Supplementary information

Material and Method

TERRA knockdown

Hela Cells were plated in six-well plates (1.5×10^5 HeLa cells per well) and cultured 24h for transfection. Cells were transfected with Telo LNA gapmer (TAACCCTAACCCTAAC) and control gapmer (CACGTCTATACACCAC) at the final concentration of 50nM using Lipofectamine 3000 (Invitrogen), as previously described. Media was replaced 24h after transfection, and 72h post transfection cells were collected for RNA extraction and used for immuno-FISH.

Figure S1

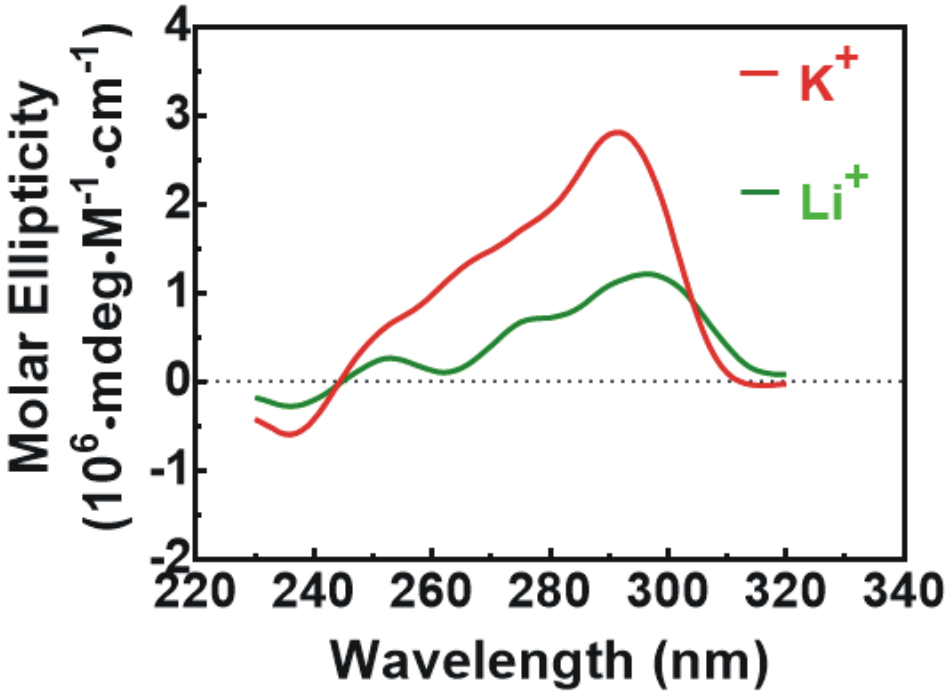


Figure S1. CD spectra of Tel21. The DNA was incubated in 150 mM KCl (Red line) or 150 mM LiCl (Green line). The data was obtained with a 0.5 μM strand concentration (pH 7.4) at 25°C. Buffer blank correction was made for both samples.

Figure S2

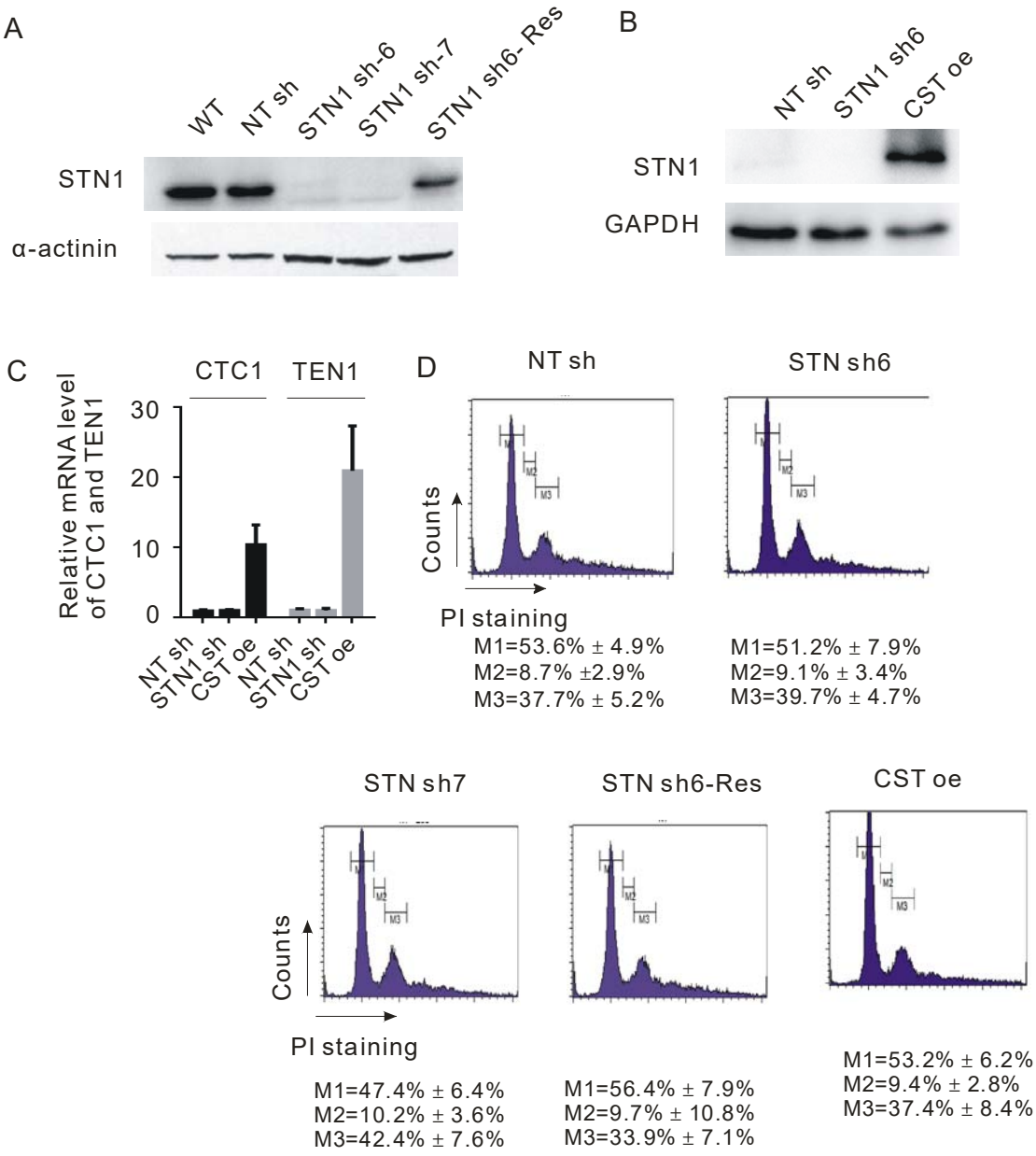
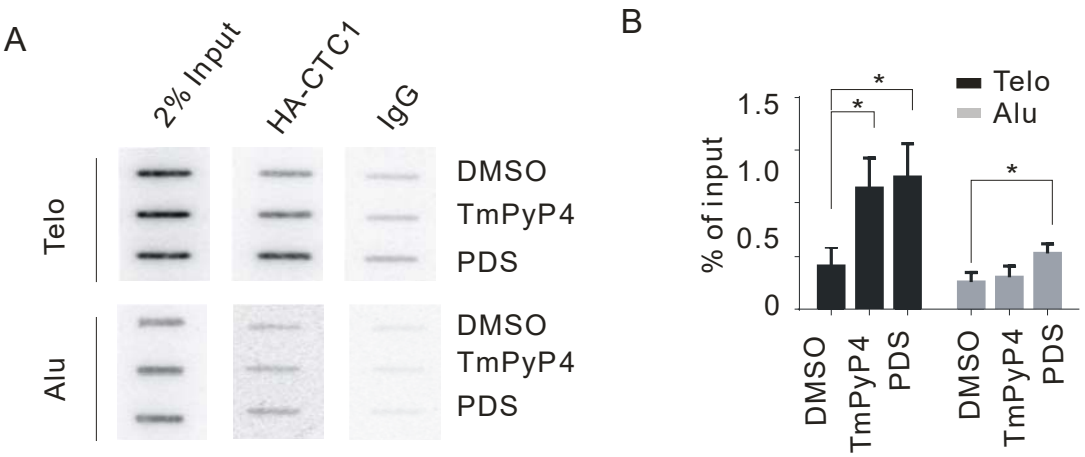


Figure S2. The expression of CST in different cell lines. (A-B) The expression of STN1 was determined by western blot using OBFC1 antibody. (C) The expression of CTC1 and TEN1 was determined by RT-PCR. (D) Cell cycle analysis was done by FACS.

Figure S3



C

		DMSO		PDS+		
		Mean	SEM	Mean	SEM	*p*-value
Telo ChIP	TPP1	0.92	0.18	1.02	0.25	0.77
	Flag-STN1	0.22	0.05	0.66	0.12	0.04
	Pol α	0.21	0.06	0.72	0.13	0.04
	Pol ε	0.39	0.16	0.74	0.10	0.15
	Pol δ	0.48	0.14	1.28	0.21	0.04
Alu ChIP	TPP1	0.38	0.12	0.45	0.16	0.75
	Flag-STN1	0.20	0.02	0.53	0.05	0.01
	Pol α	0.29	0.07	0.80	0.06	0.01
	Pol ε	0.16	0.03	0.20	0.05	0.56
	Pol δ	0.23	0.06	0.36	0.09	0.30
Tublin ChIP-PCR	TPP1	0.04	0.01	0.02	0.01	0.20
	Flag-STN1	0.02	0.01	0.02	0.01	1.00
	Pol α	0.09	0.01	0.16	0.04	0.26
	Pol ε	0.23	0.07	0.29	0.08	0.57
	Pol δ	0.17	0.04	0.17	0.06	0.97

Figure S3. CTC1 is recruited to telomeres upon G4 stabilization. ChIP was performed with HA antibody using HA-CTC1 expressing HeLa cells with and without treatment with TmPyP4 or PDS. (A) Slot blot analysis of precipitated DNA. Hybridization was with telomere probe (Telo) or Alu probe. (B) Quantification of ChIP data. Telo and Alu ChIP signals were normalized against input DNA. Data are expressed as mean ± SEM, n = 3, *P < 0.05. (C) Raw data of figure 2E. Related to Figure 2.

Figure S4

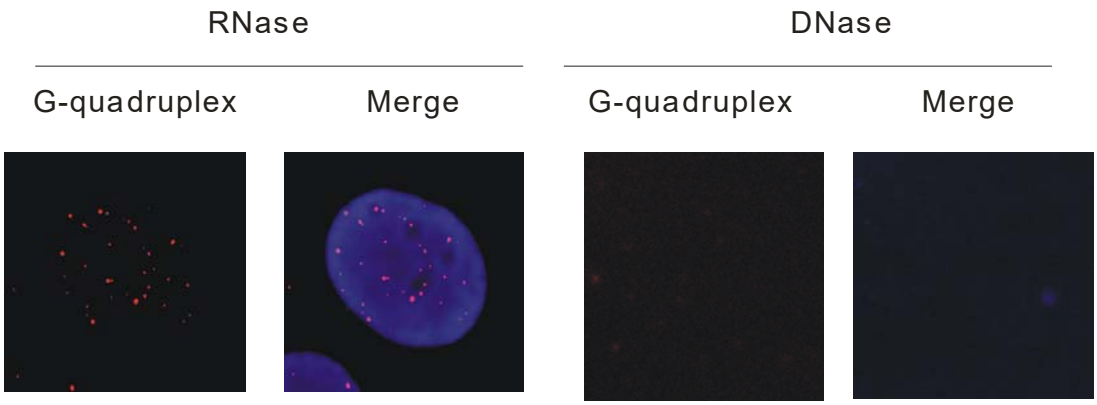


Figure S4. BG4 antibody recognizes DNA but not RNA-based G4 DNA. (A) Immunofluorescence detection of G4 (red) in HeLa cells using BG4 antibody. Cells were treated with RNase (left) or DNase (right) prior to immunolocalization and counterstained with DAPI (blue).

Figure S5

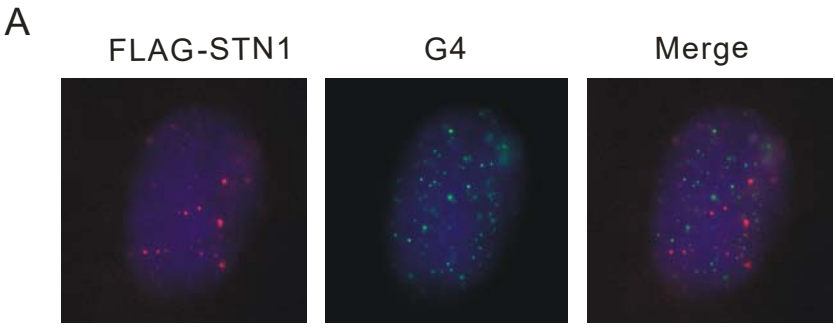


Figure S5. Representative enlarged image of FLAG-STN1 expressing cells stained with antibody to FLAG and G4. Red, FLAG-STN1; Green. G4; blue, DNA stained with DAPI. Related to Figure 3

Figure S6

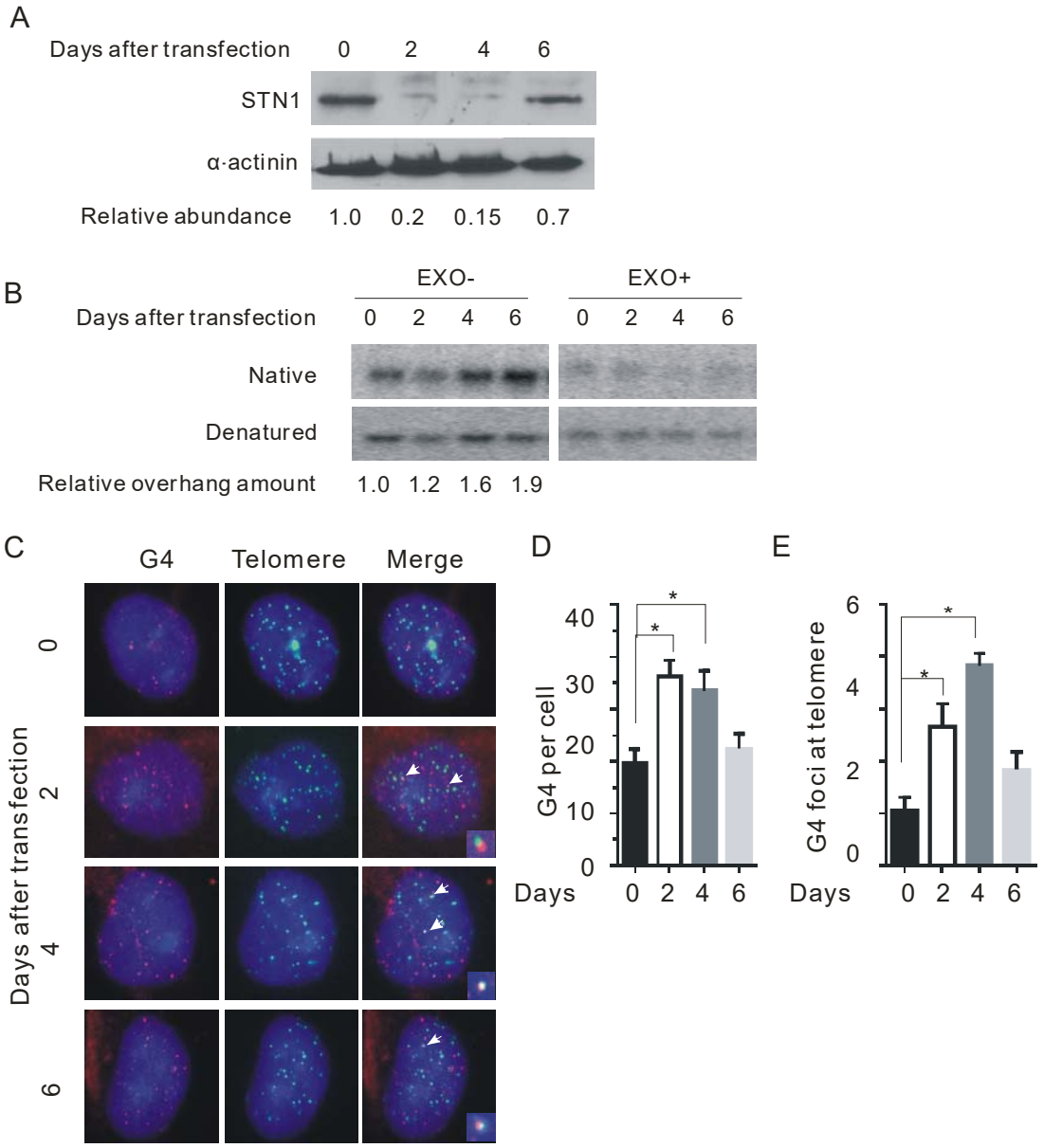


Figure S6. The increased G4 accumulation is not a result of G-overhang elongation after STN1 depletion. (A) Western blot showing STN1 knockdown by the siRNA. (B) Effect of STN1 depletion on overhang signal analyzed by in-gel hybridization (C) Localization of G4 and telomere DNA in HeLa cells at the indicated time after transfection. Red, imunolocalization of G4 DNA; Green, telomere FISH; Blue, DAPI. Arrows indicate telomere and G4 co-localization. Arrows indicate telomere and G4 co-localization. Quantification of total (D) and telomeric (E) G4 foci. Values are mean ± SEM, n = 3 experiments, * $P < 0.05$, ** $P < 0.01$.

Figure S7

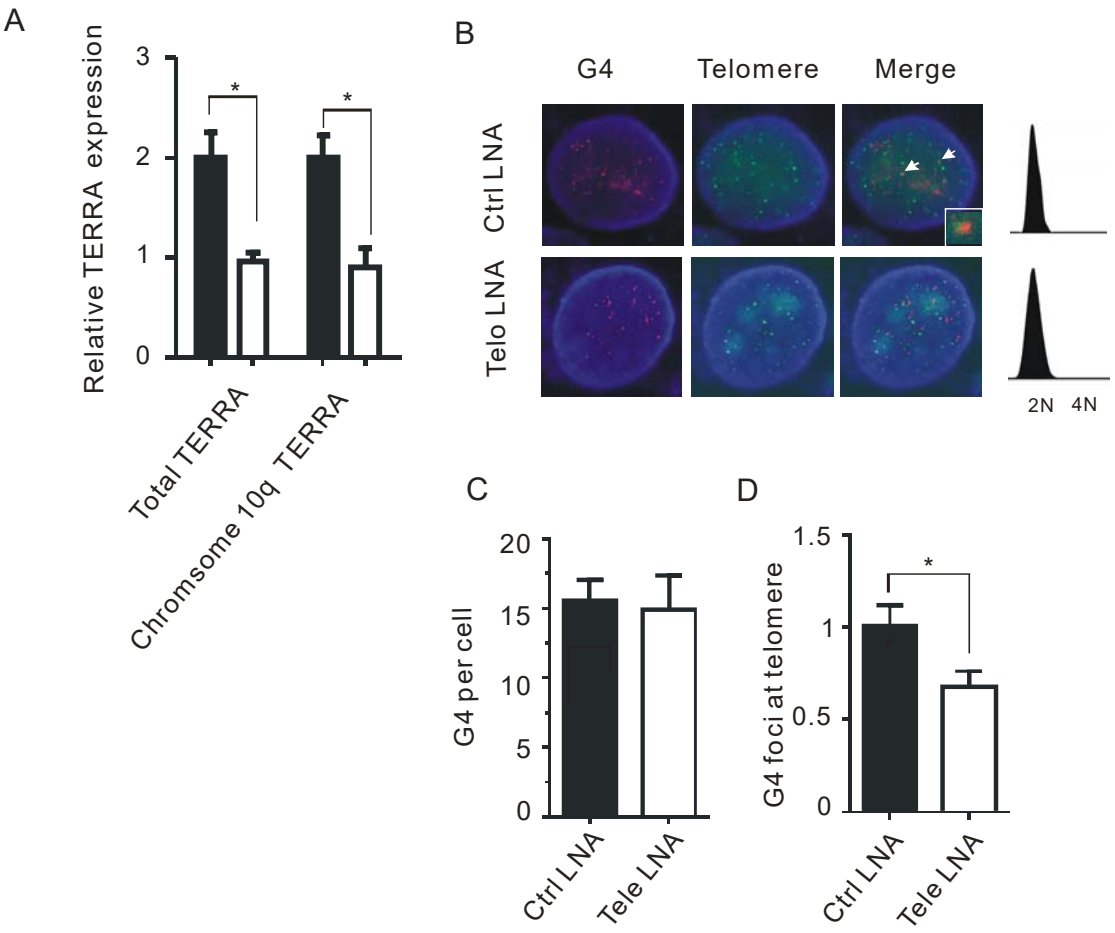


Figure S7. TERRA depletion reduces G4 formation at G1 Cells. (A) RNA was isolated from cells synchronized in G1 with a double thymidine block. The relative expression of TERRA were determined by RT-PCR. (B) Right panel: Localization of G4 and telomere DNA in HeLa cells at G1 phase. Left panel: FACS analysis showing G1 synchronization. Red, imunolocalization of G4 DNA; Green, telomere FISH; Blue, DAPI. Arrows indicate telomere and G4 co-localization. Arrows indicate telomere and G4 co-localization. Quantification of total (C) and telomeric (D) G4 foci. Values are mean ± SEM, n = 3 experiments, * $P < 0.05$, ** $P < 0.01$.

Figure S8

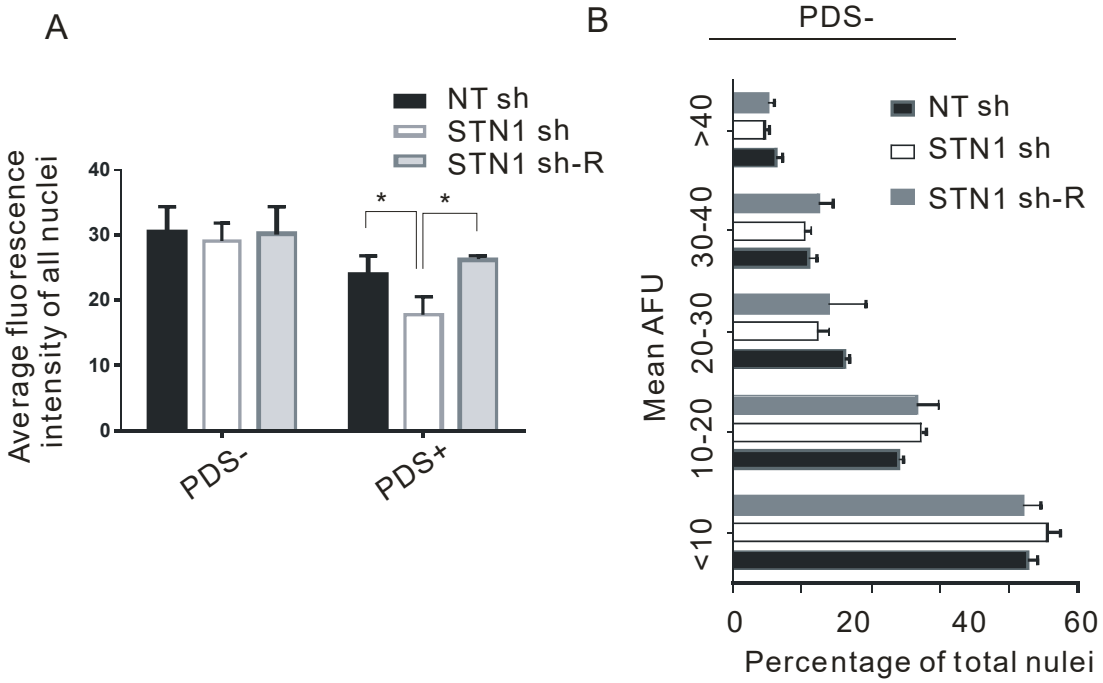


Figure S8. STN1 depletion slows bulk genomic DNA replication after G4 stabilization. (A & B) Quantification of EdU uptake based on fluorescence intensity. (A) Average fluorescence intensity values for all nuclei. The indicated HeLa cell lines were grown with/without 10 μM PDS for 24 hrs. NT sh, non target control; STN1 sh, cells depleted of STN1 with shRNA; STN1 sh-R, STN1 sh cells expressing sh-resistant FLAG-STN1. (B) Quantification of the percent of nuclei within the indicated fluorescence range. EdU labeling was in the absence of PDS treatment. AFU, arbitrary fluorescence units. Data are expressed as mean ± SEM, n = 3, *P < 0.05. Related to Figure 5.

Figure S9

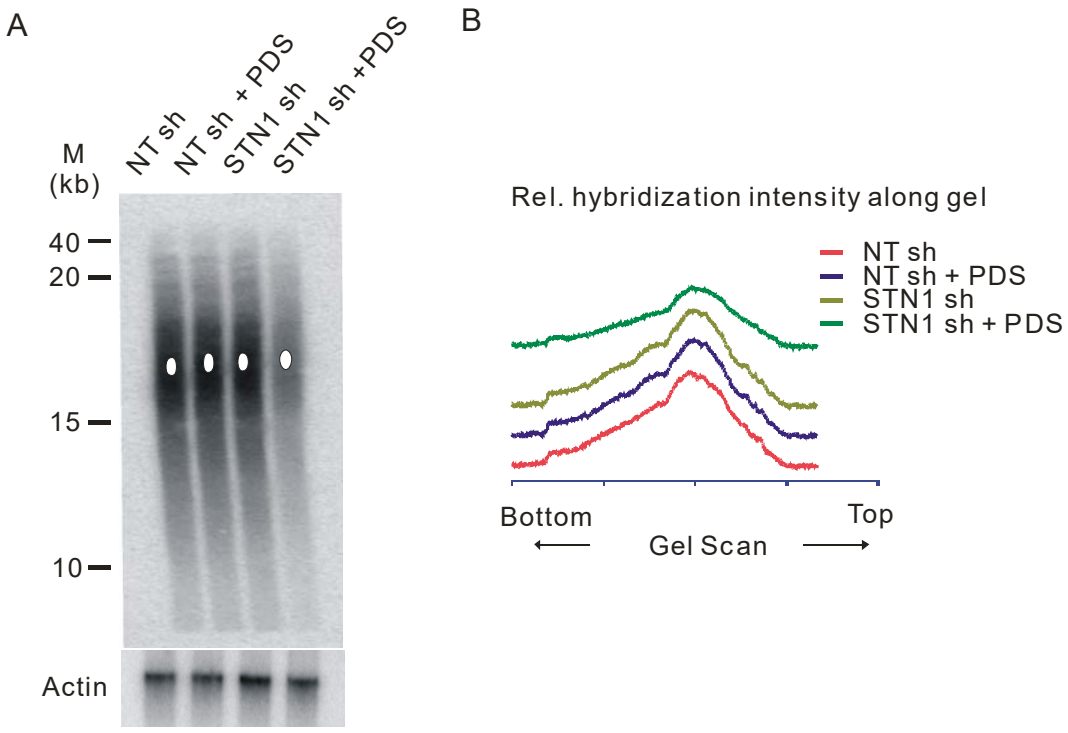


Figure S9. STN1 depletion induces telomere loss not telomere shortening after G4 stabilization. (A) Genomic DNA from pools of U2OS cells (control with NT shRNA or knockdown with STN1 shRNA) was resolved by agarose gel electrophoresis. Telomere restriction fragments were detected with ^{32}P-labeled $(TTAGGG)_3TTA$ telomere probe. M, molecular weight markers. (B) Scans showing relative intensity of hybridization signal. Scans for each sample were from top to bottom of blot. Related to Figure 6.

Figure S10

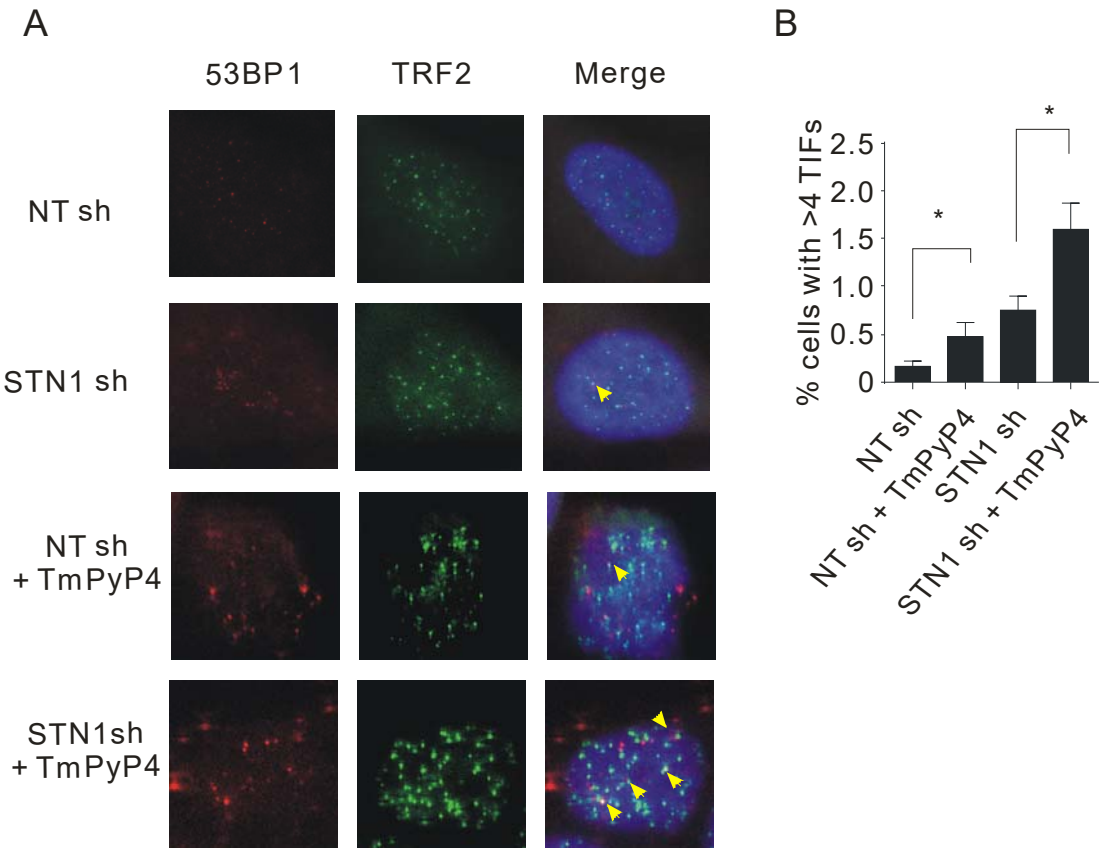


Figure S10. STN1 depletion enhances G4-induced telomere damage. (A) Immunolocalization of 53BP1 (red) and TRF2 (green) in the indicated cell lines grown with or without 50 M TmPyP4 for 48 hrs. Yellow arrowheads indicate TIFs (sites of 53BP1 colocalization with telomeres). Scale bar, 5 μm. (B) The percentage of cells with ≥4 TIFs was determined for at least 50 cells in each experiment (Mean ± SEM, n = 3 experiments, * $P < 0.05$, ** $P < 0.01$).

Figure S11

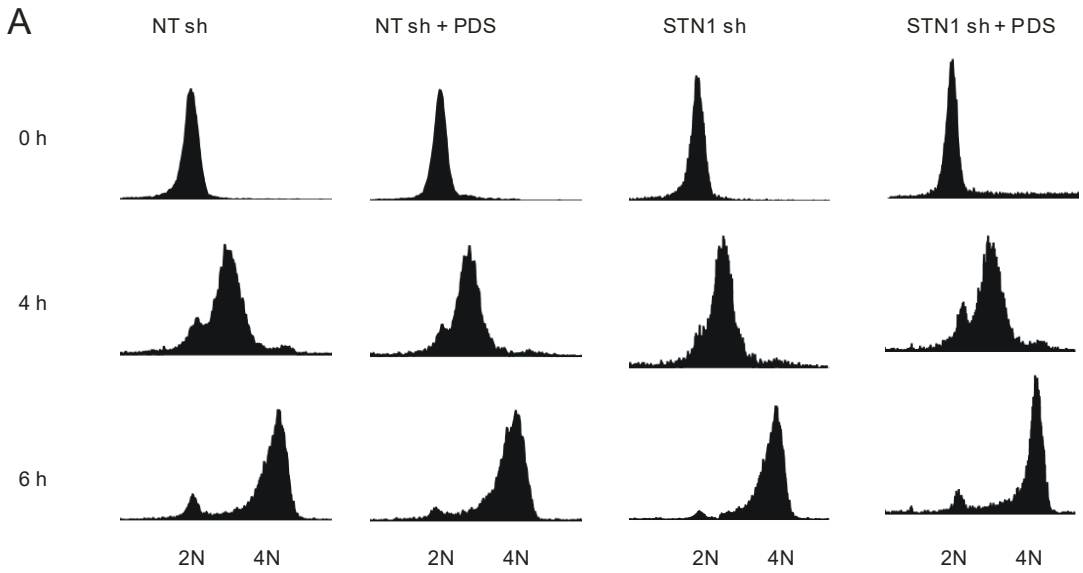


Figure S11. Synchronization of cells used to separate leading and lagging strand telomeres. (A) FACS analysis showing synchrony of cells used to collect DNA in Figure 8D.