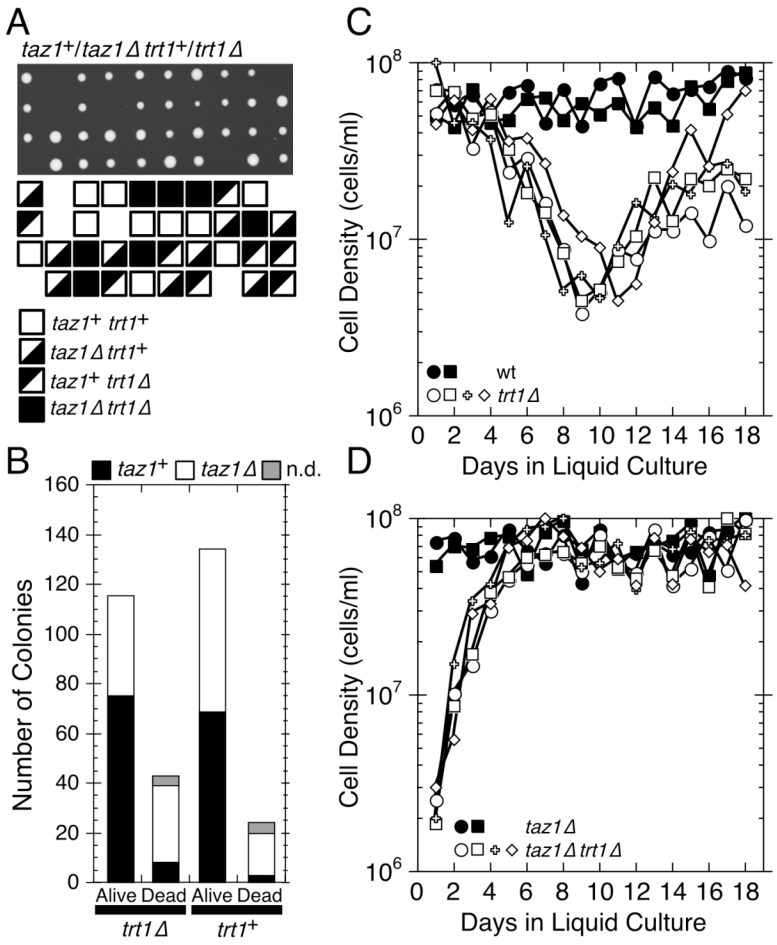
SUPPLEMENTAL MATERIALS AND METHODS

Additional details on strains used in PFGE analysis. For *taz1Δ trt1Δ rad22Δ*, agarose plugs were prepared from cells restreaked 6 times for single colonies on YES plates after generating triple mutant by genetic cross between *taz1Δ trt1Δ* survivor strain and *rad22Δ* strain. In addition, an independently restreaked single colony from 4th restreak was tested by PFGE. For *taz1Δ trt1Δ rad32Δ*, one strain was created by transforming *taz1Δ trt1Δ* with *rad32Δ* construct. This strain was subsequently restreaked on YES plates eight times before telomere structure was tested. Two additional independent *taz1Δ trt1Δ rad32Δ* strains were generated by genetic cross between *taz1Δ trt1Δ* survivor strain and *rad32Δ* strain. These strains were restreaked ten times on YES plates before telomere structure was tested. For *taz1Δ trt1Δ rad50Δ*, two independent strains were generated by genetic cross between *taz1Δ trt1Δ* survivor strain and *rad50Δ* strain. These strains were restreaked ten times on YES plates before telomere structure was tested. For *taz1Δ trt1Δ nbs1Δ*, two independent strains were generated by genetic cross between *taz1Δ trt1Δ* survivor strain and *nbs1Δ* strain. These strains were restreaked ten times on YES plates before telomere structure was tested. For *taz1Δ trt1Δ tel1Δ*, two independent strains were generated by genetic cross between *taz1Δ trt1Δ* survivor strain and *tel1Δ* strain. These strains were restreaked six times on YES plates before telomere structure was tested. For *taz1Δ trt1Δ rad3Δ*, two independent strains were generated by genetic cross between *taz1Δ trt1Δ* survivor strain and *rad3Δ* strain. These strains were restreaked seven times on YES plates before telomere structure was tested. For *taz1Δ trt1Δ pku70Δ*, two independent strains were generated by transforming *taz1Δ trt1Δ* with *pku70Δ* construct. These strains were restreaked nine times on YES plates before telomere structure was tested. For *taz1Δ trt1Δ pku70Δ tel1Δ*, two independent strains were generated by transforming *taz1Δ trt1Δ pku70Δ* with *tel1Δ* construct. These strains

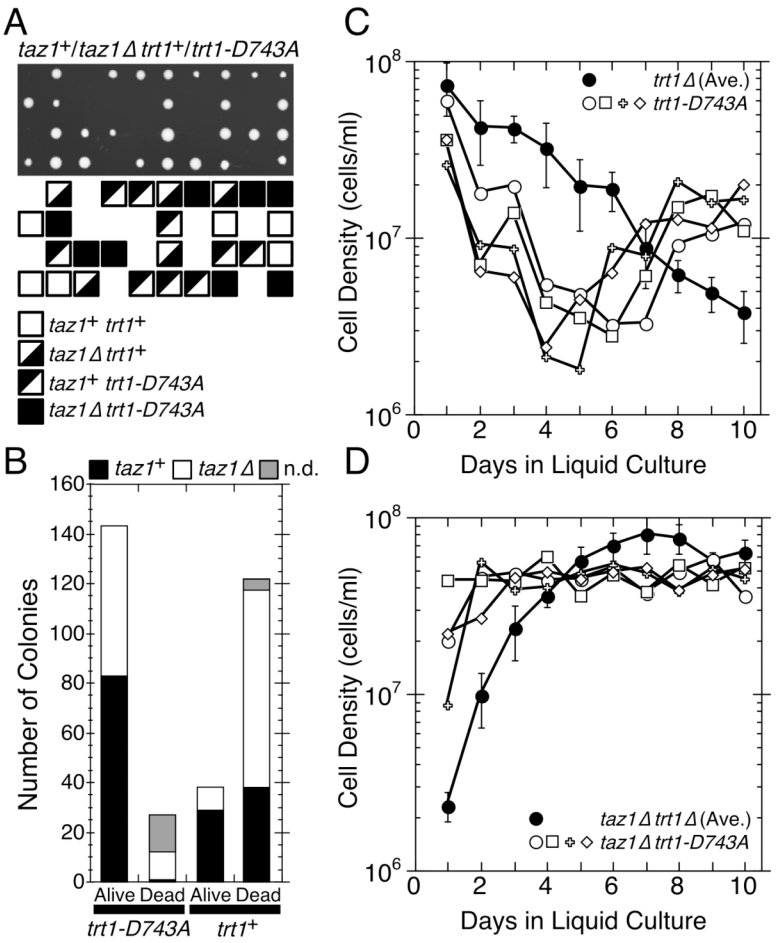
were restreaked nine times on YES plates before telomere structure was tested. For *taz1Δ trt1Δ est1Δ*, three independent strains were generated by transforming *taz1Δ trt1Δ* with *est1Δ* construct. These strains were restreaked five times on YES plates before telomere structure was tested. For *taz1Δ est1Δ*, two independent strains were generated by transforming *taz1Δ* with *est1Δ* construct. These strains were restreaked eight times on YES plates before telomere structure was tested. For *est1Δ*, one strain was generated by sporulation of the heterozygous diploid *est1Δ/est1*$^{+}$. It was restreaked eight times on YES plates before telomere structure was tested. For *taz1Δ pku70Δ est1Δ*, five independent strains were generated by transforming *taz1Δ pku70Δ* with *est1Δ* construct. These strains were restreaked nine times on YES plates before telomere structure was tested. For *taz1Δ trt1Δ rap1Δ*, four independent strains were generated by transforming *taz1Δ trt1Δ* with *rap1Δ* construct. They were restreaked ten times on YES plates before telomere structure was tested. For *rap1Δ trt1Δ*, three strains were generated by transforming *rap1Δ* with *trt1Δ* construct and additional four strains were generated by crossing *rap1Δ* strain with *trt1Δ* strain carrying *trt1*$^{+}$ plasmid. For *rap1Δ trt1Δ* strains generated by transformation, telomere structure was tested after restreaking ten times on YES plates. For *rap1Δ trt1Δ* strains generated by genetic cross, telomere structure was tested after restreaking seven times on YES plates. For Taz1 or Trt1 re-introduction experiments shown in Fig. 4 & 5, cells were restreaked on appropriate selection media plates from four to twelve times before testing for telomere structure.

SUPPLEMENTAL REFERENCES

1. **Sugawara, N. F.** 1988. DNA sequences at the telomeres of the fission yeast *S. pombe*. Ph.D. Thesis. Harvard University, Cambridge, Massachusetts.

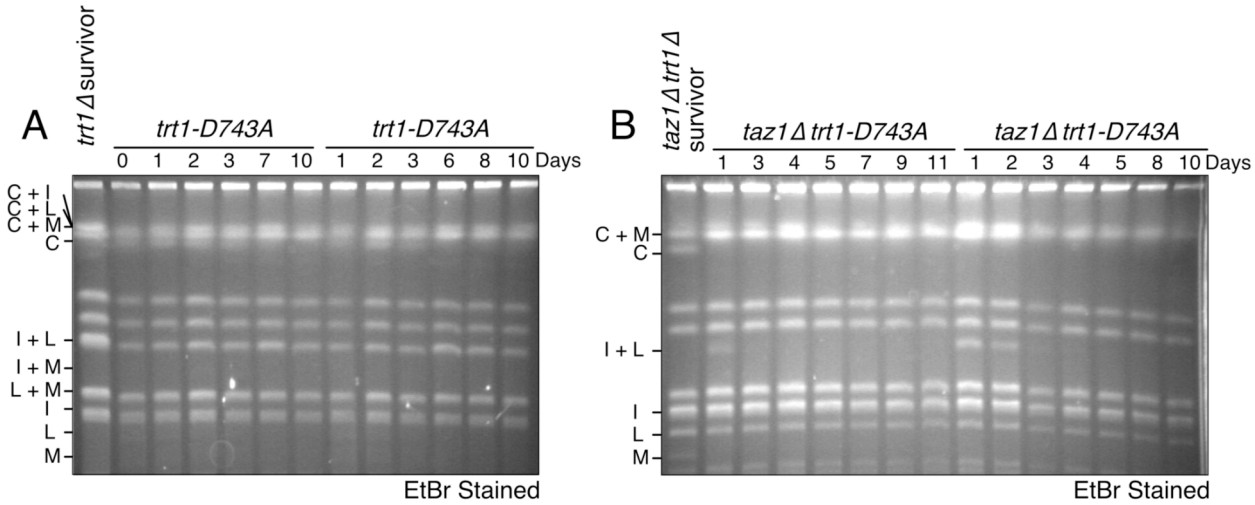


SUPPLEMENTAL FIG. S1. Growth characteristics of *trt1Δ* and *taz1Δ trt1Δ* cells after germination. (A, B) Diploid strains (CF248, CF255, CF382 or CF383) were sporulated, and the resulting tetrads were dissected and germinated on YES plates. Genotypes of cells were determined by replica plating to appropriate selective minimal plates and cell viabilities, based on the ability to form colonies, were plotted according to their genotypes. (C, D) Results of liquid cell growth experiments for wt, *trt1Δ*, *taz1Δ* and *taz1Δ trt1Δ* cells.

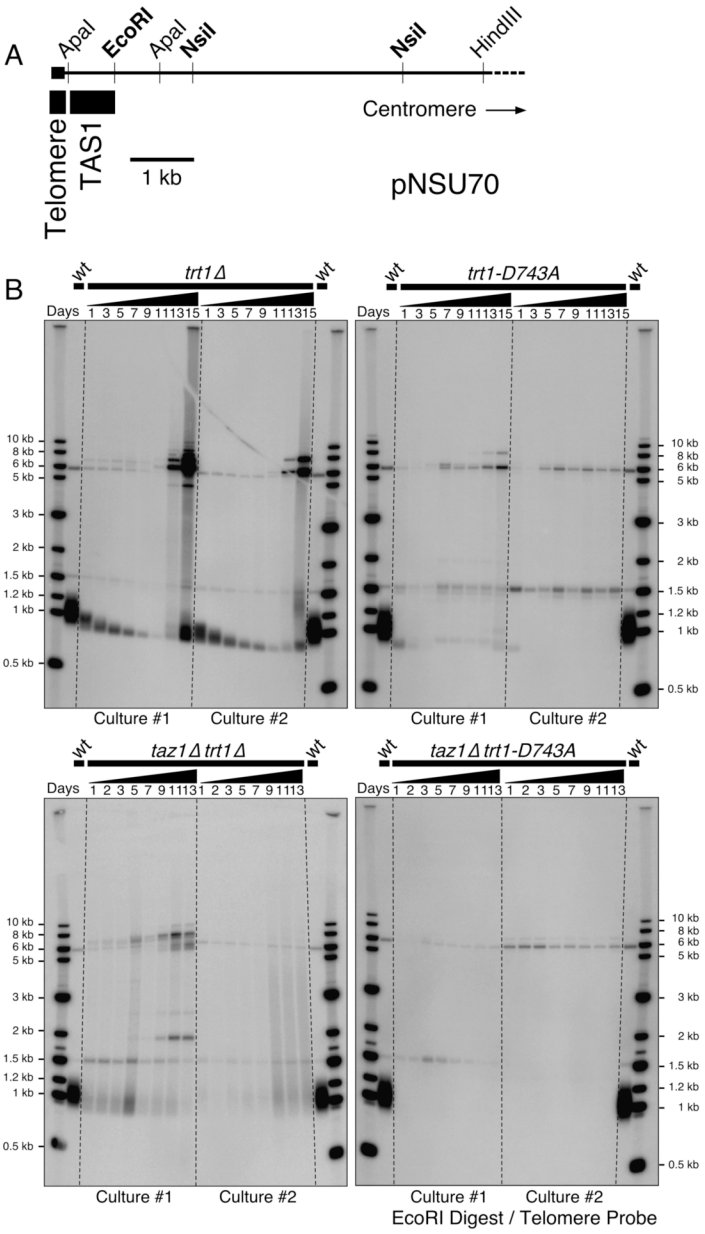


SUPPLEMENTAL FIG. S2. Growth characteristics of *trt1-D743A* and *taz1Δ trt1-D743A* cells after germination. (A, B) Diploid strains (CF658 or CF659) were sporulated and the resulting tetrads were dissected and germinated on YES plates. Genotypes of cells were determined by replica plating to appropriate selective minimum plates and cell viabilities, based on ability to form colonies, were plotted according to their genotypes. Unlike in the case of *taz1Δ*/*taz1*$^+$ *trt1Δ*/*trt1*$^+$ (Fig. S1B), spores derived from *taz1Δ*/*taz1*$^+$ *trt1-D743A*/*trt1*$^+$ diploid cells showed strong bias in the ability of spores to form colonies: 84 % of spores with *trt1-D743A* mutation were able to form colonies, while only ~24 % of spores with wild type *trt1*$^+$ gene were able to form colonies. On the other hand, the status of *taz1*$^+$ gene did not appear to affect ability of spores to form initial colonies. We are not sure why such dominant effect of Trt1-D743A on

spore viability exists. However, we note that all cells derived from *taz1Δ/taz1*$^{+}$ *trt1-D743A/trt1*$^{+}$ would be expected to carry some parental stock of both Trt1-D743A and wild type Trt1 proteins regardless of their genotypes. Therefore, the presence of very limiting amount of Trt1-D743A protein in the presence of the wild type Trt1 protein may cause an unexplained effect on the germination of *trt1*$^{+}$ spores, since the majority of these spores could not germinate and even for those few spores that were able to germinate, most of them died after only 1~2 cell divisions. (C, D) Results of cell growth experiments for *trt1-D743A* and *taz1Δ trt1-D743A* cells. For comparison, the average cell densities from 4 independent growth analysis cultures for *trt1Δ* and *taz1Δ trt1Δ* cells are also plotted. (Error bars represent standard deviations.)

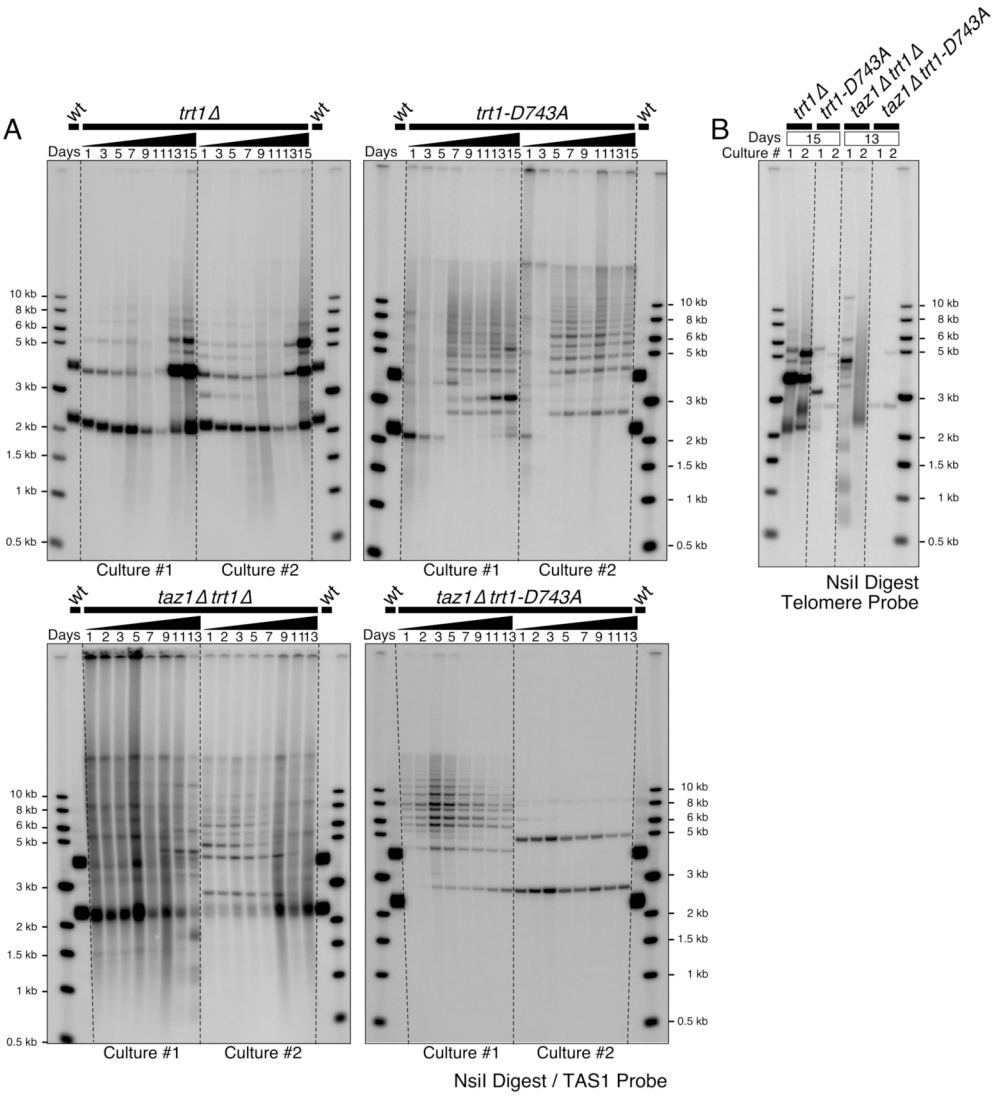


SUPPLEMENTAL FIG. S3. Non-telomeric *Not*I fragments are not affected in trt1-D743A or taz1Δ trt1-D743A cells. (A) EtBr stained agarose gels used in Fig. 3C. (B) EtBr stained agarose gels used in Fig. 3F.



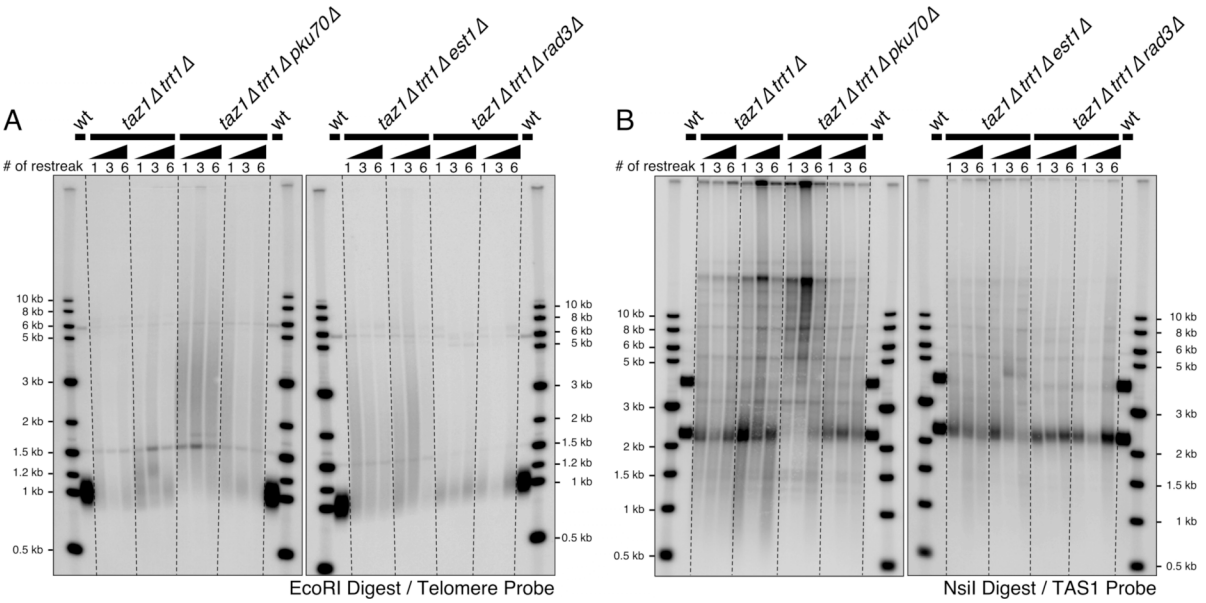
SUPPLEMENTAL FIG. S4. Telomere length analysis for serially diluted liquid cultures. (A) Restriction enzyme map of one of the telomeric and subtelomeric regions, cloned in pNSU70 (1). (B) *taz1Δ*/*taz1*$^{+}$ *trt1Δ*/*trt1*$^{+}$ or *taz1Δ*/*taz1*$^{+}$ *trt1-D743A*/*trt1*$^{+}$ diploid strains were sporulated, and *trt1Δ*, *trt1-D743A*, *taz1Δ trt1Δ*, or *taz1Δ trt1-D743A* cells were selected. For a given genotype, two independent serial liquid culture dilution series are shown. Genomic DNA samples were digested with *Eco*RI, fractionated by electrophoresis in 1 % agarose gels, transferred to nylon

membranes, and hybridized to a telomere probe derived from pNSU70 plasmid. Comparison of telomere length changes show that *trt1-D743A* cultures show accelerated shortening of a ~1 kb *Eco*RI terminal fragment compared to *trt1Δ* cultures. Survivor cells found in liquid culture experiments for *trt1-D743A* cells also showed much weaker telomere hybridization signal than survivors from *trt1Δ* liquid cultures. In addition, *trt1Δ* survivor cells from liquid cultures often contained much larger sized (4-8 kb) *Eco*RI fragments. These larger *Eco*RI fragments are likely to be caused by a loss of the telomere proximal *Eco*RI site. For *taz1Δ trt1Δ*, all liquid cultures tested showed significant hybridization signal for the telomere probe. For *taz1Δ trt1Δ* culture #1, besides the expected ~1 kb *Eco*RI fragment, we also observed strong hybridization signals for large *Eco*RI fragments. Again, these larger *Eco*RI fragments are likely to be generated by a loss of the most telomere proximal *Eco*RI site. For *taz1Δ trt1Δ* culture #2, survivor cells showed very diffused telomere signals ranging from ~1 kb to ~10 kb. In contract to *taz1Δ trt1Δ* cultures, *taz1Δ trt1Δ-D743A* survivor cultures generally showed much weaker telomere hybridization signals.



SUPPLEMENTAL FIG. S5. TAS1 structure and telomere length analysis for serially diluted liquid cultures. (A) Genomic DNA samples from *trt1Δ*, *trt1-D743A*, *taz1Δ trt1Δ*, or *taz1Δ trt1-D743A* serial liquid dilution series experiments (also used in Fig. S4) were digested with *Nsi*I, fractionated by electrophoresis in 1 % agarose gels, transferred to nylon membranes, and hybridized to a TAS1 probe derived from pNSU70 plasmid (Fig. S4A). For *trt1Δ* cells, a gradual shortening of the terminal fragment that contains telomeric repeats and the TAS1 sequence (~2.2 kb and ~4 kb) was observed from day 1 to day 11, followed by restoration of the size on day 13-15 samples. Additionally, larger sized *Nsi*I fragments were observed, possibly due to a loss of the

telomere proximal *Nsi*I site or amplification of the TAS1 repetitive sequence, as cells undergo telomere shortening. For *trt1-D743A* cells, the terminal *Nsi*I fragments shortened faster than for *trt1Δ* cells, and by days 3-5, the TAS1 signal appears to largely disappear. At days 5-7, survivors with large amplified TAS1 repeats then start to dominate the *trt1-D743A* cultures. Interestingly, not all *Nsi*I fragments detected by the TAS1 probe were detected by the telomere probe (Fig. S5B). For *taz1Δ trt1Δ* cultures, a novel hybridization pattern, not observed in *trt1Δ* cells, emerges early in the liquid culture dilution series. While a major ~2.2 kb terminal fragment is detected in both cultures, culture #1 developed novel smaller sized *Nsi*I bands. A pattern of *Nsi*I hybridization similar to the one shown in *taz1Δ trt1Δ* culture #2 was more commonly observed among independent *taz1Δ trt1Δ* liquid cultures (data not shown). For *taz1Δ trt1-D743A*, culture #1 developed a *Nsi*I fragment hybridization pattern similar to *trt1-D743A* cultures, while for culture #2, survivor cells appear to contain two distinct and sharp bands that hybridize to both TAS1 and telomere probe. We are currently unsure of the nature of survival observed for this particular culture. (B) For indicated day of liquid outgrowth, *Nsi*I digested genomic DNA samples were analyzed with a telomere probe. Results indicate that not all *Nsi*I fragments detected by the TAS1 probe can be detected by the telomere probe.



SUPPLEMENTAL FIG. S6. Telomere length and TAS1 structure are stable in *taz1Δ trt1Δ*, *taz1Δ trt1Δ pku70Δ*, *taz1Δ trt1Δ est1Δ*, and *taz1Δ trt1Δ rad3Δ* survivor cells during extensive restreaks on YES agar plates. For a given genotype, two independent strains were restreaked for indicated number of times on plates. (A) Southern blot analysis of *Eco*RI digested genomic DNA samples hybridized to a telomere probe. Diffused bands that range from ~0.8 kb to >10 kb were detected by a telomeric probe. (B) Southern blot analysis of *Nsi*I digested genomic DNA samples hybridized to a TAS1 probe. A major ~2.2 kb signal, as well as amplified larger *Nsi*I fragments were detected. For a given clone, the patterns of TAS1 are stable during restreaking processes. One of the *taz1Δ trt1Δ pku70Δ* clones appeared to have larger *Nsi*I fragments detected by the TAS1 probe. This could be due to either amplification of the TAS1 repetitive sequence or loss of telomere proximal *Nsi*I site (see Fig. S4A).

SUPPLEMENTAL TABLE S1: Strains used in this study

Figure	Short genotype	Strain	Full genotype
1B, 1C	*trt1Δ/trt1*$^+$	CF248	*h*$^+$*/h*$^-$ *leu1-32/leu1-32 ura4-D18/ura4-D18 ade6-M210/ade6-M216 his3-D1/his3-D1 trt1-2::his3*$^+$*/trt1*$^+$
1D	*taz1Δ/taz1*$^+$ *trt1Δ/trt1*$^+$	CF382	*h*$^+$*/h*$^-$ *leu1-32/leu1-32 ura4-D18/ura4-D18 ade6-M210/ade6-M216 his3-D1/his3-D1 taz1-2::ura4*$^+$*/taz1*$^+$ *trt1-2::his3*$^+$*/trt1*$^+$
2A	*taz1Δ trt1Δ*	CF458	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$
2A	*taz1Δ trt1Δ rad22Δ*	TN2783	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 or -M216 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *rad22-D2::LEU2*
2A	*taz1Δ trt1Δ rad32Δ*	TN3035	*h*$^+$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *rad32::kanMX4*
2A	*taz1Δ trt1Δ rad50Δ*	TN3037	*h*$^+$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *rad50::kanMX*
2A	*taz1Δ trt1Δ nbs1Δ*	TN3039	*h*$^+$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *nbs1::kanMX*
2A	*taz1Δ trt1Δ tel1Δ*	TN2753	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *tel1::LEU2*
2A	*taz1Δ trt1Δ rad3Δ*	TN2751	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *rad3::LEU2*
2A	*taz1Δ trt1Δ pku70Δ*	TN2599	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *pku70::kanMX4*
2A	*taz1Δ trt1Δ pku70Δ tel1Δ*	LS5634	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *pku70::hphMX tel1::kanMX4*
2A	*taz1Δ trt1Δ est1Δ*	LS4977	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *est1::kanMX*
2A	*taz1Δ est1Δ*	LS5637	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *est1::kanMX*
2A	*est1Δ*	TN4499	*h*$^+$ *leu1-32 ura4-D18 ade6-M210 his3-D1 est1::kanMX*
2A	*taz1Δ pku70Δ est1Δ*	LS6013	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *pku70::hphMX est1::kanMX*
2B-D	wt	TN2411	*h*$^-$ *leu1-32 ura4-D18 his3-D1*
2B	*taz1Δ trt1Δ rap1Δ*	LS5349 ~ 5352	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-3::LEU2 trt1-2::his3*$^+$ *rap1::ura4*$^+$
2B	*rap1Δ trt1Δ*	LS5539, 5540, 5550, 5551	*h*$^-$ *leu1-32 ura4-D18 his3-D1 rap1::ura4*$^+$ *trt1-2::his3*$^+$
2C, 2D	wt *rap1-HA*	TN4733	*h*$^+$ *leu1-32 ura4-D18 his3-D1 rap1*$^+$*::3HA-LEU2*
2C, 2D	*taz1Δ rap1-HA*	LS5470	*h*$^+$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *rap1*$^+$*::3HA-LEU2*
2C, 2D	*taz1Δ trt1Δ rap1-HA*	LS5606	*h*$^-$ *leu1-32 ura4-D18 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *rap1*$^+$*::3HA-LEU2*
3A-F	*taz1Δ/taz1*$^+$ *trt1-D743A/trt1*$^+$	CF658, CF659	*h*$^+$*/h*$^-$ *leu1-32/leu1-32 ura4-D18/ura4-D18 ade6-M210/ade6-M216 his3-D1/his3-D1 taz1-2::ura4*$^+$*/taz1*$^+$ *trt1-D743A:LEU2/trt1*$^+$
3B	*taz1*$^+$ *trt1*$^+$	TN2411	See 2B.
3B, 3C	*trt1Δ*	CF583	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 trt1-2::his3*$^+$
3E, 3F	*taz1Δ trt1Δ*	CF458	See 2A.
4B	*taz1Δ trt1Δ* + pREP81x	LS5841, 5842	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *//pREP81x*
4B	*taz1Δ trt1Δ* + pREP81-taz1$^+$	LS5843, 5844	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *//pREP81-taz1*$^+$
4B, 4C, 5D	*taz1Δ trt1Δ* + pKAN1	LS5521, 5522	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *//pKAN1*
4B, 5D	*taz1Δ trt1Δ* + pKAN-trt1$^+$	LS5523, 5524	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *//pKAN-trt1*$^+$
4B	*taz1Δ trt1Δ* + pKAN-trt1-D590A	LS5837, 5838	*h*$^-$ *leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4*$^+$ *trt1-2::his3*$^+$ *//pKAN-trt1-D590A*

TABLE S1 (continued)

Figure	Short genotype	Strain	Full genotype
4B	*taz1Δ trt1Δ* + pKAN-trt1-D743A	LS5839, 5840	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ //pKAN-trt1-D743A*
4C	*taz1Δ trt1Δ* + pKAN-trt1-ΔNsi	LS5717 ~ 5719	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ //pKAN-trt1-ΔNsi*
4C	*taz1Δ trt1Δ* + pKAN-trt1-ΔPac	LS5709, 5710, 5830	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ //pKAN-trt1-ΔPac*
4C	*taz1Δ trt1Δ* + pKAN-trt1-Δ[Nde-Xho]	LS5720 ~ 5722	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ //pKAN-trt1-Δ[Nde-Xho]*
5A	*taz1Δ trt1Δ rad3Δ* + pREP81x	LS5165, 5166	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ rad3::kanMX4 //pREP81x*
5A	*taz1Δ trt1Δ rad3Δ* + pREP81-taz1⁺	LS5167, 5168	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ rad3::kanMX4 //pREP81-taz1⁺*
5A, 5D	*taz1Δ trt1Δ rad3Δ* + pKAN1	LS5157, 5520	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ rad3::LEU2 //pKAN1*
5A, 5D	*taz1Δ trt1Δ rad3Δ* + pKAN-trt1⁺	LS5159, 5160	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ rad3::LEU2 //pKAN-trt1⁺*
5A	*taz1Δ trt1Δ rad3Δ* + pKAN-trt1-D590A	LS5161, 5162	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ rad3::LEU2 //pKAN-trt1-D590A*
5A	*taz1Δ trt1Δ rad3Δ* + pKAN-trt1-D743A	LS5163, 5164	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ rad3::LEU2 //pKAN-trt1-D743A*
5B	*taz1Δ trt1Δ est1Δ* + pREP81x	LS5375, 5376	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ est1::natMX //pREP81x*
5B	*taz1Δ trt1Δ est1Δ* + pREP81-taz1⁺	LS5377, 5378	*h⁻ leu1-32 ura4-D18 ade6-M210 his3- taz1-2::ura4⁺ trt1-2::his3⁺ est1::natMX //pREP81-taz1⁺*
5B, 5D	*taz1Δ trt1Δ est1Δ* + pKAN1	LS5368, 5518	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ est1::natMX //pKAN1*
5B, 5D	*taz1Δ trt1Δ est1Δ* + pKAN-trt1⁺	LS5369, 5370	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ est1::natMX //pKAN-trt1⁺*
5B	*taz1Δ trt1Δ est1Δ* + pKAN-trt1-D590A	LS5371, 5372	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ est1::natMX //pKAN-trt1-D590A*
5B	*taz1Δ trt1Δ est1Δ* + pKAN-trt1-D743A	LS5373, 5374	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ est1::natMX //pKAN-trt1-D743A*
5C	*taz1Δ trt1Δ pku70Δ* + pREP81x	LS5363, 5364	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ pku70::hphMX //pREP81x*
5C	*taz1Δ trt1Δ pku70Δ* + pREP81-taz1⁺	LS5365, 5366	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ pku70::hphMX //pREP81-taz1⁺*
5C, 5D	*taz1Δ trt1Δ pku70Δ* + pKAN1	LS5355, 5356	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ pku70::hphMX //pKAN1*
5C, 5D	*taz1Δ trt1Δ pku70Δ* + pKAN1-trt1⁺	LS5357, 5358	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ pku70::hphMX //pKAN-trt1⁺*
5C	*taz1Δ trt1Δ pku70Δ* + pKAN1-trt1-D590A	LS5359, 5360	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ pku70::hphMX //pKAN-trt1-D590A*
5C	*taz1Δ trt1Δ pku70Δ* + pKAN1-trt1-D743A	LS5361, 5362	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ pku70::hphMX //pKAN-trt1-D743A*
5D	*taz1Δ* + no pld	CF213	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺*
5D	wt + no pld	TN2411	See 2B.

TABLE S1 (continued)

Figure	Short genotype	Strain	Full genotype
5D	*taz1Δ trt1Δ rad3Δ* + no pld	TN2751	See 2A.
5D	*taz1Δ trt1Δ est1Δ* + no pld	LS5058	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ est1::natMX*
5D	*taz1Δ trt1Δ pku70Δ* + no pld	LS5020	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ pku70::hphMX*
5D	*taz1Δ trt1Δ* + no pld	CF458	See 2A.
5E	wt + no tag Trt1⁺	LS5420	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 trt1-2::his3⁺ //pKAN-trt1⁺*
5E	wt + Trt1-myc	CF830	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 trt1-2::his3⁺ //pKAN-trt1:Cmyc9*
5E	*taz1Δ* + no tag Trt1⁺	LS5444	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ //pKAN-trt1⁺*
5E	*taz1Δ* + Trt1-myc	LS5454	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ //pKAN-trt1:Cmyc9*
5E	*rad3Δ* + no tag Trt1⁺	LS6664	*h⁻ leu1-32 ura4-D18 ade6-M216 his3-D1 trt1-2::his3⁺ rad3::LEU2 //pKAN-trt1⁺*
5E	*rad3Δ* + Trt1-myc	LS6666	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 trt1-2::his3⁺ rad3::LEU2 //pKAN-trt1:Cmyc9*
5E	*taz1Δ rad3Δ* + no tag Trt1⁺	LS5022	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ rad3::LEU2 //pKAN-trt1⁺*
5E	*taz1Δ rad3Δ* + Trt1-myc	LS5433	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ rad3::LEU2 //pKAN-trt1:Cmyc9*
5E	*taz1Δ est1Δ* + no tag Trt1⁺	LS5240	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ est1::natMX //pKAN-trt1⁺*
5E	*taz1Δ est1Δ* + Trt1-myc	LS5423	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ est1::natMX //pKAN-trt1:Cmyc9*
5E	*pku70Δ* + no tag Trt1⁺	LS6604	*h⁻ leu1-32 ura4-D18 ade6-M216 his3-D1 trt1-2::his3⁺ pku70::hphMX //pKAN-trt1⁺*
5E	*pku70Δ* + Trt1-myc	LS6603	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 trt1-2::his3⁺ pku70::hphMX //pKAN-trt1:Cmyc9*
5E	*taz1Δ pku70Δ* + no tag Trt1⁺	LS5192	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ pku70::hphMX //pKAN-trt1⁺*
5E	*taz1Δ pku70Δ* + Trt1-myc	LS5427	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ pku70::hphMX //pKAN-trt1:Cmyc9*
5E	*taz1Δ pku70Δ* + no tag Trt1-D590A	LS5945	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ pku70::hphMX //pKAN-trt1-D590A*
5E	*taz1Δ pku70Δ* + Trt1-D590A-myc	LS5947	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ pku70::hphMX //pKAN-trt1-D590A:Cmyc9*
5E	*taz1Δ pku70Δ* + no tag Trt1-D743A	LS5949	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ pku70::hphMX //pKAN-trt1-D743A*
5E	*taz1Δ pku70Δ* + Trt1-D743A-myc	LS5951	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4⁺ trt1-2::his3⁺ pku70::hphMX //pKAN-trt1-D743A:Cmyc9*
S1A-D	*taz1Δ/taz1⁺ trt1Δ/trt1⁺*	CF382, CF383	*h⁺/h⁻ leu1-32/leu1-32 ura4-D18/ura4-D18 ade6-M210/ade6-M216 his3-D1/his3-D1 taz1-2::ura4⁺/taz1⁺ trt1-2::his3⁺/trt1⁺*
S1C	*trt1Δ/trt1⁺*	CF248, CF255	*h⁺/h⁻ leu1-32/leu1-32 ura4-D18/ura4-D18 ade6-M210/ade6-M216 his3-D1/his3-D1 trt1-2::his3⁺/trt1⁺*
S2, S3	*taz1Δ/taz1⁺ trt1-D743A/trt1⁺*	CF658, CF659	See 3A.
S4, S5	*taz1Δ/taz1⁺ trt1Δ/trt1⁺*	CF382	See 1D.
S4, S5	*taz1Δ/taz1⁺ trt1-D743A/trt1⁺*	CF658	See 3A-F.
S6	*taz1Δ trt1Δ*	CF396, CF397	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 trt1-2::his3⁺ taz1-2::ura4⁺*

TABLE S1 (continued)

Figure	Short genotype	Strain	Full genotype
S6	*taz1Δ trt1Δ pku70Δ*	CF2569, CF2570	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 trt1-2::his3⁺ taz1-2::ura4⁺ pku70::kanMX4*
S6	*taz1Δ trt1Δ est1Δ*	LS4946, LS4947	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 trt1-2::his3⁺ est1::kanMX taz1-2::ura4⁺*
S6	*taz1Δ trt1Δ rad3Δ*	TN2638, TN2639	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 trt1-2::his3⁺ taz1-2::ura4⁺ rad3::LEU2*