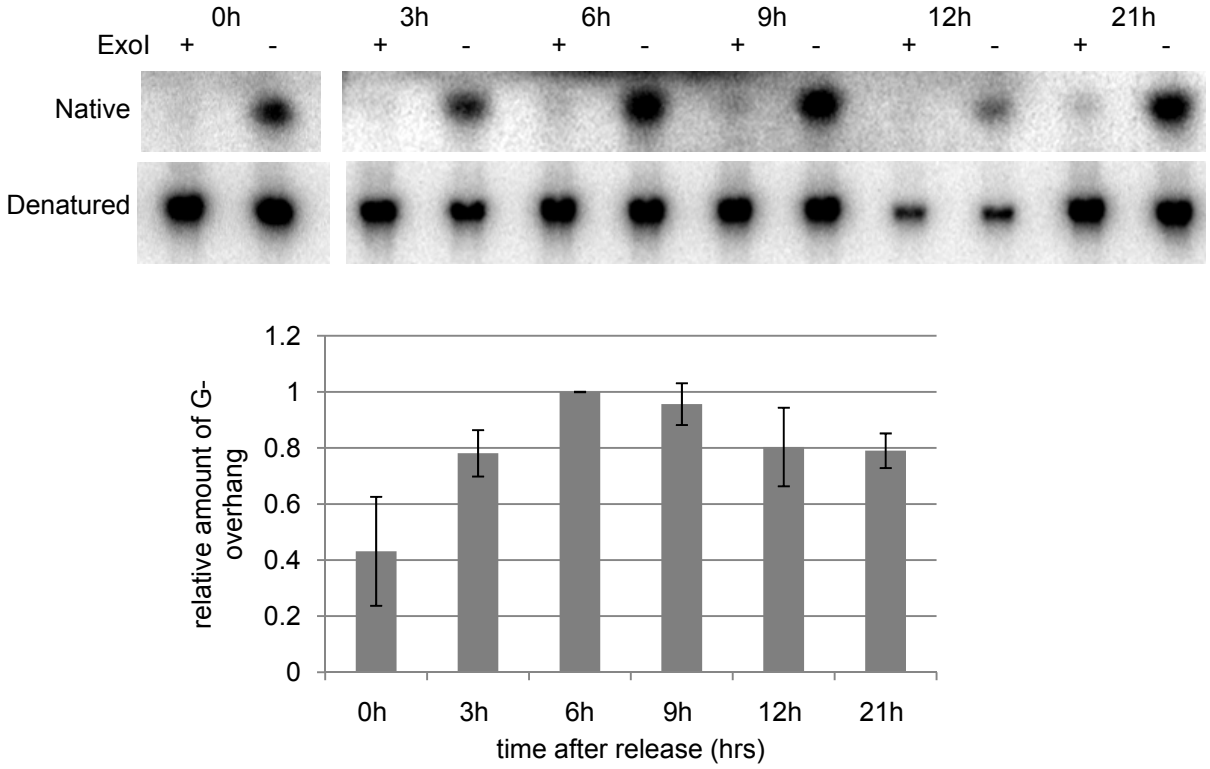

Fig. S1. Relative amount of G-overhangs in telomerase-negative cell line IMR90 during the cell cycle using non-denaturing in-gel hybridization assay. (A) DNA from G1/S synchronized IMR90 released into S phase for different hours were analyzed by non-denaturing in-gel hybridization. (B) Quantitation of relative amount of G-overhangs from three experiments. Error bars: standard deviation.

Fig. S2

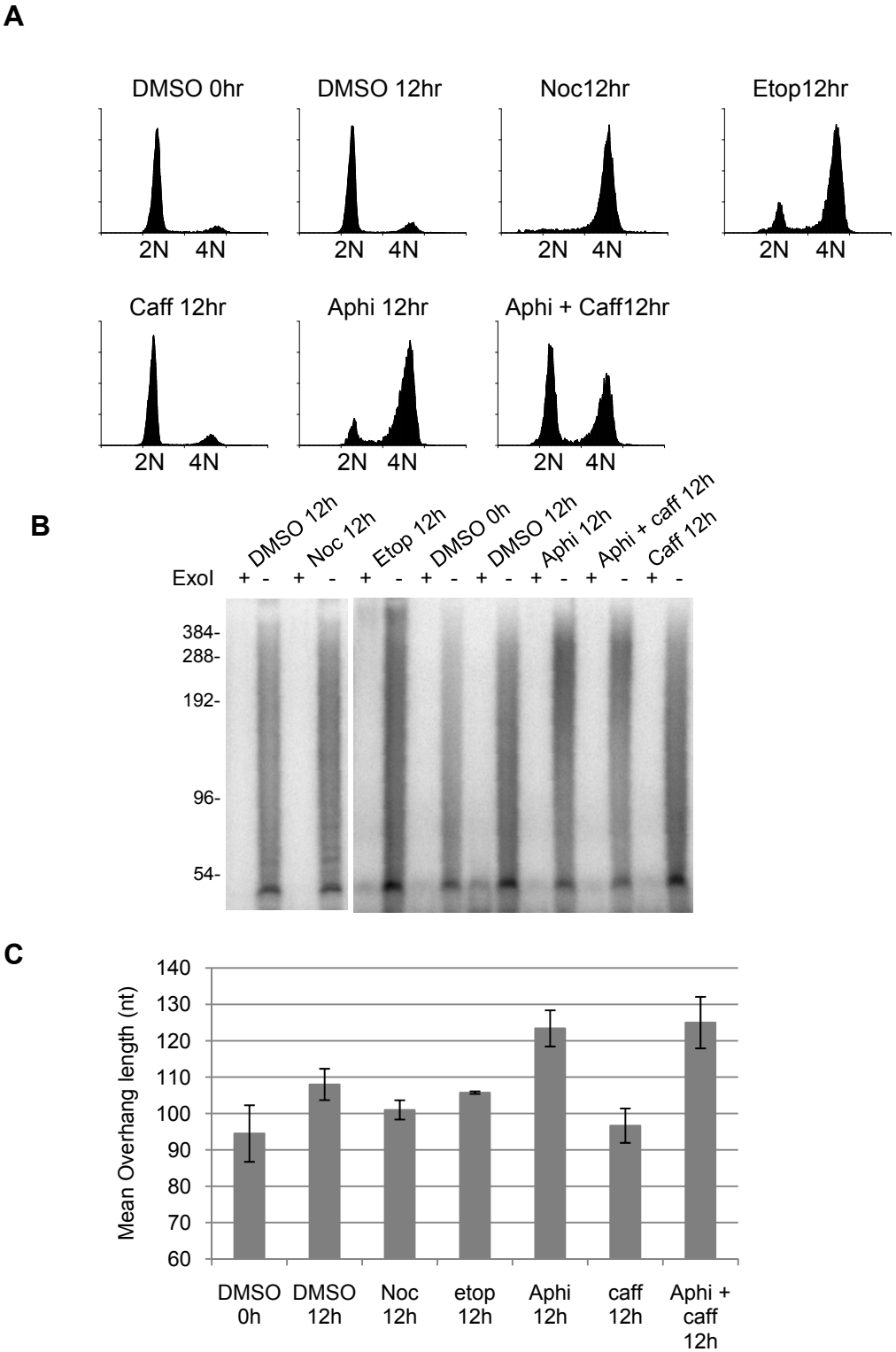

Fig. S2. The persistence of lengthened G-overhang caused by aphidicolin was not due to cell cycle arrest. HeLa was synchronized at G1/S boundary and released in S phase. Nocodazole (Noc), etoposide (Etop), or aphidicolin (Aphi) was added to media at 6.5 hrs after release. Caffeine was added 10 min before the addition of aphidicolin. Cells were collected at 12 hrs after release for FACS analysis (A) and genomic DNA was isolated for G-overhang measurement. Representative image from the overhang protection assay is shown in (B). (C) Quantitation of the weighted mean G-overhang lengths from three experiments. Error bars: standard deviation.

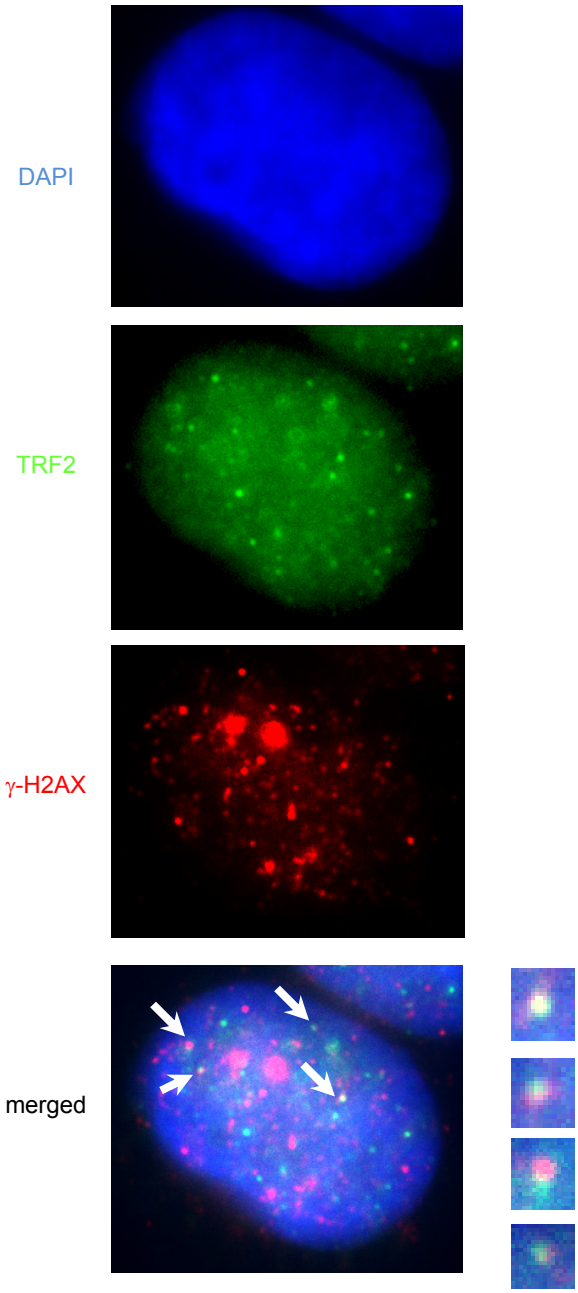

Fig. S3. Depletion of Stn1 induces DNA damage response at telomeres. HeLa was transfected with siRNA-1 of Stn1 for 72hrs and fixed for immunostaining. Cells were stained with antibodies specific for TRF2 (green) and γ-H2AX (red), and nuclei were counterstained with DAPI (blue). Cells were sectioned at 0.275 μM intervals, and co-localizations (yellow) were observed in multiple sections. Here, representative images from a single section containing co-localization of γ-H2AX with TRF2 (indicated by white arrow) is shown. Inserts show enlarged images of co-localization. More co-localizations were observed in multiple sections. Percent of cells containing TRF2/γ-H2AX co-localization is shown in Fig. 8D. Similar co-localization was also observed in HeLa depleted of Stn1 with siRNA-2.