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### ► **To cite this version:**

Camille A Mayeux, Anaïs Larue, Daniel S Oliveira, Marion Varoqui, Hélène Henri, et al.. Intricate interactions between antiviral immunity and transposable element control in *Drosophila*. 2024. <hal-04791902>

**HAL Id: hal-04791902**

**<https://hal.inrae.fr/hal-04791902v1>**

Preprint submitted on 19 Nov 2024

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# 1 **Intricate interactions between antiviral immunity and** 2 **transposable element control in *Drosophila***

3

4 Camille A Mayeux<sup>1</sup>, Anaïs Larue<sup>1,2</sup>, Daniel S. Oliveira<sup>1,3</sup>, Marion Varoqui<sup>4</sup>, H el ene Henri<sup>1</sup>, Rita  
5 Rebollo<sup>2</sup>, Natacha Kremer<sup>1</sup>, S everine Chambeyron<sup>4</sup> and Marie Fablet<sup>1,5,\*</sup>

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7 <sup>1</sup> Laboratoire de Biom etrie et Biologie Evolutive; Universit  Lyon 1; CNRS; VAS; UMR 5558,  
8 Villeurbanne, France

9 <sup>2</sup> Univ Lyon, INRAE, INSA-Lyon, BF2I, UMR 203, 69621 Villeurbanne, France

10 <sup>3</sup> Institute of Biosciences, Humanities and Exact Sciences, S o Paulo State University  
11 (Unesp), S o Jos  do Rio Preto, S o Paulo, 15054-000, Brazil

12 <sup>4</sup> Institute of Human Genetics, UMR9002, CNRS and Universit  de Montpellier, Montpellier,  
13 France

14 <sup>5</sup> Institut Universitaire de France

15 \* Corresponding author: [marie.fablet@univ-lyon1.fr](mailto:marie.fablet@univ-lyon1.fr)

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## 18 **Abstract**

19 Transposable elements (TEs) are parasite DNA sequences that are controlled by RNA  
20 interference pathways in many organisms. In insects, antiviral immunity is also achieved by  
21 the action of small RNAs. In the present study, we analyzed the impacts of an infection with  
22 *Drosophila C Virus* (DCV) and found that TEs are involved in a dual response: on the one  
23 hand TE control is released upon DCV infection, and on the other hand TE transcripts help  
24 the host reduce viral replication. This discovery highlights the intricate interactions in the  
25 arms race between host, genomic parasites, and viral pathogens.

26

## 27 **Significance statement**

28 Transposable elements (TEs) are widespread components of all genomes. They were long  
29 considered as mere DNA parasites but are now acknowledged as major sources of genetic  
30 diversity and phenotypic innovations. Using *Drosophila C virus*, here we show that TEs are  
31 at the center of defense and counter-attack between host and virus. On the one hand, TE  
32 control is released upon viral infection, and on the other hand, TE transcripts help the host  
33 reduce viral replication. To our knowledge, this is the first time such a complex host-  
34 pathogen interaction involving TEs is shown.

35

36

## 37 Introduction

38 Transposable elements (TEs) are DNA sequences considered as genomic parasites  
39 because they have the ability to move and multiply along chromosomes at the expense of  
40 the host. TEs are very diverse in structure (number of genes, length, presence of repeats,  
41 *etc.*) and abundance, from a few percent in the honeybee *Apis mellifera* (1) to ~ 80% of the  
42 recently sequenced arctic krill genome (2). Tolerance to TEs is possible thanks to epigenetic  
43 mechanisms that inhibit their activity, and there is a strong selective pressure for these  
44 mechanisms to be particularly efficient in gonads, where the genomes of the next generation  
45 lie (3–5). In insects, this control is essentially achieved through RNA interference (RNAi)  
46 pathways, and in particular *via* the piRNA pathway. piRNAs are 23-30 nt-long single-  
47 stranded RNAs that target TE sequences through sequence complementarity. They form  
48 complexes with proteins displaying RNase activity or triggering heterochromatinization (6–  
49 8).

50 RNAi is also the first line of defense against viruses in Arthropods (9–12). Antiviral immunity  
51 relies on small RNAs known as siRNAs, which are 21 nt-long single-stranded RNAs,  
52 produced by Dicer-2 from double-stranded RNA templates. Whereas siRNAs are known to  
53 play a role in the somatic control of TE activity (13, 14), the intricate interplay between TE  
54 regulation and antiviral immunity through RNAi remains largely unexplored. Upon viral  
55 infection, viral RNA genomes are converted to DNA by TE reverse transcriptases, and this  
56 process leads to the increased production of viral siRNAs, facilitating the establishment of  
57 persistent infection (15, 16). Moreover, the activation of the *mdg4* TE at the pupal stage was  
58 recently found to prime the host's antiviral immunity for adult stage (17). In addition, in a  
59 previous investigation in *Drosophila* using the Sindbis virus (SINV), which naturally infects  
60 mosquitoes, we demonstrated that the host antiviral response triggered an amplified  
61 production of TE-derived small RNAs. This resulted in the reduction of TE transcript amounts  
62 (18). In the present study, we investigated the more closely intertwined interaction between  
63 the *Drosophila* host and its natural pathogen Drosophila C virus (DCV).

64 DCV is a non-enveloped virus that belongs to the Dicistroviridae family. It has a 9.2 kb-long,  
65 single-stranded RNA genome of positive polarity (19). DCV is a natural pathogen of  
66 *Drosophila* (20, 21), and is horizontally transferred through oral infection. A few days after  
67 infection, and independently of the route of infection –either oral or systemic through  
68 experimental injections–, DCV viral particles can be detected in the fat body, trachea and

7

69 visceral muscle of the crop, midgut, hindgut, and gonads (22). Systemic DCV infection  
70 causes intestinal obstruction that leads to fly death (23). DCV was first described in *D.*  
71 *melanogaster* but it can also naturally infect *Drosophila simulans* (20). The 99 most N-  
72 terminal residues of DCV ORF1 –encoding non-structural proteins– correspond to 1A, a viral  
73 suppressor of RNA interference (VSR) that binds long double-stranded RNAs (dsRNAs) and  
74 therefore prevents Dicer2-mediated production of siRNAs (24, 25).

75 Here we show that DCV infection disrupts the regulation of TEs in a more intricate way than  
76 what was observed with SINV (18). This is particularly pronounced in the *D. melanogaster*  
77 strain, as compared to the *D. simulans* strain. In addition, we show that hosts with a higher  
78 TE load in the transcriptome display a more efficient antiviral immunity. This study highlights  
79 the prominent contribution of TEs in the immune response of *Drosophila*.

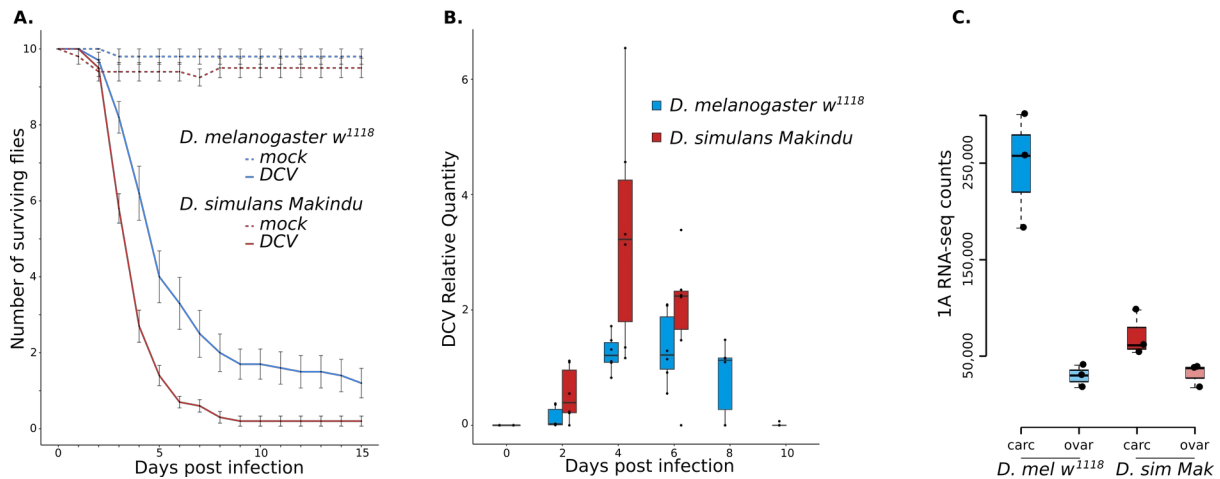
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## 81 **Results and Discussion**

82 Host response to DCV infection in *D. melanogaster*  $w^{1118}$  and *D.*  
83 *simulans* Makindu

84 We infected flies by intra-thoracic pricking (26), and followed fly mortality and DCV  
85 replication. The infection rapidly led to fly death, and significantly more rapidly in *D. simulans*  
86 Makindu (log-rank test, p-value = 0.008). On average, half of the flies were dead at 5.4 days  
87 post infection (dpi) in *D. melanogaster*  $w^{1118}$ , and 3.8 dpi in *D. simulans* Makindu (Fig. 1A).  
88 Accordingly, RT-qPCR revealed that DCV replication was 2.6-fold higher in *D. simulans*  
89 Makindu compared to *D. melanogaster*  $w^{1118}$  at 4 dpi, which corresponds to the peak of viral  
90 titer (Fig. 1B).

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91

92 **Figure 1. Response to DCV infection in *D. melanogaster w<sup>1118</sup>* and *D. simulans***  
93 **Makindu. A.** Fly survival upon DCV infection. **B.** Kinetics of DCV titers followed using RT-  
94 qPCR. **C.** Raw RNA-seq read counts mapping against 1A sequence. RNA-seq read  
95 mapping along the sequence of DCV genome is shown in Fig S1.

96 We performed RNA-seq and small RNA-seq experiments at the peak of viral titer, *i.e.*, 4 dpi  
97 in both species, in dissected ovaries and the rest of the body –hereafter called “carcasses”.  
98 DCV RNAs were highly abundant among the sequenced molecules in carcasses. We could  
99 also detect DCV RNAs in ovaries, however in much lower amounts, potentially  
100 corresponding to the presence of DCV in the muscle cells of the ovarian peritoneal sheath,  
101 as described by Ferreira *et al.* (22).

102 As previously described (23), DCV infection by pricking leads to many down-regulated genes  
103 in *D. melanogaster w<sup>1118</sup>* somatic tissues, as well as in *D. simulans* Makindu. In particular,  
104 down-regulated genes were enriched in genes related to metabolic functions, whereas the  
105 few activated genes were enriched in stress-response activity, as reported by Chtarbanova  
106 *et al.* (23), but also in immune processes (Fig S2).

107 It should be noticed that we cannot directly draw conclusions on a *D. melanogaster versus*  
108 *D. simulans* species comparison based on only one strain per species. Instead, it would  
109 require the inclusion of several strains for each species. Nevertheless, we have to  
110 acknowledge the differences in patterns between *D. melanogaster w<sup>1118</sup>* and *D. simulans*  
111 Makindu, which could correspond to several hypotheses. i) The sequencing data were  
112 produced at 4 dpi for both strain, which corresponds to a higher number of dead flies and a  
113 stronger viral replication in *D. simulans* Makindu. Therefore, 4 dpi seems to be further along  
114 in the infection process in *D. simulans* Makindu. ii) It is possible that the infection protocol

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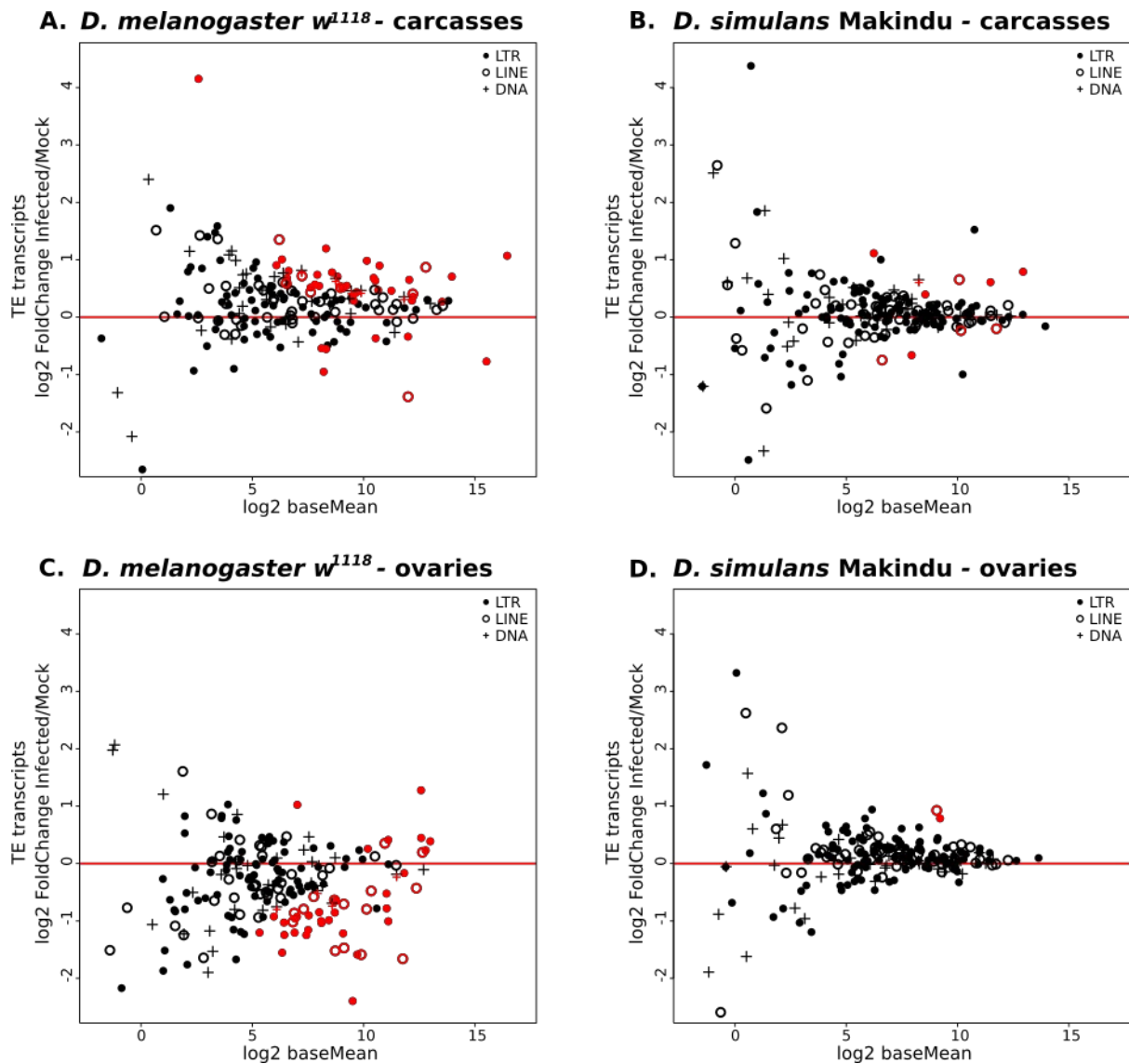
115 leads to acute infection in *D. simulans* Makindu while persistence is achieved in *D.*  
116 *melanogaster* *w*<sup>1118</sup>. iii) DCV was first described as a *D. melanogaster* pathogen. The  
117 differences between *D. melanogaster* and *D. simulans* outcomes may be due to *D. simulans*  
118 not being its preferential host, even though DCV has been found in natural samples of *D.*  
119 *simulans* (20).

120

## 121 DCV infection impacts the transcript amounts of transposable 122 elements

123 We analyzed the amounts of transcripts for 237 TE families in the carcasses of the different  
124 samples, and we found a global significant increase upon DCV infection in *D. melanogaster*  
125 *w*<sup>1118</sup> (median per TE family log<sub>2</sub> Fold Change (log<sub>2</sub>FC) = 0.28, paired Wilcoxon test: p-value  
126 = 8e-12). This is in agreement with our previous reanalysis of data produced from  
127 *D. melanogaster* *y*<sup>1</sup> fat bodies upon DCV infection, which also showed a clear increase in TE  
128 transcript amounts (18, 27). In the present data, forty three TE families are significantly  
129 upregulated upon DCV infection (DESeq2 adjusted p-value < 0.05), whereas seven are  
130 significantly down-regulated (Fig. 2A; See Fig S3 for TE family details). We could not detect  
131 significant differences across TE classes (Table S4). In *D. simulans* Makindu, there is  
132 virtually no modulation: we observed a significant increase for six TE families and a  
133 significant decrease for four TE families (Fig. 2B).

13



134

135 **Figure 2. TE transcript modulation upon DCV infection.** A. TE transcripts log<sub>2</sub>FC  
136 between DCV infected and mock conditions. Dot shapes indicate TE classes, red line is  
137 log<sub>2</sub>FC=0, *i.e.* no modulation. Red dots are TE families displaying significant differential  
138 expression at the 0.05 level for DESeq2 adjusted p-values. **A.** *D. melanogaster w<sup>1118</sup>*  
139 carcasses, **B.** *D. simulans* Makindu carcasses, **C.** *D. melanogaster w<sup>1118</sup>* ovaries, **D.**  
140 *D. simulans* Makindu ovaries.

141 In ovaries, we observed an opposite pattern for *D. melanogaster w<sup>1118</sup>* samples, with a clear  
142 decrease of TE transcript amounts: median per TE family log<sub>2</sub>FC = -0.38 (paired Wilcoxon  
143 test : p-value = 1e-9). Transcript amounts increased significantly for nine TE families  
144 whereas they significantly decreased for 41 TE families (Fig. 2C). In *D. simulans* Makindu,  
145 DCV infection had very little impact on ovarian TE transcript amounts (median per TE family  
146 log<sub>2</sub>FC = 0.10, paired Wilcoxon test : p-value = 5e-9) (Fig. 2D).

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147 In order to understand the mechanisms underlying changes in TE transcript amounts upon  
148 DCV infection, we analyzed small RNA-sequencing data from the same experimental  
149 conditions. The data showed the expected patterns in size distribution and nucleotide  
150 composition (Fig S5-S6). Ping-pong signatures were detected in all conditions, indicating  
151 that flies infected with DCV could still produce functional piRNAs (Fig S7). TE-derived  
152 siRNAs and piRNAs of both polarities clearly increased upon DCV infection in carcasses of  
153 *D. melanogaster* *w<sup>1118</sup>* and *D. simulans* Makindu, whereas we detected very low size effects  
154 in ovaries (Fig S8-S10). The increase was stronger in piRNAs compared to siRNAs (all TE  
155 families together, median log2FC: piRNAs: [0.25; 1.28], siRNAs: [0.05, 0.75], Fig S11).

156 When TE control is achieved *via* small RNA interference, the expectation is that an increase  
157 in small RNAs leads to a decrease in RNA amounts, and reciprocally. However, here, the TE  
158 families displaying small RNA increase upon infection are globally not significantly enriched  
159 in downregulated families, and this was consistent across all conditions (Table S12). Such a  
160 result reveals a dissociation between TE transcript amounts and TE-derived small RNAs in  
161 the present experimental system.

162 It has been demonstrated that viral infections in *D. melanogaster* trigger the active uptake of  
163 dsRNA molecules of viral origin in haemocytes, which fuels the siRNA machinery and allows  
164 the systemic production of antiviral siRNAs (16, 28). Using Sindbis virus (SINV), we  
165 previously proposed that dsRNA molecules of TE sequences hitchhike the uptake pathway,  
166 and are opportunistically imported within haemocytes along with viral dsRNAs. This would  
167 then allow the enhancement of TE-derived small RNA production and explain the observed  
168 systemic increase of TE-derived small RNAs, which leads to a decrease in TE transcript  
169 amounts (18). Here, we found a global increase of both TE RNAs and TE-derived small  
170 RNAs in *D. melanogaster* *w<sup>1118</sup>* carcasses. We propose that such a discrepancy is due to 1A,  
171 the VSR encoded by DCV (no VSR has been described for SINV). In the tissues where DCV  
172 is expressed, 1A is produced and binds long dsRNAs, however without any sequence  
173 specificity (24, 25, 29). Therefore, 1A mostly binds viral dsRNAs, but also other dsRNAs  
174 such as those produced from TE sequences, either due to antisense transcripts annealing to  
175 sense RNAs or to single-stranded transcripts folding back on repeated regions. Eventually,  
176 the production of TE-derived siRNAs is prevented, resulting in increased TE RNA amount.  
177 This is in agreement with the strong increase in TE transcript amounts that we previously  
178 observed in *dcr2* mutants upon SINV infection (18). Therefore, we propose that 1A is  
179 responsible for the decorrelation between TE RNA and siRNA amounts observed above. TE-  
180 derived small RNA amounts would thus result from these two mechanisms with opposed  
181 effects: the activation of the dsRNA uptake pathway leads to piRNA and siRNA increase

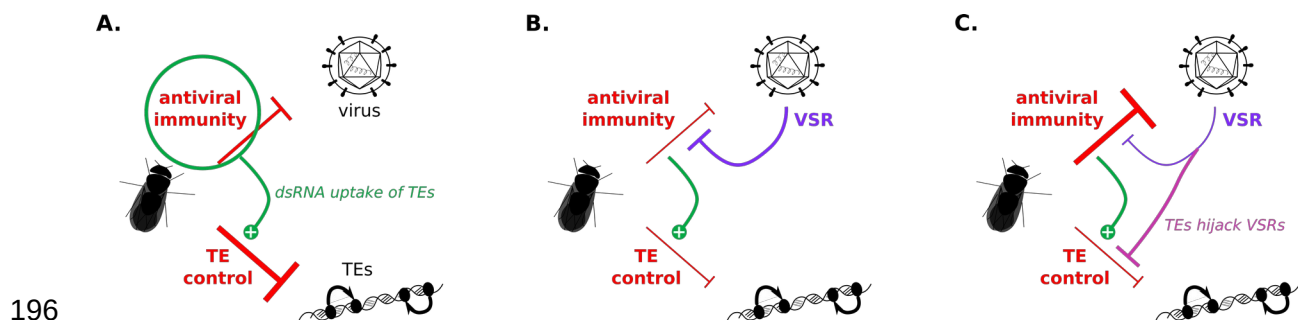
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182 while the action of 1A leads to the reduction of siRNA production. Indeed, since 1A  
183 specifically binds dsRNA molecules, it is expected to interfere mainly with siRNA production,  
184 which explains the resulting increase in small RNAs is lower for siRNAs compared to  
185 piRNAs. In the case of the previously studied SINV infection, the tripartite interaction  
186 between host, virus and TEs is simpler and only results from TEs hitchhiking the dsRNA  
187 uptake pathway. Here, the production of a VSR adds another layer of interaction. This ends  
188 up in an intricate arms race between the virus, the host and its TEs (Fig. 3). Moreover, we  
189 propose that this explains the virtual absence of any TE phenotype in *D. simulans* Makindu.  
190 Indeed, the RNA-seq data reveal that the number of reads mapping against the sequence  
191 corresponding to 1A is the highest in *D. melanogaster w<sup>1118</sup>* carcasses, and very low in  
192 ovarian samples. In carcasses, the number of reads mapping against 1A is significantly  
193 higher in *D. melanogaster w<sup>1118</sup>* compared to *D. simulans* Makindu (Fig 1C). These species-  
194 specific differences may be due to contrasted efficiencies in the production of 1A.

195



196

197 **Figure 3. Reciprocal impacts of TE control and antiviral immunity.** **A.** The fly host fights  
198 against viruses and TEs. Using SINV, we recently demonstrated that antiviral immunity  
199 enhanced TE control *via* the dsRNA uptake pathway. **B.** However, some viruses encode  
200 VSRs that inhibit antiviral immunity, such as the 1A protein produced by DCV. **C.** Here we  
201 propose that TEs hijack VSRs, which allows the release of TE control.

202

## 203 TE upregulation leads to reduced DCV titers

204 On the other side, we wondered whether TEs could modify DCV titers. Such a study is rather  
205 difficult because it requires to make TE transcript amounts vary within the same genetic  
206 background. We thus used a sophisticated transgenic *D. melanogaster* strain, which  
207 displays an inducible *piwi* knock-down in the somatic cells surrounding the ovary (30–32)

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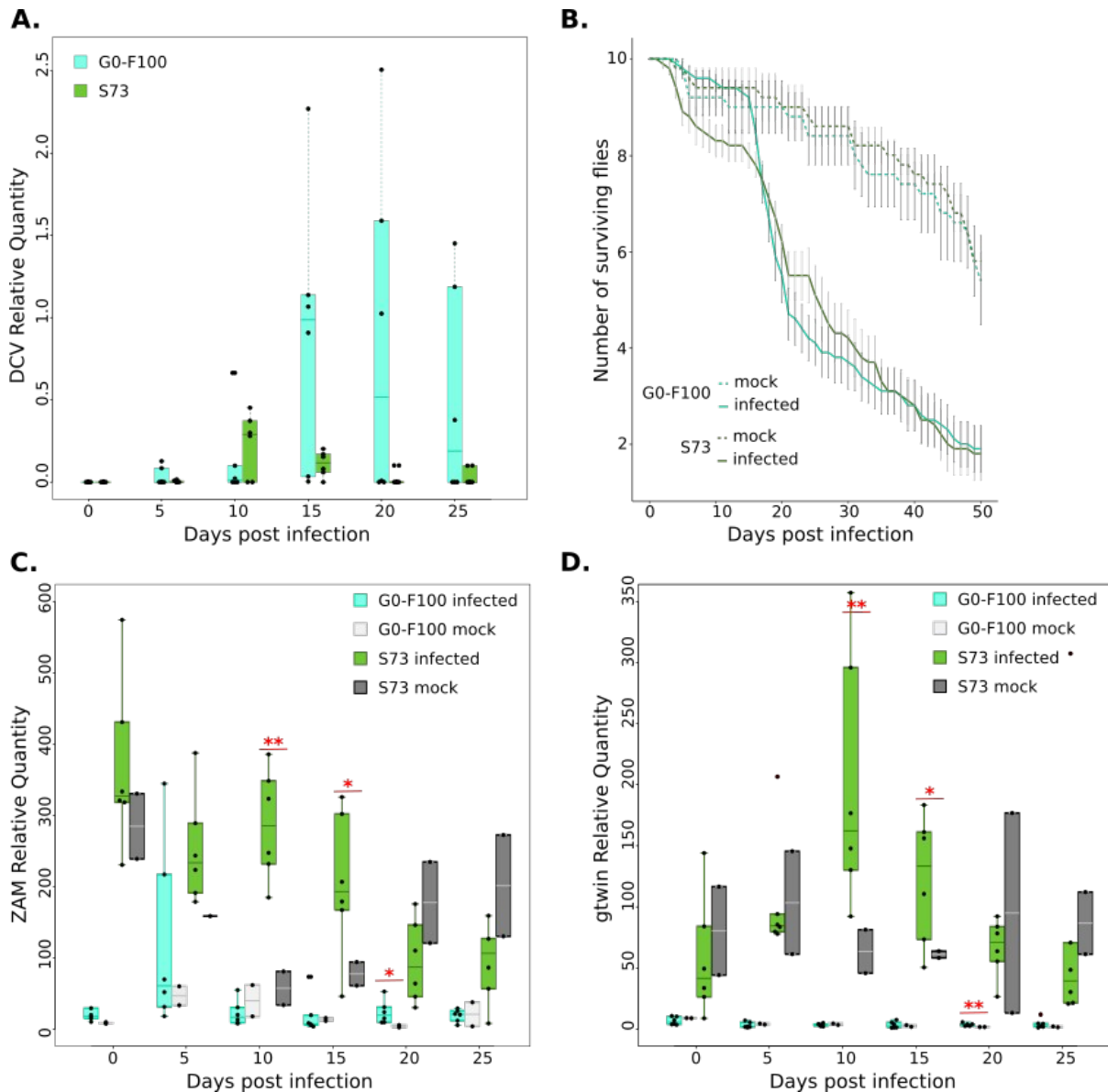
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208 [constitutive *piwi* knock-down leads to fly sterility and thus prevents from obtaining progeny  
209 (33, 34)]. The repetitive, punctual knock-down of *piwi* along 73 successive generations –  
210 hereafter named S73, S for shift in temperature causing *piwi* knock-down– led to the  
211 accumulation of TE copies from the ZAM and gtwin families (31, 32). It was accompanied by  
212 an increase in transcript amounts for these TE families, as compared to G0-F100 –no  
213 temperature shift corresponding to the original strain– whereas gene expression remained  
214 largely unaffected (Fig S13). It has to be noted that S73 is not an isogenic strain. Instead,  
215 flies are raised as large cohorts along generations, ensuring the lowest genetic drift, and  
216 resulting in pools of flies displaying distinct, low frequency TE insertions within the same  
217 genetic background. Upon intra-thoracic injection, we found that DCV replicated earlier but  
218 at strongly reduced levels in S73 compared to G0-F100 (DCV\_relative\_quantity ~ dpi +  
219 infection\_status + strain; mean strain difference = 0.23; strain effect p-value = 0.004) (Fig.  
220 4A). Fifteen days after injection, mean DCV RNA levels were 8.1-fold higher in G0-F100. Fly  
221 death rates were higher in S73 at the beginning of the infection, but the curves inverted  
222 around day 15 (log-rank test, p-value = 0.887) (Fig. 4B). Using RT-qPCR, we followed ZAM  
223 and gtwin somatic transcript amounts in both strains and both conditions (Fig. 4C and 4D).  
224 Similar to the global increase in TE transcript amounts observed in *D. melanogaster w<sup>1118</sup>*,  
225 we could detect that ZAM and gtwin transcript amounts in S73 are significantly higher upon  
226 DCV infection compared to mock samples around the peak of DCV titer.

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228

229 **Figure 4. Response to DCV infection in S73 and G0-100.** A. DCV titer in G0-F100 and  
230 S73 measured using RT-qPCR. B. Fly survival upon DCV and mock infections in G0-F100  
231 and S73. C. ZAM transcript quantification upon DCV and mock infection in G0-F100 and  
232 S73. Stars indicate significant differences between infected and mock conditions using t-  
233 tests (p-value: 0.05 \* 0.01 \*\*). D. gtwin transcript quantification upon DCV and mock infection  
234 in G0-F100 and S73. Stars indicate significant differences between infected and mock  
235 conditions using t-tests (p-value: 0.05 \* 0.01 \*\*).

236

237 We propose that the higher TE transcript amounts in S73 produce increased amounts of  
238 dsRNA molecules, which can titrate 1A. The reduction in 1A availability removes DCV  
239 protection against the fly RNAi machinery; this allows the host to produce more siRNAs  
240 against the virus and therefore set up a more efficient RNAi response. This ends up in

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241 reduced viral loads (Fig. 3).

242 Altogether, our results suggest that a higher TE load could be beneficial in case of viral  
243 infection. At a larger evolutionary scale, such results may shed a new light on *Aedes*  
244 mosquitoes, which are known as major vectors of arboviruses –which cause no harm to  
245 them–, and are described to carry a large TE load (ca half of their genome). Finally, our  
246 results uncover TEs as major players of a complex host-pathogen interaction built along  
247 long-lasting coevolution.

248

## 249 **Material and Methods**

### 250 **Drosophila strains and rearing conditions**

251 *D. simulans* Makindu strain was previously described by Akkouche *et al.* (35) and Fablet *et*  
252 *al.* (36). G0-F100 and S73 transgenic *D. melanogaster* flies were previously described by  
253 Barckmann *et al.* (30) and Mohamed *et al.* (31). All experiments were performed using 3-6  
254 day-old mated females.

255 Flies were reared on corn medium and maintained under standard laboratory conditions:  
256 12/12 L/D cycle, 25°C (or 18°C for G0-F100 and S73, in order not to induce *piwi* knock-  
257 down) and 70% RH. Chronic viral infections were eliminated by bleaching the eggs, as  
258 described previously (37), except for G0-F100 and S73. Three to 6-hour-old eggs were  
259 incubated in 50% household bleach (2.6% active chlorine) for 10 minutes, washed three  
260 times for 5 minutes in deionised water and then transferred to fresh medium for adult  
261 emergence. As expected after the treatment, we could not detect amplification  
262 corresponding to the viruses using RT-PCR (SuperScript™ IV VILO™ Master Mix (without  
263 ezDNase enzyme treatment) kit and DreamTaq DNA polymerase) and the following primers:  
264 *Drosophila A virus* (DAV): 5'- AGGAGTTGGTGAGGACAGCCCA -3' and 5'-  
265 AGACCTCAGTTGGCAGTTCGCC -3', Nora Virus (NV): 5'-  
266 ATGGCGCCAGTTAGTGACAGACCT -3' and 5'- CCTGTTGTTCCAGTTGGGTTCTGA -3',  
267 *Drosophila melanogaster* Sigma Virus (DmelSV): 5'- ATGTAACCTCGGGTGTGACAG -3' and  
268 5'- CCTTCGTTTCATCCTCCTGAG -3) (37). *rp49* was used as control (5'-  
269 CGGATCGATATGCTAAGCTGT -3' and 5'- GCGCTTGTTCGATCCGTA -3') (38). To  
270 eliminate the *Wolbachia* endosymbiotic bacteria, 3- to 6-hour-old eggs were collected and

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271 placed on fresh standard medium containing 0.25 mg/mL tetracycline hypochloride (Sigma-  
272 Aldrich), for all *Drosophila* strains. The treatment was performed during two generations and  
273 then three generations recovered on standard medium without treatment. We validated the  
274 absence of amplification by PCR using *Wolbachia* 16S primers (5'-  
275 TTGTAGCCTGCTATGGTATAACT -3' and 5'- GAATAGGTATGATTTTCATGT -3') (39),  
276 *Wolbachia* *wsp* (5'- TGGTCCAATAAGTGATGAAGAAAC -3' and 5'-  
277 AAAAATTAACGCTACTCCA -3') (40) and *FtsZ* (5'- CGAGATGGGCAAAGCGATGA -3'  
278 and 5'- ATTCCTTGCGCACCTTTCAT -3' (41)).

## 279 Virus production and quantification

280 DCV was produced and titrated in Schneider's *Drosophila* Line DL2 cells, both kindly  
281 provided by Luis Teixeira/Ewa Chrostek. DL2 cells were kept in Schneider's *Drosophila*  
282 Medium supplemented with 10% Fetal Bovine Serum, 1% penicillin-streptomycin 10,000  
283 U/mL (all Gibco). Seven days after infection (MOI: 2) in Schneider's *Drosophila* Medium  
284 supplemented with 1% penicillin-streptomycin 10,000 U/mL, the cell culture was collected  
285 and frozen at -80°C for 40 min. The culture was thawed, frozen again at -80°C for 40 min,  
286 and thawed to disrupt cells, and centrifuged at 4000 g for 10 min to remove cell debris. The  
287 supernatant was aliquoted and stored at -80°C. DCV titer was calculated by the Reed and  
288 Muench end-point calculation method: DL2 confluent cells in 96-well plates were infected  
289 with a serial 10-fold dilutions of virus suspension. The presence of active DCV was scored  
290 by cell death or clear cytopathic effects, resulting in a DCV titer of  $4.22 \times 10^9$  TCID<sub>50</sub>/mL.  
291 Similar dilutions with extracts of DL2 cells that were not inoculated with DCV did not cause  
292 any cytopathic effect in culture (42).

## 293 Virus inoculation and survival assays

294 CO<sub>2</sub>-anesthetized flies were pricked in the left pleural suture on the thorax with a 0.15 mm  
295 diameter anodized steel needle dipped into the DCV solution, as described in Martinez *et al.*  
296 (26). Flies were pricked with the 'mock' solution –extract from uninfected DL2 cells– to  
297 control for the absence of mortality in absence of DCV infection. For the survival assays, 100  
298 infected females and 50 'mock' females were kept in rich medium vials, 10 flies per vial.  
299 Flies were transferred to fresh media every 3-4 days. The number of dead flies was recorded  
300 each day after infection.

301 It has to be noted that infection experiments were performed at 25°C for *D. simulans*  
302 Makindu and *D. melanogaster* *w*<sup>1118</sup> but at 18°C for *D. melanogaster* G0-F100 and S73 in

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303 order not to induce *piwi* knock-down (30). This difference in temperature leads to differences  
304 in DCV replication kinetics and in lifespan across these experiments. Accordingly, each  
305 infected sample should be analyzed in comparison with the corresponding mock sample  
306 obtained in the exact same experimental conditions.

## 307 RNA extraction and RT-qPCR

308 To quantify DCV and TE expression, total RNAs were extracted individually for 6 infected  
309 and 2 mock flies at 0, 2, 4, 6 and 8 dpi using Qiazol and the RNeasy mini kit (Qiagen).  
310 Purified RNAs were processed using Turbo DNase (Ambion DNafree kit). Reverse  
311 transcription was performed on 4 µL of total RNAs using SuperScript IV VILO Master Mix  
312 (Invitrogen). qPCR was performed using 2 µL of cDNA, 0.5 µL of each primer (DCV : 5'-  
313 GACACTGCCTTTGATTAG -3' and 5'- CCCTCTGGGAACTAAATG -3', ZAM : 5'-  
314 CTACGAAATGGCAAGATTAATTCCAATTCC -3' and 5'-  
315 CCCGTTTCCTTTATGTCGCAGTAGCT -3', gtwin : 5'- TTCGCACAAGCGATGATAAG -3'  
316 and 5'- GATTGTTGTACGGCGACCTT -3') and 5 µL of SsoADV SYBR® Green Supermix  
317 (Bio Rad) in a QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems™) and  
318 following : 30 sec at 95 °C; 40 cycles of 95 °C for 15 sec and 58 °C for 30 sec. For  
319 standardization, we tested the *rp49*, *Adh*, and *EF1* genes, and kept *rp49* for further  
320 experiments because it displayed the highest stability across all conditions.

## 321 RNA-seq

322 Thirty pairs of ovaries were carefully and manually dissected and separated from the rest of  
323 the bodies ('carcasses') at 4 dpi. Total RNAs from 30 pairs of ovaries or 30 carcasses were  
324 extracted according to the protocol described above, using Qiazol, the RNeasy Mini Kit  
325 (Qiagen) and TurboDNase (Ambion DNafree kit) on the total amount of eluate to eliminate  
326 residual DNA. RNA quality was validated using a Bioanalyzer (Agilent). Library preparation  
327 was performed at the GenomEast platform at the Institute of Genetics and Molecular and  
328 Cellular Biology (IGBMC, France) using Illumina Stranded mRNA Prep Ligation - Reference  
329 Guide - PN 1000000124518. Libraries were sequenced as paired-end 100 base reads  
330 (HiSeq 4000: Illumina).

331 For S73 and G0-100 samples, total RNA was extracted from 3-16 h embryos using TRIzol. 4  
332 mg of total RNA was subjected to Ribo-Zero ribosomal depletion. RNA was further purified  
333 using RNA Clean & Concentrator-5. Libraries were prepared using the Illumina Stranded  
334 mRNA Prep Ligation kit and 50 nt paired-end read sequencing were performed by MGX

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29

335 sequencing services (Montpellier, France) on a SP flow cell using Novaseq 6000. The RNA-  
336 seq experiments were performed on three biological replicates.

337 Read alignment was performed using Hisat2 (43) on *D. melanogaster* and *D. simulans*  
338 reference genes available from FlyBase, versions r6.16 and r2.02, respectively, and  
339 previously masked using RepeatMasker. The reference sequence used for DCV was  
340 NC\_001834.1 from GenBank. Alignments were processed using SAMtools (44). Gene count  
341 tables were generated using eXpress (45). TE count tables were obtained using the TEcount  
342 module of TETools (46) and the same TE reference files as used in (18), and corresponding  
343 to 237 TE families. Gene and TE count tables were concatenated and then analyzed using  
344 the DESeq2 R package (version 4.3) (47). Four complete count tables were produced  
345 (*{D. melanogaster carcasses}*, *{D. melanogaster ovaries}*, *{D. simulans carcasses}*, and  
346 *{D. simulans ovaries}*). Each complete table was then analyzed by the DESeq2 procedure,  
347 ensuring that TE counts are normalized the same way as gene counts. The gene ontology  
348 (GO) analysis of RNA-seq data was performed on the differentially expressed genes with the  
349 gseGO function of the clusterProfiler package (48, 49). The GO terms were obtained using  
350 the R package org.Dm.eg.db v3.10.0 as database (50), corresponding to all *D. melanogaster*  
351 annotated genes. We converted *D. simulans* genes into their *D. melanogaster* orthologs  
352 using the orthology file from FlyBase  
353 [ftp://ftp.flybase.net/releases/FB2020\\_04/precomputed\\_files/orthologs/  
354 dmel\\_orthologs\\_in\\_drosophila\\_species\\_fb\\_2020\\_04.tsv.gz](ftp://ftp.flybase.net/releases/FB2020_04/precomputed_files/orthologs/dmel_orthologs_in_drosophila_species_fb_2020_04.tsv.gz). The GO overrepresentation  
355 analysis of biological process (BP) was performed with adjusted p-values by the FDR  
356 method, p-value cutoff at 0.05, and a minimum of 3 genes per term.

## 357 small RNA-seq

358 Ovaries of each female fly were carefully and manually dissected at 4 dpi. Small RNAs from  
359 30 pairs of ovaries or 30 carcasses were extracted using the TraPR (Trans-kingdom, rapid,  
360 affordable Purification of RISCs) small RNA Isolation kit (Lexogen), as described previously  
361 (51). The TraPR method allows the specific isolation of fully functional and physiologically  
362 relevant interfering RNAs (microRNAs, piRNAs, siRNAs and scnRNAs), by anion exchange  
363 chromatography. Size selection (18-40 nt) was performed on gel at the sequencing platform.  
364 Purified small RNAs were used for library preparation, using TruSeq Small RNA Sample  
365 Preparation Guide - PN 15004197. Libraries were sequenced on an Illumina HiSeq 4000  
366 sequencer as single-end 50 base reads.

367 Sequencing adapters were removed using cutadapt (52) -a

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368 TGG AATTCTCGGGTGCCAAGGAACTCCAGTCACTTA -m 1. Size filtering was performed  
369 using PRINTSEQ (53), in order to distinguish 21 nt-long reads (considered as siRNAs) from  
370 23-30 nt-long reads (considered as piRNAs).

371 TE count tables for sense and antisense alignments were obtained using a modified version  
372 of the TEcount module (36) of TETools (46) on the same TE reference sequences as above.  
373 Ping-pong signatures were checked using signature.py with min\_size = 23 and max\_size =  
374 30 options (54). Read count normalization using microRNA counts as supposed invariants is  
375 a common strategy. However, it has been shown that some microRNA amounts could be  
376 affected by DCV infection (55). This is why we performed three different normalization  
377 strategies : 1) normalization using all microRNA sequences annotated from FlyBase (19-39  
378 nt-long reads mapped using bowtie --best on FlyBase reference sequences dmel-all-miRNA-  
379 r6.16.fasta and dsim-all-miRNA-r2.02.fasta), 2) normalization using microRNA sequences  
380 but excluding those described to vary upon DCV infection in Monsanto-Hearnnes *et al.* (55),  
381 3) normalization using the endo-siRNAs described by Malone *et al.* (56), as we previously  
382 did in Roy *et al.* (18)). However, this last normalization procedure is only possible in  
383 *D. melanogaster* because endo-siRNA producing loci are not annotated in *D. simulans*. All  
384 normalization strategies provided similar patterns (Fig S8-S10).

385

## 386 Data availability

387 The RNA-seq and small RNA-seq data generated in this study have been submitted to the  
388 NCBI BioProject database under accession number PRJNA996035.

389

## 390 Acknowledgments

391 This work was performed using the computing facilities of Laboratoire de Biométrie Biologie  
392 Evolutive / Pôle Rhône-Alpes de bioinformatique (CC LBBE/PRABI) and the Symbiotron  
393 Pasteur platform, Laboratoire Biologie Fonctionnelle Insectes et Interactions (BF2i, Lyon).  
394 Sequencing was performed by the GenomEast platform, a member of the 'France  
395 Génomique' consortium (ANR-10-INBS-0009). We thank Matthieu Boulesteix, Miriam  
396 Merenciano, Cristina Vieira for helpful discussions. We thank Nelly Burlet, Sonia Janillon,

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397 Corinne Régis, Zainab Belgaidi, Carole Monégat, and Agnès Vallier for technical help. We  
398 thank also Clément Gilbert, Anna Zaidman-Rémy, and Feth el Zahar Haichar for helpful  
399 discussions. This work was funded by Agence Nationale de la Recherche (TEMIT grant) to  
400 M.F. and by the Agence Nationale de la Recherche (ANR-19-CE12-0012 -Top53 grant) to  
401 S.C.. M.V. was funded by CNRS-Japon PhD Joint Program.

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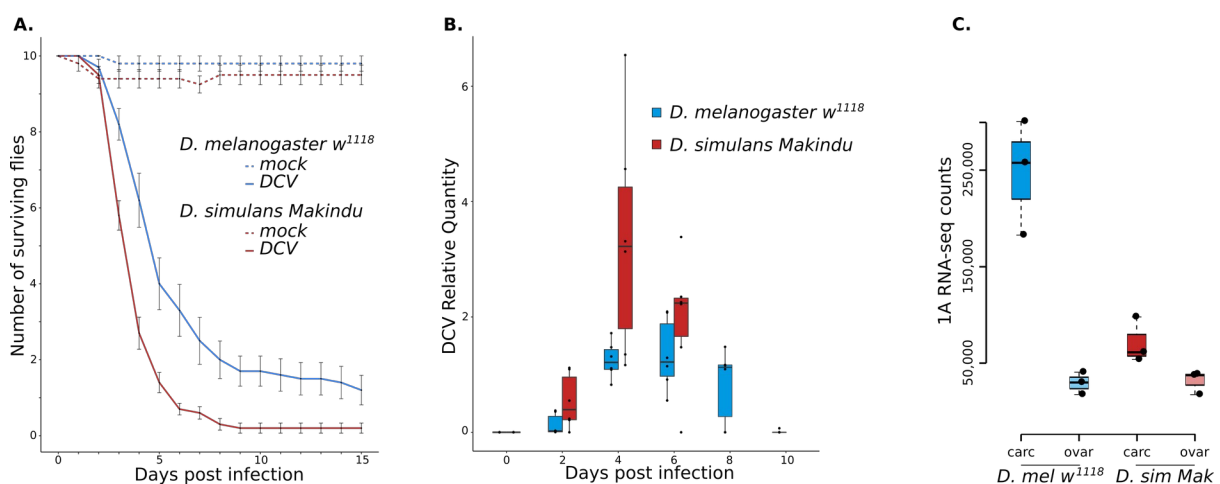
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404

## 405 Figures

405



407 **Figure 1. Response to DCV infection in *D. melanogaster w<sup>1118</sup>* and *D. simulans***  
408 **Makindu. A.** Fly survival upon DCV infection. **B.** Kinetics of DCV titers followed using RT-  
409 qPCR. **C.** Raw RNA-seq read counts mapping against 1A sequence. RNA-seq read

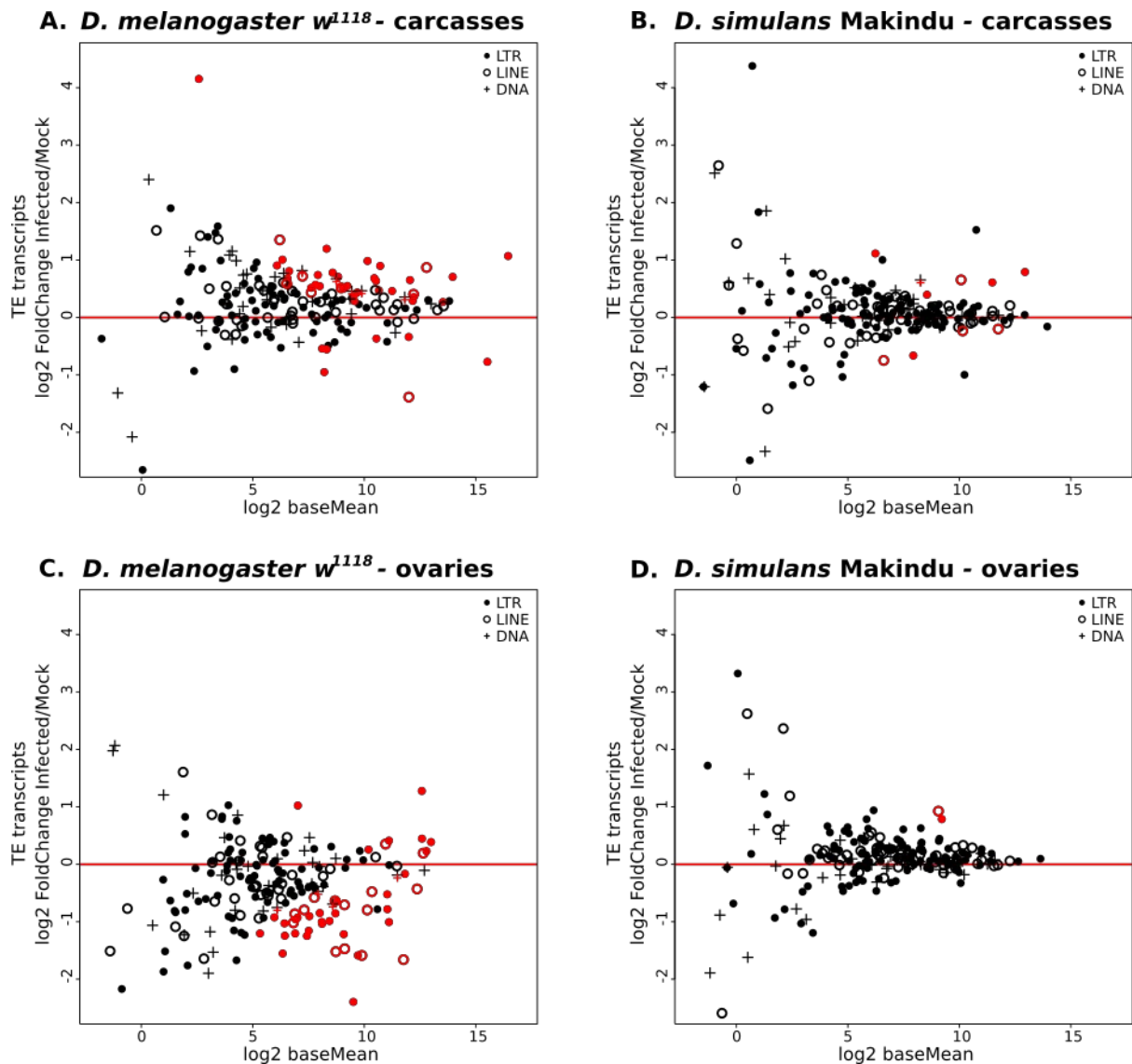
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410 mapping along the sequence of DCV genome is shown in Fig S1.

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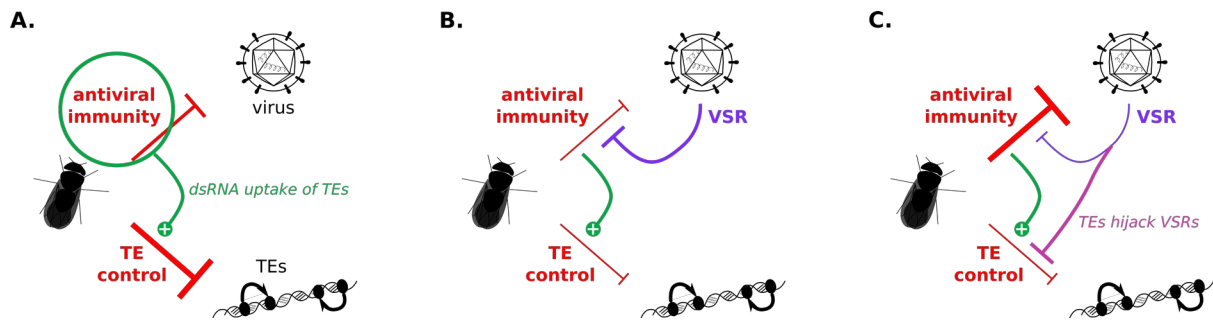
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413 **Figure 2. TE transcript modulation upon DCV infection.** A. TE transcripts log<sub>2</sub>FC  
414 between DCV infected and mock conditions. Dot shapes indicate TE classes, red line is  
415 log<sub>2</sub>FC=0, *i.e.* no modulation. Red dots are TE families displaying significant differential  
416 expression at the 0.05 level for DESeq2 adjusted p-values. A. *D. melanogaster w<sup>1118</sup>*  
417 carcasses, B. *D. simulans Makindu* carcasses, C. *D. melanogaster w<sup>1118</sup>* ovaries, D.  
418 *D. simulans Makindu* ovaries.

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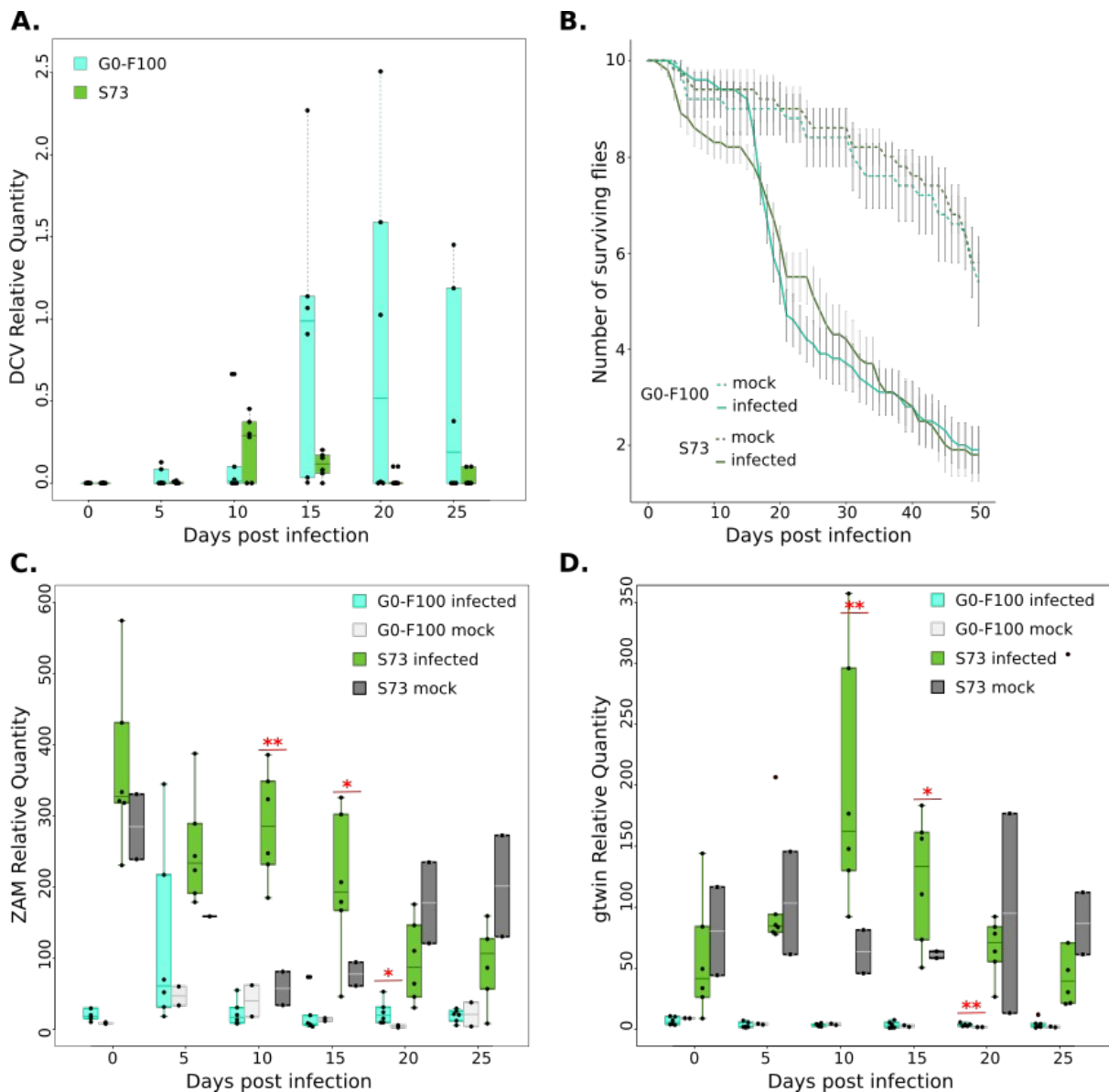


421

422 **Figure 3. Reciprocal impacts of TE control and antiviral immunity.** **A.** The fly host fights  
423 against viruses and TEs. Using SINV, we recently demonstrated that antiviral immunity  
424 enhanced TE control *via* the dsRNA uptake pathway. **B.** However, some viruses encode  
425 VSRs that inhibit antiviral immunity, such as the 1A protein produced by DCV. **C.** Here we  
426 propose that TEs hijack VSRs, which allows the release of TE control.

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429 **Figure 4. Response to DCV infection in S73 and G0-100.** **A.** DCV replication in G0-F100  
430 and S73 measured using RT-qPCR. **B.** Fly survival upon DCV and mock infections in G0-  
431 F100 and S73. **C.** ZAM transcript quantification upon DCV and mock infection in G0-F100  
432 and S73. Stars indicate significant differences between infected and mock conditions using t-  
433 tests (p-value: 0.05 \* 0.01 \*\*). **D.** gtwin transcript quantification upon DCV and mock infection  
434 in G0-F100 and S73. Stars indicate significant differences between infected and mock  
435 conditions using t-tests (p-value: 0.05 \* 0.01 \*\*).

436