Ouenzar et al., https://doi.org/10.1083/jcb.201610071

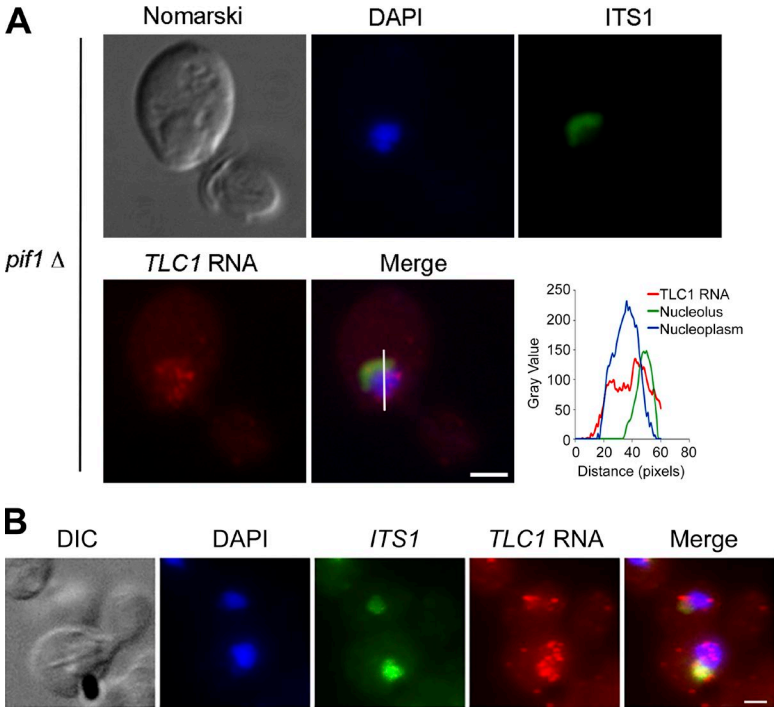

Figure S1. ***TLC1*RNA localization in *pif1Δ* and *pif1-m2* strains.** (A) Image and linescan analysis of *TLC1* RNA, ITS1 probe and DAPI of a *pif1Δ* cell in G2. Bar, 1 µm. (B) Distribution of *TLC1* RNA in a *pif1-m2* yeast cell in G2. Bar, 1 µm.

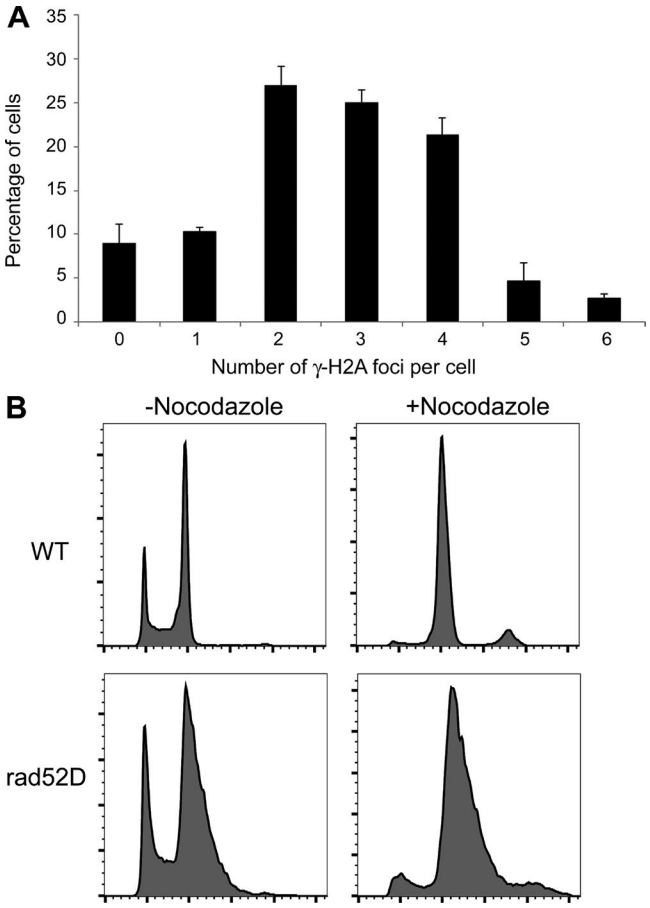

Figure S2. **Validation of bleomycin and nocodazole treatments of yeast cells.** (A) Quantification of the number of γ-H2A foci per cell in the yeast population after treatment with bleomycin; *n* = 300 cells. Error bars represent ±SD. (B) Cell cycle synchronization of WT or *rad52Δ* cells treated with nocodazole.

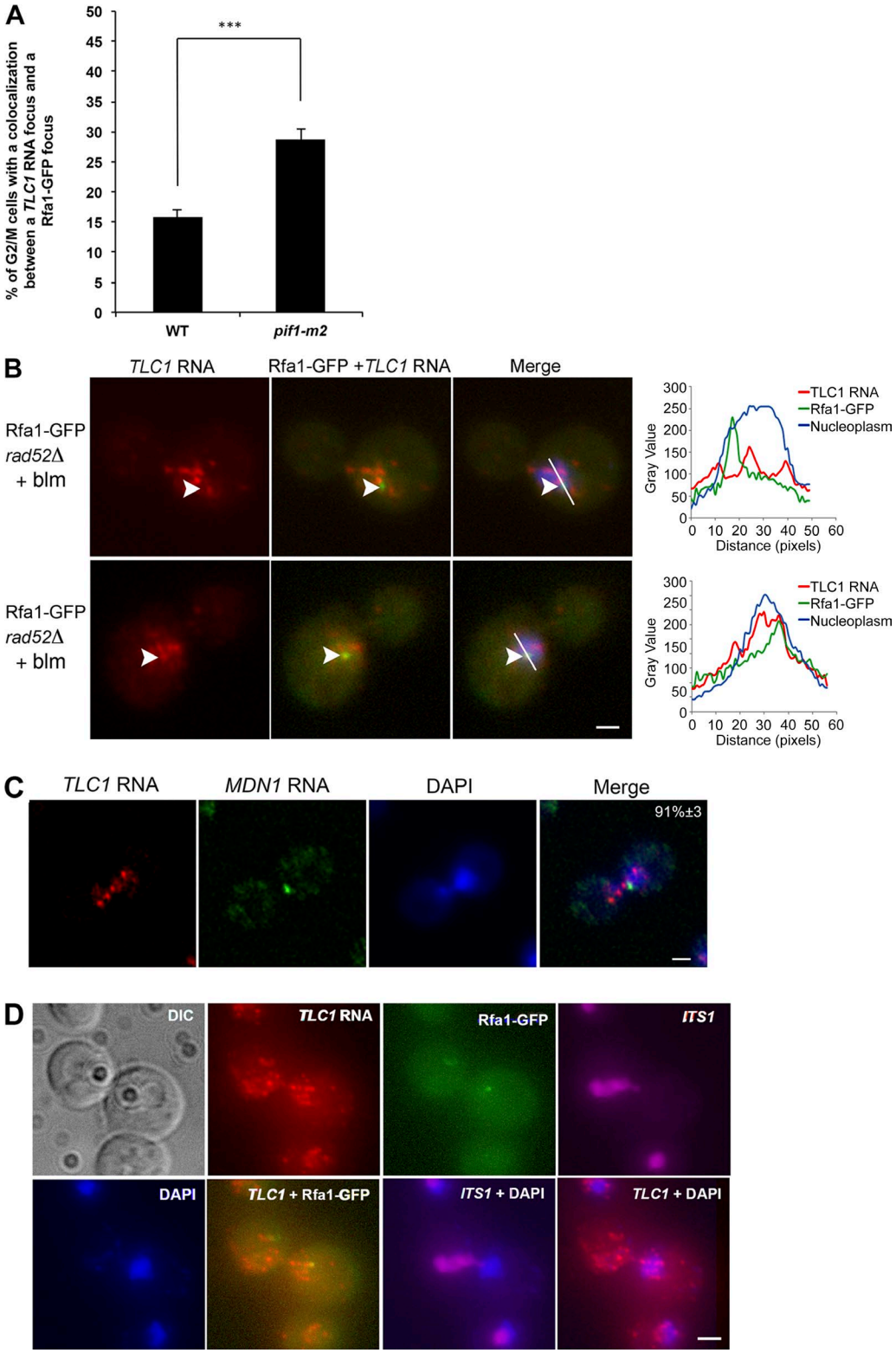

Figure S3. **Analysis of *TLC1* RNA colocalization with double-strand breaks.** (A) Quantification of colocalization events between *TLC1* RNA foci and Rfa1-GFP foci in WT and *pif1-m2* strains; $n = 45–63$ cells. Error bars correspond to ±SD. ***, $P < 0.005$ (two-tailed *t* test). (B) Linescan analysis of *TLC1* RNA, Rfa1-GFP and DAPI colocalization in the nucleus of *rad52Δ* cells. Arrowheads mark a Rfa1-GFP focus that does not colocalize (top) or colocalizes (bottom) with a *TLC1* RNA focus. (C) Quantification of *TLC1* RNA foci colocalization with a nuclear *MDN1* transcription site in G2/M cells. FISH against *TLC1* RNA and *MDN1* RNA in *rad52Δ* cells treated with bleomycin. Number represents the percentage of G2/M cells without colocalization between a *TLC1* RNA focus (red) and the *MDN1* transcription site focus (green). (D) Maximum intensity projection of *rad52Δ* yeast cell to show distribution of *TLC1* RNA foci, Rfa1-GFP focus, nucleolus (*ITS1*), and nucleoplasm (DAPI). Bars, 1 µm.

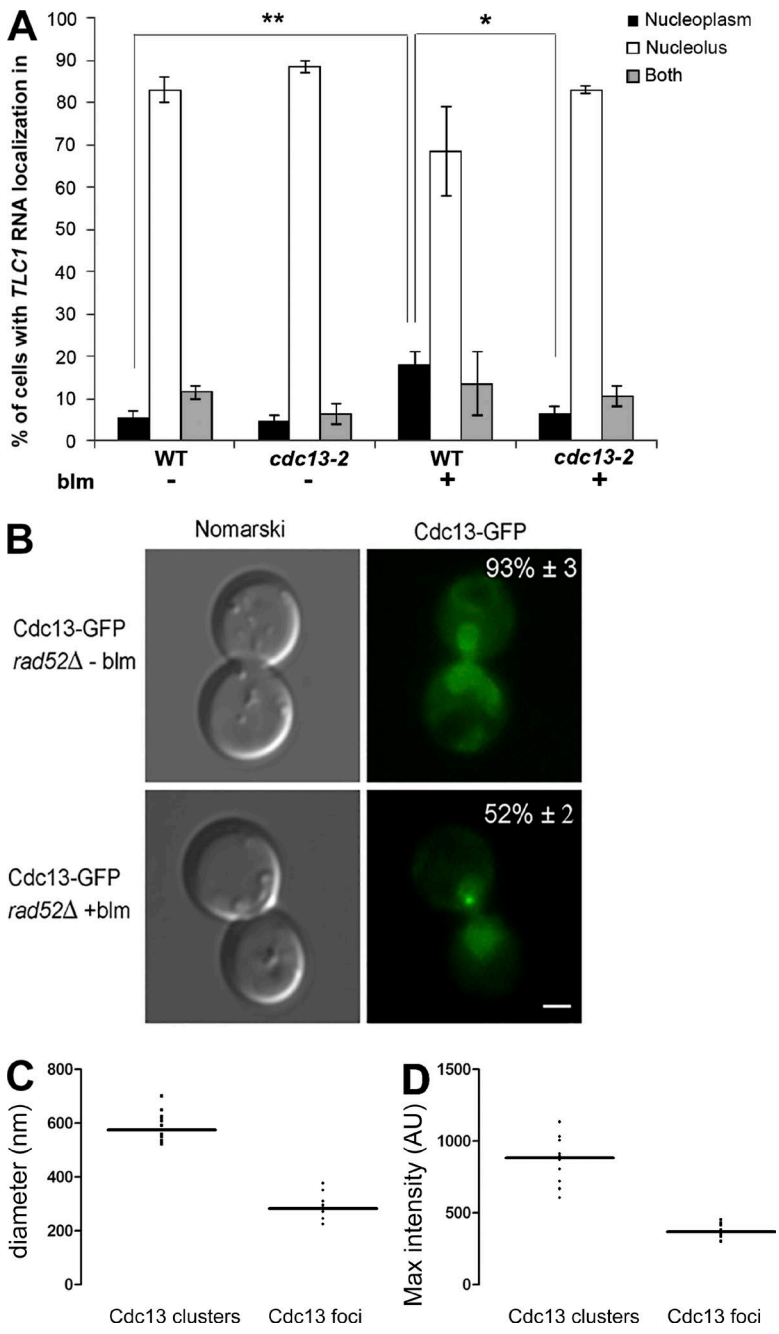

Figure S4. **Quantitative analysis of Cdc13 foci and clusters after induction of DNA damage.** (A) *TLC1* RNA localization in WT and *cdc13-2* strains before and after bleomycin treatment; $n = 300$ cells. Error bars correspond to ±SD. *, $P < 0.05$; **, $P < 0.01$ (two-tailed *t* test). (B) Formation of Cdc13-GFP foci in living yeast cells with DNA damage. Cdc13-GFP *rad52Δ* cells were treated or not with bleomycin. Quantification of each phenotype in the yeast cell population is indicated in both panels on the right. Bar, 1 µm. (C) Mean diameter of Cdc13 foci and clusters. The dot plot shows the distribution of the diameter of 10 Cdc13 foci and 13 Cdc13 clusters. Cdc13 foci have a mean diameter of 283 nm, whereas Cdc13 clusters have a mean diameter of 575 nm. (D) Mean maximal fluorescence intensities of Cdc13 foci and clusters. The dot plot shows the distribution of the maximal fluorescence intensities of 10 Cdc13 foci and 13 Cdc13 clusters. Cdc13 foci have a mean maximal fluorescence intensity of 355 arbitrary fluorescence units (AUs), whereas clusters have a mean maximal fluorescence intensity of 883 AUs.

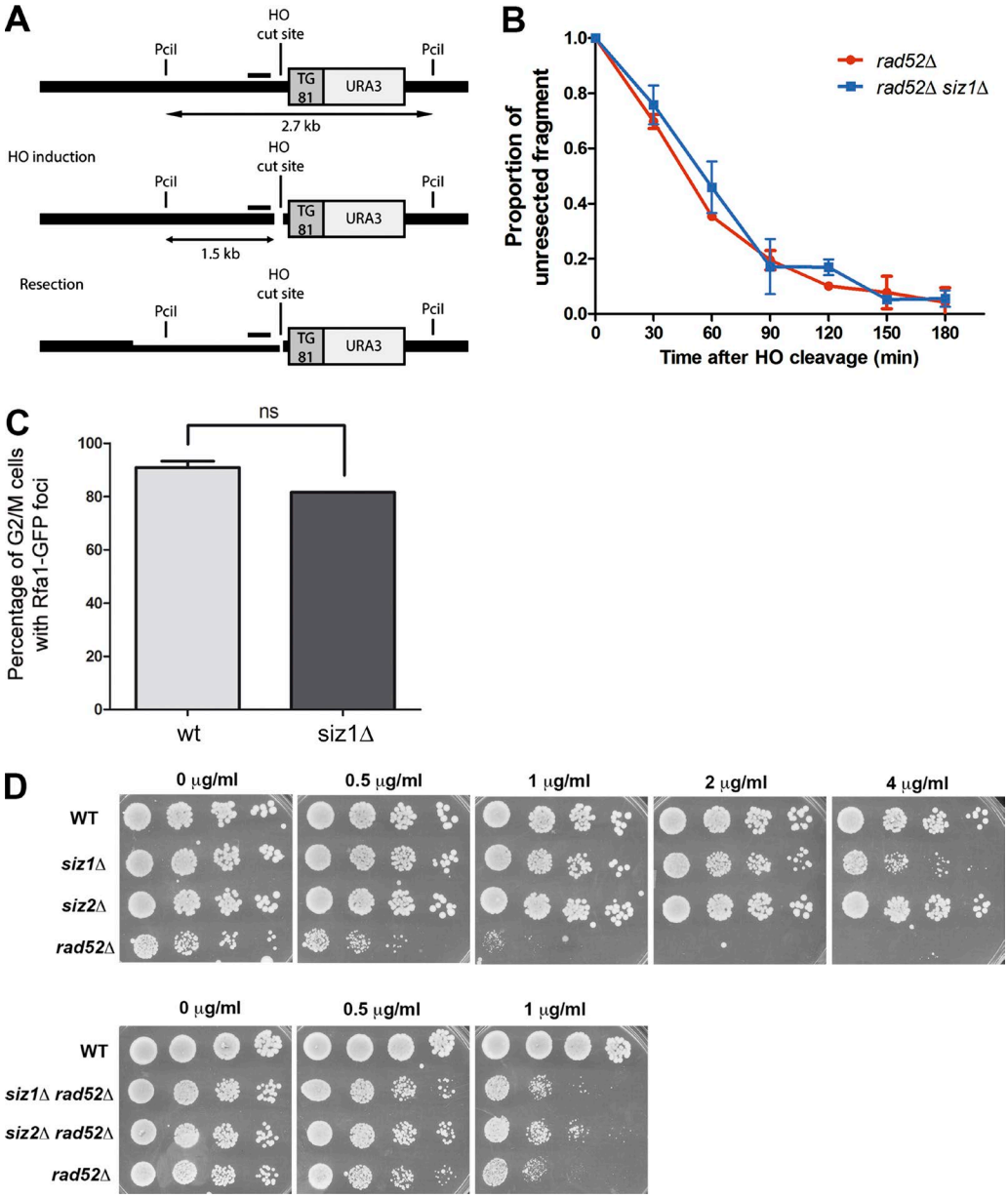

Figure S5. **Siz1 is not involved in the resection of DSBs.** (A) Schematic representation of the HO-TG_{81}-*URA3* locus used to assess resection of the HO-induced DSB. Notice that the resection is measured on the fragment without TG_{81} repeats. 5′–3′ degradation eliminates the PciI site, which results in the disappearance of the 1.5-kb fragment generated by the PciI/HO cut site. Horizontal bars indicate the position of the probe used for Southern blotting to detect the 2.7-kb and 1.5-kb fragments. (B) Kinetic of HO cut resection in *rad52Δ* and *rad52Δ siz1Δ* strains. Means from two to three independent experiments are presented. Error bars correspond to ±SD. (C) Quantification of resected DSBs marker Rfa1-GFP foci in G2/M cells from WT and *siz1Δ* strain treated with bleomycin. ns, not significant. n = 217–226 cells. Error bars correspond to ±SD. (D, top) Growth assay of *siz1Δ*, *siz2Δ* or *rad52Δ* cells on plates containing various concentrations of bleomycin. Fivefold serial dilutions were spotted on each plate, from 5×10^{-4} to 10^{-5} cells. (D, bottom) Growth assay of *siz1Δ rad52Δ*, *siz2Δ rad52Δ*, or *rad52Δ* cells on plates containing various concentrations of bleomycin. Fivefold serial dilutions were spotted on each plate, from 5×10^{-3} to 10^{-4} cells. These images are representative of at least four independent experiments.

Table S1. **Yeast strains used in this study**

Strains	Genotype	Source
W303	*Mat a, ura3-1, leu2-3, his3-11, trp1-1, ade2-1*	NA
rad52Δ	*Mat a, ura3-1, leu2-3, his3-11, trp1-1, ade2-1, rad52::TRP1*	This study
mre11Δ rad52Δ	*Mat a, ura3-1, leu2-3, his3-11, trp1-1,ade2-1, mre11::KAN rad52::TRP1*	This study
xrs2Δ rad52Δ	*Mat a, ura3-1, leu2-3, his3-11, trp1-1,ade2-1, xrs2::KAN rad52::TRP1*	This study
tel1Δ rad52Δ	*Mat a, ura3-1, leu2-3, his3-11, trp1-1,ade2-1, tel1::KAN rad52::TRP1*	This study
sml1Δ rad52Δ	*Mat a, ura3-1, leu2-3, his3-11, trp1-1,ade2-1, sml1::KAN rad52::TRP1*	This study
sml1Δ rad52Δ mec1Δ	*Mat a, ura3-1, leu2-3, his3-11, trp1-1, ade2-1, sml1 mec1::KAN rad52::TRP1*	This study
rad51Δ	*Mat a, ura3-1, leu2-3, his3-11, trp1-1, ade2-1, rad51::TRP1*	This study
Rfa1-GFP	*Mata, ura3-1, leu2-3, his3-11, trp1-1, ade2-1,RFA1-GFP::KAN*	This study
Rfa1-GFP *rad52Δ*	*Mata, ura3-1, leu2-3, his3-11, trp1-1,ade2-1,RFA1-GFP::KAN, rad52::TRP1*	This study
Rfa1-GFP *siz1Δ*	*Mata, ura3-1, leu2-3, his3-11, trp1-1, ade2-1, RFA1-GFP::TRP1, siz1::KAN*	This study
Cdc13-GFP *rad52Δ*	*Mat a, ura3-1, leu2-3, his3-11, trp1-1, ade2-1, CDC13-GFP::KAN rad52::TRP1*	This study
Cdc13-GFP Rfa1-mCherry *rad52Δ*	*Mat a, ura3-1, leu2-3, his3-11, trp1-1,ade2-1, CDC13-GFP::KAN RFA1-mCherry::TRP1 rad52::hygro*	This study
Cdc13-myc *rad52Δ*	*Mat a, ura3-1, leu2-3, his3-11, trp1-1,ade2-1, CDC13-13Myc rad52:: TRP1*	This study
Cdc13-myc *siz1Δ rad52Δ*	*Mat a, ura3-1, leu2-3, his3-11, trp1-1,ade2-1, siz1::TRP1, CDC13-13Myc::KAN rad52:: HYGRO*	This study
Cdc13-myc *rad51Δ*	*Mat a, ura3-1, leu2-3, his3-11, trp1-1,ade2-1, CDC13-13Myc rad51:: TRP1*	This study
DVL162	*MAT **a** ura3-52 ade2-101, trp1Δ-1, his3-Δ200 leu2-Δ1cdc13-Δ::LYS2CF-SUP11-TRP1/ pVL 438)*	V. Lundblad (Salk Institute, San Diego, CA)
DVL162 *rad52Δ*	*MAT **a** ura3-52 ade2-101, trp1Δ-1, his3-Δ200 leu2-Δ1cdc13-Δ::LYS2CF- SUP11-TRP1 rad52::KAN*	This study
JC1323	*Mat a, ura3-1, leu2-3 leu2-112 Rad5+, his3-11 his3-15, trp1-1,ade2-, siz1::TRP1; can1-100;*	J. Cobb (University of Calgary, Calgary, Canada)
JC1323 *rad52Δ*	*Mat a, ura3-1, leu2-3 leu2-112 Rad5+, his3-11 his3-15, trp1-1,ade2-, siz1::TRP1; can1-100; rad52::HYGRO*	This study
JC1322	*Mat a, ura3-1, leu2-3 leu2-112 Rad5+, his3-11 his3-15, trp1-1,ade2-, siz2::HIS3; can1-100;*	J. Cobb
JC1322 *rad52Δ*	*Mat a, ura3-1, leu2-3 leu2-112 Rad5+, his3-11 his3-15, trp1-1,ade2-, siz2::HIS3; can1-100; rad52::HYGRO*	This study
RDKY3615	*MAT**a** ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3*	R. Kolodner (Ludwig Institute for Cancer Research, San Diego, CA)
RDKY4343	*MATa ura3::KAN leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 pif1-m2*	R. Kolodner
RDKY3615 *rad52Δ*	RDKY3615 *rad52::TRP1*	This study
RDKY3615 *siz1Δ*	RDKY3615 *siz1::HYGRO*	This study
RDKY3615 *rad52Δ siz1Δ*	RDKY3615 *rad52::TRP1 siz1::HYGRO*	This study
RDKY3615 Rfa1-GFP	RDKY3615 *RFA1-GFP::TRP1*	This study
RDKY4343 *rad52Δ*	RDKY4343 *rad52::TRP1*	This study
RDKY4343 *siz1Δ*	RDKY4343 *siz1::HYGRO*	This study
RDKY4343 *siz1Δ rad52Δ*	RDKY4343 *siz1::HYGRO rad52::TRP1*	This study
RDKY4343 Rfa1-GFP	RDKY4343 *RFA1-GFP::TRP1*	This study
RDKY4343 Rfa1-GFP *rad52Δ*	RDKY4343 *RFA1-GFP::TRP1 rad52::HYGRO*	This study
B365-17A	*Mat trp1-1 can1-100 ura3-1 leu2-3,112 ade2::hisG-URA3-hisG his3—ade2-5′Δ--TRP1—ade2-n-his3 rad51::HIS3 rad52::TRP1*	L. Symington (Columbia University, New York, NY)
B365-7A	*Mat trp1-1 can1-100 ura3-1 leu2-3,112 ade2::hisG his3- ade2-5′Δ--TRP1—ade2-n-his3 rad51::HIS3*	L. Symington
B365-9A	*MatA trp1-1 can1-100 ura3-1 leu2-3,112 ade2::hisG his3- ade2-5′Δ--TRP1—ade2-n-his3 rad52::TRP1*	L. Symington
DDY2472	*S288C GAL-HO (leu) URA3-TG81-HOcs-LYS2 rad52::his*	D. Durocher (Lunenfeld-Tanenbaum Research Institute, Toronto, Canada)
DDY2472 *siz1Δ*	DDY2472 *siz1::KAN*	This study

Table S2. **Statistical analysis of Fig. 4 B (nucleoplasmic localization of TLC1 RNA)**

Strain	P-value
rad52Δ versus *rad52Δ mre11Δ*	0.0002
rad52Δ versus *rad52Δ xrs2Δ*	0.0001
rad52Δ versus *rad52Δ tel1Δ*	0.0002
rad52Δ versus *rad52Δ sml1Δ*	Not significant
rad52Δ versus *rad52Δ sml1Δ mec1Δ*	0.02
rad52Δ sml1Δ versus *rad52Δ sml1Δ mec1Δ*	0.13
rad52Δ versus *rad52Δ cdc13-2*	0.0013

Provided online is Table S3 in Excel, which lists all de novo telomere additions found with the Illumina sequencing approach.