Supplemental Methods

Irradiation

Radiation to cells was delivered using an XRAD 320 (Precision X-Ray Inc., N. Branford, CT) at a dose rate of 2.21 Gy/min. Radiation to mice was delivered with a Therapix DXT300 x-ray irradiator (Pantak, Inc., East Haven, CT) using 2.0 mm Al filtration (300 kV peak) at a dose rate of 1.9 Gy/min. Mice were immobilized in a custom jig that allows selective irradiation of the tumor bearing leg with shielding of the remainder of the animal. Dosimetry was performed on an annual basis using plexiglass mouse phantoms and the customized jig with embedded thermoluminescent dosimeters (three per phantom).

Gene expression analysis (supplemental)

Normalized gene expression was derived by the $2^{-\Delta\Delta C_T}$ method using the Qiagen data analysis center (Qiagen). Gene expression values were normalized by the geometric mean expression of the reference genes β-2-microglobulin, glyceraldehyde-3-phosphate dehydrogenase, and the large ribosomal protein P0. Fold change was calculated as the normalized gene expression in the MTA treated samples ($2^{-\Delta\Delta C_T}$) divided by the normalized gene expression in the vehicle treated samples ($2^{-\Delta\Delta C_T}$). Differences in gene expression were considered significant when fold regulation was ≥ 1.5 and ≤ -1.5 and $p \leq 0.05$ by paired T-test.

Cell cycle and ploidy analysis (supplemental)

Single cell suspensions were washed with PBS, fixed with 70% ethanol, and permeabilized with 0.4% Triton X (Sigma-Aldrich). Cells were incubated with anti-phospho-serine-10histone H3 antibody (Millipore, Billerica, MA) overnight, washed, and then incubated

with donkey anti-rabbit IgG conjugated to CruzFluor™ 488 (Santa Cruz Biotechnology, Dallas, TX) followed by propidium iodide (PI, Sigma-Aldrich) in PBS with 0.1% BSA and 0.5 mg/mL RNase A. DNA content and mitotic fraction were determined from duplicate experiments using a FACSCaliber cytometer (BD Biosciences, San Jose, CA) and FlowJo software (Tree Star, Inc., Ashland, OR). For polyploid analysis cells were treated as described above. Cellular DNA was stained with FxCycle Violet (Life Technologies). DNA content was determined using a FACSCanto II cytometer and FlowJo software.

γH2AX immunofluorescence (supplemental)

Cells were fixed in 2% paraformaldehyde and permeabilized with 1% Triton X-100. Following blocking with 1% BSA in PBS, cells were incubated in anti-γH2AX antibody (1:500, Millipore Corp.), washed, and then incubated with a fluorescein isothiocyanate (FITC) conjugated secondary antibody (Jackson ImmunoResearch, West Grove, PA). Nuclei were stained with 4', 6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich). Slides were mounted with VectaShield reagent (Vector Laboratories, Burlingame, CA). Slides were imaged using a Leica DM LB2 fluorescent microscope and digital images were captured with QCapture (QImaging, BC, Canada). The number of γH2AX foci per cell were manually scored in 150 cells per condition.

Mitotic catastrophe (supplemental)

Cells were fixed in ice cold methanol at -20°C for 15 min, and permeabilized with 0.5% Triton X-100. Cells were incubated with anti-α-tubulin antibody (Sigma-Aldrich) followed by a FITC-conjugated secondary antibody (Invitrogen, Carlsbad, CA). Nuclei were counterstained

with DAPI. The presence of fragmented nuclei and/or the presence of two or more distinct nuclear lobes in a single cell were scored in 150 cells per condition.

Tumor xenograft model (supplemental)

All animal procedures were institutionally approved and deemed in accordance with the guidelines of the Institute of Laboratory Animal Resources, National Research Council. Animals were fed a diet of chow and water *ad libitum*. A549 or UM-UC-3 tumor cells ($1x10^6$) were injected subcutaneously into the hind leg of 9-week-old female athymic nude mice (NCI Frederick, Frederick, MD). Tumor volumes, calculated by the formula [volume = ($width^2$ x length)/2], were measured three times weekly.

At a mean tumor volume of 172 mm^3, mice ($n \geq 8$ per treatment) were randomized to vehicle alone, MTA alone, vehicle with IR, or MTA with IR. Radiation was delivered with a Therapix DXT300 x-ray irradiator (Pantak, Inc., East Haven, CT) using 2.0 mm Al filtration (300 kV peak) at a dose of 2.92 Gy/min. Mice received a single intraperitoneal injection of MTA (1 mg/kg) or vehicle (100μl, PBS) 1 h prior to ionizing radiation (IR). For multi-fraction radiation, mice received MTA (1mg/kg) or vehicle 1 h prior to the first and third fraction of IR (4 daily fractions of 2 Gy).

Tumor growth to 1000 mm^3 was scored for each individual animal. The mean growth delay for each treatment group was calculated as the mean of the number of days for treated tumors to grow to 1000 mm^3 minus the mean of the number of days for the vehicle group to reach the same size. Standard deviations were calculated about the mean of the treated groups. Normalized tumor growth delay was defined as the time in days for tumors to grow from 172 to 1,000 mm^3 in mice exposed to combined modality minus the time in days for tumors to grow to

1,000 mm^3 in mice treated with MTA alone. The DEF was calculated as the ratio of the normalized tumor growth delay in mice treated with MTA + IR and absolute growth delay in mice treated with IR alone.

Supplemental Results

Supplemental Table: Plating efficiency with MTA or SP1 siRNA treatment

		Plating efficiency	Standard error
A549	Vehicle	0.73	0.03
	25 nM MTA	0.38	0.03
	50 nM MTA	0.21	0.06
	Control siRNA	0.66	0.08
	SP1 siRNA	0.26	0.02
UMUC3	Vehicle	0.83	0.06
	25 nM MTA	0.57	0.12
	50 nM MTA	0.15	0.03
	Control siRNA	0.85	0.03
	SP1 siRNA	0.07	0.01
BJ	Vehicle	0.159	0.03
	50 nM MTA	0.017	0.006
	Control siRNA	0.06	<0.01
	SP1 siRNA	0.03	<0.01

MTA: mithramycin-A