

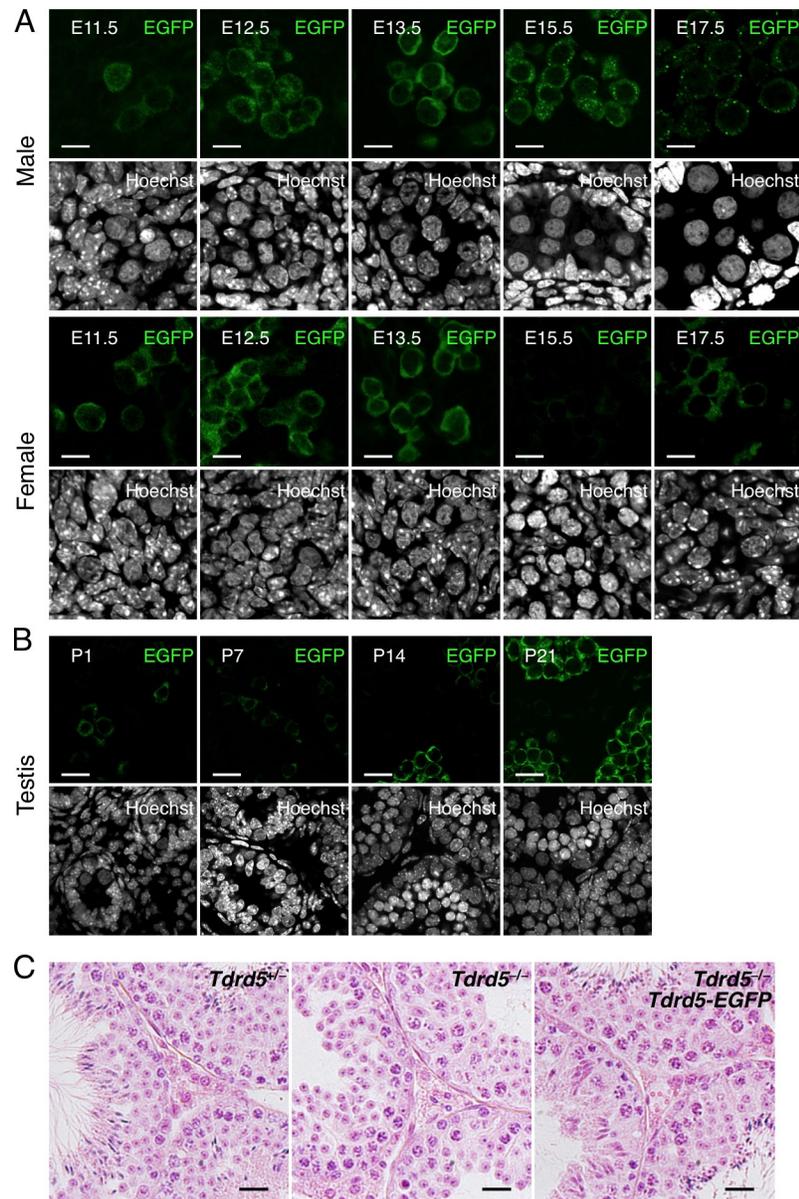
Yabuta et al., <http://www.jcb.org/cgi/content/full/jcb.201009043/DC1>

Figure S1. **TDRD5 expression in the embryonic gonads and postnatal testis, and the rescue of *Tdrd5*^{-/-} phenotype by the TDRD5-EGFP transgene.** (A) TDRD5-EGFP expression in male (top) and female (bottom) embryonic gonads at E11.5, E12.5, E13.5, E15.5, and E17.5. (B) TDRD5-EGFP expression in postnatal testes. TDRD5-EGFP is detectable at the periphery of the nucleus of type A spermatogonia at all ages. However, the signal of this stage is hardly detectable in the adult testis because the far stronger expression of TDRD5-EGFP in pachytene spermatocytes obscures the relatively weak signals of spermatogonia. (C) Histological sections of *Tdrd5*^{-/-} (left), *Tdrd5*^{-/-} (middle), and *Tdrd5*^{-/-}; *Tdrd5-EGFP*^{+/-} (right) mouse testes stained with hematoxylin and eosin. Note that the elongated spermatids and spermatozoa are properly formed in the *Tdrd5*^{-/-}; *Tdrd5-EGFP*^{+/-} mouse testis. Bars: (A) 10 μ m; (B and C) 20 μ m.

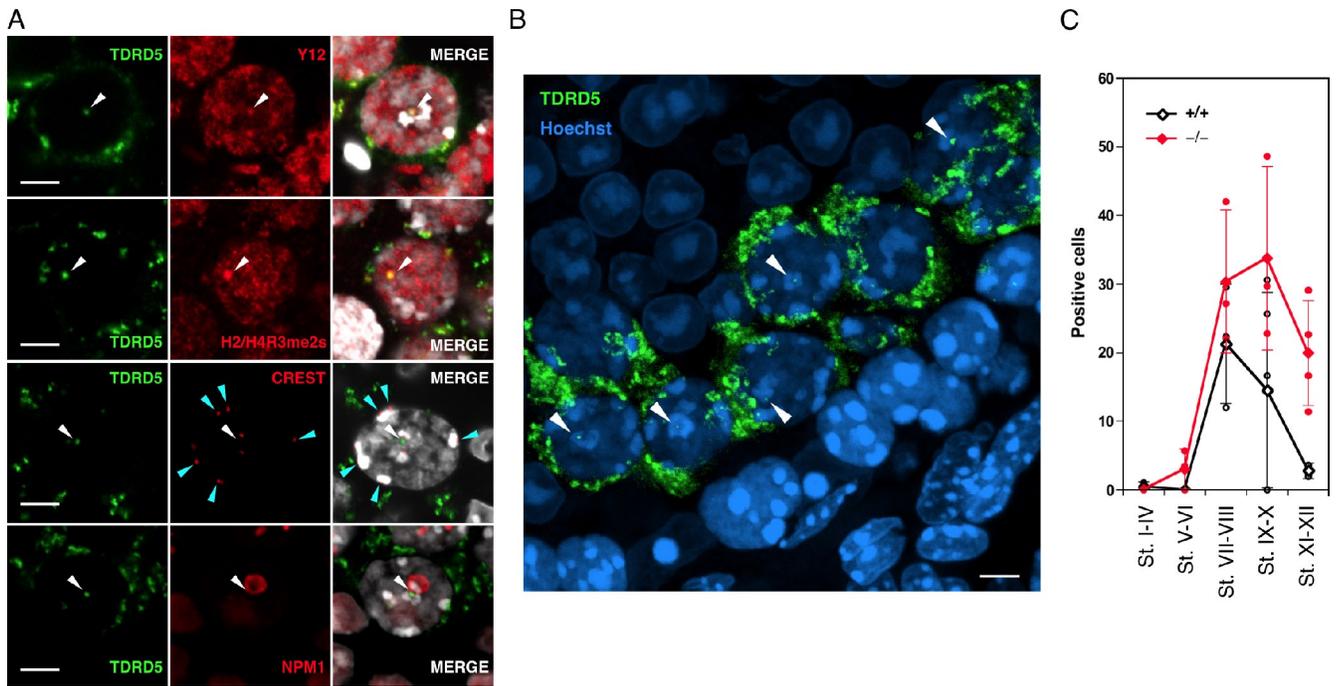
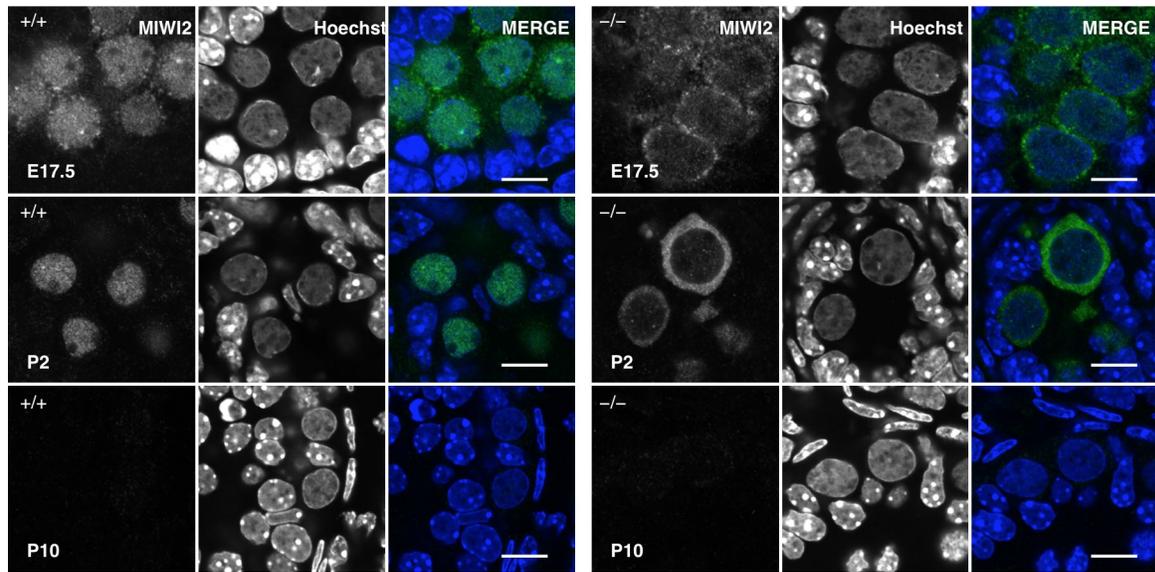


Figure S2. **T/PNBs in mid-to-late pachytene spermatocytes.** (A) Immunofluorescence analysis of T/PNBs (green, left column) co-stained with sDMA (Y12, red, middle, top), sDMA at Arg3 of histone H2/H4 (H2/H4R3me2s, red, middle, second row), centromere (CREST, red, middle, third row), and nucleolus (B23/nucleoplasmin 1 [NPM1], red, middle, bottom), respectively. White arrowheads indicate T/PNBs, whereas blue arrowheads show CREST-positive centromeres flanked by Hoechst-dense pericentromeric heterochromatin around the nuclear envelope. Bars, 5 μ m. (B) Three-dimensional projection of confocal sections of an adult seminiferous tubule in which T/PNBs (arrowheads) are observed at high frequency. Green, TDRD5-EGFP; blue, Hoechst. Bar, 10 μ m. (C) Occurrence (frequency, %) of T/PNB-positive pachytene spermatocytes at different seminiferous epithelium stages. Open black (wild type) and closed red (*Tdrd5*^{-/-}) circles represent the ratio of T/PNB-positive pachytene spermatocytes in >50 pachytene spermatocytes at the indicated stages in four independent testes. The mean values of each stage in all the testes examined are plotted as open black (wild type) and closed red (*Tdrd5*^{-/-}) squares with SD (error bars).

A



B

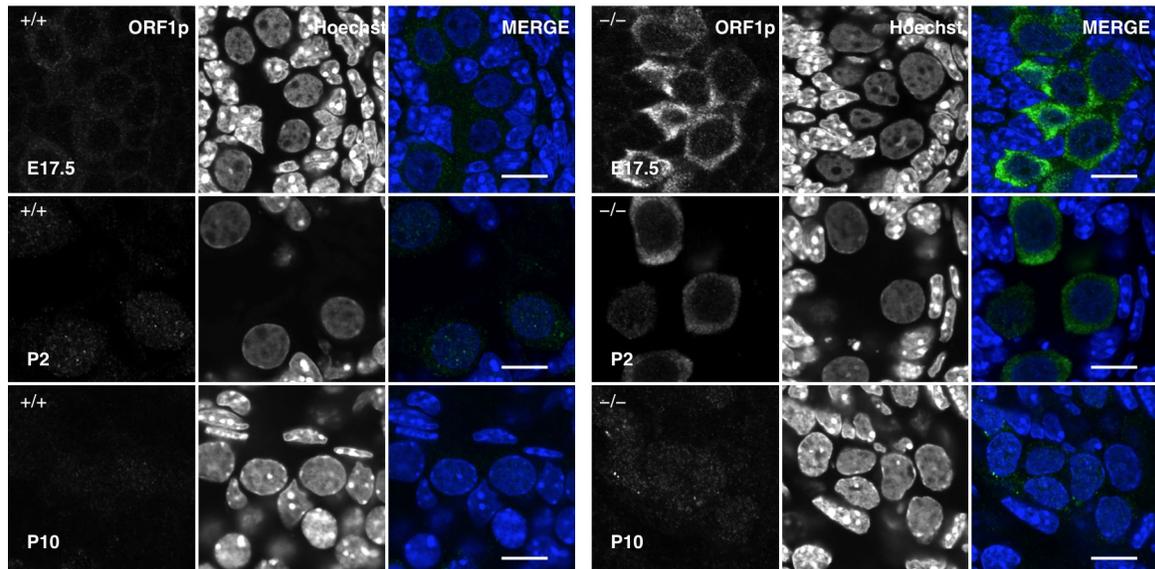


Figure S3. **MIWI2 and ORF1p expression in perinatal testes of *Tdrd5*^{+/+} and *Tdrd5*^{-/-} mice.** Immunofluorescence analysis of MIWI2 (A, left) and ORF1p (B, left) in testes at E17.5 (top), P2 (middle), and P10 (bottom) in *Tdrd5*^{+/+} (left) and *Tdrd5*^{-/-} (right) mice counterstained with Hoechst (middle). Merged images are shown on the right. Bars, 10 μ m.

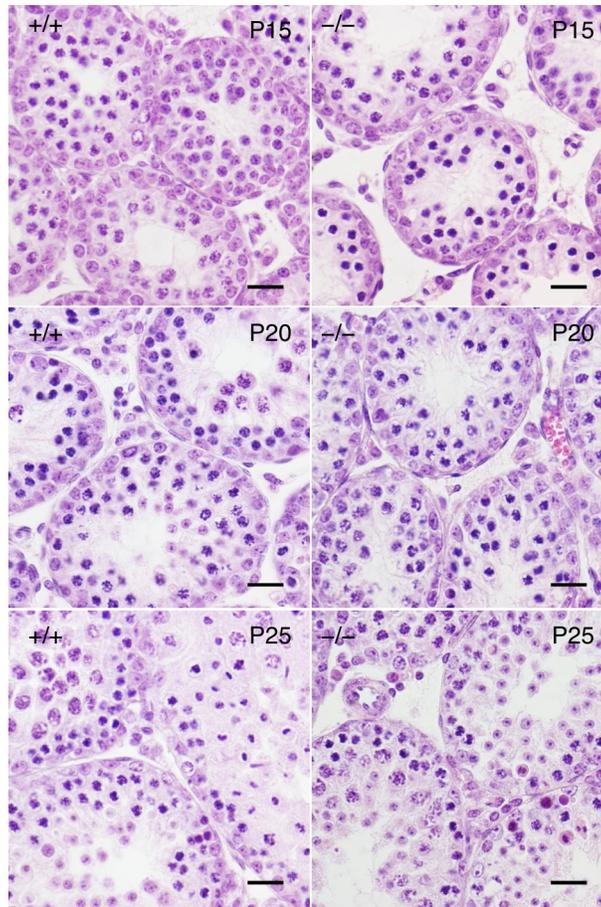


Figure S4. **First-round spermatogenesis of *Tdrd5*^{+/+} and *Tdrd5*^{-/-} animals.** Histological sections of P15 (top), P20 (middle), and P25 (bottom) *Tdrd5*^{+/+} (left) and *Tdrd5*^{-/-} (right) testes stained with hematoxylin and eosin. Bars, 20 μ m.



Figure S5. **Normal offspring from round spermatids of a *Tdrd5*^{-/-} mutant animal.** Neonatal offspring derived from round spermatids of a *Tdrd5*^{-/-} mutant.

Table S1. Primers used for RT-PCR, Q-PCR, and probe amplifications

Primer/probe	Forward	Reverse	References
Arm constructions			
<i>Tdrd5</i> -SA	5'-ACTAACGCGTCGACGACATGGTCTTTGCCTGGGTG-AAC-3'	5'-GATCTAGCTAGCGCTGTATCAAGAGCAAGAAAGTCTTGGC-3'	
<i>Tdrd5</i> -LA	5'-ATAAGAAATGCGGCCGCTGGTCTGAGTTCTCAGGACACACAGG-3'	5'-TAACGCGTCGACAAAGGCTCTTAGCCGACATCCAC-3'	
PCR genotyping			
<i>Gfp</i>	5'-GACGTAAACGGCCACAAGTT-3'	5'-AAGTCGTGCTGCTTCATGTG-3'	
<i>Tdrd5-scF</i>	5'-GGTGCTGCCGCTATACTGTAGCTGTTTC-3'		
<i>Neo-gt-1</i>	5'-CTGACCGCTTCTCTGCTTACG-3'		
<i>Tdrd5</i> -genotype1	5'-GGCCAAGACTTTCTTGCTCTTG-3'		
<i>Tdrd5</i> -genotype2	5'-TAAATGGGAGATGGCAAGAGAC-3'		
<i>Neo</i> -genotype	5'-CAAATTAAGGGCCAGCTCAITC-3'		
Southern, Northern probes			
<i>Tdrd5</i> -genotype	5'-TTTACAAAGCTATCAATCAAGCCAGAATAG-3'	5'-TACTTGGTTTTAGTCTACTGAAAAAATC-3'	
<i>Tdrd5</i> -probeA	5'-GGCGCTCTAGTGACATTCTCCGAG-3'	5'-TGGCATAATGTACCGGTCAGAG-3'	
<i>Tdrd5</i> -probeB	5'-GAGAAATGGCACAAAAGGACTG-3'	5'-TTCCGAGGGATGAGAGCTTCTG-3'	
<i>Gapdh</i>	5'-TGACATCAAGAAGGTGGTGAAG-3'	5'-CTCCTGTTATTATGGGGTCTG-3'	
<i>Actb</i> (β-actin)	5'-CAAACATCCCCAAAGTTCTAC-3'	5'-AGGGAGACCAAAGCCTTCATAC-3'	
<i>LINE1</i>	5'-TCCCAACATAGAGTCTGAG-3'	5'-AGTGGGCAGAGTATTCTGTC-3'	Bourc'his and Bestor, 2004
<i>IAP</i>	5'-TTCCGAGAACGCGAGGCACCA-3'	5'-CCCAGGACGCTCGATGGACAAG-3'	Vasicek et al., 1997
<i>SINE-B1</i>	5'-ACCCTGGCTGTCTGGAACCTCACTCTGTA-3'	5'-ATGTTTTTCATTGTAAGTCACTGATATAC-3'	Shoji et al., 2009
Minor satellite	5'-GGAAATGATAAAAACACACTGCAG-3'		Chen et al., 2003
Major satellite	5'-TATGGCGAGGAAAAGTGAAGGTTGAAAATTGAATGTCCACTGTAGGACGTGGAATATGGCAAG-3'		Chen et al., 2003
RT-PCR			
<i>Tdrd5</i>	5'-GAGAAATGGCACAAAAGGACTG-3'	5'-TGGTGTGTGAATCCTCCTTC-3'	
<i>Tdrd1</i>	5'-GCTGAGAATCTGGTGATGTGTG-3'	5'-GGAAGAGGAGAATCTGTTCTG-3'	
<i>Miwi</i>	5'-GGGAGAGGTTACAACCCAAGAC-3'	5'-ACTTCCACATCGATGACTGTCC-3'	
<i>Miwi2</i>	5'-GACCTCAAACCTGTGCCATCTC-3'	5'-GGCTAATCCAGACTTGGTCC-3'	
<i>Mili</i>	5'-CTGATCACATGCAGAGGTTGAC-3'	5'-AGCTAGCTTGTGGCATACTG-3'	
<i>Gapdh</i>	5'-ATGAATACGGCTACAGCAACAGG-3'	5'-CTCTTGCTCAGTGTCTTGCTG-3'	
Q-PCR			
<i>LINE1</i> -5'UTR	5'-GGCGAAAGGCCAAACGTAAGA-3'	5'-GGAGTGCTGCGTTCTGATGA-3'	Carmell et al., 2007
<i>LINE1</i> -ORF2	5'-GGAGGGACATTTCAITTCATCA-3'	5'-GCTGCTCTGTATTGGAGCATAGA-3'	Carmell et al., 2007
<i>IAP</i> -3'LTR	5'-GCACATGCGCAGATTATTGTT-3'	5'-CCACATTCGCCGTTACAAGAT-3'	Carmell et al., 2007
<i>IAP</i> -Gag	5'-AACCAATGCTAATTTACCTTGGT-3'	5'-GCCAATCAGCAGGCGTTAGT-3'	Carmell et al., 2007
<i>SINE-B1</i>	5'-TGAGTTCGAGGCCAGCCTGGTCTA-3'	5'-ACAGGGTTTCTCTGTGTAGCCCTG-3'	
<i>Acr</i>	5'-CTCCAGTACAGTGGACCAAG-3'	5'-TTTTCTCAGGAATGGAGGAAG-3'	
<i>Calspermin</i>	5'-TCCGAAGACAGGAATCTGTGAG-3'	5'-TGTTGGAGGGCTTGAATGTAG-3'	
<i>Creb3l4/Tisp40</i>	5'-ACACATCTGAAGATGCGAGTGAAG-3'	5'-TAAAAGGTGATAGGGAAGCCTGTG-3'	
<i>Crem</i>	5'-TCCATTGTGAATCTTGCAACC-3'	5'-AAGGCCAGCCTAAACCTACGTG-3'	
<i>Ddx4/Mvh</i>	5'-TGAAACAGTAGAGACTGAAGCCTTC-3'	5'-CCACCAAGTACAAGCTCACATTG-3'	
<i>Fhl5/Act</i>	5'-GAAATCTATGCCGCAAGTGTG-3'	5'-TTTAGTGAGAGCAAGGGCAAAG-3'	
<i>Hspa2/Hsp70-2</i>	5'-GAGCGATTGCTGATTAAGCTC-3'	5'-TAACCAAGCTCCACAGGTCAG-3'	
<i>Kif17</i>	5'-TGTCACACAGCACTGTCTCTGG-3'	5'-TGGGACAAGAAGTACCTCTCC-3'	
<i>Miwi</i>	5'-GGTGGTGGAGTGAGTGTGTTG-3'	5'-GGAATCCTATCCAGAGTGGTC-3'	
<i>Odf1/RT7</i>	5'-GTGGAAGCCGATTCTCCTGTAG-3'	5'-GACGAGAAATGCTGAAGAGCTG-3'	
<i>Arbp</i>	5'-CAAAGCTGAAGCAAAGGAAGAG-3'	5'-AATTAAGCAGGCTGACTTGGTTG-3'	
<i>Ppia</i>	5'-TTACCCATCAAACCTCCTCTG-3'	5'-AACCCAAAGAAGTTCAGTGAGAGC-3'	
<i>Prm1</i>	5'-AAGATGTCGACGACGGAGGAG-3'	5'-CGCAGGAGTTTTGATGGACTTG-3'	
<i>Prm2</i>	5'-GTAGGAGGCCACCATCACTAAGC-3'	5'-AGACATCGACATGGAATGGTG-3'	
<i>Sycp1</i>	5'-GGACAACGATTGCTAAAATTGATAGG-3'	5'-TTGGTAAAGTTGGCTCTCTTG-3'	
<i>Sycp3</i>	5'-TGAGTCTTTGAAGAAAGAACTTGAACC-3'	5'-GGTTAACAGGAACAAAATCTCCAC-3'	
<i>Tbpl1/Trf2</i>	5'-AGTAATCTCCATGTCCCACAGG-3'	5'-TTGGTCCCATATTCTCAGCATA-3'	
<i>Tnp1</i>	5'-AGGAGCATGAGGACATCAGAGG-3'	5'-ATTCCGAATTCGTACAGACTG-3'	
<i>Tnp2</i>	5'-GCTCAGGGCGAAGATACAAGTG-3'	5'-TGTGACATCATCCCAACAGTCC-3'	

References

- Bourc'his, D., and T.H. Bestor. 2004. Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. *Nature*. 431:96–99. doi:10.1038/nature02886
- Carmell, M.A., A. Girard, H.J. van de Kant, D. Bourc'his, T.H. Bestor, D.G. de Rooij, and G.J. Hannon. 2007. MIWI2 is essential for spermatogenesis and repression of transposons in the mouse male germline. *Dev. Cell*. 12:503–514. doi:10.1016/j.devcel.2007.03.001
- Chen, T., Y. Ueda, J.E. Dodge, Z. Wang, and E. Li. 2003. Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by Dnmt3a and Dnmt3b. *Mol. Cell. Biol.* 23:5594–5605. doi:10.1128/MCB.23.16.5594-5605.2003
- Shoji, M., T. Tanaka, M. Hosokawa, M. Reuter, A. Stark, Y. Kato, G. Kondoh, K. Okawa, T. Chujo, T. Suzuki, et al. 2009. The TDRD9-MIWI2 complex is essential for piRNA-mediated retrotransposon silencing in the mouse male germline. *Dev. Cell*. 17:775–787. doi:10.1016/j.devcel.2009.10.012
- Vasicek, T.J., L. Zeng, X.J. Guan, T. Zhang, F. Costantini, and S.M. Tilghman. 1997. Two dominant mutations in the mouse fused gene are the result of transposon insertions. *Genetics*. 147:777–786.