SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Mili is expressed in round spermatids. **A**, Immunostaining of a sectioned wildtype seminiferous tubule, indicating that Mili is expressed in spermatogonia (sg), spermatocytes (sc), and spermatids (st). **B**, Round spermatids were collected from the P1 (population 1) area based on cell size (forward light scattering, FSC) and cell density (side light scattering, SSC). **C**, A table showing that out of 10 million spermatogenic cells, 1.258 million round spermatids were collected. **D**, the isolated spermatids show their characteristic DAPI staining. **E**, Western blot showing that Mili is expressed in the isolated spermatids. 13 dpp wildtype and *mili* mutant testes were used as the positive and negative control, respectively. The bars in (A) and (D) are 25 μ m.

Figure S2. Tdrd1 mRNA is down-regulated in *mili* mutant during the spermatogenesis. **A**, Quantitative real-time RT-PCR showing that the levels of *tdrd1* mRNA mRNA is significant down-regulated in *mili* mutant at 7dpp and 13 dpp. **B**, Protein level of TDRD1 is also down-regulated in the *mili* mutant in both 7dpp and 13 dpp mouse testis. (n= 3). **C**, Quantitative analysis of the relative level of the Trdr1 protein as compared to GAPDH in *mili*^{+/+}, *mili*^{+/-}, and *mili*^{-/-} mice at 7 dpp and 13 dpp. Results show that the reduction of the Tdrd1 protein at these time points essentially mirrors that of Tdrd1 mRNA.

Figure S3. Mili expression and localization in not affected in Trdr1 mutants. **A**, In 18 dpp *tdrd1* wildtype testis, Mili is expressed in cytoplasm of spermatogonia and spermatocytes, and round spermatids, where it is enriched in the chromatoid body. B, Mili shows similar expression and subcellular localization pattern in 18 dpp *tdrd1* mutant testes. The bars are 25μ m.



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