Supporting Information

Farah et al. 10.1073/pnas.0902793106

SI Materials and Methods

Construction of *ade6* Alleles with the I-*Sce*I Recognition Sequence. The 80-bp ds oligonucleotides oJF166 (containing the I-*Sce*I recognition sequence) and oJF167 (containing a single base-pair difference from the I-*Sce*I recognition sequence) were inserted into the BamHI site in the *ade6*$^+$ gene of plasmid pJF63 (1) [supporting information (SI) Table S2] to give pJF150 and pJF152, respectively. A HindIII fragment from pJF150 and pJF152 containing part of the *ade6* gene with the oligonucleotide insertion was used to transform strain GP3982 to Ade$^-$ (1) to give the *ade6-3061* and *ade6-3062* alleles, respectively. Construction of the *rec12-172*::*I-SceI* allele, which directs synthesis of the I-*Sce*I endonuclease by the meiosis-specific *rec12* promoter (2, 3), was described earlier (4).

Meiotic Crosses. Single colonies were transferred to 5 ml of supplemented yeast extract liquid medium (YEL + 5S) (5) and grown at 30°C until saturated. Equal volumes (0.5 ml) of each parental culture were mixed, and the cells were washed in 0.85% NaCl and spotted on supplemented sporulation agar plates (6). The plates were incubated for 3 to 4 days at 25°C, after which the cell-ascus mixture was suspended in 1 ml of H_2O and treated with glusulase and ethanol to kill vegetative cells, essentially as described (7).

For *ade6* intragenic recombinant frequency measurements, spores were plated on supplemented yeast extract agar plates (YEA + 4S) (5) to determine the total viable spore titer and on YEA + 4S + guanine (100 μg/ml) (8) to determine the viable Ade$^+$ recombinant titer. Plates were incubated at 32°C for 3 to 4 days before counting the colonies. Reported values are the means of 3 to 36 independent experiments ± SEM.

For intergenic recombination measurements (Fig. 4), individual spore colonies were gridded onto YEA + guanine (selected Ade$^+$ colonies) or YEA + adenine (total colonies) and incubated for 3 days at 25°C. The plates were replica-plated onto properly supplemented nitrogen-base minimal agar plates (9) to score *ura4A*$^+$ and onto supplemented YEA + phloxin B plates (20 μg/ml) (10) at 37°C to score *tps16*. Ura$^+$ Tps16$^+$ diploids (< 5% of the total), detected as large cells by microscopic analysis (*mus81*$^+$ crosses) or by segregation of Ura$^-$ colonies (*mus81*Δ crosses), were omitted from the analysis.

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2. Mata J, Lyne R, Burns G, Bahler J (2002) The transcription program of meiosis and sporulation in fission yeast. *Nat Genet* 32:143–147.
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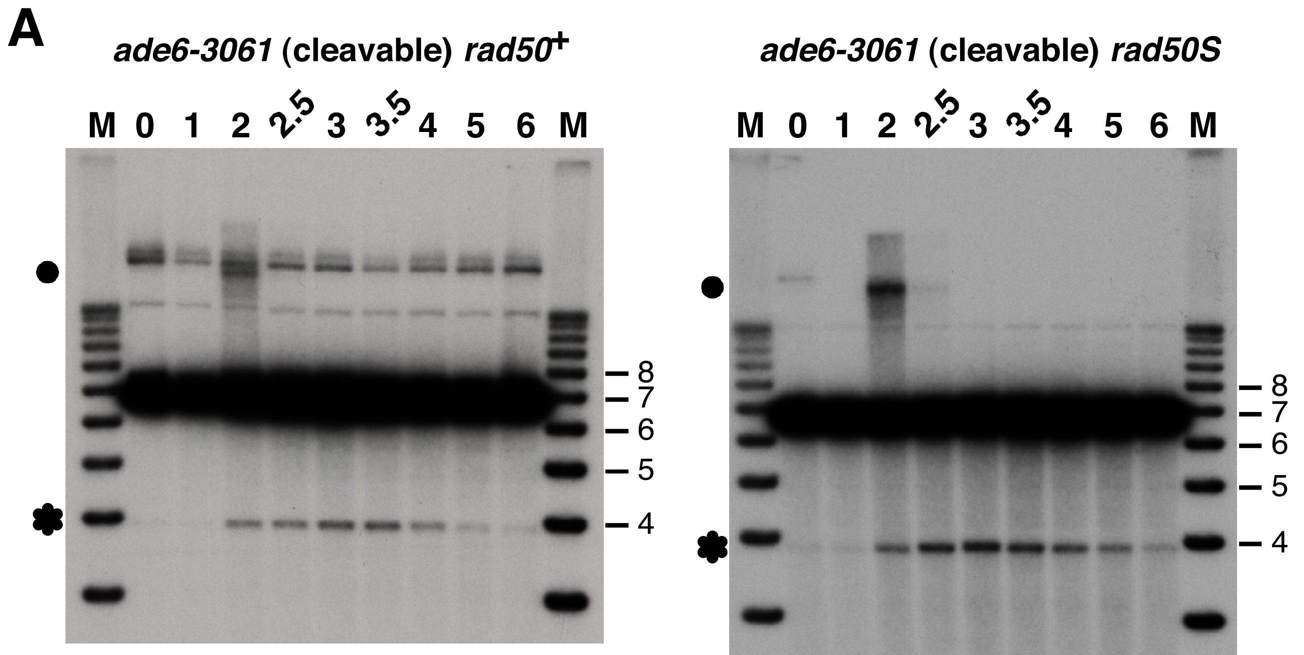

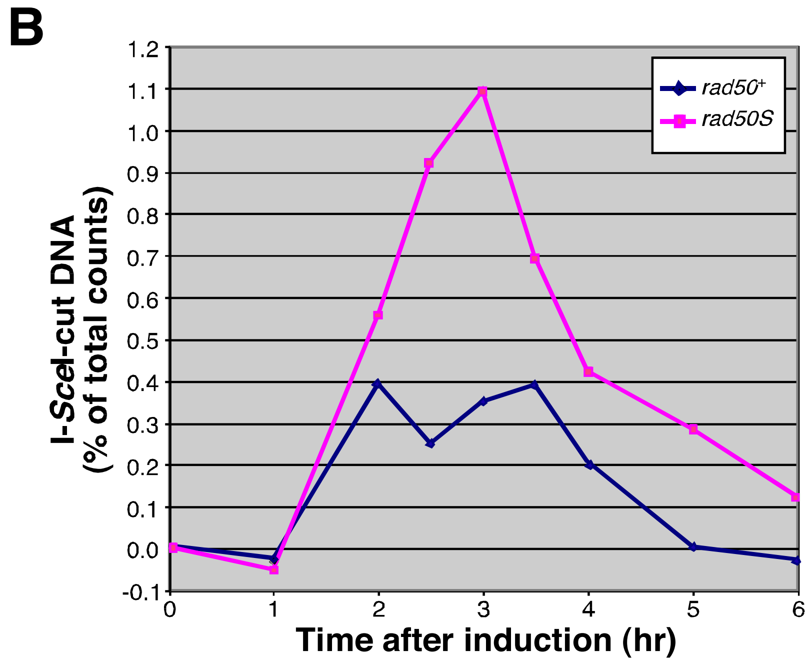

Fig. S1. (*A* and *B*) Repeat of the experiment in Fig. 3. See legend to Fig. 3 for details. There are no data for the 7-h time-point in this experiment.

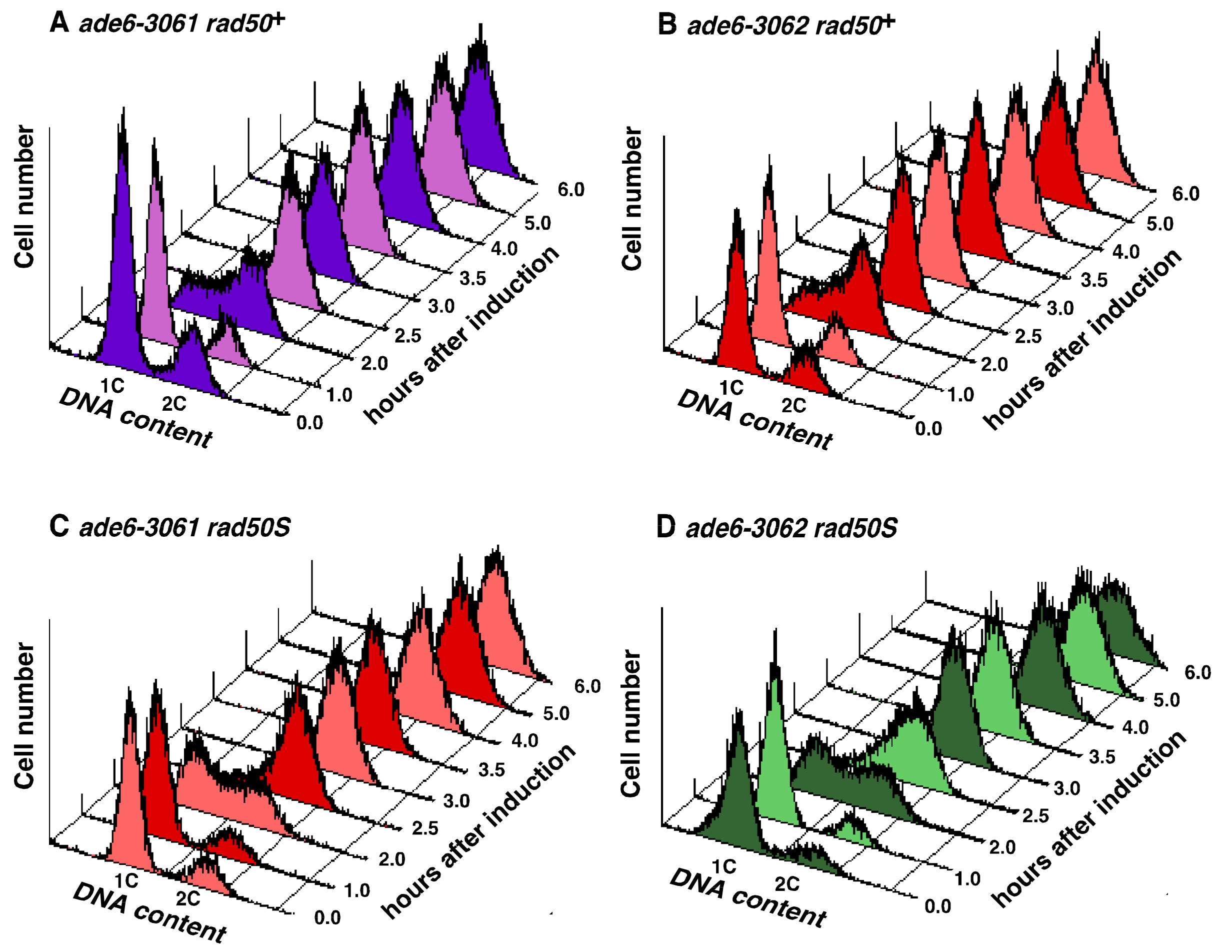

Fig. S2. (*A–D*) Bulk DNA content of synchronized *S. pombe* cells. Samples of cells from the strains meiotically induced in Fig. 3 and Fig. S3 were analyzed by flow cytometry for DNA content at the indicated times after induction. DNA replication occurred at ≈2 h in each case.

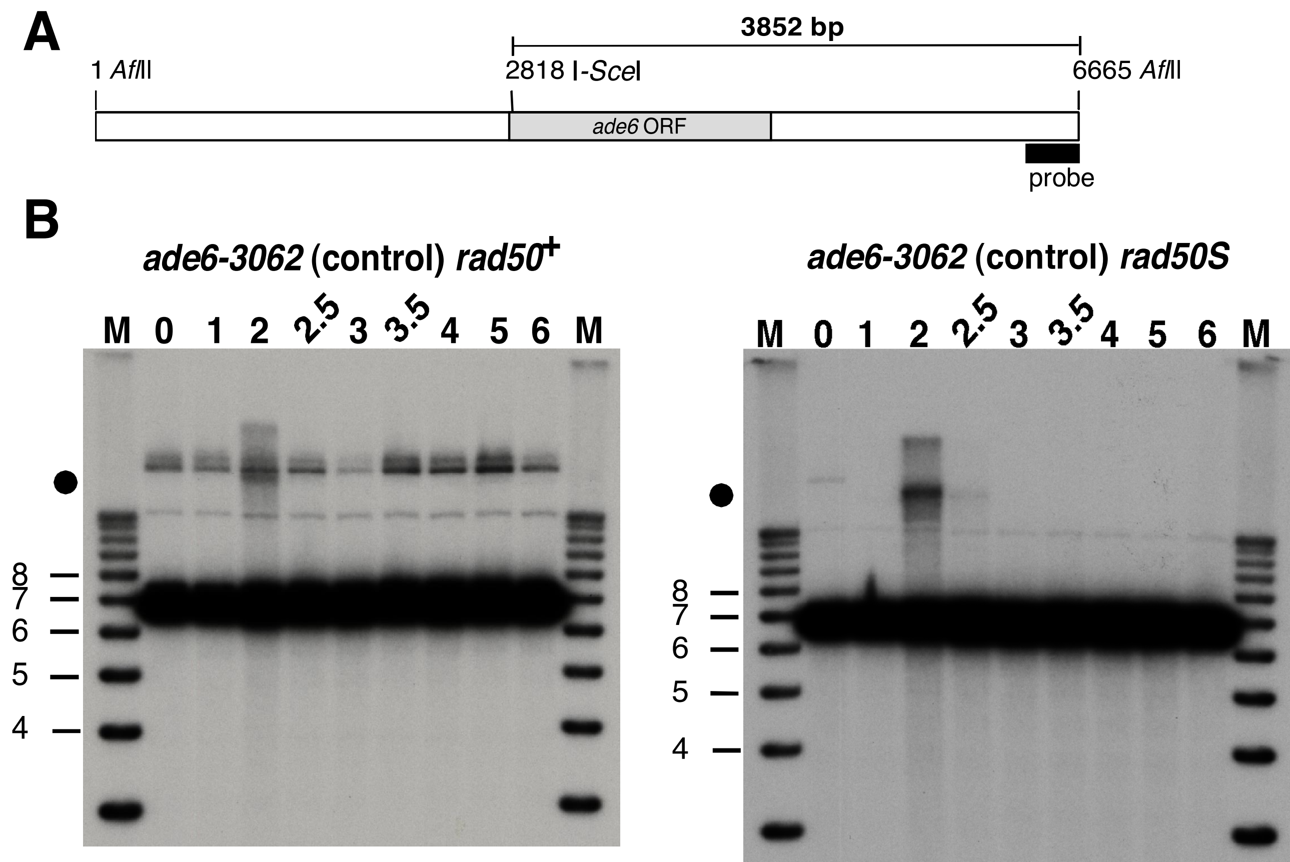

Fig. S3. DSBs are not detected at *ade6* in the presence of a mutated I-*Sce*I cut site. (*A*) Diagram of the AflII fragment on chromosome 3 containing *ade6* with the location of the probe used in (*B*). (*B*) DNA was prepared at the indicated time points (h) from strains induced for meiosis and digested with AflII. Digested DNA was separated on an agarose gel, blotted, and probed from the right as indicated in (*A*). The bullet indicates the position of replication intermediates at 2 h (see Fig. S2). (*Left*) Strain GP6314 (*ade6-3062 rad50*$^{+}$ *rec12-172*::*I-SceI*). (*Right*) Strain GP6310 (*ade6-3062 rad50S rec12-172*::*I-SceI*). Size markers (kb) are in lane M.

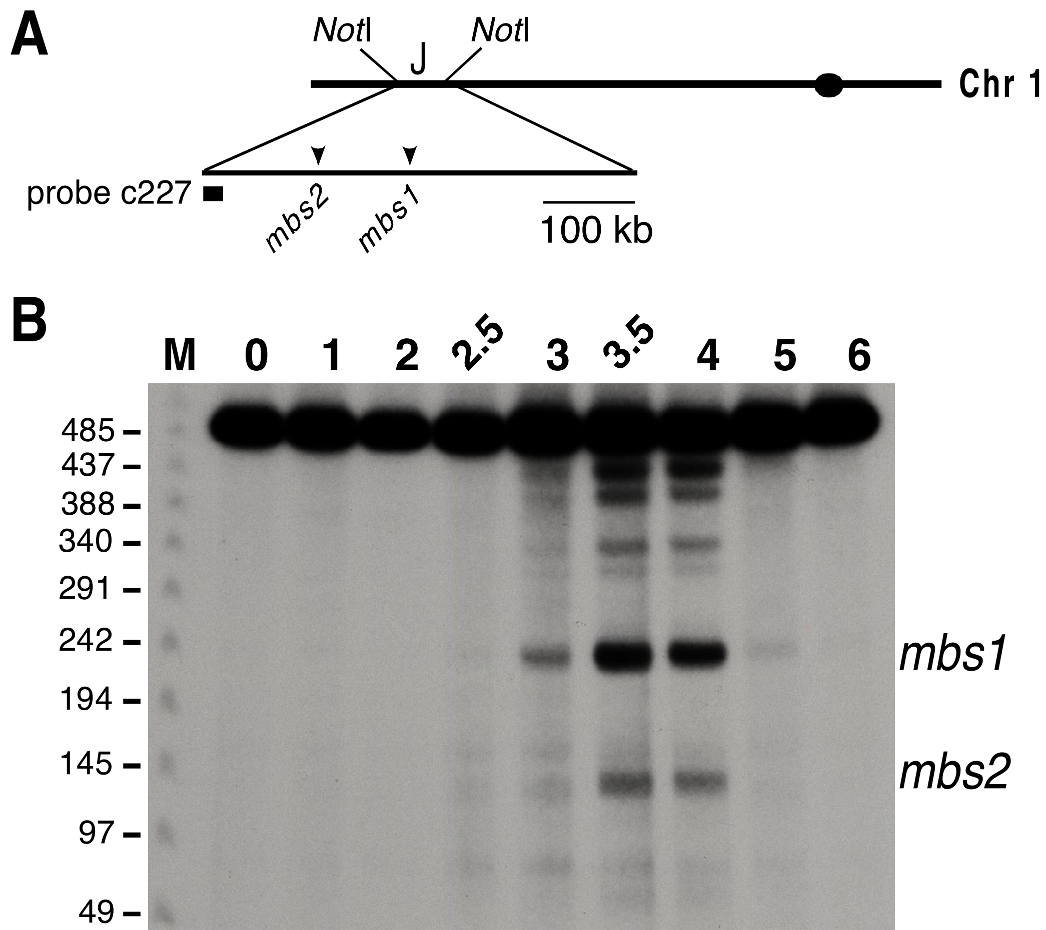

Fig. S4. Timing of the appearance and disappearance of Rec12-dependent meiotic DSBs. (*A*) Diagram of chromosome 1 with the NotI fragment J containing Rec12-dependent meiotic DNA break sites *mbs1* and *mbs2* [Young et al. (2002)]. The location of the probe used in (*B*) is indicated by a filled square. The centromere is shown as a filled circle. (*B*) DNA from strain GP6510 (h^+ *ade6-3036 pat1–114*) was prepared at the indicated time points upon meiotic induction and digested with NotI. Digested DNA was separated by pulsed-field gel electrophoresis, blotted, and probed from the left as indicated in (*A*). Size markers (kb) are in lane M.

Table S1. Low recombinant frequencies in strains with I–SceI noncuttable allele *ade6-3062*

exo1	*rad32*	*ctp1*	Recombinant frequency*	
			rad50^{+}	*rad50*Δ
+	+	+	6.1 ± 0.8 (21)	11 ± 3.1 (6)
Δ	+	+	6.7 ± 1.9 (6)	$<$18 (3)
+	*D65N*	+	7.7 ± 0.8 (6)	—
Δ	*D65N*	+	10 ± 2.6 (3)	—
+	+	Δ	$<$10 (4)	—
Δ	+	Δ	$<$5 (4)	—

*Ade^{+}/million viable spores in crosses of *ade6-3062* (I-SceI cut site) × *ade6-52*, as mean ± SEM of (*n*) crosses. Values marked with "$<$" are the upper 95% confidence limit based upon the Poisson distribution when no Ade^{+} colonies were observed in the samples plated. —, not determined.

Table S2. Oligonucleotides

Name	Sequence
oJF162	5′GATCGCATAGTCGGGTTTTTCTTTTAGTTTCAGC**TAGGGATAA** ↓ **CAGGGTAAT**TTTATAAACCCTGGTTTTGGTTTTGTAG 3′
oJF163	5′GATCCTACAAAACCAAAACCAGGGTTTATAAA**ATTACCCTGTTAT** ↓ **CCCTA**GCTGAAACTAAAAGAAAAACCCGACTATGC 3′
oJF164	5′GATCGCATAGTCGGGTTTTTCTTTTAGTTTCAGC**TAGGGA** ***G*****AACAGGGTAAT**TTTATAAACCCTGGTTTTGGTTTTGTAG 3′
oJF165	5′GATCCTACAAAACCAAAACCAGGGTTTATAAA**ATTACCCTGTT*C*TCCCTA**GCTGAAACTAAAAGAAAAACCCGACTATGC 3′

Oligonucleotides oJF162 and oJF163 were annealed to give oJF166, and oligonucleotides oJF164 and oJF165 were annealed to give oJF167; the double-strand oligonucleotides have BamHI compatible ends. For annealing, equimolar amounts were mixed in a buffer containing 100 mM NaCl, 50 mM $MgCl_2$, and 10 mM Tris·HCl (pH 8.0), heated at 85 °C for 5 min, and cooled at 25 °C for at least 20 min. Salts were removed by precipitation with ethanol in the presence of Pellet Paint (Novagen). The 18-nucleotide I-SceI recognition sequence or its noncleavable version is indicated in bold in each oligonucleotide. The vertical arrow indicates the cleavage position, and the underlined sequence, the 3′ overhang that results upon cleavage (11). The italic nucleotides in oJF164 and oJF165 represent the T→G transversion in the overhang of the wild-type sequence, resulting in a non-cleavable site (11).

Table S3. Similar gene conversion frequencies in wild type and *rad13*Δ and *rad16*Δ mutants

	Ade⁺ recombinants per 10^6 viable spores		
Mutant*	Expt. 1	Expt. 2	Mean
+	3,200	4,400	3,800
*rad13*Δ	4,900	2,700	3,800
*rad16*Δ	4,700	3,700	4,200
*rad13*Δ *rad16*Δ	3,400	3,300	3,400

*The *rad13*::*ura4*$^+$ *and rad16*::*kanMX6* mutations were in strains with the *ade6-M26* or *ade6-52* mutation.

Table S4. ***S. pombe*** **strains**

Strains	Genotype
Strains for genetic crosses	
GP1456	h^- *ade6-52 leu1-32 ura4-294 rec12-152::LEU2*
GP3982	h^+ *leu1-32 ura4-D18 rec12-172::I-SceI*
GP5157	h^+ *ade6-3061 leu1-32 ura4-D18 his3-D1 rad16::ura4*$^+$ *rec12-172::I-SceI*
GP5158	h^+ *ade6-3062 leu1-32 ura4-D18 his3-D1 rad16::ura4*$^+$ *rec12-172::I-SceI*
GP5161	h^-_{smt0} *ade6-52 leu1-32 ura4-D18 his3-D1 rad16::ura4*$^+$ *rec12-172::I-SceI*
GP5162	h^+ *ade6-3061 leu1-32 ura4-D18 rad32-D65N rec12-172::I-SceI*
GP5166	h^-_{smt0} *ade6-52 leu1-32 ura4-D18 rad32-D65N rec12-172::I-SceI*
GP5194	h^+ *ade6-3061 arg1-14 leu1-32 ura4-D18 mus81::kanMX6 rec12-172::I-SceI*
GP5195	h^+ *ade6-3061 arg1-14 leu1-32 ura4-D18 rec12-172::I-SceI*
GP5196	h^- *ade6-52 ura4A*$^+$ *leu1-32 ura4-D18 rec12-172::I-SceI tps16-23*
GP5197	h^- *ade6-52 ura4A*$^+$ *leu1-32 ura4-D18 rec12-172::I-SceI tps16-23 mus81::kanMX6*
GP5200	h^+ *ade6-3061 leu1-32 ura4-D18 his3-D1 rec12-172::I-SceI*
GP5201	h^-_{smt0} *ade6-3061 leu1-32 ura4-D18 rec12-172::I-SceI*
GP5202	h^+ *ade6-3061 leu1-32 ura4-D18 rec12-172::I-SceI*
GP5203	h^+ *ade6-3062 leu1-32 ura4-D18 his3-D1 rec12-172::I-SceI*
GP5205	h^+ *ade6-3062 leu1-32 ura4-D18 rec12-172::I-SceI*
GP5689	h^+ *ade6-3061 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *rec12-172::I-SceI*
GP5692	h^+ *ade6-3062 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *rec12-172::I-SceI*
GP5804	h^+ *ade6-3061 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *rad50::kanMX6 rec12-172::I-SceI*
GP5805	h^+ *ade6-3062 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *rad50::kanMX6 rec12-172::I-SceI*
GP5807	h^-_{smt0} *ade6-52 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *rad50::kanMX6 rec12-172::I-SceI*
GP5819	h^+ *ade6-3061 leu1-32 ura4-D18 rad50::kanMX6 rec12-172::I-SceI*
GP5821	h^+ *ade6-3062 leu1-32 ura4-D18 rad50::kanMX6 rec12-172::I-SceI*
GP5823	h^-_{smt0} *ade6-52 leu1-32 ura4-D18 rad50::kanMX6 rec12-172::I-SceI*
GP5845	h^-_{smt0} *ade6-52 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *rec12-172::I-SceI*
GP6470	h^+ *ade6-3061 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *rad32-D65N rec12-172::I-SceI*
GP6472	h^+ *ade6-3062 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *rad32-D65N rec12-172::I-SceI*
GP6475	h^-_{smt0} *ade6-52 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *rad32-D65N rec12-172::I-SceI*
GP6497	h^+ *ade6-3062 leu1-32 ura4-D18 rad32-D65N rec12-172::I-SceI*
GP6569	h^+ *ade6-3061 leu1-32 ura4-D18 ctp1::kanMX6 rec12-172::I-SceI*
GP6573	h^- *ade6-3061 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *ctp1::kanMX6 rec12-172::I-SceI*
GP6575	h^- *ade6-52 leu1-32 ura4-D18 ctp1::kanMX6 rec12-172::I-SceI*
GP6577	h^+ *ade6-52 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *ctp1::kanMX6 rec12-172::I-SceI*
GP6581	h^- *ade6-52 leu1-32 ura4-D18 exo1-1::ura4*$^+$*rec12-172::I-SceI*
GP6582	h^+ *ade6-52 leu1-32 ura4-D18 rec12-172::I-SceI*
GP6627	h^- *ade6-3061 leu1-32 ura4-D18 ctp1::kanMX6 rad50::kanMX6 rec12-172::I-SceI*
GP6628	h^- *ade6-3061 leu1-32 ura4-D18 ctp1::kanMX6 rad50::kanMX6 rec12-172::I-SceI*
GP6629	h^- *ade6-3061 leu1-32 ura4-D18 ctp1::kanMX6 rad50::kanMX6 rec12-172::I-SceI*
GP6630	h^+ *ade6-52 leu1-32 ura4-D18 ctp1::kanMX6 rad50::kanMX6 rec12-172::I-SceI*
GP6631	h^+ *ade6-52 leu1-32 ura4-D18 ctp1::kanMX6 rad50::kanMX6 rec12-172::I-SceI*
GP6632	h^+ *ade6-52 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *ctp1::kanMX6 rad50::kanMX6 rec12-172::I-SceI*
GP6633	h^+ *ade6-52 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *ctp1::kanMX6 rad50::kanMX6 rec12-172::I-SceI*
GP6636	h^+ *ade6-52 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *ctp1::kanMX6 rad50::kanMX6 rec12-172::I-SceI*
GP6638	h^- *ade6-3061 leu1-32 ura4-D18 exo1-1::ura4*$^+$ *ctp1::kanMX6 rad50::kanMX6 rec12-172::I-SceI*
Strains for meiotic induction and physical analysis	
GP6306	h^-_{smt0} *ade6-3061 rad50S rec12-172::I-SceI pat1-114*
GP6308	h^-_{smt0} *ade6-3061 rec12-172::I-SceI pat1-114*
GP6310	h^-_{smt0} *ade6-3062 rad50S rec12-172::I-SceI pat1-114*
GP6314	h^-_{smt0} *ade6-3062 rec12-172::I-SceI pat1-114*
GP6510	h^+ *ade6-3036 pat1-114*

Crosses are in Table 1 and Table S1 and Figs. 2 and 4. Genealogies are available upon request. Origins of alleles other than mating type and commonly used auxotrophic mutations are: *ade6-3061* and *ade6-3062* (*Materials and Methods*); *ade6-3036* (1); *exo1-1::ura4*$^+$ (2); *mus81::kanMX6* (3); *pat1-114* (4); *rad16::ura4*$^+$ (5); *rad32-D65N* (6); *rad50S* (1); *rad50::kanMX6* (7); *rec12-152::LEU2* (8); *rec12-172::I-SceI* (9), *ctp1::kanMX6* (10); *ura4A*$^+$ (11); *tps16-23* (12).

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2. Szankasi P, Smith GR (1995) A role for exonuclease I from *S. pombe* in mutation avoidance and mismatch correction. *Science* 267:1166–1169.
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