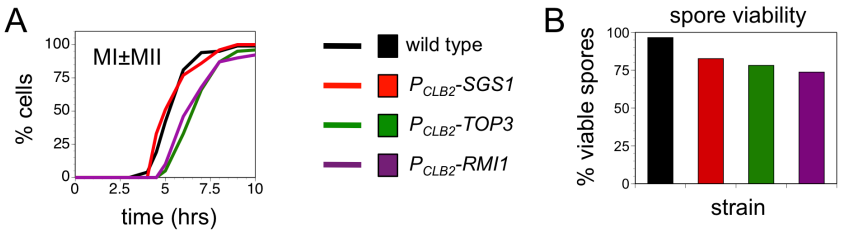

Figure S1. Timing and Efficiency of Meiotic Divisions, and Spore Viability in *P_{CLB2}-SGS1*, *P_{CLB2}-TOP3* and *P_{CLB2}-RMI1* Strains. Related to Figure 1. Shows the timing and efficiency of meiotic divisions, and spore viabilities for P_{CLB2} alleles of the STR genes.

(A) Timing and efficiency of meiotic divisions. MI±MII, cells that have completed one or both meiotic divisions.

(B) Spore viabilities. Fractions of dissected spores that are viable. Between 251 and 583 tetrads were dissected for each strain.

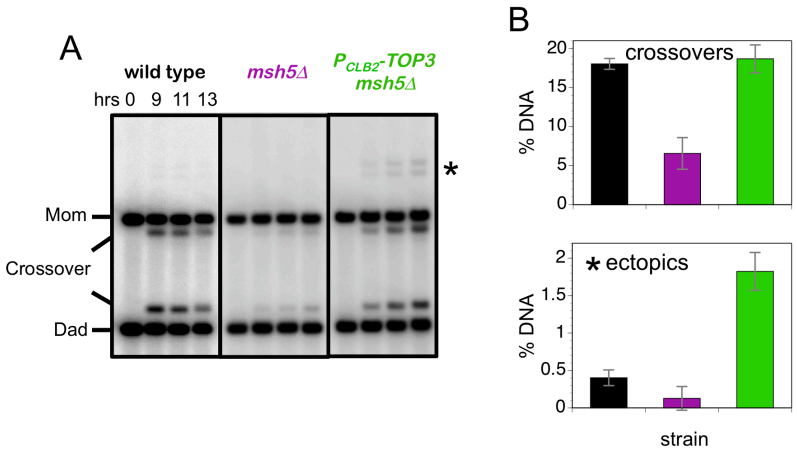

Figure S2. *P_{CLB2}-TOP3* Mutation Restores Crossing-Over in the *msh5*Δ Mutant. Related to Figure 3. Shows restoration of crossover levels in a *P_{CLB2}-TOP3 msh5* mutant.

(A) Representative Southern images of crossover analysis in indicated strains. Asterisk indicates ectopic crossover bands.

(B) Quantification of crossovers and ectopic recombination at 13hrs. Error bars show standard error.

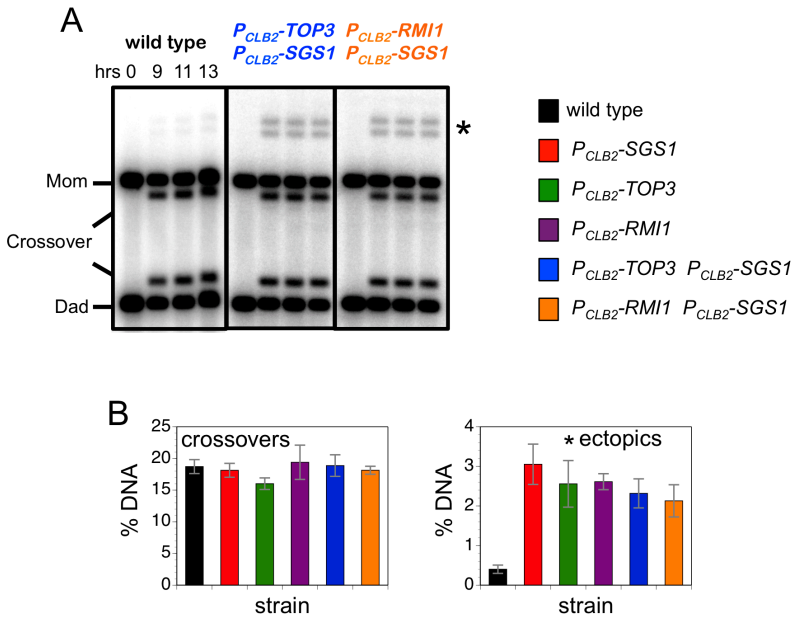

Figure S3. Meiotic Recombination in *P_{CLB2}-TOP3 P_{CLB2}-SGS1* and *P_{CLB2}-RMI1 P_{CLB2}-SGS1* Double Mutants. Related to Figure 3. Presents analysis of *P_{CLB2}-TOP3 P_{CLB2}-SGS1* and *P_{CLB2}-TOP3 P_{CLB2}-RMI1* double mutants.

(A) Representative Southern images of crossover analysis in indicated strains. Asterisk indicates ectopic crossover bands.

(B) Quantification of crossovers and ectopic recombination at 13hrs. Error bars show standard error. Data for *P_{CLB2}-SGS1, P_{CLB2}-TOP3* and *P_{CLB2}-RMI1* single mutants are from Figure 1E.

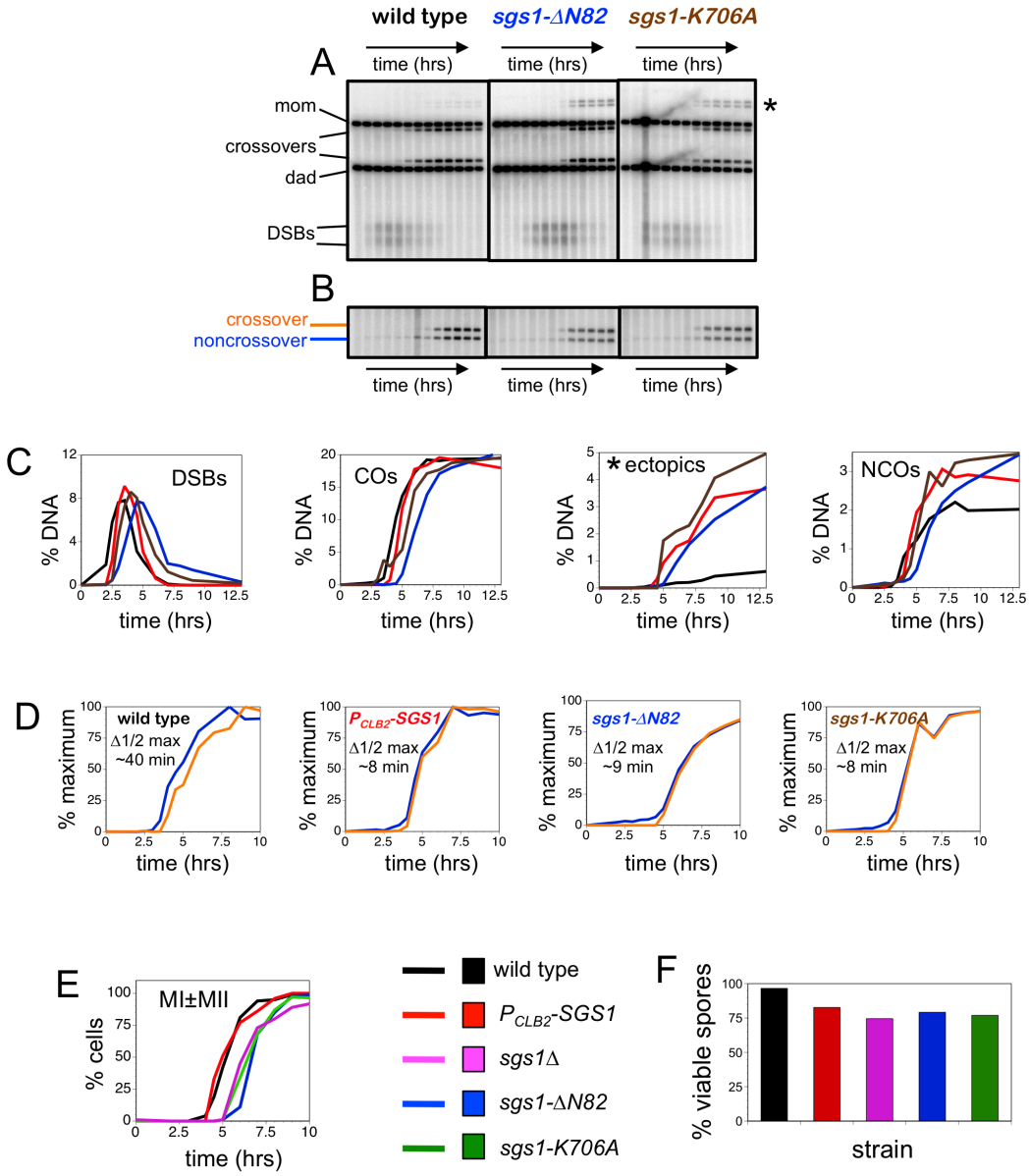

Figure S4. Sgs1-Top3 Interaction and Sgs1 Helicase Activity are Required to Suppress Aberrant Recombination During Meiosis. Related to Figure 3. Presents analysis of *sgs1* alleles defective for Top3 interaction and helicase activity.

(A) 1D Southern analysis of DSBs, allelic crossovers and ectopic crossovers (indicated by an asterisk). Time points are 0, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, and 13 hours.

(B) 1D Southern analysis to monitor noncrossover formation. Samples are from the same time courses shown in panel A.

(C) Quantitation of images shown in panels A and B. % DNA is percentage of total hybridizing DNA signal.

(D) Normalized curves to compare the timing of crossovers and noncrossovers. Δ1/2 max is the difference between the times of the half maximum values.

(E) Timing and efficiency of meiotic divisions. MI±MII, cells that have completed one of both meiotic divisions.

(F) Spore viabilities. Fractions of dissected spores that are viable. Between 100 and 568 tetrads were dissected for each strain.

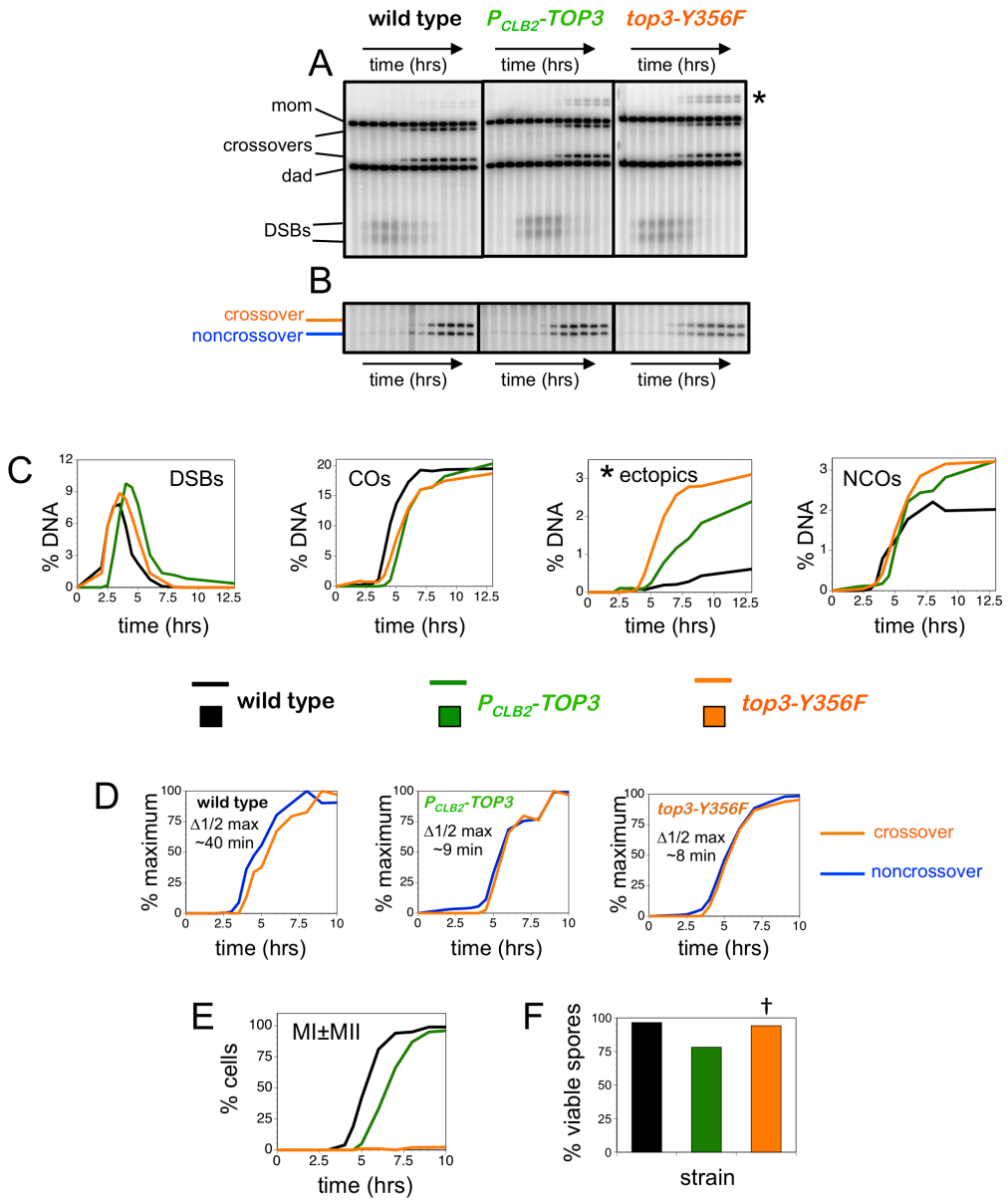

Figure S5. Comparison of Meiotic Recombination in *P_{CLB2}-TOP3* and *top3-Y356F* Strains.

Related to Figure 4. Shows additional analysis of *P_{CLB2}-TOP3* and *top3-Y356F* strains.

(A) 1D Southern analysis of DSBs, allelic crossovers and ectopic crossovers (indicated by an asterisk). Time points are 0, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, and 13 hours.

(B) 1D Southern analysis to monitor noncrossover formation. Samples are from the same time courses shown in panel A.

(C) Quantitation of images shown in panels A and B. % DNA is percentage of total hybridizing DNA signal.

(D) Normalized curves to compare the timing of crossovers and noncrossovers. ∆1/2 max is the difference between the times of the half maximum values.

(E) Timing and efficiency of meiotic divisions. MI±MII, cells that have completed one of both meiotic divisions.

(F) Spore viabilities. Fractions of dissected spores that are viable. Between 60 and 583 tetrads were dissected for each strain. † mature tetrad ascospores are very rare in sporulated *top3-Y356F* strains (<0.1% of all cells).

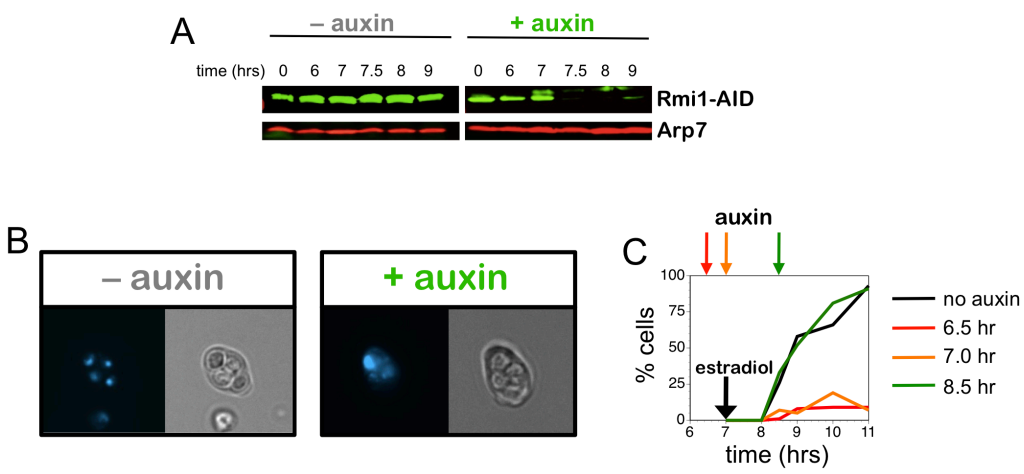

Figure S6. Rmi1 Acts Late in Meiotic Prophase to Promote Chromosome Separation.

Related to Figure 5. Presents analysis of nuclear divisions in strains carrying an Rmi1-AID degron allele. See Figure 5A for the experimental regimen.

(A) Western images showing Rmi1 levels in cells with and without addition of auxin at 7 hrs.

(B) Representative images of cells from subcultures with and without addition of auxin at 7 hrs and addition of estradiol at 7 hrs, sampled at 11 hrs. DAPI-stained and brightfield images of the same cells are shown.

(C) Quantification of nuclear divisions (2 to 4 nuclei) in subcultures with and without auxin treatment at the indicated times, following induction of Ndt80 expression at 7 hrs.

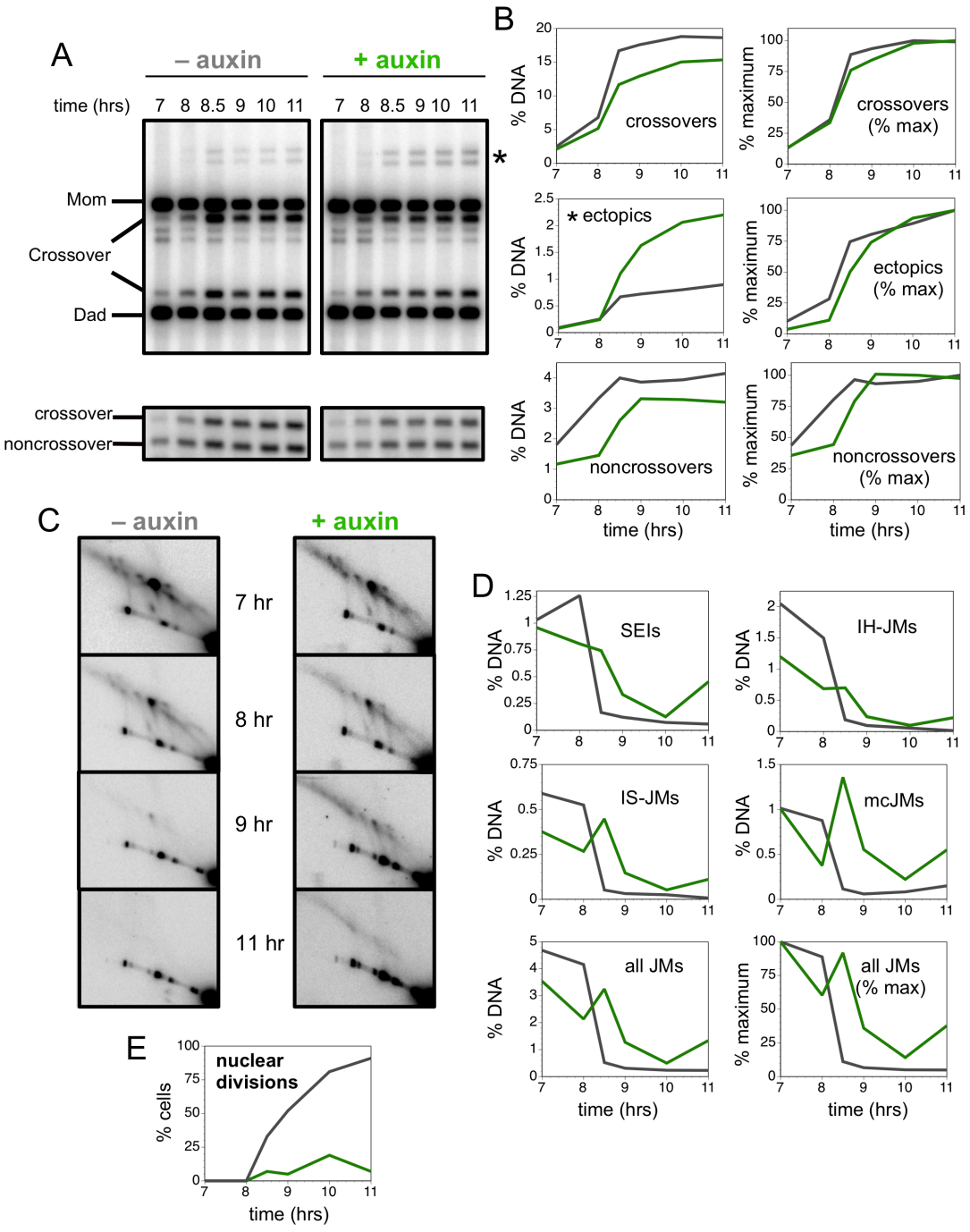

Figure S7. Late Action of Rmi1 is Required for Efficient Recombination and Suppression of Ectopic Crossovers. Related to Figure 6. Presents physical analysis of recombination in Rmi1-AID cells.

(A) Southern images of crossover (upper panels) and noncrossover (lower panels) analysis in cells with and without auxin treatment (Rmi1-AID degradation) at 7 hrs and induction of Ndt80 expression at 7 hrs (from same experiment as shown in **Supplemental Figure S6**).

(B) Quantification of allelic and ectopic crossovers, and noncrossovers from the experiment shown in panel A. Left panels show recombinants as percentage of total hybridizing DNA signal. Right panels show normalized curves to compare the timing of recombinants.

(C) Southern blot images of 2D JM analysis.

(D) Quantification of individual JM species, total JM levels and normalized JM levels in cells with and without auxin treatment.

(E) Quantification of nuclear divisions (2 to 4 nuclei) in subcultures with and without auxin treatment.

Genotype	PD:NPD:T	cM
wild type	132:3:102	26.61 ± 4.42
P_{CLB2}-SGS1	151:13:155	36.60 ± 0.25
P_{CLB2}-TOP3	139:19:114	39.53 ± 8.47
P_{CLB2}-RMI1	115:10:101	37.38 ± 7.60

Table S1. Genetic Map Distances of the *URA3–HIS4::LEU2* Interval Calculated From Tetrad Data. Related to Figure 1. Shows the tetrad data used to calculate genetic map distances and NPD ratios for the *URA3–HIS4::LEU2* interval.

Map distances and standard errors in centiMorgans (cM) were calculated from the distribution of parental ditype (PD), nonparental ditype (NPD) and tetratype (T) tetrads as described in Experimental Procedures. These data are presented in Figure 1.

Table S2. *S. cerevisiae* strains used in this study. Related to Experimental Procedures. Lists the full genotypes of yeast strains used in this study. See Excel document Table S2.