Supporting Information:

Comparison of Quantitative Mass Spectrometry Platforms for Monitoring Kinase ATP Probe Uptake in Lung Cancer

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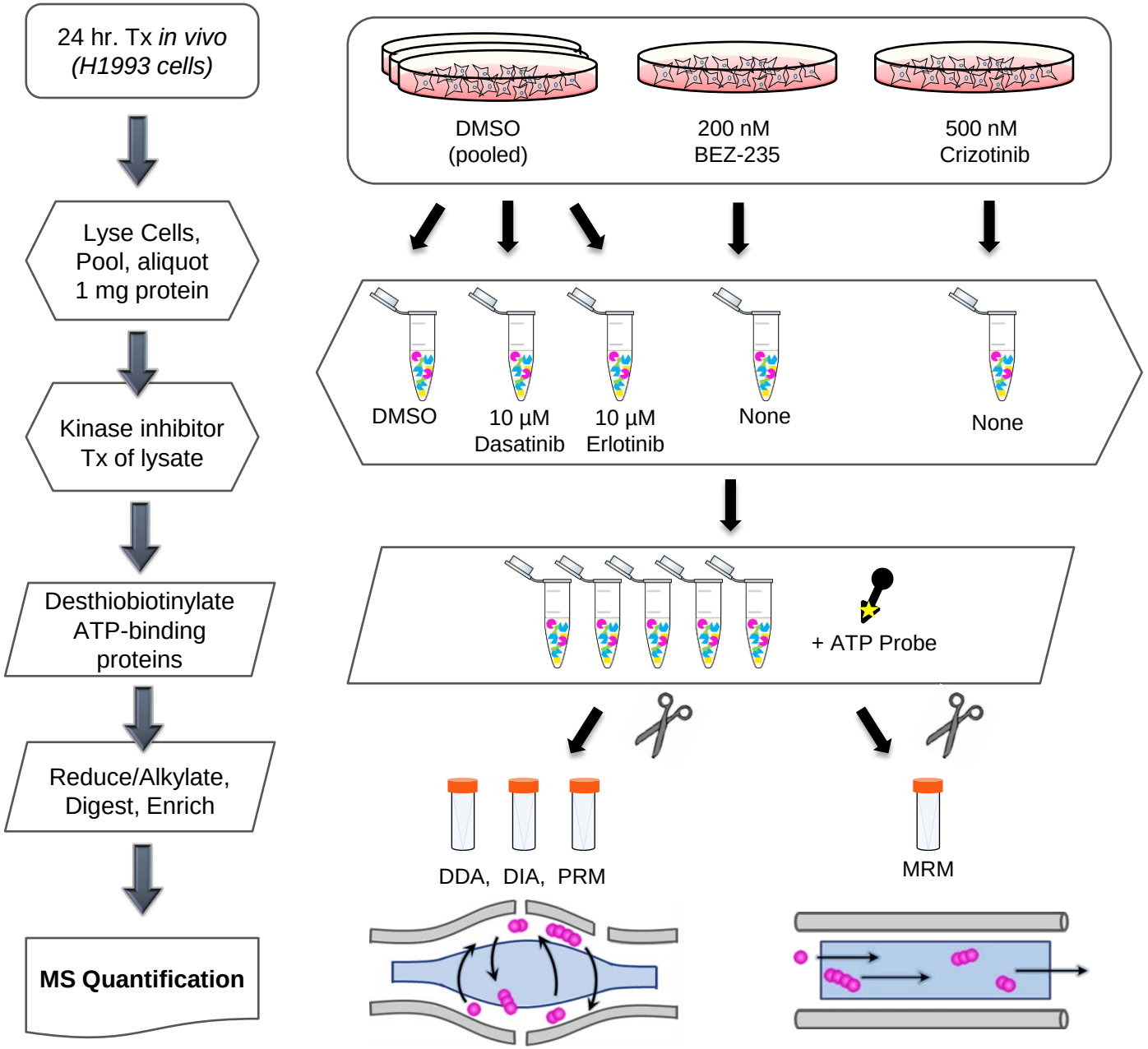
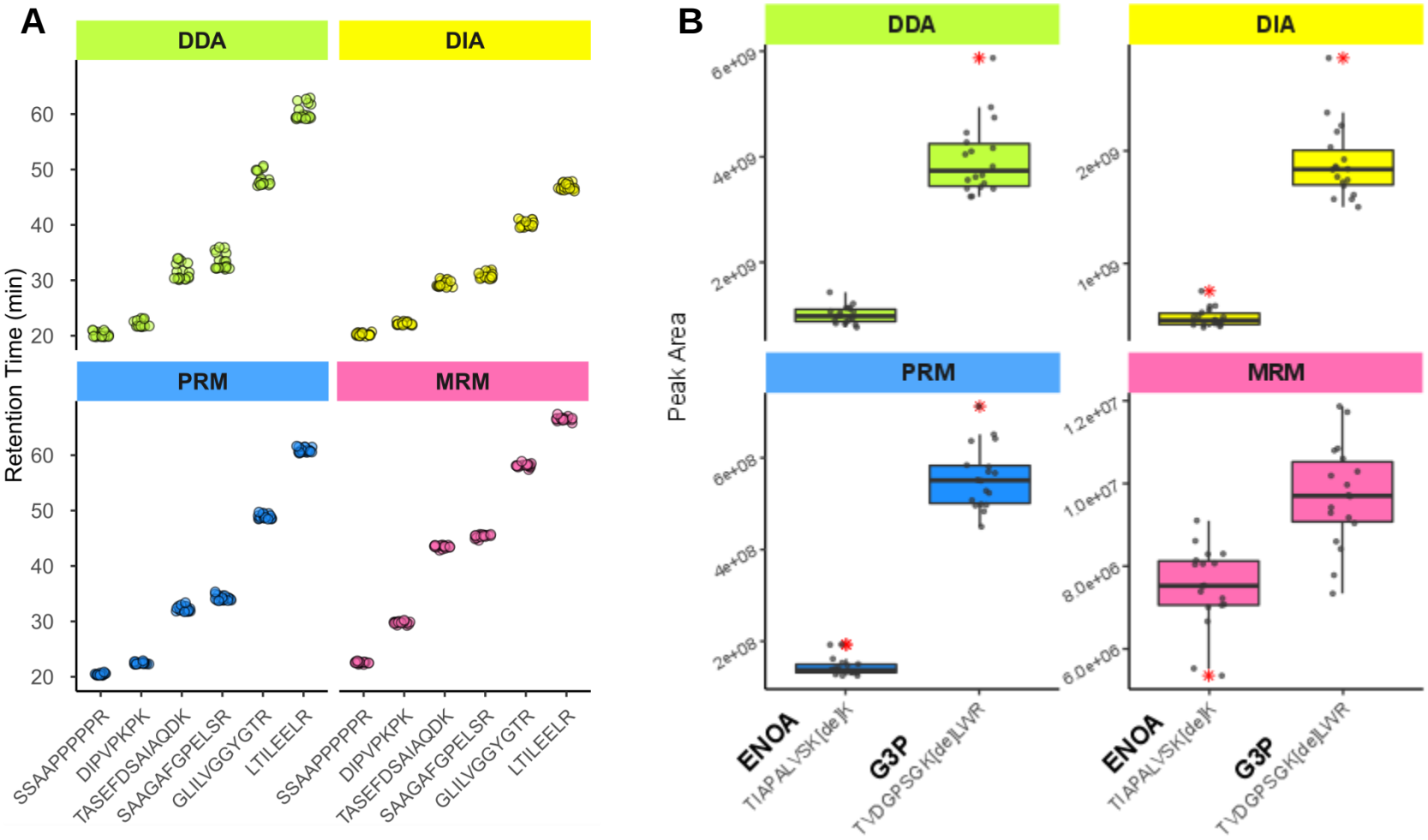
Table of Contents

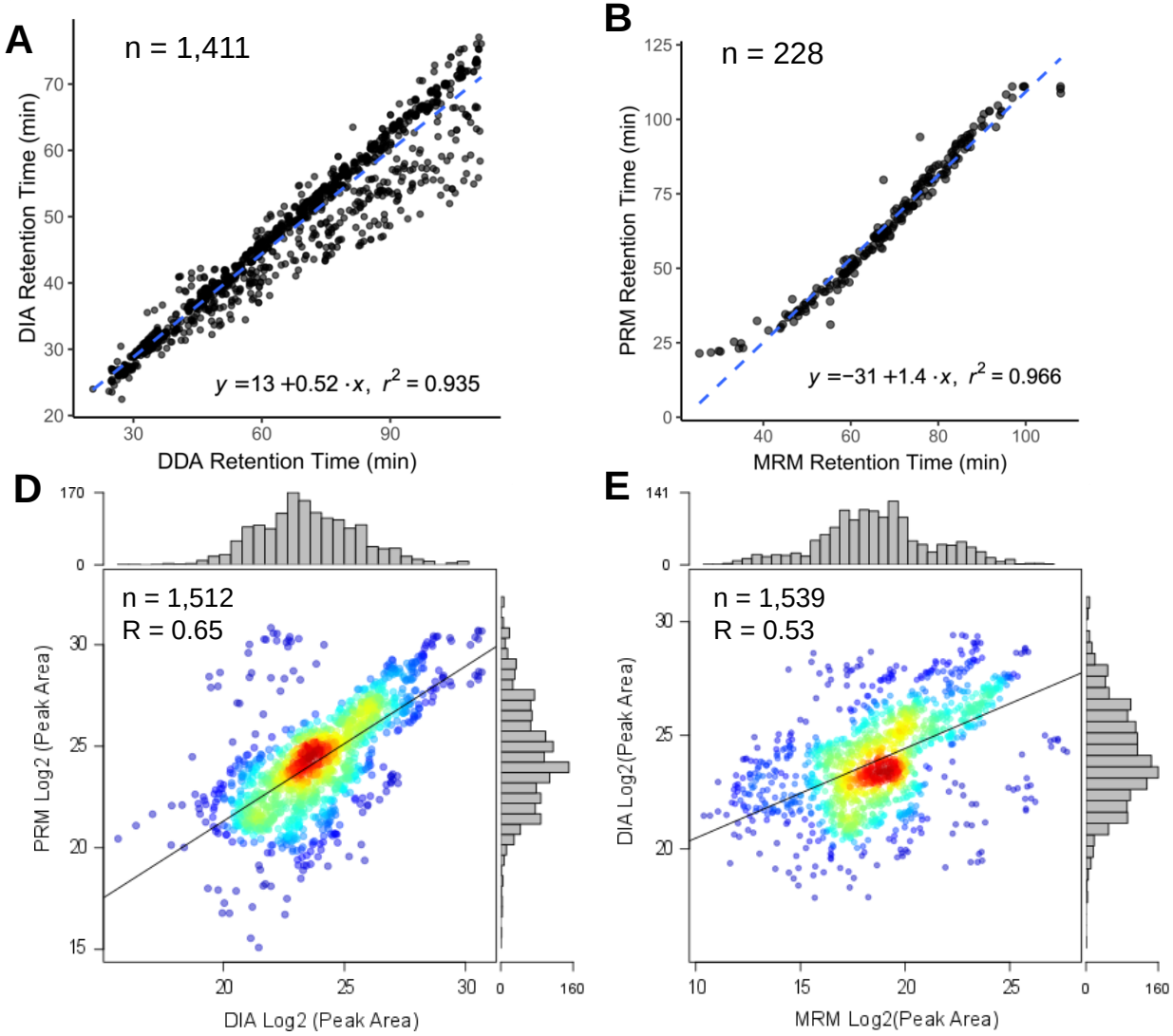
- **Table S11**. DDA analysis of H1993 cell lysate treated with Dasatinib: Log2FC, Average, Standard Deviation, N, and Standard Error
- **Table S12**. DDA analysis of H1993 cells treated with BEZ-235: Log2FC, Average, Standard Deviation, N, and Standard Error
- **Table S13**. Pinnacle Scores and Raw Peak Areas for Peptides from Kinases Only for DIA Analysis.
- **Table S14**. DIA analysis of H1993 cells treated with Crizotinib: Log2FC, Average, Standard Deviation, N, and Standard Error
- **Table S15**. DIA analysis of H1993 cell lysate treated with Erlotinib: Log2FC, Average, Standard Deviation, N, Standard Error, and Statistical Calculations
- **Table S16**. DIA analysis of H1993 cell lysate treated with Dasatinib: Log2FC, Average, Standard Deviation, N, Standard Error, and Statistical Calculations
- **Table S17**. DIA analysis of H1993 cells treated with BEZ-235: Log2FC, Average, Standard Deviation, N, Standard Error, and Statistical Calculations
- **Table S18**. Raw Peak Areas from Skyline Output for Peptides from MRM Analysis.
- **Table S19**. MRM analysis of H1993 cells treated with Crizotinib: Log2FC, Average, Standard Deviation, N, and Standard Error
- **Table S20**. MRM analysis of H1993 cell lysate treated with Erlotinib: Log2FC, Average, Standard Deviation, N, Standard Error, and Statistical Calculations
- **Table S21**. MRM analysis of H1993 cell lysate treated with Dasatinib: Log2FC, Average, Standard Deviation, N, Standard Error, and Statistical Calculations
- **Table S22**. MRM analysis of H1993 cells treated with BEZ-235: Log2FC, Average, Standard Deviation, N, Standard Error, and Statistical Calculations
- **Table S23**. Raw Peak Areas from Skyline Output for Peptides from PRM Analysis
- **Table S24**. PRM analysis of H1993 cells treated with Crizotinib: Log2FC, Average, Standard Deviation, N, and Standard Error
- **Table S25**. PRM analysis of H1993 cell lysate treated with Erlotinib: Log2FC, Average, Standard Deviation, N, Standard Error, and Statistical Calculations
- **Table S26**. PRM analysis of H1993 cell lysate treated with Dasatinib: Log2FC, Average, Standard Deviation, N, Standard Error, and Statistical Calculations
- **Table S27**. PRM analysis of H1993 cells treated with BEZ-235: Log2FC, Average, Standard Deviation, N, Standard Error, and Statistical Calculations
- **Table S28**. PRM, MRM, and DIA analysis of serial diluted desthiobiotinylated peptides from untreated H1993 cells: Average Log2 Peak Areas, Standard Deviations, and Coefficient of Variations (CVs)
- **Table S29**. DIA GeneGo enrichment analysis report.
- **Table S30**. MRM GeneGo enrichment analysis report.
- **Table S31**. PRM GeneGO enrichment analysis report.
- **Table S32**. Combined GeneGo enrichment analysis p-values.

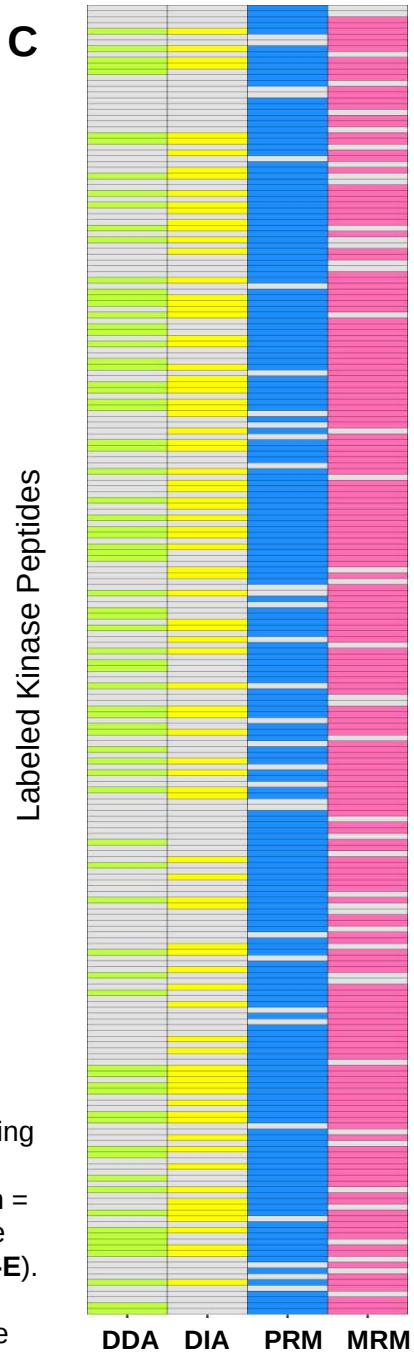


Supplemental Figure 1. Workflow diagram of sample preparation for the method comparison study.

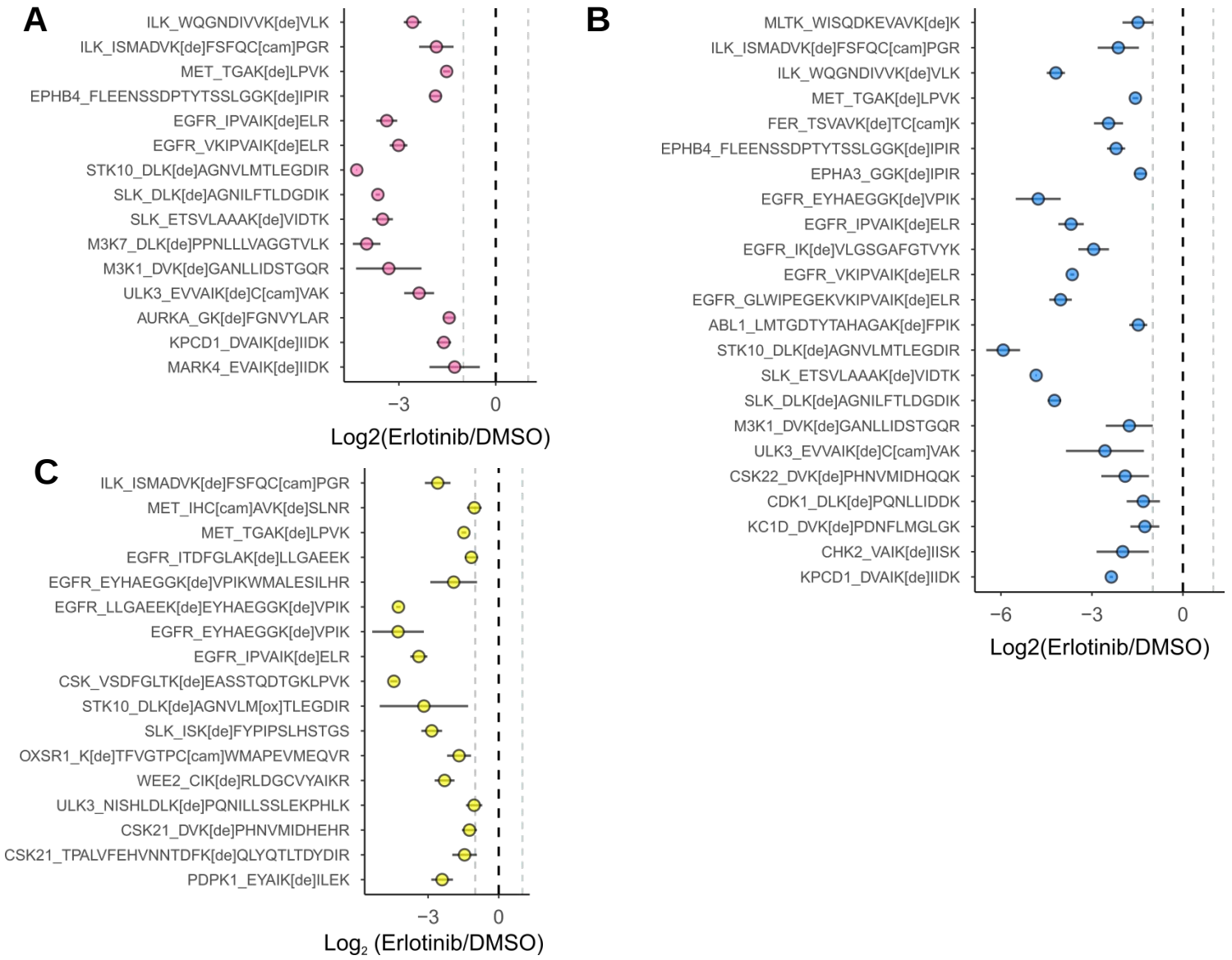


Supplemental Figure 2. Retention time stability and ion signal consistency assessed as quality control metrics. (**A**) Pierce retention time calibrator (PRTC) peptides were monitored in each LC-MS run (5 fmol/ injection) for retention time stability across the entire cohort. (**B**) Peptides from ATP-utilizing metabolic proteins, ENOA and G3P, were quantified as a control for sample preparation and peptide enrichment consistency (n = 18).

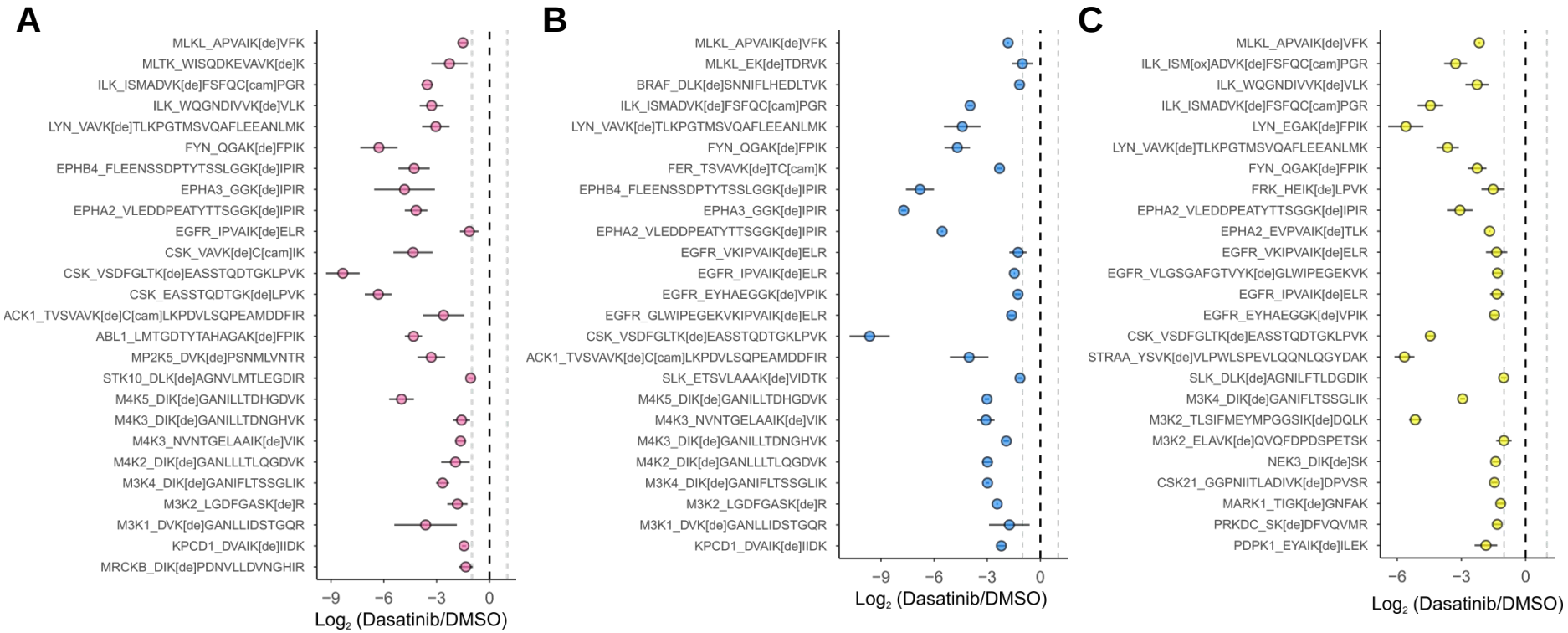




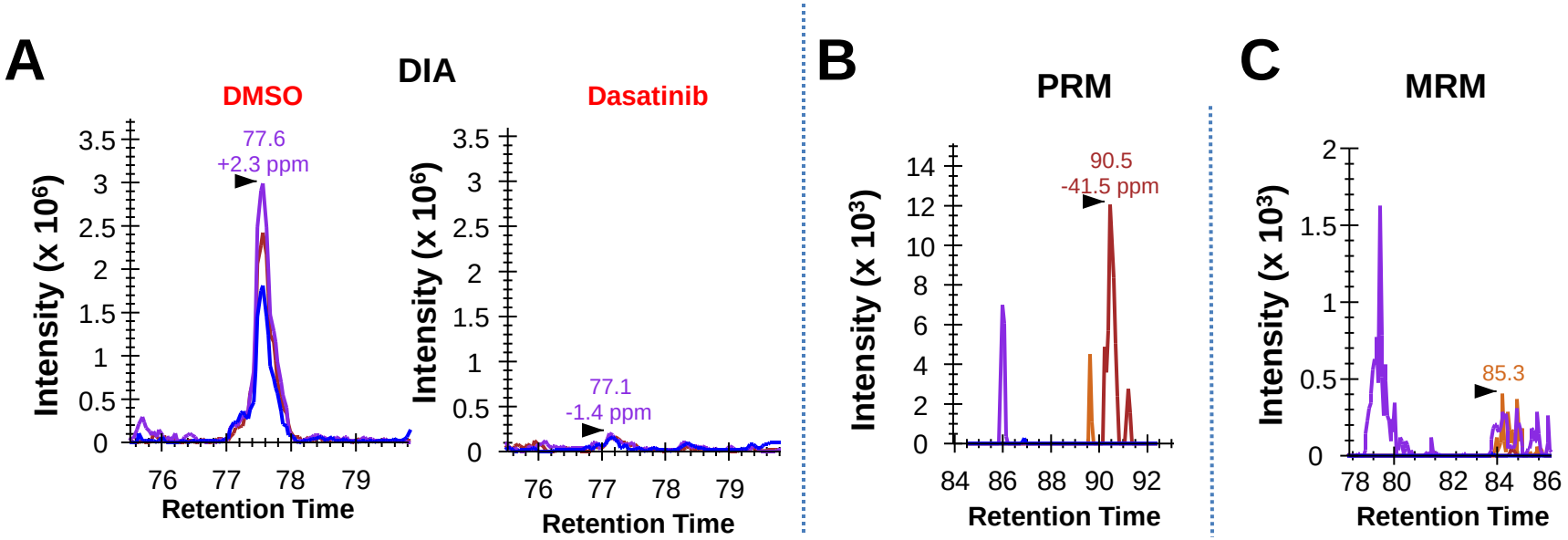
Supplemental Figure 3. Comparison of retention time (RT) correlation, peak areas, and peptide coverage across all quantitative methods. (**A**) Correlation plot of RTs from each identified peak per replicate, corresponding to a kinase peptide that was observed between both DDA and DIA (n = 1,411) in cells or lysates treated with different kinase inhibitors. Correlation plot of average RTs per kinase peptide observed by both MRM and PRM (n = 228) is shown in (**B**). Each rectangle in (**C**) represents the presence of a quantifiable desthiobiotin labeled peptide across the four datasets. Comparison of peak areas for detected kinase peptides in DIA, MRM, PRM datasets (**D-E**). In both panels, each point represents a specific peptide measurement in a single replicate and the marginal distribution of measurements across the log2 transformed peak areas or intensities is shown as histograms on the opposite axis. R values are calculated for Pearson correlation.



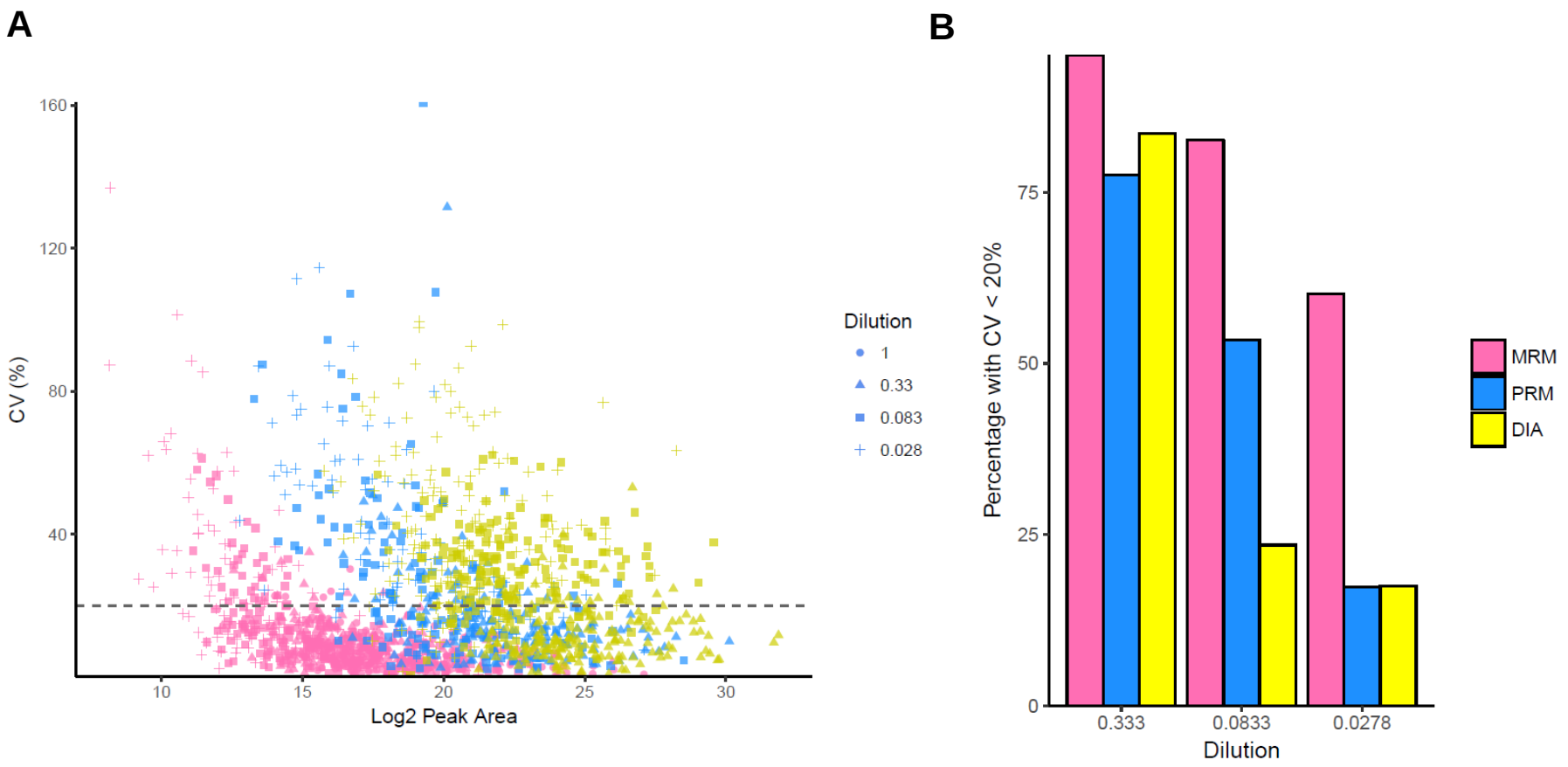
Supplemental Figure 4. Kinases downregulated by Erlotinib in each quantitative method. Desthiobiotin-labeled peptides downregulated following quantification by (**A**) MRM, (**B**) PRM, and (**C**) DIA, grouped by kinase family, are shown. Points represent the average $\log_2$(fold change) with bars indicating 95% confidence intervals. All labelled peptides with an average $\log_2$(fold change) ≤ -1 and were significantly decreasing (p-value ≤ 0.05, calculated using the student's one-tailed t-test).



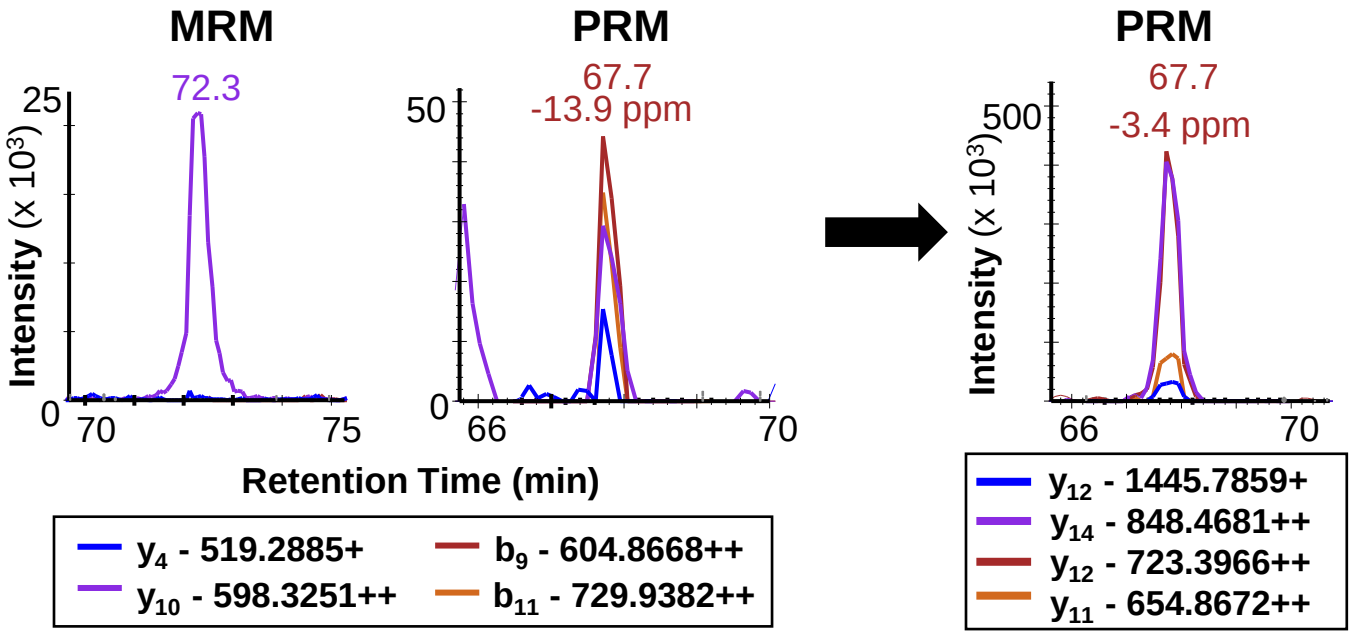
Supplemental Figure 5. Kinases downregulated by Dasatinib in each quantitative method. Desthiobiotin-labeled peptides downregulated following quantification by (**A**) MRM, (**B**) PRM, and (**C**) DIA, grouped by kinase family, are shown. Points represent the average $\log_2$(fold change) with bars indicating 95% confidence intervals. All labelled peptides with an average $\log_2$(fold change) ≤ -1 and were significantly decreasing (p-value ≤ 0.05, calculated using the student's one-tailed t-test).



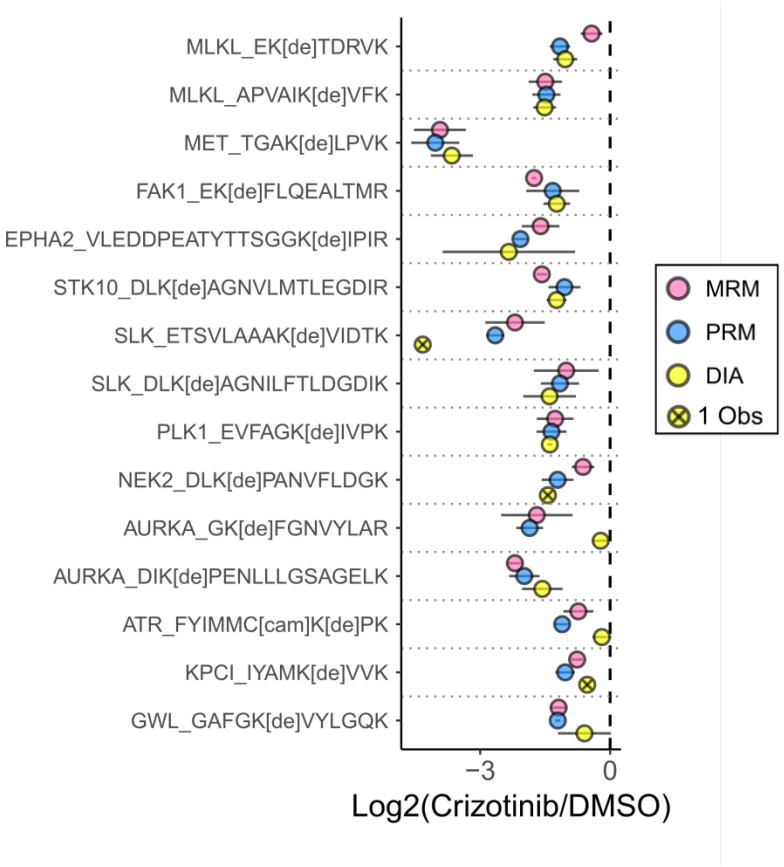
Supplemental Figure 6. DIA quantifies inhibition of STRAA_HUMAN probe labeling after Dasatinib treatment. Example peaks for a peptide (YSV**K**VLPWLSPEVLQQNLQGYDAK) from the kinase adapter protein, STRAA_HUMAN, are shown in control (DMSO) and Dasatinib treated lysates quantified by DIA (**A**). Example peaks (DMSO) from PRM (**B**), and MRM (**C**) datasets did not have quantifiable peaks in their scheduled time windows. DIA LC-MS/MS retains the ability to detect these peaks at the earlier retention time. All chromatograms were exported from Skyline.



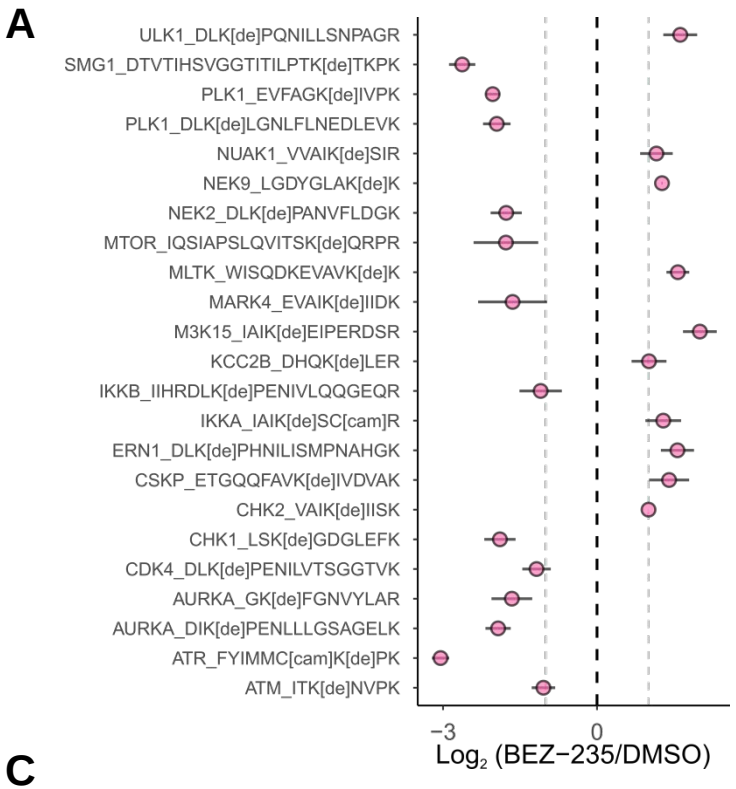
Supplementary Figure 7. Reproducibility assessment of MRM, PRM, and DIA quantification of a dilution series. A serial dilution of the desthiobiotinylated peptides was prepared from a single bulk sample and analyzed in triplicate by LC-MRM (pink), LC-PRM (blue), and LC-MS/MS with DIA (yellow). CV (%) is plotted against $\log_2$ peak area values for all measurements **(A)**, with an observable inverse relationship between $\log_2$ peak area and CV. The dotted horizontal line indicates a 20% CV cut-off. The percentage of peptides that fall below the 20% CV cut-off across the three platforms is presented for each dilution **(B)**.

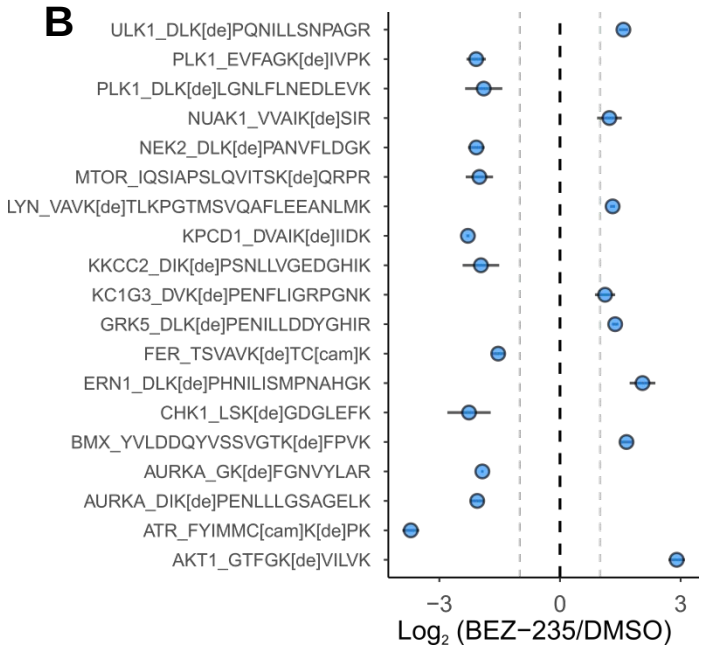


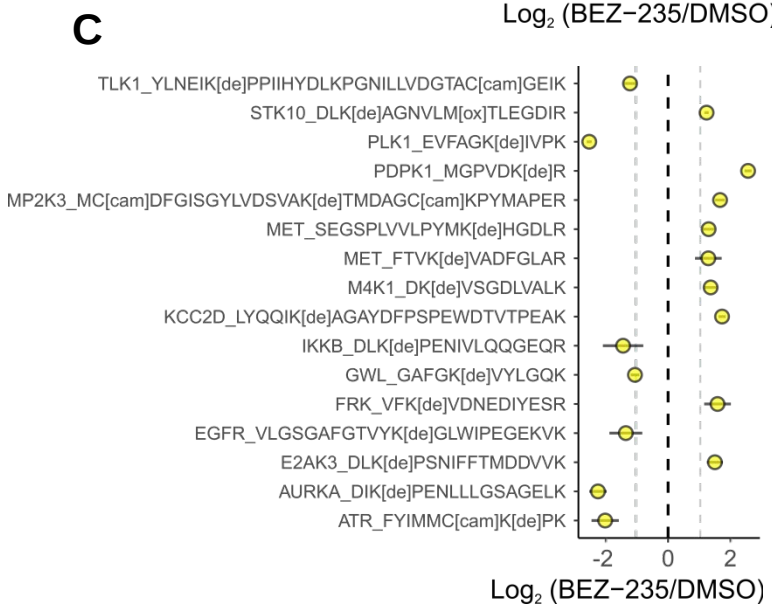
Supplementary Figure 8. PRM directs fragment ion selection for MRM assay development. Shown is an example of MRM method refinement using PRM for VQVAV**K**HLHIHTPLLDSERK from RIPK2. Fragment selection for MRM was created previously from ion trap MS/MS data and appears to be an incorrect match based on the error (-13.9 ppm) in the PRM data. Because all fragments are monitored by PRM, their selection can be changed to improve quantitation. Chromatograms were exported from Skyline.



Supplemental Figure 9. Kinases downregulated by Crizotinib across three quantitative platforms. Desthiobiotin-labeled peptides downregulated following quantification by MRM, PRM, and DIA, are shown. Points represent the average $\log_2$(fold change) with bars indicating 95% confidence intervals. All labelled peptides with an average $\log_2$(fold change) ≤ -1 and were significantly decreasing (p-value ≤ 0.05, calculated using the student's one-tailed t-test).







Supplemental Figure 10. Kinases differentially regulated by BEZ-235 in each quantitative method. Desthiobiotin-labeled peptides increasing or decreasing following quantification by MRM (**A**), PRM (**B**), and DIA (**C**) are shown grouped by kinase family. Points represent the average $\log_2$(fold change) with bars indicating 95% confidence intervals. All labeled peptides with an average $\log_2$(fold change) ≤ -1 or ≥ 1 and were significantly different (p-value ≤ 0.05, calculated using the student's two-tailed t-test).