Differential sensitivity to CDK2 inhibition discriminates the molecular mechanisms of CHK1 inhibitors as monotherapy or in combination with the topoisomerase I inhibitor SN38

Supporting Information

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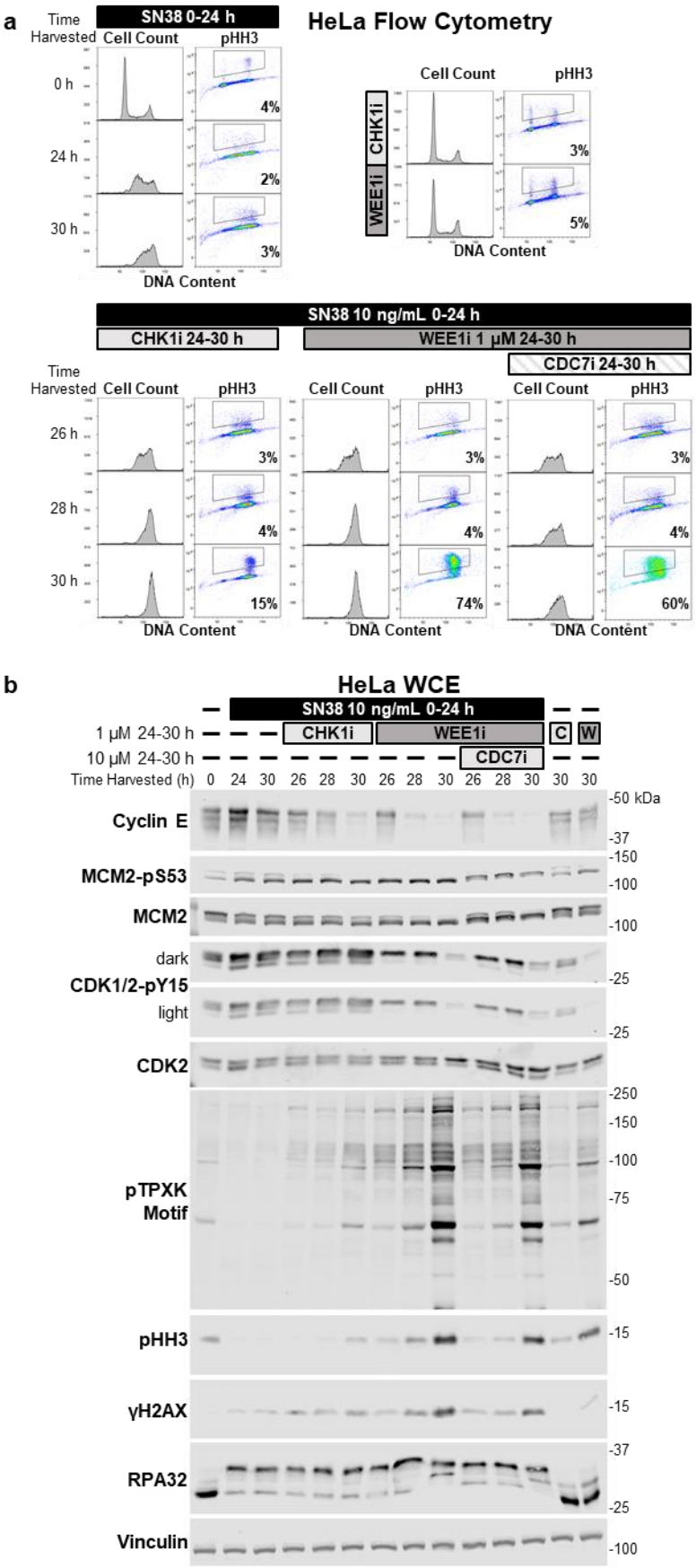

Figure S1. Kinetics of CHK1i and WEE1i-mediated activation of CDK1/2, induction of mitotic entry and DNA damage upon addition to SN38-arrested cells. (a) HeLa cells were incubated with SN38 for 24 h, then CHK1i or WEE1i were added and cells harvested at 26-30 h. Cells were immunostained with a fluorescent anti-pHH3 antibody and DNA was stained with propidium iodide, then analyzed by flow cytometry. The gates and percentages shown represent the proportion of cells positive for pHH3. (b) HeLa cells were incubated with SN38 for 24 h, then with CHK1i or WEE1i, and harvested at 26-30 h. Additionally, CDC7i was administered concurrently with WEE1i as indicated. Whole cell extracts were probed for the indicated proteins by western blot

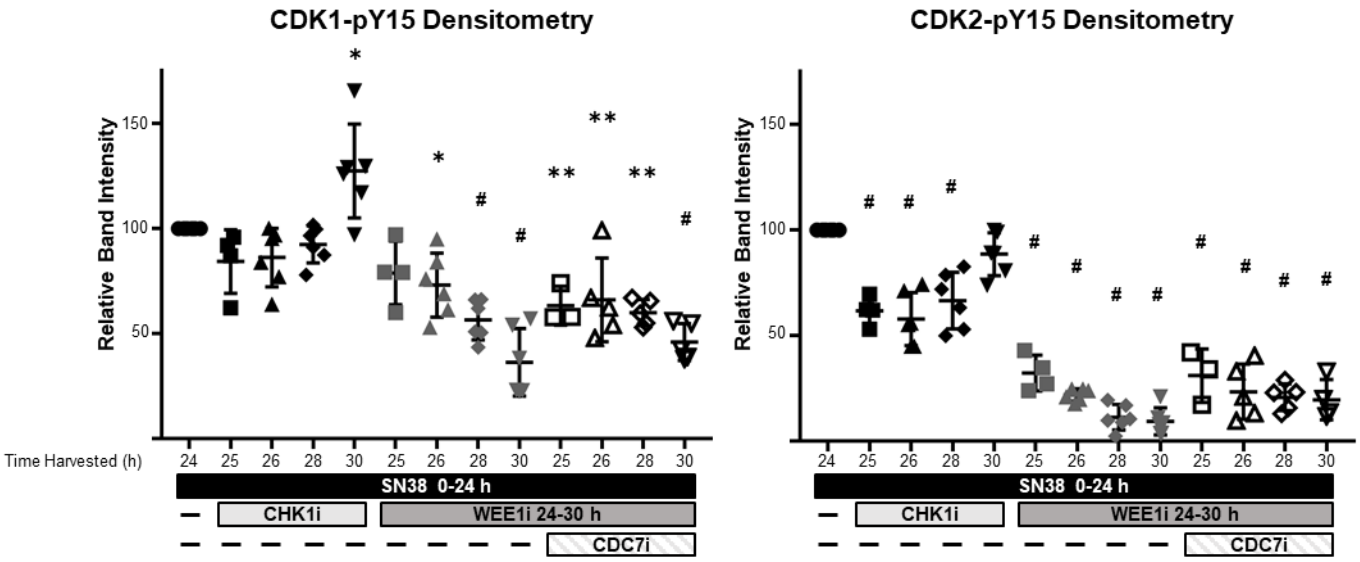

Figure S2. Densitometry of phospho-CDK1/2 blots. Scatter plots represent the mean ± SD intensity of replicate phospho-CDK blots as in Figure 5, normalized to 24 h following SN38 treatment. Significant differences from 24 h following SN38 are shown: * = p- value < 0.05, ** = p-value < 0.005; # = p-value < 0.0001, n is indicated by separate data points.

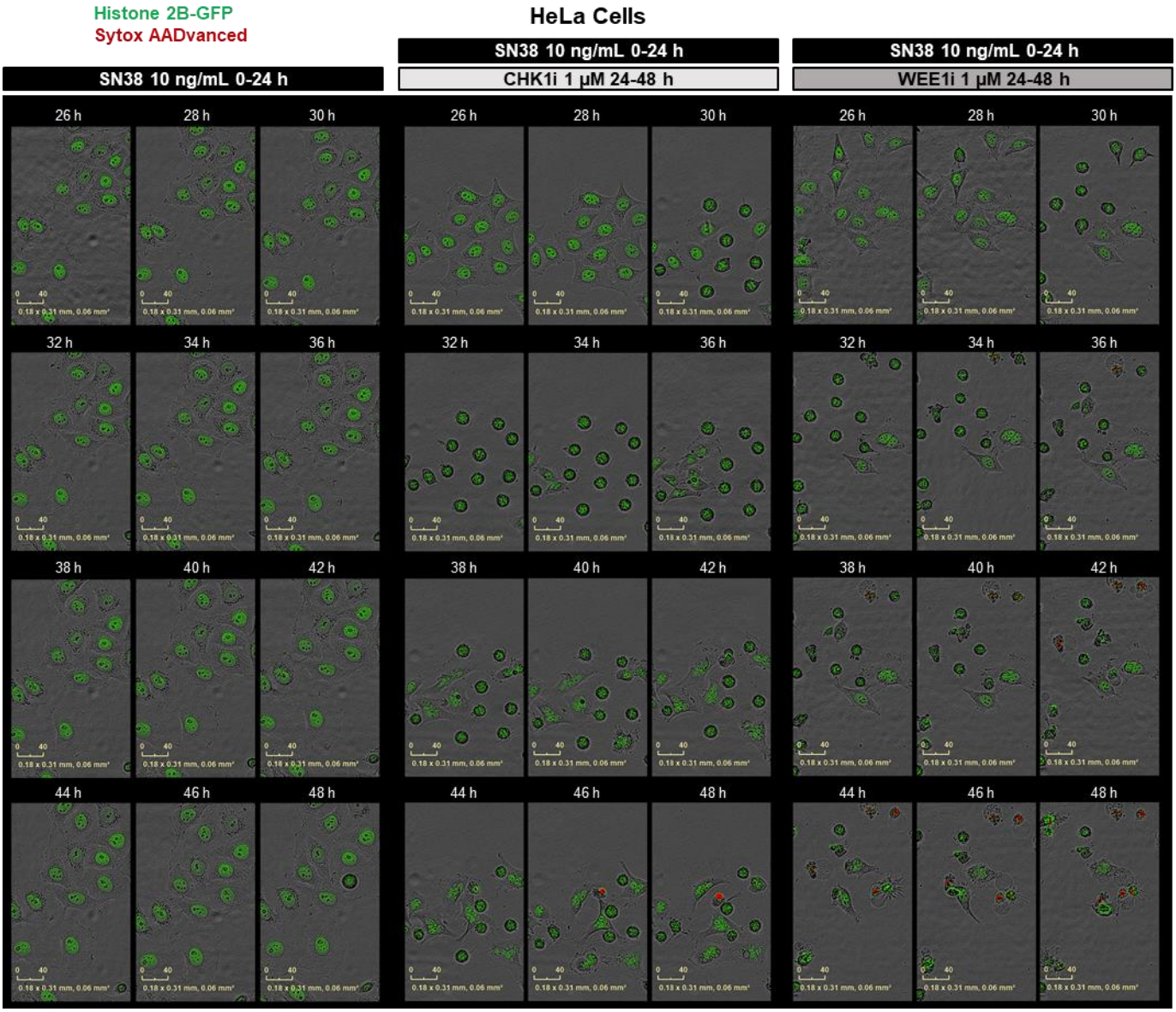

Figure S3. Images of HeLa H2B cells following addition of CHK1i, WEE1i or WEE1i plus CDC7i. HeLa cells stably expressing Histone 2B-GFP were incubated with SN38 for 24 h, then with CHK1i, WEE1i, or the combination of WEE1i and CDC7i for 24-48 h. Cells were imaged by the Incucyte Zoom system every 2 h between 26-48 h.

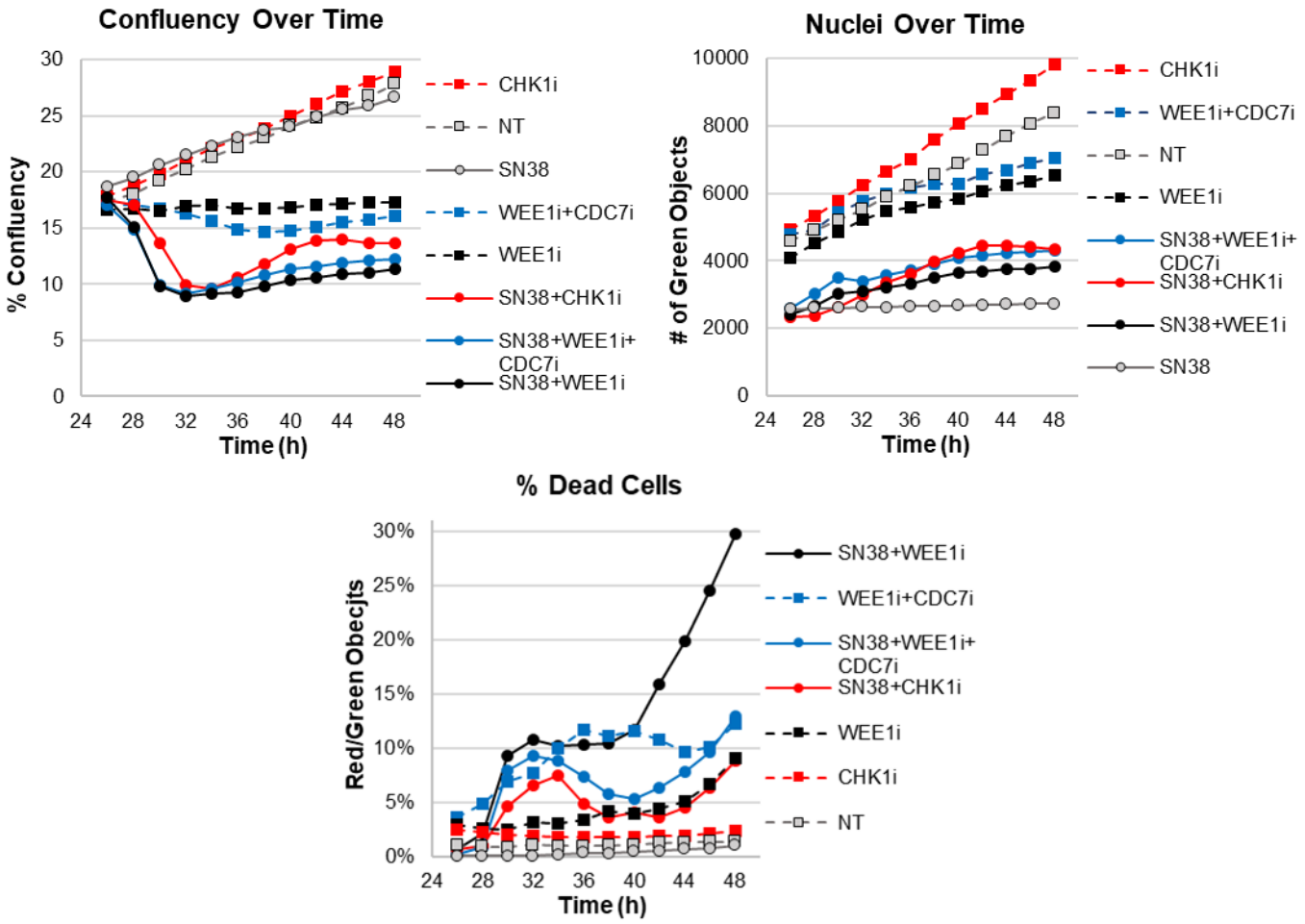

Figure S4. Quantification of events observed during the time course of HeLa cells stably expressing GFP-tagged Histone 2B. Cells were incubated as in Figure S3 and imaged every 2 h. Confluence was measured by bright field imaging of cellular area. Nuclei were measured by GFP intensity. Cell membrane permeability was measured by fluorescence of Sytox AAdvanced. Image data were processed by Incucyte Zoom software to identify objects. Data represent the mean of 2 independent replicates. The decrease in "confluency" between 28 and 32 hours reflects the cells rounding up, hence occupying less surface area on the plate.

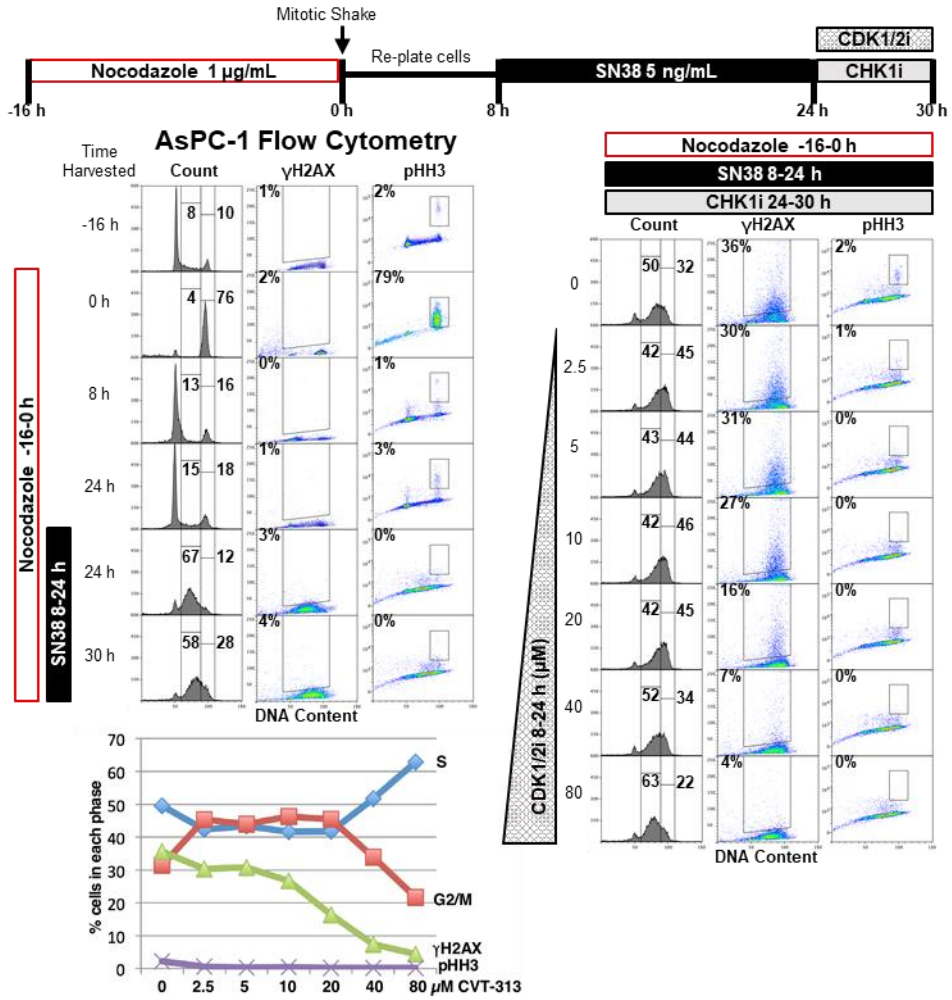

Figure S5. Impact of different concentrations of CDK1/2i on CHK1i-induced γH2AX, S phase progression and mitotic entry. Synchronized AsPC-1 cells were incubated with 5 ng/mL SN38 8-24 h after release from mitosis. CHK1i and CDK1/2i were administered 24-30 h at indicated concentrations. Cells were immunostained with fluorescent anti-pHH3 and anti-γH2AX antibodies and DNA was stained with propidium iodide then analyzed by flow cytometry. The gates, percentages, and line graph represent the proportion of cells positive for S phase DNA content, G2 DNA content, pHH3 or γH2AX. This is a repeat of the experiment in Figure 5 which used a lower concentration of SN38.

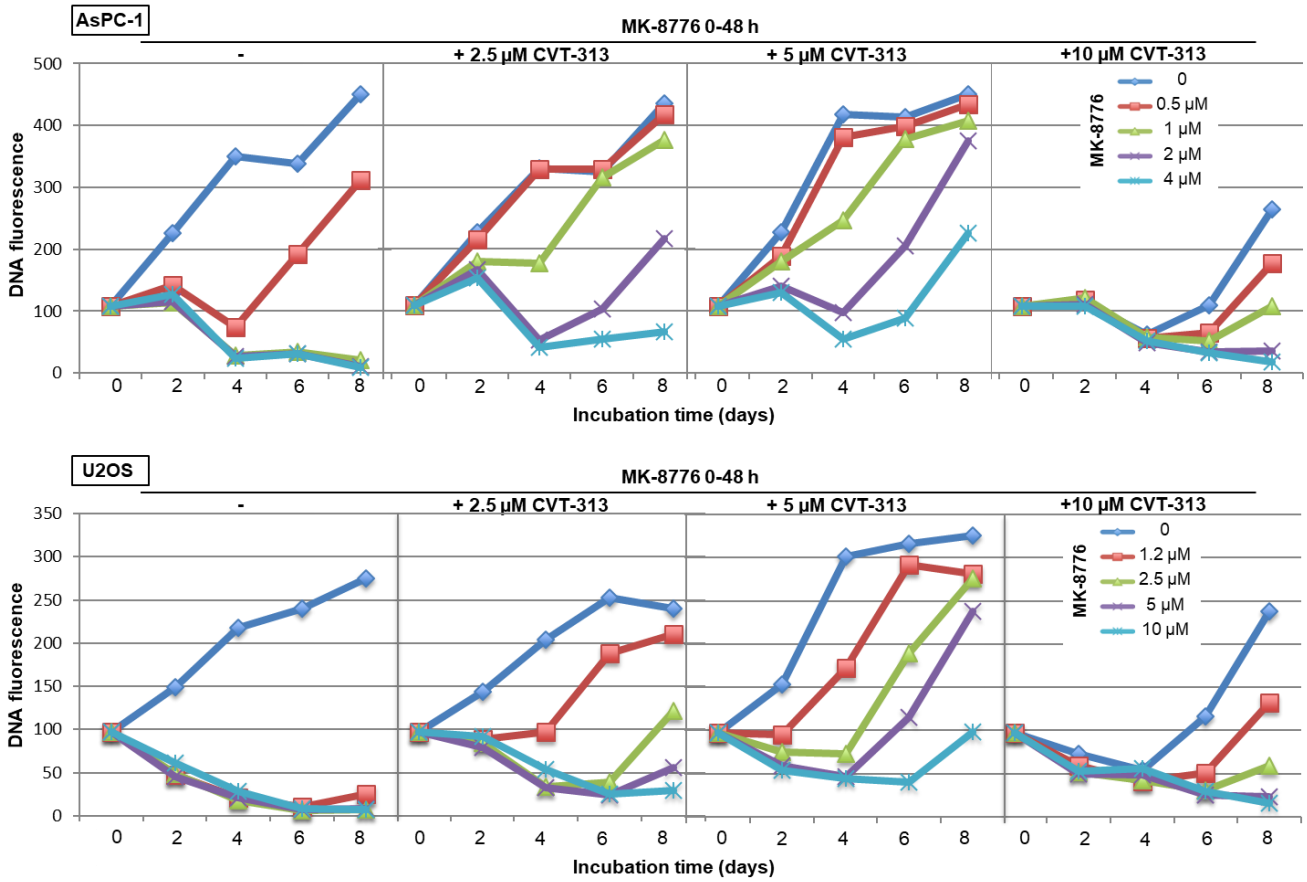

Figure S6. Low concentrations of CDK1/2i rescue cells from cytotoxicity induced by CHK1i. AsPC-1 (top) and U2OS (bottom) were incubated with the indicated concentrations of MK-8776 plus 0-10 µM CVT-313 for 48 h, at which time, the cells were washed and incubation continued in fresh media. Cells were harvested every 2 days, and analyzed for DNA content as an indication of cell number. Low concentrations of CVT-313 (2.5 and 5 µM) did not impede cell growth, but protected cells from cytotoxicity induced by MK-8776. At higher concentrations, CVT-313 itself inhibited cell growth.