Supplementary Fig. 5  Binding of telomerase RNA mutants to *Tetrahymena* TERT protein.

(A) Binding of full-length telomerase RNA to full-length TERT, measured by an immunoprecipitation assay. $^{35}$S-labelled TERT protein was translated *in vitro* in the presence of $^{32}$P-labelled RNA, and the telomerase complex was immunoprecipitated with an antibody to a FLAG tag on the TERT protein. Aliquots of the *in vitro* translation mixture ("input", left panel) or the immunoprecipitated material ("bound", right panel) were electrophoresed on 4-12% SDS-PAGE gels to visualise both TERT and the RNA simultaneously. After normalising for the amount of RNA in each input and the efficiency of each immunoprecipitation, the level of binding of each mutant was approximately equal to wild-type.

(B) Binding of full-length telomerase RNA to the TEN domain of TERT, measured by a filter-binding assay. The TEN domain of TERT (amino acids 2-191) was expressed in *E.coli* and purified. Dilutions of the protein were incubated with $^{32}$P-labelled RNA, and filtered over nitrocellulose and nylon membranes to determine the amount of protein-bound and unbound RNA respectively. WT RNA and all mutants bound to this region of TERT with a $K_d$ of $\sim20$ nM at pH 7.0.