



Supplementary Figure S2. Template-free holoenzyme activity with a trans-template RNA. (A) Secondary structure of the endogenously assembled template-less hTR (hTR Δ temp) with template RNA oligonucleotide (hTR 46-56) and aligned DNA primer T₂₁-GTTAGG. Nucleotide addition (underlined) extends the primer to form product. (B) Activity of wild-type or D868A TERT holoenzyme reconstituted in U2OS cells with hTR Δ temp and assayed with or without hTR 46-56 template, T₂₁-GTTAGG primer and the indicated nucleotide substrates parallel to the assay in Figure 1B. (C) Northern blot detection of hTR Δ temp in transfected U2OS cell extract or following TERT immunopurification (IP) for the activity assay. Note that mature hTRs migrate as multiple species due to partial folding during electrophoresis. An RNA oligonucleotide was added prior to RNA extraction for hybridization detection as a recovery control (RC).