

Dark Kinases (KRSA Report)

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Introduction

Background

The Pamstation12 instrument provides a profiling of kinase activity of cell or tissue samples. The device is loaded with either serine/threonine or tyrosine microarray chips. Each chip has 4 wells so four samples can be loaded on a single chip, and the Pamstation12 can accommodate 3 chips per run. The microarray represents 144 (STK chip) or 196 (PTK chip) reporter peptides that can be phosphorylated by serine/threonine or tyrosine kinases. The device measures the degree of the phosphorylation in real time by detecting fluorescently labeled antibodies at different exposure times. The list of peptides present in each microarray can be viewed here: [STK chip](#), [PTK chip](#)

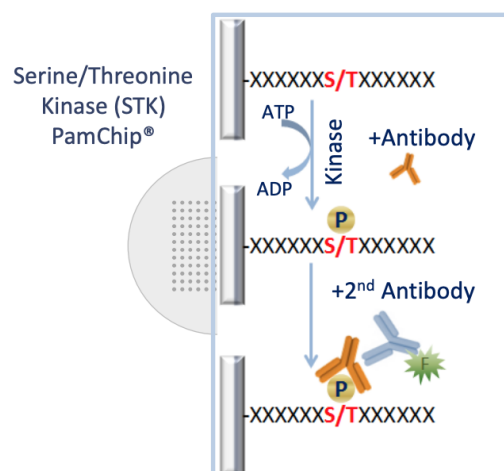
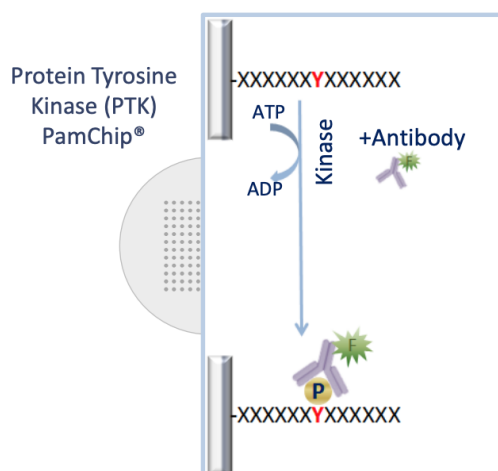
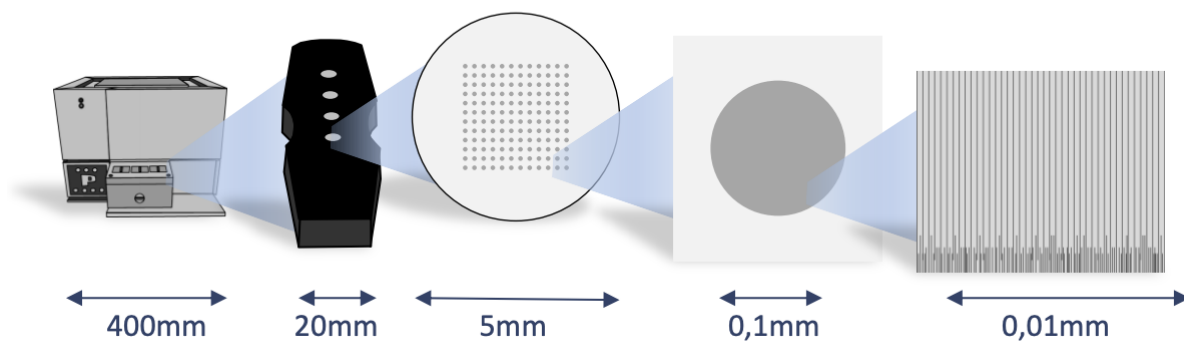
PamStation® 12

PamChip® 4
(4 arrays)

array
(144 spots)

spot
(1 peptide)

Porous membrane
(~300,00 pores)



Results

Image Analysis

The first step of analyzing the run is to convert the images taken by the PamStation of each array at different exposure times to numerical values. This is done by the Bionavigator software developed by Pamgene. The software recognizes the grid of the array with the aid of the searching algorithm (Pamgrid) to correctly identify each spot on the array. The numbers produced by this software represent the median value of the foreground pixels minus the median value of the background pixels to produce the median signal minus background (Median_SigmBg).

Reading Data

The first step will be reading the crosstab view bionavigator files (Median_SigmBg and Signal_Saturation) and defining the PamChip type (STK or PTK). The raw data is read and then transformed to be in tidy format for an easier analysis, modeling, and visualizing.

QC Initial Steps and Groups Assignments

We will perform a couple of quality control steps to deal with negative values in the data and adjust based on signal saturation (optional). Next, we will define a new column to represent the grouping. And then, we will extract end point signal values.

QC Steps and Model Fitting

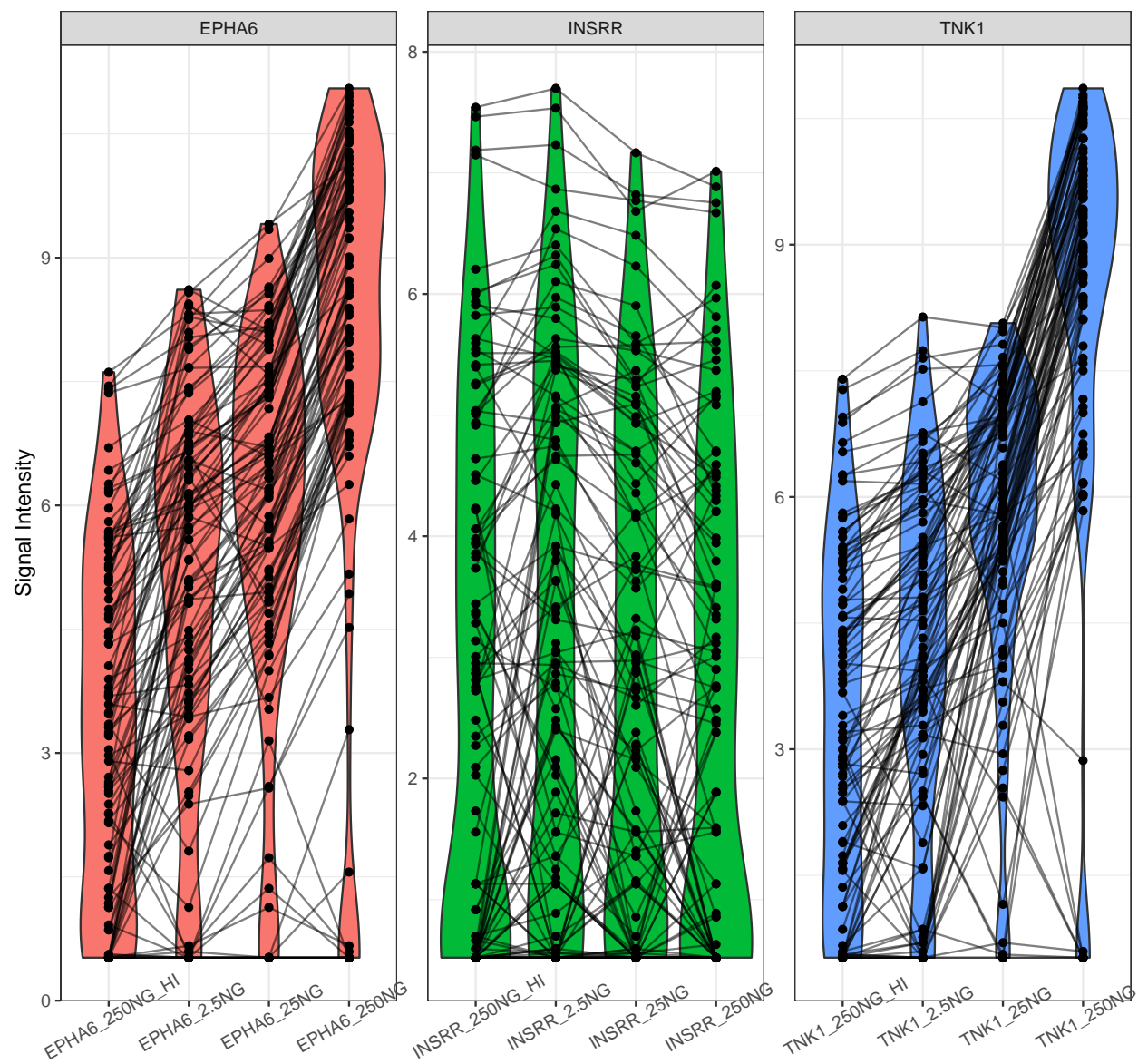
We will filter out peptides with low signals. In order to combine the values from different exposure times into a single value, a simple linear regression model of the *Median_SigmBg* as a function of exposure time is fitted. The slope of the model fit and R^2 are then used for quality control and samples comparison. The slope is also scaled by multiplying by 100 and log2 transformed (*Slope_Transformed*). We then filter out peptides with poor linear fit and reference peptides.

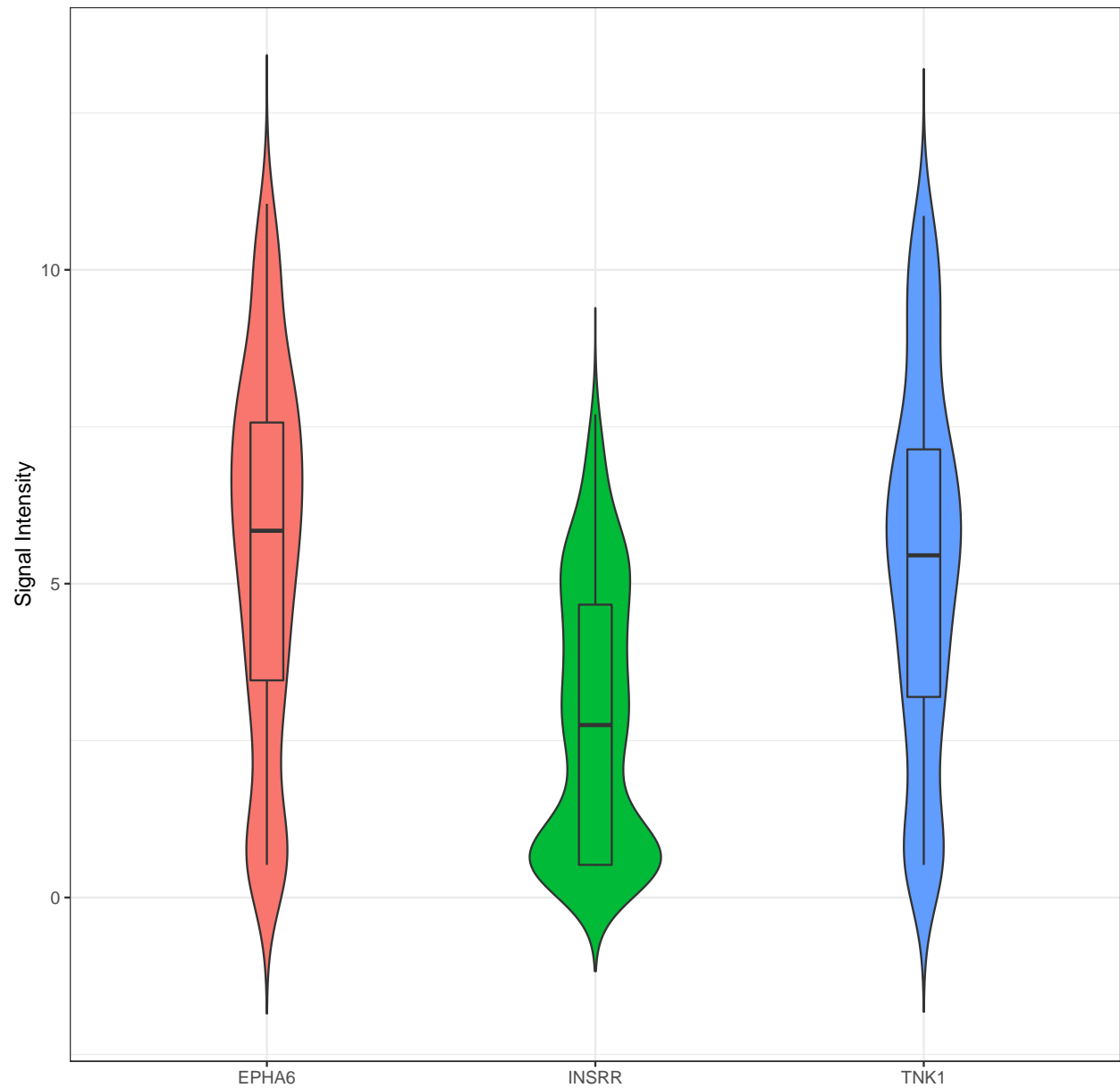
Global Signal Intensity

For a global signal intensity across all samples/groups, few figures can be plotted based on the *Slope_Transformed* values.

Global Violin Plots

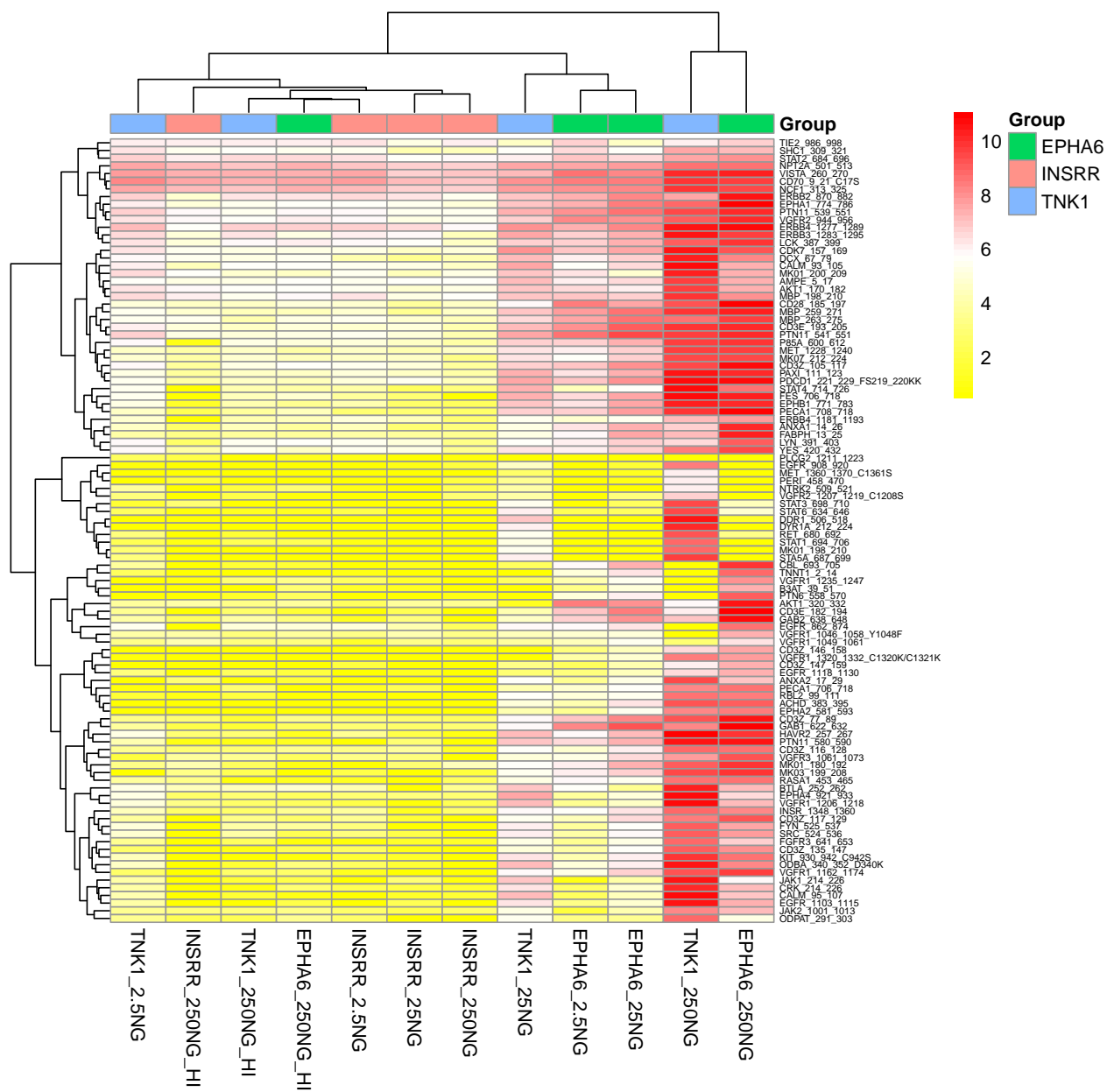
We will plot violin figures to examine global signal differences between groups/samples.



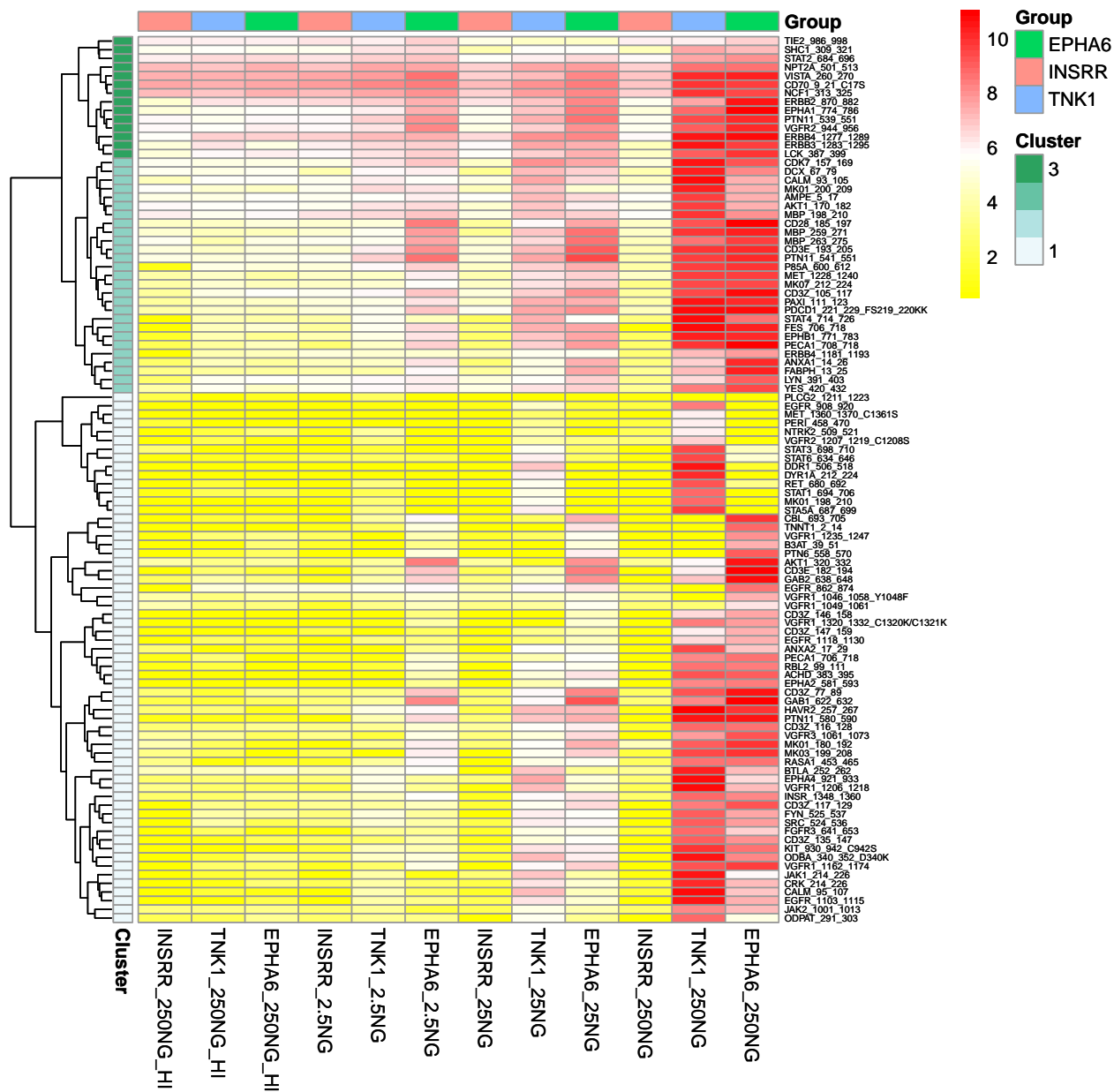


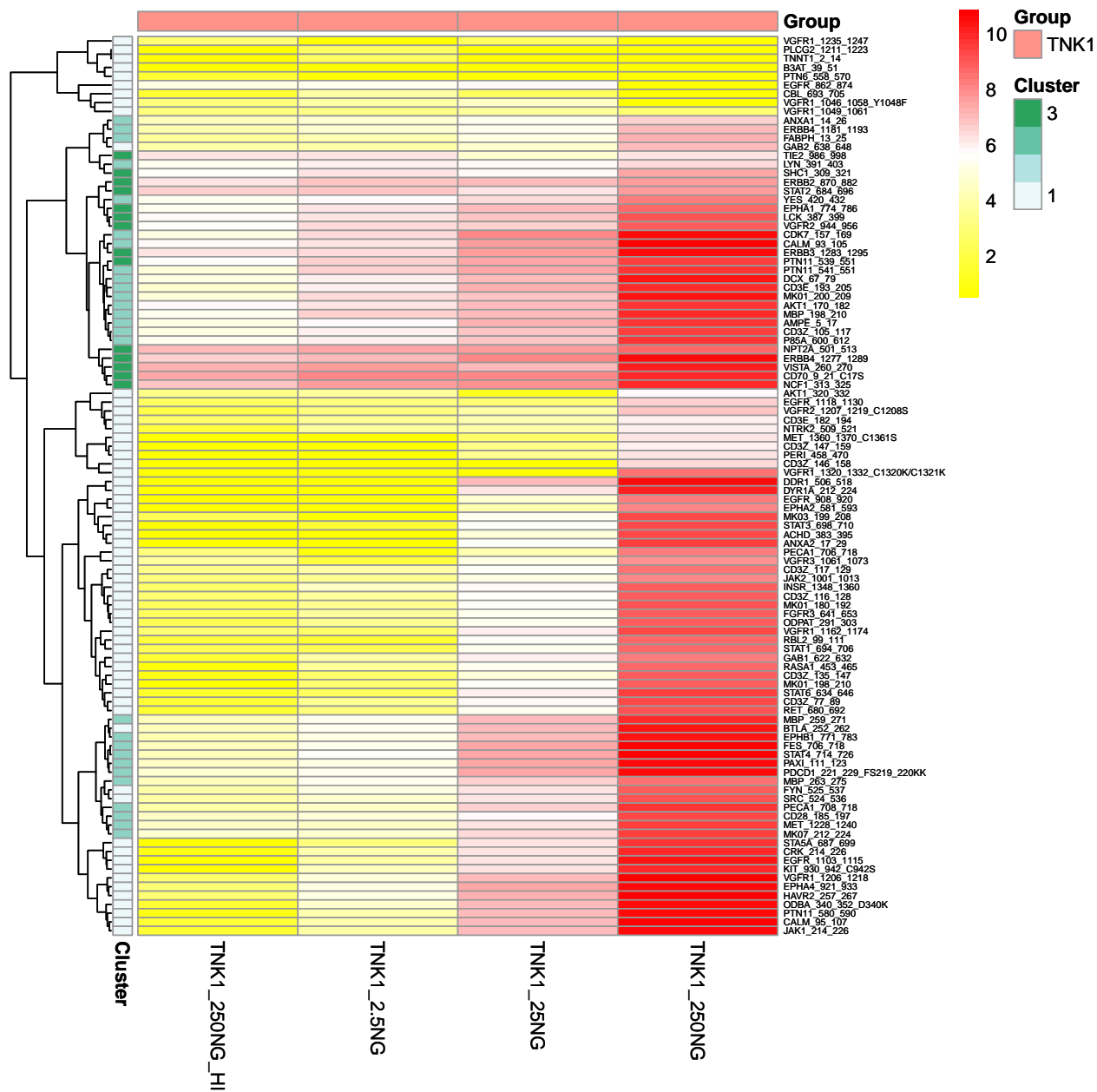
Global Heatmaps

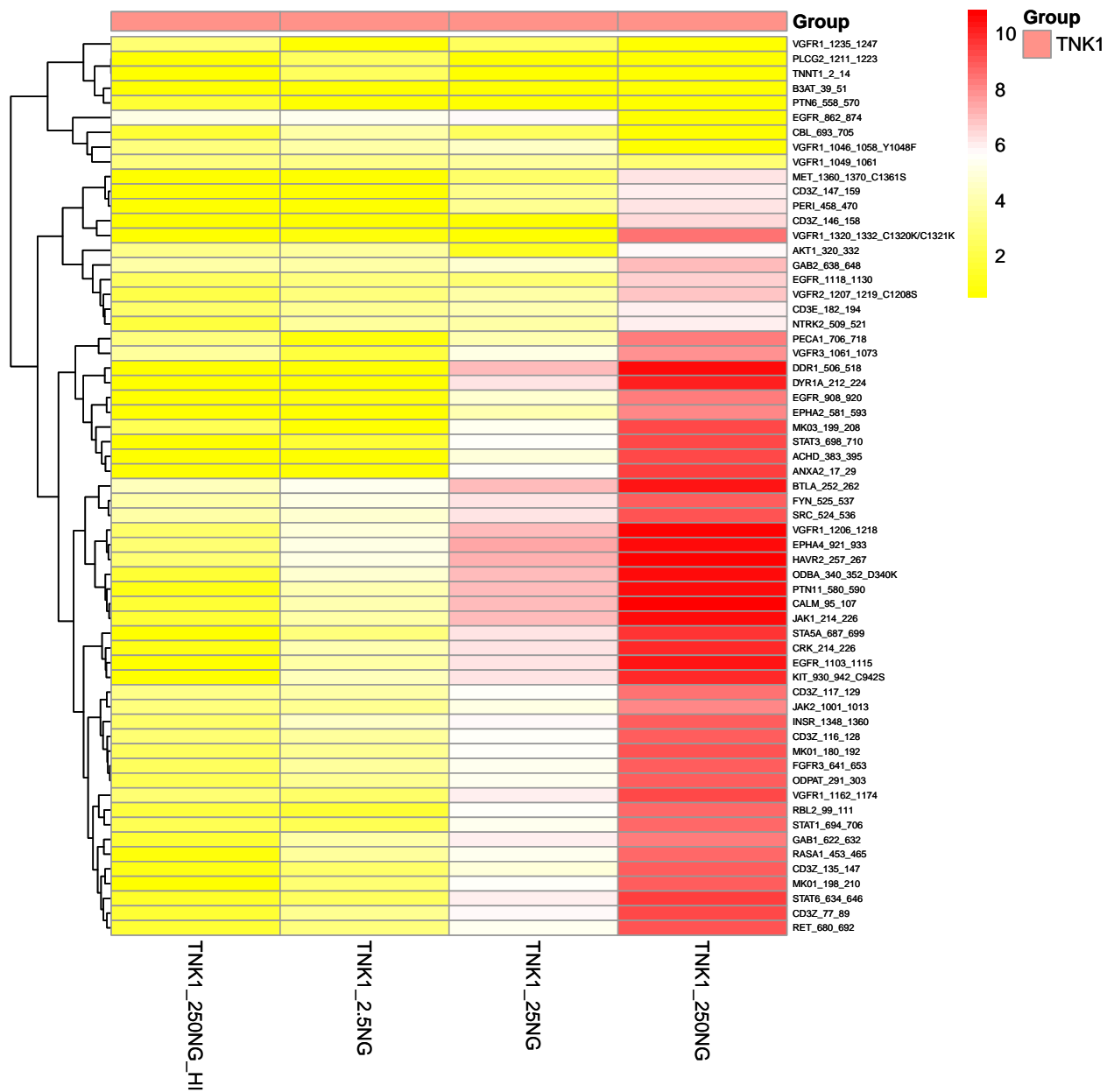
The heatmap represent all the peptides present on the chip except the positive/internal controls and peptides that failed to pass QC. The heatmaps are scaled by row to highlight the peptide signal differences across the samples. A hierarchical unsupervised clustering is applied both on the peptides and the samples to potentially group similar signatures.

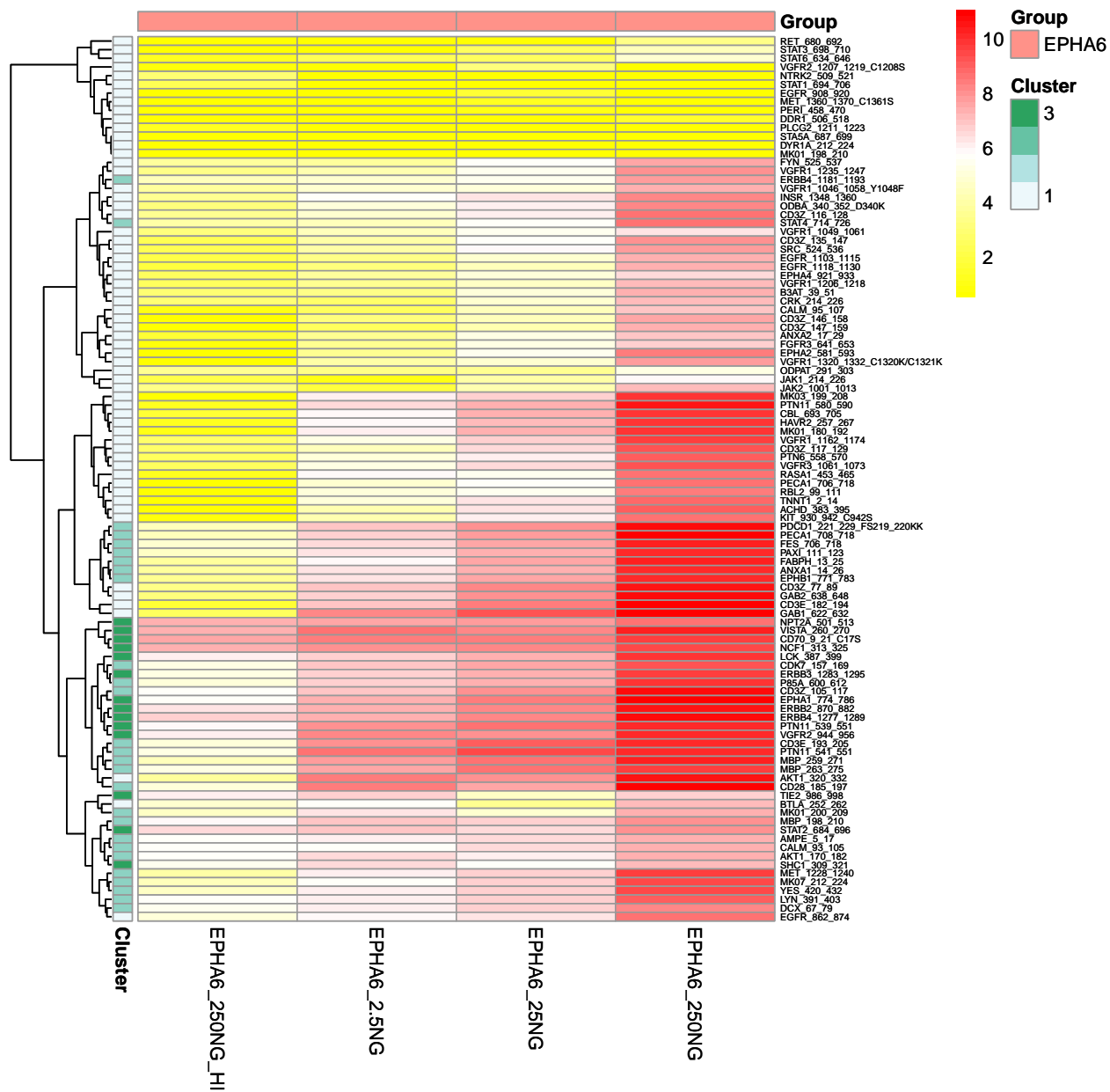


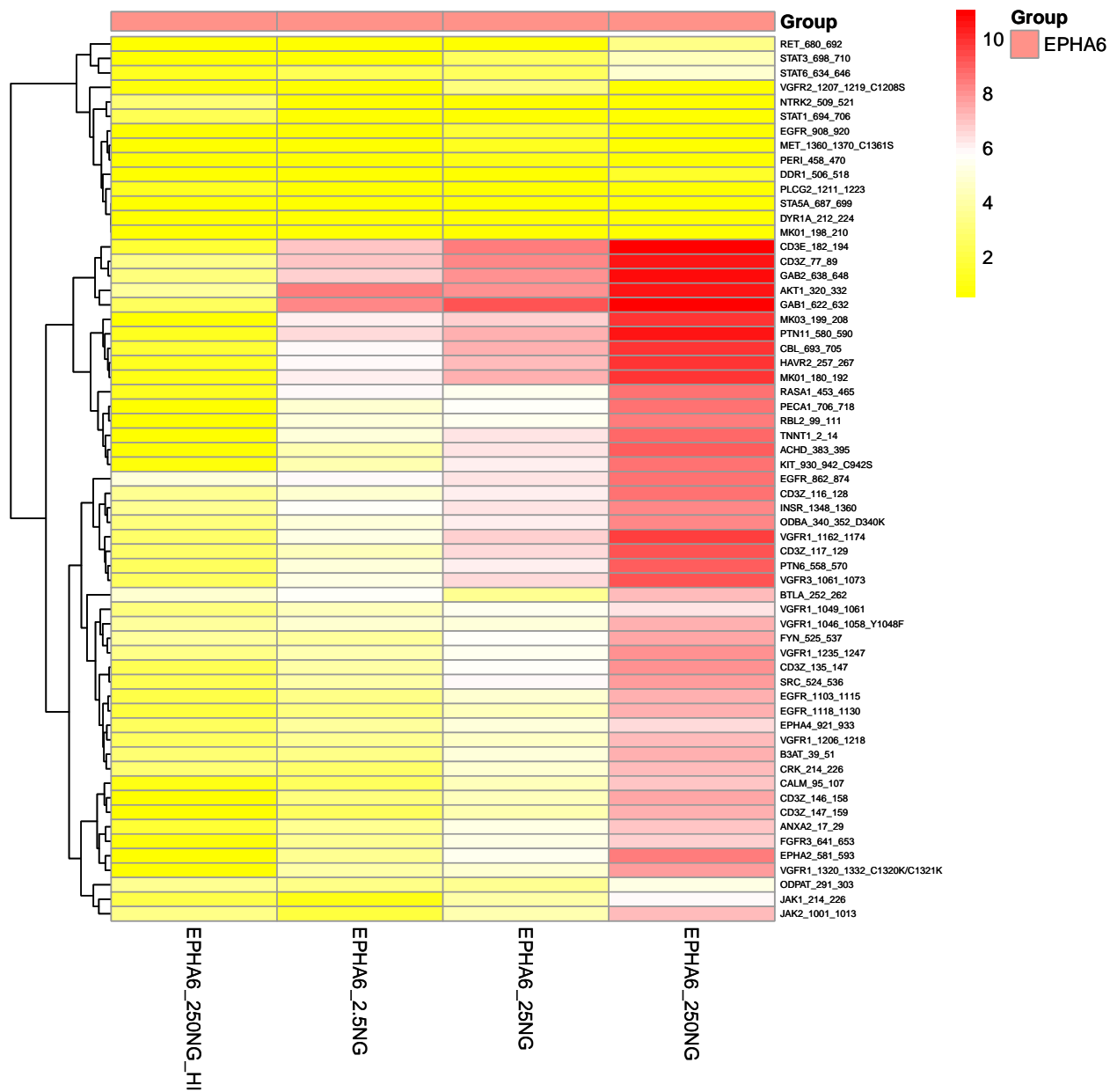


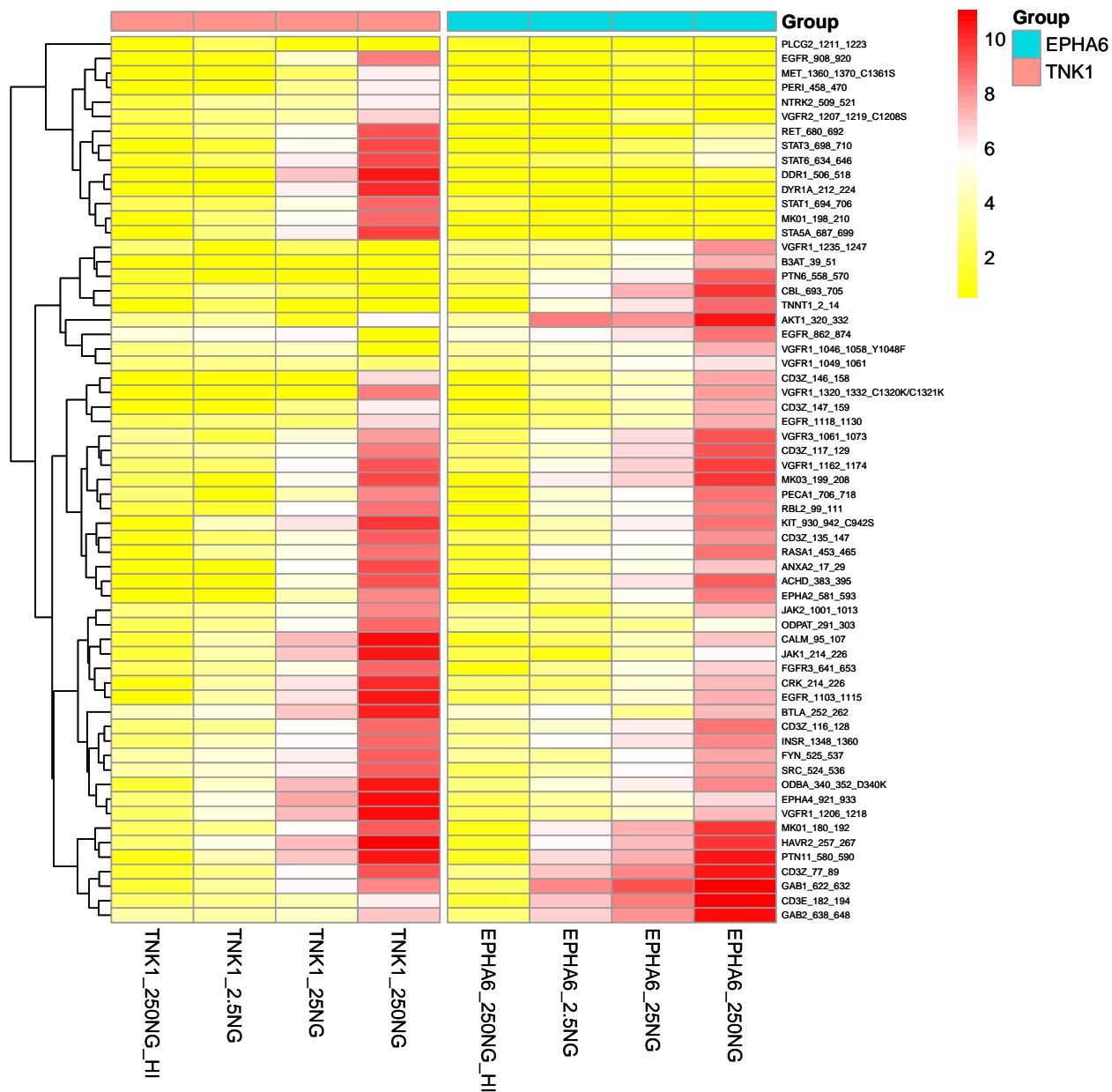












Group Comparison

To compare between samples, a two-group comparison is performed. In this case, the two group comparisons are:

- **TNK1**
- **EPHA6**

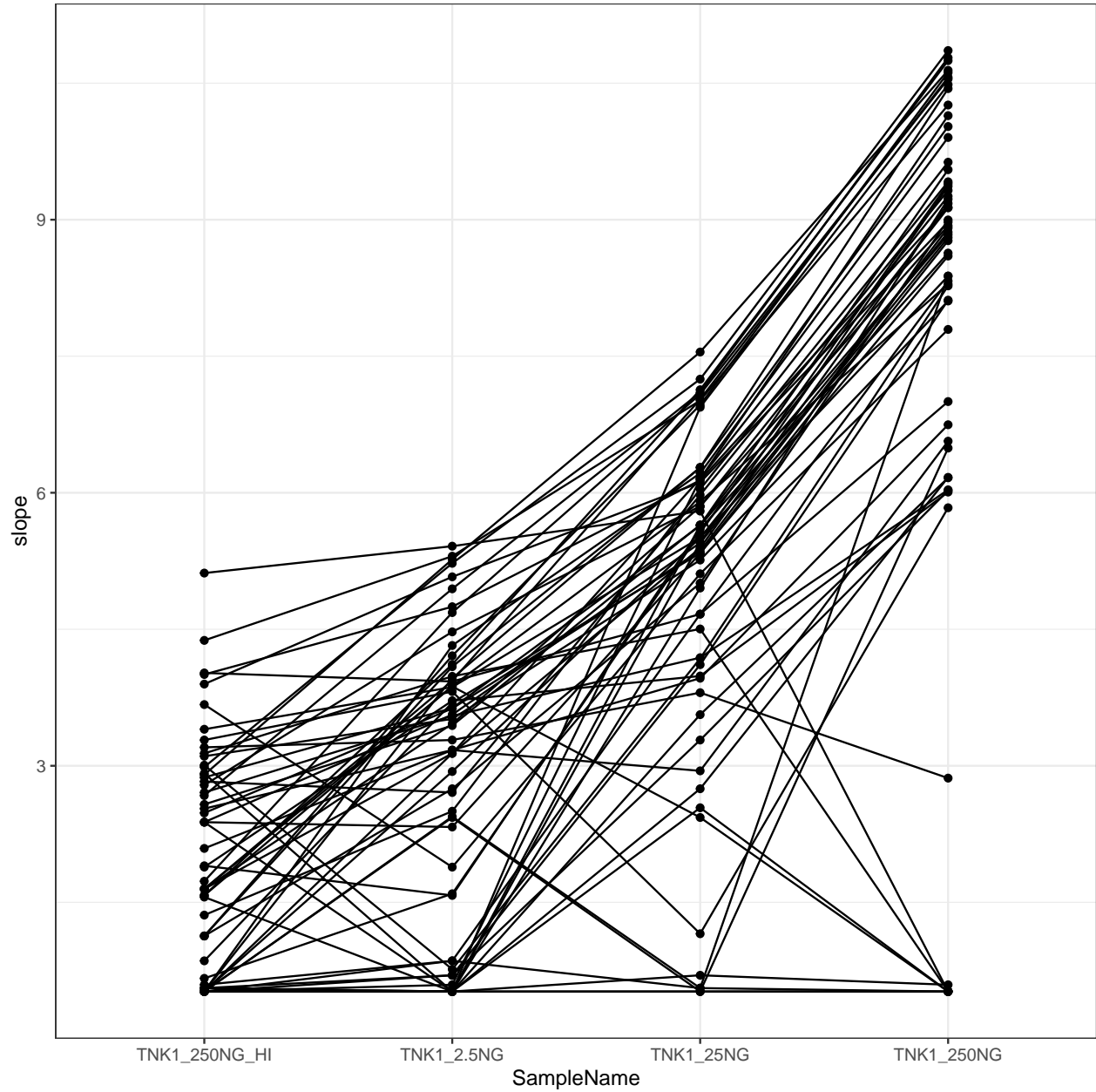
The *Slope_Transformed* ratio between each group, paired by chip, is calculated to the fold change. Based on the fold change, peptides that pass a certain fold change threshold are considered significant hits. Also, quality control steps applied in each comparison to filter out peptides that do not reach specific criteria:

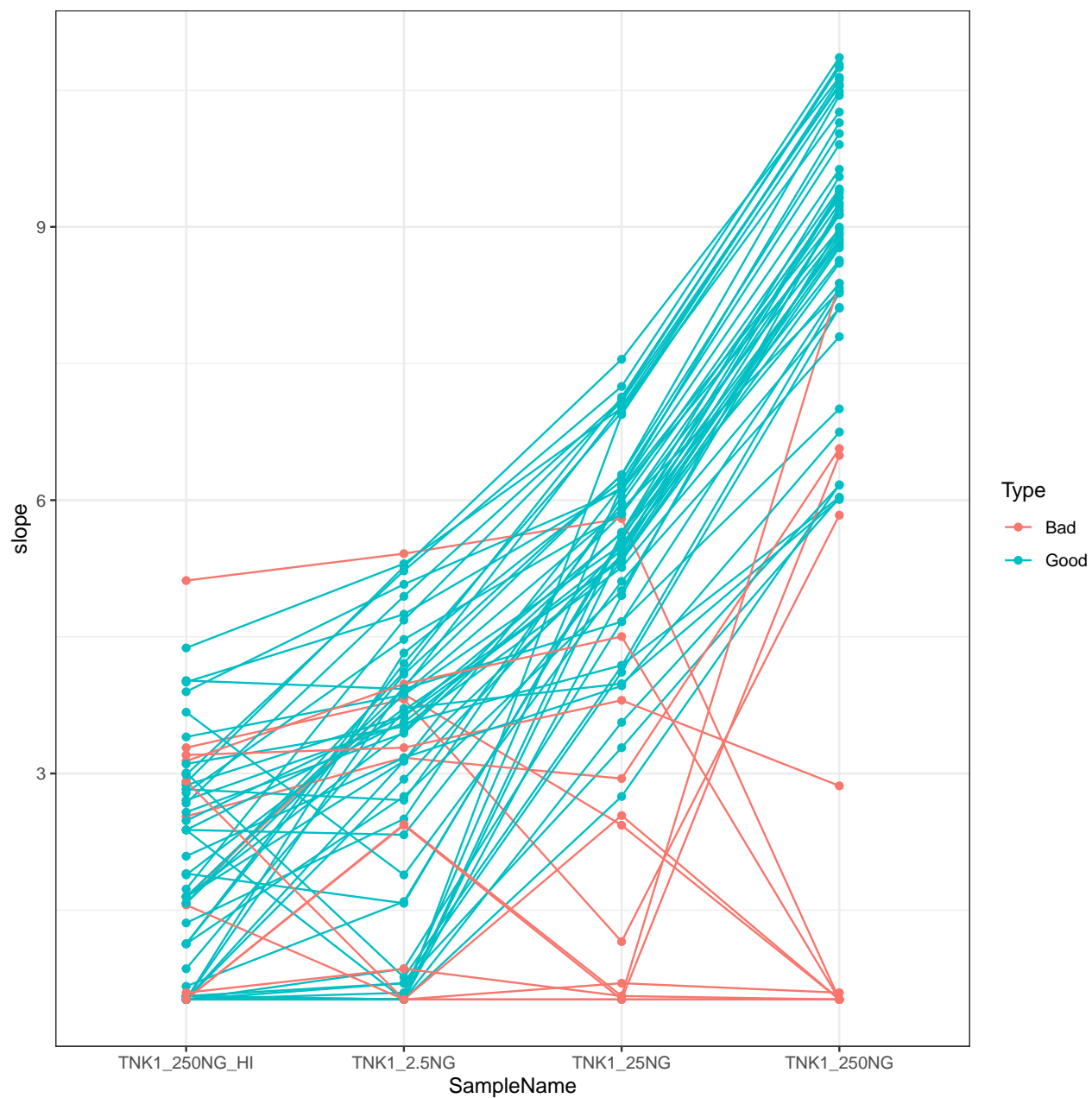
- The *Medain_SigmBg* at max exposure *100ms* must be above a certain value

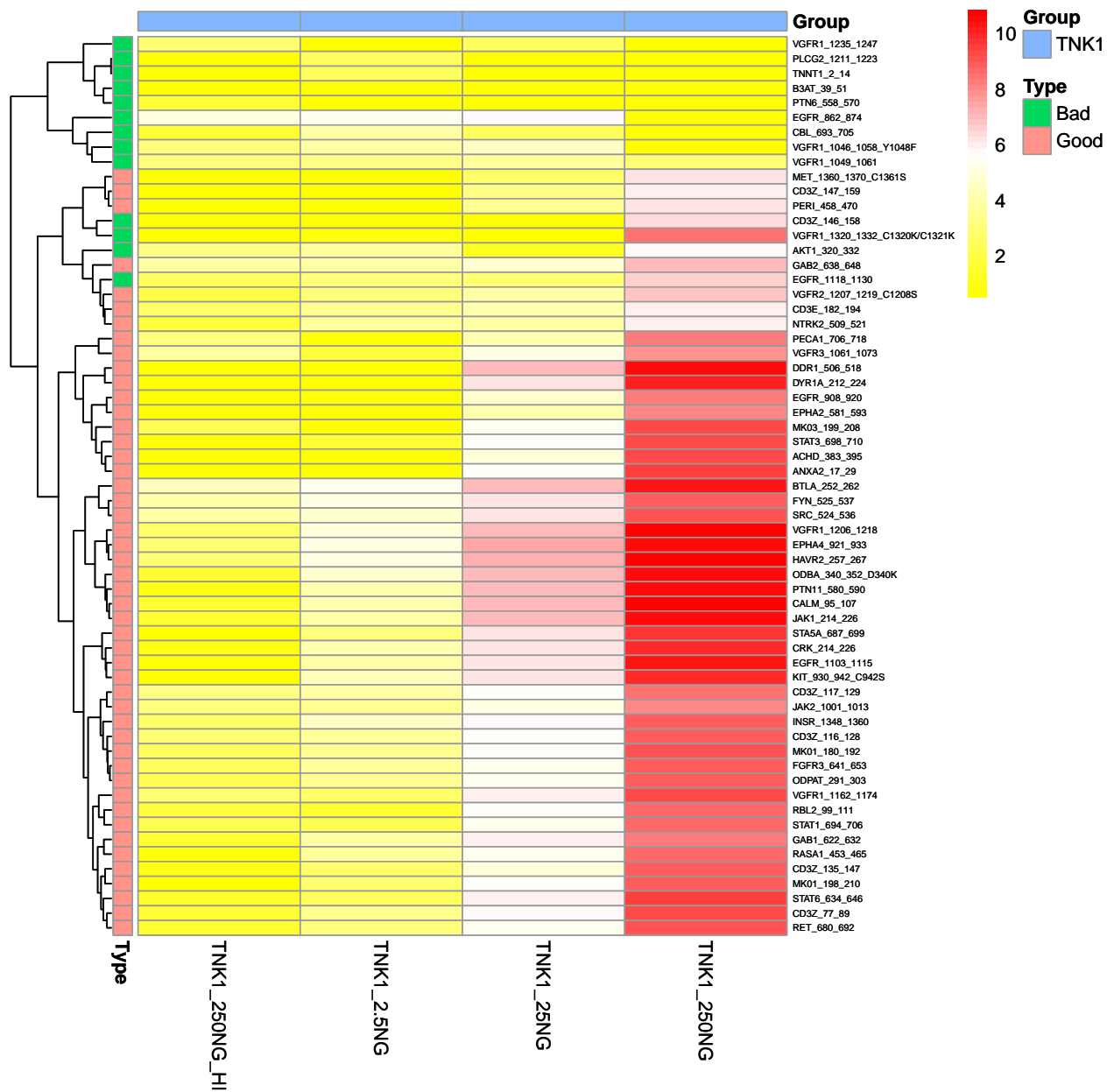
- R^2 of the linear model fit must be above a threshold value

These *Filtering Parameters* (fold change threshold, QC criteria) can be modified to adjust the stringency of the analysis. The *Filtering Parameters* that are used for this analysis:

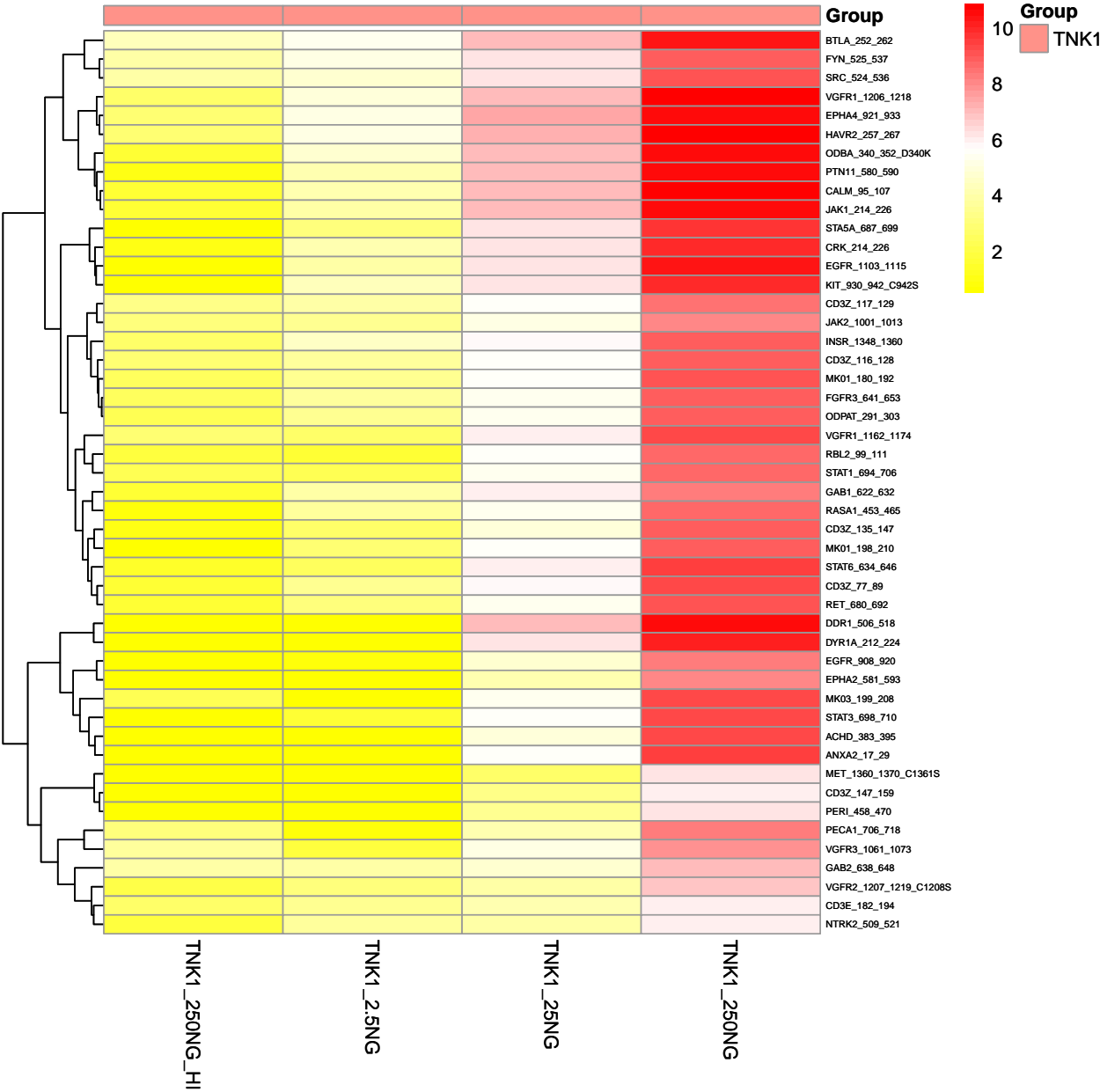
- The *Medain_SigmBg* at max exposure *100ms* must be equal or above 5
- R^2 of the linear model fit must be above or equal 0.9
- Excluded non-specific peptides
- Log fold change (LFC) cutoffs at (0.2,0.3,0.4) between IH and highest concentration



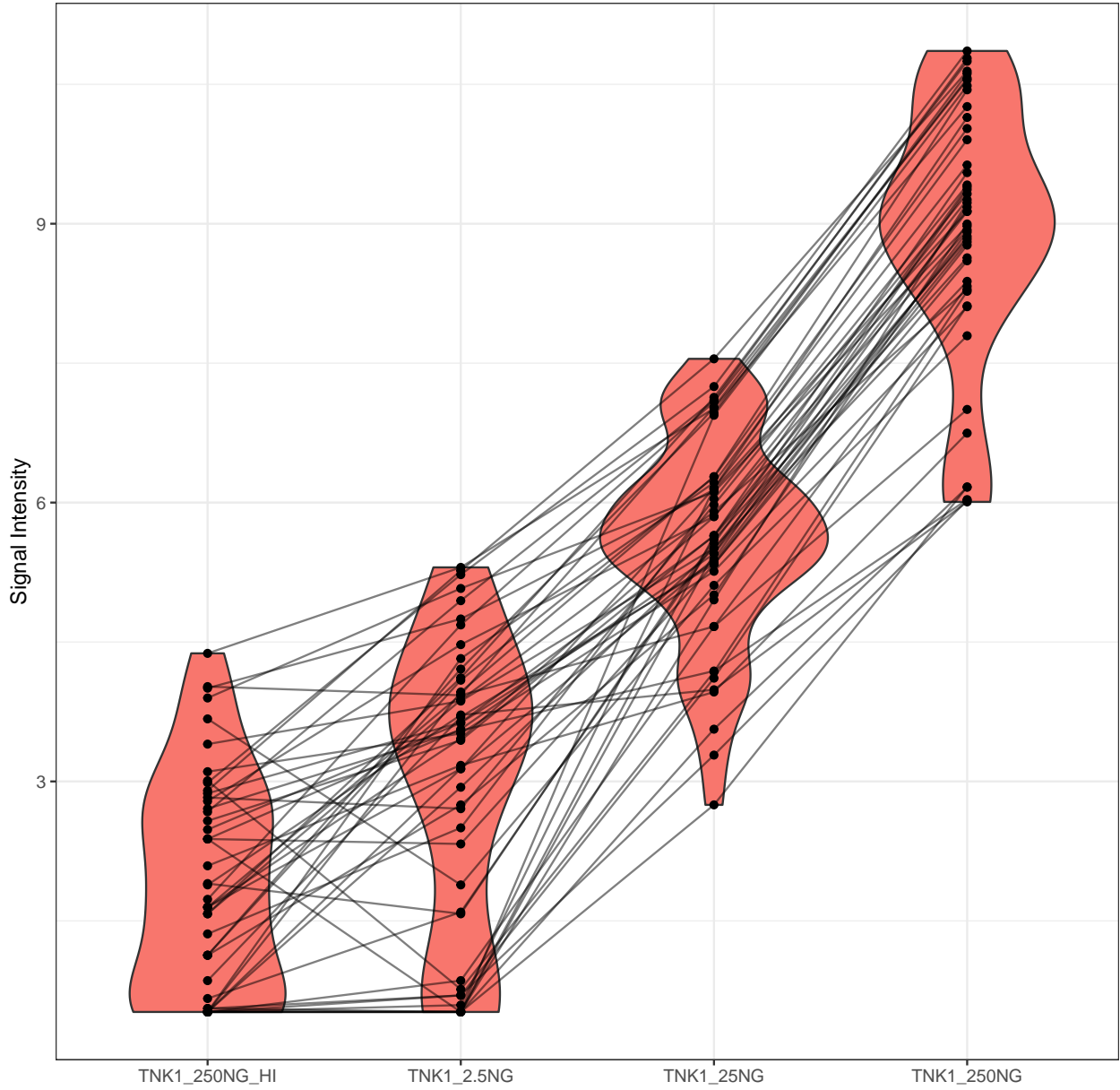


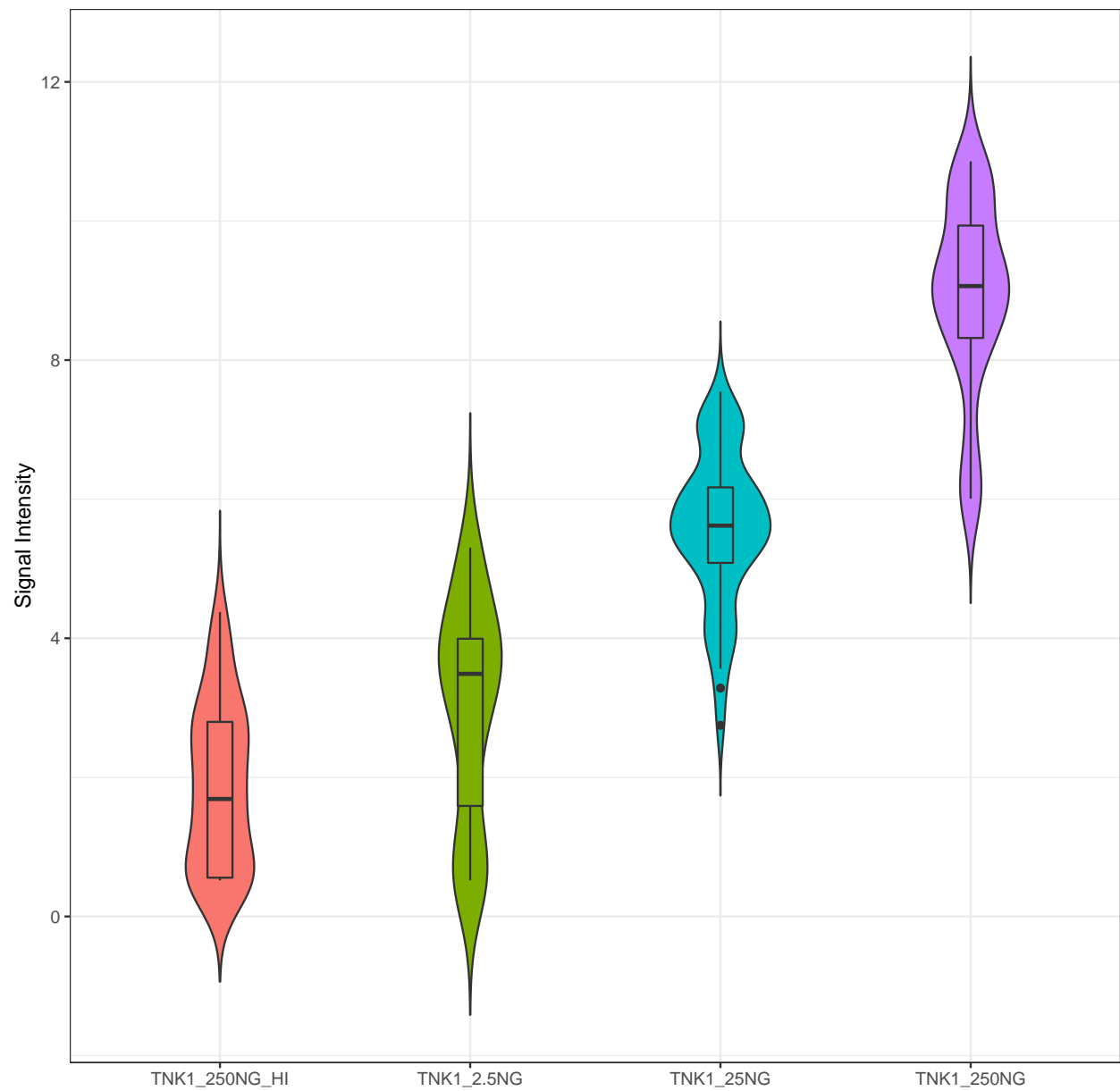


TNK1 (Heatmap)



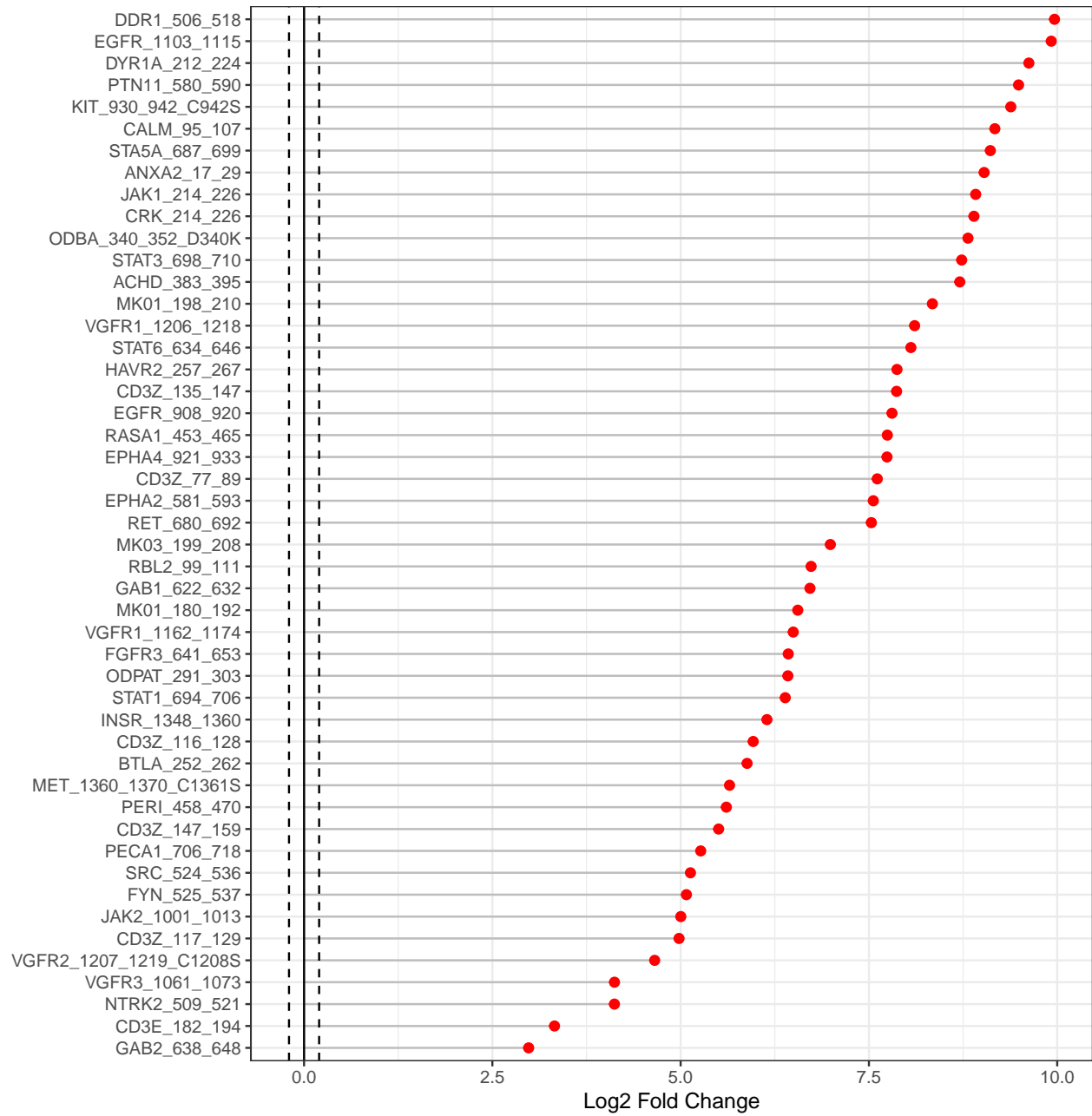
TNK1 (Violin Plot)





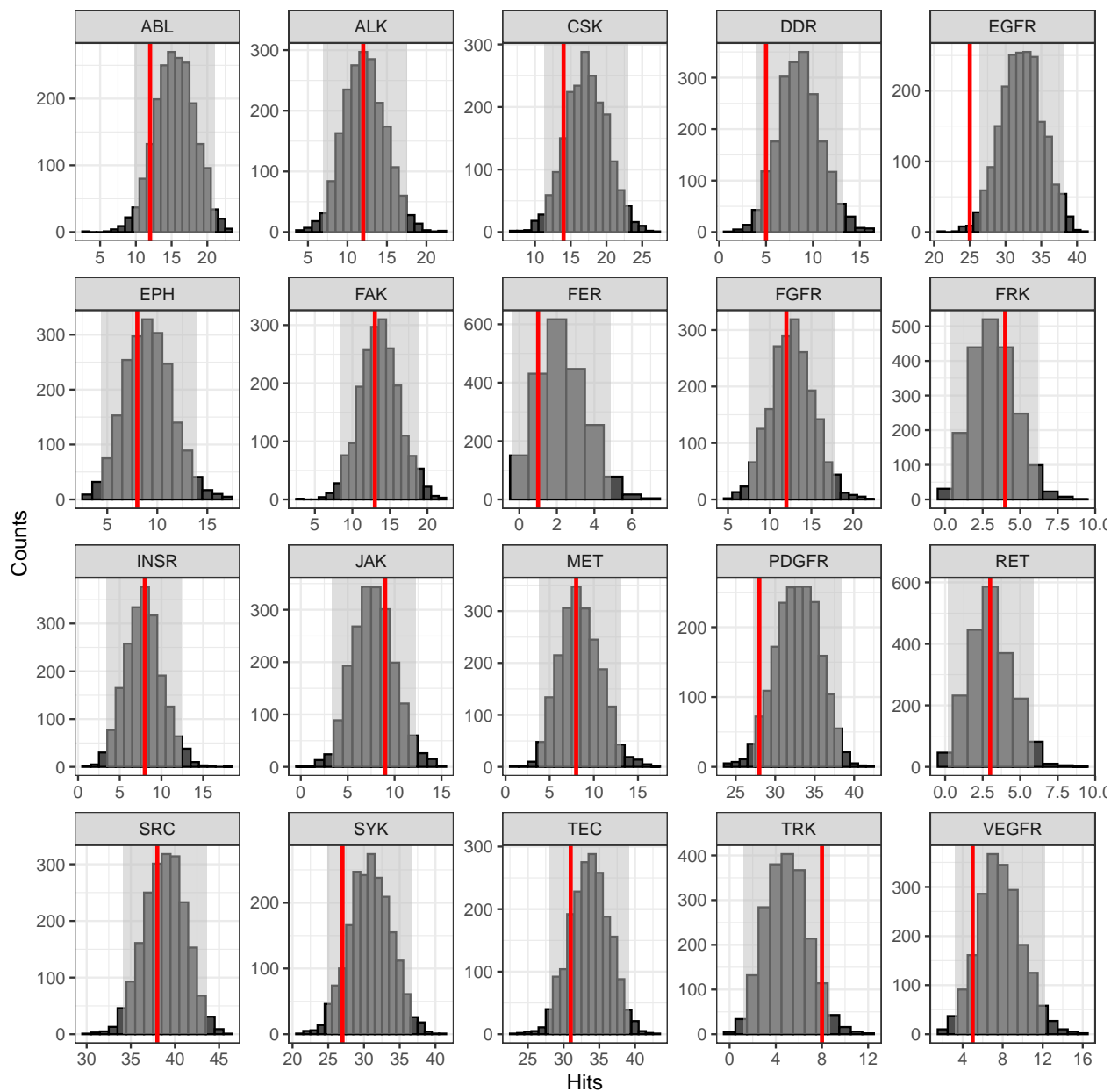
TNK1 (Waterfall Plot)

This waterfall represents the log2 fold changes between the two groups at each peptide.



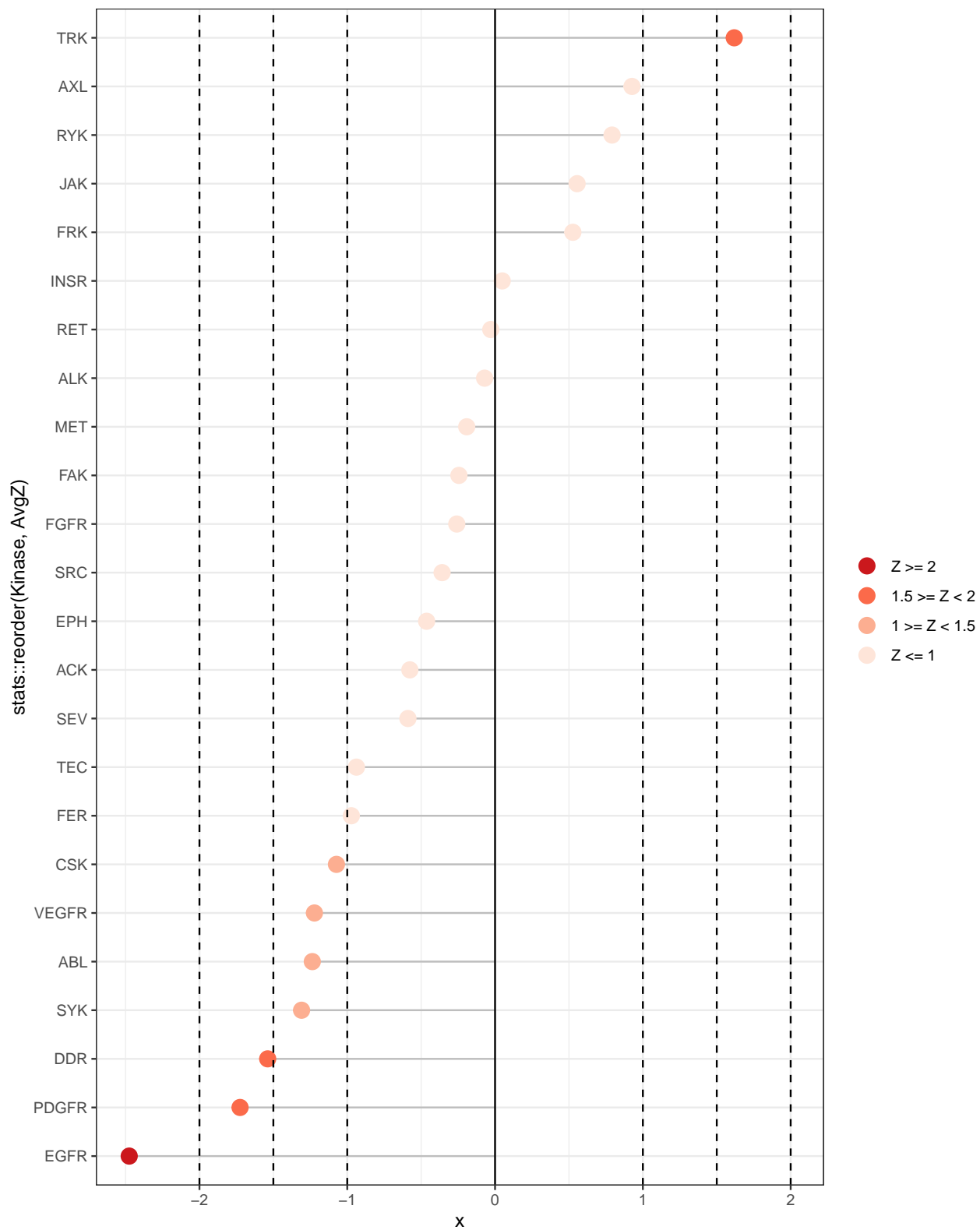
TNK1 (Upstream Kinase Analysis)

Kinase	Observed	SamplingAvg	SD	Z
EGFR	25	32.257	2.932	-2.475
PDGFR	28	32.834	2.802	-1.725
TRK	8	4.967	1.874	1.618
DDR	5	8.581	2.328	-1.538
SYK	27	30.863	2.951	-1.309
ABL	12	15.472	2.808	-1.236
VEGFR	5	7.740	2.242	-1.222
CSK	14	17.160	2.945	-1.073
FER	1	2.273	1.309	-0.972
TEC	31	33.614	2.787	-0.938
AXL	17	14.442	2.761	0.927
RYK	1	0.502	0.629	0.791
SEV	0	0.258	0.438	-0.590
ACK	0	0.250	0.433	-0.576
JAK	9	7.754	2.245	0.555
FRK	4	3.227	1.468	0.527
EPH	8	9.106	2.389	-0.463
SRC	38	38.847	2.366	-0.358
FGFR	12	12.674	2.602	-0.259
FAK	13	13.648	2.650	-0.245
MET	8	8.447	2.329	-0.192
ALK	12	12.187	2.646	-0.071
INSR	8	7.891	2.238	0.048
RET	3	3.042	1.432	-0.029



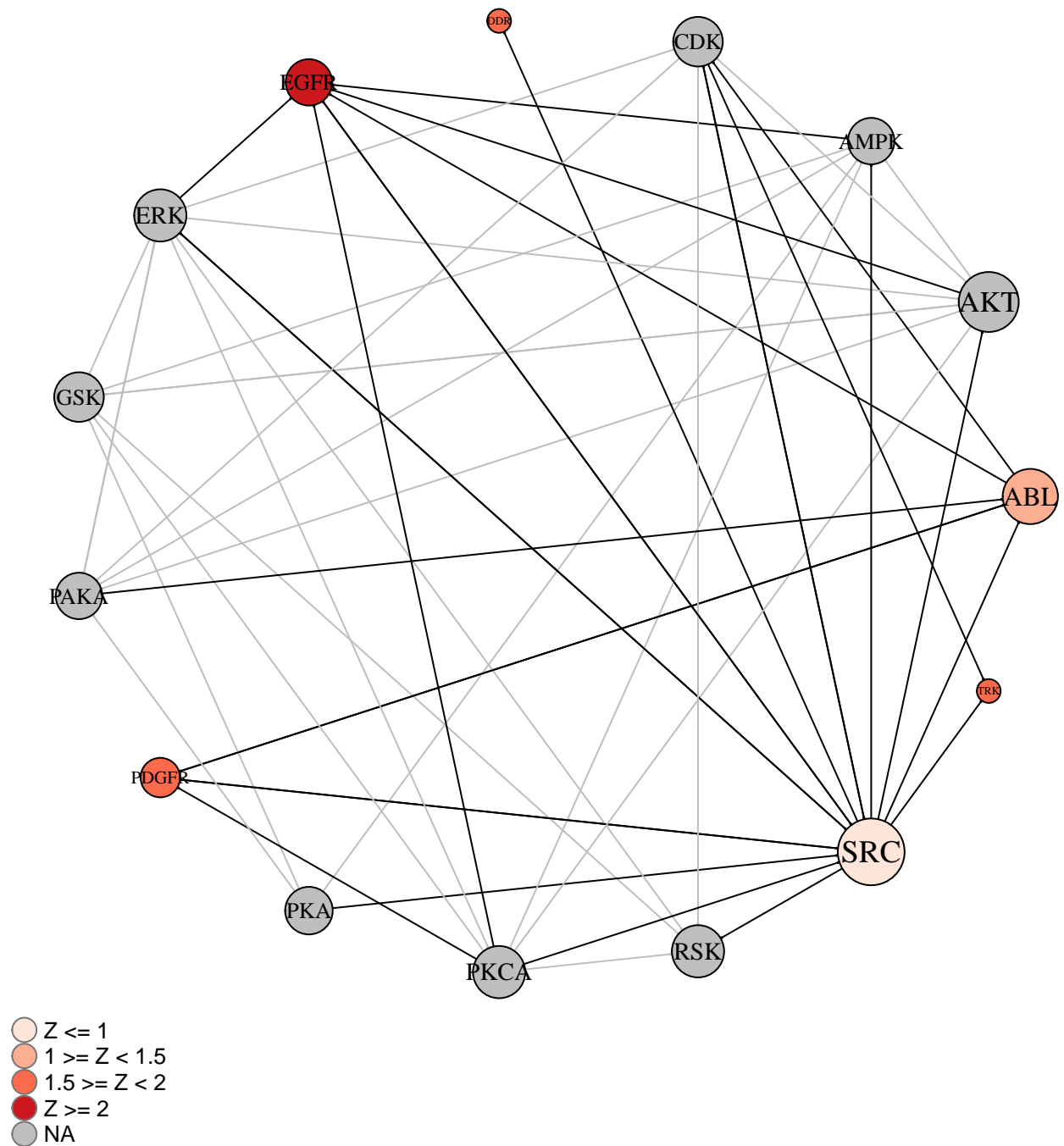
TNK1 (Z Scores Plot)

We will plot the individual and averaged Z scores using both the across and within chip analyses.

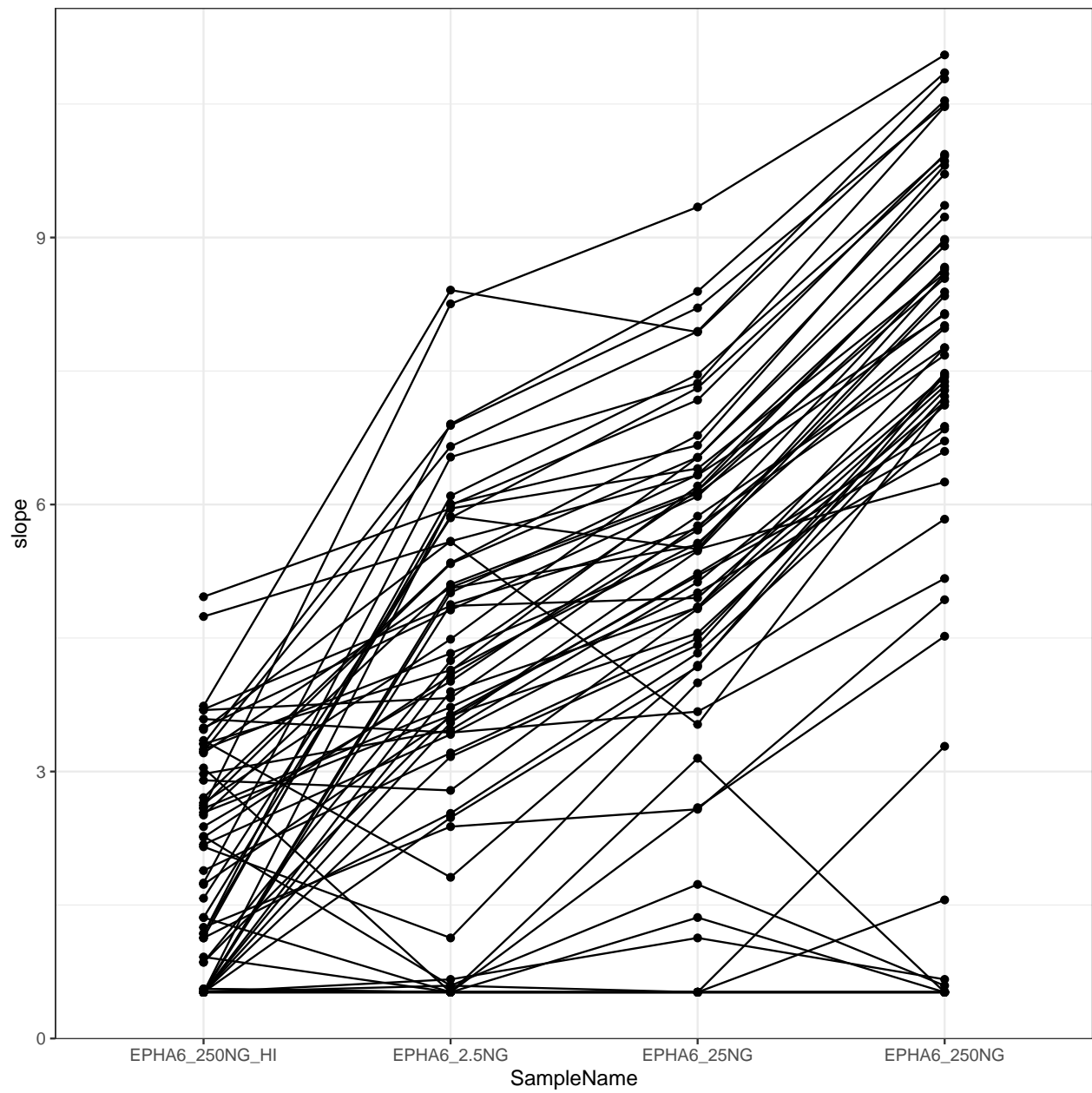


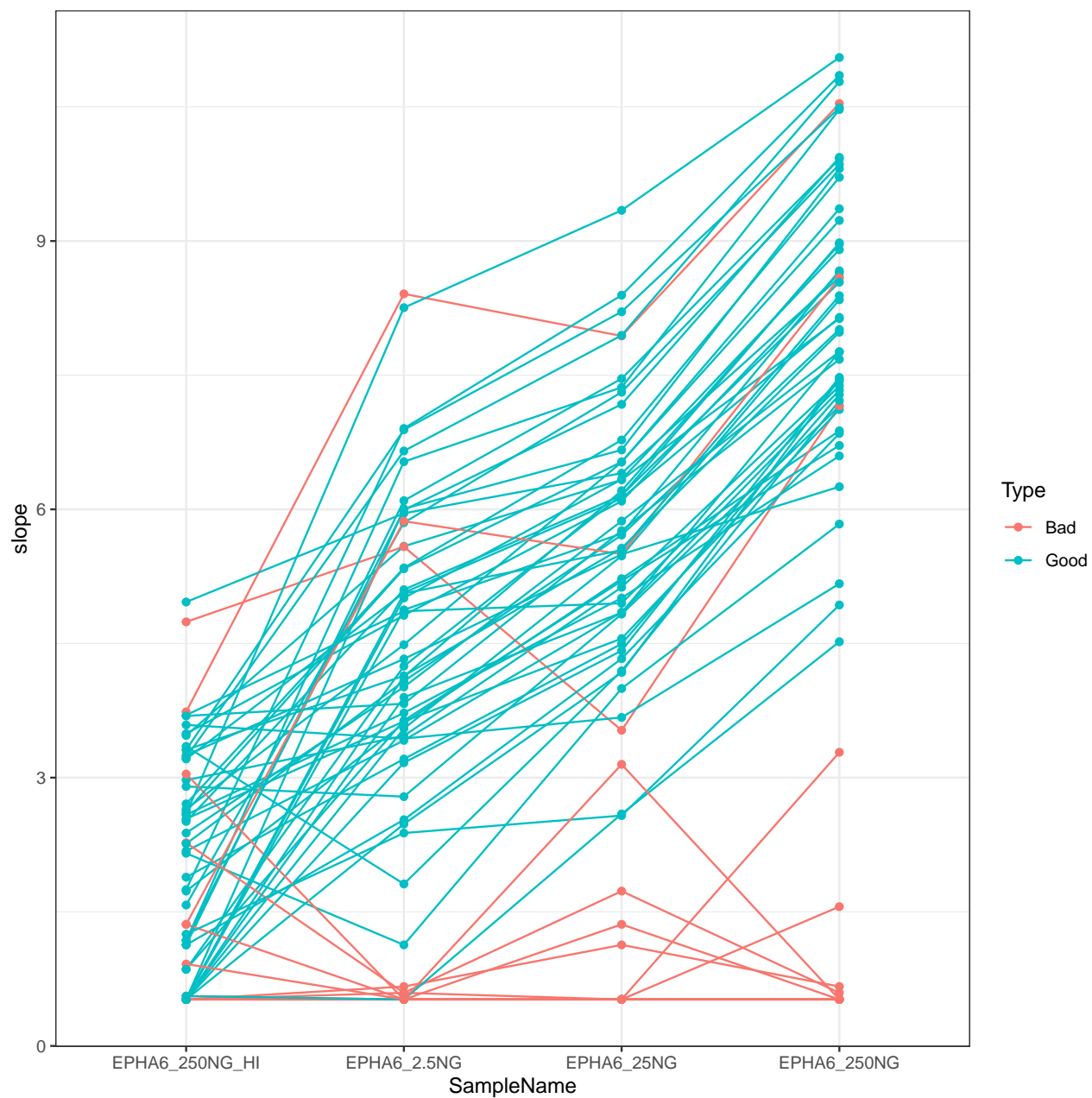
TNK1 (Ball Model Network)

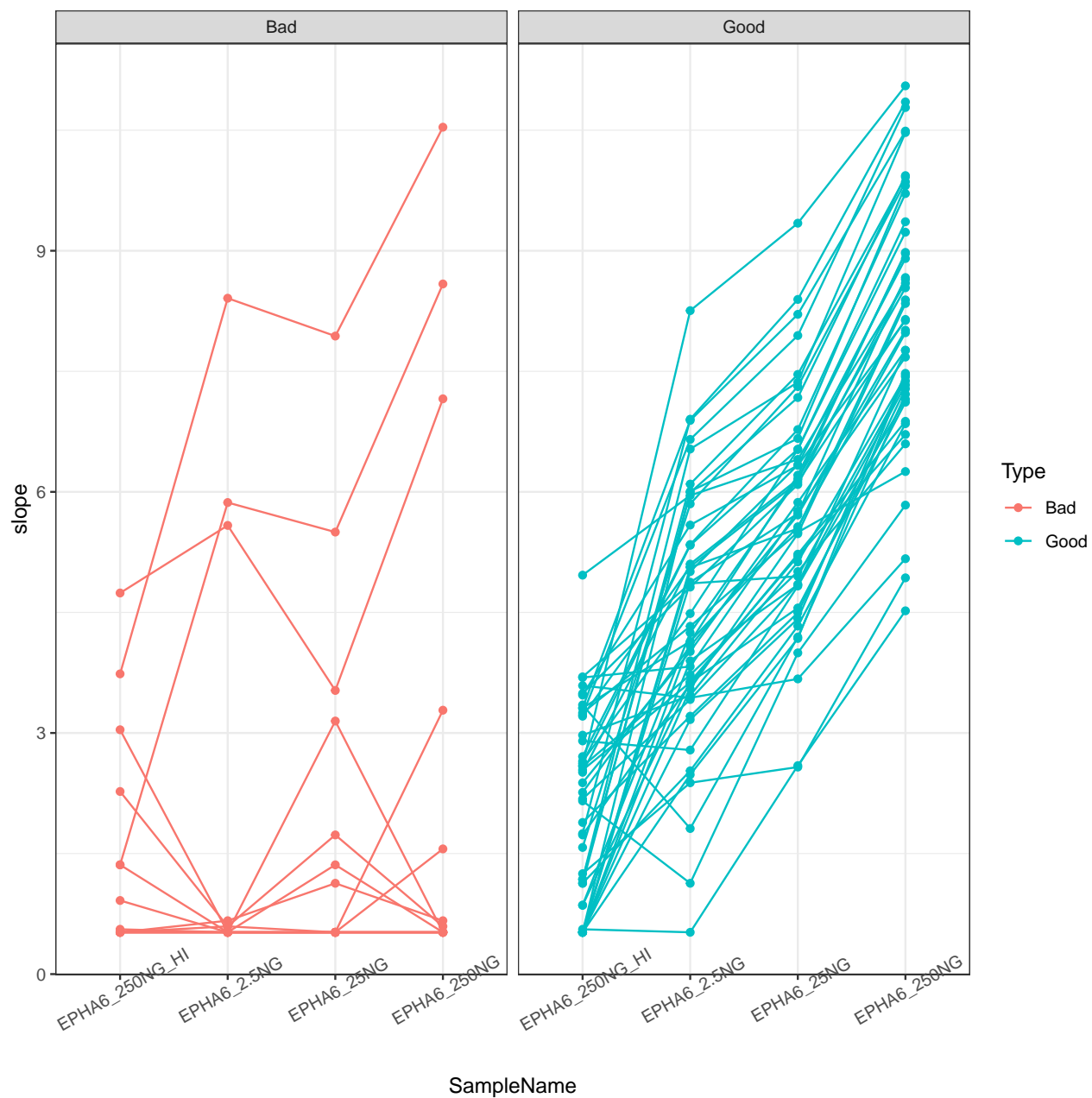
We will view the ball model network function, to generate a model representing the protein-protein interactions between kinases

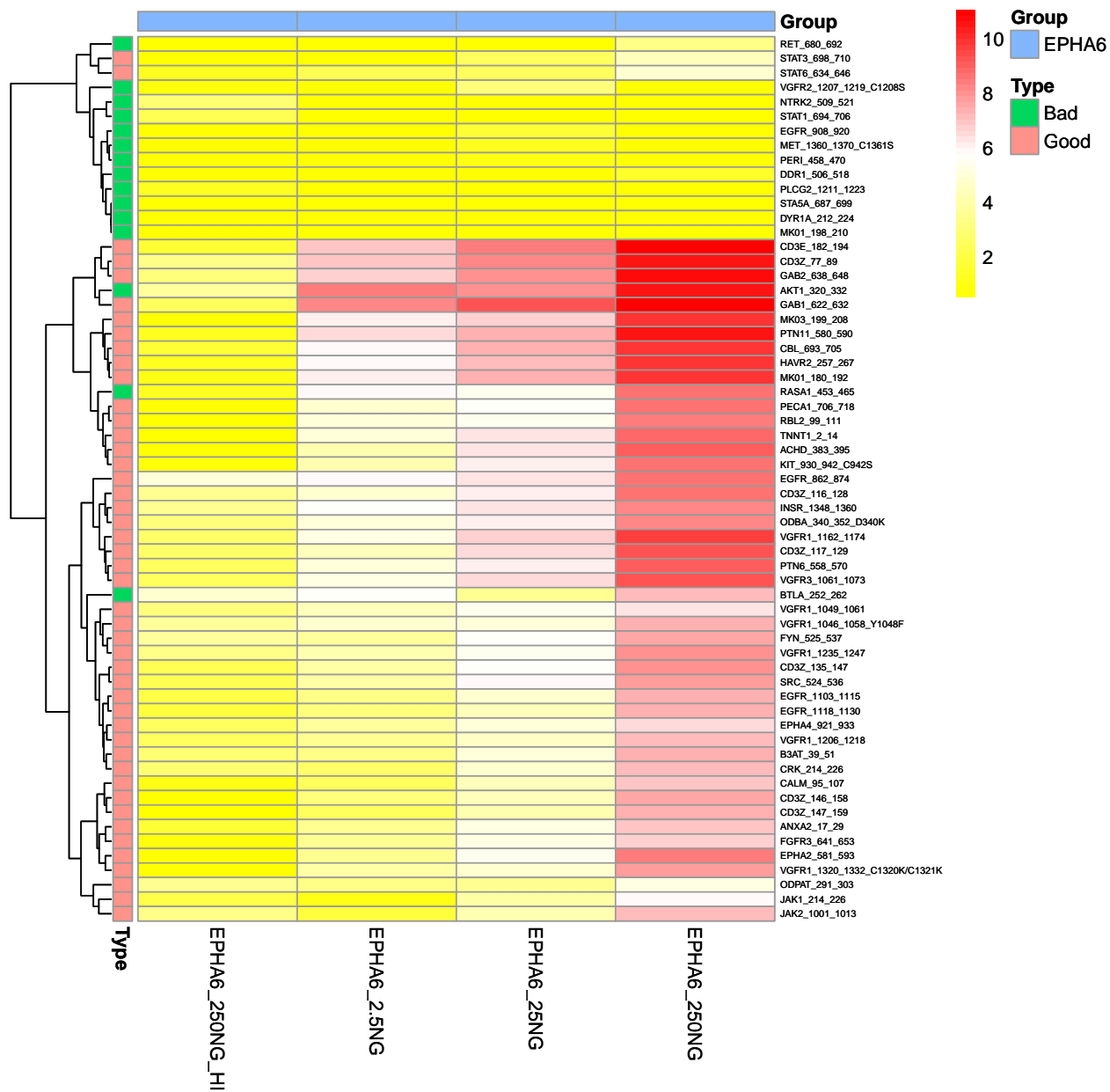


EPHA6

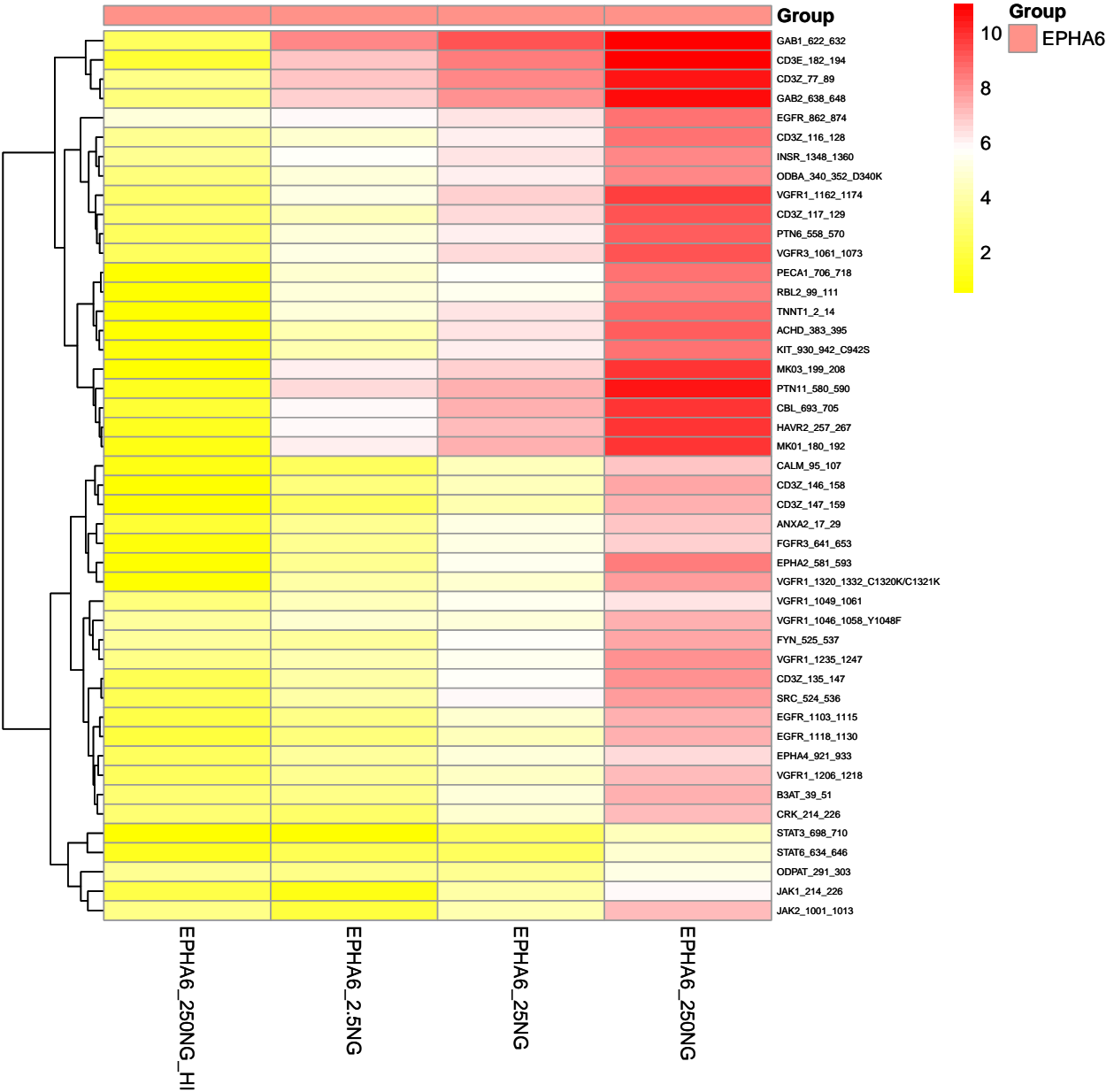




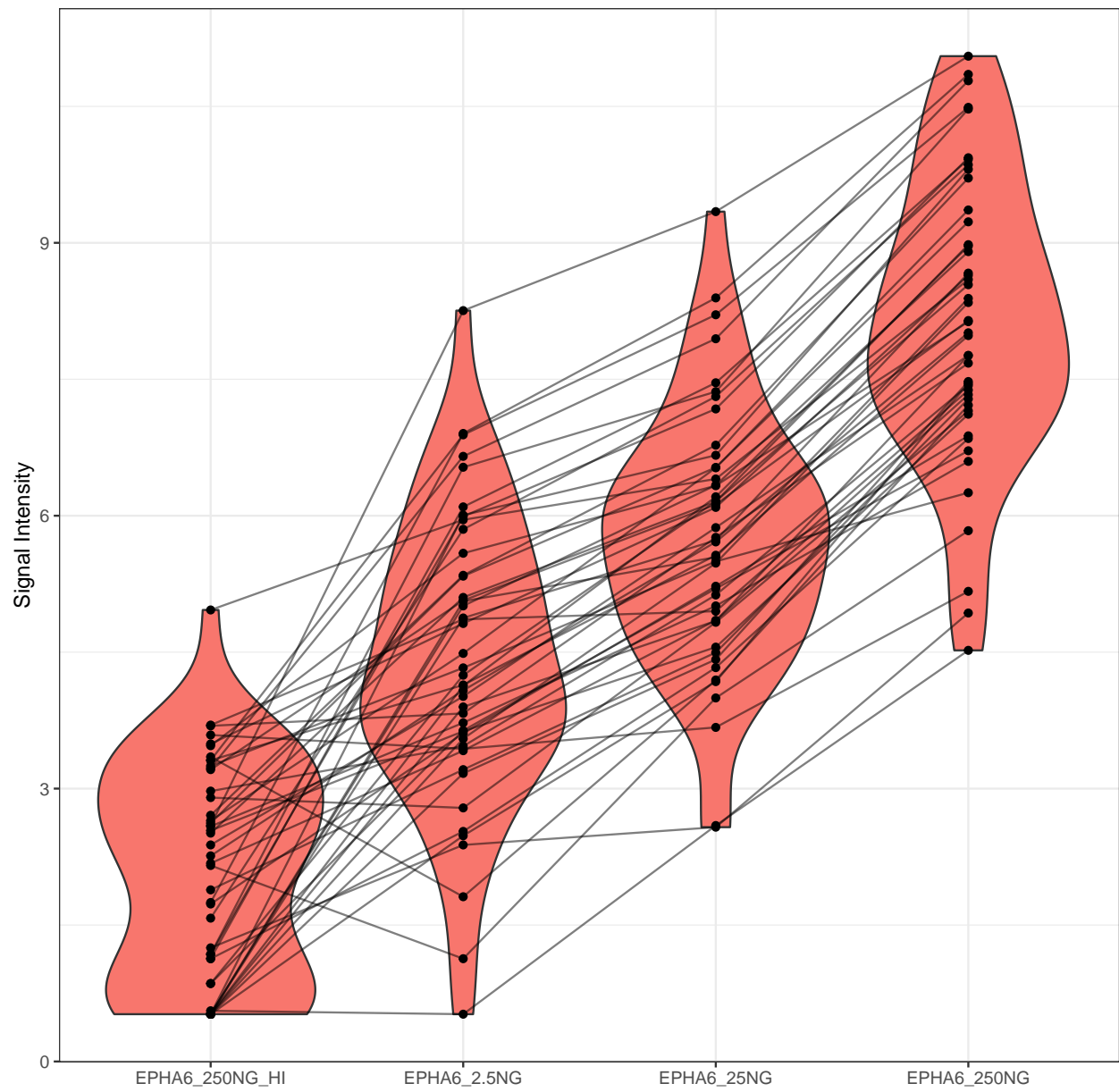


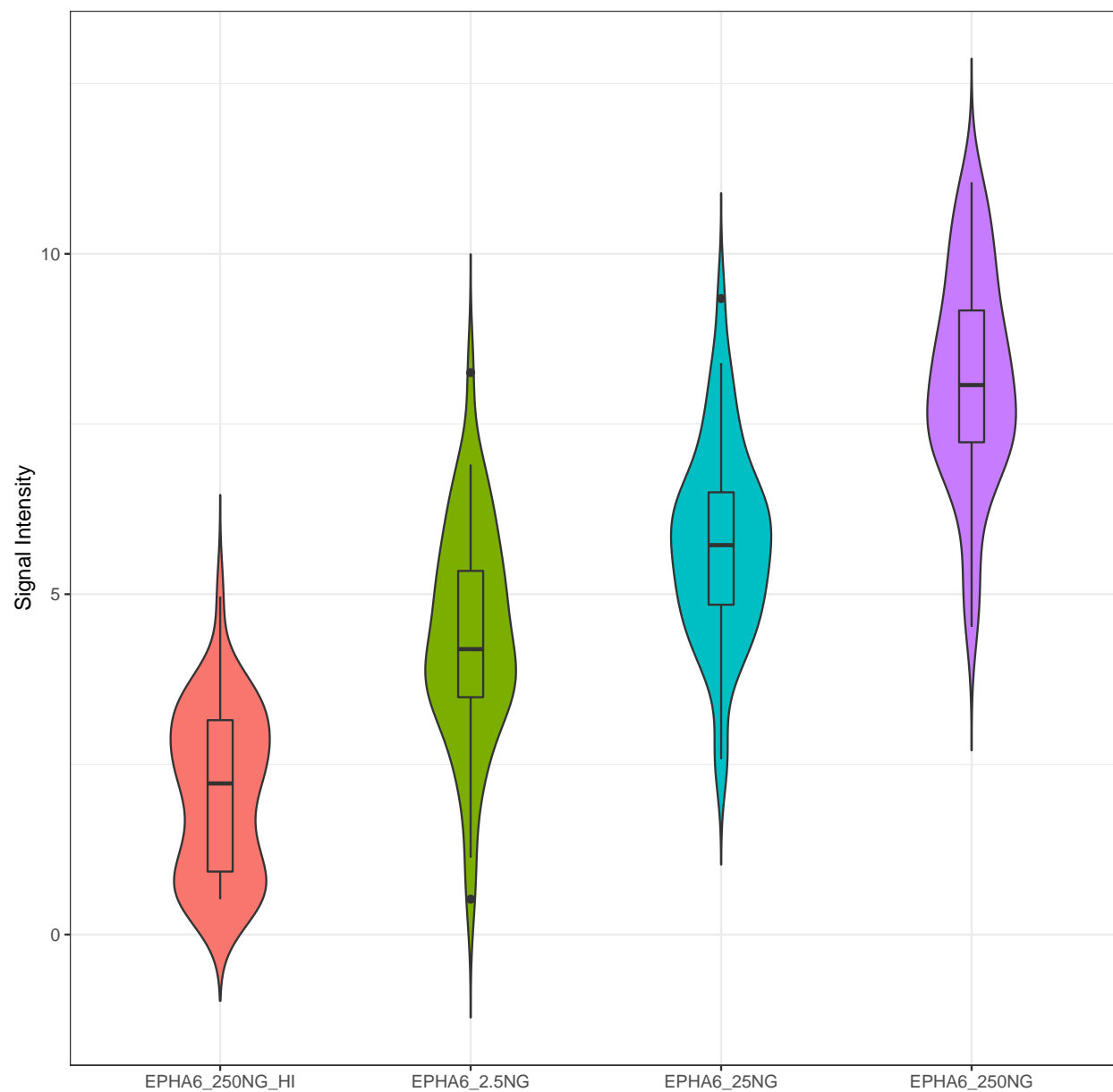


EPHA6 (Heatmap)



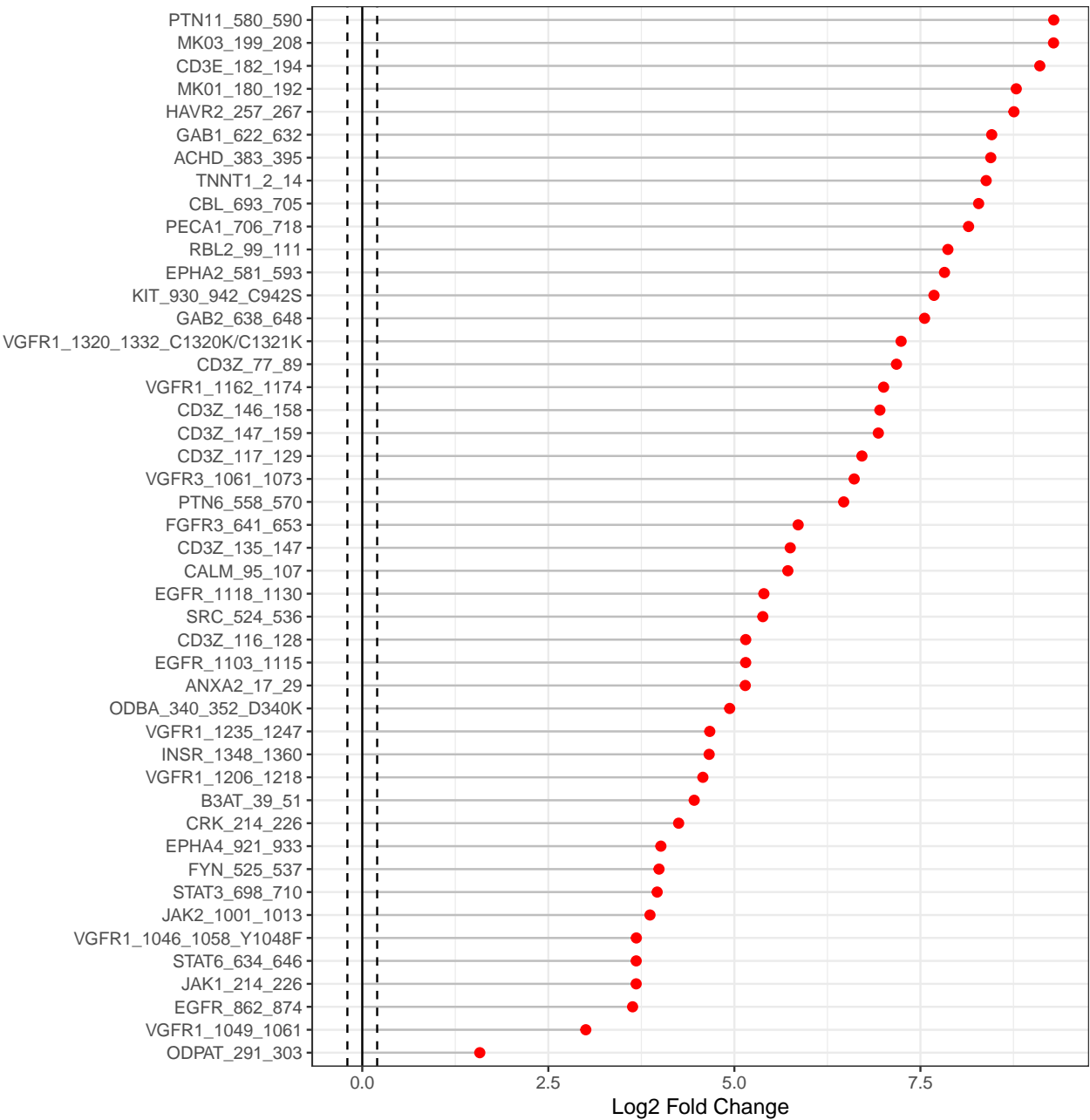
EPHA6 (Violin Plot)





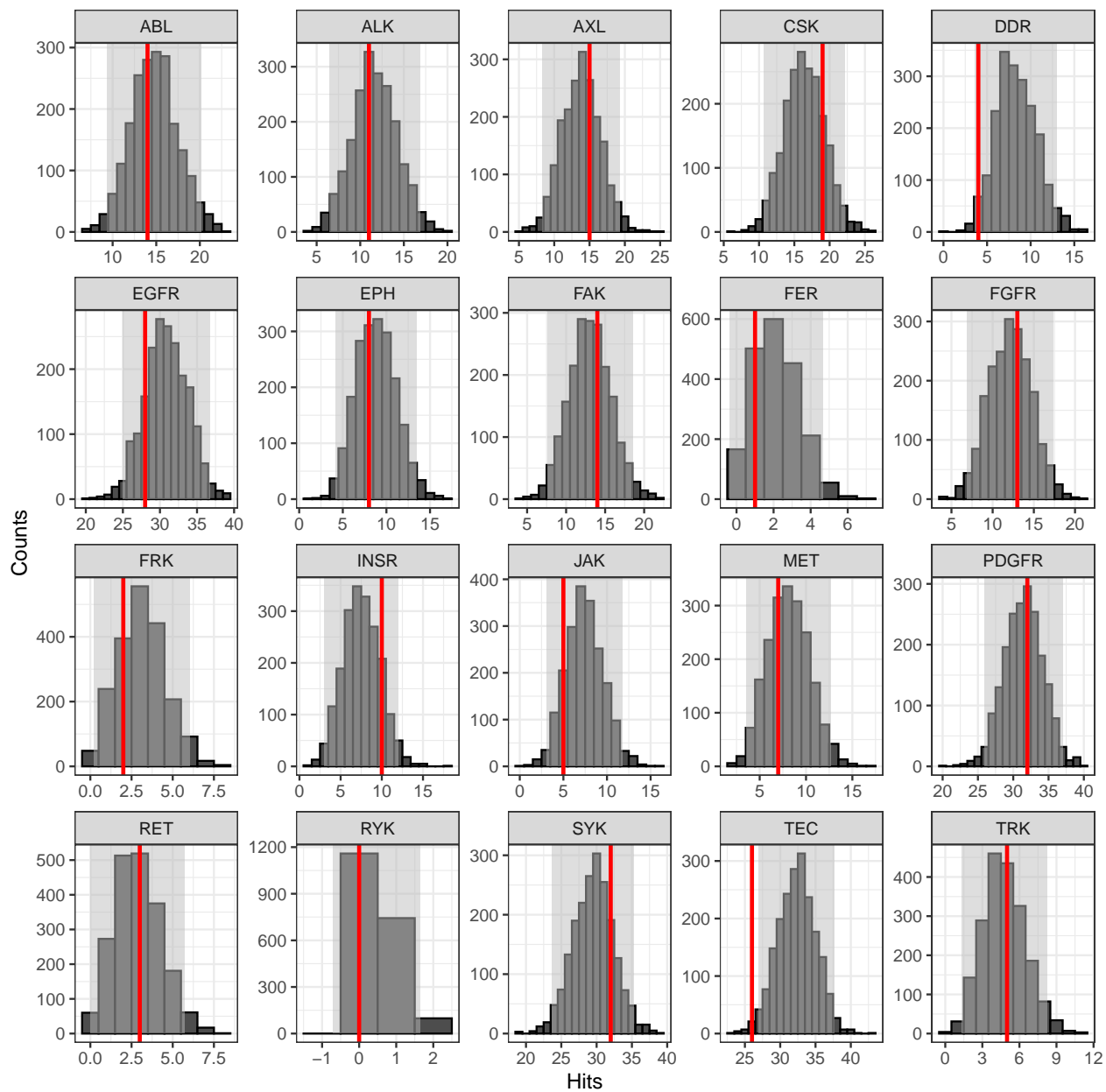
EPHA6 (Waterfall Plot)

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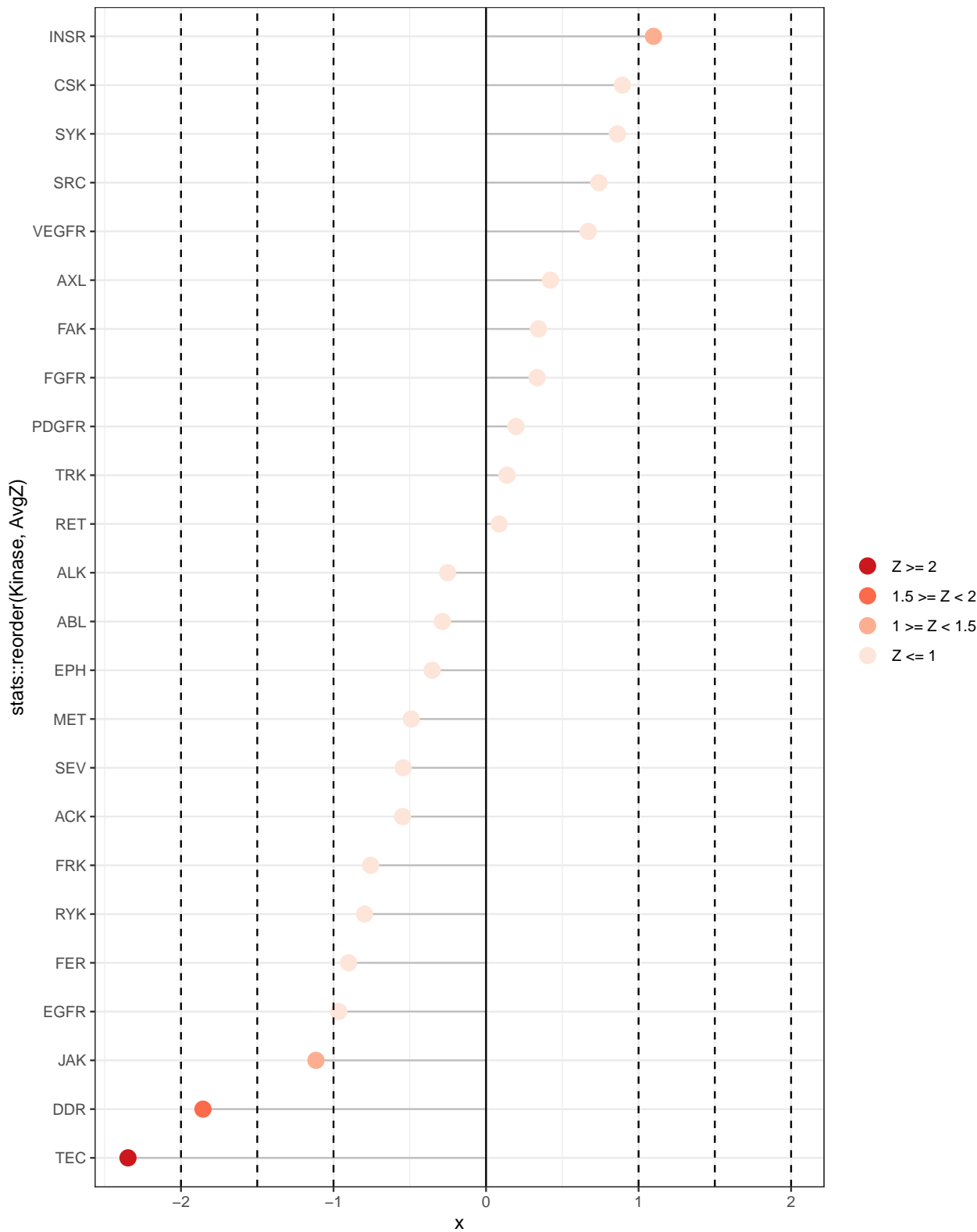
EPHA6 (Upstream Kinase Analysis)

Kinase	Observed	SamplingAvg	SD	Z
TEC	26	32.331	2.697	-2.348
DDR	4	8.340	2.339	-1.856
JAK	5	7.416	2.167	-1.115
INSR	10	7.529	2.250	1.098
EGFR	28	30.838	2.937	-0.966
FER	1	2.129	1.253	-0.901
CSK	19	16.430	2.877	0.894
SYK	32	29.513	2.886	0.862
RYK	0	0.470	0.589	-0.797
FRK	2	3.102	1.455	-0.757
SRC	39	37.272	2.337	0.740
VEGFR	9	7.526	2.198	0.671
ACK	0	0.230	0.421	-0.547
SEV	0	0.228	0.420	-0.543
MET	7	8.118	2.281	-0.490
AXL	15	13.834	2.759	0.422
EPH	8	8.821	2.323	-0.353
FAK	14	13.060	2.737	0.343
FGFR	13	12.124	2.615	0.335
ABL	14	14.774	2.703	-0.286
ALK	11	11.652	2.591	-0.252
PDGFR	32	31.458	2.767	0.196
TRK	5	4.761	1.735	0.138
RET	3	2.877	1.425	0.086



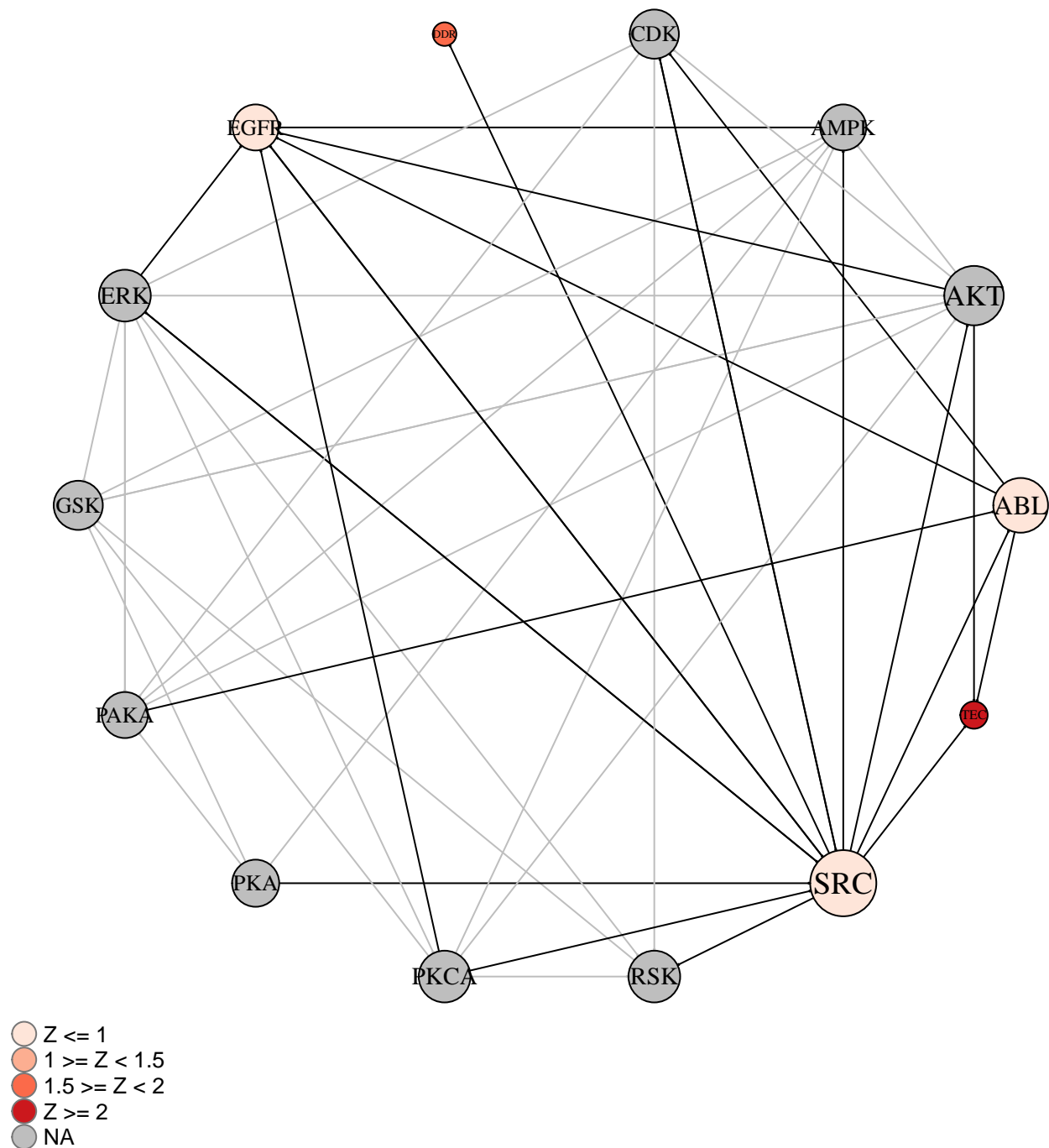
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EPHA6 (Ball Model Network)

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Session Info

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#>      tidyr      tibble      ggplot2      tidyverse      knitr      KRSA
```

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