Supporting Information

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SI Material and Methods

Electrophoretic Mobility Shift Assay. A 10-pM quantity of double-stranded oligonucleotide was end labeled with T4 DNA kinase and 50 μCi [γ-^{32}P] ATP (PerkinElmer). The unincorporated radioactivity was removed through a Sephadex G-25 (Amersham Pharmacia Biotech) spin column. Reactions were carried out in a 20-μL volume containing 4 μg nuclear extracts, 10 mM Tris·HCl (pH 7.5), 50 mM NaCl, 1 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM EDTA, 4% (vol/vol) glycerol, and 1 μg poly(dI-dC)·poly (dI-dC). Nuclear proteins were preincubated with 100-fold excess of unlabeled competitor DNA or 3 μg antibodies. After 20-min incubation at 4 °C, 0.05 pmol of [^{32}P] labeled DNA probe (~10,000 cpm) was added, and the incubation continued for another 30 min at room temperature. The reaction mixture was resolved using a 6% nondenaturing polyacrylamide gel at a constant voltage of 100 V for 90 min at 4 °C. The gel was dried, and the protein–DNA complexes were visualized by PhosphorImager analysis. Oligonucleotides (only sense strands are shown) used to generate the probe and competitor DNA were as follows:

AaET JHRE: 5′-ccatcCCACACGCGAAGacgataaaacca-3′;
Nonspecific competitor: 5′-GATCCAGATTAGGATAGCA-TATGCTACCCAGATATA-3′

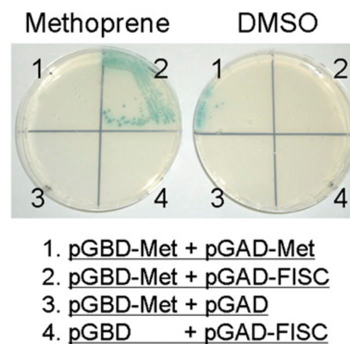

Fig. S1. AaMet-AaFISC and AaMet-AaMet interactions detected in yeast two-hybrid assays. The indicated plasmids were cotransformed into AH109 and plated on SD/-Trp/-Leu/-His/-Ade/Xα-Gal plates containing either 10^{-6} M methoprene or DMSO (solvent) only. After ~5 d, blue colonies appeared on the plate.

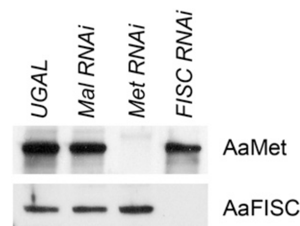

Fig. S2. Depletion of AaMet and AaFISC in adult mosquitoes by RNAi. Newly emerged adult female mosquitoes were injected with dsRNAs corresponding to *AaMet*, *AaFISC,* or bacterial *MalE* gene. Uninjected (*UGAL*) mosquitoes were also used as control. Then, 4 d after injection, midguts were collected from the mosquitoes. Protein extracts were analyzed by immunoblotting.

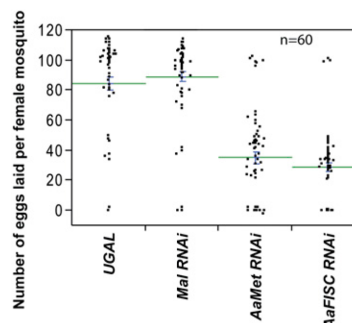

Fig. S3. RNAi-mediated knockdown of AaMet and AaFISC decreases egg deposition. Newly emerged adult female mosquitoes were injected with dsRNAs corresponding to *AaMet*, *AaFISC* or bacterial *MalE* gene. Dots represent egg counts for individual mosquitoes within 10 d after the first blood meal. Green bars represent median number of eggs oviposited from three replicates; short blue bars indicate SEs. AaMet-and AaFISC-depleted mosquitoes lay significantly fewer eggs ($P < 0.001$) than *Mal RNAi* mosquitoes and untreated control mosquitoes (*UGAL*). Data were analyzed using JMP8 software.

Fig. S4. Functional analysis of *AaET* promoter. (*A*) The 2.0-kb upstream regulatory region of *AaET* was cloned into the pGL3 basic vectors. L57 cells were transfected by the reporter plasmid and expression vectors for the indicated proteins. After transfection, cells were cultured in medium with 5×10^{-6} M JH-III or farnesol. (*B*) L57 cells were transfected by the expression vectors for AaMet and AaFISC, together with the indicated derivative reporter constructs. Activity of reporter gene was measured by dual luciferase reporter assay.

AhR/Arnt binding site	CACGCCAGC
AaET JHRE	CCACACGCGAAG
Motif 2	CACACGCACGAACGC

Fig. S5. Sequence alignment of JHRE and an AhR/Arnt binding site. AhR/Arnt is a mammalian bHLH-PAS dimer that activates the transcription of a battery of genes encoding proteins involved in xenobiotic metabolism. AhR/Arnt binding site (5′ GCGTG 3′) is from version 8.3 of TRANSFAC. JHRE sequence is also similar to motif 2 identified in JH-inducible promoters in the *Drosophila* L57 cells and in the honey bee, *Apis mellifera* (1).

1. Li Y, Zhang Z, Robinson GE, Palli SR (2007) Identification and characterization of a juvenile hormone response element and its binding proteins. *J Biol Chem* 282:37605–37617.

Fig. S6. JH response of *AaET* core promoter. Two reporter genes were constructed using the pGL3 basic reporter vector. The first gene contains a 140-bp (nt −77 to +63) core promoter of *AaET*; the second gene carries four copies of JHRE in addition to the core promoter sequence. L57 cells were transfected by the expression vectors for AaMet and AaFISC, together with one of the reporter constructs. After transfection, cells were cultured in medium with 5×10^{-6} M JH-III or farnesol.

Fig. S7. Endogenous Taiman in L57 cells affects the transactivation function of AaMet. L57 cells were diluted to 2×10^6 cells/mL in serum-free medium. A 100-μL quantity of suspension was mixed with 38 nM dsRNA corresponding to *EGFP* or *DmTaiman* and transferred to a single well of a 48-well cell culture plate. After 1 h incubation at room temperature, 200 μL medium containing 7.5% FBS was added to the cells. Three days later, the cells were transfected by the UAS×4–188-cc-Luc plasmid together with the indicated expression vectors (*A*). After transfection, cells were cultured in medium with 5×10^{-6} M of JH-III or farnesol. Dual luciferase assays were performed to measure the reporter activity. Depletion of Taiman was confirmed by Western blot analysis (*B*). Polyclonal antibodies against Taiman were a kind gift from Denise J. Montell. *Tai* dsRNA 1 and *Tai* dsRNA 2 represent samples from two independent biological replicates.