# Supporting Information

## Williams et al. 10.1073/pnas.1422948112

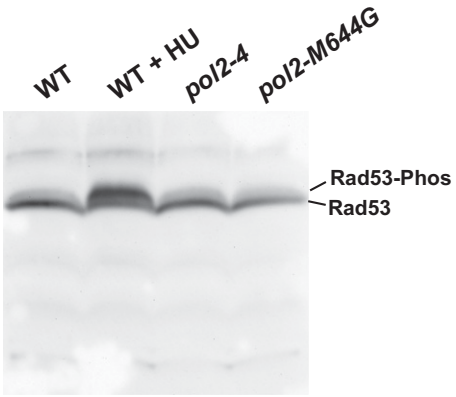


**Fig. S1.** Rad53 gel shift analysis. Phosphorylation of Rad53 was assessed by immunoblot analysis with Rad53 polyclonal antibodies. A WT strain treated with 200 mM hydroxyurea (WT + HU) served as the positive control for Rad53 phosphorylation.

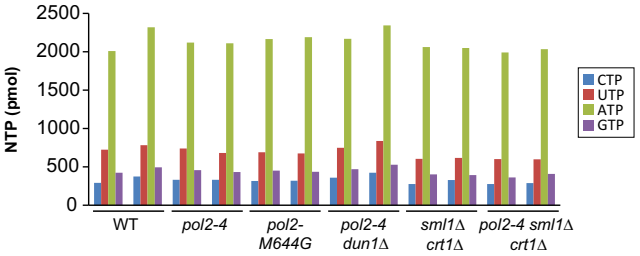


**Fig. S2.** Variation in NTP levels between samples. The NTP levels (height, mAU) normalized to NTP standards are shown for all duplicate samples.

**Table S1. Yeast strains**

| Strains* | Relevant genotype | Source |
|---|---|---|
| LW01 | *pol2Δ::kanMX msh2Δ::HIS3* (*pRS416POL2*) | Williams et al. (1) |
| LW03 | *pol2Δ::kanMX msh6Δ::HIS3* (*pRS416POL2*) | Williams et al. (1) |
| LW14 | *pol2Δ::kanMX* (*pRS416POL2*) | Williams et al. (1) |
| LW15 | *pol2Δ::kanMX dun1Δ::TRP1* (*pRS416POL2*) | This study |
| LW16 | *pol2Δ::kanMX msh6Δ::HIS3 dun1Δ::TRP1* (*pRS416POL2*) | This study |
| LW17 | *pol2Δ::kanMX rev3Δ::TRP1* (*pRS416POL2*) | This study |
| LW18 | *pol2Δ::kanMX rad30Δ::TRP1*([*pRS416POL2*) | This study |
| LW19 | *pol2Δ::kanMX mrc1Δ::HIS3* (*pRS416POL2*) | This study |
| LW21 | *pol2Δ::kanMX rad9Δ::HIS3* (*pRS416POL2*) | This study |
| LW23 | *pol2Δ::kanMX msh2Δ::HIS3 dun1Δ::TRP1* (*pRS416POL2*) | This study |
| AH7808 | *pol2Δ::kanMX sml1Δ::HIS3* (*pRS416POL2*) | This study |
| AH7905 | *pol2Δ::kanMX dun1Δ::TRP1 sm1Δ::HIS3* (*pRS416POL2*) | This study |
| AH8009 | *pol2Δ::kanMX crt1Δ::natMX* (*pRS416POL2*) | This study |
| AH8110 | *pol2Δ::kanMX dun1Δ::TRP1 crt1Δ::natMX* (*pRS416POL2*) | This study |
| AH8209 | *pol2Δ::kanMX sm1Δ::HIS3 crt1Δ::natMX* (*pRS416POL2*) | This study |
| AH8306 | *pol2::kanMX dun1Δ::TRP1 sm1Δ::HIS3 crt1Δ::natMX* (*pRS416POL2*) | This study |
| AH8403 | *pol2::kanMX dif1Δ::natMX* (*pRS416POL2*) | This study |
| AH8506 | *pol2::kanMX dun1Δ::TRP1 dif1Δ::natMX* (*pRS416POL2*) | This study |
| AH8604 | *pol2Δ::kanMX sml1Δ::HIS3 dif1Δ::natMX* (*pRS416POL2*) | This study |
| AH8702 | *pol2Δ::kanMX dun1Δ::TRP1 sml1Δ::HIS3 dif1Δ::natMX* (*pRS416POL2*) | This study |
| AH9704 | *pol2::kanMX sm1Δ::HIS3 crt1Δ::natMX dif1Δ::hygMX* (*pRS416POL2*) | This study |
| AH9806 | *pol2Δ::kanMX dun1Δ::TRP1 sm1Δ::HIS3 crt1Δ::natMX dif1Δ::hygMX* (*pRS416POL*) | This study |

*Strains were engineered from the BY4733 strain (*MATa leu2Δ0 ura3Δ0 met15Δ0 trp1Δ63 his3Δ200*), an S288C descendent (2) that we rederived via sporulation of a BY4733 × BY4734 diploid (kindly provided by Tim Formosa, University of Utah, Salt Lake City, UT). LW14 was constructed from this rederived strain by first introducing pRS416*POL2* (to provide a WT 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 (2) carrying WT *POL2* with its natural promoter. The previously described LW01 and LW03 were constructed from LW14, as were LW15, LW17, LW18, LW19, LW21, AH7808, AH8009, and AH8403. LW16 and LW23 were constructed from LW03 and LW01, respectively. AH7905, AH8110, and AH8506 were constructed from LW15. AH8209 and AH8604 were constructed from AH7808. AH8306 and AH8702 were constructed from AH7905. AH9704 was constructed from AH8209. AH9806 was constructed from AH8301.

1. Williams LN, Herr AJ, Preston BD (2013) Emergence of DNA polymerase ε antimutators that escape error-induced extinction in yeast. Genetics 193(3):751–770.
2. Brachmann CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14(2):115–132.

**Table S2. Oligonucleotides used for construction of chromosomal gene disruptions**

| Allele | PCR primer name | PCR primer sequence | PCR template* |
|---|---|---|---|
| *pol2Δ::kanMX* | Pol2-kanMXkoF | 5′-ATGATGTTTGGCAAGAAAAAAAACAACGGAGGATCTTC-CACTGCAAGATATTCAGCTGGCGAAGTTATTAGGTCTAG-AGATCTG-3′ | pUG6 (1) |
| | Pol2-kanMXkoR | 5′-TCATATGGTCAAATCAGCAATACAACTCAATAATATATC-AAAACCGTAATACTTGGCTACTACGAAGTTATATTAAGG-GTTCTCG-3′ | |
| *msh2Δ::HIS3* | Msh2U | 5′-AAAAATCTCTTTATCTGCTGACCTAACATCAAAATCCTCA-GATTAAAAGTAGATTGTACTGAGAGTGCAC-3′ | pRS413 (2) |
| | Msh2D | 5′-TTATAACAACAAGGCTTTTATATATTTCAGGTAATTATCG-TTTTCCTTTTCTGTGCGGTATTTCACACCG-3′ | |
| *msh6Δ::HIS3* | Msh6U | 5′-TTTAATTGGAGCAACTAGTTAATTTTGACAAAGCCAATT-TGAACTCCAAAGAAGTTATTAGGTCTAGAGATCTG-3′ | pRS413 (2) |
| | Msh6D | 5′-ACTTTAAAAAAAATAAGTAAAAATCTTACATACATCGTA-AATGAAAATACACGAAGTTATATTAAGGGTTCTCG-3′ | |
| *dun1Δ::TRP1* | Dun1F2TKO | 5′-ATGAGTTTGTCCACGAAAAGAGAGCACTCTGGTGATGT-AACTGACTCTTCAGATTGTACTGAGAGTGCAC-3′ | pRS414 (2) |
| | Dun1R2TKO | 5′-AGAGGCAAGATAATTCTGAGTATGTTTTGGGTATTTTAT-TGTCAGTAATTCTGTGCGGTATTTCACACCG-3′ | |
| *rev3Δ::TRP1* | Rev3U | 5′-ATTTGAGTCAATACAAAACTACAAGTTGTGGCGAAATA-AAATGTTTGGAAAGATTGTACTGAGAGTGCAC-3′ | pRS414 (2) |
| | Rev3D | 5′-TTACCAATCATTTAGAGATATTAATGCTTCTTCCCTTTGA-ACAGATTGATCTGTGCGGTATTTCACACCG-3′ | |
| *rad30Δ::TRP1* | Rev3D | 5′-TTACCAATCATTTAGAGATATTAATGCTTCTTCCCTTTGA-ACAGATTGATCTGTGCGGTATTTCACACCG-3′ | pRS414 (2) |
| | Rad30U | 5′-TAGCGCAGGCCTGCTCATTTTTGAACGGCTTTGATAAAA-CAAGACAAAGCAGATTGTACTGAGAGTGCAC-3′ | |
| *mrc1Δ::HIS3* | Mrc1ko-for | 5′-TAGCATTTCAAACACATTATGTTGGAAAAAAACCAAGA-ACAGACAAACAACTAAGGAAGTTCGTTATTCGCTTTTGA-ACTTATCACCAAATATTTTAGTGAGATTGTACTGAGAGT-GCAC-3′ | pRS413 (2) |
| | Mrc1ko-rev | 5′-CCTAGACTCGGGTGCCATCTTTTTTAATGCGACTACTTCA-AGACAGCTTCTGGAGTTCAATCAACTTCTTCGGAAAAGA-TAAAAAACCATCTGTGCGGTATTTCACACCG-3′ | |
| *rad9Δ::HIS3* | Rad9ko-for | 5′-TTTGTTCGTGGATATTTGCAACGATGAGCAATGTGAAGT-GAGCAAGATAGAGAAACGCCATAGAAAAGAGCATAGT-GAGAAAATCTTCAACATCAGGGCTAGATTGTACTGAGA-GTGCAC-3′ | pRS413 (2) |
| | Rad9ko-rev | 5′-TGGCGTGTGGGAGGATGTTCTTAGACTTAATTAAGAATC-TCTAAATTTTTTTTTATTTAATCGTCCCTTTCTATCAATTAT-GAGTTTATATATTTTTATAATTTCTGTGCGGTATTTCACACC-3′ | |
| *sml1Δ::HIS3* | sml1U | 5′-GATCTTACGGTCTCACTAACCTCTCTTCAACTGCTCAATA-ATTTCCCGCTAGATTGTACTGAGAGTGCAC-3′ | pRS413 (2) |
| | sml1D | 5′-CAGAACTAGTGGGAAATGGAAAGAGAAAAGAAAAGAG-TATGAAAGGAACTTTACTGTGCGGTATTTCACACCG-3′ | |
| *crt1Δ::natMX* | crt1MXfp | 5′-CGTTTCGTGTTGTCATGGCGATTTGGGAAAAAGTTGAAA-AAAAAAATAGCAGTAAACATGGAGGCCCAGAATACCCT-3′ | pFvL099 (3) |
| | crt1MXrp | 5′-ATATGCAACGTTATATTCTTTTTTAAATATCCCCATATACT-AATGATAGAACTTTCAGTATAGCGACCAGCATTCAC-3′ | |
| *dif1Δ::natMX* or *dif1Δ::hphMX* | dif1MXfp | 5′-AGAAACGCACGCTTTACATCACACACTAATACAGGAACA-AACAAGACTTAACATGGAGGCCCAGAATACCCT-3′ | pFvL099 (nat), pFvL100 (hph) (3) |
| | dif1MXrp | 5′-CAAGTCTGTTAAAGTCTTCTCTTGGATCCATTAACCATTGT-TTCGTGCTCCAGTATAGCGACCAGCATTCAC-3′ | |

*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 for 1 min; 30 cycles of 98° C for 10 s, 55 °C for 30 s, 72 °C for 90 s; 72 °C for 60 s. for, forward; rev, reverse.

1. Güldener U, Heck S, Fielder T, Beinhauer J, Hegemann JH (1996) A new efficient gene disruption cassette for repeated use in budding yeast. *Nucleic Acids Res* 24(13):2519–2524.
2. Brachmann CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14(2):115–132.
3. Stulemeijer IJ, et al. (2011) Dot1 binding induces chromatin rearrangements by histone methylation-dependent and -independent mechanisms. *Epigenetics Chromatin* 4(1):2.