Additional file 1：

Krüppel-homolog 1 exerts anti-metamorphic and vitellogenic functions in insects via phosphorylation-mediated recruitment of specific cofactors

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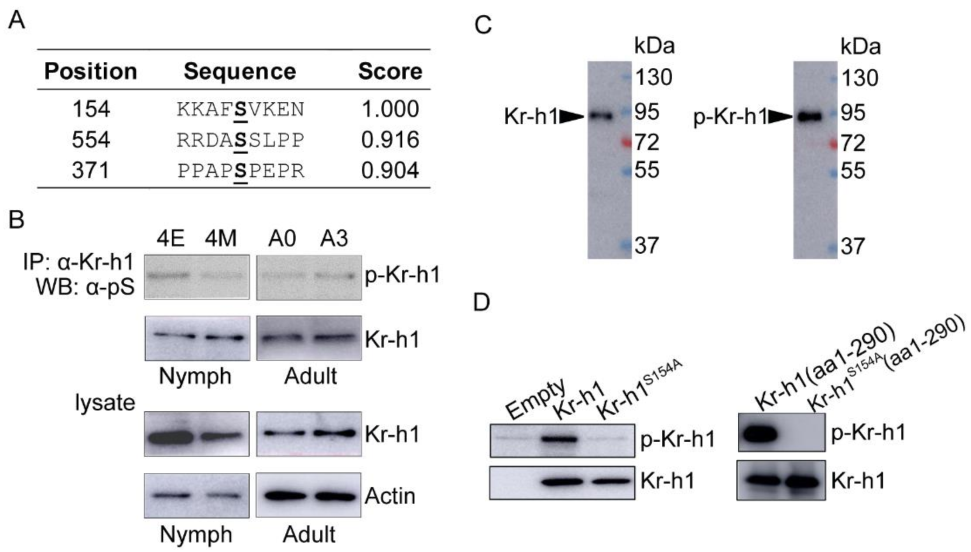
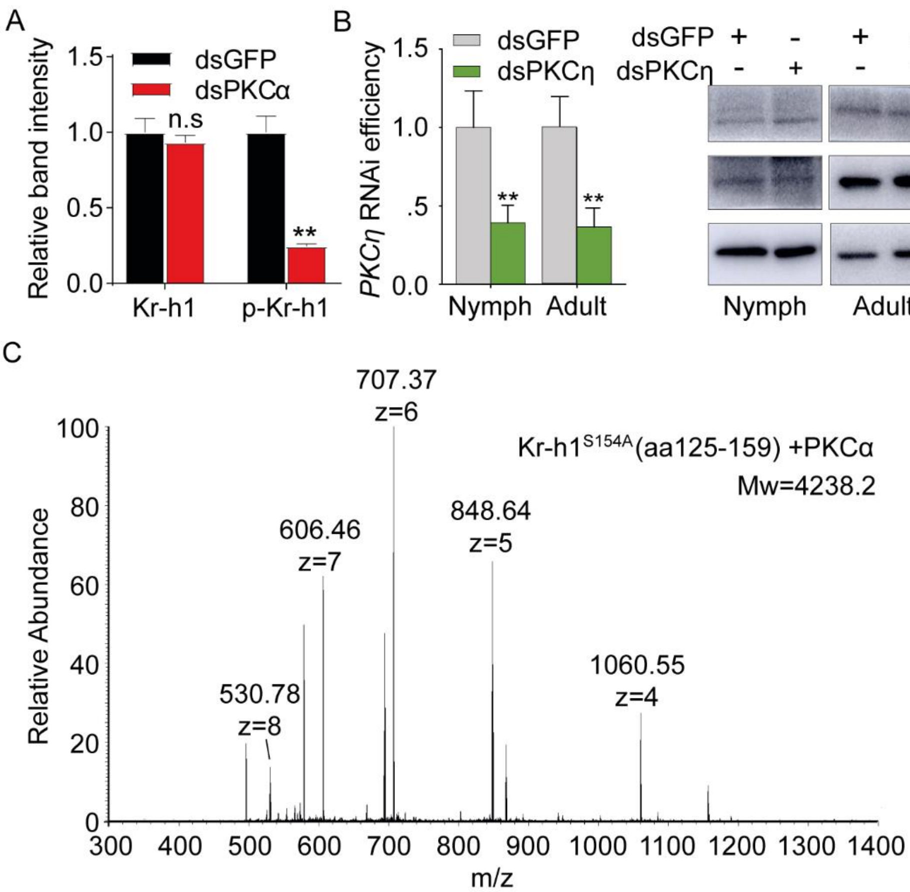
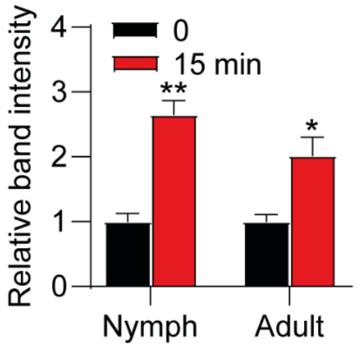


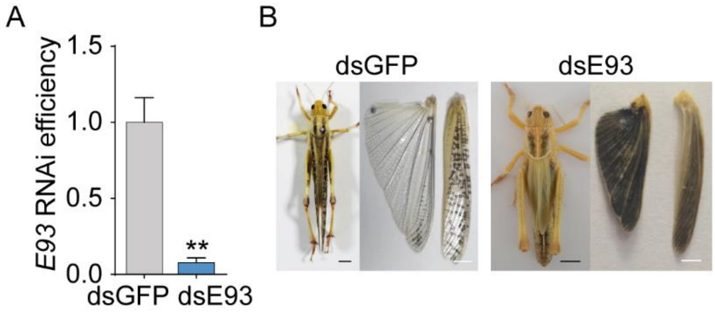
Additional file 1: Figure S1. Identification of Kr-h1 phosphorylation site. (A) Top three Kr-h1 phosphorylation sites predicted by the DISPHOS (V1.3) software. (B) Immunoprecipitation (IP) and western blot (WB) showing the abundance of Kr-h1 and phosphorylated Kr-h1 (p-Kr-h1) in the whole body of early (4E) and mid (4M) penultimate 4^{th} instar nymphs as well as the fat body of adult female locusts on day 0 (A0) and 3 (A3). α-pS, phospho-(Ser) antibody; α-Kr-h1, Kr-h1 antibody. (C) Western blots with both anti-Kr-h1and anti-p-Kr-h1 antibodies (arrows) shown in full extent with molecular weight markers (color bands). (D) Verification of phospho-Kr-h1 (Ser^{154}) antibody specificity by western blots. Left panel: Flag-Kr-h1 and mutated Kr-h1^{S154A} expressed in *Drosophila* S2 cells treated with 10 µM methoprene. Right panel: the bacterially-expressed GST-tagged peptides of Kr-h1(aa1-290) and Kr-h1^{S154A}(aa1-290) preincubated with PKCα.



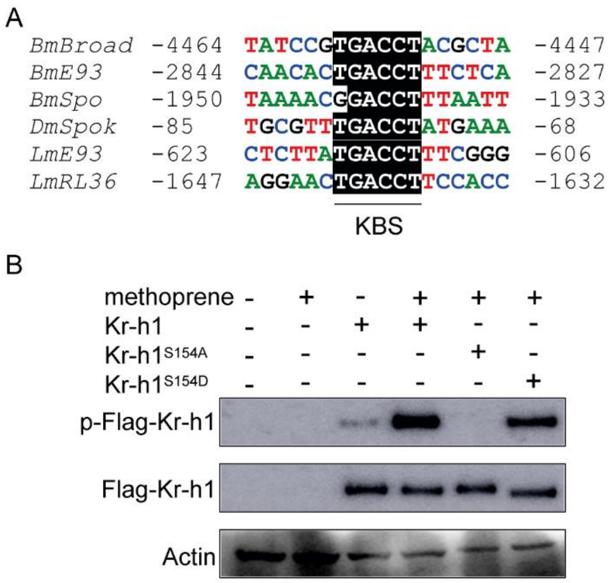
Additional file 1: Figure S2. Identification of kinase triggering Kr-h1 phosphorylation. (A) Quantitative analysis of Kr-h1 and p-Kr-h1 band intensity in western blots using protein extracts from the fat body of adult females injected with dsPKCα vs. dsGFP controls (represented by Figure 1B right panel). **, *P*<0.01. n=4. (B) Effect of *PKCη* knockdown on Kr-h1 phosphorylation. Left panel: *PKCη* knockdown efficiency. Right panels: the levels of Kr-h1 and p-Kr-h1. Nymph, the whole body of mid penultimate 4^{th} instar nymphs. Adult, the fat body of 3-day-old adult females. **, *P*<0.01. n=8. (C) LC-MS/MS analysis of mutated Kr-h1^{S154A}(aa125-159) peptide preincubated with PKCα. m/z indicates the mass to charge ratio.



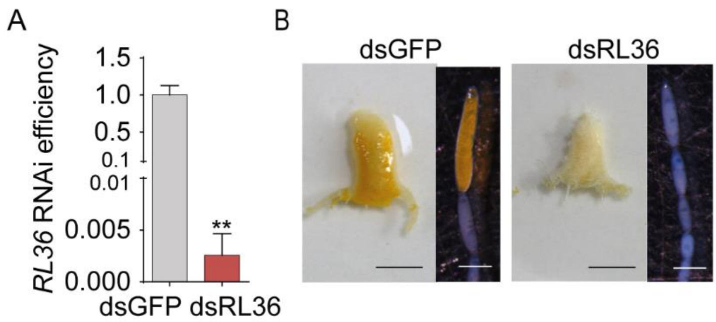
Additional file 1: Figure S3. Effect of 15-min exposure of methoprene on Kr-h1 phosphorylation. Quantitative analysis of p-Kr-h1 band intensity in western blots using the whole body of mid 5th instar nymphs and the fat body from newly-emerged adult females treated with 100 μg methoprene for 15 min (represented by Figure 2B and 2E, respectively). *, *P*<0.01; **, *P*<0.01; n=3.



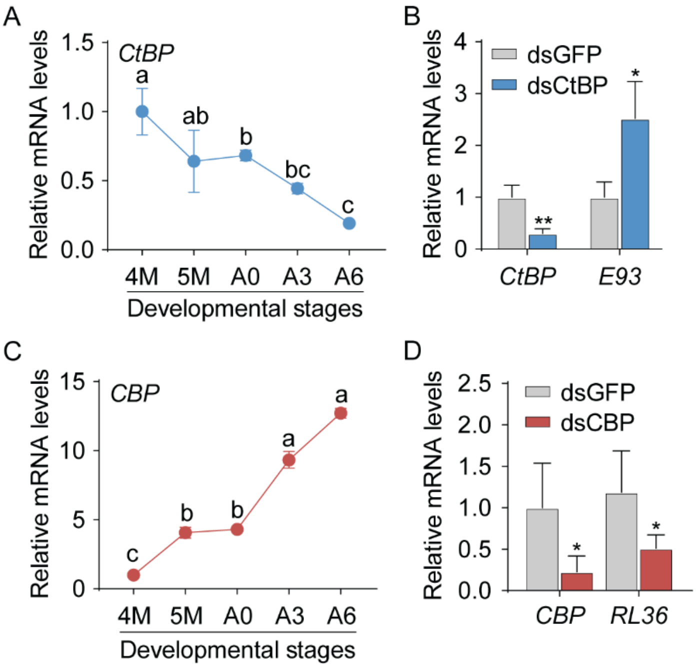
Additional file 1: Figure S4. Effect of *E93* knockdown on locust metamorphosis. (A) *E93* RNAi efficiency in the final instar nymphs. **, *P*<0.01. n=8. (B) Representative phenotypes of delayed adult eclosion (super nymphs) after *E93* knockdown. Scale bar: black, 5 mm; white, 0.5 mm.



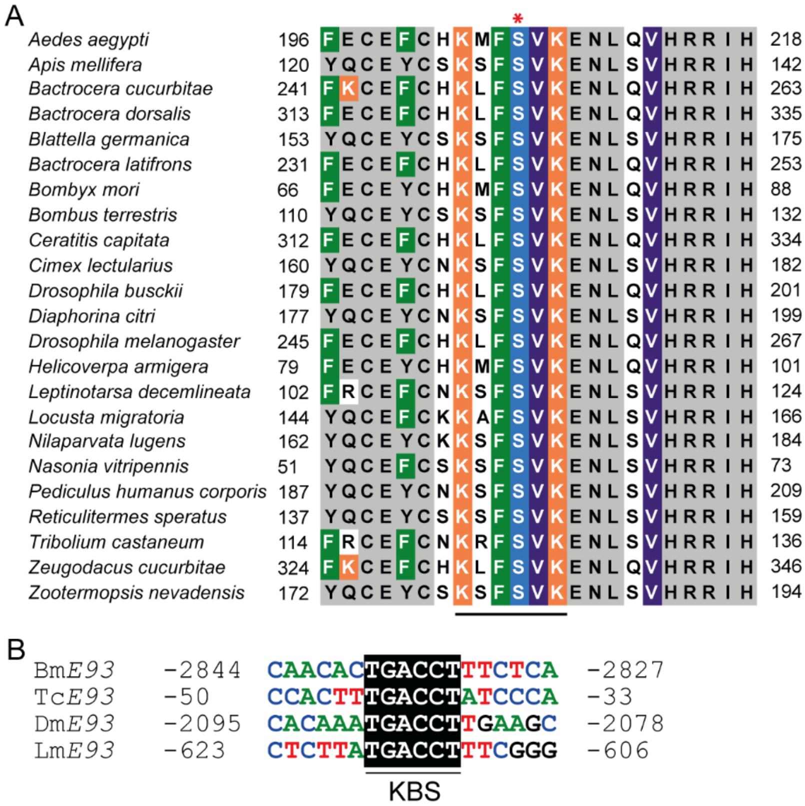
Additional file 1: Figure S5. Responsiveness of Kr-h1 phosphorylation to JH. (A) Alignment of DNA sequences containing the core KBS motif in the upstream of *Br-C*, *E93* and *Spo* from the silkworm *Bombyx mori* (*Bm*), *Spok* from *Drosophila melanogaster* (*Dm*) (references 13, 17 and 18), as well as *E93* (GenBank: MT081312) and *RL36* (GenBank: MT081313) from *Locusta migratoria* (*Lm*). (B) Western blot showing the expression of recombinant Flag-Kr-h1, Flag-Kr-h1^{S154A} and Flag-Kr-h1^{S154D} in *Drosophila* S2 cells with or without 10 μM methoprene treatment. p-Kr-h1, phospho-Kr-h1 (Ser^{154}) antibody. Flag-Kr-h1, Kr-h1 antibody.



Additional file 1: Figure S6. Effect of *RL36* knockdown on locust reproduction. (A) *RL36* RNAi efficiency in the fat body of 6-day-old adult females. **, $P<0.01$. n=8. (B) Representative phenotypes of ovaries and primary oocytes after *RL36* knockdown. Scale bar: black, 1 cm; white, 1 mm.



Additional file 1: Figure S7. Effect of *CtBP* or *CBP* knockdown on *E93* or *RL36* expression. (A) Developmental profiles of *CtBP* in the whole body of mid 4th (4M) and 5th (5M) instar nymphs as well as adult females on day 0 (A0), 3 (A3) and 6 (A6). Means labeled with different letters indicate significant difference at *P*<0.05. n=8. (B) Effect of *CtBP* knockdown on the expression of *E93* in mid 4th instar nymphs. *, *P*<0.05 and **, *P*<0.01. n=8. (C) Developmental dynamics of *CBP* in the fat body of 4M and 5M instar nymphs as well as A0, A3 and A6 adult females. Means labeled with different letters indicate significant difference at *P*<0.05. n=8. (D) Effect of *CBP* knockdown on the expression of *RL36* in the fat body of 3-day-old adult females. *, *P*<0.05. n=8.



Additional file 1: Figure S8. Alignment of the 3rd zinc-finger domain of Kr-h1 and the partial promoter sequences of *E93* with KBS motifs. (A) Alignment of the 3rd zinc-finger domain of Kr-h1 from 23 insect species with available cDNA sequences in NCBI database. Numbers donate starting and ending positions. Asterisk indicates the conserved serine residues homology to Ser^{154} of locust Kr-h1. Underlined amino acids indicate the conserved motif recognized by PKC. (B) Alignment of DNA sequences containing the core KBS motif in the promoters of *E93* from *Bombyx mori* (Bm), *Tribolium castaneum* (Tc) (GenBank: XM_015983777), *Drosophila melanogaster* (Dm) (FlyBase: FBgn0264490) and *Locusta migratoria* (Lm) (GenBank: MT081312).

Additional file 1: Table S1. Primers used for cloning and gene expression

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
Kr-h1	ATGGTGGGCTACTTCAACGG	TTACGAGGCGCCCGCGTAGT
Kr-h1(aa1-290)	ATGGTGGGCTACTTCAACGG	GCTGCAGATGGTGCACTTGT
Kr-h1(aa89-312)	TCCTTCTGCCAGAAGACGTT	GGGGTGCTGCTGCTCCGAGT
Kr-h1(aa291-591)	GAGACGTTCGCCTCCAAGAA	TTACGAGGCGCCCGCGTAGT
CtBP	ATGGACAAGCGCAAGATGCT	TTAATGTACTTCTGATGGCTC
CBP	GATGGAGTGGATGTATGCTT	TTAACCAGGAGGATTTCCTTTC
BmKr-h1	ATGGAATCATTACCTATTTT	CTATGATTCTGTAGCTGGCG
DmKr-h1	ATGGTTTACTATTCCGCCA	CTAGGAGGCCTTGGCGAA
TcKr-h1	ATGCCGGAAATGGTCGGT	ACGACGCTCCTGCATATTG

Additional file 1: Table S2. Primers used for site-directed mutagenesis

Mutation	Primer sequence (5' to 3')
Kr-h1^{S154A}-F	AAGGCCTTCGCCGTCAAGGAGAACCTGAGCGTGCACCGGCG
Kr-h1^{S154A}-R	TCTCCTTGACGGCGAAGGCCTTCTTGCAGAACTCGCACTGG
Kr-h1^{S371A}-F	GCCGGCGCCGGCGCCGGAGCCCCGCCTCGAACCCCGCCTCG
Kr-h1^{S371A}-R	GGGCTCCGGCGCCGGCGCCGGCGGCGGAGGTAGGTGCTGTT
Kr-h1^{S554A}-F	CGCGACGCCGCCTCGCTGCCGCCCCGCAAGCGCTGCAAGG
Kr-h1^{S554A}-R	GGCAGCGAGGCGGCGTCGCGGCGGTCGGGCGACGCGGGGG
Kr-h1^{S154D}-F	AAGGCCTTCGACGTCAAGGAGAACCTGAGCGTGCACCGGC
Kr-h1^{S154D}-R	TCTCCTTGACGTCGAAGGCCTTCTTGCAGAACTCGCACTGGT
BmKr-h1^{S76A}-F	AAATGTTTGCTGTGAAAGAAAATTTGCAAGTACACCGTC
BmKr-h1^{S76A}-R	TCTTTCACAGCAAACATTTTATGGCAATATTCACATTCA
BmKr-h1^{S76D}-F	AAATGTTTGATGTGAAAGAAAATTTGCAAGTACACCGTC
BmKr-h1^{S76D}-R	TCTTTCACATCAAACATTTTATGGCAATATTCACATTCA
DmKr-h1^{S255A}-F	CACAAGCTGTTCGCCGTGAAGGAGAACCTCCAGGTGCAC
DmKr-h1^{S255A}-R	CTTCACGGCGAACAGCTTGTGGCAGAACTCGCACTCGAA
DmKr-h1^{S255D}-F	CACAAGCTGTTCGACGTGAAGGAGAACCTCCAGGTGCAC
DmKr-h1^{S255D}-R	CTTCACGTCGAACAGCTTGTGGCAGAACTCGCACTCGAA
TcKr-h1^{S124A}-F	AGCGATTCGCCGTTAAAGAAAACTTGAGCGTTCATCGAAGA
TcKr-h1^{S124A}-R	TCTTTAACGGCGAATCGCTTATTGCAAAATTCGCAACGGAA
TcKr-h1^{S124D}-F	AGCGATTCGACGTTAAAGAAAACTTGAGCGTTCATCGAAGA
TcKr-h1^{S124D}-R	TCTTTAACGTCGAATCGCTTATTGCAAAATTCGCAACGGAA

Additional file 1: Table S3. Primers used for qRT-PCR, RNAi and ChIP

	Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
qRT-PCR	*Kr-h1*	AGTGCCAGGTGTGCTCCAAGA	CGAACGACTTGCCGCAGATGT
	E93	CAGGCTGGCGATGACAACA	AGTCCGATGGCGTGCTACT
	RL36	ACGAATGTGTGTGCCAAGC	CGTCCGTCAAGACTAAAGGG
	PKCα	AAGGCTCGGTTGTGGAACA	AGGAGGTTGGACTTCACGAT
	PKCη	AGCAACCAGCAACAAGAGGA	TCGTTCTGCCAGCATCACTT
	CtBP	GCGGTTGGTGCCCTAATGTG	TGAGGCAAAGGGTGGTGTCT
	CBP	TGCGTGTCAATGCCGTGATG	CTGCTGAACCTGCTCCACCA
	Rp49	CGTAAACCGAAGGGAATTGA	GAAGAAACTGCATGGGCAAT
RNAi	*Kr-h1*	GTCAAGGAGAACCTGAGCGTGC	TGCTGCTGCTCCGAGTGGCT
	PKCα	CGTTCCTCCCTTAACCCTGT	AACCTTTCCAAAGCTGCCTT
	PKCη	GCAGGCGTGTCCATCAAGTA	GGAGGTGTGAGCTTGTCTGT
	E93	AGGCTGGCGATGACAACACT	GCTGCACGGCGAGTTCCTAA
	RL36	GAAGGGACACCGGACGACAA	GTGTGTGCCAAGCCTCCTCT
	CtBP	TGGTGAACACAGCACGAGGT	ACGGAGTGTGGCACTTGGTC
	CBP	TGCGTGTCAATGCCGTGATG	CTGCTGAACCTGCTCCACCA
	GFP	CACAAGTTCAGCGTGTCCG	GTTCACCTTGATGCCGTTC
ChIP	*E93*	CCGTGGCAAGCTCGTTTT	GCACTTGGGGCAAACTGT
	RL36	GGAGAGTCAGTAGTAACTG	CAGCAGCCAAACTCTCTT