Supporting Information

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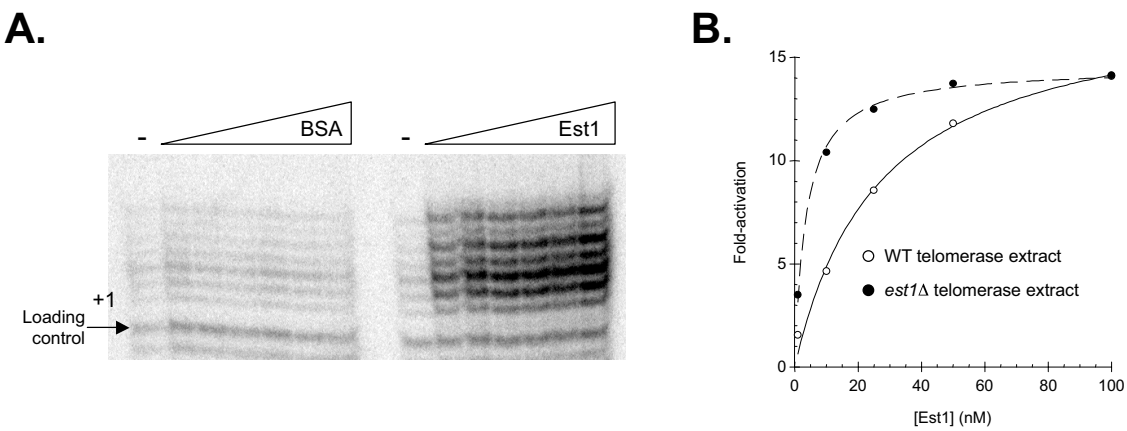

Fig. S1. Purified Est1 enhances telomerase DNA extension activity in vitro. (*A*) The Est1 effect on telomerase-mediated DNA extension was tested using an immobilized DNA substrate with a 7-base 3′-overhang and telomerase extract prepared from wild type yeast. The DNA extension reactions were supplemented with BSA or Est1 (1, 5, 10, 25, 50, 75, and 100 nM), as indicated. To serve as a loading control an end-labeled 27-base primer was added before the precipitation of the telomerase extension products. All extension products were resolved by denaturing polyacrylamide electrophoresis. The positions of the loading control and + 1 DNA extension product are marked. (*B*) The relative affinity between Est1 and telomerase produced in either wild type or *est1*Δ yeast was determined by fitting a curve to the average fold-activation vs. common Est1 protein concentration points, as shown. The *R* value for both curve fits is 0.99.

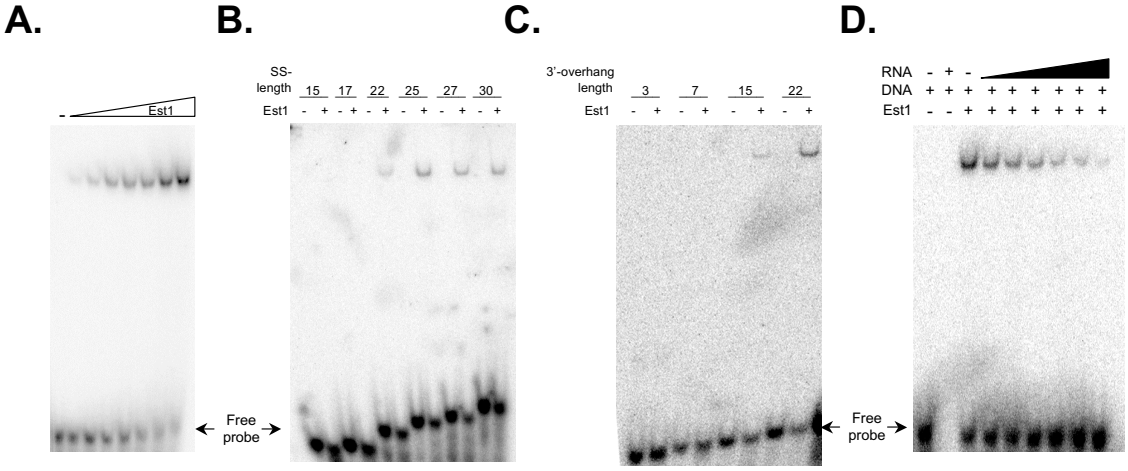

Fig. S2. Est1 binds single-stranded G-rich DNA in a DNA length-dependent manner. (*A*) The Est1 binding affinity for single-stranded G-rich DNA was determined by EMSA. The binding reactions contained a PNK end-labeled 30-nucleotide G-rich primer (50 pM) alone or with increasing Est1 levels (5, 10, 25, 50, 75, 100, and 200 nM). (*B*) The single-stranded DNA length required for Est1 (100 nM) binding was determined using primers (250 pM) with the indicated lengths. (*C*) The ability of Est1 (100 nM) to bind to hybrid DNAs (250 pM) with single-/double-stranded sections was determined using substrates with varying 3′-overhang lengths, as specified. (*D*) An RNA/DNA competition assay was used to determine if the Est1 DNA and RNA binding activities are exclusive. Est1 (100 nM) was incubated with radiolabeled 30-base single-stranded DNA primer (250 pM) alone or with increasing amounts of unlabeled TLC1 RNA (0.25, 0.5, 1.0, 2.0, 4.0, or 8.0 nM), as marked. The DNA electromobility shift assays contained the indicated Est1 protein amounts in TMG-30 buffer supplemented with BSA 200 μg/mL, 200 mg/mL poly[d(I-C)], and end-radiolabeled oligonucleotide. The single-stranded primers were based on GTGGGTGTGTGTGTG (15mer) with additions to the 5′ end accounting for the longer sequences—GG (17mer), TGTGTGG (22mer), GTGTGTGTGG (25mer), TGGTGTGTGTGG (27mer) or GGGTGGTGTGTGTGG (30mer). To create the hybrid single-/double-stranded substrates shorter complimentary oligonucleotides were annealed to the 5′ end of the G-rich 30mer. The generated 3′-overhangs varied in length from 3 bases (ACCCACACACACCCACACACACCACC), 7 bases (ACACACACCCACACACACCACCC), 15 bases (CCACACACACCACCC), or 22 bases (CACCACCC). DNA/RNA competition assays were performed as described above except the indicated amounts of unlabeled TLC1 RNA were titrated into the reactions containing radiolabeled 30mer G-rich DNA primer. After a 20-min incubation at 25 °C, the samples were resolved on a 6% native polyacrylamide gel in 1× GTG buffer (90 mM Tris, 29 mM taurine, and 0.7 mM EDTA), the polyacrylamide gel was dried and the products were visualized with a PhosphoImager.

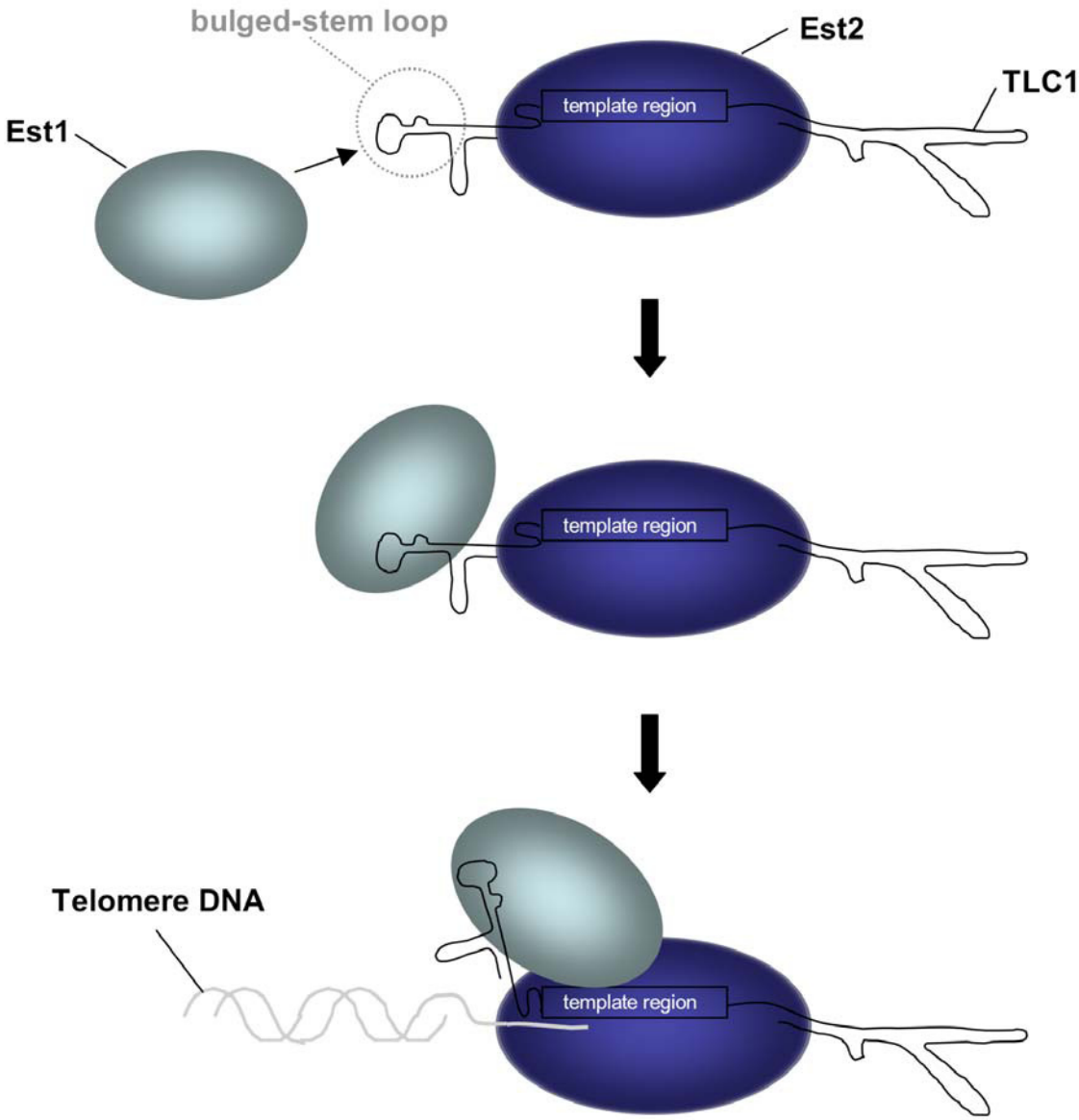

Fig. S3. Est1 stimulation of telomerase enzymatic activity is critical for proper telomere DNA lengthening. Before telomere-association the telomerase holoenzyme assembles with a telomere through Est1 and the TLC1 RNA interactions. Once telomere-bound, Est1 activates telomerase by way of direct contacts with the Est2 reverse transcriptase subunit.

MDNEEVNEECMRLFFKNARAHLDKHLTSRLTCDENAYITFRCFLDGIHR
KSTRFLEELLLKQENMYHNNNYERINDSVIPLVLKLLWLQIHEPTLQWF
EHWFHDIMRLSNRRKFRVFRIFQKKMIQFFKITHRYYYDIIEHLCAKYD
MNSVISNALFAKLNLMQYTDGLSTHEKIILNTSNPLTFSIVISLQRCVI
NLGSTHFYKTLLNKPSNKPKSVEGFEKSIRYLNIASLYLPAVGDTYFQR
AKIYLITGKFSLYFFELVRGALVRIPSKCALNNLKDFILTPDFPERRRL
MKKLAILVSKDLKGEKSFFEGQIVLQFLSIVEHTLVPQSWNASRASNCW
LLKEHLQMAALKYHSGNINVILENLAATMGSFDLMFTTRKSKEQKNKLK
YADLSERQVFFLDLSFDFIANIIDVVIKPSWQKNMEDFRYLAIIRLLMC
WIKSYRSILQYTHRHRKFCTSFALLLNDLINSPLNCSGNIYSHRPKRSY
LFREDIIFREFSCINFALTDFNDDYVYDSPDMINNIIGCPTLTKVLSPK
EECVLRIRSIIFSGMKFLEKNDTGVIWNASKYKFDLISPNIKIKRQIAL
SEISSKINVKTQQERVVSSRKVEAKRDEQQRKRAGKIAVTELEKQFANV
RRTKKLSPLPEKDGVSSELVKHAASRGRKTITGPLSSDFLSYPDEAIDA
DEDITVQVPDTPT*

Fig. S4. The identity of the Est1 protein was confirmed by mass spectrometry analysis. The masses of peptides produced by the tryptic digestion of soluble Est1 protein were measured by MALDI TOF/TOF mass spectrometry. The amino acid Est1 sequence is shown with all of the identified peptides highlighted in red. For an example of the raw data please see file:///Users/bfreeman/Documents/Microsoft%20User%20Data/Saved%20Attachments/Freeman%203–1-2007.htm.

Table S1. Summary of quantified data

Protein	RNA EMSA, %	DNA EMSA, %	Telomerase extension, %
WT Est1	100	100	100
Est1–38	30 ± 5	25 ± 9	93 ± 4
Est1–41	104 ± 3	85 ± 2	38 ± 4
Est1–42	62 ± 2	80 ± 8	110 ± 0
Est1–49	39 ± 6	70 ± 3	100 ± 1
Est1–50	30 ± 2	43 ± 6	104 ± 2
Est1–60	91 ± 1	87 ± 6	34 ± 3