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Supplementary Information

Figure S1. Increased CCNG1 expression accompanies nocodazole- and monastrol-induced mitotic arrest independent of p53 status. Asynchronous HCT116 and HCT116 $p53^{-/-}$ cells were treated with 10μM paclitaxel, 30ng/ml nocodazole or 100μM monastrol. **(a)** Cells were harvested after 16 h and stained with anti-MPM-2 antibody. The percentage of viable cells positive for MPM-2 staining is shown. **(b)** Extracts from samples of cells treated with nocodazole or monastrol were immunoblotted with anti-CCNG1 and anti-β-actin antibodies.

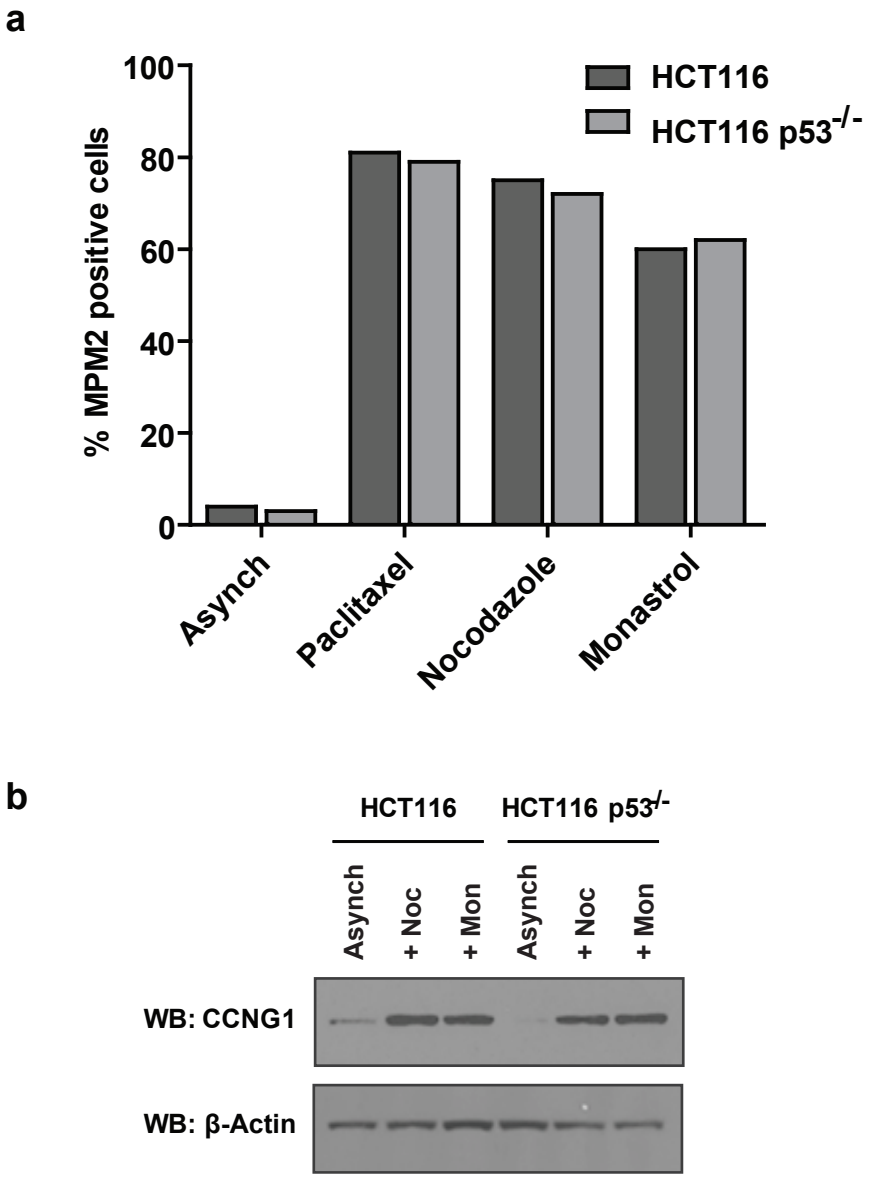
Figure S2. Reduced cell viability in paclitaxel-treated CCNG1-depleted cells is accompanied by an increase in apoptotic caspase activity. Asynchronous U2OS, HCT116 and and their isogenic p53-null counterparts exposed to CCNG1 or NT control siRNA were treated with paclitaxel as described above. **(a)** The effect of CCNG1 depletion on cell viability was assessed utilizing the CellTiter-Blue® Cell Viability Assay (Promega) over a three day period following paclitaxel treatment. Cell viability is expressed relative to the controls. **(b)** The level of apoptosis was measured using the Apo-ONE® Homogeneous Caspase-3/7 Assay (Promega) following standard protocols. Caspase activity is expressed as a ratio of the caspase-3/7 activity relative to the viability as measured in (a).Data points represent the mean of triplicate observations with the error bars representing a single standard deviation from the mean.

Figure S3. Correlation between mitotic timing and the fate of paclitaxel-treated CCNG1-depleted cells analyzed by serial time-lapse imaging. Asynchronous U2OS

cells were exposed to CCNG1 or non-targeting (NT) control siRNA as described prior to treatment with 10μM paclitaxel for 60 min. Individual cells were visualized by bright-field microscopy every 3-5 min from entry into mitotic prophase, indicated by characteristic changes in cell morphology, until anaphase, indicated by the onset of furrowing. The fate of the cells entering mitosis was visually determined and classified as: death directly from mitosis; failing cytokinesis to form a single polyploid nucleus; or completion of cytokinesis. The percentage of cells assuming each fate in CCNG1-depleted as compared to control cells was: death directly from mitosis, 8.6% (control) vs. 24.2% (siCCNG1); failing cytokinesis to form a single polyploid nucleus, 54.2% (control) vs. 30.3% (siCCNG1); completion of cytokinesis, 37.1% (control) vs. 45.4% (siCCNG1). The time taken to resolve the checkpoint was determined for cells in each cell fate category. There is a statistically significant increase in mitotic delay for paclitaxel-treated cells that die directly from mitosis or form a single polyploid nucleus following CCNG1 depletion (●) as compared to the control (▲) ($p<0.05$, Mann Whitney test). The mean is indicated with the error bars representing a single standard deviation from the mean. Results shown are typical of at least two independent experiments.

Figure S4. Increased CCNG1 expression accompanies paclitaxel-induced SAC mediated mitotic arrest in the non-cancerous RPE1 cells. Asynchronous RPE1 cells were treated with 10μM paclitaxel for 60 min. **(a)** Cells were harvested over 48 h and immunoblotted with anti-CCNG1 and anti-β-actin antibodies. β-actin is used as a loading control here and in other experiments. **(b)** Samples of the cells described in (a) were stained with mitotic phosphoprotein monoclonal antibody, MPM-2 and propidium iodide (PI) to measure DNA content, before analysis by flow cytometry. The percentage of viable cells with 4N DNA content, or positive for MPM-2 staining, is shown. CCNG1 depletion prolongs paclitaxel-induced mitotic arrest and increases drug-induced cell death. **(c)** Asynchronous RPE1 cells exposed to CCNG1 or NT

control siRNA were treated with paclitaxel, and harvested 12 h afterwards. Relative CCNG1 mRNA expression was quantitated by semi-quantitative RT-PCR. The graph shows the mean ± standard error (SEM) from triplicate experiments. **(d)** Extracts from samples of the cells described in (a) were immunoblotted with anti-CCNG1 and anti-β-Actin antibodies. **(e)** The effect of CCNG1 depletion on cell viability was assessed utilizing the CellTiter-Blue® Cell Viability Assay (Promega) over a three day period following paclitaxel treatment. Cell viability is expressed relative to the controls. Data points represent the mean of triplicate observations with the error bars representing a single standard deviation from the mean.



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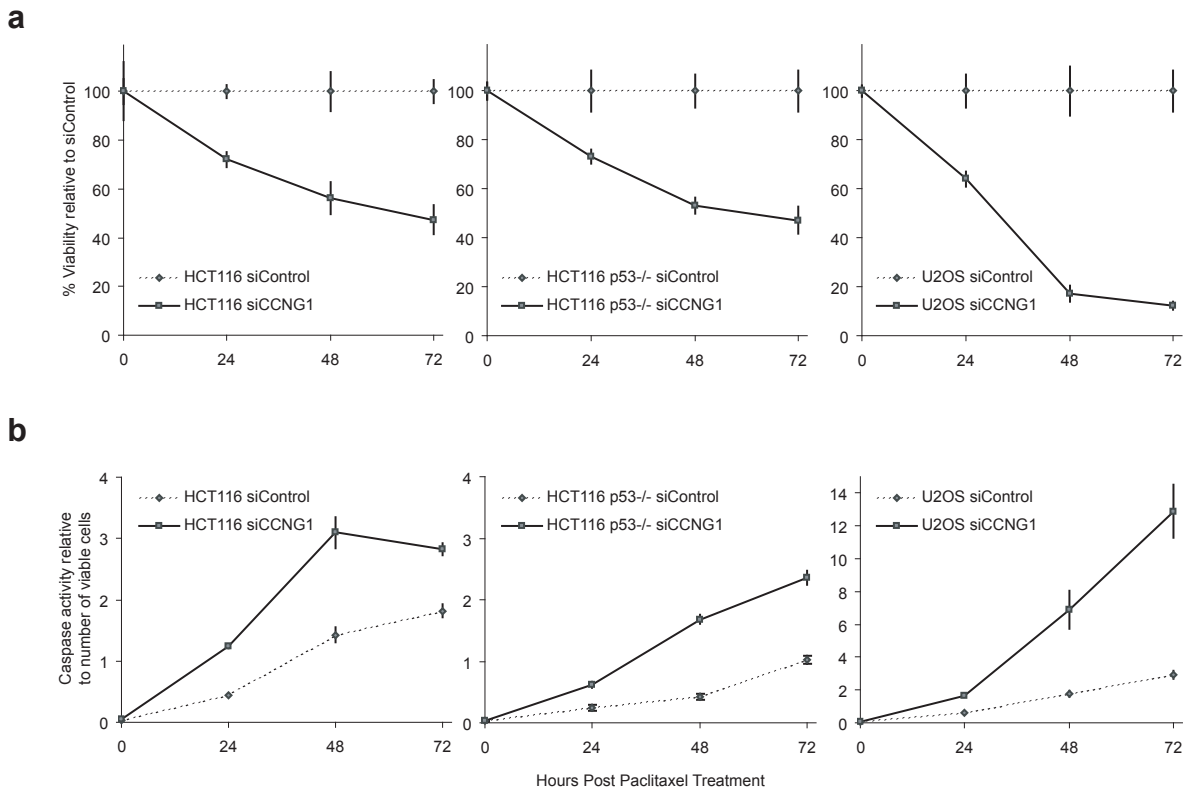

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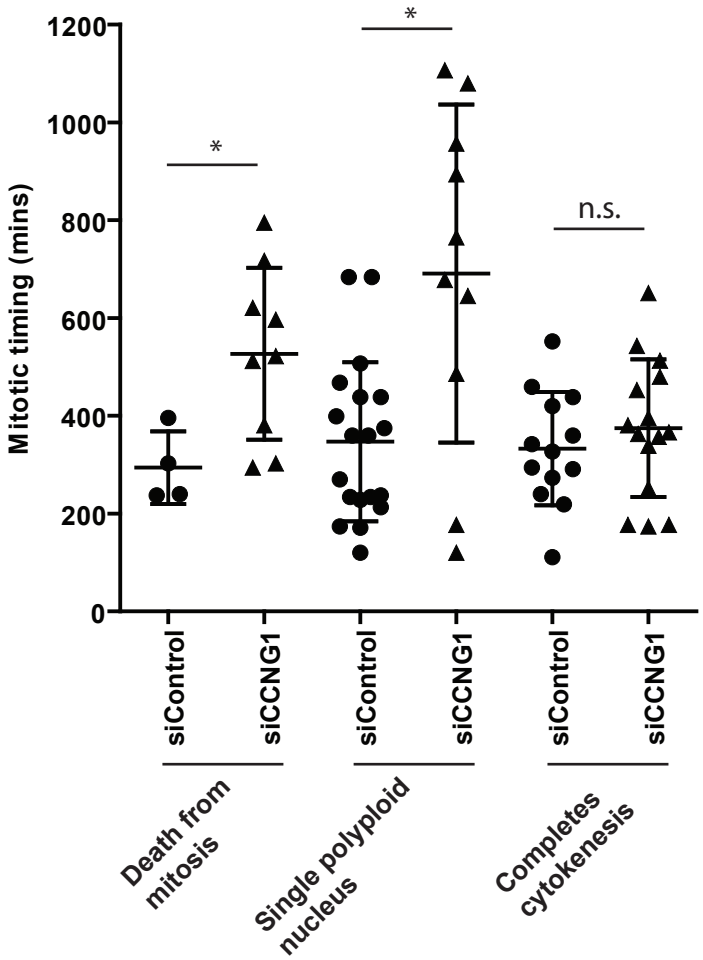

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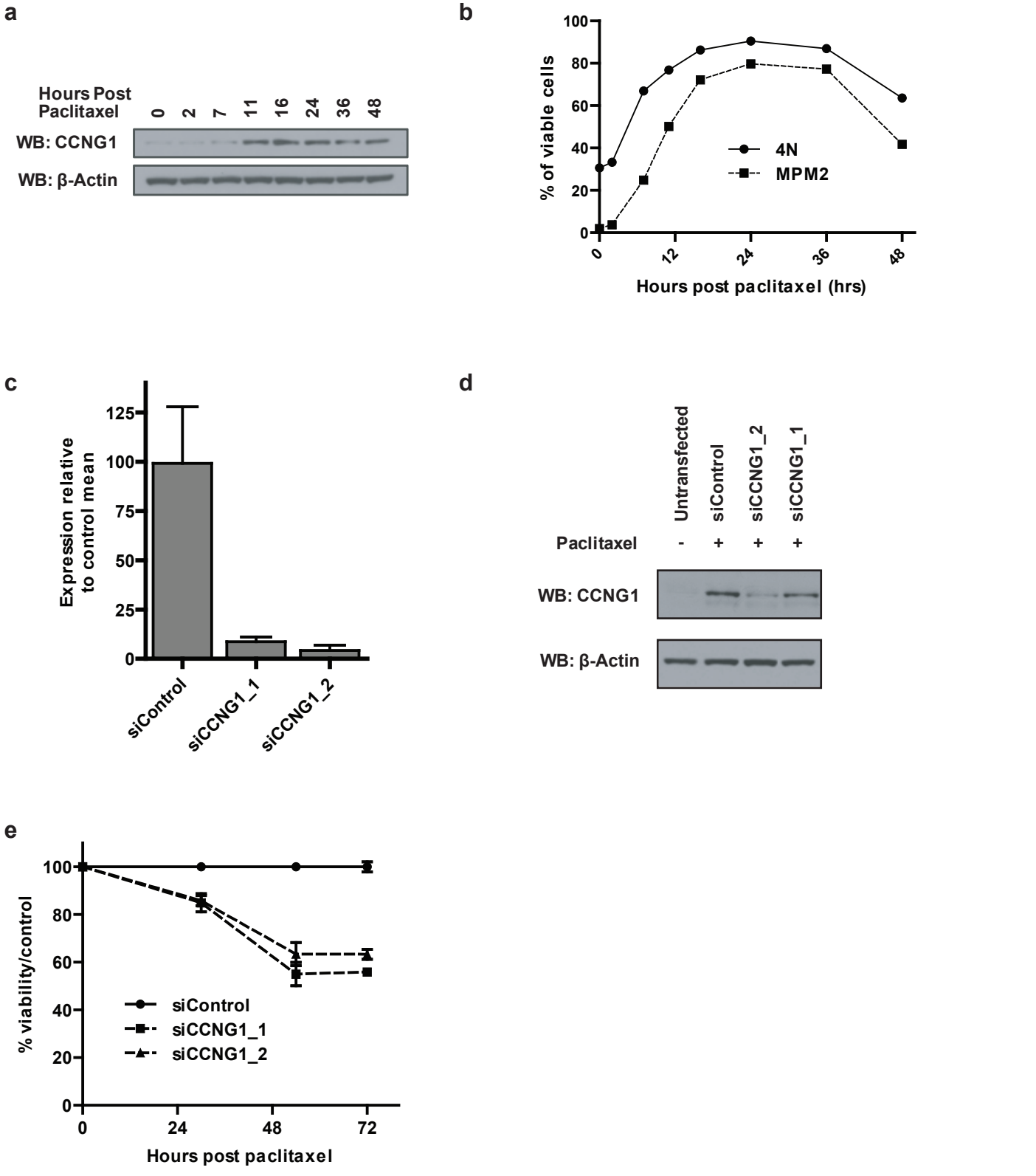

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