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Supplemental Data

RecQ Helicase, Sgs1, and XPF Family Endonuclease, Mus81-Mms4, Resolve Aberrant Joint Molecules during Meiotic Recombination

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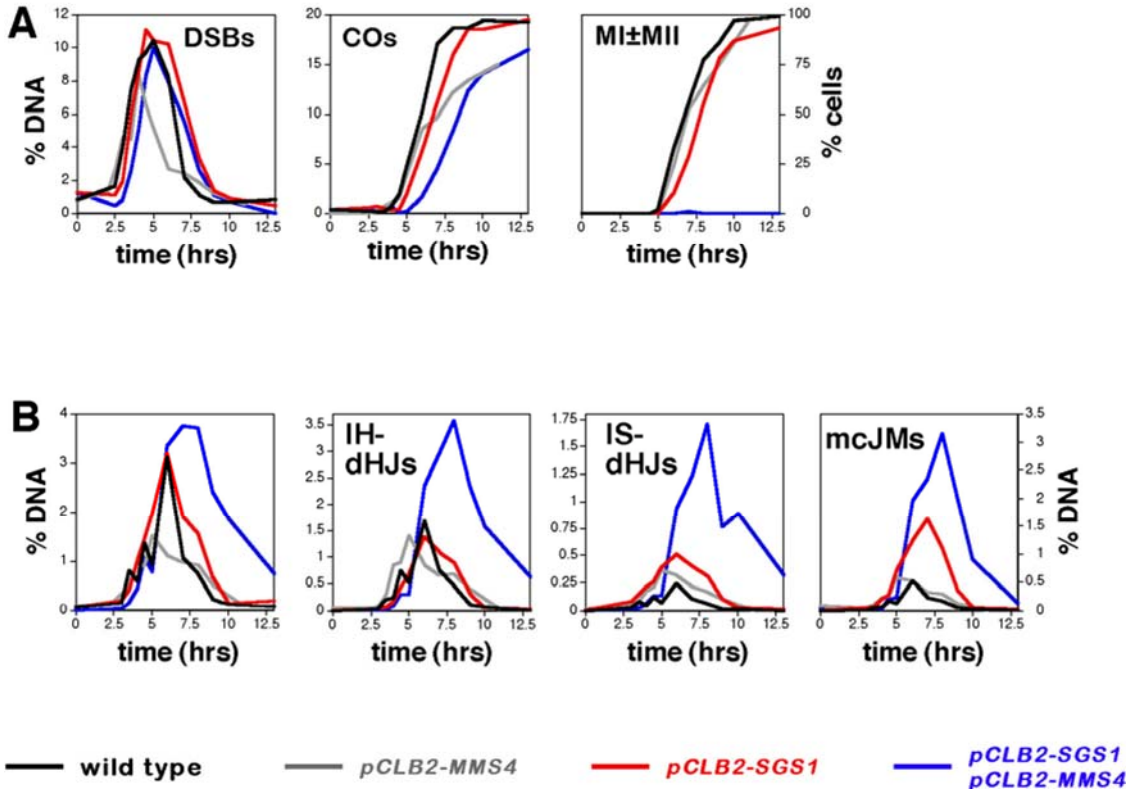

Figure S1. Independent Physical Analysis of of Recombination in Wild-Type, *pCLB2-SGS1*, *pCLB2-MMS4* and *pCLB2-SGS1 pCLB2-MMS4* Cells.

A and B. Independent cultures of wild-type, *pCLB2-SGS1*, *pCLB2-MMS4* and *pCLB2-SGS1 pCLB2-MMS4* cells were sporulated in parallel and analyzed as described in the text and Figure 3.

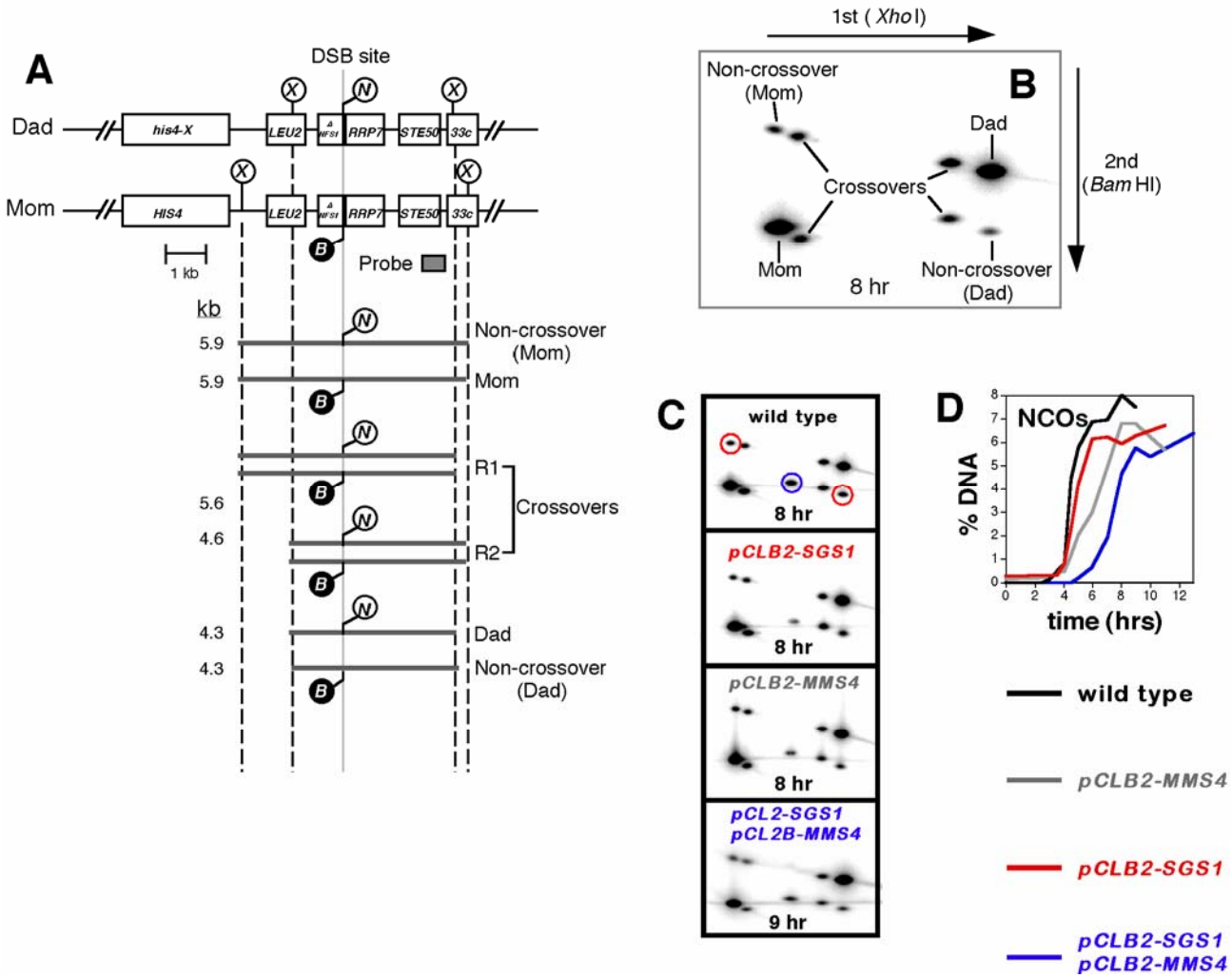

Figure S2. Noncrossover Formation in Wild-Type, *pCLB2-SGS1*, *pCLB2-MMS4* and *pCLB2-SGS1 pCLB2-MMS4* Cells.

(A) Noncrossovers at *HIS4LEU2* are detected by virtue of a *Bam*HI/*Ngo*MIV polymorphism situated immediately at the DSB site (Martini et al., 2006). Following electrophoresis of *Xho*I-digested DNA, the status of this allele is probed via in-gel digestion with *Bam*HI. The resulting products are resolved in a second dimension gel and detected by Southern analysis as shown in (B). The eight spots detected by Southern hybridization with Probe 4 correspond to those shown in (A). Four crossover products are observed because crossovers can carry either the *Bam*HI or the *Ngo*MIV allele.

(C) 2D analysis of noncrossovers. In each case a representative panel from a late time point is shown. Noncrossover species are circled in red. An internal control to monitor *Bam*HI digestion efficiency is circled in blue.

(D) Quantitative analysis of noncrossovers (NCOs). "% DNA" is percent of total hybridizing DNA. In wild-type cells, noncrossovers plateau after ~7 hrs, at ~7.5% of hybridizing DNA. *pCLB2-SGS1* cells form noncrossovers with normal timing and final levels are only slightly decreased relative to wild type.

Noncrossovers also form at slightly lower than wild-type levels in *pCLB-MMS4* cells, but levels plateau ~1 hr later. In *pCLB2-SGS1 pCLB2-MMS4* double mutants, noncrossovers form ~2 hrs later than normal at, or slightly lower than, *pCLB2-MMS4* levels. This additional delay relative to the *pCLB2-MMS4* single mutant is accounted for by the slightly delayed DSB formation in the *pCLB2-SGS1 pCLB2-MMS4* strain (see Figure 3B and Supplemental Figure S1).

Martini, E., Diaz, R.L., Hunter, N., and Keeney, S. (2006). Crossover homeostasis in yeast meiosis. Cell *126*, 285-295.

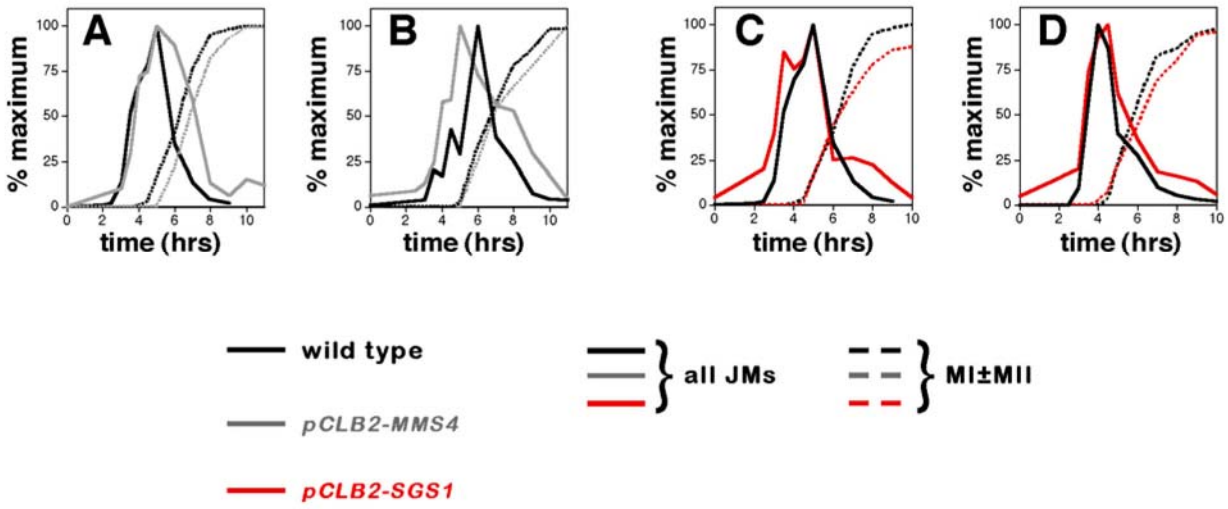

Figure S3. JM Resolution Is Delayed In *pCLB-MMS4* and *pCLB2-SGS1* mutants.

(A and B) Comparisons of the timing of JM formation and meiotic divisions (MI±MII) for two independent pairs of wild type and *pCLB-MMS4* cultures that were sporulated in parallel.

(C and D) Comparisons of the timing of JM formation and MI±MII for two independent pairs of wild-type and *pCLB2-SGS1* cultures that were sporulated in parallel.

JM formation (all JMs) and meiotic divisions (MI±MII) are expressed as percentages of their maximum values at each time point.

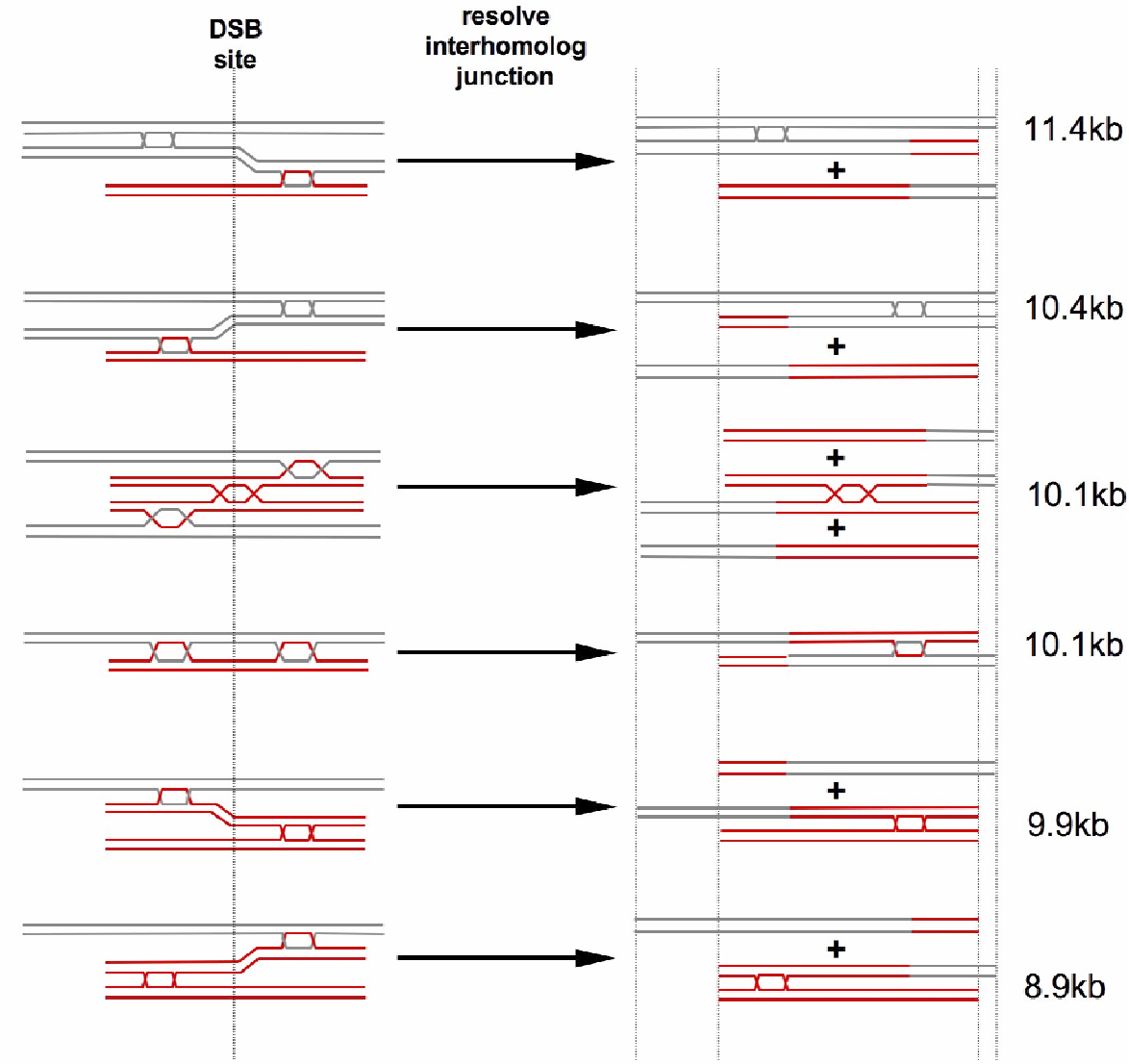

Figure S4. Potential Mechanism of rJM Formation

The four rJM species inferred from the analysis in Figure 6 could derive from the partial resolution of three- and four-chromatid mcJMs. The four possible configurations of three-chromatid mcJMs shown differ with respect to which pair of sister-chromatids is involved and the position of the interhomolog junction relative to the initiation site (DSB site). Resolution of the interhomolog dHJs produces rJMs and their reciprocal crossover products. Alternatively, an rJM could be formed via a secondary round of strand-invasion between sister-chromatids, one of which has already undergone crossing-over with a homolog (not shown; see text). Also shown is a double-IH-dHJ structure that could result from multiple strand-invasion events and could be partially resolved to give a fully recombinant dHJ structure, which is the same size as an IH-dHJ and an IH-sHJ.

Table S1. Strains used in this study.

Strain	Genotype*
NHY 1226	*MAT**a**/MATα HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoMIV) ndt80Δ::kanMX4/ndt80Δ::kanMX4*
NHY 1296	*MAT**a**/MATα HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoMIV)*
NHY 2212	*MAT**a**/MATα HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoMIV)--URA3 pCLB2-MMS4::kanMX4/pCLB2-MMS4::kanMX4*
NHY 2242	*MAT**a**/MATα HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoMIV)--URA3 pCLB2-SGS1::kanMX4/pCLB2-SGS1::kanMX4*
NHY 2273	*MAT**a**/MATα HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoMIV)--URA3 pCLB2-SGS1::kanMX4/pCLB2-SGS1::kanMX4 pCLB2-MMS4::kanMX4/pCLB2-MMS4::kanMX4*
NHY 2993	*MAT**a**/MATα HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoMIV)--URA3 pCLB2-SGS1::kanMX4/pCLB2-SGS1::kanMX4 ndt80::kanMX4/ndt80::kanMX4*
NHY 2994	*MAT**a**/MATα HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoMIV)--URA3 pCLB2-MMS4::kanMX4/pCLB2-MMS4::kanMX4 ndt80::kanMX4/ndt80::kanMX4*
NHY 2568	*MAT**a**/MATα HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoMIV)--URA3 pCLB2-SGS1::kanMX4/pCLB2-SGS1::kanMX4 pCLB2-MMS4::kanMX4/pCLB2-MMS4::kanMX4 ndt80Δ::kanMX4/ndt80Δ::kanMX4*
NHY 3170	*MAT**a**/MATα HIS4::LEU2-(New Bam)/HIS4::LEU2-(New Bam) spo11-Y135::URA3/spo11-Y135F::URA3*
NHY 3171	*MAT**a**/MATα his4-X::LEU2--URA3/HIS4::LEU2(central polymorphisms untested) spo11-Y135F::URA3/spo11-Y135F::URA3 pCLB2-SGS1::kanMX4/pCLB2-SGS1::kanMX4 pCLB2-MMS4::kanMX4/pCLB2-MMS4::kanMX4*

*All strain are also homozygous for the mutations *ura3Δ(sma-pst)* and *leu2::hisG.*