Homolog engagement controls meiotic DNA break number and distribution

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Supplementary Discussion

Meiotic arrest and steady-state DSB measurements: When DSBs were assayed at the level of a whole chromosome on pulsed-field gels, *zip3* displayed qualitatively and quantitatively different behaviors from *zip1* and *msh5*: DSBs remained at high levels in the *zip3* mutant, while *msh5* and *zip1* showed increased time-averaged DSB levels but DSBs eventually disappeared (Fig. 1b,c; Extended Data Fig. 1). It is likely that this difference is partly a consequence of qualitative differences in DSB repair defects in different *zmm* mutants, and partly a consequence of the different arrest phenotypes under the conditions of this analysis. The *zip3* mutant shows near-absolute arrest, in contrast to *zip1* and *msh5* cells, which show a delay of ~2 hr but eventually divide efficiently (Fig. 3c). In *zmm* mutants, recombination at a subset of DSBs remains arrested or delayed (e.g., ref 20), which in turn blocks or delays Ndt80 activation[9,24]. When Ndt80 becomes active, disassembly of meiotic chromosome structures and degradation of structural proteins occurs, which allows blocked recombination intermediates to proceed down alternative repair pathways, including via genetically silent recombination with sister chromatids[51,52]. Thus, the eventual progression of *zip1* and *msh5* mutants allows completion of DSB repair via pathways not available to more completely arrested *zip3* cells. This complexity underscores the difficulty in estimating total DSB numbers from direct DSB measurements, which is why the collection of complementary approaches we employ is important.

Changes in steady-state Spo11 protein levels: We evaluated whether increased DSBs could be tied to changes in steady-state Spo11 protein levels. Only a small fraction of Spo11 makes DSBs[10], so most Spo11 is not bound to oligos and under standard conditions only free Spo11 is detected in western blots[10]. In wild type, flag-tagged Spo11 was induced as cells entered meiosis, as expected, and free Spo11 was maintained at roughly constant levels while most cells were in prophase then decreased at or before the time most cells exited prophase and completed divisions, with a proteolytic fragment appearing with variable timing and at variable levels (Fig. 1d, Fig. 3a, and data not shown; compare to division kinetics in Fig. 3c). A similar pattern was observed in *msh5* and *zip1* single mutants, except that free Spo11 levels were elevated, especially at later times (Extended Data Fig. 3). In contrast, *zip3*, *ndt80*, *zip3 ndt80*, and *msh5 ndt80* strains accumulated more free Spo11 and maintained high levels through late time points with little if any of the proteolytic fragment detected (Fig. 1d, Fig. 3a, Extended Data Fig. 6). Thus, the hyperabundance and persistence of free Spo11 protein (and, in *ndt80* mutant backgrounds, persistence of Spo11-oligo complexes) clearly correlated with degree of prophase arrest but not with elevation of Spo11-oligo complexes. We conclude that, although it appears that turnover of free Spo11 is itself tied to meiotic progression, changes in this turnover cannot account for the elevated Spo11-oligo complexes. Moreover, we infer that alterations in total Spo11 levels are not the cause of increased DSB formation in *zmm* mutants and *ndt80*-arrested cells. Indeed, it was previously shown that DSB levels are relatively insensitive to elevated Spo11 protein levels in yeast and mouse[2,46].

Effects of gene conversion tract length on detection of recombination events: In principle, changes in the length and position of gene conversion tracts could affect the outcome of the two-dimensional gel analysis because gene conversions that encompass one of the flanking restriction site polymorphisms can create recombinant molecules that would score as crossovers regardless of whether they were crossovers or noncrossovers. Changes in conversion tract lengths can also affect outcomes in heteroallele recombination assays because generating a scoreable recombinant requires conversion at one but not both mutation positions[53] (see Supplementary Table 1 for more discussion). Available data are mixed with respect to effects of *zmm* mutations on tract lengths: Mancera et al. observed longer median crossover conversion tracts but shorter median noncrossover conversion tracts in *msh4* (ref 54), whereas Chen et al. observed longer median tract lengths for both crossovers and noncrossovers in *zip1*, *zip2*, and *zip4* (ref 30). Both studies used highly polymorphic hybrid strains, so it is unclear how such studies (where there may be effects of the presence of multiple mismatches in recombination intermediates[55]) extrapolate to more nearly homozygous strains such as those studied here. Nonetheless, it is plausible to consider that gene conversion tract lengths might be altered in *zmm* mutants.

We showed previously that a sequence polymorphism placed within the *his4LEU2* DSB hotspot allowed us to detect recombinant molecules in sufficient numbers to account for essentially all detectable DSBs[56]. Thus, a polymorphism very close to the DSB is efficiently incorporated into heteroduplex DNA and efficiently converted to give a scoreable outcome. Our two-dimensional gel recombination constructs are

designed on the same principles, suggesting that they also report efficiently on recombination frequencies. Thus, if tract lengths do tend to be longer in *zmm* mutants, we infer this would not significantly increase the already high efficiency of recombinant molecule detection, and thus not cause overestimation of total recombination frequencies. If anything, longer noncrossover gene conversion tracts in *zmm* might cause us to underestimate the severity of the crossover defect by causing noncrossover molecules to score as crossovers. In heteroallele recombination assays, a longer gene conversion tract favors co-conversion of the two mutations, which would render the recombination event invisible because a prototroph would not be generated[53]. Thus, if tracts are longer on average in *zmm* mutants, this would lead to an underestimate of total recombination levels at *arg4*. We conclude that it is unlikely that the *zmm* hyper-rec phenotype we observe here can be attributed solely to altered conversion tract lengths without an accompanying increase in DSB frequency. Importantly, *zip3* mutation caused little if any change in the local Spo11-oligo distributions at any of the hotspots studied here (Extended Data Fig. 4f). Thus, the altered spectra of recombination products we observed cannot be ascribed to changes in DSB locations.

Altered interhomolog vs. intersister bias cannot explain increased recombination in* zmm *mutants: It is unlikely that the increased number of interhomolog recombination products detected in *zmm* mutants reflects re-direction of DSBs that would normally have been repaired by recombination solely between sister chromatids. In meiosis, interhomolog recombination is favored over intersister recombination[57]. Because sister chromatid recombination events are genetically silent, it is difficult to estimate their numbers. Based on quantification of branched DNA recombination intermediates, estimates range from ~15% to ~33% of total recombination events involving use of the sister[51,58], but other considerations suggest the number of DSB repair events that utilize only the sister (as opposed to using both homolog and sister as templates in different stages of the recombination reaction) may be much lower[11,57,59]. However, even if we assume the highest end of the range of estimates (~one-third of total[51]), there are not enough silent sister chromatid recombination events in wild type to account for the bookkeeping in *zmm* mutants (Supplementary Table 2), even if every DSB that would have been repaired only by intersister recombination were instead re-directed to the homolog. Furthermore, Goldfarb and Lichten concluded that joint molecule formation between homologs or between sisters was affected equivalently in a *msh4* mutant[51], suggesting template choice is not strongly altered in the absence of ZMM function.

Reconciling global decreases in crossing over with a hyper-rec phenotype in* zmm *mutants: Hyper-rec behavior in *zmm* mutants seems at odds with global reduction in crossing over in tetrad analyses (e.g., ref 30), but additional considerations help reconcile this discrepancy. As noted above, the increased heteroallele recombination we observed agrees with earlier experiments[13-18], so our findings are consistent with prior studies. Moreover, reduced crossing over in our physical assays is also consistent with earlier studies at the *his4LEU2* hotspot[19,20,31,33]; this is particularly the case at *GAT1* for the *zip3* mutant (Fig. 2e), but because DSBs are more frequent at other hotspots in all *zmm* mutants tested by physical assays, we infer that ZMM deficiency reduces the per-DSB likelihood of generating a crossover at many if not all genomic locations. This per-DSB change in crossover frequency is quantitatively sufficient to more than offset the global ~1.8-fold increase in DSB numbers we infer from peak steady-state Spo11-oligo complexes, yielding a net decrease in crossover numbers. Furthermore, only cells that complete both meiotic divisions and generate four spores are typically examined in tetrad analysis, and only viable (euploid) spores can be scored. Selection bias can thus arise in tetrad analysis of mutants that exhibit meiotic arrest. In contrast, arrest is not a factor in physical analysis of bulk genomic DNA from sporulating cultures or return-to-growth heteroallele assays. We observed that *zip3* meiotic arrest is alleviated if DSB numbers are decreased by hypomorphic *spo11* mutation (Extended Data Fig. 7c). This finding, combined with the fact that DSB numbers vary widely among cells in a population[30,60], suggests that selection for a subset of cells that made relatively few DSBs may contribute to underestimating crossover (and total recombination) numbers in those *zmm* mutants that display substantial arrest.

Supplementary References

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Supplementary Table 1. Published examples of locus-specific *zmm* hyper-rec phenotypes

zmm mutation	Method*	Assay locus	Fold change vs. wild type	Reference
zip1	Heteroallele RTG	*trp1* *his4, thr1*	2–7 no change	13
zip2	Heteroallele RTG	*his4* *thr1, trp1*	~2 no change	16
zip3	Heteroallele RTG	*trp1* *his4, leu2, thr1*	~2 no change	17
zip4	Heteroallele RTG	*thr1* *his4, leu2*	~2 no change	18
msh4	NMS in tetrads	*arg4*	2.3	14
msh5	NMS in tetrads	*arg4* *his4, leu2*	6.1 no change	15
	Heteroallele RTG	*ade2, leu2, ura3*	no change	
spo16	NMS in tetrads	various	1.2–2.8	19

* "Heteroallele RTG," prototroph frequency from heteroallelic recombination in return-to-growth analysis; "NMS in tetrads," frequency of non-Mendelian segregation (gene conversion and post-meiotic segregation) in four-spore-viable tetrads.

Notes: In all cases, authors either drew no conclusion about DSB levels, or explicitly concluded that the total number of recombination initiation events was indistinguishable from wild type. Interestingly, *msh4* and *msh5* mutations caused elevated recombination at *arg4* when assayed by tetrad dissection, in contrast to our results with heteroallele RTG measurements. It is important to note that prototroph generation in heteroallele assays is more strongly dependent on the distribution of gene conversion tract lengths than is detection of non-Mendelian segregation in tetrad analysis[23,53]. This is because recombination detection in heteroallele assays requires that conversion tracts cover only one of the two mutations, since co-conversion of both mutations will not generate an Arg+ prototroph. In contrast, in tetrad dissection of diploids heterozygous for wild-type *ARG4* and an *arg4* single mutant allele, conversion tracts need only span the mutation, with no further constraint on maximum length. Thus, altered conversion tract lengths in *msh4* and *msh5* mutants[54] may obscure elevated recombination frequencies in heteroallele RTG assays.

To our knowledge, similar data have not been published for *mer3* or *pph3*, but direct DSB measurements at the *his4LEU2* hotspot are available for these and other *zmm* mutants[19,20,31-33]. In each case, data in otherwise wild-type backgrounds (i.e., not in *rad50S* or *dmc1* backgrounds) are consistent with elevated DSB numbers. However, only one of these studies suggested there might be additional DSB formation late in meiosis[31]. In the others, authors either drew no conclusion or concluded there is no change in DSB levels based on experiments in *rad50S* and/or *dmc1* backgrounds.

Supplementary Table 2. Analysis of total recombination at *CCT6*, *ERG1*, and *GAT1*

CCT6 Hotspot					
ZMM genotype	CO	NCO	DSBs	Sum	Fold Change
wild type	3.0%	2.9%	0.0%	5.9%	–
msh5	3.0%	7.4%	0.3%	10.7%	1.8
zip1	3.9%	10.5%	0.2%	14.6%	2.5
zip3	2.2%	12.5%	0.8%	15.5%	2.6

ERG1 Hotspot					
ZMM genotype	CO	NCO	DSBs	Sum	Fold Change
wild type	3.3%	1.9%	0.0%	5.2%	–
msh5	3.3%	6.3%	0.2%	9.8%	1.9
zip3	1.5%	7.1%	ND	8.8–9.4%	1.7–1.8

GAT1 Hotspot					
ZMM genotype	CO	NCO	DSBs	Sum	Fold Change
wild type	5.9%	3.2%	0.1%	9.2%	–
zip3	1.6%	6.4%	0.8%	8.8%	0.96

Data are expressed as percent of DNA. Crossovers (CO) and noncrossover gene conversions (NCO) are from two-dimensional gel electrophoresis assays; crossover values were divided by two to scale to a per-DSB equivalent. DSBs were measured at 10 hr (an equivalent time point to the crossover/noncrossover analysis) and includes half of the joint molecule signal. If DSB data were not available for a *zmm* mutant (ND) then a range of 0.2–0.8% was applied.

Supplementary Table 3. Spo11-oligo sequencing statistics

Sample	Total sequenced	Total mapped*	Uniquely mapped†
wild type sample 1	16,690,023	9,436,763 (56.5%)	8,461,015 (50.7%)
wild type sample 2	14,006,296	10,029,260 (71.6%)	9,527,314 (68.0%)
zip3 sample 1	3,760,946	2,824,442 (75.1%)	2,698,444 (71.7%)
zip3 sample 2	10,203,933	7,216,350 (70.7%)	6,952,424 (68.1%)

* Sequences that could not be mapped likely reflect sequencing errors, adaptor dimers, PCR dimers and *bona fide* Spo11-oligos derived from genomic regions that are unique to SK1 (i.e., not found in the S288C reference strain).
† Total number of reads that mapped to only one position in the genome (including mitochondrial DNA and 2μ plasmid).

Supplementary Table 5. Multiple regression results

Variable	Coefficient (standard error)	β (standard error)	t value	p value
Principal component 1	0.069 (0.011)	0.447 (0.069)	6.46	4.7e-10
Zip3 ChIP	0.461 (0.078)	0.355 (0.060)	5.87	1.2e-08
Chromosome size	0.237 (0.045)	0.284 (0.054)	5.26	2.9e-07
Rec104 ChIP	-1.55 (0.35)	-0.297 (0.068)	-4.36	1.8e-05
G+C content	-0.095 (0.021)	-0.252 (0.056)	-4.49	1.1e-05
Rec8 ChIP	-0.454 (0.14)	-0.193 (0.058)	-3.35	9.2e-04
Intercept	5.10 (1.8)	0	5e-14	1

Multiple linear regression was performed with log-fold change in Spo11-oligo density in *zip3* as the dependent variable and the indicated chromosomal features as the independent variables. Estimates of the regression coefficients and the standardized regression coefficients (β) are shown, along with t and p values based on the standardized coefficients. For this model, multiple R^2=0.430, adjusted R^2=0.415, F=29.53 on 7 and 274 degrees of freedom, and $p<2.2 \times 10^{-16}$. "Principal component 1" is the first principal component for the Rec114, Mei4, Mer2, Hop1, and Red1 ChIP data; we reduced the dimensions of the model by combining data for these proteins because of their high degree of co-linearity (see Extended Data Fig. 9d). Data were binned in 35-kb non-overlapping windows, not including sub-telomeric, pericentric, or rDNA-proximal zones. Qualitatively indistinguishable results were obtained if window sizes or window positions were varied (adjusted R^2 ranged from ~0.35 to ~0.45 for windows ranging from 20 kb to 40 kb, respectively; data not shown).

Notes: Chromosome size and the wild-type distributions of Zip3 binding and of the correlated binding of Rec114, etc., are all significant positive predictors of the degree of increase in DSB formation in *zip3* mutants. G+C content and the distributions of Rec104 and Rec8 are significant negative predictors. The regression results strongly suggest that the contributions of all of these factors are at least partially independent of one another. Including the Rec102 ChIP data did not significantly improve the fit, whether Rec104 was included (p=0.18) or excluded (p=0.65; data not shown). Note that Rec104 became a significant negative predictor of Spo11-oligo density in the context of this model, i.e., when other factors were accounted for, even though it was only weakly anti-correlated, if at all, when considered on its own (Extended Data Fig. 9b, e).

For Zip3, we used data collected 3 hr after transfer to sporulation conditions[39]. This appears to be roughly equivalent to ~4-5 hr in our time courses, based on DSB and meiotic division timing (DSBs were maximal at or before 3 hr in ref 39, vs. at ~4 hr in our study (Fig. 1c); 50% of cells completed MI by ~5 hr in ref 39, vs. at ~6.5 hr in our study (Fig. 3c)). Thus, the 3-hr Zip3 ChIP dataset represents a time when DSBs have reached maximal levels and homologs are pairing and beginning to synapse with one another, so we infer that ZMM-dependent feedback control of DSB numbers should be fully operational. At this time point, Serrentino et al. concluded that the DSB-dependent Zip3 enrichment on chromosome arms coincided principally with axis sites[39]; the positive correlation with Hop1, Red1, Rec114, Mei4, and Mer2 in our segmentation analysis agrees with this conclusion (Extended Data Fig. 9d,e). Interestingly, the Zip3 ChIP data at 4 hr showed only weak positive correlation with Spo11-oligo fold change, and the Zip3 ChIP data at 5 hr showed no significant correlation at any size scale examined (data not shown). These later time points showed a shift in the Zip3 distribution that correlated progressively better with DSB sites than with axis sites[39].

We emphasize that the regression results reveal correlations, not cause/effect relationships. For example, the negative correlation with Rec104 could indicate that domains relatively depleted for Rec104 are more sensitive to ZMM-dependent feedback, that feedback inhibition of DSBs involves local displacement of Rec104, or that increased DSBs in *zip3* and Rec104 depletion in wild type are independently correlated with some other feature(s). Moreover, if ZMM-dependent feedback does involve displacement of (some of) these proteins from chromosomes, then we anticipate that the details of the correlations may change significantly if the chromatin association is assayed at different times in wild type or mutant meiosis.

Supplementary Table 6. Yeast strains

Strain number	Genotype
SKY1465 *	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A*
SKY1708 *	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3*
SKY1843	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; zip1Δ::LEU2*
SKY1845	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; zip1Δ::LEU2*
SKY1985 †	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; msh5Δ::kanMX*
SKY2024	*MATa/MATα; ho::LYS2/''; lys2/''; ura3/''; leu2/''; arg4-Nsp/ARG4; nuc1Δ::LEU2/''; rad50-KI81::URA3/''*
SKY2025	*MATa/MATα; ho::LYS2/''; lys2/''; ura3/''; leu2/''; nuc1Δ::LEU2/''; spo11-Y135F-HA3His6::kanMX/''; rad50-KI81::URA3/''*
SKY2026 †	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; msh5Δ::kanMX*
SKY3459	*MATa/MATα; ho::LYS2/''; lys2/''; ura3/''; leu2/''; nuc1Δ::LEU2/''; spo11-HA3His6::kanMX/''; rad50-KI81::URA3/''*
SKY3472	*MATa/MATα; ho::LYS2/''; lys2/''; ura3/''; leu2/''; nuc1Δ::LEU2/''; SPO11-His6-flag3-loxP-kanMX-loxP/''; rad50-KI81::URA3/''*
SKY3621	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; pph3Δ::hphMX*
SKY3623	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; pph3Δ::hphMX*
SKY3650	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; zip4Δ::hphMX*
SKY3652	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; zip4Δ::hphMX*
SKY3660	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; ndt80Δ::kanMX*
SKY3661	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; ndt80Δ::kanMX*
SKY3662	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; ndt80Δ::kanMX; zip3Δ::hphMX*
SKY3663	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; ndt80Δ::kanMX; zip3Δ::hphMX*
SKY3666	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; dmc1Δ::hphMX*
SKY3668	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; dmc1Δ::hphMX*
SKY3671	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; msh5Δ::kanMX; dmc1Δ::hphMX*
SKY3673	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; msh5Δ::kanMX; dmc1Δ::hphMX*
SKY3684	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; YDR186c-NgoMIV; zip1Δ::LEU2*
SKY3685	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; YDR186c-SmaI; zip1Δ::LEU2*
SKY3692	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; YDR186c-NgoMIV; arg4(nde1)-Nsp; thr1A; msh5Δ::kanMX*
SKY3693	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; YDR186c-SmaI; arg4(nde1)-Bgl; CEN8::URA3; msh5Δ::kanMX*
SKY3821	*MATa/MATα; ho::LYS2/''; lys2/''; leu2/''; ura3/''; arg4-bgl/''; Spo11-6His-3FLAG-loxP-KanMX4-loxP/''; nuc1Δ::LEU2/''*
SKY3935	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; his3::hisG; SPO11-ProteinA::HIS5*
SKY3936	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; his3::hisG; SPO11-ProteinA::HIS5; zip3Δ::hphMX*
SKY3937	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; his3::hisG; SPO11-ProteinA::HIS5; zip3Δ::hphMX*
SKY4006	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; zip1Δ::LEU2; dmc1Δ::hphMX*
SKY4007	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; zip1Δ::LEU2; dmc1Δ::hphMX*
SKY4021	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; SPO11-His6-flag3-loxP-kanMX-loxP; nuc1Δ::LEU2*
SKY4022	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; SPO11-His6-flag3-loxP-kanMX-loxP; nuc1Δ::LEU2*
SKY4025	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; zip3Δ::hphMX; SPO11-His6-flag3-loxP-kanMX-loxP; nuc1Δ::LEU2*
SKY4026	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; zip3Δ::hphMX; SPO11-His6-flag3-loxP-kanMX-loxP; nuc1Δ::LEU2*
SKY4093	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; zip3Δ::hphMX*
SKY4095	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; zip3Δ::hphMX*
SKY4098	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; spo16Δ::kanMX*
SKY4100	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; spo16Δ::kanMX*

Supplementary Table 6 (continued)

SKY4227	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; zip3Δ::hphMX; dmc1Δ::hphMX*
SKY4229	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; zip3Δ::hphMX; dmc1Δ::hphMX*
SKY4292	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; msh5Δ::kanMX; SPO11-His6-flag3-loxP-kanMX-loxP; nuc1Δ::LEU2*
SKY4293	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; msh5Δ::kanMX; SPO11-His6-flag3-loxP-kanMX-loxP; nuc1Δ::LEU2*
SKY4437	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; YDR186c-NgoMIV; arg4(nde1)-Nsp; thr1A; zip3Δ::hphMX*
SKY4439	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; YDR186c-NgoMIV; arg4(nde1)-Nsp; thr1A*
SKY4440	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; YDR186c-SmaI; arg4(nde1)-Bgl; CEN8::URA3*
SKY4441	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; YGR175c-SalI; arg4(nde1)-Nsp; thr1A; zip3Δ::hphMX*
SKY4442	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; YGR175c-SpeI; arg4(nde1)-Bgl; CEN8::URA3; zip3Δ::hphMX*
SKY4443	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; YGR175c-SalI; arg4(nde1)-Nsp; thr1A*
SKY4444	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; YGR175c-SpeI; arg4(nde1)-Bgl; CEN8::URA3*
SKY4445	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; YGR175c-SpeI; arg4(nde1)-Bgl; CEN8::URA3; msh5Δ::kanMX*
SKY4446	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; YGR175c-SalI; arg4(nde1)-Nsp; thr1A; msh5Δ::kanMX*
SKY4456	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; YDR186c-SmaI; arg4(nde1)-Bgl; CEN8::URA3; zip3Δ::hphMX*
SKY4505	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; YFL021w-BamHI; arg4(nde1)-Nsp; thr1A*
SKY4506	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; YFL021w-BamHI; arg4(nde1)-Nsp; thr1A; zip3Δ::hphMX*
SKY4507	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; YFL021w-(x); arg4(nde1)-Bgl; CEN8::URA3*
SKY4508	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; YFL021w-(x); arg4(nde1)-Bgl; CEN8::URA3; zip3Δ::hphMX*
SKY4511	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; SPO11-His6-flag3-loxP-kanMX-loxP; nuc1Δ::LEU2; ndt80Δ::LEU2*
SKY4512	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; SPO11-His6-flag3-loxP-kanMX-loxP; nuc1Δ::LEU2; ndt80Δ::LEU2*
SKY4513	*MATa; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Bgl; CEN8::URA3; SPO11-His6-flag3-loxP-kanMX-loxP; nuc1Δ::LEU2; ndt80Δ::LEU2; zip3Δ::hphMX*
SKY4514	*MATα; ho::LYS2; lys2; ura3; leu2::hisG; arg4(nde1)-Nsp; thr1A; SPO11-His6-flag3-loxP-kanMX-loxP; nuc1Δ::LEU2; ndt80Δ::LEU2; zip3Δ::hphMX*
SKY4574	*MATa/MATα; ho::LYS2\"; lys2\"; ura3\"; leu2\"; his3::hisG\"; nuc1Δ::LEU2\"; SPO11-ProteinA::HIS5\"; rad50-KI81::URA3\"*
SKY4734	*MATa/MATα; ho::LYS2/"; lys2/"; leu2/"; ura3/"; arg4(nde1)-Nsp/"; Spo11-6His-3FLAG-loxP-KanMX4-loxP/"; msh5Δ::KanMX4/"; ndt80::LEU2/"*
SKY4737	*MATa/MATα; ho::LYS2/"; lys2/"; leu2/"; ura3/"; arg4-bgl/"; Spo11-6His-3FLAG-loxP-KanMX4-loxP/"; ndt80::LEU2/"*
SKY4740	*MATa/MATα; ho::LYS2/"; lys2/"; ura3/"; arg4-bgl/"; Spo11-6His-3FLAG-loxP-KanMX4-loxP/"*
SKY4768	*MATa/MATα; ho::LYS2/"; lys2/"; leu2::hisG/"; ura3/"; arg4-Nsp/ arg4-Bgl; Spo11-6His-3FLAG-loxP-KanMX4-loxP/"; nuc1Δ::LEU2/"; zip1Δ::LEU2/"*

* Strains from ref 56; † strains from ref 53; all other strains are from the current study.