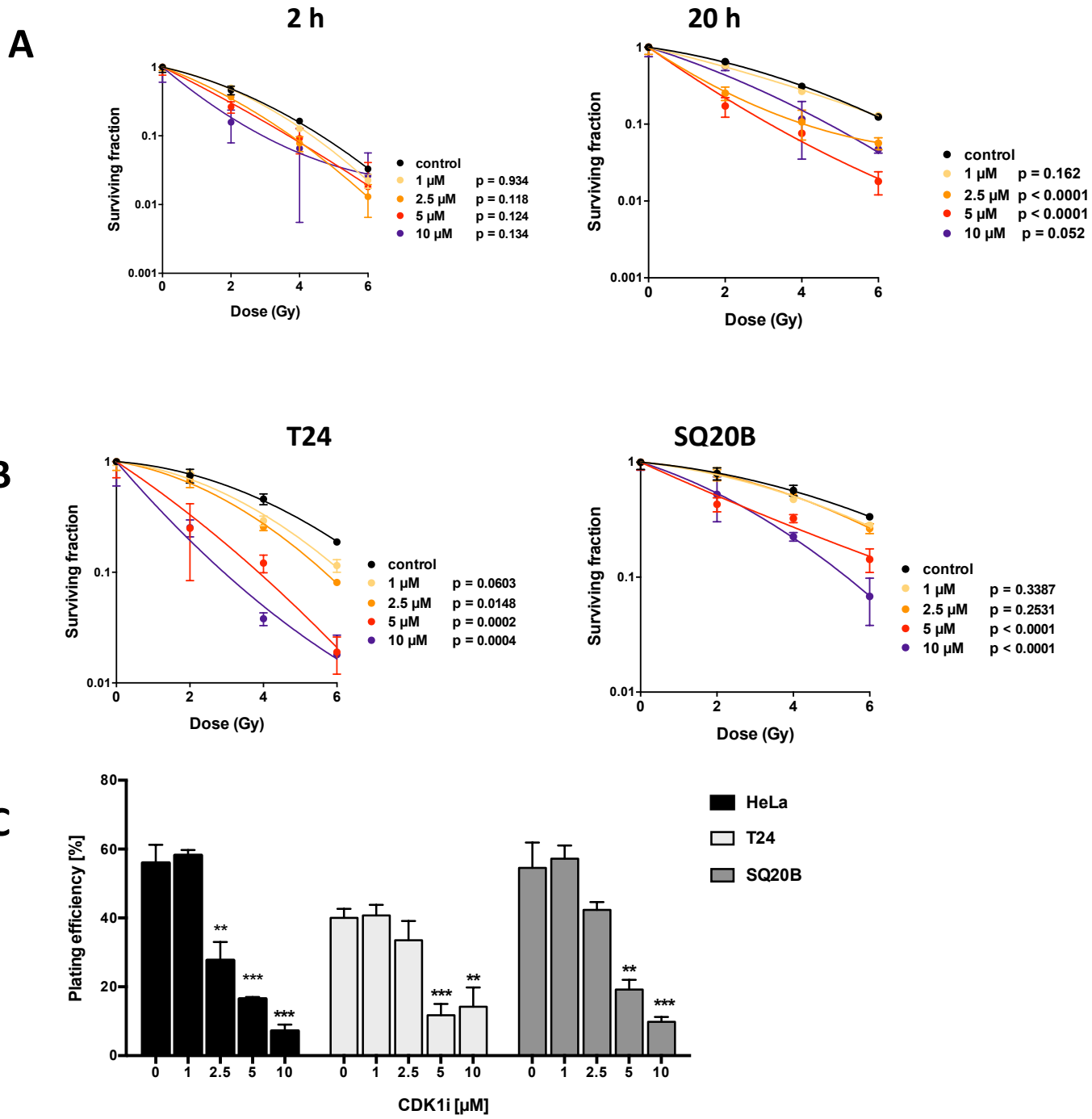
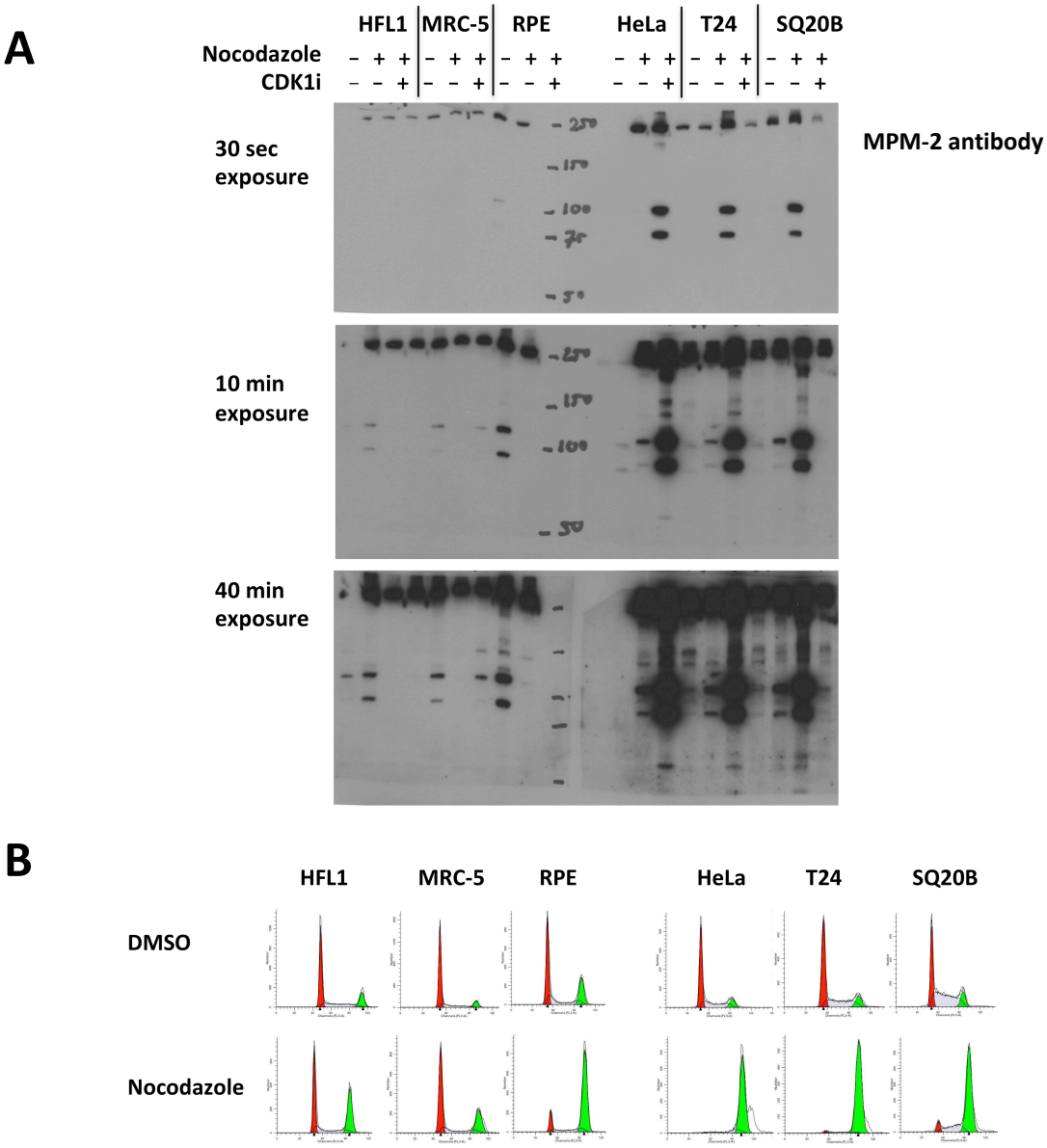



**Suppl. Fig. 1: Optimization of RO-3306 concentration and timing.**

**A.** Colony formation assay of HeLa cells treated with increasing concentrations of RO-3306 3 h after seeding. Cells were irradiated either 2 h after compound addition followed by a medium change 16 h later, or 20h after compound addition followed by a medium change immediately after irradiation. Data are presented as mean +/- SD from triplicate wells. P-values were derived by two-way ANOVA, testing whether a treatment curve was statistically different from the control curve. The higher survival fraction for 10 µM compared to 5 µM or 2.5 µM most likely results from the very low plating efficiency at this compound concentration (see panel C).

**B.** Colony formation assay of T24 and SQ20B cells treated with increasing concentrations of RO-3306 3 h after seeding followed by irradiation 20 h later. Medium was changed immediately after irradiation. Data are presented and analyzed as in (A).

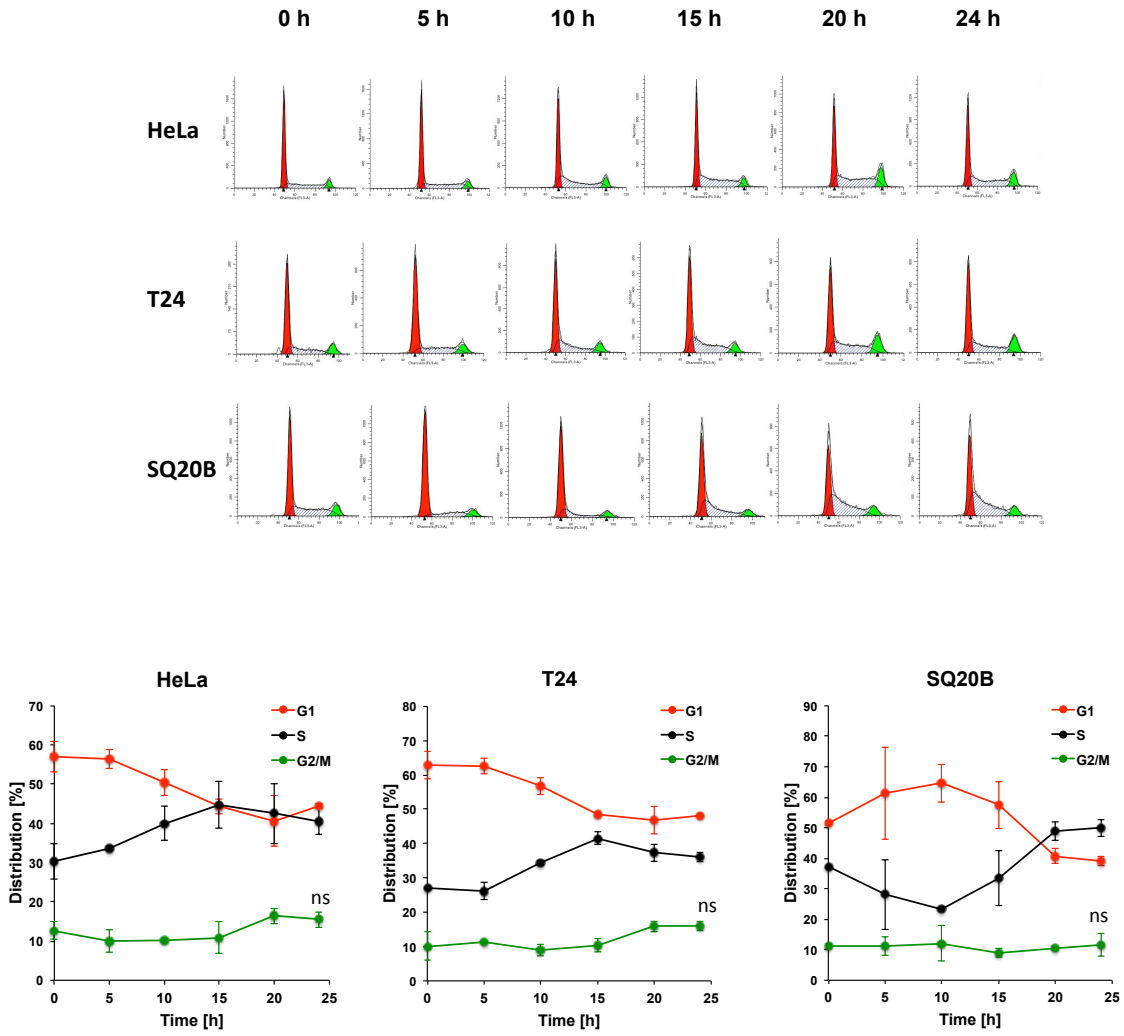
**C.** Plating efficiencies for HeLa, T24 and SQ20B cells treated with increasing concentrations of RO-3306 for 20 h. Values show mean +/- SD from triplicate wells and those that are significantly different from untreated cells are indicated (** $p < 0.01$; *** $p < 0.001$).




**Suppl Fig. 2. Biochemical confirmation of RO-3306 activity.**

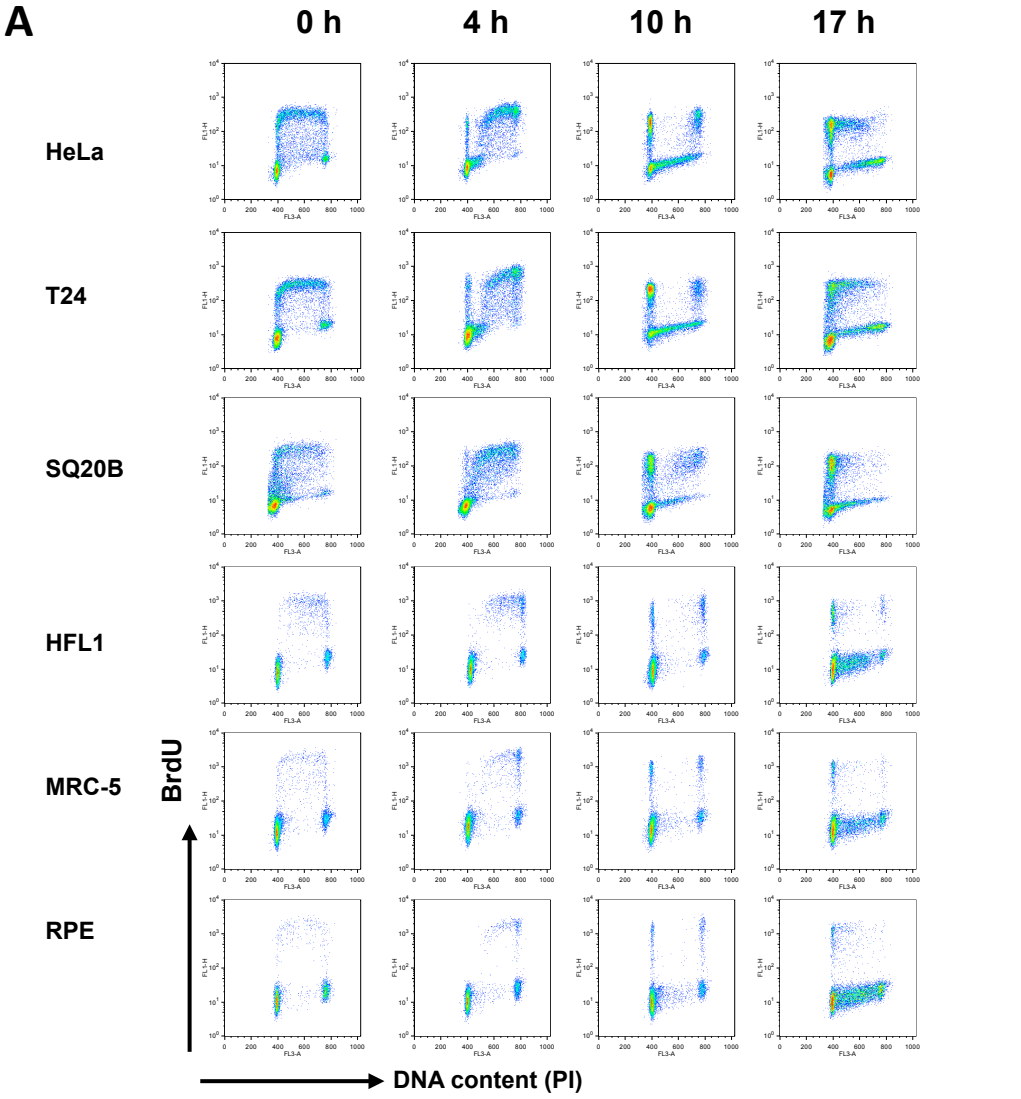
**A.** MPM-2 Western blots from Fig 2 in the main text, showing multiple exposure times. Cultures were enriched for mitotic cells by overnight treatment in nocodazole followed by 2 h of 5 µM RO-3306 in the continuous presence of nocodazole. Both floating and adherent cells were lysed and subjected to immunoblotting with the MPM-2 antibody, recognizing phosphorylated CDK1 substrates. Representative of three independent experiments.

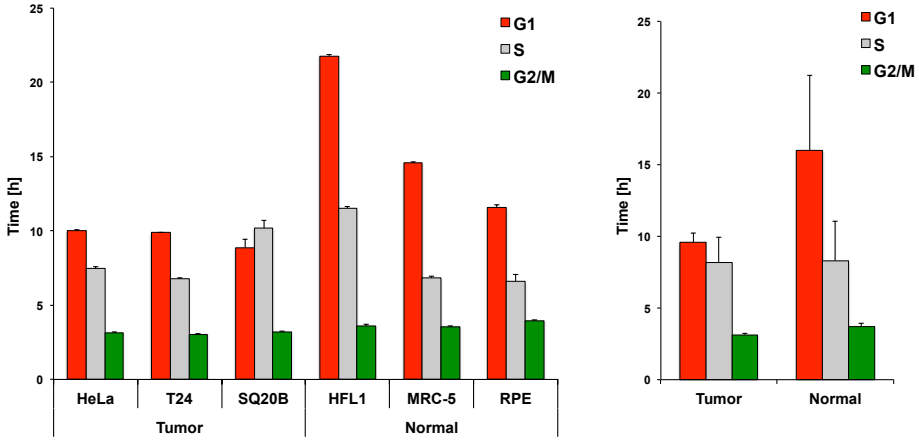
**B.** Confirmation of G2/M arrest in nocodazole-treated cells used for the Western blot analysis in panel A. Cultures were enriched for mitotic cells by overnight nocodazole treatment. Cells were then lifted, fixed in 70% ethanol and stained with propidium iodide followed by flow cytometry and curve fitting with ModFit software (red: G1; white: S; green: G2/M).




**Suppl. Fig. 3. Cell cycle progression in tumor cells**

Investigation of the cell cycle progression of untreated HeLa, T24 and SQ20B cells plated for colony formation assays. Cultures were grown for three days to 80% confluence, lifted and reseeded. Cells were then fixed for cell cycle analysis at the time points indicated. Representative histograms are shown. Graphs show the percentages in G1, S and G2/M phase as determined through ModFit curve fit analysis and plotted as mean +/- sd (n=2). No significant difference was found between the fraction of G2/M cells at t = 0 and at any of the subsequent time points (ns).

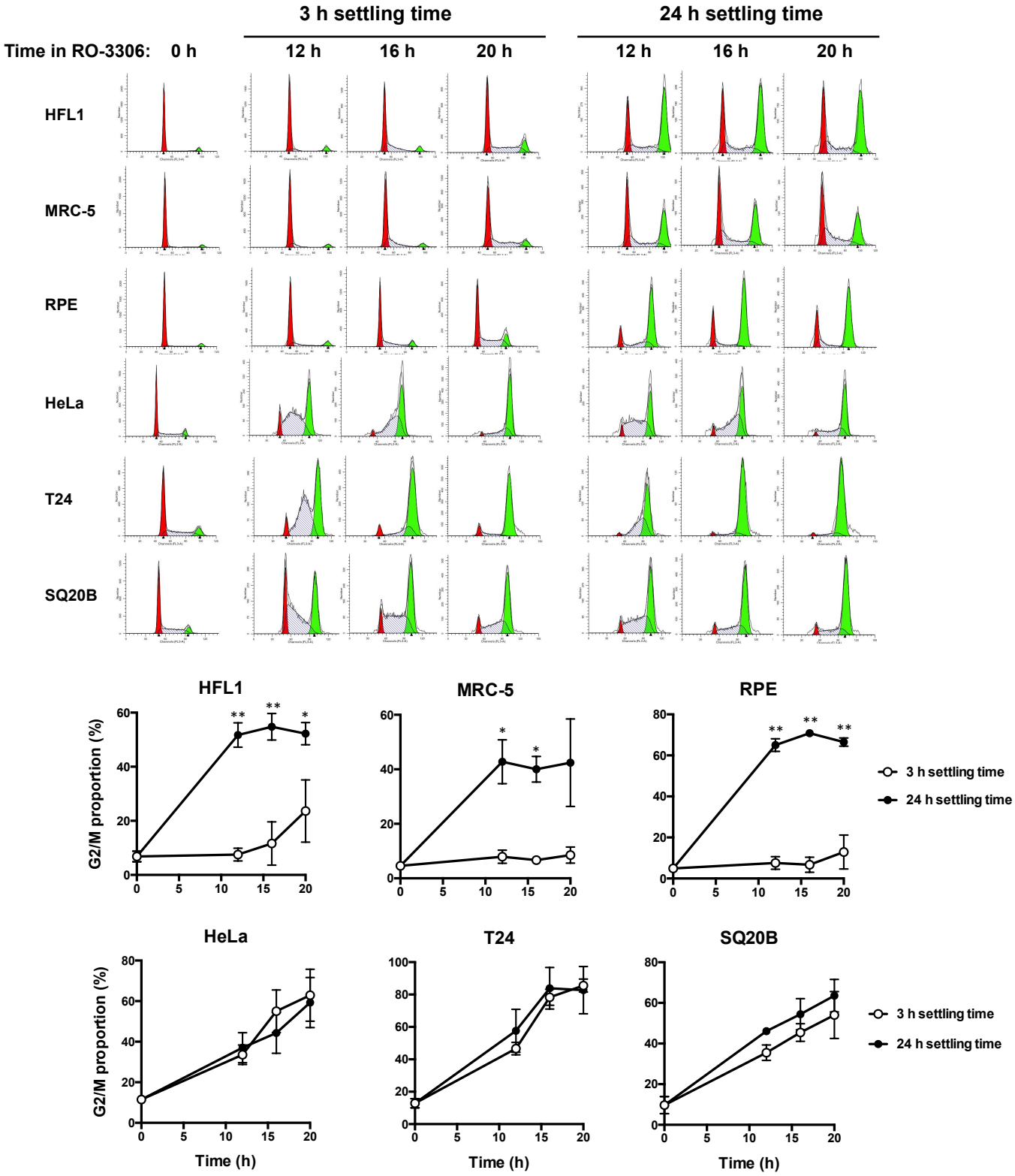


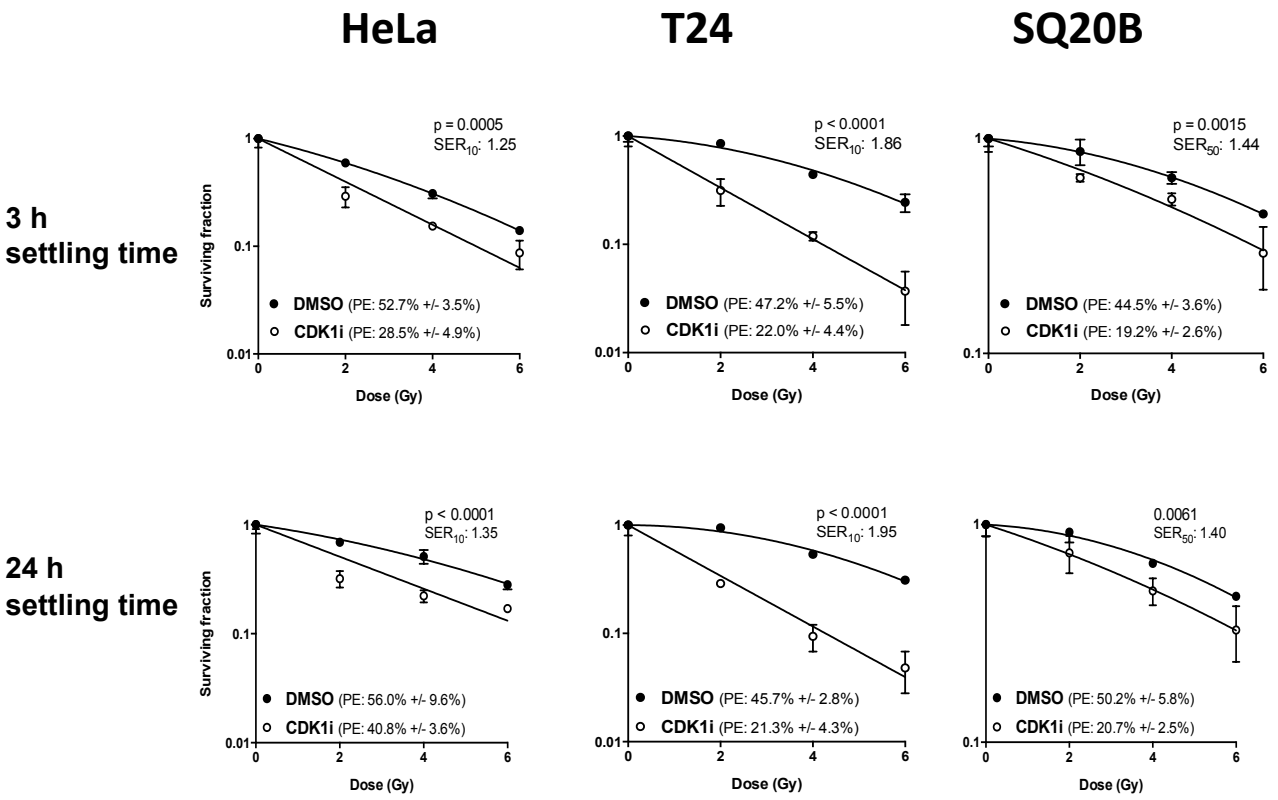

**Suppl. Fig. 4. Cycling tumor and normal cells have comparable cell cycle phase lengths**

A) Pulse chase BrdU/PI profiles of tumor and normal cells analyzed at different time points after replating. Cells were grown for three days to 80% confluence and pulse-labeled with BrdU for 30 min just prior to replating. Reseeded cells were fixed at 0 h, 4 h 10 h and 17 h after replating and stained for BrdU and DNA content.

B) Cell cycle phase duration in tumor and normal cells determined through the BrdU pulse chase experiment shown in (A). Values show the means of duplicate acquisitions +/- sd.




**Suppl. Fig. 5. CDK1 inhibitor-induced accumulation in G2/M is affected by settling time in normal cells.** Normal and tumor cell lines were grown for three days to 80% confluence, replated and treated with 5 µM RO-3306 added at 3 h or 24 h after seeding. Cell cycle profiles were analyzed at 12 h, 16 h and 20 h after RO-3306 addition. Graphs show the accumulation in the G2/M phase over time as determined through ModFit curve fit analysis and plotted as mean +/- sd from two independent experiments. Significant differences in G2/M proportion between the 3 h and 24 h settling times at equivalent RO-3306 incubation times are indicated (* $p < 0.05$; ** $p < 0.01$).




**Suppl. Fig. 6. CDK1 inhibition radiosensitizes tumor cells independently of settling time.** Clonogenic survival assay of tumor cells treated with RO-3306 at different time points after seeding followed by radiation. Cells were grown in T75 flasks for three days, lifted and plated as single cells in 6-well plates. RO-3306 (5 µM) was added either at 3 h after or 24 h after seeding, and cells were irradiated 20 h after compound addition followed by a medium change. Colonies were stained after 14 days. Representative of three independent experiments, data is presented as mean +/- SD from triplicate wells.