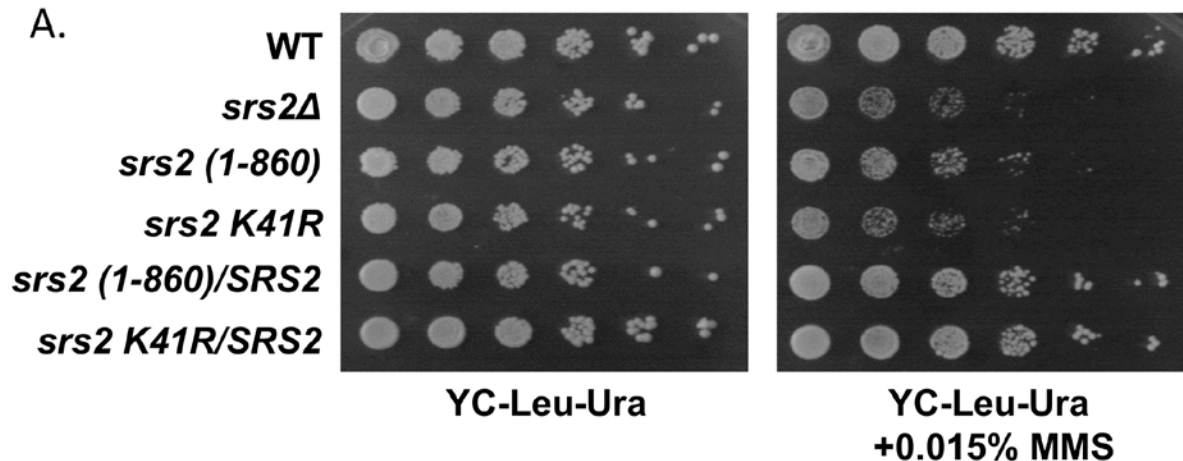


Figure S1. Expression of Srs2 domain mutant proteins. (A) Untagged proteins Srs2, *srs2*-K41R and *srs2* 875-902 $\Delta$  were detected using an antibody to the Srs2 C-terminus. (B) Srs2, *srs2* (1-860) and *srs2* (1-998) were C-terminally tagged with 13myc and detected using an anti-myc antibody. The relative expression level over either wild-type Srs2 or Srs2-myc after normalization to G6DPH is shown below the blots. (C) Protein localization was detected using a fluorescent anti-myc antibody to the myc tagged proteins. Examples are typical of 50-100 cells that were analyzed for each strain. Note that all proteins used in the experiments in the main manuscript were untagged, the tagged proteins were only used in the experiments shown here to detect protein levels and localization.



B. (CAG)<sub>70</sub> Instability on YAC in diploid strains

Strain Name	No. colonies analyzed	% Expansions	Fold over wild-type	% Contractions	Fold over wild-type
wild-type	269	1.1	1.0	2.6	1.0
<i>srs2Δ</i>	231	5.6*	5.1*	6.5*	2.5*
<i>srs2 (1-860)</i>	152	5.3*	4.7*	12.5*	4.8*
<i>srs2 (1-860)/SRS2</i>	151	3.3	2.3	2.0	0.8

\*p<0.05 compared to wild-type using Fisher's Exact Test

Figure S2. Comparison of MMS sensitivity and (CAG)<sub>70</sub> repeat instability in haploid and diploid strains. (A) Strains heterozygous for the indicated mutation or the corresponding haploid strains were serially diluted 1:5 and spotted onto plates either lacking or containing 0.015% MMS. (B) Instability of the CAG tract in the *srs2(1-860)/SRS2* diploid strain was analyzed as described in the main text, and compared to the corresponding haploid strains. Expansions, contractions, and MMS sensitivity were all suppressed by the presence of the wild-type SRS2 gene in the diploids.

### Supplemental Materials and Methods

#### Western blot analysis

Proteins were isolated as described in (1) with the following modifications: cells were resuspended in 100uL of 20% TCA prior to disruption and the resulting extract was neutralized with 50uL of 2M Tris Base. Equal volumes of extracts were loaded on 7.5% SDS page gels and semi-dry transferred to PVDF membrane; extracts from 40 OD<sub>600</sub> of cells were used for the anti-Srs2 blot, 5 OD<sub>600</sub> for the anti-myc blot. Membranes were blocked with 5% milk+PBST for 1 hour at room temperature before incubation with primary antibody in 1% milk+PBST overnight at 4°C followed by incubation with secondary antibody in 1% milk+PBST for 30 minutes at room temperature. The antibody that recognizes the Srs2 C-terminus (Santa Cruz, sc-11991) was used at 1:200 dilution. Srs2, *srs2 (1-860)* and *srs2 (1-998)* were not recognized by the sc-11991

antibody, and thus were C-terminally tagged with 13myc (2). The anti-myc antibody (Life Technologies, 13-2500) was used at 1:5000 dilution. The anti-G6DPH antibody (Sigma, A9521-1VL) was used at 1:10000 dilution. The secondary antibodies used were HRP-conjugated anti-goat (Santa Cruz, sc-2350) used at 1:5000 dilution, HRP-conjugated anti-mouse (Invitrogen, 626520) used at 1:5000 dilution, and HRP-conjugated anti-rabbit (Invitrogen, A24537) used at 1:10000 dilution. Enhanced chemiluminescence (ECL) Western blot substrate (Thermo Scientific, 32106) and Sharp autoradiography film (Denville, E3018) were used to detect protein signal. Quantification was performed in ImageJ. Two replicates were performed.

### Immunofluorescence

Early log phase cells grown in YC –Leu-Ura were fixed with 3.7% formaldehyde. Cells were spheroplasted with 50µg/ml Zymolyase (100T) in 0.1M potassium phosphate pH 7.5 at 30°C. Spheroplasts were blocked with PBST+3% BSA at room temperature for 30 minutes before incubation with primary antibody in PBST+3% BSA at 4°C overnight followed by incubation with secondary antibody at room temperature in PBST+3% BSA for 45 minutes in the dark. 15 minutes prior to the end of the secondary antibody incubation, Hoechst dye (ThermoFisher, H1398) was added to a final concentration of 2µg/ml. The anti-myc antibody was the same as above used at 1:500 dilution. The secondary antibody was an Alexa Fluor 488 conjugated goat anti-mouse antibody (ThermoFisher, A11001) used at 1:500 dilution. Images were acquired using a Zeiss microscope equipped with a Plan-Apochromat 100X 1.4 DIC oil immersion objective and Axiocam 503 mono camera. Exposure times were 50ms (DIC), 500ms (GFP), and 7000ms (DAPI). Images were processed using ZEN pro.

### MMS Sensitivity Assay

Spot assays were performed as previously described in (3) with 0.015% MMS. This data is for strains containing a CAG-70 YAC; results for strains containing a no tract or CTG-70 YAC were similar. Assays were performed with at least two replicates for each tract length.

1. Foiani, M., Marini, F., Gamba, D., Lucchini, G. and Plevani, P. (1994) The B subunit of the DNA polymerase alpha-primase complex in *Saccharomyces cerevisiae* executes an essential function at the initial stage of DNA replication. *Mol. Cell. Biol.*, **14**, 923–33.
2. Longtine, M.S., McKenzie, A., Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P. and Pringle, J.R. (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast*, **14**, 953–961.
3. Frizzell, A., Nguyen, J.H.G., Petalcorin, M.I.R., Turner, K.D., Boulton, S.J., Freudenreich, C.H. and Lahue, R.S. (2014) RTEL1 inhibits trinucleotide repeat expansions and fragility. *Cell Rep.*, **6**, 827–835.

**Table S1. Strains used in this study**

Strain	Background	Genotype	Reference
CFY765	BY4705	<i>MAT<math>\alpha</math></i> , <i>ade2<math>\Delta</math>::hisG</i> , <i>his3<math>\Delta</math>200</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>met15<math>\Delta</math>0</i> , <i>trp1<math>\Delta</math>63</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> YAC: <i>LEU2</i> No Tract <i>URA3</i>	Kerrest et al. 2009
CFY766	BY4705	<i>MAT<math>\alpha</math></i> , <i>ade2<math>\Delta</math>::hisG</i> , <i>his3<math>\Delta</math>200</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>met15<math>\Delta</math>0</i> , <i>trp1<math>\Delta</math>63</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> YAC: <i>LEU2</i> CAG-70 <i>URA3</i>	Kerrest et al. 2009
CFY1626	BY4705	<i>MAT<math>\alpha</math></i> , <i>ade2<math>\Delta</math>::hisG</i> , <i>his3<math>\Delta</math>200</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>met15<math>\Delta</math>0</i> , <i>trp1<math>\Delta</math>63</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> YAC: <i>LEU2</i> No Tract <i>URA3</i>	This study
CFY1628	BY4705	<i>MAT<math>\alpha</math></i> , <i>ade2<math>\Delta</math>::hisG</i> , <i>his3<math>\Delta</math>200</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>met15<math>\Delta</math>0</i> , <i>trp1<math>\Delta</math>63</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> YAC: <i>LEU2</i> CAG-70 <i>URA3</i>	This study
CFY925 CFY926	BY4742	<i>MAT<math>\alpha</math></i> , <i>his3<math>\Delta</math>1</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> , <i>srs2<math>\Delta</math>::KANMX6</i> YAC: <i>LEU2</i> No Tract <i>URA3</i>	Kerrest et al. 2009
CFY927 CFY928	BY4742	<i>MAT<math>\alpha</math></i> , <i>his3<math>\Delta</math>1</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> , <i>srs2<math>\Delta</math>::KANMX6</i> YAC: <i>LEU2</i> CAG-70 <i>URA3</i>	Kerrest et al. 2009
CFY2623	BY4705	<i>MAT<math>\alpha</math></i> , <i>ade2<math>\Delta</math>::hisG</i> , <i>his3<math>\Delta</math>200</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>met15<math>\Delta</math>0</i> , <i>trp1<math>\Delta</math>63</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> , <i>srs2<math>\Delta</math>::TRP1</i> YAC: <i>LEU2</i> CAG-70 <i>URA3</i>	This study
CFY1701	BY4705	<i>MAT<math>\alpha</math></i> , <i>his3<math>\Delta</math>1</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> YAC: <i>LEU2</i> CTG-70 <i>URA3</i>	Kerrest et al. 2009
CFY1712	BY4705	<i>MAT<math>\alpha</math></i> , <i>his3<math>\Delta</math>1</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> , <i>srs2<math>\Delta</math>::KANMX6</i> YAC: <i>LEU2</i> CTG-70 <i>URA3</i>	Kerrest et al. 2009
CFY2497 CFY2498	BY4705	<i>MAT<math>\alpha</math></i> , <i>ade2<math>\Delta</math>::hisG</i> , <i>his3<math>\Delta</math>200</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>met15<math>\Delta</math>0</i> , <i>trp1<math>\Delta</math>63</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> , <i>srs2-(1-860)-HYG</i> YAC: <i>LEU2</i> No Tract <i>URA3</i>	This study
CFY2087 CFY2088	BY4705	<i>MAT<math>\alpha</math></i> , <i>ade2<math>\Delta</math>::hisG</i> , <i>his3<math>\Delta</math>200</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>met15<math>\Delta</math>0</i> , <i>trp1<math>\Delta</math>63</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> , <i>srs2-(1-860)-HYG</i> YAC: <i>LEU2</i> CAG-70 <i>URA3</i>	This study
CFY2499 CFY2500	BY4705	<i>MAT<math>\alpha</math></i> , <i>his3<math>\Delta</math>1</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>ura3<math>\Delta</math>0</i> , <i>srs2-(1-860)-HYG</i> YAC: <i>LEU2</i> CTG-70 <i>URA3</i>	This study
CFY2501	BY4705	<i>MAT<math>\alpha</math></i> , <i>ade2<math>\Delta</math>::hisG</i> , <i>his3<math>\Delta</math>200</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>met15<math>\Delta</math>0</i> , <i>trp1<math>\Delta</math>63</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> , <i>srs2-(875-902<math>\Delta</math>)-HYG</i> YAC: <i>LEU2</i> No Tract <i>URA3</i>	This study
CFY2135 CFY2136	BY4705	<i>MAT<math>\alpha</math></i> , <i>ade2<math>\Delta</math>::hisG</i> , <i>his3<math>\Delta</math>200</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>met15<math>\Delta</math>0</i> , <i>trp1<math>\Delta</math>63</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> , <i>srs2-(875-902<math>\Delta</math>)-HYG</i> YAC: <i>LEU2</i> CAG-70 <i>URA3</i>	This study
CFY2503 CFY2504	BY4705	<i>MAT<math>\alpha</math></i> , <i>his3<math>\Delta</math>1</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> , <i>ura3<math>\Delta</math>0</i> , <i>can<sup>R</sup></i> , <i>srs2-(875-902<math>\Delta</math>)-HYG</i> YAC: <i>LEU2</i> CTG-70 <i>URA3</i>	This study
CFY2505	BY4705	<i>MAT<math>\alpha</math></i> , <i>ade2<math>\Delta</math>::hisG</i> , <i>his3<math>\Delta</math>200</i> , <i>leu2<math>\Delta</math>0</i> , <i>lys2<math>\Delta</math>0</i> ,	This study

CFY2506		<i>met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup>, srs2-K41R-HIS3MX6</i> YAC: <i>LEU2 No Tract URA3</i>	
CFY2305 CFY2306 CFY2307	BY4705	<i>MATα, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup>, srs2-K41R-HIS3MX6</i> YAC: <i>LEU2 CAG-70 URA3</i>	This study
CFY2507 CFY2508	BY4705	<i>MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, can<sup>R</sup>, srs2-K41R-HIS3MX6</i> YAC: <i>LEU2 CTG-70 URA3</i>	This study
CFY2509 CFY2510	BY4705	<i>MATα, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup>, srs2-(1-998)-HIS3MX6</i> YAC: <i>LEU2 No Tract URA3</i>	This study
CFY2299 CFY2300	BY4705	<i>MATα, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup>, srs2-(1-998)-HIS3MX6</i> YAC: <i>LEU2 CAG-70 URA3</i>	This study
CFY2511 CFY2512	BY4705	<i>MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, can<sup>R</sup>, srs2-(1-998)-HIS3MX6</i> YAC: <i>LEU2 CTG-70 URA3</i>	This study
CFY3025	BY4705	<i>MATα, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup>, srs2-K41R-HIS3MX6 rad51::KANMX6</i> YAC: <i>LEU2 CAG-70 URA3</i>	This study
CFY841	BY4742	<i>MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, can<sup>R</sup> rad51::KANMX6</i> YAC: <i>LEU2 CAG-70 URA3</i>	Kerrest et al., 2009
CFY2356	BY4705	<i>MATα, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup> srs2::srs2-K1081R K1089R K1142R::KANMX6</i> YAC: <i>LEU2 No Tract URA3</i>	Su et al., 2015
CFY2472 CFY2473	BY4705	<i>MATα, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup> srs2::srs2-K1081R K1089R K1142R::KANMX6</i> YAC: <i>LEU2 CAG-70 URA3</i>	Su et al., 2015
CFY4051	BY4705	<i>MATα/a, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup> srs2-(1-860)-HYG/SRS2</i> YAC: <i>LEU2 No Tract URA3</i>	This study
CFY4052	BY4705	<i>MATα/a, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup> srs2-(1-860)-HYG/SRS2</i> YAC: <i>LEU2 CAG-70 URA3</i>	This study
CFY4053	BY4705	<i>MATα/a, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup> srs2-(1-860)-HYG/SRS2</i> YAC: <i>LEU2 CTG-70 URA3</i>	This study
CFY4054	BY4705	<i>MATα/a, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup> srs2-K41R-</i>	This study

		<i>HIS3MX6 /SRS2</i> YAC: <i>LEU2</i> No Tract <i>URA3</i>	
CFY4055	BY4705	<i>MATa/a, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup> srs2-K41R-HIS3MX6 /SRS2</i> YAC: <i>LEU2 CAG-70 URA3</i>	This study
CFY4056	BY4705	<i>MATa/a, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup> srs2-K41R-HIS3MX6 /SRS2</i> YAC: <i>LEU2 CTG-70 URA3</i>	This study
CFY2600 CFY2601	BY4705	<i>MATα, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup>, mph1Δ::KANMX6</i> YAC: <i>LEU2</i> No Tract <i>URA3</i>	This study
CFY2602 CFY2603	BY4705	<i>MATα, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup>, mph1Δ::KANMX6</i> YAC: <i>LEU2 CAG-70 URA3</i>	This study
CFY1664 CFY1665	BY4705	<i>MATα, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup>, rad5Δ::KANMX6</i> YAC: <i>LEU2</i> No Tract <i>URA3</i>	This study
CFY1666	BY4705	<i>MATα, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup>, rad5Δ::KANMX6</i> YAC: <i>LEU2 CAG-70 URA3</i>	This study
CFY4104	BY4705	<i>MATa, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup> Srs2-13myc-KANMX6</i> YAC: <i>LEU2 CAG-70 URA3</i>	This study
CFY4105	BY4705	<i>MATα, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup>, srs2-(1-860)-13myc-KANMX6</i> YAC: <i>LEU2 CAG-70 URA3</i>	This study
CFY4161	BY4705	<i>MATα, ade2Δ::hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can<sup>R</sup>, srs2-(1-998)-13myc-KANMX6</i> YAC: <i>LEU2 CAG-70 URA3</i>	This study
GFY117	S288C	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1</i>	Kerrest et al., 2009
CFY3910	S288C	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2Δ::KANMX6</i>	This study
GFY177	S288C	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2-(875-902Δ)-HYG</i>	This study
GFY195	S288C	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 rad5Δ::KANMX4</i>	This study
GFY196	S288C	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 mph1Δ::KANMX4</i>	This study
GFY197	S288C	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2-K41R-HIS3MX6 rad51Δ::KANMX6</i>	This study
GFY198	S288C	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2-(875-902Δ)-</i>	This study

		<i>HYG rad51Δ::KANMX6</i>	
CFY2201	S288C	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2-(1-860)-HYG</i>	This study
CFY2301	S288C	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2-(1-998)-HIS3MX6</i>	This study
CFY2303	S288C	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2-K41R-HIS3MX6</i>	This study
CFY3078	S288C	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 exo1Δ::KANMX6</i>	This study

**Table S2. Instability Data: (CAG)<sub>70</sub> amplified from strains containing YAC CF1**

Strain	No. colonies analyzed	Expansion (#)	Expansion (%)	p-value compared to wild-type	p-value compared to <i>srs2Δ</i>
wild-type	269	3	1.1		
<i>srs2Δ</i>	231	13	5.6	0.0047	
<i>rad51Δ</i>	184	9	4.9	0.0176	0.8275
<i>srs2-(1-860)</i>	152	8	5.3	0.0207	1
<i>srs2-(875-902Δ)</i>	162	7	4.3	0.0456	0.6458
<i>srs2-K41R</i>	201	10	5.0	0.0194	0.8321
<i>srs2-(1-998)</i>	122	2	1.6	0.6493	0.0977
<i>srs2-3KR</i>	157	3	1.9	0.6739	0.1158
<i>srs2-K41R rad51Δ</i>	174	2	1.5	1	0.0178
<i>mph1Δ</i>	290	8	2.8	0.2256	0.1179
<i>rad5Δ</i>	190	4	2.1	0.4453	0.0829
<i>srs2 (1-860) /SRS2</i>	151	5	3.3	0.1425	0.3349

Strain	No. colonies analyzed	Contraction (#)	Contraction (%)	p-value compared to wild-type	p-value compared to <i>srs2Δ</i>
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wild-type	269	7	2.6		
<i>srs2</i> Δ	231	15	6.5	0.0474	
<i>rad51</i> Δ	184	25	13.6	0.0001	0.0186
<i>srs2</i> -(1-860)	152	19	12.5	0.0001	0.0649
<i>srs2</i> -(875-902Δ)	162	16	9.9	0.0016	0.2555
<i>srs2</i> -K41R	201	19	9.5	0.0018	0.2852
<i>srs2</i> -(1-998)	122	4	3.3	0.7454	0.3206
<i>srs2</i> -3KR	157	8	5.1	0.185	0.8265
<i>srs2</i> -K41R <i>rad51</i> Δ	173	3	1.7	0.7466	0.0474
<i>mph1</i> Δ	290	15	5.2	0.1322	0.5726
<i>rad5</i> Δ	190	9	4.7	0.3015	0.5287
<i>srs2</i> (1-860) /SRS2	151	3	2.0	1	0.0487



**Table S3. Fragility Data: Rate of FOA<sup>R</sup> in strains containing YAC CF1**

Strain	Repeat Length	# of Assays	Mean FOA <sup>R</sup> x 10 <sup>-6</sup>	SEM	p-value compared to wild-type	p-value compared to <i>srs2</i> Δ
wild-type	No Tract	7	2.2	0.20		
<i>srs2</i> Δ	No Tract	9	2.2	0.31	0.9639	
<i>srs2</i> -(1-860)	No Tract	5	1.9	0.28	0.4528	0.5639
<i>srs2</i> -(875-902Δ)	No Tract	5	1.8	0.32	0.2877	0.4013
<i>srs2</i> -K41R	No Tract	4	1.8	0.35	0.3467	0.4765
<i>srs2</i> -(1-998)	No Tract	3	3.4	0.23	0.0076	0.0611
<i>srs2</i> -3KR	No Tract	3	2.6	0.85	0.4844	0.5656
<i>mph1</i> Δ	No Tract	4	2.1	0.29	0.7640	0.8126
<i>rad5</i> Δ	No Tract	3	3.5	0.74	0.0396	0.0794
wild-type	CAG-70	9	9.4	1.2		
<i>srs2</i> Δ	CAG-70	16	30	3.2	0.0001	
<i>srs2</i> -(1-860)	CAG-70	4	39	5.9	<0.0001	0.2355
<i>srs2</i> -(875-902Δ)	CAG-70	5	12	1.8	0.3248	0.0062
<i>srs2</i> -K41R	CAG-70	3	22	1.2	0.0003	0.3071
<i>srs2</i> -(1-998)	CAG-70	3	26	1.6	<0.0001	0.6097
<i>srs2</i> -3KR	CAG-70	3	4.8	0.2	0.0694	0.0044
<i>mph1</i> Δ	CAG-70	7	14	1.2	0.0244	0.0040
<i>rad5</i> Δ	CAG-70	3	9.8	3.4	0.8848	0.0187
wild-type	CTG-70	7	13	1.7		
<i>srs2</i> Δ	CTG-70	9	25	3.2	0.0070	
<i>srs2</i> -(1-860)	CTG-70	10	17	1.6	0.0614	0.0397
<i>srs2</i> -(875-902Δ)	CTG-70	6	9.6	0.87	0.1899	0.0021
<i>srs2</i> -K41R	CTG-70	10	26	3.1	0.0032	0.7418
<i>srs2</i> -(1-998)	CTG-70	6	18	1.3	0.0249	0.1293

**Table S4. 2D Gel Analysis**

Strain	Origin	Genotype	Exp. #	Time point	Pause	Cone
GFY117	Kerrest et al. 2009	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1</i>	1	60'	0.72	0.35
				90'	1.27	0.41
			2	60'	1.16	0.30
				90'	1.10	0.78
			3	30'	1.40	0.53
				40'	1.62	0.37
				60'	1.42	0.66
CFY3910	This work	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2Δ::KANMX6</i>	1	40'	0.73	0.39
				60'	0.81	0.60
				90'	1.20	0.90
CFY2201	This work	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2-(1-860)-HYG</i>	1	90'	0.91	0.68
			2	50'	1.08	0.72
				60'	1.06	0.49
				90'	0.72	0.67
CFY2301	This work	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2-(1-998)-HIS3</i>	1	50'	0.78	0.75
				60'	0.80	0.49
				90'	0.77	0.66
			2	40'	0.95	0.56
				50'	1.13	0.55
				60'	0.92	0.77
GFY177	This work	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2-(875-902Δ)-HYG</i>	1	60'	0.49	0.48
			2	40'	0.73	0.50
				50'	0.89	0.43
				60'	1.15	0.78
GFY198	This work	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2-(875-902Δ)-HYG rad51Δ::KANMX6</i>	1	60'	1.17	0.53

			1	90'	1.09	0.78
CFY2303	This work	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2-K41R-HIS3</i>	1	60'	1.09	0.55
			2	50'	1.11	1.22
			3	40'	0.75	0.84
				50'	1.02	0.91
				60'	1.00	0.89
				90'	0.80	0.91
GFY197	This work	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 srs2-K41R-HIS3 rad51Δ::KANMX6</i>	1	60'	0.62	0.45
			2	50'	1.07	0.24
			3	50'	1.20	0.68
			3	60'	1.04	0.68
			3	90'	0.61	0.67
GFY195	This work	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 rad5Δ::KANMX6</i>	1	60'	0.91	0.79
			2	50'	1.22	1.33
			2	60'	1.06	1.16
			2	90'	1.04	1.03
			3	60'	0.87	0.65
CFY3078	This work	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 exo1Δ::KANMX6</i>	1	40'	0.55	1.21
				50'	0.81	0.90
				60'	0.65	1.09
				90'	0.39	1.14
GFY196	This work	<i>MATa ura3Δ851 leu2Δ1 his3Δ200 trp1Δ63 ade2-opal SUP4 arg2Δ::CTG98-TRP1 mph1Δ::KANMX6</i>	1	60'	1.01	0.85
				90'	0.99	0.93