Supplemental Methods

Clonogenic survival assay

Cell survival was evaluated by the colony formation assay in the breast cancer cell lines as previously described (1, 2). Briefly, cells were seeded and treated with the indicated doses of drugs (Lapatinib, ABT 888 or vehicle) following which the plates were left undisturbed. Three weeks following treatment, colonies were fixed with 70% ethanol, stained 1% methylene blue (Sigma) and number of positive colonies were counted (>50 cells). Survival fraction was calculated as follows: (number of colonies for treated cells/number of cells plated)/(number of colonies for corresponding control/number of cells plated). Experiments were performed at least in triplicate.

Cell Viability

Cell viability was measured using the ATP-Lite 1 step luminescence assay (Perkin Elmer) following the manufacturer’s directions. Briefly, 1000 cells in exponential phase were seeded per well in a 96 well plate and treated or transfected as required. Following the treatment period, cells were processed according to the manufacturer’s instructions and luminescence was assayed using a plate reader (BioTek).

Chromosomal homologous recombination mediated repair analysis

Breast cancer cell lines stably expressing the DRGFP repair substrate were treated as required and subsequently transfected with either an empty vector, ISce-1 expression vector to measure HR-mediated repair capacity, or a GFP expression vector to measure transfection efficiency. % GFP positive cells were detected by flow cytometry. Two days after transfection with ISceI expression plasmid or empty vector, cells were subjected to two-color fluorescence analysis, which revealed the percentage of GFP+ cells relative to the total cell number. For each analysis, 100,000 cells were processed. All transfections
were performed using Lipofectamine. HR relative to total transfected cells was
determined by division of the % GFP + cells from each ISce-1 transfection by the %
GFP+ cells from a parallel GFP transfection. 7-Aminoactinomycin D (7-AAD,
Invitrogen) was used as well to control for cell viability.

**Immunoblotting**

Immunoblotting was performed as described previously (1). Briefly, cell lysates
were prepared using radioimmunoprecipitation lysis buffer (150mM NaCl, 50mM Tris,
pH 8.0, 5mM EDTA, 0.5% sodium deoxycholate, 0.1% SDS, 1.0% Nonidet P-40)
supplemented with protease and phosphatase inhibitor cocktails (Sigma) and subjected to
SDS-PAGE analysis. All antibodies were used at dilutions recommended by the
manufacturer: HER2 (Cell Signaling, catalog # 2165), IKKα (Cell Signaling, catalog #
2682), phospho p65 (Cell Signaling, catalog # 3033), p65 (Cell Signaling, catalog #
8242), IκBα (Cell Signaling, catalog # 4814) and cMYC (Cell Signaling, catalog #
5605). β-Actin (Santa Cruz Biotechnology, catalog # sc47778) levels were also analyzed
as loading control.
References