SUPPLEMENTARY MATERIAL

Functional analysis of cancer-associated DNA polymerase ε variants in *Saccharomyces cerevisiae*

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Supplementary Table S1. Genotypes of yeast strains used for construction of *pol2-x* mutants and mutation rate measurements.

Strain	Relevant chromosomal mutation	Genotype
Haploid strains		
TM30	WT	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::LEU2*
TM44	WT	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP*
DK039a/DK039b	*pol2-F139L*	*MATa ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-X CAN1::LEU2 pol2-F139L*
SB042/SB044	*pol2-R252H*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::LEU2 pol2-R252H*
SB046/SB051	*pol2-R252H*	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP pol2-R252H*
SI022/SI023	*pol2-D290V*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::LEU2 pol2-D290V*
SI024/SI026	*pol2-D290V*	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP pol2-D290V*
DK046/DK047	*pol2-P301H*	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-X CAN1::LEU2 pol2-P301H*
SB112/SB113	*pol2-P301H*	*MATa ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-X can1Δ::loxP pol2-P301H*
DK048	*pol2-F382S*	*MATα ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-X can1Δ::loxP pol2-F382S*
DK049/DK050	*pol2-F382S*	*MATa ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-X CAN1::LEU2 pol2-F382S*
SB052/SB053	*pol2-V426L*	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP pol2-V426L*
SB054/SB055	*pol2-V426L*	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP pol2-V426L*
SI030/SI031	*pol2-L439V*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::LEU2 pol2-L439V*
SI032/SI033	*pol2-L439V*	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP pol2-L439V*
SB063/SB065	*pol2-P451R*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::LEU2 pol2-P451R*
SB066/SB068	*pol2-P451R*	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP pol2-P451R*
SB069/SB071	*pol2-S474F*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::LEU2 pol2-S474F*
SB073/SB074	*pol2-S474F*	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP pol2-S474F*

Supplementary Table S1. Genotypes of yeast strains used for construction of *pol2-x* mutants and mutation rate measurements (Continued)

Strain	Relevant chromosomal mutation	Genotype
DK051a/DK051b	*pol2-R778W*	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-X CAN1::LEU2 pol2-R778W*
SB075/SB077	*pol2-A979V*	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::LEU2 pol2-A979V*
SB079/SB081	*pol2-A979V*	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP pol2-A979V*
DK055a/DK055b	*pol2-D1757N*	*MATa ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-X CAN1::LEU2 pol2-D1757N*
SB083/SB084	*mlh1*Δ	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::LEU2 mlh1Δ::hphMX4*
SB086/SB087	*mlh1*Δ	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP mlh1Δ::hphMX4*
SB088/SB089/ SB090/SB091	*pol2-R252H mlh1*Δ	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::LEU2 pol2-R252H mlh1Δ::hphMX4*
SB092/SB093/ SB094	*pol2-R252H mlh1*Δ	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP pol2-R252H mlh1Δ::hphMX4*
SI003/SI004/ SI005/SI006	*pol2-R778W mlh1*Δ	*MATα ade5-1 lys2-InsE$_{A14}$trp1-289 his7-2 leu2-3,112 ura3-X CAN1::LEU2 pol2-R778W mlh1Δ::hphMX4*
SB095/SB096/ SB097/SB098	*pol2-A979V mlh1*Δ	*MATa ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4 CAN1::LEU2 pol2-A979V mlh1Δ::hphMX4*
SB099/SB100/ SB101/SB102	*pol2-A979V mlh1*Δ	*MATα ade5-1 lys2-InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52 can1Δ::loxP pol2-A979V mlh1Δ::hphMX4*
Diploid strains		
TM63/SB539/ SB540	WT	*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 CAN1::LEU2/can1Δ::loxP*
SB555/SB556	*pol2-R252H/POL2*	*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 CAN1::LEU2/can1Δ::loxP pol2-R252H/POL2*
SI531/SI533	*pol2-D290V/POL2*	*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 CAN1::LEU2/can1Δ::loxP pol2-D290V/POL2*

Supplementary Table S1. Genotypes of yeast strains used for construction of *pol2-x* mutants and mutation rate measurements (Continued)

Strain	Relevant chromosomal mutation	Genotype
SI527/SI528/ SI529/SI530	*pol2-D290V/pol2-D290V*	*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 CAN1::LEU2/can1Δ::loxP pol2-D290V/pol2-D290V*
SI505/SI507	*pol2-P301H/POL2*	*MATa/MATα ade5-1/ade5-1 lys2-InsE$_{A14}$/lys2-InsE$_{A14}$ trp1-289/trp1-289 his7-2/his7-2 leu2-3,112/leu2-3,112 ura3-X/ura3-X CAN1::LEU2/can1Δ::loxP pol2-P301H/POL2*
SB557/SB558/ SB559	*pol2-P301H/pol2-P301H*	*MATa/MATα ade5-1/ade5-1 lys2::InsE$_{A14}$/lys2::InsE$_{A14}$ trp1-289/trp1-289 his7-2/his7-2 leu2-3,112/leu2-3,112 ura3-X/ura3-X CAN1::LEU2/can1Δ::loxP pol2-P301H/pol2-P301H*
SI509/SI511	*pol2-F382S/POL2*	*MATa/MATα ade5-1/ade5-1 lys2-InsE$_{A14}$/lys2-InsE$_{A14}$ trp1-289/trp1-289 his7-2/his7-2 leu2-3,112/leu2-3,112 ura3-X/ura3-52 CAN1::LEU2/can1Δ::loxP pol2-F382S/POL2*
SI514/SI516	*pol2-F382S/pol2-F382S*	*MATa/MATα ade5-1/ade5-1 lys2-InsE$_{A14}$/lys2-Tn5-13 trp1-289/trp1-289 his7-2/his7-2 leu2-3,112/leu2-3,112 ura3-X/ura3-X CAN1::LEU2/can1Δ::loxP pol2-F382S/pol2-F382S*
SI522/SI526	*pol2-L439V/POL2*	*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 CAN1::LEU2/can1Δ::loxP pol2-L439V/POL2*
SI518/SI519/ SI520/SI521	*pol2-L439V/pol2-L439V*	*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 CAN1::LEU2/can1Δ::loxP pol2-L439V/pol2-L439V*
SB542/SB545	*pol2-P451R/POL2*	*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 CAN1::LEU2/can1Δ::loxP pol2-P451R/POL2*
SB547/SB548	*pol2-P451R/pol2-P451R*	*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 CAN1::LEU2/can1Δ::loxP pol2-P451R/pol2-P451R*
SB549/SB551	*pol2-S474F/POL2*	*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 CAN1::LEU2/can1Δ::loxP pol2-S474F/POL2*
SB552/SB553	*pol2-S474F/pol2-S474F*	*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 CAN1::LEU2/can1Δ::loxP pol2-S474F/pol2-S474F*
SB560/SB561	*mlh1Δ::hphMX4/mlh1Δ:: hphMX4*	*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 CAN1::LEU2/can1Δ::loxP mlh1Δ::hphMX4/mlh1Δ::hphMX4*
SB562/SB563	*pol2-R252H/POL2 mlh1Δ::hphMX4/mlh1Δ:: hphMX4*	*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 CAN1::LEU2/can1Δ::loxP pol2-R252H/POL2 mlh1Δ::hphMX4/mlh1Δ::hphMX4*

Supplementary Table S2. Plasmid, restriction site, and method used to construct *pol2* mutants.

Human POLE variant	Yeast Pol2 mimic	Plasmid	Restriction site	Mutation in truncated or full-length *POL2* after integration	Construction method[a]
F104L	F139L	YIpDK1	*Bam*HI	Full-length	1
R231H	R252H	YIpDK1	*Bgl*II	Truncated	2
D275V	D290V	YIpDK1	*Bam*HI	Full-length	1
P286H	P301H	YIpDK1	*Bam*HI	Full-length	1
N336S	N351S	YIpDK1	*Bam*HI	Full-length	1
F367S	F382S	YIpDK1	*Bam*HI	Full-length	1
V411L	V426L	YIpDK1	*Bgl*II	Truncated	2
L424V	L439V	YIpDK1	*Bam*HI	Full-length	1
P436R	P451R	YIpDK1	*Bgl*II	Truncated	2
S459F	S474F	YIpDK1	*Bgl*II	Truncated	2
R762W	R778W	p173	*Age*I	Full-length	1
A966V	A979V	p173	*Bsr*GI	Truncated	2
D1752N	D1757N	p174	*Age*I	Full-length	1

[a]Refers to the two methods described in the Materials and Methods section.

Supplementary Table S3. Spontaneous mutation rates in haploid yeast strains with *pol2* mutations mimicking human *POLE* variants.

Human POLE variant	Yeast *POL2* allele	Can^R mutation		His^+ reversion		Lys^+ reversion	
		Mutation rate ($x10^{-8}$)[a]	Fold increase	Mutation rate ($x10^{-8}$)[a]	Fold increase	Mutation rate ($x10^{-8}$)[a]	Fold increase
WT	*POL2*	23 (17-32)	1.0	1.3 (0.76-1.9)	1.0	31 (25-49)	1.0
F104L	*pol2-F139L*	21 (18-23)	0.91	1.2 (0.94-1.5)	0.92	26 (24-34)	0.84
R231H	*pol2-R252H*	18 (16-21)	0.78	0.78 (0.58-0.95)	0.60	36 (33-46)	1.2
D275V	*pol2-D290V*	**53 (44-55)**	**2.3**	**4.9 (4.2-5.9)**	**3.8**	**42 (36-44)**	**1.4**
P286H	*pol2-P301H*	**290 (250-440)**	**13**	**29 (25-37)**	**22**	**51 (40-63)**	**1.6**
N336S	*pol2-N351S*	21 (19-25)	0.91	1.6 (1.3-2.4)	1.2	32 (30-36)	1.0
F367S	*pol2-F382S*	**380 (300-490)**	**17**	**16 (8.7-22)**	**12**	56 (30-140)	1.8
V411L	*pol2-V426L*	28 (25-32)	1.2	**2.4 (2.0-3.3)**	**1.8**	30 (28-35)	0.97
L424V	*pol2-L439V*	**120 (82-140)**	**5.2**	**11 (9.6-16)**	**8.5**	32 (27-36)	1.0
P436R	*pol2-P451R*	**120 (98-150)**	**5.2**	**12 (11-16)**	**9.2**	**40 (36-49)**	**1.3**
S459F	*pol2-S474F*	**680 (560-800)**	**30**	**35 (30-47)**	**27**	**71 (62-87)**	**2.3**
R762W	*pol2-R778W*	21 (17-25)	0.91	1.0 (0.59-1.6)	0.77	26 (22-29)	0.84
A969V	*pol2-A979V*	21 (19-28)	0.91	1.4 (0.87-1.7)	1.1	31 (27-34)	1.0
D1752N	*pol2-D1757N*	21 (15-32)	0.91	1.0 (0.78-1.4)	0.77	22 (20-27)	0.71

[a]Mutation rates are given as the median of at least 18 independent cultures, with 95% confidence intervals in parentheses. Fold increase is shown relative to the wild-type haploid (WT). Bold text indicates $p < 0.05$ by Wilcoxon-Mann-Whitney compared to WT. Data from this table were used to generate Figure 2.

Supplementary Table S4. Summary of published tumor sequencing data used to calculate the frequency of DNA binding cleft variants.

Human *POLE* variant	Exon	No. times reported[a]	No. tumors analyzed[b]	Incidence per 10,000 tumors
D275V	9	1	13,283	0.75
P286H	9	1	13,283	0.75
P286R	9	160	13,159	120
F367S	11	5	7,079	7.1
L424V	13	3	13,424	2.2
P436R	13	6	13,424	4.5
S459F	14	18	11,831	15

[a]Number of times the variant has been reported in sporadic CRC and EC.
[b]Total number of tumors from studies where the corresponding exon was sequenced.

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Supplementary Table S5. P values (Fisher's exact test) for comparison of frequencies of individual *POLE* variants.

POLE Variant	D275V	P286H	L424V	P436R	F367S	S459F
P286H	1					
L424V	0.62	0.62				
P436R	0.12	0.12	0.51			
F367S	**0.022**	**0.022**	0.13	0.53		
S459F	**<0.0001**	**<0.0001**	**0.0003**	**0.0069**	0.14	
P286R	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Data used for analysis are in Supplementary Table S4. P values indicating statistically significant differences are in bold.

Supplementary Table S6. Spontaneous mutation rates in diploid yeast strains with *pol2* mutations mimicking human *POLE* variants.

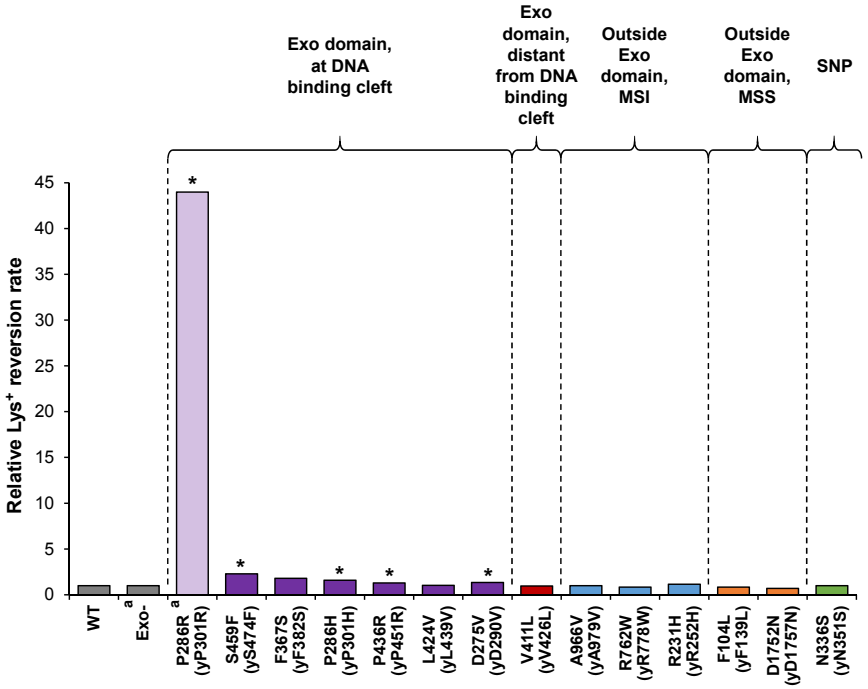
Human POLE variant	Yeast *POL2* alleles	Can^R mutation		His^+ reversion		Lys^+ reversion	
		Mutation rate ($x10^{-8}$)[a]	Fold increase	Mutation rate ($x10^{-8}$)[a]	Fold increase	Mutation rate ($x10^{-8}$)[a]	Fold increase
WT	*POL2*/*POL2*	20 (15-24)	1.0	1.1 (0.70-1.7)	1.0	22 (17-26)	1.0
D275V	*pol2-D290V/POL2*	21 (19-23)	1.0	**2.7 (2.0-3.1)**	**2.5**	16 (13-18)	0.73
	pol2-D290V/pol2-D290V	**40 (37-43)**	**2.0**	**5.3 (4.4-5.9)**	**4.8**	22 (19-28)	1.0
P286H	*pol2-P301H/POL2*	**130 (110-150)**	**6.5**	**10 (9.4-13)**	**9.1**	30[b] (27-38)	0.68[b]
	pol2-P301H/pol2-P301H	**270 (240-280)**	**14**	**24 (21-27)**	**22**	49[b] (40-52)	1.1[b]
F367S	*pol2-F382S/POL2*	**230 (190-310)**	**12**	**24 (17-37)**	**22**	59[b] (49-76)	1.3[b]
	pol2-F382S/pol2-F382S	**530 (420-710)**	**27**	**50 (32-69)**	**45**	**66 (37-83)**	**3.0**
L424V	*pol2-L439V/POL2*	**68 (53-82)**	**3.4**	**5.1 (4.5-6.4)**	**4.6**	17 (16-22)	0.77
	pol2-L439V/pol2-L439V	**120 (100-140)**	**6.0**	**13 (11-15)**	**12**	20 (18-22)	0.91
P436R	*pol2-P451R/POL2*	**82 (74-97)**	**4.1**	**7.0 (6.5-9.6)**	**6.4**	20 (17-25)	0.91
	pol2-P451R/pol2-P451R	**180 (150-200)**	**9.0**	**16 (14-19)**	**15**	24 (21-31)	1.1
S459F	*pol2-S474F/POL2*	**500 (370-640)**	**25**	**27 (24-37)**	**25**	**30 (26-31)**	**1.4**
	pol2-S474F/pol2-S474F	**1000 (850-1300)**	**50**	**92 (70-120)**	**84**	**93 (80-120)**	**4.2**

[a]Mutation rates are given as the median of at least 18 independent cultures, with 95% confidence intervals in parentheses. Fold increase is shown relative to the wild-type diploid (WT). Bold text indicates $p < 0.05$ by Wilcoxon-Mann-Whitney compared to WT. Data from this table were used to generate Figure 5.
[b]Strain contains two copies of the *lys2-InsE$_{A14}$* allele (Supplementary Table 1). The fold increase shown is the fold increase in the Lys^+ reversion rate over wild-type divided by two.

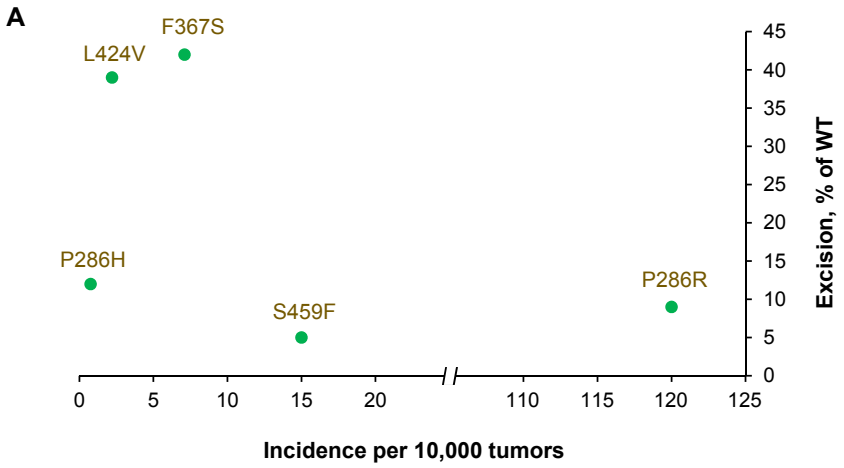
Supplementary Table S7. Spontaneous mutation rates in yeast strains with *pol2-R252H, -R778W* and *-A979V* mutations in MMR-proficient or MMR-deficient (*mlh1*Δ) background.

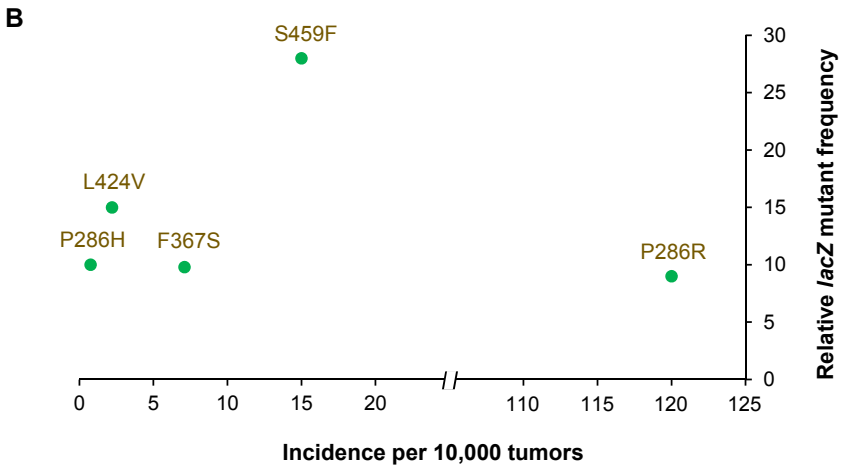
Ploidy	*POL2* allele	*MLH1* allele	Can^R mutation		His^+ reversion		Lys^+ reversion	
			Mutation rate ($x10^{-8}$)[a]	Fold increase	Mutation rate ($x10^{-8}$)[a]	Fold increase	Mutation rate ($x10^{-8}$)[a]	Fold increase
n	*POL2*	*MLH1*	15 (14-20)	1.0	0.92 (0.64-1.3)	1.0	26 (18-30)	1.0
	pol2-R252H	*MLH1*	23 (15-31)	1.5	0.90 (0.69-2.1)	0.98	32 (28-34)	1.2
	pol2-R778W	*MLH1*	15 (13-19)	1.0	0.59 (0.48-0.73)	0.64	14 (14-15)	0.54
	pol2-A979V	*MLH1*	17 (13-34)	1.1	0.93 (0.65-1.2)	1.0	21 (18-24)	0.81
	POL2	*mlh1*Δ	440 (380-490)	29	97 (57-110)	110	130000 (120000-170000)	5000
	pol2-R252H	*mlh1*Δ	**970 (930-1100)**	**65**	**150 (140-160)**	**160**	160000 (140000-260000)	6200
	pol2-R778W	*mlh1*Δ	530 (440-650)	35	100 (91-110)	110	140000 (130000-160000)	5400
	pol2-A979V	*mlh1*Δ	410 (390-470)	27	99 (90-110)	110	170000 (140000-190000)	6500
2n	*POL2*/*POL2*	*MLH1*/*MLH1*	17 (16-23)	1.0	1.2 (0.87-1.6)	1.0	18 (16-22)	1.0
	pol2-R252H/POL2	*MLH1*/*MLH1*	18 (15-21)	1.1	1.2 (1.0-1.7)	1.0	20 (17-24)	1.1
	POL2/*POL2*	*mlh1*Δ/*mlh1*Δ	450 (410-520)	26	110 (98-140)	92	120000 (100000-130000)	6700
	pol2-R252H/POL2	*mlh1*Δ/*mlh1*Δ	**820 (710-1200)**	**48**	**150 (140-170)**	**130**	140000 (120000-160000)	7800

[a]Mutation rates are given as the median of at least 18 independent cultures, with 95% confidence intervals in parentheses. Fold increase is shown relative to the wild-type haploid or diploid. Bold text indicates $p < 0.05$ by Wilcoxon-Mann-Whitney compared to *mlh1*Δ haploids or *mlh1*Δ/*mlh1*Δ diploids. Data from this table were used to generate Figure 6.

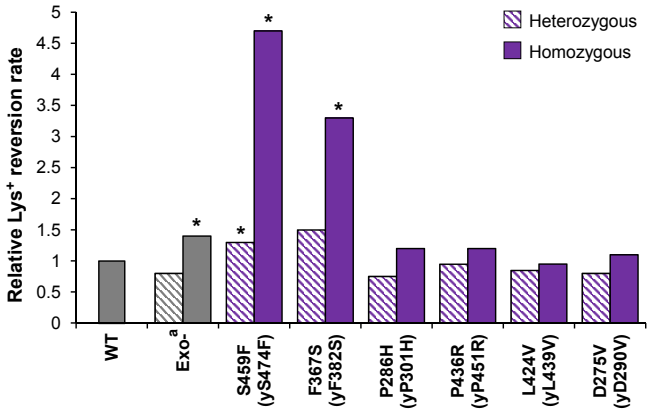


Supplementary Figure S1. Effect of cancer-associated Polε variants on the rate of *lys2-InsE$_{A14}$* reversion in haploid yeast strains. Relative mutation rates are shown compared to wild-type (WT). All experimental details and symbols are as in Figure 2. Asterisks indicate $p < 0.05$ by Wilcoxon-Mann-Whitney compared to WT. Data are from Supplementary Table S3. [a]Data from (17).

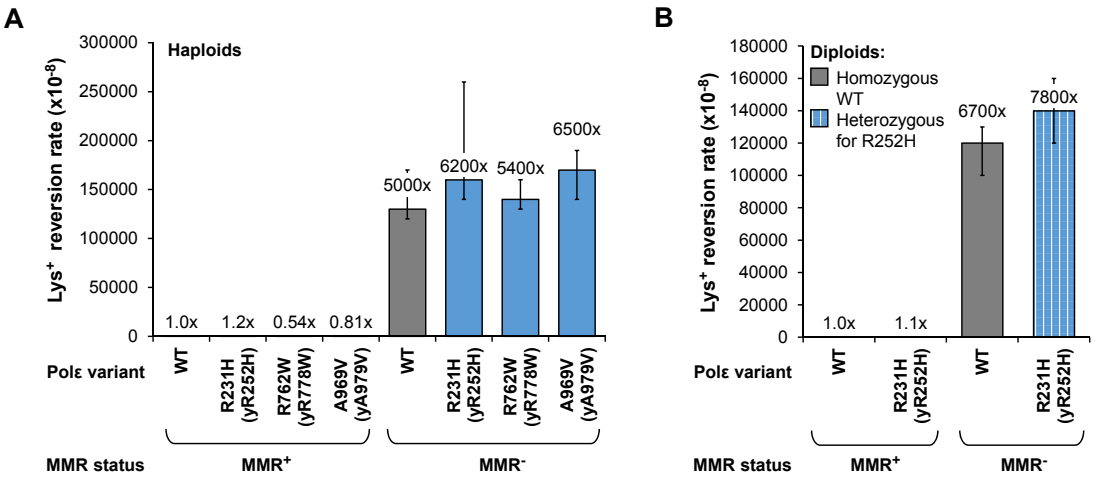




Supplementary Figure S2. Lack of correlation between *in vitro* effects of Polε mutations and their frequency in tumors. [A] Relationship between exonuclease activity defect and variant frequency. [B] Relationship between *in vitro* error rate and variant frequency. Exonuclease activity and *in vitro* error rates were measured in (14) using the N-terminal fragment of human Polε containing the exonuclease and DNA polymerase domains. The variant frequency in tumors was determined as described in Figure 4.



Supplementary Figure S3. Effect of cancer-associated Polε variants on the rate of *lys2-InsE$_{A14}$* reversion in diploid yeast strains. The strains contained one copy of the *lys2-InsE$_{A14}$* reporter allele and a nonrevertible mutation at the same position of the *LYS2* gene in the homologous chromosome. All other experimental details and symbols are as in Figure 5. Relative mutation rates are shown compared to wild-type (WT). Asterisks indicate $p < 0.05$ by Wilcoxon-Mann-Whitney compared to WT. Data are from Supplementary Table S6. [a]Data from (17).



Supplementary Figure S4. Polε variants found in MMR-deficient tumors do not affect the rate of *lys2-InsE$_{A14}$* reversion in MMR-proficient or MMR-deficient background. The reversion rate was measured in haploid [A] or diploid [B] yeast strains containing indicated chromosomal *pol2* mutations. The MMR defect was mimicked by deleting the yeast *MLH1* gene. Mutation rates are given as the median for at least 18 independent cultures, with error bars indicating 95% confidence intervals. Fold increase in mutation rate relative to the strain with wild-type *POL2* and *MLH1* genes is shown above each bar. Data are from Supplementary Table S7.