

Su and Freudenreich, Supplementary Figures

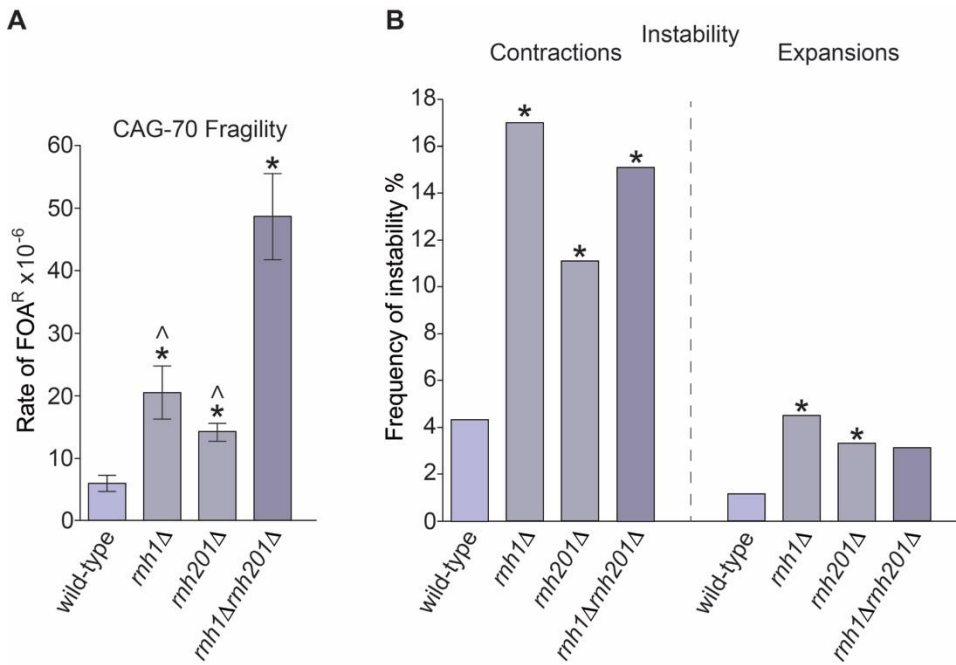


Figure S1. RNase H1 and RNase H2 single mutant phenotypes. (A) Rate of FOAR x 10⁻⁶ in indicated mutants; *p<0.05 compared to wild-type, ^p<0.05 compared to *rnh1Δrnh201Δ*, by *t* test. Average of at least 3 experiments +/- SEM is shown (see also Table S1). (B) CAG-70 expansion and contraction frequencies in indicated mutants, *p<0.05 compared to wild-type, no statistical significance of single mutants compared to *rnh1Δrnh201Δ* was detected, by Fisher's exact test (see also Table S4).

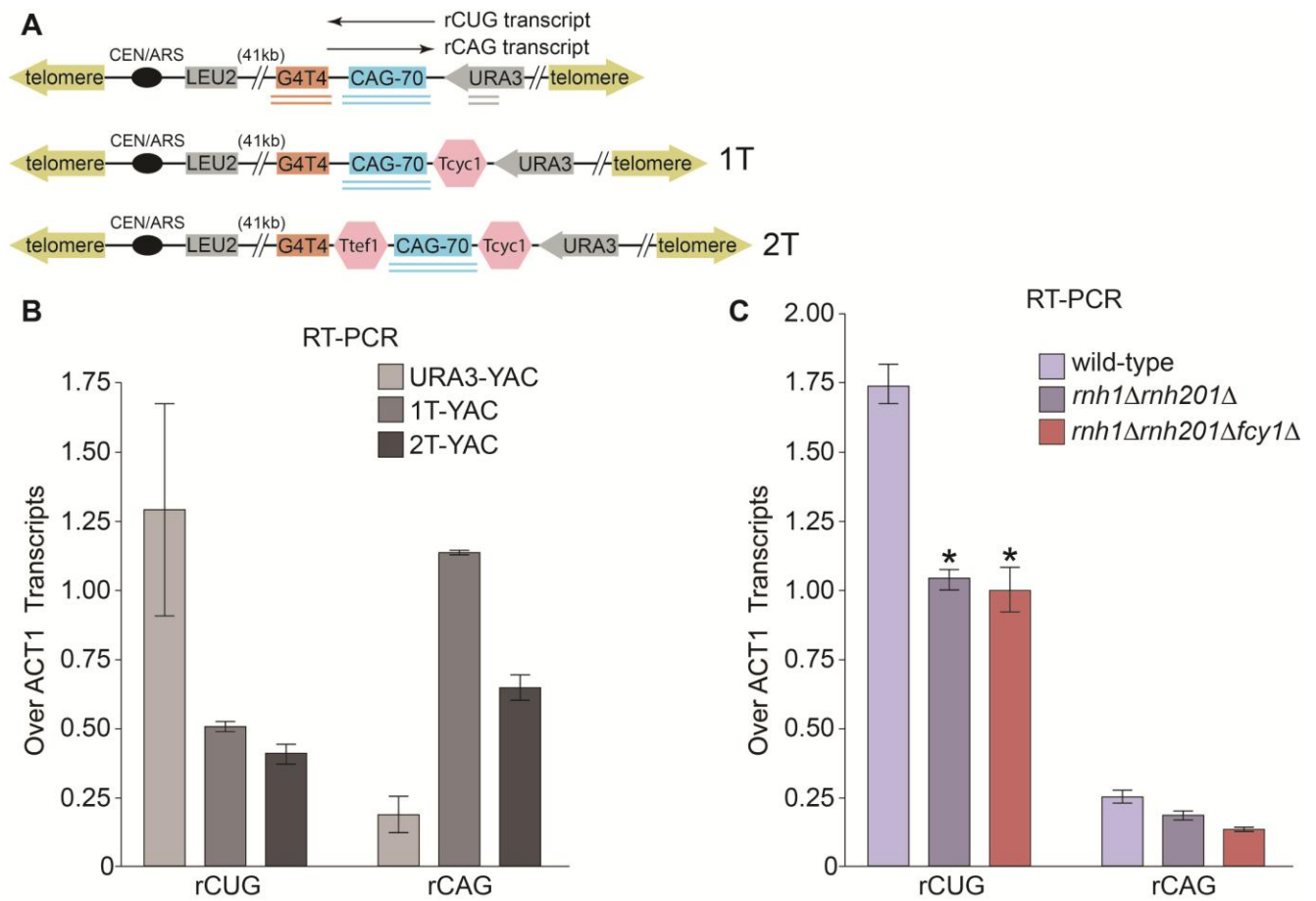


Figure S2. Determination of transcription through CAG repeats by reverse transcription (RT)-PCR, shows decreased *URA3* read-through transcription levels after terminator addition. (A) Scheme of different YACs. *URA3* transcription direction is indicated by the arrow. rCUG, CUG repeat RNA from *URA3* read-through transcription (there is 198 bp and no detected terminator in between *URA3* and CAG); rCAG, CAG repeat RNA from cryptic transcription from left side of CAG-70 repeats. Results using the *URA3*-YAC and 2 terminator (2T)-YAC are shown in main Figure 1. Double underlines indicate the qPCR amplicons used in the RT-PCR, DRIP, and ChIP experiments. (B) and (C) Quantitative PCR signals were normalized to the RNA signal from an actively transcribed gene *ACT1*. Each bar represents the mean of two experiments from two independent biological replicates +/-SEM.

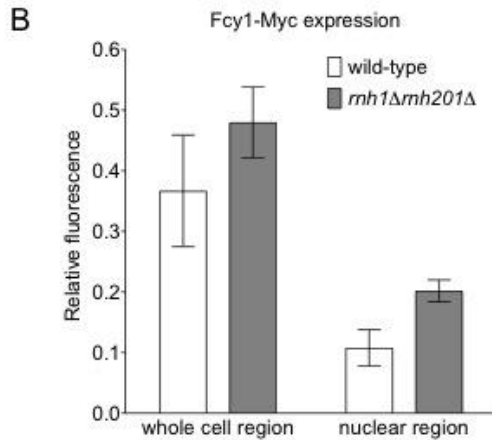
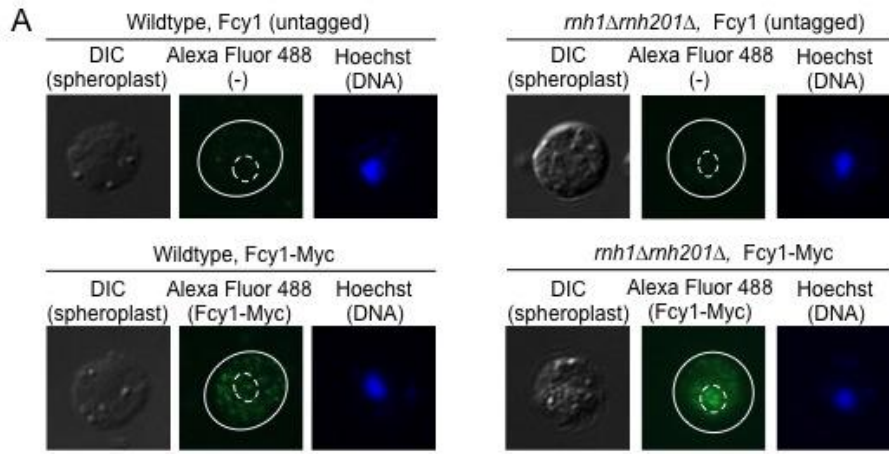


Figure S3. Fcy1 nuclear expression increases in the absence of RNase H Enzymes. (A) Immunofluorescent staining in indicated strains. Mouse anti-Myc (9E10) was detected with Alex Fluor 488 conjugated anti-mouse antibody. The fluorescent signal in green was detected and quantified with a Zeiss microscope and Zen software. A white solid circle outlines the spheroplast periphery according to the DIC picture on the left, and a white dashed circle outlines the nuclear region according to the Hoechst staining of nuclear DNA in blue. (B) Quantification of relative fluorescence in the indicated strains with Fcy1-expression is expressed as a ratio of Alexa Fluor over Hoechst intensity, with background subtracted. Seven wild-type spheroplasts and eight *rnh1Δrnh201Δ* spheroplasts were evaluated.

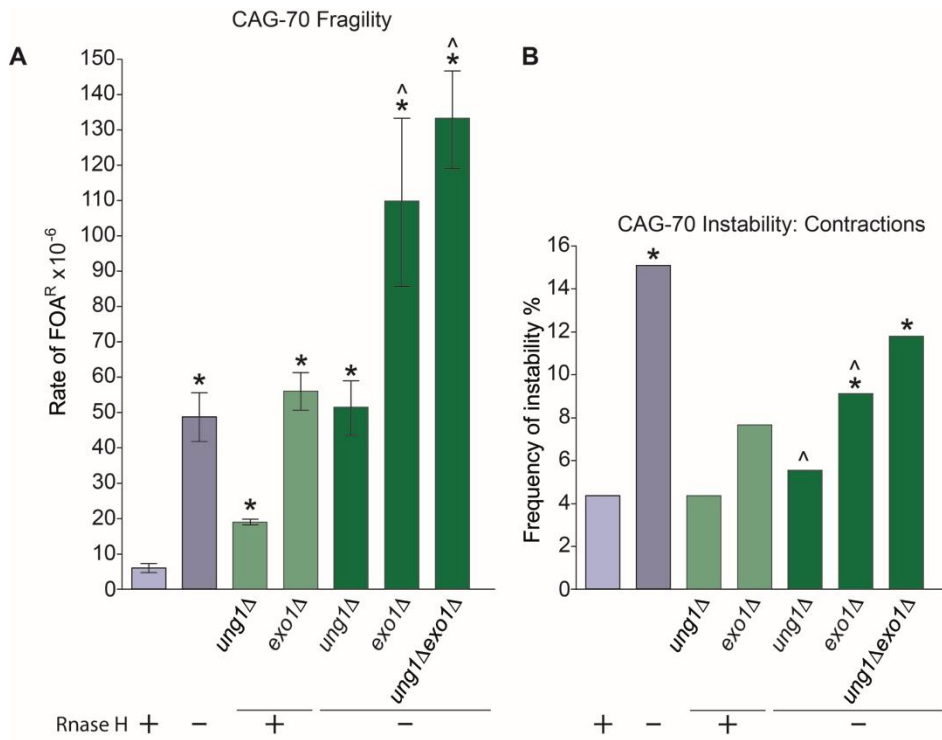


Figure S4. Deletion of Exo1 in the *rnh1Δrnh201Δ* strain exhibits an additive increase in CAG repeat fragility, but a suppression of contractions. (A) Rate of FOA^R x 10⁻⁶ in indicated mutants; *p<0.05 compared to wild-type, ^p<0.05 compared to *rnh1Δrnh201Δ*, by *t* test. Average of at least 3 experiments +/- SEM is shown (see also Table S1). (B) CAG-70 contraction frequencies in indicated mutants, *p<0.05 compared to wild-type, ^p<0.05 compared to *rnh1Δrnh201Δ*, by Fisher's exact test (see also Table S4).

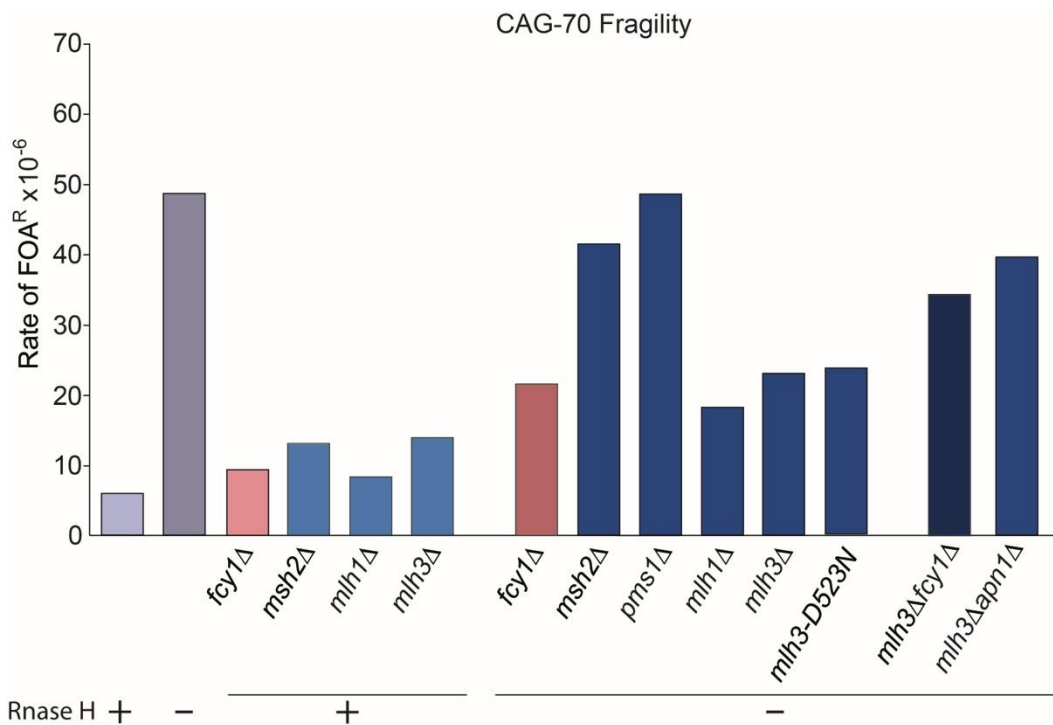


Figure S5. CAG-70 fragility in MMR-defective mutants; data adjusted to only show FOA^R due to YAC end loss. The rates of FOA^R for the indicated strains (Table S1) were adjusted by the percentage of loss of *URA3*, as assessed by PCR analysis (Table S5) to account for the higher rate of point mutations in some of the MMR-defective strains. The trends and conclusions remain the same with or without this adjustment.

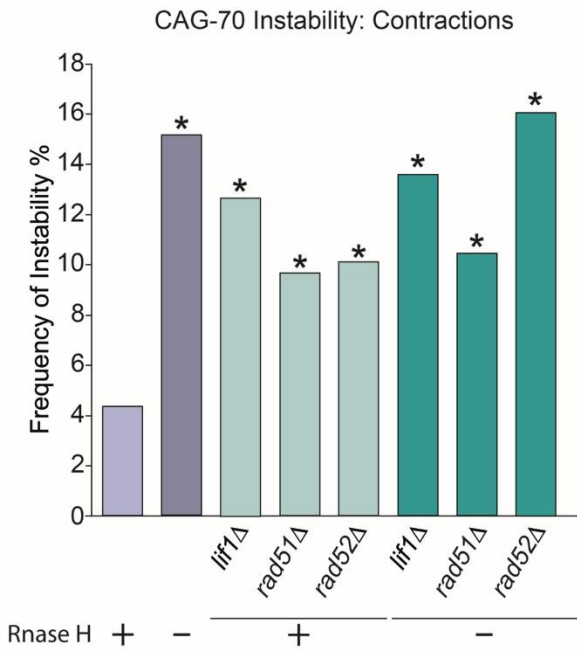


Figure S6. HR or NHEJ repair is not the primary cause of R-loop-induced CAG repeat contractions. CAG-70 contraction frequencies in indicated mutants, * $p < 0.05$ compared to wild-type, no statistical significance compared to *rnh1*Δ*rnh201*Δ was detected, by Fisher's exact test (see also Table S4).

Su and Freudenreich, Supplementary Tables

Supplementary Table 1. Fragility analysis of CAG-70 repeats on the URA3-YAC

Strains	Rate of FOA ^R (X10 ⁻⁶) ± <i>S.E.M</i>	Fold over wt	No. of Assays	p-value to wt	p-value to <i>rnh1Δ</i> <i>rnh201Δ</i>	other p-values
wild-type ¹	6.0 ±1.3	-	3	-	-	-
<i>rnh1Δ</i>	20.4 ±4.4	3.4	3	0.03	0.03	-
<i>rnh201Δ</i>	14.1 ±1.4	2.4	3	0.01	8.0 X10 ⁻³	-
<i>rnh1Δrnh201Δ</i>	48.7 ±6.9	8.1	3	3.7X10 ⁻³	-	-
cytosine/cytidine deaminase						a, to <i>fcy1Δ</i> b, to <i>cdd1Δ</i>
<i>fcy1Δ</i>	9.4±2.5	1.6	3	0.28	-	-
<i>cdd1Δ</i>	18.5±5.9	3.1	3	0.11	-	-
<i>rnh1Δrnh201Δfcy1Δ</i>	21.6±2.6	3.6	3	5.8X10 ⁻³	0.02	0.03 ^a
<i>rnh1Δrnh201Δcdd1Δ</i>	50.6±3.9	8.4	3	4.0X10 ⁻⁴	0.83	0.01 ^b
BER genes						c, to <i>ung1Δ</i> d, to <i>apn1Δ</i> e, to <i>exo1Δ</i>
<i>ung1Δ</i>	19.0±0.6	3.2	3	8.0X10 ⁻⁴	-	-
<i>apn1Δ</i>	11.5±1.3	1.9	4	0.03	-	-
<i>exo1Δ</i>	55.8±5.2	9.3	3	8.0X10 ⁻⁴	-	-
<i>rnh1Δrnh201Δung1Δ</i>	50.9±7.4	8.5	3	4.0X10 ⁻³	0.84	0.01 ^c
<i>rnh1Δrnh201Δapn1Δ</i>	60.6±5.4	10.1	3	6.0X10 ⁻⁴	0.24	1.0X10 ^{-4d}
<i>rnh1Δrnh201Δexo1Δ</i>	112.2±13.5	18.7	3	1.4X10 ⁻³	0.01	0.02 ^e
<i>rnh1Δrnh201Δung1Δapn1Δ</i>	56.8±7.7	9.5	3	2.9X10 ⁻³	0.48	8.3X10 ^{-3c} 1.1X10 ^{-3d}
<i>rnh1Δrnh201Δung1Δexo1Δ</i>	108.9±13.5	18.2	3	1.6X10 ⁻³	0.02	2.7X10 ^{-3c} 0.02 ^e
NER genes						k, to <i>rad1Δ</i> l, to <i>rad2Δ</i> m, to <i>rad14Δ</i> n, to <i>rad26Δ</i> o, to <i>rad1Δrad2Δ</i>
<i>rad1Δ</i>	6.9±0.7	1.2	3	0.56	-	-
<i>rad2Δ</i>	19.9±3.0	3.3	3	0.01	-	-
<i>rad14Δ</i>	21.1±3.2	3.5	4	0.08	-	-
<i>rad26Δ</i>	14.1±1.1	2.4	3	9.4X10 ⁻³	-	-
<i>rad1Δrad2Δ</i>	18.5±5.6	3.1	3	0.09	-	0.11 ^k 0.83 ^l
<i>rnh1Δrnh201Δrad1Δ</i>	48.5±3.5	8.1	3	3.0X10 ⁻⁴	0.98	3.0X10 ^{-4k}
<i>rnh1Δrnh201Δrad2Δ</i>	65.4±9.4	10.9	3	3.3X10 ⁻³	0.23	0.01 ^l
<i>rnh1Δrnh201Δrad14Δ</i>	79.4±4.0	13.2	3	1.0X10 ⁻⁴	0.02	2.0X10 ^{-4m}
<i>rnh1Δrnh201Δrad26Δ</i>	46.1±3.6	7.7	3	5.0X10 ⁻⁴	0.97	1.1X10 ⁻³ⁿ
<i>rnh1Δrnh201Δrad1Δrad2Δ</i>	37.3±5.71	6.2	3	5.9X10 ⁻³	0.27	0.08 ^o
MMR genes						f, to <i>mlh1Δ</i> g, to <i>mlh3Δ</i> h, to <i>msh2Δ</i> i, to <i>pms1Δ</i> j, to <i>mlh1Δmlh3Δ</i> z, to <i>rnh1Δrnh201ΔMLH3-wt</i> <i>SK1</i>
<i>msh2Δ</i>	17.4±2.2	2.9	3	0.01	-	-

<i>pms1Δ</i>	18.1±2.0	3.0	3	6.8X10 ⁻³	-	-
<i>mlh1Δ</i>	11.2±2.2	1.9	4	0.12	-	-
<i>mlh3Δ</i>	15.4±2.3	2.6	3	0.03	-	-
<i>mlh1Δmlh3Δ</i>	13.1±3.0	2.2	3	0.09	-	0.61 ^f 0.58 ^g
<i>rnh1Δrnh201Δmsh2Δ</i>	45.8±4.2	7.6	5	4.0X10 ⁻⁴	0.71	2.7X10 ^{-3h}
<i>rnh1Δrnh201Δpms1Δ</i>	50.4±9.6	8.4	3	0.01	0.89	0.03 ⁱ
<i>rnh1Δrnh201Δmlh1Δ</i>	23.2±1.6	3.9	5	3.0X10 ⁻⁴	3.4X10 ⁻³	2.5X10 ^{-3f}
<i>rnh1Δrnh201Δmlh3Δ</i>	23.7±5.4	4.0	4	0.04	0.03	0.27 ^g
<i>rnh1Δrnh201Δmlh3-D523N</i>	23.8±2.0	4.0	3	1.7X10 ⁻³	0.03	1.9X10 ^{-3z}
<i>rnh1Δrnh201ΔMLH3-wt(SK1)</i>	42.3±1.6	7.1	3	1.0X10 ⁻⁴	0.42	-
<i>rnh1Δrnh201Δmlh1Δmlh3Δ</i>	30.7±9.4	5.1	3	0.06	0.19	0.15 ^j
<i>rnh1Δrnh201Δmlh1Δfcy1Δ</i>	38.3±1.7	6.4	3	1.0X10 ⁻⁴	0.22	3.0X10 ^{-4f} 7.0X10 ^{-4a}
<i>rnh1Δrnh201Δmlh1Δapn1Δ</i>	23.1±1.8	3.9	3	1.5X10 ⁻³	0.02	0.01 ^f 3.3X10 ^{-3d}
<i>rnh1Δrnh201Δmlh3Δfcy1Δ</i>	34.3±2.6	5.7	3	6.0X10 ⁻⁴	0.12	5.4X10 ^{-3g} 2.4X10 ^{-3a}
<i>rnh1Δrnh201Δmlh3Δapn1Δ</i>	39.9±5.6	6.7	3	4.1X10 ⁻³	0.38	0.02 ^g 2.3X10 ^{-3d}
NHEJ/HR/SSA genes						p, to <i>lif1Δ</i> q, to <i>rad51Δ</i> r, to <i>rad52Δ</i>
<i>lif1Δ</i>	12.3±0.4	2.1	3	0.01	-	-
<i>rad51Δ²</i>	25.8±5.2	4.3	4	0.28	-	-
<i>rad52Δ²</i>	25.3±6.7	4.2	4	0.10	-	-
<i>rnh1Δrnh201Δlif1Δ</i>	50.2±8.4	7.9	3	8.7X10 ⁻³	0.90	0.01 ^p
<i>rnh1Δrnh201Δrad51Δ</i>	44.4±3.8	7.4	3	6.0X10 ⁻⁴	0.62	0.24 ^q
<i>rnh1Δrnh201Δrad52Δ</i>	113.0±17.7	18.8	3	2.0X10 ⁻³	0.01	1.4X10 ^{-3r}

1, wild-type data are from (1). 2, data from (2). S.E.M: standard error of mean; Statistics by *t* test.

Supplementary Table 2. Fragility analysis of no tract (CAG-0) on the URA3-YAC

Strains	Rate of FOA ^R (X10 ⁻⁶) ± S.E.M	Fold over wt	No. of Assays	p-value to wt	p-value to <i>rnh1Δrnh201</i> <i>Δ</i>	other p-values z, to <i>rnh1Δrnh201Δ</i> , CAG-70 URA3-YAC
wild-type ¹	2.2±0.5	-	3	-	-	-
<i>rnh1Δrnh201Δ</i>	13.1±1.3	6.0	6	1.9X10 ⁻³	-	2.0X10 ^{-4z}
cytosine/cytidine deaminase						a, to <i>fcy1Δ</i>
<i>fcy1Δ</i>	4.2±1.6	1.8	4	0.36	-	-
<i>rnh1Δrnh201Δfcy1Δ</i>	8.7	4.0	3	0.15	0.18	0.27 ^a

1, wild-type data are from (1). S.E.M: standard error of mean; Statistics by *t* test.

Supplementary Table 3. Fragility analysis of CAG-70 repeats on the terminator-YACs

Strains	Terminator number	Rate of FOA ^R (X10 ⁻⁶) ± S.E.M	Fold over wt	No. of Assays	p-value to wt, same YAC	p-value to URA3-YAC
wild-type	1T	2.6±0.8	-	4	-	0.07
<i>rnh1Δrnh201Δ</i>	1T	20.4±3.1	7.8	3	0.01	0.02
wild-type	2T	3.5±0.8	-	4	-	0.15
<i>rnh1Δrnh201Δ</i>	2T	22.3±3.3	6.4	3	5.7X10 ⁻³	0.03

S.E.M: standard error of mean; Statistics by *t* test.

Supplementary Table 4. Instability analysis of the CAG-70 repeat tract on the URA3-YAC (or terminator-YACs if specified)

Strains	Total rxns.	Contractions					Expansions				
		#	%	Fold over wt	p-value to wt	other p-values y, to <i>rnh1Δrnh201Δ</i>	#	%	Fold over wt	p-value to wt	other p-values y, to <i>rnh1Δrnh201Δ</i>
wild-type ¹	460	20	4.3	-	-	-	5	1.1	-	-	-
wild-type 1T YAC ³	236	15	6.4	-	-	0.27 to wt, URA3-YAC	2	0.9	-	-	1.0 to wt, URA3-YAC
wild-type 2T YAC ³	238	12	5.0	-	-	0.70 to wt, URA3-YAC	3	1.3		-	0.70 to wt, URA3-YAC
<i>rnh1Δ</i>	156	25	16.0	3.7	1.0X10 ⁻⁴	0.88 ^y	7	4.5	4.1	7.7 X10 ⁻³	0.58 ^y
<i>rnh201Δ</i>	180	20	11.1	2.6	3.1X10 ⁻³	0.28 ^y	6	3.3	3.0	0.03	1.0 ^y
<i>rnh1Δrnh201Δ</i>	192	29	15.1	3.5	1.0X10 ⁻⁴	-	6	3.1	2.8	0.07	-
<i>rnh1Δrnh201Δ</i> 1T YAC ³	76	6	7.9	1.2	0.61	0.24 to <i>rnh1Δrnh201Δ</i> , URA3-YAC	2	2.6	2.9	0.25	0.26 to <i>rnh1Δrnh201Δ</i> , URA3-YAC
<i>rnh1Δrnh201Δ</i> 2T YAC ³	143	9	6.3	1.5	0.65	0.01 to <i>rnh1Δrnh201Δ</i> , URA3-YAC	3	2.1	1.9	0.40	0.68 to <i>rnh1Δrnh201Δ</i> , URA3-YAC
cytosine/cytidine deaminase											
<i>fcy1Δ</i>	192	13	6.8	1.6	0.24	a, to <i>fcy1Δ</i> b, to <i>cdd1Δ</i>	2	1.0	0.9	1.0	a, to <i>fcy1Δ</i> b, to <i>cdd1Δ</i>
<i>cdd1Δ</i>	143	11	7.7	1.8	0.13	-	3	2.1	1.9	0.40	-
<i>rnh1Δrnh201Δ fcy1Δ</i>	125	6	4.8	1.1	0.81	5.3X10 ^{-3y} 0.63 ^a	4	3.2	2.9	0.10	1.0 ^y 0.22 ^a
<i>rnh1Δrnh201Δ cdd1Δ</i>	146	16	11.0	2.5	7.6X10 ⁻³	0.33 ^y 0.42 ^b	4	2.7	2.5	0.23	1.0 ^y 1.0 ^b
<i>rnh1Δrnh201Δ fcy1Δcdd1Δ</i>	96	7	7.3	1.7	0.2919	0.06 ^y 1.0 ^a , 1.0 ^b	3	3.1	2.8	0.14	1.0 ^y 0.3 ^a , 0.69 ^b
BER genes											
<i>ung1Δ</i>	144	7	4.9	1.1	0.83	c, to <i>ung1Δ</i> d, to <i>apn1Δ</i> e, to <i>exo1Δ</i>	5	3.5	3.2	0.06	c, to <i>ung1Δ</i> d, to <i>apn1Δ</i> e, to <i>exo1Δ</i>
<i>apn1Δ</i>	144	11	7.6	1.8	0.13	-	3	2.1	1.9	0.40	-
<i>exo1Δ</i>	144	11	7.6	1.8	0.13	-	1	0.7	0.6	1.0	-
<i>rnh1Δrnh201Δ ung1Δ</i>	144	8	5.6	1.3	0.50	7.5X10 ^{-3y}	5	3.5	3.2	0.06	1.0 ^y
<i>rnh1Δrnh201Δ apn1Δ</i>	103	6	5.8	1.4	0.60	0.02 ^y	2	1.9	1.8	0.62	0.72 ^y
<i>rnh1Δrnh201Δ exo1Δ</i>	142	13	9.2	2.1	0.03	0.13 ^y	8	5.6	5.1	3.4X10 ⁻³	0.28 ^y
<i>rnh1Δrnh201Δ ung1Δapn1Δ</i>	111	6	5.4	1.3	0.61	0.01 ^y	2	1.8	1.6	0.63	0.71 ^y

<i>rnh1Δrnh201Δung1Δfcy1Δ</i>	47	4	8.5	2.0	N/D	N/D	2	4.3	3.9	N/D	N/D
<i>rnh1Δrnh201Δung1Δexo1Δ</i>	144	17	11.8	2.7	2.3X10 ⁻³	0.43 ^y	3	2.1	1.9	0.40	0.73 ^y
NER genes						k, to <i>rad1Δ</i> l, to <i>rad2Δ</i> m, to <i>rad14Δ</i> n, to <i>rad26Δ</i> o, to <i>rad1Δrad2Δ</i>					k, to <i>rad1Δ</i> l, to <i>rad2Δ</i> m, to <i>rad14Δ</i> n, to <i>rad26Δ</i> o, to <i>rad1Δrad2Δ</i>
<i>rad1Δ</i>	144	11	7.6	1.8	0.04	-	1	0.7	0.6	1.0	-
<i>rad2Δ</i>	144	4	2.8	0.6	0.47	-	2	1.4	1.3	0.63	-
<i>rad14Δ</i>	153	13	8.5	2.0	0.06	-	10	6.5	5.9	7.0X10 ⁻⁴	-
<i>rad26Δ</i>	80	7	8.8	2.0	0.10	-	3	3.8	3.5	0.10	-
<i>rad1Δrad2Δ</i>	141	12	8.5	2.0	0.08	0.83 ^k , 0.04 ^l	4	2.8	2.6	0.23	0.21 ^k , 0.44 ^l
<i>rnh1Δrnh201Δrad1Δ</i>	140	8	5.7	1.3	0.50	7.7X10 ^{-3y} 0.64 ^k	4	2.9	2.6	0.22	0.22 ^y 0.64 ^k
<i>rnh1Δrnh201Δrad1Δapn1Δ</i>	112	8	7.1	1.6	0.22	0.045 ^y , 1 ^k 1 ^d	1	0.9	0.8	1	0.27 ^y , 1 ^k 0.63
<i>rnh1Δrnh201Δrad2Δ</i>	168	14	8.3	1.9	0.07	0.05 ^y 5.0X10 ^{-3l}	5	3.0	2.7	0.14	0.14 ^y 0.6 ^l
<i>rnh1Δrnh201Δrad14Δ</i>	144	18	12.5	2.9	1.2X10 ⁻³	0.53 ^y 0.34 ^m	4	2.8	2.5	0.23	0.23 ^y 0.17 ^m
<i>rnh1Δrnh201Δrad26Δ</i>	91	10	11.0	2.5	0.02	0.46 ^y 1.0 ⁿ	4	4.4	4	0.05	0.73 ^y 1.0 ⁿ
<i>rnh1Δrnh201Δrad1Δrad2Δ</i>	143	9	6.3	1.5	0.37	0.01 ^y 0.50 ^o	5	3.5	3.2	0.06	0.06 ^y 1.0 ^o
<i>rnh1Δrnh201Δrad1Δapn1Δ</i>	112	7	6.3	1.5	0.45	0.03 ^y , 0.81 ^d 0.32 ^k	1	0.9	0.8	1	1.0 ^y , 0.63 ^d 1.0 ^k
<i>rnh1Δrnh201Δrad2Δapn1Δ</i>	94	5	5.3	1.2	0.59	0.02 ^y , 0.60 ^d 0.32 ^l	2	2.1	1.9	0.33	1.0 ^y , 1.0 ^d 0.65 ^l
MMR genes						f, to <i>mlh1Δ</i> g, to <i>mlh3Δ</i> h, to <i>msh2Δ</i> i, to <i>pms1Δ</i> j, to <i>mlh1Δmlh3Δ</i> z, to <i>rnh1Δrnh201ΔMLH1-wt(SK1)</i>					f, to <i>mlh1Δ</i> g, to <i>mlh3Δ</i> h, to <i>msh2Δ</i> i, to <i>pms1Δ</i> j, to <i>mlh1Δmlh3Δ</i> z, to <i>rnh1Δrnh201ΔMLH1-wt(SK1)</i>
<i>msh2Δ</i>	144	19	13.2	3.1	6.0X10 ⁻⁴	-	8	5.6	5.1	3.7X10 ⁻³	-
<i>pms1Δ</i>	144	9	6.3	1.5	0.37	-	7	4.9	4.4	0.01	-
<i>mlh1Δ</i>	192	14	7.3	1.7	0.13	-	6	3.1	2.8	0.07	-
<i>mlh3Δ</i>	150	12	8.0	1.9	0.10	-	3	2.0	1.8	0.43	-
<i>mlh1Δmlh3Δ</i>	95	7	7.4	1.7	0.20	1.0 ^f 0.81 ^g	0	0.0	0.0	0.59	0.18 ^f 0.28 ^g
<i>rnh1Δrnh201Δmsh2Δ</i>	238	30	12.6	2.9	1.0X10 ⁻⁴	0.48 ^y 0.88 ^h	18	7.6	6.9	1.0X10 ⁻⁴	1.0 ^y 0.53 ^h
<i>rnh1Δrnh201Δpms1Δ</i>	143	16	11.2	2.6	4.4X10 ⁻³	0.33 ^y 0.15 ⁱ	13	9.1	8.3	1.0X10 ⁻⁴	0.03 ^y 0.17 ⁱ
<i>rnh1Δrnh201Δmlh1Δ</i>	236	40	16.9	3.9	1.0X10 ⁻⁴	0.69 ^y 3.2X10 ^{-3f}	10	4.2	3.9	7.1X10 ⁻³	0.62 ^y 0.62 ^f
<i>rnh1Δrnh201Δmlh3Δ</i>	136	6	4.4	1.0	1	1.8X10 ^{-3y} 0.23 ^g	5	3.7	3.3	0.05	0.78 ^y 0.48 ^g
<i>rnh1Δrnh201Δmlh3-D523N</i>	143	10	7.0	1.6	0.27	0.25 ^y 0.30 ^z	4	2.8	2.5	0.23	1.0 ^y 1.0 ^z
<i>rnh1Δrnh201ΔMLH3-wt(SK1)</i>	144	16	11.1	2.6	4.6X10 ⁻³	0.33 ^y	4	2.8	2.5	0.23	1.0 ^y
<i>rnh1Δrnh201Δmlh1Δmlh3Δ</i>	142	18	12.7	2.9	1.1X10 ⁻³	0.63 ^y 0.28 ^j	3	2.1	1.9	0.40	0.74 ^y 0.28 ^j
<i>rnh1Δrnh201Δmlh1Δfcy1Δ</i>	140	14	10.0	2.3	0.02	0.19 ^y 0.43 ^f , 0.31 ^a	4	2.9	2.6	0.22	1.1 ^y 0.28 ^f , 0.24 ^a
<i>rnh1Δrnh201Δmlh1Δapn1Δ</i>	112	11	9.8	2.3	0.03	0.20 ^y 0.52 ^f , 0.65 ^d	4	3.6	3.3	0.08	1.0 ^y 1.0 ^f , 0.70 ^d
<i>rnh1Δrnh201Δmlh3Δfcy1Δ</i>	134	14	10.4	2.4	0.01	0.25 ^y 0.54 ^g , 0.31 ^a	5	3.7	3.4	0.05	0.77 ^y 0.48 ^g , 0.13 ^a
<i>rnh1Δrnh201Δmlh3Δapn1Δ</i>	95	10	10.5	2.4	0.02	0.36 ^y 0.50 ^g , 0.49 ^d	1	1.0	0.9		0.43 ^y 1.0 ^g , 1.0 ^d
NHEJ/HR/SSA genes						p, to <i>lif1Δ</i> q, to <i>rad51Δ</i>					p, to <i>lif1Δ</i> q, to <i>rad51Δ</i>

						r, to <i>rad52Δ</i>					r, to <i>rad52Δ</i>	
<i>lif1Δ</i> ²	95	12	12.6	2.9	5.7X10 ⁻³	-	6	6.3	5.7	4.8X10 ⁻³	-	
<i>rad51Δ</i> ²	187	18	9.6	2.2	0.02	-	4	2.1	1.9	0.29	-	
<i>rad52Δ</i> ²	188	19	10.1	2.4	0.01	-	4	2.1	1.9	0.29	-	
<i>rnh1Δrnh201Δ</i> <i>lif1Δ</i>	96	13	13.5	3.1	1.6X10 ⁻³	0.86 ^y 1.0 ^p	2	2.1	1.9	0.35	0.72 ^y 0.19 ^p	
<i>rnh1Δrnh201Δ</i> <i>rad51Δ</i>	96	12	12.5	2.9	5.9X10 ⁻³	0.60 ^y 0.54 ^q	2	2.1	1.9	0.35	0.72 ^y 1.0 ^q	
<i>rnh1Δrnh201Δ</i> <i>rad52Δ</i>	168	27	16.1	3.7	1.0X10 ⁻⁴	1.0 ^y 5.0X10 ^{-4r}	11	6.5	6.0	5.0X10 ⁻⁴	0.14 ^y 0.06 ^r	

1, Wild-type data are combined data from (1, 2); 2, Data are from (3); 3, 1T and 2T refer to YACs containing 1 terminator or 2 terminators, respectively. "Total rxns" means total reactions done (total number of clones checked). N/D in p-value column means the sample size (total rxns) is lower than 50 clones, therefore not enough to achieve statistical accuracy. Statistics by Fisher's exact test.

Supplementary Table 5. PCR analysis of *URA3* presence in FOA-resistant colonies.

Strain	Presence of <i>URA3</i>		Total number of FOA ^R colonies ¹	Percent end loss (no <i>URA3</i>)
	number	percent		
wild-type	0	0%	20	100%
<i>rnh1Δrnh201Δ</i>	0	0%	20	100%
<i>fcy1Δ</i>	0	0%	30	100%
<i>rnh1Δrnh201Δfcy1Δ</i>	0	0%	20	100%
<i>apn1Δ</i>	0	0%	18	100%
<i>rnh1Δrnh201Δapn1Δ</i>	1	5.5	18	94.5%
<i>rnh1Δrnh201Δung1Δ</i>	0	0%	20	100%
<i>rnh1Δrnh201Δ apn1Δung1Δ</i>	0	0%	16	100%
<i>rnh1Δrnh201Δ exo1Δung1Δ</i>	0	0%	20	100%
<i>msh2Δ</i>	8	26.7%	30	73.3%
<i>pms1Δ</i>	N/D	N/D	N/D	N/D
<i>mlh1Δ</i>	8	26.7%	30	73.3%
<i>mlh3Δ</i>	2	6.7%	30	93.3%
<i>mlh1Δ mlh3Δ</i>	5	16.7%	30	83.3%
<i>rnh1Δrnh201Δmsh2Δ</i>	3	10%	30	90%
<i>rnh1Δrnh201Δpms1Δ</i>	1	3.3%	30	96.7%
<i>rnh1Δrnh201Δmlh1Δ</i>	6	20%	30	80%
<i>rnh1Δrnh201Δmlh3Δ</i>	1	3.3%	30	96.7%
<i>rnh1Δrnh201Δmlh1Δmlh3Δ</i>	4	13.3%	30	86.7%
<i>rnh1Δrnh201Δmlh1Δfcy1Δ</i>	0	0%	30	100%
<i>rnh1Δrnh201Δmlh3Δfcy1Δ</i>	0	0%	29	100%
<i>rnh1Δrnh201Δmlh3Δapn1Δ</i>	0	0%	30	100%
<i>rnh1Δrnh201Δrad1Δrad2Δ</i>	0	0%	30	100%
<i>rnh1Δrnh201Δrad14Δ</i>	0	0%	30	100%

1, Only one FOA^R colony per plate was tested, assuring that each event was independent (For 10-colony assays,

each culture plated on each FOA-Leu plate is from an individual parent colony). Highlighted area contains data for MMR defective mutants. PCR methods and primers used for checking *URA3* locus are listed in supplementary methods. Data is for the CAG-70 *URA3*-YAC.

Supplementary Table 6. Primers used in this study

Locus	Primer name	Oligonucleotide sequence
Cross CAG (instability)	NewCAGfor	CCTCAGCCTGGCCGAAAGAAAAGAAA
	NewCAGrev	CAGTCACGACGTTGTAAAACGACGG
Cross CAG (qPCR)	T7-20B	GAATTCGAGCTCCACCGCGG
	CTG rev2	CCCAGGCCTCCAGTTTGC
Cross G4T4 (qPCR)	G4T4 right 65bp	CCTGTCGTGCCAGTGTATAC
	G4T4 left 90bp	GTGGCCAGGACCCAACGCTG
URA3 internal 1 (qPCR)	Yif5 CAG for	GGGTCAACAGTATAGAACCGTG
	Yif5-CAG rev	TCAAATATGCTTCCAGCCTGC
MMR1 internal (qPCR)	MMR1 internal for	GCCCTAAGACTAGACTGGCAC
	MMR1 internal rev	GCAGAAGTTGGCTCCTCTTC
ACT1 internal (qPCR)	ACT1for3	TCCAGATGGTCAAGTCATCA
	ACT1rev3	TCGGCAATACCTGGGAACAT
URA3 internal 2 (check presence)	URA3 for2	TGCTGCTACTCATCCTAG
	URA3 rev	TCCAGCCTGCTTTTCTGTA

Supplementary Table 7. Yeast strains used in this study.

Strain Number	Strain Background	Genotype	Reference
CFY1161 CFY765	BY4705	<i>MATα</i> , <i>ade2Δ</i> :: <i>hisG</i> , <i>his3Δ200</i> ; <i>leu2Δ</i> , <i>lys2Δ</i> , <i>met15Δ</i> , <i>trp1Δ63</i> , <i>ura3Δ</i> , <i>can^R</i> ; YAC: <i>ade3-2p</i> , <i>LEU2</i> , CAG-0, <i>URA3</i> ,	(1, 2)
CFY1162 CFY766	BY4705	<i>MATα</i> , <i>ade2Δ</i> :: <i>hisG</i> , <i>his3Δ200</i> , <i>leu2Δ</i> , <i>lys2Δ</i> , <i>met15Δ</i> , <i>trp1Δ63</i> , <i>ura3Δ</i> , <i>can^R</i> ; YAC: <i>ade3-2p</i> , <i>LEU2</i> , CAG-70, <i>URA3</i> ,	(1, 2)
CFY3620, 3621	BY4705	CFY765 <i>mh1Δ</i> :: <i>HisMX6</i>	This study
CFY3412	BY4705	CFY765 <i>mh201Δ</i> :: <i>TRP1</i>	This study
CFY3641	BY4705	CFY3620 <i>mh201Δ</i> :: <i>TRP1</i>	This study
CFY3036, 3037	BY4705	CFY766 <i>mh1Δ</i> :: <i>HisMX6</i>	This study
CFY3413	BY4705	CFY766 <i>mh201Δ</i> :: <i>TRP1</i>	This study
CFY3418,	BY4705	CFY3036 <i>mh201Δ</i> :: <i>TRP1</i>	This study

3419			
CFY3684, 3685	BY4705	CFY765 <i>fcy1Δ::natNT2</i>	This study
CFY3686, 3687	BY4705	CFY3641 <i>fcy1Δ::natNT2</i>	This study
CFY3655, 3656	BY4705	CFY766 <i>fcy1Δ::natNT2</i>	This study
CFY3657, 3658	BY4705	CFY3618 <i>fcy1Δ::natNT2</i>	This study
CFY3701, 3702	BY4705	CFY766 <i>cdd1Δ::HPH</i>	This study
CFY3703, 3704	BY4705	CFY3418 <i>cdd1Δ::HPH</i>	This study
CFY3705, 3706	BY4705	CFY3657 <i>cdd1Δ::HPH</i>	This study
CFY3707	BY4705	CFY766 <i>ung1Δ::KanMX6</i>	This study
CFY3916	BY4705	CFY766 <i>apn1Δ::KanMX6</i>	This study
CFY3866, 3867	BY4705	CFY766 <i>exo1Δ:: KanMX6</i>	This study
CFY3708, 3709	BY4705	CFY3418 <i>ung1Δ::HPH</i>	This study
CFY3863, 3874	BY4705	CFY3418 <i>apn1Δ::KanMX6</i>	This study
CFY3868, 3869	BY4705	CFY3148 <i>exo1Δ:: KanMX6</i>	This study
CFY3864, 3875	BY4705	CFY3708 <i>apn1Δ::KanMX6</i>	This study
CFY3870, 3871	BY4705	CFY3708 <i>exo1Δ::KanMX6</i>	This study
CFY3715, 3716	BY4705	CFY766 <i>msh2Δ::KanMX6</i>	This study
CFY3855, 3856	BY4705	CFY766 <i>pms1Δ::KanMX6</i>	This study
CFY3752, 3753, 3754	BY4705	CFY766 <i>mlh1Δ::natNT2</i>	This study
CFY3933, 3934	BY4705	CFY766 <i>mlh3Δ::KanMX6</i>	This study
CFY3939	BY4705	CFY3752 <i>mlh3Δ::KanMX6</i>	This study
CFY3719, 3720	BY4705	CFY3418 <i>msh2Δ::KanMX6</i>	This study
CFY3797,	BY4705	CFY3418 <i>pms1Δ::KanMX6</i>	This study

3824, 3825			
CFY3755, 3756, 3757	BY4705	CFY3418 <i>mlh1Δ::natNT2</i>	This study
CFY3935, 3936	BY4705	CFY3418 <i>mlh3Δ::KanMX6</i>	This study
CFY4005, 4006	BY4705	CFY3418 <i>mlh3Δ::natNT2</i>	This study
CFY3937, 3938	BY4705	CFY3755 <i>mlh3Δ::KanMX6</i>	This study
CFY3758, 3759	BY4705	CFY3757 <i>fcy1Δ::KanMX6</i>	This study
CFY4000, 4001, 4002	BY4705	CFY3657 <i>mlh3Δ::KanMX6</i>	This study
CFY4029, 4030	BY4705	CFY4005 <i>mlh3Δ:: natNT2::SK1-mlh3D523N-KanMX6</i>	This study
CFY4027, 4028	BY4705	CFY4005 <i>mlh3::NAT::SK1-MLH3wt-KanMX6</i>	This study
CFY3876	BY4705	CFY3757 <i>apn1Δ::KanMX6</i>	This study
CFY4123, 4124	BY4705	CFY3935 <i>apn1Δ::HPH</i>	This study
CFY3809	BY4705	CFY766 <i>rad1Δ::HPH</i>	This study
CFY3710	BY4705	CFY766 <i>rad2Δ::KanMX6</i>	This study
CFY3961	BY4705	CFY766 <i>rad14Δ::KanMX6</i>	This study
CFY4046, 4047	BY4705	CFY3418 <i>rad26Δ::KanMX6</i>	This study
CFY3841, 3842	BY4705	CFY3710 <i>rad1Δ::HPH</i>	This study
CFY3839, 3840	BY4705	CFY3418 <i>rad1Δ::HPH</i>	This study
CFY3688	BY4705	CFY3418 <i>rad2Δ::KanMX6</i>	This study
CFY3843, 3844, 3845	BY4705	CFY3688 <i>rad1Δ::HPH</i>	This study
CFY4309	BY4705	CFY3688 <i>apn1Δ::HPH</i>	This study
CFY4311, 4312	BY4705	CFY3683 <i>rad1Δ::HPH</i>	This study
CFY3962	BY4705	CFY3418 <i>rad14Δ::KanMX6</i>	This study
CFY4048, 4049	BY4705	CFY3418 <i>rad26Δ::KanMX6</i>	This study
CFY2920, 2921	BY4705	CFY1162 <i>lif1Δ::His3MX6</i>	(3)
CFY3798	BY4705	CFY3418 <i>lif1Δ::KanMX6</i>	This study

CFY3659, 3660	BY4705	CFY3418 <i>rad51Δ::natNT2</i>	This study
CFY3730	BY4705	CFY3730 <i>rad52Δ::KanMX6</i>	This study
CFY3555	BY4705	<i>MATα, ade2Δ:: hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can^R</i> ; YAC: <i>ade3-2p, LEU2, CAG-70-Tcyc1 –URA3</i>	This study
CFY3580	BY4705	<i>MATα, ade2Δ:: hisG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, can^R</i> ; YAC: <i>ade3-2p, LEU2, Ttef1-CAG-70-Tcyc1 –URA3</i>	This study
CFY3644, 3645	BY4705	CFY3555 <i>rnh1Δ::HisMX6, rnh201Δ::TRP1</i>	This study
CFY3646	BY4705	CFY3580 <i>rnh1Δ::HisMX6, rnh201Δ::TRP1</i>	This study
CFY3801	BY4705	CFY766 Fcy1-1Myc/KanMX6	This study
CFY3803, 3804	BY4705	CFY3418 Fcy1-1Myc/KanMX6	This study
CFY3966, 3967, 3968	BY4705	CFY3580 Fcy1-1Myc/KanMX6	This study
CFY3807, 3808	BY4705	CFY3646 Fcy1-1Myc/KanMX6	This study

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