Reduced Level of Ribonucleotide Reductase R2 Subunits Increases Dependence on Homologous Recombination Repair of Cisplatin-Induced DNA Damage

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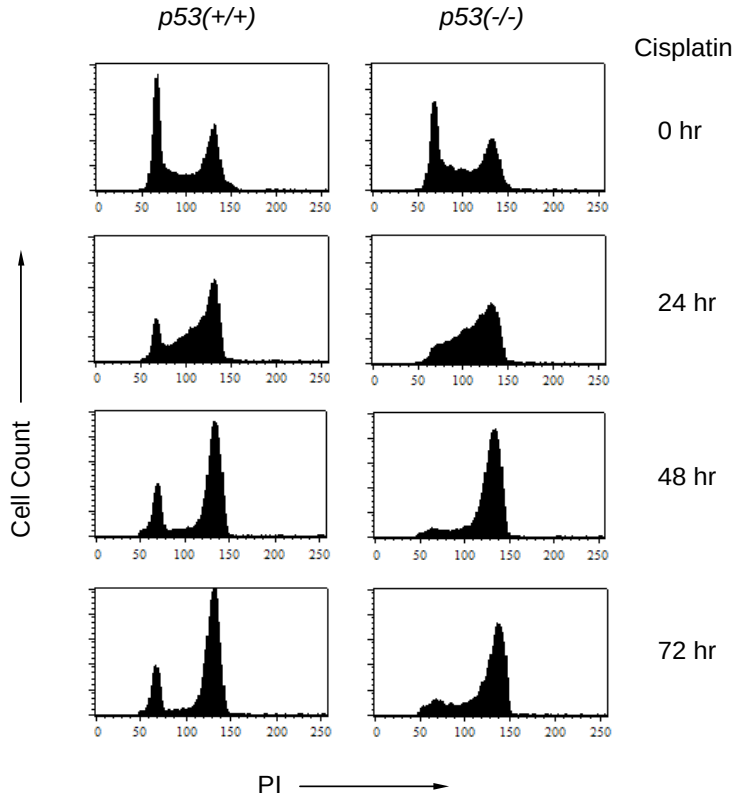
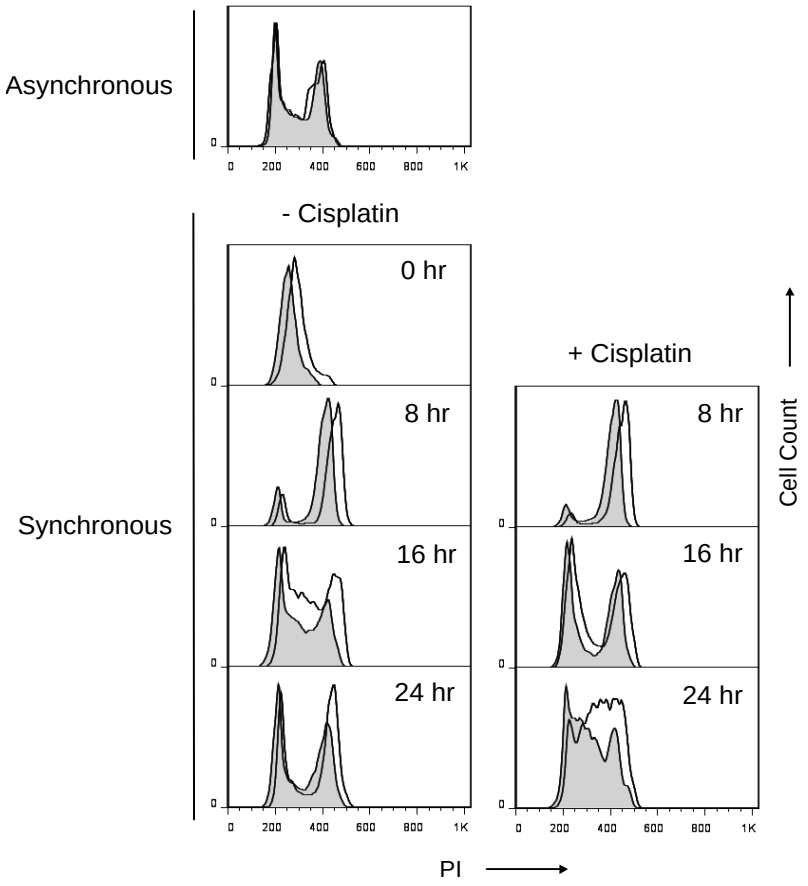
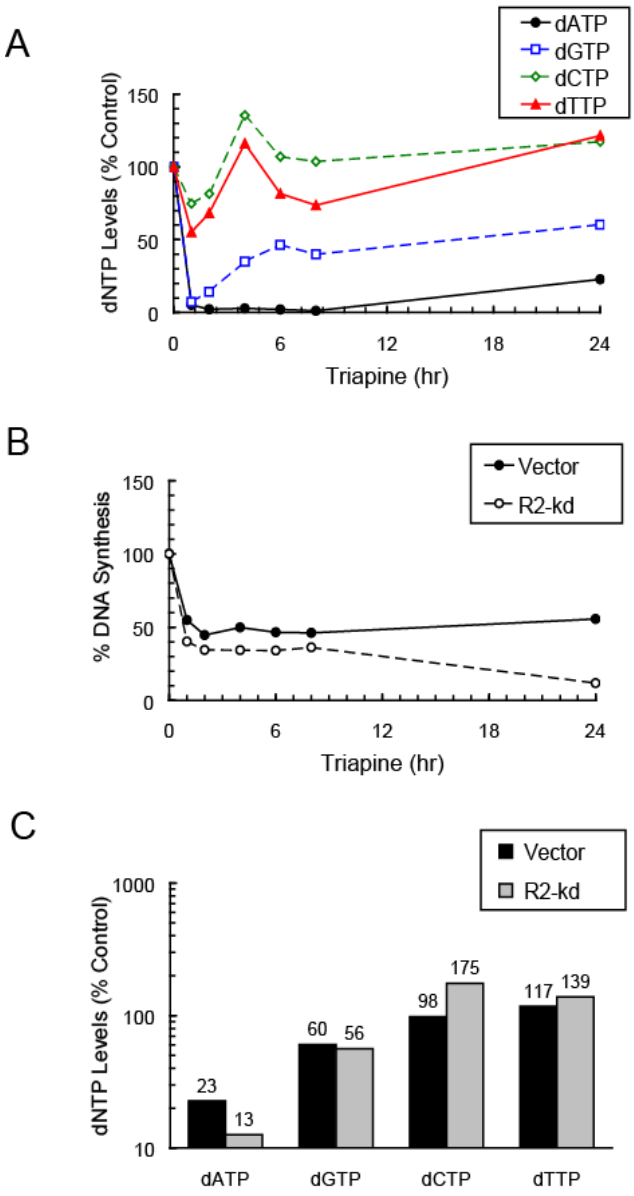
Molecular Pharmacology



Supplemental Fig. 1. Cell cycle distribution of p53(+/+) and p53(-/-) HCT116 cells following cisplatin treatment. Cells were treated continuously with 20 μM cisplatin for 0, 24, 48, and 72 hr. Subsequently cells were collected, fixed, stained with PI, and analyzed for DNA content by flow cytometry.



Supplemental Fig. 2. Cell cycle progression of synchronous p53(-/-) HCT116 cells in the presence of cisplatin. Vector (Solid-outline) and R2-knockdown (grey-shaded) cells were synchronized at early S phase. One hr prior to the release from the block, cells were left untreated or treated with 15 μM cisplatin. Cells were then released from the block by incubation with fresh medium in the absence (left column) or the continuous presence (right column) of 15 μM cisplatin. Cells were then collected at 0, 8, 16, and 24 hr following release from the block. Cells were fixed, stained with PI, and analyzed for DNA content by flow cytometry. Untreated, asynchronous cells exhibited a typical 2N-4N DNA content indicating a G1-S-G2/M cell cycle profile for comparison. Async, asynchronous cells; Sync, synchronous cells. Hr indicates the time after the release from the block.



Supplemental Fig. 3. Effects of Triapine on dNTP levels and replicative DNA synthesis in asynchronous p53(-/-) HCT116 cells. A. Triapine caused rapid and prolonged suppression of the levels of dATP and dGTP in vector cells. Cells were treated with 1 μM Triapine for the indicated times and collected for measurements of dNTPs. Each of the dNTP levels is expressed as a percentage of that of the respective untreated control at the indicated time. B. Time course of suppression of replicative DNA synthesis by Triapine. Cells pre-labeled with [14C]- thymidine were treated with 1 μM Triapine for the indicated times. During the last hr, cells were pulsed with 1 μCi/ml of [3H]-thymidine, washed, lysed, and measured for incorporation of labeled thymidine to determine the percentage of DNA synthesis as previously described (Lin et al., 2007). C. Triapine produced a greater reduction in the level of dATP in R2-knockdown cells. Cells were treated with 1 μM Triapine for 24 hr and collected for measurements of dNTPs. Each of the dNTP levels is expressed as a percentage of the respective untreated control, which is indicated above the bar graph.