Supplementary Data, Efimova et al.

Supplementary Methods

Cell lines Following selection in the presence of G418 and puromycin and flow cytometry sorting, the stable MCF7$^{Tet\text{-}On}$ GFP-IBD cell line was established. The third passage after sorting, corresponding to the sixth passage after obtaining the MCF7 Tet-On Advanced® cell line, was frozen in liquid nitrogen as a stock. For all experiments, MCF7$^{Tet\text{-}On}$ GFP-IBD cells were passaged no more than 8-10 weeks, after which time they were discarded and renewed from the frozen stock.

shRNA knockdowns. Sigma MISSION shRNA targeting and non-targeting control plasmids were used according to manufacturer's instructions. Lentivirus-containing supernatant was produced using HEK293T cells and applied three times to MCF7$^{Tet\text{-}On}$ GFP-IBD cells. 24 h post-infection, cells were seeded onto glass bottom Fluoro Dishes (World Precision Instruments) and grown for 2 d in 1 μg/ml doxycycline. At least 2 shRNA constructs targeting different sequences of corresponding mRNA were evaluated for each gene.

qPCR gene expression analysis. qPCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems). PCR primers were as follows: hsCDKN1A-F: GGACAGCAGAGGAAGACC, hsCDKN1A-R: TGGTAGAAATCTGTCATGCTG, hsGAPDH-F: TGCACCACCAACTGCTTAGC, hsGAPDH-R: GGCATGGACTGTGGTCATGAG.

BrdU incorporation, clonogenic assay, PI staining. BrdU incorporation was carried out using the FITC-BrdU Flow Kit (BD Biosciences) as per manufacturer's instructions. Clonogenic assays, PI staining and crystal violet staining were performed according to

standard protocols.

***In vitro* PARP activity assay.** Nuclear extract (500 ng) from MCF7$^{Tet\text{-}On}$ GFP-IBD cells treated with IR were incubated with 1 μM ^{32}P-NAD^{+} in a reaction mixture containing 10 mM Tris-HCl pH 8.0, 15 mM KCl, 7 mM $MgCl_2$, 6% glycerol, 1 mM DTT and 1 mM PMSF in a volume of 20 μl. The reaction was incubated at 30° C for 30 min and was stopped by the addition of SDS-PAGE sample buffer. The proteins were separated on SDS-PAGE, analyzed by autoradiography, and quantified by phosphoimaging.

Quantification of foci number and size. Images were acquired using an Olympus DSU spinning disk confocal microscope with DIC and back-thinned EMCCD camera with Slidebook v4.2. Image stacks through entire nuclei in ten random fields of cells were imaged using 0.33 μm z-steps with a 100X Planapo TIRFM 1.45 NA objective. Intensification and exposure time were kept constant for all time points (intensification = 127, exposure time = 600 msec) except for 24 h time points that required a shorter exposure time (500 msec) due to the presence of large IRIF. MCF7$^{Tet\text{-}On}$ GFP-IBD cells were irradiated using a Nordion Gammacell ^{67}Co irradiator operating at a dose rate of 1 Gy/min.

ImageJ and plug-in 3D Objects Counter were used to quantify IRIF size, number, and intensity. Foci smaller than 15 voxels (0.12 μm^3) or larger than 10,000 voxels (>85 μm^3) were excluded from analysis as they were likely noise or merged foci. ≥30 nuclei were analyzed for each time point and results were averaged to determine the mean size, number, and intensity of IRIF. Results are reported as mean ± SEM. Significance was determined by Student's t-test.

Immunofluorescence. For immunofluorescence, antibodies used were against phospho-H2AX S139 (Millipore), MDC1, $p21^{Cip1/WAF1}$ (Abcam), and 53BP1 (Novus Biologicals), detected by Texas Red goat-anti-rabbit IgG (Vector Laboratories) and Alexa Fluor 594 goat-anti-mouse IgG (Invitrogen). Images were captured on a Nikon Eclipse E800 microscope using a Nikon DXM1200C digital color camera. For confocal colocalization studies images were acquired using 0.4 μm z-steps with a 60X objective through entire nuclei on an Olympus DSU spinning disk confocal microscope and back-thinned EMCCD camera controlled by Slidebook v4.2 software. Selected image slices through the middle of cell nuclei from each z-stack were interrogated for colocalization analysis. Pearson's correlation and Overlap coefficient were determined using ImageJ software.

SA-ß-Gal assay. The SA ß-Gal assay was performed using the Senescence beta-Galactosidase Staining Kit (Cell Signaling). Images were captured on a Zeiss Axiovert 200M and Zeiss Axiocam color digital camera controlled by OpenLab software with a 20x objective.

Supplementary Figure Legends

Supplementary Figure 1. Diagram of 53BP1 IRIF binding domain reporter which includes a glycine-arginine rich (RG) motif, tandem Tudor (T) domains, a nuclear localization sequence (NLS), and two BRCT domains. The GFP-IBD construct incorporates a tetracycline-responsive element (TRE), green fluorescent protein (GFP) and the 53BP1 RG motif, tandem Tudor domains, and NLS.

Supplementary Figure 2. Confocal colocalization of GFP-IBD with endogenous, full-length 53BP1, γH2AX and MDC1 at IRIF in cells treated with 6 Gy in 3h after IR. Nuclei indicated by DAPI staining (blue). Scale bar, 10μm. Pearson's correlation and Overlap coefficient are, respectively, 0.91 and 0.94 for GFP-IBD compared to endogenous 53BP1, 0.77 and 0.84 for GFP-IBD compared to γH2AX and 0.76 and 0.87 for GFP-IBD compared to MDC1.

Supplementary Figure 3. Ionizing radiation-induced PARP activity in $MCF7^{Tet\text{-}On}$ GFP-IBD cells is decreased by ABT-888 treatment. Cells were treated with 5 μM ABT-888 for 30 min, exposed to 6 Gy and allowed to recover for the indicated time. Nuclear extracts from treated cells were incubated with 1 μM ^{32}P-NAD^+ at 30° C and reactions were resolved by PAGE. Incorporation of [^{32}P]ADP-ribose was assessed by PhosphorImager analysis.

Supplementary Figure 4. ABT-888 prevent**s** resolution of IRIF. $MCF7^{Tet\text{-}On}$ GFP-IBD cells were treated with 6 Gy (③, circles), or 6 Gy + ABT-888 (⑤, squares) and recorded

by time-lapse live-cell imaging. Foci number was determined at different time points by automated image analysis. Mean ±SEM.

Supplementary Figure 5. ABT-888 affects the size of IRIF. MCF7$^{Tet\text{-}On}$ GFP-IBD cells were treated with 6 Gy ± ABT-888 and IRIF size was determined at 1, 3, 6 and 24 h (mean ± SD, IR, white bars; IR + ABT-888, grey bars).

Supplementary Figure 6. IR + ABT-888 increases SA-βGal activity in cells deficient for p53. p53 mutant breast cancer cell lines MDA-MB-231 and MDA-MB-435 and the isogenic pair of colon carcinoma cell lines HCT116-p53wt and HCT116-p53null were treated with 6 Gy ± ABT-888, fixed at 7 d and stained for SA-βGal activity. Scale bar, 20 μm.