Expanded View Figures

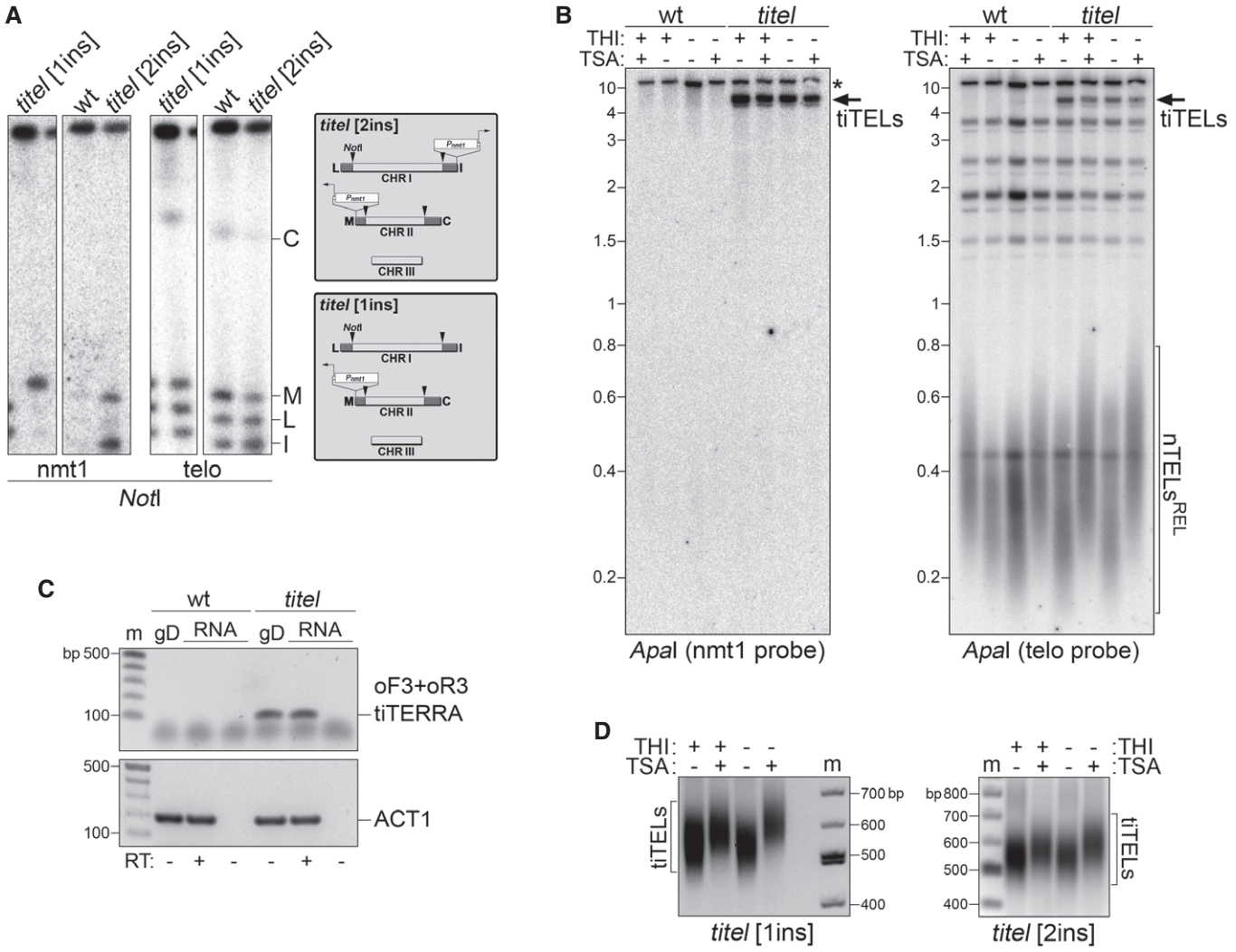

Figure EV1. Genomic organization and functional characterization of tiTELs.

A Pulsed-field gel electrophoresis (PFGE) analysis of Pnmt1 insertion (ins) in tiTEL strains. Genomic DNA from wt cells (CAF13) and cells carrying one tiTEL (CF545) or two tiTELs (CAF110) maintained in YES medium was digested with *Not*I, separated by PFGE, and hybridized sequentially using nmt1 and telomeric probes. The positions and nomenclature of the four *Not*I terminal restriction fragments entering the gel are indicated on the right. Genomic organization of tiTEL strains is sketched on the right.

B Genomic DNA was extracted from wt (CAF13) cells and cells carrying two tiTELs (CAF110) cultured for 24 h in EMM with THI and TSA as indicated. DNA was digested with *Apa*I and hybridized sequentially using nmt1 and telomeric probes. The asterisk indicates a band corresponding the endogenous *nmt1*+. TiTELs and natural telomeres (nTELs) are indicated. REL refers to telomeric sequences released in the lower part of the gel. Marker molecular weights are on the left in kilobases.

C Control RT–PCR experiments showing the specificity of oF3 + oR3 oligonucleotides. Nucleic acids were prepared from wt cells (CAF13) and cells carrying one tiTEL (CAF545) cultured for 24 h in EMM without THI. gD indicates genomic DNA, RT indicates whether or not samples were treated with reverse transcriptase. ACT1 PCR was performed to assure presence of genomic DNA and cDNA templates. Marker (m) molecular weights are on the left in base pairs.

D Telo-PCR analysis of tiTELs using genomic DNA from cells carrying one tiTEL (CAF545, on the left) or two tiTELs (CAF110, on the right) cultured for 24 h in EMM with THI and TSA as indicated. Marker (m) molecular weights are shown in base pairs.

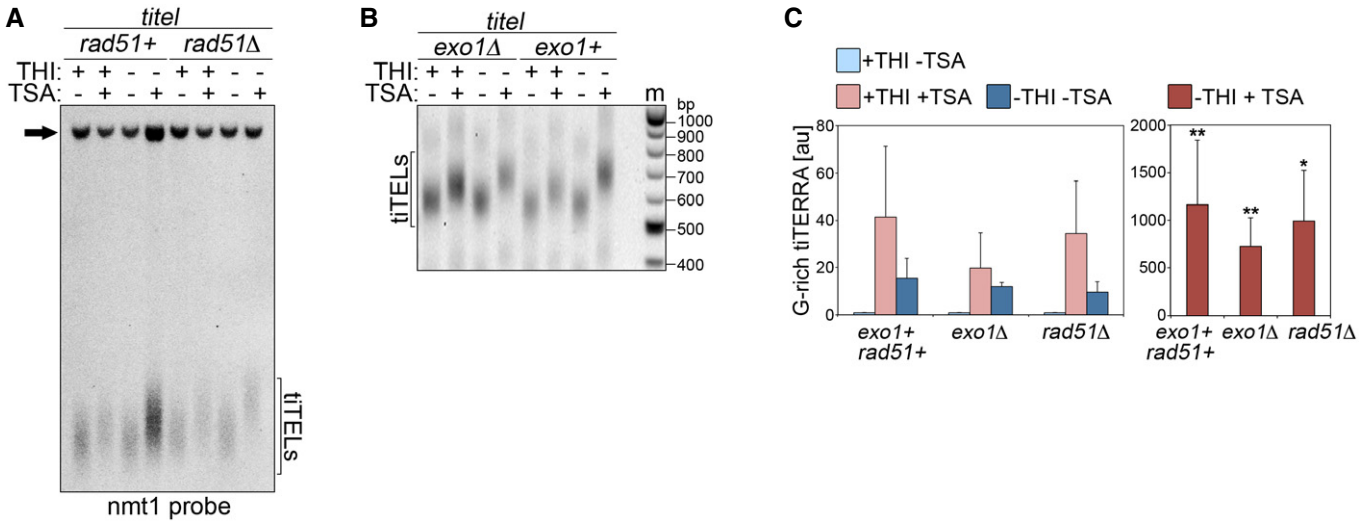

Figure EV2. Rad51 and Exo1 are not required for tiTEL elongation.

A Genomic DNA was extracted from cells carrying one tiTEL either *rad51*+ (CAF545) or *rad51*Δ (CAF550) cultured for 24 h in EMM with THI and TSA as indicated. DNA was digested with *Hind*III and Southern blot-hybridized with nmt1 probes. The black arrow points to the *nmt1*+ sequence on chromosome III.

B Telo-PCR analysis of tiTELs in cells carrying one tiTEL either *exo1*+ (CAF545) or *exo1*Δ (CAF655) cultured for 24 h in EMM with THI and TSA as indicated. Marker (m) molecular weights are on the right in base pairs.

C qRT–PCR analysis of total tiTERRA levels in cells as in (A) and (B). Values are expressed as fold increase over uninduced *exo1*+ *rad51*+ strain (CAF545). Bars and error bars are averages and SD from 3 independent experiments. *$P < 0.05$, **$P < 0.01$ (relative to uninduced parental; two-tailed Student's *t*-test).

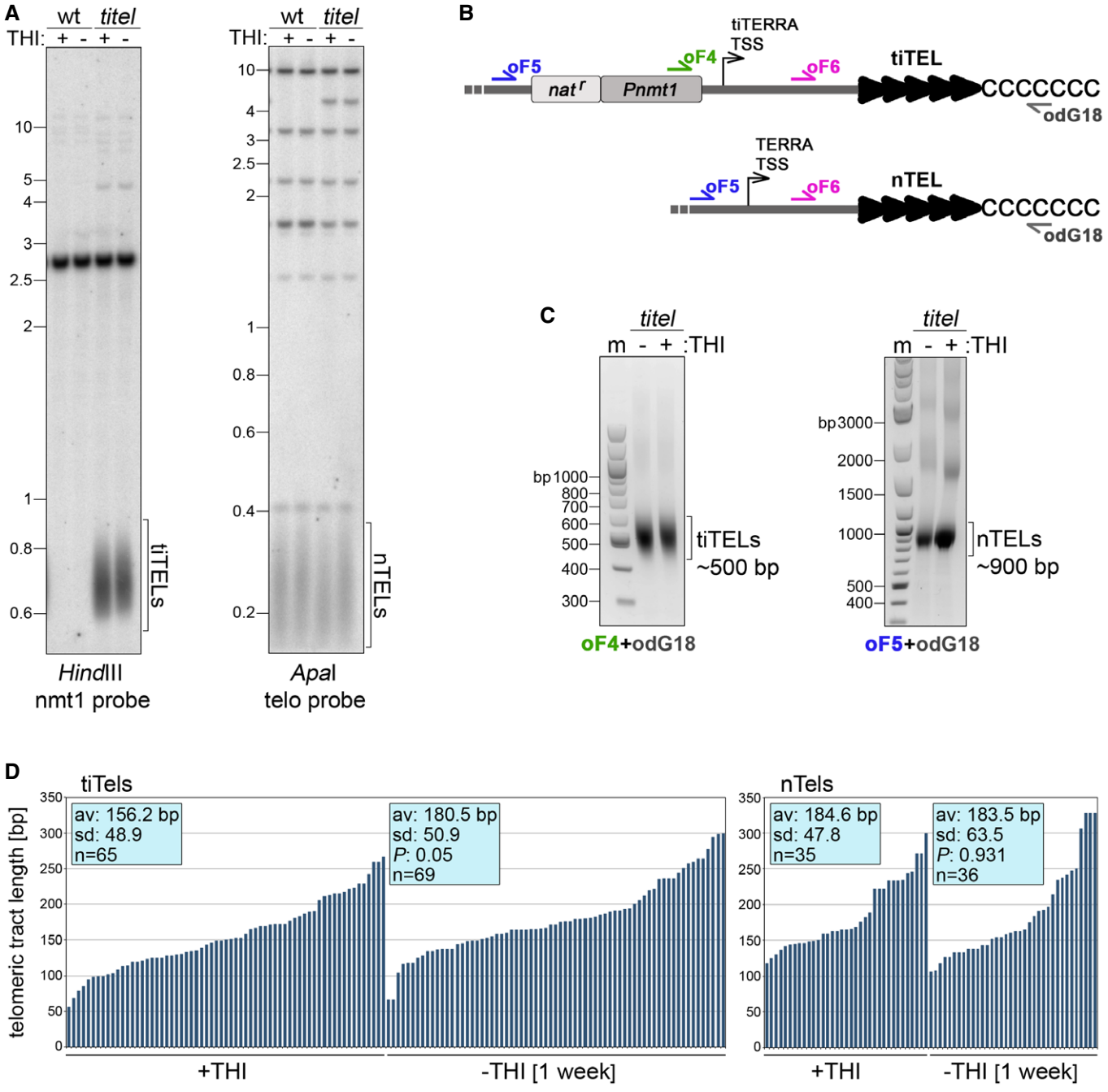

Figure EV3. Sequencing of tiTELs.

A Genomic DNA was isolated from wt cells (CAF13) and cells carrying two tiTELs (CAF110) cultured for 7 days in EMM with or without THI. DNA was digested and hybridized as indicated. Marker molecular weights are on the left in kilobases.

B Sketch showing the positions of oligonucleotides used for Telo-PCR amplification and sequencing of tiTELs and natural telomeres from the same cell. Telo-PCRs were performed using oF4 or oF5 in combination with the G-rich oligonucleotide odG18. Sequencing was performed with oF6.

C Examples of Telo-PCR products using genomic DNA from tiTEL cells as in (A) and amplified using the indicated oligonucleotides. Marker (m) molecular weights are on the left in base pairs.

D Telomere PCR products corresponding to tiTELs (on the left) or nTELs (on the right) from tiTEL cells as in (A) were cloned and sequenced. Blue bars represent individual telomeres plotted against their length in base pairs (*y*-axis). Average telomere length of the sequenced population (av) and standard deviations (SD) are indicated along with *P*-values (−THI vs. +THI) calculated with the two-tailed Student's *t*-test.

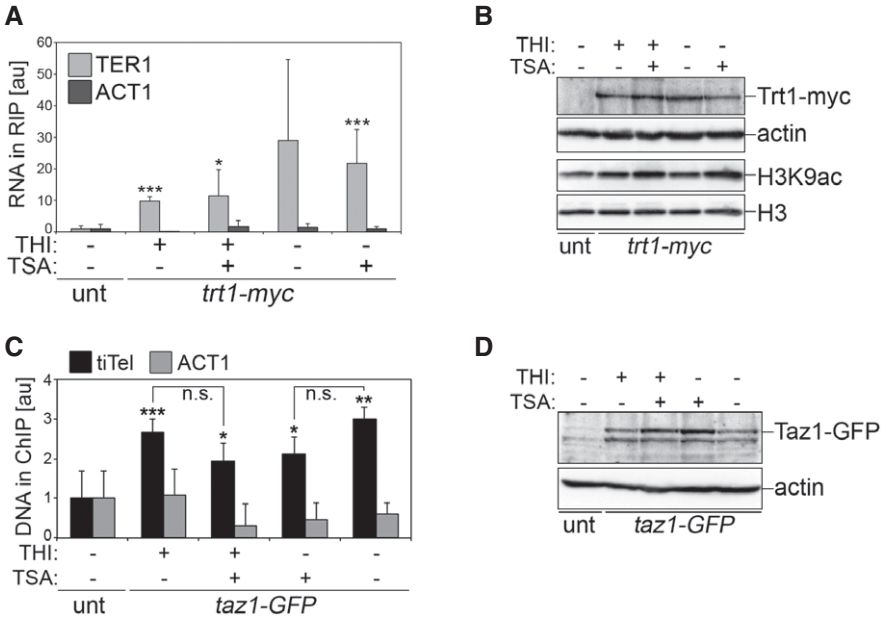

Figure EV4. Control experiments for tiTERRA RIPs and binding of Taz1-GFP to tiTELs.

A RIP experiments performed using anti-myc antibodies and extracts from cells carrying one tiTEL and expressing Trt1-myc (CAF610) cultured for 24 h in EMM with THI and TSA as indicated. TER1 (positive control) and ACT1 (negative control) in input and immunoprecipitated material were quantified by qRT–PCR. Values correspond to fraction of input RNA expressed as fold increase over an untagged (unt) control tiTEL strain (CAF545). Bars and error bars are averages and SD from three independent experiments. $*P < 0.05$, $***P < 0.001$ (relative to unt; two-tailed Student's *t*-test).

B Western blot analysis of Trt1-myc and histone H3 acetylated at lysine 9 (H3K9ac) levels in cells as in (A). Actin and total H3 serve as loading controls. The accumulation of H3K9ac in TSA+ cells confirms the effectiveness of TSA. The two upper panels and the two lower panels are from two independent membranes.

C ChIP experiments were performed using anti-GFP antibodies and extracts from cells carrying one tiTEL and expressing Taz1-GFP (MKSP1781) cultured for 24 h in EMM with THI and TSA as indicated followed by tiTEL qPCR. ACT1 qPCRs were performed to control for specificity. Values correspond to fraction of input DNA expressed as fold increase over an untagged control tiTEL strain (CAF545). Bars and error bars are averages and SD from three independent experiments. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (relative to unt; two-tailed Student's *t*-test).

D Western blot analysis of Taz1-GFP protein levels in cells as in (C). Actin serves as a loading control.