Table S1. Frequency of deletions in testis, sperm and liver, Related to Figures 1, 2, 4 and S2

Strain background	Tissue	Hotspot pair	Mouse and genotype	No. haploid genomes	No. deletions	Frequency (10^{-6})
B6	testis	Chr1 adult	1794 *Atm*$^{-/-}$	5400000	67	12.41
			*1793 *Atm*$^{+/+}$	5400000	0	<0.19
			*2018 *Atm*$^{-/-}$	2066667	8	3.87
			*2017 *Atm*$^{+/-}$	2066667	0	<0.48
			*2066 *Atm*$^{+/-}$	1600000	0	<0.63
			*2067 *Atm*$^{+/+}$	2266668	0	<0.44
			*2656 *Atm*$^{-/-}$	1400000	6	4.29
			*2657 *Atm*$^{+/-}$	3200000	0	<0.31
			*2732 *Atm*$^{+/-}$	6400000	2	0.31
			*2970 *Atm*$^{+/-}$	2000000	0	<0.50
			*3162 *Atm*$^{+/+}$	6400000	0	<0.16
			*3312 *Spo11*$^{-/-}$	6400000	0	<0.16
			*3345 *Spo11*$^{-/-}$	6400000	0	<0.16
			*3424 *Spo11*$^{-/-}$	1600000	1	0.63
			Total *Atm*$^{-/-}$	8866667	81	9.14
			Total *Atm*$^{+/-}$	15266667	2	0.13
			Total *Atm*$^{+/+}$	14066668	0	<0.071
			Total *Spo11*$^{-/-}$	14400000	1	0.07
B6	testis	Chr1 juvenile	*1 *Atm*$^{-/-}$	3200000	24	7.50
			*2 *Atm*$^{+/-}$	3200000	0	<0.31
			*3 *Atm*$^{+/+}$	3200000	0	<0.31
B6	liver	Chr1 adult	3991 *Atm*$^{-/-}$	10000000	0	<0.10
	testis			2400000	30	12.50
F1 B6/DBA	testis	Chr1 adult	2654 *Atm*$^{-/-}$	3600000	7	1.94
			2675 *Atm*$^{-/-}$	5200000	30	5.77
			2674 *Atm*$^{+/-}$	3200000	0	<0.31
			2647 *Atm*$^{+/+}$	3200000	0	<0.31
			Total *Atm*$^{-/-}$	8800000	37	4.20
B6	testis	Chr19 adult	*1793 *Atm*$^{+/+}$	5400000	2	0.37
			*2018 *Atm*$^{-/-}$	266667	2	7.50
			*2017 *Atm*$^{+/-}$	400000	0	<0.25
			*2066 *Atm*$^{+/-}$	1600000	0	<0.63
			*2067 *Atm*$^{+/+}$	1866667	0	<0.54
			*2656 *Atm*$^{-/-}$	3200000	7	2.19
			*2657 *Atm*$^{+/-}$	3200000	1	0.31
			2733 *Atm*$^{-/-}$	4800000	29	6.04
			*2732 *Atm*$^{+/-}$	4800000	3	0.63
			2734 *Atm*$^{-/-}$	400000	2	5.00
			2731 *Atm*$^{+/-}$	400000	0	<2.50
			2780 *Atm*$^{-/-}$	400000	2	5.00
			2779 *Atm*$^{+/-}$	400000	0	<2.50
			2971 *Atm*$^{-/-}$	2000000	5	2.50
			*2970 *Atm*$^{+/-}$	2000000	0	<0.50
			*3162 *Atm*$^{+/+}$	6400000	0	<0.16

			*3312 *Spo11*$^{-/-}$	6400000	0	<0.16
			*3345 *Spo11*$^{-/-}$	6400000	0	<0.16
			*3424 *Spo11*$^{-/-}$	1600000	0	<0.63
			Total *Atm*$^{-/-}$	11066667	47	4.25
			Total *Atm*$^{+/-}$	12800000	4	0.31
			Total *Atm*$^{+/+}$	13666667	2	0.15
			Total *Spo11*$^{-/-}$	14400000	0	<0.069
B6	testis	Chr19 juvenile	*1 *Atm*$^{-/-}$	1600000	11	6.88
			*2 *Atm*$^{+/-}$	3200000	0	<0.31
			*3 *Atm*$^{+/+}$	3200000	1	0.31
B6	sperm	Chr19 adult	3776 *Atm*$^{+/-}$	6400000	7	1.09
			3834 *Atm*$^{+/+}$	3200000	0	<0.31

Littermates are grouped. * The same mice used for both hotspot pairs.

Table S2. Deletions detected in *Atm*$^{-/-}$ with the analyses performed, Related to Figures 1, 2, 4, 5 and 6

Hotspot pair	Strain background (primer set)[a]	No. PCR reactions	DNA (ng) seeded in PCR	No. haploid genomes	Total No. deletions	No. deletions with insertions	No. deletions with mutations at junctions[c]	Analyses performed
Chr1	B6 (b6)	168	25	1400000	16	0	2	frequency, breakpoint distribution, microhomology, insertions, recurring deletions
		640	50	10666667	89	12	3	frequency, breakpoint distribution, microhomology, insertions, recurring deletions
		72	100	2400000	29	3	0	breakpoint distribution, insertions, microhomology, recurring deletions
				Total Chr1 from B6	134	15	5	
	B6 (b6/dba)	48	50	640000	2	1	0	insertions, microhomology, recurring deletions
	B6/DBA (b6/dba)	528	50	8800000	37	2	0	frequency, breakpoint distribution, microhomology, insertions, recurring deletions
	B6/DBA (b6)	144	50	2400000	12	1	1	insertions, microhomology, recurring deletions
				Total Chr1	185	19	6	
Chr19	B6	8	10	266667	1	0	0	breakpoint distribution, microhomology, insertions, recurring deletions
		760	50	12666667	58[b]	8	5	frequency, breakpoint distribution, microhomology, insertions, recurring deletions
				Total Chr19	59[b]	8	5	

[a] For the Chr1 hotspot pair, two different primer sets were used as indicated.

[b] For one deletion event, one breakpoint could not be precisely mapped.

[c] Deletions with short 1–3-bp non-templated insertions at the breakpoint junction and/or point mutations or indels up to 10 bp from breakpoint junctions.

Table S3. Double cutting and deletion frequencies per meiosis in *Atm*$^{-/-}$, Related to Figures 1, 2, S1 and S2

Hotspot pair		Hotspot strength (RPM)	No. DSBs per chromatid at hotspot (DSB frequency per meiosis)[a]	Expected double cutting frequency per meiosis	Deletion frequency per meiosis[b]	Expected double cutting frequency divided by deletion frequency
Chr1	Hotspot left	53	0.03975	27.7×10^{-4}	0.36×10^{-4}	77
	Hotspot right	93	0.06975			
Chr19	Hotspot left	41	0.03075	15.9×10^{-4}	0.17×10^{-4}	94
	Hotspot right	69	0.05175			
All 1–5 kb hotspot pairs (n=1023)		–	–	0.60115[c]	0.00801 (Chr1)[d] 0.00645 (Chr19)[d]	

[a] DSB frequency was estimated from SPO11-oligo counts (RPMs) by equating 3000 DSBs in *Atm*$^{-/-}$ to one million reads. Note: we considered that there are about 300 DSBs per meiotic cell in wild type and an ~10-fold increase in *Atm*$^{-/-}$.

[b] Deletion frequency calculated per meiotic genome.

[c] For each hotspot pair separated by 1–5 kb in *Atm*$^{-/-}$ on autosomes, the expected double cutting frequency per meiosis was calculated, and the frequencies were summed up.

[d] The total estimated deletion frequency per meiosis was calculated from the expected double cutting frequency at each hotspot pair relative to the double cutting and deletion frequency at Chr1 or Chr19 and summed up.

Table S4. Recurring deletions at the Chr1 hotspot pair in B6 inbred and B6 × DBA F1 hybrid mice, Related to Figure 5B

Microhomology	Microhomology length (bp)	No. deletions	Deletion (bp)	Strain
–	0	2	1416	B6
CT	2	2	1868	B6, DBA/B6
AGCC	4	2	1943	B6, DBA/B6
ACCCT	5	2	2372	B6
AGACA	5	2	2816	B6, DBA/B6
CACTGC	6	2	2371	B6
GGTTTAGA	8	8	2371	B6(6), DBA/B6(2)
AGCCAGTCA	9	2	1624	B6, DBA/B6
	Median 6 bp			
Inverted microhomology	**Microhomology length (bp)**	**No. deletions**	**Deletion/Insertion (bp)**	**Strain**
CACTGCCCTGGA	12*	2	2437/356	B6
		24 of total 185 deletions		

* A recurring deletion with inverted insertion with a 12-bp microhomology at one insert breakpoint; also see **Figure S6E**.

Table S5. Insertions derived from other hotspots, Related to Figures 6 and S6

Hotspot pair	Insert origin	Insert start	Insert end	SPO11 activity at insert origin		Insert length (bp)	Deletion length (bp)	Microhomology at insert breakpoints (left/right)
				Atm$^{-/-}$	*Atm*$^{+/+}$			
Chr1	Chr3	116341240	116341350	cluster	no	111	2343	CAC/none
	Chr6	116019448	116019796	hotspot	cluster	349	2341	CACT/A
	Chr6	83540780	83540885	hotspot	cluster	106	1967	AG/none
	Chr7	41695870	41695945	hotspot	hotspot	76	1596	GTA/none
	Chr8	87936133	87936219	hotspot	hotspot	87	1513	(GATT insertion)/none
	Chr9	56053260	56053364	cluster	no	105	1671	C/TG
	Chr12	91711551	91711567	hotspot	hotspot	17	3326	CAAAGTGTCTG/TCT
	ChrX	69476686	69476920	hotspot	hotspot	235	2108	AT/none
Chr1 B/D	Chr9	55392465	55392589	no	cluster	125	2399	C/A
Chr19	Chr16	35066202	35066440	hotspot	hotspot	239	2005	none/none
	Chr17	78359789	78359826	hotspot	hotspot	38	2307	CA/none
	Chr17	80408805	80408819	hotspot pair	hotspot pair	15	1995	T/CAT

Hotspot pair	Insert from the non-PAR ChrY	Insert start	Insert end	SPO11 activity at insert origin		Insert length (bp)	Deletion length (bp)	Microhomology at insert breakpoints (left/right)
				Atm$^{-/-}$	*Atm*$^{+/+}$			
Chr1	insert 1* (2 of 26 repeats)	28785715	28785921	hotspot	hotspot	207	2253	A/G
		58748224	58748430	cluster	hotspot			
		90475833	90476039	no	hotspot**			
	insert 2* (1 of 15 repeats)	90476619	90476713	no	hotspot**	95	1939	AAACA/G
	insert 3 (3 of 26 repeats)	5658645	5658703	hotspot	cluster	59	1500	none/none
		5944098	5944156	hotspot	cluster			

* Inserts 1 and 2 are part of the same repeat; both match 12 copies of the repeat.

** Same hotspot.

Chr19	Insert from Chr1	88533343	88533493	microsatelite repeat $(CTT)_{27}$	148	2643	ACTGAGCTATCT/CCTCCTCCT

Table S6. Insertions derived from the hotspot pair, Related to Figures 6 and S6

Hotspot pair	Hotspot left or right	Insert length (bp)	Deletion length (bp)	Distance between deletion and insertion breakpoints (bp)	Microhomology at insert breakpoints (left/right)
Chr1	left	709	2590	4	none/GAAGG
	left	22	2411	28	none/AGA
	right	343	2334	11	TCCAGGGCAGTGC/none
	right	356	2437	0	TCCAGGGCAGTG/none
	right	356	2437	0	TCCAGGGCAGTG/none
Chr1 B/D	left	493	2025	40	TTG/AAAG
	right	302	2465	56	TCCAGGGCAGT/ATTC
	right	216	2399	3	GT/T
Chr19	left	204	2591	0	none/C
	right	231		3	CTCTCTC/none
	left	130	1361	0	none/ATAGCT
	left	132	1949	0	none/TAGCT
	right	16	2063	44	GCTCT/A

Table S7. Frequency of microdeletions in individual amplicons, Related to Figure 7

Genotype	Amplicon	No. reads	No. reads with deletions (unique[a])	Frequency (adjusted[b]) (10^{-5})	P value[c] versus *Atm*$^{-/-}$ (adjusted[b])	P value[c] *Spo11*$^{-/-}$ (adjusted[b])
Atm$^{-/-}$	1	3611491	60 (36)	1.66		
	2	3072566	65 (39)	2.12		
	3	3714378	50 (43)	1.35		
	4	3240515	41 (31)	1.27		
	Total	13638950	216 (149)	1.58		
Atm$^{+/-}$	1	3615027	9 (7)	0.25	<0.0001	0.0161
	2	4036817	4 (4)	0.10	<0.0001	0.0825
	3	5334246	17 (16)	0.32	<0.0001	0.0592
	4	4704066	10 (10)	0.21	<0.0001	0.2799
	Total	17690156	40 (37)	0.23	<0.0001	0.4670
Atm$^{+/+}$	1	5099332	23 (8)	0.45 (0.20)	<0.0001 (<0.0001)	0.0004 (0.0387)
	2	4402709	53 (12)	1.20 (0.73)	0.002 (<0.0001)	<0.0001 (0.0104)
	3	3360847	6 (4)	0.18	<0.0001	0.6158
	4	3253036	5 (4)	0.15	<0.0001	0.1313
	Total	16115924	87 (28)	0.54 (0.33)	<0.0001 (<0.0001)	<0.0001 (0.0173)
Spo11$^{-/-}$	1	3339211	1 (1)	0.03	<0.0001	
	2	2915459	8 (7)	0.27	<0.0001	
	3	4485773	6 (6)	0.13	<0.0001	
	4	3568282	12 (9)	0.34	<0.0001	
	Total	14308725	27 (23)	0.19	<0.0001	

[a] Total unique deletions obtained in that amplicon; note that the same deletion can be obtained independently in the overlapping amplicon.

[b] Adjusted frequencies and P values take into account potential jackpot events, in which 14 reads were obtained for the same microdeletion in amplicon 1, and 22 for another microdeletion in amplicon 2. Because these microdeletions were not obtained or were obtained just once in other amplicons they are considered to have arisen from PCR overamplification.

[c] Chi-square test.