Supplemental Materials

Molecular Biology of the Cell

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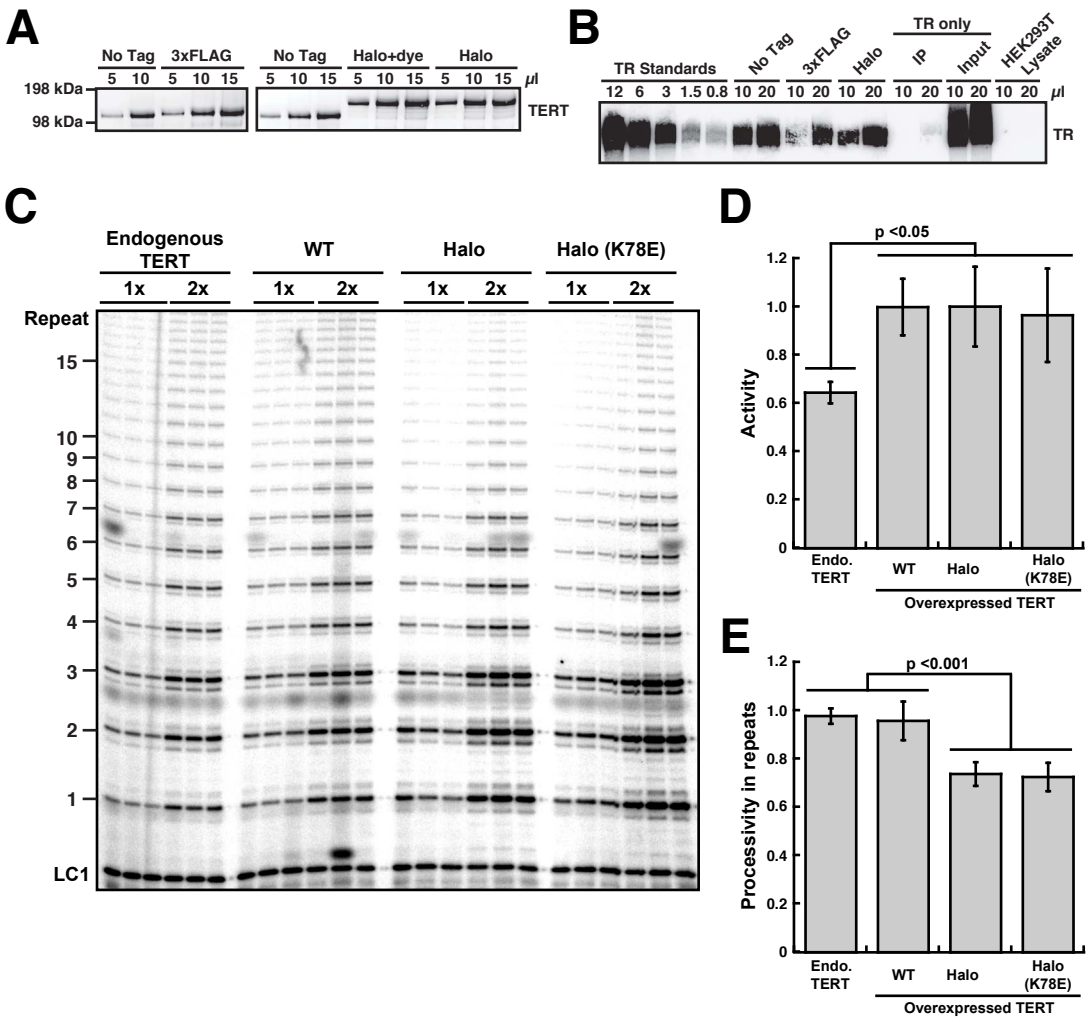
Supplemental Figure 1. Characterization of HeLa cell lines stably over-expressing various TERT proteins.(A) Western blot of telomerase immuno-purified from HEK293T cells over-expressing various untagged and tagged TERT proteins and TR, probed with an anti-TERT antibody. (B) Northern blot of RNA extracted from immuno-purified telomerase variants, probed with three TR probes. TR only input and IP were derived from HEK293T cells overexpressing only TR but not TERT, to control for non-specific TR purification. Standards are *in vitro* transcribed full-length TR. (A) and (B) are independent replicates of the data presented in Figure 1 A, B.(C) Direct telomerase extension assay at 150 mM KCl of telomerase variants immuno-purified from equal number of parental HeLa cells (Endogenous TERT), and cells over-expressing WT-TERT, HaloTag-TERT, or HaloTag-TERT (K78E). LC1, labeled DNA loading control. (D) Quantification of telomerase activity.All activities were normalized to the loading control and the activity of telomerase purified from cells over-expressing WT-TERT was set to 1.To compare the activities of telomerase from cells overexpressing TERT, Halo-TERT and Halo-TERT (K78E) activities were additionally normalized to the amount of TERT protein relative to WT-TERT detected by Western blot (see Fig. 2B). Since endogenous TERT could not be detected in the Western blot, we did not adjust for the amount of TERT and the comparison to the cell lines over-expressing TERT therefore reflects the amount of telomerase activity per cell(n = 5, Mean ± SD, t-test). (E) Quantification of processivity of telomerase purified from HeLa cells over-expressing various TERT alleles using the decay method (n = 5, Mean ± SD, t-test).

Supplemental Figure 2. Short "probing" interactions persist in the presence of Imetelstat. (A-C) Survival probability plots of stationary TERT trajectories at telomeres, Cajal bodies and other nuclear locations from cells treated with (A) no drug, (B) 2 µM mismatched control oligonucleotide, and (C) 2 µM imetelstat ($n_{Cajal\ Body}$ = 500-1000, $n_{Telomere}$ = 500-1000, $n_{Nuclear}$> 10000). Lines represent the fit of the respective dataset to a double-exponential decay (Survival probability = $A*e^{(-kfast*t)}+B*e^{(-kslow*t)}$). (D-E) Quantification of the (D) slow and

(E)fast offrate constant derived from the double exponential decay fit of the data in (A-C) at Cajal bodies, telomeres, and other nuclear locations, after treatment with (N) no drug, (C) mismatched control oligonucleotide, or (I) imetelstat. (F) Quantification of the fraction of particles that decay with the slow off-rate derived from the double exponential decay fit of the data in (A-C) Cajal bodies, telomeres, and other nuclear locations, after treatment with (N) no drug, (C) mismatched control oligonucleotide, or (I) imetelstat (n = 3, Mean, t-test).

Supplemental Figure 1



Supplemental Figure 2