Supplement S1

Role of the Srs2-Rad51 Interaction Domain in Crossover Control

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2 Supplementary Tables

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Table S1. List of yeast 2-Hybrid plasmids used in this study.

Plasmid ID	Plasmid Name	Plasmid Description
pWDH383	pEG202-Rad51	Full-length *S. cerevisiae RAD51* cloned into bait vector (pEG202)
pWDH857	pEG202-STOP	pEG202 bait vector with tandem stop codons introduced at *Eco*RI site a few base pairs downstream of the LexA domain; used as negative control
pWDH821	pJG4-5-Srs2	Full-length *S. cerevisiae SRS2* cloned into prey vector (pJG4-5)
pWDH856	pJG-4-5-STOP	pJG4-5 prey vector with tandem stop codons introduced at *Eco*RI site a few base pairs downstream of the B42/HA domain; used as negative control
pWDH896	pJG4-5-srs2-F891A	Site-directed mutagenesis to introduce *srs2-F891A* mutation using pWDH821 as a template

Table S2. List of *S. cerevisiae* used in this study. Information provided in parentheses in the Description/Background column refers to which assay the strain was used for in this study. All strains listed are from the Heyer and Jinks-Robertson laboratories, unless indicated as otherwise in the Source column.

Strain ID	Description/Background	Relevant Genotype	Source
WDHY424	EGY48 (yeast two-hybrid)	*MATa trp1 ura3-52 his3 LEU2::plexAop6-LEU2*	Dr. Brent
WDHY3429	W303 *WT*	*MATα ade2-1 can1-100 his3∆200 leu2-3,112 trp1-1 ura3::TRP1 RAD5*	
WDHY3491	W303 *srs2Δ*	*MATα ade2-1 can1-100 his3(Δ200 or -11,15) leu2-3,112 trp1-1 ura3::TRP1 RAD5 srs2Δ::KanMX*	
WDHY3459	W303 *srs2-F891A*	*MATα ade2-1 can1-100 his3Δ200 leu2-3,112 trp1-1 ura3::TRP1 RAD5 srs2-F891A*	
WDHY4189	W303 *rad55Δ*	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 RAD5 ura3::loxP rad55Δ::HphMX*	
WDHY4248	W303 *rad55Δ srs2Δ*	*MATa ade2-1 can1-100 his3(Δ200 or -11,15) leu2-3,112 trp1-1 RAD5 ura3::loxP rad55Δ::HphMX srs2Δ::KanMX*	
WDHY4501	W303 *rad55Δ srs2-F891A*	*MATα ade2-1 can1-100 his3(Δ200 or -11,15) leu2-3,112 trp1-1 ura3::TRP1 RAD5 srs2F891A rad55Δ::HphMX*	
CY8564 [WDHY3514]	W303 *rad18Δ*	*MATα rad18::LEU2*	Dr. Liberi
CY8563 [WDHY3515]	W303 *rad18Δ srs2Δ*	*MATα ADE2 rad18::LEU2 srs2::KanMX4*	Dr. Liberi
WDHY5112	W303 rad18*Δ srs2-F891A*	*rad18::LEU2 srs2-F891A RAD5*	
SJR3099 [WDHY4365]	W303 *WT* (gap repair)	*MATa ADE2 RAD5 can1::his3Δ3',18 leu2-3,112 his3Δ ura3-1 trp1 mlh1Δ::KanMX*	
SJR4172 [WDHY4364]	W303 *srs2Δ* (gap repair)	*MATa ADE2 RAD5 can1::his3Δ3',18 leu2-3,112 his3Δ ura3-1 mlh1Δ::KanMX srs2Δ::HphMX*	
SJR4191 [WDHY4362]	W303 *mph1Δ* (gap repair)	*MATα ADE2 RAD5 can1::his3Δ3',18 leu2-3,112 his3Δ ura3-1 trp1 mlh1Δ::KanMX mph1::hisG*	
SJR4148 [WDHY4361]	W303 *srs2-F891A* (gap repair)	*MATα ADE2 RAD5 can1::his3Δ3',18 leu2-3,112 his3Δ ura3-1 trp1 mlh1Δ::KanMX srs2-F891A*	
SJR4163 [WDHY4366]	W303 *mph1Δ srs2-F891A* (gap repair)	*MATα ADE2 RAD5 can1::his3Δ3',18 leu2-3,112 his3Δ ura3-1 trp1 mlh1Δ::KanMX mph1::hisG srs2-F891A*	
SJR2157 [WDHY3893]	SJR strain *WT* (gap repair)	*MATα ade2-101 his3Δ200 ura3-Nhe lys2ΔRV::hisG leu2-R can1::his3ΔKpn,18 mlh1Δ::kan Gal+*	

SJR3297	SJR strain *srs2*Δ (gap repair)	*MATα ade2-101oc his3Δ200 ura3-Nhe lys2ΔRV::hisG leu2-R can1::his3ΔKpn, 19 mut mlh1Δ::kan Gal+ srs2Δ::hyg*	
WDHY3920	SJR strain srs2-F891A (gap repair)	*MATα ade2-101 his3Δ200 ura3-Nhe lys2ΔRV::hisG leu2-R can1::his3ΔKpn,18 mlh1Δ::kan Gal+ srs2-F891A*	
tGI354 [WDHY4602]	JKM139 *WT* (physical recombination)	*ho hml::ADE1 MATa-inc hmr::ADE1 ade1 leu2-3,112 lys5 trp1::hisG ura3-52 ade3::GAL::HO arg5,6::MATa::HphMX*	Dr. Haber
tGI383 [WDHY4603]	JKM139 *srs2*Δ (physical recombination)	*ho hml::ADE1 MATa-inc hmr::ADE1 ade1 leu2-3,112 lys5 trp1::hisG ura3-52 ade3::GAL::HO arg5,6::MATa::HphMX srs2::LEU2*	Dr. Haber
WDHY4634	JKM139 *srs2-F891A* (physical recombination)	*ho hml::ADE1 MATa-inc hmr::ADE1 ade1 leu2-3,112 lys5 trp1::hisG ura3-52 ade3::GAL::HO arg5,6::MATa::HphMX srs2-F891A*	
WDHY4943	JKM139 *mph1*Δ (physical recombination)	*ho hml::ADE1 MATa-inc hmr::ADE1 ade1 leu2-3,112 lys5 trp1::hisG ura3-52 ade3::GAL::HO arg5,6::MATa::HphMX mph1Δ::KanMX4*	
WDHY4944	JKM139 *mph1*Δ *srs2-F891A* (physical recombination)	*ho hml::ADE1 MATa-inc hmr::ADE1 ade1 leu2-3,112 lys5 trp1::hisG ura3-52 ade3::GAL::HO arg5,6::MATa::HphMX srs2-F891A mph1Δ::KanMX4*	
SJR1486 [WDHY4841]	SJR1486 *WT* (inverted repeat assay)	*MATα ade2-101oc his3Δ200 ura3(Nhe)-HIS3::intron::cβ2 IR-ura3(Nhe) lys2ΔRV::hisG leu2-K-lys2Δ5'-lys2Δ3'-LEU2 Gal+*	
SJR1704 [WDHY4842]	SJR1486 *srs2*Δ (inverted repeat assay)	*MATα ade2-101oc his3Δ200 ura3(Nhe)-HIS3::intron::cβ2 IR-ura3(Nhe) lys2ΔRV::hisG leu2-K-lys2Δ5'-lys2Δ3'-LEU2 srs2Δ::kan Gal+*	
WDHY4931	SJR1486 *srs2-F891A* (inverted repeat assay)	*MATα ade2-101oc his3Δ200 ura3(Nhe)-HIS3::intron::cβ2 IR-ura3(Nhe) lys2ΔRV::hisG leu2-K-lys2Δ5'-lys2Δ3'-LEU2 Gal+ srs2-F891A*	
WDHY2067	W303 *sgs1*Δ (tetrad analysis)	*MATa can1-100 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 sgs1::HIS3 RAD5*	
WDHY2100	W303 *rad54*Δ (tetrad analysis)	*MATα can1-100 ADE2 his3-11,15 leu2-3,112 trp1-1 ura3-1 met17-s rad54::LEU2 RAD5*	

* All gap repair strains were transformed with *Bss*HII linearized pSR987 (gap repair substrate repaired through chromosomal *his3*Δ*3'* donor sequence).

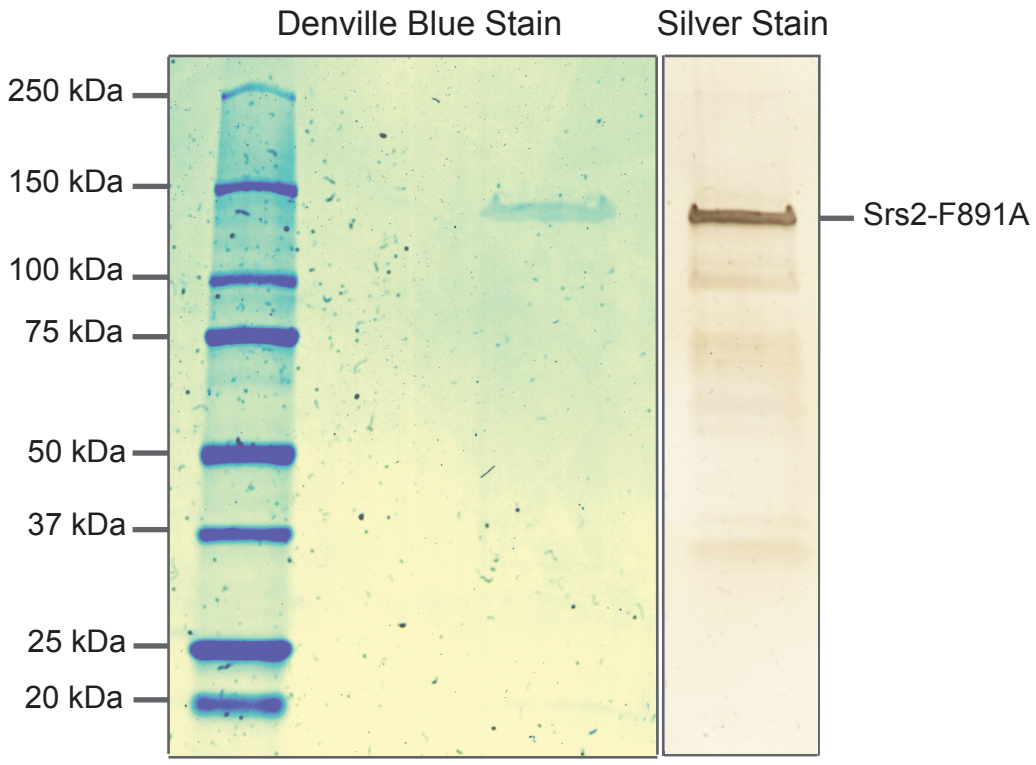
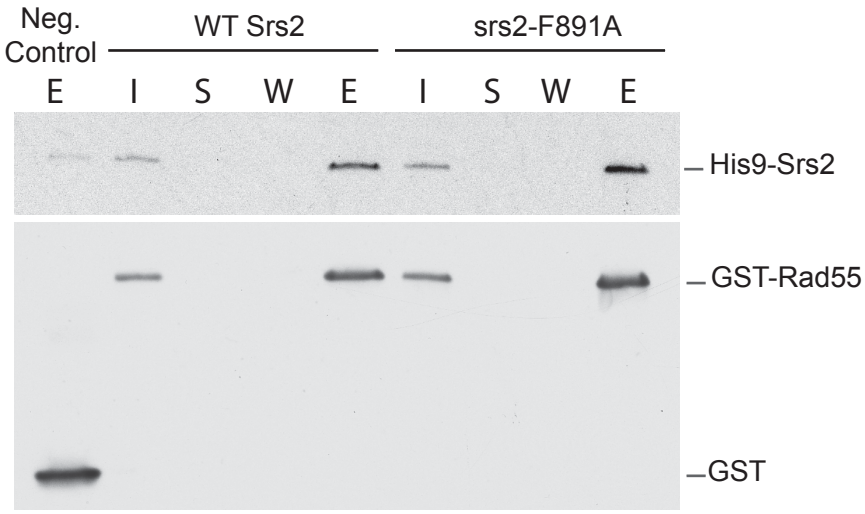


Figure S1. srs2-F891A protein purification. The final purified srs2-F891A protein was analyzed via Denville Blue and silver staining. Silver staining shows small amounts of Srs2 protein degradation products.

A



B

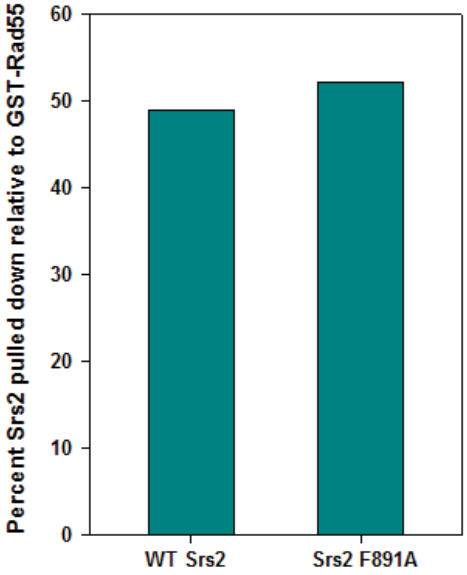


Figure S2. Srs2-Rad55 interaction is unaffected in the *srs2-F891A* mutant. A) Pull-down with 200 nM GST–Rad55–His6–Rad57 and 50 nM His9-tagged Srs2 (WT or F891A). 200 nM pure GST protein was used as negative control. I, input (10%); S, supernatant (10%); W, wash (3.6%); E, eluate (100%). B) Quantitation of the Srs2 bands normalized to the GST-Rad55 bands.

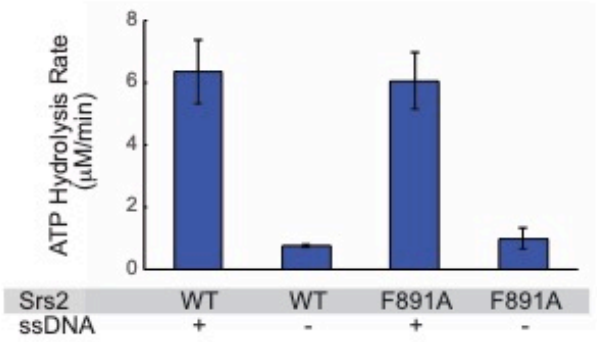


Figure S3. srs2-F891A exhibits wild-type levels of ATPase activity. ATP hydrolysis rates of WT and mutant srs2-F891A are indicated in the presence and absence of ssDNA. Shown are means ± 1 sd from n=3.

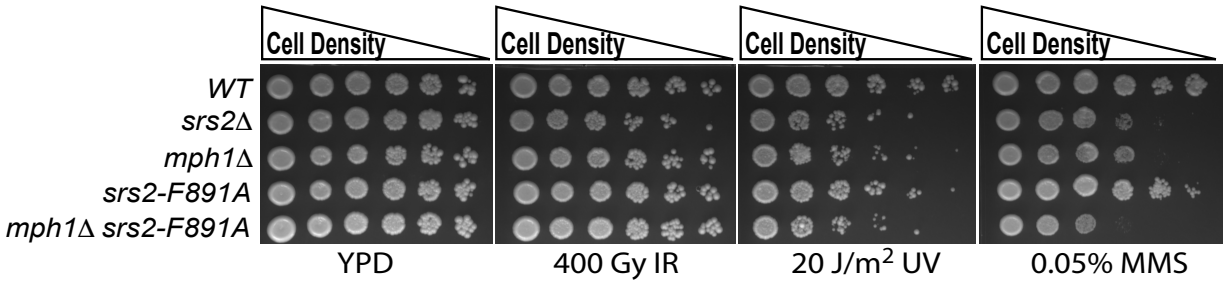
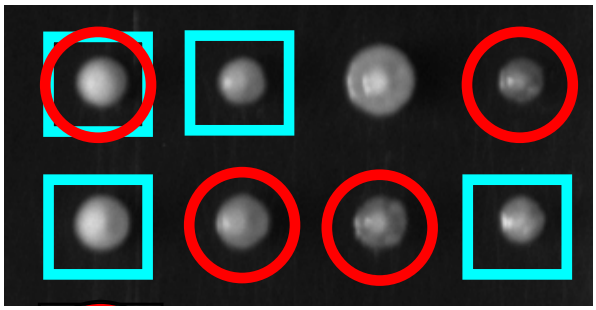


Figure S4. DNA damage sensitivity analysis of *W303* strains. Indicated strains (corresponding to WDHY 4361-4362 and WDHY 4364-4366 strains listed in Table S2) were treated with UV, IR, and MMS to examine sensitivity to DNA damage. The plates were incubated at 30˚C for 2 days prior to imaging.

A

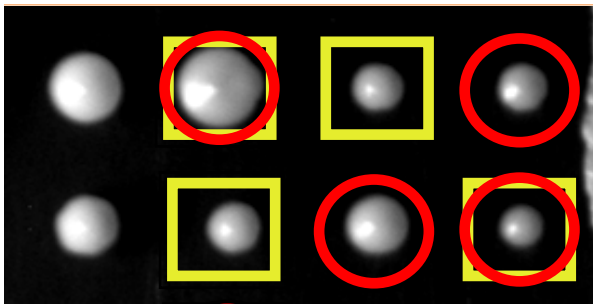


WDHY 3507 (*srs2-F891A*) x WDHY 2100 (*rad54Δ::LEU2*)

rad54Δ

srs2-F891A

B



WDHY 3459 (*srs2-F891A*) x WDHY 2067 (*sgs1Δ::HIS3*)

sgs1Δ

srs2-F891A

Figure S5. Unlike *srs2Δ*, *srs2-F891A* exhibits no genetic interaction with *rad54Δ* or *sgs1Δ*. A) Diploid *W303* heterozygous for *srs2-F891A* and *rad54Δ* was sporulated and the resulting spores were genotyped through presence of auxotrophic markers or sequence analysis. The overlapping red circle and blue square represents a double mutant spore. No synthetic lethality or growth defects were observed in the double mutants. B) Diploid *W303* heterozygous for *srs2-F891A* and *sgs1Δ* was sporulated and the resulting spores were analyzed as described above. The overlapping red circles and yellows square represent two double mutant spores. No synthetic lethality or growth defects were observed in the double mutants.