Materials & Methods

Cellular proteins were denatured in a 1% SDS + 2-mercaptoethanol buffer solution and diluted in five 2-fold serial dilutions in dilution lysis buffer. Serially diluted lysates were arrayed on nitrocellulose-coated slides (Grace Bio-Labs) by the Quanterix (Aushon) 2470 Arrayer (Quanterix Corporation). A total of 5808 spots were arrayed on each slide including spots corresponding to serially diluted (1) standard lysates, and (2) positive and negative controls prepared from mixed cell lysates or dilution buffer, respectively.

Each slide was probed with a validated primary antibody plus a biotin-conjugated secondary antibody. Antibody validation for RPPA is described in the RPPA Core website: https://www.mdanderson.org/research/research-resources/core-facilities/functional-proteomics-rppa-core/antibody-information-and-protocols.html. Signal detection was amplified using an Agilent GenPoint staining platform (Agilent Technologies) and visualized by DAB colorimetric reaction. The slides were scanned (Huron TissueScope, Huron Digital Pathology) and quantified using customized software (Array-Pro Analyzer, Media Cybernetics) to generate spot intensity.

Relative protein level for each sample was determined by RPPA SPACE (developed by MD Anderson Department of Bioinformatics and Computational Biology, https://bioinformatics.mdanderson.org/public-software/rppaspace/, Shehwana et al., 2022) by which each dilution curve was fitted with a logistic model. RPPA SPACE fits a single curve using all the samples (i.e., dilution series) on a slide with the signal intensity as the response variable and the dilution steps as the independent variable. The fitted curve is plotted with the signal intensities, both observed and fitted, on the y-axis and the $\log_2$ concentration of proteins on the x-axis for diagnostic purposes. The protein concentrations of each set of slides were then normalized for protein loading. Correction factor was calculated by (1) median-centering across samples of all antibody experiments; and (2) median-centering across antibodies for each sample. Results were then normalized across RPPA sets by replicates-based normalization as described (Akbani et al. 2014).

Details of the RPPA platform as performed by the RPPA Core are described in Siwak et al., 2019.

References

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