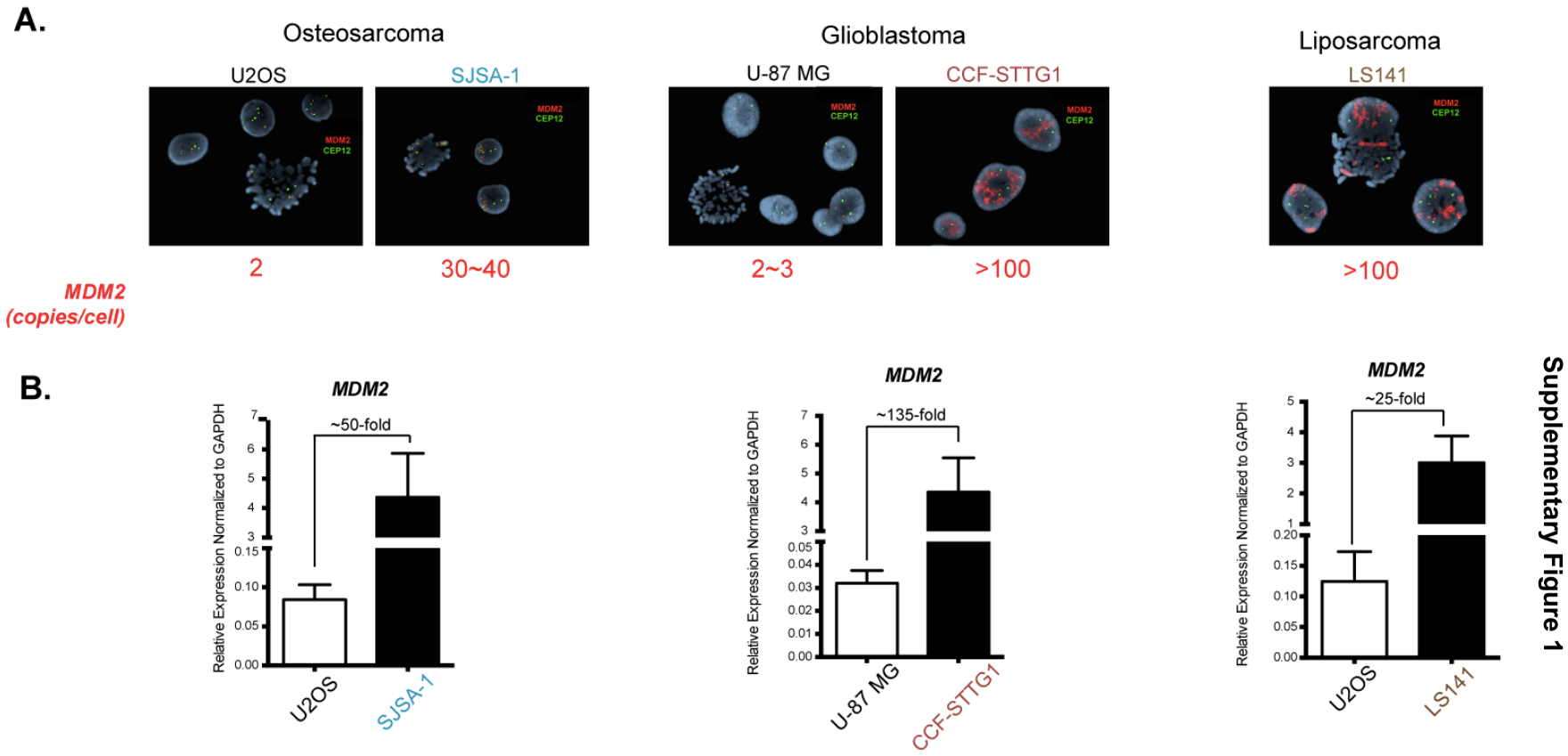
Supplementary Figure 1. Tumor lines with *MDM2* amplification overexpress Mdm2 mRNA and protein.

(A) *MDM2* gene copy number in cells was determined by fluorescence *in situ* hybridization. The *MDM2* gene (red) was probed and counted in 200 interphase cells per line. Centromere-associated probes (green) were used as a reference. Representative FISH images are shown.

(B) The expression levels of *MDM2* in U2OS, SJSA-1, U-87 MG, CCF-STTG1, and LS141 cells were determined by qPCR. Comparisons across relevant cell lines revealed that cells harboring *MDM2* gene amplification had significantly upregulated levels of *MDM2* transcripts ranging from ~25-135 fold, depending on cell type.

(C) Immunoblot analysis confirmed that Mdm2 protein was overexpressed in a manner roughly parallel to that of gene copy number and transcript level. From left to right across each lane subtending the indicated cell lines, 25, 50, and 100 μg of total protein was resolved by SDS-PAGE.



Supplementary Figure 1

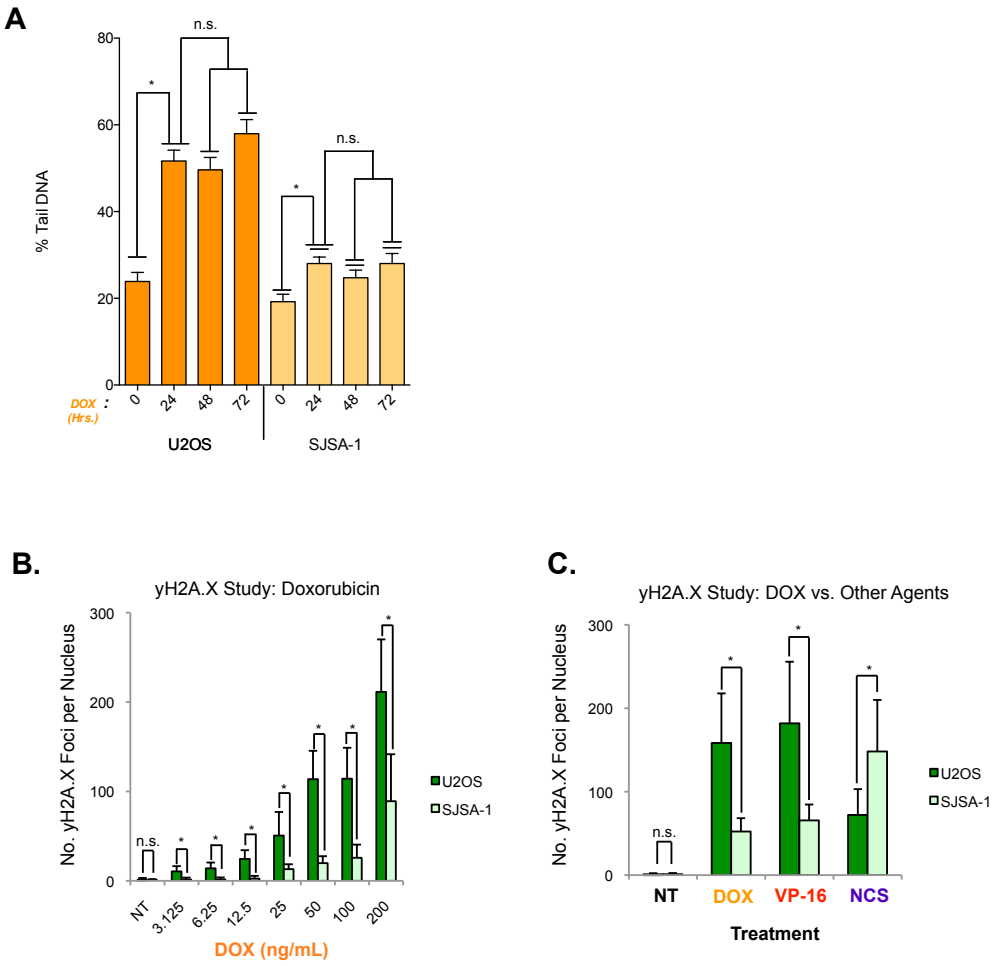
Supplementary Figure 2. The extent of double-strand DNA breaks induced by drug treatments is concordant between neutral comet assay and quantification of yH2A.x foci.

(A) Neutral comet assay quantification of double-strand DNA breaks arising from treatment with 100 ng/mL doxorubicin at 24, 48, and 72H. A representative study is shown. Data are mean ± S.E.M. n.s. = not significant.

(B) Quantification of yH2A.X foci arising in U2OS and SJSA-1 cells after treatment with 48H doxorubicin at the indicated doses. Foci from 50 individual nuclei were counted. A representative study is shown. Data are mean mean ± S.D. n.s. = not significant.

(C) Quantification of yH2A.x foci arising in U2OS and SJSA-1 cells after 48H treatment with 100 ng/mL doxorubicin, 3 μM etoposide or 200 ng/mL neocarzinostatin. Foci from 50 individual nuclei were counted. A representative study is shown. Data are ± S.D. n.s = not significant.

Supplementary Figure 2



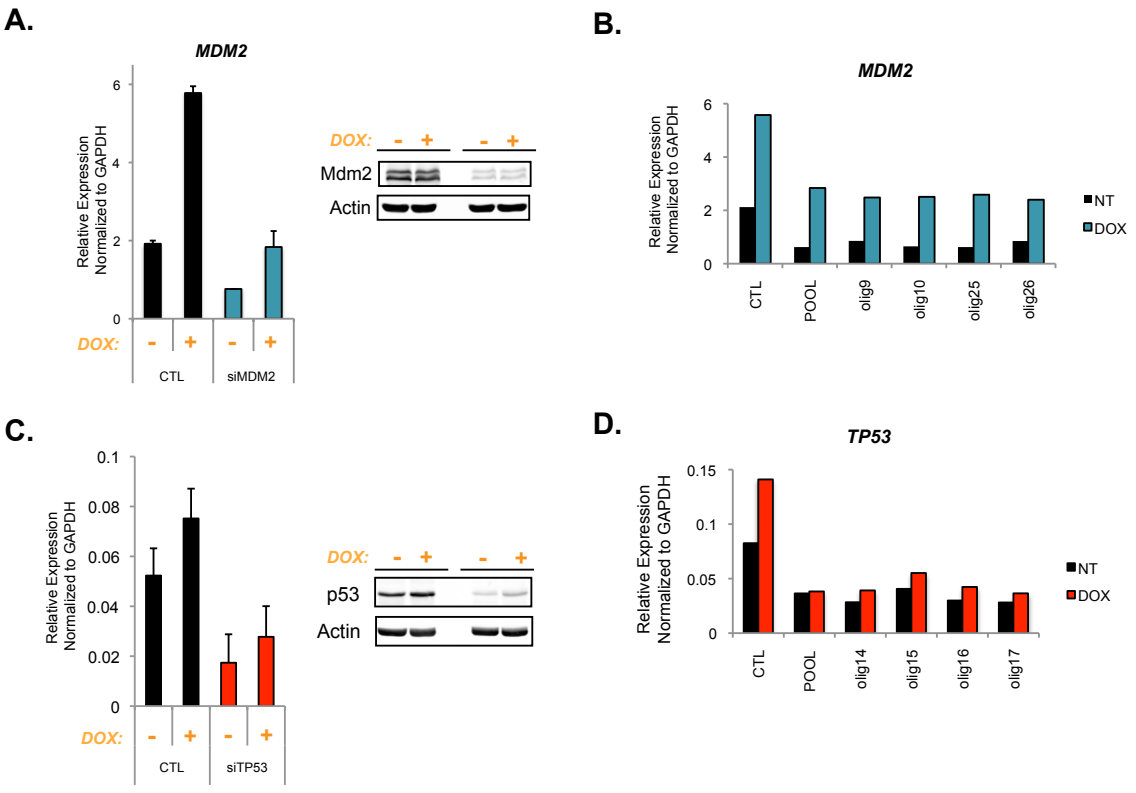
Supplementary Figure 3. Immunblot and qPCR interrogations of siRNA quality and individual oligonucleotide controls for knockdown of MDM2 and TP53 to address off-target effects

(A) qPCR (left) and immunoblot analysis (right) of *MDM2* knockdown in SJSA-1 cells in the absence of treatment and after 48H treatment with 100 ng/mL doxorubicin

(B) qPCR analysis of the extent of *MDM2* knockdown in SJSA-1 cells using pooled and individual siRNA oligonucleotides.

(C) qPCR (left) and immunoblot analysis (right) *TP53* knockdown in SJSA-1 cells in the absence of treatment and after 48H treatment with100 ng/mL doxorubicin.

(D) qPCR analysis of the extent of *TP53* knockdown in SJSA-1 cells using pooled and individual siRNA oligonucleotides.



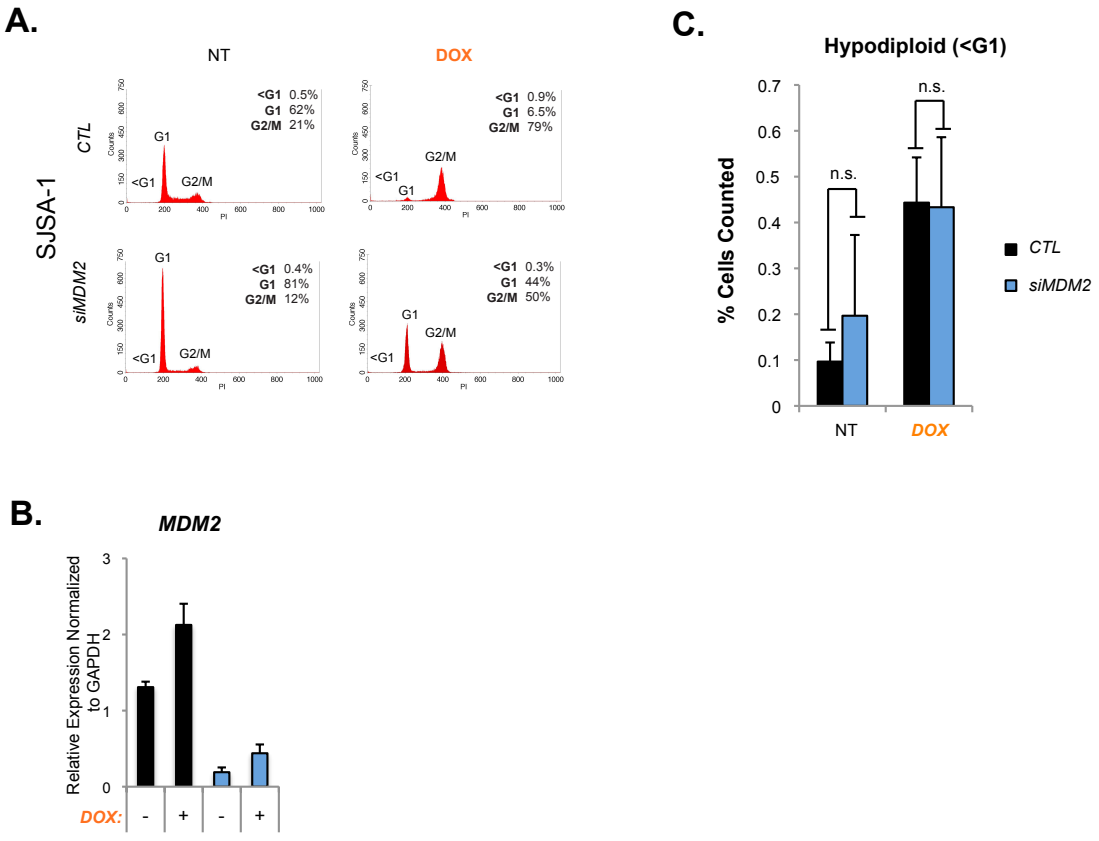
Supplementary Figure 4. Knockdown of MDM2 alone in tumor cells does not increase apoptosis compared to treatment with topoll inhibitors

(A) Flow cytometry analysis of cell cycle profiles obtained in SJSA-1 cells transfected with negative control siRNA (CTL) and treated with 100 ng/mL doxorubicin for 48H (CTL + doxorubicin) versus cells transfected with siRNA to *MDM2*. A representative experiment is shown.

(B) qPCR analysis of *MDM2* knockdown by siRNA in presence and absence of doxorubicin treatment corresponding to experiments described in (A). Data are mean of three experiments ± S.D.

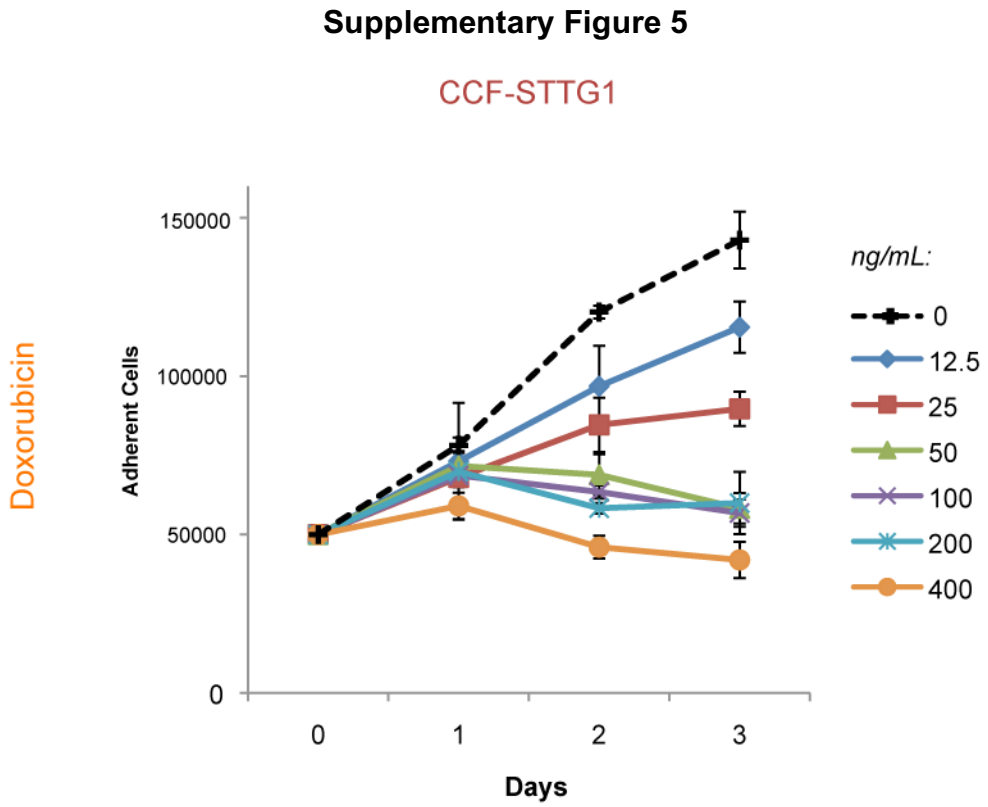
(C) Quantification of percent of hypodiploid cells counted in experiments described in (A). Data are mean of three experiments ± S.D. n.s. = not signification

Supplementary Figure 4



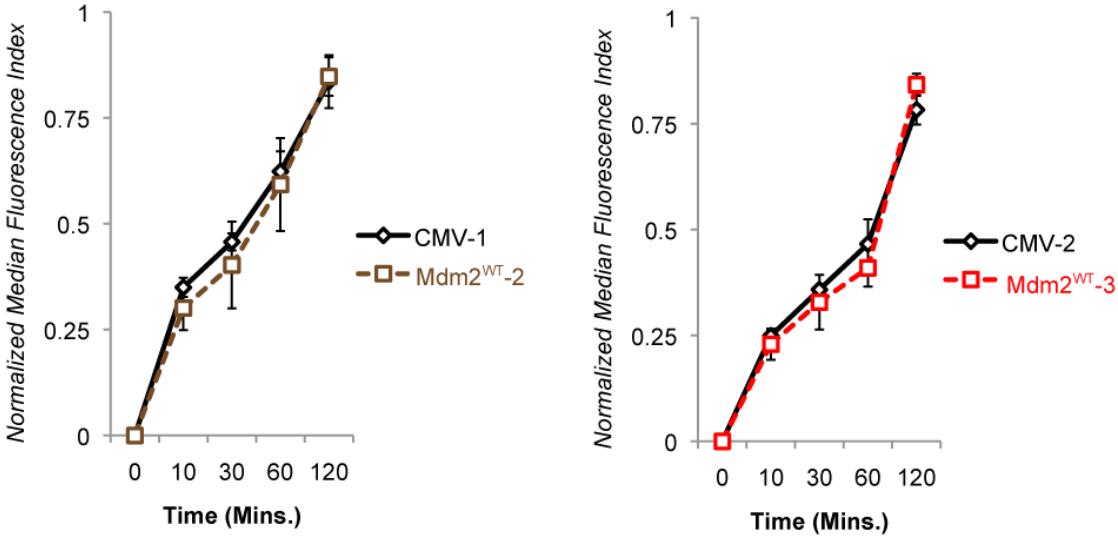
Supplementary Figure 5. Relevant doses of doxorubicinorubicin in CCF-STTG1 cells are revealed by proliferation assay. $5x10^4$ cells were seeded into medium containing the indicated doses of doxorubicin and allowed to incubate for up to 3 days. Adherent cells were counted each day beginning at day 1 post-seeding as described. Data are mean ± S.E.M.

Supplementary Figure 5



Supplementary Figure 6. Overexpression of Mdm2 in stable clones of U2OS cells does not affect doxorubicin uptake. Mdm2^{WT}-2 and Mdm2^{WT}-3 cells were seeded into medium containing 2 μg/mL doxorubicin and allowed to incubate at 37ºC for up to 120 minutes as described. Cells were harvested at the indicated timepoints and analyzed for intracellular doxorubicin accumulation by flow cytometry. NMFI was calculated and plotted against time. Data are mean ± S.E.M.

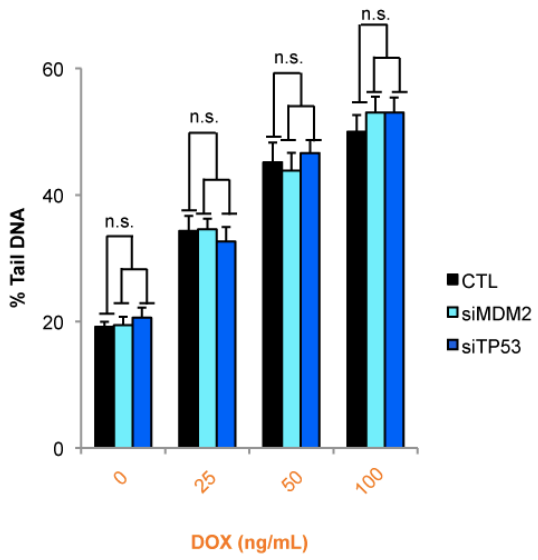
Supplementary Figure 6



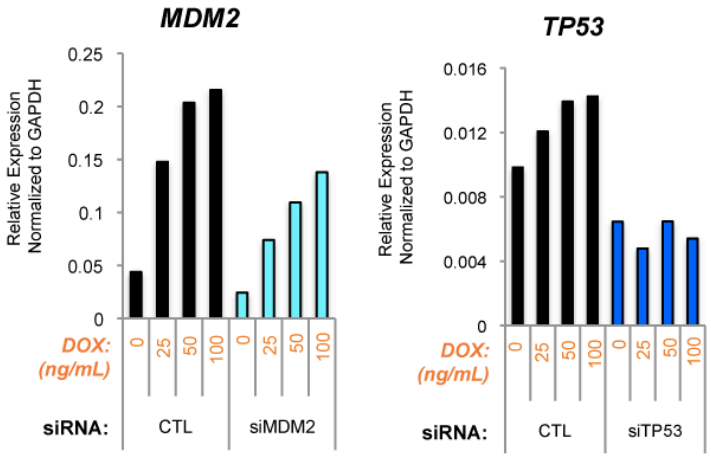
Supplementary Figure 7. p53 plays no role in the extent of DNA damage achieved by topoisomerase II inhibition. (A) U2OS cells were transfected with either control, *TP53-,* or *MDM2*-directed siRNA and treated with the indicated doses of doxorubicin for 48H. The extent of double-strand DNA breaks arising from this treatment was quantified by neutral comet assay. A representative study is shown. Data are mean ± S.E.M. n.s. = not significant. (B) Corresponding quantification of the extent of *MDM2* and *TP53* knockdown by qPCR.

Supplementary Figure 7

A.



B.

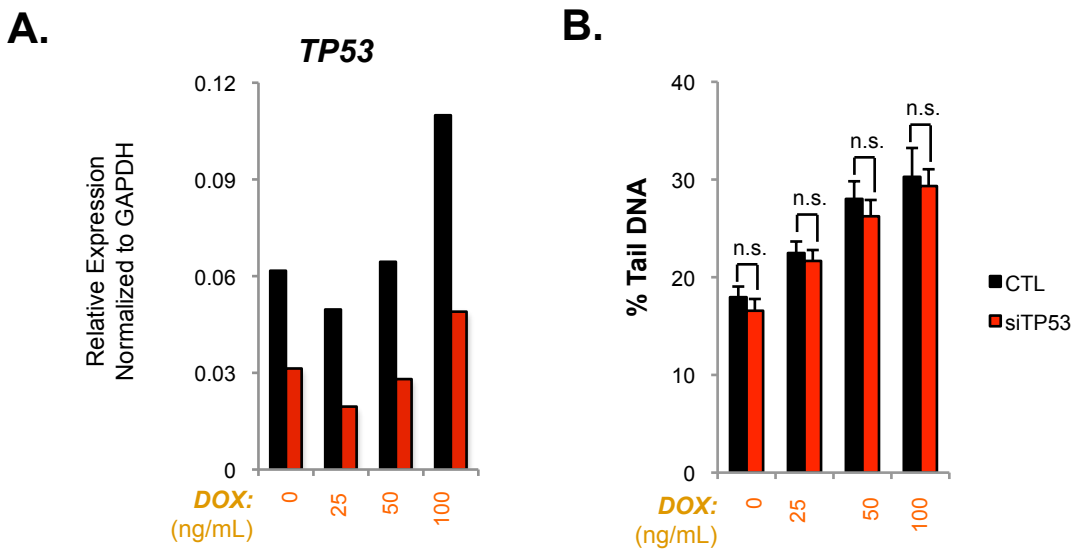


Supplementary Figure 8. Further reduction of p53 levels in SJSA-1 by siRNA to TP53 does not affect the extent of double strand DNA breaks induced by doxorubicin.

(A) SJSA-1 cells were transfected with either control of *TP53*-directed siRNA and treated with the indicated doses of doxorubicin for 48H. The extent of *TP53* knockdown was quantified by qPCR.

(B) Corresponding quantification of double-strand DNA breaks arising from doxorubicin treatment in either the control or *TP53*-knockdown setting by neutral comet assay. A representative experiment is shown. Data are mean ± S.E.M. n.s. = not significant.

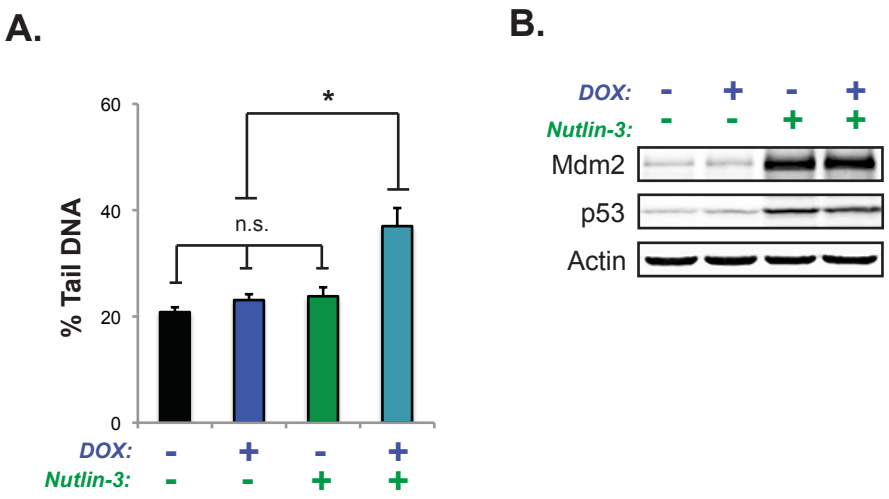
Supplementary Figure 8



Supplementary Figure 9. Co-treatment of SJSA-1 cells with Nutlin-3 and doxorubicin produces an increase in double-strand breaks.

(A) SJSA-1 cells were treated with 25 ng/mL doxorubicin, 10 μg Nutlin-3, or both for 48 hours. The extent of double-strand DNA breaks was assessed by Neutral Comet Assay. Data are mean ± S.E.M. n.s. = not significant. * = $p<0.05$.

(B) Immunoblot corresponding to experiment in (A) showing levels of Mdm2 and p53 at baseline and following each of the treatments described.



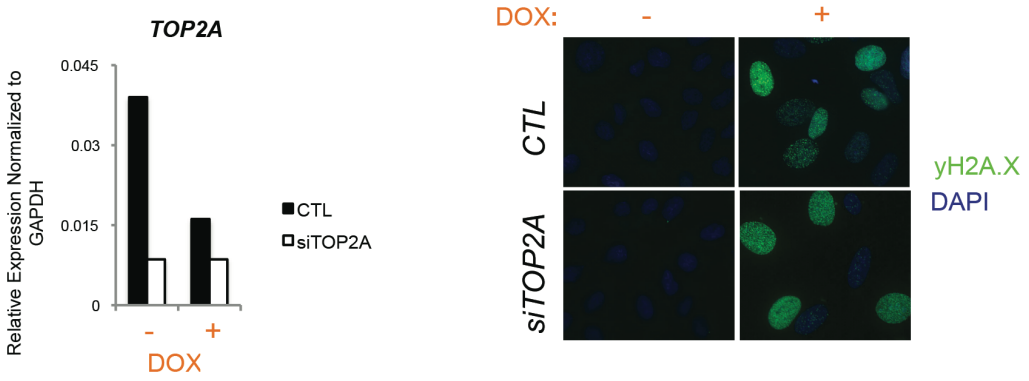
Supplemental Figure 10. DNA damage with topoisomerase II poisons is not dependent on topoisomerase IIα levels.

(A) The effect of knockdown of *TOP2A* on the ability of doxorubicin to induce DNA double-stranded breaks was studied using a siRNA approach in U2OS cells. *Left*: The extent of *TOP2A* knockdown by siRNA in the untreated and doxorubicin-treated setting was quantified by qPCR. *Right*: DNA double-stranded breaks secondary to doxorubicin treatment (100 ng/mL, 48H) in the control- or *TOP2A*-directed siRNA settings were assessed by immunofluorescent staining for phospho-γH2A.X. A representative experiment is shown. (B) The experiment described in (A) was carried out in SJSA-1 cells. A representative experiment is shown. (C) Immunoblot analysis comparing the levels of topoisomerase IIα in U2OS and SJSA-1 cells in both the basal setting and during sustained doxorubicin treatment (100 ng/mL).

Supplemental Figure 10

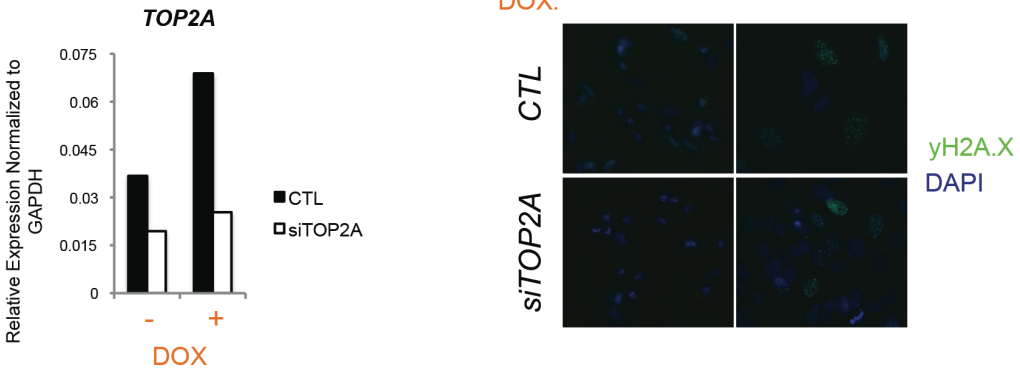
A.

U2OS



B.

SJSA-1



C.