RESEARCH ARTICLE

Figures and figure supplements

Cdc13 is predominant over Stn1 and Ten1 in preventing chromosome end fusions

Zhi-Jing Wu *et al*

Figure 1. Successive passages of single-chromosome yeast strains SY14 and SY15 do not display growth change. (**A**) Schematic of single chromosome structure in yeast strains SY14 and SY15. Single linear and circular chromosomes of the SY14 and SY15 strains are respectively aligned in the outer and inner rings. The single circular chromosome of SY15 lacks telomeres of Chr X-R and Chr XVI-L in SY14. (**B,C**) Growth analysis of the SY14 (**B**) and SY15 (**C**) strains. Several clones of the SY14 and SY15 strains were re-streaked on YPD plates 63 times at intervals of two days. (**D,E**) Growth curves of the SY14 (**D**) and SY15 (**E**) clones at the 3rd and 63rd re-streaks. Error bars represent standard deviation (s.d.), n = 3. (**F**) Telomere southern blotting assay of

Figure 1 continued on next page

Figure 1 continued

the SY14 cells at different passages (labeled on top). At each passage, three independent clones were examined. The genomic DNA of the SY14 cells was digested by XhoI and subjected to Southern hybridization with a telomere-specific TG_{1-3} probe.

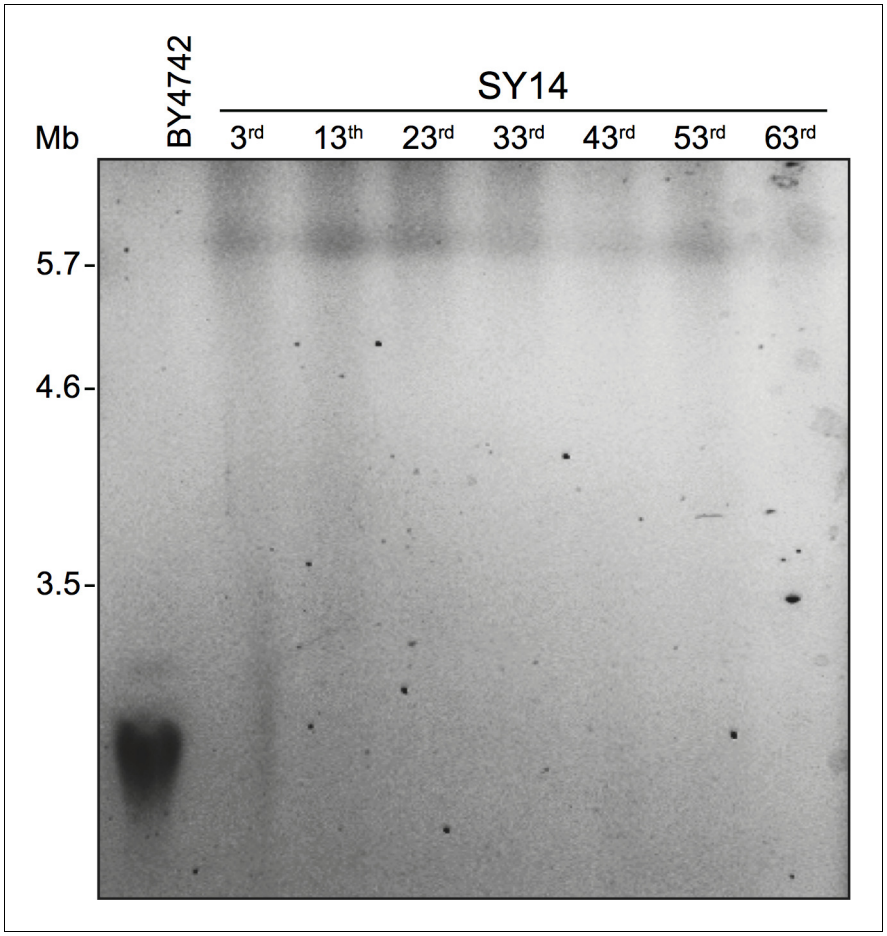


Figure 1—figure supplement 1. The single chromosome in SY14 remains intact during the passages. Fresh clones of the SY14 strain at different re-streaks (labeled on top) were inoculated into YPD and cultured overnight at 30˚C, and pulsed-field gel electrophoresis (PFGE) analysis was performed. Wild-type strain BY4742 is a control.

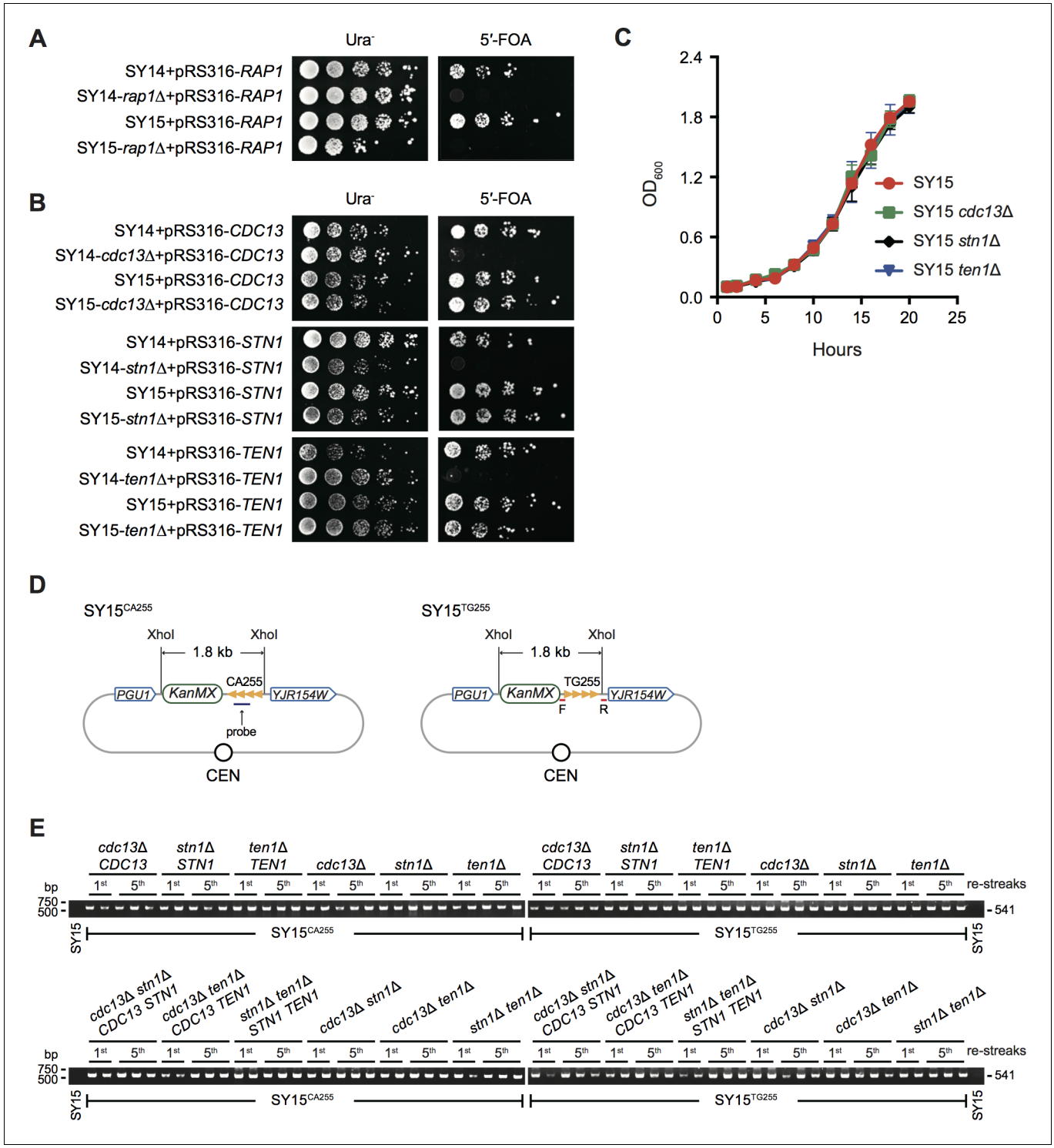


Figure 2. CST complex is not essential in single-circular-chromosome yeast strain SY15. (**A**) Growth analysis of *rap1Δ* mutants. SY14 *rap1Δ RAP1* and SY15 *rap1Δ RAP1* cells were spotted with five-fold dilutions on Ura⁻ medium (left panel) to select for the presence of pRS316-*RAP1* plasmid, and on 5′-FOA medium (right panel) to select for eviction of pRS316-*RAP1* plasmid. (**B**) Cell growth assay of *cdc13Δ*, *stn1Δ* and *ten1Δ* cells. SY14 *cdc13Δ CDC13*, SY15 *cdc13Δ CDC13*, SY14 *stn1Δ STN1*, SY15 *stn1Δ STN1*, SY14 *ten1Δ TEN1* and SY15 *ten1Δ TEN1* cells were spotted with five-fold dilutions on Ura⁻ medium (left panel) to select for the presence of pRS316 plasmids that contained a wild-type CST gene, and on 5′-FOA medium (right panel) to select for eviction of pRS316 plasmids. (**C**) Growth analysis of SY15 *cdc13Δ*, SY15 *stn1Δ* and SY15 *ten1Δ* cells. Three clones of each strain were examined. Error bars represent standard deviation (s.d.), n = 3. (**D**) Schematic representation of TG255/CA255 sequence insertion in the genomic loci of $SY15^{CA255}$ and $SY15^{TG255}$ strains. In single-circular-chromosome yeast SY15, a 255 bp telomeric sequence in both orientations (named CA255 or TG255, tandem

Figure 2 continued on next page

Figure 2 continued

orange triangles) is inserted between the *PGU1* and *YJR154W* genes. The TG probe for Southern blotting (***Figure 2—figure supplement 1***) and primer pairs for PCR-sequencing (**E**) are indicated in purple and red, respectively. The *KanMX* gene serves as a genetic marker for the integration of the telomeric tracts. The XhoI sites are used for restriction digestion in Southern blotting examining the insertions, which were ~1.8 kb in both $SY15^{CA255}$ and $SY15^{TG255}$ strains. This figure is not precisely drawn to scale. (**E**) Analysis of non-terminal telomere sequences by PCR. $SY15^{CA255}$ and $SY15^{TG255}$ strains (indicated at the bottom of each panel) were passaged on plates five times at intervals of two days. The genomic DNA was isolated from the 1st and 5th re-streaks (labeled at the top of each panel). Primers (5'-TCGACATCATCTGCCCAGAT-3' and 5'-AGTTCGAACTAGGGTAATTG-3') were used to amplify the DNA fragments flanking inserted telomeric sequence, and the PCR products were examined on agarose gels. Two or three independent clones of each genotype were examined.

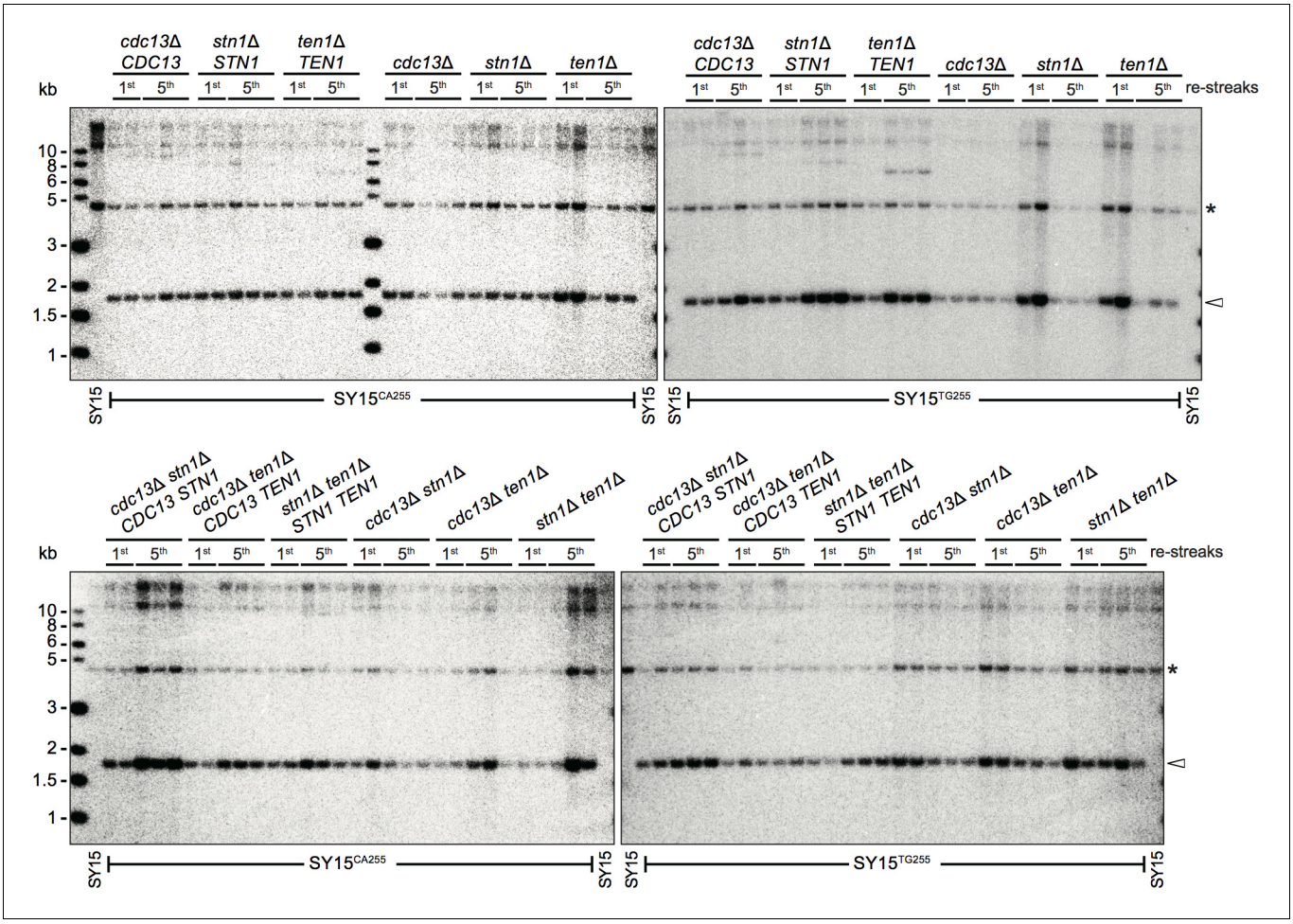


Figure 2—figure supplement 1. Southern blotting to determine the insertion of TG255/CA255 sequence in SY15^{CA255} and SY15^{TG255} strains. SY15^{CA255} and SY15^{TG255} strains (labeled at the top of each panel) were passaged on plates five times at intervals of two days. The genomic DNA was isolated from the 1st and 5th re-streaks (labeled at the top of each panel), and digested by XhoI and subjected to a Southern blotting analysis using a TG_{1-3} probe. At each passage, two or three independent clones were examined. The arrow at right indicates insertion and asterisk marks a non-specific band.

A

SY15^{CA255} *cdc13*Δ-1st

TTTTTCTAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTT
TTCGCCTCGACATCATCTGCCCAGATGCGAAGTTAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGATACTAACGCC
GCCATCCAGTTTAAACGAGCTCGAATTCGAGCTCGGTACCCACACCACACCCACACCCACACACCACACACCACACCCACACACCACACCCACACCCACACACCACACCCA
CACCACACCCACACACCCACACCCACACACCACACCCACACACCACACCCACACACCACACCCACACACCACACCCACACACCACACCCACACCACACCCACACACCACACCC
ACACACCACACCCACACCACACCCACATCCACACACCCACACACCCACACACCCACACCCACACCCACACACTCTCTCACATCTACCTCTACTCTGGGAATTCCTCGAGCCT
TCGCCATAGTTCGTGATGAAAACTTTACGGTACGTCAGACTTTAGATATTGA

SY15^{CA255} *cdc13*Δ-5th

Clone #1

TTTTTCTAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTT
TTCGCCTCGACATCATCTGCCCAGATGCGAAGTTAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGATACTAACGCC
GCCATCCAGTTTAAACGAGCTCGAATTCGAGCTCGGTACCCACACCACACCCACACCCACACACCACACACCACACCCACACACCACACCCACACCCACACACCACACCCA
CACCACACCCACACACCCACACCCACACACCACACCCACACACCACACCCACACACCACACCCACACACCACACCCACACACCACACCCACACCACACCCACACACCACACCC
ACACACCACACCCACACCACACCCACATCCACACACCCACACACCCACACACCCACACCCACACCCACACACTCTCTCACATCTACCTCTACTCTGGGAATTCCTCGAGCCT
TCGCCATAGTTCGTGATGAAAACTTTACGGTACGTCAGACTTTAGATATTGA

Clone #2

TTTTTCTAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTT
TTCGCCTCGACATCATCTGCCCAGATGCGAAGTTAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGATACTAACGCC
GCCATCCAGTTTAAACGAGCTCGAATTCGAGCTCGGTACCCACACCACACCCACACCCACACACCACACACCACACCCACACACCACACCCACACCCACACACCACACCCA
CACCACACCCACACACCCACACCCACACACCACACCCACACACCACACCCACACACCACACCCACACACCACACCCACACACCACACCCACACCACACCCACACACCACACCC
ACACACCACACCCACACCACACCCACATCCACACACCCACACACCCACACACCCACACCCACACCCACACACTCTCTCACATCTACCTCTACTCTGGGAATTCCTCGAGCCT
TCGCCATAGTTCGTGATGAAAACTTTACGGTACGTCAGACTTTAGATATTGA

Clone #3

TTTTTCTAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTT
TTCGCCTCGACATCATCTGCCCAGATGCGAAGTTAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGATACTAACGCC
GCCATCCAGTTTAAACGAGCTCGAATTCGAGCTCGGTACCCACACCACACCCACACCCACACACCACACACCACACCCACACACCACACCCACACCCACACACCACACCCA
CACCACACCCACACACCCACACCCACACACCACACCCACACACCACACCCACACACCACACCCACACACCACACCCACACACCACACCCACACCACACCCACACACCACACCC
ACACACCACACCCACACCACACCCACATCCACACACCCACACACCCACACACCCACACCCACACCCACACACTCTCTCACATCTACCTCTACTCTGGGAATTCCTCGAGCCT
TCGCCATAGTTCGTGATGAAAACTTTACGGTACGTCAGACTTTAGATATTGA

B

SY15^{TG255} *cdc13*Δ-1st

TTTTTCTAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTT
TTCGCCTCGACATCATCTGCCCAGATGCGAAGTTAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGATACTAACGCC
GCCATCCAGTTTAAACGAGCTCGAATTCCCAGAGTAGAGGTAGATGTGAGAGAGTGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTGTGGGTGTGTGGATGTGGGTGTGGT
GTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTG
GTGGTGTGGGTGTGGTGTGGGTGTGGTGTGTGGGTGTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGGGTGTGGTGTGGGTGTG
GGTGTGTGGGTGTGGTGTGGGTGTGGTGTGTGGGTGTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGGGTGTGGTGTGGGTGTG
GGTACCGAGCTCGAATTCCTCGAGCCTTCGCCATAGTTCGTGATGAAAACTTTACGGTACGTCAGACTTTAGATATTGA

SY15^{TG255} *cdc13*Δ-5th

Clone #1

TTTTTCTAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTT
TTCGCCTCGACATCATCTGCCCAGATGCGAAGTTAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGATACTAACGCC
GCCATCCAGTTTAAACGAGCTCGAATTCCCAGAGTAGAGGTAGATGTGAGAGAGTGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTGTGGGTGTGTGGATGTGGGTGTGGT
GTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTG
GGTGTGTGGGTGTGGTGTGGGTGTGGTGTGTGGGTGTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGGGTGTGGTGTGGGTGTG
GGTACCGAGCTCGAATTCCTCGAGCCTTCGCCATAGTTCGTGATGAAAACTTTACGGTACGTCAGACTTTAGATATTGA

Clone #2

TTTTTCTAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTT
TTCGCCTCGACATCATCTGCCCAGATGCGAAGTTAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGATACTAACGCC
GCCATCCAGTTTAAACGAGCTCGAATTCCCAGAGTAGAGGTAGATGTGAGAGAGTGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTGTGGGTGTGTGGATGTGGGTGTGGT
GTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTG
GGTGTGTGGGTGTGGTGTGGGTGTGGTGTGTGGGTGTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGGGTGTGGTGTGGGTGTG
GGTACCGAGCTCGAATTCCTCGAGCCTTCGCCATAGTTCGTGATGAAAACTTTACGGTACGTCAGACTTTAGATATTGA

Clone #3

TTTTTCTAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTT
TTCGCCTCGACATCATCTGCCCAGATGCGAAGTTAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGATACTAACGCC
GCCATCCAGTTTAAACGAGCTCGAATTCCCAGAGTAGAGGTAGATGTGAGAGAGTGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTGTGGGTGTGTGGATGTGGGTGTGGT
GTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTG
GGTGTGTGGGTGTGGTGTGGGTGTGGTGTGTGGGTGTGGGTGTGGTGTGTGGGTGTGGTGTGTGGGTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGGGTGTGGTGTGGGTGTG
GGTACCGAGCTCGAATTCCTCGAGCCTTCGCCATAGTTCGTGATGAAAACTTTACGGTACGTCAGACTTTAGATATTGA

Figure 2—figure supplement 2. The full sequences of the clones of SY15^{CA255} *cdc13*Δ (**A**) and SY15^{TG255} *cdc13*Δ (**B**) cells at different passages were shown as representatives. The sequences comprise part of the *KanMX* marker (in green), a 255 bp telomeric tracts (in orange) and the upstream sequence of gene *YJR154W* (in grey). The XhoI restriction site is underlined. All the sequences of insertions in the progenies of either SY15^{CA255} *cst*Δ or SY15^{TG255}*cst*Δ mutants are the same, indicating that they are very stable. Thus other results are omitted.

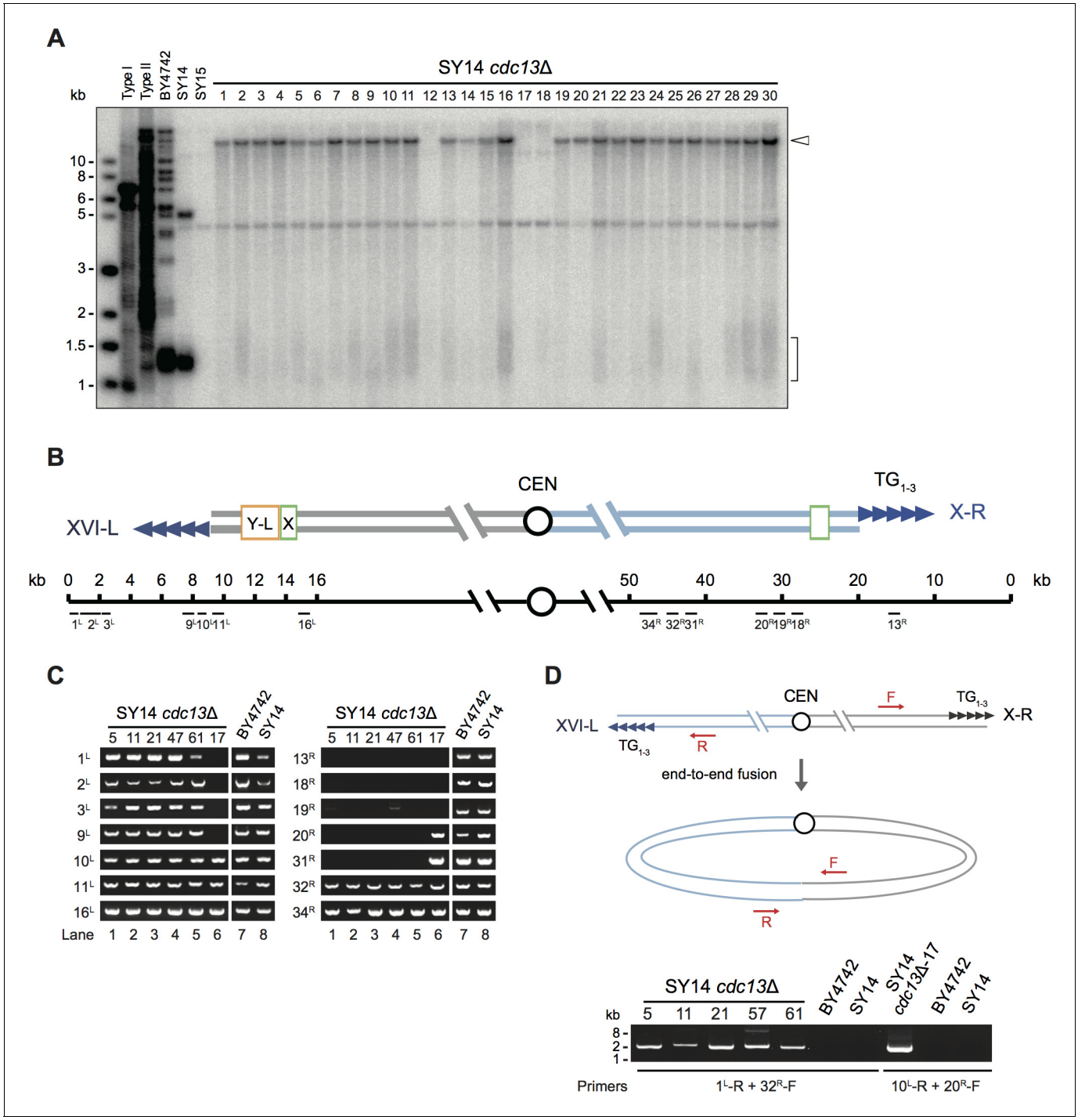


Figure 3. Survivors of SY14 *cdc13Δ* mutant contain a circularized chromosome. (**A**) Telomere Southern blotting analysis. 30 independent SY14 *cdc13Δ* colonies (labeled on top) were randomly picked, and their DNA was subjected to a telomere Southern blotting analysis to examine telomere structure. The bracket indicates Y′ telomere signals and the open arrowhead indicates the band of ~15 kb emerged in most of the clones except clones 12, 17 and 18. (**B**) Schematic representation of two chromosome arms of XVI-L and X-R in SY14 strain. Boxes in light green and yellow adjacent to telomeres (tandem blue triangles) represent subtelomeric X element and Y′-L element respectively. The numbers above the schematic line (chromosome) indicate the distance to the corresponding telomeric TG_{1-3} sequences of XVI-L and X-R (not in precise scale). Black bars labeled 1^{L}-16^{L} or 13^{R}-34^{R} (under the schematic line) indicate the position of PCR primers that were used to examine either chromosomal end erosion. (**C**) Examples of PCR mapping results that define the borders of telomere erosion in SY14 *cdc13Δ* survivors. The primer pairs (shown in (**B**)) are indicated on left in each panel. The clone numbers of SY14 *cdc13Δ* are indicated on top in each panel. Primer sequences are listed in ***Supplementary file 1***. (**D**) PCR examination of chromosome end-to-end fusion. Different pairs of primers (indicated at the bottom) were used to amplify the DNA fragments flanking the fusion points. The clone numbers of SY14 *cdc13Δ* are indicated on top.

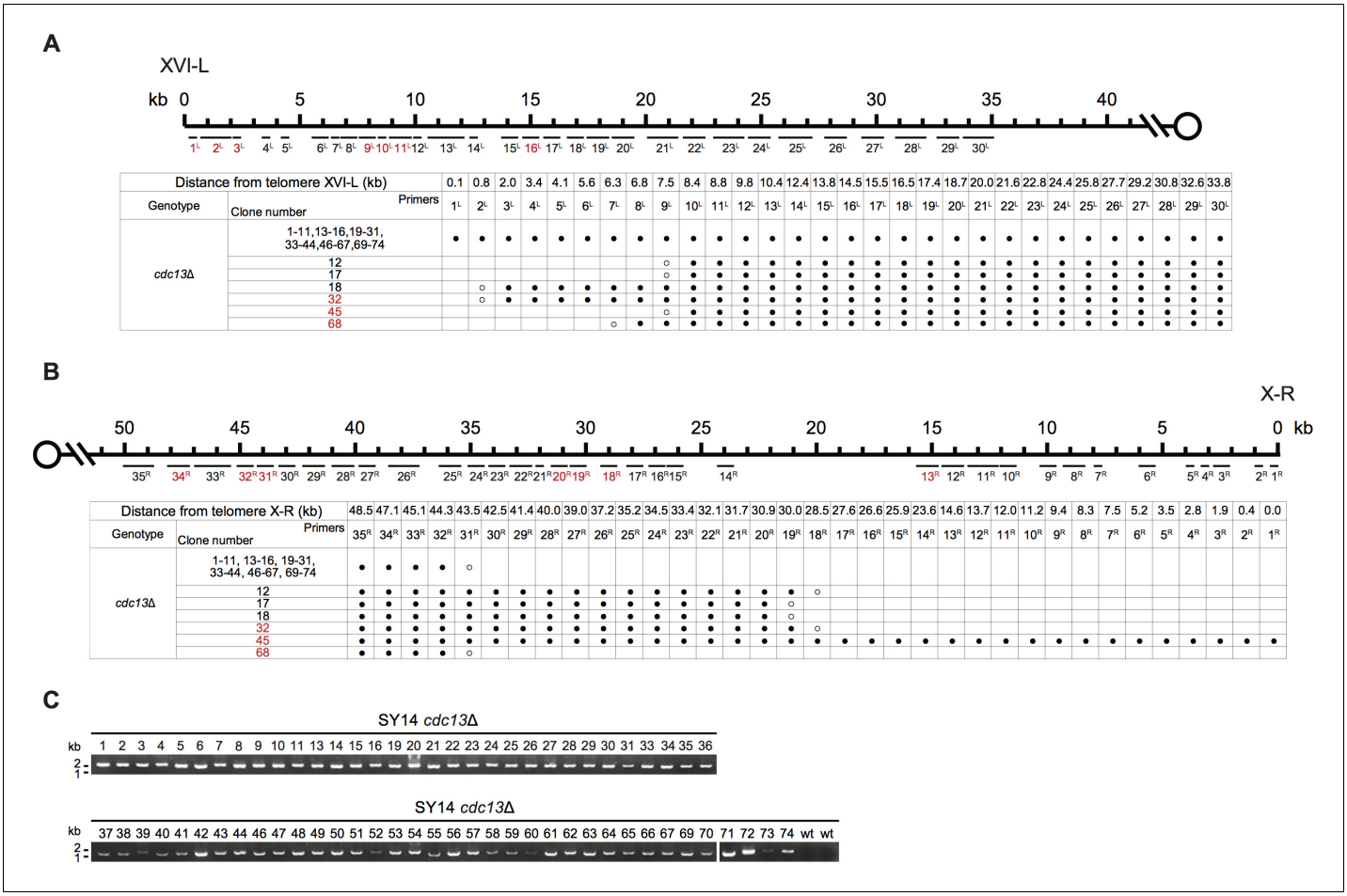


Figure 3—figure supplement 1. Borders of erosion and rTG Type of SY14 *cdc13Δ* survivors are defined by mapping and PCR amplification. (**A**) Upper panel, schematic diagram of the subtelomeric region of 0–40 kb proximal to Chr XVI-L telomeric TG_{1-3} sequence is shown. Primer pairs (No. 1^L to 30^L) are aligned and indicated at their corresponding subtelomeric loci. Lower panel, all 74 clones of SY14 *cdc13Δ* survivors are listed on left, primer pairs are listed on top, primers in red are primer pairs used in ***Figure 3B***; solid circles mean positive PCR products, and open circles mean no PCR products with corresponding primer pairs. The clone numbers in red are not-identified (NI) survivors. (**B**) Upper panel, schematic diagram of the subtelomeric region of 0–50 kb proximal to Chr X-R telomeric TG_{1-3} sequence is shown. Primer pairs (No. 1^R to 35^R) are aligned and indicated at their corresponding subtelomeric loci. Lower panel, all 74 clones of SY14 *cdc13Δ* survivors are listed on left, primer pairs are listed on top, primers in red are primer pairs used in ***Figure 3B***; solid circles mean positive PCR products, and open circles mean no PCR products with corresponding primer pairs. The clone numbers in red are not-identified (NI) survivors. (**C**) Primer pairs of 1^L-R and 32^R-F were used to amplify the DNA fragments flanking the fusion points in SY14 *cdc13Δ* survivors. PCR products were examined on agarose gels. The genotype and clone numbers were indicated on top.

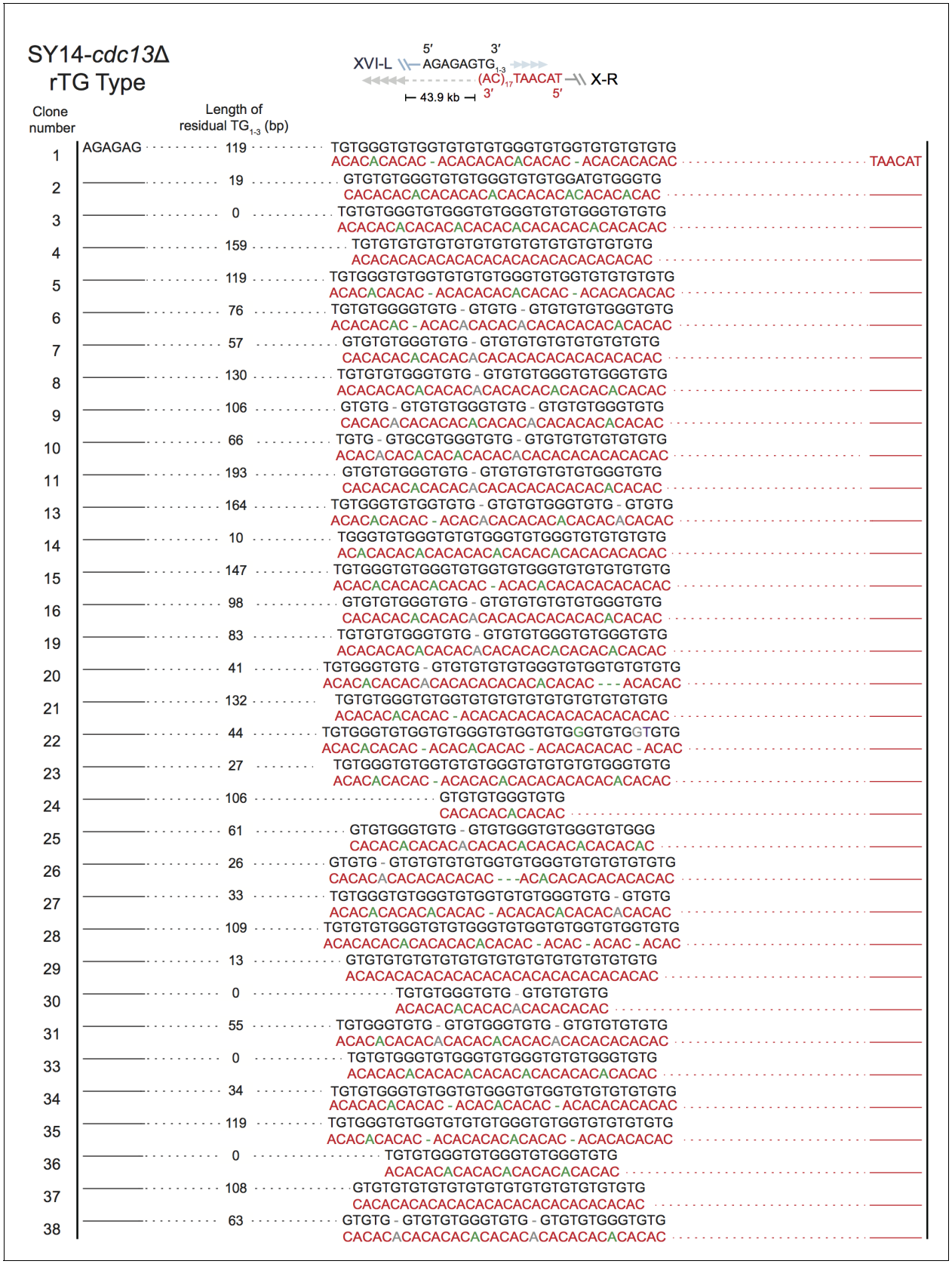


Figure 3—figure supplement 2. Fusion junctions in most SY14 *cdc13Δ* survivors contain TG sequences (rTG Type). The sequences at the junction of 68 independent clones (labeled on left) of SY14 *cdc13Δ* survivors are determined: ***Figure 3—figure supplement 2***, clone 1 to 38; ***Figure 3—figure***

Figure 3—figure supplement 2 continued on next page

Figure 3—figure supplement 2 continued

supplement 3, clone 39-74. Top panel depicts the fusion points of rTG Type survivors. In each clone, sequence in black indicates the sequence of Chr XVL-L, sequence in red indicates the sequence of Chr X-R, bases in green are mis-paired, bases in grey or dashes are deleted. In each clone, the length of the residual TG_{1-3} sequence proximal to junction point of Chr XVI-L is also shown.

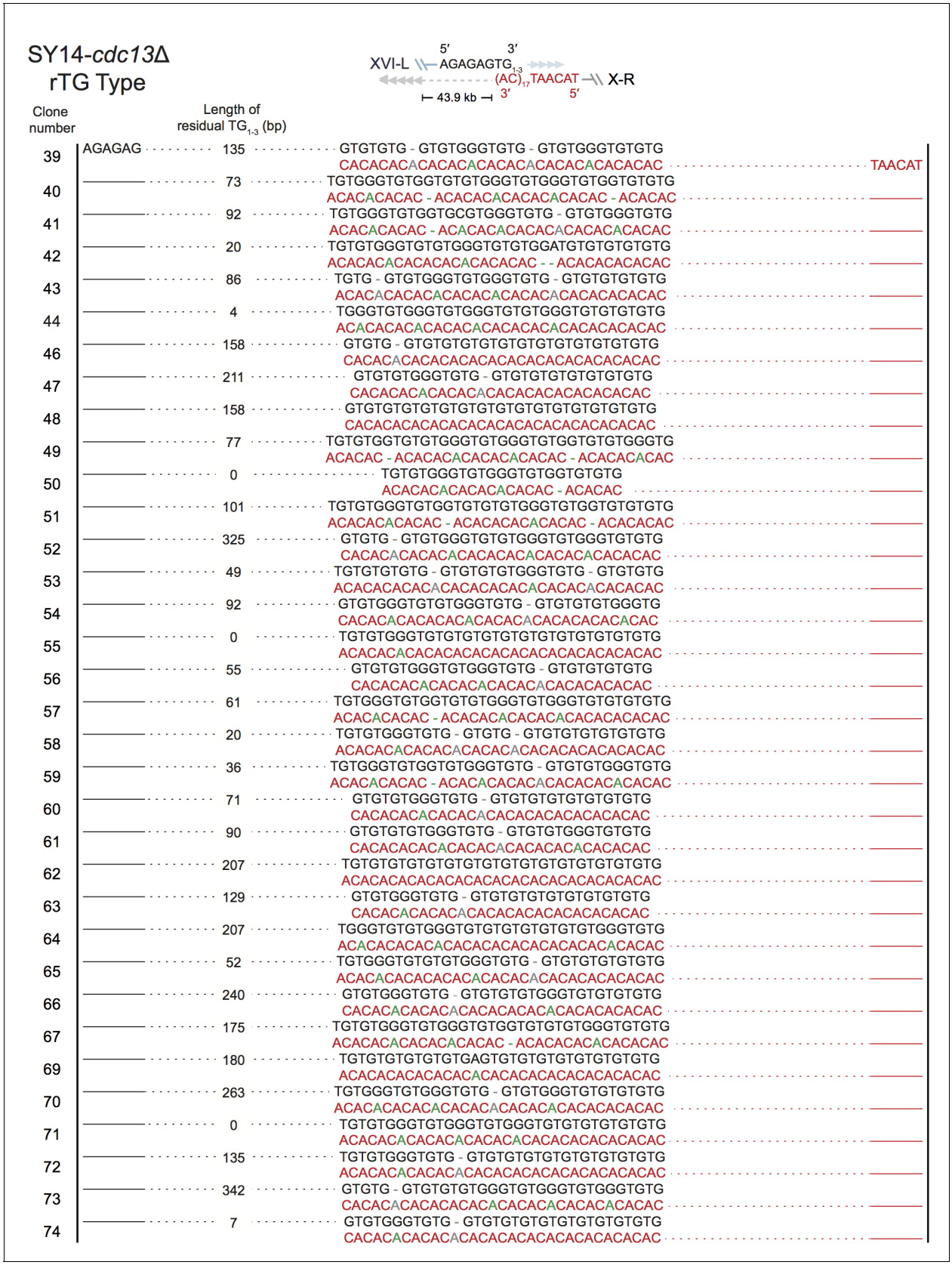


Figure 3—figure supplement 3. Fusion junctions in most SY14 *cdc13Δ* survivors contain TG sequences (rTG Type).

Non-TG Type	Fusion junction sequence	Genotype	Clone number
1	XVI-L 5′ CCACTCTATCTCCATCTGACGAAAGAGTCAAC 3′ ⊢8.3 kb⊣ X-R ⊢30.0 kb⊣ 3′ ATAGAGGAATGCTACTTTCTCAGTTGGAATAA 5′	*cdc13*Δ	12
2	XVI-L 5′ TGCTGAAGCTGTTTTCAACTATGGTGACTTCACCACCATGTTGACTGGTATT 3′ ⊢8.2 kb⊣ X-R ⊢30.1 kb⊣ 3′ TCGGCAAAAACTGATGCCACTAAAGTGATGAGCTAACTGGCCATAAAGCCCA 5′	*cdc13*Δ	17
3	XVI-L 5′ TACCGACTCCAAC 3′ ⊢1.7 kb⊣ X-R ⊢30.4 kb⊣ 3′ GATGTTGGAGATG 5′	*cdc13*Δ	18

Figure 3—figure supplement 4. Fusion junction sequences of non-TG Type survivors derived from SY14 *cdc13*Δ mutants. The genotype and clone numbers are listed on right. Sequences at the fusion junctions of three independent clones are shown. According to the fusion sequences, they were be classified into three types (listed on left).

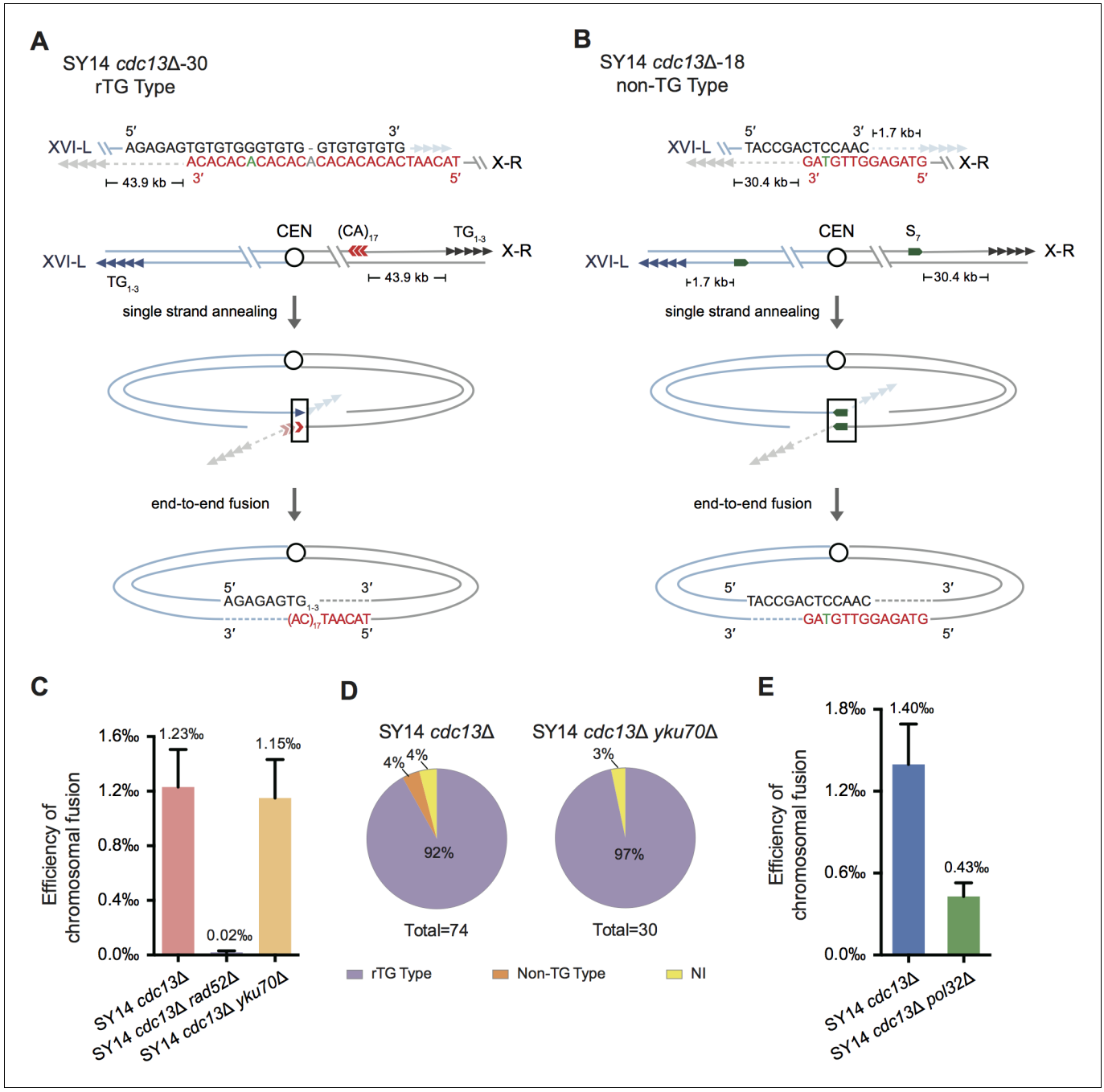


Figure 4. Chromosome fusion of SY14 *cdc13Δ* cells is nearly eliminated in the absence of Rad52. (**A**) Schematic of rTG Type survivors in SY14 *cdc13Δ*. In SY14 *cdc13Δ* clone 30, the fusion region of TG_{1-3} sequence (in black) is in Chr XVI-L, and the $(CA)_{17}$ region (in red) locates 43.9 kb away from of Chr X-R. Bases in green are mis-paired, bases in grey or dashes are deleted. (**B**) Schematic of non-TG Type survivors in SY14 *cdc13Δ*. In SY14 *cdc13Δ* clone 18, the fusion sequence of CTCCAAC (in black) is 1.7 kb away from Chr XVI-L telomere, and the fusion sequence of GTTGTAG (in red) is 30.4 kb away from of Chr X-R telomere. Bases in green are mis-paired. (**C**) Quantification of survivor generation rates of SY14 *cdc13Δ* (1.23‰), SY14 *cdc13Δ rad52Δ* (0.02‰) and SY14 *cdc13Δ yku70Δ* (1.15‰) cells. Error bars represent standard deviation (s.d.), n = 3. (**D**) Percentage of rTG Type, non-TG Type and not-identified (NI) survivors in SY14 *cdc13Δ* (n = 74) and SY14 *cdc13Δ yku70Δ* (n = 30) strains. (**E**) Quantification of survivor generation rates of SY14 *cdc13Δ* (1.40‰) and SY14 *cdc13Δ pol32Δ* (0.43‰) cells. Error bars represent standard deviation (s.d.), n = 3.

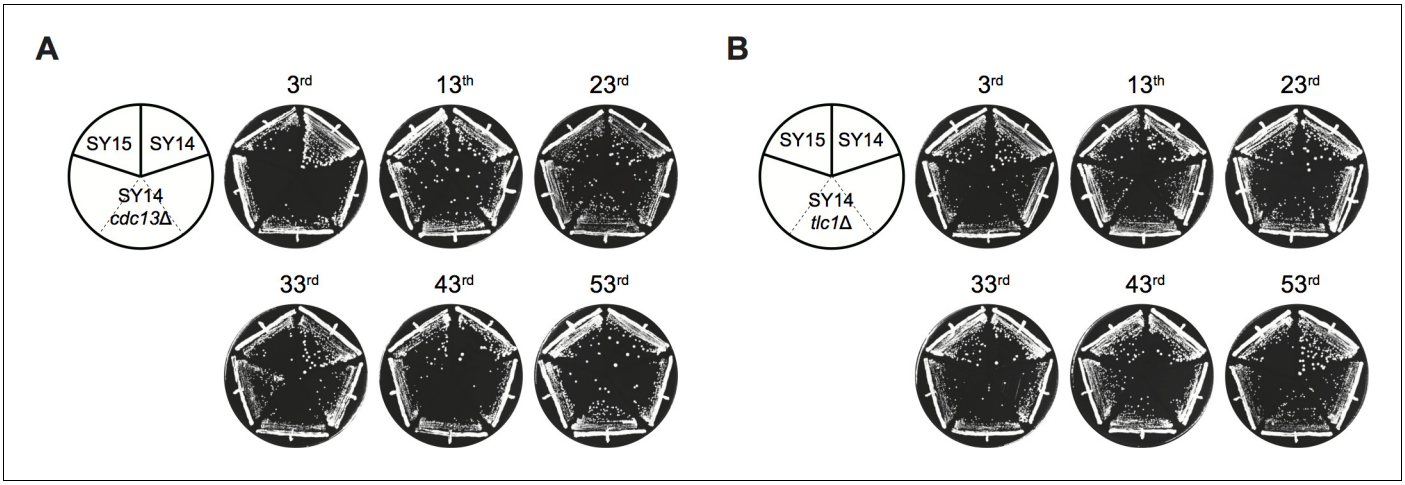


Figure 4—figure supplement 1. Survivors harboring circular chromosome maintain a stable genome. Growth analysis of SY14 *cdc13*Δ (**A**) and SY14 *tlc1*Δ (**B**) survivors. A few clones of each strain were re-streaked at intervals of three days on YPD plates for 53 times.

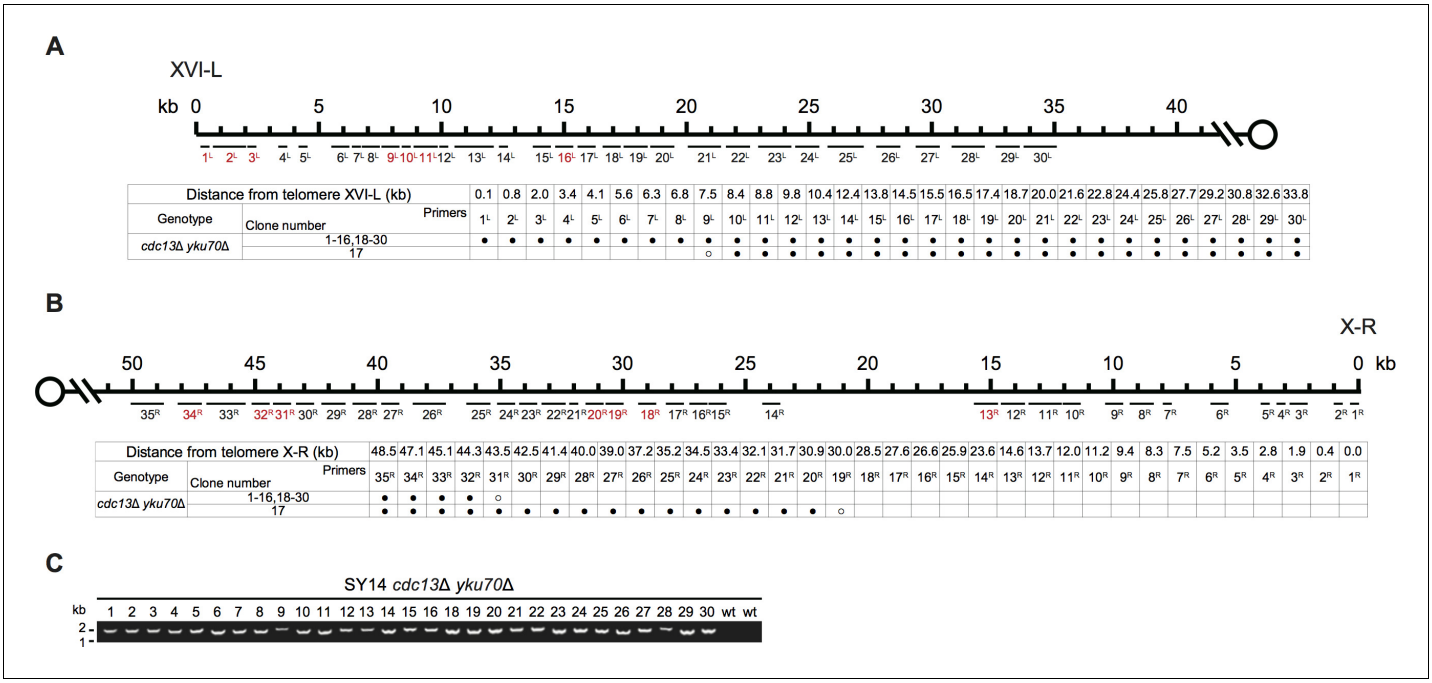


Figure 4—figure supplement 2. Borders of erosion (**A** and **B**) and rTG Type (**C**) of SY14 *cdc13Δ yku70Δ* survivors are defined by mapping and PCR amplification.

Figure 4—figure supplement 3. Fusion junctions of rTG Type in SY14 *cdc13Δ yku70Δ* survivors. The sequences at the junction of 29 independent clones (labeled on left) of SY14 cdc13Δ *yku70Δ* survivors are determined. In each clone, the length of the residual TG_{1-3} sequence proximal to junction point of Chr XVI-L is also shown.

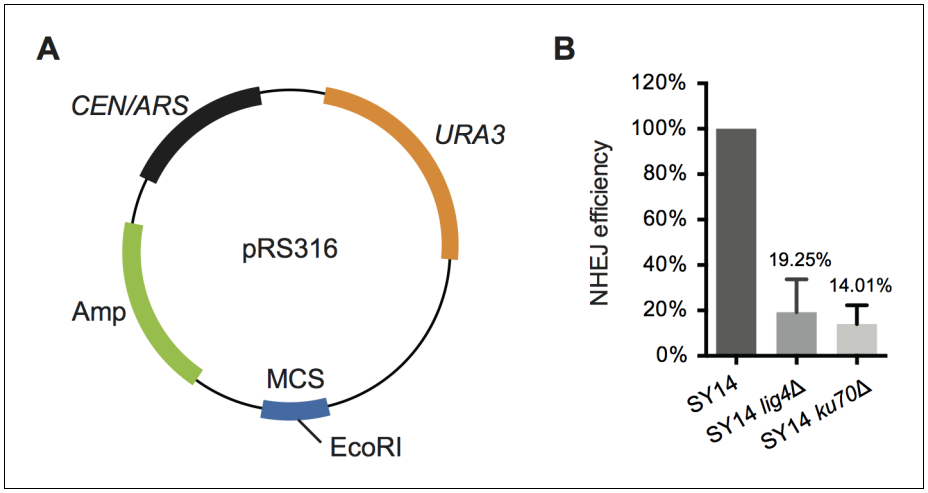


Figure 4—figure supplement 4. NHEJ pathway is still functional in single-linear-chromosome yeast SY14. (**A**) Map of the test plasmid substrate pRS316. The ampicillin marker, CEN/ARS cassette, *URA3* marker and the multiple cloning site (MCS) containing EcoRI recognition sites are shown in green, black, orange and blue, respectively. (**B**) NHEJ efficiency of SY14, SY14 *lig4*Δ and SY14 *yku70*Δ strains. Data are the average of three independent experiments normalized to SY14, which was set to 100%. The error bars indicate the standard deviations (s.d.), n = 4.

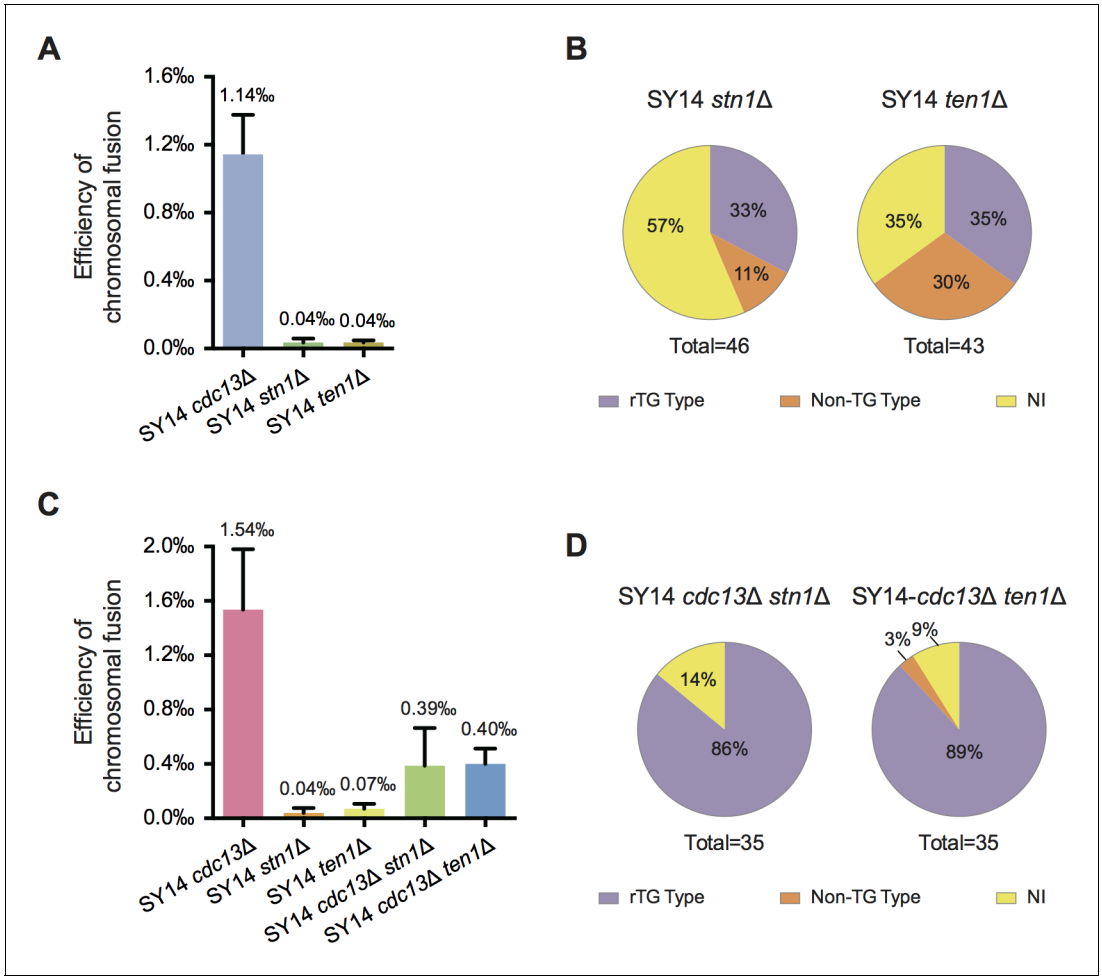
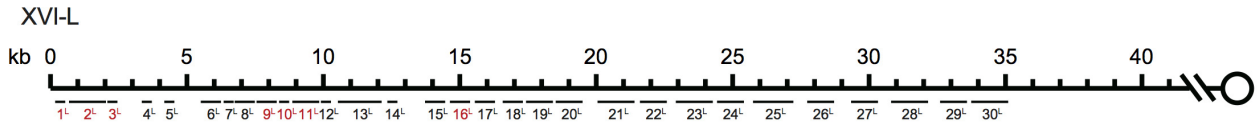


Figure 5. Chromosome fusion frequency in either SY14 *stn1*Δ or SY14 *ten1*Δ cells is much lower than that in SY14 *cdc13*Δ cells. (**A**) Quantification of survivor generation rates of SY14 *cdc13*Δ (1.14‰), SY14 *stn1*Δ (0.04‰) and SY14 *ten1*Δ (0.04‰) cells. Error bars represent standard deviation (s.d.), n = 3. (**B**) Percentage of rTG Type, non-TG Type and not-identified (NI) survivors in SY14 *stn1*Δ (n = 46) and SY14 *ten1*Δ (n = 43) cells. (**C**) Quantification of survivor generation rates of SY14 *cdc13*Δ (1.54‰), SY14 *stn1*Δ (0.04‰), SY14 *ten1*Δ (0.07‰), SY14 *cdc13*Δ *stn1*Δ (0.39‰) and SY14 *cdc13*Δ *ten1*Δ (0.40‰) cells. Error bars represent standard deviation (s.d.), n = 3. (**D**) Percentage of rTG Type, non-TG Type and not-identified (NI) survivors in SY14 *cdc13*Δ *stn1*Δ (n = 35) and SY14 *cdc13*Δ *ten1*Δ (n = 35) cells.



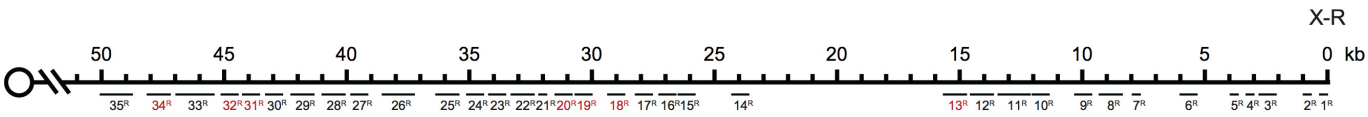
A — *stn1Δ*

Distance from telomere XVI-L (kb)	0.1	0.8	2.0	3.4	4.1	5.6	6.3	6.8	7.5	8.4	8.8	9.8	10.4	12.4	13.8	14.5	15.5	16.5	17.4	18.7	20.0	21.6	22.8	24.4	25.8	27.7	29.2	30.8	32.6	33.8
Primers / Clone number	1L	2L	3L	4L	5L	6L	7L	8L	9L	10L	11L	12L	13L	14L	15L	16L	17L	18L	19L	20L	21L	22L	23L	24L	25L	26L	27L	28L	29L	30L
6, 8,12,16,20,21,22, 25,26,28,30,32,41,42,43	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
1			○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
2		○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
3									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
4									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
5									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
7	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
9	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
10									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
11	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
13	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
14									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
15	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
17	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
18									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
19,46									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
23		○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
24									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
27								○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
29	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
31	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
33	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
34	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
35									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
36	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
37	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
38	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
39	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
40									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
44	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
45									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

B — *ten1Δ*

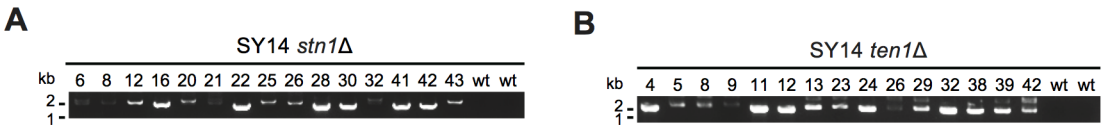
Distance from telomere XVI-L (kb)	0.1	0.8	2.0	3.4	4.1	5.6	6.3	6.8	7.5	8.4	8.8	9.8	10.4	12.4	13.8	14.5	15.5	16.5	17.4	18.7	20.0	21.6	22.8	24.4	25.8	27.7	29.2	30.8	32.6	33.8
Primers / Clone number	1L	2L	3L	4L	5L	6L	7L	8L	9L	10L	11L	12L	13L	14L	15L	16L	17L	18L	19L	20L	21L	22L	23L	24L	25L	26L	27L	28L	29L	30L
4,5,8,9,11,12,13, 23,24,26,29,32,38,39,42	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
1,2									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
3									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
6,10,37									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
7											○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
14											○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
15											○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
16	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
17	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
18	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
19,21,22,25,34									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
20				○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
27											○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
28											○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
30											○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
31											○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
33								○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
35									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
36									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
40											○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
41											○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
43											○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

Figure 5—figure supplement 1. PCR mapping of the borders of Chr XVI-L erosion in SY14 *stn1Δ* (**A**) and *ten1Δ* (**B**) survivors.



	Genotype	Distance from telomere X-R (kb)	48.5	47.1	45.1	44.3	43.5	42.5	41.4	40.0	39.0	37.2	35.2	34.5	33.4	32.1	31.7	30.9	30.0	28.5	27.6	26.6	25.9	23.6	14.6	13.7	12.0	11.2	9.4	8.3	7.5	5.2	3.5	2.8	1.9	0.4	0.0
		Clone number \ Primers	35R	34R	33R	32R	31R	30R	29R	28R	27R	26R	25R	24R	23R	22R	21R	20R	19R	18R	17R	16R	15R	14R	13R	12R	11R	10R	9R	8R	7R	6R	5R	4R	3R	2R	1R
A	*stn1Δ*	6,8,12,16,20,21,22, 25,26,28,30,32,41,42,43	●	●	●	●	○																														
		1	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○
		2	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																		
		3	●	○																																	
		4	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																		
		5	●	○																																	
		7	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																		
		9	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																			
		10	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																		
		11	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																			
		13	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																			
		14	●	○																																	
		15	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○						
		17	●	●	●	●	●	●	●	●	●	●	○																								
		18	●	○																																	
		19,46	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																		
		23	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○		
		24	●	●	●	●	●	●	●	●	●	●	○																								
		27	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○
		29	●	●	●	●	●	●	●	●	●	●	○																								
		31	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																		
		33	●	●	●	●	●	●	●	●	●	●	○																								
		34	●	●	●	●	●	●	●	●	●	●	○																								
		35	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																				
		36	●	●	●	●	●	●	●	●	●	○																									
		37	●	●	●	●	●	●	●	●	●	○																									
		38	●	●	●	●	●	●	●	●	●	○																									
		39	●	●	●	●	●	●	●	●	○																										
		40	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																				
		44	●	●	●	●	●	●	●	●	●	●	○																								
		45	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																				
B	*ten1Δ*	4,5,8,9,11,12,13,23, 24,26,29,32,38,39,42	●	●	●	●	○																														
		1,2	●	●	●	●	●	●	●	●	●	●	○																								
		3	●	●	●	●	●	●	●	●	●	●	○																								
		6,10,37	●	●	●	●	●	●	●	●	●	●	○																								
		7	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																		
		14	●	●	●	●	●	●	●	●	●	●	○																								
		15	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○													
		16	●	●	●	●	●	●	●	●	○																										
		17	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○						
		18	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																		
		19,21,22,25,34	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																		
		20	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
		27	●	●	●	●	●	●	●	●	●	●	○																								
		28	●	●	●	●	●	●	●	●	●	●	○																								
		30	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																	
		31	●	●	●	●	●	●	●	●	●	●	●	○																							
		33	●	●	●	●	●	●	●	●	●	●	●	○																							
		35	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																		
		36	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																		
		40	●	●	●	●	●	●	●	●	●	●	○																								
		41	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																	
		43	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																	

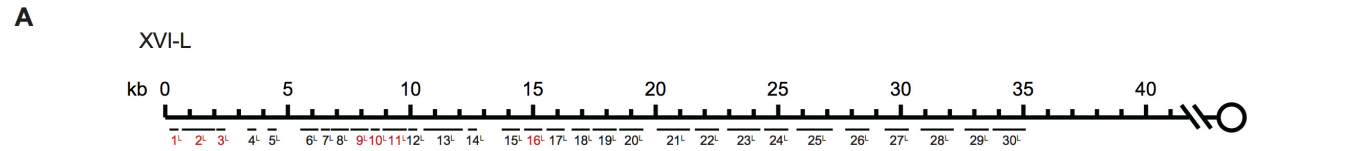
Figure 5—figure supplement 2. PCR mapping of the borders of Chr X-R erosion in SY14 *stn1*Δ (**A**) and *ten1*Δ (**B**) survivors.



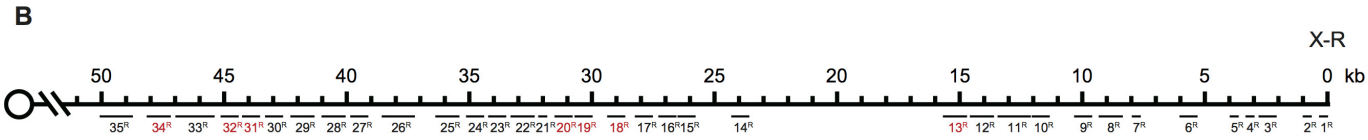
C

Non-TG Type	Fusion junction sequence	Genotype	Clone number
1	XVI-L 5′ ATGCCACTACCACT 3′ ⊢1.6 kb⊣ GATGGTGAGGATGT 5′ X-R ⊢30.5 kb⊣ 3′	*stn1Δ*	2
2	XVI-L 5′ AGAGTCATCACTGGTGTCCCATGGTACTCTACCAGATTGAGACCGGCTATCTC 3′ ⊢ 8.1 kb ⊣ TAGTGACCACAAGGCACCATGAGATGGTCTAATTTCGGCCGATAGAGAAGTCG 5′ X-R ⊢30.2 kb⊣ 3′	*stn1Δ*	19 46
3	XVI-L 5′ ATTATGCAAGGGCTGTCAA 3′ ⊢1.3 kb⊣ GTACCCGACGGTTCTTCGC 5′ X-R ⊢2.1 kb⊣ 3′	*stn1Δ*	23
4	XVI-L 5′ CAACTATGGTGACTTCACCACCATGTTGACTGGTATT 3′ ⊢ 8.2 kb ⊣ ACCGCTAAAGTGGTGGTACAACTGACCATAAAGTCCA 5′ X-R ⊢36.2 kb⊣ 3′	*stn1Δ*	24
5	XVI-L 5′ CAACTATGGTGACTTCACCACCATGTTGACTGGTATT 3′ ⊢ 8.2 kb ⊣ ACCGCTAAAGTGGTGGTACAACTGACCATAAAGTCCA 5′ X-R ⊢36.2 kb⊣ 3′	*ten1Δ*	1 2
6	XVI-L 5′ GAGACCTACCCAGTTGAGATTGCTGAAGCTGTTTT 3′ ⊢ 8.2 kb ⊣ ATGGGTGGACTTTAACGGTTCCGACAAAAACGGCC 5′ X-R ⊢36.2 kb⊣ 3′	*ten1Δ*	3
7	XVI-L 5′ TCTGACGAAAGAGTCAACTTGGTCGAATTGGGTGTTTACGTCTCCGATAT 3′ ⊢ 8.3 kb ⊣ CTTTCCCAGTTAAACTAACTTAACCGACAAATGCAAAGACTATAACCGCG 5′ X-R ⊢36.1 kb⊣ 3′	*ten1Δ*	6 10 37
8	XVI-L 5′ AGAGTCATCACTGGTGTCCCATGGTACTCTACCAGATTGAGACCGGCTATCTC 3′ ⊢ 8.1 kb ⊣ TAGTGACCACAAGGCACCATGAGATGGTCTAATTTCGGCCGATAGAGAAGTCG 5′ X-R ⊢30.2 kb⊣ 3′	*ten1Δ*	19 21 22 25 34
9	XVI-L 5′ TGCTGAAGCTGTTTTCAACTATGGTGACTTCACCACCATGTTGACTGGTATT 3′ ⊢ 8.2 kb ⊣ TCGGCAAAAACTGATGCCACTAAAGTGATGAGCTAACTGGCCATAAAGCCCA 5′ X-R ⊢30.1 kb⊣ 3′	*ten1Δ*	35
10	XVI-L 5′ CTATCTAAGGACGGTATCTACACTGCTATTCCAA 3′ ⊢ 8.1 kb ⊣ TTCCTACCATAAATGTGCCGATAGGGTTGGAGAT 5′ X-R ⊢30.3 kb⊣ 3′	*ten1Δ*	36

Figure 5—figure supplement 3. Determination of rTG Type survivors by PCR (**A** and **B**) and fusion junction sequences of non-TG Type survivors (**C**) in SY14 *stn1Δ* and SY14 *ten1Δ* mutants.

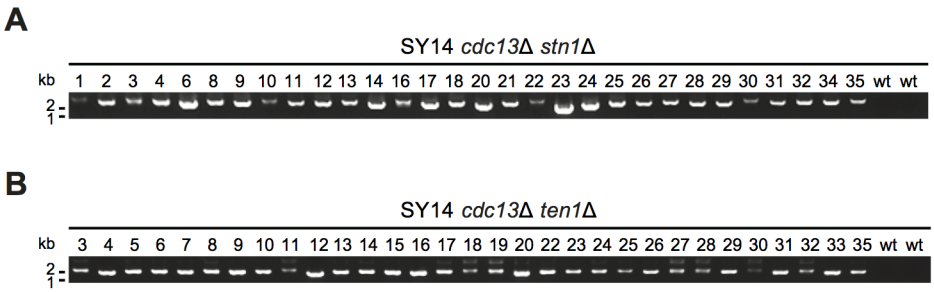


Genotype	Clone number \ Primers	1L	2L	3L	4L	5L	6L	7L	8L	9L	10L	11L	12L	13L	14L	15L	16L	17L	18L	19L	20L	21L	22L	23L	24L	25L	26L	27L	28L	29L	30L
Distance from telomere XVI-L (kb)		0.1	0.8	2.0	3.4	4.1	5.6	6.3	6.8	7.5	8.4	8.8	9.8	10.4	12.4	13.8	14.5	15.5	16.5	17.4	18.7	20.0	21.6	22.8	24.4	25.8	27.7	29.2	30.8	32.6	33.8
cdc13Δ stn1Δ	1-4,6,8-14, 16-18,20-32,34,35	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	5									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	7									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	15											○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	19		○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	33	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
cdc13Δ ten1Δ	3-20,22-33,35	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	1		○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	2	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	21									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	34	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●



Genotype	Clone number \ Primers	35R	34R	33R	32R	31R	30R	29R	28R	27R	26R	25R	24R	23R	22R	21R	20R	19R	18R	17R	16R	15R	14R	13R	12R	11R	10R	9R	8R	7R	6R	5R	4R	3R	2R	1R
Distance from telomere X-R (kb)		48.5	47.1	45.1	44.3	43.5	42.5	41.4	40.0	39.0	37.2	35.2	34.5	33.4	32.1	31.7	30.9	30.0	28.5	27.6	26.6	25.9	23.6	14.6	13.7	12.0	11.2	9.4	8.3	7.5	5.2	3.5	2.8	1.9	0.4	0.0
cdc13Δ stn1Δ	1-4,6,8-14, 16-18,20-32,34,35	●	●	●	●	○																														
	5	●	●	●	●	○																														
	7	●	●	●	●	○																														
	15	●	●	●	●	○																														
	19	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	33	●	●	●	○																															
cdc13Δ ten1Δ	3-20,22-33,35	●	●	●	●	○																														
	1	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	2	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○								
	21	●	●	●	●	●	●	●	●	●	●	○																								
	34	●	●	●	●	○																														

Figure 5—figure supplement 4. PCR mapping of the borders of Chr XVI-L erosion (**A**) and Chr X-R erosion (**B**) in SY14 *cdc13Δ stn1Δ* and *cdc13Δ ten1Δ* survivors.



C

Non-TG Type	Fusion junction sequence	Genotype	Clone number
1	XVI-L 5′ AGAGTCATCACTGGTGTCCCATGGTACTCTACCAGATTGA 3′ 8.1 kb 36.3 kb 3′ TAGTGGCCACACGGTACCATGAGATGATCTAACTACCCAC 5′ X-R	*cdc13Δ ten1Δ*	21

Figure 5—figure supplement 5. Determination of rTG Type survivors by PCR (**A**) in *cdc13Δ stn1Δ* and *cdc13Δ ten1Δ* survivors and fusion junction sequences of non-TG Type survivors (**B**) derived from SY14 *cdc13Δ ten1Δ* mutants.

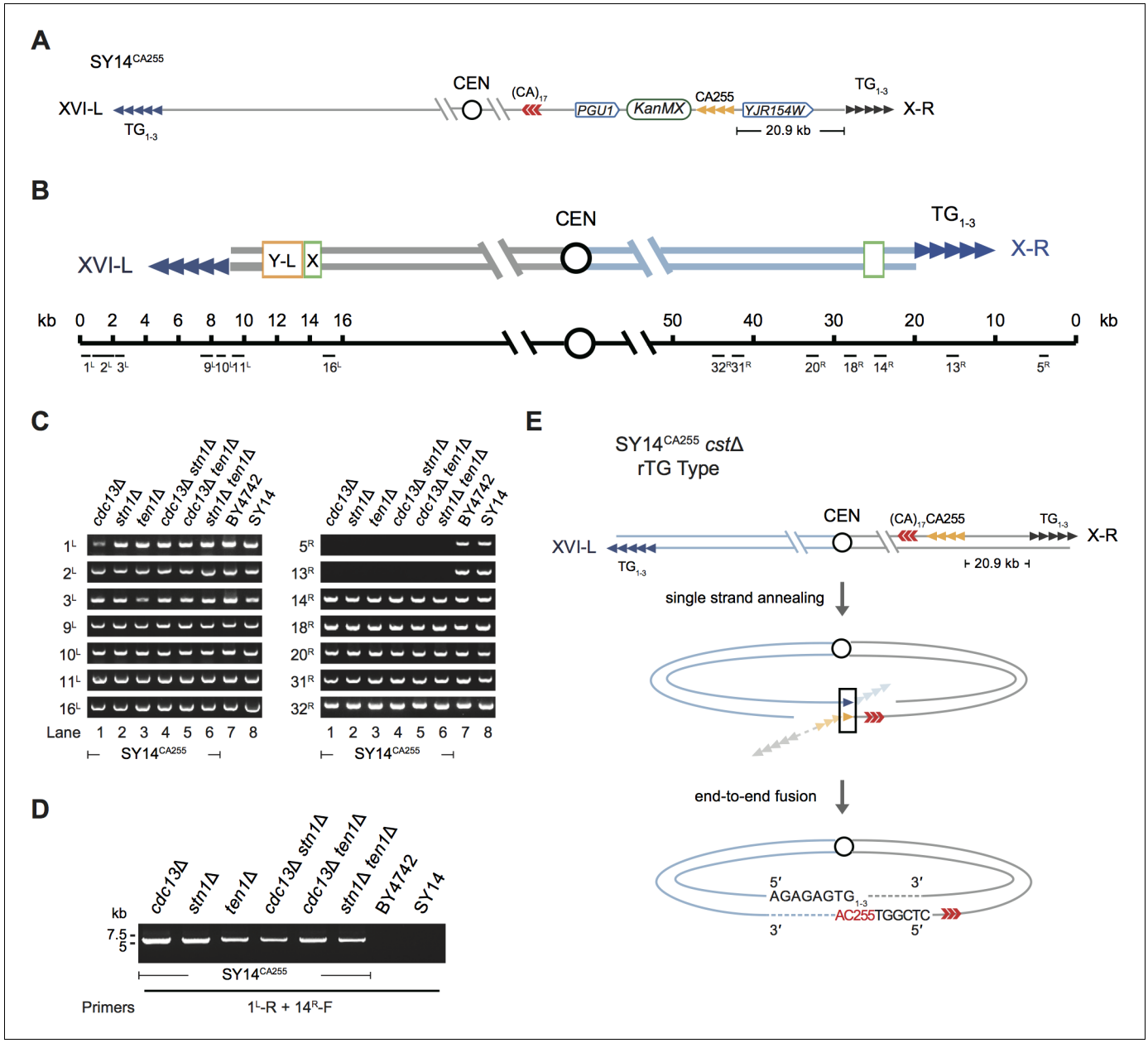


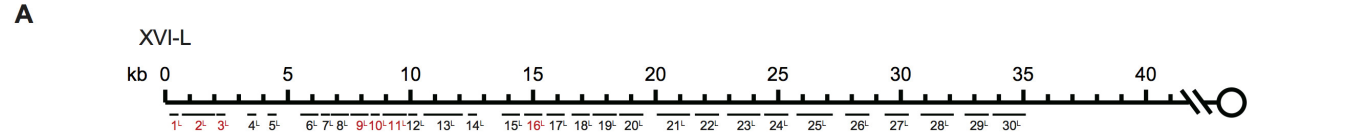
Figure 5—figure supplement 6. SY14^{CA255} *cstΔ* survived cells utilize CA255 sequence for chromosomal circularization. (**A**) Schematic representation of SY14^{CA255} strain. A 255 bp telomeric sequence (named CA255, tandem orange triangles) is inserted between the *PGU1* and *YJR154W* genes. These two genes are located at the right arm of Chr X, and the inserted telomere sequence is 20.9 kb away from telomeric TG_{1-3} sequence (tandem grey triangles) in SY14. The *KanMX* gene serves as a genetic marker for the integration of the telomeric tracts. This figure is not precisely drawn to scale. (**B**) Schematic representation of two chromosome arms of XVI-L and X-R in SY14 strain. Boxes in light green and yellow adjacent to telomeres (tandem blue triangles) represent subtelomeric X element and Y′-L element respectively. The numbers above the schematic line (chromosome) indicate the distance to the corresponding telomeric TG_{1-3} sequences of XVI-L and X-R (not in precise scale). Black bars labeled 1 ^{L}-16^{L} or 5 ^{R}-32^{R} (under the schematic line) indicate the position of PCR primers that were used to examine either chromosomal end erosion. (**C**) Examples of PCR mapping results that define the borders of telomere erosion in SY14^{CA255} *cstΔ* survivors. The primer pairs (shown in (**B**)) are indicated on left in each panel. The clone numbers of SY14^{CA255} *cstΔ* are indicated on top in each panel. Primer sequences are listed in ***Supplementary file 1***. (**D**) PCR examination of chromosome end-to-end fusion. A pair of primers (indicated at the bottom) were used to amplify the DNA fragments flanking the fusion points. The genotypes of SY14^{CA255} *cstΔ* are indicated on top. (**E**) Schematic of rTG Type survivors in SY14^{CA255} *cstΔ* cells. The CA255 region and '5′-$(CA)_{17}$-3′' repeat are shown in orange and red, respectively.

Figure 6. Telomerase inactivation in SY14 cells results in senescence and survivor formation. (**A**) Senescence assay in liquid medium. The growth (OD_{600}) of SY14 (green), SY14 *tlc1*Δ (three clones in pink, red and orange), SY15 (black) and SY15 *tlc1*Δ (blue) strains were monitored every 24 hr for 16 days. (**B**) Telomeric Southern blotting assay. Genomic DNA of the SY14 *tlc1*Δ and SY15 *tlc1*Δ strain examined in (**A**) were digested by XhoI and subjected to a Southern blotting analysis. The bracket indicates Y′ telomere signals. (**C**) Senescence assay of the SY14 *tlc1*Δ and SY15 *tlc1*Δ strains on solid medium. After eviction of the pRS316-*TLC1* plasmid in SY14 *tlc1*Δ *TLC1* or SY15 *tlc1*Δ *TLC1* strains by 5′-FOA selection, two independent SY14 *tlc1*Δ and SY15 *tlc1*Δ clones were re-streaked eight times to allow survivors to form. SY14 and SY15 were controls. (**D**) Telomere Southern blotting analysis of SY14 *tlc1*Δ survivors obtained on solid medium. 50 independent survivor clones (labeled 1 to 50 on top) were randomly picked, and their

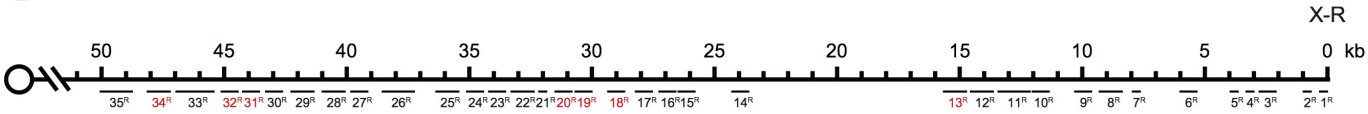
Figure 6 continued on next page

Figure 6 continued

genomic DNA was subjected to Southern blotting assay with a telomeric TG_{1-3} probe. The clone numbers in red are non-TG Type survivors. The clone numbers in blue are not-identified survivors. The bracket indicates Y′ telomere signals and the open arrowhead indicates the new band of ~15 kb emerged in the majority of survivors.

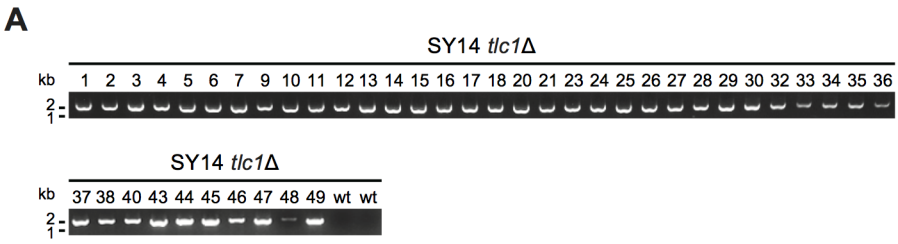


Distance from telomere XVI-L (kb)		0.1	0.8	2.0	3.4	4.1	5.6	6.3	6.8	7.5	8.4	8.8	9.8	10.4	12.4	13.8	14.5	15.5	16.5	17.4	18.7	20.0	21.6	22.8	24.4	25.8	27.7	29.2	30.8	32.6	33.8
Genotype	Primers / Clone number	1L	2L	3L	4L	5L	6L	7L	8L	9L	10L	11L	12L	13L	14L	15L	16L	17L	18L	19L	20L	21L	22L	23L	24L	25L	26L	27L	28L	29L	30L
tlc1Δ	1-7,9-18,20,21, 23-30,32-38,40,43-49	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	8	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	19	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	22									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	31									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	39									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	41									○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	42																○	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	50	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●



Distance from telomere X-R (kb)		48.5	47.1	45.1	44.3	43.5	42.5	41.4	40.0	39.0	37.2	35.2	34.5	33.4	32.1	31.7	30.9	30.0	28.5	27.6	26.6	25.9	23.6	14.6	13.7	12.0	11.2	9.4	8.3	7.5	5.2	3.5	2.8	1.9	0.4	0.0
Genotype	Primers / Clone number	35R	34R	33R	32R	31R	30R	29R	28R	27R	26R	25R	24R	23R	22R	21R	20R	19R	18R	17R	16R	15R	14R	13R	12R	11R	10R	9R	8R	7R	6R	5R	4R	3R	2R	1R
tlc1Δ	1-7,9-18,20,21, 23-30,32-38,40,43-49	●	●	●	●	○																														
	8	●	●	●	●	●	●	●	●	●	●	○																								
	19	●	●	●	●	●	●	●	●	●	●	○																								
	22	●	●	●	●	●	●	●	●	●	●	○																								
	31	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																		
	39	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																		
	41	●	●	●	●	●	●	●	●	●	●	○																								
	42	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○																	
	50	●	●	●	●	●	●	●	●	●	●	●	●	●	○																					

Figure 6—figure supplement 1. PCR mapping of the borders of Chr XVI-L erosion (**A**) and Chr X-R erosion (**B**) in SY14 *tlc1Δ* survivors.



B

Non-TG Type	Fusion junction sequence	Genotype	Clone number
1	XVI-L 5′ TGCTGAAGCTGTTTTCAACTATGGTGACTTCACCACCATGTTGACTGGTATT 3′ ⊢ 8.2 kb ⊣ X-R TCGGCAAAAACTGATGCCACTAAAGTGATGAGCTAACTGGCCATAAAGCCCA 5′ ⊢30.1 kb⊣ 3′	*tlc1*Δ	31 39
2	XVI-L 5′ CAACTATGGTGACTTCACCACCATGTTGACTGGTATT 3′ ⊢ 8.2 kb ⊣ X-R ACCGCTAAAGTGGTGGTACAACTGACCATAAAGTCCA 5′ ⊢36.2 kb⊣ 3′	*tlc1*Δ	22 41

Figure 6—figure supplement 2. Determination of rTG Type survivors by PCR (**A**) and fusion junction sequences of non-TG Type survivors (**B**) of SY14 *tlc1*Δ mutants.

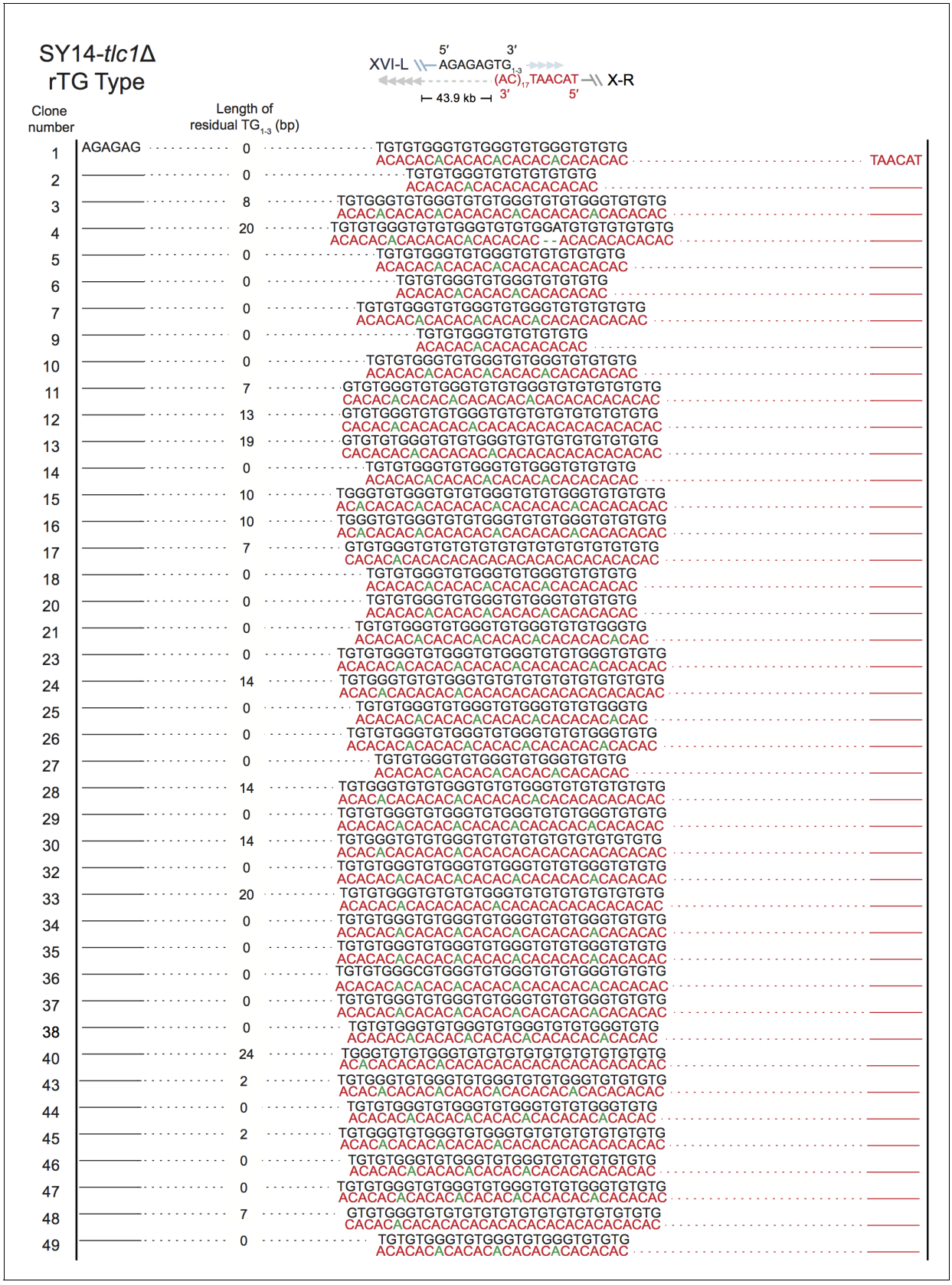

SY14-tlc1Δ
rTG Type
5′ 3′
XVI-L AGAGAGTG1-3
(AC)17TAACAT X-R
43.9 kb 3′ 5′
Clone number
Length of residual TG1-3 (bp)
1 AGAGAG 0 TGTGTGGGTGTGGGTGTGGGTGTGTG ACACACACACACACACACACACACAC TAACAT
2 0 TGTGTGGGTGTGTGTGTGTG ACACACACACACACACACAC
3 8 TGTGGGTGTGGGTGTGTGGGTGTGTGGGTGTGTG ACACACACACACACACACACACACACACACAC
4 20 TGTGTGGGTGTGTGGGTGTGTGGATGTGTGTGTGTG ACACACACACACACACACACAC - - ACACACACACAC
5 0 TGTGTGGGTGTGGGTGTGTGTGTGTG ACACACACACACACACACACACACACAC
6 0 TGTGTGGGTGTGGGTGTGTGTG ACACACACACACACACACACAC
7 0 TGTGTGGGTGTGGGTGTGGGTGTGTGTGTG ACACACACACACACACACACACACACACACAC
9 0 TGTGTGGGTGTGTGTGTG ACACACACACACACACAC
10 0 TGTGTGGGTGTGGGTGTGGGTGTGTGTG ACACACACACACACACACACACACACAC
11 7 GTGTGGGTGTGGGTGTGTGGGTGTGTGTGTGTG CACACACACACACACACACACACACACACACAC
12 13 GTGTGGGTGTGTGGGTGTGTGTGTGTGTGTGTG CACACACACACACACACACACACACACACACAC
13 19 GTGTGTGGGTGTGTGGGTGTGTGTGTGTGTGTG CACACACACACACACACACACACACACACACACAC
14 0 TGTGTGGGTGTGGGTGTGGGTGTGTGTG ACACACACACACACACACACACACACACAC
15 10 TGGGTGTGGGTGTGTGGGTGTGTGGGTGTGTGTG ACACACACACACACACACACACACACACACACAC
16 10 TGGGTGTGGGTGTGTGGGTGTGTGGGTGTGTGTG ACACACACACACACACACACACACACACACACAC
17 7 GTGTGGGTGTGTGTGTGTGTGTGTGTGTGTGTG CACACACACACACACACACACACACACACACACAC
18 0 TGTGTGGGTGTGGGTGTGGGTGTGTGTG ACACACACACACACACACACACACACACAC
20 0 TGTGTGGGTGTGGGTGTGGGTGTGTGTG ACACACACACACACACACACACACACACAC
21 0 TGTGTGGGTGTGGGTGTGGGTGTGTGGGTG ACACACACACACACACACACACACACACACAC
23 0 TGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTGTG ACACACACACACACACACACACACACACACACAC
24 14 TGTGGGTGTGTGGGTGTGTGTGTGTGTGTGTGTG ACACACACACACACACACACACACACACACACACAC
25 0 TGTGTGGGTGTGGGTGTGGGTGTGTGGGTG ACACACACACACACACACACACACACACAC
26 0 TGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTG ACACACACACACACACACACACACACACACAC
27 0 TGTGTGGGTGTGGGTGTGGGTGTGTG ACACACACACACACACACACACACACAC
28 14 TGTGGGTGTGTGGGTGTGTGGGTGTGTGTGTGTG ACACACACACACACACACACACACACACACACAC
29 0 TGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTGTG ACACACACACACACACACACACACACACACACAC
30 14 TGTGGGTGTGTGGGTGTGTGTGTGTGTGTGTGTG ACACACACACACACACACACACACACACACACACAC
32 0 TGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTGTG ACACACACACACACACACACACACACACACACAC
33 20 TGTGTGGGTGTGTGTGGGTGTGTGTGTGTGTGTG ACACACACACACACACACACACACACACACACACAC
34 0 TGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTGTG ACACACACACACACACACACACACACACACACAC
35 0 TGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTGTG ACACACACACACACACACACACACACACACACAC
36 0 TGTGTGGGCGTGGGTGTGGGTGTGTGGGTGTGTG ACACACACACACACACACACACACACACACACAC
37 0 TGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTGTG ACACACACACACACACACACACACACACACACAC
38 0 TGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTG ACACACACACACACACACACACACACACACAC
40 24 TGGGTGTGTGGGTGTGTGTGTGTGTGTGTGTG ACACACACACACACACACACACACACACACACACAC
43 2 TGTGGGTGTGGGTGTGGGTGTGTGGGTGTGTGTG ACACACACACACACACACACACACACACACACACAC
44 0 TGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTG ACACACACACACACACACACACACACACACACAC
45 2 TGTGGGTGTGGGTGTGGGTGTGTGTGTGTGTGTG ACACACACACACACACACACACACACACACACACAC
46 0 TGTGTGGGTGTGGGTGTGGGTGTGTGTGTGTG ACACACACACACACACACACACACACACACACAC
47 0 TGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTGTG ACACACACACACACACACACACACACACACACAC
48 7 GTGTGGGTGTGTGTGTGTGTGTGTGTGTGTGTG CACACACACACACACACACACACACACACACACAC
49 0 TGTGTGGGTGTGGGTGTGGGTGTGTG ACACACACACACACACACACACACACACAC

Figure 6—figure supplement 3. Fusion junctions of rTG Type in SY14 *tlc1*Δ survivors. The sequences at the junction of 42 independent clones (labeled on left) of SY14 *tlc1*Δ survivors are determined. In each clone, the length of the residual TG_{1-3} sequence proximal to junction point of Chr XVI-L is also shown.

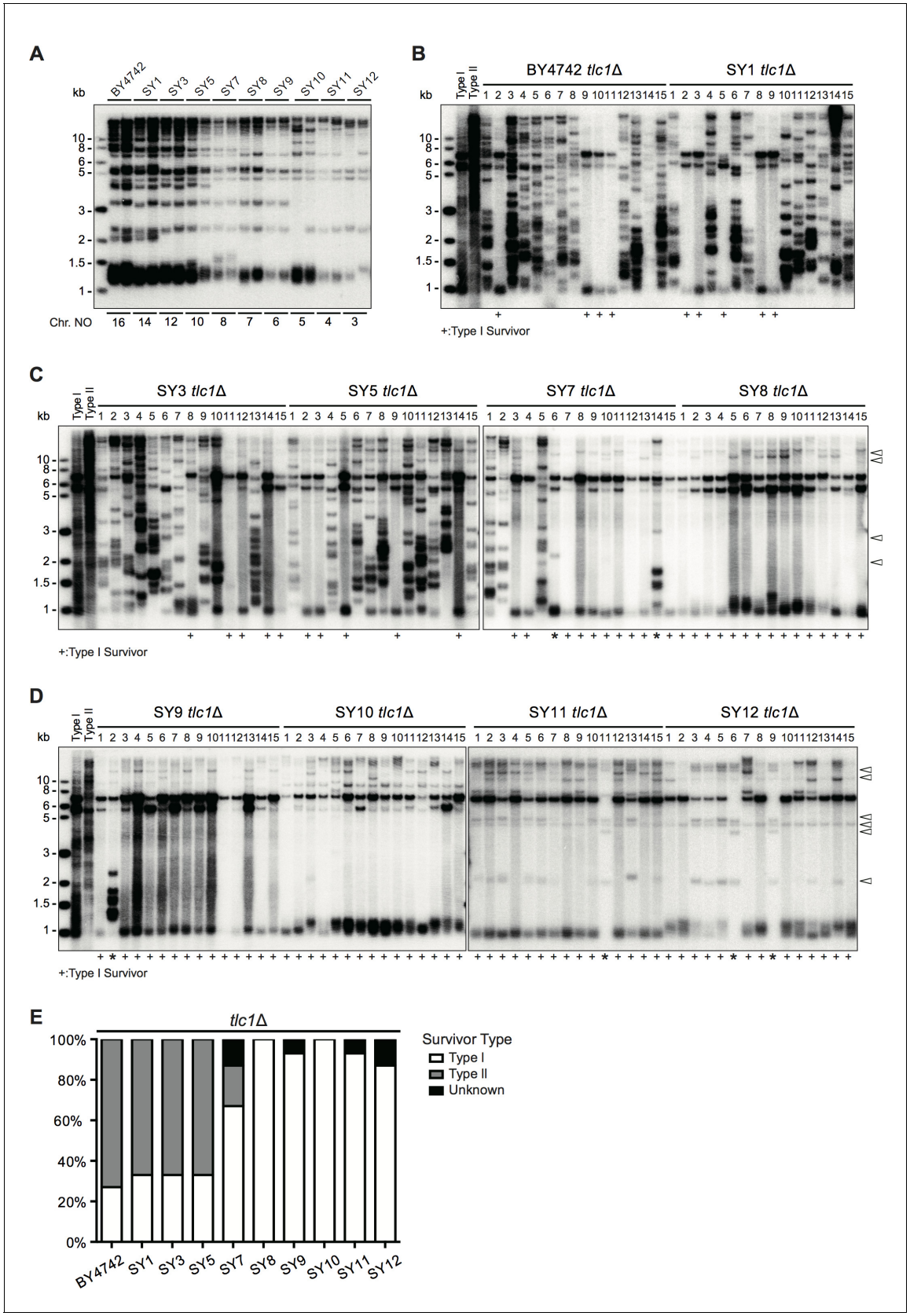


Figure 6—figure supplement 4. Telomerase null cells may experience inter- and/or intra-chromosome fusions to survive with the decrease of chromosome numbers in budding yeast. (**A**) Telomere Southern blotting analysis. The genomic DNAs from the strains (labeled on top) with different

Figure 6—figure supplement 4 continued on next page

Figure 6—figure supplement 4 continued

numbers of chromosomes (labeled at bottom) were digested by XhoI and subjected to Southern hybridization. Two independent clones of each strain were examined. (**B-D**) Telomere Southern blotting analysis of *tlc1*Δ survivors obtained on plates. Fifteen independent survivor clones (1 to 15, labeled on top of each panel) of each strain were randomly picked, and their telomere structures were examined by Southern blotting with a TG_{1-3} probe. '+" at the bottom indicates Type I survivors. '*" marks the survivors which are not typical Type I or Type II. Open arrows at the right of the panels indicate distinct bands. (**E**) Percentage of Type I, Type II and unknown survivors in SY *tlc1*Δ strains with different numbers of chromosomes. Y-axis, percentage of different kinds of survivors; X-axis, SY *tlc1*Δ strains with different numbers of chromosomes.

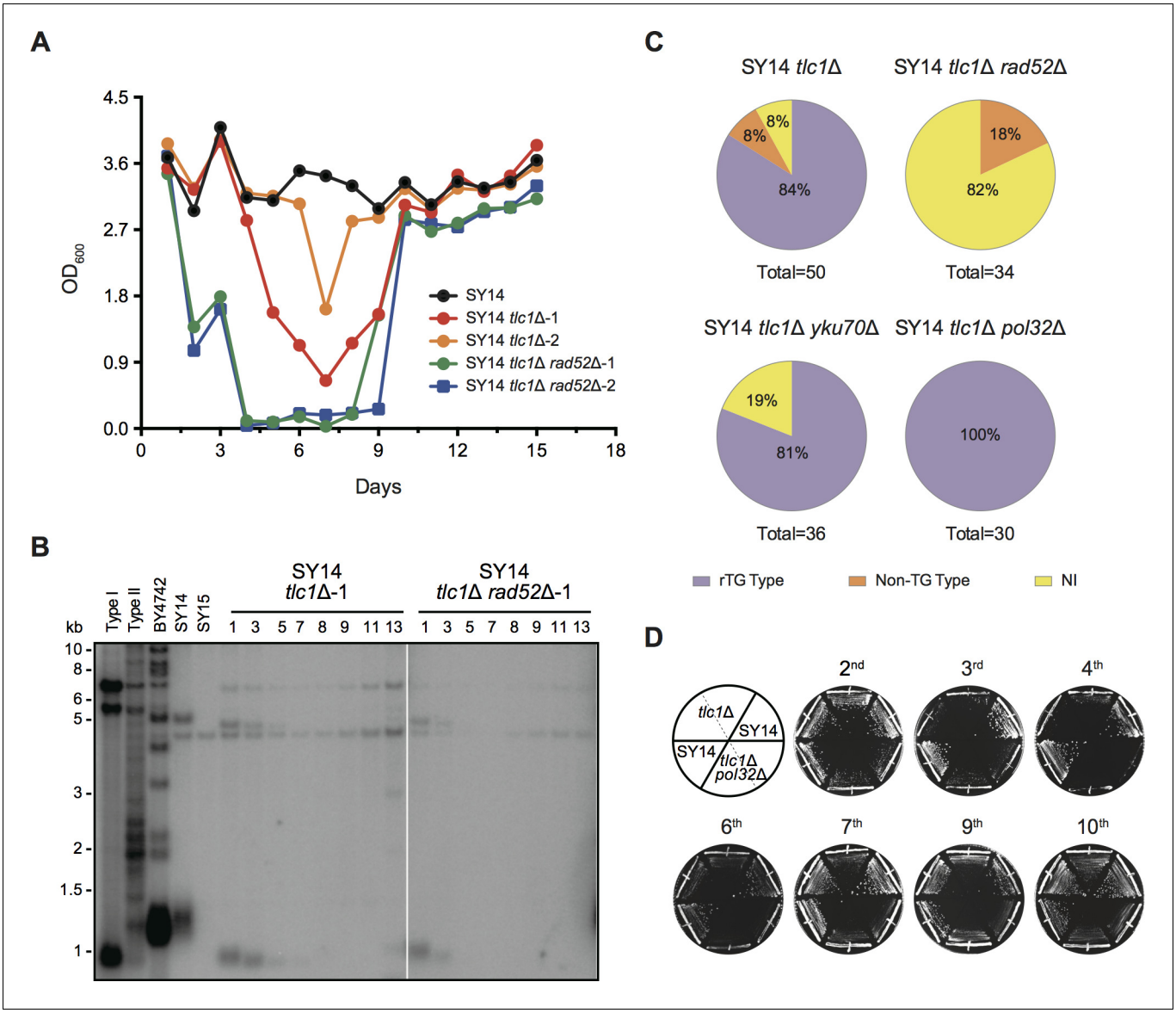
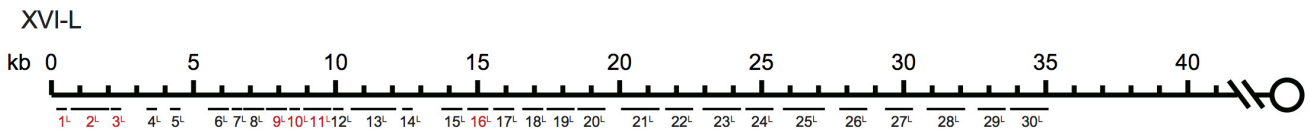
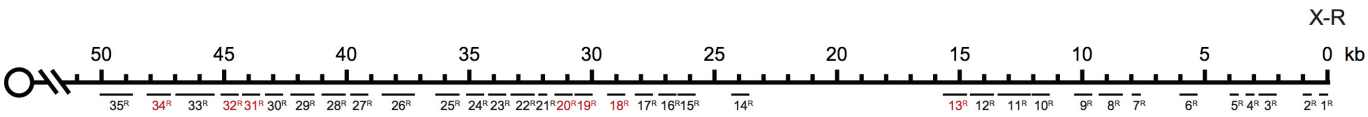


Figure 7. Survivors in SY14 *tlc1*Δ have a circular chromosome. (**A**) Senescence assay in liquid medium. The growth (OD_{600}) of SY14 (black), SY14 *tlc1*Δ (red, orange) and SY14 *tlc1*Δ *rad52*Δ (green, blue) strains were monitored every 24 hr for 15 days. (**B**) Telomere Southern blotting analysis of SY14 *tlc1*Δ and SY14 *tlc1*Δ *rad52*Δ survivors. Genomic DNA of the SY14 *tlc1*Δ and SY14 *tlc1*Δ *rad52*Δ strains assayed in (A) were digested by XhoI and subjected to a Southern blotting analysis. (**C**) Percentage of rTG Type, non-TG Type and not-identified (NI) survivors in SY14 *tlc1*Δ (n = 50), SY14 *tlc1*Δ *rad52*Δ cells (n = 34), SY14 *tlc1*Δ *yku70*Δ (n = 36) and SY14 *tlc1*Δ *pol32*Δ (n = 30). (**D**) Senescence assay of the SY14 *tlc1*Δ and SY14 *tlc1*Δ *pol32*Δ strains on solid medium. After eviction of the pRS316-*TLC1* plasmid in SY14 *tlc1*Δ *TLC1* or SY14 *tlc1*Δ *pol32*Δ *TLC1* strains by 5′-FOA selection, two independent SY14 *tlc1*Δ and SY14 *tlc1*Δ *pol32*Δ clones were re-streaked ten times to allow survivors to form. SY14 was a control.



Distance from telomere XVI-L (kb)		0.1	0.8	2.0	3.4	4.1	5.6	6.3	6.8	7.5	8.4	8.8	9.8	10.4	12.4	13.8	14.5	15.5	16.5	17.4	18.7	20.0	21.6	22.8	24.4	25.8	27.7	29.2	30.8	32.6	33.8
Genotype	Primers / Clone number	1L	2L	3L	4L	5L	6L	7L	8L	9L	10L	11L	12L	13L	14L	15L	16L	17L	18L	19L	20L	21L	22L	23L	24L	25L	26L	27L	28L	29L	30L
tlc1Δ rad52Δ	1	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	2	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	3	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	4	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	5	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	6	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	7	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	8													○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	9													○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	10	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	11													○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	12	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	13,14												○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	15																○	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	16																○	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	17	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	18	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	19	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	20	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	21	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	22																○	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	23,24,25,26													○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	27																○	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	28																○	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	29	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	30	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	31	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	32	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	33	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	34	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

Figure 7—figure supplement 1. PCR mapping of the borders of Chr XVI-L erosion in SY14 *tlc1Δ rad52Δ* survivors.



Distance from telomere X-R (kb)		48.5	47.1	45.1	44.3	43.5	42.5	41.4	40.0	39.0	37.2	35.2	34.5	33.4	32.1	31.7	30.9	30.0	28.5	27.6	26.6	25.9	23.6	14.6	13.7	12.0	11.2	9.4	8.3	7.5	5.2	3.5	2.8	1.9	0.4	0.0
Genotype	Clone number / Primers	35R	34R	33R	32R	31R	30R	29R	28R	27R	26R	25R	24R	23R	22R	21R	20R	19R	18R	17R	16R	15R	14R	13R	12R	11R	10R	9R	8R	7R	6R	5R	4R	3R	2R	1R
tlc1Δ rad52Δ	1	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○													
	2	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○													
	3	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○													
	4	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○													
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	13,14	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○	
	15	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○	
	16	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○	
	17	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○								
	18	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○								
	19	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○								
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	23,24,25,26	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○													
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	33	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○													
	34	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○													

Figure 7—figure supplement 2. PCR mapping of the borders of Chr X-R erosion in SY14 *tlc1Δ rad52Δ* survivors.

Non-TG Type	Fusion junction sequence	Genotype	Clone number
1	XVI-L 5′ GATCGCCCGTGCTATA 3′ ⊢10.0 kb⊣ ⊢1.0 kb⊣ 3′ GGCGCGAGATAGGAAA 5′ X-R	*tlc1*Δ *rad52*Δ	13
			14
2	XVI-L 5′ GTATCGAACGACCGCA 3′ ⊢12.1 kb⊣ ⊢23.9 kb⊣ 3′ TTGCTGGTGTATAATA 5′ X-R	*tlc1*Δ *rad52*Δ	23
			24
			25
			26

Figure 7—figure supplement 3. Fusion junction sequences of non-TG Type survivors derived from SY14 *tlc1*Δ *rad52*Δ mutants.

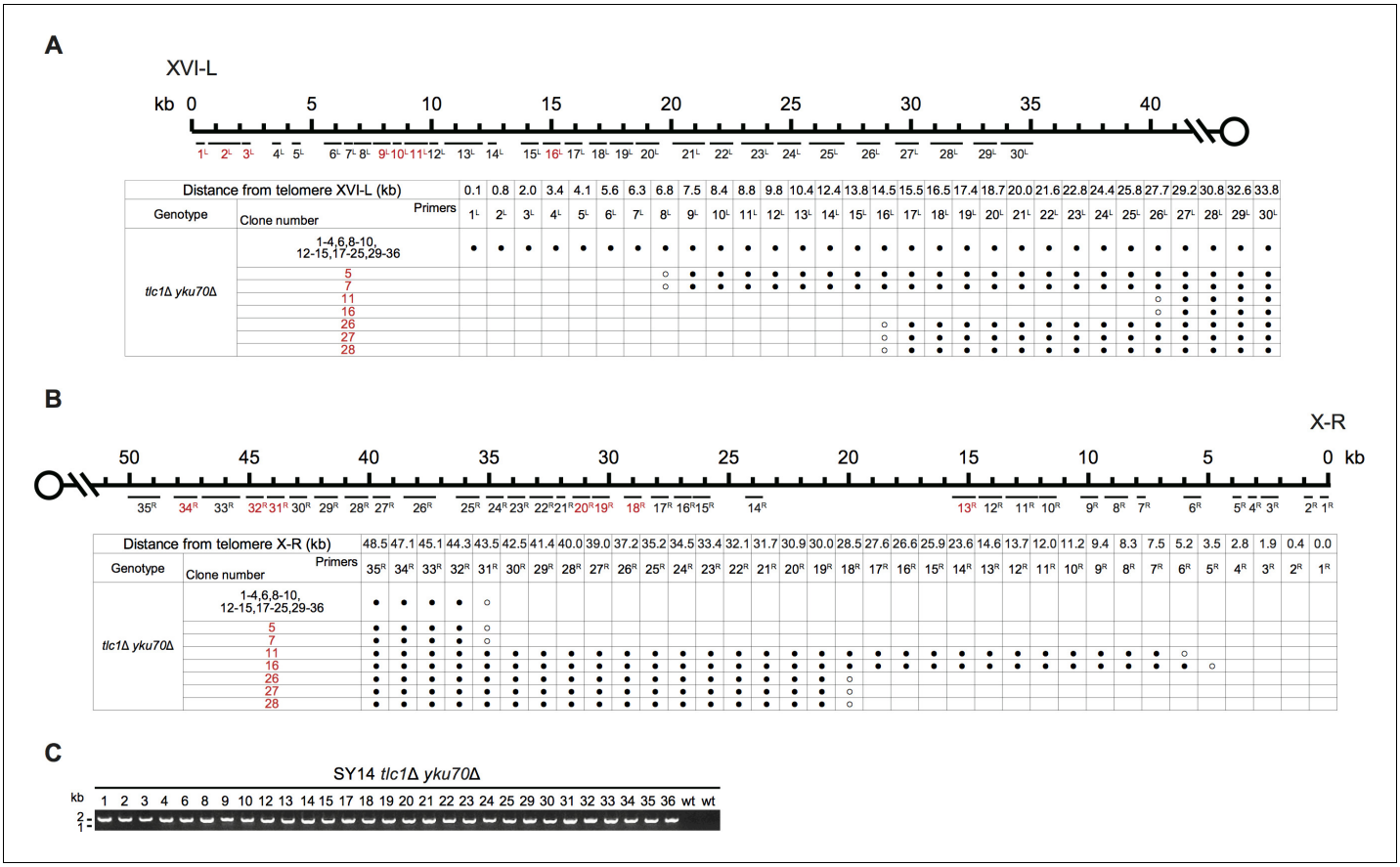

Figure 7—figure supplement 4. Borders of erosion (**A** and **B**) and rTG Type (**C**) of SY14 *tlc1Δ yku70Δ* survivors are defined by mapping and PCR amplification.

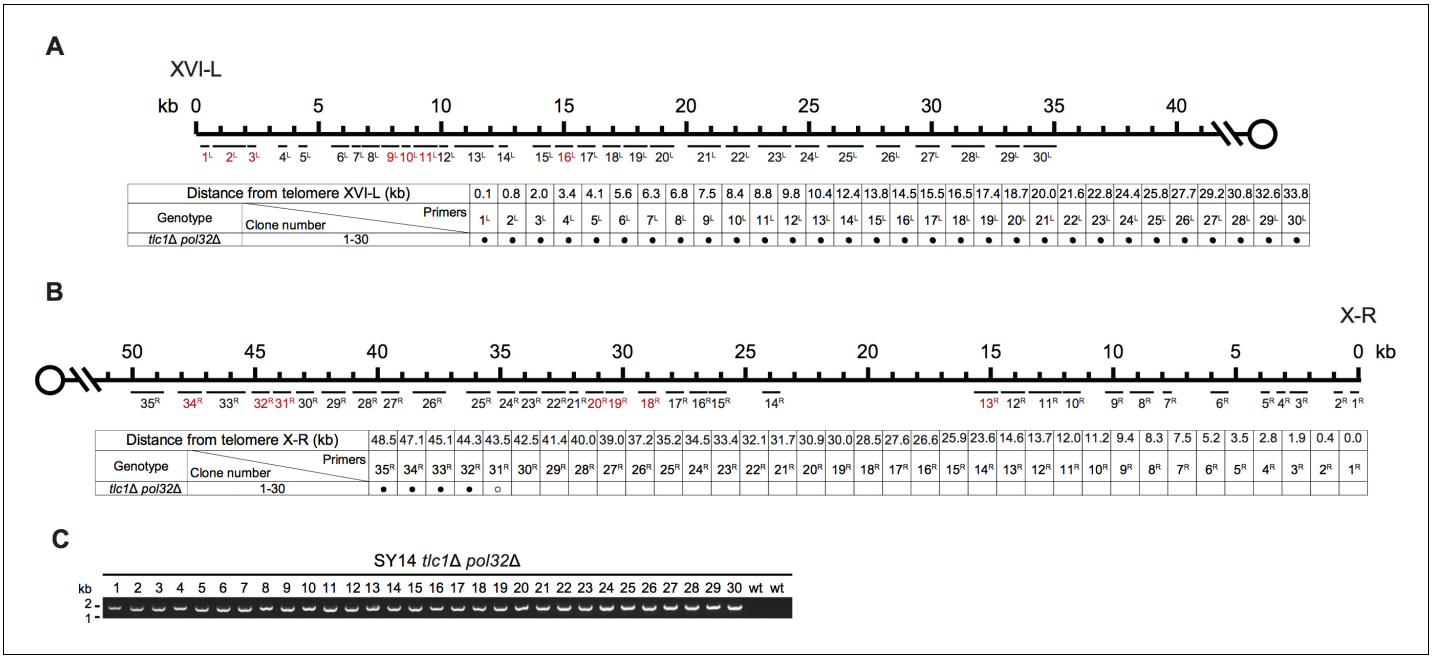

Figure 7—figure supplement 5. Chromosomal circularization in SY14 *tlc1*Δ cells is independent of Pol32. (**A-C**) Borders of erosion (**A and B**) and rTG Type (**C**) of SY14 *tlc1*Δ *pol32*Δ survivors are defined by mapping and PCR amplification.