Supplementary Information

Host serum-circulating flavivirus NS1 co-opts mosquito miRNA processing to suppress early immune defense

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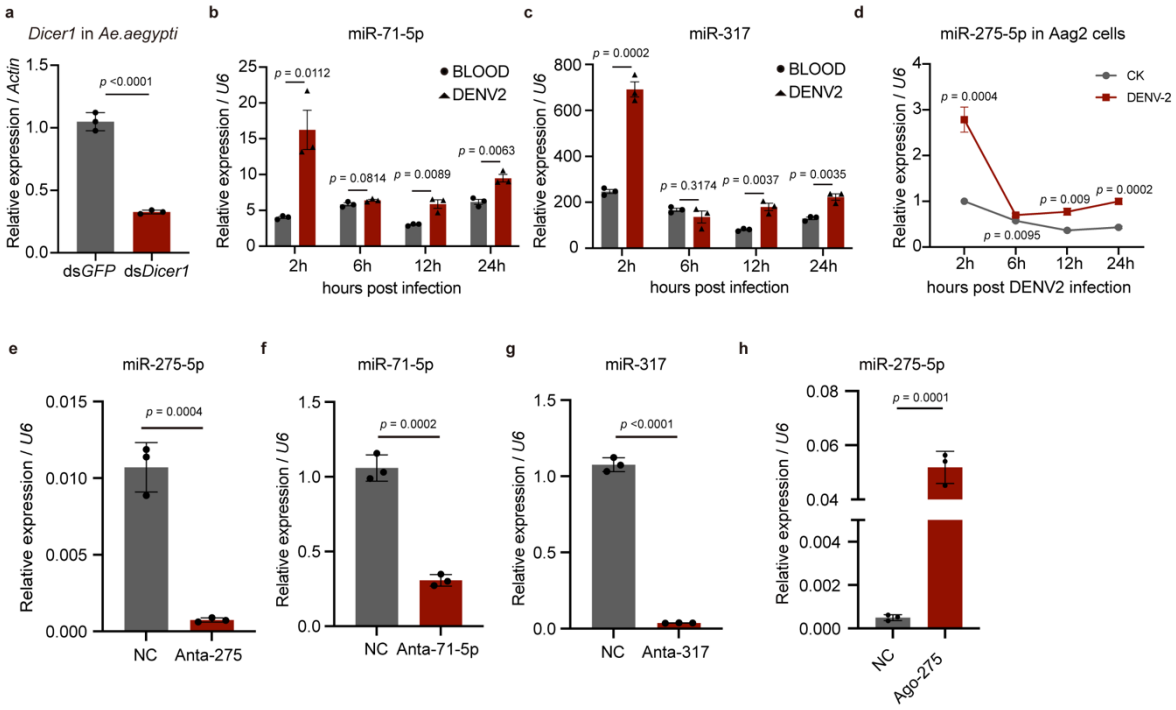
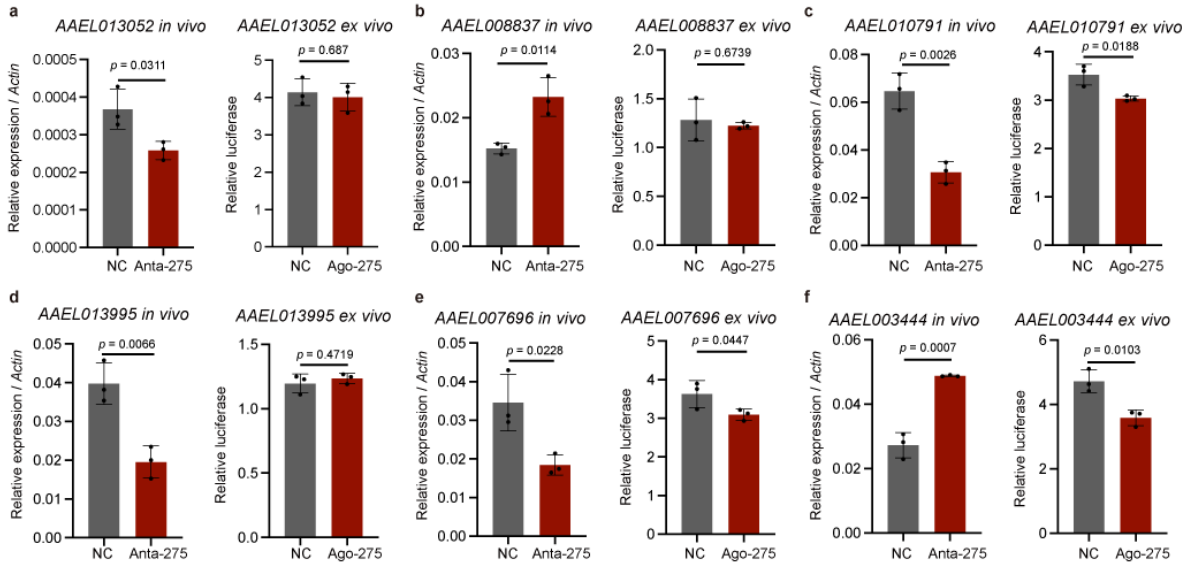


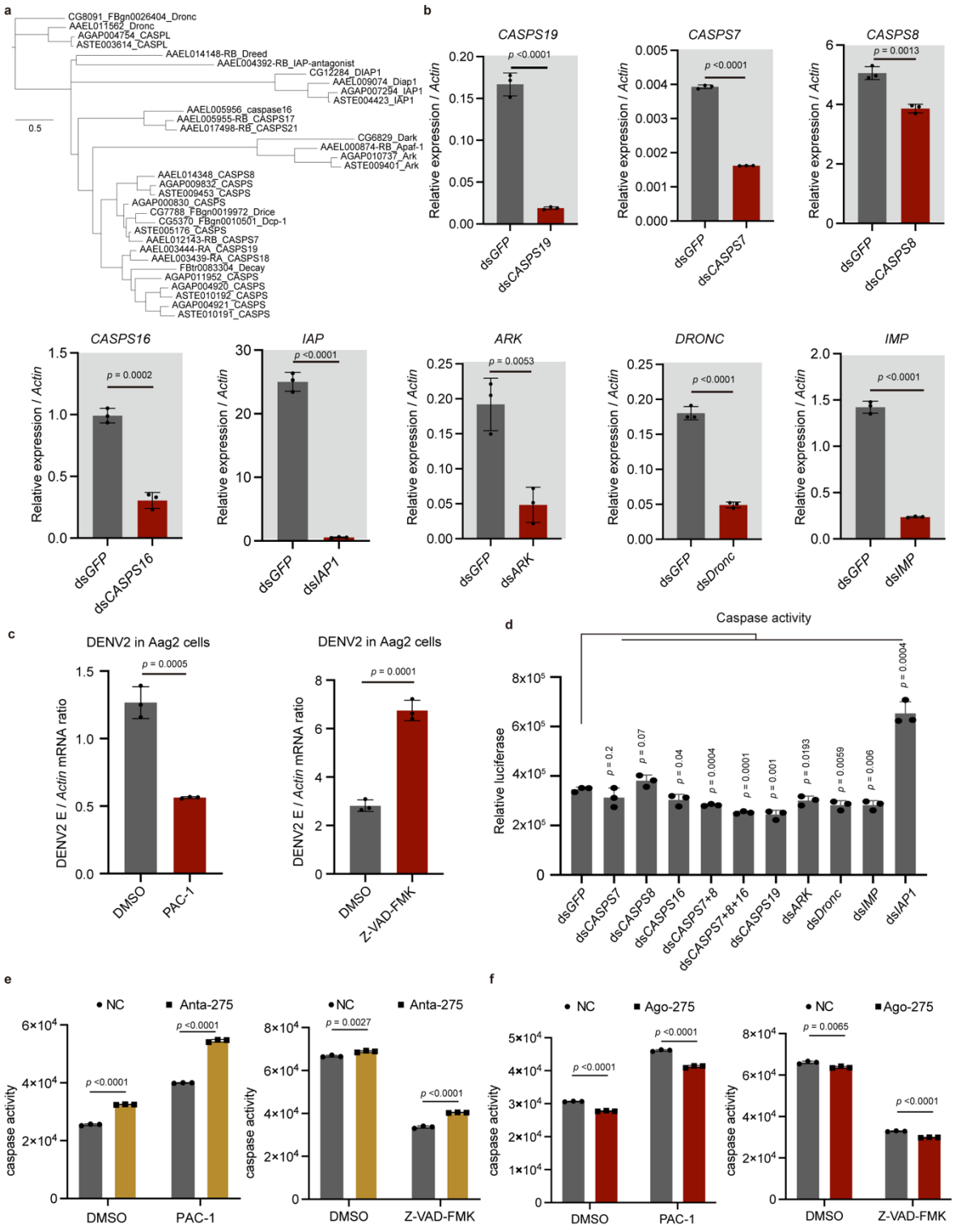
Supplementary Fig. 1| Induction and functional relevance of *Ae. aegypti* mosquito miRNAs following DENV2 infection.

a, qPCR quantification of *Dicer1* mRNA abundance after injection of *GFP* or *Dicer1* dsRNA in mosquitoes (n=20). **b,c,** qPCR quantification of miR-71-5p and miR-317 in mosquito midguts at different time points after feeding with uninfected or DENV2-infected blood. **d,** Temporal expression profile of miR-275-5p in Aag2 cells infected with DENV2, measured by qPCR at 2, 6, 12, and 24 h post infection. CK, uninfected blood. **e, f, g,** qPCR quantification of miR-275-5p, miR-71-5p, and miR-317 levels in mosquito midguts (n=20) following antagomir injection. NC represents a negative control for antagomir with irrelevant sequences. **h,** Validation of miR-275-5p overexpression in mosquitoes injected with miR-275-5p agomir, quantified by qPCR. The experiment was repeated at least twice with similar results. Data are presented as mean ± SD. Statistical significance was determined by two-tailed Student's *t*-test. Source data are provided as a Source Data file.



Supplementary Fig. 2| miR-275-5p inhibits *CASPS19* expression.

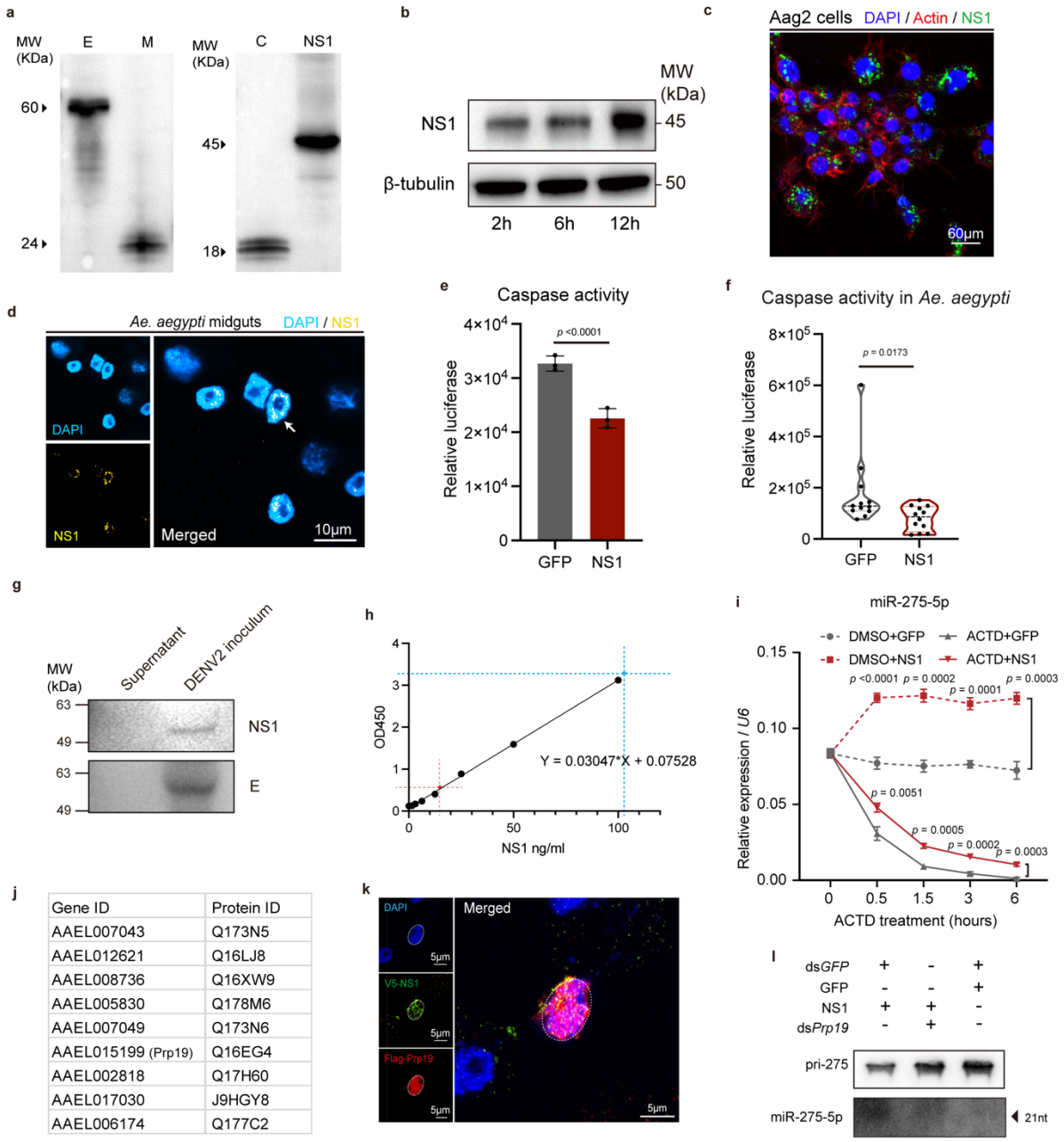
a-f, *Ex vivo* dual-luciferase reporter assays and *in vivo* analyses indicate that the immune-related candidate genes identified through computational prediction are not direct targets of miR-275-5p. NC: negative controls. The experiment was repeated three times with similar results. Data are presented as mean ± SD. Statistical significance was determined by two-tailed Student's *t*-test. Source data are provided as a Source Data file.



Supplementary Fig. 3| CASPS19 is involved in mosquito cell apoptosis.

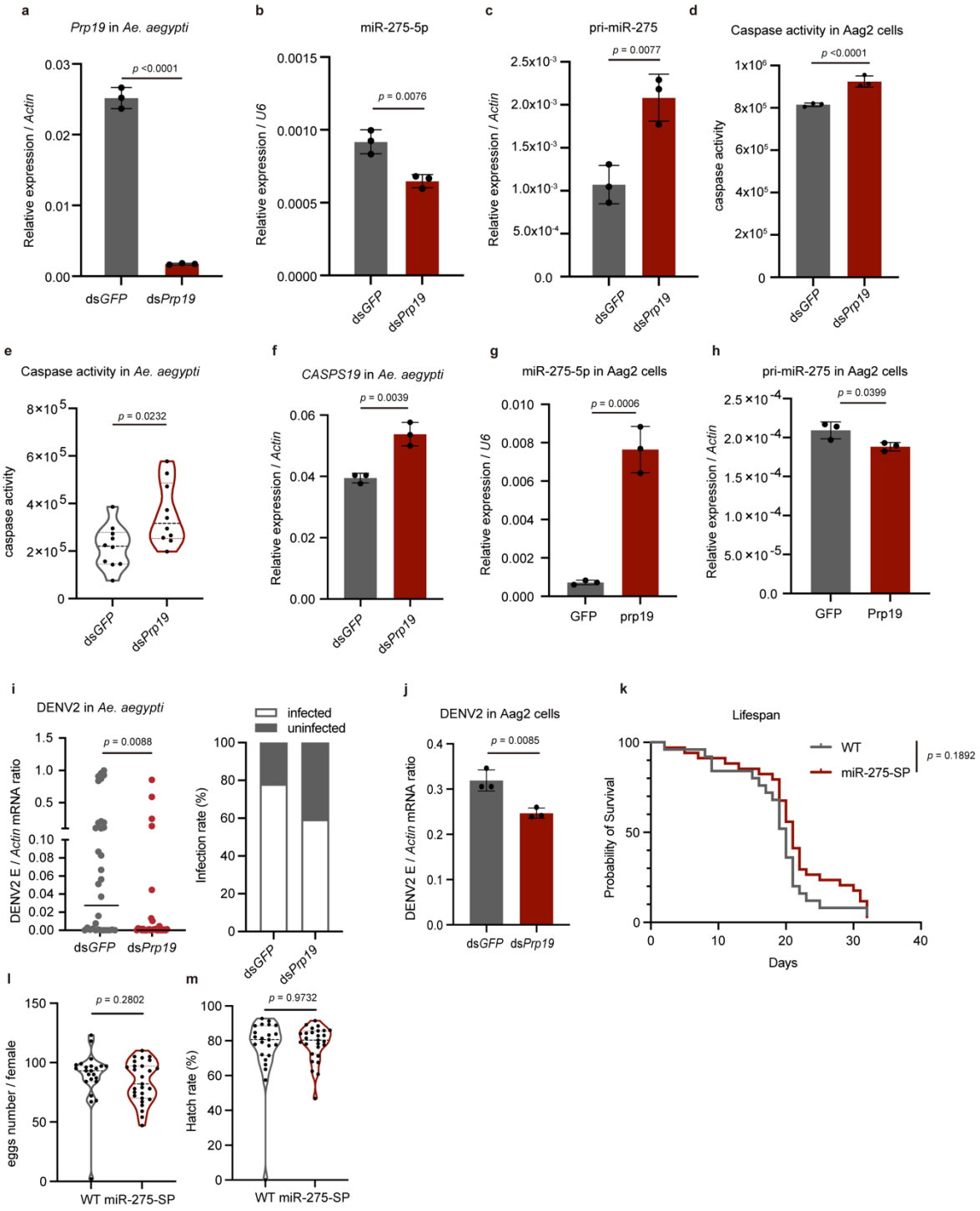
a, Phylogenetic tree of mosquito caspase proteins. **b,** qPCR validation of *CASPS19*, *CASPS7, CASPS8, CASPS16, ARK, DRONC,* and *IMP* silencing efficiency in mosquitoes. **c,** Aag2 cells were pretreated with the caspase activator PAC-1 or the pan-caspase inhibitor Z-VAD-FMK

(10 μM each) for 12 h, followed by DENV2 infection. Viral load was quantified by qPCR. **d,** Measurement of caspase activity in the cells after knockdown of the indicated cell apoptotic genes. ds*CASPS7+8* refers to the simultaneous interference with both *CASPS7* and *CASPS8*. ds*CASPS7+8+16* refers to the simultaneous interference with *CASPS7*, *CASPS8* and *CASPS16*. **e,f,** Aag2 cells were treated with PAC-1 or Z-VAD-FMK for 12 h, followed by transfection with either miR-275-5p antagomir (Anta-275, e) or agomir (Ago-275, f). Cells were subsequently infected with DENV2 for 48 h. Caspase activity was measured at 2dpi. The experiment was repeated at least twice with similar results. Data are mean ± SD, statistical significance was determined by two-tailed Student's *t*-test. Source data are provided as a Source Data file.



Supplementary Fig. 4| The DENV NS1 protein interacts with Prp19 to promote miR-275-5p expression at early infection and engages the host miRNA processing machinery. **a,** Western blot analysis of DENV2 proteins detected with an anti-V5 antibody. **b,** Western blot analysis of NS1 protein in Aag2 cells at indicated time points post incubation with NS1 (4 μg/ml). **c**, Confocal immunofluorescence images of Aag2 cells showing NS1 localization (green) 2 h after incubation with NS1. Actin (red) and nuclei (DAPI, blue) are shown. **d,** Confocal images of mosquito midguts showing NS1 localization (yellow) in mosquito midgut epithelial cells 2 h after feeding on NS1-containing blood. DAPI (blue). The right panel

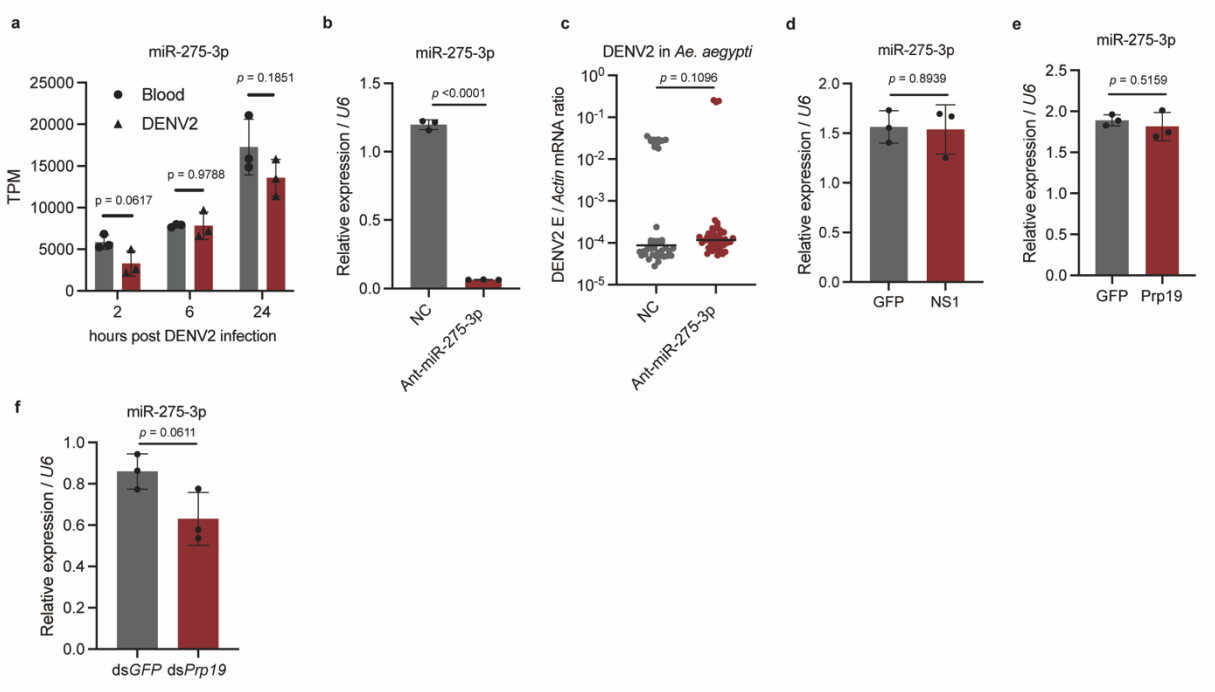
shows a magnified view of the merged image. n= 6 or 8 midguts per group. **e,** Caspase activity in Aag2 cells incubated with NS1 or GFP for 12 h. **f,** Caspase activity in mosquito midgut lysates at 12 h after feeding on GFP- or NS1-containing blood. n=12 per group. Each dot represents one mosquito. **g,** Western blot detection of NS1 in the DENV2 inoculum. **h,** Standard curve for NS1 protein quantification. Absorbance (OD450) is plotted against the known concentrations of recombinant NS1 protein. The data were fitted with linear regression (R^2=0.999). Red and blue asterisks indicate absorbance values for the 100-fold and 10-fold diluted virus inoculum, respectively. **i,** qPCR analysis of miR-275-5p abundance in Aag2 cells pretreated with dactinomycin (10 μg/ml) for the indicated periods followed by 2 h incubation with GFP or NS1 protein. **j,** Identification of NS1-interacting proteins in Aag2 cells. **k,** Confocal immunofluorescent images showing co-localization of NS1 (green) and Prp19 (red) in Aag2 cells. The right panel displays a magnified view of the merged image. **l,** Northern blot analysis of primary transcript and mature miR-275-5p. The experiment was repeated at least twice with similar results. Data are presented as mean ± SD or median (f). Statistical significance was determined by two-tailed Student's *t*-test or Mann-Whitney test (f). Source data are provided as a Source Data file.



Supplementary Fig. 5| NS1 protein interacts with Prp19 to promote miR-275-5p biogenesis and facilitate viral infection.

a, qPCR analysis of *Prp19* abundance in mosquitoes injected with ds*Prp19*. **b,c**, qPCR analysis of mature miR-275-5p and its primary transcript (pri-miR-275) in *Prp19*-depleted Aag2 cells. **d,e**, Caspase activity in *Prp19*-depleted Aag2 cells (d) and mosquito midguts (e). n=10 per group. Each dot represents one mosquito. **f,** *CASPS19* transcript abundance in

Prp19-depleted cells was measured by qPCR. **g,h,** qPCR quantification of miR-275-5p and pri-miR-275 in Aag2 cells transfected with Prp19. **i,** DENV2 RNA abundance was measured by qPCR in *Prp19*-depleted mosquitoes at 7 dpi. Infection rates are displayed to the right. n= 32 per group. Infection rates are displayed to the right. Each dot represents one mosquito. **j,** Viral RNA abundance was quantified by qPCR in *Prp19*-depleted Aag2 cells. **k,** Lifespan of miR-275-SP (n=33) and wild type (n=24) mosquitoes. **l,** Fecundity of female mosquitoes. Number of eggs laid per female by individual WT (n=23) and miR-275 sponge (SP, n=27) females. **m,** Egg hatching rate. Percentage of eggs hatched from WT (n=23) and miR-275-SP (n=27) lines. The experiment was repeated at least twice with similar results. Data are presented as mean ± SD (a-d, f-h, j) or median (e, i, l, m). Statistical significance was determined by Student's *t*-test (a-d, f-h, j), Mann-Whitney test (e, i, l, m) and Log-rank (Mantel-Cox) test (k). Source data are provided as a Source Data file.



Supplementary Fig. 6| miR-275-3p does not contribute detectably to DENV infection phenotypes.

a, Temporal expression profile of miR-275-3p abundance after DENV2 infection. Mosquitoes were infected with DENV2 and subjected to sRNA sequencing at the indicated time points. The line graph depicts mean normalized miR-275-3p abundance across three biological replicates. **b,** miR-275-3p abundance in mosquitoes injected with a miR-275-3p–specific antagomir (Ant-miR-275-3p). **c,** Viral loads in mosquitoes injected with the indicated antagomirs and subsequently fed with DENV2-infected blood meal. Viral titers were measured at 7 days post-infection (dpi). n = 42 or 45 mosquitoes per group. Each dot represents an individual mosquito. **d,** qPCR quantification of miR-275-3p transcript abundance in mosquitoes fed blood supplemented with GFP or NS1 protein. **e,** qPCR quantification of miR-275-3p in Aag2 cells transfected with Prp19 or GFP for 48 h. **f,** qPCR quantification of miR-275-3p in *Prp19*-depleted Aag2 cells. Data are presented as mean ± SD or median (c). Statistical significance was determined by two-tailed Student's *t*-test and Mann-Whitney test (c). Source data are provided as a Source Data file.

Supplementary Table 1 | miR-275-5p target prediction in *Aedes aegypti* using the multi-algorithm and multi-model strategy.

Gene ID	Gene Description	mfe (kcal/mol)	Target site
AAEL013052	Ubiquitin carboxyl-terminal hydrolase isozyme	-24.4	CGCGCUA---AGCAGGA----ACCG----AGAC ||||||| |||:||| |||: |||| GUGUGGUUUUUCGCCCUUAGUUGGGAGUUUUUGC
AAEL008837	Ubiquitin-conjugating enzyme E2R2	-23.6	CGCGCUAAGCAGGAACCGAGAC :||||| :||||:::||: ACGCGA--UGUCCGAUGCA
AAEL010791	Autophagy-specific protein	-20.4	CGCGCUAAG----CAGGAACCGAGAC :|||||||| ||:::|||:|||: CUGUGGUUUUAAGGUACGUGGGUCUU
AAEL013995	Autophagy related gene	-22.6	CGCGCU----AAGCA---GGA--ACCGAGAC |:|||| ||||| ||| |||||: GGGUGACCCCUUCGUAAGCCUUGUGGUUA
AAEL007696	REL1	-23.7	CGCGCUAAGCAGGAA-CCGAGAC |||||| ||||||| ||: || GCGCGA--UGUUUUUAGGA-CUA
AAEL003444	Caspase (short)	-26.1	CGCGCUAAGCAGGAACCGAGAC |||||||||||:: |||||: GUGUGAUUCGUAA---GUUCUA

Supplementary Table 2 | Primers and oligoes used in this study.

Primers	Sequence (5'-3')
Cy5-pri-275	AACACGGCAGCAAUCAACGAGUGCCAGGUGACUCCAGCAG AGUAUCCUUUCGAUUUCGCGCGCUAAGCAGGAACCGAGAC UUUGUCAUUUGCUAGCAGUCAGGUACCUGAAGUAGCGCGC GUGAUCAAACAGGAAGCGUCAUCAAGCGACAACAGAGGCG UGAAAAUCGCUA
qPCR-U6-F	AGAGAAGATTAGCATGGCCCCTGC
qPCR-miR-275-5p-F	CGCGCTAAGCAGGAACCGAGACAAA
qPCR-DENV-2-F	CAGATCTCTGATGAATAACCAACG
qPCR-DENV-2-R	CATTCCAAGTGAGAATCTCTTTGTCA
qPCR-DENV-4-F	TTGTCCTAATGATGCTGGTCG
qPCR-DENV-4-R	TCCACCTGAGACTCCTTCCA
qPCR-ZIKV-F	GGAGAAGAAGAGACGAGGCG
qPCR-ZIKV-R	GATATGGCCTCCCCAGCATC
qPCR-Actin-F	GAACACCCAGTCCTGCTGACA
qPCR-Actin-R	TGCGTCATCTTCTCACGGTTAG
qPCR-Dicer1-F	AGCAATCAACAGTACGCGGA
qPCR-Dicer1-R	GGCGTTTGACAGTCCAACAC
qPCR-miR-125-5p	TCCCTGAGACCCTAACTTGTGA
qPCR-miR-71-5p	AGAAAGACATGGGTAGTGAGAT
qPCR-miR-988-3p	CCCCTTGTTGCAAACCTCACGC
qPCR-*CASPS19*-F	TCGGTCATGGCCAAAAGGAA
qPCR-*CASPS19*-R	TGCTCGCCATGTGACATCAT
qPCR-IAP1-F	CGGAACCAAGCTACGACACA
qPCR-IAP1-R	GACTGGTCCATTCGATGCCA
qPCR-IMP-F	CATGGAAGCAACAACGGTGG
qPCR-IMP-R	ACTGAGAACGCCTTCCATCG
qPCR-DRONC-F	CCACGGATAAGGGCGGTATG

Primers	Sequence (5'-3')
qPCR-DRONC-R	AGAATCCGGTGGCTTTGAGG
qPCR-ARK-F	GAGTTCTACGCCGAGTTGCT
qPCR-ARK-R	CGGTTGTTGTACTGGCTTGC
qPCR-CASPS7-F	ATTCTACTCGTGGCGCAACA
qPCR-CASPS7-R	CGAAAGTCAGCAGGGTCAGT
qPCR-CASPS8-F	CGGTGTCGGACGTTAGTAGG
qPCR-CASPS8-R	CCGGAATTGTGGCAAAAGCA
qPCR-CASPS16-F	GATGCTACTCGGACTGCCAC
qPCR-CASPS16-R	CGTTTGGATGAATGTGGTGCC
qPCR-Prp19-F	AGCCTTGGCTACCCTCAAAC
qPCR-Prp19-R	GAATGACCTCGGCGCTCATA
qPCR-pri275-FP	GTCATTTGCTAGCAGTCAGGTACCTG
qPCR-pri275-RP	GGTTTGCCCGGGTTTTCGATTTCTAGC
qPCR-pre275-FP	GTATCCTTTCGATTTCGCGC
qPCR-pre275-RP	TCCTGTTTGATCACGCGCGC
qPCR-Drosha-F	AGAAACTGGCCGAGCTTACC
qPCR-Drosha-R	GCGGTTGATAAGGAGCCTGT
qPCR-Pasha-F	GAATCCAGCCCCTCCACTTC
qPCR-Pasha-R	CCTCCTCCTCCAAATCGTCG