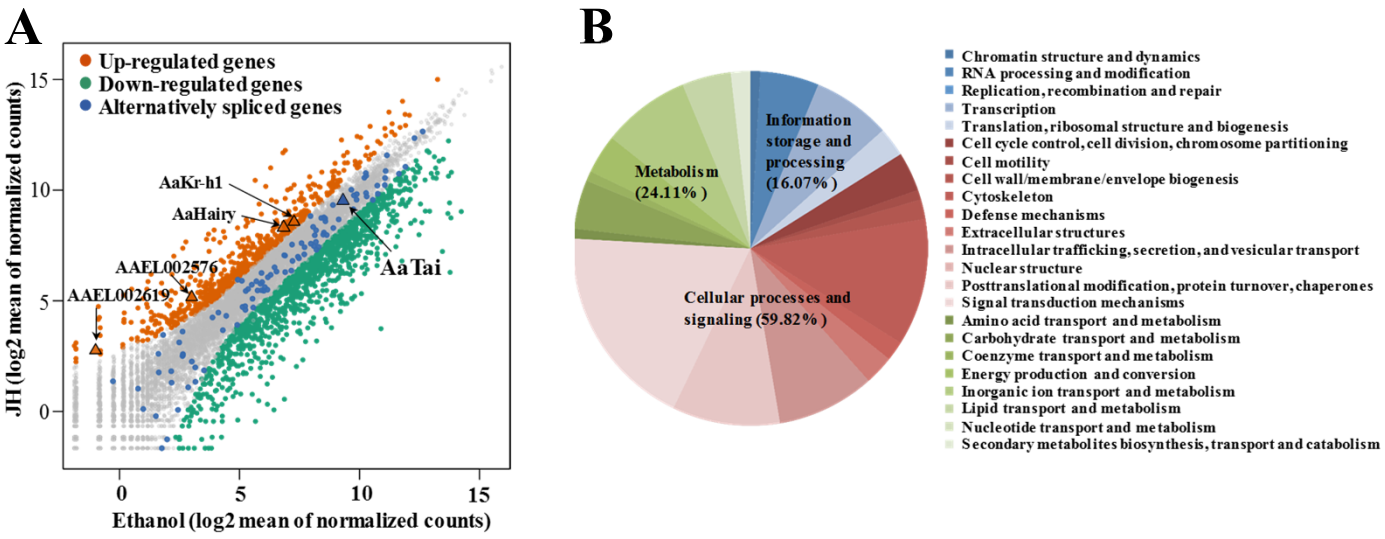

Figure S1. RNA-Seq analysis of differential splicing. (*A*) Transcriptomic changes induced by the JH treatment. Normalized reads from DESeq2 were averaged within each group. Genes showing significant changes in mRNA abundance (Fold change ≥2, $p<0.05$) are colored in orange (up-regulated) and green (down-regulated). Differentially spliced genes identified by rMATS are labeled with blue dots. (*B*) Functional groups of the differentially spliced genes in response to JH. The pie chart shows the percentage of genes that fall within each functional group by annotation using the eggNOG database.

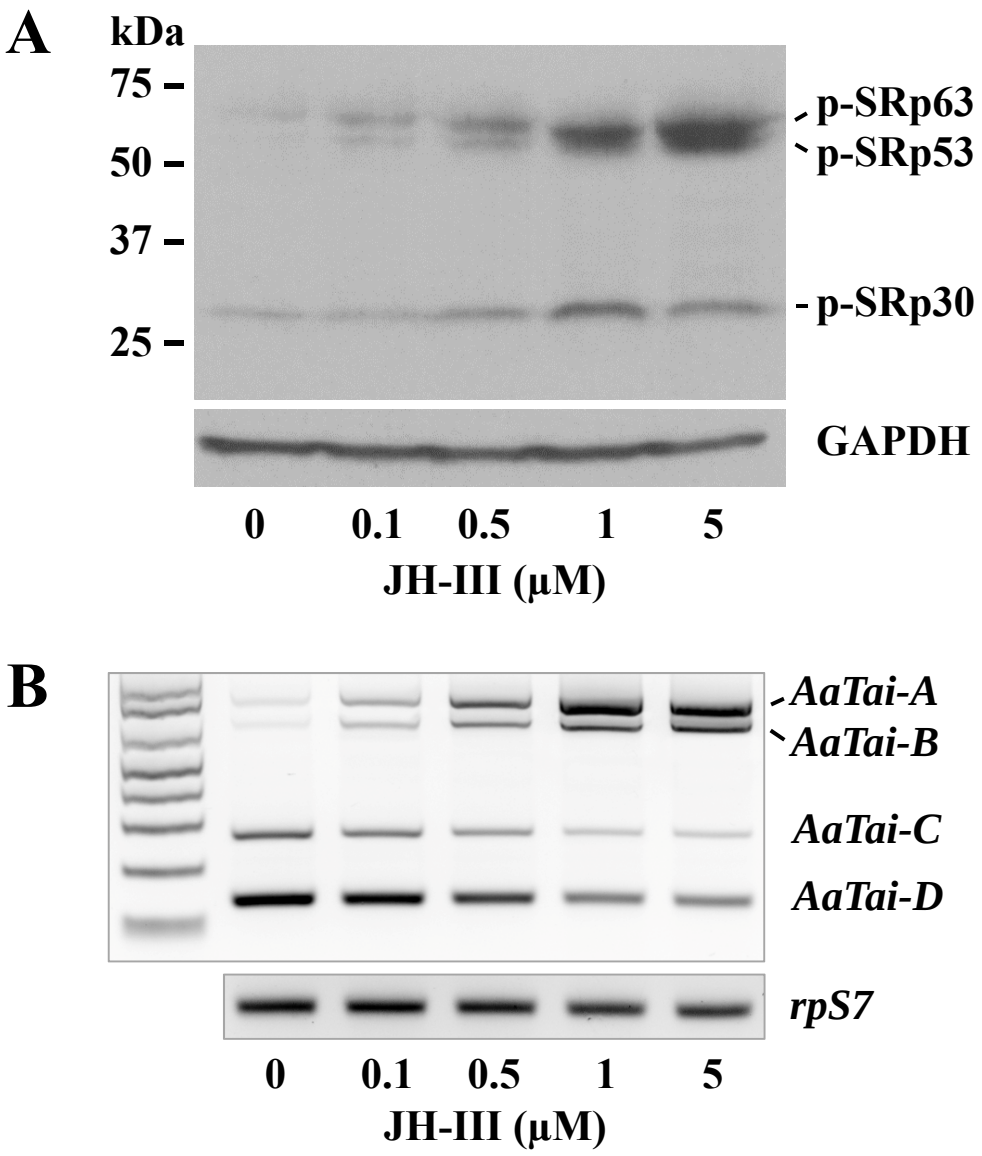

Figure S2. Dosage effect of JH on the phosphorylation of SRSFs and the splicing of *taiman*. Fat bodies were isolated from female mosquitoes at 30 min PE and were cultured *in vitro* for 4 h with JH-III at the indicated concentrations. (A) The phosphorylation of SRSFs was detected by immunoblotting. (B) The mRNA levels of individual *AaTai* isoforms were assessed by using RT-PCR.

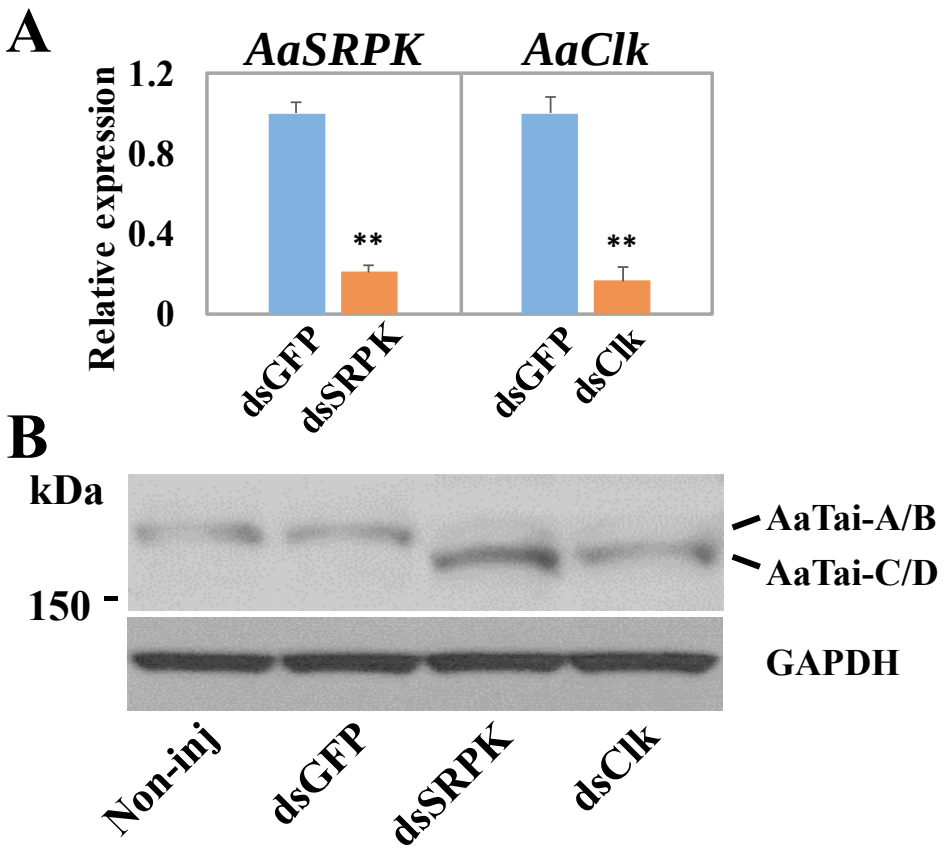

Figure S3. Knockdown of *AaSRPK* or *AaClk* thwarts the generation of AaTai-A/B proteins in the previtellogenic fat body. DsRNAs of *AaSRPK* or *AaClk* were injected into newly emerged female mosquitoes. (*A*) The knockdown efficiencies for *AaSRPK* and *AaClk* were evaluated by using quantitative RT-PCR at 96 h PE before *in vitro* fat body culture. DsRNA corresponding to the green fluorescent protein gene (dsGFP) was used as a control. ** denotes statistical significance ($p<0.01$). (*B*) The AaTai isoforms in the fat body of the AaSRPK- and AaClk-depleted mosquitoes were examined by immunoblotting with an antibody that recognizes the common region of AaTai isoforms. Note that the AaTai isoforms were switched from A/B to C/D in these mosquitoes, compared with the non-injected and dsGFP-injected controls.

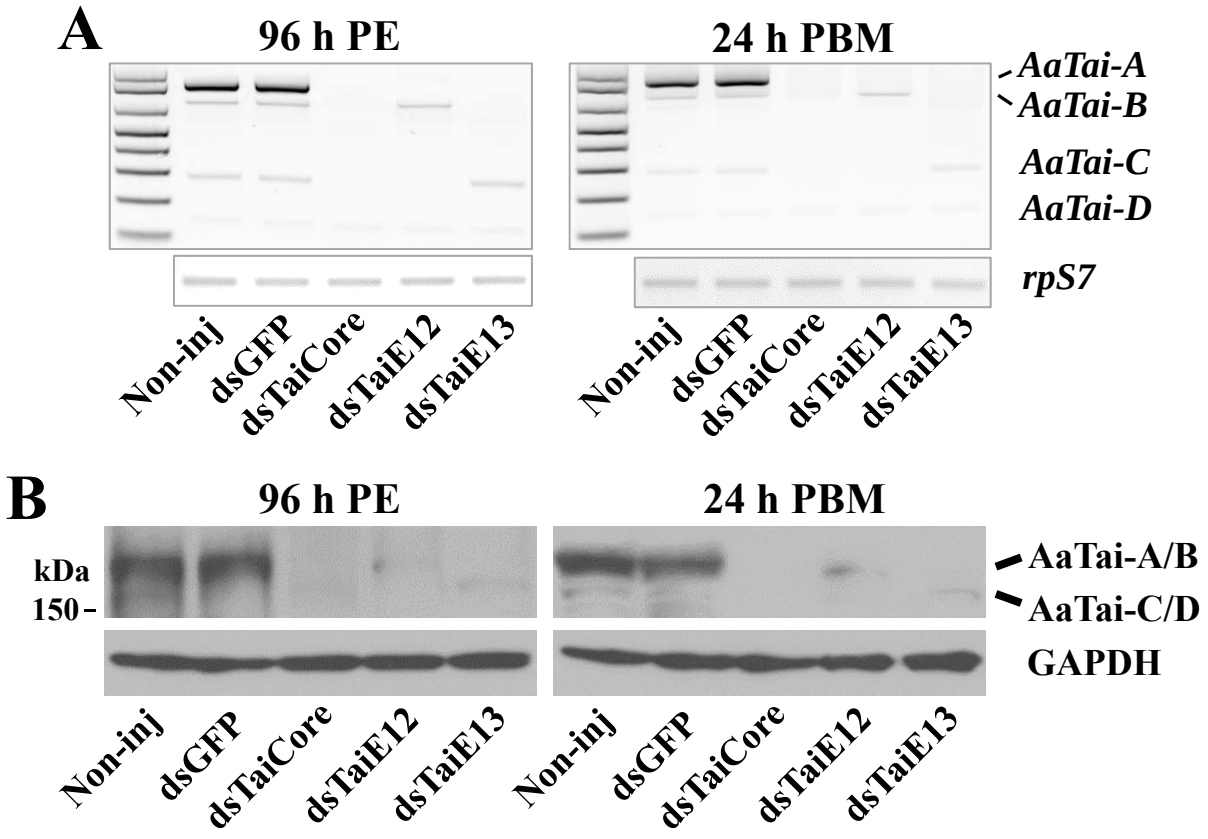

Figure S4. AaTai-A and AaTai-B are depleted in the fat body by the dsRNA for the exon 13 of *AaTai*. Newly emerged female mosquitoes were injected with the dsRNAs that specifically targeted the common region (TaiCore), exon 12 (TaiE12) and exon 13 (TaiE13) of *AaTai*. (*A*) The mRNA levels of four AaTai isoforms at 96 h PE and 24 h PBM were examined by RT-PCR. *RpS7* gene was used as an internal control. (B) The protein levels of AaTai isoforms in the fat body were examined by immunoblotting using the antibody that recognized the common region of AaTai. GAPDH was used as a loading control.

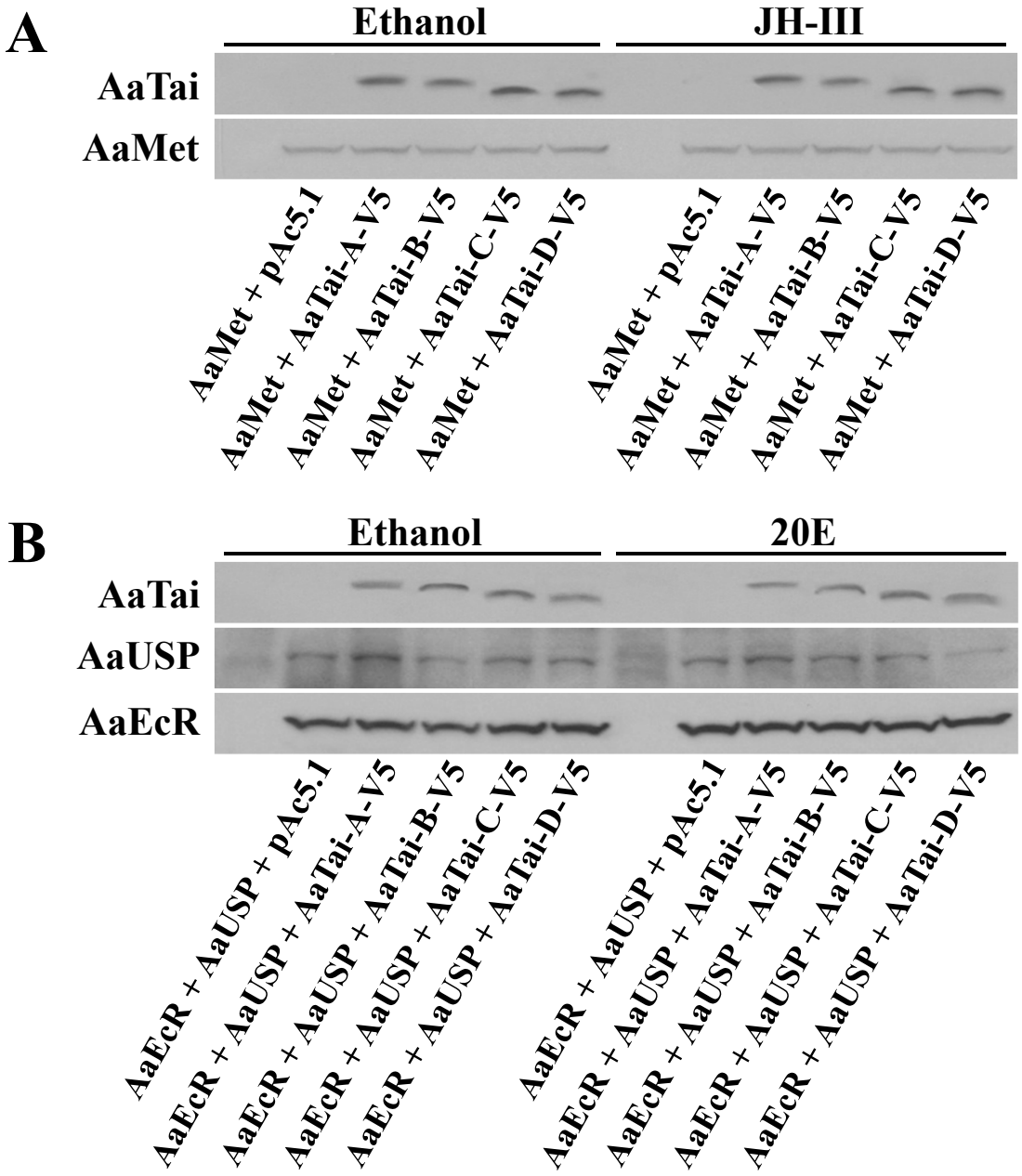

Figure S5. The AaTai isoforms are expressed at comparable levels in the transfected cells in the luciferase reporter assay. *Drosophila* L57 cells were transfected with reporter constructs and expression vectors as shown in Fig. 6, and were treated with JH-III (*A*) or 20E (*B*). The protein amounts of AaMet, AaTai, AaEcR, and AaUSP were analyzed by immunoblotting. Equal amounts of proteins were loaded in each lane. A V5 antibody was used to detect the V5-tagged AaTai isoforms. The antibodies for AaMet, AaEcR, and AaUSP were described in the Materials and Methods section.

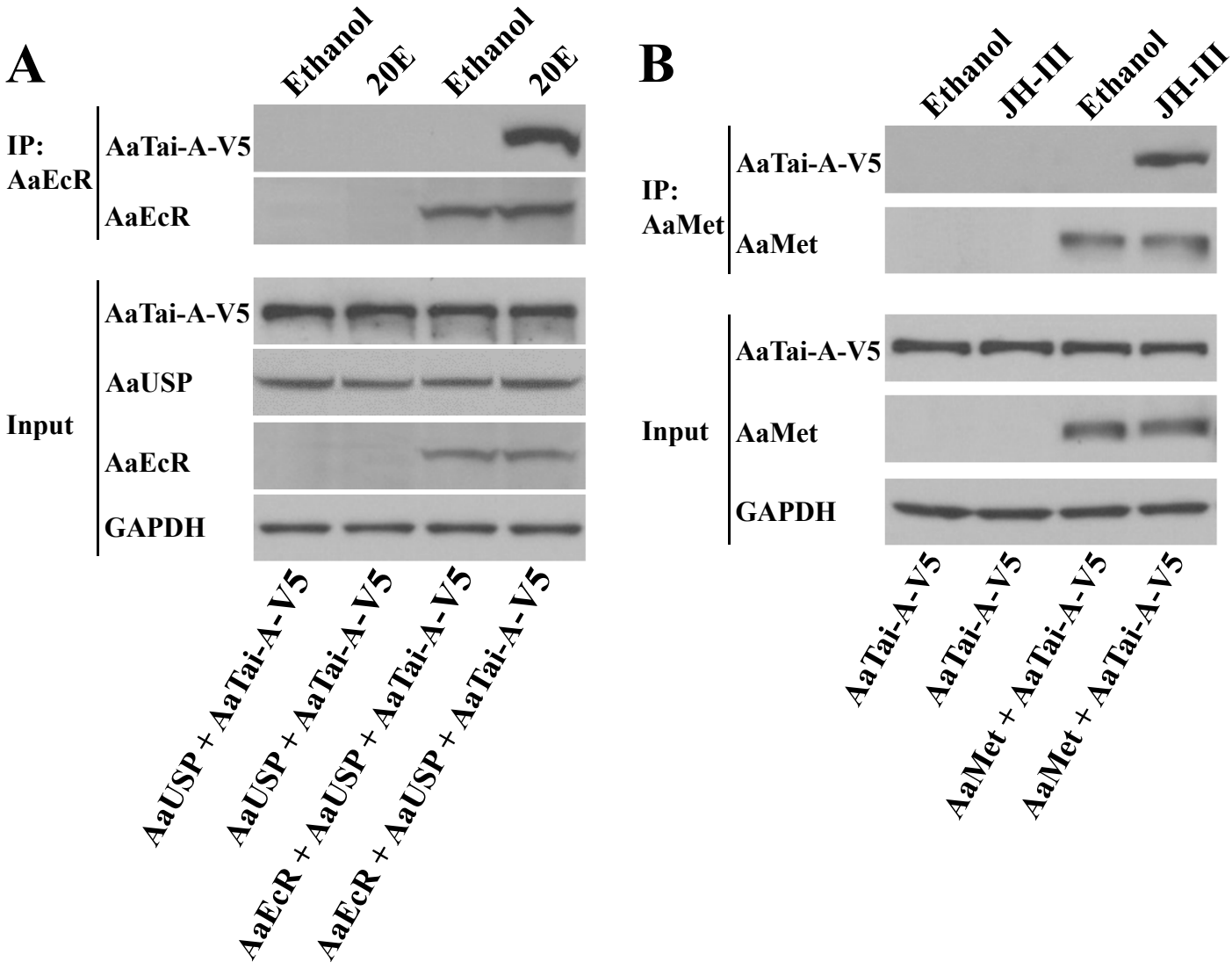

Figure S6. AaTai-A specifically interacts with AaEcR or AaMet. (*A*) AaTai-A and AaUSP were overexpressed in L57 cells with or without AaEcR. After 20E treatment, immunoprecipitation was performed using the cell lysates and anti-AaEcR antibody, followed by immunoblotting using anti-V5 antibody. (*B*) AaTai-A was overexpressed in L57 cells with or without AaMet. After JH treatment, immunoprecipitation was performed using the cell lysates and anti-AaMet antibody. The pellets were analyzed by immunoblotting using anti-V5 antibody.

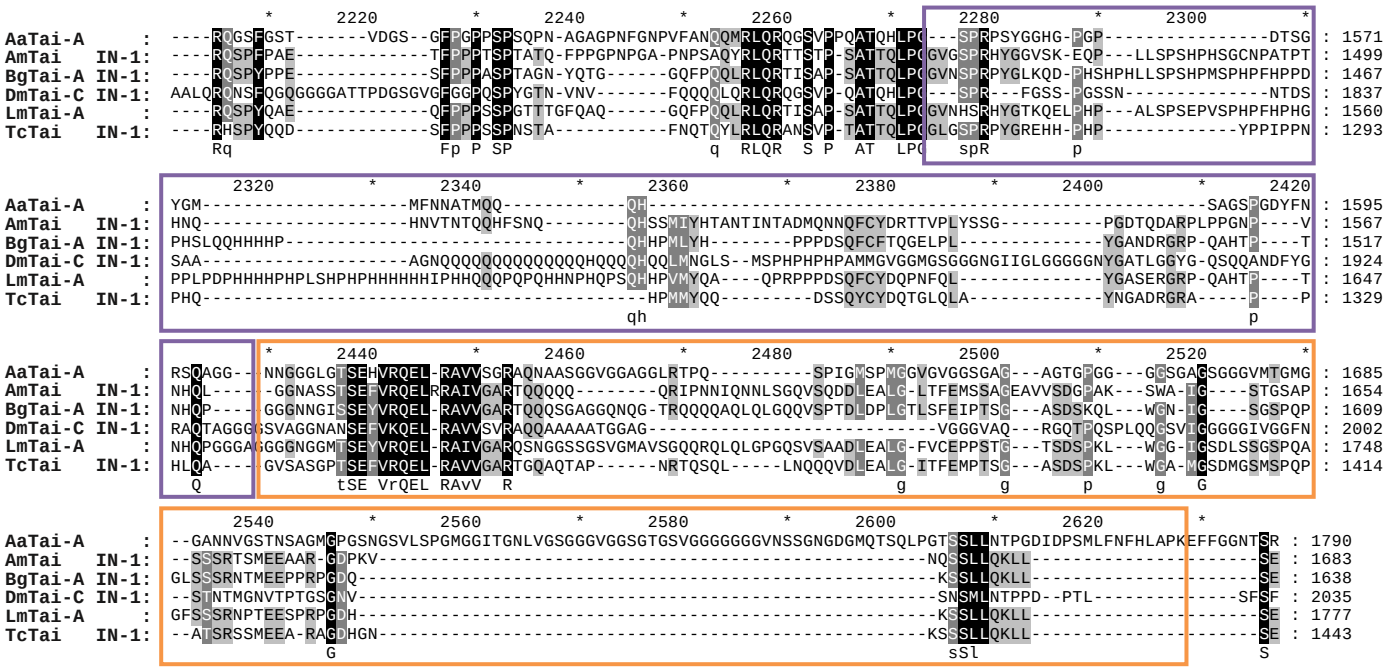

Figure S7. Alignment of Taiman proteins from several insect species. The sequence of AaTai-A was used in this analysis as it includes the constitutive exons and the alternative exons E12 and E13. Other Taiman sequences containing INDEL-1 (IN-1) are from *Apis mellifera* (*Am*, GenBank Accession XP_006563176), *Blattella germanica* (*Bg*, GenBank Accession CDO33883), *Drosophila melanogaster* (*Dm*, GenBank Accession NP_001188746), *Locusta migratoria* (*Lm*, GenBank Accession ANG56297) and *Tribolium castaneum* (*Tc*, GenBank Accession XP_967666). The IN-1 regions of Taiman in these insect species correspond to the E12 of AaTai-A, which is marked in the purple box. The protein sequence encoded by the E13 of AaTai-A is framed by the orange box. Note the highly conserved region within E13. MAFFT program (http://mafft.cbrc.jp/alignment/software) was used to perform this alignment.

Table S1. Juvenile hormone-induced phosphorylation of protein factors implicated in pre-mRNA splicing

Uniprot ID	Protein Name	Gene Name	Peptide Sequence*	Normalized Ratio JH vs Control
Q176A9	Arginine/serine-rich splicing factor (SRp30)	AAEL006473	RRGT(ph)PTYSPVQR	10.6
Q16YF1	Serine/threonine-protein kinase PRP4-like protein	AAEL008556	KKS(ph)EEFFIIDEPSPMDSK	5.78
Q172Z4	Splicing factor 45 (SRp45)	AAEL007239	SGFAGRPS(ph)SDDEEEFRPGLK	1.98
Q17IT0	Splicing factor yt521-b (SRp63)	AAEL002272	S(ph)RSRSPQASSTADTSTETK	2.01
Q16M17	Splicing factor, arginine/serine-rich 18 (SRp53)	AAEL012459	NQQNNAS(ph)DEEDYASGSSR	2.24
Q16YK1	SRSF protein kinase (SRPK)	AAEL008507	LLDDAEGYS(ph)SDEREGEQEGREDYCR	5.97

* ph-phosphorylation. For SILAC phosphoproteome analysis, the digested samples were subjected to a titanium dioxide enrichment step. The enriched fractions were examined to measure the phosphopeptide change, while the flow-through samples were used to measure the protein level change. SILAC quantitation was performed using the Matrix Science Mascot Distiller Quantitation toolbox (http://www.matrix-science.com/home.html). Quantitation was based on the relative intensities of the extracted ion chromatograms of the peptide precursors. The AaMet and AaTai proteins (phosphorylated or un-phosphorylated) were not detected in the mass spectral analysis. This was probably caused by dynamic range issues in the mass spectrometer and was also due to the low abundance of AaMet and AaTai in the Aag2 cells.

Table S2. Primers for RT-PCR, the synthesis of dsRNAs, and qRT-PCR

Primer	Forward Sequence (5'-3')	Reverse Sequence (5'-3')	Assay
AaTai	CAAGCAACTCAGCATCTACCA	AGCCTAAACACGTACGAACAA	RT-PCR
dsSRPK	TAATACGACTCACTATAGGGAGGGTAGTTTAGAGAACGGAGATGTG	TAATACGACTCACTATAGGGAGTCGTCGCGGCAATAGTTATC	RNAi
dsClk	TAATACGACTCACTATAGGGAGGGATCTGAAGCCGGAGAATATC	TAATACGACTCACTATAGGGAGGCTTCACCTAGTGTGATTCTGT	
dsTaiCore	TAATACGACTCACTATAGGGAGGGAAATCCCTTCAACAACAATCC	TAATACGACTCACTATAGGGAGGGTAGATGCTGAGTTGCTTGA	
dsTaiE12	TAATACGACTCACTATAGGGAGGATCGCCACGTCCCTCGTACG	TAATACGACTCACTATAGGGAGCAGCTTGACTGCGATTGAAGTA	
dsTaiE13	TAATACGACTCACTATAGGGAGAAAGCCCGATCGGAATGTCG	TAATACGACTCACTATAGGGAGAAATTGAATAGCATTGACGGATCG	
dsGFP	TAATACGACTCACTATAGGGGCTGTTAAAAGTGGATGATGATAC	TAATACGACTCACTATAGGGAATCGGCACCTTGGTAGAACGATC	
AaSRPK	GACAGGTGCTGGAAGGATTAG	CAGGCCAACTTTCGGATGTA	qRT-PCR
AaClk	TGCTGGACTGGTTCGATTAC	GGGTAGGGTTCGTAGTTGTTT	
AaTaiCore	ACTCGAGCCAATAGCGTAGTA	GCTGAGGACTGAGTTGTGTTC	
AaTaiE13	GGGCTGGGCACTTCGGAACAC	CTCCGCCAACACCGCCACTT	
AaKr-h1	TTCTCGCAACAACAGCAACATCCG	TCATCAGATCCATTGACGCTGGGT	
Aa2576	CTCGTGGGAATGGGCATCTT	AAGTAACCGTTGCGAGGGAG	
AaE74B	GCCACTCAGGAATATCTGGAAC	GGAAAGTGGGACGATGGATAAC	
AaE75A	CATCATCAGTCGTCTTCCCTATC	CGACTCTCGCATGATGTAGTT	
AaVg	GCAGGAATGTGTCAAGCGTGA	ACGAGGACGAAGAATCGGAAG	
RpS7	TCAGTGTACAAGAAGCTGACCGGA	TTCCGCGCGCGCTCACTTATTAGATT	

Dataset S1. Differentially expressed mRNA detected after the juvenile hormone treatment

Dataset S2. Alternative splicing events detected after the juvenile hormone treatment