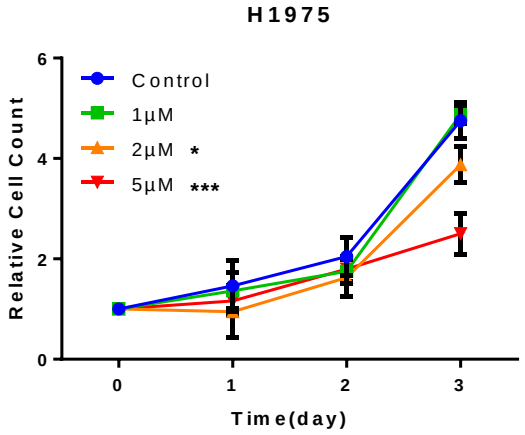
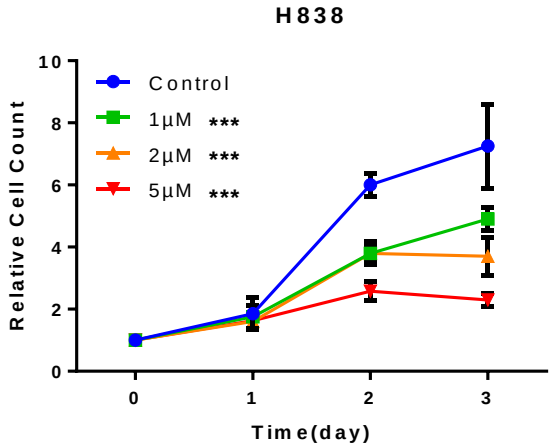
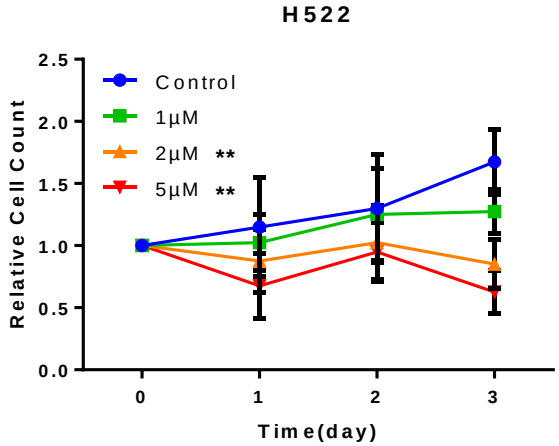
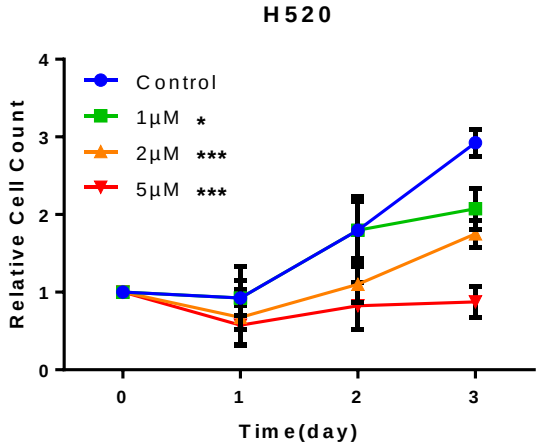
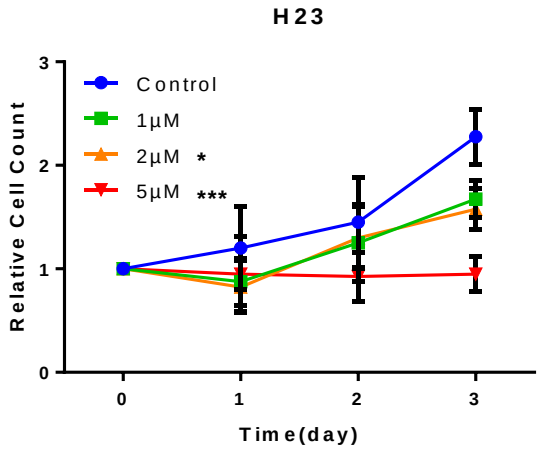
Supplementary Material



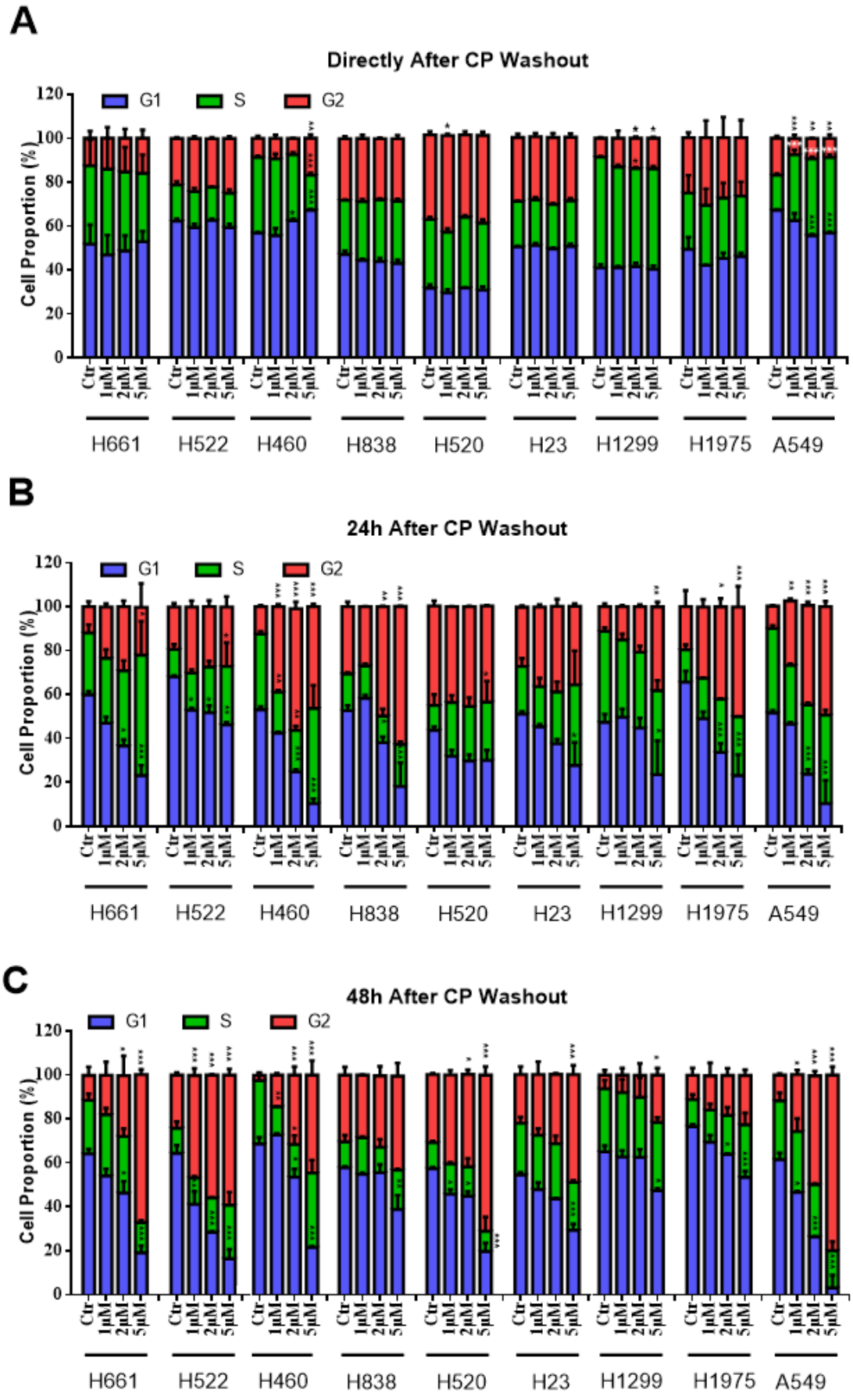




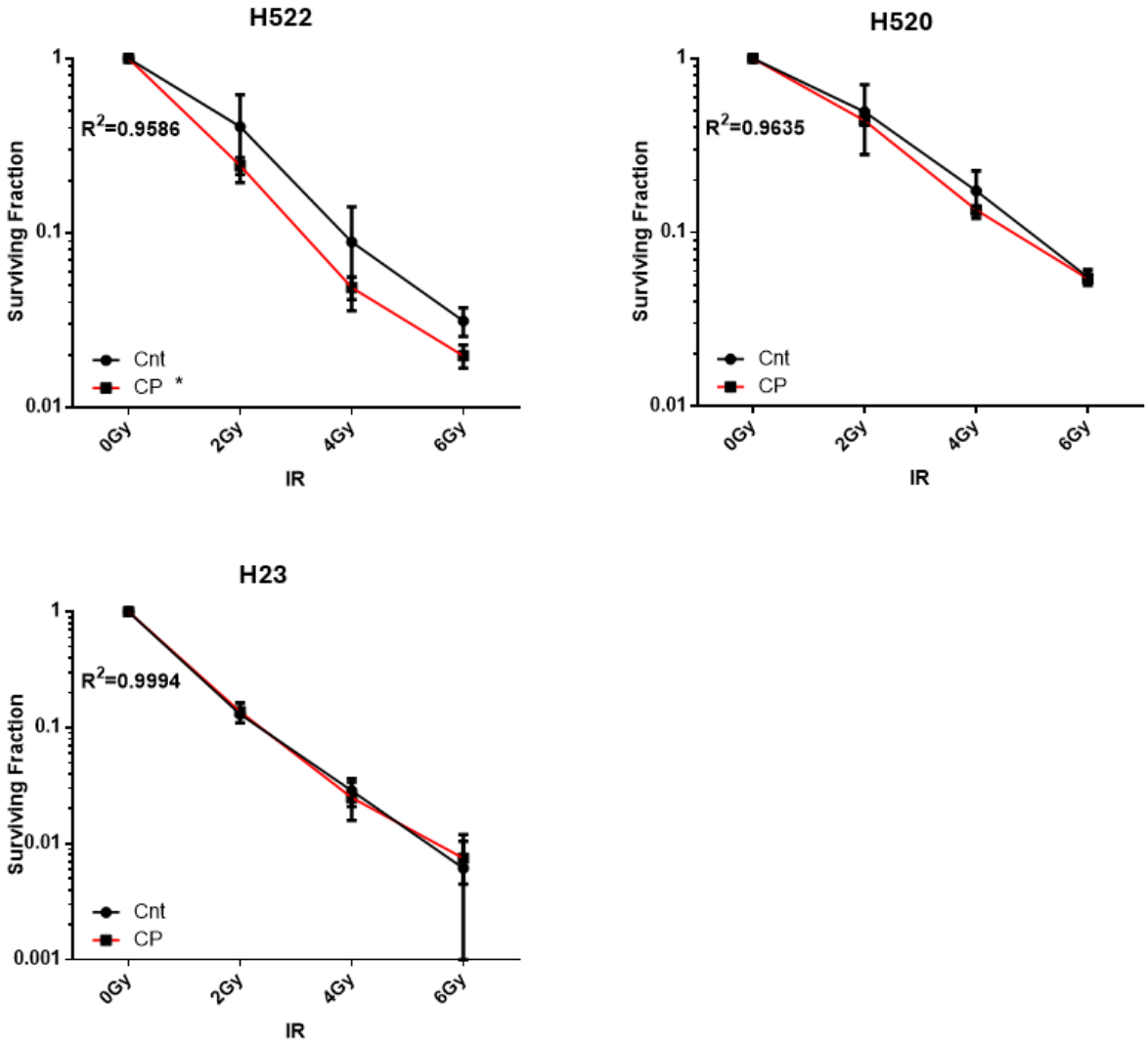




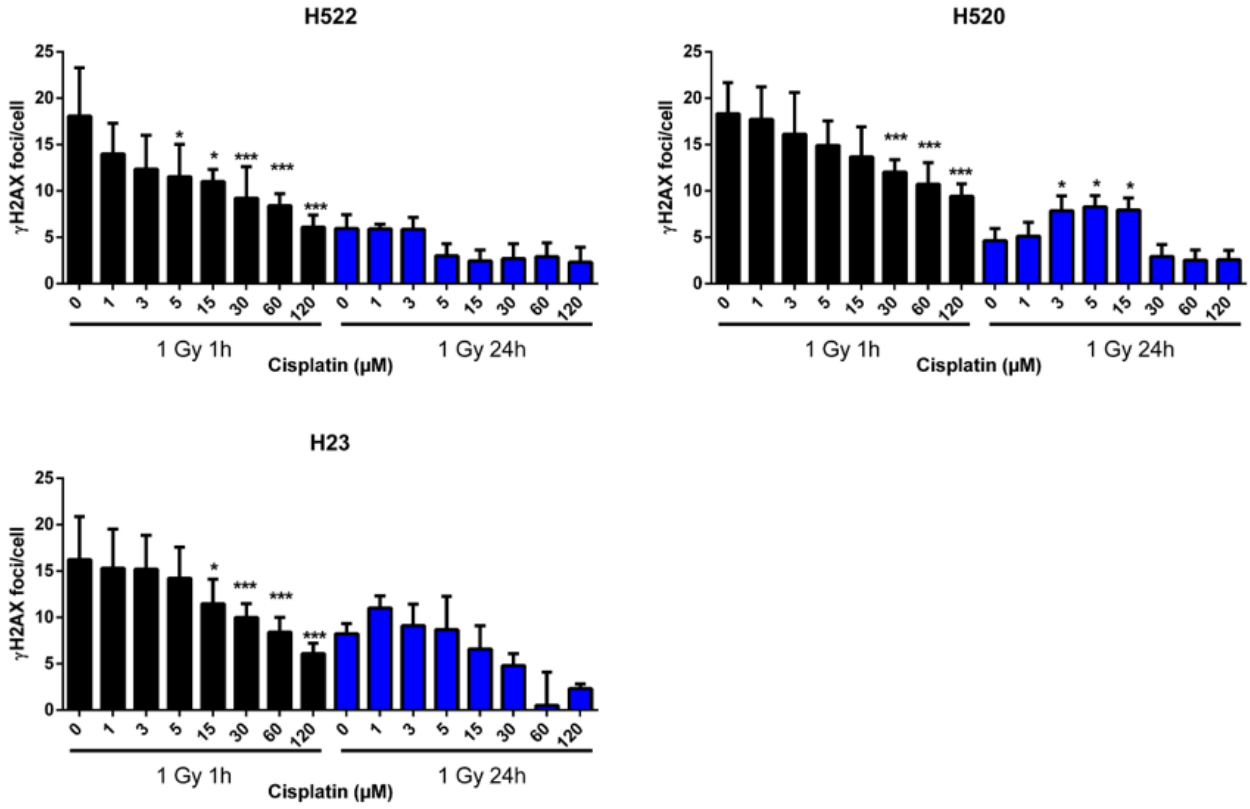
Supplement figure S1: Effect of cisplatin on proliferation of human NSCLC cell lines. NSCLC cell lines (H1975, H838, H522, H520 and H23) were exposed to cisplatin concentrations of 0, 1, 2 and 5 µM for four hours, and the number of cells was counted at 24, 48 and 72 hours after CP washout. The data presented herein signifies the mean ± standard deviation of three to four independent experiments. Statistically significant differences compared with corresponding control cells (not treated with cisplatin) are shown (*P < 0.05, **P < 0.01, ***P < 0.001).


A
Directly After CP Washout
G1
S
G2
Cell Proportion (%)
120
100
80
60
40
20
0
Ctr
1μM
2μM
5μM
H661
H522
H460
H838
H520
H23
H1299
H1975
A549
B
24h After CP Washout
C
48h After CP Washout

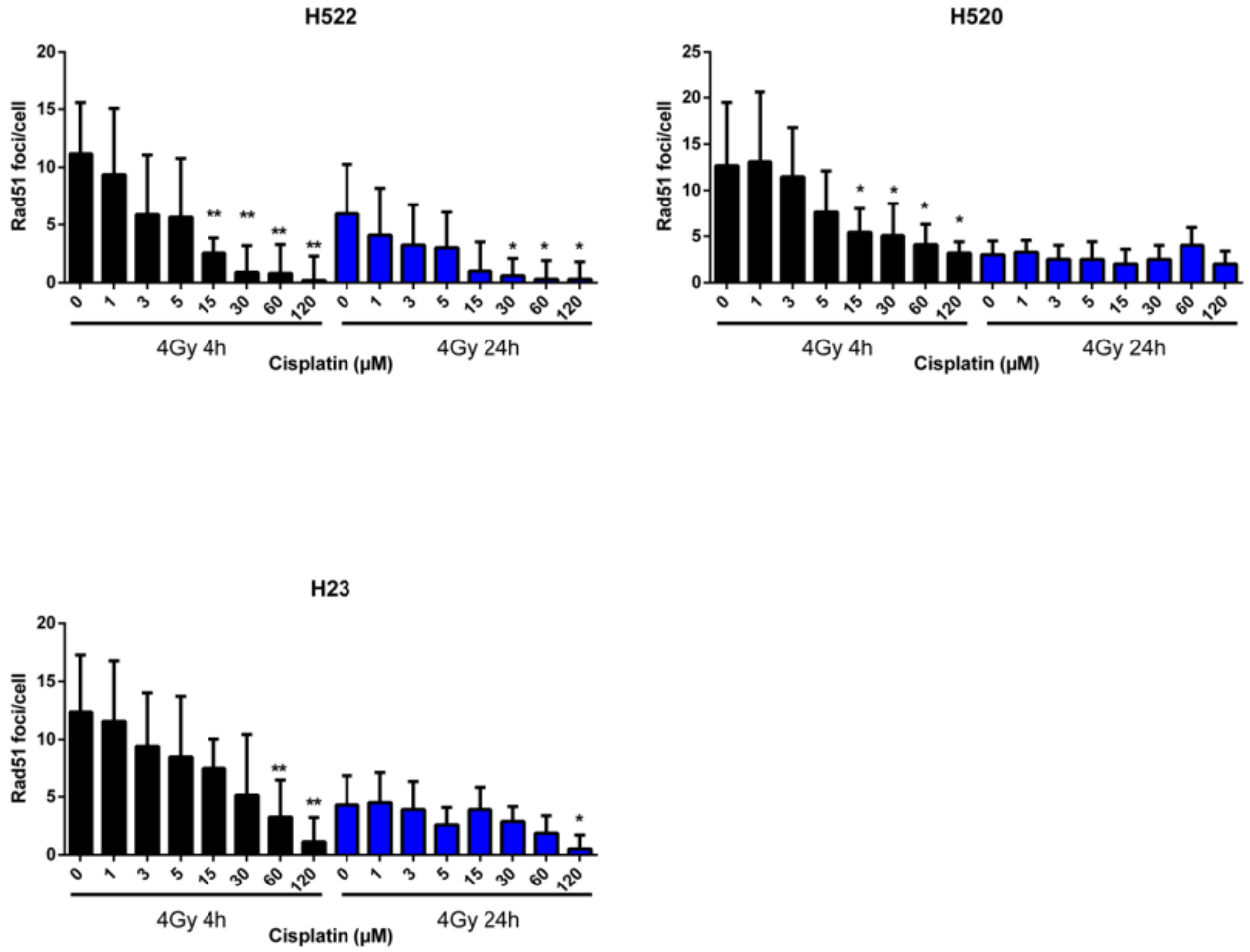
Supplement figure S2: Effect of cisplatin on human NSCLC cell cycle distribution. NSCLC cell lines were exposed to CP (0, 1, 2 and 5 μM) and fixed at 0h (A), 24h (B) and 48 h after CP washout (C). Data represents mean ± SD of 3–4 independent experiments are shown. Statistically significant differences compared with corresponding control cells (not treated with cisplatin) are shown (*P < 0.05, **P < 0.01, ***P < 0.001).



Supplement Figure S3: Effect of Cisplatin on human NSCLC clonogenic survival. NSCLC cells (H522, H520, H23) were plated for clonogenic survival assays after exposure to cisplatin (2 µM) for 4h. After allowing the cells to attach, the colonies were irradiated with 0, 2, 4 and 6 Gy and left for incubation for 10 - 18 days. Data represents mean ± SD of 3–4 independent experiments. Statistically significant differences compared with corresponding control cells (not treated with cisplatin) using nonlinear regression (curve fit) and globally shared R2 values are shown (*P < 0.05, **P < 0.01).



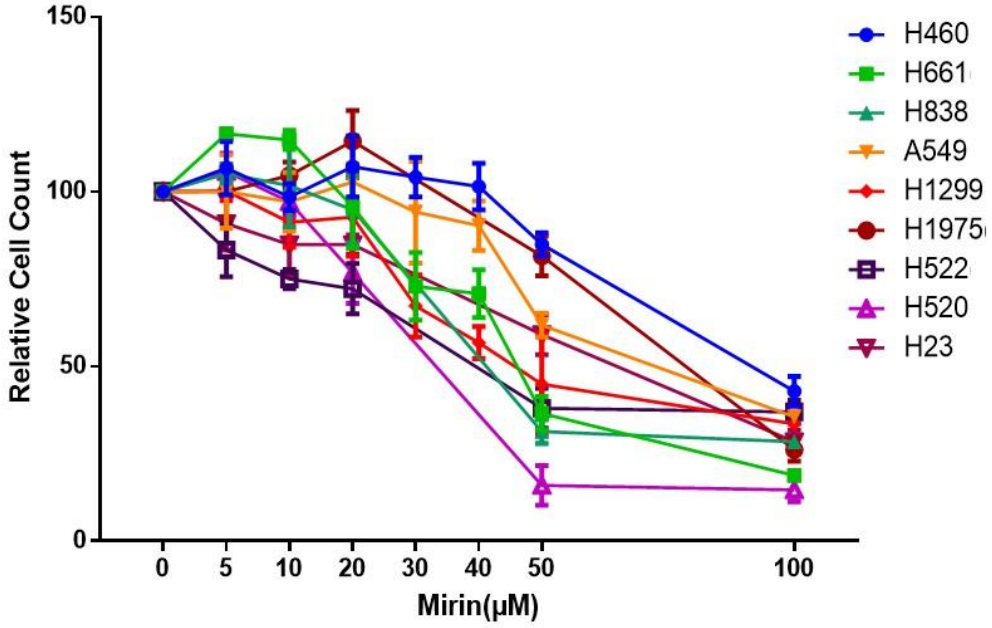
Supplement figure S4: Effect of Cisplatin on NHEJ repair signalling of NSCLCs. Cells plated for immunofluorescence assays after exposure to cisplatin (1 to 120 μM), were irradiated with 0 Gy and 1 Gy followed by fixations at 1 h, 4 h and 24 h and γH2AX foci were scored. Data represents mean ± SD of 3–4 independent experiments are shown. Statistically significant differences compared with corresponding control cells (not treated with cisplatin) using student's t test are shown (*P < 0.05, **P < 0.01, ***P < 0.001).



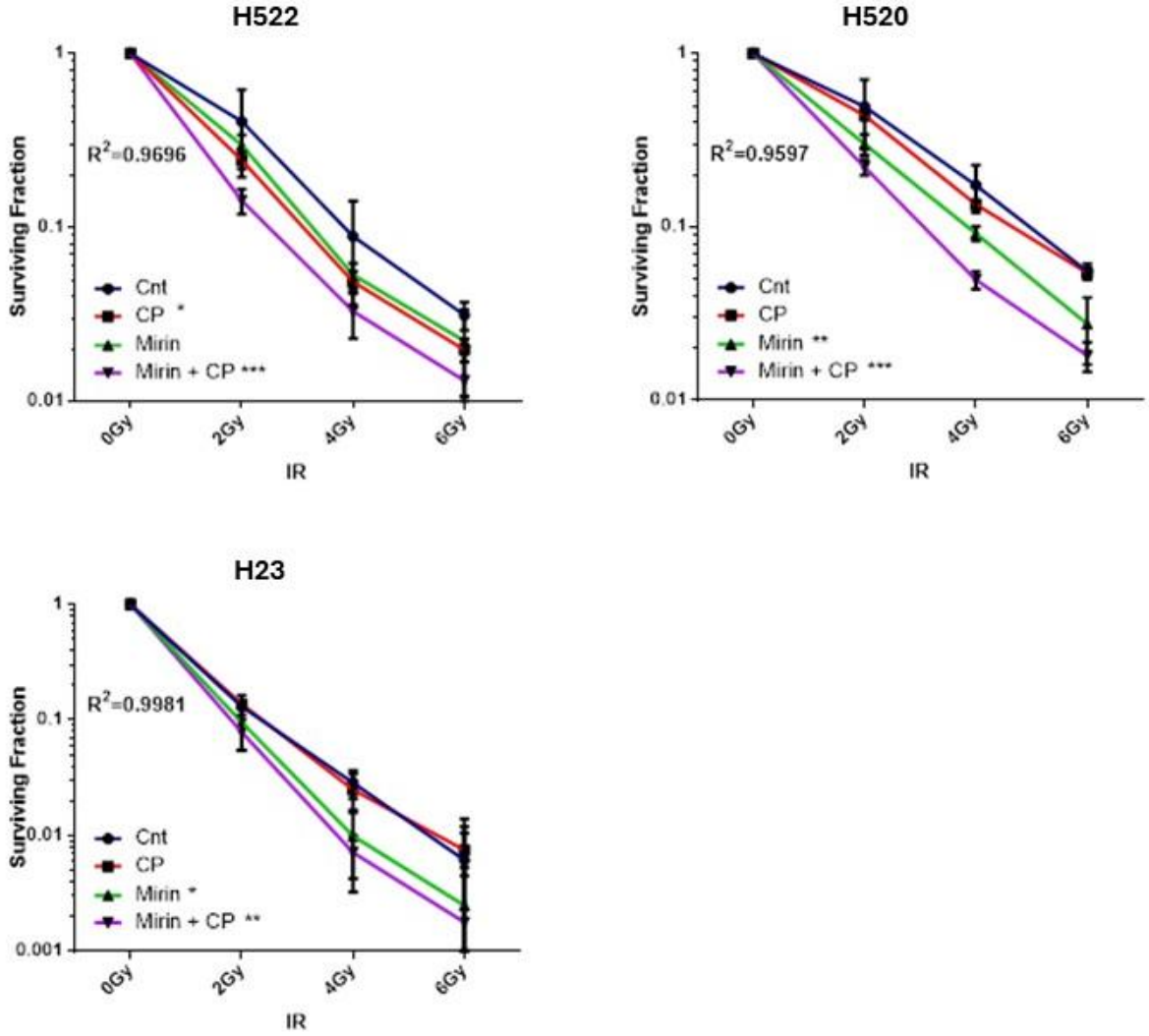
Supplement figure S5: Effect of cisplatin on HR repair of NSCLCs. After exposure to cisplatin (1 to 120 μM), cells were plated for immunofluorescence assays, irradiated with 0 Gy and 4 Gy followed by fixations at various times thereafter (1 h, 4 h and 24 h) and RAD51 foci were scored. The data represents mean ± SD of 3–4 independent experiments. Statistically significant differences compared with corresponding control cells (not treated with cisplatin) using student's t test are shown (*P < 0.05, **P < 0.01, ***P < 0.001).

Supplementary Table S1: Pt-GpG Adduct Values at 24h of CP and CP+Mirin treatment groups. (Mean ± SD, n=3). P values (student's T test) = ns (>0.05), ** (<0.05), *** (<0.01).

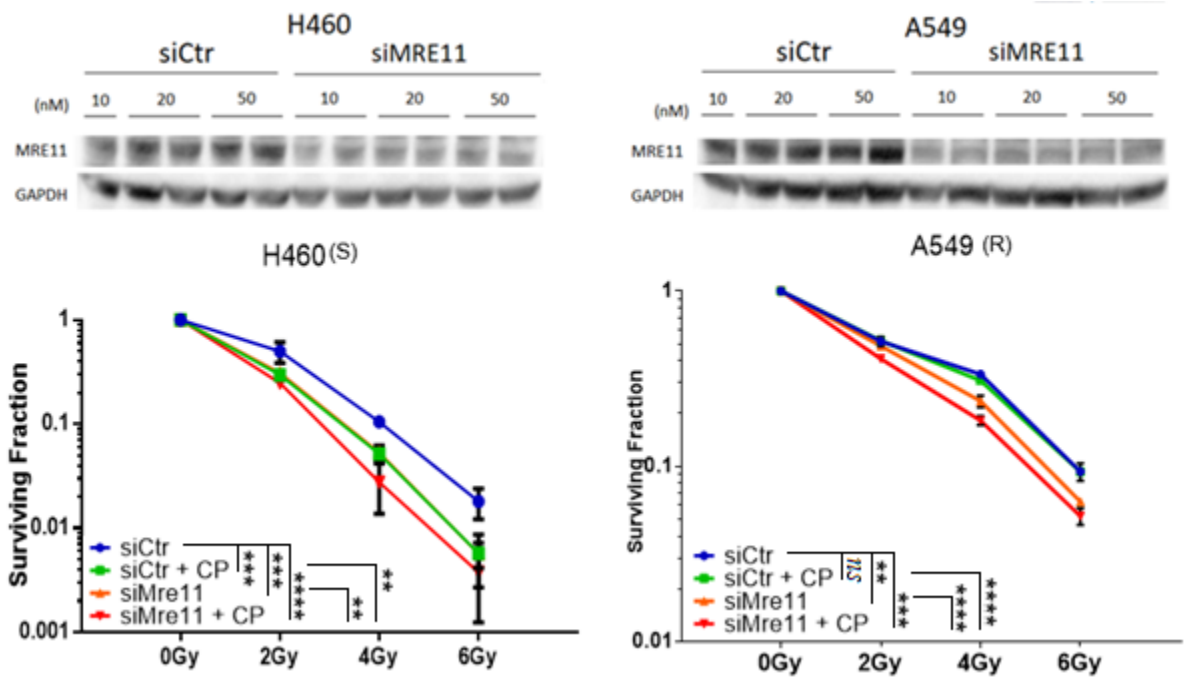
Cell Line	Adduct Level at 24h CP Only	Adduct Level at 24h CP+Mirin	P Value		
H661(S)	0.45 ±0.12	0.27 ±0.12	ns	↓	
H522(S)	0.81 ±0.42	0.59 ±0.17	***	↓	
H460(S)	0.34 ±0.01	0.33 ±0.01	ns	↓	
H520(R)	0.42 ±0.04	1.01±0.13	***	↑	
H23(R)	0.50 ±0.08	1.09 ±0.11	***	↑	
H838(R)	0.78 ±0.03	0.85±0.02	**	↑	
H1299(R)	0.11 ±0.01	0.34 ±0.03	***	↑	
H1975(R)	0.34 ±0.22	0.43 ±0.24	ns	↑	
A549(R)	0.23 ±0.02	0.71 ±0.02	***	↑	



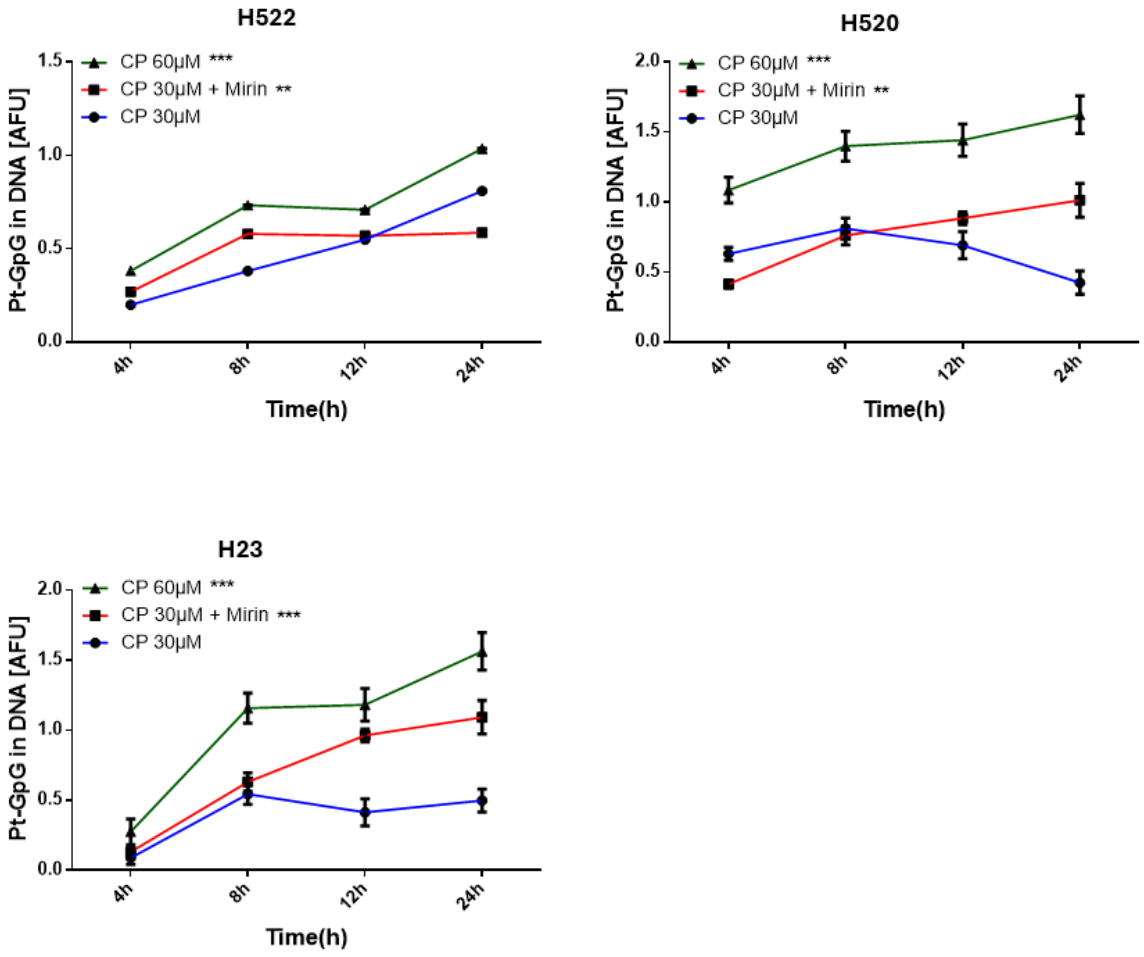
Supplementary figure S6: Effect of Mirin on human NSCLC cell proliferation. Human NSCLC cells were exposed to 0–100 μM concentrations of Mirin for 48h and viable cells were counted. Data represent mean + SD of 3–4 independent experiments.



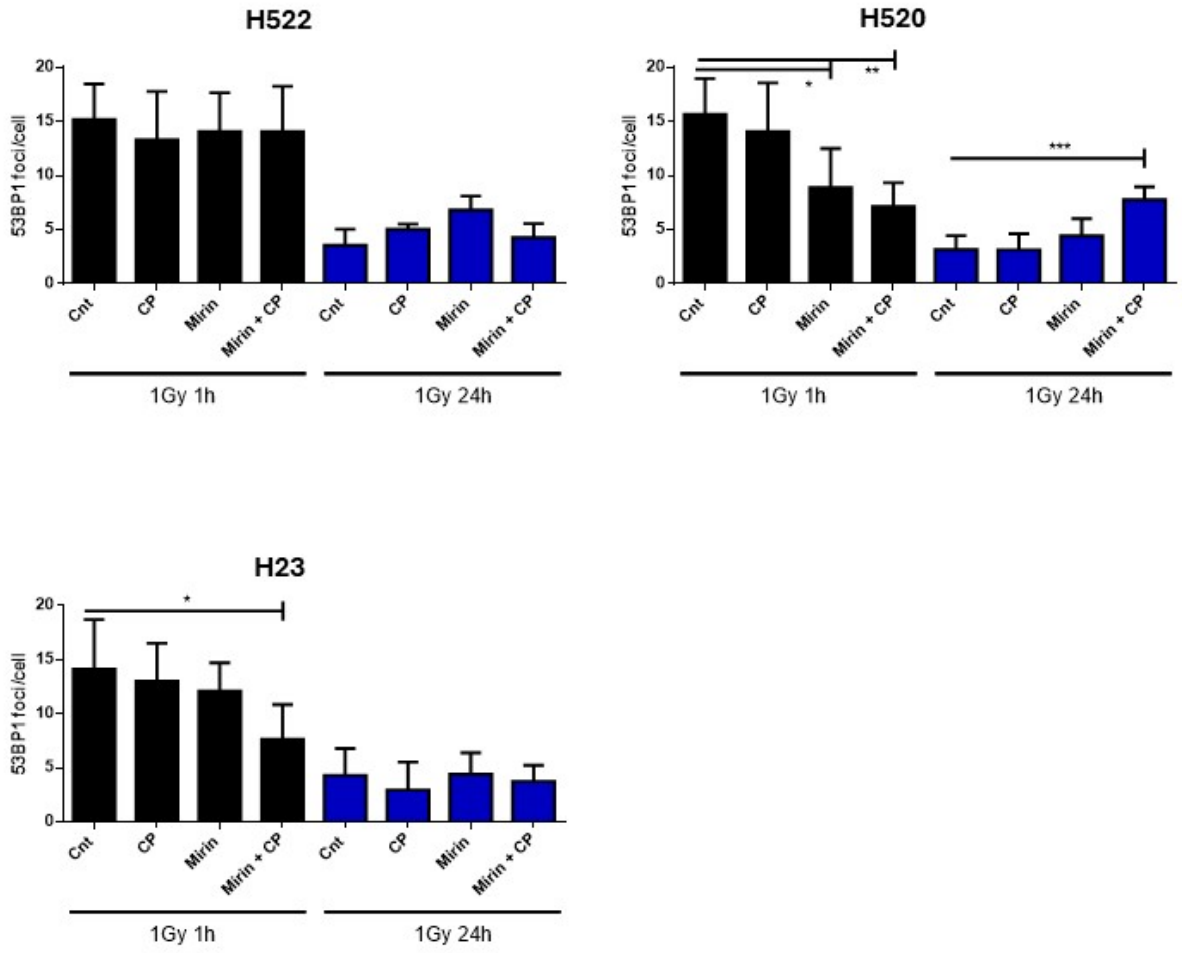
Supplement Figure S7: Effect of cisplatin, mirin, and combined treatment on human NSCLC radiation sensitivity. NSCLC cell lines (H522, H520 and H23) were treated with 20 µM mirin for 20 h followed by 2 µM cisplatin treatment for 4 h plated for clonogenic survival assays into mirin containing media for further 24 h Mirin treatment. After allowing the cells to attach for 4-5 h, the colonies were irradiated with 0, 2, 4, 6 Gy and left for incubation with mirin containing media for 24 h. The media was replaced next day and colonies were left for incubation for 10-18 days. Mean $\pm$ SD of 3–5 independent experiments are shown. Statistically significant differences compared with corresponding control cells (not treated with cisplatin) using non-linear regression (curve fit) and globally shared R2 values are shown (*P < 0.05, **P < 0.01, ***P < 0.001).



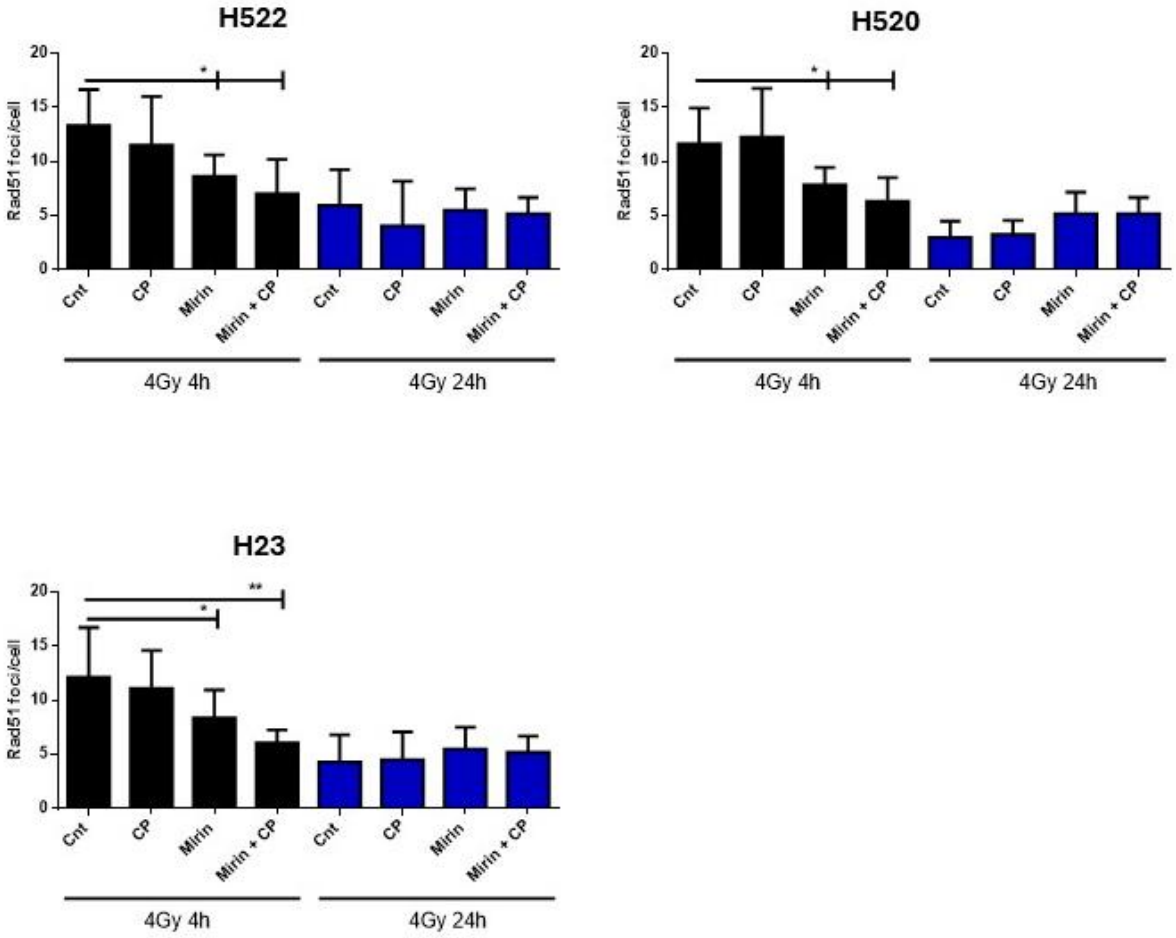
Supplementary Figure S8: Effect of siRNA transfection against MRE11 on clonogenic survival of H460 and A549 cells. Cells were transfected with 20 nM siRNA against MRE11 for 5 h followed by 48h of incubation. Cells then were treated with 2 µM cisplatin for 4h and plated for clonogenic survival assays. After allowing the cells to attach for 4-5 h, the cells were irradiated with 0, 2, 4, 6 Gy and left for incubation for 10-18 days. Representative results of 3–5 independent experiments are shown. Statistically significant differences among every exposition group when compared with siCtr and among themselves are shown (*P < 0.05, **P < 0.01, ***P < 0.001).



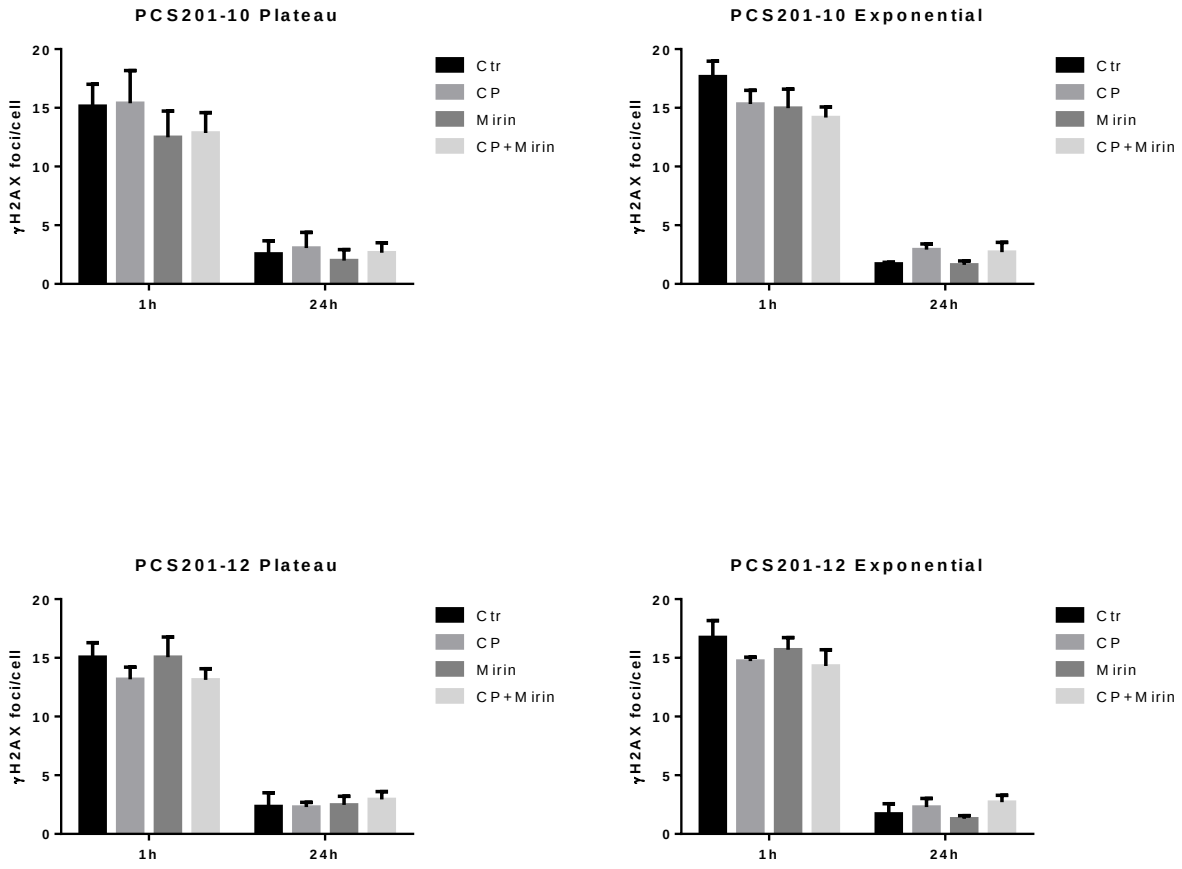
Supplementary figure S9: CP-DNA adduct kinetics after Cisplatin and Combined treatment in NSCLCs. NSCLC cell lines (H522, H520 and H23) were plated for immunofluorescence assays and were treated with 20 µM Mirin for 20 h followed by 30 µM and 60 µM cisplatin treatment for 4h and fixed at 4, 8, 12 and 24h. Mean ± SD of 3–4 independent experiments are shown. Statistically significant differences compared with CP 30µM are shown (*P < 0.05, **P < 0.01, ***P < 0.001, n=3).



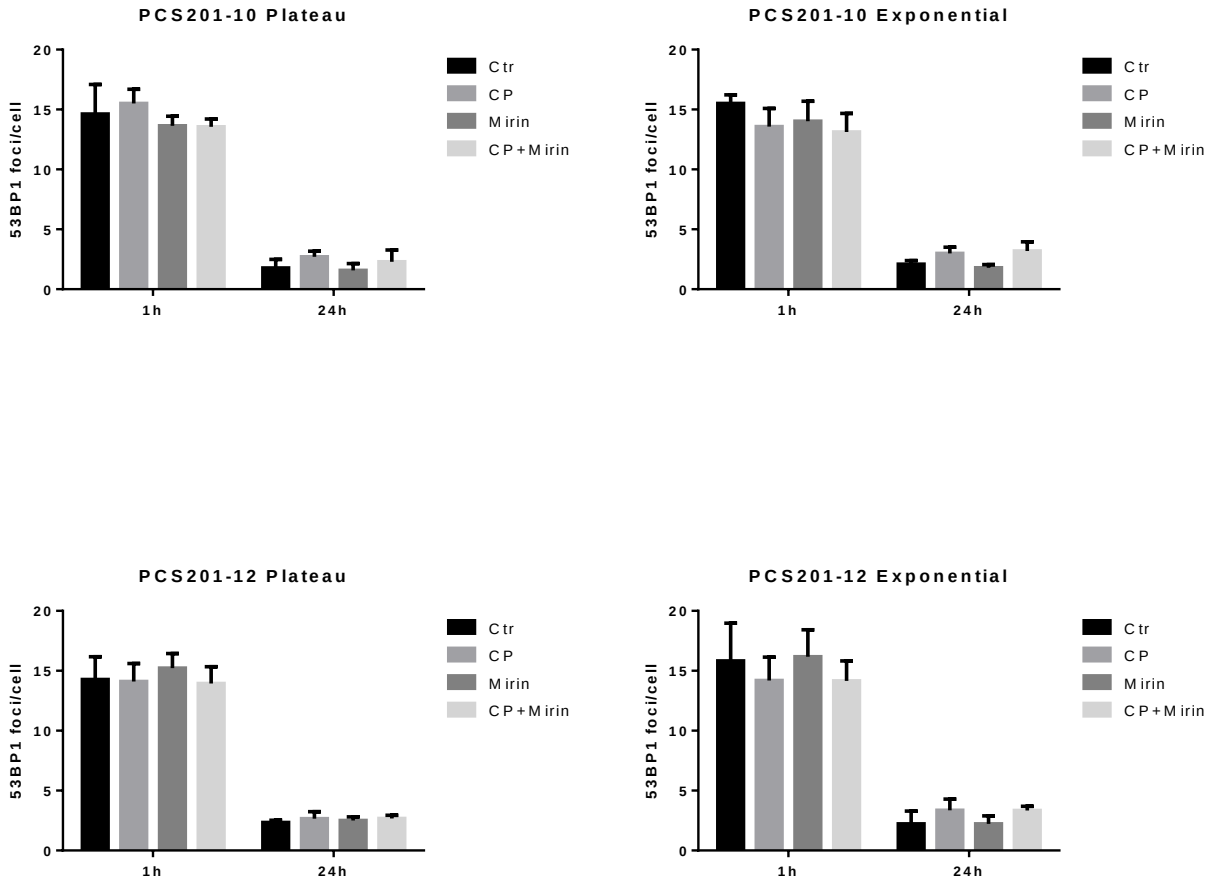
Supplementary figure S10: Effect of cisplatin, mirin and combined treatment on 53BP1 repair signalling. Human NSCLC cells were plated for immunofluorescence assays and were treated with 20 μM Mirin for 20 h followed by 2 μM cisplatin treatment for 4h and were irradiated with 0, 1 Gy prior to fixation at 1 h and 24 h, and 53BP1 foci were scored. Mean ± SD of 3–4 independent experiments are shown. Statistically significant differences compared with corresponding control cells (not treated with Cisplatin) using student's t test are shown (*P < 0.05, **P < 0.01, ***P < 0.001).



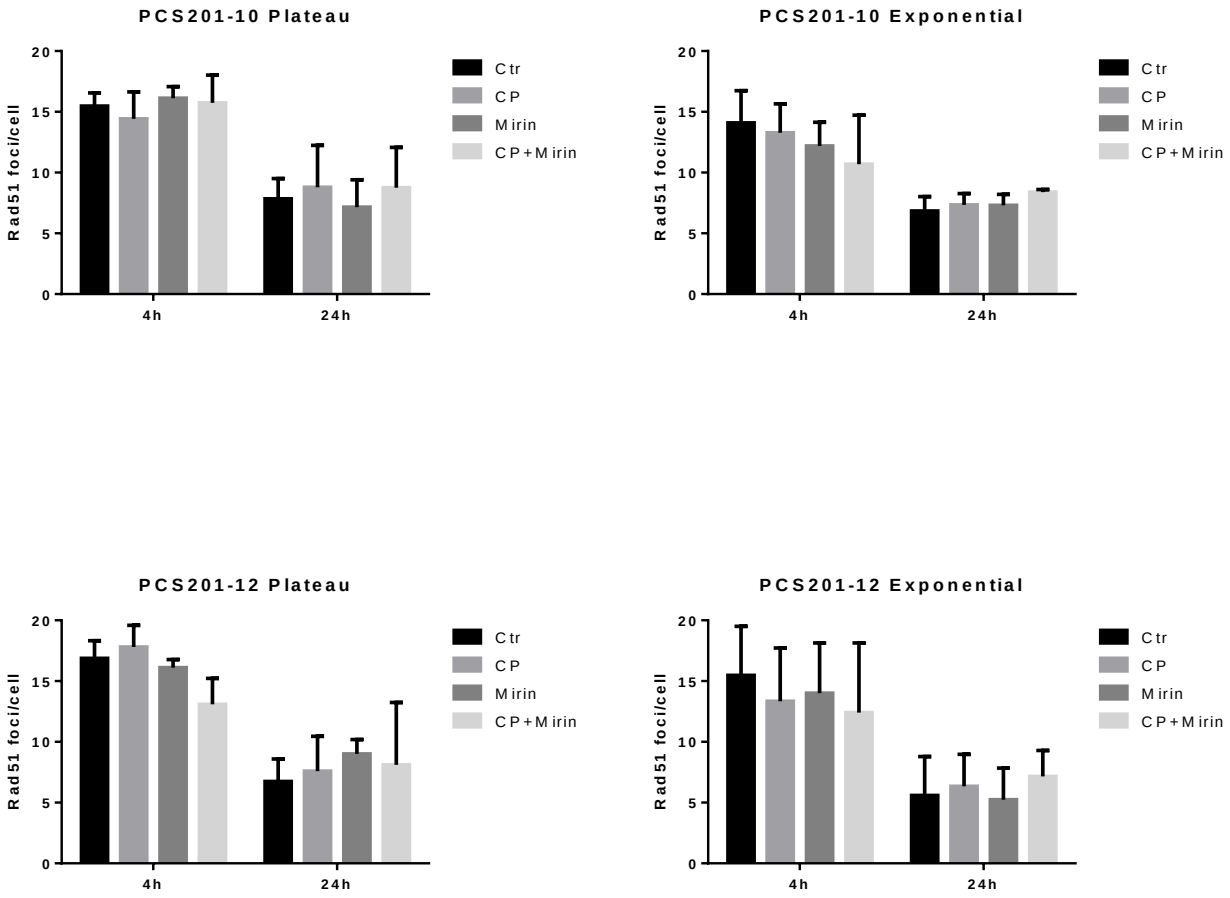
Supplementary figure S11: Effect of cisplatin, mirin and combined treatment on 53BP1 repair signalling. Human NSCLC cells were plated for immunofluorescence assays and were treated with 20 µM Mirin for 20 h followed by 2 µM cisplatin treatment for 4h and were irradiated with 0, 1 Gy prior to fixation at 4 h and 24 h, and Rad51 foci were scored. Mean ± SD of 3–4 independent experiments are shown. Statistically significant differences compared with corresponding control cells (not treated with Cisplatin) using student's t test are shown (*P < 0.05, **P < 0.01, ***P < 0.001).



Supplementary figure S12: Effect of Cisplatin, Mirin and combined treatment on γH2AX foci formation in HDFN and HDFA. Plateau – and Exponentially growing Human DFP and DFAwere plated for immunofluorescence assays and were treated with 20μM Mirin for 20h followed by 2μM cisplatin treatment for 4h and were irradiated with 0, 1 Gy prior to fixation at 1h and 24h. Mean ± SD of 3–4 independent experiments are shown. Statistically significant differences compared with corresponding control cells (not treated with cisplatin) are shown.



Supplementary figure S13: Effect of Cisplatin, Mirin and combined treatment on 53BP1 foci formation in HDFN and HDFA. Plateau – and exponentially growing Human DFP and DFA cells were plated for immunofluorescence assays and were treated with 20µM Mirin for 20h followed by 2µM cisplatin treatment for 4h and were then irradiated with 0, 1 Gy prior to fixation at 1h and 24h. Representative results of 3–4 independent experiments are shown. Statistically significant differences compared with corresponding control cells (not treated with cisplatin) are shown.



Supplementary figure S14: Effect of Cisplatin, Mirin and combined treatment on Rad51 foci formation in HDFN and HDFA. Plateau – and exponentially growing Human DFP and DFA cells were plated for immunofluorescence assays and were treated with 20 µM Mirin for 20 h followed by 2 µM cisplatin treatment for 4 h and were then irradiated with 0, 4 Gy prior to fixation at 4 h and 2 4h. Representative results of 3–4 independent experiments are shown. Statistically significant differences compared with corresponding control cells (not treated with cisplatin) are shown (*P < 0.05, **P < 0.01).