SUPPLEMENTAL MATERIAL AND METHODS

Protein Extraction

Protein amounts for all samples were quantified using a standard BCA assay (Pierce, Rockford, IL). Each of the eight biological replicates was treated individually. Equal amounts of protein from six different passages of DLD-1 (P1-P6) were pooled to create a diploid reference sample.

Sample Preparations for Proteomic Analysis

Samples were enzymatically digested using an in-solution protocol where 100 µg of each protein sample were incubated with 150 µL of 100 mM triethylammonium bicarbonate (TEAB), 100 µL HPLC water, and 15 µL of 100 mM dithiothreitol (DTT) for 1 h at 65 °C. Reduced cysteine residues were alkylated with 30 µL of 100 mM iodoacetamide (IAA) at ambient temperature for 20 min in the dark. Protein samples were then digested with 2 µL trypsin gold (1 µg/µL) (Promega, Madison, WI) at 37 °C for 3 h followed by incubation with an additional 2 µL of trypsin gold overnight at 30 °C. All digested protein samples were desalted using C18 tips (Pierce, Rockford, IL) and dried using a vacuum concentrator prior to labeling with dimethyl reagents.

Samples were resuspended in 100 mM TEAB. “Light” labeling reagent (CH_2O, 4%, 20 µL) was added to the pooled DLD-1 samples and “Heavy” labeling reagent (CD_2O, 4%, 20 µL) was added to each DLD-1+13 clone, followed by the addition of borane pyridine (600 mM, 20 µL). Samples were incubated with agitation for 1 h. The process was

repeated once before quenching the reaction with 8 µL of 10% formic acid (FA). “Light” and “Heavy” labeled samples were combined in a 1:1 ratio.

SCX fractionation was completed using a SCX Spin Tip Sample Preparation Kit (Protea Biosciences, Morgantown, WV). The SCX spin tips were conditioned and the combined protein samples were loaded according to manufacturer’s instructions. After adding 200 µL of the appropriate ammonium formate buffer (20, 50, 80,100, 150, 250, 500 mM with 10% ACN at pH 3) the sample was centrifuged for 4 min at 4,000 rpm. Fractions, including the flow through, were dried and desalted with C18 ZipTips (Millipore, Billerica, MA). The desalted peptides were resuspended in 0.1% FA with 5% ACN.

Mass Spectrometry Analysis

All samples were analyzed using liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) on a nano-Acquity ultra performance LC system (100x100 C18 BEH column, Waters) coupled to a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). LC system solvents were 0.1% FA (A) and 0.1% FA in ACN (B). Peptide sample separations were performed with a linear gradient from 5-50% B for 105 min followed by an organic wash and aqueous re-equilibration at a flow rate of 1000 nL/min with a total run time of 120 minutes. The mass spectrometer was operated in a Top 12 data dependent mode with automatic switching between MS and MS/MS. Source ionization parameters were as follows: spray voltage, 2.2 kV; capillary temperature, 280 °C; and s-lens level, 50.0. Full scan MS mode (400-1800 *m/z*) was operated at a resolution of 70,000 with automatic gain

control (AGC) target of $1x10^6$ ions and a maximum ion transfer (IT) of 20 ms. Ions selected for MS/MS were subjected to the following parameters: resolution 17,500; AGC $1x10^5$ ions; maximum IT 80 ms; 2.0 m/z isolation window; normalized collision energy 28.0; underfill ratio 1.0%; and dynamic exclusion of 40.0 s. All samples were run with duplicate injections.

Mass Spectrometric Data Analysis

All raw files were analyzed using the MaxQuant (1.3.0.5) software platform. The files were searched using the Andromeda (1.3.0.5) search engine against UniProt (72390 entries) and decoy human databases. The following MaxQuant parameters were used: trypsin gold was selected as the enzyme; two missed cleavages were allowed; precursor mass tolerance was set to 7ppm; fragment mass tolerance was set to 50ppm; dynamic modifications included protein N-terminal acetylation, N-terminal glutamine deamidation, methionine oxidation and carbamidomethylation of cysteine was set as a fixed modification. The false discovery rate (FDR) was set to 0.01 for peptides and proteins. Proteins identified were required to contain a “unique or razor” peptide with a minimum length of six amino acids for identification. For quantification, light and heavy dimethyl labels (K and N-term) were selected. Relative peptide and protein quantification were performed automatically by MaxQuant with “Requantify,” “Filter labeled amino acids” and “Match between runs” features enabled. MaxQuant reported the “Heavy”/”Light” protein group ratios.