Supplementary Table 1. Yeast strains

Strain	Relevant genotype
SJR2571	*apn1Δ::TRP1*
SJR2712	*ntg1Δ::loxP-TRP1-loxP ntg2Δ::loxP-hyg-loxP*
SJR2653	*apn1Δ::TRP1 mag1Δ:: hyg*
SJR2752	*apn1Δ::TRP1 ogg1Δ::loxP-URA3Kl-loxP*
SJR2750	*apn1Δ::TRP1 ung1Δ::loxP-hyg-loxP*
SJR2726	*apn1Δ::loxP-URA3Kl-loxP ntg1Δ::loxP-TRP1-loxP ntg2Δ::loxP-hyg-loxP*
SJR2651	*apn1Δ::TRP1 rev3Δ::hyg^R*
SJR2794	*apn1Δ::loxP-URA3Kl-loxP ntg1Δ::loxP ntg2Δ::loxP-hyg-loxP ung1Δ::loxP-TRP1-loxP*
SJR2780	*apn1Δ::loxP-URA3Kl-loxP ntg1Δ::loxP ntg2Δ::loxP-hyg-loxP rev3Δ::TRP1*
SJR2817	*apn1Δ::loxP-TRP1-loxP ntg1Δ::loxP ntg2Δ::loxP-hyg-loxP*

All strains were derived from SJR2391 [*MATα ura3-52 ade2-101$_{oc}$ trp1Δ1 lys2Δ::nat leu2-K:TetR'-Ssn6:LEU2 his4Δ::TET-lys2ΔA746F (kan^R)*] [1]. Deletion of DNA repair/bypass genes was accomplished by one-step disruption with PCR-generated fragments. These fragments contained one of the following individual selective sequences flanked by appropriate locus-specific targeting sequences: *hyg* [2], *TRP1* [3], *loxP-URAKl-loxP* [4], *loxP-hyg-loxP* (pSR955), or *loxP-TRP1-loxP* (pSR954). pSR955 or pSR954 were derived by replacing the *kan* marker of pUG6 [5] with a *Bgl*II/*Sac*I fragment containing a *hyg* marker (from hphMX4 [2]) or a *TRP1* marker

(from pFA6-TRP1 [3]), respectively. When applicable, subsequent Cre/LoxP-mediated deletion of the selective marker was carried out according to described protocols [4].

1. Kim, N., Abdulovic, A. L., Gealy, R., Lippert, M. J., and Jinks-Robertson, S. Transcription-associated mutagenesis in yeast is directly proportional to the level of gene expression and influenced by the direction of DNA replication. *DNA Repair* **6**, 1285 (2007).
2. Goldstein, A. L. and McCusker, J. H. Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast* **15**, 1541 (1999).
3. Longtine, M.S. et al. Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast* **14**, 953 (1998).
4. Gueldener, U., Heinisch, J., Koehler, G. J., Voss, D., and Hegemann, J. H. A second set of loxP marker cassettes for Cre-mediated multiple gene knockouts in budding yeast. *Nucleic Acids Res.* **30**, e23 (2002).
5. Guldener, U., Heck, S., Fielder, T., Beinhauer, J., and Hegemann, J. H. A new efficient gene disruption cassette for repeated use in budding yeast. *Nucleic Acids Res.* **24**, 2519 (1996).

Supplementary Table 2. Effect of Dut1 overexpression on mutation rates

Genotype	*TET-lys2ΔA746* reversion rate x 10^{-8} (95% CI)		*CAN1* forward mutation rate x 10^{-8} (95% CI)	
	pRS426	pRS426-*GAL1-DUT1*	pRS426	pRS426-*GAL1-DUT1*
apn1	23.0 (14.1-28.0)	15.1 (5.69-29.7)	99.2 (95.1-122)	106 (55.7-171)
apn1 ntg1 ntg2	171 (143-281)	17.1 (16.3-21.4)	224 (199-308)	159 (106-287)

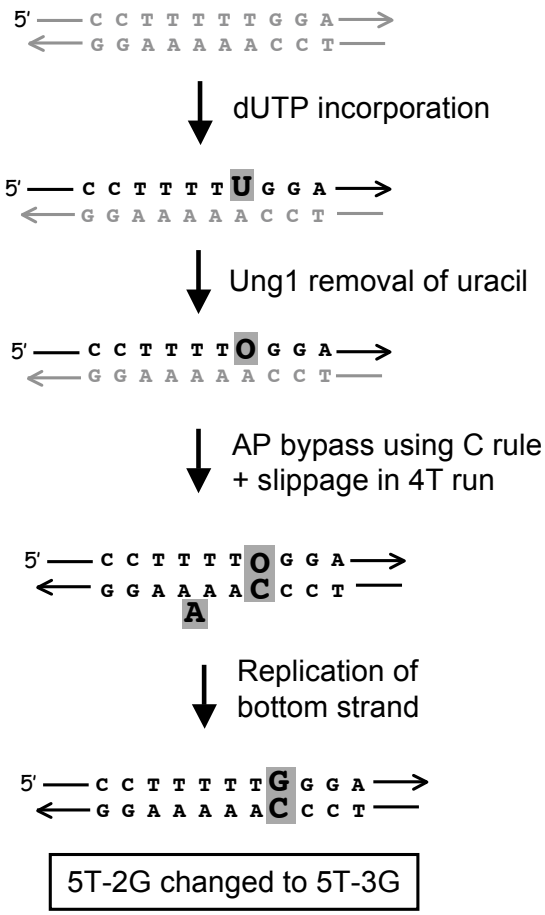
Approximately five primary Ura^+ transformants were inoculated directly into SC-Ura liquid medium supplemented with 2% glycerol, 2% ethanol, and 2% galactose. The following day, parallel 1 ml cultures were started in the same medium using ~500,000 cells of the overnight culture. After 4 days growth at 30^o, appropriate dilutions were plated on SCD-Ura to determine the number of plasmid-containing cells, on SCD-Ura-Lys to select Lys^+ revertants, or on SCD-Ura-Arg supplemented with 60 μg/ml L-canavanine sulfate (Sigma) to select *can1* mutants. CI = confidence interval.

Supplementary Table 3. Complex mutations at the 5T2G hotspot

Strain genotype	Lys^+ rate x 10^{-8} (95% CI)	Complex mutations at the 5T2G hotspot	
		Number/total	Rate (x 10^{-8})
WT	4.28 (3.35-6.74)	0/117	<0.037*
apn1	14.5 (12.4-18.1)	8/127	0.91
apn1 ntg1 ntg2	150 (125-192)	21/94	34
apn1 ntg1 ntg2 + Dox	1.19 (0.949-1.32)	0/89	<0.013*
apn1 ntg1 ntg2 rev3	5.14 (2.65-10.2)	0/92	<0.057*
apn1 ntg1 ntg2 ung1	8.15 (6.52-14.8)	0/89	<0.092*

Dox = doxycycline; CI = confidence interval

* rate calculated assuming 1 event



Supplementary Figure 1. Model for complex insertions at the 5T2G hotspot. Black lines and letters correspond to newly-synthesized DNA. O = AP site.