

Supplementary Information for
Wolbachia reduces virus infection in a natural population

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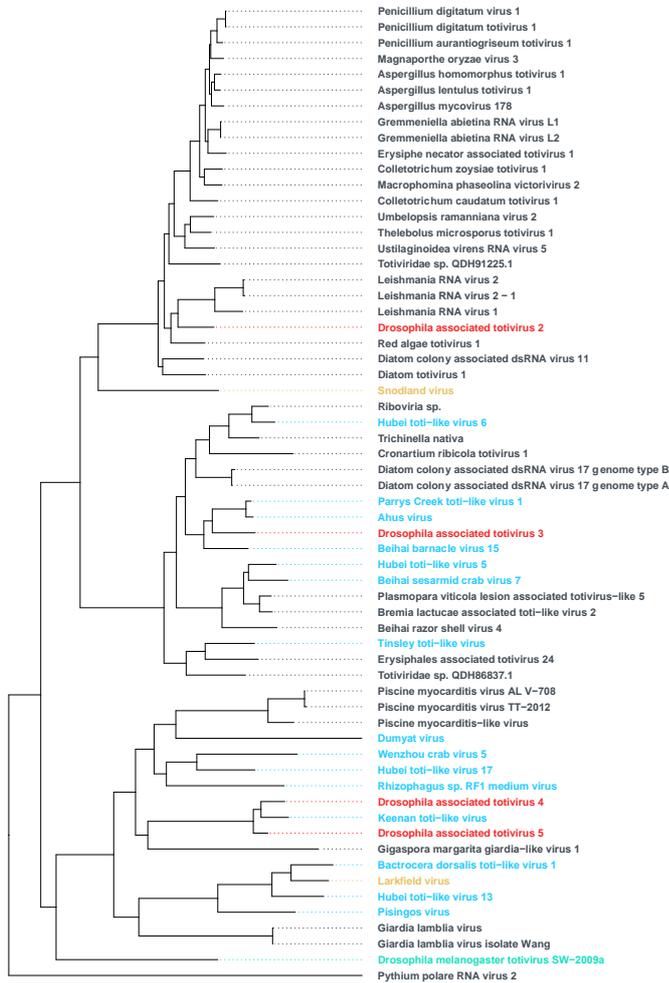
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** contributed equally

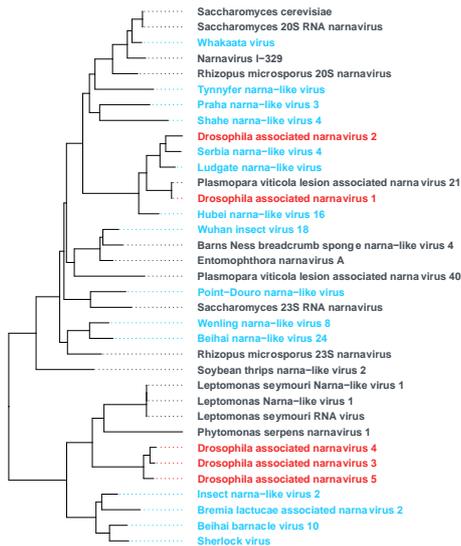
Supplementary Figure 1. Phylogenies of novel and known viruses. Maximum likelihood phylogenies were reconstructed from their predicted RDRP protein sequences. Additional taxa were included on the basis of the top DIAMOND blastx matches of *Drosophila* viruses queried against the NCBI non-redundant protein 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
 Non-*D. melanogaster* specific *Drosophila* virus
 Arthropod-associated virus

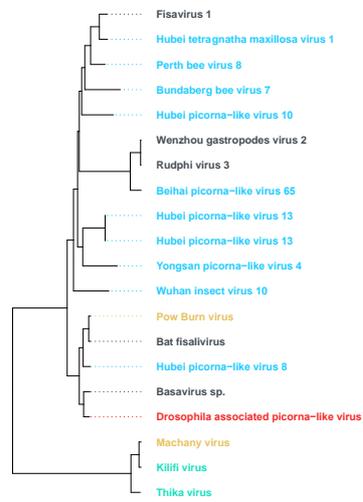
Totiviridae



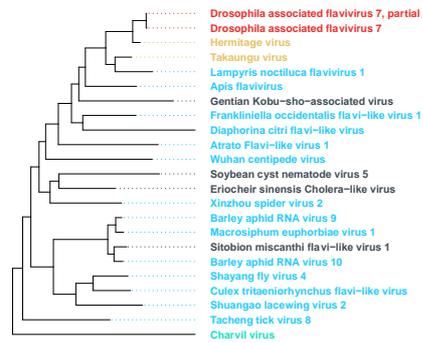
Narnaviridae



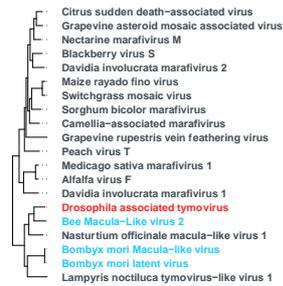
Picornavirales



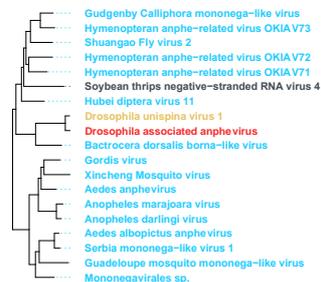
Flaviviridae



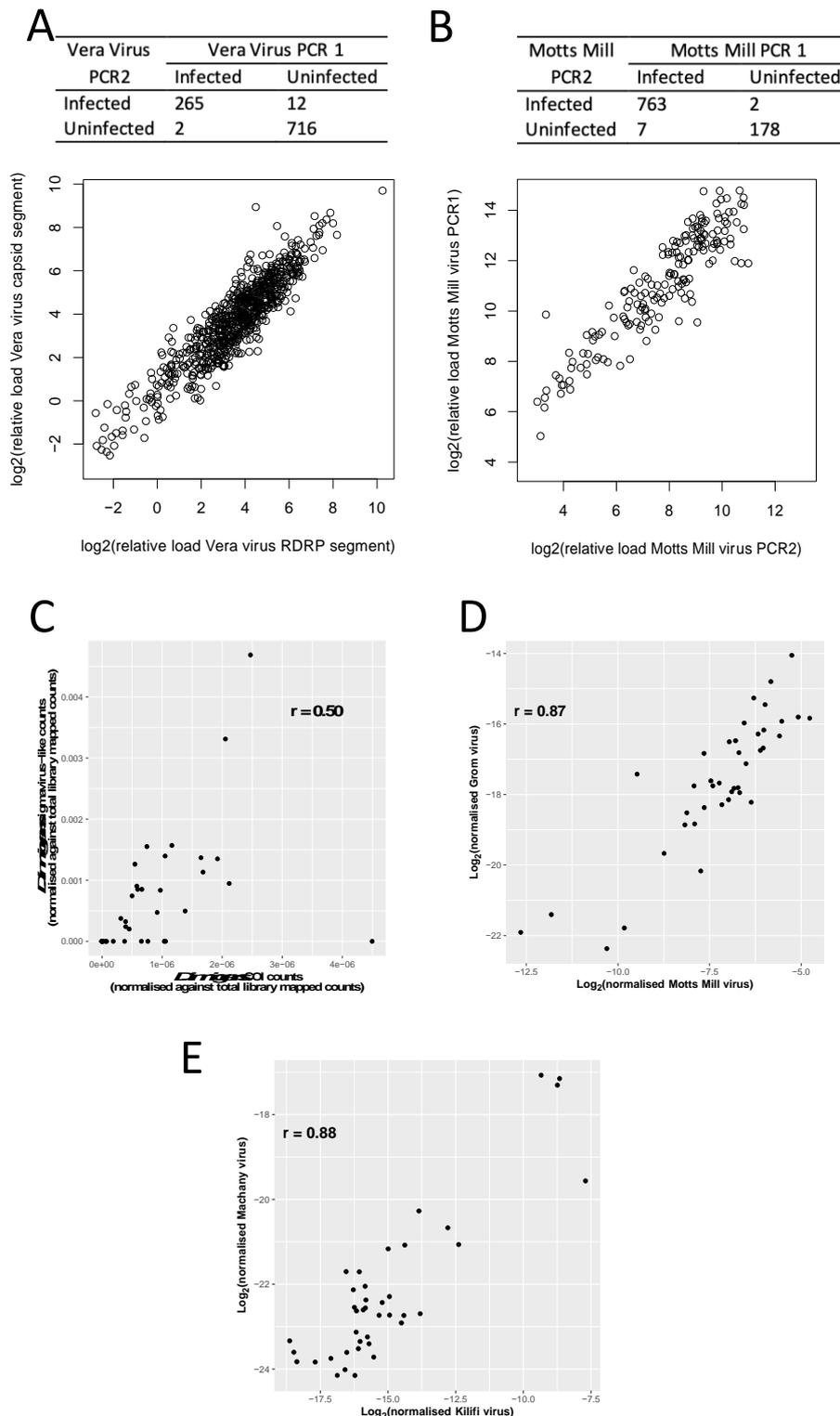
Tymoviridae



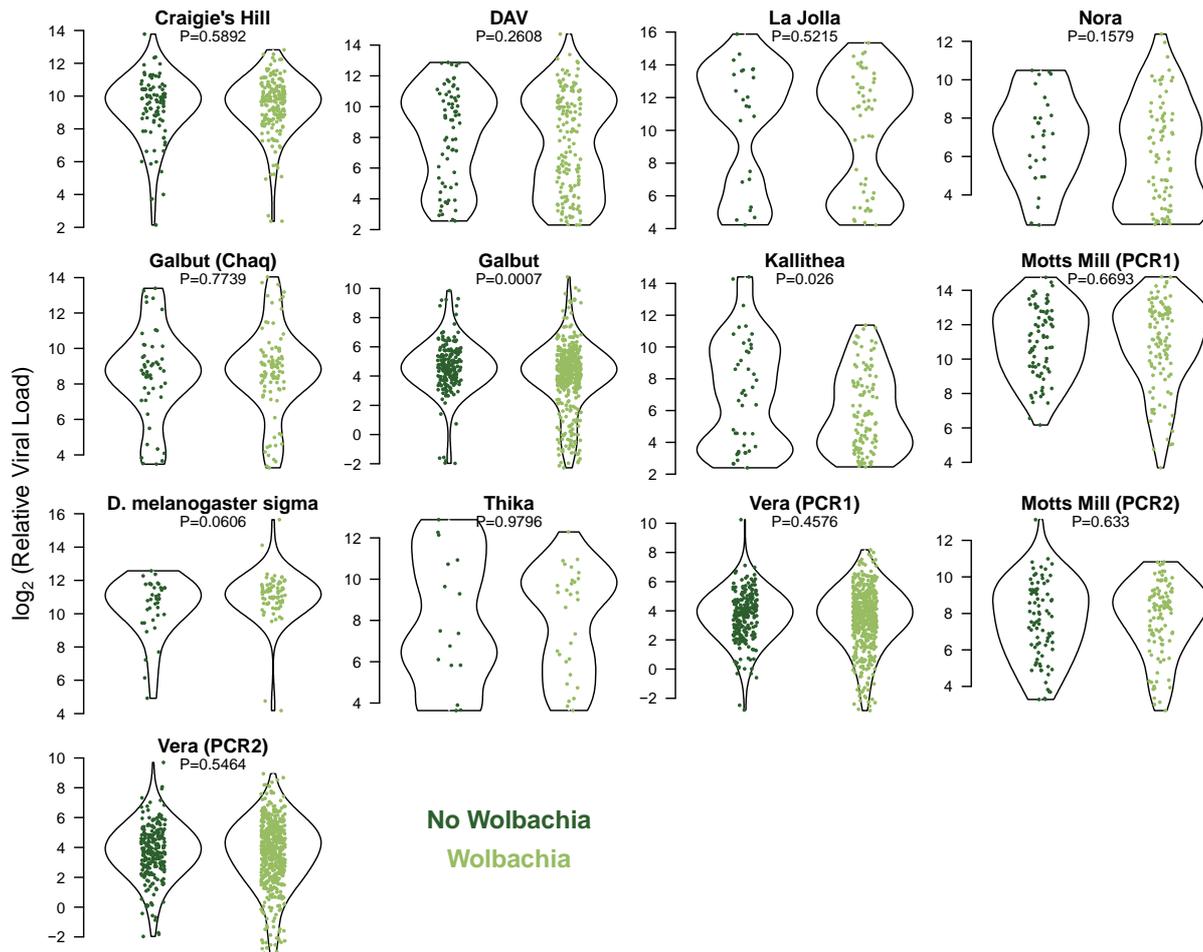
Mononegavirales



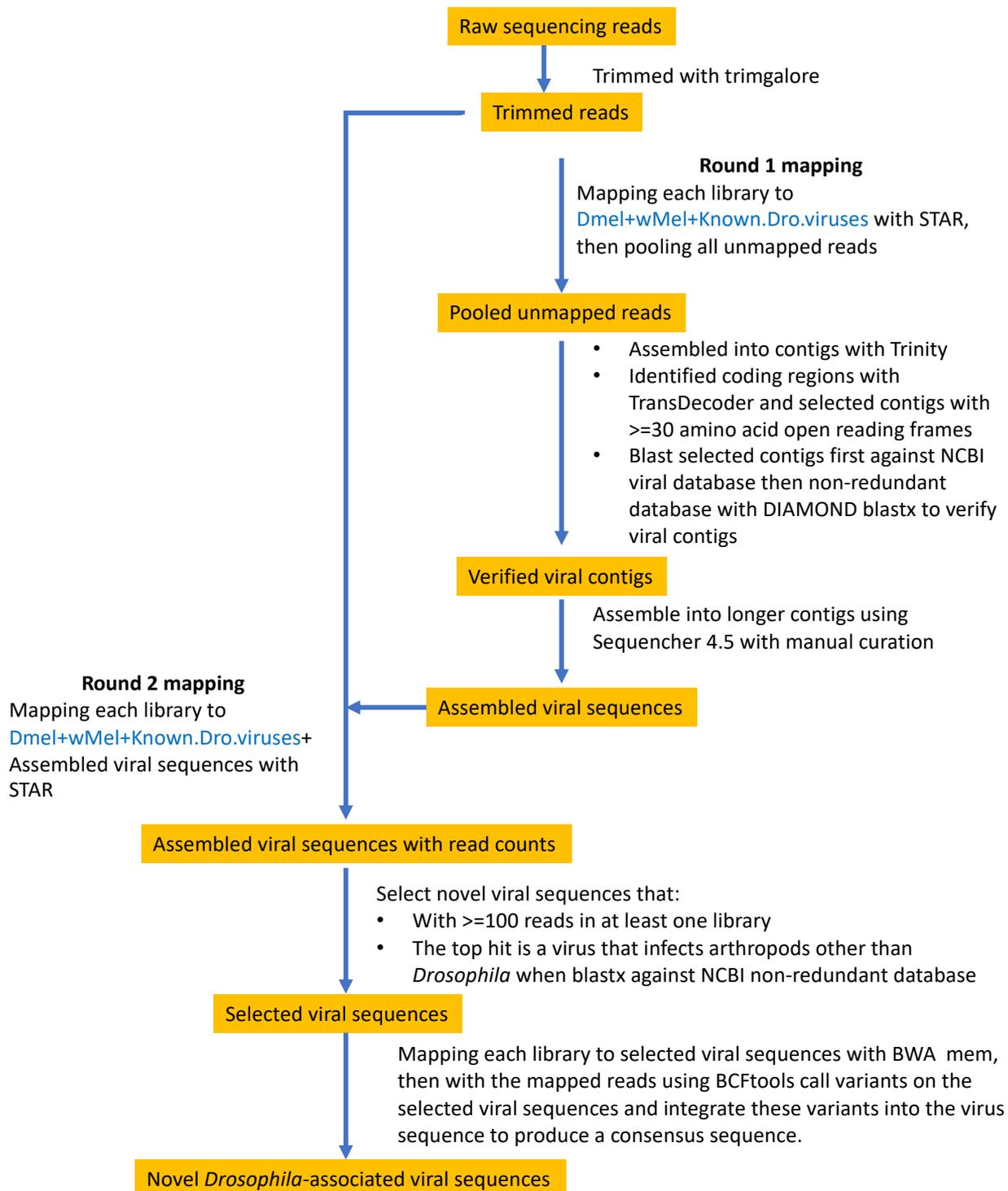
Supplementary Figure 2. Correlation of PCR assays and reads of mapped to different sequences. (A and B) Infection status (table) and viral load (plot, only infected samples) of two different segments of the vera virus genome (panel A) or regions of the Motts Mill genome (panel B). PCRs performed on individual 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 the *D. immigrans* cytochrome oxidase I gene (*COI*). (D) The number of reads mapping to Grom virus compared to 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 *wMel* 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		
<i>D. melanogaster</i> 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	CCTATTTGCGGGGCATTTTCG		
Nora virus	Nora1_Fw	AACGAGGAGCGATTGACGAG	85	103
	Nora1_Rv	TTGCTTCCAATTGCGCTGAC		
Thika virus	Thika3_Fw	CGTGGTGGTACAGGCAAAAC	113	105
	Thika3_Rv	TATGGGTCAGGTTTCGGGTAA		
Vera virus	Vera_capsid_Fw	GAGGGGTAGAGGCGGTTTTTC	96	97
	Vera_capsid_Rv	GGCATTGTTCTCGCTCTCT		