Supplementary Information

Reduced Rif2 and no Mec1 targets short telomeres for elongation rather than double-strand break repair

Jean S. McGee[1,2], Jane A. Phillips[1,2], Angela Chan[1], Michelle Sabourin[1], Katrin Paeschke[1], Virginia A. Zakian[1,3]

Department of Molecular Biology

Princeton University

Princeton NJ 08544[1]

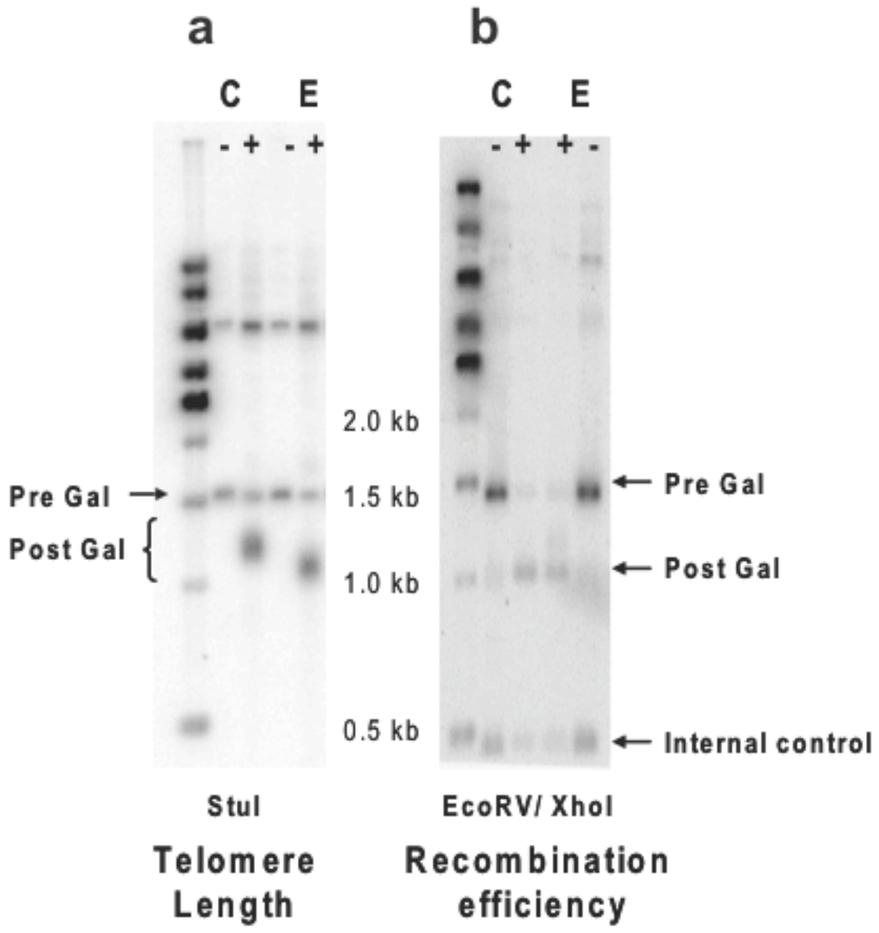
co-first authors[2]

Corresponding author[3]

e mail: vzakian@princeton.edu

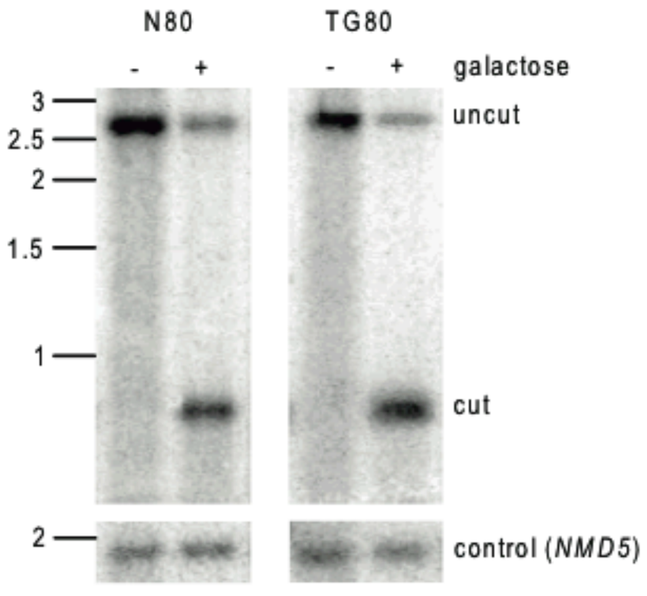
phone: 609-258-6770

fax: 609-258-1701



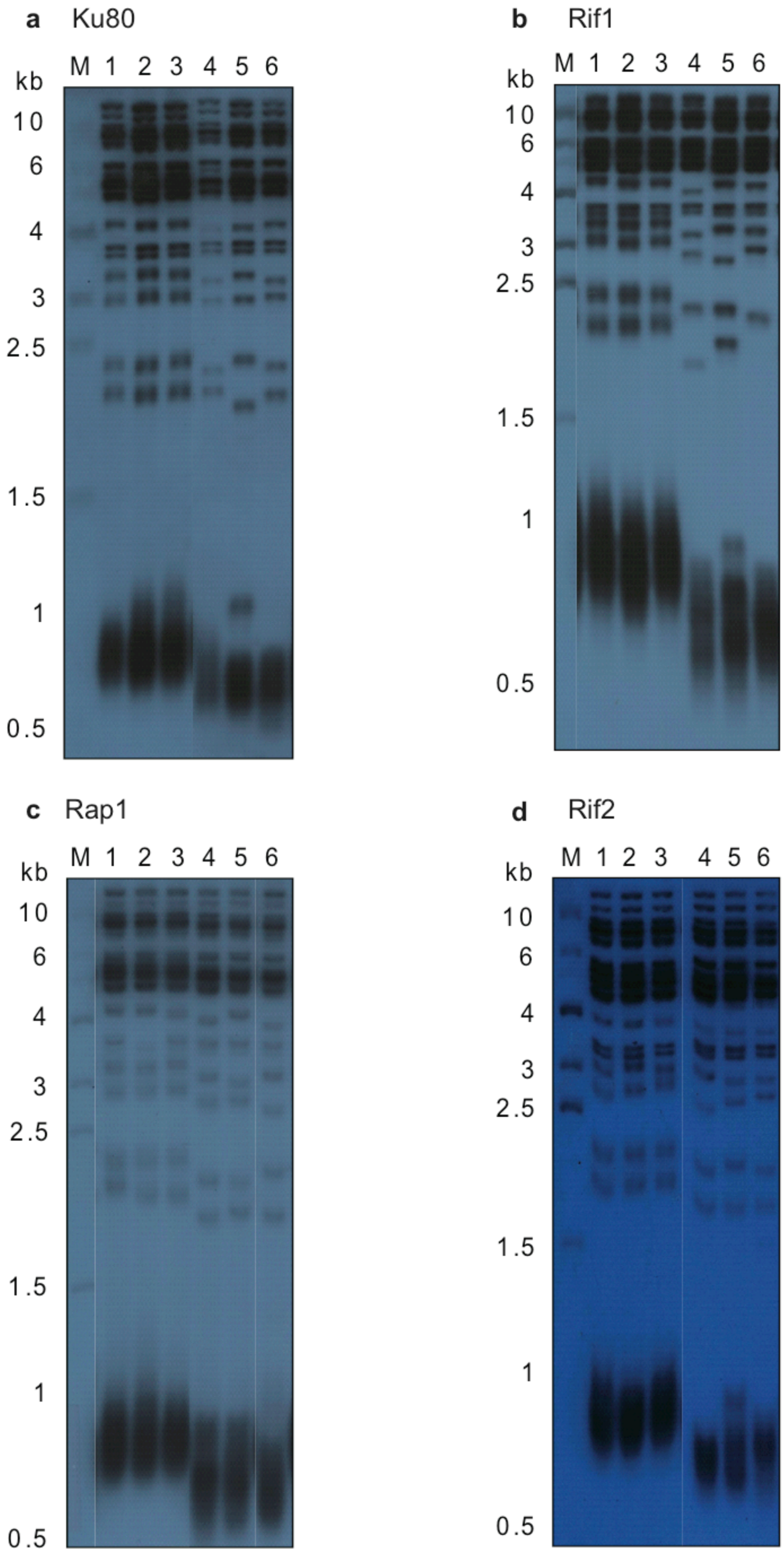
Supplementary Figure 1

Supplementary Figure 1: Representative Southern analysis after telomere recombination. C, control and E, experimental strain before (-) and after (+) galactose induction of FLP expression. DNA was prepared after 0 or 3 hrs of galactose addition. DNA was digested with *Stu*I or with *EcoR*V and *Xho*I, separated on agarose gels, and analyzed by Southern hybridization. (**a**) Amount of telomeric DNA on the VII-L telomere after galactose induction was ~100 or ~300 bps in, respectively, the control and experimental strains. (**b**) The restriction fragment generated from the VII-L telomere that has (post-Gal) or has not (pre-Gal) undergone recombination is indicated. In both of these representative experiments, recombination efficiency was ~70%.



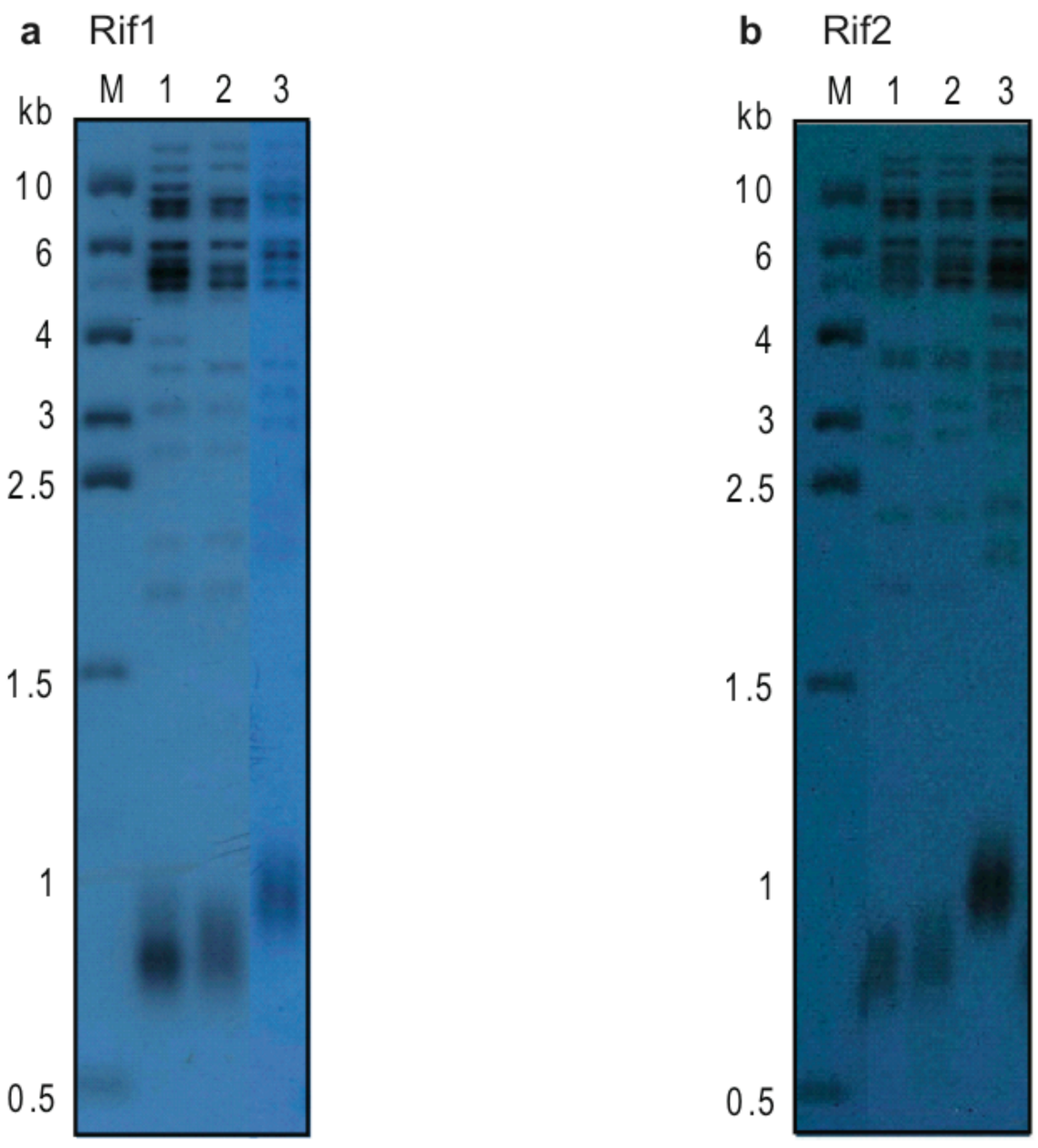
Supplementary Figure 2

Supplementary Figure 2: Efficiency of *HO* cutting. Cleavage at the *HO* recognition site was monitored by Southern blotting, as in[24]. Concurrent with all ChIP experiments, samples were taken before and after galactose addition to determine the efficiency of *HO* cleavage. DNA was digested with *Eco*RV and probed with radiolabeled DNA complementary to sequence centromere proximal to the *HO* site. Representative blots are shown for both the TG80-HO and N80-HO strains. "Uncut" bands mark the size of the intact chromosome before *HO* cleavage; "cut" bands mark the position of the shortened VII-L after *HO* cleavage. The unrelated *NMD5* gene was probed as a loading control.



Supplementary Figure 3

Supplementary Figure 3: Telomeres of various strains shorten to similar extents. Aliquots from the same cell cultures used for ChIP (**Fig. 5**, panels **b-e**) were also used to examine telomere length. DNA was *Pst*I-*Xho*I digested and subjected to electrophoresis in a 1% agarose gel and then analyzed by Southern hybridization using a P^{32}-labeled telomeric $C_{1-3}A/TG_{1-3}$ probe. Molecular weight markers are in kilobase pairs. Representative blots from three independent spore clones from *TLC1* (lanes 1-3) and *tlc1Δ* (lanes 4-6) cells to determine telomere lengths from the same cells used to detect telomeric DNA associated with Yku80-MYC (**a**), Rif1-MYC (**b**), endogenous Rap1 (**c**) or Rif2-MYC (**d**). All lanes are from the same blot but some lanes that are not relevant to this experiment were excluded.



Supplementary Figure 4

Supplementary Figure 4: Telomeres in *tlc1*Δ, *tlc1*Δ *rif1*Δ, and *tlc1*Δ *rif2*Δ cells shorten to similar extents. Aliquots from the same cell cultures used for ChIP and subsequent telomere PCR (**Fig. 6**, panel **b**) were also used to verify that telomeres in *tlc1*Δ versions of *rif1*Δ and *rif2*Δ cells shorten to a similar extent as telomeres in *RIF1 RIF2 tlc1*Δ cells. DNA was processed and analyzed as described in (**Supplementary Fig. 3**). Molecular weight markers are in kilobase pairs. Representative blots from different spore clones are shown for *tlc1*Δ (lane 1), *tlc1*Δ, *rif1*Δ (lane 2, panel **a**) or *rif2*Δ (lane 2, panel **b**) and WT (lane 3). Different lanes were aligned using Photoshop to exclude samples not processed or further analyzed.

Supplementary Table 1: Yeast strains

Strain	Genotype	
Lev220	W303 1-A MATa *leu2::LEU2-GAL10-FLP1 [cir°] adh4::FRT-URA3-TEL270-TEL270-FRT-TEL* [4]	
Lev187	W303 1-A MATa *leu2::LEU2-GAL10-FLP1 [cir°] adh4::FRT-URA3-FRT-TEL* [4]	
MS125	Lev220 *bar1Δ::KAN* [8]	Experimental
MS632	MS125 Mre11-13MYC-*TRP1*	Experimental
MS680	MS632 but Lev187 VII-L telomere	Control
MS669	MS125 Rad50-13MYC-*TRP1*	Experimental
MS691	MS669 but Lev187 VII-L telomere	Control
MS648	MS125 Xrs2-13MYC-*TRP1*	Experimental
MS651	MS648 but Lev187 VII-L telomere	Control
MS407	MS125 Tel1-3HA [8]	Experimental
MS716	MS407 *sml1Δ*::*HIS3 mec1*-3HA-*TRP1*-3HA (*mec1Δ*)	Experimental
MS685	MS125 *sml1Δ*::*HIS3* Mec1-3HA,	Experimental
MS701	Lev187 *bar1Δ::KAN sml1Δ*::*HIS3* Mec1-3HA	Control
AC121	MS685 *tel1Δ::HIS3*	Experimental
AC126	MS701 *tel1Δ::HIS3*	Control
AC130	MS125 RFA1-13MYC-TRP1	Experimental
AC127	AC130 but Lev187 VII-L telomere	Control
YPH501	*MAT*a/*MAT*α *ura3-52/ura3-52 lys2-801_amber/lys2-801_amber ade2-101_ochre/ade2-101_ochre trp1-Δ63/trp1-Δ63 his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1*	
JSM1	YPH501 *tlc1Δ::LEU2/TLC1, RIF1-Gly8-Myc9::TRP1/ RIF1-Gly8-Myc9::TRP1*	
JSM2	YPH501 *tlc1Δ::LEU2/TLC1, RIF2-Gly8-Myc9::TRP1/ RIF2-Gly8-Myc9::TRP1*	
JSM3	YPH501 *tlc1Δ::LEU2/TLC1, KU80-Gly8-Myc18::TRP1/ KU80-Gly8-Myc18::TRP1*	
JSM4	YPH501 *tlc1Δ::LEU2/TLC1*	
JSM5	YPH501 *tlc1Δ::LEU2/TLC1, rif1Δ::HIS3/RIF1, TEL1-3HA/TEL1-3HA*	
JSM6	YPH501 *tlc1Δ::LEU2/TLC1, rif2Δ::TRP1/RIF2, TEL1-3HA/TEL1-3HA*	
YAB285	W303a *matΔ RAD5 ade2Δ lys2Δ leu2::Gal-HO mnt2::LYS2 adh4::ADE2-TG80-HOsite* [24]	
YAB1083	W303a *matΔ RAD5 ade2Δ lys2Δ leu2::Gal-HO mnt2::LYS2 adh4::ADE2-N80-HOsite* [24]	
JP404	YAB285 *bar1Δ::nat sml1Δ::HIS3 MEC1-HA3*	
JP393	YAB1083 *bar1Δ::nat sml1Δ::HIS3 MEC1-HA3*	
JP397	YAB285 *bar1Δ::nat Rfa1-Myc13::TRP1*	
JP398	YAB1083 *bar1Δ::nat Rfa1-Myc13::TRP1*	
JP370	YAB285 *CDC13-Myc9::TRP1 bar1Δ::nat*	
JP373	YAB1083 *CDC13-Myc9::TRP1 bar1Δ::nat*	

Supplementary Table 2: Primer sequences

Primer	Sequence
VI-R Forward	5’-ATC ATT GAG GAT CTA TAA TC-3’
VI-R Reverse	5’-CTT CAC TCC ATT GCG-3’
XV-L Forward	5’-TAA CCC TGT CCA ACC TGT CT-3’
XV-L Reverse	5’-ATA CTA TAG CAT CCG TGG GC-3’
ARO1 Forward	5’-TCG TTA CAA GGT GAT G-3’
ARO1 Reverse	5’-AAT AGC GGC AAC AAC-3’
VII-L (FRT) Forward	5'- TGA TAT GTG TTA CGC AGA ATA C-3'
VII-L (FRT) Reverse	5'-TGA GAA GCA CCG CAA TG-3'
RPL11A Forward	5’-TCA CAT CCA CGT GAC CAG TT-3’
RPL11A Reverse	5’-AAC TTT CGC ATA GCT GAG TGG-3’
TELVIR-88	5’-AAA TGA GGA CTG GGT CAT GG-3’ [10]
ChXVL(-88)	5’-TGG TTA AAT GGG GCA GGG TA-3’
dG_{18}-BamHI	5’-CGG GAT CCG_{18}-3’ [10]
FTM7	5’-TGG TTT CTT GTC TGG TTT CTC AAC -3’
RTM7	5’-GAA TAC GCT GGT TTG CAT AAA GG -3’
Bar1D-F	5’-ATT TAA TTC TAG TGG TTC GTA TCG CCT AAA ATC ATA CCA AAA TAA AAA GAG TGT CTA GAA GGG TCA TAT ACG TAC GCT GCA GGT CGA CGG A-3’
Bar1D-R	5’-GCT TTC CAT GTA TTA AAA ATG ACT ATA TAT TTG ATA TTT ATA TGC TAT AAA GAA ATT GTA CTC CAG ATT TCC ATC GAT GAA TTC GAG CTC GT-3’
Sml1D-F	5’- TGT CTT ATC TGC TCC TTT GTG ATC TTA CGG TCT CAC TAA CCT CTC TTC AAC TGC TCA ATA ATT TCC CGC TCG TTT CGG TGA TGA C-3’
Sml1D-R	5’-TAG TAG GAC GAG AGT CCC TGA AAA GAA GGG TAT CTA AGA GAA GAA AAG AAC AGA ACT AGT GGG AAA TGG ATT CCT GAT GCG GTA TTT TCT CCT-3’
Rfa1Myc-F	5’-CCT ACA CAG CTT GAA TTA CAG GGC TGA AGC CGA CTA TCT TGC CGA TGA GTT ATC CAA GGC TTT GTT AGC TCG GAT CCC CGG GTT AAT TAA-3’
Rfa1Myc-R	5’-GAA ATA GAA GTT TTT TTT TTA CAT TTC TCA TAT GTT ACA TAG ATT AAA TAG TAC TTG ATT ATT TGA TAC AGA ATT CGA GCT CGT TTA AAC-3’
rif1D::HIS3 5’	5’-CGA AAG TAA TAA CGC AGT CTT AGA GCG ATT TGA GGC AAT TCG TCG GCA TCA GTA CCT AAG GAG ATA AAA GTG CTC TTG GCC TCC TCT AG-3’
rif1D::HIS3 3’	5-CTT TCT TCC ACA GAT ATT CCG TTC AAG TCG CCC GGT GTG AAC CCT CTC AAT CTG GCC ACT AAC CTT CTC GAC TCG TTC AGA ATG ACA CG-3’
rif2D::TRP1 5’	5’-CAA TTT CAA TAA GTT AGA ACA ATA TAT GTA CGA ATA TAG ATA TAA ATA CGA ACG TGG TTA GTA TAT AGA GAC AGG CAA GTG CAC AAA CAA TAC-3’
rif2D::TRP1 3’	5’-CTT TTC CAA AGG AGT TGC CAT CTC TTT GTA TTG TTC GAA CTC TTT CAA AAG ACC TTG GTA ATT TAT CTA TCA TGG GAA GCA TTT AAT AGA ACA GC-3’

Supplementary Methods:

Strains: Gene disruptions were confirmed by both Southern analysis and phenotype. Functionality of epitope tagged proteins was determined by appropriate monitors such as telomere length, growth rates, and/or DNA damage sensitivity. Tel1 and Mec1 were epitope tagged with three HA epitopes between amino acids 798 and 799 or 736 and 737, respectively[18]. The *SML1* gene was deleted in the strain expressing Mec1-HA and replaced with *HIS3* prior to tagging since an intermediate in the tagging protocol is functionally *mec1Δ*, and *mec1Δ* cells are inviable unless *SML1* is deleted[57]. Mre11, Rad50, Xrs2, and Rfa1 were each tagged at their C-termini with thirteen MYC epitopes using PCR (all primers are listed in **Supplementary Table 2**) and the pFA6a-13MYC-TRP1 plasmid as described[58]. For all epitope tagged Lev220-derived strains, a control strain was generated by mating the tagged experimental strain with Lev187 (*adh4*::FRT-*URA3*-FRT-telomere) from[4] and selecting spore products with the Lev187 VII-L telomere. Both versions of the DSB strains contain a *LYS2* marker on the telomere-proximal side of the *HO* recognition site. Prior to carrying out experiments, cells were maintained on solid media containing glucose but lacking lysine to select against cells that had been cleaved at the *HO* site due to leaky expression of *HO*.

Protein content at short telomeres: Experiments to determine protein composition of telomeres shortened from their ends were carried out as follows. First, the diploid YPH501 *tlc1*Δ*::LEU2/TLC1*[45] was sporulated and dissected to obtain *tlc1*Δ*::LEU2* spore clones that were then mated to derivatives of the haploid strain YPH499 containing MYC tagged *RIF1*, *RIF2*, or *KU80* [8] to generate three diploid strains, each of which was heterozygous for one of the tagged genes. As described previously[8], proteins were tagged

with either nine (Rif1 and Rif2) or eighteen (Yku80) MYC epitopes at their carboxyl termini with an eight glycine linker inserted between the ORF and the epitopes, which improve the function of tagged proteins[59]. The diploids were sporulated, dissected, and then spore clones mated to obtain the final diploid strains used in the analysis (JSM1, JSM2, and JSM3; **Supplementary Table 1**) that were heterozygous for *TLC1* and homozygous for the tagged protein. Strain JSM4 (**Supplementary Table 1**), which is heterozygous for the *TLC1* deletion but had no tagged protein, was used to determine Rap1 levels.

For experiments to determine the effects of Rif genes on Tel1 binding to telomeres of different lengths, the W303a derived KRY22 strain, which contains Tel1-HA (tagged as described above) was backcrossed six times to YPH500 to generate a haploid strain containing Tel1-HA in the YPH background. This strain was mated with a *tlc1*Δ*::LEU2* spore clone that was obtained by dissection of JSM4 to generate a diploid in the YPH501 background that was heterozygous for both the *TLC1* deletion and TEL1-HA. The diploid was sporulated to obtain *tlc1*Δ*::LEU2* TEL1-HA spore clones which were then mated to otherwise isogenic *rif1*Δ*::HIS3* or *rif2*Δ*::TRP1* cells to obtain a diploid that was heterozygous for all three genes. After sporulation and dissection, spore clones were identified and then mated to generate strains that were homozygous for Tel1-HA, heterozygous for the *TLC1* deletion, and heterozygous for either *rif1*Δ (JSM5) or *rif2*Δ (JSM6, **Supplementary Table 1**). Prior to the experiment, the final diploid strains (JSM5 and JSM6) were streaked at least ten times to equilibrate telomeres to wild type lengths. *RIF1* and *RIF2* deletions were made by transformation with a PCR-generated fragment using *HIS3* or *TRP1* as a template (from pRS303 or pRS304 plasmid) and

primers for sequences flanking *RIF1* or *RIF2* respectively (see **Supplementary Table 2** for primer sequences).

Synchrony methods: For galactose induction and cell synchronization in both the induced short telomere and induced DSB strains, cells were grown at 30°C in rich media plus 2% raffinose to an OD_{660} of 0.25, the yeast pheromone alpha factor (Princeton University) was added to a final concentration of 160 μM, and cells were cultured until at least 90% of the cells were unbudded. Dry galactose (Sigma) was added to a final concentration of 1%, and cells were returned to 30°C for an additional 3 hr. Cells were then transferred to rich medium plus glucose and containing alpha factor for 15 min before the alpha factor was removed by filtration and cells released into the cell cycle at 24°C by the addition of protease (Sigma; 150 μg/ml final concentration). Samples were taken at least every 15 min and processed for flow cytometry, Southern blot analysis to determine per cent recombination or per cent DSB formation, and ChIP.

For experiments examining protein content at telomeres shortened from their ends, the appropriately epitope tagged diploid strain was sporulated and dissected to identify *tlc1*Δ::*LEU2* and *TLC1* spores. Spore clones were grown for a total of ~25-30 generations in log phase growth in rich medium. 1/50 of the final culture was used to prepare genomic DNA for Southern blot analysis; the rest of the sample was used for ChIP. For telomere PCR, the diploid strain was sporulated and dissected to identify *tlc1*Δ*::LEU2* spores containing either *RIF1* or *rif1*Δ*::HIS3* and *tlc1*Δ*::LEU2* spores containing either *RIF2* or *rif2*Δ*::TRP1*. Spore clones were grown for a total of ~25-30 generations in log phase growth in rich medium and processed for ChIP as described below.

Telomere PCR: Samples were processed with minor modifications of described methods[10]. The DNA in the ChIP and input samples (30 µl ChIP and 10 µl input) was first denatured at 95°C for 5 minutes, then tailed with terminal deoxynucleotide transferase (New England Biolabs) in the presence of dCTP (Invitrogen) at 37°C for 30 min. Poly C-tailed DNA (10 µl for both ChIP and input) was PCR amplified using Platinum *Taq* DNA polymerase High Fidelity (Invitrogen) with a primer specific for the subtelomeric region of chromosome VI-R or XV-L (TELVIR-88 and ChXVL(-88) respectively, **Supplementary Table 2**) and an oligo dG primer (dG_{18}-BamHI, **Supplementary Table 2**) complementary to the poly dC-tail with the PCR conditions as described[10]. The PCR products were resolved on a 3% w/v MetaPhor (Lonza) agarose gel along with a 1 Kb Plus DNA Ladder (Invitrogen). The lengths of the telomere PCR products were determined by the AlphaImager 3400 Molecular Weight Analysis program, which automatically locates the peak density of a band. Telomere lengths were determined by subtracting 114 bp for VI-R or 113 bp for XV-L of non-telomeric sequences from the average length of the telomere PCR band. For all experiments, an aliquot of the telomere PCR products were gel-purified, cloned and sequenced to verify that they contained telomeric repeats. Of the 100 sequenced clones, 90% contained telomeric DNA while 10% had no insert.

Chromatin immuno-precipitation (ChIP): All ChIPs were performed as described[8,54-55]. Anti-sera were anti-MYC (Clontech monoclonal Ab #631206), anti-HA (Santa Cruz, monoclonal Ab #SC7392X), anti-H2A phosphoS129 (Abcam polyclonal Ab #ab15083) or an affinity purified polyclonal anti-Rap1 serum[56]. The amount of DNA in ChIP and input samples was quantitated using real-time PCR (BioRad iCycler). For induced DSB

experiments, primers FTM7 and RTM7 were used to amplify a unique sequence centromere-proximal to the TG80-HO or N80-HO recognition site on chromosome VII-L as described[24]. In most cases, the per cent IP was normalized to the amount of the non-telomeric *ARO1* sequence in the immuno-precipitate and input samples. However, for the Rfa1-Myc and the H2AX experiments, results are presented as per cent immuno-precipitate (amount of target sequence in IP/amount in input sample).

For all ChIP experiments, samples from each time point were amplified in duplicate or triplicate to obtain an average value for each sample. Each synchrony was repeated at least three times. For determining protein content at telomeres shortened from their ends, ChIPs were carried out on three or more independent spore clones for each genotype. Data are presented as the mean plus or minus one standard deviation (as indicated by error bars). Where applicable, a two-tailed Student's t-test was used to determine statistical significance. A p-value ≤ 0.05 was considered significant.