Genome Destabilizing Mutator Alleles Drive Specific Mutational Trajectories in Saccharomyces cerevisiae

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ABSTRACT In addition to environmental factors and intrinsic variations in base substitution rates, specific genome-destabilizing mutations can shape the mutational trajectory of genomes. How specific alleles influence the nature and position of accumulated mutations in a genomic context is largely unknown. Understanding the impact of genome-destabilizing alleles is particularly relevant to cancer genomes where biased mutational signatures are identifiable. We first created a more complete picture of cellular pathways that impact mutation rate using a primary screen to identify essential Saccharomyces cerevisiae gene mutations that cause mutator phenotypes. Drawing primarily on new alleles identified in this resource, we measure the impact of diverse mutator alleles on mutation patterns directly by whole-genome sequencing of 68 mutation-accumulation strains derived from wild-type and 11 parental mutator genotypes. The accumulated mutations differ across mutator strains, displaying base-substitution biases, allele-specific mutation hotspots, and break-associated mutation clustering. For example, in mutants of POL32 and the Cdc13–Stn1–Ten1 complex, we find a distinct subtelomeric bias for mutations that we show is independent of the target sequence. Together our data suggest that specific genome-instability mutations are sufficient to drive discrete mutational signatures, some of which share properties with mutation patterns seen in tumors. Thus, in a population of cells, genome-instability mutations could influence clonal evolution by establishing discrete mutational trajectories for genomes.

E Volutionary analysis and laboratory studies show that mutation rates vary across chromosomes in unperturbed cells in association with factors such as base composition, repair efficiency, transcription, or replication timing (reviewed in Nishant et al. 2009). More recently, observations of mutation clustering in long stretches of single-stranded DNA (ssDNA) have been posited as conserved mutational mechanisms operating in tumors and methylmethane sulfonate (MMS)-treated yeast cells (Roberts et al. 2012). However, the influence of specific mutations on mutational patterns in the genome is usually not well characterized. Nonetheless, the spectrum of mutations will strongly influence the rates at which important phenotypes evolve. Recent work in Escherichia coli nicely demonstrates this concept by showing that different mutator alleles more potently adapt to different antibiotics based on the penetrance of the resistance mutations conferred by each mutator (Couce et al. 2013). A handful of studies in models such as Caenorhabditis elegans and Saccharomyces cerevisiae have linked mutator alleles to mutation patterns on a genome-wide scale (e.g., dog-1 deficiency mutating G-quadruplexes, mismatch-repair-deficiency mutating homopolymers, DNA polymerase δ variants preferentially mutating lagging strands) (Cheung et al. 2002; Larrea et al. 2010; Zanders et al. 2010). However, the influence of most mutator alleles on mutation patterns is not understood at this level. Thus, not only the mutation rate increase conferred by a mutator allele, but also its unique mutational signature (i.e., preferred type of mutation and bias to a genomic context, if any) generates its effect on genome stability and evolution.

Tumor genomes accumulate alterations during oncogenesis. The accumulation of mutations, aneuploidies, or epigenetic changes frequently exceeds the normal rate of such events due to a predisposing mutation or environmental
state that increases genetic instability. This in turn acts as an enabling characteristic of oncogenesis in most tumors (Hanahan and Weinberg 2011). Increased genetic instability, either in the form of increased mutation rate or chromosome instability (CIN), may promote selection of oncogenic cells from pre-oncogenic populations by increasing the likelihood that the requisite constellation of oncogenic mutations will occur in fewer cell divisions (Stratton et al. 2009; Loeb 2011). Thus, early genome-destabilizing mutations have the potential to shape the evolutionary trajectory of precancerous cells and intratumoral heterogeneity in large tumor cell populations.

Analysis of tumor genomes has uncovered discrete mutational signatures operating within and between tumor types (Nik-Zainal et al. 2012; Alexandrov et al. 2013b). These powerful computational approaches have demonstrated that multiple mutational processes operate simultaneously in tumors (Nik-Zainal et al. 2012; Alexandrov et al. 2013a). Mutational signatures can result from environmental exposure to genotoxins such as ultraviolet light or components of cigarette smoke (Pleasance et al. 2010a,b). Other signatures show the signs of endogenous processes such as the inappropriate action of APOBEC family cytosine deaminases (Nik-Zainal et al. 2012; Alexandrov et al. 2013b; Burns et al. 2013). Indeed, APOBECs from various sources are sufficient to induce breast-cancer-like patterns of mutations (i.e., clustered hypermutation of C:G basepairs or “kataegis”) when expressed ectopically in yeast (Lada et al. 2012; Alexandrov et al. 2013b; Taylor et al. 2013). Importantly, mutant alleles in genomestability factors such as the DNA repair proteins BRCA1 and BRCA2 also correlate with a characteristic pattern of mutations (Nik-Zainal et al. 2012).

Screens in yeast have identified dozens of genome-destabilizing “mutator” alleles that function in a handful of cellular pathways (Huang et al. 2003; Smith et al. 2004). However, these efforts have focused on nonessential gene deletions available from the yeast knockout collection and thus are missing the fraction of mutants encoded by essential genes. In this study we probe mutant alleles of >500 essential yeast genes for those that increase the forward mutation rate, highlighting the role of the DNA replication machinery in suppressing mutation accumulation. Analyzing whole genome sequences for 68 mutation accumulation lines derived from 12 parental genotypes, reveals examples of mutator-allele-associated base-substitution biases, clustered mutations, and locus-specific patterns of mutations. These observations show that different genome destabilizing lesions can result in discrete mutational trajectories for a genome and thus, for a population of cells, could influence clonal evolution.

Materials and Methods

Yeast growth, microscopy, and fluctuation analyses

Strains used are listed in Supporting Information, Table S7. Yeast were grown on rich media except for CAN1, URA3 fluctuation, or LEU2 recombination analyses, which were conducted as described (Lang and Murray 2008; Stirling et al. 2012). Rates per generation were calculated from at least 12 (recombination rate) or 18 (mutation rate) independent cultures using the FALCOR program (Hall et al. 2009). For microscopy, logarithmic cultures in synthetic medium were shifted to 37°C for 2 hr mounted on concanavalin-coated slides, imaged using Metamorph (Molecular Devices), and scored using ImageJ (rsbweb.nih.gov/ij/) as described (Stirling et al. 2012).

Mutation accumulation

Overnight cultures of parent clones were diluted into parallel cultures in 96 DeepWell plates. Cultures saturated over 3 days were diluted 10,000-fold in fresh medium and the process was repeated until ~195 generations elapsed. Temperature sensitivity (ts) was confirmed by spot assays at 37°C for the ts-alleles to remove wells with revertants. For evolved wild type (WT), rad52Δ, tsa1Δ, and ts-wells, single colonies were isolated and genomic DNA was prepared using two rounds of phenol-chloroform extraction and ethanol precipitation with an intervening RNaseA step (75 μg/ml RNaseA at 37°C, 30 min).

Sequencing, data analysis, and mutation identification

Whole-genome sequencing was done using the Illumina HiSeq2000 platform. Sequence files are deposited at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) (no. PRJNA219315). After whole-genome sequencing on 12 parental strains and 4–6 evolved progeny strains, postquality control reads were aligned to the S. cerevisiae reference genome (University of California Santa Cruz version sacCer2) using Burrows-Wheeler Aligner 0.5.7 (Li and Durbin 2009), using default parameters. For each pair of parent–progeny, joint variant calling was carried out using SAMtools 0.1.13 utilities (Li et al. 2009), with the parameters −C50 to decrease false calls from reads with excessive mismatches to the reference. A score based on log ratio of genotype likelihoods was used to identify variants in the progeny, with a threshold of 20 (Li et al. 2009). Positions of variants determined in the parental strains were ignored in our analysis regardless of their quality score to reduce false positive calls. The remaining variants were annotated with SnpEff 3.0 (Cingolani et al. 2012), using the Ensembl sacCer2.61 database (Flicek et al. 2013). Copy-number variants were detected using a modified version of CNAnseq developed at the Michael Smith Genome Sciences Centre, as described (Shah et al. 2006; Jones et al. 2010). As an additional quality control, we manually checked variants with Phred-scaled variant quality scores <20 using the Integrated Genomics Viewer (Broad Institute) and discarded those with poor read support.

Base substitution and flanking nucleotide bias

Base substitution biases were calculated using the Fisher exact test to compare with a wild-type mutation spectrum.
derived from the literature (Lynch et al. 2008; Lang et al. 2013). As in the literature, each mutation, regardless of strand, is expressed as a change originating in a C or T (Nik-Zainal et al. 2012). A Holm–Bonferroni approach was applied to Fisher’s test $P$-values to correct for multiple hypothesis testing; corrected $P$-values are reported.

### Mutation clustering analysis

Clustering was assessed essentially as described (Roberts et al. 2012). Intermutation distances for variants in the genome of a single isolate were calculated and those appearing within a 100-kb window were tested statistically. For the purposes of this analysis, mutations within 10 bp were considered “complex mutations” and treated as a single event, thus not constituting clusters unto themselves. Using the intermutation distance (i.e., cluster size) in base pairs, the number of mutations observed in a candidate cluster and the probability of finding a mutation at a given location in the genome, we calculated the cumulative probability on a negative binomial distribution of observing the intermutation distance at random. The probability of identifying a mutation at a given location was determined by dividing the total number of mutations $(n)$ for a particular genotype by the number of base pairs sequenced for that strain (no. of genomes sequenced $\times 12,162,995$ bp). Calculations were done using Matlab V7.12.0.635. To account for multiple hypothesis testing, we divided our original significance threshold ($P < 0.01$) by the number of putative clusters analyzed (103), establishing a corrected significance threshold of $P < 9.7 \times 10^{-5}$.

### Results and Discussion

#### Systematic identification of mutator alleles in essential genes

Our previous efforts to catalog yeast CIN genes revealed a bias toward essential genes; thus we reasoned that there were likely to be unrecognized essential genes operating to suppress mutations. We surveyed 813 alleles in 525 essential genes ($\sim$50% of total) (Ben-Aroya et al. 2008; Breslow et al. 2008; Li et al. 2011) for increased $CAN1$ mutation frequencies, identifying 47 mutator alleles in 38 genes (Figure 1A). Only those alleles that retested as mutators across five independent patches are included (Table S1). To assess the sensitivity of our assay, we quantified mutation rates for alleles of 33 mutator genes (Figure 1B). Fluctuation analysis revealed that all but two alleles had a twofold or higher increase in mutation rate at 30°, suggesting that our primary screen was as sensitive to changes in mutation rate as previous screens and that our retesting effectively removed false positives (Huang et al. 2003).

Combining our dataset with the *Saccharomyces* Genome Database phenotype term “mutation frequency: increased,” we compiled the list of yeast genes with reported mutator phenotypes (Table S1). These 127 genes represent a community survey of $>5000$ yeast genes ($\sim$85% of total) for mutator phenotypes, thus most cellular pathways that suppress mutations are probably represented. Functional enrichment analysis of the mutator alleles highlights the dominant role of the DNA repair and replication machinery in suppressing mutations (Figure 1C). Gene Ontology analysis showed that essentially all DNA repair pathways are significantly enriched (i.e., $P < 10^{-8}$ for base-excision repair, nucleotide excision repair, mismatch repair, break-induced replication, homologous recombination, and nonhomologous end joining). Nearly 90% of the mutators likely function at least partially in the nucleus, consistent with more direct mechanisms in preventing a mutator phenotype compared with CIN, for which $\sim$40% of CIN genes function outside the nucleus (Stirling et al. 2011). Mutants affecting the nucleotide pool, oxidative stress response, or mitochondrion function make up the only nonnuclear functional groups, consistent with the established potential for these pathways to influence nuclear genome integrity indirectly through their effects on oxidative base damage and Fe-S cluster formation (Huang et al. 2003; Veatch et al. 2009). A total of 72 of 127 mutator alleles compiled here also have a reported CIN phenotype, highlighting the considerable overlap of the two phenotypes. Nonetheless, this list of essential mutator alleles enhances the resource of genome-destabilizing mutations available in yeast.

#### Determining the mutation spectra driven by different mutator alleles

The CAN1 marker used as a primary screen for mutator alleles has been sequenced extensively to describe the mutational spectrum of mutator alleles (Huang et al. 2003). However, CAN1 only tests mutations in $\sim$1.8 kb of the $\sim$12-Mb yeast genome and isolating CANV strains requires loss-of-function mutations, biasing the mutation spectrum toward frame-shifts, stop codons, and a subset of amino acid substitutions. Therefore, it is plausible that different mutator alleles will selectively drive mutation in the genome based on a set of parameters that may or may not be represented in CAN1. A well-characterized example of this phenomenon is the association of mismatch repair deficiency with increased mutation rates in homopolymeric or microsatellite sequences (Zanders et al. 2010). Thus, while CAN1 is a convenient marker for identifying mutator alleles, analysis of diverse sequence contexts is more likely to reveal any allele-specific locus biases that exist.

To explore the mutational spectrum for diverse mutator alleles in a whole-genome context we devised a mutation accumulation experiment. Eleven mutator alleles were chosen for this analysis: two strong and well-characterized mutators from the literature, namely deletions of the homologous recombination protein *RAD52* and the peroxiredoxin *TSA1* (Huang and Kolodner 2005; Mortensen et al. 2009), and nine mutator alleles identified in our primary screen of essential genes (Figure 1). The alleles were chosen partly to represent diverse cellular pathways [e.g., homologous...
recombination (rad52Δ), oxidative stress tolerance (tsa1Δ), splicing (snu114-60), transcription (taf12-W486*), mitochondrial function (mas1-1), and telomere capping (stn1-13) and also acknowledge the inherent bias toward DNA replication by querying different perturbations in replication machinery (i.e., orc2-1, rfc2-1, mcm7-ts, pol1-ts, and pol2-12). For each strain, a single parental clone was split into parallel cultures, grown for ~195 generations by repeated dilution, and single colonies were derived from the endpoint cultures. Genomic DNA from the parent and four to six independently evolved progeny was whole-genome sequenced for a total of 12 starting strain backgrounds, producing 80 haploid whole-yeast-genome sequences at an average of 57-fold sequence coverage (schematized in Figure 2A; Table S2). Subtracting the parental genotype from the evolved strains identifies accumulated variants. This experiment captured single-nucleotide variants (SNVs), copy-number variants (CNVs, i.e., deletions or amplifications of large chromosomal regions), small insertion/deletion mutations (indels), and a few other types of chromosomal rearrangements (Figure 2B and Table S3). Based on published mutation rates (Lang and Murray 2008; Lynch et al. 2008), we predicted zero to two mutations per WT genome and observed a slightly higher but comparable rate (median = 2.5 mutations/genome; range, two to four mutations). In contrast, the mutator alleles produced ~2- to 10-fold more mutations on average (Figure 2B). SNVs were the most frequently identified mutation. One limitation of short read sequencing is difficulty in detecting indels between ~15 and 50 bp. However, the indels we did detect were
were usually segmental changes internal to the chromosome. These CNVs were not observed in the WT isolates but 7 of 11 mutator allele sets accumulated at least one predicted CNV during the experiment, including whole chromosome gains and segmental gains and losses (Table S3 and Table S4). Subtelomeric gains and losses were the most common type of CNV, presumably because large deletions in other parts of the genome would be lethal to a haploid cell and because telomeres represent repetitive, difficult-to-replicate regions prone to mutation (Nishant et al. 2010). Other types of structural variants were rarely detected but included large insertions, deletions, and inversions (Table S3 and Table S4).

Copy-number variants linked to hyperrecombination

In analyzing CNVs, we noted some common features of segmental changes internal to the chromosome. These CNVs were usually <20 kb, were invariably flanked by repetitive sequences (e.g., HXT6/HXT7 paralogues, ENA1/ENA2/ENA5 paralogues, RUF5-1/RUF5-2 ncRNAs, and Ty1 retrotransposons) and often contained an origin of replication. The nature of the CNVs indicated that unscheduled recombination events were likely taking place in these mutator strains, potentially linked to defective replication fork progression. Indeed some CNVs showed direct evidence of recombination (e.g., homozygosity of HXT6 and HXT7 paralogues, Figure 3A) and others are known to be recombination hotspots (St Charles and Petes 2013). It is worth noting that additional events would almost certainly be evident in diploids where larger deletions or intrachromosomal rearrangements, lethal to the haploid cells in our experiment, could be tolerated.

Together these observations led us to hypothesize that many observed CNVs could be due to increases in homologous recombination. Using a plasmid-based assay for direct repeat recombination, we found that strains with the most segmental CNVs (i.e., stn1-13, pol1-ts, and tsaiΔ) had significant increases in direct repeat recombination (Figure 3B). Consistently, several of these strains (e.g., POL1, and TSA1 mutants) have reported increases in Rad52 foci, indicative of ongoing DNA repair, whereas the Rad52 foci status of stn1-13 is unknown (Ragu et al. 2007; Stirling et al. 2012). We scored a subset of mutator alleles from our screen for Rad52-YFP foci and found that stn1-13, along with other replication and transcription mutants, cause significant increases in Rad52 foci (Figure 3C and Table S1; Stirling et al. 2012). We suggest that some of the identified CNVs are driven by inappropriate recombination events due to increased DNA damage. While surprising for a circular plasmid given the role of Stn1 in telomere capping, the observed increase in plasmid-based recombination rate in stn1-13 cells supports its recently described role in global DNA replication fork restart via interactions with the POLα complex (Stewart et al. 2012).

Varied base-substitution patterns originating in different mutator alleles

We pooled accumulated mutations for a given parental genotype to assess allele-associated mutation signatures. Variants were classified as originating in the pyrimidine base to simplify SNVs into six categories (i.e., C to T or T to C transition and T to A, T to G, C to A, and C to G transversions) (Figure 4A and Table S5). Since we did not accumulate enough mutations in our WT strain to confidently set a baseline, we relied on the whole-genome and marker sequencing data compiled in the literature to set a standard of the yeast mutational spectrum (Figure 4B) (Lynch et al. 2008; Lang et al. 2013).

Even combining all the genomes sequenced for a given strain, we accumulated relatively few base changes for each strain and could not universally predict mutation biases. Previous studies with similar numbers of variants have focused on mutators that create very well-defined signatures (e.g., indels in homopolymeric sequence for mismatch repair defects) (Zanders et al. 2010; Ma et al. 2012). Other studies have used strains with extremely high mutation rates due to
multiple mutator alleles (Larrea et al. 2010). Remarkably, we did observe significant shifts in the observed mutation type for rfc2-1, pol1-ts, and stn1-13 alleles using Fisher’s exact test (Holm–Bonferroni corrected $P < 0.05$) (Figure 4B). rad52Δ exhibited candidate variation in the Fisher test but did not meet our significance threshold after correction for multiple hypothesis testing (Figure 4B). The driving force of mutation bias for rfc2-1 and stn1-13 appears to be a proportionate increase in transition frequency, while the spectrum of pol1-ts is more complex (e.g., double the number of T to A transversions). Additional refinement and analyses of the context of base-substitution bias in such genome-wide studies will require larger numbers of variants and a continual refinement of the true WT mutation spectrum.

Limiting analysis to strains with >50 genic SNVs, we also observed a weak transcriptional strand bias in rad52Δ mutations (i.e., increased C to T mutations in the transcribed strand), suggesting that inability to complete transcription-associated recombination may lead to an error-prone mechanism of repair in these cells (Figure S1). While we do not understand the mechanisms in each case, as has been seen in analysis of tumor genomes (Nik-Zainal et al. 2012), our data suggest that transcription could have a significant allele-specific influence on mutation spectra.

Regional and functional genomic elements dictate mutation position

Biased mutation position could reflect specific chromatin environments or functional features of the genome that interact with a mutator allele to drive mutations in a specific locus. Relationships with features such as transcription, nucleotide content, or replication timing have previously been noted in cancer and model organisms (Lang and Murray 2011; Drier et al. 2013). We first analyzed the relative position of mutations along the length of chromosomes irrespective of sequence features, normalizing mutation positions to the position along the chromosome from 0 to 100%. Kolmogorov–Smirnov tests showed that only the

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**Figure 3** Linking segmental copy-number changes to recombination. (A) Example of segmental deletion in pol1-ts. Integrative Genomics Viewer of mapped reads shows a deletion encompassing ARS432, flanked by the HXT6/HXT7 paralogues. Loss of sequence heterogeneity between HXT6 and -7 (dotted boxes) supports a deletion by intrachromosomal recombination. (B) Direct repeat recombination rates in selected mutator alleles. Error bars represent 95% confidence intervals. (C) Rad52-YFP foci accumulation in selected mutator alleles. Blue bars were significantly different from WT ($P < 0.01$). Error bars are the standard error of the mean.
**stn1-13** and **pol1-ts** mutation patterns differed significantly from an idealized distribution and from other alleles patterns (Figure 4C). The **stn1-13** and **pol1-ts** mutation profiles showed hotspots of mutation in subtelomeric regions not seen in other alleles. For **stn1-13** this bias was expected since previous work demonstrated that **stn1-13** cells accumulate ssDNA in telomeric regions (Grandin et al. 1997).

We investigated potential correlations between mutation position in a given mutator allele and a variety of functional features of the genome. Generally, we did not see significant associations with GC content or with transcribed regions of the genome. The one exception to this was for **stn1-13**, which showed increased mutations outside of genes, likely associated with mutation bias to gene-poor subtelomeres (Figure S2 and see below). Similar to observations in human evolutionary studies and in cancer genomes, we found that mutations in 5 of 11 mutators occur in regions with later average replication timing compared to the rest of the genome (Figure S2) (Raghuraman et al. 2001; Stamatoyannopoulos et al. 2009; Lang and Murray 2011; Drier et al. 2013). Possible explanations include accumulation of damage prone ssDNA in late replicating regions (Stamatoyannopoulos et al. 2009) or may relate to the fact that some translesion polymerases are not expressed until late in S phase (Waters and Walker 2006). It has been suggested that late in replication error-prone translesion synthesis becomes more important to complete replication of DNA associated with stalled replisomes (Lang and Murray 2011). These observations support the notion that late replicating regions may be sensitized sites that are revealed in certain mutator allele genetic backgrounds.

**Increases in mutation clustering and region-specific mutation rates**

Mutation clustering may arise due to the coordination of mutational events or as part of hotspots of mutation. It has been observed that yeast cells accumulate clusters of mutations when treated with the DNA alkylating chemical MMS and that tumor cells accumulate clusters of mutations in a variety of contexts, some related to the action of cytosine deaminases (Nik-Zainal et al. 2012; Roberts et al. 2012; Burns et al. 2013). However, the contribution of mutator alleles to these phenomena has not been widely assessed.

We defined 28 mutation clusters across the 68 evolved genomes that were unlikely to have arisen by chance (P < 9.7 × 10−5) using the criteria laid out in the literature for MMS-induced mutation clusters, namely, any two or more SNV, indel, or complex mutations within 100 kb of each other that meet our significance threshold (Roberts et al. 2012). WT, **mcm7-ts**, and **pol2-12** did not produce any clusters, while the other mutator genomes contained between one and nine mutation clusters (Figure S1 and Table S6). Typically clusters contained two to three mutations but there were examples of larger clusters in **pol1-ts**, **stn1-13**, and **mas1-1** (Figure S1). Significantly clustered mutations

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*Figure 4* Mutator allele-driven biases in mutation type and position. (A) Base substitution types across mutator allele backgrounds. Shown are the raw number of each transversion (reds) and transition (blues) detected in the mutator genomes. (B) Proportion of mutation types. Selected data from A, expressed as a proportion, and its comparison with a WT spectrum (Lynch et al. 2008, 2013), reveal significant variations from the WT. P-values represent Holm–Bonferroni corrected results of a Fisher’s exact test (*<0.05). (C) Mutation position as a percentage of chromosome length. Relative mutation position along the 16 chromosomes in **stn1-13** and **pol1-ts** differ significantly from **rad52** (Kolmogorov–Smirnov \( P < 0.05\)). The x-axis (mutation rank) reflects the position of each mutation in an ordered list, expressed as a proportion of the total.
spanned as few as 21 bp and as many as 126 kb, with a median of \(~3.6\) kb (Figure S1). One candidate mechanism for mutation clustering in the absence of genotoxins or APOBECs, is exposure of resected ssDNA during prolonged repair reactions. Local mutation rates increase in this scenario and we found that at least \(\text{pol1-ts}\) and \(\text{stn1-13}\) have an increased frequency of DNA breaks as measured by \(\text{Rad52}\) foci (Figure 3C) (Hicks et al. 2010; Nik-Zainal et al. 2012; Roberts et al. 2012; Stirling et al. 2012). In support of this model, for nearly all clusters, the mutation density fell within the range of expected resection length for repair of a DNA double-strand break (i.e., up to \(~10\) kb) (Chung et al. 2010).

To examine cluster position, we generated rainfall plots (Nik-Zainal et al. 2012). Strikingly, clusters of mutations in \(\text{stn1-13}\) and \(\text{pol1-ts}\) strains occurred almost exclusively in subtelomeric regions, suggesting that the observed telomeric bias may relate to clusters (Figure 5A). At least for \(\text{STN1}\), this observation supports literature showing that uncapped telomeres lead to hypermutability in association with ssDNA and that \(\text{stn1-13}\) accumulates ssDNA in telomeres (Grandin et al. 1997; Yang et al. 2008). It is notable that some clusters in \(\text{mas1-1}\) or \(\text{rfc2-1}\) were near repetitive sequences known to be recombination hotspots (e.g., Ty elements, \(\text{ENA1,2,5}\) gene cluster) that may be more likely to initiate inappropriate repair reactions involving resection (Table S6) (St Charles and Petes 2013). Alternatively, many of these clusters occurred adjacent to predicted CNVs (Figure 5A), suggesting that, as in other systems, break-associated mutation clustering might be driving the observed pattern (Hicks et al. 2010; Deem et al. 2011; Roberts et al. 2012; Drier et al. 2013). Indeed, break-induced replication itself has been found to be highly error prone and could contribute to clustering in the mutator genomes (Deem et al. 2011).

Overall, mutation accumulation and sequencing suggested that local mutation rates were likely higher in subtelomeres for \(\text{pol1-ts}\) and \(\text{stn1-13}\) strains. While we did not observe a subtelomeric bias for mutations in other strains, providing the essential control for the specificity of this observation to \(\text{pol1-ts}\) and \(\text{stn1-13}\), subtelomeres do exhibit lower variant scores due to their repetitive nature. Moreover, we could not determine whether the primary subtelomeric sequence or the chromosomal context itself was driving mutations. Therefore, we directly tested mutation position biases by introducing the counterselectable \(\text{URA3}\) marker into a subset of mutator lines at loci increasing in distance from the Tel VI-L (Figure 5B). This system enabled us to measure the mutation rate at the same target sequence, \(\text{URA3}\), in different chromosomal contexts (Lang and Murray 2011). Fluctuation analysis of \(\text{URA3}\) positioned at 15, 84, or 112 kb from Tel VI-L showed dramatic distance-associated decreases in mutation rate for \(\text{stn1-13}\) and \(\text{pol1-ts}\) but not for the \(\text{rad52}\) strain, which showed an identical rate regardless of the marker position (Figure 5B). The presence of mutation prone subtelomeres in \(\text{STN1}\) and \(\text{POL1}\) mutants is consistent with the function of \(\text{STN1}\) in telomere protection and in recruitment of the \(\text{POL}\alpha\)-primase complexes to subtelomeres for efficient replication (Yang et al. 2008; Wang et al. 2012).
Conclusion

We identified dozens of essential mutator genes which contribute to a catalog of mutator alleles that define an expanded suite of such cellular mechanisms that suppress mutations. In addition, this work takes a powerful approach to defining mutation spectra for mutator alleles: whole-genome sequencing. These data suggest that mutational signatures vary in an allele-specific fashion and are influenced by several key features: (1) concurrent chromosome instability related to increases in recombination; (2) replication timing, chromosomal context; and (3) break-associated mutation clustering.

Building on this work, and the existing literature on mutator allele-driven mutation signatures (e.g., mismatch repair, DNA polymerase δ) (Larrea et al. 2010; Zanders et al. 2010), will require both broader studies of more mutator conditions (e.g., genotypes, environment, ploidy) to identify novel signatures, and deeper analysis of larger numbers of variants for a single mutator to improve statistical power. These analyses will be important because tumors are almost certainly influenced by the interaction between specific early genome destabilizing mutations or genotoxins and correspondingly sensitized loci within the genome. In addition to increasing the rate of mutation, mutator alleles introduce base substitution biases or broader locus-associated biases including mutation clustering. Recent detailed analysis of mutation patterns across 21 breast tumors revealed mutation clusters associated with a mutational process termed “kataegis” that is associated with cytosine deamination by APOBEC family proteins (Nik-Zainal et al. 2012; Taylor et al. 2013). Our data demonstrate that specific mutator genotypes are sufficient to generate mutation clusters in the absence of genotoxins or APOBECs, probably via break-associated hypermutability as seen in models and in human cancers (Roberts et al. 2012; Drier et al. 2013). Expanding mutation pattern studies in model systems may therefore be key to mechanistically connect specific predisposing genotypes to aspects of mutational signatures seen in tumor genomes.

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Genome Destabilizing Mutator Alleles Drive Specific Mutational Trajectories in *Saccharomyces cerevisiae*

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**Figure S1** Strand mutational bias and mutation clustering. (A) Shown are C>T mutations, the dominant mutation type, occurring in genes for the six most mutated alleles (i.e. >50 SNVs detected in transcribed regions). The binomial probability, after Holm-Bonferroni correction, that more C>T mutations occur on the non-transcribed strand is shown above the data for each allele. *indicates that rad52Δ has a bias toward C>T on the transcribed strand (p<0.05) and that pol1-ts has a bias toward C>T on the non-transcribed stand (p>0.95). (B) Mutations per significant cluster for each allele. (C) Mutation cluster size. Shown on a log scale with the average mutations/kb noted above.
Figure S2  Genomic properties of mutated regions. (A) Proportion of mutations occurring in genes versus intergenic regions. A significant decrease (Fisher test p<0.01) of mutations in genes is seen in stn1-13. (B) Replication times flanking detected mutations are from (Raghuraman et al. 2001). Wilcoxon ranksum test p-values compared to the genome average are indicated above. The dotted line transecting the boxplots indicate the median time of the genome. Boxplots were produced in R. The black line indicates the median value, the box boundaries indicate the 1st and 3rd quartile and the whiskers indicate 1.5x the interquartile range.
Table S1 Compiled list of yeast mutator alleles. This list includes essential mutators identified in our study, the results of the genome-wide screen for non-essential mutator alleles (Huang et al., 2003) and the compilation of literature reported phenotypes compiled at www.yeastgenome.org.

| ORF ID | Gene | Source | Rad52 foci | CIN phenotype* | Group |
|--------|------|--------|------------|----------------|-------|
| YIL150C | MCM10 | This study | van Pel et al., 2013 | GCR, ALF | DNA replication |
| YBR202W | MCM7 | This study | None reported | MCM, Chromosome Loss | DNA replication |
| YBR060C | ORC2 | This study | None reported | CTF x2, GCR x4 | DNA replication |
| YNL261W | ORC5 | This study | None reported | CTF | DNA replication |
| YBL035C | POL12 | This study | van Pel et al., 2013 | GCR | DNA replication |
| YOL146W | PSF3 | This study | Stirling et al., 2012 | CTF, GCR, ALF | DNA replication |
| YJL194W | CDC6 | This study | Stirling et al., 2012 | CTF, GCR | DNA replication |
| YBL023C | MCM2 | This study | Stirling et al., 2012 | CTF, GCR | DNA replication |
| YLR274W | MCM5 | This study | Stirling et al., 2012 | CTF, chromosome loss, GCR | DNA replication |
| YML065W | ORC1 | This study | Stirling et al., 2012 | CTF, ALF | DNA replication |
| YGL113W | SLD3 | This study | Stirling et al., 2012 | CTF | DNA replication |
| YOL094C | RFC4 | This study | Stirling et al., 2012 | GCR, ALF | DNA replication |
| YJL173C | RFA3 | This study | Stirling et al., 2012 | None reported | DNA replication |
| YNL262W | POL2 | This study | Stirling et al., 2012 | None reported | DNA replication |
| YJR006W | POL31 | This study | Stirling et al., 2012 | CTF, ALF | DNA replication |
| YNL102W | POL1 | This study | Stirling et al., 2012 | CTF x2, GCR | DNA replication |
| YJR068W | RFC2 | This study | Stirling et al., 2012 | CTF, CTF(o/e), GCR | DNA replication |
| YDL164C | CDC9 | This study | Stirling et al., 2012 | GCR, ALF | DNA replication |
| YDR062W | LCB2 | This study | None reported | None reported | Miscellaneous |
| YOL114W | NOP8 | This study | None reported | None reported | Miscellaneous |
| YIL118W | RHO3 | This study | None reported | None reported | Miscellaneous |
| YGL098W | USE1 | This study | None reported | None reported | Miscellaneous |
| YMR308C | PSE1 | This study | None reported | CTF, GCR | DNA replication |
| YLR163C | MAS1 | This study | None reported | GCR | DNA replication |
| YDL120W | YFH1 | This study | None reported | None reported | DNA replication |
| YER012W | PRE1 | This study | None reported | CTF, CTF(o/e), ALF | DNA replication |
| YKL210W | UBA1 | This study | None reported | None reported | DNA replication |
| YDR082W | STN1 | This study | None reported | None reported | DNA replication |
| YGL169W | SUA5 | This study | None reported | None reported | DNA replication |
| YLR010C | TEN1 | This study | None reported | None reported | DNA replication |
| YKL173W | SNU114 | This study | None reported | CTF, GCR | DNA replication |
| YGR274C | TAF1 | This study | None reported | CTF, GCR | DNA replication |
| Yeast Gene ID | Protein Name | Reference | Function | Notes |
|--------------|--------------|-----------|----------|-------|
| YDR145W      | TAF12        | This study | None reported | Transcription |
| YMR005W      | TAF4         | This study | None reported | Transcription |
| YLR115W      | CFT2         | This study | None reported | Transcription |
| YBR088C      | POL30        | This study / www.yeastgenome.org | None reported | DNA replication AND repair |
| YDL102W      | POL3         | This study / www.yeastgenome.org | None reported | DNA replication AND repair |
| YML032C      | RAD52        | Huang et al., 2003 | None reported | DNA repair |
| YMR224C      | MRE11        | Huang et al., 2003 | None reported | DNA repair |
| YHR120W      | MSH1         | Huang et al., 2003 | None reported | DNA repair |
| YML060W      | OGG1         | Huang et al., 2003 | None reported | DNA repair |
| YML061C      | PIF1         | Huang et al., 2003 | None reported | DNA repair |
| YCR066W      | RAD18        | Huang et al., 2003 | None reported | DNA repair |
| YNL250W      | RAD50        | Huang et al., 2003 | None reported | DNA repair |
| YDR078C      | SHU2         | Huang et al., 2003 | None reported | DNA repair |
| YLR376C      | PSY3         | Huang et al., 2003 | None reported | DNA repair |
| YIL132C      | CSM2         | Huang et al., 2003 | None reported | DNA repair |
| YML021C      | UNG1         | Huang et al., 2003 | None reported | DNA repair |
| YMR167W      | MLH1         | Huang et al., 2003 | None reported | DNA repair |
| YKL113C      | RAD27        | Huang et al., 2003 | None reported | DNA repair |
| YER095W      | RAD51        | Huang et al., 2003 | None reported | DNA repair |
| YGL163C      | RAD54        | Huang et al., 2003 | None reported | DNA repair |
| YDR076W      | RAD55        | Huang et al., 2003 | None reported | DNA repair |
| YDR004W      | RAD57        | Huang et al., 2003 | None reported | DNA repair |
| YOR144C      | ELG1         | Huang et al., 2003 | None reported | DNA repair |
| YIL116W      | HIS5         | Huang et al., 2003 | None reported | Miscellaneous |
| YDL162C      | YDL162C      | Huang et al., 2003 | None reported | Miscellaneous |
| YLR154C      | RNH203       | Huang et al., 2003 | None reported | Miscellaneous Oxidative Stress / Mito |
| YMR038C      | CCS1         | Huang et al., 2003 | None reported | Oxidative Stress / Mito |
| YJR104C      | SOD1         | Huang et al., 2003 | None reported | Oxidative Stress / Mito |
| YHR206W      | SKN7         | Huang et al., 2003 | None reported | Oxidative Stress / Mito |
| YMR166C      | YMR166C      | Huang et al., 2003 | None reported | Oxidative Stress / Mito |
| YML007W      | YAP1         | Huang et al., 2003 | None reported | Oxidative Stress / Mito |
| YML028W      | TSA1         | Huang et al., 2003 | None reported | Oxidative Stress / Mito |
| YPR023C      | EAF3         | www.yeastgenome.org | None reported | Chromatin |
| YDR174W      | HMO1         | www.yeastgenome.org | None reported | Chromatin |
| YDL042C      | SIR2         | www.yeastgenome.org | None reported | Chromatin |
| YKL114C      | APN1         | www.yeastgenome.org | None reported | DNA repair |
| YBL019W      | APN2         | www.yeastgenome.org | None reported | DNA repair |
| YOL090W | MSH2 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YCR092C | MSH3 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YDR097C | MSH6 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YEL062W | NPR2 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YAL015C | NTG1 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YOL043C | NTG2 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YCR014C | POL4 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YPL022W | RAD1 | www.yeastgenome.org | None reported | ALF | DNA repair |
| YML095C | RAD10 | www.yeastgenome.org | None reported | BiMx2, ALF | DNA repair |
| YBR114W | RAD16 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YGR258C | RAD2 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YJR035W | RAD26 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YDR030C | RAD28 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YER171W | RAD3 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YDR419W | RAD30 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YER162C | RAD4 | www.yeastgenome.org | None reported | LOH | DNA repair |
| YHL006C | SHU1 | www.yeastgenome.org | None reported | ALF | DNA repair |
| YDR092W | UBC13 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YBR223C | TDP1 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YLR135W | SLX4 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YDR440W | DOT1 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YPL024W | RMI1 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YBR274W | CHK1 | www.yeastgenome.org | None reported | GCR | DNA repair |
| YPL194W | DDC1 | www.yeastgenome.org | None reported | CT, BiM, ALF | DNA repair |
| YOR005C | DNL4 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YDL101C | DUN1 | www.yeastgenome.org | None reported | ALF, LOH | DNA repair |
| YKL032C | IXR1 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YDL200C | MGT1 | www.yeastgenome.org | None reported | None reported | DNA repair |
| YOR368W | RAD17 | www.yeastgenome.org | None reported | BiM, ALF | DNA repair |
| YER173W | RAD24 | www.yeastgenome.org | None reported | BiM, ALF | DNA repair |
| YLR032W | RAD5 | www.yeastgenome.org | None reported | BiM, ALF, GCR | DNA repair |
| YDL059C | RAD59 | www.yeastgenome.org | None reported | BiM, ALF | DNA repair |
| YDR217C | RAD9 | www.yeastgenome.org | None reported | BiM, ALF | DNA repair |
| YDR369C | XRS2 | www.yeastgenome.org | None reported | BiM, ALF, LOH | DNA repair |
| YPR019W | MCM4 | www.yeastgenome.org | None reported | ALF | DNA replication |
| YBR087W | RFC5 | www.yeastgenome.org | None reported | CTF, GCR, ALF | DNA replication |
| YNL072W | RHH201 | www.yeastgenome.org | None reported | BiM, ALF, LOH | DNA replication |
| YGR180C | RNR4 | www.yeastgenome.org | None reported | ALF | DNA replication |
| YBR252W | DUT1 | www.yeastgenome.org | None reported | None reported | DNA replication |
| YKL067W | YNK1 | www.yeastgenome.org | None reported | None reported | DNA replication |
| Yeast ID | Protein | Website                  | Function(s) Reported | Genetic Phenotypes | Notes |
|---------|---------|--------------------------|----------------------|--------------------|-------|
| YEL019C | MMS21   | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | DNA replication AND repair |
| YDR288W | NSE3    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | Stirling et al., 2012 | DNA replication AND repair |
| YMR190C | SGS1    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | Alvaro et al., 2007 | DNA replication AND repair |
| YLR383W | SMC6    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | DNA replication AND repair |
| YLR234W | TOP3    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | DNA replication AND repair |
| YAL040C | CLN3    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | CTF, ALF           | Miscellaneous |
| YBR278W | DPB3    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | Miscellaneous |
| YDR113C | PDS1    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | Miscellaneous |
| YGL255W | ZRT1    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | Miscellaneous |
| YOR330C | MIP1    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | Oxidative Stress / Mitochondria |
| YPR103W | PRE2    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | Proteolysis |
| YJR001W | PRE3    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | Proteolysis |
| YBR173C | UMP1    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | Proteolysis |
| YOR157C | PUB1    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | Proteolysis |
| YMR039C | SUB1    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | Transcription |
| YGL127C | SPT10   | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | Transcription |
| YGL115W | ASF1    | [www.yeastgenome.org](http://www.yeastgenome.org) | None reported        | None reported      | Transcription |

*CIN phenotypes defined as: MCM = minichromosome maintenance; ALF = MATa-like faker; BiM = Bimater; CTF = chromosome transmission fidelity; LOH = loss-of-heterozygosity; GCR = gross chromosomal rearrangement.

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Stirling, P. C. *et al.* *Genes Dev.* **26**, 163-175 (2012).

van Pel, D. M. *et al.* *G3 (Bethesda)* **3**, 273-282 (2013).
| Library ID | Average coverage | Strain | ID       | Type |
|------------|------------------|--------|---------|------|
| A21529     | 70.47965081      | mas1 evolved A3 -33 | PH_125 | MA   |
| A21530     | 60.87233789      | mas1 evolved B3 -34 | PH_126 | MA   |
| A21531     | 54.56920917      | mas1 evolved C3 -35 | PH_127 | MA   |
| A21532     | 43.6662041       | mas1 evolved D3 -36 | PH_128 | MA   |
| A21533     | 49.92637791      | mas1 evolved E3 -37 | PH_129 | MA   |
| A21534     | 75.52079405      | mas1 evolved G3 -38 | PH_130 | MA   |
| A21500     | 48.38892651      | mas1-1 start -4     | PH_96  | Parent |
| A21545     | 50.36829555      | mcm7 evolved 1-D5 -49 | PH_141 | MA   |
| A21546     | 65.33676102      | mcm7 evolved 1-E5 -50 | PH_142 | MA   |
| A21541     | 48.77270326      | mcm7 evolved 2-A5 -45 | PH_137 | MA   |
| A21542     | 67.70084456      | mcm7 evolved 2-B5 -46 | PH_138 | MA   |
| A21544     | 21.66170081      | mcm7 evolved 2-E5 (2) -48 | PH_140 | MA   |
| A21503     | 59.43827413      | mcm7-ts 1-start -7   | PH_99  | Parent |
| A21502     | 55.42162961      | mcm7-ts 2-start -6   | PH_98  | Parent |
| A21547     | 65.12670362      | orc2-1 evolved A6 -51 | PH_143 | MA   |
| A21548     | 65.82275321      | orc2-1 evolved B6 -52 | PH_144 | MA   |
| A21550     | 77.0362934       | orc2-1 evolved E6 (2) -54 | PH_146 | MA   |
| A21551     | 68.78371861      | orc2-1 evolved G6 (2) -55 | PH_147 | MA   |
| A21552     | 66.65805956      | orc2-1 evolved H6 (2) -56 | PH_148 | MA   |
| A21504     | 68.55303057      | orc2-1 start -8      | PH_100 | Parent |
| A21571     | 78.71104085      | pol1-ts evolved A10 -75 | PH_167 | MA   |
| A21572     | 48.7945061       | pol1-ts evolved B10 -76 | PH_168 | MA   |
| A21573     | 62.53451087      | pol1-ts evolved C10 -77 | PH_169 | MA   |
| A21574     | 57.48445895      | pol1-ts evolved D10 -78 | PH_170 | MA   |
| A21575     | 57.25541198      | pol1-ts evolved E10 -79 | PH_171 | MA   |
| A21576     | 53.63426617      | pol1-ts evolved F10 -80 | PH_172 | MA   |
| A21508     | 50.30710695      | pol1-ts start -12    | PH_104 | Parent |
| A21565     | 48.78465966      | pol2-12 evolved A9 -69 | PH_161 | MA   |
| A21567     | 59.59276947      | pol2-12 evolved C9 -71 | PH_163 | MA   |
| A21568     | 28.62454106      | pol2-12 evolved D9 -72 | PH_164 | MA   |
| A21569     | 48.65173271      | pol2-12 evolved G9 -73 | PH_165 | MA   |
| A21570     | 31.62489223      | pol2-12 evolved H9 -74 | PH_166 | MA   |
| A21507     | 34.25342304      | pol2-12 start -11    | PH_103 | Parent |
| A21583     | 70.22598774      | rad52D evolved A12 -87 | PH_179 | MA   |
| A21584     | 60.00374115      | rad52D evolved B12 -88 | PH_180 | MA   |
| A21585     | 71.64434409      | rad52D evolved C12 -89 | PH_181 | MA   |
| A21586     | 60.06868078      | rad52D evolved D12 -90 | PH_182 | MA   |
| A21587     | 67.21625902      | rad52D evolved E12 -91 | PH_183 | MA   |
| A21588     | 66.58819676      | rad52D evolved F12 -92 | PH_184 | MA   |
| A21510     | 54.49184428      | rad52D start -14     | PH_106 | Parent |
| ID     | Value          | Description                        | Parent ID | Parent Description |
|--------|----------------|------------------------------------|-----------|--------------------|
| A21553 | 55.87855135    | rfc2-1 evolved A7 -57             | PH_149    | MA                 |
| A21554 | 62.2958519     | rfc2-1 evolved B7 -58             | PH_150    | MA                 |
| A21555 | 43.77071135    | rfc2-1 evolved C7 -59             | PH_151    | MA                 |
| A21556 | 51.57940776    | rfc2-1 evolved D7 -60             | PH_152    | MA                 |
| A21558 | 61.35926783    | rfc2-1 evolved H7 -62             | PH_154    | MA                 |
| A21559 | 53.22563888    | rfc2-1 start -9                   | PH_101    | Parent             |
| A21560 | 61.83213143    | snu114-60 evolved A8 -63          | PH_155    | MA                 |
| A21561 | 76.22786227    | snu114-60 evolved B8 (2) -64      | PH_156    | MA                 |
| A21562 | 75.62629163    | snu114-60 evolved C8 (1) -65      | PH_157    | MA                 |
| A21563 | 53.97748497    | snu114-60 evolved E8 -66          | PH_158    | MA                 |
| A21564 | 56.82287191    | snu114-60 evolved F8 -67          | PH_159    | MA                 |
| A21565 | 64.85522318    | snu114-60 evolved H8 (1) -68      | PH_160    | MA                 |
| A21566 | 47.09060297    | snu114-60 start -10               | PH_102    | Parent             |
| A21567 | 66.71080839    | stn1 evolved 1-G2(2) -32          | PH_124    | MA                 |
| A21568 | 78.985569      | stn1 evolved 2-A2 -27             | PH_119    | MA                 |
| A21569 | 43.95146387    | stn1 evolved 2-B2 -28             | PH_120    | MA                 |
| A21570 | 37.45304741    | stn1 evolved 2-G2(2) -31          | PH_123    | MA                 |
| A21571 | 31.20172972    | stn1-13 1-start -3                | PH_95     | Parent             |
| A21572 | 36.66158112    | stn1-13 2-start -2                | PH_94     | Parent             |
| A21573 | 49.622076      | taf12 evolved A4 -39              | PH_131    | MA                 |
| A21574 | 72.13596281    | taf12 evolved B4 -40              | PH_132    | MA                 |
| A21575 | 62.48621642    | taf12 evolved C4 -41              | PH_133    | MA                 |
| A21576 | 86.62640704    | taf12 evolved D4 -42              | PH_134    | MA                 |
| A21577 | 54.69510295    | taf12 evolved E4 -43              | PH_135    | MA                 |
| A21578 | 54.92883869    | taf12 evolved F4 -44              | PH_136    | MA                 |
| A21579 | 50.42151321    | taf12_W486* start -5              | PH_97     | Parent             |
| A21580 | 35.0507503     | tsa1D evolved A11 -81             | PH_173    | MA                 |
| A21581 | 57.84830838    | tsa1D evolved B11 -82             | PH_174    | MA                 |
| A21582 | 61.80048215    | tsa1D evolved C11 -83             | PH_175    | MA                 |
| A21583 | 47.00550159    | tsa1D evolved D11 -84             | PH_176    | MA                 |
| A21584 | 73.7593676     | tsa1D evolved E11 -85             | PH_177    | MA                 |
| A21585 | 47.85698427    | tsa1D evolved F11 -86             | PH_178    | MA                 |
| A21586 | 67.50977673    | tsa1D start -13                   | PH_105    | Parent             |
| A21587 | 58.88429461    | WT evolved A1 -21                 | PH_113    | MA                 |
| A21588 | 52.58702687    | WT evolved B1 -22                 | PH_114    | MA                 |
| A21589 | 54.645945      | WT evolved C1 -23                 | PH_115    | MA                 |
| A21590 | 54.1512431     | WT evolved D1 -24                 | PH_116    | MA                 |
| A21591 | 58.41025882    | WT evolved E1-25                  | PH_117    | MA                 |
| A21592 | 67.32363211    | WT evolved F1-26                  | PH_118    | MA                 |
| A21593 | 45.1548396     | WT single start -1                | PH_93     | Parent             |

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### Table S3  Summary of mutation accumulation experiment

|                       | WT | orc2-1 | pol2-12 | snl1-13 | mas1-1 | taf12-486* | rad52Δ | mcm7-ts | rfc2-1 | snu114-60 | pol1-ts | tsa1Δ |
|-----------------------|----|--------|---------|---------|--------|-----------|--------|--------|--------|-----------|---------|-------|
| Mutation accumulation genomes sequenced | 6  | 5      | 6       | 4       | 6      | 6         | 6      | 6      | 5      | 6         | 6       | 5     |
| SNV                   |    |        |         |         |        |           |        |        |        |           |         |       |
| GC change             | 5  | 13     | 42      | 19      | 59     | 58        | 86     | 53     | 27     | 47        | 59      | 81    |
| AT change             | 8  | 9      | 14      | 14      | 32     | 20        | 35     | 22     | 15     | 15        | 39      | 30    |
| Indel                 |    |        |         |         |        |           |        |        |        |           |         |       |
| -1 fs                 | 2  | 3      | 3       | 1       | 5       | 3         | 3      | 10     | 5      | 5         | 8       | 2     |
| +1 fs                 | 0  | 0      | 2       | 1       | 4       | 6         | 6      | 2      | 1      | 0         | 9       | 4     |
| 2 - 230 bp            | 1  | 1      | 1       | 6       | 1       | 7         | 3      | 2      | 2      | 2         | 8       | 2     |
| Complex (within 10bp) |    |        |         |         |        |           |        |        |        |           |         |       |
| Whole chromosome      | 0  | 0      | 1       | 2       | 1       | 0         | 0      | 1      | 2      | 0         | 4       | 0     |
| Sub-telomeric         | 0  | 0      | 3       | 7       | 2       | 0         | 0      | 0      | 2      | 0         | 11      | 4     |
| Segmental             | 0  | 0      | 0       | 1       | 0       | 0         | 0      | 0      | 2      | 0         | 6       | 1     |
| Other Struc. Variant  |    |        |         |         |        |           |        |        |        |           |         |       |
| Total Events detected | 17 | 27     | 67      | 55      | 108     | 98        | 133    | 92     | 62     | 72        | 155     | 126   |
| Fold increase in WGS mutation | 1.00 | 1.59 | 3.94 | 3.24 | 6.35 | 5.76 | 7.82 | 5.41 | 3.65 | 4.24 | 9.12 | 7.41 |
| Fold increase in CAN | 1.00 | 6.36 | 16.64 | 11.48 | 3.65 | 7.91 | 14.74 | 9.83 | 5.44 | 7.72 | 12.35 | 12.63 |
| Mutations per genome  | 2.83 | 5.40 | 11.17 | 13.75 | 18.00 | 16.33 | 22.17 | 15.33 | 12.40 | 12.00 | 25.83 | 25.20 |
| Fold increase per genome | 1.00 | 1.91 | 3.94 | 4.85 | 6.35 | 5.76 | 7.82 | 5.41 | 4.38 | 4.24 | 9.12 | 8.89 |
Table S4  Details of mutations detected in whole genome sequencing.

*Mutation clusters are labelled in blue highlighting.
**In combination with the strain name, each mutation has a unique ID. Occasionally, a series of large CNVs covering almost an entire chromosome were seen, most likely representing whole chromosome gains. These are numbered consecutively here (e.g. CNV1a, 1b, 1c, etc.) but were treated as single events for our analysis in Table S3.

Table S4 is available for download as an Excel file at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.159806/-/DC1.
Table S5  Analysis of SNV mutation type.

| Pooled SNV | rfc2-1 | pol1-ts | stn1-13 | rad52Δ | snu114-60 | taf12-W486* | pol2-12 | mas1-1 | mcm7-ts | orc2-1 | tsa1Δ | WT (Lynch et al., 2008; Lang et al., 2013) |
|------------|--------|---------|---------|--------|-----------|-------------|---------|--------|---------|--------|-------|----------------------------------------|
| C>A        | 1      | 16      | 5       | 17     | 9         | 14          | 9       | 24     | 16      | 3      | 23    | 503                                    |
| C>G        | 10     | 12      | 3       | 29     | 14        | 14          | 16      | 15     | 9       | 3      | 19    | 308                                    |
| T>A        | 4      | 16      | 3       | 11     | 4         | 9           | 5       | 7      | 10      | 3      | 8     | 148                                    |
| T>G        | 1      | 5       | 0       | 10     | 5         | 5           | 3       | 9      | 5       | 1      | 7     | 118                                    |
| C>T        | 16     | 31      | 11      | 40     | 24        | 30          | 17      | 20     | 28      | 7      | 39    | 513                                    |
| T>C        | 10     | 18      | 11      | 14     | 6         | 5           | 6       | 16     | 7       | 5      | 15    | 192                                    |

|             | Uncorrected p-values | Corrected p-value |
|-------------|----------------------|-------------------|
|             | 0.0002                | 0.0027            |
|             | 0.0036                | 0.0359            |
|             | 0.0041                | 0.0373            |
|             | 0.0142                | 0.1135            |
|             | 0.1220                | -                 |
|             | 0.1727                | -                 |
|             | 0.2230                | -                 |
|             | 0.2630                | -                 |
|             | 0.2724                | -                 |
|             | 0.3019                | -                 |
|             | 0.4768                | -                 |
|             | -                    | -                 |

Uncorrected p-values:

Corrected p-values:

- Uncorrected p-values:
  - C>A: 0.0002
  - C>G: 0.0036
  - T>A: 0.0041
  - T>G: 0.0142
  - C>T: 0.1220
  - T>C: 0.1727

- Corrected p-values:
  - C>A: 0.0027
  - C>G: 0.0359
  - T>A: 0.0373
  - T>G: 0.1135
  - C>T: -
  - T>C: -
| Strain       | Cluster ID             | p-value      | Size (bp) | Number of mutations |
|--------------|------------------------|--------------|-----------|---------------------|
| orc2-1       | orc2-1 cluster 1       | 1.94E-06     | 57756     | 3                   |
| snu114-60    | snu114-60 cluster 5    | 1.04E-07     | 454       | 2                   |
| rad52Δ       | rad52Δ cluster13       | 3.90E-05     | 33842     | 3                   |
| taf12-W486*  | taf12-W486* cluster 7  | 2.16E-05     | 4958      | 2                   |
| tsa1Δ        | tsa1Δ cluster 18       | 7.83E-05     | 6264      | 2                   |
| tsa1Δ        | tsa1Δ cluster 20       | 4.10E-05     | 4528      | 2                   |
| tsa1Δ        | tsa1Δ cluster 3        | 4.46E-05     | 4721      | 2                   |
| stn1-13      | stn1-13 cluster 1      | 9.24E-42     | 372       | 10                  |
| stn1-13      | stn1-13 cluster 2      | 3.42E-09     | 86        | 2                   |
| stn1-13      | stn1-13 cluster 3      | 1.63E-18     | 10291     | 7                   |
| stn1-13      | stn1-13 cluster 4      | 1.44E-22     | 115       | 5                   |
| mas1-1       | mas1-1 cluster 13      | 5.38E-09     | 72        | 2                   |
| mas1-1       | mas1-1 cluster 14      | 3.94E-05     | 6308      | 2                   |
| mas1-1       | mas1-1 cluster 2       | 3.54E-07     | 595       | 2                   |
| mas1-1       | mas1-1 cluster 7       | 2.19E-09     | 77644     | 6                   |
| rfc2-1       | rfc2-1 cluster 1       | 8.86E-07     | 1618      | 2                   |
| rfc2-1       | rfc2-1 cluster 3       | 2.50E-06     | 30185     | 3                   |
| rfc2-1       | rfc2-1 cluster 4       | 1.78E-06     | 26911     | 3                   |
| rfc2-1       | rfc2-1 cluster 5       | 2.50E-06     | 2721      | 2                   |
| pol1-ts      | pol1-ts cluster 1      | 1.06E-18     | 4983      | 7                   |
| pol1-ts      | pol1-ts cluster 10     | 8.23E-30     | 470       | 8                   |
| pol1-ts      | pol1-ts cluster 11     | 8.53E-10     | 21        | 2                   |
| pol1-ts      | pol1-ts cluster 13     | 1.41E-07     | 288       | 2                   |
| pol1-ts      | pol1-ts cluster 17     | 2.25E-07     | 364       | 2                   |
| pol1-ts      | pol1-ts cluster 2      | 3.24E-07     | 6814      | 3                   |
| pol1-ts      | pol1-ts cluster 4      | 5.95E-09     | 126611    | 7                   |
| pol1-ts      | pol1-ts cluster 5      | 8.76E-20     | 216       | 5                   |
| pol1-ts      | pol1-ts cluster 9      | 2.49E-08     | 120       | 2                   |

**AVERAGE** | 9.82E-06 | 14619 | 4
**Table S7  Yeast strains and plasmids used in this study.**

| Plasmid name | Relevant features | Source |
|--------------|------------------|--------|
| LNA          | LEU2-5′-LEU2-3′, TRP1, CEN | Andres Aguilera (Also used in Stirling et al., 2012) |

| Strain name | Relevant genotype | Source |
|-------------|------------------|--------|
| BY4741      | MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 | Open biosystems |
| ts-alleles  | BY4741, YFEG::KanMX | Li et al., 2011 |
| DAMP alleles | BY4741 YFEG::KanMX | Breslow et al., 2008 |
| PSY1090     | MATa ura3Δ0 leu2Δ0 his3Δ1 rad52Δ::KanMX | This study |
| PSY1087     | MATa ura3Δ0 leu2Δ0 his3Δ1 tsα1Δ::KanMX | This study |
| PSY366      | BY4741, Rad52-YFP::URA3 | Stirling et al., 2012 |
| PSY1111     | MATa can1Δ::STE2pr-SpHis5 lyp1Δ RAD52-YFP::URA3 rfa3-313::KanMX | Stirling et al., 2012 |
| PSY1137     | MATa can1Δ::STE2pr-SpHis5 lyp1Δ RAD52-YFP::URA3 taf12-486::KanMX | Stirling et al., 2012 |
| PSY1133     | MATa can1Δ::STE2pr-SpHis5 lyp1Δ RAD52-YFP::URA3 pol2-12::KanMX | Stirling et al., 2012 |
| PSY1110     | MATa can1Δ::STE2pr-SpHis5 lyp1Δ RAD52-YFP::URA3 stn1-13::KanMX | Stirling et al., 2012 |
| PSY1125     | MATa can1Δ::STE2pr-SpHis5 lyp1Δ RAD52-YFP::URA3 rfa4-40::KanMX | Stirling et al., 2012 |
| PSY1120     | MATa can1Δ::MFA1pr-HIS3::LEU2 lyp1Δ LYS2 his3Δ1 sld3-ts::URA3 RAD52-YFP::KanMX | Stirling et al., 2012 |
| GL2         | BY4741, aad6Δ::URA3 | Lang and Murray, 2011 |
| GL15        | BY4741, bst1Δ::URA3 | Lang and Murray, 2011 |
| GL21        | BY4741, hxt10Δ::URA3 | Lang and Murray, 2011 |
| PSY1157     | BY4741, aad6Δ::URA3, pol1-ts::KanMX | This study |
| PSY1151     | BY4741, bst1Δ::URA3, pol1-ts::KanMX | This study |
| PSY1153     | BY4741, hxt10Δ::URA3, pol1-ts::KanMX | This study |
| PSY1167     | BY4741, aad6Δ::URA3, stn1-13::KanMX | This study |
| PSY1168     | BY4741, bst1Δ::URA3, stn1-13::KanMX | This study |
| PSY1163     | BY4741, hxt10Δ::URA3, stn1-13::KanMX | This study |
| PSY1655     | BY4741, aad6Δ::URA3, rad52Δ::KanMX | This study |
| PSY1656     | BY4741, bst1Δ::URA3, rad52Δ::KanMX | This study |
| PSY1658     | BY4741, hxt10Δ::URA3, rad52Δ::KanMX | This study |

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