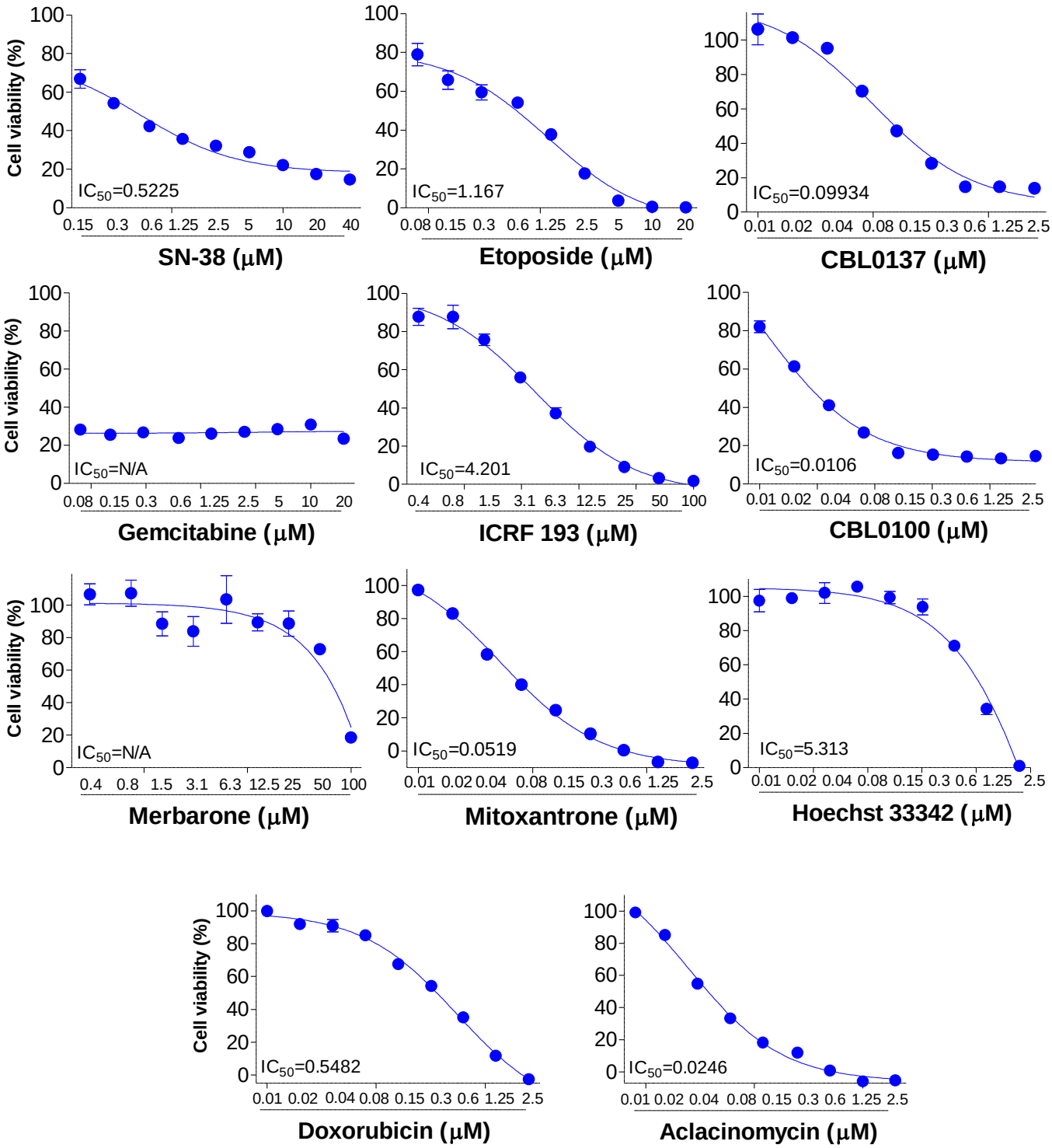


Figure S1. HeLa cells cytotoxicity induced by different DNA-targeting small molecules after 48 hours incubation. Cell viability was determined using resazurin-based assay (error bars represent the mean of at least three replicates ± SD). 50% toxic concentration of an inhibitor (IC_{50}) was calculated using GraphPad Prism 5 software (95% confidence interval) by nonlinear regression with an inhibitory dose-response model.

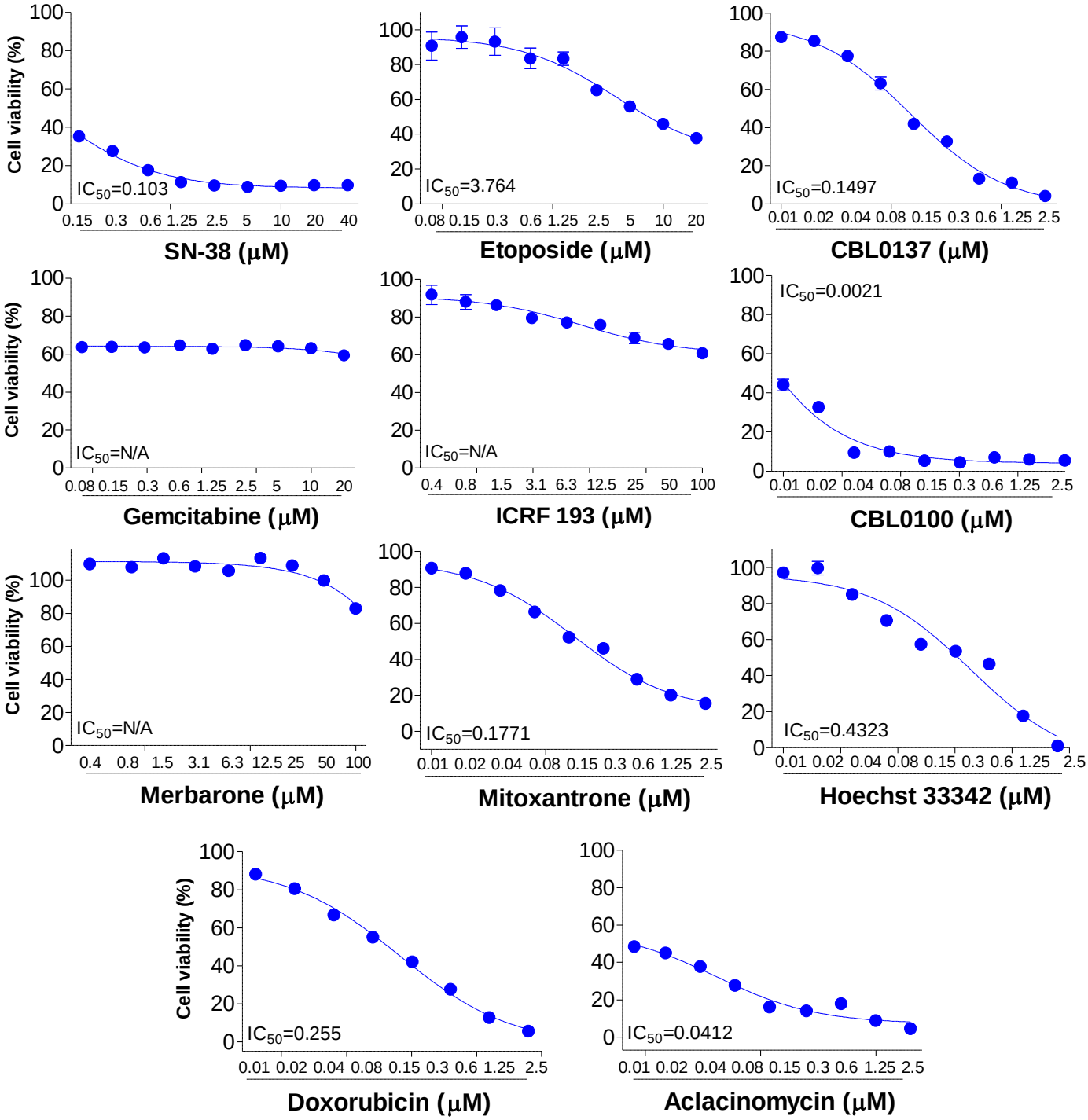


Figure S2. HCT116 cells cytotoxicity induced by different DNA-targeting small molecules after 48 hours incubation. Cell viability was determined using resazurin-based assay (error bars represent the mean of at least three replicates ± SD). 50% toxic concentration of an inhibitor (IC_{50}) was calculated using GraphPad Prism 5 software (95% confidence interval) by nonlinear regression with an inhibitory dose-response model.

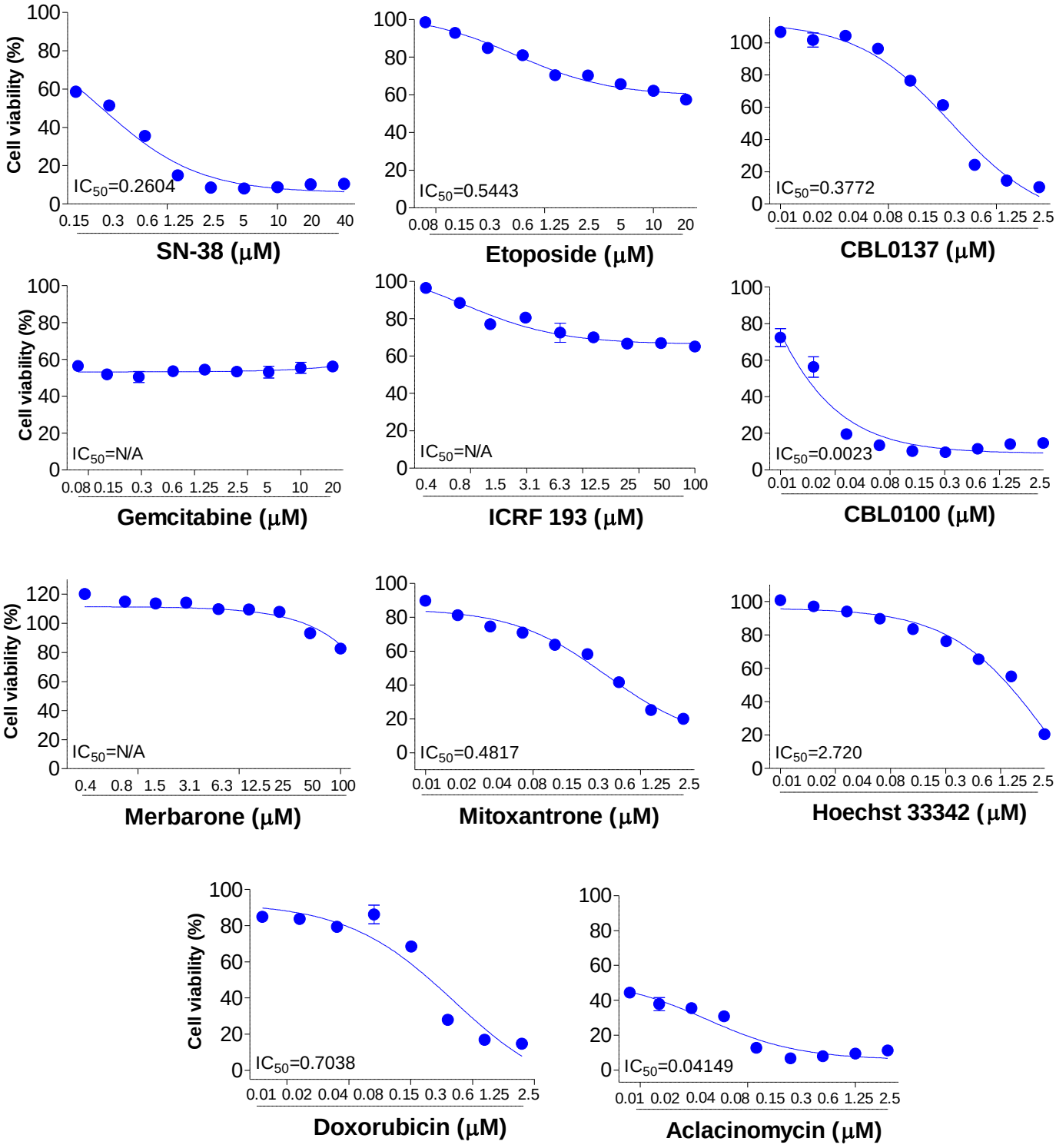


Figure S3. MCF7 cells cytotoxicity induced by different DNA-targeting small molecules after 48 hours incubation. Cell viability was determined using resazurin-based assay (error bars represent the mean of at least three replicates ± SD). 50% toxic concentration of an inhibitor (IC_{50}) was calculated using GraphPad Prism 5 software (95% confidence interval) by nonlinear regression with an inhibitory dose-response model.

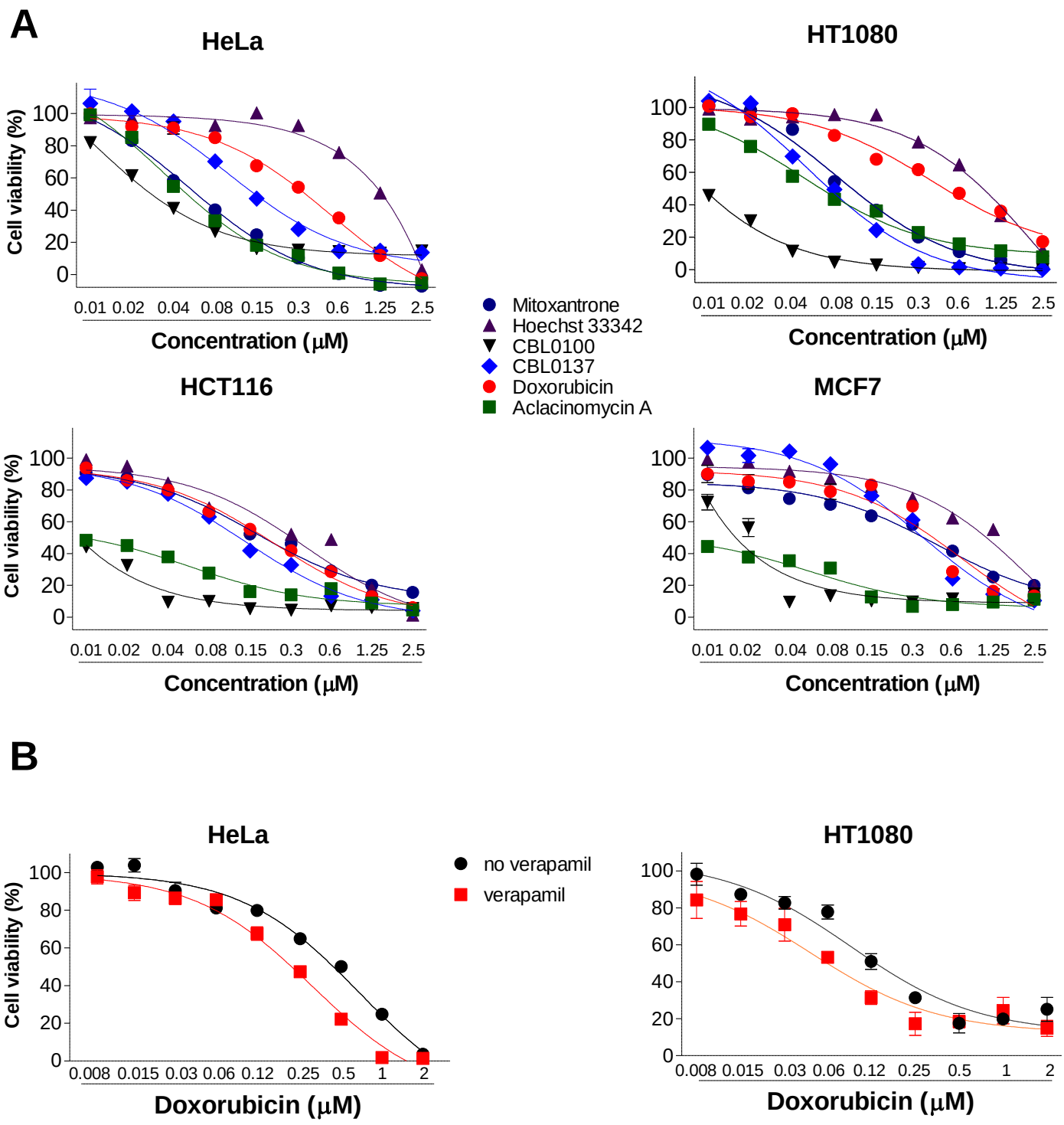


Figure S4. Comparative cytotoxicity of HT1080, HeLa, MCF7 and HCT116 cells induced by different DNA-binding small molecules (**A**), as well as Doxorubicin cytotoxicity in the presence and absence of MDR inhibitor, verapamil (20μM) (**B**) after 48 hours incubation for HT1080 and HeLa cells. Cell viability was determined using resazurin-based assay (error bars represent the mean of at least three replicates ± SD).

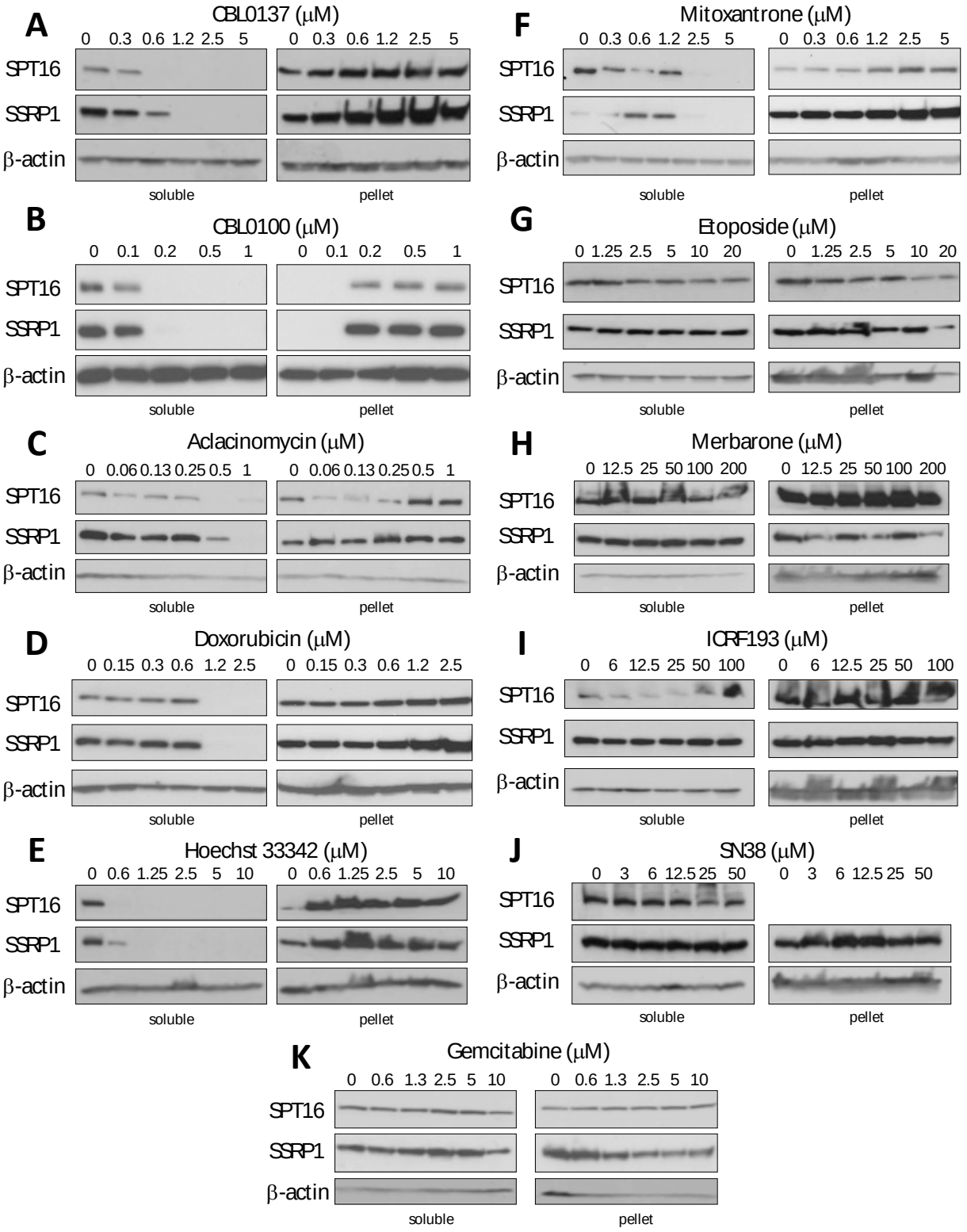


Figure S5. Ability of DNA-targeting compounds to induce c-trapping. Representative immunoblots of soluble fractions (left side) and chromatin pellets (right side) extracts of HeLa cells treated for 24 hours with compounds inducing c-trapping (**A-F**): CBL0137 (**A**), CBL0100 (**B**) Aclacinomycin A (**C**), Doxorubicin (**D**), Hoechst 33342 (**E**) and Mitoxantrone (F) as well as c-trapping non-inducing compounds (**G-K**): Etoposide (**G**), Merbarone (**H**), ICRF-193 (I), SN-38 (**J**) and Gemcitabine (**K**).

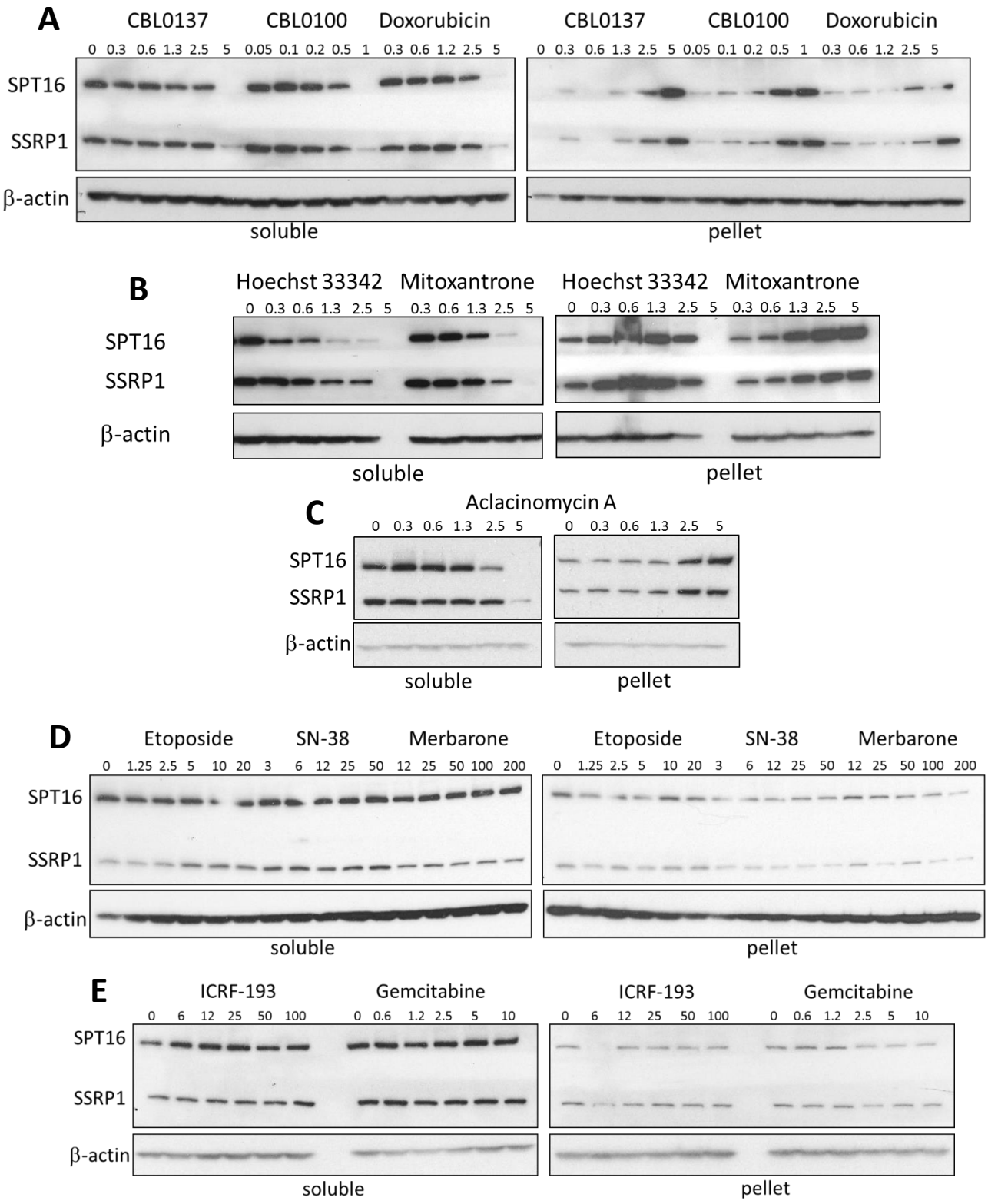


Figure S6. Ability of DNA-targeting compounds to induce c-trapping. Representative immunoblots of soluble fractions (left side) and chromatin pellets (right side) extracts of HCT116 cells treated for 24 hours with inducing c-trapping (**A-C**): CBL0137, CBL0100, Doxorubicin (**A**), Hoechst 33342, Mitoxantrone (**B**) and Aclacinomycin A (**C**), as well as c-trapping non-inducing compounds (**D-E**): Etoposide, SN-38, Merbarone (**D**), ICRF-193 and Gemcitabine (**E**).

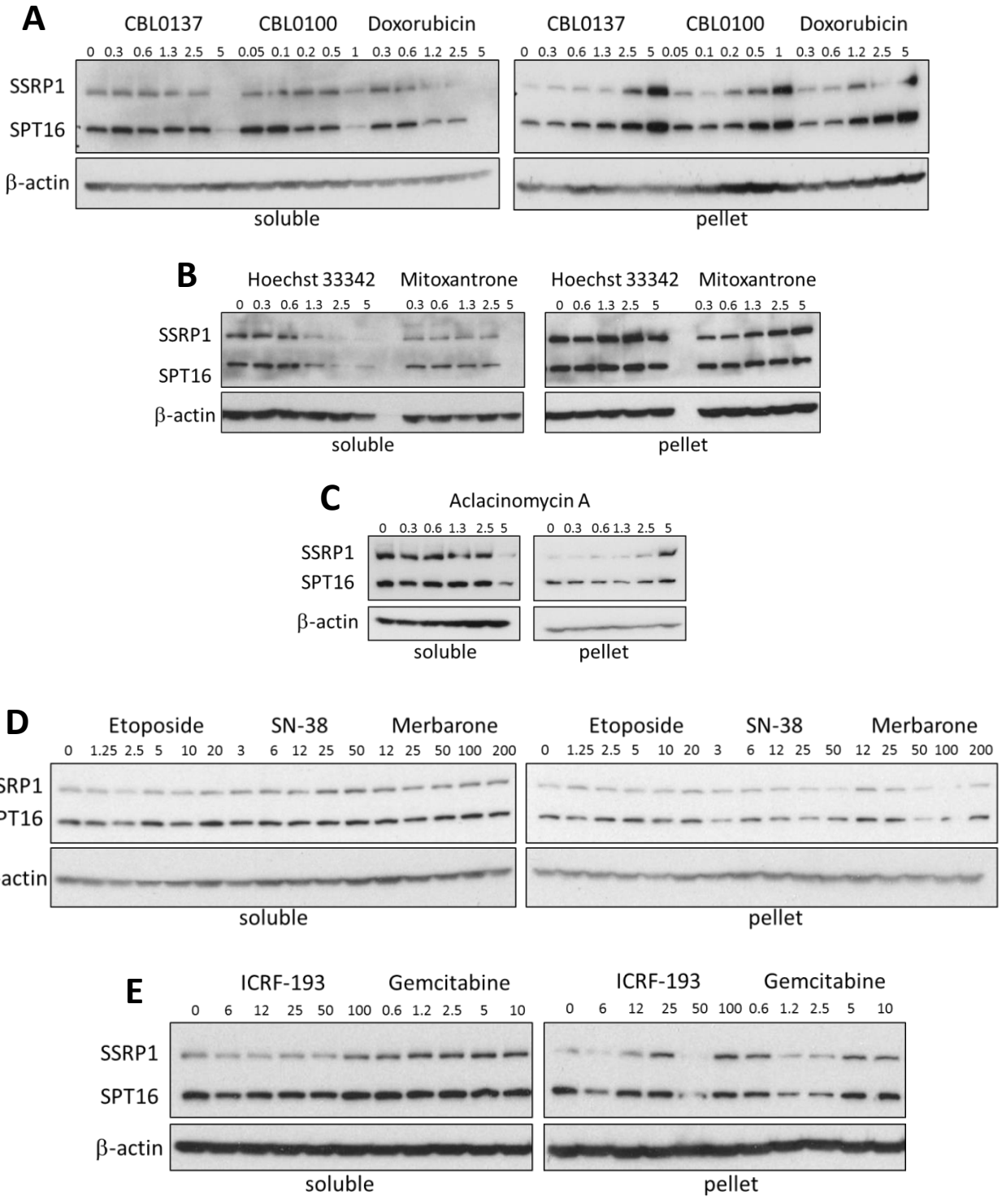


Figure S7. Ability of DNA-targeting compounds to induce c-trapping. Representative immunoblots of soluble fractions (left side) and chromatin pellets (right side) extracts of MCF7 cells treated for 24 hours with inducing c-trapping (**A-C**): CBL0137, CBL0100, Doxorubicin (**A**), Hoechst 33342, Mitoxantrone (**B**) and Aclacinomycin A (**C**), as well as c-trapping non-inducing compounds (**D-E**): Etoposide, SN-38, Merbarone (**D**), ICRF-193 and Gemcitabine (**E**).

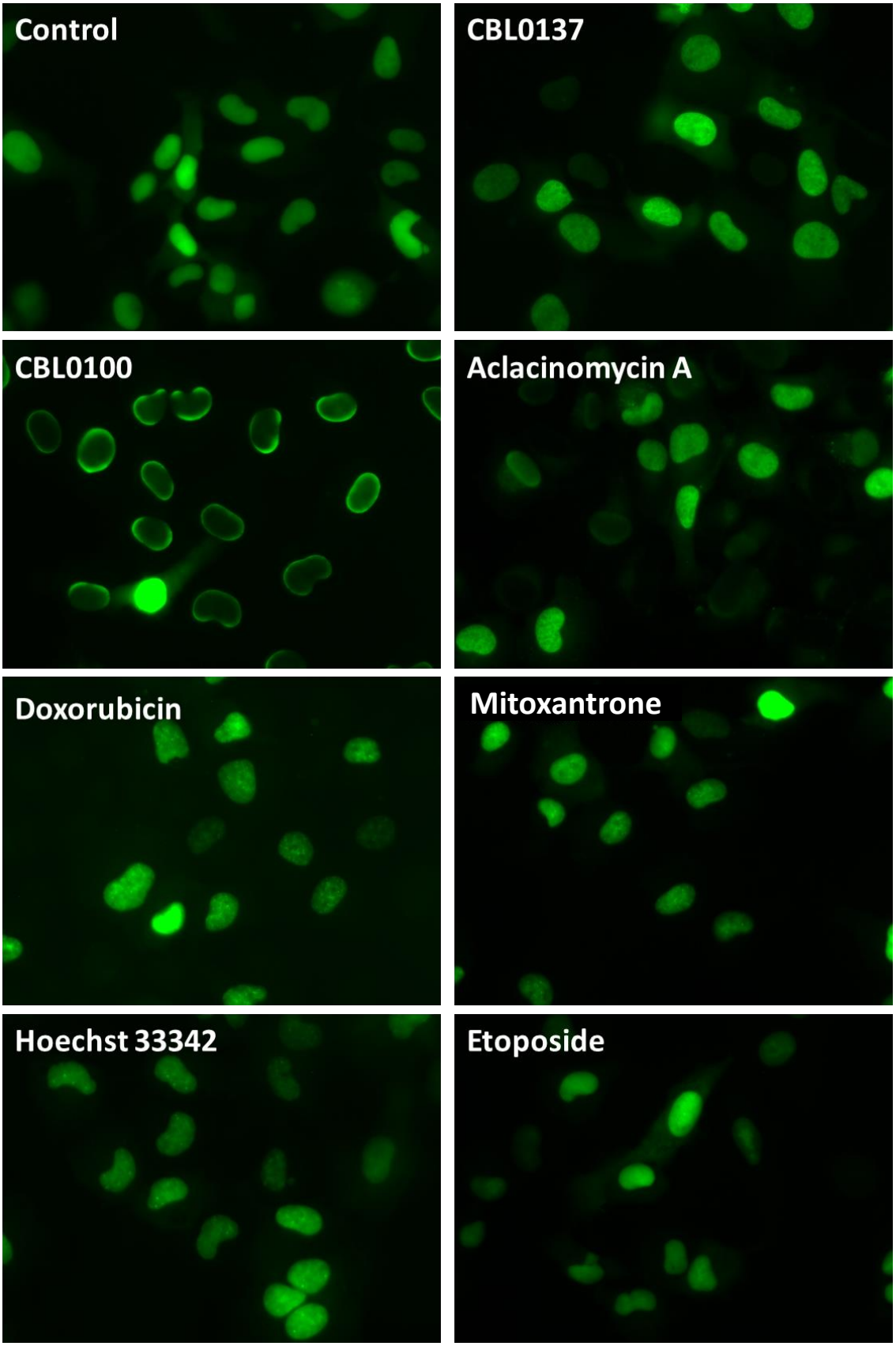


Figure S8. Common patterns of c-trapping (if present) in cell population. Imaging of HT1080 GFP-SSRP1 cells treated with 3μM of each compound for 1 hour, except etoposide (5 μM for 24 hours). Cells were fixed with 4% paraformaldehyde for 10 minutes. Images were taken with 40X lens. Exposition time was adjusted to fit majority of cells per field of view.

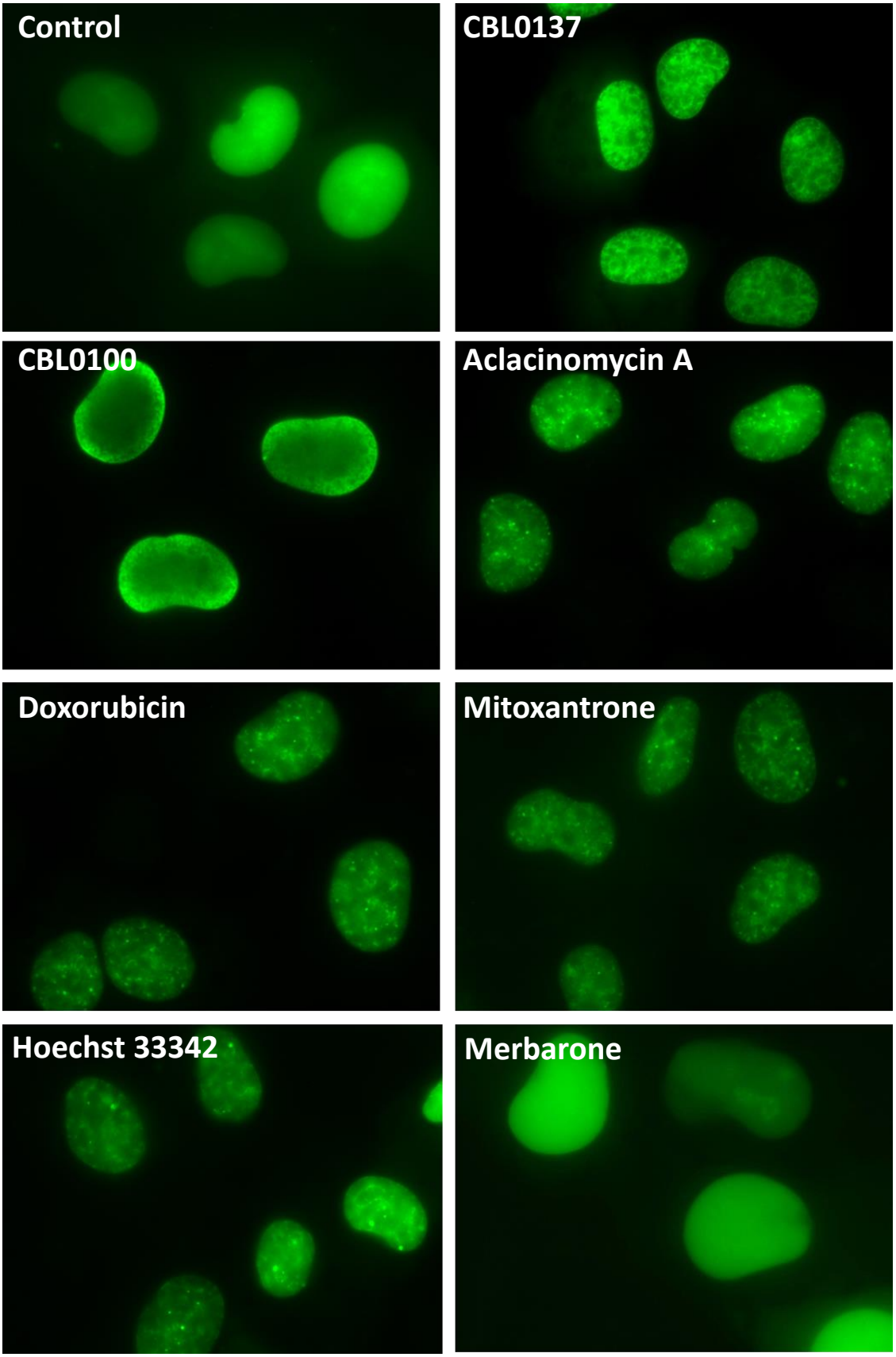


Figure S9. Common patterns of c-trapping (if present) in cell population. Imaging of HT1080 GFP-SSRP1 cells treated with 3μM of each compound for 1 hour, except merbarone (50 μM for 24 hours). Cells were fixed with 4% paraformaldehyde for 10 minutes. Images were taken with 100x oil X lens. Exposition time was adjusted to fit majority of cells per field of view.

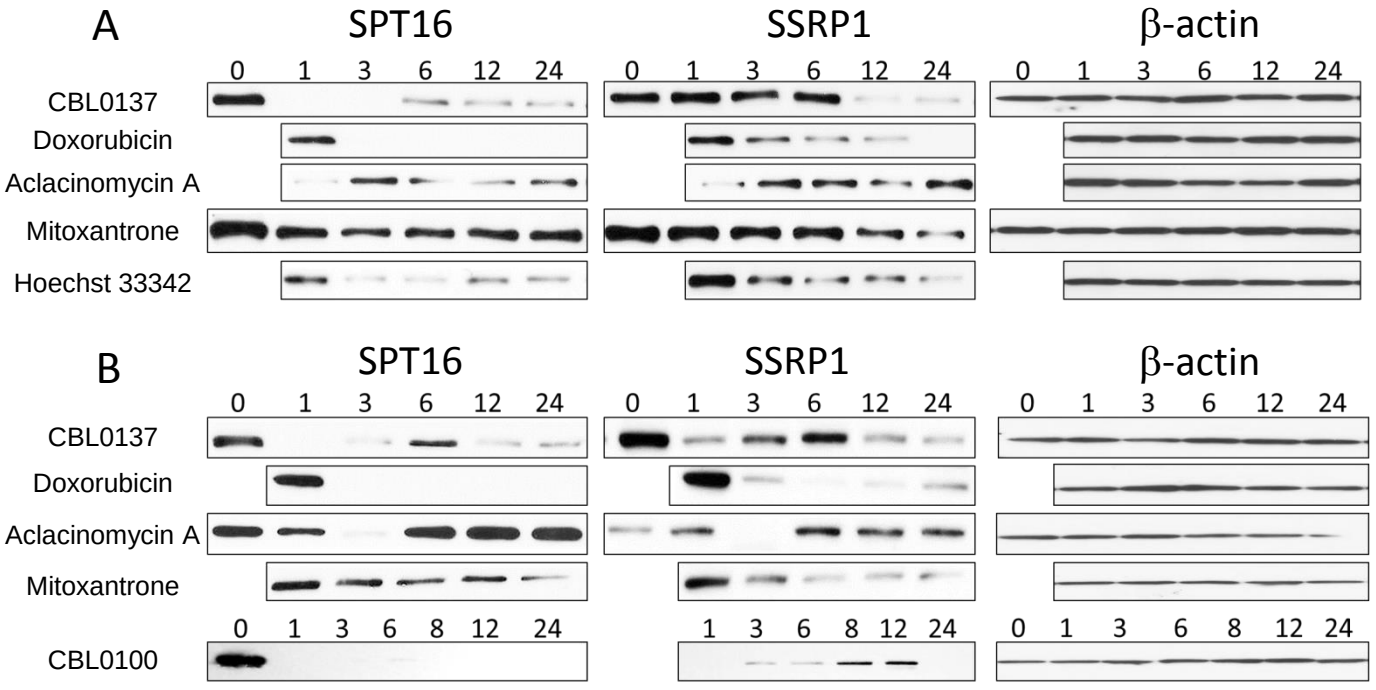


Figure S10. Time-dependent effect of treatment with 2μM of DNA-binding compounds on FACT subunits (SPT16 and SSRP1) distribution. Representative immunoblots of soluble fractions (**A**) of HeLa and (**B**) HT1080 cells.

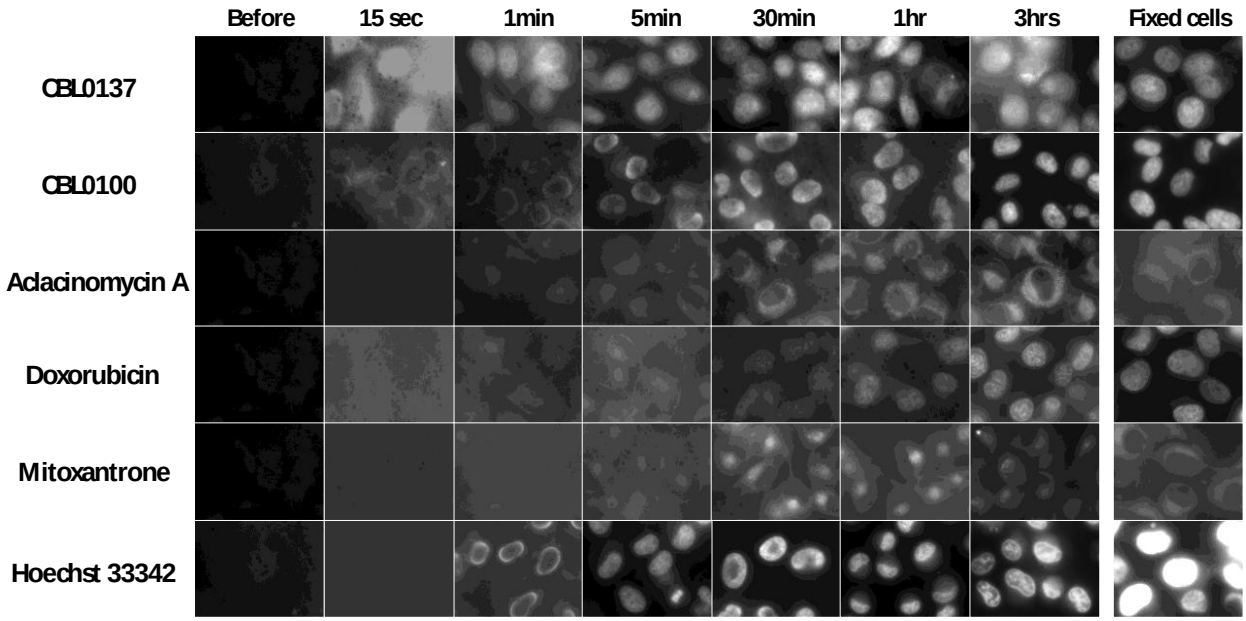


Figure S11. Kinetic of nuclear accumulation of compounds monitored using compound auto-fluorescence. All drugs were used at 5µM. Cell fixed with 4% paraformaldehide were used to assess maximal possible auto-fluorescence. The kinetics of compound accumulation in cell nuclei are very close to the kinetics of c-trapping for all compounds with the exception of Aclacinomycin A and Mitoxantrone, which have little to no nuclear fluorescence[1].

[1]Egorin MJ, Clawson RE, Ross LA, Schlossberger NM, Bachur NR. Cellular accumulation and disposition of aclacinomycin A. Cancer Res **1979**; 39:4396-400; Smith PJ, Sykes HR, Fox ME, Furlong IJ. Subcellular distribution of the anticancer drug mitoxantrone in human and drug-resistant murine cells analyzed by flow cytometry and confocal microscopy and its relationship to the induction of DNA damage. Cancer Res **1992**; 52:4000-8.

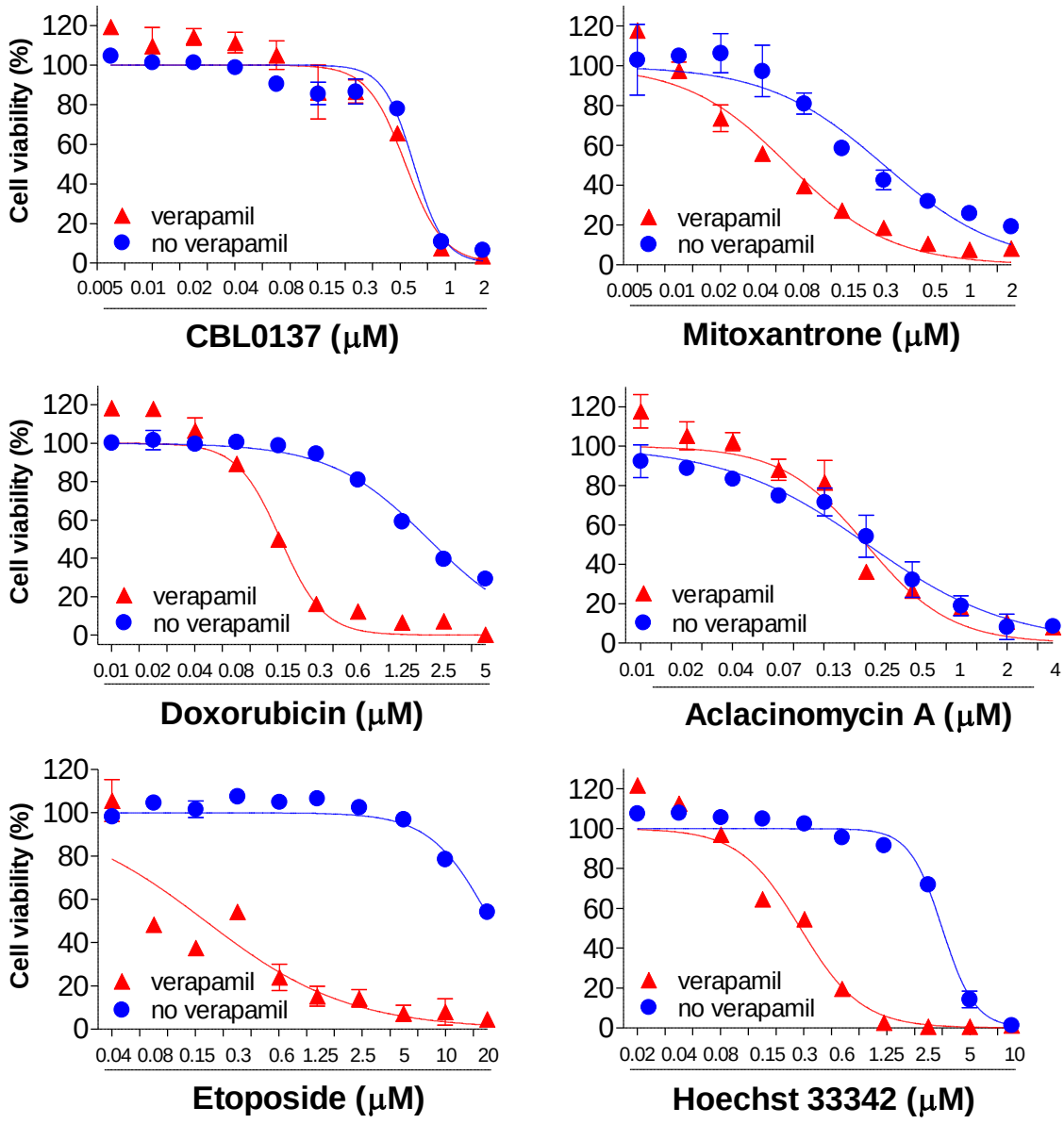


Figure S12. Differential toxicity of the DNA-targeting compounds to HL60/VCR cells overexpressing Multi-Drug Transporters (MDR) in the presence and absence of MDR inhibitor, Verapamil (20μM) within 48 hours incubation. Cell viability was determined using resazurin-based assay (error bars represent the mean of at least three replicates ± SD). The toxicity of the compounds which are substrates of MDR (Mitoxantrone, Doxorubicin, Hoechst 33342, Etoposide) is increased in the presence of Verapamil; while the toxicity of the compounds, which are not MDR substrates (CBL0137, CBL0100, Aclacinomycin A). Three latter compounds cause c-trapping during the first 1-15 minutes of incubation, much faster than the former compounds, Mitoxantrone or Doxorubicin (>1 hour). The fast kinetic of c-trapping of established MDR substrate Hoechst 33342[2].

[2]Van den Berg Van Saparoea HB, Lubelski J, Van Merkerk R, Mazurkiewicz PS, Driessen AJ. Proton motive force-dependent Hoechst 33342 transport by the ABC transporter LmrA of Lactococcus lactis. Biochemistry **2005**;44:16931-8) may be explained by high-affinity DNA binding (Bazhulina NP, Nikitin AM, Rodin SA, Surovaya AN, Kravatsky YV, Pismensky VF, *et al.* Binding of Hoechst 33258 and its derivatives to DNA. Journal of biomolecular structure & dynamics **2009**;26:701-18.

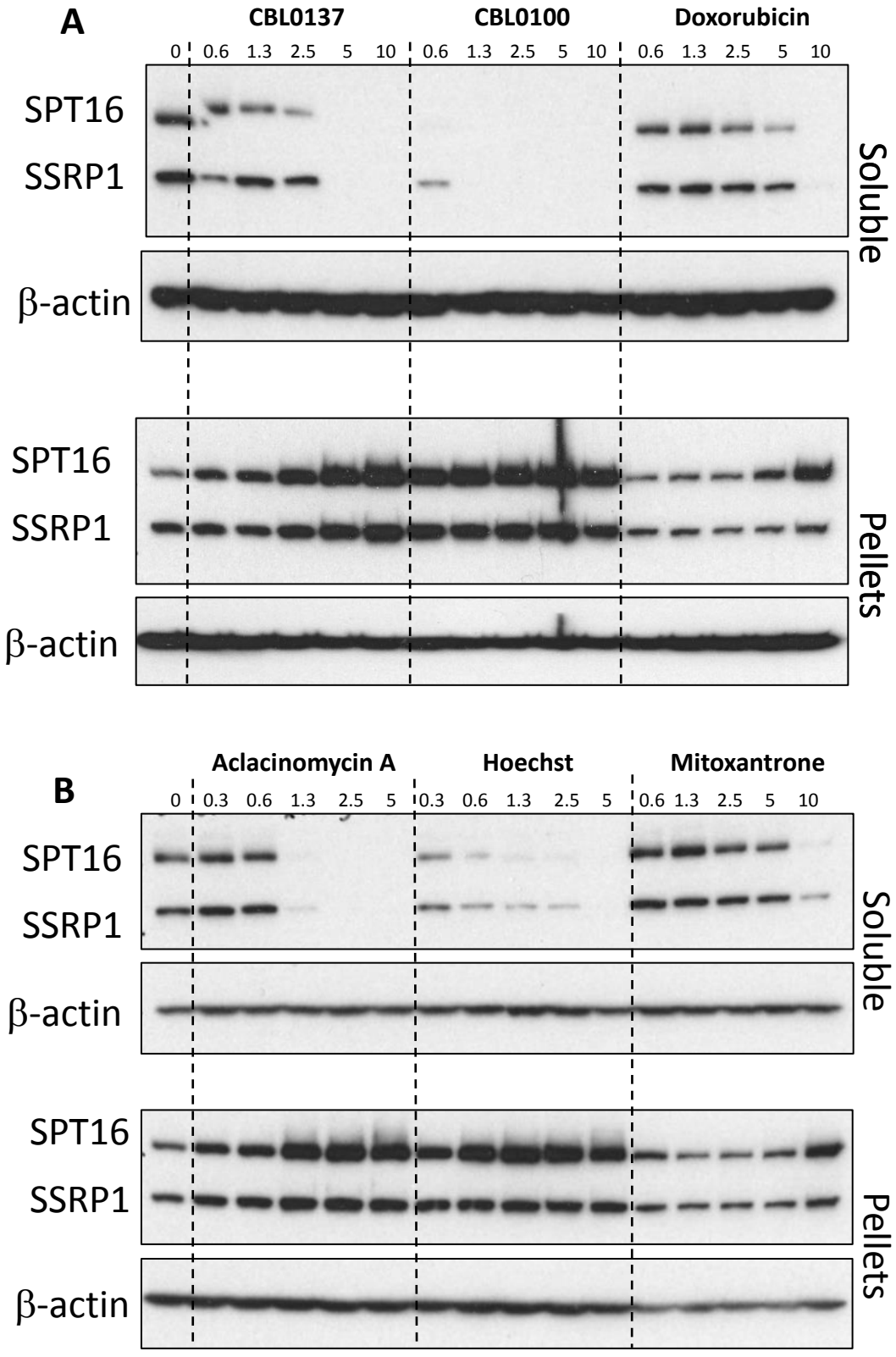


Figure S13. Ability of DNA-binding compounds to induce c-trapping. Representative immunoblots of soluble fractions (up) and chromatin pellets (down) extracts of HCT116 cells treated with c-trapping inducing compounds for 30 min: CBL0137, CBL0100 (**A**) and Aclacinomycin A (**B**), as well as for 60 min: Doxorubicin (**A**), Hoechst 33342, and Mitoxantrone (**B**).

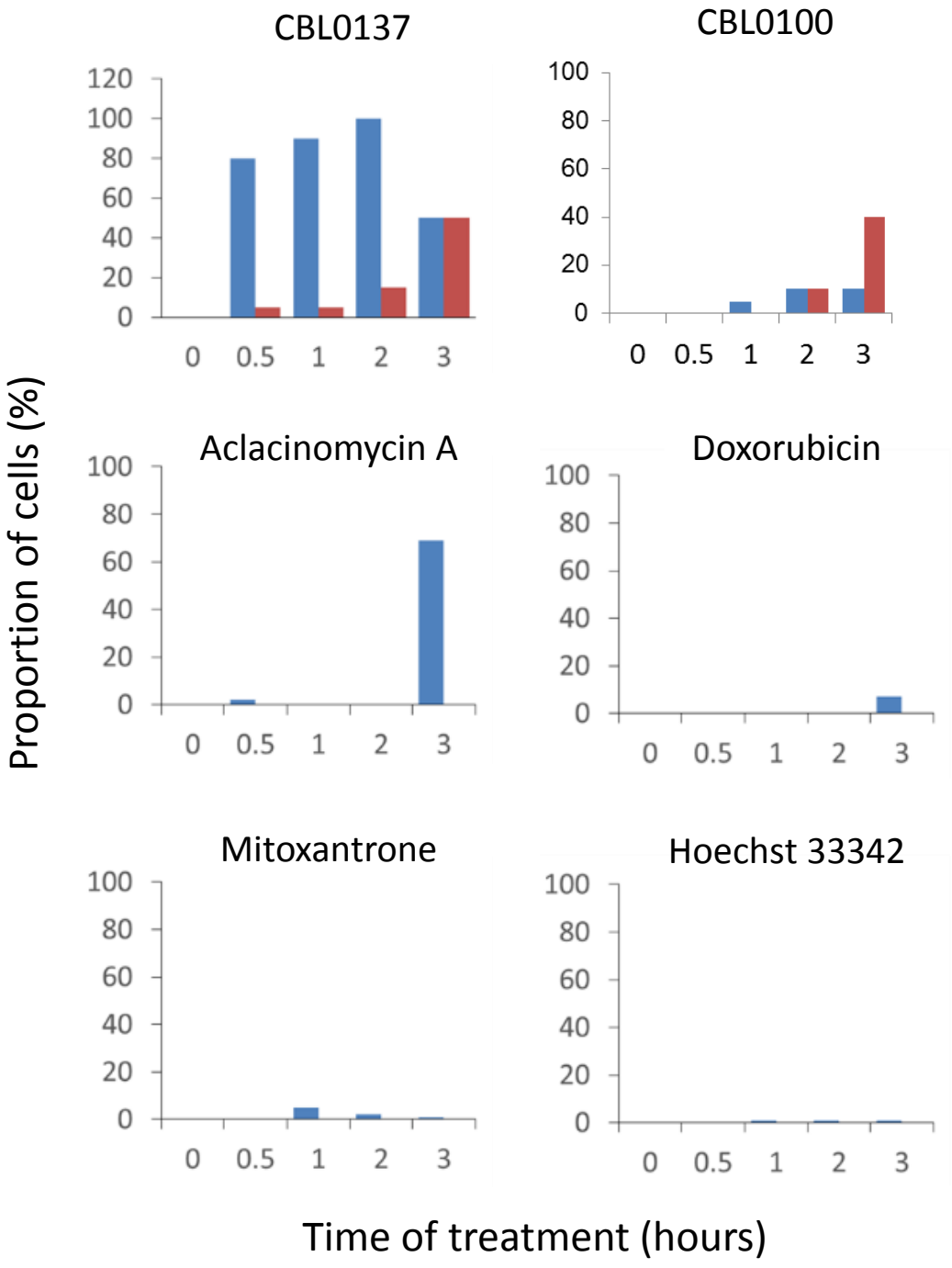


Figure S14. Quantitation of histones H4 eviction from chromatin in HeLa cells treated with 10μM of different drugs, for the indicated periods of time. Blue bars – proportion of cells with histones around nucleoli, red bars – inside nucleoli.

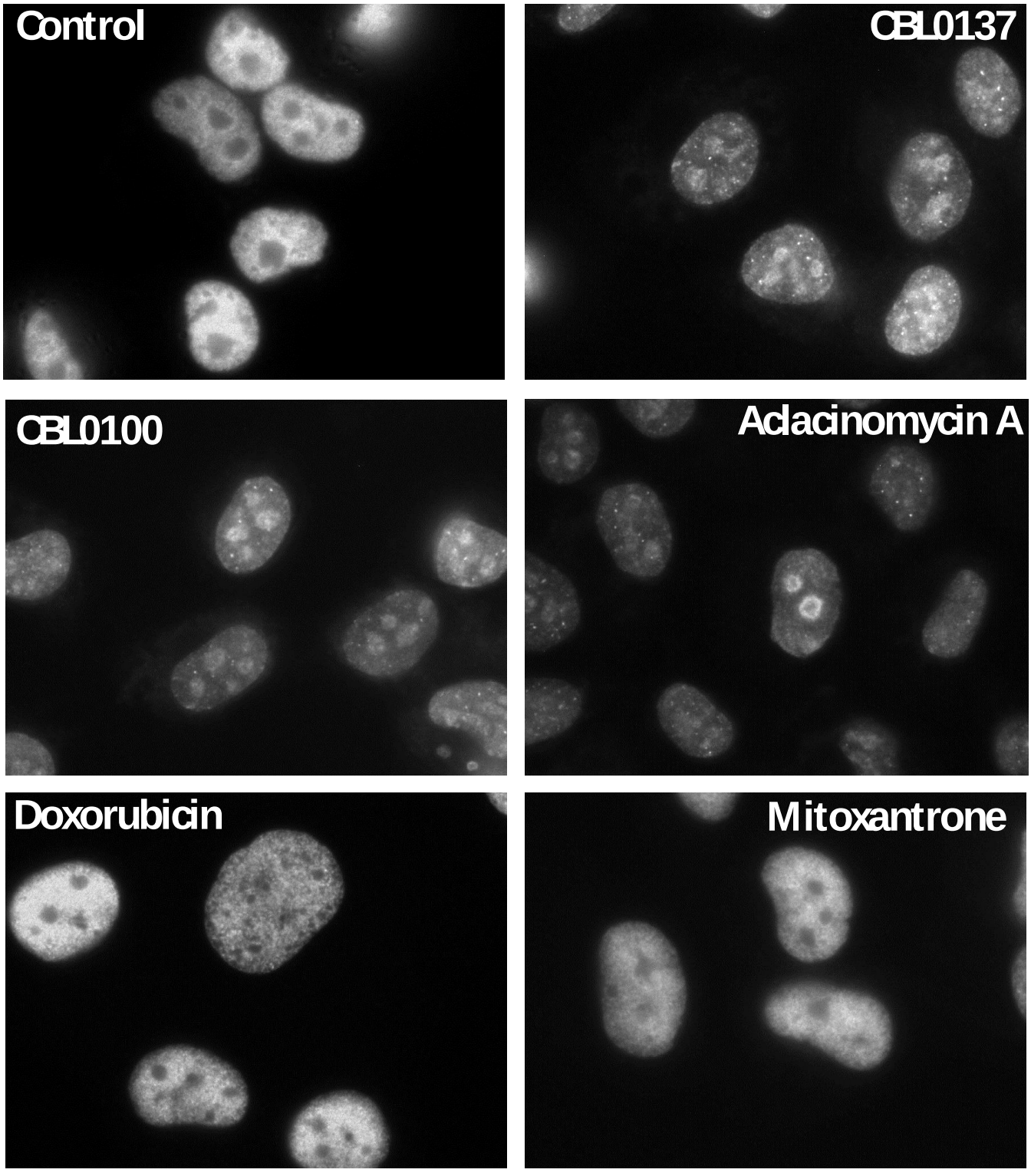


Figure S15. Immunofluorescent staining of histone H3 in HT1080 cells treated with 5µM of the indicated compounds for 3 hours.

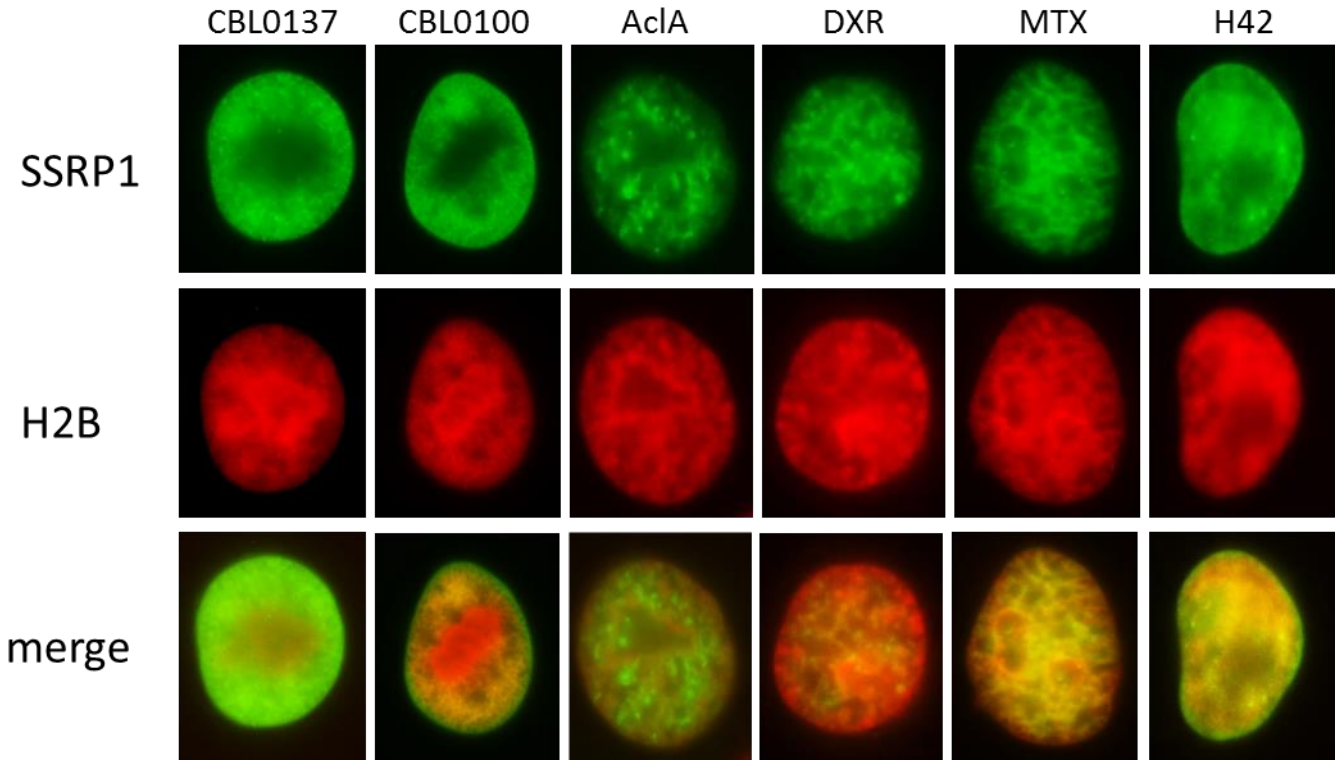


Figure S16. Different patterns of SSRP1 distribution in the process of c-trapping caused by CBL0137, CBL0100, Aclacinomycin A (AclA), Doxorubicin (DXR), Mitoxantrone (MTX) and Hoechst 33342 (H42). Photographs of typical (present in more than 90% of cells) nuclei of HT1080 cells expressing GFP-tagged SSRP1 and mCherry tagged histone H2B. Cells were treated with 10μM of the compounds for 1.5 hours.

A

Compound	Feature	AMI (Strength of colocation)	BMI (Absence of colocation)	Peak Height	p-value	Percentage Near Features	Total Features	Average Feature Length
Aclacynomycin A	Z-DNA Motif	178.3	0	37.1	0	90.00%	447021	15
	Short Tandem Repeat	11.4	0.1	3.2	0	98.90%	3267154	14
	Mirror Repeat	42.2	0.1	9.1	0	99.10%	2047527	48
	Inverted Repeat	0	0	0.3	0.7	99.90%	7020354	21
	G-Quadruplex Motif	28.9	0.5	7.5	0	90.70%	387265	26
	Direct Repeat	74.8	0.3	16.5	0	98.70%	1622866	34
	A-Phased repeat	0.3	1.3	1.3	0.34	74.50%	439104	24
CBL0100	Z-DNA Motif	407.4	0	94.7	0	99.00%	447021	15
	Short Tandem Repeat	35.5	0	9.1	0	99.80%	3267154	14
	Mirror Repeat	83.4	0.1	20.4	0	99.90%	2047527	48
	Inverted Repeat	0	0.4	0.2	0.62	100.00%	7020354	21
	G-Quadruplex Motif	4	15	3	0	94.20%	387265	26
	Direct Repeat	109.1	0	27.5	0	99.90%	1622866	34
	A-Phased repeat	0	3.6	0	1	72.20%	439104	24
CBL0137	Z-DNA Motif	423.5	0	94.6	0	98.70%	447021	15
	Short Tandem Repeat	36.2	0	8.9	0	99.80%	3267154	14
	Mirror Repeat	85.7	0	20	0	99.70%	2047527	48
	Inverted Repeat	0	0	0.2	0.67	100.00%	7020354	21
	G-Quadruplex Motif	2.5	17.8	2.4	0	93.60%	387265	26
	Direct Repeat	111.4	0	26.9	0	99.90%	1622866	34
	A-Phased repeat	0.1	3.5	0.7	0.6	72.00%	439104	24

B

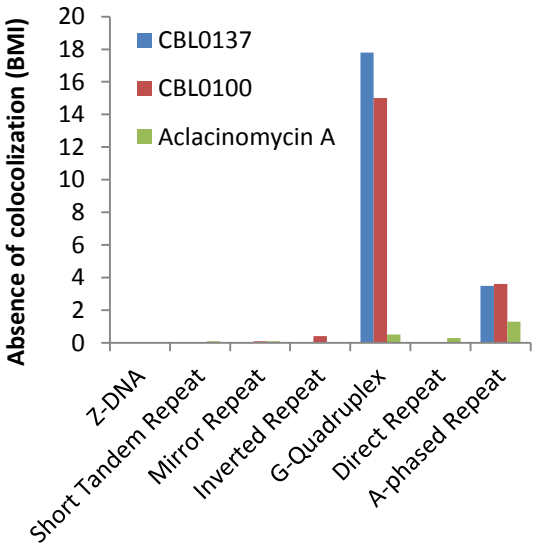


Figure S17. Analyses of colocalization of SSRP1 defined by ChIP-seq and human genome regions prone to transition to non-B DNA structures based on non-B DNA database (Non-B DB v2.0) defined using ColoWeb software. (**A**) Summary of ColoWeb analysis for novel peaks appeared in cells treated with aclacinomycin A or curaxins. (**B**) Absence of colocolozation of SSRP1 peaks and different types of human genome regions prone to non-B DNA transitions. BMI – below median integral – index used by ColoWeb to measure absence of colocalization.

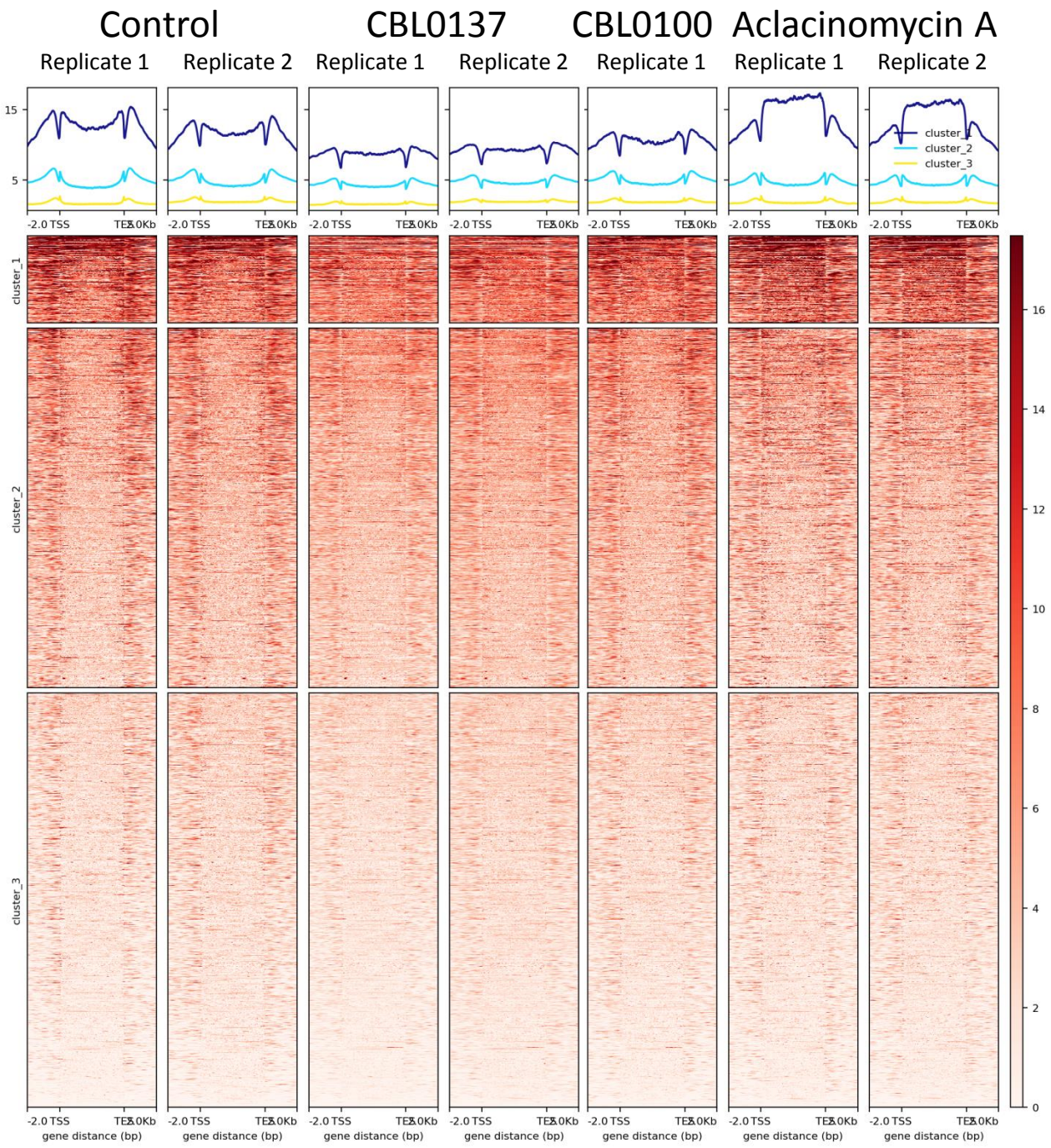


Figure S18. Heatplots and average gene profiles of SSRP1 distribution over all genes in HT1080 cells. Two replicates for each samples used for ChIP-seq are shown.

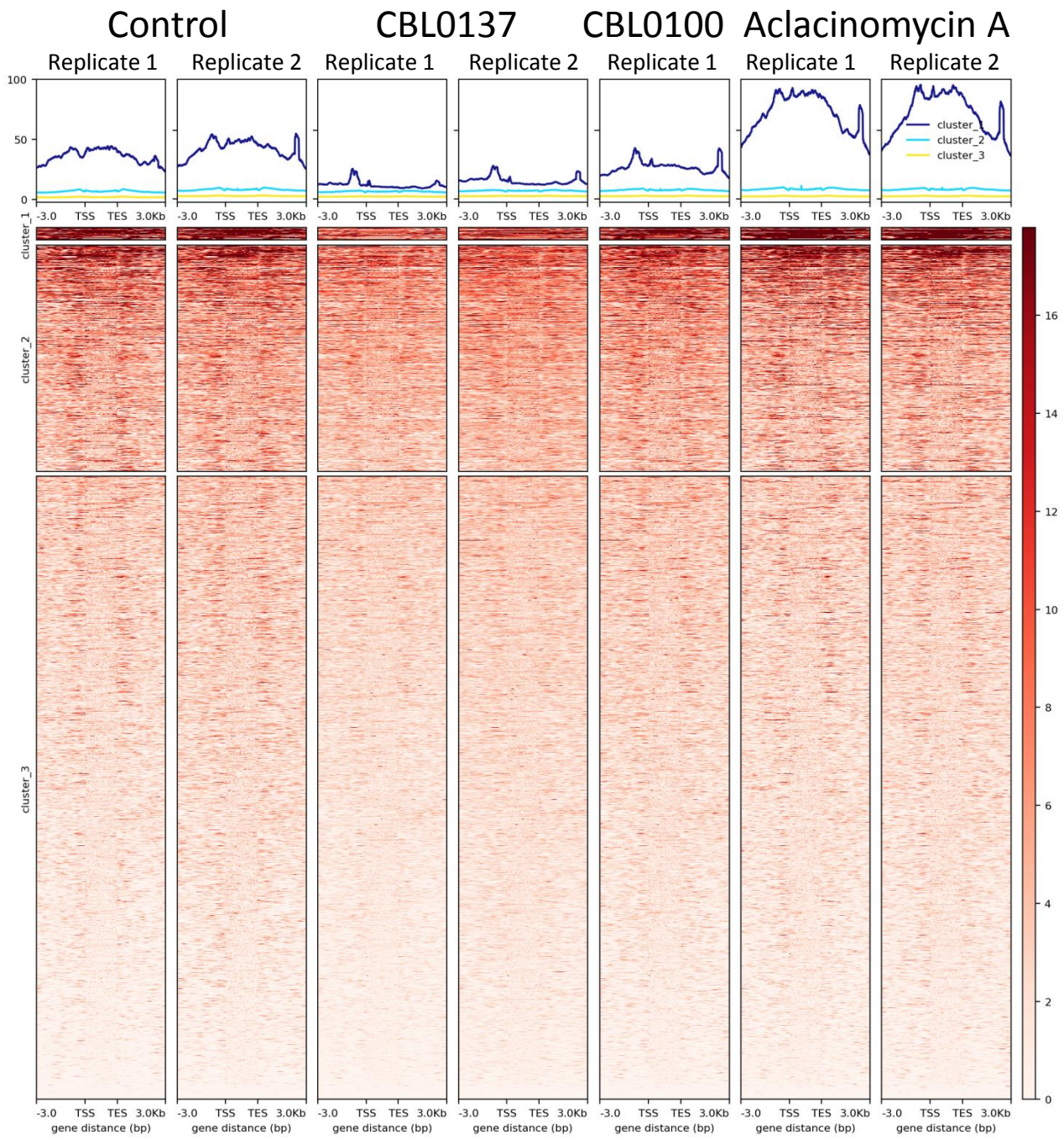


Figure S19. Heatplots and average gene profiles of SSRP1 distribution over non-coding RNAs in HT1080 cells. Two replicates for each samples used for ChIP-seq are shown.

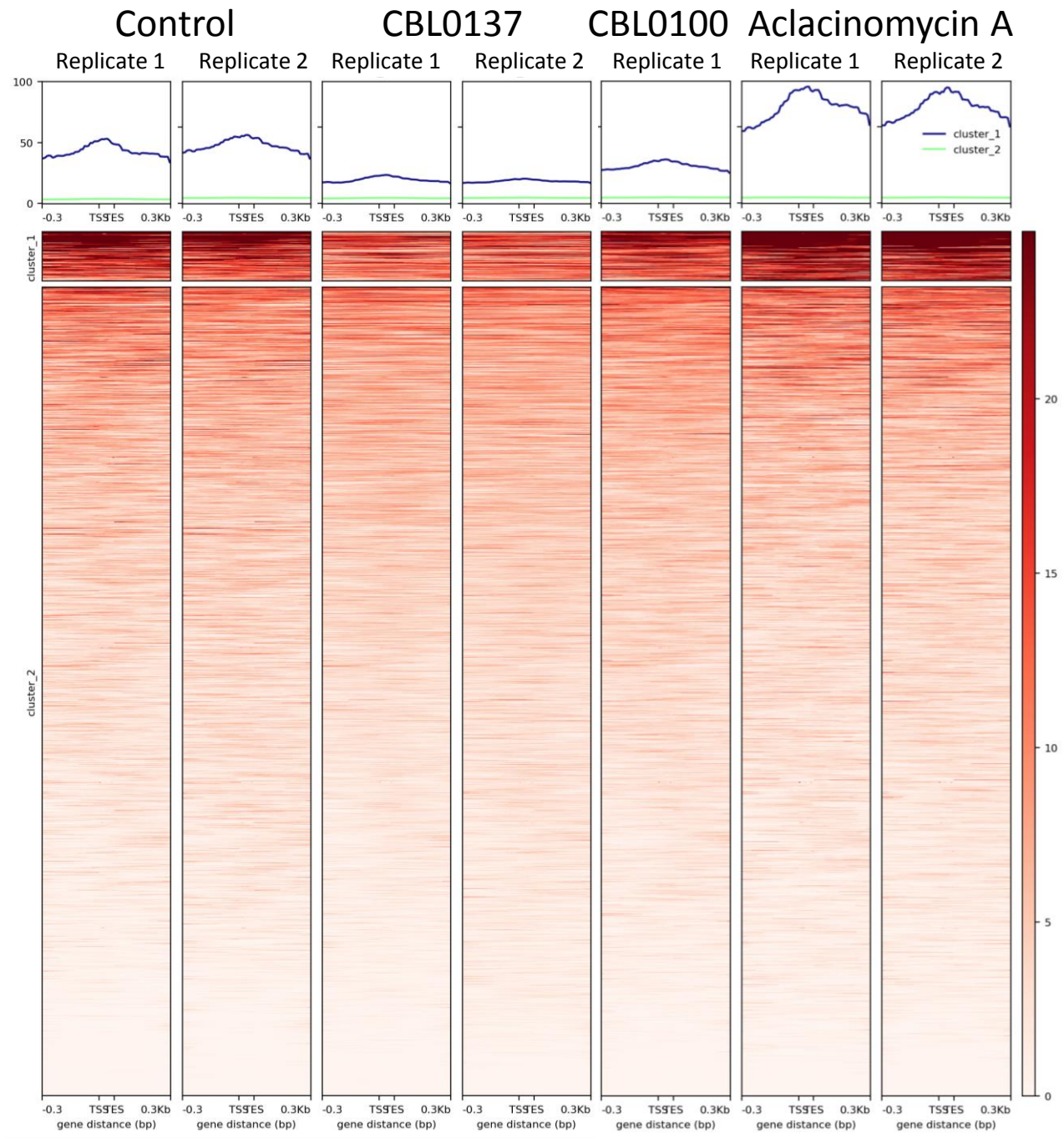
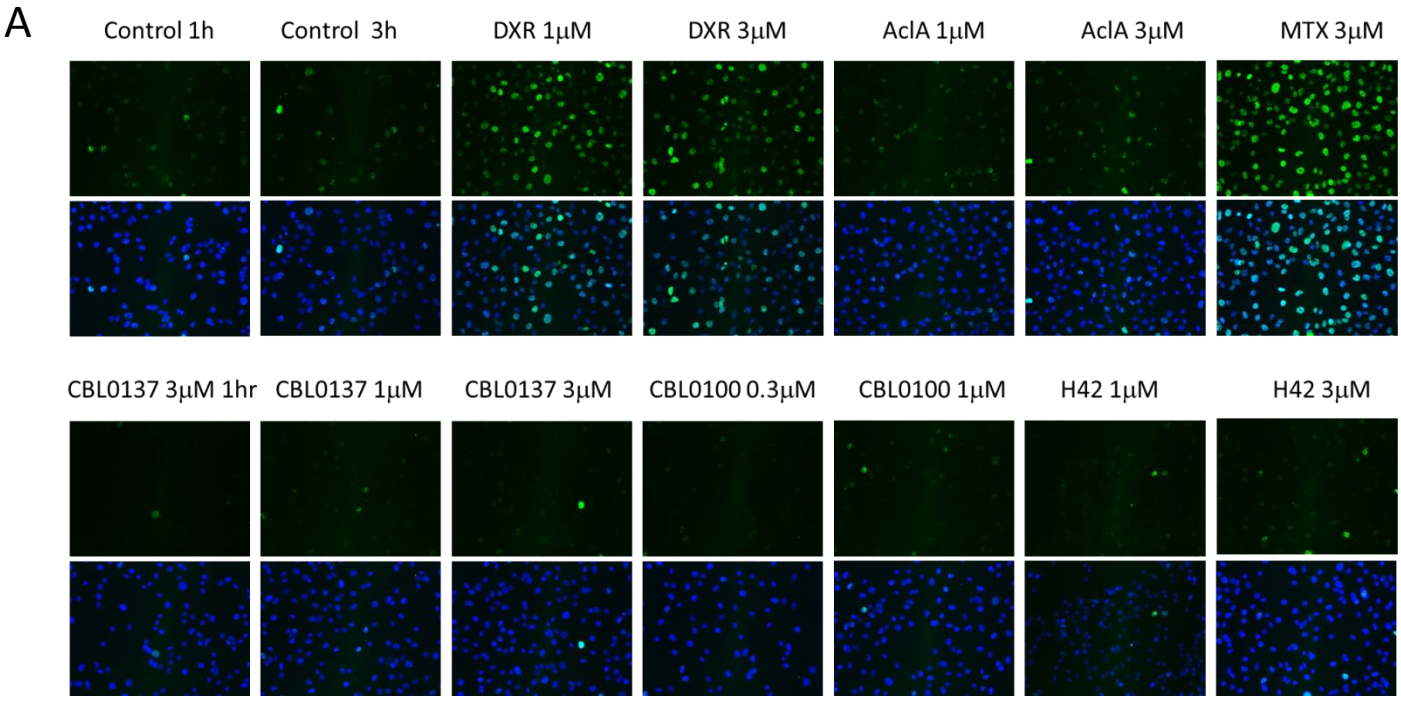


Figure S20. Heatplots and average gene profiles of SSRP1 distribution over miRNAs in HT1080 cells. Two replicates for each samples used for ChIP-seq are shown.



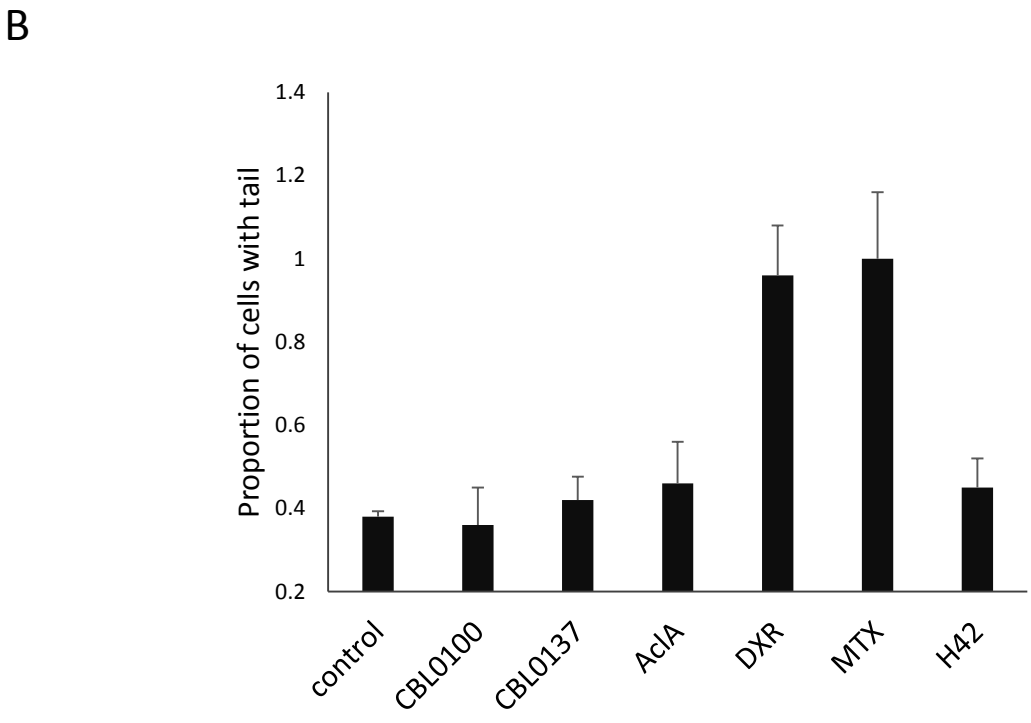


Figure S21. Comparison of CBL0100, CBL0137, Aclacinomycin A (AclA), Doxorubicin (DXR), Mitoxantrone (MTX) and Hoechst 33342 (H42) compounds ability to induce DNA breaks. (**A**) Staining of HT1080 cells for γH2AX (green) and DNA (blue). Cell were treated with the compounds for 1 hour (where indicated) or 3 hours (all the rest). (**B**) Quantitation of the result of comet assay run in alkali conditions after treatment of HeLa cells with 3µM of the compounds for 3 hours. Bars – mean of 10 fields of view ±SD.

Compounds	Cytotoxicity		C-trapping	Histone eviction (in cells)			DNA damage		Destbilization of nucleosome (cell-free conditions)	MNase sensitivity
	IC50 (μM)	IC90 (μM)	EC50 (μM)	H1 Emax (μM)	H2B (μM)	H4 (μM)	γH2AX (%)	Comet (Tail Moment)	EC50 (μM)	
CBL0137	0.15	1.25	2.14	5.83	5	5	0	4	20	–
CBL0100	0.03	0.15	0.98	2.60	2.5	1	0	0	7.8	–
Aclacinomycin A	0.05	0.23	1.76	35.00	20	10	10	8	5	–
Doxorubicin	0.4	1.25	2.95	16.67	40	20	56	55	4	–
Mitoxantrone	0.06	0.26	3.00	36.67	NA	NA	78	60	4	–
Hoechst 33342	2.2	1.9	2.74	50.00	NA	NA	4	12	35	–
RANKS										
CBL0137	4.0	4.5	3.0	2.0	2.0	2.0	5.5	5.0	5.0	1.5
CBL0100	1.0	1.0	1.0	1.0	1.0	1.0	5.5	6.0	4.0	1.5
Aclacinomycin A	2.5	2.0	2.0	4.5	3.0	3.0	3.0	4.0	3.0	2.5
Doxorubicin	5.0	4.5	5.6	3.0	4.0	4.0	2.0	1.5	1.5	2.5
Mitoxantrone	2.5	3.0	5.6	4.5	5.5	5.5	1.0	1.5	1.5	5.0
Hoechst 33342	6.0	6.0	5.6	6.0	5.5	5.5	4.0	3.0	6.0	6.0
CORRELATION COEFFICIENT (r)										
Correlation with cytotoxicity (IC50)	1.00	0.97	0.72	0.53	0.57	0.57	-0.12	-0.49	0.31	0.47
Correlation with cytotoxicity (IC90)	0.97	1.00	0.75	0.50	0.59	0.59	-0.07	-0.46	0.37	0.52
Correlation with c-trapping	0.72	0.75	1.00	0.62	0.90	0.90	-0.66	-0.90	-0.23	0.74
P-VALUE										
Correlation with cytotoxicity (IC50)	0.00	0.00	0.09	0.38	0.24	0.24	0.82	0.32	0.48	0.35
Correlation with cytotoxicity (IC90)	0.00	0.00	0.07	0.39	0.21	0.21	0.87	0.35	0.48	0.28
Correlation with c-trapping	0.09	0.07	0.00	0.23	0.02	0.02	0.16	0.02	0.68	0.48

Figure S22. Correlation analyses of different effects of DNA-binding compounds. IC50 and IC90 are 50% and 90% toxic concentrations for HeLa cells obtained in cytotoxicity experiments shown on Figure S1. EC50 – concentration of drugs causing relocalization of 50% of FACT from soluble to pellet fractions of HeLa cells during 30 (CBL0137, CBL100, Aclacinomycin A, Hoechst 33342) and 60 (Doxorubicin, Mitoxantrone) minutes incubation. Mean of concentrations of SSRP1 and SPT16 subunits defined for soluble and pellet fractions. H1 Emax – concentration of drugs causing maximal accumulation of H1 inside nucleoli upon 2 hours incubation. H2B and H4 – concentration of drugs at which accumulation of these histones around nucleoli started to be observed upon 2 hours incubation. γH2AX - proportion of cells in population with positive staining. Comet – proportion of cells in population with tail moment > than in control cells. NA – effect was not achieved.