Supporting Information for

"Targeted Proteomic Analysis Revealed Kinome Reprogramming during Acquisition of Radioresistance in Breast Cancer Cells"

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Table of Contents:

Supplementary Experimental Conditions:

Cell Culture

MCF-7 and MDA-MB-231 cells, as well as their paired radioresistant lines were cultured in Dulbecco's modified eagle medium (DMEM). The culture medium was supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and penicillin (100 IU/mL). The cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Approximately 5×10^6 cells were harvested by trypsinization, washed twice with cold PBS, and lysed with CelLytic M cell lysis reagent (Sigma) containing 1% protease inhibitor cocktail by incubating on ice for 30 min. The lysates were centrifuged at 9,000g at 4°C for 20 min, and the resulting supernatants were collected. For SILAC labeling experiments, the cells were cultured in SILAC medium containing unlabeled lysine and arginine, or [$^{13}C_6$, $^{15}N_2$]-lysine and [$^{13}C_6$]-arginine, for at least five cell doublings.

LC-PRM Analysis

All LC-PRM experiments were carried out on a Q Exactive Plus quadrupole-Orbitrap mass spectrometer coupled with an EASY-nLC 1000 system (Thermo Fishier Scientific). The samples were automatically loaded onto a 4-cm trapping column (150 μm i.d.) packed with ReproSil-Pur 120 C18-AQ resin (5 μm in particle size and 120 Å in pore size, Dr. Maisch GmbH HPLC) at a flow rate of 3 μL/min. The trapping column was coupled to a 20-cm fused silica analytical column (PicoTip Emitter, New Objective, 75 μm i.d.) packed with ReproSil-Pur 120 C18-AQ resin (3 μm in particle size and 120 Å in pore size, Dr. Maisch GmbH HPLC). The peptides were then resolved using a 140-min linear gradient of 9-38% acetonitrile in 0.1% formic acid and at a flow rate of 300 nL/min. The spray voltage was 1.8 kV. Peptide ions were collisionally activated in the HCD cell

at a normalized collision energy of 29 to yield MS/MS, which was recorded in the Orbitrap analyzer at a resolution of 17,500 with an AGC target of 1×10^5.

The linear predictor of empirical retention time (RT) from normalized RT (iRT)[1] for targeted peptides of kinases was determined by the linear regression of RTs of BSA standard peptides obtained for the current chromatography setup.[2-5] This RT-iRT linear relationship was re-defined between every eight LC-MS/MS runs by injecting another BSA tryptic digestion mixture. The targeted precursor ions were monitored in scheduled PRM mode with an 8-min retention time window.

All raw files were processed using Skyline (version 3.5) for the generation of extracted-ion chromatograms and for peak integration[6]. Six most abundant y ions found in MS/MS acquired from shotgun proteomic analysis were chosen for peptide identification and quantification, where a mass accuracy of 20 ppm or better was imposed for fragment ions during the identification of peptides in the Skyline platform. The targeted peptides were first manually checked to ensure that the chromatographic profiles of multiple fragment ions derived from the light and heavy forms of the same peptide could be overlaid. The data were then processed to ensure that the distribution of the relative intensities of multiple transitions associated with the same precursor ion is correlated with the theoretical distribution in the MS/MS acquired from shotgun proteomic analysis. The sum of peak areas from all transitions of light or heavy peptides was used for quantification.

Western blot

Cells were cultured in 6-well plates and lysed at 50-70% confluency following the aforementioned procedures. The concentrations of the resulting protein lysates were determined by using Bradford Assay (Bio-Rad). The whole cell lysate for each sample (10 μg) was denatured

by boiling in Laemmli loading buffer and resolved by using SDS-PAGE. Subsequently, the proteins were transferred onto a nitrocellulose membrane at 4°C for 2 h. The resulting membrane was blocked with PBS-T (PBS with 0.1% Tween 20) containing 5% milk (Bio-Rad) at room temperature for 2 h. The membrane was subsequently incubated with primary antibody at 4°C overnight and then with secondary antibody at room temperature for 1 h. After thorough washing with PBS-T, the HRP signal was detected using Pierce ECL Western Blotting Substrate (Thermo).

Human CHK1 (Cell Signaling Technology, 2360S, 1:2000 dilution) and TAF9 (Proteintech, 10544-1-AP, 1:2500 dilution) antibodies were employed as the primary antibodies. Horseradish peroxidase-conjugated anti-rabbit IgG and IRDye® 680LT Goat anti-Mouse IgG were used as secondary antibodies. Membranes were also probed with anti-Actin antibody (Cell Signaling #4967, 1:10000 dilution) and anti-GAPDH antibody (Santa Cruz Biotechnology, sc-32233, 1:5000 dilution) to verify equal protein loading.

References:

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Figure S1. Uncropped Western blot images.