**Supplementary Data**

**Supplementary Materials and Methods**

**Supplementary Table 1:** Chemicals, cells, constructs and antibodies used in the study.

|  |  |  |
| --- | --- | --- |
|  | **Cat number / Reference** | **Manufacturer / Source** |
| **Chemicals** |  |  |
|  | Doxorubicin | D1515 | Sigma-Aldrich |
|  | Etoposide | E1383 | Sigma-Aldrich |
|  | ICRF-193 | I4659 | Sigma-Aldrich |
|  | Gemcitabine | G6423 | Sigma-Aldrich |
|  | Merbarone | M2070 | Sigma-Aldrich |
|  | Aclacinomycin A | BML-AW8655-0005 | Enco |
|  | Mitoxantrone | CAS 65271-80-9 | Santa Cruz |
|  | SN-38 | 2684 | TOCRIS |
|  | Hoechst 33342 | H3570 | Thermo Fisher |
|  | CBL0100 |  |  Incuron, LLC |
|  | CBL0137 |  |
|  | Verapamil | V4629 | Sigma-Aldrich |
|  | 9-Aminoacridine | 92817 | Sigma-Aldrich |
|  | Resazurin | R7017 | Sigma-Aldrich |
| **Cells** |  |  |
|  | HeLa | CCL-2 | ATCC |
|  | HT1080 | CCL-121 | ATCC |
|  | HCT116 | CCL-247 | ATCC |
|  | MCF7 | HTB-22 | ATCC |
|  | HL60/VCR  | (Rahman et al., 1992) |  |
| **Constructs** |  |  |
|  | C-GFP-SSRP1 | (Gasparian et al., 2011) | Gurova Lab cloning |
|  | GFP-tagged SSRP1 deletion mutants | (Safina et al., 2017) | Gurova Lab cloning |
|  | SPT16-GFP | (Safina et al., 2017) | GeneCopoeia |
|  | mCherry-H1.5 | (Leonova et al., 2017, under review) | GeneCopoeia |
|  | pH2B\_mCherry\_IRES\_puro2 | Addgene plasmid # 21045 | A gift from Daniel Gerlich |
|  | mOrange2-H4-23 | Addgene plasmid #57964 | A gift from Michael Davidson |
| **Antibodies** |  |  |
|  | γH2AX (IF 1:200) | 9947 | Cell Signaling |
|  | anti-SSRP1 (WB 1: 4000) | 609702 | BioLegend |
|  | anti-Spt16 (WB 1: 4000) | 607002 | BioLegend |
|  | H3 (IF 1:200) | 4499 | Cell Signaling |
|  | H3 (WB 1: 10000) | 05-928 | EMD Millipore |
|  | Anti-β-Actin (1:20000) | A1978 | Sigma-Aldrich |
|  | anti-mouse (1:4000) | sc2005 | Santa Cruz |
|  | anti-rabbit (1:4000) | sc2004 | Santa Cruz |

**Cytotoxicity**

HeLa, MCF7, HCT116 and HT1080 cells (3x103 cells/well) were seeded in 96-well plates for overnight adhesion. HL60/VCR suspension cells (5x103 cells/well) were seeded in 96-well plates and treated the same day. 50M of 9-Aminoacridine was used as a positive control for complete cell death. Cells were treated for 48 h with a range of drug concentrations. Cell viability was determined with resazurin saline solution. Fluorescence was measured at 560Ex/590Em using Tecan Infinite 200 PRO reader.

**DNA damage**

H2AX (Cell Signaling, cat# 9947) and comet assay (Cell Biolabs, Inc., cat# STA-355) were used to detect the ability of the compounds to induce cellular DNA breaks. HT1080 cells were treated with compounds (1 or 3µM) for 1 or 3 h followed by staining for H2AX using standard immunofluorescent staining protocol (see below).HeLa cells treated with 3M compounds for 6 h were combined with Comet Agarose at a 1:10 ratio and applied to a preheated OxiSelect™ 96-Well Comet Slide at 37°C for 15 min followed by an hour immersion in pre-chilled lysis buffer at 4°C in the dark. Before alkaline electrophoresis, the slide was immersed in pre-chilled alkaline solution for 30 minutes at 4°C in the dark.Alkaline electrophoresis was performed at 1 volt/cm (300mA) for 15-30 min. The slide was then washed with pre-chilled ddH2O and cold 70% ethanol and dried. The staining was performed using Vista Green DNA dye. Quantitation was performed to determine the proportion of cells with tails as an average of two replicates ±SD.

**Extraction of soluble and chromatin-bound proteins from cells**

Cells were lysed on ice using 1x Cell Culture Lysis Reagent (Promega, cat# E1531) containing protease inhibitors (1:25, Roche, cat# 1838145). Extracts were centrifuged at 10,000 rpm for 10 min at 4oC to obtain the soluble fraction. The chromatin-bound proteins were obtained by resuspension of the remaining pellets in 1x Cell Culture Lysis Reagent followed by sonication three times for 30 sec each using the Bioruptor UCD-200, Diagenode. Protein concentrations were measured using Quick Start Bradford 1x Dye Reagent (Bio-Rad, cat# 500-0205). Equal amounts of protein were run on gradient 4-20% precast gels (Invitrogen) and transferred to Immobilon-P membrane (Millipore).

**Western Blot Analysis**

Membranes were blocked with 5% non-fat milk-TBS-T buffer for 1 hour and incubated overnight with primary antibodies. The following primary antibodies were used: anti-SSRP1 (1: 4000; BioLegend, cat# 609702), anti-Spt16 (1: 4000; BioLegend, cat# 607002), and H3 (1: 10000; EMD Millipore, cat# 05-928). Anti-β-Actin (1:20000; Sigma, cat# A1978) was used to verify equal protein loading and transfer. After hybridization to a horseradish peroxidase-conjugated anti-mouse (1:4000; Santa Cruz, cat# sc2005) or anti-rabbit (1:4000; Santa Cruz, cat# sc2004) secondary antibodies, washed blots were incubated with ECL solution (Western Lightning® Plus-ECL, Enhanced Chemiluminescence Substrate; Perkin Elmer, cat# NEL104001EA) visualized using autoradiography film (Denville Scientific).

**Immunofluorescent staining and fluorescent microscopy**

For immunofluorescent staining cells were fixed with 4% paraformaldehyde, 0.1% Triton X100 in PBS, followed by blocking with PBS containing 3% BSA, and staining with appropriate antibodies (Suppl. Table 1) in the 0.6% BSA, 0.05% Triton X100 in PBS solution. Washing was done with 0.1% Triton X100 in PBS. Fluorescent images of live and fixed cells as well as compound auto-fluorescence were obtained with a Zeiss Axio Observer A1 inverted microscope with N-Achroplan 100×/1.25 oil lens, Zeiss MRC5 camera, and AxioVision Rel.4.8 software.

**Micrococcal nuclease digestion**

Effect of the compounds on nucleosome stability within chromatin was analyzed using HeLa cell nuclei with the drugs, followed by assessment of chromatin sensitivity to digestion with micrococcal nuclease (MNase; New England BioLabs, cat# M0247S), preferentially digesting protein-free DNA. Drugs (100µM) were incubated for 15 minutes with nuclei of HeLa cells, followed by MNase digestion and DNA extraction (Ultra Pure Phenol:Chloroform:Isoamyl Alcohol; Invitrogen, cat# 15593-031). DNA was further precipitated with ethanol 100%, washed with ethanol 70%, dried and dissolved in 20µl of TE buffer. Agarose 1.5% gel electrophoresis was performed at 80v.

**Statistical Analyses**

IC50, IC90 and EC50 values were obtained using GraphPad Prism 5.02 software by nonlinear regression with an inhibitory dose-response model. The statistical significance of correlation between different effects of DNA-binding compounds was assessed using either Pearson (for measured parameters) or Spearman (for ranked parameters) correlation coefficients using GraphPad Prism 5.02 software.

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