Figure S1. Related to Figure 1

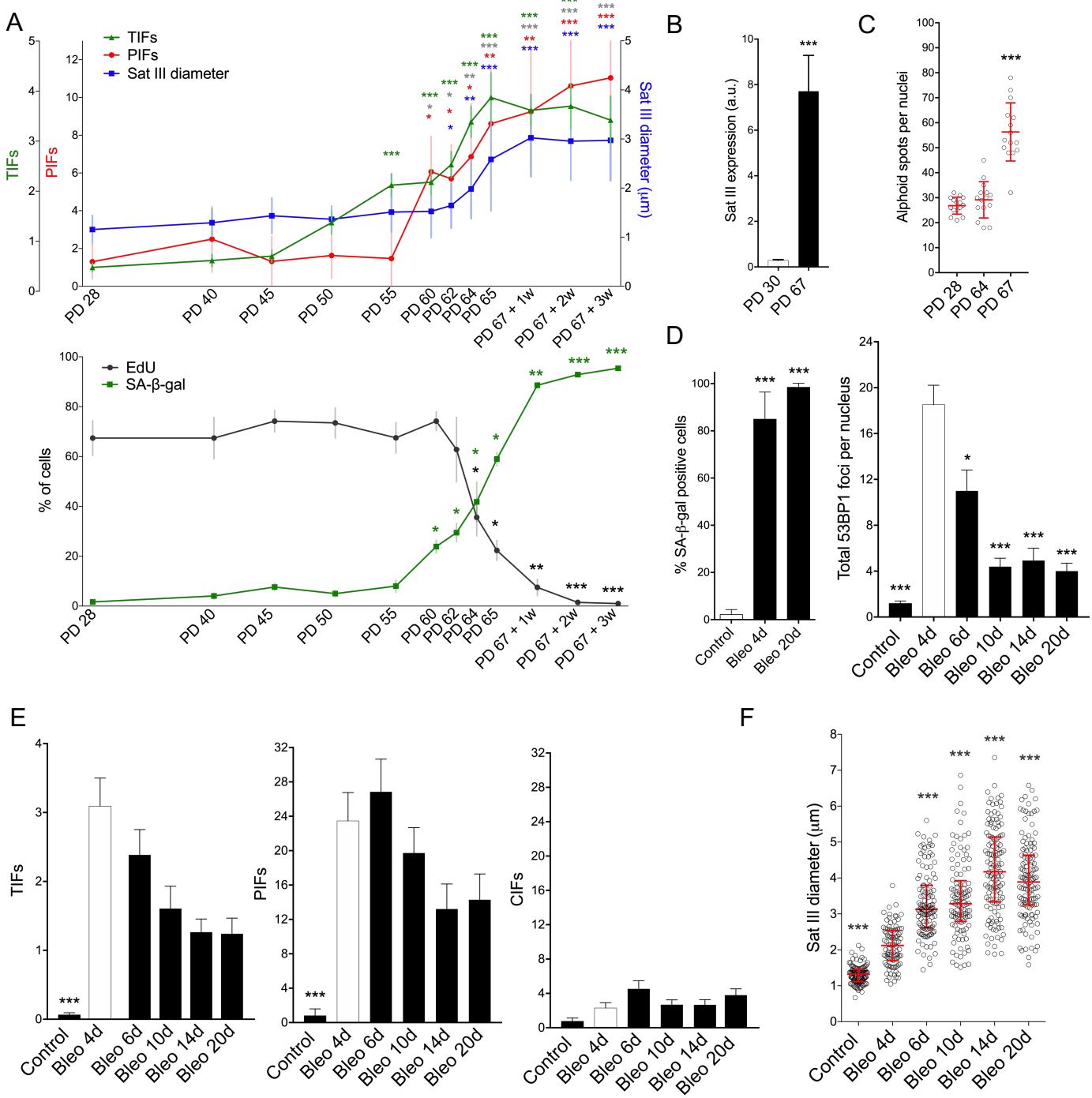

Figure S1. Pericentromeric heterochromatin is unstable during replicative senescence

(A) MRC-5 cells collected at different population doublings (PDs) until senescence. Top panel, telomeric damage (TIFs), pericentromeric damage (PIFs) and opening of Sat III (represented as the diameter of the Sat III PNA signal in μm) were estimated from immunofluorescent confocal microscopy images by the colocalization of 53BP1 antibody and a PNA probe with the corresponding repeat element. TIFs represent the number of colocalizations per nucleus, while PIFs show the percentage of PNA signal colocalising with 53BP1. Bottom panel shows the percentage of cells incorporating EdU (1 μM for 24 h) and the percentage of cells positive for senescence associated β-galactosidase. Data represent mean ± SD of three biological replicates. Statistical analyses were performed using the Kruskal-Wallis test (*P < 0.01; **P < 0.001; ***P < 0.0001). (B) Sat III expression of young and senescent MRC-5 cells was performed by qPCR. Mean ± SD of three biological replicates is shown. Statistical analyses were performed using the Mann-Whitney U-test (***P < 0.0001). (C) Number of alphoid spots per nucleus of three independent experiments. Error bars indicate mean ± SD. (D) Young MRC-5 PD 28 were treated with 10 μg/ml bleomycin for 4 days. After that time, fresh media without bleomycin was added to the culture and kept for further 20 days. The percentage of cells displaying the SA-β-galactosidase marker and the mean 53BP1 foci per nuclei was estimated. (E) TIFs, PIFs and CIFs and Sat III opening (F) of the conditions described above. Data represent mean ± SD of three biological replicates. Statistical analyses were performed using the Kruskal-Wallis test (***P < 0.0001).

Figure S2. Related to Figure 1 and 2

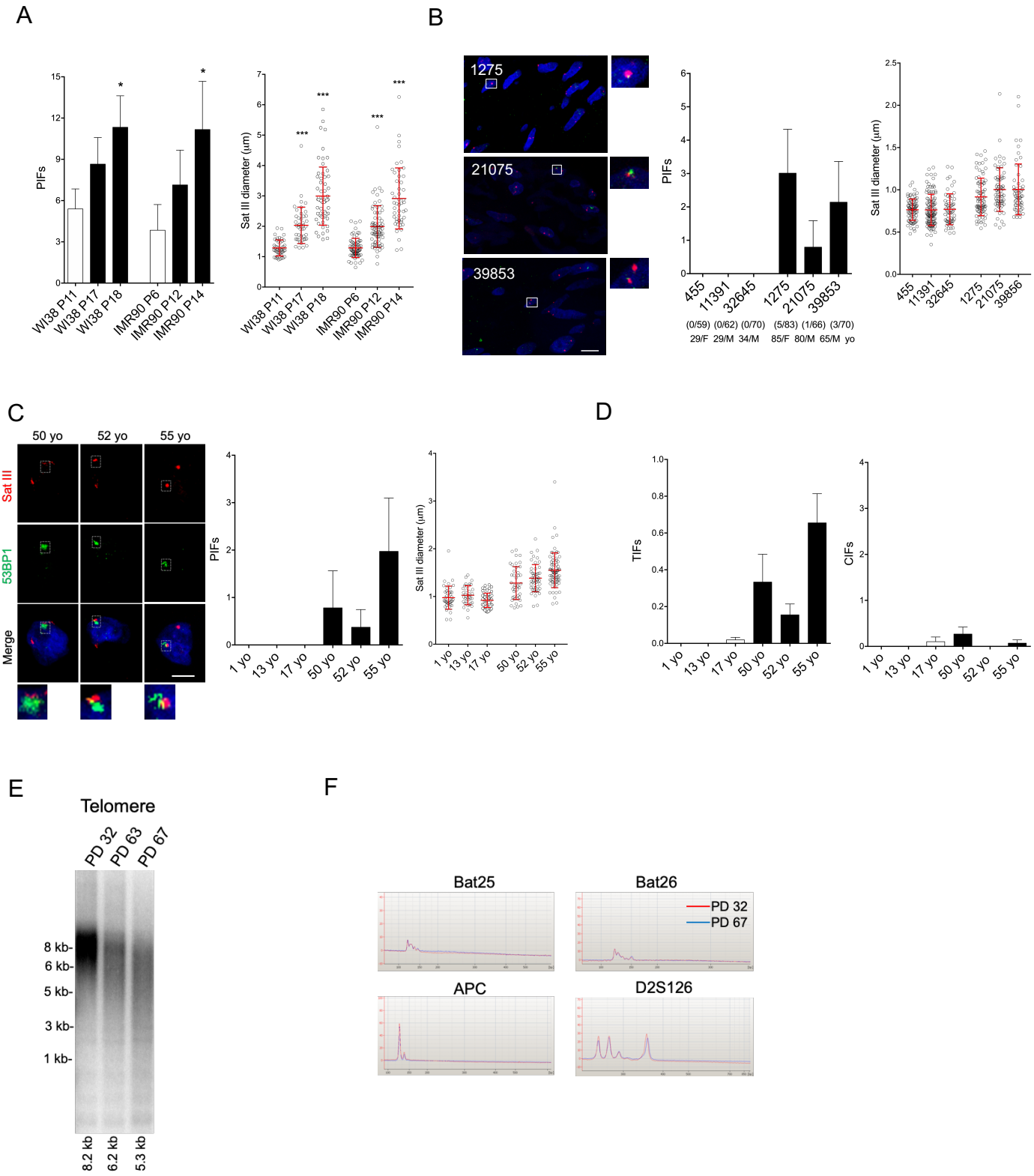

Figure S2. Pericentromeric instability is a hallmark of senescence
(A) PIFs and Sat III opening of WI-38 and IMR-90 human fibroblasts. Senescence phenotype was evident at passage 18 (P18) and 14 (P14) respectively. (B) Immuno-histochemistry of dermal skin stained with a Sat III PNA probe in red and the 53BP1 protein in green. Graphs show the number of PIFs and Sat III opening. The number of colocalization events and fibroblast analysed are indicated in parenthesis; the age and sex of the donor is shown at the bottom of the graph. Error bars represent mean ± SD. (C) PIFs, Sat III opening and (D) TIFs and CIFs of mesenchymal stem cells of different ages. Data represent mean ± SD of at least 80 cells. (E) Telomere length analysis of young (PD 32), pre-senescent (PD 63) and senescent (PD 67) cells digested with *Hinf*I and *Rsa*I. The size of the main intensity peak is indicted at the bottom of the gel. (F) Size distribution of microsatellites from young and senescent cells using a Bioanalyzer instrument. yo = year old. Scale bar = 10 μm.

Figure S3. Related to Figure 3

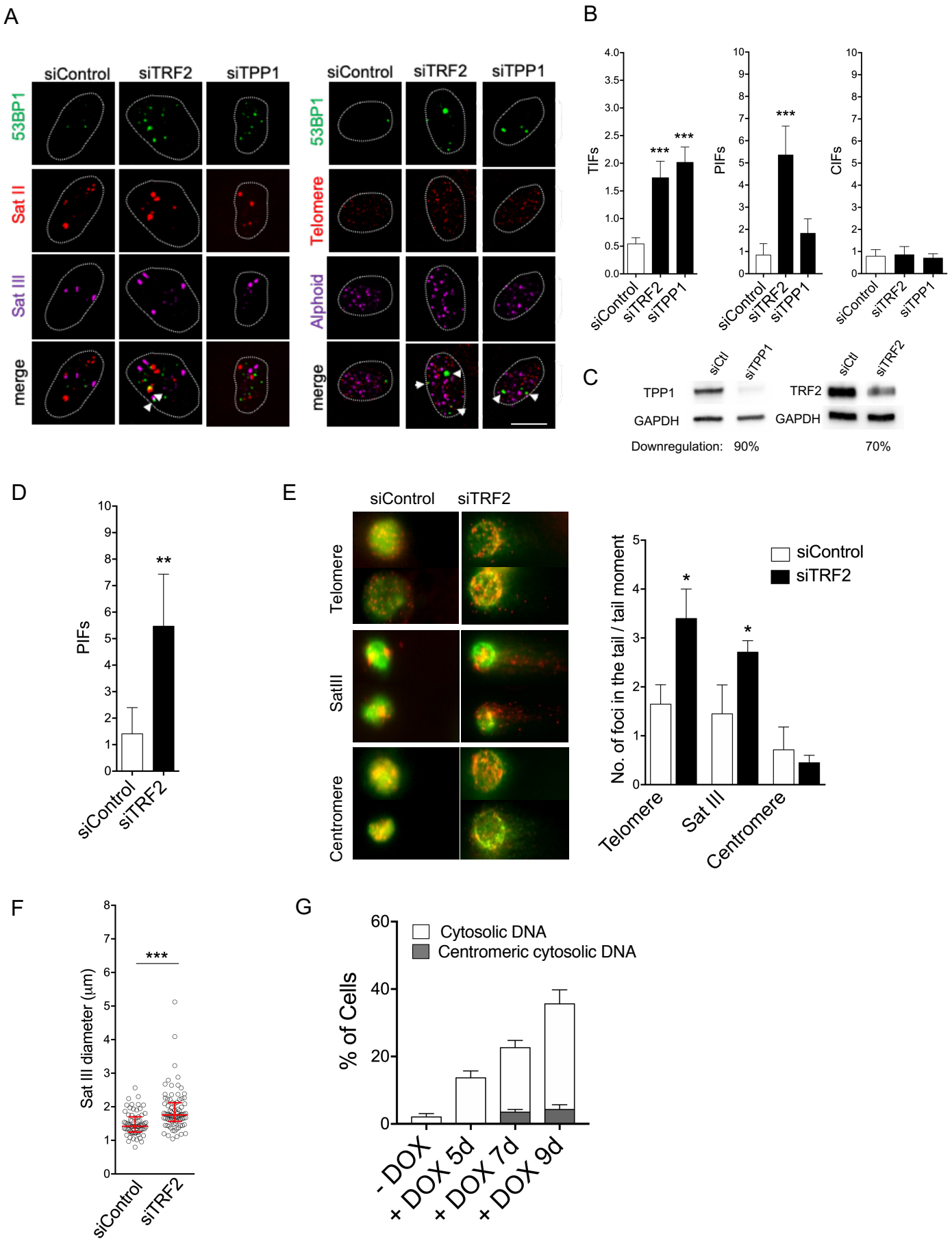

Figure S3. TRF2 controls PCH stability

(A) Representative confocal images and (B) quantification of telomere (TIFs), pericentromeric (PIFs) and centromeric (CIFs) damage in young (PD 28) MRC-5 cells. Cells were treated for three days with the indicated siRNA. PIFs was measured by combining Sat II and Sat III PNA probes. Data represent mean ± SD of three biological replicates. Statistical analyses were performed using the Kruskal-Wallis test (***$P < 0.0001$). Scale bar = 10 μm. (C) Western blotting of MRC-5 cells transfected with the indicated siRNA for 72 h and corresponding to the experiment in *B*. (D) PIF quantification of young MRC-5 cells with TRF2 depletion by siRNA. (E) Neutral comet assay of MRC-5 cells (PD 28) with three-day incubation of siControl or siTRF2. DNA was visualized with YOYO-1 fluorescent dye (green) whilst the PNA staining is shown in red. Quantification was performed by counting the number of PNA foci in the tail of the comet normalized by the tail moment (tail length × DNA signal in the tail/total DNA signal. Error bars represent SEMs of at least 80 cells. *$P < 0.01$; two-tailed Student's *t*-test. (F) Compaction of Sat III. Error bars represent mean ± SD of three independent experiments. Statistical analyses were performed using the Mann-Whitney U-test (***$P < 0.0001$). (G) Cytosolic quantification of alphoid DNA in HeLa cells with TRF2 downregulation. Cells were incubated with doxycycline (DOX) at a final concentration of 1 μg/ml for 7 or 9 days to induce the expression of an shRNA against TRF2. Error bars represent mean ± SD of three independent experiments. Scale bar = 10 μm.

Figure S4. Related to Figure 4

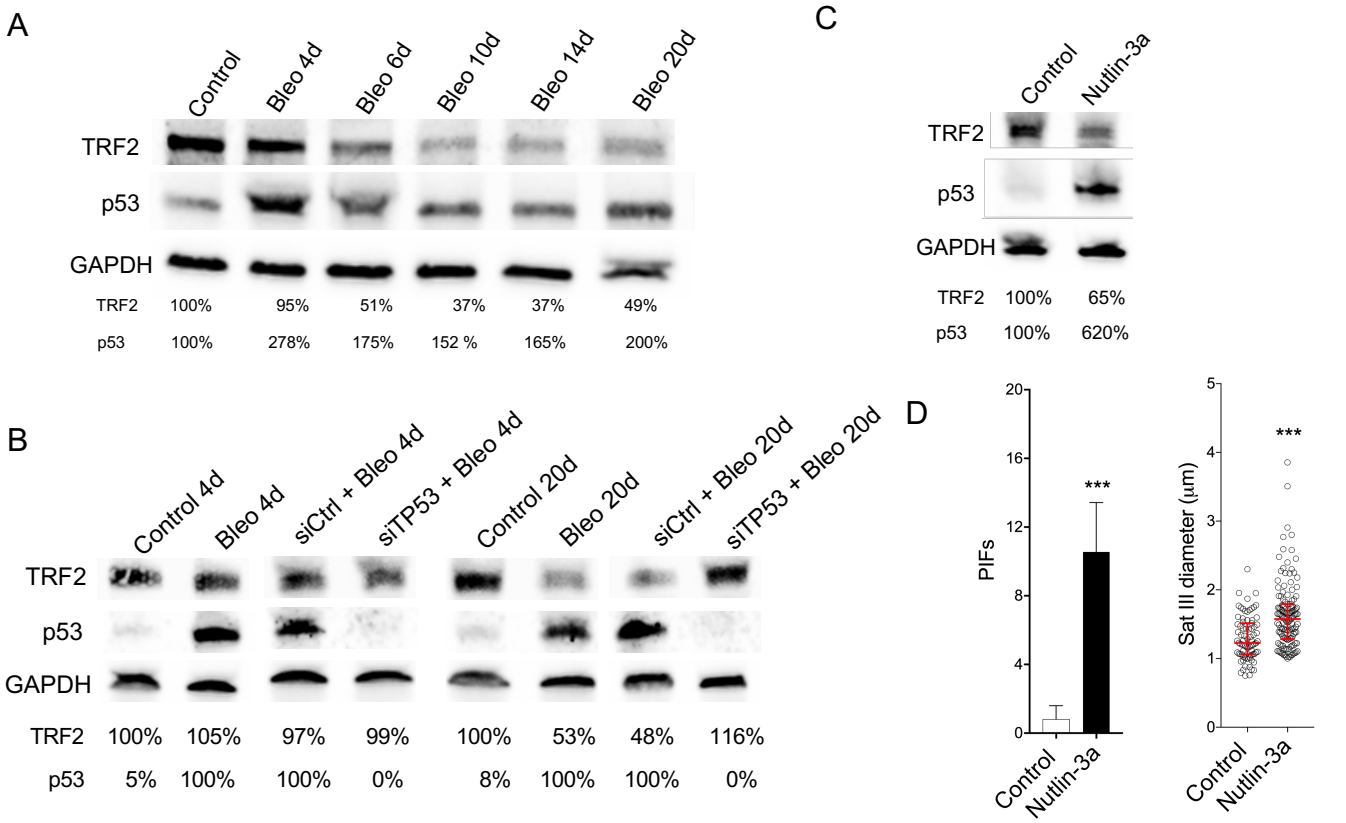

Figure S4. TP53-dependent downregulation of TRF2

(A) Western blotting showing the expression of p53 and TRF2 in MRC-5 cells treated with bleomycin as indicated in Figure 1SD and (B) with the indicated siRNA transfections for 72 h. (C) Expression of TRF2 and p53, (D) PIFs and Sat III opening of young MRC-5 cells (PD 30) treated with 10 μM Nutlin-3a for 48 h. Error bars represent mean ± SD of three independent experiments. Statistical analyses were performed using the Mann-Whitney U-test (***$P < 0.0001$).

Figure S5. Related to Figure 5

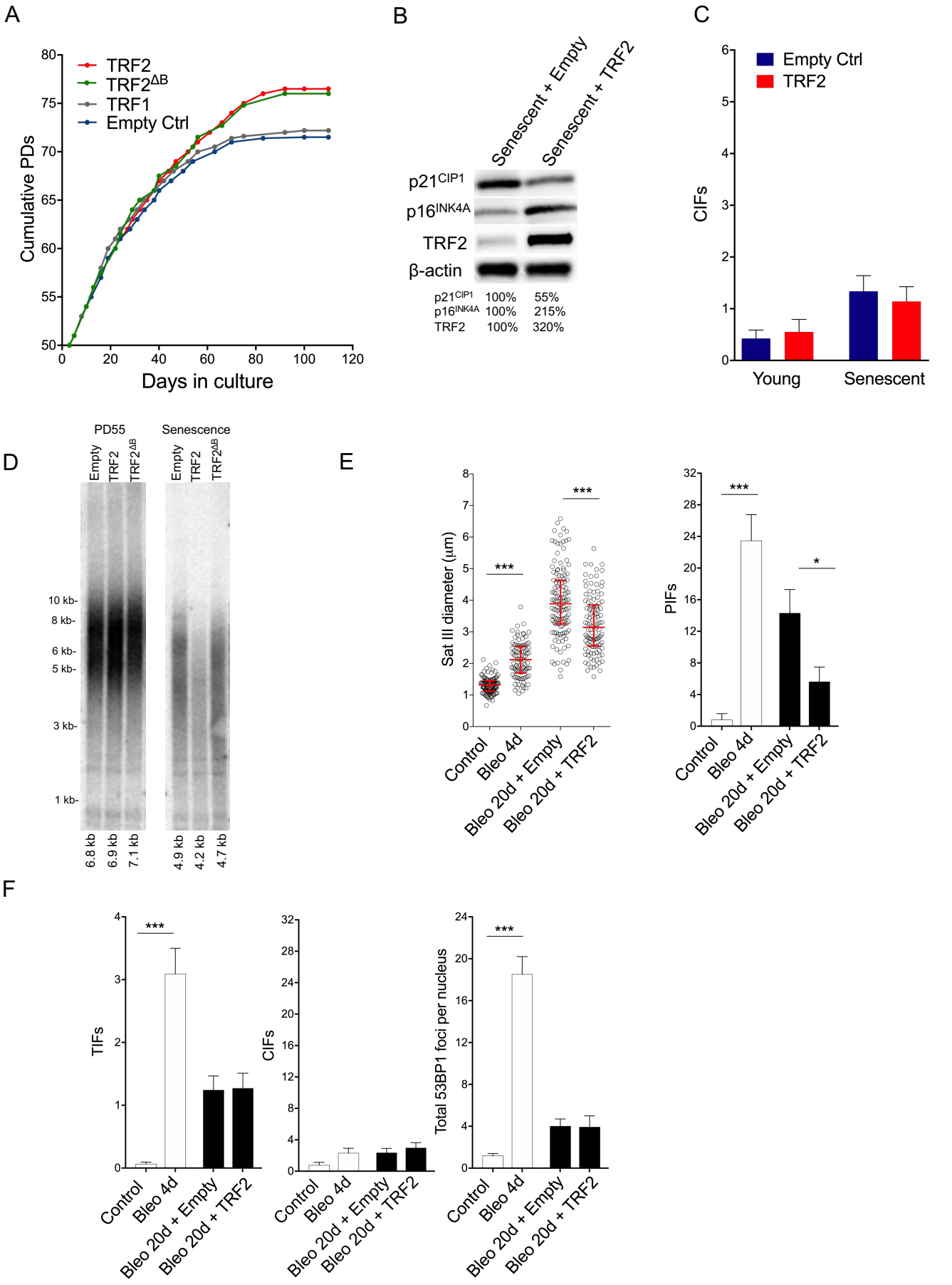

Figure S5. TRF2 is required for the stability of PCH during replicative senescence

(A) Growth curve of MRC-5 cells transduced at PD 50 with lentivirus containing an empty vector, TRF1, the full-length TRF2 or the truncation TRF2$^{\Delta B}$ form. Cells entered into senescence at PD 71 for control and TRF1 transduced cells and at PD 75 for TRF2 and TRF2$^{\Delta B}$ cells. The cells were allowed to grow until senescence at 5% oxygen. (B) Western blotting of senescent cells expressing TRF2 or a control sequence. (C) CIF analysis of control and TRF2-transduced cells. (D) Telomere length analysis of MRC-5 cells at PD 55 and at senescence. The size of the main intensity peak is indicted at the bottom of the gel. Error bars show the mean ± SD of three independent experiments. (E) Opening, PIFs, (F) TIFs, CIFs and total damage of young MRC-5 cells exposed to 10 μg/ml bleomycin for 4 days follow by 20 days with fresh media without bleomycin. Transduction with an empty vector or TRF2 expressing vector was performed after the 4 days of bleomycin treatment.

Figure S6. Related to Figure 5

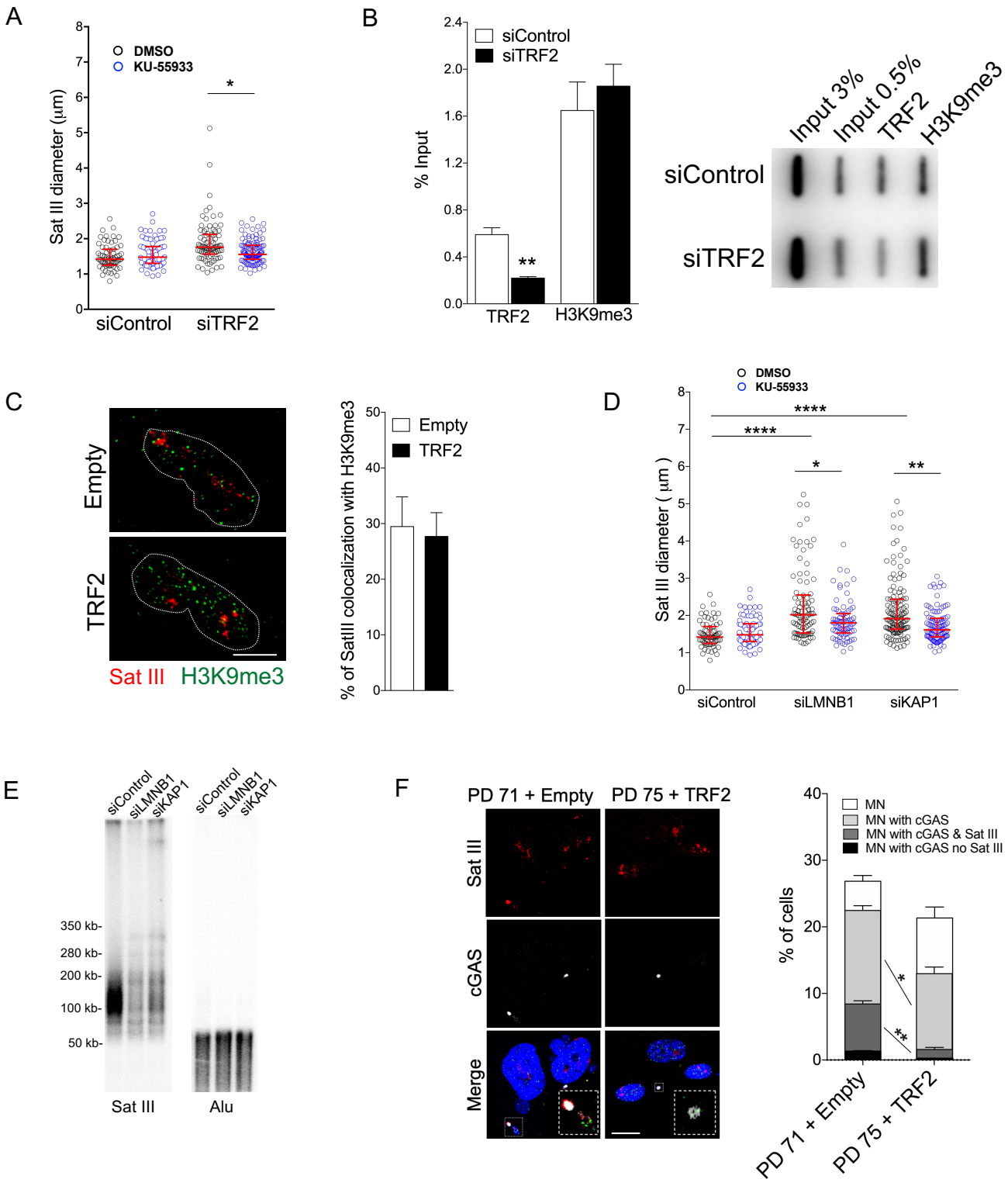

Figure S6. ATM activation is important for pericentromeric DNA opening
(A) Sat III opening of young MRC-5 exposed to 72 h with the indicated siRNA. KU-55933 was added to the media one day before the cells were harvested at a final concentration of 10 μM. (B) Slot blot showing ChIP experiments performed in TRF2 depleted MRC-5 cells. The membrane was hybridized with a Sat III probe. Enrichment in relation to the signal of the input is shown, error bars represent SEM of 3 biological replicates. (C) Sat III PNA probe colocalizing with H3K9me3 in MRC-5 senescent cells expressing TRF2 or an empty vector control. Error bars depict SD of n=3; scale bar = 10 μm. (D) Sat III opening of young MRC-5 (PD 30) exposed to 72 h with the indicated siRNA. KU-55933 was added to the media 24 h before harvesting at a final concentration of 10 μM. Data represent mean ± SD of three biological replicates. Statistical analyses were performed using the Kruskal-Wallis test (*P < 0.01; **P < 0.001; ****P < 0.00001). (E) Size distribution of young MRC-5 cells exposed to 72 h siRNA incubation. (F) Confocal images of cells stained with cGAS and a Sat III probe in MRC-5 senescent cells transduced with TRF2-expressing lentivirus or control. The percentage of nuclei containing at least one micronucleus (MN) is shown. Data represent mean ± SD of three biological replicates.

Figure S7. Related to Figure 6

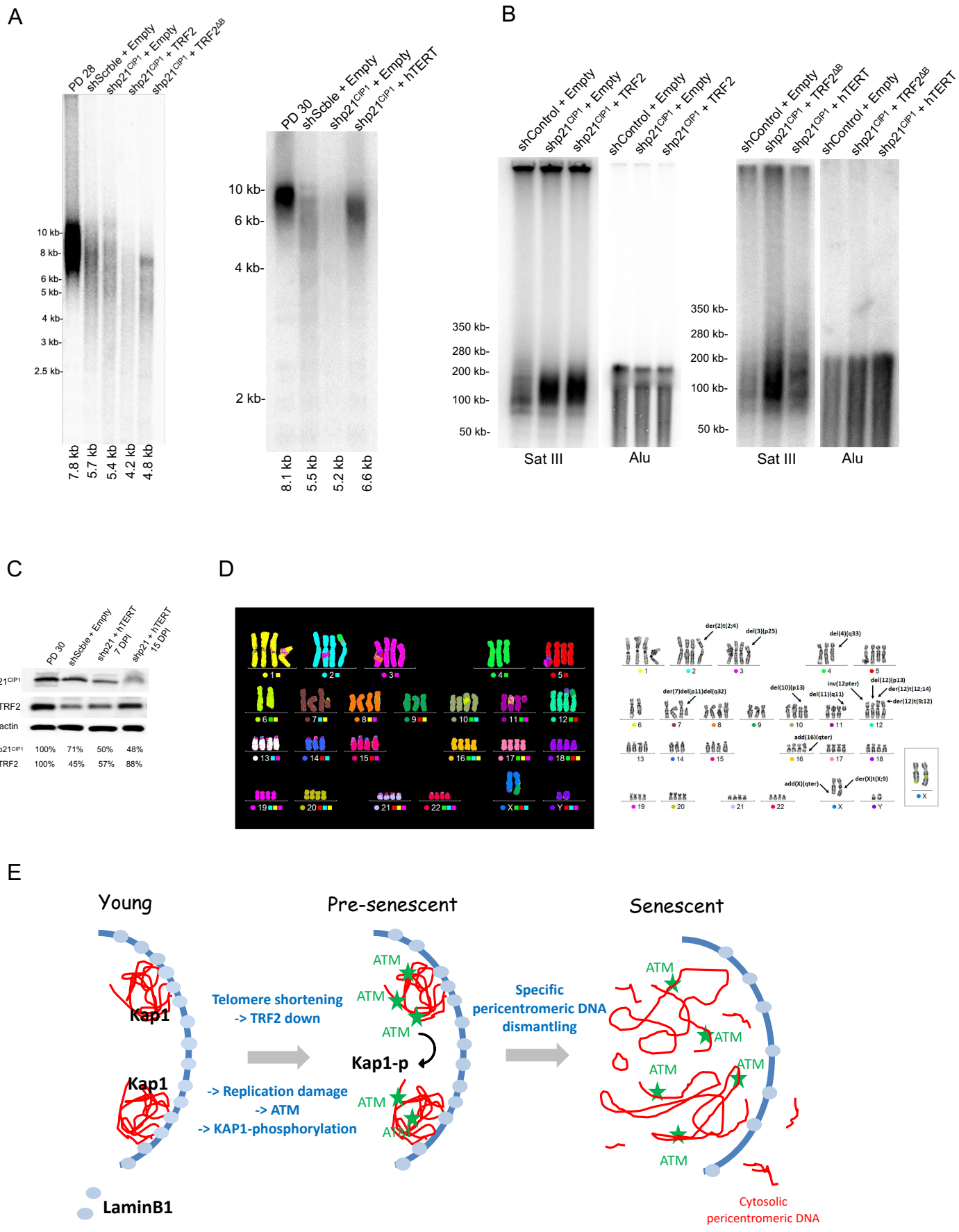

Figure S7. PCH stability in post-senescence cells

(A) Telomere length analysis and (B) pericentromeric size distribution by Southern blotting of post-senescent cells transduced with the full length TRF2, the truncated form TRF2$^{\Delta B}$ or hTERT and control conditions. Cells were collected 10 days post-infection. The size of the main intensity peak for telomere length is indicted at the bottom of the gels. (C) Immunoblotting of post-senescent MRC-5 cells expressing hTERT at different days post-infection (DPI). Protein levels were calculated in relation to young MRC-5 cells (PD 30). (D) Example of a metaphase spread from shp21^{CIP1} + Empty transduced cells. Identification of chromosome aberrations assessed by combining multi-color FISH staining (left panel) together with Inverted DAPI-Banding (right panel) is indicated with arrows. PCH rearrangements involving both homologues of chromosome X in the same metaphase spread stained with alphoid pancentromeric probe (green-FITC) and Satellite III probe (red-Cy3) (small box). (F) Model of specific PHC dismantling at senescence onset.