Figure S1.

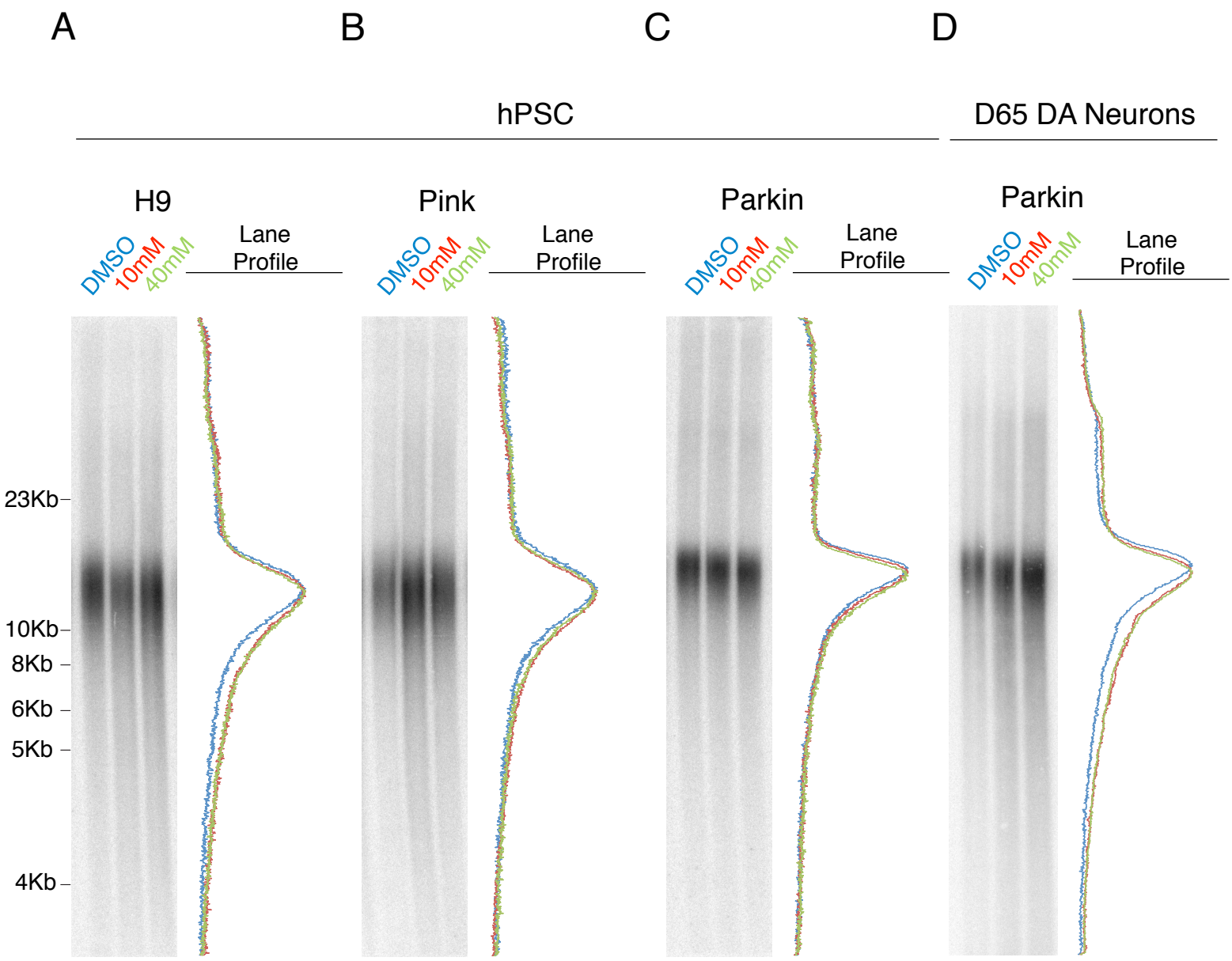
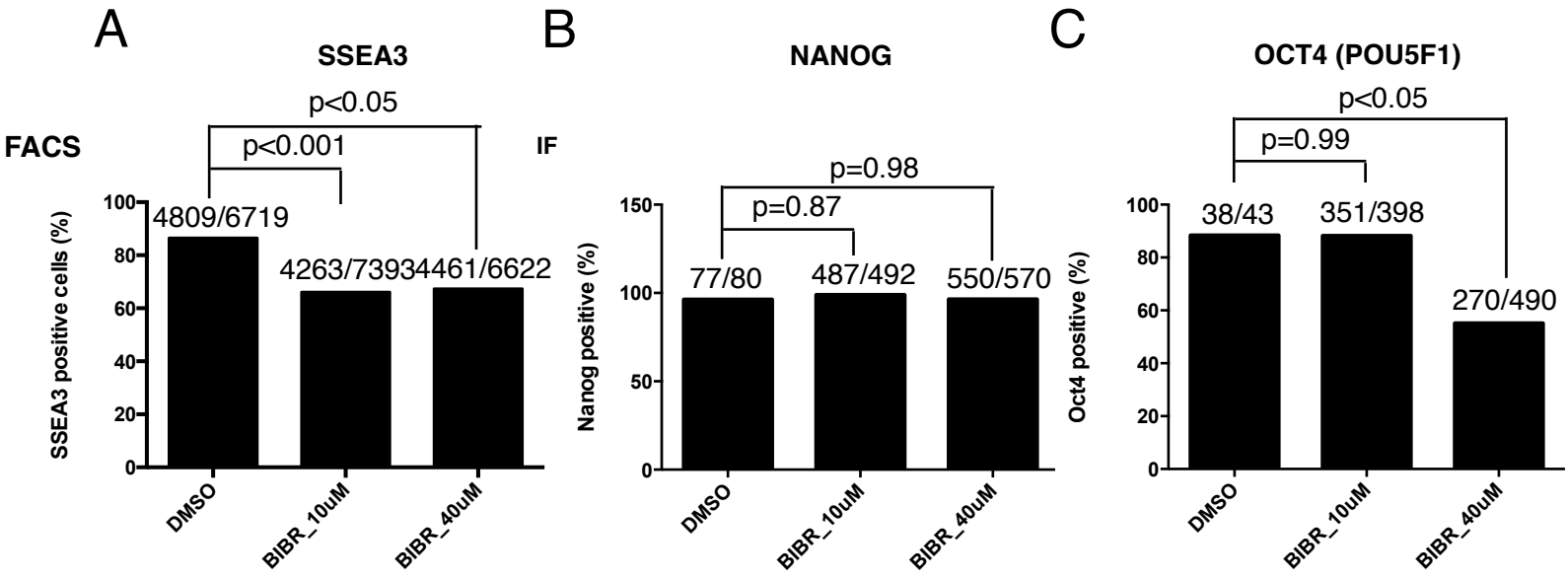

A
B
C
D
hPSC
D65 DA Neurons
H9
Pink
Parkin
Parkin
DMSO
10mM
40mM
Lane Profile
23Kb
10Kb
8Kb
6Kb
5Kb
4Kb

Figure S1. Telomere shortening by Southern blot of telomeric restriction fragments upon pharmacological inhibition of telomerase in hPSC and hPSC-derived neurons, related to Figure 2 and 3. Southern blot of MboI/AluI-digested DNA from H9 (A), Pink (B) and Parkin (C) hPSC cell lines treated with DMSO or BIBR1532 (10 and 40 μM) for 14-16 days. Day 65 DA neurons derived from Parkin line treated with DMSO or BIBR1532 (10 and 40 μM) for 14 days before the differentiation protocol and during the differentiation protocol until day 18 (D) Telomeric fragments were separated by gel electrophoresis and hybridized with a telomeric radiolabelled probe. The lane profile on the right of the blot shows 32P signal distribution for each of the untreated or treated samples, with the color code indicated ontop of each lane. Signal distribution is represented as a percentage of the maximum grey value recorded for each lane.

Figure S2.

Pluripotency markers



Cell Cycle analysis

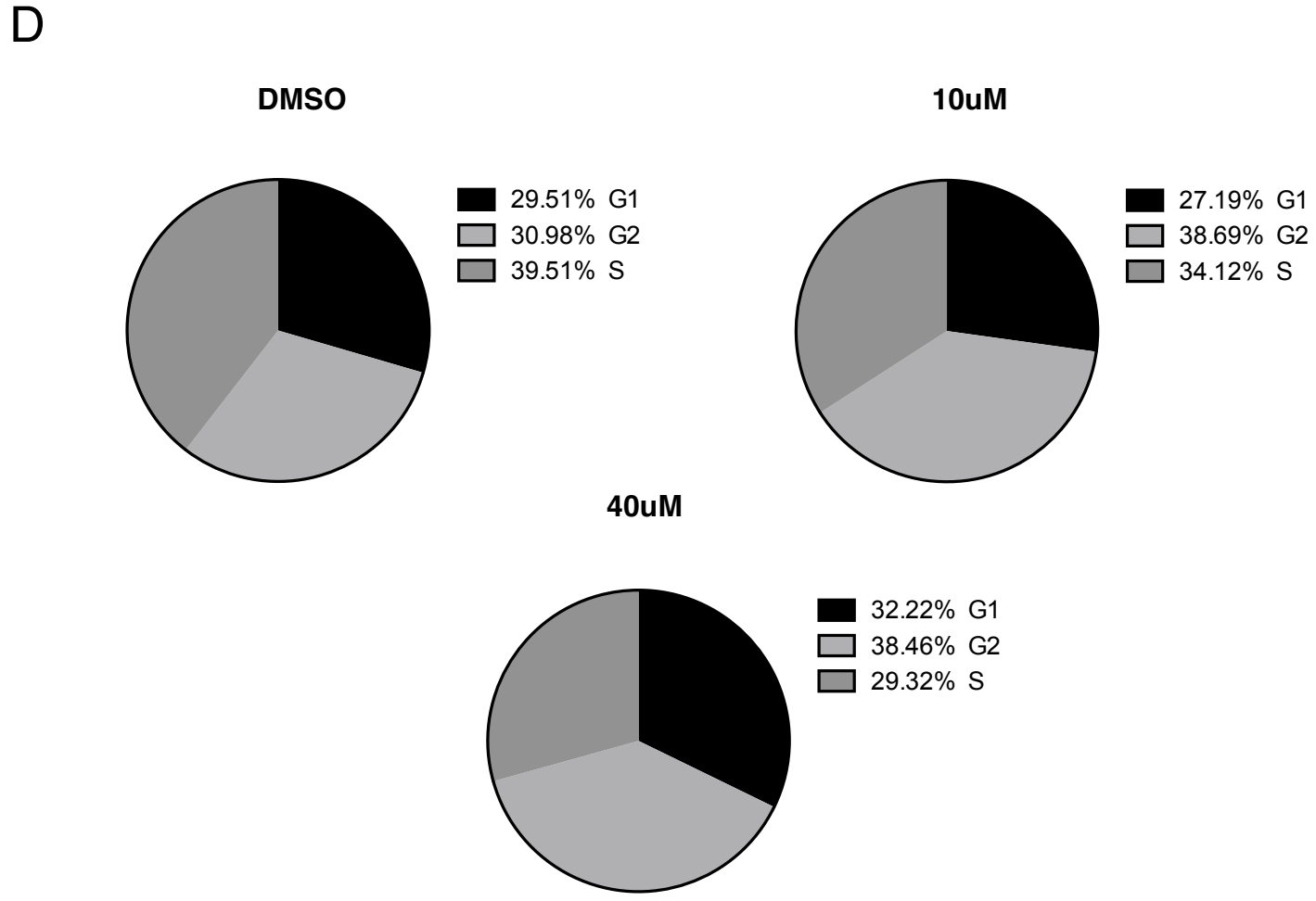


Figure S2. Functional consequences of telomerase pharmacological downregulation in the ES stage, related to Figure 2. H9 cells were treated with two different concentrations of BIBR1532 telomerase inhibitor and DMSO as a control for two weeks and several functional assays were performed (A) Analysis of the pluripotency marker SSEA3 measured by FACS. Numbers above bars represent the number of positive nuclei over the total number of nuclei analyzed. Expression of NANOG (B) and OCT4 (POU5F1) (C) pluripotency markers measured by immunofluorescence. Numbers above bars represent the number of positive nuclei over the total number of nuclei analyzed (E) FACS-based cell cycle analysis on propidium iodide (PI)-stained cells was used to determine the percentage of cells in a given cell cycle phase.

Figure S3.

Day30

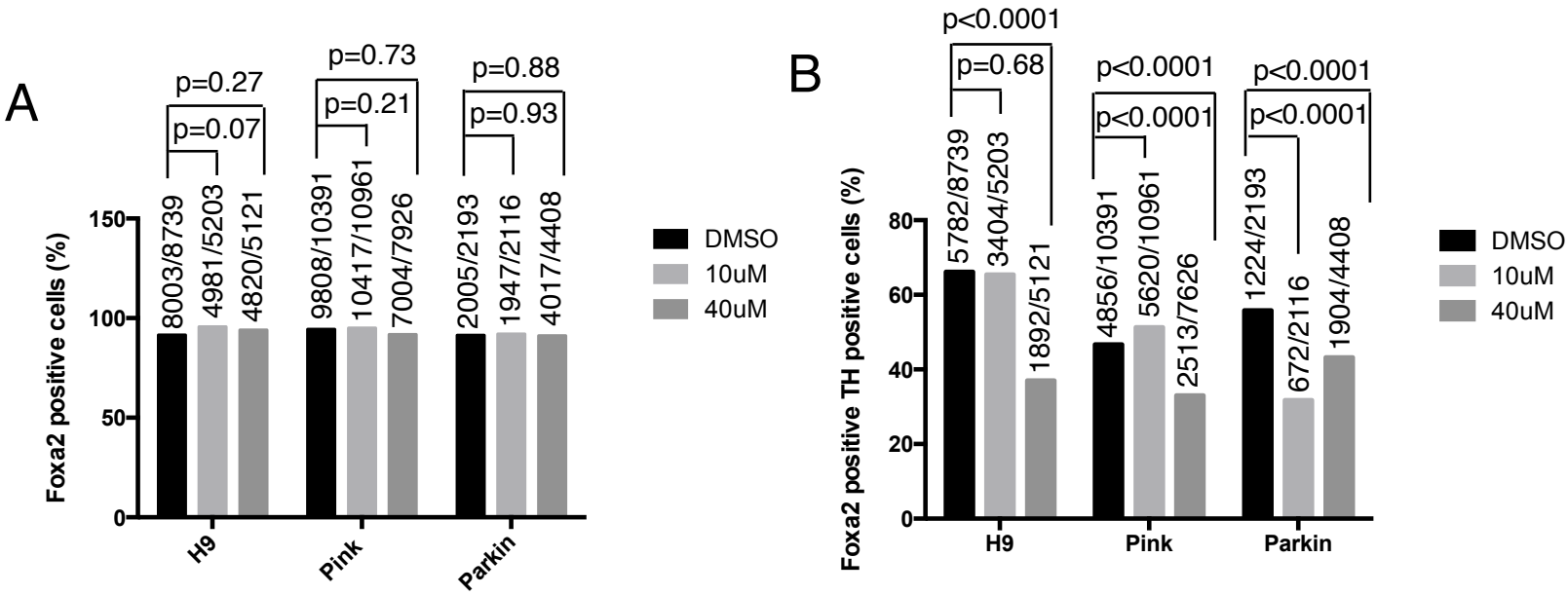
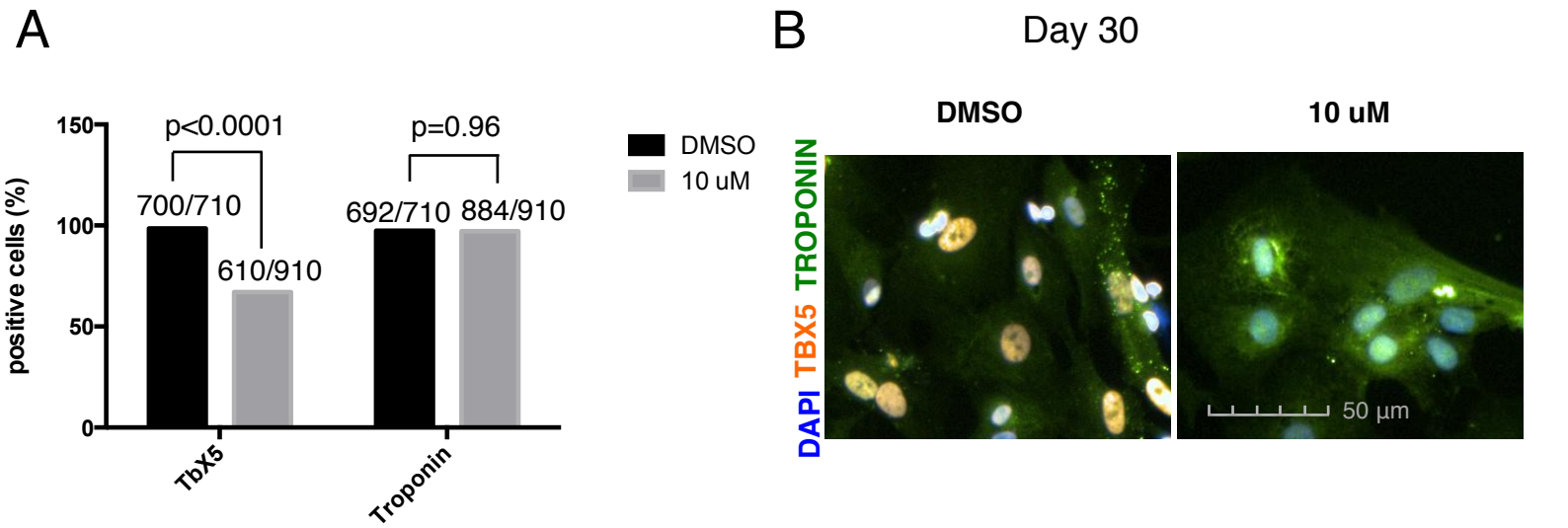


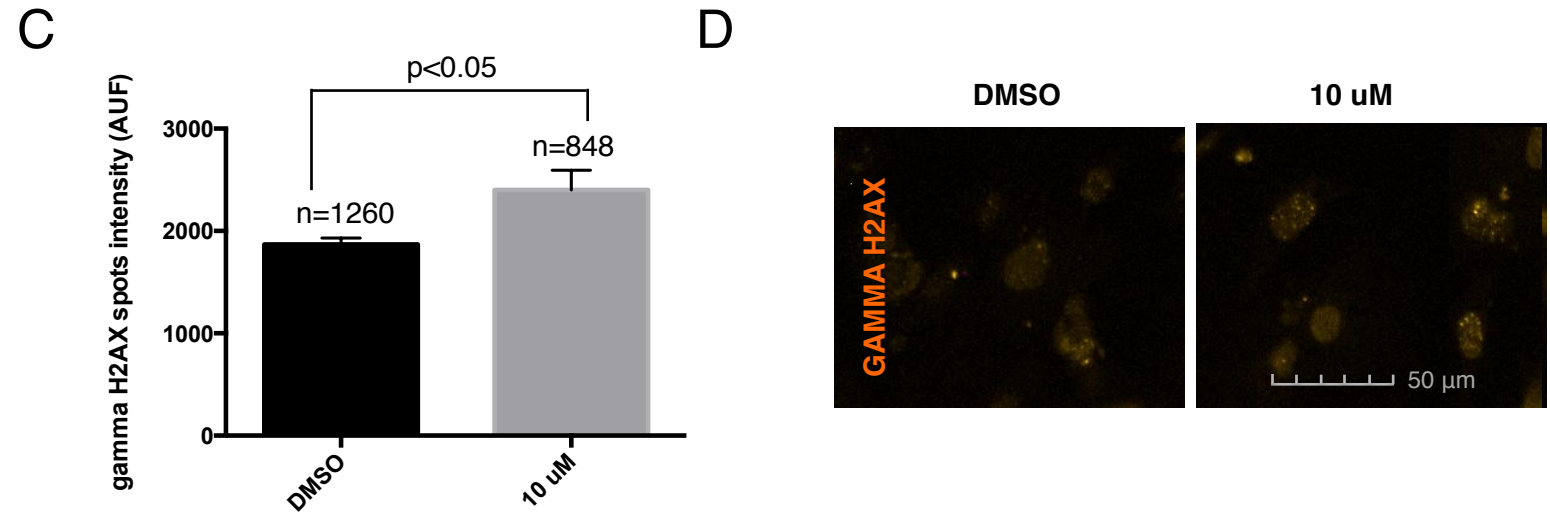
Figure S3. Analysis of mDA markers at day 30 of the mDA differentiation protocol, related to Figure 4. Quantitative analysis of FOXA2 (A) and FOXA2 and TH double positive cells (B) in the hES cell line H9 and two PD iPSC cell lines, PINK1 and PARKIN- derived neurons at day 30 of the mDA differentiation protocol. Cells were treated with two different concentrations (10 and 40 μM) of the telomerase inhibitor BIBR1532 and DMSO was used as a control. Numbers above bars represent the number of positive nuclei over the total number of nuclei analyzed.

Figure S4.

Cardiomyocytes markers



DNA damage



Telomere length

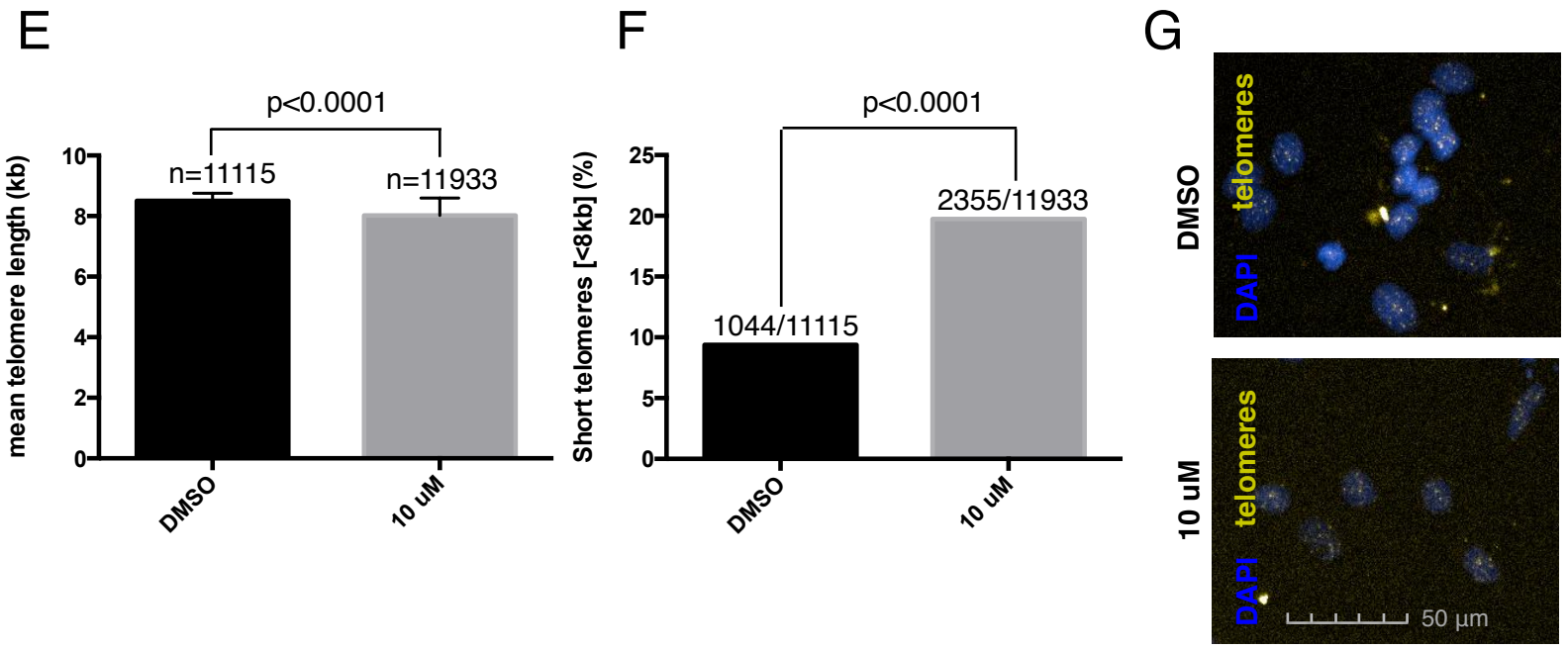


Figure S4. Aging-associated phenotypes in mature cardiomyocytes treated with the telomerase inhibitor, related to Figure 3. (A) Quantitative analysis of the cardiomyocytes markers TBX5 and TROPONIN. Numbers above bars represent the number of positive nuclei over the total number of nuclei analyzed (B) Representative images of the immunofluorescence of TBX5 (red) and TROPONIN (green) (C) Quantitative analysis of DNA damage measured by mean gamma H2AX intensity per nucleus (AUF). Bars are represented as mean ± SEM. Numbers above bars represent the number of nuclei analyzed (D) Representative images of gamma H2AX immunofluorescence (E) Quantification of the mean telomere length ± SEM measured by HT Q-FISH. Numbers above bars indicate the number of telomere spots quantified (F) Percentage of short telomeres (<8kb) measured by HT QFISH. Numbers above bars indicate the number of short telomeres out of the total number of telomeres (G) Representative HT Q-FISH images. Nuclei are stained with DAPI (blue). Telomeres are stained with CY3 (yellow).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Statistical Analyses

Student's t-test was used to calculate the statistical significance of the mean telomere length, mean gamma H2AX intensity and average number of dendrites per cell, between the treated and the control cells. Chi-squared test was used to assess the statistical differences of the proportion between treated cells and control in the following variables: percentage of short telomeres, MitoSOX, FOXA2, TH, SSEA3, NANOG, OCT4 and TBX5 positive cells. Prism (version 6.0a; GraphPad) was used for data presentation and analysis.

Quantitative RT–PCR primers

Gene	**Sequence**
TERT-F	5'-TGTGCACCAACATCTACAAG-3'
TERT-R	5'-GCGTTCTTGGCTTTCAGGAT-3'
TERC-F	5′-CTAACCCTAACTGAGAAGGGCGTA-3′
TERC-R	5′-GGCGAACGGGCCAGCAGCTGACATT-3′
GAPDH-F	5'-ATGTTCGTCATGGGTGTGAA-3'
GAPDH-R	5'-AGGGGTGCTAAGCAGTTGGT-3'