

Additional File – Supplemental Information:

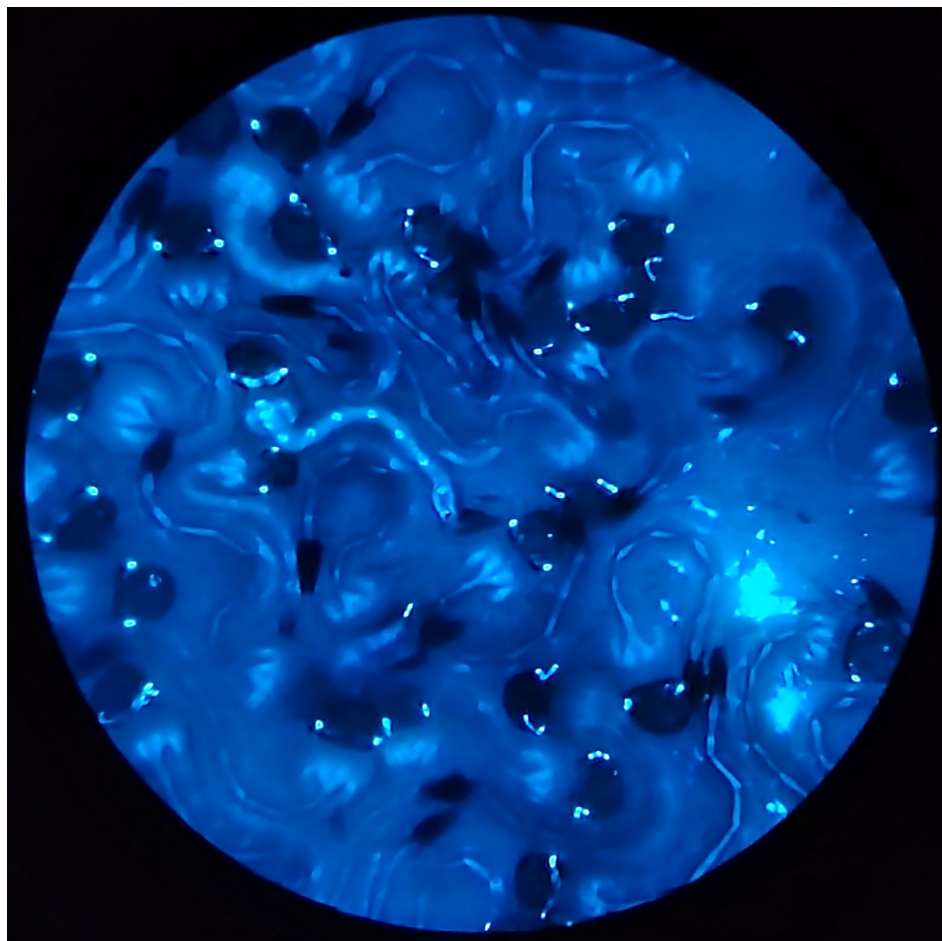
Supplemental Figure S1: ECFP eye marker expression in $\Delta R2D2^{+/-}$ L2/L3 (G1) larvae of *Ae. aegypti*.

Supplemental Figure S2: Effects of monoallelic *r2d2* KO on mosquito fecundity and fertility.

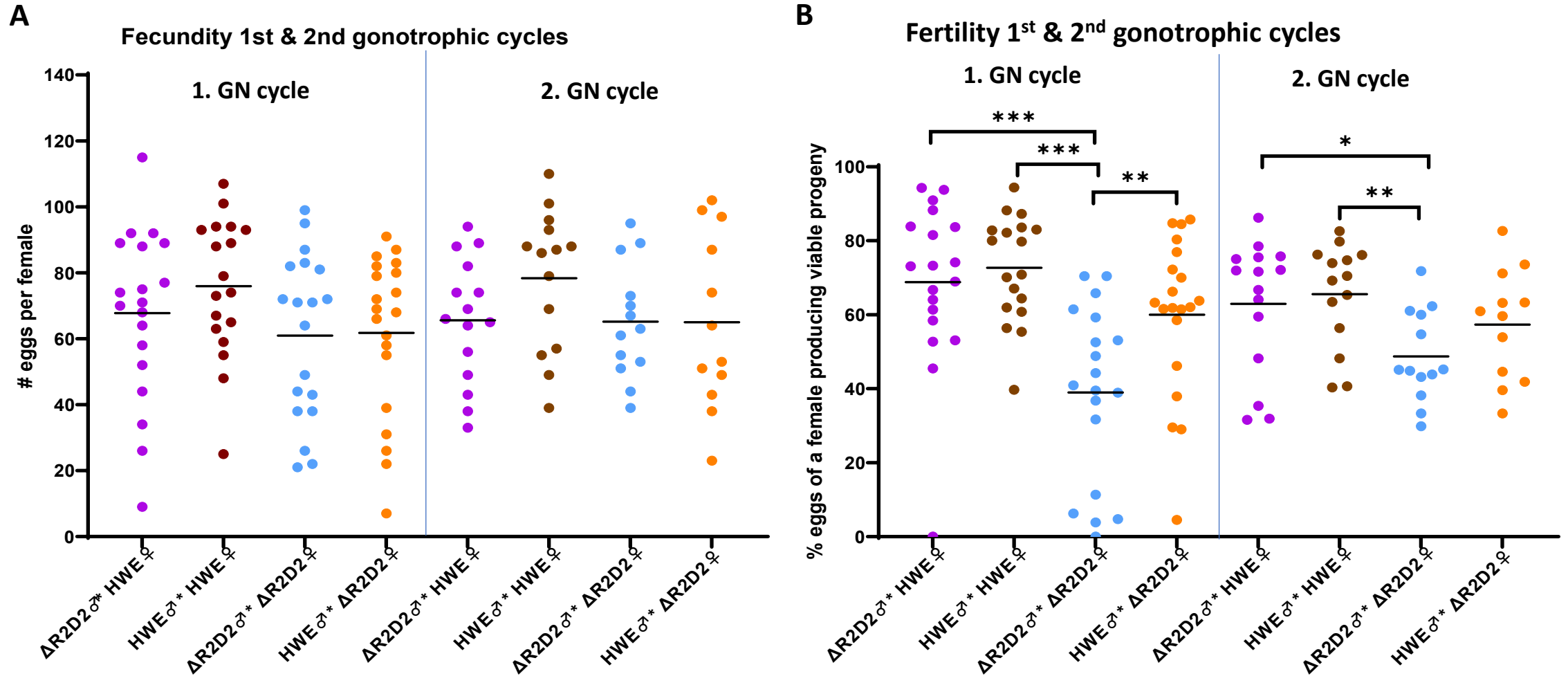
Supplemental Figure S3: Relative *dcr2* expression levels in MAYV infected $\Delta R2D2^{+/-}$ and HWE females at 4 hpi, 4 dpi, and 10 dpi.

Supplemental Figure S4: Relative mCherry expression levels in MAYV infected $\Delta R2D2^{+/-}$ females as analyzed by RT-qPCR.

Supplemental Table S1. List of oligonucleotide primers used for the study.

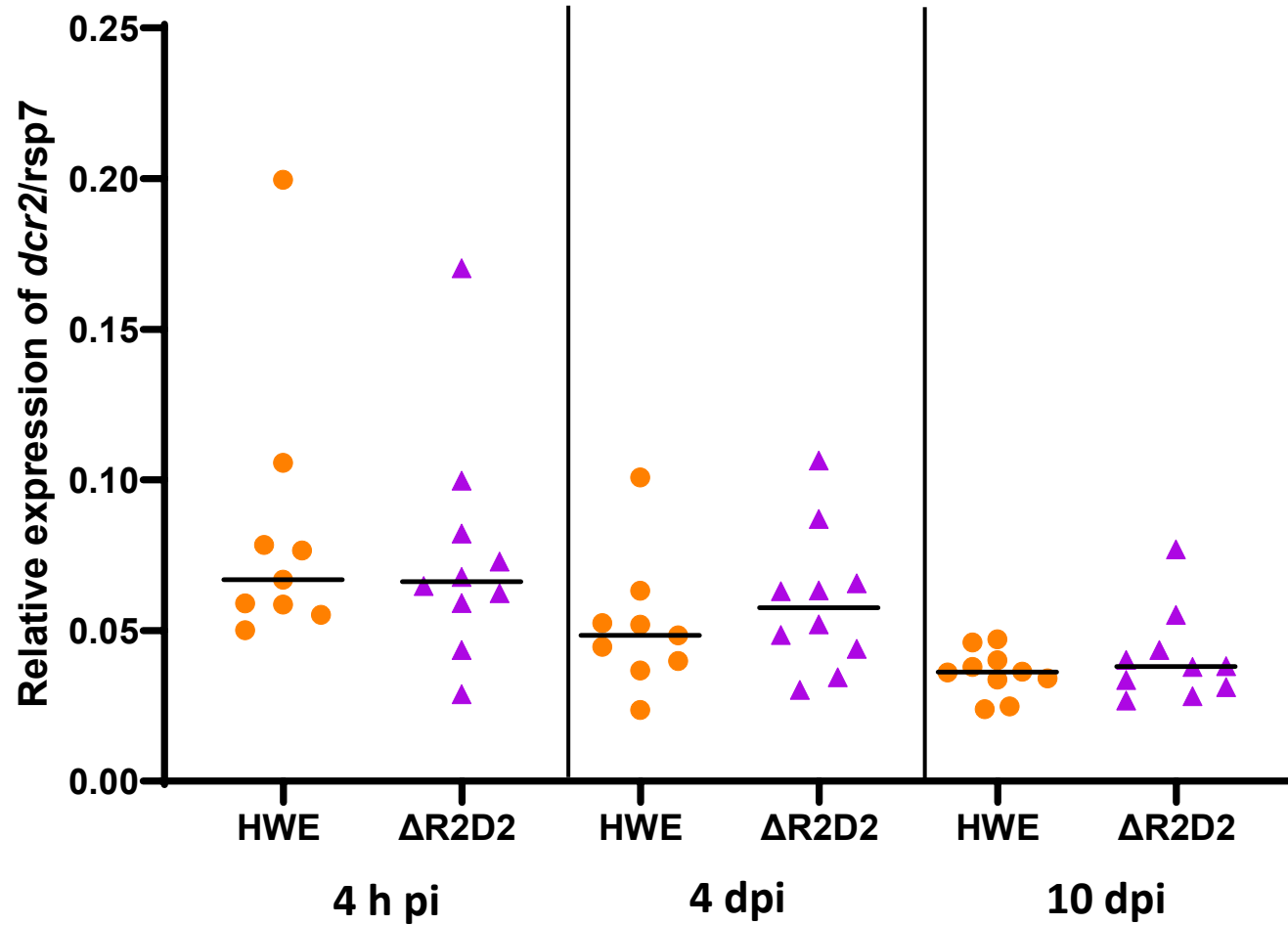


Supplemental Figure S1: ECFP eye marker expression in $\Delta R2D2^{(+/-)}$ L2/L3 (G1) larvae of *Ae. aegypti* (M2, bloodfeed #1; see **Table 1**) resulting from male G0 survivors (harboring the “ $\Delta R2D2$ -U6” donor plasmid), which had been backcrossed to the HWE recipient strain of *Ae. aegypti*.

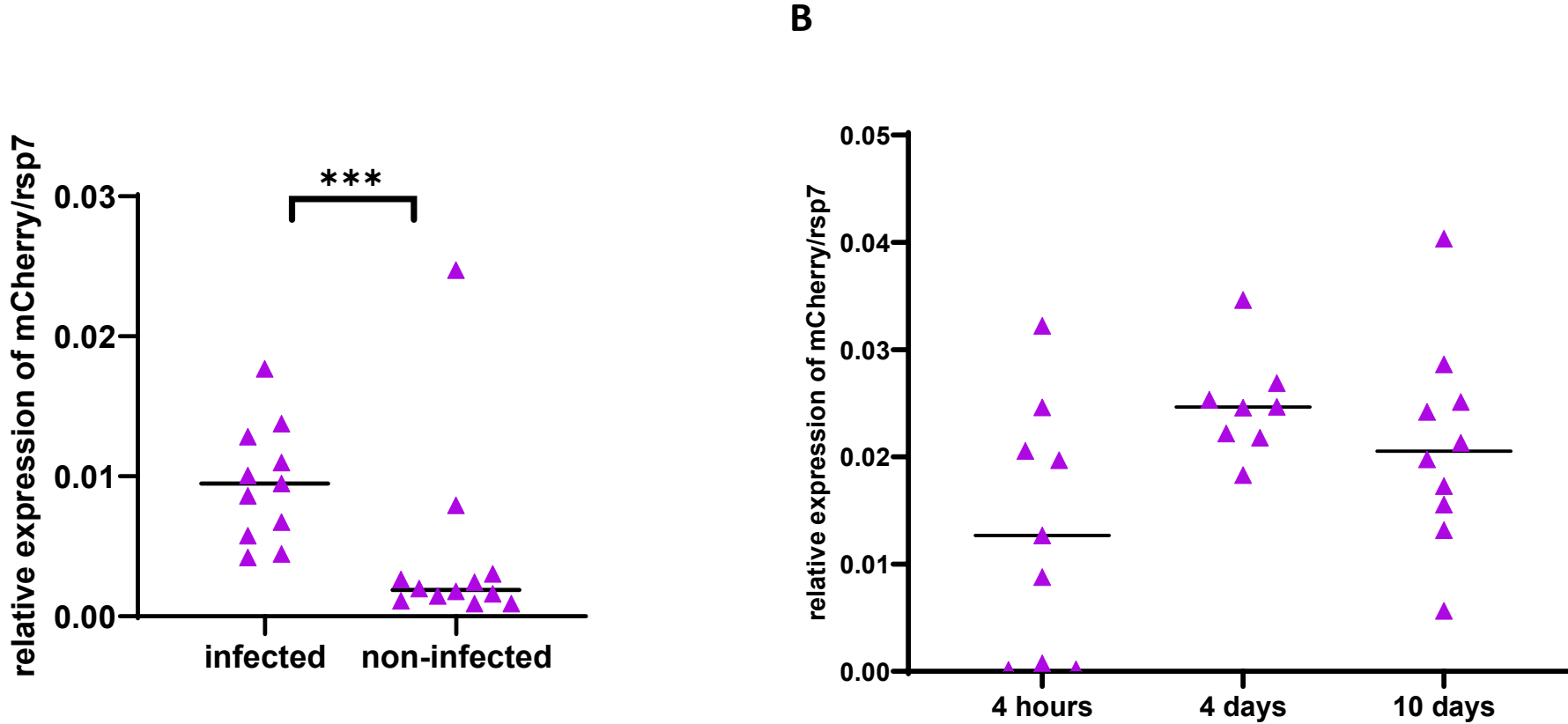


Supplemental Figure S2: Effects of monoallelic *r2d2* KO on mosquito fecundity and fertility. **(A)** Fecundity over two gonotrophic cycles of individual $\Delta R2D2^{+/-}$ and HWE females (n=20) when outcrossed or intercrossed. Each data point represents the number of eggs per gonotrophic cycle produced by a single female. **(B)** Fertility over two gonotrophic cycles of individual $\Delta R2D2^{+/-}$ and HWE females (n=20) when outcrossed or intercrossed. Each data point represents the relative quantity of pupae developed from all eggs a single female produced during the first or second gonotrophic cycle. Bars represent mean values. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.0001$; Mann-Whitney U-test.

dcr2 expression time course in MAYV infected mosquitoes



Supplemental Figure S3: Relative *dcr2* expression levels in MAYV infected $\Delta R2D2^{+/-}$ and HWE females at 4 hpi, 4 dpi, and 10 dpi (virus titer in the bloodmeal: $\sim 10^7$ PFU/mL). Each data point represents a whole-body female. Expression levels of *dcr2* were normalized to the average expression of *rsp7*. Bars indicate median values. Statistical test: Mann-Whitney U-test.



Supplemental Figure S4: Relative mCherry expression levels in MAYV infected $\Delta R2D2^{+/-}$ females as analyzed by RT-qPCR (**A**) at 4 dpi with MAYV; (**B**) at 4 hpi, 4 dpi, and 10 dpi with MAYV. Each data point represents a whole-body female, which had acquired a MAYV containing bloodmeal (titer in the bloodmeal: $\sim 10^7$ PFU/mL) or a similar bloodmeal containing no virus. mCherry expression levels were normalized to the average expression of *rsp7*. Bars indicate median values. *** = $p = 0.0010$; Mann-Whitney U-test.

Supplemental Table 1. List of oligonucleotide primers used for the study

Primer name	Purpose	Primer sequence 5'-3'	Amplicon
MAYV_RTq_F	MAYV detection	ATAGACGACCTGCAGTCA	196 bp
MAYV_RTq_R	MAYV detection	TGATAGACTGCCACCTCT	196 bp
r2d2_RTq_F	r2d2 expression	GTCCGCCGGGAATTCACAAGA	170 bp
r2d2_RTq_R	r2d2 expression	GTAGGTCCGTATGATGTGC	170 bp
rsp7_RTq_F AAEL009496	rsp7 house-keeping gene	GCAGACCACCATGAACACA	64 bp
rsp7_RTq_R AAEL009496	rsp7 house-keeping gene	CACGTCCGGTCAGCTTCTTG	64 bp
mCherry_RTq_F	mCherry reporter	CTGAGGTCAAGACCACCTACAAG	114 bp
mCherry_RTq_R	mCherry reporter	GTACTGTTCCACGATGGTGTAGTC	114 bp
dcr2_RTq_F	dcr2 expression	CATCTACTTGCCGACCGGAG	153 bp
dcr2_RTq_R	dcr2 expression	GAATTCGGCCTGCTGCTTAG	153 bp
r2d2_dsRNA_F	dsRNA production	<u>TAATACGACTCACTATAGGGG</u> GCAAGAG CTGTGTGTGTCCA*	450 bp
r2d2_dsRNA_R	dsRNA production	<u>TAATACGACTCACTATAGGGG</u> AATCATCTC GTTGCACGCTG	450 bp

*underlined: T7 RNA promoter sequence.