

Supplemental Data

Cisplatin in Combination with MDM2 Inhibition Downregulates Rad51 Recombinase in a Bimodal Manner to Inhibit Homologous Recombination and Augment Tumor Cell Kill

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Molecular Pharmacology

Supplemental Table S1. Statistical analysis by post hoc Tukey of Rad51 mRNA expression following exposure of A2780 cells to cisplatin, Nutlin-3 or the combination

Control vs. Cisplatin	Diff = -0.0543	95% CI = -0.2426 to 0.1341	p = 0.7939
Control vs. Nutlin-3	Diff = -0.2853	95% CI = -0.4736 to -0.0969	p = 0.0056
Control vs. Combination	Diff = -0.6130	95% CI = -0.8014 to -0.4246,	p = 0.0000
Cisplatin vs. Nutlin-3	Diff = -0.2310	95% CI = -0.4193 to -0.0426,	p = 0.0184
Cisplatin vs. Combination	Diff = -0.5587	95% CI = -0.7471 to -0.3704	p = 0.0000
Nutlin-3 vs. Combination	Diff = -0.3278	95% CI = -0.5161 to -0.1394	p = 0.0023

Results were subjected to one-way ANOVA and then Tukey post hoc. CI, confidence interval.

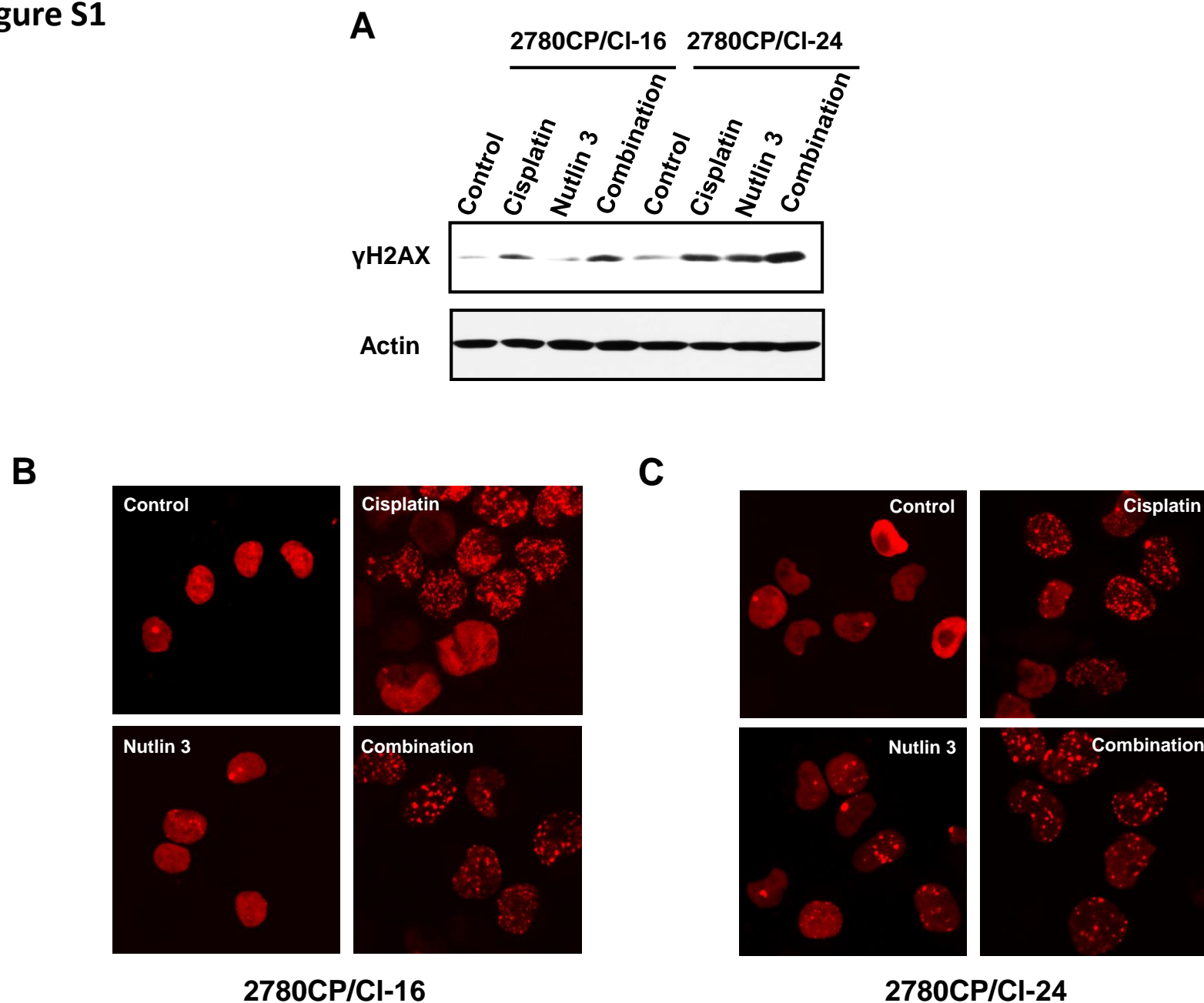
Supplemental Table S2. Statistical analysis by post hoc Tukey of Rad51 mRNA expression following exposure of A2780 p53-proficient (+/+) cells to cisplatin, Nutlin-3 or the combination

Control vs. Cisplatin	Diff = -0.0571	95% CI = -0.2891 to 0.1749	p = 0.8578
Control vs. Nutlin-3	Diff = -0.2601	95% CI = -0.4921 to -0.0281	p = 0.0291
Control vs. Combination	Diff = -0.6163	95% CI = -0.8483 to -0.3842	p = 0.0001
Cisplatin vs. Nutlin-3	Diff = -0.2030	95% CI = -0.4350 to 0.0290	p = 0.0880
Cisplatin vs. Combination	Diff = -0.5591	95% CI = -0.7912 to -0.3271	p = 0.0002
Nutlin-3 vs. Combination	Diff = -0.3561	95% CI = -0.5881 to -0.1241	p = 0.0051

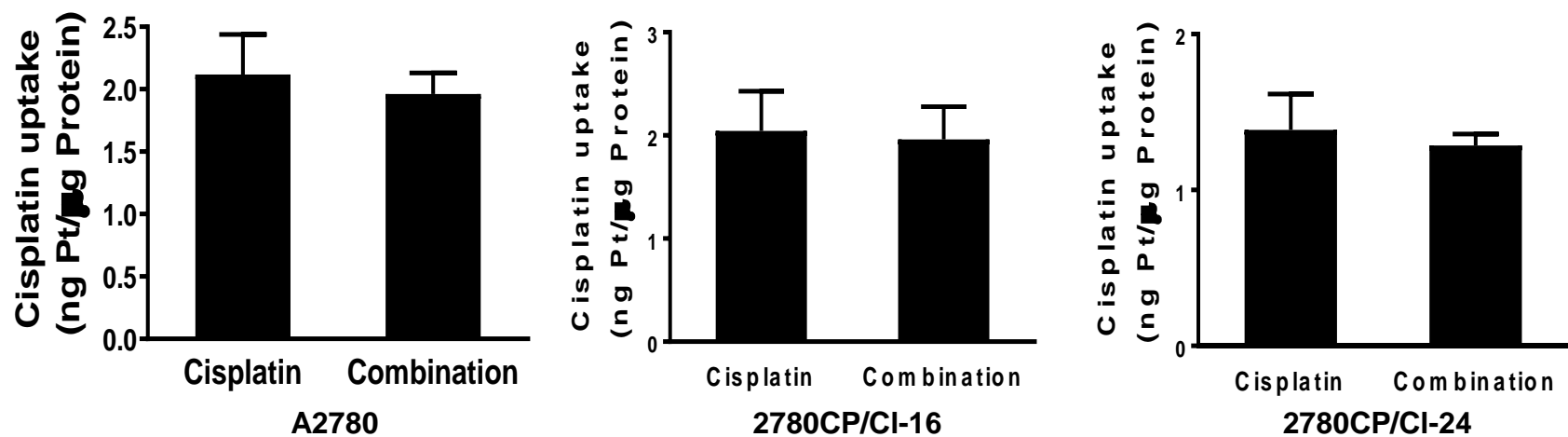
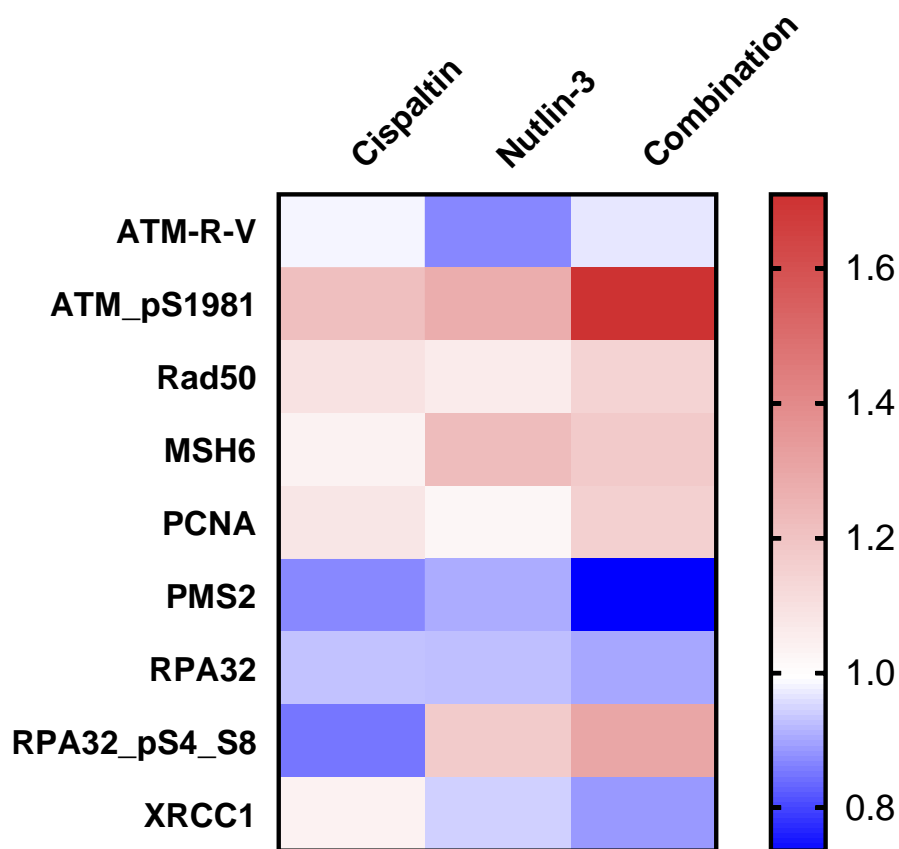
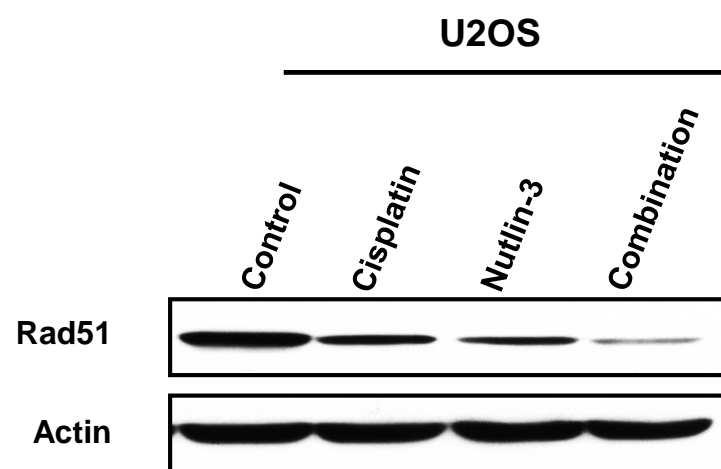
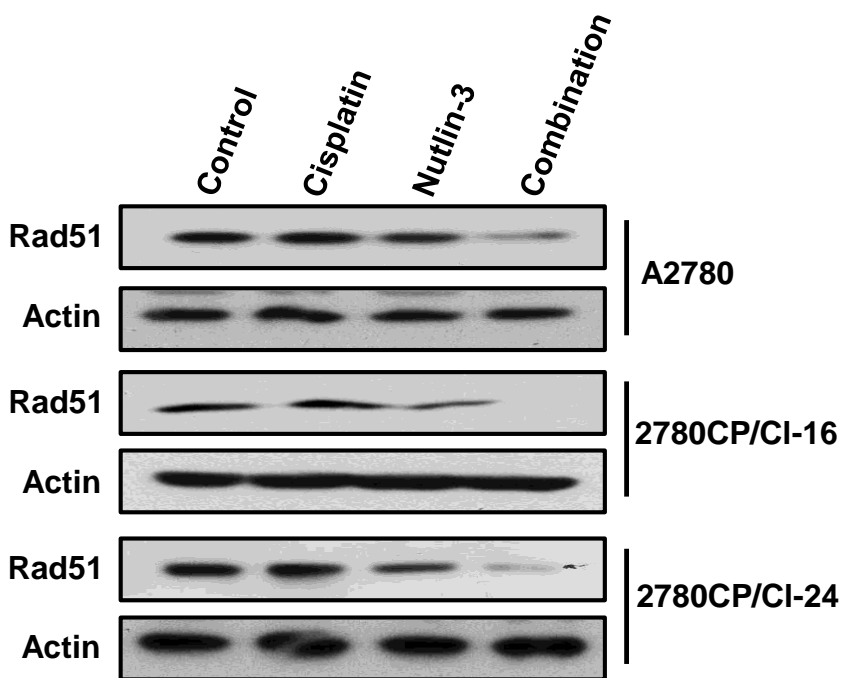
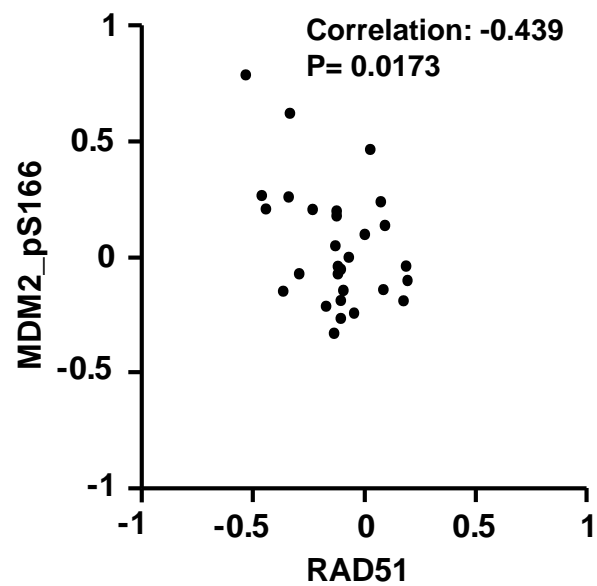
Results were subjected to one-way ANOVA and then Tukey post hoc. CI, confidence interval.

Supplemental

Figure S1



Supplemental Figure S1 Legend. (A) A2780 cells were treated with cisplatin, Nutlin-3 or the combination for 24 hours, collected and analyzed by immunoblot with the indicated antibody. Drug concentrations were as follows: 2780CP/CI-16 (cisplatin, 5 μ M; Nutlin-3, 2 μ M) and 2780CP/CI-24 (cisplatin, 5 μ M; Nutlin-3, 4 μ M). (B) 2780CP/CI-16 cells stably transfected with DNA damage reporter plasmid were treated with cisplatin, Nutlin-3 or combination for 24 hours, cells were then fixed and DNA damage foci monitored using confocal microscopy. (C) 2780CP/CI-24 were treated as in Fig. S1B to monitor DNA damage foci.

Fig. S2**A****B****C****D****E**

Supplemental Figure S2 Legend. (A) A2780, 2780CP/C1-16 and 2780CP/C1-24 cells were treated with cisplatin (200 μ M) for 2 hours or pretreated with Nutlin-3 for 24 hours following 2 hours cisplatin (200 μ M), cells were then collected and intracellular cisplatin concentration determined by atomic absorption. The results are shown as mean \pm SD of three independent experiments. (B) A2780 cells treated for 24 hours with cisplatin (1 μ M), Nutlin-3 (0.5 μ M) or the combination were subjected to RPPA analysis, and the proteins associated with DNA repair pathways and identified by Ingenuity Pathway Analysis are shown as fold change vs. control. (C) U2OS cells were treated with cisplatin (7.5 μ M), Nutlin-3 (0.5 μ M) or combination for 24 hours, cells were then harvested and analyzed using immunoblot with indicated antibodies. (D) Rad51 levels in A2780 (cisplatin, 1 μ M and Nutlin-3, 0.2 μ M), 2780CP/C1-16 (cisplatin, 5 μ M and Nutlin-3, 0.5 μ M) and 2780CP/C1-24 (cisplatin, 5 μ M and Nutlin-3, 0.5 μ M) were determined by Western blot following drug exposure for 24 hr. (E) Correlation between MDM2-pS166 and RAD51 from TCPA database using information from the category ‘MDACC ovary level 4 cell lines.’ The values shown are expressed as Log 2.