Supplementary Figure Legends

**Supplementary Figure 1.** Comparison of assays for measuring CHK1 potentiation of cytotoxic agents in vitro. **A,** Determination of CCT244747 GI\(_{50}\) alone or in combination with a fixed concentration (GI\(_{50}\)) of gemcitabine or SN38 in SW620 colon tumor cells in vitro. The potentiation index (PI, ratio of SRB GI\(_{50}\) of CCT244747 alone : Combination GI\(_{50}\)) was 4.0 and 14.8 for SN38 and gemcitabine, respectively (see Table 1 for mean±SD values). Survival curves for combinations were corrected to 100%. **B,** Determination of SN38 or gemcitabine GI\(_{50}\) alone or in combination with a fixed concentration of CCT244747 (1µM) in SW620 colon tumor cells in vitro. The potentiation index (PI, ratio of SRB GI\(_{50}\) of genotoxic alone : genotoxic + 1µM CCT244747) was 1.4 and 3.2 for gemcitabine and SN38, respectively. PI values for several independent experiments as shown in B were 1.2±0.058 and 2.9±0.33 (mean±SD, n=4) for SN38 and gemcitabine, respectively.

**Supplementary Figure 2.** A constrained, scaffold docking model of CCT244747 in the ATP pocket of human CHK1 realized using the SAR-020106 X-ray crystal structure (PDB 2ym8).

**Supplementary Figure 3** Characterisation of the effects of minimally toxic concentrations of CCT244747 alone or in combination with genotoxic agents (+) in HT29 and SW620 colon cancer cell lines. **A,** HT29 cells were treated with SN38 (100nM) or CCT244747 alone or in combination for 24h. **B,** SW620 cells were treated with gemcitabine (200nM) or CCT244747 alone or in combination for 24h. Cells were pre-treated with CCT244747 alone 1h prior to cytotoxic exposure. Protein expression was assessed by western blotting (40µg per lane) as described in Figure 2 and Materials and Methods.

**Supplementary Figure 4.** Effects of scheduling and exposure times on the ability of two different CHK1 inhibitors to potentiate gemcitabine cytotoxicity in SW620 cells *in vitro.* **A,** Effect of different contact times on the capacity of SAR-020106 to enhance gemcitabine cytotoxicity in SW620 cells. Gemcitabine was continuously present (96h) at an IC\(_{50}\) concentration and SAR-020106 was added at the start of treatment over a range of concentrations for the intervals shown (i.e. 0-T\(_1\), 0-T\(_2\), 0-T\(_3\), etc). Growth delay and potentiation were measured using an SRB assay as described in Materials and Methods. Potentiation Index (PI) was expressed as a percentage of the maximum PI. Values are mean±SE, for n=3-5 independent experiments. **B,** Treatment of synchronized SW620 cells with gemcitabine and SAR-020106. Cells were
synchronized with nocodazole (100ng/ml x 16h). Eight hours following release (at G1/S boundary) cells were treated with a fixed concentration of gemcitabine (GI_{50}) for 96h combined with different concentrations of SAR-020106 for 0-96, 0-48 or 0-24h. Values are mean±SE for 3 independent determinations. **C**, Effects of contact time on the potentiation of gemcitabine cytotoxicity in synchronized compared to asynchronous SW620 cells. See **B** and Materials and Methods for further details. Values are mean±SE, n=3-5. **D**, Effects of different contact times and treatment schedules on the ability of CCT244747 to enhance gemcitabine cytotoxicity in SW620 cells. Values are mean±SE, for 3-7 independent experiments.

**Supplementary Figure 5.** Effects of acute CCT244747 administration on non-tumor bearing GEMM mouse body weights. CCT244747 was administered at a dose of 150mg/kg p.o. x 7days and mouse body weights were measured daily. Results are mean±SD body weights for 5 mice. There was no apparent body weight loss, toxicity or morbidity following this treatment and hemizygous MYC-N tumor bearing GEMM mice were therefore treated with 100mg/kg CCT244747 p.o. x 7 days (see Figure **5**).