Supporting Information

Cell Culture and Drug Treatment. All cell lines were grown at 37 °C under a humidified atmosphere of 5% (vol/vol) CO_2. The testicular germ cell tumor lines (NTera2 and Tera2), and breast cancer cell line MDA-MB-231 were purchased from ATCC. The testicular germ cell tumor, GCT27 was a gift from Prof. John Turchi (Indiana University, School of Medicine). An NTera2 HMGB4$^{-/-}$ cell line was generated via CRISPR-Cas9 mediated knockout. NTera2 and NTera2 HMGB4$^{-/-}$ cells were maintained in high glucose Dulbecco's modified Eagle medium (DMEM) from ATCC and MDA-MB-231 was maintained in Gibco DMEM. Media were supplemented with 10% (wt/vol) fetal bovine serum, 10 U/ml penicillin, and 10 mg/ml streptomycin. Cisplatin (*cis*-diamminedichloroplatinum(II), Strem), solutions were freshly prepared for each treatment in 100% PBS.

CRISPR/Cas9-Mediated Gene Editing. We targeted HMGB4 via SpCas9-mediated genome editing in human testicular embryonic carcinoma cells (NTera2). To do so, we designed an sgRNA-targeting exon 5 of the human HMGB4 gene. The designed sgRNA strands were phosphorylated and cloned into the pSpCas9(BB)-2A-GFP plasmid (Addgene: PX458). The insertion into the plasmid was validated by DNA sequencing. To measure the editing efficiency of the vector, we transfected NTera2 cells with the plasmid. After 48 h, GFP positive cells were placed into 96 well plates in triplicate for a 3-week expansion by fluorescence assisted cell sorting (FACS). Cells were functionally assessed by qRT-PCR and Western blot for HMGB4 gene and protein levels respectively.

Transfection. NTera2, NTera2 HMGB4$^{-/-}$, or MDA-MB-231 cells were seeded 24 h prior to transfection. Cells were transfected with pcDNA3.1 encoding human HMGB4 using TransIT-LT1 (Mirus Bio LLC) transfection reagent (TR) according to manufacturer's protocol. Functional studies including qRT-PCR, Western blot, and MTT were performed 48 h post-transfection.

shRNA Interference. For XPB (ERCC3) knockdown, *E. coli* glycerol stock containing the lentiviral plasmid vector pLKO.1-puro (SHCLNG-NM_000122) was purchased from

Sigma-Aldrich and amplified following the manufacturer's protocol. The glycerol stock was streaked onto an LB-agar-ampicillin plate and incubated at 37 °C overnight. The following day, a single colony was picked and dipped into 5 mL terrific broth (TB) culture medium containing ampicillin (100 μg/mL) using a sterile pipet tip and cultured overnight on a shaker at 37 °C. The bacteria were spun down (3000 rpm, 4 °C, and 20 min) and a mini-prep (Qiagen) was performed to isolate the pure plasmid. NTera2 or NTera2 HMGB4$^{-/-}$ cells were transfected with the plasmid using TransIT-LT1 (Mirus Bio LLC) transfection reagent (TR). Briefly, cells were seeded on a 100 mm petri dish and cultured to ~70-80% confluence. The medium was replaced with fresh medium on the day of transfection. Just before the transfection, the plasmid DNA (12 μg) was mixed with TR (45 μL) in 1.5 mL OPTI-MEM medium and incubated for 30 min at room temperature. The mixture was then added to the cells, mixed gently and incubated for 48 h. The medium was aspirated and 10 mL fresh medium containing 2 μg/mL puromycin was added. A puromycin selection was performed for 2 days. After that the transfected cells were grown in fresh RPMI medium without puromycin and allowed to expand.

Cellular Cytotoxicity. The potency of cisplatin was evaluated in NTera2, NTera2 HMGB4$^{-/-}$, MDA-MB-231, and MDA-MB-231^{HMGB4} cells by using the MTT assay. Cells (2 x 10^4) were seeded in a 96-well plate and incubated at 37 °C in 5% (vol/vol) CO_2 overnight. Various concentrations of cisplatin (0.03-50 μM) were added to each well followed by incubation for 72 h. The medium was removed and 200 μL of an 0.05 mg/mL solution of MTT in DMEM was added to each well and incubated for 2-4 h, after which the medium was aspirated and 100 μL DMSO was added to dissolve the resulting purple formazan. The absorbance of the solution was determined at 570 and 650 nm and a plot of absorbance versus concentration generated dose-response curves. The IC_{50} values were interpolated from the dose dependent plots.

Apoptosis Measurement by Flow Cytometry. Apoptosis experiments were performed using the apoptosis/dead cell (APC-Annexin V/SYTOX green) detection protocol. The

manufacturer's protocol was followed to carry out this experiment. Briefly, untreated and treated cells (5×10^5) were suspended in 1x Annexin binding buffer (100 µL) (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4), then 5 µL of APC-Annexin V and 2 µL of SYTOX green were added to each sample and incubated at 37 °C for 15 min, after which more binding buffer (150 µL) was added while gently mixing. The samples were kept on ice prior to being read on the FACSCalibur-HTS flow cytometer (BD Biosciences) and 20,000 events per sample were acquired. Cell populations were analyzed using the FlowJo software (Tree Star).

Cell Cycle Measurement by Flow Cytometry. In order to examiner the cell cycle, flow cytometry studies were carried out to monitor the DNA content. NTera2 and NTera2 HMGB4$^{-/-}$ cells were incubated with and without the cisplatin (4 µM) treatment for 24-72 h at 37 °C. Cells were harvested from adherent cultures by trypsinization. Following centrifugation at 1000 rpm for 5 min, cells were washed with PBS, and then fixed with 70% (vol/vol) ethanol in PBS after respective cisplatin incubation periods. Fixed cells were collected by centrifugation at 2500 rpm for 3 min, washed with PBS and centrifuged as before. Cellular pellets were re-suspended in 50 µg/ml propidium iodide (Sigma) in PBS for nucleic acids staining and treated with 100 µg/ml RNaseA (Sigma). DNA content was measured on a FACS Calibur-HTS flow cytometer (BD Biosciences) using laser excitation at 488 nm and 20,000 events per sample were acquired. Cell cycle profiles were analyzed using the ModFit software.

Cell-Free Extracts (CFEs). The NTera2 cells used were from the American Type Culture Collection and NTera2 HMGB4$^{-/-}$ were derived from NTera2 through CRISPR/Cas9 mediated knockout. Whole CFEs from the two cell lines were prepared by the previously published protocol of Manley *et al.* (1) and stored at -80 °C until use.

Excision Assay Substrate Preparation. The 1,2-d(GpG) cross-linked substrates were 146-bp linear duplexes obtained by ligating the cisplatin-modified 5' ^{32}P-labeled oligomer d(TTCACCG*G*CCTTCC) with four other overlapping oligomers, following the established protocol (2). The guanine sites, designated with asterisks, were platinated

with the *cis*-diammineplatinum(II) moiety. The oligomer (0.5 µg) was then labeled in a 50 µl solution containing [γ^{32}P]ATP(6000 Ci/mmol;1Ci = 37 GBq) and mixed with 1.0 µg of each of the other four oligomers, which were phosphorylated with T4 PNK containing 10 mM nonradioactive ATP. The mixture was precipitated with ethanol in the presence of oyster glycogen and the oligomers were resuspended in 15 µl of annealing buffer, which contained 20 mM Tris HCl (pH 7.4), 50 mM NaCl, and 2 mM $MgCl_2$. The mixture was heated at 70 °C for 5 min and cooled gradually over 4 h to 22 °C. To the annealed solution was added 10 x ligation buffer (NEB) to achieve an ATP concentration of 0.2 mM. The volume was adjusted to 50 µl with water, T4 DNA ligase was added, and ligation was carried out at 16 °C for 20 h. The DNA was separated on a 6-8% (wt/vol) polyacrylamide denaturing gel (TE buffer, 32-20 mA for 2.5 h) and the full-length fragment was located by autoradiography, excised, and isolated from the gel using the crush and soak method(3) followed by ethanol precipitation. The DNA was resuspended in 50 µl of annealing buffer, heated to 70 °C, and cooled gradually (2-3 hr) to 22 °C.

Excision Assay. The excision reaction buffer contained 25 mM HEPES (pH 7.9), 45 mM KCl, 4.4 mM $MgCl_2$, 2 mM ATP, 0.4 mM dithiothreitol, 0.1 mM EDTA, 2.5 % (vol/vol) glycerol, and 200 µg/ml of bovine serum albumin. NTera2 or NTera2 HMGB4$^{-/-}$ CFE (50 µg), 30 pM substrate, and 250 ng of pBR322 as carrier DNA were mixed in the excision reaction buffer. The reaction was carried out at 30 °C for 75 min. Adding SDS up to 1% and proteinase K to 0.4 mg/ml stopped the reaction. After proteinase K digestion and phenol/chloroform extraction, the DNA was separated on 8-10% (wt/vol) polyacrylamide denaturing gels (TE buffer, 32-20 mA for 2.5 h). Each gel was dried on a Labconco gel drier and exposed to a phosphor storage screen overnight before being imaged using a Storm840 imager (Amersham Biosciences).

Western Blot. Cells (1 × 10^6 cells) were incubated with or without cytotoxic agent (2 µM) for respective time points at 37 °C. Cells were washed with PBS, scraped into SDS-PAGE loading buffer (64 mM Tris-HCl (pH 6.8)/9.6% glycerol (vol/vol)/2% SDS (wt/vol)/5% (vol/vol) β-mercaptoethanol/0.01% Bromophenol Blue), and incubated at

95 °C for 10 min. Whole cell lysates were resolved by 4-20% (wt/vol) sodium dodecylsulphate polyacylamide gel electrophoresis (SDS-PAGE; 200 V for 25 min) followed by electro-transfer to polyvinylidene difluoride membranes, PVDF (350 mA for 1 h). Membranes were blocked in 5% (wt/vol) bovine serum albumin in PBST (PBS/0.1% Tween 20) and incubated with the appropriate primary antibodies (Cell Signalling Technology and Santa Cruz). After incubation with horseradish peroxidase-conjugated secondary antibodies (Cell Signalling Technology), 1:1000, immuno-complexes were detected with the ECL detection reagent (BioRad) and analyzed using UVP biospectrum imaging system fitted with a chemiluminescence filter.

Quantitative (q)RT-PCR. Total RNA was isolated with the Qiagen RNAeasy RNA isolation kit (Hilden, Germany) and cDNA was synthesized with the Applied Biosystems reverse-transcription-kit (Foster City, CA, USA). qRT-PCR was performed in a Light cycler 480 II thermo cycler with SYBR Green PCR Mastermix (Roche). All primers (Table S1) were separated by at least one intron on the genomic DNA to exclude their amplification. PCR reactions were checked by including no-RT controls, by omission of templates, and by melting curves. Standard curves were generated for each gene. Relative quantification of gene expression was determined by comparison of threshold values. All samples were analyzed in duplicate at two different dilutions. All results were normalized to beta-actin.

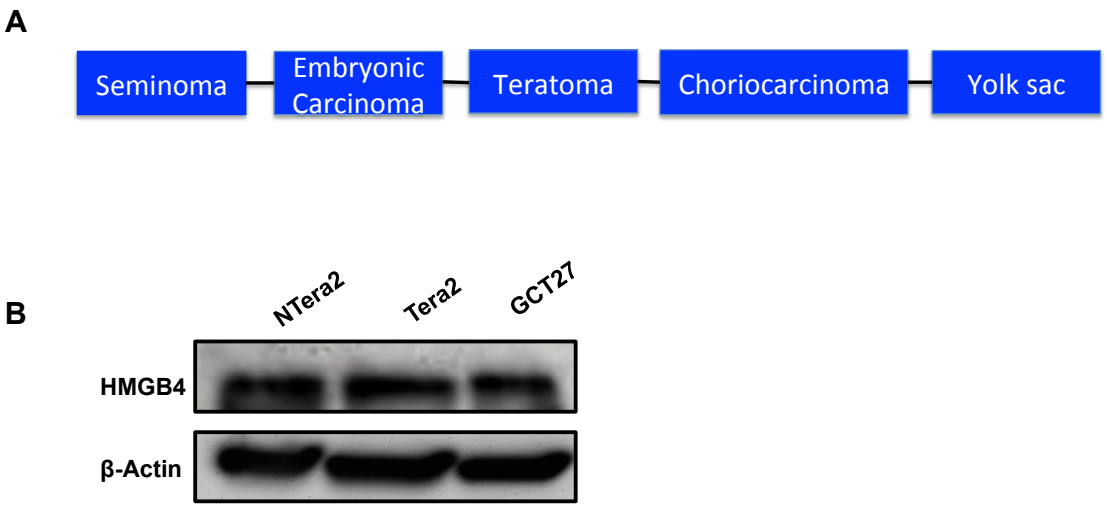
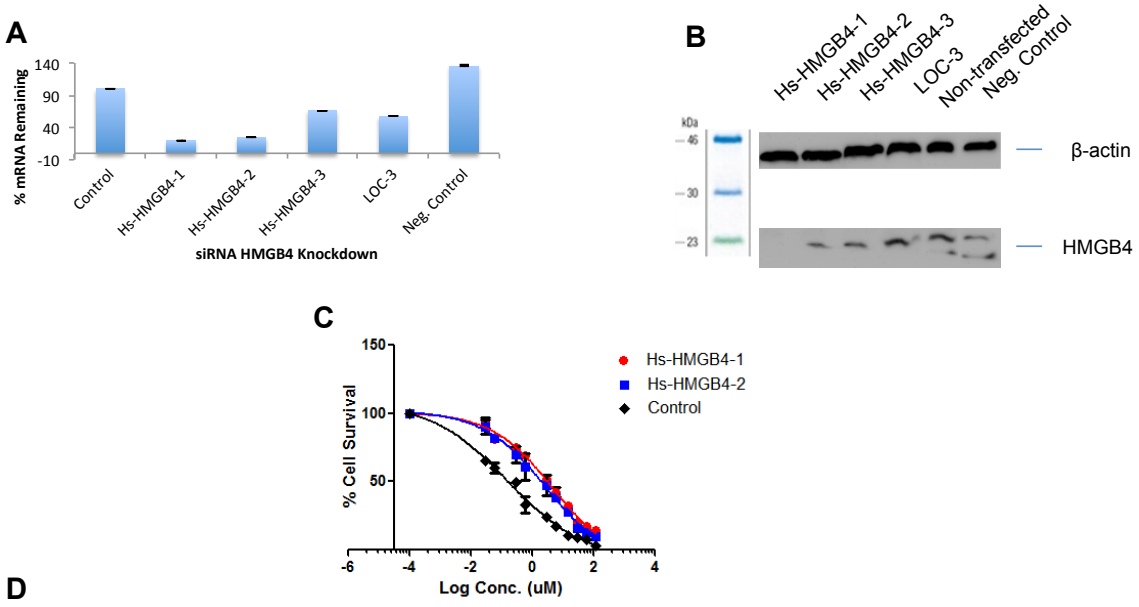


Figure S1. (A) A schematic representation of histopathological classification of TGCTs. TGCTs are classified as seminoma and non-seminoma, with non-seminoma comprising embryonic carcinoma, teratoma, choriocarcinoma and yolk sac. (B) Immunoblotting of HMGB4 from cell lysates of Tera2, a cell line derived from a metastatic embryonic carcinoma TGCT; NTera2, a Tera2 cell line implanted in nude mice and subsequently grown in culture, and GCT27, a teratoma. β-actin was used as a loading control.



Cell line	Cisplatin (IC_{50})	Fold
Ntera2	0.89 ± 0.83 μM	1.0
NTera2 $HMGB4^{KD}$ (Hs-HMGB4-1)	4.20 ± 1.22 μM	~4.5
NTera2 $HMGB4^{KD}$ (Hs-HMGB4-2)	3.52 ± 0.73 μM	~3.5

Figure S2. (A) mRNA level of HMGB4 after short-interfering RNA (siRNA) transfection of NTera2 cells (Hs-HMGB4-1, Hs-HMGB4-2, Hs-HMGB4-3, LOC-3, and Neg. control are siRNA sequences purchased from Qiagen, USA). (B) HMGB4 protein levels following siRNA transfection. (C) Dose response curves correlating HMGB4 levels with sensitivity by cell viability measurement using the MTT assay. (D) Quantification of IC_{50} values extrapolated from dose response curves.

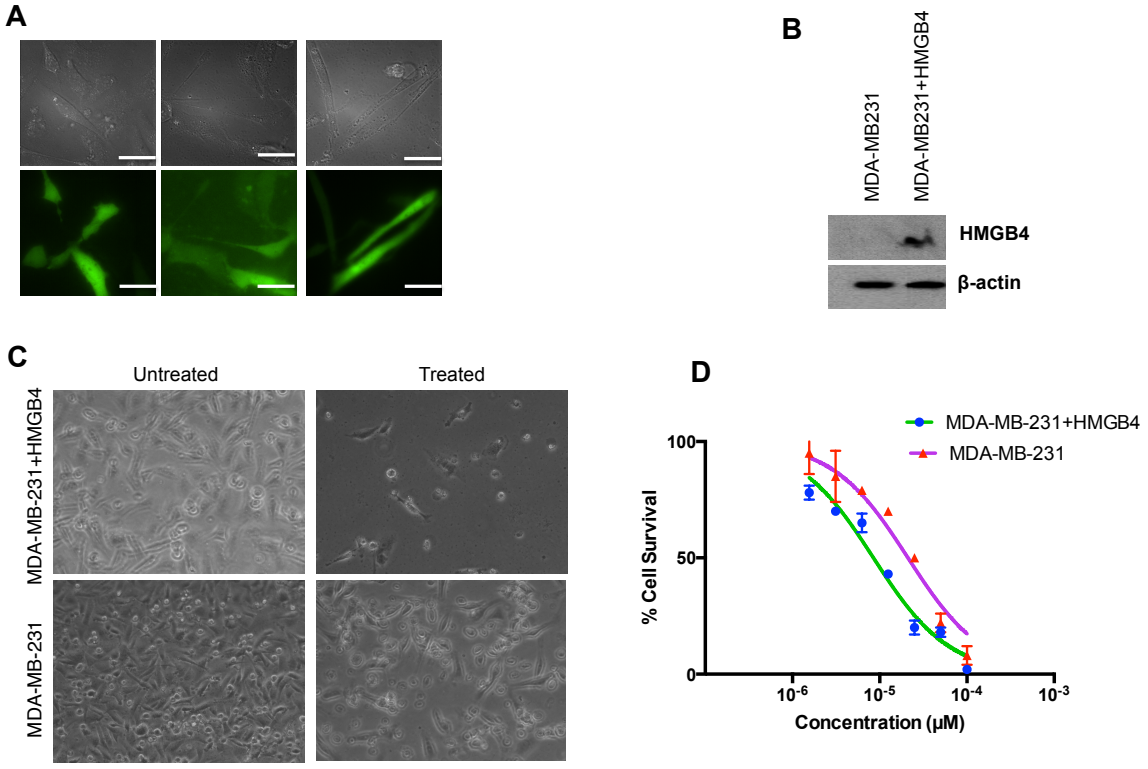


Figure S3. (A) Co-transfection of pcDNA-HMGB4 and pTurbo GFP plasmids into breast cancer cell, MDA-MB-231. Fluorescent cells are a measure of transfection efficiency. (B) HMGB4 protein level 48 h after pcDNA-HMGB4 transfection into MDA-MB-231cells. (C) DIC images of cisplatin treated (10 μM, 72 h) or untreated MDA-MB-231/MDA-MB-231+HMGB4 cells. (D) Dose response curves of cisplatin treated MDA-MB-231/MDA-MB-231+HMGB4 cells.

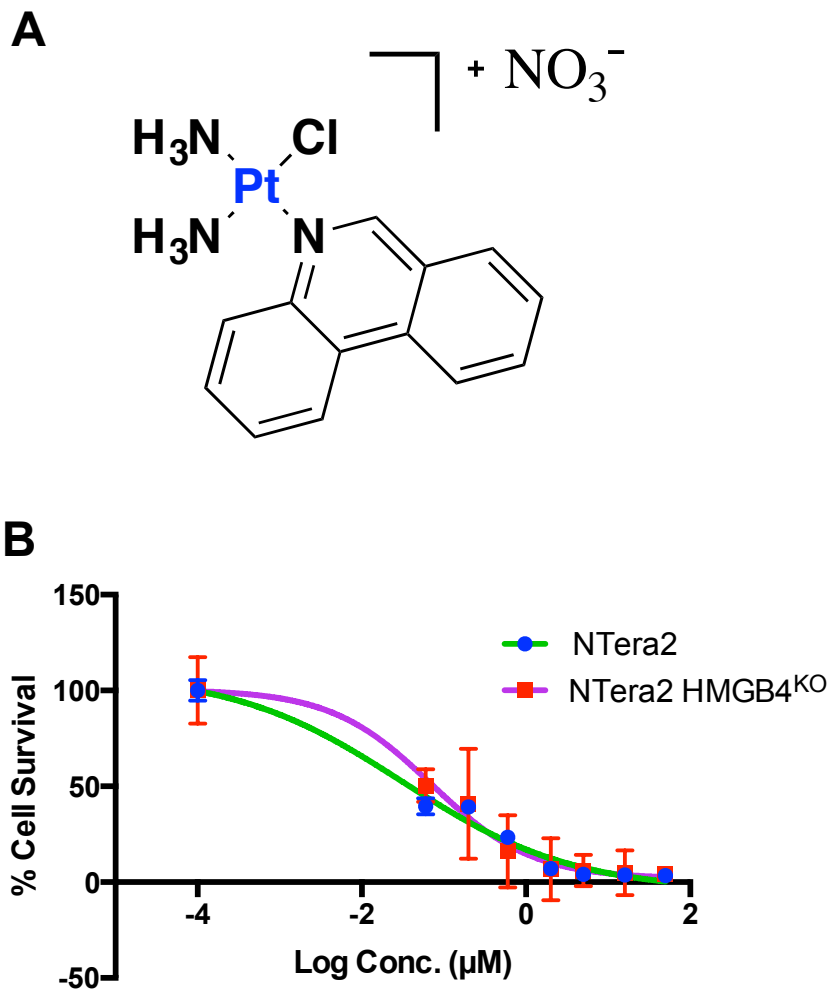


Figure S4. (A) Chemical structure of phenanthriplatin (B) Dose response curves of phenanthriplatin treated NTera2/ NTera2 HMGB4$^{-/-}$ cells.

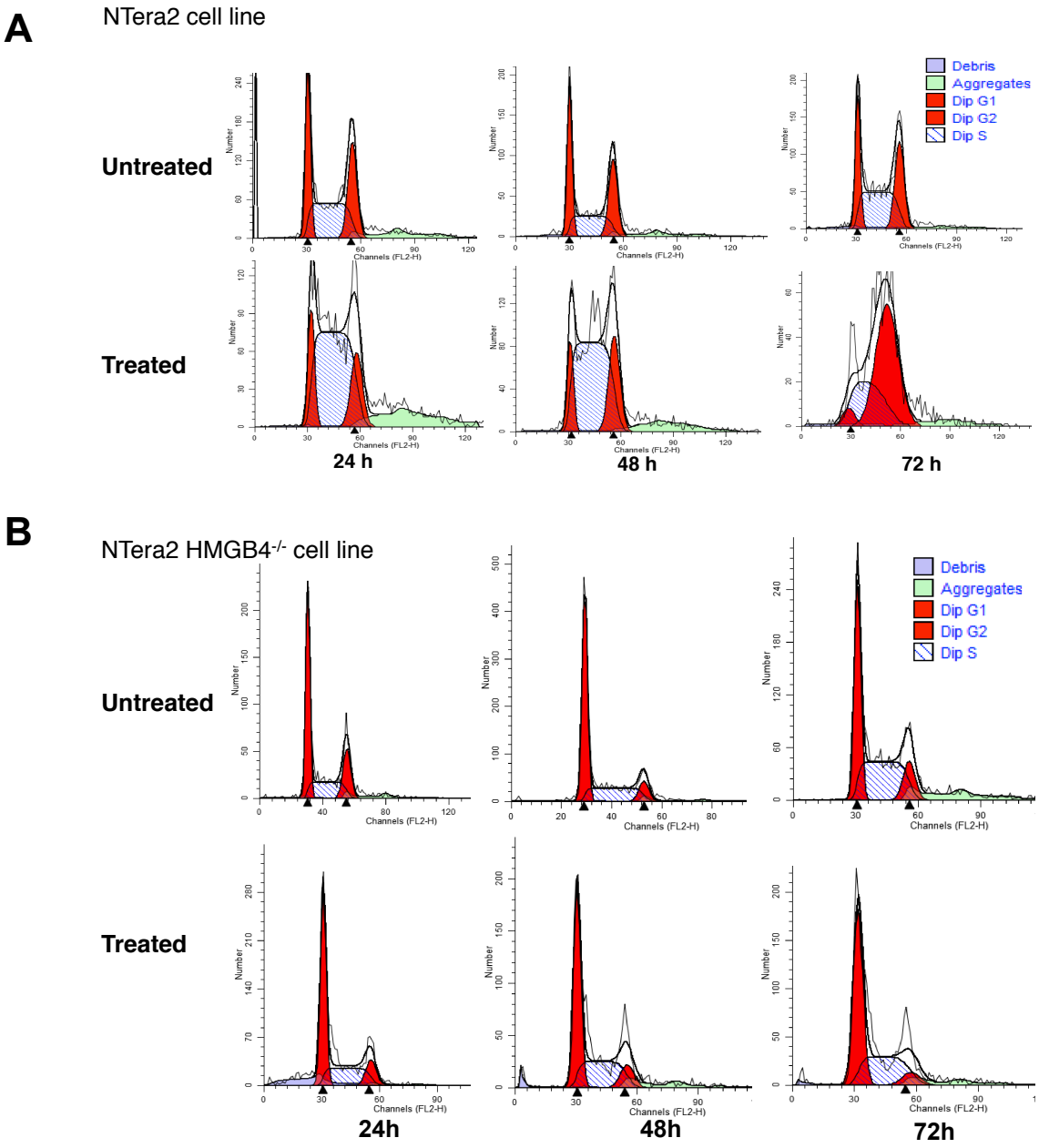


Figure S5. (A) Histogram representing the different phases of the cell cycle for NTera2 cells in the absence and presence of cisplatin (2 μM) over the course of 72 h. (B) Histogram representing the different phases of the cell cycle for NTera2 HMGB4$^{-/-}$ cells in the absence and presence of cisplatin (2 μM) over the course of 72 h. Quantification of cell cycle phases are derived from histogram plots in A and B for G_0/G_1 (Dip G1), S (Dip S), and G_2/M (Dip G2) phase.

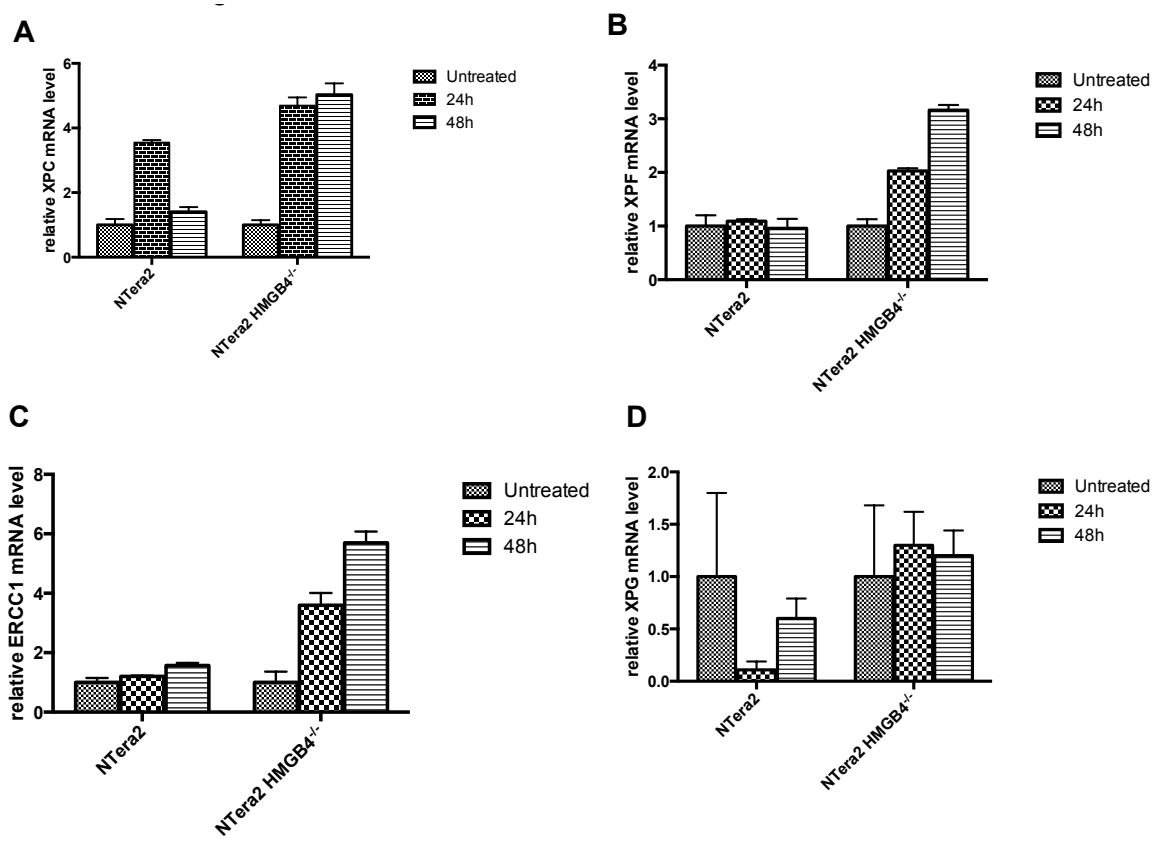


Figure S6. (A), XPC; (B), XPF; (C), ERCC1 and (D), XPG mRNA transcripts in NTera2 and NTera2 HMGB4$^{-/-}$ cells. Cells were treated with cisplatin (2 μM) or untreated and RNA was isolated at different time points for reverse transcriptase polymerase chain reaction studies using primers for the four respective proteins.

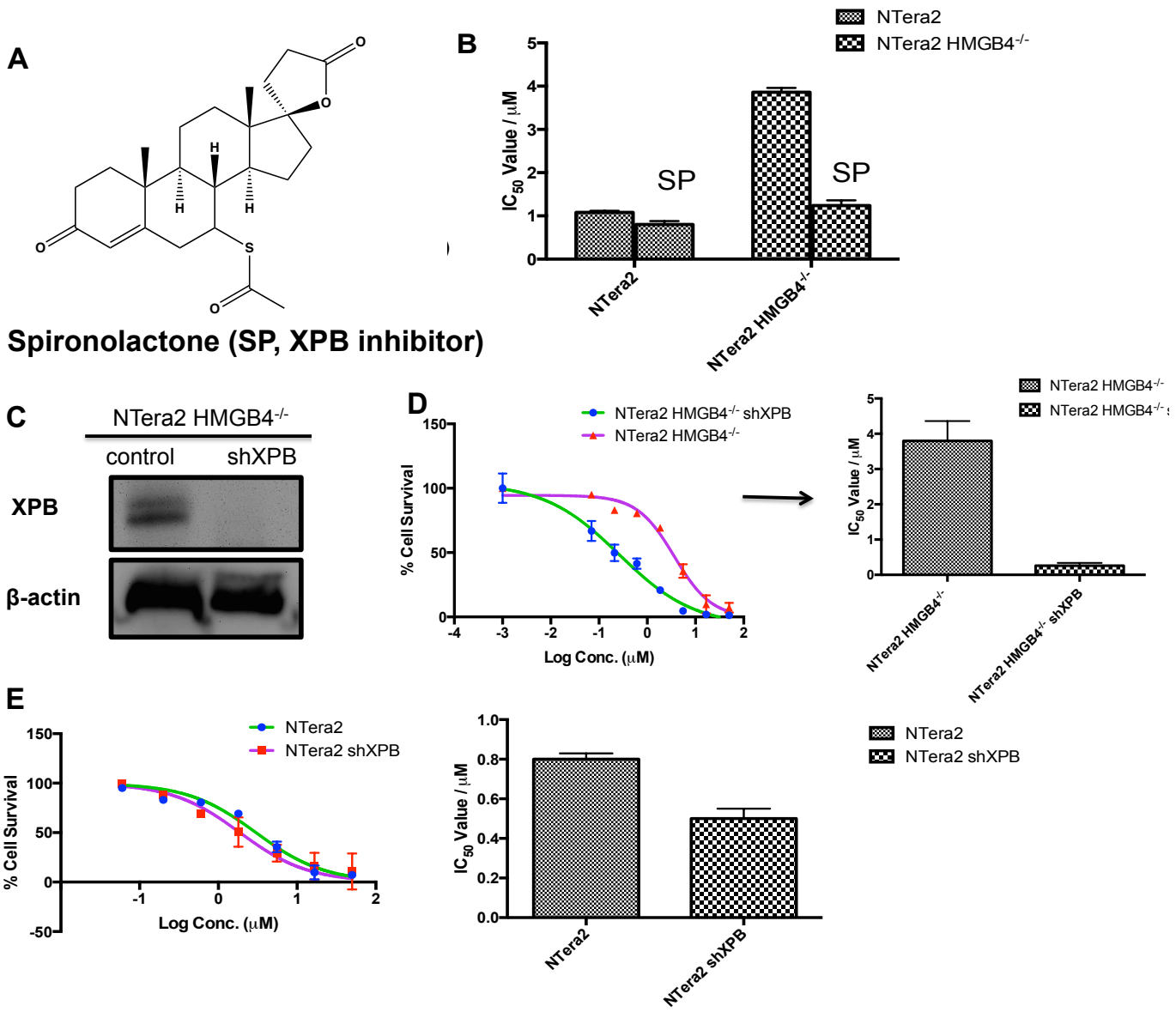


Figure S7. (**A**) Chemical structure of spironolactone, an inhibitor of XPB. (**B**) Quantification of IC_{50} values from dose response curves obtained by pretreatment of NTera2 or NTera2 HMGB4$^{-/-}$ cells with spironolactone (SP, 60 µM) followed by cisplatin in a dose dependent manner. (**C**) Evidence that shRNA targets and downregulates human XPB in NTera2 HMGB4$^{-/-}$. Western blot shows depletion of XPB protein after targeting XPB gene with shRNA. (**D**) Comparative cytotoxicity of NTera2 HMGB4$^{-/-}$ and NTera2 HMGB4$^{-/-}$shXPB treated with cisplatin. Dose response curves (left) and IC_{50} values (right). (**E**) Dose response curves of cisplatin treated NTera2 vs. NTera2 shXPB cells (left); quantification of IC_{50} values (right).

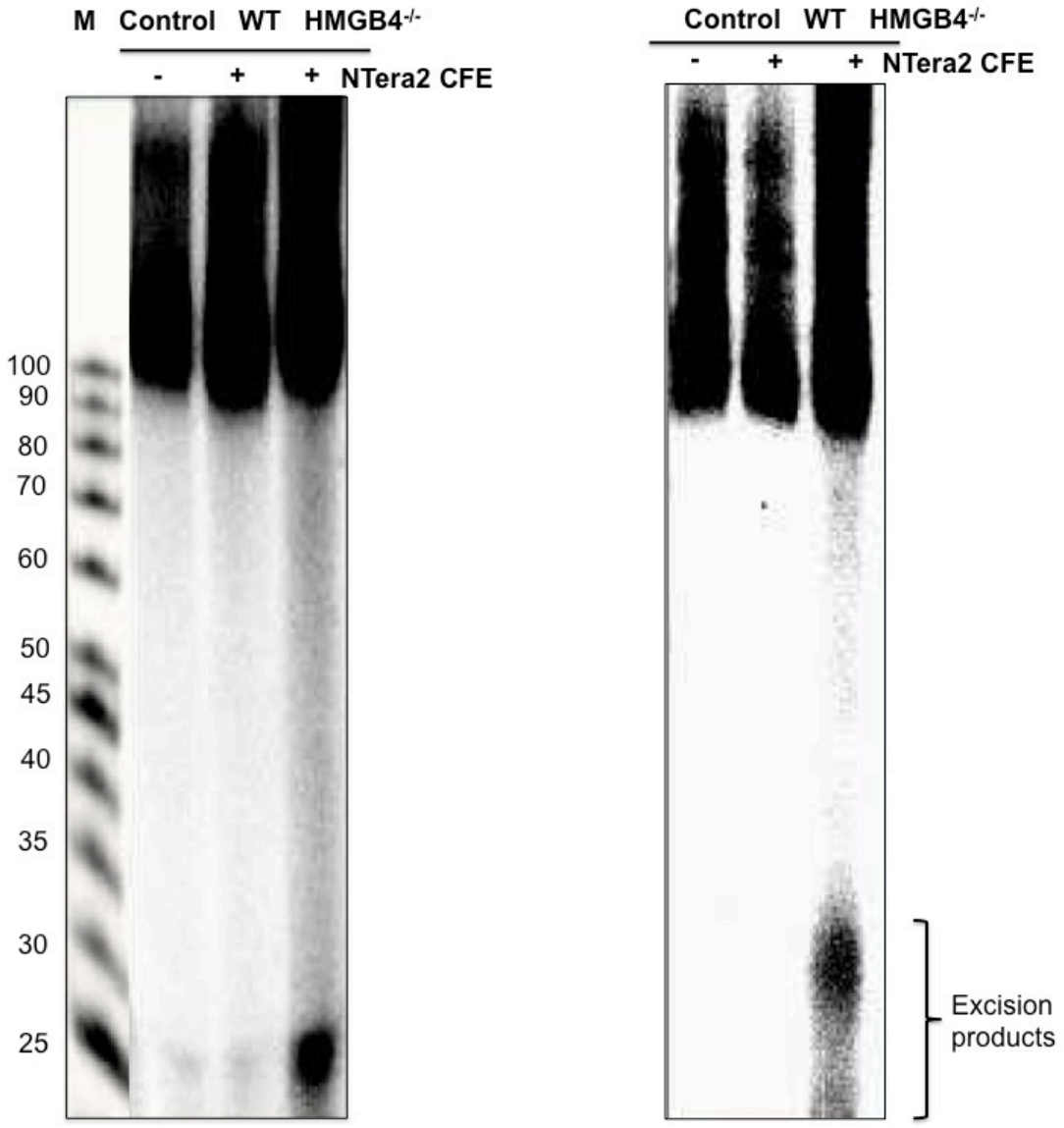


Figure S8. Excision of 1,2-d(GpG) cisplatin cross-links by excinuclease activity in testicular cancer cell extracts (two independent extracts). The substrate (146 bp, Pt-DNA probe) was incubated with CFEs from NTera2 and NTera2 HMGB4$^{-/-}$ cells. The reaction was run for 75 min and the excision products were resolved on 10 % (wt/vol) polyacrylamide denaturing gels (TE buffer, 55-46 mA for 5 h). The position of the main excision product, 25-30 nt, is indicated by a curly bracket.

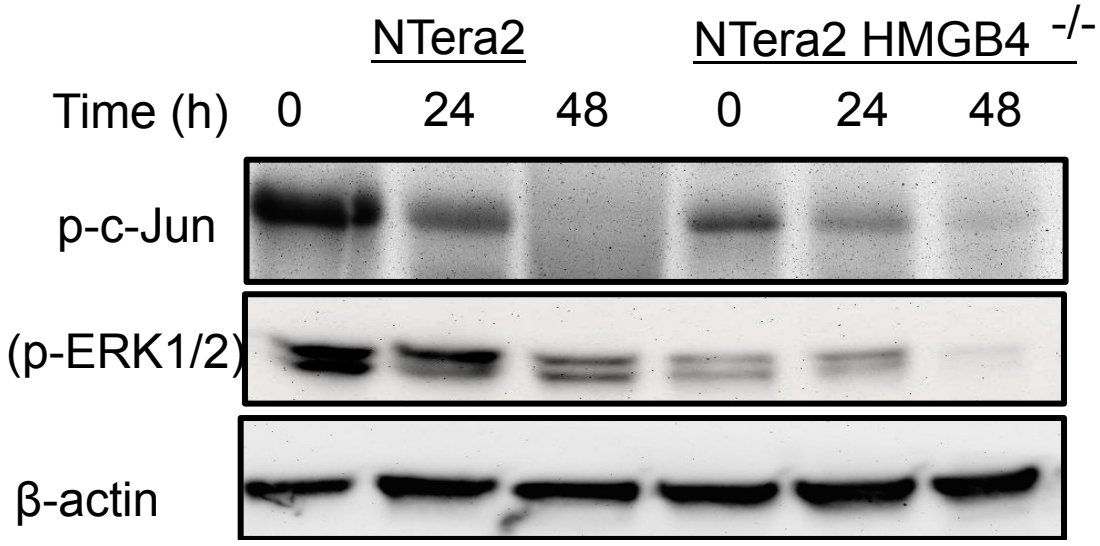


Figure S9. Western blot showing MAPK pathway protein expression in NTera2 and NTera2 HMGB4$^{-/-}$ cells following treatment with cisplatin (2 μM) at the indicated time points. Whole cell lysates were resolved by SDS-PAGE and analyzed by immunoblotting against p-c-Jun, p-ERK1/2, and β-actin (loading control).

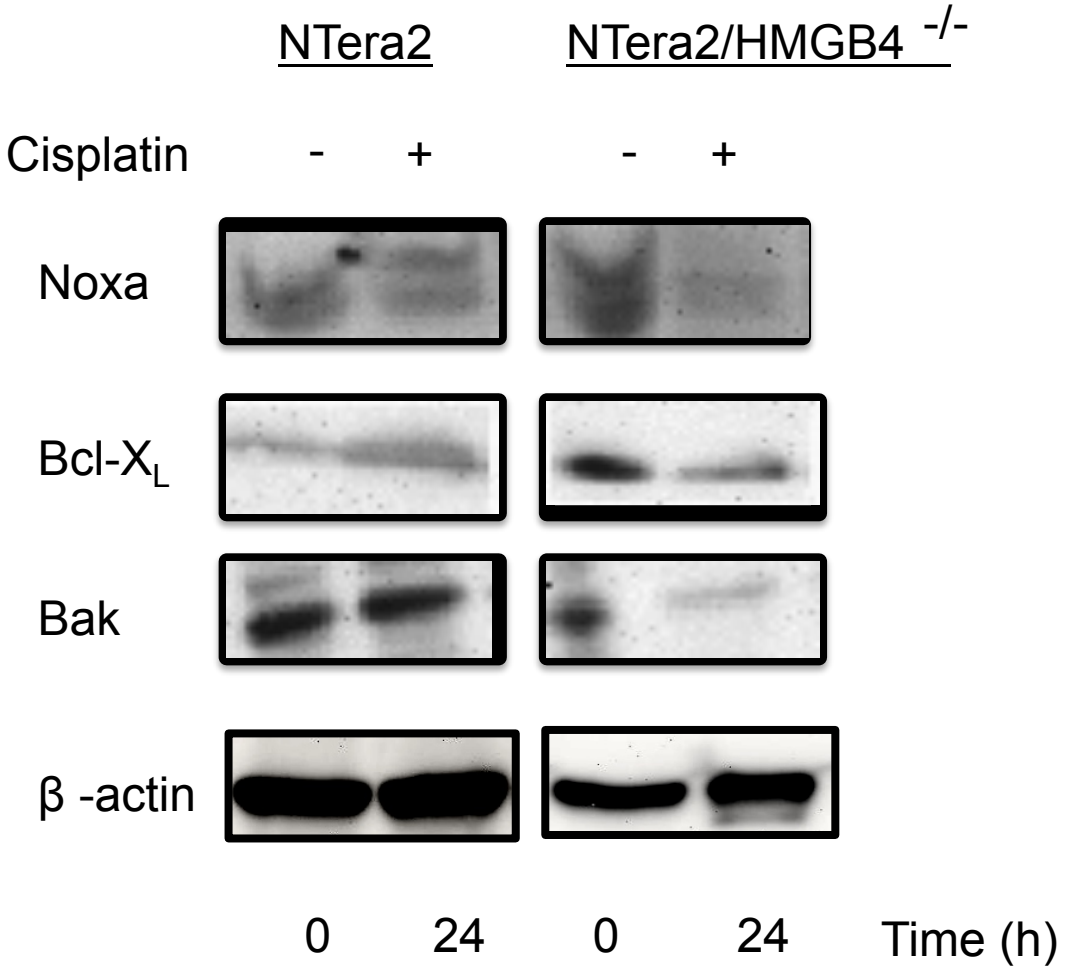


Figure S10. Western blots showing protein expression in NTera2 and NTera2 HMGB4$^{-/-}$ cells following treatment with cisplatin (2 μM) for 24 h. Whole cell lysates were resolved by SDS-PAGE and analyzed by immunoblotting against Noxa, Bcl-X_L, Bak, and β-actin (loading control).

Table S1. Primers for quantitative reverse transcriptase polymerase chain reactions.

Gene	Primer sequence
XPB (ERCC3)	Fwd: 5'-GATTGAGCCGGAAGTCCCC-3'; Rev: 5'- TGGATTTCTTCTTGTCGCGGT-3'
XPC	*Fwd: 5'-GCTGGGTGACGTGAGAGAAA-3';* *Rev: 5'-GGTGAACCTTGTGTGTGTCC-3'*
XPF	*Fwd: 5'-TCTGGATCCTTTGTGGCACC-3';* *Rev: 5'-AGCCAACCTGAATTCTGACCA-3'*
XPG	*Fwd: 5'-ACTGGAAAACCGTGGCTTCT-3';* *Rev: 5'-AATGCTAATATCAACAGCCAGGA-3'*
ERCC1	*Fwd: 5'-GGGATGAGAACGTAGACGCC-3';* *Rev: 5'ATAAGGGCTTGGCCACTCCAG-3'*
HMGB4	Fwd: 5'-*CCCCTCTTTCGGCTCACATT-3'; Rev: 5'-CCAACACCTGTGGGCATTTG-3'*
β-actin	*Fwd: 5'-ACAGGTAAGCCCTGGCTGCC-3';* *Rev: 5'-AGCACTGTGTTGGCGTACAG-3'*

References

1. Manley JL, Fire A, Cano A, Sharp PA, & Gefter ML (1980) DNA-dependent transcription of adenovirus genes in a soluble whole-cell extract. *Proc Natl Acad Sci U S A* 77(7):3855-3859.
2. Huang JC, Zamble DB, Reardon JT, Lippard SJ, & Sancar A (1994) HMG-domain proteins specifically inhibit the repair of the major DNA adduct of the anticancer drug cisplatin by human excision nuclease. *Proc Natl Acad Sci U S A* 91(22):10394-10398.
3. Reardon JT & Sancar A (2006) Purification and characterization of Escherichia coli and human nucleotide excision repair enzyme systems. *Methods Enzymol* 408:189-213.