**Drosophila** microRNA modulates viral replication by targeting a homologue of mammalian cJun

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**Abstract**

MicroRNAs (miRNAs) are important regulators of biological processes, including host–virus interaction. This study investigated the involvement of *Drosophila melanogaster* miR-8-5p in host–virus interaction. *Drosophila* flies and cells challenged with *Drosophila* C virus (DCV) were found to have lower miR-8-5p abundance compared to uninfected samples. Lowering miR-8-5p abundance by experimental inhibition of the miRNA led to an increase in viral accumulation, suggesting that the observed decrease in the miR-8-5p abundance during DCV infection enhances viral replication. miR-8-5p putative targets were identified and included dJun, a transcription factor gene whose mammalian homologue cJun is induced by various viruses through kinase activation. Increasing miR-8-5p abundance using miR-8-5p mimics resulted in a decrease in dJun and GFP reporter levels. Furthermore, when the putative target in dJun was mutated, addition of miR-8-5p mimics did not result in the same antagonistic effect on dJun. These results show negative regulation of dJun by miR-8-5p and suggest that an miRNA-mediated pathway is involved in dJun regulation during viral infection. To analyse the role of dJun during DCV infection, dJun was knocked down in cells prior to DCV infection. Knockdown of dJun decreased DCV replication, providing evidence that dJun up-regulation is concomitant with miR-8-5p down-regulation during DCV infection and supports viral replication. These results highlight the role of miRNA in regulating the transcription factor gene dJun and uncover a previously unrecognized mechanism by which dJun is regulated during host–virus interaction.

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**INTRODUCTION**

MicroRNAs (miRNAs) are small non-coding RNAs that regulate mRNA transcript stability and protein translation [1]. Produced by a spectrum of organisms, miRNAs are canonically derived from RNA polymerase II transcription of protein-coding or non-coding regions of the genome [2]. In animals, the primary miRNA (pri-miRNA) transcript undergoes sequential cleavage by Drosha and then Dicer (Dcr) to liberate the ~22 nt long mature double-stranded miRNA–miRNA* duplex [3–5]. The miRNA–miRNA* duplex is then loaded onto the RNA-induced silencing complex (RISC), whereby the duplex is unwound [6]. One strand, traditionally called the star strand (miRNA*), is removed from the complex, leaving behind the other strand to guide the RISC to its targets [7].

Binding of the miRNA seed region (second to eighth nucleotide from the 5’-end) to complementary sequences in an mRNA is sufficient for miRNA recognition [8–10], and thereby enables modulation of gene expression [11]. With 28,645 miRNAs recorded in the latest miRNA registry miRBase [12], and with a single miRNA being able to target many genes [9, 13–15], miRNAs regulate almost all important eukaryotic cellular processes [16–18]. In the context of host–virus interaction, functional analyses of miRNAs show their diverse and divergent roles. While some miRNAs inhibit virus accumulation [19, 20], others facilitate virus proliferation [21–24]. miRNA regulation of viral replication can occur by direct targeting of viral or host mRNA [17, 25–29].

The vinegar fly *Drosophila melanogaster* has been used extensively as a model organism for a range of biological phenomena, including host–virus interaction, due to the high degree of conservation between *Drosophila*, arthropod vectors of arboviruses like mosquitoes, and mammals [30–34]. Recently, we demonstrated the effect of infecting *Drosophila* with its natural pathogen, *Drosophila* C virus (DCV), a positive-sense RNA virus belonging to the *Cripaviruses* genus of the *Dicistroviridae* family [35], on the *Drosophila* miRNA profile. DCV infection results in changes in the levels of *Drosophila* miRNAs, including a decrease in the abundance of both strands of the miR-8 duplex: miR-8-
3p and the less abundant and traditionally designated miRNA* of miR-8, miR-8-5p [36]. Down-regulation of the more abundant Drosophila miR-8-3p homologue in Bombyx mori (bmo-miR-8) has already been shown to benefit viral infection [37].

In this study, we explored the function of miR-8-5p in host-virus interaction. The results show that the observed decrease in the levels of miR-8-5p during DCV infection supports viral replication. Further, results show that miR-8-5p has a direct antagonistic effect on the transcription factor Drosophila Jun (dJun). dJun is the Drosophila homologue of the mammalian cJun [38], a gene whose induction by a wide range of viruses [39–48] leads to enhanced viral replication [39, 41].

RESULTS

miR-8-5p has lower abundance during DCV infection of flies and cells

Recently, we showed that DCV infection of Drosophila ORC flies results in differential abundance of miRNAs, including miR-8-5p [36]. To test the generality of the lowered miR-8-5p abundance in DCV-infected flies, Drosophila wild-type w1118 flies were infected with either PBS (mock) or DCV and harvested at 3 days post-infection (p.i.). Analysis of miR-8-5p abundance showed that w1118 DCV-infected flies had lower miR-8-5p abundance by an average of 60 % than those injected with PBS (paired Student’s t-test, **P<0.01, Fig. 1). To analyse whether a change in miR-8-5p also occurs in cultured cells, Drosophila S2 cells were treated with either PBS or DCV at an m.o.i. of 5. Cells were harvested 2 days p.i. and analysed for miR-8-5p levels. miR-8-5p abundance was, on average, 80 % lower in DCV-infected cells than in control cells (paired Student’s t-test, *P<0.05, Fig. 1). As in whole flies, the abundance of miR-8-5p in cells was lower in DCV-infected cells. This indicates that the response of S2 cells to DCV, in the context of miR-8-5p regulation, is consistent with that of the whole flies, and they can therefore be used as a model for the whole flies.

Lower miR-8-5p abundance increases DCV replication

Since DCV infection results in a decrease in miR-8-5p levels in both flies and cells, the significance of reduced miR-8-5p for DCV infection was examined using synthetic miR-8-5p inhibitors. Inhibitors are antisense oligonucleotides that bind to complementary miRNA sequences to potently and irreversibly inhibit miRNA function [49, 50]. Cells were transfected with synthetic scrambled sequence (control) or synthetic antisense miR-8-5p sequence (miR-8-5p inhibitor). Cells were then infected with DCV at an m.o.i. of 5. Inhibition of miR-8-5p led to an increase in virus genome copies by an average of 400 % (paired Student’s t-test, *P<0.05, Fig. 2). This suggests that the observed decrease in miR-8-5p levels during DCV infection of flies and cells supports viral infection.

miR-8-5p targets dJun, the Drosophila homologue of mammalian cJun

To gain an insight into how how miR-8-5p modulates virus accumulation, putative targets of miR-8-5p were predicted using DIANA-microT-CDS [51] and confirmed using RNAhybrid [52]. Among these, Jun-related antigen (Jra), classified by DAVID [53] as having functions in the negative regulation of immune response, was found to have two putative miR-8-5p binding sites in its 3’-UTR (Fig. 3a, b). Jra is also known as dJun because it is the Drosophila homologue for mammalian cJun, a gene that is activated rapidly by a wide array of stimuli, including viral infection [38].

Treating cells with miRNA mimics and then quantitatively analysing the transcript levels of the predicted targets is one of the direct methods used to test miRNA:gene interaction [54]. To experimentally assess whether miR-8-5p indeed targets dJun, assays using synthetic miR-8-5p mimics were

![ Fig. 1. miR-8-5p is down-regulated during DCV infection. Wild-type w1118 flies (left panel) and S2 cells (right panel) were infected with PBS (mock) or DCV. Levels of miR-8-5p normalized to U6 were quantified using RT-qPCR. miRNA level in mock-treated samples was set to 100 % (grey bars) and the miRNA relative abundance in DCV-treated samples was as depicted (black bars). Data include three independent experiments. Bars represent the mean and the lines represent the SEM (paired Student’s t-test, **P<0.01, *P<0.05). ](image-url)
performed. S2 cells were transfected with either synthetic scrambled sequence duplexes or synthetic duplexes that mimic miR-8-5p. The transcript abundance of the predicted target, dJun, was then measured. Addition of miR-8-5p mimics reduced the transcript levels of dJun by an average of 60% (paired Student's t-test, **P<0.01, Fig. 3c), suggesting interaction between miR-8-5p and dJun. To assess whether the repression of dJun seen in miR-8-5p-mimic-treated cells is a result of a direct interaction between miR-8-5p and dJun, a reporter assay was employed. With the rationale that binding of miRNA will repress the expression of its target and also the gf or luciferase cloned upstream of the target [55, 56], a 298 bp long fragment of dJun 3′-UTR was cloned into a previously constructed pIZ/GFP plasmid [57] (Fig. S1, available in the online Supplementary Material). In miRNA–target interaction studies using a reporter gene and a mimic, use of a cell line from a different species circumvents the artefact effect on the target sequence of miRNAs endogenous to the organism of interest. The plasmid pIZ/GFP-dJun was co-transfected with...
control and miR-8-5p mimics into Spodoptera frugiperda (Sf9) cells and changes in gfp transcripts were quantified by RT-qPCR. In miR-8-5p-transfected cells, the gfp transcript was reduced by an average of 70% compared to cells transfected with the control mimic (paired Student’s t-test, *P<0.05, Fig. 3d). To confirm that the interaction between miR-8-5p and dJun is sequence-dependent, four nucleotides in each of the two predicted miR-8-5p binding sites in dJun 3’-UTR were mutated (Fig. S2). The mutated dJun insert was cloned into pIZ/GFP and the plasmid was then co-transfected with control and miR-8-5p mimics into Sf9 cells. Analysis of gfp transcripts showed no difference between control-treated and mimic-treated cells (Paired Student’s t-test, P>0.05, Fig. 3e), providing evidence that the miR-8-5p repression of dJun occurs through direct and sequence-dependent interaction.

**dJun levels increase during DCV infection**

Having shown that DCV infection lowers miR-8-5p, and that miR-8-5p in turn negatively regulates dJun, we posited that DCV infection would increase the abundance of dJun. To test this, flies were challenged with either PBS (control) or DCV and harvested at 3 days p.i., and dJun transcript abundances were then measured by RT-qPCR. The dJun transcript was on average 200% higher in DCV-infected flies compared to mock-infected flies (paired Student’s t-test, *P<0.05, Fig. 4a). This indicates that, concomitant to lowering of miR-8-5p during infection, dJun is up-regulated in the presence of DCV.

**dJun supports DCV infection**

To examine whether the increase in dJun mRNA levels during DCV infection is biologically relevant in the context of insect–virus interaction, we tested the effect of decreasing dJun on DCV replication. Cells were transfected with either non-specific double-stranded RNA (nsdsRNA) as a control or double-stranded dJun (dsdJun) to knock down dJun expression. Changes in dJun as a result of dsdJun treatment were measured and found to be on average 50% lower in dsdJun-treated cells compared to nsdsRNA-treated cells (Fig. S3). Cells were subsequently infected with DCV and analysis of DCV genomic RNA in the control and treated cells show that DCV accumulation is on average 60% lower in cells with lower dJun (paired Student’s t-test, **P<0.01, Fig. 4b). This shows that cells with a decrease in the abundance of the dJun transcript have lower DCV genome abundance.

**DISCUSSION**

Understanding the host factors, regulatory pathways and mechanisms involved in viral infection is useful for designing interventions that may interfere with the replication of viruses, including medically important ones such as Zika virus, dengue virus and West Nile virus. The more abundant strands of miRNA duplexes are known to be modulators of viral replication [17, 25–29]. For example, the more abundant Bombyx mori miR-8, which is homologous to Drosophila miR-8-3p, has been experimentally shown to affect Bombyx mori nucleopolyhedrosis virus (BmNPV) accumulation [37]. However, the role of the less abundant miR-8 (miR-8-5p) in host–virus interaction is still unknown. Generally, it is less clear whether miRNA* strands have the same widespread role during viral infection. In this study, we used the Drosophila–DCV model system to examine the role of the less abundant miR-8-5p during infection of the host by its natural pathogen. This study found that miR-8-5p levels decrease during DCV infection of whole flies and cells, and that this decrease impacts positively on viral replication. These results indicate that the observed miR-8-5p decrease during DCV infection is a mechanism that is supportive of viral replication. dJun was computationally predicted and experimentally validated as a target of miR-8-5p. Its protein product, Jun (dJun) is the transcription factor and is up-regulated by a variety of physiological and pathophysiological stimuli, such as growth factors, cytokines and UV radiation, Jun is activated primarily by JNK phosphorylation of Jun’s conserved N-terminal phosphorylation sites [59, 60]. The activated Jun...
cJun is activated during infection by a range of viruses in mammals. These viruses include positive-sense single-stranded RNA viruses (such as dengue virus [39] and Japanese encephalitis virus [47]), negative-sense single-stranded RNA viruses (influenza virus [42, 43] and vesicular stomatitis virus [46]) and DNA viruses (herpes simplex virus [40, 41], Epstein–Barr virus [44] and varicella-zoster virus [45]). Activation or increase in cJun/cJun results in enhanced replication of viruses. In contrast, blocking the pathway that leads to activation of cJun lowers dengue virus [39] and herpes simplex virus [41] yields. In this study, the transcript levels of dJun increased during DCV infection. This is consistent with previous studies that showed the effect of viral replication on Jun transcript and its protein.

Whereas all the previous host–virus interaction studies focusing on Jun have shown that regulation of the gene is affected through phosphorylation of Jun by signal transduction pathways, the results of this study show that Jun is also regulated through the miRNA pathway during viral infection. Our results thus uncover a previously unrecognized mechanism by which Jun is regulated during host–virus interaction (Fig. 5). The abundance levels of the genes are determined not only by the rate of mRNA transcription, but also by the rate of mRNA degradation. In the case of Jun in Drosophila–DCV, DCV infection results in activation of the JNK pathway [65]. It is conceivable that while autoregulation of Jun, which occurs as a result of its phosphorylation, increases the gene’s transcription [64], the down-regulation of miR-8-5p seen during DCV infection of Drosophila is a mechanism to ensure that the increase in Jun transcription is complemented by a decrease in Jun degradation by miR-8-5p. This would mean that different cellular activities targeting Jun are occurring. On the one hand, this raises the question of how each regulatory mechanism contributes to Jun abundance, and on the other hand, it opens up the possibility that the regulation seen in mammalian cJun during various viral infections may not only occur due to kinase activation, but also because of changes in miRNAs. There are many existing studies that separately show that cJun is regulated during viral infection and that miRNAs are regulated during viral infection. For example, Japanese encephalitis virus infection of mice activates cJun [47] and down-regulates miR-200c-3p [66]. Analysis of cJun and miR-200c-3p sequences shows putative binding sites for cJun: miR-200c-3p (data not shown). The mammalian cJun also has putative binding sites for miR-200b-5p, the mammalian homologue of Drosophila miR-8-5p [67], as well as several other miRNAs (data not shown). This merits a review of the different mechanisms leading to regulation of Jun, as well as of other genes known to change during viral infection of host, with particular attention being paid to miRNA’s role.

\[\text{cJun} \rightarrow \text{miR-200c-3p} \rightarrow \text{Jun} \]

**Fig. 5.** Proposed model for dJun regulation during DCV infection. Our results suggest that dJun up-regulation during viral infection is a miR-8-5p-mediated pathway that subsequently supports viral accumulation. How dJun increases viral accumulation is, however, not yet understood. Black arrows show our current findings, with numbers corresponding to figures in the text. In the context of miR-8-5p: (1) DCV infection results in miR-8-5p down-regulation; (2) conversely, miR-8-5p down-regulation results in higher DCV accumulation; (3) miR-8-5p negatively regulates dJun, such that its down-regulation results in increased dJun. In the context of dJun, (4a) DCV infection results in increased dJun transcription and (4b) increased dJun enhances DCV accumulation. Grey arrows indicate previous knowledge of dJun regulation during DCV infection; DCV infection results in JNK phosphorylation and JNK phosphorylation leads to increased dJun transcription. The contribution of regulation by JNK phosphorylation and miR-8-5p to the net increase in dJun is still unknown. There may also be other miRNAs that are involved, which are as yet unidentified. In mammals, it is known that infection by various viruses leads to JNK phosphorylation and, consequently, increased Jun transcription. It is also known that miRNA (specifically miR-216b) can regulate cJun. Whether the same miRNA regulation occurs during mammalian viral infection remains to be studied.

Knockdown of dJun decreased DCV replication. This is consistent with previous studies showing the reciprocal relationship between Jun and viruses, and Jun modulation of viral replication. Presently, even with all the studies showing the effect of Jun on virus infection, the mechanism by which Jun regulation affects viral replication is still not understood. The positive correlation between Jun and viral replication could be due to the induction of host factors required for viral replication. For example, cJun activation increases transcription of RACK1 [68], a cellular factor required for DCV and hepatitis B infection [69]. Alternatively, increased Jun could be contributing towards a more conducive intracellular environment for viruses, as in the case when host antiviral factors and mechanisms are repressed. Apoptosis is one such antiviral response that Jun regulates, either positively or negatively [70]. While the apoptotic pathway may be employed by viruses to enable release and infection of other cells, the pathway is also a mechanism used by hosts to limit time and cellular machinery for viral replication [71]. In sensory neurons, in partnership with ebi, cJun represses the transcription of hid, a major component of the apoptosis pathway [72]. In mammary glands, deletion of cJun reduces survivin (BIRC5), an inhibitor of apoptosis [73]. It is possible that in the context of host–virus interaction, the increase in Jun allows for decrease in apoptosis.
Investigation into the targets of Jun during viral infection will hopefully uncover the mechanism by which Jun carries out its viral modulation function. Because Drosophila only has one homologue of Jun, in contrast to the four homologues in mammals, the use of the model organism will remove the difficulty of tracking gene multiplicity, and will therefore greatly simplify and aid Jun–virus interaction research [64, 74, 75].

Beyond showing the role of miR-8-5p in virus infection of hosts and miR-8-5p targeting of clun, this study brings together miRNAs and transcription factors, the two largest families of gene regulators [76]. In essence, the study shows that a transcription factor that is well-known to autoregulate its own transcription is also regulated by another regulator, an miRNA, specifically during viral replication. The co-regulation of clun by itself and miRNA is consistent with the results of a recent study on prolonged endoplasmic reticulum (ER) stress. Upon treatment with tunicamycin, clJun is regulated by miR-216b [77]. The fact that the same type of network motif occurs during viral replication and ER stress shows the importance of co-regulation by transcriptional and post-transcriptional mechanisms. In the context of host–virus interaction, this also shows that viruses are able to exploit the different regulatory pathways in a coordinated fashion to enable efficient viral replication. This suggests that in the design of interventions to preclude viral infections, the common target of different regulatory nodes should be focused on.

**METHODS**

**Flies, cells and viruses**

Drosophila melanogaster wild-type w1118 was maintained on a standard cornmeal diet at 25 °C with 12 h light/dark cycles. Flies were treated to remove any virus or Wolbachia infections by dechorionation of eggs and treatment with tetracycline, respectively, as previously described [78, 79]. Absence of Wolbachia and DCV was confirmed by PCR and RT-PCR, respectively.

Drosophila S2 cells were grown in Schneider’s medium (Thermo Fisher Scientific) supplemented with 10% (v/v) fetal bovine serum (FBS) (Thermo Fisher Scientific) and penicillin–streptomycin (Thermo Fisher Scientific). Spodoptera frugiperda Sf9 cells were grown in SF-900 medium (Thermo Fisher Scientific). Both cell cultures were grown at 27 °C.

Drosophila C virus isolate EB (DCV Eb) [80, 81] was prepared, purified and titred as previously described [80, 81].

**Viral infections**

Flies were challenged with either control (phosphate buffer saline, PBS) or DCV by injecting 50.6 nl of PBS or PBS with 1 × 10⁶ DCV IU ml⁻¹ into the upper lateral part of the abdomen of carbon dioxide-anaesthetized 4–7-day-old male flies using needles pulled from borosilicate glass capillaries and a Nanoject II microinjector (Drummond Scientific). At 3 days post-injection, five flies from each treatment were collected. At least three independent biological replicates were performed.

S2 cells were seeded at 1 × 10⁶ cells per well in a six-well plate for an hour. The medium was removed and replaced with 1 ml serum-free medium (SFM) containing either PBS or DCV at an m.o.i. of 5. Plates were placed on a rocker for an hour, after which 1 ml Schneider medium supplemented with 20% FBS was added to each well. The cells were incubated at 27 °C for 2 days and then harvested. Three independent biological replicates were performed.

**RNA extraction**

Harvested flies were homogenized using TissueLyser II (Qiagen) in tubes containing 1 ml Ribozol (Amresco) and three 3 mm beads (Sigma-Aldrich). Homogenization was performed for 90 s at 30 Hz s⁻¹. For cells, the medium was removed and replaced with 1 ml Ribozol to lyse them. Total RNA of the samples were extracted as per the manufacturer’s protocol, with the exception that 900 µl isopropanol was added to each sample.

**miRNA quantification**

RNA from the whole fly and cell samples was polyA tailed and reverse-transcribed using QuantimiR (System Biosciences) supplied reagents as per System Biosciences’s instructions. The QuantimiR-generated cDNAs, along with Platinum SYBR green qPCR SuperMix-UDG (Thermo Fisher Scientific) were then used for reverse-transcription quantitative PCR (RT-qPCR) for miRNA as per the manufacturer’s instructions. U6 was used as the reference gene. Forward primer was supplied by QuantimiR, whereas miR-8-5p and U6-specific reverse primers were synthesized by Thermo Fisher Scientific. The primer sequences are listed in Table S1. A Rotor-Gene 6000 thermal cycler (Qiagen) was used with the following profile: 90 °C for 2 min, 95 °C for 10 min, and 40 cycles repeats of 95 °C for 15 s and 60 °C for 1 min. This was followed by a standard melt curve analysis to confirm that only the expected product had been amplified. The abundance of miR-8-5p relative to the host reference gene was determined using qGENE software and analysed using GraphPad Prism. Three independent biological replicates with duplicate technical replicates for each sample were performed.

**Virus accumulation in miRNA inhibitor-treated cells**

Synthetic miR-8-5p inhibitor and control scramble inhibitor (sequences listed in Table S1) were synthesized by GenePharma. S2 cells were seeded at 1 × 10⁶ cells per well in a six-well plate for an hour. The medium was removed and replaced with 1 ml transfection medium, which included SFM. Cellfectin II (Thermo Fisher Scientific) and control inhibitor or inhibitor. Each mixture was prepared by separately mixing 6 µl Cellfectin with 94 µl SFM and 5 µl 20 µM control inhibitor or inhibitor with 95 µl SFM. The Cellfectin–SFM and control inhibitor–SFM or inhibitor–SFM were then mixed together and incubated at room temperature for 30 min. After incubation, 800 µl SFM was added onto the mixture to make 1 ml transfection medium. The transfection medium was transferred onto the cell-culture wells. Transfection proceeded on a
rock for 5 h, after which 1 ml 20% FBS Schneider medium with either no DCV (control) or with DCV at an m.o.i. 5 was added onto the wells. The cells were then incubated at 27°C for 2 days. After incubation, the medium was removed and replaced with 1 ml Ribozol for subsequent RNA extraction, as described above. RNA extracts were then DNase-treated (New England Biolabs) as per the manufacturer’s protocol. cDNAs were synthesized using the DNase-treated RNA as a template, random primers (New England Biolabs) and Superscript III (Thermo Fisher Scientific). Synthesized cDNAs were used as a template for RT-qPCR to quantify DCV. DCV was normalized using ribosomal protein RpL32 (RpL32) as the reference gene. The primers for DCV and RpL32 were as listed in Table S1. The abundance of DCV relative to the host reference gene was determined using qGENE software and analysed using GraphPad Prism. Each transfection was performed at least three times to produce biological replicates.

miR-8-5p target analysis

Putative targets were predicted using DIANA-microT-CDS [51], which is recognized as one of the best prediction tools [82–84]. Putative targets were further screened by looking at the gene ontologies and functional annotations of the predicted targets using DAVID [53, 85]. Once dJun was identified as a putative target of interest, RNAhybrid [52] was used to confirm the DIANA-microT-CDS results and identify other possible binding sites in the miRNA.

dJun quantification in mimic-treated cells

The synthetic control scrambled sequence mimic and miR-8-5p mimic (sequences listed in Table S1) were synthesized by GenePharma. Cells were prepared and transfected with control mimics or mimics as per the protocol used for analysing virus accumulation in inhibitor-treated cells, with modifications. Briefly, S2 cells were seeded for an hour. The medium was removed and replaced with 1 ml transfection medium containing control inhibitor or inhibitor (prepared as described above). The cells were placed on a rocker for 5 h, after which 1 ml 20% FBS Schneider media was added onto the wells. The cells were then incubated at 27°C. After 48 h, 6 µl control inhibitor or inhibitor was added onto the wells. Cells were harvested after an additional 24 h incubation. The medium was removed and replaced with 1 ml Ribozol for subsequent RNA extraction, DNase-treatment, cDNA synthesis and qPCR, as described above. RpL32 was used as the reference gene. The primers for dJun and RpL32 are as listed in Table S1. The abundance of DCV relative to the host reference gene was determined using qGENE software and analysed using GraphPad Prism. At least three independent biological replicates were performed.

Assaying reporter GFP in pIZ/GFP-dJun and mimic-co-transfected cells

A dJun sequence with 4 nt mutations in each of the two predicted sites (Fig. S2) corresponding to the miRNA seed region binding site was synthesized and cloned into pIZ-GFP by Thermo Fisher Scientific. Sf9 cells were co-transfected with mutated pIZ/GFP-dJun and control mimic or mimic as above. Cells were harvested at 48 hours p.i., and the RNA was processed and the GFP quantified as above. Three biological replicates per experiment were performed.

dJun levels in flies

Flies were challenged with either control (PBS) or DCV as described above, harvested at 3 days p.i., and processed for RT-qPCR, as above. dJun was quantified using RpL32 as the reference gene. The primers are listed in Table S1. Relative dJun levels were calculated using qGENE and analysed using GraphPad Prism. At least three independent biological replicates were performed.

DCV in dJun-knocked down cells

Double-stranded RNA of GFP and dJun were prepared as described previously [80] using the primers listed in Table S1. To analyse for dJun knockdown, S2 cells were treated with 1 µg GFP dsRNA (non-specific double-stranded RNA, nsdsRNA, as control) or dJun dsRNA as described [86]. At 2 days p.i., cells were harvested and RNA was extracted and prepared for RT-qPCR for dJun, as above. To determine the effect of dJun knockdown in cells, S2 cells were treated with 1 µg GFP or dJun dsRNA as described [86] and infected with DCV at an m.o.i. 0.1. At 2 days p.i., 1 µg dJun dsRNA was retransfected into the cells. Cells were harvested 24 h afterwards. RNA was extracted and prepared for RT-qPCR for dJun to analyse dJun knockdown and
DCV accumulation, as above. Relative \textit{dlun} and DCV levels were calculated using qGENE and analysed using GraphPad Prism. At least three independent biological replicates per experiment were performed.

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Conflicts of interest
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Ethical statement
This research did not require ethical approval.

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