

Figure S1 Validations of germ cell population purity. (A) Percentage of targeted cell types based on Giemsa staining for spermatogonia/early prophase I (LZ), pachytene/diplotene (PD), secondary spermatocyte (SS), and round spermatid (RS) populations from reciprocal F₁ males (white bars, *domesticus*^{LEWES X} X chromosome; black bars, *musculus*^{PWK X} X chromosome). Data are presented as genotype means + 1 SE. (B) Autosomal genes that are expressed in different germ cell types are enriched in the correct germ cell populations from reciprocal F₁ males. The cell population in which each gene should be most highly expressed is indicated with a red box. All genes were enriched in the expected cell populations with the exception of *Acrv1*, which was equally enriched in the SS and RS populations. Expression was normalized relative to *Ubc* (ΔC_T) and adjusted to produce positive values by setting the lowest expression level to zero. Data are presented as genotype means + 1 SE.

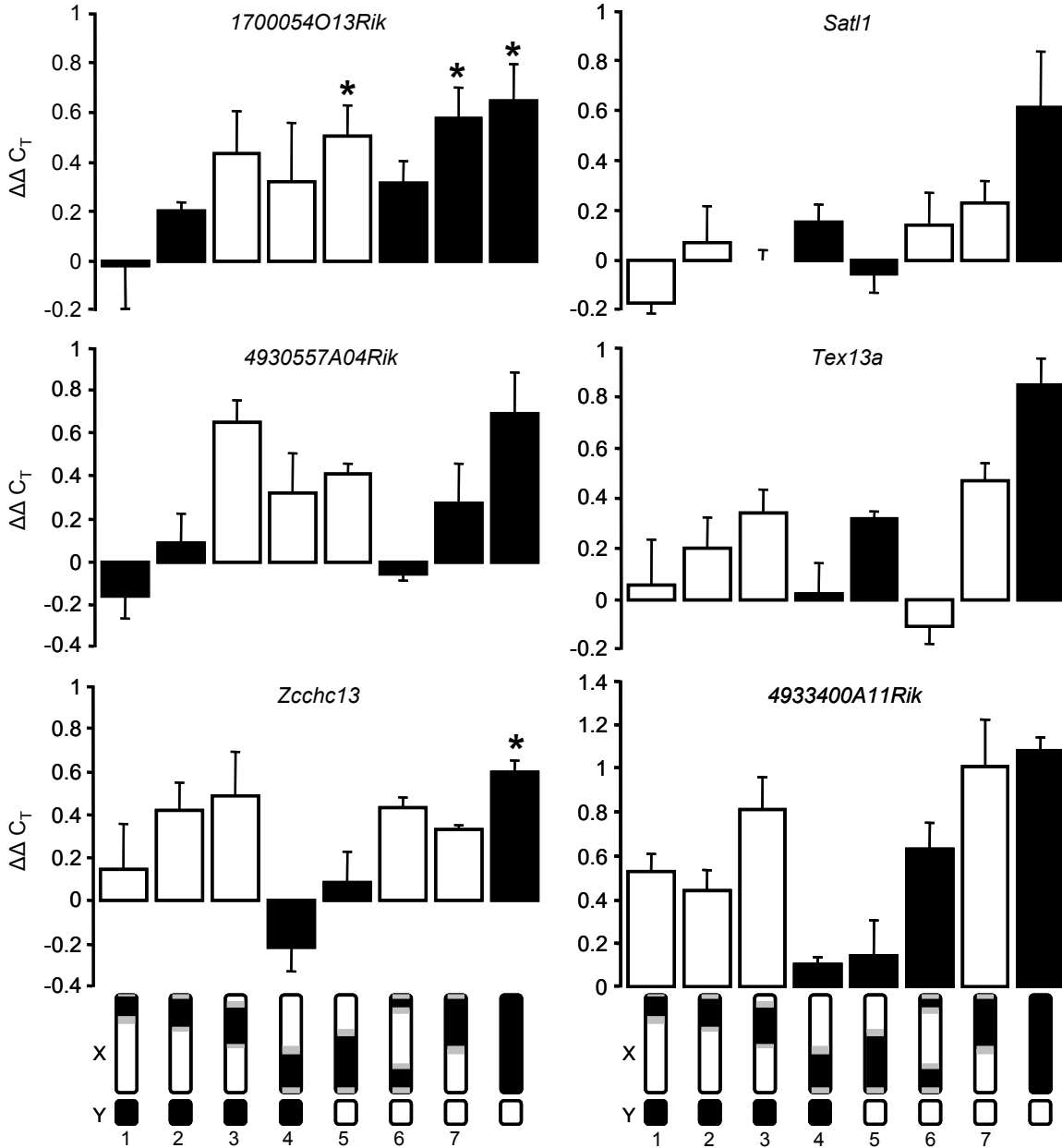


Figure S2 Relative expression of six X-linked genes in the testes of F_1 males. $\Delta\Delta C_T$ is the normalized difference in expression between experimental genotypes (shown on the X axis) and the fertile F_1 . X introgression genotypes are numbered 1-7; black bars denote the *musculus*^{PWK} allele at a given locus, white bars denote the *domesticus*^{LEWES} allele. Error bars are ± 1 SE. Tests for significant differences between experimental and control genotypes were performed on ΔC_T values with ANOVA followed by Dunnett *post hoc* tests, * $P \leq 0.05$.

Table S1 Primers used in this study

Gene	Chr.	Primer name	Primer sequence	Amplicon size (bp)	Reference
<i>4930557A04Rik</i>	X	A04Rik F	KGCTGATGAGACTCCAGGAT	163	<i>a</i>
		A04Rik R	TCCACCAACAAGGGAGTCAT		
<i>Efhc2</i>	X	Efhc2 F	CATCCTGGGTTGCTTTTGAT	136	<i>a</i>
		Efhc2 R	TGAATGGTGTCTCTCAAGG		
<i>Actrt1</i>	X	Actrt1 F	CTCAAAAATGGTCTGCAACAGC	164	<i>b</i>
		Actrt1 R	TCTTGATAGGGTTCCCTCAAA		
<i>1700013H16Rik</i>	X	H16Rik F	GCAATGGCACAGCTTAATGA	68	<i>a</i>
		H16Rik R	TCCTCATTCCATTGCTCAAA		
<i>Fmr1</i>	X	Fmr1 F	GGAAAAGCCAGACAGCGTAG	165	<i>a</i>
		Fmr1 R	CCTGTGCCATCTGCCTACT		
<i>4930468A15Rik</i>	X	A15Rik F	CACTGGCAATGGCAACTCTA	162	<i>a</i>
		A15Rik R	GCCTGCGATAACTCCTGGTA		
<i>Dmrtc1a</i>	X	Dmrtc1a F	GAGACGCCTGGTTGAGAGAC	104	<i>a</i>
		Dmrtc1a R	TCTTCCAGTGCGAACTCCT		
<i>Zcchc13</i>	X	Zcchc13 F	GTGAACTGCAGCAAGACGAG	100	<i>a</i>
		Zcchc13 R	GGGGTTACTAAGCGGTAGCC		
<i>Rps6ka6</i>	X	Rps6ka6 F	AAGAACGCAGCAACGTTAT	94	<i>a</i>
		Rps6ka6 R	AAACTGGCTCTCCCTCTCC		
<i>Sat11</i>	X	Sat11 F	AACCATTGGATTTGCCATGT	153	<i>a</i>
		Sat11 R	TCGGTGTGATGGCTATCTG		
<i>1700008I05Rik</i>	X	I05Rik F	AAAGCCAATTCGTGGAGACAAAT	192	<i>b</i>
		I05Rik R	TGGGAGAGATGCAGAATATCCA		
<i>Tex13a</i> (<i>1700025D03Rik</i>)	X	Tex13a F	TGTCACCAGACCTGAAGCAG	169	<i>a</i>
		Tex13a R	CTTACTGCATTCCGGGAGAGC		
<i>Ammecr1</i>	X	Ammecr1 F	GCTTTTCTGCTCGGTGTCTC	143	<i>a</i>
		Ammecr1 R	CAACCTCCGGTAGGTAGGTG		
<i>4933400A11Rik</i>	X	A11Rik F	AAATGGGTATCAGGCAGCAC	170	<i>a</i>
		A11Rik R	CAGTGGGCCATTAAGCATGT		
<i>Acrv1</i>	9	Acrv1 F	TGAGTACACCACTTCCAAGCA	60	<i>c</i>
		Acrv1 R	AAGCACATGTGTGGCAATTT		
<i>Protamine 1 (Prm1)</i>	16	Prot1 F	ACAAAATTCACCTGCTCACA	129	<i>d</i>
		Prot1 R	CATCTGCTCCTGCTTTTGCT		

Gene	Chr.	Primer name	Primer sequence	Amplicon size (bp)	Reference
<i>Hormad1</i>	3	Hormad1 F	CTGCTGACACCAAGAAAGCA	148	<i>a</i>
		Hormad1 R	CCTGGTGGTTGTAATCTGG		
<i>Hist1h1t (H1t)</i>	13	Hist1h1t F	GTTGGCTCTCCTTTAGTTTTCACTT	128	<i>a</i>
		Hist1h1t R	GCCTTTTAGATGAAGGTTTCTCCT		
<i>Igf1r</i>	7	Igf1r F	CGGTGACTTCTGCTCAAATG	150	<i>a</i>
		Igf1r R	AATGGCGGATCTTCACGTAG		
<i>Ubc</i>	5	Ubc F	AGCCCAGTGTTACCACCAAG	118	<i>a</i>
		Ubc R	CTAAGACACCTCCCCATCA		
<i>beta-actin (actb1)</i>	5	B-actin F	CTGTATCCCCTCCATCGTG	167	<i>a</i>
		B-actin R	CTTCTCCATGTCGTCACAGT		

a, this study; *b*, Coquet *et al.* 2009; *c*, Åkerfelt *et al.* 2008; *d*, Okada *et al.* 2007

Supporting Literature Cited

- ÅKERFELT, M., E. HENRIKSSON, A. LAIHO, A. VIHERRAARA, K. RAUTOMA *et al.*, 2008 Promoter ChIP-chip analysis in mouse testis reveals Y chromosome occupancy by HSF2. *Proc. Natl. Acad. Sci. USA* 105: 11224-11229.
- COCQUET, J., P. J. I. ELLIS, Y. YAMAUCHI, S. K. MAHADEVIAH, N. A. AFFARA *et al.*, 2009 The multicopy gene *Sly* repressed the sex chromosomes in the male mouse germline after meiosis. *PLoS Biology* 7: e1000244.
- OKADA, Y., G. SCOTT, M. K. RAY, Y. MISHINA, and Y. ZHANG, 2007 Histone demethylase JHDM2a is critical for *Tnp1* and *Prm1* transcription and spermatogenesis. *Nature* 450: 119-123.