**Supplementary Legends**

**Figure S1.** *In vitro* profiling of SCH900776 and its selected analogs. A, *in vitro* kinase assay, data from Eurofins KinaseProfiler service for SCH900776, MU378 and MU380. B, Comparative efficacy of SCH900776 and indicated analogs (in both enantiomeric forms) in U2OS cells. Cells were treated with HU overnight (1 mmol/L) prior to treatment with indicated concentrations of the CHK1 inhibitors for 2 hours. Cells were allowed to grow in drug free medium post drug treatment for 72 hours and then assessed for cell survival by WST assay. Data represented as mean values ± SD from 3 different experiments. C, Cells immediately harvested after the treatment were analyzed by western blot for phosphorylation of CHK1 at pS296 and β-actin as a loading control.

**Figure S2.** Analogs of MU380 with the N-trifluoromethylpyrazol pharmacophore. A, Schematic representation of the chemical structures. B, U2OS cells were treated first with HU (1 mmol/L) overnight followed by treatment with indicated concentrations of the individual compounds. Cells were allowed to grow in drug free medium post drug treatment for 72 hours and then assessed for cell survival by WST assay. C, Efficacy of the CHK1 inhibitors in U2OS cells in the presence of metabolite MU379. Cells were treated with HU overnight (1 mmol/L) prior to treatment with CHK1 inhibitors (2 µmol/L) and increasing concentrations of MU379 for 2 hours. Cells were allowed to grow in drug free medium post drug treatment for 48 hours and then assessed for cell survival by WST assay. Data represents means of three independent experiments normalized as % of control (vehicle). P-value for comparison between groups (P<0.05), \* ~ HU+SCH900776 vs. HU+SCH900776+MU379, # ~HU+MU380 vs. HU+MU380+MU379.

**Figure S3.** Dot plots of flow cytometric analysis using H2AX ( A) and Annexin V staining (B) related to Figure 2D, E. Percentages of positively stained cells are represented as mean (n=3). C, U2OS cells were depleted of CHK1 using lentiviral delivery of shRNA. Cell lysates of control shGFP-U2OS and CHK1 depleted shCHK1-U2OS cells were analyzed for total CHK1 levels. shGFP-U2OS and shCHK1-U2OS cells were treated with HU (1 mmol/L) overnight or left untreated and then harvested after 48 hours for cell survival by WST assay.

**Figure S4.** Dose response curves of relative viability of the tested cell lines treated with CHK1 inhibitors at indicated concentrations in combination with HU in the concentration range shown on the x-axis (related to Figure 3A).

**Figure S5.** Dynamics of cytotoxic effects of CHK1 inhibitors alone and in combination with HU. Bioimpedance was measured using xCELLigence® system for MDCK (A) and DU145 (C) cell lines shown as cell index (CI) over the indicated time interval. Cell lines were treated with HU (0.3 mmol/L for MDCK and 0.2 mmol/L for DU-145) for 24 hours and then for 2 hours with CHK1 inhibitors (4 µmol/L). Data are shown from the point of media exchange and normalized to non-treated controls. Quantification of cell index relative to control at time 48 hours after media exchange for MDCK (B) and DU 145 (D) cell lines. E, A representative Hela.S-FUCCI trace of cells treated as indicated (GEM 0.5 µmol/L, CHK1 inhibitors 0.5 µmol/L). Red, G1-phase ~ mKO-hCdt1 (30/120), green, S/G2/M-phase ~ mAG-hGeminin (1/110). Acquired data were analyzed using TrackMate FIJI plugin. F, Times between cell divisions were extracted from obtained tracks and median value for each treatment was calculated. Length of cell phases G1 (G) and S/G2/M (H) were calculated for individual cells.

**Figure S6.** Dose response curves of relative viability of the tested cell lines treated with CHK1 inhibitors at indicated concentration in combination with GEM in concentration range shown on the x-axis (related to Figure 3B).

**Figure S7.** Dose response curves of relative viability of the tested cell lines treated with CHK1 inhibitors at indicated concentration in combination with irradiation in the dose range shown on the x-axis.

**Figure S8.** Western blot analysis of total CHK1 and p-CHK1 (S296) levels in a panel of selected cell lines after indicated treatments. Cell lines were treated with GEM at corresponding EC50 values (see Table S2) for 24 hours. CHK1 inhibitors SCH900776 and MU380 at concentration 4 µmol/L were added for 2 hours where indicated, after the following recovery in drug-free media for 2 hours, cells were harvested for analysis.

**Figure S9**. Drug retention analysis. A, Unsupervised hierarchical clustering of sensitivity ratio for MU380 and SCH900776 obtained from Figure 3A, B with drug retention index (DRI) determined by measurement of the active efflux of specific fluorescent substrates (JC1 and mitoxantrone) and specific inhibitor of BCRP protein (fumitremorgin C). DRI is defined as: median fluorescence of individual probe divided by median fluorescence of probe in combination with drug efflux inhibitor (FTC). Data are shown as means in log scale from at least three biological replicates. B, Correlation sensitivity ratio for MU380 and SCH900776 determined from Figure 3A, B and DRI (Spearman’s correlation). Significant correlation (p<0.05) are highlighted in red.

**Figure S10**. MU380 induces CHK1 pS345 in mice hair follicles. Skin samples were harvested 0.5, 2, 8 and 24 hours after dosing (30 mpk), fixed, sectioned and stained with anti-CHK1 pS345. A, Representative images from skin sections. Scale bars represent 100 µm. B, Slides were scanned and analyzed using image analysis approach. Data are presented as % of high positive CHK1 pS345 nuclei (≥0.75 of maximum DAB signal). C, Table summarizing number of identified objects plotted in B. D, Extended data for Fig. 5C.

**Figure S11.** A, Bioluminescent signal of SHO mice bearing MiaPaCa2 luc xenografts treated with GEM (150 mpk), SCH900776 (25 mpk), or MU380 (25 mpk) alone or in indicated combinations on days 7, 12 and 17 (n=3-5). CHK1 inhibitors were administrated 4 hrs after GEM treatment. Data are mean and s.e.m.,\* ~ p<0.05 vs. vehicle. B, Weight of MiaPaCa2 luc tumor-bearing animals (mean ± S.D.). C, Table summarizing number of identified objects plotted in Fig. 5D. D, Representative images of γH2AX staining in MiaPaCa2 luc tumor sections. Mice were treated with single bolus as described above and tumors were dissected 24 hrs after the treatment.