Supplementary information

Establishment of a versatile cell line for juvenile hormone signaling analysis in *Tribolium castaneum*

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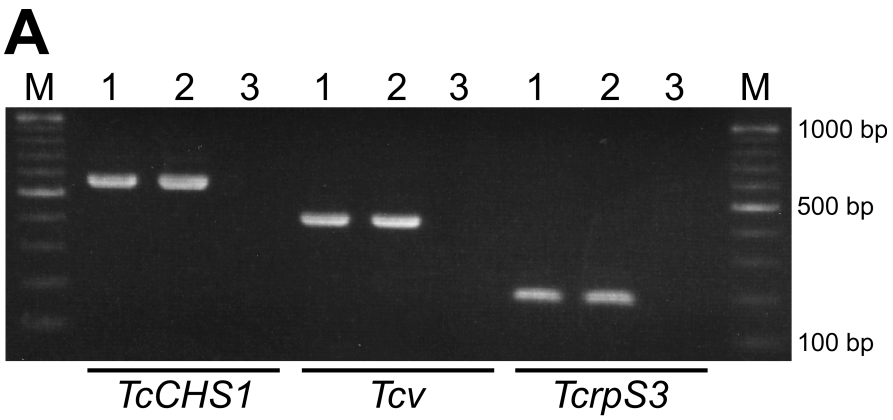
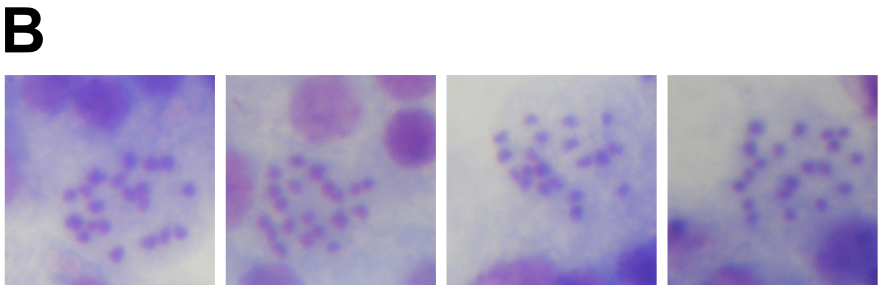
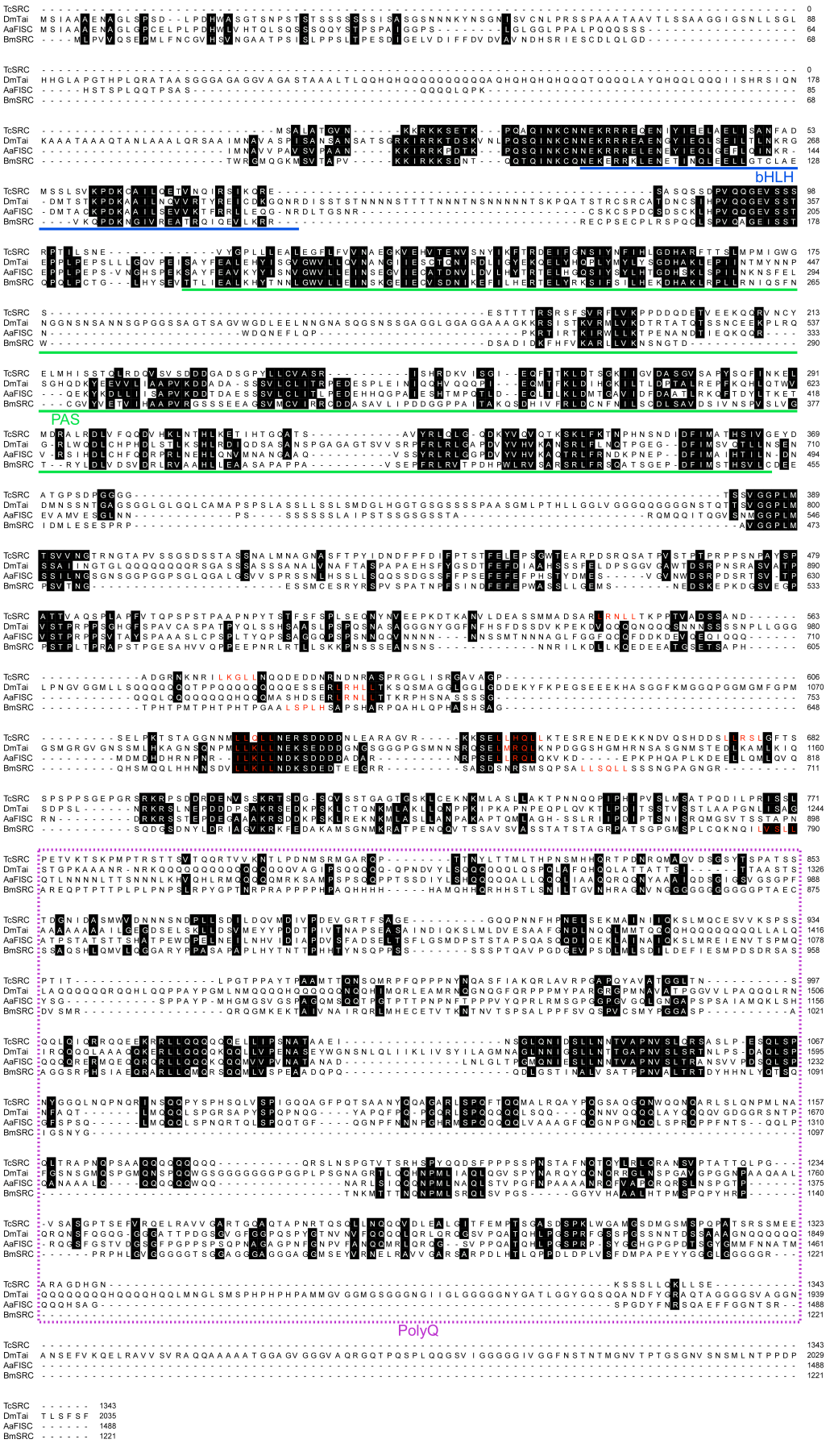

A
M 1 2 3 1 2 3 1 2 3 M
1000 bp
500 bp
100 bp
TcCHS1
Tcv
TcrpS3


B

Supplementary Figure S1. Characterization of the Tc81 cell line. (A) PCR characterization of Tc81 cells. Genomic DNA was extracted from whole bodies of *T. castaneum* larvae and Tc81 cells using DNAzol Reagent (Invitrogen) and amplified by PCR with primer sets specific for 3 representative *T. castaneum* genes. Primers and PCR conditions were the same as described by Goodman et al.[1] (M) 100 bp DNA ladder; (1) PCR product amplified from larval genomic DNA; (2) PCR product amplified from Tc81 cell genomic DNA; (3) PCR product amplified from water (no template negative control). *TcCHS1*, *chitin synthase 1*; *Tcv*, *tryptophan oxygenase V*; *TcrpS3*, *ribosomal protein S3*. (B) Typical metaphase chromosomes of Tc81 cells. Karyotype analysis of Tc81 cells was performed essentially according to the methods of Mitsuhashi[2].

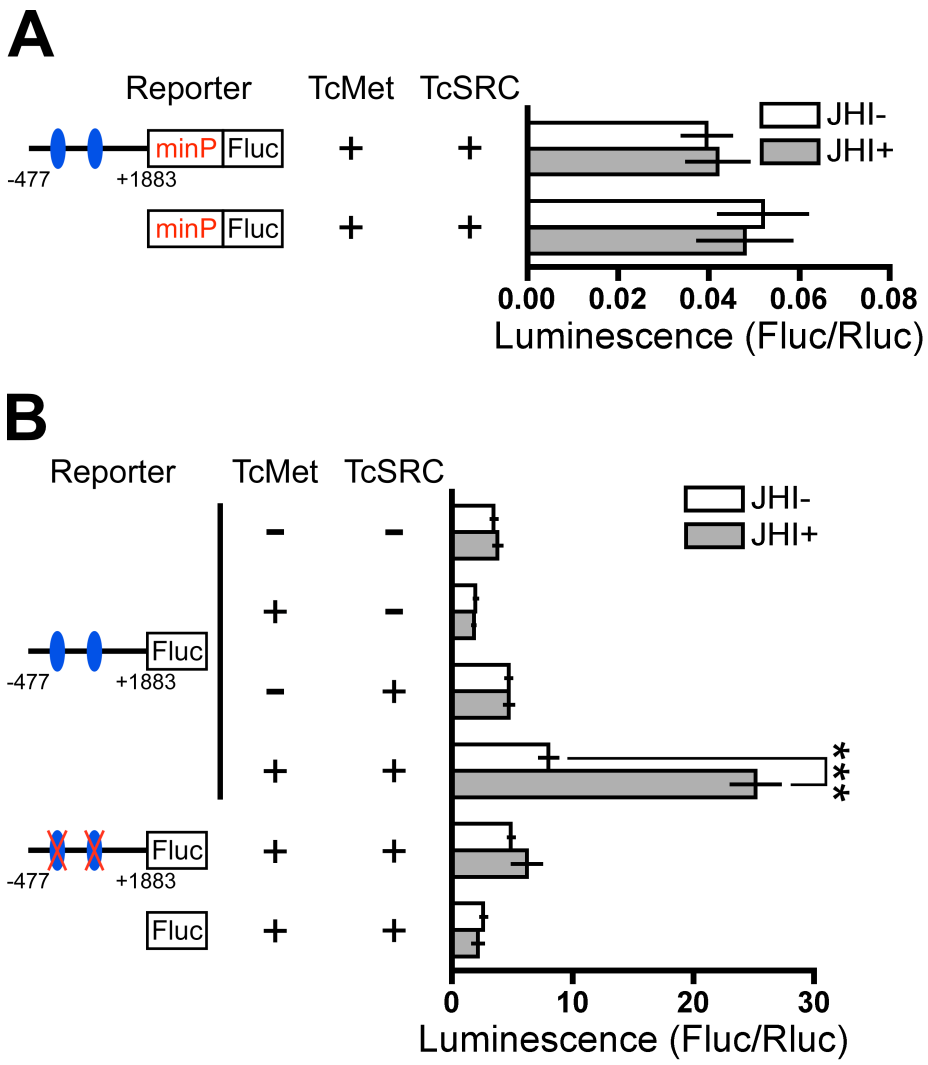
1 Goodman, C. L., et al. A cell line derived from the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae). *In Vitro Cell. Dev. Biol. Anim.* **48**, 426-433 (2012).

2 Mitsuhashi, J. Compositions of salt solutions and culture media. in *Invertebrate Tissue Culture Methods* (ed Mitsuhashi, J.). 401-420 (Springer Lab Manual, Tokyo 2002).

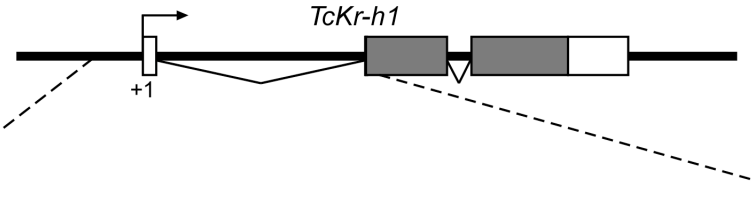

bHLH
PAS
PolyQ

Supplementary Figure S2. Alignment of SRC protein sequences. Alignment of the predicted amino acid sequences of TcSRC with *D. melanogaster* Taiman (DmTai, AAG16637), *A. aegypti* FISC (AaFISC, ABE99837), and *B. mori* SRC (BmSRC, AB703620). The bHLH and PAS domains are indicated by lines, red letters represent the LXXLL motifs, and the polyQ domains are boxed (purple)[3]. Black shading indicates identical amino acid residues.

3 Zhu, J. S., Chen, L., Sun, G. Q. & Raikhel, A. S. The competence factor beta Ftz-F1 potentiates ecdysone receptor activity via recruiting a p160/SRC coactivator. *Mol. Cell. Biol.* **26**, 9402-9412 (2006).


A
Reporter
TcMet
TcSRC
minP
Fluc
-477
+1883
+
+
JHI-
JHI+
0.00
0.02
0.04
0.06
0.08
Luminescence (Fluc/Rluc)
B
Reporter
TcMet
TcSRC
-
+
Fluc
-477
+1883
JHI-
JHI+
***
0
10
20
30
Luminescence (Fluc/Rluc)

Supplementary Figure S3. Functional analysis of *TcMet*, *TcSRC*, and *k*JHRE in mammalian HEK293 cells and *Drosophila* S2 cells. (A) The *k*JHRE-reporter vector (-477 to +1883, pGL4.14) was modified for mammalian cells; the mammalian minimal promoter (minP) (pGL4.27, Promega) was inserted into the region between *k*JHRE and the firefly luciferase gene. HEK293 cells were cotransfected with the modified *k*JHRE-reporter vector (-477 to +1883 and minP, pGL4.14) and a plasmid expressing the full ORF of *TcMet* and/or *TcSRC*. Cells were then treated with 0.1 μM JH III for 24 h. (B) S2 cells were cotransfected with a *k*JHRE-reporter vector (-477 to +1883, pGL4.14) and a plasmid expressing the full ORF of *TcMet* or *TcSRC* in the same manner as that applied for Tc81 cells. Cells were then treated with 0.1 μM JH III for 24 h. Xs indicate mutations in the *k*JHREc reporter. (A, B) Reporter activity was examined using a dual-luciferase reporter assay system. Data represent means ± SDs (n = 3). Data were analyzed by Student's *t*-tests (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; not indicated, $P > 0.05$).



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CAACTTTTGGGACCTGACCCAGGCCACGCATAAAATTAATTAACCCATCGATCGTTGC
TATGATAACGCACAACAATTCAAATAAAAACCAACCGCCAAACGCTCCTCTTTATCGAC
TCGCATAATAATCGCGATACCGTCGCTTGCAAAATATGTTTATTAACACAAACGAATCC
AAAATCCCTAGTACTTCGCCTCCACGTGCCGTTTCAACACGGGCCAGACTGTTCGGCA
*k*JHREc

TCTAGTGGGCGAGAAGAGTGCGGGGAAGGACGCAAACATCCGATCGGCCCTCATCG
GCACCCACCGCAACAGCCGAACGCGCGCGAGGTAAACATTTAGTTTGATTTTAGCCC

+1 Transcription start site

AACCAAGTGTAGCAGTGAGAAGAAAAATTGCCATTGAATAACACCACCAAGTGCTATT
TCACTATCATTCAATTGGACACAACCCCATGGCACACACAGGTCTCCAAACCTGAGAA
ATTGTAAGTTTTTATTTTACTTATCAAAAATTTTCTTTTTTTCTGGGCTTGGGTTGGTAC
GGCTTGCATCGCCACCGCGATAAGGGTAAAGTATCAAAAATGTGACAGGACGCGTCA
AAAAACGGCCAAAATGTCATTGAGTAAATAAAATGCGTGCGTTGTGGGCCGGCGCGG
GTGTTTGGGGGGCGCAGGGGGCGCTCTAACTCACGACCTACGTCTCTCTAGGTTGTC
GAGGTCGGTGATCGTTGTAAAAAACCGGCCGACACTCCACGTGGAGGCGAATGACTT
*k*JHREc

GGGCACGCACGCTCGTGCCTCTAACTCAGCCTTGTCCTATAGCGAGTGAGCGGCCTC
GCTTTAAACGCCACAGTTGTTCGCCTCGATGCATCGCCTCCTCGTGCCGTTCGTTTAG
TTGTTTTGGCCTGACCAATCAACGTTTATTATAAATAGAACGCGTTGCCTTCTGTCATTT
ATTATAAACAATCGGCGGCCGTTTTGACGTTTTTTCGCTCATCATGACCTACCTTGCGA
TTGAATTGGGGTGCGTTCGTTGACTCCGGGTTGTCTTTTCGGGGGGTCAAAAACCGC
CCGCTACCCCCACCTACGCAATGCACACGCACGGGGCGGGGCCCCATCATCAACGC
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GGTGATAAGGTGCAGGAGGTTTTTATCAAAAATTTATTCATGAGGTGCAAGGAGGAAC
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TTGACTTGGTATTTTTAGCATAATATTATACGTAACTTGATAATGAACTGCATGAAATCG
CTCATTCGGTACTTGCAGTCATGCGAATTTAACGGTTATAAAGGGTGAGTTGTTTAAGA
ATTTAAATACTCAATGTAAATAATCAAATGGTTGAGTCAATTGTAAGATCAAAGTAACTG
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CAAAAATTAATCAGGTGATAAGTGTATGATTTTCAAGTACAAAAATAACGTCACCATTCT
GTAGGAGAAATTTTTTCTTTAATTTTTTCTCGATCAAAGAGGTCTCTGACTGAAATTAAG
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CAGGCAACTATTAGATTACATTTTAAGAAATTTTGCGTAATAATTGAGTTTTTGCTGAAT
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GTTCTAGTTTTATAAAAACAGGGTAAAAAAACTAAAAGTATAGGAAAGGTTTAATTAAAG
TCCGTGAAAATTACCAAGTCAAAATTTTTGATGATAAAATTGATAACAACTCATGATGGT
CGCCTGAAGCACAAATGTTTTGCAAAATTTCATGAATCGTTGTTCATTTTGAAGCTTGA
AACTCATTTATTTCGCTAATTTTTGTTAATAAATTATTATTCCGAAATAGTGAGAATTGTT

Translation start site

TGTTTCGTTTCAGAGACTCCTTGGCAA**ATG**CCGGAAATGGTCGGTTATTACACCGAAG
ACCCCTTGGCCATAGCGCCTGTTCCTACCGTTGACGAAGCCCGTCTTTCAGTAAAGAA
AGTCGTCTGCAGCCCCG +1883

Exon 1

Exon 2

Supplementary Figure S4. Nucleotide sequences of the upstream region and first intron of *TcKr-h1*. The sequences are shown below the genomic structure of *TcKr-h1*. Boxed sequences indicate exons, bolded text indicates the translation start site (ATG), and red sequences represent *k*JHREcs. Numbers indicate distances from the transcription start site.

Supplementary 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′)
pCR4Blunt-TOPO_TcSRC_RTPCR	PCR, ligation	RLM RACE cDNA (whole body)	TcSRC_RTPCR_FW	GCAGCGAGTCGACGACGACGACG
			TcSRC_RTPCR_RV	CCATGACCTGGTCCAGGATGTCCG
pCR4Blunt-TOPO_TcSRC_5'RACE	RLM RACE	RLM RACE cDNA (whole body)	TcSRC_5'RACE_RV1	GGAAATAACTTTGTCCCTGTGCGAAATCCTG
		TcSRC_5'RACE 1st PCR product	TcSRC_5'RACE_RV2	CCGACACCGACACTTGGTCTCTCAG
pCR4Blunt-TOPO_TcSRC_3'RACE	RLM RACE	RLM RACE cDNA (whole body)	TcSRC_3'RACE_FW1	GAACAGCATGCACCACCAGCGAACG
		TcSRC_3'RACE 1st PCR product	TcSRC_3'RACE_FW2	CTCAAGTGGATTCTGGTTCCTACACCTCG
pCR4Blunt-TOPO_TcSRC_ORF	PCR, ligation	RLM RACE cDNA (whole body)	TcSRC_ORF_FW	ATGAGCGCCTTGGCCACCGGTGTC
			TcSRC_ORF_RV	TCACTCGGACAGCAGCTTCTGCAGCAG
pBIND_TcMet	PCR, ligation	pGEM-T_TcMet *	TcMet_pBIND_FW	TTTGGATCCGTATGTTAGCTCCTTGTGAACTCTATAC
			TcMet_pBIND_RV	AAAACGCGTTCATACTTTGTTACGAAGTAGTTGGTC
pBIND_TcSRC	PCR, ligation	pCR4Blunt-TOPO_TcSRC_ORF	TcSRC_pBIND_FW	TTTGCGGCCGCAATGAGCGCCTTGGCCACCG
			TcSRC_pBIND_RV	AAAGGTACCTCACTCGGACAGCAGCTTCTG
pBIND_TcMet Δ GAL4DBD	Inverse PCR	pBIND_TcMet	TcMet_pBINDiPCR_FW	ATGTTAGCTCCTTGTGAACTCTATACCAACTC
			TcMet_pBINDiPCR_RV	CTTTCAGGAGGCTTGCTTCAAGCTGGC
pBIND_TcSRC Δ GAL4DBD	Inverse PCR	pBIND_TcSRC	TcSRC_pBINDiPCR_FW	ATGAGCGCCTTGGCCACCGGTGTC
			TcSRC_pBINDiPCR_RV	CTTTCAGGAGGCTTGCTTCAAGCTGGC
pACT_TcMet	PCR, ligation	pGEMT_TcMet	TcMet_pACT_FW	TTTGGATCCGTATGTTAGCTCCTTGTGAACTCTATAC
			TcMet_pACT_RV	AAAACGCGTTCATACTTTGTTACGAAGTAGTTGGTC
pACT_TcSRC	PCR, ligation	pCR4Blunt-TOPO_TcSRC_ORF	TcSRC_pACT_FW	TTTGCGGCCGCAATGAGCGCCTTGGCCACCG
			TcSRC_pACT_RV	AAAGGTACCTCACTCGGACAGCAGCTTCTG
pACT_TcMet Δ VP16AD	Inverse PCR	pACT_TcMet	TcMet_pACTiPCR_FW1	ATGTTAGCTCCTTGTGAACTCTATACCAACTC
			TcMet_pACTiPCR_RV1	CTTTCAGGAGGCTTGCTTCAAGCTGGC
pACT_TcSRC Δ VP16AD	Inverse PCR	pACT_TcSRC	TcSRC_pACTiPCR_FW1	ATGAGCGCCTTGGCCACCGGTGTC
			TcSRC_pACTiPCR_RV1	CTTTCAGGAGGCTTGCTTCAAGCTGGC
pACT_TcMet_C-teminal_VP16AD	PCR, ligation	pACT_TcMet Δ VP16AD	TcMet_pACTiPCR_FW2	GGTACCTGAATAACTAAGGCCGCTTCCC
			TcMet_pACTiPCR_RV2	TACTTTGTTACGAAGTAGTTGGTCATGCTGATATATC
		pACT	VP16AD_FW1	ATGAAGCTACTGTCTTCTATCGAACAAGCATGC
			VP16AD_RV1	TTATCCCGGACCCGGGGAATCCCC
pACT_TcSRC_C-teminal_VP16AD	PCR, ligation	pACT_TcSRC Δ VP16AD	TcSRC_pACTiPCR_FW2	GGTACCTGAATAACTAAGGCCGCTTCCC
			TcSRC_pACTiPCR_RV2	CTCGGACAGCAGCTTCTGCAGCAGC
		pACT	VP16AD_FW1	ATGAAGCTACTGTCTTCTATCGAACAAGCATGC
			VP16AD_RV1	TTATCCCGGACCCGGGGAATCCCC

* Minakuchi et al. *Dev. Biol.* **325**, 341-350 (2009).

Supplementary Table S2. List of primers and templates used for dsRNA and qPCR

Target gene and primer name	template	Nucleotide sequence (5′ to 3′)
dsRNA		
TcMet		
TcMet_dsRNA_FW	pGEM-T_TcMet*	GGATCCTAATACGACTCACTATAGGGACGACCAGGGAACTGTTGAAAG
TcMet_dsRNA_RV		GGATCCTAATACGACTCACTATAGGCGACGGTTCGGTTTGTTGTGTTAC
TcSRC		
TcSRC_dsRNA_FW	pCR4Blunt-TOPO_	GGATCCTAATACGACTCACTATAGGCTAAGCCGCCCACGGTCG
TcSRC_dsRNA_RV	TcSRC_ORF	GGATCCTAATACGACTCACTATAGGGACAGAGGTAGTAGATCGCGTC
MalE		
MalE_dsRNA_FW	pMAL-c4E (NEB)	TGATTGCTGCTGACGGGGGT
MalE_dsRNA_RV		TTTCTGGGCGTTTTCCATAGTGG
qPCR		
TcKr-h1		
TcKrh1_qPCR_FW	cDNA	GTTTGCTCCAAGGGGTTCACG
TcKrh1_qPCR_RV		GGTTGTAGCCGAAGGATTTGCC
TcMet		
TcMet_qPCR_FW	cDNA	CATTGCAGGTTATATGACTGAGGAAGTGT
TcMet_qPCR_RV		GAGTAAACGGTAACATGATGATCCTTTGCT
TcSRC		
TcSRC_qPCR_FW	cDNA	ACGAGACCGTGGAGGAGAAGCA
TcSRC_qPCR_RV		AACTTTGTCCCTGTGCGAAATCC
TcRp49		
TcRp49_qPCR_FW	cDNA	CAGGCACCAGTCTGACCGTTATG
TcRp49_qPCR_RV		GCTTCGTTTTGGCATTGGAGC

* Minakuchi et al. *Dev Biol* **325**, 341-350 (2009).

Supplementary 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_-477/+1883	Gateway system	Genomic DNA	TcKrh1_Pro_F1	AAAAAGCAGGCTNNGTTGCACTCGCTAATTACCTCAACCAGATAAC
			TcKrh1_Pro_R1	AGAAAGCTGGGTNCGGGGCTGCAGACGACTTTCTTTACTG
pGL4.14_-477/+106	Inverse PCR	pGL4.14_-477/+1883	TcKrh1_ProiPCR_F1	ATCAAGATCTGGCCTCGGCGGCCAAG
			TcKrh1_ProiPCR_R1	AATTTCTCAGGTTTGGAGACCTGTGTGTGC
pGL4.14_+106/+1883	Inverse PCR	pGL4.14_-477/+1883	TcKrh1_ProiPCR_F2	GTAAGTTTTTATTTTACTTATCAAAAATTTTCTTTTTTTCTGGGCTTGG
			TcKrh1_ProiPCR_R2	ATCCTCGAGGCTAGCGAGCTCAGGTAC
pGL4.14_-477/+1883_M1	Inverse PCR	pGL4.14_-477/+1883	TcKrh1_ProiPCR_F3	TTTAAACCGTTTCAACACGGGCCAGACTGTTC
			TcKrh1_ProiPCR_R3	TTTAAAAAGTACTAGGGATTTTGGATTCGTTTGTGTTAATAAAC
pGL4.14_-477/+1883_M2	Inverse PCR	pGL4.14_-477/+1883	TcKrh1_ProiPCR_F4	TTTAAAAATGACTTGGGCACGCACGCTCGTG
			TcKrh1_ProiPCR_R4	TTTAAAGAGTGTCGGCCGGTTTTTTACAACGATC
pGL4.14_-477/+1883_M3	Inverse PCR	pGL4.14_-477/+1883_M1	TcKrh1_ProiPCR_F5	TTTAAAAATGACTTGGGCACGCACGCTCGTG
			TcKrh1_ProiPCR_R5	TTTAAAGAGTGTCGGCCGGTTTTTTACAACGATC
pGL4.14_-477/+106_M1	Inverse PCR	pGL4.14_-477/+106	TcKrh1_ProiPCR_F6	TTTAAACCGTTTCAACACGGGCCAGACTGTTC
			TcKrh1_ProiPCR_R6	TTTAAAAAGTACTAGGGATTTTGGATTCGTTTGTGTTAATAAAC
pGL4.14_+106/+1883_M1	Inverse PCR	pGL4.14_+106/+1883	TcKrh1_ProiPCR_F7	TTTAAAAATGACTTGGGCACGCACGCTCGTG
			TcKrh1_ProiPCR_R7	TTTAAAGAGTGTCGGCCGGTTTTTTACAACGATC

Red letters indicate mutated nucleotides