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Supporting Information

Chromatin Regulates Genome Targeting with Cisplatin

Emmanouil Zacharioudakis[+], Poonam Agarwal[+], Alexandra Bartoli, Nathan Abell, Lavaniya Kunalingam, Valérie Bergoglio, Blerta Xhemalce, Kyle M. Miller, and Raphaël Rodriguez**

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Author Contributions

E.Z. Performed all the experiments unless stated otherwise: Equal
P.A. Performed all the experiments unless stated otherwise: Equal
N.A. Performed the RNA-Seq experiment: Supporting
A.B. Supervision: Supporting
V.B. Provided cell line: Supporting
B.X. Supervision: Supporting
L.K. Validation: Supporting
K.M. Formal analysis: Lead
R.R. Formal analysis: Lead.

Supporting Information

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METHODS

Cell lines and culture conditions. U2OS cells and HCT116 were purchased from ATCC and cultured in standard conditions using DMEM supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin and incubated at 37 °C with 5% CO_2. A2780 cells (cisplatin sensitive) were purchased from Sigma-Aldrich (#93112519) and maintained in RPMI-1640 medium containing 2 mM L-glutamine and 10% FBS. HCT116 *RAD18* KO cells were kindly provided by Junjie Chen's Lab (MD Anderson) and grown in DMEM medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin.

Cell viability assays. Cell viability assays were carried out by plating U2OS cells (2,000 cells per well) in 96-well plates. Cells were treated with the relevant drug for 72 h, then incubated with CellTiter-Blue® (20 µL/well) for 1 h before recording fluorescence (560(20) Ex/590(10) Em) using a PerkinElmer Wallac 1420 Victor^2 Microplate Reader.

Drugs and inhibitors. Picoplatin, APPA and APP were prepared in the laboratory as described in Synthesis. Suberoylanilide hydroxamic acid (SAHA), MS-275 and hydroxyurea (HU) were purchased from Sigma and cisplatin was purchased from Tocris. Stock solutions of APPA, picoplatin, and cisplatin were prepared at a concentration of 10 mM in DMF for cell viability assay. A fresh stock solution of 1 mM in 0.9% w/v NaCl was freshly prepared for APPA and cisplatin for use in cell imaging and pull-down experiments.

***In vitro* reaction of hairpin DNA with APPA and DIBO (Dibenzocyclooctyne)-Alexa 488.** Hairpin (hp) DNA (5′-AAAACCAAAAATTTTTTTTTGGTTTT-3′) was diluted in 10 mM Na_2PO_4, pH 7.0, 100 mM $NaNO_3$, 1 mM $Mg(NO_3)_2$ (80 µM) and heated up at 90 °C for 5 mins, then left to cool down at room temperature overnight. A stock solution of APPA at a concentration of 640 µM in 0.9% w/v NaCl was freshly prepared and reacted with an equal volume of hairpin DNA solution (typically 8 nmol). The reaction of hp with APPA was performed at 37 °C for 18 h. Unbound APPA and salts were removed using a Sephadex G-25 Medium size exclusion resin (GE Healthcare) on laboratory prepared spin columns (BioRad). Platinated DNA (hp-Pt) was reacted with DIBO-Alexa 488 (Life Technologies; #C-10405; 2.5 µl, 1.25 mM) at room temperature for 3 h. Unreacted DIBO-Alexa 488 was removed by Sephadex G-25 Medium size columns and further desalting was achieved by means of C18 ZipTips.

MALDI-TOF mass spectrometry analysis. The Alexa 488 labeled DNA-Pt was diluted (1:9) to the matrix solution (1.7 mg of ammonium citrate to 200 µL of a saturated solution of 3-hydroxypicolinic acid (3-HPA) in acetonitrile/water (1:1 (vol/vol)). The mixture was deposited on the MALDI plate and left to dry slowly at room temperature. A MALDI-TOF/TOF UltrafleXtreme mass spectrometer (Bruker Daltonics, Bremen) was used for the experiment. Mass spectra were

obtained in linear positive ion mode. All data were processed using the FlexAnalysis software package (Bruker Daltonics).

Dot blot assay. U2OS cells were cultured in 15 cm dishes and treated with APP or APPA for 3 h. Total genomic DNA was isolated from cells using DNeasy Blood and Tissue kit (Qiagen; #69506) according to the manufacturer protocol. DNA was reacted with Alexa Fluor® 488 alkyne using a click reaction buffer according to the manufacturer protocol (ThermoFisher Scientific; C10337). Labled DNA was mechanically sheared using a bioruptor (Diagenode), 15 mins, 10 sec on/off at 4 °C. DNA was then purified using QIAquick PCR purification kit (Qiagen, #28106) according to the manufacturer protocol. 2 μg of total DNA were loaded on Hybond nylon membranes (GE Healthcare). Samples were air dried and visualized using a Bio-Rad Molecular Imager ChemiDoc XRS+ system.

Immunofluorescence analysis and microscopy. U2OS cells were treated with APPA and/or SAHA at ~70 % confluence. After treatments, cells were washed with PBS and pre-extracted with CSK buffer (10 mM Pipes, pH 7.0, 100 mM NaCl, 300 mM sucrose, 3 mM $MgCl_2$, and 0.7% Triton X-100) twice for 3 mins. Then, cells were washed with PBS and fixed with 2% PFA for 13 mins. *In cellulo*, APPA click-labeling with Alexa Fluor® 488 alkyne (Life Technologies; #A10267) was performed based on our previously published procedure.[1] Cells were blocked and incubated for 1 h at room temperature with primary antibodies as indicated; PCNA (Abcam; ab18197), TRF1 (Abcam; ab10579), CENP-A (Abcam; ab13939), fibrillarin (Abcam; ab4566), FLAG (Sigma; monoclonal anti-FLAG M2). The RAD18 (Abcam; ab57447) antibody was incubated for 16 h at 4 °C. After incubation with primary antibodies, cells were washed with PBS and incubated with the appropriate mouse or rabbit secondary antibodies (ThermoFisher Scientific; #A-21236, #A-21245, #A-11037, #A-11005, #A-11001) in the blocking solution. After PBS washes, coverslips were dipped in water and mounted on glass slides using Citifluor™ AF2 (Biovalley), Vectashield containing DAPI (Vector laboratories) or Hoechst 33258 to visualize cell nuclei. Images were taken with Leica SP8 inverted confocal microscope, Delta Vision inverted widefield microscope or Fluoview 1000 confocal microscope (Olympus). Data were analyzed with ImageJ.

RNA-Seq. Total RNA was extracted from untreated cells or treated with APPA, SAHA or in combination of SAHA and APPA using RNeasy Mini Kit (Qiagen, #74106) following the manufacturer's protocol. Residual DNA was removed by DNase I on column digestion. RNA concentration was determined using Nanodrop and subjected to RNA-seq library preparation and deep sequencing at the NGS facility, MD Anderson Cancer Center. All datasets were analyzed with FastQC to confirm a lack of sequencing abnormalities. No adapter contamination was detected. rRNA and tRNA sequences were filtered, and remaining sequences were aligned to the most recent build of the human genome (hg38) using Tophat2/Bowtie2 with sensitive parameters. Alignments with a mapping quality score of less than 5 or that were flagged as secondary were removed and

files sorted and indexed. Read counts per gene were calculated from the remaining alignments using HTSeq with the Gencode v21 comprehensive genome annotation, and results were exported into a raw counts expression matrix. Differentially expressed genes were identified using edgeR with default parameters except for two modifications: first, a gene was required to have an expression value of at least 1 count per million reads in at least three samples to be tested and second, a differentially expressed gene was required to have both an absolute fold change of 3 or greater and a statistically significant FDR-adjusted P-value < 0.01. All final results were exported to Excel and all downstream plotting and analysis was performed with custom scripts in R with the ggplot2 graphics package. The heatmap was generated by hierarchically clustered by row and column using Pearson correlation as a distance metric, and mean centered and normalized by row.

Quantitative real-time PCR. U2OS cells were treated with cisplatin or APPA for 3 h. Total RNA was extracted using RNeasy Mini kit (Qiagen, #74106) and treated with RNase- free DNase I following manufacturer's protocol. From 2 µg of total RNA from each sample, cDNA was synthesized using SuperScript III first-strand synthesis system (Invitrogen). To analyze mRNA expression levels of candidate genes, gene-specific qPCR primers were designed as tabulated below:

Gene Name	Primer sequence
CXCL8	F' TCCTGATTTCTGCAGCTCTGT
	R' AAATTTGGGGTGGAAAGGTT
IL11	F' GGACAGGGAAGGGTTAAAGG
	R' CTCAGCACGACCAGGACC
AREG	F' ACGAACCACAAATACCTGGC
	R' TTTCACTTTCCGTCTTGTTTTG
EREG	F' AGGAGGATGGAGATGCTCTG
	R' CACAGTTGTACTGAGGACTGCC
RRAD	F' CAACAAGAGCGACCTGGTG
	R' CCGCTGATGTCTCAATGAACT
ATOH8	F' TCAGCTTCTCCGAGTGTGTG
	R' ACAGTGGTGGCCTTGGTCTT
UCN2	F' CTGCCTTACCCCAGAAGCA
	R' ACTCTGCCCAACATCAGGAC
DRD1	F' AGCGAAGTCCACATTCCAAG
	R' ATGTCTTCTCGCTCCTCCAA
HTR2A	F' CCGCTTCAACTCCAGAACTAA
	R' GAATCGTCCTGTAGCCCAAA
BMF	F' AAGGTTGTGCAGGAAGAGGA
	R' CAGTGCATTGCAGACCAGTT

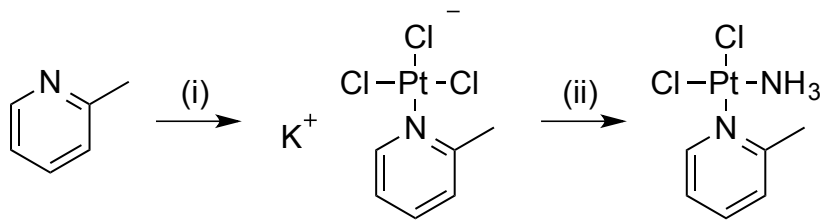
For normalization, the qPCR primer pair for GAPDH (glyceraldehyde-3-phosphate dehydrogenase) F'- CAATGACCCCTTCATTGACC and R'-GATCTCGCTCCTGGAAGAT were used. Quantitative PCR (qPCR) analyses were performed on the StepOnePlus Real-Time PCR system (Applied Biosystems) using SYBR green master mix (Applied Biosystems) with the above indicated primers.

DNA pull-down assay. U2OS cells were treated with APPA alone or in combination with SAHA. After treatment, total genomic DNA of each sample was purified using DNeasy Blood and Tissue kit (Qiagen; #69506). Pure link RNaseA (Invitrogen) was used to remove RNA during genomic DNA extraction. Click reaction was performed on the isolated DNA using Biotin-PEG4 alkyne (Sigma-Aldrich; #764213) and incubated for 1 h protected from light at room temperature. The click reaction was quenched using 4 mM EDTA. The DNA was fragmented up to ~100-350 bp size using bioruptor (Diagenode) and purified using the QIAquick PCR purification kit (Qiagen; #28106). To capture the biotin tagged APPA-DNA conjugates, each sample was incubated with Dynabeads® MyOne™ Streptavidin T1 (Invitrogen, #65602) followed by washing with a buffer containing 1 M NaCl, 5 mM Tris-HCl, pH 7.5 and 0.5 mM EDTA. Beads were then washed with 8 M urea followed by three washes (washing buffer with 100 mM NaCl). After washing, beads were incubated in 1.8 M thiourea for 48 h at 37 °C. DNA was purified using QIAquick PCR purification kit (Qiagen) and quantified using Qubit.

Cell lysis and Immunobloting. Cells were washed once with PBS and lysed in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100). For western blot, samples were briefly sonicated followed by boiling in SDS sample buffer and separated by SDS-PAGE gels. Proteins were transferred to nitrocellulose membrane (GE Healthcare) and western blotting was performed following standard protocols. Western blots were detected by chemiluminescence (GE Healthcare Amersham ECL prime) using a Bio-Rad Molecular Imager ChemiDoc XRS+ system. The primary antibodies used for western blotting: H2AX (Millipore; #07-627), γH2AX [pSer139] (Novus Biologicals; NB100-384), histone H4 (Abcam; ab7311), acetyl-histone H4 (Lys16) (Cell Signaling; #8804), acetyl-histone H4 (Millipore; #06-866), PCNA (Santa Cruz Biotech; PC10), RAD18 (Cell Signaling; #21000), PARP (Cell Signaling; #9542), β-tubulin (Abcam; ab6046), RPA32 (Abcam; ab2175), p-RPA32 (S4/S8) (Bethyl Laboratories; A300-245A-5), Chk1 (Santa Cruz Biotechnology; sc-8408), p-Chk1 (S345) (Cell Signaling; #133D3), cleaved Caspase-3 (Cell Signaling; #9664P), p-RPA32 (S33) (Bethyl Laboratories; A300-246A). Secondary antibodies used were: anti-rabbit IgG, HRP-linked (Cell Signaling; #7074), anti-mouse IgG, HRP-linked (Cell Signaling; #7076).

Flag-Polη. The DNA fragment encoding Flag-Polη was extracted from pCDNA3.1/Hygro Flag-Polη plasmid and cloned in pTRIP plasmid to obtain pTRIP-CMV-Flag-Polη.[2] Lentivirus particles were produced in 293FT cells by co-transfection of pTRIP-CMV-Flag-Polη, pVSVg and P8.91. U2OS cells were transduced in optiMEM (Gibco) with 5 µg/mL protamine sulfate with lentivirus particles at MOI: 8 for 24 h.

SYNTHESIS. All starting materials were purchased from commercial sources and used without further purification, or purified according to *Purification of Laboratory Chemicals* (Armarego, W.L.F., Chai, C.L.L. 5th edition). Solvents were dried under standard conditions. Reactions were monitored by thin-layer chromatography (TLC) using TLC silica gel coated aluminum plates 60F-254 (Merck). Column chromatography was performed using Merck silica gel 60, 0.040-0.063 mm (230-400 mesh). NMR spectroscopy was performed on Bruker 300 or 500 MHz apparatus equipped with a cryoprobe. Spectra were run in $CDCl_3$, or DMF-d_7 at 298 K unless otherwise stated. Molecular structures have been characterized using a comprehensive dataset including ^{1}H- and ^{13}C-NMR spectra (1D and 2D experiments). ^{1}H chemical shifts are expressed in ppm using the residual non-deuterated solvents as internal standard ($CDCl_3$ ^{1}H, 7.26 ppm) and (DMF-d_7 ^{1}H, 8.03, 2.92, 2.75 ppm). The following abbreviations are used: s, singlet; d, doublet; dd, double doublet; t, triplet; td, triplet doublet; q, quartet; m, multiplet; bs, broad singlet. ^{13}C chemical shifts are expressed in ppm using the residual non-deuterated solvents as internal standard ($CDCl_3$ ^{13}C, 77.16 ppm) and (DMF-d_7 ^{13}C, 163.15, 34.89, 29.76 ppm). Exact masses were recorded on a LCT Premier XE (Waters) equipped with an ESI ionization source and a TOF detector and on a Q-TOF 6540 (Agilent).



Synthesis of picoplatin. (i) K_2PtCl_4, NMP, 60 °C, 4 h, 63%. (ii) KCl (2.5 N), $CH_3CO_2NH_4$, NH_4OH (2.5 N), 45 °C, 1 h, 49%.

K[$PtCl_3$(2-picoline)] was prepared according to a previously published procedure.[3] To a suspension of K_2PtCl_4 (300 mg, 0.72 mmol) in *N*-methyl-2-pyrrolidone (1.2 ml) was added a solution of commercially available 2-picoline **1** (74 mg, 0.79 mmol) in *N*-methyl-2-pyrrolidone (0.9 ml) portionwise. The rate of the addition was 20% of the solution per 30 mins. After addition of the first portion, the reaction mixture was immersed in an oil bath and stirred at 60 °C for 4 h. Then, the mixture was allowed to reach room temperature, followed by addition of dichloromethane (9 ml). The precipitants KCl and K[$PtCl_3$(2-picoline)] were collected by filtration and washed with dichloromethane (3 × 1 ml) and diethyl ether (3 × 1 ml). The product was dried under reduced pressure to afford the titled compound as a mixture with KCl (250 mg, 63%) as a yellow solid. ^{1}H NMR (500 MHz, DMF-d_7): δ 8.99 (d, *J* = 6.0 Hz, 1H), 7.72 (t, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 7.5 Hz, 1H), 7.22 (t, *J* = 6.0 Hz, 1H), 3.24 (s, 3H).

Picoplatin was prepared according to a previously published procedure.[3] To a solution of K[$PtCl_3$(2-picoline)]/KCl (231 mg, 0.42 mmol) dissolved in a KCl solution (0.33 ml, 2.5 N) was added ammonium acetate (163 mg, 2.12 mmol)

diluted in an ammonium hydroxide solution (0.84 ml, 2.5 N). The resulting mixture was stirred in the dark at 45 °C for 1 h. The precipitate was collected by filtration and was washed with water (2 × 1 ml) and acetone (2 × 1 ml). The product was dried under reduced pressure to afford **picoplatin** (78 mg, 49%) as a yellow solid. ^{1}H NMR (500 MHz, DMF-d_7): δ 9.02 (d, *J* = 6.0 Hz, 1H), 7.86 (t, *J* = 7.5 Hz, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.34 (t, *J* = 6.0 Hz, 1H), 4.39 (br s, 3H), 3.18 (s, 3H). HRMS (ESI-TOF) calcd. for $C_6H_{10}Cl_2N_2NaPt^+$ $[M+Na]^+$ 397.9766, found: 398.9744.

Methyl 4-chloropicolinate (2). Compound **2** was prepared according to a modified procedure.[4] To a suspension of the commercially available 4-chloro-pyridine-2-carboxylic acid **1** (5.0 g, 31.84 mmol) in dichloromethane (135 ml) at 0 °C was added oxalyl chloride (4.8 g, 38.21 mmol), followed by a slow addition of catalytic amount of dimethylformamide (0.55 ml). The resulting mixture was stirred at room temperature for 2 h. After this time, the mixture was concentrated to dryness under reduced pressure. The solid residue was solubilized in methanol (55 ml) and was stirred at room temperature for another 16 h. The mixture was concentrated to dryness under reduced pressure, and the residue re-suspended with 5% aq. $NaHCO_3$. The product was extracted with EtOAc (2 × 20 ml). The combined organic layer was washed with brine (2 × 10 ml), dried over anhydrous $MgSO_4$, filtered and concentrated to dryness under reduced pressure to afford **2** (4.0 g, 74%) as a beige solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.63 (d, *J* = 5.0 Hz, 1H), 8.12 (dd, *J* = 2.0, 0.5 Hz, 1H), 7.48 (dd, *J* = 5.0, 2.0 Hz, 1H), 4.00 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.7, 150.7, 149.3, 145.5, 127.2, 125.7, 53.3. HRMS (APPI) calcd. for $C_7H_7ClNO_2^+$ $[M+H]^+$ 172.0160, found: 172.0156.

(4-Chloropyridin-2-yl)methanol (3). Compound **3** was prepared according to a modified procedure.[5] To a mixture of methanol (24 ml) and tetrahydrofuran (14 ml) were added **2** (4.1 g, 23.87 mmol) and calcium chloride (10.5 g, 95.48 mmol). The reaction mixture was cooled to 0 °C. Then, sodium borohydride (1.8 g, 47.74 mmol) was added portionwise. The resulting mixture was stirred at room temperature for 24 h. Then, the same amounts of methanol, tetrahydrofuran, calcium chloride, and sodium borohydride were added following the same procedure, and the reaction mixture was stirred for 24 h. After this time, water (80 ml) was added to the reaction mixture, which was stirred for 2 h. The product was extracted with EtOAc (3 × 180 ml). The combined organic layer was washed with brine (100 ml), dried over $MgSO_4$ and concentrated to dryness under reduced pressure to afford **3** (3.0 g, 87%) as a pale white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.38 (d, *J* = 5.5 Hz, 1H), 7.34 (s, 1H), 7.18 (dd, *J* = 5.5, 2.0 Hz, 1H), 4.71 (s, 2H), 4.19 (br s, 1H).^{13}C NMR (75 MHz, $CDCl_3$): δ 161.6, 149.5, 145.0, 122.8, 121.1, 64.2. HRMS (ESI-TOF) calcd. for $C_6H_6ClNNaO^+$ $[M+Na]^+$ 166.0036, found: 166.0028.

(4-Azidopyridin-2-yl)methanol (4). Sodium azide (1.3 g, 20.89 mmol) was added to a mixture of **3** (1.0 g, 6.96 mmol) in dimethylformamide (10.5 ml) and

water (0.52 ml). The resulting mixture was stirred at 85 °C for 14 d. After this time, water (5 ml) was added and the product was extracted with EtOAc (3 × 7 ml). The combined organic layer was washed with brine (7 ml), dried over anhydrous $MgSO_4$, filtered and concentrated to dryness under reduced pressure to afford **4** (634 mg, 60%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.42 (d, *J* = 5.5 Hz, 1H), 6.97 (dd, *J* = 2.0, 0.5 Hz, 1H), 6.83 (dd, *J* = 5.5, 2.0 Hz, 1H), 4.72 (s, 2H), 3.93 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.8, 149.9, 149.7, 113.0, 110.7, 64.3. HRMS (APPI) calcd. for $C_6H_7N_4O^+$ $[M+H]^+$ 151.0614, found: 151.0601.

4-Azido-2-(chloromethyl)pyridine (5). Compound **4** (398 mg, 2.65 mmol) was solubilized in dry chloroform (3 ml) at 0 °C, followed by the dropwise addition of thionyl chloride (946 mg, 7.95 mmol). The reaction mixture was allowed to reach room temperature and was stirred for 16 h. The pH was adjusted to 8 by slow addition of saturated aq. $NaHCO_3$. The product was extracted with chloroform (3 × 3 ml). The combined organic layer was washed with brine (4 ml), dried over anhydrous $MgSO_4$, filtered and concentrated to dryness under reduced pressure to afford **5** (313 mg, 70%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.48 (d, *J* = 5.5 Hz, 1H), 7.14 (d, *J* = 2.0 Hz, 1H), 6.89 (dd, *J* = 5.5, 2.0 Hz, 1H), 4.64 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.7, 150.8, 150.0, 113.5, 113.1, 46.3. HRMS (APPI) calcd. for $C_6H_6ClN_4^+$ $[M+H]^+$ 169.0276, found: 169.0284.

2-((4-Azidopyridin-2-yl)methyl)isoindoline-1,3-dione (6). Potassium phthalimide (378 mg, 2.04 mmol) and **5** (313 mg, 1.85 mmol) were suspended in a solution of dimethylformamide (2 ml). The reaction mixture was stirred at room temperature for 16 h. After this time, the mixture was concentrated to dryness under reduced pressure. The solid residue was washed with water (2 × 2 ml) and collected by filtration to yield **6** (483 mg, 93%) as a beige solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.44 (d, *J* = 5.5 Hz, 1H), 7.92-7.86 (m, 2H), 7.77-7.71 (m, 2H), 6.91 (d, *J* = 2.0 Hz, 1H), 6.84 (dd, *J* = 5.5, 2.0 Hz, 1H), 4.98 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.2, 157.4, 151.1, 149.6, 134.3, 132.2, 123.7, 113.0, 112.2, 42.9. HRMS (ESI-TOF) calcd. for $C_{14}H_{10}N_5O_2^+$ $[M+H]^+$ 280.0829, found: 280.0817.

(4-azidopyridin-2-yl)methanamine (7). To a solution of **6** (712 mg, 2.55 mmol) in tetrahydrofuran (2.7 ml) and methanol (2.7 ml) was added dropwise a solution of hydrazine hydrate (140 mg, 2.8 mmol) in methanol (0.85 ml). The reaction mixture was stirred at room temperature for 16 h. After this time, the mixture was concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography (dichloromethane/methanol, 95:5) to afford **7** (139 mg, 36%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.46 (d, *J* = 5.5 Hz, 1H), 6.98 (d, *J* = 2.0 Hz, 1H), 6.82 (dd, *J* = 5.5, 2.0 Hz, 1H), 3.97 (s, 2H), 1.77 (br s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.0, 150.7, 149.4, 112.4, 111.5, 47.7. HRMS (APPI) calcd. for $C_6H_8N_5^+$ $[M+H]^+$ 150.0774, found: 150.0764.

2-Aminomethylpyridine (dichloro) platinum (II) azide. (APPA, 8). Compound **7** (139 mg, 0.93 mmol) was solubilized in water (9.3 ml) and the pH was adjusted to 6 by slow addition of HCl (1 N). To the resulting solution was added a solution of K_2PtCl_4 (386 mg, 0.93 mmol) in water (9.3 ml). The mixture was stirred at room temperature for 3 h. A yellow/orange precipitate formed as the reaction took place and the pH dropped to 1. The pH was adjusted to 6 by addition of NaOH (1 N). After completion of the reaction, the precipitate was collected by filtration and washed with water (2 × 2 ml) and ethanol (2 × 2 ml). The solid residue was dried in a dessicator to yield **APPA** (211 mg, 54%) as a yellow-orange solid. ^{1}H NMR (500 MHz, DMF-d_7): δ 9.10 (d, *J* = 6.5 Hz, 1H, H_1), 7.51 (d, *J* = 2.0 Hz, 1H, H_4), 7.30 (dd, *J* = 6.5, 2.0 Hz, 1H, H_2), 6.23 (br s, 2H, NH), 4.33 (t, *J* = 6.0 Hz, 2H, H_6). ^{13}C NMR (125 MHz, DMF-d_7): δ 168.7 (C_5), 151.9 (C_1), 149.3 (C_3), 115.8 (C_2), 113.1 (C_4), 54.1 (C_6). ^{195}Pt (107 MHz, DMF-d_7): δ 2086. HRMS (ESI-TOF) calcd. for $C_7H_8Cl_2N_5O_2Pt^-$ $[M+HCO_2H-H]^-$ 458.9708, found: 458.9725.

2-Aminomethylpyridine (dichloro) platinum (II) (APP). APP was prepared according to a previously published procedure.[6] The commercially available 2-picolylamine (78 mg, 0.72 mmol) was solubilized in water (7.2 ml) and the pH was adjusted to 6 by slow addition of HCl (1 N). To the resulting solution was added a solution of K_2PtCl_4 (300 mg, 0.72 mmol) in water (7.2 ml). The mixture was stirred at room temperature for 4 h. A yellow precipitate formed as the reaction took place and the pH dropped to 1. The pH was adjusted to 6 by addition of NaOH (1 N). After completion of the reaction, the precipitate was collected by filtration and washed with water (2 × 2 ml) and ethanol (2 × 2 ml). The solid residue was dried in a dessicator to yield **APP** (111 mg, 41%) as a yellow solid. ^{1}H NMR (300 MHz, DMF-d_7): δ 9.25 (d, *J* = 6.5 Hz, 1H), 8.19 (td, *J* = 7.5, 1.5 Hz, 1H), 7.73 (d, *J* = 7.5 Hz, 1H), 7.53 (t, *J* = 6.5 Hz, 1H), 6.25 (br s, 2H), 4.37 (t, *J* = 6.0 Hz, 2H).

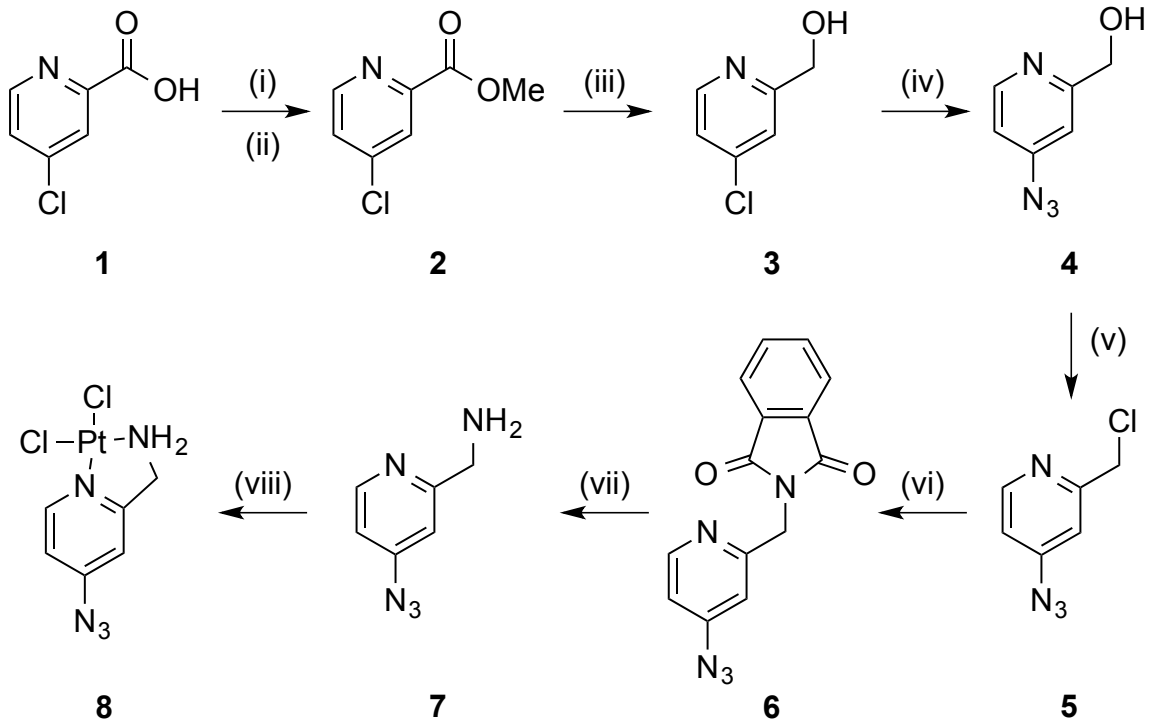
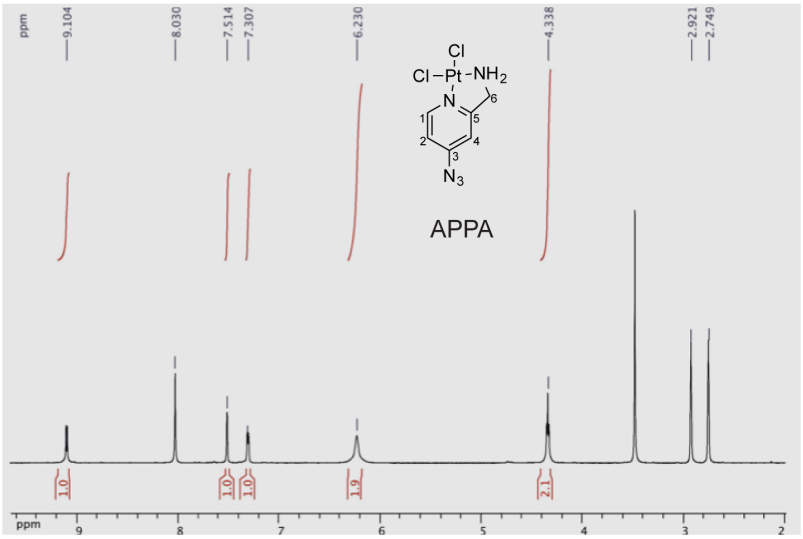


Figure S1. Synthetic route to APPA. (i) $(COCl)_2$, DCM, cat. DMF, rt, 2 h. (ii) MeOH, rt, 16 h, 74%. (iii) $CaCl_2$, $NaBH_4$, MeOH:THF (1.6:1), 0 °C to rt, 48 h, 87%. (iv) NaN_3, DMF:H_2O (20:1), 85 °C, 14 d, 60%. (v) $SOCl_2$, $CHCl_3$, 0 °C to rt, 16 h, 70%. (vi) Potassium phthalimide, DMF, rt, 16 h, 93%. (vii) NH_2NH_2•H_2O, THF:MeOH (1:1), rt, 16 h, 36%. (viii) K_2PtCl_4, H_2O, rt, 3 h, 54%.

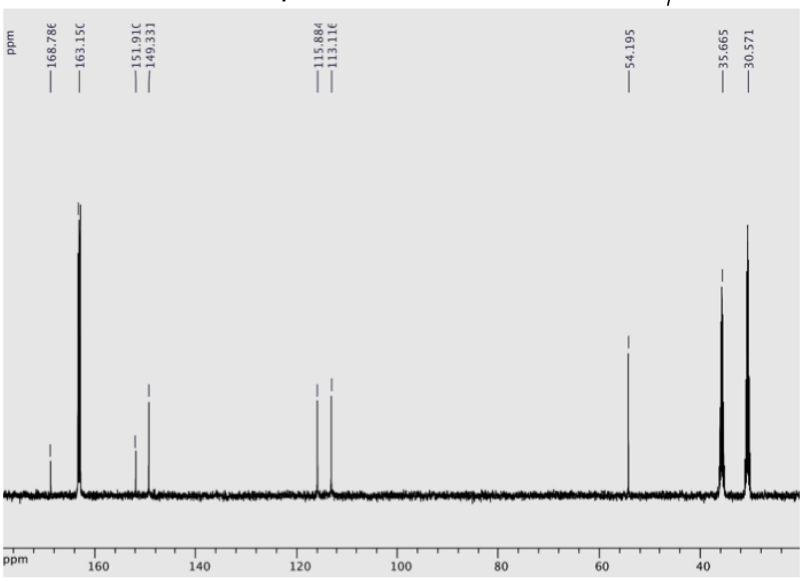
A

^{1}H NMR spectrum of APPA in DMF-d$_7$



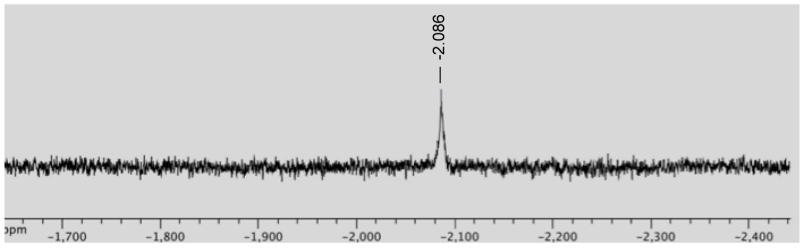
B

^{13}C NMR spectrum of APPA in DMF-d$_7$



C

^{195}Pt NMR spectrum of APPA in DMF-d$_7$



D

HRMS of APPA

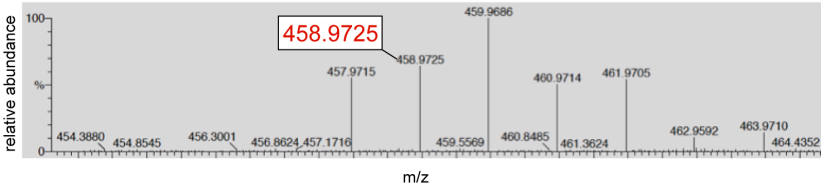


Figure S2. Spectral data of APPA. (A) ^{1}H NMR. (B) ^{13}C NMR. (C) ^{195}Pt NMR. (D) High Resolution Mass Spectrum.

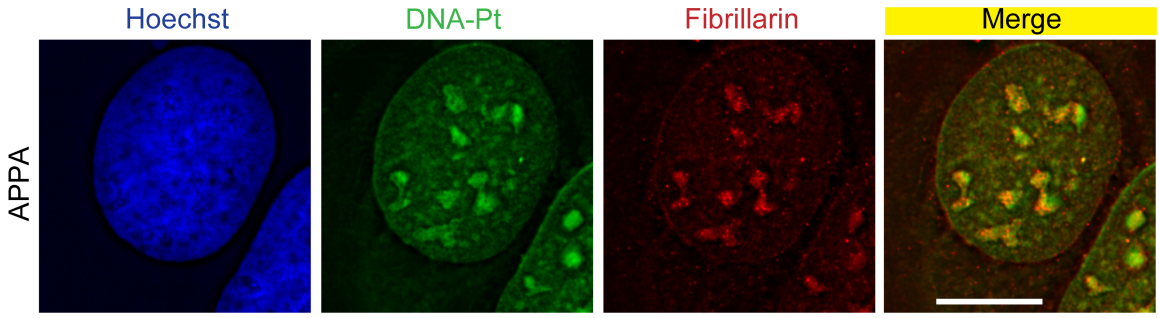


Figure S3. APPA accumulates in nucleoli. Visual detection of labeled DNA-Pt by fluorescence microscopy in osteosarcoma U2OS cells showing colocalization with the nucleolar marker fibrillarin. Cells were treated with APPA (250 μM for 3 h). Scale bar, 20 μm.

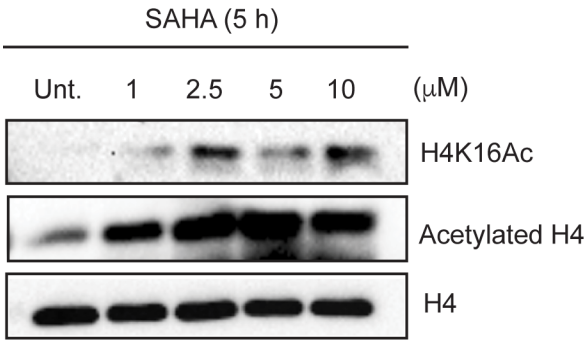


Figure S4. SAHA induces hyperacetylation of H4. Western blot analysis of osteosarcoma U2OS cells treated as indicated.

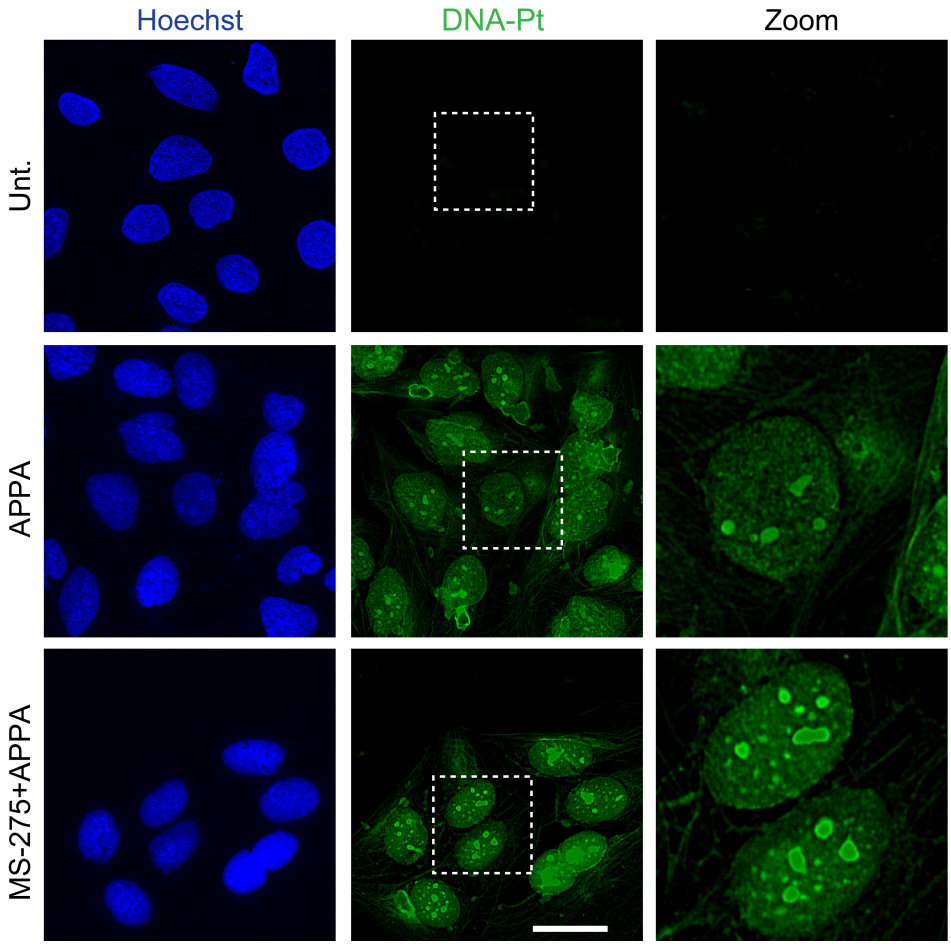


Figure S5. MS-275 induces the formation of DNA-Pt foci. Visual detection of labeled DNA-Pt by fluorescence microscopy in U2OS cells. Cells were treated with APPA (250 μM for 3 h) and MS-275 (5 μM for 5 h) as indicated. Zoomed images are 3×. Scale bar, 20 μm.

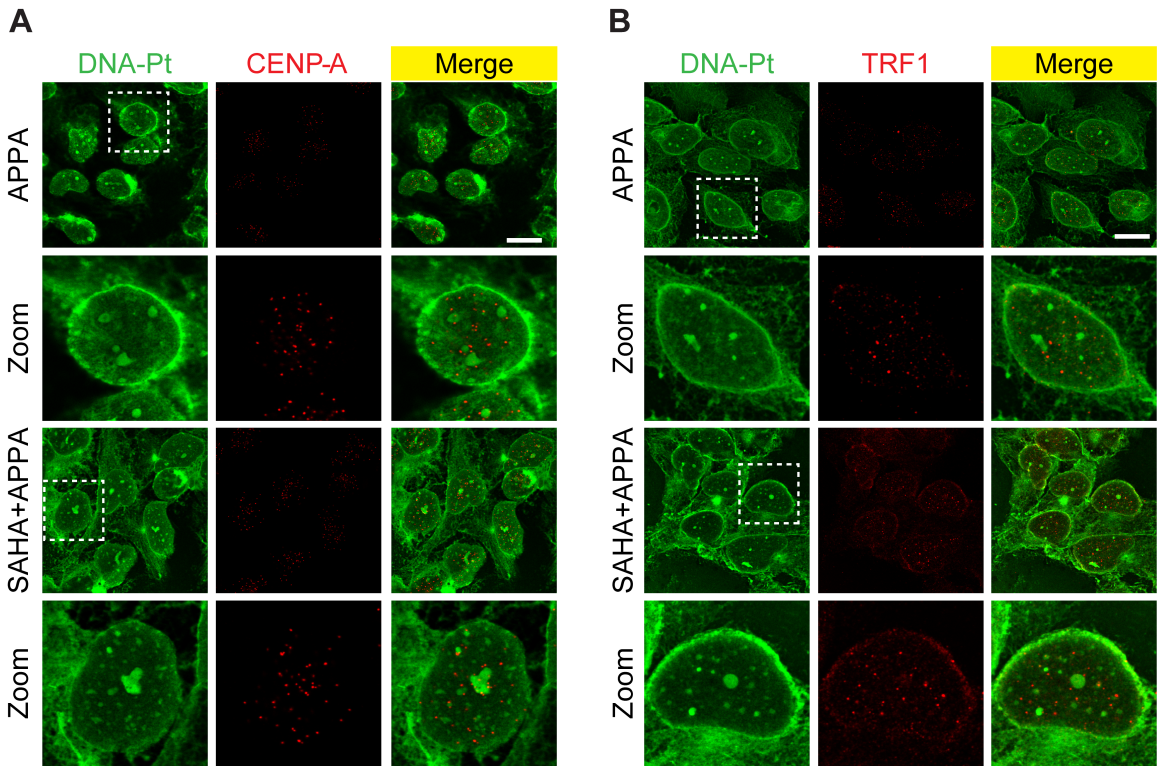


Figure S6. DNA-Pt foci do not predominently form at centromere and telomeres. Visual detection of labeled DNA-Pt by fluorescence microscopy in osteosarcoma U2OS cells co-stained with markers of centromeres and telomeres. (A) Centromeric marker CENP-A. (B) Telomeric marker TRF1. Cells were treated with APPA (250 μM for 3 h) and SAHA (2.5 μM for 5 h) as indicated. Zoomed images are 3×. Scale bar, 20 μm.

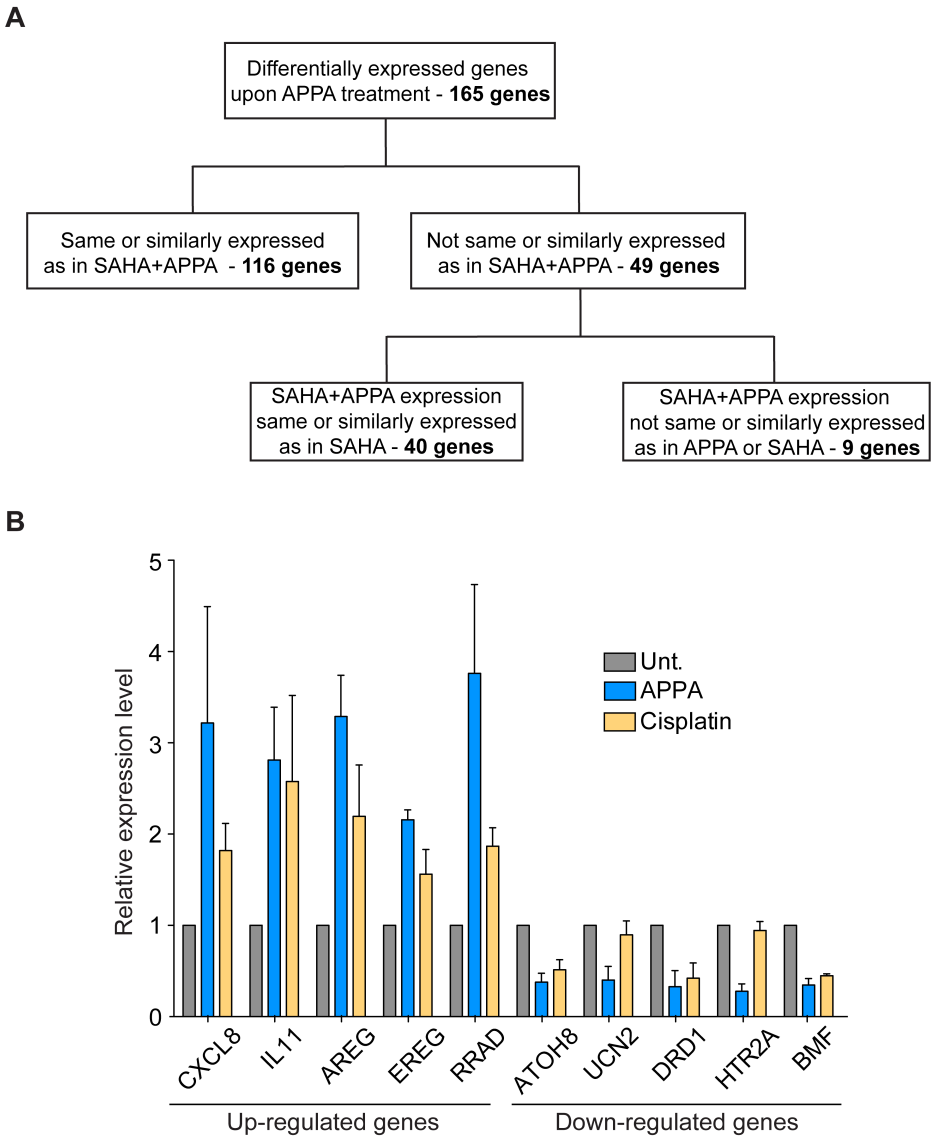


Figure S7. Transcriptional analysis in cells treated with platinum drugs. (A) Tree diagram representing the 165 differentially expressed genes from APPA vs Unt. cells identified by RNA-Seq. Comparisons of gene sets were performed between the differentially expressed genes in APPA to SAHA or SAHA+APPA treatments. Of these genes, 116 genes had the same or similar expression profiles in SAHA+APPA. Of the other 49 genes, 40 had the same or similar gene expression profiles as in SAHA and only 9 genes displayed a gene expression pattern that was not observed in either APPA or SAHA treatments alone. Differentially expressed genes are defined as fold change > 3 and FDR < 0.01 in Unt. vs APPA. Similar expression is defined as being within 1 fold change of SAHA+APPA values either up- or down-regulated. (B) Cisplatin and APPA treatment induce similar transcriptional effects. Analysis of top up- and down-regulated genes in APPA or cisplatin treated cells by qPCR. Histogram represents data from three independent experiments. Cells were treated with APPA (250 μM for 3 h) or cisplatin (10 μM for 3 h). Error bars represent s.e.m.

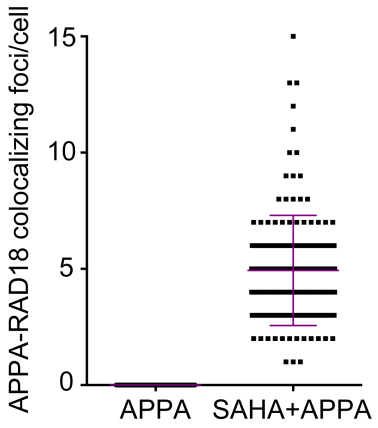


Figure S8. Quantification of DNA-Pt and RAD18 colocalizing foci in U2OS cells treated as indicated in **Figure 4A**. 50 cells were scored per condition; *n* = 3; Foci were independently counted by two individuals in double blinded experiments. Error bars represent mean ±SD.

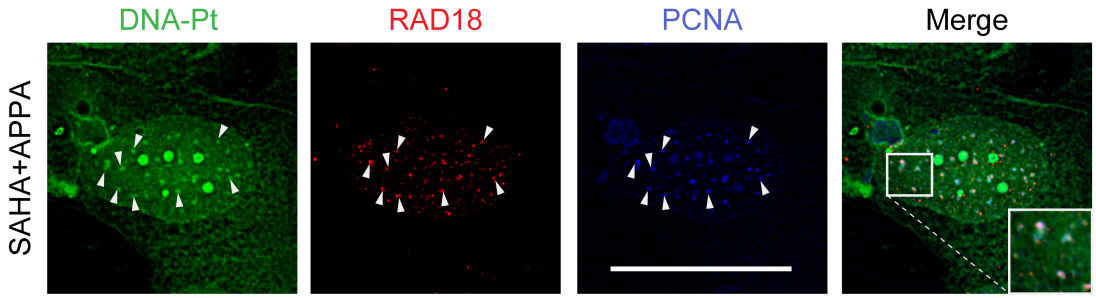


Figure S9. DNA-Pt colocalize with PCNA and RAD18. Visual detection of DNA-Pt, RAD18 and PCNA by fluorescence microscopy in osteosarcoma U2OS cells. Cells were treated with APPA (250 μM for 3 h) and SAHA (2.5 μM for 5 h) as indicated. Scale bar, 20 μm.

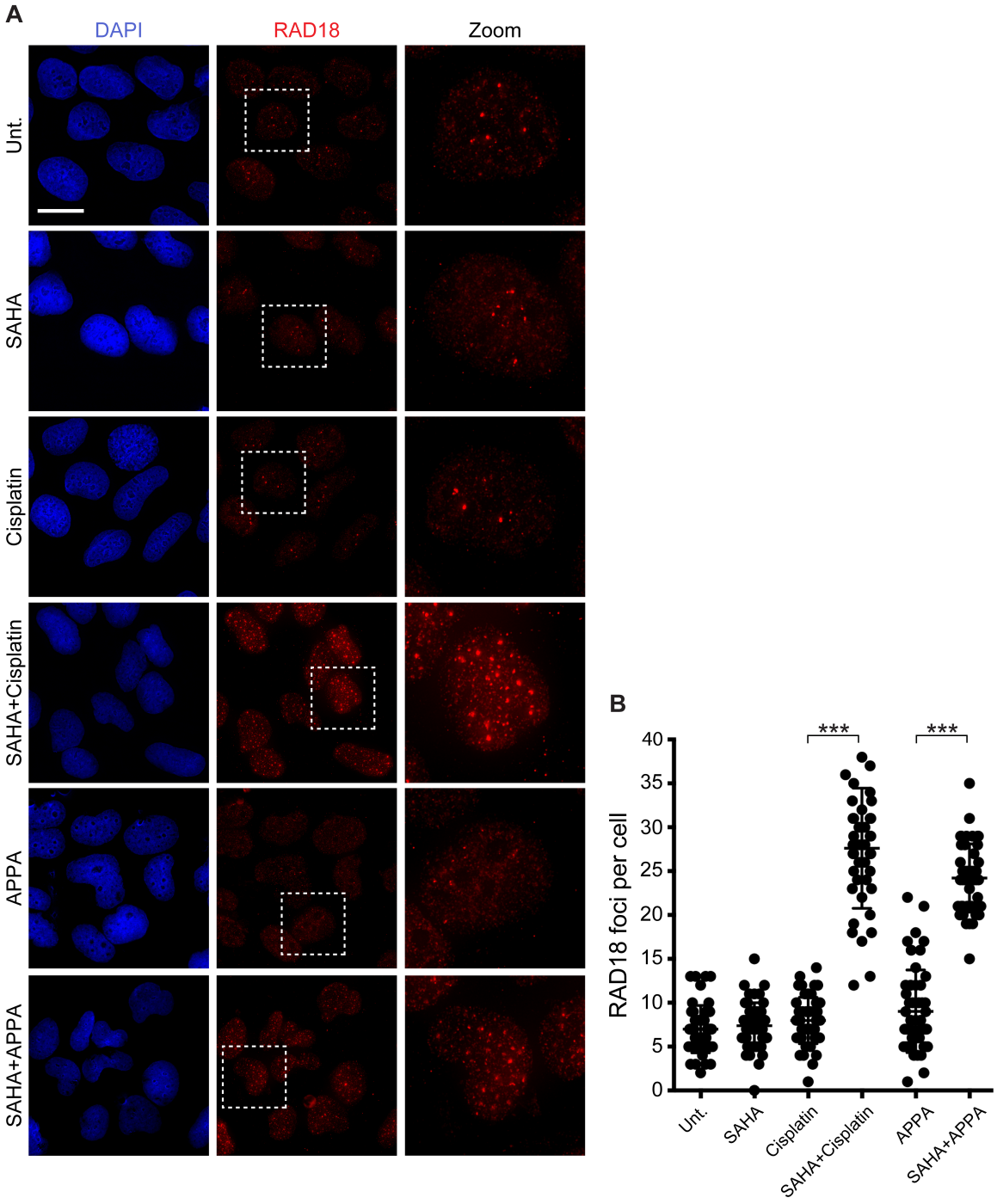


Figure S10. SAHA and platinum drugs increase the number of RAD18 foci. (A) Visual detection of RAD18 by fluorescence microscopy in osteosarcoma U2OS cells. Cells were treated with cisplatin (10 μM for 3 h), APPA (250 μM for 3 h) and SAHA (2.5 μM for 5 h) as indicated. Zoomed images are 3×. Scale bar, 20 μm. (B) Quantification of A. 25 cells were scored per condition; n = 2; Foci were independently counted by two individuals in double blinded experiments. Error bars represent mean ±SD; ***P < 0.001, Student's t-test.

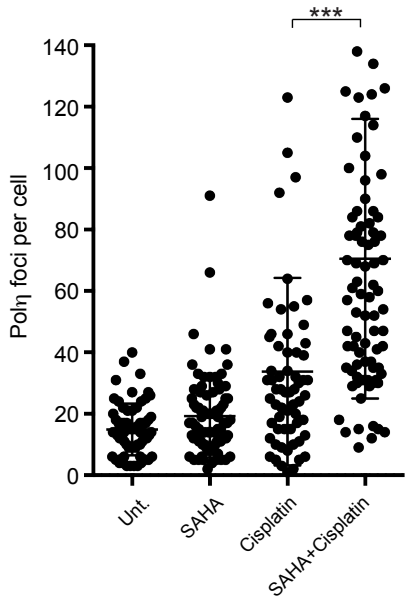


Figure S11. Quantification of Polη foci in U2OS cells treated as indicated in **Figure 4C**. > 25 Polη positive cells were scored per condition; $n = 2$; Foci were computationally scored using Fiji software and the Analyze Particles function.[7] Error bars represent mean ±SD; ***$P < 0.001$, Student's *t*-test.

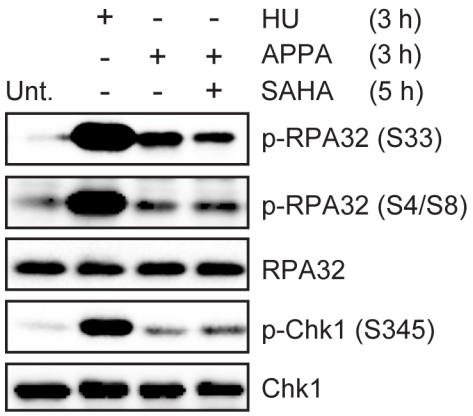


Figure S12. SAHA and APPA induce similar replication stress compared to APPA. Western blot analysis of osteosarcoma U2OS cells treated as indicated showing markers of replication stress. Cells were treated with HU (2 mM), APPA (250 μM) and SAHA (2.5 μM) as indicated.

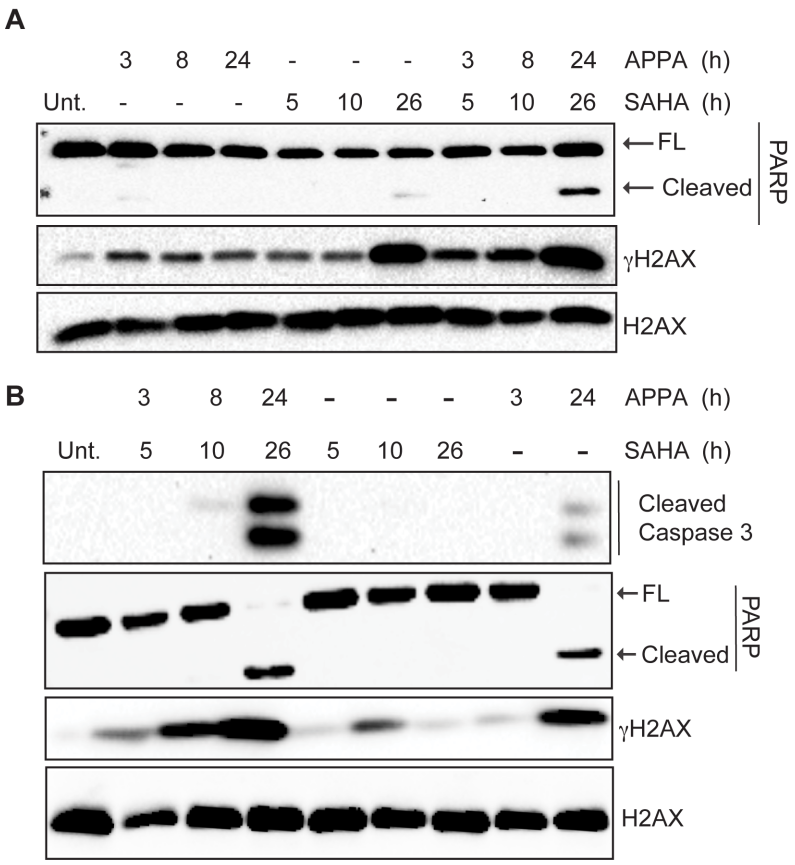


Figure S13. SAHA and APPA induce apoptosis signaling. Western blot analysis of cancer cells treated as indicated showing apoptotic markers. (A) Osteosarcoma U2OS cells. (B) Ovarian A2780 cells. Cells were treated with APPA (250 μM) and SAHA (2.5 μM) as indicated.

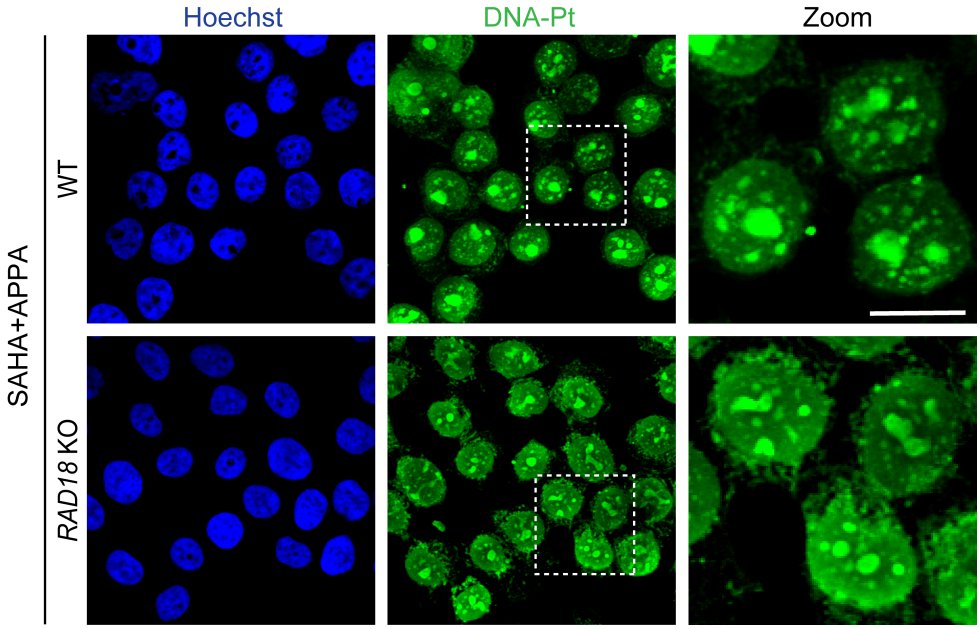


Figure S14. *RAD18* KO does not prevent the formation of DNA-Pt foci. Visual detection of labeled DNA-Pt by fluorescence microscopy in colon carcinoma HCT-116 WT and HCT-116 *RAD18* KO cells. Cells were treated with APPA (250 μM for 3 h) and SAHA (2.5 μM for 5 h). Zoomed images are 4×. Scale bar, 10 μm.

table S1. Unbiased screening of drugs in combination with APPA.

Small molecule	Biological target/phenotype	Concentration/time of treatment	Reference	Supplier
Taxol	β-tubulin/stabilizes microtubules	1 μM for 6 h	*8*, *9*	Tocris (1097)
5-Azacytidine	DNMT1 and DNMT3/induce DNA hypomethylation	10 μM for 24 h	*10*, *11*, *12*	Tocris (3842)
Pyridostatin	G-quadruplex motif/alters gene transcription	10 μM for 6 h	*1*	In-house
KU55933	ATM/disrupts the signaling and repair of DSBs	20 μM for 4 h	*1*	Tocris (3544)
NU7441	DNA-PK/disrupts the signaling and repair DSBs	2 μM for 4 h	*1*	Tocris (3712)
GW7647	USP1/enhancing TLS and Fanconi anemia activity	50 μM for 3 h	*13*	Tocris (1677)
JQ1	BET bromodomains/inhibits BET-dependent transcription	1 μM for 5 h	*14*	Tocris (4499)
SAHA	HDACs/induces chromatin relaxation	2.5 μM for 5 h	*15*	Sigma (SML0061)
MS-275	HDACs/induces chromatin relaxation	5 μM for 5 h	*16*	Sigma (EPS002)
Garcinol	p300 and PCAF (HATs)	10 μM for 24 h	*17*	Tocris (4827)
Remodelin	NAT10/alters microtubule nucleation	10 μM for 24 h	*18*	In-house
Tranylcypromine	LSD1/BHC110	10 μM for 24 h	*19*, *20*, *21*	Tocris (3852)
JIB-04	Pan Jumonji HDMTs inhibitor	10 μM for 24 h	*22*	Tocris (4972)
SGC0946	DOT1L (HMT)	10 μM for 24 h	*23*	Tocris (4541)
DZNep	Pan HMTs inhibitor	10 μM for 24 h	*24*, *25*	Tocris (4703)

table S2. RNA-Seq analysis. Differentially expressed genes in APPA, SAHA and SAHA+APPA treatments compared to untreated. Differentially expressed genes are defined as fold change > 3 and FDR < 0.01. Data represent gene expression analysis performed from two independent experiments.

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