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Supplementary Information for

Defining the impact of mutation accumulation on replicative lifespan in yeast using cancer-associated mutator phenotypes

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Supplementary Text

S1. *The can1Δ/CAN1 genotype increases RLS.*

It has been previously shown that diploid cells in the BY4743 background live longer than isogenic haploid cells (BY4742 or BY4741) (1), and our data are consistent with this (BY4743 median RLS = 34.5, n = 660). We also observed that *CAN1* hemizygous strains (designated WT for our studies) were longer-lived than BY4743, the diploid background strain (Fig. S2 and S3) (WT median RLS = 39, n = 761; BY4743 median RLS = 34.5, n = 660, $p < 0.001$, Wilcoxon rank sum test), as has been previously reported for loss of *CAN1* in haploid yeast (2). Our data suggests that this longevity phenotype depends on gene dosage in diploids. Our strict use of *CAN1::natMX/can1Δ::HIS3* strains eliminates this potential source of variation in our aging measurements.

S2. *Variability in lifespans of strains with low mutation burdens.*

To estimate the mutation burden at the beginning of lifespan analysis, we sequenced three independent clones of mother cells isolated in an identical manner as those used in our lifespan cohorts. As controls, we sequenced the genomes of three independent isolates of the parental strains, AH2601 and AH2801. We found that AH2601 isolates shared nine clonal mutations that segregated in AH2601-derived strains. AH2801 isolates had no common mutations. Every AH2601 and AH2801 isolate also carried 5-13 unique variants, which evidently arose during the propagation of the strain. Unique mutations, such as these, were passed on to the haploid spores used for mating and contributed to the clonal mutation burdens in our diploid strains, along with any additional mutations that arose in the few haploid divisions prior to mating.

Clonal mutations affect all cells in all lifespan cohorts and will have the largest potential impact on survival demographics. Of course, mutations continually arise in our mutator lines at a rate determined by the mutator alleles. Subclonal variants arising during the initial propagation of the diploid strain were fixed by isolating single colonies from frozen stocks. These may influence lifespan of individual cohorts. Subclonal variants that arose after this single cell bottleneck will be largely private to individual cells within the cohort and likely generate greater variability within survival cohorts. However, the probability of deleterious mutations and synthetic interactions increases as a function of aggregate mutation burden. Thus, conceivably, there may be a level of mutation burden

where every cell carries private lifespan-limiting mutations, giving the appearance of a uniform effect across the entire population.

WT, *MSH6/msh6Δ*, *POL2/pol2-4*, and *POL2/pol2-4 MSH6/msh6Δ* strains derived from our mating experiments exhibited similar median lifespans, mutation rates, and mutation burdens as the WT parental strain (yML046) used to construct AH2601 and AH2801 (Table S1). Importantly, the WT strain derived from AH2601 (AH3251) contained all nine AH2601 mutations (six nonsynonymous changes), suggesting that none of these variants in the heterozygous state influence lifespan. Strains lacking Msh6 (*msh6Δ/msh6Δ*) or Polε proofreading (*pol2-4/pol2-4* and *pol2-4/pol2-4 MSH6/msh6Δ*) displayed a 10-fold increase in mutation rate relative to WT (Fig. S1*B*) and a fractional increase in mutation burden (Fig. S1*C*). Seven out of the nine isolates of these three genotypes displayed moderate declines in median lifespan even though deficiencies in Polε proofreading or Msh6 failed to reduce lifespan in haploids. The genotypes of the two strains with normal lifespans, yML262 (*msh6Δ/msh6*Δ) and yML268 (*pol2-4/pol2-4 MSH6/msh6Δ*) argues that reduction in RLS in the other strains derives from specific mutations rather than the *msh6Δ/msh6Δ* or *pol2-4/pol2-4* mutator genotypes, per se.

A review of the history of each strain and their genomic sequence supports the hypothesis that the lifespan reductions in many of these strains are due to passenger mutations inherited from the parental strain and not the mutator genotype. yML268 is derived from the same AH2801 culture from which we isolated long-lived WT (yML279), *MSH6/msh6Δ* (yML280, yML281), and *POL2/pol2-4 MSH6/msh6Δ* (yML267, yML271, and yML274) strains. A different AH2801 culture served as the source for short-lived *msh6Δ/msh6Δ* (yML337 and yML338), *pol2-4/pol2-4* (yML329 and yML332), and *pol2-4/pol2-4 MSH6/msh6Δ* strains (yML326). All of these related strains carried two nonsynonymous mutations (*yel020c-A58V*, and *vid30-P18Q*). Deletion of *VID30* has been previously reported to reduce chronological lifespan (3, 4). Although the reductions in RLS observed in yML338, yML329, and yML332 were similar, yML337 and yML326 were even shorter-lived. yML337 carried two additional heterozygous nonsynonymous mutations while yML326 had a different set of five heterozygous nonsynonymous mutations. These additional mutations could lower lifespan in an additive fashion or potentially interact epistatically with *yel020c-A58V* or *vid30-P18Q*. The remaining short-lived *pol2-4/pol2-4 MSH6/msh6Δ* strains (AH4703 and AH4713) came from a different AH2801 culture and shared two different heterozygous nonsynonymous mutations (*ade6-G445R* and *stt4-K1223N*) and one homozygous mutation (*bcp1-A4D*). Variation in

lifespan was also observed among the *POL3/pol3-01* strains (AH3203, AH3228, AH3240, and AH3241), which were all derived from the same AH2601 culture. One strain (AH3228) had a significantly shorter median lifespan than the others and carried three unique heterozygous nonsynonymous mutations (*hnm1-W372L*, *ypl068C-N98I*, *mms1-K861Q*). Taken together, these observations suggest that relatively small numbers of heterozygous mutations, acting alone or in combination, can reduce diploid yeast lifespan.

S3. *Short-lifespans of POL3/pol3-01 MSH6/msh6Δ strains*

Clonal mutation did not explain reduced RLS in the six *POL3/pol3-01 MSH6/msh6Δ* strains. These strains exhibited similar mutation rates as the *POL3/pol3-01* strains. Some of these strains had higher clonal mutation burdens, which likely indicates that a WT and a strongly mutagenic *pol3-01 msh6Δ* spore were mated to generate those strains. All six strains were isolated from the same AH2601 culture. Two nonsynonymous mutations segregated among these strains, but neither variant was present in all six isolates. Reduced lifespan in *POL3/pol3-01 MSH6/msh6Δ* cells may be due to a shared mutation missed by our analysis, different clonal mutations specific to each strain, or the *POL3/pol3-01 MSH6/msh6Δ* genotype itself. The observation that AH2601 is short-lived (Fig.4) suggests that the genotype exerts an unexplained dominant negative effect on lifespan.

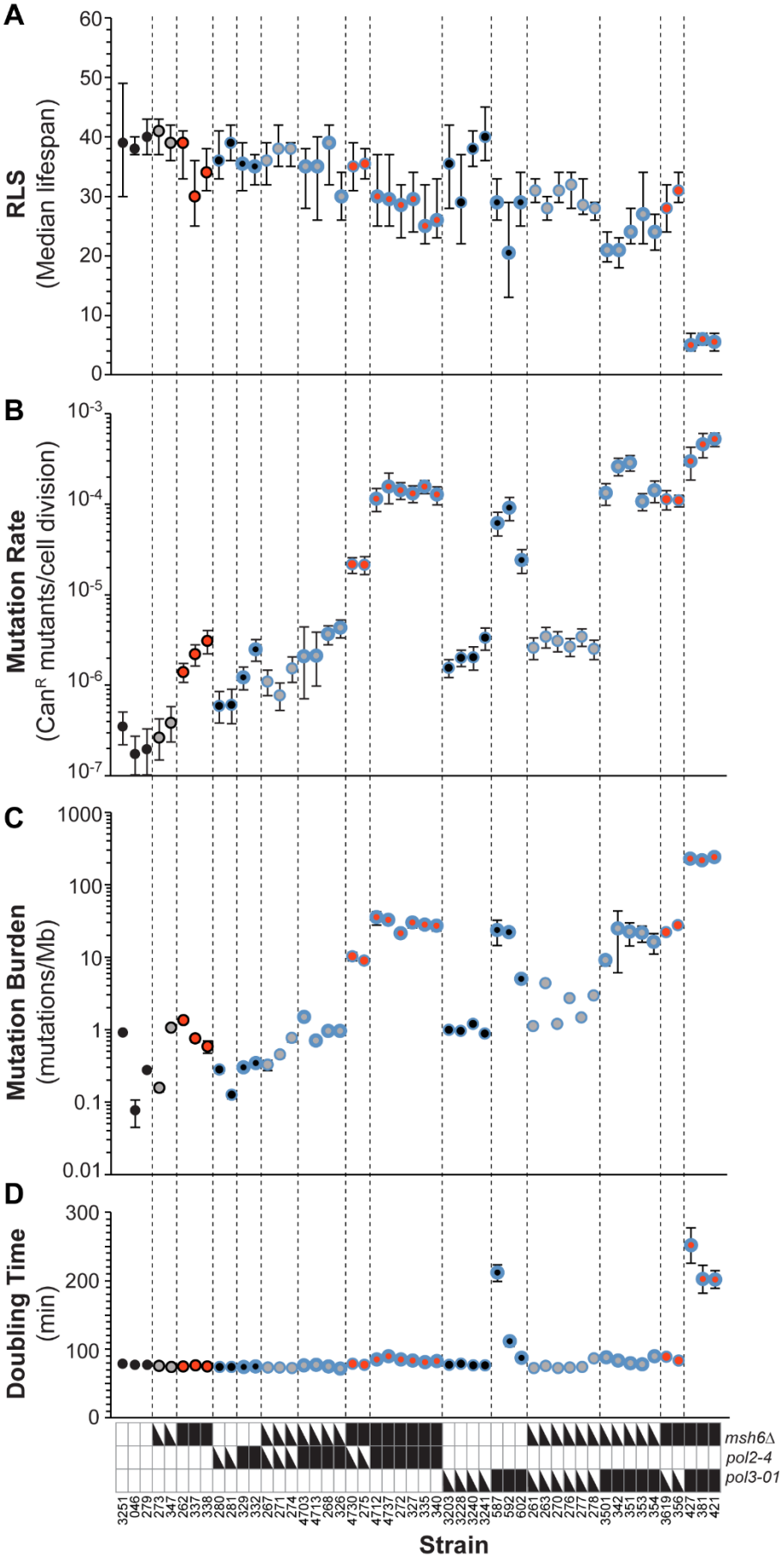
A
RLS
(Median lifespan)
60
50
40
30
20
10
0
B
Mutation Rate
(Can^R mutants/cell division)
10^-3
10^-4
10^-5
10^-6
10^-7
C
Mutation Burden
(mutations/Mb)
1000
100
10
1
0.1
0.01
D
Doubling Time
(min)
300
200
100
0
msh6Δ
pol2-4
pol3-01
3251 046 279 273 347 262 337 338 280 281 329 332 267 271 274 4703 4713 268 326 4730 275 4712 4737 272 327 335 340 3203 3228 3240 3241 587 592 602 261 263 270 276 277 278 3501 342 351 353 354 3619 356 427 381 421
Strain

Fig. S1. Lifespan, mutation rates, and mutation burden of polymerase proofreading and/or mismatch repair defective diploid mutator yeast. Genotypes and strain identification numbers are represented schematically in the grid at the bottom: empty squares, homozygous WT allele; filled right triangle, heterozygous, mutator allele, filled squares, homozygous mutation. The fill color of the circles indicate MMR status: black, WT; gray, *MSH6/msh6Δ*; red, *msh6Δ/msh6Δ*. The color and thickness of the line around the circles indicate proofreading status: thin black line, WT; thin blue line, *POL3/pol3-01* or *POL2/pol2-4*; thick blue line, *pol3-01/pol3-01* or *pol2-4/pol2-4*. All data for these plots can be found in Dataset S1. A) Median RLS of diploid mutator strains separated by genotype. Error bars, 95% confidence intervals (CI). B) Mutation rates of diploid mutators. All strains were hemizygous for *CAN1* (*CAN1::natMX/can1Δ::HIS3*), which facilitated canavanine-resistance (Can^R) mutation rate measurement. Error bars, 95% CI. Mutation rates compared to WT using the log-rank test; p-values were corrected for multiple testing ($p < 0.001$ for all strains except *MSH6/msh6Δ*). C) Mutation burdens (SNVs/Mb) of diploid mutator yeast measured using whole-genome sequencing. Sequenced populations were age-matched to cells analyzed in RLS. Three independent isolates sequenced for each strain. Error bars, standard error of the mean (SEM). D) Peak doubling time (minutes/cell division). Error bars, SEM.

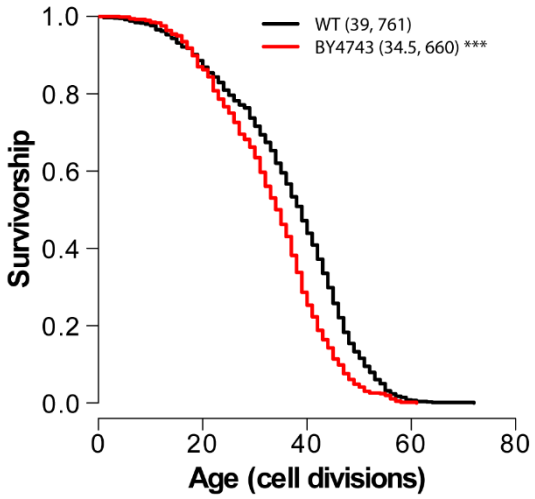

Fig. S2. RLS of AH0401 (WT) and BY4743 (median RLS and mother's dissected in parentheses). *** = $p < 0.001$, Wilcoxon rank sum test.

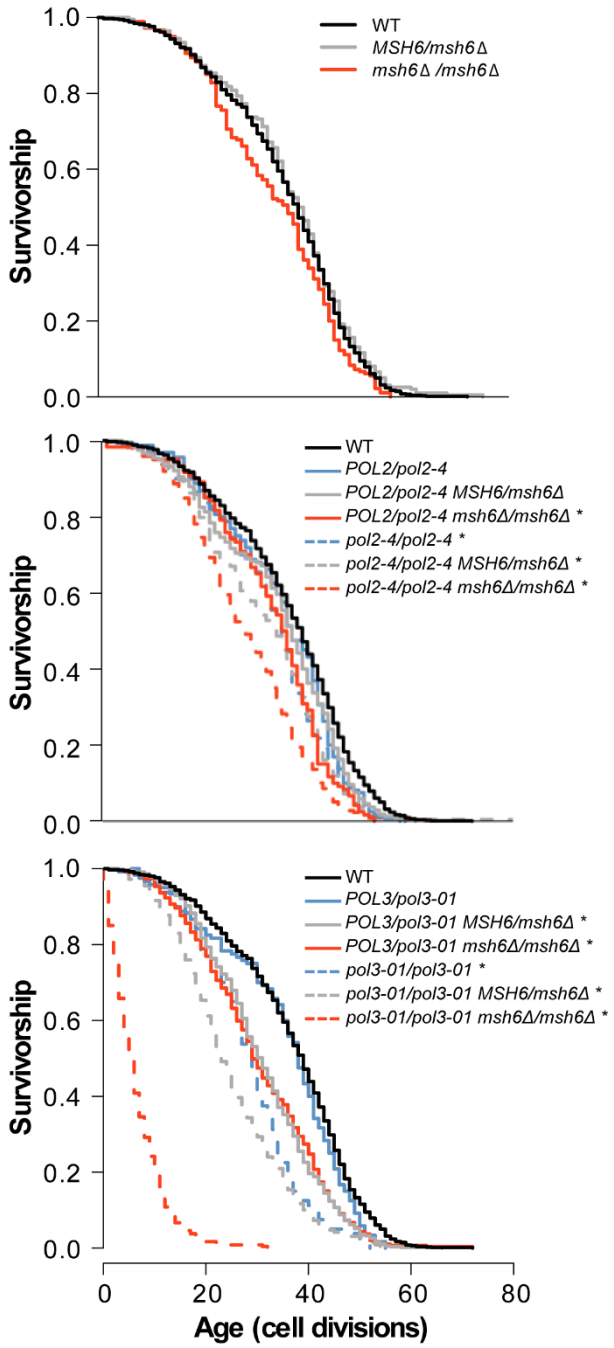

Fig. S3. RLS of diploid mismatch repair and polymerase proofreading deficient mutator strains. (*A*) *MSH6* deficient (*msh6Δ*) mutators. (*B*) Polε proofreading-deficient (*pol2-4*) strains with and without *MSH6*. (*C*) Polδ proofreading-deficient (*pol3-01*) strains with and without *MSH6*. * = $p < 0.05$, Wilcoxon rank sum test, Bonferroni-corrected.

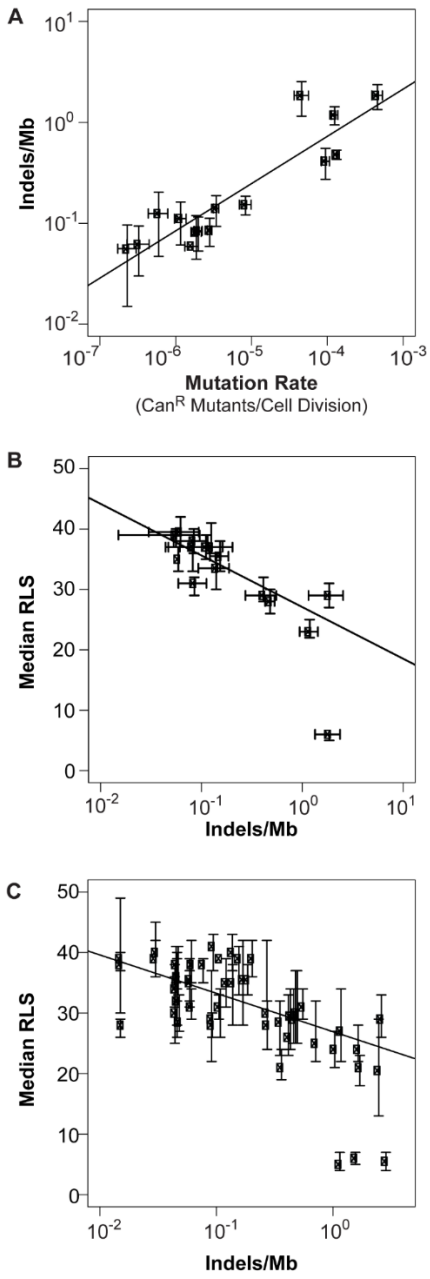

Fig. S4. Decreased lifespan negatively correlates with increased insertion/deletion mutations in diploid yeast. (*A*) Correlation between mutation rate and indels/Mb. Horizontal error bars = 95% CIs. Vertical error bars, SEM. Linear model adjusted R-squared = 0.80, $p < 0.001$. (*B*) Median RLS as a function of indels/Mb. Horizontal error bars, 95% CIs. Vertical error bars, SEM. Linear model adjusted R-squared = 0.70, $p < 0.001$. (*C*) Median RLS as a function of indels/Mb in diploid mutators. Instead of pooling on the basis of mutator genotype, individual mutator strains were plotted. Error bars, SEM. Linear model adjusted R-squared = 0.43, $p < 0.001$. For *B* and *C*, linear regression modeling was performed after excluding the outlying dattum (*pol3-01/pol3-01 msh6Δ/msh6Δ*).

Table S1. Yeast strains.

Strain	Parent Strain	Relevant genotype	Source
AH0401	BY4743	*CAN1::natMX/can1Δ::HIS3*	Herr et al., 2014
AH2601	AH0401	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 POL3/URA3::pol3-01*	This study
AH2801	AH0401	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 POL2/pol2-4::URA3*	Kennedy et al., 2015
AH3203	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/MSH6 POL3/URA3::pol3-01*	This study
AH3228	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/MSH6 POL3/URA3::pol3-01*	This study
AH3240	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/MSH6 POL3/URA3::pol3-01*	This study
AH3241	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/MSH6 POL3/URA3::pol3-01*	This study
AH3251	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/MSH6 POL3/POL3*	This study
AH3501	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 URA3::pol3-01/URA3::pol3-01*	This study
AH3619	AH2601	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 POL3/URA3::pol3-01*	This study
AH4703	AH2801	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 pol2-4::URA3/pol2-4::URA3*	This study
AH4712	AH2801	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 pol2-4::URA3/pol2-4::URA3*	This study
AH4713	AH2801	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 pol2-4::URA3/pol2-4::URA3*	This study
AH4730	AH2801	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 POL2/pol2-4::URA3*	This study
AH4737	AH2801	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 pol2-4::URA3/pol2-4::URA3*	This study
yML033*	AH0401	MA1, CAN1::natMX/can1Δ::HIS3	This study
yML034*	AH0401	MA2, CAN1::natMX/can1Δ::HIS3	This study
yML036*	AH0401	MA7, *CAN1::natMX/can1Δ::HIS3*	This study
yML037*	AH0401	MA9, *CAN1::natMX/can1Δ::HIS3*	This study
yML038*	AH0401	MA4, *CAN1::natMX/can1Δ::HIS3*	This study
yML039*	AH0401	MA6, *CAN1::natMX/can1Δ::HIS3*	This study
yML040*	AH0401	MA10, *CAN1::natMX/can1Δ::HIS3*	This study
yML041*	AH0401	MA5, *CAN1::natMX/can1Δ::HIS3*	This study
yML042*	AH0401	MA11, *CAN1::natMX/can1Δ::HIS3*	This study
yML043*	AH0401	MA8, *CAN1::natMX/can1Δ::HIS3*	This study
yML044*	AH0401	MA3, *CAN1::natMX/can1Δ::HIS3*	This study
yML261	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 POL3/URA3::pol3-01*	This study
yML262	AH2601	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 POL3/POL3*	This study
yML263	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 POL3/URA3::pol3-01*	This study
yML267	AH2801	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 POL2/pol2-4::URA3*	This study
yML268	AH2801	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 pol2-4::URA3/pol2-4::URA3*	This study
yML270	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 POL3/URA3::pol3-01*	This study
yML271	AH2801	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 POL2/pol2-4::URA3*	This study
yML272	AH2801	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 pol2-4::URA3/pol2-4::URA3*	This study
yML273	AH2801	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 POL2/POL2*	This study
yML274	AH2801	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 POL2/pol2-4::URA3*	This study

yML275	AH2801	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 POL2/pol2-4::URA3*	This study
yML276	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 POL3/URA3::pol3-01*	This study
yML277	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 POL3/URA3::pol3-01*	This study
yML278	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 POL3/URA3::pol3-01*	This study
yML279	AH2801	*CAN1::natMX/can1Δ::HIS3 MSH6/MSH6 POL2/POL2*	This study
yML280	AH2801	*CAN1::natMX/can1Δ::HIS3 MSH6/MSH6 POL2/pol2-4::URA3*	This study
yML281	AH2801	*CAN1::natMX/can1Δ::HIS3 MSH6/MSH6 POL2/pol2-4::URA3*	This study
yML326	AH2801	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 pol2-4::URA3/pol2-4::URA3*	This study
yML327	AH2801	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 pol2-4::URA3/pol2-4::URA3*	This study
yML329	AH2801	*CAN1::natMX/can1Δ::HIS3 MSH6/MSH6 pol2-4::URA3/pol2-4::URA3*	This study
yML332	AH2801	*CAN1::natMX/can1Δ::HIS3 MSH6/MSH6 pol2-4::URA3/pol2-4::URA3*	This study
yML335	AH2801	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 pol2-4::URA3/pol2-4::URA3*	This study
yML337	AH2801	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 POL2/POL2*	This study
yML338	AH2801	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 POL2/POL2*	This study
yML340	AH2801	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 pol2-4::URA3/pol2-4::URA3*	This study
yML342	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 URA3::pol3-01/URA3::pol3-01*	This study
yML347	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 POL3/POL3*	This study
yML351	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 URA3::pol3-01/URA3::pol3-01*	This study
yML353	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 URA3::pol3-01/URA3::pol3-01*	This study
yML354	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/msh6Δ::LEU2 URA3::pol3-01/URA3::pol3-01*	This study
yML356	AH2601	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 POL3/URA3::pol3-01*	This study
yML381	AH2601	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 URA3::pol3-01/URA3::pol3-01*	This study
yML421	AH2601	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 URA3::pol3-01/URA3::pol3-01*	This study
yML427	AH2601	*CAN1::natMX/can1Δ::HIS3 msh6Δ::LEU2/msh6Δ::LEU2 URA3::pol3-01/URA3::pol3-01*	This study
yML587	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/MSH6 URA3::pol3-01/URA3::pol3-01*	This study
yML592	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/MSH6 URA3::pol3-01/URA3::pol3-01*	This study
yML602	AH2601	*CAN1::natMX/can1Δ::HIS3 MSH6/MSH6 URA3::pol3-01/URA3::pol3-01*	This study

* = mutation accumulation line

Table S2. Primers.

Primer Name	Sequence
AH2601 Strain Construction	
MSH6GU	TTTAATTGGAGCAACTAGTTAATTTTGACAAAGCCAATTTGAACTCCAAAAGATTGTACTGAGAGTGCAC
MSH6GD	ACTTTAAAAAAAATAAGTAAAAATCTTACATACATCGTAAATGAAAATACCTGTGCGGTATTTCACACCG
POL3U	ATATTGAGCACTTGCTATTAAGCATTAATCTTTATACATATACGCACAGCAAGATTGTACTGAGAGTGCAC
pldr6	AAACCACAACGGCATCGTGC
Genotyping Assays	
pol2-4U	CCTTCTTTGCTAACAGATAAGATTT
pol2-S5	ATAACACTCTCAGGGGACAAGTATAT
msh6-upstream	CCTTCTTTGCTAACAGATAAGATTT
msh6-downstream	CAGCTATTAATGTTCAACTTATTCC
Illumina Adapter Primers	
MWS51	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
MWS55	5'/Phos/GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
Illumina I5 index primers for QPCR	
P5	AATGATACGGCGACCACCGAGATCTACAC
I5index1	AATGATACGGCGACCACCGAGATCTACACTATAGCCTACACTCTTTCCCTACACGACG
I5index2	AATGATACGGCGACCACCGAGATCTACACATAGAGGCACACTCTTTCCCTACACGACG
I5index3	AATGATACGGCGACCACCGAGATCTACACCCTATCCTACACTCTTTCCCTACACGACG
I5index4	AATGATACGGCGACCACCGAGATCTACACGGCTCTGAACACTCTTTCCCTACACGACG
I5index5	AATGATACGGCGACCACCGAGATCTACACAGGCGAAGACACTCTTTCCCTACACGACG
I5index6	AATGATACGGCGACCACCGAGATCTACACTAATCTTAACACTCTTTCCCTACACGACG
Illumina I7 Index Primers for QPCR	
I7index1	CAAGCAGAAGACGGCATACGAGATACAAGCTGTGACTGGAGTTCAGACGTGTGC
I7index2	CAAGCAGAAGACGGCATACGAGATAAACATCGTGACTGGAGTTCAGACGTGTGC
I7index3	CAAGCAGAAGACGGCATACGAGATACATTGGGTGACTGGAGTTCAGACGTGTGC
I7index4	CAAGCAGAAGACGGCATACGAGATACCACTGGTGACTGGAGTTCAGACGTGTGC
I7index5	CAAGCAGAAGACGGCATACGAGATAACGTGAGTGACTGGAGTTCAGACGTGTGC
I7index6	CAAGCAGAAGACGGCATACGAGATCGCTGATGTGACTGGAGTTCAGACGTGTGC
I7index7	CAAGCAGAAGACGGCATACGAGATCAGATCTGTGACTGGAGTTCAGACGTGTGC

I7index8	CAAGCAGAAGACGGCATACGAGATATGCCTAGTGACTGGAGTTCAGACGTGTGC
I7index9	CAAGCAGAAGACGGCATACGAGATCTGTAGCGTGACTGGAGTTCAGACGTGTGC
I7index10	CAAGCAGAAGACGGCATACGAGATAGTACAAGTGACTGGAGTTCAGACGTGTGC
I7index11	CAAGCAGAAGACGGCATACGAGATCATCAAGGTGACTGGAGTTCAGACGTGTGC
I7index12	CAAGCAGAAGACGGCATACGAGATAGTGGTCGTGACTGGAGTTCAGACGTGTGC
I7index13	CAAGCAGAAGACGGCATACGAGATAACAACCGTGACTGGAGTTCAGACGTGTGC
I7index14	CAAGCAGAAGACGGCATACGAGATAACCGAGGTGACTGGAGTTCAGACGTGTGC
I7index15	CAAGCAGAAGACGGCATACGAGATAACGCTTGTGACTGGAGTTCAGACGTGTGC
I7index16	CAAGCAGAAGACGGCATACGAGATAAGACGGGTGACTGGAGTTCAGACGTGTGC
I7index17	CAAGCAGAAGACGGCATACGAGATAAGGTACGTGACTGGAGTTCAGACGTGTGC
I7index18	CAAGCAGAAGACGGCATACGAGATACACAGAGTGACTGGAGTTCAGACGTGTGC
I7index19	CAAGCAGAAGACGGCATACGAGATACAGCAGGTGACTGGAGTTCAGACGTGTGC
I7index20	CAAGCAGAAGACGGCATACGAGATACCTCCAGTGACTGGAGTTCAGACGTGTGC
I7index21	CAAGCAGAAGACGGCATACGAGATACGCTCGGTGACTGGAGTTCAGACGTGTGC
I7index22	CAAGCAGAAGACGGCATACGAGATACGTATCGTGACTGGAGTTCAGACGTGTGC
I7index23	CAAGCAGAAGACGGCATACGAGATACTATGCGTGACTGGAGTTCAGACGTGTGC
I7index24	CAAGCAGAAGACGGCATACGAGATAGAGTCAGTGACTGGAGTTCAGACGTGTGC
I7index25	CAAGCAGAAGACGGCATACGAGATAGATCGCGTGACTGGAGTTCAGACGTGTGC
I7index26	CAAGCAGAAGACGGCATACGAGATAGCAGGAGTGACTGGAGTTCAGACGTGTGC
I7index27	CAAGCAGAAGACGGCATACGAGATAGTCACTGTGACTGGAGTTCAGACGTGTGC
I7index28	CAAGCAGAAGACGGCATACGAGATATCCTGTGTGACTGGAGTTCAGACGTGTGC
I7index29	CAAGCAGAAGACGGCATACGAGATATTGAGGGTGACTGGAGTTCAGACGTGTGC
I7index30	CAAGCAGAAGACGGCATACGAGATCAACCACGTGACTGGAGTTCAGACGTGTGC
I7index31	CAAGCAGAAGACGGCATACGAGATCAAGACTGTGACTGGAGTTCAGACGTGTGC
I7index32	CAAGCAGAAGACGGCATACGAGATCAATGGAGTGACTGGAGTTCAGACGTGTGC
I7index33	CAAGCAGAAGACGGCATACGAGATCACTTCGGTGACTGGAGTTCAGACGTGTGC
I7index34	CAAGCAGAAGACGGCATACGAGATCAGCGTTGTGACTGGAGTTCAGACGTGTGC
I7index35	CAAGCAGAAGACGGCATACGAGATCATACCAGTGACTGGAGTTCAGACGTGTGC
I7index36	CAAGCAGAAGACGGCATACGAGATCCAGTTCGTGACTGGAGTTCAGACGTGTGC
I7index37	CAAGCAGAAGACGGCATACGAGATCCGAAGTGTGACTGGAGTTCAGACGTGTGC

I7index38	CAAGCAGAAGACGGCATACGAGATCCGTGAGGTGACTGGAGTTCAGACGTGTGC
I7index39	CAAGCAGAAGACGGCATACGAGATCCTCCTGGTGACTGGAGTTCAGACGTGTGC
I7index40	CAAGCAGAAGACGGCATACGAGATCGAACTTGTGACTGGAGTTCAGACGTGTGC
I7index41	CAAGCAGAAGACGGCATACGAGATCGACTGGGTGACTGGAGTTCAGACGTGTGC
I7index42	CAAGCAGAAGACGGCATACGAGATCGCATACGTGACTGGAGTTCAGACGTGTGC
I7index43	CAAGCAGAAGACGGCATACGAGATCTCAATGGTGACTGGAGTTCAGACGTGTGC
I7index44	CAAGCAGAAGACGGCATACGAGATCTGAGCCGTGACTGGAGTTCAGACGTGTGC
I7index45	CAAGCAGAAGACGGCATACGAGATCTGGCATGTGACTGGAGTTCAGACGTGTGC
I7index46	CAAGCAGAAGACGGCATACGAGATGAATCTGGTGACTGGAGTTCAGACGTGTGC
I7index47	CAAGCAGAAGACGGCATACGAGATGACTAGTGTGACTGGAGTTCAGACGTGTGC
I7index48	CAAGCAGAAGACGGCATACGAGATGAGCTGAGTGACTGGAGTTCAGACGTGTGC
I7index49	CAAGCAGAAGACGGCATACGAGATGATAGACGTGACTGGAGTTCAGACGTGTGC
I7index50	CAAGCAGAAGACGGCATACGAGATGCCACATGTGACTGGAGTTCAGACGTGTGC
I7index51	CAAGCAGAAGACGGCATACGAGATGCGAGTAGTGACTGGAGTTCAGACGTGTGC
I7index52	CAAGCAGAAGACGGCATACGAGATGCTAACGGTGACTGGAGTTCAGACGTGTGC
I7index53	CAAGCAGAAGACGGCATACGAGATGCTCGGTGTGACTGGAGTTCAGACGTGTGC
I7index54	CAAGCAGAAGACGGCATACGAGATGGAGAACGTGACTGGAGTTCAGACGTGTGC
I7index55	CAAGCAGAAGACGGCATACGAGATGGTGCGAGTGACTGGAGTTCAGACGTGTGC
I7index56	CAAGCAGAAGACGGCATACGAGATGTACGCAGTGACTGGAGTTCAGACGTGTGC
I7index57	CAAGCAGAAGACGGCATACGAGATGTCGTAGGTGACTGGAGTTCAGACGTGTGC
I7index58	CAAGCAGAAGACGGCATACGAGATGTCTGTCGTGACTGGAGTTCAGACGTGTGC
I7index59	CAAGCAGAAGACGGCATACGAGATGTGTTCTGTGACTGGAGTTCAGACGTGTGC
I7index60	CAAGCAGAAGACGGCATACGAGATTAGGATGGTGACTGGAGTTCAGACGTGTGC
I7index61	CAAGCAGAAGACGGCATACGAGATTATCAGCGTGACTGGAGTTCAGACGTGTGC
I7index62	CAAGCAGAAGACGGCATACGAGATTCCGTCTGTGACTGGAGTTCAGACGTGTGC
I7index63	CAAGCAGAAGACGGCATACGAGATTCTTCACGTGACTGGAGTTCAGACGTGTGC
I7index64	CAAGCAGAAGACGGCATACGAGATTGAAGAGGTGACTGGAGTTCAGACGTGTGC
I7index65	CAAGCAGAAGACGGCATACGAGATTGGAACAGTGACTGGAGTTCAGACGTGTGC
I7index66	CAAGCAGAAGACGGCATACGAGATTGGCTTCGTGACTGGAGTTCAGACGTGTGC
I7index67	CAAGCAGAAGACGGCATACGAGATTGGTGGTGTGACTGGAGTTCAGACGTGTGC

I7index68	CAAGCAGAAGACGGCATACGAGATTTCACGCGTGACTGGAGTTCAGACGTGTGC
I7index69	CAAGCAGAAGACGGCATACGAGATAACTCACGTGACTGGAGTTCAGACGTGTGC
I7index70	CAAGCAGAAGACGGCATACGAGATAAGAGATGTGACTGGAGTTCAGACGTGTGC

Dataset S1

Median replicative lifespans, mutation rates, mutation burdens, and doubling times of diploid strains.

Dataset S2

Whole genome sequencing of diploid strains.

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