Supporting Information

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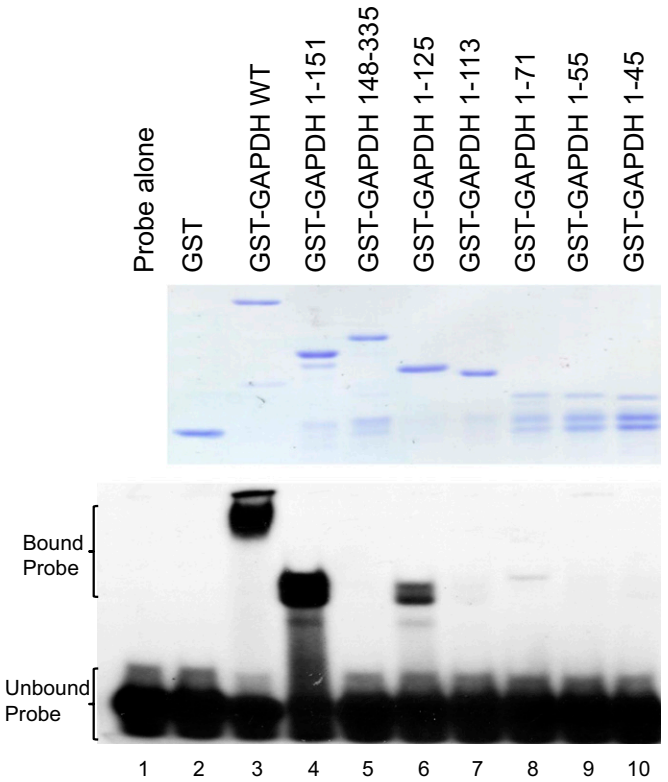

Fig. S1. Binding of different GAPDH fragments to telomeric DNA oligonucleotides. *Upper*, Coomassie blue staining of ~1.0 μg indicated purified proteins. *Lower*, electrophoretic mobility shift assay. Purified proteins as indicated were individually incubated with radioisotope-labeled telomeric DNA oligonucleotide followed by electrophoresis and autoradiography. Data are representative of three similar experiments.

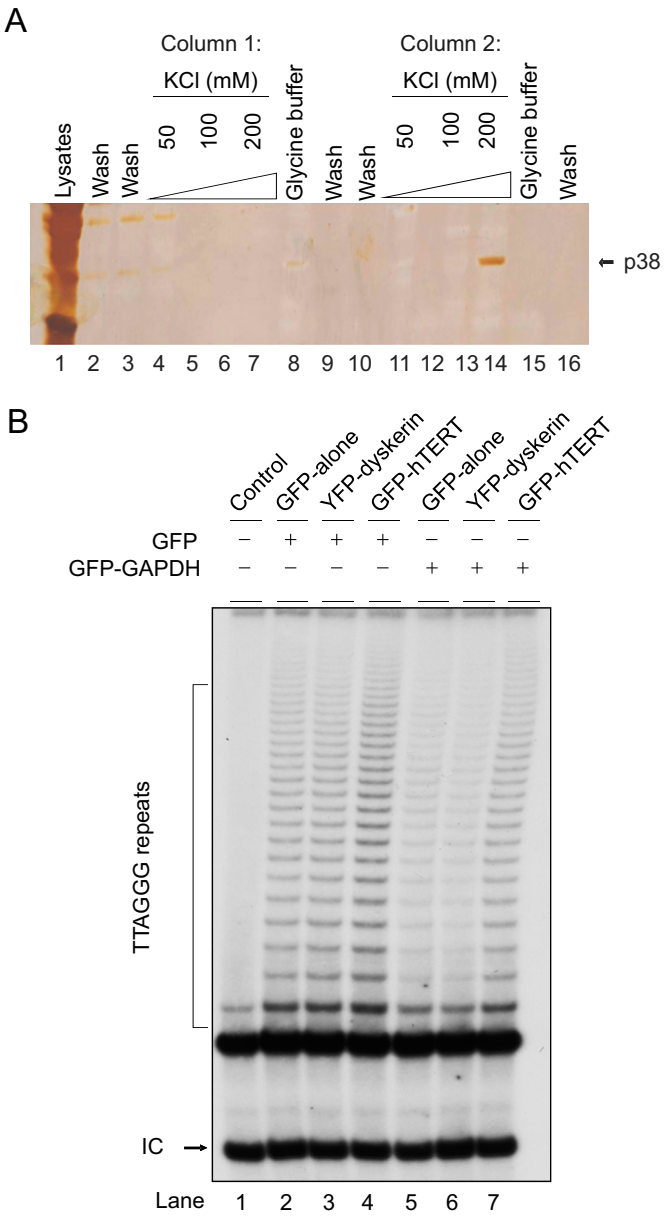

Fig. S2. hTERT sequence pulls down GAPDH and reverses GAPDH inhibition of telomerase activity. (*A*) Pull-down assay demonstrating a physical interactive complex of GAPDH and GST–hTERT fragment. GST–hTERT 423–538 and GST–hTERT 423–658 were incubated with breast cancer cell lysates, and after extensive washing, proteins bound to the GST–hTERT proteins were eluted with increasing concentrations of salt. A 38-kDa protein from the GST–hTERT 423–658 was specifically eluted. (*B*) Effect of transient expression of GFP–GAPDH, GFP–GAPDH plus dyskerin, and GFP–GAPDH plus hTERT in MCF7 cells on telomerase activity. Telomerase activity was measured by TRAP 48 h after transfection of cells with the construct combinations indicated.

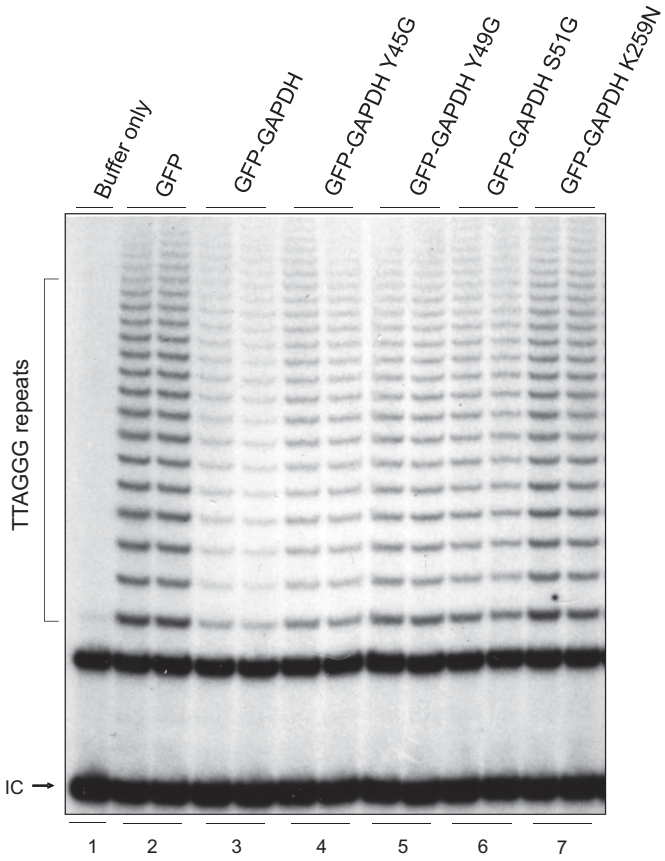

Fig. S3. Effect of stable expression of GFP, GFP–GAPDH, GFP–GAPDHY45G, GFP–GAPDHY49G, GFP–GAPDHS51G, and GFP–GAPDHK259N on telomerase activity of MCF7 cell cultures. Telomerase activity was measured by TRAP assay approximately 6 wk after transfection. Telomerase products and internal controls (ICs) are indicated. Data are representative of four similar experiments.

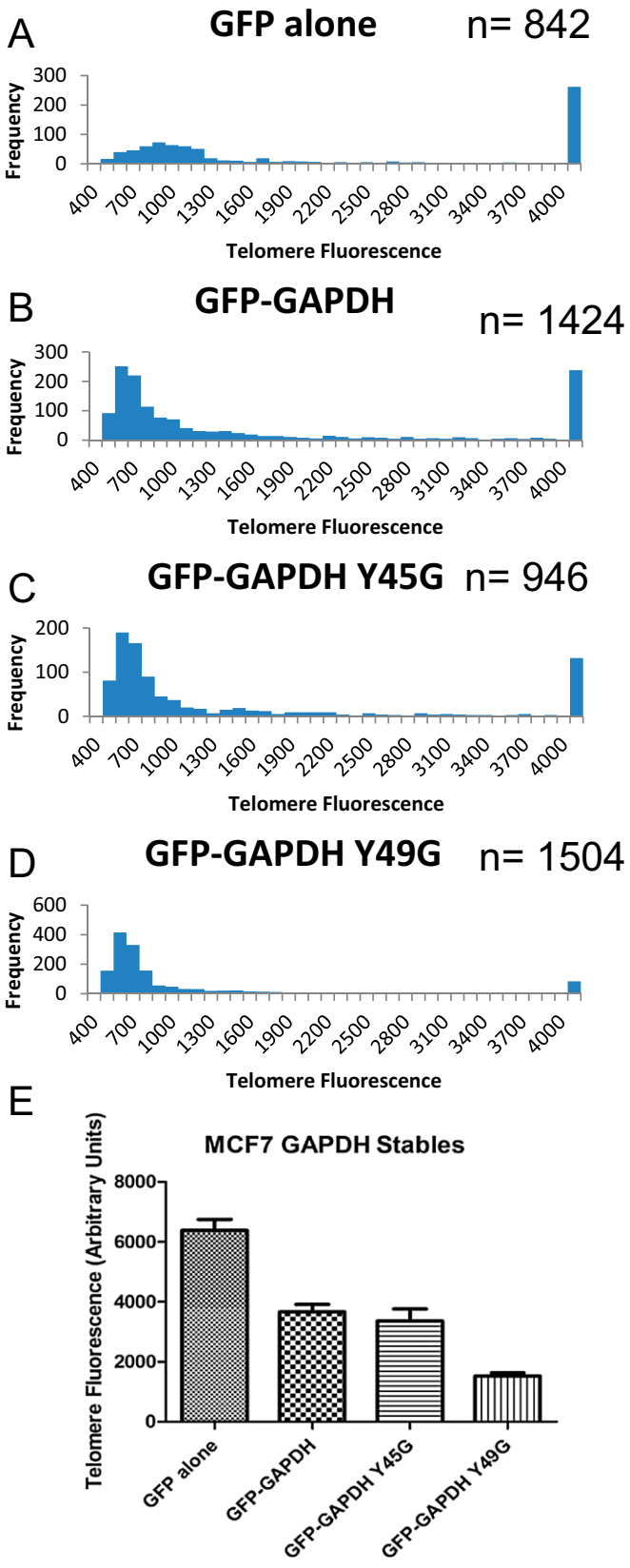

Fig. S4. Effects of GAPDH and GAPDH mutants on telomere length. (*A*) GFP control. (*B*) Wild-type GFP–GAPDH. (*C*) GFP–GAPDH Y45G. (*D*) GFP–GAPDH Y49G. (*E*) Graphical depiction of mean telomere signal frequency in MCF7 cell lines stably expressing indicated fusion proteins as determined by telomere Q-FISH ~10 wk posttransfection. Data are means ± SD of three experiments.

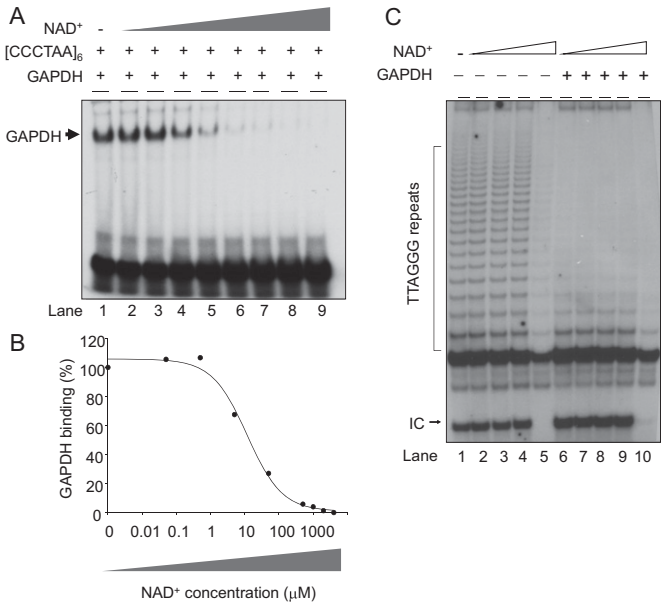

Fig. S5. NAD^+ blocked GAPDH binding telomeres but did not inhibit telomerase activity. (*A*) NAD^+ inhibits binding of GAPDH to telomeric DNA. Erythrocyte GAPDH was incubated with radiolabeled $[CCCTAA]_6$ and NAD^+. Lanes 1–9: 0, 50 nM, 0.5 μM, 5 μM, 50 μM, 0.5 mM, 1 mM, 2 mM, and 4 mM NAD^+, respectively). (*B*) Scatter plot of densitometric values of bands corresponding to DNA bound GAPDH. The line of best fit, generated using SigmaPlot for Windows software version 10.0 (Systat), was used to estimate 50% inhibition of the GAPDH–DNA interaction by NAD^+. (*C*) Effect of NAD^+ on GAPDH-mediated inhibition of telomerase activity in vitro. Cancer cell lysates were incubated with (+) and without (–) erythrocyte GAPDH for 30 min during the substrate elongation step of the TRAP assay, in the presence of increasing amounts of NAD^+ (0.0, 0.1, 1.0, 10.0, and 100.0 pmol, respectively). NAD^+ was preincubated with GAPDH before addition of cell lysates.

Table S1. Identification of a 38-kDa protein to be GAPDH from GST–hTERT pull down

Proteins used in the pull-down assay	Experiment samples	Protein bound, kDa	No. of peptides resolved	GAPDH coverage by mass spectrometry, %
GST	—	—	—	—
GST–hTERT 423–538	—	—	—	—
GST–hTERT 423–658	93–7	38	12	59
GST–hTERT 423–658	93–14	38	12	51
GST–hTERT 423–658	95–4	38	12	56

—, Not detected.