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Supplemental Data

Mus81/Mms4 Endonuclease and Sgs1 Helicase Collaborate to Ensure Proper Recombination Intermediate Metabolism during Meiosis

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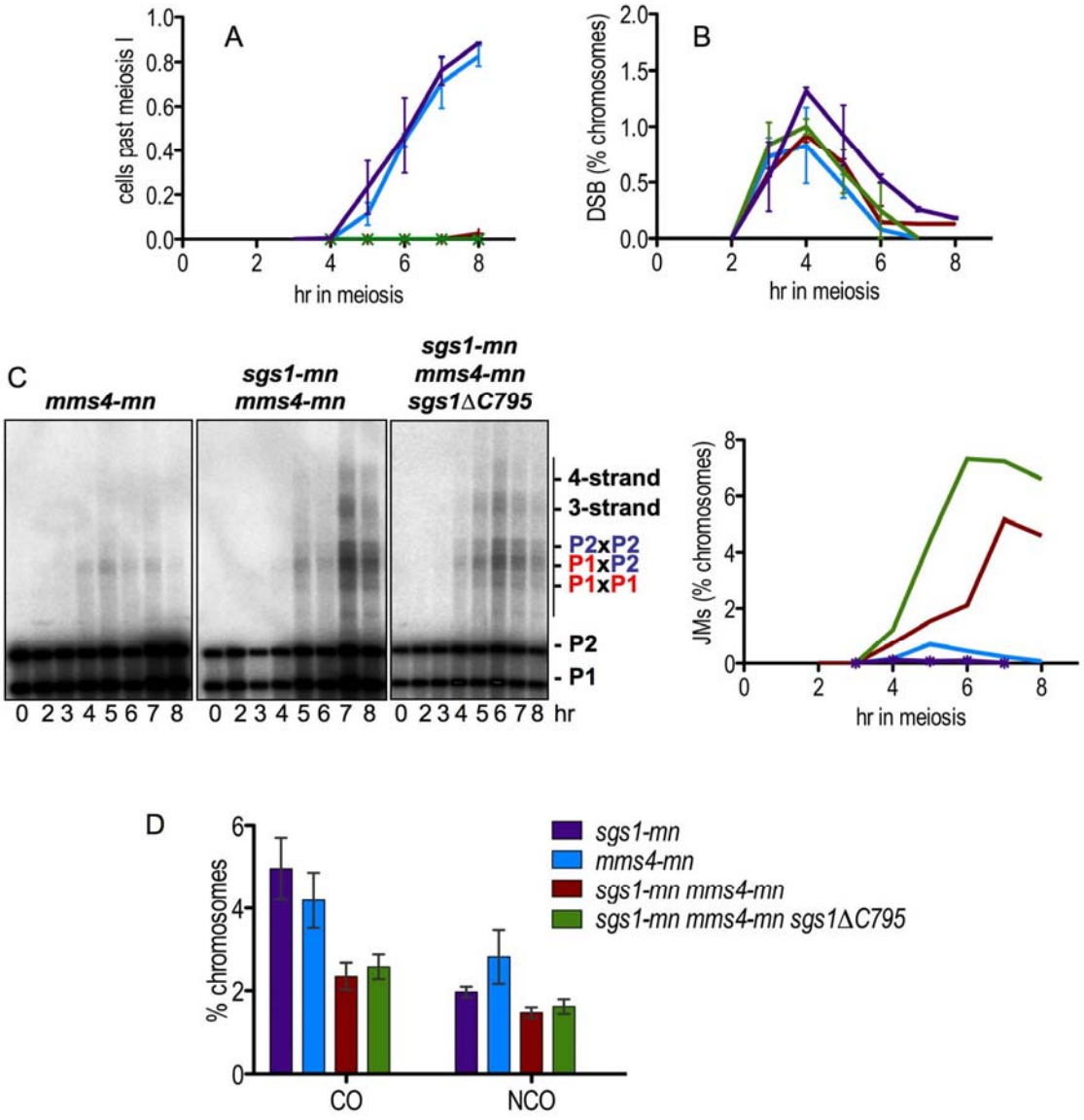
Table S1.

Strain	Relevant genotype	
MJL2984		*his4::URA3-tel-arg4-EcPal+9 leu2-R* *HIS4 leu2-R::URA3-tel-ARG4*
MJL3166	MJL2984+	*natMX::pCLB2-3HA-SGS1* *natMX::pCLB2-3HA-SGS1*
MJL3019	MJL2984+	*mus81Δ::KanMX* *mus81Δ::KanMX*
MJL3181	MJL2984+	*natMX-pCLB2-3HA-SGS1 mus81Δ::KanMX* *natMX-pCLB2-3HA-SGS1 mus81Δ::KanMX*
MJL3164	MJL2984+	*ndt80Δ::KanMX* *ndt80Δ::KanMX*
MJL3243	MJL2984+	*natMX-pCLB2-3HA-SGS1 kanMX-pGAL1-MUS81 ura3-pGPD1-(GAL4-ER)-URA3* *natMX-pCLB2-3HA-SGS1 mus81Δ::KanMX ura3Δ*
MJL3269	MJL2984+	*MUS81-3FLAG::HphMX* *mus81Δ::KanMX*
MJL3270	MJL2984+	*kanMX-pGAL1-MUS81-3FLAG ura3-pGPD1-(GAL4-ER)-URA3* *mus81Δ::KanMX ura3Δ*
MJL3244	MJL2984+	*kanMX-pGAL1-3HA-SGS1 mus81Δ::KanMX ura3-pGPD1-(GAL4-ER)-URA3* *natMX-pCLB2-3HA-SGS1 mus81Δ::KanMX ura3Δ*
MJL3298	MJL2984+	*natMX-pCLB2-3HA-SGS1 mus81Δ::KanMX spo11Y135F-3HA* *natMX-pCLB2-3HA-SGS1 mus81Δ::KanMX spo11Y135F-3HA*
MJL2442		*his4::URA3-arg4-EcPal+9 LEU2* *HIS4 leu2::URA3-ARG4*
MJL3091	MJL2442+	*natMX-pCLB2-3HA-SGS1* *natMX-pCLB2-3HA-SGS1*

MJL3109	MJL2442+	*kanMX-pCLB2-3HA-MMS4* --- *kanMX-pCLB2-3HA-MMS4*
MJL3110	MJL2442+	*natMX-pCLB2-3HA-SGS1* *kanMX-pCLB2-3HA-MMS4* --- --- *natMX-pCLB2-3HA-SGS1* *kanMX-pCLB2-3HA-MMS4*
MLJ3122	MJL2442+	*natMX-pCLB2-3HA-SGS1* *kanMX-pCLB2-3HA-MMS4* *TRP1* --- --- --- *sgs1Δ::natMX* *kanMX-pCLB2-3HA-MMS4* *TRP1::sgs1ΔC795*
MJL3144	MJL2442+	*natMX-pCLB2-3HA-SGS1* *kanMX-pCLB2-3HA-MMS4* *spo11Y135F-3HA* --- --- --- *natMX-pCLB2-3HA-SGS1* *kanMX-pCLB2-3HA-MMS4* *spo11Y135F-3HA*

Table S1. Strains used in this study.
All strains are *MAT*a/*MAT*α SK1 diploids and are homozygous for *lys2 ho::LYS2 arg4Δ ura3Δ*, except where otherwise indicated. Diploids are derived from MJL2984 (Jessop et al., 2005) or from MJL2442 (Allers and Lichten, 2001), as indicated.

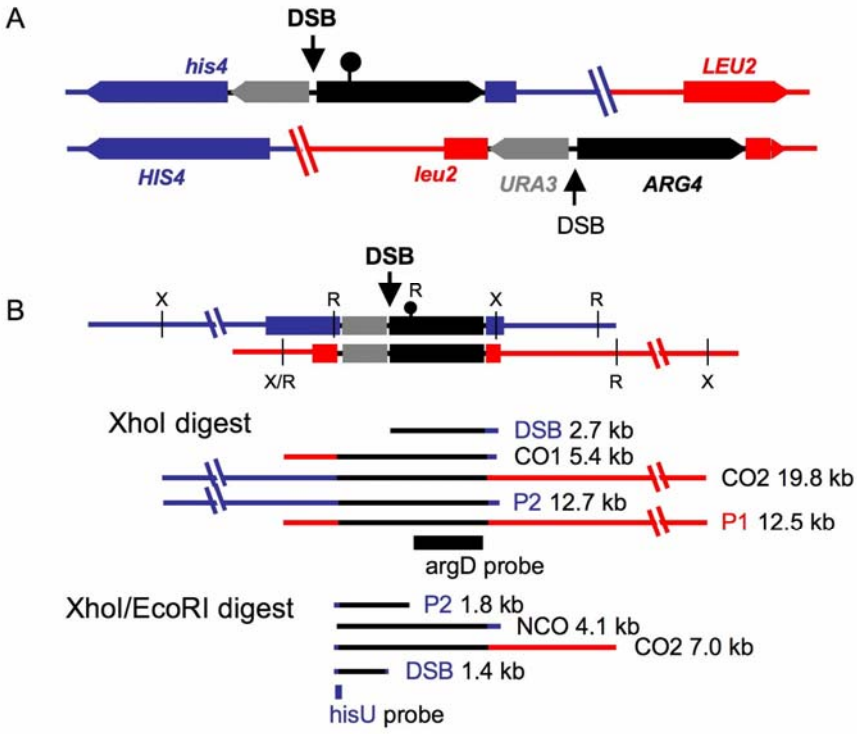
Supplementary Figure 1



Supplementary Figure 1. Meiotic recombination and nuclear divisions in *sgs1-mn mms4-mn* and *sgs1-mn sgs1ΔC795 mms4-mn* mutants
The strains used in this figure contain a version of the recombination interval in which *URA3* and *ARG4* are in head-to-tail orientation, and which lacks the 65 nt telomere sequence (Allers and Lichten, 2001). DSBs, JMs and recombinants are reduced relative to those seen with the recombination interval illustrated in Figure 2 and Supplementary Figure 2. Digestion and probing strategies are similar to those shown in Figure 2 and Supplementary Figure 2.
A. Meiotic nuclear divisions in *sgs1-mn* (purple, MJL3091), *mms4-mn* (light blue, MJL3109), *sgs1-mn mms4-mn* (dark red, MJL3110) and *sgs1-mn sgs1ΔC795 mms4-mn* (green, MJL3122) mutants. Cells with 2 nuclei or greater were scored as having transited meiosis I.

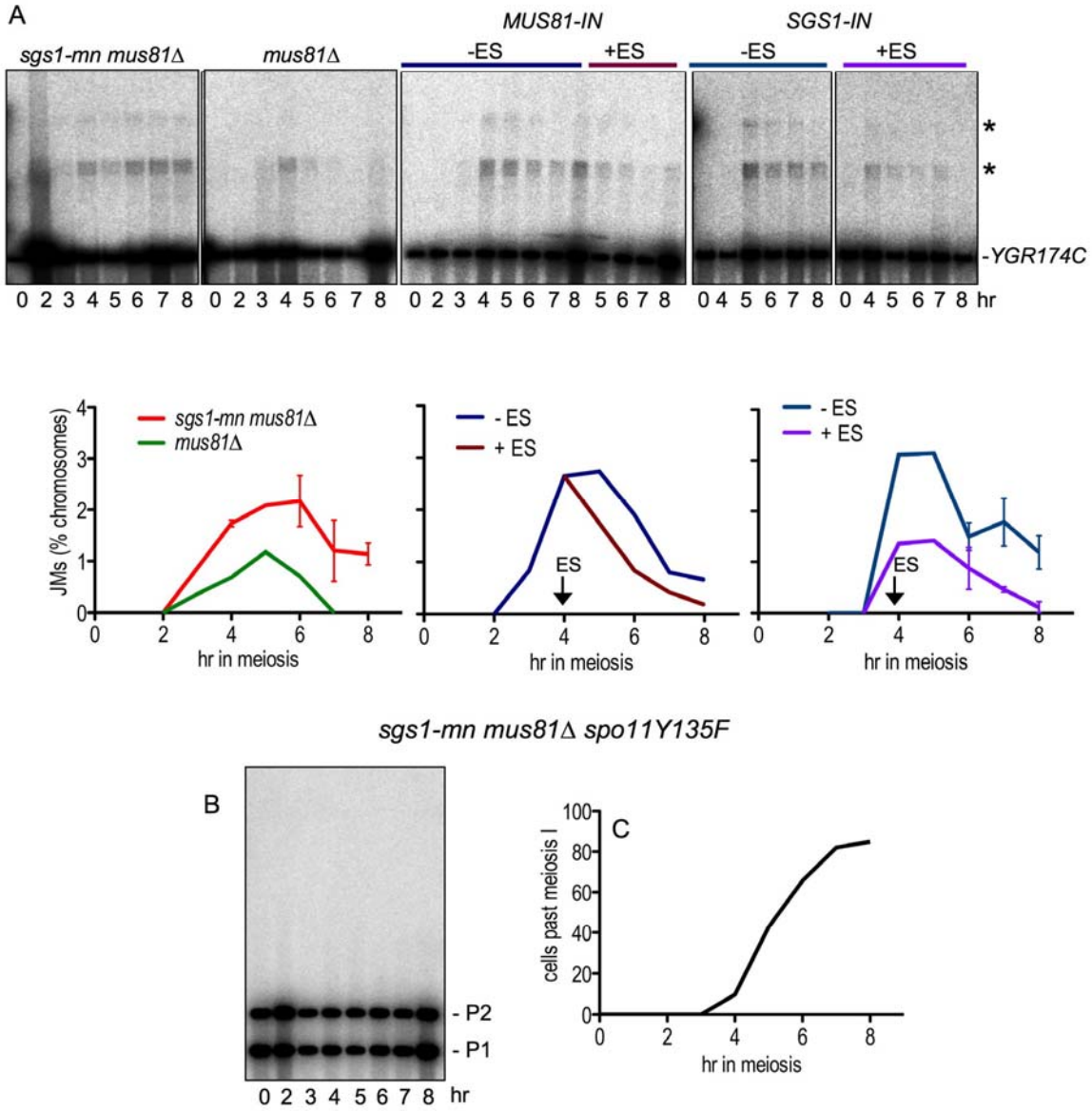
B. DSB formation and repair. DNA was isolated from sporulating cultures at the indicated time points, digested with *Xho*I, and southern blots were probed with argD to detect DSBs within the *URA3-ARG4* recombination interval. DSB frequencies are plotted as a percentage of insert containing DNA. Symbols are as in 1A.
C. JMs accumulate in *sgs1-mn mms1-mn* mutants. Left--Southern blots of DNA prepared from wild type and mutant strains, digested with *Xmn*I and probed with argD. Vertical bar on right denotes region of blot used for JM frequency measurements. Right--Quantification of total JM frequencies, plotted as a percent of total insert sequences. Symbols are as in 1A.
D. CO and NCO recombinant levels. Products were quantified from Southern blots of DNA digested with *Xho*I/*Eco*RI, probed with hisU (similar to Supplementary Figure 2). The average of 7 and 8 hour samples, calculated from 2 independent measurements for each strain, are plotted.
All plots report mean ± s. e. m.

Supplementary Figure 2



Supplementary Figure 2. Illustration of digests used to measure DSBs, COs and NCOs.
A. Recombination interval. For details, see legend to Figure 2.
B. Digests and probes used to detect DSBs, COs and NCOs. Restriction fragments produced by an *Xho*I (X) digest are probed with DNA from *ARG4* (black bar, argD) to detect DSBs and CO products. Restrictions fragments produced by a double digest with *Xho*I and *Eco*RI (R) are probed with DNA from *HIS4* (blue bar, hisU) to detect CO products and NCO gene convertants where the *arg4* allele containing the palindrome is converted to *ARG4*.

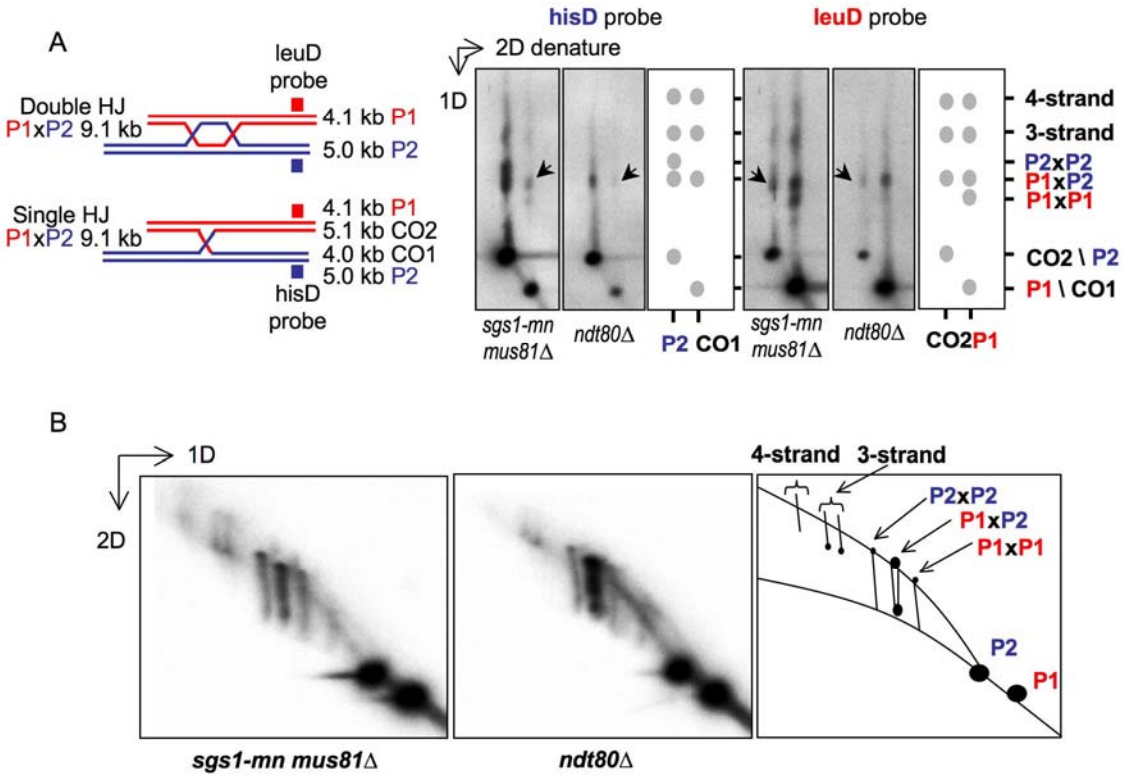
Supplementary Figure 3



Supplementary Figure 3. JM accumulation at native DSB hotspot in *sgs1 mus81* is relieved by *MUS81* or *SGS1* and requires meiosis-induced DSBs. **A**. JM accumulation at a native DSB hotspot. DNA from indicated time points (*sgs1-mn mus81*Δ, MJL3181, red; *mus81*Δ, MJL3019, green; *sgs1-mn mus81*Δ/*MUS81-IN*, MJL3243, without estradiol—dark blue, with estradiol at 4hr—dark red; *3XHA-sgs1-mn/3XHA-SGS1-IN mus81*Δ, MJL3244, without estradiol—blue, with estradiol at 4 hr—light purple) was digested with *Xmn*I, displayed on Southern blots, and probed with *YGR174C* sequences. JM species are indicated by an asterisk (⋆). About 8% of chromosomes have a DSB at *YGR175C* (R. Shroff, C. Buhler and M.L. unpublished result). Similar results were obtained with *sgs1-mn mms4-mn* (MJL3110, data not shown).

Quantification of total JMs species, plotted as a % of total lane signal (% of chromosomes *VII*) is shown.
B. JM formation and accumulation in *sgs1-mn mus81*Δ requires meiotic recombination. DNA from indicated time points from a DSB-negative, *sgs1-mn mus81*Δ *spo11-Y135F* strain (MJL3298) was digested with *Xmn*I, displayed on Southern blots, and probed with argD to detect JMs in the *URA3-ARG4* recombination interval. Similar results were obtained with *sgs1-mn mms4-mn spo11-Y135F* (MJL3144, data not shown).
C. Nuclear division proceeds in cells lacking Sgs1, Mus81/Mms4 and meiotic recombination. DSB-negative *sgs1-mn mus81*Δ *spo11Y135F* cells were sporulated, samples were stained with DAPI and nuclei counted. Cells with at least 2 nuclei were scored as having transited meiosis I. Similar results were obtained with *sgs1-mn mms4-mn spo11Y135F* (data not shown).
All plots report mean ± s. e. m.

Supplementary Figure 4



Supplementary Figure 4. JMs contain single HJs.
A. 2-dimensional native/denaturing electrophoresis of *Xmn*I digested DNA from 8 hr samples of *sgs1-mn mus81*Δ or *ndt80*Δ strains. Schematic of expected sizes for component strands in *Xmn*I digests probed with hisD or leuD if JMs contain one or two HJs. JMs containing an even number of HJs (here referred to as dHJs) will contain only parental-length single DNA strands. JMs with an odd number of HJs (here referred to as sHJs) will contain CO- and parental-length single DNA strands. JMs that contain an even number of HJs, but where one chromatid has undergone a crossover (referred to as rJMs, Oh *et al.* 2008) will also contain CO- and parental-length DNA strainds. The first electrophoresis dimension was run under native conditions; the gel was then rotated 90-degrees and run under DNA-denaturing conditions. The Southern blot was probed with hisD (left), stripped and re-probed with leuD (right). CO strands are frequently observed at the interhomolog-JM position (arrows).

While this restriction enzyme/probe combination cannot unambiguously determine if these CO strands derive from sHJ-JMs or from rJMs, digests using other restriction enzymes identify sHJ-JMs as the origin of the majority of CO strands in these intermediates (L. J., Anuradha Sourirajan and M. L., unpublished data).

B. 2-dimensional native/native electrophoresis of *Xmn*I digested DNA from 8 hr samples of *sgs1-mn mus81*Δ or *ndt80*Δ strains. The Southern blot was probed with argD. The interpretative panel shows the inferred identity of JM species; see also (Oh et al., 2007).

Supplemental References

Allers, T., and Lichten, M. (2001). Differential timing and control of noncrossover and crossover recombination during meiosis. Cell *106*, 47-57.
Jessop, L., Allers, T., and Lichten, M. (2005). Infrequent co-conversion of markers flanking a meiotic recombination initiation site in *Saccharomyces cerevisiae*. Genetics *169*, 1353-1367.
Oh, S. D., Lao, J. P., Hwang, P. Y., Taylor, A. F., Smith, G. R., and Hunter, N. (2007). BLM ortholog, Sgs1, prevents aberrant crossing-over by suppressing formation of multichromatid joint molecules. Cell *130*, 259-272.
Oh, S. D., Lao, J. P., Taylor, A. F., Smith, G. R., and Hunter, N. (2008) RecQ helicase, Sgs1, and XPF-family endonuclease, Mus81-Mms4, resolve aberrant joint molecules during meiotic recombination. Mol Cell, *in press*.