# Monitoring cell surface N-glycoproteome dynamics by quantitative proteomics reveals mechanistic insights into macrophage differentiation


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## Running Title

Cell surface glycoproteome dynamics

## Supplemental Tables

**Table S1: Cell surface proteome mapping by on-bead digestion and elution with trypsin, related to Figures 1,2 and 3.**

**Table S2: Cell surface proteome mapping by on-bead digestion and elution with PNGase F, related to Figure 1.**

**Table S3: Plasma membrane proteome remodeling during monocyte to macrophage differentiation, related to Figures 4 and 5**

**Table S4: Influence of kinase inhibitors on monocyte to macrophage differentiation process, related to Figure 6**

**Table S5: Kinobeads kinase inhibitor profiles, related to Results**

## Supplemental Figure Legends

**Figure S1: Reproducibility of cell surface proteome mapping and comparison of trypsin-protocol vs. PNGase F-protocol vs. whole-Proteome analyses, related to Figure 1.**

A. Representative comparison of estimated abundances of identified plasma membrane proteins in two biological replicates of the plasma membrane proteome mapping on YT cells. Abundance estimation is based on the $\log_{10}$ average MS-Signal of the three most intense tryptic peptides. Coefficient of correlation $R^2$:0.87. B. Average overlap of identified proteins in two biological replicates of plasma membrane proteome mappings of cell lines and primary cell types (n=18, standard deviation in percentage). C. Comparison of Trypsin- and PNGase F protocol on plasma membrane protein and number of unique peptide identification. D. Number of identified plasma membrane annotated proteins in cell surface proteome enrichment and published whole proteome analysis samples. E. As in D but for plasma membrane annotated protein with annotated transmembrane domains. F. Comparison of cell surface proteome enrichment and published whole proteome analysis on number of unique peptide identifications of plasma membrane proteins with annotated transmembrane domains. G. Density plots of abundance estimations before and after normalization of trypsin samples. H. As in G but showing PNGase F data. I. Principal component analysis based on identified cell surface proteins and their abundances in additional cell surface proteome mappings for 4 cell lines with 4 replicates. J. Cumulative increase in numbers of identified cell surface proteins per number of replicates. K. Number and abundance of all identified proteins colored according to number of annotated glycosylations. L**.** Abundance of identified cell surface proteins colored according to number of annotated glycosylations. Small pie chart showing relative fraction of non-glycosylated cell surface proteins with or without annotated transmembrane domains.

**Figure S2: The cell surface proteomes of primary cell types are strikingly different from immortalized cells and reflect lower metabolism and higher functional specialization, related to Figure 3.**

A. Fractional abundance of plasma membrane proteome subgroups on the cell surface of cell lines and primary cell types. Fractional abundances represent the summed abundance estimations for each subgroup relative to the summed abundance estimations of all identified plasma membrane proteins. Prominent plasma membrane proteome subgroups are ATP-binding cassette transporters (ABC = magenta), cluster of differentiation proteins (CD = blue), G-protein coupled receptors (GPCR = green), Integrins (ITG = yellow), solute carrier transporters (SLC = orange) and all other plasma membrane proteins in light grey. B. Same data set as in A. but illustrating identified numbers of proteins.

**Figure S3: Time resolved dynamics in the plasma membrane proteome reflect biological and cell type environment differences between monocytes and macrophages, related to Figures 4 and 5.**

A. Illustration of all identified significantly regulated plasma membrane proteins during differentiation from monocytic to macrophage-like cells. Standard deviations in Replicate 1 are represented as error bars. B. As in A but illustrating all identified significantly regulated plasma membrane kinases. C. Heat map of average $\log_2$ relative abundances for significantly regulated plasma membrane proteins during differentation. Up-regulation is represented by red colors while down-regulation is represented by blue colors. A significantly regulated protein was specified to have a Benjamini-Hochberg corrected p-value < 0.01 and a $\log_2$ relative fold change >= 2 or <= -2 at least once in the time course. Protein clustering by row dendrogram (clustering: UPGMA, distance: euclidian, ordering: average value, normalization: Z-score). D. As in C but showing the subset of proteins early up-regulated during the differentiation time course.

**Figure S4: THP-1 differentiation in presence of dasatinib results in changed cellular morphology associated with modified cell surface markers and reduced phagocytotic activity, related to Figure 6.**

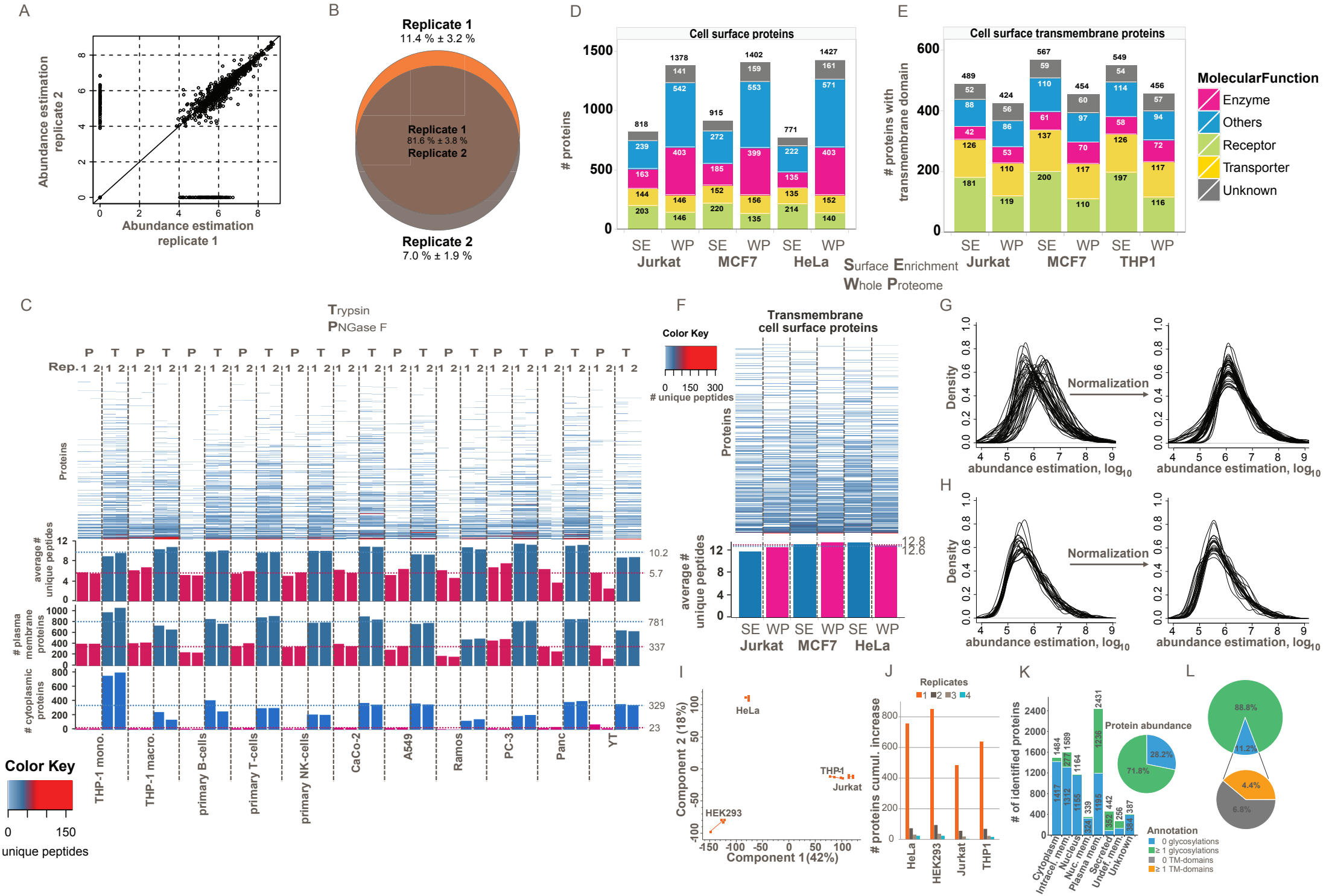
A. Differentiation of THP-1 cells over 48h with PMA in presence of 1µM kinase inhibitors sunitinib, dasatinib and imatinib in comparison to untreated control. B. Proteins with significantly altered abundance in THP-1 cells differentiated in presence or absence of sunitinib.

**Figure S5: Targeted kinases by marketed kinase inhibitors, related to Results.**

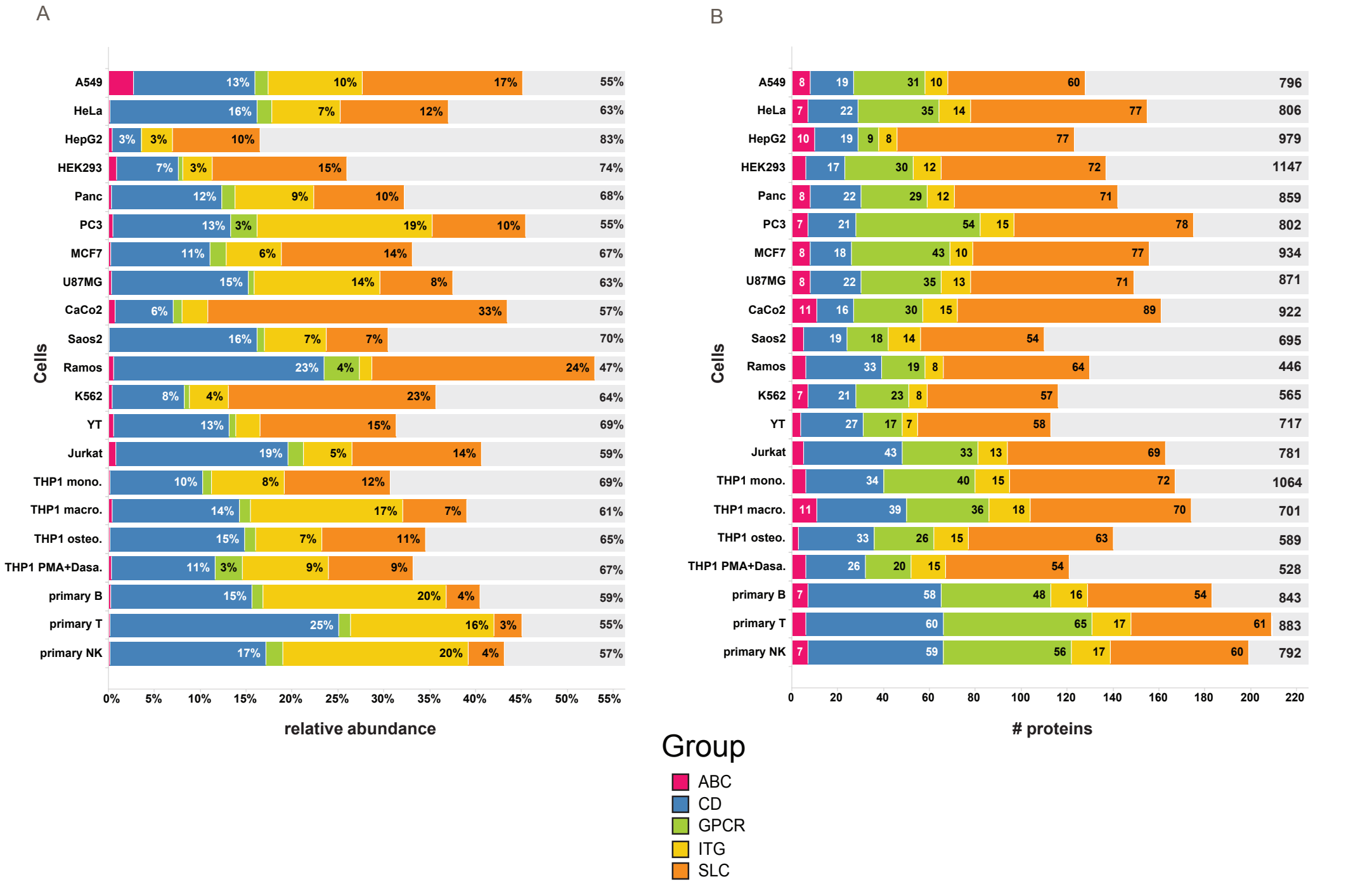
Heatmap of targeted kinases by the kinase inhibitors imatinib, sunitinib and dasatinib evaluated by kinobeads assay with $pIC_{50} \geq 6$.

## Supplemental Figures

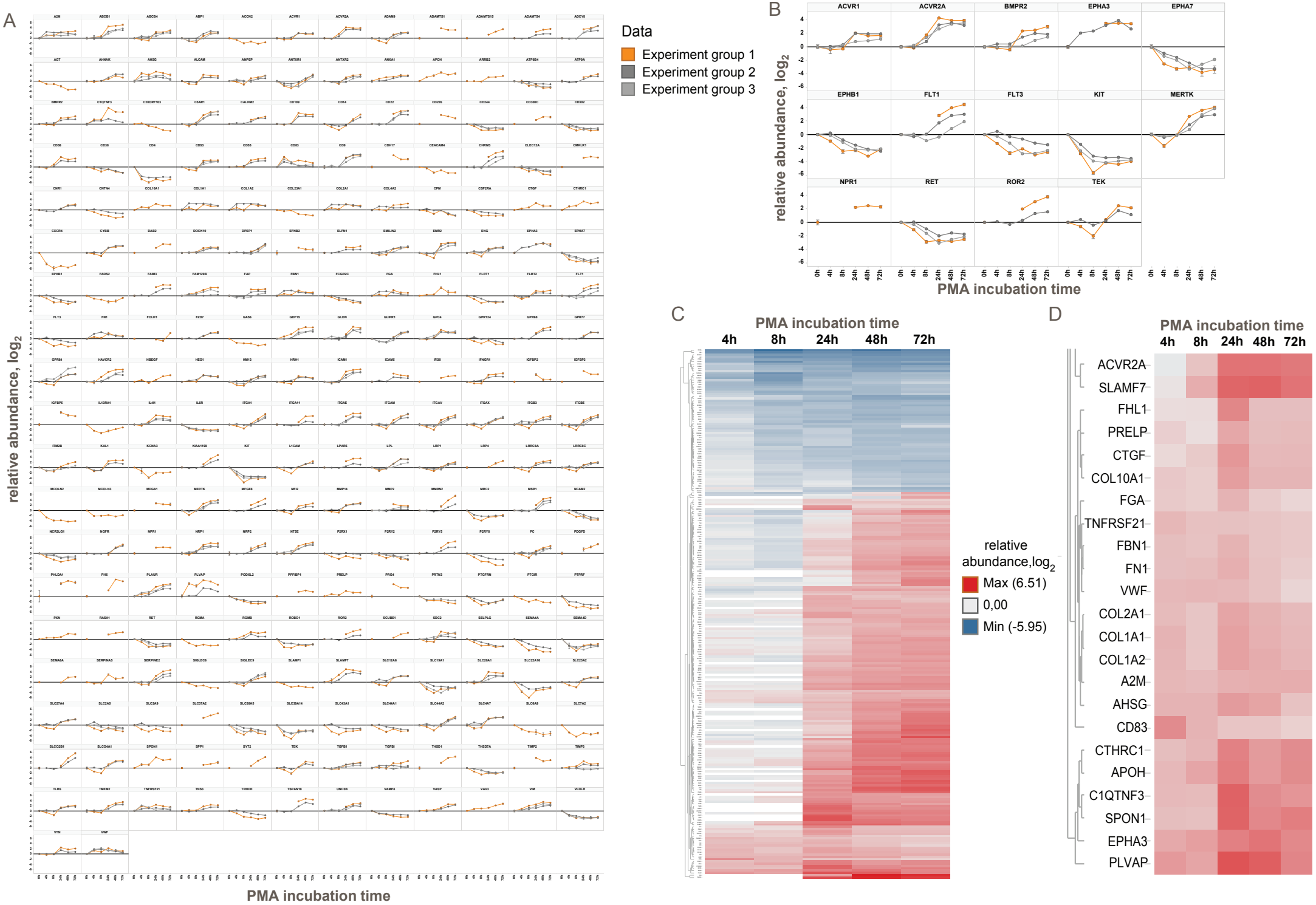
# supplemental Fig. S1



# supplemental Fig. S2

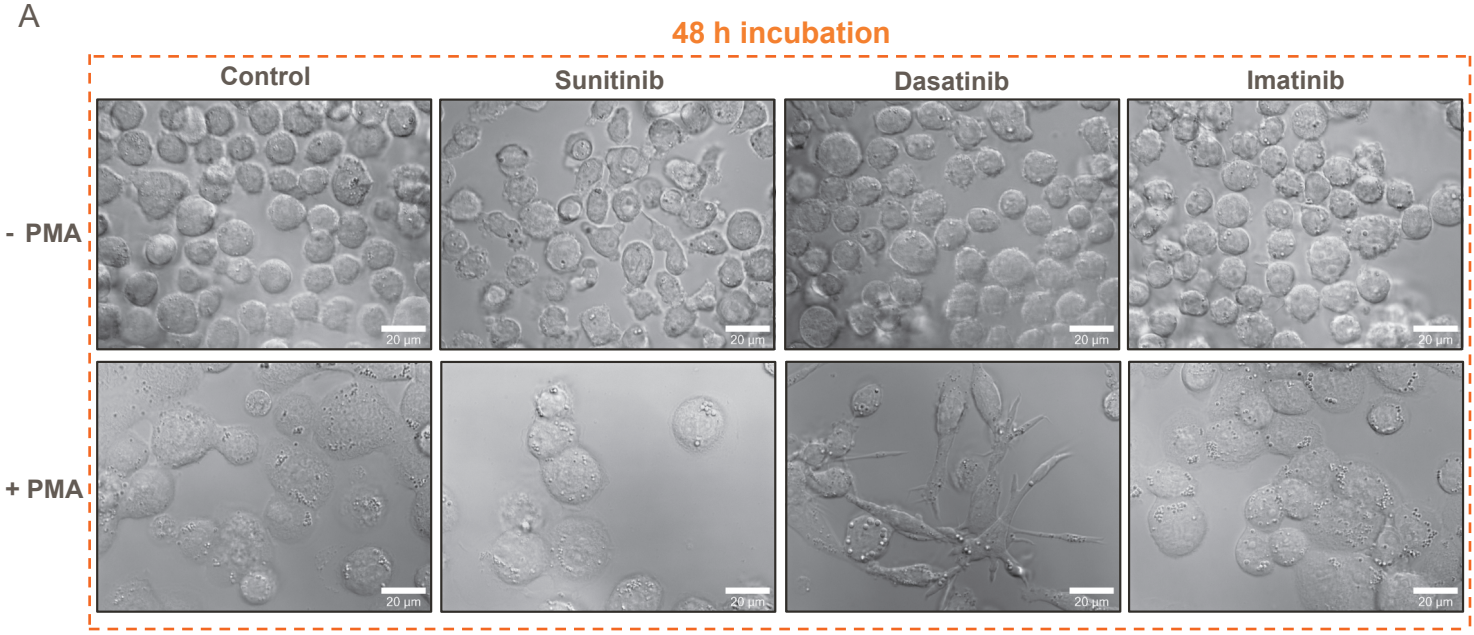


# supplemental Fig. S3



supplemental Fig. S4

A

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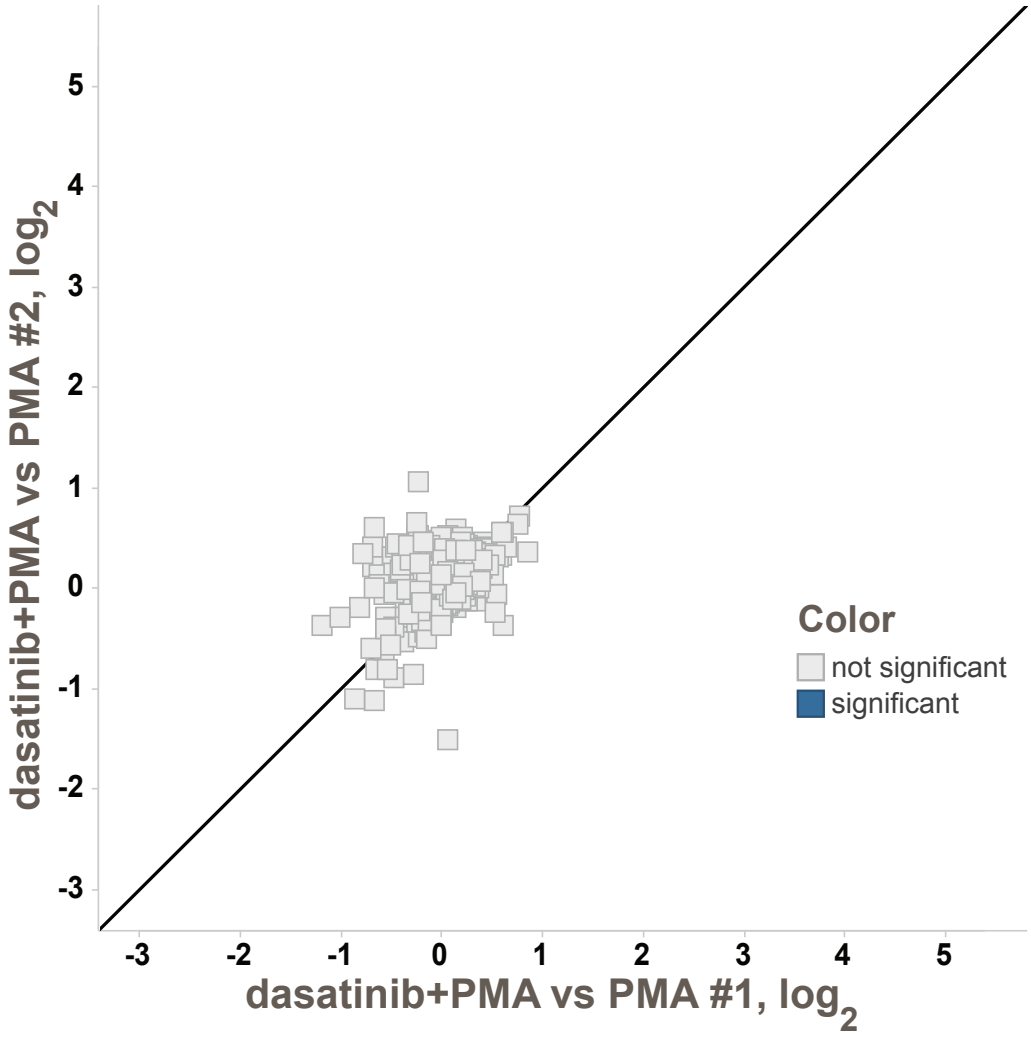

supplemental Fig. S5