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online data

SUPPLEMENTARY ONLINE DATA

Human cells enter mitosis with damaged DNA after treatment with pharmacological concentrations of genotoxic agents

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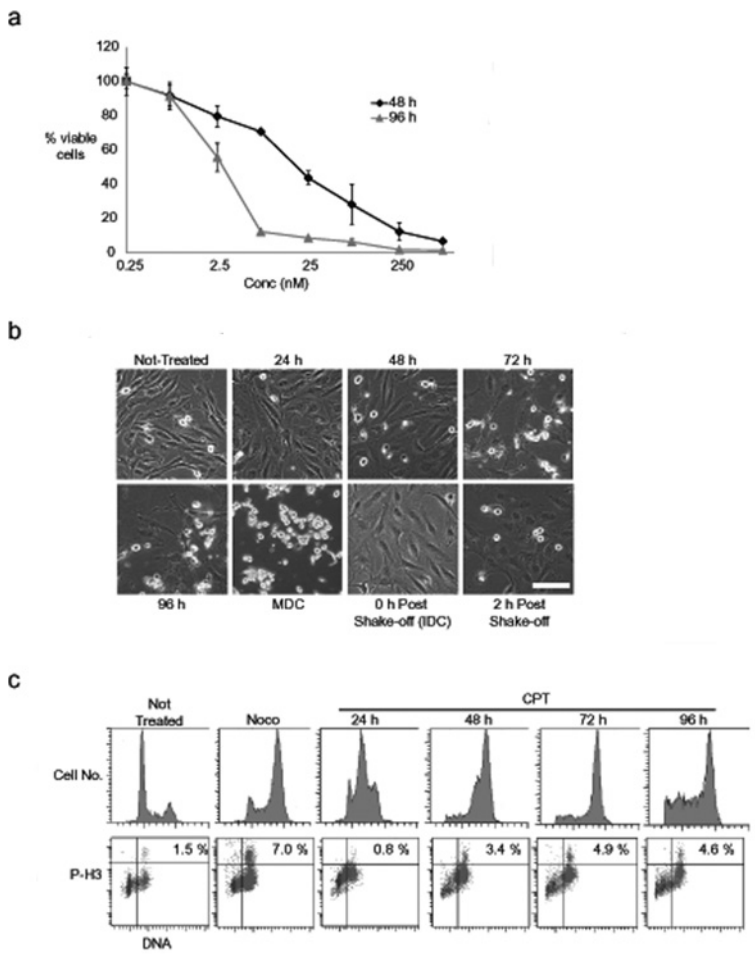

Figure S1 M059K cells treated with CPT are alive at 48 h and enter mitosis

(**a**) Human fibroblastic glioma cells (M059K) were treated with graded concentrations of CPT for either 48 h (◆) or 96 h (▲) and viability was measured by the MTT assay. Results are mean±S.E.M. percentages of live cells from triplicate experiments. M059K cells were either not treated or treated with 25 nM CPT and observed by phase-contrast light microscopy at 24, 48, 72 and 96 h. Rounded cells were visible in non-treated samples, but rarely at 24 h after treatment and commonly by 48 h after treatment. Rounded cells were collected after mechanical shake-off (MDC) leaving behind flattened interphase cells (IDC). New rounded cells appeared within 2 h in the adherent culture. Scale bar, 100 μm. (**c**) Cells were either not treated (NT) or treated with nocodazole (Noco) or 25 nM CPT for 24, 48, 72 and 96 h. Samples were analysed by flow cytometry for DNA content and for phospho-Ser^{10} histone H3 signals. The percentage of cells positive for phospho-Ser^{10} histone H3 (P-H3) is listed in the upper right-hand quadrant. DNA content was determined by propidium iodide staining.

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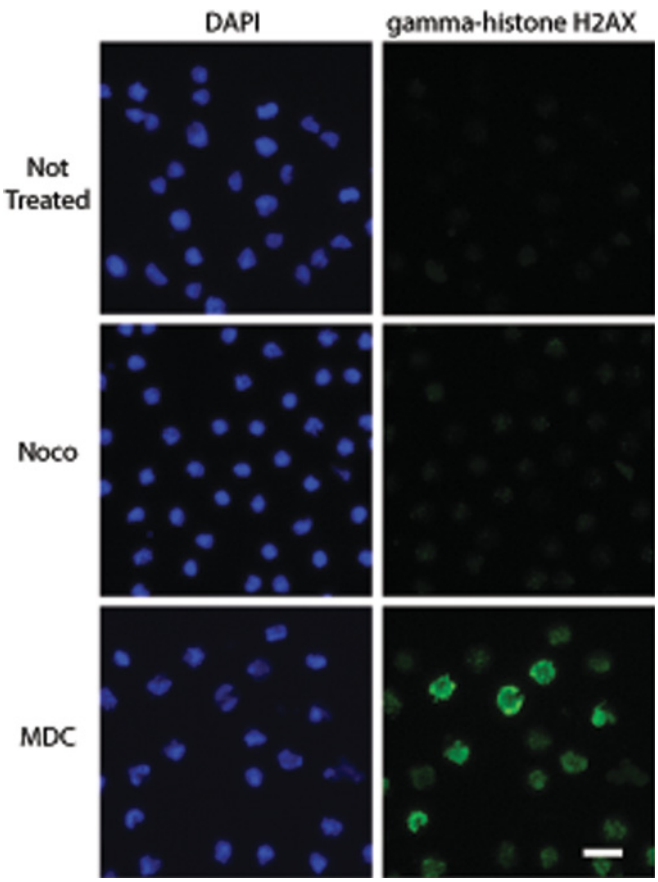

Figure S2 MDCs can be distinguished from nocodazole-induced mitotic cells by γH2AX staining

Cells were not treated, treated with nocodazole (Noco) or treated with CPT. Mitotic cells were collected after nocodazole or CPT treatment by mechanical shake-off, plated on to coverslips and stained with DAPI (left-hand panels) and γH2AX antibodies (right-hand panels). Cells were observed by immunofluorescence microscopy under identical detection conditions. Scale bar, 10 μm.

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