Figure S1

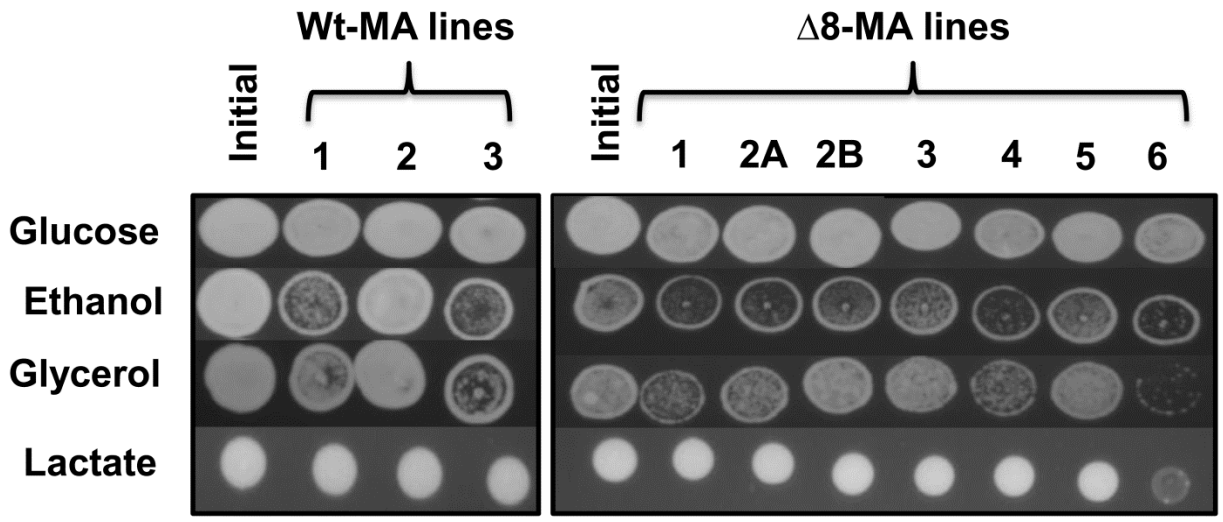

Figure S1. Spotting assay using respiratory substrates for initial and all MA lines. Wt and Δ8 strains were analyzed on plates containing 2% glucose (Glu), ethanol (EtOH), glycerol (Gly) and lactic acid (Lac), respectively, to analyze the function of mitochondria. Left panel shows the growth of Wt lines and right panel of Δ8 lines. All MA lines were able to utilize all respiratory substrates excluding the Δ8 MA line 6, which showed poor growth on glycerol and lactic acid plates.

Table S1. Yeast strains used in this study.

Strain	Designation of Wt and mutant cells	Genotype	Source
BY4741	Wt	MAT a *his3 leu2 met15 ura3*	ATCC
GY25	Δ3Gpx	MAT a *his3 leu2 met15 ura3* Δ*gpx1:URA3,* Δ*gpx2:HIS3,* Δ*gpx3: KAN*	Avery et al., 2001
GY14	Δ5Prx	MATa *his3 leu2 met15 ura3* Δ*tsa1:KAN*, Δ*tsa2:LEU2*, Δ*dot5:MET15*, Δ*ahp1:HIS3*, Δ*prx1:URA3*	Wong et al., 2004
GY150	Δ6 (all ΔPrx +ΔGpx1)	MATa *his3 leu2 met15 ura3* Δ*gpx3:KAN, URA3*, Δ*tsa1:KAN*, Δ*tsa2:LEU2*, Δ*dot5:MET15*, Δ*ahp1:HIS3*, Δ*prx1:URA3*	This study
GY29	Δ7 (all ΔPrx+2 ΔGpx1,3)	MATa *his3 leu2 met15 ura3* Δ*gpx1:URA3*, Δ*gpx3:KAN*, Δ*tsa1:KAN*, Δ*tsa2:LEU2*, Δ*dot5:MET15*, Δ*ahp1:HIS3*, Δ*prx1:URA3*	Fomenko et al., 2011
GY100	Δ8 (all ΔPrx+all ΔGpx)	MATa *his3 leu2 met15 ura3* Δ*tsa1:KAN*, Δ*tsa2:LEU2*,Δ*dot5:MET15*, Δ*ahp1:HIS3*, Δ*prx1:URA3*, Δ*gpx2:HYG*, Δ*gpx1:URA3*, *Δgpx3:KAN*	Fomenko et al., 2011
GY151	Δ8 (all ΔPrx+all ΔGpx)	MATa *his3 leu2 met15 ura3* Δ*tsa1:KAN*, Δ*tsa2:LEU2*,Δ*dot5:MET15*, Δ*ahp1:HIS3*, Δ*prx1:URA3*, Δ*gpx2:HYG*, Δ*gpx1:NAT, Δgpx3:KAN*	This study

Figure S2

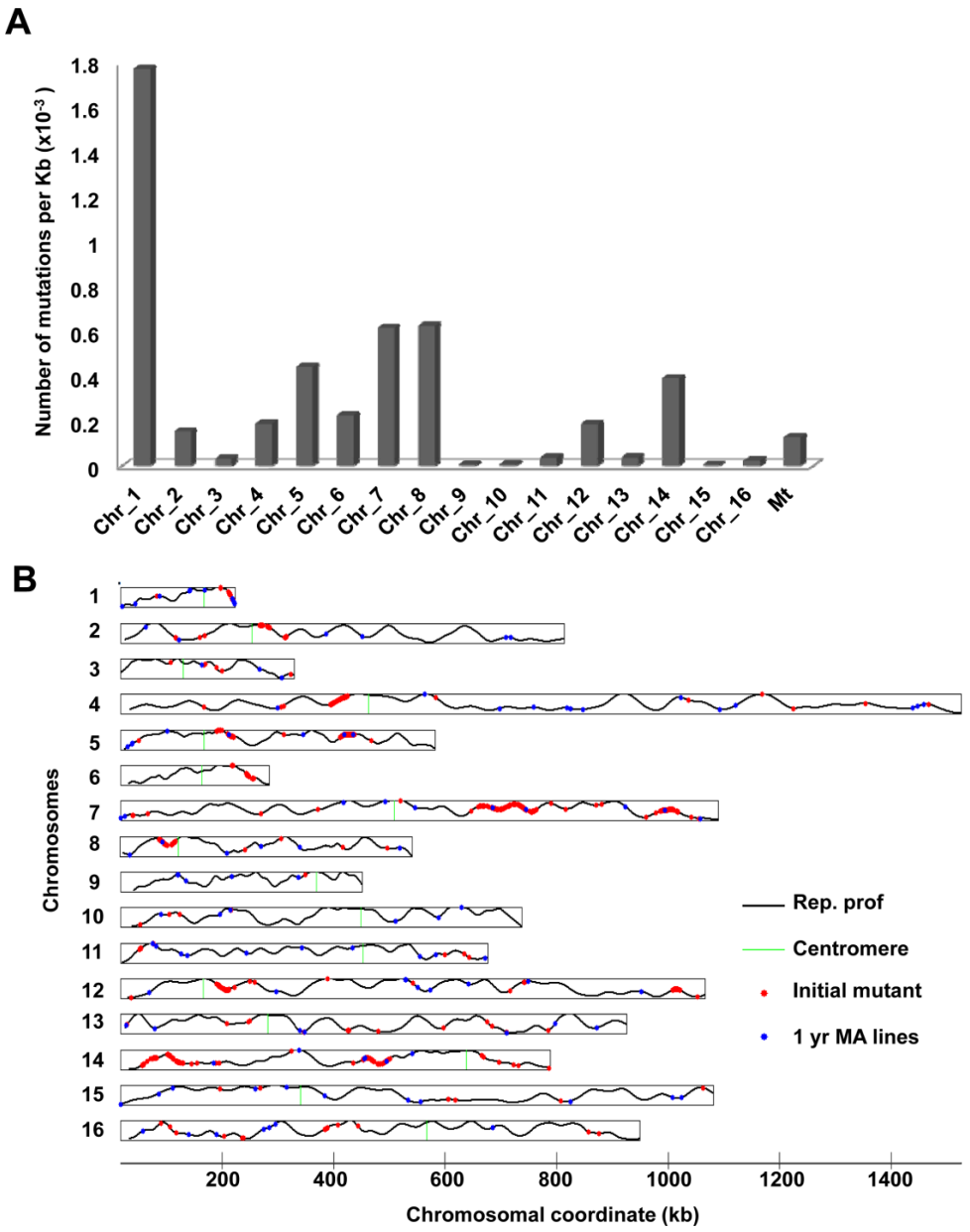

Figure S2. Chromosome distribution of mutations in the original Δ8 strain. A) Number of mutations was normalized according to chromosome length and plotted for each chromosome. **B**) Distribution of initial mutations (red dots) together with mutations identified after the long- term growth (blue dots) per chromosome shown with replication timing (Koren *et al*. 2010).

Table S2. Lifespan analysis of MA lines.

MA-line	MA-line Mean	MA-line N	Initial Mean	Initial N	% Change	Ranksum P-Value
1	4.4	20	9.2	20	52.2	0.011
2A	7	20	9.2	20	23.9	0.26
2B	6.9	20	9.2	20	25.0	0.23
3	6.3	20	9.2	20	31.5	0.14
4	4.3	20	9.2	20	53.3	0.011
5	6.4	20	9.2	20	30.4	0.13
6	6.4	20	9.2	20	30.4	0.13
Wt-1	15.8	20	20.7	20	23.7	0.11
Wt-2	16.6	20	20.7	20	19.8	0.13
Wt-3	19.8	20	20.7	20	4.6	0.79

Figure S3

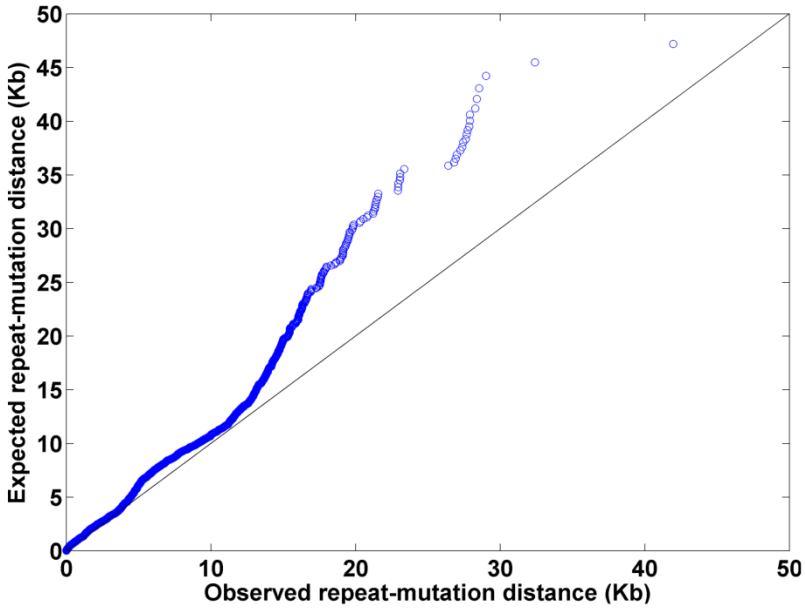

Figure S3. Observed versus expected distances between repeats and mutations in the initial Δ8 strain. Variable tandem repeats were extracted from Vinces *et al.* 2009 (intergenic repeats) and Verstrepen *et al.* 2005 (intragenic repeats) for our analysis. Blue circles are the observed versus expected distances between mutations in the initial strain and their nearest repeat. The expected distances between mutations and repeats were calculated by averaging over 100 randomized locations of mutations. The diagonal represents equal expected and observed distances. Only repeats of length of 5 bp or more and a repeat number of 3 or more were used for this analysis.

Table S3. Primers used for RNA-seq library preparation.

3‘ adapter	AppAGATCGGAAGAGCACACGTCT/3ddC/
RT-primer	pGATCGTCGGACTGTAGAACTCTGAACCTGTCGGTGGTCGCCGTATCATT/iSp18/GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
PCR forward	AATGATACGGCGACCACCGACAGGTTCAGAGTTCTACAGTCCGACGATC
PCR reverse primers (indexed)	CAAGCAGAAGACGGCATACGAGAT**CGTGAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**ACATCG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**GCCTAAG**TGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**TGGTCA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**CACTGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**ATTGGC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**GATCTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**TCAAGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**CTGATC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**AAGCTA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**AAGCTA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**TACAAG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Figure S4

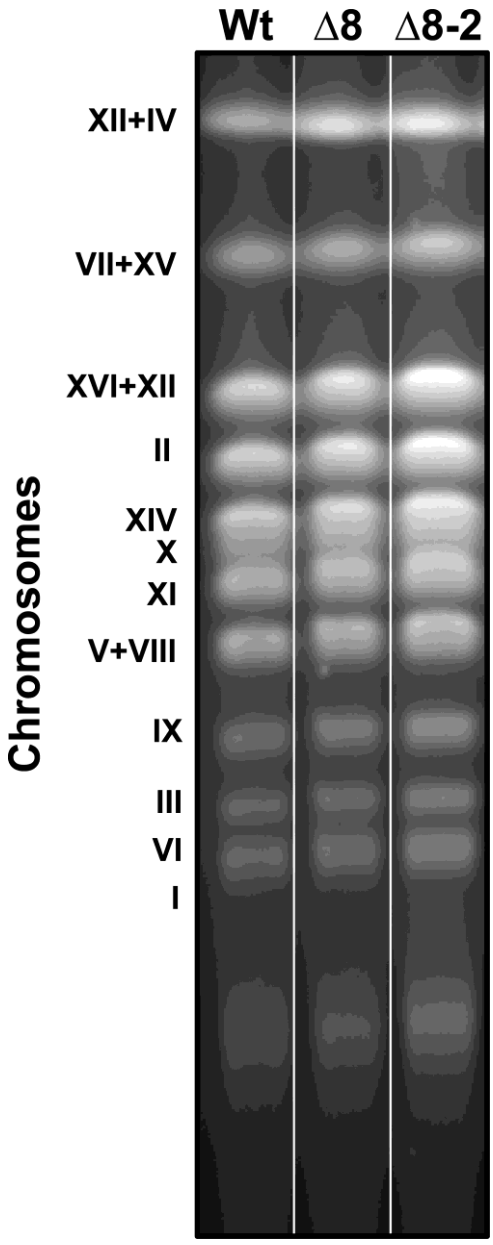

Figure S4. Pulse-field gel analysis showing chromosome size comparison between Wt and Δ8 isolates. PFGE was conducted using a BioRad Contour-clamped homogeneous electric field (CHEF) Mapper XA system and genomic samples were prepared using the CHEF genomic DNA plug kit. Agarose-embedded chromosomal DNA preparation and running conditions were performed according to the kit manual.

Figure S5

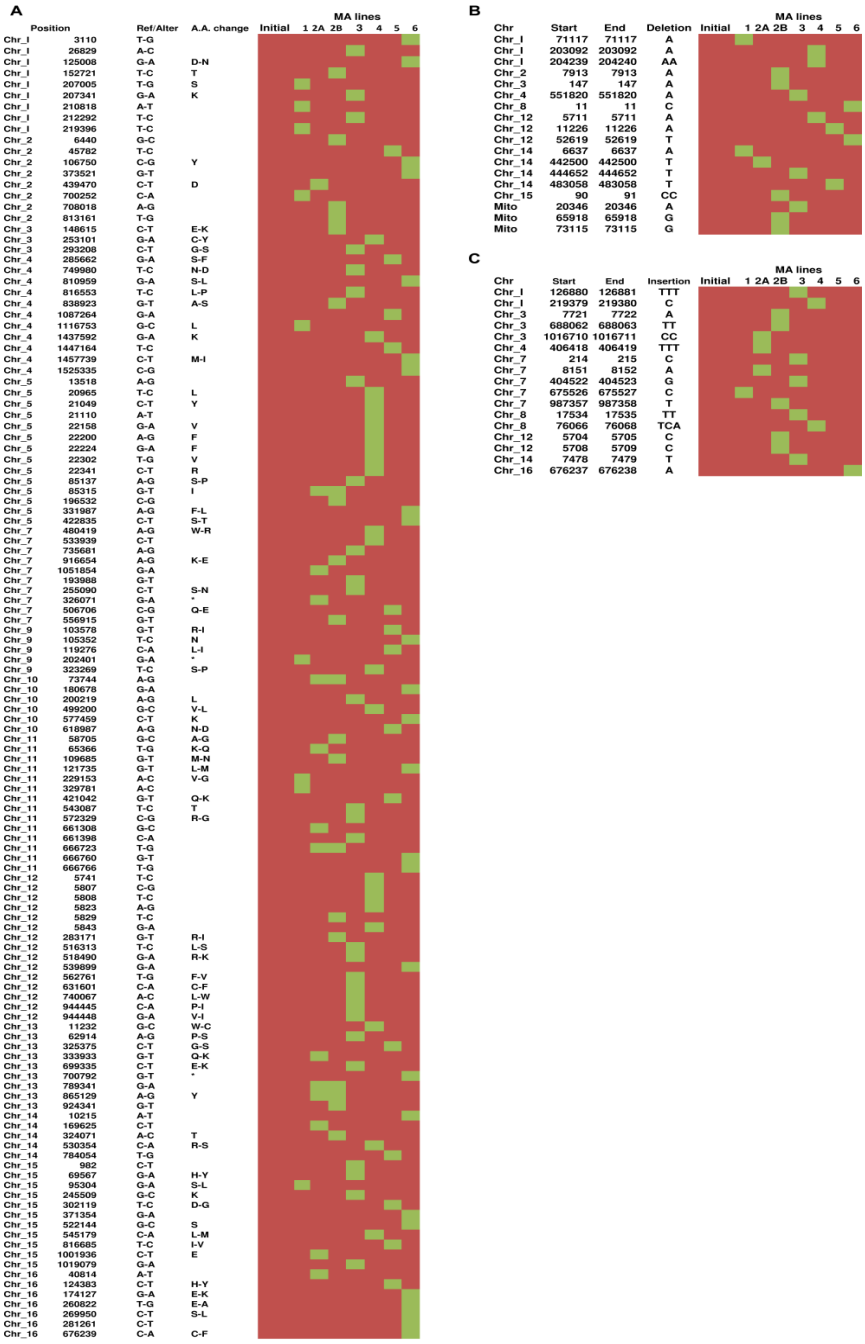

Figure S5. List of point mutations and indels in Δ8 MA lines following the long-term growth experiment. Green shows the presence of mutations with positions for each MA line represented by each column. Red shows the absence of mutations for this position for the indicated MA line. **A**) Point mutations. **B**) Deletions. **C**) Insertions.

Figure S6

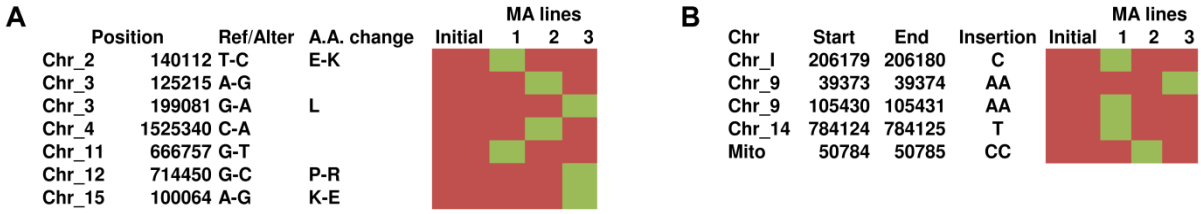

Figure S6. List of point mutations and indels in Wt MA lines following the long-term growth experiment. Same representation is shown here for Wt MA lines as in Figure S5.

Figure S7

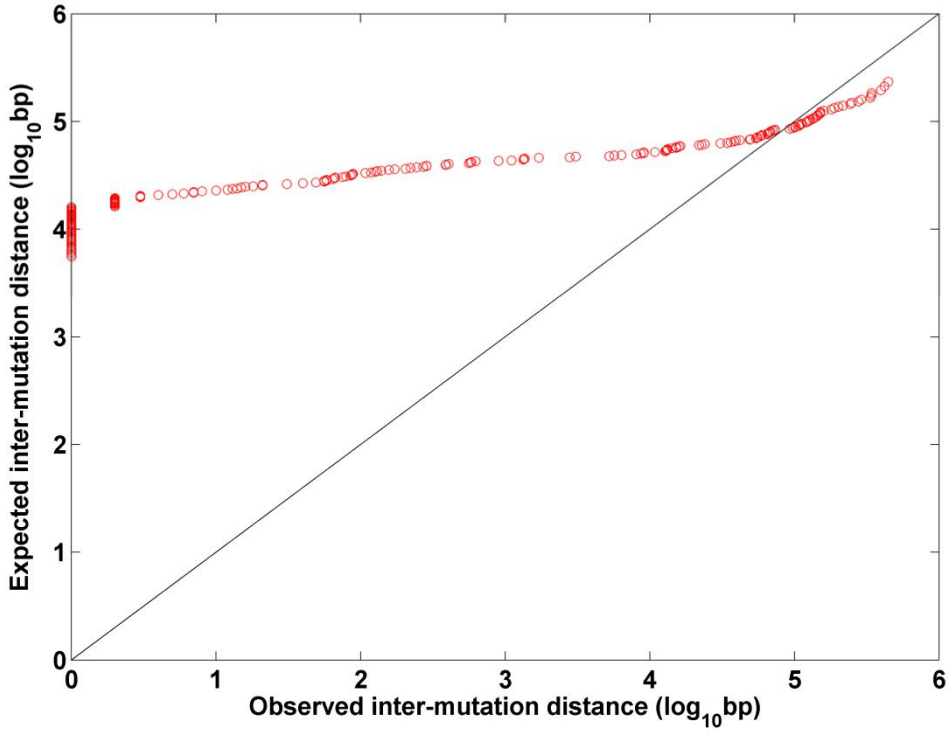

Figure S7. Clustering mutations in the new Δ8 isolate. Observed versus expected distances between adjacent mutations were analyzed as described in Figure 3B.

Figure S8

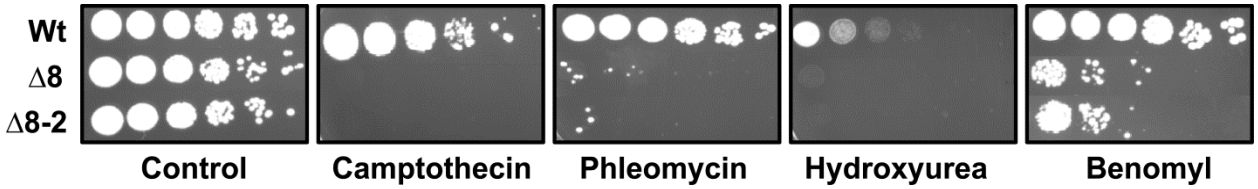

Figure S8. Sensitivity of Δ8 and Wt cells to DNA damage agents. Ten-fold dilution of Wt and Δ8 cells grown on YPD plates containing 15 µg/ml camptothecin (replication inhibitor), 150 mM hydroxyurea (replication inhibitor), 0.3 µg/ml phleomycine (double strand break inducer) or 15 µg/ml benomyl (microtubule poison).

Files S1-S2 are available for download as Excel files at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.169243/-/DC1.

File S1. List of mutations and indels in the initial Δ8 strains. Excel spreadsheet shows the position and nucleotide change of point mutations and indels in the Δ8 strains. If the mutation is in the coding region, gene was indicated on the right of the each position together with amino acid change. Genes without amino acid change indicates silent mutations.

File S2. List of genes and expression values analyzed in Wt and mutant strains. Log 2 value of each gene was clustered and analyzed to yield expression profile.

References

Koren, A., H. J. Tsai, I. Tirosh, L. S. Burrack, N. Barkai *et al*, 2010 Epigenetically-inherited centromere and neocentromere DNA replicates earliest in S-phase. PLoS Genet. **6:** e1001068.

Verstrepen, K. J., A. Jansen, F. Lewitter and G. R. Fink, 2005 Intragenic tandem repeats generate functional variability. Nat. Genet. **37:** 986-990.

Vinces, M. D., M. Legendre, M. Caldara, M. Hagihara and K. J. Verstrepen, 2009 Unstable tandem repeats in promoters confer transcriptional evolvability. Science **324:** 1213-1216.