Exo1-protected DNA nicks direct crossover formation in meiosis

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ABSTRACT
In most sexually reproducing organisms crossing over between chromosome homologs during meiosis is critical for the viability of haploid gametes. Most crossovers that form in meiosis in budding yeast result from the biased resolution of double Holliday Junction (dHJ) intermediates. This dHJ resolution step involves the actions Rad2/XPG family nuclease Exo1 and the Mlh1-Mlh3 mismatch repair endonuclease. At present little is known about how these factors act in meiosis at the molecular level. Here we show that Exo1 promotes meiotic crossing over by protecting DNA nicks from ligation. We found that structural elements in Exo1 required for interactions with DNA, such as bending of DNA during nick/flap recognition, are critical for its role in crossing over. Consistent with these observations, meiotic expression of the Rad2/XPG family member Rad27 partially rescued the crossover defect in exo1 null mutants, and meiotic overexpression of Cdc9 ligase specifically reduced the crossover levels of exo1 DNA binding mutants to levels approaching the exo1 null. In addition, our work identified a role for Exo1 in crossover interference that appears independent of its resection activity. Together, these studies provide experimental evidence for Exo1-protected nicks being critical for the formation of meiotic crossovers and their distribution.
INTRODUCTION

Cells in meiosis undergo a single round of DNA replication followed by reductional and equational chromosomal divisions to produce haploid gametes. In most eukaryotes, including budding yeast and humans, the accurate segregation of homologous chromosomes during the first reductional division (Meiosis I) requires the formation of crossovers between homologs. Physical linkages created by crossovers and sister chromosome cohesions distal to the crossover site are critical for proper segregation of chromosome pairs during Meiosis I (Maguire, 1974; Hunter, 2015; Zickler and Kleckner, 2015). The inability to establish these physical connections can lead to improper chromosome segregation and aneuploidy, and in humans is thought to be an important cause of birth defects and miscarriages (Hassold and Hunt, 2001; Nagaoka et al., 2012; Hunter, 2015).

In baker's yeast crossover formation in meiotic prophase is initiated through the genome-wide formation of roughly 150 to 200 Spo11-induced double-strand breaks (DSBs; Keeney et al., 1997; Pan et al., 2011). These breaks are resected in a 5' to 3' direction to form 3' single-stranded tails (Cao et al., 1990; Padmore et al., 1991). Strand exchange proteins coat the single stranded tails and promote their invasion into homologous sequences in the unbroken homolog (Hunter, 2015). In the major crossover pathway (Class I), the resulting D-loop intermediate is stabilized by ZMM proteins including Zip2-Zip4-Spo16 and Msh4-Msh5 to form a single end invasion intermediate (SEI; Figure 1A; Hunter and Kleckner, 2001; Fung et al., 2004; Borner et al., 2004; Lynn et al., 2007; De Muyt et al., 2018). This recombination intermediate forms concomitantly with the synaptonemal complex, a structure that is thought to remove chromosomal tangles and interlocks during the homology search process (Padmore et al., 1991; Sym et al., 1993; de Boer and Heyting, 2006). DNA synthesis from the SEI, followed by second-end capture, results in the formation of the double-Holliday junction intermediate (dHJ). The dHJ is thought to be stabilized by Msh4-Msh5 and resolved in a biased orientation to form ~90 crossovers (COs) in the yeast genome that are distributed so that they are evenly spaced (crossover interference) and every homolog pair receives at least one crossover (Figure 1A;
How dHJs are resolved in a biased manner to form crossovers is a major unanswered question. Investigators have suggested that the presence of nicks in dHJs ensures biased resolution by creating asymmetric structures that are resolved to form crossover-only products (reviewed in Machin et al., 2020). In support of such ideas, whole genome sequencing of hDNA tracts formed in meiosis inferred a model in which meiotic crossover resolution is biased towards DNA synthesis tracts (Martini et al., 2011; Marsolier-Kergoat et al., 2018). In this model nicks maintained at the ends of synthesis tracts could direct biased and asymmetric cleavage of the dHJ by recruiting a nick-binding protein that acts in the resolution mechanism. However, such a model is inconsistent with a denaturing gel analysis of dHJs that form at a meiotic hotspot in S. cerevisiae; this work showed that all single strands within the dHJs are continuous (Schwacha et al., 1994; 1995). It is also inconsistent with recent work in S. cerevisiae showing that a vast majority of crossovers initiated at another hotspot displayed evidence of branch migration, with about half of the COs having formed from dHJs located on one side of the initiating double-strand break. In such a situation, nicks should not be present in positions that direct biased resolution (Ahuja et al., 2021). Thus, it remains unclear if nicks participate in meiotic crossover formation.

What factors act in the biased resolution of dHJs? The MMR endonuclease Mlh1-Mlh3 and the XPG/Rad2 family nuclease Exo1 have been shown to act in meiotic crossover resolution, with mlh3Δ and exo1Δ single and double mutant strains displaying similar crossover defects in crossing over (Khazanehdari and Borts, 2000; Zakharyevich et al., 2010; 2012). Biochemical analyses of Mlh1-Mlh3 indicate that its endonuclease activity is required for its role in crossover formation, but not as a structure-specific nuclease that symmetrically cleaves Holliday junctions (Nishant et al., 2008; Rogacheva et al., 2014; Ranjha et al., 2014; Manhart et al., 2017). Exo1 acts in many steps in DNA metabolism, including creating 3’ single-stranded
ends for homologous recombination, telomere maintenance, DNA mismatch repair, DNA replication, and crossover-specific dHJ resolution in meiosis. Exo1 contains an N-terminal Rad2/XPG nuclease domain that is conserved in Rad2/XPG family members and an unstructured C-terminal tail that interacts with the mismatch repair factors Msh2 and Mlh1 (Tishkoff et al., 1997; Tran et al., 2001). In vitro studies demonstrated that Exo1 displays a robust and processive 5’ to 3’ exonuclease on the ends of a double-strand break, and on gapped and nicked duplex DNA. In addition, it displays 5’ flap endonuclease activity (Kunkel and Erie, 2015; Goellner et al., 2015; Szankasi and Smith, 1992; Fiorentini et al., 1997; Lee and Wilson, 1999; Tran et al., 2002; Genschel and Modrich, 2003; Zakharyevich et al., 2010).

In meiosis exo1Δ strains display a defect in the 5’ to 3’ resection of Spo11-induced DSBs and a meiotic crossover defect. In fact, resection is reduced in exo1Δ to an average of 270 nt compared to 800 nt in wild-type. Despite showing these defects, exo1Δ mutants display wild-type timing and levels of meiotic recombination intermediates, including dHJs (Zakharyevich et al., 2010). Genetic analysis showed that disruption of a conserved Mlh1-Interaction Protein sequence (MIP box) in the Exo1 C-terminal domain conferred intermediate defects in meiotic crossing over, suggesting that Exo1 promotes meiotic crossovers through interactions with Mlh1 and possibly other factors (Amin et al., 2001; Argueso et al., 2003; Tran et al., 2004; 2007; Zakharyevich et al., 2010). Curiously, an exo1 mutation (D173A) that disrupts a metal binding site critical for nuclease function was shown to have only a minimal impact on meiotic crossing over. Together these analyses suggested that Exo1’s interactions with Mlh1-Mlh3, but not its nuclease function, are critical for crossover formation (Abdullah et al., 2004; Zakharyevich et al., 2010; Keelagher et al., 2011).

The studies outlined above in addition to recent biochemical analyses have led to the proposal that Mlh1-Mlh3 interacts with Exo1, Msh4-Msh5 and the DNA polymerase processivity factor PCNA for biased resolution of double Holliday junctions (Cannavo et al., 2020; Sanchez et al., 2020; Kulkarni et al., 2020). This proposal suggests that DNA signals are present in dHJ intermediates that are critical for such resolution; however, these studies have not provided
direct evidence for such signals. Here we provide genetic evidence that Exo1 acts to protect DNA from being ligated in recombination intermediates during the formation of crossover products. We also show that it plays a critical role in ensuring that meiotic crossovers are widely spaced for proper chromosome segregation in the Meiosis I division. These observations provide evidence for dynamic and distinct roles for Exo1 in both crossover placement and for maintaining a nicked recombination intermediate for the resolution of dHJs into crossovers.

RESULTS

Mutations in metal coordinating and active site residues in Exo1 do not disrupt meiotic crossing over.

The crystal structure of human Exo1 with 5’ recessed DNA (PDB #3QE9) identified two metals in the catalytic site of the Exo1-DNA structure, with residue D171 assisting D173 in coordinating one metal, and residue D78 coordinating the other, to hydrolyze the phosphodiester backbone of DNA (Figures 1B and S1; Orans et al., 2011; Mueser et al., 1996; Hwang et al., 1998; Feng et al., 2004; Shi et al., 2017). While the exo1-D173A mutation in baker’s yeast was shown to disrupt Exo1 nuclease activity (Tran et al., 2002), mutation of other amino acids that coordinate the catalytic metals was not performed. Mutation of other nucleases that act through a two-metal catalysis mechanism suggested that altering a single metal binding residue does not fully ablate function and could create novel functions, perhaps because a water molecule can substitute as a ligand (Schiltz et al., 2019). For example, work by Lee et al. (2002) showed that the human exo1-D78A and exo1-D173A mutant proteins display nuclease activities, though at levels significantly lower than the wild-type protein.

In baker’s yeast meiosis, mutation of a single metal binding residue (exo1-D173A) caused a disruption in the 5’ to 3’ resection steps of meiotically induced DSBs, but only minor, if any defects in meiotic crossing over, suggesting that Exo1’s nuclease functions were not required in this step (Abdullah et al., 2004; Zakharyevich et al., 2010). We purified exo1-D173A from baculovirus infected Sf9 cells (Materials and Methods), but were unable to purify a full
length variant (exo1-D78A,D173A) expected to disrupt both metal binding sites (Figure S2). We tested the nuclease activity of exo1-D173A on a 2.7 kb pUC18 substrate containing four pre-existing nicks (Figure S2A) as well as supercoiled plasmids. As shown in Figure S2A and C, exo1-D173A was deficient for exonuclease activity on the substrate containing four pre-existing nicks. However, exo1-D173A displayed a weak DNA nicking activity on closed circular DNA similar to that seen for Mlh1-Mlh3 (~10% nicking of pUC18 at 20 nM exo1-D173A compared to ~20% nicking at 20 nM Mlh1-Mlh3), suggesting that a role for Exo1 nuclease activity in crossover resolution was not fully resolved (Manhart et al., 2017). In contrast, wild-type Exo1 did not display such nicking activity, consistent with previous work showing that human Exo1 displayed little or no endonuclease activity on blocked-end DNA substrates (Figure S2B; Lee et al., 2002). Interestingly, the addition of a mutation predicted to be critical for DNA binding, G236D (see below), decreased the nicking activity of the exo1-D173A protein by about two-fold, consistent with previous studies indicating that Exo1 nuclease activity was dependent on its DNA binding activity (Figure S2D; Orans et al., 2011).

To test the effect of mutations in the Exo1 catalytic site we made D78A, D171A, and D173A mutations (Group I, Figure 1B) in combination to disrupt coordination of both metals. We also mutated residues in Exo1 which interact with and position DNA in an orientation to be cleaved (Orans et al., 2011). These residues (H36, K85, R92, K121, Group II) contribute to the fraying of the duplex DNA bases away from its complement and reside within an α4-α5 helical arch microdomain that forms part of the Exo1 active site (Figures 1B, S1). This microdomain is important for catalysis and also defines substrate specificity throughout the flap endonuclease (FEN) superfamily and consequently Exo1 5’ flap binding (Ceska et al., 1996; Devos et al., 2007; Gloor et al., 2010; Orans et al., 2011). Within this region R92 has been shown to be a critical residue for Exo1 catalysis; it interacts with the scissile bond on the DNA to position it adjacent to the catalytic metal core, and the R92A mutation dramatically decreased nuclease activity of human Exo1 in vitro to similar levels of the D173A metal-coordinating mutation (Orans
et al., 2011). K121 (R in human Exo1) is part of the α5 helix and coordinates passage of the DNA substrate through the active site.

We analyzed meiotic crossing over by tetrad analysis at four consecutive intervals on Chromosome XV (104.9 cM map distance in wild-type, 52 cM in exo1Δ) and at one interval (CEN8-THR1) on Chromosome VIII (~39% single crossovers in wild-type, 20% in exo1Δ; Figures 2A and 3A; Thacker et al., 2011). These two chromosomal regions showed defects in crossing over similar to those seen previously (exo1Δ, ~2-fold decreased; mlh3Δ, ~2-fold; msh5Δ, ~3-fold; exo1Δ mus81Δ, ~12-fold) and confirmed the epistatic relationship between exo1Δ and mlh3Δ (Figure 2B; Argueso et al., 2004; Nishant et al., 2008; Zahkaryevich et al., 2012; Al-Sweel et al., 2017). As shown in Figures 2B and 3B and Tables S1 and S2, disruption of either one or both metal binding sites of Exo1 (Group I) had minor if any effects on meiotic crossing over. There was a small crossover (<10%) reduction in some of the catalytic mutants compared to wild-type; this reduction could result from defects in DNA binding that result from perturbation of the active site. In fact, the human exo1-D78A mutant protein showed defects in binding to DNA flap structures (Lee et al., 2002). In addition, the exo1-H36E, exo1-K85A/E, exo1-R92A and exo1-K121A/E mutations (Group II) had very modest, if any effect on meiotic crossing over compared to wild-type, suggesting that coordination of the scissile bond for catalysis within the active site is not critical for crossing over. The dramatic loss of nuclease activity seen with human Exo1 bearing K85A, R92A or K185A mutations (Orans et al., 2011; Li et al., 2019) further supports the dispensability of Exo1 catalytic activity for crossing over. These observations indicate that the critical function(s) of Exo1 in meiotic crossover resolution are not catalytic in nature.

**Mutation of DNA binding domains of Exo1 reveal a DNA binding role for Exo1 in meiotic crossing over.**

The structure solved by Orans et al. (2011) revealed that Exo1 makes key contacts with DNA through several defined domains (Figure 1B). For example, G236 (Group IV) is one of several
residues in a helix-two turn-helix motif that coordinates a metal ion and forms hydrogen bonds with DNA backbone oxygen residues to stabilize an interaction with Exo1 and the pre-nick duplex DNA. This conserved motif is only slightly modified from observed FEN-1 structures (Ceska et al., 1996; Feng et al., 2004) and is presumed to facilitate exonuclease processivity as the protein moves along the DNA backbone (Pelletier et al., 1996; Orans et al., 2011). K185 is part of a small hairpin loop between strands β6 and β7 and is also thought to be critical for recognition of duplex DNA (Orans et al., 2011; Li et al., 2019). The K185A mutation has been shown to diminish Exo1 nuclease activity several fold in vitro, and confer elevated sensitivity to DNA-damaging agents, likely due to a defect in binding duplex DNA (Li et al., 2019). A crucial component of Rad2/XPG members is the hydrophobic wedge (Figure 1B, Group III), a structurally conserved domain which induces a sharp bend at a ds-ssDNA junction, and gives the enzyme family its specificity for gapped/nicked DNA substrates (Orans et al., 2011, Chapados et al., 2004). Several hydrophobic residues within the wedge motif displace the non-substrate strand, as well as two lysine residues which appear to coordinate this portion of the non-substrate strand (Figure 1B).

As shown in Figure 2B and 3B and Tables S1 and S2, the exo1-K185E and exo1-G236D mutations conferred significant decreases in crossover formation (68 cM, 29.1% tetratype in exo1-G236D and 73 cM, 24.5% tetratype in exo1-K185E) in the URA3-HIS3 and CEN8-THR1 intervals, respectively. Interestingly, the hydrophobic wedge mutations exo1-S41E (58.6 cM, 28.4% tetratype), and exo1-F58E (69.9 cM, 27.8% tetratype) also conferred crossover defects with double mutation combinations (exo1-K185E,G236D-24.2% tetratype; exo1-S41E,F58E-24.6% tetratype) conferring more severe phenotypes. We then made a series of double and triple mutants that included a catalytic, DNA binding, and Mlh1-interacting (MIP) mutations (Figure 3B; Table S1). Combining groups did not confer crossover phenotypes equivalent to the exo1Δ, and including a catalytic mutation (-D171A, -D173A) with any single DNA binding mutation that conferred a crossover phenotype did not further impair crossover formation. However, a triple mutation, exo1-R92A,K121A,K185A (24.3% tetratype) conferred a more
severe phenotype than the single mutations, and another triple mutation, \( \text{exo1-D173A,K185E,G236D} \) (22.4% tetratype), conferred a phenotype very close to the \( \text{exo1}\Delta \), also suggesting that catalytic mutations could impact DNA binding as indicated above (Figure 3B).

The data collected from assaying double and triple mutants validated the results of single catalytic and DNA binding mutations, identified DNA binding mutants that confer a near \( \text{exo1}\Delta \) crossover phenotype, and showed that the Exo1 active site is relatively insensitive to mutation for crossover formation. These observations also indicated that the decrease in crossover frequency seen in single mutants is compounded in multiple mutant combinations (Figure 3B).

We then examined the spore viability of \( \text{exo1} \) mutant strains. The \( \text{exo1}\Delta \) strain showed a tetrad spore viability pattern (74% spore viability; 4, 2, 0 viable tetrads > 3, 1) consistent with Meiosis I non-disjunction (Figures 2B; S3; Ross-Macdonald and Roeder, 2004; Abdullah et al., 2004). However, decreases in meiotic crossing over and spore viability did not correlate in the \( \text{exo1} \) strains. For example, \( \text{exo1} \) mutants with very similar defects in crossing over showed spore viabilities that ranged from 89% (\( \text{exo1-G236D, exo1-MIP} \)) to 71 to 73% (\( \text{exo1-K185E,G236D, exo1-K185E,MIP} \)). A plausible explanation for these differences is that the \( \text{exo1} \) mutations display other phenotypes in addition to meiotic crossover phenotypes. In fact, some of the \( \text{exo1} \) mutations analyzed above conferred defects in DNA repair, as measured by sensitivity to methyl-methane sulfonate (MMS). However, the MMS phenotypes did not correlate with defects in meiotic crossing over (Figure S4). For example, the \( \text{exo1-D78A, exo1-D171A, and exo1-D173A} \) catalytic mutations conferred stronger MMS sensitivities compared to their nearly wild-type meiotic CO phenotypes. Similar disparities between DNA repair and CO phenotypes were seen for the active site mutations \( \text{exo1-K85E and exo1-K121A} \), the DNA binding mutant \( \text{exo1-K185E} \) and the MLH interacting mutant \( \text{exo1-MIP} \). This analysis suggested that the lack of correlation between spore viability and crossover phenotype seen in \( \text{exo1} \) mutants was likely complicated by their defects in DNA repair. Further support for this idea was seen by the lack of a 4, 2, 0 viable tetrads > 3, 1 pattern in the \( \text{exo1} \) mutant alleles, though this pattern was clearly displayed by \( \text{exo1}\Delta \) (Figure S3). One explanation for this lack of
a pattern in *exo1* mutants with strong crossover defects is that the DNA repair defects in these mutants conferred a pleiotropic decrease in spore viability, obscuring a Meiosis I non-disjunction phenotype. Another potential explanation (discussed below) is that *exo1Δ* strains show increased disjunction as the result of defects in crossover positioning (genetic interference, see below). Together, these observations provide evidence that Exo1 contains distinct DNA repair and meiotic CO functions and DNA binding by Exo1, but not its nuclease activity, is critical for meiotic CO resolution.

**Expression of RAD27 in meiosis partially complements the crossover defect in exo1 null strains.**

The Rad2 family of nucleases consists of four evolutionarily conserved members: *RAD2/XPG* in yeast/humans respectively, *EXO1/EXO1, RAD27/FEN-1*, and *YEN1/GEN1*. While all four have distinct roles in DNA metabolism, three members, Exo1, Rad2, and Rad27, possess both 5′→3′ exo- and 5′ flap endo-nuclease activity, and Yen1 appears to act exclusively as an endonuclease (Sun et al., 2003, Ip et al., 2008; Tomlinson et al., 2010). In yeast, *RAD27* shares the highest sequence similarity with *EXO1*, suggesting functional overlap. In fact, previous studies have shown that *EXO1* can complement some *RAD27* functions, and the *exo1Δ rad27Δ* double mutant is inviable (Tishkoff et al., 1997, Xie et al., 2001; Qiu et al., 1999). While the substrate preferences of Rad2 family proteins vary, all have been shown to bind nicked, gapped, and/or blunt end DNA, with a particular affinity for single- to double-stranded DNA junctions. They all appear to induce a sharp bend in the DNA substrate upon protein binding (Lee and Wilson, 1999; Genschel and Modrich, 2003; Orans et al., 2011). These observations structurally demonstrate how *RAD2* family proteins can share redundant capacities for endo- and exo-nucleolytic functions.

We reasoned that a protein that mimicked the DNA binding affinity for similar DNA substrates could complement this function in cells lacking Exo1. We therefore tested the ability for Rad27 to complement the meiotic function of Exo1. We did not observe complementation by
RAD27 expressed through its native promoter, but upon placing RAD27 under control of the EXO1 promoter (pEXO1-RAD27) we saw significant increases in crossing over on both Chromosomes VIII (from 21.5% to 29.9% tetratype; Figure 4A; Table S1B) and XV (Figure 4B; 54 cM map distance in exo1Δ to 72 cM exo1Δ containing pEXO1-RAD27), likely due to the high levels of meiotic expression of the EXO1 promoter (Figure S5; Brar et al., 2012). Efforts were made to improve exo1Δ complementation by fusing a MIP domain, or the entire C-terminus of Exo1 to Rad27 to create a functional Mlh1 interaction; however, they were unsuccessful.

We reasoned that if Rad27 complemented the meiotic role of Exo1 by binding a specific DNA substrate based on structural similarity divorced from catalytic activity, inactivating Rad27 through mutation of a metal-coordinating aspartic acid D179 (Shen et al., 1996; Gary et al., 1999) would not impact its ability to effect higher crossover frequencies. Indeed, exo1Δ cells expressing pEXO1-RAD27 or pEXO1-rad27-D179A showed similar levels of crossover complementation. This observation encouraged us to further test our hypothesis by making five additional rad27 mutations based on previous biochemical and structural characterization of the human homolog of Rad27, FEN-1. These included rad27-R101A; equivalent to FEN1-R100A, of which the mutant FEN-1 protein exhibited a strong catalytic defect but remained competent for flap binding and bending (Song et al., 2018), and rad27-R105A and rad27-K130A, equivalent to FEN-1-R104A and FEN-1-K132A, of which the mutant FEN-1 proteins exhibited 20- and 5-fold reductions in flap cleavage but were not characterized for flap binding or bending (Tsutakawa et al., 2017). Two other mutations were analyzed based on Exo1 and Rad27 homology: rad27-A45E, which aligns to a mutation in the Exo1 hydrophobic wedge (exo1-S41E, Group III, Figure 1B), and rad27-H191E, which aligns to a mutation in the Exo1 DNA binding domain (exo1-K185E, Group IV). As shown in Figure 4A, rad27-R101A, rad27-R105A and rad27-K130A, which coordinate the scissile bond for catalysis, complemented the crossover defect in exo1Δ, consistent with the phenotypes exhibited by exo1 Group II mutations. Interestingly, the rad27-A45E and rad27-H191E mutations were defective in exo1Δ
complementation, as predicted for their requirements in flap bending and stabilizing the DNA backbone, respectively.

We also tested if RAD27 expression from the EXO1 promoter could improve meiotic crossover functions of exo1 strains bearing mutations within (exo1-K185E) or outside of the DNA binding domain (exo1-MIP). As shown in Figure 4C, meiotic crossing over in exo1-K185E, but not exo1-MIP, was increased in cells containing pEXO1-RAD27. These observations are consistent with Rad27 being able to substitute for Exo1 DNA binding functions because improved complementation by pEXO1-RAD27 was seen in a DNA binding mutant (exo1-K185E) but not in a mutant predicted to be functional for DNA binding (exo1-MIP), but defective in interacting with other crossover factors.

Finally, we saw no complementation of meiotic crossing over by pEXO1-RAD27 in strains lacking functional Mlh1-Mlh3 (mlh3Δ), indicating that Rad27 complementation was specific to Exo1 function. This observation differs from observations made by Arter et al. (2018), who found that expression of the Rad2/XPG nuclease Yen1 complemented crossover defects in both exo1Δ and mlh3Δ strains. One explanation for the Yen1 complementation phenotype is that Yen1 Holliday junction resolvase activity could bypass Mlh1-Mlh3-Exo1 dependent dHJ resolution steps.

**Meiotic crossover phenotype of exo1 DNA binding mutants is significantly reduced when Cdc9 ligase is overexpressed in meiosis.**

Reyes et al. (2021) et al. recently showed that overexpression of the budding yeast ligase Cdc9 disrupted DNA mismatch repair through the premature ligation of replication-associated nicks that act as critical repair signals. If the role of Exo1 in meiotic recombination involved nick binding/protection, then we reasoned that meiotic overexpression of CDC9, the budding yeast DNA ligase involved in DNA replication, could lead to premature ligation of DNA synthesis-associated nicks critical for maintaining biased resolution. We posited that some exo1 DNA binding mutants that maintained near wild-type levels of crossing over might be especially
susceptible to Cdc9 overexpression. During meiosis CDC9 expression appears to be low relative to HOP1, whose expression increases dramatically in meiotic prophase and remains high through dHJ resolution (~6hrs in meiosis; Figure S5). We thus expressed CDC9 under control of the HOP1. As shown in Figure 4D we saw no disruption of crossing over in exo1 mutants that contained intact DNA binding domains (EXO1, exo1-MIP, exo1-D173A) or in a mutant (exo1-K85E) predicted to be defective in steps post-DNA bending (Orans et al., 2011). However, we saw modest to severe losses of crossing over in exo1 DNA binding mutant hypomorphs. As shown in Figure 4D, pHOP1-CDC9 reduced single crossovers in exo1-K185A from 35.3 to 31.3% and in exo1-K61E from 35.1 to 25.2%. These data, in conjunction with the RAD27 complementation experiments, provide evidence for a nick protection role for Exo1 in crossover formation.

**Interference analysis suggests a role for Exo1 prior to crossover resolution.**

While expression of RAD27 under the EXO1 promoter (pEXO1-RAD27 plasmid) could partially complement CO defects in exo1Δ strains, it did not improve the meiotic spore viability or MMS resistance seen in exo1Δ strains (Figures 4B). We performed crossover interference analysis to determine if exo1Δ strains showed defects in addition to those seen in DSB resection and CO resolution. As described below, we found that exo1Δ strains displayed crossover interference defects that were not complemented by the pEXO1-RAD27 plasmid.

First, we analyzed exo1Δ strains bearing pEXO1-RAD27 for defects in crossover interference on chromosome XV using the Malkova method, which calculates genetic distances between intervals in the presence and absence of a neighboring crossover (Figure 5; Table S3; Malkova et al., 2004; Martini et al., 2006). These measurements are presented as a ratio, wherein 0 indicates complete interference and 1 indicates no interference. Three pairs of intervals (URA3-LEU2-LYS2, LEU2-LYS2-ADE2, LYS2-ADE2-HIS3) were tested for interference. In all three interval pairs tested, exo1Δ displayed a loss of interference compared to wild-type. Most strikingly, two intervals that displayed strong interference in wild-type strains
(Malova ratios of 0.48 at URA3-LEU2-LYS2 and 0.43 at LEU2-LYS2-ADE2) displayed a complete loss of interference in exo1Δ (1.28 and 0.84 respectively). These results are reminiscent of the interference defects observed previously in msh4Δ and msh5Δ (Ross-Macdonald and Roeder, 1994; Hollingsworth et al., 1995; Novak et al., 2001; Nishant et al., 2010; Figure 5). Interestingly, a lack of interference was observed in all three intervals in the exo1Δ strain containing pEXO1-RAD27 (Malkova ratios of 1.41, 0.90, and 0.81 in Intervals I, II, III, respectively; Figure 5), supporting the idea that RAD27 expression in meiosis could complement only Exo1’s crossover functions.

The interference defect seen in exo1Δ (all three intervals showed a lack of interference) was stronger than that seen in the mlh3Δ strain (two intervals showed a lack of interference), suggesting a role for Exo1 in promoting interference independent from its association with Mlh1-Mlh3 in crossover resolution. To determine if the early resection role of Exo1 (Zahkaryevich et al., 2010) could account for this interference function, exo1-D171A,D173A and exo1-D78A,D173A catalytic mutants were analyzed for interference defects (Figure 5). Strikingly, these mutants displayed interference similar to or stronger than wild-type. In fact, the interference defect observed in exo1Δ was not recapitulated in any of the exo1 alleles tested.

Interference was also measured using the COC (Coefficient of Coincidence) method (Papazian, 1952; Table S3A). COCs measure the double crossover rate compared to the expected rate in the absence of interference. The COC ratios were consistent with the Malkova ratio analysis, supporting the idea that loss of interference in exo1Δ was not recapitulated in any of the mutant alleles. Together the data indicate a previously uncharacterized role for Exo1 in establishing crossover interference and suggest that the pro-interference role of Exo1 is either more robust than the pro-crossover role or involves specific contact or interaction sites that were not examined in this study (see Discussion).

Genetic interactions involving Msh4-Msh5, Mlh1-Mlh3 and Exo1 also support roles for Exo1 in crossover interference.
The finding that exo1Δ showed defects in crossover interference encouraged us to determine if we could identify genetic interactions involving factors that interact with Exo1 and play roles in crossover interference. To initiate this work we analyzed exo1-F447A,F448A (referred to as exo1-MIP), which contains mutations in an Mlh1-interacting peptide box (MIP) that disrupt both Mlh1-Exo1 interactions and meiotic crossing over (Tran et al., 2007; Zakharyevich et al., 2010).

In the spore autonomous fluorescence assay we found that the exo1-MIP mutation conferred intermediate defects in CO formation (33.3% single crossovers (tetratype) compared to 37.5% in wild-type) when both this allele and MLH3 were present in two copies (Figure S6; Table S4). However, when both exo1-MIP and MLH3 were present in single copies, we observed a two-fold reduction in CO levels (to 22.6% tetratype) that approached levels seen in mlh3Δ (Figure S6). This observation confirmed interactions between Mlh1-Mlh3 and Exo1 and encouraged us to use gene dosage as an approach to identify additional genetic interactions involving Exo1 using mlh3 alleles, mlh3-42 and mlh3-54, that confer defects in Mlh3-mediated mismatch repair (MMR) but do not disrupt crossing over. Previous work showed that the mlh3 alleles disrupted Mlh1-Mlh3 interactions (Al-Sweel et al., 2017). We reduced the gene dosage of eleven meiotic genes from two to one and measured crossing over at the CEN8-THR1 interval on chromosome VIII (Figure S6; Table S4). SGS1 and RMI1 were included because they encode components of a Sgs1-Top3-Rmi1 complex that acts as a pro-crossover factor in meiotic recombination (Jessop et al., 2006; Zakharyevich et al., 2012; Kaur et al., 2015).

As shown in Figure S6 and Table S4, we observed defects for both mlh3 alleles in crossing over when the gene dosage of EXO1, MSH4, or MSH5 was reduced to one copy. For MLH1, we observed such dosage effects with only the mlh3-54 allele, and for SGS1 and RMI1, with only the mlh3-42 allele (Figure S6). Interestingly, the residues mutated in mlh3-54 mapped to the Mlh1-Mlh3 dimerization interface whereas residues mutated in mlh3-42 mapped to the distal periphery of the dimerization interface (Dai et al., 2021). While this observation might help explain the different effect of gene dosage for MLH1 in mlh3-42 and mlh3-54 backgrounds, it is
unclear why the _mlh3-42_ allele disrupts the stability of Mlh1-Mlh3 or why it showed gene dosage interactions with _SGS1_ and _RMI1_.

_mlh3_ allele-specific interactions were not observed when reducing dosage for a group of ZMM family genes (_ZIP1, ZIP3, ZIP4, SPO16, MER3_) which are thought to act upstream of Mlh1-Mlh3 to stabilize early recombination intermediates and promote CO outcomes (Agarwal and Roeder, 2000; Snowden et al., 2004; Borner et al., 2004; Kolas et al., 2005; Argueso et al., 2004; Shinohara et al., 2008; Hatkevich and Sekelsky, 2017). As shown in Figure S6, a reduction of gene dosage for _ZIP1_ and _SPO16_ did not alter crossing over in any _MLH3_ background, and a reduction of dosage for _ZIP3_ and _MER3_ led to CO decreases in _MLH3, mlh3-42_, and _mlh3-54_ backgrounds. _ZIP4_ fit a somewhat similar pattern to _ZIP3_ and _MER3_, but statistical significance was mixed, with significance for haploinsufficiency seen in only the _mlh3-42_ background. Together, these studies support a model in which Msh4-Msh5, Mlh1-Mlh3, and Exo1 form a group that participates in crossover interference (Santucci-Darmanin et al., 2002; Santucci-Darmanin et al., 2000; Zakharyevich et al., 2010; Krishnaprasad et al., 2021).

**Msh5 DNA interactions and foci are not dependent on Exo1.**

Crossover interference involves the recruitment of ZMM proteins which stabilize and identify a set of dHJs for Class I crossover resolution. Among this class of factors is Msh4-Msh5, which stabilizes SEIs after strand invasion (Boerner et al., 2004). During meiosis, the Msh4-Msh5 complex binds _in vivo_ to DSB hotspots, chromosome axes, and centromeres (Krishnaprasad et al., 2021). We previously showed Msh5 can bind resected DSB structures _in vivo_ in a mutant defective in strand invasion (_dmc1Δ_ mutant; Krishnaprasad et al., 2021). Meiotic DSB resection by Exo1 results in the formation of extensive 3’ overhangs that can promote strand invasion and joint molecule formation stabilized by ZMM proteins (Zakharyevich et al., 2010). However, previous studies have shown that in _exo1Δ_, joint molecule formation is normal, though there is a roughly 50% reduction in crossovers (Khazanehdari and Borts, 2000; Tsubouchi and Ogawa, 2000; Zakharyevich et al., 2010). Since interference and crossover formation is significantly
reduced in msh5Δ, an explanation for the interference defect in exo1Δ is that Msh4-Msh5 recruitment to recombination intermediates is compromised due to reduced resection of DSBs (Zahkaryevich et al., 2010). To address this, we analysed Msh5 binding in an exo1Δ mutant using a combination of ChIP-qPCR and cytological methods.

We performed ChIP-qPCR analysis of Msh5 binding in exo1Δ at the representative DSB hotspots (BUD23, ECM3, CCT6), chromosomal axes (Axis I, Axis II and Axis III), centromeres (CENIII, CENVIII), and the DSB coldspot (YCRO93W; Krishnaprasad et al., 2021). Enhanced Msh5 binding was observed in exo1Δ at some of the representative DSB hotspots (ECM3, CCT6) at 4h and 5h relative to the wild-type (Figure 6A). Msh5 binding at the axes and centromeres in exo1Δ was similar to wild-type from 3-5 hrs (Figure 6A).

Msh5 binding in exo1Δ was also analysed by cytological analysis of Msh5 foci (Figure 6B). The average numbers of Msh5 foci per cell in exo1Δ at 3 hrs (34), 4 hrs (45) and 5 hrs (48) were comparable to the number of Msh5 foci in wild-type at the same time points (33, 42, and 48 respectively) (Figure 6C). However, measurement of the foci intensity showed that the Msh5 foci appeared brighter in exo1Δ (Figure 6C). These observations support the ChIP-qPCR data showing enhanced Msh5 binding in exo1Δ mutants, especially at DSB hotspots. Together the ChIP and Msh5 localization studies suggest that Msh4-Msh5 localization is not dependent on either the long-range resection activity of Exo1 or interaction with Exo1. This information, in conjunction with interference analysis of exo1 nuclease defective mutants supports a direct role for Exo1 in establishing interference.

**DISCUSSION**

In this study we identified a critical function for Exo1 in meiotic crossing over dependent on its ability to bind to nicked/flapped DNA structures. This conclusion is supported by the finding that meiotic expression of the structurally similar RAD2 family nuclease Rad27 can partially compensate for the loss of crossovers in the absence of Exo1, and that meiotic overexpression of the Cdc9 ligase conferred a significant crossover defect in exo1 DNA binding domain
mutants. Based on these observations we propose that Exo1 acts in meiotic crossover formation by binding to nicks/flaps analogous to those created during lagging strand DNA synthesis (Figure 7). In contrast to the functions of Rad27 and Exo1 during replication, which cleave 5’ flaps in mechanisms that facilitates ligation of the resulting nick (Balakrishnan and Bambara, 2013), the Exo1/Rad27 meiotic crossover function occurs independently of nuclease activity. Such a nuclease-independent activity likely serves to protect nicks or flaps in recombination intermediates from premature ligation, ensuring their incorporation into a resolution mechanism. In addition, a nick/flap bound Exo1 could act to recruit Mlh1-Mlh3 to the dHJ. In support of this idea, work by Manhart et al. (2017) showed that the presence of Mlh1-Mlh3 polymer at a nicked strand can direct the endonuclease to cut the opposite strand, providing a possible mechanism for how biased resolution could occur.

**Incorporating nick-protection with models of dynamic dHJs.**

A role for a nicked recombination intermediate in forming meiotic crossovers has been proposed for many years, with a summary of a few studies provided below. 1. Electron microscopy studies of Holliday junction structures purified from yeast cultures in pachytene failed to reveal open centers expected of fully ligated junctions (Bell and Byers, 1983), though the structure of dHJs in vivo is not well understood, and so we cannot exclude the presence of factors that allow centers in fully ligated junctions to open. 2. Nicked HJs are favorable substrates for resolution by resolvase proteins in vitro (Fricke et al., 2005), and nicked HJs comprise a large proportion of Holliday junction structures observed in mutants defective in the structure-selective nucleases Yen1 and Mms4-Mus81, suggesting that they represent mitotic recombination intermediates (Garcia-Luis and Machin, 2014). 3. Whole genome sequencing of meiotic spore progeny inferred that the resolution of dHJs is biased towards new DNA synthesis tracts, implying that these tracts contain distinguishing features such as nicks (Marsolier-Kergoat et al., 2018). 4. Biochemical studies have led to models in which nicks persisting during dHJ formation could provide a substrate for continued loading of MMR/replication factors implicated in dHJ resolution.
(e.g. RFC, PCNA, Msh4-Msh5; Kulkarni et al., 2020; Cannavo et al., 2020). Furthermore, Kulkarni et al. (2020) and Cannavo et al. (2020) showed that PCNA, which is loaded onto primer template junctions during DNA replication, promotes nicking by Msh4-Msh5 and Mlh1-Mlh3. The above observations, however, are challenging to reconcile with observations in S. cerevisiae indicating that single strands of DNA within dHJs appear to be continuous (at least at the resolution of denaturing alkaline gels; Schwacha and Kleckner, 1994, 1995) and dHJs are much more dynamic than predicted based on the canonical DSB repair model (Marsolier-Kergoat et al., 2018; Peterson et al., 2020; Ahuja et al., 2021; Figure 7A). However, it is possible that nicked recombination intermediates are not detected because they are transient, yet able to provide the signals critical for crossover formation, such as loading of PCNA.

dHJs have often been portrayed as static intermediates, constrained to the location of the initiating DSB (Figure 7A). While the nick protection mechanism proposed here can be understood in the context of a canonical model in which Exo1 recruits Mlh1-Mlh3 to nick the single-stranded DNA opposite the Exo1 protected nick (Figure 7A), recent work indicated that dHJs undergo significant branch migration in vivo. Recently Marsolier-Kergoat et al. (2018), Peterson et al. (2020), and Ahuja et al. (2021) showed in meiosis that one or both junctions of the dHJ can move independently or in concert prior to resolution. Marsolier-Kergoat et al. (2018) estimated the frequency of branch migration to be on the order of 28%, and Ahuja et al. (2021), based on a detailed analysis of a well-defined recombination hotspot containing a high density of single nucleotide polymorphisms, inferred that ~50% of crossovers occurred in locations where both HJs are located on one side of the initiating DSB, with a much higher number of crossovers showing some migration.

How can nick protection be incorporated into crossover mechanisms that involve branch migration of HJs? One possibility is that nicks are translocated through "nick translation" (Marsolier-Kergoat et al., 2018). For certain types of branch migration, this mechanism would push the nicks to a new dHJ location, allowing bias to be maintained (Figure 7B, upper panel). In one such model (Marsolier-Kergoat et al., 2018), Exo1 nick protection would occur when DNA
synthesis encounters a 5’ end and resolution by Mlh1-Mlh3 would occur (Figure 7B). Alternatively, Mlh1-Mlh3 could nick at a distance from the Exo1-protected nick (Peterson et al., 2020, Figure 7B, lower panel), which could be reconciled based on previous studies showing that MLH proteins form polymers on DNA and can make multiple nicks on DNA (Hall and Kunkel, 2001; Manhart et al., 2017; Kim et al., 2019). In the Marsolier-Kergoat (2018) model, the synthesis of new DNA tracts has been hypothesized to be followed by processing of the resultant 5’ flap to create a nick. Though appealing, this model needs to be balanced with our findings that the catalytic activity of Rad27 is not necessary to rescue crossing over in an exo1Δ strain.

A key aspect of extensive branch migration is that it should prevent DNA nicks from serving as substrates for biased resolution because they locate away from the resolution site. To reconcile this observation with our analysis of Exo1, such nicks could act as substrates for the activation of an Mlh1-Mlh3 polymer (Figure 7C). Previous work showed that Mlh1-Mlh3 requires a large DNA substrate for nuclease activation and that polymerization barriers impeded its nuclease activity (Manhart et al., 2017). As such, branch migration may provide a way to move the dHJ from a constrained state that is occupied by factors that establish the dHJ such as Msh4-Msh5. In such a model, the signaling imposed by the binding of Exo1 to nicks could act across a distance, and through an initial Exo1-Mlh1-Mlh3 interaction, allowing the Mlh1-Mlh3 polymer to occupy the comparatively unconstrained DNA away from the invasion site (Figure 7C). Thus, we may consider the Exo1-nick interaction site as a nucleation point for Mlh1-Mlh3. This would add asymmetry to the polymer and ensure that Mlh1-Mlh3 nicks in a biased manner. We illustrate this within the context of a model presented by Manhart et al. (2017), in which Mlh1-Mlh3 requires polymerization across multiple kilobases to be catalytically active to cleave Type II Holliday junctions. Variations of such a model have been presented by Kulkarni et al. (2020). These models would also provide an explanation for the importance of Exo1-Mlh1-Mlh3 interactions during meiotic crossing over (but see below). In this model, we see Exo1-nick interactions as a means of guarding essential nicks from premature ligation. This would ensure
that the dHJ remains “flexible” if needed for Mlh1-Mlh3 polymerization and activation. These models are not mutually exclusive, and further work is required to understand how resolution factors interact with mobile and static dHJs.

An additional challenge with the models presented in Figure 7 is that while Exo1 and FEN-1 bind flap structures to coordinate tail removal and ligation steps, the endonuclease activities of these proteins do not appear to be required for crossover resolution. However, the finding that ligase overexpression can disrupt crossing over in exo1 DNA binding hypomorphs suggests that a ligatable nick serves as a critical recombination intermediate. One possibility is that there is a coordinated displacement of Exo1 by Mlh1-Mlh3 that induces Mlh1-Mlh3 nicking on the opposite strand. In such a model there could be other processing events that removal 5’ tails such as one involving Msh2-Msh3 recognition of the flap, followed by endonuclease cleavage by Rad1-Rad10 (Sugawara et al., 1997). It is also worth noting that studies in which we observed complementation of the exo1Δ strain with the pEXO1-rad27-D179A plasmid contained native RAD27 that could act to remove 5’ tails.

Does Exo1 direct Mlh1-Mlh3 nicking? A coordinated set of steps are required in meiotic recombination to promote Exo1 mediated resection of DSBs, D-loop formation, DNA polymerase mediated synthesis of the invading 3’ strand, Exo1 protection of flaps/nicks, and ligation of cleaved dHJs. The transitions between these steps are likely to proceed through mechanisms that involve post-translational modifications (e.g. Bhagwat et al., 2021). Recent studies have shown that Exo1 has a key role in the activation of Mlh1-Mlh3 through Cdc5 Kinase (Sanchez et al., 2020), and a protein association/mass spectrometry study (Wild et al., 2019) suggested that Mlh1-Mlh3 meiotic interactions with Exo1 are dynamic. However, we and others have shown that the exo1-MIP mutant defective in Mlh1 interactions displays an intermediate defect in meiotic crossing over (Figure S6; Zahkaryevich et al. 2010), suggesting the possibility of other factors/structures facilitating Mlh1-Mlh3 endonuclease activation. Consistent with this, Mlh1-Mlh3 foci appear to form in meiotic prophase in the absence of Exo1 (Sanchez et al., 2020) and RAD27 complementation of the exo1Δ crossover defect was not
complete and did not improve crossover interference (Figure 4). One mechanism consistent with the above observations is that a DNA structure or protein barrier forms during meiotic recombination that activates the Mlh1-Mlh3 endonuclease, analogous to that seen for activation of Type I restriction enzymes through head-on collision of two translocating enzymes. (Szczelkun, 2002). Understanding how these transitions occur will require both in vitro reconstruction studies using purified proteins and novel in vivo approaches to identify nicks in dHJ intermediates.

A role for Exo1 in promoting genetic interference

In baker’s yeast the ZMM factor Zip3 has been shown to be an early marker for crossover designation and interference, prior to the formation of physical crossovers, and previous work has suggested that crossover interference and crossover assurance are carried out as distinct functions by the ZMMs (Shinohara et al., 2008). These observations indicate that crossover interference is established prior to dHJ resolution (reviewed in Zhang et al., 2014). Interestingly, while mlh3Δ mutants lose dHJ resolution bias, residual interference in mlh3Δ mutants suggest that biased resolution is not required for interference. In contrast, a more severe loss of crossover interference in exo1Δ (Figure 5) suggests a role beyond preserving resolution bias by protecting nicks, analogous to ZMM proteins which designate crossovers and assure interference on the maturing dHJ. The interference role for Exo1 was also reflected in spore viability patterning, as only the full exo1Δ displayed a viability pattern consistent with non-disjunction. While it is not possible to determine precisely how crossover patterning is disrupted in our exo1Δ data, the strong interference defect and clear non-disjunction pattern seen in exo1Δ strains is consistent with ZMM proteins that work early in imposing interference. The nature of this role remains unclear, as none of the exo1 alleles tested showed the interference defect seen in exo1Δ, and in fact some exo1 mutants showed increased interference. While Exo1 has been observed to interact with Msh2 through a Msh2-interacting-peptide (SHIP) box, direct interaction with Msh4-Msh5 has not been characterized (Goellner et al., 2018). A link
between Exo1 and Msh4-Msh5 is also discouraged by the finding that Msh4-Msh5 localization is not dependent on Exo1 (Figure 6). This observation and previous work showing that joint molecule formation occurs at wild-type levels in \textit{exo1Δ} mutants (Zakharyevich et al., 2010) suggest that the interference defect seen in \textit{exo1Δ} mutants does not reflect the defective loading of Msh4-Msh5 to recombination intermediates.

Could the interference defect seen in \textit{exo1Δ} mutants reflect a defect in resection of DSBs? The enhanced Msh5 association with chromosomes in \textit{exo1Δ} could be interpreted as stabilizing DSB repair intermediates that would normally be eliminated and thus contribute to an interference defect. Several points argue against this idea: 1. \textit{exo1Δ} has reduced crossovers despite increased binding of Msh5 (Figure 6; Khazanehdari and Borts, 2000; Tsubouchi and Ogawa, 2000; Zakharyevich et al., 2010). 2. Msh5 enrichment in \textit{exo1Δ} could reflect compensatory/ homeostatic mechanisms to ensure crossover formation when there is a defect in the processing of recombination intermediates (e.g. Cole et al., 2012). 3. As indicated above, a large number of \textit{exo1} mutants containing mutations in catalytic and DNA binding domains (Figure 5) maintain crossover interference, consistent with defects in DSB binding domains not being the cause of the interference defect seen in \textit{exo1Δ} mutants. 4. We obtained evidence for a set of genetic interactions involving Exo1, Mlh1-Mlh3 Msh4-Msh5 and Sgs1-Top3-Rmi1 (Figure S6) consistent with Exo1 interaction with genes that are thought to function at both early and later stages in the meiotic crossover resolution pathway. Teasing apart how Exo1 coordinates roles in crossover selection and resolution is critical for understanding how biased resolution of dHJs occurs.

**MATERIALS AND METHODS**

**Exo1 homology model.** The crystal structure of human Exo1 in complex with 5’ recessed DNA (amino acids 2 to 356; Orans et. al., 2011) was used to map residues in yeast Exo1 critical for function. A homology model was constructed (Figure 1B) using the Phyre2 software (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index). The predicted structure was
aligned to human Exo1 (PDB ID: 3QEB) using Pymol (https://pymol.org/2/). Metal binding residues mutated in this study were D78, D171, and D173. Active site residues mutated were H36, K85, R92, K121. Hydrophobic wedge residues mutated were S41, F58, and K61 and DNA binding residues mutated were K185 and G236. For Figure S1 the Exo1 protein sequence from *S. cerevisiae* was submitted to the BLASTP server at NCBI and run against the landmark database. Protein sequences of Exo1 homologs from different model organisms were analyzed and a multiple-sequence alignment was generated with MAFFFT using default settings (Katoh et al., 2018).

**Purification of Exo1.** Exo1-FLAG variants (Exo1, exo1-D173A, exo1-G236D, exo1-D173A,G236D) were purified from pFastBac1 constructs (Table S6) in the baculovirus/Sf9 expression system as described by the manufacturer (Invitrogen) with the following modifications (Nicolette et al., 2010). Briefly, 250 ml of Sf9 cell pellet was resuspended in 7.5 mL of a buffer containing 50 mM Tris pH 7.9, 1 mM EDTA, 0.5 mM PMSF, 0.5 mM β-mercaptoethanol, 20 μg/mL leupeptin, and 0.25x Halt protease inhibitor cocktail (Thermo). The suspension was incubated on ice for 15 min, after which NaCl was added to a final concentration of 100 mM and glycerol was added to a concentration of 18 % (v/v) and incubated on ice for 30 min. The cells were centrifuged at 30,000xg for 30 min. The cleared lysate was applied to a 2 mL SP Sepharose Fast Flow column at a rate of ~15 mL/hr. The column was washed with 10 mL of a buffer containing 50 mM Tris pH 7.9, 10 % glycerol, 100 mM NaCl, 0.5 mM PMSF, 5 mM β-mercaptoethanol, and 6.7 μg/mL leupeptin. Exo1 variant was eluted with the above buffer containing 700 mM NaCl. Fractions containing Exo1 protein variant were pooled and applied to 0.3 mL of M2 anti-FLAG agarose beads (Sigma) in batch, incubating with rotation for ~1.5 hours at 4 ºC. Unbound protein was isolated by centrifugation at 2,000 RPM for 5 min in a swinging bucket centrifuge at 4 ºC. The resin was resuspended in 7 mL of buffer containing 20 mM Tris pH 7.9, 150 mM NaCl, 10 % glycerol, 0.1 % NP40, 0.5 mM PMSF, 0.5 mM β-mercaptoethanol, 6.7 μg/mL leupeptin, and one-third of a Complete Protease Tablet
(Roche) for every 100 mL of buffer and flowed into an empty column at ~15 ml/hr, allowing to pack. The column was then washed with 0.6 ml of the above buffer excluding the NP40 (wash buffer II). Exo1-FLAG variants were eluted using wash buffer II containing 0.1 mg/mL 3x-FLAG peptide (Sigma). After applying elution buffer, the flow was stopped after the first three fractions were collected and incubated for ~1 hr before resuming flow and collecting fractions. Fractions containing Exo1 variant were pooled, flash frozen in liquid nitrogen, and stored at -80 ºC. All purification steps were performed at 4 ºC. Protein concentration was determined by the method of Bradford (1976).

**Endonuclease assays.** Exo1 endonuclease reactions were performed on supercoiled 2.7 kb pUC18 or 4.3 kb pBR322 DNA (Invitrogen), or pUC18 DNA nicked by incubation with Nt.BstNBI (New England Biolabs; Rogacheva et al., 2014; Manhart et al., 2017). Briefly, 20 μl reactions (0 to 30 nM Exo1 or mutant derivative) were assembled in a buffer containing 20 mM HEPES-KOH pH 7.5, 20 mM KCl, 0.2 mg/ml BSA, 1% glycerol, and 5 mM MgCl2 unless otherwise indicated. Reactions (37°C, 1 hr) were stopped by the addition of a stop mix solution containing final concentrations of 0.1 % SDS, 14 mM EDTA, and 0.1 mg/ml Proteinase K (New England Biolabs) and incubated at 37 ºC for 20 min. Products were resolved by 1.2% agarose gel containing 0.1 μg/mL ethidium bromide. Samples were prepared and gels were run as described previously (Manhart et al., 2017). Gel quantifications were performed using GelEval (FrogDance Software, v1.37) using negative control reactions as background.

**Media and yeast strains.** *S. cerevisiae* SK1 yeast strains used in this study (Table S5) were grown at 30°C in either yeast extract peptone- dextrose (YPD) or synthetic complete media supplemented with 2% glucose (Rose et al., 1990). When required, geneticin (Invitrogen, San Diego) or nourseothricin (Werner BioAgents, Germany) were added to media at recommended concentrations (Goldstein and McCusker, 1999). Meiotic crossing over was analyzed in the SK1 isogenic background using spore-autonomous assays to measure crossing over in the *CEN8*
THR1 interval on Chromosome VIII (SKY3576/SKY3575 parental diploids, Thacker et al., 2011) and in the SK1 congenic EAY1108/EAY1112 background (four intervals on Chromosome XV, Argueso et al., 2004). Sporulation media was prepared as described (Argueso et al., 2004).

**Strain constructions.** Mutant alleles were transformed into *S. cerevisiae* with integration plasmids, geneXΔ::KANMX PCR fragments or on CEN6-ARSH4 and 2μ plasmids using standard techniques (Gietz et al., 1995; Rose et al., 1990). To confirm integration events, genomic DNA from transformants was isolated as described previously (Hoffman and Winston, 1987). Transformants bearing EXO1::KANMX and exo1::KANMX mutant derivatives were screened for integration by analyzing DNA fragments created by PCR using primers AO4061 and AO3838. Integration of exo1 alleles was confirmed by DNA sequencing of the DNA fragments created by PCR using primers AO3666 and AO3399 (Table S7). To confirm integration of geneXΔ::KANMX mutations, primers that map outside of the geneXΔ::KANMX PCR fragment were used (Table S7). At least two independent transformants for each genotype were made.

**exo1 integrating and EXO1, RAD27 and CDC9 expression plasmids.**

Plasmids created in this study are shown in Table S6 and the oligonucleotide primers used to make plasmids are shown in Table S7. Genes expressed in plasmids are from the SK1 strain background (Kane and Roth, 1974).

pEAI422 (4.7 KB; exo1Δ::KANMX) was built using HiFi DNA Assembly (New England Biolabs). It contains a complete deletion of the EXO1 open reading frame but retains 280 bp of 5' flanking and 340 bp of flanking 3' sequence. This plasmid was digested with SpeI and SmaI to release the exo1Δ::KANMX fragment prior to transformation.

pEAI423 (7.2KB; EXO1-KANMX) contains the entire EXO1 gene with ~300 bp of promoter sequence and ~500 bp of sequence downstream of the stop codon linked to the KANMX marker. In this construct, there are ~300 base pairs of immediate downstream
sequence to retain the small gene of unknown function that is immediately found after EXO1, followed by KANMX, followed by downstream homology. pEAI423 was created using HiFi assembly of the following DNA fragments: 1. BamH1 digested pUC18. 2. An EXO1 gene fragment made by PCR-amplifying SK1 genomic DNA with primers AO4030 and AO4031. 3. A KANMX gene fragment made by PCR-amplifying plasmid pFA6 (Bahler et al., 1998) with AO4032 and AO4033. 4. Downstream EXO1 sequences made by PCR-amplifying SK1 genomic DNA with AO4034 and AO4035. Integration of this construct confers a wild-type EXO1 genotype. Derivatives of pEAI423 containing mutations in EXO1 were constructed with the Q5 mutagenesis kit (New England Biolabs) using pEAI423 as template and the oligonucleotides shown in Table S7. The sequence of the entire open reading frame of EXO1 in wild-type and mutant constructs was confirmed by DNA sequencing in the Cornell Bioresource Center using primers AO275, AO643, AO694, AO804, AO2383, AO3886, AO4028. pEAI423 and mutant derivatives were digested with SpeI and NheI to introduce EXO1::KANMX or exo1::KANMX fragments into SKY3576 and SKY3575 by gene replacement.

pEAA726 (10.5 KB; MLH3, CEN6-ARSH4, URA3) an MLH3 complementation vector, was created by ligating a BamHI-Sall MLH3-KANMX fragment from pEAA636 into the pRS416 (ARS/CEN, URA3; Christianson et al., 1992) backbone digested with BamHI and Sall.

pEAA722 (6.4 KB; RAD27, CEN6-ARSH4, URA3), a RAD27 complementation vector, was constructed in two steps. First, a fragment of the RAD27 gene containing 259 bp upstream and 300 bp downstream sequence was created by PCR amplification of SK1 genomic DNA using primers AO4707 + AO4708. The resulting fragment was digested with SpeI + KpnI and ligated into pRS416 digested with SpeI + KpnI to create pEAA722.

pEAA715 (7.8 KB; EXO1, CEN6-ARSH4, URA3) was constructed in two steps. First, a fragment of the EXO1 gene containing 400 bp upstream and downstream sequence was created by PCR amplification of SK1 genomic DNA using primers AO4631 and AO4636. The resulting fragment was digested with SpeI + KpnI and ligated into pRS416 digested with SpeI + KpnI to create pEAA715.
pEAA720 (6.8 KB), a pEXO1-RAD27 (EXO1 promoter driving RAD27 expression), CEN6-ARSH4, URA3 vector, was constructed by HiFi assembly (New England Biolabs) using the following fragments: 1. pRS416 (CEN6-ARSH4, URA3) digested with Kpnl + XbaI. 2. EXO1 promoter region (400 bp immediately upstream ATG) amplified from the SK1 genome using AO4643 + AO4644. 3. The entire RAD27 ORF amplified from the SK1 genomic DNA using AO4645 + AO4637. 4. The EXO1 downstream region (400 bp immediately downstream of the stop codon) amplified from the SK1 genomic DNA using AO4638 + AO4636. rad27 mutant alleles were constructed with the Q5 mutagenesis kit (New England Biolabs) using pEAA720 as template. The oligonucleotides used to make the alleles are shown in Table S7). All RAD27 plasmid constructs were confirmed by DNA sequencing.

pEAM327 (9.3 KB), a CDC9, 2µ, URA3 plasmid, was constructed in two steps. First a fragment of the CDC9 ORF, containing 1000 bp upstream and 400 bp downstream sequence was created by PCR amplification of SK1 genomic DNA using primers AO4783 and AO4784. The resulting fragment was digested with HindIII and Kpnl and then ligated to pRS426 (2µ, URA3) backbone also digested with HindIII and Kpnl to create pEAM327.

pEAM329 (8.8 KB) is a 2µ, URA3 plasmid that expresses CDC9 from the HOP1 promoter (pHOP1-CDC9). It was constructed through HiFi assembly using the following fragments: 1. A DNA backbone was created by PCR amplification of pEAM327 using primers AO4837 and AO4838; the resulting DNA fragment lacks the CDC9 promoter. 2. A 500 bp DNA fragment of the HOP1 promoter (up until the HOP1 start codon) was created by PCR amplification of SK1 genomic DNA using primers AO4839 and AO4840. The two fragments were then assembled using HiFi Assembly to create pEAM329, which was confirmed by DNA sequencing.

**Tetrad analysis.** Diploids derived from EAY1108/EAY1112 were sporulated using the zero-growth mating protocol (Argueso et al., 2003). Briefly, haploid parental strains were patched together, allowed to mate overnight on complete minimal plates, and then struck onto selection
plates to select for diploids. The resulting diploids were then transferred from single colonies to sporulation plates where they were incubated at 30°C for 3 days. Tetrad s were dissected on minimal complete plates and then incubated at 30°C for 3–4 days. Spore clones were replica-plated onto relevant selective plates and assessed for growth after an overnight incubation. Genetic map distances were determined by the formula of Perkins (1949). Interference calculations from three-point intervals were conducted as described (de los Santos et al., 2001; Novak et al., 2001; Shinohara et al., 2003). Statistical analysis was done using the Stahl Laboratory Online Tools (https://elizabethhousworth.com/StahlLabOnlineTools/) and VassarStats (http://faculty.vassar.edu/lowry/VassarStats.html) and the Handbook of Biological Statistics (http://udel.edu/mcdonald/statintro.html).

Interference was measured by the Malkova method (Malkova et al., 2004). This method measures cM distances in the presence and absence of a neighboring crossover. The ratio of these two distances denotes the strength of interference, with a value closer to 1 indicating a loss of interference. Significance in the distribution of tetrads was measured using a G test (McDonald, 2014) and values of p<0.05 were considered indicative of interference. The coefficient of coincidence (C.O.C) was also measured for each interval by calculating the ratio of observed vs expected double crossovers.

**Spore-autonomous fluorescence assay.** We analyzed crossover events between spore-autonomous fluorescence reporter constructs at the CEN8-THR1 locus on Chromosome VIII (SKY3576, SKY3575; Thacker et al., 2011). To produce diploid strains for analysis in the spore autonomous fluorescence assay, haploid yeasts of opposite mating types were mated by patching together on YPD from freshly streaked colonies and allowed to mate for 4 hrs, and then transferred to tryptophan and leucine dropout minimal media plates to select for diploids. Diploids grown from single colonies were patched onto sporulation plates and incubated at 30°C for approximately 72 hours. Diploid strains containing ARS-CEN or 2µ plasmids were also grown on selective media to maintain the plasmids until just prior to patching onto sporulation
plates. Spores were treated with 0.5% NP40 and briefly sonicated before analysis using the Zeiss AxioImager.M2. At least 500 tetrads for each genotype were counted to determine the % tetratype. Two independent transformants were measured per allele. A statistically significant difference from wild-type and exo1Δ controls based on χ² analysis was used to classify each allele as exhibiting a wild-type, intermediate, or null phenotype. We applied a Benjamini-Hochberg correction at a 5% false discovery rate to minimize α inflation due to multiple comparisons.

**Sensitivity to methyl-methane sulfonate.** Yeast strains were grown to saturation in YPD liquid media, after which they diluted in water and spotted in 10-fold serial dilutions (undiluted to 10⁻⁵) onto YPD media containing 0.04% MMS (v/v; Sigma). Plates were photographed after a 2-day incubation at 30°C.

**Haploinsufficiency screen.** We created knockout transformation PCR fragments consisting of a KANMX4 antibiotic resistance marker flanked by 300 bp of upstream and downstream homology with respect to the open reading frame (ORF) of each gene of interest. These cassettes were amplified by PCR from genomic preps of the appropriate strains from the *Saccharomyces* genome deletion project (Giaever et al., 2014). In this collection, each ORF has been replaced with KANMX4.

EAY3486 (Table S5), a *mlh3Δ* strain carrying a gene encoding a cyan fluorescent protein (CFP) on chromosome VIII, was transformed with the PCR amplified knockout cassette. Cells were then plated on YPD-G418 plates and grown at 30°C for three days. At least two independent transformants were verified by confirming resistance to G418 and PCR amplification of using genomic preps of G418 resistant transformants. For PCR verification, primers annealing 350 bp upstream and downstream of the ORF of the gene of interest were utilized to ensure integration at the proper locus. Haploids were then mated to four *MLH3* strains each carrying a gene encoding a red fluorescent protein (RFP) on chromosome VIII. These four
strains are as follows: EAY3252 (MLH3), EAY3255 (mlh3Δ), EAY3572 (mlh3-42), and EAY3596 (mlh3-54). Diploids were isolated by selecting on media lacking tryptophan and leucine and analyzed in the spore-autonomous fluorescence assay described below.

Our criteria for allele-specific interactions was one in which there was little to no change in percent tetratype in either an MLH3 and mlh3Δ background, but there was a significant drop of percent tetratype in either mlh3-42 or mlh3-54 backgrounds. Significance was assessed by \( \chi^2 \) test between haplosufficient and haploinsufficient conditions. To minimize \( \alpha \) inflation due to multiple comparisons, we applied a Benjamini-Hochberg correction at a 5% false discovery rate (Benjamini and Hochberg, 1995).

**Chromatin immunoprecipitation.** Yeast strains KRY753, KTY756, KTY757, NHY1162 and NHY1168 used in the ChiP-qPCR and Msh5 localization analyses (Figure 6) are all derivatives of the *S. cerevisiae* SK1 strain. The *exo1Δ:: KanMX4* marker in KTY753, KTY756 and KTY757 was created using homologous recombination based gene knockout approach in the NHY1162/1168 background (Martini et al., 2006). The transformed colonies were verified by PCR using primers designed for the *EXO1* flanking regions. Msh5 ChIP was performed using polyclonal Msh5 antibody (generated in rabbit) and Protein A Sepharose beads (GE Healthcare) on synchronized meiotic cultures as described in Krishnaprasad et al. (2021). The immunoprecipitated DNA was collected at 3h, 4h, and 5h post entry into meiosis and used for ChIP-qPCR. The DNA enrichment for the Msh5 ChIP-qPCR was estimated with reference to the input at each time point. Msh5 enrichment data for the wild-type was from Krishnaprasad et al. (2021). ChIP-qPCR was performed on two independent biological replicates of Msh5 immunoprecipitated DNA samples from *exo1Δ* (3h, 4h, and 5h). Msh5 binding was analyzed at representative DSB hotspots (*BUD23, ECM3, CCT6*), axes (*Axis I, Axis II, Axis III*), centromeres (*CENIII, CENVIII*), and DSB coldspot (*YCR093W*). Chromosomal coordinates for these regions and the primer sets used for the qPCR are described in Krishnaprasad et al. (2021).
Cytological analysis of Msh5 foci.

Chromosome spreads (3h, 4h and 5h) were prepared from synchronized meiotic cultures (3, 4 and 5hr) as described (Bishop, 1994; Shinohara et al., 2008; Challa et al., 2019). Msh5 staining was performed using primary antibody against Msh5 (Shinohara et al., 2008) at 1:500 dilution, followed by secondary antibody (Alexa fluor 488, Thermo Fisher Scientific) at 1:1500 dilution. The Msh5 stained samples were imaged using an epi-fluorescence microscope (BX51, Olympus) with a 100X objective (NA,1.3). Images were captured by the CCD camera (CoolSNAP, Roper) processed using iVision (Sillicon) software. To quantify Msh5 focus intensity, the mean fluorescence of a whole nucleus was quantified with Fiji (ImageJ). The final fluorescence intensity of Msh5 was normalized with DAPI intensity for each nucleus. Fluorescence intensity refers to pixel intensity per unit area on chromosome spreads.

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COMPETING INTERESTS

The authors declare no competing interests.

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FIGURE LEGENDS

Figure 1. Metal binding, active site interactions, and DNA contact sites of Human Exo1 based on the crystal structure of the Exo1-5' recessed DNA complex. A. Canonical model showing roles for Msh4-Msh5, Mlh1-Mlh3, and Exo1 in meiotic crossover resolution. See text for details. B. Close-up of the Exo1 active site (adapted from Orans et al. (2011) using crystal structure PDB #3QEA). We highlight the following residues which were mutated in this study (Figure S1): Group I; acidic residues (D78, D171, D173) which coordinate the two metal ions. Group II; residues that are part of the α4-α5 helical arch involved in fraying (H36, K85, K121) and coordinating the scissile bond adjacent to the catalytic metals that interact with the active site (R92). Group III; S41, F58, K61, which are part of a hydrophobic wedge which induces the sharp bend in DNA at the site of a nick. Group IV; K185, G236, residues that interact with duplex DNA (K185, G236). Group V; residues (F447, F448) in a region of Exo1 that interact with Mlh1. The exo1-F447A,F448A allele is abbreviated in the text as exo1-MIP.

Figure 2. Meiotic crossover phenotypes in exo1 mutant strains. A. Genetic markers on chromosome XV spanning the CENXV-HIS3 interval in the EAY1108/1112 strain background (Argueso et al., 2004). The solid circle indicates the centromere. Distances between markers in KB and cM are shown for wild-type (not drawn to scale). B. Cumulative genetic distance (cM) in wild-type (WT) and exo1 strains. Genetic map distances for the URA3-HIS3 interval of chromosome XV in wild-type and the indicated mutant strains. Each bar is divided into sectors corresponding to genetic intervals in the URA3-HIS3, as measured from tetrads (T). The spore viability data obtained from tetrad analysis are shown, with the complete data set presented in Figure S3. The asterisks indicate the number of genetic intervals (0-4) that are distinguishable from wild-type in the indicated genotypes as measured using standard error calculated by Stahl Laboratory Online Tools (https://elizabethhousworth.com/StahlLabOnlineTools/; Table S2).
Figure 3. Crossing over for the indicated exo1 strains was measured in the 20 cM CEN8 to THR1 interval on Chr. XV using a spore-autonomous fluorescence assay (Thacker et al., 2011). A. The spore autonomous fluorescence assay was used to measure single meiotic crossover events (tetratypes) in the chromosome VIII CEN8-THR1 interval (Thacker et al., 2011). B. Single meiotic crossover events in the indicated strains. Mutations are separated into categories based on disruption of specified functions outlined in Figure 1B. EXO1 and exo1Δ levels are indicated by green and red dashed lines, respectively. *, statistically distinguishable from EXO1 and exo1Δ; -, distinguishable from EXO1, but indistinguishable from exo1Δ. See Table S1 for the complete data set.

Figure 4. RAD27 expressed from the EXO1 promoter can restore crossover functions to exo1Δ strains. A. pEXO1-RAD27, ARS-CEN (pEAA720), the indicated mutant rad27 derivatives (pEAA724, pEAA727-731), and an empty ARS-CEN vector (pRS416), were transformed into an exo1Δ strain and examined for crossing over at the CEN8-THR1 locus. The rad27 mutations were grouped (I, metal-coordinating; II, active-site; III, hydrophobic wedge; IV, duplex DNA) like those presented for Exo1 (Figure 1B). Significance (*p<0.05; **p<0.01) compared to the exo1Δ strain containing an empty vector was determined using a two-tailed Fisher’s Exact Test. B. The pEXO1-RAD27 plasmid pEAI482 was transformed into exo1Δ strains (with pEXO1, ARS CEN (pEAI483) and an empty ARS-CEN vector (pLZ259) as controls) to measure crossing over in the URA3-HIS3 interval in the EAY1108/1112 background. Asterisks indicate the number of genetic intervals that are distinguishable from the exo1Δ containing the empty vector, as measured using standard error calculated through Stahl Laboratory Online Tools (https://elizabethhousworth.com/StahlLabOnlineTools/; Table S2). C. mlh3Δ and the indicated exo1 strains were transformed with pEXO1-RAD27 (pEAA720), pEXO1-rad27-D179A (pEAA724) and empty vector (pRS416), and examined for crossing over at the CEN8-THR1 locus. Significance (*p<0.05) compared to the exo1Δ strain containing an empty vector (panel A) was determined using a two-tailed Fisher’s Exact Test. D. CDC9
overexpression in meiosis disrupts the crossover functions of exo1 DNA binding mutants. Strains with the indicated exo1 genotypes (Table S5) were transformed with a 2µ URA3 vector containing no insert (empty 2µ, pRS426) or CDC9 expressed from the HOP1 promoter (pHOP1-CDC9, 2µ, pEAM329) and then assessed for meiotic crossing over in the CEN8-THR1 interval. Significance is shown between each empty vector-pHOP1-CDC9 pair using a two-tailed Fisher’s Exact Test, with ** indicating p<0.01.

Figure 5. Interference Analysis for pairs of adjacent genetic intervals on Chromosome XV in the EAY1108/EAY1102 strain background. Crossover interference was analyzed on Chromosome XV by measuring centimorgan (cM) distances in the presence and absence of a neighboring crossover (Malkova et al., 2004; Martini et al., 2006; Tables S3A, S3B). Malkova interference is presented as a ratio of cM crossover absent/cM crossover present. *Dashes indicate no detectable positive interference. Significance of differences in tetrad distribution was assessed using a G test. Statistically significant p values (p <0.05) suggest the presence of interference (I) in the genetic interval (Tables S3B).

Figure 6. Msh5 localization to chromosomes in wild-type and exo1Δ strains. A. ChIP-qPCR analysis of Msh5 binding at DSB hotspots (BUD23, ECM3, and CCT6), centromere regions (CEN III, CEN VIII) and axis regions (Axis I, Axis II, Axis III) relative to DSB coldspot (YCR093W) in wild-type and exo1Δ at 3, 4, and 5 hrs after transfer of cells to sporulation media (see Krishnaprasad et al., 2021 for region assignment). The samples are normalized using input and plotted after dividing with the cold spot value. Error bars represent the standard deviation from two independent biological replicates. B. Representative images of Msh5 staining of chromosome spreads of wild-type and the exo1 mutant cells at 5-hr incubation in sporulation media. Msh5, green; DAPI, blue. Bar indicates 2 µm. C, top; number of Msh5 foci was counted in Msh5-focus positive spreads at the indicated times. At each time point, 30 nuclei were counted. Mean+/- standard deviation of three independent time courses are shown. C, bottom;
relative ratio of Msh5 intensity to DAPI intensity was quantified. At each time point, 30 Msh5-positive nuclei were analyzed. Mean+/- standard deviation of three independent time courses are shown.

**Figure 7. Models for biased resolution of double Holliday junctions.** A. Canonical model. In the major interference-dependent crossover pathway, a D-loop intermediate is stabilized by ZMM proteins including Msh4-Msh5 to form a single end invasion intermediate. DNA synthesis from the SEI, followed by second-end capture, results in the formation of the dHJ intermediate which is stabilized by Msh4-Msh5. Biased resolution of the two junctions results in crossover formation. In this model, Exo1 protection of the nick/flap structure recruits Mlh1-Mlh3 to nick the DNA strand opposite the Exo1 protected nick. B. dHJ resolution through limited branch migration, focusing on models adapted from Marsolier-Kergoat et al. (2018; upper panel) and Peterson et al. (2020; lower panel). In these models one or both junctions of the dHJ move prior to resolution. In our adaptation of the Peterson et al. (2020) model, Exo1-protection of nicks recruits Mlh1-Mlh3 as in panel A. In our adaptation of the Marsolier-Kergoat et al. (2018) model, Exo1 protects nicks made by nick translation (resolution independent nicks) and recruits Mlh1-Mlh3 as in panel A. C. dHJ resolution through extended branch migration (Ahuja et al., 2021). Branch migration creates a substrate for Mlh1-Mlh3 polymerization (Manhart et al., 2017). In such a model, the signaling imposed by the binding of Exo1 to nicks acts at a distance. Mlh1-Mlh3 is recruited by Exo1 and forms a polymer with a specific polarity that can displace other factors or be activated upon interaction with such factors. The polymer is activated to introduce a nick on one strand of the duplex DNA on Type II dHJs when it forms a critical length required for stability. See text for details.
Fig. 1

I. Metal-coordinating residues

II. Active-site interactors

III. Hydrophobic wedge

IV. Duplex DNA interactors

V. C-terminal Mlh1 interaction (MIP)

ZMM proteins stabilize SEIs

Biased resolution
Fig. 2

I. Metal coordinating

III. Wedge

IV. DNA binding

V. Mih1 interaction

A

B

URA3-LEU2
LEU2-LYS2
LYS2-ADE2
ADE2-HIS3

URA3-HIS3: 395 KB, 100.9 cM
TRP1

Chr. XV (1,095 KB)

130 43 59 167 KB
22.8 27.5 12.9 37.2 cM

deo2::hisG his3::hisG

% Spore Viability

0 20 40 60 80 100
Fig. 3

A

B

I. Metal Coordinating Residues
II. Active Site Interactors
III. Hydrophobic Wedge Components
IV. Duplex DNA Interactors
V. Mlh-1 Interaction

Class Combinations

% single crossovers (tetatype)
Fig. 4
% Spore viability | Malkova interference ratios
---|---
**wild-type** 96% | TRP1: 0.48 0.43 0.90
| URA3 LEU2 LYS2 ADE2 HIS3 1.28 0.84 1.62
| TRP1 1.41 0.90 0.81
| URA3 LEU2 LYS2 ADE2 HIS3 0.46 0.58 1.08
| TRP1 1.29 0.97 2.16
| URA3 LEU2 LYS2 ADE2 HIS3 0.54 0.17 0.65
| TRP1 0.46 0.15 0.56
| URA3 LEU2 LYS2 ADE2 HIS3 0.25 0.44 1.03
| TRP1 0.46 0.15 0.56
| URA3 LEU2 LYS2 ADE2 HIS3 0.25 0.44 1.03
| TRP1

**exo1Δ** 74% | pEXO1-RAD27
---|---
**mlh3Δ** 71% | exo1-D78A,D173A
**msh5Δ** 33% | exo1-S41E
**exo1-G236D** 86%
**Fig. 6**

(A) DNA-fold enrichment with respect to DSB coldspot over time in sporulation media (hrs).

(B) Merged, DAPI, and Msh5 images showing the distribution of Msh5 focus number and relative Msh5 intensity.

(C) Graphs depicting the relative Msh5 focus number and intensity over time in sporulation media (hrs).
A. Nick directed without branch migration

B. Nick directed with branch migration

C. Extensive branch migration coupled with Mlh1-Mlh3 polymerization initiating from Exo1 protected nick

Fig. 7
Figure Legends

Figure S1. Alignment of Exo1 protein sequences from *S. cerevisiae* (accession # NP_014676), *S. pombe* (NP_596050.1), *H. sapiens* (NP_003677), *M. musculus* (NP_036142) and *D. melanogaster* (NP_477145). Sequence alignment of Exo1 from different species. Triangles indicate mutations made in this study. See Materials and Methods for sequence alignment details.

Figure S2. Nuclease activity of Exo1 on plasmid substrates. A. Nuclease activity of Exo1 (WT) and exo1-D173A (DA; Materials and Methods) on a 2.7 kb pUC18 plasmid with four pre-existing nicks. DNA products were resolved by native agarose gel. Exo1 is present at 6 nM, 12 nM, and 24 nM in lanes 2-4, and exo1-D173A is present at 20 and 40 nM in lanes 5-6. B. Exo1 does not show nuclease activity on supercoiled (cc) 2.7 kb pUC18 plasmid. Exo1 is present at 1 nM and 10 nM in lanes 2 and 3, respectively, and exo1-D173A is present at 20 nM in lane 4. C. Titration of exo1-D173A endonuclease activity on supercoiled (cc) pBR322 substrate. D. Titration of exo1-D173A, exo1-G236D and exo1-D173A,G236D endonuclease activity on a supercoiled pBR322 substrate.

Figure S3. Spore viability profile of wild-type and the indicated exo1 strains in the EAY1108/EAY1112 strain background. The percent of tetrads with 4, 3, 2, 1, and 0 viable spores are shown from the dissections presented in Figure 2 as well as the total number of tetrads dissected and the overall spore viability.

Figure S4. Sensitivity of exo1 mutants to the DNA damaging agent MMS. *Wild-type* and the indicated exo1 mutants (Figure 2A) were spotted in 10-fold serial dilutions onto YPD and YPD media containing 0.04% MMS (Materials and Methods). Plates were photographed after a
2-day incubation at 30°C. In the bottom most panel an exo1Δ strain (EAY4778) was transformed with an ARS-CEN vector containing no insert (pRS416), EXO1 (pEAA715) or RAD27 expressed from the EXO1 promoter (pEXO1-RAD27, pEAA720).

Figure S5. mRNA seq and ribosome profiling of EXO1, RAD27, CDC9 and HOP1 expression in SK1 meiosis. Data obtained from Brar et al. (2012). RPKM= Reads per kilobase of coding sequence per million mapped reads.

Figure S6. Haploinsufficiency analysis shows genetic interactions between MLH3 and MLH1, EXO1, MSH4, MSH5, SGS1, and RMI1, but not between MLH3 and ZIP1, ZIP3, ZIP4, SPO16, and MER3. A. The mlh3-42 and mlh3-54 mutations analyzed in haploinsufficiency analysis map onto the C-terminal domain of MLH3. Each allele confers defects in Mlh1-Mlh3 interactions and Mlh3-dependent DNA mismatch repair, but do not confer strong defects in meiotic crossing over (Al-Sweel et al., 2017). B. Strains containing one or two copies of EXO1, MLH3, or the exo1-MIP mutations were analyzed for crossing over in the 20 cM CEN8 to THR1 interval using a spore-autonomous fluorescence assay (Thacker et al., 2011). C. A haploinsufficiency screen identified EXO1, MLH1, MSH4, MSH5, SGS1, and RMI1 interactions with MLH3. Strains containing one or two copies of EXO1, MLH1, MSH4, MSH5, SGS1 and RMI1 were analyzed for crossing over in wild-type, mlh3Δ and mlh3-42 and mlh3-54 strains (Materials and Methods). D. Haploinsufficiency of ZIP1, ZIP3, ZIP4, and MER3 conferred decreases in crossover frequencies that were not mlh3 alleles-specific, and haploinsufficiency of SPO16 did not affect CO frequency. Crossing over was also measured in the 20 cM CEN8 to THR1 interval. Significance was assessed by χ² test between haplosufficient and haploinsufficient conditions. To minimize α inflation due to multiple comparisons, we applied a Benjamini-Hochberg correction at a 5% false discovery rate.
Fig. S2
Fig. S3

Viable spores per tetrad class

WT
exo1Δ
mh3Δ
exo1Δ, mh3Δ
msh5Δ
exo1Δ, msh5ΔΔ
exo1Δ, pEXO1 - RAD27
exo1 Δ, D78A/D173A
exo1 Δ, D71A/D73A
exo1 Δ, S41E
exo1 Δ, F58E
exo1 Δ, K185E
exo1 Δ, G236D
exo1 Δ, K185E, G236D
exo1 Δ, MIP
exo1 Δ, K185E, MIP
exo1 Δ, D78A/D173A
exo1 Δ, D71A/D73A
exo1 Δ, S41E
exo1 Δ, F58E
exo1 Δ, K185E
exo1 Δ, G236D
exo1 Δ, K185E, G236D
exo1 Δ, MIP
exo1 Δ, K185E, MIP

Controls

I. Catalytic
II. Wedge
III. DNA binding
IV. DNA binding
V. MIP

I. Metal binding
III. Hydrophobic wedge

Viable spores
- 4
- 3
- 2
- 1
- 0

tetrads dissected

|       | 501 | 486 | 210 | 238 | 151 | 133 | 261 | 297 | 395 | 262 | 244 | 333 | 541 | 410 | 411 | 406 |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| % viable | 96  | 74  | 71  | 74  | 33  | 48  | 66  | 83  | 79  | 74  | 87  | 85  | 86  | 71  | 89  | 73  |
| Phenotype      | MMS Phenotype | Meiotic CO Phenotype | MMS-CO correlation? |
|---------------|---------------|----------------------|---------------------|
| wild-type     | +++           | +++                  | Yes                 |
| exo1-D78A     | +             | +++                  | No                  |
| exo1-D173A    | ++            | +++                  | Yes                 |
| exo1-D171A    | ++            | +++                  | Yes                 |
| exo1-D173A, D171A | ++      | +++                  | Yes                 |
| wild-type     | +++           | +++                  | Yes                 |
| exo1-D78A, D173A | +       | +++                  | No                  |
| exo1-D173A, D171A | ++      | +++                  | Yes                 |
| exo1-R92A     | ++            | +++                  | Yes                 |
| exo1-K86E     | +             | +++                  | No                  |
| exo1-K121A    | -             | +++                  | No                  |
| exo1-S41E     | +             | +/-                  | Yes                 |
| exo1-F58E     | ++            | +/-                  | No                  |
| exo1-K185E    | ++            | +/-                  | No                  |
| exo1-G236D    | +             | +/-                  | Yes                 |
| exo1-MIP      | +++           | +                    | No                  |
| exo1.Δ + pEXO1-RAD27 | +++   | +++                  | No                  |
| exo1.Δ + pEXO1 | +++           | +++                  | No                  |
| empty vector  | -             | -                    | -                   |

II. Active site

**I. Catalytic/metal binding**

**II. Active site**

**III. Hydrophobic wedge**

**IV. Duplex DNA Mlh1-interaction**

**RAD27 complementation**
Meiotic expression profiles from Brar et al., 2012

mRNA

Ribosome Profiling

Fig. S5
Fig. S6
Table S1A. Spore Autonomous Meiotic Crossover Analysis of exo1 mutants.

| Allele | %Tetraply | Tetrams Counted | Phenotype |
|--------|-----------|-----------------|-----------|
| EXO1/EXO1 | 39.0 | 1071 | + |
| EXO1/exo1Δ | 37.9 | 1071 | + |
| exo1Δ/exo1Δ | 20.0 | 1054 | - |

**Metal Binding (Group 1)**

| Allele | %Tetraply | Tetrams Counted | Phenotype |
|--------|-----------|-----------------|-----------|
| exo1-D171A/exo1Δ | 38.9 | 517 | + |
| exo1-D171A,D173A/exo1Δ | 39.1 | 511 | + |
| exo1-D78A/exo1-D78A | 39.7 | 531 | + |
| exo1-D173A/exo1-D173A | 37.4 | 519 | + |
| exo1-D78A,D173A/exo1-D78A,D173A | 36.4 | 544 | + |

**Active Site DNA Interactors (Group II)**

| Allele | %Tetraply | Tetrams Counted | Phenotype |
|--------|-----------|-----------------|-----------|
| exo1-H36E/exo1Δ | 35.4 | 506 | + |
| exo1-K85A/exo1Δ | 34.8 | 526 | + |
| exo1-K85E/exo1Δ | 34.5 | 533 | + |
| exo1-R92A/exo1Δ | 34.8 | 506 | + |
| exo1-R121A/exo1Δ | 37.7 | 605 | + |
| exo1-R121E/exo1Δ | 34.5 | 765 | + |

**Hydrophobic Wedge (Group III)**

| Allele | %Tetraply | Tetrams Counted | Phenotype |
|--------|-----------|-----------------|-----------|
| exo1-S41E/exo1Δ | 28.4 | 506 | INT |
| exo1-F58E/exo1Δ | 27.8 | 507 | INT |
| exo1-K61A/exo1Δ | 35.1 | 525 | + |
| exo1-K61E/exo1Δ | 35.1 | 507 | + |
| exo1-S41E,F58E/exo1Δ | 24.6 | 504 | INT |
| exo1-S41E,K61E/exo1Δ | 24.5 | 506 | - |

**Duplex DNA Interactors (Group IV)**

| Allele | %Tetraply | Tetrams Counted | Phenotype |
|--------|-----------|-----------------|-----------|
| exo1-K185A/exo1Δ | 35.4 | 720 | + |
| exo1-K185E/exo1Δ | 24.5 | 649 | INT |
| exo1-G236D/exo1-G236D | 29.9 | 521 | INT |
| exo1-G236D/exo1Δ | 29.1 | 515 | INT |
| exo1-G236D,K185E/exo1Δ | 24.2 | 508 | - |

**Mlh1-Interacting (MIP, Group V)**

| Allele | %Tetraply | Tetrams Counted | Phenotype |
|--------|-----------|-----------------|-----------|
| exo1- F447A,F448A/exo1-F447A,F448A | 33.3 | 547 | INT |
| exo1- F447A,F448A/exo1Δ | 26.2 | 519 | INT |

**Double and Triple Mutants**

| Allele | %Tetraply | Tetrams Counted | Phenotype |
|--------|-----------|-----------------|-----------|
| exo1-D171A,G236D/exo1Δ | 31.1 | 552 | INT |
| exo1-D173A,G236D/exo1Δ | 32.7 | 618 | + |
| exo1-D173A,G236D/ exo1-D173A,G236D | 35.7 | 532 | + |
| exo1-D173A,K185E,G236D/exo1Δ | 22.4 | 553 | - |
| exo1-G236D,F447A,F448A/exo1Δ | 25.1 | 617 | INT |
| exo1-K185E,F447A,F448A/exo1Δ | 24.8 | 572 | INT |
| exo1-D173A,G236D,F447A,F448A/exo1Δ | 26.6 | 500 | INT |
| exo1-R92A,R121A,K185A/exo1Δ | 24.3 | 535 | - |

Homzygous mutations were made by crossing two independently constructed strains with the exo1 variants in the SKY3576 (containing cyan fluorescent protein; Table S5) and SKY3575 (containing red fluorescent protein) backgrounds. Heterozygous mutations were made by crossing two independently constructed strains with exo1 variants in the SKY3576 and EAY4151 (exo1Δ) backgrounds. Diploid strains were induced for meiosis and % tetratype in the
CEN8-THR1 interval was measured, by determining the total tetratypes/sum of tetratypes and parental ditypes). At least 500 tetrads were counted for each allele, and unless indicated (*one transformant analyzed), at least two transformants were analyzed for each background. Significance was assessed by Fisher’s exact test between mutant and wild-type EXO1 and exo1Δ tetraply type values. To minimize α inflation due to multiple comparisons, we applied a Benjamini-Hochberg correction at a 5% false discovery rate. +, indistinguishable from wild-type; -, indistinguishable from exo1Δ; INT, distinguishable from both wild-type and exo1Δ.

Table S1B. Spore autonomous assay: pEXO1-RAD27 complementation of exo1Δ and mlh3Δ strains

| Genotype                  | Plasmid           | %Tetratype | Tetrads Counted | Phenotype |
|---------------------------|-------------------|------------|-----------------|-----------|
| exo1Δ/exo1Δ              | EXO1              | 34.1       | 557             | +         |
| exo1Δ/exo1Δ              | empty vector      | 21.5       | 512             | -         |
| exo1Δ/exo1Δ              | RAD27             | 22.6       | 1032            | -         |
| exo1Δ/exo1Δ              | pEXO1-RAD27       | 29.9       | 521             | +         |
| exo1Δ/exo1Δ              | pEXO1-rad27-D179A | 28.8       | 510             | +         |
| exo1Δ/exo1Δ              | pEXO1-rad27-A45E  | 22.4       | 511             | -         |
| exo1Δ/exo1Δ              | pEXO1-rad27-R101A | 29.7       | 542             | +         |
| exo1Δ/exo1Δ              | pEXO1-rad27-R105A | 28.7       | 521             | +         |
| exo1Δ/exo1Δ              | pEXO1-rad27-K130A | 28.9       | 505             | +         |
| exo1Δ/exo1Δ              | pEXO1-rad27-H191E | 24.0       | 530             | -         |
| mlh3Δ/mlh3Δ              | MLH3              | 35.6       | 508             | +         |
| mlh3Δ/mlh3Δ              | empty vector      | 22.5       | 528             | -         |
| mlh3Δ/mlh3Δ              | pEXO1-RAD27       | 21.5       | 512             | -         |
| mlh3Δ/mlh3Δ              | pEXO1-rad27-D179A | 19.9       | 513             | -         |
| exo1-K185E/exo1Δ         | empty vector      | 25.4       | 1538            | N/A       |
| exo1-K185E/exo1Δ         | pEXO1-RAD27       | 29.0       | 1541            | N/A       |
| exo1-F447A,F448A/exo1Δ   | empty vector      | 30.1       | 512             | N/A       |
| exo1-F447A,F448A/exo1Δ   | pEXO1-RAD27       | 29.7       | 526             | N/A       |

Diploids of the indicated genotype that contain markers to measure crossing over in the CEN8-THR1 interval (Table S5) were transformed with the indicated plasmids (pEAA715-EXO1, URA3, CEN6-ARSH4; pRS416-URA3,CEN6-ARSH4; pEAA722-RAD27, URA3, CEN6-ARSH4; pEAA720-pEXO1-RAD27, URA3, CEN6-ARSH4; pEAA724-pEXO1-rad27-D179A, URA3, CEN6-ARSH4; pEAA727-rad27-A45E, URA3, CEN6-ARSH4; pEAA728-rad27-R101A, URA3, CEN6-ARSH4; pEAA729-rad27-R105A, URA3, CEN6-ARSH4; pEAA730-rad27-K130A, URA3, CEN6-ARSH4; pEAA731-rad27-H191E, URA3, CEN6-ARSH4) and selected for plasmid retention. The resulting strains were induced for meiosis and % tetraply type (single crossovers) in the CEN8-THR1 interval was measured, by determining the total tetratypes/sum of tetratypes and parental ditypes. At least 500 tetrads were counted for each allele/plasmid combination, and at least two transformants were analyzed for each condition. Significance (presented in Figure 4A, C) was assessed by Fisher’s Exact Test between exo1Δ strains containing pRS416 (empty vector) and test conditions with the indicated plasmids. To minimize α inflation due to
multiple comparisons, we applied a Benjamini-Hochberg correction at a 5% false discovery rate. The significance of % tetratype in exo1-K185E and exo1-F447A,F448A (MIP) strains containing pRS416 (empty vector) and pEAA720 (pEXO1-RAD27) was determined using Fisher’s exact test. N/A, not applicable.

Table S1C. Effect of pHOP1-CDC9 expression on meiotic crossing over in exo1 strains.

| Genotype          | Plasmid          | %Tetratype | Tetrads Counted |
|-------------------|------------------|------------|-----------------|
| EXO1/exo1Δ        | empty vector     | 41.3       | 520             |
| EXO1/exo1Δ        | pHOP1-CDC9       | 41.0       | 528             |
| exo1Δ/exo1Δ       | empty vector     | 21.6       | 519             |
| exo1Δ/exo1Δ       | pHOP1-CDC9       | 22.2       | 543             |
| exo1-MIP/exo1Δ    | empty vector     | 30.1       | 512             |
| exo1-MIP/exo1Δ    | pHOP1-CDC9       | 30.2       | 540             |
| exo1-K61E/exo1Δ   | empty vector     | 35.1       | 521             |
| exo1-K61E/exo1Δ   | pHOP1-CDC9       | 25.2       | 514             |
| exo1-K85E/exo1Δ   | empty vector     | 36.2       | 1529            |
| exo1-K85E/exo1Δ   | pHOP1-CDC9       | 33.3       | 1530            |
| exo1-K185A/exo1Δ  | empty vector     | 35.3       | 1536            |
| exo1-K185A/exo1Δ  | pHOP1-CDC9       | 31.3       | 1583            |
| exo1-D173A/exo1Δ  | empty vector     | 38.9       | 501             |
| exo1-D173A/exo1Δ  | pHOP1-CDC9       | 38.5       | 509             |

Diploids of the annotated genotype were transformed with the indicated plasmid (pRS426-URA3, 2µ; pEAM329-pHOP1-CDC9, URA3, 2µ) and selected for diploidy and plasmid retention. Diploid strains were induced for meiosis and % Tetratype in the CEN8-THR1 interval was measured by determining the total tetratypes/sum of tetratypes and parental ditypes. At least 500 tetrads were counted for each allele/plasmid combination, and at least two transformants were analyzed for each condition. Significance was assessed by Fisher’s exact test between pRS426 value and pEAM329 value and is shown in Figure 4D.
Table S2. Genetic map distances (cM) and the distribution of parental and recombinant progeny for the EAY1108/EAY1112 strain background in WT, *mlh3Δ*, *msh5Δ*, and *exo1* strains on Chromosome XV.

| Relevant genotype | Number analyzed | cM | PD | TT | NPD | Number analyzed | cM (95% CI) | Parental | Recombinant |
|-------------------|----------------|----|----|----|-----|----------------|-------------|----------|-------------|
| **URA3-LEU2:**    |                |    |    |    |     |                |             |          |             |
| wild-type         | 501            | 22.4+/−1.5 | 292 | 206 | 3   | 2285           | 22.2 (19.8-23.2) | 1794 | 491         |
| exo1Δ            | 486            | 11.2+/−1.4 | 392 | 91  | 3   | 2510           | 9.6 (8.5-10.8)  | 2267 | 241         |
| exo1-K185E       | 333            | 17.1+/−2.5 | 227 | 96  | 12  | 1549           | 18.1 (16.2-20.1) | 1269 | 280         |
| exo1-G236D       | 541            | 15.8+/−1.6 | 388 | 129 | 6   | 2676           | 14.2 (12.9-15.6) | 2296 | 380         |
| exo1-K185E G236D | 410            | 16.3+/−1.5 | 286 | 122 | 2   | 2409           | 15.5 (14.1-17.0) | 2033 | 374         |
| exo1-MIP         | 411            | 14.8+/−1.6 | 304 | 104 | 3   | 1915           | 12.8 (11.4-14.4) | 1669 | 246         |
| exo1-K185E MIP   | 406            | 14.9+/−1.6 | 300 | 103 | 3   | 4036           | 13.7 (11.3-14.3) | 1934 | 246         |
| exo1-D79A D173A  | 297            | 18.9+/−2.1 | 200 | 94  | 3   | 1760           | 16.2 (14.4-17.9) | 1471 | 284         |
| exo1-D171A D173A | 395            | 18.4+/−1.2 | 250 | 145 | 0   | 2049           | 18.9 (17.2-20.6) | 1662 | 387         |
| exo1-F586        | 244            | 16.6+/−1.5 | 163 | 81  | 0   | 1112           | 19.4 (17.1-21.9) | 896  | 216         |
| exo1-S41E        | 262            | 10.3+/−1.2 | 208 | 54  | 0   | 1362           | 11.1 (9.5-12.9)  | 1211 | 151         |
| mhl3Δ           | 210            | 13.6+/−2.7 | 208 | 39  | 3   | 1191           | 10.5 (8.8-12.4)  | 1066 | 125         |
| msh5Δ           | 151            | 10.9+/−1.7 | 118 | 33  | 0   | 1111           | 8.5 (6.9-10.2)   | 1017 | 94          |
| exo1Δ mus81Δ    | 133            | 2.8+/−0.9  | 128 | 6   | 0   | 1767           | 1.2 (0.7-1.8)    | 1745 | 21          |
| exo1Δ msh3Δ     | 238            | 11.1+/−1.3 | 184 | 52  | 0   | 1221           | 10.4 (8.7-12.2)  | 1092 | 127         |
| exo1Δ pRAD27 (EXO1 Promoter) | 261 | 12.3+/−1.3 | 197 | 64  | 0   | 1433           | 12.6 (10.9-14.4) | 1253 | 180         |
| exo1Δ pEXO1      | 206            | 21.9+/−2.5 | 127 | 79  | 2   | 1072           | 19.4 (17.1-21.9) | 864  | 208         |
| exo1Δ pEmpty Vector | 220        | 12.5+/−2.3 | 175 | 43  | 2   | 1149           | 10.2 (8.5-12.1)  | 1032 | 117         |
| **LEU2-LYS2:**   |                |    |    |    |     |                |             |          |             |
| wild-type         | 501            | 28.7+/−1.5 | 233 | 264 | 4   | 2285           | 27.6 (25.7-29.4) | 1655 | 630         |
| exo1Δ            | 486            | 11.1+/−1.1 | 377 | 108 | 1   | 2510           | 11.9 (10.6-13.2) | 2210 | 298         |
| exo1-K185E       | 333            | 21.2+/−2.9 | 272 | 56  | 7   | 1549           | 9.8 (8.4-11.4)   | 1397 | 152         |
| exo1-G236D       | 541            | 17.3+/−1.6 | 372 | 145 | 6   | 2676           | 16.2 (14.8-17.7) | 2242 | 434         |
| exo1-K185E G236D | 410            | 15.1+/−1.5 | 296 | 112 | 2   | 2409           | 13.6 (12.2-15.0) | 2080 | 327         |
| exo1-MIP         | 411            | 13.9+/−1.6 | 312 | 96  | 3   | 1915           | 12.5 (11.0-14.0) | 1676 | 239         |
| exo1-K185E MIP   | 406            | 16.3+/−1.2 | 274 | 132 | 0   | 1934           | 16.3 (14.7-18.0) | 1619 | 315         |
| exo1-D79A D173A  | 297            | 27.6+/−2.3 | 153 | 140 | 4   | 1706           | 24.7 (22.6-26.7) | 1322 | 433         |
| exo1-D171A D173A | 395            | 22.7+/−1.2 | 216 | 179 | 0   | 2049           | 21.9 (20.1-23.7) | 1601 | 448         |
| exo1-F586        | 244            | 14.9+/−1.8 | 176 | 67  | 1   | 1112           | 15.5 (13.4-17.7) | 940  | 172         |
| exo1-S41E        | 262            | 13.4+/−2.0 | 202 | 58  | 2   | 1362           | 13.4 (11.7-15.4) | 1179 | 183         |
| mhl3Δ           | 210            | 14.3+/−2.0 | 155 | 54  | 1   | 1191           | 13.1 (11.2-15.1) | 1035 | 156         |
| genotype          | value 1  | value 2  | value 3  | value 4  | value 5  | value 6  | value 7  | value 8  | value 9  | value 10 | value 11 | value 12 | value 13 | value 14 | value 15 | value 16 |
|-------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| msh5::Δ          | 151      | 9.6+/−1.7| 122      | 29       | 0        | 1111     | 9.5 (7.9-11.4)| 1005     | 106      |
| exo1::Δ mus81::Δ | 133      | 3.4+/−1.1| 126      | 9        | 0        | 1767     | 2.6 (1.9-3.4) | 1722     | 45       |
| exo1::Δ mih3::Δ  | 238      | 10.4+/−1.3| 187      | 49       | 0        | 1221     | 11.5 (9.7-13.4)| 1079     | 140      |
| exo1::Δ + pRAD27 (EXO1 Promoter) | 261      | 17.8+/−2.1| 178      | 81       | 2        | 1433     | 15.2 (13.4-17.2)| 1215     | 218      |
| exo1::Δ + pEXO1  | 206      | 26.2+/−2.9| 114      | 91       | 3        | 1072     | 22.7 (20.2-25.3)| 829      | 243      |
| exo1::Δ + pEmpty Vector | 220      | 12.7+/−1.9| 169      | 50       | 1        | 1149     | 12.5 (10.7-14.6)| 1005     | 144      |

**LYS2-ADE2:**

wild-type          | 501      | 14.8+/−1.2| 358      | 142      | 1        | 2285     | 14.8 (13.4-16.3)| 1947     | 338      |
| exo1::Δ          | 486      | 7.3+/−1.1| 425      | 59       | 2        | 2510     | 6.6 (5.7-7.7) | 2342     | 166      |
| exo1-K185E       | 333      | 12.3+/−1.8| 267      | 67       | 1        | 1549     | 10.3 (8.8-11.9)| 1390     | 159      |
| exo1-G236D       | 541      | 11.8+/−1.4| 420      | 99       | 4        | 2676     | 10.0 (8.9-11.2)| 2409     | 267      |
| exo1-K185E G236D | 410      | 6.6+/−1.3| 366      | 42       | 2        | 2409     | 5.9 (4.9-6.9) | 2266     | 141      |
| MIP              | 411      | 7.8+/−0.9| 347      | 64       | 0        | 1915     | 7.4 (6.3-8.7) | 1773     | 142      |
| exo1-K185E, MIP  | 406      | 8.3+/−0.9| 339      | 67       | 0        | 1934     | 7.7 (6.5-9.0) | 1785     | 149      |
| exo1-D79A,D173A  | 297      | 12.1+/−1.5| 230      | 66       | 1        | 1760     | 11.3 (9.8-12.8)| 1557     | 198      |
| exo1-D171A,D173A | 395      | 8.9+/−1.0| 325      | 70       | 0        | 2049     | 10.2 (8.9-11.5)| 1641     | 208      |
| exo1-F58E        | 244      | 11.7+/−1.3| 148      | 64       | 0        | 1112     | 14.7 (12.7-17.0)| 948      | 164      |
| exo1-S41E        | 262      | 8.6+/−1.2| 217      | 45       | 0        | 1362     | 8.1 (6.7-9.7) | 1251     | 111      |
| mih3::Δ         | 210      | 5.5+/−1.1| 187      | 23       | 0        | 1191     | 5.1 (3.9-6.5) | 1130     | 61       |
| msh5::Δ         | 151      | 4.6+/−1.3| 137      | 14       | 0        | 1111     | 3.7 (2.7-5.0) | 1070     | 41       |
| exo1::Δ mus81::Δ | 133      | 0+/−0     | 133      | 0        | 0        | 1763     | 1.4 (0.9-2.0) | 1738     | 24       |
| exo1::Δ mih3::Δ  | 238      | 6.6+/−1.6| 210      | 25       | 1        | 1221     | 6.1 (4.8-7.5) | 1145     | 74       |
| exo1::Δ + pRAD27 (EXO1 Promoter) | 261      | 8.0+/−1.1| 219      | 42       | 0        | 1433     | 8.6 (7.2-10.2)| 1310     | 123      |
| exo1::Δ + pEXO1  | 208      | 15.8+/−2.5| 152      | 54       | 2        | 1072     | 14.2 (12.1-16.4)| 920      | 152      |
| exo1::Δ + pEmpty Vector | 220      | 5.4+/−1.0| 196      | 24       | 0        | 1149     | 6.5 (5.2-8.1) | 1074     | 75       |

**ADE2-HIS4:**

wild-type          | 501      | 39+/−2.1| 170      | 319      | 12       | 2285     | 35.1 (33.1-37.1)| 1483     | 802      |
| exo1::Δ          | 486      | 24.8+/−2.1| 295      | 181      | 10       | 2510     | 20.9 (19.3-22.5)| 1985     | 523      |
| exo1-K185E       | 333      | 22.5+/−2.3| 203      | 131      | 1        | 1549     | 20.7 (18.7-22.8)| 1229     | 320      |
| exo1-G236D       | 541      | 23.8+/−2.2| 339      | 171      | 13       | 2676     | 20.0 (18.6-21.6)| 2139     | 537      |
| exo1-K185E G236D | 410      | 23.3+/−2.1| 254      | 149      | 7        | 2409     | 20.1 (18.5-21.7)| 1923     | 484      |
| exo1-MIP         | 411      | 23.2+/−1.7| 235      | 173      | 3        | 1915     | 21.6 (19.8-23.5)| 1501     | 414      |
Mutants are isogenic derivatives of EAY1108/EAY1112. Genetic intervals correspond to the genetic distance calculated from tetrads +/- one standard error. Standard error was calculated using the Stahl Laboratory Online Tools website (https://elizabethhousworth.com/StahlLabOnlineTools/). For single spore analysis, data are shown as 95% confidence intervals around the recombination frequency. For tetrad analysis the centimorgan (cM) map distance was calculated using the formula of Perkins (1949): $\frac{50(\text{TT}+(6\text{NPD})/\text{(PD+TT+NPD}}$. To compare to the tetrad data, recombination frequencies obtained from single spores (Parental/(Parental+Recombinant)) were multiplied by 100 to yield genetic map distances (cM).

| Mutant                        | Chromosome Position | Recombination Frequency |
|-------------------------------|---------------------|-------------------------|
| exo1-K185E,MIP                | 406                 | 23.4 +/- 2.1            |
| exo1-D78A,D173A               | 297                 | 41.6 +/- 3.2            |
| exo1-D171A,D173A              | 395                 | 35.2 +/- 2.7            |
| exo1-F58E                     | 244                 | 26.6 +/- 2.5            |
| exo1-S41E                     | 262                 | 26.3 +/- 3.2            |
| msh5.1                       | 210                 | 22.9 +/- 3.1            |
| msh5.1                       | 151                 | 16.9 +/- 3.4            |
| exo1.1 mus81.1               | 133                 | 2.2 +/- 0.9             |
| exo1.1 msh3.1                | 238                 | 25.8 +/- 3.1            |
| exo1.1 + pRAD27 (EXO1 Promoter) | 261          | 33.7 +/- 4.0            |
| exo1.1 + pEXO1               | 208                 | 36.1 +/- 3.3            |
| exo1.1 + pEmpty Vector       | 220                 | 23.2 +/- 2.4            |

**Genetic Intervals**

- For single spore analysis, data are shown as 95% confidence intervals around the recombination frequency.
- For tetrad analysis, the centimorgan (cM) map distance was calculated using the formula of Perkins (1949): $\frac{50(\text{TT}+(6\text{NPD})/\text{(PD+TT+NPD}}$. To compare to the tetrad data, recombination frequencies obtained from single spores (Parental/(Parental+Recombinant)) were multiplied by 100 to yield genetic map distances (cM).
Table S3A. Interference measurements on Chromosome XV.

| Intervals | I   | II  | III | # tetrads | Interference |
|-----------|-----|-----|-----|-----------|--------------|
| **wild-type** |     |     |     |           |              |
| Malkova   | 0.48| 0.43| 0.90| 501       | Intervals I, II |
| C.O.C     | 0.66| 0.52| 0.92|           |              |
| **exo1Δ**  |     |     |     |           |              |
| Malkova   | 1.28| 0.84| 1.62| 486       | No intervals |
| C.O.C     | 1.10| 0.78| 1.10|           |              |
| **exo1-D171A,D173A** | |     |     |           |              |
| Malkova   | 0.37| 0.53| 0.40| 395       | All intervals |
| C.O.C     | 0.50| 0.63| 0.58|           |              |
| **exo1-D78A, D173A** | |     |     |           |              |
| Malkova   | 0.54| 0.17| 0.65| 297       | All intervals |
| C.O.C     | 0.59| 0.28| 0.9 |           |              |
| **exo1-S41E** |     |     |     |           |              |
| Malkova   | 0.46| 0.15| 0.56| 262       | Intervals I, II |
| C.O.C     | 0.56| 0.19| 0.73|           |              |
| **exo1-F58E** |     |     |     |           |              |
| Malkova   | 0.60| 0.30| 0.78| 244       | Interval II   |
| C.O.C     | 0.79| 0.21| 0.99|           |              |
| **exo1-G236D** | |     |     |           |              |
| Malkova   | 0.25| 0.44| 1.03| 541       | Intervals I, II |
| C.O.C     | 0.33| 0.53| 0.93|           |              |
| **exo1-K185E** | |     |     |           |              |
| Malkova   | 0.47| 0.40| 1.70*| 333       | Intervals I, II |
| C.O.C.    | 0.60| 0.49| 1.2 |           |              |
| **exo1-G236D,K185E** | |     |     |           |              |
| Malkova   | 0.50| 0.82| 1.19| 410       | Intervals I   |
| C.O.C     | 0.64| 0.98| 0.89|           |              |
| **exo1-MIP** |     |     |     |           |              |
| Malkova   | 0.92| 0.48| 0.59| 411       | Interval II   |
| C.O.C.    | 0.66| 0.58| 0.69|           |              |
| **exo1Δ + pEXO1-RAD27** | |     |     |           |              |
| Malkova   | 1.41*| 0.90| 0.81| 261       | No intervals |
| C.O.C     | 1.3 | 0.97| 0.84|           |              |
| **mlh3Δ**  |     |     |     |           |              |
| Malkova   | 0.46| 0.58| 1.08| 210       | Interval I    |
| C.O.C     | 0.63| 0.66| 0.96|           |              |
| **msh5Δ**  |     |     |     |           |              |
| Malkova   | 1.29| 0.97| 2.16*| 151       | No intervals |
| C.O.C     | 1.1 | 1.12| 1.84|           |              |
The Malkova ratio and coefficient of coincidence (COC, ratio of double crossovers observed/double crossovers expected) were performed for the indicated genotypes in the EAY1108/EAY1112 strain background (Materials and Methods, strains listed in Table S5). These methods were performed for intervals I (\textit{URA3-LEU2-LYS2}), II (\textit{LEU2-LYS2-ADE2}), and III (\textit{LYS2-ADE2-HIS3}). 0 = Absolute Interference; 1= No interference. Significance of differences in tetrad distribution was assessed using a G test. Differences in distribution with \( p<0.05 \) were considered to be significant evidence of interference. Intervals with ratios significantly above 1 were observed and denoted with * to indicate potential negative interference. Detailed analysis of the Malkova ratio calculation is presented in Table S3B.
Table S3B. Detailed calculations of Malkova ratios presented in Figure 5 and Table S3A.

| wild-type | URA3-LYS2 | LEU2-LYS2 | LYS2-ADE2 | ADE2-HIS3 | U-L-K | CDC | Significant Interference (G-test) Malkova |
|----------|-----------|-----------|-----------|-----------|-------|-----|-----------------------------------|
| Reference Interval | PD | 58:19:1 | PD | 98:13:2 | 130:102:1 | PD | 114:236:6 | PD | 114:55:1 | K-A-H | 0.899864366 | 0.921 | No |
| Measured interval | cM | 15.8 | cM | 32.2 | 23.2 | cM | 33.2 | 38.3 | cM | 17.9 | |
| TT-NPD | 155:73:1 | TT-NPD | 184:74:0 | 228:45:00 | TT-NPD | 103:36:62 | 56:61:5 | TT-NPD | 244:87:0 | |
| 18.9 | cM | 19.9 | 7.6 | cM | 17.5 | 40.9 | 10.2 | No |
| p <0.05 | p <0.05 | p <0.05 | p <0.05 | p <0.05 | 0.117 | |
| Ratio | 0.52792926 | 0.42807143 | 0.32327586 | 0.52792926 | 1.06789512 | 0.73184335 |

| exo1A | URA3-LYS2 | LEU2-LYS2 | LYS2-ADE2 | ADE2-HIS3 | U-L-K | CDC | Significant Interference (G-test) Malkova |
|-------|-----------|-----------|-----------|-----------|-------|-----|-----------------------------------|
| Reference Interval | PD | 306:96:0 | PD | 306:96:0 | 320:49:02 | PD | 320:97:1 | 263:144:8 | PD | 263:32:00 | K-A-H | 0.616713382 | 0.21 | No |
| Measured interval | cM | 11 | cM | 19.7 | 7.7 | cM | 11.9 | 23.8 | cM | 5.4 | |
| TT-NPD | 71:22:01 | TT-NPD | 96.22:01 | 96:13:00 | TT-NPD | 48.13:00 | 32.27:02 | TT-NPD | 162:27:02 | |
| 14.9 | cM | 12.8 | 6 | cM | 10.7 | 6 | cM | 10.2 | |
| p | 0.18 | p | 0.82 | 0.598 | p | 0.856 | 0.34 | p | 0.534 | |
| Ratio | 1.35454545 | 0.77922079 | 0.89959696 | 1.34453782 | Ratio | 1.88688888 |

| exo1-D17A.A173A | LEU2-LYS2 | LYS2-ADE2 | ADE2-HIS3 | U-L-K | CDC | Significant Interference (G-test) Malkova |
|----------------|-----------|-----------|-----------|-------|-----|-----------------------------------|
| Reference Interval | PD | 104:146:0 | PD | 104:112:0 | 160:50:0 | PD | 166:159:0 | 134:178:13 | PD | 134:48:00 | K-A-H | 0.39620876 | 0.58 | Yes |
| Measured interval | cM | 29.2 | cM | 25.9 | 11.6 | cM | 24.5 | 39.4 | cM | 13.2 | |
| TT-NPD | 112:33:00 | TT-NPD | 146:30:00 | 150:22:00 | TT-NPD | 56:20:00 | 462:20:00 | TT-NPD | 191:22:00 | |
| 11.4 | cM | 3.2 | 5.6 | cM | 14.3 | 15.7 | cM | 5.2 | |
| p <0.05 | p <0.05 | p <0.05 | p <0.05 | p <0.05 | 0.05 | |
| Ratio | 0.39041046 | 0.35521236 | 0.42756862 | 0.58367347 | 0.59847716 | Ratio | 0.595393935 |

| exo1-D7BA.A173A | URA3-LYS2 | LEU2-LYS2 | LYS2-ADE2 | ADE2-HIS3 | U-L-K | CDC | Significant Interference (G-test) Malkova |
|----------------|-----------|-----------|-----------|-----------|-------|-----|-----------------------------------|
| Reference Interval | PD | 84:112:4 | PD | 84:86:0 | 95:57:01 | PD | 95:131:4 | 77:142:11 | PD | 77:27:01 | K-A-H | 0.64673419 | 0.9 | Yes |
| Measured interval | cM | 54 | cM | 22.5 | 20.6 | cM | 33.7 | 45.2 | cM | 15.7 | |
| TT-NPD | 69:29:00 | TT-NPD | 116:25:00 | 135:69:00 | TT-NPD | 58:09:00 | 26:30:00 | TT-NPD | 153:90:00 | |
| 14.4 | cM | 14.9 | 3.1 | cM | 6.7 | 21.1 | cM | 10.2 | |
| p <0.05 | p <0.05 | p <0.05 | p <0.05 | p <0.05 | 0.191 | |
| Ratio | 0.42362841 | 0.66322222 | 0.15045454 | 0.10881506 | 0.64398531 | Ratio | 0.64956153 |
| exo1-541E | Reference Interval | URA3-LEU2 | LEU2-lys2 | lys2-ade2 | ade2-lys3 | U-L-K | 0.465201798 | 0.56 |
|-----------|----------------|------------|-----------|----------|----------|------|----------------|------|
| Measured Interval | LEU2-lys2 | URA3-LEU2 | LEU2-lys2 | lys2-ade2 | ade2-lys3 | ade2-lys3 | U-L-K | 0.150525374 | 0.2 |
| PD | 155:11:02 | 156:47:00 | 156:43:00 | 156:58:02 | 127:33:7 | 127:32:0 | K-A-H | 0.565 | 0.73 |
| cm | 15.1 | 11.6 | 10.6 | 15.7 | 28.8 | 10 |
| Tt+PD | 45:07:03 | 35:07:03 | 56:02:02 | 32:13:00 | 10:13:00 | 0.3 |
| cm | 6.6 | 5.8 | 5.8 | 6.3 | 5.6 |
| p | 0.059 | <0.05 | 0.059 | 0.059 | 0.059 |
| Ratio | 0.43053858 | 0.16037569 | 0.16037569 | 0.16037569 | 0.16037569 |

| exo1-585E | Reference Interval | URA3-LEU2 | LEU2-lys2 | lys2-ade2 | ade2-lys3 | U-L-K | 0.601776062 | 0.79 |
|-----------|----------------|------------|-----------|----------|----------|------|----------------|------|
| Measured Interval | LEU2-lys2 | URA3-LEU2 | LEU2-lys2 | lys2-ade2 | ade2-lys3 | ade2-lys3 | U-L-K | 0.238795831 | 0.21 |
| PD | 111:51:01 | 111:65:00 | 125:51:00 | 125:51:00 | 96:86:3 | 96:53:00 | K-A-H | 0.77904519 | 0.99 |
| cm | 17.5 | 16.6 | 14.5 | 17.9 | 28.3 | 12.6 |
| Tt+PD | 65:16:00 | 52:16:00 | 62:06:02 | 51:06:02 | 33:24:00 | 01:24:00 |
| cm | 9.9 | 11.8 | 4.4 | 5.3 | 21.1 | 10.4 |
| p | 0.095 | <0.05 | <0.05 | <0.05 | <0.05 | 0.336 |
| Ratio | 0.56571429 | 0.32334842 | 0.74353044 | 0.74353044 | 0.74353044 |

| exo1-526D | Reference Interval | URA3-LEU2 | LEU2-lys2 | lys2-ade2 | ade2-lys3 | U-L-K | 0.429567932 | 0.33 |
|-----------|----------------|------------|-----------|----------|----------|------|----------------|------|
| Measured Interval | LEU2-lys2 | URA3-LEU2 | LEU2-lys2 | lys2-ade2 | ade2-lys3 | ade2-lys3 | U-L-K | 0.42606719 | 0.53 |
| PD | 251:132:6 | 251:120:5 | 289:84:3 | 289:129:6 | 274:140:10 | 274:140:10 | K-A-H | 0.347903968 | 0.938 |
| cm | 21.8 | 21.8 | 19.9 | 19.5 | 23.6 | 23.6 |
| Tt+PD | 125:12:00 | 136:11:01 | 135:14:01 | 87:15:00 | 69:15:00 | 150:32:02 |
| cm | 4.4 | 6.7 | 6.7 | 7.4 | 24 | 12 |
| p | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.728 |
| Ratio | 0.2037037 | 0.22643216 | 0.49067005 | 0.37648718 | 0.10394915 | 0.105263158 |

| exo1-518E | Reference Interval | URA3-LEU2 | LEU2-lys2 | lys2-ade2 | ade2-lys3 | U-L-K | 0.472062763 | 0.6 |
|-----------|----------------|------------|-----------|----------|----------|------|----------------|------|
| Measured Interval | LEU2-lys2 | URA3-LEU2 | LEU2-lys2 | lys2-ade2 | ade2-lys3 | ade2-lys3 | U-L-K | 0.396495574 | 0.49 |
| PD | 173:71:9 | 173:82:6 | 181:36:2 | 181:74:19 | 189:95:1 | 189:35:21 | K-A-H | 1.702957359 | 1.2 |
| cm | 24.7 | 26.0 | 14.2 | 25.3 | 16.1 | 10 |
| Tt+PD | 66:12:01 | 80:12:01 | 85:08:01 | 83:09:01 | 36:28:03 | 98:29:02 |
| cm | 11.4 | 9.7 | 7.5 | 6.7 | 34.3 | 16.1 |
| p | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.24 |
| Ratio | 0.46153146 | 0.49258606 | 0.52698001 | 0.26462123 | 1.70881152 | 1.61 |

| exo1-518E 0236D | Reference Interval | URA3-LEU2 | LEU2-lys2 | lys2-ade2 | ade2-lys3 | U-L-K | 0.503982534 | 0.64 |
|----------------|----------------|------------|-----------|----------|----------|------|----------------|------|
| Measured Interval | LEU2-lys2 | URA3-LEU2 | LEU2-lys2 | lys2-ade2 | ade2-lys3 | ade2-lys3 | U-L-K | 0.817063861 | 0.98 |
| PD | 194:90:2 | 194:100:2 | 264:00:02 | 264:100:2 | 22:15:06 | 22:15:06 | K-A-H | 1.138678712 | 0.69 |
| cm | 8.9 | 9.6 | 5.3 | 13.6 | 22.7 | 8 |
| Tt+PD | 102:22:00 | 92:22:00 | 62:12:00 | 32:12:00 | 29:14:01 | 141:13:02 |
| cm | 0.05 | <0.05 | 0.518 | 0.796 | 0.78 | 0.09 |
| Ratio | 0.5 | 0.50793051 | 0.74042757 | 0.98668688 | 0.97004547 | 4.0365877 |
### exo1-MIP

| Reference Interval | URA3-LEU2 | LEU2-LYS2 | LYS2-ADE2 | ADE2-NRS3 | U-L-K | 0.917190776 | 0.66 | No |
|-------------------|-----------|-----------|-----------|-----------|-------|-------------|-----|-----|
| Measured Interval | LEU2-LYS2 | URA3-LEU2 | LYS2-ADE2 | LEU2-LYS2 | ADE2-NRS3 | LYS2-ADE2 | L-K-A | 0.482655502 | 0.58 | Yes |
| PD                | 222.82.0  | PD        | 222.87.2  | 256.55.00 | PD     | 256.67.3  | 190.153.3 | PD     | 190.45.00 | K-A-H | 0.592906961 | 0.69 | No |
| cM                | 13.5       | cM        | 15.9      | 8.8       | cM     | 15.2      | 24.7      | cM     | 8.6      |
| TT+NPD            | 90.14.03   | TT+NP0D  | 92.17.01  | 91.09.00  | TT+NP0D| 56.89.00  | 45.29.00  | TT+NP0D| 157.19.00 |
| cM                | 15         | cM        | 11.5      | 4.5       | cM     | 6.9       | 15.4      | cM     | 5.4      |
| p                 | 0.06       | p         | 0.075     | 0.00      | p      | <0.05     | 0.066     | p      | 0.063     |
| **Ratio**         | 1.11111111 | Ratio     | 0.72327044 | 0.51193634 | Ratio  | 0.4539737 | 0.6234178 | Ratio  | 0.5625    |

### exo1-A pEXO1-RAD27

| Reference Interval | URA3-LEU2 | LEU2-LYS2 | LYS2-ADE2 | ADE2-NRS3 | U-L-K | 1.411630037 | 1.3 | No |
|-------------------|-----------|-----------|-----------|-----------|-------|-------------|----|-----|
| Measured Interval | LEU2-LYS2 | URA3-LEU2 | LYS2-ADE2 | LEU2-LYS2 | ADE2-NRS3 | LYS2-ADE2 | L-K-A | 0.904978749 | 0.97 | No |
| PD                | 14139.02  | PD        | 14139.00  | 1429.00   | PD     | 14998.2   | 1238.11   | PD     | 12327.00  | K-A-H | 0.809081386 | 0.84 | No |
| cM                | 16.8       | cM        | 10.4      | 8.1       | cM     | 18.3      | 34.5      | cM     | 9        |
| TT+NP0D           | 37.27.00   | TT+NP0D  | 56.27.00  | 70.13.00  | TT+NP0D| 29.13.00  | 27.13.02  | TT+NP0D| 98.15.00  |
| cM                | 21.1       | cM        | 16.3      | 7.8       | cM     | 15.5      | 29.8      | cM     | 8.6      |
| p                 | 0.054      | p         | 0.129     | 0.982     | p      | 0.702     | 0.604     | p      | 0.617     |
| **Ratio**         | 1.283583738 | Ratio    | 1.56730785 | 0.96295295 | Ratio  | 0.5403454  | 0.86376812 | Ratio  | 0.7555504  |

### mls1A

| Reference Interval | URA3-LEU2 | LEU2-LYS2 | LYS2-ADE2 | ADE2-NRS3 | U-L-K | 0.481415992 | 0.63 | Yes |
|-------------------|-----------|-----------|-----------|-----------|-------|-------------|-----|-----|
| Measured Interval | LEU2-LYS2 | URA3-LEU2 | LYS2-ADE2 | LEU2-LYS2 | ADE2-NRS3 | LYS2-ADE2 | L-K-A | 0.535056197 | 0.66 | No |
| PD                | 12047.01  | PD        | 12032.03  | 1386.00   | PD     | 13650.01  | 1196.05   | PD     | 11916.00  | K-A-H | 1.091273485 | 0.96 | No |
| cM                | 18.9       | cM        | 16.1      | 6.3       | cM     | 15        | 22.2      | cM     | 5.97     |
| TT+NP0D           | 3507.00    | TT+NP0D  | 4807.00   | 5130.00   | TT+NP0D| 1904.00   | 1507.01   | TT+NP0D| 6703.00   |
| cM                | 8.3        | cM        | 6.4       | 3.6       | cM     | 8.7       | 28.3      | cM     | 3.3      |
| p                 | 0.23       | p         | <0.05     | 0.572     | p      | 0.53      | 0.699     | p      | 0.969     |
| **Ratio**         | 0.523516348 | Ratio    | 0.39751553 | 0.59016393 | Ratio  | 0.56      | 1.27477477 | Ratio  | 0.86777219 |

### mls5A

| Reference Interval | URA3-LEU2 | LEU2-LYS2 | LYS2-ADE2 | ADE2-NRS3 | U-L-K | 1.289669665 | 1.104 | No |
|-------------------|-----------|-----------|-----------|-----------|-------|-------------|-----|-----|
| Measured Interval | LEU2-LYS2 | URA3-LEU2 | LYS2-ADE2 | LEU2-LYS2 | ADE2-NRS3 | LYS2-ADE2 | L-K-A | 0.968335907 | 1.118 | No |
| PD                | 6025.00   | PD        | 6025.00   | 16813.00  | PD     | 11025.00  | 4633.00   | PD     | 976.00    | K-A-H | 2.157353014 | 1.841 | No* |
| cM                | 8.7        | cM        | 10.3      | 5.4       | cM     | 9.3       | 12.9      | cM     | 7.08     |
| TT+NP0D           | 2408.00    | TT+NP0D  | 2206.00   | 2703.00   | TT+NP0D| 1303.00   | 1505.02   | TT+NP0D| 3107.00   |
| cM                | 12.5       | cM        | 13.5      | 5         | cM     | 9.4       | 38.6      | cM     | 9.2      |
| p                 | 0.757      | p         | 0.783     | 0.903     | p      | 0.99      | <0.05     | p      | 0.824     |
| **Ratio**         | 1.28863979 | Ratio    | 1.22126214  | 0.92323593 | Ratio  | 1.51075269 | 3.0155625 | Ratio  | 1.26943503 |

*Potential negative interference*
Legend, Table S3B.
Crossover interference was analyzed using the Malkova method (Malkova et al., 2004; Martini et al., 2006) for chromosome XV. For each genetic interval, tetrads were divided based on the presence or absence of a recombination event in a reference interval. For each reference interval, the map distance was measured in the adjacent intervals, thus obtaining two map distances for each interval. The significance of differences in tetrad distribution was assessed using a G test. Differences in distribution, with p<0.05, were considered to be evidence of interference. The data are presented as the average ratio of the two map distances in each neighboring interval, with a smaller ratio indicating stronger interference. An interval was considered to have a “loss of positive interference” phenotype when both adjacent intervals displayed no detectable positive interference. Ratios significantly greater than 1 are indicated with * to denote potential negative interference. TT, tetratype; NPD, nonparental ditype; PD, parental ditype.
Table S4. Analysis in diploid strains containing haploinsufficiency of EXO1, MLH1, MSH4, MSH5, SGS1, RMI1, ZIP1, ZIP3, ZIP4, SPO16, MER3 genes in mlh3-42 and mlh3-54 strain backgrounds.

| Relevant genotype                      | % tetraploidy | tetrads counted | Phenotype |
|----------------------------------------|---------------|-----------------|-----------|
| Haploinsufficiency tests                |               |                 |           |
| EXO1/EXO1, MLH3/MLH3                   | 37.5          | 550             | +         |
| exo1Δexo1Δ, MLH3/MLH3                  | 17.8          | 549             | -         |
| EXO1/EXO1, mlh3Δ/mlh3Δ                 | 20.6          | 1037            | -         |
| EXO1/exo1Δ, MLH3/MLH3                  | 37.3          | 509             | +         |
| EXO1/EXO1, MLH3/mlh3Δ                  | 39.9          | 1042            | +         |
| EXO1/exo1Δ, MLH3/mlh3Δ                 | 36.3          | 1050            | +         |
| exo1-MIP/exo1-MIP, MLH3/MLH3           | 33.3          | 547             | +/-       |
| exo1-MIP/exo1Δ, MLH3/MLH3              | 26.2          | 519             | +/-/-     |
| exo1-MIP/exo1-MIP, MLH3/mlh3Δ          | 26.6          | 516             | +/-/-     |
| exo1-MIP/exo1Δ, MLH3/mlh3Δ             | 22.6          | 1006            | -         |
| exo1-MIP/EXO1, MLH3/mlh3Δ              | 35.3          | 1022            | +         |
| Haploinsufficiency tests with mlh3 alleles |           |                 |           |
| Controls                               |               |                 |           |
| MLH3/mlh3Δ                             | 39.9          | 1042            | +         |
| mlh3Δ/mlh3Δ                            | 20.6          | 1037            | -         |
| mlh3-42/mlh3Δ                          | 38.6          | 1058            | +         |
| mlh3-54/mlh3Δ                          | 37.4          | 1039            | +         |
| MLH3-interacting genes                 |               |                 |           |
| MLH3/mlh3Δ, EXO1/exo1Δ                 | 38.0          | 549             | +         |
| mlh3Δ/mlh3Δ, EXO1/exo1Δ                | 18.9          | 519             | -         |
| mlh3-42/mlh3Δ, EXO1/exo1Δ              | 29.1          | 518             | +/-/-     |
| mlh3-54/mlh3Δ, EXO1/exo1Δ              | 29.4          | 527             | +/-/-     |
| MLH3/mlh3Δ, MLH1/mlh1Δ                 | 39.5          | 591             | +         |
| mlh3Δ/mlh3Δ, MLH1/mlh1Δ                | 17.0          | 575             | -         |
| mlh3-42/mlh3Δ, MLH1/mlh1Δ              | 35.2          | 449             | +         |
| mlh3-54/mlh3Δ, MLH1/mlh1Δ              | 29.7          | 416             | +/-/-     |
| MLH3/mlh3Δ, MSH4/msh4Δ                 | 41.4          | 517             | +         |
| mlh3Δ/mlh3Δ, MSH4/msh4Δ                | 18.5          | 497             | -         |
| mlh3-42/mlh3Δ, MSH4/msh4Δ              | 33.7          | 517             | +/-/-     |
|                |        |     |     |     |
|----------------|--------|-----|-----|-----|
| *mlh3-54/mlh3Δ, MSH4/msh4Δ* | 30.4   | 529 | +/− |     |
| *MLH3/mlh3Δ, MSH5/msh5Δ*     | 35.7   | 533 | +   |     |
| *mlh3Δ/mlh3Δ, MSH5/msh5Δ*    | 23.4   | 519 | −   |     |
| *mlh3-42/mlh3Δ, MSH5/msh5Δ*  | 29.1   | 519 | +/− |     |
| *mlh3-54/mlh3Δ, MSH5/msh5Δ*  | 31.9   | 516 | +/− |     |
| **STR complex components**    |        |     |     |     |
| *MLH3/mlh3Δ, SGS1/sgs1Δ*     | 38.6   | 521 | +   |     |
| *mlh3Δ/mlh3Δ, SGS1/sgs1Δ*    | 23.7   | 515 | −   |     |
| *mlh3-42/mlh3Δ, SGS1/sgs1Δ*  | 31.6   | 537 | +/− |     |
| *mlh3-54/mlh3Δ, SGS1/sgs1Δ*  | 35.1   | 533 | +   |     |
| **ZMM factors**               |        |     |     |     |
| *MLH3/mlh3Δ, ZIP1/zip1Δ*     | 36.3   | 518 | +   |     |
| *mlh3Δ/mlh3Δ, ZIP1/zip1Δ*    | 17.9   | 518 | −   |     |
| *mlh3-42/mlh3Δ, ZIP1/zip1Δ*  | 37.3   | 251 | +   |     |
| *mlh3-54/mlh3Δ, ZIP1/zip1Δ*  | 36.6   | 520 | +   |     |
| *MLH3/mlh3Δ, ZIP3/zip3Δ*     | 31.7   | 489 | +/− |     |
| *mlh3Δ/mlh3Δ, ZIP3/zip3Δ*    | 18.9   | 534 | −   |     |
| *mlh3-42/mlh3Δ, ZIP3/zip3Δ*  | 30.0   | 523 | +/− |     |
| *mlh3-54/mlh3Δ, ZIP3/zip3Δ*  | 27.9   | 542 | +/− |     |
| *MLH3/mlh3Δ, ZIP4/zip4Δ*     | 35.1   | 510 | +   |     |
| *mlh3Δ/mlh3Δ, ZIP4/zip4Δ*    | 17.8   | 495 | −   |     |
| *mlh3-42/mlh3Δ, ZIP4/zip4Δ*  | 29.2   | 511 | +/− |     |
| *mlh3-54/mlh3Δ, ZIP4/zip4Δ*  | 31.3   | 514 | +/− |     |
| **MLH3/mlh3Δ, MER3/mer3Δ**   |        |     |     |     |
| *MLH3/mlh3Δ, MER3/mer3Δ*     | 30.3   | 538 | +/− |     |
| *mlh3Δ/mlh3Δ, MER3/mer3Δ*    | 21.0   | 520 | −   |     |
| *mlh3-42/mlh3Δ, MER3/mer3Δ*  | 28.5   | 549 | +/− |     |
| *mlh3-54/mlh3Δ, MER3/mer3Δ*  | 30.6   | 518 | +/− |     |
| *MLH3/mlh3Δ, SPO16/spo16Δ*  | 41.2   | 529 | +   |     |
Strains with the indicated relevant genotypes (Table S5) containing the \textit{THR1::m-Cerulean-TRP1} and \textit{CEN8::tdTomato-LEU2} markers on chromosome VIII were induced for meiosis and $\%$ tetratype in the \textit{CEN8-THR1} interval was measured by determining the total tetratypes/sum of tetratypes and parental ditypes. At least two transformants were analyzed for each background. Significance was assessed by $\chi^2$ test between mutant and wild-type \textit{EXO1} \textit{and exo1Δ} tetratype values. To minimize $\alpha$ inflation due to multiple comparisons, we applied a Benjamini-Hochberg correction at a 5% false discovery rate. +, indistinguishable from WT; -, indistinguishable from \textit{exo1Δ}; +/-, distinguishable from both wild-type and \textit{exo1Δ}.

| Genotype                        | Tetratypes | Ditypes | Significance |
|---------------------------------|------------|---------|--------------|
| \textit{mlh3Δ/mlh3Δ, SPO16/spo16Δ} | 21.1       | 515     | -            |
| \textit{mlh3-42/mlh3Δ, SPO16/spo16Δ} | 36.7       | 542     | +            |
| \textit{mlh3-54/mlh3Δ, SPO16/spo16Δ} | 37.6       | 515     | +            |
### Table S5. Strains used in this study.

#### A. exo1 mutant analysis

| Strain            | Genotype                                                                                   | Purpose                                      |
|-------------------|-------------------------------------------------------------------------------------------|----------------------------------------------|
| **A. exo1 mutant** | **analysis, spore autonomous, SK1 isogenic background**                                   |                                              |
| SKY3576           | MATα, ho::LYS2, lys2, ura2, leu2::hisG, trp1::hisG, THR1::m-Cerulean-TRP1                  | Integration of exo1 mutant alleles           |
| SKY3575           | MATα, ho::LYS2, lys2, ura3, leu2::hisG, trp1::hisG, CEN8::tdTomato-LEU2                    | Integration of exo1 mutant alleles           |
| EAY4149-EAY4150   | Same as SKY3576, but exo1Δ::KANMX                                                          | exo1Δ negative control                       |
| EAY4151-EAY4153   | Same as SKY3575, but exo1Δ::KANMX                                                          | exo1Δ negative control                       |
| EAY4154-EAY4156   | Same as SKY3576, but EXO1::KANMX                                                          | EXO1::KANMX control                          |
| EAY4157-EAY4159   | Same as SKY3575, but EXO1::KANMX                                                          | EXO1::KANMX control                          |
| EAY4160-EAY4162   | Same as SKY3576, but exo1-D78A::KANMX                                                      | D78A Mutant                                  |
| EAY4163-EAY4164   | Same as SKY3575, but exo1-D78A::KANMX                                                      | D78A Mutant                                  |
| EAY4165-EAY4167   | Same as SKY3576, but exo1-D173A::KANMX                                                     | D173A Mutant                                  |
| EAY4168-EAY4170   | Same as SKY3575, but exo1-D173A::KANMX                                                     | D173A Mutant                                  |
| EAY4171-EAY4172   | Same as SKY3576, but exo1-G236D::KANMX                                                     | G236D Mutant                                  |
| EAY4173-EAY4174   | Same as SKY3576, but exo1-G236D::KANMX                                                     | G236D Mutant                                  |
| EAY4175-EAY4177   | Same as SKY3576, but exo1-D78A,D173A::KANMX                                                | D78A,D173A mutant                             |
| EAY4178           | Same as SKY3576, but exo1-D78A,D173A::KANMX                                                | D78A,D173A mutant                             |
| EAY4179           | Same as SKY3576, but exo1-D173A,G236D::KANMX                                               | D173A,G236D mutant                            |
| EAY4180-EAY4181   | Same as SKY3576, but exo1-D173A,G236D::KANMX                                               | D173A,G236D mutant                            |
| EAY4182-EAY4184   | Same as SKY3576, but exo1-F447A,F448A::KANMX                                               | F447A,F448A mutant (MIP)                      |
| EAY4185-EAY4187   | Same as SKY3576, but exo1-F447A,F448A::KANMX                                               | F447A,F448A mutant (MIP)                      |
| EAY4510-EAY4511   | Same as SKY3576, but exo1-D171A::KANMX                                                     | D171A mutant                                  |
| EAY4512-EAY4513   | Same as SKY3576, but exo1-D171A,D173A::KANMX                                               | D171A,D173A mutant                            |
| EAY4514-EAY4515   | Same as SKY3576, but exo1-R92A::KANMX                                                      | R92A mutant                                   |
EAY4516-EAY4517  Same as SKY3576, but exo1-K121A::KANMX  K121A mutant
EAY4518-EAY4519  Same as SKY3576, but exo1-K121E::KANMX  K121E mutant
EAY4520-EAY4521  Same as SKY3576, but exo1-K185A::KANMX  K185A mutant
EAY4522-EAY4523  Same as SKY3576, but exo1-K185E::KANMX  K185E mutant
EAY4524-EAY4525  Same as SKY3576, but exo1-D173A,G236D::KANMX  D173A,G236D mutant
EAY4526-EAY4527  Same as SKY3576, but exo1-G236D,F447A,F448A::KANMX  G236D,F447A,F448A::KANMX
EAY4528-EAY4529  Same as SKY3576, but exo1-D173A,G236D,F447A,F448A::KANMX  D173A,G236D,F447A,F448A::KANMX
EAY4530-EAY4531  Same as SKY3576, but exo1-K185E,F447A,F448A::KANMX  K185E,F447A,F448A::KANMX
EAY4532-EAY4533  Same as SKY3576, but exo1-D173A,K185E,G236D::KANMX  D173A,K185E,G236D::KANMX
EAY4534-EAY4535  Same as SKY3576, but exo1-D171A,G236D::KANMX  D171A,G236D::KANMX
EAY4536-EAY4537  Same as SKY3576, but exo1-K185E,G236D::KANMX  K185E,G236D::KANMX
EAY4538-EAY4539  Same as SKY3576, but exo1-R92A,K121A,K185A::KANMX  R92A,K121A,K185A::KANMX
EAY4805-EAY4806  Same as SKY3576, but exo1-H36E::KANMX  H36E mutant
EAY4807-EAY4808  Same as SKY3576, but exo1-S41E::KANMX  S41E mutant
EAY4809-EAY4812  Same as SKY3576, but exo1-F58E::KANMX  F58E mutant
EAY4813-EAY4814  Same as SKY3576, but exo1-K61A::KANMX  K61A mutant
EAY4815-EAY4817  Same as SKY3576, but exo1-K61E::KANMX  K61E mutant
EAY4818-EAY4820  Same as SKY3576, but exo1-K85A::KANMX  K85A mutant
EAY4821-EAY4822  Same as SKY3576, but exo1-K85E::KANMX  K85E mutant
EAY4881-EAY4882  Same as SKY3576, but exo1-S41E,F58E::KANMX  S41E,F58E::KANMX
EAY4883-EAY4884  Same as SKY3576, but exo1-S41E,K61E::KANMX  S41E,K61E::KANMX

B. exo1 mutant analysis, tetrad analysis, SK1 congenic background

EAY1108  MATa, trp1:hisG leu2:hisG ho::hisG ura3 lys2 URA3::CENXV LEU2::CENXV, LYS2 insertion at position 505193 on chromosome XV
EAY1112  MATalpha, ura3, trp1::hisG, leu2::hisG, lys2, ho::hisG, ade2::hisG, his3A::hisG, TRP1::CENXV
EAY1281  Same as EAY1108 but msh5::NATMX
EAY1282  Same as EAY1112 but msh5::NATMX
EAY1847  Same as EAY1108 but mlh3::KANMX
EAY1848  Same as EAY1112 but mlh3::KANMX
| EAY4778 | Same as EAY1108 but exo1Δ::KANMX |
| EAY4779 | Same as EAY1112 but exo1Δ::KANMX |
| EAY4780 | Same as EAY1112 but D171A,D173A::KANMX |
| EAY4781 | Same as EAY1112 but D171A,D173::KANMX |
| EAY4782 | Same as EAY1112 but exo1-185E::KANMX |
| EAY4783 | Same as EAY1112 but exo1-G236D::KANMX |
| EAY4784 | Same as EAY1112 but exo1-S41E::KANMX |
| EAY4785 | Same as EAY1112 but exo1-F58E::KANMX |
| EAY4786 | Same as EAY1112 but exo1Δ::KANMX |
| EAY4787 | Same as EAY1108 but exo1Δ::KANMX |
| EAY4788 | Same as EAY1112 but exo1Δ::KANMX |
| EAY4789 | Same as EAY1108 but exo1Δ::KANMX |
| EAY4790 | Same as EAY1112 but exo1-MIP::KANMX |
| EAY4791 | Same as EAY1112 but exo1-MIP, K185E::KANMX |
| EAY4792 | Same as EAY1112 but exo1-G236D,D173A::KANMX |
| EAY4793 | Same as EAY1112 but exo1-G236D::KANMX |

C. Msh5 ChIP-qPCR and localization studies, SK1 isogenic background

| NHY1162 | MATα, ho::hisG, leu::hisG, ura3(ΔSma-Pst), his4X::LEU2-(NgoMIV)::URA3 | ChIP-qPCR, Msh5 localization |
| NHY1168 | MATα, ho::hisG, leu2::hisG, ura3(ΔSma-Pst), HIS4::LEU2-(BamH1) | ChIP-qPCR, Msh5 localization |
| KTY753 | MATα/MATα, ho::hisG/ho::hisG, leu2::hisG/leu2::hisG, ura3(ΔSma-Pst)/ura3(ΔSma-Pst), his4-X::LEU2-(NgoM IV)::URA3/HIS4::LEU2-(BamH1), exo1Δ::KanMX4/exo1Δ::KanMX4 | ChIP-qPCR, Msh5 localization |
| KTY756 | MATα, ho::hisG, leu2::hisG, ura3(ΔSma-Pst), his4-X::LEU2-(NgoM IV)::URA3, exo1Δ::KanMX4 | ChIP-qPCR, Msh5 localization |
| KTY757 | MATα, ho::hisG, leu2::hisG, ura3(ΔSma-Pst), HIS4::LEU2-(BamH1), exo1Δ::KanMX4 | ChIP-qPCR, Msh5 localization |

D. Haploinsufficiency studies, SK1 isogenic background

| EAY3252 | MATalpha, ho::hisG, ura3, leu2::hisG, trp1::hisG, ADE2, HIS4, | MLH3 control for haploinsufficiency screen |
| Strain | Description |
|--------|-------------|
| CEN8Tomato::LEU2, MLH3, lys214::insE-A14 |  |
| EAY3255 | MATalpha, ho::hisG, urea3, leu2::hisG, trp1::hisG, ade2, his4xB, CEN8Tomato::LEU2, mlh3Δ::NATMX, lys214::insE-A14 | mlh3Δ::NATMX control for haploinsufficiency screen |
| EAY3572 | Same as EAY3255, but mlh3-R552A,D553A,K555A,D556A::KANMX | mlh3-42 mutant |
| EAY3596 | Same as EAY3255, but mlh3-R552A,D553A,K555A,D556A::KANMX | mlh3-54 mutant |
| EAY3486 | MATa, ho::LYS2; lys2; urea3; leu2::hisG; trp1::hisG; THR1::m-Cerulean-TRP1; mlh3Δ::NATMX | Integration of mutant alleles |
| EAY4645-EAY4647 | Same as EAY3486 but exo1Δ::KANMX | mlh3Δ, exo1Δ double mutant |
| EAY4556-EAY4557 | Same as EAY3486 but exo1-MIP::KANMX | mlh3Δ, exo1-MIP double mutant |
| EAY4648-EAY4650 | Same as EAY3486 but mlh1Δ::KANMX | mlh3Δ, mlh1Δ double mutant |
| EAY4622-EAY4624 | Same as EAY3486 but msh4Δ::KANMX | mlh3Δ, msh4Δ double mutant |
| EAY4625-EAY4627 | Same as EAY3486 but msh5Δ::KANMX | mlh3Δ, msh5Δ double mutant |
| EAY4654-EAY4656 | Same as EAY3486 but sgs1Δ::KANMX | mlh3Δ, sgs1Δ double mutant |
| EAY4657-EAY4659 | Same as EAY3486 but rmi1Δ::KANMX | mlh3Δ, rmi1Δ double mutant |
| EAY4631-EAY4633 | Same as EAY3486 but zip1Δ::KANMX | mlh3Δ, zip1Δ double mutant |
| EAY4637-EAY4639 | Same as EAY3486 but zip3Δ::KANMX | mlh3Δ, zip3Δ double mutant |
| EAY4640-EAY4642 | Same as EAY3486 but zip4Δ::KANMX | mlh3Δ, zip4Δ double mutant |
| EAY4643-EAY4644 | Same as EAY3486 but mer3Δ::KANMX | mlh3Δ, mer3Δ double mutant |
| EAY4628-EAY4630 | Same as EAY3486 but spo16Δ::KANMX | mlh3Δ, spo16Δ double mutant |
| Plasmid     | Markers                        | Purpose                                |
|-------------|--------------------------------|----------------------------------------|
| pUC18       | amp<sup>R</sup>                | Exo1 endonuclease assay substrate      |
| pBR322      | amp<sup>R</sup>                | Exo1 endonuclease assay substrate      |
| pRS416      | amp<sup>R</sup>, URA3, CEN6-ARSH4 | Empty vector control                   |
| pLZ259      | amp<sup>R</sup>, NATMX, CEN6-ARSH4 | Empty vector control                   |
| pRS426      | amp<sup>R</sup>, URA3, 2µ       | Empty vector control                   |
| pEAI422     | amp<sup>R</sup>, KANMX         | Integration of exo1Δ-KANMX             |
| pEAI423     | amp<sup>R</sup>, KANMX         | Integration of EXO1-KANMX              |
| pEAI442     | amp<sup>R</sup>, KANMX         | Integration of exo1-H36E               |
| pEAI471     | amp<sup>R</sup>, KANMX         | Integration of exo1-S41E               |
| pEAI472     | amp<sup>R</sup>, KANMX         | Integration of exo1-F58E               |
| pEAI473     | amp<sup>R</sup>, KANMX         | Integration of exo1-K61A               |
| pEAI474     | amp<sup>R</sup>, KANMX         | Integration of exo1-K61E               |
| pEAI444     | amp<sup>R</sup>, KANMX         | Integration of exo1-K85A               |
| pEAI475     | amp<sup>R</sup>, KANMX         | Integration of exo1-K85E               |
| pEAI476     | amp<sup>R</sup>, KANMX         | Integration of exo1-S41E,F58E          |
| pEAI478     | amp<sup>R</sup>, KANMX         | Integration of exo1-S41E,K61E          |
| pEAI424     | amp<sup>R</sup>, KANMX         | Integration of exo1-D78A               |
| pEAI445     | amp<sup>R</sup>, KANMX         | Integration of exo1-R92A               |
| pEAI446     | amp<sup>R</sup>, KANMX         | Integration of exo1-K121A              |
| pEAI448     | amp<sup>R</sup>, KANMX         | Integration of exo1-K121E              |
| pEAI447     | amp<sup>R</sup>, KANMX         | Integration of exo1-D171A              |
| pEAI425     | amp<sup>R</sup>, KANMX         | Integration of exo1-D173A              |
| pEAI450     | amp<sup>R</sup>, KANMX         | Integration of exo1-K185A              |
| pEAI451     | amp<sup>R</sup>, KANMX         | Integration of exo1-K185E              |
| pEAI426     | amp<sup>R</sup>, KANMX         | Integration of exo1-G236D              |
| pEAI437     | amp<sup>R</sup>, KANMX         | Integration of exo1-F447A,F448A (MIP)  |
| pEAI427     | amp<sup>R</sup>, KANMX         | Integration of exo1-D78A,D173A         |
| pEAI449     | amp<sup>R</sup>, KANMX         | Integration of exo1-D171A,D173A        |
| pEAI456     | amp<sup>R</sup>, KANMX         | Integration of exo1-D171A,G236D        |
| pEAI436     | amp<sup>R</sup>, KANMX         | Integration of exo1-D173A,G236D        |
| pEAI458     | amp<sup>R</sup>, KANMX         | Integration of exo1-D173A,G236D,F447A,F448A (MIP) |
| pEAI452     | amp<sup>R</sup>, KANMX         | Integration of exo1-G236D,F447A,F448A (MIP) |
| pEAI467     | amp<sup>R</sup>, KANMX         | Integration of exo1-K185E,F447A,F448A (MIP) |
| pEAI460     | amp<sup>R</sup>, KANMX         | Integration of exo1-D173A,K185E,G236D  |
| pEAI461     | amp<sup>R</sup>, KANMX         | Integration of exo1-K185E,G236D        |
| pEAI466     | amp<sup>R</sup>, KANMX         | Integration of exo1-R92A,K121A,K185A   |
| pEAA715     | amp<sup>R</sup>, URA3, CEN6-ARSH4, EX01 | EXO1 complementation                 |
| pEAA483     | amp<sup>R</sup>, NATMX, CEN6-ARSH4, EX01 | EXO1 complementation                 |
| pEAA726     | amp<sup>R</sup>, URA3, CEN6-ARSH4, MLH3 | MLH3 complementation                 |
| Vector | Description | Notes |
|--------|-------------|-------|
| pEAA636 | amp<sup>R</sup>, HIS3, CEN6-ARSH4, MLH3, KANMX | MLH3 complementation |
| pEAA722 | amp<sup>R</sup>, URA3, CEN6-ARSH4, RAD27 | RAD27 expression, native promoter |
| pEAA720 | amp<sup>R</sup>, URA3, CEN6-ARSH4, pEXO1-RAD27 | RAD27 expression under EXO1 promoter |
| pEAI482 | amp<sup>R</sup>, NATMX, CEN6-ARSH4, pEXO1-RAD27 | Expression of RAD27 under EXO1 promoter |
| pEAA727 | amp<sup>R</sup>, URA3, CEN6-ARSH4, pEXO1-rad27-A45E | rad27-A45E expression under EXO1 promoter |
| pEAA728 | amp<sup>R</sup>, URA3, CEN6-ARSH4, pEXO1-rad27-R101A | rad27-R101A expression under EXO1 promoter |
| pEAA729 | amp<sup>R</sup>, URA3, CEN6-ARSH4, pEXO1-rad27-R105A | rad27-R105A expression under EXO1 promoter |
| pEAA730 | amp<sup>R</sup>, URA3, CEN6-ARSH4, pEXO1-rad27-K130A | rad27-K130A expression under EXO1 promoter |
| pEAA724 | amp<sup>R</sup>, URA3, CEN6-ARSH4, pEXO1-rad27-D179A | rad27-D179A expression under EXO1 promoter |
| pEAA731 | amp<sup>R</sup>, URA3, CEN6-ARSH4, pEXO1-rad27-H191E | rad27-H191E expression under EXO1 promoter |
| pEAM327 | amp<sup>R</sup>, URA3, 2µ, CDC9 | CDC9 expression, native promoter |
| pEAM329 | amp<sup>R</sup>, URA3, 2µ, pHOP1-CDC9 | Overexpression of CDC9 under the HOP1 promoter |
| pFB-EXO1-FLAG | amp<sup>R</sup>, Gm<sup>R</sup>, EXO1-FLAG | EXO1 expression from pFastBac (From Michael Liskay) |
| pFB-exo1-D173A-FLAG | amp<sup>R</sup>, Gm<sup>R</sup>, exo1-D173A-FLAG | exo1-D173A expression from pFastBac (From Michael Liskay) |
| pEAE422 | amp<sup>R</sup>, Gm<sup>R</sup>, exo1-G236D-FLAG | exo1-G236D expression from pFastBac |
| pEAE423 | amp<sup>R</sup>, Gm<sup>R</sup>, exo1-D173A-G236D-FLAG | exo1-D173A,G236D expression from pFastBac |
| Primer  | Sequence (lowercase indicates bases being mutated) | Purpose                  |
|---------|---------------------------------------------------|--------------------------|
| AO257  | GGAGCTCGAAAAAACTGAAG                                 | EXO1 Sequencing          |
| AO643  | CGGATGTGATGTGAGAACTG                             | EXO1 Sequencing          |
| AO694  | CCTGCGCGGGTTGCAATGAT                              | EXO1 Sequencing          |
| AO804  | AGAAAGGCTTCTTACTTCAACC                            | EXO1 Sequencing          |
| AO2383 | GAGACGGTCACAGCTTGTCT                             | EXO1 Sequencing          |
| AO3397 | ATATACCTGAAAGACAGAGACTG                          | EXO1 Sequencing          |
| AO3398 | TAGTGACAAATCAGTGGAGACAGAA                         | EXO1 Sequencing          |
| AO3399 | GGAATAATCAACTGATAATGACCT                         | EXO1 Sequencing          |
| AO3400 | ACCAGACACATCATATTGATAAT                          | EXO1 Sequencing          |
| AO3401 | CCCAGTCTCAACTACTACACAAAT                        | EXO1 Sequencing          |
| AO3402 | CAAATCACGGAAAGCCATCACTGC                        | EXO1 Sequencing          |
| AO3666 | ATGGGATTTTAAATTTTTCTTTCTTTGTTTTGTACT            | PCR Amplification of EXO1 |
| AO3838 | TGTTGCGGAGAGCAGACAAATTC                         | PCR Amplification of EXO1 |
| AO4061 | TTTAAATTTTTTTTCTTTTTATAGGGCCATTAGTTTTGTACT      | PCR Amplification of EXO1 |
| AO4583 | TGCAATGCGTAgaAGAGCAGCCT                          | exo1 mutagenesis, H36E   |
| AO4584 | TAGGCATCAATGCTTACAC                             | exo1 mutagenesis, H36E   |
| AO4585 | AGCAGCCTGagaATGCGATTTAG                         | exo1 mutagenesis, S41E   |
| AO4586 | CTATGTCAGCATGCTAC                              | exo1 mutagenesis, S41E   |
| AO4587 | GTACCCTCGGagaTTCTAAAGAGATTTAG                   | exo1 mutagenesis, F58E   |
| AO4588 | TTATCAGTTTTTTCTCCTTTG                          | exo1 mutagenesis, F58E   |
| AO4589 | GTTTTTTCTAGcaAGATTAGTTTTAGTTGAAAACC             | exo1 mutagenesis, K61A   |
| AO4590 | GTTTTTTCTAGGacaAGATTTAGTTTATTTGAAAAC             | exo1 mutagenesis, K61E   |
| AO4591 | TGGAGGTACTTATCATGTG                            | exo1 mutagenesis, K61A/E |
| AO4592 | ATGGTGTCTTGGGCTGTAATAGG                        | exo1 mutagenesis, D78A   |
| AO4593 | TACGGGTCGACTTTATTTATGAGGTGGTTTCC               | exo1 mutagenesis, D78A   |
| AO4594 | TGCCATTCGAGTTCAATGCTAG                          | exo1 mutagenesis, K85A   |
| AO4595 | TCACCATCGAAGCACAATCAGG                         | exo1 mutagenesis, K85A   |
| AO4596 | ATCCGAGTTGGAAATGCTACTG                         | exo1 mutagenesis, K85E   |
| AO4597 | GGACTATTTTCTGCAAGAGCAGCA                       | exo1 mutagenesis, K85E   |
| AO4598 | TGCCATTGACTTTTTCTCTTTG                        | exo1 mutagenesis, K85A   |
| AO4599 | GGACTATTTTCTGGCAAGAGACTG                      | exo1 mutagenesis, K85A/E |
| AO4600 | GACTTTTTTTAAATGCTACTG                         | exo1 mutagenesis, K121A  |
| AO4601 | GACTTTTTAAATGCTACTG                            | exo1 mutagenesis, K121E  |
| AO4602 | ATAGCATTTTTCTTTTTTTTGTGCCACAGG                 | exo1 mutagenesis, K121A/E|
| AO4603 | AATATCGAGCAGCTTTCTGCACAC                      | exo1 mutagenesis, D171A  |
| AO4604 | ATTTCCTGCACATTGTTTCACCA                       | exo1 mutagenesis, D171A  |
| AO4605 | GGAAGATTTCTGcaTCCGGTCTGCTC                   | exo1 mutagenesis, D173A  |
| AO4606 | GATATTATCCTTGCACATG                         | exo1 mutagenesis, D173A  |
| AO4607 | TGCTCCTCGTCTCGGAGATGT                       | exo1 mutagenesis, D171A,D173A|
| AO4608 | gaagcTTCGGATATTATCCTTTGAC                        | exo1 mutagenesis, D171A,D173A|
| AO4609 | ACGTCTCATTAGCgcTTAGGAAACGCTGTATTACT          | exo1 mutagenesis, K185A  |
| AO4610 | ACGTCTCATTAGCgaATCTAGGAGTAATGTGTTTTGTACT      | exo1 mutagenesis, K185E  |
| AO4611 | CCAGATCTGGAAGAGGCAAG                          | exo1 mutagenesis, G236D  |
| AO4612 | ATCCATTGTTAGTACAGAATCCG                        | exo1 mutagenesis, G236D  |
AO3885  AAGAAGCAAGctgcAATAAAACCCTCCATGACTG  exo1 mutagenesis, F447A,F448A (MIP)
AO3886  GTATCCTCAACGTTTCTTG  exo1 mutagenesis, F447A,F448A (MIP)
AO4908  GTTTTTATTgaaGTAAAGCAAGAAC  rad27 mutagenesis, A45E
AO4909  TGATAGAGACATAGAAGGC  rad27 mutagenesis, A45E
AO4910  GTTGACAAAGgtcTCTTCAAGAGGGTGG  rad27 mutagenesis, R101A
AO4911  TCATAGATTGCCATGCTTGG  rad27 mutagenesis, R101A
AO4912  GCTTTGTGCAACTCATGAG  rad27 mutagenesis, R105A
AO4913  AAGATTGGTGgctGTCTCCAAAGAGC  rad27 mutagenesis, R105A
AO4914  CTTTCTCTCTCTATCTCTTTTC  rad27 mutagenesis, K130A
AO4915  AAGACTGACTGTTATAGAACACCCTTC  mlh3∆::NATMX disruption primer set
AO4916  GCTAAGCTCATTCGATTGTAAC  mlh3∆::NATMX disruption primer set
AO4917  CTAGAATCTCTATTTTTTTTGACATTATTTGTACT  mlh3∆::NATMX disruption primer set
AO4918  AAAAAAATGTAATTGCACTAGC  mlh3∆::NATMX disruption primer set
AO4919  CGTTTGTTTTCGGCTTGC  mlh1∆::KANMX disruption primer set
AO4920  TCAAAATACTACAATATGATATTAAGATAATTGAGTTAAAA  mlh1∆::KANMX disruption primer set
AO4921  GAAATGCGAAATGTGAAGGAAG  sgs1∆::KANMX disruption primer set
AO4922  AGCTGATGCAGCGTT  zip3∆::KANMX disruption primer set
AO4923  AAAAGTCAGGTGCTTTTTAAAACAC  zip3∆::KANMX disruption primer set
AO4924  TCAATCTTGTAGAAAACGCTGTG  zip3∆::KANMX disruption primer set
AO4925  GTCATCCTCAGAGCTTCTCTATTTTTTTTGACATTATTTGTACT  mlh3∆::NATMX disruption primer set
AO4926  AAAAAAATGTAATTGCACTAGC  mlh3∆::NATMX disruption primer set
AO4927  CGTTTGTTTTCGGCTTGC  mlh1∆::KANMX disruption primer set
AO4928  TCAAAATACTACAATATGATATTAAGATAATTGAGTTAAAA  mlh1∆::KANMX disruption primer set
AO4929  GAAATGCGAAATGTGAAGGAAG  sgs1∆::KANMX disruption primer set
AO4930  AGCTGATGCAGCGTT  zip3∆::KANMX disruption primer set
AO4931  AAAAGTCAGGTGCTTTTTAAAACAC  zip3∆::KANMX disruption primer set
AO4932  TCAATCTTGTAGAAAACGCTGTG  zip3∆::KANMX disruption primer set
AO4066  GTGTACATAGCGTGCTTGG  zip3Δ::KANMX disruption primer set
AO4197  ATGAGTGAAATCCATTTTCTTTTG  zip4Δ::KANMX disruption primer set
AO4198  GGTGACTGGTTCAGG  zip4Δ::KANMX disruption primer set
AO4199  TTTGGTTCAAGAAGAAATGGAAGG  zip4Δ::KANMX disruption primer set
AO4200  CGTAACCTTTATGTATTTAAACC  zip4Δ::KANMX disruption primer set
AO4071  TCTTCTTCATGCGCCCTCAT  mer3Δ::KANMX disruption primer set
AO4072  GAATGAATTACTAATCTCATTCGATTC  mer3Δ::KANMX disruption primer set
AO4073  TGGTTTTATGCGCTTCTTTCAC  mer3Δ::KANMX disruption primer set
AO4074  GCCGGCAAGTTATCCTAT  spo16Δ::KANMX disruption primer set
AO4096  CAGAAGTGATGTGCTCATGG  spo16Δ::KANMX disruption primer set
AO4097  CACCGACTGACAGGC  spo16Δ::KANMX disruption primer set
AO4098  GAAGCTCAGGCCTCTGC  spo16Δ::KANMX disruption primer set
AO4099  CTTTAAAAACAGGATCCGAAGAG  spo16Δ::KANMX disruption primer set