

A functional requirement for sex-determination M/m locus region

***lncRNA* genes in *Aedes aegypti* female larvae**

Supplementary Files:

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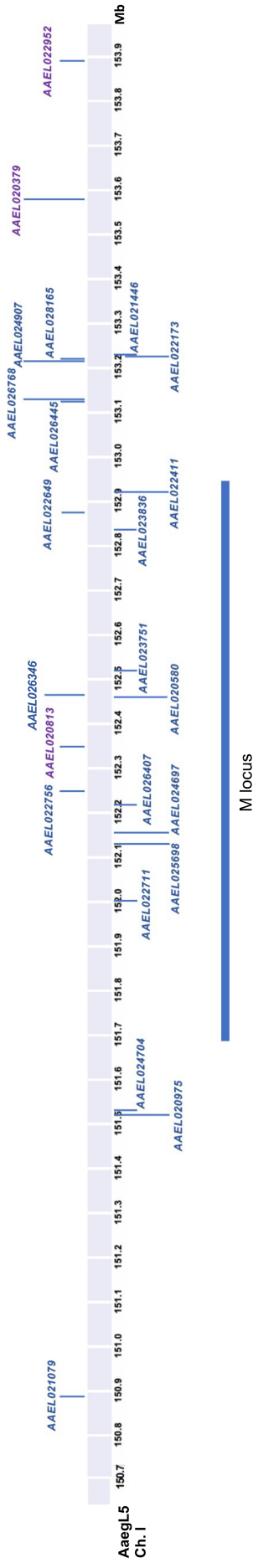
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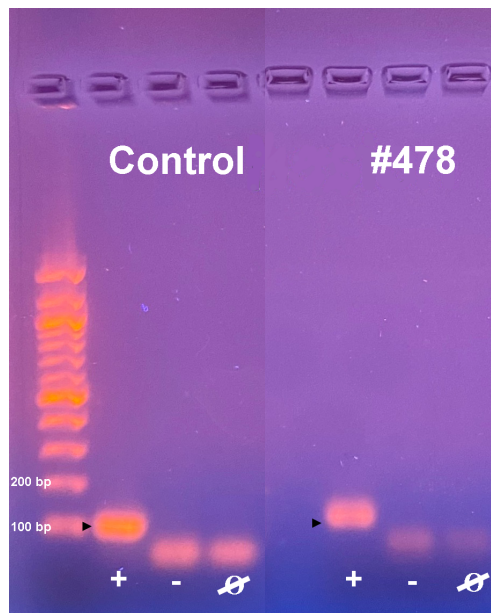
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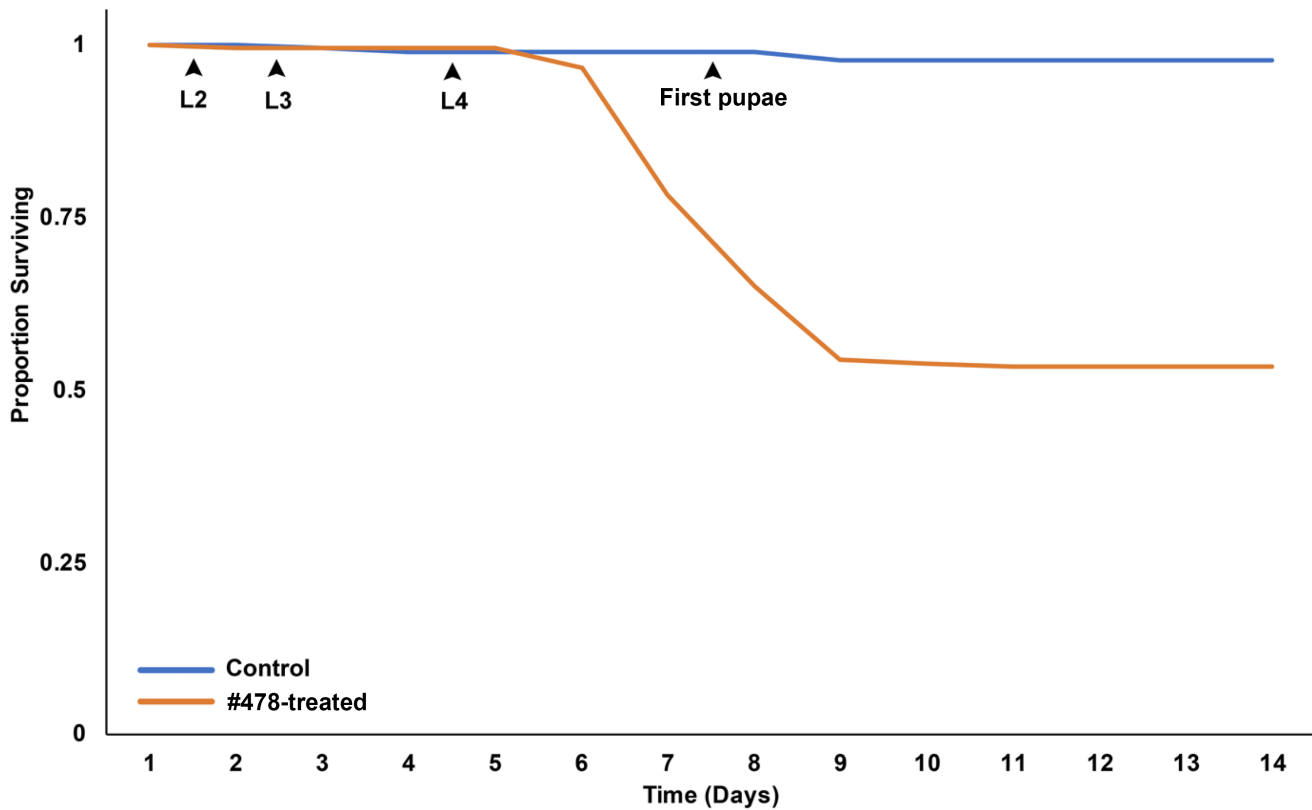


Supplementary Fig. S1. Chromosomal locations of M/m locus region genes containing target sites corresponding to the siRNAs identified in the female-specific lethal screen. Genes shown above the chromosomal schematic are on the forward strand, while those noted below it are on the reverse strand. The genes targeted by larvicide #478 are highlighted in purple. *AAEL027827* (Chromosome 1: 154,564,311-154,565,137 forward strand) and *AAEL027761* (Chromosome 1: 172,956,231-172,956,668 Mb, forward strand) also contain target sites, but are not pictured here. The predicted boundaries of the M (dark blue) locus⁷ are indicated.



Supplementary Fig. S2. Confirmation of shRNA expression in recombinant yeast. cDNA was prepared from total RNA which was extracted from the control or #478 recombinant yeast strains. The cDNA subsequently served as template in PCR reactions in which forward primers corresponding the 3' end of the control (left) or #478 (right) shRNA hairpins and a reverse primer corresponding to the terminator amplified a fragment of ~100 bp (see DNA standard ladder at far left). Amplicons were visualized on an agarose gel stained with ethidium bromide. Negative controls included amplifications with cDNA prepared from yeast strains that had not been transformed with shRNA expression plasmids (marked by – sign), as well as a PCR reactions in which no cDNA had been added (marked by knot symbol; unused primers are visible in both of these negative controls). One of two biological replicate experiments that yielded similar results is shown here.

Survival Curve - #478 - Treated Larvae



Supplementary Fig. S3. Larval survival following treatment with yeast interfering RNA larvicide #478.

A survival curve for larvae that fed on yeast larvicide #478 is shown. Although larvae that were treated with control interfering RNA yeast survived, nearly half of larvae treated with yeast larvicide #478 died prior to pupation. Data were compiled from nine replicate container trials per control or #478 treatment (n = 180 larvae total per treatment). L1 = first instar, L2 = second instar, L3 = third instar, L4 = fourth instar.

siRNA #	Target Sequence	Corresponding M/m Locus Region <i>lncRNA</i> Genes
470	GGUUUACUAAAAUCACUUUCCUUG	AAEL026346
487	GUCACUAAGCUCUAUAAUCAAAUA	AAEL022649
511	GUUUUUUUAUGUUUGCAUCAACAGUU	AAEL023836
525	UGGCAAUUAUCCAAGAACAUCUAC	AAEL028165
526	AAUUUAUCCAAGAACAUCUACAUCU	AAEL028165
540	AAAGUGCAUCAUGUGAUAAAUCGA	AAEL021079
542	AUUAUGAACAACAUGUUUAAAUA	AAEL027827
553	GGCUAUGCAAACCAAUUCAAAUCA	AAEL022711

Supplementary Table S1. Female-specific siRNA larvicides that target single M/m locus region *lncRNA* genes. A number of siRNAs identified in the screen have perfectly conserved 25 bp target sites that are found in singular *lncRNA* genes at or tightly linked to the M/m locus.

siRNA #	Target Sequence	Corresponding M/m Locus Region <i>lncRNA</i> Genes	Corresponding <i>lncRNA</i> Genes outside M/m Locus Region
474	AGAAUCUUCUJACAAUCACUGCCUC	AAEL020580	AAEL024146
478	GACUAAUGUCUGGAAUJAGUAUAAA	AAEL020379, AAEL020813, AAEL022952	
504	CAUCCAACCUUCAAGCGAAUCAGTG	AAEL026407	AAEL021597, AAEL022807, AAEL026655
505	AUUGAGACUUACCAACUGAUCAGUU	AAEL024697	AAEL021470, AAEL027259
506	CAAGUGAAAUAACAUCAAGAUUU	AAEL022756	AAEL024428, AAEL022640
509	GAUAAAGCAUUCAUUCCGCUACUUA	AAEL025698	AAEL021884
514	AGCAGAAAGAUUGAAAUUAAUACCA	AAEL022411, AAEL027761	AAEL023838
516	AGCGUUGAAAAUCUAUAAAAACCU	AAEL026768, AAEL026445	
517	AGCGAUGGAAGAUUGUAAAAUCGA	AAEL026768, AAEL026445	
518	AGUCAGGGUUUAAUUCAUUGUUCGA	AAEL021446, AAEL022173, AAEL023751, AAEL024907, AAEL028165	AAEL022531, AAEL027422
559	AACCAGAAUCGGUAACCUAAAUUGU	AAEL024704, AAEL020975	

Supplementary Table S2. Female-specific siRNA larvicides that target multiple *A. aegypti lncRNA*

genes. A number of siRNAs identified in the screen have perfectly conserved 25 bp target sites that are found in transcripts which correspond to multiple *lncRNA* genes, at least one of which resides in the M/m locus region.

Chromosomal Location	<i>lncRNA</i> Gene	Type
Chromosome 1: 150,998,186-150,999,313 forward strand	AAEL021079	Intragenic (AAEL017331)
Chromosome 1: 151,645,556-151,646,218 reverse strand	AAEL020975	Intragenic (AAEL017331)
Chromosome 1: 151,659,254-151,659,981 reverse strand	AAEL024704	Intragenic (AAEL017331)
Chromosome 1: 152,107,761-152,108,630 reverse strand	AAEL022711	Intragenic (AAEL021838)
Chromosome 1: 152,238,688-152,239,180 reverse strand	AAEL025698	Intragenic (AAEL021838)
Chromosome 1: 152,265,957-152,266,593 reverse strand	AAEL024697	Intergenic
Chromosome 1: 152,315,810-152,316,472 reverse strand	AAEL026407	Intergenic
Chromosome 1: 152,350,018-152,350,537 forward strand	AAEL022756	Intergenic
Chromosome 1: 152,441,886-152,442,268 forward strand	AAEL020813	Intergenic
Chromosome 1: 152,552,655-152,554,770 reverse strand	AAEL020580	Intergenic
Chromosome 1: 152,555,446-152,556,373 forward strand	AAEL026346	Intergenic
Chromosome 1: 152,609,464-152,609,987 reverse strand	AAEL023751	Intergenic
Chromosome 1: 152,908,968-152,909,549 reverse strand	AAEL023836	Intergenic
Chromosome 1: 152,962,655-152,995,751 forward strand	AAEL022649	Intergenic
Chromosome 1: 152,998,785-152,999,264 reverse strand	AAEL022411	Intergenic
Chromosome 1: 153,191,089-153,192,260 forward strand	AAEL026445	Intragenic (AAEL014760)
Chromosome 1: 153,195,585-153,196,259 forward strand	AAEL026768	Intragenic (AAEL014760)
Chromosome 1: 153,276,374-153,276,935 forward strand	AAEL024907	Intergenic
Chromosome 1: 153,284,611-153,285,229 forward strand	AAEL028165	Intergenic
Chromosome 1: 153,287,383-153,289,863 reverse strand	AAEL022173	Intergenic
Chromosome 1: 153,296,153-153,298,858 reverse strand	AAEL021446	Intergenic
Chromosome 1: 153,621,362-153,621,744 forward strand	AAEL020379	Intergenic
Chromosome 1: 153,923,572-153,923,954 forward strand	AAEL022952	Intergenic
Chromosome 1: 154,564,311-154,565,137 forward strand	AAEL027827	Intragenic (AAEL008071)
Chromosome 1: 172,956,231-172,956,668 forward strand	AAEL027761	Intergenic

Supplementary Table S3. Chromosomal locations of M/m locus region *A. aegypti* lncRNA genes

identified in the screen. siRNAs that induced significant death in females correspond identically to 25 bp target sites in 24 M/m locus *lncRNA* genes, the identification numbers and chromosomal locations of which are shown. For intragenic *lncRNA* genes, the genes in which the lncRNA coding sequences are contained are noted.

siRNA #	Target sequence	M/m Locus Region <i>lncRNA</i> Genes
471	GGUUUACUAAAAUCACUUUUCUTG	AAEL026346, AAEL022070
473	CACUUUUCUUGUAAUCCUCAACCAA	AAEL022070
475	GUGCUAUACUUGGAACUUGGCGATT	AAEL022114, AAEL020130, AAEL024877
476	AGAUUUCUCAGUCAACCGAAAU CGA	AAEL022114, AAEL020130, AAEL024877
477	CGAGGACUAAUGUCUGGAAUUAGTA	AAEL020813, AAEL020379, AAEL022952
479	GGAUACCGGUGGACAUUCUAUCUGG	AAEL024877, AAEL020130, AAEL022114
480	UGAAAGGAAUUUACGGAAGAACATT	AAEL022112, AAEL020185
481	GAACCAUUGUCAACAUCGAUAGUGC	AAEL028699
482	AACCAGUACUGAAUUGUCAGAAAGA	AAEL022894, AAEL025485
484	ACCAACUUUAUACAAAGAAAAGGTC	AAEL022321, AAEL025316
485	GUGGUGGUAAUUAUAGACGAAUCAA	AAEL025316
488	AGAUGAACCAUACGCUAGAACAAG	AAEL022649, AAEL014822
489	GUAGUAUCAAUUGAAUUAUUAUUGCT	AAEL017331
492	GAUGAUUUCGAACAGAAUUUUCUGA	AAEL014760
499	GAUGUUGAUGUACAGGUUAUUUCTA	AAEL011832
500	GGACCAACUUUUACUUCAGAUAGA	AAEL011832
503	GGAUCAUUCUCCCAUGCCAUCAA	AAEL026407
507	CUUAGUGGAAUUGAAUUGUAGAUAC	AAEL025579, AAEL027372
508	AUUGUAGAUACCUUGGAUGAAUAAA	AAEL025579, AAEL027372
510	AAGAAUACAAAUCAUCUGCUACCAA	AAEL021974
512	GUAUGAGACAUGACUAAAAAUGTT	AAEL022650
513	GAAGAAACUCUAUUGAUCACAUGAA	AAEL021077
515	GCAGCAUGAUUUCAAUACUUCTACTA	AAEL026785
520	GAUAAAUGUUACUAAAGCUAUUCAA	AAEL023283
524	GGCAAUUUAUCCAAGAACAUCUACA	AAEL028165
527	AAACGAGAAUUUGUGGAAUAGUTG	AAEL026346
528	AAACGAGAAUUUGUGGAAUAGUTG	AAEL026346
541	AGCAUUUGGACAAAUCAAUACAGTA	AAEL021079
543	CAUGUUUAAAUAAAUAAAAAAGCAT	AAEL027827
544	GAGACUGCAAAGAACGUUACUATA	AAEL028113
552	GAGAGAUCCAGUCCAGUUUAAUCGA	AAEL025669

Supplementary Table S4. siRNAs that had no significant impact on male:female ratios. A number of siRNAs corresponding to M/m locus region *lncRNA* genes were evaluated in the screen and found to have no significant impact on male or female *A. aegypti* larval survival.