Supplementary Information

Cadmium is a mutagen that acts by inhibiting mismatch repair

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Supplementary Fig. 1

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Supplementary Fig. 2

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Supplementary Table 1

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Supplementary Table 2

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Supplementary Table 3

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Supplementary Table 4

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Supplementary Methods

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Supplementary Note

Supplementary Note A.

MATERIALS AND METHODS

Yeast strains. Most of the yeast strains were isogenic with CG379[1] (Note CG379 the same as AMY125 in[2]). Each strain was *MATα ade5-1 his7-2 leu2-3,112 trp1-289 ura3-52*. The *his7-2* is a –1 frameshift mutation creating a run of 7 adenines, which reverts primarily by +1 changes[3]. The strains also carried mutant *lys2* alleles which were used as mutation or recombination reporters: (i) frameshift reporters -- long homonucleotide runs described in[4] -- *lys2-A*$_{14}$ (A14 run, reverts via –1), *lys2-A*$_{12}$ (A12 run, reverts via +1), *lys2-A*$_{10}$ (A10 run, reverts via –1); (ii) interchromosomal recombination strains ALE100 and ALE101 and intrachromosomal ALE1000 and ALE1001 recombination strains are described in[5]. Strains carried 5'-truncated *lys2* sequence and the *LEU2* gene that were integrated into the same chromosome II as a direct repeat with the *lys2::HS-D* allele (intrachromosomal reporter) or into the chromosome III next to natural *LEU2* gene (interchromosomal reporter). Four isogenic strains that differed in genetic background from CG379 were obtained from Dr. Sue Jinks-Robertson (SJR-strains in Fig. 2 and Supplementary Table 2). Each of these strains carried a homonucleotide run of 10 nucleotides, A, T, G or C, at the same position in the *LYS2* gene[6]. The Pol δ proofreading-deficient mutant strains (*pol3-01*) have been described[7,8]. Proofreading-deficient Pol ε (*pol2-4*) strains in our collection appeared to be slightly more resistant to cadmium compared with all other isogenic strains. Therefore, we obtained fresh *pol2-4* isolates using the replacing construct described in[1]. Fresh *pol2-4* isolates were indistinguishable from all other isogenic strains, therefore we explain increased resistance phenomenon by acquiring a secondary mutation. Deletion-replacement isolates of

MMR-genes and other genes were created by transferring G418-resistance cassette (G418-R) from strains that were included in the complete set of haploid deletion strains purchased from ResGen Invitrogen Corporation (http://www.resgen.com/products/YEASTD.php3). We used the deletion set based on BY4741 (*MATa his3-D1 leu2-D0 met15-D0 ura3-D0*). G418-R was amplified from genomic DNA of corresponding deletion strain with PCR primers homologous to intergenic regions 100-300 nt upstream and downstream the replaced ORF. PCR products carrying G418-R flanked by 100-300 regions surrounding the targeted ORF were used to transform wild type yeast strains to G418-resistance. The transformants arose primarily by homologous recombination replacing, the targeted ORF with the G418-R. The resulting deletions were verified by PCR and by phenotype. The sequences of the PCR primers are available upon request. Two independent isolates of each mutant were used in the study. Isogenic diploids were obtained by transforming haploid yeast strains with the plasmid YEpHO (carrying *LEU2* and HO-endonuclease). HO-endonuclease caused mating type switching within the cell population followed by mating and diploid formation.

A spot-test for mutagenesis in yeast A14 homonucleotide run (*lys2-A14*) was used to detect strong mutagenic effects. Approximately 10^8 yeast cells were plated onto 100 mm plates with synthetic complete (SC) or, in the case of exposure to $FeCl_3$, with YPD medium. A 24 mm filter paper circle was placed at the center and 200 µl of the test-solution was applied to the filter. Plates were incubated at 30°C for 3-4 days. The concentration of the agent that caused growth inhibition in the vicinity (5-10 mm) of the

filter circle, but allowed normal growth at larger distance was chosen for mutagenesis testing. (Note: in the case of 500 mM solutions of $MnCl_2$ and $ZnSO_4$, growth inhibition was barely detectable only in the close vicinity of the disk). These plates were replica plated to media without lysine. After 4-5 days plates with selective media were scored and responses were documented by scanning and storing photo-images. The appearance of increased density of revertant colonies compared with control was indicative of mutagenesis. Spot-tests were repeated 3-4 times for each chemical.

References for Supplementary Note A.

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Supplementary Note B.

STUDIES WITH HUMAN CELLS

Since MMR is conserved in eukaryotes, it is possible that chronic exposure to low, biologically relevant levels of cadmium may also inhibit MMR in human cells. For example, low levels of cadmium (0.2 - 2 μM) were recently reported to increase carcinogenic transformation frequency of a mouse embryonic cell line by non-lethal doses of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)[1]. Previously it was shown that mammalian cells lacking MMR are able to tolerate MNNG and other alkylating agents by avoiding "futile cycles of repair" or by preventing apoptosis. As a result MNNG-induced mutagenesis in MMR deficient cells is greatly increased (reviewed in [2]). We suggest that this might be due to inhibition of MMR by cadmium, thereby increasing both MNNG tolerance and mutagenesis, which would increase the likelihood of mutations and cell transformation. In order to evaluate possible effects of cadmium on MMR in human cells we examined its impact on microsatellite stability in two MMR-proficient human cells lines exposed to sub-lethal concentrations of 0.2 and 1 μM cadmium for 3 weeks (approximately 25 doublings). To detect short deletion/insertion events, variations in the size of mononucleotide repeat BAT26 were evaluated by small-pool PCR using an approach similar to that described by[3]. Cadmium exposure resulted in a small, but statistically significant increase in the frequency of mutant alleles (data not shown). However, we did not detect any changes in the size of BAT26 using standard, large-pool PCR of DNAs from clones derived from subsets of the cell populations treated with 1 μM of cadmium (data not shown). The absence of a strong increase, as found for yeast, may reflect biological features of the cell lines used. The difference in results obtained with

the two techniques might be due to a higher sensitivity of small-pool PCR in detecting microsatellite changes in the fraction of cells with inhibited MMR, especially since the increase found with small-pool PCR is small. We suggest that more extensive studies with several cell lines will be required to determine the effect of cadmium on microsatellite instability in human cells.

References for Supplementary Note B.

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Supplementary Figure 1

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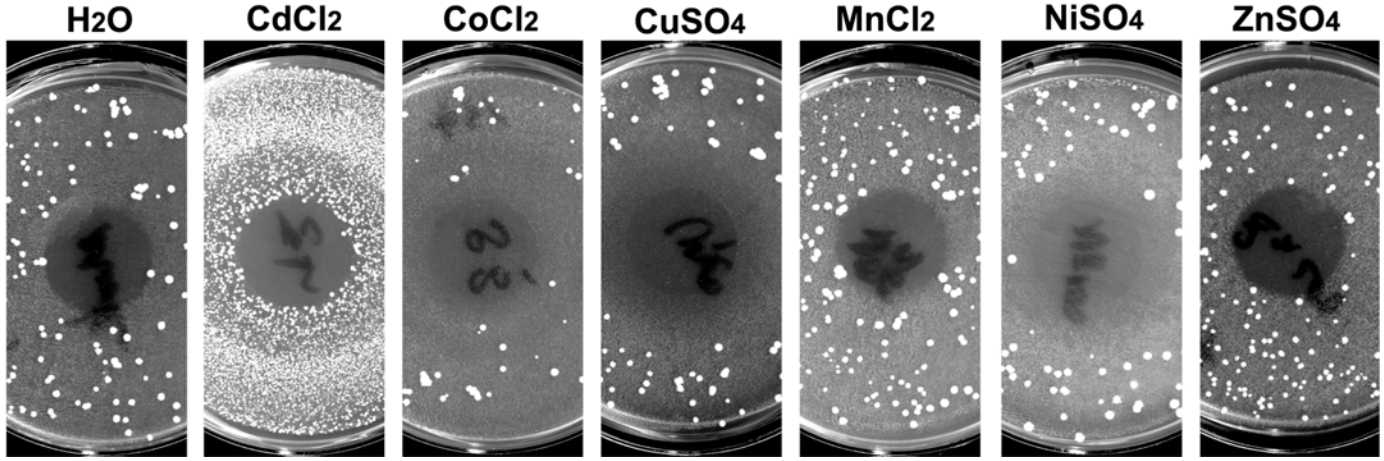

Spot-test of mutagenesis caused by divalent metal ions in a yeast A14 homonucleotide run allele *lys2-A_{14}*. Spot-tests were performed as described in Supplementary Note A. 200 μl of a solution was added onto the circular filter at the center of the plate. Salt concentrations were chosen that caused growth inhibition in the vicinity of the filter: $CdCl_2$ - 12.5 mM, $CoCl_2$ – 250 mM; $CuSO_4$ – 375 mM, $MnCl_2$ – 500 mM; $NiSO_4$ – 500 mM; $ZnSO_4$ – 500 mM. Each revertant colony corresponds to a spontaneous or induced mutant.

Supplementary Figure 2

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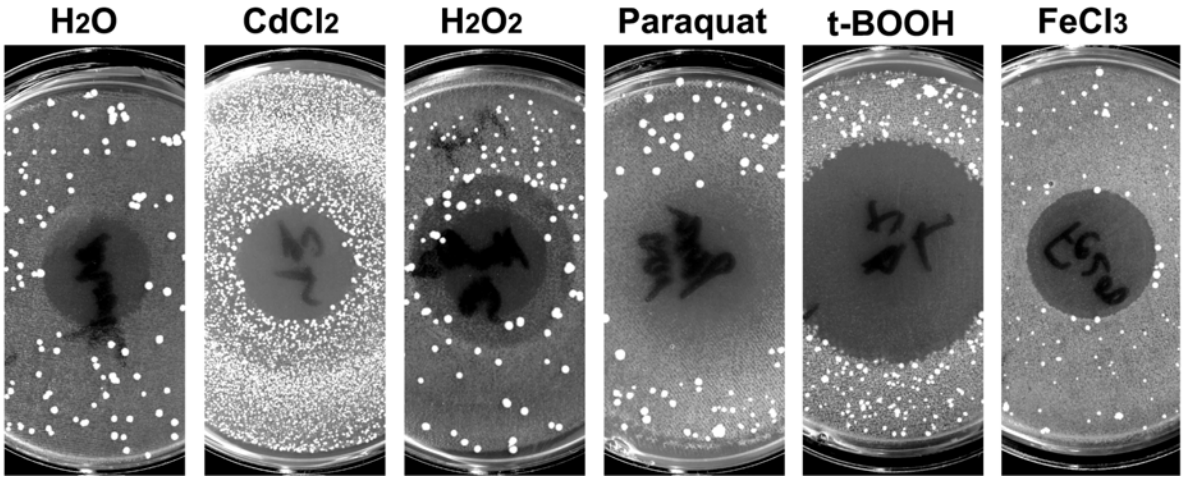

Spot-tests of mutagenesis in *lys2-A$_{14}$* microsatellite by agents causing oxidative damage. Spot-tests were performed as described in Supplementary Note A. 200 µl of the following solutions were applied: $CdCl_2$ – 12.5 mM; H_2O_2 – 4.4 M; paraquat – 500 mM; t-BOOH (tert-butyl hydroperoxide) – 200 mM; $FeCl_3$ – 500mM. (Note: negative control (H_2O) and positive control ($CdCl_2$) are represented by images of the same plates as on Supplementary Fig. 1.

Supplementary Table 1. Effects of chronic exposure of yeast to $CdCl_2$ on the mutability of specific reporters, viability and formation of petite mutants.

Strain	Reporter	$CdCl_2$ (μM)	Mutation in the reporters					Petite mutants (%)		Viability (%)
			Fold increase over untreated	Rate (median)	95% CI for rate	Frequency (median)	95% CI for frequency	Median	95% CI	
WT, A14 (E134)	lys2-A_{14}	0	1.0	3.6E-07	2.8E-07 - 6.1E-07	1.2E-06	7.9E-07 - 2.5E-06	2.8	2.4 - 4.3	100
		0.5	11	3.9E-06	1.8E-06 - 9.2E-06	2.4E-05	7.7E-06 - 6.4E-05	2.3	1.5 - 3.3	87
		1	84	3.0E-05	2.3E-05 - 4.0E-05	2.3E-04	1.7E-04 - 3.4E-04	2.6	1.9 - 4.4	90
		3	1094	3.9E-04	3.2E-04 - 5.2E-04	4.0E-03	3.3E-03 - 5.2E-03	85	79 - 91	78
		5	2186	7.8E-04	6.2E-04 - 9.1E-04	7.9E-03	6.1E-03 - 9.1E-03	92	83 - 93	65
	his7-2	0	1.0	1.2E-08	3.9E-09 - 2.8E-08	1.2E-08	3.9E-09 - 2.8E-08	N/A		
		0.5	2.7	3.2E-08	1.9E-08 - 5.1E-08	1.3E-08	3.2E-08 - 1.3E-07			
		1	10	1.2E-07	9.8E-08 - 1.5E-07	2.2E-08	3.8E-08 - 4.7E-07			
		3	17	2.0E-07	1.2E-07 - 2.8E-07	5.1E-07	2.4E-07 - 8.0E-07			
		5	48	5.7E-07	2.5E-07 - 1.4E-06	1.6E-06	5.6E-07 - 4.5E-06			
	CAN1	0	1.0	3.8E-07	3.5E-07 - 1.9E-06	1.0E-06	8.5E-07 - 7.7E-06			
		0.5	1.1	4.2E-07	2.6E-07 - 5.6E-07	1.1E-06	5.7E-07 - 1.7E-06			
		1	1.4	5.5E-07	2.5E-07 - 8.4E-07	1.3E-06	5.5E-07 - 2.5E-06			
		3	4.5	1.7E-06	8.3E-07 - 2.4E-06	6.1E-06	2.4E-06 - 9.1E-06			
		5	60	2.3E-05	2.4E-06 - 6.1E-05	9.7E-05	7.6E-06 - 2.5E-04			

Strain	Reporter	$CdCl_2$ (μM)	Mutation in the reporters					Petite mutants (%)		Viability (%)
			Fold increase over untreated	Rate (median)	95% CI for rate	Frequency (median)	95% CI for frequency	Median	95% CI	
WT, A12 (E133)	lys2-A_{12}	0	1.0	1.5E-07	9.2E-08 - 1.3E-06	4.2E-07	2.3E-07 - 6.8E-06	3.3	1.9 - 5.2	100
		0.5	0.94	1.4E-07	7.5E-08 - 2.2E-07	3.8E-07	1.8E-07 - 7.4E-07	4.2	2.1 - 4.5	108
		1	4.3	6.4E-07	1.4E-07 - 1.6E-06	2.7E-06	3.9E-07 - 8.0E-06	3.3	2.9 - 6.6	108
		3	90	1.3E-05	8.9E-06 - 3.1E-05	9.3E-05	6.1E-05 - 2.4E-04	86	79 - 94	77
		5	196	2.9E-05	1.9E-05 - 3.4E-05	2.1E-04	1.3E-04 - 2.4E-04	91	83 - 98	65
	his7-2	0	1.0	2.8E-09	8.5E-11 - 1.6E-08	2.8E-09	8.5E-11 - 1.6E-08			
		0.5	14	4.1E-08	2.2E-08 - 6.8E-08	1.7E-08	1.5E-08 - 1.6E-07			
		1	23	6.6E-08	3.7E-08 - 7.9E-08	1.3E-07	5.7E-08 - 1.8E-07			
		3	78	2.2E-07	1.3E-07 - 6.1E-07	6.1E-07	3.2E-07 - 2.3E-06			
		5	163	4.6E-07	2.6E-07 - 1.1E-06	1.3E-06	5.4E-07 - 4.5E-06			
								N/A		
	CAN1	0	1.0	2.7E-07	1.4E-07 - 4.6E-07	9.4E-07	4.0E-07 - 1.8E-06			
		0.5	0.72	2.0E-07	1.3E-07 - 2.7E-07	6.2E-07	3.7E-07 - 8.6E-07			
		1	1.8	5.1E-07	2.6E-07 - 1.1E-06	2.0E-06	8.6E-07 - 5.5E-06			
		3	8.1	2.2E-06	1.2E-06 - 4.3E-06	1.1E-05	5.8E-06 - 2.5E-05			
		5	65	1.8E-05	5.4E-06 - 4.3E-05	1.2E-04	3.1E-05 - 3.1E-04			
WT, A10 (E26)	lys2-A_{10}	0	1.0	8.2E-08	4.8E-08 - 1.0E-07	2.1E-07	1.1E-07 - 2.7E-07	3.0	1.5 - 4.6	100
		0.5	3.7	3.1E-07	2.4E-07 - 4.3E-07	1.1E-06	7.6E-07 - 1.6E-06	3.1	1.3 - 10	76
		1	89	7.3E-06	5.5E-06 - 8.5E-06	4.9E-05	3.6E-05 - 5.9E-05	2.6	1.5 - 4.9	79
		3	616	5.0E-05	4.3E-05 - 8.0E-05	4.1E-04	3.4E-04 - 7.0E-04	87	85 - 93	79
		5	1637	1.3E-04	9.8E-05 - 1.8E-04	1.1E-03	7.9E-04 - 1.6E-03	94	88 - 100	75
	his7-2	0	1.0	1.8E-08	8.6E-09 - 3.3E-08	1.8E-08	8.6E-09 - 3.3E-08			
		0.5	5.1	9.2E-08	6.3E-08 - 1.3E-07	9.2E-08	6.3E-08 - 1.3E-07			
		1	3.7	6.7E-08	3.7E-08 - 1.1E-07	1.4E-07	4.9E-08 - 2.6E-07			
		3	9.1	1.6E-07	7.4E-08 - 5.4E-07	4.1E-07	1.3E-07 - 1.9E-06			
		5	23	4.2E-07	3.4E-07 - 6.5E-07	1.0E-06	7.4E-07 - 1.9E-06			
								N/A		
	CAN1	0	1.0	2.6E-07	2.3E-07 - 4.0E-07	1.0E-06	7.8E-07 - 1.6E-06			
		0.5	1.6	4.1E-07	2.6E-07 - 1.0E-06	1.5E-06	8.3E-07 - 4.7E-06			
		1	1.5	3.9E-07	2.0E-07 - 5.2E-07	1.4E-06	6.7E-07 - 2.0E-06			
		3	5.7	1.5E-06	1.1E-06 - 5.7E-06	7.0E-06	4.9E-06 - 3.4E-05			

Strain	Reporter	$CdCl_2$ (μM)	Mutation in the reporters					Petite mutants (%)		Viability (%)
			Fold increase over untreated	Rate (median)	95% CI for rate	Frequency (median)	95% CI for frequency	Median	95% CI	
WT-SJR, 10G (SJR938)	lys2-10G	0	1.0	4.6E-07	4.2E-07 - 6.6E-07	1.8E-06	1.7E-06 - 2.6E-06	1.7	1.0 - 2.3	100
		0.5	2.8	1.3E-06	5.0E-07 - 4.4E-06	6.3E-06	2.1E-06 - 2.7E-05	1.8	0.41 - 2.3	123
		1	7.9	3.6E-06	2.7E-06 - 5.6E-06	2.1E-05	1.5E-05 - 3.5E-05	1.6	1.0 - 3.1	137
		3	160	7.3E-05	5.5E-05 - 8.6E-05	6.4E-04	4.6E-04 - 7.3E-04	2.1	0.64 - 2.7	124
		5	326	1.5E-04	1.0E-04 - 1.8E-04	1.3E-03	8.6E-04 - 1.7E-03	4.0	2.2 - 6.5	105
		10	451	2.1E-04	1.7E-04 - 7.6E-04	1.8E-03	1.4E-03 - 7.1E-03	8.3	3.8 - 18	61
		20	934	4.3E-04	3.2E-04 - 6.2E-04	3.7E-03	2.7E-03 - 5.3E-03	28	19 - 33	27
WT-SJR, 10A (SJR939)	lys2-10A	0	1.0	7.0E-08	4.2E-08 - 1.1E-07	1.4E-07	5.4E-08 - 2.7E-07	1.1	0.8 - 1.9	100
		1	2.1	1.4E-07	8.0E-08 - 2.4E-07	1.4E-07	1.5E-07 - 3.3E-07	1.1	0.0 - 1.3	105
		1	3.3	2.3E-07	7.4E-08 - 4.4E-07	7.2E-07	1.3E-07 - 1.7E-06	1.0	0.42 - 2.6	112
		3	80	5.5E-06	4.3E-06 - 7.1E-06	3.3E-05	2.5E-05 - 4.6E-05	2.8	1.5 - 4.8	110
		5	141	9.8E-06	7.8E-06 - 1.3E-05	7.2E-05	5.1E-05 - 8.2E-05	3.7	1.9 - 6.3	95
		10	398	2.8E-05	1.6E-05 - 3.6E-05	1.8E-04	9.0E-05 - 2.5E-04	12	2.6 - 18	73
		20	856	6.0E-05	4.5E-05 - 6.1E-05	4.0E-04	3.1E-04 - 4.3E-04	22	16 - 40	85
WT-SJR, 10T (SJR980)	lys2-10T	0	1.0	3.9E-07	3.6E-07 - 6.7E-07	1.3E-06	1.1E-06 - 2.9E-06	1.5	0.0 - 1.7	100
		0.5	1.7	6.6E-07	2.8E-07 - 7.2E-07	2.6E-06	9.7E-07 - 2.7E-06	0.67	0.0 - 1.8	86
		1	2.0	7.9E-07	4.5E-07 - 1.3E-06	3.2E-06	1.5E-06 - 5.3E-06	0.38	0.0 - 2.2	96
		3	29	1.1E-05	8.0E-06 - 1.5E-05	7.4E-05	4.9E-05 - 1.0E-04	1.6	0.0 - 3.4	90
		5	82	3.2E-05	2.8E-05 - 3.6E-05	2.3E-04	1.9E-04 - 2.8E-04	2.7	0.9 - 3.9	79
		10	162	6.3E-05	4.2E-05 - 1.3E-04	4.7E-04	3.0E-04 - 1.0E-03	9.8	5.4 - 14	78
		20	262	1.0E-04	7.9E-05 - 2.1E-04	7.5E-04	6.0E-04 - 1.8E-03	28	16 - 39	64
WT-SJR, 10C (SJR981)	lys2-10C	0	1.0	3.3E-07	2.1E-07 - 4.8E-07	1.1E-06	5.0E-07 - 1.7E-06	2.5	1.3 - 2.9	100
		0.5	2.4	7.9E-07	3.1E-07 - 9.3E-07	3.4E-06	8.8E-07 - 3.9E-06	1.1	0.0 - 3.1	103
		1	3.0	1.0E-06	4.7E-07 - 1.3E-06	4.5E-06	1.7E-06 - 5.6E-06	1.7	0.47 - 2.3	103
		3	77	2.6E-05	1.8E-05 - 3.2E-05	1.9E-04	1.3E-04 - 2.5E-04	2.2	0.80 - 3.9	98
		5	229	7.6E-05	4.3E-05 - 1.5E-04	6.1E-04	3.4E-04 - 1.2E-03	3.8	1.9 - 12	100
		10	676	2.3E-04	1.6E-04 - 6.0E-04	1.9E-03	1.2E-03 - 5.3E-03	14	12.0 - 17	90
		20	1128	3.8E-04	2.4E-04 - 4.5E-04	3.3E-03	2.0E-03 - 3.9E-03	22	17 - 35	24

Strain	Reporter	$CdCl_2$ (μM)	Mutation in the reporters					Petite mutants (%)		Viability (%)
			Fold increase over untreated	Rate (median)	95% CI for rate	Frequency (median)	95% CI for frequency	Median	95% CI	
msh2, A14 (YH1026 & YH1027)	lys2-A_{14}	0	1.0	1.4E-03	9.5E-04 - 1.7E-03	1.2E-02	7.8E-03 - 1.6E-02	6.1	3.9 - 8.1	100
		0.5	0.9	1.2E-03	9.8E-04 - 1.4E-03	1.1E-02	7.4E-03 - 1.3E-02	7.0	3.4 - 15	77
		1	1.1	1.4E-03	9.9E-04 - 2.6E-03	1.2E-02	7.8E-03 - 2.6E-02	11	7.0 - 21	87
		3	1.2	1.6E-03	8.9E-04 - 2.7E-03	1.2E-02	7.8E-03 - 2.1E-02	99	95 - 100	73
		5	1.1	1.4E-03	1.1E-03 - 2.0E-03	1.1E-02	7.1E-03 - 1.5E-02	85	41 - 100	67
	his7-2	0	1.0	3.3E-06	1.4E-06 - 7.1E-06	1.6E-05	5.8E-06 - 3.6E-05			
		0.5	2.0	6.7E-06	3.2E-06 - 1.1E-05	3.6E-05	1.5E-05 - 6.2E-05			
		1	1.8	5.9E-06	7.0E-07 - 1.4E-05	3.0E-05	2.0E-06 - 8.1E-05			
		3	1.5	4.8E-06	7.8E-07 - 8.6E-06	2.1E-05	2.2E-06 - 4.1E-05			
		5	2.3	7.5E-06	3.6E-06 - 1.1E-05	3.3E-05	1.4E-05 - 5.3E-05			
								N/A		
	CAN1	0	1.0	1.9E-05	4.8E-06 - 1.1E-04	1.2E-04	2.5E-05 - 9.4E-04			
		0.5	0.33	6.2E-06	4.3E-06 - 8.9E-06	3.3E-05	2.1E-05 - 5.0E-05			
		1	0.57	1.1E-05	6.4E-06 - 3.4E-04	5.6E-05	3.5E-05 - 3.1E-03			
		3	0.64	1.2E-05	8.2E-06 - 8.2E-05	6.2E-05	3.8E-05 - 5.8E-04			
		5	0.57	1.1E-05	3.0E-06 - 2.0E-04	5.1E-05	1.2E-05 - 1.4E-03			
msh3, A14 (YH1028 & YH1029)	lys2-A_{14}	0	1.0	2.4E-06	1.3E-06 - 4.2E-06	1.3E-05	6.8E-06 - 2.5E-05	2.1	0.46 - 2.7	100
		0.5	3.0	7.2E-06	3.8E-06 - 1.3E-05	4.5E-05	2.2E-05 - 9.1E-05	2.2	1.5 - 4.8	107
		1	25	6.0E-05	5.0E-05 - 7.8E-05	4.8E-04	4.0E-04 - 6.4E-04	3.7	0.84 - 6.3	105
		3	233	5.5E-04	4.4E-04 - 8.0E-04	5.5E-03	4.4E-03 - 8.4E-03	88	80 - 92	92
		5	515	1.2E-03	1.0E-03 - 1.8E-03	1.2E-02	1.0E-02 - 1.8E-02	93	84 - 100	96
	his7-2	0	1.0	9.0E-08	4.6E-08 - 7.2E-07	2.0E-07	6.6E-08 - 3.2E-06			
		0.5	0.94	8.5E-08	4.3E-08 - 1.5E-07	1.7E-07	6.2E-08 - 3.6E-07			
		1	1.3	1.2E-07	8.1E-08 - 1.8E-07	2.2E-07	1.3E-07 - 3.8E-07			
		3	3.3	2.9E-07	1.4E-07 - 5.4E-07	7.2E-07	2.5E-07 - 1.7E-06			
		5	6.5	5.8E-07	3.2E-07 - 2.4E-06	1.3E-06	5.3E-07 - 9.0E-06			
								N/A		
	CAN1	0	1.0	7.6E-07	4.6E-07 - 1.9E-06	3.3E-06	1.7E-06 - 1.1E-05			
		0.5	0.67	5.1E-07	4.1E-07 - 6.1E-07	1.9E-06	1.5E-06 - 2.5E-06			
		1	1.1	8.3E-07	6.1E-07 - 2.9E-06	3.1E-06	2.5E-06 - 1.5E-05			
		3	4.4	3.4E-06	2.2E-06 - 1.4E-04	1.6E-05	1.0E-05 - 1.2E-03			
		5	65	4.9E-05	1.1E-05 - 2.6E-04	3.2E-04	6.0E-05 - 2.2E-03			

Strain	Reporter	$CdCl_2$ (μM)	Mutation in the reporters					Petite mutants (%)		Viability (%)
			Fold increase over untreated	Rate (median)	95% CI for rate	Frequency (median)	95% CI for frequency	Median	95% CI	
msh6, A14 (YH1030 & YH1031)	lys2-A_{14}	0	1.0	6.5E-05	6.1E-05 - 9.6E-05	5.0E-04	4.6E-04 - 7.5E-04	1.6	0.30 - 2.2	100
		0.5	1.3	8.2E-05	5.8E-05 - 2.5E-04	6.5E-04	4.4E-04 - 2.3E-03	1.3	0.39 - 4.0	101
		1	3.2	2.1E-04	9.7E-05 - 1.7E-03	1.8E-03	8.0E-04 - 1.9E-02	2.7	0.30 - 4.6	95
		3	11	6.8E-04	3.8E-04 - 8.8E-04	6.5E-03	3.4E-03 - 8.8E-03	73	68 - 79	89
		5	12	7.9E-04	6.1E-04 - 1.1E-03	7.2E-03	5.4E-03 - 1.1E-02	96	92 - 98	85
	his7-2	0	1.0	1.6E-07	1.2E-07 - 3.8E-07	2.7E-07	1.6E-07 - 1.0E-06	N/A		
		0.5	1.5	2.4E-07	1.0E-07 - 4.6E-07	5.3E-07	9.7E-08 - 1.3E-06			
		1	0.7	1.1E-07	5.0E-08 - 8.2E-07	1.4E-07	3.3E-08 - 2.5E-06			
		3	1.8	2.9E-07	1.8E-07 - 3.8E-07	5.5E-07	2.4E-07 - 8.3E-07			
		5	3.8	6.0E-07	2.9E-07 - 2.2E-06	1.2E-06	4.0E-07 - 6.5E-06			
	CAN1	0	1.0	3.7E-06	3.3E-06 - 4.2E-06	1.8E-05	1.5E-05 - 2.0E-05			
		0.5	1.0	3.7E-06	2.9E-06 - 1.6E-05	1.8E-05	1.4E-05 - 9.7E-05			
		1	1.4	5.1E-06	2.5E-06 - 6.0E-05	2.6E-05	1.2E-05 - 4.6E-04			
		3	1.2	4.5E-06	2.5E-06 - 6.8E-06	2.1E-05	1.0E-05 - 3.3E-05			
		5	6.2	2.3E-05	4.8E-06 - 6.7E-05	2.3E-05	4.8E-06 - 6.7E-05			
2n, pol3-01, A14 (YH984 & YH986)	lys2-A_{14}	0	1.0	6.0E-05	8.3E-06 - 2.2E-04	4.0E-04	3.1E-05 - 1.6E-03	0.0	0.0 - 2.5	100
		0.5	4.6	2.8E-04	3.6E-05 - 8.8E-04	2.1E-03	2.2E-04 - 7.5E-03	2.3	0.0 - 17	95
		1	34	2.0E-03	1.4E-03 - 2.3E-03	1.8E-02	1.3E-02 - 2.1E-02	21	0.0 - 29	95
		3	53	3.2E-03	2.3E-03 - 4.4E-03	2.8E-02	2.1E-02 - 3.7E-02	31	7.0 - 41	88
		5	70	4.2E-03	2.4E-03 - 1.0E-02	3.6E-02	2.1E-02 - 9.2E-02	42	31 - 72	80
	his7-2	0	1.0	1.7E-05	9.5E-06 - 5.7E-05	9.2E-05	4.4E-05 - 3.6E-04	N/A		
		1	2.6	4.5E-05	1.1E-05 - 1.2E-04	2.4E-04	4.6E-05 - 6.8E-04			
		1	8.7	1.5E-04	1.2E-04 - 2.2E-04	9.7E-04	7.1E-04 - 1.4E-03			
		3	8.0	1.4E-04	6.0E-05 - 1.9E-04	7.6E-04	2.4E-04 - 1.1E-03			
		5	14	2.4E-04	8.9E-05 - 3.0E-04	1.3E-03	4.8E-04 - 2.0E-03			

Strain	Reporter	$CdCl_2$ (μM)	Mutation in the reporters					Petite mutants (%)		Viability (%)
			Fold increase over untreated	Rate (median)	95% CI for rate	Frequency (median)	95% CI for frequency	Median	95% CI	
2n, WT, A14 (YH990 & YH991)	lys2-A_{14}	0	1.0	3.6E-07	2.1E-07 - 6.9E-07	1.2E-06	6.1E-07 - 2.7E-06	0.0	0.0 - 1.1	100
		0.5	6	2.3E-06	1.3E-06 - 6.2E-06	1.2E-05	6.0E-06 -3.8E-05	0.0	0.0 - 1.1	98
		1	78	2.8E-05	1.4E-05 - 6.4E-05	2.2E-04	1.1E-04 - 5.6E-04	0.53	0.50 - 0.95	93
		3	1450	5.2E-04	3.9E-04 - 5.8E-04	5.4E-03	4.1E-03 - 6.2E-03	18	11.0 - 21	87
		5	2104	7.5E-04	6.1E-04 - 9.5E-04	7.9E-03	6.3E-03 - 1.0E-02	91	89 - 94	73
	his7-2	0	1.0	3.6E-08	1.2E-08 - 1.5E-07	8.1E-08	1.2E-08 - 4.6E-07	N/A		
		0.5	2.2	7.9E-08	2.9E-08 - 2.5E-07	2.1E-07	5.8E-08 - 9.8E-07			
		1	3.5	1.2E-07	5.7E-08 - 2.5E-07	4.1E-07	1.4E-07 - 1.0E-06			
		3	33	1.2E-06	5.5E-07 - 2.0E-06	6.1E-06	2.5E-06 - 1.1E-05			
		5	24	8.6E-07	4.1E-07 - 2.5E-06	3.5E-06	1.5E-06 - 1.3E-05			
pol2-4, A14 (YH1052 & YH1053)	lys2-A_{14}	0	1.0	3.9E-07	3.2E-07 - 4.7E-07	1.5E-06	1.2E-06 - 1.9E-06	2.6	1.8 - 3.6	100
		0.5	49	1.9E-05	1.5E-05 - 2.6E-05	1.6E-04	1.1E-04 - 2.1E-04	2.0	1.4 - 3.5	92
		1	865	3.4E-04	1.9E-04 - 6.4E-04	3.6E-03	2.0E-03 - 7.3E-03	8.3	4.9 - 13	95
		3	2578	1.0E-03	7.6E-04 - 1.6E-03	1.1E-02	8.4E-03 - 1.9E-02	92	90 - 95	94
		5	4950	2.0E-03	1.2E-03 - 2.4E-03	2.3E-02	1.3E-02 - 2.9E-02	92	76 - 95	85
	his7-2	0	1	4.4E-08	2.7E-08 - 6.2E-08	1.1E-07	5.4E-08 - 1.7E-07	N/A		
		0.5	9	3.9E-07	1.1E-07 - 1.3E-06	1.2E-06	1.7E-07 - 5.2E-06			
		1	190	8.4E-06	1.8E-06 - 2.6E-05	5.2E-05	8.7E-06 - 1.9E-04			
		3	279	1.2E-05	8.1E-06 - 6.8E-05	7.2E-05	4.6E-05 - 5.2E-04			
		5	230	1.0E-05	7.2E-06 - 3.3E-05	5.4E-05	3.8E-05 - 2.1E-04			
	CAN1	0	1.0	1.3E-06	8.6E-07 - 3.8E-06	5.9E-06	3.5E-06 - 2.1E-05			
		0.5	3.8	5.1E-06	3.3E-06 - 7.5E-05	2.8E-05	1.7E-05 - 6.3E-04			
		1	30	4.1E-05	1.8E-05 - 4.9E-05	3.1E-04	1.2E-04 - 3.8E-04			
		3	60	8.0E-05	2.0E-05 - 1.6E-04	6.2E-04	1.3E-04 - 1.3E-03			
		5	133	1.8E-04	1.5E-04 - 4.9E-04	1.5E-03	1.2E-03 - 4.5E-03			

Supplementary Table 2. Mutation rates in the yeast *lys2-A$_{14}$* microsatellite during chronic exposure to hydrogen peroxide and paraquat.

		Mutation in lys2-A$_{14}$					Petite mutants (%)		
Agent	Concentration (mM)	fold increase over untreated	Rate (median)	95% CI for rate	Frequency (median)	95% CI for frequency	Median	95% CI	Viability (%)
Control	0	1.0	3.1E-07	2.2E-07 - 4.3E-07	8.0E-07	5.6E-07 - 1.3E-06	4.2	2.3 - 6.0	100
H_2O_2	0.5	1.3	4.1E-07	2.3E-07 - 7.7E-07	1.1E-06	5.2E-07 - 2.7E-06	16	6.3 - 23	73
	1	1.2	3.6E-07	2.6E-07 - 1.2E-06	1.0E-06	6.5E-07 - 4.9E-06	4.3	2.5 - 5.4	10
	1.5	0.9	2.8E-07	1.8E-07 - 2.7E-06	7.5E-07	4.5E-07 - 1.3E-05	3.4	2.3 - 4.6	1.1
Paraquat	0.05	1.0	3.0E-07	1.6E-07 - 4.2E-07	8.6E-07	3.5E-07 - 1.2E-06	0.87	0.0 - 3.4	108
	0.1	0.6	1.9E-07	1.2E-07 - 2.8E-07	3.9E-07	3.1E-07 - 7.4E-07	1.8	0.0 - 3.2	105
	0.5	1.2	3.8E-07	2.1E-07 - 1.4E-06	9.8E-07	5.5E-07 - 5.2E-06	69	47.1 - 88	100 very slow growth

Colonies were growing significantly slower in the presence of 0.5 mM of paraquat (not shown), while less than 1% cells formed colonies on the media with 1mM of paraquat, indicating biological effect in the tested range of concentrations.

Supplementary Table 3. Effects of chronic exposure of yeast to $CdCl_2$ on recombination in yeast.

Strain	$CdCl_2$ (μM)	Recombination					Petite mutants (%)		Viability (%)
		Fold increase over untreated	Rate (median)	95% CI for rate	Frequency (median)	95% CI for frequency	Median	95% CI	
Interchromosomal recombination (ALE100 & ALE101)	0	1.0	1.0E-07	7.5E-08 - 1.4E-07	3.1E-07	2.1E-07 - 4.2E-07	2.9	2.1 - 5.2	100
	0.5	1.3	1.3E-07	7.5E-08 - 1.8E-07	3.5E-07	1.9E-07 - 5.3E-07	2.4	1.7 - 4.9	100
	1	1.4	1.5E-07	9.6E-08 - 3.7E-07	4.1E-07	2.4E-07 - 1.3E-06	4.3	2.8 - 4.9	103
	3	2.8	2.9E-07	1.9E-07 - 2.3E-06	9.0E-07	5.5E-07 - 1.1E-05	94	75 - 97	73
	5	3.6	3.8E-07	2.6E-07 - 9.2E-06	1.0E-06	6.6E-07 - 5.6E-05	94	88 - 96	73
Intrachromosomal recombination (ALE1000 & ALE1001)	0	1.0	8.4E-06	4.4E-06 - 1.7E-05	4.2E-05	2.1E-05 - 9.7E-05	ND		100
	0.5	1.0	8.0E-06	6.0E-06 - 1.4E-05	3.8E-05	2.7E-05 - 7.6E-05			96
	1	1.0	8.0E-06	5.3E-06 - 2.1E-05	3.8E-05	2.3E-05 - 1.2E-04			89
	3	1.0	8.7E-06	4.0E-06 - 1.7E-05	4.3E-05	1.8E-05 - 8.6E-05			86
	5	1.1	9.2E-06	4.0E-06 - 5.9E-05	3.3E-05	1.3E-05 - 1.9E-04			90

Supplementary Table 4 Spectrum of *can1* mutations.

	wild type				*pol2-4*			
	0 μM $CdCl_2$*		5 μM $CdCl_2$		0 μM $CdCl_2$		5 μM $CdCl_2$	
	mutation	occurrence	mutation	occurrence	mutation	occurrence	mutation	occurrence
Base substitution	C→A C→T C→G G→A G→T A→G T→A	4/20 2/20 1/20 4/20 3/20 1/20 1/20 total 16/20 (80%)	C→A G→T G→A	2/24 2/24 1/24 total 5/24 (21%)	C→G C→T G→A G→T A→T	1 1 2 6 4 total 14/18 (78%)	C→A C→T G→T G→A A→G	1 3 5 4 1 total 14/30 (47%)
Frameshift	T_4→T_3 T_3→T_2	1/20 1/20 total 2/20 (10%)	C_3→C_2 G_4→G_3 A_6→A_5 A_5→A_4 A_1→A_0 T_6→T_7 T_4→T_3	4 1 6 1 1 2 4 total 19/24 (79%)	A_4→A_3 T_6→T_7	1 2 total 3/18 (17%)	T_6→T_7 T_5→T_6 T_3→T_4 A_4→A_5 T_6→T_5 A_6→A_5 A_5→A_4 total 16/30 (53%)	5 2 1 2 2 3 1
Deletion‡‡	$\Delta_{1324-1339}$ (16, 8)	1/20 (5%)		0/24 (0%)	$\Delta_{276-302}$ (27, 8)	1 1/18 (0.06%)		0/30 (0%)
Complex	TTCTC →CTCT CTG	1/20 (5%)		0/24 (0%)		0/18 (0%)		0/30 (0%)

Nucleotide coordinates of the wild-type *CAN1* gene are used with the A of the ATG as nucleotide 1. Deletion mutations are designated by Δ_{x-y} (*a, b*), where *x* is the nucleotide coordinate of the first nucleotide of the wild-type sequence that is deleted, and *y* is the nucleotide coordinate of the last nucleotide that is deleted; *a* - length (nt) of the deletion; *b* - length (nt) of the associated short repeat sequences. Data for 0 μM of $CdCl_2$ are from Jin *et al*. *Proc. Natl. Acad. Sci. USA* 98, 5122-5127 (2001)