Supporting Information

Stecklein et al. 10.1073/pnas.1203326109

SI Methods

DNA Foci Formation Assay. Cells were grown in eight-well chamber slides and treated as indicated. Fixative was 4% paraformaldehyde with 3% sucrose on ice for 30 min. Permeabilizer was 0.1% Triton X-100 in PBS for five minutes at room temperature. Cells were blocked with blocking buffer [1% BSA (fraction V), 3% goat serum, and 0.1% Triton X-100] for 2 h at 4 °C. Primary antibodies were diluted in blocking buffer [BRCA1 Ab1 (1:200), γ-H2AX (1:1,000)] and incubated on fixed/permeabilized cells overnight at 4 °C. Secondary antibody was goat anti-mouse Alexa 594 (Invitrogen/Molecular Probes) diluted in blocking buffer at 1:1,000. Nuclei were counterstained with DAPI (Sigma-Aldrich) at 50 ng/mL for 30 min. Foci formation was quantitated using the Zeiss Pascal confocal software by normalizing the fluorescent signal for γ-H2AX or BRCA1 to the DAPI signal.

Homologous Recombination and Nonhomologous End Joining. HeLa cells were transfected with the DR–GFP reporter plasmid, selected with 5 μg/mL puromycin, and cloned. The I-SceI or empty control vector was electroporated into the HeLa–DR–GFP cells (1 μg/1 × 10^5) cells using the Amaxa nucleofector system (Lonza) and cells were immediately plated into 250 nM 17-AAG or DMSO. After 24 h, the drug was removed and replaced with fresh media for an additional 24 h. Cells were trypsinized and HR was measured by assessing number of GFP+ cells on a BD LSR II flow cytometer. A fraction of the treated cells were used to determine the HR/NHEJ ratio as previously described (1). Digested products were visualized and quantitated using the UVP BioImaging System (UVP).

Adenoviruses and Adenoviral Production. The coding region of the I-SceI endonuclease with two 5′ nuclear localization signals and an HA tag was excised from the pCBASce plasmid as an XbaI/MscI fragment and cloned into pShuttle-CMV, which had been digested with XbaI and EcoRV. The pShuttle-CMV-I-SceI plasmid was then digested with PmeI, desalted by Qiaex, and then electroporated into *Escherichia coli* strain BJ5183 along with pAdEasy1 to allow homologous recombination to occur (2). The resultant vector was PacI linearized and was transfected into AD293 cells. Adenoviral supernatants were produced by freeze/thaw and sonication and were titered using the Adeno-X Rapid Titer Kit (Clontech).

Antibodies. Mouse anti-Actin (MAB1501; Upstate/Millipore), mouse anti-BARD1 (ab50984; Abcam), mouse anti-BRCA1 (Ab1) (OP92; Calbiochem/EMD Biosciences), rabbit anti-BRCA1 (C-20) (sc-642; Santa Cruz Biotechnology), mouse anti-phospho-Histone H2A.× (Ser139) (γ-H2AX) (05–636; Upstate/Millipore), mouse anti-HSP90 (05–594; Upstate/Millipore), mouse anti-HSP90 (ADI-SPA-830; Stressgen), mouse anti-HSP70 (610608; BD Biosciences), mouse anti-HSP27 (2402; Cell Signaling Technology), rabbit anti-HSF1 (4356; Cell Signaling Technology), mouse anti-Ubiquitin (13–1,600; Invitrogen), mouse anti-CHK1 (2360; Cell Signaling Technology), rabbit anti-pCHK1^{S345} (2,341; Cell Signaling Technology), rabbit anti-cyclin B1 (sc-752; Santa Cruz Biotechnology), rabbit anti-H3 (9715; Cell Signaling Technology), rabbit anti-pH3 (9701; Cell Signaling Technology), anti-HA (H3663; Sigma-Aldrich), Alexa-488 anti-pH3^{S28} (558610; BD Biosciences), anti-rabbit HRP-linked (7074; Cell Signaling Technology), and anti-mouse HRP-linked (7076; Cell Signaling Technology).

Cell Cycle, DNA Synthesis, and Apoptosis Assays. HCC1937 and HCC1937^{BRCA1} cells were treated with 250 nM 17-AAG or vehicle for indicated times. For cell cycle analysis, cells were trypsinized and resuspended 1 mL of 0.9% NaCl, and vortexed gently during dropwise addition of 2.5 mL 90% cold ethanol. After 30 min of fixation, cells were stained with 50 μg/mL propodium iodide and treated with RNase A for 30 min. For studies examining mitotic entry, cell cycle staining was completed as described, with the addition of Alexa-488 anti-pH3^{S28} (558610; BD Biosciences) staining step per the manufacturer's instructions. DNA synthesis and TUNEL staining was performed using the Click-iT EdU cell proliferation assay (Invitrogen) and the Click-iT TUNEL assay (Invitrogen) as described by the manufacturer. Annexin staining assay was done with V450 Annexin V (BD Biosciences; 560506) per manufacturer supplied protocol.

1. Nakanishi K, et al. (2005) Human Fanconi anemia monoubiquitination pathway promotes homologous DNA repair. *Proc Natl Acad Sci USA* 102:1110–1115.

2. He TC, et al. (1998) A simplified system for generating recombinant adenoviruses. *Proc Natl Acad Sci USA* 95:2509–2514.

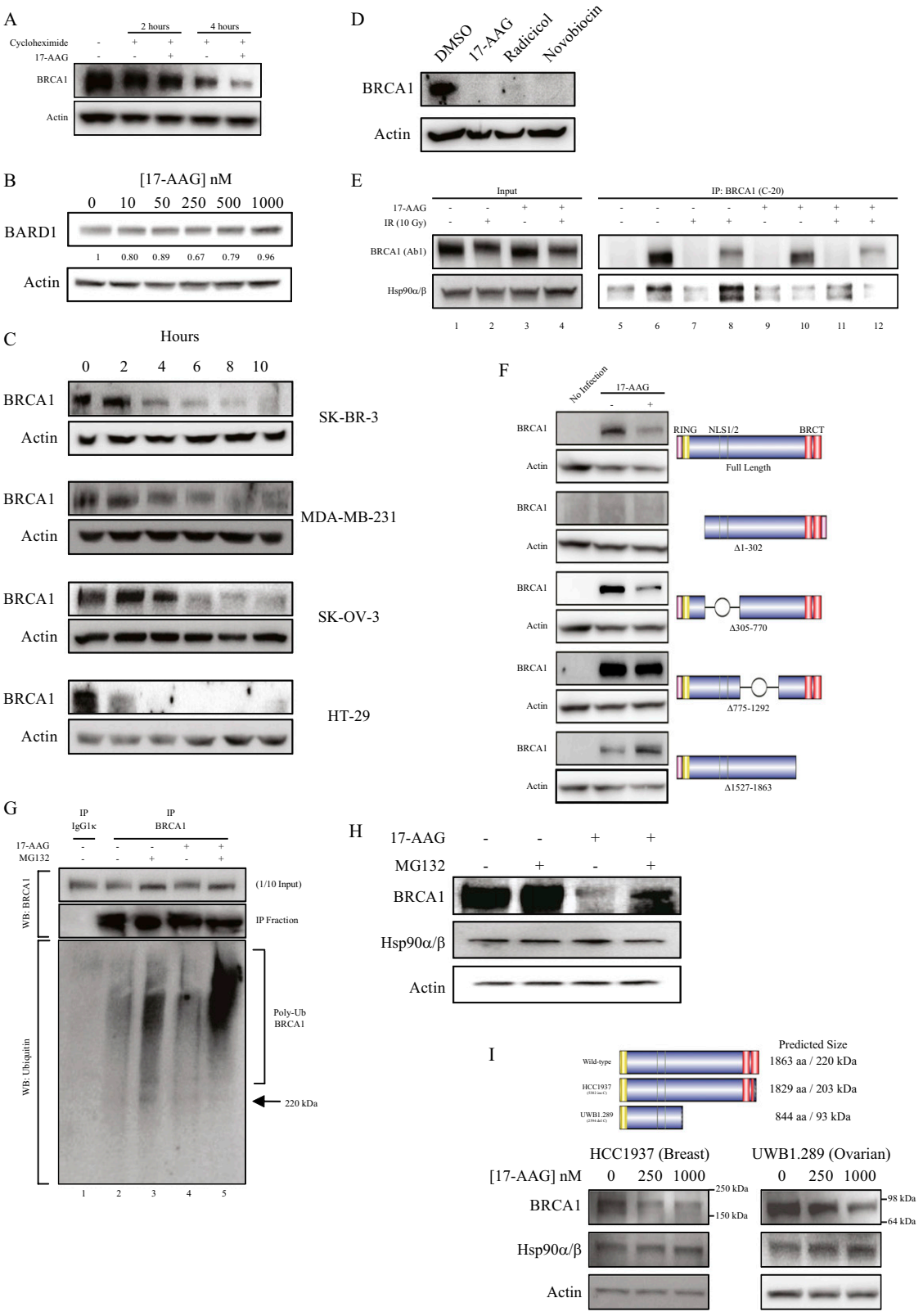

Fig. S1. Inhibition of Hsp90 induces BRCA1 degradation. (*A*) Western blot of HCC1937^{BRCA1} cells treated with 25 μg/mL cycloheximide in the presence or absence of 1 μM 17-AAG for the indicated duration. (*B*) Western blots of MCF7 cells treated with indicated concentrations of 17-AAG for 8 h. (*C*) Western blots of SK–BR-3 and MDA–MB-231 breast cancer cells, SK-OV-3 ovarian cancer cells, and HT-29 colorectal cancer cells treated with 250 nM 17-AAG for the indicated duration. (*D*) Western blots of MCF7 cells treated with DMSO, 250 nM 17-AAG, 2 μM radicicol, or 2 mM novobiocin for 8 h. (*E*) Immunoprecipitation of BRCA1 in MCF7 cells 4 h after treatment with 250 nM 17-AAG, 10 Gy IR or both. (*F*) MCF7 cells were infected with BRCA1 wild-type or indicated deletion mutant-encoding adenoviruses at an MOI of 250. After 48 h, 1 μM 17-AAG or vehicle was added for 8 h and total protein was used for Western blot. (*G*) MCF7 cells were

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treated with vehicle (-) or 250 nM 17-AAG in the presence or absence of 10 μM MG132 for 2 h. Cells were lysed and a BRCA1 monoclonal antibody or isotype control antibody was used to immunoprecipitate BRCA1. Proteins were electrophoresed and probed for BRCA1 (top two blots) or with an ubiquitin antibody (bottom blot) to detect polyubiquitinated BRCA1. (*H*) Western blots of MCF7 cells pretreated with vehicle (-) or 10 μM MG132 for 1 h and then treated with additional vehicle or 250 nM 17-AAG for 8 h. (*I*) Western blots of HCC1937 and UWB1.289 cells treated with indicated concentrations of 17-AAG for 8 h.

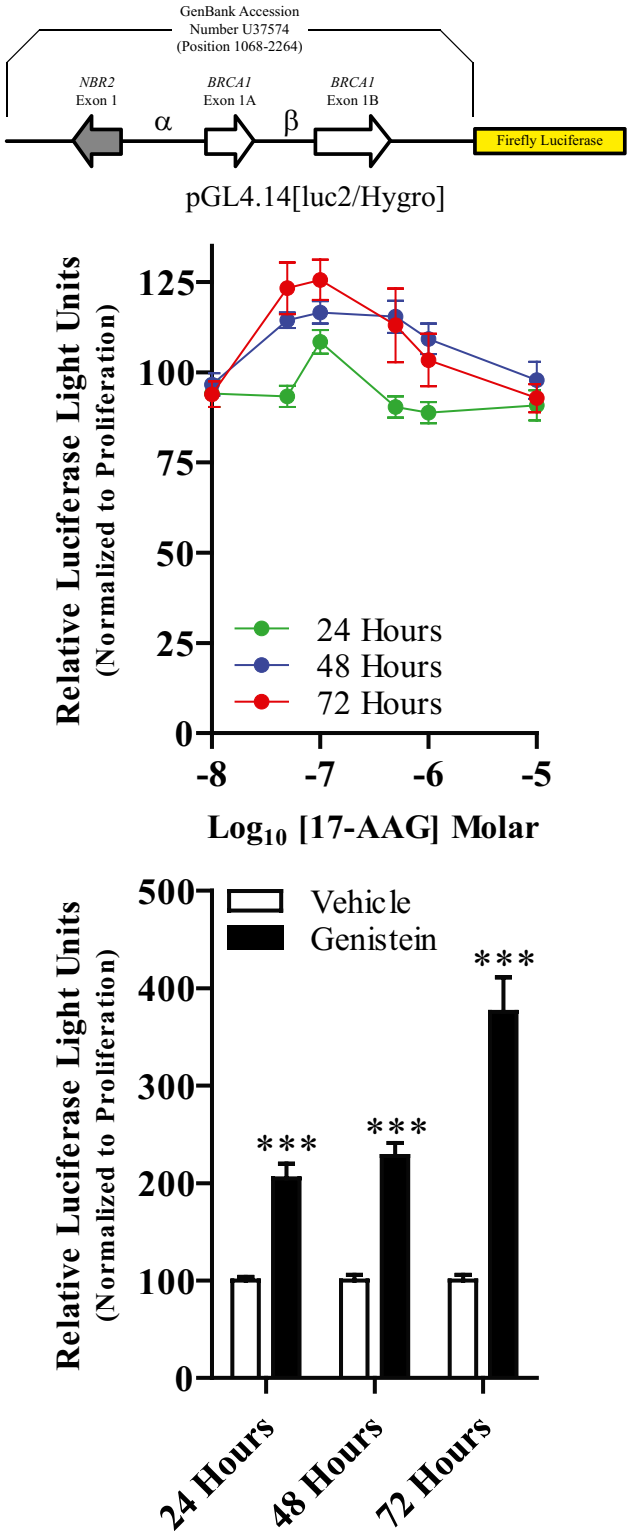

Fig. S2. Inhibition of Hsp90 results in modest changes in *BRCA1*-promoter activity. MCF7 cells with a stably integrated *BRCA1* promoter-driven Firefly luciferase reporter were treated with the indicated dose of 17-AAG for 24, 48, or 72 h. Experiments were conducted in parallel with MTS viability assays to account for cytotoxicity. Each point was normalized to relative cell number, and then to the vehicle (DMSO) -treated sample. Error bars represent SEM of three independent experiments, each performed in duplicate. *Bottom* illustrates the response of this reporter system to 10 μM genistein [a known inducer of the *BRCA1* promoter (1)] over the same time period. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

1. Fan S, Meng Q, Auborn K, Carter T, Rosen EM (2006) BRCA1 and BRCA2 as molecular targets for phytochemicals indole-3-carbinol and genistein in breast and prostate cancer cells. *Br J Cancer* 94:407–426.

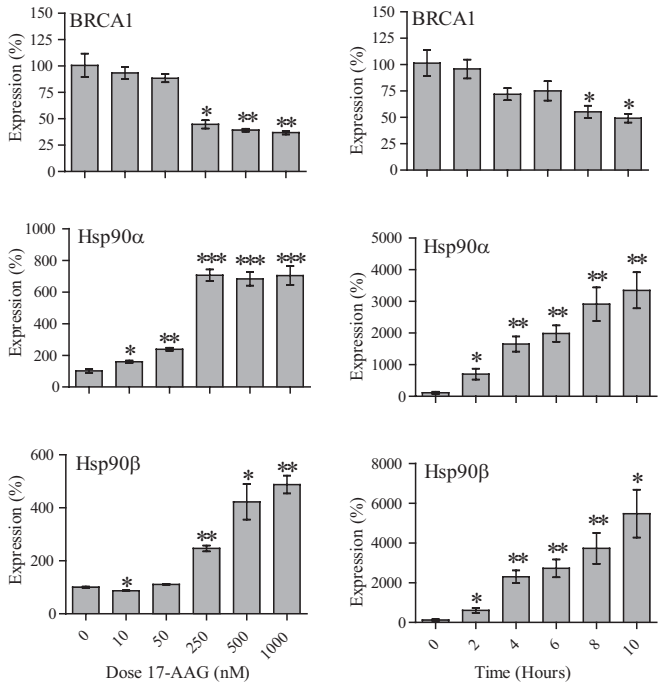

Fig. S3. Inhibition of Hsp90 leads to a delayed loss of BRCA1 mRNA. MCF7 cells were treated with the indicated dose of 17-AAG for 8 h (*Left*) or with 250 nM 17-AAG for the indicated duration (*Right*) and total cellular RNA was isolated and used for qRT–PCR analysis of BRCA1, Hsp90-α, and Hsp90-β mRNA expression. Error bars represent SEM of triplicate measurements. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

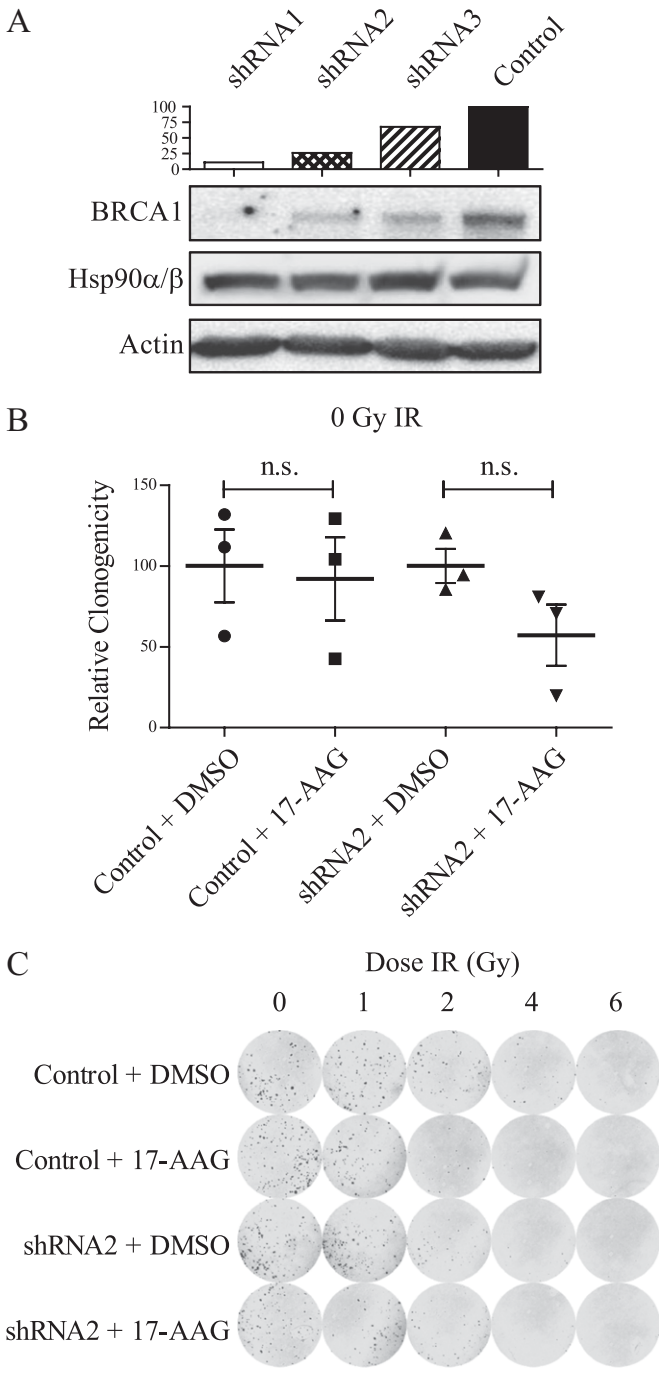

Fig. S4. BRCA1 expression mediates sensitivity to 17-AAG and is associated with the ability of 17-AAG to sensitize cells to ionizing radiation. (*A*) Western blots of MCF7 cells infected with lentiviruses expressing control or BRCA1-targeting shRNAs. The graph above the BRCA1 blot indicates the ratio of BRCA1 band intensity of each BRCA1-targeting lentivirus compared with the control lentivirus, normalized to actin loading control. (*B*) Relative clonogenic potential of each population in the absence of IR. (*C*) Representative images of MCF7 clonogenic growth 10 d after exposure to 0–6 Gy of IR. $n = 3$. n.s., not significant.

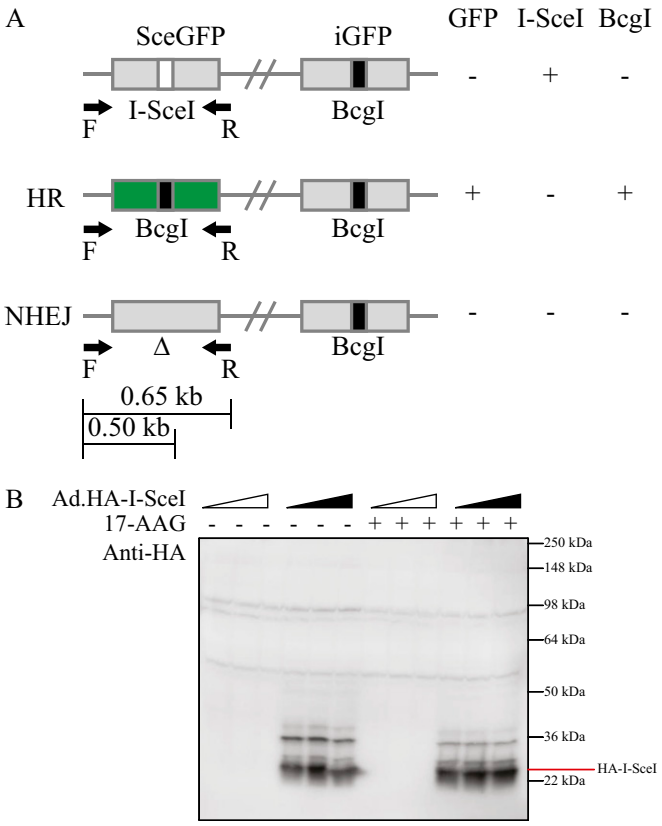

Fig. S5. HR/NHEJ functional reporter assay. (*A*) Schematic of the integrated DR–GFP reporter before and after HR or NHEJ. (*B*) HeLa cells were infected with an adenovirus encoding HA-tagged I-SceI (closed triangles) or an empty adenovirus (open triangles) and plated as described for the HR/NHEJ assay. Total protein was isolated, electrophoresed, and probed with an anti-HA antibody.

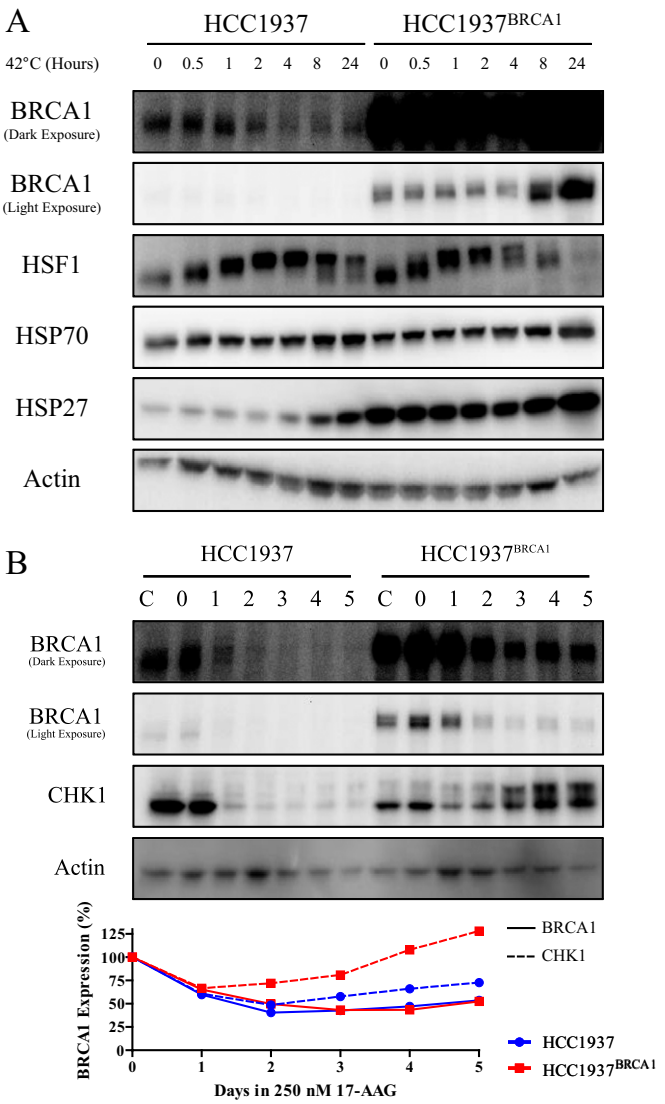

Fig. S6. Effect of BRCA1 expression the heat shock response and CHK1 stability. (*A*) HCC1937 and HCC1937^{BRCA1} cells were heat shocked (42 °C) for the time indicated. Total protein was isolated, electrophoresed, and probed with the indicated antibodies. (*B*) HCC1937 and HCC1937^{BRCA1} cells were grown in 250 nM 17-AAG for the indicated number of days (*C* indicates no 17-AAG, "0" indicates vehicle control). Total protein was isolated, electrophoresed, and probed with the indicated antibodies.

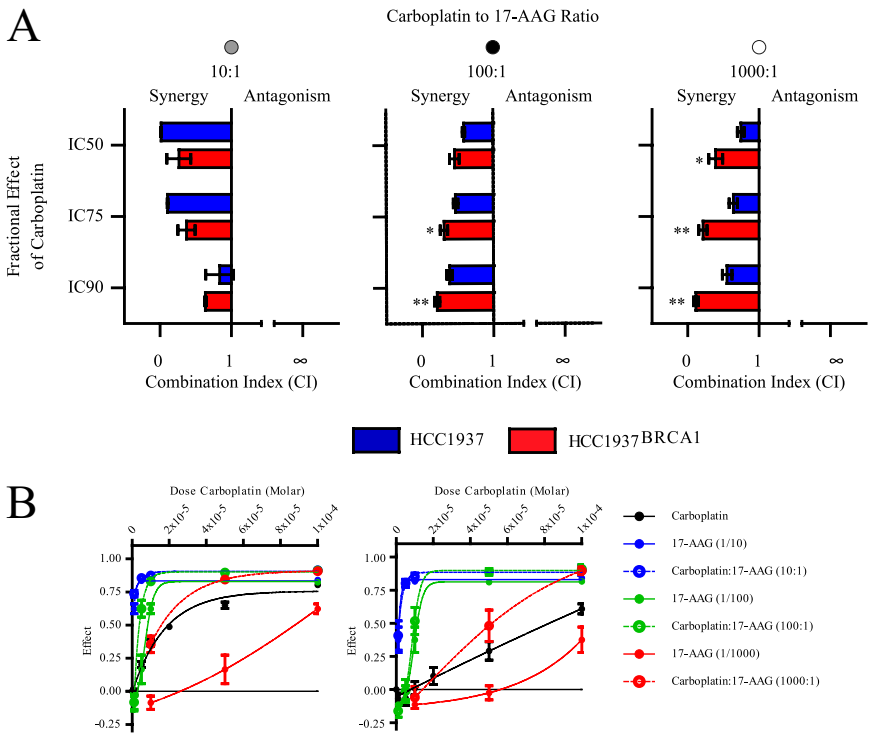

Fig. S7. (*A*) Combination index for HCC1937 and HCC1937^{BRCA1} cells at equal ratio doses and three response levels. Error bars represent SEM of three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Student's *t* test) represents comparison between HCC1937 and HCC1937^{BRCA1} combination index at specified ratio and response level. (*B*) Dose-effect curves for carboplatin at dose specified on X axis, 17-AAG at 1/10, 1/100, or 1/1,000 of dose specified on X axis, or carboplatin in combination with 17-AAG at 10:1, 100:1, or 1,000:1. Error bars represent SEM of three independent experiments.

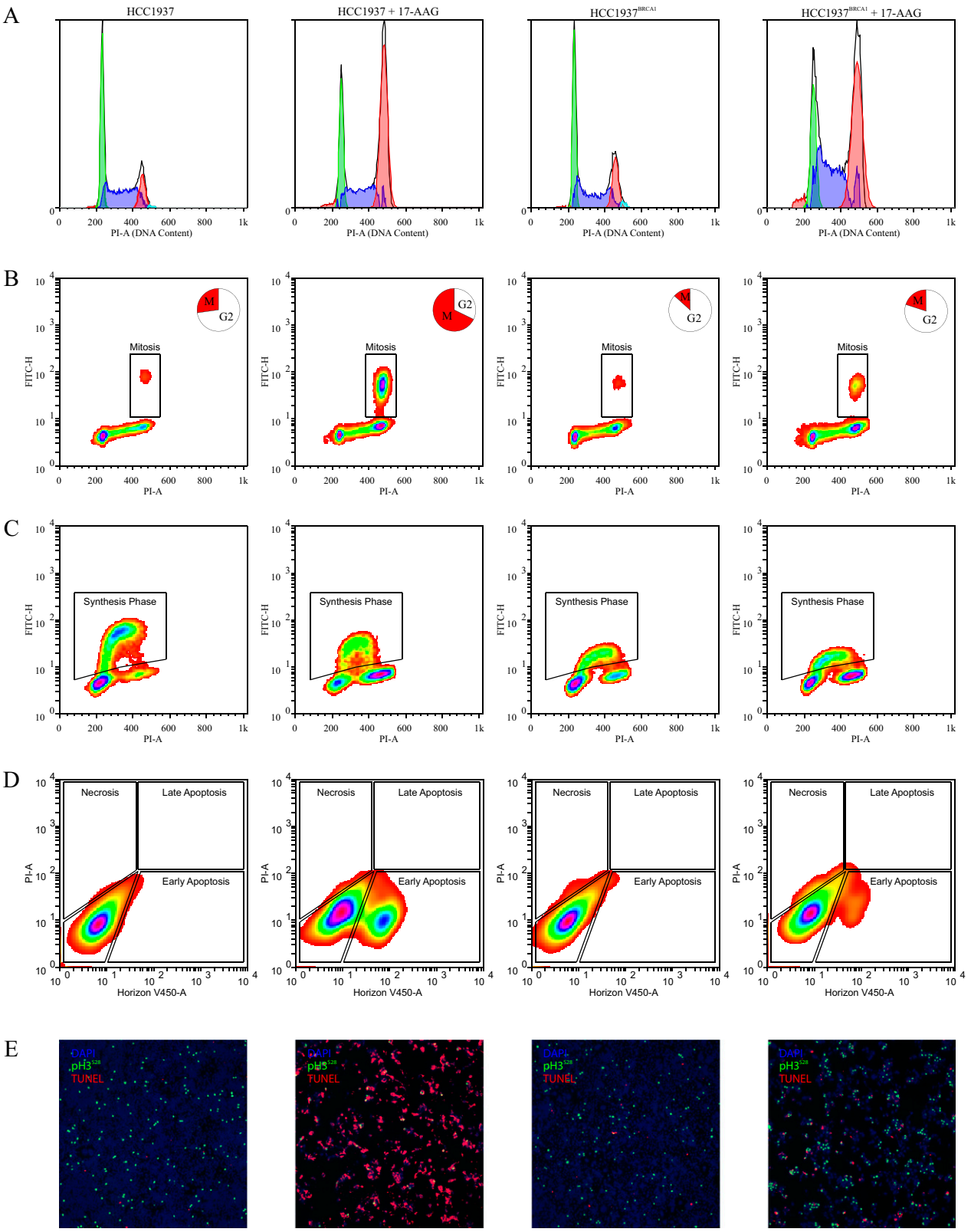

Fig. S8. Representative flow cytometric or high-content imaging data for graphs presented in Fig. 4.