Supplementary Materials and Methods

Subcellular fractionation and Western blotting. Nuclear and cytoplasmic fractions were prepared using the NE-PER® Nuclear and Cytoplasmic Extraction reagents (Thermo Scientific) as per manufacturer's instruction. Protein concentration was quantitated using the BCA protein assay kit (Pierce). Equal amounts of protein were loaded in each lane and separated on a 4-12% Bis-Tris NuPAGE® gel (Invitrogen), then transferred onto a nitrocellulose membrane. Membranes were probed with the following primary antibodies: mouse anti-γH2AX (phosphoS139) (Abcam), rabbit anti-RAD51 (Abcam), rabbit anti-BRCA1 (Cell Signaling Technology), mouse anti-Lamin A/C (Cell Signaling Technology), mouse anti-α-tubulin (Sigma-Aldrich). The blots were incubated with either horseradish peroxidase (HRP)-labeled secondary antibodies: goat anti-rabbit-HRP or goat anti-mouse-HRP (Bio-Rad); or infrared secondary antibodies: IRDye 800CW goat anti-rabbit IgG or IRDye 680RD goat anti-mouse IgG (LI-COR Biosciences). HRP antibody target proteins were detected by incubating with Supersignal West Pico chemiluminescent substrate and exposing the blot to x-ray film. Protein bands were quantitated with Image J software (NIH, Bethseda, MD). Infrared signals were detected using the Odyssey CLx infrared imaging system and bands quantified using Image Studio software (LI-COR Biosciences).

Confocal Microscopy and foci analysis. Image acquisition was performed with a Nikon A1R+ confocal imaging system (Nikon Instruments Inc., Melville, NY) using a galvano scanner and a Nikon CFI Apochromat TIRF 60x/1.49 oil objective lens. DAPI and TRITC were excited with a 405 nm and 561 nm solid-state lasers respectively, and fluorescence emissions were collected using 425-475 nm and 570-620 bandpass filters, respectively. TRITC emissions were collected using a GaAsP detector that has higher sensitivity. Images were acquired from randomly selected fields as a z-

stack with step size of 0.5 μm (DU145) or 0.4 μm (RWPE1) to give a total of 25 slices. For each selected field of view, two images were taken, a DAPI image and a γH2AX image. A minimum of 50 cells were imaged per sample. All images were acquired under the same laser power, camera gain and offsets settings and at the scale factor of 0.21 μM/pixel. Images were saved as ND2 files and maximum intensity projection image were obtained from the z-stacks. Image processing and foci analysis were performed using Fiji software (19). Briefly, the DAPI image was thresholded to obtain a binary nuclei mask. Binary γH2AX foci image were obtained by thresholding the corresponding γH2AX maximum intensity projection image and applying a watershed algorithm to separate foci that were touching. Minimum threshold values were chosen that produced a processed image that is visually similar to the original image. The nuclei mask was applied to the γH2AX foci image to demarcate the foci signals of each individual nucleus. Particle analysis was performed for each individual nucleus with minimum particle size of 0.1 μm^2 to be included. Nucleus with $\geq$ 10 foci was considered positive for DNA damage. For visualization, a standardized adjustment of brightness and contrast was applied to the whole image using Photoshop post-analysis.

PARP assay. PARP activity was assessed using the HT PARP in vivo Pharmacodynamic Assay II kit (Trevigen) which measures net PAR levels in cells extracts. Briefly, cells were treated and extracts prepared using cell lysis buffer supplemented with PMSF and protease inhibitor. Cell extracts are then incubated overnight at 4°C with PAR monoclonal antibody immobilized onto 96-wells plate to capture cellular PAR. A serial dilution of PAR (1000 – 10 pg/ml) was included to generate a PAR standard curve. An anti-PAR polyclonal rabbit antibody was added to the wells for 2 h at 22°C as the detecting antibody followed by incubation with a goat anti-rabbit IgG-HRP secondary antibody for 1 h at 22°C. A chemiluminescent HRP substrate was added and

RLU was measured immediately on a microplate reader (BioTek). PAR levels in the cell extracts were determine using the PAR standard curve and expressed as pg/mg of protein extract.

Histone acetylation assay. Histone proteins were extracted from treated cells using EpiSeeker Histone Extraction kit (Abcam) as per the manufacturer's instruction. Equal amount of protein per sample were separated on a 12% Bis-Tris NuPAGE® gel and transferred onto a nitrocellulose membrane. Membranes were probed with rabbit polyclonal Histone H4ac (pan-acetyl) antibody (Active Motif) and mouse monoclonal Histone H4 antibody (Cell Signaling). Following incubation with the appropriate infrared secondary antibodies, histone proteins were imaged using Odyssey CLx imaging system to detect for acetylation of histone H4.

Comet assay. Alkaline comet assay were performed using the CometAssay® Reagent Kit (Trevigen). Cells were harvested and approximately 1 x 10^3 cells were immobilized in low melting point agarose onto pre-coated Trevigen CometSlide™. Following cell lysis at 4°C for 1 h, slides are immersed in alkaline unwinding solution, pH>13 (200 mM NaOH, 1 mM EDTA) for 20 min at RT in the dark to unwind and denature the DNA. The slides are subjected to electrophoresis in alkaline electrophoresis solution, pH>13 at 4°C for 30 min at constant voltage of 21V. Slides were dried and DNA stained with SYBR® Gold for visualization using an epifluorescent microscope. % Tail DNA of 100 cells per sample was scored at random using the Cometscore Pro (TriTek Corp.) software.