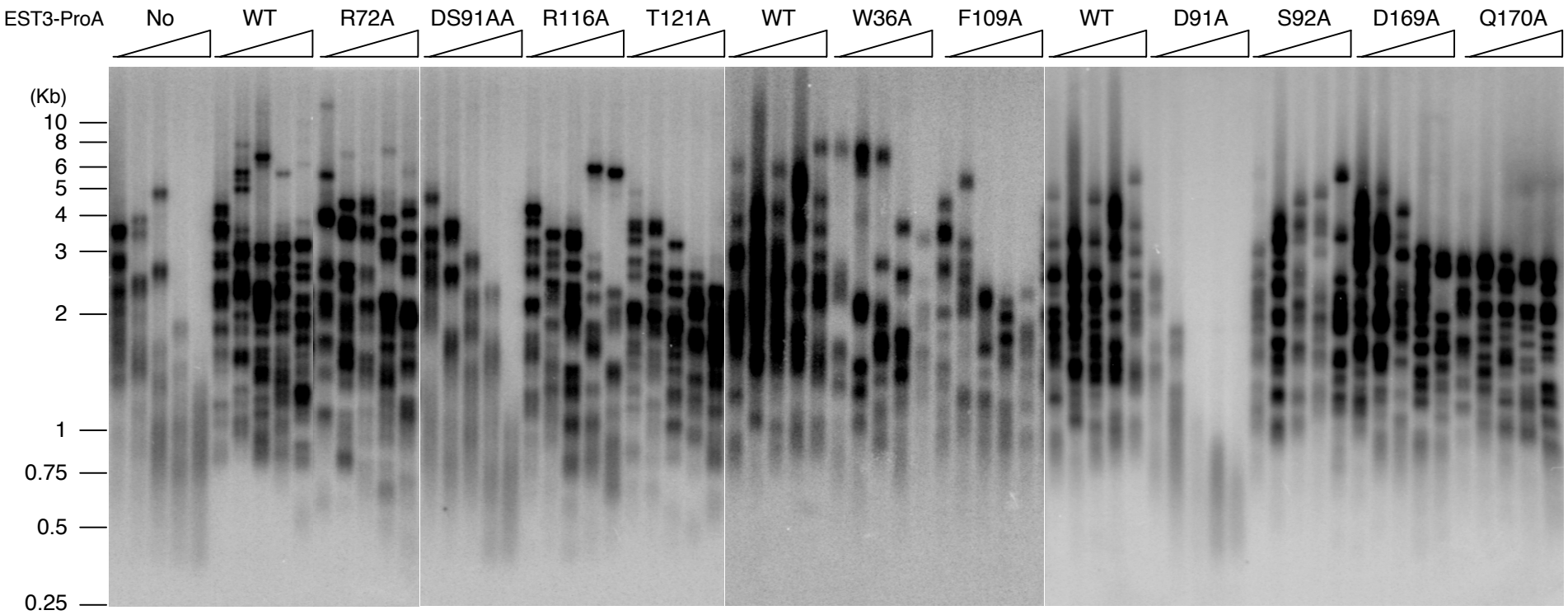
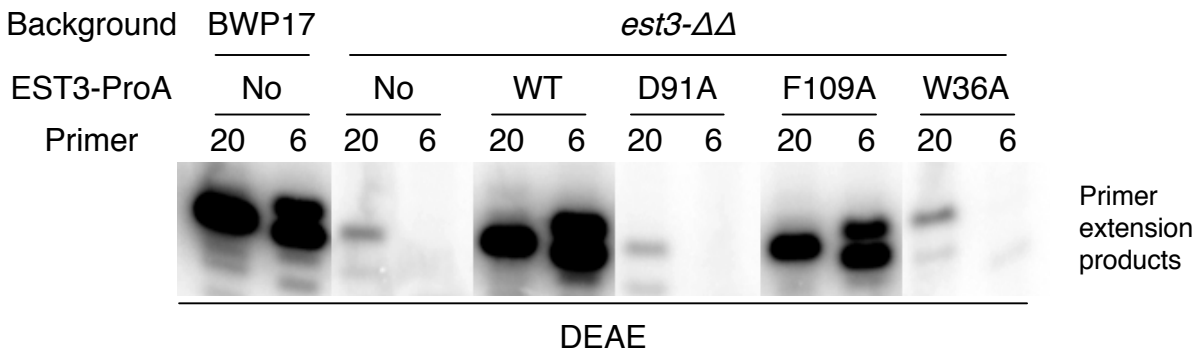
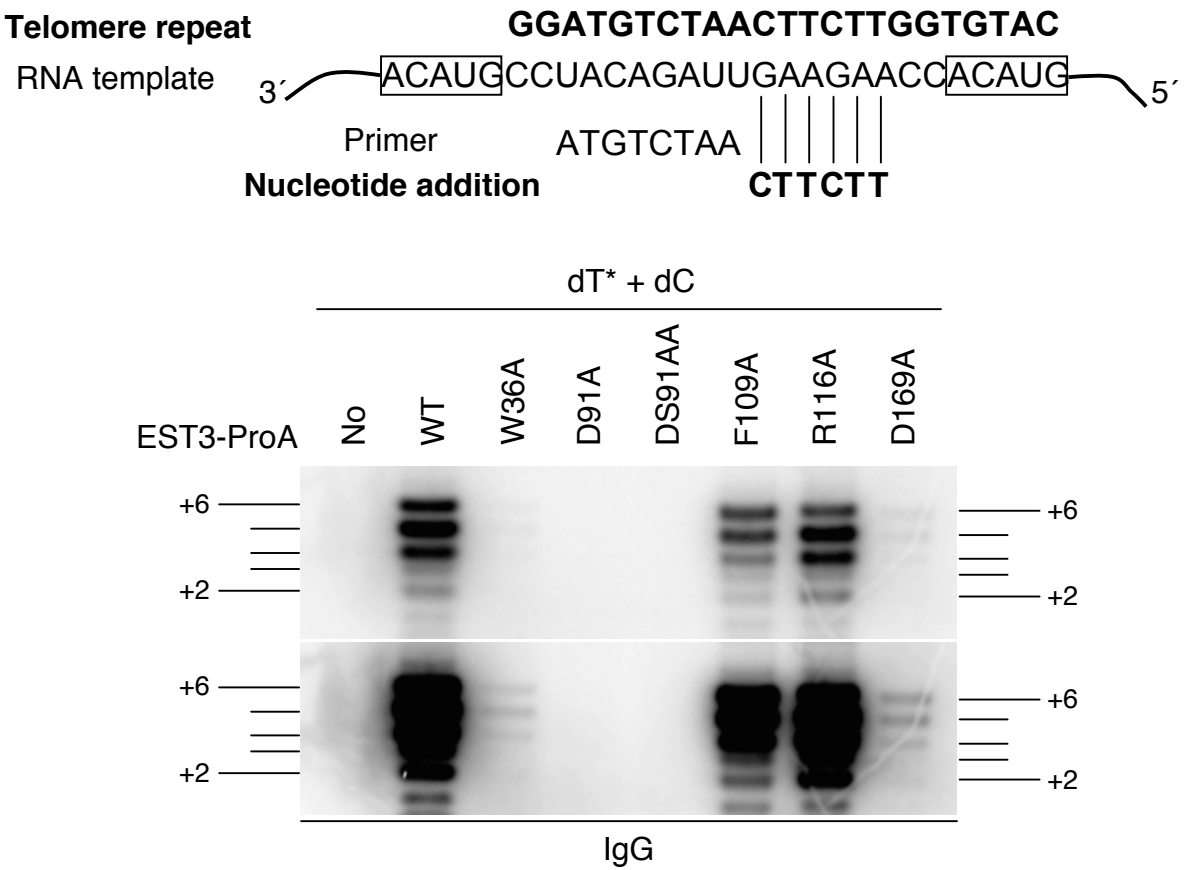


Supplementary Fig. 1. *Effects of Candida EST3 mutations on telomere lengths.*

Telomeres from successive streaks of *est3Δ/est3Δ* strains with different mutant alleles of protein A-tagged *EST3* were analyzed by Southern blotting. The chromosomal DNAs were derived from streaks 4, 8, 12, 16 and 20 of each indicated strain. One streak corresponds to ~ 25 generations of growth. The results are representative of at least two independent clones.

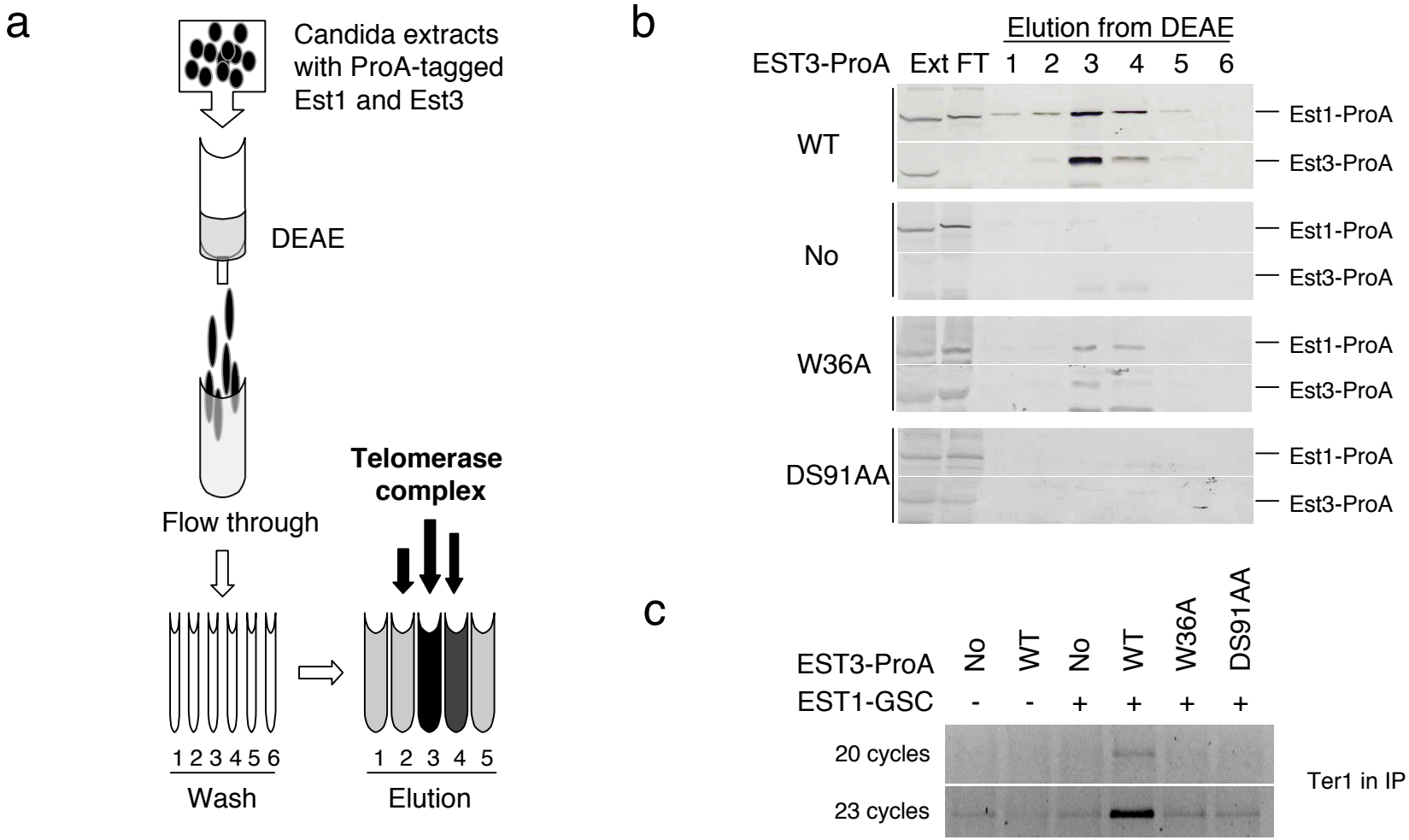


Supplementary Fig. 2. *Effects of Candida EST3 mutations on telomerase activity.*
Telomerase in the BWP17, *est3Δ/est3Δ* (*est3-ΔΔ*), and *est3-ΔΔ* strain reconstituted with various *EST3* alleles were isolated by DEAE chromatography and tested for primer extension activity using primers P6 or P20 and 0.2 μM labeled dTTP.

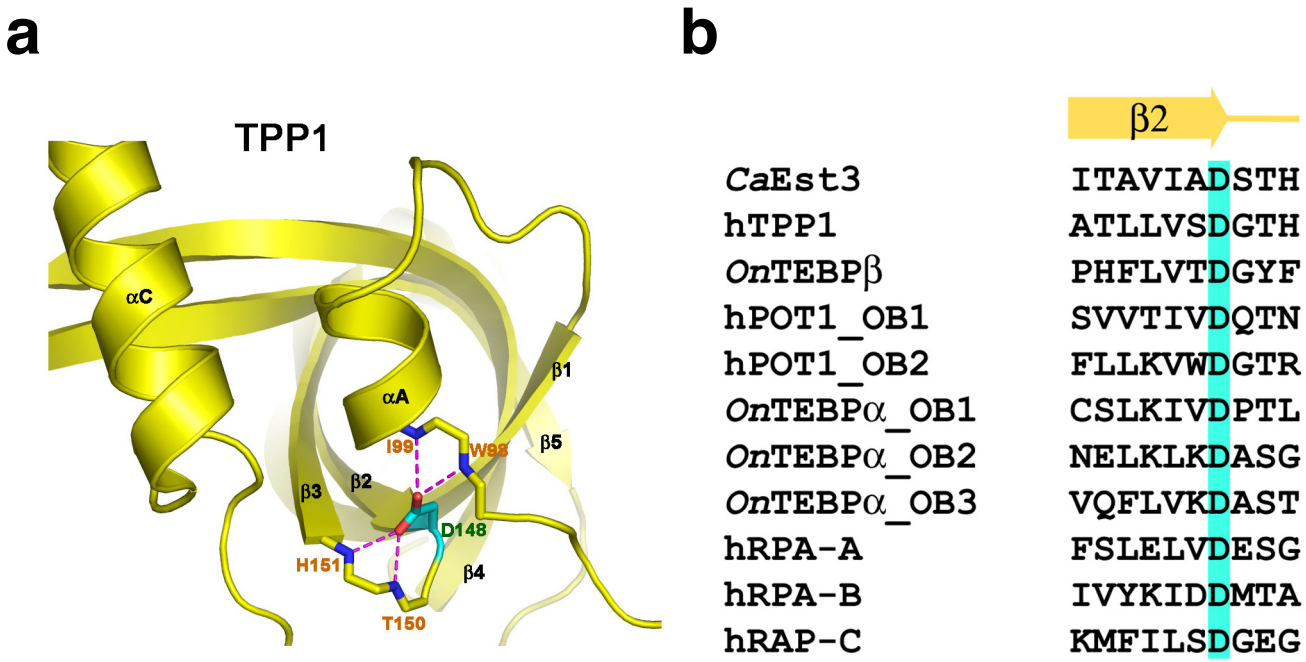


Supplementary Fig. 3. *Effects of Candida EST3 mutations on telomerase processivity.*

Telomerase from the indicated strains were isolated by IgG-Sepharose pull down and subjected to primer extension analysis using an 8-nt primer as well as 50 μM unlabeled dCTP (dC) and 0.2 μM labeled dTTP(dT*). The sizes of the products relative to the input primer are indicated by horizontal lines to the left and right of the panel. The bottom panel is an overexposure of the top panel.



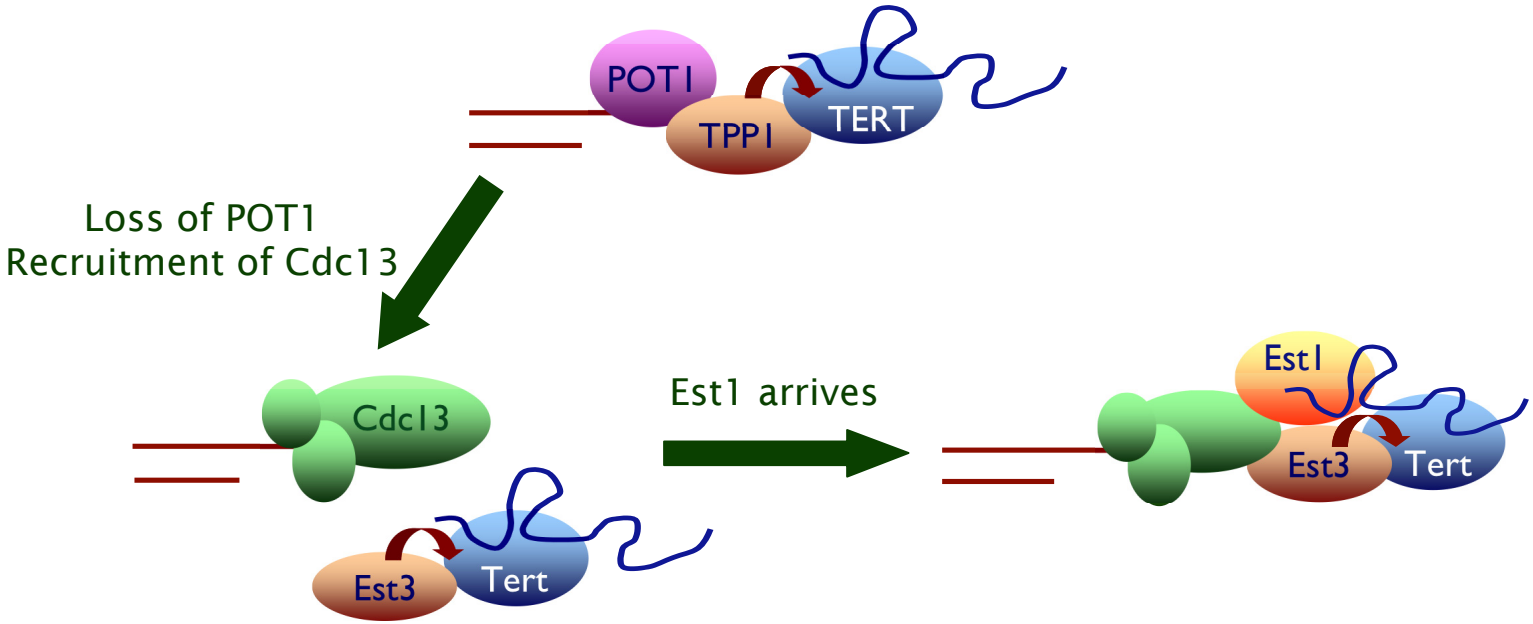
Supplementary Fig. 4. *The association of Est1 with telomerase in strains containing EST3 mutants.*
(a) A schematic illustration of the DEAE chromatography used to purify partially the telomerase complex is shown. Typically, telomerase will bind to the DEAE column and be eluted in fraction 3 or 4 of the high salt step.
(b) Extracts from the indicated strains were subjected to DEAE chromatography, and the levels of Est1 and Est3 in various fractions detected using antibodies directed against protein A. The strains contain various alleles of protein A-tagged *EST1* and *EST3* as follows: No, *est3Δ/est3Δ EST1*-ProA; WT, *est3Δ/est3Δ EST3*-ProA *EST1*-ProA; W36A, *est3Δ/est3Δ EST3-W36A*-ProA *EST1*-ProA; DS91AA, *est3Δ/est3Δ EST3-DS91AA*-ProA *EST1*-ProA.
(c) Extracts from the indicated strains were treated with Streptavidin-Agarose to precipitate GSC-tagged Est1. Total RNAs on the beads were isolated by proteinase K digestion and ethanol precipitation, followed by RT-PCR analysis of Ter1 as described elsewhere. Thermocycling was performed for 20 or 23 cycles to ensure the linearity of the signals. The strains contain various combinations of GSC-tagged *EST1* and protein A-tagged *EST3*, as follows: No, *est3Δ/est3Δ EST1*-GSC; WT, *est3Δ/est3Δ EST3*-ProA *EST1*-GSC; W36A, *est3Δ/est3Δ EST3-W36A*-ProA *EST1*-GSC; DS91AA, *est3Δ/est3Δ EST3-DS91AA*-ProA *EST1*-GSC.



Supplementary Fig. 5. *The conservation of an Asp residue in OB fold proteins and its structural role.*

(a) The backbone structure of TPP1 is shown in a ribbon representation. Residue D146, located at the end of β2, is shown as sticks that make four hydrogen bonding interactions with residues in the L2-3 loop (T150 and H151) and residues near αA (W98 and I99).

(b) A local alignment of sequences near the end of β2 from many OB fold proteins (including Est3, TPP1, POT1, TEBPβ and RPA) illustrates the conservation of this aspartic acid residue.



Supplementary Fig. 6. *A possible evolutionary scenario for yeast telomere binding proteins and telomerase.*
The precursor of TPP1/Est3 may be part of the telomeric protein complex and a stimulatory factor for telomerase. During budding yeast evolution, this precursor may be lost from the telomeres and its function in telomere protection and telomerase activation taken up by the CST complex and telomerase-bound Est3, respectively. See text for more detailed explanations.