Supporting Information

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SI Materials and Methods

***Drosophila* and *Bombyx* Stocks.** All *Drosophila* stocks were reared at 25 °C on a standard cornmeal and yeast diet including agar under a 12-h:12-h light:dark cycle (1). Wild-type *yw* was used as indicated. *Phm-Gal4::UAS-GFP/Tm6B* (gift from Michael B. O'Connor, University of Minnesota, Minneapolis) was used to specifically drive gene expression in *Drosophila* PG. Transgenic stock *UAS-Kr-h1* (120052) (2) was obtained from the Kyoto Stock Center (DGRC). *UAS-Kr-h1 RNAi* line (HMC03086) was obtained from the National Institute of Genetics at Japan (NIG-Fly; https://shigen.nig.ac.jp/fly/nigfly/). *UAS-white RNAi* (BLM33623) (3) and enhancer-trap line *Kr-h1*^{10642} with a P-element insertion (BLM12380) (4, 5) were obtained from the Bloomington *Drosophila* Stock Center (BDSC). In addition, the *Bombyx* larvae strain *Dazao* was reared on fresh mulberry leaves at 25 °C with a cycle of 12-h light:12-h dark.

***Drosophila* and *Bombyx* Cell Lines.** *Drosophila* S2 cell line, originally derived from embryos, was cultured in Schneider's medium (Life Technologies) supplemented with 10% FBS (Gibco) and standard cell culture antibiotics (Gibco) at 27 °C. The *Bombyx* embryo-derived cell line BmE was cultured in Grace's medium (Life Technologies) containing 10% FBS and standard cell culture antibiotics (Gibco) at 27 °C.

Developmental Timing. For RNAi, overexpression, and mutant assays in *Drosophila*, six to eight female flies were allowed to lay eggs at 25 °C for 8–12 h in a vial containing standard food. After keeping the vial at 25 °C for 24 h AEL, 40–50 L1 (the first-larval instar) larvae with large size were transferred to a new vial containing standard food and reared at 25 °C. Under this condition, control larvae generally develop to pupariation at around 120 h AEL. The numbers of pupariation in *Drosophila* and larval–pupal transition in *Bombyx* were recorded every 8 h. In addition, under the condition indicated above, *Bombyx* larvae feed for 7 d during the last (the fifth) larval instar and then start wandering, corresponding to the beginning of larval–pupal transition.

RNA Extraction and qRT-PCR. Total RNA samples were extracted from *Drosophila* brain–RG complexes or S2 cells and *Bombyx* PG or BmE cells at different time points or under different conditions as indicated. cDNAs were synthesized with 100-ng to 2-μg total RNA using the M-MLV reverse transcriptase kit (Promega). qRT-PCR was performed using a 7500 FastReal Time PCR System (Applied Biosystems) and a SYBR Premix Ex Taq kit (Takara). *α-tubulin at 84B* (*α-tub84B*; NM_057424) was used as an internal control in *Drosophila* and eukaryotic translation initiation factor 4A (*Eif-4a*; NM_001043911) was used as an internal control in *Bombyx*. All experiments were independently performed with at least three biological replicates and the relative mRNA expression levels were calculated using the $2^{-\Delta\Delta CT}$ method. All primers used for qRT-PCR are listed in Table S1.

JHM Application and Ecdysteroid Feeding Experiments. For JHM treatment in *Drosophila*, conditioned food containing methoprene as a JHM was prepared by mixing 10 μL ethanol or 10 μL ethanol dissolved JHM (500 mg/mL; Sigma) with 490 μL deionized distilled water (ddH_2O) and 25 mg dry yeast. At 24 h AEL, 40–50 *Drosophila* L1 larvae were transferred into new vials containing conditioned food supplemented with different chemicals. *Bombyx* larvae on the second day of the fifth instar were smeared on the body wall with JHM (20 μg per larva) or acetone as control. In addition, JHM applications on cultured cells and ex vivo cultured tissues were performed as previously described (6). In addition, for ecdysteroid feeding experiments, we separately mixed 10 μL ethanol, 10 μL ecdysone (50 mg/mL; Shanghai yuanye Bio-Technology), or 10 μL 20E (50 mg/mL; Sigma) with 490 μL ddH_2O and 25 mg dry yeast, and spread them on standard food in a vial. Beginning from 48 h AEL, 40–50 larvae were transferred to a new vial containing standard food supplemented with different chemicals. The progression to pupariation in *Drosophila* and larval–pupal transition in *Bombyx* were then scored.

Ecdysteroid Measurement. Ecdysteroid titer was measured by using EIA approach (7, 8). For ecdysteroid measurement in *Drosophila* larvae, 20 larvae at 48 h AEL or 5 larvae at 96 h AEL were cleaned with ddH2O and collected in a clean tube. Then, ecdysteroids were extracted from whole animals using 1 mL ethanol, as described previously (7). The pooled supernatants were evaporated using a SpeedVac and redissovled in 1 mL EIA buffer (Cayman Chemical). For ecdysteroid measurement in *Bombyx* larvae, 20 μL hemolymph from five larvae on the fifth day (2 d before wandering as a marker of larval–pupal transition) of the fifth-larval instar for the control were preserved in 2 mL PBS buffer, and then 20 μL mixture was dissolved in 1 mL sample diluents (BIOHJ). Subsequently, ecdysteroid titer was estimated according to the manufacturer's instructions of the EIA kit (Cayman Chemical or BIOHJ). Absorbance was measured at 410 nm for Cayman kit or 450 nm for BIOHJ kit on BioTek H4 microplate reader.

RNA in Situ Hybridization. Digoxygenin (DIG)-labeled *DmKr-h1* probes were synthesized using DIG RNA-labeling mix (Roche) and T7 RNA polymerase (Promega). The brain–RG complexes were isolated from *Drosophila* larvae at various time points. Then, the samples were fixed with 4% paraformaldehyde and digested with proteinase K at a concentration of 5 μg/mL for 75 s at 37 °C. RNA in situ hybridization was performed as previously described (9), and the signals were imaged using a fluorescence microscope (Olympus). Primers for preparing DIG probes are listed in Table S1.

Immunostaining. Brain–RG complexes from *Drosophila* larvae at various time points were dissected in PBS and fixed with 4% paraformaldehyde for 30 min at room temperature. Tissues were washed with 0.5% PBT (PBS with 0.5% Triton-X 100) for three times and then incubated at 4 °C overnight with primary antibodies against DmSpok or DmKr-h1. The guinea pig anti-DmSpok antibody was a gift from Michael B. O'Connor, University of Minnesota, Minneapolis (1:500). Rabbit anti–DmKr-h1 (1:1,000) antibody was prepared by ChinaPeptides. Then, the samples were incubated with the following secondary antibodies, goat anti-guinea pig Alexa Fluor 594 (1:1,000; Abcam) or goat anti-rabbit Alexa Fluor 594 (1:1,000; Life Technologies), and nuclei were visualized by DAPI (1:1,000; Life Technologies) staining. Finally, the samples were mounted in Vectashield mounting buffer after being washed in PBS buffer three times with 10 min per time. Fluorescence signals were captured by confocal microscopy (Olympus) at the excitation wavelengths of 488 nm and 594 nm.

EdU Staining. EdU staining in *Drosophila* larvae was performed with the commercial EdU Kit (RIBOBIO) according to the manufacturer's protocol. Briefly, brain–RG complexes were isolated from third instar larvae and cultured for 2 h with 50 μM EdU in Schneider's *Drosophila* medium at room temperature.

Tissues were fixed in 4% formaldehyde for 30 min and then incubated with Apollo dye for 1 h. Tissues were stained with DAPI, washed three times for 10 min in PBS, and mounted in Vectashield. Fluorescence signals were captured by confocal microscopy (Olympus) at the excitation wavelengths of 488 nm and 594 nm.

Western Blotting and Antibodies. Total proteins were isolated from *Drosophila* S2 cells or *Bombyx* BmE cells with Nonidet P-40 lysis buffer (Beyotime), and Western blotting was performed following the protocol reported previously (10). The primary antibodies used in this study were listed as follows: Rabbit anti-BmKr-h1 (1:1,000), mouse anti-V5 (1:1,000; Abcam), mouse anti-RFP (1:1,000; Abcam), and mouse antitubulin (1:10,000; Beyotime). Rabbit anti–BmKr-h1 antibody was prepared by Zoonbio Biotechnology.

Dual Luciferase Assay. The ORF of *DmKr-h1* and red fluorescent protein gene (*RFP*)-fused *BmKr-h1* were subcloned into pAC5.1 containing V5 tag (Life Technologies) and pSL1180 (Life Technologies) vectors for gene overexpression, respectively. The promoters for steroidogenic enzyme genes of *Drosophila* and *Bombyx* were subcloned into a pGL3-Basic vector (Promega). KBS mutated and deleted promoters of *DmSpok* and *BmSpo* were obtained according to the protocol of the MutanBEST Kit (TaKaRa) by using the primers listed in Table S1. The newly constructed vectors were cotransfected with pAC5.1–DmKr-h1 vector into S2 cells or with pSL1180-BmKr-h1 vector into BmE cells. Forty-eight hours after transient transfection, dual luciferase assay experiments were performed as described previously (11). A pRL-TK vector containing the *Renilla* luciferase gene was used as an internal control. In addition, cells that were transfected with different promoter constructs were treated for 48 h with JHM at a concentration of either 10 μg/mL per well for S2 cells or 1 μg/mL per well for BmE cells, and were collected to perform dual luciferase assay.

ChIP. According to the manufacturer's instructions for the EZ-ChIP Immunoprecipitation Kit (Millipore), cells overexpressing V5-tagged DmKr-h1 or RFP-tagged BmKr-h1 were fixed with 37% formaldehyde to cross-link chromatin, and then sonicated to shear into DNA fragments of 200–1,000 bp in length. Complexes were immunoprecipitated and separately enriched with 1 μg antibody against IgG, V5, BmKr-h1, and RFP (Abcam). The purified DNA from the immunoprecipitated chromatin was used for PCR analysis. The PCR products were electrophoresed in 2% agarose gels. The primers amplifying the specific region covering the potential KBS for Kr-h1 are listed in Table S1.

EMSA. DNA oligonucleotides containing the consensus binding sites for Kr-h1 were labeled with Biotin at the 5′-end and annealed to produce double-strand probes. Recombinant DmKr-h1 and BmKr-h1 were purified from *Escherichia coli* strain BL21 (DE3) competent cells (TransGen). EMSA experiments were performed following the manufacturer's instructions of the EMSA/Gel-Shift Kit (Beyotime) and as we reported previously (11). Briefly, normal binding reaction was performed in a 10-μL reaction mixture containing 20 pM probes and different amounts of purified recombinant Kr-h1 proteins (1, 3, 5, and 7 μg). For competition assays, before adding the labeled intact probes, the reaction mixture containing 6 μg purified recombinant Kr-h1 protein was incubated with different amount of unlabeled competitor probes (i.e., intact, KBS-mutation, or KBS-deletion), including 0-fold, 1-fold (20 pM), 5-fold (100 pM), 25-fold (500 pM), or 50-fold (1,000 pM). For antibody-based EMSA analysis, nuclear extracts were harvested from *Drosophila* S2 cells overexpressing V5-tagged DmKr-h1 by NE-PER nuclear and cytoplasmic extraction (Thermo Scientific Pierce). The reaction mixtures containing about 2-μg nucleoproteins and 20-pM probes were incubated with 2-μg tag antibody (Sigma). All reaction mixtures were finally electrophoresed in 5% (wt/vol) polyacrylamide gels. Related primers are listed in Table S1.

BSP Analysis. As described previously (12), the extracted genomic DNA was treated with bisulfite according to the manufacturer's instructions using the EpiTect Bisulfite Kit (Epigentek). By using ZymoTaq DNA polymerase (Zymo research), bisulfite-treated genomic DNA was amplified by touchdown PCR with the primers listed in Table S1 and cloned into pMD19-T vector for further sequencing. Touchdown PCR cycles were programmed as follows (13): predenaturation at 95 °C for 5′; followed by 20 cycles in which 95 °C for 30 min, varying annealing temperatures (decreasing by 1 °C per two cycles from 65 °C to 55 °C) for 30 min, and 72 °C for 30 min; After this, followed by a further 30 cycles in which 95 °C for 30 min, 54 °C for 30 min, and 72 °C for 30 min; Finally, the PCR was ended with a final extension at 72 °C for 5 min. In addition, 5 mg DNA methylation inhibitor Aza (Sigma) was dissolved in PBS with 20-mM final concentration, and 50 μM Aza were applied to treat cells for 48 h (14).

Statistical Analysis. Statistical analysis was performed by using the Student's *t* test. Data are presented as the mean ± SE of three independent biological replicates and $P < 0.05$ was accepted as significant; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

1. Li Q, Gong Z (2015) Cold-sensing regulates *Drosophila* growth through insulin-producing cells. *Nat Commun* 6:10083.
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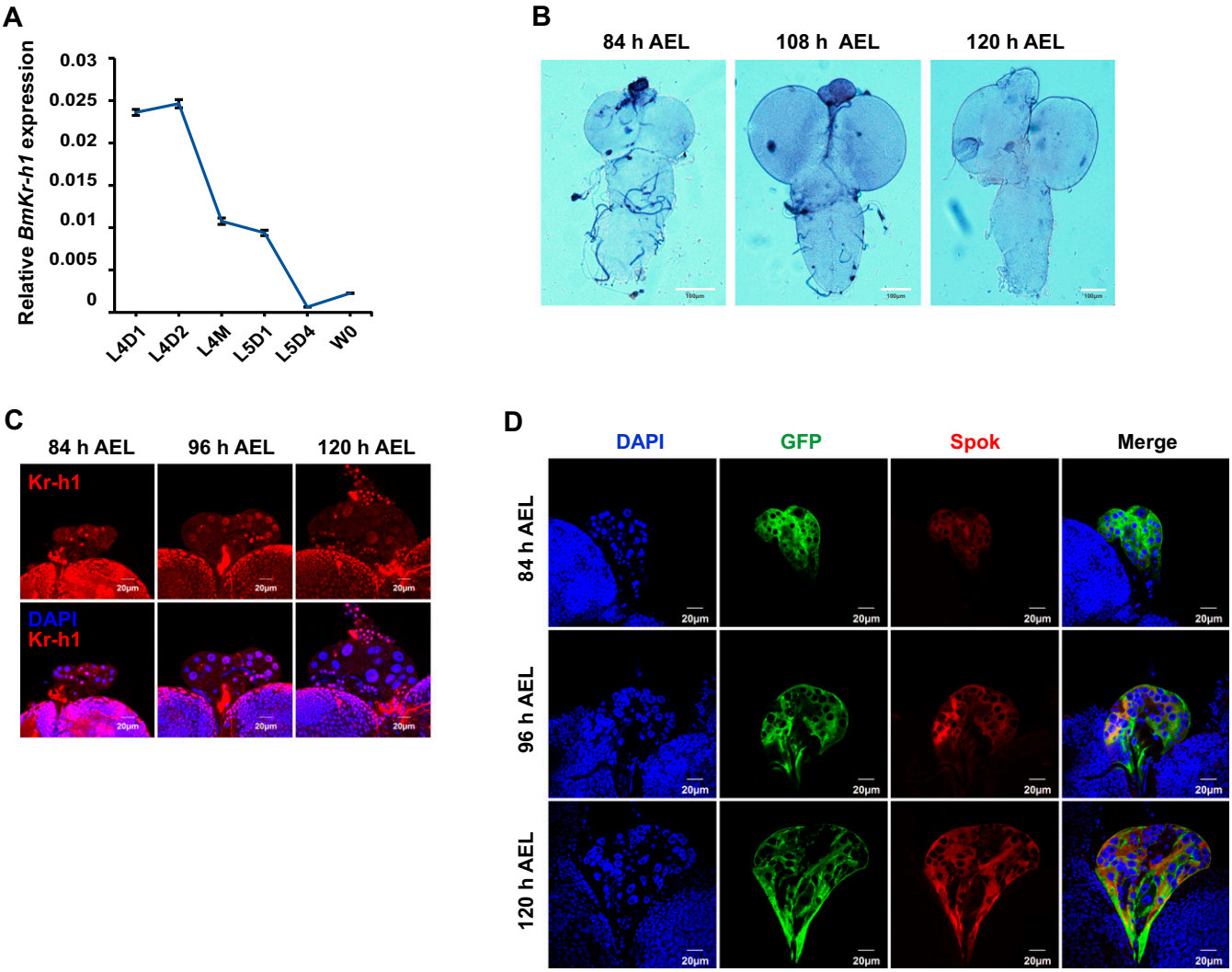

Fig. S1. Expression profiles of *Kr-h1* and *Spok* in *Drosophila* and/or *Bombyx* PGs. (*A*) Expression profiles of *BmKr-h1* in *Bombyx* PG during larval development. D1, first day; D2, second day; D4, fourth day; L4, fourth-larval instar; L5, fifth-larval instar; M, molting; W0, just wandering. (*B*) *DmKr-h1* mRNA level was measured by RNA in situ hybridization in *Drosophila* PG during the third-larval instar. (*C*) Developmental changes of DmKr-h1 protein level in *Drosophila* PG during the third-larval instar. The antibody against DmKr-h1 was used. DAPI (blue), DmKr-h1 (red). (*D*) Developmental changes of DmSpok protein level in the *Drosophila* PG during the third-larval instar. The anti-DmSpok antibody was used. DAPI (blue), GFP (green), DmSpok (red). Values are represented as the mean ± SE (error bars). For the significance test: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. controls. [Scale bars, 100 μm (*B*) and 20 μm (*C* and *D*).]

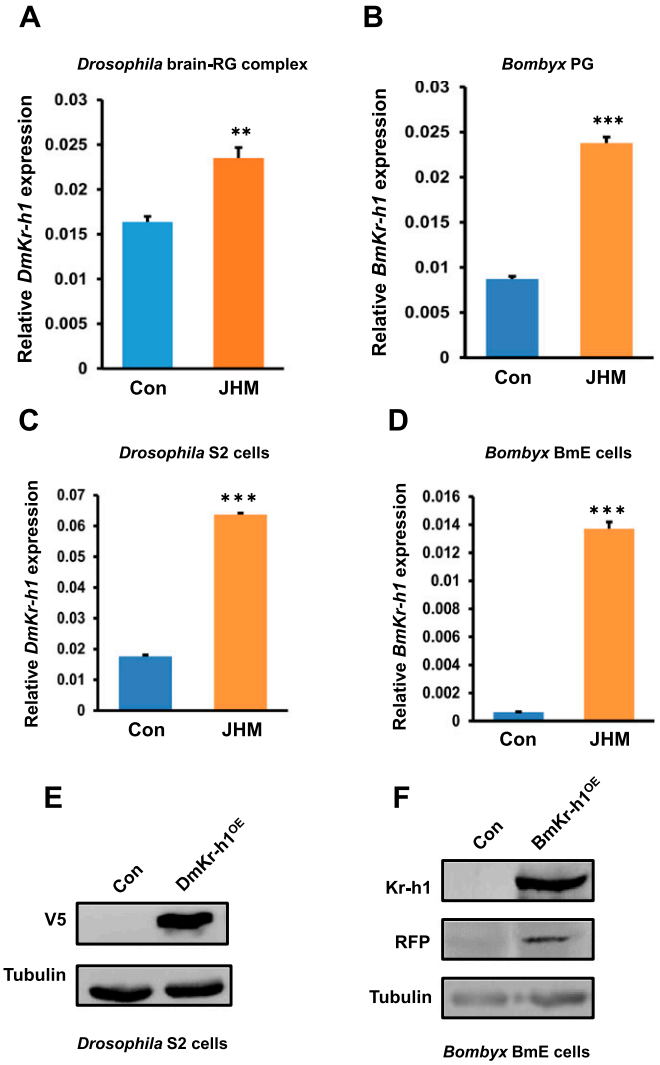

Fig. S2. *Kr-h1* expression changes in *Drosophila* and *Bombyx* PGs or cultured cells following specific treatments. (*A* and *B*) Relative *Kr-h1* mRNA levels were detected in ex vivo-cultured *Drosophila* brain–RG (*A*) and *Bombyx* PG (*B*) following a treatment with JHM methoprene for 6 h. (*C* and *D*) JHM treatment induces *Kr-h1* mRNA transcription in *Drosophila* S2 cells (*C*) and *Bombyx* BmE cells (*D*). (*E* and *F*) V5-tagged DmKr-h1 and RFP-tagged BmKr-h1 were overexpressed in S2 cells (*E*) and BmE cells (*F*), respectively. Values are represented as the mean ± SE (error bars). For the significance test: $^{**}P < 0.01$, $^{***}P < 0.001$ vs. controls.

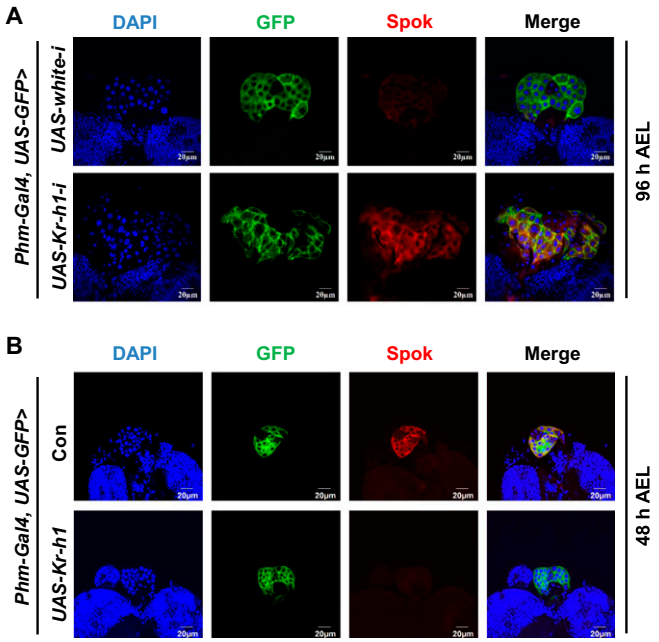

Fig. S3. Level of *DmKr-h1* expression in the *Drosophila* PG affects DmSpok protein level. (*A*) Change of DmSpok protein level in the PG of *Drosophila* larvae with knockdown of *DmKr-h1* in the PG at 96 h AEL. The anti-DmSpok antibody was used. (*B*) Change of DmSpok protein level in the PG of *Drosophila* larvae with *DmKr-h1* overexpression in the PG at 48 h AEL. DAPI (blue), GFP (green), DmSpok (red). (Scale bars, 20 μm.)

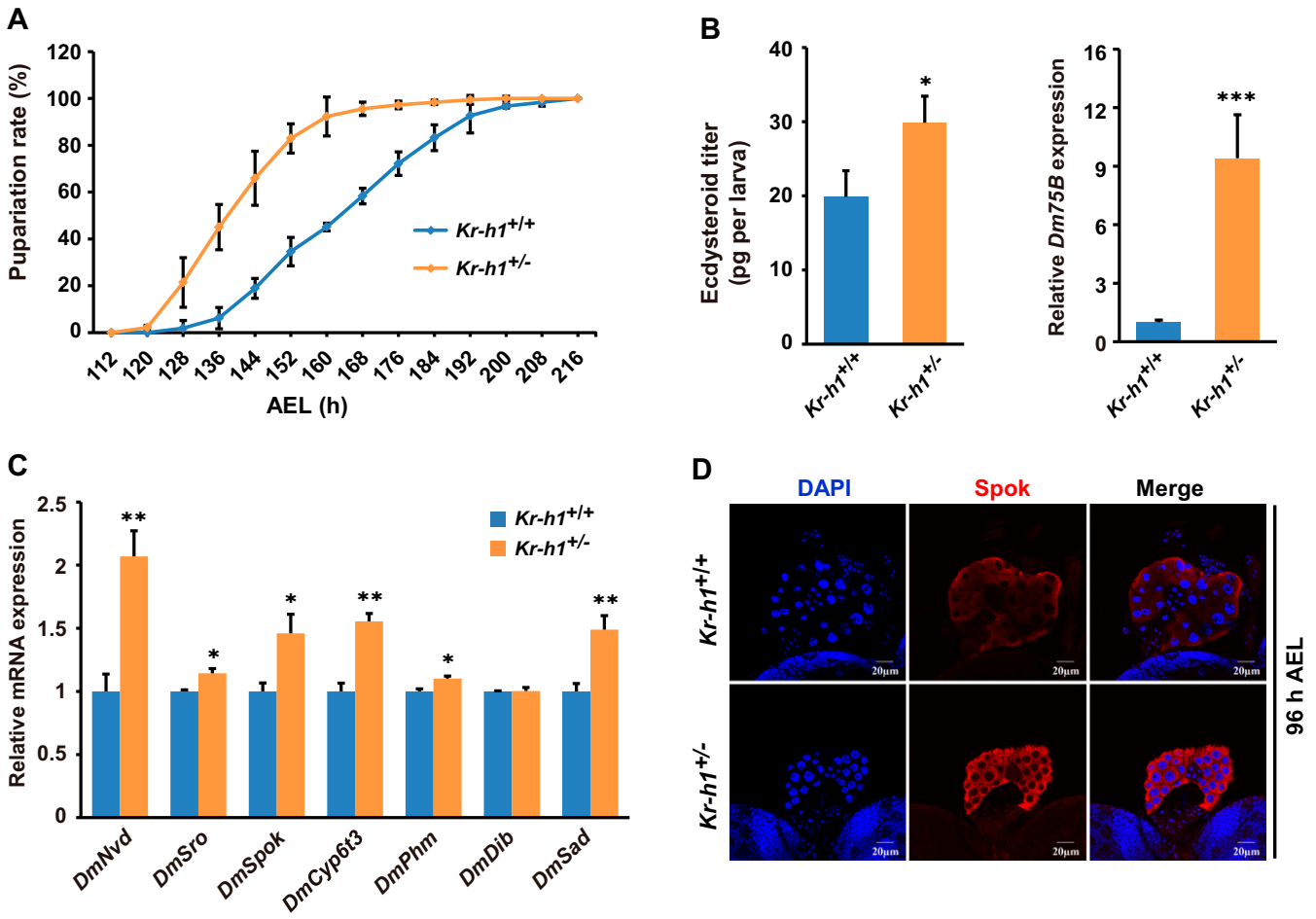

Fig. S4. *DmKr-h1* heterozygotes promotes ecdysone biosynthesis and induces premature pupariation in *Drosophila*. (*A*) Pupariation rates of *DmKr-h1*$^{+/-}$ heterozygotes *Drosophila* larvae compared with control. (*B*) Increased ecdysone synthesis in whole body and increased *DmE75B* transcription in the fat body of *DmKr-h1*$^{+/-}$ heterozygotes. (*C*) mRNA levels of steroidogenic enzyme genes are up-regulated in *DmKr-h1*$^{+/-}$ heterozygotes brain–RG complex. (*D*) Reduction of *DmKr-h1* expression in *DmKr-h1*$^{+/-}$ heterozygotes at 96 h AEL elevates DmSpok protein levels. DAPI (blue), GFP (green), DmSpok (red). Values are represented as the mean ± SE (error bars). For the significance test: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. controls. (Scale bars, 20 μm.)

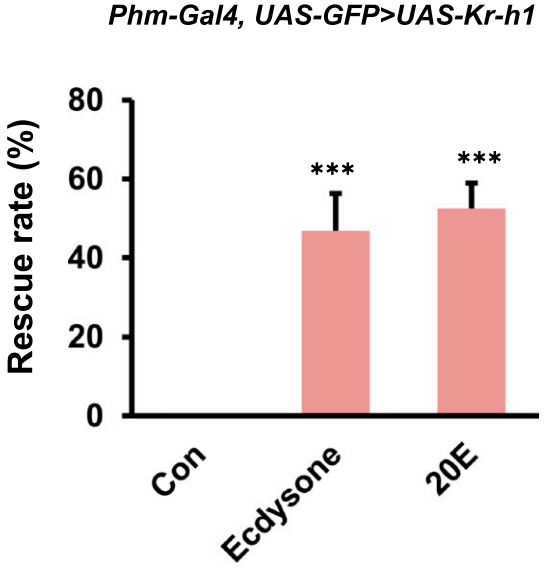

Fig. S5. Rate of the rescue following ecdysone or 20E application. *Drosophila* larvae overexpressing *DmKr-h1* in the PG were continuously fed with ecdysone or 20E at the beginning of 48 h AEL and their pupariation rates were estimated. Values are represented as the mean ± SE (error bars). For the significance test: ***$P < 0.001$ vs. controls.

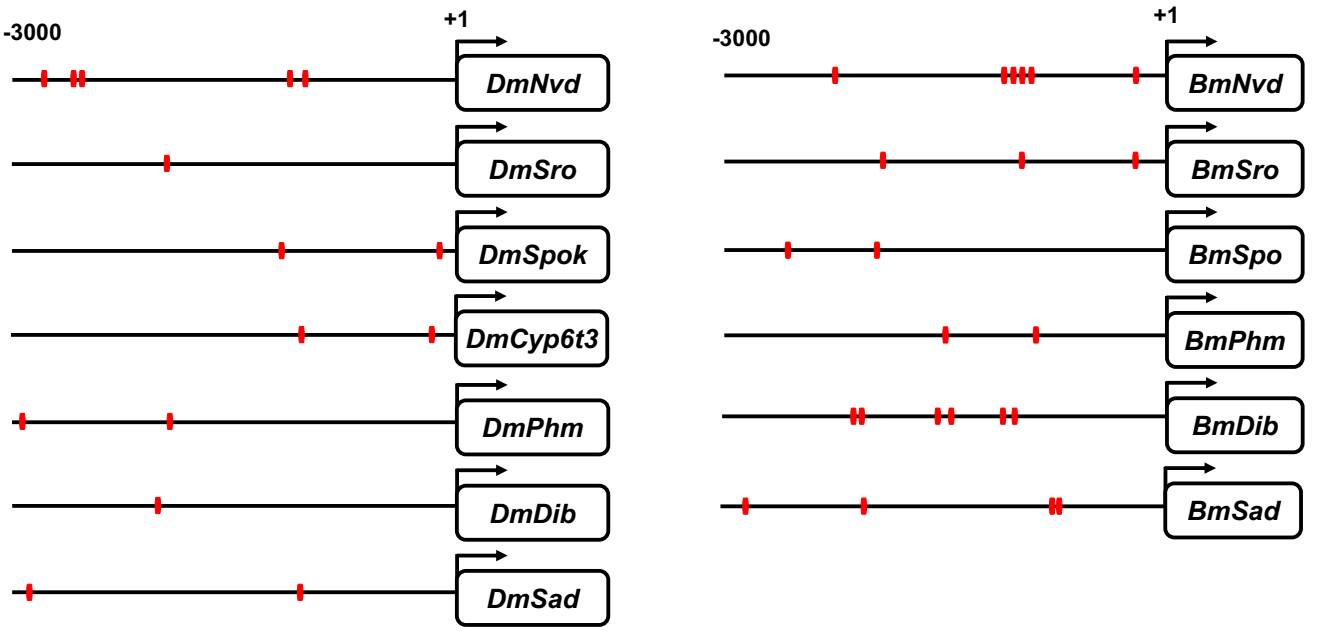

Fig. S6. Schematic of the potential KBSs for Kr-h1 in the promoters of *Drosophila* and *Bombyx* steroidogenic enzyme genes. Potential KBSs are indicated in red.

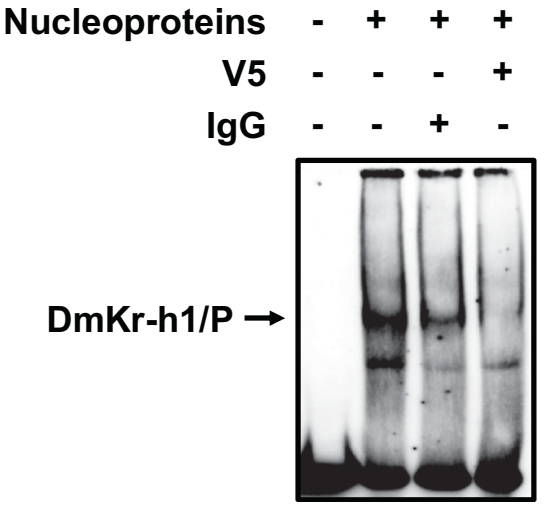

Fig. S7. Nucleoproteins from *DmKr-h1*–overexpressing S2 cells directly bind to the proximal KBS of the *DmSpok* promoter. Coincubating nucleoproteins from S2 cell overexpressing V5-tagged DmKr-h1 with a specific antibody against V5-tag results in loss of the binding band.

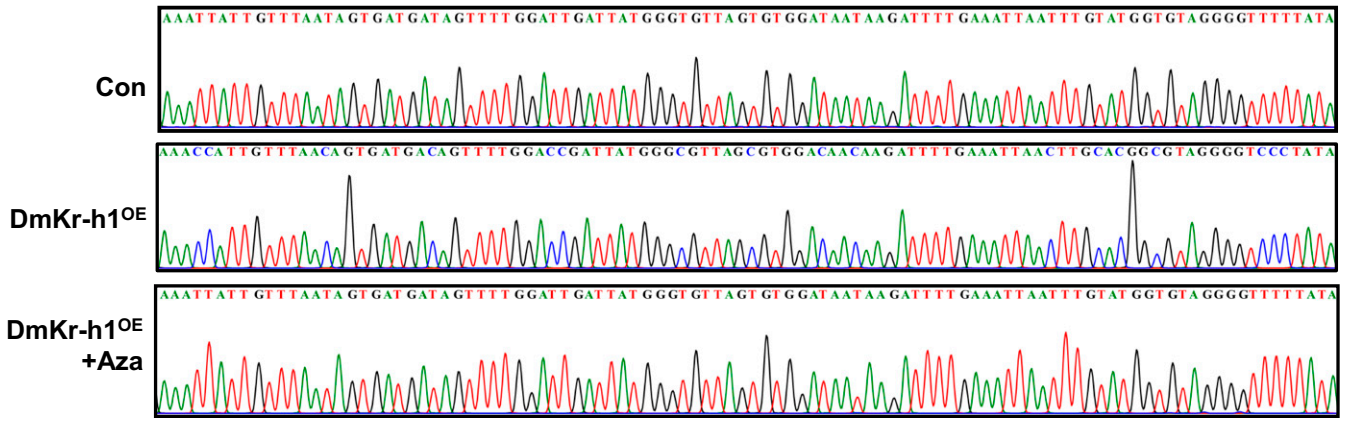

Fig. S8. Bisulfite sequencing PCR analysis of the *DmSpok* promoter. Genomic DNA was extracted from S2 cells of control, *DmKr-h1* overexpression (OE), or *DmKr-h1* overexpression with the treatment of DNA methylation inhibitor Aza, and subsequently treated with bisulfite. The promoter of the *DmSpok* gene was cloned and then sequenced.

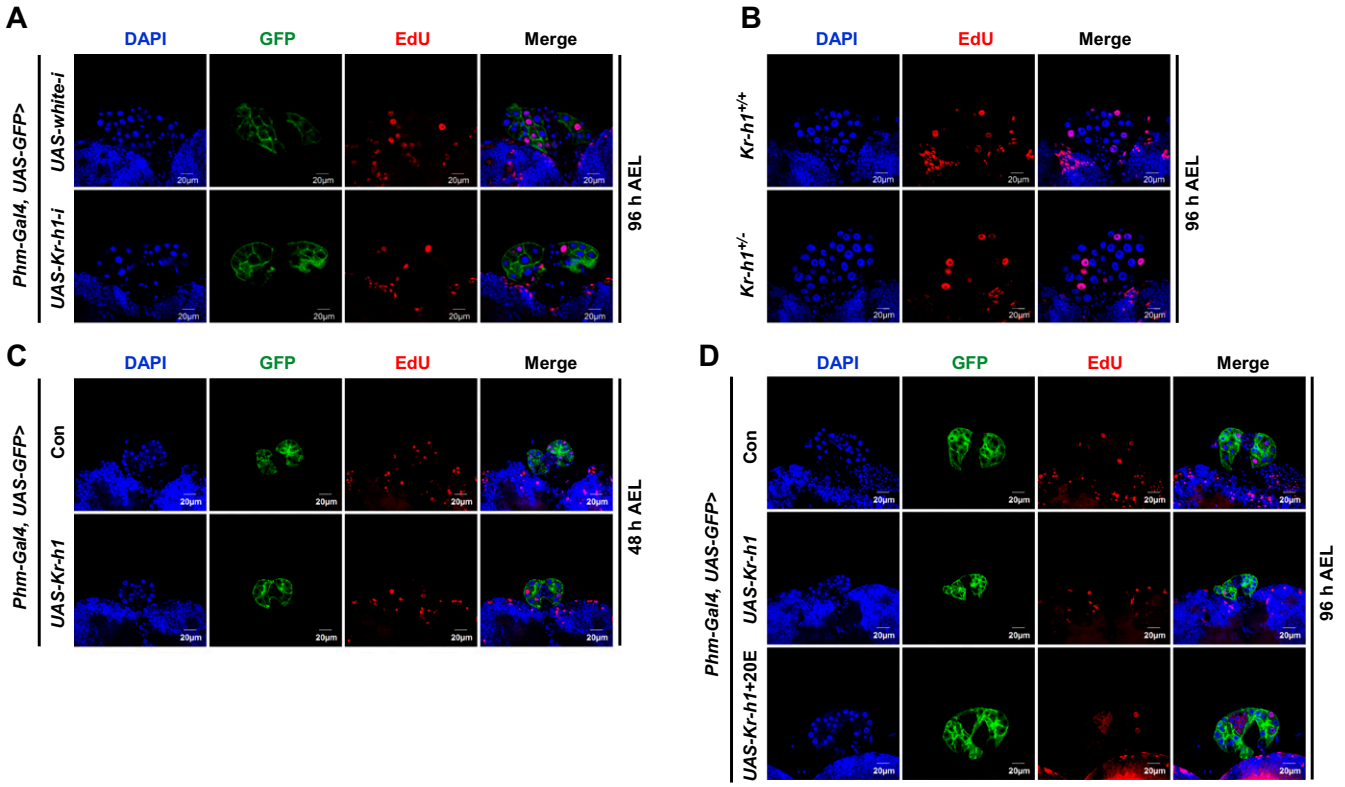

Fig. S9. *Drosophila DmKr-h1* has no effect on DNA replication in PG cells. (*A*) PG-specific knockdown of *DmKr-h1* has no effect on DNA replication in *Drosophila* PG cells and PG size. Brain–RG complex of *Drosophila* larvae with knockdown of *DmKr-h1* in the PG at 96 h AEL was stained with EdU to detect DNA replication. (*B*) DNA replication and PG size was not affected in *Kr-h1*$^{+/-}$ heterozygotes at 96 h AEL. (*C*) DNA replication and PG size was not affected by PG-specific overexpression of *DmKr-h1* at 48 h AEL. (*D*) Feeding 20E rescued the decrease in PG size induced by *DmKr-h1* overexpression in the PG at 96 h AEL. DAPI (blue), EdU (red), GFP (green). (Scale bars, 20 μm.)

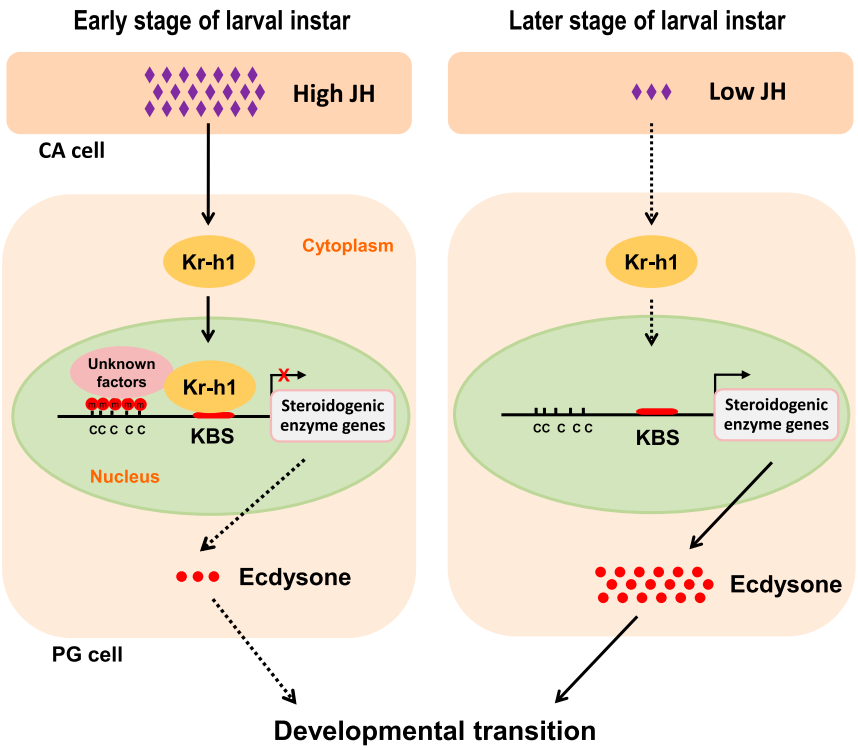

Fig. S10. Proposed model of direct repression of Kr-h1–mediated JH signaling on insect ecdysone biosynthesis.

Table S1 The primers used in this study

Primer name	Primer sequence
qRT-PCR	
DmAlpha-Tub84B -F	5′ GCTGTTCCACCCCGAGCAGCTGATC 3′
DmAlpha-Tub84B-R	5′ GGCGAACTCCAGCTTGGACTTCTTGC 3′
DmKr-h1-F	5′ CCGAATACGACATAACAGCC 3′
DmKr-h1-R	5′ CCCGATTTCCGTGAATATGT 3′
DmNvd-F	5′ TGGGGGAGATTGACGATACA 3′
DmNvd-R	5′ ATGGGGACCACCTCGCTTTC 3′
DmSro-F	5′ TCCTTATGCCGCCTCCAA 3′
DmSro-R	5′ GTGTTGCTCCGCACTGAA 3′
DmCyp6t3-F	5′ GGTGTGTTTGGAGGCACTG 3′
DmCyp6t3-R	5′ GGTGCACTCTCTGTTGACGA 3′
DmSpok-F	5′ TATCTCTTGGGCACACTCGCTG 3′
DmSpok-R	5′ GCCGAGCTAAATTTCTCCGCTT 3′
DmPhm-F	5′ GCTCAGGCAGTCAAAGGAA 3′
DmPhm-R	5′ CTCGAACGGCTAGGAAGTG 3′
DmDib-F	5′ GGAGGAGAATGTTAGGAATGG 3′
DmDib-R	5′ ACTCGGAAGCACTCTCCTGGC 3′
DmSad-F	5′ CTGTTCTTCATGGAGGGCGC 3′
DmSad-R	5′ CAACAGGGGCAGTTCGTAG 3′
DmE75B-F	5′ ATTGCCCCTCCTACTCCAT 3′
DmE75B-R	5′ AGCGTGAACTTATCGTCCTG 3′
BmEif-4a-F	5′ TTCGTACTGGCTCTTCTCGT 3′
BmEif-4a-R	5′ CAAAGTTGATAGCAATTCCCT 3′
BmKr-h1-F	5′ ACCCATACTGGCGAGCGACCAT 3′
BmKr-h1-R	5′ CCTCTCCTTTGTGTGAATACGACGG 3′
BmNvd-F	5′ GACGGATGGGCAGCAAAT 3′
BmNvd-R	5′ GTGCCGCCGACCGCAAGATT 3′
BmSro-F	5′ CCGTTAGCCGTCTTGTAGC 3′
BmSro-R	5′ CGGTTTCTATTCCTTTGTGC 3′
BmSpo-F	5′ GGACATCCGATCCTTCATCT 3′
BmSpo-R	5′ TCTTCGTGTAGCACCCTGAG 3′
BmPhm-F	5′ GAGATGGGAGTCGCAGGTGT 3′
BmPhm-R	5′ CAAAGTATGCGTCTGTGCCT 3′
BmDib-F	5′ ATTTCTTCAACAACCCCAGC 3′
BmDib-R	5′ ACATTTCGTCCTGGCATCTC 3′
BmSad-F	5′ TTATGGACGGAGAGGATTGG 3′
BmSad-R	5′ TCGTATTCTGGCGTGGGCTT 3′
RNA in suit hybridization	
DmKr-h1-F	5′ TAATACGACTCACTATAGGGACACAAGCAGAGGTAGGAAG 3′
DmKr-h1-R	5′ TAATACGACTCACTATAGGGTGAGACCGTTAGAAGAGGAA 3′
ChIP PCR	
BmSpo-F	5′ TTAGAAACATCAAACTGTTGATGC 3′
BmSpo-R	5′ GTGCAATGGTGTTACCTAACTGAA 3′
DmSpok-F	5′ ATATTCGCCTTGTTTGGT 3′
DmSpok-R	5′ CGCAATAGCGAATAGCAC 3′
EMSA probe	
probe-Biotin-DmSpok-F	5′ Biotin-TTTATTTTAAATATGCGTTTGACCTATGAAATTTTATTGTTGTTGGAAAG 3′
probe-DmSpok-F	5′ TTTATTTTAAATATGCGTTTGACCTATGAAATTTTATTGTTGTTGGAAAG 3′
probe-DmSpok-R	5′ CTTTCCAACAACAATAAAATTTCATAGGTCAAACGCATATTTAAAATAAA 3′
probe-DmSpok-mutation-F	5′ TTTATTTTAAATATGCGTTTTTTTTATGTTTTTTTATTGTTGTTGGAAAG 3′
probe-DmSpok-mutation-R	5′ CTTTCCAACAACAATAAAAAAACATAAAAAAAACGCATATTTAAAATAAA 3′
probe-DmSpok-deletion-F	5′ TTTATTTTAAATATGCGTTTATGTTTATTGTTGTTGGAAAG 3′
probe-DmSpok-deletion-R	5′ CTTTCCAACAACAATAAACATAAACGCATATTTAAAATAAA 3′
probe-Biotin-BmSpo-F	5′ Biotin-GTTGATGCTGATACCGAATAAAACGGACCTTTAATTGCAAAGCTAAAAGT 3′
probe-BmSpo-F	5′ GTTGATGCTGATACCGAATAAAACGGACCTTTAATTGCAAAGCTAAAAGT 3′
probe-BmSpo-R	5′ ACTTTTAGCTTTGCAATTAAAGGTCCGTTTTATTCGGTATCAGCATCAAC 3′
probe-BmSpo-mutation-F	5′ GTTGATGCTGATACCGAATAAAACGTTTTTTTAATTTTTTAGCTAAAAGT 3′
probe-BmSpo-mutation-R	5′ ACTTTTAGCTAAAAAATTAAAAAAACGTTTTATTCGGTATCAGCATCAAC 3′
probe-BmSpo-deletion-F	5′ GTTGATGCTGATACCGAATAAAACGTTAATTGAGCTAAAAGT 3′
probe-BmSpo-deletion-R	5′ ACTTTTAGCTCAATTAACGTTTTATTCGGTATCAGCATCAAC 3′
Bisulfite sequencing PCR	
DmSpok-F	5′ GTTAGAGTGGGAATGGCAAACAG 3′
DmSpok-R	5′ TCGGGTCGATCTAACCGTATCCA 3′

Table S1 Cont.

Primer name	Primer sequence
Kr-h1 overexpression	
pAC5.1-DmKr-h1-F	5′ GGGGTACCATGGTTTACTATTCCGCCAACC 3′
pAC5.1-DmKr-h1-R	5′ CCGCTCGAGGGAGGCCTTGGCGAACTGA 3′
pCold-sumo-DmKr-h1-F	5′ GGGGTACCATGGTTTACTATTCCGCCAACC 3′
pCold-sumo-DmKr-h1-R	5′ CCGCTCGAGCTAGGAGGCCTTGGCGAACTGA 3′
pSL1180-BmKr-h1-F	5′ CGCGGATCCATGATAGGTGACGAGGAGCG 3′
pSL1180-BmKr-h1-R	5′ CGCGGATCCTGATTCTGTAGCTGGCGGAG 3′
pGEX-4T-1-BmKr-h1-F	5′ CGCGGATCCATGATAGGTGACGAGGAGCG 3′
pGEX-4T-1-BmKr-h1-R	5′ AAATATGCGGCCGCCTATGATTCTGTAGCTGGCGGAG 3′
Promoter cloning for luciferase activity assays	
DmNvd-F	5′ CGGGGTACCAAGTACGTGACCTCCCGTGAAAC 3′
DmNvd-R	5′ CTAGCTAGCATTATTAATAAGTATCTATAATAATAGTAAAATG 3′
DmSro-F	5′ CGGGGTACCTCGCTCCCACTTCAGCAGACA 3′
DmSro-R	5′ CTAGCTAGCATCGCCGCTTCTCGAGTAACTGAAA 3′
DmCyp6t3-F	5′ GGGGTACCCCTCTAACTATCTGGTTATTCCTAGTACTG 3′
DmCyp6t3-R	5′ TCCCCCGGGGGTGTCTATAAGATATAAGATAGAATATTATAACTATGAA 3′
DmSpok-F	5′ CCGCTCGAGATTTGCTTGCTTGTGTGAGTAA 3′
DmSpok-R	5′ GATCTCGAGTTTCAGCCTTAGTAAATAGTTC 3′
DmSpok-1524-F	5′ CCGCTCGAGCGTCCAACATATCGTCCAAT 3′
DmSpok-750-F	5′ CCGCTCGAGCTCTCTTGTATTGTCTTGTA 3′
DmSpok-523-F	5′ CCGCTCGAGGAGTGGGAATGGCAAACAGT 3′
DmSpok-166-F	5′ CCGCTCGAGGCTTTATTATTTAGGCACCA 3′
DmSpok-65-F	5′ CCGCTCGAGTTATTGTTGTTGGAAAGGGT 3′
DmPhm-F	5′ GGGGTACCACCTTGACACATTTTTCTGC 3′
DmPhm-R	5′ CCGCTCGAGCACTTTCGATTTCCTCCTGCTCTCA 3′
DmDib-F	5′ GGGGTACCGAAACACTGTGCAATTAGCGC 3′
DmDib-R	5′ CTAGCTAGCTCGTTACTCTGGTCTTTTCGC 3′
DmSad-F	5′ CGGGGTACCTAAAGCGTCACACAATGGTAGCAACAATAAC 3′
DmSad-R	5′ CTAGCTAGCCGGTGTACGTCACGGTGGCAGC 3′
BmNvd-F	5′ TCCCCCGGGTGCGTTTTATAAAGATTTAAGAGA 3′
BmNvd-R	5′ CCGCTCGAGTTTGTTTGTTTGAATTTGAG 3′
BmSro-F	5′ TCCCCCGGGGAGGGTATCCCAGAA 3′
BmSro-R	5′ CCGCTCGAGCGCGCTGACTATTTAGAATA 3′
BmSpo-F	5′ TCCCCCGGGGGCGGTAGATTCAGAGCGA 3′
BmSpo-R	5′ CCGCTCGAGACTTATATTTGGATATAAGA 3′
BmPhm-F	5′ TCCCCCGGGCGCAGTGTGACTACACATCT 3′
BmPhm-R	5′ CCGCTCGAGATTACCTTAAATTAAAAATA 3′
BmDib-F	5′ TCCCCCGGGAGAGGGTGAGGAGGCAGAGT 3′
BmDib-R	5′ CCGCTCGAGGTTTCCTAAACTGAAGCATT 3′
BmSad-F	5′ CGGGGTACCGAGATGTGCAAAATCGCCTTCCAAATTA 3′
BmSad-R	5′ CCGCTCGAGGTCGATATATTTGTGTAAGC 3′
BmSpo-mutation-F	5′ TTTTTTTAATTTTTTAGCTAAAAGTGGCGTGTTAAGCAAAA 3′
BmSpo-mutation-R	5′ CGTTTTATTCGGTATCAGCATCAAC 3′
BmSpo-deletion-F	5′ AGCTAAAAGTGGCGTGTTAAGCAAAA 3′
BmSpo-deletion-R	5′ CGTTTTATTCGGTATCAGCATCAAC 3′
DmSpok-mutation-F	5′ TTTTTATGTTTTTTTATTGTTGTTGGAAAGGGTTTTTGTATA 3′
DmSpok-mutation-R	5′ AAACGCATATTTAAAATAAA 3′
DmSpok-deletion-F	5′ TTTATTGTTGTTGGAAAGGGTTTTTGTATA 3′
DmSpok-deletion-R	5′ AAACGCATATTTAAAATAAA 3′

F, forward primer; R, reverse primer.