Supplemental Materials and Methods

Cell culture- LNCaP and C4-2 cells were maintained in improved minimum essential media (IMEM) supplemented with 5% ΔFBS (heat-inactivated FBS). LAPC4 cells were maintained in Iscove's modified Dulbecco's medium supplemented with 10% ΔFBS. 22Rv1 and PC3 cells were maintained in Dulbecco's modified Eagle's media supplemented with 10% ΔFBS. All media were supplemented with 2 mmol/L of L-glutamine and 100 units/mL penicillin-streptomycin. For hormone deficient conditions, media used was appropriate phenol red-free media supplemented with 5 or 10% charcoal dextran-treated serum (CDT). DHT was obtained from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in ethanol. N-acetylcysteine was obtained from Sigma-Aldrich, dissolved in water, and applied 1 hour prior to any additional treatments. MDV3100 and Nu7441 were obtained from Selleckchem (Houston, TX, USA) and were dissolved in DMSO. Ionizing radiation (IR) was delivered by a Panatek orthovoltage X-ray irradiator calibrated daily using a Victoreen dosimeter. UV was delivered by a Stratalinker UV Crosslinker. All experiments were seeded onto poly-L-lysine coated plates and allowed to adhere for 24 hours, at which point media was changed and cells treated as indicated.

Xenograft treatment- Mice assigned to the castration group were surgically castrated on the first day of treatment (day 1). Mice assigned to the IR group were treated with 2 consecutive daily doses of 2 Gy IR (day 1, 2: 4 Gy total IR). Mice assigned to the combination castration & IR group were surgically castrated on the first day of treatment (day 1), allowed 48 hours to recover, and then treated with 2 consecutive daily doses of 2 Gy IR (day 4, 5).

Antibodies used in immunfluorescence, ChIP, and immunoblot assays- Antibodies used for immunofluorescence were as follows: phosho-Histone H2A.X (Ser 139) (Millipore, #05-636), 53BP1 (Novus Biologicals, #100-304), and Rad51 (GeneTex, #70230). Antibody used for ChIP assays were as follows: AR (N-20, directed against amino acids 1-20 by Bethyl Laboratories, and validated by immunoblot, IP, IHC, and ChIP to perform identically to commercial antisera directed against this epitope). Antibodies used for immunoblots were as follows: DNA-PKcs (Ab-4, cocktail) (Thermo Scientific, #423-P0), DNA-PKcs (phospho S2056) (Abcam, #18192), Ku70 (Thermo Scientific, #329-P0),

ATM (Cell Signaling Technology, #2873), phospho-ATM (Ser1981) (Cell Signaling Technology, #5883S), Ran (BD Biosciences, #610340), and Lamin B (Santa Cruz, #6217).

Comet assay- Briefly, treated or untreated cells were collected, suspended in ice cold PBS at10^5 cells/ml, mixed with molten LMAgarose (1:10 ratio) and spread on CometSlide. After the agarose solidified, the slides were successively placed in lysis solutions (Trevigen) and then subjected to TAE electrophoresis. Cells were fixed with 70% ethanol and stained with SYBR Green. Comet tail moments were then assessed using COMETscore.v1.5 (AutoCOMET.com, Sumerduck, VA) image processing software as described by the manufacturer. Data is reported as tail moments, which assesses the fluorescence intensity in the tail relative to the head while accounting for the relative area of both dipoles. Quantification of the tail moments from each condition was calculated from a minimum of 100 cells for each data point.

Clonogenic survival analysis- Cells were seeded at low densities in hormone proficient or hormone deficient media and treated as described. After 14 days cells were fixed in ice-cold 100% ethanol and stained with crystal violet. Colonies with >50 cells were counted and set relative to the calculated plating efficiency for each treatment condition.