Supplementary Tables

Supplementary Table 1: Noncrossover frequency at *Psmb9*.

Queried Polymorphism [b]	♀ Chromosome				♂ Chromosome			
	B10.A		SGR		B10.A		SGR	
	Non-crossovers (genomes)	Frequency ($x10^{-3}$) (95% CI)[a]	Non-crossovers (genomes)	Frequency ($x10^{-3}$) (95% CI)	Non-crossovers (genomes)	Frequency ($x10^{-3}$) (95% CI)	Non-crossovers (genomes)	Frequency ($x10^{-3}$) (95% CI)
-87	2 (4739)	0.42 (0.05-1.52)	n.d.		0 (8981)	<0.0001 (0.00-0.33)	n.d.	
BsrFI	65 (18994)	3.42[c] (2.64-4.36)	3 (19286)	0.16[c] (0.03-0.45)	37 (64563)	0.57[c] (0.40-0.79)	2 (58681)	0.034[c] (0.004-0.012)
38	118 (17736)	6.65 (5.51-7.97)	2 (8805)	0.23 (0.03-0.82)	103 (64563)	1.60 (1.30-1.93)	1 (58681)	0.017 (0.0004-0.095)
➤70	119 (16478)	7.22 (5.98-8.64)	0 (1258)	<0.0008 (<2.38)	111 (70405)	1.58 (1.30-1.90)	0 (58681)	<0.00002 (<0.051)
➤87	26 (7255)	3.58 (2.34-5.25)	n.d.		23 (15736)	1.46 (0.93-2.019)	n.d.	
StyI	0 (4739)	<0.0002 (<0.78)	n.d.		0 (21577)	<0.00005 (0.00-0.13)	n.d.	
SphI	0 (4739)	<0.0002 (<0.78)	n.d.		0 (25082)	0.08 (0.01-0.29)	n.d.	

[a]Confidence interval (CI) calculations here and in the text are from http://vassarstats.net/ using the Wilson procedure with continuity correction.
[b]Polymorphisms correspond in order to the asterisks in Fig. 1h and Supplementary Figure 1.
[c]Note the 10 to 30-fold higher conversion frequency on the B10.A chromosome compared to the SGR chromosome.
Red arrowhead indicates polymorphisms overlapping the position of the $PRDM9^{wm7}$ binding site.

Supplementary Table 2: Primer and oligonucleotide information.

a. *Psmb9* hotspot primer sequences.

Psmb9 primers	Sequence	Strain specificity	Marker
881U21	CTGTGCTGGGTAGTTTTGGGC	B10, R209, B10.A, SGR	
10225L21	GGAAACTGGAACTGGAGTCTG	B10, R209, B10.A, SGR	
7716U21	CCTGATTTCCTTCGGTGACTA	B10, R209	
3457L19	GCCCCTGGATGGGTTGTAA	B10, R209	
1297U20A	ACAGTCTCTCCTCTGCTTTC	B10.A	
1296U21S	GACAGTCTCTCCTCTGCTTTA	SGR	
1308U19	TCTGCTTTCCTGGACACGG	B10	
1308U19b	TCTGCTTTACTGGACATGT	R209	
1820L21	GAGCCACAAGGCAGTAAGAGC	B10.A, SGR	
2020L17A	GGCAGACCCACCGAAAT	B10.A	*-87*
2020L17S	GGCAGACCCACCGAAAC	SGR	*-87*
2092U16b10	GTTGTGCAAGATGCCT	B10, B10.A	*Bsr*FI
2092U16R209	GTTGTGCAAGATGCCG	R209, SGR	*Bsr*FI
2128U18A	ACAGCTTCAAGGGCTCTC	B10.A	*38*
2128U18S	ACAGCTTCAAGGGCTCTG	SGR	*38*
2160U18b10	CACGTGGATCCAGGGAAT	B10.A	*70*
2160U17sgr	ACGTGGATCCAGGGAAC	SGR	*70*
2176U17b10	AGAACTTTGACCATTAC	B10.A	*87*
2176U17sgr	AGAACTTTGACCATTAT	SGR	*87*
2296U25S7H	gaggcaaTTTTTTTTTTTTTTAATCTTGCCTC	B10, SGR	*Sty*I
2296U25A7H	aaggcaaTTTTTTTTTTTTTTAAATCTTGCCTT	R209, B10.A	*Sty*I
SphU17S	GGCTGGTTTCAGGGCAT	B10, SGR	*Sph*I
SphU17A	GGCTGGTTTCAGGGCAG	R209, B10.A	*Sph*I
3044L14b10	ACAAACCATTGCCC	B10.A	

3044L15sgr	TACAAACCATTGCCT	SGR
3402L20	AATAAAGGGCTAGCTGTGCA	B10, SGR
3402L20b	AATAAAGGGCTGGCTGTGCC	R209, B10.A
4623L20	ATTTGAGAGTGGGAGGGGAT	B10, SGR
4623L20b	ATTTGAGAGTGAGAGGGGAC	R209, B10.A

b. *Psmb9* primer combinations.

PCR	Hybrid	Specificity	Annealing Temp	Forward primer	Reverse primer
1st	B10 x R209	All	60°C	881U21	10225L21
2nd	B10 x R209	All	61°C	7716U21	3457L19
2nd	B10 x R209	B10	61°C	1308U19	3402L20
2nd	B10 x R209	R209	59°C	1308U19b	3402L20b
2nd	B10 x R209	Noncrossover B10→R209 at *Bsr*FI	63°C	2092U16R209	3402L20
2nd	B10 x R209	Noncrossover R209→B10 at *Bsr*FI	63°C	2091U17B10	3402L20b
2nd	B10 x R209	Noncrossover B10→R209 at *Sty*I	62°C	2296U25A7H	3402L20
2nd	B10 x R209	Noncrossover R209→B10 at *Sty*I	62°C	2296U25S7H	3402L20b
2nd	B10 x R209	Noncrossover B10→R209 at *Sph*I	64°C	SphU17A	3402L20
2nd	B10 x R209	Noncrossover R209→B10 at *Sph*I	64°C	SphU17S	3402L20b
1st	B10.A x SGR	All	60°C	881U21	10225L21
2nd	B10.A x SGR	All	62°C	1297U20A 1296U21S	1820L21
2nd	B10.A x SGR	B10.A	60°C	1297U20A	3402L20b
2nd	B10.A x SGR	SGR	60°C	1296U21S	3402L20
2nd	B10.A x SGR	Crossover B10.A-SGR	63°C	1297U20A	3402L20
2nd	B10.A x SGR	Crossover SGR-B10.A	63°C	1296U21S	3402L20b
2nd	B10.A x SGR	Noncrossover B10.A→SGR at *-87*	63°C	1297U20A	2020L17S
2nd	B10.A x SGR	Noncrossover SGR→B10.A at *-87*	63°C	1296U21S	2020L17A

2nd	B10.A x SGR	Noncrossover B10.A→SGR at *Bsr*FI	63°C	2092U16R209	3402L20b
2nd	B10.A x SGR	Noncrossover SGR→B10.A at *Bsr*FI	63°C	2091U17B10	3402L20
2nd	B10.A x SGR	Noncrossover B10.A→SGR at *38*	63°C	2128U28S	3402L20b
2nd	B10.A x SGR	Noncrossover SGR→B10.A at *38*	63°C	2128U28A	3402L20
2nd	B10.A x SGR	Noncrossover B10.A→SGR at *70*	62°C	2161U17SGR	3402L20b
2nd	B10.A x SGR	Noncrossover SGR→B10.A at *70*	62°C	2160U18B10	3402L20
2nd	B10.A x SGR	Noncrossover B10.A→SGR at *87*	55°C	2176U17SGR	3044L14B10
2nd	B10.A x SGR	Noncrossover SGR→B10.A at *87*	55°C	2176U17B10	3044L14SGR
2nd	B10.A x SGR	Noncrossover B10.A→SGR at *Sty*I	62°C	2296U25S7H	3402L20b
2nd	B10.A x SGR	Noncrossover SGR→B10.A at *Sty*I	62°C	2296U25A7H	3402L20
2nd	B10.A x SGR	Noncrossover B10.A→SGR at *Sph*I	64°C	SphU17S	3402L20b
2nd	B10.A x SGR	Noncrossover SGR→B10.A at *Sph*I	64°C	SphU17A	3402L20

c. *A3* hotspot primer sequences.

A3 primers	Sequence	Strain specificity
A3f600	TGAAGCTCACTATTCTGTGCAA	Universal
A3f943	GAAATCTCCCAGAGCACAGG	Universal
A3r3588	AATGTGACAGGAGAGGAGCTGACCA	Universal
A3r3938	ATGTGGAGAGGCCAGCGCTCA	Universal
A3r6000	GAGACACAATTCAGGTCCTGA	Universal
Bf3	ATAAGCACGTATTTGAGGCC	A/J
Df3-1	AAGCACGTGTTTGAGGCG	DBA/2J
Bf4-1	CAGCAGCTGAGTTAAAACT	A/J
Df4-1	CAGCAGCTGAGTTAAAACA	DBA/2J
Dr5465	GTGTCACATTTCAGTTGATGC	DBA/2J
Br4020	TCTCCAACAGTGGGGGAT	A/J
Dr4020	TCTCCAACAGTGGGGGAC	DBA/2J
Br3938	GCTACCTTCTAAAAGTGGA	A/J

d. *A3* primer combinations.

PCR	Specificity	Annealing Temperature	Forward primer	Reverse primer
1st	Universal	60°C →58°C	A3f600	A3r6000
2nd	A/J → DBA/2J	64°C→63°C→62°C	Bf3	Dr5465
2nd	DBA/2J → A/J	64°C→63°C→62°C	Df3-1	Br4020
3rd	A/J → DBA/2J	62°C	Bf4-1	Dr4020
3rd	DBA/2J → A/J	62°C	Df4-1	Br3938
2nd	A/J → Universal	64°C→63°C→62°C	Bf3	A3r3938
2nd	DBA/2J → Universal	64°C→63°C→62°C	Df3-1	A3r3938
3rd	A/J → Universal	62°C	Bf4-1	A3r3588
3rd	DBA/2J → Universal	62°C	Df4-1	A3r3588
4th	Universal	61°C →59°C	A3f943	A3r3588

e. *A3* oligo sequences for genotyping.

Allele Build 37	A/J oligo	DBA/2J oligo
1:161954686	AGCACCATGGAGCGGCTG	AGCACCACGGAGCGGCTG
1:161955037	TGCATGTAAGTAAAGGGC	TGCATGTTGGTAAAGGGC
1:161955139*	GTCTCCACGCCTCAGCAT	CTCTCCATGCCTCAGCAT
1:161955306	AATAATGATCCAGTCTTT	AATAATGGTCCAGTCTTT
1:161955342	TTCTTCTTTGTGAATTTT	TTGTTCTCTGTGAATTTT
1:161955432	ATGCATCACGTGCATCTC	ATGCATCCCGTGCATCTC
1:161955480	TTGCCATCCCCAGGGACT	TTGCCATCCCTAGGGACT
1:161955488	CAGGGACTACACGGTTAT	TAGGGACTGCACGGTTAT
1:161955604	ATAAAGCCTGGGGCTATA	ATAAAGC-TCAATCTTAGA
1:161955784	AGAGTCTCAAGCAATCCA	AGAGTCTCAAACAATCCA
1:161955792	CAATCCACACTGACCTTG	CAATCCATACTGACCTTG
1:161955851**	TCACAGACGTGCCCTGAC	TCACAGATGTCCCCTTAC
1:161955880*	CCAGAAGCTGCTATCATG	CCAGAAGCTGCTACCATG
1:161955885*	CATGCGCCACACCCACAG	CATGTTCGCCACACCCAC
1:161955887*	ACACCCACAGCCACACCC	
1:161955968	CATCGTTCCAATCCCCTA	CATCGTTACAATCCCCTA
1:161956144	TGTTGCTTCATGGGTTTG	TGTTGCTGCATGGGTTTG
1:161956261	CAGAAGTTTTTGAGTTCG	CAGAAGTCTTTGAGTTCG
1:161956354	GAGATGACTCTAGAATGT	GAGATGAATCTAGAATGT
1:161956547	ATGTGTATTTCCTAATAC	ATGTGTAGTTCCTAATAC
1:161956666	CTATGAGTAAAATTGAGT	CTATGAGTGAAATTGAGT
1:161956882	CACGGTTCTTGTGGGCTT	CACCGTTTTTGTGGGCTT

*Probes new to this study. All other probes were identical to those used in the previous study[18]. **The A/J oligo has a mismatch at the 3rd polymorphism with the A/J genomic sequence (TCACAGACGTGCCCTTAC).

f. Southwestern: Primers used for generating probes across the *A3* hotspot.

Probe	Primer	Sequence	Strain
1	Xb1524U20	gct cta gaGGTGCAAGAACACAGTTTCA	A/J, DBA
	Xb1814L18	gct cta gaGCTCGGTGCTGGAGGTAC	A/J, DBA
2	Xb1752U19	gct cta gAATAACAAGGGTTGGCAAG	A/J, DBA
	Xb2018L20	gct cta gACACCCTCTTCTGGACTCTT	A/J, DBA
3	Xb1978U21	gct cta gaTTGTGAGTCTATCAATGCATC	A/J, DBA
	Xb2246L21	gct cta gaCAATTAACTTTGCTGGTTTGT	A/J, DBA
4	Xb2206U21	gct cta gACCACCTAGTGTGCTAGTGTG	A/J, DBA
	Xb2408L17A	gct cta gaCATGGTAAGGGCACGTC	A/J
	Xb2408L17D	gct cta gaCATGGTAAGGGGACATC	DBA
5	Xb2242U23	gct cta gAACAACAAACCAGCAAAGTTAAT	A/J, DBA
	Xb2501L20	gct cta gaGTCTAGGACAAGGCATGAGA	A/J, DBA
6	Xb2465U16	gct cta gaCACAGGAAGCCGATCA	A/J, DBA
	Xb2741L20	gct cta gAATGCCAGATGGTATCCCTA	A/J, DBA
7	Nh2687U16	aat tcg cta GCCATCCCCTGTTGCT	A/J, DBA
	Nh2937L21	aat tcg cta GCTGGAATGGAAAGAACTCAT	A/J, DBA
8	Nh2919U19	aat tcg cta gcAATGTTCTTCCGGGTCTCA	A/J, DBA
	Nh3180L21	aat tcg cta gCAGTGACACCAGGACTCAAAC	A/J, DBA

g. Southwestern: Oligos annealed to generate probes at the *A3* PRDM9 binding site.

Oligo	Sequence	Strain
A3_AU	CGTGCCCTTACCATGTCCAGAAGCTGCTATCATGCGCCACACCCACAGCCACAC	A/J
A3_AL	TGGGTGTGGCTGTGGGTGTGGCGCATGATAGCAGCTTCTGGACATGGTAAGGGC	A/J
A3_BU	CGTGCCCTGACCATGTCCAGAAGCTGCTACCATGCGCCACACCCACAGCCACAC	B6
A3_BL	TGGGTGTGGCTGTGGGTGTGGCGCATGGTAGCAGCTTCTGGACATGGTCAGGGC	B6
A3_DU	TGTCCCCTTACCATGTCCAGAAGCTGCTACCATGTTCGCCACACCCACAGGAAG	DBA
A3_DL	CGGCTTCCTGTGGGTGTGGCGAACATGGTAGCAGCTTCTGGACATGGTAAGGGG	DBA

Supplementary Table 3: Probability of detecting a crossover or a noncrossover in single oocytes.

Exp.	Hybrid	n_{tot}	n_0	n_1	n_2	n_2/n_1	*p*	P_{1CO}	P_{2CO}	$n_{0\ expected}$
1	B10 x R209	48	2	9	37	4.111	0.671	0.892	0.450	1
2	B10 x R209	48	5	6	37	6.167	0.726	0.925	0.527	0
3	B10 x R209	32	2	7	23	3.286	0.637	0.868	0.406	1
4	B10.A x SGR	51	3	10	38	3.500	0.646	0.875	0.478	1
5	B10.A x SGR	90	0	19	71	3.737	0.656	0.882	0.431	1
6	B10.A x SGR	69	2	8	59	7.375	0.748	0.937	0.560	0

Exp., experiment number.
n_{tot}, total number of oocytes tested.
n_0, n_1, n_2, number of oocytes with observed amplification of zero, one, and both parental alleles.
p, probability of amplifying a specific chromatid based on a *B(4,p)* distribution, which is equivalent to the probability of detecting a noncrossover involving a single chromatid.
P_{1CO}, probability of amplifying at least one of chromatid from a crossover.
P_{2CO}, probability of amplifying both chromatids from a crossover.
$n_{0\ expected}$, estimate of expected n_0 based on the value of *p*. The excess of oocytes with no observed amplification (i.e., n_0) is likely due to a lack of amplifiable DNA, such that these oocytes were not further considered in frequency calculations. For each individual experiment, oocytes were collected from one single 25 to 30-day-old mouse.