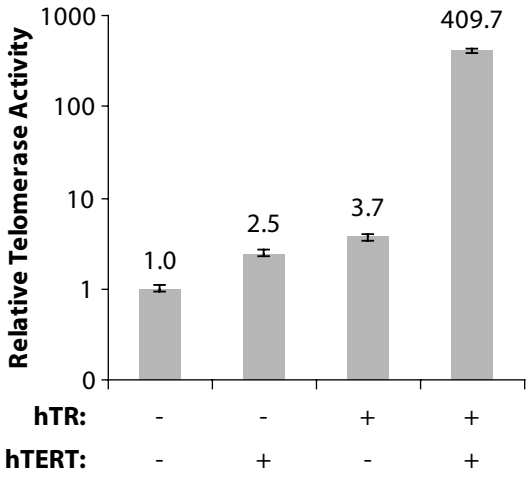
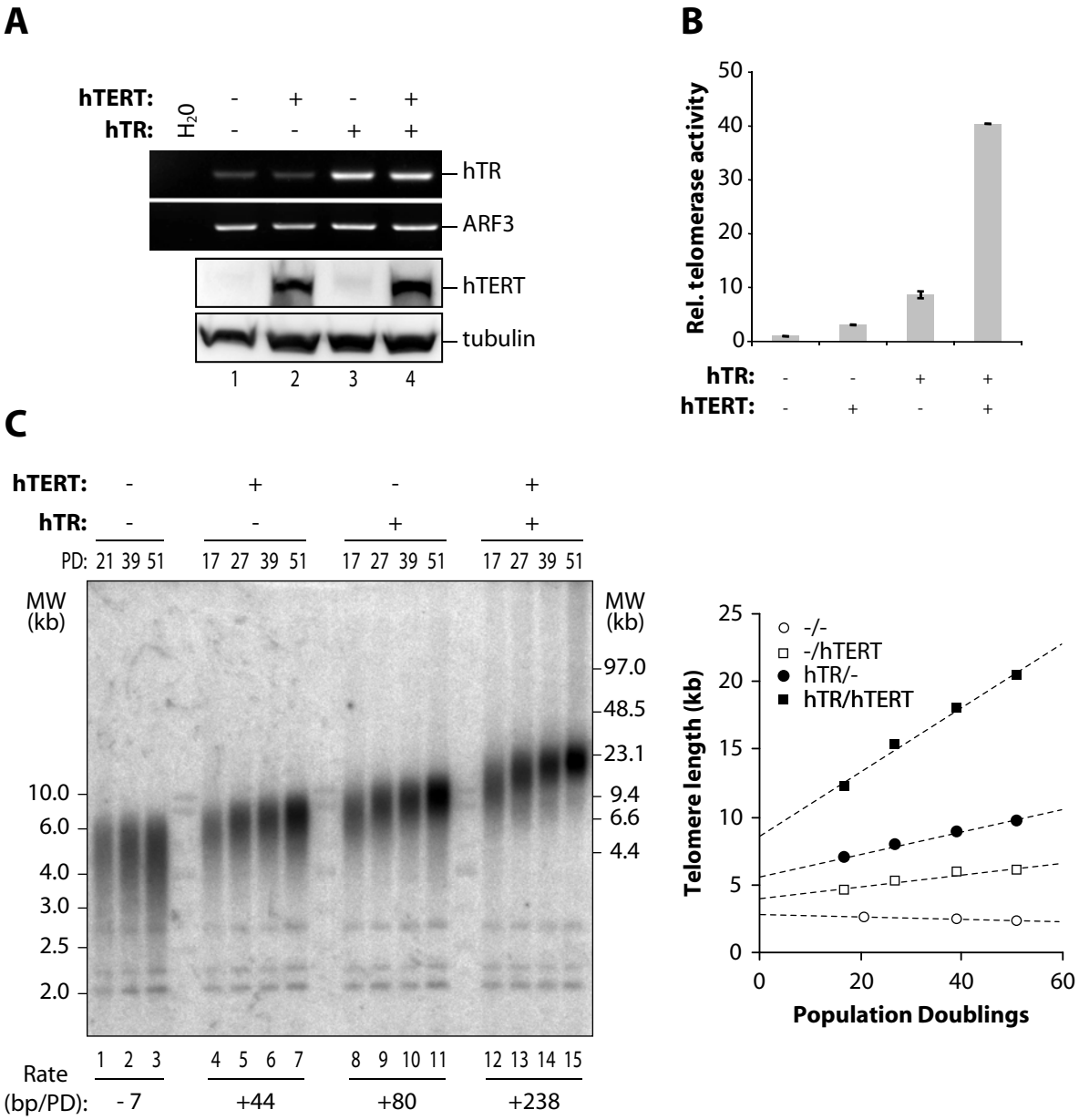


Cristofari & Lingner - Supplementary Figure 1



Cristofari & Lingner -Supplementary Figure 2

Supplementary Figure Legends

Supplementary Figure 1. Massive increase of telomerase activity upon transient overexpression of hTR and hTERT in Hela cells. Telomerase activity was determined by RQ-TRAP. RNase treatment of extracts reduced the signal to below the detection limit (not shown). Each bar represents the mean ± SD of at least four measurements. Transfection efficiency was estimated >90% from a separate transfection done with a GFP-encoding plasmid.

Supplementary Figure 2. Cooperative effects on telomere length of long-term hTR and hTERT overexpression in HT1080 cells. Cells were transduced with retroviral vectors encoding hTR or hTERT or the respective empty vectors. Cell populations were analyzed after double selection. (**A**) Overexpression of telomerase core components. RT-PCR amplification (upper panels) of hTR or ARF3 as a loading control. RT-PCR conditions were ensured to be in the dynamic range and RT-dependent (not shown). Immunoblot (lower panels) with anti-hTERT R484 antibody or with anti-tubulin antibody as a loading control. (**B**) Telomerase activity as determined by RQ-TRAP. RNase treatment of extracts reduced the signal to below the detection limit (not shown). Each bar represents the mean ± SD of at least four measurements. (**C**) Genomic blot of telomeric restriction fragments (TRFs) in transduced HT1080 cell populations at the indicated population doublings (PD) separated by pulse-field gel electrophoresis (PFGE). The rate of elongation is indicated below in base pairs per PD (bp/PD) for this gel. The graph shows median telomere length at the indicated cell population doubling. Telomere length was deduced by subtracting 2kb for subtelomeric sequences from the median TRF length obtained from the gel.

Supplementary Table

		Median	IQR	n	Ratio
TRF1	-/-	6810	1793-13710	97	10.9
	hTR/hTERT	73932	39339-130202	95	
TRF2	-/-	4197	1043-34947	58	8.8
	hTR/hTERT	36797	16976-80198	69	

Supplementary Table 1. Quantification of TRF1 and TRF2 immunofluorescence signals in nuclear foci. Median refers to the median fluorescence intensity of TRF1 or TRF2 foci per cell. IQR, interquartile range; n, number of cells analyzed; ratio, ratio of median fluorescence in super-telomerase (hTR/hTERT) cells over control (-/-) cells.

Supplementary Methods

Quantification of TRF1 and TRF2 immunofluorescence signals

Pictures were taken with a wide field Zeiss Axioplan microscope. Acquisition time was identical for all fields and set such as the TRF1 and TRF2 signal were never saturated. For a given field 25 Z-stack pictures were taken with a blue filter (excitation 365 nm, emission BP 445/50 nm) or a red filter (excitation BP 546/12 nm, emission BP 575-640 nm). Analysis was performed with ImageJ free software (http://sb.info.nih.gov/ij/). A single picture was generated by maximum intensity projection of a given stack. Blue (DNA) and red (TRF1/2 dots) channels were separated. For every cell in a field, the blue channel was used to produce a mask and to exclude signals outside of the nucleus. An arbitrary threshold was applied to the red channel to eliminate diffuse nuclear signal thus taking only into account signals which were concentrated in foci. The threshold was chosen to reduce background to the minimum while avoiding cells with no signal at all. The same threshold was applied to all cells. The TRF1 or TRF2 fluorescence levels correspond to the total intensity of all TRF1 or TRF2 dots per cell. A semi-automated script was used and is available upon request.