Supplementary Material

Supplementary Figure Legends

Supplementary Figure S1. Analysis of Ura+His+ recombinants. A. Box-plot comparing the frequency of Ura+ and Ura+His+ recombinants. Note that the scale is logarithmic and that the frequency of the Ura+His+ is approximately three orders of magnitude lower. **B.** PCR across the *ura4*⁺ locus with same primers form Figure 1C. Shown are pre-recombinants (e.g. intact *ura-his-ura*) and post recombinants Ura+ and Ura+His+. Only when the break is induced can Ura+His+ be recovered. **C.** PCR to check the his3+ locus on chromosome 2 in WT (*his3*⁺), Pre (*ura4::ura'-his-'ura, his3-D1*) and induced recombinants Ura+His+. The top gel labeled "PCR across *his3*⁺ locus" is PCR with primers upstream and downstream the his3⁺ open reading frame. Note that the WT (*his3*⁺) has a longer fragment than both pre and post recombinants which should be *his3-D1* locus has not been converted to *his3*⁺. The bottom gel labeled "PCR within the *his3*⁺ ORF" is PCR with primers within the *his3*⁺ ORF. Note that all samples produce the same band indicating that they all have an intact *his3*⁺ ORF.

Supplementary Figure S2. Sensitivity of strains to MMS. Strains of the indicated genotypes were grown in YES at 32°C overnight then 5X serial dilutions were spotted onto YES plates or YES+0.008%MMS. Plates were incubated at 32°C for 4 days. Note that both the *rad52* Δ and *rad51* Δ mutant strains remain sensitive indicating that they did not acquire a suppressor.

Α



Supplementary Figure S2. Sensitivity of mutants to MMS



Identifier	Genotype	Source
FY 1828	h+ his3-D1 leu1-32	Forsburg
RCP 24	h+ ura4::ura4-his3-HO-ura4 his3-D1 leu1-32	Forsburg
RCP 71	h ⁻ ∆rad52::kanMX4-Bioneer ura4::ura4-his3-HO-ura4 his3-D1	This
	leu1-32 ade6-M210/216?	Study
RCP 228	h ⁻ smt-0 ∆rhp51::kanMX4-Bioneer ura4::ura4-his3-ura4 leu1-	Forsburg
	32 ade6-M216/210 his3-D1	
RCP 178	h ⁻ ∆pku70::kanMX ura4::ura4-his3-HO-ura4 leu1-32 his3-D1	This
	ade6-M210	Study
RCP 256	h+ ∆rad52::kanMX4-Bioneer ∆rad51::kanMX4-Bioneer	This
	ura4::ura4-his3-ura4 leu1-32 his3-D1 ade6-M216/210	Study
RCP 258	h ⁻ Δrad52::kanMX4-Bioneer Δpku70::kanR ura4::ura4-his3-	This
	HO-ura4 leu1-32 his3-D1 ade6-M210	Study
RCP 275	h ⁻ smt-0 ∆rad51::kanMX4-Bioneer ∆pku70::kanR ura4::ura4-	This
	his3-ura4 his3-D1 leu1-32 ade6-M216/210	Study
RCP 124	h+ ura4::ura4-his3-HO-ura4 his3-D1 leu1-32/ pREP81X-HO	This
		Study
RCP 81	h ⁻ ⊿rad52::kanMX4-Bioneer ura4::ura4-his3-HO-ura4 his3-D1	This
	leu1-32 ade6-M210/216 /pREP81X-HO	Study
RCP 267	h ⁻ smt-0 ∆rad51::kanMX4-Bioneer ura4::ura4-his3-ura4 leu1-	This
	32 his3-D1 ade6-M216/210 /pREP81X-HO	Study
RCP 371	h ⁻ ∆pku70::kanMX ura4::ura4-his3-HO-ura4 leu1-32 his3-D1	This
	ade6-M210 /pREP81X-HO	Study
RCP 268	h+ ∆rad52::kanMX4-Bioneer ∆rad51::kanMX4-Bioneer	This
	ura4::ura4-his3-ura4 leu1-32 his3-D1 ade6-M216/210	Study
	/pREP81X-HO	
RCP 288	h ⁻ ∆rad51::kanMX4-Bioneer ∆pku70::kanMX ura4::ura4-his3-	This
	ura4 leu1-32 ade6-M216/210 ura4-D18 his3-D1 /pREP81X-	Study
	НО	
RCP377	h ∆pku70::kanMX ∆rad52::kanMX-bioneer ura4::ura4-his3-	This
	HO-ura4 leu1-32 his3-D1 ade6-M210 /pREP81X-HO	Study

Supplementary Table S1. Strains used in this study.

Supplementary Table S2. Descriptive statistics for spontaneous breaks when cells were released in Edinburgh Minimal Media and platted on EMM-Uracil with Phloxin B. Recombinants per 10⁵ cells.

	Ν	Mean		Std. Deviation	
	Statistic	Statistic	Std. Error	Statistic	
WT	36	30.70	4.20	25.20	
⊿rad52	30	14.98	3.61	19.77	
⊿rad51	19	175.14	22.64	98.69	
⊿pku70	18	146.32	34.41	146.00	
⊿rad52⊿rad51	24	18.82	5.41	26.48	
⊿rad52⊿pku70	25	5.65	1.24	6.18	
⊿rad51⊿pku70	17	192.82	58.79	242.39	

Supplementary Table S3. Descriptive statistics for spontaneous breaks when cells were released in Edinburgh Minimal Media and plated on EMM-Uracil without Phloxin B. Recombinants per 10⁵ cells.

	Ν	Mean		Std. Deviation	
	Statistic	Statistic	Std. Error	Statistic	
WT	20	24.94	8.40	37.58	
⊿rad52	29	14.74	3.41	18.34	
⊿rad51	10	156.81	27.77	87.81	
⊿pku70	18	154.81	23.48	99.63	
∆rad52∆rad51	15	19.72	4.08	15.81	
∆rad52∆pku70	25	4.89	0.74	3.69	
⊿rad51⊿pku70	12	157.03	42.46	147.07	

Supplementary Table S4. Descriptive statistics for induced breaks when cells were released in Edinburgh Minimal Media. Recombinants per 10⁴ cells.

	N	Mean		Std. Deviation
	Statistic	Statistic	Std. Error	Statistic
WT + pREP81X-HO w/ Thiamine	26	154.64	33.34	170.01
<i>∆rad5</i> 2 + pREP81X-HO w/ Thiamine	50	10.77	1.06	7.52
<i>∆rad51</i> + pREP81X-HO w/ Thiamine	33	18.76	3.36	19.31
<i>∆pku70</i> + pREP81X-HO w/ Thiamine	35	370.07	92.24	545.70
<i>∆rad52∆rad51</i> + pREP81X-HO w/ Thiamine	29	3.66	1.68	9.04
<i>∆rad52∆pku70</i> + pREP81X-HO w/ Thiamine	30	5.28	0.83	4.53
<i>∆rad51∆pku70</i> + pREP81X-HO w/ Thiamine	29	6.84	0.57	3.07
WT + pREP81X-HO w/o Thiamine	47	3045.98	259.99	1782.37
<i>∆rad5</i> 2 + pREP81X-HO w/o Thiamine	34	99.87	10.95	63.84
<i>∆rad51</i> + pREP81X-HO w/o Thiamine	46	35.12	5.27	35.76
<i>∆pku70</i> + pREP81X-HO w/o Thiamine	26	3684.68	344.18	1754.99
<i>∆rad52∆rad51</i> + pREP81X-HO w/o Thiamine	39	0.46	0.079	0.49
<i>∆rad52∆pku70</i> + pREP81X-HO w/o Thiamine	36	235.55	29.21	175.24
<i>∆rad51∆pku70</i> + pREP81X-HO w/o Thiamine	28	13.54	4.7	24.63
WT + pREP81X-Vector w/o Thiamine	50	5.67	1.27	8.95
<i>∆rad5</i> 2 + pREP81X-Vector w/o Thiamine	28	0.79	0.20	1.06
<i>∆rad51</i> + pREP81X-Vector w/o Thiamine	19	7.68	2.72	11.85
<i>∆pku70</i> + pREP81X-Vector w/o Thiamine	N/A			
<i>∆rad52∆rad51</i> + pREP81X-Vector w/o Thiamine	20	0.14	0.02	0.11
<i>∆rad52∆pku70</i> + pREP81X-Vector w/o Thiamine	35	0.77	0.24	1.41
<i>∆rad51∆pku70</i> + pREP81X-Vector w/o Thiamine	20	2.84	0.84	3.77