Supplementary Material for

A *Saccharomyces cerevisiae* Ribonuclease H2 interaction network functions to suppress genome instability

By

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Supplementary Figures.

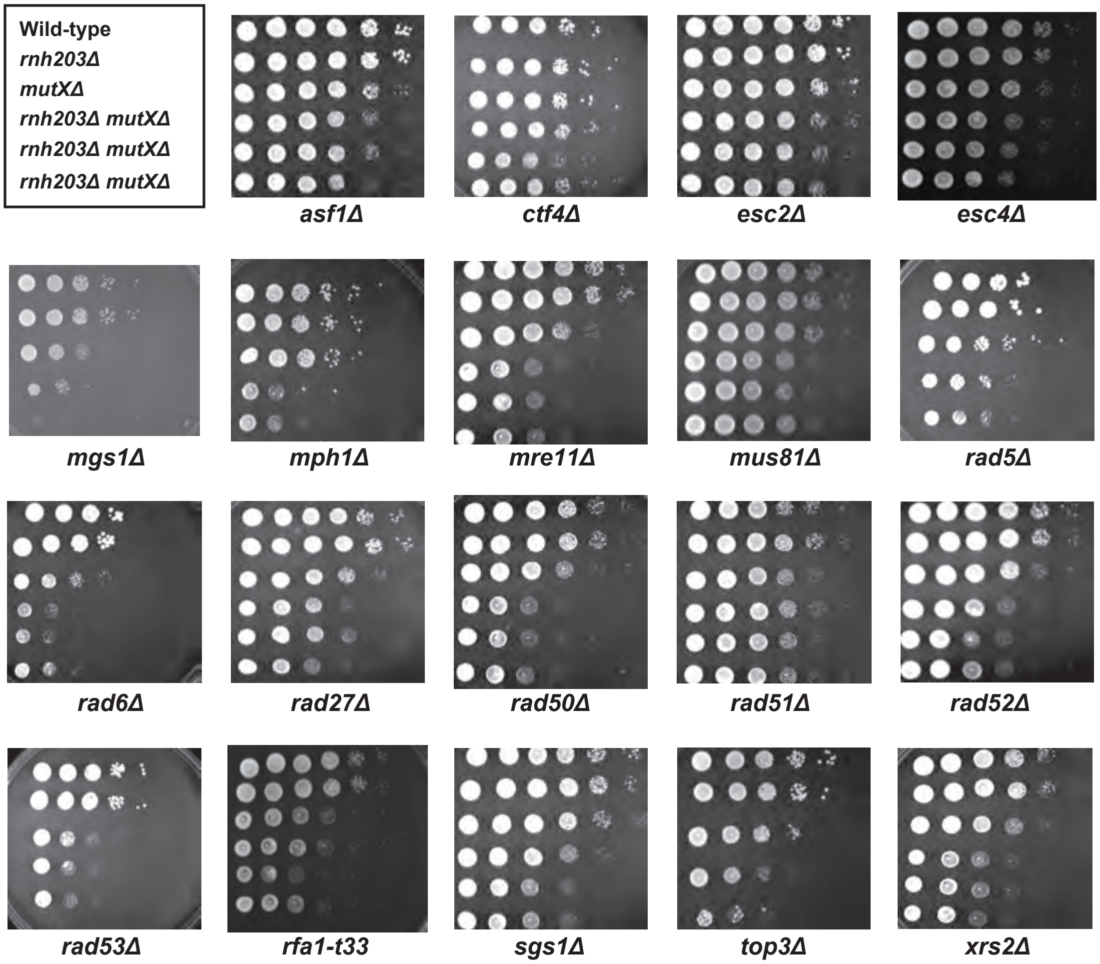
Figure S1. Analysis of growth phenotypes caused by RNase H2 defects in combination with different DNA metabolism defects. Cultures were diluted to either 1 × 10^6 cells/mL or 1 × 10^5 cells/mL and then 2 µl from ten-fold serial dilutions were spotted in each row. Strains were spotted onto each plate as follows: top row - wild-type, second row - the *rnh203Δ* single mutant, third row - the query single mutant (*mutXΔ*), fourth through sixth rows - independent isolates of the double mutant.

Figure S2. RNase H2 defects do not affect growth of some DNA metabolism mutants. Cultures were diluted to either 1 × 10^6 cells/mL or 1 × 10^5 cells/mL and then 2 µl from ten-fold serial dilutions were spotted in each row. Strains were spotted onto each plate as follows: top row - wild-type, second row - the *rnh203Δ* single mutant, third row - the query single mutant (*mutXΔ*), fourth through sixth rows - independent isolates of the double mutant.

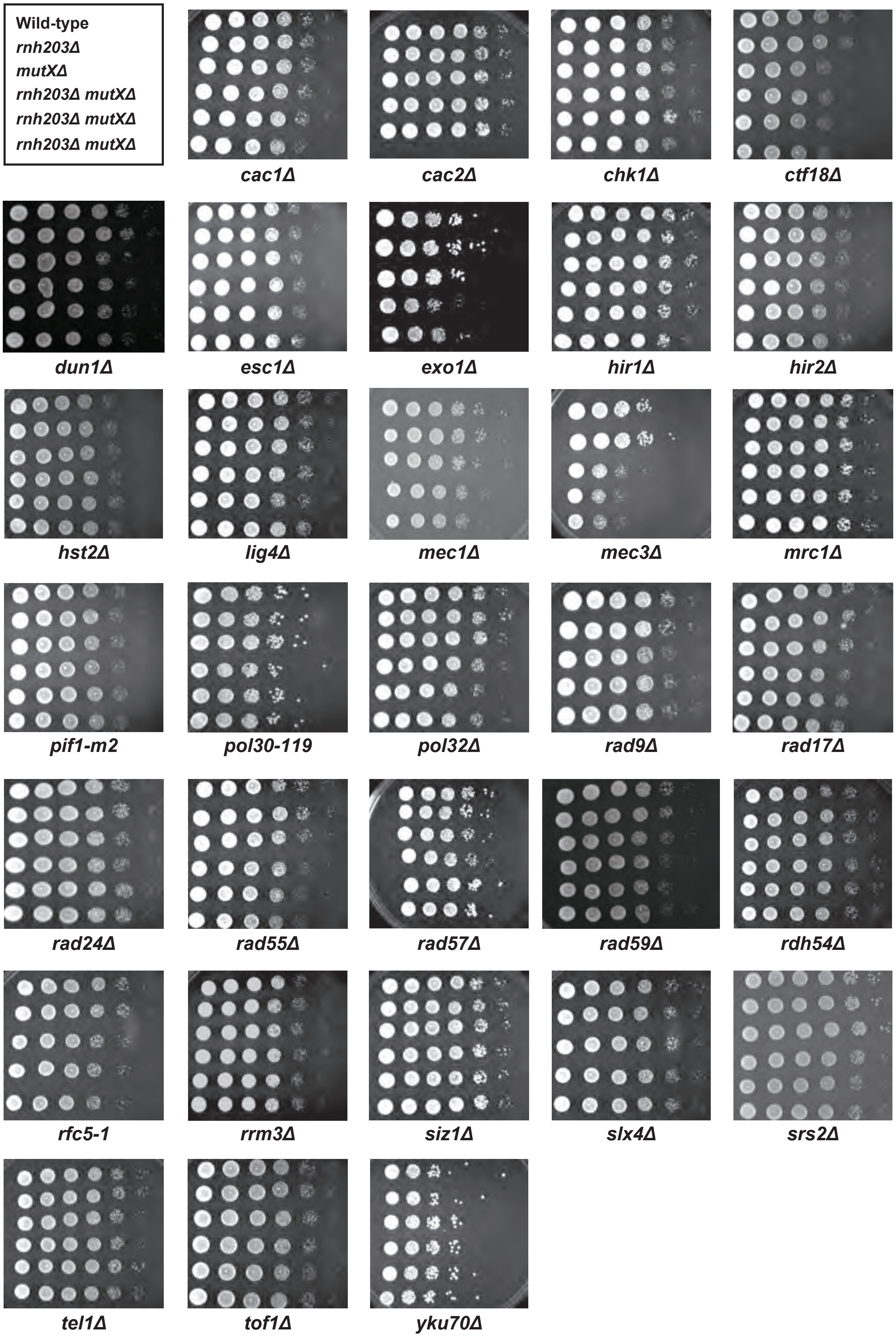
Figure S3. RNase H2 defects in combination with some DNA metabolism defects can cause the accumulation of cells with an aberrant morphology phenotype. The budding index was measured to determine percentage of cells in each phase of the cell cycle and percentage of cells with an aberrant morphology phenotype. Data for the indicated mutant strains are presented as histograms with error bars indicating the standard deviation.

Figure S4. RNaseH2 defects do not cause an aberrant morphology phenotype in combination with some DNA metabolism defects. The budding index was measured to determine percentage of cells in each phase of the cell cycle and percentage of cells with an aberrant morphology phenotype. Data for the indicated mutant strains are presented as histograms with error bars indicating the standard deviation.

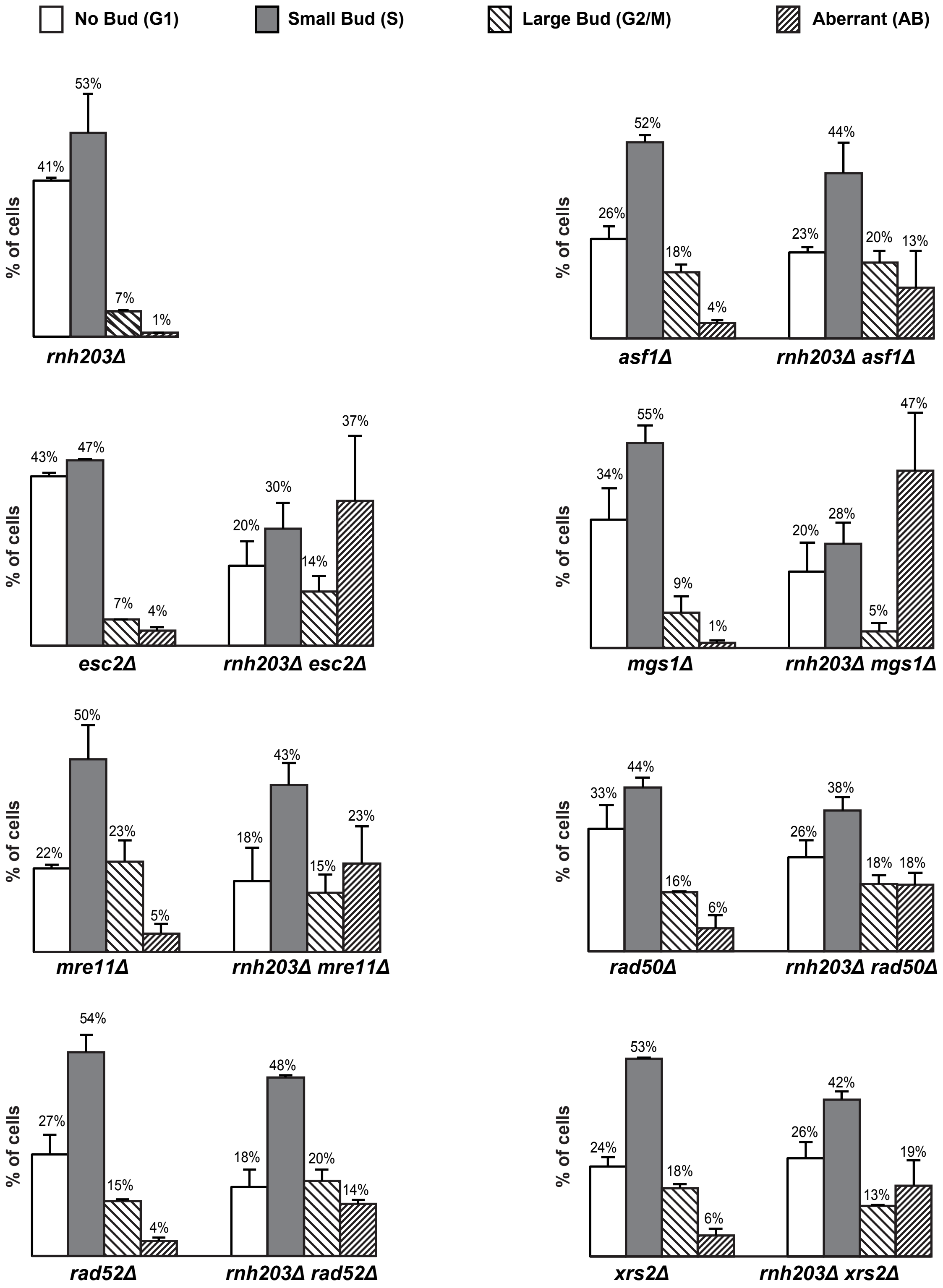
Supplemental Figure 1.



Supplemental Figure 2.



Supplemental Figure 3.



Supplemental Figure 4.

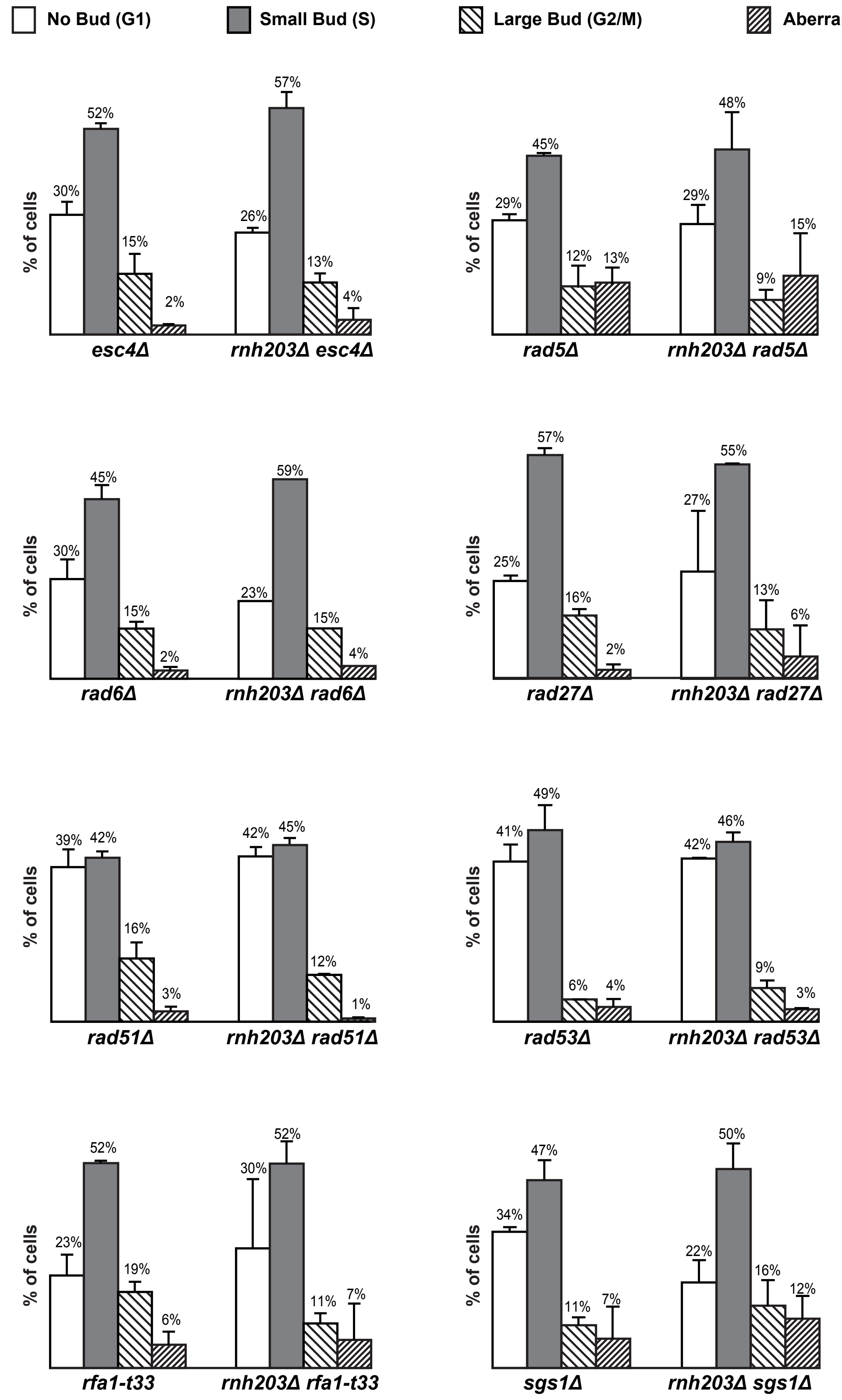


Table S1. Effect of RNase H2 defects on mutation rates.

Genotype (RDKY #)	***Can^r***	***hom3-10***	***lys2ΔBgl***	***GCR***
Wild-type (7239)	6.0 [0.7-12.5] × 10^{-7} (1)	1.9 [1.4-40.1] × 10^{-9} (1)	6.8 [1.7-25.5] × 10^{-9} (1)	3.6 [1.9-14.0] × 10^{-10} (1)
rnh201Δ (7235)	3.6 [2.2-9.8] × 10^{-7} (0.6)	4.3 [2.9-6.3] × 10^{-9} (2.3)	1.1 [0.6-2.3] × 10^{-8} (1.6)	4.7 [2.4-14.0] × 10^{-10} (1.3)
rnh202Δ (7237)	3.7 [2.4-5.6] × 10^{-7} (0.6)	5.7 [3.6-8.8] × 10^{-9} (3.0)	1.1 [0.7-1.9] × 10^{-8} (1.6)	3.3 [2.2-7.1] × 10^{-10} (0.9)
rnh203Δ (7209)	3.2 [2.2-3.9] × 10^{-7} (0.5)	7.5 [2.9-10.6] × 10^{-9} (3.9)	1.0 [0.7-1.5] × 10^{-8} (1.5)	2.0 [0.0-8.9] × 10^{-10} (0.5)

All strains are isogenic to RDKY7239 (*MATα, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2ΔBgl, hom3-10, ade2::hisG, ade8, hxt13::URA3*). The numbers in parentheses ( ) are the fold increase relative to RDKY7239. Numbers in brackets [ ] represent the 95% confidence intervals.

Table S2. Mutation spectrum analysis of an *rnh203*Δ mutant strain.

Genotype	***Mutation****	***Frequency†***	***%***	***Rate***
Wild-type	Single BS	60/94	63.8	35×10^{-8}
	GC to TA	19/94	20.2	11×10^{-8}
	GC to AT	15/94	16.0	8.8×10^{-8}
	GC to CG	15/94	16.0	8.8×10^{-8}
	AT to TA	7/94	7.4	4.1×10^{-8}
	AT to CG	2/94	2.1	1.2×10^{-8}
	AT to GC	2/94	2.1	$1.2 X 10^{-8}$
	-1 Frameshift	15/94	16.0	8.8×10^{-8}
	+1 Frameshift	2/94	2.1	1.2×10^{-8}
	Double BS	1/94	1.1	0.6×10^{-8}
	Double BD	0/94	<1.1	$<0.6 \times 10^{-8}$
	Large Deletion	12/94	10.6	5.8×10^{-8}
	Large Insertion	0/94	<1.1	$<0.6 \times 10^{-8}$
	Complex	4/94	6.4	3.5×10^{-8}
rnh203Δ	Single BS	47/82	57.3	18×10^{-8}
	GC to TA	12/82	14.6	4.7×10^{-8}
	GC to AT	6/82	7.3	2.3×10^{-8}
	GC to CG	14/82	17.1	5.5×10^{-8}
	AT to TA	6/82	7.3	2.3×10^{-8}
	AT to CG	6/82	7.3	2.3×10^{-8}
	AT to GC	3/82	3.7	1.2×10^{-8}
	-1 Frameshift	19/82	23.2	7.4×10^{-8}
	+1 Frameshift	3/82	3.7	1.2×10^{-8}
	Double BS	0/82	<1.2	$<0.4 \times 10^{-8}$
	Double BD	4/82	4.9	1.6×10^{-8}
	Large Deletion	3/82	2.4	1.2×10^{-8}
	Large Insertion	2/82	2.4	0.8×10^{-8}
	Complex	4/82	6.1	1.6×10^{-8}

* BS, Base Substitution; BD, Base Deletion; Large Deletion or insertion, Deletions or insertions of greater than 2 bases; Complex, Multiple base substitution and/or frameshift mutations. All Double BD were found in short dinucleotide repeat sequences.

† The number of times each type of mutation occurred is listed under frequency.

Table S3. Effect of an RNase H2 mutation on the rate of accumulating GCRs.

	Wild-type		***rnh203Δ***	
Genotype	***RDKY #***	***GCR Rate***	***RDKY #***	***GCR Rate***
Wild-type	7239	3.6 [1.9-14.0] $\times 10^{-10}$ (1)	7209	2.0 [0.0-8.9] $\times 10^{-10}$ (0.5)
dun1Δ	7240	8.9 [3.1-62.0] $\times 10^{-8}$ (247)	7242	1.2 [0.9-3.0] $\times 10^{-7}$ (333)
hir1Δ	7261	7.0 [5.0-40.1] $\times 10^{-10}$ (2)	7263	5.2 [4.5-7.9] $\times 10^{-10}$ (1)
mec1Δ	7302	2.1 [0.8-5.8] $\times 10^{-8}$ (58)	7304	2.0 [0.1-8.3] $\times 10^{-8}$ (55)
mec3Δ	7277	< 2.4 [1.9-2.8] $\times 10^{-9}$ (6)	7304	5.7 [2.3-11.5] $\times 10^{-9}$ (15)
mgs1Δ	7281	< 5.3 [4.6-10.1] $\times 10^{-10}$ (1)	7283	< 1.1 [0.8-1.5] $\times 10^{-9}$ (3)
mph1Δ	7286	1.1 [0.6-5.6] $\times 10^{-9}$ (3)	7288	< 1.2 [1.1-2.8] $\times 10^{-9}$ (3)
mrc1Δ	7298	4.3 [1.6-23.0] $\times 10^{-9}$ (12)	7300	2.1 [1.0-9.6] $\times 10^{-9}$ (6)
pif1-m2	7314	3.1 [2.2-4.7] $\times 10^{-8}$ (86)	7312	2.6 [1.7-5.6] $\times 10^{-8}$ (72)
rad5Δ	7336	2.4 [1.1-98.5] $\times 10^{-9}$ (7)	7338	< 2.6 [1.8-9.9] $\times 10^{-9}$ (7)
rad17Δ	7324	4.9 [3.2-10.2] $\times 10^{-9}$ (14)	7326	6.9 [4.8-24.8] $\times 10^{-9}$ (19)
rad27Δ	7332	1.1 [0.7-2.0] $\times 10^{-7}$ (306)	7335	2.2 [0.7-3.1] $\times 10^{-7}$ (611)
rad51Δ	7344	3.5 [1.5-7.9] $\times 10^{-9}$ (10)	7346	3.7 [1.8-21.6] $\times 10^{-9}$ (10)
rad51Δ rad59Δ	7497	1.2 [0.6-2.7] $\times 10^{-8}$ (33)	7499	1.6 [0.2-3.7] $\times 10^{-8}$ (44)
rad52Δ	7348	6.0 [0.3-10.4] $\times 10^{-8}$ (167)	7350	7.1 0.5-17.2] $\times 10^{-8}$ (197)
rad53Δ	7370	7.4 [3.7-10.2] $\times 10^{-9}$ (21)	7372	5.4 [2.4-8.2] $\times 10^{-9}$ (15)
rad55Δ	7374	3.2 [1.5-16.5] $\times 10^{-9}$ (9)	7376	5.3 [3.3-16.7] $\times 10^{-9}$ (15)
rad59Δ	7382	4.5 [1.7-27.0] $\times 10^{-9}$ (13)	7383	1.8 [0.3-3.5] $\times 10^{-8}$ (50)
rfa1-t33	7397	1.2 [0.9-1.7] $\times 10^{-7}$ (325)	7399	8.6 [5.3-11.4] $\times 10^{-8}$ (239)
rrm3Δ	7410	1.4 [0.9-5.5] $\times 10^{-9}$ (4)	7412	2.2 [1.3-3.1] $\times 10^{-9}$ (6)
siz1Δ	7418	1.9 [0.8-5.4] $\times 10^{-9}$ (5)	7420	4.7 [1.7-10.3] $\times 10^{-9}$ (13)
slx4Δ	7426	1.8 [0.6-3.0] $\times 10^{-8}$ (37)	7428	1.8 [1.3-4.3] $\times 10^{-8}$ (38)
srs2Δ	7430	6.1 [4.9-38.0] $\times 10^{-10}$ (2)	7432	6.5 [5.6-14.9] $\times 10^{-10}$ (2)
top1Δ	8143	7.6 [1.6-56.6] $\times 10^{-10}$ (2)	8141	5.6 [2.5-30.1] $\times 10^{-10}$ (3)
top3Δ	7442	1.5 [1.2-27.0] $\times 10^{-9}$ (4)	7444	1.8 [1.3-6.2] $\times 10^{-9}$ (5)
yku70Δ	7450	8.9 [6.5-16.4] $\times 10^{-10}$ (3)	7452	1.1 [0.8-1.3] $\times 10^{-9}$ (3)

All strains are isogenic to RDKY7239 (*MATα, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2ΔBgl, hom3-10, ade2::hisG, ade8, hxt13::URA3*). The numbers in parentheses ( ) are the fold increase relative to RDKY7239. Numbers in brackets [ ] represent the 95% confidence intervals.

Table S4**.** *S. cerevisiae* strains constructed for the present study. All strains in RDKY7239 background.

RDKY #	Genotype
3615	*MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2::hisG ade8 yel069c::URA3*
7239	*MATalpha ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2::hisG ade8 yel069c::URA3*
7210	RDKY7239 *asf1::HIS3*
7501	RDKY7239 *asf1::HIS3 rad51::HPH*
7503	RDKY7239 *asf1::HIS3 rad51::HPH rnh203::KANMX4*
7212	RDKY7239 *asf1::HIS3 rnh203::KANMX4*
7214	RDKY7239 *cac1::TRP1*
7216	RDKY7239 *cac1::TRP1 rnh203::KANMX4*
7218	RDKY7239 *cac2::TRP1*
7219	RDKY7239 *cac2::TRP1 rnh203::KANMX4*
7221	RDKY7239 *chk1::HIS3*
7223	RDKY7239 *chk1::HIS3 rnh203::KANMX4*
7225	RDKY7239 *ctf18::TRP1*
7227	RDKY7239 *ctf18::TRP1 rnh203::KANMX4*
7229	RDKY7239 *ctf4::KANMX4*
7231	RDKY7239 *ctf4::KANMX4 rnh203::HIS3*
7240	RDKY7239 *dun1::HIS3*
7242	RDKY7239 *dun1::HIS3 rnh203::KANMX4*
7244	RDKY7239 *esc1::HIS3*
7245	RDKY7239 *esc1::HIS3 rnh203::KANMX4*
7248	RDKY7239 *esc2::HIS3*
7507	RDKY7239 *esc2::HIS3 rad51::HPH*
7509	RDKY7239 *esc2::HIS3 rad51::HPH rnh203::KANMX4*
7250	RDKY7239 *esc2::HIS3 rnh203::KANMX4*
8145	RDKY7239 *esc2::HIS3 top1::HPH*
8147	RDKY7239 *esc2::HIS3 top1::HPH rnh203::KANMX4*
7252	RDKY7239 *esc4::HIS3*
7254	RDKY7239 *esc4::HIS3 rnh203::KANMX4*
7256	RDKY7239 *exo1::TRP1*
7258	RDKY7239 *exo1::TRP1 rnh203::KANMX4*
7261	RDKY7239 *hir1::HIS3*
7263	RDKY7239 *hir1::HIS3 rnh203::KANMX4*
7265	RDKY7239 *hir2::HIS3*
7267	RDKY7239 *hir2::HIS3 rnh203::KANMX4*
7269	RDKY7239 *hst2::HIS3*
7271	RDKY7239 *hst2::HIS3 rnh203::KANMX4*
7290	RDKY7239 *lig4::HIS3*
7292	RDKY7239 *lig4::HIS3 rnh203::KANMX4*
7302	RDKY7239 *mec1::TRP1 sml1::KANMX4*
7304	RDKY7239 *mec1::TRP1 sml1::KANMX4 rnh203::HIS3*
7277	RDKY7239 *mec3::HIS3*
7279	RDKY7239 *mec3::HIS3 rnh203::KANMX4*
7281	RDKY7239 *mgs1::KANMX4*
7283	RDKY7239 *mgs1::KANMX4 rnh203::HIS3*
7286	RDKY7239 *mph1::KANMX4*
7511	RDKY7239 *mph1::KANMX4 rad51::HPH*
7513	RDKY7239 *mph1::KANMX4 rad51::HPH rnh203::HIS3*
7288	RDKY7239 *mph1::KANMX4 rnh203::HIS3*
7298	RDKY7239 *mrc1::TRP1*
7300	RDKY7239 *mrc1::TRP1 rnh203::KANMX4*
7273	RDKY7239 *mre11::HIS3*
7275	RDKY7239 *mre11::HIS3 rnh203::KANMX4*

7294	RDKY7239 *mus81::TRP1*
7296	RDKY7239 *mus81::TRP1 rnh203::KANMX4*
7312	RDKY7239 *pif1-m2*
7314	RDKY7239 *pif1-m2 rnh203::KANMX4*
7318	RDKY7239 *pol30-119::LEU2*
7316	RDKY7239 *pol30-119::LEU2 rnh203::KANMX4*
7320	RDKY7239 *pol32::TRP1*
7322	RDKY7239 *pol32::TRP1 rnh203::KANMX4*
7324	RDKY7239 *rad17::HIS3*
7326	RDKY7239 *rad17::HIS3 rnh203::KANMX4*
7328	RDKY7239 *rad24::HIS3 rnh203::KANMX4*
7330	RDKY7239 *rad24::HIS3 rnh203::KANMX4*
7332	RDKY7239 *rad27::HIS3*
7334	RDKY7239 *rad27::HIS3 rnh203::KANMX4*
7336	RDKY7239 *rad5::KANMX4*
7338	RDKY7239 *rad5::KANMX4 rnh203::HIS3*
7340	RDKY7239 *rad50::HIS3*
7342	RDKY7239 *rad50::HIS3 rnh203::KANMX4*
7344	RDKY7239 *rad51::HIS3*
7498	RDKY7239 *rad51::HIS3 rad59::TRP1*
7499	RDKY7239 *rad51::HIS3 rad59::TRP1 rnh203::KANMX4*
7346	RDKY7239 *rad51::HIS3 rnh203::KANMX4*
7348	RDKY7239 *rad52::HIS3*
7351	RDKY7239 *rad52::HIS3 rnh203::KANMX4*
7370	RDKY7239 *rad53::HIS3 sml1::TRP1*
7373	RDKY7239 *rad53::HIS3 sml1::TRP1 rnh203::KANMX4*
7374	RDKY7239 *rad55::TRP1*
7376	RDKY7239 *rad55::TRP1 rnh203::KANMX4*
7378	RDKY7239 *rad57::HIS3*
7379	RDKY7239 *rad57::HIS3 rnh203::KANMX4*
7382	RDKY7239 *rad59::TRP1*
7383	RDKY7239 *rad59::TRP1 rnh203::KANMX4*
7385	RDKY7239 *rad6::HIS3*
7387	RDKY7239 *rad6::HIS3 rnh203::KANMX4*
7389	RDKY7239 *rad9::HIS3*
7391	RDKY7239 *rad9::HIS3 rnh203::KANMX4*
7393	RDKY7239 *rdh54::TRP1*
7395	RDKY7239 *rdh54::TRP1 rnh203::KANMX4*
7397	RDKY7239 *rfa1-t33*
7399	RDKY7239 *rfa1-t33 rnh203::KANMX4*
7401	RDKY7239 *rfc5-1::TRP1*
7403	RDKY7239 *rfc5-1::TRP1 rnh203::KANMX4*
7235	RDKY7239 *rnh201::HIS3*
7237	RDKY7239 *rnh202::TRP1*
7233	RDKY7239 *rnh203::HIS3*
7209	RDKY7239 *rnh203::KANMX4*
7209	RDKY7239 *rnh203::KANMX4*
7410	RDKY7239 *rrm3::TRP1*
7412	RDKY7239 *rrm3::TRP1 rnh203::KANMX4*
7414	RDKY7239 *sgs1::HIS3*
7515	RDKY7239 *sgs1::HIS3 rad51::HPH*
7517	RDKY7239 *sgs1::HIS3 rad51::HPH rnh203::KANMX4*
7416	RDKY7239 *sgs1::HIS3 rnh203::KANMX4*
8149	RDKY7239 *sgs1::HIS3 top1::HPH*
8151	RDKY7239 *sgs1::HIS3 top1::HPH rnh203::KANMX4*
7418	RDKY7239 *siz1::TRP1*
7420	RDKY7239 *siz1::TRP1 rnh203::KANMX4*
7422	RDKY7239 *slx1::KANMX4*
7424	RDKY7239 *slx1::KANMX4 rnh203::HIS3*

7426	RDKY7239 *slx4::HIS3*
7428	RDKY7239 *slx4::HIS3 rnh203::KANMX4*
7430	RDKY7239 *srs2::HIS3*
7432	RDKY7239 *srs2::HIS3 rnh203::KANMX4*
7434	RDKY7239 *tel1::HIS3*
7436	RDKY7239 *tel1::HIS3 rnh203::KANMX4*
7438	RDKY7239 *tof1::HIS3*
7440	RDKY7239 *tof1::HIS3 rnh203::KANMX4*
8143	RDKY7239 *top1::HPH*
8141	RDKY7239 *top1::HPH rnh203::KANMX4*
7442	RDKY7239 *top3::KANMX4*
7445	RDKY7239 *top3::KANMX4 rnh203::HIS3*
7446	RDKY7239 *xrs2::HIS3*
7448	RDKY7239 *xrs2::HIS3 rnh203::KANMX4*
7450	RDKY7239 *yku70::TRP1*
7452	RDKY7239 *yku70::TRP1 rnh203::KANMX4*