Supporting Information

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SI Materials and Methods

cDNA Cloning of *BmMet1*, *BmMet2*, and *BmSRC*. Primers were designed for cloning cDNAs of *BmMet1*, *BmMet2,* and *BmSRC* by using information obtained from the *Bombyx mori* genome (Table S1). Fragments of these genes were amplified by PCR using first-strand cDNAs prepared from the prothoracic glands or epidermis of *B. mori* larvae and the RTPCR_FW and RTPCR_RV primers (Table S1). Their 5′- and 3′-end sequences were obtained by 5′ RACE and 3′ RACE with the 5′ RACE and 3′ RACE primers (Table S1), respectively, using a SMART RACE cDNA amplification kit (Clontech) and GeneRacer kit (Invitrogen). The full-length cDNA sequences of these genes then were obtained by combining the sequence data from the RT-PCR and RACE analyses. Their full ORFs were amplified by PCR from the cDNAs using the ORF_F and ORF_R primers (Table S1), subcloned into the pGEM-T Easy vector (Promega), and sequenced.

Chemicals. Juvenile hormones (JH) JH I and JH II were purchased from SciTech, and JH III was from Sigma-Aldrich. Methoprene (juvenile hormone analog [JHA]) was obtained from SDS Biotech, and farnesoic acid and methyl farnesoate were from Echelon Biosciences. Aliquots of stock methanol solutions of these compounds were dried under a stream of nitrogen and dissolved in the culture medium by sonication. Cycloheximide was obtained from Sigma-Aldrich and dissolved in DMSO before use.

Quantitative Real-Time PCR. Quantitative real-time PCR (qPCR) analysis was performed essentially as described previously (1). Total RNA was extracted from *B. mori* tissues or cells by using an RNeasy Plus mini kit (Qiagen) and was used to synthesize cDNAs with a PrimeScript RT reagent kit (Takara Bio). The primers designed to amplify both isoforms of the promoter region of *B. mori* Krüppel homolog 1 gene (*BmKr-h1*) are shown in Table S2. *BmRp49* was used as the internal reference (Table S2). The reaction was carried out in a 10-μL reaction volume containing template cDNA derived from 1 ng of total RNA, SYBR Premix Ex Taq (Takara Bio), and 0.2 μM of each primer by using a LightCycler 480 real-time thermal cycler (Roche). The PCR conditions were 95 °C for 5 min and 55 cycles of 95 °C for 5 s and 60 °C for 20 s. The relative molarities of the gene transcripts were calculated by a crossing-point analysis using standard curves generated from a plasmid containing a fragment of each gene. The expression levels of *BmKr-h1α*, *BmKr-h1β*, and *BmKr-h1* were normalized against those of *BmRp49*.

Developmental Expression Profile of *BmKr-h1* in *B. mori* Larvae. *B. mori* larvae (Kinsyu × Showa strain) were reared at 25 °C under 12 h light/dark conditions. Larvae were sampled from day 0 of the third instar to day 6 of the fifth instar, and the epidermis of each larva was dissected. The level of *BmKr-h1* expression in the epidermis was measured by qPCR.

Allatectomy and JHA Treatment. Corpora allata were removed, using fine forceps under a microscope, from *B. mori* larvae (Kinsyu × Showa strain) at day 0 of the fourth instar, and the larvae were allowed to recover for 3 h. Two microliters of an acetone solution of JHA (0.5 μg/μL) or 2 μL of acetone only was applied topically to the dorsal abdomen of the allatectomized larvae. After 12 h, the epidermis of each larva was dissected, and the level of *BmKr-h1* expression in the epidermis was measured by qPCR.

Cell Culture. The NIAS-Bm-aff3 cell line, derived from the fat body of *B. mori* (2, 3), was maintained at 25 °C in IPL-41 medium (Gibco, Invitrogen) containing 10% (vol/vol) FBS (Cell Culture Technologies). The HEK293 cell line was maintained at 37 °C in a 5% CO_2 atmosphere in minimum essential medium eagle (Sigma-Aldrich) supplemented with 10% FBS and nonessential amino acids (Gibco, Invitrogen).

Construction of Reporter Plasmids. Genomic DNA was extracted from a silkworm of the Daizo p50 strain using a conventional method (4). The 5′-flanking and first intron regions (−4741 to +968 or −4741 to +116) of *BmKr-h1* were amplified from the genomic DNA by using KOD FX DNA polymerase (Toyobo) with a forward primer containing attB1 and Kozak sequences (BmKrh1Pro_F) and a reverse primer containing an attB2 sequence (BmKrh1_Pro_R1 or BmKrh1_Pro_R2) (Table S3). The amplified PCR products were inserted into a pGL4.14 luciferase reporter plasmid (Promega) modified for the Gateway system according to the supplier's instructions (Invitrogen). A KOD Plus mutagenesis kit (Toyobo) was used for the construction of modified reporter plasmids. Reporter plasmids carrying deleted *BmKr-h1* 5′-flanking regions were constructed from plasmid pGL4.14_−4741/+116 by inverse PCR with the appropriate BmKrh1_ProiPCR primer sets (Table S3). Reporter plasmids carrying deleted *BmKr-h1* basal promoter regions and 3× core region of the JH response element (JHRE) of *BmKr-h1* (*k*JHREc) were constructed from plasmid pGL4.14_−2165/+116 and −49/+116 by inverse PCR with the appropriate BmKrh1_ProiPCR primer sets (Table S3). For the construction of reporter plasmids carrying the *BmA3* or *Bmhsp70* promoter, the basal promoter region of *BmKr-h1* (−49 to +116) was removed from plasmid pGL4.14_−2165/+116 & −49/+116 by inverse PCR with the BmKrh1_ProiPCR_F13 and BmKrh1_ProiPCR_R24 primers. The amplified fragment was ligated with the *BmA3* or *BmhspP70* promoter DNA that was amplified by PCR from the plasmid pBacA3GAL4/3×P3DsRed or pBacMCS hsp7GAL4/3×P3DsRed (5) with the appropriate primer sets (Table S3). Reporter plasmids carrying mutated *BmKr-h1* 5′-flanking regions were constructed from plasmid pGL4.14_−2165/+116 by inverse PCR with the appropriate BmKrh1_ProiPCR primer sets (Table S4).

Construction of Expression Plasmids. To construct plasmids expressing the GAL4 DNA-binding domain (GAL4DBD) or VP16 activation domain (VP16AD) fused to the N terminus of BmMet1, BmMet2, or BmSRC in HEK293 cells, the full ORFs of the cDNAs were amplified by PCR from plasmids pGEMT_BmMet1, pGEMT_BmMet2, and pGEMT_BmSRC, respectively, by using the appropriate pBIND primers and pACT primers (Table S1). The amplified fragments were ligated into BamHI- and MluI-digested pBIND plasmids (Promega). A plasmid expressing GAL4DBD fused to the N terminus of VP16AD was constructed similarly (Table S1). To construct plasmids expressing native BmMet2 and BmSRC in HEK293 cells, GAL4DBD was deleted from plasmids pBIND_BmMet2 and pBIND_BmSRC by inverse PCR with the pBINDiPCR primers (Table S1). Plasmids for expressing *BmUSP*, *BmARNT*, *BmHIF-1α*, or *BmTimeless* in HEK293 cells were constructed as follows. The full ORFs of these cDNAs were amplified by PCR from full-length cDNA clones using the appropriate GW_FW primers containing attB1 and Kozak sequences and GW_RV primers containing an attB2 sequence (Table S1). The amplified

fragments were inserted into the pDONR 221 plasmid (Invitrogen) and then into the pcDNA3.2/V5-DEST vector (Invitrogen).

Analysis of JHRE in *Kr-h1* Homologs of Other Insect Species. Genomic databases of *Tribolium castaneum*, *Apis mellifera*, *Nasonia vitripennis*, *Acyrthosiphon pisum*, and *Drosophila melanogaster* were searched for *Kr-h1* homologs with the tblastn program (http://blast.ncbi.nlm.nih.gov/) by using the BmKr-h1 sequence as the query (GenBank accession no. BAJ05087). The sequences of the start sites of transcription or translation in *Kr-h1* were used to identify putative *k*JHREc sequences from 5′-flanking regions.

1. Shinoda T, Itoyama K (2003) Juvenile hormone acid methyltransferase: A key regulatory enzyme for insect metamorphosis. *Proc Natl Acad Sci USA* 100:11986–11991.
2. Imanishi S, Akiduki G, Haga A (2002) Novel insect primary culture method by using newly developed media and extra cellular matrix. *In vitro Cell Dev Biol* 38:16-A.
3. Takahashi T, et al. (2006) Calreticulin is transiently induced after immunogen treatment in the fat body of the silkworm *Bombyx mori*. *J Insect Biotechnol Sericology* 75:79–84.
4. Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Vol 2, pp 9.14–9.19.
5. Uchino K, et al. (2006) Evaluating promoter sequences for trapping an enhancer activity in the silkworm *Bombyx mori*. *J Insect Biotechnol Sericology* 75:89–97.

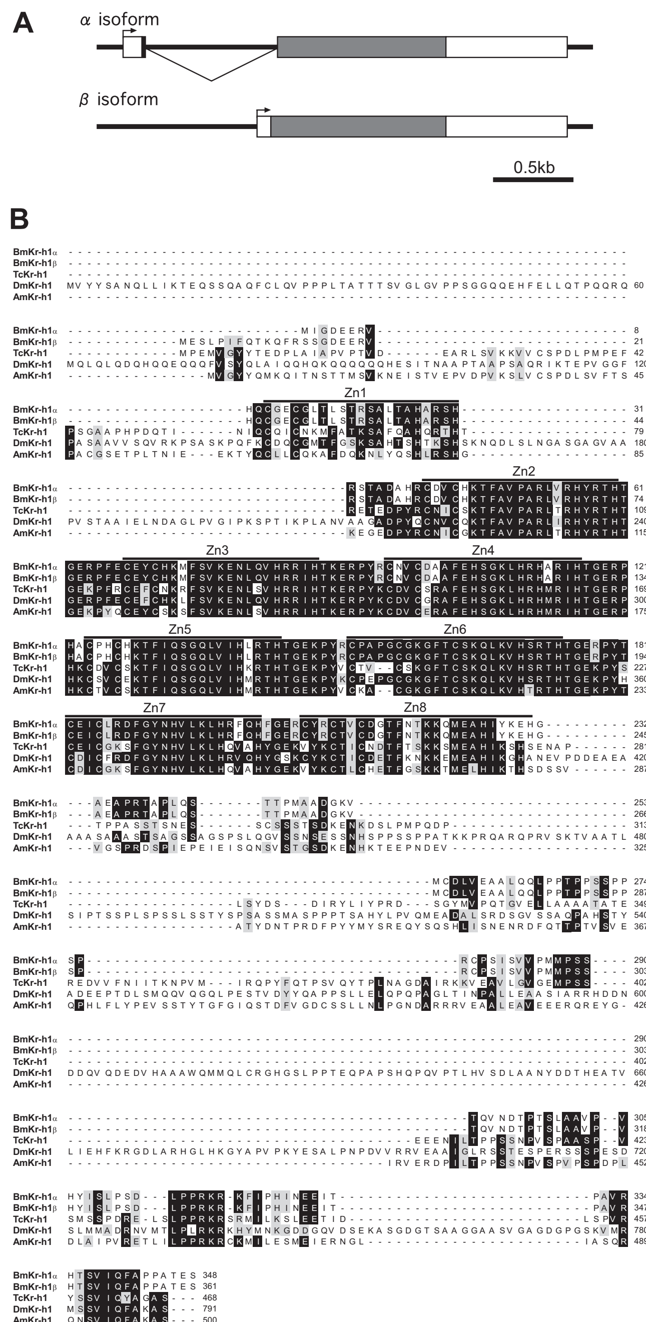

Fig. S1. (*A*) Schematic representation of the genomic structure of *BmKr-h1* and alignment of Kr-h1 sequences from other insect species. Genomic structures of *BmKr-h1α and BmKr-h1β*. Putative exons are boxed, and ORFs are shaded. Arrows indicate predicted transcription start sites. (*B*) Alignment of predicted amino acid sequences of BmKr-h1α and BmKr-h1β with the sequences of *T. castaneum* Kr-h1 (TcKr-h1, GenBank accession no. NP_001129235), *D. melanogaster* Kr-h1 (DmKr-h1, GenBank accession no. NP_477467), and *A. mellifera* Kr-h1 (AmKr-h1, GenBank accession no. AB642243). Black and light gray shading indicate identical and similar amino acid residues, respectively. The eight C_2H_2 zinc finger domains are indicated by the symbol "Zn."

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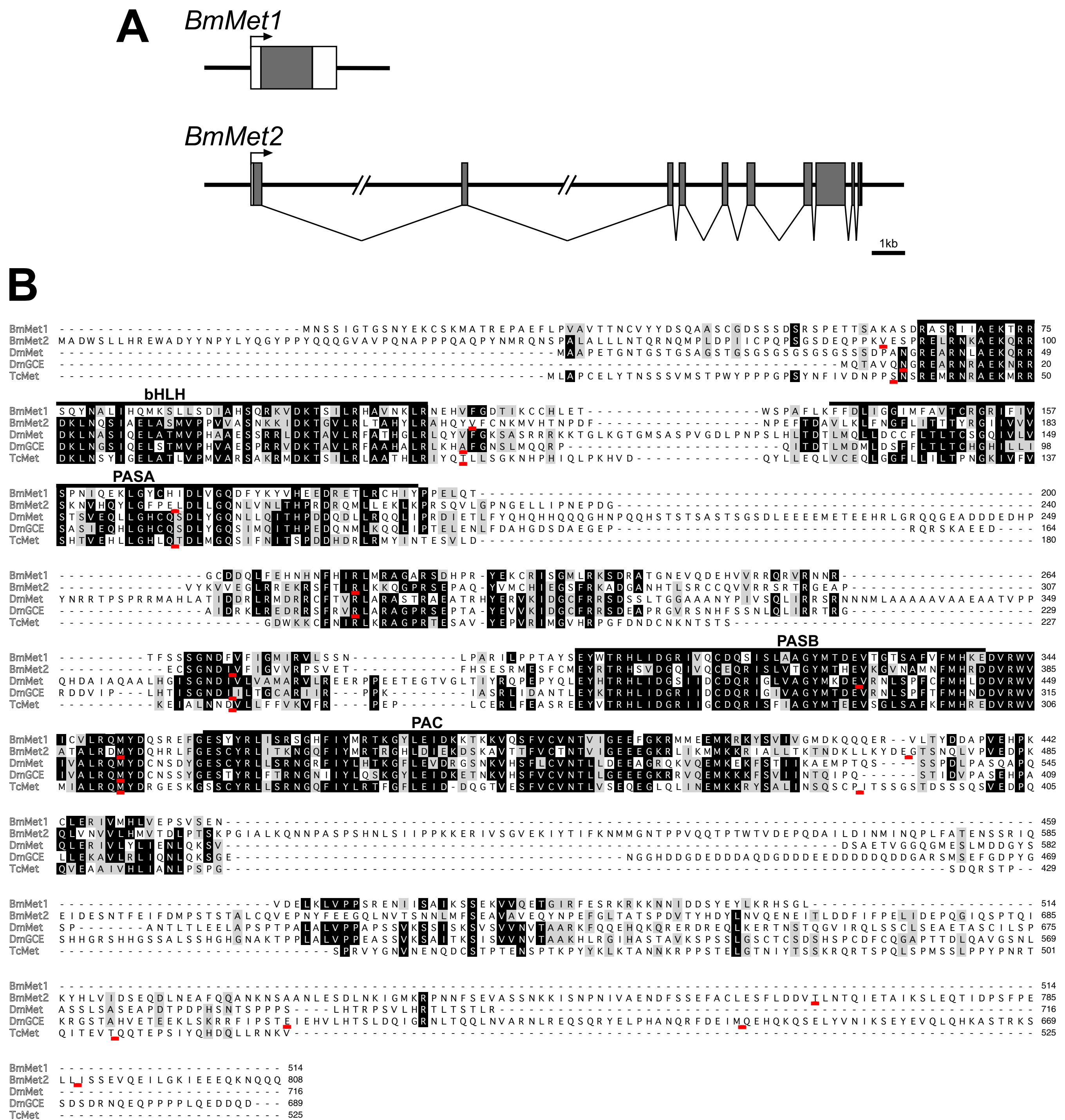

Fig. S2. Schematic representation of the genomic structures of *BmMet1* and *BmMet2* (*A*) and alignment of Met sequences from other insect species (*B*). (*A*) Genomic structures of *BmMet1* and *BmMet2*. Putative exons are boxed, and ORFs are shaded. (*B*) Alignment of the predicted amino acid sequences of BmMet1 and BmMet2 with the sequences of *D. melanogaster* Met (DmMet, GenBank accession no. NP_511126) and GCE (DmGCE, GenBank accession no. NP_511160) and *T. castaneum* Met (TcMet, GenBank accession no. BAG71980). Black and light gray shading indicate identical and similar amino acid residues, respectively. The bHLH, PASA, PASB, and PAC domains (1) are indicated. Red bars mark the positions of introns in the ORFs. The positions of introns were inferred from the insect genomic databases KAIKObase (http://sgp.dna.affrc.go.jp/KAIKObase/), BeetleBase (http://beetlebase.org/), and FlyBase (http://flybase.org/).

1. Baumann A, Fujiwara Y, Wilson TG (2010) Evolutionary divergence of the paralogs *Methoprene tolerant* (*Met*) and *germ cell expressed* (*gce*) within the genus *Drosophila*. *J Insect Physiol* 56:1445–1455.

Fig. S3. Mutation analysis to identify the critical sequences of *k*JHRE. NIAS-Bm-aff3 cells were cotransfected with mutant *k*JHRE reporter plasmids carrying firefly luciferase and a reference reporter plasmid carrying *Renilla* luciferase and were treated with 10 μM JHA for 24 h. (*Right*) Reporter activity was measured by using the Dual-Luciferase reporter assay system. Data represent means ± SD (n = 3). Means with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$). (*Left*) In the *k*JHRE reporter plasmid (−2165 to −2025 and −49 to +116, pGL4.14), GTG and CAC were changed to AAA and TTT, respectively (*A*), GAG and CTC to TTT and AAA, respectively (*B*), and GCG and CGC to TTT (*B*), as indicated. The leftmost symbols indicate the names of constructs corresponding to those in Table S3, and the colored circles (red, blue, and green) represent the locations of mutations.

Fig. S4. Analysis of the basal promoter region of *BmKr-h1*. NIAS-Bm-aff3 cells were cotransfected with the indicated reporter plasmids and a reference reporter plasmid and were treated with 10 μM JHA for 24 h. Reporter activity was measured using the Dual-Luciferase reporter assay system. Data represent means ± SD (n = 3). Means with the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$). Some data were analyzed by the Student's t test (**$P < 0.01$, ***$P < 0.001$). (*A*) In the *k*JHRE reporter (−2165 to −2025 and −49 to +116, pGL4.14), the basal promoter region (−49 to +116) was truncated. (*B*) In the *k*JHRE reporter, the basal promoter region was exchanged with the basal promoter region of *BmA3* (GenBank accession no. AF422795, +50 to +138) and *BmHsp70* (GenBank accession no. AB035326, −162 to +11).

Table S1. List of primers used for cDNA cloning and construction of expression plasmids

Plasmid name	Construction method	PCR template	Primer name	Nucleotide sequence (5′ to 3′)
pGEMT_BmMet1_RTPCR	PCR, ligation	5′ SMART RACE cDNA (fifth prothoracic glands)	BmMet1_RTPCR_FW	ATGAACTCGTCTATTGGGACAGGATCGAAT
			BmMet1_RTPCR_RV	TTACAATCCACTGTGCCTTTTTAAATACTCATAGCTG
pGEMT_BmMet1_5′RACE	5′-SMART RACE	5′ SMART RACE cDNA (fifth prothoracic glands)	BmMet1_5′RACE	CGTGAATCTGAAGAAGAGTCTCCGCAG
pGEMT_BmMet1_3′RACE	3′-SMART RACE	3′ SMART RACE cDNA (fifth prothoracic glands)	BmMet1_3′RACE	TCCAAAGTGTCTTGAGCGGATTGTAATG
pGEMT_BmMet1_ORF	PCR, ligation	cDNA	BmMet1_ORF_FW	ATGAACTCGTCTATTGGGACAGGATCGAAT
			BmMet1_ORF_RV	TTACAATCCACTGTGCCTTTTTAAATACTCATAGCTG
pDONR_BmMet1	PCR, Gateway system	pGEMT_BmMet1	BmMet1_GW_FW	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGAACTCGTCTATTGGGAC
			BmMet1_GW_RV	AGAAAGCTGGGTCTTACAATCCACTGTGCCTTT
pBIND_BmMet1	PCR, ligation	pGEMT_BmMet1	BmMet1_pBIND_FW	TTTGGATCCGAATGAACTCGTCTATTGGGACAGGA
			BmMet1_pBIND_RV	AAAACGCGTTTACAATCCACTGTGCCTTTTTAAATACT
pGEMT_BmMet2_RTPCR	PCR, ligation	cDNA	BmMet2_RTPCR_FW	AACCTGGTGAATCTGACTCATCCG
			BmMet2_RTPCR_RV	CGCTTTCCCTCCTCTTCTCCAATAA
pGEMT_BmMet2_5′RACE	5′-SMART RACE	5′ SMART RACE cDNA (fifth prothoracic glands)	BmMet2_5′RACE	GGTCCCTGCTTCTTCAACCTGATGG
pGEMT_BmMet2_3′RACE	3′-SMART RACE	3′ SMART RACE cDNA (fifth prothoracic glands)	BmMet2_3′RACE	GCGAACAGCGTATCTCGTTGGTCAC
pGEMT_BmMet2_ORF	PCR, ligation	cDNA	BmMet2_ORF_FW	ATGGCTGATTGGTCTCTGCTGCACC
			BmMet2_ORF_RV	TTATTGTTGTTGGTTTTTCTGTTCCTCTTC
pDONR_BmMet2	PCR, Gateway system	pGEMT_BmMet2	BmMet2_GW_FW	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGGCTGATTGGTCTCTGCT
			BmMet2_GW_RV	AGAAAGCTGGGTCTTATTGTTGTTGGTTTTTCT
pBIND_BmMet2	PCR, ligation	pGEMT_BmMet2	BmMet2_pBIND_FW	TTTGGATCCGAATGGCTGATTGGTCTCTGCTGC
			BmMet2_pBIND_RV	AAAACGCGTTTATTGTTGTTGGTTTTTCTGTTCCTCT
pBIND_BmMet2ΔGAL4DBD	Inverse PCR	pBIND_BmMet2	BmMet2_pBINDiPCR_FW	ATGGCTGATTGGTCTCTGCTGCACCG
			BmMet2_pBINDiPCR_RV	CTTTCAGGAGGCTTGCTTCAAGCTGGC
pACT_BmMet2	PCR, ligation	pGEMT_BmMet2	BmMet2_pACT_FW	TTTGGATCCGAATGGCTGATTGGTCTCTGCTGC
			BmMet2_pACT_RV	AAAACGCGTTTATTGTTGTTGGTTTTTCTGTTCCTCT
pBIND_VP16AD	PCR, ligation	pACT (Promega)	VP16AD_ pBIND_FW	TTTGGATCCGAATGAAGCTACTGTCTTCTATCGAACAA
			VP16AD_ pBIND_RV	AAAGATATCTTATCCCGGACCCGGGGAAT
pGEMT_BmSRC_RT-PCR	PCR, ligation	RLM RACE cDNA (epidermis)	BmSRC_RTPCR_FW	GGCACTCAAACATTACACCAATAACCTCGG
			BmSRC_RTPCR_RV	CCAGTTGAAAGATTGTATGTTCCTAAGTAACGGTC
pGEMT_BmSRC_5′RACE	RLM RACE	RLM RACE cDNA (epidermis)	BmSRC_5′RACE_RV1	GTTTCGCATGATCTTTCTCATGTAAAATCGAAAATATCG
		BmSRC_5′RACE first PCR product	BmSRC_5′RACE_RV2	CCTATATAGTTCTGTTCTTTCGTGAAGAATAAACTCC
pGEMT_BmSRC_3′RACE	RLM RACE	RLM RACE cDNA (epidermis)	BmSRC_3′RACE_FW1	GGGTTTTATTGGAAATTAATTCGAAAGGTGAAATAGAGTG
		BmSRC_3′RACE first PCR product	BmSRC_3′RACE_FW2	GTATCAGATAATATCAAGGAGTTTATTCTTCACGAAAGAAC
pGEMT_BmSRC_ORF	PCR, ligation	cDNA	BmSRC_ORF_FW	ATGCTGCCCGTGGTTCAATCCGAGCC
			BmSRC_ORF_RV	TCACCGTCCGCCGCCTCCGC
pDONR_BmSRC	PCR, Gateway system	pGEMT_BmSRC	BmSRC_GW_FW	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGCTGCCCGTGGTTCAATCC
			BmSRC_GW_RV	AGAAAGCTGGGTCTCACCGTCCGCCGCCTCC
pBIND_BmSRC	PCR, ligation	pGEMT_BmSRC	BmSRC_pBIND_FW	TTTGGATCCATGCTGCCCGTGGTTCAATCC
			BmSRC_pBIND_RV	AAAACGCGTTCACCGTCCGCCGCCTCC
pBIND_BmSRCΔGAL4DBD	Inverse PCR	pBIND_BmSRC	BmSRC_pBINDiPCR_FW	ATGCTGCCCGTGGTTCAATCCGAGCC
			BmSRC_pBINDiPCR_RV	CTTTCAGGAGGCTTGCTTCAAGCTGGC
pACT_BSRC	PCR, ligation	pGEMT_BmSRC	BmSRC_pACT_FW	TTTGGATCCATGCTGCCCGTGGTTCAATCC
			BmSRC_pACT_RV	AAAACGCGTTCACCGTCCGCCGCCTCC
pDONR_BmUSP	PCR, Gateway system	Full-length cDNA clone (fwdP12_FL5_C16)	BmUSP_GW_FW	AAAAAGCAGGCTNNATGTCGAGCGTGGCGAAGAA
			BmUSP_GW_RV	AGAAAGCTGGGTNCTACATGATGTTGGTGTCGA
pDONR_BmARNT	PCR, Gateway system	Full-length cDNA clone (fmaV30399)	BmARNT_GW_FW	AAAAAGCAGGCTNNATGTCTGCCGTAGCTCCAAC
			BmARNT_GW_RV	AGAAAGCTGGGTNTTACTCAAAGTTCGAATTAA
pDONR_BmHIF-1α	PCR, Gateway system	Full-length cDNA clone (fprW24_F_E04)	BmHIF-1α_GW_FW	AAAAAGCAGGCTNNATGTCTACGAAACCAGCAAA
			BmHIF-1α_GW_RV	AGAAAGCTGGGTNCTACCCAAGTTTGACGTCAT
pDONR_BmTimeless	PCR, Gateway system	Full-length cDNA clone (fdpeP16_F_G06)	BmTimeless_GW_FW	AAAAAGCAGGCTNNATGGAATGGGTTTTAAGAAG
			BmTimeless_GW_RV	AGAAAGCTGGGTNTCAGAAATTGGGCGCAAATT

Table S2. List of primers used for quantitative RT-PCR

Target gene and primer name	Nucleotide sequence (5′ to 3′)
BmKr-h1	
BmKrh1_qRT-PCR_FW	ACCCATACTGGCGAGCGACCAT
BmKrh1_qRT-PCR_RV	CCTCTCCTTTGTGTGAATACGACGG
BmKr-h1α	
BmKrh1α_qRT-PCR_FW	CACAACCTACGCCAACATTAGAAACG
BmKrh1α_qRT-PCR_RV	ACTGATGAACTCGCTCCTCGTCAC
BmKr-h1β	
BmKrh1β_qRT-PCR_FW	GAAACAATTTCGTTCTTCAGGTGACG
BmKrh1β_qRT-PCR_RV	TCGTGCGTGTGCTGTAAGCG
BmRp49	
BmRp49_qRT-PCR_FW	CAGGCGGTTCAAGGGTCAATAC
BmRp49_qRT-PCR_RV	TGCTGGGCTCTTTCCACGA

Table S3. List of primers, methods, and templates used for construction of reporter plasmids

Reporter plasmid name	Construction method	PCR template	Primer name	Nucleotide sequence (5′ to 3′)
pGL4.14_−4741/+968	Gateway system	Genomic DNA	BmKrh1_Pro_F2	AAAAAGCAGGCTNNGTCTCTATTGGTGGTTCCAGCAC
			BmKrh1_Pro_R1	AGAAAGCTGGGTNGCACTGATGAACTCGCTCCTCGTCAC
pGL4.14_-4741/+116	Gateway system	Genomic DNA	BmKrh1_Pro_F2	AAAAAGCAGGCTNNGTCTCTATTGGTGGTTCCAGCAC
			BmKrh1_Pro_R2	AGAAAGCTGGGTNAATGATGATTTGGATGCGAAGCGT
pGL4.14_−2165/+116	Inverse PCR	pGL4.14_−4741/+116	BmKrh1_ProiPCR_F8	CAAGTGTGGGGAGACGGCGAGAGG
			BmKrh1_ProiPCR_R1	ATCCTCGAGGCTAGCGAGCTCAGGTAC
pGL4.14_−1978/+116	Inverse PCR	pGL4.14_−4741/+116	BmKrh1_ProiPCR_F1	CGTACCGTCTACCTACCGTGTTTGTGGTG
			BmKrh1_ProiPCR_R1	ATCCTCGAGGCTAGCGAGCTCAGGTAC
pGL4.14_-−165/−1999 and −49/+116	Inverse PCR	pGL4.14_−2165/+116	BmKrh1_ProiPCR_F13	CGCGAGCATTCCGGGCCGGGA
			BmKrh1_ProiPCR_R6	TGGATGGAATGTCATAGCTGGATGTGTGCG
pGL4.14_−2165/−2025 and −49/+116	Inverse PCR	pGL4.14_−2165/+116	BmKrh1_ProiPCR_F13	CGCGAGCATTCCGGGCCGGGA
			BmKrh1_ProiPCR_R10	TGCGGTGTGAAGGAGATGGGGTGGG
pGL4.14_−2165/−2045 and −49/+116	Inverse PCR	pGL4.14_−2165/+116	BmKrh1_ProiPCR_F13	CGCGAGCATTCCGGGCCGGGA
			BmKrh1_ProiPCR_R11	GTGGGGCAAGCGGTAGCGTTCGACA
pGL4.14_−2165/−2068 and −49/+116	Inverse PCR	pGL4.14_−2165/+116	BmKrh1_ProiPCR_F13	CGCGAGCATTCCGGGCCGGGA
			BmKrh1_ProiPCR_R12	CACGTGGAGGCCCACCGCGGTT
pGL4.14_−2165/−2079 and −49/+116	Inverse PCR	pGL4.14_−2165/+116	BmKrh1_ProiPCR_F13	CGCGAGCATTCCGGGCCGGGA
			BmKrh1_ProiPCR_R42	CCACCGCGGTTTCGAAGCGCGC
pGL4.14_3×JHRE core and −49/+116	Inverse PCR	pGL4.14_−2165/−2025 and −49/+116	BmKrh1_ProiPCR_F52	CACGTGGGCCTCCACGTGCGCGAGCATTCCGGGCCG
			BmKrh1_ProiPCR_R38	GAGGCCCACGTGGAGGCCATCCTCGAGGCTAGCGAGCTC
pGL4.14_−2105/−2068 and −49/+116	Inverse PCR	pGL4.14_−2165/−2068 and −49/+116	BmKrh1_ProiPCR_F21	CACACGCGCGCTTCGAAACCGC
			BmKrh1_ProiPCR_R1	ATCCTCGAGGCTAGCGAGCTCAGGTAC
pGL4.14_−2165/−2025 and −29/+116	Inverse PCR	pGL4.14_−2165/−2025 and −49/+116	BmKrh1_ProiPCR_F23	AGTCTTCAGTTGAGCGATCAAGCGACG
			BmKrh1_ProiPCR_R10	TGCGGTGTGAAGGAGATGGGGTGGG
pGL4.14_−2165/−2025 and −19/+116	Inverse PCR	pGL4.14_−2165/−2025 and −49/+116	BmKrh1_ProiPCR_F24	TGAGCGATCAAGCGACGGCAGTCG
			BmKrh1_ProiPCR_R10	TGCGGTGTGAAGGAGATGGGGTGGG
pGL4.14_−2165/−2025 and −49/+92	Inverse PCR	pGL4.14_−2165/−2025 and −49/+116	BmKrh1_ProiPCR_F9	ATCAAGATCTGGCCTCGGCGGCCAAG
			BmKrh1_ProiPCR_R13	TAACGTTTCTAATGTTGGCGTAGGTTGTGATTCA
pGL4.14_−2165/−2025 and −49/+26	Inverse PCR	pGL4.14_−2165/−2025 and −49/+116	BmKrh1_ProiPCR_F9	ATCAAGATCTGGCCTCGGCGGCCAAG
			BmKrh1_ProiPCR_R15	ACTCGACCGACAGTCTGCGGCC
pGL4.14_−2165/−2025 and BmA3	Inverse PCR (vector)	pGL4.14_−2165/−2025 and −49/+116	BmKrh1_ProiPCR_F13	CGCGAGCATTCCGGGCCGGGA
			BmKrh1_ProiPCR_R24	TTTACGCGTTGCGGTGTGAAGGAGATGG
	PCR (insert)	pBacBmA3dGAL4/3×P3DsRed	BmA3d_FW	TTTACGCGTGAAAAACAAACTGGAGCCGATA
			BmA3d_RV	TTTAGATCTCTTAGTAAATGAGAACCGACTC
pGL4.14_−2165/−2025 and BmHSP70	Inverse PCR (vector)	pGL4.14_−2165/−2025 and −49/+116	BmKrh1_ProiPCR_F13	CGCGAGCATTCCGGGCCGGGA
			BmKrh1_ProiPCR_R24	TTTACGCGTTGCGGTGTGAAGGAGATGG
	PCR (insert)	pBacBmhsp70GAL4/3×P3DsRed	Bmhsp70d_FW	TTTACGCGTCTACGCTGCGATTGGATAGA
			Bmhsp70d _RV	TTTAGATCTCGTCTGCTTCGTATTGCGTT

Table S4. List of primers used for construction of mutated reporter plasmids

Reporter plasmid name	Primer name	Nucleotide sequence (5′ to 3′)
pGL4.14_–2165/+116_M1	BmKrh1_ProiPCR_F25	CAAAAAAAGGGAGACGGCGAGAGGAGTTGAG
	BmKrh1_ProiPCR_R1	ATCCTCGAGGCTAGCGAGCTCAGGTAC
pGL4.14_–2165/+116_M2	BmKrh1_ProiPCR_F31	CAAAAATGGGGAGACGGCGAGAGGAGTTGAG
	BmKrh1_ProiPCR_R1	ATCCTCGAGGCTAGCGAGCTCAGGTAC
pGL4.14_–2165/+116_M3	BmKrh1_ProiPCR_F32	CAAGTAAAGGGAGACGGCGAGAGGAGTTGAG
	BmKrh1_ProiPCR_R1	ATCCTCGAGGCTAGCGAGCTCAGGTAC
pGL4.14_–2165/+116_M4	BmKrh1_ProiPCR_F26	TTTGCGCTCTGTTGTTGAGAGTTCACACGC
	BmKrh1_ProiPCR_R16	CGTAGACTCAACTCCTCTCGCCGTC
pGL4.14_–2165/+116_M5	BmKrh1_ProiPCR_F27	TTTTTGCGCGCTTCGAAACCGCGGTG
	BmKrh1_ProiPCR_R17	AACTCTCAACAACAGAGCGCGTGCG
pGL4.14_–2165/+116_M6	BmKrh1_ProiPCR_F33	TTTACGCGCGCTTCGAAACCGCGGTG
	BmKrh1_ProiPCR_R17	AACTCTCAACAACAGAGCGCGTGCG
pGL4.14_–2165/+116_M7	BmKrh1_ProiPCR_F34	CATTTGCGCGCTTCGAAACCGCGGTG
	BmKrh1_ProiPCR_R17	AACTCTCAACAACAGAGCGCGTGCG
pGL4.14_–2165/+116_M8	BmKrh1_ProiPCR_F28	AAAGGCCTCCACGTGTCGAACGCTAC
	BmKrh1_ProiPCR_R18	CGCGGTTTCGAAGCGCGCGTGT
pGL4.14_–2165/+116_M9	BmKrh1_ProiPCR_F29	TTTAAAACGAACGCTACCGCTTGCCCCAC
	BmKrh1_ProiPCR_R19	GAGGCCCACCGCGGTTTCGAAG
pGL4.14_–2165/+116_M10	BmKrh1_ProiPCR_F35	TTTGTGTCGAACGCTACCGCTTGCCCCAC
	BmKrh1_ProiPCR_R19	GAGGCCCACCGCGGTTTCGAAG
pGL4.14_–2165/+116_M11	BmKrh1_ProiPCR_F36	CACAAAACGAACGCTACCGCTTGCCCCAC
	BmKrh1_ProiPCR_R19	GAGGCCCACCGCGGTTTCGAAG
pGL4.14_–2165/+116_M12	BmKrh1_ProiPCR_F30	TTTCCCATCTCCTTCACACCGC
	BmKrh1_ProiPCR_R20	GGGCAAGCGGTAGCGTTCGACAC
pGL4.14_–2165/+116_M13	BmKrh1_ProiPCR_F13	CGCGAGCATTCCGGGCCGGGA
	BmKrh1_ProiPCR_R21	GCGAAAAAAAGGAGATGGGGTGGGGCAAGCG
pGL4.14_–2165/+116_M14	BmKrh1_ProiPCR_F13	CGCGAGCATTCCGGGCCGGGA
	BmKrh1_ProiPCR_R22	GCGAAATGAAGGAGATGGGGTGGGGCAAGCG
pGL4.14_–2165/+116_M15	BmKrh1_ProiPCR_F13	CGCGAGCATTCCGGGCCGGGA
	BmKrh1_ProiPCR_R23	GCGGTAAAAAGGAGATGGGGTGGGGCAAGCG
pGL4.14_–2165/+116_M16	BmKrh1_ProiPCR_F37	CAAGTGTGGGTTTACGGCGAGAGGAGTTGAGTCTACG
	BmKrh1_ProiPCR_R1	ATCCTCGAGGCTAGCGAGCTCAGGTAC
pGL4.14_–2165/+116_M17	BmKrh1_ProiPCR_F38	TTTAGGAGTTGAGTCTACGCACGCGCTC
	BmKrh1_ProiPCR_R25	GCCGTCTCCCCACACTTG
pGL4.14_–2165/+116_M18	BmKrh1_ProiPCR_F39	TTTTTGAGTCTACGCACGCGCTCTGTTGTTG
	BmKrh1_ProiPCR_R26	CTCTCGCCGTCTCCCCACACTTG
pGL4.14_–2165/+116_M19	BmKrh1_ProiPCR_F40	TTTTCTACGCACGCGCTCTGTTGTTGAGAG
	BmKrh1_ProiPCR_R27	AACTCCTCTCGCCGTCTCCCCACA
pGL4.14_–2165/+116_M20	BmKrh1_ProiPCR_F41	AAATGTTGTTGAGAGTTCACACGCGCGCTTC
	BmKrh1_ProiPCR_R28	CGCGTGCGTAGACTCAACTCCTCTC
pGL4.14_–2165/+116_M21	BmKrh1_ProiPCR_F42	TTTTTTTCACACGCGCGCTTCGAAACCGC
	BmKrh1_ProiPCR_R29	AACAACAGAGCGCGTGCGTAGACTCAA

Table S4. Cont.

Reporter plasmid name	Primer name	Nucleotide sequence (5′ to 3′)
pGL4.14_–2165/+116_M22	BmKrh1_ProiPCR_F43	AAACACGTGTCGAACGCTACCGCTTGC
	BmKrh1_ProiPCR_R30	GCCCACCGCGGTTTCGAAGCGC
pGL4.14_–2165/+116_M23	BmKrh1_ProiPCR_F13	CGCGAGCATTCCGGGCCGGGA
	BmKrh1_ProiPCR_R36	GCGGTGTGAAGTTTATGGGGTGGGGCAAGCGGTAGC
pGL4.14_–2165/+116_M24	BmKrh1_ProiPCR_F44	CAAGTGTGGGGAGACGTTTAGAGGAGTTGAGTCTACGCACGCGC
	BmKrh1_ProiPCR_R1	ATCCTCGAGGCTAGCGAGCTCAGGTAC
pGL4.14_–2165/+116_M25	BmKrh1_ProiPCR_F45	TTTACGCGCTCTGTTGTTGAGAGTTCACACGCGCGC
	BmKrh1_ProiPCR_R31	TAGACTCAACTCCTCTCGCCGTCTCC
pGL4.14_–2165/+116_M26	BmKrh1_ProiPCR_F46	CGCATTTTTTCTGTTGTTGAGAGTTCACACGCGCGC
	BmKrh1_ProiPCR_R31	TAGACTCAACTCCTCTCGCCGTCTCC
pGL4.14_–2165/+116_M27	BmKrh1_ProiPCR_F47	TTTTTTTTTCGAAACCGCGGTGGGCCTCCA
	BmKrh1_ProiPCR_R32	TGTGAACTCTCAACAACAGAGCGCGTG
pGL4.14_–2165/+116_M28	BmKrh1_ProiPCR_F48	TTTTGTGGGCCTCCACGTGTCGAACG
	BmKrh1_ProiPCR_R33	GTTTCGAAGCGCGCGTGTGAACTCTC
pGL4.14_–2165/+116_M29	BmKrh1_ProiPCR_F49	TTTTACCGCTTGCCCCACCCCATCTCC
	BmKrh1_ProiPCR_R34	TTCGACACGTGGAGGCCCACCG
pGL4.14_–2165/+116_M30	BmKrh1_ProiPCR_F50	TTTTTGCCCCACCCCATCTCCTTCACAC
	BmKrh1_ProiPCR_R35	GTAGCGTTCGACACGTGGAGGCC
pGL4.14_–2165/+116_M31	BmKrh1_ProiPCR_F13	CGCGAGCATTCCGGGCCGGGA
	BmKrh1_ProiPCR_R37	AAAGTGTGAAGGAGATGGGGTGGGGCAA
pGL4.14_–2165/+116 _transversion 1	BmKrh1_ProiPCR_F59	TGTGGCCTCCACGTGTCGAACGCTAC
	BmKrh1_ProiPCR_R18	CGCGGTTTCGAAGCGCGCGTGT
pGL4.14_–2165/+116 _transversion 2	BmKrh1_ProiPCR_F60	TTACTCCACGTGTCGAACGCTACCGCTT
	BmKrh1_ProiPCR_R39	CACCGCGGTTTCGAAGCGCGCG
pGL4.14_–2165/+116 _transversion 3	BmKrh1_ProiPCR_F61	AGACACGTGTCGAACGCTACCGCTTGC
	BmKrh1_ProiPCR_R30	GCCCACCGCGGTTTCGAAGCGC
pGL4.14_–2165/+116 _transversion 4	BmKrh1_ProiPCR_F62	ACAGTGTCGAACGCTACCGCTTGCCCCAC
	BmKrh1_ProiPCR_R19	GAGGCCCACCGCGGTTTCGAAG
pGL4.14_–2165/+116 _transversion 5	BmKrh1_ProiPCR_F63	CACTGTTCGAACGCTACCGCTTGCCCCAC
	BmKrh1_ProiPCR_R19	GAGGCCCACCGCGGTTTCGAAG
pGL4.14_–2165/+116 _transversion 6	BmKrh1_ProiPCR_F64	GATAACGCTACCGCTTGCCCCACCCC
	BmKrh1_ProiPCR_R40	CACGTGGAGGCCCACCGCGGTT
pGL4.14_–2165/+116 _transition 1	BmKrh1_ProiPCR_F65	ACAGGCCTCCACGTGTCGAACGCTAC
	BmKrh1_ProiPCR_R18	CGCGGTTTCGAAGCGCGCGTGT
pGL4.14_–2165/+116 _transition 2	BmKrh1_ProiPCR_F66	AATCTCCACGTGTCGAACGCTACCGCTT
	BmKrh1_ProiPCR_R39	CACCGCGGTTTCGAAGCGCGCG
pGL4.14_–2165/+116 _transition 3	BmKrh1_ProiPCR_F67	TCTCACGTGTCGAACGCTACCGCTTGC
	BmKrh1_ProiPCR_R30	GCCCACCGCGGTTTCGAAGCGC
pGL4.14_–2165/+116 _transition 4	BmKrh1_ProiPCR_F68	TGTGTGTCGAACGCTACCGCTTGCCCCAC
	BmKrh1_ProiPCR_R19	GAGGCCCACCGCGGTTTCGAAG
pGL4.14_–2165/+116 _transition 5	BmKrh1_ProiPCR_F69	CACACATCGAACGCTACCGCTTGCCCCAC
	BmKrh1_ProiPCR_R19	GAGGCCCACCGCGGTTTCGAAG
pGL4.14_–2165/+116 _transition 6	BmKrh1_ProiPCR_F70	CTAAACGCTACCGCTTGCCCCACCCC
	BmKrh1_ProiPCR_R40	CACGTGGAGGCCCACCGCGGTT

Red letters indicate mutated nucleotides.