Supporting Information

Mertz et al. 10.1073/pnas.1422934112

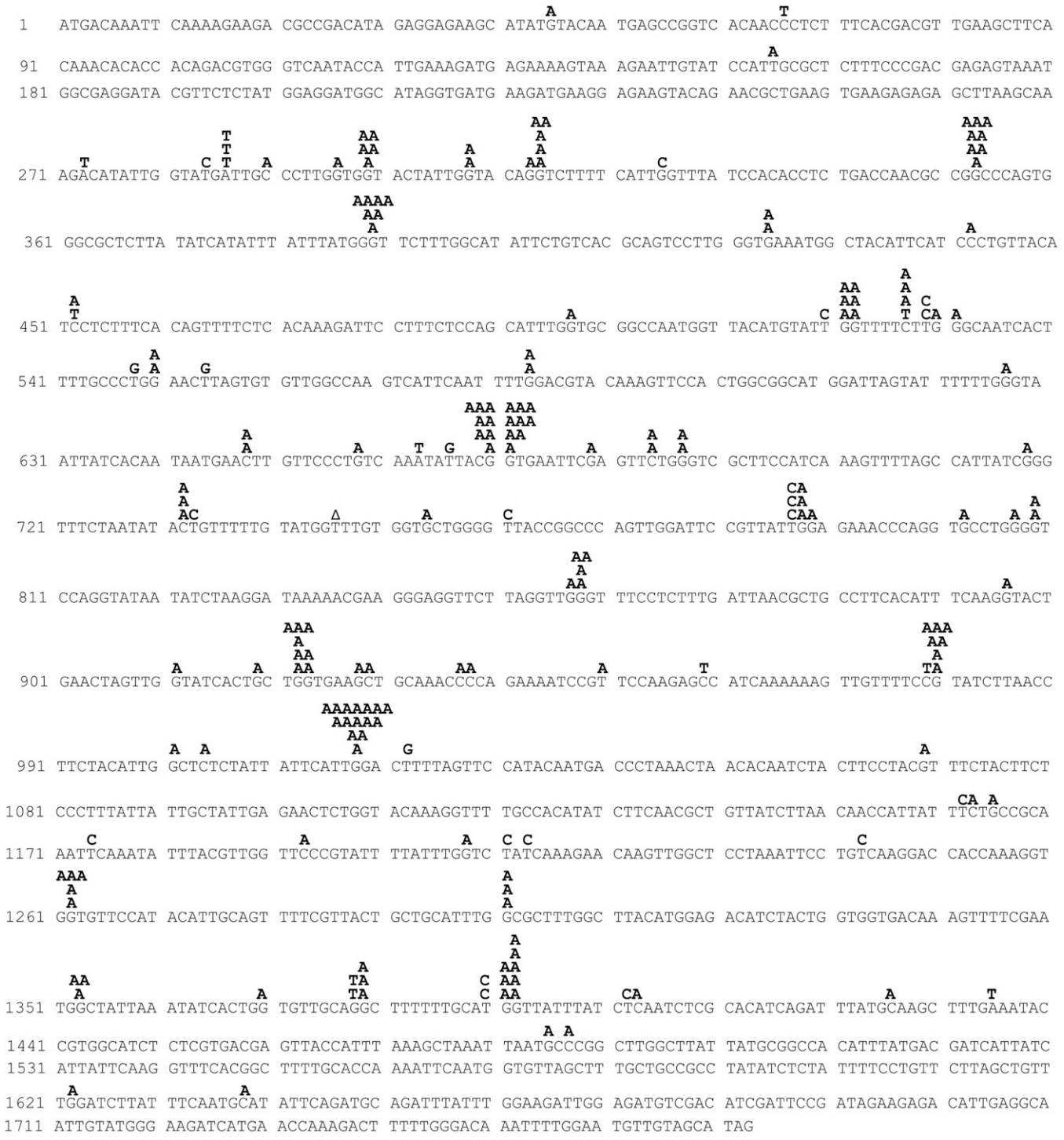
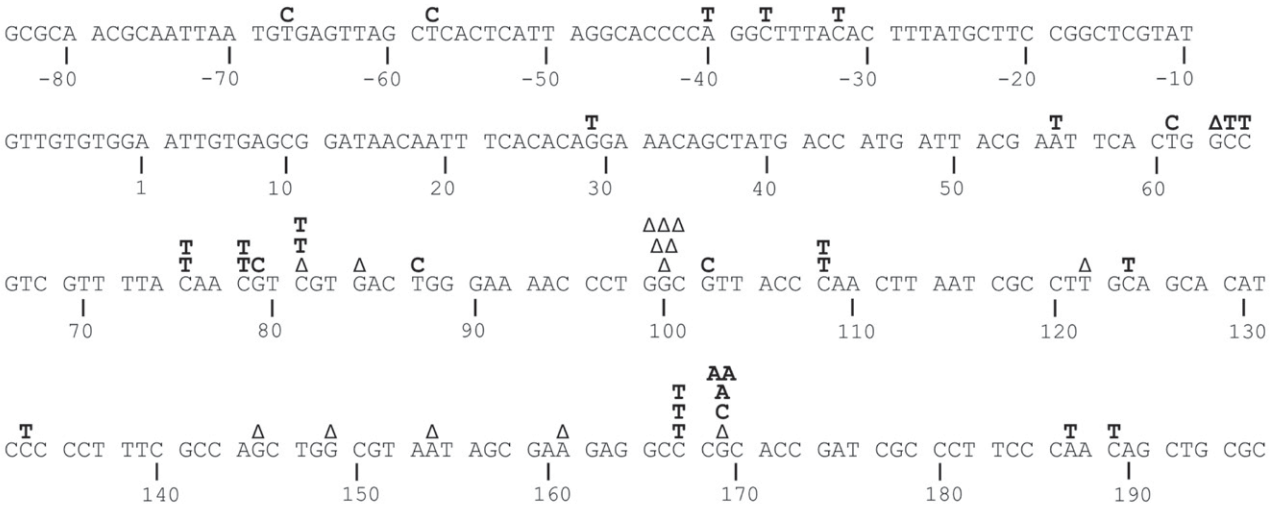


Fig. S1. Spectrum of spontaneous *can1* mutations in the *msh6 pol3-R696W* strain containing pPOL3. Mutations (bold text) are displayed above the coding sequence of *CAN1* (plain text). Nucleotides are numbered from the start of the *CAN1* ORF. The data are summarized in Table 1 and Fig. 4*D*.

A. Wild-type Polδ (100 μM dNTPs)



B. R696W Polδ (100 μM dNTPs)

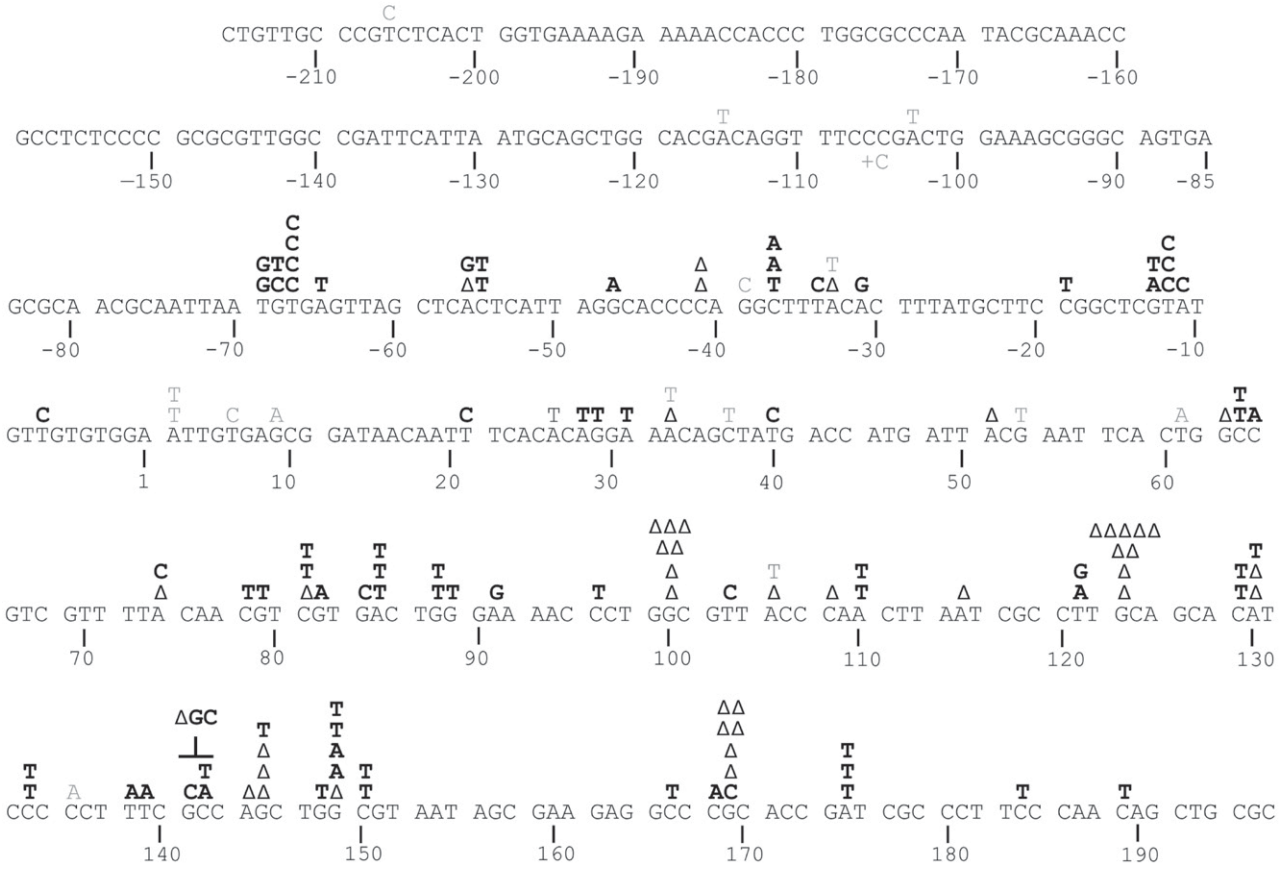
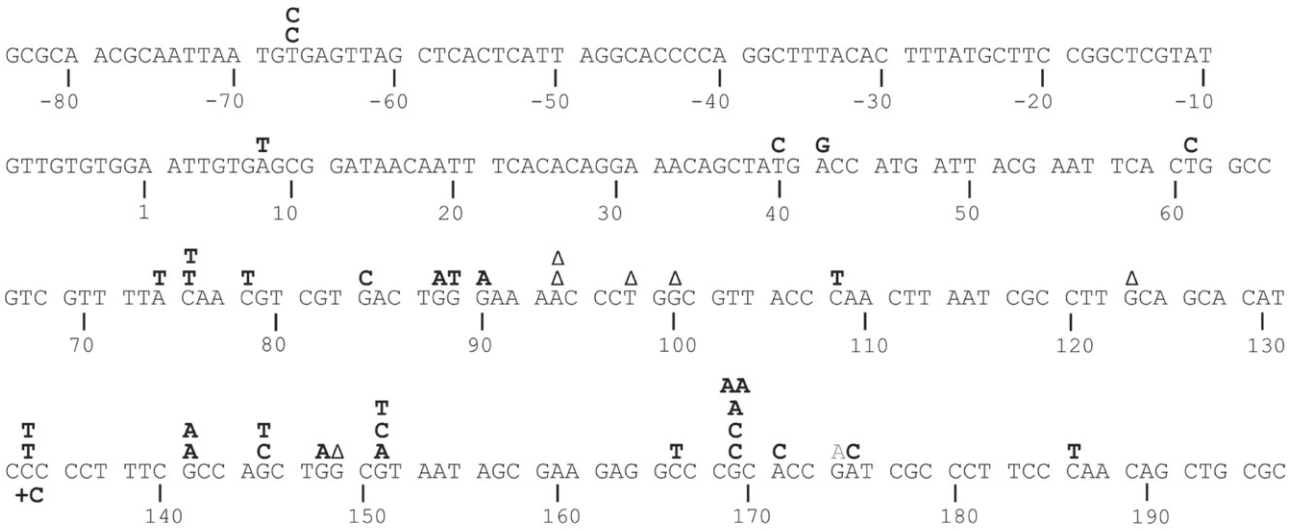


Fig. S2. (continued)

C. Wild-type Pol δ (S-phase dNTP concentrations)



D. R696W Pol δ (S-phase dNTP concentrations)

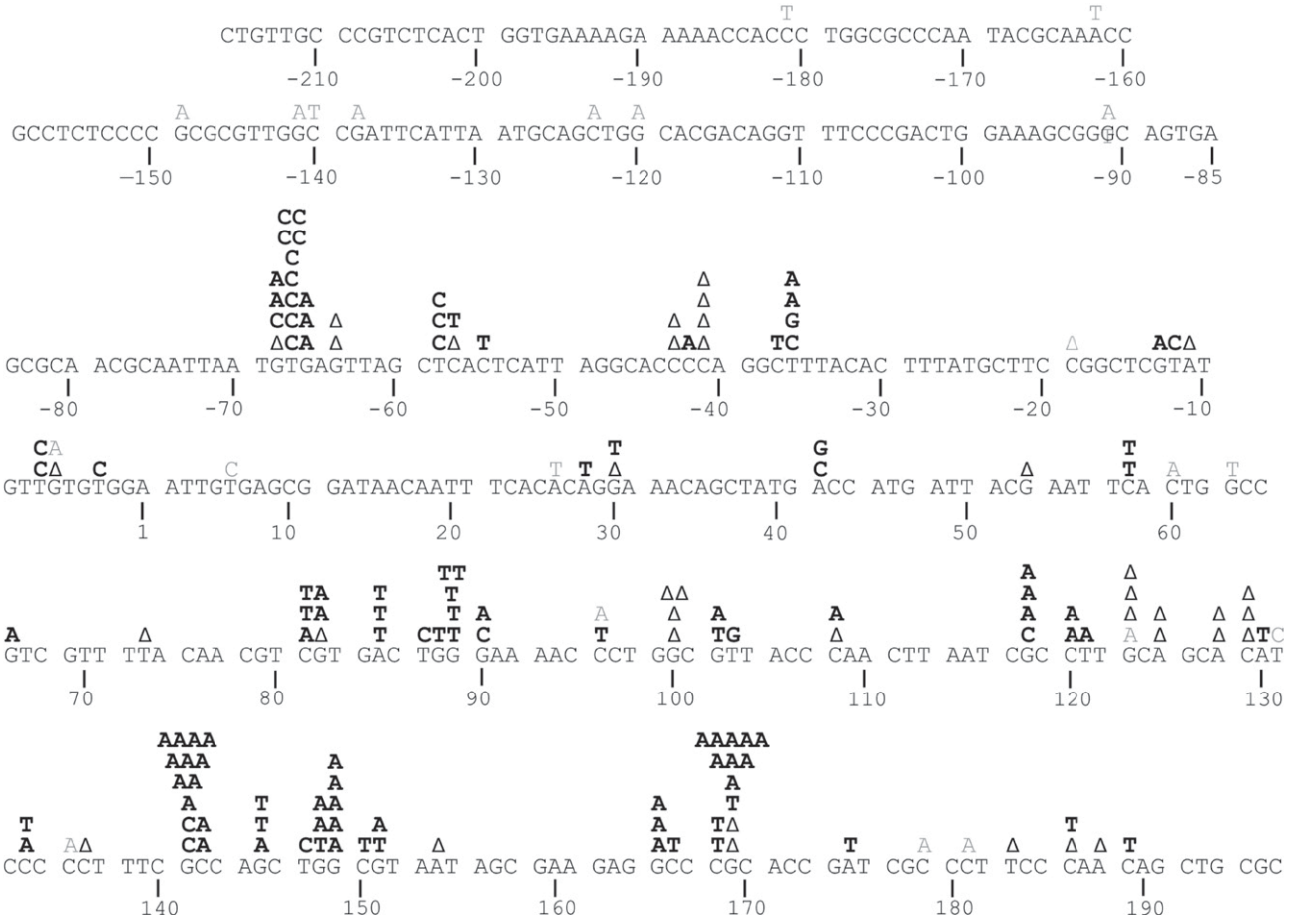


Fig. S2. (continued)

E. R696W Polδ (dNTP concentrations three-fold higher than estimated for S-phase)

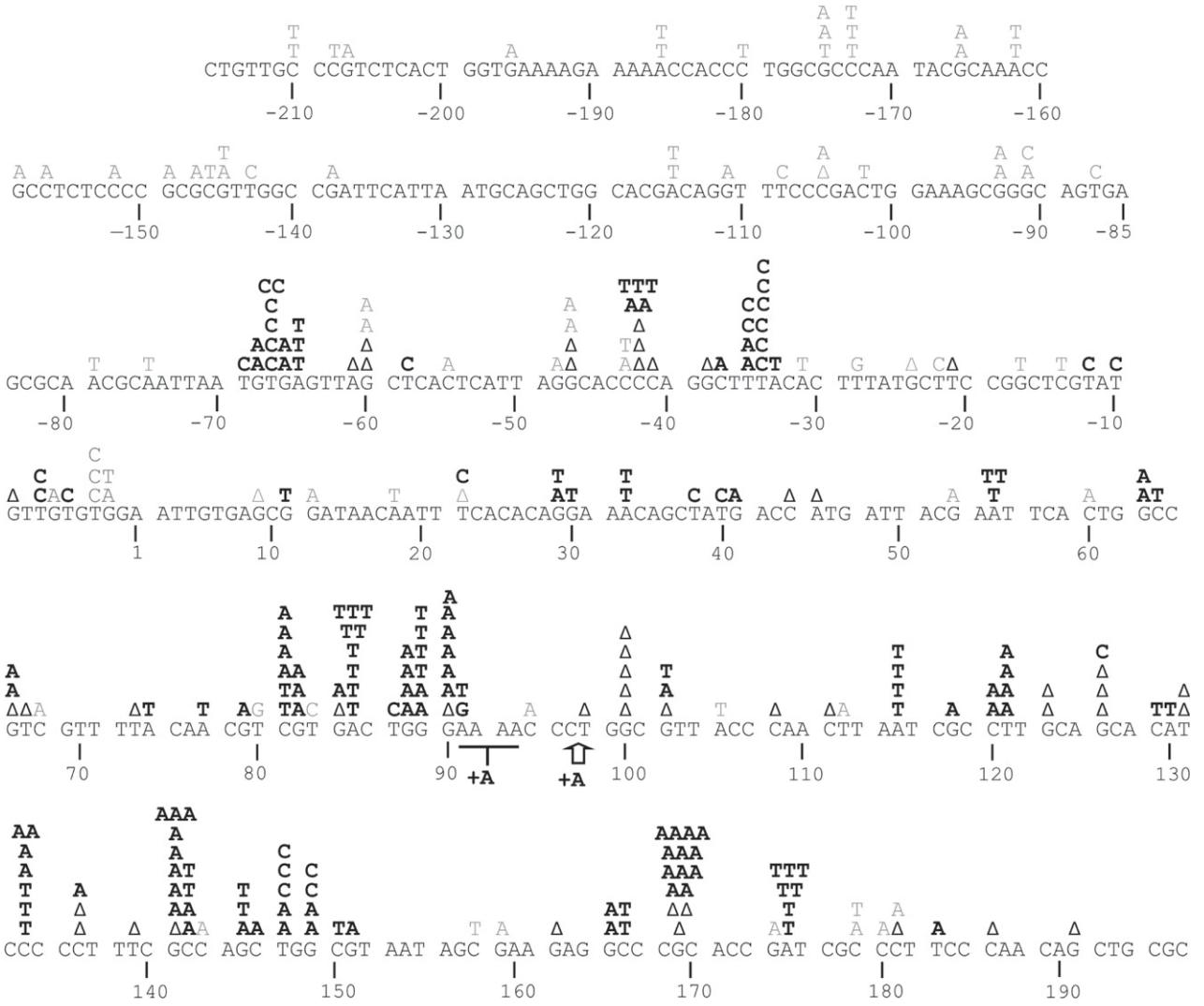


Fig. S2. Spectra of mutations generated by wild-type Polδ and Polδ-R696W in vitro at the indicated dNTP concentrations. Base substitutions and deletions (open triangles) are displayed above the *lacZ* sequence. Insertions are shown under the *lacZ* sequence. Detectable mutations are shown in black, bold text. Silent mutations are shown in plain, gray text. The region corresponding to nucleotides −210 to −85 contains no sites at which mutations can be detected. Sequencing of wild-type Polδ synthesis products revealed no mutations in this region. Silent mutations in this region are depicted in the Polδ-R696W spectra. In addition to the point mutations depicted in *A*–*E*, the following large deletions were found: wild-type Polδ (100 μM dNTPs) −177 to 127; wild-type Polδ (S-phase dNTPs) −168 to 108; and Polδ-R696W (S-phase dNTPs) −96 to 123, −83 to 117, and 148–228.

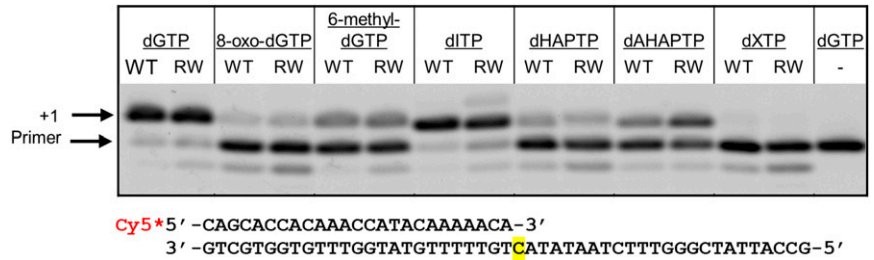


Fig. S3. Incorporation of noncanonical dNTPs by wild-type Polδ (WT) or Polδ-R696W (RW). Reactions were performed with 2.5 nM Polδ, 24 nM Cy5-labeled DNA substrate, 40 mM Tris·HCl (pH 7.8), 125 mM NaAc, 8 mM MgAc, 1 mM DTT, 0.2 mg/mL BSA, 4% (wt/vol) polyethyleneglycol 8000, and the indicated dNTP at 100 μM for 50 s at 30 °C. The DNA substrate is shown below the gel, with the first templating base highlighted in yellow. Reaction products were separated on a 12% (wt/vol) polyacrylamide gel under denaturing conditions. The arrows indicate the position of the unextended primer. dHAPTP, 2′-deoxy-6-hydroxylaminopurine 5′-triphosphate; dXTP, 2′-deoxyxanthosine 5′-triphosphate.

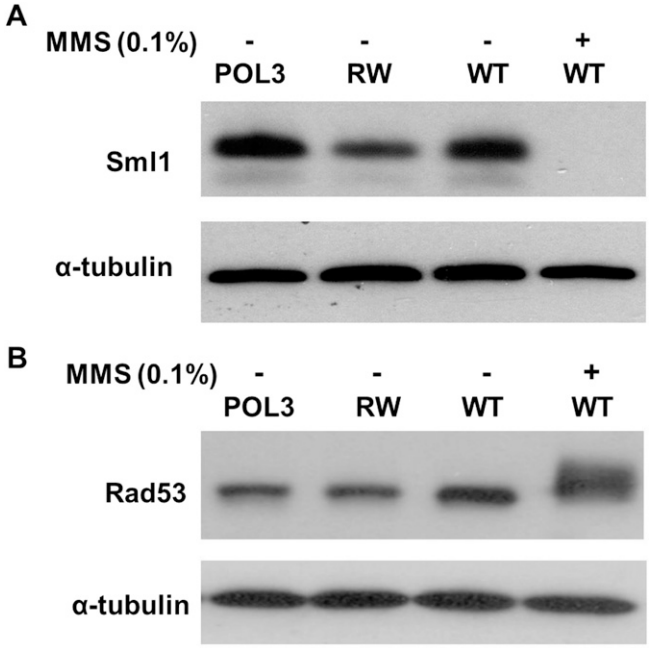


Fig. S4. Western blot analysis of proteins that modulate RNR activity. Immunoblots were conducted using whole-cell extracts from a wild-type strain containing pPOL3 (POL3), a *pol3-R696W* strain containing pPOL3 (RW), and a wild-type yeast strain without pPOL3 (WT) using the indicated primary antibodies. Experimental and loading control blots in each panel are from the same gel. A wild-type strain treated with the DNA damaging agent methyl methanesulfonate (MMS), which activates the DNA damage checkpoint, was used as a control. (*A*) *pol3-R696W* strains have reduced levels of RNR inhibitor Sml1. (*B*) *pol3-R696W* strains do not show detectable Rad53 phosphorylation.

Table S1. Effect of MMR, proofreading, and RNR metabolism deficiencies upon the mutator phenotype of haploid strains expressing *pol3-R696W*

Genotype*	Can^R mutation rate ($\times 10^{-7}$)[†]	Fold change[‡]
WT[§]	2.3 (1.6–2.7)	
WT + pPOL3[§]	2.6 (2.1–3.8)	
WT	2.7 (2.3–3.2)	
WT + pPOL3	2.6 (1.9–4.0)	1
*msh6*Δ	38 (32–41)	14
*msh6*Δ + pPOL3	35 (24–44)	13
pol3-R696W + pPOL3[§]	54 (44–65)	21
pol3-R696W + pPOL3	59 (48–70)	23
*pol3-R696W msh6*Δ + pPOL3	1,500 (850–1,900)	580
*dun1*Δ + pPOL3	3.8 (2.9–4.6)	1.5
*sml1*Δ + pPOL3	5.5 (4.9–6.6)	2.1
*crt1*Δ + pPOL3	5.8 (4.8–7.0)	2.2
*dun1*Δ *sml1*Δ *crt1*Δ + pPOL3	7.3 (5.5–11)	2.8
*pol3-R696W dun1*Δ + pPOL3	3.7 (2.1–5.3)	1.4
*pol3-R696W sml1*Δ + pPOL3	61 (50–71)	23
*pol3-R696W dun1*Δ *sml1*Δ + pPOL3	35 (30–45)	13
*pol3-R696W dun1*Δ *sml1*Δ *crt1*Δ + pPOL3	97 (88–110)	37
*pol3-R696W dun1*Δ + pDUN1 + pPOL3	57 (48–71)	22
WT + pPOL3.GST[§]	2.9 (2.4–3.9)	1
WT + pPOL3.GST-5DV[§]	15 (12–18)	5.2
WT + pPOL3.GST-R696W[§]	230 (200–270)	79
WT + pPOL3.GST-5DV,R696W[§]	1,200 (1,100–1,500)	410
*dun1*Δ + pPOL3.GST	3.9 (2.8–4.4)	1.3
*dun1*Δ + pPOL3.GST-R696W	9.7 (7.5–10)	3.3

*All haploid yeast strains used for mutation rate measurements are derived from 1B-D770. Full genotypes are described in Table S4. The majority of strains have *LEU2* integrated downstream of *CAN1*.
[†]Values are medians for at least 18 cultures. The 95% confidence limits are shown in parentheses.
[‡]Fold change in the mutation rate relative to the wild-type control.
[§]These strains have an unmodified *CAN1* allele.

Table S2. Mutator effects of the *pol3-R696W* allele in MMR-proficient and MMR-deficient diploid strains

Genotype*	Can^R mutation rate ($\times 10^{-7}$)[†]	Fold change[‡]
POL3/POL3	2.3 (2.0–3.0)	1
POL3/pol3-R696W	22 (17–26)	9.6
*msh6*Δ/*msh6*Δ	41 (31–56)	18
*POL3/pol3-R696W msh6*Δ/*msh6*Δ	470 (280–630)	200

The Can^R mutation rates were measured in diploid strains with a single copy of *CAN1* (Fig. 1*A*).
*Full genotypes are described in Table S4.
[†]Mutation rates are medians for at least 18 cultures. The 95% confidence limits are shown in parentheses.
[‡]Fold change in the mutation rate relative to the wild-type diploid.

Table S3. Fidelity of in vitro synthesis by Polδ-R696W at dNTP concentrations threefold higher than estimated for S-phase *pol3-R696W* cells containing pPOL3

	R696W Polδ (threefold S-phase dNTP concentration)	
	No.	ER ($\times 10^{-5}$)
Base substitutions	172	86
Transitions	100	68
A→G (A-dCTP)	1	2.7
G→A (G-dTTP)	55	190
C→T (C-dATP)	16	41
T→C (T-dGTP)	28	68
Transversions	72	28
A→C (A-dGTP)	0	<3.8
A→T (A-dATP)	33	73
C→A (C-dTTP)	17	55
C→G (C-dCTP)	0	<5.0
G→C (G-dGTP)	3	9.4
G→T (G-dATP)	10	30
T→A (T-dTTP)	9	35
T→G (T-dCTP)	0	<3.0
Frameshifts	47	18
Minus 1	45	17
Plus 1	2	0.74
Large deletions	0	
Total	219	
lacZ mutant frequency	0.084	

The data are based on analysis of 179 *lacZ* mutants, 82 of which contained more than one mutation. Only phenotypically detectable *lacZ* mutations are listed. The error rate (ER) for individual mutation types were calculated as described in *Materials and Methods*. Silent mutations were excluded from the ER calculation. The location of individual mutations in the *lacZ* sequence is shown in Fig. S2. The background mutation frequency for unfilled M13mp2 gapped substrate was 0.0016.

Table S4. Yeast strains used in this study

Strain	Relevant chromosomal mutation*	Plasmid	*CAN1* allele	Genotype
Haploid strains used to produce data				
1B-D770	WT	—	*CAN1*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4*
TM20	WT	pPOL3	*CAN1*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 [GAL1-POL3]*
TM19	*pol3-R696W*	pPOL3	*CAN1*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 pol3-R696W [GAL1-POL3]*
TM30	WT	—	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2*
TM42	WT	pPOL3	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 [GAL1-POL3]*
TM43	*msh6Δ*	pPOL3	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 msh6Δ::HygB [GAL1-POL3]*
TM34	*pol3-R696W*	pPOL3	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 pol3-R696W [GAL1-POL3]*
TM36	*pol3-R696W msh6*	pPOL3	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 pol3-R696W msh6Δ::HygB [GAL1-POL3]*
TM52	*dun1Δ*	pPOL3	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 dun1Δ::KanMX [GAL1-POL3]*
TM51	*sml1Δ*	pPOL3	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 sml1Δ::HygB [GAL1-POL3]*
TM69	*crt1Δ*	pPOL3	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 crt1Δ::KanMX [GAL1-POL3]*
TM70	*dun1Δ sml1Δ crt1Δ*	pPOL3	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 dun1Δ::KanMX sml1Δ::HygB crt1Δ::NatMX [GAL1-POL3]*
TM56	*pol3-R696W dun1Δ*	pPOL3	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 pol3-R696W dun1Δ::KanMX [GAL1-POL3]*
TM58	*pol3-R696W sml1Δ*	pPOL3	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 pol3-R696W sml1Δ::HygB [GAL1-POL3]*
TM59	*pol3-R696W dun1Δ sml1Δ*	pPOL3	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 pol3-R696W dun1Δ::KanMX sml1Δ::HygB [GAL1-POL3]*
TM68	*pol3-R696W dun1Δ sml1Δ crt1Δ*	pPOL3	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 pol3-R696W dun1Δ::KanMX sml1Δ::HygB crt1Δ::NatMX [GAL1-POL3]*
TM71	*pol3-R696W dun1Δ*	pPOL3 + pDUN1	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 pol3-R696W dun1Δ::KanMX [DUN1] [GAL1-POL3]*
TM72	WT	pPOL3.GST	*CAN1*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 [GAL1-GST-POL3]*
TM73	WT	pPOL3.GST-5DV	*CAN1*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 [GAL1-GST-POL3-5DV]*
TM74	WT	pPOL3.GST-R696W	*CAN1*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 [GAL1-GST-POL3-R696W]*
TM75	WT	pPOL3.GST-5DV,R696W	*CAN1*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 [GAL1-GST-POL3-5DV,R696W]*
TM76	WT	pPOL3.GST	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 dun1Δ::KanMX [GAL1-GST-POL3]*
TM77	WT	pPOL3.GST-R696W	*CAN1::Kl.LEU2*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::Kl.LEU2 dun1Δ::KanMX [GAL1-GST-POL3-R696W]*
Diploid strains used to produce data				
TM63	WT	—	*CAN1::Kl.LEU2/ can1::loxP*	*MATa/MATα ade5-1/ade5-1 lys2-Tn5-13/lys2-InsE$_{A14}$ trp1-289/ trp1-289 his7-2/his7-2 leu2-3,112/leu2-3,112 ura3-4/ura3-52 can1Δ::loxP/CAN1::Kl.LEU2*
TM44 × TM34	*POL3/pol3-R696W*	—	*CAN1::Kl.LEU2/ can1::loxP*	*MATa/MATα ade5-1/ade5-1 lys2-Tn5-13/lys2-InsE$_{A14}$ trp1-289/ trp1-289 his7-2/his7-2 leu2-3,112/leu2-3,112 ura3-4/ura3-52 can1Δ::loxP/CAN1::Kl.LEU2 POL3/pol3-R696W*
TM45 × TM43	*msh6Δ/msh6Δ*	—	*CAN1::Kl.LEU2/ can1::loxP*	*MATa/MATα ade5-1/ade5-1 lys2-Tn5-13/lys2-InsE$_{A14}$ trp1-289/ trp1-289 his7-2/his7-2 leu2-3,112/leu2-3,112 ura3-4/ura3-52 can1Δ::loxP/CAN1::Kl.LEU2 msh6Δ::HygB/msh6Δ::KanMX*

Table S4. Cont.

Strain	Relevant chromosomal mutation*	Plasmid	*CAN1* allele	Genotype
TM45 × TM36	*POL3/pol3-R696W msh6Δ/msh6Δ*	—	*CAN1::Kl.LEU2/can1::loxP*	*MATa/MATα ade5-1/ade5-1 lys2-Tn5-13/lys2-InsE$_{A14}$ trp1-289/trp1-289 his7-2/his7-2 leu2-3,112/leu2-3,112 ura3-4/ura3-52 can1Δ::loxP/CAN1::Kl.LEU2 POL3/pol3-R696W msh6Δ::HygB/msh6Δ::KanMX*
TM64	WT	pPOL3	*CAN1::Kl.LEU2/can1::loxP*	*MATa/MATα ade5-1/ade5-1 lys2-Tn5-13/lys2-InsE$_{A14}$ trp1-289/trp1-289 his7-2/his7-2 leu2-3,112/leu2-3,112 ura3-4/ura3-52 can1Δ::loxP/CAN1::Kl.LEU2* [*GAL1-POL3*]
TM44 × TM34	*POL3/pol3-R696W*	pPOL3	*CAN1::Kl.LEU2/can1::loxP*	*MATa/MATα ade5-1/ade5-1 lys2-Tn5-13/lys2-InsE$_{A14}$ trp1-289/trp1-289 his7-2/his7-2 leu2-3,112/leu2-3,112 ura3-4/ura3-52 can1Δ::loxP/CAN1::Kl.LEU2 POL3/pol3-R696W* [*GAL1-POL3*]
TM46 × TM34	*pol3-R696W/pol3-R696W*	pPOL3	*CAN1::Kl.LEU2/can1::loxP*	*MATa/MATα ade5-1/ade5-1 lys2-Tn5-13/lys2-InsE$_{A14}$ trp1-289/trp1-289 his7-2/his7-2 leu2-3,112/leu2-3,112 ura3-4/ura3-52 can1Δ::loxP/CAN1::Kl.LEU2 POL3/pol3-R696W* [*GAL1-POL3*]
Strains used in construction of diploid strains				
E134	WT	—	*CAN1*	*MATα ade5-1 lys2::InsE$_{A14}$ trp-289 his7-2 leu2-3,112 ura3-52*
TM39	WT	—	*can1Δ::loxP*	*MATα ade5-1 lys2::InsE$_{A14}$ trp-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP*
TM46	*pol3-R696W*	pPOL3	*can1Δ::loxP*	*MATα ade5-1 ys2-InsE$_{A14}$ trp-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP* pol3-R696W [*GAL1-POL3*]
TM44	WT	pPOL3	*can1Δ::loxP*	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP* [*GAL1-POL3*]
TM45	*msh6Δ*	—	*can1Δ::loxP*	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP msh6Δ::KanMX*

Details of strain construction are provided in *Materials and Methods*.
*WT, wild type.