Supporting Information

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SI Materials and Methods

In Situ Hybridization. Probes were amplified from testis cDNA by PCR and cloned into the pCRII-TOPO vector (Invitrogen) using the following primers: 3′ UTR of *Mov10l1*, forward primer—5′-CCAGGCTCTCCAAAAGTGAG-3′; reverse primer—5′-AAG-GTCAGTCCAAGGAAGCA-3′; C-terminal region of *Mov10l1*, forward primer—5′-GGCCTATGGGTTGAATGTGT-3′; and reverse primer—5′-GATGGGCTTCTCCCTTCAC-3′.

Histological and Immunohistological Examination. For histology, tissues were fixed in Bouin's solution or with 4% paraformaldehyde (PFA) in PBS, embedded in paraffin wax, cut into 5-μm thick sections, and stained with hematoxylin and eosin (H&E). For immunofluorescence staining, primary antibodies were used in 1:400 dilutions: anti-LINE ORF1p antibody (kindly provided by Sandra L. Martin, University of Colorado School of Medicine), anti-Mili antibody (Abcam), and anti-Miwi antibody (Abcam). Anti-SCP1 and anti-SCP3 antibodies (Novus Biologicals) were used in 1:200 dilutions. For detection of apoptotic cells, testis sections were deparaffinized and then treated with TUNEL reagent (in situ cell death detection kit, Roche). Nuclei were counterstained with DAPI.

RNA Analysis. Total RNA was extracted from tissues using TRIzol reagent according to the manufacturer's instructions (Invitrogen), For real-time PCR, 1 μg RNA was reverse-transcribed using SuperScript II reverse transcriptase (Invitrogen) and random hexamer primers and then used as a template for SybrGreen real-time PCR. Primers for full-length *Mov10l1* were 5′-GACTTTC-CAGCCGCTTCTT-3′ and 5′-TGCATGGATCTCCTCTAGCC-3′; for *Champ*, 5′-GTACCACGGACCAGCCAGA-3′ and 5′-GA-AGAACTCCTTATCTCGTGCTTT-3′; for *Csm*, 5′-TGCGGT-GCATATAACCCATT-3′ and 5′-ATGGTAGAACAGGCGTG-AGG-3′; and for the exon 20 splice variant (E20-SV), 5′-AAA-TATGATGGAATTAACCATGAGAC-3′ and 5′-AGAGCCG-AGTGGGACCTG-3′. For microarray analysis, RNA from testes of two to three mice was pooled for each time point. Microarray analysis was then performed by the University of Texas Southwestern Microarray Core Facility using Illumina's Mouse-6 V2 BeadChip. Expression data of *Mov10l1*, *Mov10*, *Mili*, and *Hspa2* (Fig. 1 *A* and *B*; Fig. S5*C*) was from the Gene Expression Omnibus (GEO), National Center for Biotechnology Information (NCBI) Reference Series GES640, GES829, and GES830. For Northern blotting, 8 μg total testis RNA was denatured for 5 min at 70 °C in a buffer containing 50% formamide and 10 mM EDTA. After electrophoresis on a 17% polyacrylamide gel, RNA was transferred onto a Hybond N membrane (Amersham) in 0.5× Tris/Borate/EDTA (TBE) buffer at 80 V for 1 h. Hybridization was then at 39 °C with ^{32}P-labeled Star-Fire oligonucleotide probes (Integrated DNA Technologies) directed against let-7a and a subset of piRNAs. For detection of total piRNAs, total RNA extracted from testes was dephosphorylated at the 5′ ends with antarctic phosphatase (New England Biolabs) and then end-labeled using T4 polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP. One microgram of radio-labeled RNA was separated on a 17% denaturating PAGE, and signals were detected with x-ray film.

Generation of Conditional Mov10l1 Knockout Mice. For generation of the *Mov10l1* targeting vector, 5′ arm, 3′ arm, and the exon 20 containing the knockout arm were PCR amplified from 129SvEv genomic DNA and cloned into the pGKNEO-F2L2DTA plasmid (Addgene plasmid 13445). The targeting vector was then linearized and electroporated into 129SvEv-derived ES cells. A total of 100 neomycin-resistant ES cell clones were isolated and analyzed for homologous recombination by Southern blotting. Three clones with a properly targeted *Mov10l1* allele were injected into 3.5-d-old C57BL/6 blastocystes, and high-percentage chimeric male mice were then crossed to C57BL/6 females. We achieved successful germline transmission with all three clones and kept mice from two clones as independent lines until identical phenotypes could be confirmed for both lines. Heterozygous *Mov10l1*$^{neo/+}$ mice were intercrossed with hACTB:FLPe transgenic mice to remove the neomycin resistance cassette, and global deletion of exon 20 was then achieved by breeding *Mov10l1*$^{+/fl}$ mice to CAG-*Cre* transgenic mice. Mouse genotypes were determined by PCR using tail DNA and primers specific to the *Mov10l1* locus. To test for fertility, six male *Mov10l1*$^{-/-}$ mice were bred with wild-type and *Mov10l1*$^{+/-}$ females, respectively, over a 5-mo mating period.

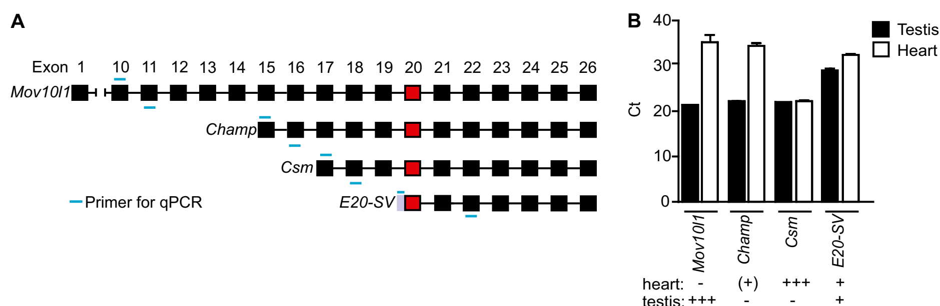

Fig. S1. Selective expression of full-length *Mov10l1* in spermatogonic cells. (*A*) Schematic depiction of the exon structure of mouse full-length *Mov10l1* and two known splice variants of the *Mov10l1* gene, *Champ* (cardiac helicase activated by MEF2 protein) and *Csm* (cardiac-specific isoform of *Mov10l1* gene). EST data suggested the existence of another splice variant starting at exon 20 (E20-SV). Exon 20 contains the putative helicase domain (red). (*B*) mRNA expression of the known and predicted isoforms of the *Mov10l1* gene was determined in heart and testis lysates by real-time PCR. The primer pair for full-length *Mov10l1* was isoform-specific, as were the primers for the potential splice variant starting at exon 20 (forward primer was located in the predicted 5′ UTR). Primers for *Champ* also detected full-length *Mov10l1* and primers for *Csm* also detected *Champ* and full-length *Mov10l1*. Primer locations are indicated in *A* with blue lines. Because testis expression with *Champ* or *Csm* primers did not change compared with using isoform-specific *Mov10l1* primers, all of the expression presumably came from the *Mov10l1* full-length transcript. In contrast, heart expression increased with *Csm* primers as compared with *Mov10l1* or *Champ* primers, suggesting that *Csm* is the predominantly expressed transcript in the heart. Results are presented as threshold cycles (Ct). +++, strong expression; +, weak expression; −, absent.

Fig. S2. Generation of *Mov10l1* knockout mice. (*A*) Design of the *Mov10l1* targeting vector and allele. *LoxP* sites were inserted into the introns flanking exon 20, which contains the putative helicase domain (red). The neomycin resistance cassette (NEO) was removed in the mouse germline by breeding heterozygously floxed mice to hACTB::FLPe transgenic mice, and global deletion of exon 20 was then achieved by breeding *Mov10l1*$^{+/fl}$ mice to CAG-*Cre* transgenic mice. (*B*) Detection of wild-type and floxed *Mov10l1* allele by Southern blot analysis with a 5′ probe upstream and a 3′ probe downstream of the floxed region. Genomic DNA from embryonic stem cells with a homologously integrated *Mov10l1* knockout construct was digested with XbaI. (*C*) Detection of *Mov10l1* cDNA from wild-type (WT) and *Mov10l1*$^{-/-}$ (KO) mice by PCR with primer pairs targeting exons (E) 17/18 and exons 20/22, respectively. (*D*) Radioactive in situ hybridization with a probe directed against the C terminus of *Mov10l1*. Testis section of 15-d and 7-wk-old mice, respectively, was exposed for 1 wk to the S^{35}-labeled probe. (*E*) No compensatory up-regulation of Moloney leukemia virus 10 (*Mov10*) in *Mov10l1*$^{-/-}$ mice. Expression of *Mov10* at postnatal days 10, 12, and 14. Total RNA from testes of two to three mice was pooled for each time point, and expression was then determined by microarray analysis. (*F*) Testis weight of *Mov10l1*$^{-/-}$ mice compared with *Mov10l1*$^{+/-}$ mice. $n \geq 3$ for each time point; $^{*}P < 0.05$ and $^{**}P < 0.01$ by ANOVA with Bonferroni posttest.

Fig. S3. *Mov10l1*$^{-/-}$ testis at different time points compared with control. (*A*–*H*) H&E-stained transverse sections of *Mov10l1*$^{-/-}$ testes versus *Mov10l1*$^{+/-}$ testes at different ages. Until P14, seminiferous tubules of *Mov10l1*$^{-/-}$ and *Mov10l1*$^{+/-}$ mice appeared similar. Starting at P15, *Mov10l1*$^{-/-}$ mice showed degenerating spermatocytes (*H*, arrowheads). (*I* and *J*) Feulgen staining of testis sections from 3-mo-old mice. (*K*–*N*) TUNEL staining of sections from testis of 5-wk-old *Mov10l1*$^{+/-}$ and *Mov10l1*$^{-/-}$ mice, respectively. (*K* and *L*) Red channel with the apoptotic nuclei. (*M* and *N*) Overlay with DAPI-stained nuclei (blue).

Fig. S4. Determination of mRNA expression of *Mili* and *Miwi*. mRNA expression of *Mili* and *Miwi* at postnatal days 10, 12, and 14 was determined by real-time PCR. Data were calculated relative to expression of *Mili*/*Miwi* in *Mov10l1*$^{+/-}$ mice at P10 by the 2-ΔΔCT method using *Gapdh* expression as the housekeeping gene. n = 3–4 mice per time point.

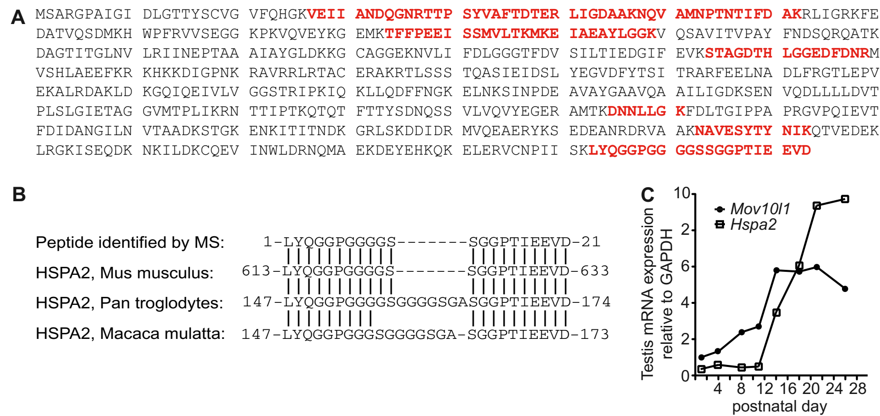

Fig. S5. Identification of HSPA2 as interaction partner of MOV10L1 by mass spectrometry (MS). (*A*) Sequence of murine HSPA2 (gene ID, 15512); the peptides that were identified by MS are in red. (*B*) To verify that HSPA2 was derived from mouse testis and not from COS cells, which originate from the African green monkey (*Chlorocebus sabaeus*), we performed a BLAST search with the longest identified (by MS) HSPA2-specific peptide (LYQGGPGGGGSSGGPTIEEVD); this peptide showed solely 100% identity with rat and mouse HSPA2 (*Mus musculus*, gene ID 15512) and merely partial identity with the HSPA2 from chimpanzee (*Pan troglodytis*, gene ID 450139) or rhesus monkey (*Macaca mulatta*, gene ID 574271). The genome of the African green monkey is not yet sequenced, but chimpanzee, rhesus monkey, and African green monkey have a common ancestor, which compared with mouse obviously had an insertion in the analyzed HSPA2 peptide. (*C*) Relative expression of *Hspa2* and *Mov10l1* mRNA in testes of C57BL/6 mice during postnatal development (NCBI GEO Series, GES640).

Dataset S1. Genes up-regulated in Mov10l1$^{-/-}$ mice

Dataset S1

Gene expression in *Mov10l1*$^{-/-}$ mice versus *Mov10l1*$^{+/-}$ mice was determined at P10, P12, and P14 by microarray analysis (Illumina's Mouse-6 V2 BeadChip) of pooled testis RNA from two to three mice for each time point. Depicted are the genes (target ID, transcript #, accession #, probe ID, gene name) that were >2 times up-regulated in *Mov10l1*$^{-/-}$ mice versus *Mov10l1*$^{+/-}$ mice at P14.

Dataset S2. Genes down-regulated in Mov10l1$^{-/-}$ mice

Dataset S2

Gene expression in *Mov10l1*$^{-/-}$ mice versus *Mov10l1*$^{+/-}$ mice was determined at P10, P12, and P14 by microarray analysis (Illumina's Mouse-6 V2 BeadChip) of pooled testis RNA from two to three mice for each time-point. Depicted are the genes (target ID, transcript #, accession #, probe ID, gene name) that were >50% down-regulated in *Mov10l1*$^{-/-}$ mice versus *Mov10l1*$^{+/-}$ mice at P14.