Characterization of cardiac glycoside natural products as potent inhibitors of DNA double-strand break repair by a whole cell double immunofluorescence assay.

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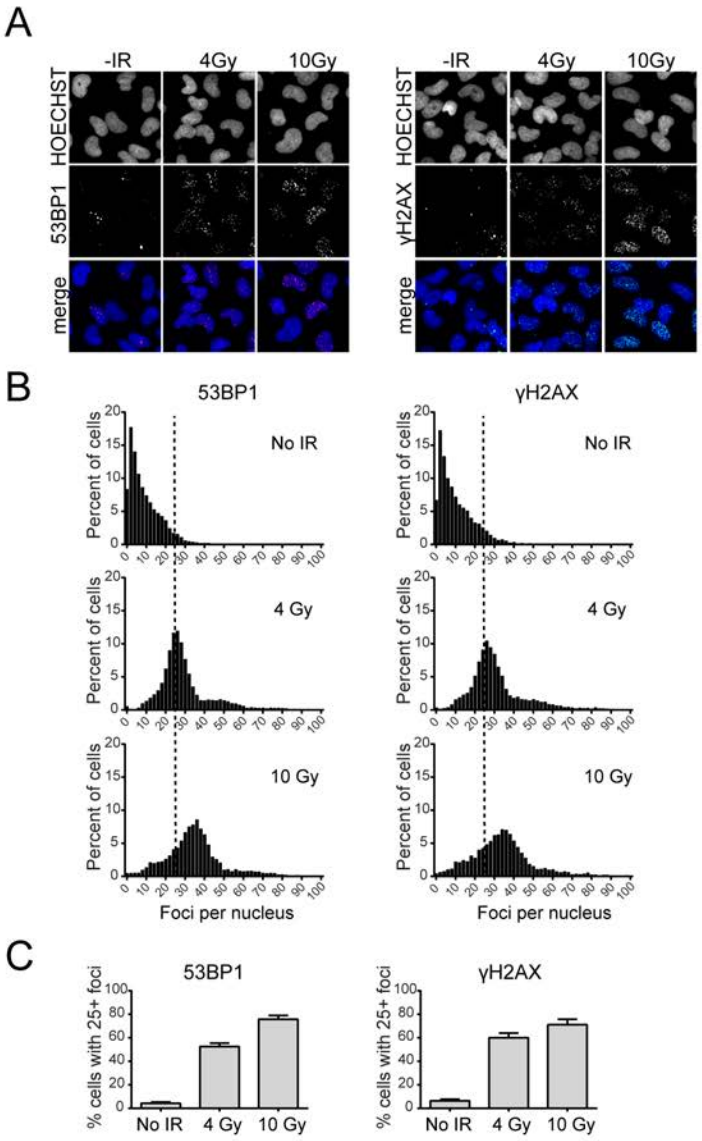
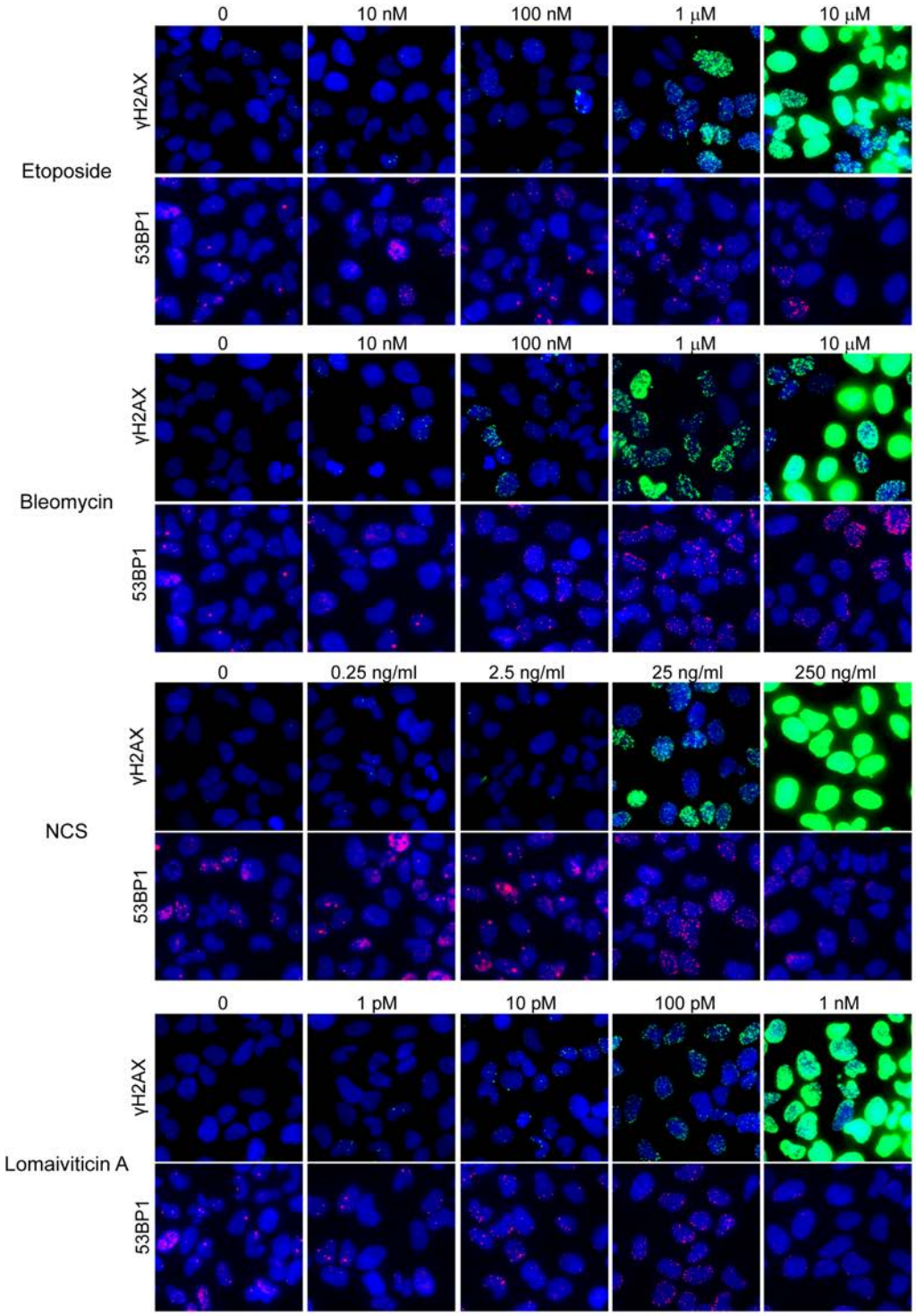
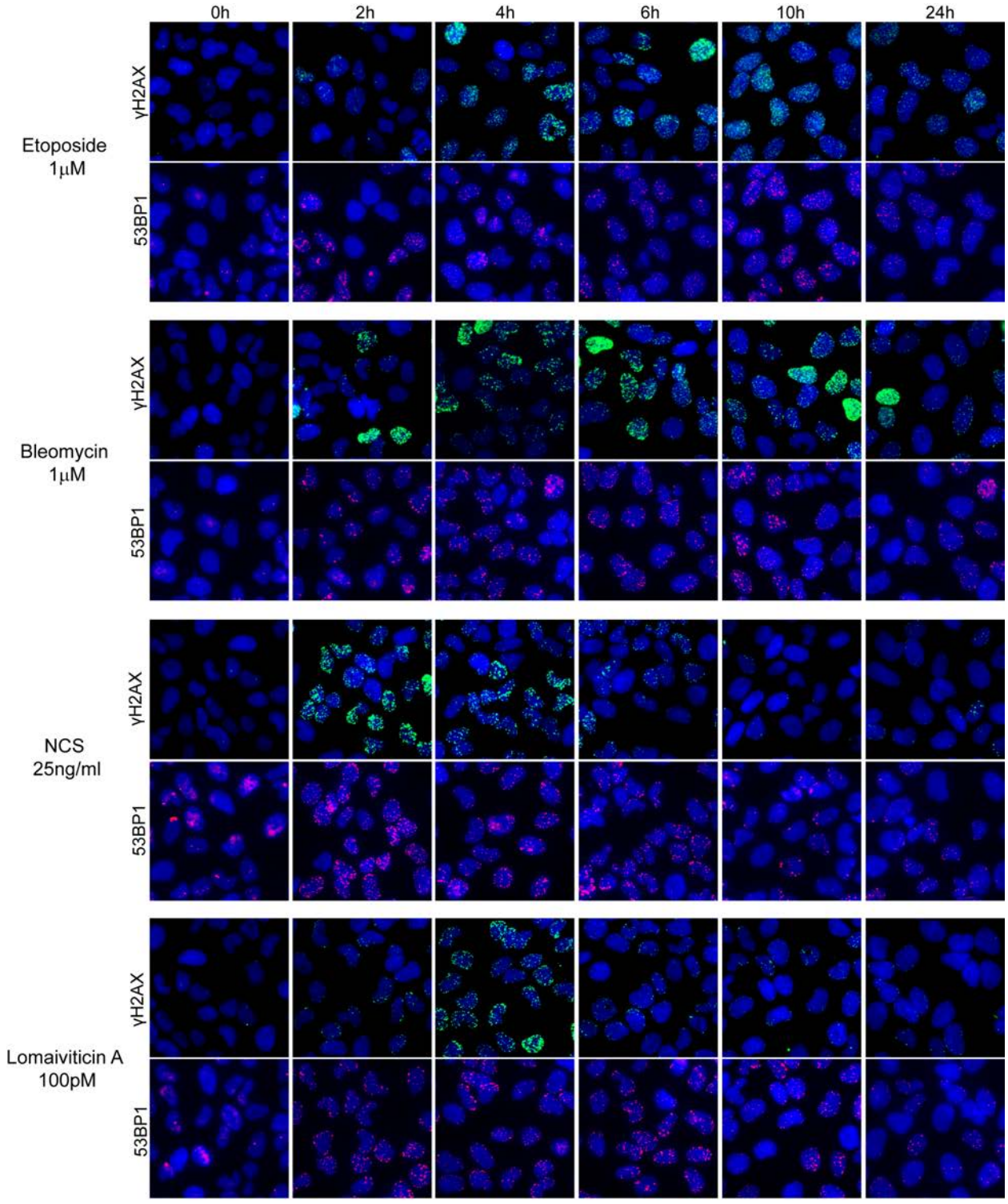
Supplementary Figures



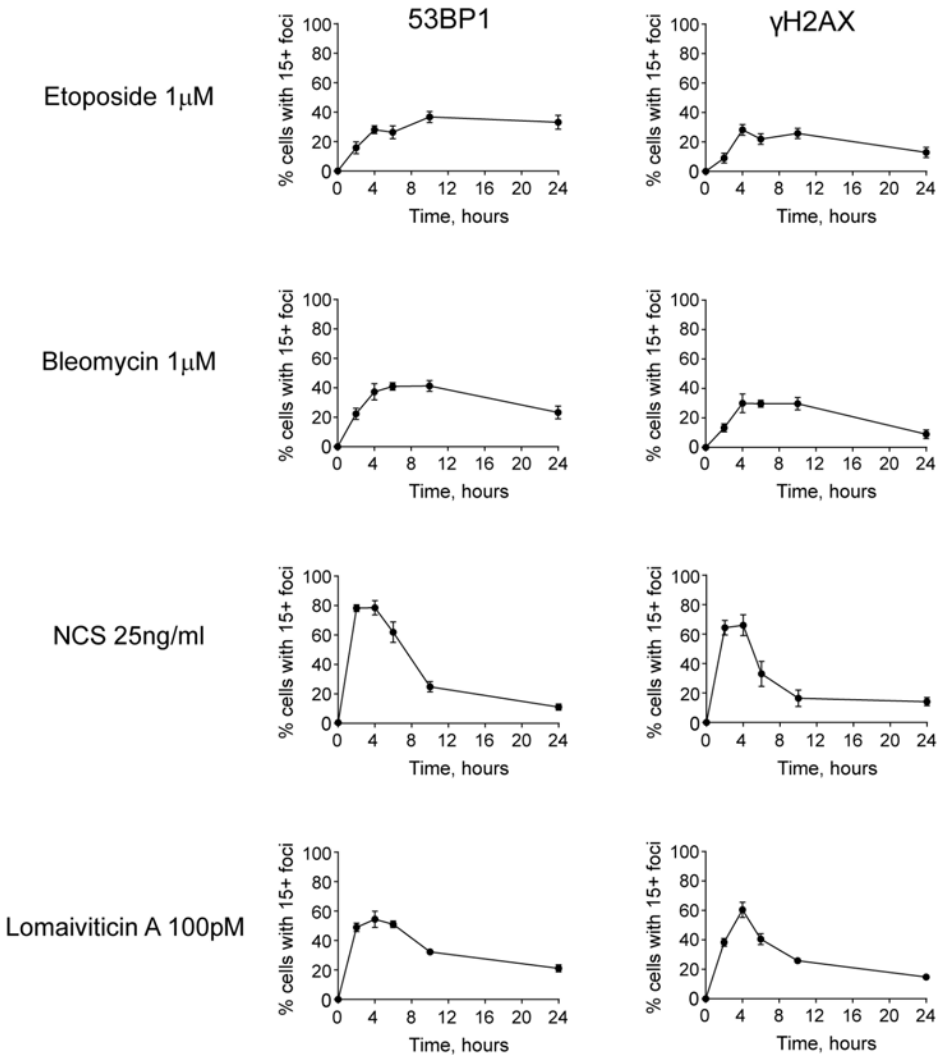
Supplementary Figure 1. Characterization of 53BP1 and γH2AX foci in T98G cells exposed to 4 or 10 Gy IR. (A) Raw InCell 2200 images of 53BP1 and γH2AX foci. (B) Distribution of cell population as a function of 53BP1 and γH2AX foci number. Histograms for control cells (No IR) and cells subjected to 4 Gy or 10 Gy IR and analyzed 5 h post-irradiation are shown. Dashed line represents 25 foci threshold. (C) Percent of cells with >25 53BP1 or γH2AX foci after treatment with 4 or 10 Gy IR.



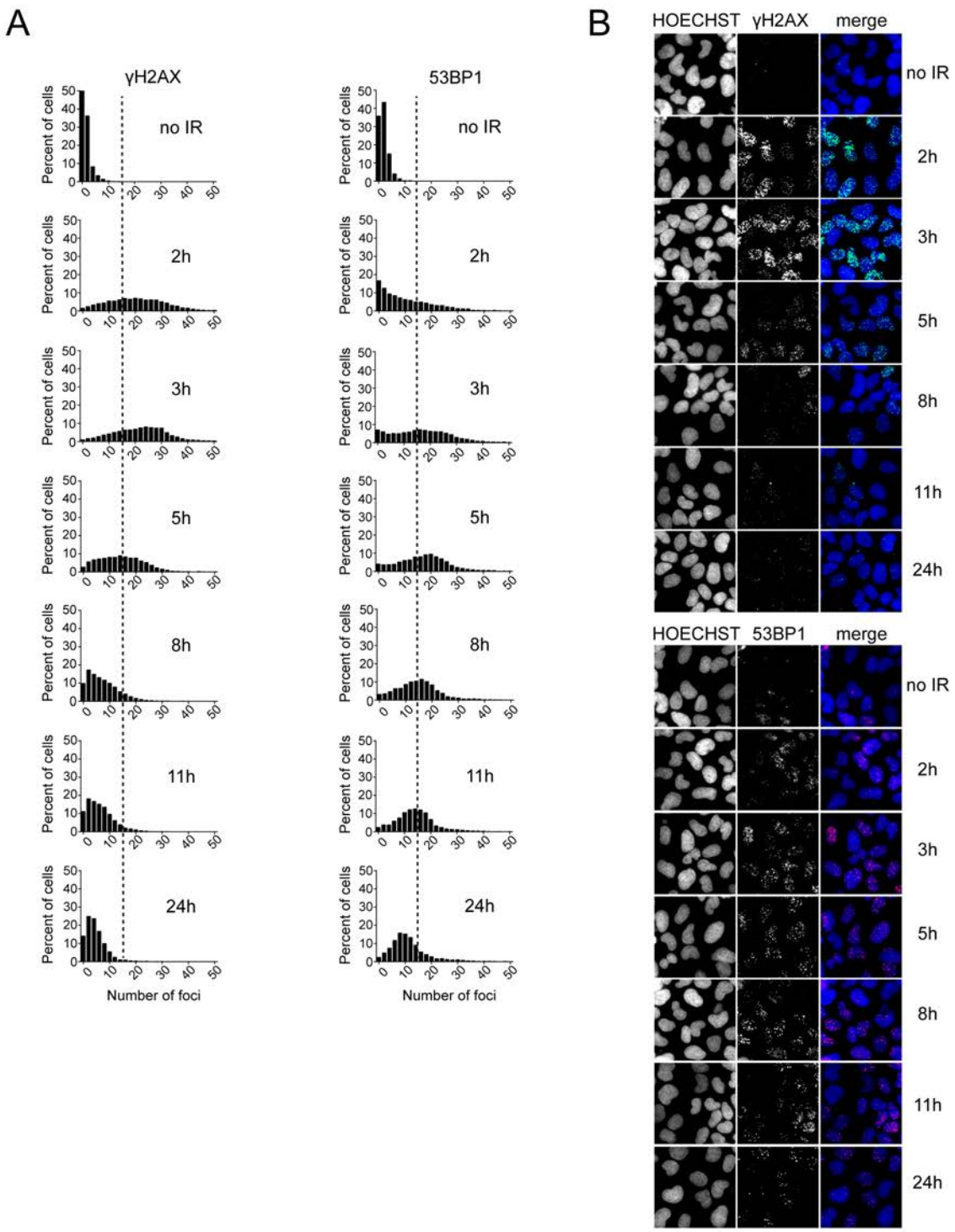
Supplementary Figure 2. Analysis of 53BP1 and γH2AX images in cells treated with DNA-damaging agents. Raw 53BP1 and γH2AX IF images of cells subjected to 4 h drug treatments are shown. Drug concentrations used for the treatment are indicated.



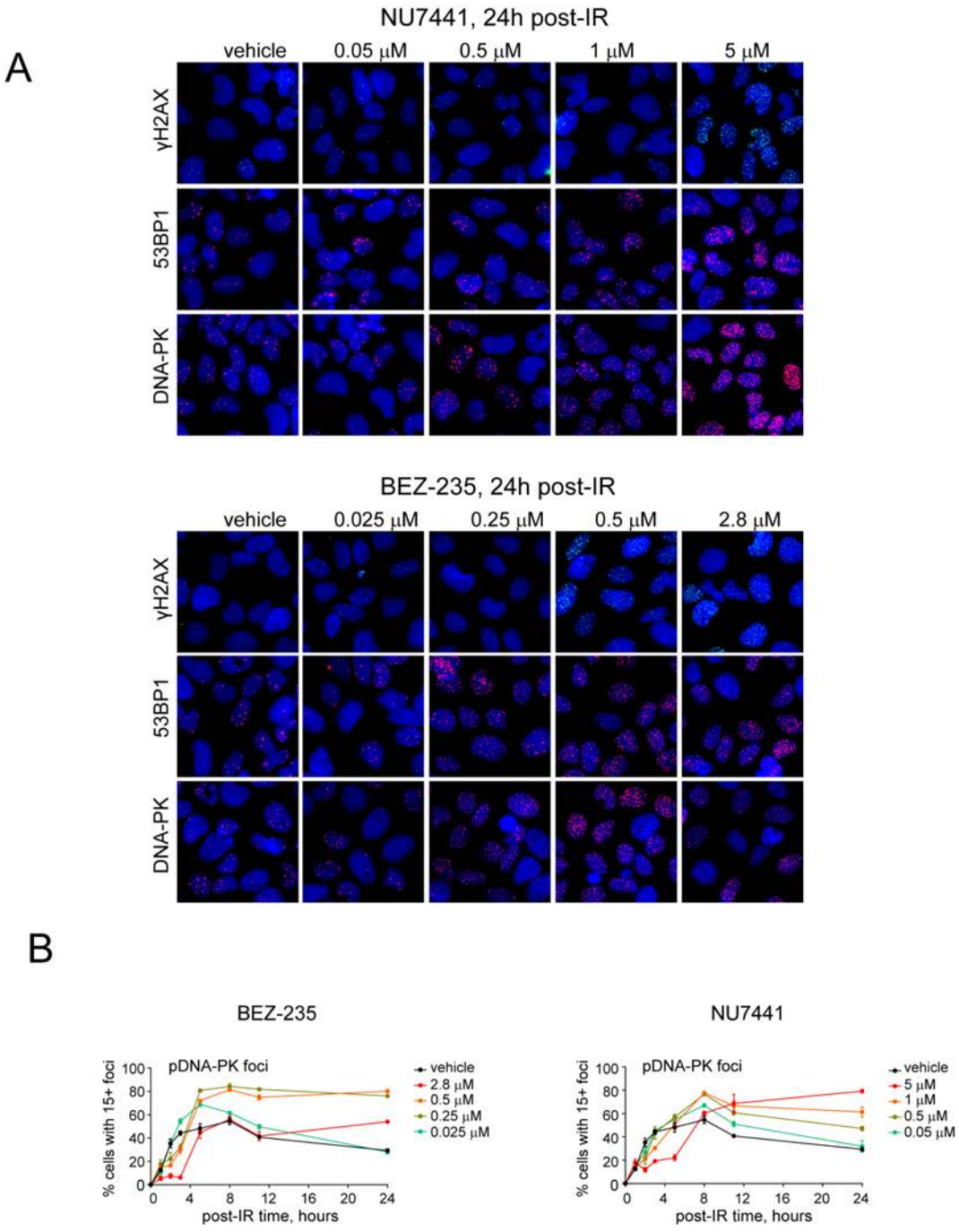
Supplementary Figure 3. Timecourse of 53BP1 and γH2AX foci formation and resolution in cells treated with DNA-damaging agents. Raw 53BP1 and γH2AX IF images are shown. Drug doses and treatment times are indicated.



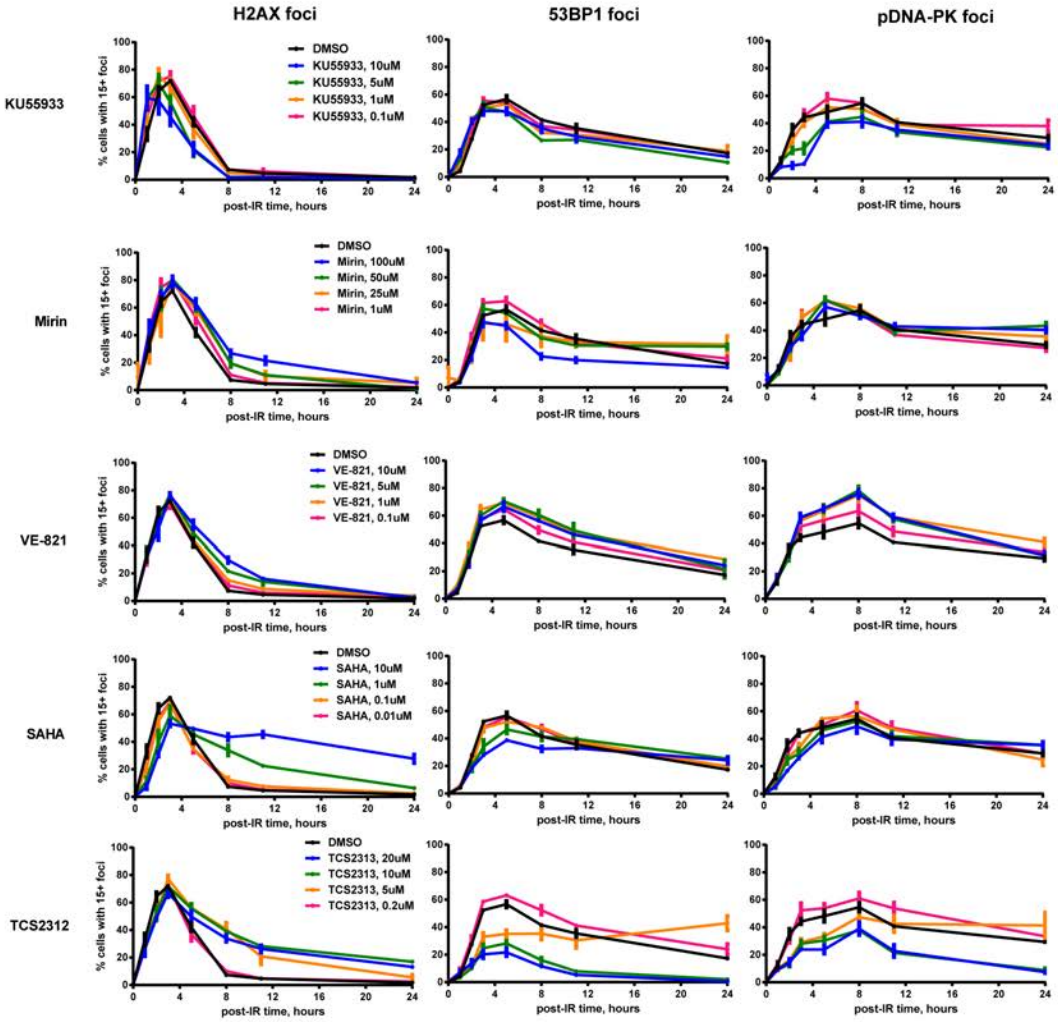
Supplementary Figure 4. Quantification of 53BP1 and γH2AX foci in cells treated with DNA-damaging agents. Percent of cells with >15 foci is shown as a function of drug treatment time.



Supplementary Figure 5. Timecourse of 53BP1 and γH2AX foci formation and resolution in cells subjected to 10 Gy irradiation. (A) Distribution of cell population as a function of γH2AX or 53BP1 foci numbers at indicated post-irradiation time points. Dashed line represents a 15 foci threshold. (B) Raw images of γH2AX (top) and 53BP1 (bottom) foci at the indicated times post-IR.



Supplementary Figure 6. γH2AX, 53BP1 and DNA-PK foci patterns in cells treated with Nu7441 and BEZ-235 DNA repair inhibitors. (A) Raw foci images of cells pre-treated with indicated doses of NU7441 or BEZ-235 for 1 h prior to irradiation, and then analyzed 24 h post-IR. (B) Percent of cells with >15 foci as a function of post-irradiation time. Cells were pre-treated with drugs for 1 h prior to irradiation. Drug concentrations used for treatment are indicated.



Supplementary Figure 7. γH2AX, 53BP1 and DNA-PK foci kinetics in cells treated with known DNA repair inhibitors. Cells were pre-treated with drugs for 1 h prior to irradiation. Percent of cells with >15 foci is shown as a function of post-IR time. Assayed drug doses are indicated.

A

53BP1 15+ Foci (Relative to NU7441)	Z'	S/B	CV pos control	CV neg control
NIH_plate 1	0.47	3.14	0.1	0.08
NIH_plate 2	0.53	3.45	0.06	0.19
ENZO FDA_plate 1	0.52	2.97	0.05	0.16
ENZO FDA_plate 2	0.69	3.46	0.04	0.11
Yale Procured Drugs	0.59	2.63	0.03	0.13
GenPlus_plate 3	0.59	2.89	0.05	0.12
GenPlus_plate 2	0.69	3.46	0.04	0.1
GenPlus_plate 1	0.69	3.55	0.04	0.12

γH2AX 15+ Foci (Relative to NU7441)	Z'	S/B	CV pos control	CV neg control
NIH_plate 1	0.66	8.36	0.07	0.22
NIH_plate 2	0.7	8.99	0.07	0.19
ENZO FDA_plate 1	0.56	7.61	0.09	0.27
ENZO FDA_plate 2	0.68	8.71	0.07	0.18
Yale Procured Drugs	0.65	7.04	0.07	0.19
GenPlus_plate 3	0.71	7.58	0.05	0.26
GenPlus_plate 2	0.66	8.94	0.08	0.16
GenPlus_plate 1	0.8	12.95	0.05	0.22

B

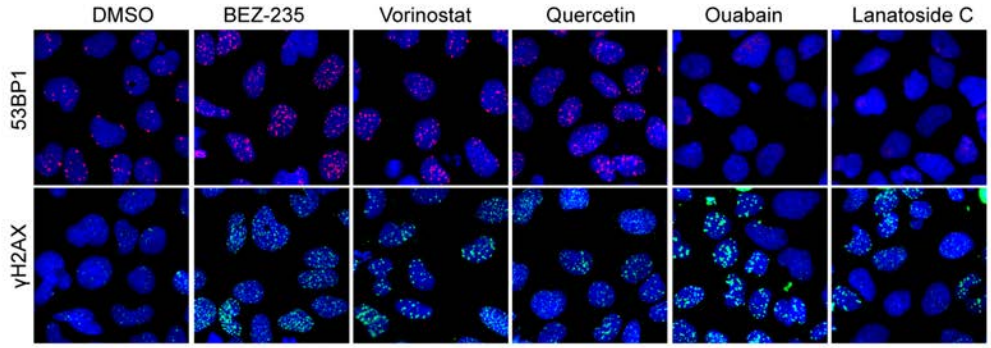
53BP1 15+ Foci (Relative to BEZ-235)	Z'	S/B	CV pos control	CV neg control
NIH_plate 1	0.71	3.46	0.05	0.08
NIH_plate 2	0.57	3.66	0.05	0.18
ENZO FDA_plate 1	0.46	2.8	0.06	0.16
ENZO FDA_plate 2	0.61	3.55	0.06	0.11
Yale Procured Drugs	0.55	2.68	0.05	0.12
GenPlus_plate 3	0.59	2.92	0.05	0.12
GenPlus_plate 2	0.68	3.56	0.05	0.1
GenPlus_plate 1	0.72	3.81	0.04	0.12

γH2AX 15+ Foci (Relative to BEZ-235)	Z'	S/B	CV pos control	CV neg control
NIH_plate 1	0.52	7.05	0.1	0.28
NIH_plate 2	0.51	7.52	0.12	0.19
ENZO FDA_plate 1	0.38	5.21	0.12	0.25
ENZO FDA_plate 2	0.54	7.1	0.11	0.18
Yale Procured Drugs	0.39	5.82	0.14	0.19
GenPlus_plate 3	0.4	5.75	0.12	0.26
GenPlus_plate 2	0.56	7.12	0.1	0.16
GenPlus_plate 1	0.73	10.67	0.06	0.22

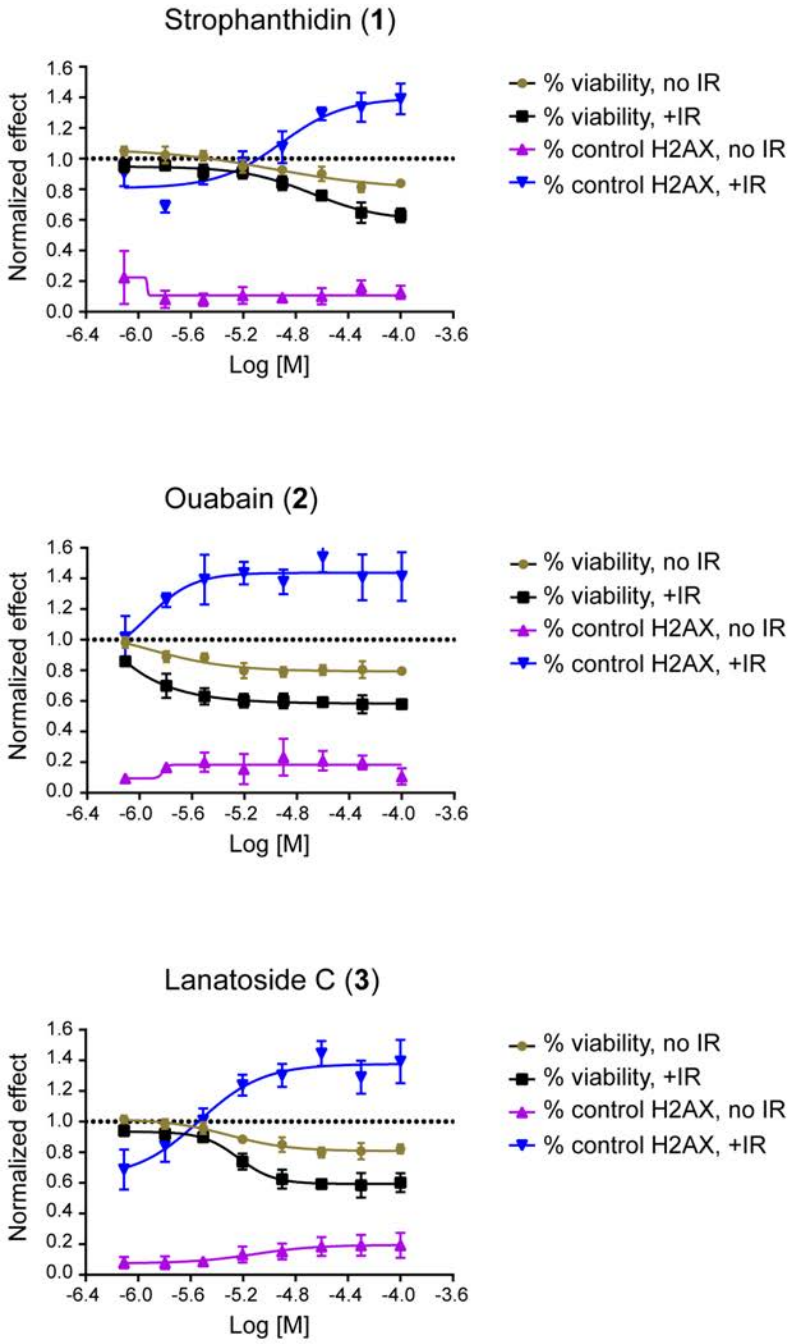
Supplementary Figure 8. Summary of screen statistics. (A) Z prime (Z'), signal-to-background (S/B) and coefficient of variation (CV) calculated from the mean and standard deviations of negative vehicle control and positive NU7441 control samples for each screening plate. (B) Same as A, but BEZ-235 samples were used as positive control.

DNA cleavers, replication/synthesis inhibitors					
drug name	mechanism	53BP1 %effect in the screen	gH2AX %effect in the screen	library	notes
bleomycin	DNA cleaver	90%	80%	ENZO FDA	
		90%	17%	Microsource	
Mitoxantrone	topoisomerase 2 inhibitor	-40%	30%	ENZO FDA	
Mitomycin	DNA crosslinker	72%	-18%	ENZO FDA	
		no effect	no effect	Microsource	
camptothecin	topoisomerase 1 inhibitor	35%	no effect	Microsource	
		75%	no effect	ENZO FDA	
		88%	no effect	ENZO FDA	
teniposide	NDNA crosslinker, topo 2 inhibitor	35%	60%	Yale procured	
etoposide	topoisomerase 2 inhibitor	63%	no effect	ENZO FDA	
doxorubicin	DNA intercalator, topo 2 inhibitor	-50%	no effect	ENZO FDA	60% viability, cells look apoptotic
		-30%	40%	NIH	
daunorubicin	DNA intercalator, topo 2 inhibitor	-50%	15%	ENZO FDA	60% viability, cells look apoptotic
epirubicin	DNA intercalator, topo 2 inhibitor	-40%	15%	NIH	70% viability, cells look apoptotic
idarubicin	DNA intercalator, topo 2 inhibitor	-40%	-10%	NIH	65% viability, cells look apoptotic
		-50%	-16%	ENZO FDA	
irinotecan	topoisomerase 1 inhibitor	no effect	no effect	NIH	
topotecan	topoisomerase 1 inhibitor	45%	no effect	NIH	
		no effect	no effect	ENZO FDA	
mitoxantrone	topoisomerase 2 inhibitor	-40%	30%	ENZO FDA	
cisplatin	DNA binding, crosslinking	40%	no effect	Microsource	
carboplatin	DNA binding, interference with DDR	35%	no effect	Microsource	
		no effect	no effect	ENZO FDA	
oxaliplatin	DNA crosslinking, DNA synthesis inhibition	no effect	no effect	ENZO FDA	
cytarabine	DNA synthesis inhibition	60%	no effect	Microsource	
		36%	no effect	ENZO FDA	
gemcitabine	nucleoside analog, RNR inhibitor	75%	no effect	ENZO FDA	
fluorouracil	pyrimidine analog, thymidilate synthase inhibitor, DNA replication inhibitor	no effect	no effect	Microsource	
		no effect	no effect	ENZO FDA	
hydroxyurea	ribonucleotide reductase inhibitor	no effect	no effect	Microsource	

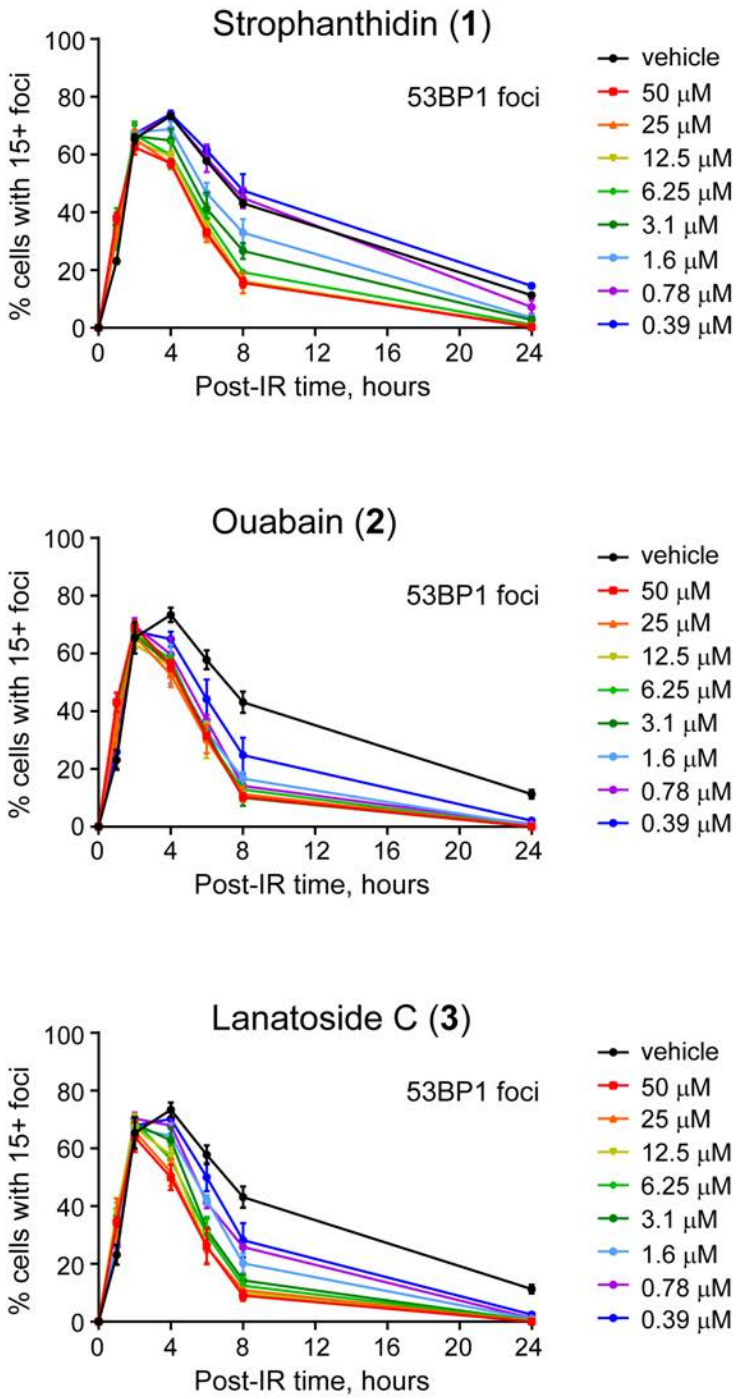
Supplementary Figure 9. Identification of know DNA damaging agents as screen actives. Table summarizes relative percent effect for 53BP1 and γH2AX foci phenotypes for drugs known to induce DNA damage via indicated mechanisms.



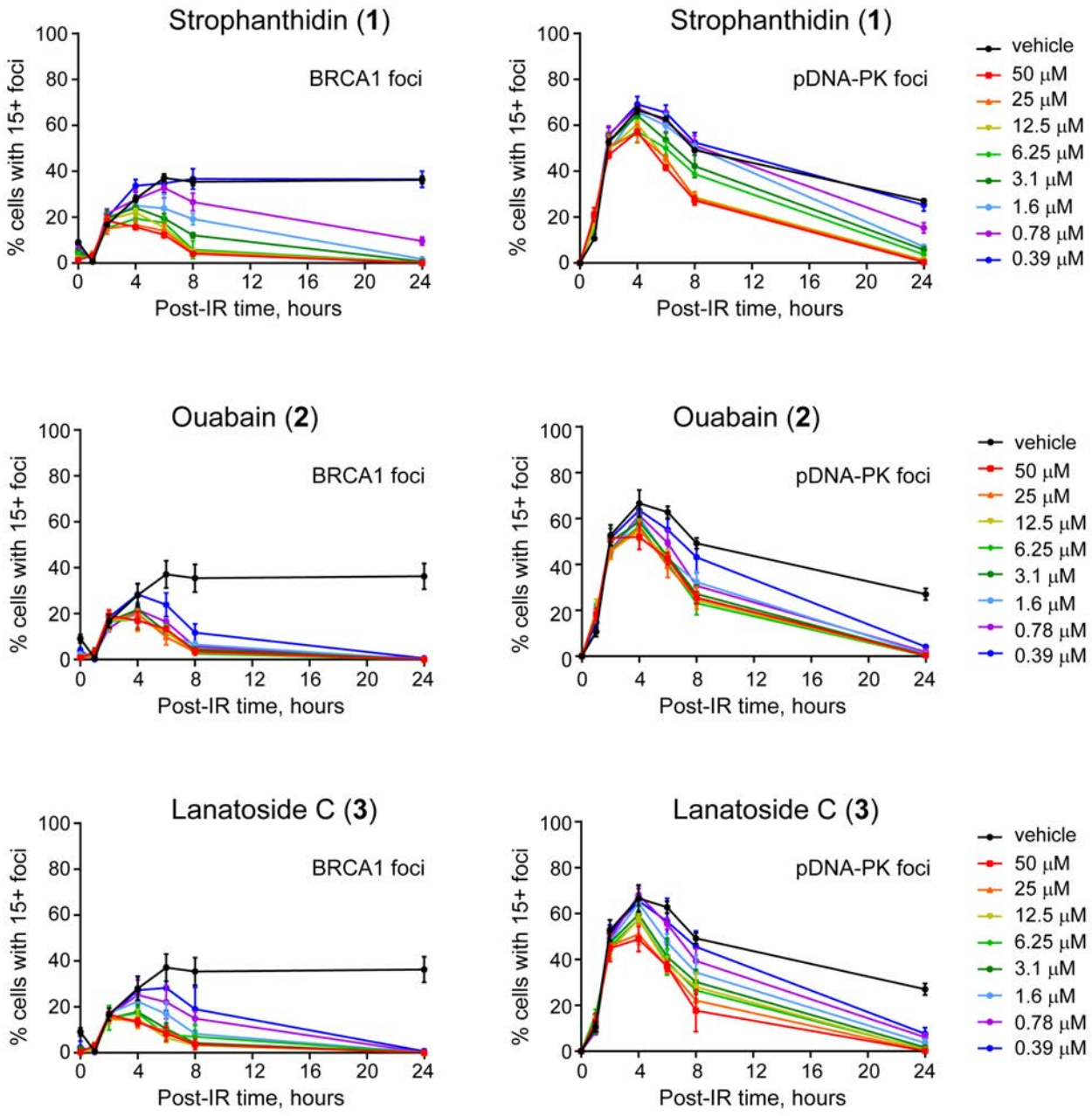
Supplementary Figure 10. Representative 53BP1 and γH2AX foci images for selected screen actives. Shown are raw IF images of cells pre-treated with indicated library compounds, subjected to 10 Gy IR, and analyzed 24 h post-irradiation.



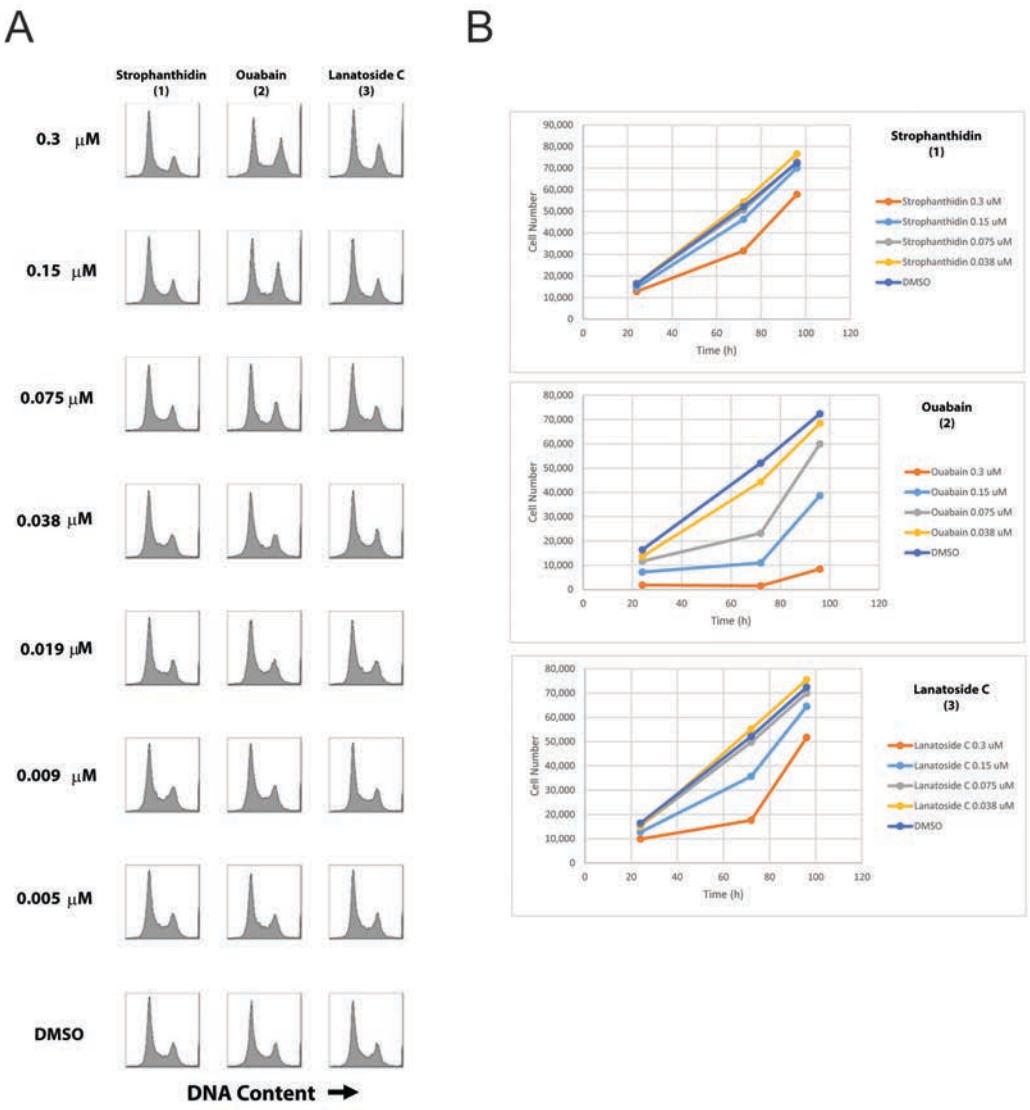
Supplementary Figure 11. Effect of selected hits on cell viability and γH2AX foci numbers in U2OS cells with or without IR treatment. Dose curves for cells subjected to 10 Gy IR (+IR) and un-irradiated cells (no IR) are shown for Strophanthidin (1), Ouabain (2) and Lanatoside C (3) screen actives. Data was normalized relative to negative (vehicle) control



Supplementary Figure 12. 53BP1 foci kinetics in cells treated with selected hit compounds and subjected to 10 Gy IR. Percent of cells with >15 foci is shown as a function of post-irradiation time, drug concentrations used for dose response analysis are indicated.



Supplementary Figure 13. DNA-PK and BRCA1 foci kinetics in cells treated with selected hit compounds and subjected to 10 Gy IR. Percent of cells with >15 foci is shown as a function of post-irradiation time, drug concentrations used for dose response analysis are indicated.



Supplementary Figure 14. Cell cycle and growth rates analysis of cells treated with selected hit compounds. (A) Representative cell cycle phase histograms for cells treated with Strophanthidin (1), Ouabain (2) and Lanatoside C (3). Drug concentrations used for dose response analysis are indicated. (B) Growth curves for cells treated with various concentrations of Strophanthidin (1), Ouabain (2) and Lanatoside (3), grown in 96-well microplates. Cell numbers are shown based on enumeration of H33342-stained nuclei after cellular fixation and staining.