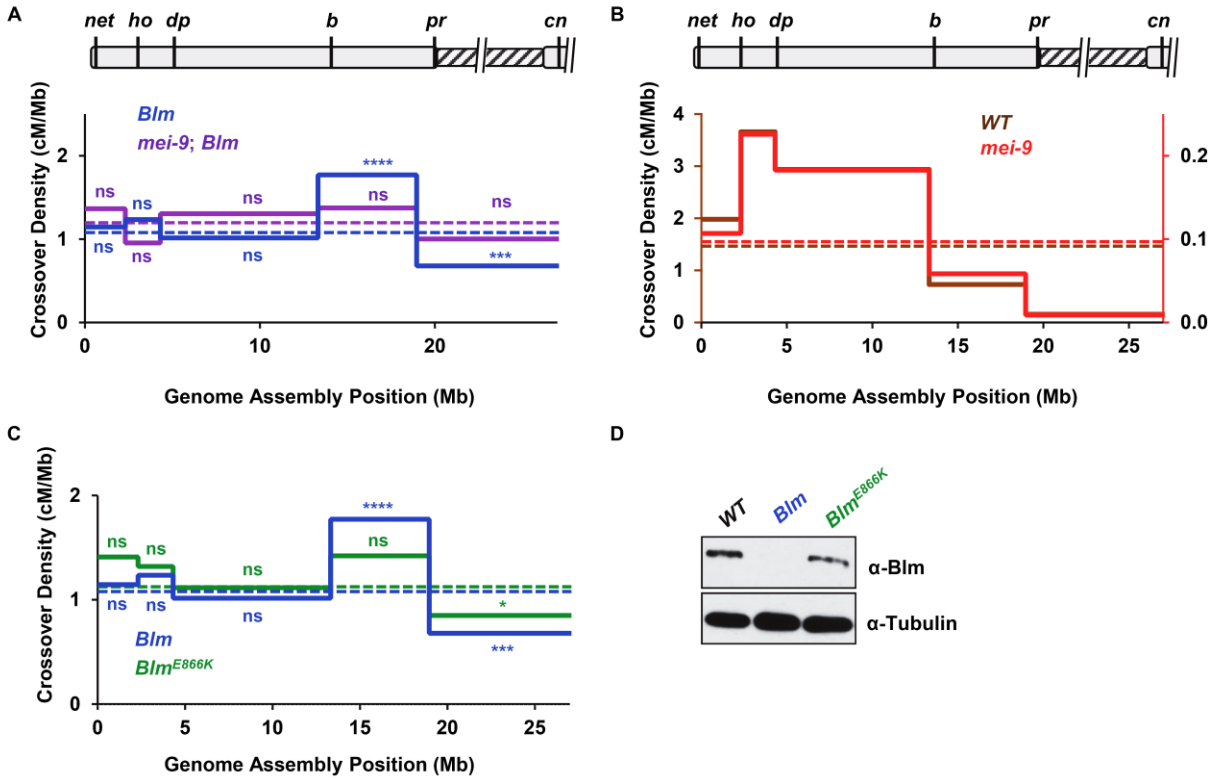


Figure S1. Crossover distributions of various genetic backgrounds. Related to Figure 1. Crossover distribution on *2L* in various mutants. (A) *Blm* single mutants (*n*=1171) compared to *mei-9*; *Blm* double mutants (*n*=1047). Dotted line is average density across the region assayed. (B) Wild type (*n*=4222) compared to *mei-9* single mutants (*n*=2433). Note that *mei-9* is graphed on a second Y-axis with a different scale. (C) *Blm* null mutants compared to Blm^{E866K} helicase-dead mutants (*n*=1136). (D) Western blot showing Blm protein in wild-type, *Blm* null (the genotype used in other experiments but without post-recombination expression of Blm), and Blm^{E866K}. Indicators of statistical significance in A-C are for chi-squared test on observed number of crossovers versus expected number if proportional to physical size: ns, $P>0.05$; *$P < 0.05$; ***$P <0.001$; ****$P <0.0001$ after correction for multiple comparisons.

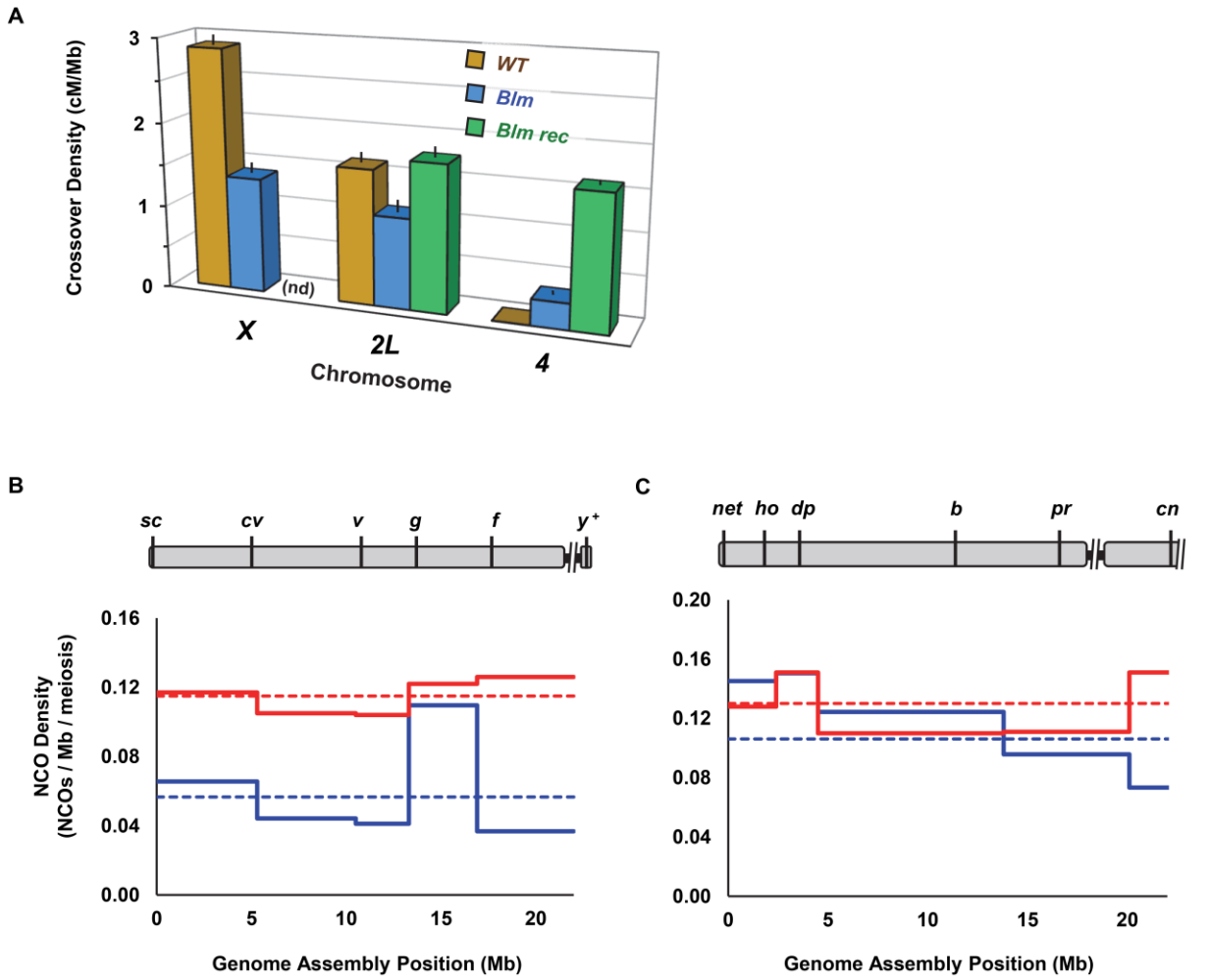
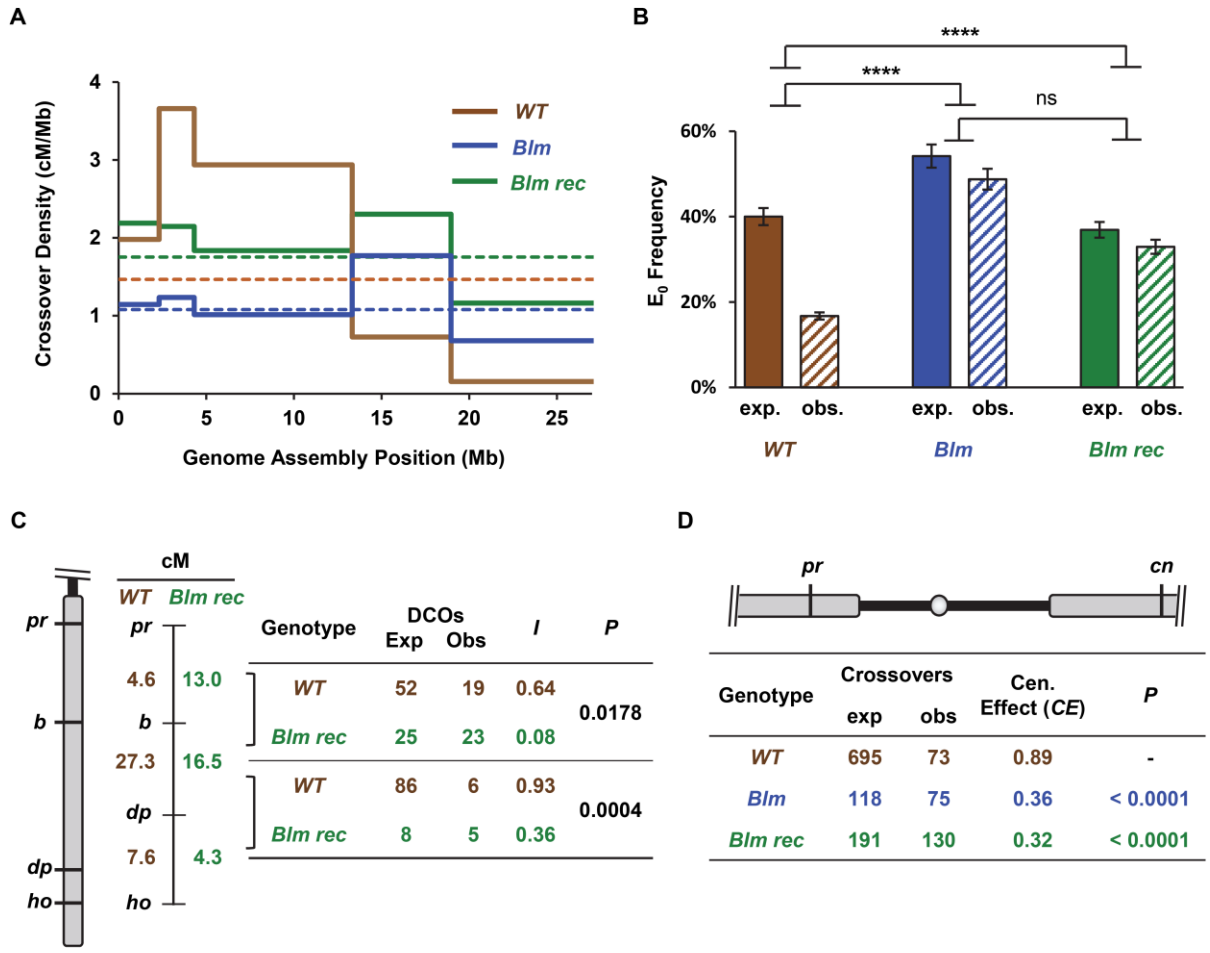


Figure S2. Crossover density and distribution of noncrossovers. Related to Figure 2. (A) Average crossover density across each chromosome arm assayed. The *X* was not assayed in *Blm rec* double mutants. Error bars are 95% confidence intervals. (B,C) Distribution of non-crossover gene conversion events on the *X* chromosome (A) and chromosome *2L* (B). Data from Comeron *et al.* [S8] (red lines) were provided as maximum likelihood probabilities of noncrossover gene conversion per meiosis in 100 kb windows. We took the mean probability across each of our crossover intervals and converted to noncrossovers per Mb per meiosis. Data from Miller *et al.* [S9] (blue lines) were actual number of noncrossovers detected among 196 meioses. We summed the number observed in each of our crossover intervals, multiplied by four to account for the recovery of only one of the four chromatids per meiosis, multiplied by 1.89 to correct for the number not observed because they did not include a SNP (using the Miller *et al.* estimation of 549 expected but only 291 observed; note that their SNP density was much lower on the *X* chromosome, so the correction here probably underestimates the true frequency), and then converted to noncrossovers per Mb per meiosis. Neither of these distributions is significantly different from the expectation of a distribution proportional to physical length, and they are not significantly different from one another (by G-test for goodness of fit, using the actual number of events counted in the Miller *et al.* dataset [34 on the *X*, 78 on *2L*] and the expected number observed in 196 progeny based on the Comeron *et al.* values).



Genotype	DCOs Exp	DCOs Obs	*I*	*P*
WT	52	19	0.64	0.0178
Blm rec	25	23	0.08	
WT	86	6	0.93	0.0004
Blm rec	8	5	0.36	

Genotype	Crossovers exp	Crossovers obs	Cen. Effect (*CE*)	*P*
WT	695	73	0.89	-
Blm	118	75	0.36	< 0.0001
Blm rec	191	130	0.32	< 0.0001

Figure S3. Analysis of *Blm rec* double mutants. Related to Figure 3. (A) Crossover distribution on *2L*. Data for wild-type (*WT*) and *Blm* are the same as in Figure 2B. (B) Crossover assurance on *2L*. Analysis is identical to that presented in Figure 3A, except these data are from the *2L* region we analyzed. Although this analysis is limited to one arm instead of one chromosome, consideration of karyotype evolution in different *Drosophila* species [S10] suggests that crossover assurance may have evolved using the arm as the unit of measurement. Excluding the *pr*-*cn* interval did not change the outcome of this analysis. (C) Interference. Analysis is identical to that presented in Figure 1B, except these data are from *2L*. Because of the reduction in crossovers in *Blm* single mutants, there were not enough predicted double crossovers to measure interference in these mutants. (D) Centromere effect. Data for *WT* and *Blm* are the same as in Figure 1C.

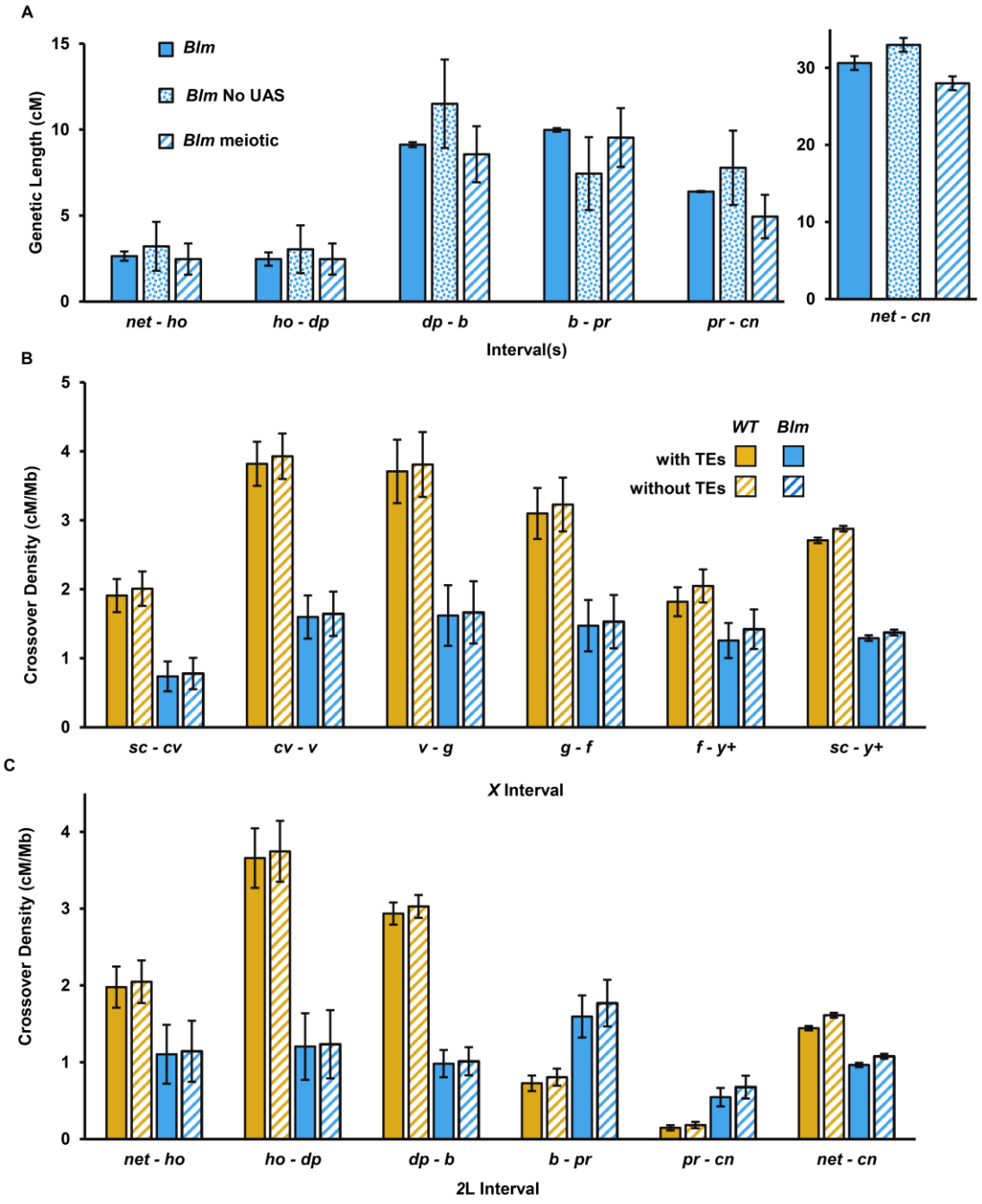


Figure S4. Effects of *Blm* genetics and including or excluding transposable elements. Related to Figure 2. (A) Different *Blm* genotypes. Graphs show genetic distance (cM) for *2L* intervals (right: sum of all intervals). *Blm* = with *UASp::Blm* and *matα::GAL4* to express Blm after recombination is completed (*n* = 1171). This is the genotype used in all other experiments; *Blm* No UAS = *Blm* without the *UAS::Blm* expression system (*n* = 591, since most embryos fail to develop); *Blm* meiotic = *Blm* (with *UAS::Blm*) adjusted by subtracting the mitotic crossovers recovered in *Blm mei-P22* mutants (*n* = 1659). Raw data are in Table S2. (B,C) Graphs show crossover density when TEs are included (solid) or excluded (hatched) for (B) *X* chromosome and (C) *2L*. Error bars are 95% confidence intervals. Raw data are in Table S3.

Progeny		Maternal Genotype						
		WT	*Blm*	*mei-9*	*mei-9*; *Blm*	*Blm mei-P22*	Blm^{E866K}	*Blm rec*
Parental		2376	844	2366	720	1602	806	705
SCO	1 (*net-ho*)	176	23	6	23	4	32	31
	2 (*ho-dp*)	290	22	11	15	2	26	29
	3 (*dp-b*)	1099	89	40	104	14	96	136
	4 (*b-pr*)	154	104	8	74	10	74	103
	5 (*pr-cn*)	39	61	2	84	24	72	87
DCO	1-2	1	1	0	0	0	1	0
	1-3	11	4	0	7	0	2	11
	1-4	10	2	0	0	0	0	7
	1-5	2	1	0	1	0	2	0
	2-3	6	1	0	1	0	0	5
	2-4	7	2	0	2	0	0	7
	2-5	13	2	0	1	0	1	2
	3-4	19	5	0	1	0	8	19
	3-5	17	7	0	9	0	6	21
	4-5	2	2	0	3	3	7	8
TCO		0	1	0	2	0	2	9
n		4222	1171	2433	1047	1659	1136	1181

Table S1. Meiotic crossovers on chromosome *2L*. Related to Figures 1 and 2. Each row lists the number of total progeny from parental and single (SCO), double (DCO), or triple (TCO) crossover classes for each genotype listed at the top of each column. The bottom row lists the total number of progeny in each genotype. Overall crossover densities from these data are shown in Figures 1A, S2A, and S4. Distributions are graphed in Figures 2B and S4C.

Progeny		Maternal Genotype	
		WT	*Blm*
Parental		2133	4415
SCO	1 (*sn*-*v*)	346	147
	2 (*v*-*g*)	305	175
	3 (*g*-*os*)	300	211
DCO	1-2	4	5
	1-3	22	11
	2-3	5	7
TCO		0	0
n		3088	4953

Table S2. Meiotic crossovers on chromosome *X* used to measure interference. Related to Figure 1. Each row lists the number of total progeny from parental and single (SCO), double (DCO), or triple (TCO) crossover classes for each genotype listed at the top of each column. The bottom row lists the total number of progeny in each genotype. These data are summarized in Figure 1B.

Progeny Classes		Maternal Genotype	
		WT	*Blm*
Parental		1015	801
SCO	1(*sc-cv*)	148	37
	2 (*cv-v*)	333	73
	3 (*v-g*)	162	40
	4 (*g-f*)	155	47
	5 (*f-y*$^+$)	166	70
DCO	1-2	5	2
	1-3	18	2
	1-4	20	1
	1-5	29	1
	2-3	25	4
	2-4	44	3
	2-5	26	10
	3-4	6	2
	3-5	14	2
	4-5	10	4
TCO		3	0
n		2179	1099

Table S3. Meiotic crossovers on chromosome *X*. Related to Figure 2. Each row lists the number of total progeny from parental and single (SCO), double (DCO), or triple (TCO) crossover classes for each genotype listed at the top of each column. The bottom row lists the total number of progeny in each genotype. Overall crossover densities from these data are shown in Figures S2A and S4. Crossover distributions are shown in Figures 2A and S4B.

Progeny		Maternal Gentoype		
		WT	*Blm*	*mei-P22 Blm*
Parental	+ +	1728	1716	1648
	ci sv	1384	1380	1597
Crossover	*ci* +	0	6	0
	+ *sv*	0	4	0
	n	3112	3106	3245

Table S4. Meiotic crossovers on chromosome *4*. Related to Figure 2. Each row lists the number of total progeny from parental (*P*) and crossover (CO) classes for each genotype listed at the top of each column. The bottom row lists the total number of progeny in each genotype. For P and CO, the + symbol indicates wild-type for a marker, while the gene name indicates mutant for a marker, in the order along the chromosome (*ci sv*). These data are summarized in Figure 2C and S2A.

Supplemental Experimental Procedures

***Drosophila* stocks.** Flies were maintained on standard medium at 25°C. Heteroallelic and homozygous experimental flies for null mutations that have been previously described: Blm^{N1} and Blm^{D2} (ref 20); $mei\text{-}9^{a}$ (ref 28). The Blm^{E866K} mutant is heteroallelic for Blm^{N1} and Blm^{D3} (E866K, in the DEAH motif) [S1]. Most embryos from *Blm* null females die [S2], making it difficult to get enough progeny to score in recombination experiments. To overcome this lethality, we used transgenes that express Blm protein after the conclusion of meiotic recombination [S3]. We also scored progeny of *Blm* mutants that did not have this transgene; the results were similar with and without the transgene (Figure S3).

Pre-meiotic germline crossovers are significantly elevated in *Blm* mutant males [S4]. To determine whether this is true in the female germline, we eliminated meiotic DSBs with mutations in *mei-P22* (Figure S4). There were a small number of crossovers that presumably occurred in the pre-meiotic germline; correcting for these did not affect our conclusions.

Genetic assays. *X*-chromosome NDJ was determined by mating virgin females with *y cv v f / T(1:Y)*B^{S} males and scoring for viable exceptional progeny: *XXY* females have *Bar* eyes. *X*O males have Bar^{+} eyes and the phenotypes associated with the *y cv v f* chromosome. NDJ rates and statistical comparisons were done as in Zeng *et al*. [S5].

Crossovers on chromosome *2L* were measured by crossing virgin *net* $dpp^{d\text{-}ho}$ *dp b pr cn* /+ females (1-5 days old) of desired mutant backgrounds to *net* $dpp^{d\text{-}ho}$ *dp b pr cn* homozygous males and scoring the progeny for all markers. Crossovers on the *X* were measured by crossing virgin *y sc cv v g f* y^{+}/ + females (1-5 days old) crossed to *y sc cv v g f / Y* males. Crossovers on chromosome *4* were measured by crossing virgin ci^{1} sv^{n} / + females in the described genetic backgrounds to ci^{1} sv^{n} males and scoring the progeny. All crossovers on chromosome *4* were verified in progeny tests (for those that were fertile) and through PCR using the following primers: ci^{1}: GCTAATGATGATGCTTCAATCTGC and GGACACATGCTGTGCTTCTACAG; sv^{n}: CAGGTGTTCATTTACCGTCATCC and GCTACCAGGCGAATTCTAC. Primer combinations resulted in an amplified product in the presence of the wild-type allele of each respective gene.

Virgin *sn v* g^{2} $Df(1)os^{0}$, $upd1^{os\text{-}o}$ / + females of various genetic backgrounds were mated with *sn v* g^{2} $Df(1)os^{0}$, $upd1^{os\text{-}o}$ males and the subjected values were counted. Crossover values were used to calculate coefficient of coincidence (*c*) and interference (*I*) [S6]. Fisher's exact test (two-tailed) was used to determine significance between observed and expected double-crossover frequencies (GraphPad QuickCalcs; http://www.graphpad.com/quickcalcs/).

To analyze recombination histories of exceptional progeny, virgin *y sc cv v f* y^{+}/+ (Cross I) or *y sc cv v g f* y^{+} / + (Cross II) in a *Blm* mutant background were crossed to *y sc cv v f / T(1:Y)*B^{S} (Cross I) or *y sc cv v g f / T(1:Y)*B^{S} (Cross II) males. Virgin exceptional *XXY* female progeny with *Bar* eyes were collected and crossed to *y sc cv v f* (Cross I) or *y sc cv v g f* (Cross II) males. Assessment of the exceptional progeny genotype was performed as previously described [S7].

Recombination calculations. Genetic distances are expressed in centiMorgans (cM), which is 100 *(R / n), where R is number of recombinant progeny in an interval (including single crossover, double-crossover, etc.) and n is total number of progeny scored. 95% confidence intervals were calculated from variance, as in Stevens [S7].

Molecular distances are from the positions of genetic markers on the *Drosophila melanogaster* reference genome, release 6.12. In cases where specific mutations were not previously described, we used the midpoint of the gene. Release 6 adds several Mb of sequence to the right end of *X* and *2L* and the left end of *2R*. These pericentromeric sequences have a high transposable element (TE) content (24.2% of the assembled sequence between *pr* and *cn*). Meiotic crossovers are partially or completely excluded from TEs in yeast, maize, and mice[31-33]. We do not know whether this is true for *Drosophila*[14,34], so we calculated crossover density and the centromere effect with TEs included and with TEs excluded (Figure S4). We did this for chromosome *4* crossovers also (22.3% TE). We used the calculations without TEs in the main text, but including TE distances did not affect the conclusions. To compare crossover density between genotypes we conducted G tests for goodness of fit, and χ^2 for post-hoc tests in each interval.

For crossover assurance analysis, the equations of Weinstein[35] were applied to the *X* crossover dataset to convert the number of observed parental and single, double, and triple crossover progeny to bivalent exchange ranks (E_0, E_1, E_2, and E_3). Expected numbers of each exchange rank were obtained from a Poisson distribution in which the average number of events was the average number of crossovers on this chromosome per meiosis (total cM/50) and the Poisson random variable was equal to the exchange rank. The number of observed and expected was compared using two-tailed Fisher's Exact Test.

SDS-PAGE and immunoblotting. Young virgin females (<3 days old) of desired genotypes were fattened overnight with yeast paste and males. 25 ovaries of each genotype were dissected in 1xPBS and homogenized using a pestle in 100µl of 1x protein sample buffer diluted with water from 4x stock concentration (10% glycerol, 2% SDS, 0.1% bromophenol blue, 0.3 ml 1M Tris-HCl pH 6.8). 5µl of DTT was added to each sample prior to heating at 95°C for 5 minutes. 10µl of each sample was loaded into 6% SDS-Polyacrylamide gels, and proteins were resolved at 120V. Proteins were transferred to PVDF (Bio-Rad) overnight at 35V. After transfer, the membrane was incubated for 1 hour with blocking solution (5% milk in 1xPBST). The membrane was then incubated with primary antibodies diluted in blocking solution overnight in 4°C and subsequently washed 3x for 7 minutes in 1xPBST. The membrane was incubated with secondary antibody at 1:10,000 in blocking solution for 45 minutes at room temperature prior to incubation with chemiluminescent substrate (Thermo Scientific) for 5 minutes. The membrane was then exposed to film.

Blm protein was detected using rabbit α-Blm antibody generated against amino acids 536-635 in the N-terminal half of Blm at a concentration of 1:10,000. Mouse α-tubulin was used at 1:20,000 (Sigma). Secondary goat α-rabbit or mouse HRP-conjugated antibodies (Santa Cruz) were used at 1:10,000.

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