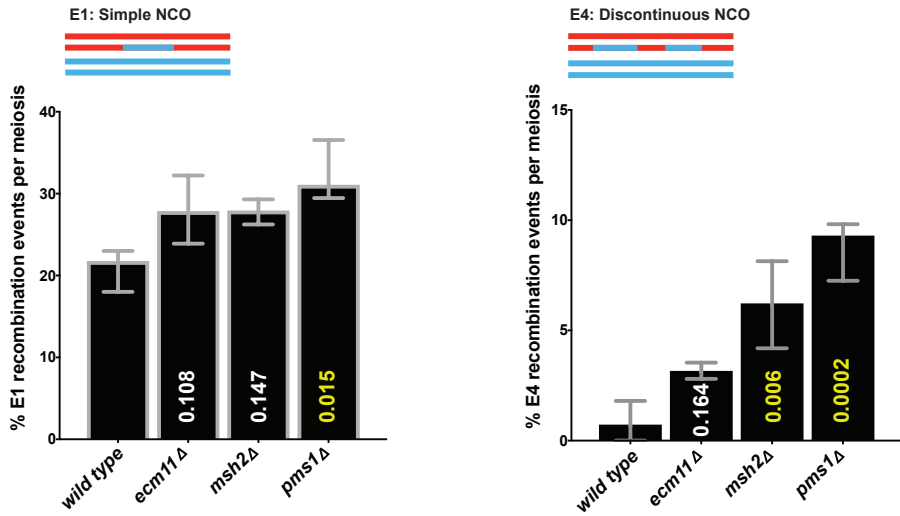


Figure S1

A



B

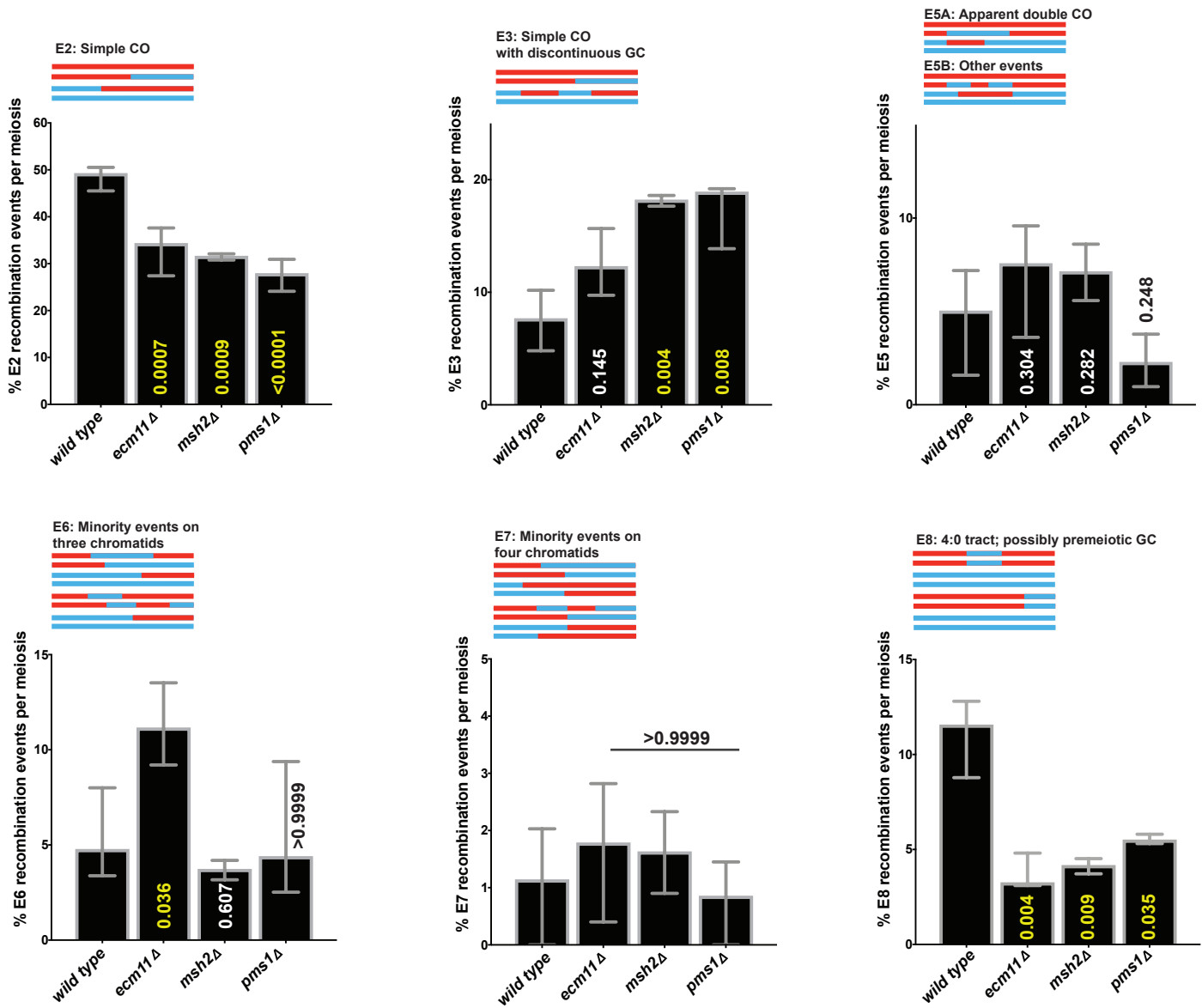


Figure S1. Genome-wide sequence analysis of *ecm11* meiotic products indicates a role for SC central element in limiting both majority and minority interhomolog recombination events.

(Relates to Figure 2)

Bar graphs indicate the proportion of specific interhomolog recombination events found in four wild type, five *ecm11*, two *msh2*, and three *pms1* Octad Rec-Seq datasets; median and range values for each strain are plotted. *P* values obtained by Fisher's Exact test (indicated in yellow if significant, white if not significant) indicate whether the average proportion of a particular class of recombination events in each mutant is significantly different from the average proportion found in wild type. Recombination event categories are described in Methods, and data plotted in these graphs is listed in Table 2. Note chromosome 7 recombination events were excluded for all octads, due to chromosome 7 disomy in two wild-type samples.

Figure S2

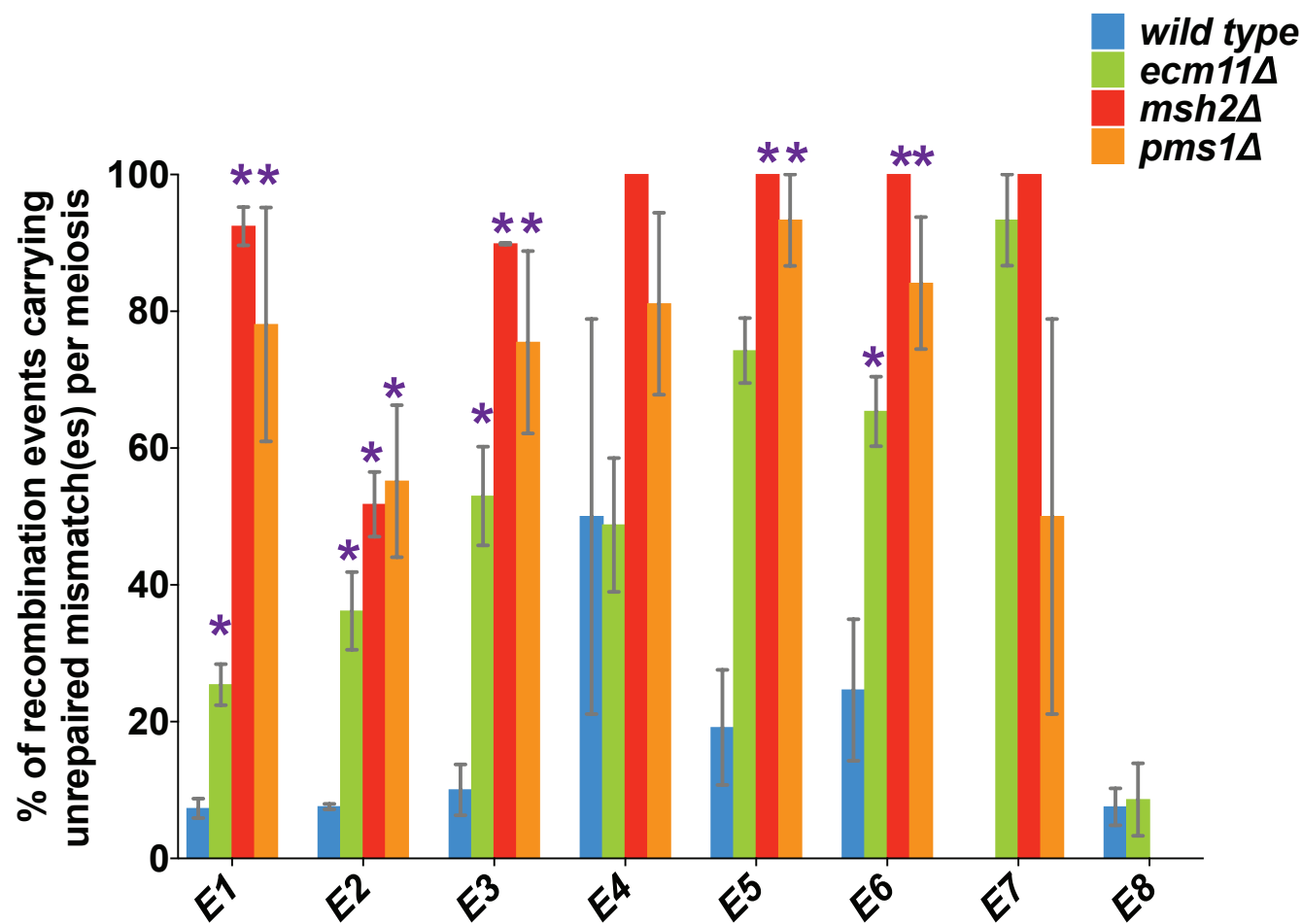


Figure S2. *ecm11* mutants exhibit an increased number of unrepaired mismatch-carrying events in several categories of recombination.

(Relates to Figure 3)

Column graph plots the median and range percentage of specific classes of recombination events (indicated on the x axis) that display unrepaired mismatches in four wild-type (blue), five *ecm11* (green), two *msh2* (red), and three *pms1* (orange) octads. For each class of recombination event, strains showing significant differences relative to wild type are labeled with a purple asterisk (two-tailed *P* values obtained using a Fisher's Exact test). The proportion of E1 recombination events with unrepaired mismatches for *ecm11*, *msh2*, and *pms1* are significantly increased relative to wild type ($P=0.045$, <0.0001 , <0.0001 respectively). The proportion of E2 and E3 recombination events with unrepaired mismatches is also significantly increased in *ecm11*, *msh2*, and *pms1* ($P <0.0001$ for E2 in each strain; $P=0.04$, <0.0001 , 0.0007 respectively, for E3). The proportion of E5 recombination events with unrepaired mismatches is not significantly different between wild-type and *ecm11* ($P=0.69$), but is significantly different between wild-type and *msh2*, or *pms1* ($P=0.0006$, and 0.028 respectively). Finally, the proportion of E6 recombination events with unrepaired mismatches is significantly different from wild-type in *ecm11*, *msh2*, and *pms1* ($P=0.045$, 0.007 , 0.019 , respectively). Note chromosome 7 recombination events for two wild-type octads were excluded from the analysis due to chromosome 7 disomy.

Figure S3

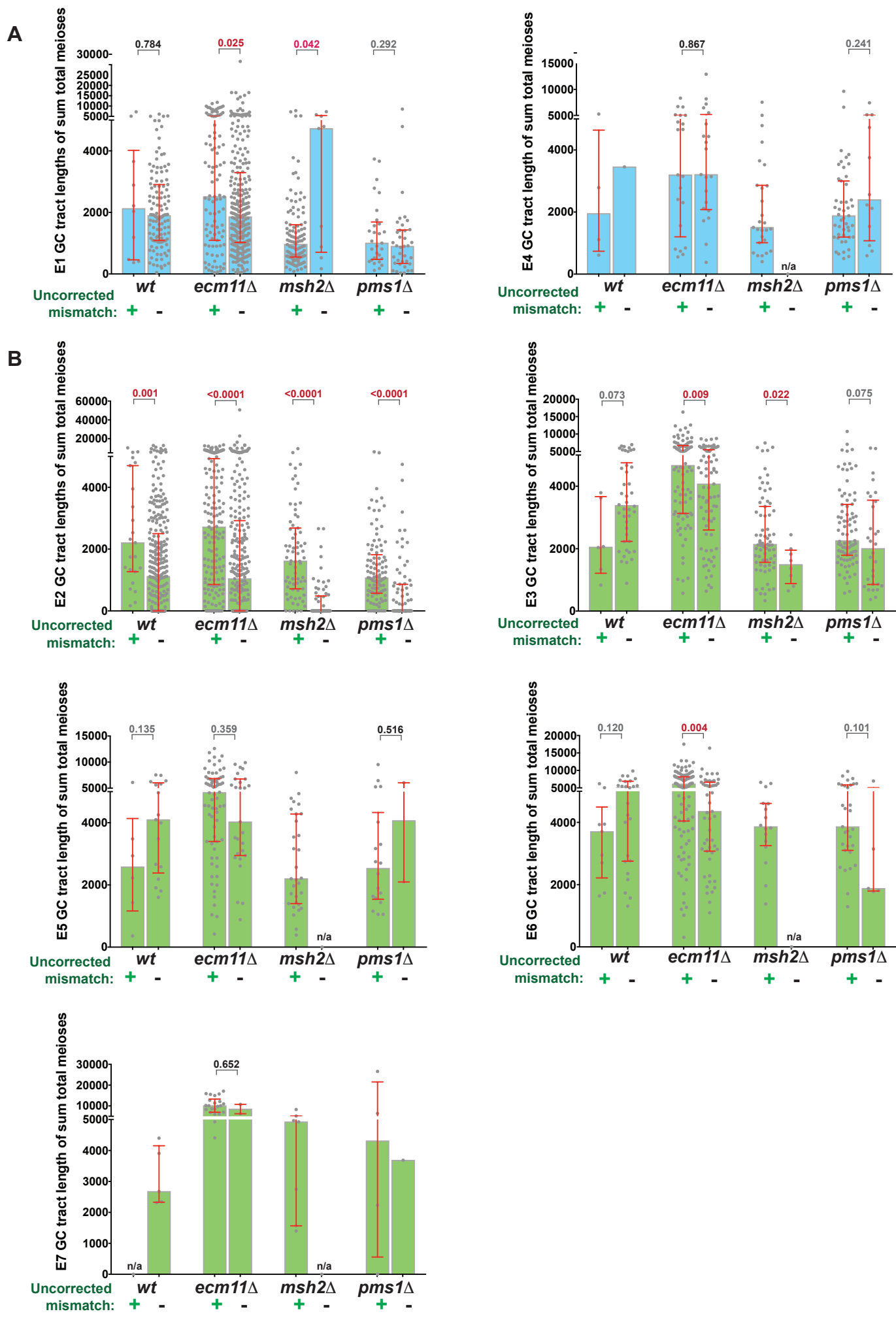


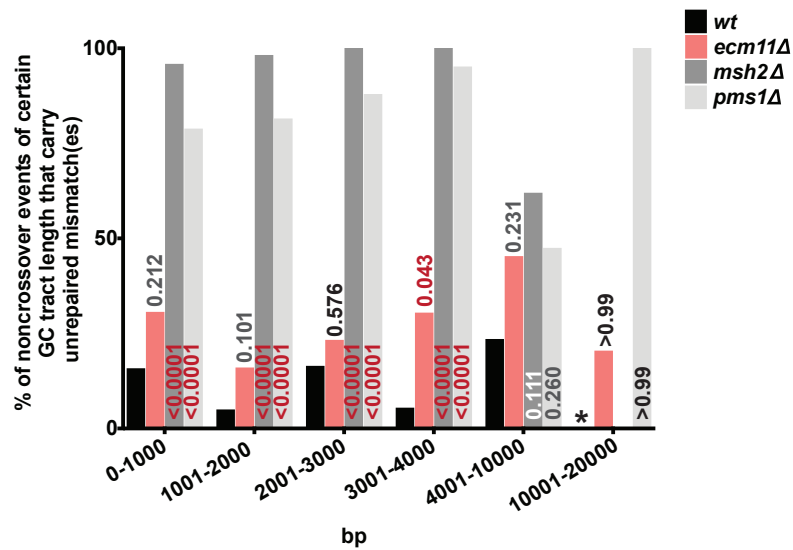
Figure S3. Gene conversion tracts with unrepaired mismatches often average longer than their mismatch-free counterparts in *ecm11* mutants.

(Relates to Figure 4)

Each circle on the scatterplots represents a gene conversion tract length (# nucleotides) associated with a noncrossover (light blue shading, **A**) or crossover (green shading, **B**) class of recombination events. Total gene conversion tract lengths with each type of recombination signature (E1-E7) from four wild-type, five *ecm11*, two *msh2*, and four *pms1* meioses (Octad Rec-Seq datasets) are plotted in separate columns depending on whether the event carries an unrepaired mismatch (indicated below with a + or -; strains listed on the *x* axis). Height of the shaded area indicates a median value for each group, while red error bars indicate interquartile range. Two-tailed *P* values using a Mann-Whitney test are indicated above the error bars (red if significant). Note chromosome 7 recombination events for two wild-type octads were excluded from these analyses, due to chromosome 7 disomy.

Figure S4

A



B

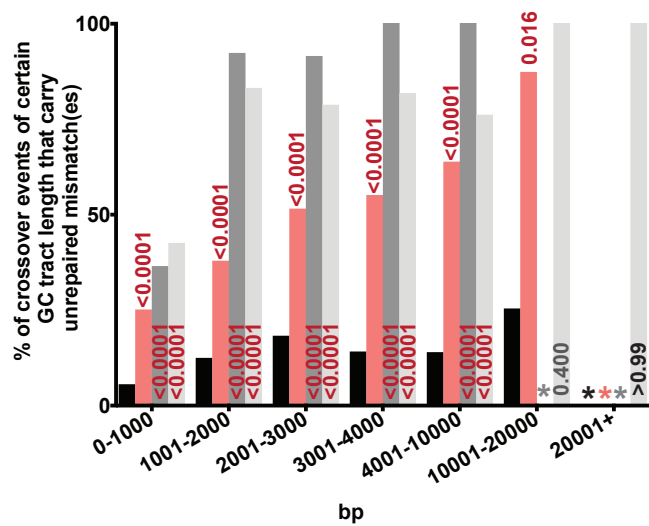


Figure S4. Elevated frequency of unrepaired mismatches in *ecm11* mutants regardless of gene conversion tract length.

(Relates to Figure 5, Table S2)

Column graphs plot the percentage of unrepaired mismatch-carrying noncrossover (**A**) or crossover (**B**) events with gene conversion tracts of distinct length ranges (indicated on the *x* axis). Data plotted is consolidated from all genome-wide recombination events identified in four wild-type (black bars), five *ecm11* (pink bars), two *msh2* (gray bars) and three *pms1* (silver bars) meioses (Octad Rec-Seq datasets). For each category of tract length, a Fisher's Exact test was used to determine whether the proportion of unrepaired mismatch-carrying recombination events is significantly different in any of the mutant strains relative to wild-type (*P* values are indicated on the graph; red indicates statistical significance). The proportion of noncrossovers carrying an unrepaired mismatch in *ecm11* is only significantly greater than wild-type in the 3001-4000 tract length category, but the proportion of crossovers carrying an unrepaired mismatch in *ecm11* is significantly greater than wild-type for every tract length category. The raw data plotted in (**A**, **B**) is given in Table S2. Note chromosome 7 recombination events for two wild-type octads were excluded from these analyses, due to chromosome 7 disomy.

Figure S5

SNP density among total events (regardless of unrepaired mismatch)

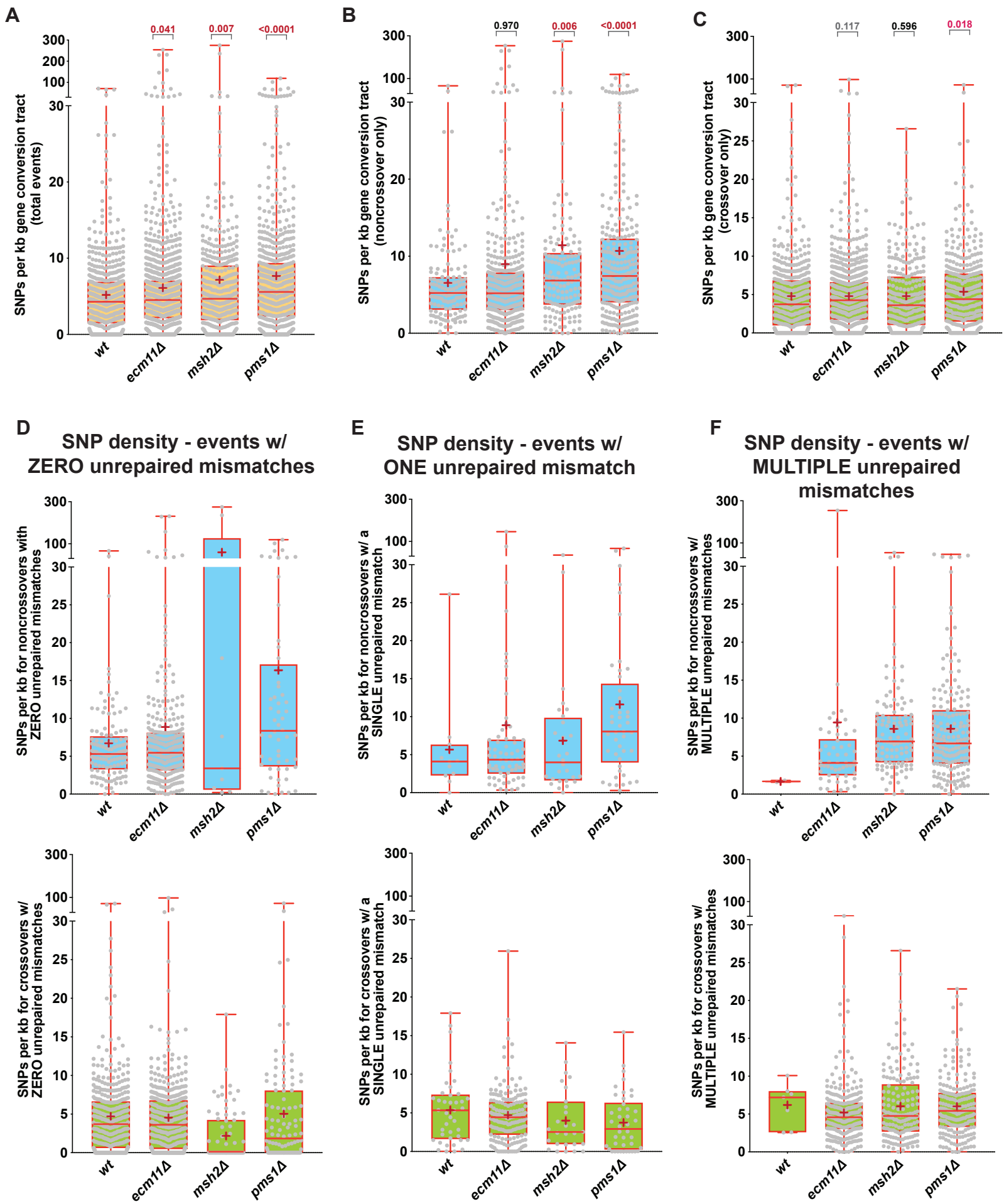
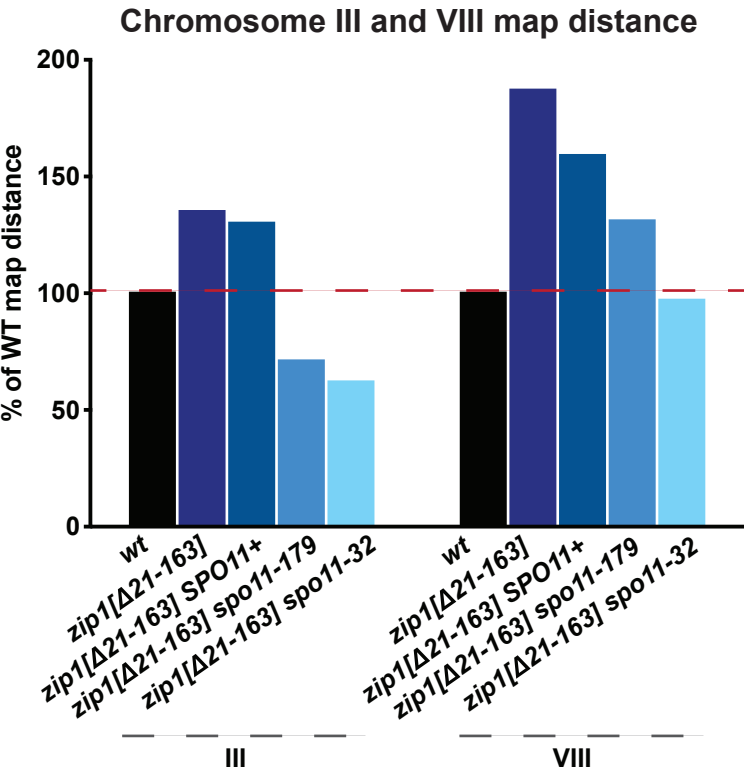


Figure S5. SNP density in *ecm11* gene conversion tracts is similar to wild type.

Grey circles in each box whisker plot represents a single nucleotide polymorphism (SNP) density measurement (SNP per kilobase) for individual gene conversion tracts in four wild-type, five *ecm11*, two *msh2* and three *pms1* meioses. The shaded box encompasses the 25th to 75th percentile (middle 50%) of values, red bar within the shaded area indicates the median, red vertical bars indicate the range, and red + indicates the mean for each genotype. Graphs in (A-C) plot SNP density values for total interhomolog recombination events regardless of the presence of an unrepaired mismatch (A, yellow shading), for all noncrossover (E1 + E4) events regardless of an unrepaired mismatch (B, blue shading), and for all crossover (E2, E3, E5, E6, E7) events regardless of an unrepaired mismatch (C, green shading). *P* values to described statistical differences between mutant and wild type SNP densities, calculated using the Mann Whitney test. are indicated above mutant columns; see Results narrative for additional *P* values calculated after particular high SNP density outliers are removed (relevant for the total *ecm11* and *msh2* noncrossover tract comparisons). Graphs in (D-F) plot SNP density values of noncrossover (blue shading, upper) or crossover (green shading, lower) events that display zero (D), one (E), or more than one (F) unrepaired mismatch. For *ecm11*, the population of tracts with a single unrepaired mismatch do not show a strongly significant increase in SNP density relative to the population of tracts with zero unrepaired mismatches (*P* calculated using the Mann Whitney test = 0.05 for crossovers, *P* = 0.07 for noncrossovers), and the population of tracts with multiple unrepaired mismatches do not show a strongly significant increase in SNP density relative to the population of tracts with a single unrepaired mismatch (*P* = 0.15 for crossovers, *P* = 0.96 for noncrossovers). Note chromosome 7 recombination events for two wild-type octads were excluded from these analyses, due to chromosome 7 disomy.

Figure S6

A



B

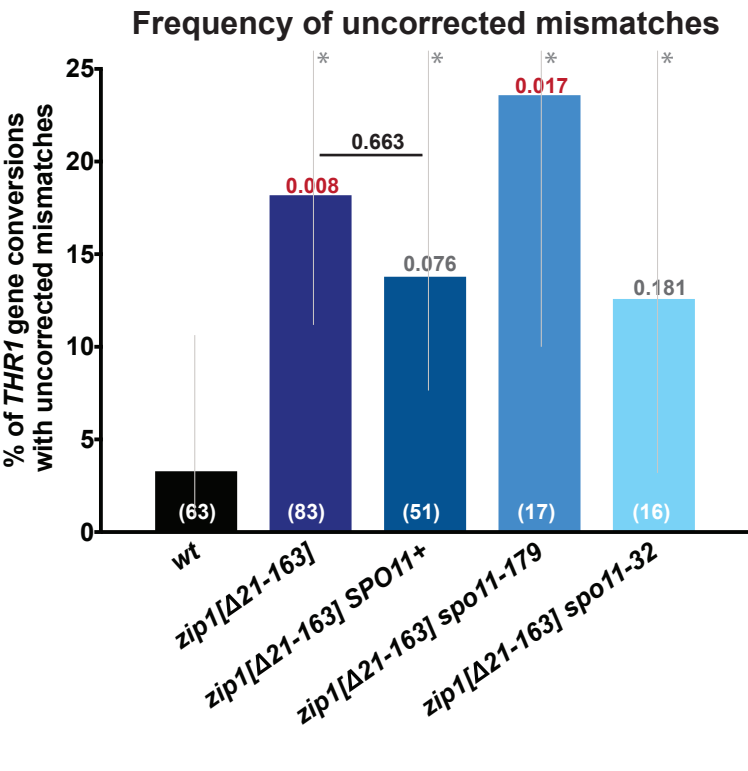


Figure S6. The mismatch repair defect apparent in SC-deficient mutants is not likely due to saturation of mismatch repair machinery caused by elevated DNA double strand breaks.

Graph in (A) plots the genetic map length of chromosome III (left cluster) and chromosome VIII (right cluster), in *zip1[Δ21-163]* diploids homozygous for either *SPO11+* (navy bar) or two different *spo11* hypomorphic alleles (light blue bars). Five genetic markers spanning chromosome III and four genetic markers, including *THR1/thr1-4*, along the length of chromosome VIII were used to calculate map distances (See Table S1 for full genetic map data). Summed genetic map distances between all intervals on III and VIII are displayed as a percentage of the wild-type value. A reduction in the genetic map length relative to both *zip1[Δ21-163] SPO11+* and wild type is observed in each strain carrying a *spo11* hypomorphic allele. Graph in (B) plots the percentage of *THR1* gene conversion-associated tetrads with a phenotypically sectoried colony on SC-Thr (a non-conventional gene conversion event (post-meiotic segregation) reflecting an unrepaired mismatch at the *THR1* locus), for each of the *zip1[Δ21-163]* strains homozygous for *spo11* reduction-of-function alleles as well as the control (*SPO11+*) strain. The total number of tetrads (meioses) observed with nonmendelian segregation (conventional or non-conventional) at *THR1* is indicated (in white) at the bottom of the corresponding bar for each strain. A two-tailed *P* value for each set of mutant data compared with wild type, calculated using a Fisher's Exact test, is indicated above individual columns (values in red are considered statistically significant, grey not quite significant, and black not significant). All three CRISPR-Cas9 manipulated *zip1[Δ21-163]* strains display elevated frequencies of post-meiotic segregation at *THR1*, although the increase is statistically significant (using Fishers Exact) only in the *zip1[Δ21-163] spo11-179* strain. The minimal and maximal values defining 95% confidence intervals for each proportion are indicated by grey bars; *the maximal values for *zip1[Δ21-163]* carrying *SPO11+*, *spo11-179* and *spo11-32* alleles are 26%, 47%, 36%, respectively. *P* values from one-way ANOVA analysis of variance, followed by the Dunnett's test for multi-comparisons of every mean to the wild type control, are as follows (left to right, beginning with *zip1[Δ21-163]*):, *P*=0.030, 0.300, 0.095, 0.750. See Table 4 for additional detail regarding the frequency of gene conversion at *THR1* and characteristic features of post-meiotic segregation events in these strains.

Figure S7

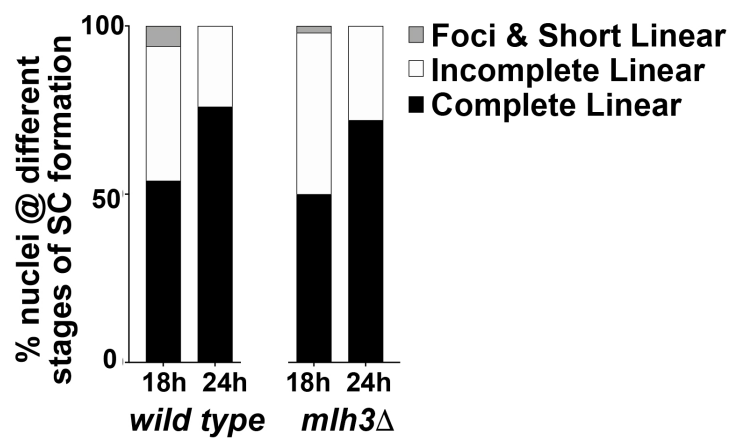
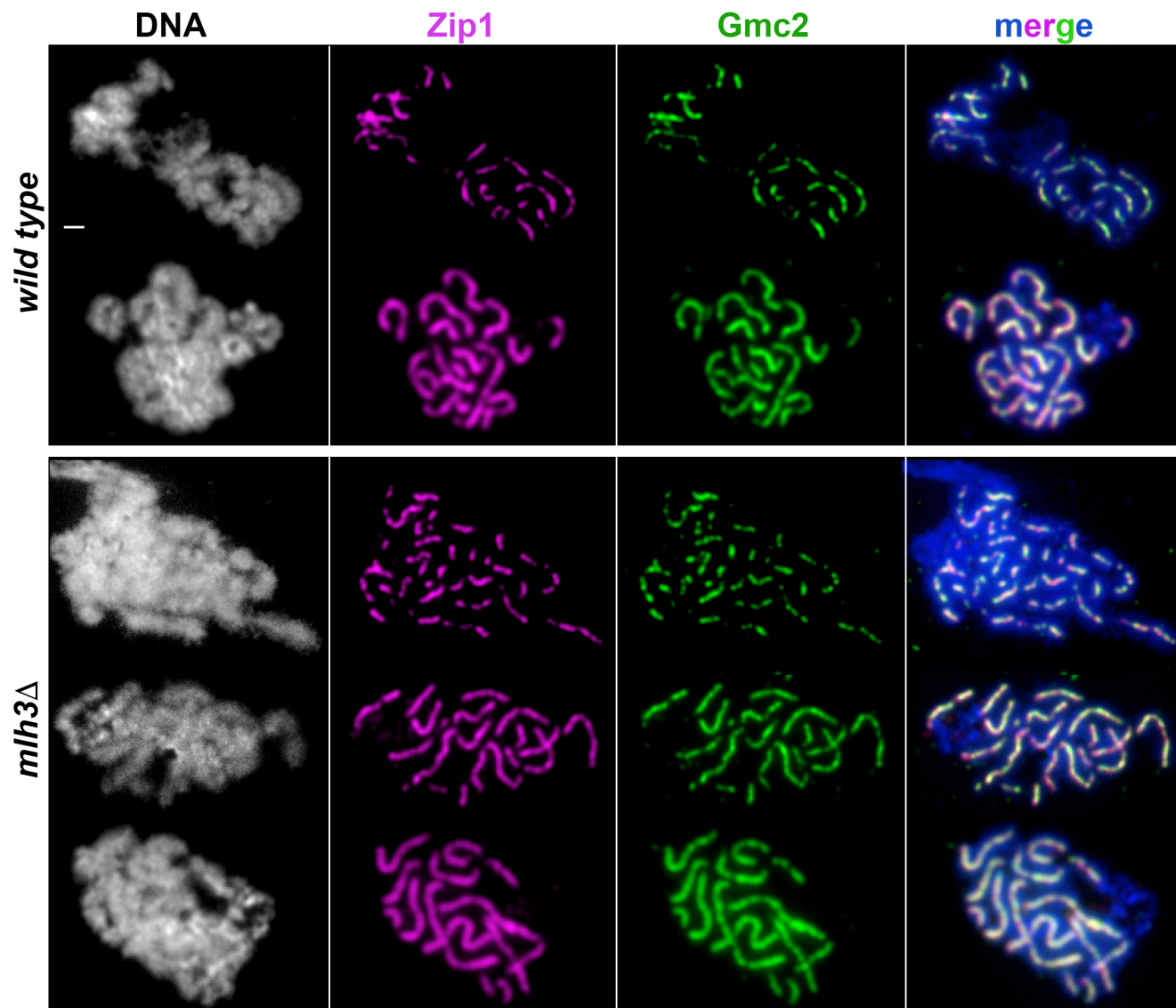
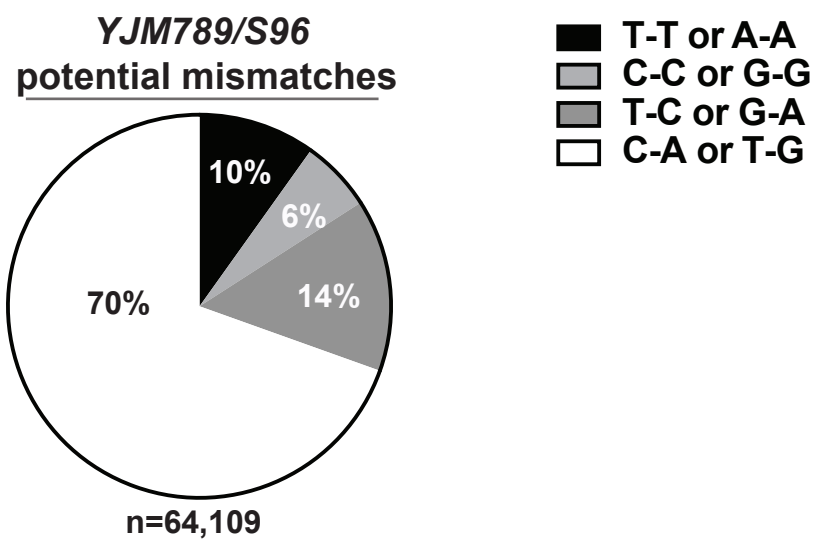


Figure S7. SC assembles with normal timing in *mlh3* mutant meiocytes.

Images show examples of surface spread mid-meiotic prophase nuclei from *MLH3* (top two rows) and *mlh3* mutant (bottom three rows) strains. Both strains are missing the transcription factor Ndt80, to ensure that all sporulating cells, which progress through meiosis at variable rates in this strain background, halt progression at late prophase with maximal SC length. For each genotype, structures consisting of coincident Zip1 (magenta) and Gmc2 (green) were evaluated in 50 nuclei at 18 and 24 hours after placement in sporulation media. As indicated in the stacked bar graph, wild-type and *mlh3* strains at both time points displayed similar proportions of nuclei with complete SC (dark shading, second, fourth and fifth row of example images), intermediate SC (white shading, top and third row of example images) or only SC protein foci on chromatin (grey shading). Scale bar, 1 μ m.

Figure S8



Unrepaired mismatches observed:

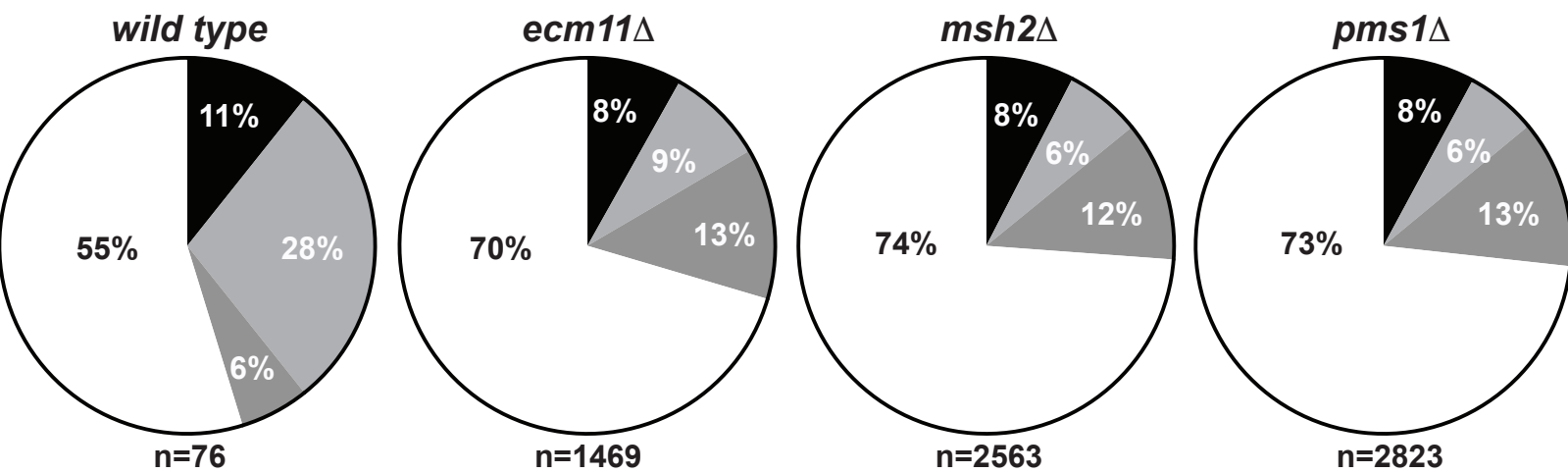


Figure S8. All mismatch types appear equally susceptible to repair failure in *ecm11*, *msh2* and *pms1* meioses.

Pie graphs display the proportion of distinct types of mismatches within each population. The upper graph gives the proportion of every potential meiotic heteroduplex mismatch type, based on the 64,109 SNPs present between the YJM789 and S96 genomic sequences. The lower graphs display the proportion of each mismatch type among PMS events (unrepaired mismatches) in four wild-type, five *ecm11*, two *msh2* and three *pms1* octad Rec-Seq datasets. Data from individual octads within each genotype are summed. Mismatch types were identified using the YJM and S96 nucleotide sequence at the coordinate of each unrepaired mismatch. If the DNA sequence coordinate in “mom” within an octad bears a “T” (the YJM nucleotide), whereas its cognate “daughter” instead bears an “A” (the S96 nucleotide), the meiotic heteroduplex is inferred to have carried an A-A or a T-T mismatch. Classes include A-A/T-T (black), C-C/G-G (light grey), T-C/G-A (dark grey), and C-A/T-G (white). Percentages are indicated over corresponding shaded components of the graphs, and below the graph is indicated the total number of mismatches classified (n) for each genotype. Note chromosome 7 recombination events for two wild-type octads were excluded from these analyses due to chromosome 7 disomy.

Table S1. Genetic map distances of strains analyzed for post-meiotic segregation at *THR1*

GENOTYPE (STRAIN)	INTERVAL (CHROMOSOME)	PD	TT	NPD	TOTAL	cM (± SE)	%WT	cM by chrM	%WT by chrM	viability
WT (K842) *	<i>HIS4-CEN3</i> (III)	584	528	10	1122	26.2 (1.1)	100	103.7 (III)	100	97%
	<i>CEN3-MAT</i> (III)	708	416	4	1128	19.5 (0.9)	100			
	<i>MAT-RAD18</i> (III)	412	676	18	1106	35.4 (1.3)	100	75.2 (VIII)	100	
	<i>RAD18-HMR</i> (III)	651	454	8	1113	22.6 (1.0)	100			
	<i>SPO11-SPO13</i> (VIII)	453	630	33	1116	37.1 (1.6)	100			
	<i>SPO13-THR1</i> (VIII)	913	180	2	1095	8.8 (0.7)	100			
	<i>THR1-LYS2</i> (VIII)	490	590	8	1088	29.3 (1.0)	100			
ecm11Δ (K857) *	<i>HIS4-CEN3</i> (III)	464	375	5	844	24.0 (1.1)	92	134.2 (III)	129	92%
	<i>CEN3-MAT</i> (III)	416	431	13	860	29.6 (1.4)	152			
	<i>MAT-RAD18</i> (III)	263	495	29	787	42.5 (2.0)	120	115.8 (VIII)	154	
	<i>RAD18-HMR</i> (III)	315	457	25	797	38.1 (1.9)	169			
	<i>SPO11-SPO13</i> (VIII)	342	446	40	828	41.4 (2.2)	112			
	<i>SPO13-THR1</i> (VIII)	480	272	8	760	21.1 (1.4)	240			
	<i>THR1-LYS2</i> (VIII)	216	469	53	738	53.3 (2.7)	182			
ecm11[K5R,K101R] (K1123/K1124)	<i>HIS4-CEN3</i> (III)	181	229	5	415	31.2 (1.9)	119	160.5 (III)	155	93%
	<i>CEN3-MAT</i> (III)	175	227	11	413	35.5 (2.5)	182			
	<i>MAT-RAD18</i> (III)	105	259	27	391	53.8 (3.6)	152	130.7 (VIII)	174	
	<i>RAD18-HMR</i> (III)	157	240	15	412	40.0 (2.8)	177			
	<i>SPO11-SPO13</i> (VIII)	117	275	30	422	53.9 (3.5)	145			
	<i>SPO13-THR1</i> (VIII)	243	134	3	380	20.0 (1.8)	227			
	<i>THR1-LYS2</i> (VIII)	95	242	29	366	56.8 (3.9)	194			
gmc2Δ (K906) *	<i>HIS4-CEN3</i> (III)	224	237	7	468	29.8 (1.9)	114	154.4 (III)	149	92%
	<i>CEN3-MAT</i> (III)	212	247	9	468	32.2 (2.1)	165			
	<i>MAT-RAD18</i> (III)	139	280	23	442	47.3 (3.0)	134	120.3 (VIII)	160	
	<i>RAD18-HMR</i> (III)	119	311	15	445	45.1 (2.5)	200			
	<i>SPO11-SPO13</i> (VIII)	188	251	19	458	39.9 (2.8)	108			
	<i>SPO13-THR1</i> (VIII)	239	180	7	426	26.1 (2.1)	297			
	<i>THR1-LYS2</i> (VIII)	103	277	28	408	54.3 (3.5)	185			
zip1[Δ21-163] (AF6) **	<i>HIS4-CEN3</i> (III)	263	311	10	584	31.8 (1.8)	121	139.9 (III)	135	89%
	<i>CEN3-MAT</i> (III)	242	331	13	586	34.9 (1.9)	179			
	<i>MAT-RAD18</i> (III)	208	329	17	554	38.9 (2.2)	110	124.5 (VIII)	166	
	<i>RAD18-HMR</i> (III)	231	320	11	562	34.3 (1.9)	152			
	<i>SPO11-SPO13</i> (VIII)	182	332	44	558	53.4 (3.2)	144			
	<i>SPO13-THR1</i> (VIII)	325	194	3	522	20.3 (1.4)	231			
	<i>THR1-LYS2</i> (VIII)	162	329	34	525	50.8 (3.0)	173			
ecm11Δ zip1[Δ21-163] (AF5)	<i>HIS4-CEN3</i> (III)	230	252	11	493	32.3 (2.1)	123	143.1 (III)	138	93%
	<i>CEN3-MAT</i> (III)	186	306	11	503	37.0 (2.1)	190			
	<i>MAT-RAD18</i> (III)	187	274	14	475	37.7 (2.4)	106	120.9 (VIII)	161	
	<i>RAD18-HMR</i> (III)	193	272	12	477	36.1 (2.2)	160			
	<i>SPO11-SPO13</i> (VIII)	144	308	30	482	50.6 (3.1)	136			
	<i>SPO13-THR1</i> (VIII)	276	177	2	455	20.8 (1.4)	236			
	<i>THR1-LYS2</i> (VIII)	145	280	28	453	49.5 (3.2)	169			
msh2Δ (AM4003)	<i>HIS4-CEN3</i> (III)	62	77	3	142	33.5 (3.9)	128	118.6 (III)	114	78%
	<i>CEN3-MAT</i> (III)	98	43	1	142	17.3 (2.8)	89			
	<i>MAT-RAD18</i> (III)	36	97	4	137	44.2 (4.2)	125	73.5 (VIII)	98	
	<i>RAD18-HMR</i> (III)	85	55	2	142	23.6 (3.4)	104			
	<i>SPO11-SPO13</i> (VIII)	59	82	4	145	36.6 (4.2)	99			
	<i>SPO13-THR1</i> (VIII)	120	22	0	142	7.8 (1.5)	88			
	<i>THR1-LYS2</i> (VIII)	64	77	1	142	29.2 (2.8)	100			
pms1Δ (AM4004)	<i>HIS4-CEN3</i> (III)	14	7	0	21	16.7 (5.1)	64	107.6 (III)	104	76%
	<i>CEN3-MAT</i> (III)	12	10	0	22	22.7 (5.3)	116			
	<i>MAT-RAD18</i> (III)	9	11	2	22	52.3 (17.5)	148	85.2 (VIII)	113	
	<i>RAD18-HMR</i> (III)	15	7	0	22	15.9 (5.0)	70			
	<i>SPO11-SPO13</i> (VIII)	5	14	1	20	50.0 (13.7)	135			
	<i>SPO13-THR1</i> (VIII)	17	5	0	22	11.4 (4.5)	130			
	<i>THR1-LYS2</i> (VIII)	11	10	0	21	23.8 (5.5)	81			
zip1[Δ21-163] SPO11+ (K1765) (CRISPR <i>SPO11</i> allele)	<i>HIS4-CEN3</i> (III)	186	207	9	402	32.5 (2.4)	124	134.2 (III)	129	92%
	<i>CEN3-MAT</i> (III)	156	243	6	405	34.4 (2.0)	176			
	<i>MAT-RAD18</i> (III)	160	213	13	386	37.7 (2.8)	106	60.6 (VIII)	159	
	<i>RAD18-HMR</i> (III)	195	191	7	393	29.6 (2.2)	130			
	<i>SPO13-THR1</i> (VIII)	233	142	2	377	20.4 (1.6)	232			
	<i>THR1-LYS2</i> (VIII)	124	244	10	378	40.2 (2.5)	137			
zip1[Δ21-163] spo11-179 (K1830) (CRISPR <i>spo11</i> allele)	<i>HIS4-CEN3</i> (III)	460	167	0	627	13.3 (0.9)	51	73.7 (III)	71	82%
	<i>CEN3-MAT</i> (III)	426	205	2	633	17.1 (1.1)	87			
	<i>MAT-RAD18</i> (III)	288	334	6	628	29.5 (1.5)	83	50.0 (VIII)	131	
	<i>RAD18-HMR</i> (III)	465	162	2	629	13.8 (1.1)	61			
	<i>SPO13-THR1</i> (VIII)	523	94	0	617	7.6 (0.7)	86			
	<i>THR1-LYS2</i> (VIII)	189	407	19	615	42.4 (2.1)	144			
zip1[Δ21-163] spo11-32 (K1767) (CRISPR <i>spo11</i> allele)	<i>HIS4-CEN3</i> (III)	821	188	6	1015	11.0 (0.9)	42	64.2 (III)	62	65%
	<i>CEN3-MAT</i> (III)	814	185	9	1008	11.9 (1.1)	61			
	<i>MAT-RAD18</i> (III)	448	539	10	997	30.0 (1.2)	85	36.9 (VIII)	97	
	<i>RAD18-HMR</i> (III)	824	173	9	1006	11.3 (1.1)	50			
	<i>SPO13-THR1</i> (VIII)	906	97	0	1003	4.8 (0.5)	55			
	<i>THR1-LYS2</i> (VIII)	429	558	14	1001	32.1 (1.3)	109			

GENOTYPE (STRAIN)	INTERVAL (CHROMOSOME)	PD	TT	NPD	TOTAL	cM (± SE)	%WT	cM by chrM	%WT by chrM	viability
zip3Δ (K926) **	HIS4-CEN3 (III)	323	161	10	494	22.4 (2.1)	85	87.1 (III)	84	81%
	CEN3-MAT (III)	328	167	5	500	19.7 (1.6)	101			
	MAT-RAD18 (III)	319	154	10	483	22.2 (2.1)	63			
	RAD18-HMR (III)	306	174	8	488	22.8 (2.0)	101			
	SPO11-SPO13 (VIII)	336	120	7	463	17.5 (1.9)	47	35.8 (VIII)	48	
	SPO13-THR1 (VIII)	429	44	1	474	5.3 (0.9)	60			
	THR1-LYS2 (VIII)	369	98	4	471	13.0 (1.5)	44			
msh4Δ (K852) *	HIS4-CEN3 (III)	375	96	1	472	10.8 (1.1)	41	53.4 (III)	51	71%
	CEN3-MAT (III)	425	51	1	477	6.0 (0.9)	31			
	MAT-RAD18 (III)	276	184	7	467	24.2 (1.9)	68			
	RAD18-HMR (III)	352	116	0	468	12.4 (1.0)	55			
	SPO11-SPO13 (VIII)	365	89	3	457	11.7 (1.4)	32	30.4 (VIII)	40	
	SPO13-THR1 (VIII)	423	27	0	450	3.0 (0.6)	34			
	THR1-LYS2 (VIII)	319	129	2	450	15.7 (1.4)	54			
zip3Δ msh4Δ (AM3658/AM3659) **	HIS4-CEN3 (III)	353	194	10	557	22.8 (1.9)	87	92.3 (III)	89	79%
	CEN3-MAT (III)	368	193	8	569	21.2 (1.7)	109			
	MAT-RAD18 (III)	344	177	11	532	22.8 (2.0)	64			
	RAD18-HMR (III)	330	210	12	552	25.5 (2.0)	113			
	SPO11-SPO13 (VIII)	360	179	8	547	20.8 (1.8)	56	42.5 (VIII)	57	
	SPO13-THR1 (VIII)	464	64	1	529	6.6 (0.9)	75			
	THR1-LYS2 (VIII)	392	129	5	526	15.1 (1.5)	52			
ecm11Δ msh4Δ (K882) *	HIS4-CEN3 (III)	358	51	0	409	6.2 (0.8)	24	49.6 (III)	48	58%
	CEN3-MAT (III)	383	28	1	412	4.1 (1.0)	21			
	MAT-RAD18 (III)	237	153	5	395	23.2 (2.0)	66			
	RAD18-HMR (III)	292	105	4	401	16.1 (1.8)	71			
	SPO11-SPO13 (VIII)	339	56	0	395	7.1 (0.9)	19	36.5 (VIII)	49	
	SPO13-THR1 (VIII)	341	35	0	376	4.7 (0.8)	53			
	THR1-LYS2 (VIII)	244	118	11	373	24.7 (2.8)	84			
mlh3Δ (K854) *	HIS4-CEN3 (III)	663	285	2	950	15.6 (0.9)	60	63.1 (III)	61	86%
	CEN3-MAT (III)	764	185	3	952	10.7 (0.8)	55			
	MAT-RAD18 (III)	526	398	11	935	24.8 (1.3)	70			
	RAD18-HMR (III)	725	213	2	940	12.0 (0.8)	53			
	SPO11-SPO13 (VIII)	604	330	10	944	20.7 (1.2)	32	31.4 (VIII)	42	
	SPO13-THR1 (VIII)	871	67	0	938	3.6 (0.4)	41			
	THR1-LYS2 (VIII)	648	282	2	932	15.8 (0.9)	54			
mlh3Δ zip3Δ (K1046)	HIS4-CEN3 (III)	156	44	2	202	13.9 (2.5)	53	74.4 (III)	72	74%
	CEN3-MAT (III)	145	63	4	212	20.5 (3.1)	105			
	MAT-RAD18 (III)	135	60	2	197	18.3 (2.6)	52			
	RAD18-HMR (III)	121	74	2	197	21.7 (2.7)	96			
	SPO11-SPO13 (VIII)	143	55	1	199	15.3 (2.1)	41	40.8 (VIII)	54	
	SPO13-THR1 (VIII)	172	19	0	191	5.0 (1.1)	57			
	THR1-LYS2 (VIII)	126	59	3	188	20.5 (3.1)	70			
mlh3Δ msh4Δ (K1049)	HIS4-CEN3 (III)	292	51	1	344	8.3 (1.3)	32	52.8 (III)	51	54%
	CEN3-MAT (III)	308	44	0	352	6.3 (0.9)	32			
	MAT-RAD18 (III)	211	128	4	343	22.2 (2.1)	63			
	RAD18-HMR (III)	234	110	0	344	16.0 (1.3)	71			
	SPO11-SPO13 (VIII)	243	90	2	335	15.2 (1.7)	41	37.5 (VIII)	50	
	SPO13-THR1 (VIII)	291	25	1	317	4.9 (1.2)	56			
	THR1-LYS2 (VIII)	231	80	5	316	17.4 (2.4)	59			
ecm11Δ mlh3Δ (AM3896/ AM3897/K888) *	HIS4-CEN3 (III)	836	296	10	1142	15.6 (1.0)	60	87.9 (III)	85	89%
	CEN3-MAT (III)	839	302	8	1149	15.2 (1.0)	78			
	MAT-RAD18 (III)	527	464	34	1025	32.6 (1.7)	92			
	RAD18-HMR (III)	635	381	21	1037	24.5 (1.4)	108			
	SPO11-SPO13 (VIII)	689	401	22	1112	24.0 (1.4)	65	75.7 (VIII)	101	
	SPO13-THR1 (VIII)	794	213	8	1015	12.9 (1.0)	147			
	THR1-LYS2 (VIII)	439	473	45	957	38.8 (2.0)	132			
K. lactis ZIP1 (AS1)	HIS4-CEN3 (III)	285	207	13	505	28.2 (2.3)	108	101.5 (III)	98	77%
	CEN3-MAT (III)	308	211	9	528	25.1 (1.9)	129			
	MAT-RAD18 (III)	310	181	12	503	25.1 (2.2)	71			
	RAD18-HMR (III)	316	188	8	512	23.1 (1.9)	102			
	SPO11-SPO13 (VIII)	281	196	25	502	34.5 (2.9)	93	66.0 (VIII)	88	
	SPO13-THR1 (VIII)	374	99	0	473	10.5 (0.9)	119			
	THR1-LYS2 (VIII)	310	157	7	474	21.0 (1.9)	72			
mlh3Δ K. lactis ZIP1 (AS19)	HIS4-CEN3 (III)	317	208	13	538	26.6 (2.1)	102	99.0 (III)	95	72%
	CEN3-MAT (III)	320	220	10	550	25.5 (1.9)	131			
	MAT-RAD18 (III)	321	200	8	529	23.4 (1.8)	66			
	RAD18-HMR (III)	327	195	9	531	23.5 (1.9)	104			
	SPO11-SPO13 (VIII)	289	212	20	521	31.9 (2.6)	86	59.7 (VIII)	79	
	SPO13-THR1 (VIII)	412	85	0	497	8.6 (0.8)	98			
	THR1-LYS2 (VIII)	352	137	9	498	19.2 (2.0)	66			

At least some data for strains marked * or ** were previously published in (VOELKEL-MEIMAN et al. 2016)*, or (VOELKEL-MEIMAN et al. 2019)**

Table S1. Genetic map distances of strains analyzed for post-meiotic segregation at *THR1*.

(Relates to Figures 1, 7, S6)

Map distances were calculated using tetrad analysis as described previously (VOELKEL-MEIMAN *et al.* 2019) in strains heterozygous for the following nine alleles: *his4-260,519*, *hphMX@CEN3*, *MATa*, *ADE2@RAD18*, *natMX@HMR* (on chromosome III); *TRP1+@SPO11*, *spo13::URA3+*, *thr1-4*, *LYS2@210kb* (on chromosome VIII). Four spore-viable tetrads with no more than seven gene conversion (non-2:2) events were included in calculations. See Tables 1 and 4 for *THR1* gene conversion and post-meiotic segregation frequencies. Table indicates map distances and their corresponding percentages of the wild-type values for individual intervals, and the corresponding percentage of wild-type cumulative chromosomal map distance for chromosome III and VIII. Some data from this table is plotted in Figure S6. At least some of the data for strains marked * or ** were previously published in (VOELKEL-MEIMAN *et al.* 2016)*, or (VOELKEL-MEIMAN *et al.* 2019)**.

Table S2. The frequency of unrepaired mismatches among recombination events with different gene conversion tract lengths

A	% of noncrossover GC tracts of different lengths (n) that carry an unrepaired mismatch						
	0-1000 bp	1001-2000bp	2001-3000bp	3001-4000bp	4001-10,000bp	10,001-20,000bp	20,000+bp
<i>wt</i>	15.4 (26)	4.6 (44)	16.0 (25)	5.0 (20)	23.1 (13)	n/a (0)	n/a (0)
<i>ecm11</i>	30.2 (96)	15.6 (109)	22.9 (70)	30.0 (40)	44.9 (98)	20.0 (10)	0 (1)
<i>msh2</i>	95.5 (66)	97.8 (45)	100.0 (17)	100.0 (7)	61.5 (13)	n/a (0)	n/a (0)
<i>pms1</i>	78.4 (125)	81.1 (74)	87.5 (40)	94.7 (19)	47.1 (17)	100.0 (1)	n/a (0)

B	% of crossover GC tracts of different lengths (n) that carry an unrepaired mismatch						
	0-1000 bp	1001-2000bp	2001-3000bp	3001-4000bp	4001-10,000bp	10,001-20,000bp	20,000+bp
<i>wt</i>	5.2 (136)	12.0 (83)	17.9 (56)	13.7 (51)	13.6 (59)	25.0 (4)	n/a (0)
<i>ecm11</i>	24.7 (186)	37.5 (104)	51.1 (88)	54.7 (106)	63.5 (342)	86.9 (46)	0 (2)
<i>msh2</i>	36.1 (97)	91.9 (99)	91.1 (45)	100.0 (30)	100.0 (38)	n/a (0)	n/a (0)
<i>pms1</i>	42.2 (121)	82.8 (87)	78.3 (60)	81.4 (43)	75.8 (33)	100.0 (1)	0 (1)

Table S2. Frequency of unrepaired mismatches among recombination events with different gene conversion tract lengths.

(Relates to Figure S4)

The percentage of unrepaired mismatch-carrying noncrossover (**A**) or crossover (**B**) events with specific gene conversion tract lengths. Data is consolidated from all genome-wide recombination events identified in four wild-type, five *ecm11*, two *msh2* and three *pms1* meioses (Octad Rec-Seq datasets). For each category of gene conversion tract lengths, a Fisher's Exact test was used to determine whether the proportion of mismatch-carrying recombination events is significantly different in any of the mutant strains relative to wild-type (*P* values are indicated on the Figure S4 graphs; red indicates statistical significance).

Table S3. The per-nucleotide frequency of unrepaired mismatches in wild-type, *ecm11*, *msh2*, and *pms1* meioses

A NONCROSSOVER EVENTS							
	Unrepaired mismatch - containing GC tract length (cumulative bp)	Total unrepaired mismatches among all events	Unrepaired mismatches per bp of GC tract, for mismatch-carrying events	Fishers Exact, two sided <i>P</i>	Mismatch-free GC tract length (cumulative bp)	Unrepaired mismatches per bp of GC tract, for all events	Chi square with Yates Correction, two sided <i>P</i>
<i>wild type</i>	35309	17	4.81E-04		243858	6.09E-05	
<i>ecm11</i>	416823	255	6.12E-04	0.428	844793	2.02E-04	<0.0001
<i>msh2</i>	207732	802	3.86E-03	<0.0001	30807	3.36E-03	<0.0001
<i>pms1</i>	345315	1194	3.46E-03	<0.0001	99791	2.68E-03	<0.0001

B CROSSOVER EVENTS							
	Unrepaired mismatch - containing GC tract length (cumulative bp)	Total unrepaired mismatches among all events	Unrepaired mismatches per bp of GC tract, for mismatch-carrying events	Chi square with Yates Correction, two sided <i>P</i>	Mismatch-free GC tract length (cumulative bp)	Unrepaired mismatches per bp of GC tract, for all events	Chi square with Yates Correction, two sided <i>P</i>
<i>wild type</i>	127351	59	4.63E-04		789499	6.44E-05	
<i>ecm11</i>	2270528	1214	5.35E-04	0.311	1322763	3.38E-04	<0.0001
<i>msh2</i>	494207	1761	3.56E-03	<0.0001	37193	3.31E-03	<0.0001
<i>pms1</i>	605311	1629	2.69E-03	<0.0001	145097	2.17E-03	<0.0001

Table S3. Per-nucleotide frequency of unrepaired mismatches in wild-type, *ecm11*, *msh2*, and *pms1* meioses.

(Relates to Figure 5)

Table lists the per-nucleotide frequency of unrepaired mismatches identified among the sum total of noncrossover (A) and crossover (B) recombination events in four wild type, five *ecm11*, two *msh2*, and three *pms1* Octad Rec-Seq datasets. In the columns to the left of the vertical midline, an unrepaired mismatch per-nucleotide frequency *exclusive to mismatch-carrying events* was calculated by dividing the total number of unrepaired mismatches found in a given genotype by the cumulative gene conversion tract length of events carrying an unrepaired mismatch in that strain. In columns on the right of the midline, the mismatch per nucleotide frequency for *all events* was calculated by dividing the total number of unrepaired mismatches by the cumulative gene conversion tract length of all events. Chi square with Yates Correction analysis found that defective mismatch repair events are significantly more frequent among both noncrossover and crossover events in *ecm11* relative to wild-type. However, among those select recombination events susceptible to inefficient mismatch repair, the unrepaired mismatch frequency is not significantly different between *ecm11* and wild-type (according to a Fisher's Exact test or Chi square with Yates Correction). See Figure 5 legend for additional statistical analysis. Note chromosome 7 recombination events for two wild-type octads were excluded from these analyses, due to chromosome 7 disomy.

Table S4. Strains used in this study

GENOTYPE	
YAM1252	<u>lys2ΔNhe</u> <u>his4-260,519</u> <u>leu2-3,112</u> <u>MATα</u> <u>trp1-289</u> <u>ura3-1</u> <u>thr1-4</u> <u>ade2-1</u> lys2 Δ Nhe his4-260,519 leu2-3,112 MAT α trp1-289 ura3-1 thr1-4 ade2-1
K842	<u>lys2ΔNhe</u> <u>HIS4</u> <u>leu2-3,112</u> <u>hphMX4@CEN3</u> <u>MATα</u> <u>ADE2@RAD18</u> <u>natMX4@HMR</u> lys2 Δ Nhe his4-260,519 leu2-3,112 CEN3 MAT α RAD18 HMR <u>trp1-289</u> <u>ura3-1</u> <u>TRP1MX4@SPO11</u> <u>spo13::URA3</u> <u>THR1</u> <u>210kb</u> <u>ade2-1</u> trp1-289 ura3-1 SPO11 SPO13 thr1-4 LYS2@210kb ade2-1
K857	K842 homozygous <i>ecm11::LEU2</i>
K1123/ K1124	K842 homozygous <i>ecm11</i> [K5R, K101R]
K906	K842 homozygous <i>gmc2::kanMX4</i>
AF6	K842 homozygous <i>zip1</i> [Δ 21-163]
AF5	AF6 <i>ecm11::kanMX4</i>
AM4003	K842 homozygous <i>msh2::kanMX4</i>
AM3697	<u>lys2ΔNhe</u> <u>HIS4</u> <u>leu2-3,112</u> <u>hphMX4@CEN3</u> <u>MATα</u> <u>ADE2@RAD18</u> <u>natMX4@HMR</u> LYS2 his4-260,519 leu2-3,112 CEN3 MAT α RAD18 HMR <u>trp1-289</u> <u>URA3</u> <u>TRP1MX4@SPO11</u> <u>THR1</u> <u>210kb</u> <u>msh2::kanMX4</u> <u>ade2-1</u> trp1-289 ura3-1 SPO11 thr1-4 LYS2@210kb msh2::kanMX4 ade2-1
AM3696	AM3697 homozygous <i>MSH2 pms1::kanMX4 natMX4@HMR</i>
AM4004	K842 homozygous <i>pms1::kanMX4</i>
Octad Rec-Seq experiments	
AM3740	<u>YJM789 (YEM045 isolate)</u> <u>MATα</u> <u>ho::his6</u> <u>AMN1[A1103T]</u> YJMS96 MAT α ho::his6 AMN1
AM3735	AM3740 homozygous <i>ecm11::kanMX4</i>
AM3736	AM3740 homozygous <i>msh2::hphMX4</i>
AM3738	AM3740 homozygous <i>pms1::hphMX4</i>
K926	K842 homozygous <i>zip3::kanMX4</i>
K852	K842 homozygous <i>msh4::kanMX4</i>
AM3658/ AM3659	K842 homozygous <i>zip3::kanMX4 msh4::LEU2</i>
K882	K857 homozygous <i>msh4::kanMX4</i>
K854	K842 homozygous <i>mlh3::kanMX4</i>
K1046	K842 homozygous <i>mlh3::LEU2 zip3::kanMX4</i>
K1049	K842 homozygous <i>mlh3::LEU2 msh4::kanMX4</i>
AM3896/ AM3897/ K888	K857 homozygous <i>mlh3::kanMX4</i>

AS1	K842 homozygous <i>K. lactis ZIP1</i>
AS19	AS1 homozygous <i>mlh3::kanMX4</i>
K1765	AF6 homozygous <i>SPO11</i> (<i>SPO11</i> inserted by CRISPR cas9 <i>spo11::kanMX4</i> haploid parent strains)
K1830	AF6 homozygous <i>spo11-179</i> (<i>spo11-N137K, K317I, T365S</i>) (<i>spo11-179</i> inserted by CRISPR cas9 <i>spo11::kanMX4</i> haploid parent strains)
K1767	AF6 homozygous <i>spo11-32</i> (<i>spo11-L254H</i>) (<i>spo11-32</i> inserted by CRISPR cas9 <i>spo11::kanMX4</i> haploid parent strains)
AM5083	YAM1252 homozygous <i>ndt80::LEU2</i>
AM5086	AM5083 homozygous <i>mlh3::hphMX</i>

Table S4. Strains used in this study.

Strains are of the BR1919-8B background (ROCKMILL AND ROEDER 1998), except for those listed under the heading “Octad Rec Seq Experiments”, which are derived from a cross between YJM789 and S96 genetic backgrounds (WEI *et al.* 2007; ANDERSON *et al.* 2011).

Table S5. Spore viabilities of hybrid strains.

Genotype (strain)	Tetrads dissected	4 spore viable	3 spore viable	2 spore viable	1 spore viable	0 spore viable	Spore viability (%)
wild type (AM3740)	66	40	15	6	3	2	83
<i>ecm11</i> (AM3735)	155	34	60	36	18	7	65
<i>msh2</i> (AM3736)	102	16	16	30	29	11	49
<i>pms1</i> (AM3738)	92	17	18	28	21	8	54