Supporting Information

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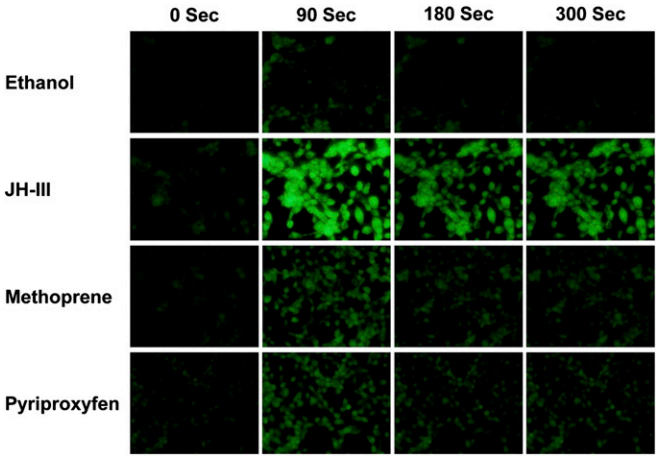

Fig. S1. JH-induced increase in intracellular calcium concentration in Aag2 cells. The calcium indicator Fluo-8 AM was added to the culture medium 45 min before Aag2 cells were stimulated with the indicated chemicals (1 μM) or ethanol (0.1%). Images were captured at 0, 90, 180, and 300 s after the treatments. The experiment was performed three times with similar results.

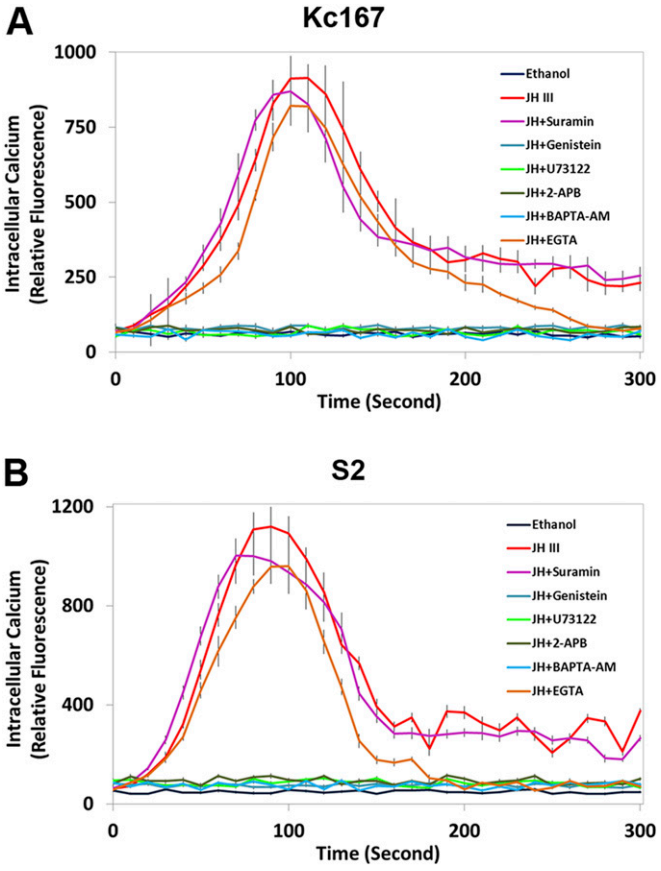

Fig. S2. The JH-induced increase in intracellular calcium requires the function of PLC in *Drosophila* Kc167 (*A*) and S2 (*B*) cells. Cells were treated with 1 μM of JH-III and the indicated chemicals that blocked the PLC–Ca^{2+} signaling pathway. Ethanol was used as a negative control. Intracellular calcium was measured using the fluorescent calcium marker Fluo-8 AM. Results are the mean ± SD of three independent experiments.

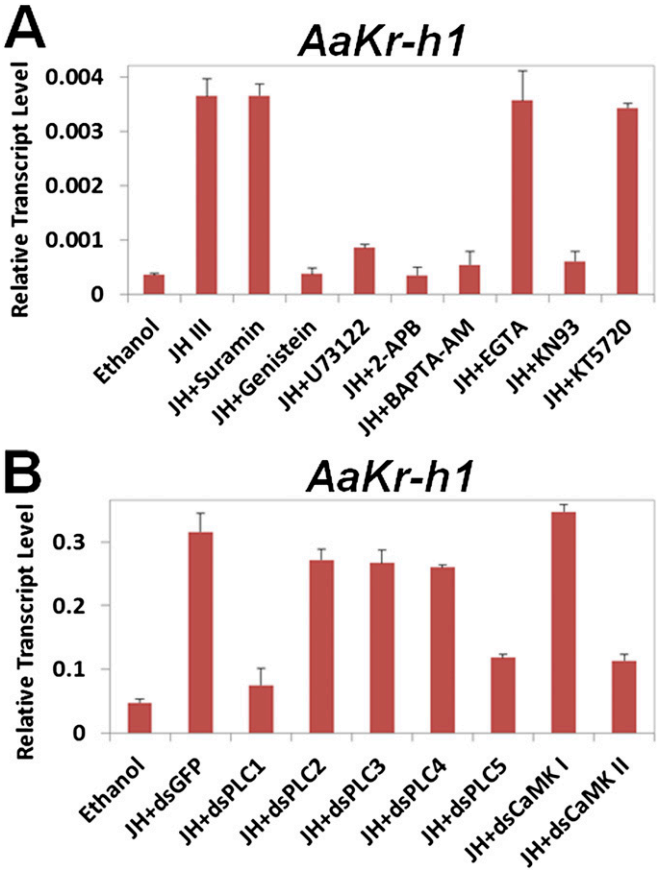

Fig. S3. Disruption of the PLC/Ca^{2+}/CaMKII pathway represses the JH-induced expression of *AaKr-h1* in Aag2 cells. (*A*) After cells were preincubated with the indicated inhibitors for 45 min, 1 μM JH-III was added to the culture medium. The cells were collected for RNA extraction 1 h after the addition of JH. The mRNA levels of *AaKr-h1* were determined by using real-time PCR. Results are the mean ± SD of three independent experiments. (*B*) Aag2 cells were transfected with dsRNAs against GFP (a negative control), individual PLC isoforms, CaMKI, or CaMKII. Two days after the transfection, cells were treated with 1 μM JH-III for 1 h. The mRNA transcripts of *AaKr-h1* were analyzed by real-time PCR. Results are the mean ± SD of three independent experiments.

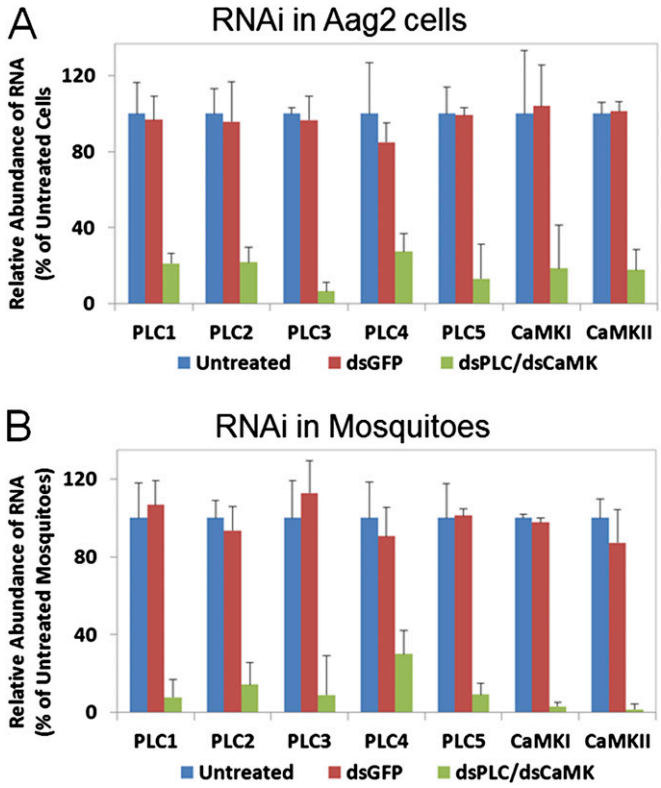

Fig. S4. RNAi-mediated knockdown of PLC isoforms and CaMKII. (*A*) DsRNAs for GFP, individual PLC isoforms, CaMKI, and CaMKII were added to the cultured Aag2 cells. Cells were collected 48 h after the addition of dsRNA. RNAi efficiency was assessed by real-time PCR. The amount of the dsRNA-targeted transcript in the untreated cells was set as 100%. Results are the mean ± SD of at least three independent experiments. (*B*) DsRNAs were injected into newly emerged female mosquitoes. RNAi efficiency was evaluated 72 h after injection. The amount of the dsRNA-targeted transcript in the untreated mosquitoes was set as 100%. Results are the mean ± SD of at least three independent experiments.

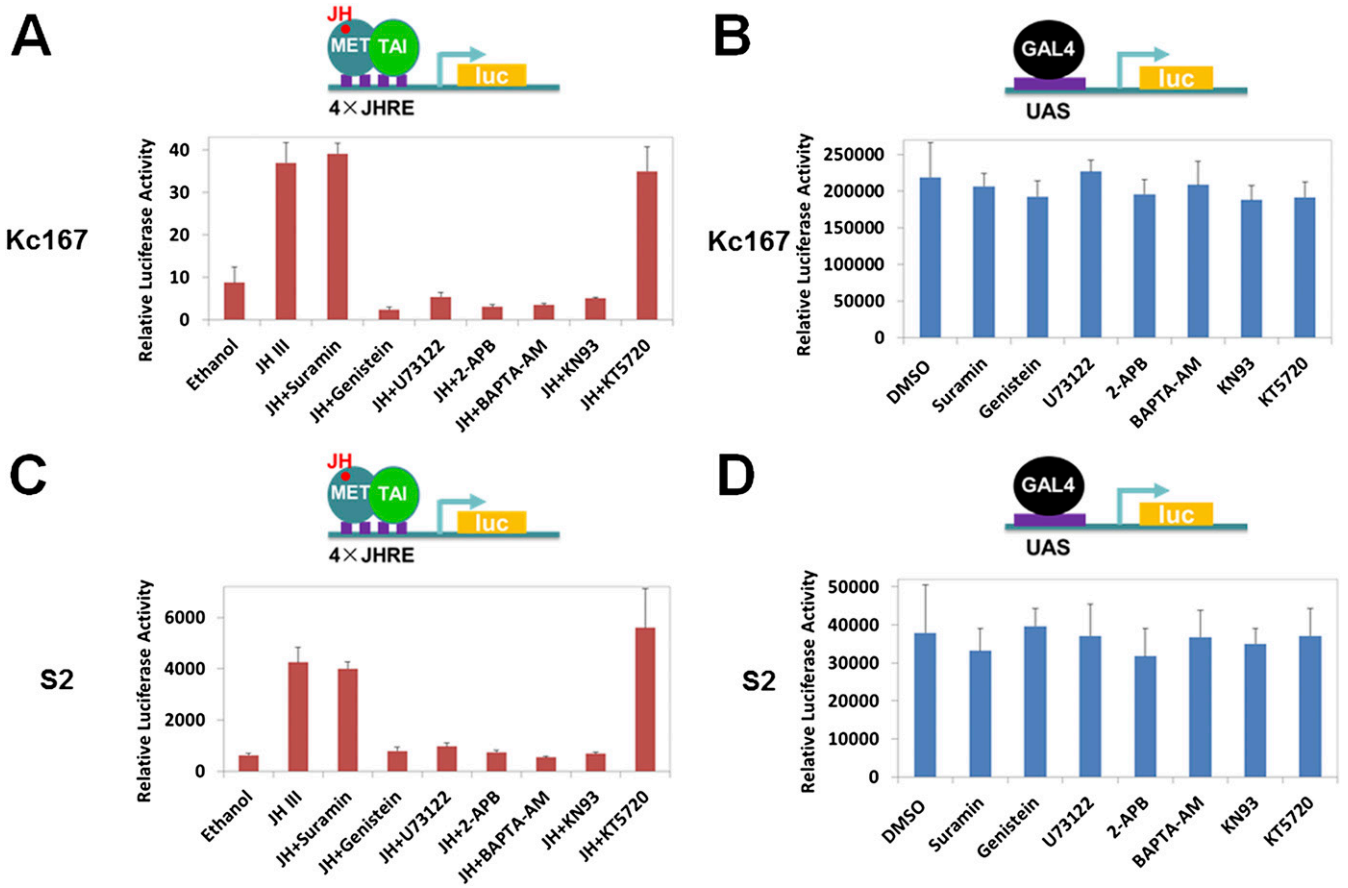

Fig. S5. The PLC/Ca^{2+}/CaMKII pathway modulates the MET/TAI-mediated gene expression in *Drosophila* cells. (*A* and *C*) Kc167 (*A*) and S2 (*C*) cells were transfected with expression plasmids for AaMET and AaTAI, together with a 4×JHRE-luc firefly luciferase reporter construct and a constitutively expressing Renilla luciferase construct. Transfected cells were incubated with indicated inhibitors for 1 h followed by treatment with 1 μM JH-III for 4 h. Results are expressed as the ratio of firefly to Renilla luciferase activity. Results are the mean ± SD of at least three independent experiments. (*B* and *D*) Kc167 (*B*) and S2 (*D*) cells were transfected with an expression vector for GAL4, together with a 4×UAS-luc firefly luciferase reporter construct and a constitutively expressing Renilla luciferase construct. Transfected cells were treated with the indicated inhibitor and JH-III as described for *A* and *C*.

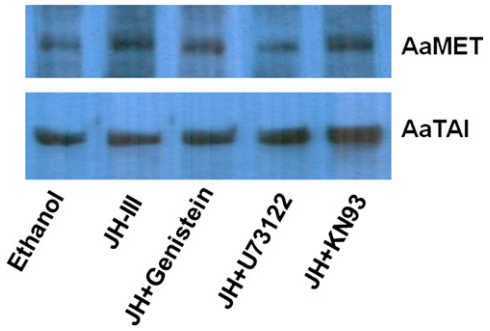

Fig. S6. The protein levels of AaMET and AaTAI are not affected by the PLC pathway. Abdomens from newly emerged mosquitoes were cultured in vitro with Genistein, U73122, or KN93 for 1 h. After 1 μM JH-III was added to the medium, the culture continued for additional 3 h. The amounts of AaMET and AaTAI in the cultured fat bodies were analyzed by Western blot.

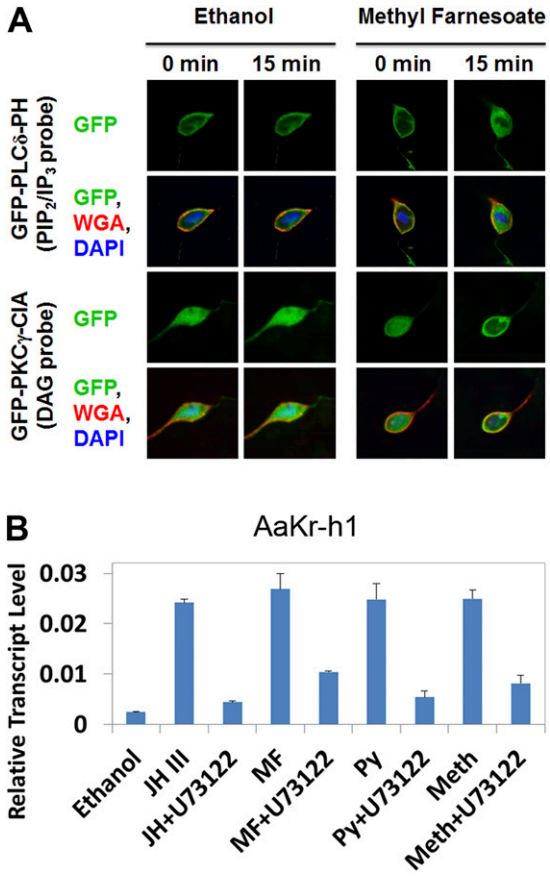

Fig. S7. MF regulates the expression of JH target genes via the PLC pathway. (*A*) MF stimulates the PLC-mediated hydrolysis of PIP_2. Aag2 cells were transfected with plasmids encoding GFP-PLCδ-PH or GFP-PKCγ-C1A and were stained with DAPI (blue) and WGA (red). The PLCδ PH domain binds to PIP_2 and IP_3 with high affinity. The PKCγ C1A domain has a high affinity for DAG. Subcellular translocation of the GFP reporters after treatment with 1 μM MF was captured using a confocal microscope at 1,000× magnification. Representative images are shown. (*B*) Fat bodies from newly emerged mosquitoes were cultured in vitro with the PLC inhibitor U73122 (1 μM) for 1 h. Then JH-III, MF, pyriproxyfen (Py), or methoprene (Meth) was added to the culture medium to a final concentration of 1 μM, and the fat bodies were cultured for another hour and collected for RNA extraction. Expression of *AaKr-h1* was analyzed by real-time PCR. Results are the mean ± SD of three independent experiments.

Table S1. Isoforms of phospholipase C and calcium/calmodulin-dependent protein kinases

Gene	Accession number	Description
PLC1	AAEL004431	*Ae. aegypti* phospholipase Cγ
PLC2	AAEL008246	*Ae. aegypti* phospholipase Cε
PLC3	AAEL009380	*Ae. aegypti* phospholipase Cβ
PLC4	AAEL017393	*Ae. aegypti* phospholipase C, PH-PLC β domain
PLC5	AAEL010124	*Ae. aegypti* phospholipase C, PI-PLC γ domain
CaMKI	AAEL011441	*Ae. aegypti* calcium/calmodulin-dependent protein kinase I
CaMKII	AAEL013824	*Ae. aegypti* calcium/calmodulin-dependent protein kinase II

Table S2. Primers used in ChIP, qRT-PCR, and RNAi assays

Assay	Primer	Forward sequence (5′–3′)	Reverse sequence (5′–3′)
ChIP	Kr-h1-Pro	TTCCGCGGCCAGTCCTCGACAAA	CTCTCTGCTGCTGCTGCTCACTGA
	Kr-h1-CDS	TTCTGGAATGTGGATTGTTGA	CCTTTGCTTTCGTTCACTCA
	ET-Pro	GTTTTGAAATTACCCATCCCACACG	GTCCATTCCTATGATGCGGATTCTT
	ET-CDS	GTAAGGATTCTTGCCAGGGAGACTC	ATCCATTGGCGAACAGTGGACAC
RT-PCR	Kr-h1	TTCTCGCAACAACAGCAACATCCG	TCATCAGATCCATTGACGCTGGGT
	ET	AATACAGATCCTGCGGCCTA	CCTCACCCGCAGTATAATGG
	AAEL002576	CTCGTGGGAATGGGCATCTT	AAGTAACCGTTGCGAGGGAG
	AAEL002619	AGCCCCAACTTGTGTGTAGG	CTTCTGGGTGTGGTGGTCTC
	PLC1	AGCTCGGTTTCGACGACTTT	TTCCGAGCGGATCGTTTTGT
	PLC2	CCACGCTCTCAACTGGTTTG	TTCGCGTGGGACACACTATG
	PLC3	GCCAAGGAACGATCGGACAA	TCTGCTGCTTCTGCACTCCCG
	PLC4	CGAGCCGAACAAGTTCAACG	AGTGTGACAGCGGTTGATCC
	PLC5	CCAAAGAGACTGAAGCGGGT	CACGTGACAACTGGTGCTTG
	CaMKI	GGAACAGGAGCGTTTTCGGA	ATCGCTTCAGCACACGGATT
	CaMKII	GCGGCCAAGATCATCAACAC	CGGTCACCAGATCGAACACT
	GFP	TAATACGACTCACTATAGGGGCTGTTAAAAGTGGATGATGATAC	TAATACGACTCACTATAGGGAATCGGCACCTTGGTAGAACGATC
	RpS7	TCAGTGTACAAGAAGCTGACCGGA	TTCCGCGCGCGCTCACTTATTAGATT
RNAi	dsPLC1	TAATACGACTCACTATAGGGAGAAGCTCATGACATCGCCCAA	TAATACGACTCACTATAGGGAGCTCCGGGAGCTTCTTGTGTT
	dsPLC2	TAATACGACTCACTATAGGGAGTCAGCGACGATGAATACGAGGAT	TAATACGACTCACTATAGGGAGCGATGAACATTGGTAACAGGGAT
	dsPLC3	TAATACGACTCACTATAGGGAGAACTGGACAGCGTCAAGAAGAA	TAATACGACTCACTATAGGGAGCACCGTTTCAAGCGTTACAATC
	dsPLC4	TAATACGACTCACTATAGGGAGACTTAATGTCGGAGGATAACCCA	TAATACGACTCACTATAGGGAGGGTGCTGGTGGAGATGATTGTT
	dsPLC5	TAATACGACTCACTATAGGGAGAAACAGGCTACCACGTTGCTGA	TAATACGACTCACTATAGGGAGAGAACGCTTATCCAACTCACTC
	dsCaMKI	TAATACGACTCACTATAGGGAGCGGGTCAGGCATTTGCTGTTAA	TAATACGACTCACTATAGGGAGACTGCTTTGCCGTATGGCTTTT
	dsCaMKII	TAATACGACTCACTATAGGGAGCAACATCCGAACATTGTCAGGC	TAATACGACTCACTATAGGGAGCTGGTGGCGAGCATCGTAGTGA
	dsGFP	TAATACGACTCACTATAGGGGCTGTTAAAAGTGGATGATGATAC	TAATACGACTCACTATAGGGAATCGGCACCTTGGTAGAACGATC