

Supporting Information

Mayoral et al. 10.1073/pnas.1420131112

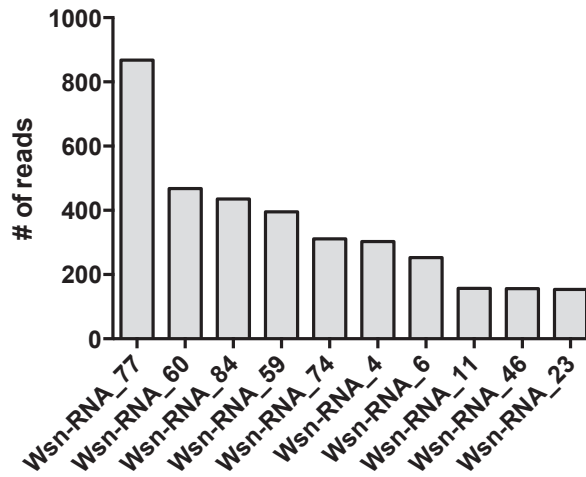


Fig. S1. Ten most abundant *WsnRNAs* from deep sequencing of small RNAs from Aag2 cells infected with *Wolbachia-wMelPop-CLA*.

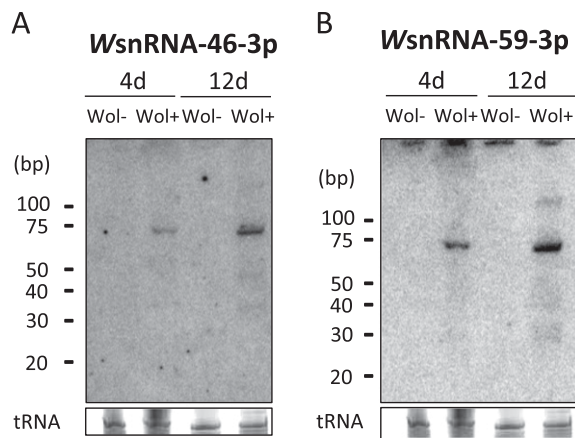


Fig. S2. The antisense mature *WsnRNA-46-3p* and *WsnRNA-59-3p* are not detected in adult mosquitoes. *WsnRNA-46-3p* (A) and *WsnRNA-59-3p* (B) after Northern blotting from 4- and 12-d-old *A. aegypti* mosquitoes with *Wolbachia-wMelPop-CLA* (Wol⁺) and without (Wol⁻). tRNA is shown as loading control.

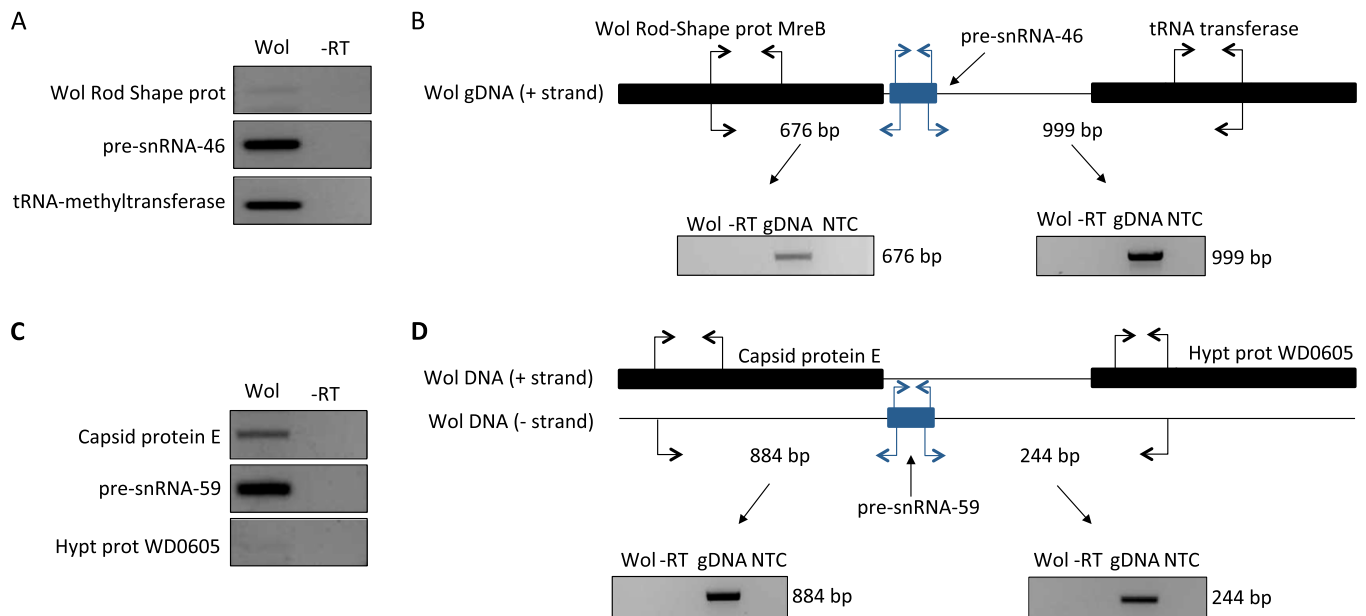


Fig. S5. *WsnRNAs* are transcribed independent of the flanking genes. (A) RT-PCR analysis of expression of pre*WsnRNA-46* and the flanking genes *Wolbachia* Rod-Shape protein *MreB* and tRNA-methyltransferase. (B) RT-PCR using internal primers of the rod-shape protein *MreB* and pre*WsnRNA-46* or tRNA-methyltransferase and pre*WsnRNA-46*. Genomic DNA was included as positive control. (C) RT-PCR analysis of expression of pre*WsnRNA-59* and the flanking genes Capsid protein E and hypothetical protein WD0605. (D) RT-PCR using internal primers of the Capsid protein E and pre*WsnRNA-59* or hypothetical protein WD0605 and pre*WsnRNA-59*. Genomic DNA was included as positive control.

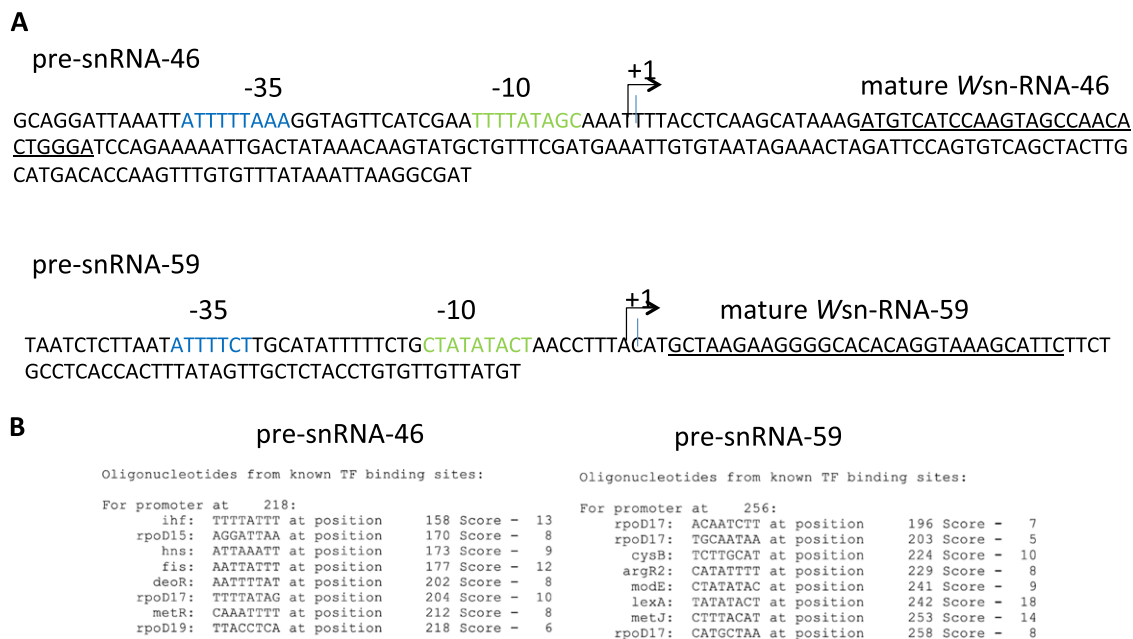


Fig. S6. TATA box, initiation transcription site, and transcription factors prediction upstream of pre*WsnRNAs-46* (A) and pre*WsnRNA-59* (B).

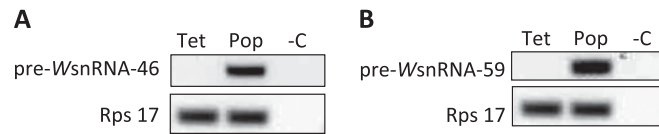


Fig. S7. preWsnRNAs are transcribed from the *Wolbachia* genome. PCR amplification of preWsnRNA-46 (A) and preWsnRNA-59 (B) from genomic DNA extracted from Tet and Pop mosquitoes. -C, negative control. Rps17 is shown as control.

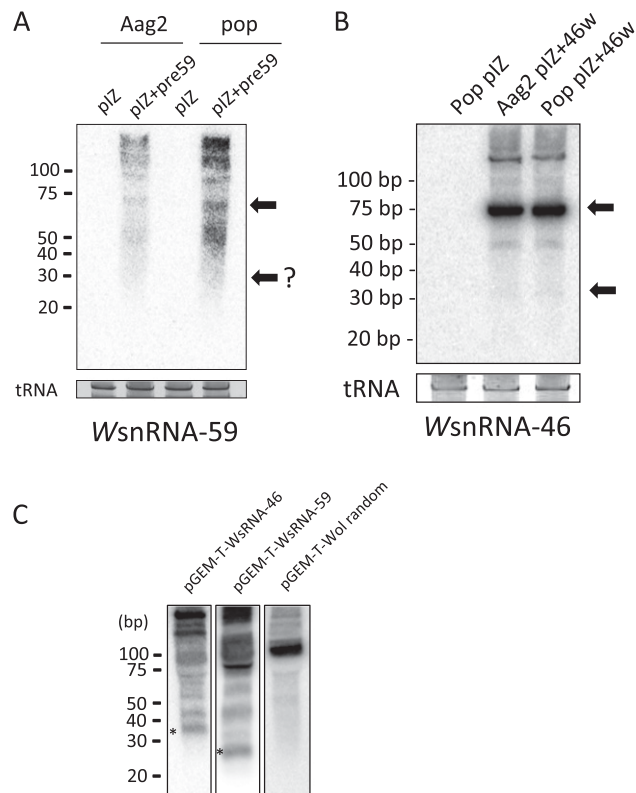
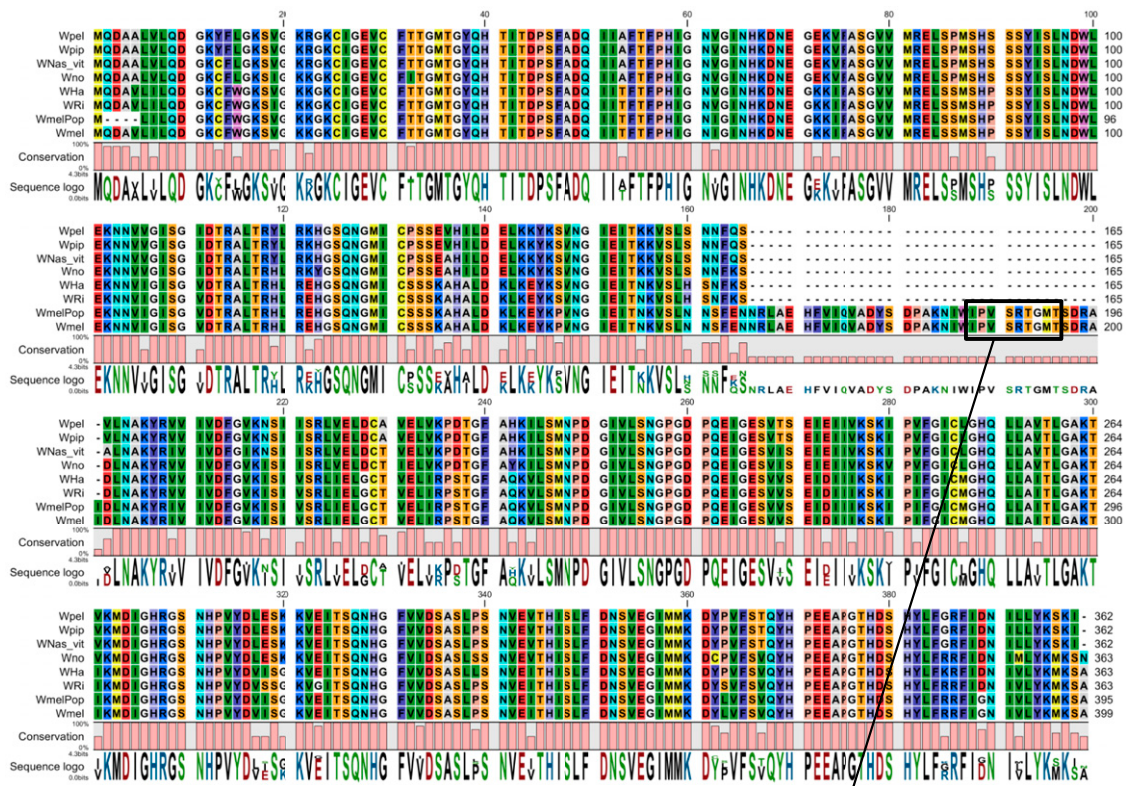


Fig. S8. preWsnRNAs sequences are recognized, transcribed, and sliced by the prokaryotic machinery of *E. coli*. Northern blot analysis of WsnRNA-46 (A) and WsnRNA-59 (B) 3 d after the transfection of the piZ/V5 constructs containing preWsnRNAs into Aag2 and Pop cells. (C) Northern blot analysis of WsnRNA-46 and WsnRNA-59 after overnight expression in *E. coli* transformed with preWsnRNA-46 and preWsnRNA-59 using pGEM-T-Easy.

A *Wol* Carbamoyl-phosphatase synthase (*carA*)



B



Target sequence of WsnRNA-46

mfe: -31.6 kcal/mol	
p-value: undefined	
position 562	
target 5' A	CAC A C 3'
UUCCAGUGU	GC AC UGGA UGACAU
AGGGUCACA	CG UG ACCU ACUGUA
miRNA 3'	AC A A 5'

Predicted secondary structure of the 109 bp *Wolbachia* Palindromic Element (WPE) inserted in the Carbamoyl-phosphatase synthase (*carA*) gene

Fig. S9. (Continued)

E *Wol* UDP-N-acetylmuramoylalanine--D-glutamate ligase (*muR D*)

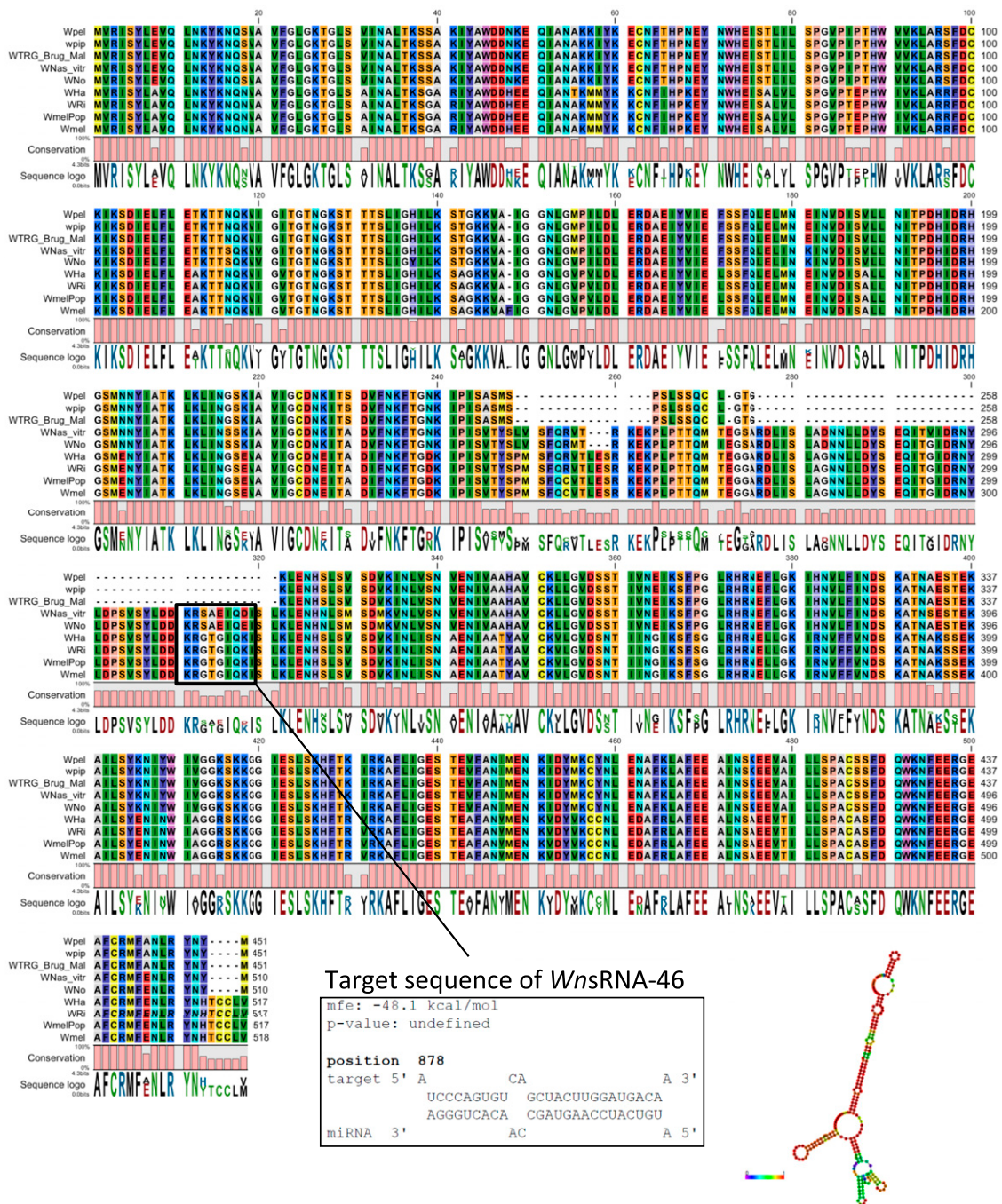
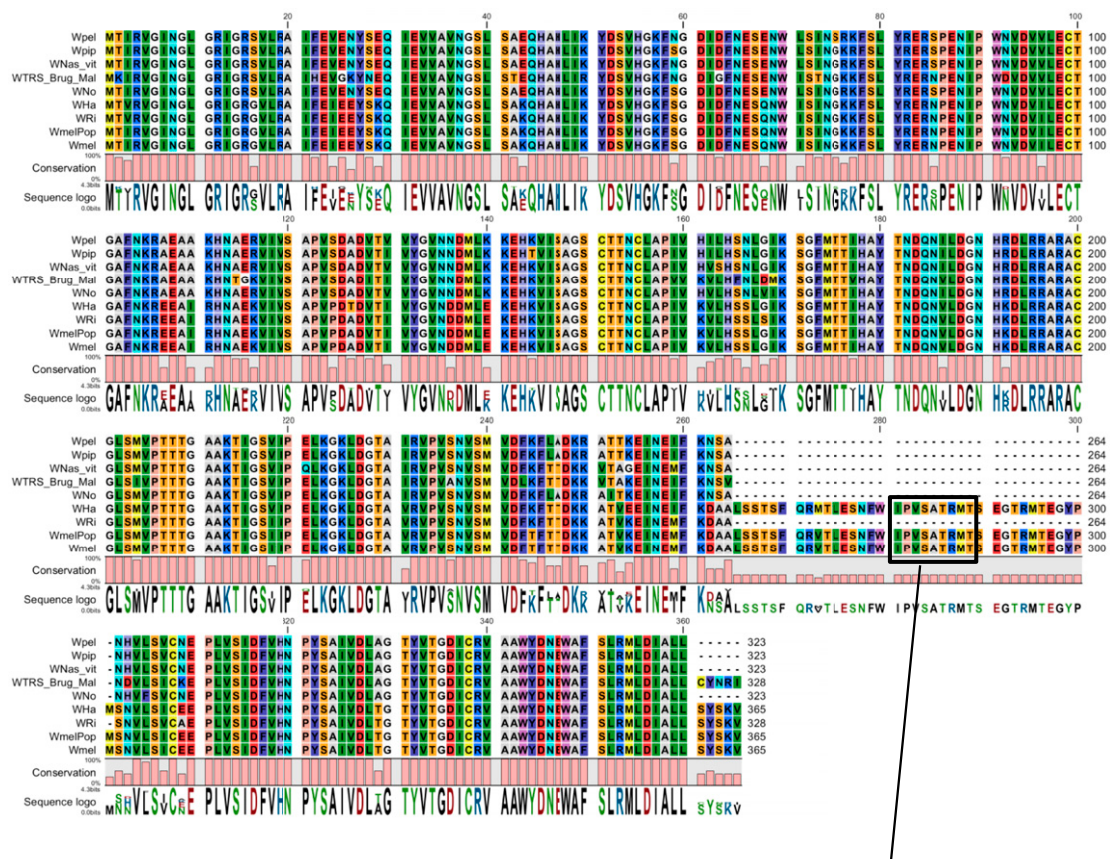


Fig. S9. (Continued)

F Wol Glycerinaldehyde 3-phosphatase dehydrogenase (*Gpdh_C*)



Target sequence of *WnsRNA-46*

```
mfe: -44.1 kcal/mol
p-value: undefined

position 841
target 5' A CA C C 3'
        UCCAGUGU GCUACU GGAUGACAU
        AGGGUCACA CGAUGA CCUACUGUA
miRNA 3' AC A 5'
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Fig. S9. *WnsRNA-46* targets inserted palindromic sequences in *Wolbachia* target genes. (A) Alignment of the *Wolbachia* gene carbamoyl-phosphatase (*carA*) in different *Wolbachia* strains and its putative targeted sequence. (B) Secondary structure prediction of the palindromic sequences inserted in the *carA* gene by using RNAfold. (C) Alignment of the *Wolbachia* gene Geranyltransferase (*IspB*) in different *Wolbachia* strains and its putative targeted sequence. (D) Alignment of the *Wolbachia* gene UDP-*N*-acetylmuramoylalanine-*D*-glutamate ligase (*murD*) and its putative targeted sequence. (E) Alignment of the *Wolbachia* gene tRNA modification GTPase (*TrmE*) in different *Wolbachia* strains and its putative targeted sequence. (F) Alignment of the *Wolbachia* gene Glycerinaldehyde 3-phosphatase dehydrogenase (*Gpdh_C*) in different *Wolbachia* strains and its putative targeted sequence.

Table S1. Sequence identity of pre*WnsRNA-46* and pre*WnsRNA-59* in different strains of *Wolbachia*

	wAlbB	wAna	wBm	wBol1	wMel	wMelPop	wMelPopCLA	wMelCS	wOo	wOv	wPip	wRi	wUni	wVitB	wWil
pre <i>miRNA59</i> , %	No	100	*	‡	100	100	100	100	No	*	*	100	*	*	*
pre <i>miRNA46</i> , %	No	88 [‡]	†	†	100	100	100	100	No	*	†	88 [‡]	†	†	100
<i>Wolbachia</i> supergroup	B	A	D	B	A	A	A	A	C	C	B	A	A	A	A

wAlbB, *Aedes albopictus*; wAna, *Drosophila ananassae*; wBm, *Brugia malayi*; wBol1, *Hypolimnas bolina*; wMel, *Drosophila melanogaster*; wMelPop, *Drosophila melanogaster*; wMelPopCLA, *Drosophila melanogaster*; wMelCS, *Drosophila melanogaster*; wOo, *Onchocerca ochengi*; wOv, *Onchocerca volvulus*; wPip, *Culex quinquefasciatus* Pel; wRi, *Drosophila simulans*; wUni, *Muscidifurax uniraptor*; wVitB, *Nasonia vitripennis*; wWil, *Drosophila willistoni*.

*Just very small fragment homology.

†Homology to less than half the sequence.

‡High homology to more than half the sequence.

Table S2. Target prediction for *WsnRNA-46* and *WsnRNA-59* in *Wolbachia* strain *wMelPop* genome using NCBI blast and RNAhybrid for predictions

<i>WsnRNA</i>	Potential target genes in <i>Wolbachia</i>	Region	RNAhybrid	Mfe, kcal/mol
<i>WsnRNA-46</i>	UDP- <i>N</i> -acetylmuramoylalanine- <i>D</i> -glutamate ligase	CDS	Position 878 target 5' A CA A 3' UCCCAGUGU GCUACUUGGAUGACA AGGGUCACA CGAUGAACCUACUGU miRNA 3' AC A 5'	-48.1
	Hypothetical protein gb AE017196.1 :227855-228025	CDS	Position 17 target 5' A CA A 3' UCCCAGUGU GCUACUUGGAUGACA AGGGUCACA CGAUGAACCUACUGU miRNA 3' AC A 5'	-48.1
	Hypothetical protein gb AE017196.1 :395766-396101	CDS	Position 266 target 5' A A C C 3' UCCCAGUGUU GCUACUUG AUGACA AGGGUCACAA CGAUGAAC UACUGU miRNA 3' C C A 5'	-43.5
	Glyceraldehyde 3-phosphate dehydrogenase	CDS	Position 841 target 5' A CA C C 3' UCCCAGUGU GCUACU GGAUGACAU AGGGUCACA CGAUGA CCUACUGUA miRNA 3' AC A 5'	-44.1
	Carbamoyl-phosphate synthase, small subunit	CDS	Position 658 target 5' A G A CA A 3' UCC AGU GU GCUACUUGGAUGACA AGG UCA CA CGAUGAACCUACUGU miRNA 3' G AC A 5'	-39.9
	Geranyltranstransferase	CDS	Position 581 target 5' A CA C C 3' UCCAGUGU GCUACUUG AUGACA AGGGUCACA CGAUGAAC UACUGU miRNA 3' AC C A 5'	-38.1
	tRNA modification GTPase TrmE	CDS	Position 1117 target 5' A CA C C 3' UCCAGUGU GCUACUUG AUGACAU AGGGUCACA CGAUGAAC UACUGUA miRNA 3' AC C 5'	-38.5
<i>WsnRNA-59</i>	Isoleucyl-tRNAs	CDS	Position 1092 target 5' A CG ACUG G CAUAC GGA G 3' GA GU GC UUGUG UGCUCCU UUUGGU CU CG UG GACAC ACGGGGA GAAUCG miRNA 3' UA AAA A 5'	-27.1
	Prophage LambdaW4	CDS	Position 344 target 5' A ACAUACA G ACA G 3' GAAUGCUIUACU CC UU CUUAGC CUUACGAAAUGG GG AA GAAUCG miRNA 3' ACACACG G 5'	-30.6
	ATP-dependent Clp protease, ATP-binding subunit ClpX	CDS	Position 854 target 5' A A A UCAA G 3' GAAUGCUIUAC UG UGU CCU CUUACGAAAUG AC ACA GGA miRNA 3' G CGG AGAAUCG 5'	-25.5
	Ankyrin domain protein (WD0596)	CDS	Position 344 target 5' A ACAUACA G ACA G 3' GAAUGCUIUACU CC UU CUUAGC CUUACGAAAUGG GG AA GAAUCG miRNA 3' ACACACG G 5'	-30.6

Mfe, minimum free energy.

Table S3. Target prediction for WsnRNA-46 and WsnRNA-59 in *A. aegypti* using NCBI blast and RNAhybrid for predictions

WsnRNA	Potential target genes in <i>A. aegypti</i>	Region	RNAhybrid	Mfe, kcal/mol	
WsnRNA-46	Dynein heavy chain AAEL007225-RA	CDS	Position 3662 target 5' U A C GAC CAAAG U 3' UCU AGU G GCU ACUUGGAUGACAU AGG UCA C CGA UGAACCUACUGUA miRNA 3' G AAC 5'	-30.3	
	AMP-dependent ligase AAEL006823-RA	CDS	Position 1261 target 5' A CGACAUC G A 3' CCGG GGCUACUUGGAUGA G GGUC CCGAUGAACCUACU U miRNA 3' AG ACAA G A 5'	-35.3	
	Zinc finger proteína AAEL013028-RA	CDS	Position 928 target 5' C AU A A G G A UG C 3' UCCCAGU G UG CU G UUGGA UG GU AGGGUCA C AC GA U AACCU AC UA miRNA 3' A C G UG 5'	-26.2	
	Hypothetical protein AAEL009280-RA	CDS	Position 959 target 5' A CAGCA AUCAGA G 3' UUCAGU GUUGG ACUUGGAUGACAU GGGUCA CAACC UGAACCUACUGUA miRNA 3' A GA 5'	-35.6	
	Swiprosin AAEL006006-RA	CDS	Position 178 target 5' G AAAC A UC G 3' UCGG G UGGCUACUUGGA UGGCG GGUC C ACCGAUGAACCU ACUGU miRNA 3' AG A A A 5'	-33.2	
	Selenoprotein G AAEL005523-RA	CDS	Position 125 target 5' A AUU CCGACCGAUCUC A 3' CUCGGU GUU GGCUACUUGGAUGG GGGUCA CAA CCGAUGAACCUACU miRNA 3' A GUA 5'	-34.5	
	Farnesyltransferase alpha subunit/rab geranylgeranyl transferase alpha subunit AAEL000421	CDS	Position 4 target 5' A CGACGGA C A A 3' CCU AG UG UGG CUACUUGGAUGAUA GGG UC AC ACC GAUGAACCUACUGU miRNA 3' A A A 5'	-33.9	
	Rreceptor protein tyrosine phosphatase AAEL013105-RA	CDS	Position 1168 target 5' U A C CGUACUA C 3' UCC CG UG CUACUUGGAUGAU AGG GU AC GAUGAACCUACUG miRNA 3' C AACC UA 5'	-27.4	
	Inositol 1,4,5-trisphosphate receptor AAEL003608-RA	CDS	Position 2396 target 5' A UUAC G C 3' UAGU UGGC UUGGAUGACAU GUCA ACCG AACCUACUGUA miRNA 3' AGG CA AUG 5'	-29.1	
	ATP-dependent Clp protease, ATP-binding subunit ClpX	CDS	Position 854 target 5' A A A UCAA G 3' GAAUGCUIUAC UG UGU CCU CUUACGAAAUG AC ACA GGA miRNA 3' G CGG AGAAUCG 5'	-25.5	
	WsnRNA-59	Hypothetical proteína AAEL000859	CDS	Position 456 target 5' C ACUGGG GCUU U C 3' UGCU GCC UGUGCCCUUCUU GC ACGA UGG ACACGGGGAAGAA CG miRNA 3' CUU AA AC U 5'	-39.3
		Serine protease inhibitor AAEL011777	CDS	Position 923 target 5' C GGAAAG AUG G 3' GC GUGUGCCCUUC AG CG CACACGGGGAAG UC miRNA 3' CUUA AAAUGGA AA G 5'	-33.7

