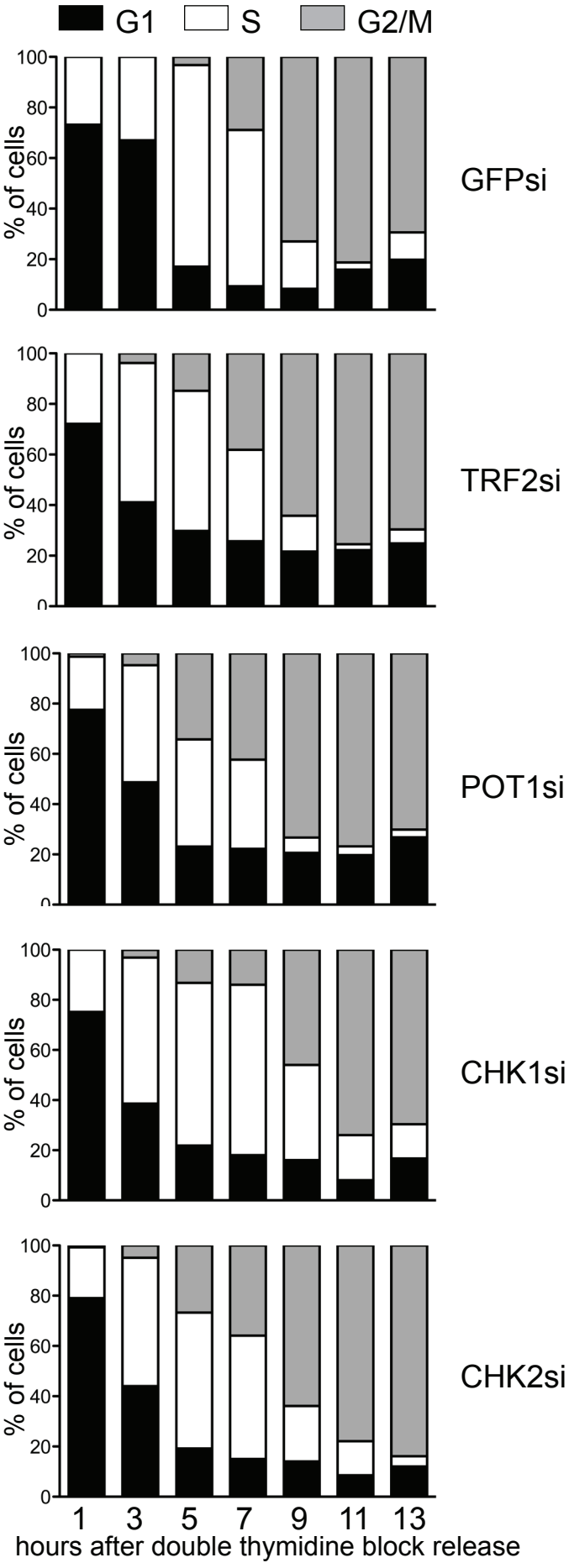


Figure S1. U2OS synchronous cell cycle progression. U2OS cells transfected with TRF2, POT1, CHK1, CHK2 or control GFP siRNAs were grown for 48 hours before synchronization by double thymidine block and release. Cells collected at the indicated times after release were processed by FACS analyses of DNA content. Similar patterns of cell cycle distribution were obtained in at least two independent experiments.

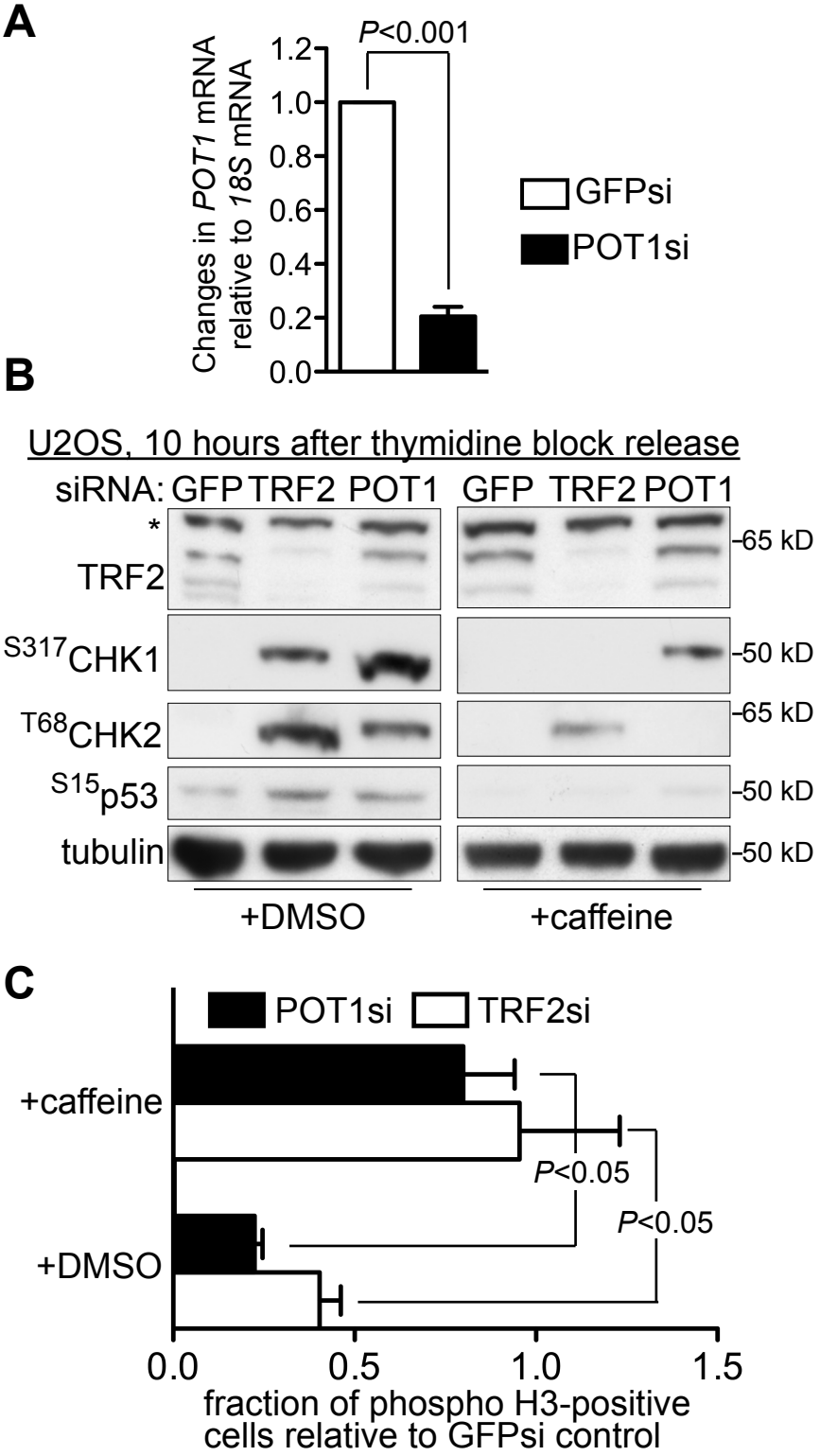
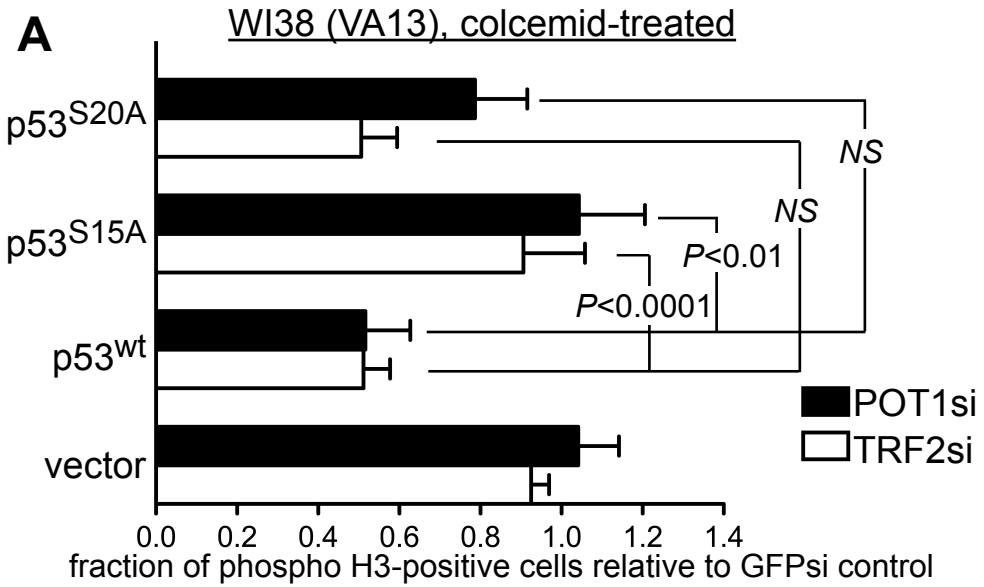
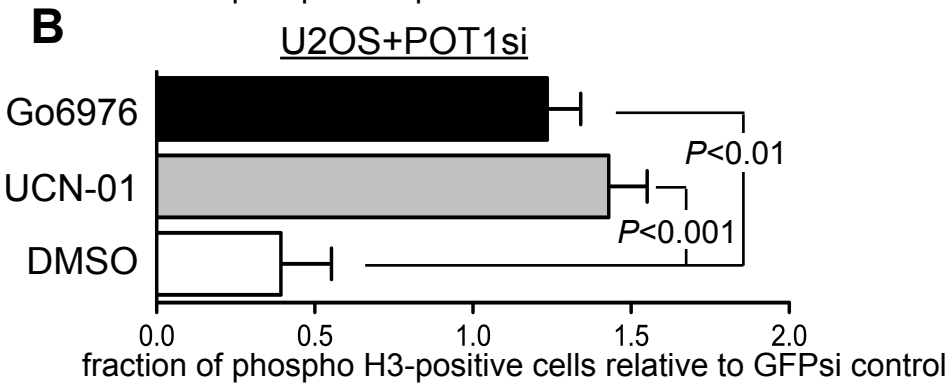
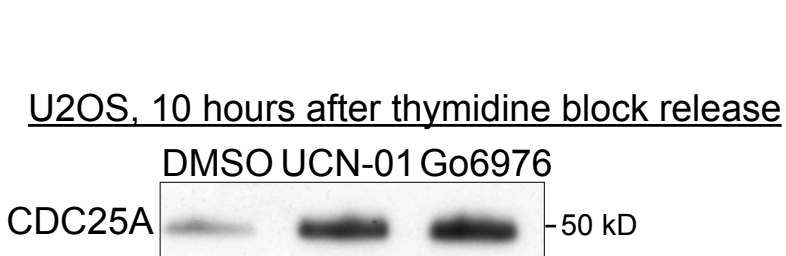


Figure S2. A. Real-time RT-PCR analysis of siRNA-mediated POT1 depletion. U2OS cells transfected with POT1 or control GFP siRNA were grown for 24 hours. mRNA was isolated and the levels of *POT1* transcript were determined using real-time RT-PCR. The *P* value was calculated using an unpaired two-tailed t test. **B.** Caffeine treatment abrogates the G2/M checkpoint response to telomeres uncapped through siRNA-mediated inhibition of TRF2 or POT1. U2OS cells transfected with TRF2, POT1 or control GFP siRNAs were grown for 48 hours before synchronization by double thymidine block and release in fresh media for 10 hours. Caffeine or solvent (DMSO) were added to the media 4 hours prior to collection. Cell extracts were prepared and immunoblotted as shown. **C.** U2OS cells treated as in (A) were stained with propidium iodide and an antibody against phosphorylated histone H3-Ser 10 and analysed by flow cytometry. n=10,000 cells were analyzed for each sample. Error bars represent SD of two independent experiments. *P* values were calculated using an unpaired two-tailed t test.







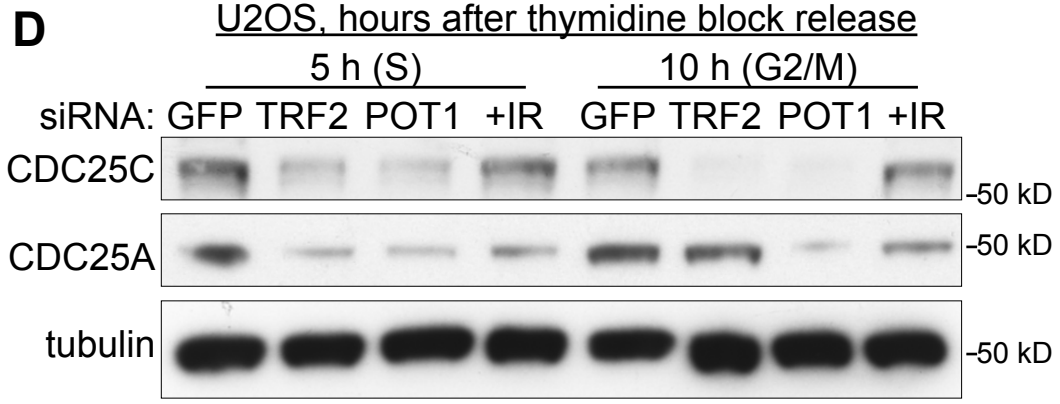


Figure S3. A. Ser15 residue of p53, a target for ATM/ATR-dependent phosphorylation, is required to prevent mitotic entry of WI38(VA13) human cells with uncapped telomeres. WI38(VA13) cells were transfected with p53-encoding constructs or vector alone, 24h after treatment with TRF2, POT1 or control GFP siRNAs. Twenty four hours later cells were treated with colcemid for 3 hours, stained with propidium iodide and an antibody against phosphorylated histone H3-Ser 10 and analysed by flow cytometry. N=10,000 cells were analyzed for each sample. Error bars represent SD of at least two independent experiments. *P* values were calculated using an unpaired two-tailed t test. **B.** CHK1 chemical inhibition rescues the G2/M arrest in POT1-depleted cells. U2OS cells transfected with POT1 or control GFP siRNAs were grown for 48 hours before synchronization by double thymidine block and release in fresh media for 10 hours. UCN-01 and Go6976 inhibitors, or DMSO control were added to the media 4 hours before collection. Cells were stained with propidium iodide and an antibody against phosphorylated histone H3-Ser 10 and analysed by flow cytometry. N=10,000 cells were analyzed for each sample. Error bars represent SD of two independent experiments. *P* values were calculated using an unpaired two-tailed t test. **C.** U2OS cells were synchronized by double thymidine block and release in fresh media for 10 hours. UCN-01 and Go6976 inhibitors, or DMSO control were added to the media 4 hours before collection. Cell extracts were immunoblotted as shown. **D.** Telomere damage induced by TRF2 depletion leads to CDC25C reduction in G2/M and CDC25A/C reduction in S. CDC25C is not downregulated by exposure to IR either in S or G2/M**.** U2OS cells transfected with control GFP, TRF2 or POT1 siRNAs were grown for 48 hours before synchronization by double thymidine block and release. Extracts prepared at the indicated times after release were immunoblotted as shown. Tubulin was used as a loading control. As controls, U2OS cells at 5 or 10 hours after release from double thymidine block were exposed to 10 Gy of IR, allowed to recover for 2 hours and collected for cell extract preparation.

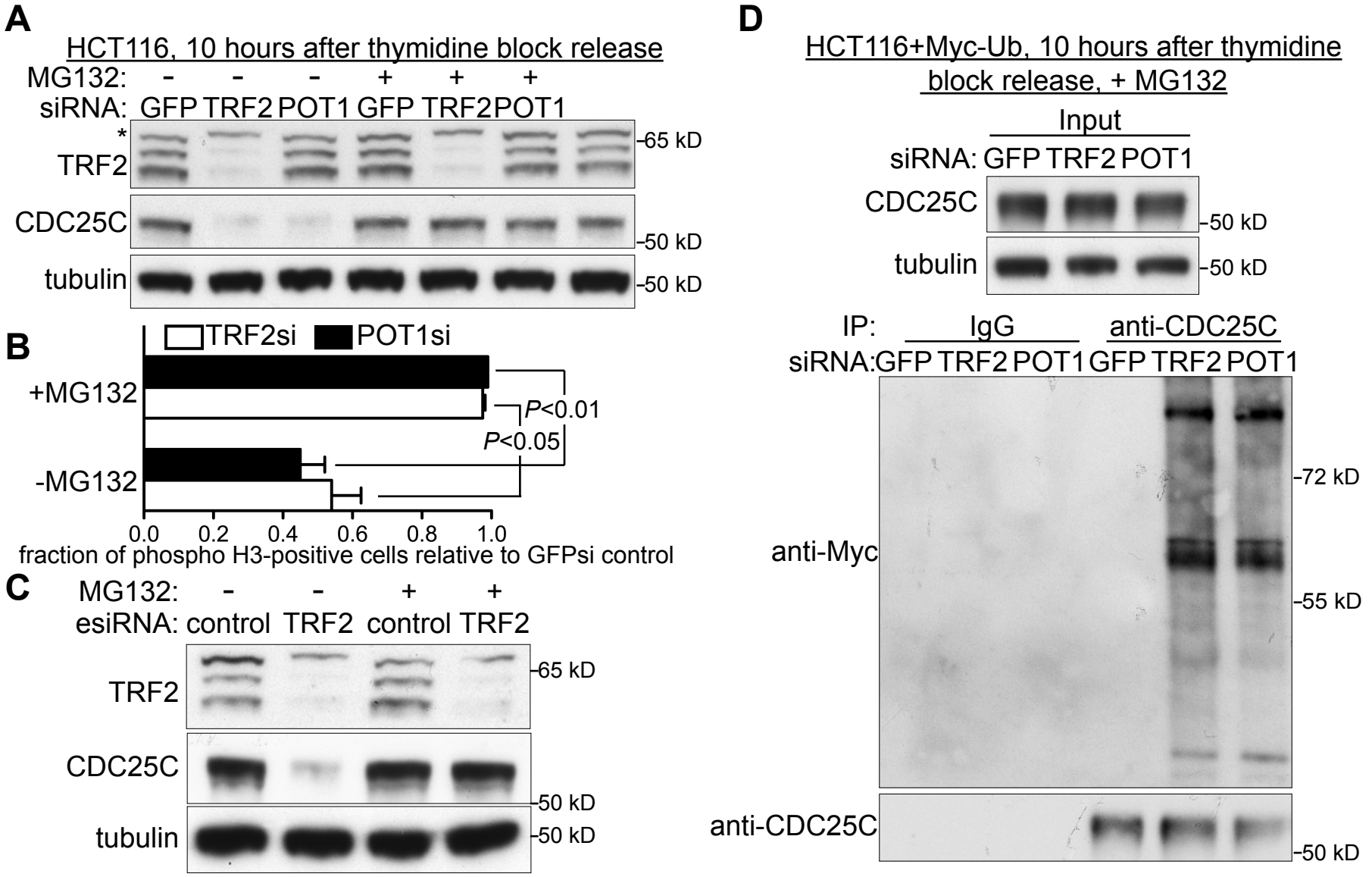


Figure S4. A. Proteasome-mediated destruction of the CDC25C prevents mitotic entry in the presence of uncapped telomeres in p53-proficient HCT116 cells. HCT116 cells transfected with TRF2, POT1 or control GFP siRNAs were grown for 48 hours before synchronization by double thymidine block and release in fresh media for 10 hours. Proteasome inhibitor (MG132) was added to the media 3 hours prior to collection. Cell extracts were prepared and immunoblotted as shown. Extracts from cells exposed to 10 Gy of IR were used as a control. *, non-specific band. **B.** HCT116 cells treated as in **A.** were stained with propidium iodide and an antibody against phosphorylated histone H3-Ser 10 and analysed by flow cytometry. N=10,000 cells were analyzed for each sample. Error bars represent SD of three independent experiments. *P* values were calculated using an unpaired two-tailed t test. **C.** HCT116 cells transfected with esiRNA for TRF2 (Sigma) or control were processed as in **A. D.** TRF2 or POT1 inhibition triggers CDC25C ubiquitylation in HCT116 cells. HCT116 cells were treated with TRF2, POT1 or control GFP siRNAs for 24 hours before transfection with Myc-ubiquitin expressing construct. Twenty four hours later cells were synchronized by double thymidine block and release in fresh media for 10 hours. Proteasome inhibitor (MG132) was added to the media 3 hours prior to collection. Cell extracts used as input and proteins immunoprecipitated with IgG control or anti-CDC25C antibody were immunoblotted as shown.

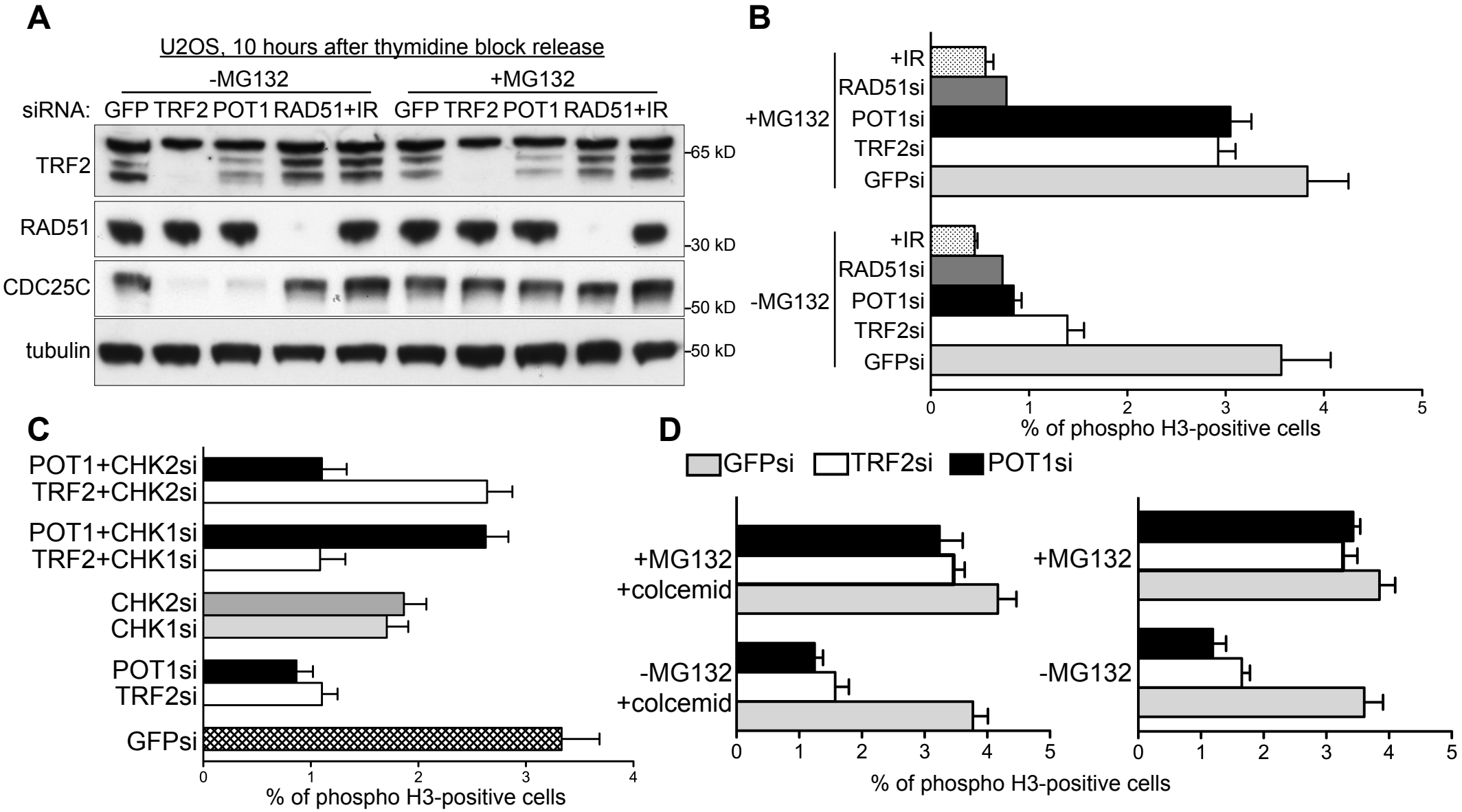


Figure S5. A. Chronic DNA damage induced by RAD51 depletion does not lead to CDC25C degradation in G2/M and the ensuing mitotic entry block is not rescued by proteasome inhibition**.** U2OS cells transfected with indicated siRNAs for 48 hours were synchronized by double-thymidine block and released in fresh media for 10 hours. Proteasome inhibitor (MG132) was added to the media 3 hours prior to collection. As controls, U2OS cells at 10 hours after release from double thymidine block were exposed to 10 Gy of IR, allowed to recover for 2 hours and collected for cell extract preparation. **B.** U2OS cells treated as in **A.** were stained with propidium iodide and an antibody against phosphorylated histone H3-Ser 10 and analysed by flow cytometry. N=10,000 cells were analyzed for each sample. Error bars represent SD of three independent experiments. **C.** Absolute values for the flow cytometry analyses of percentages of phosphorylated histone H3-Ser 10 stained cells shown in Fig. 4C and Fig. 5D.