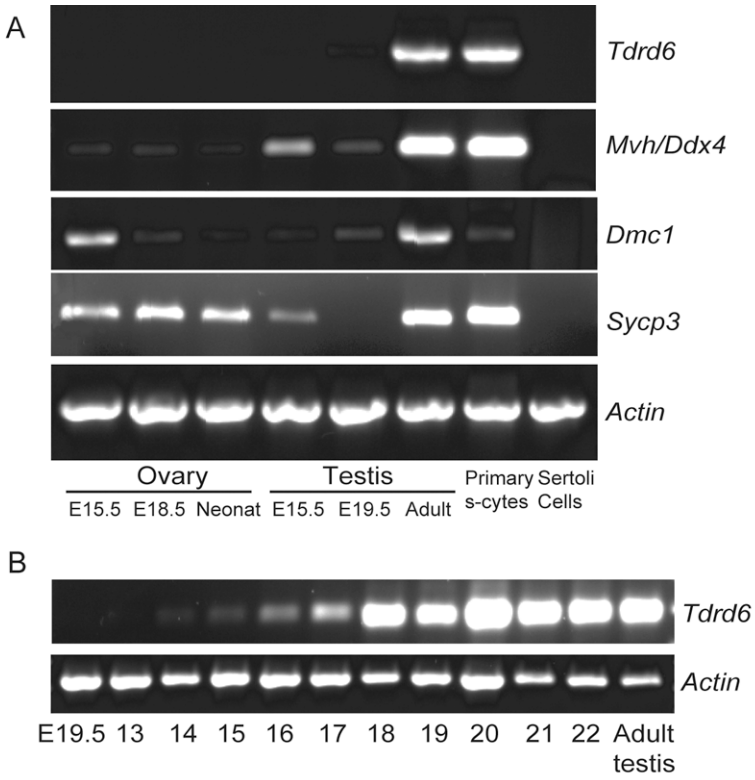
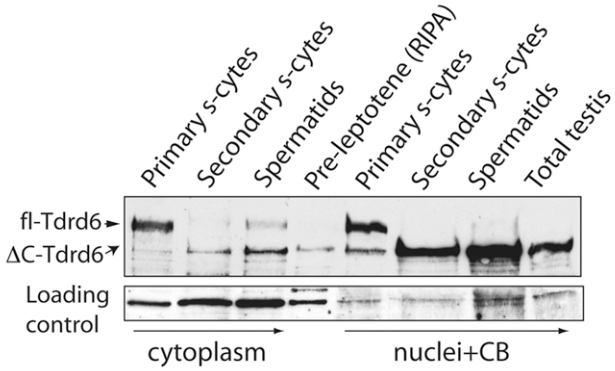
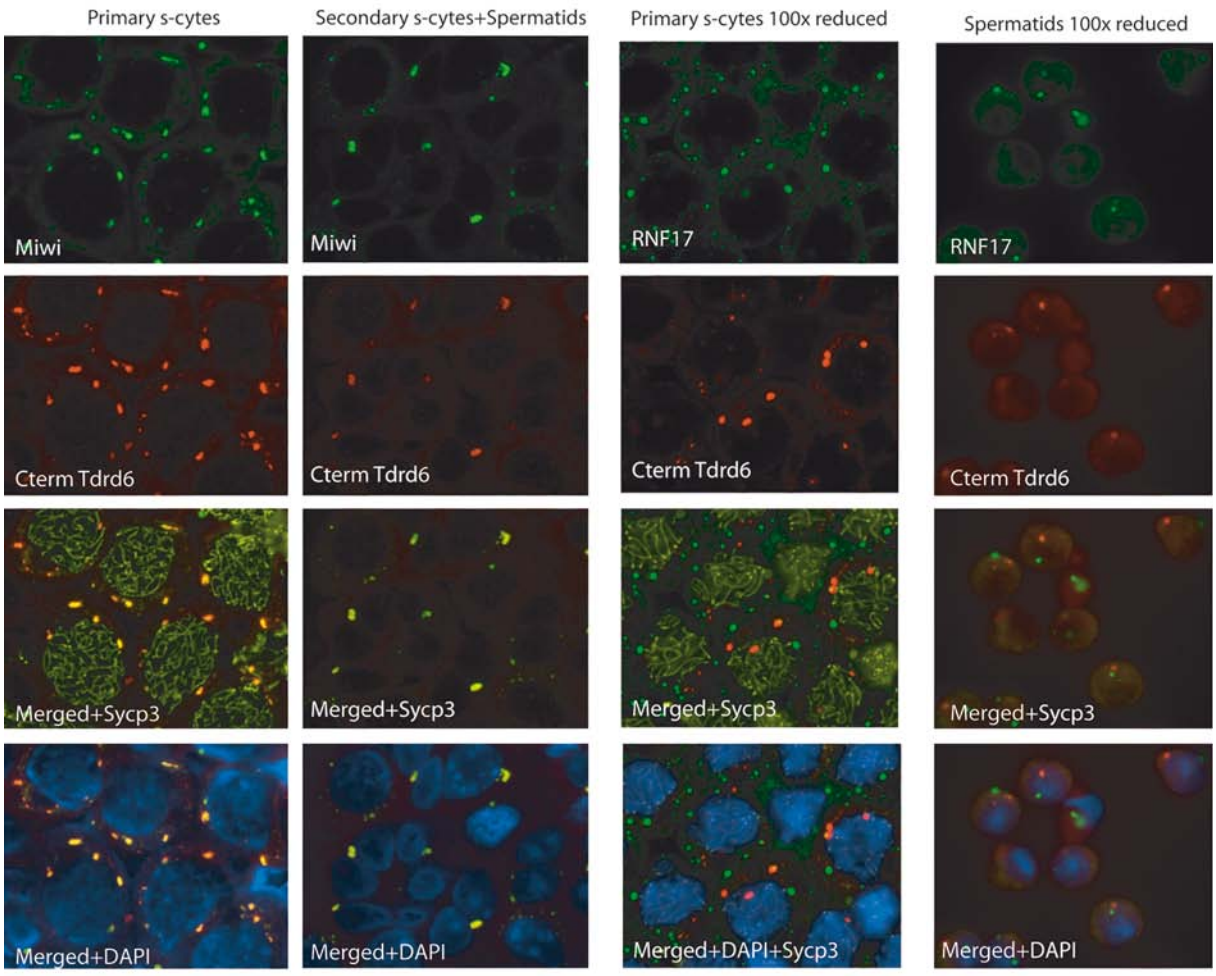


Supplementary Fig. 1 Analysis of Tdrd6 expression . (A) Comparison of Tdrd6 expression and other germ cell-specific genes in male and female gonads. The label at the bottom of each lane indicates the tissue or cell type used for total RNA isolation and the age of the mouse at the time of isolation. E15.5, E18.5 and E19.5 designate embryos at 15.5, 18.5 and19.5 days post coitum (dpc), respectively; Neonat - newborn female; Primary s-cytes - sorted primary spermatocytes (B) Time-course of Tdrd6 mRNA expression in juvenile and adult testis assayed by RT-PCR. Beta actin was used as loading control.



Suppl. Fig. 2 Immunoblot analysis of Tdrd6 protein expression in cytoplasmic or nuclear extract. Spermatocyte and spermatid populations from adult testis were separated by FASC sorting. Tdrd6 expression in 10 microg cytoplasmic and 5 microg nuclear extract (including CBs, which co-sediment with nuclei) was analysed with anti-265 antibody.



Suppl. Fig.3 Tdrd6 co-localizes with Miwi and not with RNF17 loci. Cytospun primary spermatocytes or secondary spermatocytes and spermatids were co-stained with either anti-Miwi or anti-RNF17 (green) and anti-Cterm antibodies (red). Synaptonemal complexes on chromosomes are marked with a-Sycp3 (blue) and nuclei are stained with DAPI.

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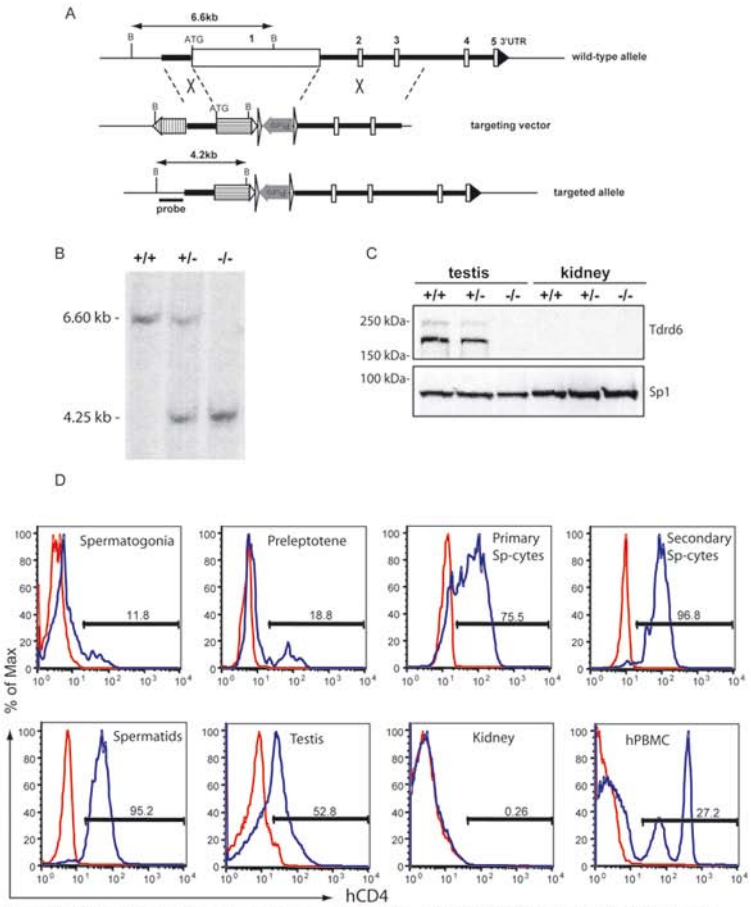
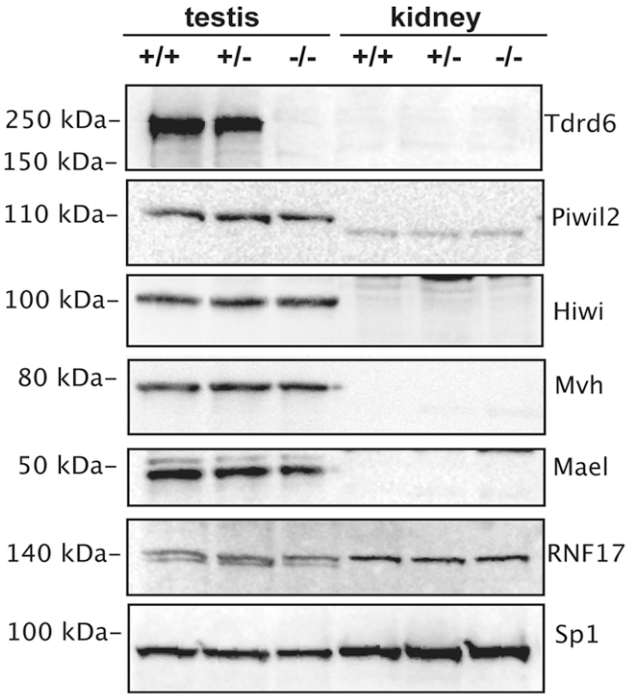
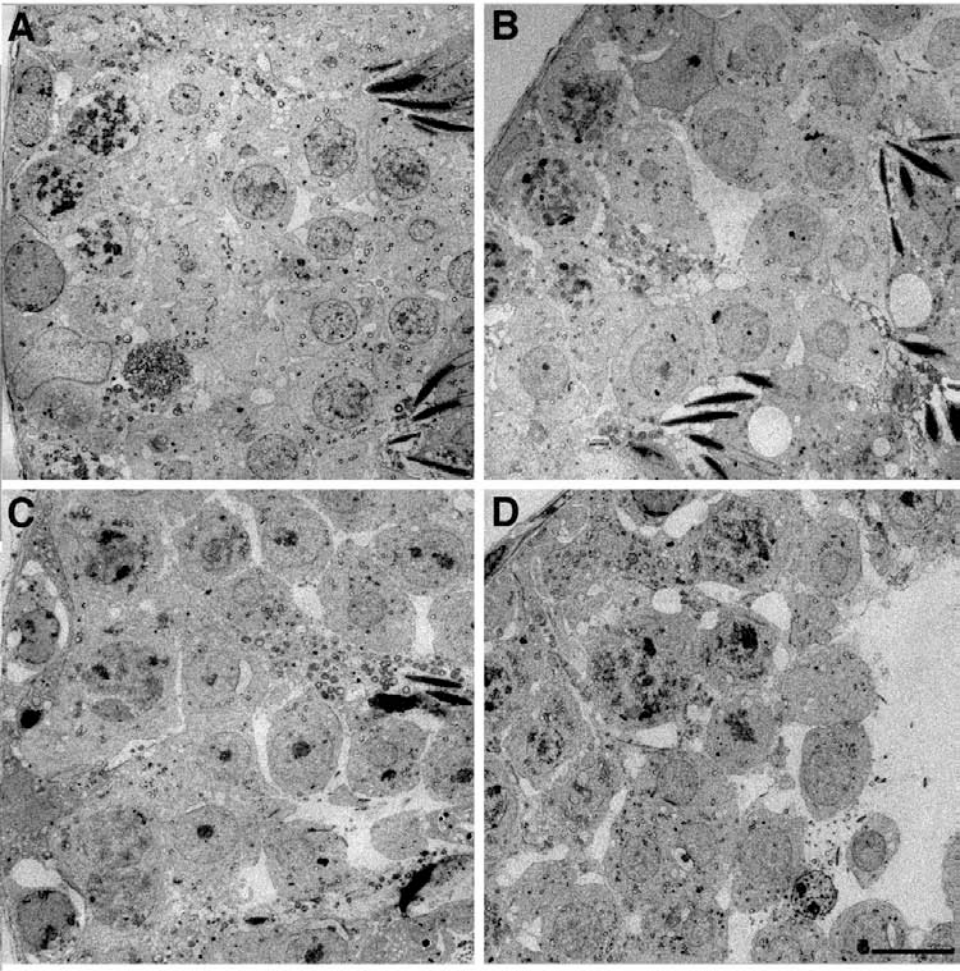


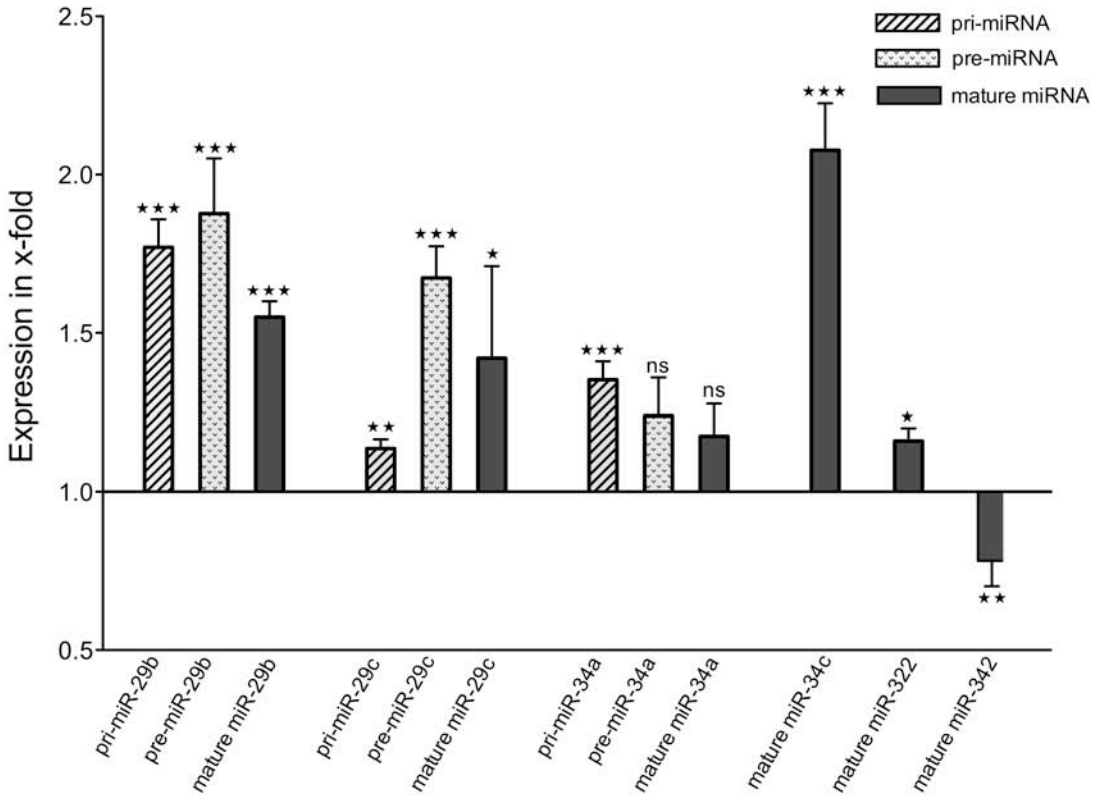
Suppl. Fig. 4. Targeted disruption of the Tdrd6 gene and generation of Tdrd6 null/hCD4 knock-in allele. (A) Schematic representation of the wild-type allele, the targeting vector and the mutated allele. The numbered white boxes (1-5) denote the five coding exons of the gene. The targeting vector includes the PGKpuro gene (puromycin resistance) for positive selection and the PGK-DTA (diphteria toxin) gene for negative selection as well as the hCD4 gene in frame with the Tdrd6 5'UTR and ATG (start) codon. (B) Southern blot analysis of representative offspring from heterozygous mating. The wild-type allele produces a 6.6 kb BglII product, while the disrupted allele gives rise to a 4.25 kb band with a 1.4 kb 5'-end hybridization probe. (C) Western blot analysis of the Tdrd6 in testes of the same offspring using antibodies against the N-terminus of Tdrd6. Lysates (10 mg of protein per lane) of the testes or kidney were loaded on the gel. The +/+, +/– and –/– indicate wildtype, heterozygous and homozygous mutant testes or kidney, respectively. Sp1 was used as loading control. (D) FACS analysis of anti-hCD4 stained total testis cell suspension and sorted germ cell subpopulations from Tdrd6+/– mice. Kidney cell suspension and human peripheral blood monocytes (hPBMC), which normally express CD4, were used as controls.



Suppl. Fig. 5. Immunoblot of nuclear extracts from testis and kidney from Tdrd6+/+, Tdrd6+/- and Tdrd6-/- mice. Extracts were probed with specific antibodies for chromatoid body markers Tdrd6, PiwiL2, Mvh, Mael, Hiwi, and for control Rnf17 and Sp1.



Suppl. Fig. 6. Electron microscopy of seminiferous tubules. In stage I seminiferous tubules of Tdrd6+/- (A) and Tdrd6-/- mice (B) elongated spermatids are apparent. Due to a block in spermiogenesis, stage V seminiferous tubules of Tdrd6-/- mice (D) lack elongated spermatids, while they are present in Tdrd6+/- mice (C) (bar 10 μm).



Supplemental Figure 7 Differentially expressed precursor and mature miRNAs in testes of Tdrd6-/- mice. Analysis was performed with total testis RNA from 18 days old Tdrd6-/- and Tdrd6+/- mice. Precursors (Pri- and pre-miRNAs) were analyzed for miR-29b, miR-29c and miR-34a. Mature miRNAs were analyzed using primer for miR-29b, miR-29c, miR-34a, miR-34c, miR-322 and miR-342. The values for the Tdrd6+/- control were set to 1.0 and the respective increase or decrease in Tdrd6-/- mice are represented as mean +/- SEM. (*** P value < 0.001, ** P value 0.001 to 0.01, * P value 0.01 to 0.05, ns not significant P value > 0.05)

Supplemental Table 1: PCR primer used in this study

ShArm forward	5'- GAATTCTTCAAGGATAAGCTCAACGTGGAGAA
ShArm Reverse	5'- CGCCAACCGAGAGCT
LongArm forward	5'- GAAAATTTAAATACAAAGAAGTTAGCTCAGATTTG
LongArm reverse	5'- CAGTTTAAACGCTAACATGCTAACACTA
hCD4 reverse	5'- GGGAAGCTGTACAGGTCAGTTCCA
Tdrd6ShArmgeno forward	5'- GGCTGCTACCAGTCGCGAGTC
Tdrd6genoWT reverse	5'- ATGGAGGAGCAACACATCCAGGAC
+ Tdrd6 RT-fwd	5'- GAAGGAAGGAAGTGCGGGAGC
Tdrd6 RT-rev	5'- CACCTCCACACGCCTCCTCC
Cterm-fwd BglII	5'- GCCAGAtctGGAAGGAAGTGCG
C-term-revXhoI	5'- ATGCTCGAGTCATATGTTCAGC
Nterm-fwdBglII	5'-TCGAGATCTACTCCGGGGCTGC
Nterm-revXhoI	5'-CCTCGAGGGGCAGGTGGAAGA
Actin-F (qRT-PCR)	5'- AGGCATTGCTGACAGGATGCAG
Actin-R (qRT-PCR)	5'- AGCACTTGCGGTGCACGATG
GAPDH-F (qRT-PCR)	5'- GAGAAACCTGCCAAGTATGATG
GAPDH-R (qRT-PCR)	5'- GGAGTTGCTGTTGAAGTCGC
MP-F (miR-qRT-PCR)	5'- TGTCAGGCAACCGTATTCACC
MP-R (miR-qRT-PCR)	5'- CGTCAGATGTCCGAGTAGAGG
5S rRNA-F (miR-qRT-PCR)	5'- GCCCGATCTCGTCTGATCT
5S rRNA-R (miR-qRT-PCR)	5'- AGCCTACAGCACCCGGTATT
RT-miR-29b-1(miR-qRT-PCR)	5'- TGTCAGGCAACCGTATTCACCGTGAGTGGTAACACT
RT-miR-29c(miR-qRT-PCR)	5'- TGTCAGGCAACCGTATTCACCGTGAGTGGTTAACCG
RT-miR-34a(miR-qRT-PCR)	5'- TGTCAGGCAACCGTATTCACCGTGAGTGGTACAACC
RT-miR-34c(miR-qRT-PCR)	5'- TGTCAGGCAACCGTATTCACCGTGAGTGGTCCTGGC
RT-miR-322(miR-qRT-PCR)	5'- TGTCAGGCAACCGTATTCACCGTGAGTGGTTCCAAA
RT-miR-342(miR-qRT-PCR)	5'- TGTCAGGCAACCGTATTCACCGTGAGTGGTCCTGGC
Short-miR-29b1R(miR-qRT-PCR)	5'- CGTCAGATGTCCGAGTAGAGGGGGAACGGCGTAGCACCATTTGAAATCAG
Short-miR-29c-R(miR-qRT-PCR)	5'- CGTCAGATGTCCGAGTAGAGGGGGAACGGCGTAGCACCATTTGAAATCGG
Short-miR-34a-R(miR-qRT-PCR)	5'- CGTCAGATGTCCGAGTAGAGGGGGAACGGCGTGGCAGTGTCTTAGCTCC
Short-miR-34c-R(miR-qRT-PCR)	5'- CGTCAGATGTCCGAGTAGAGGGGGAACGGCGAATCACTAACCACACAGC
Short-miR-322-R(miR-qRT-PCR)	5'- CGTCAGATGTCCGAGTAGAGGGGGAACGGCGCAGCAGCAATTCATGTTT
Short-miR-342-R(miR-qRT-PCR)	5'- CGTCAGATGTCCGAGTAGAGGGGGAACGGCGAGGGGTGCTATCTGTGAT
Pri-miR-29b1-F(miR-qRT-PCR)	5'- TGTAAGCCTCGTGCTCACTG
Pri-miR-29b1-R(miR-qRT-PCR)	5'- GAAGGTGAAGTCCGTGCAAT
Pre-miR-29b1-F(miR-qRT-PCR)	5'- AGGAAGCTGGTTTCATCTGG
Pre-miR-29b1-R(miR-qRT-PCR)	5'- AGAACACTGATTTCAAATG
Pri-miR-29c-F(miR-qRT-PCR)	5'- AGCAAGGAAGGGGTAAGAGC
Pri-miR-29c-R(miR-qRT-PCR)	5'- ACCGACTGGTGGTGTTCTTC

Pre-miR-29c-F(miR-qRT-PCR)	5’- ATCTCTTACACAGGCTGACC
Pre-miR-29c-R(miR-qRT-PCR)	5’- TCCCCCTACATCATAACCGA
Pri-miR-34a-F(miR-qRT-PCR)	5’- TCCTGACTCAGCCTCCTTGT
Pri-miR-34a-R(miR-qRT-PCR)	5’- TGACACACTCCTCCAGCACT
Pre-miR-34a-F(miR-qRT-PCR)	5’- CCAGCTGTGAGTAATTCTTT
Pre-miR-34a-R(miR-qRT-PCR)	5’- ACAATGTGCAGCACTTCTAG

Mmu-miR	GeneID	Fold	Genomic Locus	Mmu-miR- Cluster	features	IG/IT
-29c	39300	4,3	1:196,863,234-196,863,828	-29b-2, -29c		IG
-30a*	39306	2,7	1:123,279,108-23,279,178			IG
-128	39210	1,8	1:130,098,938-130,099,007			IT
-466a-3P	38815	1,9	2: 10,388,571-10,438,791	-466a, -466b, -4667a, -669, -297a, -297c, -466b-1, -466d		IT
-466a-5P	35833	4,1				IT
-466b-3P	39949	2,3				IT
-466b-5P	38810	1,7				IT
-467a	38825	1,6				IT
-200a	38505	2,5	4:155,428,014-155,429,859	-200a, -200b, -429		IT
-200b	38723	2,5				IT
-30e*	39308	3,1	4:120,442,139-120,445,302	-30c-, 30e		IT
-34a*	39306	1,3	4:149,442,563-149,442,664			IG
-339-5P	39327	2,1	5:139,845,604-139,845,699			IT
-29b-1	38569	1,9	6: 31,012,660-31,013,093	-29a, -29b-1	CpG,TSS	IT
-489	38535	2,4	6: 3,671,301-3,672,003	-489, -653		IG
-148a	35857	1,9	6: 51,219,811-51,219,909		CpG,TSS	IG
-202	38506	2,8	7:147,143,588-147,143,659		TSS	IG
-140	35847	2,4	8:110,075,144-110,075,213			IT
-191	39255	0,7	9:108,470,650-108,471,192	-191, 425	CpG	IG
-34c*	39350	1,8	9: 50,911,139-50,911,750	-34a, -34c	CpG,TSS	IG
-125b	35830	0,7	9: 41,390,009-41,390,085		TSS	IT
-451	39370	1,6	11: 77,886,507-77,886,743	-144, -451		IG
-497	35866	3,0	11: 70,048,219-70,048,637	-195, -497		IG
-21	35883	2,3	11: 86,397,569-86,397,660			IT
-152	35861	2,0	11: 96,711,707-96,711,779		CpG,TSS	IT
-301a	38983	1,6	11: 86,926,506-86,926,591		CpG.TSS	IT
-379	38528	2,0	12:110,947,270-110,982,005	-134, -154, -299, -300, -323, -329, -369, -377,-376a, -376b, -376c, -379, -380, -381, -382, -409, -410, -411 -412, -453, -476, -485, -487b, -494, -495, -496, -539,-541, -543, -544, -654, -666, -667, -668, -679, -758		IG
-487b	38377	1,8				IG
-494	39394	2,5				IG
-203	38328	2,1	12:113,369,091-113,369,166		CpG,TSS	IG
-342-3P	38991	0,6	12:109,896,830-109,896,928			IT
-7a	38644	2,8	13: 58,494,140-58,494,247			IG
-15a	35864	1,8	14: 62,250,717-62,250,947	-15a, -16-1		IG
-17*	38715	1,9	14:115,442,893-115,443,728	-17, -18a, -19a, -19b-1, -20a, -92a-1	CpG,TSS	IG
-19a	38326	2,1			CpG,TSS	IG
-125a	39208	0,6	17: 17,967,152-17,967,843	Let-7e, -99b, -125a	TSS	IG
-204	38727	0,7	19: 22,825,095-22,825,162			IT
-101b	38961	2,8	19: 29,209,769-29,209,865			IT
-362-3P	38773	2,5	X: 6,814,809-6,825,623	-188, -362, -500, -501, -532		IT
-18b	38720	1,7	X: 50,094,870-50,095,744	-18, -19b, -20b, 92a, -106a, -363	TSS	IG
-20b	38708	2,2			TSS	IG
-106a	38708	1,5			TSS	IG
-201	38725	2,2	X: 65,241,271-65,241,626	-201, -547		IG
-322	35904	1,7	X: 50,401,174-50,407,526	-322, -351, -450a, -450b, -503, -542	CpG,TSS	IG
-351	38995	2,7			CpG,TSS	IG
-503	38849	2,2			CpG,TSS	IG
-463	38807	2,9	X: 64,029,932-63,067,200	-463, -470, -471, -472, -473a, -473b, -880, -881, -883a, -883b, -871, -878, -889		IG
-470	38833	1,5				IG

-471	38834	3,3				IG
-472	38683	3,7				IG
-878-5P	38687	2,0				IG
-881*	40027	2,3				IG
-743b-3P	38681	2,1				IG
-883a-5P	40153	3,4				IG
-883b-3P	38698	1,8				IG
-465a-5P	38533	4,1	X: 64,079,130-64,092,300	-465a, -465b-1, -465b-2, -465c-1, -465c-2		IG
-465b-5P	38810	1,7				IG
-361	35910	1,5	X:110,188,433-110,188,502			IT
-805	39885	2,0	MT:16,115-16,209			IG

Supplemental Table 2

Differentially expressed miRNAs in testes of 18 days old Tdrd6$^{-/-}$ mice. The genomic loci and feature sets used to annotate miRNAs are available through the miRBase database (http://microrna.sanger.ac.uk). * indicates minor miR* sequences; IG: intragenic; IT: intergenic.

Supplemental Experimental Procedures

Generation and genotyping of Tdrd6 deficient mice

To generate the *Tdrd6*^{null} allele we disrupted the first of the five exons (Chromosome 17; ENSEMBLE gene ID ENSMUSG00000040140), which contains 95% of the Tdrd6 cDNA sequence. Homologous genome sequences amplified by PCR (Table S1) were cloned into the targeting vector pTV-Uni [1]: The final targeting vector consisted of: (i) the Diphtheria toxin A gene for negative selection; (ii) a 5' arm of homology encompassing 1.4 kb upstream of the start codon; (iii) a truncated human CD4 cDNA; (iv) a synthetic intron and bovine growth hormone polyadenylation sequence; (v) a loxP flanked puromycin resistance gene; and (vi) a "long arm" of homology of 5 kb downstream of downstream of exon 1 of the *Tdrd6* locus. The linerized targeting vector was electroporated into R1 W4/129S6 embryonic stem (ES) cells (Taconic Inc.). ES cell clones that survived puromycin selection were analyzed by PCR and the absence of random integration was confirmed by Southern Blotting. One positive clone was injected into a C57BL/6 blastocyst and the resulting chimeras were crossed with C56BL/6 mice and transmitted the mutant allele through the germline. Genomic DNA from the progeny was analyzed by PCR (primers see Table S1) and by Southern Blotting according to standard procedures [2]. Briefly, tail DNA was isolated, digested with *Bgl*II, separated by agarose gel electrophoresis and transferred to Hybond-N^{+} nylon (Amersham) membrane. The DNA was crosslinked to the membrane with UV light and hybridized with 1.4kb fragment of α-^{32}P-dCTP-labeled probe prepared from a region 200 bp upstream of the Tdrd6 start codon. Homozygous mice were obtained by crossing heterozygous males to either heterozygous females or homozygous females. The mouse colony is maintained in a mixed genetic background of 129 and C57BL/6.

Histological analysis, TdT-mediated dUTP nick end labeling (TUNEL) assay.

Testes were dissected, weighed, fixed in either Bouin's solution (Sigma) or 3.7% paraformaldehyde overnight at 4°C, and cut into 6 µm paraffin sections. Periodic acid-Schiff (PAS) was used for histological analysis on paraffin sections according to standard

protocols. Apoptosis was analyzed by TUNEL labeling using the TACS 2 TdT-DAB kit (Trevigon Inc.).

Northern analysis, RT-PCR and qPCR

Testes were collected at the indicated mouse age and RNA was isolated using an RNAeasy kit (Qiagen Inc., Valencia, CA) or for qPCR extracted with Trizol reagent (Invitrogen). RNA preparations were treated with DNAse (Promega) for 30 minutes at 37°C. RNA isolated from adult Smc1β-/- mice or from cultured Sertoli cells was used as control. Northern blots were performed by standard techniques [3]. Probes hybridizing to Smc1β and L32 mRNA were isolated as previously described [4]. A 400bp fragment of Tdrd6 produced by PCR from pcDNA3.1-3xHA-Tdrd6 (see TnT section) with primers Tdrd6-RT-fwd and Tdrd6-RT-rev (Table S1) was used as a template for generating randomly α[^{32}P]-dCTP-labeled probe against this sequence, using the Stratagene PrimeIt DNA labelling kit. Radiolabelled probes were purified using prepacked Sephadex G50 columns (NICK columns, Amersham). Northern hybridization signals were acquired on PhosphorImager (Molecular Dynamics, Sunnyvale, CA). RT-PCR reactions were performed with the One-Step Kit from Qiagen according to the manufacturer's recommendations. For primer sequences refer to Table S2.

Total cDNA was prepared from 1 μg of RNA using SuperScript II Reverse Transcriptase (Invitrogen). For real-time qPCR reactions poly-dT primers (Roche) were used. Quantitative PCR was carried out using QuantiTect SYBR Green PCR Kit from Qiagen. Actin and GAPDH were used for normalization. Primers for qPCR were used as described [5] and are listed in Table S1.

Southern Blot analyses of retrotransposon gene methylation

Five μg genomic DNA were digested over night at 37° C with either MspI or HpaII restriction enzymes (NEB Inc.). The digested DNA was subjected to 1% agarose gel electrophoresis, and was then was transferred to an Hybond-N+ charged nylon membrane (Amersham). The L1 probe corresponded to nucleotides 515–1,628 of the L1 sequence (GenBank accession no. M13002) [6]. The probe was random-prime labeled with ^{32}P-dCTP using Prime-It RmT Random Primer Labeling Kit (Stratagene).

Hybridizations were carried out overnight at 65°C.

Sucrose cushion centrifugation

Decapsulated testes from adult C57BL/6 mice were Dounce-homogenized in 10 mM Hepes, pH 7.6, 25 mM KCl , 0.15 mM spermine, 0.5 mM spermidine, 1 mM EDTA, 2 M sucrose and 10 % glycerol. The homogenate was diluted with homogenization buffer and the sample layered over a cushion of the same buffer of one third of the sample volume, as described in [7]. Samples were centrifuged for 30 min at 25,000 x g in an SW55 rotor (Beckman, Mountain View, CA). Fractions were collected manually starting from the top of the gradient. The pellet containing nuclei was resuspended in a 9:1 (v/v) mixture of homogenization buffer and glycerol, homogenized with a Teflon pestel in a Dounce homogenizer and centrifuged under the same conditions to obtain pure nuclei.
Five percent of each fraction was loaded for immunoblot.

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