## Supplementary Information for

# Wolbachia reduces virus infection in a natural population

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**Supplementary Figure 1. Phylogenies of novel and known viruses**. Maximum likelihood phylogenies were reconstructed from their predicted RDRP protein sequences. Additional taxa were included on the basis of the top DIAMOND blastx matches of *Drosophila* viruses queried against the NCBI non-redundant protein database (the host species was not used as a criterion for inclusion). Taxa in green and orange are viruses previously described as being associated with *D. melanogaster* or other species of *Drosophila* respectively. Taxa in red are new *D. melanogaster* associated viruses described in this study. Taxa in blue were associated with other species of arthropods.

### Known D. melanogaster virus Novel Drosophila virus specific *Drosophila* virus

Totiviridae



Peniciliium digitatum virus 1 Peniciliium digitatum totivirus 1 Peniciliium aurantiogriseum totivirus 1 Magnaporthe oryzae virus 3 Aspergillus homomorphus totivirus 1 Aspergillus michulus totivirus 1 Aspergillus mycovirus 178 Gremmeniella abietina RNA virus L1 Gremmeniella abietina RNA virus L2 Erysiphe necorio associated totivirus 1 Colletotrichum zoysiae totivirus 1 Macrophomina phaseolina victorivirus 1 Umbelopsis ramanniana virus 2 Thelebolus microsporus totivirus 1 Ustilaginoidea virens RNA virus 5 Totiviridae sp. QDH91225.1 Toriviridae sp. ODH91225.1 Leishmania RNA virus 2 Leishmania RNA virus 2 Drosophila associated torivirus 2 Red algae totivirus 1 Diatom colony associated dsRNA virus 11 Diatom totivirus 1 Riboviria sp. Hubel tott-tikk virus e Trichinella nativa Cronartium ribicola totivirus 1 Diatom colony associated dsRNA virus 17 genome type B Diatom colony associated dsRNA virus 17 genome type A Parrys Creek toti-like virus 1 Drosophila associated totivirus 3 Huboi toti-liko virus 5 Beihai sesarmid crab virus 7 Plasmopara viticola lesion associated totivirus-like 5 Bremia lactucae associated toti-like virus 2 Beihai razor shell virus 4 Tinsley toti-like virus Erysiphales associated totivirus 24 Totiviridae sp. QDH86837.1 Piscine myocarditis virus AL V-708 Piscine myocarditis virus TT-2012 Piscine myocarditis-like virus Dumyat virus Wenzhou crab virus 5 Hubei toti-like virus 17 Rhizophagus sp. RF1 m Drosophila associated totivirus 4 Drosophila associated totivirus 5 Gigaspora margarita giardia-like virus 1 Bactrocera dorsalis toti-Larkfield virus Hubei toti-like virus 13 Pisingos virus Giardia lamblia virus Giardia lamblia virus isolate Wang Pythium polare RNA virus 2



Saccharomyces cerevisiae Saccharomyces 20S RNA narnavirus Narnavirus I-329 Rhizopus microsporus 20S narnavirus Tynnyfer narna-like virus Praha narna-like virus 3 Shahe narna-like virus 4 Drosophila associated narnavirus 2 Serbia narna-like virus 4 Ludgate narna-like virus Ludgate narna-like virus Plasmopara viticola lesion associated narna virus 21 Drosophila associated narnavirus 1 Hubei narna-like virus 16 Wuhan insect virus 18 Wuhan insect virus i o Barns Ness breadcrumb spong e narna-like virus 4 Entomophthora narna virus A Plasmopara viticola lesion associated narna virus 40 Point-Douro narna-like virus Saccharomyces 23S RNA narnavirus Reindia name-fike virus 34 Behlar name-fike virus 24 Rhizopus microsporus 235 namavirus Soybean thröp sama-like virus 2 Leptomonas saymouri RNA virus Leptomonas seymouri RNA virus Phytomonas serpens namavirus 1 Drosophila associated namavirus 4 Drosophila associated namavirus 5 Insecti nama-fike virus 2 Bremis lactucae associated namavirus 2 Behlar bamache virus 10 Sherlock virus



## Flaviviridae



Drosophila associated flavivirus 7, partial Drosophila associated flavivirus 7 Takaungu virus Lampyris noctiluca flavivirus 1 Gentian Kobu-sho-associated virus Frankliniella occidentalis flavi-Diaphorina citri flavi-like virus Atrato Flavi-like virus 1 Wuhan centipede virus Soybean cyst nematode virus 5 Eriocheli sinensis Cholera-like virus Xinzhou spider virus 2 Barley aphid RNA virus 9 Sitobion miscanthi flavi-like virus 1 Barley aphid RNA virus 10 Shayang fly virus 4 Culex tritaeniorhynchus fla Shuangao lacewing virus 2 us 2 a tick virus 8 Charvil virus

### Ту viridad

- Citrus sudden death-associated virus Grapevine asteroid mosaic associated virus Nectarine marafivirus M Blackberry virus S Davidia involucrata marafivirus 2 Matze rayado fino virus Switchgrass mosaie virus Sorghum bicolor marafivirus Camellia-associated marafivirus Camellia-associated marafivirus Peach virus T Medicago sativa marafivirus 1 Altafa virus F Davida involucrata marafivirus 1 Davida involucrata marafivirus 1 Davida involucrata marafivirus 1 Bee Macuel-Like virus 2 Nasturium officinale macula-like virus 1 Bombys mori Macuel-like virus 1 Ē Ŀ F ŀĽ
- rE.
- Ŀ Lampyris noctiluca tymovirus-like virus 1

udgenby Calliphora mononega–like virus lymenopteran anphe-related virus OKIAV73 huangao Fly virus 2 lymenopteran anphe-related virus OKIAV72 -C \_ Soybean thrips negative-stranded RNA virus 4 us 11 Hubel diptera virus 11 Drosophila unispina virus 1 Drosophila associated anphevirus Bactrocera dorsalis borna-like viru -[ F Gordis virus Xincheng Mos nphevirus  $\Gamma_{c}$ 

# es marajoara viru: es darlingi virus bopictus anphevi tononega-like viru

Supplementary Figure 2. Correlation of PCR assays and reads of mapped to different sequences. (A and B) Infection status (table) and viral load (plot, only infected samples) of two different segments of the vera virus genome (panel A) or regions of the Motts Mill genome (panel B). PCRs performed on individual flies. Viral load is measured by quantitative PCR relative to the Rpl32 mRNA. In Panel A the Y axis corresponds to primers that amplify the capsid protein gene (GenBank accession number: MT742172.1) and the X axis primers that amplify the RDRP gene (accession number: MT742171.1). (C) The number of reads mapping to a newly-discovered virus related to the Drosophila immigrans sigmavirus compared to the number of reads mapping to Motts Mill virus. (E) Reads mapping to Machany virus compared to Kilifi virus. Read counts are normalised against the total number of mapped reads in the library. Pearson correlation coefficients are shown. In panels C-E each point represents a library made from a pool of flies



**Supplementary Figure 3. The viral load in individual flies with or without** *Wolbachia.* Vera and Motts Mill viruses were repeated with two sets of PCR primers. Only virus-infected flies are shown. P values are from a one-way ANOVA. Viral loads are expressed as viral RNA copy number relative to the *Drosophila* gene *Rpl32.* The data on Galbut virus is the same as that presented in the main text.



Supplementary Figure 4. Schematic of bioinformatics analysis.



Legend

Dmel: *D. melanogaster* genome wMel: *Wolbachia* strain *w*Mel genome Known.Dro.viruses: all viral sequences in GenBank where the host is in the genus *Drosophila*  **Supplementary Table 1. PCR primer sequences.** Primer efficiency was estimated using a serial dilution of the template.

Virus	Primer Name	Sequence (5'-3')	Product Length (bp)	Primer Efficiency (%)
Galbut (Chaq)	Chaq2 Fw	TGAAATCACCATGCGTGTGC	105	105
	Chaq2_Rv	TCGTTAGCGCTCCACTTCTC		
Craigies Hill	C.Hill1_Fw	TAMCATCCAGCTGTTGCCAC	116	101
	C.Hill1_Rv	TTGCTGGTGAACCACCCATT		
DAV	DAV2_Fw	SATGGTTCAAGGACCCCGTC	83	99
	DAV2_Rv	TCTTGGGCATTGCACTGTAG		
D. melanogaster sigmavirus	Dromel.sigma3_Fw	AAAGATCTGGCAGTGAGCCC	80	102
	Dromel.sigma3_Rv	TCTCCTTACAGCCGAGATGA		
Vera virus	Vera_RDRP_Fw	AACCATTTCCGAGCGTAACCA	74	98
	Vera_RDRP_Rv	TTGATAGACGCTTGTGCCGC		
Galbut virus	Galbut1_Fw	CTCCGACACAAGTCGTCCAG	116	99
	Galbut1_Rv	GAGCAACTGCTTTCAGGCAC		
Motts Mill virus	MottsMill1_Fw	GAAGGTGGTTTTTGGGCGTT	118	92
	MottsMill1_Rv	AAGGCCAGATATAGCGCGAC		
Kallithea virus	Kallithea3_Fw	TTTCAGGGCACTCAGTAGCG	83	107
	Kallithea3_Rv	GCAACCTTTGCCACCTACAC		
La Jolla virus	LaJolla1_Fw	CGGACCAGAGTGTAGCCAAG	112	101
	LaJolla1_Rv	AGTGCCATCCAYCGATTTGT		
Motts Mill virus	MottsMill2_Fw	GAGACWTGGCAGTCTTTGGA	105	96
	MottsMill2_Rv	CCTATTTGCGGGGGCATTTCG		
Nora virus	Nora1_Fw	AACGAGGAGCGATTGACGAG	85	103
	Nora1_Rv	TTGCTTCCAATTGCGCTGAC		
Thika virus	Thika3_Fw	CGTGGTGGTACAGGCAAAAC	113	105
	Thika3_Rv	TATGGGTCAGGTTCGGGTAA		
Vera virus	Vera_capsid_Fw	GAGGGGTAGAGGCGGTTTTC	96	97
	Vera_capsid_Rv	GGCATTGTTCCTCGCTCTCT		