Supporting Information

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SI Experimental Procedures

Clones and Antibodies. ***Mammalian expression constructs.*** Two isoforms of mouse Tudor domain-containing 12 (TDRD12) are identified in the databases: one short form encoding for 407 aa, previously identified as Ecat8 (ES-cell associated transcript 8) (1) and a predicted longer isoform (Uniprot accession no. Q9CWU0) encoding for 1,215 aa, which is a C-terminal extension of the shorter isoform. The longer isoform is orthologous to the fly Tdrd12 protein SoYB (2). We confirmed the existence of this longer form in fetal and adult mouse testes total RNA by RT-PCR (Fig. S1*A*). The full-length cDNA for mouse *Tdrd12* was amplified from mouse testes, and a mammalian expression construct was generated by inserting the open reading frame into the pCIneo-HA vector (3). Expression constructs for HA-tagged versions of hAGO2 (3), MILI, MIWI2, and MIWI (4) are already described.

Bombyx *Tdrd12 cloning.* Using the *Drosophila* Tdrd12 ortholog SoYb (2) as a query, we mined a de novo assembled PolyA+ transcriptome of BmN4 cells and identified a unique cDNA encoding *Bombyx mori* Tdrd12 (BmTdrd12). It is predicted to encode an ∼200-kDa protein (1,759 aa), which we confirmed by our Western blot analysis of BmN4 cell lysates using the anti-BmTdrd12 antibodies (Fig. S2*A*). Our efforts to express the full-length protein as an HA-tagged version in BmN4 cells failed. An alignment with *Drosophila* SoYb indicates that ∼250 aa at the N terminus of BmTdrd12 is not conserved. An expression construct lacking 1–259 aa was expressed robustly in BmN4 cells and is referred to in this study as HA-BmTdrd12 (260–1,759 aa) (Fig. 2 and Fig. S2*B*). Based on its interaction with the Piwi protein Siwi and its localization within nuages of transfected BmN4 cells (Fig. 2), we conclude that HA-BmTdrd12 reflects a functional tagged version of the *Bombyx* protein, useful for cell-culture studies. Despite the lack of conservation with *Drosophila* proteins, we note that the N-terminal extension of BmTdrd12 (1–259 aa) is conserved in other Lepidopterans like the Monarch butterfly (*Danus plexippus*), displaying 68% similarity and 46% identity over this 259-aa region.

BmTdrd12 protein sequence (the 1–259 aa, in bold, is absent in our HA-BmTdrd12 construct). **MASDYYQVEILHYLNPNLIWVEVLNSPNEISFEQLGVYGILPIDASLDVERPGLKLQRSEDWMPATAILMKNIFQNLEQVWFSPTHIDRRSSIFDNNIHKYGELIIKKNGVQLYLSKELVKAGLATEDPCQFHQYMSLGKIKTKLSNTETRAVIKNLEEYYRKSSKPKELWQKSVHQNTSIFHAGERLQALTVKNLERHNNRQNIMLLENKLKDLEQCKGSDEVSLGRGVCRVPSNKSEMVMLTNKRLKNRLELLSKIN**MKSDATDAVKATKRNFSGDGQRKNFENDFESDDESVKKVSIANTINTSDGSANVVDKLLDEKQIDNVFNNKKQICYTESTRRNPVKKAACIVYGPPSINIDKLPLKEAPKMTKTVKWTPHVDCDKEASEVSFGDVDSHVKLDVKNLDKFHEIADRIEIEKTIPVDVNIHKDLYDSMINNKNESESKIMETANLKTEMKNLRKSSILQSKLKQFDKFNVSSNSAASESSTKSSMDSSRISDEDDLSSDDEMSEIMETFKLNLATPKKSEAKHTIDHIEVNNTKLNANPFKNLDGSKSVFVDKLTSPVLLVHTKRNNKVQPCSLLRDVPFGTSIHVVLRNMGIKHPTRLQTVSWGTILRGLSTFLISPPRSGKTMGYLPAVCRLVRDFRKESPDSCGPKCIIVCATSKSVSEVERISKMLLGLEDKVFACYSGMDNLSVTTALLNGCDLLICTPKSIVRLLQNDLSVDLRDLTTFVVDDCERISDVYSNEVKYVLYEIKNMLKNRVNKELKVQIVVASRIWCDFLEPIVLKAPDSVVCIGAFQELILYSKISTTVDFLRPENKIANVLQFIDSVQGPKRTVVVCRADNEVKAVESSLRYNNRVVFACDNTMNIHDLYNLNVVWGDFEDPTLGPILVCCDSNLVHLNVTDASYLIHYSLPALFSTFCKRFSVLNDNYPSIFKNESRDLKVKVLMDESNVEQLPKILNFLKRCTENVPKILDEVSEKILNEKDLAKVKDLVPLCDNLLSLGICPDTWNCTERHRIFKECDSPADWIPKNGVVTFQILYFHSAVMYSARLLSNTVDGETTKYPQTYSTLSLKMGMYFSKESSRRLHGIPMVGDVCAVSKKQNFFIRCQVVKIISFYKNGNPNYVVIKLIDEEKFEQSRDIYLYHLPDEFKDMKTYVVQVRLANIQPQDKDITFSCLAKNELEKIVEKNEDLFMRGHVAMSVGSCIFVDTLEACLDLSSLSETVVRHNFKQELLNAHAVPNPKHLSILEEMCEKSGLIVKAVTNEQVVPKPIPVLPAAQWAHLEDDLSSVYLASVEDMDKLFVRLVKFESCMKLLNIEINKYVSENTVPLDGSNVGDIVLAKFPDDSMYERARIDHIYSEDKVKCFFVDQGDWRDVSTNDLATITENFITQLPFQAIECRLIGIRPFGEQWTEFSTNWFSDHCFEDAKGNLKHLYVKHFTKEKADCTGGHKYGVALIDTYTNEDIIVNQLLIDLNLAKENVDEIAYLSEIKCNKTVLNNDATVDEEEGSLSGVSEPESNINVPLDKVFLKPPIRSVPLVDSDNETSDSDTWQINRPEDFKALFMRTRPESSKIIPMITANEVQNNADGETSKDTSTILEEKGQLPEKVKDDELKLSKPKICWSQNKNTVKLKILIAGIEDYKLKIEDRAVAFSANHCDVEYGFKLELYGVVDVNKSRHSNKGQYVLVTMTKLMCRNWLALTKEGDSQKWIVYDVDTIEASSDEEVYRDDTLEVIKNIHNTNNGSDSEDDDFLDDVS.

BmN4 cell-expression constructs. Constructs expressing *Bombyx* Siwi and Ago3 were described previously (5). The cDNA encoding BmTdrd12 or its deletions were cloned into the pBEMBL-HA vector to express the following proteins: HA-BmTdrd12 (260–1,759 aa), HA-BmTdrd12-A (260–1,619 aa), HA-BmTdrd12-B (260–1,282 aa), and HA-BmTdrd12-C (1,407–1,759 aa). Introduction of point mutations into predicted ATP-binding or ATP-hydrolysis sites of BmTdrd12 was performed by PCR with overlapping primers carrying the required mutations in the respective motifs: ATP-binding (GKT→GAT; K630A) and ATP hydrolysis (DDCE→AACE; D735A, D736A).

Antibodies. For antibody production, cDNAs corresponding to the following amino acids were cloned into the pETM-11 vector [European Molecular Biology Laboratory (EMBL) Protein Expression Facility]: *Bombyx* BmTdrd12 (920–1,099 aa), mouse MIWI2 (1–212 aa), and mouse TDRD12 (131–398 aa). Insoluble antigens were purified from inclusion bodies and solubilized in 8 M urea. After dilution with adjuvant (1:1), rabbits were injected for polyclonal antibody production. Antibodies were purified with antigen immobilized on filter strips.

Other antibodies used were: anti-MILI mouse monoclonal antibody (13E-3) (4), anti-TDRD1 (6), and *Bombyx* Siwi and Ago3 rabbit antibodies (5). The anti-L1ORF1p was a kind gift from Sandra Martin, University of Colorado School of Medicine, Aurora, CO (7). Commercial antibodies were purchased as follows: rabbit anti-HA (Santa Cruz), anti-Myc (EMBL Monoclonal Antibody Core Facility), anti-Actin for detecting *Bombyx* Actin (Santa Cruz; cat. no. SC-1616-R).

Cell-Culture Experiments. To verify the quality of rabbit polyclonal antibodies to mouse TDRD12, mammalian HEK293T cell cultures (in 6-cm dishes) were transfected (Lipofectamine and Plus Reagent; Invitrogen) with 5 μg of expression plasmids for various proteins. Cell lysates were resolved by 10% (wt/vol) SDS/PAGE, and proteins were analyzed by Western blotting to detect tagged proteins with anti-tag antibodies or tested for cross-reactivity with purified anti-TDRD12 antibodies. The purified antibody specifically detected only HA-tagged mouse TDRD12 and no other controls like HA-MILI, MIWI, MIWI2, or Myc-TDRD1.

The BmN4 *Bombyx mori* (silkworm) ovarian cells were transfected (Fugene; Roche) with 2 μg of expression plasmids for immunoprecipitation experiments. After 48 h posttransfection, BmN4 cells were lysed in 50 mM Tris·HCl, pH 8.0, buffer containing 150 mM NaCl, 0.5% Triton X-100, 0.1% Nonidet P-40, and protease inhibitor (Complete Protease Inhibitor; Roche) in

glass tissue homogenizer, and the cell lysate was centrifuged at 16,000 × *g* for 10 min at 4 °C. The supernatant was incubated for 3 h with HA-affinity matrix (Roche) or Protein G Sepharose (GE Healthcare) preincubated with antibodies. The immunoprecipitates were separated by SDS/PAGE and analyzed by Western blot.

Mouse Mutants. ***Generation of the targeted*** **Tdrd12** ***mutant.*** To disrupt the in vivo function of *Tdrd12*, we designed a targeting vector to replace exons 3 and 4 (which encode the first tudor domain) with a *β-geo* cassette (β-galactosidase and neomycin resistance fusion gene), by promoter trap selection (Fig. S3*A*). Homologous recombination removes ∼50% of the tudor domain, and any mutant transcript would undergo nonsense-mediated decay due to the resulting frame-shift. We PCR-amplified the 5′ homology arm (4.4 kb) using KOD plus (Toyobo) with primers 77010-LA-S13341 and 7701010-LA-AS17705. The 3′ arm (7 kb) was amplified using the 77010-SA-S19831 and 77010-AS26839(X) primers. The IRES *β-geo* cassette was inserted between the two PCR-amplified homology arms. For negative selection, the diphtheria toxin-A cassette was placed downstream of the 3′ arm. The targeting vector was linearized with NotI and introduced into RF8 ES cells by electroporation. We identified clones with homologous recombination by PCR. Primer 77010-LA-S12295 was designed to bind to sequence upstream of the 5′ arm, and the antisense primer (β-*geo* screening AS2) recognizes the *β-geo* cassette, yielding a 4.9-kb fragment for the targeted allele. PCR-positive clones were genotyped by Southern blotting with a 3′ probe (523 bp), which was amplified using primers m77010gU28402 and m77010gL28925. ES cell genomic DNA was extracted with Puregene Cell Lysis Solution (Gentra Systems), digested with SpeI, separated on a 0.8% agarose gel, and transferred to a nylon membrane. The Southern probe detected 15.7-kb and 9.2-kb fragments from the wild-type and targeted alleles, respectively (Fig. S3*B*). Presence of the targeted allele was further confirmed by detection of a 4.9-kb PCR fragment (Fig. S3*C*). The targeted ES cells were then used to generate mutant mice by standard procedures. Animals were maintained in C57BL/6;129S4/SvJae mixed genetic background.

Once correctly targeted clones were identified, cells and mice were genotyped using a three-primer PCR assay. The common primer m77010-gU17547 was designed to anneal to intron 2 of both the wild-type and targeted loci. Primer m77010-gL18297 binds to intron 3 and together with primer m77010-gU17547 amplifies a 774-bp wild-type fragment. The *β-geo* screening AS2 primer amplifies a 250-bp fragment from the targeted allele in combination with primer m77010-gU17547. All primers used for creation and analysis of the targeted *Tdrd12* mutant are listed in Table S2. All animal experiments were conducted according to national, European and internal EMBL regulations.

Generation and characterization of ENU-induced mutant mouse for Tdrd12. The *repro23* mutant mice were produced by ENU-induced mutagenesis in the ReproGenomics Program of The Jackson Laboratory (8). A total of 587 male F_2 mice were obtained by intercross of heterozygous (+/*repro23*) F_1 mice, derived from the cross between *repro23*/*repro23* homozygous female and JF1/Ms (+/+) male mice. The *repro23* locus has previously been mapped on a 2.2-Mb region of mouse chromosome 7 (8). By genotyping seven microsatellite markers on this region, we found no recombination between the *repro23* locus and a microsatellite marker *D7Mit78* whereas at least one recombination was found between the *repro23* locus and the remaining 6 microsatellite marker. Therefore, the *repro23* critical region was narrowed down to the 0.9-Mb interval between *D7Mit225* and *D7Mok1*.

Genomic DNA and cDNA synthesized from testis RNA of the mutant and normal mice were used for sequencing of the 13 candidate genes in the 0.9-Mb critical region. The PCR and RT-PCR products covering entire coding regions of these genes were sequenced by using ABI PRISM 310 Genetic Analyzer (Applied Biosystems). By comparing the sequences between normal and *repro23*/*repro23* mice, a nucleotide substitution of C to A resulting in nonsense mutation was found in exon 8 of the *Tdrd12* gene. No other mutation was found in the remaining genes.

Total RNA was isolated from testes using TRIzol regent (Invitrogen), reverse-transcribed into cDNA using oligo dT primers and PrimeScript RTase (Takara), and subjected for PCR reactions using primers for *Tdrd12* gene (TGTTCGAGGTCCTGGTGCTG and GCTTCCACTTGGGTAGTTGCTC) or L1 (GGCGAAAGGCAAACGTAAGA and GGAGTGCTGCGTTCTGATGA) and IAP (AACCAATGCTAATTTCACCTTGGT and GCCAATCAGCAGGCGTTAGT) retrotransposones.

Immunoprecipitations and Nucleic Acid Analyses. Immunoprecipitations and 5′-end labeling were as previously described (4). Germ cells were purified from testes and bisulfite conversion of the genomic DNA, and bisulfite analyses were performed as reported (9).

Northern Blot Analysis of Transposon Expression. Total RNA was extracted from testes of wild-type, heterozygous and homozygous *Tdrd12* mutant mice using TRI reagent according to the manufacturer's guide (MRC Inc; cat. no. TR 118) and treated with DNaseI (Fermentas). Approximately 10 μg of DNaseI-treated RNA was analyzed on denaturing formamide agarose gel and transferred onto Nylon membrane (Amersham; Hybond N+) through capillary transfer for 16 h in 20× SSC solution (For 1 L: 175 g NaCl, 88.2 g Na Citrate dehydrate). After transfer was complete, membrane was taken out and UV cross-linked with 120 mJ/cm^2 in Stratagene "cross linker". Cross-linked membrane was washed with 2× SSC once. Prehybridization was done at least for 2 h in Church buffer [0.25 M Na Phosphate buffer, pH 7, 2.1 mM EDTA, 1% (wt/vol) BSA, 7% (wt/vol) SDS, Filter solution] at 65 °C.

Radiolabeled L1 probe was prepared from LINE1 DNA fragment (515 bp–1,680 bp; GenBank accession no. M13002) (10) using Random prime DNA labeling kit (Roche; cat no. 1004760001). Radiolabeled IAP probe was similarly prepared from the IAP DNA (GenBank accession no. AF 303453). Probes were denatured at 95 °C for 5 min and hybridized with membrane in Church buffer at 65 °C overnight with continuous mixing. Next day washing was performed at 65 °C as followed: twice 15 min each with buffer-1 (2× SSC, O.1% SDS), twice 15 min each with buffer-2 (0.2× SSC, 0.1%SDS), and finally with 0.1× SSC at room temperature for 5 min. Membrane was wrapped in plastic film and exposed to Phosphor Imaging screen and scanned with typhoon scanner (GE Healthcare).

Immunofluorescence. Hematoxylin/eosin staining was performed on paraffin sections of testes samples. Immunofluorescence analysis was performed on O.C.T. (Tissue-Tek Cryo-OCT compound; Fisher Scientific) embedded testes cryosections. After incubation with primary antibodies, slides were washed and further incubated with Alexa 488- or Alexa 555-coupled secondary antibodies (Invitrogen). Samples were then examined by fluorescence microscopy (Olympus BX61), and images were captured using CCD (Olympus DP70).

BmN4 cells were grown on coverslips, and endogenous Piwi proteins or transfected proteins were detected as reported previously (5). Confocal images of the indirect fluorescence were recorded using a Leica TCS SP2 AOBS inverted microscope.

Small RNA Libraries and Bioinformatics. Small RNA libraries from immunoprecipitated RNAs were prepared by using the NEB kit [NEBNext Multiplex Small RNA Library Prep Set for Illumina (indexes 1–12); cat. no. E7300]. For total small RNA libraries from P0 testes of *Tdrd12*$^{+/-}$ and *Tdrd12*$^{-/-}$ animals, small RNAs

(~14–40 nt) were gel purified by urea-PAGE and library was prepared as above. Small RNA libraries were sequenced for 50 cycles with Illumina Genome Analyzer IIx (EMBL Gene core facility).

After sequencing, reads were processed to remove adapter sequences, followed by sorting based on the barcode information. These reads were then mapped to the mouse (July 2007, NCBI37/mm9 assembly) or *Bombyx mori* genome (11). Only perfectly mapping reads were retained for further analysis. Genome annotations were extracted based on those defined by Entrez. For plotting repeat small RNA density, reads were mapped to transposon consensus allowing up to a maximum of three mismatches as previously described (12). Density plots were displayed after normalization of all libraries to 1 million reads.

The following proteins were used in the sequence alignment showing aromatic cage residues in tudor domains (Fig. 1*C*): human Staphylococcal nuclease domain-containing 1 (SND1) (GI:77404396); *Drosophila* Tudor (GI:19550189); mouse TDRD1 (GI:268607546); *Drosophila* CG31755 (SoYb) (GI:386769395), CG11133 (BoYb) (GI:24668815); *Bombyx mori* Tdrd12 (see *SI Experimental Procedures*); and mouse TDRD12 (Uniprot accession no. Q9CWU0).

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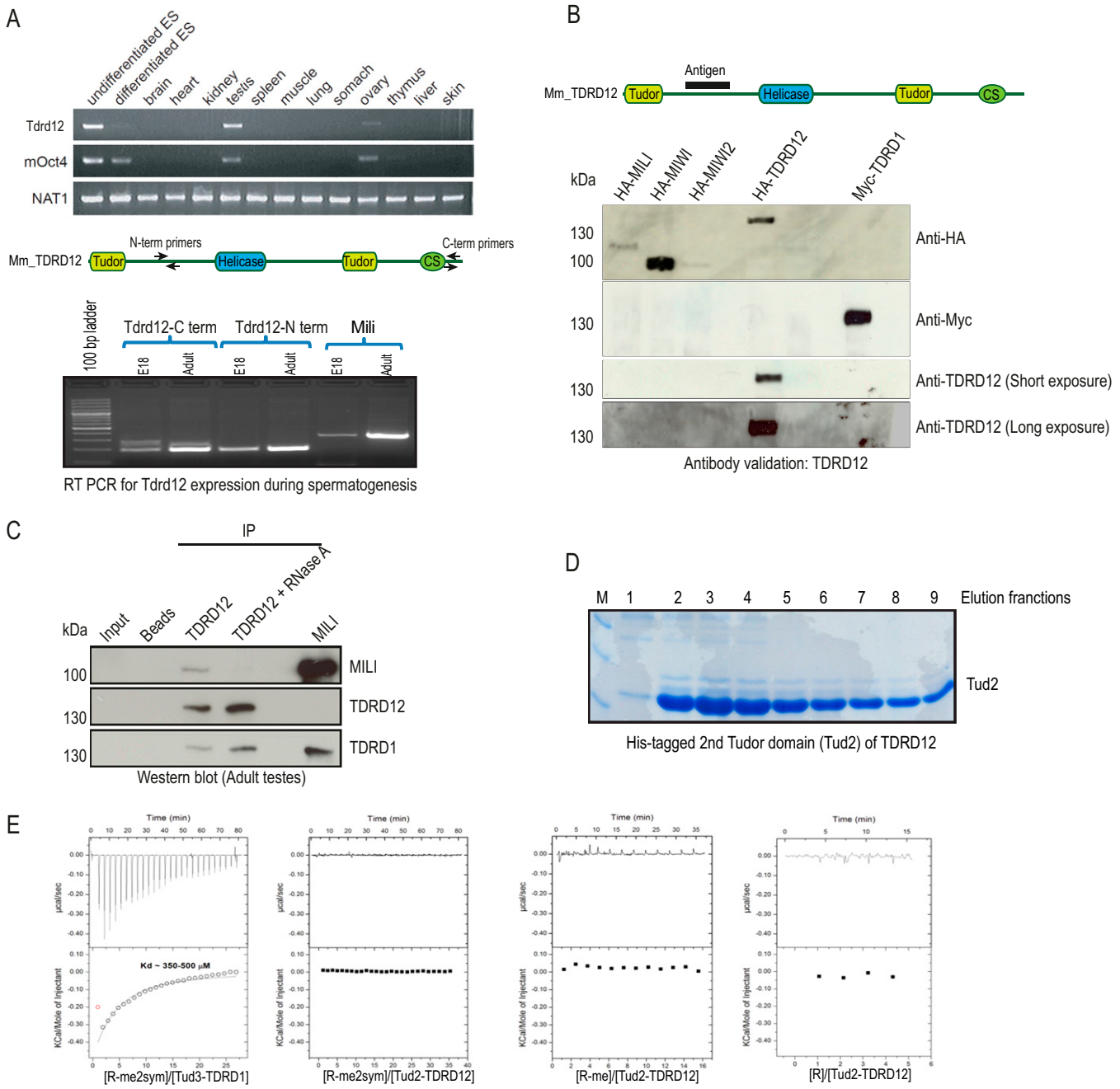

Fig. S1. *Tdrd12* expression in mouse tissues and its characterization. (*A*) RT-PCR expression analysis of *Tdrd12* expression in indicated adult mouse tissues or embryonic stem (ES) cells (*Upper*). *Lower* shows RT-PCR expression analysis of *Tdrd12* expression in embryonic and adult mouse testes using primer pairs at N-terminal (Tdrd12-N term) and C-terminal (Tdrd12-C term) regions of *Tdrd12* coding region. (*B*, *Upper*) A cartoon depicting the domain organization of mouse TDRD12. The region used as an antigen for raising rabbit polyclonal antibodies is indicated. (*Lower*) To test the specificity of the purified anti-TDRD12 antibodies, blots containing various proteins (HA- or Myc-tagged) expressed in HEK293T cells were probed with anti-TDRD12 antibodies. Only HA-TDRD12 was recognized by the antibodies, confirming its specificity. (*C*) Protein complexes were immunoprecipitated (IP) from adult mouse testes extracts and probed by Western analysis for the indicated proteins. When indicated, purified complexes were treated with RNaseA. This is a repetition of the experiment shown in Fig. 1*B*. (*D*) Coomassie blue stained SDS PAGE gel for the purified second tudor domain (Tud2) of mouse TDRD12 expressed in *E.coli*. (*E*) Isothermal calorimetry (ITC) measurements of indicated TDRD1 or TDRD12 tudor domains with unmodified and methylated arginine residues. No interaction was detected with Tud2 of TDRD12, while the positive control (Tud3 of TDRD1) shows high micromolar-range affinity for sDMA (me2sym), as previously reported (1).

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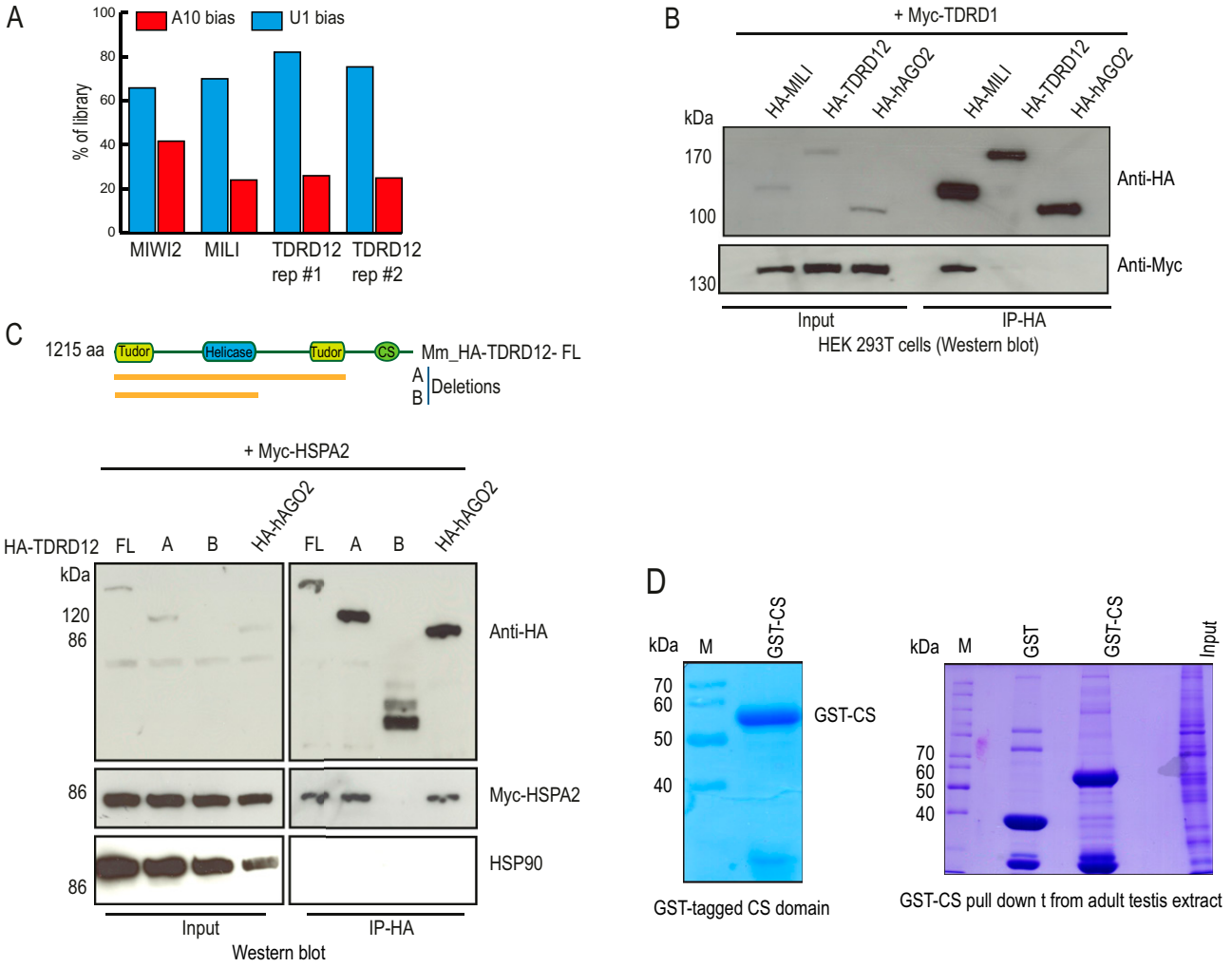

Fig. S2. Characterization of mouse TDRD12. (*A*) The nucleotide bias for a uridine at first position (1U) or adenosine at 10th position (A10) of reads in the libraries is given. (*B*) HA- and Myc-tagged constructs were co-transfected in HEK293 cells and immunoprecipitated with HA affinity beads and probed for the co-immunoprecipitated Myc-tagged protein. As expected, TDRD1 interacts with MILI, while TDRD12 did not show an association with TDRD1. Human AGO2 (hAGO2) was used as negative control. (*C*) HA- and Myc-tagged constructs were co-transfected in HEK293T cell. Protein complexes were immunoprecipitated with HA-affinity beads and probed for co-imunoprecipitated Myc-tagged HSPA2 (HSP70) or endogenous HSP90 proteins. HSPA2 nonspecifically interacts with both mouse TDRD12 and hAGO2, while HSP90 does not interact with either full length mouse TDRD12 or its deletion variants. The CS domain in other proteins is shown to associate with the molecular chaperones HSP70 and HSP90 (1). But our experiments do not support such a role for the domain in TDRD12. (*D*) Coomassie-stained SDS-PAGE of purified GST-tagged CS domain of mouse TDRD12 expressed in BL21 *E.coli* cells (*Left*). Pull-down experiment conducted with adult mouse testes extracts using GST alone or GST-CS peptide (*Right*). None of the associated bands were revealed to be HSP90 or HSP70, as determined by MS/MS.

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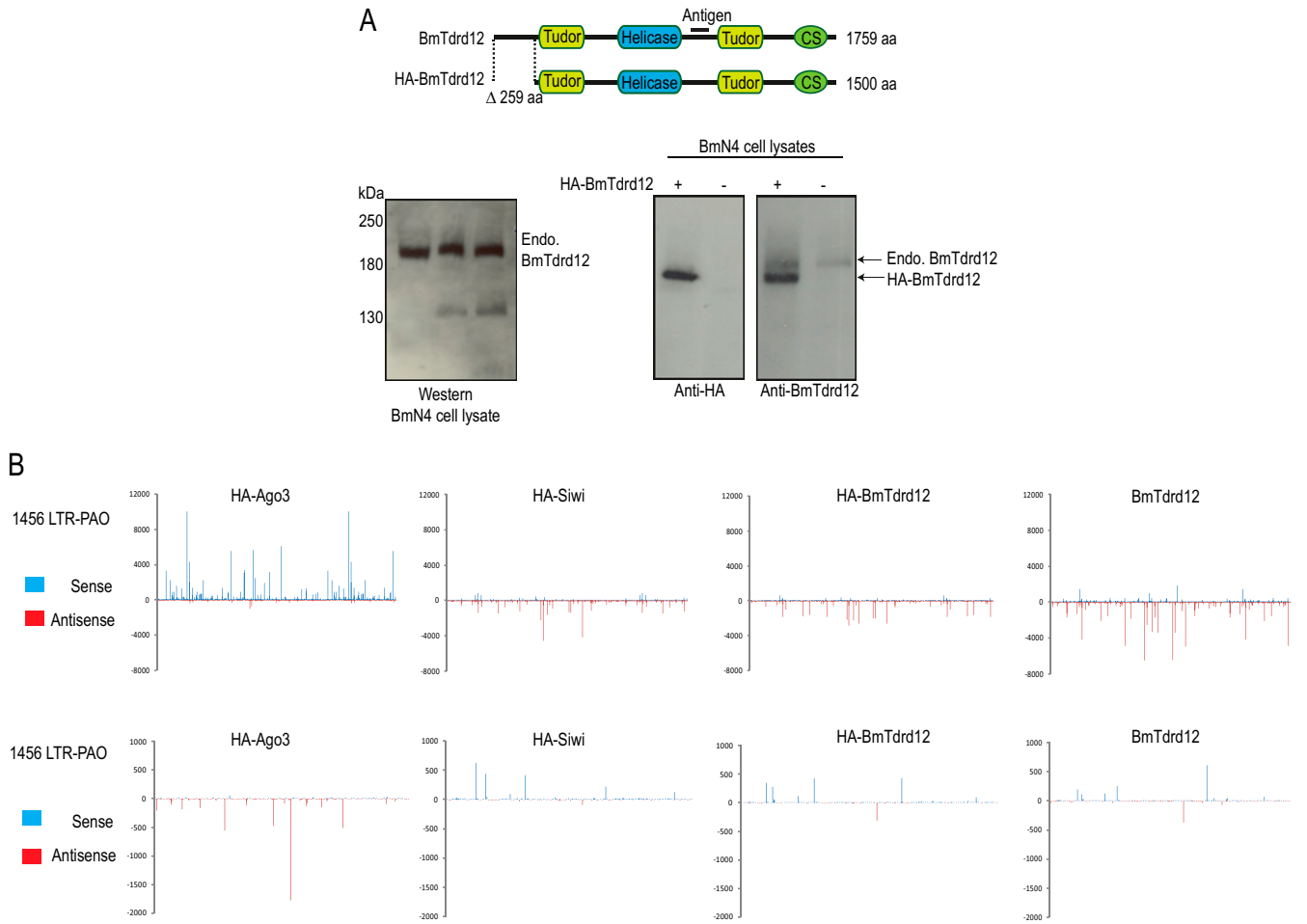

Fig. S3. Characterization of *Bombyx mori* Tdrd12. (*A*) Domain organization of predicted *Bombyx mori* Tdrd12 (BmTdrd12). The cDNA for BmTdrd12 corresponds to a protein encoding 1759 aa. In this study, an HA-tagged version of BmTdrd12 that lacks the N-terminal 259 aa was used, as we failed to express the tagged full-length construct. Western blot analysis with purified BmTdrd12 antibodies detects a band of expected size (~200 kDa, expected from 1759 aa) in BmN4 cell lysates (*Left*). The antibodies also recognize HA-BmTdrd12 expressed in BmN4 cells as an additional faster-migrating band (*Right*). (*B*) Sequence analysis of the strand-orientation of piRNA reads in small RNA libraries prepared from HA-tagged BmTdrd12, endogenous BmTdrd12, HA-Siwi and HA-Ago3 complexes on indicated *Bombyx mori* transposon consensus sequences. The strand-orientation of reads from the BmTdrd12 and Siwi libraries are the same.

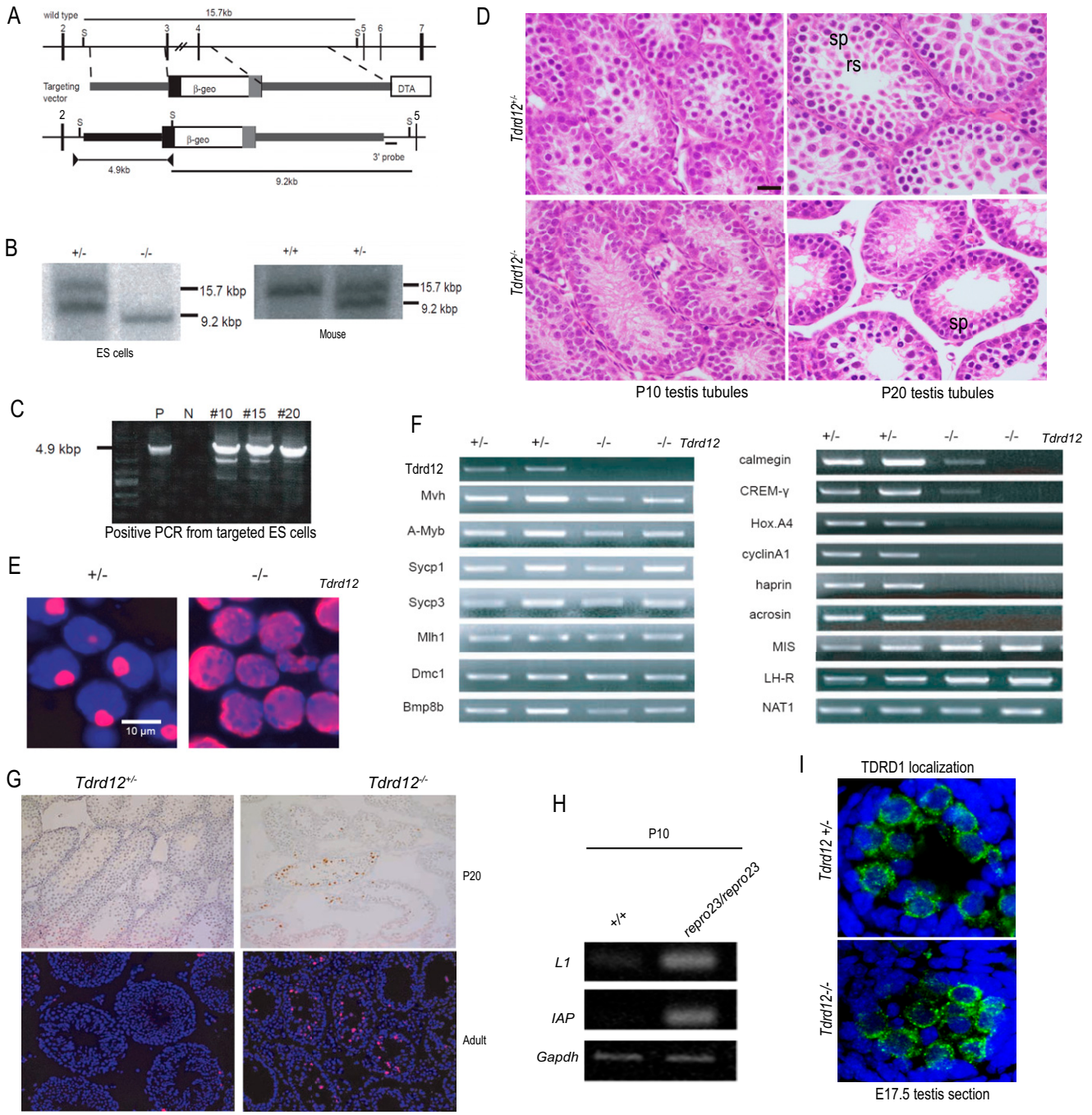

Fig. S4. Generation of the targeted *Tdrd12* mutant. (*A*) Schematic showing the targeted disruption of the mouse *Tdrd12* locus in RF8 ES cells. By homologous recombination, the targeting vector replaced exon 3 and 4 (coding for the first tudor domain) with a fusion cassette consisting of β-galactosidase and neomycin resistance gene (β-geo). The replacement of exon 3 and 4 with targeting vector results in gene deletion, as transcripts from the mutant allele contains nonsense codons, leading to NMD. The diphtheria toxin A cassette was used for negative selection. The SpeI (S) restriction sites are indicated used for positive clone screening by southern blotting. Note that an additional SpeI site is brought in the targeting vector. (*B*) Presence of the recombined locus can be detected by Southern blotting with genomic DNA from ES cells or targeted mice. Wild-type allele gives a 15.7-kb fragment, while targeted allele generates in a 9.2-kb fragment. (*C*) Presence of targeted allele is also confirmed by a PCR reaction to amplify a 4.9-kb product (see scheme in *A*). The primers are anchored on the genomic DNA and the β-geo cassette from the targeting vector. (*D*) Hematoxylin and eosin staining of 10-d- and 20-d-old animal testis of the indicated *Tdrd12* genotypes. Spermatogenic defect is hardly apparent in the P10 *Tdrd12* mutant, even though we already noted transposon activation at this early stage (see Fig. 3*D*). (*E*) Testis sections were stained for the phosphorylated form of H2AX (γ-H2AX) and DAPI (for DNA). The XY body (sex body) seen in pachytene spermatocytes is visible in control heterozygous *Tdrd12* mutants, but not in homozygous mutants. (*F*) RT-PCR analysis of genes specifically expressed during various stages of spermatogenesis. Gene normally expressed in haploid round spermatids are not detected in the *Tdrd12* mutant. (*G*) TUNEL staining to detect apoptotic cells in P20 (IHC, brown staining) and adult animals (IF, red staining) of indicated genotypes. Note the abundant presence of such cells in the *Tdrd12* mutant. (*H*) Expressions of retrotransposons in *repro23* mice testes. Expression of both L1 and IAP retrotransposons markedly increased in *repro23/repro23* in P10 testis. (*I*) Unaffected localization of TDRD1 in embryonic testes of indicated *Tdrd12* genotypes.

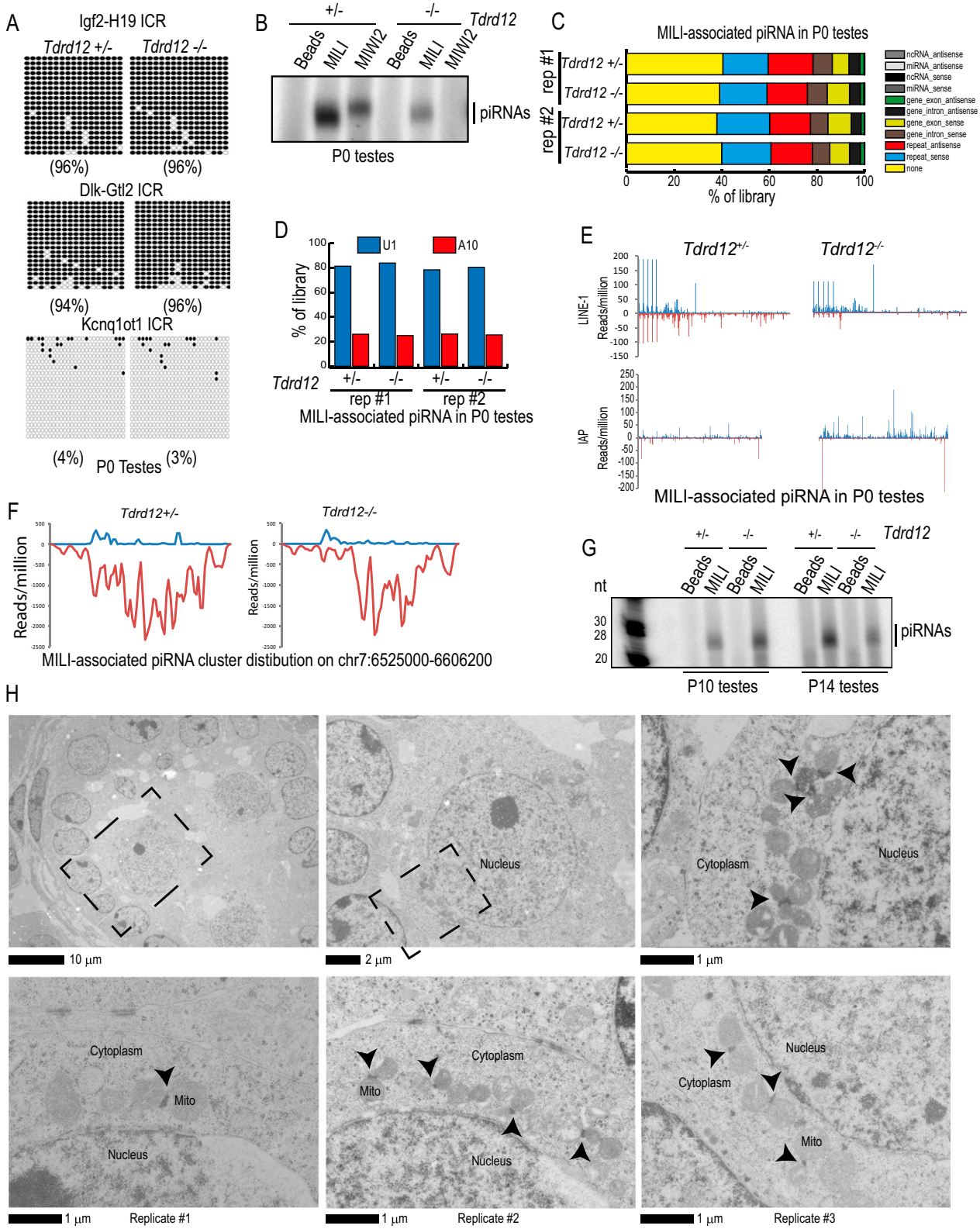

Fig. S5. Analysis of *Tdrd12* mutants. (*A*) Promoter CpG DNA methylation (indicated as filled circles) on three imprinted genomic loci was quantified (given in percentages) by bisulfite sequencing. Methylation of imprinted loci is unaffected in *Tdrd12* mutants. (*B*) Immunoprecipitation of Piwi proteins present in P0 testes of indicated animals. Associated small RNAs were radiolabelled and visualized. Note the absence of MIWI2-bound piRNAs in the *Tdrd12* mutant. This experiment is a biological replicate of the experiment shown in Fig. 4*D*. (*C*–*F*) Analysis of MILI-associated reads from P0 testes. (*C*) Genome annotations, (*D*) nucleotide preference at indicated positions of the reads, (*E*) mapping to transposon consensus, and (*F*) mapping to an embryonic piRNA cluster. (*G*) Pre-pachytene piRNAs are still associated with MILI in the 10-d-old (P10) and P14 *Tdrd12* mutants. (*H*) Electron micrographs showing unaffected presence of electron-dense intermitochondrial cement (arrowheads) in *Tdrd12* mutant fetal testis. Three biological replicates of the mutant were analysed. Mito, mitochondria.

Table S1. List of all deep sequencing datasets used in this study

Library	Organism	Age	Genotype	Mapped reads
MILI IP # rep1	Mouse	P0	*Tdrd12* $^{+/-}$	16473692
MILI IP # rep1	Mouse	P0	*Tdrd12* $^{-/-}$	15024728
MILI IP # rep2	Mouse	P0	*Tdrd12* $^{+/-}$	19246231
MILI IP # rep2	Mouse	P0	*Tdrd12* $^{-/-}$	15722298
Total small RNA (14-40 nt)	Mouse	P0	*Tdrd12* $^{+/-}$	12393343
Total small RNA (14-40 nt)	Mouse	P0	*Tdrd12* $^{-/-}$	16113332
MILI IP	Mouse	E18	Wild type	16549061
MIWI2 IP	Mouse	E18	Wild type	12590475
TDRD12 IP # rep1	Mouse	E18	Wild type	9071424
TDRD12 IP # rep2	Mouse	E18	Wild type	10209745
HA-BmTdrd12 IP	*Bombyx mori* cell line, Bmn4	N/A	N/A	10213582
BmTDRD12 IP (endogenous)	*Bombyx mori* cell line, Bmn4	N/A	N/A	12018857

Table S2. List of selected primers used in this study

Primer name	Sequences
Primers used for characterizing Tdrd12 targeted mouse mutant	
MvhVg2	CCAAAAGTGACATATATACCC
MvhVas3	TTGGTTGATCAGTTCTCGAG
CREM-Upper	GATTGAAGAAGAAAAATCAGA
CREM-Lower	CATGCTGTAATCAGTTCATAG
Dmc1-Upper	TTCGTACTGGAAAAACTCAGCTGTATC
Dmc1-Lower	CTTGGCTGCGACATAATCAAGTAGCTCC
HoxA4-Upper	TGAGCGCTCTCGAACCGCCTATACC
HoxA4-Lower	GATGGTGGTGTGGGCTGTGAGTTTG
Mlh1-Upper	AGGAGCTGATGCTGAGGC
Mlh1-Lower	TTTCATCTTGTCACCCGATG
Sycp-Upper	ATGGAGAAGCAAAAGCCCTTC
Sycp-1Lower	TTTCTGCTTCAGTTCAGATTC
CyclinA1-Upper	ATGCATCGCCAGAGCTCCAAGAG
CyclinA1-Lower	GGAAGTGGAGATCTGACTTGAGC
Sycp-3-Upper	GGTGGAAGAAAGCATTCTGG
Sycp-3-Lower	CAGCTCCAAATTTTTCCAGC
A-Myb-Upper	AAGAAGTTGGTTGAACAACACGG
A-Myb-Lower	AGGAAGTAACTTAGCAATCTCGG
Calmegin-Upper	ATATGCGTTTCCAGGGTGTTGGAC
Calmegin-Lower	GTATGCACCTCCACAATCAATACC
NAT1-U10	GAGTCGGAGCTCTATGGAGGTGG
NAT1-L21	GCCCGAAGAAGCACTGAAACGAGAA
Bmp-8b-Upper	CGCAACATGGTAGTCCAGGC
Bmp-8b-Lower	GGATACTGAAGAGCCTGAGC
MIS-S	TTGGTGCTAACCGTGGACTT
MIS-AS	GCAGAGCACGAACCAAGCGA
m77010-U362	CTGGTGCAGGGCTCTGATTAAGTC
m77010-L1148	TTTACACAGCCGTTCTTCTCGTCA
Acrosin-S	CGGAGTCTACACAGCCACCT
Acrosin-AS	GCATGAGTGATGAGGAGGTT
Haprin-S	CCAGAACATGAGACAGAGAG
Haprin-AS	AGCAACTTCCTGAGCATACC
LH-R-S	TGCAACCTCCTCAATCTGTC
LH-R-AS	AGCGTGGCAACCAGTAGGCT
m77010-gU17547	ACTGTTGCTAATGGGAGCTACTGT
m77010-gL18297	GGGCAGTATCTTTCCTGAGTCATA
bGeo-screening-AS6	GCGGAATTCTCTAGAGTCCAGATC
77010-LA-S13341	AGGATGGTTAGGCAAGGTTCTCTTACTC
77010-LA-AS17705	GCACTTCAGCTCCTGGCAGTACACCACA
77010-SA-S19831	TGTGAAGACATCCAGCGCAGGTTGACTT
77010-AS26839(X)	GGCTCGAGTCCACCTCATACCTGA
m77010-gU28402	GGGGAAGATTTTTATTGTTGCTA
m77010-gL28925	AAGAGTTGTAGGCACTGCAAACAC
bgeo-screening-AS2	GACCTTGCATTCCTTTGGCGAGAG
Primers for cloning Tdrd12 antigen	
RPoligo820	CATGCCATGGAGAAGCCTGTGACATTGCACATTG
RPoligo821	ATTCTTATGCGGCCGCTTACTTCTTCAGGTCTGCCGACAG
Primers for cloning Tudor domain of Tdrd12	
RRoligo261	CATGCCATGGGACACATTAGGGTAATACCTTTTTACAT
RRoligo262	ATTCTTATGCGGCCGCTCAGTCAGCTGGCTTAACCCTACAA
Primers for Tdrd12 RTPCR	
RRoligo331	CTTCAGTGCCTGGGTAGGAG
RRoligo332	TCCTCCCAGTGGTCAAAGTC
RRoligo333	TGTGTACTGGCCAACCAAAA
RRoligo334	CTGAGGTCCCATCAGCTCTC