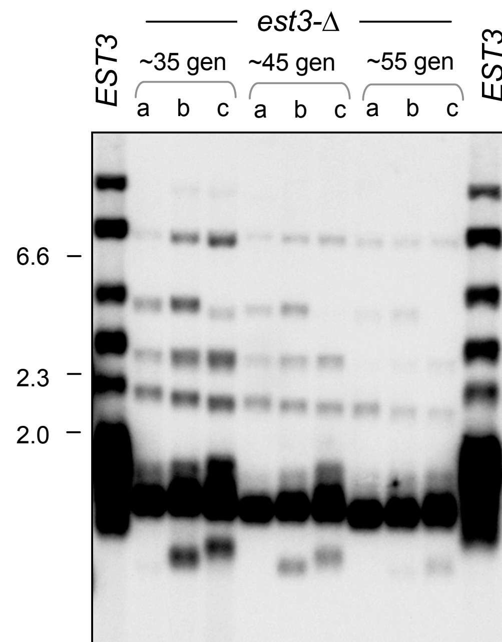

Figure S1

Genomic DNA was prepared from 3 newly generated isolates (a, b, and c) of an *S. castellii est3*-Δ strain propagated for 35 to 55 generations, as well as from the parental *EST3* strain, digested with *Hind*III, and Southern blotted to monitor telomeric restriction fragments, which were detected with a radiolabeled $(TCTGGGTG)_{10}$ oligomer.

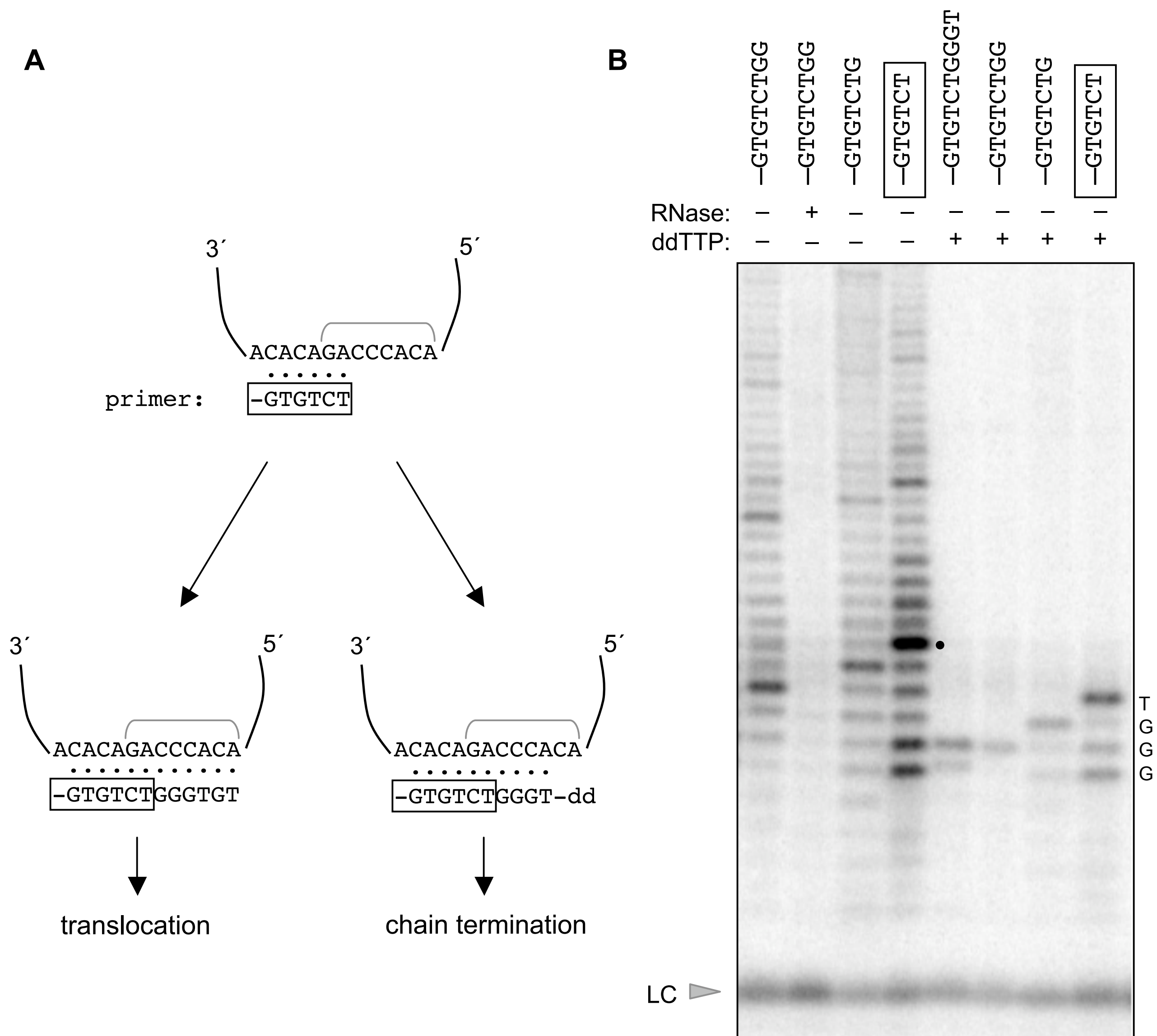

Figure S2

(**A**) A schematic of the *S. castellii* telomerase RNA template region, showing the expected products with primer 4, under standard assay conditions (which should allow multiple rounds of elongation on the template, with an 8-nucleotide periodicity, with the initial pause occurring after the addition of six nucleotides; indicated by a black dot in part **B**) or in the presence of ddTTP (which should only permit the addition of four nucleotides).

(**B**) Telomerase activity from a wild type *S. castellii* strain was assessed with the indicated primers, in the presence/absence of RNaseA or ddTTP.

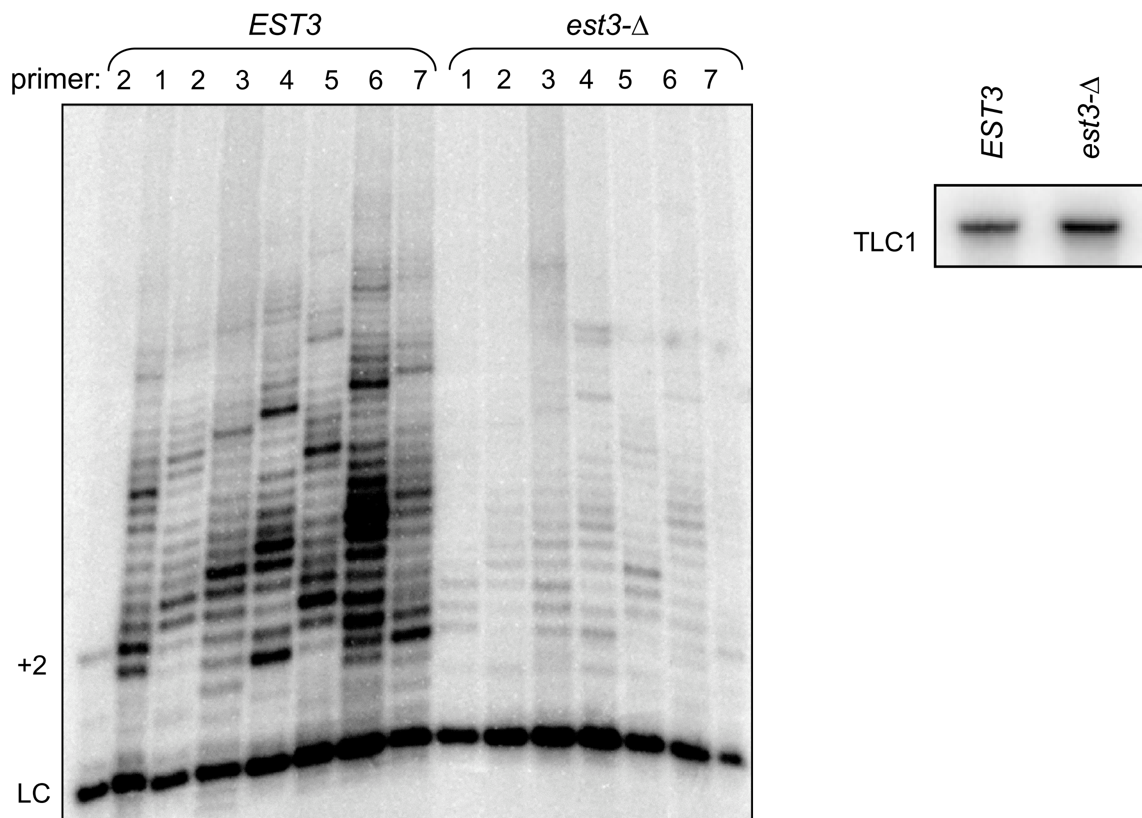

Figure S3

Telomerase activity from wild type and *est3*-Δ extracts were examined with seven primers that span the template region of the *S. castellii* telomerase RNA; lane 1, telomerase activity in the presence of ddTTP. Northern analysis indicates the levels of the *S. castellii* TLC1 RNA in the wild type and *est3*-Δ eluates. In this experiment, low levels of enzyme activity capable of undergoing multiple rounds of elongation can be detected in the *est3*-Δ extract, which is consistent with the results in Figure 2B demonstrating that catalytically active enzyme is present in *est3*-Δ extracts. The difference between the results in Figure 2A versus the results shown here is presumably due to the increased detection of reaction products in this particular experiment.

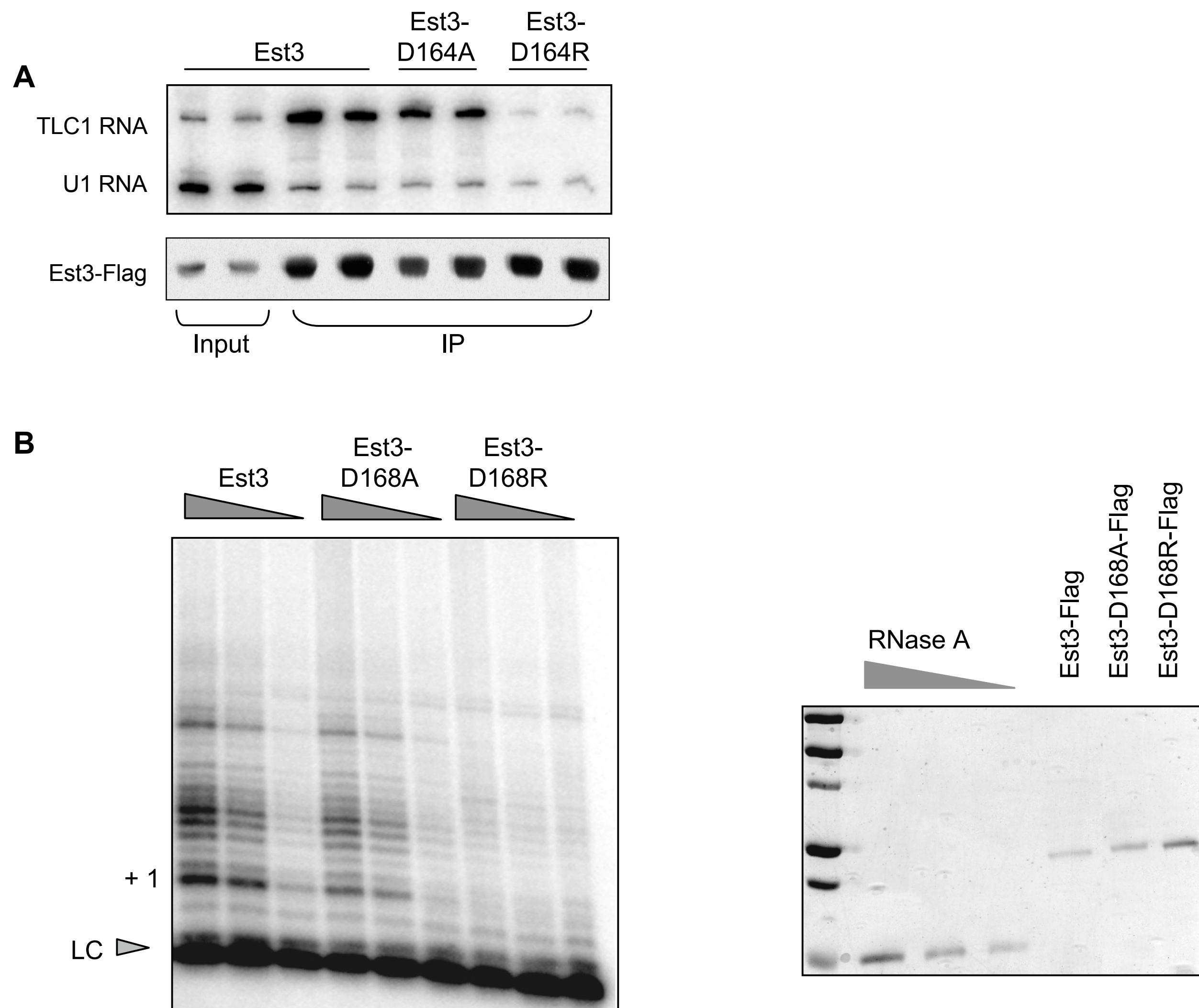

Figure S4

(**A**) Extracts from *S. cerevisiae* strains expressing Est3-Flag, Est3-D164A-Flag and Est3-D164R-Flag were immunoprecipitated (IP) with anti-FLAG antibody. IPs were analyzed by northern blotting to detect the TLC1 telomerase RNA or the unrelated U1 RNA, and by western blotting to detect Est3-Flag, as described previously (Lee et al. 2008). Input samples for the northern and western analysis are 2% and 10%, respectively.

(**B**) Telomerase extracts prepared from an *est3*-Δ strain of *S. castellii* were assayed for activity with primer 4, in the presence of 1 µl, 0.1 µl and 0.01 µl of wild type *S. castellii* Est3-Flag, Est3-D168A-Flag and Est3-D168R-Flag, where 1 µl of wild type Est3 protein is equivalent to ~ 100 nM; the protein concentration of the wild type Est3 protein was estimated by comparison with the RNaseA (0.5, 0.25 and 0.125 µg) standards shown in the Coomassie-stained SDS-PAGE gel on the right. The *S. castellii* D168 residue is the equivalent of the *S. cerevisiae* D164 residue shown in part (**A**).

Lee, J.S., Mandell, E.K., Tucey T.M. Morris, D.K. and Lundblad, V. (2008) *NSMB*, **15**, 990-997

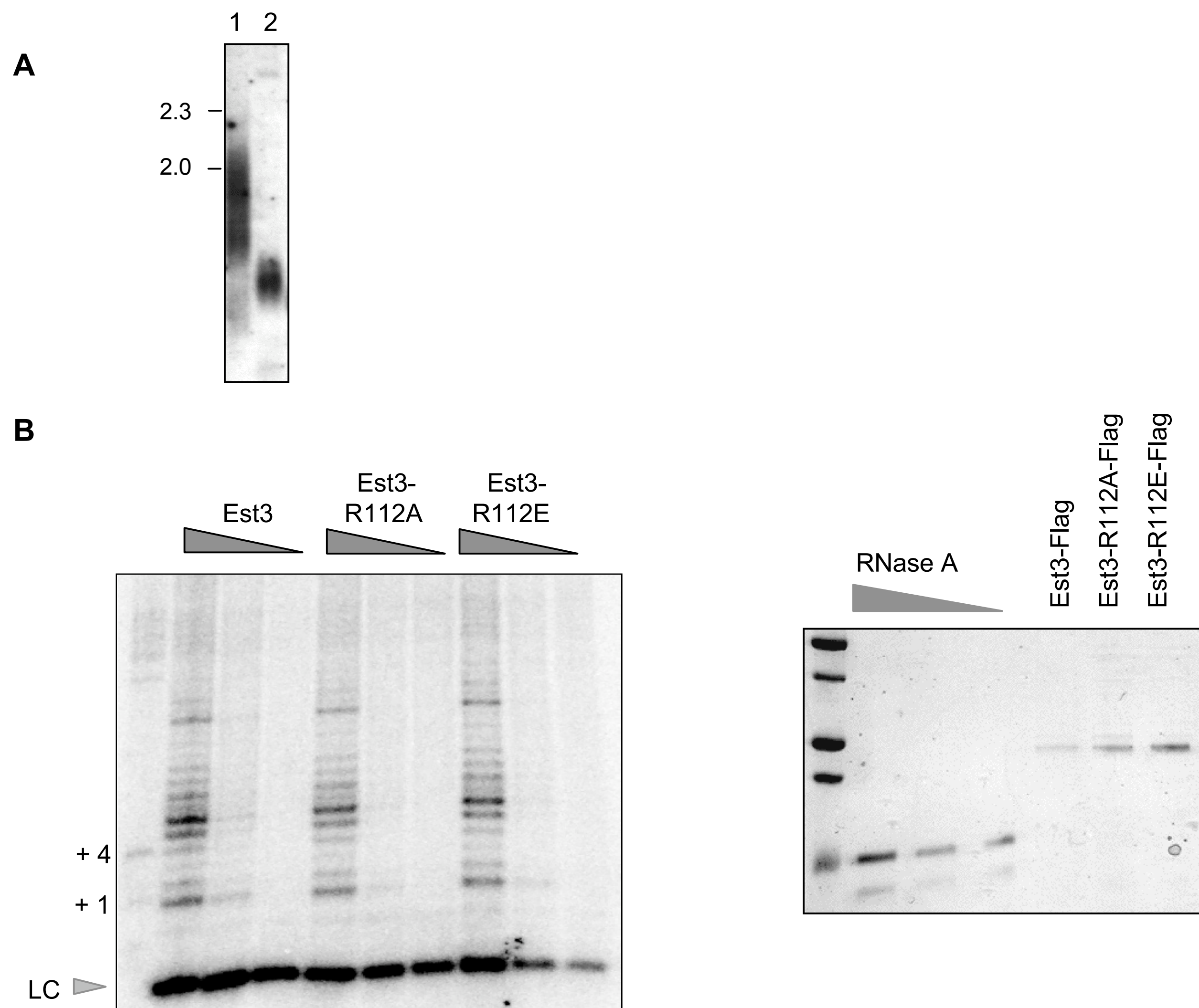

Figure S5

(**A**) Telomere length of an *est3*-Δ strain of *S. castellii*, expressing wild type *EST3* (lane 1) or *est3-R112A* (lane 2); genomic DNA was digested with *Hind*III This gel was run under conditions that emphasized resolution of telomeric bands in the ~2 kb range.

(**B**) Telomerase extracts prepared from an *est3*-Δ strain of *S. castellii* were assayed for activity with primer 4, with 10-fold serial dilutions of wild type *S. castellii* Est3, Est3-R112A and Est3-R112E protein, as shown in the Coomassie-stained SDS-PAGE gel on the right. Lane 1, wild type telomerase activity with primer 4, in the presence of ddTTP.

(A)

S. castellii:

	est3-Δ / EST3
GGGTGTCTGGGTGTCT	0.53 +/- 0.12
GGTGTCTGGGTGTCTG	0.38 +/- 0.07
GTGTCTGGGTGTCTGG	0.34 +/- 0.05

3′-ACACAGACCCACA-5′

(B)

C. albicans:

	est3-Δ / EST3
TGTCTAACTTCT	< 0.05
GTCTAACTTCTT	< 0.05
TCTAACTTCTTG	~ 1
CTAACTTCTTGG	~ 1

3′-ACUGCCUACAGAUUGAAGAACCACAUG-5′

Figure S6

A comparison of the template regions from (**A**) the *S. castellii* telomerase RNA, aligned with primers 2 - 4 used in in this work and (**B**) the *C. albicans* telomerase RNA (Hsu et al. 2007a), aligned with a subset of primers; the full length primer sequences are shown. The ratio of telomerase activity in *est3*-Δ versus *EST3* strains of *C. albicans* is taken from previously published data (Hsu et al. 2007b). A conserved region that is shared in common with the two telomerase RNA templates is shown in red, and the portion of each telomeric primer which is capable of annealing with the template is indicated in blue. The 3' boundary of the *S. castellii* telomerase RNA template is based on an alignment with the comparable region of the *S. cerevisiae* TLC1 RNA, whereas the 5' boundary was experimentally determined based on the pattern of incorporation with various primers, in the presence of dideoxynucleotide analogs (data not shown), as well as the location of the pausing pattern in the standard elongation assay (Figure 1 and Supplementary Figure S2).

Hsu, M., McEachern, M.J., Dandjinou, A.T., Tzfati, Y., Orr, E., Blackburn, E.H. and Lue, N.F. (2007a). *Proc. Natl. Acad. Sci. USA,* **104**, 11682-11687.

Hsu, M., Yu, E.Y., Singh, S.M. and Lue, N.F. (2007b) *Eukaryot. Cell,* **6**, 1330-1338.