Supplemental Material for

Histone methyltransferase PRDM9 is not essential for meiosis in male mice

Ondrej Mihola, Florencia Pratto, Kevin Brick, Eliska Linhartova, Tatyana Kobets, Petr Flachs, Chris L. Baker, Radislav Sedlacek, Kenneth Paigen, Petko M. Petkov, R. Daniel Camerini-Otero, and Zdenek Trachtulec

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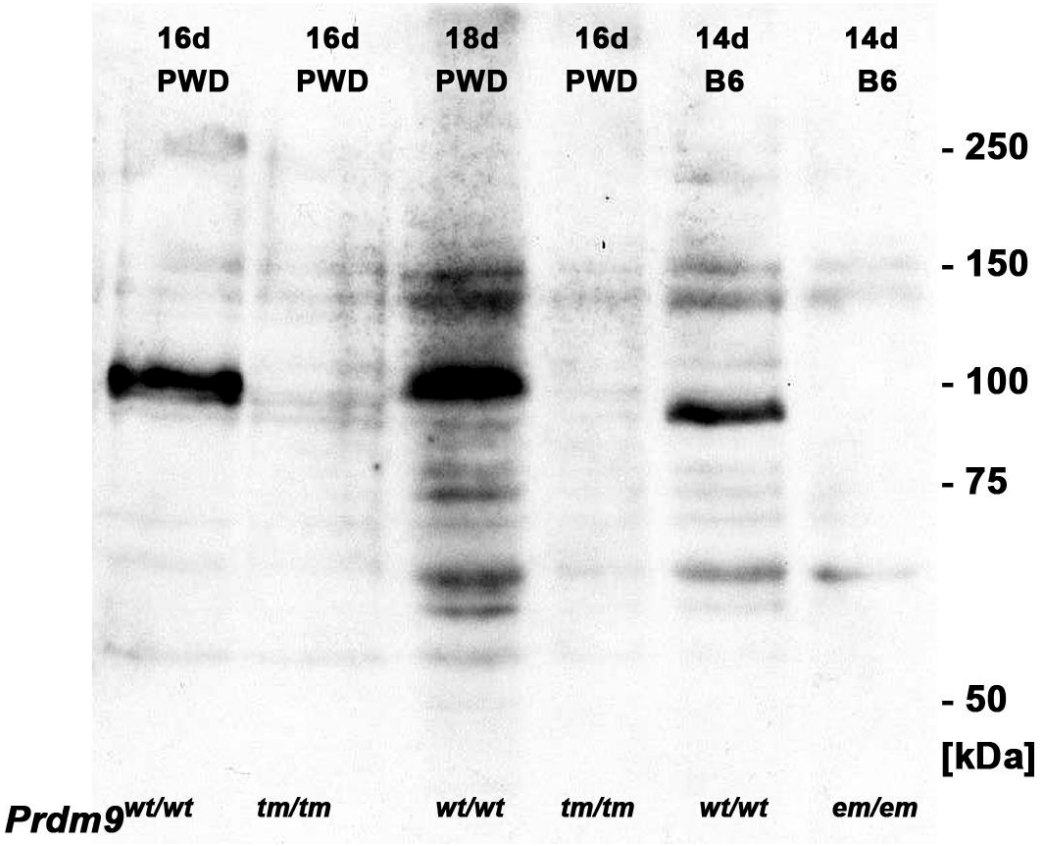
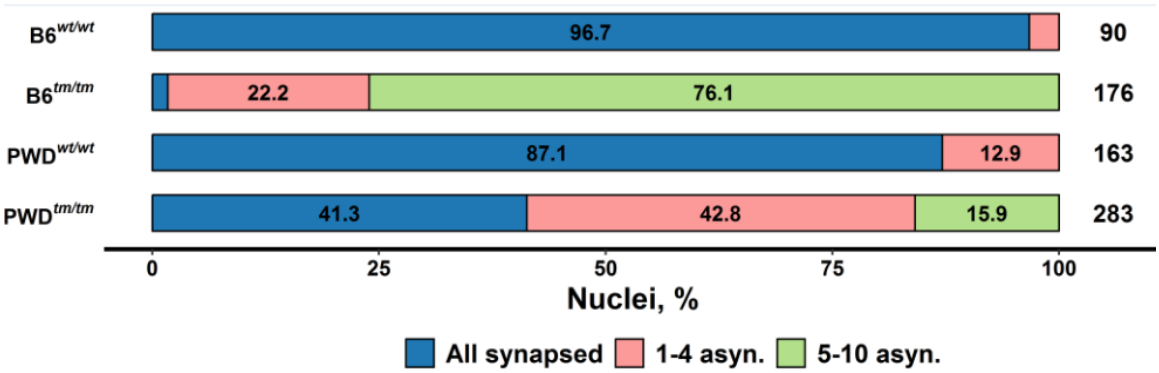
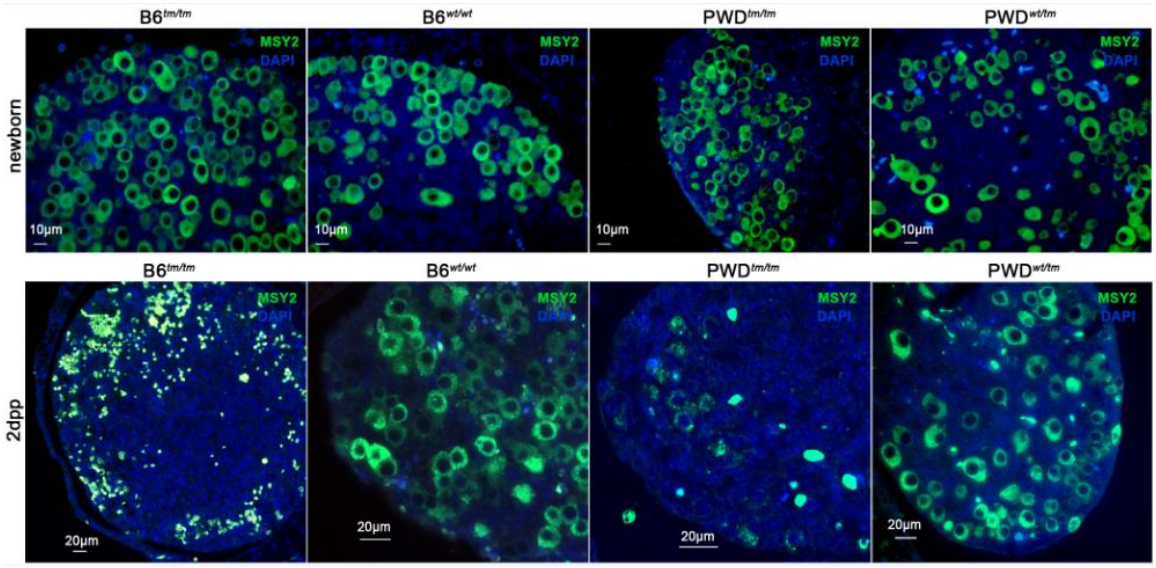


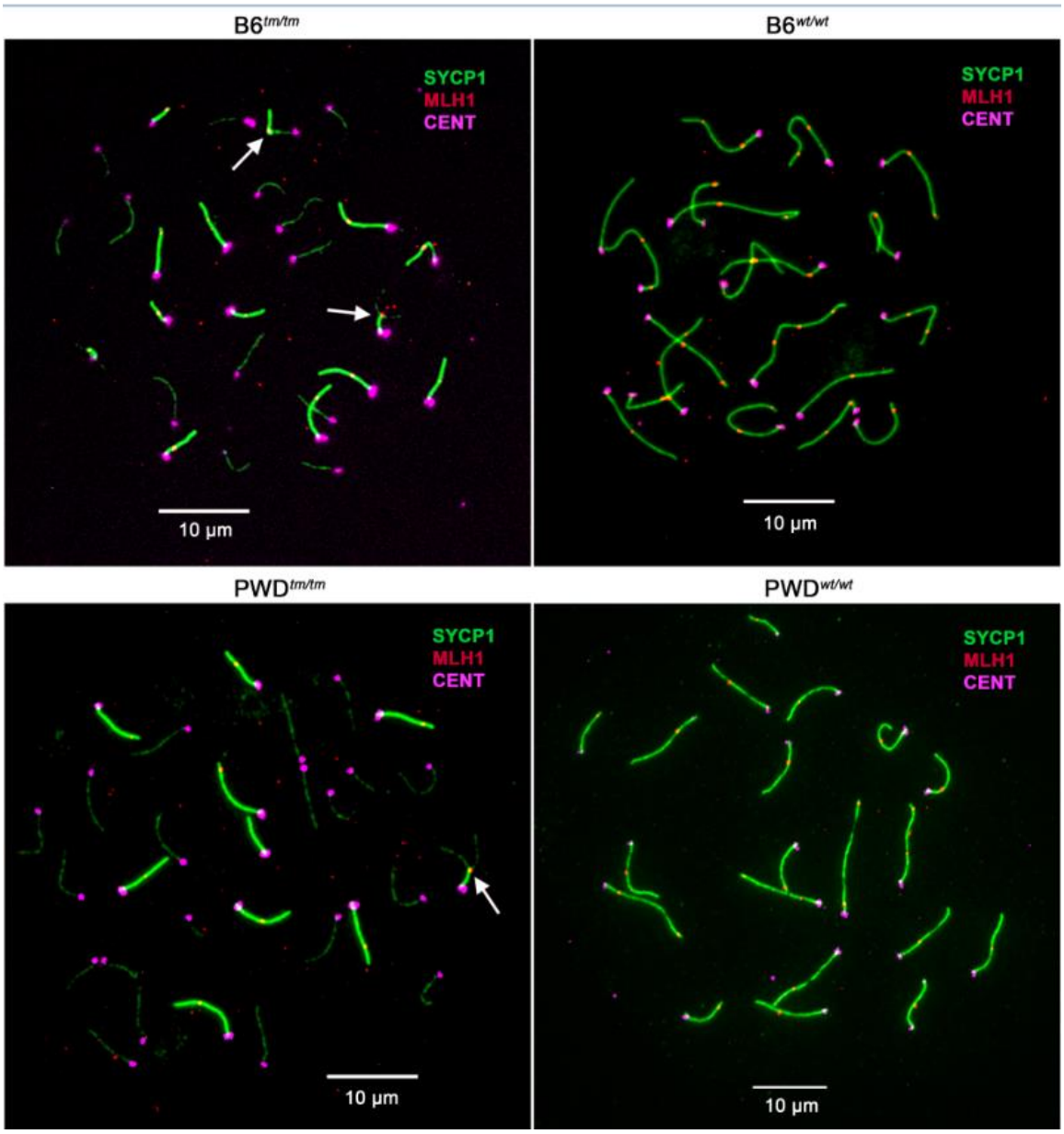
Supplemental Figure S1. No PRDM9 protein is detected in PWD$^{tm/tm}$ or B6$^{em/em}$ male mice. Western blotting with anti-PRDM9 antibody. Testicular proteins were isolated from males of the indicated age (d, days post partum), background, and *Prdm9* genotype. Both lane 2 and 4 contain proteins from 16d PWD$^{tm/tm}$ (two different males). Note that the membrane is overexposed to show differences in loading. The PWD allele of PRDM9 contains one more zinc-finger than the B6 allele (Mihola et al. 2009) and thus has a higher molecular weight.



Supplemental Figure S2. Extensive full synapsis of pachytene chromosomes in male PWD mice lacking functional PRDM9. Only spermatocytes with more than nine synapsed chromosomes were counted (using immunolabeling for SYCP3, SYCP1, and yH2AFX). Three classes were discerned ("All 19 synapsed autosomes" represents "normal" pachytene cell). The numbers of the analyzed nuclei are given on the right.



Supplemental Figure S3. Oocyte attrition increases due to loss of PRDM9 (see also Fig. 3C,D). Ovarian sections were immunostained for oocyte cytoplasm (MSY2=YBX2) and counterstained for nuclei (DAPI). The number of oocytes was similar in all tested newborn females, but dropped two days after birth (2dpp) in both types of *Prdm9*-deficient mice (B6$^{tm/tm}$ and PWD$^{tm/tm}$) compared to littermate controls (B6$^{wt/wt}$ and PWD$^{wt/tm}$). Note that most labeled cells in B6$^{tm/tm}$ ovaries are not MSY2-positive but infiltrating erythrocytes autofluorescent due to hemoglobin, as deduced from their size and biconcave shape.



Supplemental Figure S4. Crossover nodules are present in the oocytes of *Prdm9*-deficient B6$^{tm/tm}$ and PWD$^{tm/tm}$ newborn females. Chromosome spreads of pachytene nuclei from newborn females (immunostained for SYCP1, synaptonemal complex protein 1; MLH1, mutL homolog 1; CENT, centromere). Bright SYCP1 signal indicates synapsis. In PWD$^{tm/tm}$ and B6$^{tm/tm}$ mice, crossovers (MLH1 foci) occur on synapsed parts of partially asynapsed chromosomal pairs (arrows) as well as on fully synapsed chromosomes (orange foci).

Breeding schema for:
((B6$^{wt/em}$ × PWD$^{wt/wt}$) × PWD$^{wt/wt}$)N3F1$^{em/em}$

Step 1:

Intercross		♀B6$^{wt/em}$	
		wt	**em**
♂PWD$^{wt/wt}$	**wt**	(♀B6 × PWD)$^{wt/wt}$	(♀B6 × PWD)$^{wt/em}$
	wt	(♀B6 × PWD)$^{wt/wt}$	(♀B6 × PWD)$^{wt/em}$

Step 2:

Backcross		♀(♀B6 × PWD)$^{wt/em}$	
		wt	**em**
♂PWD$^{wt/wt}$	**wt**	(♀B6 × ♀PWD)N2$^{wt/wt}$	(♀B6 × PWD)N2$^{wt/em}$
	wt	(♀B6 × ♀PWD)N2$^{wt/wt}$	(♀B6 × PWD)N2$^{wt/em}$

Step 3:

Backcross		♀(B6 × PWD)N2$^{wt/em}$	
		wt	**em**
♂PWD$^{wt/wt}$	**wt**	(B6 × PWD)N3$^{wt/wt}$	(B6 × PWD)N3$^{wt/em}$
	wt	(B6 × PWD)N3$^{wt/wt}$	(B6 × PWD)N3$^{wt/em}$

Step 3:

Intercross		♀(♀B6 × PWD)N3$^{wt/em}$	
		wt	**em**
♂(♀B6 × PWD)N3$^{wt/em}$	**wt**	(♀B6 × PWD)N3F1$^{wt/wt}$	(♀B6 × PWD)N3F1$^{wt/em}$
	em	(♀B6 × PWD)N3F1$^{wt/em}$	**(♀B6 × PWD)N3F1$^{em/em}$**

Supplemental Figure S5. Breeding schema for the preparation of (B6$^{wt/em}$ × PWD) × PWD)N3F1$^{em/em}$ males (Punnett squares). Only the color-coded offspring is used in the next step.

Breeding schema for: **(B6 × PWD)F7$^{em/em}$**

Step 1:

Intercross		♀PWD$^{wt/wt}$	
		wt	**wt**
♂B6$^{wt/em}$	**wt**	(♀PWD × ♂B6)$^{wt/wt}$	(♀PWD × ♂B6)$^{wt/em}$
	em	(♀PWD × ♂B6)$^{wt/wt}$	(♀PWD × ♂B6)$^{wt/em}$

Step 2:

Backcross		♀(♀PWD × ♂B6)$^{wt/em}$	
		wt	**em**
♂PWD$^{wt/wt}$	**wt**	(♀PWD × ♂B6)N2$^{wt/wt}$	(♀PWD × ♂B6)N2$^{wt/em}$
	wt	(♀PWD × ♂B6)N2$^{wt/wt}$	(♀PWD × ♂B6)N2$^{wt/em}$

Step 3:

Intercross		♀(♀PWD × ♂B6)N2$^{wt/em}$	
		wt	**em**
♂(♀PWD × ♂B6)N2$^{wt/em}$	**wt**	(♀PWD × ♂B6)F3$^{wt/wt}$	(♀PWD × ♂B6)F3$^{wt/em}$
	em	(♀PWD × ♂B6)F3$^{wt/em}$	(♀PWD × ♂B6)F3$^{em/em}$

Step 4:

Intercross		♀(♀B6 ×♂PWD)F1$^{wt/em}$	
		wt	**em**
♂(♀PWD × ♂B6)F3$^{em/em}$	**em**	(♀B6 × ♂PWD)F4$^{wt/em}$	(♀B6 × ♂PWD)F4$^{em/em}$
	em	(♀B6 × ♂PWD)F4$^{wt/em}$	(♀B6 × ♂PWD)F4$^{em/em}$

Step 5-6:

Intercross		♀(♀B6 × ♂PWD)$^{wt/em}$Fn	
		wt	**em**
♂(♀B6 × ♂PWD)Fn$^{em/em}$	**em**	(♀B6 × ♂PWD)F(n+1)$^{wt/em}$	(♀B6 × ♂PWD)F(n+1)$^{em/em}$
	em	(♀B6 × ♂PWD)F(n+1)$^{wt/em}$	(♀B6 × ♂PWD)F(n+1)$^{em/em}$

Step 7:

Intercross		♀(♀B6 × ♂PWD)F6$^{wt/em}$	
		wt	**em**
♂(♀B6 × ♂PWD)F6$^{em/em}$	**em**	(♀B6 × ♂PWD)F7$^{wt/em}$	(♀B6 × ♂PWD)F7$^{em/em}$
	em	(♀B6 × ♂PWD)F7$^{wt/em}$	**(♀B6 × ♂PWD)F7$^{em/em}$**

Supplemental Figure S6. Breeding schema for the preparation of (B6 × PWD)F7$^{em/em}$ males. In Steps 5 to 7, fertile males (B6 × PWD)Fn$^{em/em}$ were crossed with heterozygous littermate females (B6 × PWD)Fn$^{wt/em}$.

Breeding schema for: (♀B6.PWD-ChrX.1 × PWD)F1$^{tm/tm}$

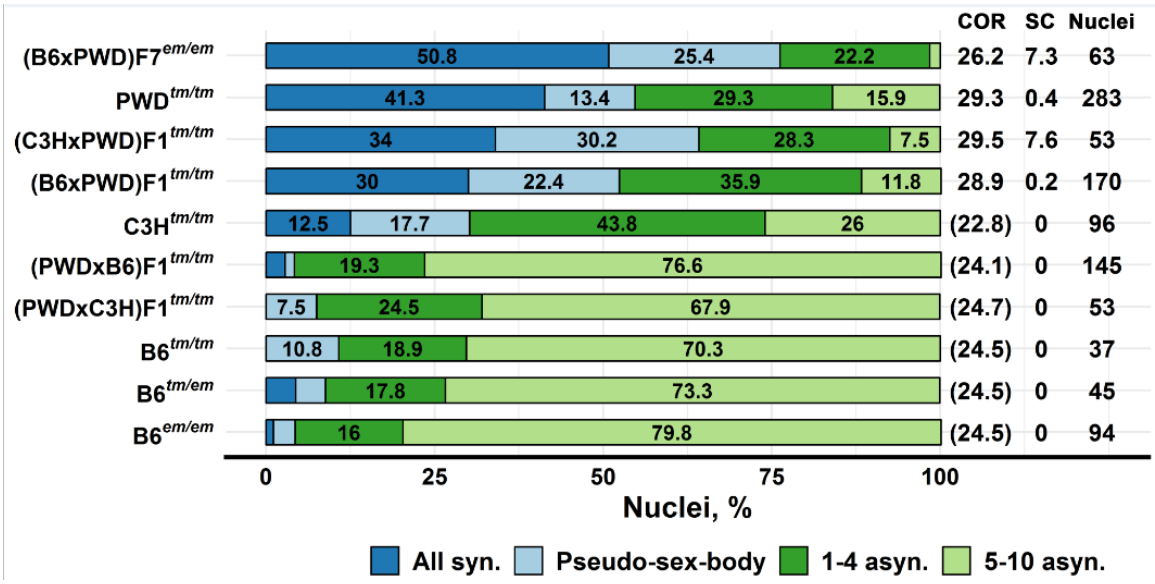
Step 1:

Intercross		♀B6.PWD-ChrX.1$^{wt/wt}$	
		wt	**tm**
♂B6$^{wt/tm}$	**wt**	(♀B6.PWD-ChrX.1 × B6)$^{wt/wt}$	(♀B6.PWD-ChrX.1 × B6)$^{wt/tm}$
	wt	(♀B6.PWD-ChrX.1 × B6)$^{wt/wt}$	(♀B6.PWD-ChrX.1 × B6)$^{wt/tm}$

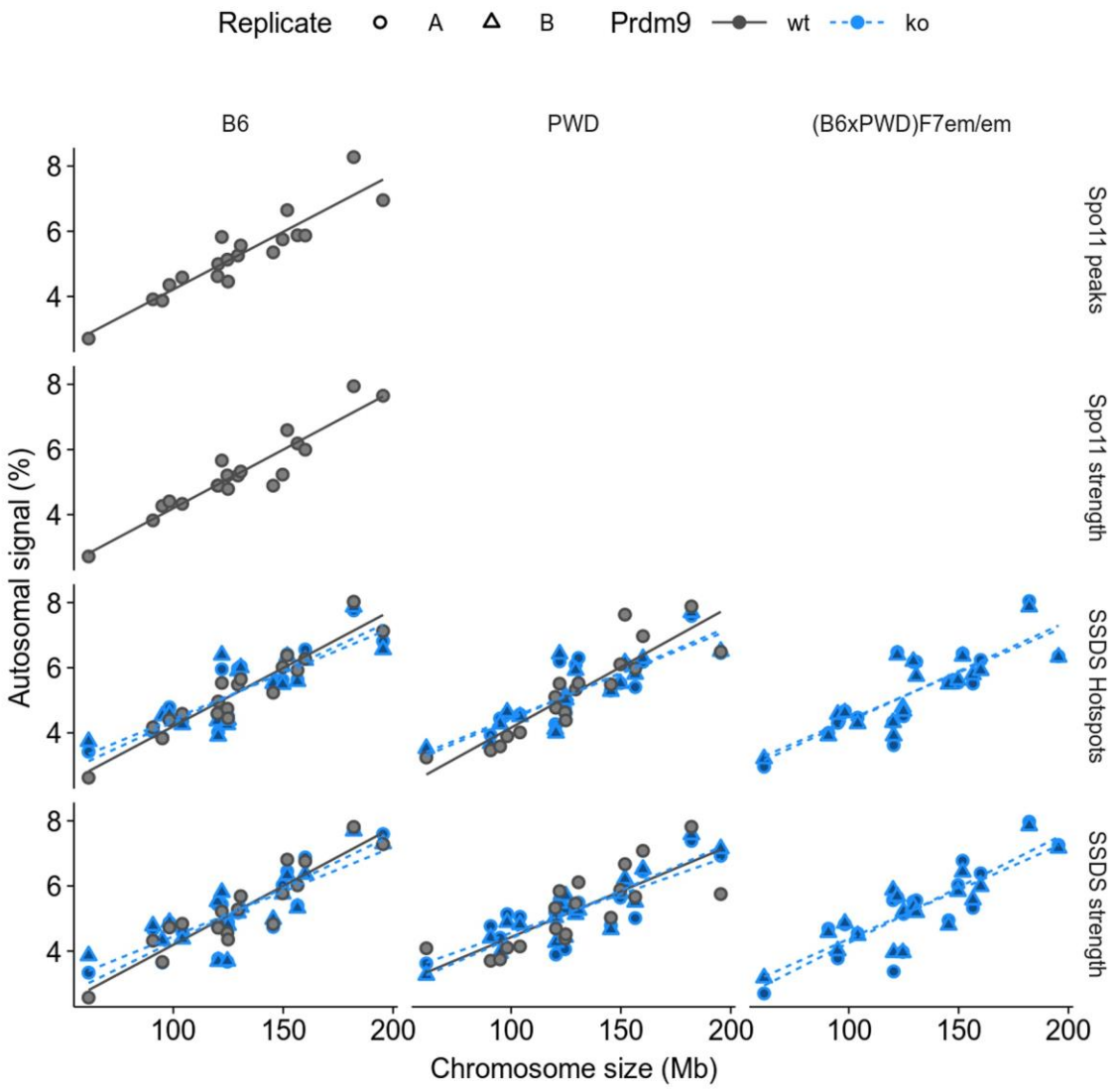
Step 2:

Intercross		♀(♀B6.PWD-ChrX.1 × B6)$^{wt/em}$	
		wt	**tm**
♂PWD$^{wt/tm}$	**wt**	(♀B6.PWD-ChrX.1 × PWD)F1$^{wt/wt}$	(♀B6.PWD-ChrX.1 × PWD)F1$^{tm/wt}$
	tm	(♀B6.PWD-ChrX.1 × PWD)F1$^{wt/tm}$	**(♀B6.PWD-ChrX.1 × PWD)F1$^{tm/tm}$**

Supplemental Figure S7. Breeding schema for the preparation of (B6.PWD-ChrX.1 × PWD)F1$^{tm/tm}$ males. In Step 1, the *Prdm9*^{tm} allele was introduced into B6 subconsomic carrying a part of mouse chromosome X from PWD. In step 2, intersubspecific F1 male hybrids were made.



Supplemental Figure S8. Synapsis and crossover rate in pachytene spermatocytes (PS) of *Prdm9*-deficient males. All PS are divided into four classes: full autosomal synapsis with sex body encompassing just X and Y (All syn.), sex body including one to two partially asynapsed autosome(s) positive for both SYCP1 and yH2AFX (Pseudo-sex-body), one to four asynapsed (SYCP1 negative and yH2AFX positive) autosomes (1-4 asyn.), and five to ten asynapsed autosomes (5-10 asyn.). PS with more than ten asynapsed autosomes were considered zygotene. The spermcounts (SC, in millions), crossover rates (COR, autosomal MLH1 counts per PS), and numbers of PS nuclei analyzed for synapsis are given on the right; the numbers in parentheses refer to the CORs of *Prdm9*$^{wt/wt}$ males. Note that the percentage of all synapsed chromosomes may differ between this figure and other figures and tables, because not all (but nearly all) nuclei could be sorted into these four classes.



Supplemental Figure S9. Meiotic DSBs are less frequent on shorter chromosomes. SSDS and Spo11-oligo strength are independent measures of DSB frequency. SSDS hotspots are from this report and Smagulova et al., 2016. Spo11 oligo sequencing data are from Lange et al., 2016. Empty panels are because Spo11-oligo data are only available for B6 mice. Only the signals at called peaks in each dataset are used. Blue dashed lines are from mice lacking functional PRDM9.

Supplemental Table S1. Mouse crossover rates (autosomal MLH1 foci per normal pachytene spermatocyte)

Mouse background (female parent shown first) and genotype	Number of mouse (reference)	Number of cells	MLH1 - mean	MLH 1 - SD
Total B6	(Balcova et al. 2016)	**78**	**24.5**	**0.8**
PWD$^{wt/wt}$	1	31	29.9	2.7
PWD$^{wt/wt}$	2	17	28.9	2.1
PWD$^{wt/wt}$	3	21	28.8	2.0
Total PWD	(Balcova et al. 2016)	**69**	**29.3**	**0.6**
PWD$^{tm/tm}$	1	17	29.7	3.0
PWD$^{tm/tm}$	2	20	30.0	1.6
PWD$^{tm/tm}$	3	33	29.3	2.1
Total PWD$^{tm/tm}$		**70**	**29.6**	**0.3**
C3H$^{wt/wt}$	1	70	23.2	1.6
C3H$^{wt/wt}$	2	59	22.3	1.8
Total C3H$^{wt/wt}$		**129**	**22.8**	**0.7**
(PWD x C3H)F1$^{wt/wt}$	1	58	25.1	2.1
(PWD x C3H)F1$^{wt/wt}$	2	70	24.3	2.2
(PWD x C3H)F1$^{tm/wt}$	**3**	**74**	**24.8**	**2.3**
Total (PWD x C3H)F1-*Prdm9*$^{wt/wt}$		**128**	**24.7**	**0.6**
(C3H x PWD)F1$^{tm/tm}$	1	56	29.6	2.0
(C3H x PWD)F1$^{tm/tm}$	2	49	29.5	2.8
(C3H x PWD)F1$^{tm/tm}$	3	47	29.3	2.3
Total (C3H x PWD)F1$^{tm/tm}$		**152**	**29.5**	**0.2**
(B6 x PWD)F7$^{em/em}$	1	63	26.4	2.3
(B6 x PWD)F7$^{em/em}$	2	72	25.7	1.9
(B6 x PWD)F7$^{em/em}$	3	53	26.7	1.7
Total (B6 x PWD)F7$^{em/em}$		**188**	**26.2**	**0.5**
(B6 x PWD)F1$^{tm/tm}$	1 - pool of 2	16	28.8	1.8
(B6 x PWD)F1$^{tm/tm}$	2 - pool of 2	25	28.9	2.0
Total (B6 x PWD)F1$^{tm/tm}$		**41**	**28.9**	**0.1**
Total (B6 x PWD)F1$^{wt/wt}$	(Bhattacharyya et al. 2013)	**46**	**27.1**	**2.5**
Total (B6 x PWD)F1$^{tm/wt}$	1	**23**	**26.4**	**2.4**
(B6.PWD-ChrX.1 x PWD)F1$^{tm/tm}$	1- pool of 2	**15**	**28.9**	**1.4**
Total B6.PWD-ChrX.1s$^{wt/wt}$	(Balcova et al. 2016)	**285**	**22.2**	**0.5**
(PWD x B6)$^{wt/tm}$	1- pool of 2	18	25.0	1.2

(PWD x B6)$^{wt/tm}$	2- pool of 2	37	24.3	2.1
Total (PWD x B6)$^{wt/tm}$		**55**	**24.5**	**1.8**
Total (PWD x B6)F1$^{wt/wt}$	(Bhattacharyya et al. 2013)	**41**	**24.1**	

SD, standard deviation (SD for each total shown in bold was counted from the means of individuals or pools)

Supplemental Table S2. Fertility parameters of *Prdm9*-deficient males

Genotype	Diff.s.	n	TW ± SD	rTW ± SD	SC ± SD*
PWD$^{tm/tm}$	31.4	9	41 ± 9	2.1 ± 0.4	0.11 ± 0.13
PWD$^{tm/tm}$	11.7	6	44 ± 4	2.3 ± 0.6	0.59 ± 0.86

Diff.s., differential segment encompassing *Prdm9* (Mb of non-PWD haplotype); n, number of males; TW, weight of paired testicles in mg; SD, standard deviation; rTW relative testis weight (TW adjusted to body weight, mg/g); SC, epididymal spermcount (millions); *, $P = 0.133$ (Wilcoxon test).

Supplemental Table S3. Sample quality metrics and analysis of DMC1-ChIP SSDS libraries from *Prdm9*-deficient testes

Library (genotype)	**Replicate**	**SSDS fragments (x 10^6)**	**Hotspots**	**SpoT (%)**
B6$^{tm/tm}$	A	7	13,862	74
B6$^{tm/tm}$	B	6	16,866	59
PWD$^{tm/tm}$	A	20	22,310	59
PWD$^{tm/tm}$	B	4	10,428	64
(B6 x PWD)F7$^{em/em}$	A	13	14,898	24
(B6 x PWD)F7$^{em/em}$	B	7	11,301	33

*SpoT (Signal Percentage of Tags) = 100 x (ssDNA fragments in hotspots) / (Total ssDNA fragments).

Supplemental Table S4. Details and statistics of male fertility parameters

Background (female parent shown first) and *Prdm9* genotype	n	TW (mg)	±	SD	rTW (mg/g)	±	SD	SC (million)	±	SD	nP (%)	±	SD	CO rate	Ferti-lity
PWD$^{wt/wt}$	12	127	±	8	6.4	±	0.4	24.8	±	6.7	94	±	10	29.3	F
PWD$^{tm/tm}$	30	45*	±	5	2.3*	±	0.3	0.42*	±	0.45	40*	±	24	29.6	S
B6$^{tm/tm}$	5	55	±	5	1.7	±	0.1	0.00	±	0.00	2			N.a.	S
B6$^{em/em}$	4	53	±	1	1.9	±	0.1	0.00	±	0.00	1	±	1	N.a.	S
B6$^{em/tm}$	4	54	±	5	2	±	0.1	0.00	±	0.00	2			N.a.	S
(((B6$^{em/B6}$xPWD)xPWD)N3)F1$^{em/em}$	7	61	±	21	2.3	±	0.6	1.5	±	4	N.d.			N.d.	N.d.
(((PWKxB6$^{tm/B6}$)xPWK)N3)F1$^{tm/tm}$	8	78	±	18	4	±	1	5.3	±	5.6	N.d.			N.d.	N.d.
C3H$^{wt/wt}$	5	160	±	13	5.1	±	0.6	18	±	2	99	±	1	22.7	F
C3H$^{tm/tm}$	6	51*	±	4	1.6*	±	0.1	0.00*	±	0.00	9*	±	3	N.a.	S
(B6 x PWD)F1$^{em/tm}$	7	59	±	8	2.3	±	0.3	0.12	±	0.2	35	±	6	N.d.	S
(B6 x PWD)F1$^{tm/tm}$	10	60	±	7	2.4	±	0.3	0.19	±	0.3	37	±	17	28.9**	S
(PWD x B6)F1$^{tm/tm}$	4	61	±	1	1.9	±	0.5	0.00*	±	0.00	2*	±	2	N.a.	S
(B6 x PWD)F1$^{wt/wt}$	11	101	±	12	4.1	±	0.5	4.5	±	6.2	38	±	9	27.1**	F
(PWD x B6)F1$^{wt/wt}$	5	60*	±	8	2.3*	±	0.4	0.00*	±	0.00	18	±	8	24.2**	S
(B6 x PWD)F1$^{tm/wt}$	15	156	±	19	6.3	±	1.1	32	±	19	78	±	10	26.4**	F
(PWD x B6)F1$^{wt/tm}$	9	57*	±	7	3.5*	±	0.4	0.05*	±	0.07	36*	±	21	24.5**	‡
(B6.PWD-ChrX.1 x PWD)F1$^{tm/tm}$	9	79	±	8	3.1	±	0.4	0.6	±	1	N.d.			28.9	N.d.
(B6.PWD-ChrX.1s x PWD)F1$^{em/tm}$	4	62*	±	9	2.4*	±	0.2	0.00*	±	0.00	N.d.			N.a.	S
(B6 x PWD)F7$^{em/em}$	8	64	±	18	3.2	±	0.8	7.3	±	4.0	46	±	10	26.4	F
(C3H x PWD)F1$^{tm/tm}$	6	88	±	15	3.0	±	0.7	7.6	±	5.6	49	±	7	29.6†	F
(PWD x C3H)F1$^{tm/tm}$	6	59*	±	3	2.0*	±	0.1	0.00*	±	0.00	3*	±	1	N.a.	S
(C3H x PWD)F1$^{wt/tm}$	6	187	±	6	6.1	±	0.7	29	±	9	88			N.d.	F
(PWD x C3H)F1$^{tm/wt}$	5	143*	±	37	4.4*	±	1.4	25	±	7	54	±	4	24.7†	N. d.

(C3H x PWD)F1$^{wt/wt}$	5	182	±	9	5.8	±	0.7	23	±	5	83			N.d.	F
(PWD x C3H)F1$^{wt/wt}$	7	145*	±	19	4.4*	±	0.8	17	±	10	39	±	7	24.8†	F

^{tm}, the *Prdm9*^{tm101Ymat} null allele; ^{em}, the *Prdm9*^{em1Fore} null allele; n, number of males; TW, weight of paired testicles; SD, standard deviation; rTW relative testis weight (same as TW/BW); SC, epididymal spermcount (millions); nPS, % normal (full synapsis) pachytene spermatocytes (of all pachynema); COR, cross-over rate (autosomal MLH1 foci/cell); F, fertile (produced pups),; S, sterile (no pups and/or SC=0); N.a., not applicable (cell arrest); N.d., not determined; *, significant difference ($p<0.05$) in comparison to the value in the same column and the previous row (of the same color); **, COR of (PWD x B6)F1 differ from (B6 x PWD)F1 ($p=0.008$); †, COR of (PWD x C3H)F1 versus (C3H x PWD)F1 differ ($p=0.0008$); ‡, few pups, see Flachs et al. 2012