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Supplementary Information for

A DNA virus-encoded immune antagonist fully masks the potent antiviral activity of RNAi in Drosophila

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Supplementary Text

Construction of mutant IIV6 Δ340R

Recombinant IIV6 lacking 340R (IIV6 Δ340R) was generated by homologous recombination. To this end, *Drosophila* S2 cells were transfected with a recombination template plasmid containing the GFP transgene fused to 14 N-terminal amino acids of 340R, flanked by homology arms at both the 5' and 3' ends (Fig. 1A). When transfected into non-infected cells, this plasmid did not express GFP, suggesting that the flanking sequences does not contain a functional promoter element or that viral proteins are required to activate the promoter region. However, GFP was readily detectable in IIV6 infected cells transfected with the recombination template plasmid. Transferring culture supernatant from these cells to naive uninfected cells again resulted in GFP expression, strongly suggesting that homologous recombination between IIV6 and the template plasmid has successfully occurred. The mutant virus was then purified away from the wildtype (WT) virus by repeated serial dilution on S2 cells. The 340R gene was indeed replaced by GFP in the mutant virus, as established by diagnostic PCR using primers flanking the recombination site and GFP specific primers (SI Appendix, Fig. S1 A, B) as well as by Sanger sequencing. Absence of WT virus in the mutant virus stock was verified by PCR using primers specific for 340R (SI Appendix, Fig. S1). We previously showed that IIV6 340R suppresses RNAi using reporter assays in cells in which 340R was expressed as a transgene or using cell-free biochemical assays in the presence of recombinant 340R (1). Using a Dicer assay in extracts from S2 cells infected with either WT or Δ340R mutant virus, we verified that 340R is responsible for suppression of RNAi in IIV6 infected cells (SI Appendix, Fig. S1C).

Supplementary Material and methods

Cells and virus

Drosophila S2 cells (Invitrogen) and DCV, CrPV and IIV6 were propagated as described (2, 3). Where indicated, UV inactivated IIV6 was used (2). IIV6 titers were determined by end-point dilution on S2, with cultures passaged at day 7, and visually inspected 5 days later (2). $TCID_{50}$ values were calculated according to the Reed and Muench method.

Construction of mutant virus

To produce IIV6 Δ340R, a donor template for homologous recombination was generated. The left homology arm (LHA), including the first 14 aa of the 340R coding sequence, amplified from DNA isolated from IIV6 infected S2 cells using primers WB304 and WB310 and ligated into *Sac*I and *Xma*I digested pGEM3Z plasmid (Promega). The 340R right homology arm (RHA) was amplified using primers WB311 and WB305 and inserted into pGEM3Z-LHA using *Xma*I and *Xba*I restriction sites. Finally, the eGFP sequence was amplified using primers WB312 and WB313, and the amplicon was inserted into the pGEM3Z-LHA+RHA backbone plasmid using the *Xma*I restriction site. Colony PCR was used to confirm the correct orientation of the eGFP insertion. All individual plasmids were validated by Sanger sequencing.
S2 cells were seeded at a density of 3.5×10^5 cells per well in a 24-well plate and transfected the next day with 0.5 µg of recombination plasmid pGEM3Z-LHA-340R14-eGFP-RHA using Effectene Transfection Reagent (Qiagen). One day post transfection, cells were infected with IIV6 at an MOI of 1. The eGFP signal became visible at two days post infection and 100 µL supernatant from eGFP positive wells was collected, passed through a 0.2 µm syringe filter, and subjected to serial dilutions in a 96-well plate, in which S2 cells were seeded at a density of 2.5×10^4 cells per well. Cells were scored for cytopathic effect (CPE) and GFP at 7 and 14 dpi and culture supernatant (of the highest dilution that scored positive) was harvested, filtered, and subjected to end-point dilution to eliminate WT virus that might remain present. The same procedure was performed for a third round after which absence of WT IIV6 was verified by PCR. A final round of selection by serial dilution was used to generate IIV6 Δ340R, which was validated by a series of diagnostic PCRs using primers flanking, and internal to 340R and GFP (SI Appendix, SI Appendix, Fig. S1).

Flies

50 nl of undiluted virus stock (IIV6 wildtype virus: titer 6.34×10^8 $TCID_{50}$/ml) or an equivalent of the 340R mutant virus was injected into the thorax of adult flies (4). The following genotypes were used: w^{1118} wildtype control flies, *Dcr-2*^{L811fsX} and *AGO2*^{414} flies (5, 6). Three pools of five flies were harvested at indicated time points, frozen, and stored until processing. As insufficient numbers of homozygous miR-275-305 knockout (KO) flies (7) were obtained, trans-heterozygotes were generated by crossing miR-275-305 KO flies with w^{1118} control flies or with flies carrying the *cuc*^{1} P-element insertion (a miR-275-305 allele (7)) or the Df(2L)ED483 deficiency which deletes ~153 kb of DNA including the miR-275-305 cluster.

Plasmids

Plasmids encoding RNAi suppressor proteins have been described previously (1, 3, 8, 9). To analyze the effect of viral RNAi suppressors on miRNA expression, S2 cells were seeded in a 6-well plate at a density of 3.0×10^6 cells per well and transfected the next day with 1 µg of plasmid using Effectene Transfection Reagent (Qiagen). Cells were harvested 3 days post transfection followed by RNA isolation for Northern blot analysis.
miR-305 reporters were made by cloning DNA oligonucleotides containing two fully complementary target sites for either miR-305-5p, miR-305-3p or a scrambled miR-305-3p sequence into the 3' UTR of pMT-GL3 that was digested by *Sac*II and *Pme*I. The effect of IIV6 on miR-305 mediated silencing was analyzed by seeding 5.0×10^4 S2 cells per well in a 96 wells plate and infecting them the next day with IIV6 WT or IIV6 Δ340R at an MOI of 5. The cells were transfected 6 hours after infection using Effectene Transfection Reagent and were

harvested for luminescence measurements at 48 hpi (experiment 1) or 24 hpi using the Dual-Luciferase Reporter Assay System (Promega).

PCR.
Cells were harvested by centrifugation for 5 min at 400 g, and pellets were resuspended in 200 µL PBS, and treated with proteinase K. DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen) and PCRs were performed using Phusion Polymerase, according to the manufacturers' instructions.
Flies were homogenized in 180 µl PBS, and DNA was isolated using the QIAamp DNA Blood Mini Kit. 30 ng of DNA was used as input for qPCR to quantify IIV6 DNA copies, using the GoTaq qPCR system (Promega) and primers for IIV6 161L (Figure 1B) and IIV6 193R (Figure 5) using rp49 as internal normalization control. Reactions were run on a Light Cycler 480 (Roche) and analyzed using the $2^{-\Delta\Delta Ct}$ method. The day 0 sample was used as input to normalize the qPCR data.

Northern blot
Northern blots were performed as described before (10). Where applicable, cells were transfected with dsRNA targeting Dcr-1 or as a control GFP. dsRNA was generated as described (11). β-elimination of total RNA was performed as described before (12), using the same RNA samples as used to prepare small RNA sequencing libraries. 10 µg of RNA in 47.5 µl nuclease-free water was oxidized by addition of 12.5 µl 200 mM $NaIO_4$ and 40 µl 5x borate buffer, followed by incubation at room temperature for 30 min. As a control, water was added to the RNA instead of $NaIO_4$. The unreacted $NaIO_4$ was quenched by addition of 10 µl glycerol and incubation at room temperature for another 10 min. To induce β-elimination, 10 µl of 500 mM NaOH was added, followed by incubation at 45 °C for 90 min. The RNA was purified by ethanol precipitation and migration of small RNAs was analyzed by Northern blotting as describe before (10). Oligonucleotide sequences are provided in SI Appendix, Table S1.

Stemloop qRT-PCR
Stemloop qRT-PCR assays were based on the method of (13), with adaptations. S2 cells were infected with IIV6 (WT or ∆340R) at an MOI of 1. RNA was isolated at 72 hpi using RNA-Solv reagent (Omega Biotek) and 100 ng was used as input for reverse transcription using SuperScript II Reverse Transcriptase (Thermo Scientific) and stemloop primers specific for miR-305-5p, miR-305-3p and U6. RNA oligonucleotides were used as standard curves for absolute quantification of miR-305-5p and miR-305-3p. A 10-fold dilution series of each RNA oligonucleotide was added to 100 ng RNA from HeLa cells, which do not express miR-305, and analyzed in parallel to IIV6 infected samples. qPCR was performed using the GoTaq qPCR system (Promega) with a universal primer detecting the stemloop and a primer specific to the miRNA or, as a normalization control, U6 RNA.

Dicer assay
Dicer assay was performed as described before (2) in extracts from S2 cells infected with either IIV6 WT or ∆340R at an MOI of 1 (harvested at 2 dpi).

Electrophoretic mobility shift assay (EMSA)
RNA oligonucleotides (Table S1) were ordered from Sigma-Aldrich. Recombinant proteins and EMSAs have been described previously (1). Signal intensity on the films was quantified using ImageJ. Kd values were determined by fitting association curves by non-linear regression (one-phase association) using GraphPad Prism, version 5.03.

Small RNA sequencing and analyses.
S2 cells were infected with IIV6 WT or ∆340R mutant virus at an MOI of 1. RNA was isolated at 72 hpi using RNA-Solv reagent (Omega Biotek). The small RNAs were size selected by loading 25 µg of total RNA on a 15% acrylamide/7M urea/0.5x TBE gel and excising the small RNAs ranging from 19-24 nt using radioactively labeled size markers, run in lanes flanking the

samples, as rulers. The RNA was purified from gel by overnight incubation in 0.3 M NaOAc, followed by ethanol precipitation. The pellet was dissolved in 11 µl nuclease-free water, of which 5µl was used as input for small RNA library preparation using the TruSeq kit (Illumina) according to the manufacturer's recommendations. After the final PCR amplification, the libraries were size purified from a 6% acrylamide/1x TBE gel by overnight incubation in 0.3M NaOAc, and ethanol precipitation. The samples were quantified using the Agilent 2100 Bioanalyzer System and pooled libraries were sequenced in an Illumina Hiseq4000 machine by Plateforme GenomEast (Strasbourg, France).

For the analysis of viral small RNAs, clipped reads were mapped to the IIV6 genome (GenBank accession AF303741.1) or to specific IIV6 genes using Bowtie (Galaxy tool version 1.1.2) allowing 1 mismatch. Mapped reads were normalized to library size (expressed as reads per million, RPM) and to the average of four viral transcripts (193R, 205R, 206R and 251L, relative transcript levels measured by qRT-PCR). For the genome distribution, the number of 5' ends of all vsiRNAs at each position were plotted. miRNA levels were analyzed by mapping the clipped reads to the *Drosophila* genome (dm6 build; GCA_000001215.4) without allowing mismatches. The genome coordinates for mature miRNAs (dme.gff3 coordinates from miRbase on dm6 build) were intersected with the mapped reads to find all mature miRNAs in the datasets (using bedtools intersect intervals version 2.27.0; -wa).

The miRNA heat map was based on all mature microRNA sequences with a minimal normalized count of 100 reads per million in the sum of all libraries. For hierarchical clustering of miRNAs, the mean expression level of uninfected, IIV6 WT and ∆340R virus infected S2 cells were determined. Then fold changes of infected libraries compared to the uninfected control were calculated, log2 transformed and used as input for multiple experiment viewer (MEV version 4.9.0, Tm4) (14). An average linkage clustering was performed using Pearson Correlation with 'gene tree' and 'optimize gene leaf order' enabled. The heat map was generated with Microsoft Excel 2010 using the conditional formatting tool. The log-2 transformed fold change of individual libraries compared to the mean of the uninfected libraries was used as input. Individual microRNAs were sorted according to the output of the MEV clustering.

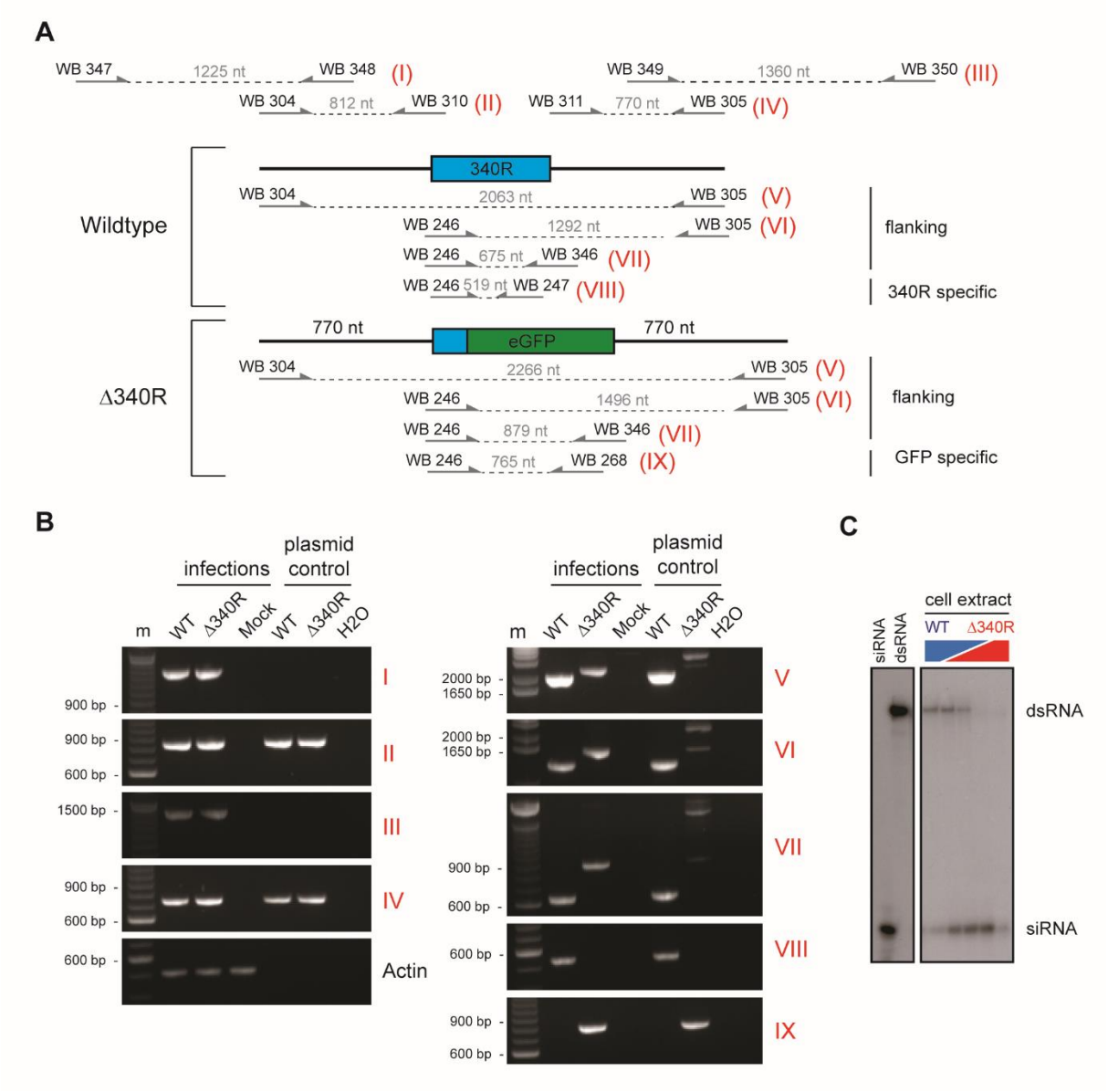

Fig. S1. Characterization of IIV6 Δ340R mutant virus.
(**A**) Overview of the primers used for PCR-based characterization of IIV6 Δ340R mutant virus. Indicated are the relative positions and expected sizes of the PCR products. (**B**) PCR to validate the accuracy of homologous recombination and to verify absence of wildtype (WT) virus in the IIV6 Δ340R virus stock. PCR was performed on DNA of S2 cells infected with the indicated virus stocks at 3 dpi. Both a 340R-WT plasmid containing left and right homology arms as well as the final Δ340R-eGFP recombination plasmid served as template controls. M, size marker with selected sizes indicated. (**C**) Dicer assay in extracts of S2 cells infected with IIV6 WT or Δ340R. Cells were infected with the indicated viruses at an MOI of 1.0 and cell extract was prepared at 2 dpi. Processing of synthetic, radiolabelled dsRNA into siRNAs was analyzed on a 12% denaturing polyacrylamide gel. The dsRNA substrate and synthetic, radiolabelled siRNAs were run alongside as size markers at different locations of the same gel.

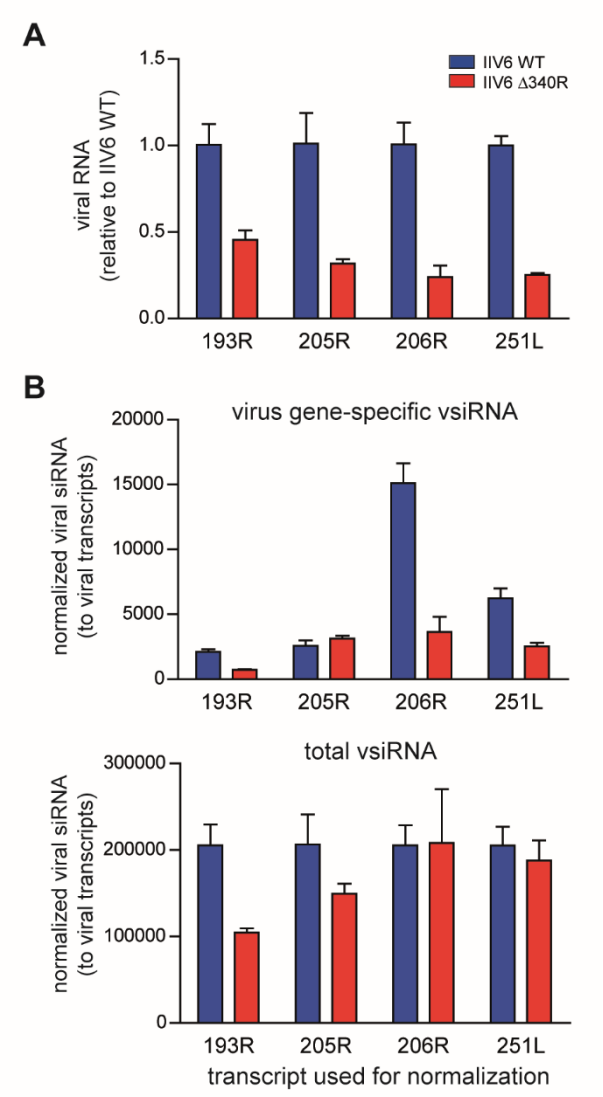

Fig. S2. vsiRNA levels normalized to transcript levels of four individual IIV6 genes.
(**A**) Transcript levels of four viral genes in the samples used to prepare sequencing libraries, assessed by RT-qPCR. Data are normalized to the house-keeping gene rp49 and expressed relative to IIV6 WT infection (blue bars, means +/- SD of three biological replicates). (**B**) Number of vsiRNAs from specific viral genes (top panel) or total vsiRNAs (lower panel), normalized to library size and to the transcript levels of the indicated viral genes from A (mean +/- SD of three biological replicates).

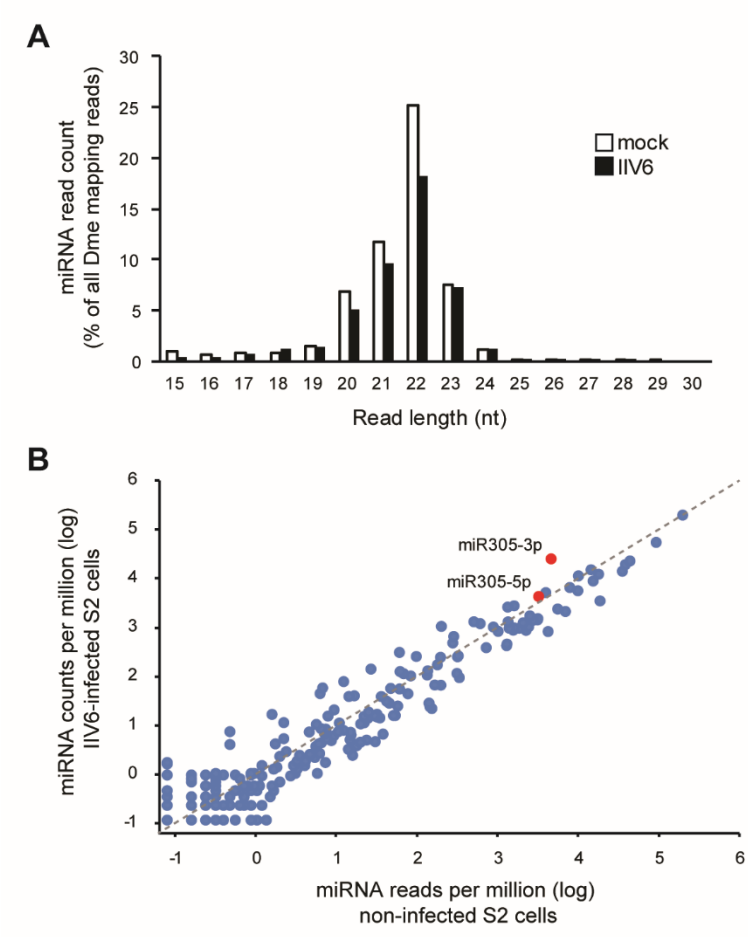

Fig. S3: miRNA levels in IIV6 infected *Drosophila* S2 cells from Kemp *et al* (15).
(**A**) miRNA read counts in non-infected and IIV6 infected S2 cells. Small RNA reads were mapped to the *Drosophila* genome, intersected with the position of pre-microRNAs, and plotted according to their size. (**B**) As for (A), but genome-mapped small RNAs were intersected with the positions of mature microRNAs. For individual miRNAs, the normalized read counts (per million mapped reads) in non-infected and IIV6 infected cells were plotted. miR-305-5p and 3p are indicated by red symbols. The dashed diagonal line reflects equal read counts in non-infected and IIV-6 infected cells.

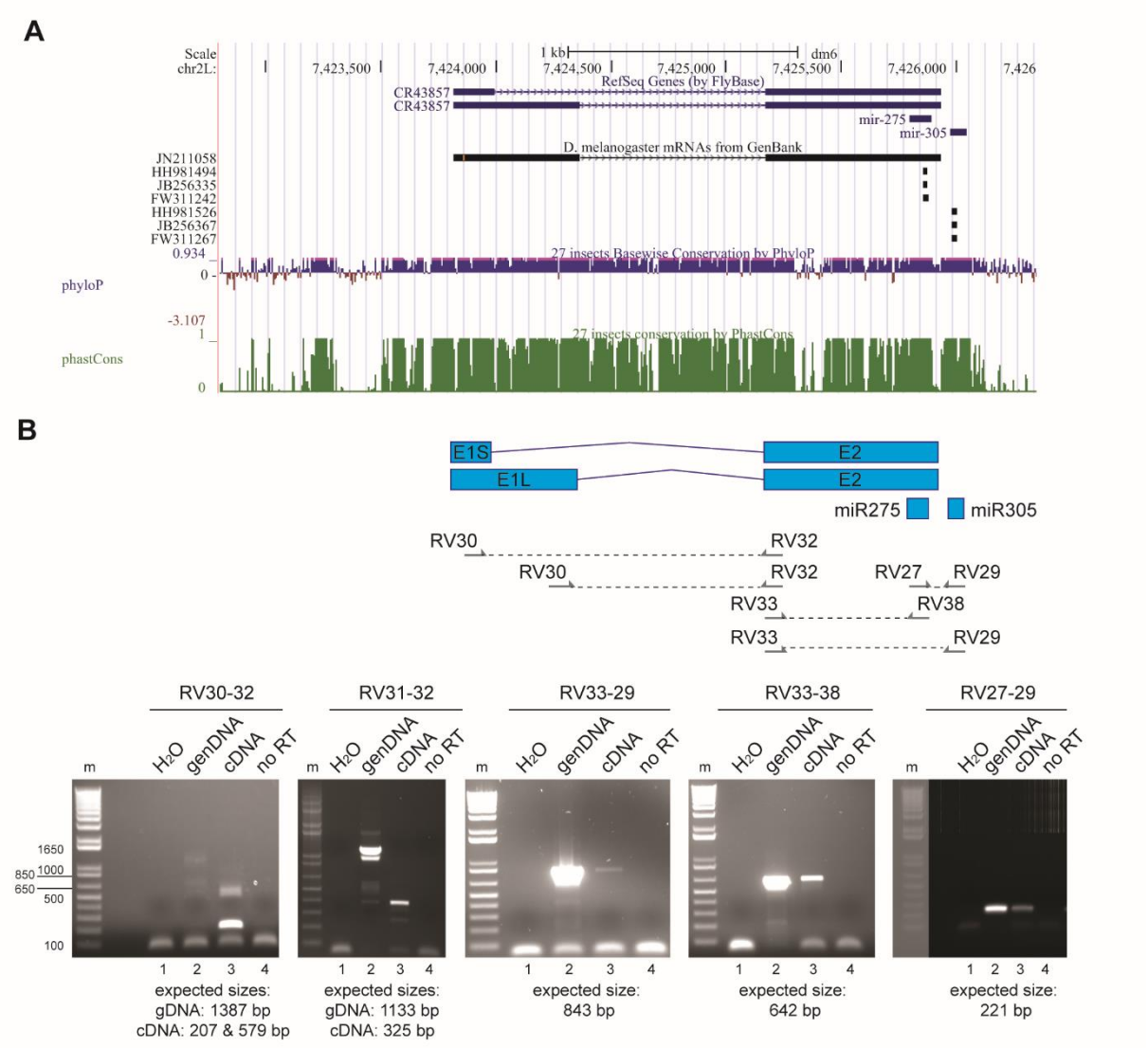

Fig. S4. Organization of miR-275-305 precursor locus.
(**A**) Genome browser screenshot of the miR-275-305 locus**.** (**B**) PCR analyses of the miRNA locus on genomic DNA (genDNA), cDNA from w^{1118} adult flies. Reactions in absence of reverse transcriptase (no RT) are included to verify absence of DNA contamination in RNA preparations. Locations of the primers are drawn schematically. M, size marker (1kb plus). The marker on agarose gel RV27-29 is a higher exposure from the same gel. Although miR-275 clearly overlaps with non-coding transcript CR43857, the genome assembly is ambiguous as to whether miR-305 is part of this transcript. RT-PCR analyses verified that miR-275 and miR-305 are indeed expressed from a single transcript.

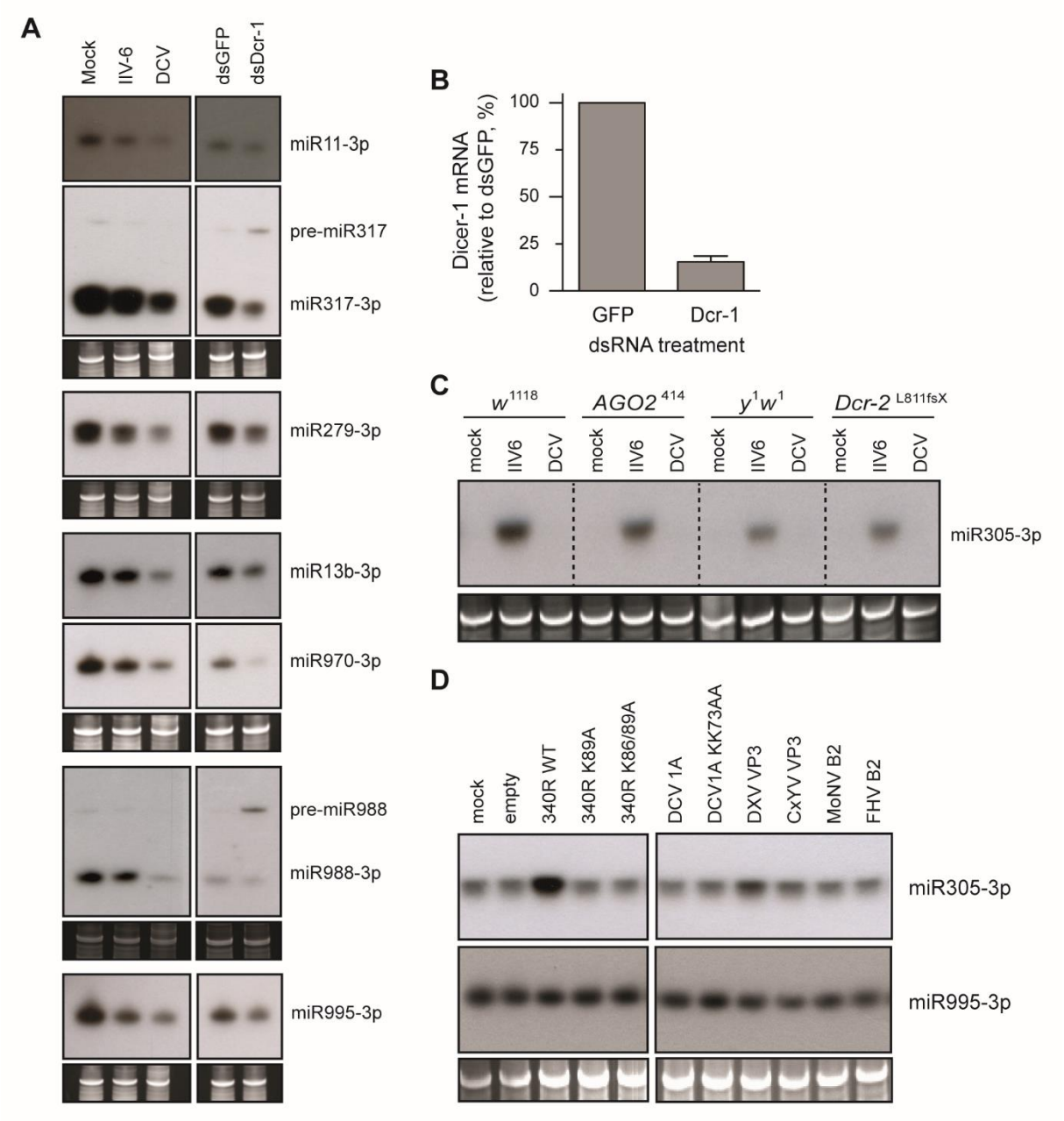

Fig. S5. IIV6 induces miR305-3p in an RNAi independent manner.
(**A**) Northern blots of selected miRNAs during Drosophila C virus (DCV) and IIV6 infection of *Drosophila* S2 cells at 3 days post infection. As controls, non-infected cells treated with dsRNA targeting Dicer-1 (dsDcr-1) or GFP (dsGFP, control) were run on the same gel. Ethidium bromide stained rRNA was used as a loading control. The miR-279-3p image was obtained by re-probing the miR-275-3p blot (Fig. 3B); for clarity the ribosomal RNA image is shown here again. The miR-11-3p image was obtained by re-probing the miR-317-3p blot, and the miR-13b-3p image by re-probing the miR-970-3p blot. (**B**) Quantification of Dicer-1 mRNA in S2 cells treated with dsRNA targeting GFP or Dicer-1, used for the northern blots in (A). Data are normalized to rp49 and expressed relative to GFP dsRNA-treated cells. Bars are means and SD of 2 biological replicates. (**C**) Northern blot analysis of miR-305-3p in wildtype control flies (w^{1118} and y^1w^1) and RNAi mutant flies with the indicated genotypes at 3 dpi. (**D**) Northern blot of miR-305-3p and miR-995-3p in S2 cells transfected with expression plasmids encoding the indicated viral suppressors of RNAi. For IIV6 340R and DCV 1A, mutants in the double-stranded RNA binding domain were included. Cells were harvested at 3 days after transfection and analyzed by northern blot. The panels are split to reflect that the samples were loaded at different locations of the same gel.

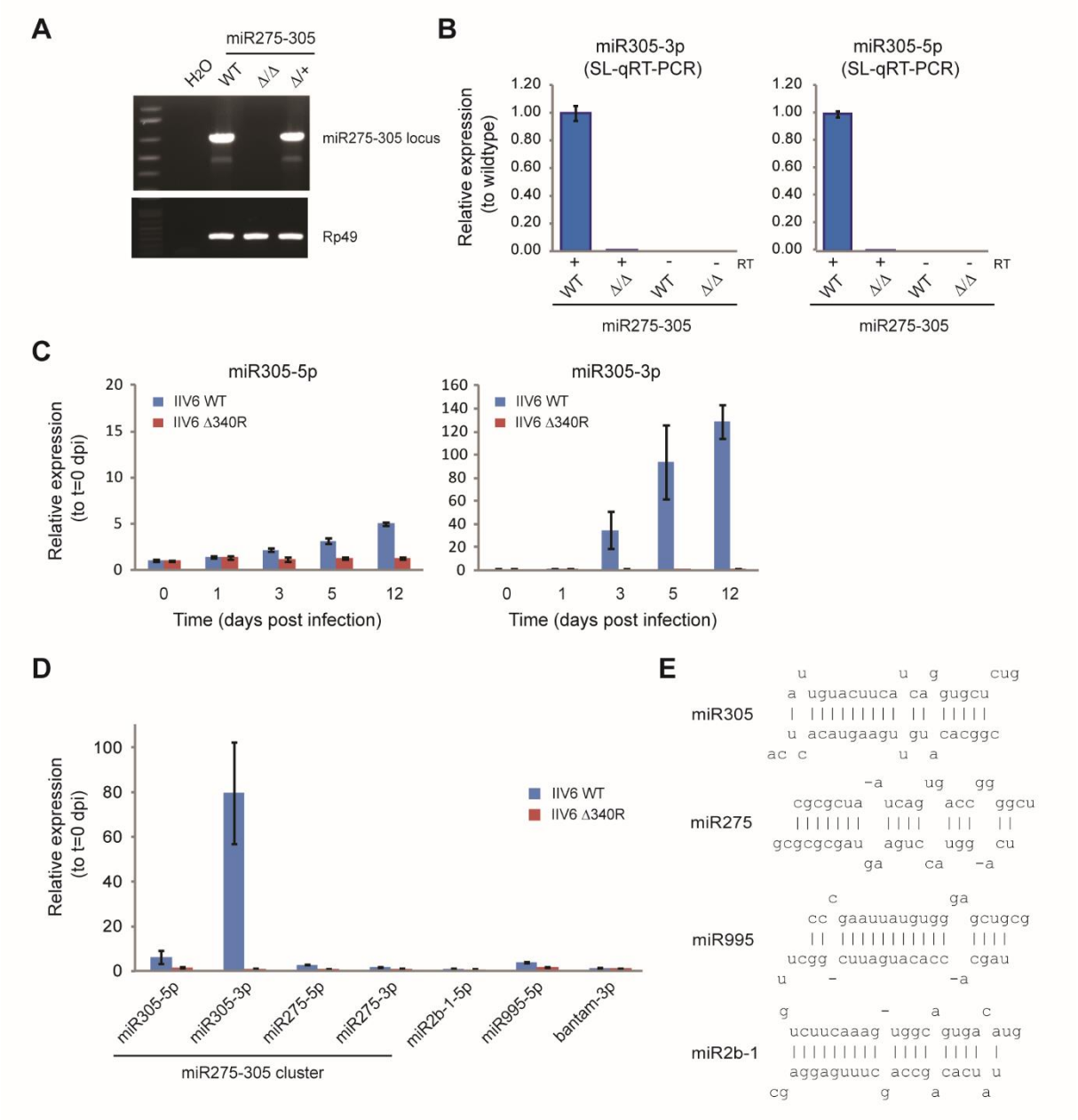

Fig. S6. Quantification of miRNAs by stemloop qRT-PCR in wildtype and Δ340R mutant IIV6 infected cells. (**A**) PCR analysis of the miR-275-305 locus in wildtype (WT, *w*^{1118}) flies and flies heterozygous (Δ/+) and homozygous (Δ/Δ) for a deletion of the miR-275-305 locus (7). rp49 was used as an internal control. (**B**) Validation of stemloop (SL)-qRT-PCR assays for miR-305-3p (left panel) and 5p (right panel) on WT flies (genotype *w*^{1118}) and flies deficient for the miR-275-305 locus (Δ/Δ). Data are normalized to WT flies for each miRNA. Data represent means and SD of 2 replicates of 5 pooled flies. (**C**) miR-305-5p (left panel), miR-305-3p (right panel), and U6 snRNA were quantified by SL-qRT-PCR in three pools of 5 *w*^{1118} flies infected with IIV6 WT or Δ340R, harvested at the indicated time points. Data were normalized to U6 snRNA and expressed relative to t=0 dpi. (**D**) Multiplex SL-qRT-PCR for the clustered miR-275 and miR-305 and the indicated miRNAs at 12 dpi. Data are means and SD from three pools of 5 *w*^{1118} flies. Data are expressed relative to t=0 for each miRNA. (**E**) Sequence and predicted structure of 5p:3p duplexes of miR-305, miR-275, miR-995, and miR-2b-1, used in gel shift assays of Figure 4.

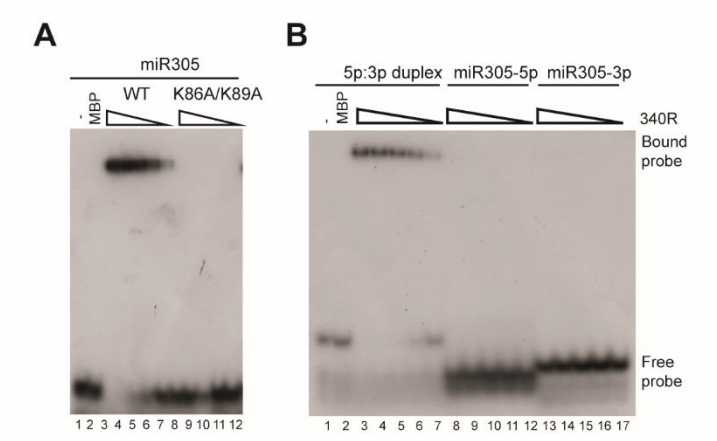

Fig. S7. IIV6 340R binds miR305 duplexes, but not mature single-stranded miRNAs, in a dsRNA-binding domain dependent manner.
(**A**) Electrophoretic mobility shift assay (EMSA) of miR-305 duplex with 340R WT (lanes 3-7) and 340R K87A/K89A mutant (1) (lanes 8-12; less sample had been loaded in lane 10). (**B**) EMSA of miR-305 5p:3p duplex (lanes 1-7), miR-305-5p (lanes 8-12), and miR-305-3p (lanes 13-17) with 340R WT. Two-fold dilutions of recombinant protein were tested, starting from a concentration of 3.2 μM. Incubations in buffer only (-) and MBP were included as controls, as indicated.

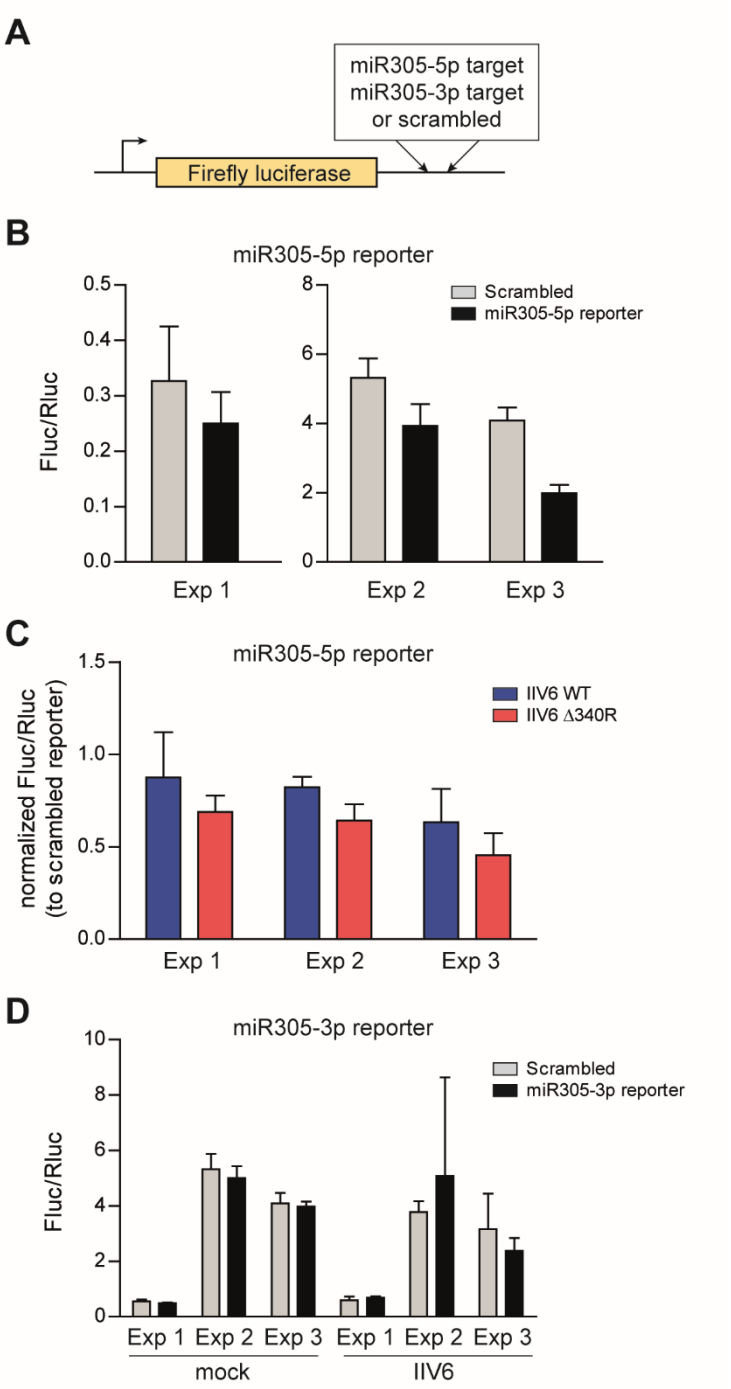

Fig. S8. IIV6 infection affects miRNA function in a 340R dependent manner.
(A) Schematic representation of miRNA reporters. Two perfectly complementary target sites were introduced into the 3' untranslated region of the firefly luciferase (Fluc) reporter gene. A plasmid encoding *Renilla* luciferase (Rluc) was included as normalization control. (**B-D**) Fluc over Rluc ratios in cells transfected with the indicated reporters in non-infected *Drosophila* S2 cells (mock) or cells infected IIV6 WT or Δ340R, as indicated. Data from three independent experiments (Exp 1-3) are presented, each experiment performed in three replicate wells. Means and SD are presented. Experiment 1 was harvested at 48 hpi, experiments 2 and 3 at 24 hpi. Data in (C) are normalized to the reporter with the scrambled target site. The difference in miR-305-5p activity between IIV6 WT and Δ340R infected cells was analyzed using a paired Student's *t*-test on the means of each experiment ($P < 0.001$).

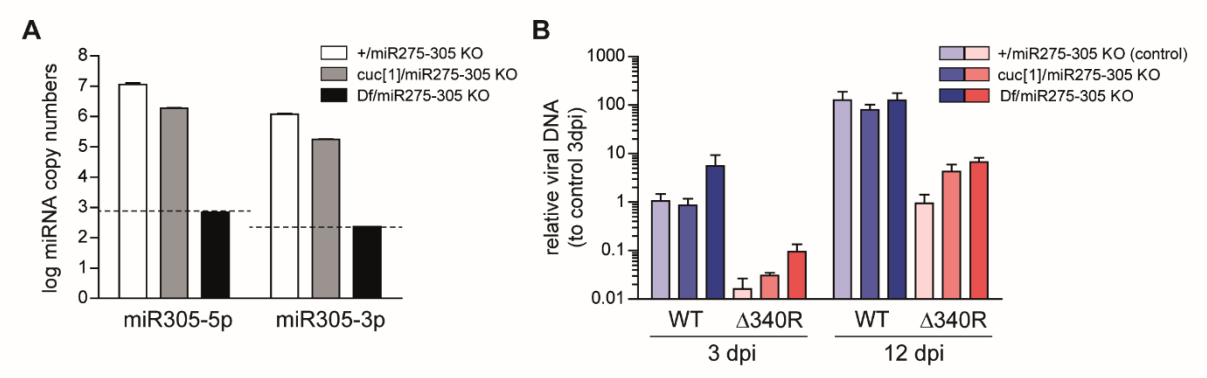

Fig. S9. IIV6 infection in flies lacking the miR-275-305 cluster.
(**A**) Quantification of miR305-5p and 3p in trans-heterozygous offspring of crosses of miR-275-305 knockout (KO) flies with wildtype flies (+, genotype w^{1118}), or flies carrying the miR-275-305 allele cuc^{1} or the Df(2L)ED483 deficiency spanning the miR275-305 locus. Horizontal dashed lines indicate the detection limit for each miRNA. Means of 2 pools of 10 flies are presented. (**B**) IIV6 viral DNA quantification in flies with the indicated genotypes infected with IIV6 WT or Δ340R at 3 and 12 dpi. qPCR data were normalized to the housekeeping gene rp49 and presented relative to the control cross infected with IIV6 WT at 3dpi. Means and SD of 3 pools of 5 female flies are presented.

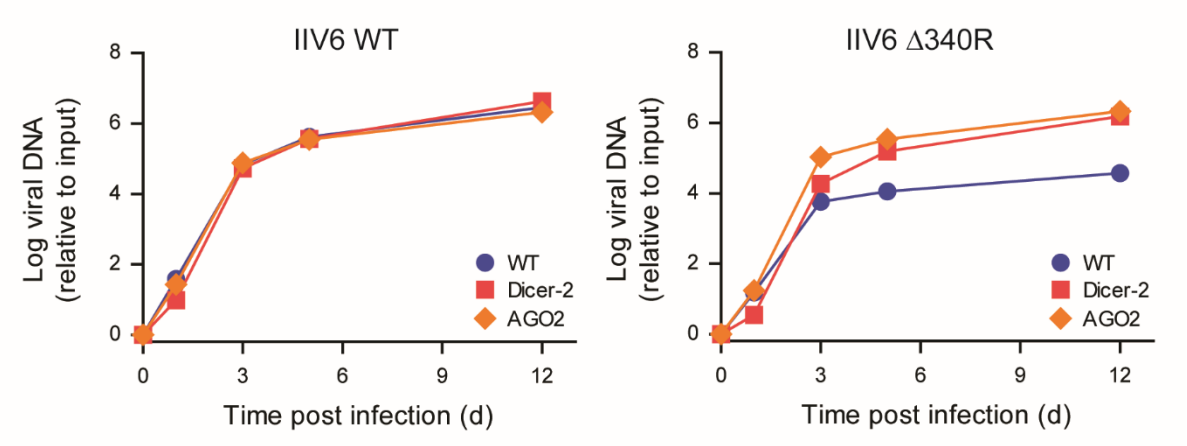

Fig. S10. Replication kinetics of IIV6 WT and Δ340R in wildtype (w^{1118}), *Dicer-2*, and *AGO2* mutant flies. The same data as presented in Fig. 5B were plotted, but now grouped by virus genotype. qPCR data were normalized to the housekeeping gene rp49 and presented relative to a sample taken directly after inoculation (input). The data were log transformed and presented as means and SD of three biological replicates of 5 flies.

Supplemental table 1. Oligonucleotide sequences

Template generation and diagnostic PCR for IIV6 Δ340R (restriction sites underlined)

Code	Name	Sequence (5′- 3′)
WB246	ORF 340R Fwd (XbaI)	AGTTCTAGAaacATGGAAAAACAAAAAGATAACGTAACTTC
WB247	ORF 340R Rev (XbaI)	GGTTCTAGAAATATCGTCTATATCAACAGAGTCTTC
WB268	eGFP Rev (NotI)	GGTGCGGCCGCTTACTTGTACAGCTCGTCCATGC
WB304	pGEM-3Z_ORF340R_UD (SacI) Fwd	AGTGAGCTCGCAGCCCATGCCCTGCATC
WB305	pGEM-3Z_ORF340R_UD (XbaI)Rev	GGTTCTAGAGCATCTTTTGAAGAAAGCTTTG
WB310	pGEM-3Z_Up+ORF340R_14 (XmaI) Rev	GGTCCCGGGCTCTTCTTTAGTAGAAGTTACGTTATCTTTTTG
WB311	pGEM-3Z_Do-ORF340R_14 (XmaI) Fwd	AGTCCCGGGAATTTTTAATGACGTATCATTAAAAATTTATTC
WB312	GFP (XmaI) Fwd	AGTCCCGGGGTGAGCAAGGGCGAGGAGC
WB313	GFP (XmaI) Rev	GGTCCCGGGTTACTTGTACAGCTCGTCCATGCC
WB346	340R-D	GAAAAATTGTGGTTATTAAGTCGAATATCTGC
WB347	ORF 337L-Rev	GTCTTGATGATGAAGGAACTAGATGC
WB348	ORF 335L-Fwd	GACCGGCTTAATTGTCATATCTATAAG
WB349	ORF 344R-Fwd	GAAGCTGAAACACCAGTGAAAGG
WB350	ORF 346R-Rev	CGATTCATCATTAAAATTATATTTTCGA
RVR88	Actin42A-19 Fwd	GCGTCGGTCAATTCAATCTT
RVR89	Actin42A-386 Rev	CTTCTCCATGTCGTCCCAGT

qPCR

Code	Name	Sequence (5′- 3′)	Reference
WB100	IIV6 65880 Fwd (161L)	CCATCGGATCTGTATGATGTTGCAC	
WB101	IIV6 65999 Rev (161L)	TGGCACTGTTATTGTAGATGAGGT	
WB102	IIV6 82935 Fwd (193R)	CACAACCAAGATTTGGATCACAACCA	
WB103	IIV6 83069 Rev (193R)	ACACGAAGAATGACCACAAGGA	
RVR105	Dcr1-Fwd	TCCATCTCCCAGTTTTACCG	
RVR106	Dcr1-Rev	GTCCCGCATCGATATCATCT	
RVR129	Rp49 Fwd (RpL32)	ATGACCATCCGCCCAGCATAC	(16)
RVR130	Rp49 Rev (RpL32)	CTGCATGAGCAGGACCTCCA	(16)

Primers to generate T7-flanked templates for in vitro transcription

Code	Name	Sequence (5′- 3′)
RVR5	T7-dmDCR1_A Fwd	TAATACGACTCACTATAGGGAGACTATCTCAGTTGCGAGGTTGG
RVR6	T7-dmDCR1_A Rev	TAATACGACTCACTATAGGGAGAGGGTATGTCTTTCAGTTCGTCC
RVR19	T7-GFP 125 Fwd	TAATACGACTCACTATAGGGAGAAGCTGACCCTGAAGTTCATCTG

RVR20	T7-GFP 561 Rev	TAATACGACTCACTATAGGGAGAGGTGTTCTGCTGGTAGTGGTC

Probes for northern blots

Code	Name	Sequence (5′- 3′)
WB325	miR995-3p	AAGCCGAATCATGTGGTGCTA
WB326	miR279-3p	TTAATGAGTGTGGATCTAGTCA
WB328	miR988-3p	GCGTGAGGTTTGCAACAAGGGG
WB329	miR317-3p	ACTGGATACCACCAGCTGTGTTCA
WB330	miR970-3p	ATAGCCGCGTGTGTCTTATGA
WB333	miR305-3p	TGAGTGTACTTCAACATGTGCCG
WB334	miR305-5p	CAGAGCACCTGATGAAGTACAAT
WB336	miR11-3p	GCAAGAACTCAGACTGTGATG
WB351	miR275-3p	CGCGCGCTACTTCAGGTACCTGA
WB352	miR13b-3p	ACTCGTCAAAATGGCTGTGATA
GO-690	IIV6 siRNA probe (-) 196578	AGTTTGCCGATCCTGTATGTA
GO-691	IIV6 siRNA probe (-) 91868	ATATTCAAATACCCGACCTCG

miR275-miR305 locus

Code	Name	Sequence (5′- 3′)
RV27	miR275-Fwd	CTTGCGCGCTAATCAGTGAC
RV29	miR305-rev	TTCAACATGTGCCGGGTTAC
RV30	E1S-Fwd	GTCGTTCGTTTCGCACCAAA
RV31	E1L-Fwd	TCAACGTGGTTTCTGGAGCT
RV32	E2-rev	TTCGGAGCGTAGTATTGGCC
RV33	E2-Fwd	TATCAAGGGTCTGCCGAACG
RV38	miR275-rev	GTCACTGATTAGCGCGCAAG

Stemloop qPCR

Code	Name	Sequence (5′- 3′)
RV048/BP56	SL miR305-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCAGAGC
RV050/BP54	SL miR305-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGAGTG
RV056	SL U6 snRNA	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAAAT
RV052	SL miR275-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGCCCC
RV054	SL miR275-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGCGCG
RV058	SL miR2b-1-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCATGTC
RV060	SL miR995-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGCAGC
PM324	SL-bantam-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACATCAGC

RV049	F-miR305-5p	CGGCGCATTGTACTTCATCAGGT
RV051	F-miR305-3p	CGGAATCGGCACATGTTGAAGTA
RV068	F-U6 snRNA	CGCAAAATCGTGAAGCGTTC
RV053	F-miR275-5p	CGGAATCGCGCTAATCAGTGACC
RV055	F-miR275-3p	CGGCGCTCAGGTACCTGAAGTAG
RV059	F-miR2b-1-5p	CGGAATGTCTTCAAAGTGGCAGT
RV061	F-miR995-5p	CGGAATCCCGAATTATGTGGGAG
PM325	F-bantam-3p	GCCCGCTGAGATCATTTTGAAAG
RV065/PM323	R-univ-SL-qPCR	GTGCAGGGTCCGAGGT

RNA oligonucleotides

Name	**Sequence (5′- 3′)**
miR305-5p	AUUGUACUUCAUCAGGUGCUCUG
miR305-3p	CGGCACAUGUUGAAGUACACUCA
miR995-5p	CCCGAAUUAUGUGGGAGCUGCG
miR995-3p	UAGCACCACAUGAUUCGGCUU
siR-305-5p	AGUGUACUUCAACAUGUGCCGUG
siR-305-3p	GAGCACCUGAUGAAGUACAAUCA

miR-305 reporters

Code	**Name**	**Sequence (5′- 3′)**
GO-693	scrambled miRNA target site Fw	AATGACCGCTAACGTGGTACCTGTAATGTCTGACCCTGCGAGCTAA CGTGGTACCTGTAATGTCCCGCGGCTGCGTTT
GO-694	scrambled miRNA target site Rv	AAACGCAGCCGCGGGACATTACAGGTACCACGTTAGCTCGCAGGGT CAGACATTACAGGTACCACGTTAGCGGTCATTGC
GO-695	Dme-miR-305-3p target site Fw	AATGACCTGAGTGTACTTCAACATGTGCCGTGACCCTGCGATGAGT GTACTTCAACATGTGCCGCCGCGGCTGCGTTT
GO-696	Dme-miR-305-3p target site Rv	AAACGCAGCCGCGGCGGCACATGTTGAAGTACACTCATCGCAGGGT CACGGCACATGTTGAAGTACACTCAGGTCATTGC
GO-697	Dme-miR-305-5p target site Fw	AATGACCCAGAGCACCTGATGAAGTACAATTGACCCTGCGACAGAG CACCTGATGAAGTACAATCCGCGGCTGCGTTT
GO-698	Dme-miR-305-5p target site Rv	AAACGCAGCCGCGGATTGTACTTCATCAGGTGCTCTGTCGCAGGGT CAATTGTACTTCATCAGGTGCTCTGGGTCATTGC

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