGTTCCATCCCTGCAT	CGGCATATCCGGCGAATAGA
AAEL012941	TAATACGACTCACTATAGGGG CTTGCTGTTGCCGATTTCA	TAATACGACTCACTATAGGGA TGAAACACGTCTCCCTCCG	CGAGAAGCAGAAGGGGAACA	CCGTCAGGAAGGGGCTAATC
AAEL009614	TAATACGACTCACTATAGGGA TTTCATCGGTCGGAGCAGG	TAATACGACTCACTATAGGGT GACACTGTAGAATCGGCGG	GAACACGAGGAAGCCTGTGA	CGCGATGGAGGTATCGAACA
AAEL009739	TAATACGACTCACTATAGGGA ATGAAAAGCAAAGCCCGCA	TAATACGACTCACTATAGGGA GCGACGTTGGTTTGTGTCT	AAGGCACCGAGCAAATCGTA	TGGAGTGACGAGGTTGCAAT A
AAEL000590	TAATACGACTCACTATAGGGT TGAATGGCTACGGCGAAGA	TAATACGACTCACTATAGGGC TGGGCTTGTGGACATTCCT	GAGTCCAGTGTGTGACCCCA AG	TCAACTTCTGCACCGTCTCC
AAEL008683	TAATACGACTCACTATAGGGT GATCACCAGCACGGACTTC	TAATACGACTCACTATAGGGT CAAAGTTCCACCTCGGCTC	TCGACTTCGCTGCTTGACAT	CCGAGACAGATGGCAACTT
AAEL012588	TAATACGACTCACTATAGGG AACGCTGGAACATCCAGGAG	TAATACGACTCACTATAGGGG TTCTCTGCCAGGAAGGCAT	GAAAGACCAGGGCAATCGGA	TTGATGTGACACAGAGCCCG

Table S3. Primers for dsRNA, siRNA synthesis, sequences for probes and qPCR

Primers for dsRNA synthesis			**Primers for qPCR**	
Gene ID	**Upper primer**	**Lower primer**	**Upper primer（qPCR）**	**Lower primer(qPCR)**
AAEL009242	TAATACGACTCACTATAGGGC GACTGAACGTGGATGGTC	TAATACGACTCACTATAGGGC ACGCGACATTTGCTATCCG	GTTGCCAGAACCACTACGGA	CTTCCTCGACCGGAACCAAA
AAEL001217	TAATACGACTCACTATAGGG CCATCGACTTTGCCGAGGAG	TAATACGACTCACTATAGGGA CCATCAGGCTCATCACACC	AGGCGCTGCTAAAACAAATC G	ATGGCTGTGCGTGCATTTTG
AAEL023762	TAATACGACTCACTATAGGGC ATCCGCATCTGAATTCGCC	TAATACGACTCACTATAGGGT TCGCTTCTGCGTTATTTTGCT	GGATGACTACTCGTCGGATGT	AATGGCGAATTCAGATGCGG
AAEL003489	TAATACGACTCACTATAGGG GAACTCAGTGGCCAGGAACA	TAATACGACTCACTATAGGG GTAGTGCGACTGGAAGTGCT	GTCAAGCATTCTTCGCCGTC	AGCACTCGAAGCAACACAGA
AAEL000730	TAATACGACTCACTATAGGG ATGGAAGTTACGGGACAGGC	TAATACGACTCACTATAGGG AGAGAGCTCGTCTTTTCGGC	CGATGCCGTGCTAAATGTGG	CGAGGTGGTGGTGACCTCGTT TT
AAEL003248	TAATACGACTCACTATAGGGC GAGAAAACCCAGCTTCCCA	TAATACGACTCACTATAGGGT GACCGTTACATGAGTGGGC	CCGTTTCCCTATCCACCACC	ATTGCAATCCGAGGCCTTCA
AAEL011124	TAATACGACTCACTATAGGGA AATCAGTTTGAAGGAGTTG	TAATACGACTCACTATAGGGC CAGCAGTTTCAGCTTGAAC	AGACCGCCTCGAAAAAGACT	GAAGGCGCCACTTTTTGTGG
AAEL020680	TAATACGACTCACTATAGGGT GACGGAATCGGAATGTCC	TAATACGACTCACTATAGGGT GACGGAATCGGAATGTCCC	ATGATTACCAATGCATCGA	CGTGAACATCGTGGAACCTCT
AAEL007476	TAATACGACTCACTATAGGGT GGACAAGGAACAACAGCGA	TAATACGACTCACTATAGGGG TCGACCAGCGTACCATCTG	GCTGGTCCCTCGTCTTCTTC	TAGCGACAGGTGCCCATTTT
AAEL007701	TAATACGACTCACTATAGGGG AAGGTGAATCCGGTGCGTA	TAATACGACTCACTATAGGGA CGGCAAAAATGCGAAGGAG	CATGCCGGAATCTCGAGTGA	GCATGCGTTTTCCTCCACAG
AAEL002078	TAATACGACTCACTATAGGGC GGATTTCAGCCCACAAACG	TAATACGACTCACTATAGGGA CGCTCATCGACAGACAACT	TTGCCGCAGAAGATCCTACC	GAAGTCCTCGTAGCTGAGGC
AAEL007797	TAATACGACTCACTATAGGGG CCGGACAGACAAGGATACG	TAATACGACTCACTATAGGGT GTGCAGCCAACATTCTTCG	AAACGGAGGACAACGTCTGC	TCCCGTTACTACCGGAACCA
AAEL003680	TAATACGACTCACTATAGGGA AGGGTGTAGCCGGTTTGAG	TAATACGACTCACTATAGGGA GGAGATCGGTCACAACACG	CAGAGCTGGAACCCCAAACT	CAACCTTGCGGGATGAATGC
AAEL000523	TAATACGACTCACTATAGGGG CCGTTCTCTCCTCACCATT	TAATACGACTCACTATAGGGG CCGTTCTCTCCTCACCATT	TTCCTCGCCTCAAATGGCC	TGGAGCTGGCCAGTGGAATC

Table S3. Primers for dsRNA, siRNA synthesis, sequences for probes and qPCR

Primers for dsRNA synthesis			**Primers for qPCR**	
Gene ID	**Upper primer**	**Lower primer**	**Upper primer（qPCR）**	**Lower primer(qPCR)**
AAEL004861	TAATACGACTCACTATAGGGA AAAGAACCTTCAGCCGCCA	TAATACGACTCACTATAGGGA CACATCGGACATTTCGGGC	CGGAAGCTCTCCTAGCATCG	ATTCGTTGCACTCTGGTGGA
AAEL001610	TAATACGACTCACTATAGGGG CAGGAAGAGAAGTGGGGTC	TAATACGACTCACTATAGGGG ACTGACTTAGCGCTTGTGC	GGTCGATTGCGATGCTGTTG	CTTCGTTACCGAGTACGCCA
AAEL008854	TAATACGACTCACTATAGGGA ACGCCAGCTGGGTAAATCA	TAATACGACTCACTATAGGGC TCCAGTGCTGCCTCGATAG	TCGCAGCTACGTCAAGAAGG	ACTTGCTCGATCTTTGCCCA
AAEL027780	TAATACGACTCACTATAGGGA CGTTGCATCGATATGTGGC	TAATACGACTCACTATAGGGG AGGCGTTTCTTCATCCGTC	ACGGTCGATGAGGAACCATC	TAGCTGTGGGAACACTCCAG
AAEL011413	TAATACGACTCACTATAGGGA TCAATGCGCCCAAGTTTCG	TAATACGACTCACTATAGGGG AGAAGCCTCAACGACCTCC	TTATCCGGAACAGTAGCGGC	GACCCGGAAGCACCTGAAG
AAEL003009	TAATACGACTCACTATAGGGC GAGATGTTGGTTCCCCCTC	TAATACGACTCACTATAGGGC TGGTCGAGGGAAGATTGGG	GCTGTCGAAAAGTGCCAAGG	CTTGATGCCACTCTCGGGAA
AAEL007227	TAATACGACTCACTATAGGGT GATCCTGACGATAACGCCG	TAATACGACTCACTATAGGGC GGATCGAAGTTACCCCTCG	ACAGCTATGGCCTAGACGGA	TCCATGTCTACGCACACACC
AAEL007167	TAATACGACTCACTATAGGGC GCAGAATGTCGAGGTGCTA	TAATACGACTCACTATAGGGG CAGCTTGCTGGTGGAAAAT	TCGTTCCTAAACCGTGAGGC	CGTACCGGAATCATTTGCCG
AAEL011580	TAATACGACTCACTATAGGGT GTTGATGGTTCGCTTTCTGC	TAATACGACTCACTATAGGGC TCTTCGATCTCCTGTTCCCG	TTTGAAGAACTCGGCTGCGA	CGTATCCGACCGGAACTCAT
AAEL002020	TAATACGACTCACTATAGGGT CATGCACCGAATGTGTGGA	TAATACGACTCACTATAGGGT CCAATCGAGCAGTTGACCG	CGAGCCGGTCGATATGTTCA	ATGATGAGCGTCACCAGCAA
AAEL011668	TAATACGACTCACTATAGGGC CGAGCGTGATCGAGTACAA	TAATACGACTCACTATAGGGC GTCAACGATCTTGGCCCTA	GTAGTAGAGTCGCCACCAGC	TCTGTTGCTAACGGTGGCTT
AAEL013965	TAATACGACTCACTATAGGGT CGACGAAGTCGTACGGAAG	TAATACGACTCACTATAGGGG ATACCTGTTGGTGCCGGAG	CACTGCTCGGAAGGGTTCAT	CACTGCTCGGAAGGGTTCAT
AAEL003104	TAATACGACTCACTATAGGGA GAAGAAACCCACCCGTTCG	TAATACGACTCACTATAGGGT TCTTCGCGGGAGGTCTTTC	ACTGAAGGCCCGTGTAACAG	CCTGATCACTATCGGCCTCG
AAEL001778	TAATACGACTCACTATAGGGC TACCGTGGAATGGCCAACT	TAATACGACTCACTATAGGGC ACACCGCAAATCGCACATC	GCTAGGAAGCGCAAGCAATC	GCTTTGCGCTGGTGCTTTAT

Table S3. Primers for dsRNA, siRNA synthesis, sequences for probes and qPCR

Primers for dsRNA synthesis			**Primers for qPCR**	
Gene ID	**Upper primer**	**Lower primer**	**Upper primer（qPCR）**	**Lower primer(qPCR)**
AAEL006839	TAATACGACTCACTATAGGG CTTTGACCGCACAATGCAGG	TAATACGACTCACTATAGGGC GCGGTCGATACTGTAGTCTT	AAAGCTGCCTGGTGAAACAC	TTCCATCAGATTCGGCACCA
AAEL000570	TAATACGACTCACTATAGGGC GACGTGAAACCGGACAAAAT G	TAATACGACTCACTATAGGGG AAAGATTCTTCCTCCGGCGT	AATTCGACGACGTGAAACCG	AATTCGACGACGTGAAACCG
AAEL012337	TAATACGACTCACTATAGGGA GCACATTCCACAGCAGTCA	TAATACGACTCACTATAGGGT CTTGCACTCGGTTTCGAGG	CATCGAAGGAAGTCGCCACA	GTAGAACACCAGCCACACCA
AAEL004356	TAATACGACTCACTATAGGGC GGGTGTCTCCTCATCATGG	TAATACGACTCACTATAGGGA CGAAGCGGTTTAAGCTGGA	AACGACTCAAGCGGAACGAT	CGGTACTCGTGATCGACTGG
AAEL009874	TAATACGACTCACTATAGGGA GCGCTAGGTAGGGTCTACG	TAATACGACTCACTATAGGGG GACAGCTGCGATTCACCT	AAATATCCTCCGCCAGTCCG	CAATGACCAGATGTGTGCCG
AAEL004697	TAATACGACTCACTATAGGGC TGTATCCTGACGCCACTCC	TAATACGACTCACTATAGGGA ATTCTTCGTCGGTGAGCGT	ACTCATCTCGTATGCATATCA	GTTCTCCCAAGGAGTGTCGG
AAEL011927	TAATACGACTCACTATAGGGG AGTGCCTCCTTTGTTCGGA	TAATACGACTCACTATAGGGC GACGTTTCCTGTGCCAATG	CCAACCAGGTTCACGATGGA	ACCTCCTCTTGGGACTCGAA
AAEL005288	TAATACGACTCACTATAGGGC TTCGCAGAGTCGGTCCAAA	TAATACGACTCACTATAGGGA TAGTGTGACCACGAGCACC	GTCAACATTGTCGGCACTCG	CGAAGAATTCGTCCTCCGGG
AAEL002345	TAATACGACTCACTATAGGGC TGCCAGGACGAATTCCAGT	TAATACGACTCACTATAGGGT GTACAAACAGGGTCCGTGG	GCAAGTTGCGGGAAGTGTTT	GCAAGTTGCGGGAAGTGTTT
AAEL005646	TAATACGACTCACTATAGGGG GAAGTGGCTGCAACACCTA	TAATACGACTCACTATAGGGC TGGCAGCAGTAGACGGTAA	GGACGGTACACTTGCTTGGT	GTGGAAAAATCGACCCCTGC
AAEL003343	TAATACGACTCACTATAGGGC GCTCGATGTCTCACGATCT	TAATACGACTCACTATAGGGT CTTGCACGGTGCTAACGAT	TAGTCGCGATTCGTACACGG	CGGTATCCGACAACGATGCT
AAEL011279	TAATACGACTCACTATAGGGT GACTCCCCAGACGAGTTCT	TAATACGACTCACTATAGGGT TCCGGTGGGGTCAAAAGAG	ACTCCGGATGCCATTTCTGG	ATCGTGCCGAAGGGTAACTG
AAEL012105	TAATACGACTCACTATAGGGG GCGAGAAACGTTCTTGAGC	TAATACGACTCACTATAGGGT GCGGCAGATTACGGTTGTA	TTGTGCGGAAGTCCACTTGA	GCCGGCAAGGATTGTTGTTT
AAEL014545	TAATACGACTCACTATAGGGC TAGACGGAGGACGGCAGGA	TAATACGACTCACTATAGGGT CGCGTCATATCATACACTCCG	AGCATCCTCATTAGACGGCG	TATGGAGTTTCCCATCGGAG

Table S3. Primers for dsRNA, siRNA synthesis, sequences for probes and qPCR

Primers for dsRNA synthesis			**Primers for qPCR**	
Gene ID	**Upper primer**	**Lower primer**	**Upper primer（qPCR）**	**Lower primer(qPCR)**
AAEL005267	TAATACGACTCACTATAGGGC GATGGCTGCTCAATTCTG	TAATACGACTCACTATAGGGT ATCGATGGCTCGAAGTCGC	GCCAGTAAGGGAAGTGGTCC	TAAAACTAGGCTCGTGGCGG
AAEL001357	TAATACGACTCACTATAGGGC GAAGGTGAAAGTGGAGCCT	TAATACGACTCACTATAGGGT TGGCGCTTGAATCGCTTTC	CAACGGGAATGGAAGCGTTG	TCGCTGCAATTCAGTCTCGT
AAEL023913	TAATACGACTCACTATAGGGC GGTCCGGACTTACACACTG	TAATACGACTCACTATAGGGA CGGGTCACTAGCATCGTC	AGTGAAGAACCACAACGGCA	GCACAGACAGGGCGAGATTA
AAEL014030	TAATACGACTCACTATAGGGG CGGCGATTTGAAGATTCCC	TAATACGACTCACTATAGGGT CGGATTCAAGACGGAGTGC	CGGATTACGGGTGCATCTCA	CGACCAGGCCTATAACGACC
AAEL007527	TAATACGACTCACTATAGGGG GGAATTGCCATAGGACCGA	TAATACGACTCACTATAGGGA CGAAGCTTCCATTCGGGTT	TAGTTGGTGATGGCGGGAAC	TAGTTGGTGATGGCGGGAAC
AAEL006589	TAATACGACTCACTATAGGGT CGGCCAAAATTACGCGTTG	TAATACGACTCACTATAGGGC TGCCACCGATGAGGGATTT	TCATAATGCGCGTCCCAAGT	TCATAATGCGCGTCCCAAGT
AAEL010831	TAATACGACTCACTATAGGG GAAGGGAATGCAAACGACCG	TAATACGACTCACTATAGGG CGCAGTTTTCGCAGAGACAC	CGTCTTCCTGGGTGTGGATG	TTGCTAGTTGGGGTGCTTCC
AAEL011179	TAATACGACTCACTATAGGGA AGCAGGGTCAGGTGTGATG	TAATACGACTCACTATAGGGT GCATTCGCGGTGTTTGTTT	ACAACCGGCAGTCGGATAAG	CAGGACCGGCAAAATGTGTG
AAEL001933	TAATACGACTCACTATAGGGG CCATCGGATGCAAACAACC	TAATACGACTCACTATAGGGG GCGAGGGTAGCAGATTCTC	TCCCCTTCATCATGCACACC	GATGAGCACACAGAGGGACC
AAEL001459	TAATACGACTCACTATAGGGA TGCTCAACCCTGTCCGATG	TAATACGACTCACTATAGGGC CAAAGGACCACATCCTCCC	TTCACCAGCTGGCAAAGGAA	AGCTTCCTCGATCACGCATT
AAEL010211	TAATACGACTCACTATAGGGA GCTTCCTCGATCACGCATT	TAATACGACTCACTATAGGGG CGACGACTGGAGATCTGTT	GTTGGGAAAAGCTCACGCTG	CTTTCCGTTCGTTGTGCTGG
AAEL017329	TAATACGACTCACTATAGGGC GAGTGCCAAGTTTGTGGTG	TAATACGACTCACTATAGGGA GTAGCAAGCCACGTGGAAA	GGCAGGGATCATTCCCGTAG	GGCAGGGATCATTCCCGTAG
AAEL004689	TAATACGACTCACTATAGGGC CCTGCCAACGTCCATACTT	TAATACGACTCACTATAGGGT CATTCAATGCGTCCAGCCA	ACTACAGCCTCCAAGCATTC	GCTGTGAGGTTGTTGACACC
AAEL004697(*AaHRD1* dsRNA1)	TAATACGACTCACTATAGGGC TGTATCCTGACGCCACTCC	TAATACGACTCACTATAGGGA ATTCTTCGTCGGTGAGCGT	ACTCATCTCGTATGCATATCA	GTTCTCCCAAGGAGTGTCGG

Table S3. Primers for dsRNA, siRNA synthesis, sequences for probes and qPCR

Primers for dsRNA synthesis			**Primers for qPCR**	
Gene ID	**Upper primer**	**Lower primer**	**Upper primer（qPCR）**	**Lower primer(qPCR)**
AAEL004697(*AaHRD1* dsRNA2)	TAATACGACTCACTATAGGGT GCATTCCGGCCCATGTATT	TAATACGACTCACTATAGGGA AGTACTAATGGCGCTCCCG	ACTCATCTCGTATGCATATCA	GTTCTCCCAAGGAGTGTCGG
AAEL004697(*AaHRD1* dsRNA3)	TAATACGACTCACTATAGGGT TGGGTTCATTCGCGTGGTA	TAATACGACTCACTATAGGGT AGCATGCAATGGGGAAGCA	ACTCATCTCGTATGCATATCA	GTTCTCCCAAGGAGTGTCGG
AAEL004514 *(AaSel1L)*	TAATACGACTCACTATAGGGA CAACGCACAAGCGATGTTC	TAATACGACTCACTATAGGGT GGCGTGGATTCGTATTGCT	GAGTTGGAACCTCCACAGGG	GTTCGAACCGTCCTTCACCT
AALC636_030691 (*AablHRD1*)	TAATACGACTCACTATAGGGG CTGAACATTCTGCGAACCC	TAATACGACTCACTATAGGGG AGGTAAACGGGCCACCAAT	CCTCAAGTCGTTCCACTGGT	CAATAGTCCAGCCACTCGCA
AALC636_009275 (*AablSel1L*)	TAATACGACTCACTATAGGGT AGGATGGCTTCGGAACAGC	TAATACGACTCACTATAGGGG CCTCCTCTCCGGCTACTC	AGGGCGATGTACAAGCTCAG	TTGGCCGCCTGACTGAAATA
CPIJ003290(*CqHRD1*)	TAATACGACTCACTATAGGGC ATTTGCATCATCTGCCGGG	TAATACGACTCACTATAGGGG CTCGTTACCTTCCATTGCG	AGTCCAACCCAAGTATGGCG	GCTCCATCAGGTGCTCGAAT
AAEL005856	TAATACGACTCACTATAGGGG GCGTGGGAAAGTCTACCAA	TAATACGACTCACTATAGGGT TCACCAGCTGATCTACGGC	TTACCAAGGGCGGAATCGTC	CGTACACTCCAGATCGCTCC
AAEL010716	TAATACGACTCACTATAGGGA AAATTCCACGGCAACCTGC	TAATACGACTCACTATAGGGG CTCTGCTCCTTGACGAAGA	ACCCTGATGGAGCTGGGTAT	TCCAAACAGTTTTTGCGCCC
AAEL004523	TAATACGACTCACTATAGGGC AACCTTCTCGGAGTGTGGG	TAATACGACTCACTATAGGGA TGATGGTGACTGCCAGCAA	ACTATGGACAGCCATCACGC	TTCGAAGCCAGGATGACACG
AAEL013989	TAATACGACTCACTATAGGGC CGGAGGTTCCAGCAATCTG	TAATACGACTCACTATAGGGA TCTTCCCCGGAACGTACTCA	GATCTCCCACAAAGCCCACA	GTGTAGAAACGCCACATGCC
AAEL005471	TAATACGACTCACTATAGGGA TCGCCAAGATCTACGAGCC	TAATACGACTCACTATAGGGT GGTTCTCAACGCATCTCGT	TTTCCTGTCGCCGACGTTTA	AATCGGATCGAGTCCTTGGC
AAEL013226	TAATACGACTCACTATAGGGA TCTTTGGTGCCCTGTTGGT	TAATACGACTCACTATAGGGT TTTGCCCTTTCCTCGGTGT	GGCTCAAATCGAATGTGCCG	ACTTTGAAGCCATCAGGGCA

The probes for Taqman qPCR

Gene ID	**Upper primer（qPCR）**	**Lower primer(qPCR)**	**Probes**
A. aegypti actin	GAACACCCAGTCCTGCTGACA	TGCGTCATCTTCTCACGGTTAG	5'FAM-AGGCCCCGCTCAACCCGAAG-3'TAMRA

A. albopictus actin	CGGAAGAGCACCCAGTTCTC	TGTGTCATCTTCTCGCGGTTAG	
C. quinquefasciatus actin	AGTCCAACCCAAGTATGGCG	GCTCCATCAGGTGCTCGAAT	
DENV2	CATTCCAAGTGAGAATCTCTTTGTCA	CAGATCTCTGATGAATAACCAACG	5'FAM-ATGCTGAAACGCGAGAGAAACCGC-3'TAMRA
ZIKV	CCGCTGCCCAACACAAG	CCACTAACGTTCTTTTGCAGACAT	5'FAM-AGCCTACCTTGACAAGCARTCAGACACTCAA-3'TAMRA
JEV	CTGGTCCATAGGGAGTGGTTTC	CTCCACGCTGTGCTCGAA	

Sequences for siRNA synthesis

Gene	**Upper primer (SiRNA)**	**Lower primer (SiRNA)**
HsHRD1 #1	GCAUGGCAGUCCUGUACAUTT	AUGUACAGGACUGCCAUGCTT
HsHRD1#2	UCAUCUGCCGAGAAGAGAUTT	AUCUCUUCUCGGCAGAUGATT
HsSel1L#1	GCCAGGAUGAAUCCUUAGATT	UCUAAGGAUUCAUCCUGGCTT
HsSel1L#2	GCAAUCUAAUAGCCCACAUTT	AUGUGGGCUAUUAGAUUGCTT

Sequences for qPCR of *Hs*HRD1/*Hs*Sel1L

Gene name	**Upper primer (qPCR)**	**Lower primer (qPCR)**
HsHRD1	CATTTTCCATACCAGCTGCC	GCTGAGGCAAGAGTGGTG
HsSel1L	AGACAGCCTCAAGAGCCAAG	AATGGCGGTCAAAGCTGGTT

Sequences for *Hs*GAPDH/*Mus*GAPDH

Gene name	**Upper primer (qPCR)**	**Lower primer (qPCR)**
HsGAPDH	AGCCTCAAGATCATCAGCAATG	ATGGACTGTGGTCATGAGTCCTT

MusGAPDH	TCAACAGCAACTCCCACTCTTCCA	ACCCTGTTGCTGTAGCCGTATTCA

Supplementary Table 4

Table S4. Sequences of primers for plasmids construction

Plasmids	Upper primer（PCR）	Lower primer（PCR）	Upper primer（PCR）	Lower primer（PCR）
pET28a-*Aa*HRD1-△TM-His	GTCGACAAGCTTGCGGCCGCA TGGTCAAGATTTACACACTTCC	TGGTGGTGGTGGTGCTCGAG ATCAGAGCTCATCCTGGAAC		
pXJ-NS1-HA	TAGGGCGAATTCGCGGCCGCA CCATGGATAGTGGTTGCGTTGT	AACATCGTATGGGTAGGATC CGGCTGTGACCAAGGAGT		
pXJ-NS2A-HA	TAGGGCGAATTCGCGGCCGCA CCATGGGAGTCAAAGTTCTGTT TGCCCTGATCTGC	AGCGTAATCTGGAACATCGT ATGGGTAGGATCCCCTTTTCT TGCTGGTTCTTGAG	TTTGCCCTGATCTGCATCGCT GTGGCCGAGGCCGTGTACTCC ATTGAAGGAGAAAGAAGAG	
pXJ-NS2B-HA	TAGGGCGAATTCGCGGCCGCA CCATGGGAGTCAAAGTTCTGTT TGCCCTGATCTGC	CATCGTATGGGTAGGATCCCC GTTGTTTCTTCACTTC	TTTGCCCTGATCTGCATCGCT GTGGCCGAGGCCGTGACTAG CTGGCCACTAAATG	
pXJ-NS3-HA	GCGAATTCGCGGCCGCACCAT GGCTGGAGTATTGTGG	CATCGTATGGGTAGGATCCCT TTCTTCCAGCTGCAAACT		
pXJ-NS4A-HA	TAGGGCGAATTCGCGGCCGCA CCATGTCCCTGACCCTGAACCT AA	GAACATCGTATGGGTAGGAT CCTCTCTGCTTTTCTGGTTCT GG		
pXJ-NS4B-HA	TAGGGCGAATTCGCGGCCGCA CCATGGGAGTCAAAGTTCTGTT TGCCCTGATCTGC	AACATCGTATGGGTAGGATC CCCTTCTCGTGTTGGTTGT		
pXJ-NS5-HA	CTATAGGGCGAATTCGCGGCCG CACCATGGGAACTGGCAACAT A	AACATCGTATGGGTAGGATC CCAGGACTCCTGCCTCTT		
pXJ-*Hs*HRD1-Myc	TATAGGGCGAATTCGCGGCCGC ACCATGTTCCGCACGGCA	ATAAGATCTGGTACCCTCGA GTCACAGATCCTCTTCTGAG ATGAGTTTTTGTTCGGAT		AGATGAGTTTTTGTTCGGATC CGTGGGCAACAGGAGACTC
pXJ-*Hs*HRD1-C329S-Myc	TATAGGGCGAATTCGCGGCCGC ACCATGTTCCGCACGGCA	GGACATCCATACGGGAGGTG GGGCAGGTCT	AGACCTGCCCCACCTCCCGTA TGGATGCC	AGATGAGTTTTTGTTCGGATC CGTGGGCAACAGGAGACTC
pXJ-*A.a*HRD1-Flag	GCGAATTCGCGGCCGCACCAT GAGAGCCATCGGAATTTCA	GATCTGGTACCCTCGAGCTA CTTATCGTCGTCATCCTTGTA ATCGGATCCGTCGGAGGACA TCCTTGAGCCAAA		
pXJ-NS4A-Flag	GGCGAATTCGCGGCCGCACCA TGTCCCTGACCCTGAACCTAA	GTCGTCATCCTTGTAATCGG ATCCTCTCTGCTTTTCTGGTT CTGGA		

Table S4. Sequences of primers for plasmids construction

Plasmids	Upper primer（PCR）	Lower primer（PCR）	Upper primer（PCR）	Lower primer（PCR）
pXJ-NS4A-K20R-Flag	GGCGAATTCGCGGCCGCACCATGTCCCTGACCCTGAACCTAA	TGTCCAGTGCGTCTCTTGCCCGCTGAGTCATGAAAGT	ACTTTCATGACTCAGCGGGCAAGAGACGCACTGGACA	GTCGTCATCCTTGTAATCGGATCCTCTCTGCTTTTCTGGTTCTGGA
pXJ-NS4A-K80R-Flag	GGCGAATTCGCGGCCGCACCATGTCCCTGACCCTGAACCTAA	ACATTCCCAGGGTCATCCGCCCTATACCCCTTCCGCTC	GAGCGGAAGGGGTATAGGGCGGATGACCCTGGGAATGT	GTCGTCATCCTTGTAATCGGATCCTCTCTGCTTTTCTGGTTCTGGA
pXJ-NS4A-K125R-Flag	GGCGAATTCGCGGCCGCACCATGTCCCTGACCCTGAACCTAA	GTCGTCATCCTTGTAATCGGATCCTCTCTGCCGTTCTGGTTCTGG		
pUb-*A.a*HRD1-Myc	CTCAACAGAGATTTCAACGAATCATGCGTGCCATCGGAATATC	CTCAACATCATCTCTCGATTACAGATCCTCTTCAGAGATGAGTTTCTGCTCATCAGAGCTCATCCTGGAA		
pUb-*A.a*HRD1-C328S-Myc	CTCAACAGAGATTTCAACGAATCATGCGTGCCATCGGAATATC	GCAGAATGTTCAACCGGGATGTTGGACAAGTTTGCTGCCG	CGGCAGCAAACTTGTCCAACATCCCGGTTGAACATTCTGC	TTCAGAGATGAGTTTCTGCTCATCAGAGCTCATCCTGGAACC
pUb-NS4A-3XFlag	CAACAGAGATTTCAACGAATCATGAGTCTCACCCTGAATC	CTCAACATCATCTCTCGATTACTTGTCGTCGTCGTCTTTATAATCGATGTCGTGATCTTTGTAATC		ATTACTTGTCGTCGTCGTCTTTATAATCGATGTCGTGATCTTTGTAATC
pUb-NS4A-K20R-3XFlag	CAACAGAGATTTCAACGAATCATGAGTCTCACCCTGAATC	GGCGTCGCGGGCCCGCTGGGTCATGAACGT	ACGTTCATGACCCAGCGGGCCCGCGACGCC	CCGTCGTGATCCTTGTAGTCGCGCT
pUb-NS4A-K80R-3XFlag	CAACAGAGATTTCAACGAATCATGAGTCTCACCCTGAATC	CATGCCCAGGGTCATTCGGCCGATACCCCTGC	GCAGGGGTATCGGCCGAATGACCCTGGGCATG	CCGTCGTGATCCTTGTAGTCGCGCT
pUb-NS4A-K125R-3XFlag	CAACAGAGATTTCAACGAATCATGAGTCTCACCCTGAATC	GGCGAATTCGCGGCCGCACCATGTCCCTGACCCTGAACCTAA		
pUb-NS4A-T2A-Puro	TATAAAGACGACGACGACAAGGAGGGCCGCGGCAGCCTGC	TGCTCAACATCATCCGATTATCAGGCACCGGGCTTGCGGG	TAATCGGATGATGTTGAGCAATAA	CTTGTCGTCGTCGTCTTTATAATCG
pUb-NS4A-△N40-3XFlag	CAACAGAGATTTCAACGAATCATGTATAACCACGCACTTAGCGA	CCGTCGTGATCCTTGTAGTCGCGCTGCTTTTCTGGCTCGG		
pUb-NS4A-△C40-3XFlag	CAACAGAGATTTCAACGAATCATGAGTCTCACCCTGAATC	CCGTCGTGATCCTTGTAGTCACAACACATGCCCAGGGTC		
pUb-NS4A-△41-87-3XFlag	CAACAGAGATTTCAACGAATCATGAGTCTCACCCTGAATC	AAATCGAGGCGGTAATGATAGCACGTCCTCCTGCCTCA	TGAGGCAGGAGGACGTGCTATCATTACCGCCTCGATTT	CCGTCGTGATCCTTGTAGTCGCGCT

Table S4. Sequences of primers for plasmids construction

Plasmids	Upper primer（PCR）	Lower primer（PCR）	Upper primer（PCR）	Lower primer（PCR）
pUb-*Aa*SRα-Myc	CTCAACAGAGATTTCAACGAA TCATGTTGGATTTGTTCACCAT	TCTTCAGAGATGAGTTTCTG CTCCTTCATCAACGCATGTAC GA		
pMT-*A.a*HRD1-V5	GATCTAGATCGGGGTACCATGC GTGCCATCGGAATATC	TTCGAAGGGCCCTCTAGACT CGAGATCAGAGCTCATCCTG GAAC		
pMT-*A.a*HRD1-C328S-V5	GATCTAGATCGGGGTACCATGC GTGCCATCGGAATATC	GCAGAATGTTCAACCGGGAT GTTGGACAAGTTTGATGCCG	CGGCAGCAAACTTGTCCAAC ATCCCGGTTGAACATTCTGC	TTCGAAGGGCCCTCTAGACTC GAGATCAGAGCTCATCCTGGA AC
pAC-*A.a*HRD1-V5	AGTCCAGTGTGGTGGAATTCAT GCGTGCCATCGGAATATC	CGAAGGGCCCTCTAGACTCG AGATCAGAGCTCATCCTGGA AC		
pAC-*A.a*HRD1-C328S-V5	AGTCCAGTGTGGTGGAATTCAT GCGTGCCATCGGAATATC	AGTCCAGTGTGGTGGAATTC ATGCGTGCCATCGGAATATC		
pMT-GFP-V5	GATCTAGATCGGGGTACCATGG TGAGCAAGGGCGAGGAGCT	GAAGGGCCCTCTAGACTCGA GCTTGTACAGCTCGTCCATG		
pMT-*Aa*SRα-V5	GGGATCTAGATCGGGGTACCAT GTTGGATTTGTTCACCAT	TTCGAAGGGCCCTCTAGACT CGAGCTTCATCAACGCATGT ACGA		
pMT-*Aa*SRP54-Myc	GGGATCTAGATCGGGGTACCAT GGTTTTAGCGGATCTCGGTCG	GAGATGAGTTTTTGTTCAGA CTTACCGAATCCGCCCATCA		
AAV-eGFP	/	/		
pUb-JEV-NS4A/K79R-3XFlag	AACAGGATTTCAACGAATCATG TCAGCTGTCAGTTTTATCGAAG	CCGTCGTGATCCTTGTAGTC CCTTTGTTTTTCAGGCTCTG		
pMT-*Cq*HRD1-V5	GATCTAGATCGGGGTACCATGC GTGCCCTCGGGATA	TTCGAAGGGCCCTCTAGACT CGAGGTTGGCTCCAGACGA GGCGG		
pMT-*Cq*HRD1-C328S-V5	GATCTAGATCGGGGTACCATGC GTGCCCTCGGGATA	GTGCGCAGAATGTTCAACCG AGAGGTCGGGCACGTCTGCT	AGCCGACGTGCCCGACCTCTC GGTTGAACATTCTGCGCAC	TTCGAAGGGCCCTCTAGACTC GAGGTTGGCTCCAGACGAGG CGG

SI reference

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