Supporting Information

Liu et al. 10.1073/pnas.1416278111

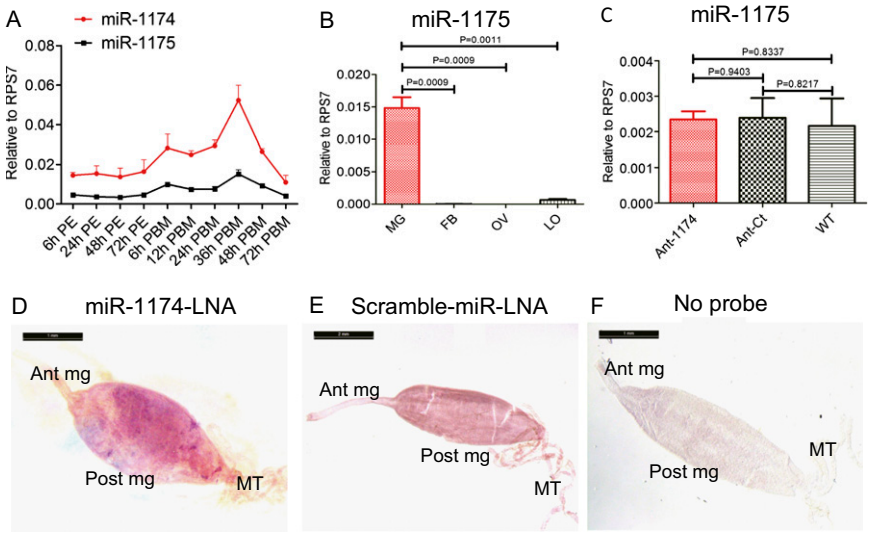

Fig. S1. Expression profiles of miR-1174 and miR-1175 in *Aedes aegypti*. (*A*–*C*) Quantitative real-time (qRT) PCR. (*A*) Expression profiles of miR-1174 and miR-1175 in the midgut during reproductive cycles of female *A. aegypti* mosquitoes. PE, h posteclosion; PBM, h post blood meal. (*B*) Tissue-specific expression of miR-1175. (*C*) miR-1175 levels in treated, as in *B*. Error bars depict ±SEM (*D*–*F*) Whole-mount in situ localization of miR-1174 in the digestive system of the female *Aedes* mosquito. (*D*) In situ hybridizations using a 5′ and 3′ digoxigenin (DIG)-labeled locked nucleic acid-modified DNA oligonucleotide (LNA) complementary to miR-1174 (miR-1174–LNA). (*E*) The Scramble-miR negative control LNA (Scramble-miR–LNA). (*F*) Control untreated mosquito. The digestive system of female mosquitoes consists of anterior midgut (Ant mg), posterior midgut, and stomach (Post mg). The excretory system attached to the digestive system is represented by the Malpighian tubules (MT).

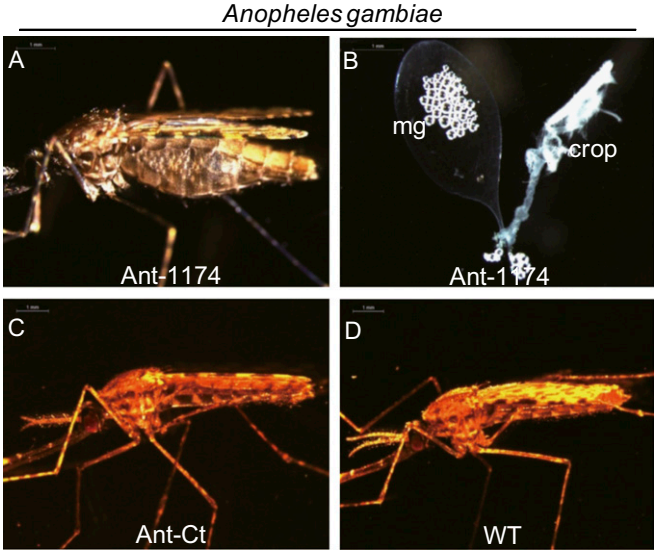

Fig. S2. Effect of miR-1174 silencing in female *Anopheles gambiae* mosquitoes maintained on 10% (wt/vol) sugar solution. (*A*–*D*) Female *A. gambiae* mosquitoes. (*A*) A female mosquito with the bloated-abdomen phenotype after Ant-1174 injection. (*B*) Digestive system isolated from a female mosquito with the bloated-abdomen phenotype. Note an extremely extended crop. mg, midgut. (*C*) A female mosquito injected with control scramble antagomir probe (Ant-Ct). (*D*) Untreated WT mosquito.

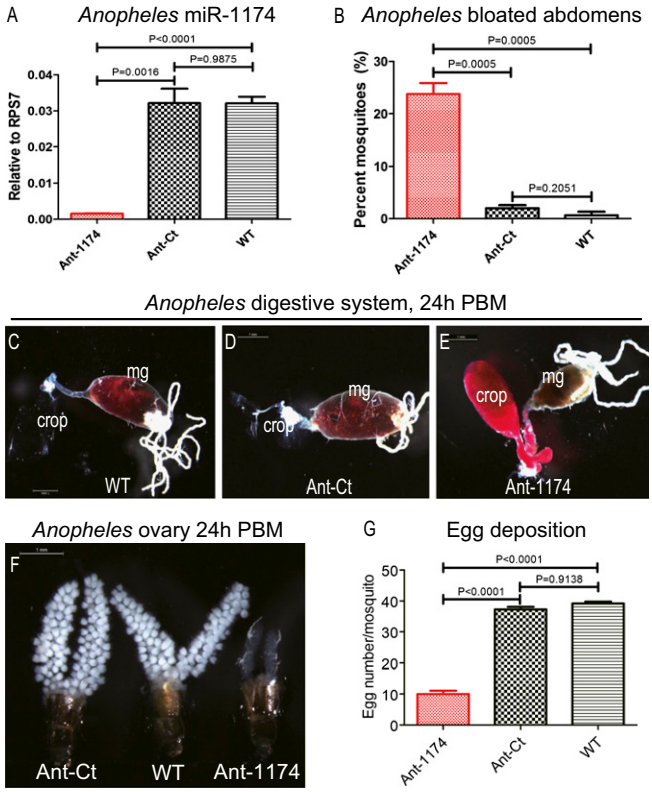

Fig. S3. Effect of miR-1174 silencing in female *A. gambiae* mosquitoes. (*A*) miR-1174 level after antagomir silencing (Ant-1174) compared with the effect of scrambled control antagomir (Ant-Ct) in female *Anopheles* mosquitoes. WT shows miR-1174 level in untreated mosquitoes. qRT-PCR; error bars depict ±SEM (*B*) Percentage of the bloated-abdomen phenotype in female mosquitoes injected with Ant-1174, Ant-Ct, H_2O, or untreated WT. (*C–E*) Isolated digestive systems from mosquitoes 24 h after blood-feeding. (*C*) Untreated WT. (*D*) Ant-Ct–injected. (*E*) Ant-1174–injected. mg, midgut. (*F*) Ovaries isolated 24 h PBM from female mosquitoes injected with Ant-Ct, untreated WT, and Ant-1174. (*G*) Number of eggs laid by female mosquitoes injected with Ant-1174, Ant-Ct, or untreated WT.

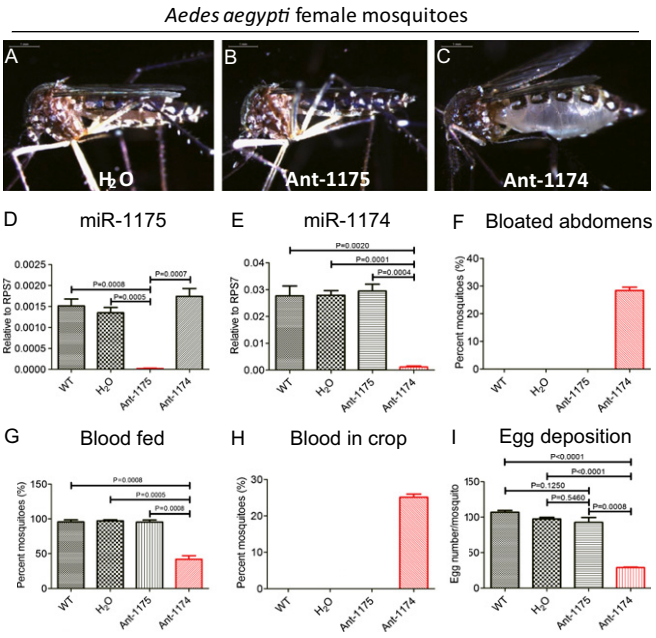

Fig. S4. Silencing of miR-1175 does not exhibit any obvious phenotypes in female *A. aegypti* mosquitoes. (*A*–*C*) Female mosquitoes injected with H_2O, Ant-1175, or Ant-1174 and maintained on 10% sugar solution for 3 d. Note the lack of bloated-abdomen phenotype in Ant-1175–injected mosquito. (*D*) miR1175 levels after injections of H_2O, Ant-1175, or Ant-1174. miR-1175 level in WT mosquitoes serves as a control. qRT-PCR; error bars depict ±SEM. (*E*) miR-1174 levels after injections of H_2O, Ant-1175, or Ant-1174. miR-1174 level in WT mosquitoes serves as a control. qRT-PCR; error bars depict ±SEM. (*F*) Percentage of the bloated-abdomen phenotype female mosquitoes injected with H_2O, Ant-1175, or Ant-1174. WT mosquitoes serve as a control. (*G*) Percentage of blood-fed mosquitoes after indicated treatments. (*H*) Percentage of mosquitoes with blood in crop in experiment *G*. (*I*) Number of eggs laid by female mosquitoes after indicated treatments.

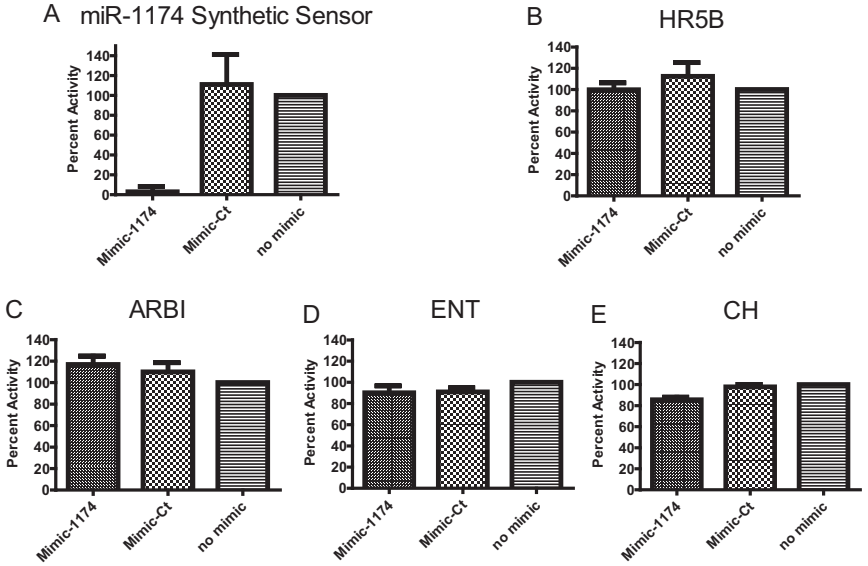

Fig. S5. miR-1174 directly targets AAEL002510, Serine hydroxymethyltransferase 3′UTR in vitro. (*A*) miR-1174 synthetic sensor containing three sites complementary to miR-1174 with four nucleotide linkers between each miR-1174 binding site used as a positive control. (*B*) AAEL012079, Heat Repeat Containing 5B. (*C*) EL001779, Apoptosis-related Bax inhibitor. (*D*) AAEL005411, Equilibrative nucleoside transporter. (*E*) AAEL010558, Conserved hypothetical. Data represent the percentage activity (Δ Fold Activity × 100) average ± SEM of triplicate samples.

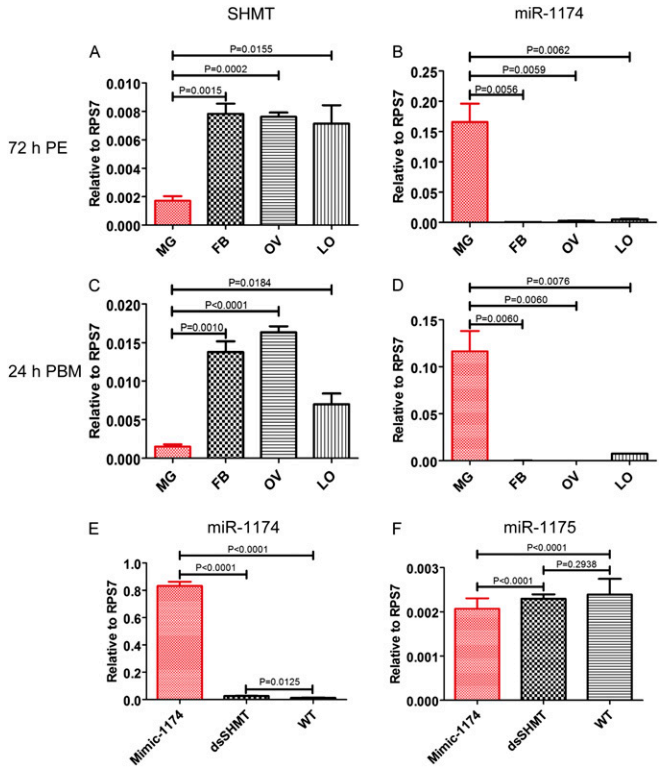

Fig. S6. Expression levels of *serine hydroxymethyltransferase (SHMT)* transcript and miR-1174 in different tissues of female *A. aegypti*. Tissues were collected from mosquitoes at 72 h posteclosion (PE) (*A* and *B*) and at 24 h PBM (*C* and *D*). qRT-PCR; error bars depict ±SEM. (*E* and *F*) miR-1174 and miR-1175 levels in females used in the experiment in Fig. 4*C*. qRT-PCR; error bars depict ±SEM.

Table S1. Top five targets selected for Dual Luciferase Reporter Assay validation

Gene ID	Ortholog ID	Gene name	Abbreviation	Species	Programs
AAEL002510	AGAP004900	Serine hydroxymethyl transferase	SHMT	Aae	IN; TS; PITA; MR; RH
AAEL012079	AGAP002215	Heat Repeat Containing 5B	HR5B	Aae	IN; TS; PITA; MR; RH
AAEL001779	AGAP005775	Apoptosis-related Bax inhibitor	ARBI	Aga	IN; TS; PITA; MR; RH
AAEL005411	AGAP003892	Equilibrative nucleoside transporter	ENT	Aae, Aga	IN; TS; PITA; MR
AAEL010558	AGAP000964	Conserved hypothetical	CH	Aae, Aga	IN; TS; PITA; MR

Abbreviation, gene name abbreviation; Species, species in which the target was detected; Programs, programs that detected a gene target: Aae, *A. aegypti*; Aga, *A. gambiae*; IN, "in-house"; TS, TargetScan; PITA, Probability of Interaction by Target Accessibility; MR, miRanda; RH, RNAhybrid.