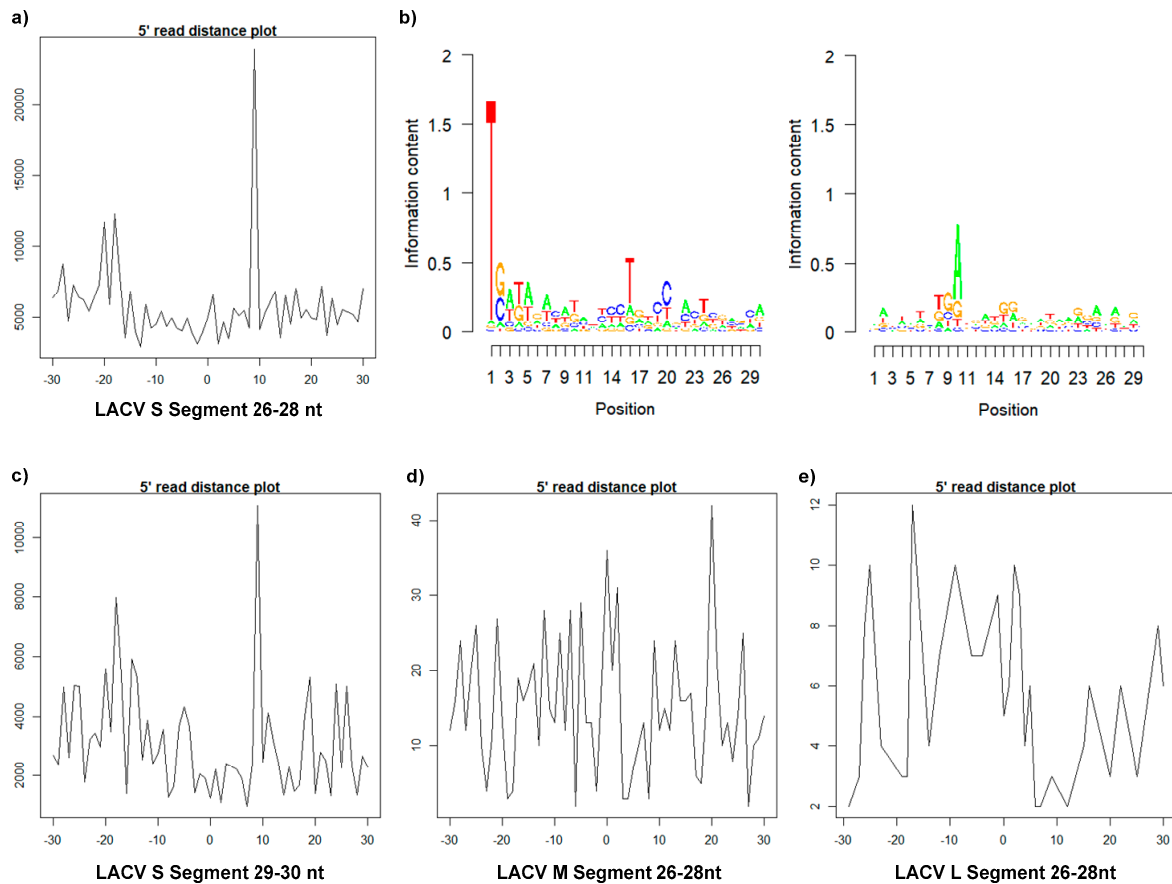


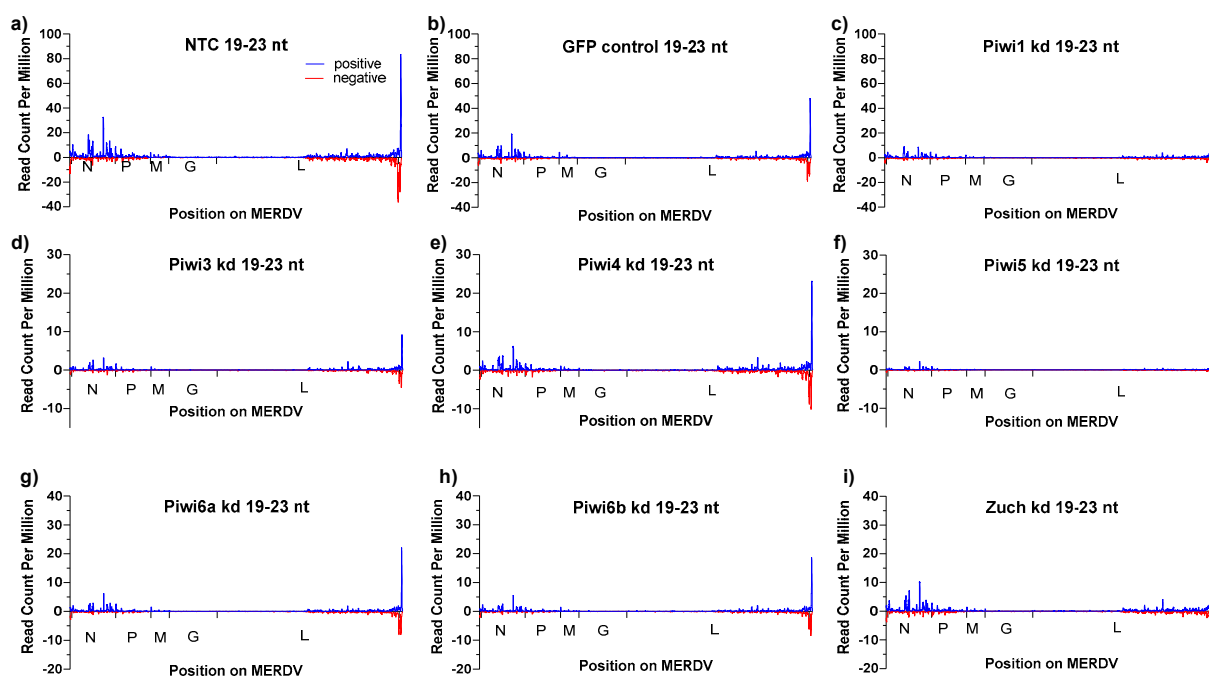
Supplementary Table S1. *Aedes aegypti*, *Drosophila melanogaster*, *Culex quinquefasciatus*, and *Culex tarsalis* PIWI genes used for phylogenetic tree analysis.

Species	Gene	Accession Number
<i>Aedes aegypti</i>	AePiwi1	AAEL008076
	AePiwi2	AAEL008098
	AePiwi3	AAEL013692
	AePiwi4	AAEL007698
	AePiwi5	AAEL013233
	AePiwi6	AAEL013227
	AePiwi7	AAEL006287.1
	AeAgo3	AAEL007823
	AeZuc	AAEL011385
<i>Drosophila melanogaster</i>	DmPiwi	NM_057527
	DmAgo3	EF211827
	DmZuc	NM_135686.3
<i>Culex quinquefasciatus</i>	CqPiwi1	XM_001844015.2
	CqPiwi3	XM_038255983.1
	CqPiwi4	XM_038251629.1
	CqPiwi5	XM_038251859.1
	CqPiwi6a	XM_038251857.1
	CqPiwi6b	XM_038258148.1
	CqPiwi7	XM_038266499.1
	CqZuc	XM_001870711.2
	CqAgo3	XM_038254040.1
<i>Culex tarsalis</i>	CtPiwi1	mRNA6102*
	CtPiwi4	mRNA2582*
	CtPiwi5	mRNA13996*
	CtPiwi6	mRNA4897*
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	CtZuc	mRNA10624*
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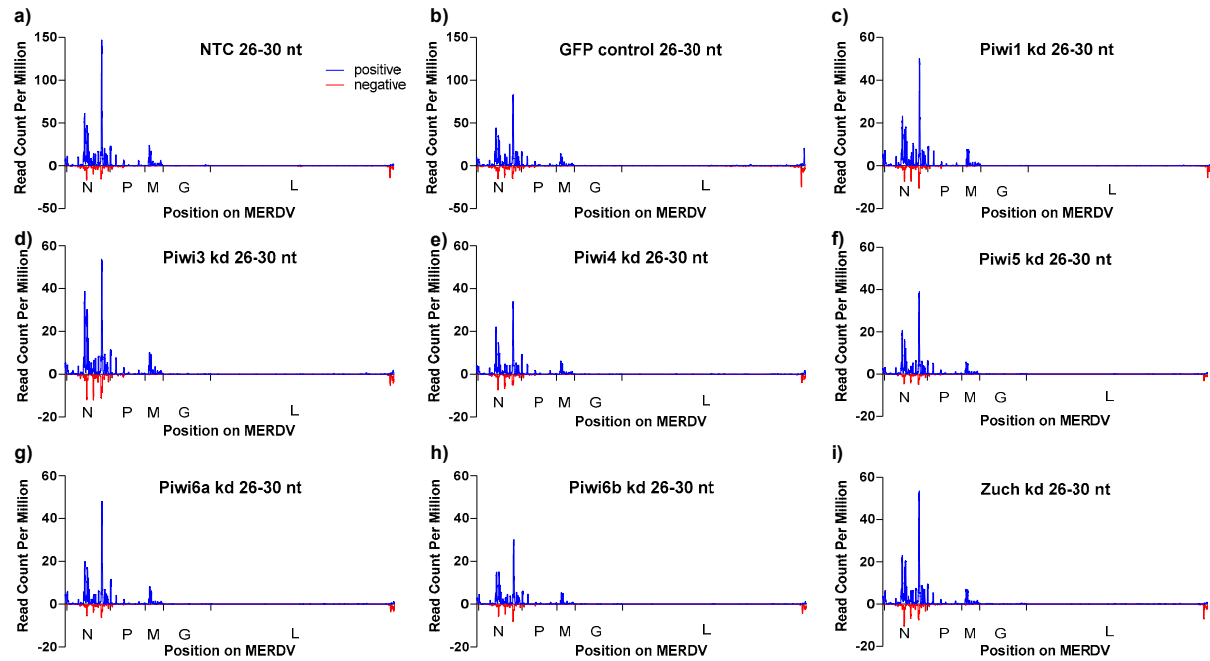
*Internal identifier – sequences are available in Supplemental Material S1.



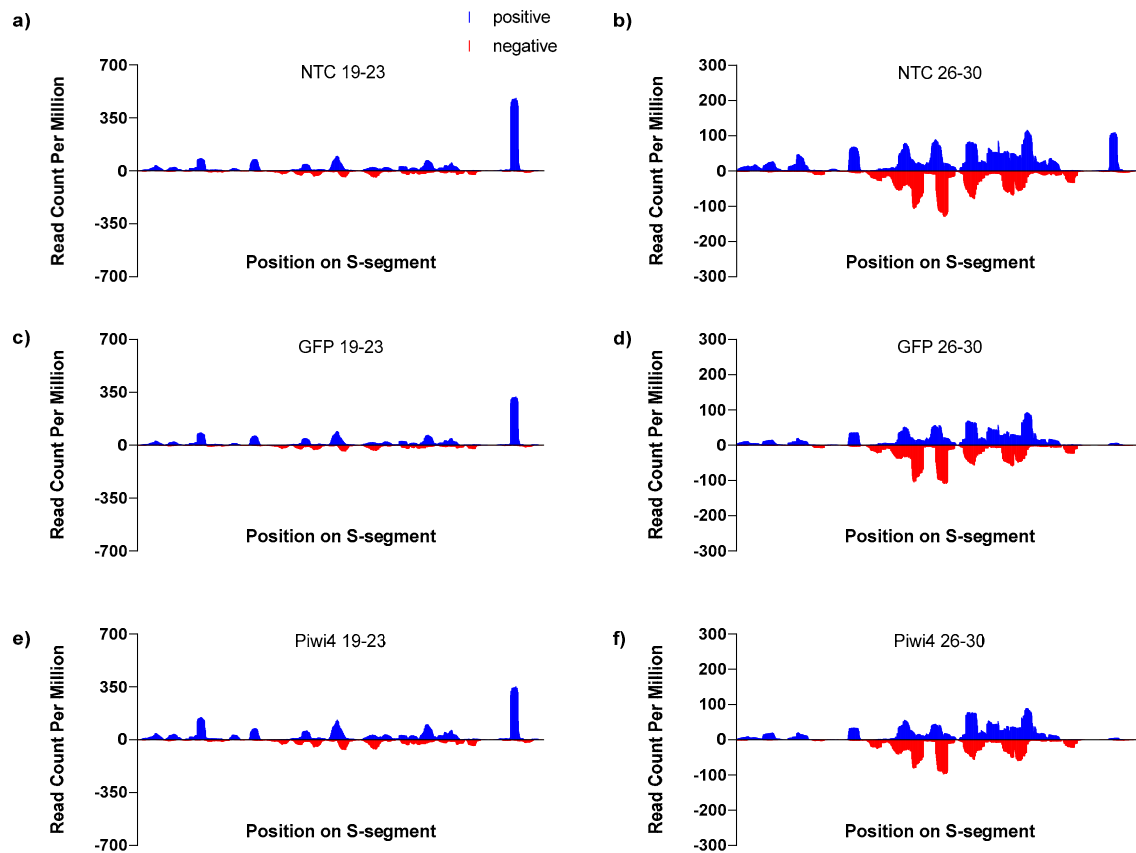
Supplemental Figure S1. Virus-derived sRNA reads in *Cx. quinquefasciatus* cells. sRNA libraries were sequenced from Hsu cells 2 dpi with LACV and aligned to the S segment (a, b, c), LACV M segment (d), LACV L segment (e). Distance plots were generated from LACV-derived 26-28 nt sRNA reads for the LACV S (a), LACV M (d), and LACV L (e) segment, or LACV derived 29-30 nt sRNA reads for the LACV S segment (c). Nucleotide biases of 29-30 nt sRNA reads derived from antisense (left) and sense (right) for LACV S segment are also shown (b).



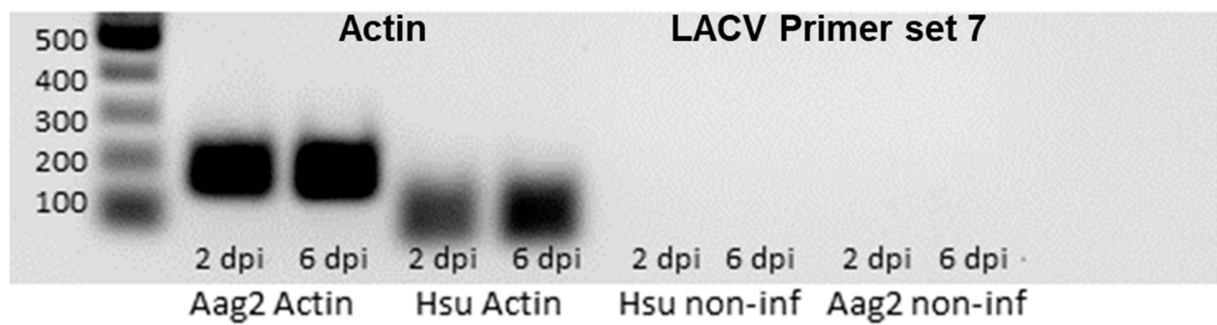
Supplemental Figure S2. 19-23 nt sRNA reads mapped to MERDV genome. Hsu cells were treated with either no dsRNA (a), GFP dsRNA (b), Piwi1 dsRNA (c), Piwi3 dsRNA (d), Piwi4 dsRNA (e), Piwi5 dsRNA (f), Piwi6a dsRNA (g), Piwi6b dsRNA (h), or Zuc dsRNA (i).



Supplemental Figure S3. 26-30 nt sRNA reads mapped to MERDV genome. Hsu cells were treated with either no dsRNA (a), GFP dsRNA (b), Piwi1 dsRNA (c), Piwi3 dsRNA (d), Piwi4 dsRNA (e), Piwi5 dsRNA (f), Piwi6a dsRNA (g), Piwi6b dsRNA (h), or Zuc dsRNA (i).



Supplemental Figure S4. sRNA reads mapped to LACV S-segment. 19-23nt (a,c,e) and specifically 26-30 nt (b,d,f) sRNA reads from LACV-infected Hsu cells were mapped along the LACV genome S segment. LACV-infected Hsu cells were treated with either no dsRNA (a,b), GFP dsRNA (c,d), or Piwi4 dsRNA (e,f).



Supplemental Figure S5. LACV vDNA forms in Hsu and Aag2 cells. The housekeeping gene actin is seen at 2- and 6- dpi in Aag2 and Hsu cells, corresponding to DNA extractions from Figure 10. Hsu and Aag2 cells were left uninfected, and primer set 7 was used in PCR reactions, but no product was detected.

Supplemental Material S1: *Culex tarsalis* PIWI coding sequences.

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>Zucchini_mRNA10624

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>Ago3_mRNA4166

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tgccgcgacactttccggccggggcgggctcagttcattcaagcgtgctgaagcagccgctggagtcgggtcgttcgggtccgacc

>Sequence alignment of CtPiwi7 and CtPiwi8 using CLUSTAL O(1.2.4) multiple sequence alignment (97.3% nucleotide identity). Numbers (60, 120, etc.) indicate nucleotide position of the last nucleotide on the respective line.

Piwi7	60	atgcggttc	cgga	cgga	catt	tcgga	tgtggtg	cgaacgc	ggc	cggcggat	tgtcaggtg
Piwi8	60	atgcggttc	cgga	cgga	catt	tcgga	tgtggtg	cgaacgc	ggc	cggcggat	tgtcaggtg
		*****	*****	***		*****				*****	*****
Piwi7	120	acgaagcac	ggaact	tcggg	acggc	agatccgcct	g	acggc	gaactact	tccgggtgatg	
Piwi8	120	acgaagcac	ggaact	tcggg	acggc	agatccgcct	t	acggc	gaactact	tccgggtgatg	
		*****		*****				*****		*****	
Piwi7	180	cataccgac	ggggagg	ccatg	tccagt	accgggtgg	acttt	tgtgc	cgagattg	agagt	
Piwi8	180	cataccgac	ggggagg	ccatg	tccagt	accgggtgg	acttt	tgtgc	cgagattg	agagt	
		*****		*****				*****		*****	
Piwi7	240	gtgagggcg	cggaagg	cgttg	atgcacc	agctgaag	ccgacc	ctcgga	ccgatc	ctgttc	
Piwi8	140	gtgagggcg	cggaagg	cgttg	atgcacc	agctgaag	ccgacc	ctcgga	ccgatc	ctgttc	
		*****		*****				*****		*****	
Piwi7	300	gacggaggt	tccatc	tttatg	agccggg	acaagacgc	ggagcg	aggagag	tgaaattacg		
Piwi8	300	gacggaggt	tccatc	tttatg	agccggg	acaagacgc	ggagcg	aggagag	tgaaattacg		
		*****		*****				*****		*****	

Piwi7 360 acgaaggaaatgcagtcccagcaggagtagcctgggtgaagatcaagaagggtcggagtgggt
 Piwi8 360 acgaaggaaatgcagtcccagcaggagtagcctgggtgaagatcaagaagggtcggagtgggt

 Piwi7 420 gactggacgtcggagatggcgctgaccgtgctcaacttgatcaaccgacgcggcatggga
 Piwi8 420 gactggacgtcggagatggcgctgaccgtgctcaacttgatcaaccgacgcggcatggga

 Piwi7 480 ggacttcggctgcagcaaatcggacggaacttcttcgaccccaatgggaagggtccgactc
 Piwi8 480 ggacttcggctgcagcaaatcggacggaacttcttcgaccccaatgggaagggtccgactc

 Piwi7 540 gcggagttcgggctggaactctaccccggtacataaccagcatccggcagcacgagcag
 Piwi8 540 gcggagttcgggctggaactctaccccggtacataaccagcatccggcagcacgagcag

 Piwi7 600 aacattctgatgtgctgcggaagtgcgcacaaaggatcatgcgggaggacacctgctacgga
 Piwi8 600 aacattctgatgtgctgcggaagtgcgcacaaaggatcatgcgggaggacacctgctacgga

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 Piwi7 720 ctaggcactgtggtgatggcgatctacggccggaacaacacgtacaccgtgagcgaatgtg
 Piwi8 720 ctgggaactgtggtgatggcgatctacggccggaacaacacgtacaccgtgagcgaatgtg
 ** ** *****
 Piwi7 780 gagttcgccacgacgccggaacattcgttcgagacggcaaacgggagaatgaccttcgtg
 Piwi8 780 gagttcgccacgacgccggaacattcgttcgagacggcaaacgggaaggatgaccttcgtg

 Piwi7 840 gagtactaccggagtcagtacaacatcgatcatccgggatccgcggcagccgatgctgggtg
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 Piwi7 900 tcgcggggccagggtcgcgggacgtacgggcccgaaggccggagctggtgtactctggtgccg
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 Piwi7 960 gagctgatgcgcgcgacccgggtgacggatgagatgcggaggaactttactttgatgcgc
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 Piwi7 1020 gccatggcggaccacacccggtgacgcccggatcggcgcatgctgcggctagaggcggttc
 Piwi8 1020 gccatggcggaccacacccgactcactccggatcggcgcatgctgcggctgaggcggttc

 Piwi7 1080 aaccagcgctgcacgacacccccgaaagtatcgaaatcttcgcttctggcagacggaa
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 Piwi7 1140 ctgcaccgcccggctggtggaactccgggcccgcgttctgcacccagagaagatcctgttc
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 Piwi7 1200 agcgagcagcagctgaatatgtcgctcattccggacgactccgagtggtggcagatggcc
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 Piwi7 1260 ttccggaacaaccagatgtaccgctcggagcagctccagaactggttcctgatggttcg
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 Piwi7 1320 cgcaactgggtcgtaaccgcgatccgcagctgatcatgtgcatcgtagcaacgataaa
 Piwi8 1317 cgcaactgggtcgtaaccgcgatccgcagctgatcatgtgcatcgtagcaacgataaa

 Piwi7 1380 gcggaccgctacgcggcgatcaagaccaagtgtgctgcgtcgaacgagcgatcgccaccag
 Piwi8 1377 gcggaccgctacgcggcgatcaagaccaagtgtgctgcgtcgaacgagcgatcgccaccag

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Piwi7 1440 gtcatcaaggccaagacgatcacgccgaaggggtggcaacgtgcgccacgttgatgtcgggtg
Piwi8 1437 gtcatcaaggccaagacgatcacgccgaaggggtggcaacgtgcgccacgttgatgtcgggtg
*****
Piwi7 1500 gcgaccaagggtcgccatccagatgaactgcaagatcggcggaatcccgtggatcgtgacg
Piwi8 1497 gcgaccaagggtcgccatccagatgaactgcaagatcggcggaatcccgtggatcgtgacg
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Piwi7 1560 aacccactgcgatcgatcatggtgatcggttacgacgtctgtcacgacactcggaaccga
Piwi8 1557 aacccactgcgatcgatcatggtgatcggttacgacgtctgtcacgacactcggaaccga
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