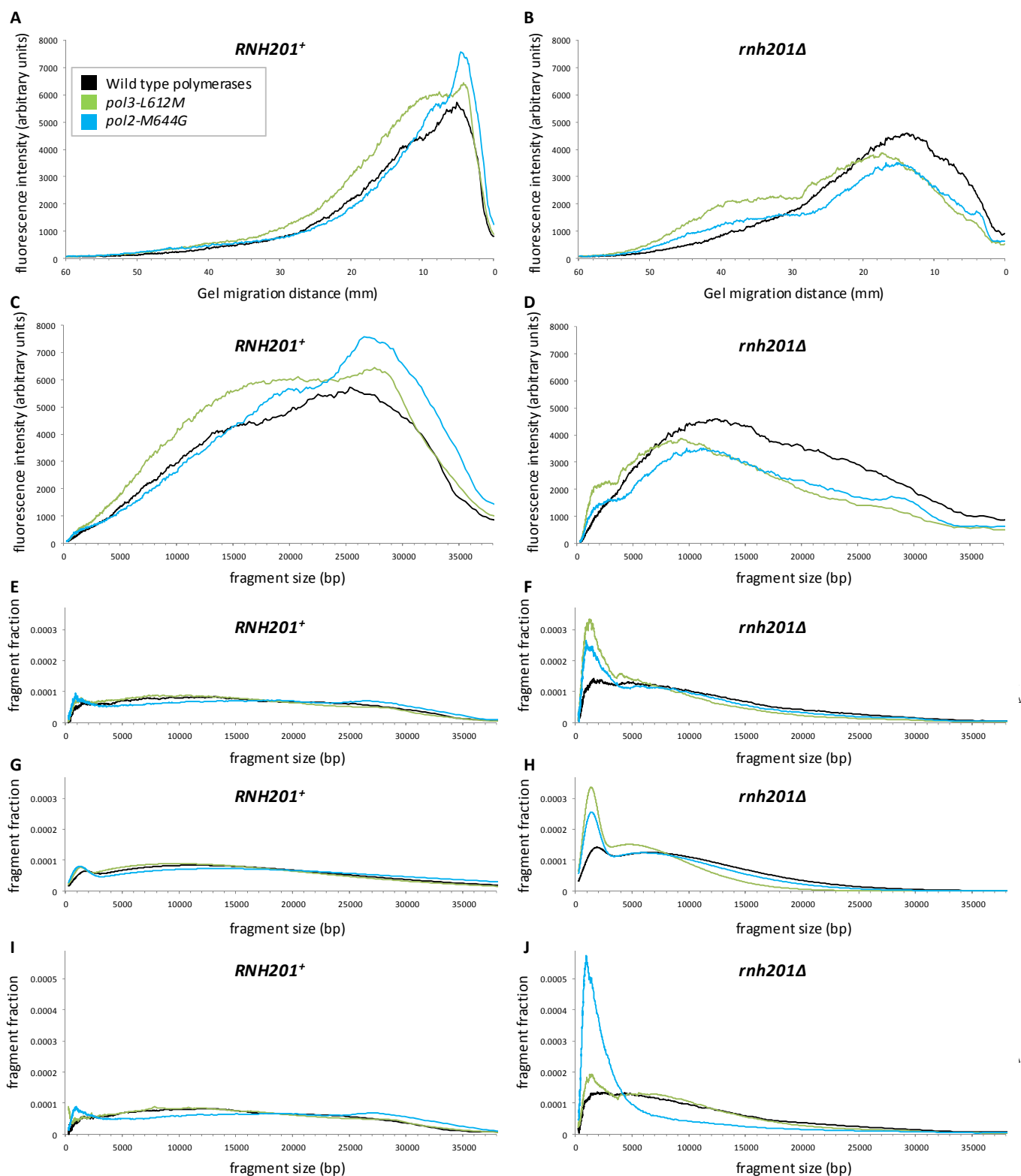**Supplemental Information**

Ribonucleotides Are Signals for Mismatch Repair

of Leading-Strand Replication Errors

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Figure S1. Quantifying ribonucleotide incorporation using alkaline-hydrolysis data, Related to Figure 1. Curves are derived from strains with wild type polymerases (black), the L612M Pol δ mutator allele (green), or the M644G Pol ε mutator allele using data from one of four independent experiments. Panels A-H refer to lagging strand data (Probe A in Fig. 1C). Panels I and J refer to

leading strand data (Probe B in Fig. 1C). (**A**) Fluorescence intensity curves for one representative alkaline-hydrolysis experiment with $RNH201^+$ strains (Fig. 1C, Probe A, $RNH201$ = +). (**B**) As per panel A, but for $rnh201\Delta$ strains (Figure 1C, Probe A, $RNH201$ = -). (**C**) An exponential transformation (constructed from ladder positions) is applied to convert gel migration distances from panel A into fragment sizes. (**D**) As per panel C, but for starting values from panel B. (**E**) Total intensities in each fragment size bin (panel C) are converted to average intensities per bin by dividing by bin width. The results are normalized to average fragment fractions by dividing by total lane intensity. (**F**) As per panel E, but with starting values from panel D. (**G**) Least squares regression of panel E data versus the sum of two Weibull distributions. The area under each curve yields the fraction of fragments in high- and low- mobility peaks. (**H**) As per panel G, but versus curves from panel F. (**I**) As per panel E, but with Probe B (leading strand). Analyses were performed as in panels A-H. (**J**) As per panel F, but with Probe B (leading strand). When comparing panels F and J, note that the fraction of fragments in the high mobility (Fig.1C) peaks track with *in vitro* ribonucleotide incorporation rates (Fig. 1A).

Table S1. *pol2-M644G msh3Δ* **mutation rates in two** *URA3* **orientations ±** *MSH6* **and/or** *RNH201*, **Related to Figure 2**

pol2-M644G msh3,6Δ/msh3Δ			mutation rate (x10^{-7})	95% confidence	5-FOA^R mutants	no *URA3* change	*URA3* mutations	Transitions		Frameshifts	
								#	rate	#	rate
URA3 orientation 1	RNH201	msh3Δ	1.6	(1.1-2.5)	122	15	109	9	0.12	50	0.66
		msh3,6Δ	110	(88-140)	173	16	166	67	43	56	36
		correction factors							**360x**		**54x**
	rnh201Δ	msh3Δ	11.0	(8.8-14)	137	17	120	19	1.5	26	2.1
		msh3,6Δ	250	(170-350)	164	18	154	64	98	67	100
		correction factors							**64x**		**49x**
	RNH201/rnh201Δ correction factor ratios								**5.6**		**1.1**
	p-values								≤ 0.001		0.430
URA3 orientation 2	RNH201	msh3Δ	2.90	(2.2-3.9)	125	17	108	4	0.093	88	2.0
		msh3,6Δ	280	(220-370)	158	16	144	34	60	80	140
		correction factors							**650x**		**69x**
	rnh201Δ	msh3Δ	15	(11-21)	139	21	118	3	0.32	33	3.6
		msh3,6Δ	280	(210-360)	122	22	102	30	69	54	120
		correction factors							**210x**		**35x**
	RNH201/rnh201Δ correction factor ratios								**3.1**		**2.0**
	p-values								0.10		0.015

For comparison, all rates are listed by strain in Table S6. All rates are multiplied by 10^7. C.f. stands for correction factor. Correction factors are compared in Fig. 2A, listed as "*msh3,6Δ/msh3Δ*". Strain origins and related references may be found in Table S5.

Table S2. *pol2-M644G* mutation rates in two *URA3* orientations ± *MMR* and/or *RNH201*, Related to Figure 2

pol2-M644G msh3,6Δ/wt MMR			mutation rate (x10⁻⁷)	95% confidence	5-FOA^R mutants	no *URA3* change	*URA3* mutations	Transitions		Frameshifts	
								#	rate	#	rate
URA3 orientation 1	*RNH201*	wt MMR	1.7	(1.3-2.3)	342	105	237	24	0.12	12	0.060
		msh3,6Δ	110	(88-140)	173	16	166	67	43	56	36
		correction factors							**360x**		**600x**
	rnh201Δ	wt MMR	7.6	(4.3-10)	128	4	125	25	1.5	7	0.42
		msh3,6Δ	250	(170-350)	164	18	154	64	98	67	100
		correction factors							**66x**		**250x**
	RNH201/rnh201Δ correction factor ratios								**5.4**		**2.4**
	p-values								≤ 0.001		0.083
URA3 orientation 2	*RNH201*	wt MMR	0.83	(0.71-0.96)	246	123	124	36	0.12	14	0.047
		msh3,6Δ	280	(220-370)	158	16	144	34	60	80	140
		correction factors							**500x**		**3,000x**
	rnh201Δ	wt MMR	14	(9.8-20)	138	0	138	5	0.51	7	0.71
		msh3,6Δ	280	(210-360)	122	22	102	30	69	54	120
		correction factors							**140x**		**170x**
	RNH201/rnh201Δ correction factor ratios								**3.7**		**17**
	p-values								0.058		≤ 0.001

pol2-M644G msh2Δ/wt MMR			mutation rate (x10⁻⁷)	95% confidence	5-FOA^R mutants	no *URA3* mutations	*URA3* mutations	Transitions		Frameshifts	
								#	rate	#	rate
URA3 orientation 1	*RNH201*	wt MMR	1.7	(1.3-2.3)	342	105	237	24	0.12	12	0.060
		msh2Δ	180	(120-270)	333	35	306	92	50	162	88
		correction factors							**420x**		**1,500x**
	rnh201Δ	wt MMR	7.6	(4.3-10)	128	4	125	25	1.5	7	0.42
		msh3,6Δ	250	(170-350)	164	18	154	64	98	67	100
		correction factors							**66x**		**250x**
	RNH201/rnh201Δ correction factor ratios								**6.3**		**6.0**
	p-values								≤ 0.001		0.009
URA3 orientation 2	*RNH201*	wt MMR	0.83	(0.71-0.96)	246	123	124	36	0.12	14	0.047
		msh2Δ	180	(100-220)	254	38	224	83	59	85	60
		correction factors							**480x**		**1,300x**
	rnh201Δ	wt MMR	14	(9.8-20)	138	0	138	5	0.51	7	0.71
		msh3,6Δ	280	(210-360)	122	22	102	30	69	54	120
		correction factors							**140x**		**170x**
	RNH201/rnh201Δ correction factor ratios								**3.6**		**7.3**
	p-values								0.066		0.004

Some data is repeated from previous tables and between panels. For comparison, all rates are listed by strain in Table S6. All rates are multiplied by 10⁷. C.f. stands for correction factor. Correction factors are compared in Fig. 2A, listed as "*msh3,6Δ*/wt MMR" (above) and "*msh2Δ*/wt MMR" (below). Strain origins and related references may be found in Table S5.

Table S3. *pol3-L612M* mutation rates in two *URA3* orientations ± *MSH6* and/or *RNH201*, **Related to Figure 2**

pol3-L612M *msh6Δ*/wt MMR			mutation rate ($\times 10^{-7}$)	95% confidence	5-FOA^R mutants	no *URA3* change	*URA3* mutations	Transitions #	rate	Frameshifts #	rate
URA3 orientation 1	RNH201	wt MMR	1.4	(1.1-1.8)	245	72	173	112	0.64	20	0.11
		msh6Δ	170	(130-230)	313	14	310	271	150	6	3.3
		correction factors						**230x**		**29x**	
	rnh201Δ	wt MMR	1.4	(0.97-2.1)	151	28	123	60	0.56	22	0.20
		msh6Δ	180	(130-260)	142	13	132	117	150	8	10
		correction factors						**270x**		**50x**	
	RNH201/rnh201Δ correction factor ratios							**0.9**		**0.6**	
	p-values							0.370		0.230	
URA3 orientation 2	RNH201	wt MMR	2.30	(2.0-2.7)	243	45	198	87	0.82	34	0.32
		msh6Δ	160	(130-210)	354	27	335	241	110	5	2.3
		correction factors						**130x**		**7.0x**	
	rnh201Δ	wt MMR	1	(0.84-1.5)	188	45	143	45	0.26	23	0.13
		msh6Δ	180	(140-230)	70	4	71	57	150	1	2.6
		correction factors						**570x**		**19x**	
	RNH201/rnh201Δ correction factor ratios							**0.23**		**0.37**	
	p-values							≤ 0.001		0.021	

For comparison, all rates are listed by strain in Table S6. All rates are multiplied by 10^7. Correction factors are compared in Fig. 2B, listed as "*msh6Δ*/wt MMR". Strain origins and related references may be found in Table S5.

Table S4. Mutation rates in a wild type polymerase background for two *URA3* orientations ± *MMR* and/or *RNH201*, Related to Figure 2

wild type pols *msh3,6Δ*/wt MMR			mutation rate (x10⁻⁷)	95% confidence	5-FOAᴿ mutants	no *URA3* change	*URA3* mutations	Transitions #	Transitions rate	Frameshifts #	Frameshifts rate
URA3 orientation 1	*RNH201*	wt MMR	0.2	(0.14-0.23)	211	70	141	47	0.040	11	0.0094
		msh3,6Δ	8	(6.1-11)	228	63	170	38	1.4	100	3.6
		correction factors							**34x**		**380x**
	rnh201Δ	wt MMR	0.3	(0.22-0.46)	251	95	156	32	0.041	27	0.034
		msh3,6Δ	10	(6.5-14)	298	92	206	76	2.4	98	3.2
		correction factors							**60x**		**92x**
	RNH201/rnh201Δ correction factor ratios								**0.6**		**4.1**
	p-values								0.083		≤ 0.001
URA3 orientation 2	*RNH201*	wt MMR	0.21	(0.16-0.27)	257	99	158	43	0.035	14	0.011
		msh3,6Δ	8	(6.1-10)	258	79	186	32	0.97	113	3.4
		correction factors							**28x**		**300x**
	rnh201Δ	wt MMR	1	(0.43-0.75)	273	62	211	32	0.075	14	0.033
		msh3,6Δ	12	(8.8-17)	244	49	196	37	1.8	99	4.9
		correction factors							**24x**		**150x**
	RNH201/rnh201Δ correction factor ratios								**1.1**		**2.0**
	p-values								0.38		0.069

wild type pols *msh2Δ*/wt MMR			mutation rate (x10⁻⁷)	95% confidence	5-FOAᴿ mutants	no *URA3* change	*URA3* mutations	Transitions #	Transitions rate	Frameshifts #	Frameshifts rate
URA3 orientation 1	*RNH201*	wt MMR	0.2	(0.14-0.23)	211	70	141	47	0.040	11	0.0094
		msh2Δ	7	(3.5-15)	195	15	180	41	1.5	118	4.4
		correction factors							**38x**		**460x**
	rnh201Δ	wt MMR	0.3	(0.22-0.46)	251	95	156	32	0.041	27	0.034
		msh3,6Δ	10	(6.5-14)	298	92	206	76	2.4	98	3.2
		correction factors							**60x**		**92x**
	RNH201/rnh201Δ correction factor ratios								**0.63**		**5.1**
	p-values								0.17		≤ 0.001
URA3 orientation 2	*RNH201*	wt MMR	0.21	(0.16-0.27)	257	99	158	43	0.035	14	0.011
		msh2Δ	10	(6.7-13)	193	12	181	23	1.1	113	5.6
		correction factors							**32x**		**490x**
	rnh201Δ	wt MMR	1	(0.43-0.75)	273	62	211	32	0.075	14	0.033
		msh3,6Δ	12	(8.8-17)	244	49	196	37	1.8	99	4.9
		correction factors							**24x**		**150x**
	RNH201/rnh201Δ correction factor ratios								**1.3**		**3.3**
	p-values								0.29		0.007

Some data is repeated from previous tables and between panels. For comparison, all rates are listed by strain in Table S6. All rates are multiplied by 10^7. Correction factors are compared in Fig. 2C, listed as "*msh3,6Δ*/wt MMR" (above) and "*msh2Δ*/wt MMR" (below). Strain origins and related references may be found in Table S5.

Table S5. Strains used in this study, Related to Figures 1 and 2 and Table 1

strain	strain origin	ploidy	polymerase alleles	other genotype notes	data set origin
h001	see reference and dissected from diploid d011 or d041 or d081	haploid	wild type	URA3 OR1	expanded from (Nick McElhinny et al., 2010a)
h002	see reference and dissected from diploid d012 or d042 or d082	haploid	wild type	URA3 OR2	expanded from (Nick McElhinny et al., 2010a)
h011	see reference and dissected from diploid d011	haploid	wild type	msh2Δ URA3 OR1	expanded from (Nick McElhinny et al., 2010a)
h012	see reference and dissected from diploid d012	haploid	wild type	msh2Δ URA3 OR2	expanded from (Nick McElhinny et al., 2010a)
h041	dissected from diploid d041 or d081	haploid	wild type	msh3,6Δ URA3 OR1	this study
h042	dissected from diploid d042 or d082	haploid	wild type	msh3,6Δ URA3 OR2	this study
h051	see reference (SNM106)	haploid	wild type	rnh201Δ URA3 OR1	from (Clark et al., 2011)
h052	see reference (SNM114)	haploid	wild type	rnh201Δ URA3 OR2	from (Clark et al., 2011)
h081	dissected from diploid d081	haploid	wild type	msh3,6Δ rnh201Δ URA3 OR1	this study
h082	dissected from diploid d082	haploid	wild type	msh3,6Δ rnh201Δ URA3 OR2	this study
h201	see reference (DTA201) and dissected from diploid d211	haploid	pol2-M644G	URA3 OR1	expanded from (Pursell et al., 2007)
h202	see reference (DTA202) and dissected from diploid d212	haploid	pol2-M644G	URA3 OR2	expanded from (Pursell et al., 2007)
h201ML	SNM82 (Nick McElhinny et al., 2010b)	haploid	pol2-M644L	URA3 OR1	this study
h202ML	SNM90 (Nick McElhinny et al., 2010b)	haploid	pol2-M644L	URA3 OR2	this study
h211	DTA201 and dissected from diploid d211	haploid	pol2-M644G	msh2Δ URA3 OR1	from (Lujan et al., 2012)
h212	DTA202 and dissected from diploid d212	haploid	pol2-M644G	msh2Δ URA3 OR2	from (Lujan et al., 2012)
h221	see reference (DTA201Δmsh3) and dissected from diploid d281	haploid	pol2-M644G	msh3Δ URA3 OR1	from (Clark et al., 2011)
h222	see reference (DTA202Δmsh3) and dissected from diploid d282	haploid	pol2-M644G	msh3Δ URA3 OR2	from (Clark et al., 2011)
h231	dissected from diploid d281	haploid	pol2-M644G	msh6Δ URA3 OR1	this study
h232	dissected from diploid d282	haploid	pol2-M644G	msh6Δ URA3 OR2	this study
h241	dissected from diploid d281	haploid	pol2-M644G	msh3,6Δ URA3 OR1	this study
h242	dissected from diploid d282	haploid	pol2-M644G	msh3,6Δ URA3 OR2	this study
h251	see reference (SNM120) and dissected from diploid d281	haploid	pol2-M644G	rnh201Δ URA3 OR1	expanded from (Nick McElhinny et al., 2010b)
h252	see reference (SHM127) and dissected from diploid d282	haploid	pol2-M644G	rnh201Δ URA3 OR2	expanded from (Nick McElhinny et al., 2010b)
h251ML	transformation of h201ML with HYG-R with RNH201 flanking DNA (Nick McElhinny et al., 2010b)	haploid	pol2-M644L	rnh201Δ URA3 OR1	this study
h252ML	transformation of h201ML with HYG-R with RNH201 flanking DNA (Nick McElhinny et al., 2010b)	haploid	pol2-M644L	rnh201Δ URA3 OR2	this study
h261	see reference and dissected from diploid d281	haploid	pol2-M644G	msh3Δ rnh201Δ URA3 OR1	expanded from (Clark et al., 2011)

h262	see reference and dissected from diploid d282	haploid	*pol2-M644G*	*msh3Δ rnh201Δ URA3 OR2*	expanded from *(Clark et al., 2011)*
h271	see reference and dissected from diploid d281	haploid	*pol2-M644G*	*msh6Δ rnh201Δ URA3 OR1*	from *(Clark et al., 2011)*
h272	see reference and dissected from diploid d282	haploid	*pol2-M644G*	*msh6Δ rnh201Δ URA3 OR2*	from *(Clark et al., 2011)*
h281	dissected from diploid d281	haploid	*pol2-M644G*	*msh3,6Δ rnh201Δ URA3 OR1*	expanded from *(Clark et al., 2011)*
h282	dissected from diploid d282	haploid	*pol2-M644G*	*msh3,6Δ rnh201Δ URA3 OR2*	expanded from *(Clark et al., 2011)*
h301	see reference (DTA301) and dissected from diploid d341 or d371	haploid	*pol3-L612M*	*URA3 OR1*	expanded from *(Nick McElhinny et al., 2008; Nick McElhinny et al., 2010a)*
h302	see reference (DTA302) and dissected from diploid d342 or d372	haploid	*pol3-L612M*	*URA3 OR2*	expanded from *(Nick McElhinny et al., 2008; Nick McElhinny et al., 2010a)*
h331	dissected from diploid d341 or d371	haploid	*pol3-L612M*	*msh6Δ URA3 OR1*	this study
h332	dissected from diploid d342 or d372	haploid	*pol3-L612M*	*msh6Δ URA3 OR2*	this study
h351	see reference (JSW11) and dissected from diploid d371	haploid	*pol3-L612M*	*rnh201Δ URA3 OR1*	this study
h352	see reference (JSW15) and dissected from diploid d372	haploid	*pol3-L612M*	*rnh201Δ URA3 OR2*	this study
h371	dissected from diploid d371	haploid	*pol3-L612M*	*msh6Δ rnh201Δ URA3 OR1*	this study
h372	dissected from diploid d372	haploid	*pol3-L612M*	*msh6Δ rnh201Δ URA3 OR2*	this study
d011	also known as ySNM_1025	diploid	wild type	*msh2Δ/MSH2 URA3 OR1*	from *(Nick McElhinny et al., 2008)*
d012	also known as ySNM_1028	diploid	wild type	*msh2Δ/MSH2 URA3 OR2*	from *(Nick McElhinny et al., 2008)*
d041	derived from mating of h031 and h021	diploid	wild type	*msh3,6Δ/MSH3,6 URA3 OR1*	this study
d042	derived from mating of h032 and h022	diploid	wild type	*msh3,6Δ/MSH3,6 URA3 OR2*	this study
d081	derived from mating of h041 and h051	diploid	wild type	*msh3/MSH3 msh6Δ/MSH6 rnh201Δ/RNH201 URA3 OR1*	this study
d082	derived from mating of h042 and h052	diploid	wild type	*msh3/MSH3 msh6Δ/MSH6 rnh201Δ/RNH201 URA3 OR2*	this study
h021	also known as DTA101Δmsh3	haploid	wild type	*msh3Δ URA3 OR1*	from *(Clark et al., 2011)*
h022	also known as DTA102Δmsh3	haploid	wild type	*msh3Δ URA3 OR2*	from *(Clark et al., 2011)*
h031	dissected from d031	haploid	wild type	*msh6Δ URA3 OR1*	this study
h032	dissected from d032	haploid	wild type	*msh6Δ URA3 OR2*	this study
d031	transformation of ySNM_1001 with *TRP1* with *MSH6* flanking DNA	diploid	wild type	*msh6Δ/MSH6 URA3 OR1*	this study
d032	transformation of ySNM_1005 with *TRP1* with *MSH6* flanking DNA	diploid	wild type	*msh6Δ/MSH6 URA3 OR2*	this study
ySNM 1001	transformation of DTA101 with plasmid YEpHO	diploid	wild type	*URA3 OR1*	from *(Nick McElhinny et al., 2008)*
ySNM 1005	transformation of DTA102 with plasmid YEpHO	diploid	wild type	*URA3 OR2*	from *(Nick McElhinny et al., 2008)*
d341	derived from mating of h331 and h321	diploid	*pol3-L612M*	*msh3,6Δ/MSH3,6 URA3 OR1*	this study
d342	derived from mating of h332 and h322	diploid	*pol3-L612M*	*msh3,6Δ/MSH3,6 URA3 OR2*	this study
d371	derived from mating of h331 and h351	diploid	*pol3-L612M*	*msh6Δ/MSH6 rnh201Δ/RNH201 URA3 OR1*	this study

d372	derived from mating of h332 and h352	diploid	pol3-L612M	msh6Δ/MSH6 rnh201Δ/RNH201 URA3 OR2	this study
h331	dissected from d331	haploid	pol3-L612M	msh6Δ URA3 OR1	this study
h332	dissected from d332	haploid	pol3-L612M	msh6Δ URA3 OR2	this study
d331	transformation of ySNM_1018 with TRP1 with MSH6 flanking DNA	diploid	pol3-L612M	msh6Δ/MSH6 URA3 OR1	this study
d332	transformation of ySNM_1021 with TRP1 with MSH6 flanking DNA	diploid	pol3-L612M	msh6Δ/MSH6 URA3 OR2	this study
h321	also known as DTA301Δmsh3	haploid	pol3-L612M	msh3Δ URA3 OR1	this study
h322	also known as DTA302Δmsh3	haploid	pol3-L612M	msh3Δ URA3 OR2	this study
d211	derived from mating of DTA201 and h211	diploid	pol2-M644G	msh2Δ/MSH2 URA3 OR1	this study
d212	derived from mating of DTA202 and h212	diploid	pol2-M644G	msh2Δ/MSH2 URA3 OR2	this study
d281	derived from mating of h241 and h251	diploid	pol2-M644G	msh3,6Δ/MSH3,6 rnh201Δ/RNH201 URA3 OR1	this study
d282	derived from mating of h242 and h252	diploid	pol2-M644G	msh3,6Δ/MSH3,6 rnh201Δ/RNH201 URA3 OR2	this study
h241	dissected from d241	haploid	pol2-M644G	msh3,6Δ URA3 OR1	this study
h242	dissected from d242	haploid	pol2-M644G	msh3,6Δ URA3 OR2	this study
d241	derived from mating h231 and h221	diploid	pol2-M644G	msh3,6Δ/MSH3,6 URA3 OR1	this study
d242	derived from mating h232 and h222	diploid	pol2-M644G	msh3,6Δ/MSH3,6 URA3 OR2	this study
h231	derived from dissection of d231	haploid	pol2-M644G	msh6Δ URA3 OR1	this study
h232	derived from dissection of d232	haploid	pol2-M644G	msh6Δ URA3 OR2	this study
d231	derived from dissection and back cross of d231.5	diploid	pol2-M644G	msh6Δ/MSH6 URA3 OR1	this study
d232	derived from dissection and back cross of d232.5	diploid	pol2-M644G	msh6Δ/MSH6 URA3 OR2	this study
d231.5	derived from mating h201 and h031	diploid	pol2-M644G/POL2	msh6Δ/MSH6 URA3 OR1	this study
d232.5	derived from mating h202 and h032	diploid	pol2-M644G/POL2	msh6Δ/MSH6 URA3 OR2	this study

All strains are descended from Δ|(-2)|-7B-YUNI300 (Pavlov et al., 2001) and have the following genotypic markers (homozygous in diploids): *CAN1, his7-2, leu2-Δ::kanMX, ura3-Δ::, trp1-289, ade2-1, lys2-ΔGG2899-2900. OR1* and *OR2* refer to *URA3* orientations at the *AGP1* locus on *Saccharomyces cerevisiae* chromosome 3: the *URA3* coding strand is the leading strand template in *OR1* and the lagging strand template in *OR2*.

Table S6. Mutation rates, Related to Figure 2

Polymerase	Locus	RNH201	MMR alleles	mutation rate (x10⁻⁷)	95% confidence	5-FOA^R mutants	wild type URA3	URA3 mutations	Transitions count	Transitions rate	Frameshifts count	Frameshifts rate
pol2-M644G	URA3 OR1	+	wt	1.7	(1.3-2.3)	342	105	237	24	0.12	12	0.060
		+	msh6Δ	110	(82-150)	219	26	205	130	65	12	6.0
		Δ	wt	7.6	(4.3-10)	128	4	125	25	1.5	7	0.42
		Δ	msh6Δ	170	(130-220)	141	10	133	82	99	7	8.4
	URA3 OR2	+	wt	0.83	(0.71-0.96)	246	123	124	36	0.12	14	0.047
		+	msh6Δ	54	(40-75)	181	38	149	79	24	10	3.0
		Δ	wt	14	(9.8-20)	138	0	138	5	0.51	7	0.71
		Δ	msh6Δ	81	(57-110)	134	20	126	65	39	3	1.8
	URA3 OR1	+	msh3Δ	1.6	(1.1-2.5)	122	15	109	9	0.12	50	0.66
		+	msh3,6Δ	110	(88-140)	173	16	166	67	43	56	36
		Δ	msh3Δ	11	(8.8-14)	137	17	120	19	1.5	26	2.1
		Δ	msh3,6Δ	250	(170-350)	164	18	154	64	98	67	100
	URA3 OR2	+	msh3Δ	2.9	(2.2-3.9)	125	17	108	4	0.093	88	2.0
		+	msh3,6Δ	280	(220-370)	158	16	144	34	60	80	140
		Δ	msh3Δ	15	(11-21)	139	21	118	3	0.32	33	3.6
		Δ	msh3,6Δ	280	(210-360)	122	22	102	30	69	54	120
	OR1	+	msh2Δ	180	(120-270)	333	35	306	92	50	162	88
	OR2	+	msh2Δ	180	(100-220)	254	38	224	83	59	85	60
pol3-L612M	URA3 OR1	+	wt	1.4	(1.1-1.8)	245	72	173	112	0.64	20	0.11
		+	msh6Δ	170	(130-230)	313	14	310	271	150	6	3.3
		Δ	wt	1.4	(0.97-2.1)	151	28	123	60	0.56	22	0.20
		Δ	msh6Δ	180	(130-260)	142	13	132	117	150	8	10
	URA3 OR2	+	wt	2.3	(2.0-2.7)	243	45	198	87	0.82	34	0.32
		+	msh6Δ	160	(130-210)	354	27	335	241	110	5	2.3
		Δ	wt	1.1	(0.84-1.5)	188	45	143	45	0.26	23	0.13
		Δ	msh6Δ	180	(140-230)	70	4	71	57	150	1	2.6
wild type polymerases	URA3 OR1	+	wt	0.2	(0.14-0.23)	211	70	141	47	0.040	11	0.0094
		+	msh3,6Δ	8.1	(6.1-11)	228	63	170	38	1.4	100	3.6
		Δ	wt	0.3	(0.22-0.46)	251	95	156	32	0.041	27	0.034
		Δ	msh3,6Δ	10	(6.5-14)	298	92	206	76	2.4	98	3.2
	URA3 OR2	+	wt	0.21	(0.16-0.27)	257	99	158	43	0.035	14	0.011
		+	msh3,6Δ	7.8	(6.1-10)	258	79	186	32	0.97	113	3.4
		Δ	wt	0.64	(0.43-0.75)	273	62	211	32	0.075	14	0.033
		Δ	msh3,6Δ	12	(8.8-17)	244	49	196	37	1.8	99	4.9
	OR1	+	msh2Δ	7.2	(3.5-15)	195	15	180	41	1.5	118	4.4
	OR2	+	msh2Δ	10	(6.7-13)	193	12	181	23	1.1	113	5.6
pol2-M644GL	OR1	+	wt	0.39	(0.30-0.52)	87	39	49	8	0.036	10	0.045
	OR1	Δ	wt	0.50	(0.37-0.66)	83	31	52	17	0.10	7	0.042
	OR1	+	wt	0.61	(0.53-0.71)	101	33	69	17	0.10	18	0.11
	OR1	Δ	wt	0.50	(0.45-0.56)	93	46	47	27	0.15	4	0.022

All rates are multiplied by 10^7. Strain origins and related references may be found in Table S5.

Table S7. Mutation rates in a *pol2-M644L* background for two *URA3* orientations ± *RNH201*, Related to Figure 2

pol2-M644L		mutation rate (x10⁻⁷)	95% confidence	5-FOAᴿ mutants	no *URA3* change	*URA3* mutations	±1-base Indels #	±1-base Indels rate	2-5 bp Deletions #	2-5 bp Deletions rate
RNH201	*URA3* OR1	0.39	(0.30-0.52)	87	39	49	10	0.045	3	0.0049
	URA3 OR2	0.50	(0.37-0.66)	83	31	52	7	0.042	1	0.0060
	combined	0.44	(0.33-0.58)	170	70	101	17	0.043	4	0.0052
rnh201Δ	*URA3* OR1	0.61	(0.53-0.71)	101	33	69	18	0.11	9	0.0060
	URA3 OR2	0.50	(0.45-0.56)	93	46	47	4	0.022	1	0.0054
	combined	0.55	(0.49-0.63)	194	79	116	22	0.048	10	0.0057
increase upon *RNH201* deletion								**1.1x**		**1.9x**

For comparison, some rates are listed by strain in Table S6. All rates are multiplied by 10^7. "Combined" rates are geometric means of rates from two *URA3* orientations (mutation rates from forward mutation experiments are lognormally distributed). Fold-increases in rates upon *RNH201* deletion are compared in Fig. 2D. Strain origins and related references may be found in Table S5.

Supplemental References

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