Supporting Information

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



Fig. 52. 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 preWsnRNA-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 preWsnRNA-46 or tRNA-methyltransferase and preWsnRNA-46. Genomic DNA was included as positive control. (*C*) RT-PCR analysis of expression of preWsnRNA-59 and the flanking genes Capsid protein E and hypothetical protein WD0605. (*D*) RT-PCR using internal primers of the Capsid protein E and preWsnRNA-59 or hypothetical protein WD0605 and preWsnRNA-59. Genomic DNA was included as positive control.

Α

pre-snRNA-46 -35 -10 +1 mature Wsn-RNA-46 GCAGGATTAAATTATTTTTAAAGGTAGTTCATCGAATTTTATAGCAAATTTTACCTCAAGCATAAAG<u>ATGTCATCCAAGTAGCCAACA</u> <u>CTGGGA</u>TCCAGAAAAATTGACTATAAACAAGTATGCTGTTTCGATGAAATTGTGTAATAGAAACTAGATTCCAGTGTCAGCTACTTG CATGACACCAAGTTTGTGTTTATAAATTAAGGCGAT

pre-snRNA-59

-35 -10 +1 mature Wsn-RNA-59 TAATCTCTTAATATTTTCTGCATATTTTCTGCTATAACCTTTACAACCTTTACAT<u>GCTAAGAAGGGGGCACACAGGTAAAGCATTC</u>TTCT GCCTCACCACTTTATAGTTGCTCTACCTGTGTTGTTATGT

В		pre-snRNA-4	6	pre-snRNA-59 Oligonucleotides from known TF binding sites:		
	Oligonucleotides	from known TF bindi	ng sites:			
	For promoter at ihf: TTTT rpoID5: AGGA hns: ATTA fis: AATT deoR: AATT rpoIT7: TTTT metR: CAAA	218: ATTT at position TTAA at position AATT at position TTAT at position TTAT at position ATTG at position	158 Score - 13 170 Score - 8 173 Score - 9 177 Score - 12 202 Score - 8 204 Score - 10 212 Score - 8	For promoter at 256: rpoD17: ACAATCAT at position 196 Score - 7 rpoD17: TGCAATAA at position 203 Score - 5 cysB: TCTTGCAT at position 224 Score - 10 argR2: CATATTAT at position 249 Score - 8 modE: CTATATAC at position 241 Score - 9 lexA: TATATACT at position 242 Score - 18 metJ: CTTTACAT at position 253 Score - 14		





Fig. 57. 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.

Α

Wol Carbamoyl-phosphatase synthase (carA)



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

Fig. S9. (Continued)



Wol Geranyltranferase (IspB)



Fig. S9. (Continued)

S A L

D

Wol tRNA modification GTPase (TrmE)



Fig. S9. (Continued)





Fig. S9. (Continued)

F Wol Glyceraldehyde 3-phosphatase dehydrogenase (Gpdh_C)



Target sequence of WnsRNA-46



Fig. S9. *Ws*nRNA-46 targets inserted palindromic sequences in *Wolbachia* target genes. (*A*) Alignment of the *Wolbachia* gene carbamoyl-phosphatase synthase (*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 Geranyltranferase (*IspB*) in different *Wolbachia* strains and its putative targeted sequence. (*B*) Alignment of the *Wolbachia* gene UDP-*N*-acetylmuramoylalanine-D-glutamate ligase (*muR D*) and its putative targeted sequence. (*E*) Alignment of the *Wolbachia* gene Granyltranferase (*IspB*) in different *Wolbachia* strains and its putative targeted sequence. (*E*) Alignment of the *Wolbachia* gene UDP-*N*-acetylmuramoylalanine-D-glutamate ligase (*muR D*) and its putative targeted sequence. (*E*) Alignment of the *Wolbachia* gene Granyltranferase (*IspB*) in different wolbachia strains and its putative targeted sequence. (*E*) Alignment of the *Wolbachia* gene Granyltranferase (*IspB*) in different *Wolbachia* strains and its putative targeted sequence. (*E*) Alignment of the *Wolbachia* gene Glyceraldehyde 3-phosphatase dehydrogenase (*Gpdh_C*) in different *Wolbachia* strains and its putative targeted sequence.

Table S1. Sequence identity of preWsnRNA-46 and preWsnRNA-59 in different strains of Wolbachia

	wAlbB	wAna	wBm	wBol1	wMel	wMelPop	wMelPopCLA	wMelCS	wOo	wOv	wPip	wRi	wUni	wVitB	wWil
premiRNA59, %	No	100	*	+	100	100	100	100	No	*	*	100	*	*	*
premiRNA46, %	No	88 [‡]	+	+	100	100	100	100	No	*	+	88 [‡]	†	t	100
Wolbachia supergroup	В	Α	D	В	А	А	А	А	С	С	В	А	Α	А	А

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
prediction	S

WsnRNA	Potential target genes in Wolbachia	Region	RNAhybrid	Mfe, kcal/mo
WsnRNA-46	UDP-N-acetylmuramoylalanine-D-glutamate ligase	CDS	Position 878	-48.1
			target 5' A CA A 3'	
			UCCCAGUGU GCUACUUGGAUGACA	
			AGGGUCACA CGAUGAACCUACUGU	
			miRNA 3' AC A 5'	
	Hypothetical protein gb AE017196.1 :227855–228025	CDS	Position 17	-48.1
			target 5' A CA A 3'	
			UCCCAGUGU GCUACUUGGAUGACA	
			AGGGUCACA CGAUGAACCUACUGU	
			miRNA 3' AC A 5'	
	Hypothetical protein gb AE017196.1 :395766–396101	CDS	Position 266	-43.5
			target 5' A A C C 3'	
			UCCCAGUGUU GCUACUUG AUGACA	
			AGGGUCACAA CGAUGAAC UACUGU	
			miRNA 3' C C A 5'	
	Glyceraldehyde 3-phosphate dehydrogenase	CDS	Position 841	-44.1
	algeraldengde 5 phosphate dengdrogendse	605	target 5' \triangle C \triangle C C 3'	
	Carbamovi-phosphato synthaso, small subunit	CDS	Position 658	20.0
	Cal balloyi-phosphate synthase, sinal subulit	CDJ	target 5' \wedge \cap \wedge \wedge \wedge 3'	-59.9
	Gorapyltranstransforaça	CDS	Decition ES1	20.1
	Geranyitranstransferase	CD3	Fosition 581 target $F' \wedge C \wedge C \cap C'$	-50.1
	tona diffection CTDess True	CDC	MIRNA 3' AC C A 5'	20 F
	IRNA mouncation Grease time	CDS		-20.2
14/ DNIA 50		656		27.4
WSNRNA-59	Isoleucyi-trinas	CDS	Position 1092	-27.1
			target 5' A CG ACUG G CAUAC GGA G 3'	
			GA GU GC UUGUG UGCUCCU UUUGGU	
			mirna 3' ua aaa a 5'	
	Prophage LambdaW4	CDS	Position 344	-30.6
			target 5' A ACAUACA G ACA G 3'	
			GAAUGCUUUACU CC UU CUUAGC	
			CUUACGAAAUGG GG AA GAAUCG	
			miRNA 3' ACACACG G 5'	
	ATP-dependent Clp protease, ATP-binding subunit ClpX	CDS	Position 854	-25.5
			target 5' A A A UCAA G 3'	
			GAAUGCUUUAC UG UGU CCU	
			CUUACGAAAUG AC ACA GGA	
			miRNA 3' G CGG AGAAUCG 5'	
	Ankyrin domain protein (WD0596)		Position 344	-30.6
			target 5' A ACAUACA G ACA G 3'	
			GAAUGCUUUACU CC UU CUUAGC	
			CUUACGAAAUGG GG AA GAAUCG	
			miRNA 3' ACACACG G 5'	

Mfe, minimum free energy.

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Table S3.	Target prediction for	WsnRNA-46 and WsnRNA-59 in	A. aegypti using NCBI blast	and RNAhybrid for predictions
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<i>W</i> snRNA	Potential target genes in A. aegypti	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' GAAUGCUUUAC 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 UGUGCCCCUUCUU 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 GUGUGCCCCUUC AG CG CACACGGGGAAG UC miRNA 3' CUUA AAAUGGA AA G 5'	-33.7

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Ta	ble	S3.	Cont.
	DIC		

PNAS PNAS

WsnRNA	Potential target genes in A. aegypti	Region	RNAhybrid	Mfe, kcal/mol
	Serine protease AAEL002593	CDS	Position 258 target 5' A GUA A U A 3' AAUGU ACCUGUGUGCC UUUC UG GC UUACG UGGACACACGG GAAG AU CG miRNA 3' C AAA G A 5'	-34.1
	Organic anion transporter AAEL010917	CDS	Position 407 target 5′ U A G 3′ GAAUGCUUUACC GC CUUUCU CUUACGAAAUGG CG GGAAGA miRNA 3′ ACACA G AUCG 5′	-28.7
	Arsenite inducuble RNA associated protein aip-1 AAEL009931	CDS	Position 686 target 5' A GCA C 3' AGUGU CC GUGUGCCCCUUC AGU UUACG GG CACACGGGGAAG UCG miRNA 3' C AAAU A AA 5'	-34.7
	Myosin-VIIa AAEL001220	CDS	Position 5645 target 5' A CG GA C AGUGAAGG U 3' G GCU ACCUG G GUCC UUUUAGC U CGA UGGAC C CGGG AGAAUCG miRNA 3' C UA AA A A GA 5'	-29.6
	Glucose transporter AAEL006264	CDS	Position 1511 target 5' C A GGUGU A 3' UGU GCC UGUGUGC UCCUUUUU GC ACG UGG ACACACG GGGAAGAA CG miRNA 3' CUU AAA U 5'	-30.3
	U520 protein AAEL011187-RA		Position 1286 target 5' A UC G GA GGG C 3' GAA UGCU CUGUGUGCCCC C AGC CUU ACGA GACACACGGGG G UCG miRNA 3' AAUG AA AA 5'	-37.5

Mfe, minimum free energy.