Supplemental Material to:

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Telomerase enzymatic component hTERT shortens long
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Supplementary Figure Legends

S-Fig. 1. Detect telomerase activity and expressions of hTERT wild-type, mutant and hTR. The TRAP assay was performed to evaluate the telomerase activity, qRT-PCR for mRNA level and Western blot analysis for protein expression. IMR90 was used as a reference to calculate fold change of hTERT mRNA and telomerase activity. Telomerase positive cell lines HT1080 and MDA231 were used as positive controls.
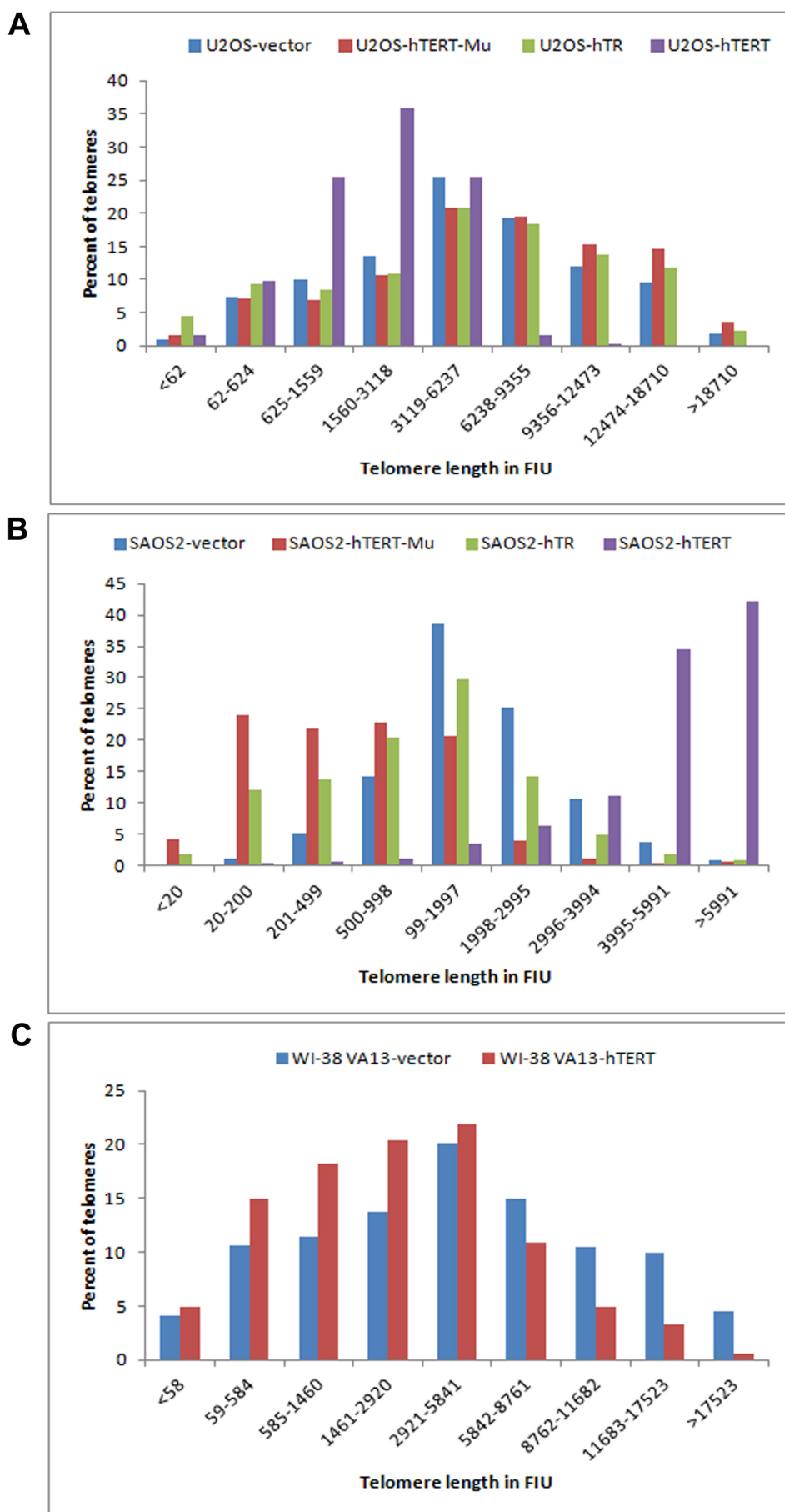
S-Fig. 2. Effect of hTERT over-expressing on telomere length. Histograms to analyze effects of telomere length in indicated cell lines (for main **Fig. 1**).

S-Fig. 3. hTERT shortens long telomeres in ALT+ cancer cells. Empty vectors or hTERT were expressed in ALT+ U2OS (**A**) and SAOS2 (**C**) cells. Correlation between the average TL at specific chromosomal ends in vector control cells and percent of TL change at the corresponding chromosomal ends in hTERT over-expressing cells. Each dot represents a chromosomal end. 30 cells were analyzed per cell line. **B**. TRF assays were performed to quantitative telomere length. Genomic DNA was loaded to gel and transferred to a Nylon membrane followed by telomeric DNA probe hybridization.

S-Fig. 4. Localizations of hTERT at long telomeres. ALT SAOS2 cells were processed for indirect immunofluorescence and telomeric DNA FISH. Images were captured with a 100x objective. hTERT (HA, green) localized at telomeres including those telomeres with high signal intensity (telomeric PNA probe, red). An enlarged co-localization focus of hTERT and a telomere with high signal intensity is shown in pictures on the right corner.
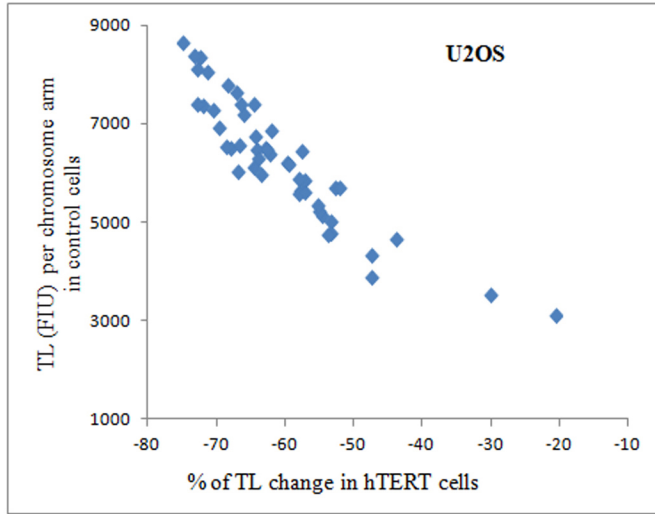
S-Fig. 5. Overexpression of hTERT does not induce telomere dysfunctional foci. IMR90 cells transduced with empty vector, hTERT or POT1-shRNA were immunostained with anti-γ-H2AX mouse monoclonal antibody (*red*) together with anti-TRF1 antibody (*green*). The nuclei were counterstained with DAPI. POT1-shRNA is used as a positive control.
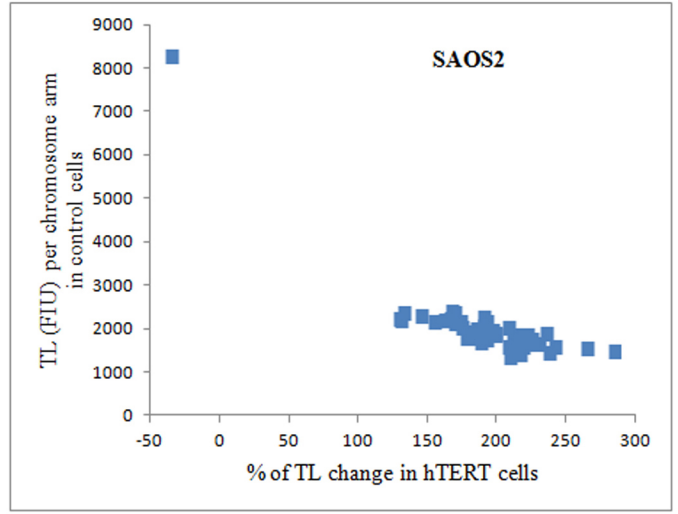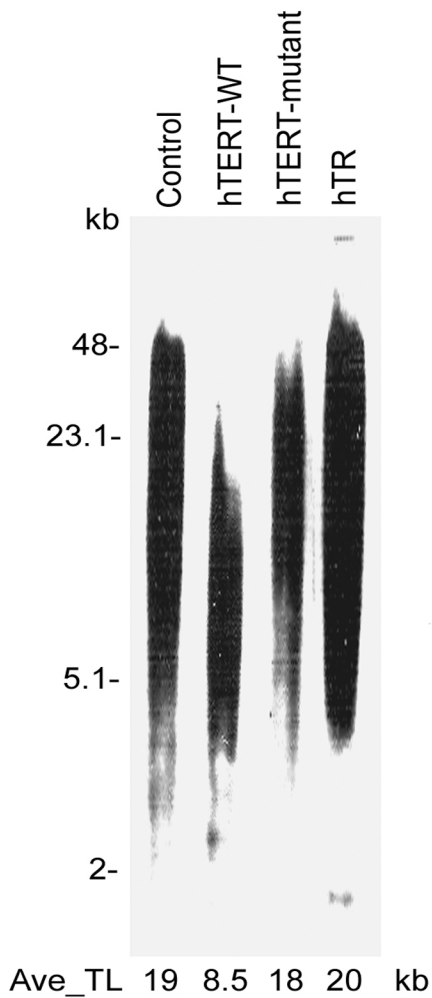
S-Fig. 1

A

	hTERT mRNA	Telomerase activity
	(fold)	(fold)
U2OS	1	1
U2OS-hTERT	7.5	6.5
U2OS-hTERT-Mu	8	1
SAOS2	1	1
SAOS2-hTERT	8.7	8.1
SAOS2-hTERT-Mu	9	1
IMR90	1	1
IMR90-hTERT	7.5	8
WI38	1	1
WI38-hTERT	7	7.2
WI38 VA13	1	1
WI38 VA13-hTERT	7	7
HT1080	10	9.5
MDA231	10	9.2

B

S-Fig. 2

S-Fig 3

A

C

B

S-Fig. 4

SAOS2-hTERT

| HA (hTERT) | Telomeric DNA | Merge |

S-Fig. 5

| γH2AX | TRF1 | Merge |

IMR90-vector

IMR90-hTERT

POT1-shRNA