THE HSP82 MOLECULAR CHAPERONE CAN PROMOTE A SWITCH BETWEEN UNEXTENDABLE AND EXTENDABLE TELOMERE STATES

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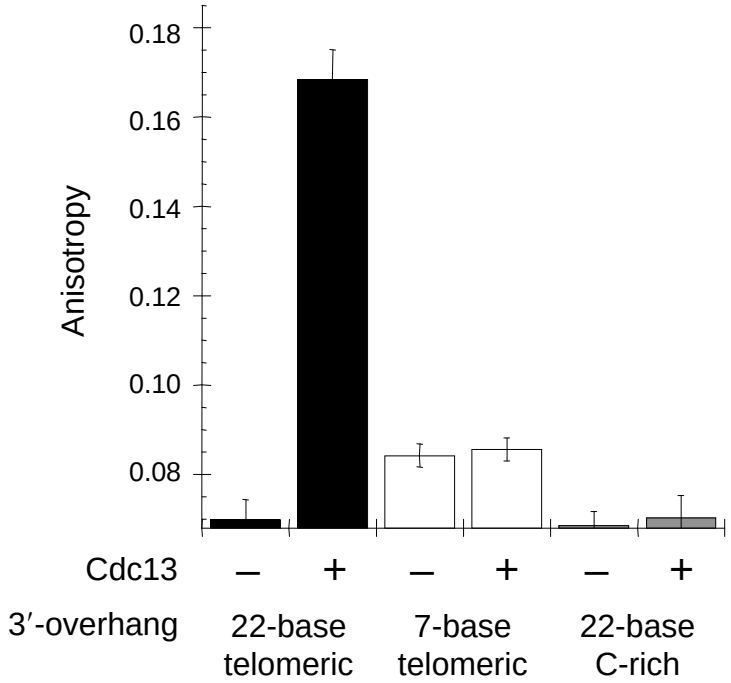
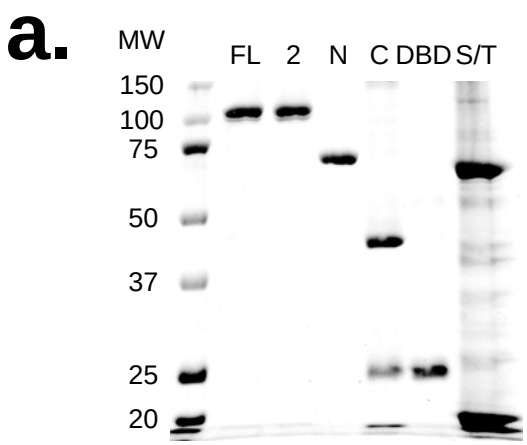
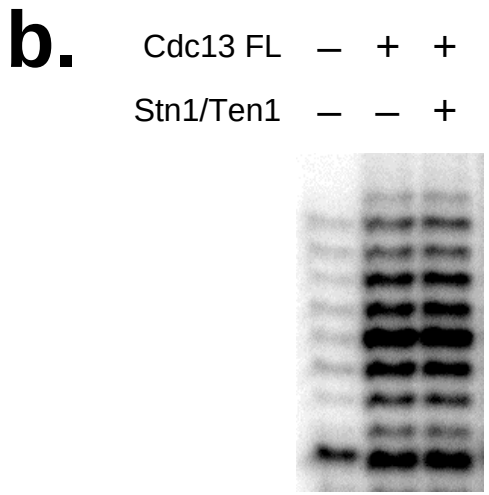
Supplementary Figure 1

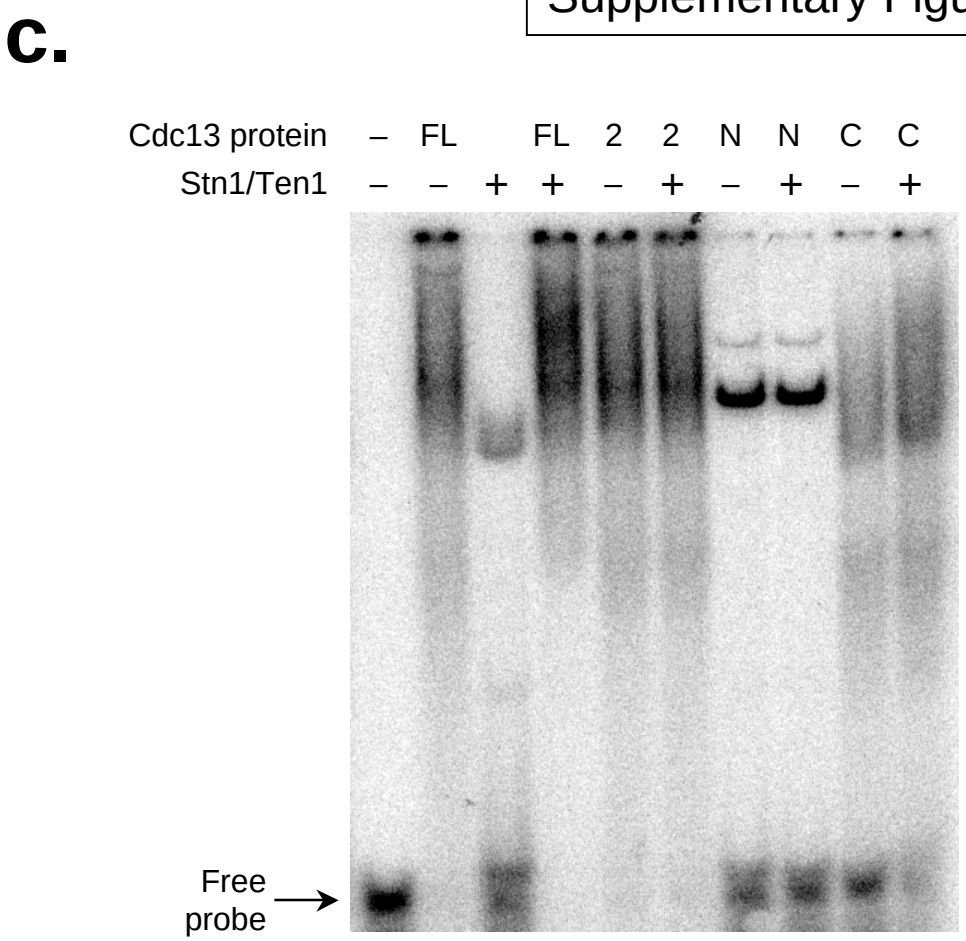


Supplementary Figure 1. Cdc13 displays DNA binding specificity. Fluorescence anisotropy with a fluorescein-labeled DNA substrates was used to detect DNA association. The anisotropy of the DNA oligonucleotides alone or in the presence of Cdc13 (100 nM) is shown. The 22-base and 7-base telomeric DNA substrates were prepared as described in Materials and Methods and the 22-base C-rich substrate was prepared with a fluorescein-labeled C-rich oligonucleotide (12.5 nM; fl-CACCCACACACACACCCCACCACACACACC) and a primer complimentary to the 5'-section of the fluorescein-labeled C-rich oligonucleotide (TGTGGGTG). All anisotropy data represent average values (mean +/- s.d.) from 5 independent assays.

Supplementary Figure 2

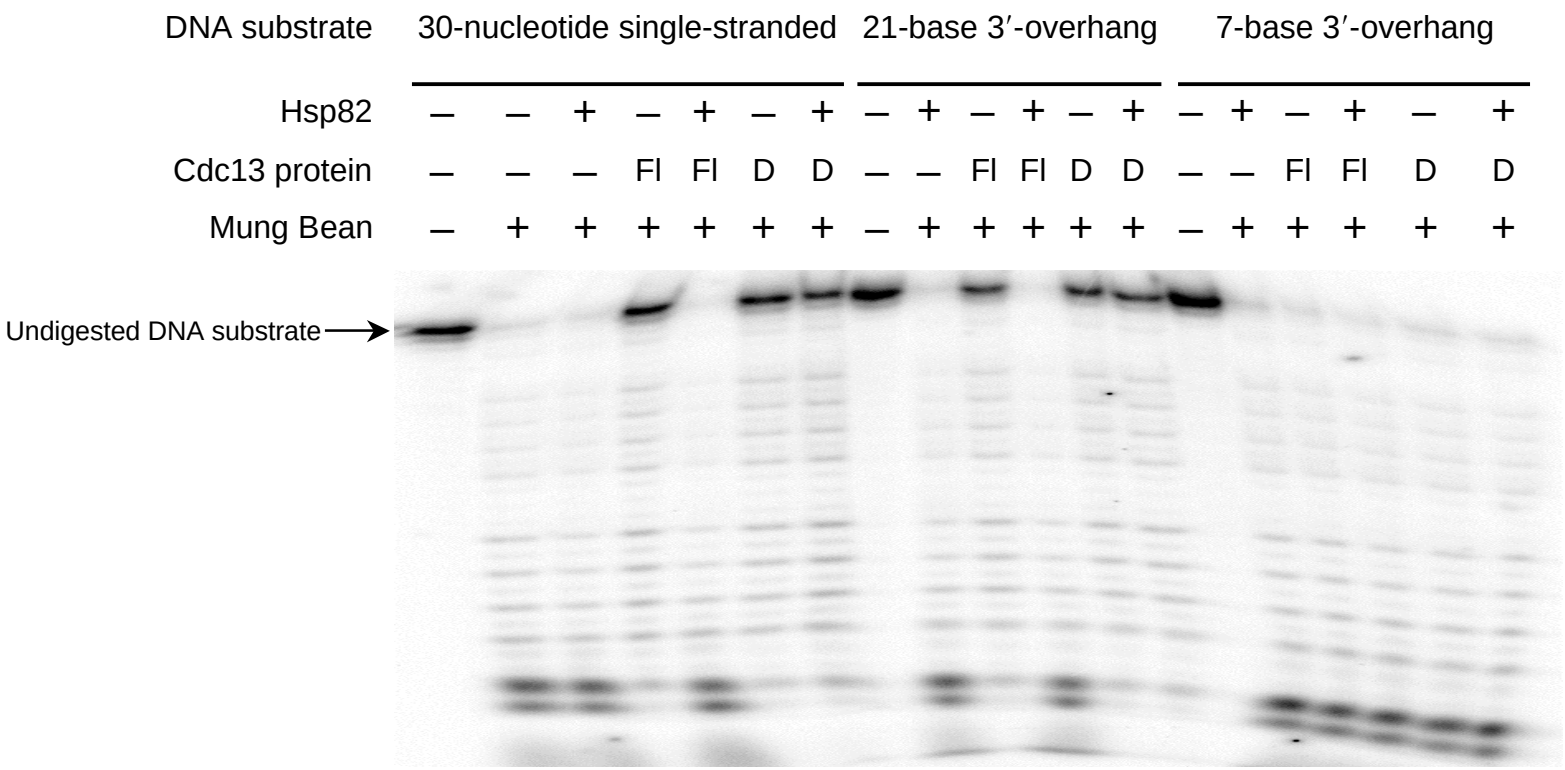






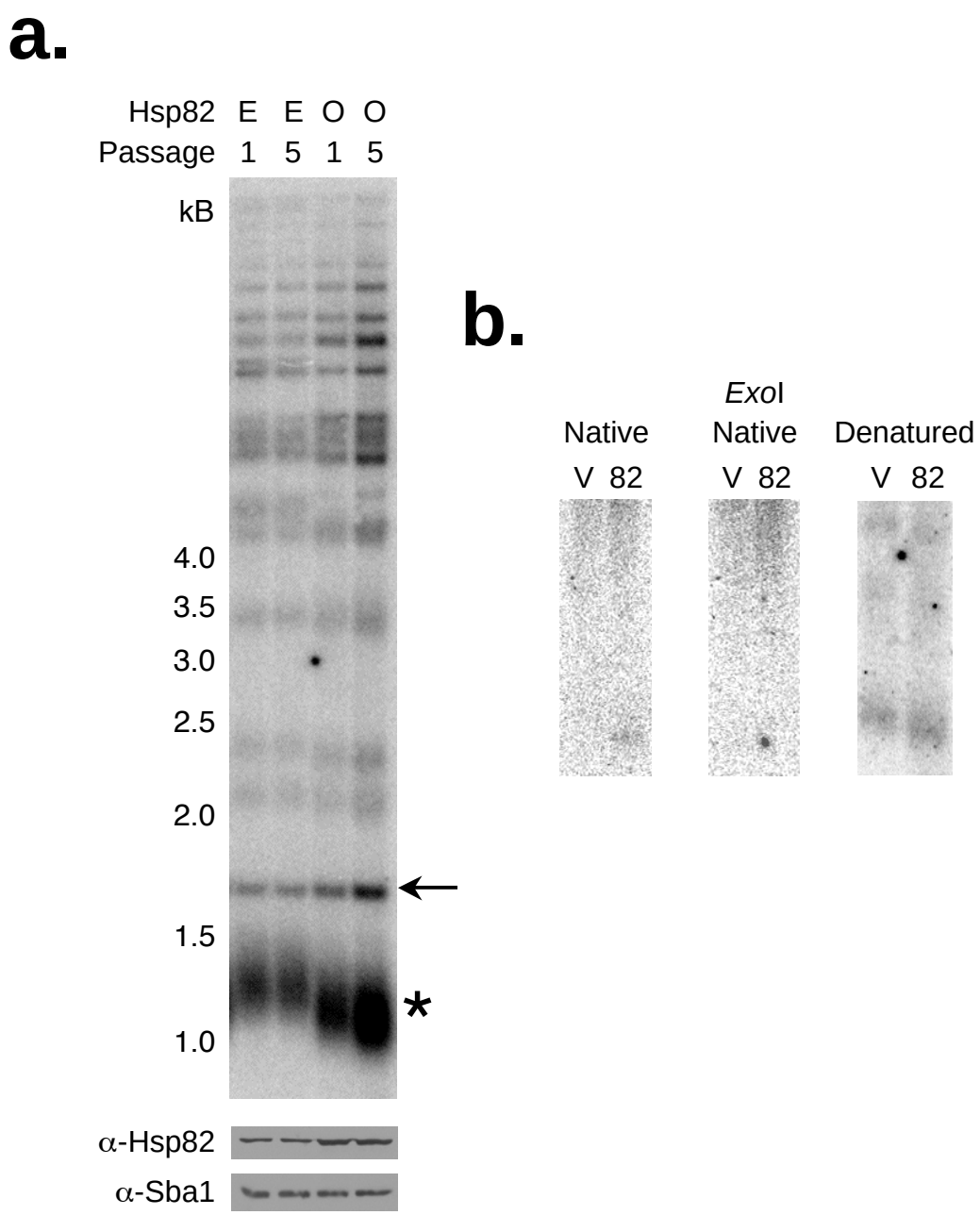
Supplementary Figure 2. Stn1/Ten1 does not alter the ability of Cdc13 full-length to stimulate telomerase activity using a 7-base 3'-overhang DNA substrate and the Cdc13 carboxyl-terminus harbors an Stn1/Ten1 interaction surface. (**a**) Recombinant purified (1 μg) Cdc13 full-length (FL), Cdc13-2, amino-terminus (N), carboxyl-terminus (C) or DNA binding domain (DBD) fragments and Stn1/Ten1 (S/T) was resolved on a 12% SDS-PAGE and stained with Coomassie Blue. (**b**) Telomerase-mediated DNA extension of a 7-base 3'-overhang DNA substrate unsupplemented or supplemented with Cdc13 (250 nM) or Cdc13 and Stn1/Ten1 (250 nM). (**c**) The abilities of the Cdc13 protein derivatives (100 nM) to form a DNA-bound complex with Stn1/Ten1 (100 nM) was determined using the electro-mobility shift assay with a radio-labeled 15-base 3'-overhang telomeric oligonucleotide.

Supplementary Figure 3



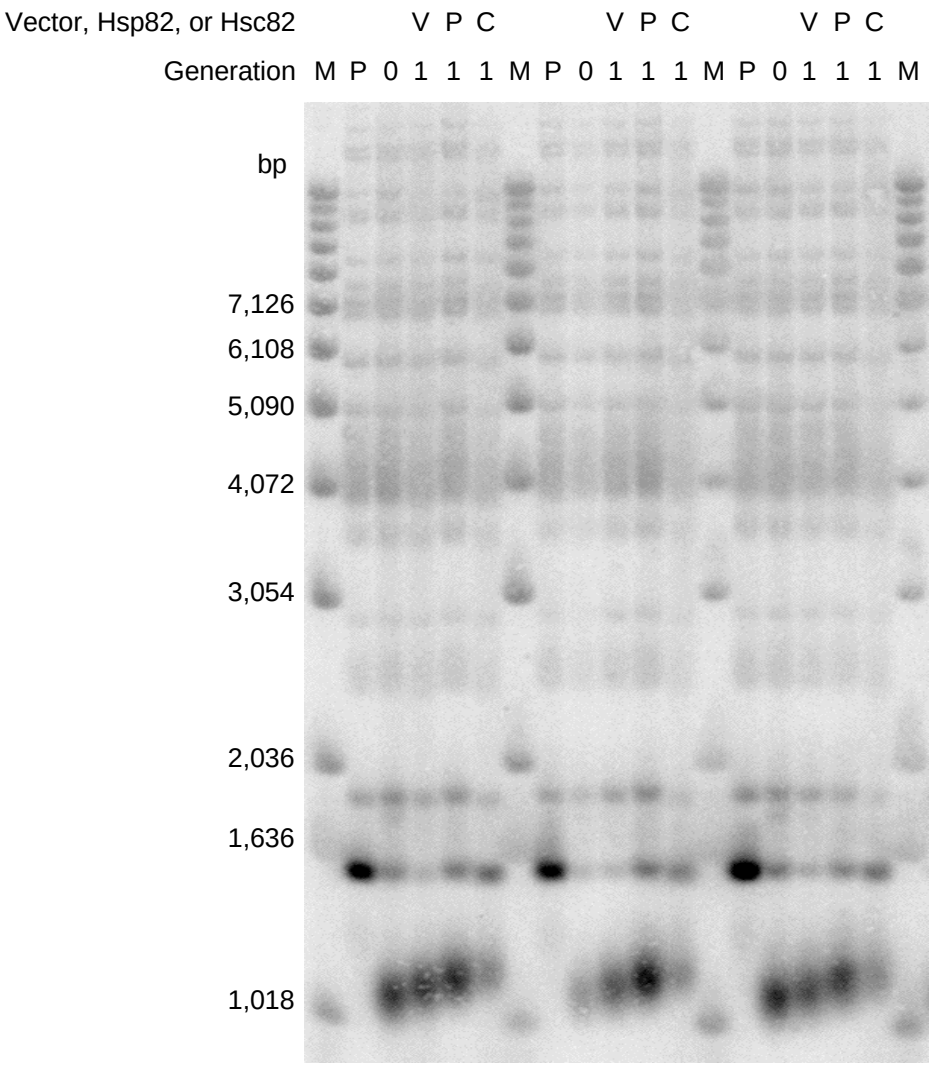
Supplementary Figure 3. The ability of full-length Cdc13 to protect DNA from nuclease digestion is disrupted by Hsp82 whereas the protection capacity of the DNA binding domain of Cdc13 is unaffected by Hsp82. Radio-labeled 30-nucleotide single-stranded telomeric DNA (250 pM) or hybrid single-/double-stranded DNA substrates (250 pM) with either 21- or 7-base 3'-overhangs were treated with Mung bean nuclease alone or after incubation (2 min r.t.) with full-length Cdc13 (Fl) or the DNA binding domain (DBD) of Cdc13 (100 nM). All three substrates use the radio-labeled 30-nucleotide single-stranded DNA. The hybrid 3'-overhang substrates are formed with oligonucleotides complimentary to the 5' end of the 30-nucleotide single-stranded DNA (see Material and Methods). Following nuclease addition the reactions were incubated 12 min at 30°C, the reactions were stopped by the addition of formamide/NaOH loading buffer and boiling for 5 min, the samples were resolved on a 12% denaturing polyacrylamide gel, the polyacrylamide gel was dried and the products were visualized with a PhosphoImager. The 7-base 3'-overhang substrate likely displays no apparent protection by either full-length Cdc13 or the DBD since it is not bound by either Cdc13 protein derivative.

Supplementary Figure 4



Supplementary Figure 4. Hsp82 overexpression reduces telomere DNA length and telomere end protection. (**a**) Genomic DNA was isolated from yeast with endogenous Hsp82 (E) or ectopically overexpressed Hsp82 (O) levels after 1 or 5 passages. Telomeric DNA (asterisk marks the position of the Y' subtelomeric fragments) and a non-telomeric, internal DNA fragment of chromosome IV (arrow) were visualized by Southern blot analysis. The endogenous (E) and ectopically overexpressed Hsp82 (O) protein levels were determined by western blot analysis, as a loading control the protein levels for the cochaperone Sba1 are shown. To achieve the ~2-fold increase in Hsp82 the heterotypic Glyceraldehyde-3-Phosphate Dehydrogenase (*GPD*) promoter was used to drive expression from a high-copy 2μ plasmid (pRS423-*GPD-HSP82*). The endogenous Hsp82 protein levels (17 μ M) and the potential feedback inhibition on the endogenous HSP82 promoter by elevated Hsp82 protein likely contribute to the relatively modest steady-state increase in Hsp82 protein. (**b**) Analysis of telomeric DNA end structure. Detection of telomeric G-strand 3'-overhangs was accomplished by a native Southern blot assay[28]. Genomic DNA was prepared from yeast transformants (vector (V) or pRS423-*GAL1-HSP82* (82)) that were grown at 30°C for two generations in galactose containing media. Samples were split and mock (Native) treated or digested with *Exo*I (*Exo*I) followed by digestion with *Xho*I and analyzed by in-gel hybridization as previously described[21]. Single-stranded TG_{1-3} 3'-overhang DNA was detected using a radio-labeled telomere CA probe. The total TG_{1-3} signal (Denatured) in the mock treated sample was detected following denaturation and reprobing of the gel.

Supplementary Figure 5



Supplementary Figure 5. Hsp82 overexpression increases DNA extension from an inducibly truncated telomere. Genomic DNA was isolated from yeast with endogenous Hsp82 (V) or ectopically overexpressed Hsp82 (P) or Hsc82 (C) levels after a single cell cycle. To avoid potential effects on capping activities Hsp82 or Hsc82 was expressed from the *GAL1* promoter. Wild type (LEV336) yeast transformants (pRS425-*GAL1* (V), pRS425-*GAL1-HSP82* (P) or pRS425-*GAL1-HSC82* (C)) growing exponentially in the selective raffinose (2% v/v) media were treated with α-factor (10 μg mL^{-1}) to synchronize the cells in G1, the media was supplemented with galactose (1% v/v final) to induce *FLP1* and chaperone expression; prior to growth in galactose an aliquot was removed for genomic DNA extraction ('preflip' or P sample). Following a 3 h incubation at 30°C, a sample was removed for genomic DNA extraction (0 generation), the remaining culture was clarified by a brief centrifugation, the cells were washed, resuspended in glucose containing media and allowed to double as determined by cell counting using a hemocytometer. The recovered genomic DNAs were digested with *Stu*I, the products were resolved on a 25 cm 1% (w/v) agarose gel along with DNA markers (M) and the DNA was transferred to Immobilon-Ny^{+} membrane (Millipore Inc.). The terminus of the left arm of chromosome VII (*i.e.*, extending telomere) was identified following hybridization in Ekono buffer (ISC BioExpress Inc.) using a radioactive probe produced by random prime labeling of the *Hind*III-*Stu*I restriction fragment DNA of the sp242 vector[22].