Supporting Information

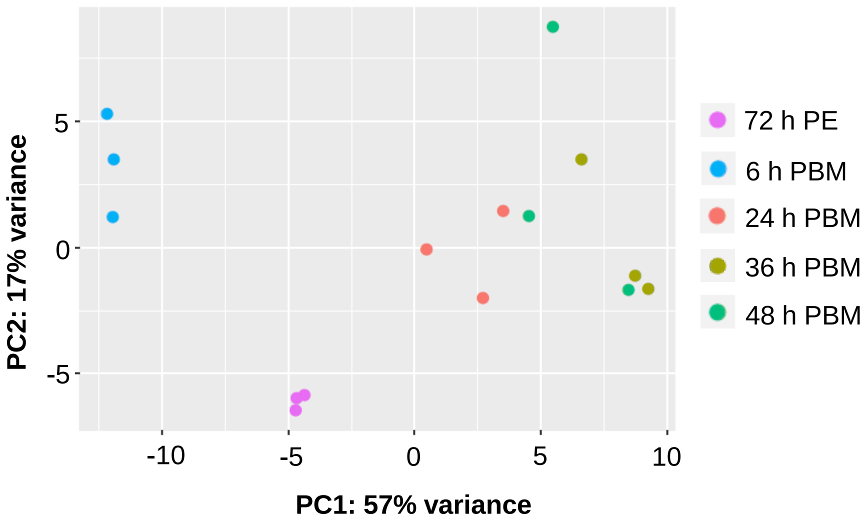

Fig. S1. Principle component analysis (PCA) of three biological replicates of small RNA libraries (DESeq2 package).

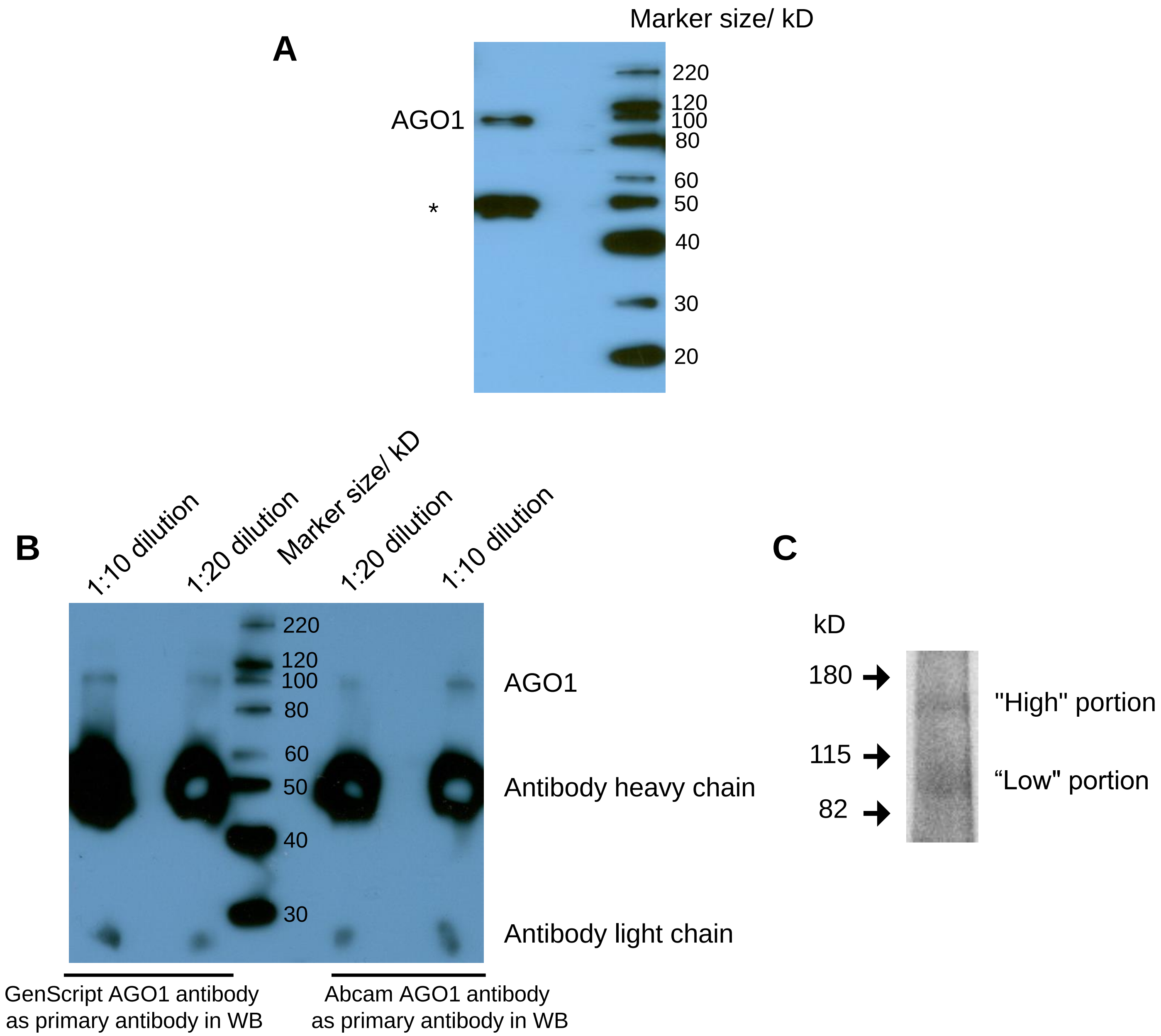

Fig. S2. Examination of AGO1 antibody specificity. (*A*) AGO1 detection in fat body lysates with the *Drosophila* antibody Abcam ab5070 in western blot (*, non-specific band). (*B*) AGO1 immunoprecipitation using the GenScript AGO1 antibody. Diluted elution was probed by either the GenScript AGO1 antibody or the Abcam ab5070 antibody in western blot. (*C*) AGO1-RNA complexes in "High" and "Low" portions during CLIP-seq library preparation.

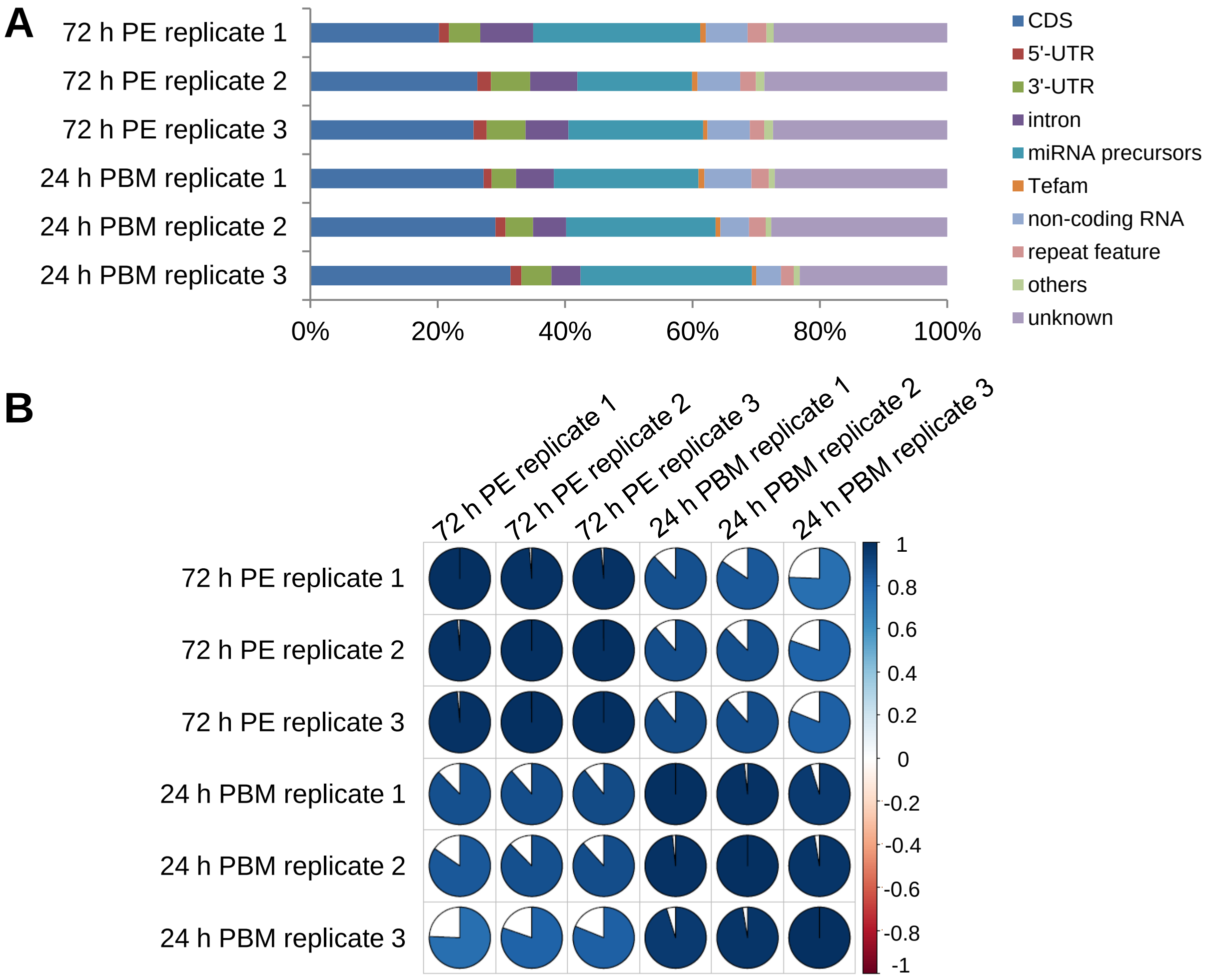

Fig. S3. Overall statistics of AGO1 CLIP-seq library. (*A*) mapping statistics for different annotation categories in AGO1 CLIP-seq libraries; non-coding RNA includes rRNA, tRNA, snRNA and snoRNA. (*B*) sample-wise pearson correlations of AGO1 CLIP-seq libraries based on normalized counts of mature miRNAs.

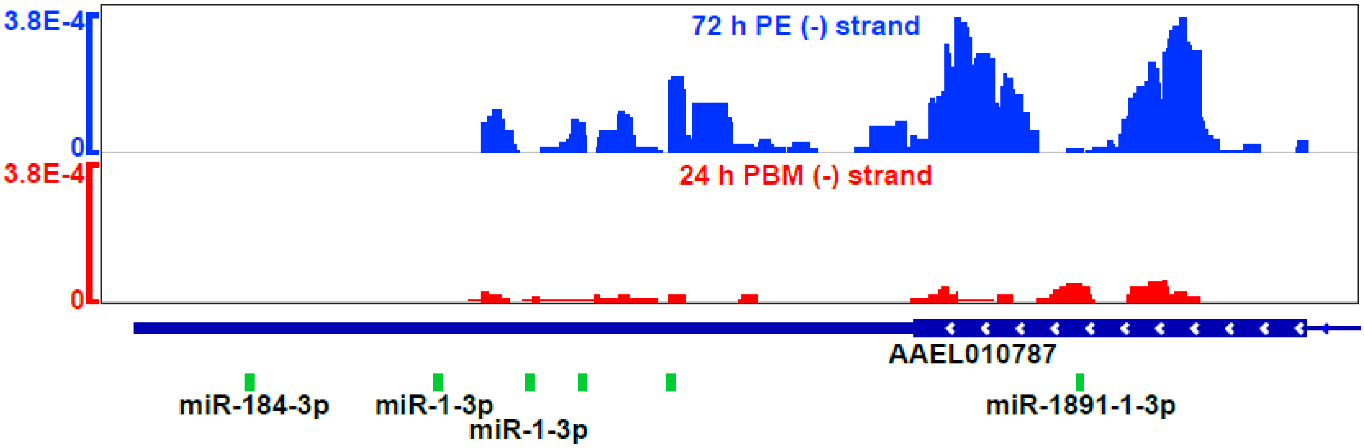

Fig. S4. Example of non-distinct 3' UTR peaks of AAEL010787.

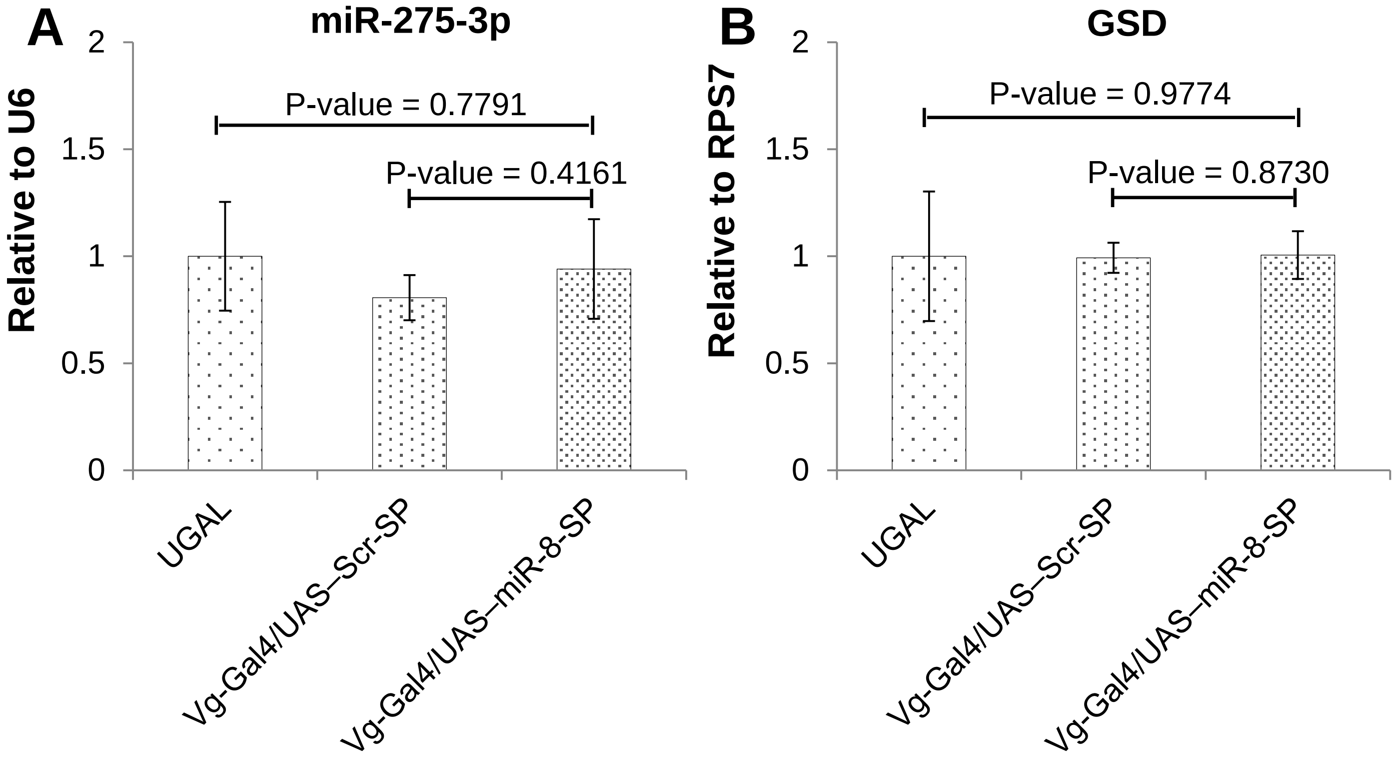

Fig. S5. Quantitative PCR examination of miR-275-3p (*A*) and GSD (*B*) in UGAL, Vg-Gal4/UAS-Scr-SP and Vg-Gal4/UAS-miR-8-SP. Expression levels were normalized to that of U6 for miR-275-3p and that of RPS7 for GSD. Three independent biological replicates were performed. Average of relative expression was taken as 1 from UGAL wild type mosquitoes. Statistical significance was calculated using unpaired t-tests, and expression levels were represented as mean±SD. GSD, AAEL006834, glutamate semialdehyde dehydrogenase.

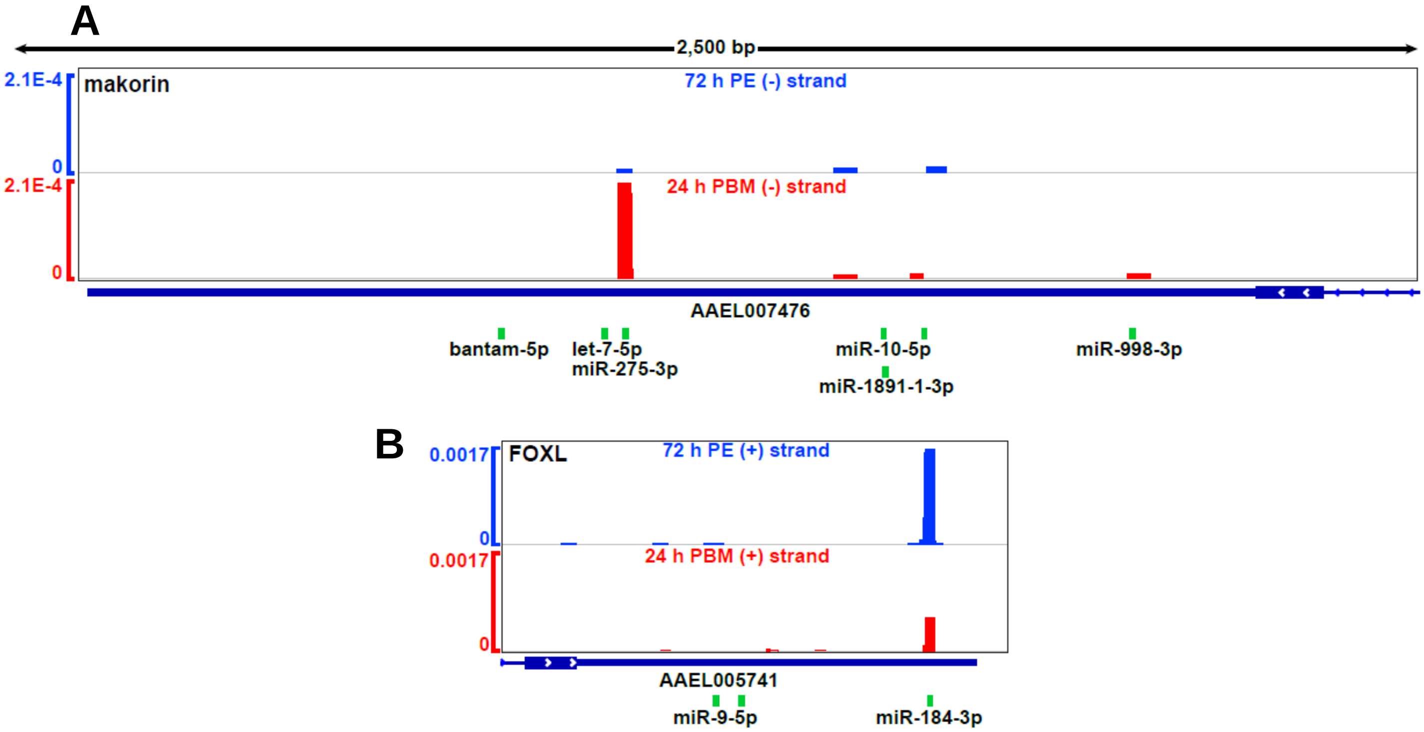

Fig. S6. IGV illustrations of AGO1 CLIP-seq 3' UTR signals for AAEL007476 ((*A*), makorin) and AAEL005741 ((*B*), FOXL).

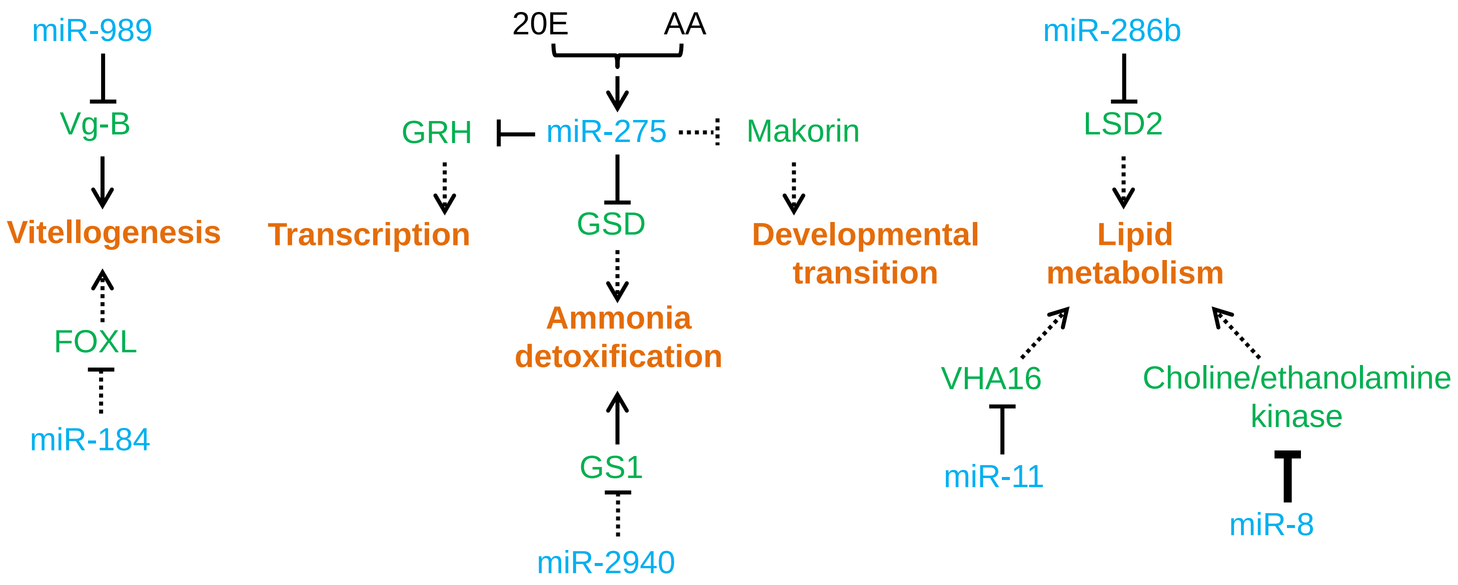

Fig. S7. A proposed miRNA regulatory network in multiple physiological processes in the fat body during the vitellogenic period. Validated miRNA-mRNA interactions from CLIP-seq analyses and the luciferase assay (Fig. 4), as well as previously demonstrated links to physiological processes, are marked with solid lines, while target interactions validated by CLIP-seq only, and proposed connections to physiology, are indicated by dashed lines. The miR-8 - Choline/ethanolamine kinase interaction, validated in CLIP-seq analyses, the luciferase assay and *in vivo* transgenic miR-8 sponge technique, is indicated by a bold solid line. Gene list: Vg-B, AAEL006138, Vitellogenin isoform B; FOXL, AAEL005741, Forkhead-box transcription factor L; GRH, AAEL000577, DNA binding protein elf-1, orthologue of D. melanogaster grainy head; GSD, AAEL006834, glutamate semialdehyde dehydrogenase; GS1, AAEL001887, glutamine synthetase 1; Makorin, AAEL007476; LSD2, AAEL006820, lipid storage droplets surface binding protein 2; VHA16, AAEL000291, V-type proton ATPase 16-kDa proteolipid subunit; choline/ethanolamine kinase, AAEL008853.

Table S1. Mapping statistics of small RNA libraries

Sample	Number of reads	Number of genome aligned	Percentage of aligned reads
72 h PE rep1	23766505	23284580	97.97%
72 h PE rep2	24942979	24297250	97.41%
72 h PE rep3	20776502	20289771	97.66%
6 h PBM rep1	14604621	13616527	93.23%
6 h PBM rep2	21410522	20820661	97.24%
6 h PBM rep3	16406287	15168215	92.45%
24 h PBM rep1	17559341	17017053	96.91%
24 h PBM rep2	22789102	22013117	96.59%
24 h PBM rep3	13390993	12887694	96.24%
36 h PBM rep1	8857422	8558678	96.63%
36 h PBM rep2	22896024	22284705	97.33%
36 h PBM rep3	19140626	18666414	97.52%
48 h PBM rep1	15840623	15385978	97.13%
48 h PBM rep2	30654177	29670297	96.79%
48 h PBM rep3	26209301	25603175	97.69%
Total	299245025	289564115	96.76%

Table S2. miRNA expression profiles in seven clades of unsupervised hierarchical clustering (Fig. 1*B*)*

Clade	miRNA ID	72 h PE	PBM				Expression pattern
			6 h	24 h	36 h	48 h	
V	miR-184	22551.6	18142	13212.9	16932.5	19812	72 h PE peak
V	miR-11-3p	7341.5	8072	7341.2	6408.1	5221.1	72 h PE peak
V	miR-100	2972.9	2155.1	3281.3	1785.8	1747.6	72 h PE peak
V	miR-306-5p	2051.6	2115.3	1943.3	1785.3	1594	72 h PE peak
V	miR-9c-5p	1599.8	1591.1	1092.7	1080.5	948.3	72 h PE peak
V	miR-11-5p	1345.7	1287.2	840.3	967.9	1111.9	72 h PE peak
V	miR-125-5p	556.7	443.1	466.8	446.4	434.6	72 h PE peak
V	miR-998	187.2	188.4	181.8	105.6	86.1	72 h PE peak
V	miR-9b	157.7	177.3	141.2	99.4	102.8	72 h PE peak
V	miR-308-5p	115.3	118.8	87.5	74.3	98.9	72 h PE peak
V	miR-988-3p	108.8	103.3	63.2	53.1	67.3	72 h PE peak
V	miR-9c-3p	87.9	88.5	69.3	59.7	52.2	72 h PE peak
V	miR-iab-4-5p	37.4	35.8	24.9	33.8	33.9	72 h PE peak
V	miR-306-3p	8.7	6	3.1	3.2	9.3	72 h PE peak
V	miR-988-5p	2.4	2.5	1.9	1.6	2.2	72 h PE peak
VII	bantam-3p	15059.9	17800.6	15626.8	14006.6	14306.3	6 h PBM peak
VII	let-7	1272	1394.3	1261.7	1277.3	1273.9	6 h PBM peak
VII	miR-252-5p	875.2	1113.3	963.4	651.9	1019.8	6 h PBM peak
VII	miR-278-5p	118.4	278.5	58.1	60	131	6 h PBM peak
VII	miR-92a-3p	10.3	176.9	10.6	9.2	19.7	6 h PBM peak
VII	miR-252-3p	10.6	14.6	10.5	8.2	9.8	6 h PBM peak

VII	miR-79-3p	5.4	7.4	6.9	3.8	4.3	6 h PBM peak
I	miR-8-3p	123223	127328.5	130780.2	119923.3	109098	24 h PBM peak
I	miR-276-3p	4794.8	5589.9	8010.8	7522.3	6857.2	24 h PBM peak
I	miR-275-3p	1397.5	3306.9	6617	4219.6	3513.4	24 h PBM peak
I	miR-263a-5p	1572.3	1154.6	3519.2	3283.9	2592.2	24 h PBM peak
I	miR-279	1495.5	1755.7	1927.2	1459.2	1136.6	24 h PBM peak
I	miR-34-5p	638.2	937.3	1531.1	659.4	1168	24 h PBM peak
I	miR-305-5p	400.9	852.5	1270	1030.8	770.2	24 h PBM peak
I	miR-996	575.6	685.8	695.2	632.7	549.8	24 h PBM peak
I	miR-999	352.6	386	409	390.3	342	24 h PBM peak
I	miR-989	184.6	0.5	622.4	54.3	223.4	24 h PBM peak
I	miR-305-3p	23.5	252.8	307.8	73.1	64.6	24 h PBM peak
I	miR-1	64.5	139.8	233.4	87.3	151.2	24 h PBM peak
I	miR-263a-3p	30.7	32.3	115.7	67	69.8	24 h PBM peak
I	miR-275-5p	5.9	130.9	124.9	8.8	8.9	24 h PBM peak
I	miR-276-5p	25.2	46.3	74.9	53.2	38.7	24 h PBM peak
I	miR-980-3p	26.5	34.8	42.1	38.5	27	24 h PBM peak
I	miR-308-3p	25.6	45.7	37.2	30.3	19.1	24 h PBM peak
I	miR-33	19.5	29.3	41.9	37.9	16.3	24 h PBM peak
I	miR-282-5p	13.2	13.6	14.1	13.5	12.8	24 h PBM peak
II	miR-317	4305.5	3292.2	5389.7	15057.4	7871.8	36 h PBM peak
II	miR-970	455.4	556.8	511.3	564.6	418.4	36 h PBM peak
II	miR-286b	2.8	0.6	166.3	1017.5	281.3	36 h PBM peak
II	miR-2945-3p	204.6	215.7	216.1	241.3	188.8	36 h PBM peak
II	miR-1889-5p	26.8	40.7	86.3	176.4	62.5	36 h PBM peak
II	miR-1890	43.9	46.3	35	53.9	47.3	36 h PBM peak
II	miR-2944b-5p	0.1	0.1	8.2	80.6	34.4	36 h PBM peak
II	miR-2a-5p	6.4	6	8.3	11.4	7.7	36 h PBM peak
II	miR-2944b-3p	0.7	0.5	1	11.2	3.3	36 h PBM peak
II	miR-993	2.5	2.8	1.9	4.4	3.4	36 h PBM peak
IV	miR-2940-3p	4955.1	5543.5	5160.4	7166	8106.1	48 h PBM peak
IV	miR-281-5p	3107	3284.9	1870.6	3628.6	18603.1	48 h PBM peak
IV	miR-10	1395.1	1100.5	1106.8	1210.5	1721.5	48 h PBM peak
IV	miR-277-3p	785.4	656	844.8	1018.1	982.8	48 h PBM peak
IV	miR-2941	68.8	1.2	581.4	1100.5	1622.9	48 h PBM peak
IV	miR-2c	538.9	565.3	520.4	558.7	670.1	48 h PBM peak
IV	miR-2a-3p	534.1	553.4	508.2	579.9	677.2	48 h PBM peak
IV	miR-2b	538.2	563.9	518.4	558	669	48 h PBM peak
IV	miR-71-3p	434.2	412.3	430.1	828	702.3	48 h PBM peak
IV	miR-9a	289.3	323.4	317.1	316.4	364	48 h PBM peak
IV	miR-34-3p	228.6	152.3	176.8	209	308.3	48 h PBM peak
IV	miR-278-3p	144.5	145.9	182.1	193.9	185.8	48 h PBM peak
IV	miR-92b-3p	145.3	76.3	115.7	141.6	265.4	48 h PBM peak
IV	miR-309a	0.7	0.3	28.5	327.6	256.8	48 h PBM peak
IV	miR-375	23.8	16.1	44	272.6	256.5	48 h PBM peak
IV	miR-316	89.8	88.5	96	119.6	167.3	48 h PBM peak
IV	miR-283	60.5	68.9	133.2	118.1	145.9	48 h PBM peak

IV	miR-2946	13.5	0.3	80.7	157.3	236.9	48 h PBM peak
IV	miR-309b-3p	0.5	0.3	24.4	259.9	197.1	48 h PBM peak
IV	miR-957	43.6	40.4	35.8	63.8	79.5	48 h PBM peak
IV	miR-133	33.3	35.6	31.5	65.5	70.8	48 h PBM peak
IV	miR-87	34.5	34.2	34.3	41.1	60.5	48 h PBM peak
IV	miR-281-3p	14	16	11.3	19.5	84.2	48 h PBM peak
IV	miR-981	12.5	12.7	11	16.2	16	48 h PBM peak
IV	miR-263b-5p	13	11.8	11.2	14.7	14.8	48 h PBM peak
IV	miR-137	10.7	9.6	11.9	11.3	13.3	48 h PBM peak
IV	miR-307	4.7	4.7	5	6.5	8.3	48 h PBM peak
IV	miR-13-5p	3.7	4.2	5.6	6.4	6.3	48 h PBM peak
IV	miR-1000	3.1	3.4	3.2	7.9	6.6	48 h PBM peak
IV	miR-210	2	3.1	1.9	2.8	5.9	48 h PBM peak
IV	miR-929	2.1	2.2	1.8	2.9	3.3	48 h PBM peak
IV	miR-927	2	1.5	1.6	2.4	3.4	48 h PBM peak
III	miR-14	6355.9	7176	6398.4	6854.7	7196	
III	miR-8-5p	2336.8	4777	3555.8	4902.8	4910.4	
III	miR-12-5p	411.8	582	512.4	728.2	720.6	
III	miR-2940-5p	66.1	139.8	120.2	141.7	175	
III	miR-13-3p	82.1	109.9	89.8	104.5	99.9	
III	miR-190	40.3	50.9	37	49.5	48.6	
III	miR-315-5p	9.6	13.2	11.8	17.4	15.2	
VI	bantam-5p	1070.2	1102.6	1141.4	991.7	1401.6	
VI	miR-31	169	187.6	162.7	155.9	203	
VI	miR-1891	60.8	70	84.3	54.3	89.2	
VI	miR-1174	1.3	103.2	16.3	9.9	119.3	
VI	miR-71-5p	11.6	23.8	27.3	15.3	21.3	
VI	miR-12-3p	12.5	23.2	14.2	15.4	20.1	
VI	miR-1175-3p	0.5	20.3	4.2	2.6	33.9	
VI	miR-1175-5p	0.2	13.9	2.3	1.1	26.3	
VI	miR-7	2.3	9.4	7.7	3.5	7.9	
VI	miR-1889-3p	3.2	5.2	3.3	3.4	5	

*Abundance is represented by averages of normalized values from three biological replicates.

Table S3. Mapping statistics of AGO1 CLIP-seq libraries

	72 h PE			24 h PBM		
	rep1	rep2	rep3	rep1	rep2	rep3
Initial reads	21903299	30358134	14017387	49991023	27871705	13223180
Reads >15nt post adaptor trim	14264580	20304524	9361612	33420720	17777337	9829347
Bowtie2 stats						
aligned 0 time	6938446	8741320	3294900	18274983	8476858	2952817
aligned exactly 1 time	1212214	1920106	927267	1985002	1884686	1720546
aligned >1 times	6113920	9643098	5139445	13160735	7415793	5155984
Overall alignment rate	51.36%	56.95%	64.80%	45.32%	52.32%	69.96%

Table S4. Total lengths of 5' UTR, CDS and 3' UTR in AaegL3.3 gene set

	AaegL 3.3 annotations	
	Estimated lengths (nt)	Percentage
5' UTR	2,790,392	8.71%
CDS	24,176,559	75.46%
3' UTR	5,070,301	15.83%
Total	32,037,252	100%

Table S5. The five miRNA families with most identified targets

miRNA family	Number of targets in Dataset S4	miRNA abundance rank	
		72 h PE + 24 h PBM	full time course
miR-279, miR-286b, miR-2945-3p, miR-996	13	15th*	15th*
miR-11-3p, miR-13-3p, miR-2a-3p, miR-2b, miR-2c	11	4th**	5th**
miR-14	11	6th	6th
miR-316	11	49th	51st
miR-8-3p	8	1st	1st

*, ranking of most abundant miRNA individual of the family, miR-279

**, ranking of most abundant miRNA individual of the family, miR-11-3p

Table S6. Significantly enriched GO terms (P-value < 0.05) in ontology of biological process for 140 3' UTR peaks harboring 7mer miRNA seeds

GO ID	Gene Count	GO term
GO:0046394	5	carboxylic acid biosynthetic process
GO:0016053	5	organic acid biosynthetic process
GO:0044711	13	single-organism biosynthetic process
GO:0008652	4	cellular amino acid biosynthetic process
GO:1901607	4	alpha-amino acid biosynthetic process
GO:0044283	6	small molecule biosynthetic process
GO:0044699	35	single-organism process
GO:0009064	3	glutamine family amino acid metabolic process
GO:0009084	3	glutamine family amino acid biosynthetic process
GO:0006650	3	glycerophospholipid metabolic process
GO:0046486	3	glycerolipid metabolic process
GO:0044763	27	single-organism cellular process
GO:0009108	4	coenzyme biosynthetic process
GO:0051188	4	cofactor biosynthetic process
GO:0044765	13	single-organism transport

Table S7. Summary of miRNAs interacting with specific COG functional groups

Functional category	Functional group	Interacting miRNAs*
Information storage and processing	[K] Transcription	bantam-5p, miR-14, miR-184, miR-275-3p, miR-286b
Cellular processes and signaling	[T] Signal transduction mechanisms	miR-11-3p, miR-286b, miR-305-5p, miR-316
	[U] Intracellular trafficking, secretion, and vesicular transport	miR-9c-3p, miR-11-3p, miR-252-5p, miR-286b, miR-1889-5p, miR-2940-3p
	[O] Posttranslational modification, protein turnover, chaperones	miR-8-3p, miR-14, miR-71-3p, miR-275-3p, miR-286b, miR-316, miR-317, miR-2940-5p
Metabolism	[C] Energy production and conversion	miR-8-5p, miR-11-3p, miR-277-3p, miR-317
	[I] Lipid transport and metabolism	bantam-3p, miR-8-3p, miR-71-3p, miR-252-5p, miR-277-3p, miR-283, miR-286b, miR-316, miR-2940-3p
	[E] Amino acid transport and metabolism	miR-31, miR-263a-5p, miR-275-3p, miR-316, miR-2940-5p
	[F] Nucleotide transport and metabolism	miR-8-5p, miR-275-3p, miR-316, miR-317
	[P] Inorganic ion transport and metabolism	bantam-5p, miR-14, miR-71-3p, miR-252-5p, miR-998, miR-1889-5p
	[H] Coenzyme transport and metabolism	miR-14, miR-31, miR-316
	[G] Carbohydrate transport and metabolism	miR-286b
	[Q] Secondary metabolites biosynthesis, transport and catabolism	miR-31

*One miRNA name is listed to represent a unique 7mer seed family. miR-11-3p represents the seed family of miR-11-3p, miR-13-3p, miR-2a-3p, miR-2b and miR-2c. Similarly, miR-286b is listed as that of miR-279, miR-286b, miR-2945-3p and miR-996, while miR-275-3p is for miR-275-3p and miR-306-5p.

Supplementary datasets

Dataset S1. Raw and normalized expression value (RPM) for 124 *Ae. aegypti* mature miRNAs in miRBase v21 from three biological replicates of small RNA libraries.

Dataset S2. miRDeep2 output of candidate loci for new miRNAs from fifteen small RNA libraries.

Dataset S3. Peak calling output from three biological replicates of *Ae. aegypti* fat body AGO1 CLIP-seq libraries at 72 h PE and 24 h PBM.

Dataset S4. DESeq2 output of 3' UTR peaks containing miRNA 7mer complementary sites, normalized by mRNA transcript abundance. Functional groups and categories were assigned based on EggNOG database v4.5 for 140 3' UTR peaks harboring 7mer miRNA seeds.

Dataset S5. DNA/RNA oligo sequences.