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Saw1 Localizes to Repair Sites but is not Required for Recruitment of Rad10 to Repair Intermediates Bearing Short Non-Homologous 3' Flaps during Single-Strand Annealing in *S. cerevisiae*

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Abstract

SAW1 is required for efficient removal by the Rad1-Rad10 nuclease of 3' non-homologous DNA ends (flaps) formed as intermediates during two modes of double-strand break repair in *S. cerevisiae*, single-strand annealing (SSA) and synthesis-dependent strand annealing (SDSA). Saw1 was shown *in vitro* to bind flaps with high affinity, but displayed diminished affinity when flaps were short (< 30 deoxynucleotides [nt]), consistent with it not being required for short flap cleavage. Accordingly, this study, using *in vivo* fluorescence microscopy showed that *SAW1* was not required for recruitment of Rad10-YFP to DNA double-strand breaks (DSBs) during their repair by SSA when the flaps were ~10 nt. In contrast, recruitment of Rad10-YFP to DSBs when flaps were ~500 nt did require *SAW1* in G1 phase of cell cycle. Interestingly, we observed a substantial increase in colocalization of Saw1-CFP and Rad10-YFP at DSBs when short flaps were formed during repair, especially in G1, indicating significant recruitment of Saw1 despite there being no requirement for Saw1 to recruit Rad10. Saw1-CFP was seldom observed at DSBs without Rad10-YFP. Together these results support a model in which Saw1, and Rad1-Rad10 are recruited as a complex to short and long flaps in all phases of cell cycle, but that Saw1 is only required for recruitment of Rad1-Rad10 to DSBs when long flaps are formed in G1.

Keywords

Saw1; Rad10; Double-strand break repair; Single-strand annealing

Introduction

DSB repair pathways are genetically complex with many genes sharing overlapping functions or functioning in more than one subpathway [1–3]. Synthesis-dependent strand annealing (SDSA) normally repairs DSBs, but single-strand annealing (SSA) is an alternate pathway that can repair DSBs occurring between direct repeats that appear throughout the genome [4]. In SSA, DSBs between repeats are resected in a 5' → 3' fashion to create 3'

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single-strands that can be annealed by Rad52 in a process that is generally *RAD51*-independent [5]. Unique DNA sequence originally located between the repeats becomes two overhanging 3'-flaps that are cleaved by Rad1-Rad10 nuclease prior to ligation, thereby permanently eliminating the unique sequences and one repeat [6]. In higher eukaryotes, numerous diseases are associated with such DNA repeat instability, including breast cancer and acute myeloid leukemia [7,8].

In *Saccharomyces cerevisiae* SSA requires *SAW1*, mutation of which was found to bear epistatic relationships with other mutations that affect SSA, such as *rad1*, *slx4*, *msh2* and *rad52* among others [9]. Saw1 protein interacts physically *in vitro* with Rad1, Msh2, Msh3, Rad52, Rad51 and Slx4 [9,10]. Analysis by chromatin immunoprecipitation revealed that *SAW1* is required to recruit Rad1 to SSA repair sites [11]. Saw1 has intrinsic affinity for flap and splayed arm DNAs, and binding of Saw1 to Rad1-Rad10 increases the affinity of Rad1-Rad10 for 3'-flaps [11]. Further, *SAW1* is required for Rad10 recruitment during SDSA [12], which, like SSA, requires Rad1-Rad10 [13].

Prior evidence suggests Saw1 functions primarily at flaps longer than 30 nt since *in vitro* Saw1 shows diminished affinity for 3'-flaps shorter than ~30 deoxynucleotides (nt), and promotes binding of Rad1-Rad10 to flaps 30 nt or longer [11]. However, Rad1-Rad10 binds 3'-flaps *in vitro* without Saw1 [14,15]. Plasmid-based assays showed a reduced requirement for both *SAW1* and *RAD1* when flaps are short [9,16]. Intriguingly, in strains where SSA requires processing of 20 nt flaps the *saw1* mutant displayed a slightly higher SSA efficiency than the *rad1* mutant, while efficiency in a *saw1 rad1* double mutant indicated that *rad1* is epistatic to *saw1* [9]. These reports show Saw1 facilitates binding of Rad1-Rad10 to 3'-flaps, but suggest this function cannot be carried out when flaps are short, and that Rad1-Rad10 can function without Saw1.

Additionally, there is genetic evidence for a separate mechanism for removal of short flaps as removal of flaps of 10 or fewer nt is synergistically reduced in the *rad1 pol3-01* double mutant compared with the *pol3-01* or *rad1* single mutants, although the efficiency in *pol3-01* is only slightly higher than in *rad1 pol3-01* [16]. This supports a model in which flaps of ~10 nt or fewer are largely removed by the exonuclease activity of DNA polymerase δ instead of Rad1-Rad10, but that polymerase δ cannot subserve Rad1-Rad10 function in every context. Since Saw1 is implicated in recruitment of Rad1-Rad10 to flaps, but Saw1 binds poorly to short 3'-flaps that Rad1-Rad10 can repair to a limited extent, it is of interest to determine *in vivo* the extent to which Rad1-Rad10 is recruited to short flaps and the extent to which recruitment is *SAW1*-dependent. We demonstrate *in vivo* that Saw1 localizes to SSA intermediates with Rad1-Rad10, but that the extent of the requirement of Saw1 for Rad10 recruitment is influenced by 3'-flap length and phase of the cell cycle.

Materials and Methods

Cloning of yeast strains containing SSA substrates giving short or long flaps

HIS3 (663 bp) genes were integrated immediately flanking the I-*SceI* site in YER186C in strain PF025-7A (Table 1) using adaptamer-mediated PCR and standard gene transplacement (Fig. S1). Transformants were selected on Synthetic Complete agar lacking

histidine (SC-his agar), screened by PCR, and sequenced for the *HIS3::I-SceI::HIS3* cassette which contained no mutations. The resulting strain (PF177, “short flap” strain) was crossed with PF148-17D, PF150-74A, LSY401 and W4259-3B giving rise to PF160-13B, PF158-7D, PF179-9A and PF156-16C, respectively. PF193-30A derived from the LSY401 cross and represents an outcrossed short flap wild-type strain. PF177, PF160-13B, PF158-7D, PF179-9A and PF156-16C were used in microscopy experiments. The presence of *rad52::HIS5* was confirmed by sensitivity to gamma-irradiation (400 Gy).

“Long flap” strains were prepared analogously but contain 500 bp of endogenous chromosomal DNA sequence on each side of *I-SceI* but between the *HIS3* repeats (Fig. S1). The originally isolated transformant (PF178) was crossed with PF148-17D, PF229, LSY401 and LSY715 giving rise to PF196-4B, PF230-68B, PF187-23A and PF189-17A, respectively. PF191-41C derived from the LSY401 cross and represents an outcrossed long flap wild-type strain. To confirm there were no phenotypic anomalies in the originally transformed short and long flap strains, microscopy controls were conducted comparing them to isogenic outcrossed isolates (PF193-30A and PF191-41C), which showed no differences between the original and outcrossed strains (Fig. S2).

Microscopy

Strains bear chromosomally integrated copies of the Tetracycline repressor fused with RFP (TetR-RFP) and 224 tandem copies of the Tetracycline operator (tetO) near the *I-SceI* restriction site at iYER186 as described [17]. The site is cut by *I-SceI* with 60–70% efficiency in asynchronously growing cells [18,17]. Both the Rad10-YFP and Saw1-CFP genes have been shown to be functional, exhibiting efficiencies of SSA and SDSA that are comparable to wild-type strains in a plasmid-based DSB repair assay [12,19]. Strains were transformed with plasmid pWJ1320, containing the *I-SceI* gene under control of the *GAL1* promoter and an *ADE2* selection marker as described [20]. Transformants were plated on SC-ade medium containing 2% raffinose for selection and subsequently propagated in SC-ade with raffinose liquid medium at 23 °C. Overnight cultures were diluted to 0.1 OD₆₀₀ and incubated (3 h). DSBs were induced by adding galactose to 2% (w/v) and incubating the cultures (30 min) prior to preparation for microscopy as described [12].

Microscopy was performed on a Zeiss AxioImager M1 microscope outfitted as described [12]. Integration time was (800 ms, Rad10-YFP) and (400 ms, TetR-RFP) for experiments in which only YFP and RFP were being imaged. Integration times were (400 ms, Rad10-YFP), (200 ms, Saw1-CFP) and (200 ms, TetR-RFP) for experiments with YFP, CFP and RFP. Foci were counted by inspecting images from each focal plane of the Z-stack contrast enhanced as described [12]. Cells were classified as “G1” if they contained two nuclei which had separated to opposite sides of a dividing cell, or that contained only one nucleus and no bud. The presence of the bud was determined from the Differential Interference Contrast (DIC) images; the location of the nucleus was determined by the presence of the Rad10-YFP pan-nuclear signal. Cells were classified as “S/G2” if they contained a bud and only one nucleus. Cells were classified as “M” if they contained a bud and two nuclei that were in contact with each other. Unless otherwise noted, at least 100 cells were analyzed per experimental condition per trial. Graphs report percentages deriving from sums of foci

counts from three or more independent trials and were normalized to differing background foci counts from differences in visual acuity between researchers analyzing the data. Unless otherwise noted, error bars represent exact binomial confidence intervals at the 95% confidence level. Unless otherwise noted Fisher's exact test was applied (one-tailed) to key data comparisons and significant differences are indicated ("n.s." indicates $0.05 < P$, "*" indicates $0.01 < P < 0.05$, "**" indicates $0.001 < P < 0.01$; "****" indicates $P < 0.001$). Images were prepared using Adobe Photoshop and Illustrator (Adobe Systems, Mountain View, CA).

Quantitative PCR

PF177, PF160-13B, PF178 and PF196-4B were cultured and DSB-induced as described for microscopy. At intervals, aliquots (3 mL) were harvested and genomic DNA extracted using standard protocols, storing samples at -20°C . DNA was subjected to quantitative PCR (qPCR) using the RealMasterMix Fast SYBR kit (5 PRIME) on a SmartCycler II system (Cepheid) using manufacturer's instructions (version 2.0d software), but scaling reactions to 25 μL . A 3-step cycling protocol was used: 1 cycle (95°C , 2 min), 45 cycles [$(95^{\circ}\text{C}$, 15 sec), (65°C , 25 sec), (72°C , 30 sec)], 1 cycle (72°C , 2 min). Optical reads were acquired during annealing and product purity assayed by melt curve and agarose gel electrophoresis. Short flap substrates were amplified with primers 5'-d(GAGTCACTGCCAGGTATCGT) and 5'-d(CGAAGCAGTGTATGAGCACAGTGAG) giving a 1182 bp product. Long flap substrates were amplified using primers 5'-d(GCAGGCAAGATAAACGAAGGCAAAG) and 5'-d(CTTACTATCCTTTCCCAGAACGAAATC) giving a 716 bp product. Results were compared to a *SER3* reference amplified with primers 5'-d(TGAAGCCTTTCTCAACGGG) and 5'-d(GCACAGTGTTCTCTTGGTCG) giving a 1042 bp product. Primer efficiencies were established via standard means [21]. Averaged C_q values were used to calculate relative copy numbers using the Livak 2^{-C_q} method [21]. Error bars represent standard deviations of C_q averages error-propagated according to standard methods [22]. P-values were calculated using a T-test (GraphPad Prism 6.0). Key data comparisons and significant differences are indicated ("n.s." indicates $0.05 < P$, "*" indicates $0.01 < P < 0.05$, "**" indicates $0.001 < P < 0.01$; "****" indicates $P < 0.001$).

Results

Recruitment of Rad10 to SSA repair sites does not require SAW1 when 3'-flaps are short

To determine whether the genetic requirement for *SAW1* in recruitment of Rad1-Rad10 to SSA sites depends on the lengths of the 3'-flaps, we prepared strains of *S. cerevisiae* containing an inducible DSB site flanked by *HIS3* gene sequences (663 bp). Flanking *HIS3* repeats were either abutted with the *I-SceI* site (short flap), or spaced 500 nt away (long flap) (Fig. S1). In short flap substrates, the *I-SceI* cleavage site itself becomes the 3' overhanging flaps of ~10 nt, whereas in long flap substrates, flaps are ~500 nt. Strains also contained a fluorescently labeled *RAD10* gene (Rad10-YFP) and a DSB site near a DNA array constitutively bound by fluorescently-labeled TetR-mRFP (DSB-RFP) [19]. Strains were either wild-type or *saw1* deletion mutants.

Results from DSB induction experiments show Rad10-YFP is recruited to DSB-RFP repair sites similarly in wild-type and *saw1* short flap strains (Fig. 1a). Following DSB formation we observed a Rad10-YFP/DSB-RFP colocalized focus in 19% of dividing (S/G2/M) wild-type cells and in 13% of dividing *saw1* cells with background levels of only ~5.3% in each of the corresponding uninduced controls. Similarly, we observed a colocalized focus in 19% of DSB-induced non-dividing (G1) wild-type and in 15% of DSB-induced non-dividing *saw1* mutant cells (G1) with background levels of 3.8% and 7.0% in uninduced cells, respectively.

In contrast, in long flap strains, we clearly observed that Rad10-YFP recruitment to DSB-RFP repair sites was *SAWI*-dependent in non-dividing G1 wild-type cells as DSB induction gave a Rad10-YFP-DSB-RFP colocalized focus in 23% of the cells, but only 10% of *saw1* mutant cells contained a colocalized focus (Fig. 1b). Background levels of colocalized foci were slightly higher in uninduced *saw1* mutant cells (9.1%) than in uninduced wild-type cells (3.3%). Intriguingly, in dividing cells we did not observe that Rad10-YFP recruitment was *SAWI*-dependent, as DSB induction led to a colocalized focus in nearly 20% of the wild-type cells, and in 14% of *saw1* cells versus 7.4% and 4.1%, respectively in uninduced controls. Classification of the phase of cell cycle in our experiments is systematic since it is based on readily identifiable hallmarks of cell cycle progress (e.g. presence of a bud, presence of one versus two nuclei in the bud, etc.). Presumably, however, a small percentage of S phase cells could be oriented “bud side down” on the slides and misclassified as G1 since the cells only contain one nucleus in fluorescence images with no visible bud in the corresponding DIC images. We don’t believe this to represent more than possibly a few percent of the cells, however, since prior work with inducible DSB sites that were not flanked by DNA repeats (which induces SDSA, accepted not to occur in G1) exhibited only a modest background of Rad10-YFP/DSB-RFP colocalized foci in a few percent of G1 cells [12,23,19].

As a control we examined whether the genetic dependencies of focus formation were consistent with repair by SSA. Since flap formation would require annealing of homologous single strands by Rad52, but not Rad51, Rad10-YFP recruitment is expected to be dependent on *RAD52* but not *RAD51* [5]. Our results show that colocalized focus formation is dependent on *RAD52*, but independent of *RAD51*, consistent with Rad10-YFP localization to short and long flaps created during SSA (Fig.1a and 1b).

We also examined the influence of *SAWI* on the efficiency of SSA repair product formation in short and long flap substrates. We isolated genomic DNA from DSB-induced samples and quantitated repair product formation using qPCR. We found no significant difference in repair product formation in wild-type and *saw1* mutant cells with short flap substrates (Fig. 2a), but found significantly reduced levels in *saw1* with long flap substrates (Fig. 2b). Gel analysis of qPCR samples corroborated these findings, and additionally confirmed that PCR products were chiefly of the expected SSA repair product sizes (Fig. 2c & 2d).

Saw1-CFP colocalizes with Rad10-YFP at DSB sites with both short and long 3’-flaps

To further investigate repair factor recruitment to short and long flaps we examined simultaneous localization of Rad10-YFP and Saw1-CFP to DSBs in wild-type strains. As

previously observed [12], Saw1-CFP localizes to DSBs with Rad10-YFP but exhibited low abundance and extensive photobleaching which prevented carrying out timelapse experiments since data from successively imaged fields is impossible to analyze. Therefore, to characterize the independent behavior of Saw1 we counted triple colocalized foci (Rad10-YFP/Saw1-CFP/DSB-RFP), double colocalized foci (Saw1-CFP/DSB-RFP, Rad10-YFP/DSB-RFP and Saw10-CFP/Rad10-YFP) and single Rad10-YFP foci or Saw1-CFP foci in fields of cells imaged 30 minutes after DSB induction (Fig. 3).

In the short flap strain, triple colocalized foci were highly induced by DSB formation as we observed that 24% of the dividing cells (S/G2/M) contained triple foci following DSB induction versus only 5% in uninduced controls (Fig. 3a). Likewise G1 cells exhibited an increase in triple foci following DSB induction as we observed triple foci in 37% of the induced G1 cells while only observing triple foci in 15% of uninduced cells (Fig. 3a). S/G2/M cells exhibited a significantly greater abundance of Rad10-YFP/DSB-RFP double colocalized foci (46%) in comparison to triple foci (24%) following DSB induction (Fig. 3a). There was no significant increase in Rad10-YFP/DSB-RFP foci in G1 cells (21% induced versus 15% uninduced) (Fig. 3a). We observed few Saw1-CFP/Rad10-YFP colocalized foci not localized to DSB-RFP sites and only low levels of Saw1-CFP/DSB-RFP colocalized foci, Saw1-CFP single foci and Rad10-YFP single foci (Fig. 3a and 3b).

In the long flap strain, triple colocalized foci increased significantly to 53% from 35% in S/G2/M cells following DSB induction. Similarly, in G1, triple foci increased significantly to 36% from 16% (Fig. 3c). We did not observe a significant increase in Rad10-YFP/DSB-RFP double foci in any phase of cell cycle (Fig. 3c). Only low levels of Saw1-CFP and Rad10-YFP single foci were observed (Fig. 3d). Hence, both short and long flap strains show substantial Saw1-CFP recruitment to SSA sites.

Discussion

In contrast to mammalian cells, *S. cerevisiae* utilizes SSA in all phases of cell cycle to repair double-strand breaks flanked by DNA repeats and exhibits a preference for SSA over non-homologous end joining (NHEJ) [1,24]. SSA is even preferred in G1 during which mammalian cells suppress SSA [24,25]. It has been established in *S. cerevisiae* that Saw1 recruits the Rad1-Rad10 endonuclease to repair intermediates bearing 3'-flaps, yet the precise requirements for Saw1 have not been defined, and most work to date has been accomplished *in vitro*. Herein we provide *in vivo* evidence that Rad10 is recruited to SSA sites when DNA flaps are short, but that Saw1 is not required for this recruitment. However, when flaps are long Rad10 recruitment requires Saw1 in non-dividing cells, consistent with prior reports [11,9]. Background recruitment of Rad10 to long flaps in non-dividing *saw1* cells, however, was slightly higher than in wild-type cells (Fig. 1b) possibly indicating a minor, non-productive, but persistent association of Rad1-Rad10 in the absence of Saw1, resulting in accumulation of additional background signal. Intriguingly, recruitment of Rad10 to long flaps was observed in dividing *saw1* cells, indicating Saw1 is dispensable for recruitment in S/G2/M. To our knowledge, this is the first evidence that Saw1 function is cell cycle dependent. In contrast, our prior work shows *SAW1* is required for SDSA in both dividing and non-dividing cells [12].

Interestingly, the requirement of Saw1 for recruitment of Rad1-Rad10 does not correlate with the appearance of Saw1 at repair sites. DSB formation in wild-type strains stimulated substantial induction of triple foci in all cell cycle phases, indicating that Saw1 and Rad10 are both recruited to SSA sites even when Saw1 is not required for recruitment of Rad10. Additionally, these results mirrored earlier work in which we observed that very few DSBs that contained Saw1-CFP were without Rad10-YFP, but there were many that contained Rad10-YFP without Saw1-CFP [12]. These data suggest that Saw1 and Rad1-Rad10 arrive at repair sites together as a complex but that Saw1 may depart prior to Rad1-Rad10.

There are three additional salient features regarding the triple-labeled data. First, comparing short and long flap strains, the data are surprisingly similar in non-dividing cells despite differences in the requirement for *SAW1* revealed in figures 1 and 2. Second, in dividing cells the abundance of triple foci was higher with long flaps than with short flaps, despite results in figure 1a and 1b showing that *SAW1* is not required for recruitment of Rad10 to either short or long flaps in dividing cells. Together these features indicate a more complicated role for Saw1 than simply recruiting Rad1-Rad10 to SSA repair sites in dividing cells. Third, in dividing cells, short flaps displayed a greater abundance of Rad10-YFP/DSB-RFP double foci relative to triple foci, which was opposite the trend observed for long flaps. If repair intermediates are initially bound by Saw1-Rad1-Rad10 complexes, this result suggests the Saw1-Rad1-Rad10 complex is longer-lived when 3'-flaps are long but that Saw1 dissociates more rapidly from short flaps, the latter being observed in our experiments as a relative excess of Rad10-YFP/DSB-RFP double foci over triple foci. However, it is also possible that Saw1-Rad1-Rad10 and Rad1-Rad10 complexes localize stochastically to short flap repair intermediates. If Saw1-Rad1-Rad10 and Rad1-Rad10 complexes bind stochastically, results indicate modulation of binding affinities in S/G2/M with short flaps (Fig. 4).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 2.

SSA repair product formation is not significantly impaired in a short flap *saw1* strain relative to WT, but is reduced when 3'-flaps are long. a. Quantitative PCR experiments with short flap WT (PF177, black bars) and *saw1* (PF160-13B, gray bars) strains showing relative amplification of the SSA repair product. b. Quantitative PCR experiments with long flap WT (PF178, black bars) and *saw1* (PF196-4B, gray bars) strains showing relative amplification of the SSA repair product. Error bars represent standard deviations; T-tests of pairwise comparisons are shown. c. and d. Representative agarose gels of aliquots of qPCRs from cells in a. and b. removed from the thermocycler following the indicated number of cycles. Diagrams above graphs in a. and b. depict the DNA substrate and SSA product corresponding to the graph and gel immediately below it in a. and c., and b. and d., respectively

Fig. 3.

Saw1-CFP and Rad10-YFP colocalize to sites of SSA in high abundance even with short DNA flaps. Graphs of focus counts from three DSB induction experiments in triple-labeled strains: a. and b. short flap substrate (PF158-7D); c. and d. long flap substrate (PF230-68B). a. and c.: graphs categorize foci according to the highest ordering of fluorescent markers present (Rad10-YFP, Saw1-CFP, DSB-RFP). For example, a triple focus containing YFP, CFP and RFP was classified as “CFP/YFP/RFP” and does not appear in calculations for CFP/YFP, YFP/RFP or CFP/RFP. “Induced” = galactose induction; “Uninduced” = uninduced control. b. and d.: same as a. and c. but showing abundances of uncolocalized CFP and YFP foci observed. DSB-RFP foci abundances were not plotted since nearly every cell exhibits one whether or not undergoing repair. Plotted values represent means (λ 's) from ideal Poisson distributions least squares fit to the observed distributions of foci per cell. The least squares deviations were propagated into the overall errors, which are depicted as the error bars. Statistical comparisons were carried out via a paired t_{calc} according to the NIST/SEMATECH e-handbook of Statistical Methods and then calculating p from integration of the single tail area beyond the paired t_{calc} in a Gaussian distribution. “****” indicates $p < 0.001$, “***” indicates $0.001 < p < 0.01$, “**” indicates $0.01 < p < 0.05$, “n.s.” indicates $0.05 < p$. Legend next to c. goes with a. and c.; legend next to d. goes with b. and d. Diagram in a. depicts the DNA substrate corresponding to data in a and b.; diagram in c. depicts the DNA substrate corresponding to data in c. and d.

Fig. 4. Model for recruitment of Rad1-Rad10 by Saw1 in SSA repair of a. Short flaps (~10 nt) and b. Long flaps (~500 nt). I-SceI-induced strand breaks are processed so complementary strands (black and gray thick lines) anneal at DNA repeats (gray portion of lines) forming flap intermediates. A Saw1-Rad1-Rad10 complex (blue and yellow shapes) is recruited to the flap (1 and 2), but Saw1 is not required for recruitment to short flaps and may not be present (1). Saw1 departs before Rad1-Rad10, but may depart more quickly in S/G2/M when 3'-flaps are short (3) versus long (4). Saw1 may depart prior to cleavage by Rad1-Rad10 as shown (5 and 6) or remain present until cleavage is complete. Gap filling and ligation of the nick occur (7 and 8)

Table 1

Strains used.

Name	Genotype	Where published
LSY401 ^a	<i>MAT LYS2 rad51::LEU2</i>	[26]
LSY715	<i>MAT LYS2 rad52::LEU2</i>	[27]
W4259-3B	<i>MAT ADE2 rad52::HIS5</i>	Gift from Rodney Rothstein
PF025-7A	<i>MATa LYS2 TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) I-SceI (iYER186C) Rad10-YFP</i>	[19]
PF148-17D	<i>MAT TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) I-SceI (iYER186C) Rad10-YFP saw1::LEU2</i>	Here
PF150-74A	<i>MAT TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) I-SceI (iYER186C) Rad10-YFP Saw1-CFP</i>	Here
PF156-16C	<i>MATa LYS2 TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) HIS3::I-SceI::HIS3 (iYER186C) Rad10-YFP rad52</i>	Here
PF158-7D	<i>MATa TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) HIS3::I-SceI::HIS3 (iYER186C) Rad10-YFP Saw1-CFP</i>	Here
PF160-13B	<i>MATa LYS2 TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) HIS3::I-SceI::HIS3 (iYER186C) Rad10-YFP saw1::LEU2</i>	Here
PF177	<i>MATa LYS2 TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) HIS3::I-SceI::HIS3 (iYER186C) Rad10-YFP</i>	Here
PF178	<i>MATa LYS2 TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) HIS3::500bp:I-SceI::500bp::HIS3 (iYER186C) Rad10-YFP</i>	Here
PF179-9A	<i>MATa LYS2 TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) HIS3::I-SceI::HIS3 (iYER186C) Rad10-YFP rad51::LEU2</i>	Here
PF187-23A	<i>MATa LYS2 TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) HIS3::500bp:I-SceI::500bp::HIS3 (iYER186C) Rad10-YFP rad51::LEU2</i>	Here
PF189-17A	<i>MATa LYS2 TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) HIS3::500bp:I-SceI::500bp::HIS3 (iYER186C) Rad10-YFP rad52::LEU2</i>	Here
PF191-41C	<i>MATa LYS2 TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) HIS3::500bp:I-SceI::500bp::HIS3 (iYER186C) Rad10-YFP</i>	Here
PF193-30A	<i>MATa LYS2 TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) HIS3::I-SceI::HIS3 (iYER186C) Rad10-YFP</i>	Here
PF196-4B	<i>MATa LYS2 TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) HIS3::500bp:I-SceI::500bp::HIS3 (iYER186C) Rad10-YFP saw1::LEU2</i>	Here
PF229	<i>MAT ADE2 TRP1 bar1::LEU2 Saw1-CFP</i>	Here
PF230-68B	<i>MATa RP1 TetR-mRFP (iYGL119W) URA3::tetOx224(iYER187W) HIS3::500bp:I-SceI::500bp::HIS3 (iYER186C) Rad10-YFP Saw1-CFP</i>	Here

^a All strains in this study are haploid and derivatives of W303-1A and W303-1B containing the following endogenous mutations *ade2-1 lys2 trp1-1 can1-100 his 3-11,15 leu2-3,112 ura3-1* unless otherwise noted [28]. Additionally, all strains are wild-type for *RAD5*.