**Figure S1.**

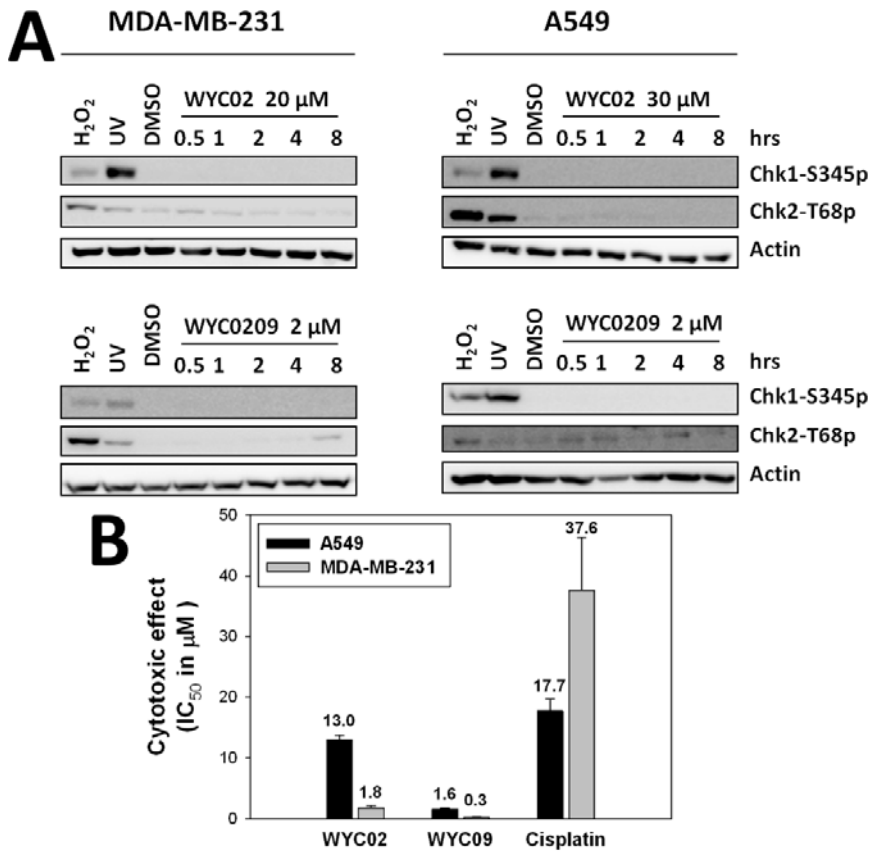



**Figure S1.** Effect of WYC on DDR in cancer cell lines. A, immunoblots showing phosphorylation of Chk1 $Ser^{345}$ (Chk1-S345p) and Chk2 $Thr^{68}$ (Chk2-T68p) in MDA-MB-231 and A549 cells following exposure to WYC02 or WYC0209 at indicated doses and times. Cells treated with 0.1 mM $H_2O_2$ and 10 J/$m^2$ UV for 1 h served as positive control. B, cytotoxicity test for WYC02, WYC0209, and cisplatin on A549 and MDA-MB-231 cells. Data are presented as mean ± SEM (n = 3) for the calculated half-maximal inhibitory concentration ($IC_{50}$).

**Figure S2.**

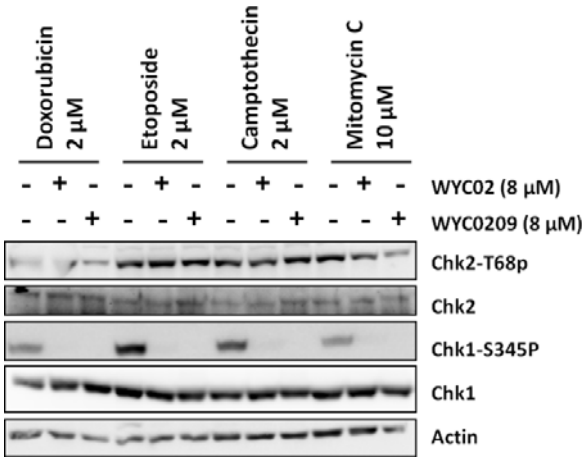



**Figure S2.** Effect of WYC on DDR induced by the therapeutic agents mentioned above. DDR in the immunoblots was determined by detecting phosphorylation of Chk1 $Ser^{345}$ (Chk1-S345p) and Chk2 $Thr^{68}$ (Chk2-T68p) following exposure of MDA-MB-231 cells to the indicated agents for 4 h with or without WYC02 or WYC0209 pretreatment.

**Figure S3.**

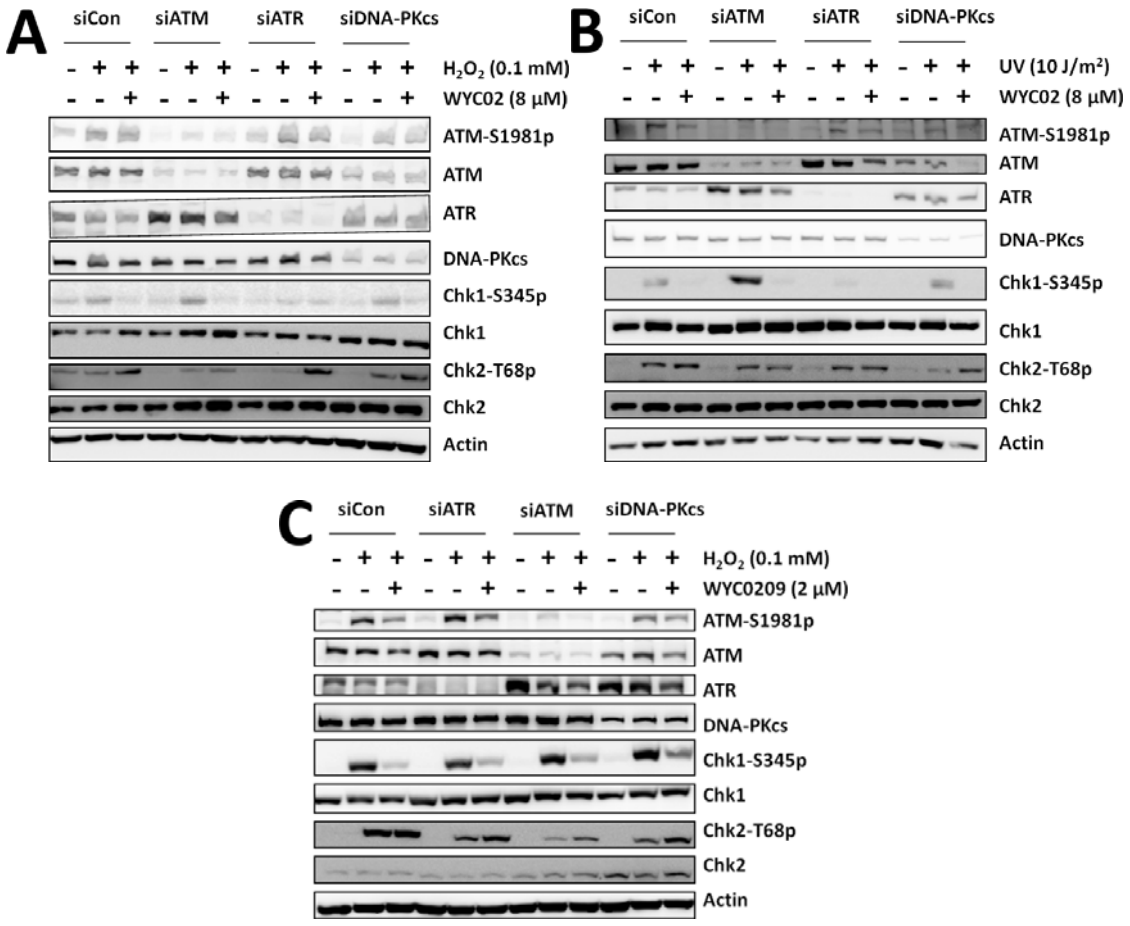



**Figure S3.** Effect of WYCs in PI3K-depleted cells. A, the inhibitory effect of WYC02 on DDR in the siRNA of ATM-, ATR-, or DNA-PKcs-transfected HEK293Tcells. Indicated siRNAs were transfected for 48 h, and incubated in the presence or absence of 8 μM WYC02 for 30 min followed by DDR induction using 0.1 mM $H_2O_2$ or B, 10 J/$m^2$ of UV irradiation for 1 h. C, the same experimental design as A was applied with 4 μM WYC0209.

**Figure S4.**

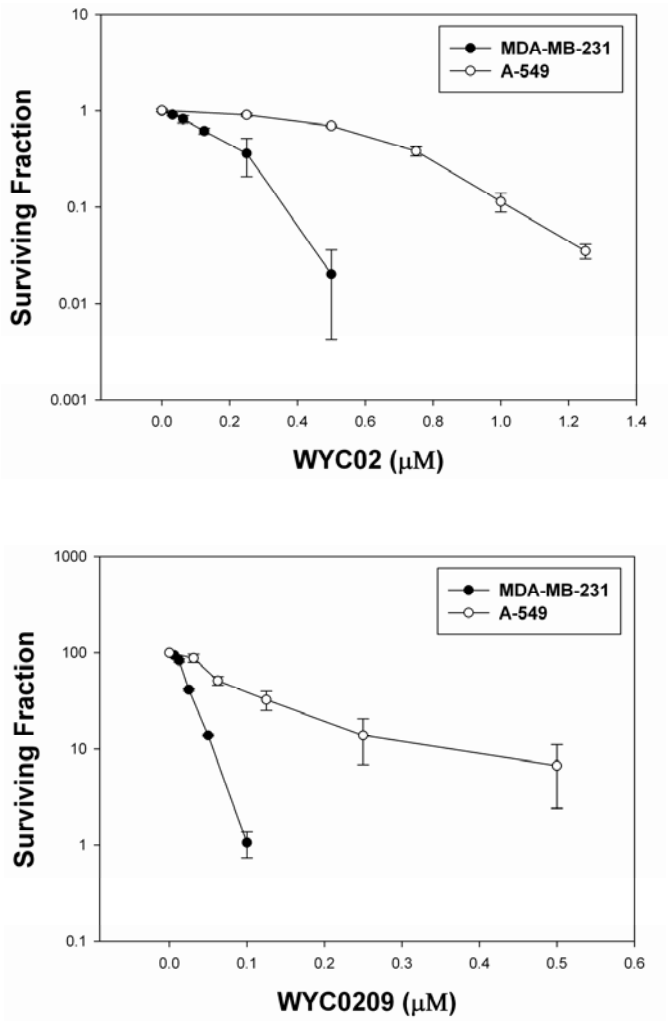
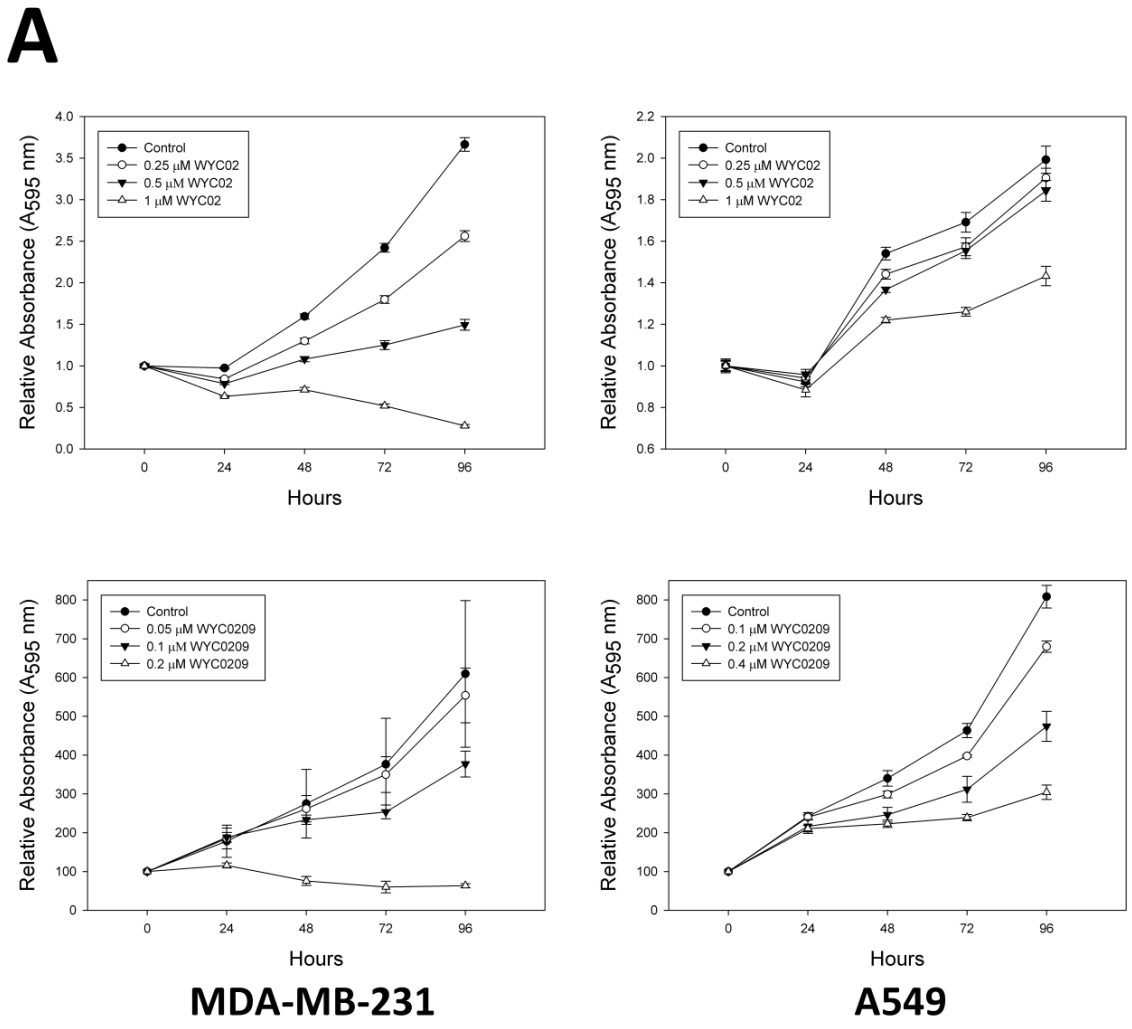
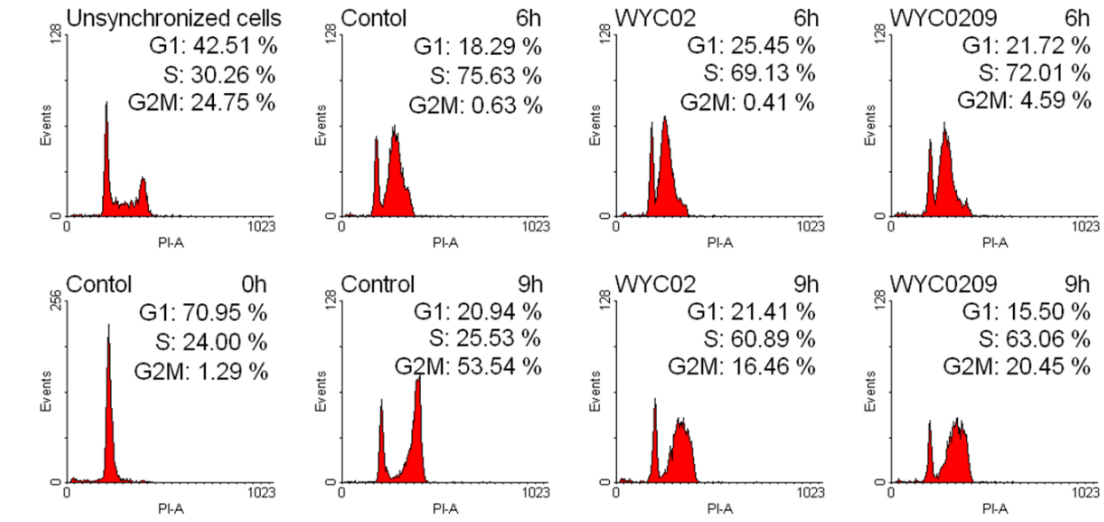



**Figure S4.** Effect of WYCs on cancer growth. Surviving fractions were calculated from the clonogenic growth of MDA-MB-231 and A549 cells following exposure to indicated WYC02 (upper) or WYC0209 (lower) concentrations for 7–10 d. Data are presented as means ± SEM (n = 3).

**Figure S5**


A
Relative Absorbance (A595 nm)
Hours
Control
0.25 μM WYC02
0.5 μM WYC02
1 μM WYC02
0.05 μM WYC0209
0.1 μM WYC0209
0.2 μM WYC0209
0.4 μM WYC0209
MDA-MB-231
A549


B
Unsynchronized cells
G1: 42.51 %
S: 30.26 %
G2M: 24.75 %
Contol 6h
G1: 18.29 %
S: 75.63 %
G2M: 0.63 %
WYC02 6h
G1: 25.45 %
S: 69.13 %
G2M: 0.41 %
WYC0209 6h
G1: 21.72 %
S: 72.01 %
G2M: 4.59 %
Contol 0h
G1: 70.95 %
S: 24.00 %
G2M: 1.29 %
Control 9h
G1: 20.94 %
S: 25.53 %
G2M: 53.54 %
WYC02 9h
G1: 21.41 %
S: 60.89 %
G2M: 16.46 %
WYC0209 9h
G1: 15.50 %
S: 63.06 %
G2M: 20.45 %
Events
PI-A

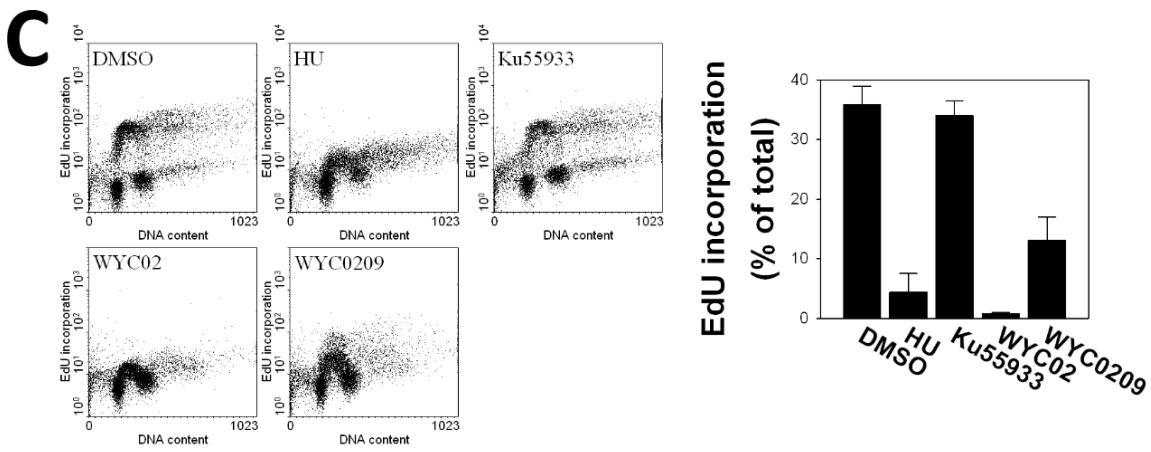



**Figure S5.** WYCs disturb DNA replication and inhibit cell growth. A, MDA-MB-231 (left) and A549 (right) cells (800–1000 cells) were seeded into each well of a 96-well plate 1 d before the experiment. MTT assay was performed at the indicated times following exposure to WYC02 (upper) or WYC0209 (lower) at indicated doses. All the insoluble purple formazan was dissolved at the end of the experiment by DMSO, and the absorbance of the colored solution was measured. B, FACS histogram for cell-cycle analysis. U2OS cells were synchronized using the double thymidine block assay. Cells were blocked by thymidine (2.5 mM) for 17 h and released in normal medium for 12 h. WYC02 (4 μM) or WYC0209 (0.4 μM) was added after release from the second run of 17 h of thymidine block. The cells were harvested at the indicated times, and cell-cycle distribution was analyzed by PI staining. C, FACS dot blot for detection of 5-ethynyl-2′-deoxyuridine (EdU) incorporated into the DNA of cultured MDA-MB-231 cells. The cells were treated with DMSO, 1 mM HU, 10 μM KU55933, 4 μM WYC02, or 0.4 μM WYC0209 for 6 h, and labeled with 10 μM EdU for 30 min before analysis. EdU was detected after it reacted with Alexa647-azide, and CellCycle 405-blue was used to stain the DNA content (Invitrogen).

**Figure S6**

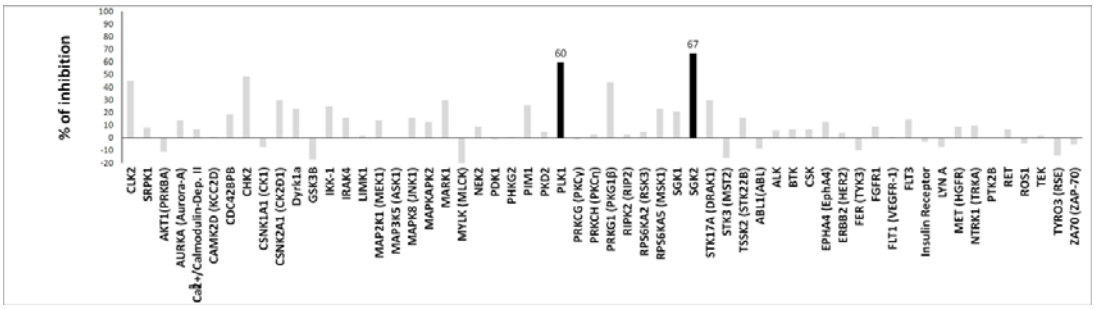



**Figure S6.** The Kinome Diversity Screen for WYC02. The *in vitro* kinase assay for indicated kinases was conducted by MDS Pharma Services. Each reaction mixture contained individual buffer, kinase, substrate, and [γ-$P^{32}$] ATP, and the reaction occurred in the presence or absence of 3 μM WYC02. Data are presented as the average percentage of inhibition to the control from 2 tests.

**Figure S7**

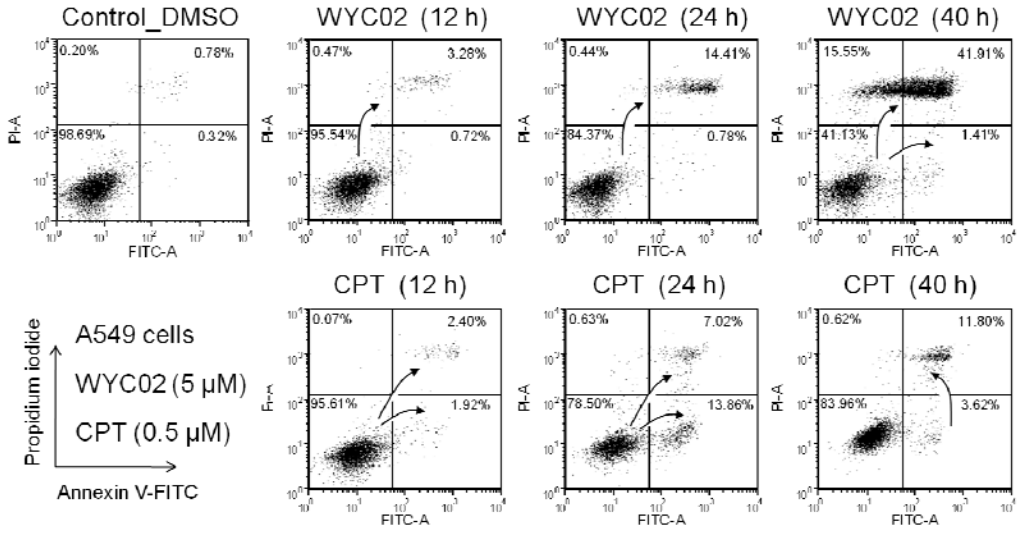



**Figure S7.** The death effect of WYC02 and camptothecin (CPT) in A549 calls. FACS dot-blot analysis for A549 cells exposed to 5 μM WYC02 or 0.5 μM CPT for indicated times. Fresh harvested cells were double-stained with propidium iodide and FITC-conjugated annexin-V, and fluorescence was analyzed immediately by flow cytometry.

**Figure S8**

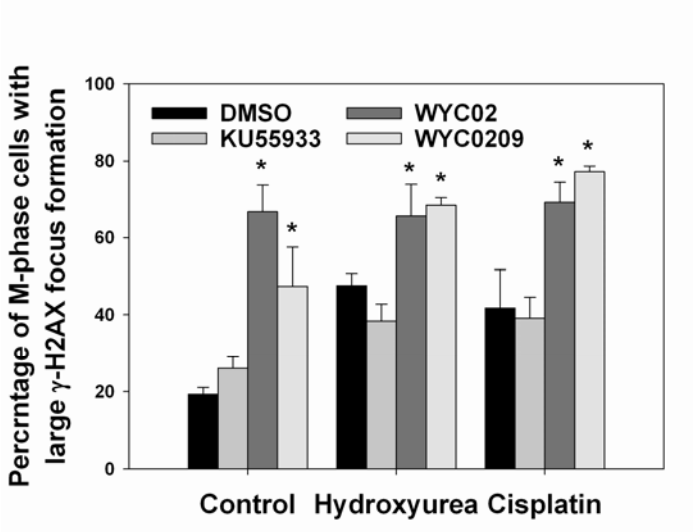



**Figure S8.** WYCs increase gamma-H2AX focus formation in M-phase cells. Quantification of the result in Figure 4C showing the focus larger than background was counted, and the data was presented as percentages of mitotic nuclei with containing at least more than 5 large foci per nucleus. The values were presented as mean ± standard error of mean (SEM) from three separate experiments, where * indicates a significant difference ($P$< 0.05) relative to control without WYCs treatment.