SUPPLEMENTAL MATERIAL for ***Tetrahymena*** **Telomerase Holoenzyme Assembly, Activation, and Inhibition by Domains of the p50 Central Hub**

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SUPPLEMENTAL FIGURE LEGENDS

FIG S1 Endogenously expressed ZZF- or FZZ-tagged p50 can be truncated by partial proteolysis. One-step affinity purifications from cell extracts of *Tetrahymena* were performed using anti-FLAG antibody resin. Eluted samples were subject to immunoblot analysis with rabbit IgG as primary antibody to detect the ZZ tag module. Mock indicates purification from a cell extract lacking tagged protein. At right, the suggested annotation of full-length and truncated p50 proteins is based on SDS-PAGE migration.

FIG S2 The N-terminal domain of p50 is sufficient for activity stimulation by p75. Activity assays were done as in Fig. 2C with the substitution of p50N30 for full-length p50.

FIG S3 C-terminal tagging of p50 increases telomere length. Telomeric restriction fragment length was assayed after denaturing gel electrophoresis by hybridization with an oligonucleotide probe complementary to the subtelomeric region of the palindromic chromosome encoding ribosomal RNA. Genomic DNA from p50-FZZ cells used a cell line created previously (19) by tag fusion to the endogenous open reading frame sequence.

FIG S4 Endogenously expressed p50N30 and p50N25 can copurify telomerase holoenzyme activity. Cell extracts expressed no tagged protein (WT) or a full-length (FL) or truncated C-terminally tagged p50. (A) Cell extract was used for immunoblot analysis with rabbit IgG as primary antibody to detect the ZZ tag module. (B) One-step affinity purifications from

Tetrahymena cell extract were performed using anti-FLAG antibody resin, with a 20 min activity assay reaction.

FIG S5 Representative negative staining EM images of p50N30-F telomerase in Peak A (left) and Peak B (right) from size-exclusion chromatography (see Fig. 5).

FIG S6 Fourier shell correlation (FSC) of the RCT 3D reconstructions of p50N30-F telomerase holoenzyme (black line) or particles lacking Teb1 (red line). Calculations were performed by splitting the data by odd and even numbers before the final round of 3D reconstruction. Both reconstructions have a resolution of ~26Å based on the FSC=0.5 criterion.

Figure S1

FLAG IP, blot for ZZ:

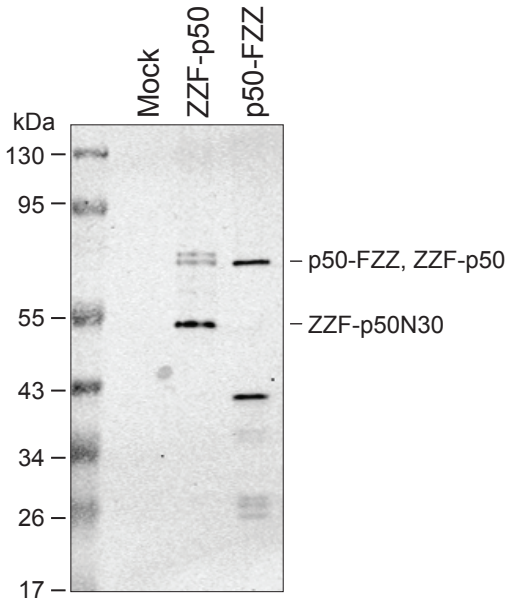


Figure S2

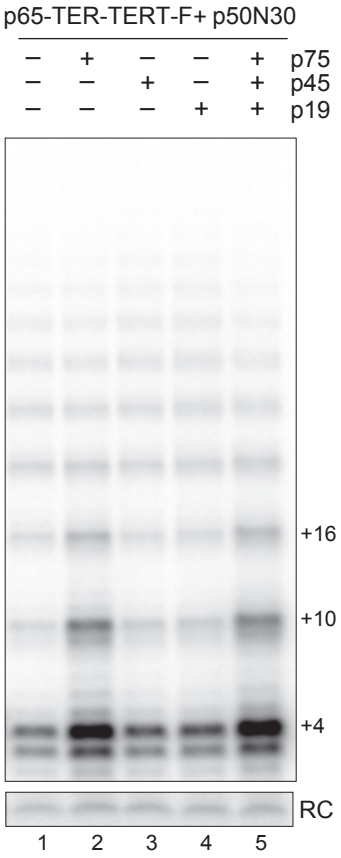


Figure S3

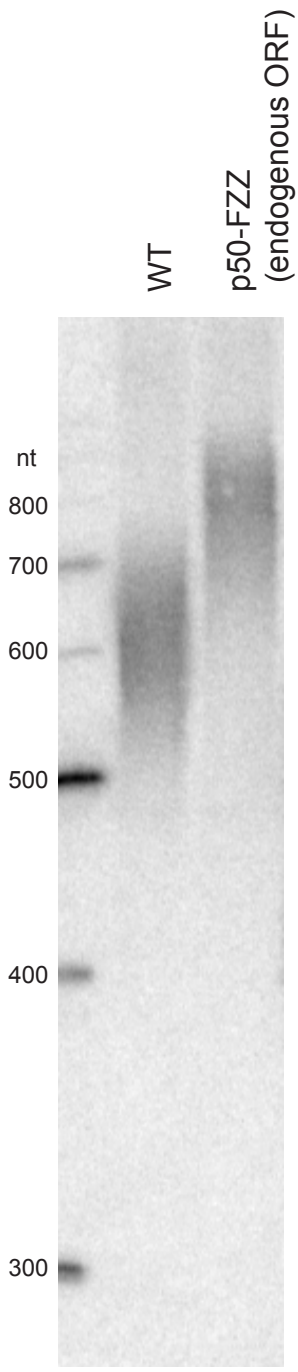
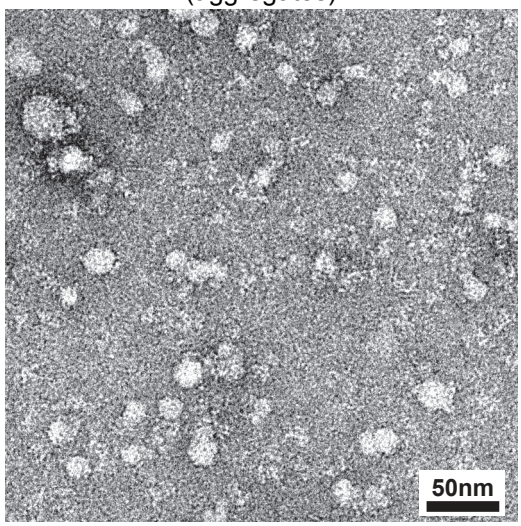


Figure S4


A
WT
FL
N30
N25
p50-FZZ
p50N30-FZZ
p50N25-FZZ
B
WT
FL
N30
N25
+16
+10
+4
RC

Figure S5

Peak A from SEC
(aggregates)



Peak B from SEC
(telomerase holoenzyme)

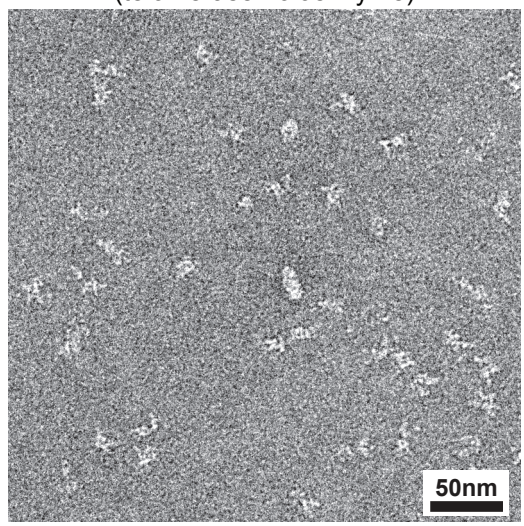
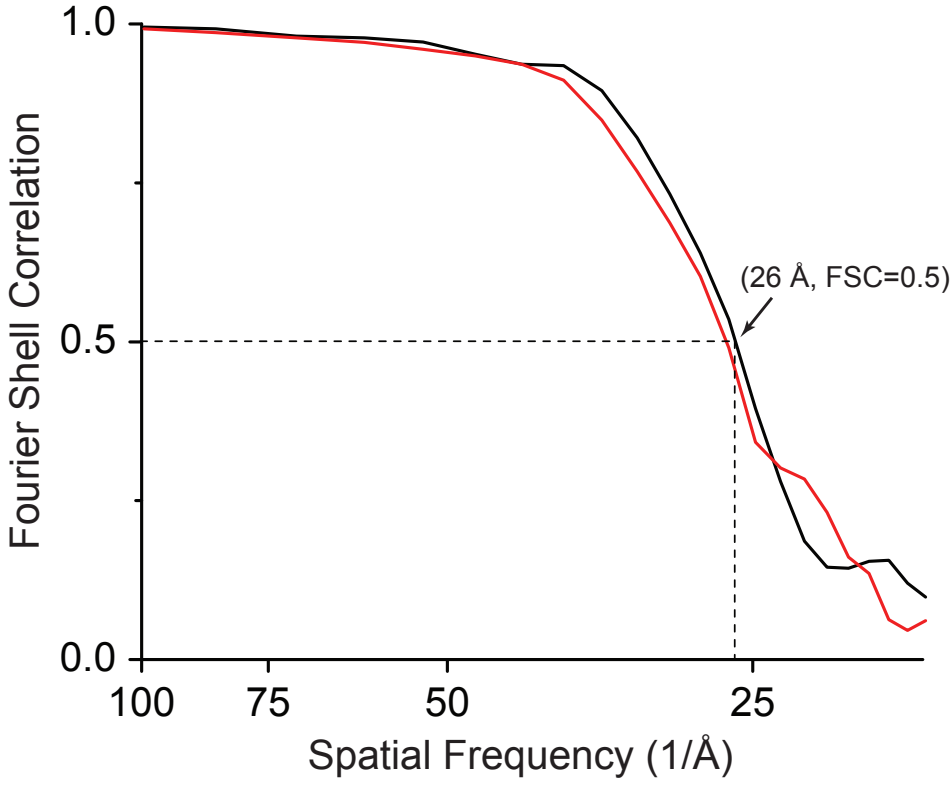


Figure S6


1.0
0.5
0.0
Fourier Shell Correlation
(26 Å, FSC=0.5)
100
75
50
25
Spatial Frequency (1/Å)