Table S1 Oligonucleotides used for site-directed mutagenesis

Allele	PCR Primer Name	PCR Primer Sequence
pol2-4	pol2-4F1	5′-CTGTGGTAATGGCATTTGCTATAGCTACCACGAAGCCGCC-3′
	pol2-4R1	5′-GGCGGCTTCGTGGTAGCTATAGCAAATGCCATTACCACAG-3′
pol2-G435C	eex51qcF	5′-ATTCTTATTTACCACAATGTTCCCAGGGTTTAAAA-3′
	eex51qcR	5′-TTTTAAACCCTGGGAACATTGTGGTAAATAAGAAT-3′
pol2-V522A	eex20qcR	5′-GAAATGTTGTTGATGGCTCAAGCTTATCAACAT-3′
	eex20qcR	5′-ATGTTGATAAGCTTGAGCCATCAACAACATTTC-3′
pol2-T850M	eex26qcF	5′-GAAATGGCGGGGATTATGTGTTTAACAGGTGCC-3′
	eex26qcR	5′-GGCACCTGTTAAACACATAATCCCCGCCATTTC-3′
pol2-K966Q	eex55qcF	5′-GAAGGAAAAGGTATACAGAAAAGATATGCTGTC-3′
	eex55qcR	5′-GACAGCATATCTTTTCTGTATACCTTTTCCTTC-3′
pol2-A1153D	eex12qcF	5′-GAAAGACTTGGATCTGATATACAAAAGATAATT-3′
	eex12qcR	5′-AATTATCTTTTGTATATCAGATCCAAGTCTTTC-3′

Mutations were introduced into pRS415POL2 or pRS415pol2-4 using the QuickChange protocol (Wang and Malcolm 1999), Phusion Polymerase (New England Biolabs), the indicated primers, and PCR conditions of 95°C, 1 min; 16x (95°C, 40 sec.; 53°C, 60 sec., 68°C, 7 min.).

Table S2 Yeast strains

Strain	Relevant Genotype	Reference
LW01[a]	*pol2::kanMX msh2::HIS3* + pRS416*POL2*	This study
LW02[a]	*pol2::kanMX msh3::MET15* + pRS416*POL2*	This study
LW03[a]	*pol2::kanMX msh6::HIS3* + pRS416*POL2*	This study
LW04[a]	*pol2::kanMX mlh1::HIS3* + pRS416*POL2*	This study
LW05[a]	*pol2::kanMX pms1::HIS3* + pRS416*POL2*	This study
LW06[a]	*pol2::kanMX mlh3::MET15* + pRS416*POL2*	This study
LW07[a]	*pol2::kanMX msh6::HIS3 msh3::LEU2* + pRS416*POL2*	This study
LW08[a]	*pol2::kanMX pms1::HIS3 mlh3::TRP1* + pRS416*POL2*	This study
LW09[a]	*pol2::kanMX msh2::HIS3 rev3::TRP1* + pRS416*POL2*	This study
LW10[a]	*pol2::kanMX msh2::HIS3 rad30::TRP1* + pRS416*POL2*	This study
BP0109[b]	*pol3::HIS3 msh2::TRP1* + pGL310 [*URA3/POL3*]	(Herr *et al.* 2011)
BP0210[b]	*pol3::HIS3 msh3::TRP1* + pGL310 [*URA3/POL3*]	This study
BP0301[b]	*pol3::HIS3 msh6::TRP1* + pGL310 [*URA3/POL3*]	This study
BP0412[b]	*pol3::HIS3 mlh1::TRP1* + pGL310 [*URA3/POL3*]	This study
BP0502[b]	*pol3::HIS3 pms1::TRP1* + pGL310 [*URA3/POL3*]	This study
LW09[b]	*pol3::HIS3 mlh3::TRP1* + pGL310 [*URA3/POL3*]	This study
LW10[b]	*pol3::HIS3 msh6::TRP1 msh3::MET15* + pGL310 [*URA3/POL3*]	This study
LW11[b]	*pol3::HIS3 pms1::TRP1 mlh3::MET15* + pGL310 [*URA3/POL3*]	This study
LW12[c]	*pol2::NAT1 msh2::MET15* + pRS416*POL2*	This study
LW13[c]	*pol3::NAT1 msh2::MET15* + pGL310 [*URA3/POL3*]	This study

[a] Strains engineered from the BY4733 strain (*MATa leu2Δ0 ura3Δ0 met15Δ0 trp1Δ63 his3Δ200*), an S288C descendent (Brachmann *et al.* 1998) that we re-derived via sporulation of a BY4733 X BY4734 diploid (kindly provided by Tim Formosa, University of Utah). The *pol2::kanMX* strains were constructed from this re-derived BY4733 strain by first introducing pRS416*POL2* (to provide a wild-type plasmid copy of *POL2*) and then replacing the entire chromosomal *POL2* gene with a *kanMX* cassette. pRS416*POL2* is the *CEN6/ARSH4/URA3* plasmid pRS416 (Brachmann *et al.* 1998) carrying wild-type *POL2* with its natural promoter.

[b] These strains were engineered from P3H3a described in (Herr *et al.* 2011).

[c] These strains were engineered from the Y7092 strain (*MATα can1Δ::STE2pr-his5 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*), an S288C descendent modified by the Boone lab to be used as a query strain in Synthetic Genetic Analysis (Tong and Boone 2007).

Table S3 Construction of chromosomal gene disruptions

Allele	PCR Primer Name	PCR Primer Sequence	PCR Template [a]
pol2Δ::kanMX	Pol2-kanMXkoF	5′-ATGATGTTTGGCAAGAAAAAAAACAACGGAGGATCTTCCACTGCAAGATATTCAGCTGGCGAAGTTATTAGGTCTAGAGATCTG-3′	pUG6 (Guldener *et al.* 1996)
	Pol2-kanMXkoR	5′-TCATATGGTCAAATCAGCAATACAACTCAATAATATATCAAAACCGTAATACTTGGCTACTACGAAGTTATATTAAGGGTTCTCG-3′	
pol2Δ::natMX	Pol2::nat1-for2	5′-AGAGCATATGATGATGAAAGAGCACATTCTATCAAGATAACACTCTCAGGGGACAAGTATACATGGAGGCCCAGAATACCCT-3′	pFvL99 (Stulemeijer *et al.* 2011)
	Pol2::nat1-rev2	5′-TTTTTTTTTTTTTTTTTTTCATGGTAAAGAGGCCATTGAACCTCGCGTTATATACTGCTTACCAGTATAGCGACCAGCATTCAC-3′	
pol3Δ::natMX	Pol3MXF	5'-ATAGATATTGAGCACTTGCTATTAAGCATTAATCTTTATACATATACGCACAGCAACATGGAGGCCCAGAATACCCT-3'	pFvL99 (Stulemeijer *et al.* 2011)
	Pol3MXR	5′-GCAAAAAGTTGTTAGCCTTTCTTAATCCTAATATGATGTGCCACCCTATCGTTTTCAGTATAGCGACCAGCATTCAC-3′	
msh2Δ::HIS3 or *msh2Δ::TRP1*	Msh2U	5′-AAAAATCTCTTTATCTGCTGACCTAACATCAAAATCCTCAGATTAAAAGTAGATTGTACTGAGAGTGCAC-3′	pRS413 or pRS414 (Brachmann *et al.* 1998)
	Msh2D	5′-TTATAACAACAAGGCTTTTATATATTTCAGGTAATTATCGTTTTCCTTTTCTGTGCGGTATTTCACACCG-3′	
msh2Δ::MET15	Msh2::Met15F	5′-AAAAATCTCTTTATCTGCTGACCTAACATCAAAATCCTGCTGGCTTAACTATGCGGCATC-3′	pRS411 (Brachmann *et al.* 1998)
	Msh2::Met15R	5′-TTATAACAACAAGGCTTTTATATATTTCAGGTAATTATGTTTACAATTTCCTGATGCGGT-3′	
msh6Δ::HIS3 or *msh6Δ::TRP1*	Msh6U	5′-TTTAATTGGAGCAACTAGTTAATTTTGACAAAGCCAATTTGAACTCCAAAGAAGTTATTAGGTCTAGAGATCTG-3′	pRS413 or pRS414 (Brachmann *et al.* 1998)
	Msh6D	5′-ACTTTAAAAAAAATAAGTAAAAATCTTACATACATCGTAAATGAAAATACACGAAGTTATATTAAGGGTTCTCG-3′	
mlh1 Δ::HIS3 or *mlh1Δ::TRP1*	Mlh1U	5′-ATAGTGATAGTAAATGGAAGGTAAAAATAACATAGACCTATCAATAAGCAATGTCTCTCAGAATAAAAGCAGATTGTACTGAGAGTGCAC-3′	pRS413 or pRS414 (Brachmann *et al.* 1998)
	Mlh1D	5′-CTCAGGAAATAAACAAAAAACTTTGGTATTACAGCCAAAACGTTTTAAAGTTAACACCTCTCAAAAACTTTACTGTGCGGT ATTTCACACCG-3′	
pms1Δ::HIS3 or *pms1Δ::TRP1*	Pms1U	5′-GAACGCGAAAAGAAAAGACGCGTCTCTCTTAATAATCATTATGCGATAAAATGTTTCACCACATCGAAAAAGATTGTACTGAGAGTGCAC-3′	pRS413 or pRS414 (Brachmann *et al.* 1998)
	Pms1D	5′-TGTATATAATGTATTTGTTAATTATATAATGAATGAATATCAAAGCTAGATCATATTTCGTAATCCTTCGACTGTGCGGTATTTCACACCG-3′	

mlh3::MET15 or *mlh3Δ::TRP1*	Mlh3KO-upstream	5′-ACATAAACCAGCGAGGCTTTCAAGGAAGAATGAACGTGAACTCGTCAACTCAAAAAGAAAAGATTGTACTGAGAGTGCAC-3′	pRS411 or pRS414 (Brachmann *et al.* 1998)
	Mlh3KO-downstream	5′-TGCATATCCGCGCAATTTAAAATGCAGGCGACAAACCTTGTTCCAGGATTAAGGTTCTCTCTGTGCGGTATTTCACACCG-3′	
msh3::MET15 or *msh3Δ::LEU2*	Msh3KO-upstream	5′-GTACTTTTGAGAGCCAAAAGCAGTGCAAATAGATTTATTTTGTTGAATCTATTAACAATAAGATTGTACTGAGAGTGCAC-3′	pRS411 or pRS415 (Brachmann *et al.* 1998)
	Msh3KO-downstream	5′-TCAGTGGATATCCAATGATAGTAATTTCGCGAGTTTATCCGTTGCTGTTATATTATCTGTGCGGTATTTCACACCG-3′	
rev3Δ::TRP1	Rev3U	5′-ATTTGAGTCAATACAAAACTACAAGTTGTGGCGAAATAAAATGTTTGGAAAGATTGTACTGAGAGTGCAC-3′	pRS414 (Brachmann *et al.* 1998)
	Rev3D	5′-TTACCAATCATTTAGAGATATTAATGCTTCTTCCCTTTGAACAGATTGATCTGTGCGGTATTTCACACCG-3′	
rad30Δ::TRP1	Rad30U	5′-TAGCGCAGGCCTGCTCATTTTTGAACGGCTTTGATAAAACAAGACAAAGCAGATTGTACTGAGAGTGCAC-3′	pRS414 (Brachmann *et al.* 1998)
	Rad30D	5′-TCATTTTTTTCTTGTAAAAAATGATAAGATGTTTTTGGAAGATGTAACTTCTGTGCGGTATTTCACACCG-3′	

[a] pRS411 was used as template for gene replacement with *MET15*; pRS413 with *HIS3;* pRS414 with *TRP1*; pRS415 with *LEU2*.

Mutations were introduced into yeast using PCR products generated with the indicated primers and template DNAs. The PCR conditions for all primers used here were: 98°C, 1 min.; 30x (98°C, 10 sec.; 55°C, 30 sec.; 72°C, 90 sec.); 72°C, 60 sec.

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