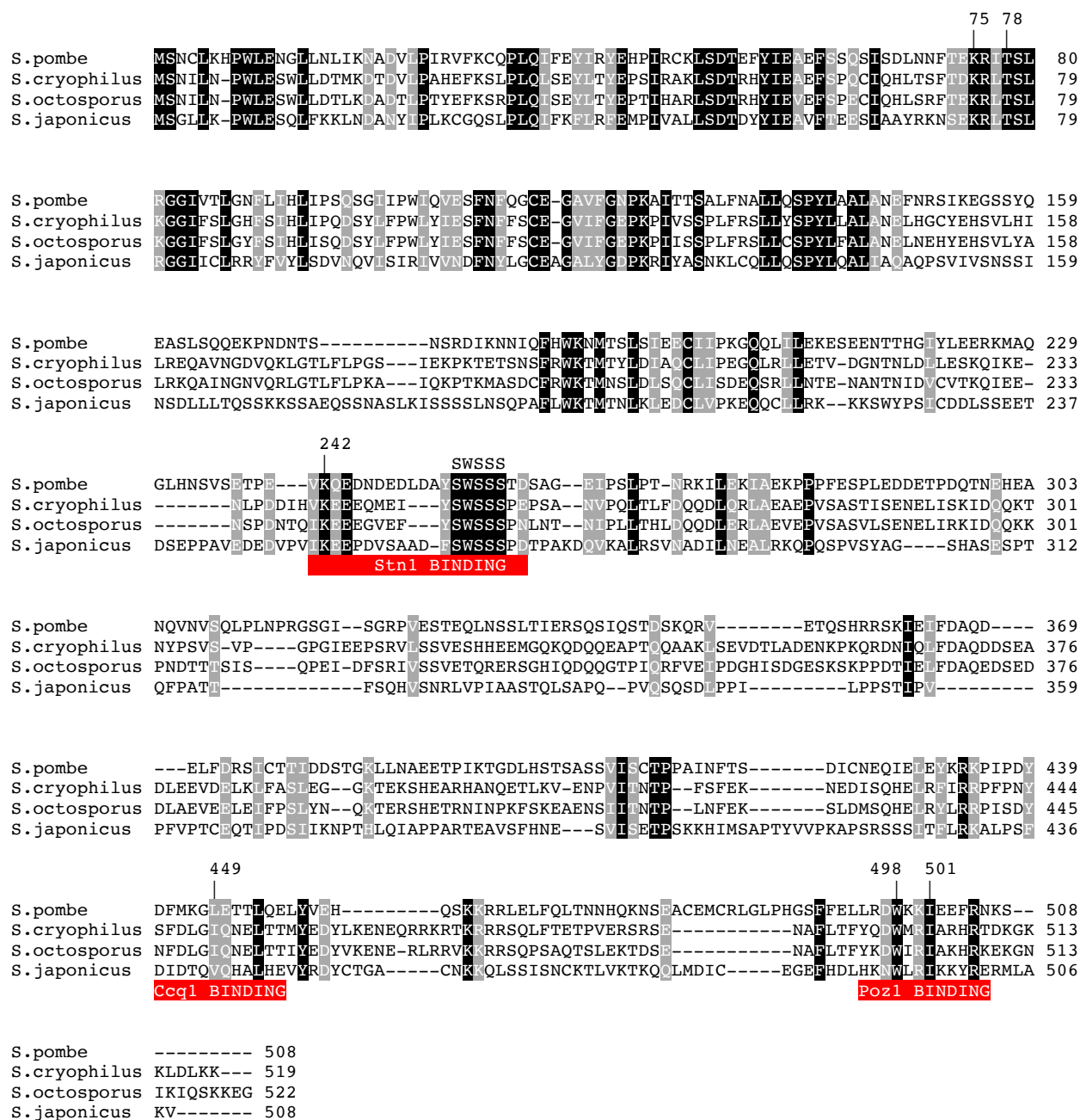
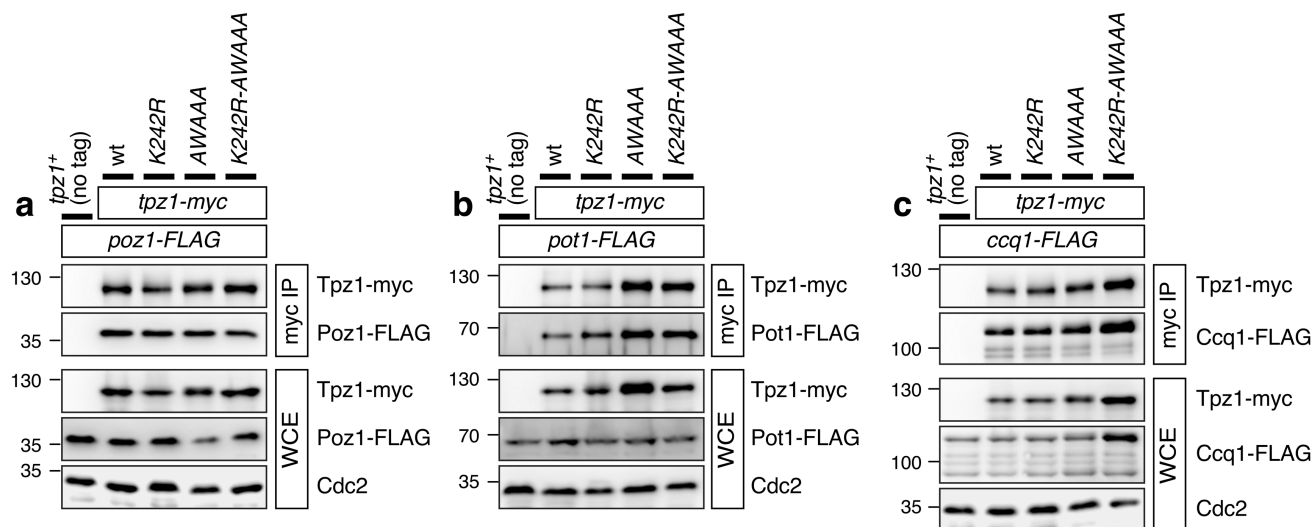
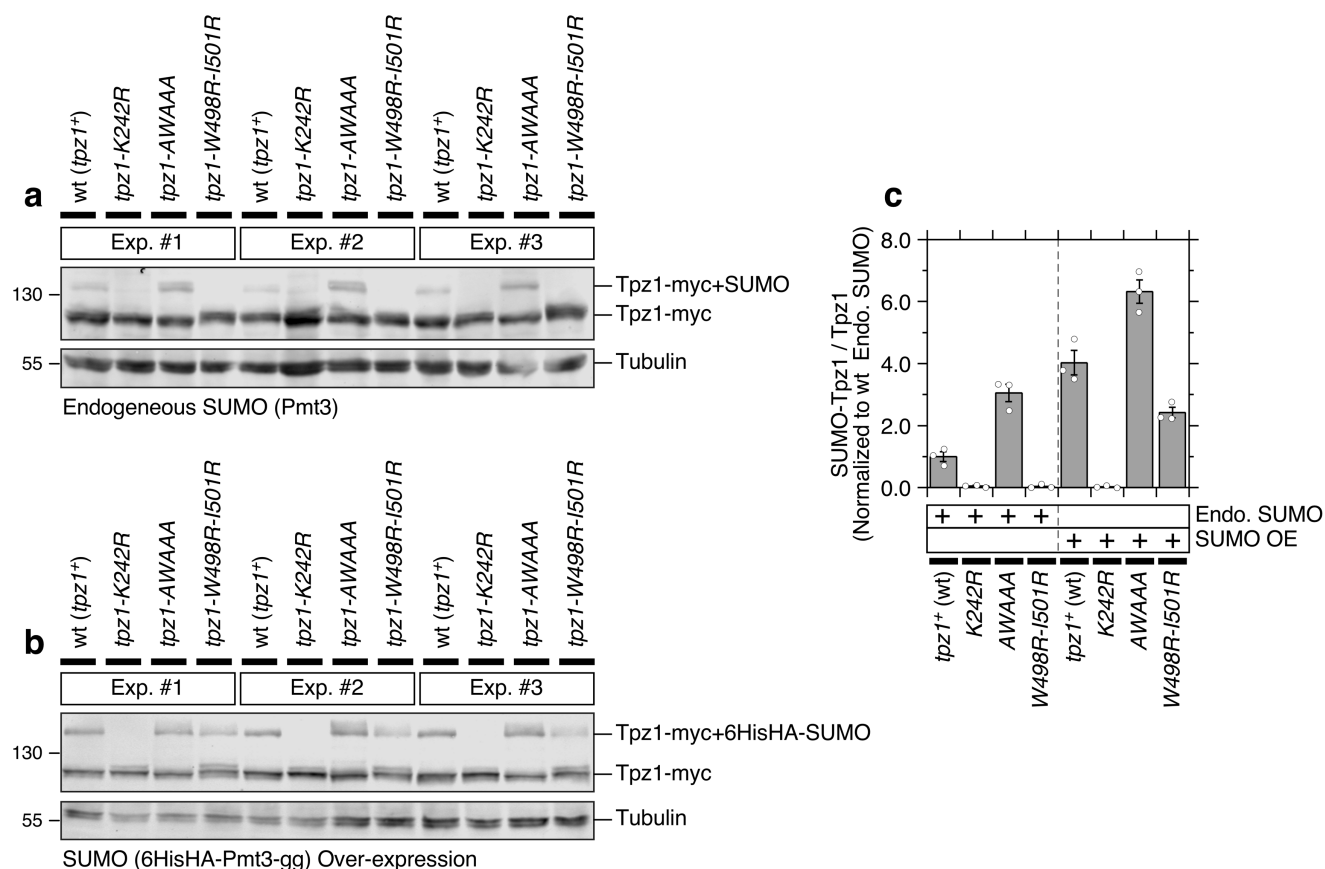




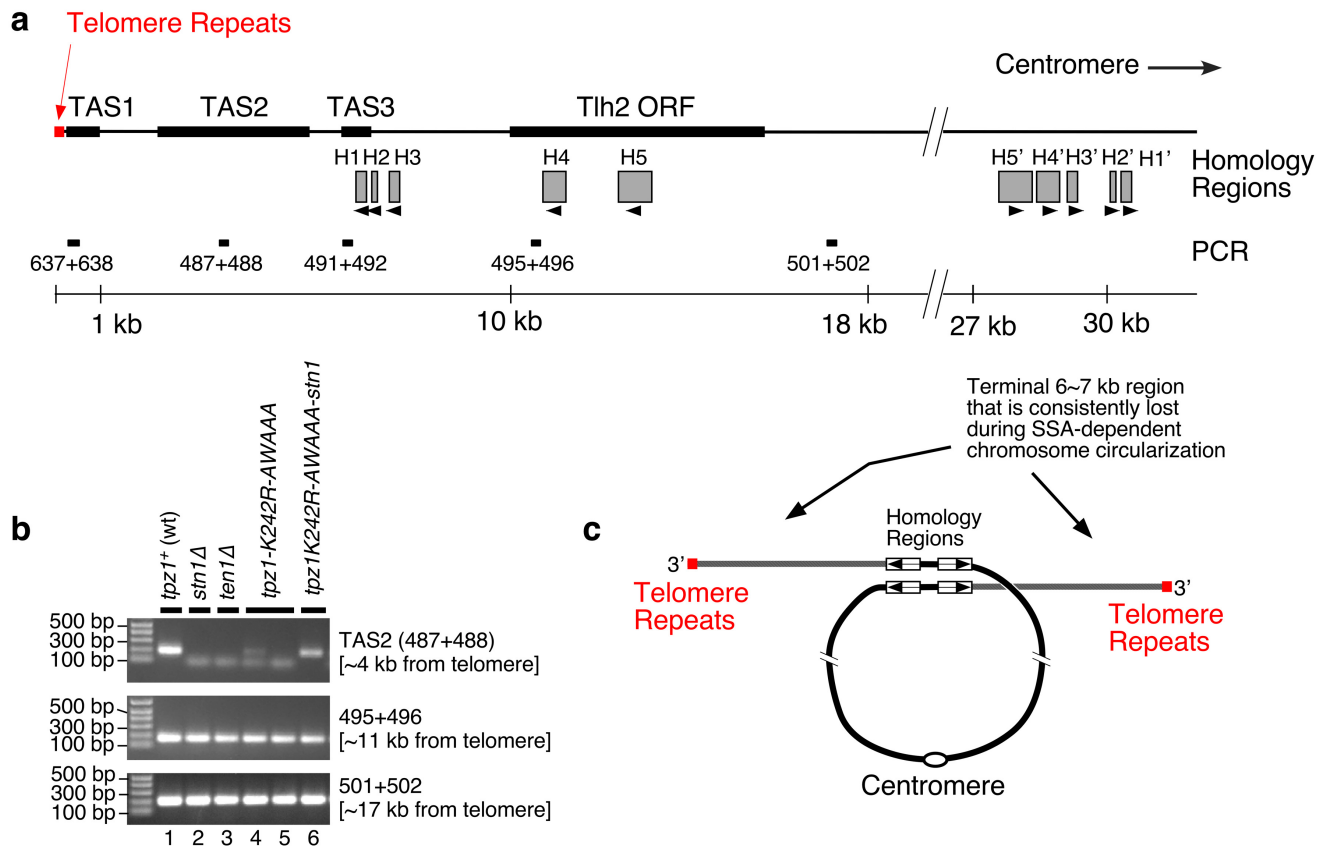
Supplementary Figure 1. Alignment of full length Tpz1 among four *Schizosaccharomyces* species. Identical residues conserved among all four species are marked black, while amino acid residues that maintain similar chemical properties among all four species are marked gray. Regions/Residues important for mediating Tpz1-Stn1, Tpz1-Ccq1, and Tpz1-Poz1 interactions are also indicated.



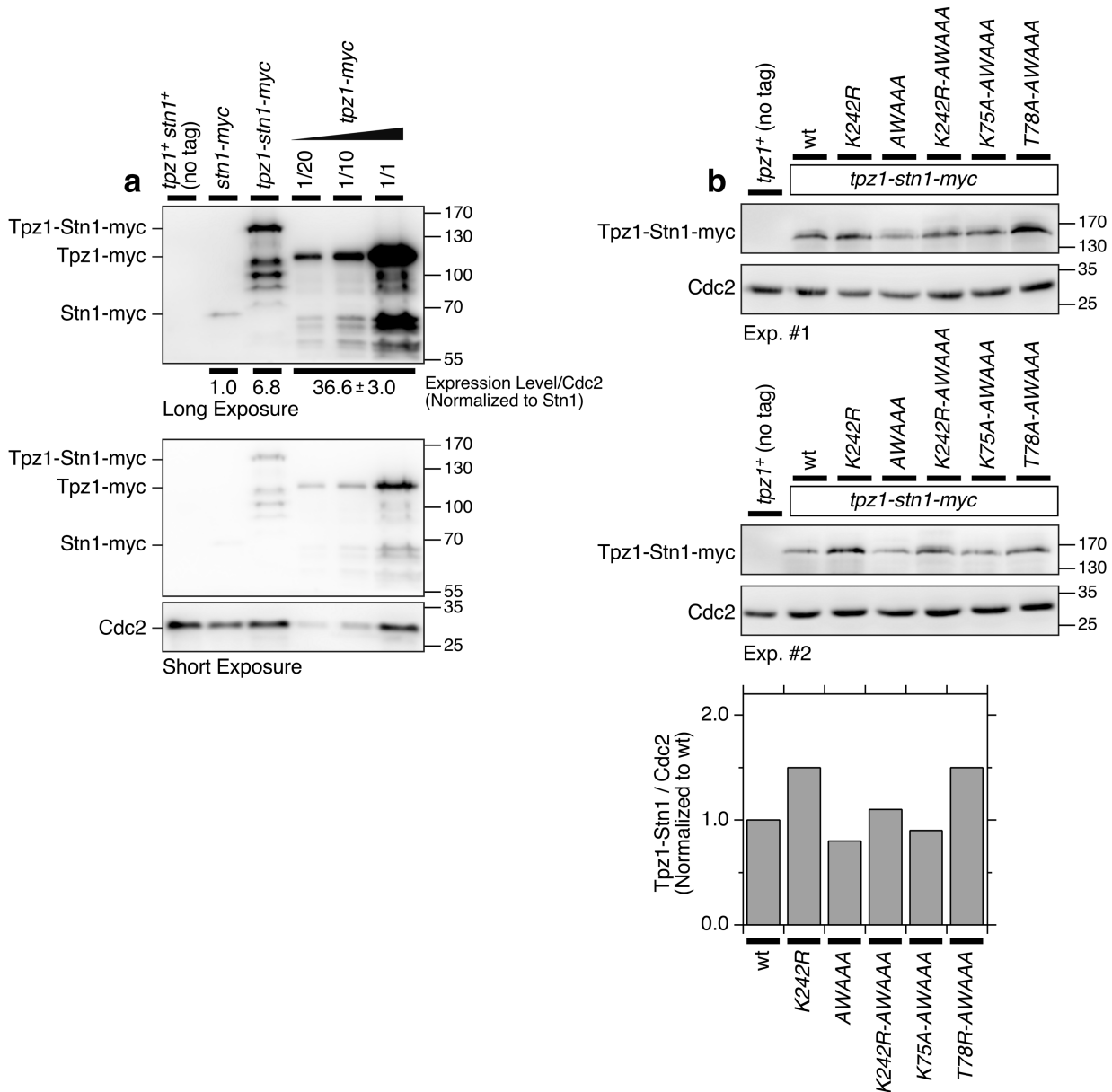
Supplementary Figure 2. Neither *tpz1*-K242R nor *tpz1*-AWAAA mutation interferes with the formation of the shelterin complex. **(a-c)** Co-IP analysis for (a) Tpz1-Poz1, (b) Tpz1-Pot1, and (c) Tpz1-Ccq1 interaction for wild-type (wt), K242R, AWAAA or K242R-AWAAA mutants of Tpz1. Cdc2 western blot served as loading control for whole cell extract (WCE). Molecular weight (kDa) of size markers are indicated.



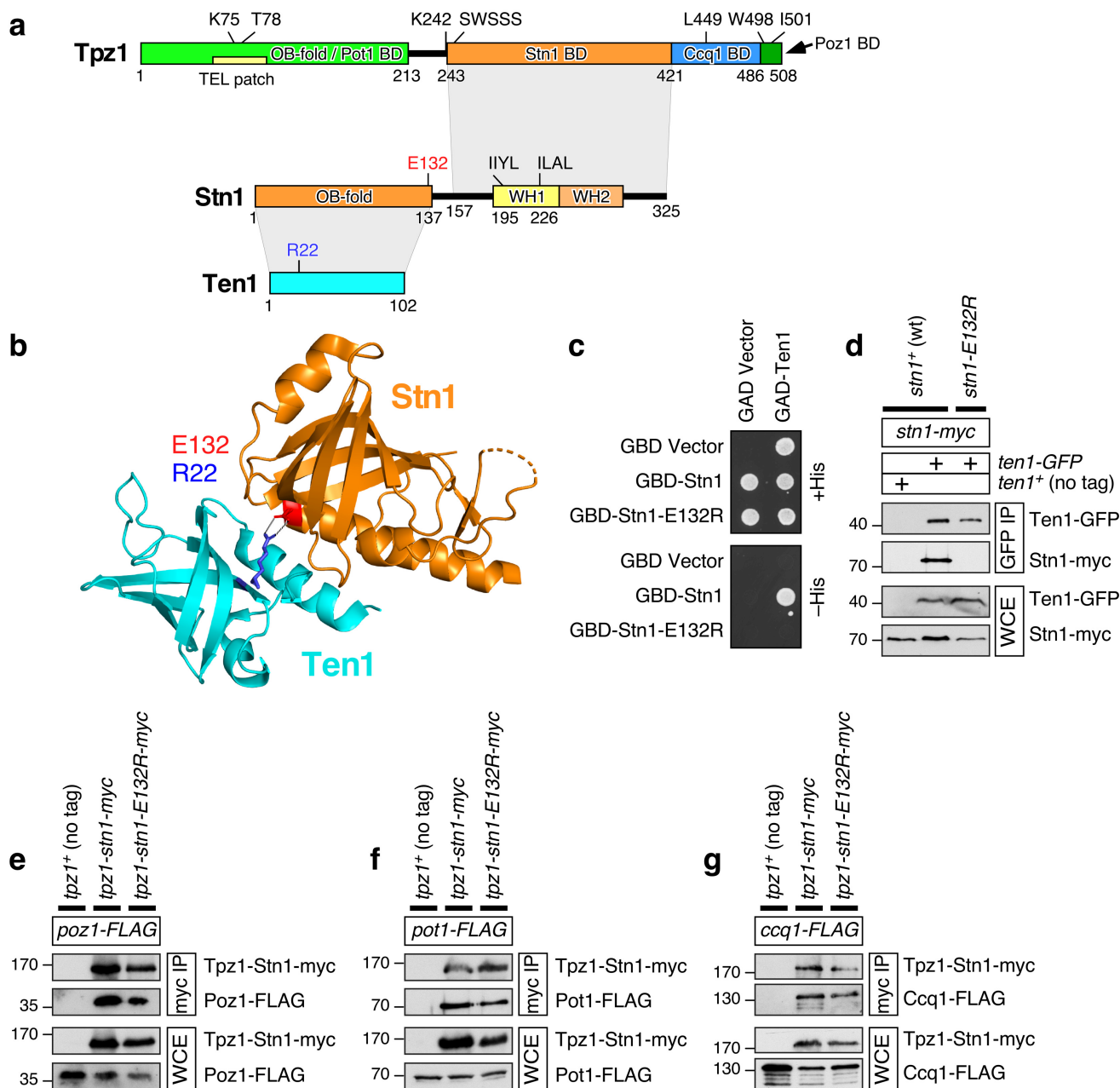
Supplementary Figure 3. Analysis of Tpz1 SUMOylation levels by western blot. Detection of Tpz1 SUMOylation in wild-type (wt) and indicated mutant alleles of Tpz1 with **(a)** endogenous SUMO or **(b)** over-expressed 6HisHA-SUMO. Molecular weight (kDa) of size markers are indicated. **(c)** Quantification of SUMO-Tpz1/Tpz1 ratio, normalized to wild-type cells with endogenous SUMO expression. Error bars represent SEM (n=3). Quantification data is included in Supplementary Data 1.



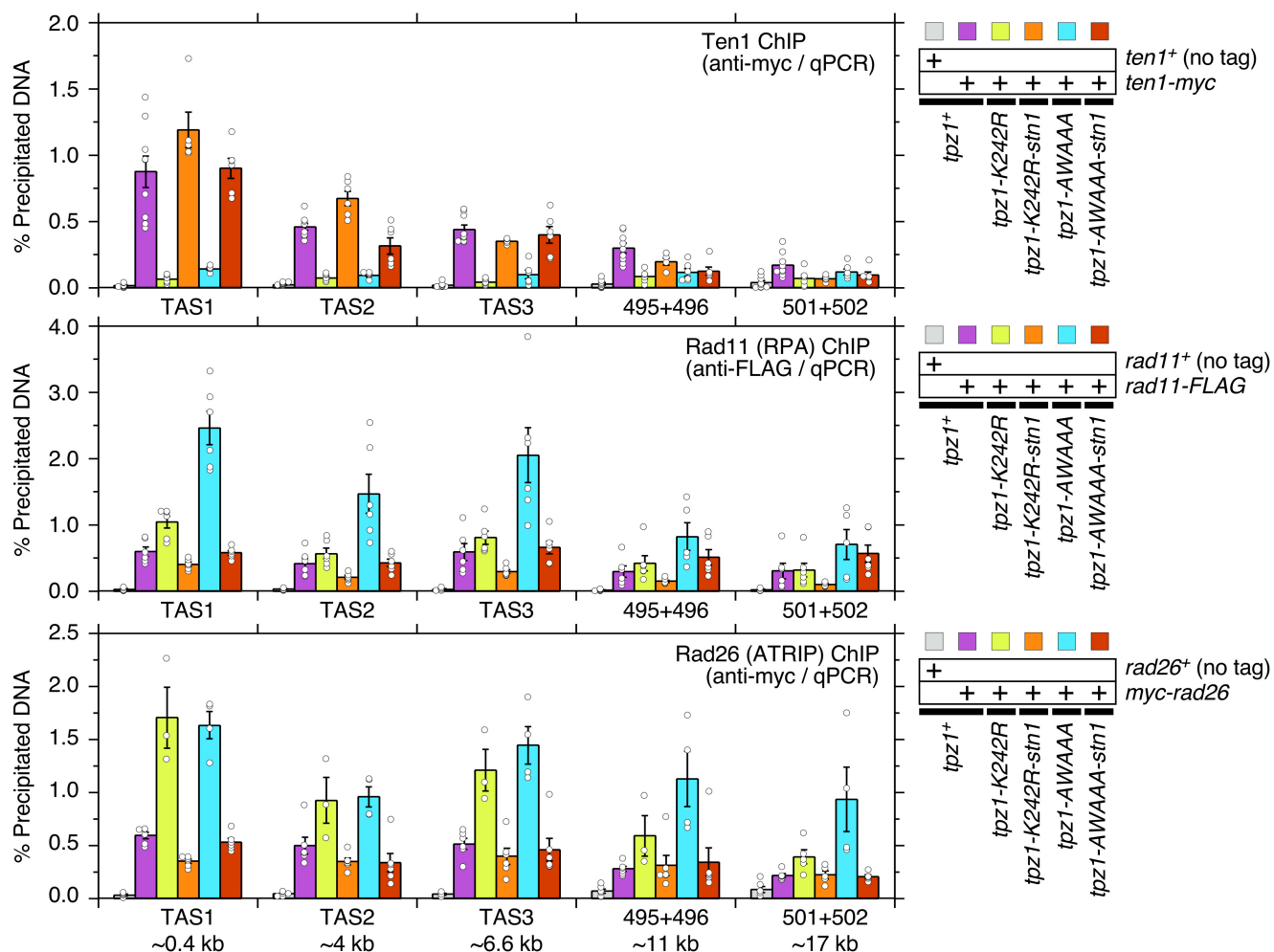
Supplementary Figure 4. Fission yeast strains carrying circular chromosomes lose sub-telomeric sequence due to SSA. **(a)** A schematic map of telomere and sub-telomere regions. Main regions of sub-telomeres¹ and previously identified homology regions utilized in generating circular chromosomes by SSA mechanism² are indicated. Locations and primer numbers for PCR products used to characterize sub-telomere regions in **(b)** or sub-telomere ChIP analysis shown in Supplementary Figure 7 are also indicated. **(b)** PCR analysis of indicated strains for sub-telomere regions as indicated. **(c)** A schematic drawing depicting loss of distal sub-telomere regions after SSA-based fusions that generate circular chromosomes in fission yeast cells defective in telomere protection.



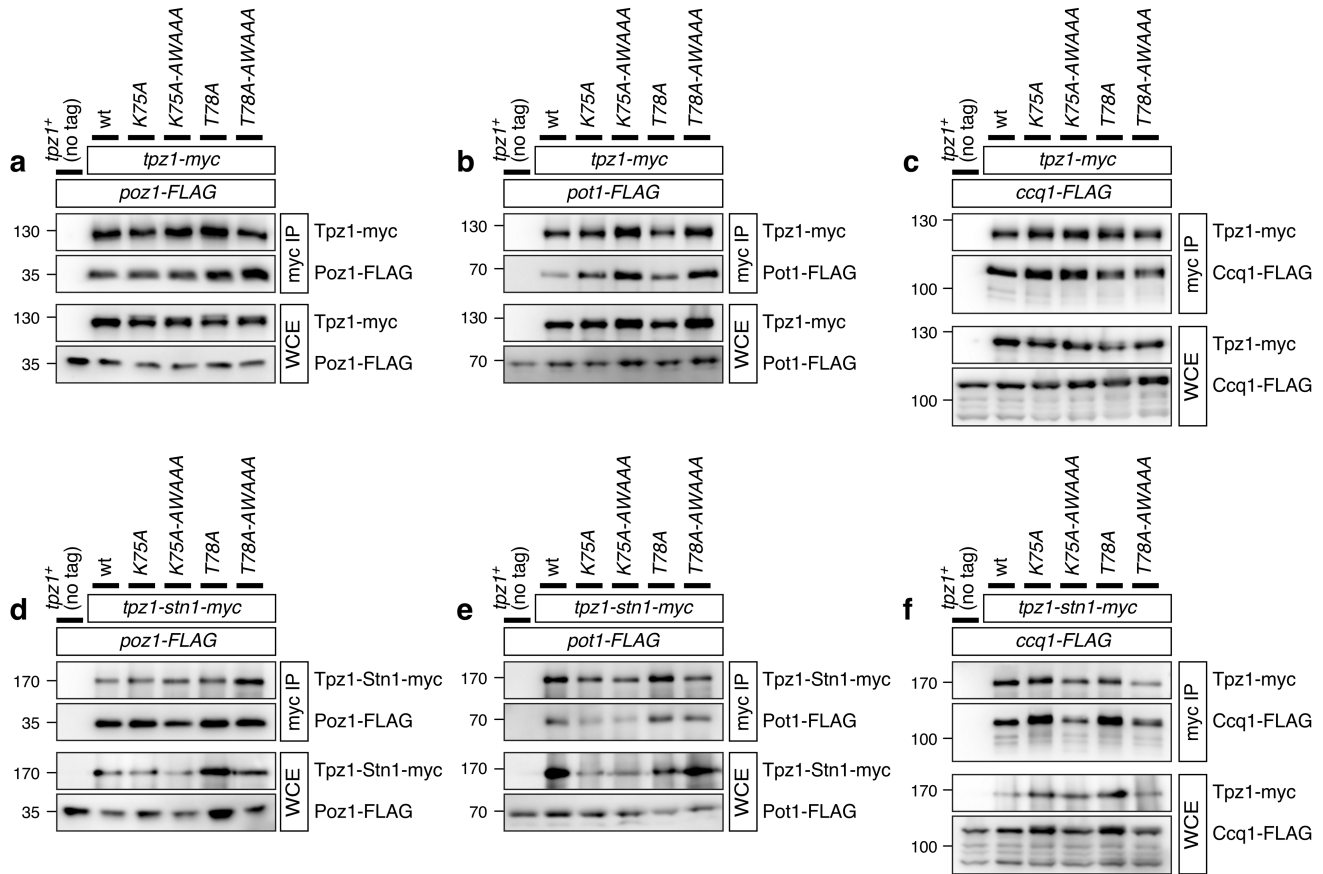
Supplementary Figure 5. Western blot analysis of Stn1, Tpz1 and Tpz1-Stn1 proteins. **(a)** Expression levels for myc-tagged Stn1, Tpz1-Stn1 and Tpz1 were analyzed by western blot. Molecular weight (kDa) of size markers are indicated. Expression levels of indicated proteins were quantified against Cdc2 loading control, and normalized to the value obtained for Stn1. For Tpz1, average of Tpz1/Cdc2 ratio among three indicated dilutions and SEM is indicated. **(b)** Comparison of Tpz1-Stn1 expression levels among wild-type (wt) and indicated tpz1 mutant alleles. Western blot results from two independent experiments are shown. Average of Tpz1-Stn1/Cdc2 ratio from two experiments shown (normalized to wt) were plotted. No major changes in expression levels for Tpz1-Stn1 fusion protein were observed among different mutants. Quantification data is included in Supplementary Data 1



Supplementary Figure 6. Additional characterization of *stn1-E132R* mutation and Tpz1-Stn1 fusion construct. **(a)** A schematic representation of Tpz1, Stn1 and Ten1. Domains previously found to mediate Tpz1-Stn1 and Stn1-Ten1 interaction are indicated with gray shaded areas^{3,4}. In addition, residues that are important for mediating Stn1-Ten1 interaction (Stn1 Glu132 and Ten1 Arg22) and possible SUMO-Interacting Motif (SIM) sequences in Stn1 (IIYL and ILAL) are indicated. For Tpz1, see Fig 1b legend for details on indicated domains and residues. **(b)** Crystal structure of Stn1-Ten1 complex⁵. Amino acid residues for Stn1 Ser16-His154 and Ten1 Asp2-Asp102 are shown (PDF ID: 3KF6). Side chains for Stn1 Glu132 and Ten1 Arg22, predicted to play a critical role in facilitating Stn1-Ten1 interaction are shown, and highlighted in red and blue, respectively. **(c, d)** *Stn1-E132R* mutation disrupts Stn1-Ten1 interaction as detected by (c) Y2H assay and (d) co-IP. **(e-g)** Based on co-IP assays, both Tpz1-Stn1 and Tpz1-Stn1-E132R fusion proteins can interact with the other shelterin subunits (e) Poz1, (f) Pot1 and (g) Ccq1. Molecular weight (kDa) of size markers are indicated.



Supplementary Figure 7. ChIP analysis of sub-telomere regions for Ten1, Rad11 and Rad26. Quantitative PCR-based ChIP analysis for indicated genetic backgrounds are carried out for Ten1, Rad11 (RPA) and Rad26^{ATRIP}. See supplementary Fig 4a for locations of PCR products within sub-telomere regions, used in ChIP assays. Plots show mean values plus/minus SEM and distribution of individual data points from at least 3 independent experiments. Raw data values and statistical analysis of ChIP assays by two-tailed Student's t-test are shown in Supplementary Data 1.



Supplementary Figure 8. Mutant constructs of Tpz1 or Tpz1-Stn1 can still form the shelterin complex. **(a-c)** Co-IP analysis for (a) Tpz1-Poz1, (b) Tpz1-Pot1, and (c) Tpz1-Ccq1 interaction for wild-type (wt), and indicated mutants of Tpz1. **(d-f)** Co-IP analysis for (d) Tpz1-Poz1, (e) Tpz1-Pot1, and (f) Tpz1-Ccq1 interaction for wild-type (wt), and indicated Tpz1 mutant versions of Tpz1-Stn1 fusion protein. Molecular weight (kDa) of size markers are indicated.

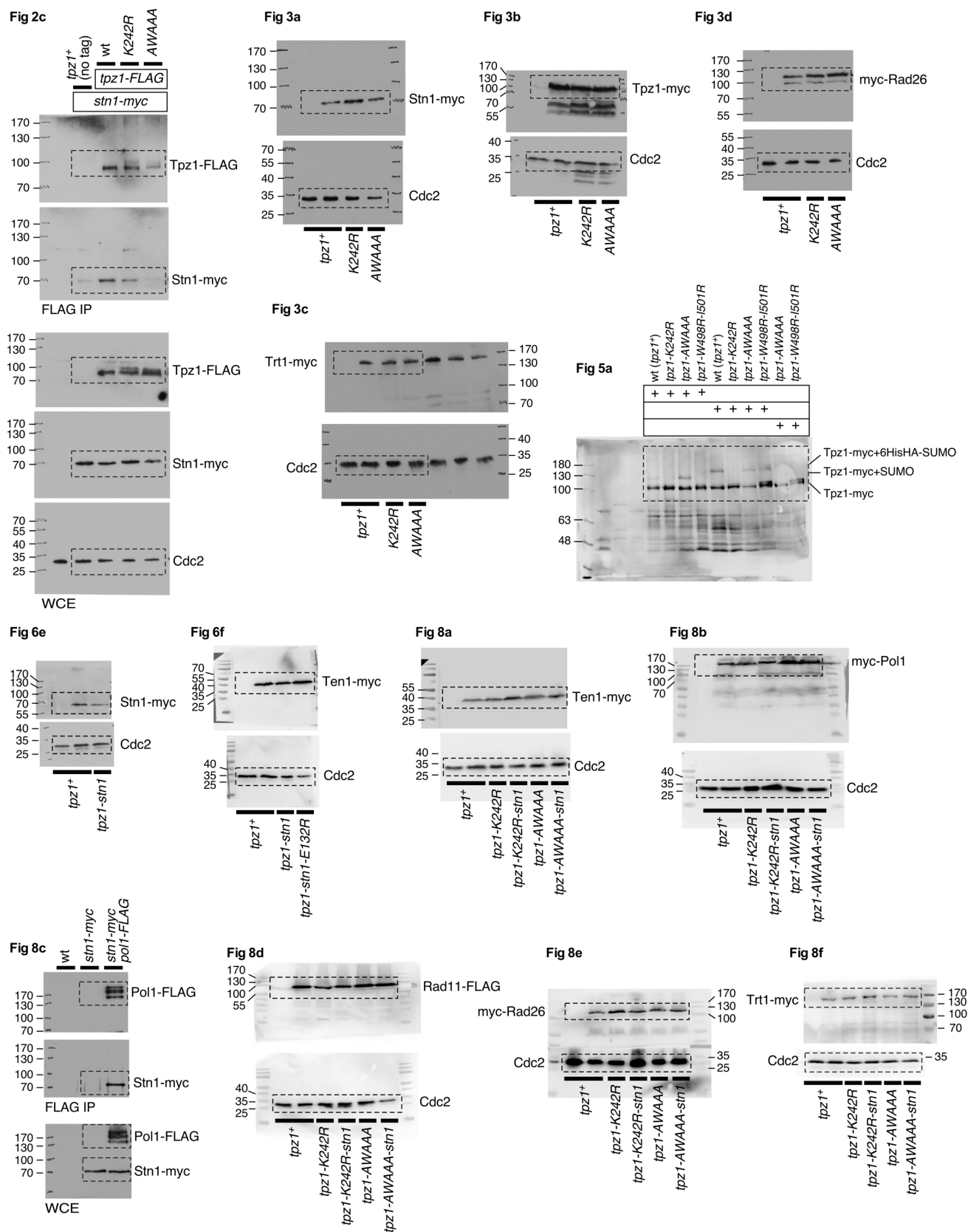


Fig 9d

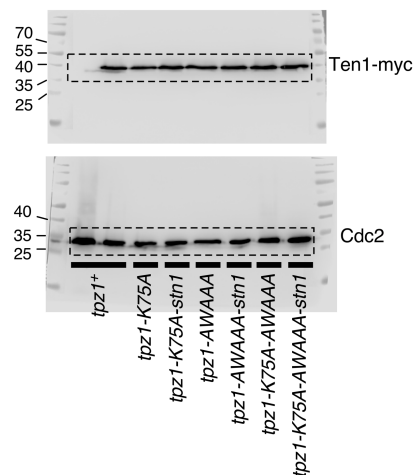
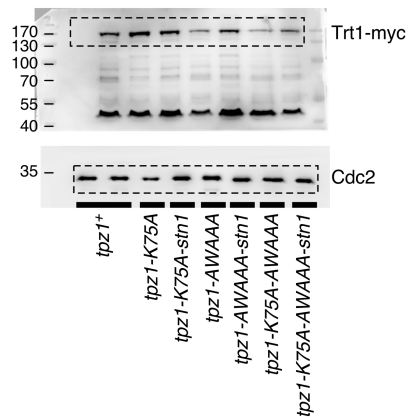
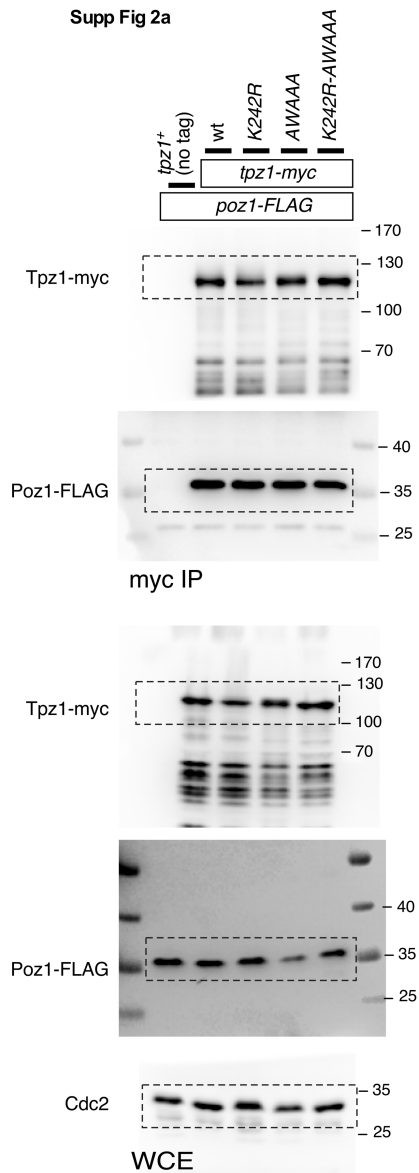


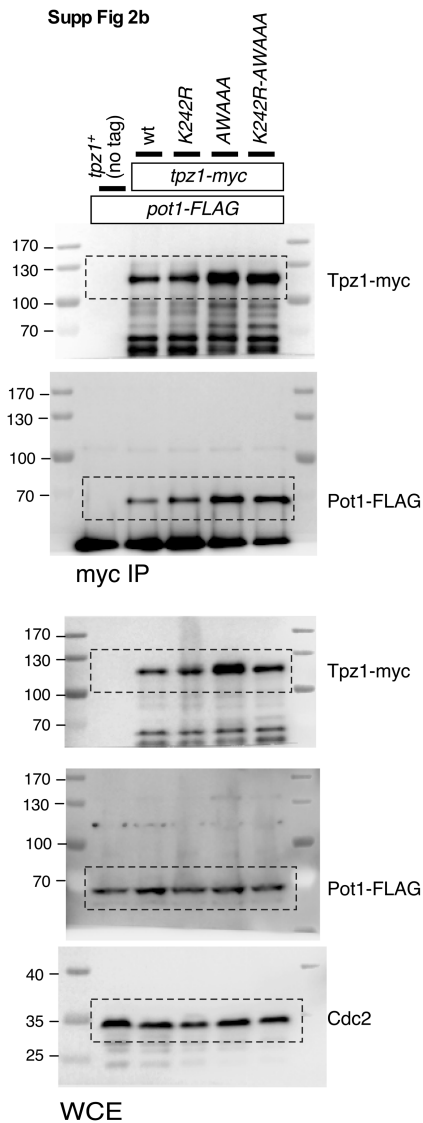
Fig 9e



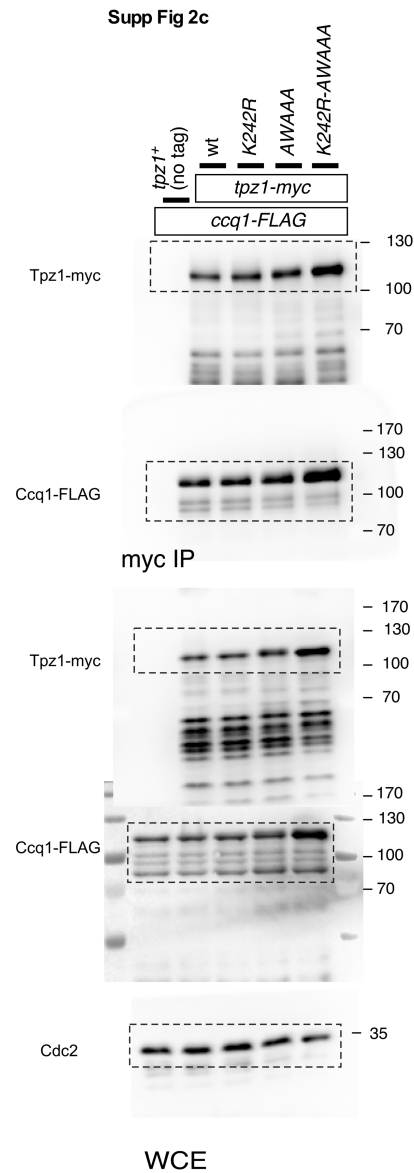
Supp Fig 2a



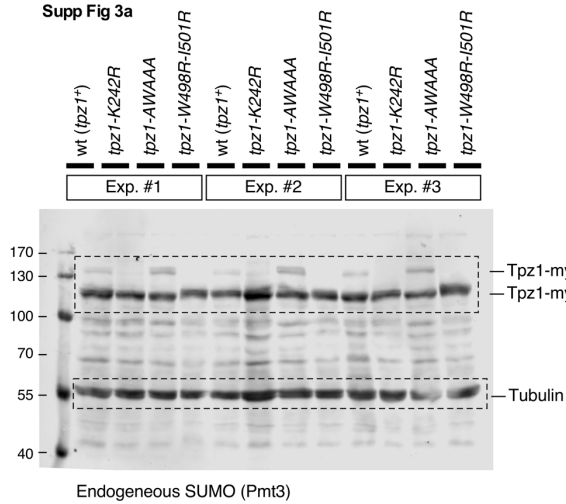
Supp Fig 2b



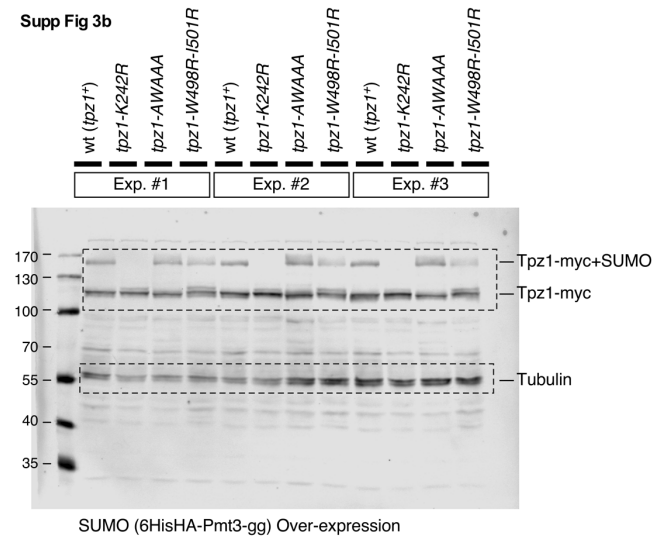
Supp Fig 2c



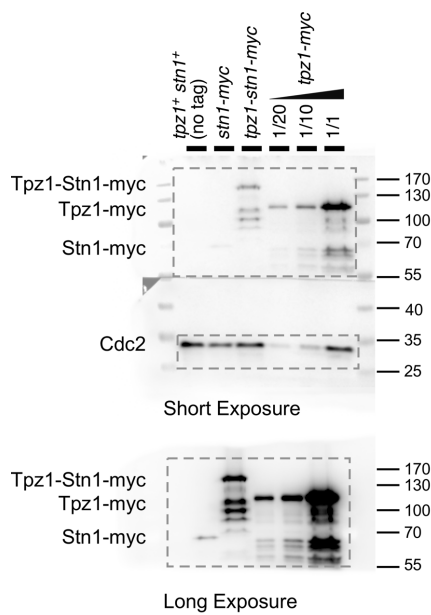
Supp Fig 3a



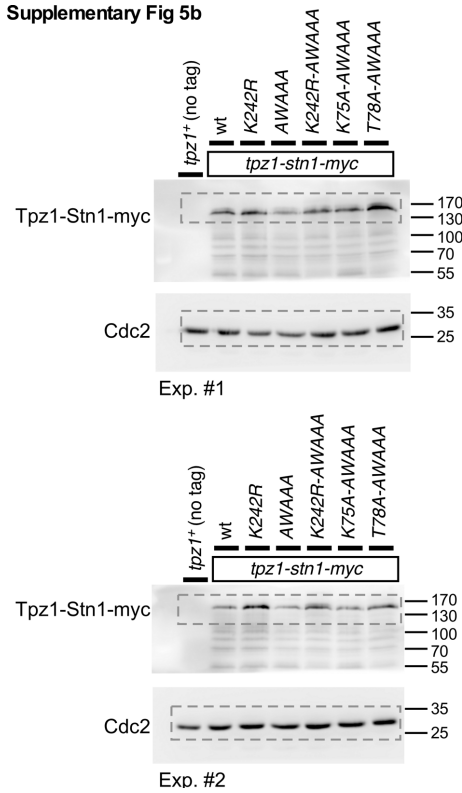
Supp Fig 3b

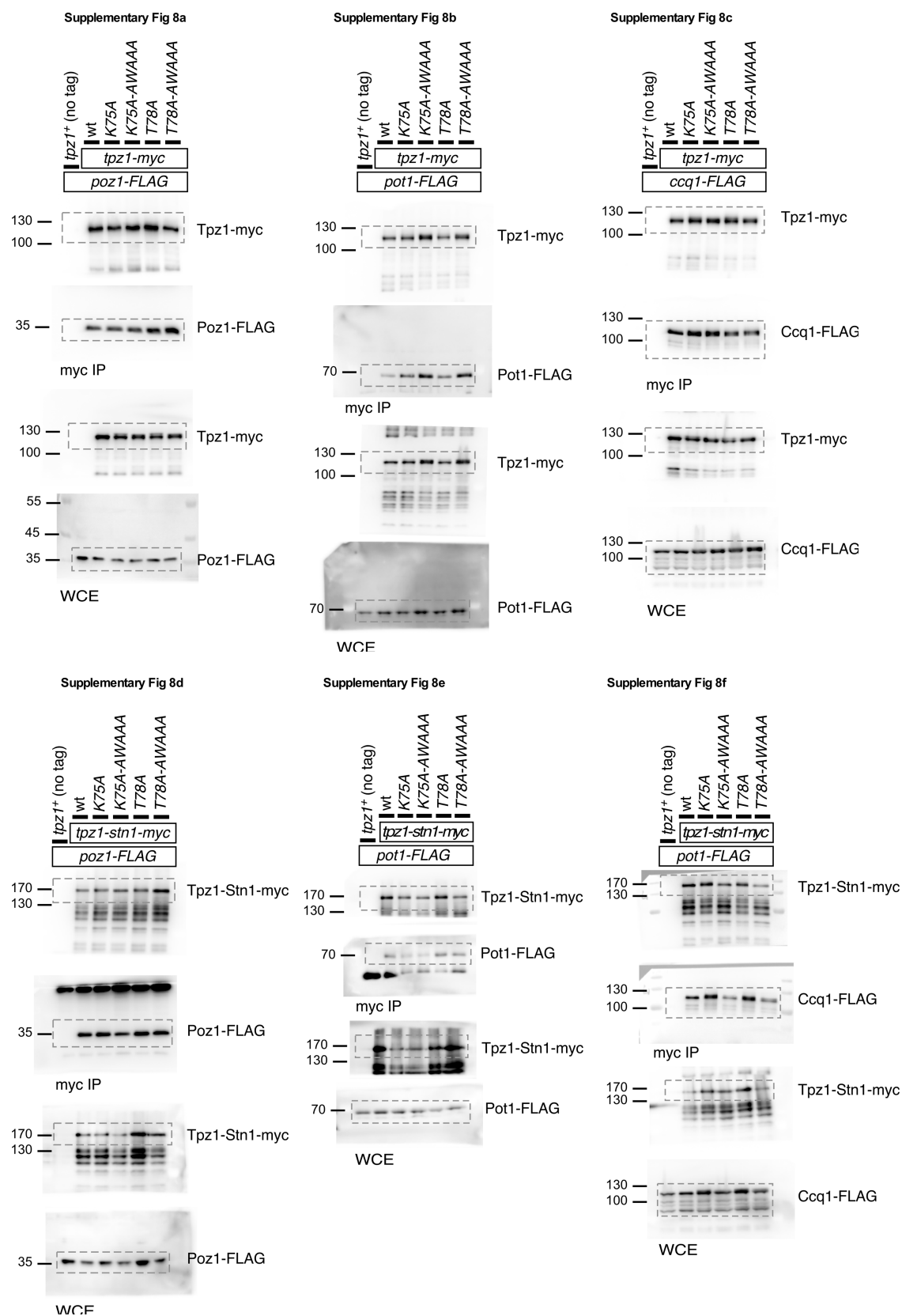


Supplementary Fig 5a



Supplementary Fig 5b





Supplementary Figure 9. Uncropped western blot gels for indicated figures. Areas of gels shown in final figures are marked with dashed boxes. Sizes of protein molecular weight markers (kDa) are also indicated.

SUPPLEMENTARY REFERENCES

1. Harland, J.L., Chang, Y.T., Moser, B.A. & Nakamura, T.M. Tpz1-Ccq1 and Tpz1-Poz1 interactions within fission yeast Shelterin modulate Ccq1 Thr93 phosphorylation and telomerase recruitment. *PLoS Genet* **10**, e1004708 (2014).
2. Wang, X. & Baumann, P. Chromosome fusions following telomere loss are mediated by single-strand annealing. *Mol Cell* **31**, 463-73 (2008).
3. Chang, Y.T., Moser, B.A. & Nakamura, T.M. Fission yeast shelterin regulates DNA Polymerases and Rad3^{ATR} kinase to limit telomere extension. *PLoS Genet* **9**, e1003936 (2013).
4. Matmati, S. et al. The fission yeast Stn1-Ten1 complex limits telomerase activity via its SUMO-interacting motif and promotes telomeres replication. *Sci Adv* **4**, eaar2740 (2018).
5. Sun, J. et al. Stn1-Ten1 is an Rpa2-Rpa3-like complex at telomeres. *Genes Dev* **23**, 2900-14 (2009).