

## Supplementary Materials for

### **Piwi suppresses transcription of Brahma-dependent transposons via Maelstrom in ovarian somatic cells**

Ryo Onishi, Kaoru Sato, Kensaku Murano, Lumi Negishi, Haruhiko Siomi, Mikiko C. Siomi\*

\*Corresponding author. Email: [siomim@bs.s.u-tokyo.ac.jp](mailto:siomim@bs.s.u-tokyo.ac.jp)

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#### **The PDF file includes:**

Figs. S1 to S5

Table S3

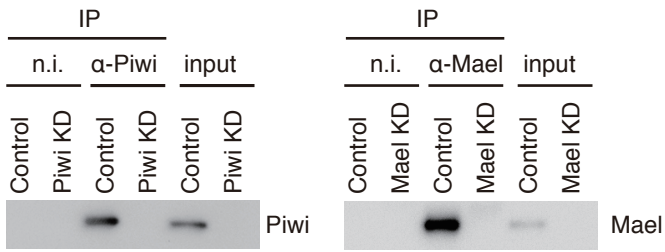
Legends for tables S1 and S2

#### **Other Supplementary Material for this manuscript includes the following:**

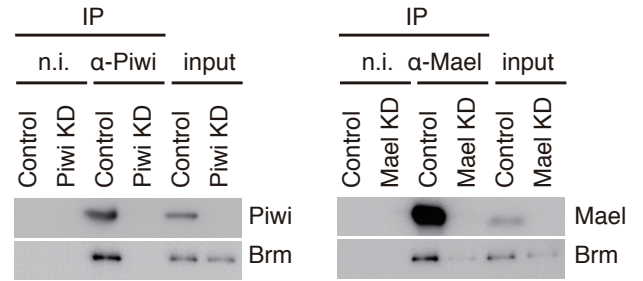
(available at [advances.sciencemag.org/cgi/content/full/6/50/eaaz7420/DC1](https://advances.sciencemag.org/cgi/content/full/6/50/eaaz7420/DC1))

Tables S1 and S2

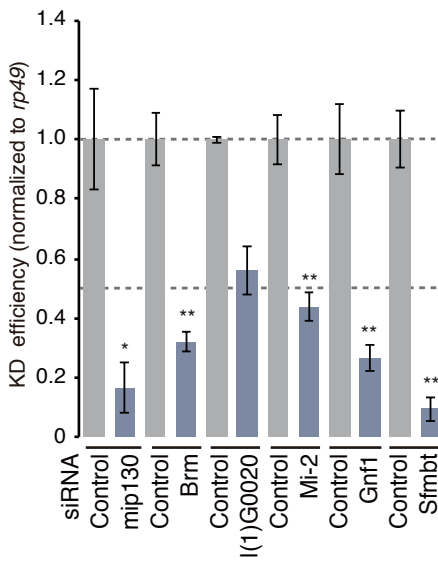
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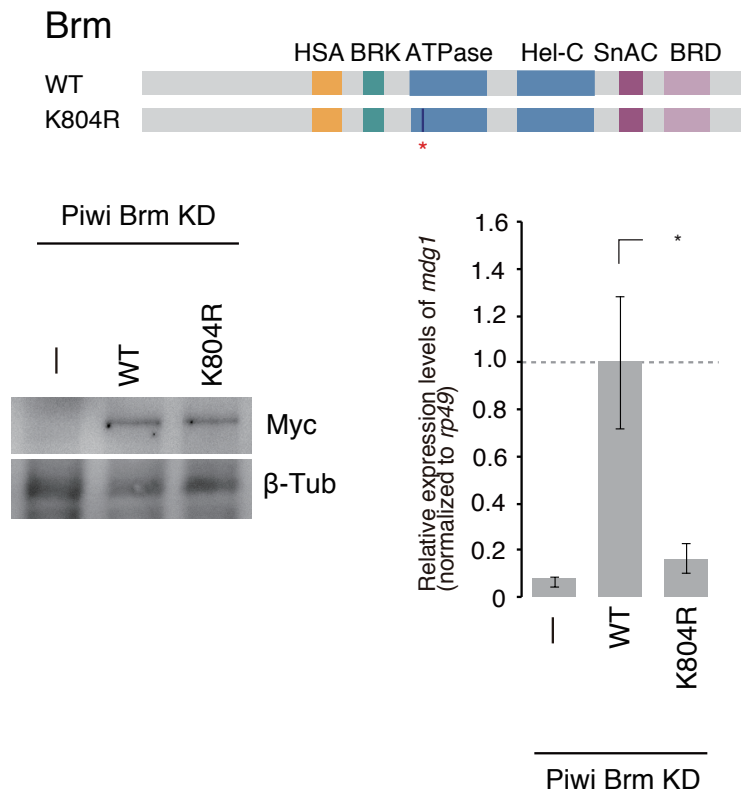
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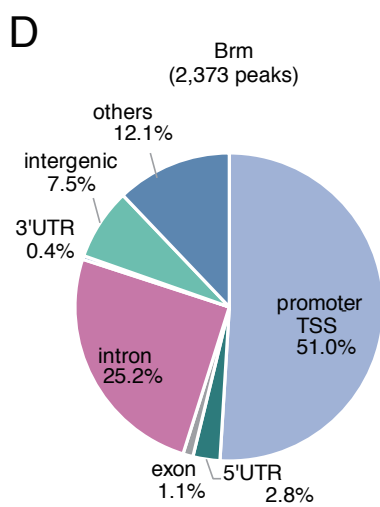
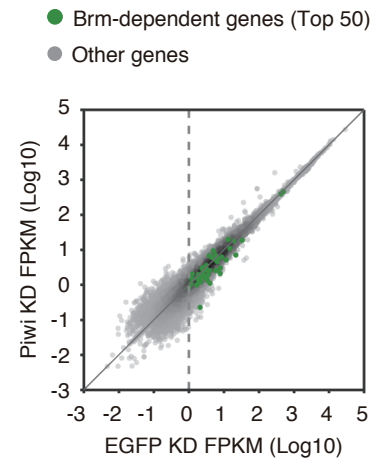
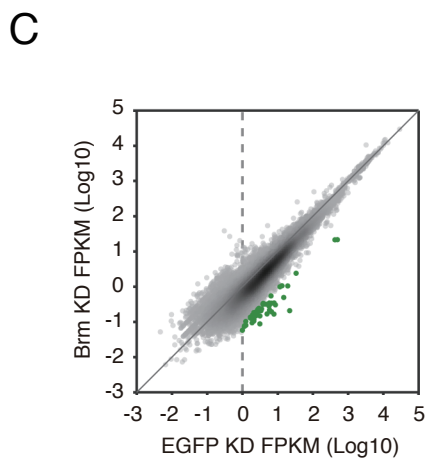
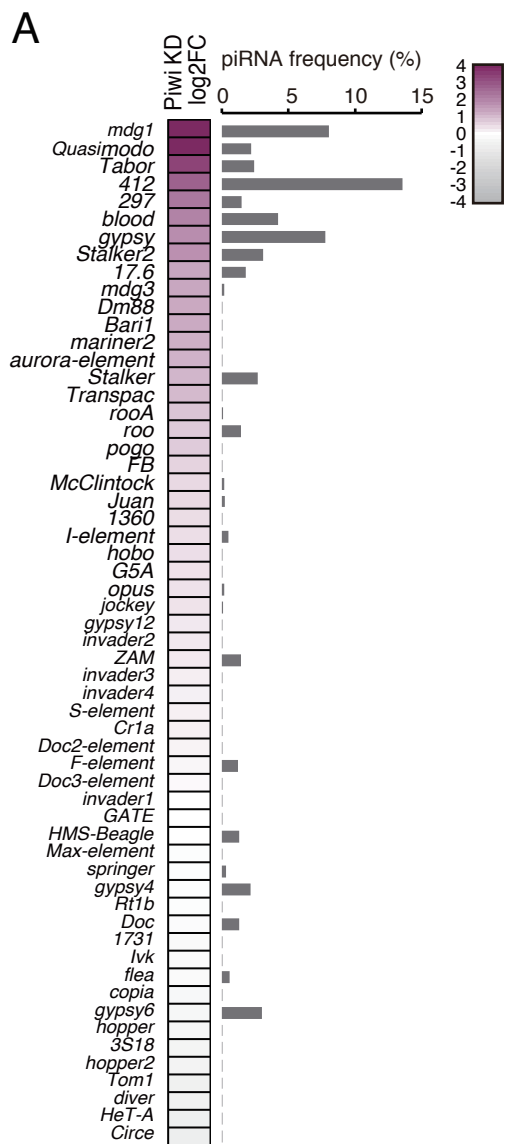


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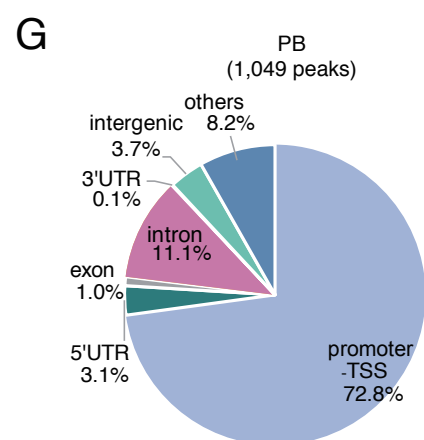
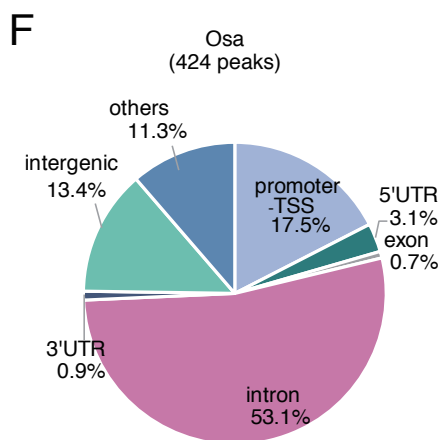
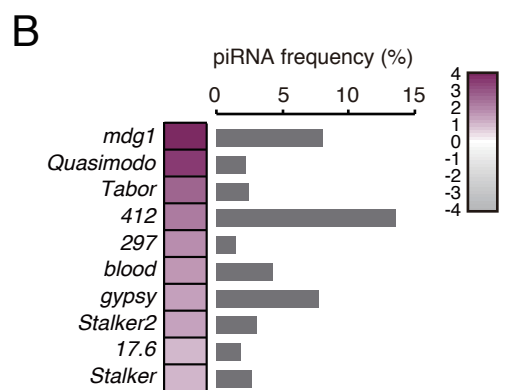
**Figure S1. Brm is a new component of the nuclear Piwi complex in OSCs. (A)**

Western blot showing specific immunoprecipitation of Piwi and Mael using anti-Piwi and anti-Mael monoclonal antibodies. **(B)** qRT-PCR analysis showing changes in the RNA levels of *mip130*, *Brm*, *I(1)G0020*, *Mi-2*, *Gnf1* and *Sfmbt* before (Control) and after RNAi-based knockdown (KD). n=3. \*, p<0.05; \*\*, p<0.01. **(C)** Western blot showing specific interaction of Piwi and Mael with Brm. **(D)** Top: Schematic representations of WT Brm and K804R point mutant Brm (red asterisk). K804R is a dominant negative form of Brm that is defective in ATP hydrolysis (29). Lower left: western blot showing protein levels of exogenous Brm and  $\beta$ -Tub (loading control). -; control. Lower right: RT-qPCR showing relative expression levels of *mdg1* in OSCs expressing WT Brm and K804R mutant Brm under conditions where Piwi and Brm were depleted. HSA; Helicase SANT-associated domain, BRK; BRM and KIS domain, ATPase; Helicase superfamily 1/2, ATP-binding domain, Hel-C; Helicase, C-terminal, SnAC; Snf2 ATP coupling domain, BRD; bromodomain, -; control. n=3. \*, p<0.05.

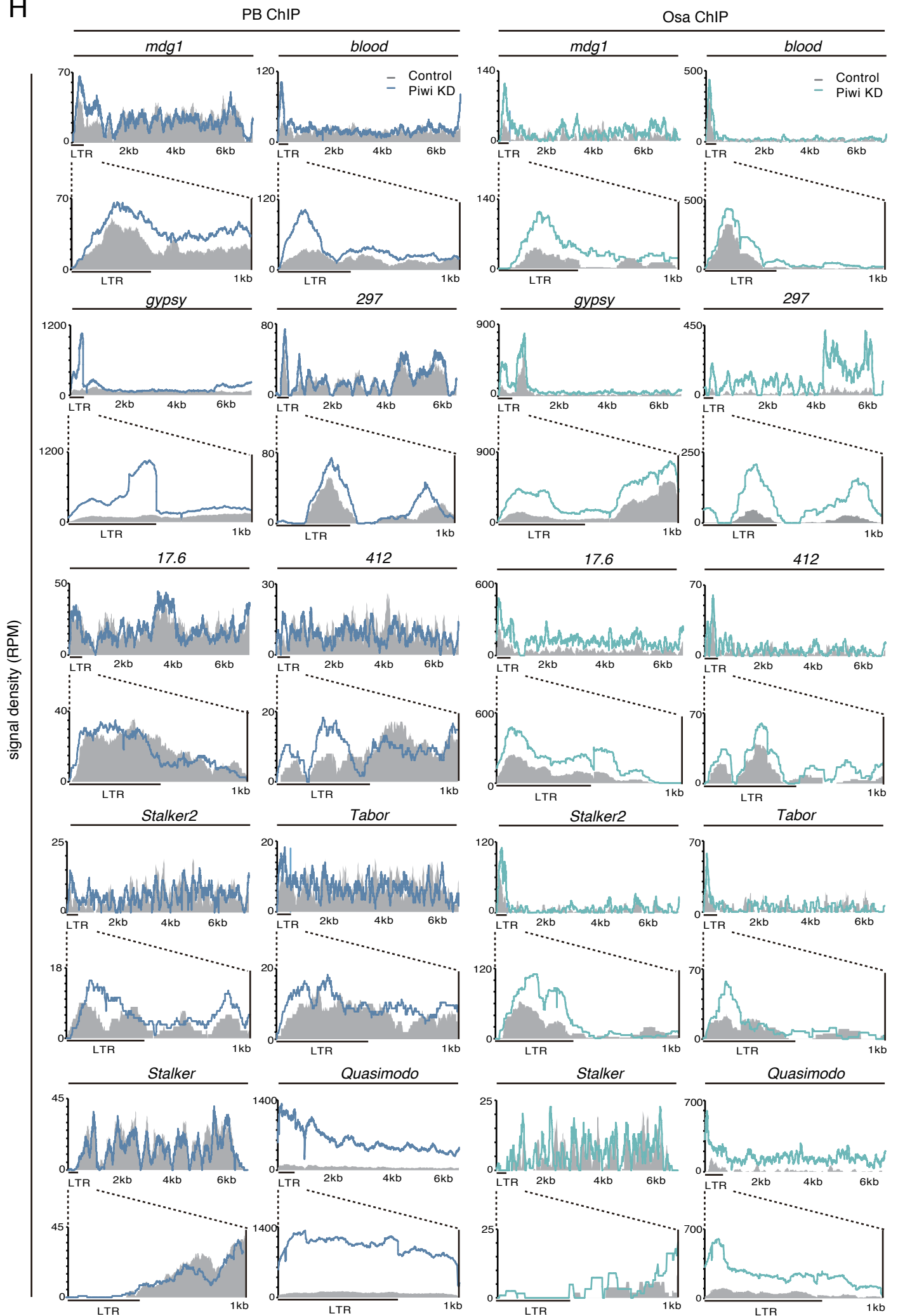


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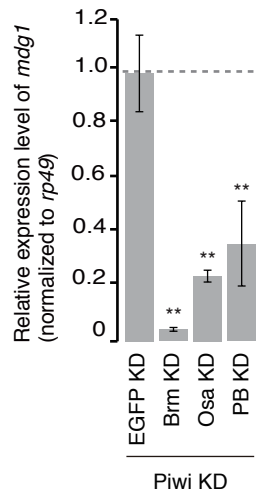
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PBAP	PB Bap170 SAYP	Bap55 Bap60 Actin



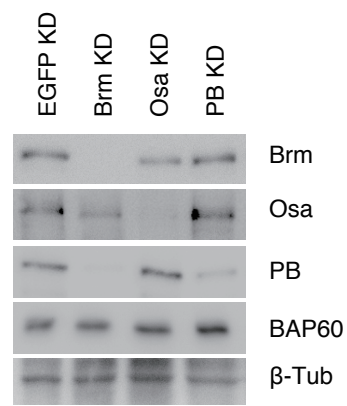
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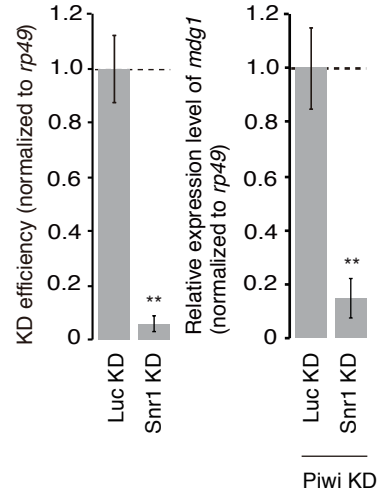
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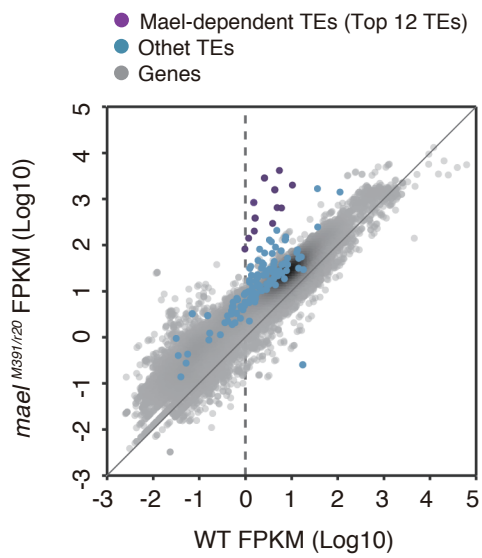
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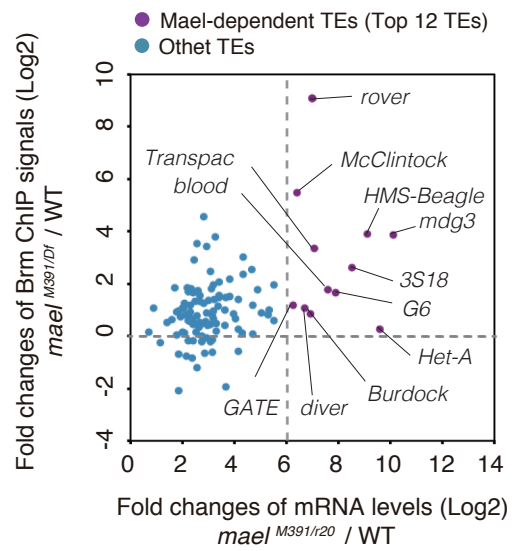
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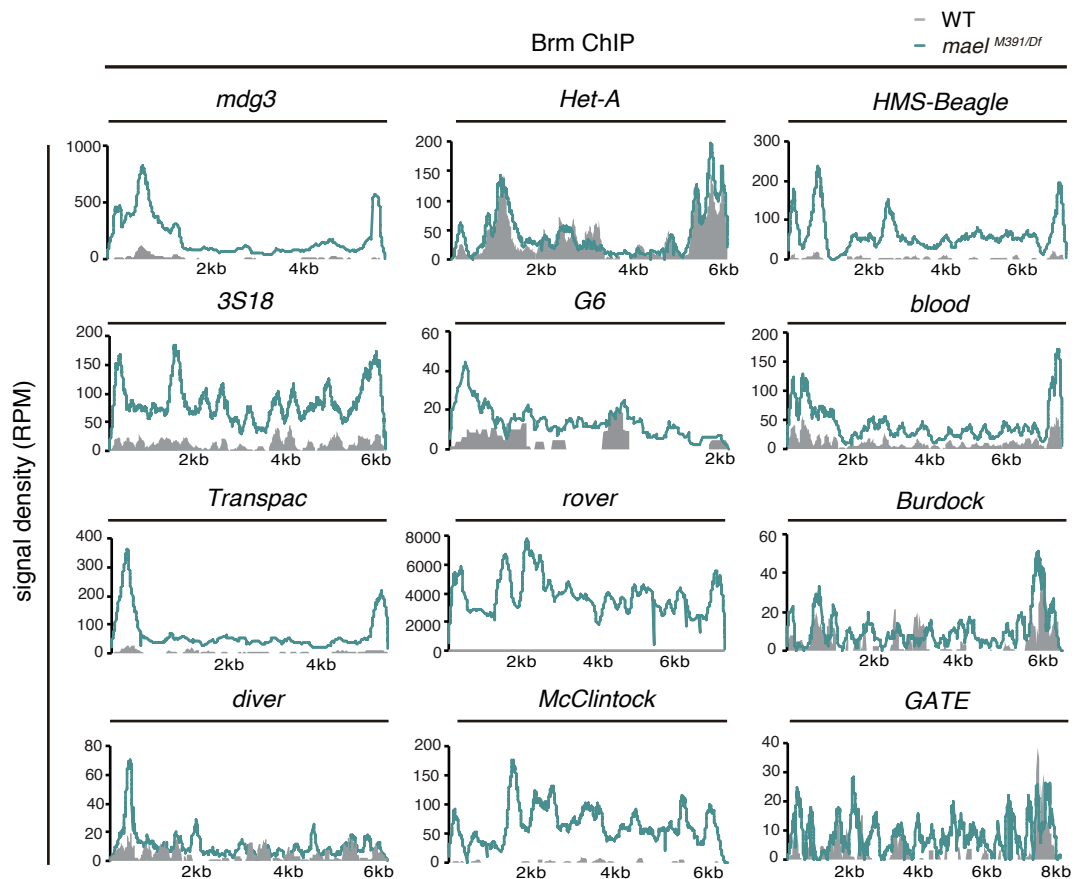
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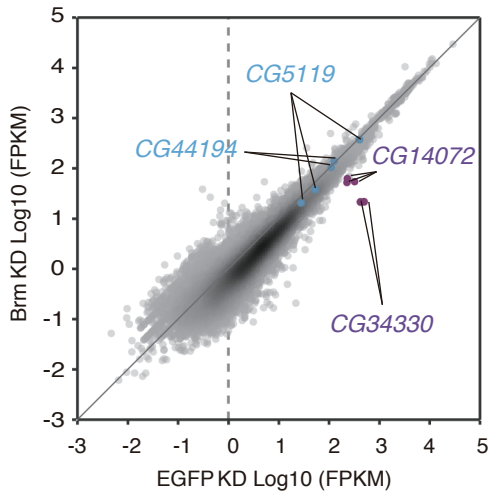


**Figure S2. Brm plays a role in the transcriptional activation of Piwi-targeted transposons.** (A) Heat map showing fold change of transposons in Piwi KD OSCs relative to those in EGFP KD OSCs. Bar graph represents piRNA frequency (%) of indicated transposons in OSCs. (B) Heat map of ten Piwi-dependent transposons from (A). (C) Scatter plots showing the expression levels of the top 50 Brm-dependent genes (FPKM>1.0) relative to EGFP KD OSCs in Brm (left) and Piwi (right) KD OSCs. Green plots represent the top 50 Brm-dependent genes and gray plots represent other TEs and genes. (D) Pie chart showing the occupancy of Brm on genomic elements. (E) Protein components of BAP and PBAP. (F) Pie chart showing the occupancy of Osa on genomic elements. (G) Pie chart showing the occupancy of PB on genomic elements. (H) Density plots for normalized PB and Osa ChIP-seq signals over the consensus sequence from Piwi-dependent TEs in control and Piwi KD OSCs (gray infill and colored lines, respectively). (I) RT-qPCR analysis showing change in RNA levels of *mdg1* in indicated depletion conditions. n=3. \*, p<0.05; \*\*, p<0.01. (J) Western blotting showing the efficiency of RNAi for Brm, Osa, and PB. The level of BAP60, another protein in both BAP and PBAP, was also detected. It is noted that depletion of Brm in OSCs affected the abundances of Osa and PB as previously reported (32).  $\beta$ -Tub; loading control. (K) RT-qPCR analysis showing change in RNA levels of *Snr1* and *mdg1* in indicated depletion conditions (left and right panels, respectively). n=3. \*\*, p<0.01. (L) Scatter plots showing the expression levels of the top 12 Mael-dependent TEs (Log2FC >6, *mdg3*, *Het-A*, *HMS-Beagle*, *3S18*, *G6*, *blood*, *Transpac*, *rover*, *Burdock*, *diver*, *McClintock*, *GATE*) relative to WT ovaries in *mael*<sup>m391/r20</sup> ovaries (19). Purple plots represent the top 12 Mael-dependent TEs, light blue plots represent other TEs and gray plots represent genes. (M) Scatter plots showing the change of normalized Brm

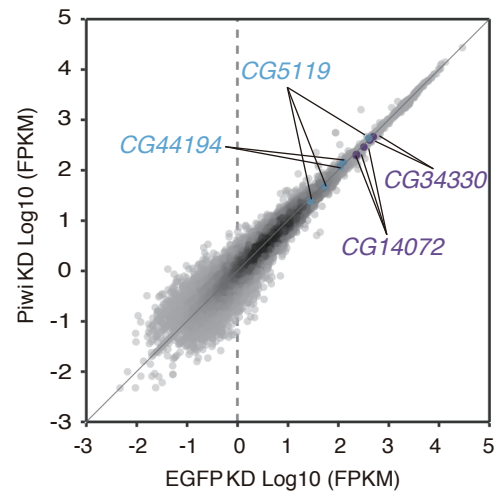
ChIP-seq signals of the top 12 Mael-dependent TEs relative to WT ovaries in *mael*<sup>m391/Df</sup> ovaries. Purple plots represent the top 12 Mael-dependent TEs, light blue plots represent other TEs. **(N)** Density plots for normalized Brm ChIP-seq signals over the consensus sequence from the top 12 Mael-dependent TEs in WT ovaries and *mael*<sup>m391/Df</sup> ovaries (gray infill and colored lines, respectively).



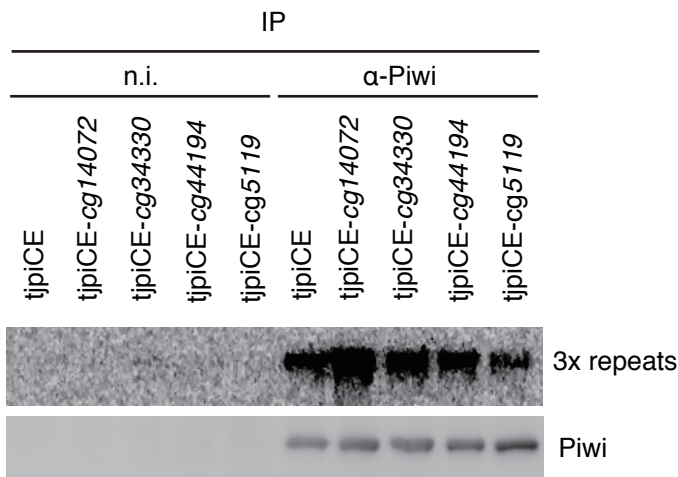
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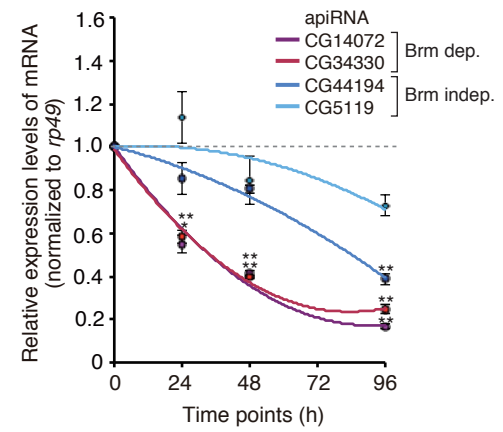
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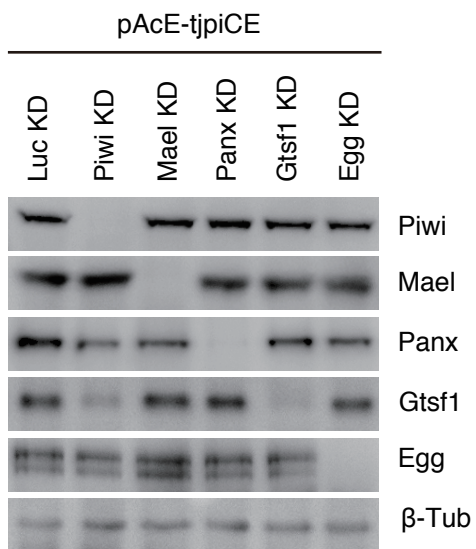
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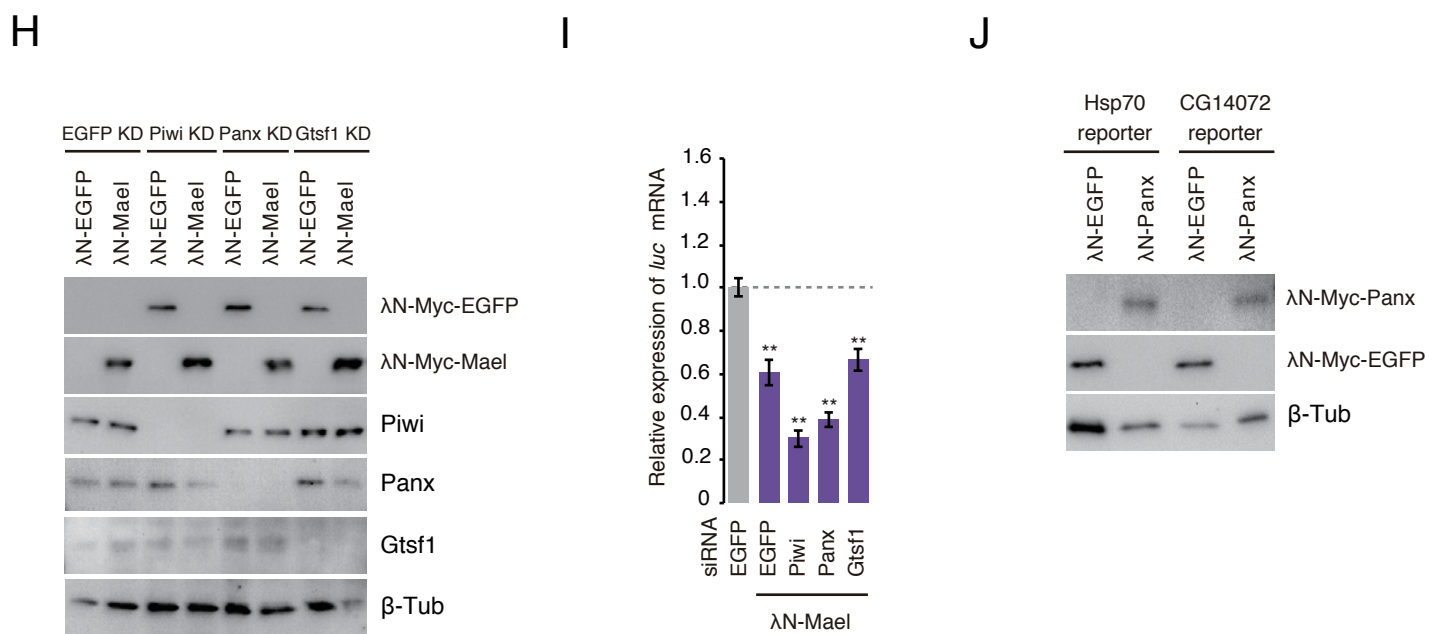
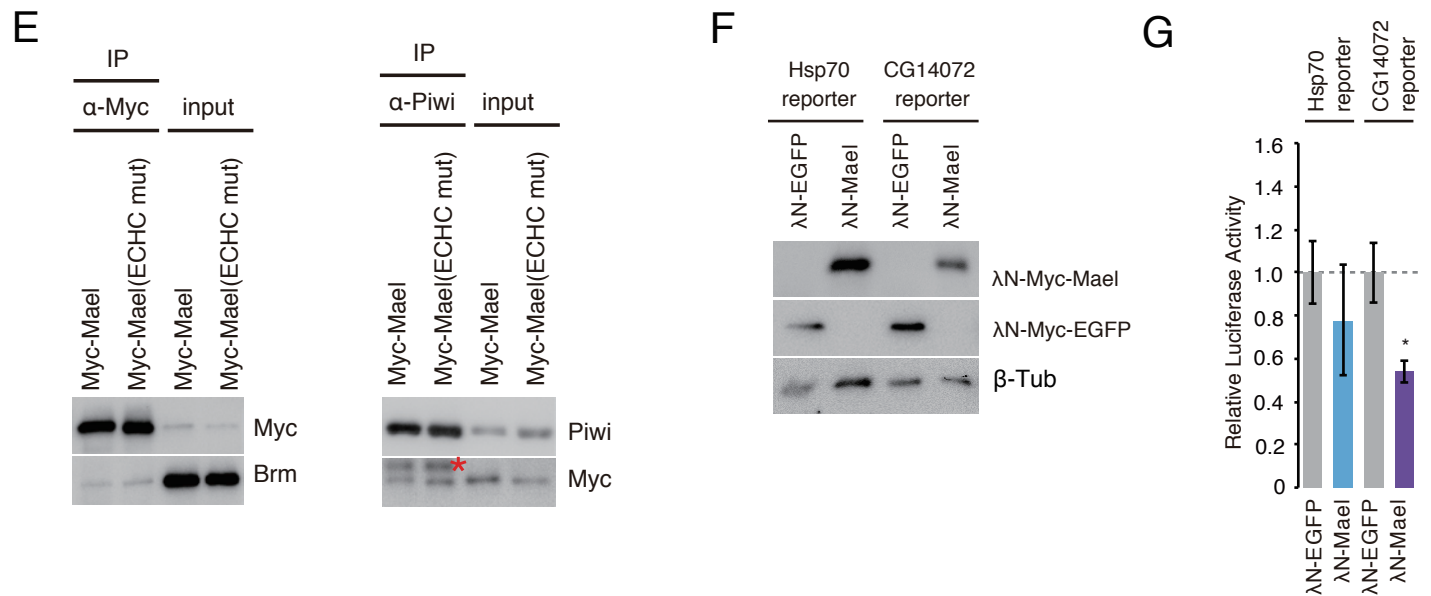
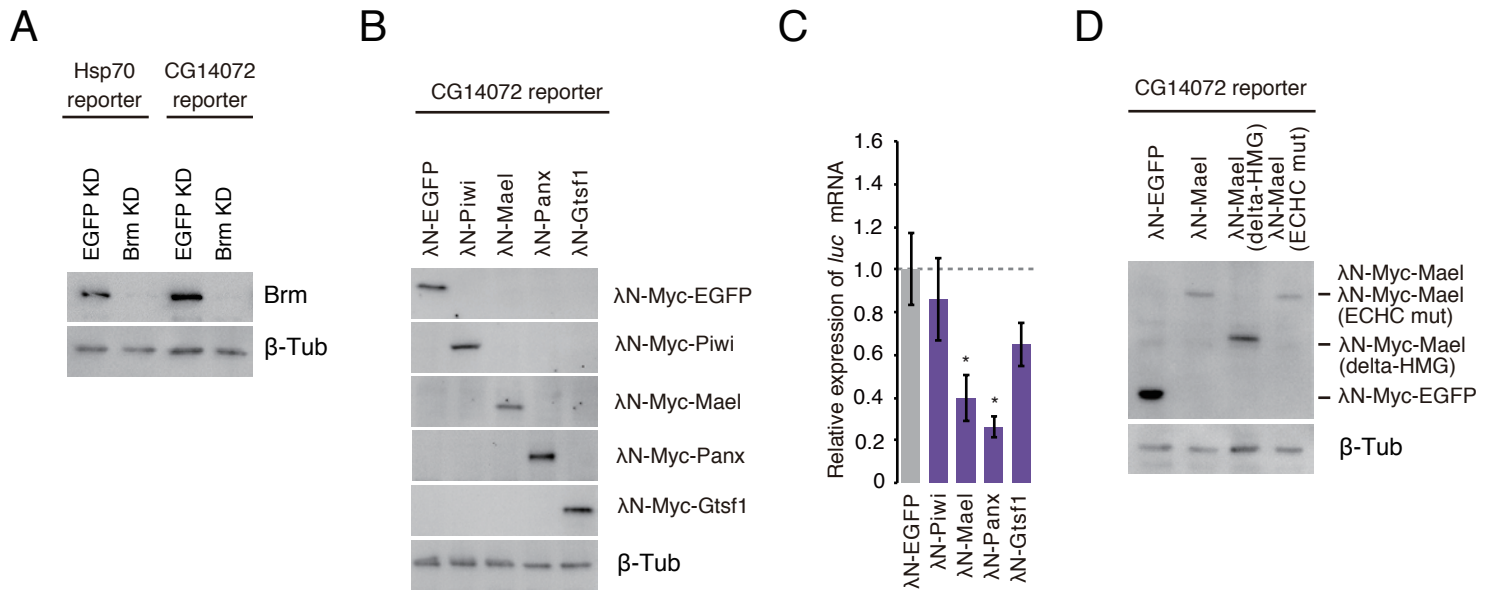
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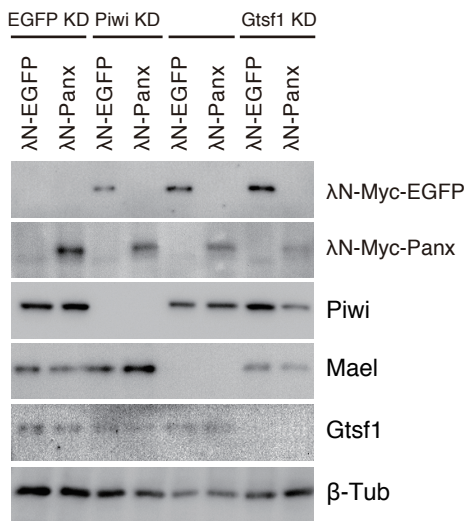
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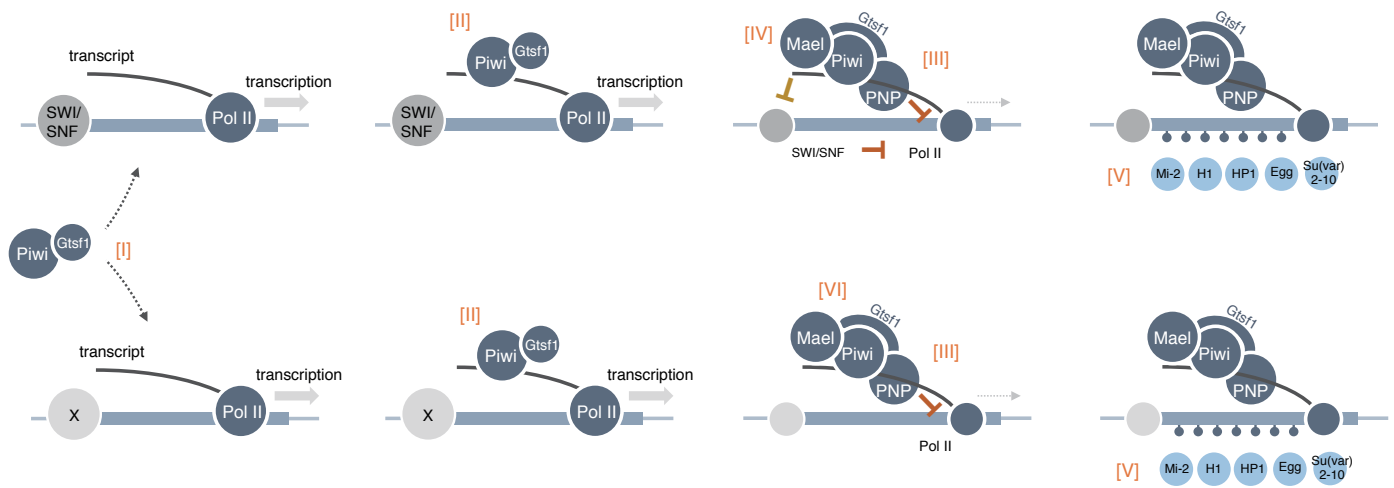
**Figure S3. Artificial piRNA-driven Piwi induces the repression of Brm-dependent genes.** **(A)** Scatter plots showing changes in the RNA levels of RefSeq transcripts in Brm-depleted OSCs. *CG14072* and *CG34330* were selected as candidates for Brm-dependent genes and *CG44194* and *CG5119* for Brm-independent genes. **(B)** Scatter plots showing changes in the RNA levels of RefSeq transcripts in Piwi-depleted OSCs. The spots corresponding to *CG14072*, *CG34330*, *CG44194*, and *CG5119* are indicated. **(C)** Northern blotting showing production and loading of non-target, *CG14072*-, *CG34330*-, *CG44194*- and *CG5119*-targeting artificial piRNAs onto Piwi in OSCs. **(D)** Polynomial approximation curve showing time-course changes in RNA levels of indicated genes after transfection of the artificial piRNA-expressing plasmid. Colored dot and bar represent the mean and error value, respectively. n=3. \*, p<0.05; \*\*, p<0.01. **(E)** Western blotting showing protein levels of Piwi, Mael, Panx, Gtsf1, Egg in apiRNA-expressing OSCs.  $\beta$ -Tub; loading control.



K

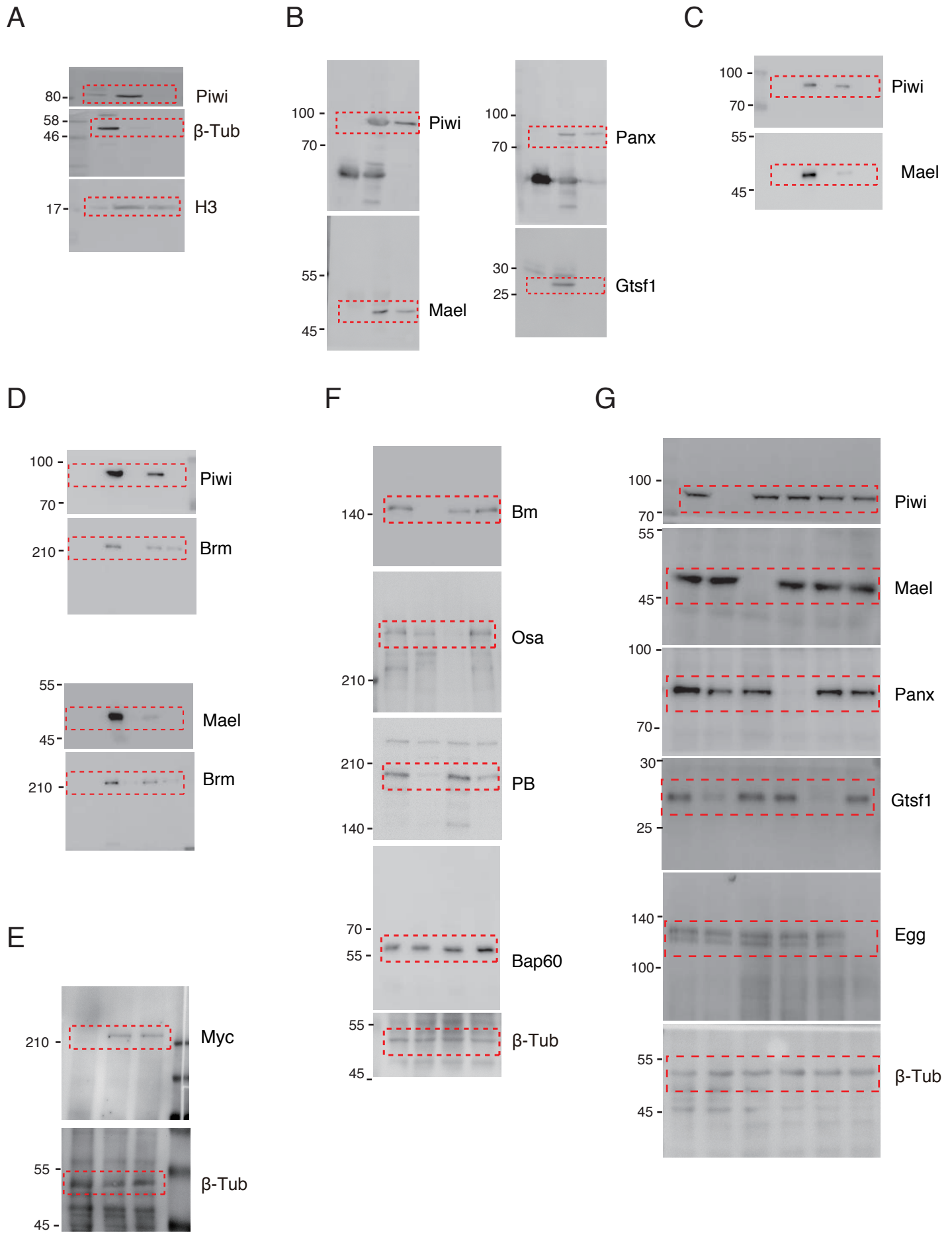


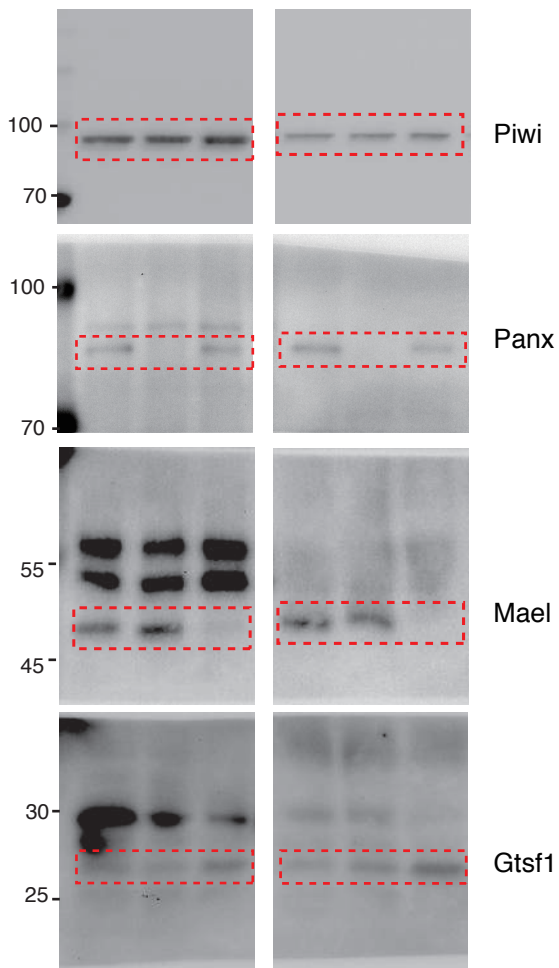
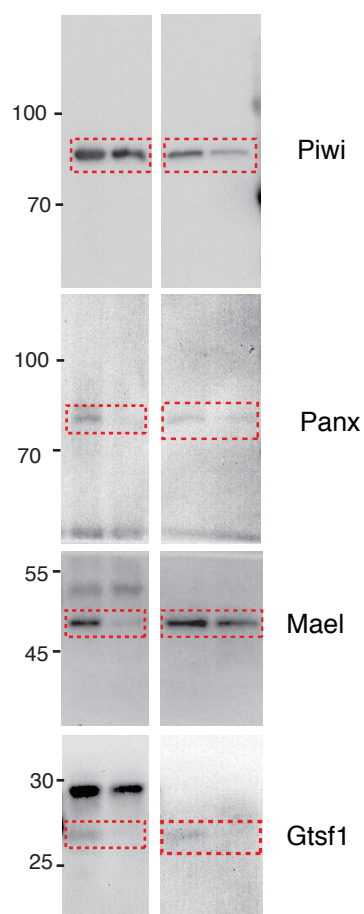
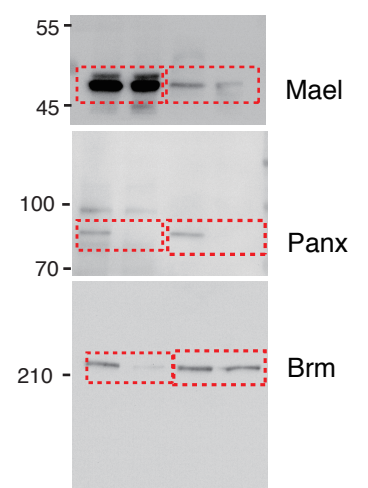
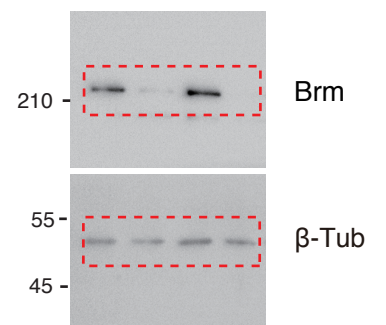
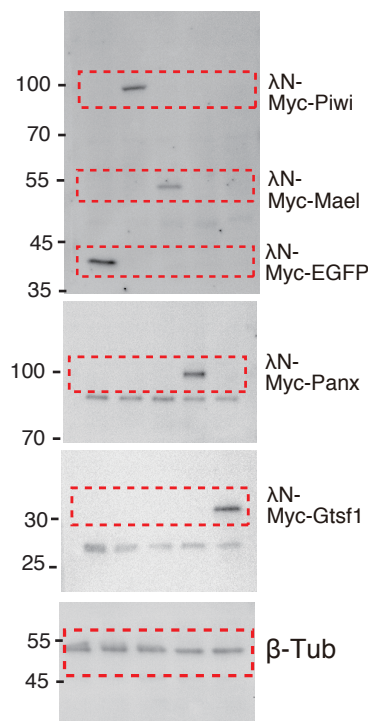
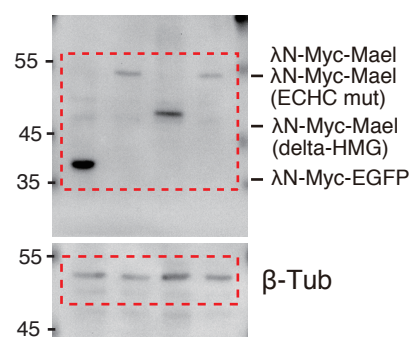
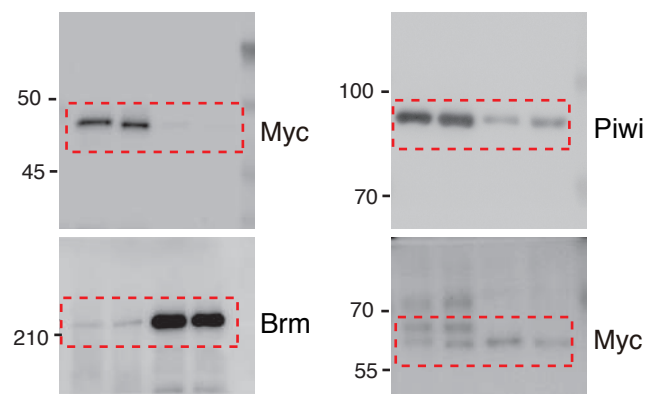
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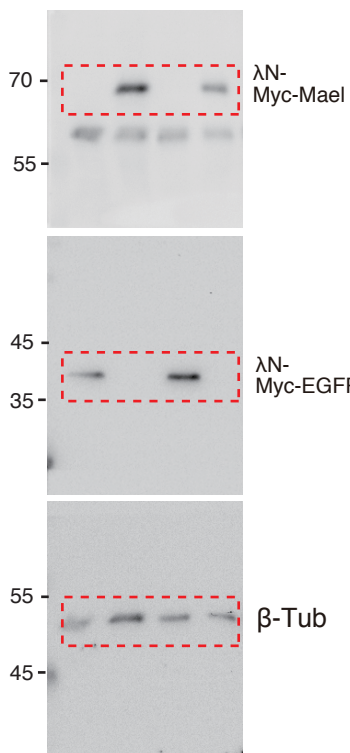
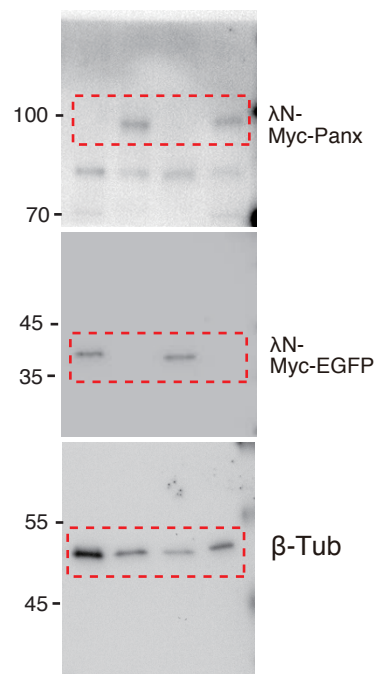
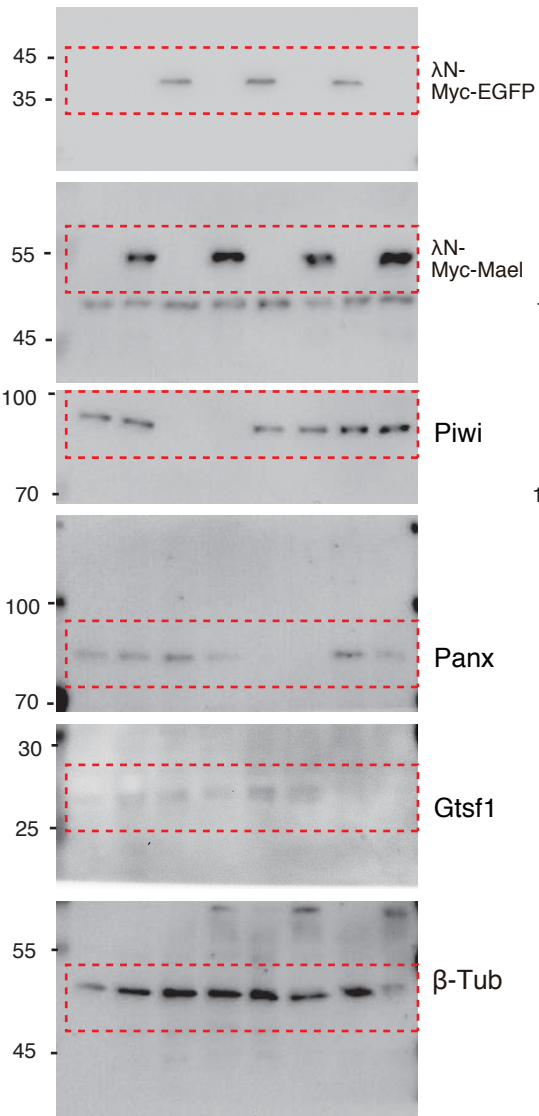
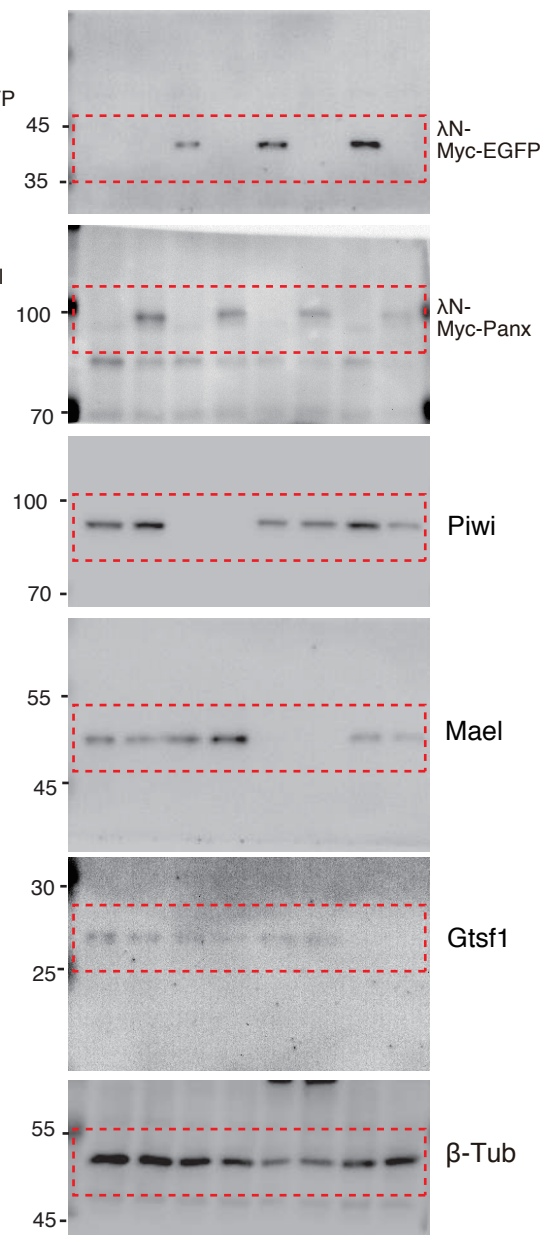
**Figure S4. Artificial tethering of Mael induces the repression of Brm-dependent genes.** (A) Western blotting showing protein levels of Brm in *luc*-reporter expressing OSCs.  $\beta$ -Tub; loading control. (B) Western blotting showing protein levels of the indicated  $\lambda$ N fusion proteins in *CG14072*-reporter-expressing OSCs.  $\beta$ -Tub; loading control. (C) qRT-PCR showing changes in the RNA levels of the luciferase reporter, 48 h after transfection of each tethering construct. *CG14072*-reporter-expressing OSCs were used. n=3. (D) Western blotting showing protein levels of  $\lambda$ N fusion EGFP and Mael wild-type and mutants in *CG14072*-reporter-expressing OSCs.  $\beta$ -Tub; loading control. (E) Western blotting showing both Mael and Mael mutant interaction with Brm and Piwi. (F) Western blotting showing protein levels of  $\lambda$ N fusion EGFP and Mael wild-type in *Hsp70*- or *CG14072*-reporter-expressing OSCs.  $\beta$ -Tub; loading control. (G) Bar graph showing relative luciferase activities of OSC lysates, 96 h after transfection of each tethering construct. *CG14072*-reporter-expressing OSCs and *Hsp70*-reporter-expressing OSCs were used. n=3. \*, p<0.05. (H) Western blotting showing protein levels of  $\lambda$ N fusion EGFP and Mael, and protein levels of Piwi, Panx, and Gtsf1 in *CG14072*-reporter-expressing OSCs before and after RNAi treatment.  $\beta$ -Tub; loading control. (I) qRT-PCR showing changes in RNA levels of luciferase reporter upon KD of each gene. 48 h after Mael tethering construct transfection. The *CG14072*-luc reporter was used. n=3. (J) Western blotting showing protein levels of  $\lambda$ N fusion EGFP and Panx in *Hsp70*-reporter or *CG14072*-reporter-expressing OSCs.  $\beta$ -Tub; loading control. (K) Western blotting showing protein levels of  $\lambda$ N fusion EGFP and Panx, and protein levels of Piwi, Mael, Panx, and Gtsf1 in *CG14072*-reporter-expressing OSCs before and after RNAi treatment.  $\beta$ -Tub; loading control. (L) Model of Piwi-piRISC-driven transcription silencing in OSCs. [I] Upon

cytoplasmic assembly, Piwi-piRISC translocates to the nucleus along with Gtsf1. [II] Piwi-piRISC then targets nascent transposon transcripts to induce silencing. [III] The PNP complex composed of Panx, Nxf2 and p15 (a.k.a. Nxt1) binds Piwi to enforce the Piwi-RNA binding. Panx initiates Pol II suppression. [IV] Mael joins the Piwi complex through Gtsf1 and suppresses Pol II by displacing SWI/SNF from the target loci. [V] Heterochromatinization is induced by chromatin modifiers. [VI] Mael has little effect on Brm-independent targets, whose transcription is activated by an unidentified factor X. Panx, but not Mael, has potential to silence transposons activated by X.



**H****I****J****K****L****M****N**



**O****Q****P****R**

**Figure S5. Uncropped gel images. Red boxes indicate cropped images that are presented in the manuscript. (A)** Uncropped images of the western blot shown in Fig. 1A. **(B)** Uncropped images of the western blot shown in Fig. 1B. **(C)** Uncropped images of the western blot shown in fig. S1A. **(D)** Uncropped images of the western blots shown in fig. S1C. **(E)** Uncropped images of the western blot shown in fig. S1D. **(F)** Uncropped images of the western blot shown in fig. S2J. **(G)** Uncropped images of the western blot shown in fig. S3E. **(H)** Uncropped images of the western blot shown in Fig. 4I. **(I)** Uncropped images of the western blot shown in Fig. 4J. **(J)** Uncropped images of the western blot shown in Fig. 4K. **(K)** Uncropped images of the western blot shown in Figure S4A. **(L)** Uncropped images of the western blot shown in Figure S4B. **(M)** Uncropped images of the western blot shown in fig. S4D. **(N)** Uncropped images of the western blot shown in fig. S4E. **(O)** Uncropped images of the western blot shown in fig. S4F. **(P)** Uncropped images of the western blot shown in fig. S4H. **(Q)** Uncropped images of the western blot shown in fig. S4J. **(R)** Uncropped images of the western blot shown in fig. S4K.

**Table S1 (separate file): List of proteins detected in LC-MS/MS analysis**

**Table S2 (separate file): GO term clusters for common proteins to the Piwi and Mael complexes**

**Table S3. Sequences of oligonucleotides**

Experiment	Primer Name	Primer sequence (Forward, Reverse)
Vector construction	pAcM-vector	GCGGCCGCTCGAGTCTAGAG,GGTACCAAGCTTGTTACAGGT
	InFusion-Brm	AACAAGCTTGGTACCATGGCCTCGCCCTCTCCGGC, GACTCGAGCGGCCGCCTAGTCCATGTCATCGTCGTC
	siBrm-Res	CTGGTTACGCTGTTGATGGACCGTAAGAAGGTTATGGGT, TTCTTACGGTCCATCAACAGCGTAACCAGCGAAATGGTT
	del-HSA	GAAGCAAGAGGATGAGGAGGGTTAC, TCATCCTCTTGCTTCTCTAGCTTCTC
	del-BRK	CAGCATTGACAGCTGTGGGAGCAAC, CAGCTGTCAATGCTGCCCTCATCAAT
	K804R	TTTGGGTAGAACCATTCAAACCATTTC, GAATGGTTCTACCCAAACCCATTTTCAT
	CG14072rev	GATCAGCATCTCCATTGCCAACAACGC, GATCGAGATGGATGGTCTACAATATGC
	tjcis-CG14072	CCATCCATCTCGATCGAGGATCCCAT, ATGGAGATGCTGATCAGCGAGCTCAG
	CG34330rev	TGATCAGCATCGACACAGCCAGTCG, GATCGAGATGCAGGGCGTGCCTCC
	tjcis-CG34330	CCCTGCATCTCGATCGAGGATCCCAT, TGTCGATGCTGATCAGCGAGCTCAG
	tjcis-vector	CTCGATCGAGGATCCCATAG,GCTGATCAGCGAGCTCAGGC
	CG5119rev	AGCTCGCTGATCAGCGCTAGTGTGCGTGCCTGTGT, GGATCCTCGATCGAGATGGCTTCTCTATACGTCGG
	CG44194rev	AGCTCGCTGATCAGCTACTTGACGTGGGCATACTT, GGATCCTCGATCGAGATGTGCTACAGAATATATCC
	10boxB-vector (CG14072)	AGCCGGTACCATGGCCGAAGACGCCAAAAACATAA, ATTGCAGATCTGAAAAATGATGTGACAGTGAAAAATGA
	CG14072 -promoter	TTCCAGATCTGCAATCTCTGTTTATTTGTCCTAAG, GCCATGGTACCGGCTGTGATTTCAATTTGGC
	10boxB-vector (Hsp70)	GGTACCATGGCCGAAGACGC, AGATCTGGAAAAATGATGTGACAG
	Hsp70 -promoter	CATTTTTCCAGATCTATCCCCCTAGAATCCCCAAA, TTCGGCCATGGTACCCAGATCCCCCAGAGTTCTCT
	λN-insert(Nhel)	ACGTGCTAGCAACATGGACGCACAAACACGACGACGTG, ACGTGCTAGCGCAGCGTAATCTGGAACATCGTATGGGTAAG
	λN-vector	CTCGAGTCTAGAGGGCCCTT, GGTACCAAGCTTGTTACAGGT
	EGFP-insert	AACAAGCTTGGTACCATGGTGGAGCAAGGGCGAGGA, CCCTCTAGACTCGAGCTTGTACAGCTCGTCCATGC
	Gtsf1-insert	AACAAGCTTGGTACCATGGTTTATTGCCCGTACAA, CCCTCTAGACTCGAGCTACTGGCGCCTTGAGTATG
	delta-HMG	TGGTACCCAGGTGGACAAGGCCAAAAG, TCCACCTGGGTACCAAGCTTGTTACAGG

Experiment	Gene	Primer sequence (Forward, Reverse)
qRT-PCR	<i>rp49</i>	CCGCTTCAAGGGACAGTATCTG, ATCTCGCCGCAGTAAACGC
	<i>mdg1</i>	AACAGAAACGCCAGCAACAGC, CGTCCCATGTCCGTTGTGAT
	<i>mip130</i>	CGTTGACAAGCCGCTATTCG, GCTGGATTTCGATCCGCCTTA
	<i>brm</i>	ACAAGAGCGCATCGAAAAGGA, CTGCGACAGTAGGAAAGCCAA
	<i>mi-2</i>	ACAGGAACATTGCGTCAGGA, TAGCCTGGCCAATACGATGG
	<i>l(1)G0020</i>	GTATTAGCTCGGGCTGTCCC, AAATGGAGACCAGGCGATGG
	<i>gnf1</i>	ACGCAGGAAAGCCGTGATAA, ATCATCCTCGCTGGATGCTG
	<i>sfmt</i>	GAAGACACCCATCGCCTACA, GTGTGCTTCACTCCAATGGC
	<i>snr1</i>	GCGGGTCATTGTTAAGCTG, CCGCACAGAGTTAATGGC
	<i>CG14072</i>	CCGTATGCCAAATTGAAATCACAGC, AGCATGGCCAGAAATCCGA
	<i>CG34330</i>	ACTCTGGGACTACGTTTGCG, ATGCGAAGTGAACGCGAGTA
	<i>CG44194</i>	CATTCTGCAGCGTTGGTGTG, GTCGCTCCAGGGAGTTTAGC
	<i>CG5119</i>	CATCTCGCTCGCAGTACAT, TGATTTGACGGAAGGGTCGG
	<i>Luc</i>	CGTCGCCAGTCAAGTAACAA, TTTCTTGCGTCGAGTTTTC

Experiment	Gene	Primer sequence (Forward, Reverse)
ChIP-qPCR	<i>CG14072</i>	CCGTATGCCAAATTGAAATCACAGC, AGCATGGCCAGAAATCCGA
	<i>CG34330</i>	ACTCTGGGACTACGTTTGCG, ATGCGAAGTGAACGCGAGTA

Experiment	siRNA name	siRNA sense	siRNA antisense
RNAi	siEGFP	GGCAAGCUGACCCUGAAGUTT	ACUUCAGGGUCAGCUUGCCTT
	siLuc	CGUACGCGAAUACUUCGATT	UCGAAGUAAUCCGCGUACGTT
	siPiwi	GCUCCCAGGCGUGAAGGUGTT	CACCUUCACGCCUGGGAGCTT
	siMael	CGCCAAGAUGUCCCAUGAUTT	AUCAUGGGACAUCUUGGCGTT
	siGtsf1	CAUAGUGAGAGAGCCCAUTT	AUUGGGCUCUCUCACUAUGTT
	siGtsf1-2	GCTCCAGCAGCACATCTTATT	UAAGAUGUGCUGCUGGAGCTT
	siPanx	CGGCUACGCUGUACAAGAATT	UUCUUGUACAGCGUAGCCGTT
	siEgg	GGUCACAAGCGUAAUAGCUTT	AGCUAAUACGCUUGUGACCTT
	siBrm	CCUACCUUAUGGAUCGAAATT	UUUCGAUCCAUAAGGUAGGTT
	siSnr1	CAAGAACGAGAGCAUGAUUTT	AAUCAUGCUCUCGUUCUUGTT
	siOsa	CGGACUCGUUGUGCAAACUGU	ACAGUUUGCACAACGAGUCCG
	siPolybromo	CGCGUCAAGAGUCUCUCCAGU	ACUGGAGAGACUCUUGACGCG
	siMip130	GGUCACUGCCAGAUUAAGATT	TCUUAUCUGGCAGUGACCTT
	siMi-2	GCCAGAGUGGCUCAUUGUUTT	AACAAUGAGCCACUCUGGCTT
	siL(1)G0020	CCAGCAGGAGCUCUGGUUATT	UAACCAGAGCUCUCUGGTT
	siGnf1	GGAAGUCAAGACUUCUAGATT	UCUAGAAGUCUUGACUUCCTT
	siSfmt	UUGGAGUGAAGCACACAUUCU	AGAAUGUGUGCUUCACUCCAA

Experiment	Probe	Probe sequence
Northern Blot	3xrepeats	GACCGAGTCAAGTTGAAAACCTATGGACCGAGTCAAGTTGAAAACCTATG