

## Supplementary Material

### Supplementary Methods

**Cell synchronization.** For synchronized cell growth, thymidine was added to 30% confluent U2OS cells to a final concentration of 2.5mM. Cells were incubated for 17 hours, washed three times with PBS and released in thymidine free complete medium for 12 hours. Thymidine was added again at 2.5mM for 17 hours. Cells were released and collected at the indicated time points. siRNA were transfected 4 hours before the first thymidine block.

**RNAi.** Stealth RNAi (siRNA) (Invitrogen) used for ING2 downregulation (siING2) was synthesized to the following sequences: siING2#1: (5'-CCAGAGAGCACUAAUUAUAGUCA-3') and siING2#2: (5'-CAA AUGGAGUUACACUCACAGUGUU-3'). Transfections were performed on 30% confluent U2OS, with 40nM siRNA duplexes using lipofectAMINE RNAimax (#13778-075, Invitrogen). Transfections were performed for 48 hours. miRNA used to establish stable clones downregulated for ING2 were synthesized to the following sequences: miING2#1: (5'-UGCUGAACACUGUGAGUGUAACUCCAGUUUGGCCACUGACUGAC UGGAGUUACUCACAGUGUU-3') and miING2#2: (5'-CCUGAACACUGUGAGUAACUCCAGUCAGUCAGUGGCCAAAACUGGAGUUACACUCACAGUGUUC-3').

**Plasmids.** pcDNA-ING2(Pedoux et al, 2005) and its truncated forms were transfected using lipofectAMINE 2000 (Invitrogen), according to the manufacturers instructions.

**Antibodies.** Antibodies used in this study were: rabbit anti ING2 (ING2#1) and rat anti-ING2 (ING2#2) (Gozani et al., 2003), MCM6 (sc-22781, Santa Cruz), cdc45 (sc-55568), ORC2 (sc-13238), PCNA (sc-7907), PARP (sc7150), Histone H3 (17-615, Upstate), actin (Sigma-Aldrich), Caspase3 active form (BD Pharmingen #557035), p-Chk1 (Ser296) (Cell signalling #2349) gamma-H2AX (Ser139) (Cell signalling #2577), BrdU (rat anti-BrdU ab-6326), IdU (mouse anti-BrdU, BD Pharmingen), FITC-conjugated BrdU antibodies (Roche) and anti single strand DNA (MAB3034, Upstate).

**Western Blotting of Whole Cell Extract (WCE)** Samples were prepared for immunoblotting by lysis in RIPA buffer (Tris-HCl 50mM pH 7.5, NaCl 150mM, NaDOC 0.5%, SDS 0.1%, NP40 1%, 1mM PMSF and protease inhibitor cocktail).

**BrdU and propidium iodide FACS analysis.** Before harvesting cells for FACS analysis they were pulsed with 100µM of BrdU for 20min. The collected cells were fixed in 70% cold ethanol for 30 minutes on ice. Cells were then denatured in 4N HCl for 30 minutes at room temperature, washed in PBS containing 0.5% Tween 20 and incubated with FITC-conjugated

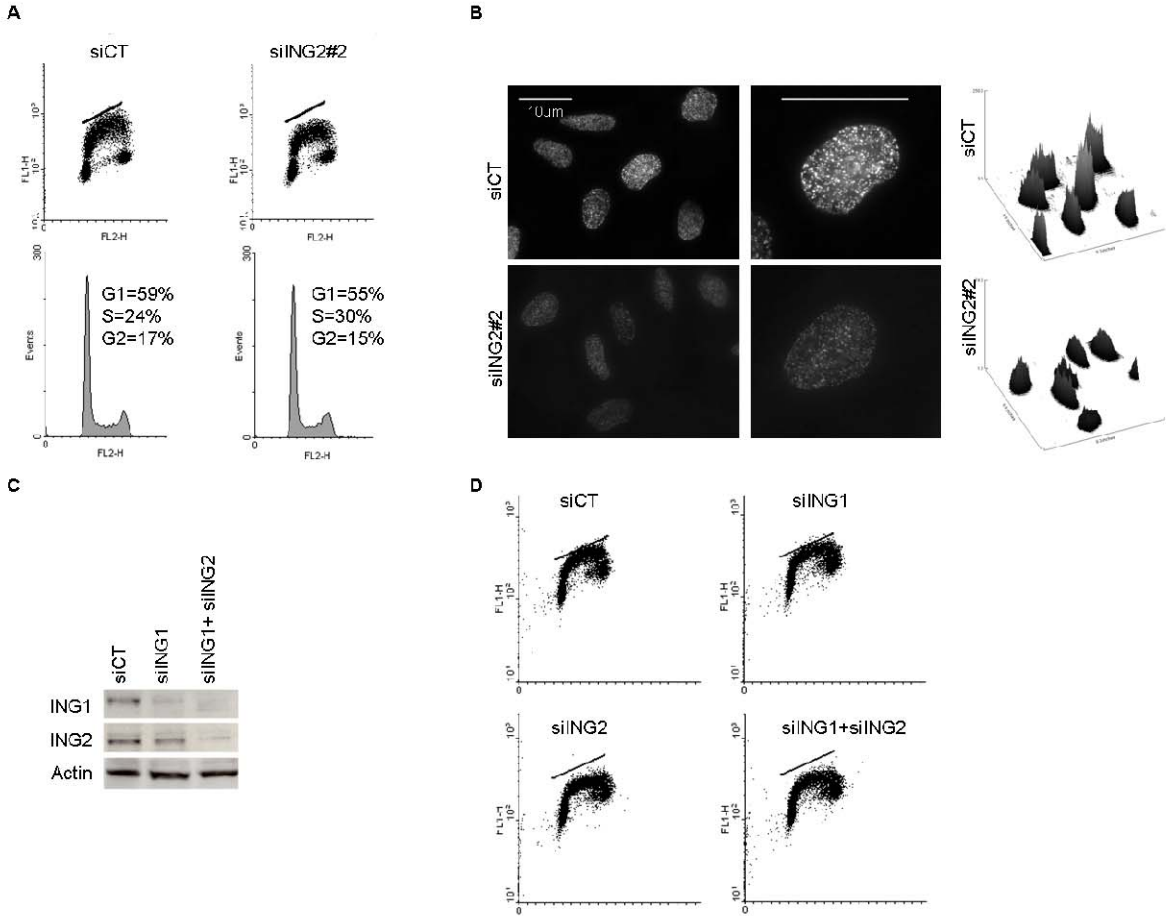
BrdU antibody in PBS containing 0.1% BSA for 30 minutes. Cells were washed in PBS, stained with Propidium Iodide (PI) and analyzed on a FACScan (BD Bioscience) and CellQuest software (BD Bioscience) to assess cell cycle distribution.

**Cell proliferation and SA- $\beta$ -Gal assays.** Cells were counted, plated at the same density, transfected with siRNA and maintained in culture for 4 days. Senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -Gal) activity was detected in hTERT MRC5 fibroblasts as previously described (Dimri et al, 1995). For cell proliferation, cells were counted the first day (T0) and at day 4 (T4). The results are the mean of three independent experiments.

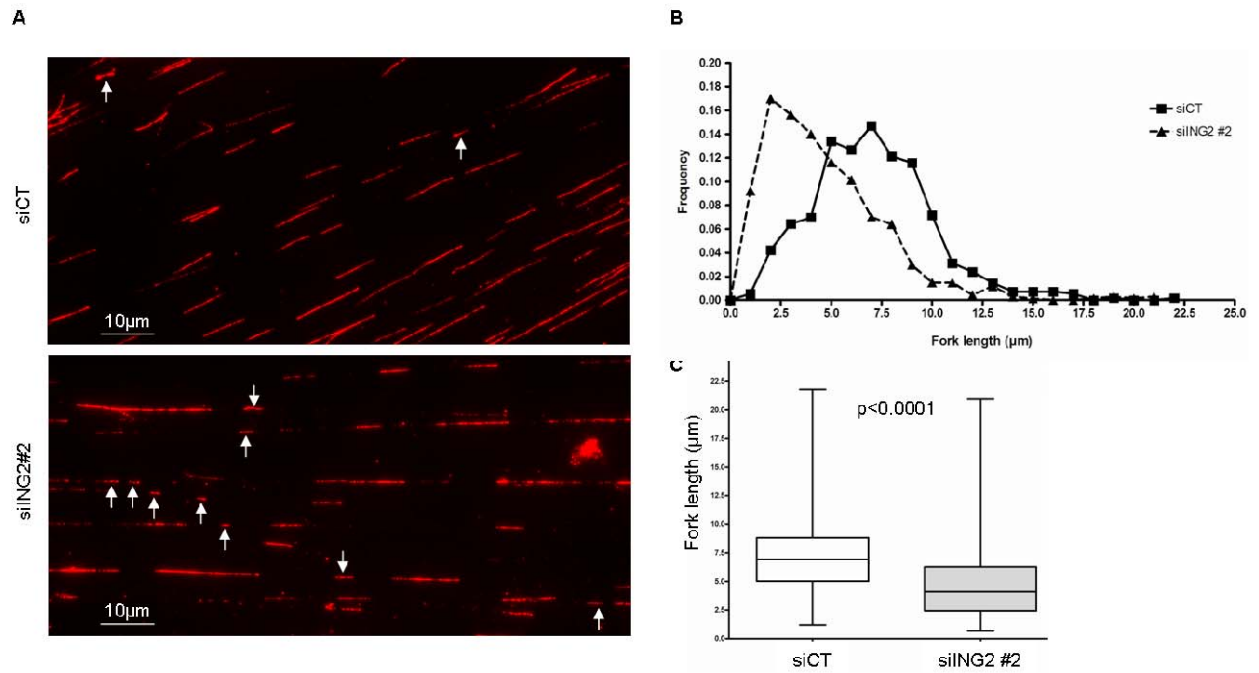
**Immunoprecipitation and pull-down experiment.** U2OS cells were collected in extraction buffer containing 50mM Tris pH 8.0, 20% glycerol, 1mM DTT, 0.1% NP40, 500mM KCl, and protease inhibitor cocktail. Cells were lysed by 3 cycles of freeze-thaw (liquid N<sub>2</sub>-37°C). Soluble lysates were incubated with protein G beads previously coupled to the PCNA antibody for 4 hours at 4°C with rocking. Beads were washed four times in the extraction buffer, SDS sample buffer was added and lysates were boiled before loading on NuPAGE® Novex 4-12% Bis-Tris Gels (# NP0329BOX, Invitrogen). For the GST pull-down experiment, ING2 full length and truncated forms were translated *in vitro* using the TNT® T7/SP6 Coupled Reticulocyte Lysate System Kit (L5020 Promega). Gluthatione Sepharose 4B was resuspended in GST buffer (50mM Tris HCl pH 8, 150mM NaCl, 0.05% NP40, PIC) and incubated with 20 $\mu$ l of ING2 translated products and 1 $\mu$ g of full length PCNA recombinant protein with GST tag (Abnova #H00005111-P01) for 2 hours at 4°C. Gluthatione was washed six times in GST buffer.

**Immunofluorescence staining of replication foci.** For visualization of DNA replication foci, 100 $\mu$ M of bromodeoxyuridine (BrdU) was incorporated into DNA for 20min. Cells were fixed with 70% cold ethanol. Cells were denatured in 1.5 M HCl for 30 min, and incubated in 5% chicken serum (Sigma Aldrich), 0.5% Tween 20, and 0.1% BSA in PBS for 20 min. Primary anti-BrdU antibody was added to the slides, and incubated for 1 h. Slides were washed with PBS-Tween 20 followed by incubation with secondary antibody (donkey anti-rat FITC, Jackson ImmunoResearch) for 1 h.

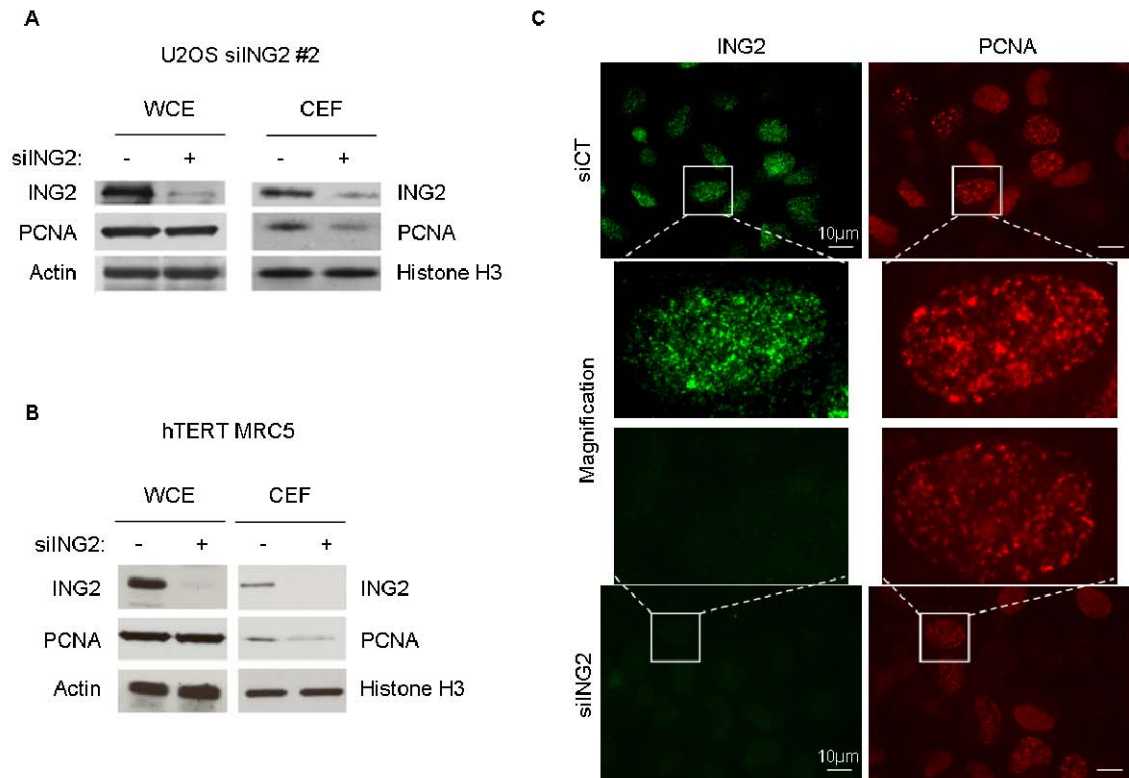
Supplementary Figures



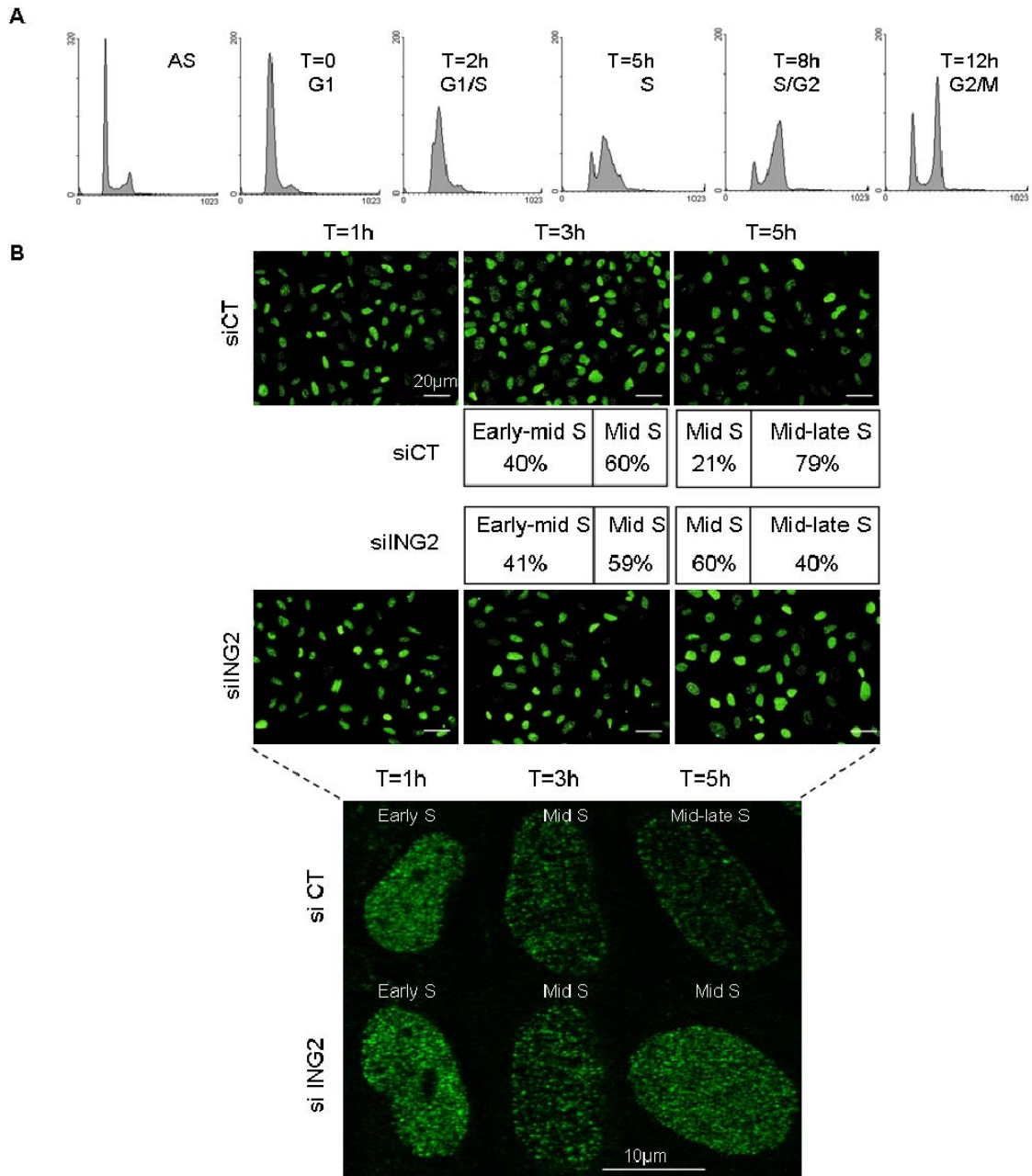
**Figure 1S: ING2 but not ING1 knockdown impairs BrdU incorporation.** (A) Asynchronous U2OS cells were transfected with siCT or siING2#2 for 48h, pulsed with BrdU for 20min and collected. FACS analysis shows BrdU incorporation profiles. (B) **Left panel:** Representative immunofluorescence images of BrdU incorporation. Pictures were acquired with the same exposure time. **Right panel:** Graphic Image J representation of BrdU intensity measured for immunostained cells shown in (B). (C) The efficiency of ING1 and ING2 downregulation was assessed by Western Blot. (D) Incorporation of BrdU performed as in (A) in cells transfected with the indicated siRNA.



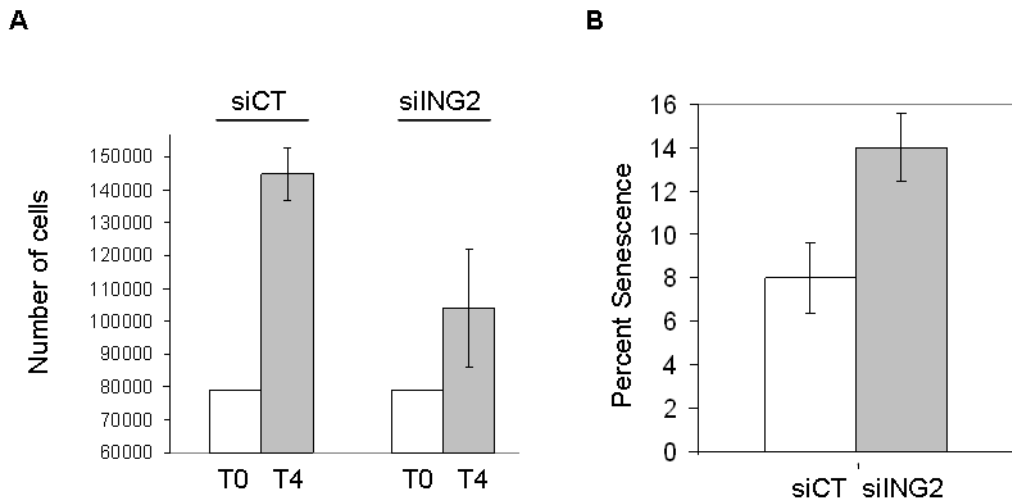
**Figure 2S: Fork progression rate decreases after siING2#2 transfection. (A)** Representative immunofluorescence images showing IdU positive tracks (red). White arrows point out short IdU positive tracks. **(B)** Fork length repartition measured in microns ( $\mu$ m) with ImageJ software for siCT (n=533) and siING2#2 (n=592). **(C)** Boxplots represent interquartile ranges and extreme values of forks length for siCT and siING2. Horizontal bar denotes the median. The p value was measured using an Anova test followed by a Mann-Whitney test.



**Figure 3S: ING2 targets PCNA to the chromatin fraction.** U2OS (A) or hTERT MRC5 cells (B) were transfected with siCT or with the indicated siING2. The expression of ING2 and PCNA was assessed in the Whole Cell Extract (WCE) and in the Chromatin Enriched Fraction (CEF). Actin and Histone H3 were used as loading controls. (C) Immunofluorescence analysis of chromatin bound(Sengupta et al, 2003) proteins in the nuclei. One nucleus is magnified for each staining. Pictures were acquired with the same exposure time.



**Figure 4S: Progression of cells in S phase.** U2OS cells were synchronized at the G1/S transition (T=0) released and collected at the indicated time points during S phase progression. **(A)** FACS analysis of Propidium Iodide incorporation shows the cell cycle of untransfected cells at each time point, compared to asynchronous cells (AS). **(B)** Before cell collection, cells were pulsed for 30 minutes with BrdU to determine between early, mid or late S phase patterns. Representative confocal immunofluorescence images of BrdU staining are shown. The percentage of cells displaying early, mid or late S phase pattern is shown at each time point. Exposure time was adjusted for siING2 cells to obtain signal intensity comparable to siCT cells.



**Figure 5S: ING2 downregulation slows down cell proliferation and increases the occurrence of senescence.** (A) hTERT MRC5 cells transfected with siCT or siING2 were counted and plated at the same density on the first day (T0). After four days of culture (T4 days), cells were counted and proliferation is represented graphically. The p value ( $p < 0.02$ ) was calculated using a student t test. (B) SA- $\beta$ -Gal assay was performed on the cells plated as in (C) and the percentage of cells stained for SA- $\beta$ -Gal from three independent experiments is represented.