Supplemental Information.

Double Holliday Junctions are Intermediates of DNA Break Repair

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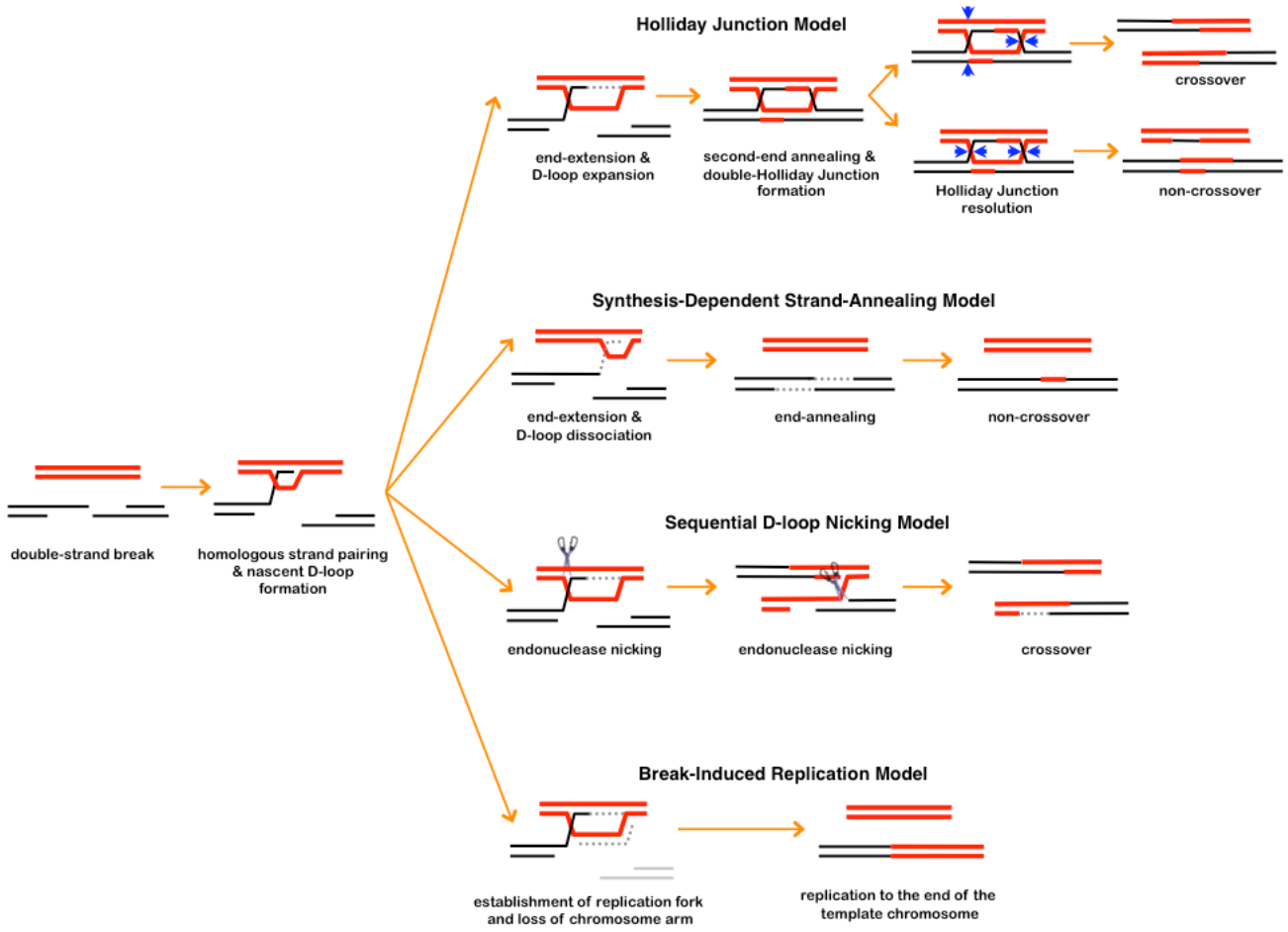
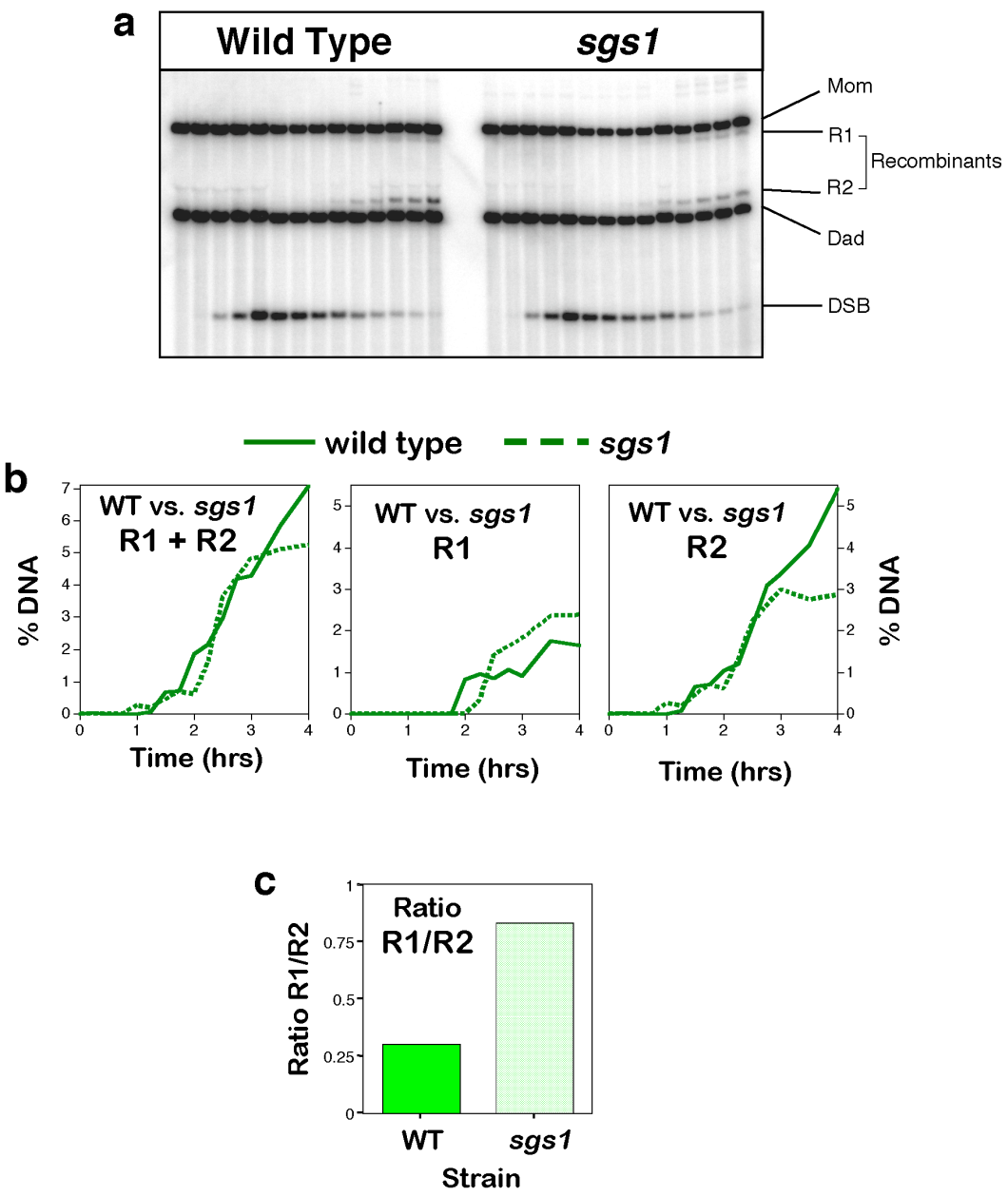
Seven figures and one table.



Supplemental Figure S1. Pathways of DSB-Repair by Homologous recombination[1-9].

The major models for the repair DNA double-strand-breaks (DSBs) by homologous recombination are shown. Initiation occurs by DSB formation and nuclease-mediated resection of the 5'-strands to form long, single-stranded tails. Nucleoprotein filaments of ssDNA and RecA-like proteins then assemble and catalyze homologous pairing and DNA strand-exchange with a template chromosome. Nascent DNA is subsequently synthesized from the invading 3'-end. The various repair pathways differentiate after this step. The canonical "DSB-Repair" model[2] designated here as the, "Holliday Junction Model", uniquely posits that double-Holliday Junctions (dHJs) are central intermediates in the repair of DNA double-strand-breaks (DSBs). In this study, dHJs are identified *in vivo* as intermediates of DSB-repair in mitotically cycling cells. Dashed lines indicate nascent DNA. Blue arrowheads indicate nucleolytic incision of Holliday Junctions. D-loop, displacement loop.



Supplemental Figure S2. Analysis of recombinants in wild-type and *sgs1* cells.

The *sgs1* allele used in this study was *sgs1-ΔC795::hphMX4* (see online **Methods**).

a, Images of 1D gels hybridized with Probe 4 showing time-course analysis of mitotic DSB repair in wild-type and *sgs1-ΔC795* cells. Samples were digested with *Xho*I as in **Fig. 1b**.

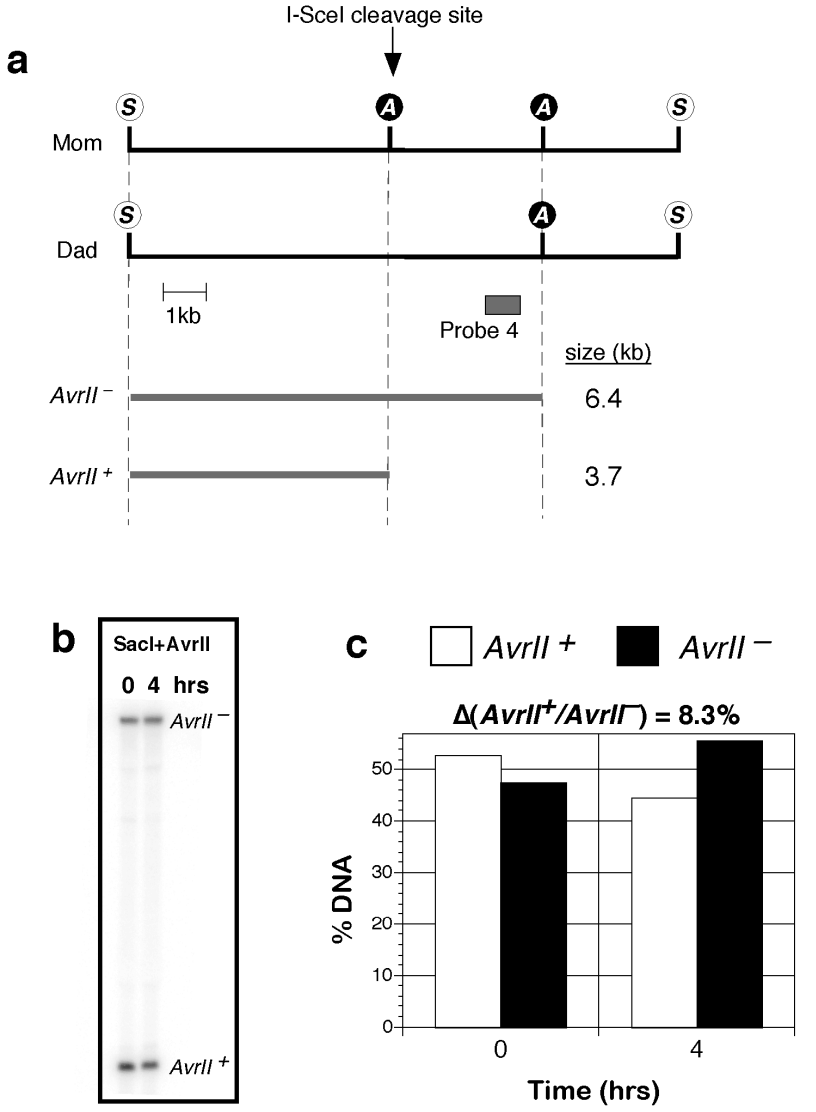
b, Quantitation of the two recombinant bands (R1+R2, R1 and R2) in wild-type and *sgs1-ΔC795* cells.

c, Final R1:R2 ratios in wild-type and *sgs1-ΔC795* cells.

If recombinant bands R1 an R2 arise exclusively from reciprocal inter-homolog crossing-over in the *HIS4LEU2-SceI* system, then the ratio of R1:R2 should equal one. However, in wild-type cells, the smaller recombinant band, R2, is greatly overrepresented relative to the larger R1. This R2 bias is likely caused by gene conversion of the most DSB-proximal *Xho*I site (specifically, the recipient "Mom" chromosome acquires the *Xho*I site located in *LEU2*; see **Fig. 1a**). Gene conversion (occurring without associated crossing-over) undoubtedly accounts for the difference between R1 and R2 (3.8%) in wild-type cells. The remaining 3.2% recombinants could be solely due to *bona fide* reciprocal crossing-over, but we cannot rule out the possibility that non-crossover gene conversion also contributes to these products, i.e. 3.2% is a *maximum* estimate of crossover levels in wild-type cells at the *HIS4LEU2-SceI* locus.

Thus, "Recombinants" in the *HIS4LEU2-SceI* system represent both crossover and non-crossover products of DSB-repair between homologs. This is in contrast to meiotic recombination at *HIS4LEU2,* where recombinants R1 and R2 accurately report interhomolog crossovers: the ratio of R1:R2 = 1 and R1+R2 matches crossover levels determined by genetic analysis[10].

Our analysis of recombinants in *sgs1* cells is consistent with the interpretation above. *sgs1* mutation has two independent effects on mitotic DSB-repair, reducing the extent of DSB resection (5'-strand degradation) and increasing the fraction of DSBs that are repaired with an associated crossover [11-13]. Reduced resection is expected to reduce the gene conversion frequency of DSB-proximal *Xho*I sites and thus the level of R2 recombinants. Consistently, the level of R2 recombinant is reduced by about half in *sgs1-ΔC795* cells (from 5.4% in wild-type to 2.9%). In contrast, the level of R1 is increased in *sgs1-ΔC795* cells relative to wild type (2.4% vs 1.6%), consistent with an increase in the fraction of DSBs that are repaired with a crossover outcome.



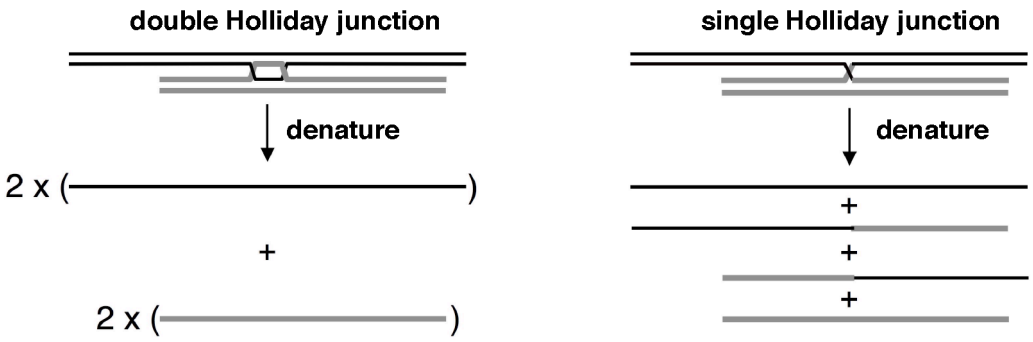
Supplemental Figure S3. Measurement of Interhomolog Recombination Levels at *HIS4LEU2-SceI*.

a, Map of the *HIS4LEU2-SceI* locus showing restriction sites used to analyze gene-conversion of a polymorphic *Avr*II site. This site is located directly at the DSB site and should always be converted during inter-homolog repair. Lollipops indicate restriction sites: S, SacI; A, *Avr*II.

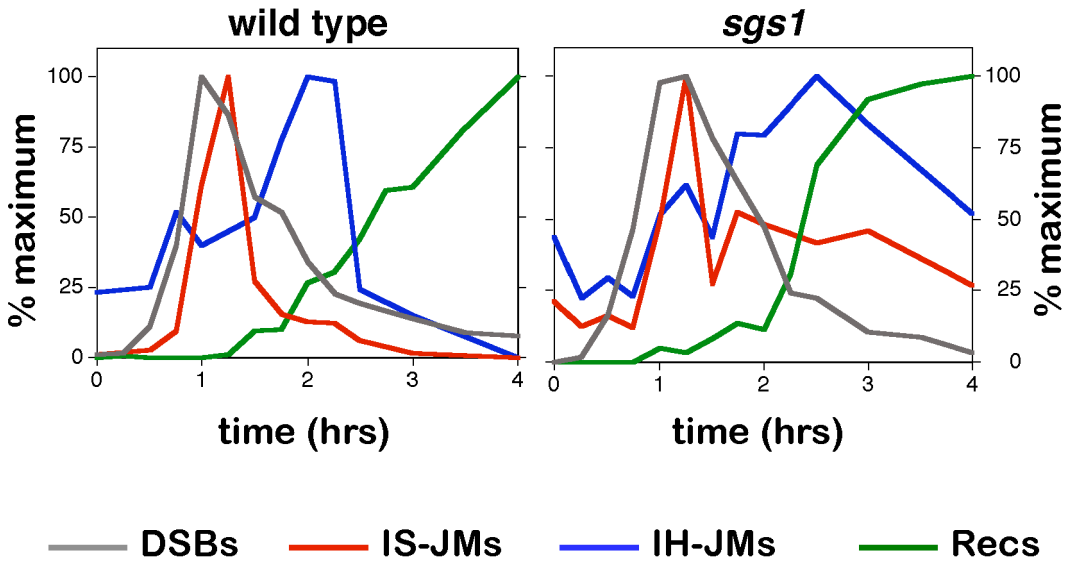
b, 1D gel Southern analysis of *Sac*I + *Avr*II digested genomic DNA from the zero and four hour time-points of the wild-type time-course shown in **Figs. 1 and 4**.

c, Quantitation of the Southern image in **b** shows that 8.3% of *Avr*II sites are converted during DSB-repair in wild-type cells. Correcting for the fact that crossing-over produces two recombinant molecules per DSB, we can estimate that inter-homolog DSB-repair at *HIS4LEU2-SceI* in wild-type cells is

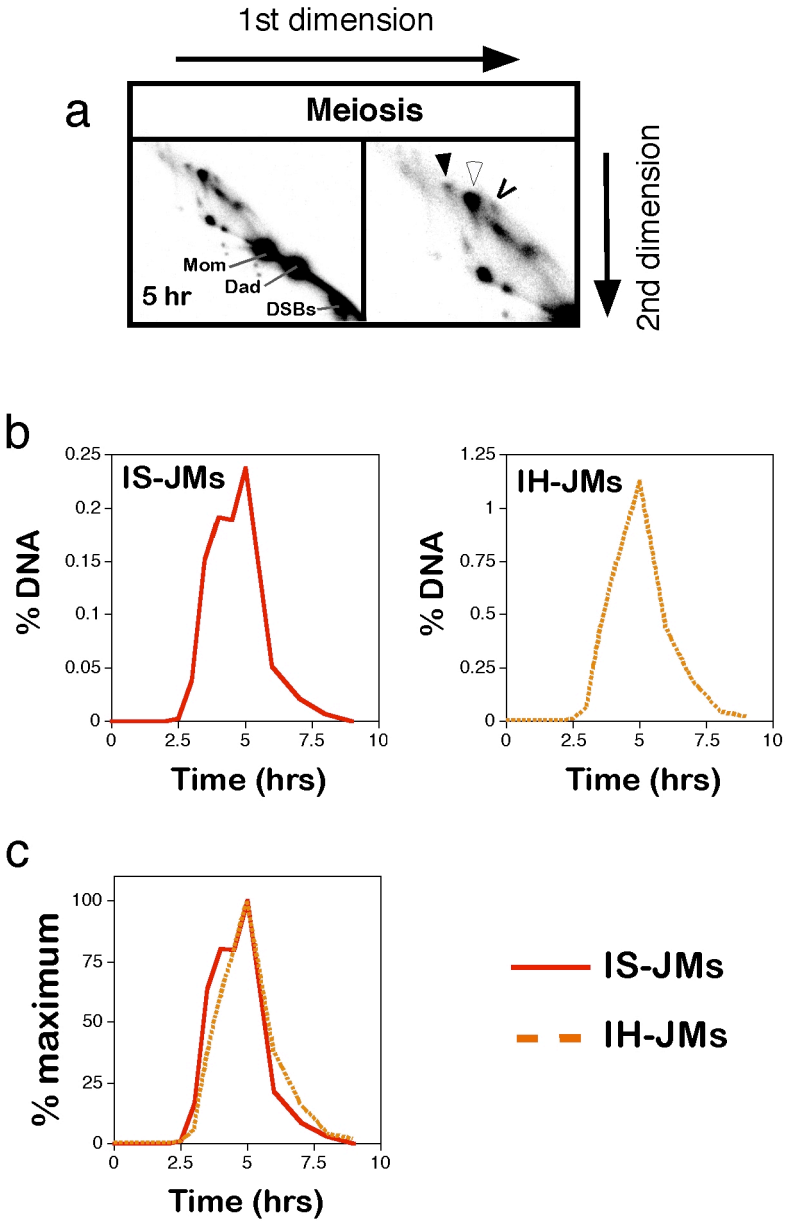
associated with crossing-over ~19% of the time (1.6% R1 recombinants/8.3% total interhomolog recombinants). This is likely an overestimation, for the reasons discussed in **Fig. S2**.



Supplemental Figure S4. Strand compositions of double-Holliday junctions and single-Holliday junctions formed between polymorphic homologous chromosomes (see analysis in **Fig. 3d**).



Supplemental Figure S5. Timing of DSB-repair intermediates in wild-type and *sgs1* cells. DSBs, JMs and Recs are expressed as percent of maximum values against time after DSB induction. Data are from the experiments shown in **Fig 4.** In these graphs, "Recs" represents R1+R2 (see **Fig. 1**).



Supplemental Figure S6. JM Levels and Timing During Meiotic Recombination.

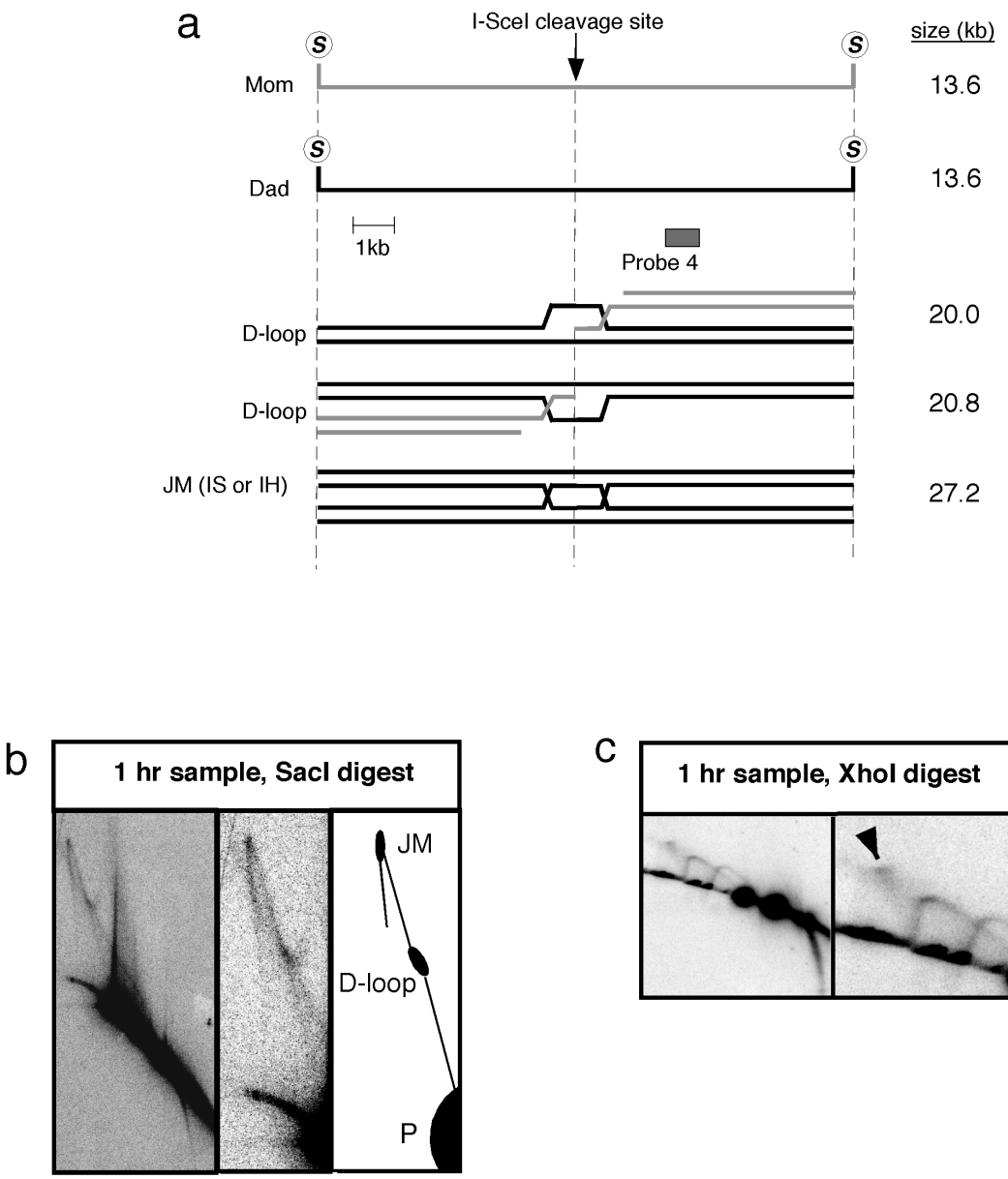
a, Image of native/native 2D gel hybridized with Probe 4 showing JMs formed at *HIS4LEU2* during meiosis. Right-hand-side panel shows a blow-up of the JM region. Arrowheads indicate JM species corresponding to those detected during mitotic DSB-repair (see **Figures 2** and **3**): black arrowhead, Mom+Mom intersister-JM; white arrowhead; Mom+Dad inter-homolog-JM; caret, Dad+Dad intersister-JM. The other prominent JM species detected during meiosis are Single-End Invasions (SEIs), which

correspond to the invasion of a homolog by only one DSB-end[2]. Discrete SEI-like signals were not detected during mitotic DSB-repair (see **Figure 2**).

b, Quantitation of meiotic inter-sister and inter-homolog JMs versus time after induction of meiosis via transfer to sporulation media.

c, Relative timing of inter-sister and inter-homolog JMs during. JMs are expressed as percent of maximum values against time following transfer to sporulation media.

Methods for analyzing meiotic recombination intermediates at the *HIS4LEU2* locus have been described[14].



Supplemental Figure S7. Recovery of JMs at *HIS4LEU2-SceI*.

a, Map of the *HIS4LEU2-SceI* locus showing flanking *Sac*I restriction sites and expected sizes of recombination intermediates formed between the corresponding *Sac*I fragments.

b, Images of native/native 2D gel analysis of *Sac*I digested DNA sample taken 1 hr after DSB-induction. The middle panel shows a magnification of the JM region and the right-hand panel is an interpretation of this image. P, parental *Sac*I fragments; D-loop, putative displacement loop intermediates; JM, Joint Molecules (inter-homolog and inter-sister species are not distinguished by *Sac*I digestion).

c, Images of native/native 2D gel analysis of *Xho*I digested DNA from the same sample as in **b**. The JM region is magnified in the right-hand panel and a black arrowhead highlights the inter-sister JM species. JM levels measured in **b** and **c** are 0.15% and 0.14% respectively. Thus, JMs are efficiently recovered within the *Xho*I fragments used in this study, i.e. JMs do not migrate beyond the *Xho*I sites in significant numbers.

Table S1. Strains used in this study.

Strain (description)	Genotype*
NHY 53 (haploid precursor)	*MAT**a** leu2::hisG ho::hisG ura3Δ(sma-pst)*
NHY 56 (haploid precursor)	*MATα leu2::hisG ho::hisG ura3Δ(sma-pst)*
MBY 249 (diploid, DSB on Dad)	*MAT**a** HIS4::LEU2"mom" lys2::URA3-pGAL1/10-SCEI* *MATα his4-X::LEU2"dad"(I-SceI) lys2::URA3-pGAL1/10-SCEI*
MBY 506 (haploid, DSB on Mom)	*MAT**a** HIS4::LEU2"mom"(I-SceI) lys2::URA3-pGAL1/10-SCEI*
MBY 514 (diploid, DSB on Mom)	*MAT**a** HIS4::LEU2"mom"(I-SceI) lys2::URA3-pGAL1/10-SCEI* *MATα his4-X::LEU2"dad" lys2::URA3-pGAL1/10-SCEI*
MBY 529 (*rad51* diploid, DSB on Mom)	*MAT**a** HIS4::LEU2"mom"(I-SceI) lys2::URA3-pGAL1/10-SCEI rad51Δ::hisG* *MATα his4-X::LEU2"dad" lys2::URA3-pGAL1/10-SCEI rad51Δ::hisG*
NHY 1876 (*sgs1* diploid, DSB on Mom)	*MAT**a** HIS4::LEU2"mom"(I-SceI) lys2::URA3-pGAL1/10-SCEI sgs1-ΔC795::hphMX4* *MATα his4-X::LEU2"dad" lys2::URA3-pGAL1/10-SCEI sgs1-ΔC795::hphMX4*

*All strains are homozygous for the mutations *leu2::hisG, ho::hisG* and *ura3Δ(sma-pst).*

References

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