

Supplemental figure legends

Figure S1

(A) Schematic representation of the Tin2DN construct. The CMV promoter is controlled by a Tetracycline operator (TetO) for expression of Tin2DN and reporter gene eGFP, contained in an IRES bicistronic vector. (B) HCA2 fibroblasts expressing inducible Tin2DN treated with (+) or without (-) Dox for 48 h. Western blot of whole cell lysates for Tin2, GFP and GAPDH (housekeeping) proteins. (C-G) HCA2 expressing inducible Tin2DN, p16 or TetR alone (CTRL) treated with Dox. (C) Merged immunofluorescence images of representative cells at each time point, taken with a DAPI (blue, nuclear counterstain) or a FITC (for GFP emission) filter. Scale bar: 20 μm . (D) Representative Tin2DN-induced stained cells for γH2AX (green), 53BP1 (red) and DAPI (blue). Graph shows percentage of cells showing more than three γH2AX and 53BP1 positive foci after 96 h induction. Scale bar: 20 μm . (E) Graph shows percentage of SA-B-GAL positive cells represented in left panel. Scale bar: 200 μm . (F) Representative images of HCA2 cells expressing histone H2B-GFP and cultured in the live cell imaging system (Incucyte). Scale bar: 200 μm . (G) Number of DDF per single cells after indicated time of Tin2DN induction. After 48 h of induction, almost all cells present at least one foci. All quantified data are the mean \pm S.D of triplicates and represent at least three independent experiments. NS: non-significant. Two-way ANOVA: * $p < 0.05$; ** $p < 0.01$.

Figure S2

BJ cells expressing inducible shGFP (control), shTin2, shTRF2 or shPOT1 were treated with Dox. (A, B) Percentage of EdU positive cells from 24 h EdU pulses. (C) After 48 h with (+) or without (-) Dox, whole cell lysates were analyzed by western blotting for Tin2 and GAPDH proteins. (D) After 48 h Dox induction (ON) or without (OFF), extracted RNA was reverse-transcribed for Sybr qPCR using TRF2 and POT1 primers. Results are compared to the control shGFP (no induction; OFF) and presented in a log₂ scale. (E) Cells were fixed and assessed for SA-B-GAL. Scale bar: 200 μm . All quantified data are the mean \pm S.D of triplicates and represent at least three independent experiments. Two-way ANOVA: ** $p < 0.01$.

Figure S3

BJ cells expressing inducible Tin2DN, shGFP, shTin2, shTRF2 or shPOT1 were treated with Dox. (A) DAPI stained cells showing genomic instability (mitotic catastrophes, chromosome bridges or micronuclei) after 144 h of Tin2DN induction; white arrows point out aberrations. Scale bar: 20 μm . (B) Cells expressing H2B-GFP cultured for live cell imaging (Incucyte). Normal mitosis is shown (32h30) preceding two latent metaphases (108h and 162h15) leading to genomic instability as chromosome bridges and micronuclei, respectively. Three different cells are shown (1st, 2nd and 3rd). NEB: Nuclear Envelope Breakdown. ANA: Anaphase. TELO: Telophase. Scale bar: 20 μm . (C) Representative images of cells stained for DAPI and telomeres probed using tFISH (green). For the indicated PDs or indicated induction, the mean telomeric fluorescence intensity per cells was measured and reported in the graph. Scale bar: 20 μm . (D) HCA2 cells were cultured to replicative senescence (young versus old left panels) or Tin2DN was induced with Dox for up to 7 d (right panels). Representative southern blot of telomeres restriction fragments length assays is presented with DNA molecular weight labels. Telomere length distributions was quantified in the gel lanes and is presented graphically. bp : base pair. (E-H) Tin2DN was induced with Dox for only 120 h (OFF 120 h) or sustained throughout the experiment. (E) Representative images of cells

stained for 53BP1 (red) and DAPI (blue), and telomeres probed using tFISH (green). Co-localisation of probes is defined as a TIF. Fluorescence intensity for the signal of 53BP1 and the telomere was linearly assessed between a and b. Scale bar: 10 μ m. **(F)** Graph shows the percentage of SA-B-GAL positive cells represented in panel. Scale bar: 200 μ m. **(G, I)** Cells were stained for 53BP1 and DAPI. Mean number of 53BP1 foci per nuclei is shown. **(H, J)** Percentage of DAPI stained cells showing genomic instability. All quantified data are the mean \pm S.D of triplicates and represent at least three independent experiments. NS: non-significant. Two-way ANOVA: ** $p < 0.01$.

Figure S4

(A-E) HCA2 cells expressing inducible Tin2DN and shGFP or shp53, or only with inducible p16. **(B)** Following 4 h with (+) or without (-) 4 Gy of X-ray radiation and treatment with Ku-55933 (ATMi), cells were lysed. **(C)** Following 4 h with (+) or without (-) the indicated radiation or treatment duration with Dox, cells were lysed. **(C-D)** Cells expressing only Tin2DN were treated with 10 μ M of Ku-55933 (+), or solvent (1:1000 DMSO) (-). **(A-C)** Western blots of whole cell lysates for p53 and GAPDH (housekeeping), and for **(B-C)** DDR-activated proteins pATM (S1981-ATM) and pCHK2 (Thr68-Chk2) as well as ATM and CHK2 proteins. **(D)** Following 72 h with (ON) or without (OFF) induction, cells were assessed for cell viability by DRAQ7 and flow cytometry. Gates were defined to include all the dead cells (DRAQ7 positive) which were not debris. **(E)** Cells were treated 72 h with Dox (ON/OFF) and with 100 ng/mL of nocodazole 4 h prior to fixation. Suspended cells were spread on glass slides, stained with DAPI (red) and telomeres were probed using tFISH (green). Graph shows percentage of cells in metaphase showing at least one end-to-end fusion. Panel shows representative cells with indicated double centromeres (white arrowheads). Scale bar: 5 μ m. **(F)** Cells expressing H2B-GFP and shp53 were cultured for live cell imaging (Incucyte) during Tin2DN induction. Individual cells undergoing defective mitosis after Tin2DN induction (two different cells; 1st and 2nd). NEB: Nuclear Envelope Breakdown. ANA: Anaphase. TELO: Telophase. Right panel shows duration of each mitosis observed at single cell level, resulting in a linear correlation. Circular diagrams indicate percentage of abnormal mitosis detected. Scale bar: 20 μ m. All quantified data are the mean \pm S.D of triplicates and represent at least three independent experiments. NS: non-significant. Two-way ANOVA: ** $p < 0.01$.

Figure S5

(A) BJ cells expressing inducible Tin2DN were cultured in serum starvation media (0.1% FBS) or in standard proliferative condition (8% FBS), and treated with Dox. At indicated times, cells were assessed for SA-B-GAL. **(B)** BJ cells expressing inducible shGFP, shTin2, shTRF2 or shPOT1 were treated 120 h with Dox (ON/OFF) and with 100 ng/mL of nocodazole 4 h prior to fixation. Panel shows representative cell spreading. Cells were stained with DAPI (red) and telomeres were probed using tFISH (green). Asterisks denote sister-chromatid fusions. Scale bar: 20 μ m. **(C-H)** BJ cells expressing inducible Tin2DN and shGFP, shRad51, shRPA, shKu80 or shLigase IV. **(C, D)** Western blot of whole cell lysates for Rad51, RPA, Ku80, Ligase IV and GAPDH (housekeeping) proteins. **(E)** Cells were irradiated with 1 Gy dose and fixed 6 h after treatment. **(E, F)** Cells were stained for 53BP1 and DAPI to quantify the mean number of 53BP1 foci per nuclei. **(F, G)** Cells were treated with Dox and fixed at indicated time points. **(G)** Percentage of DAPI stained cells showing genomic instability at indicating time points. **(H)** At indicated times, cells were assessed for neutral comet assay (detection of DSBs). Graph shows the percentage of comet tail DNA represented in panel. Scale bar: 200 μ m. All quantified data are the mean \pm S.D of

triplicates and represent at least two independent experiments. NS: non-significant. Two-way ANOVA: * $p < 0.05$; ** $p < 0.01$.

Figure S6

(A) BJ cells expressing inducible Tin2DN and shGFP, shRad51, shRPA, shKu80 or shLigase IV. Representative BJ cells during Tin2DN induction are shown (top panels). Cells stained for 53BP1 and DAPI were quantified for mean number of 53BP1 foci per nuclei (bottom panel). Telomeres were probed using tFISH (green). Foci that co-localised with telomeric probes were TIF and those that did not were non-TIF. Scale bar: 20 μm . All quantified data are the mean \pm S.D of triplicates and represent at least two independent experiments. NS: non-significant. Two-way ANOVA: ** $p < 0.01$.

Figure S7

(A, B) BJ cells were continuously cultured in DMEM media containing 8% FBS. PD and doubling time (DT) were assessed for five distinct populations, resulting in a final selection of four different populations (PD20, PD50, PD60 and PD70) with different SA signatures. (C) Cells were cultured for live cell imaging (Incucyte) to determine the proliferative rate via cell confluence reported against time 0. (D) For indicated PDs, cells were assessed for SA-B-GAL. Percentage of positive cells (divided into low or high expression categories) is reported. (E) For indicated PDs, cells were assessed for neutral comet assay (detection of DSBs). Graph shows percentage of comet tail DNA represented in panel. Scale bar: 200 μm . All quantified data are the mean \pm S.D of triplicates and represent at least two independent experiments. NS: non-significant. Two-way ANOVA: * $p < 0.05$; ** $p < 0.01$.

Figure S8

(A-B) Cells were treated with 10 μM of BrdU 24 or 72 h prior to fixation. Genomic double-stranded DNA was stained for BrdU, 53BP1 and DAPI. At indicated amounts of foci, percentage of BrdU positive and negative cells was assessed at each PD. (C-D) BJ cells expressing shGFP or shp53 were cultured for 2 days prior to experiments. Cell viability was assessed by DRAQ7 and flow cytometry. (C) Representative flow cytometry panels in which gates were defined to include all dead cells (DRAQ7 positive) that were not debris. (D) Percentage of live cells (DRAQ7 negative) is reported for each PD. All quantified data are the mean \pm S.D of triplicates and represent at least three independent experiments. NS: non-significant. Two-way ANOVA: ** $p < 0.01$.

Supplemental information

Supplementary Table 1. shRNA target sequences

| Target gene symbol | Description | Sequences* |
|--------------------------------|------------------------------------|-----------------------------|
| <i>RAD51</i> (Rad51) | Rad51 recombinase | 5'-TAGTCTGTTCTGTAAAGGGCG-3' |
| <i>RPA1</i> (RPA) | Replication protein A1 | 5'-TTCCCAATCTTTAATCCAACG-3' |
| <i>TP53</i> (p53) | Tumor protein p53 | 5'-GACTCCAGTGGTAATCTACT-3' |
| <i>XRCC5</i> (Ku80) | X-ray repair cross complementing | 5'-TTACTCATGGTAAAGCCCACG-3' |
| <i>LIG4</i> (Ligase IV) | DNA ligase 4 | 5'-TAAAGGTGTTTAGATGCGAGC-3' |
| <i>gfp</i> (GFP) | Green fluorescent protein | 5'-GCTGGAGTACAACACTACAAC-3' |
| <i>TINF2</i> (Tin2) | TERF1 interacting nuclear factor 2 | 5'-GTGCAGCTCCGTCATTACCAT-3' |
| <i>TERF2</i> (TRF2) | Telomeric repeat binding factor 2 | 5'-GCGCATGACAATAAGCAGA-3' |
| <i>POT1</i> (POT1) | Protection of telomeres 1 | 5'-GGGTGGTACAATTGTCAATGT-3' |

*Mature antisense are listed

Supplementary Table 2. Antibodies and probes

| Antibodies | Source | Identifier | Dilution |
|--|-------------------|-------------------|-----------------|
| Rabbit anti-53BP1 | Novus Biologicals | Cat#NB100-305 | 1:2000 |
| Mouse anti-H2AX (S139; γH2AX) | Millipore | Cat#JBW301 | 1:1000 |
| Mouse anti-p53 | Santa Cruz | Cat#sc-126 | 1:1000 |
| Rabbit anti-GAPDH | Cell Signaling | Cat#2118S | 1:2000 |
| Rabbit anti-pChk2 (Thr68) | Cell Signaling | Cat#2661S | 1:500 |
| Rabbit anti-Chk2 | Cell Signaling | Cat#2662 | 1:500 |
| Mouse anti-pATM (S1981) | Cell Signaling | Cat#4526S | 1:2000 |
| Mouse anti-ATM | Abcam | Cat#ab78 | 1:2000 |
| Mouse anti-Tin2 | Novus Biologicals | Cat#NB600-1522 | 1:500 |
| Mouse anti-p21 | BD Biosciences | Cat#556430 | 1:1000 |
| Rabbit anti-GFP | Abcam | Cat#ab290 | 1:1000 |
| Mouse anti-Rad51 | Abcam | Cat#ab213 | 1:1000 |
| Mouse anti-Plk1 | Abcam | Cat#ab17056 | 1:1000 |
| Rabbit anti-Ligase IV | Santa Cruz | Cat#sc-28232 | 1:1000 |
| Rabbit anti-Ku80 | Cell Signaling | Cat#2180S | 1:1000 |
| Mouse anti-RPA70 | Calbiochem | Cat#NA13 | 1:1000 |
| Mouse anti-BrdU | BD Biosciences | Cat#347580 | 1:1000 |
| Donkey anti-mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | ThermoFisher | Cat#A21202 | 1:800 |
| Donkey anti-rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | ThermoFisher | Cat#A21206 | 1:800 |
| Donkey anti-mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 | ThermoFisher | Cat#A31571 | 1:800 |
| Donkey anti-rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 | ThermoFisher | Cat#A31573 | 1:800 |
| Goat anti-mouse IgG HRP-conjugated Secondary Antibodies | Santa Cruz | Cat#sc-2005 | 1:10 000 |
| Goat anti-rabbit IgG HRP-conjugated Secondary Antibodies | Santa Cruz | Cat#sc-2030 | 1:10 000 |
| Alexa Fluor 647 Azide, Triethylammonium Salt | ThermoFisher | Cat#A10277 | 1:10 000 |
| Vysis CEP 8 SpectrumAqua Probe | Abbott | Cat#06J54-018 | 1:50 |
| Vysis LSI MYC SpectrumGreen Probe | Abbott | Cat#04N36-020 | 1:50 |

| | | | |
|--|----------|-----------|------|
| TelC-Cy3 telomere probe, C-rich/leading strand | Panagene | Cat#F1002 | 1:50 |
|--|----------|-----------|------|

Supplementary Table 3. Sybr RT-qPCR primer sequences

| Gene symbol | Sense | Description | Sequences |
|----------------------------|---------|-----------------------------------|--------------------------------|
| <i>TERF2</i> (TRF2) | Forward | Telomeric repeat binding factor 2 | 5'-CAAGGAAGCTGCTGTCATTA-3' |
| <i>TERF2</i> (TRF2) | Reverse | Telomeric repeat binding factor 2 | 5'-CCAGGAAGCGCAGCATCTTC-3' |
| <i>POT1</i> (POT1) | Forward | Protection of telomeres 1 | 5'-AAATTGATGCATATCCGTGGT-3' |
| <i>POT1</i> (POT1) | Reverse | Protection of telomeres 1 | 5'-CCCATACCCATGCTAACATCA-3' |
| <i>TBP</i> (TBP) | Forward | TATA-box binding protein | 5'-GAGCCAAGAGTGAAGAACAG-3' |
| <i>TBP</i> (TBP) | Reverse | TATA-box binding protein | 5'-ACCTTATAGGAAACTTCACATCAC-3' |

Figure S1

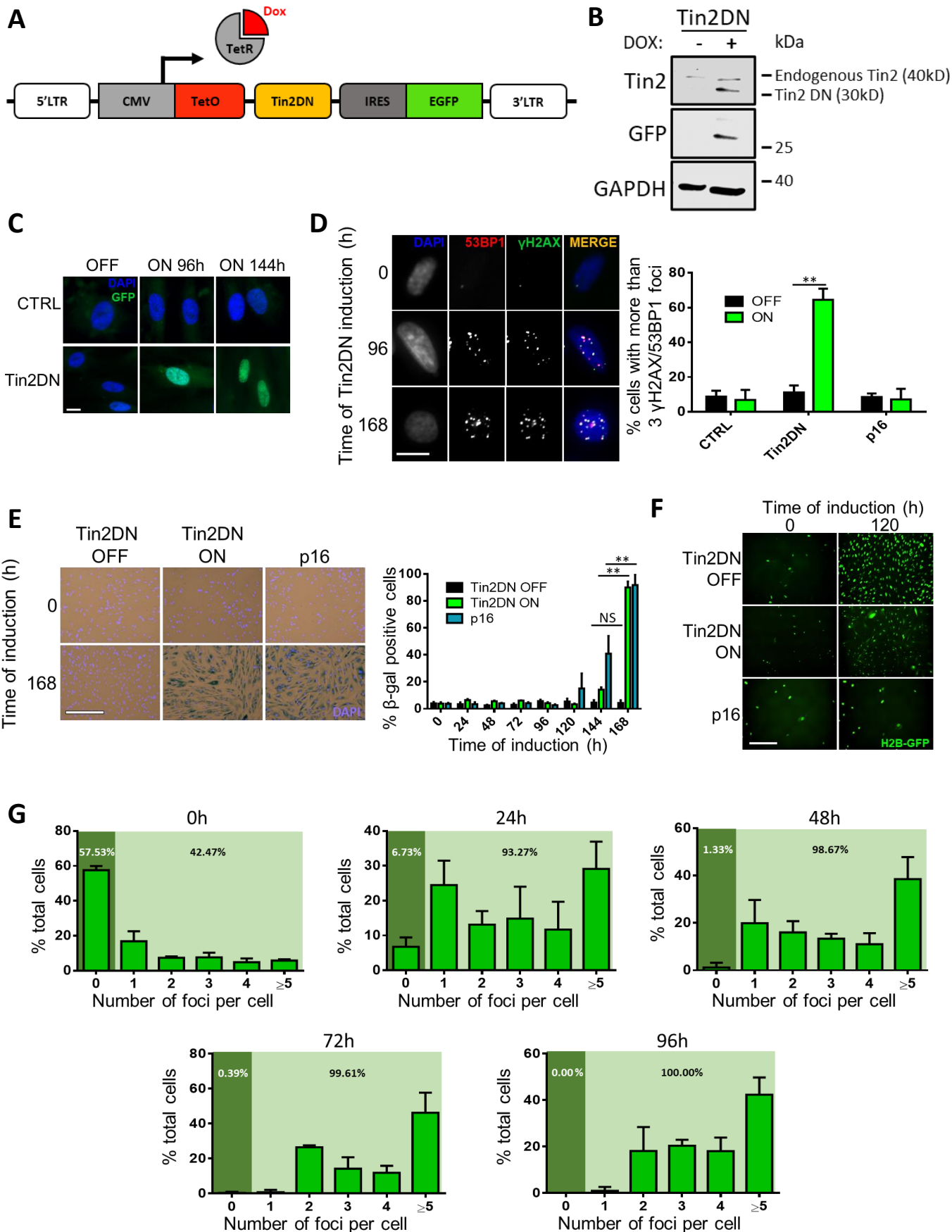


Figure S2

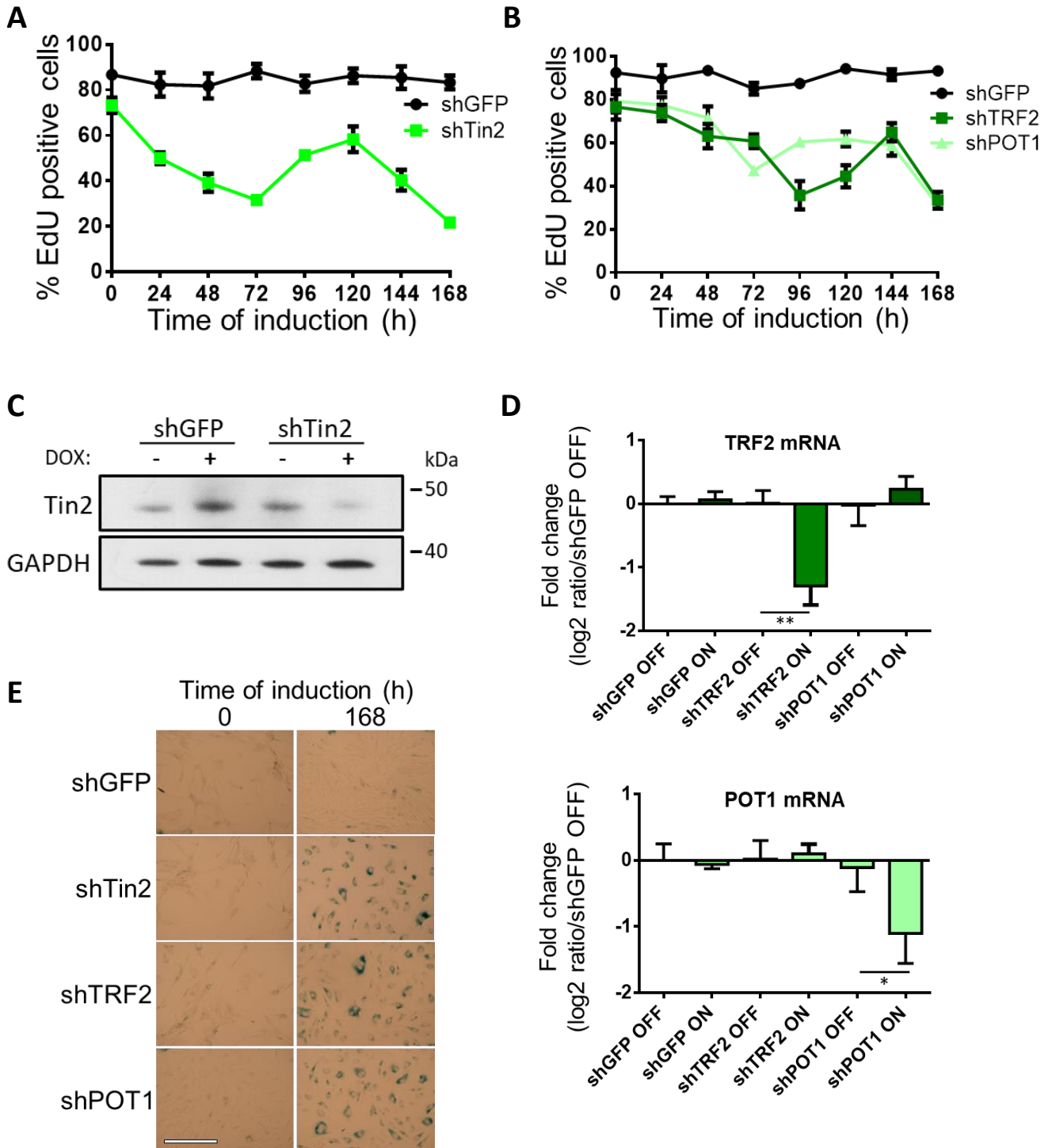
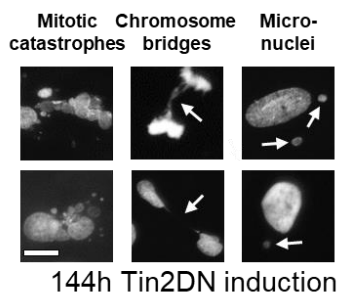
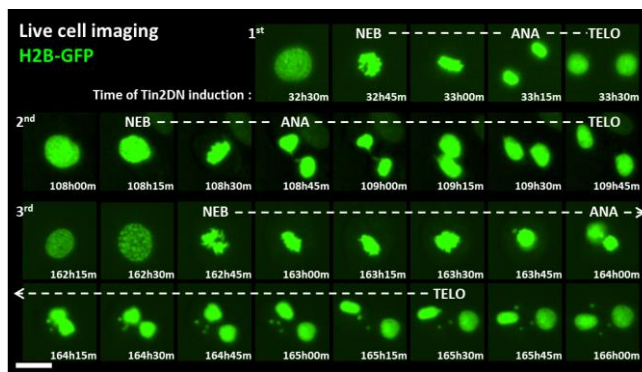


Figure S3

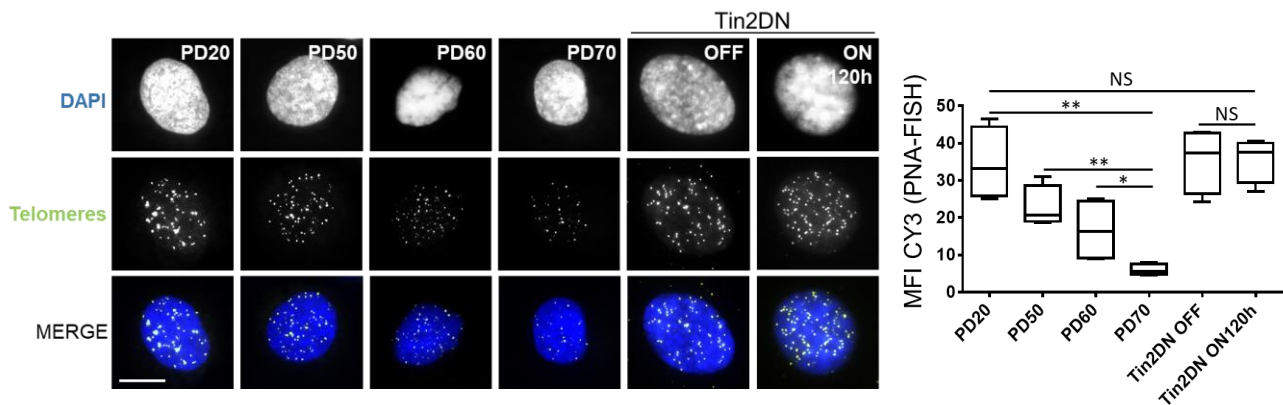
A



B



C



D

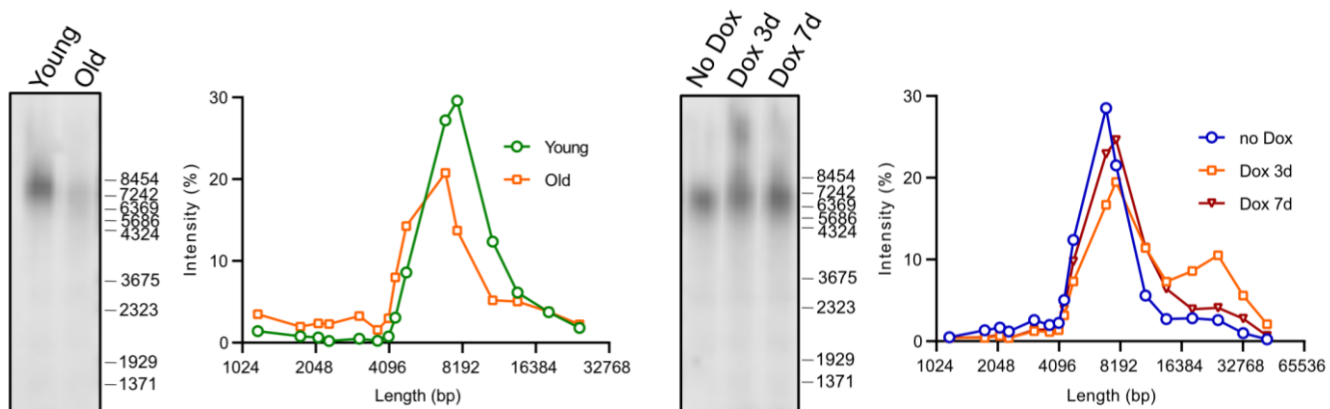


Figure S3 continued

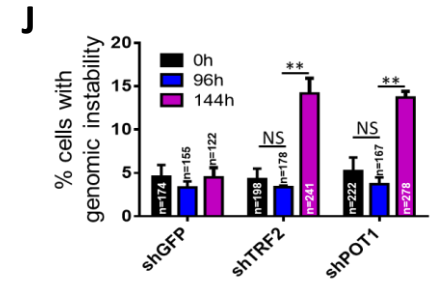
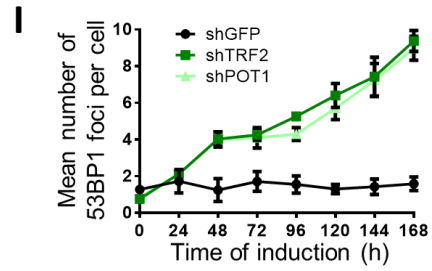
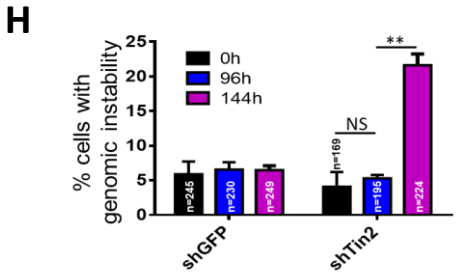
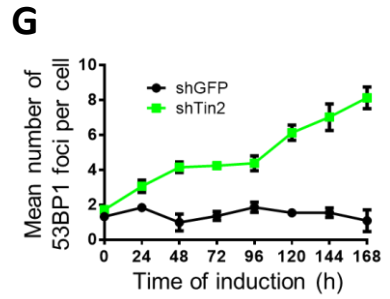
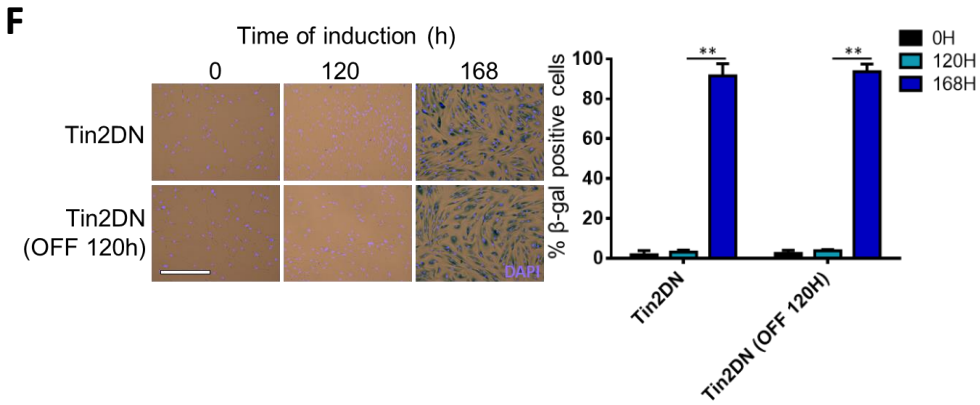
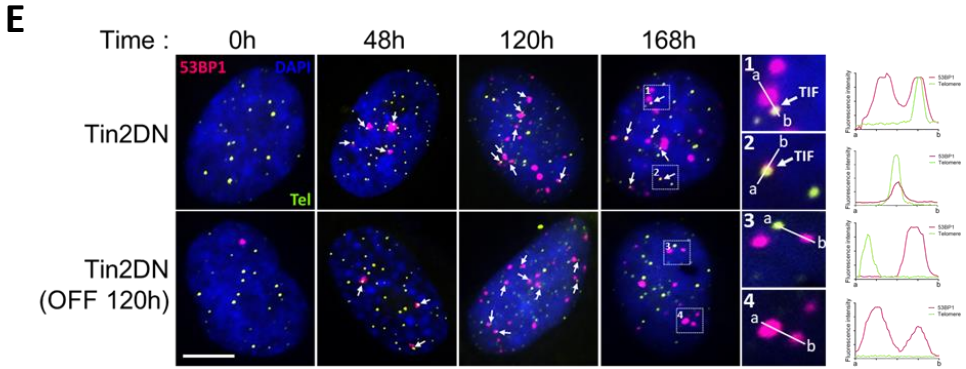


Figure S4

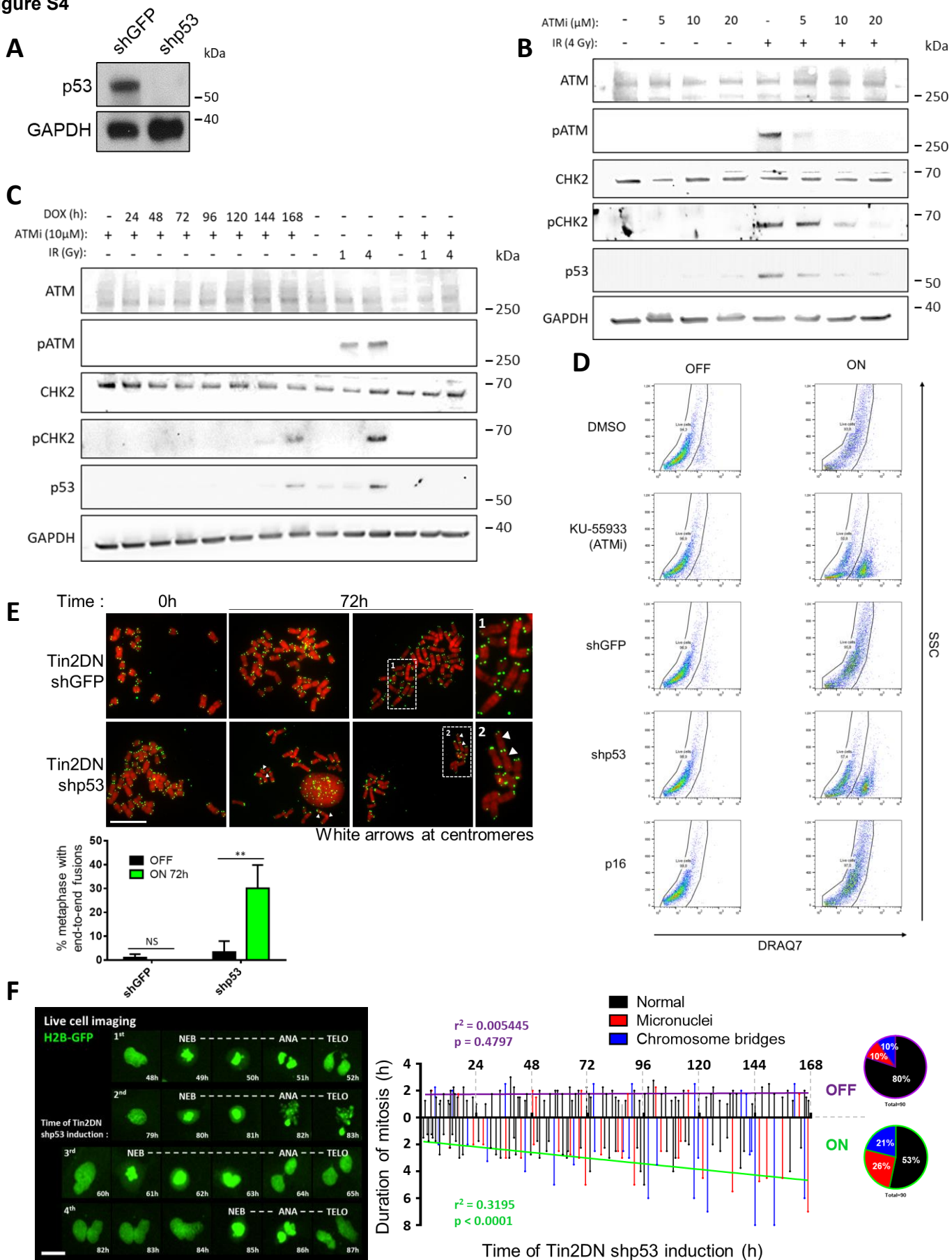


Figure S5

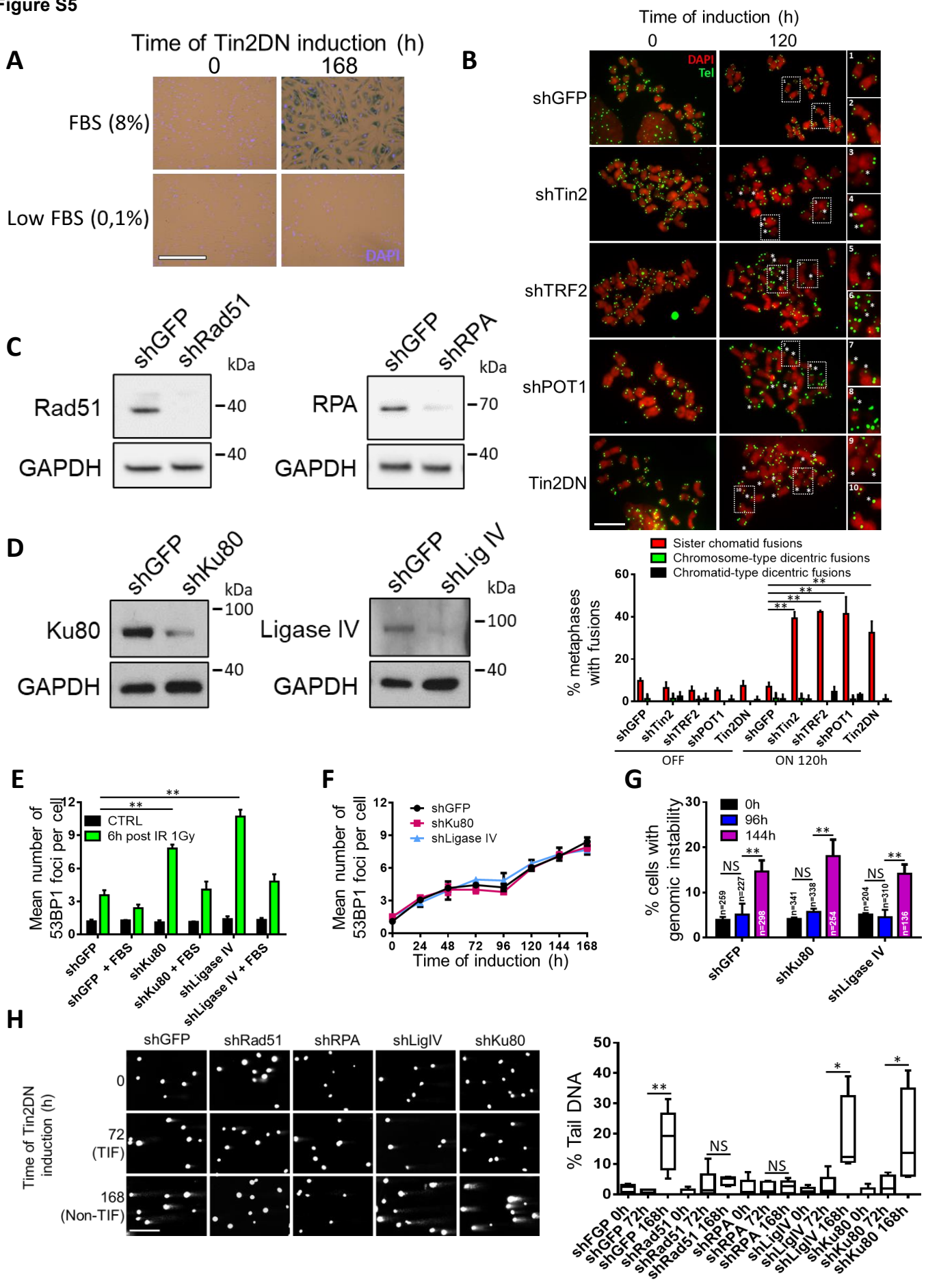


Figure S6

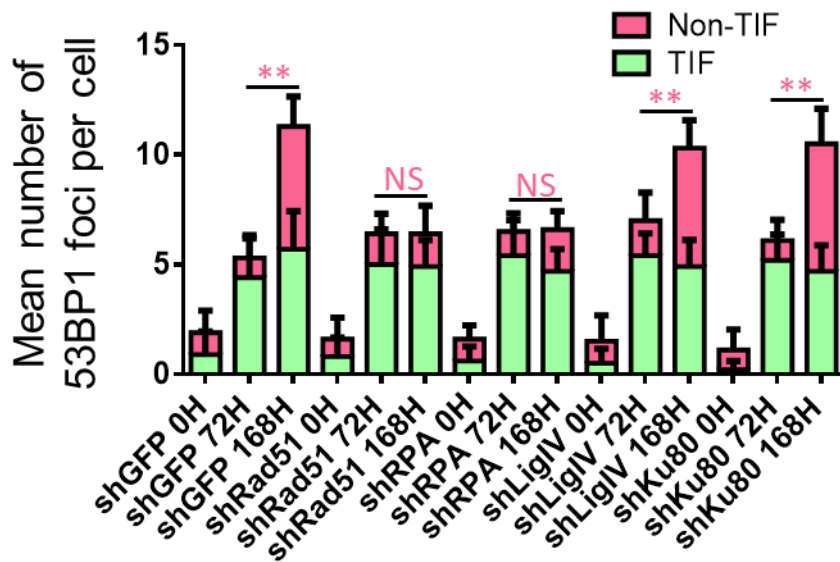
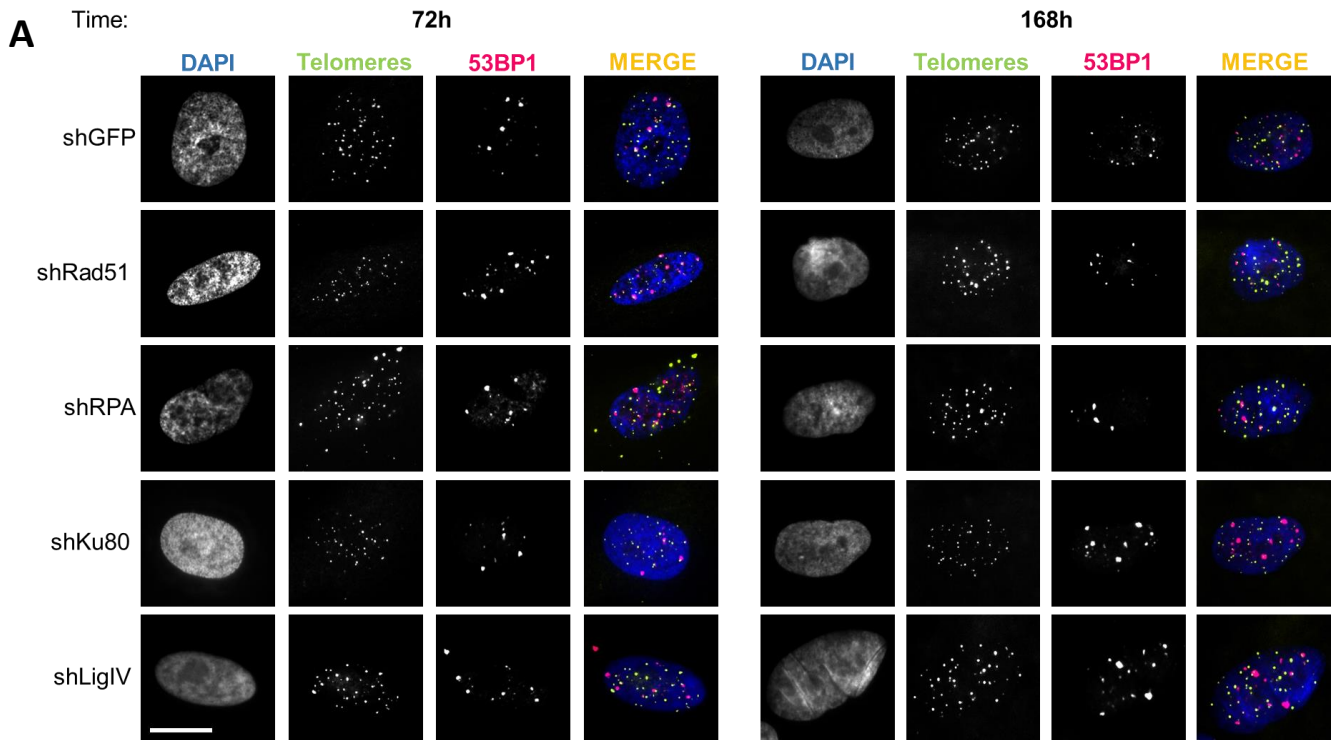


Figure S7

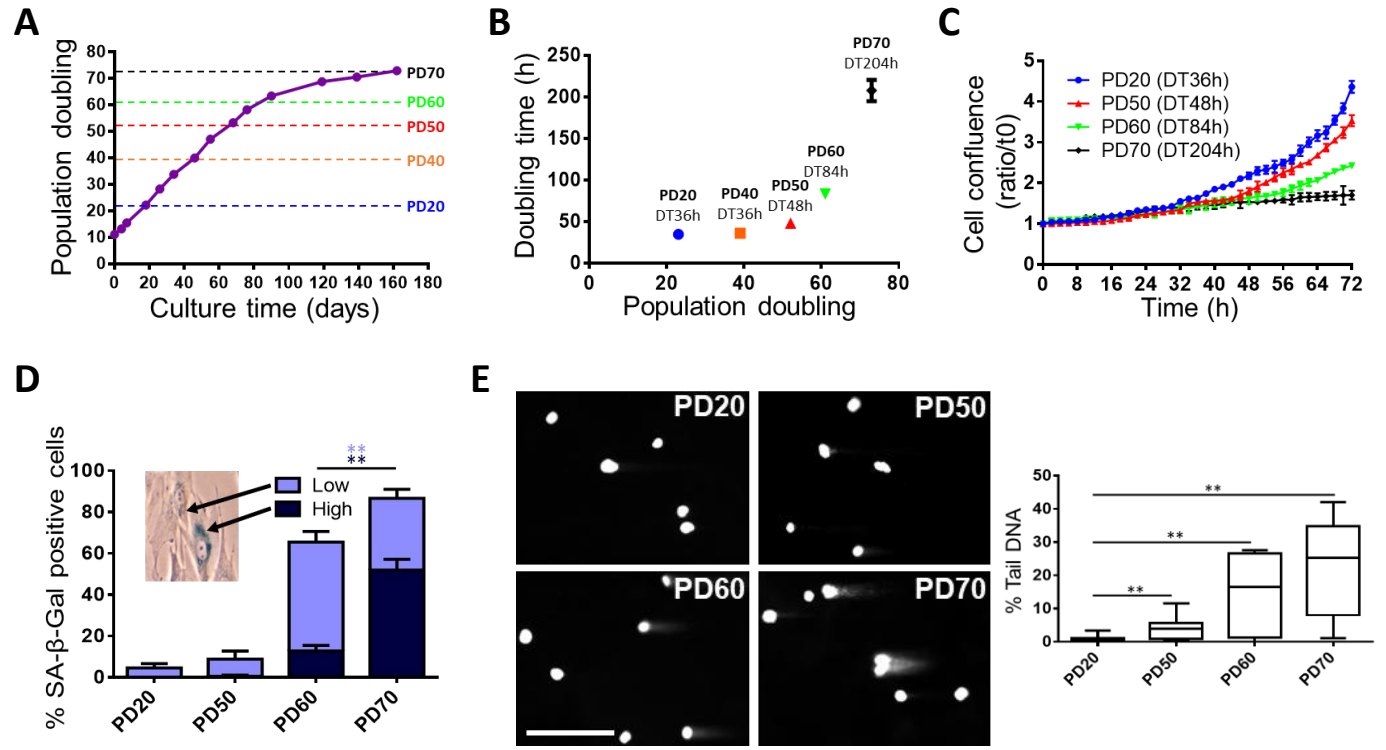


Figure S8

