Supplementary Information

Single-molecule real-time detection of telomerase extension activity

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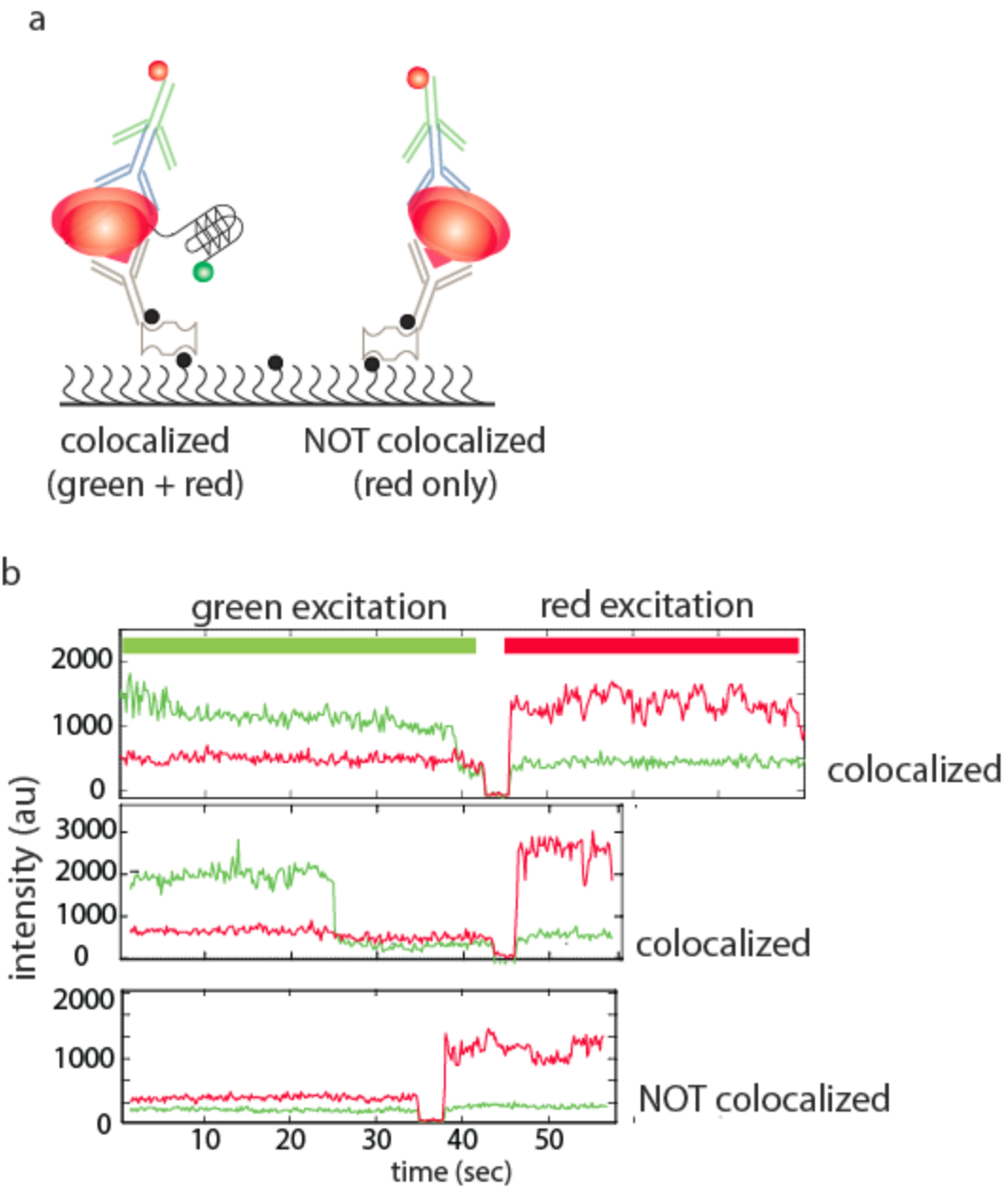
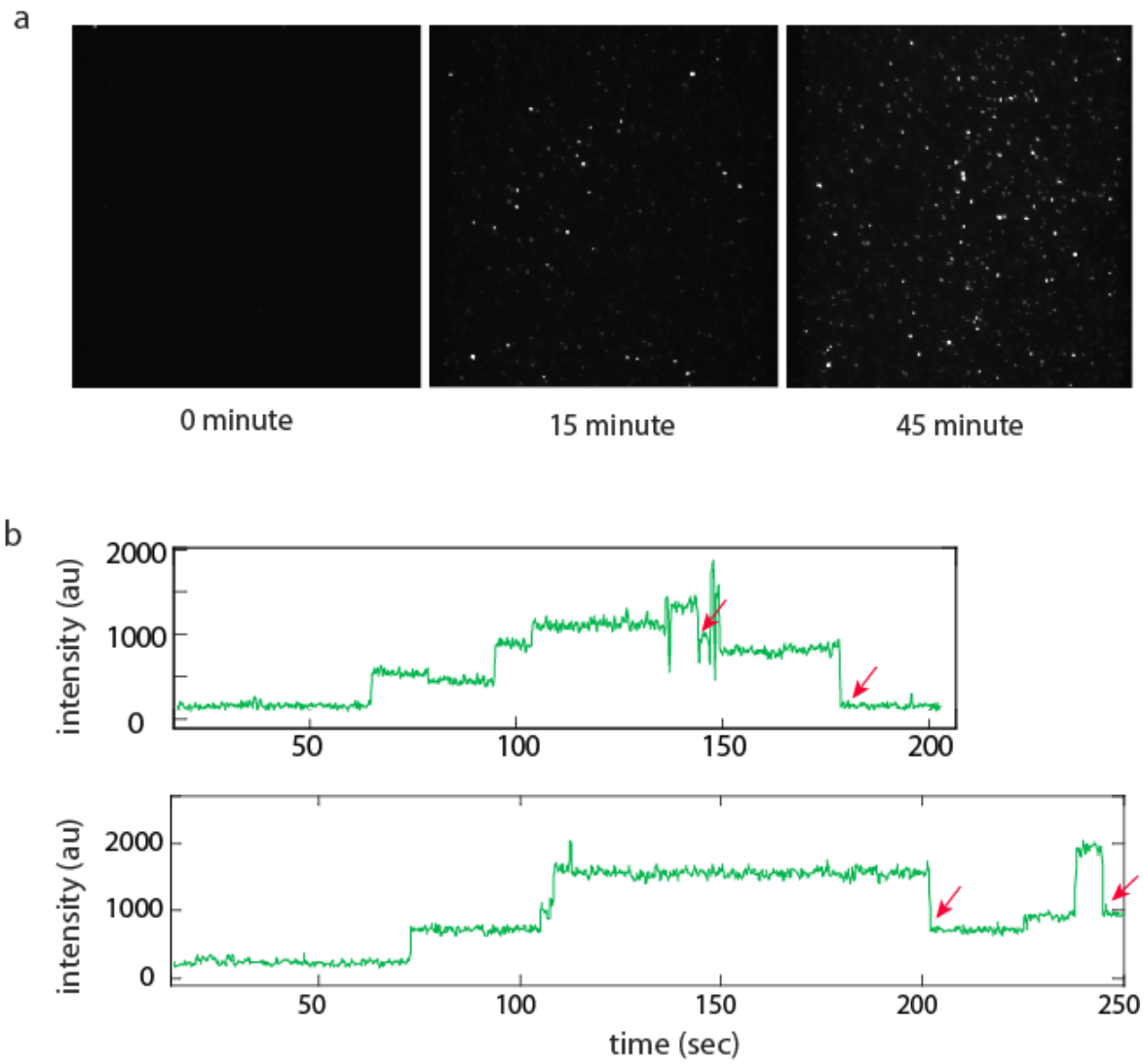
Supplementary Figure 1



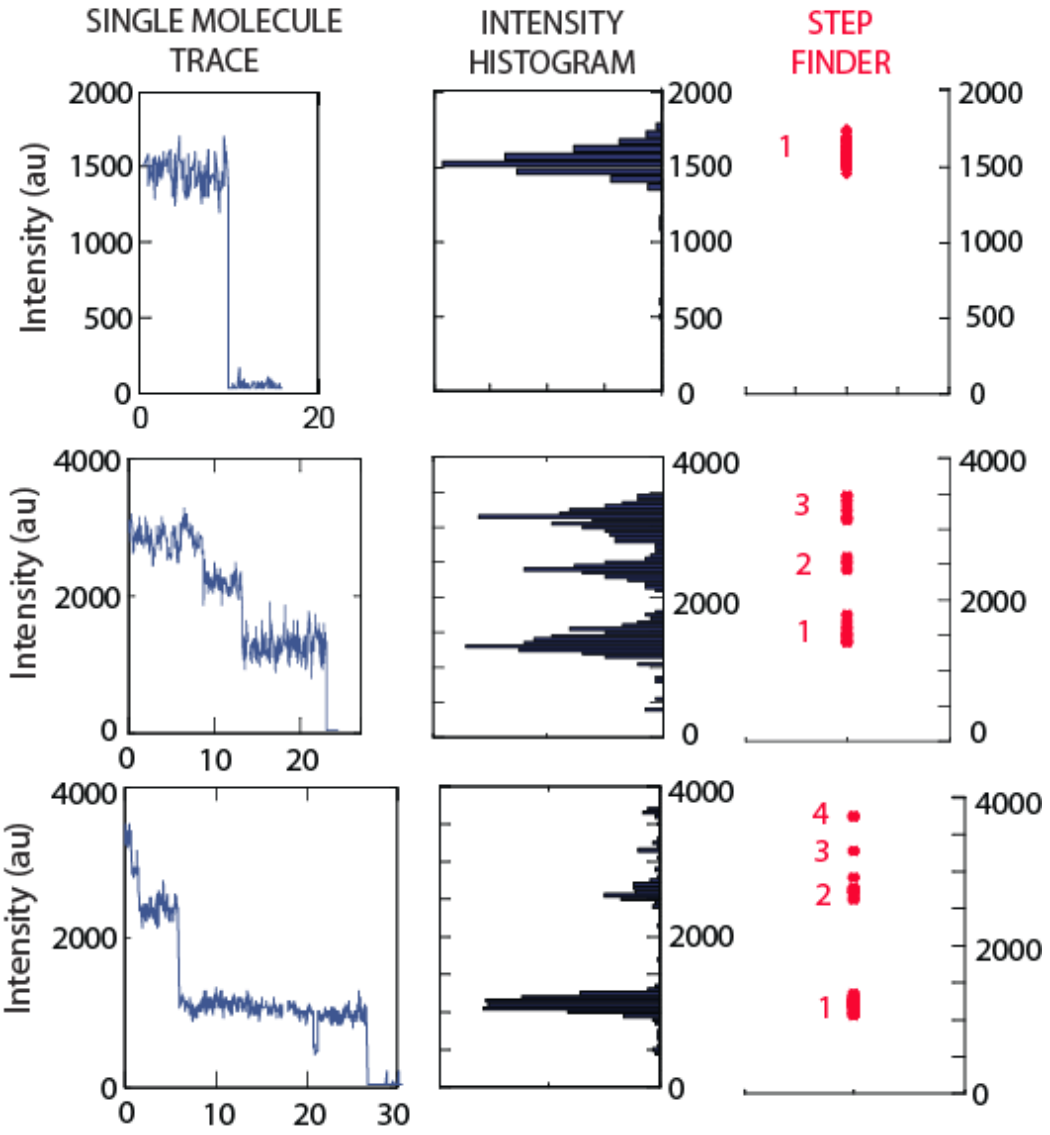
SUPPLEMENTARY FIGURE 1 (a) Colocalization between Cy3 (green) labeled DNA substrate and telomerase labeled with primary and Cy5 (red) labeled secondary antibody. (b) Single molecule traces which demonstrate colocalization (top two) and lack of colocalization (bottom). Upon sequential excitation by green and red lasers, the colocalized trace shows both green and red signals.

Supplementary Figure 2



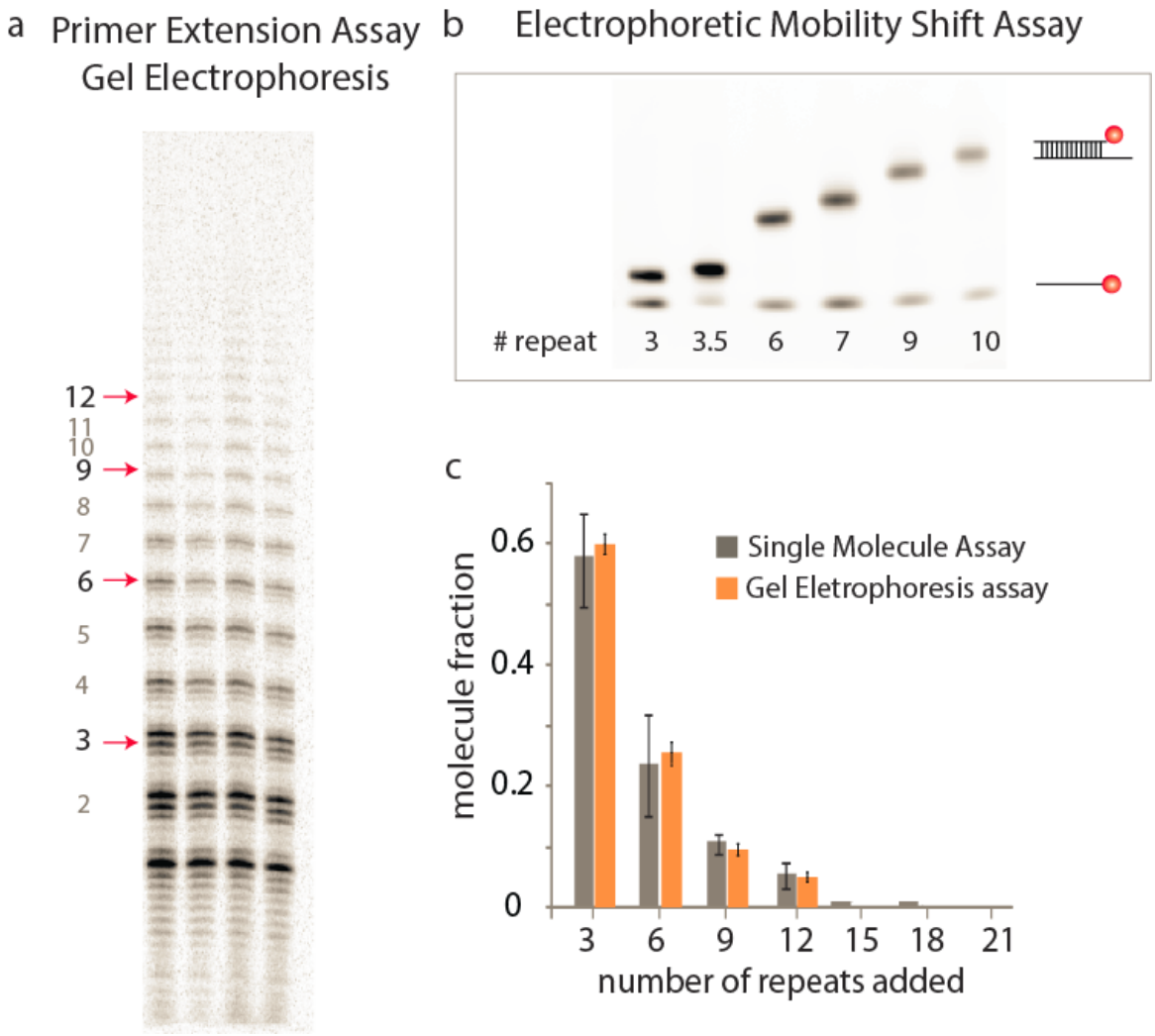
SUPPLEMENTARY FIGURE 2 (a) Fluorescence imaging surface taken at 0, 15 and 45 minutes after the initiation reaction. Number of fluorescence spots increase and the fluorescence in each spot becomes brighter over time. (b) Single molecule traces exhibit occasional blinking or photobleaching of fluorescent dyes, which makes it difficult to obtain an accurate number of extension steps.

Supplementary Figure 3



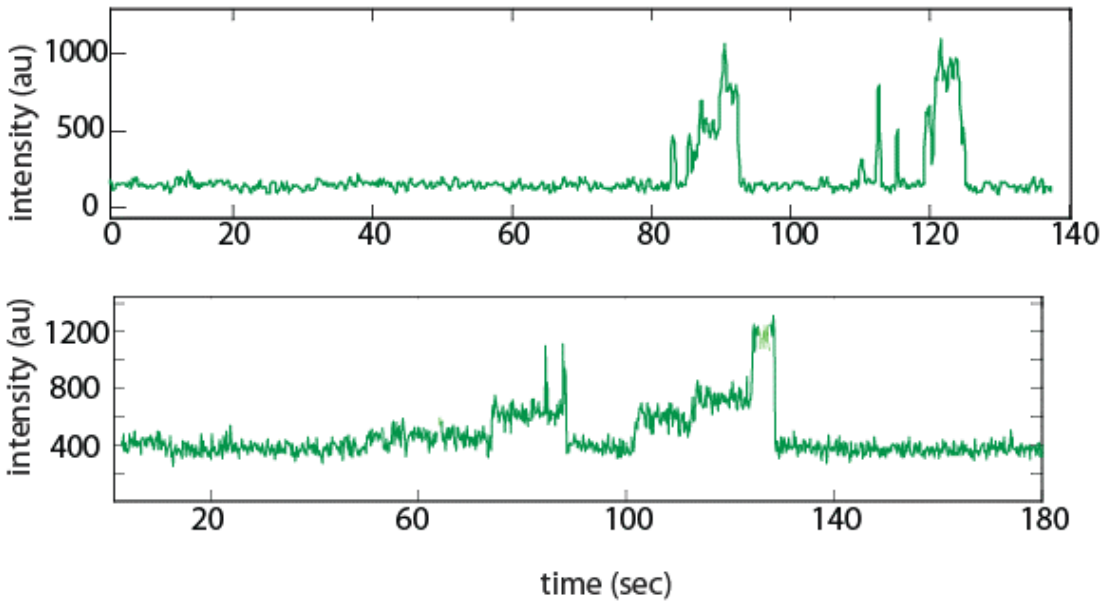
SUPPLEMENTARY FIGURE 3 The single molecule trace (left panel) obtained in the photobleaching experiment was analyzed by generating an intensity histogram (middle panel) and a simple algorithm to find a local maxima (step finder, right panel).

Supplemental Figure 4



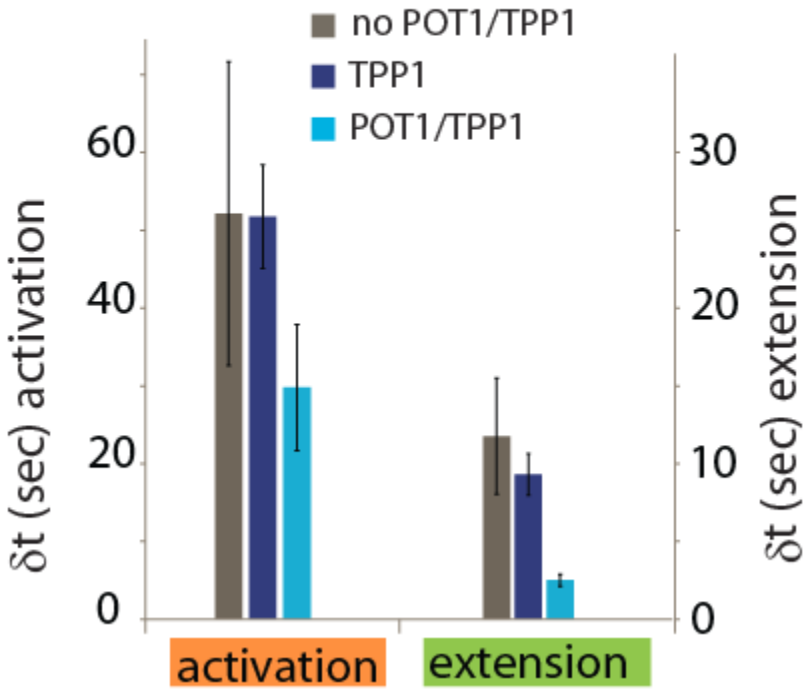
SUPPLEMENTARY FIGURE 4 (a) Primer extension assay (from four independent experiments) performed in the same condition as the single molecule experiment. The numbers TTAGGG repeats added by telomerase are marked with arrows. (b) EMSA analysis of probe binding to telomeric overhangs of various lengths (fluorescently labeled) incubated with 10x molar excess of complementary probe DNA. One tight shifted band observed for the various overhang lengths indicates that the probe binding is maximized in this condition. (c) The fraction of molecules extended by various numbers of repeats calculated from the gel analysis (orange bars; gels shown in (a)) shows high correlation with the extension data obtained in the single molecule assay (gray bar). The intensity of gel bands corresponding to 3, 6, 9 and 12 added repeats were summed and the fraction of each repeat length was calculated as the molecule fraction. The orange bars are raw data collected from single molecule assay, which is different from that shown in Figure 3. For the data presented in Fig 3E, the majority of very short products representing 2-3 added repeats were excluded from analysis because they represent abortive extension products (Latrick, C. M. & Cech, T. R. POT1-TPP1 enhances telomerase processivity by slowing primer dissociation and aiding translocation. *EMBO J* **29**, 924-933).

Supplementary Figure 5



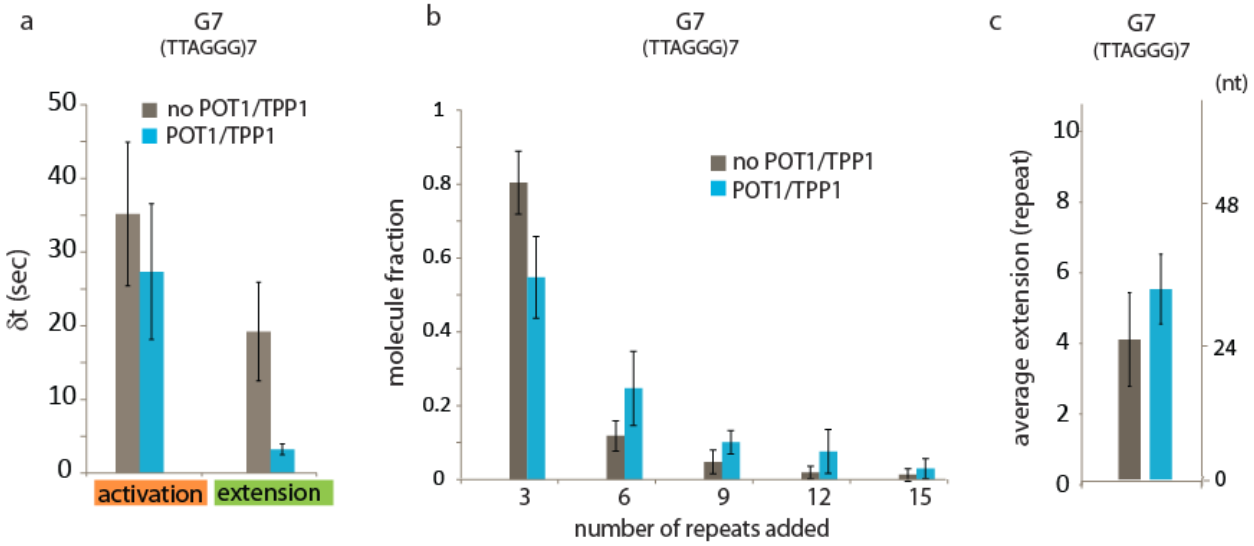
SUPPLEMENTARY FIGURE 5 Single molecule traces of telomerase extension in the presence of POT1-TPP1 exhibit multiple rounds of telomere extension.

Supplementary Figure 6



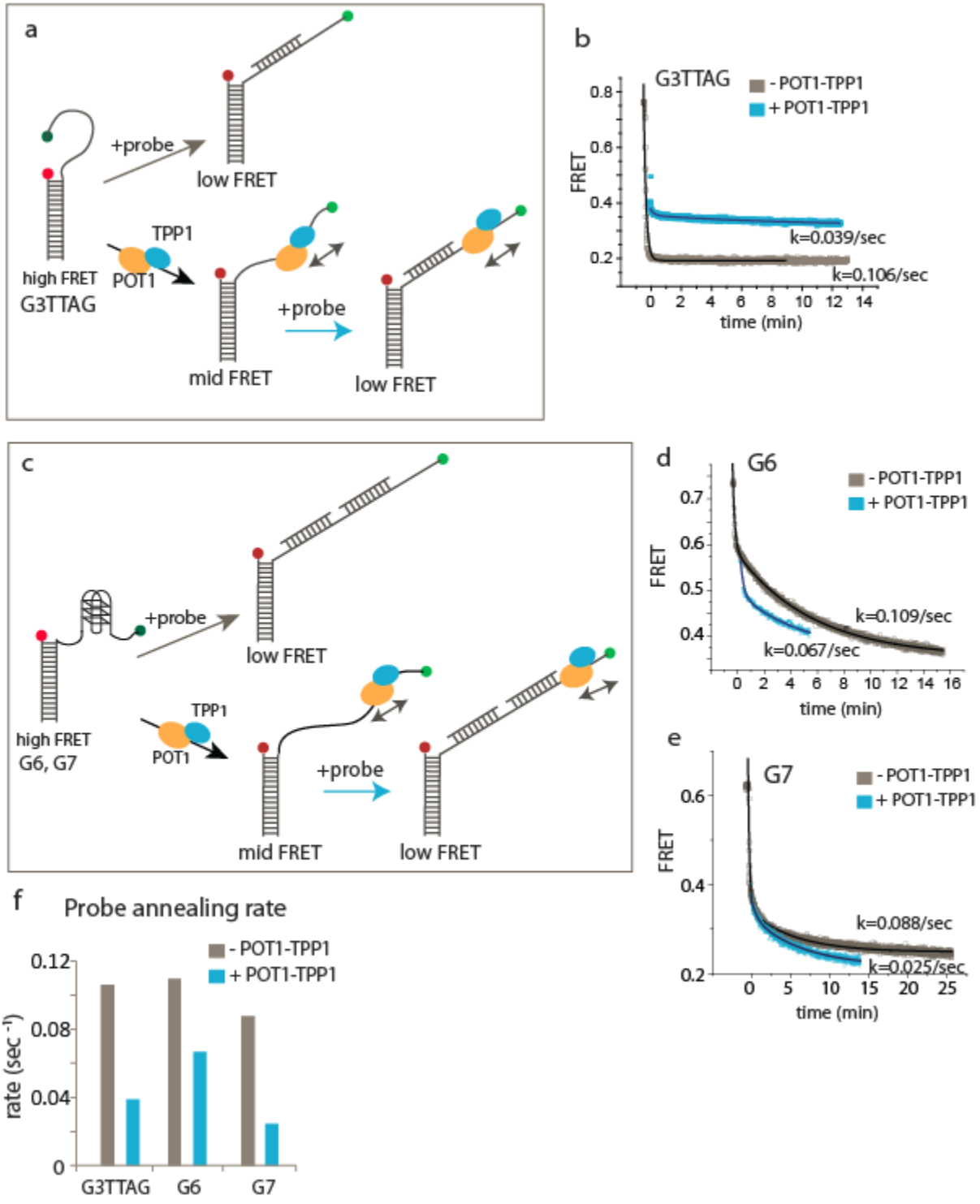
SUPPLEMENTARY FIGURE 6 TPP1 alone does not influence telomerase extension activity. The activation time and extension time for telomerase (no POT1/TPP1), telomerase+TPP1 (TPP1) and telomerase+ POT1-TPP1 (POT1/TPP1) are shown. The similar dwell times between the telomerase and telomerase +TPP1 indicates that TPP1 does not affect the telomerase activity. The 5-6 fold diminished extension time in the presence of POT1/TPP1, again shows that the accelerated extension activity only occurs in the presence of both POT1 and TPP1.

Supplementary Figure 7



SUPPLEMENTARY FIGURE 7 The accelerated extension by POT1-TPP1 is not due to unfolding of G-quadruplex. The same experiment performed with G7, seven repeats of TTAGGG in the absence and presence of POT1-TPP1 reveals similar level of difference in both times measured (a) and the extension length (b, c).

Supplementary Figure 8



SUPPLEMENTARY FIGURE 8 POT1-TPP1 does not accelerate probe binding (a,c) Schematic of probe binding assay to G3TTAG, G6 and G7 (3.5, 6 and 7 repeats of TTAGGG) substrates. (b,d,e) Ensemble FRET measurement of probe binding in the absence and presence of POT1-TPP1. (f) Probe annealing rates for all constructs.