

Supplementary Materials: The RNAi pathway is important to control Mayaro virus infection in *Aedes aegypti* but not for *Wolbachia*-mediated protection

Table S1. Oligonucleotides sequences.

Target	Description	Sequence (5'–3')	Application	Reference
RPL32 AAEL003396	RpL32 Fw	AGCCGCGTGTGTACTCTG	qPCR	[36]
	RpL32 Rv	ACTTCTTCGTCCGCTTCTTG		
MAYV	MAYV Fw	GTGGTCGCACAGTGAATCTTTC	qPCR	[10]
	MAYV Rv	CAAATGTCCACCAGGCGAAG		
Firefly luciferase U47295	FLUC Fw (T7)	taatacgactcactataggagaAACAATCCGGAAGCGACCAA	dsRNA synthesis	
	FLUC Rv (SP6)	attaggtgacactatagaagtGTACTGGCGACGTAATCCAC		
AGO2 AAEL017251	AGO2 Fw	AGATTGACAAGCAGAAAATCCAC	qPCR	[36]
	AGO2 Rv	CATTGGACGCATCAGCA		
	AGO2 Fw (T7)	taatacgactcactataggagaCAGTTCAAGCAGACGAACCA	dsRNA synthesis	
	AGO2 Rv (SP6)	attaggtgacactatagaagTGATGTAGACGCGTCCTCTG		

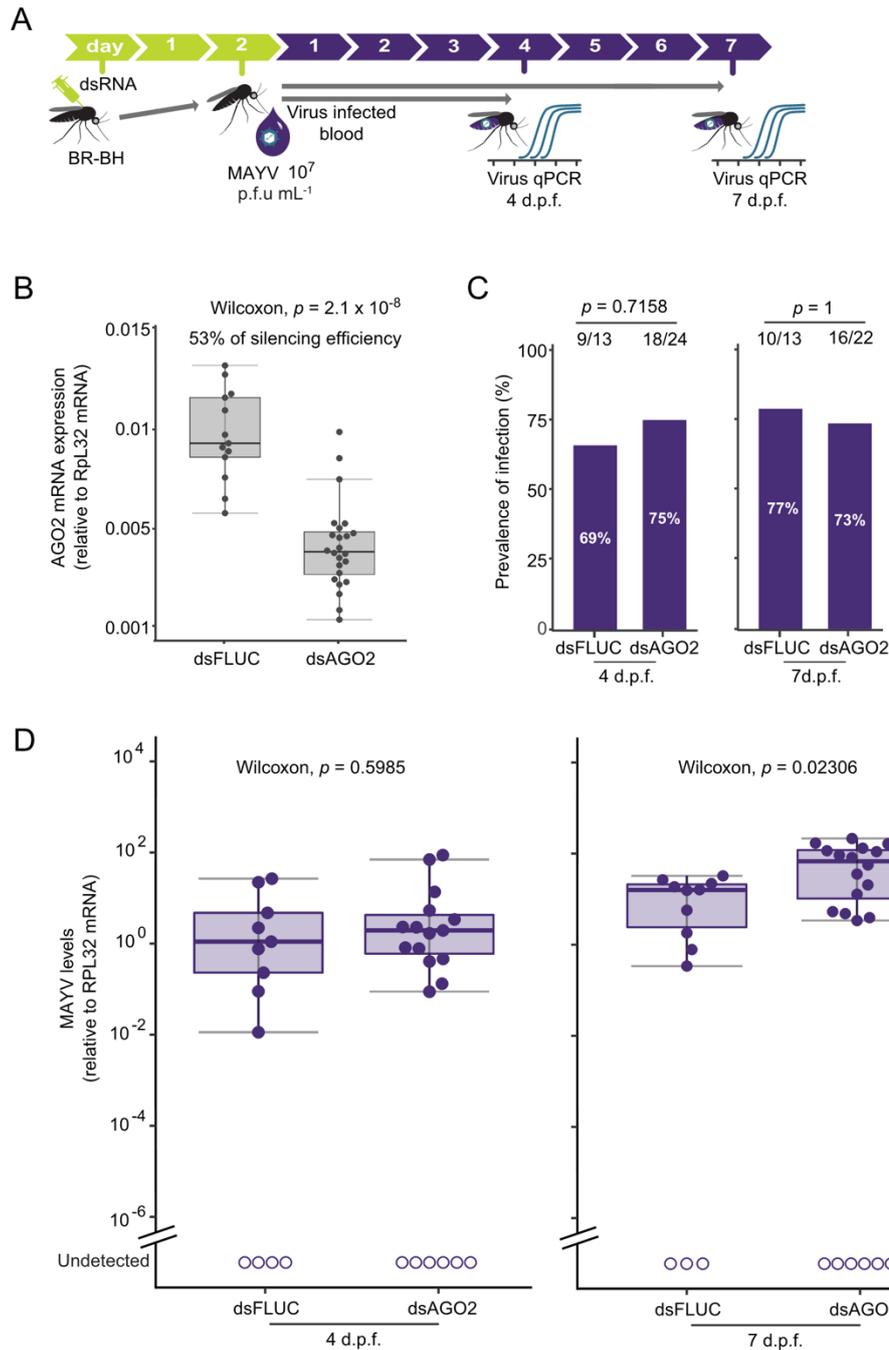


Figure S1. The effect of siRNA pathway in *Aedes aegypti* infected with lower dose of MAY (10^7 p.f.u. ml^{-1}). **(A)** Scheme of the silencing process using dsRNA. BR-BH females were intrathoracically injected with dsAGO2 and dsFLUC (control group). 2 days post microinjection, mosquitoes were fed on a blood meal containing 10^7 p.f.u. ml^{-1} of MAYV. Mosquitoes were collected at 4 and 7 d.p.f and tested individually by qPCR to detect viral RNA levels. **(B)** Mosquitoes collected at 4 d.p.f were also tested individually by qPCR for AGO2 mRNA expression for measuring the silencing efficiency. Each dot represents an individual whole mosquito. Statistical analyses were performed using the Mann-Whitney-Wilcoxon test, comparing the Ago2 expression levels. **(C)** Prevalence of infection. Total number of mosquitoes tested are indicated above each column. Statistical analyses were performed using the two-tailed Fisher's exact test. **(D)** MAYV RNA levels at 4 and 7d.p.f. Each dot represents an individual whole mosquito. Statistical analyses were performed using the Mann-Whitney-Wilcoxon test, comparing the MAYV titers of the infected mosquitoes.

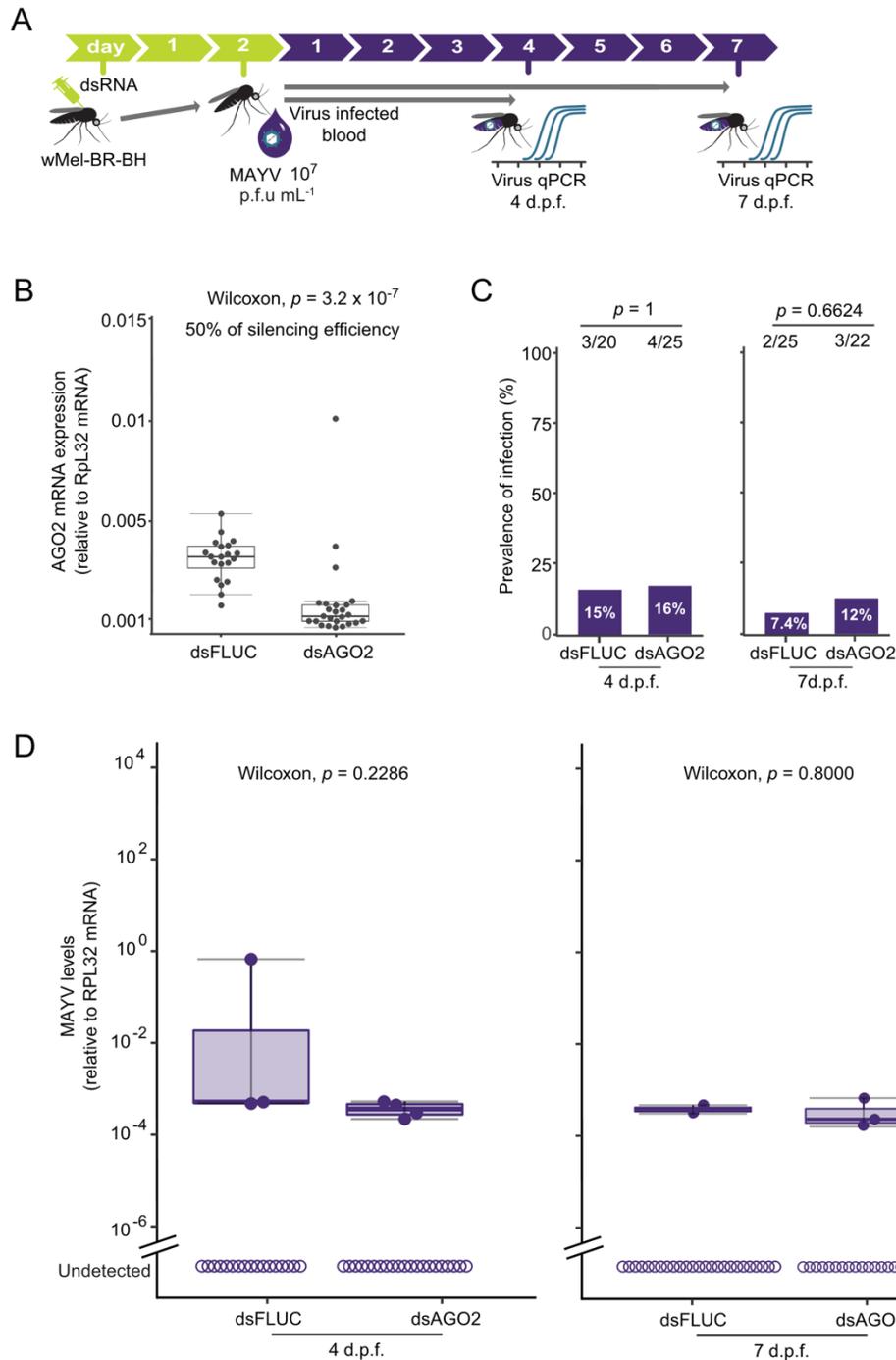


Figure S2. The effect of the mosquito siRNA pathway on *Wolbachia*-mediated MAYV protection using a lower dose of MAY (10^7 p.f.u. ml⁻¹). (A) Scheme of the silencing process using dsRNA. *wMel-BR-BH* females were intrathoracically injected with dsAGO2 and dsFLUC (control group). 2 days post microinjection, mosquitoes were fed on a blood meal containing 10^7 p.f.u. ml⁻¹ of MAYV. Mosquitoes were collected at 4 and 7 d.p.f and tested individually by qPCR to detect viral RNA levels. (B) Mosquitoes collected at 4 d.p.f were also tested individually by qPCR for AGO2 mRNA expression for measuring the silencing efficiency. Each dot represents an individual whole mosquito. Statistical analyses were performed using the Mann-Whitney-Wilcoxon test, comparing the AGO2 expression levels. (C) Prevalence of infection. Total number of mosquitoes tested are indicated above each column. Statistical analyses were performed using the two-tailed Fisher's exact test. (D) MAYV RNA levels at 4 and 7d.p.f. Each dot represents an individual whole mosquito. Statistical analyses were performed using the Mann-Whitney-Wilcoxon test, comparing the MAYV titers of the infected mosquitoes.