Supporting Information

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SI Methods

Statistical Analysis. The number of new insertions into ORFs in RO strains was calculated as the average number of new insertions [(average of 7.5-fold increase in Ty1 copy number × 32 Ty1 elements) - 32 preexisting elements = 208] multiplied by the frequency of Ty1 insertion into a non-Ty1 ORF (0.03; ref. 1) to yield a frequency of 6.24 insertions into ORFs per strain (this is actually an overestimate because the total genome size is constantly increasing as a consequence of ongoing Ty1 insertion). The probability of creating MMS sensitivity in any one strain is therefore 6.24 multiplied by the chance of creating MMS sensitivity by disrupting any nonessential ORF (103/4,644; ref. 2), to yield a probability of 0.137. The probability of creating MMS sensitivity in 6 RO strains by Ty1 disruption of ORFs is therefore $(0.137)^6 = 6.69 \times 10^{-6}$. Conversely, the probability of not creating drug sensitivity in any one RO strain is 1 - 0.137 = 0.863, and the probability of creating MMS sensitivity in at least one RO strain is $1 - (0.863)^6 = 0.587$.

Quantitative PCR Analysis. Total RNA was extracted from yeast cells as previously described (3), treated with 2 μL Turbo DNase (Ambion), and purified over an RNeasy column (Qiagen). cDNA was synthesized from 500 ng RNA using the SuperScript III kit (Invitrogen) with 50 ng random hexamers and then treated with 2U RNase H. Real-time PCR analysis was performed using 10 ng of cDNA or 1.0 ng genomic DNA prepared as described (4) with primers JB6666 (AAATGGACCGTGGTTCTGAG) and JB6667 (GTTTAGCCGTTCAGCGACTC) to amplify Ty1 and JB66689 (CTGCCGGTATTGACCAAACT) and JB6690 (CGGTGATTTCCTTTTGCATT) to amplify *ACT1*. Reactions were performed with the QuantiTect SYBR Green PCR kit (Qiagen) at 95 °C for 15 min followed by 33 cycles of 95 °C for 15 sec, 58 °C for 30 sec, and 72 °C for 30 sec. The relative amount of Ty1 DNA was calculated from the ΔC_T value of Ty1 DNA relative to the amount of *ACT*1 DNA, and then normalized to the WT signal ($\Delta\Delta C_T$).

Southern Blot Analysis. To monitor the introduction of Ty1 elements into RO Ty1 strains, Southern blotting was performed as described (5). DNA was extracted from each strain, digested with AvaI, and separated by electrophoresis. This method distinguishes introduced elements, which lack AvaI/XhoI sites and therefore form a ladder of hybridizing fragments >5.6 kb long. The small number of preexisting bands larger than 5.6 kb in the WT correspond to Ty1 elements naturally lacking AvaI/XhoI sites in their LTRs. Following transfer to nitrocellulose, the blot was probed with the PstI-PstI fragment of Ty1-H3 (nt 1138–1892), labeled with ^{32}P using random hexamer primers (6).

Pulsed-Field Gel Electrophoresis. PFGE was performed according to manufacturer's directions (Bio-Rad; CHEF-DR III system) on strains grown overnight in YPD medium at 30 °C. To assay sensitivity to HU and the mutant *POL1* allele, independent cultures were incubated in YPD medium containing 200 mM HU at 22 °C or 30 °C for 2.5 h and then recovered on YPD medium at 22 °C for 2 d. A single well isolated colony from each plate was cultured in YPD medium for 2 d at 22 °C. From each culture, 1.8×10^8 cells were suspended in 150 μL 10 mM Tris pH 7.5, 20 mM NaCl, and 50 mM EDTA pH 8.0, mixed with 15 μL 20 mg/mL lyticase (ICN Biomedicals) and an equal volume of 2% low melting point agarose (Invitrogen) and allowed to solidify. Agarose plugs were extruded into Lyticase buffer (10 mM Tris pH 7.5, 50 mM EDTA pH 8.0, 1 mg/mL lyticase) and incubated at 37 °C for 1 h. Plugs were washed in 20 mM Tris (pH 8.0) and 50 mM EDTA and incubated overnight in proteinase K reaction buffer (1% SDS, 0.2% sodium deoxycholate, 100 mM EDTA, pH 8.0, 1 mg/mL proteinase K) at 50 °C. Following four 1-h washes in a solution of 20 mM Tris, pH 8.0, and 50 mM EDTA, agarose plugs were melted, loaded onto a 1% MegaBase agarose gel (Bio-Rad) and run in 0.5× TBE buffer for 24 h at 14 °C and 200 V with a switch time of 60 sec. Chromosome bands were visualized by ethidium bromide staining.

Transformation with Gene Deletion Cassettes. Deletion cassettes were derivatives of the yeast knockout collection containing the *URA3* gene in place of the *kanMX* marker (7–9). Query constructs were created by long-range PCR with Ex Taq (Takara) and contained 1.5 kb of homology on either end of the gene disruption cassette; transformations were performed as described (9). For query constructs that led to a specific defect in colony size or number in the RO Ty1 strains after 40 h growth at 30 °C, transformants were colony purified on SC–Ura. The presence of the deletion cassette at the correct genomic locus was confirmed by PCR analysis of genomic DNA with primers flanking the query construct, employing the SfiI site within the *URA3* cassette (8) to identify disrupted target genes.

PCR Analysis. Integration of multiple Ty1 elements at the same locus was assayed by PCR of 0.05 ng genomic DNA with primers JB9492 (GGAAAGTCCACCAAGGCTTCA) and 9493 (TGGAGTGCTCAGAGGCGTTC) to detect head-to-tail insertions, or by PCR of 5.0 ng gDNA with primer 9492 alone to detect tail-to-tail insertions or primer 9493 alone to detect head-to-head insertions. PCR was performed for 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 3 min. PCR products were cloned into the TOPO-TA vector (Invitrogen) and sequenced.

Northern Blot Analysis. Total RNA was extracted from yeast cells using hot acid phenol as described (3), treated with 2 μL Turbo DNase (Ambion), and purified over an RNeasy column (Qiagen). Fifteen micrograms of RNA were separated on a 1.2% agarose gel in formaldehyde/MOPS buffer, transferred to a GeneScreen Plus membrane (NEN Life Sciences) in 10× SSC by upward capillary transfer, UV cross-linked, and dried in a vacuum oven. Membranes were probed at 68 °C in ULTRAhyb with 0.1 mg/mL herring sperm DNA (Ambion) using ^{32}P-labeled riboprobes synthesized from linear templates using the Promega riboprobe T7 kit and comprising nucleotides 5111–5218 of Ty1 or 319-1084 of *ACT1*.

Immunoblotting. Yeast proteins were purified as described (10), fractionated on a 4%–20% Tris-glycine gel, transferred to Hybond nitrocellulose membranes, and probed with Ty1 anti-VLP or anti-β-tubulin (T5168; Sigma) antisera at dilutions of 1:20,000 and 1:2,000, respectively.

Statistical Analysis of Colony Size. The sizes of 25 to 50 yeast colonies per strain were measured using Adobe Illustrator software. *P* values were determined from a 2-tailed Student *t* test to determine whether the average size of colonies from the RO lineage containing a gene deletion was significantly smaller than that of the WT strain containing the same deletion.

1. Ji H, et al. (1993) Hotspots for unselected Ty1 transposition events on yeast chromosome III are near tRNA genes and LTR sequences. *Cell* 73:1007–1018.
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7. Pan X, et al. (2006) A DNA integrity network in the yeast Saccharomyces cerevisiae. *Cell* 124:1069–1081.
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10. Gardner RG, Nelson ZW, Gottschling DE (2005) Ubp10/Dot4p regulates the persistence of ubiquitinated histone H2B: distinct roles in telomeric silencing and general chromatin. *Mol Cell Biol* 25:6123–6139.
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12. Curcio MJ, et al (2007) S-phase checkpoint pathways stimulate the mobility of the retrovirus-like transposon Ty1. *Mol Cell Biol* 27:8874–8885.

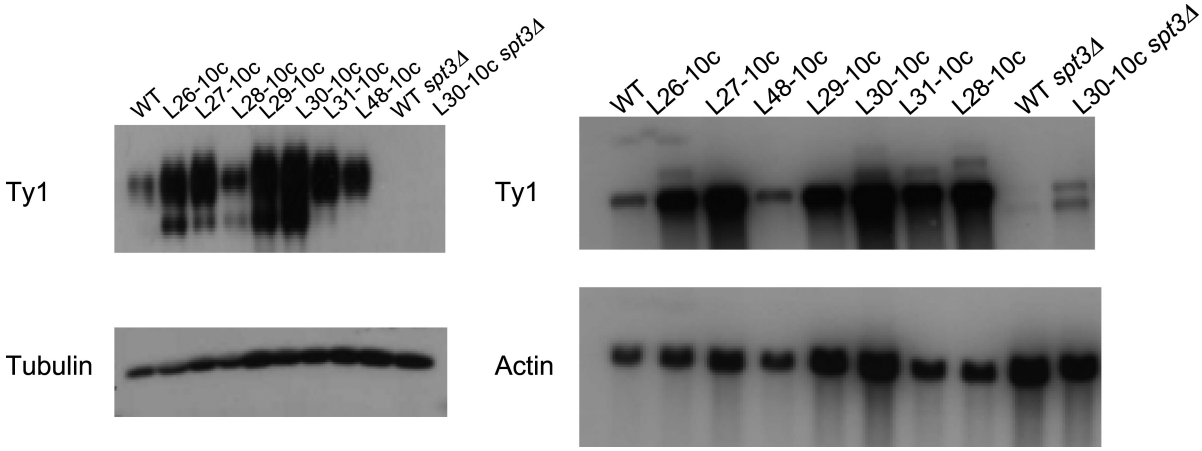
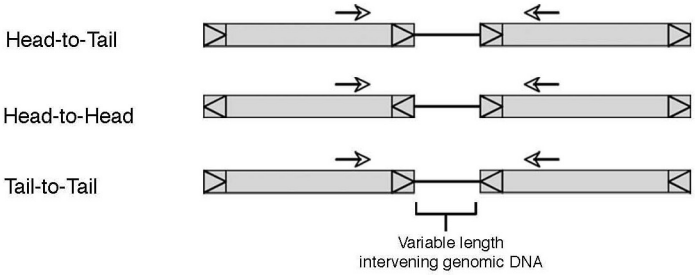
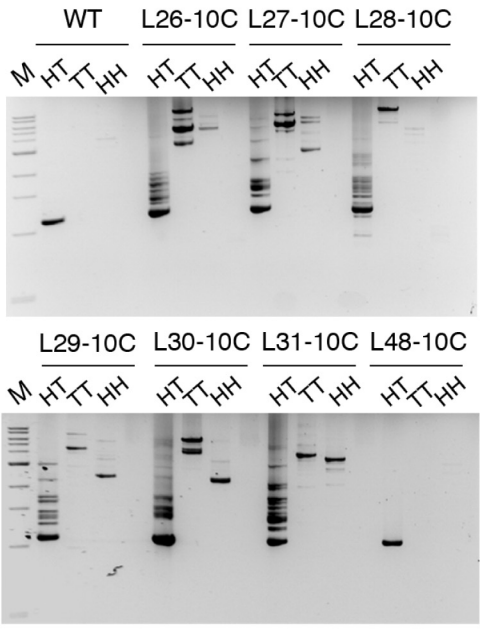


Fig. S1. (*Left*) Western blot of total protein from WT and RO Ty1 strains probed for the Ty1 Gag protein (*Top*) or β-tubulin (*Bottom*). (*Right*) Northern blot of total RNA from logarithmic cultures of WT and RO Ty1 strains probed for the Ty1 sequence (*Top*) or actin (*Bottom*).

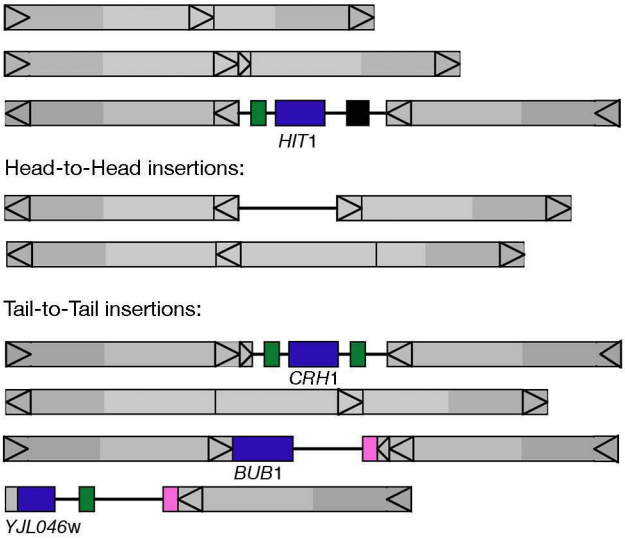
a.



b.



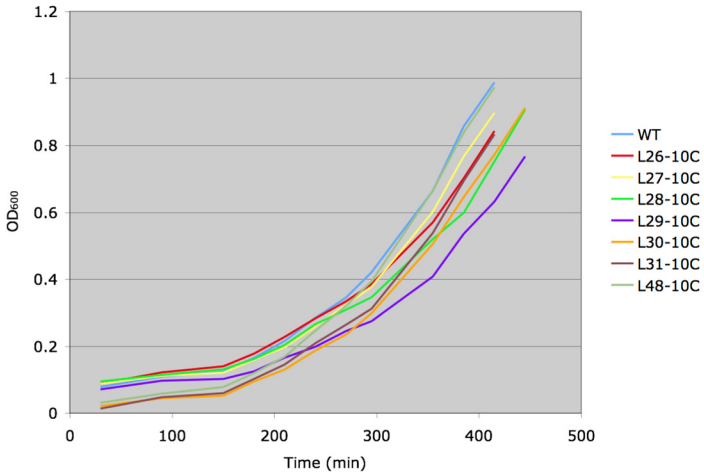
c.



Isolated from	Ty1 sequence (nt)	Intervening genomic sequence (chromosome, coordinates)
WT, L26-10C L29-10C, L31-10C	5384-5918; 1-725	None
L31-10C	5200-5918; 52-314; 52-725	None
L31-10C	5200-5918; 5918-5200	*X* 538350-540445
L29-10C	725-1; 705-725	*III* 169202-168913
L26-10C	725-1; 5918-2267; 593-725	None
L26-10C	5200-5918; 5918-5200	*VII* 876286-878928
L26-10C	725-334; 2767-5918; 1-725	
L29-10C	5200-5220; 5200-5918; 5918-5750	*VII* 873836-876063
L29-10C	5200-5220; 5918-5200	*X* 352612-354500

Fig. S2. Tandem Ty1 insertions in RO Ty1 strains. (*a*) Orientation of potential Ty1 insertions in head-to-tail, head-to-head, and tail-to-tail orientation, with the position of PCR primers at Ty1 nt 725–705 and 5200–5220 indicated by the arrows. (*b*) PCR for tandem integrations of Ty1 elements in high-copy strains in head-to-tail (*HT*, 1:100 dilution of template DNA), tail-to-tail (*TT*), and head-to-head (*HH*) orientations (M, 1 kb DNA ladder). The WT strain produces a single 1,100-bp band of head-to-tail tandem Ty1 elements. (*c*) Sequenced PCR products from WT and RO Ty1 strains. Regions of the Ty1 element in shadow are remainder of the full-length Ty1 element outside the PCR primers and are indicated to highlight the orientation of each element. Blue boxes represent ORFs in the intervening genomic DNA, green boxes represent tRNA genes, black boxes represent ARS elements, and pink boxes represent endogenous LTRs (not drawn to scale).

a.



b.

Strain	Doubling time (min) Expt 1	Doubling time (min) Expt 2
WT	97.2	92.7
L26-10C	105.8	98.6
L27-10C	95.6	88.6
L28-10C	106.8	96.3
L29-10C	99.6	109.6
L30-10C	87.1	91.8
L31-10C	84.4	86.1
L48-10C	91.6	95.2

c.

Fig. S3. Phenotypes of RO Ty1 strains. Growth curves (*a*) and doubling times (*b*) of WT and RO Ty1 strains in YPD medium as measured by OD_{600}. (*c*) Array-CGH analysis of the L31–10C strain (provided by Maitreya Dunham, Seattle, WA) shows a deletion on chromosome *III* between the *MAT* and *HMR* loci. (Scale bars: twofold increases or decreases in copy number.)

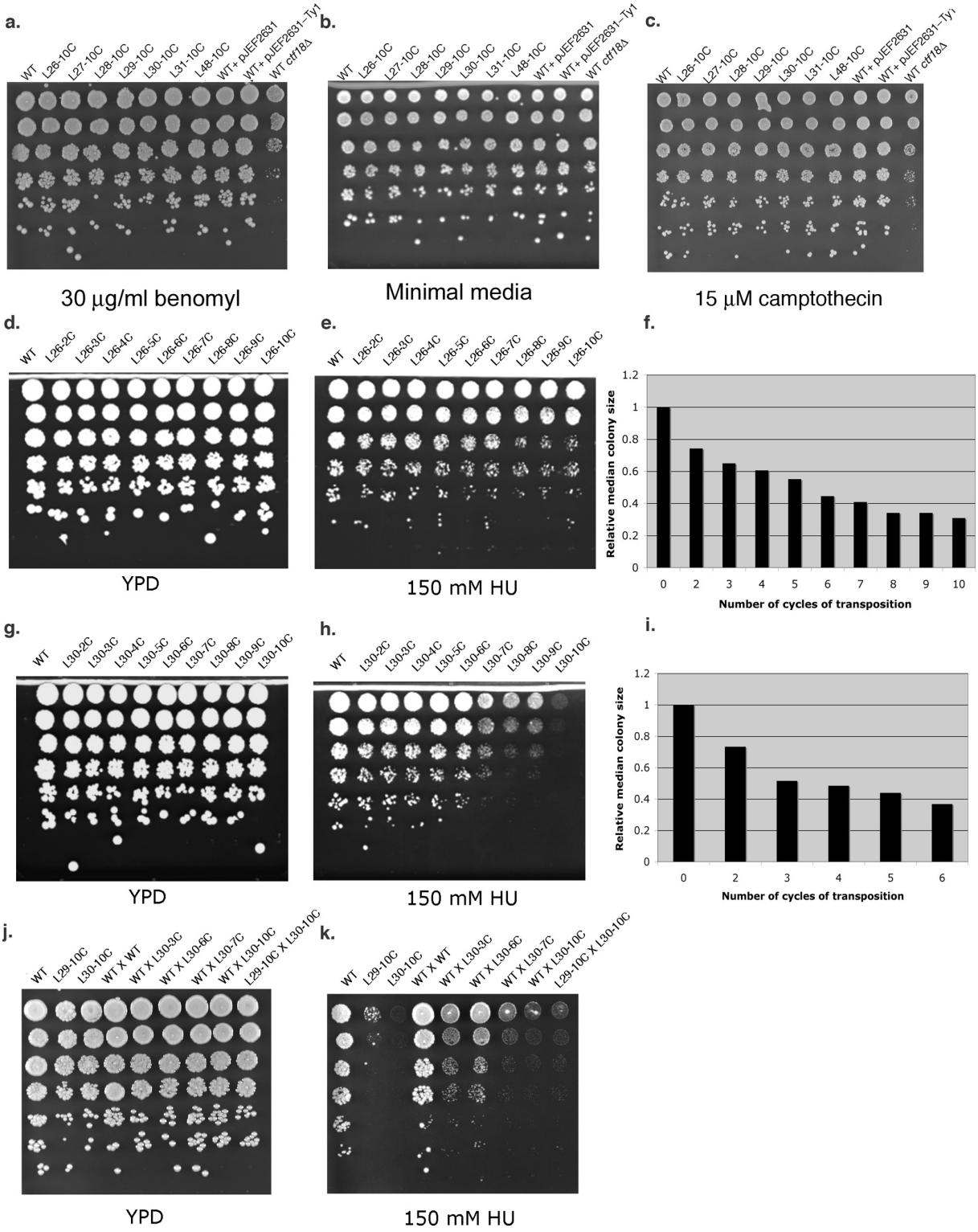


Fig. S4. To assay sensitivity of RO Ty1 strains to benomyl and camptothecin, serial dilutions of yeast strains were plated onto YPD + 30 μg/mL benomyl for 2 d (*a*), minimal media for 2 d (*b*), or minimal media + 15 μM camptothecin for 2 d (*c*). Dose-dependent increase in HU sensitivity with increasing Ty1 copy number. Serial dilutions of yeast strains were plated onto YPD (*d* and *g*) or YPD + 150 mM HU (*e* and *h*). HU sensitivity of RO Ty1 lineages L26 (*d* and *e*) and L30 (*g* and *h*) following 2 to 10 cycles of Ty1 transposition. HU sensitivity of the RO Ty1 lineages was quantified by plating individual colonies of the L26 (*f*) or L30 (*i*) lineage onto 150 mM HU and measuring the colony area of 50 isolated colonies closest to the center of the plate. HU sensitivity of haploid (WT, L29–10C, L30–10C) and heterozygous diploid RO Ty1 strains was assayed by plating serial dilutions of yeast strains onto YPD (*j*) or YPD + 150 mM HU (*k*).

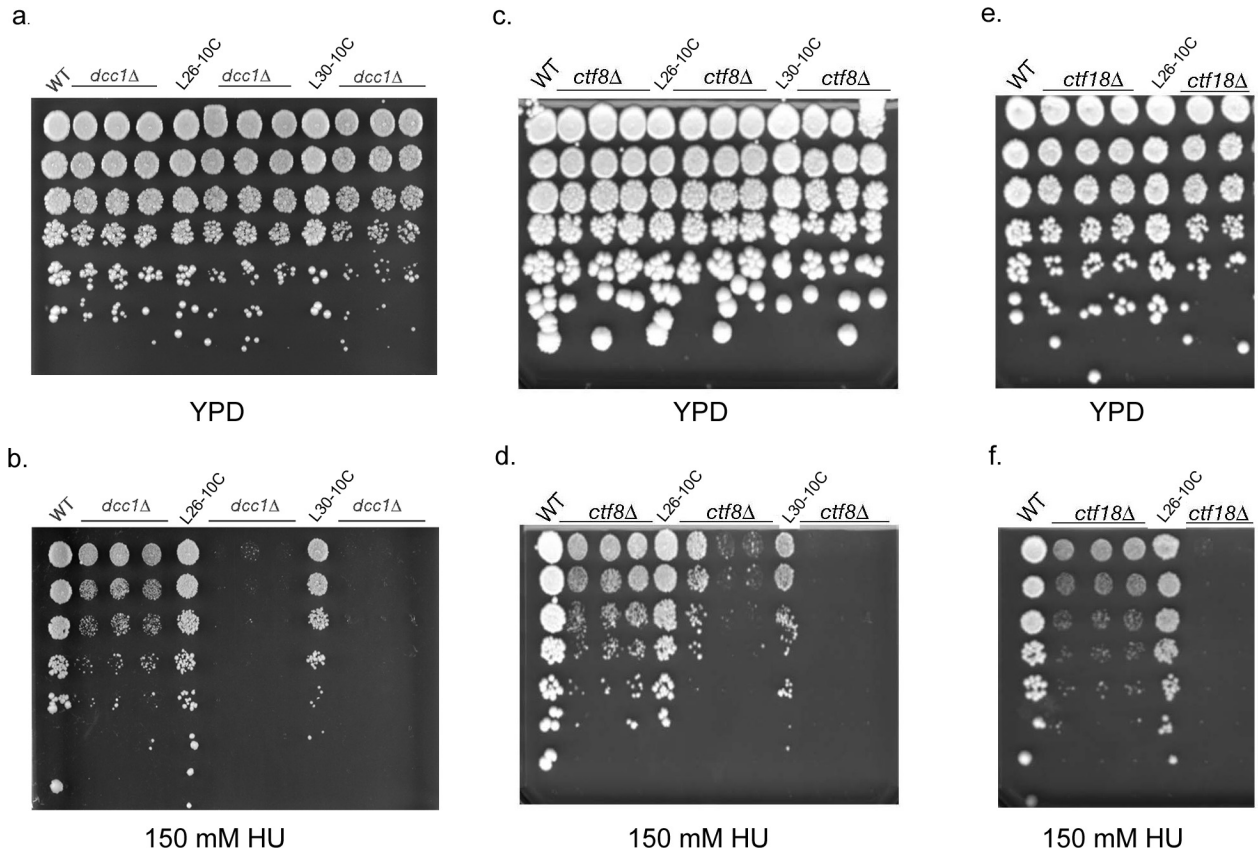


Fig. S5. Deletion of *DCC1*, *CTF8*, or *CTF18* exacerbates the sensitivity of RO Ty1 strains to HU. Serial dilutions of WT and RO Ty1 strains with and without deletion of *DCC1*, *CTF8*, and *CTF18* were plated onto YPD (*a*, *c*, and *e*) or YPD + 150 mM HU (*b*, *d*, and *f*). No transformants of L30–10C were recovered with the *CTF18* deletion.

Table S1. Sporulation efficiency of RO Ty1 strains[a]

Strain	Relative Ty1 copy number[b] (*MAT*a, *MAT*α)		Sporulation efficiency (%)		
			Day 3	Day 5	Day 7
WT × WT	1	1	3.3 ± 2.3	4.2 ± 2.6	5.1 ± 3.8
L28–10C × L28–10C	7.2	7.2	2.1 ± 1.2	4.4 ± 1.0	6.0 ± 1.2
L29–10C × L29–10C	5.5	5.5	1.0 ± 0.9	2.0	3.5 ± 0.5
L30–10C × L30–10C	9.6	9.6	0	0	0
WT × L26–10C	1	4.0	1.9 ± 1.7	2.1 ± 1.1	3.3 ± 1.9
WT × L28–10C	1	7.2	2.2 ± 0.9	3.9 ± 0.2	4.8 ± 1.7
WT × L29–10C	1	5.5	4.2 ± 2.8	6.7 ± 0.7	8.4 ± 0.9
WT × L30–10C	1	9.6	4.3 ± 2.8	4.3 ± 2.1	7.0 ± 2.4
L26–10C × L28–10C	4.0	7.2	0.9 ± 0.9	1.1 ± 0.4	2.4 ± 0.7
L26–10C × L29–10C	4.0	5.5	1.8 ± 1.2	2.3 ± 1.9	4.2 ± 2.0
L26–10C × L30–10C	4.0	9.6	0.8 ± 0.3	1.1 ± 0.1	1.6 ± 0.6

[a]When mated to haploid counterparts.
[b]Data from Fig. 1*A*.

Table S2. Transformation efficiency of WT and RO Ty1 strains with Ty1-*URA3* DNA

Strain	Relative Ty1 integration frequency $\times 10^{-5}$
WT	4.18
L26–10C	109
L27–10C	39.7
L28–10C	76.9
L29–10C	21.9
L30–10C	25.9
L31–10C	13.5
L48–10C (control strain)	5.4

Mean number of transformants for 2 to 3 independent transformations performed with linear Ty1-*URA3* DNA was divided by the mean number of transformants obtained with a circular replication-competent plasmid DNA to obtain a relative Ty1 integration frequency.

Table S3. Gene deletions affecting the growth of RO Ty1 strains

Gene deletion	Colony area (mm^2) WT strain	Colony area (mm^2) RO strain	*P* value	Relative colony area[a]
DRC signaling				
ctf8[c]	0.68 ± 0.23	0.39 ± 0.12	8.61×10^{-11}	0.583
ctf18[c,e]	0.16 ± 0.004	0.07 ± 0.002	3.52×10^{-14}	0.438
dcc1[c]	0.55 ± 0.32	0.27 ± 0.11	1.22×10^{-4}	0.494
mrc1	0.50 ± 0.24	0.22 ± 0.13	1.10×10^{-9}	0.446
tof1	0.53 ± 0.16	0.43 ± 0.18	6.35×10^{-3}	0.811
csm3[c]	0.17 ± 0.06	0.09 ± 0.04	2.16×10^{-6}	0.529
rad17	0.83 ± 0.26	0.51 ± 0.16	1.02×10^{-10}	0.616
PRR				
rad6	0.15 ± 0.06	0.09 ± 0.03	2.21×10^{-8}	0.600
Transcriptional silencing				
hst1	0.59 ± 0.21	0.35 ± 0.13	6.67×10^{-10}	0.584
hst3	0.49 ± 0.24	0.28 ± 0.11	2.70×10^{-5}	0.563
npt1[e]	0.60 ± 0.20	0.35 ± 0.15	1.16×10^{-10}	0.583
esc2	0.54 ± 0.27	0.27 ± 0.09	7.19×10^{-6}	0.513
Chromatin remodeling				
rsc1	0.15 ± 0.01	0.08 ± 0.001	7.39×10^{-7}	0.533
htz1	0.16 ± 0.004	0.08 ± 0.003	1.09×10^{-9}	0.500
DNA replication				
elg1[d]	0.19 ± 0.01	0.09 ± 0.002	1.11×10^{-9}	0.474
rad27[c,d,e]	0.24 ± 0.10	0.09 ± 0.05	6.93×10^{-15}	0.375
ydl162c[b]	0.20 ± 0.06	0.07 ± 0.03	5.70×10^{-19}	0.350
ctf4[c,d]	0.42 ± 0.18	0.32 ± 0.10	6.53×10^{-4}	0.762
rrm3[d]	0.56 ± 0.16	0.29 ± 0.11	2.76×10^{-16}	0.513
pol32[c]	0.15 ± 0.06	0.07 ± 0.04	4.38×10^{-12}	0.467
dia2	0.47 ± 0.16	0.38 ± 0.17	5.92×10^{-3}	0.809
Replication fork restart				
sgs1[c,d]	0.14 ± 0.05	0.07 ± 0.02	3.01×10^{-14}	0.500
mus81[e]	0.16 ± 0.002	0.11 ± 0.04	6.13×10^{-4}	0.688
slx8	0.13 ± 0.003	0.07 ± 0.03	1.09×10^{-7}	0.538
hex3/slx5	0.37 ± 0.20	0.20 ± 0.05	2.50×10^{-7}	0.530
hpr5[c,e]	0.13 ± 0.002	0.08 ± 0.001	1.96×10^{-9}	0.615
Other				
rpn4	0.18 ± 0.01	0.09 ± 0.002	6.39×10^{-12}	0.500

[a]The relative area of colonies from the RO strain without a gene deletion compared to the WT strain is 0.980 with a *P* value of 0.696, confirming no difference in colony size.
[b]The ydl162c deletion cassette has been demonstrated to affect the expression of the adjacent Cdc9 DNA ligase gene (7).
[c]Deletions affect not only the growth of RO Ty1 strains, but also confer supersensitivity to HU.
[d]Deletions have been shown by Scholes *et al*. to either increase or decrease the Ty1 transposition rate (11,12).
[e]Transformation with the deletion cassette produces both a reduction in colony size as well as fewer transformants in RO Ty1 strains relative to WT.
Abbreviations: DRC signaling, DNA replication checkpoint signaling; PRR, post-replication repair; DDC signaling, DNA damage checkpoint signaling; NER, nucleotide excision repair.

Table S4. Gene deletions that do not affect the growth of RO Ty1 strains

Function	Deletion
DRC signaling	*dun1*
PRR	*rad18*[a]
Transcriptional silencing	*hst2*, *hst4*, *ard1*, *nat1*, *esc1*, *sir2*, *sir3*
Chromatin remodeling	*cac2*, *asf1*, *rsc2*
Chromosome segregation	*csm1*, *csm2*, *sgo1*, *cin8*
DDC signaling	*rad9*, *chk1*
Recombination	*rad51*[a], *rad52*[a], *rad54*
Oxidative stress	*tsa1*, *lys7*
Mismatch repair	*pms1*, *msh2*
NER	*rad1*, *rad10*
Other	*rmd7*, *pmr1*, *isw2*, *rpn10*, *mms22*, *mms4*, *mph1*, *pif1*, *rtt109*, *sae2*[a], *shu1*, *slx1*, *slx4*, *rev3*, *lte1*

[a]Deletions have been shown by Scholes et al. to either increase or decrease the Ty1 transposition rate (11,12).

Table S5. Yeast strains

Strain	Genotype	Plasmid
GRF167	*MATα his3Δ200 ura3–167*	–
JB721	*MATa* GRF167	–
L26–10C. . . . L31–10C	=GRF167; 10 cycles of Ty1 transposition	–
L48–10C	=GRF167	–
GRF167 + pJEF2631	=GRF167	pJEF2631
GRF167 + pJEF2631-Ty1	=GRF167	pJEF2631-Ty1
L30–2C. . . L30–9C	=GRF167 following 2–9 cycles of Ty1 transposition	–
L26–2C. . . L26–9C	=GRF167 following 2–9 cycles of Ty1 transposition	–
Δctf18	=GRF167; *ctf18*::*URA3*	–
Δdcc1	=GRF167; *dcc1*::*URA3*	–
WT × WT	*MATa*/α JB721/GRF167	–
WT × L30–3C	*MATa*/α JB721/L30–3C	–
WT × L30–6C	*MATa*/α JB721/L30–6C	–
WT × L30–7C	*MATa*/α JB721/L30–7C	–
WT × L30–10C	*MATa*/α JB721/L30–10C	–
L30–10C × L29–10C	*MATa*/α L30–10C/L29–10C	–
WT pol1–17; L26 pol1–17	=GRF167; *pol1–17*	–
WT pol1–17 + POL1; L26 pol1–17 + POL1	=GRF167; *pol1–17*	pRS413-POL1