Hyppa and Smith “Crossover invariance…” CELL-D-09-01843R2

SUPPLEMENTAL INFORMATION INVENTORY

Supplemental data:

Supplemental Information

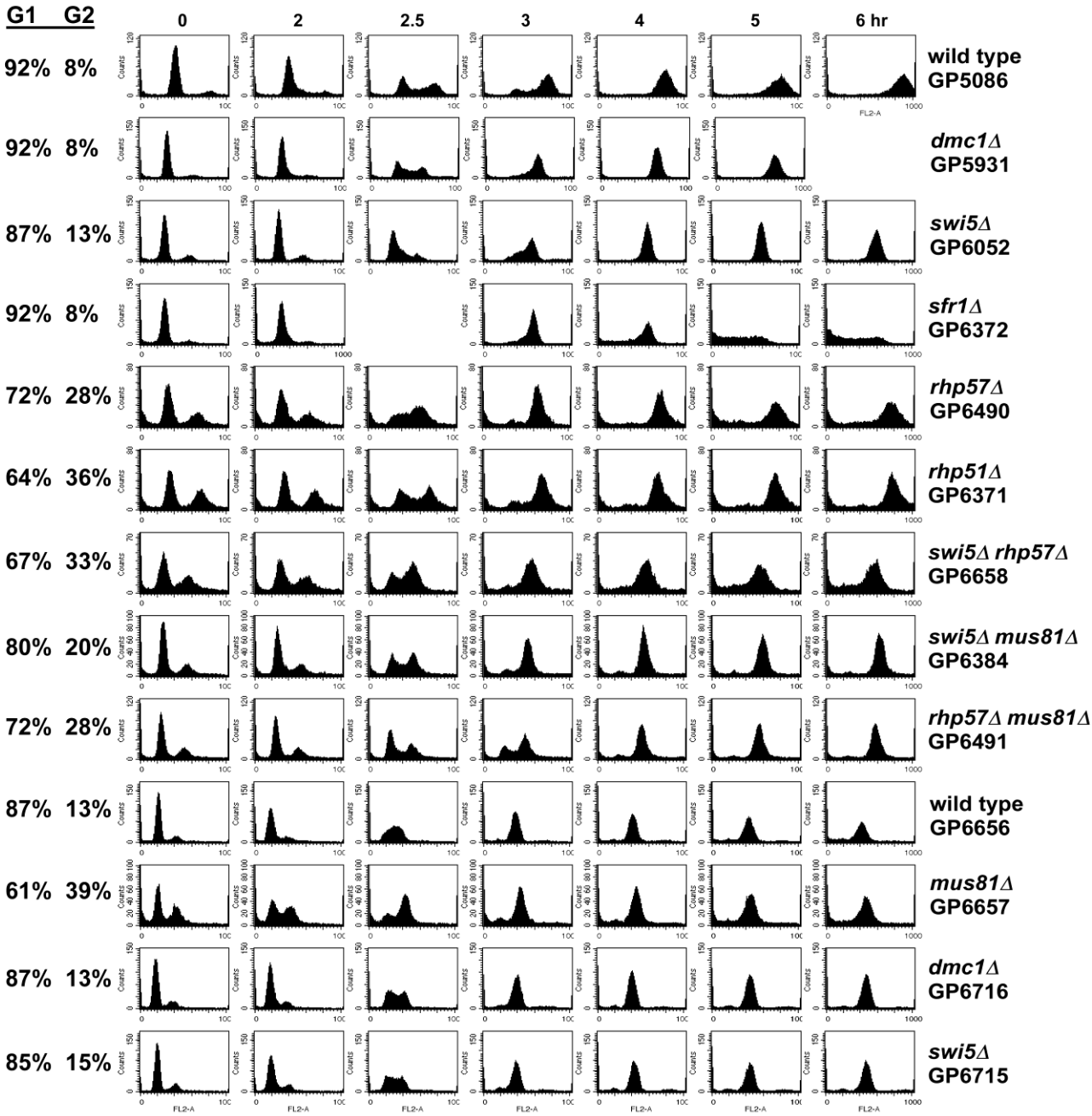

Figure S1, related to Figures 2, 3, 4, 5, S2, S3, S4, and S5. Deletions Eliminating Rad51, Its Mediator Complexes, Dmc1, or Mus81-Eme1 Do Not Interfere with Replication during Meiosis

The relative G1 and G2 DNA contents were measured by flow cytometry from the start of meiosis (0 hr) to 7 hr. Initial (0 hr) values are to the left of each row. Each experiment was performed twice with similar results. Strain numbers and relevant genotypes are indicated to the right of each row. DNA from these strains was analyzed in Figures 2 – 5, except for strains GP6656, GP6657, GP6715, and GP6716, which were analyzed in Figures S3 – S5. Note that the fraction of cells in G2 at 0 hr ranges from 8% to 39%. Although the fate of these cells is not known, if they do not enter meiosis, then the frequency of meiotic recombination intermediates would be reduced by about ½ of these values (*i.e.*, 4 – 20%), an amount too small to affect the conclusions here. No reduction would be required if these cells enter meiosis. Any reduction would not affect the ratio of IS:IH HJs in a given strain.

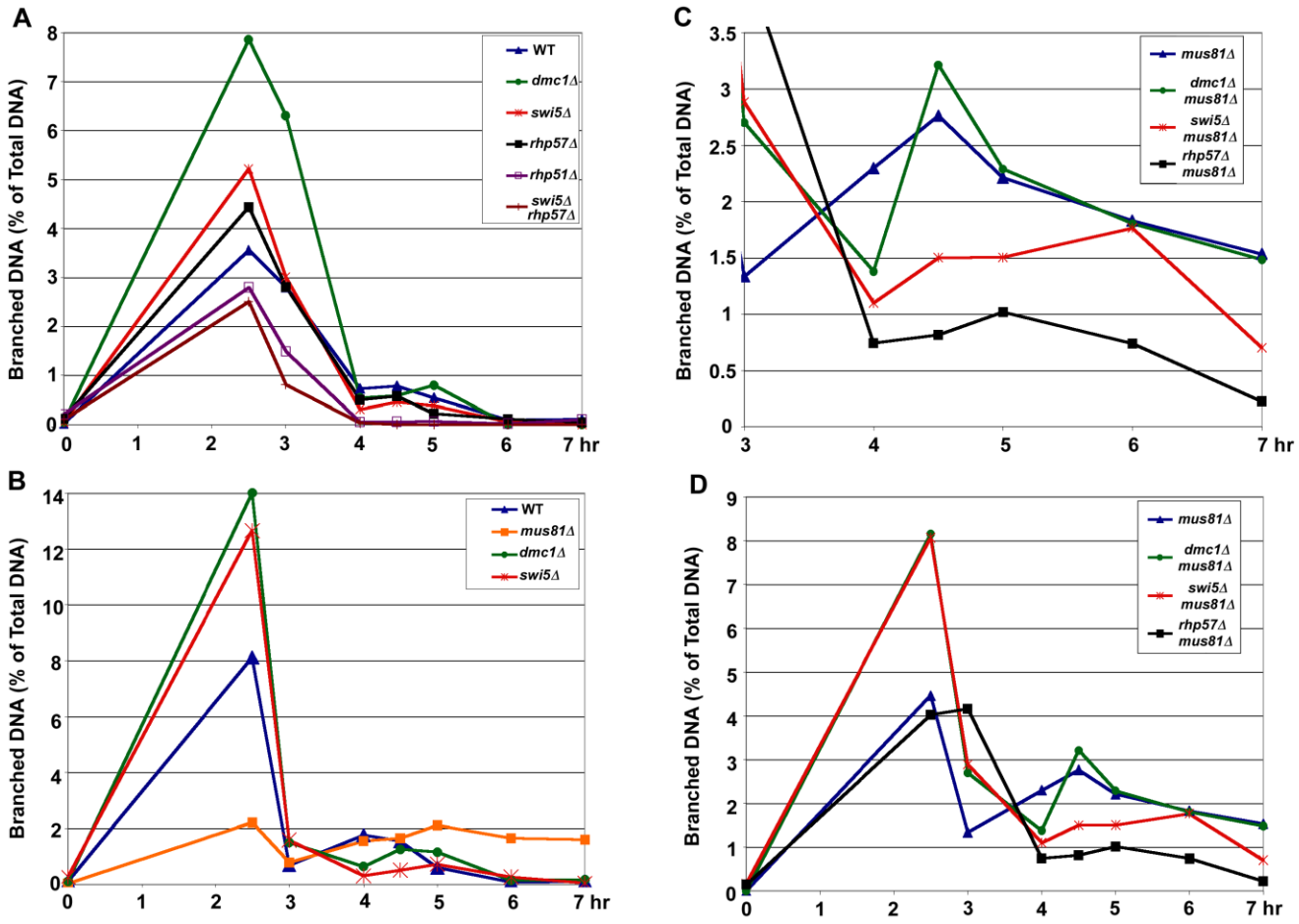

Figure S2, related to Figures 2, 3, and 4. Progression of Replication in Strand Exchange Mutants and Quantification of Accumulated Holliday Junctions at *mbs1* in the *mus81Δ* Background

(A) Entire meiotic time course of the experiment in Figure 2.

(B) Entire meiotic time course of the experiment in Figure S3. The low amount of replication intermediates in the *mus81Δ* strain is likely due to replication occurring slightly earlier than normal, as evidenced from the beginning of the shift from G1 to G2 at 2 hr in strain GP6657 (Figure S1), and to the absence of data at 2 hr. Replication levels in other *mus81Δ* strains are similar to those in wild type (Cromie et al. 2006; Figure S2D).

(C) Accumulation of HJs in *mus81Δ* derivatives supports the HJ formation defects seen in *mus81*$^{+}$ strains (Figure 2).

(D) Full meiotic time course of the experiment in part C, showing replication intermediates formed between 2 and 3 hr in *mus81Δ* strains.

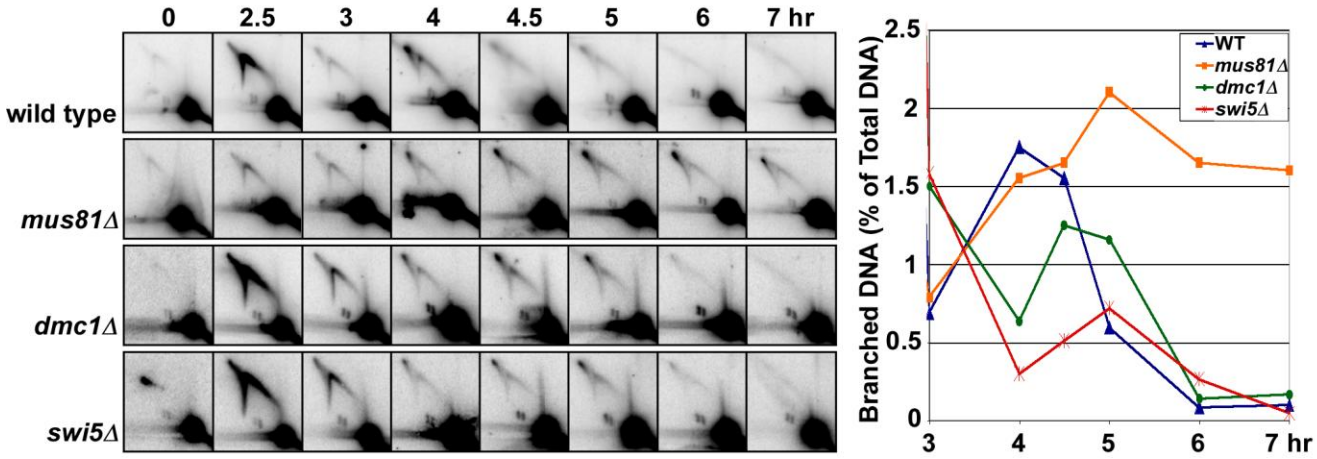

Figure S3, related to Figure 2. HJ Formation at the DSB Hotspot *ade6-3049* Parallels That at *mbs1*

DNA from meiotically induced strains was digested with *Bsr*GI and analyzed for structures related to recombination at *ade6-3049*. The formation and resolution of HJs was measured in the strains indicated, as was done in Figure 2. Each measurement is the mean of two independent meiotic inductions, and nearly all values are within 20% of their respective mean; error bars are omitted for clarity.

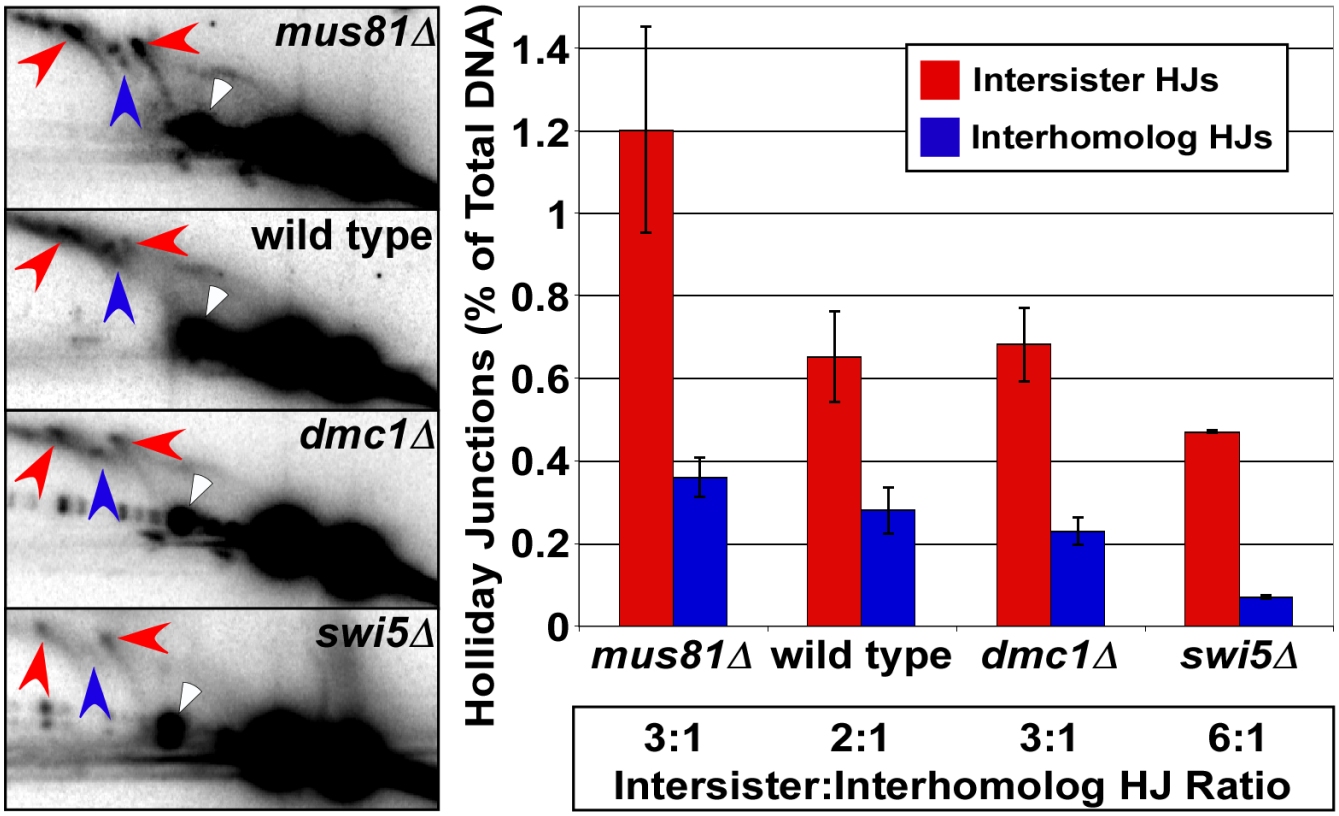

Figure S4, related to Figure 3. Intersister HJs Outnumber Interhomolog HJs at the *ade6-3049* Hotspot

The relative amounts of IH and IS HJs in the indicated mutants were determined using diploids with heterozygous restriction site markers as indicated (see Figure S5, lower right panel, for diagram). The IH and IS HJs were determined by differences in their masses, 20.6 and 16.6 kb for IS HJs, and an intermediate mass of 18.6 kb for IH HJs. Gel images from 4.5 or 5 hr (the time of maximal HJs) for the indicated mutants are shown. Red arrows indicate IS HJs; blue arrows, IH HJs; white arrows, partial digestion by either *Sca*I or *Pml*I. Quantification of 3 – 5 experiments is displayed on the bar graph; data are the mean, and error bars indicate the range or SEM. The ratio of IS to IH is given below for comparison. The frequency of IS HJs is not significantly different in wild type and *swi5Δ* ($p > 0.2$ by t-test); IH HJs are significantly reduced in *swi5Δ* ($p < 0.02$).

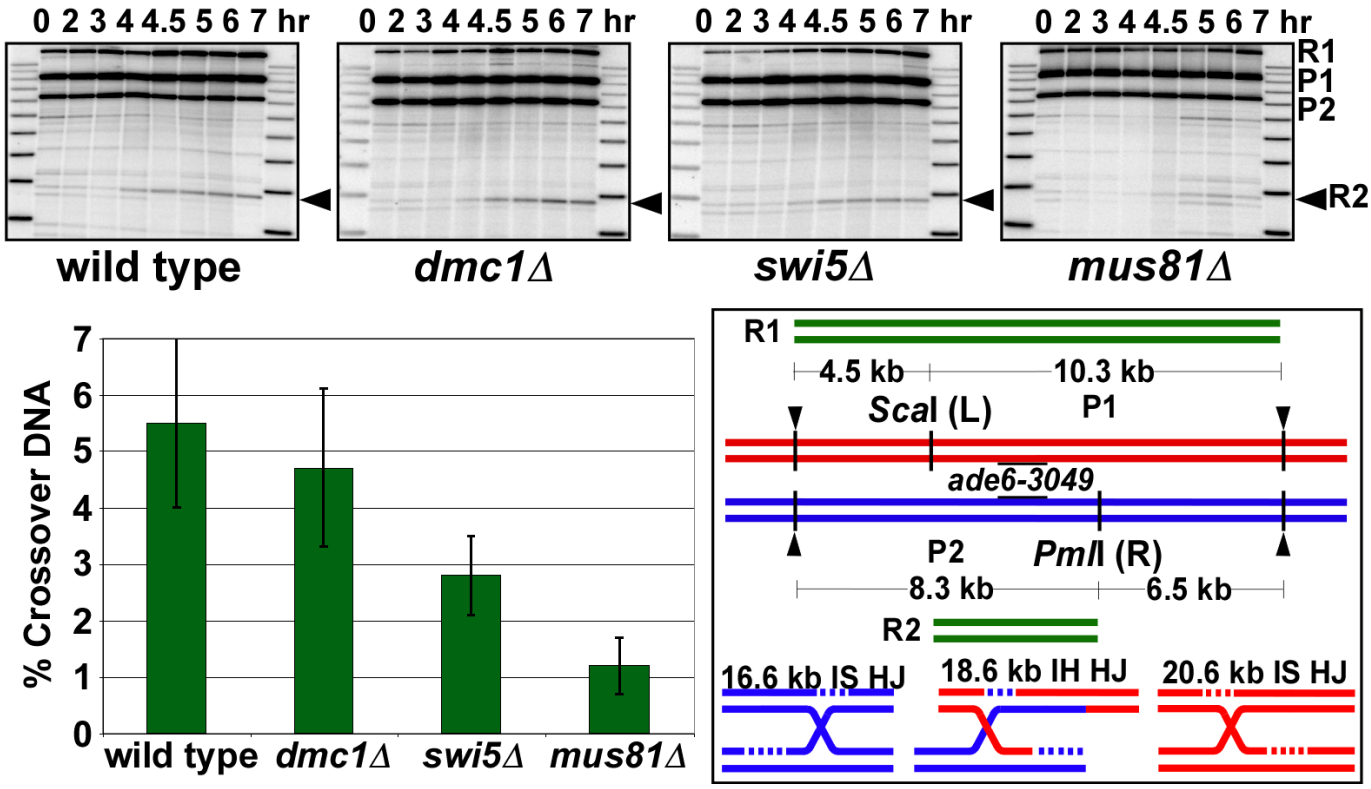

Figure S5, related to Figure 4. Crossover DNA at the *ade6-3049* Hotspot Depends on Swi5 But Not on Dmc1

Crossover DNA was measured by the accumulation of the R2 recombinant DNA fragment (black arrowhead in top panels; see diagram in lower right panel). Crossover frequency is 2 × (R2 DNA)/total DNA. Each measurement is the average of two independent meiotic inductions; error bars indicate the range.

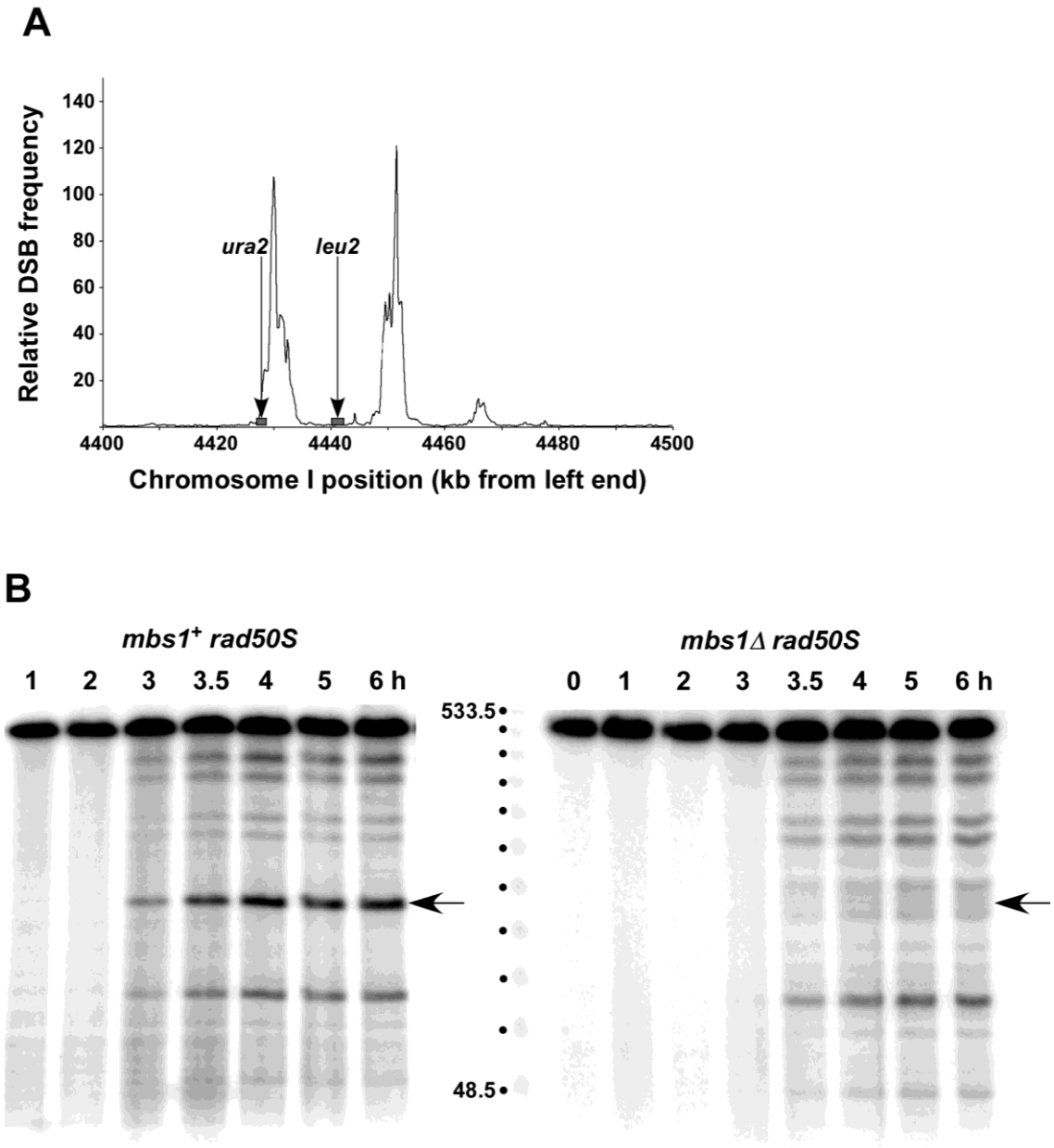

Figure S6, related to Table 1.

A. A Strong DSB Hotspot Between *ura2* and *leu2*
The DSB hotspot in the *ura2* – *leu2* genetic interval was measured using microarray analysis of Rec12(FLAG)-DNA covalent linkages in a *rad50S* strain (5 hr after meiotic induction) normalized to the genome median (Cromie et al., 2007; Hyppa et al., 2008). The locations of the *ura2* and *leu2* ORFs are indicated by grey boxes, just below the arrowheads.

B. Deletion of the *mbs1* Hotspot Strongly Reduces Local DSBs
Meiotic DNA prepared at the indicated times after meiotic induction of strains GP3062 (*mbs1*$^{+}$, left) and GP4262 (*mbs1-19,* right) was digested with *Not*I and analyzed by Southern blot hybridization. The 501 kb *Not*I J fragment was probed from the left end. The arrow indicates the position of *mbs1*, and the ladder consists of 48.5 kb increments of lambda concatemers (NEB).