My Thesis

STK analysis for GitHub

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11/16/23

Table of contents

1 Introduction

1.1 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

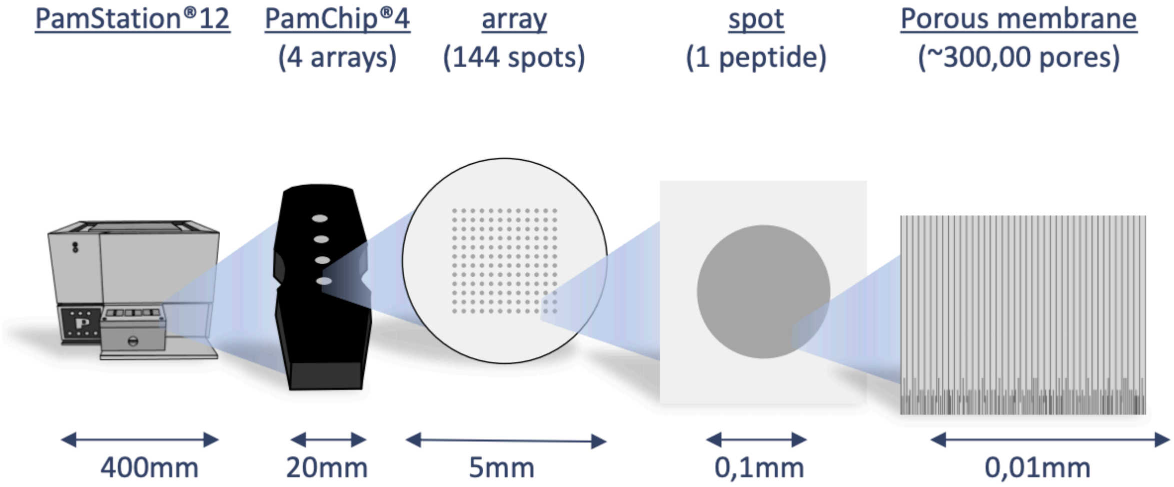


Figure 1.1: Pamgene Kinase Activity Platform

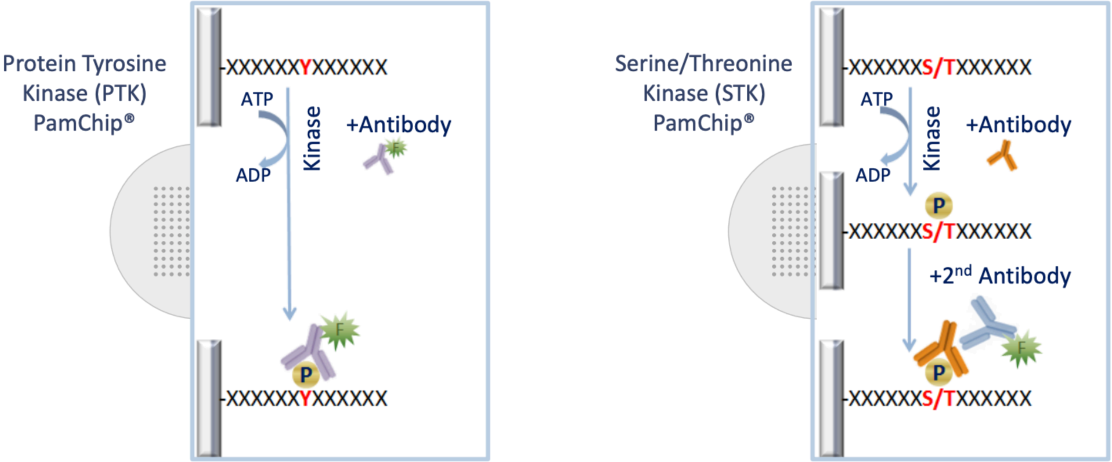
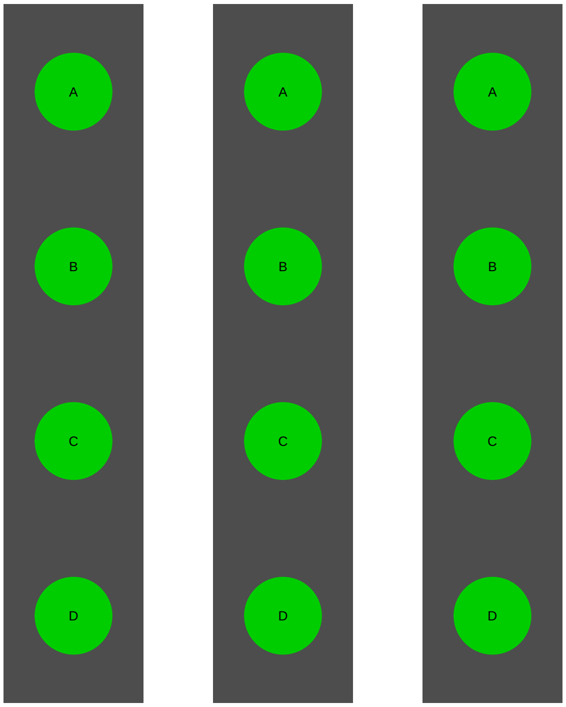


Figure 1.2: Pamgene Platform Detection

2 Run Design

Designing the placement of the samples on the chips and arrays is important to consider due to the variability across different chips and batches. During the run some wells are subject to fail and their data cannot be analyzed and shown below as red.



3 Results

3.1 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).

3.2 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.

3.3 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

3.4 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 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 references peptides.

3.5 Global Signal Intensity

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

3.5.1 Global CV Plots

We will plot the coefficient of variation on both the normal and normalized fits. This will help us to identify groups with high variation that could be explained by sample outliers.

3.5.2 Global Violin Plots

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

3.5.3 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 group potentially similar signatures.

3.6 Group Comparison

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

- AMPK KD Neuron + Untreated vs Wild Type Neuron + AICAR
- AMPK KD Neuron + Untreated vs Wild Type Neuron + Untreated
- AMPK KD Neuron + AICAR vs Wild Type Neuron + AICAR

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 *Median_SigmBg* at max exposure *100ms* must be above a certain value

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

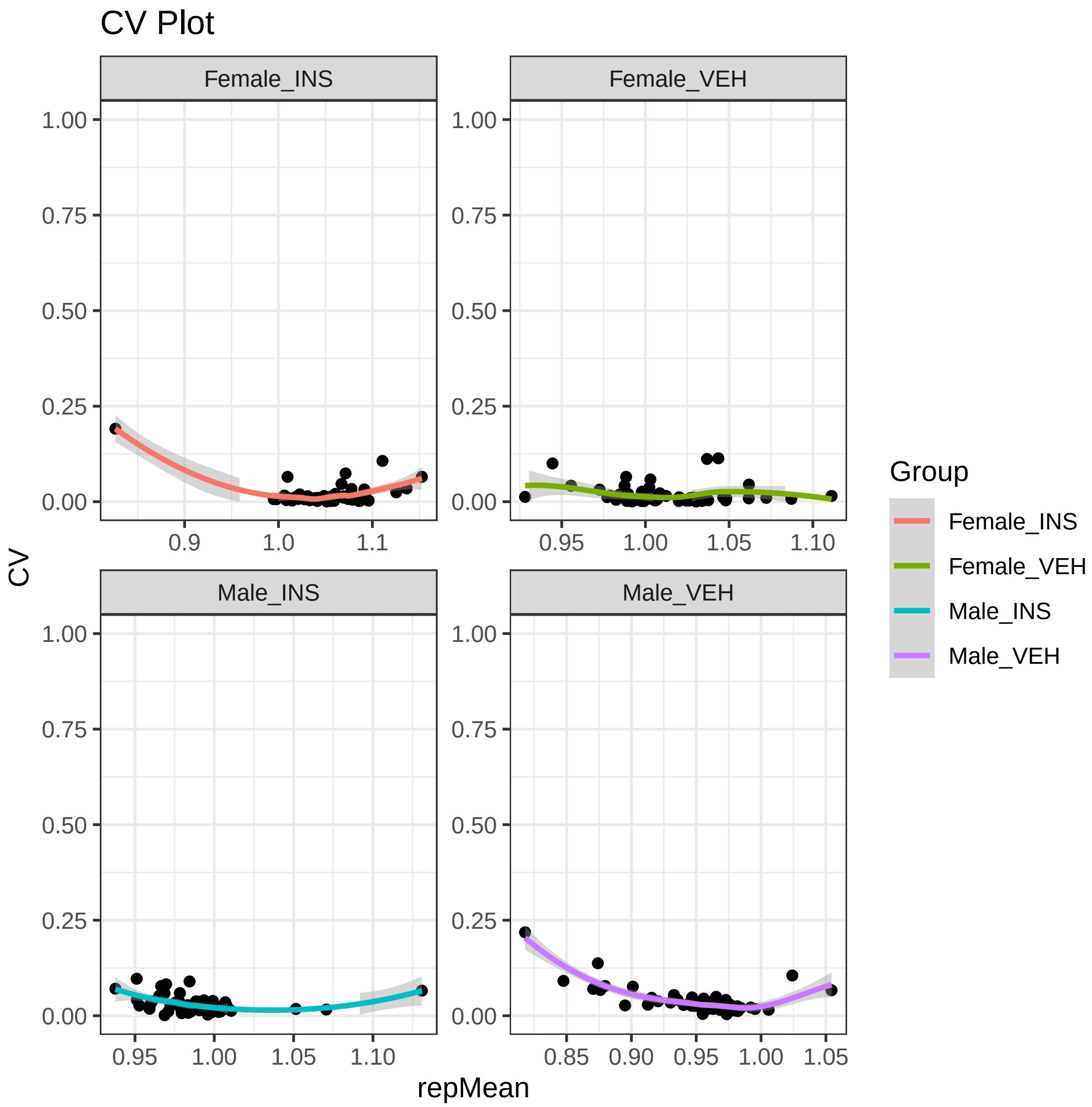


Figure 3.1: Coefficient of Variation plotted for each peptide across all 4 groups

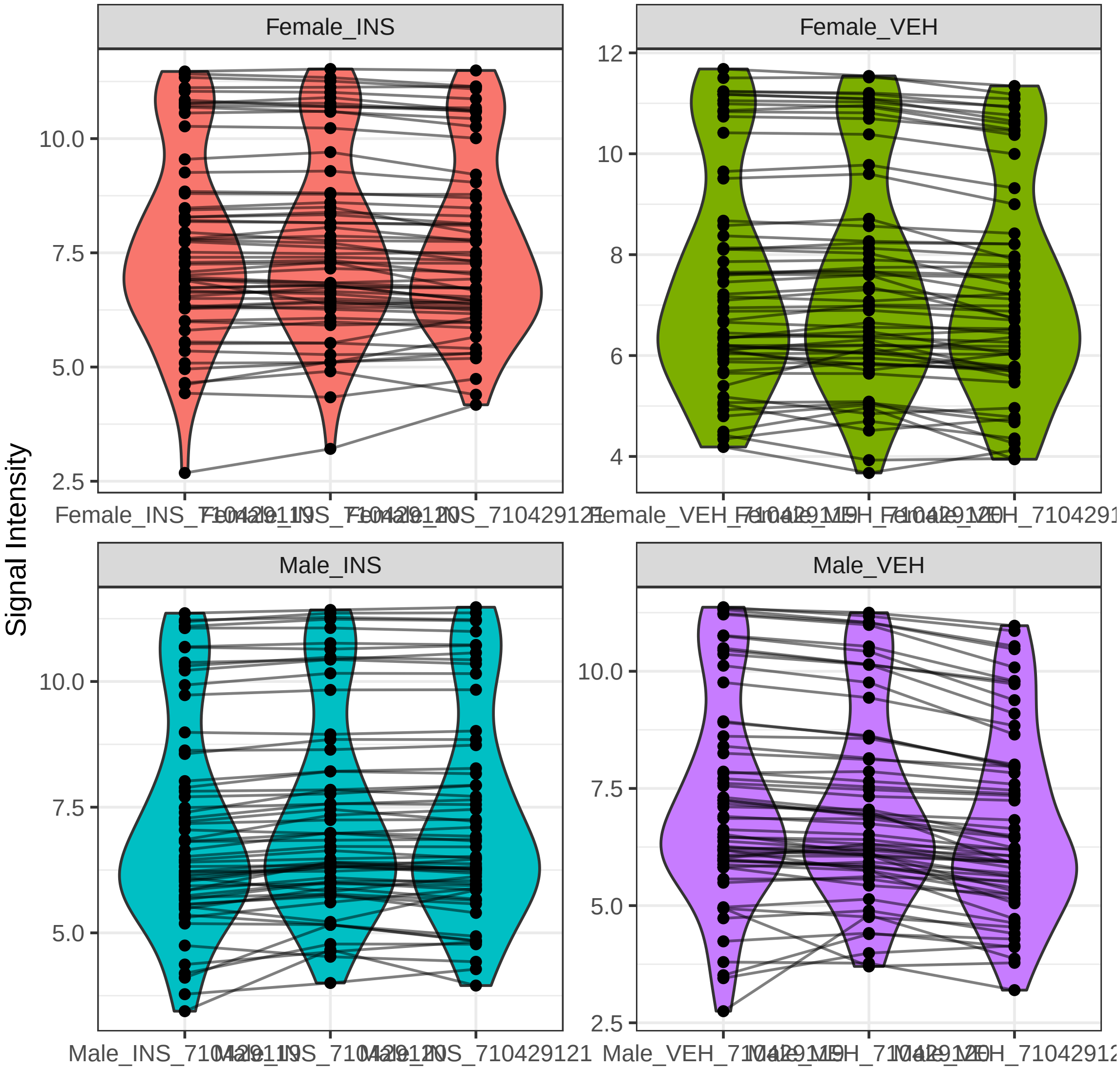


Figure 3.2: Violin Plots for signal intensity Distribution Across Groups for all replicates

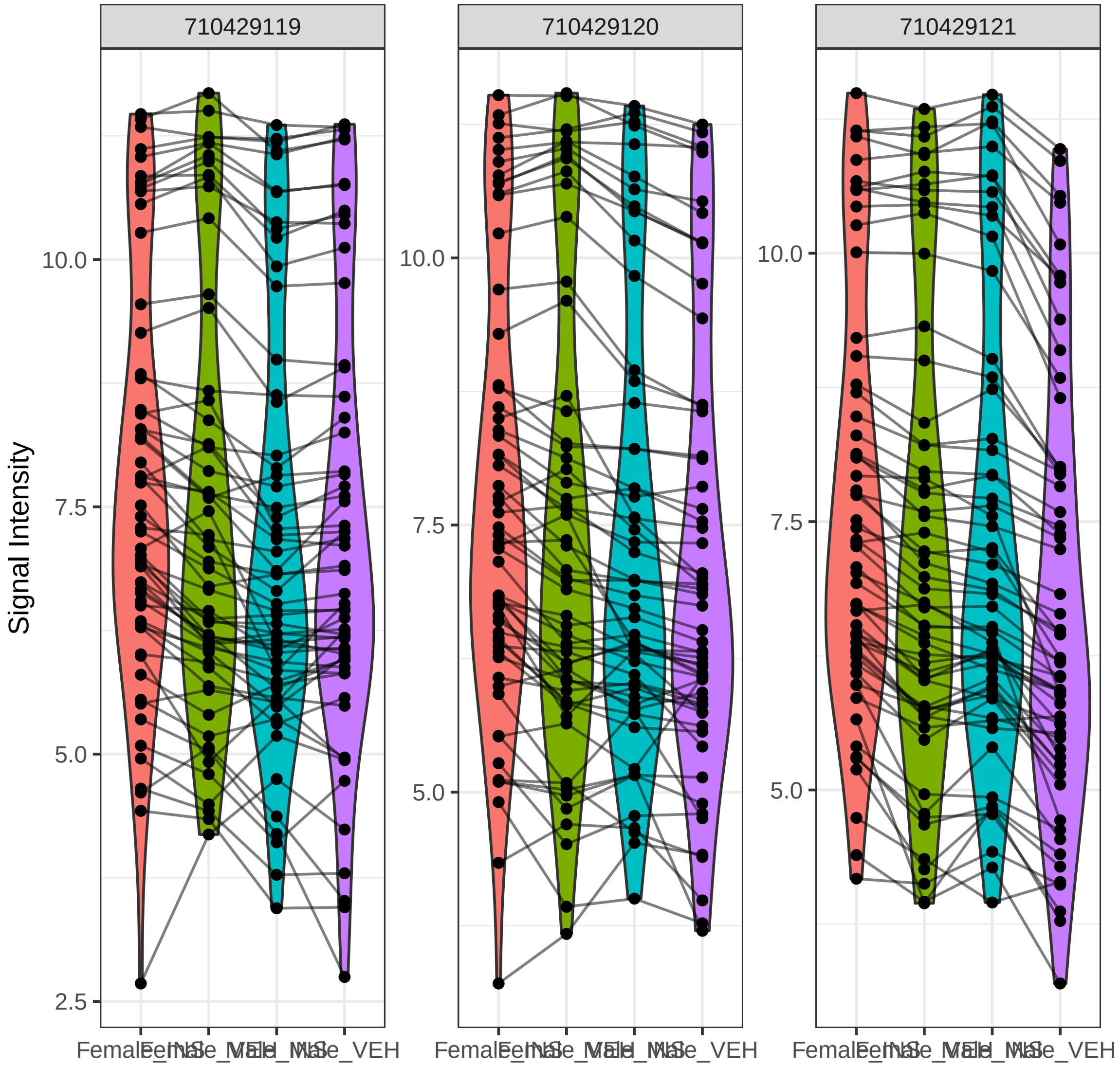


Figure 3.3: Violin Plots for signal intensity Distribution Across Chips for all replicates

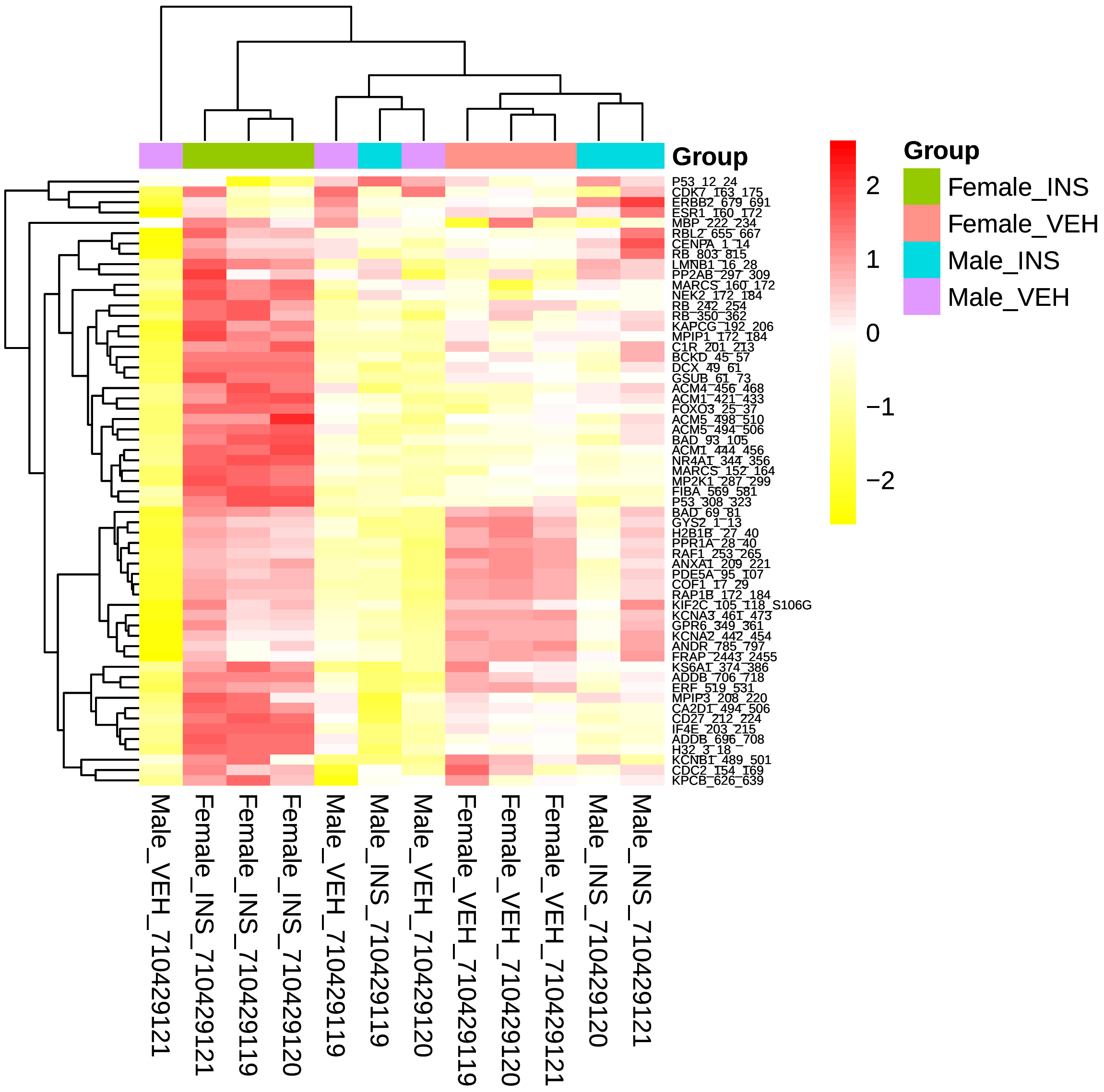


Figure 3.4: Row and chip normalized intensity values for the selected peptides

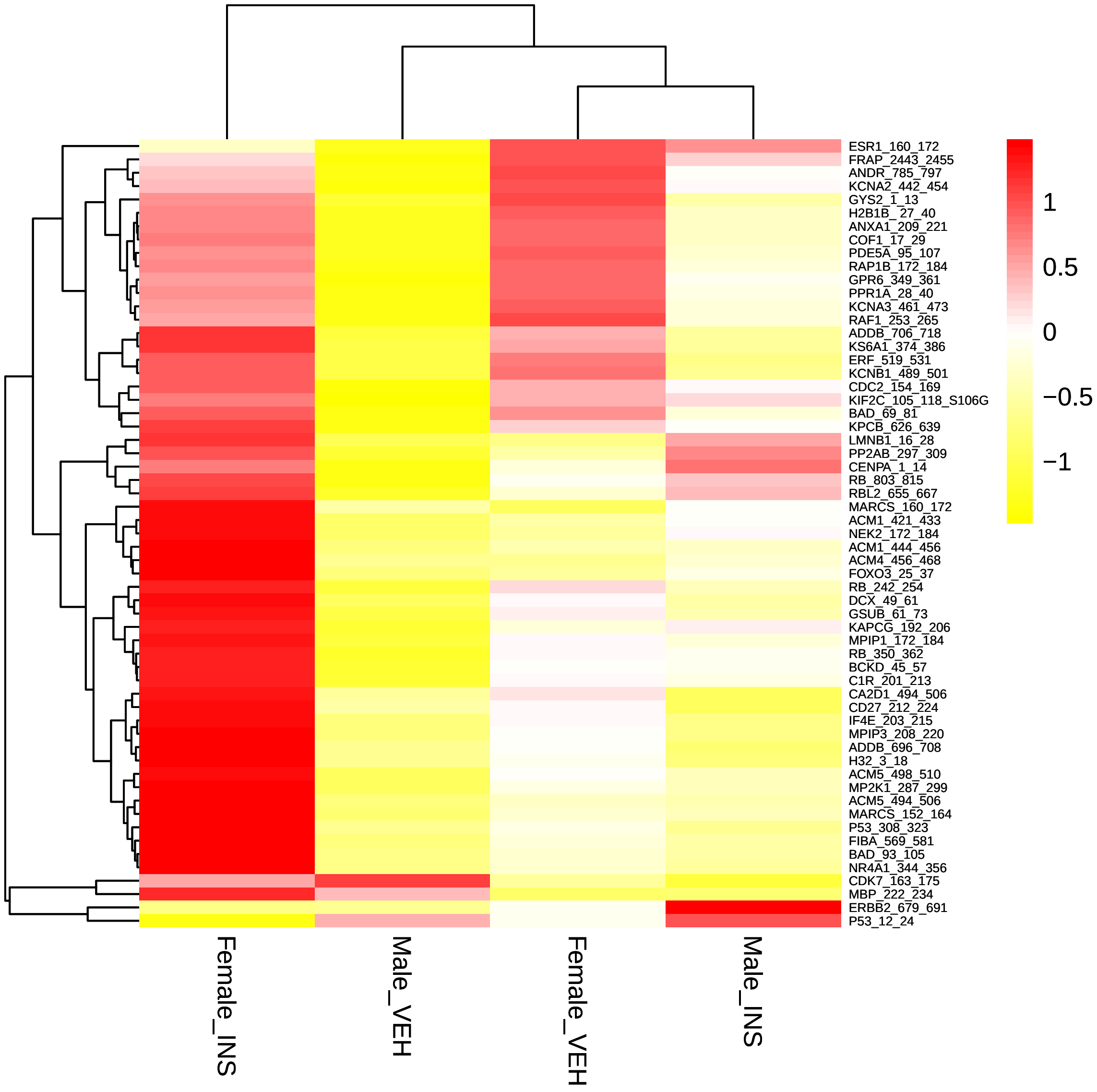
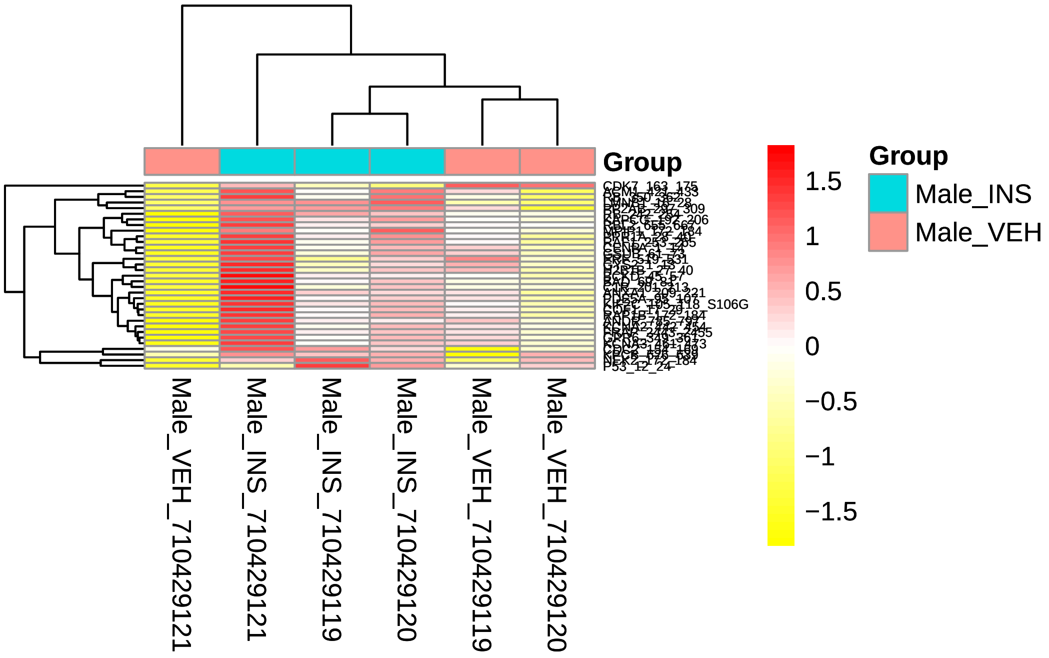


Figure 3.5: Row and group normalized intensity values for the selected peptides

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 *Median_SigmBg* at max exposure *100ms* must be equal or above 5
- R^2 of the linear model fit must be above or equal 0.8
- Log fold change (LFC) cutoffs at (0.2,0.3,0.4)



3.6.1 Male_INS vs Male_VEH

3.6.1.1 Heatmap

After applying the *Filtering Parameters* for this group comparison, only *33*/141 peptides carried forward in the analysis (i.e. *33 hits*). Below are some figures to visualize the differences between these samples for considering these *hits*.

3.6.1.2 Violin Plot

Below, the violin plot visualizes the distribution of selected peptides for the analysis.

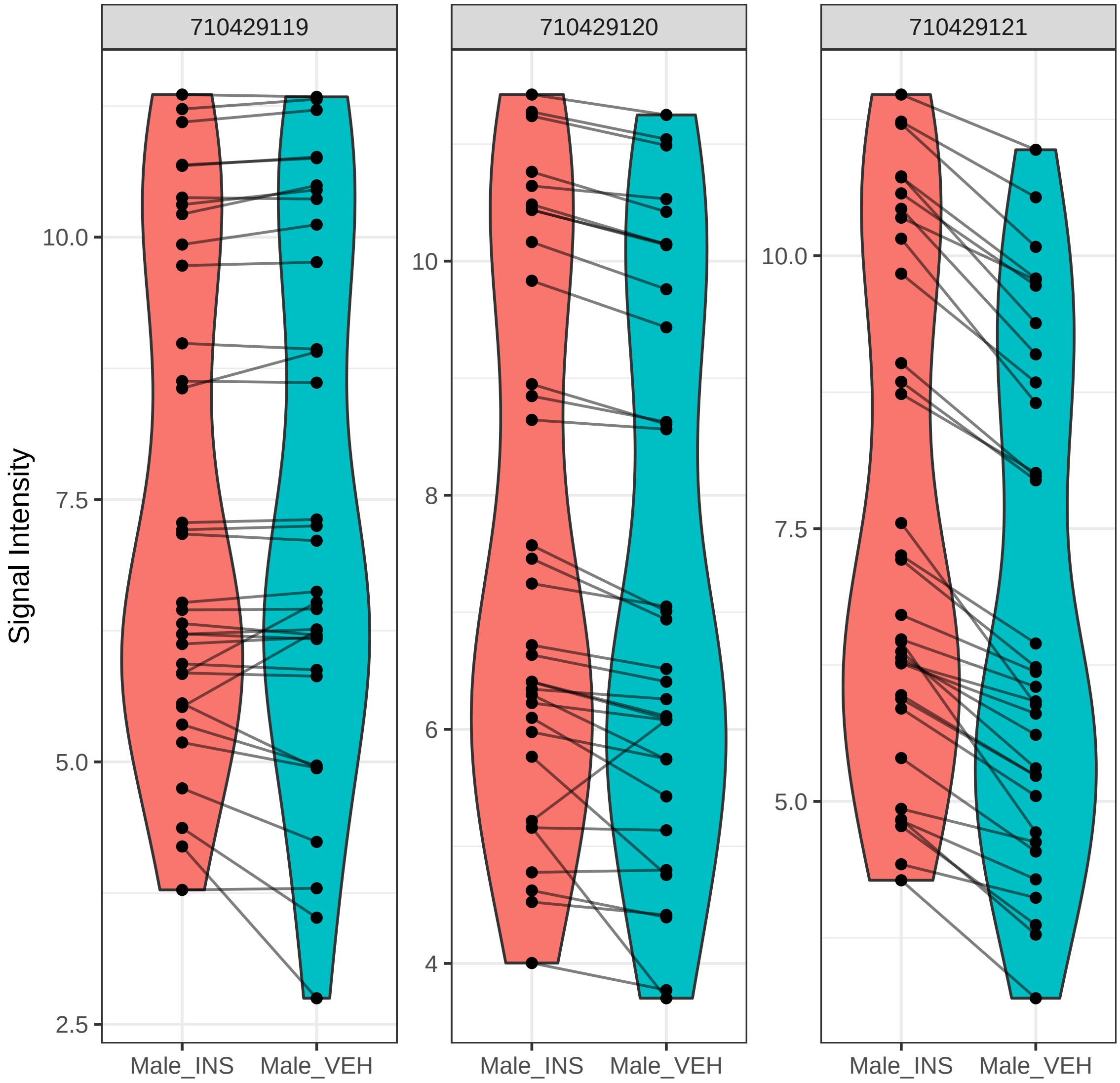


Figure 3.6: Violin plot of two groups

3.6.1.3 Waterfall Plot

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

3.6.1.4 Upstream Kinase Analysis

The lab carefully curated and mapped the kinases that can act and phosphorylate each peptide present on the chip. This was achieved by using multiple sources including GPS 3.0, Kinexus Phosphonet, PhosphoELM and PhosphoSite Plus. Based on that association between peptides and kinases, a random sampling analysis is performed for these hits. The basic idea of *KRSA* is: For each iteration (*2000* iterations performed in this analysis), the same number of hits are randomly selected from the total 141/or 193 peptides present on the chip. Predicted kinases are then mapped to this sample list of peptides and number of kinases are determined. The kinase count from the actual hits and random sampling is then compared to determine the significance.

Kinase	AvgZ
CHK1	1.9100000
DYRK	1.8966667
MST	1.7608333
BRSK	1.6058333
P38	1.5125000
VRK1	1.4366667
KHS	1.4041667
JNK	1.2366667
MARK	1.1408333
MAPKAPK	1.1250000
CK1	-0.8116667
CAMK2	-0.8275000
PKCD	-0.8291667
PAKA	-0.8300000
NMO	-0.8875000
STE11	-0.9691667
PKA	-1.2216667
RIPK	-1.3450000
PLK	-1.5150000
PKD	-1.8191667

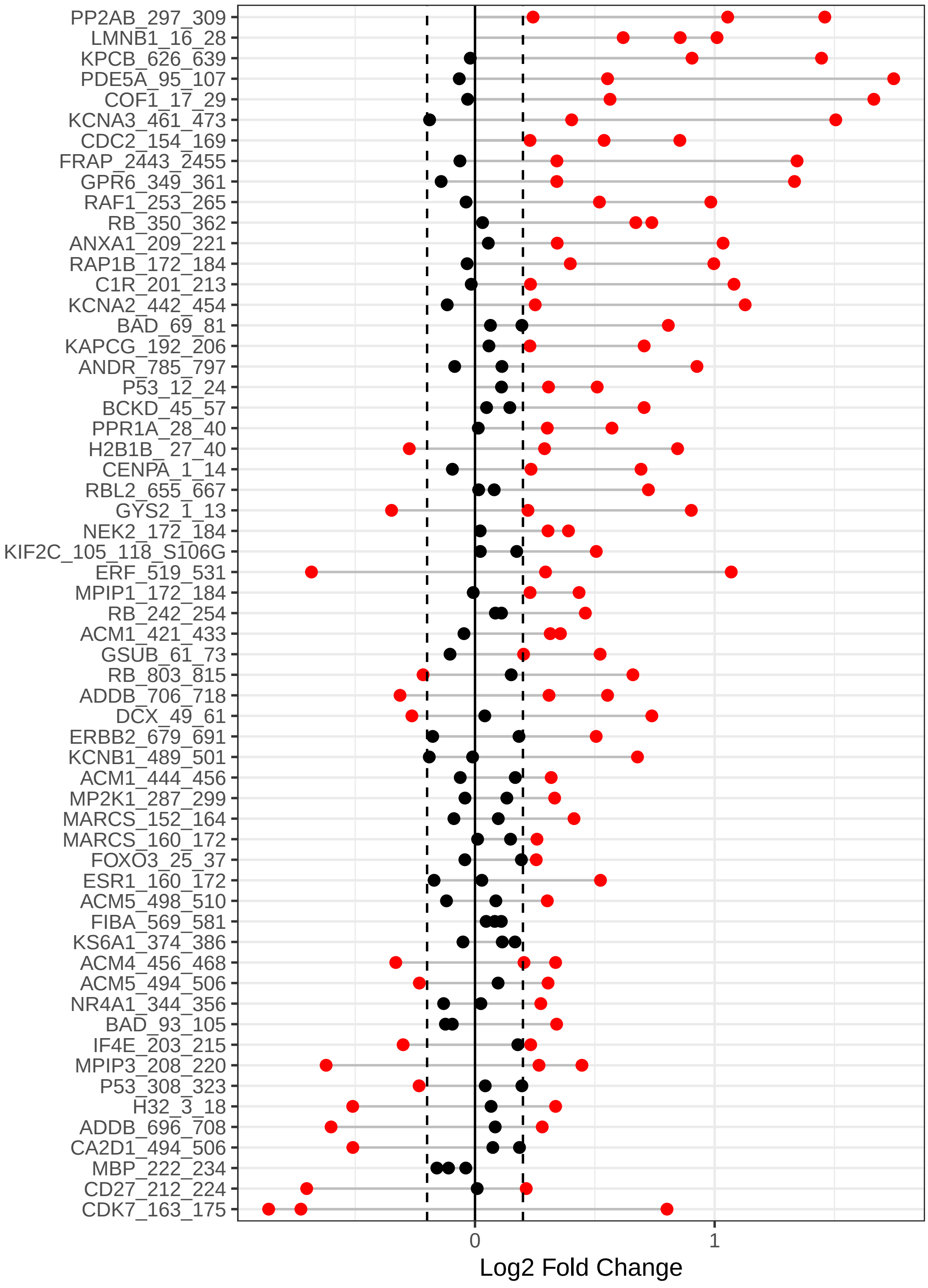


Figure 3.7: Waterfall Plot to show the distribution of change in peptide phosphorylation

Method	NumberOfPeptides
meanLFC.0.15	39
meanLFC.0.2	33
meanLFC.0.3	19
meanLFC.0.4	15
710429119.0.15	24
710429119.0.2	21
710429119.0.3	16
710429119.0.4	11
710429120.0.15	37
710429120.0.2	29
710429120.0.3	16
710429120.0.4	9
710429121.0.15	57
710429121.0.2	53
710429121.0.3	47
710429121.0.4	37

3.6.1.5 Z Scores Plot

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

3.6.1.6 Reverse KRSA Plot

We will use the reverse KRSA plot function, to plot the log2 fold change values for all peptides mapped to kinase hits. This will help us examine the activity of the kinase

3.6.1.7 Coverage Plot

To view the coverage of kinases across the full list of peptides on the chip, we will use the coverage plot function

3.6.1.8 Ball Model Network

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

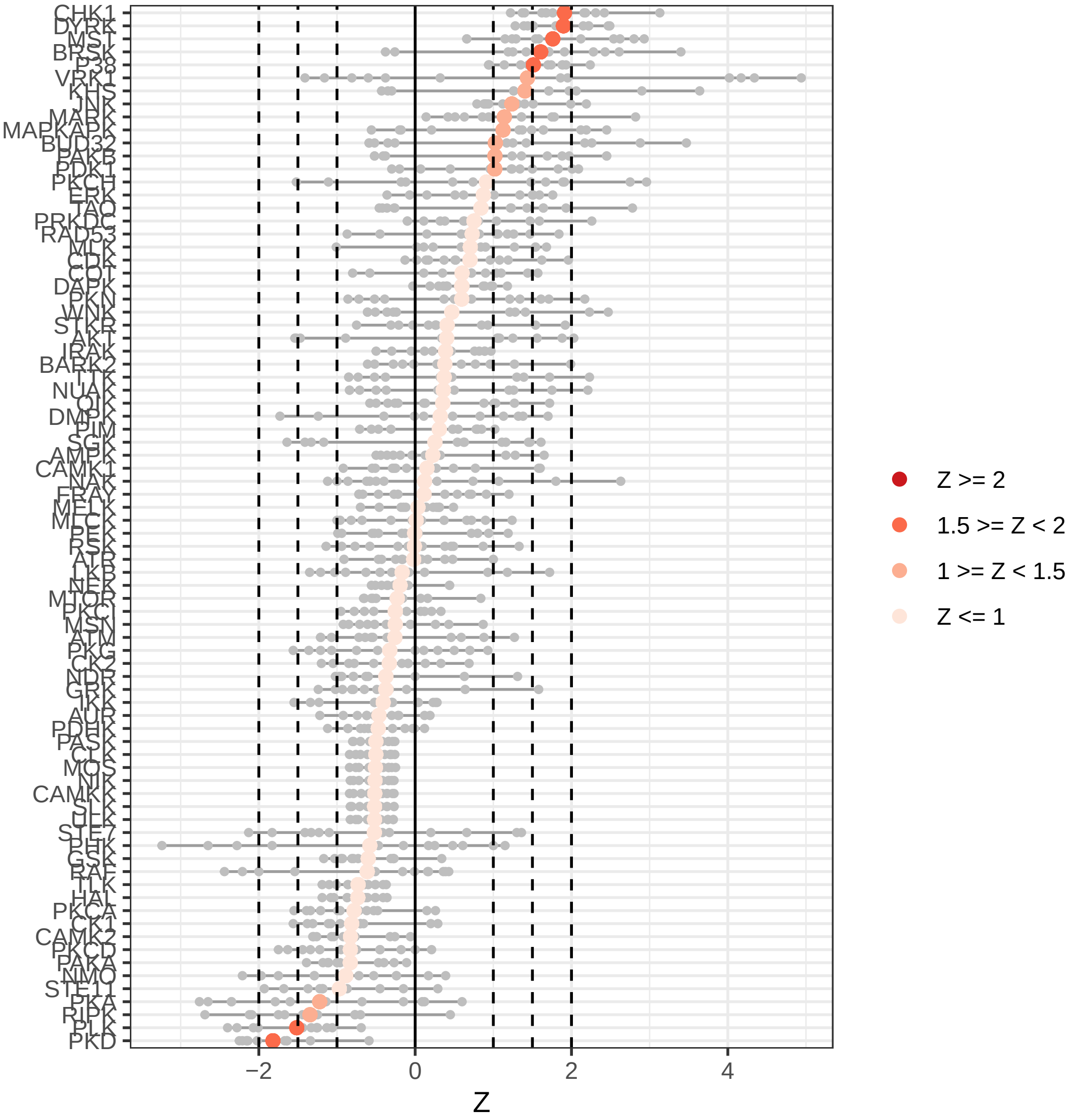


Figure 3.8: Waterfall plot of the Z Scores of each kinase family

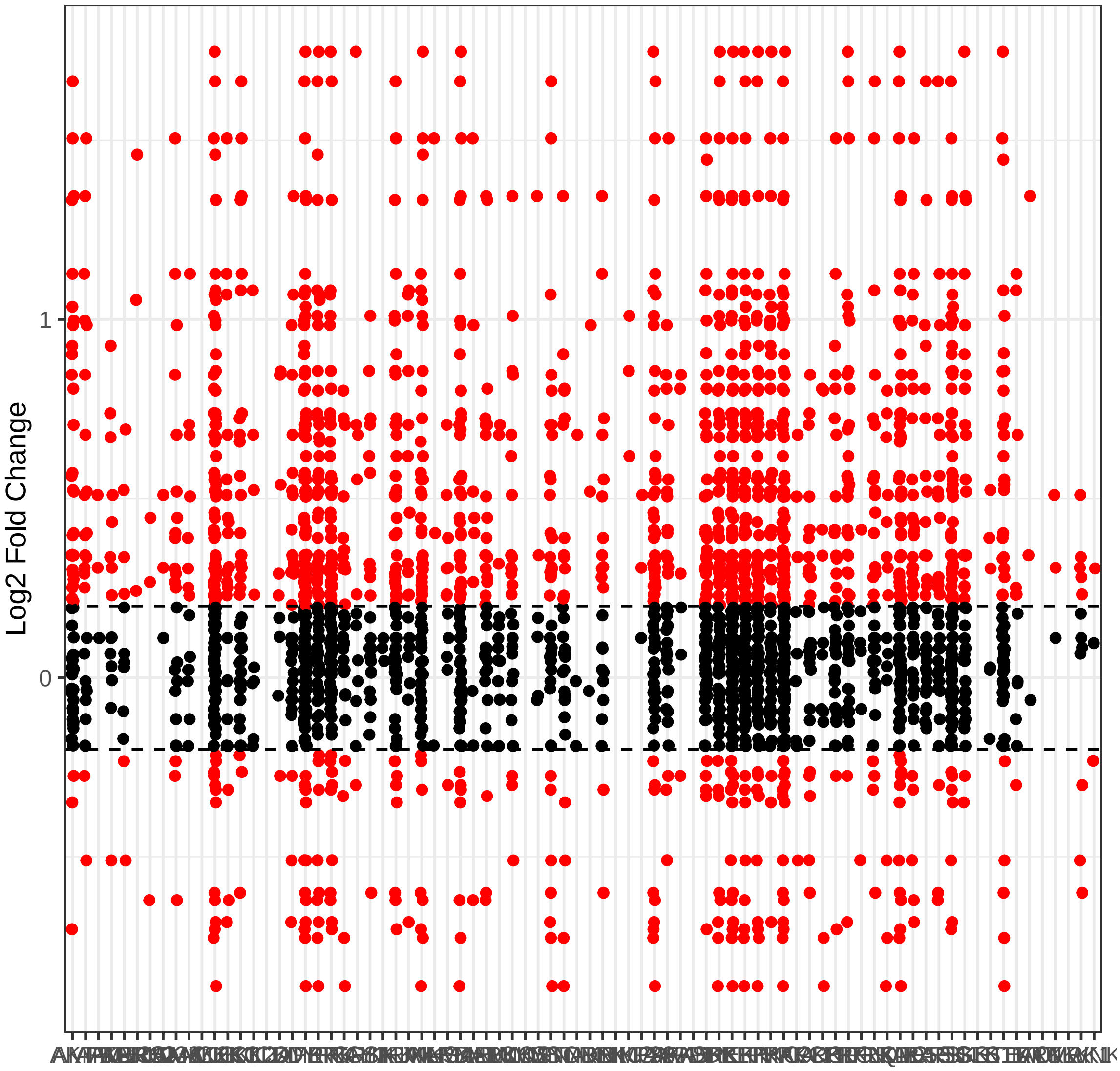


Figure 3.9: Kinase Activity summary for each kinase family based on peptide phsophorylation

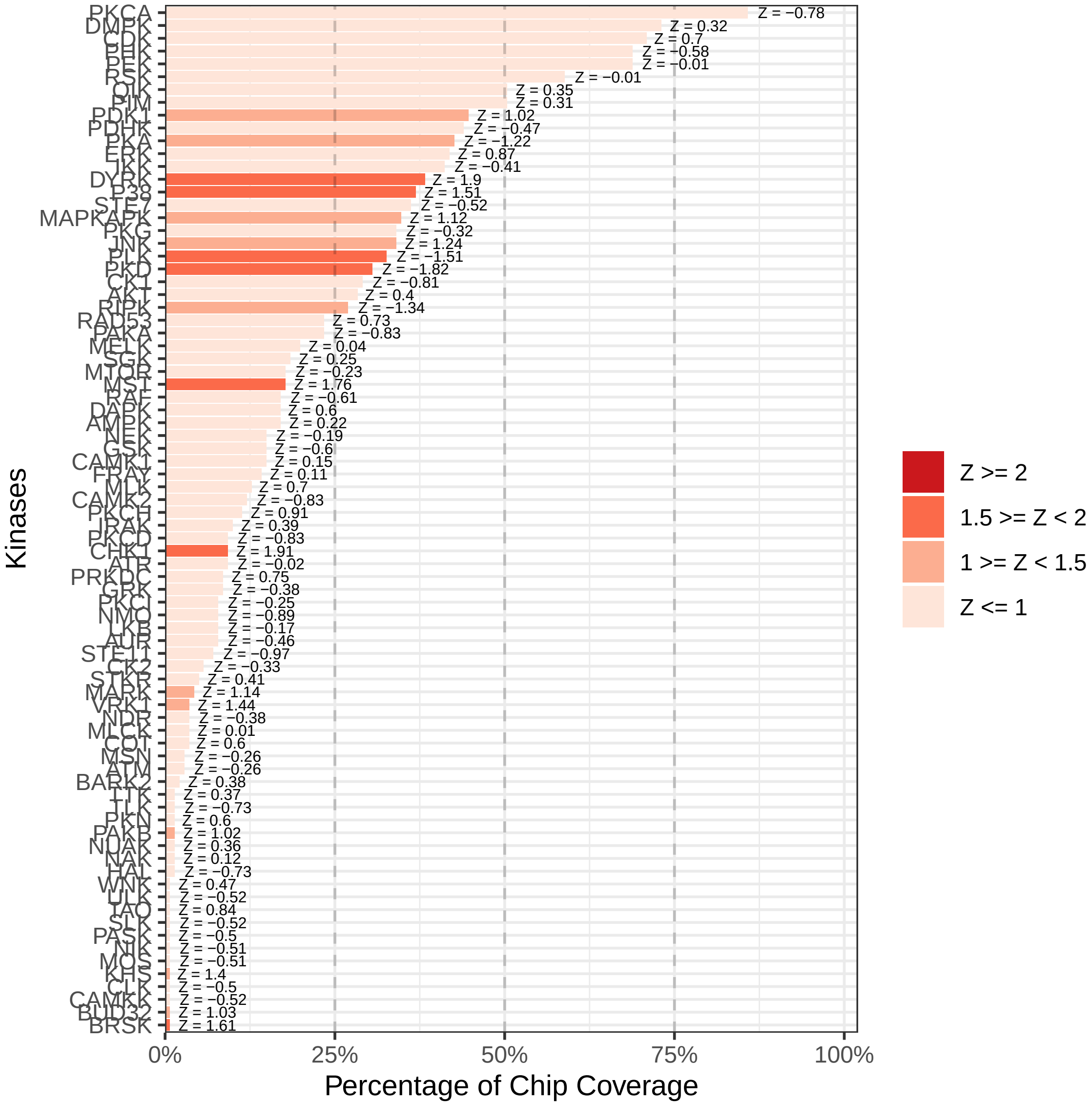
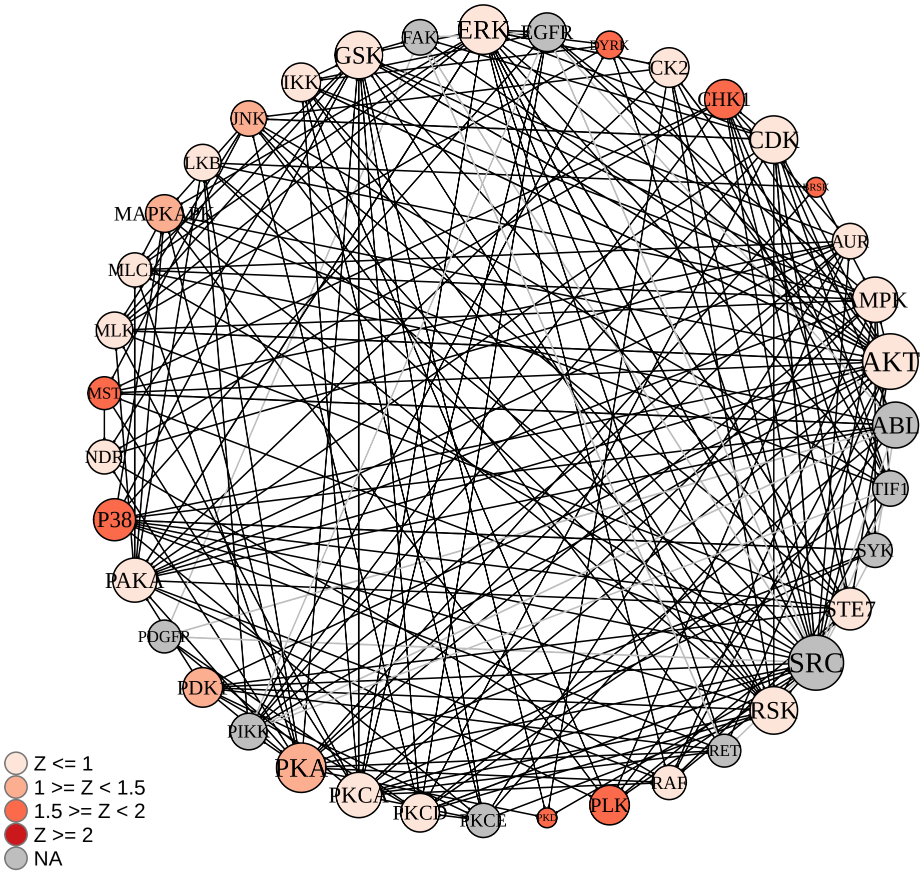


Figure 3.10: Percentge of peptides each kinase family phosphorylates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EGFR
FAK
GSK
DYRK
CK2
IKK
CHK1
JNK
CDK
LKB
BRSK
MAPKAPK
AUR
MLCK
AMPK
MLK
AKT
MST
ABL
NDR
TIF1
P38
SYK
PAKA
STE7
PDGFR
SRC
PDK
RSK
PIKK
RET
PKA
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PLK
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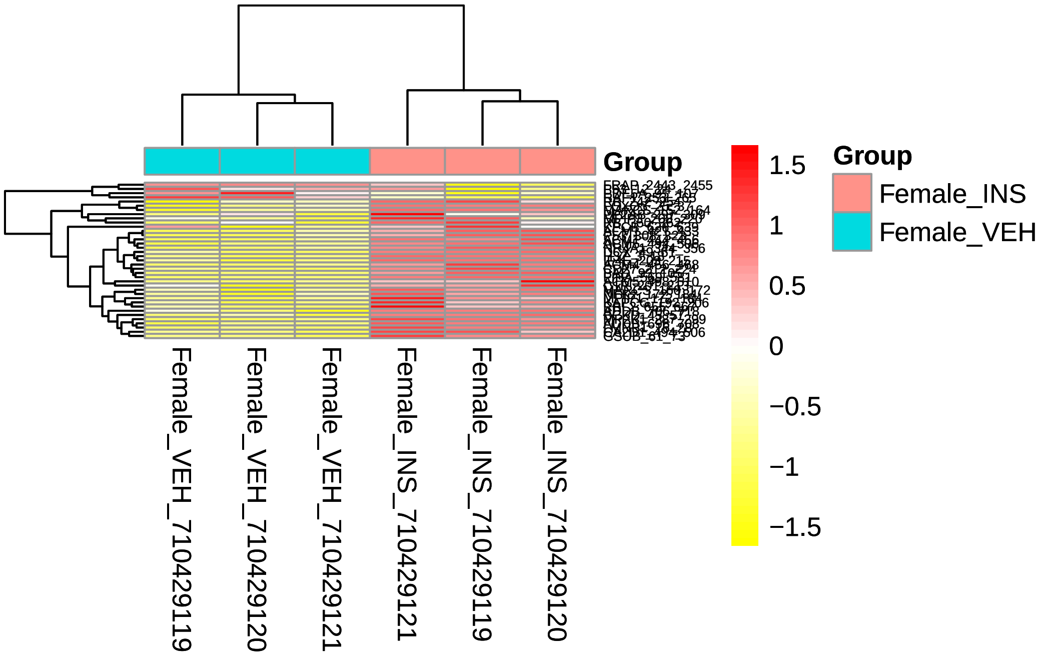
PKCE
PKD
Z <= 1
1 >= Z < 1.5
1.5 >= Z < 2
Z >= 2
NA



3.6.2 Female_INS vs Female_VEH

3.6.2.1 Heatmap

After applying the *Filtering Parameters* for this group comparison, only *37*/141 peptides carried forward in the analysis (i.e. *37 hits*). Below are some figures to visualize the differences between these samples for considering these *hits*.

3.6.2.2 Violin Plot

Below, the violin plot visualizes the distribution of selected peptides for the analysis.

3.6.2.3 Waterfall Plot

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

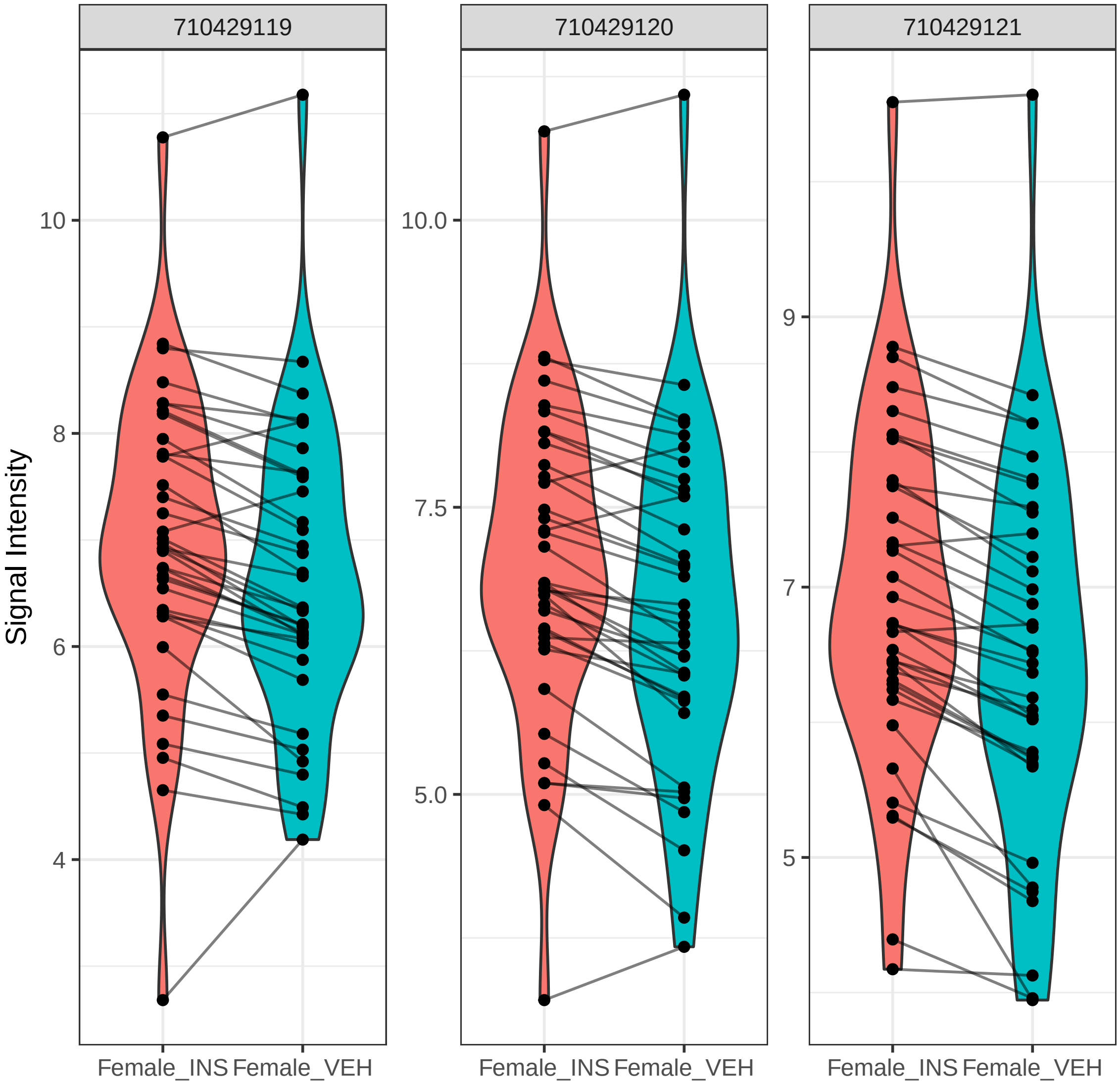


Figure 3.11: Violin plot of two groups

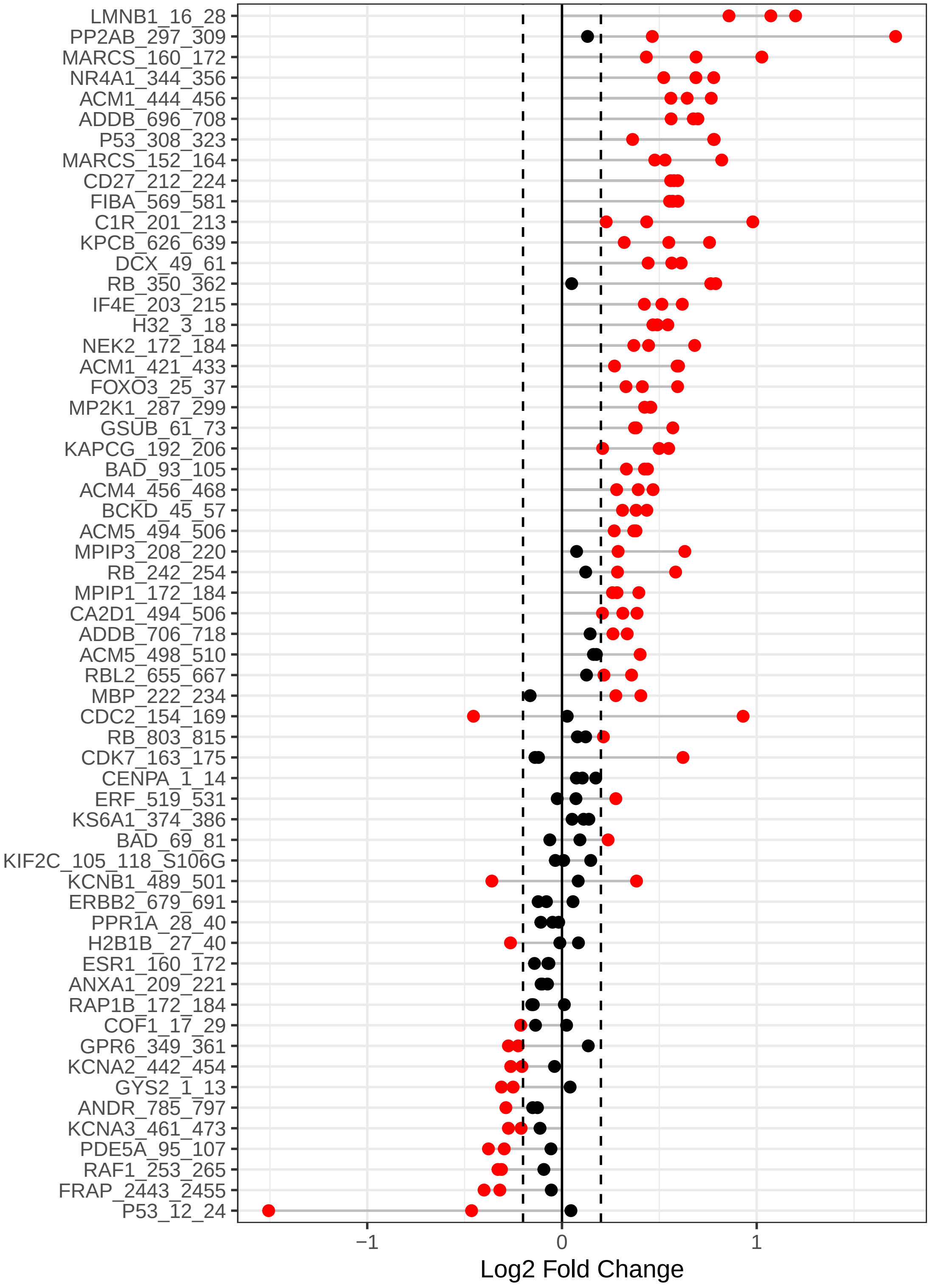


Figure 3.12: Waterfall Plot to show the distribution of change in peptide phosphorylation

3.6.2.4 Upstream Kinase Analysis

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Kinase	AvgZ
ERK	2.684167
VRK1	2.673333
MLCK	2.275000
PKN	1.958333
PKCH	1.850000
P38	1.677500
JNK	1.625000
DYRK	1.412500
MLK	1.255000
MST	1.164167
PKCD	-1.108333
STE11	-1.118333
NMO	-1.197500
RIPK	-1.203333
RSK	-1.270833
PLK	-1.298333
PHK	-1.439167
PKG	-1.561667
PKD	-1.833333
PKA	-2.137500

Method	NumberOfPeptides
meanLFC.0.15	43
meanLFC.0.2	37
meanLFC.0.3	31
meanLFC.0.4	23
710429119.0.15	42

Method	NumberOfPeptides
710429119.0.2	41
710429119.0.3	32
710429119.0.4	24
710429120.0.15	42
710429120.0.2	40
710429120.0.3	30
710429120.0.4	22
710429121.0.15	42
710429121.0.2	39
710429121.0.3	31
710429121.0.4	23

3.6.2.5 Z Scores Plot

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

3.6.2.6 Reverse KRSA Plot

We will use the reverse KRSA plot function, to plot the log2 fold change values for all peptides mapped to kinase hits. This will help us examine the activity of the kinase

3.6.2.7 Coverage Plot

To view the coverage of kinases across the full list of peptides on the chip, we will use the coverage plot function

3.6.2.8 Ball Model Network

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

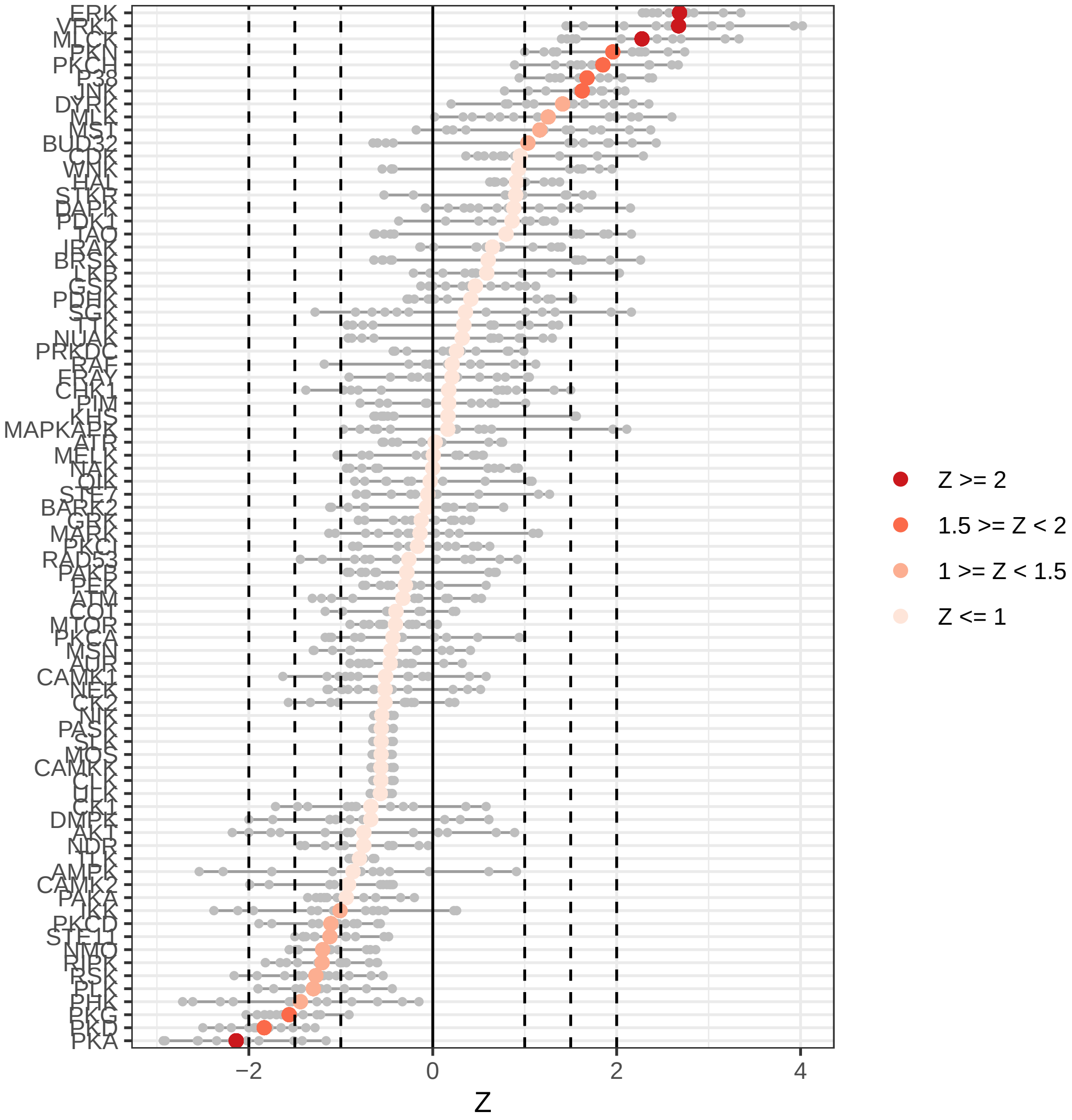


Figure 3.13: Waterfall plot of the Z Scores of each kinase family

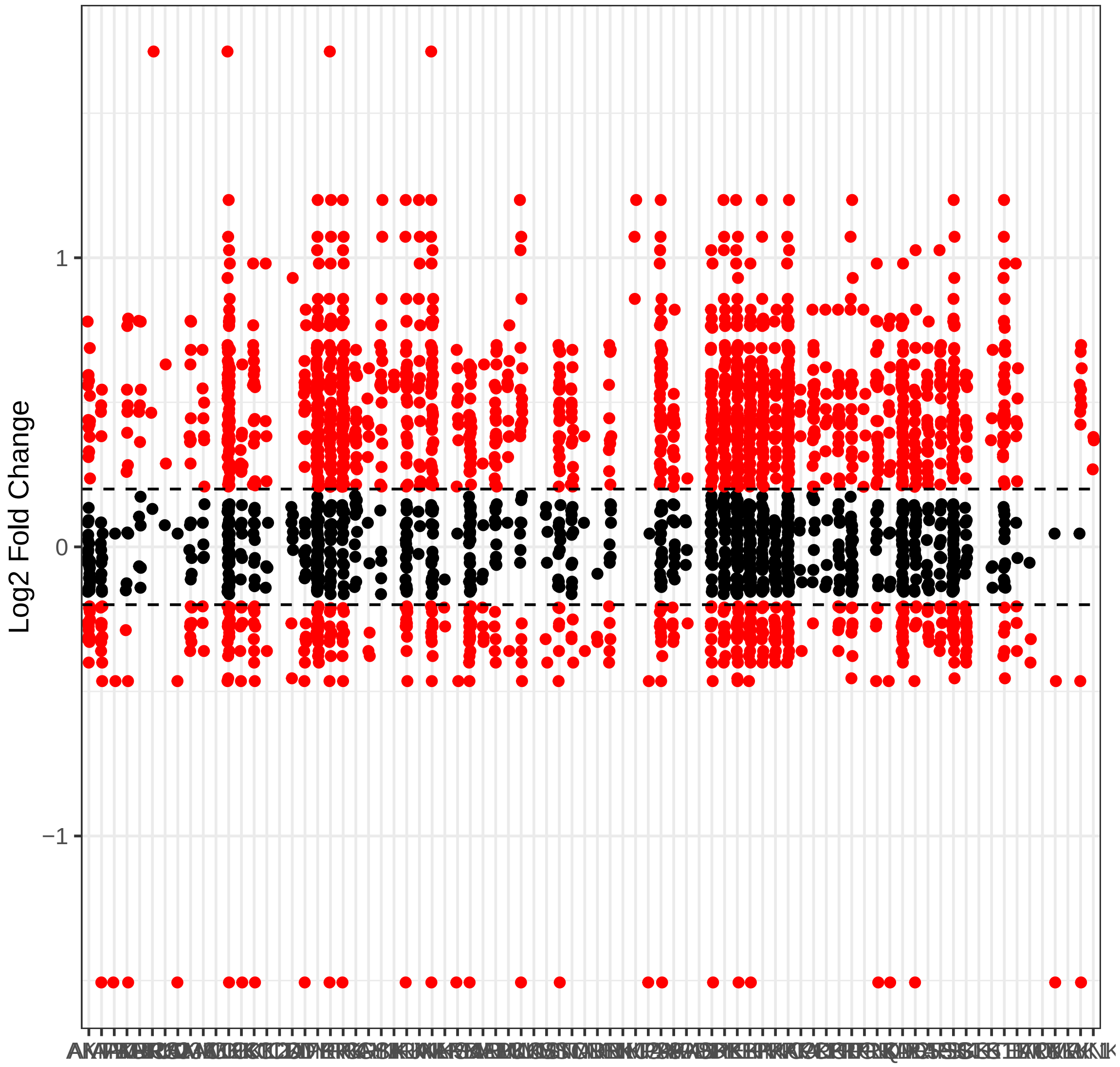


Figure 3.14: Kinase Activity summary for each kinase family based on peptide phsophorylation

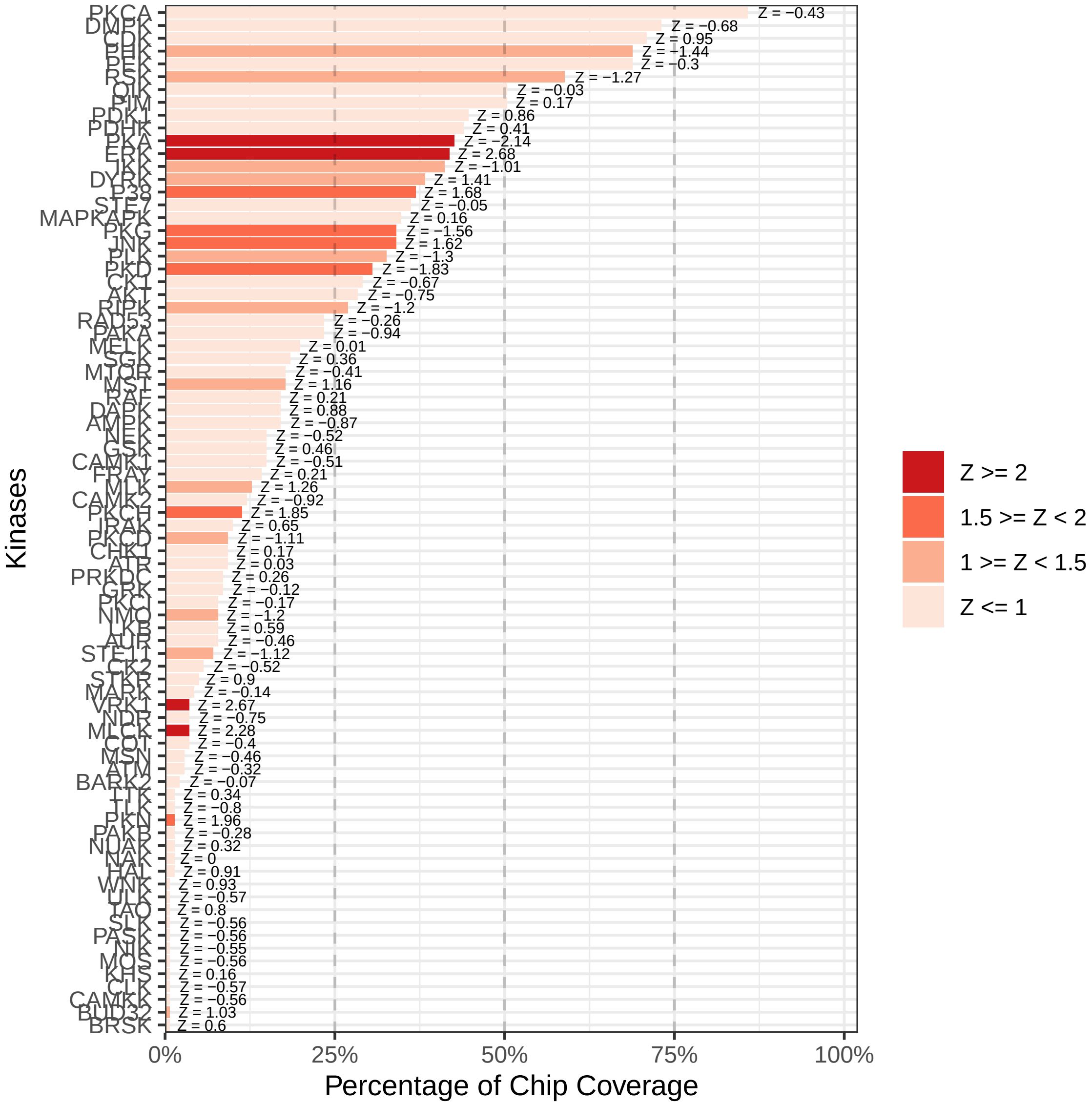
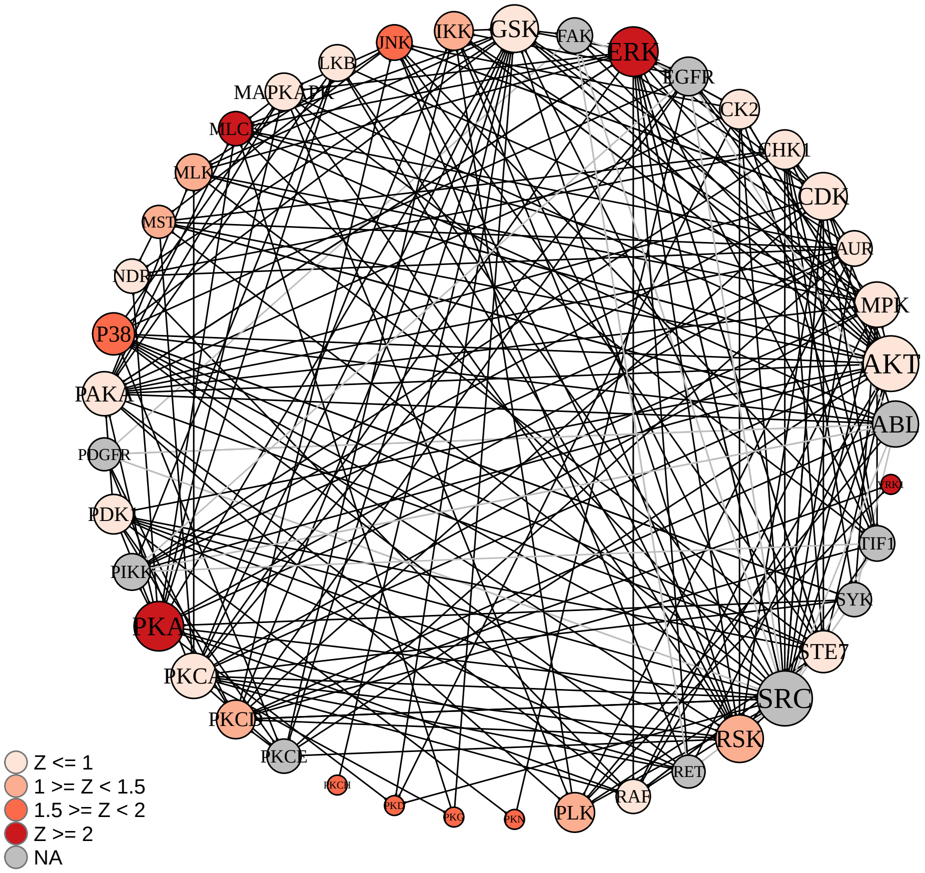


Figure 3.15: Percentge of peptides each kinase family phosphorylates


GSK
IKK
JNK
LKB
MAPKAPK
MLCK
MLK
MST
NDR
P38
PAKA
PDGFR
PDK1
PIKK
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PKCA
PKCD
PKCE
PKCH
PKD
PKG
PKN
PLK
RAF
RET
RSK
SRC
STE7
SYK
TIF1
VRK1
ABL
AKT
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AUR
CDK
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CK2
EGFR
ERK
FAK
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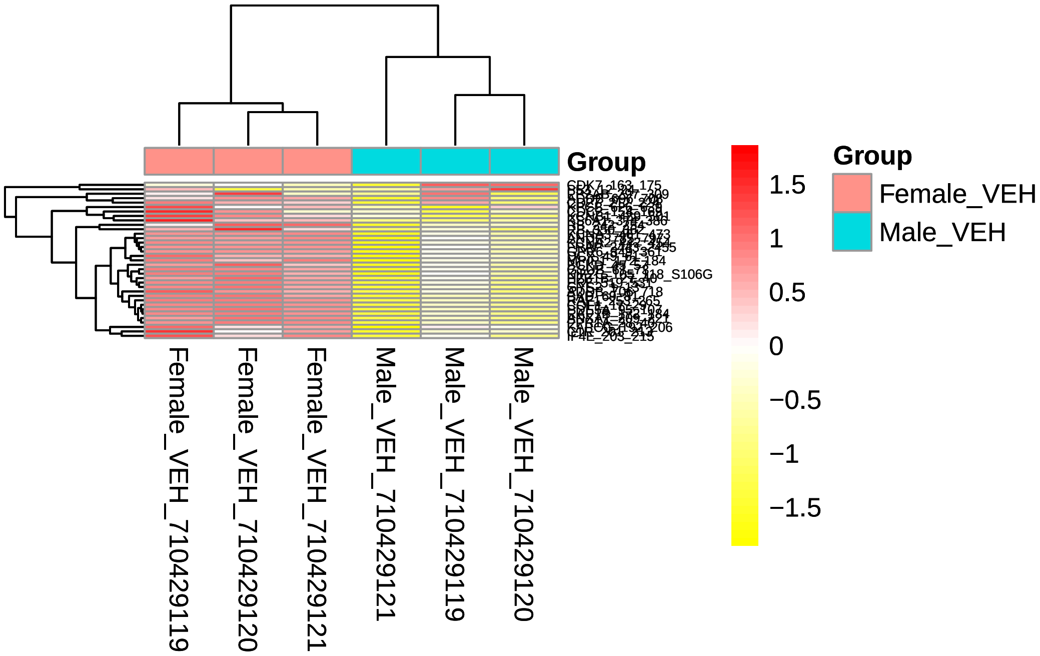
1.5 >= Z < 2
Z >= 2
NA



3.6.3 Male_VEH vs Female_VEH

3.6.3.1 Heatmap

After applying the *Filtering Parameters* for this group comparison, only *35*/141 peptides carried forward in the analysis (i.e. *35 hits*). Below are some figures to visualize the differences between these samples for considering these *hits*.

3.6.3.2 Violin Plot

Below, the violin plot visualizes the distribution of selected peptides for the analysis.

3.6.3.3 Waterfall Plot

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

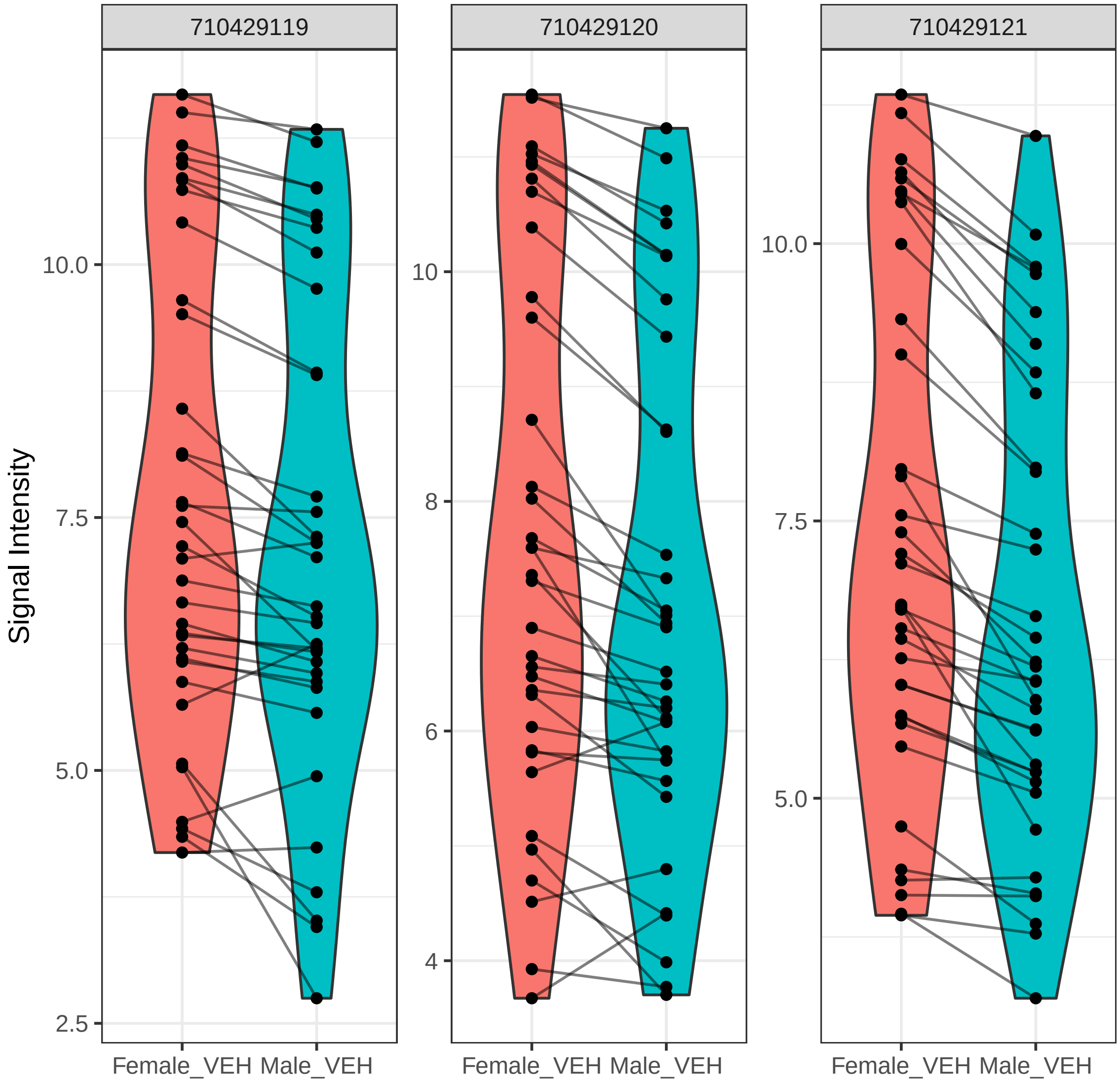


Figure 3.16: Violin plot of two groups

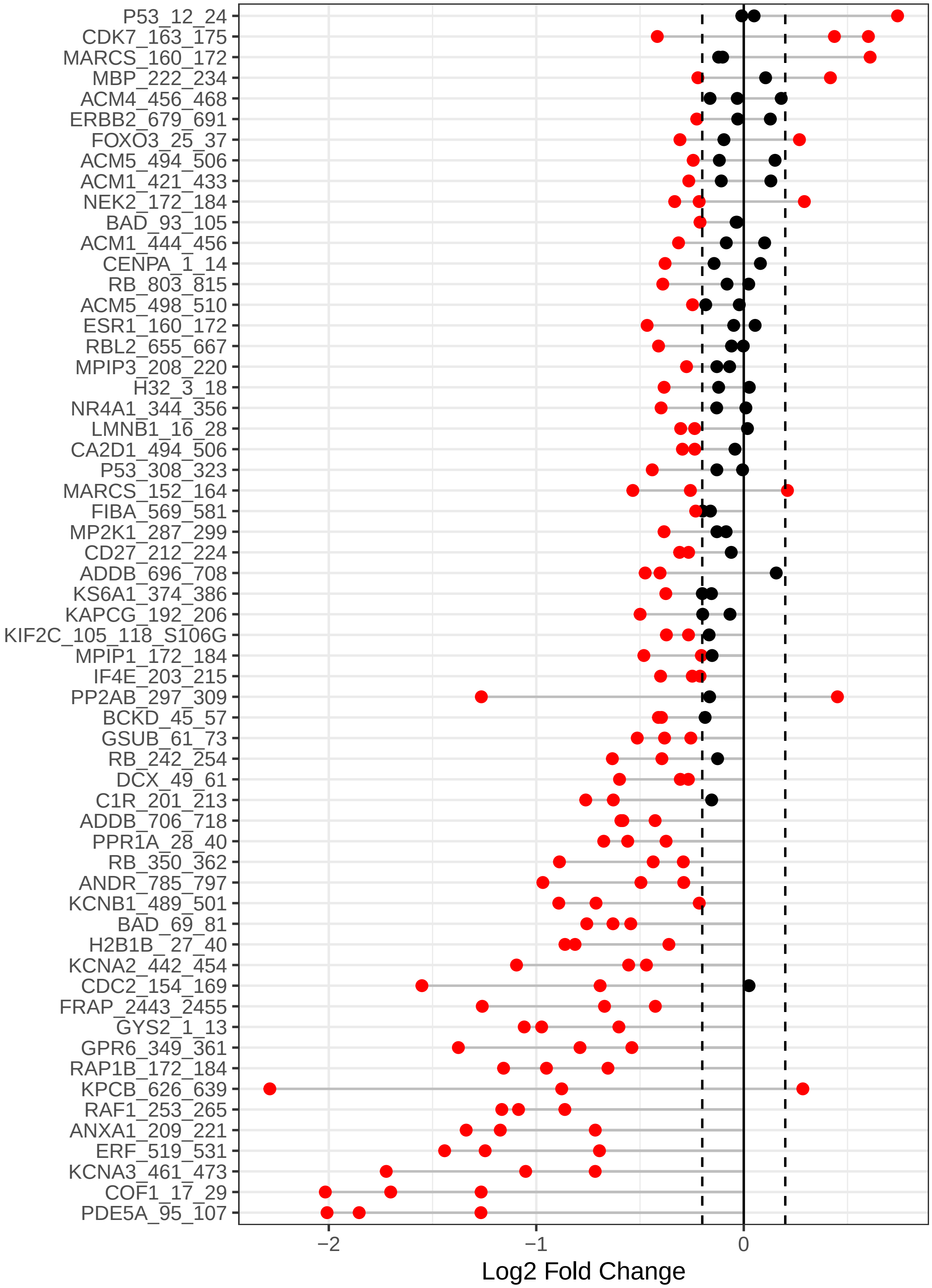


Figure 3.17: Waterfall Plot to show the distribution of change in peptide phosphorylation

3.6.3.4 Upstream Kinase Analysis

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SGK	1.9583333
MST	1.9466667
MAPKAPK	1.8716667
CHK1	1.8316667
TAO	1.7941667
KHS	1.7791667
P38	1.6225000
CDK	1.4741667
GSK	-0.7750000
TLK	-0.8166667
STE7	-0.8416667
RIPK	-1.1566667
PLK	-1.1766667
AUR	-1.2216667
STE11	-1.2366667
PKD	-1.2400000
PKCD	-1.3683333
NMO	-1.7116667

Method	NumberOfPeptides
meanLFC.0.15	47
meanLFC.0.2	35
meanLFC.0.3	26
meanLFC.0.4	21
710429119.0.15	39

Method	NumberOfPeptides
710429119.0.2	32
710429119.0.3	24
710429119.0.4	20
710429120.0.15	42
710429120.0.2	38
710429120.0.3	28
710429120.0.4	24
710429121.0.15	55
710429121.0.2	51
710429121.0.3	42
710429121.0.4	32

3.6.3.5 Z Scores Plot

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

3.6.3.6 Reverse KRSA Plot

We will use the reverse KRSA plot function, to plot the log2 fold change values for all peptides mapped to kinase hits. This will help us examine the activity of the kinase

3.6.3.7 Coverage Plot

To view the coverage of kinases across the full list of peptides on the chip, we will use the coverage plot function

3.6.3.8 Ball Model Network

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

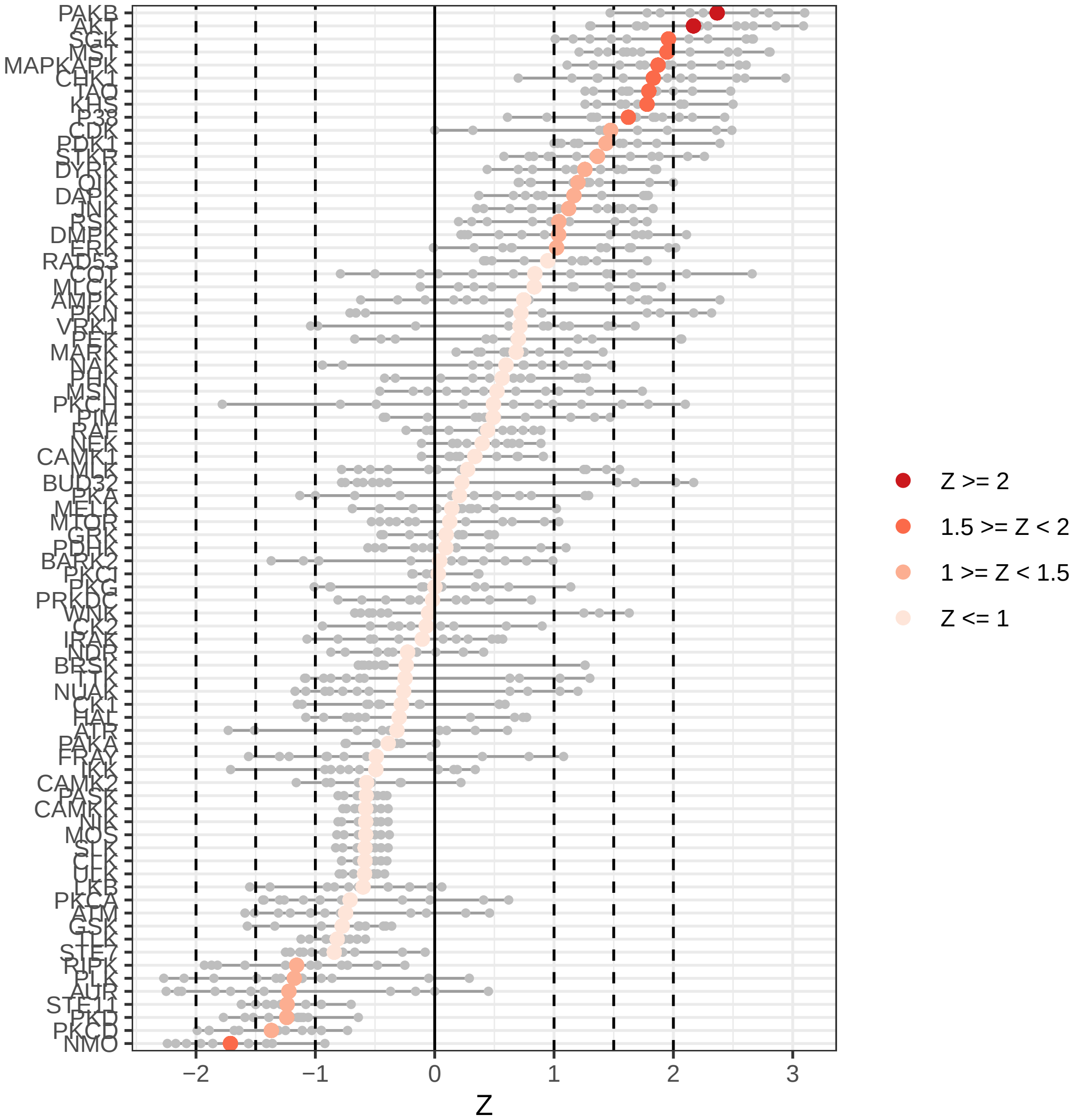


Figure 3.18: Waterfall plot of the Z Scores of each kinase family

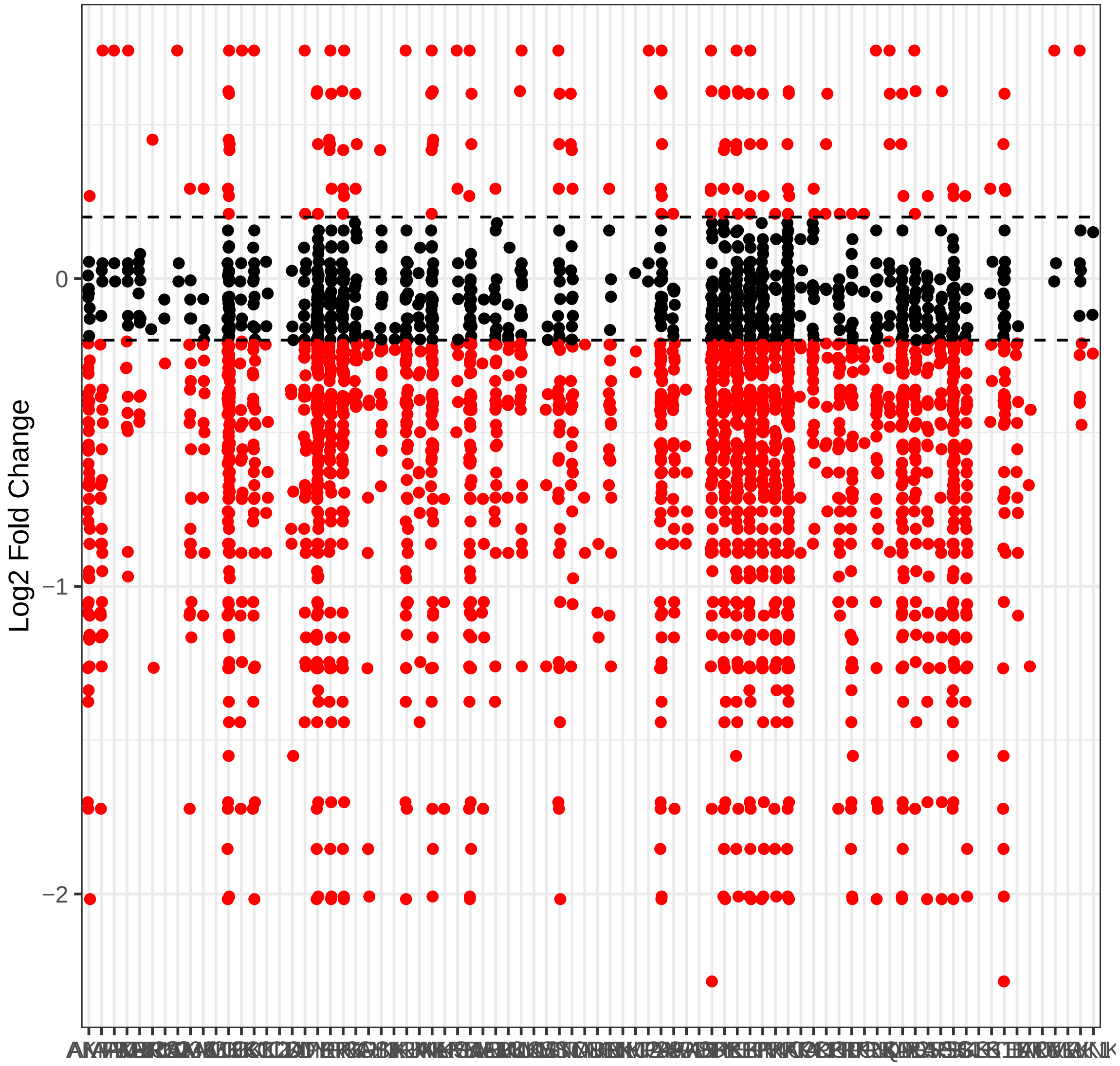


Figure 3.19: Kinase Activity summary for each kinase family based on peptide phsophorylation

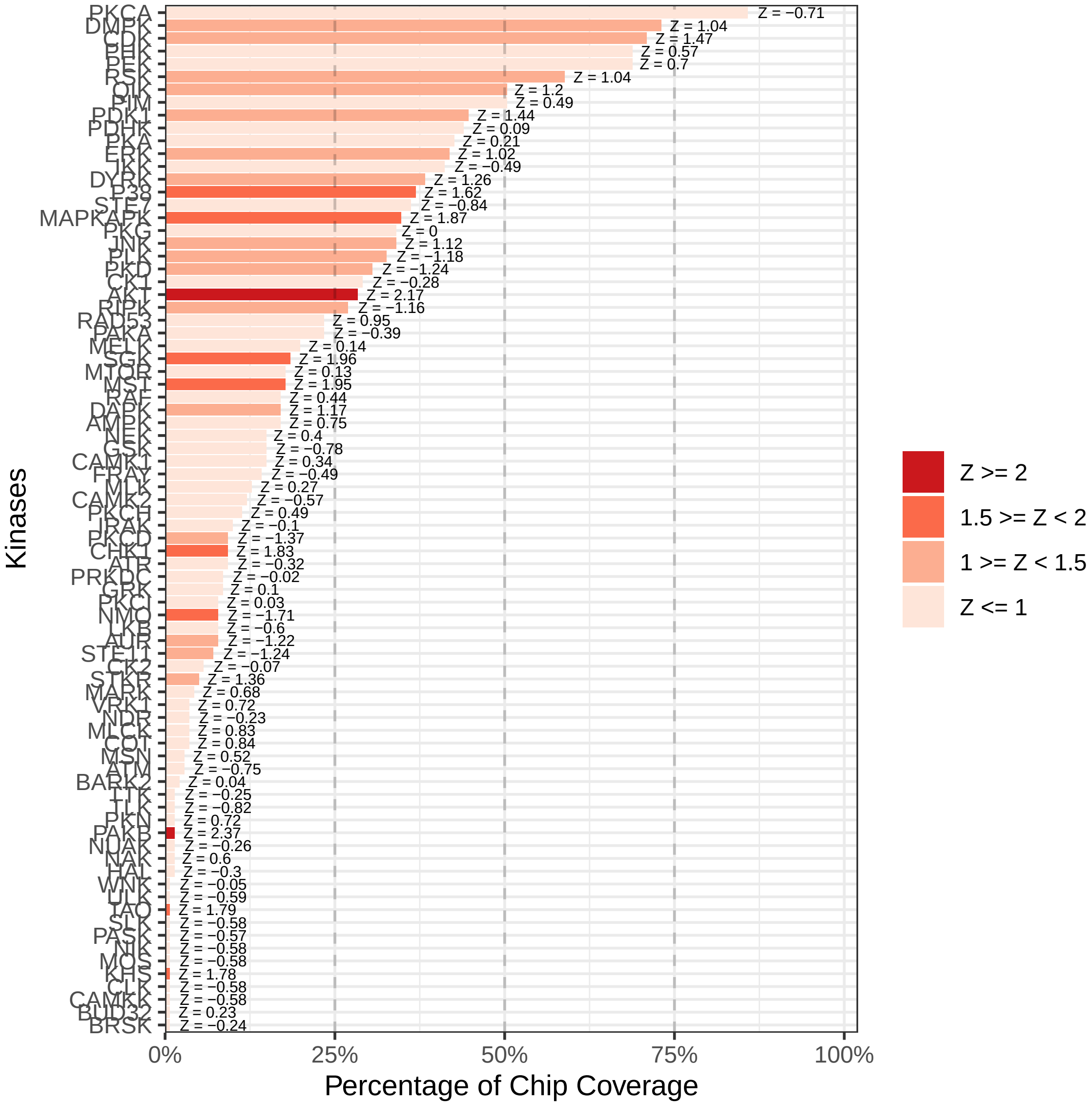
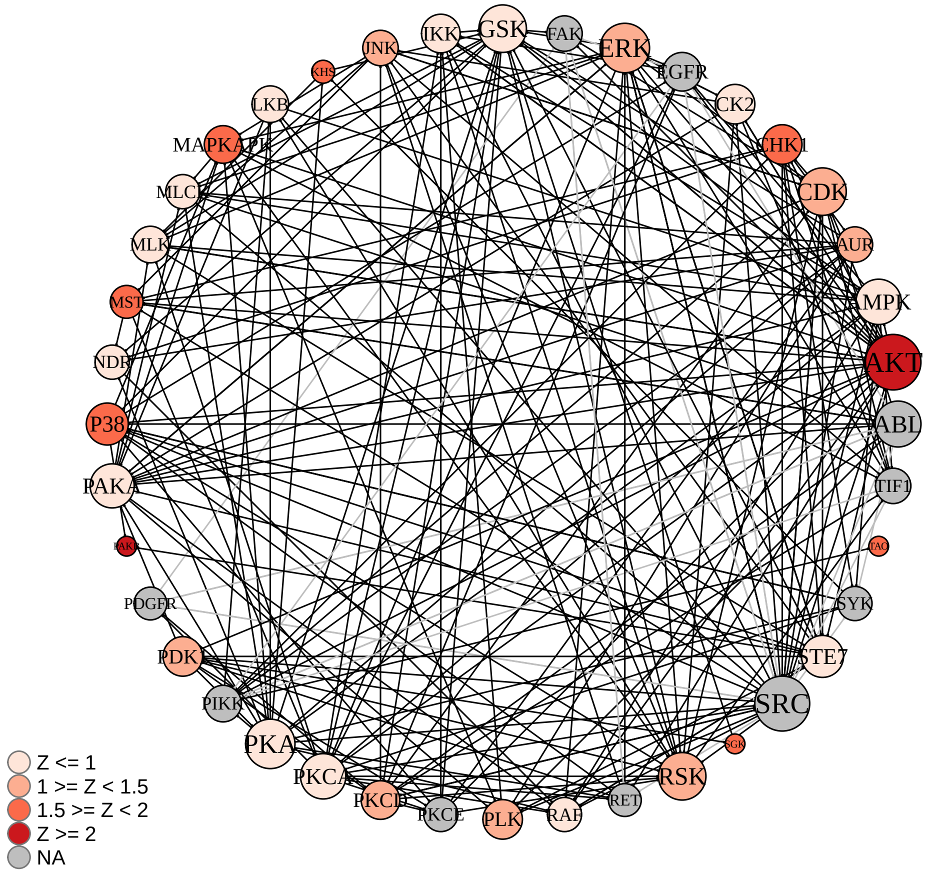


Figure 3.20: Percentge of peptides each kinase family phosphorylates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JNK
KHS
LKB
MAPKAPK
MLCK
MLK
MST
NDR
P38
PAKA
PAKB
PDGFR
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PIKK
PKA
PKCA
PKCD
PKCE
PLK
RAF
RET
RSK
SGK
SRC
STE7
SYK
TAO
TIF1
ABL
AKT
AMPK
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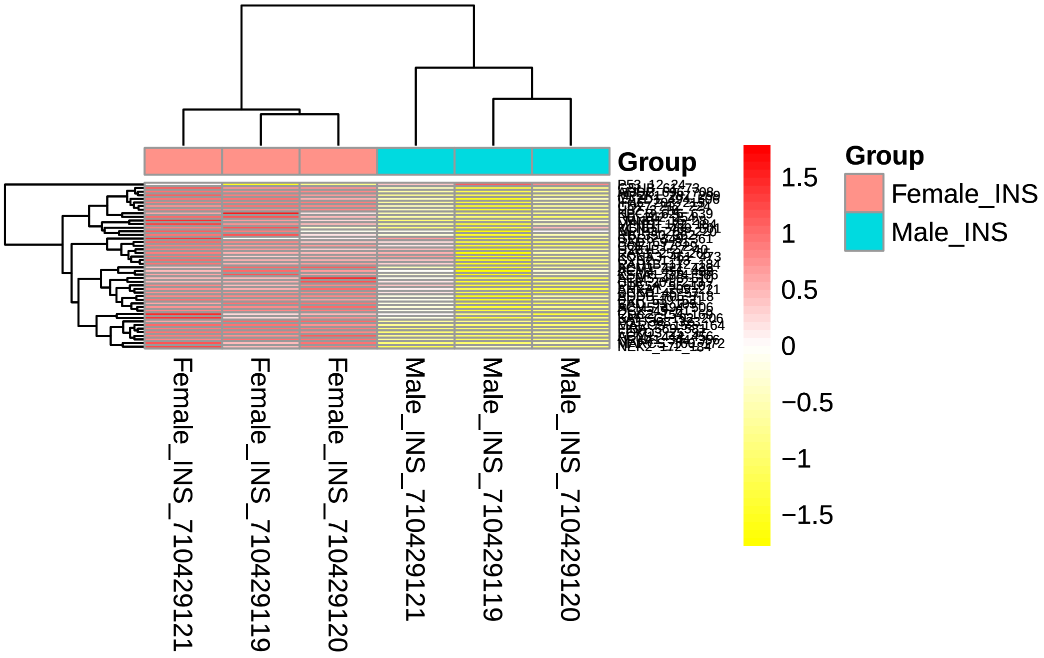
1.5 >= Z < 2
Z >= 2
NA



3.6.4 Male_INS vs Female_INS

3.6.4.1 Heatmap

After applying the *Filtering Parameters* for this group comparison, only *46*/141 peptides carried forward in the analysis (i.e. *46 hits*). Below are some figures to visualize the differences between these samples for considering these *hits*.

3.6.4.2 Violin Plot

Below, the violin plot visualizes the distribution of selected peptides for the analysis.

3.6.4.3 Waterfall Plot

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

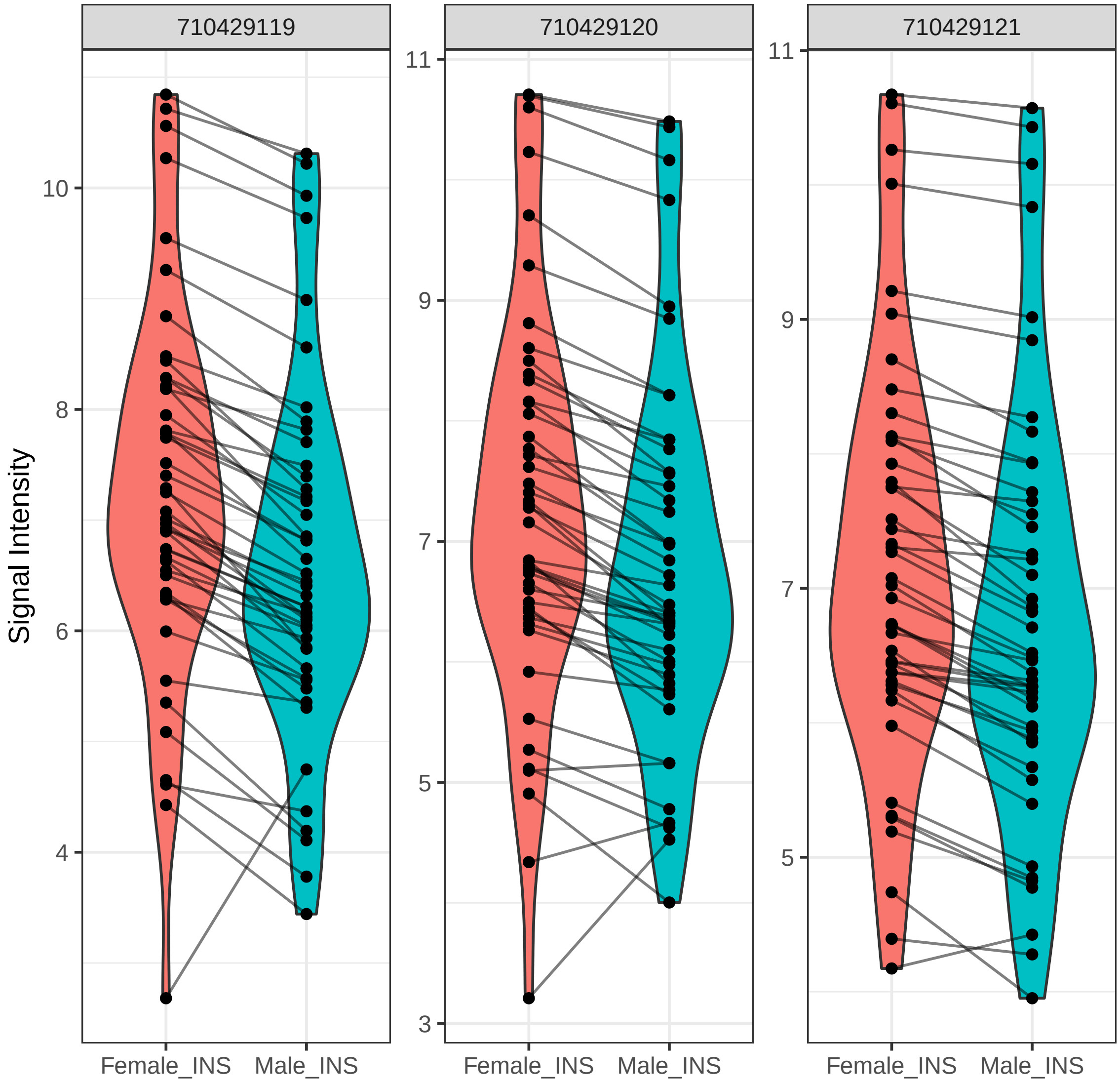


Figure 3.21: Violin plot of two groups

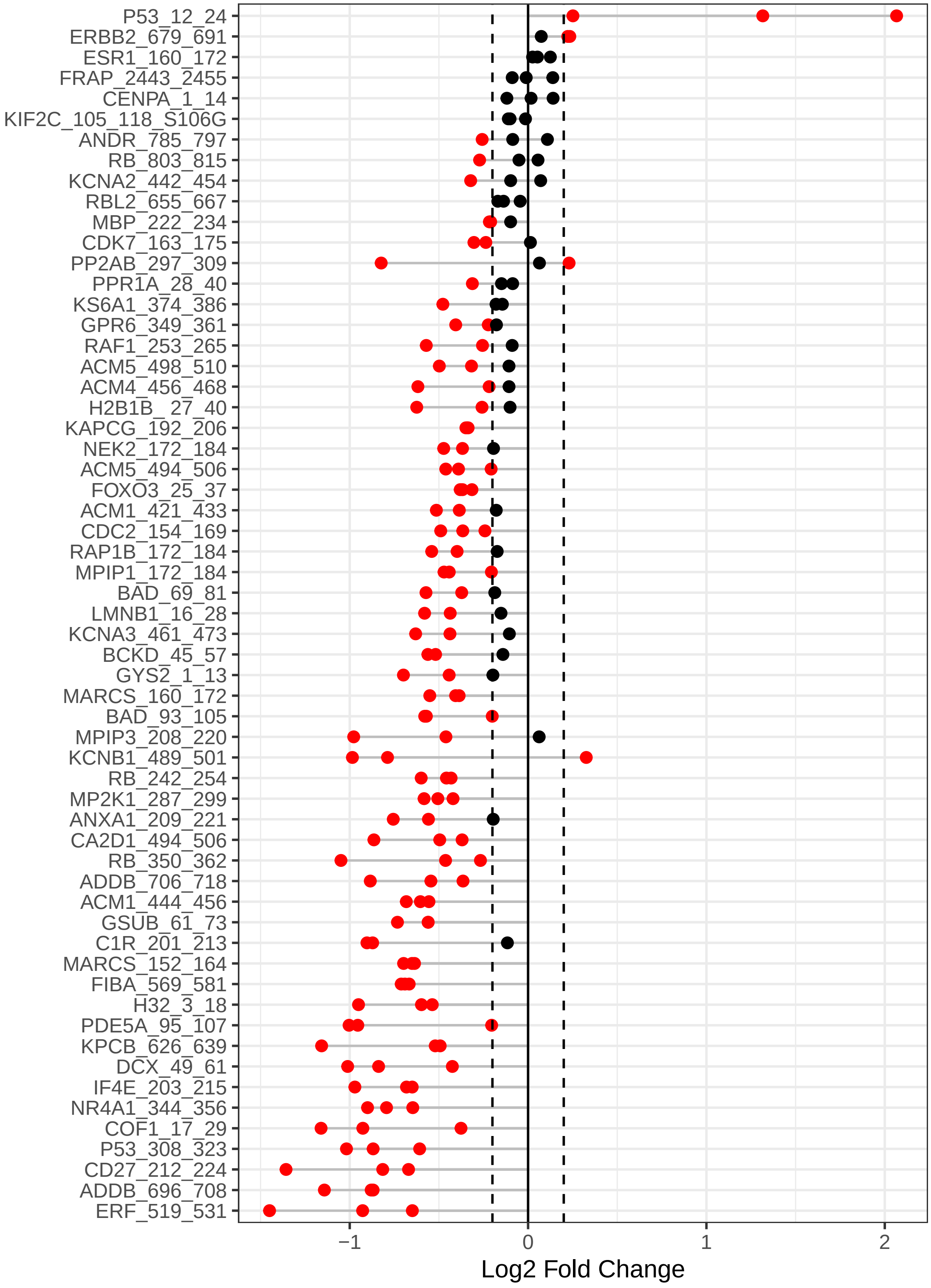


Figure 3.22: Waterfall Plot to show the distribution of change in peptide phosphorylation

3.6.4.4 Upstream Kinase Analysis

The lab carefully curated and mapped the kinases that can act and phosphorylate each peptide present on the chip. This was achieved by using multiple sources including GPS 3.0, Kinexus Phosphonet, PhosphoELM and PhosphoSite Plus. Based on that association between peptides and kinases, a random sampling analysis is performed for these hits. The basic idea of *KRSA* is: For each iteration (*2000* iterations performed in this analysis), the same number of hits are randomly selected from the total 141/or 193 peptides present on the chip. Predicted kinases are then mapped to this sample list of peptides and number of kinases are determined. The kinase count from the actual hits and random sampling is then compared to determine the significance.

Kinase	AvgZ
ERK	2.786667
VRK1	2.484167
MLCK	2.220000
PKN	2.140833
MST	2.036667
P38	1.919167
DYRK	1.695000
PKCH	1.580000
CHK1	1.551667
BUD32	1.210000
MTOR	-1.008333
CK1	-1.064167
PLK	-1.089167
NEK	-1.095000
CAMK2	-1.273333
PKA	-1.354167
RIPK	-1.450000
STE11	-1.545000
NMO	-1.645000
PKD	-2.070000

	Method	NumberOfPeptides	
meanLFC.0.15			51
meanLFC.0.2			46
meanLFC.0.3			44
meanLFC.0.4			29
710429119.0.15			51

Method	NumberOfPeptides
710429119.0.2	49
710429119.0.3	46
710429119.0.4	40
710429120.0.15	49
710429120.0.2	47
710429120.0.3	38
710429120.0.4	28
710429121.0.15	42
710429121.0.2	36
710429121.0.3	29
710429121.0.4	24

3.6.4.5 Z Scores Plot

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

3.6.4.6 Reverse KRSA Plot

We will use the reverse KRSA plot function, to plot the log2 fold change values for all peptides mapped to kinase hits. This will help us examine the activity of the kinase

3.6.4.7 Coverage Plot

To view the coverage of kinases across the full list of peptides on the chip, we will use the coverage plot function

3.6.4.8 Ball Model Network

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

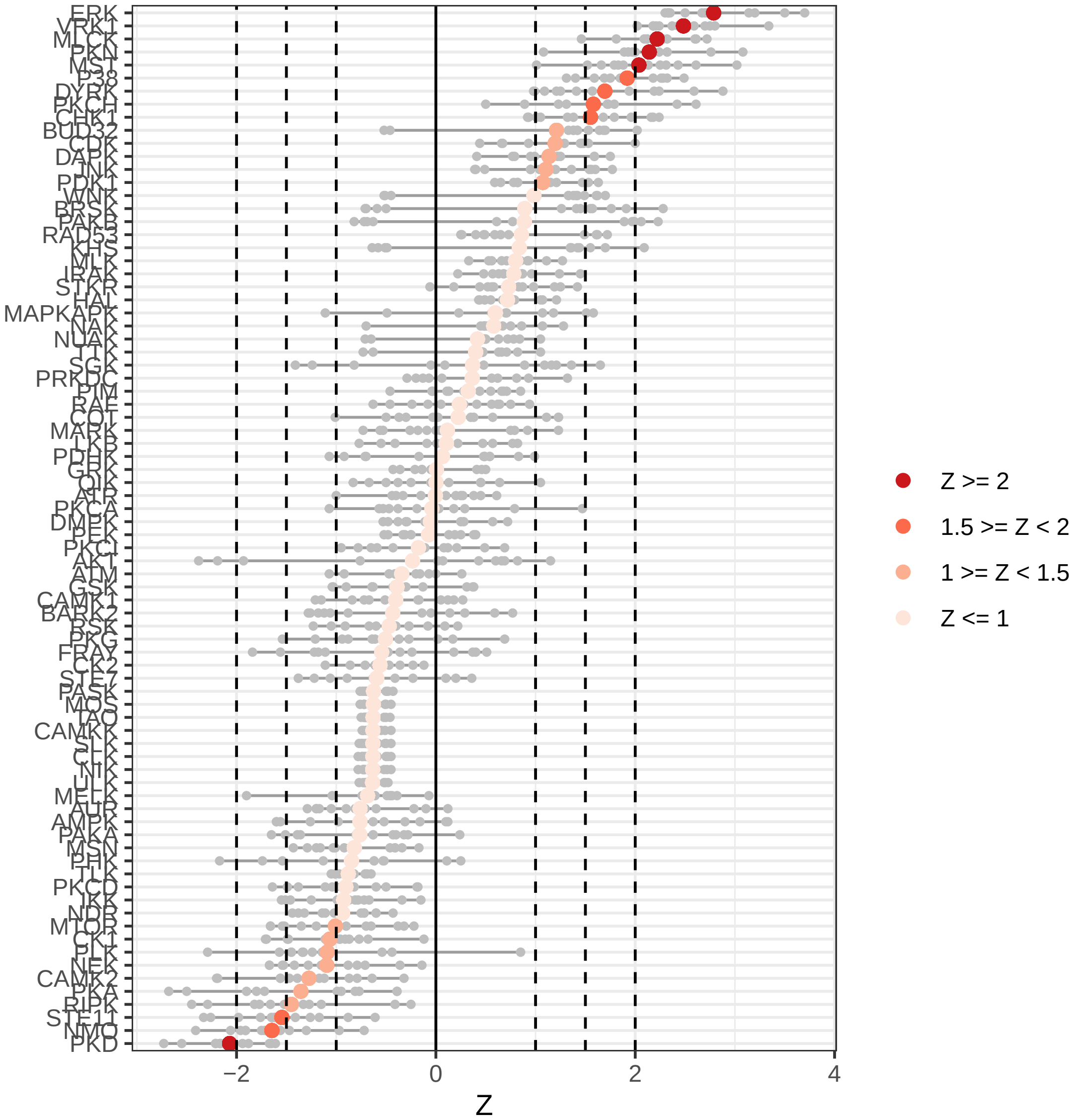


Figure 3.23: Waterfall plot of the Z Scores of each kinase family

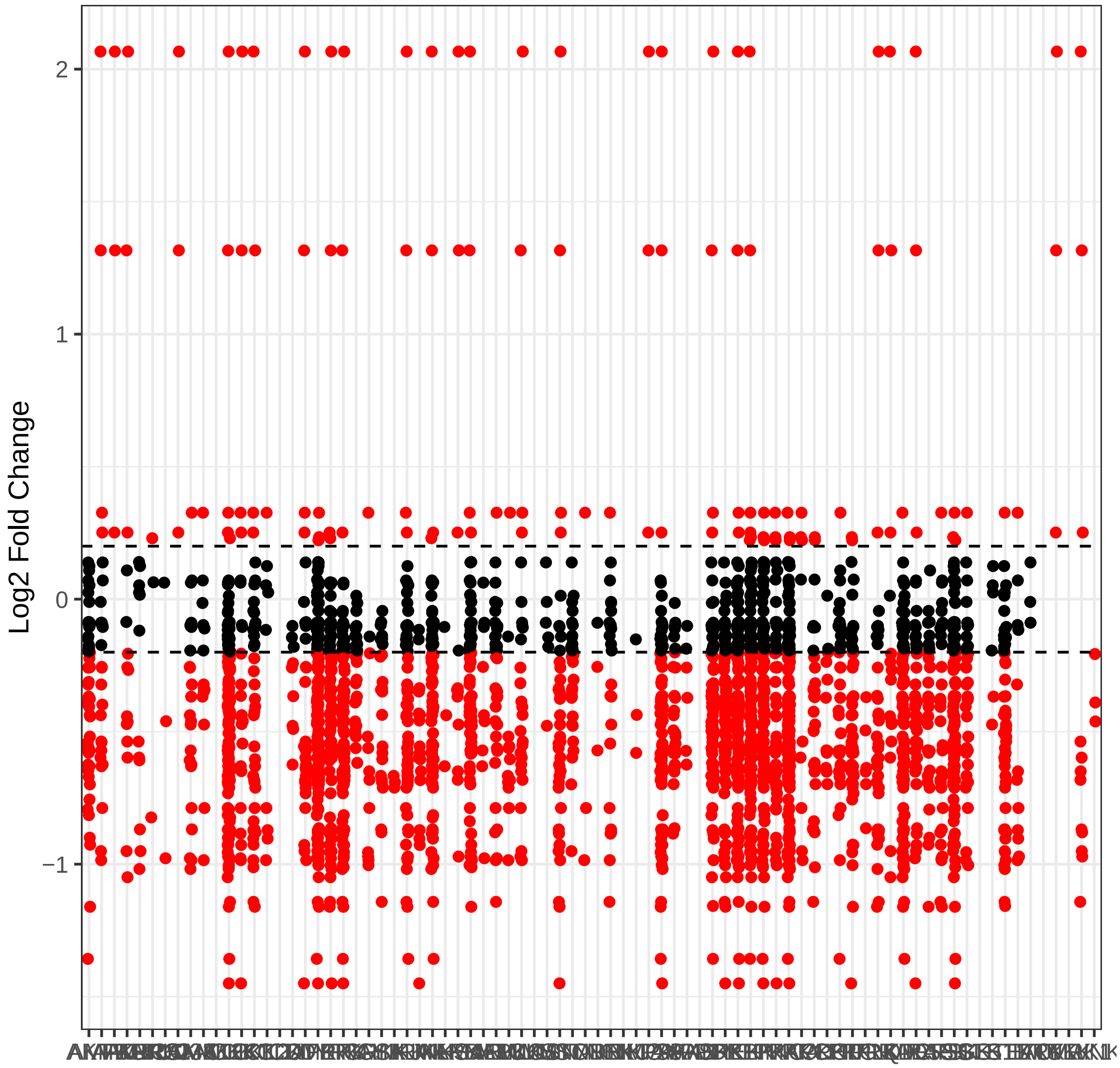


Figure 3.24: Kinase Activity summary for each kinase family based on peptide phsophorylation

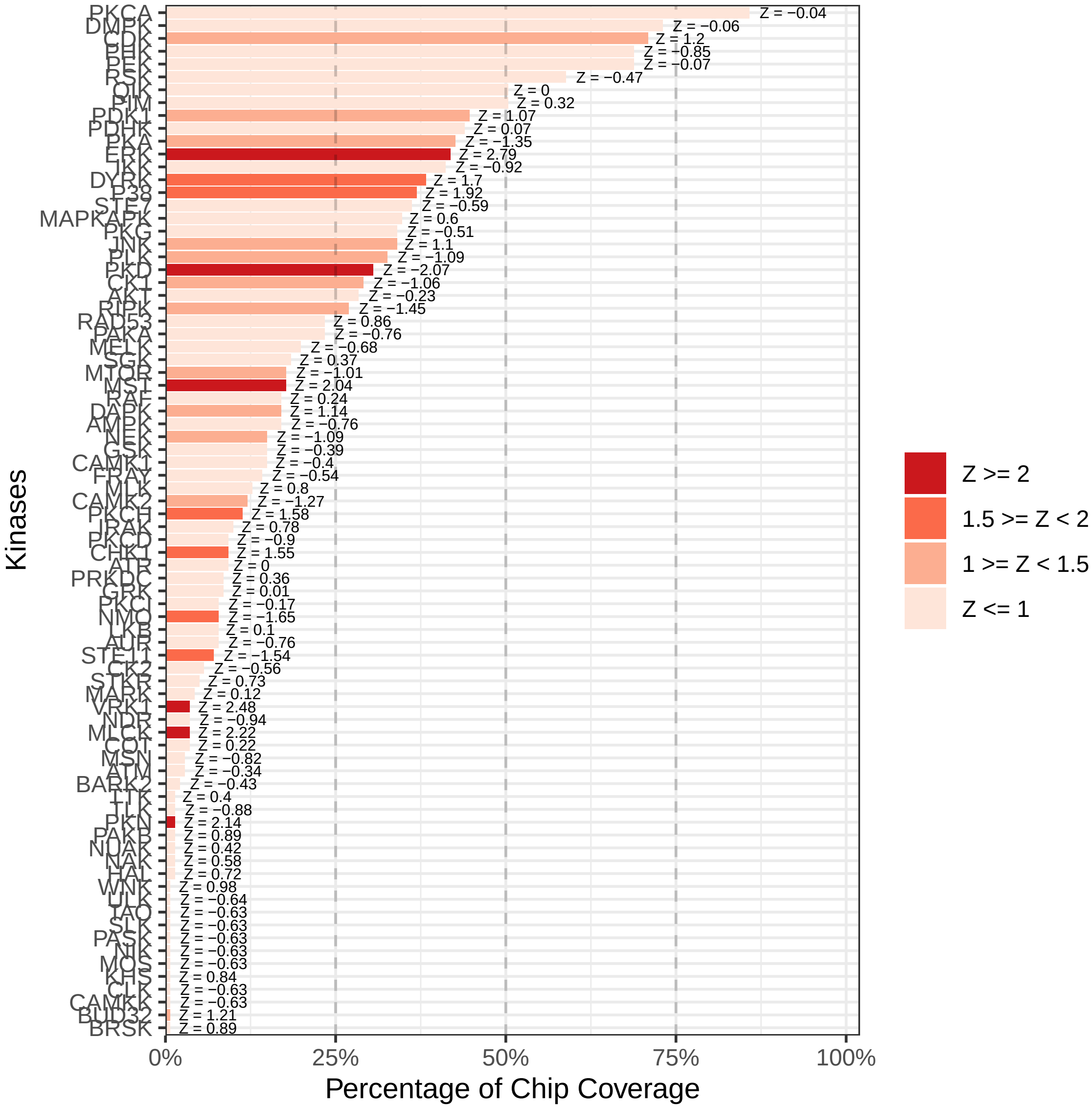
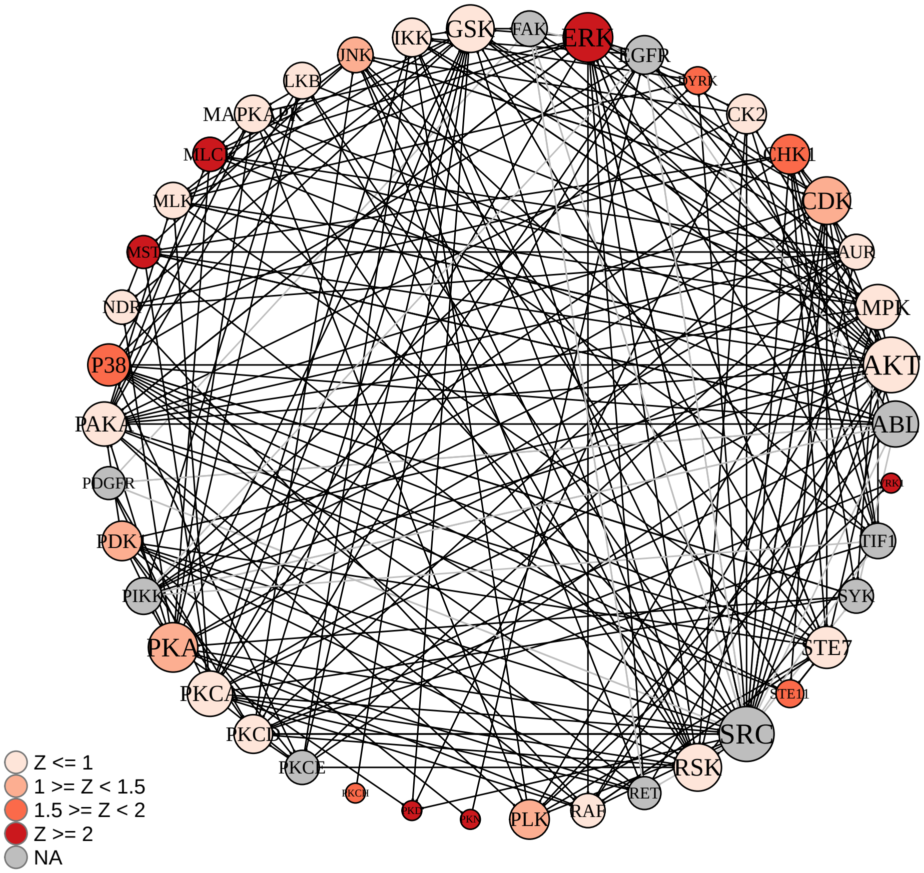


Figure 3.25: Percentge of peptides each kinase family phosphorylates


GSK
FAK
ERK
EGFR
DYRK
CK2
CHK1
CDK
AUR
AMPK
AKT
ABL
VRK1
TIF1
SYK
STE7
STE11
SRC
RSK
RET
RAF
PLK
PKN
PKD
PKCH
PKCE
PKCD
PKCA
PKA
PIKK
PDK
PDGFR
PAKA
P38
NDR
MST
MLK
MLCK
MAPKAPK
LKB
JNK
IKK
Z <= 1
1 >= Z < 1.5
1.5 >= Z < 2
Z >= 2
NA

4 Session Info

```
#> - Session info ---------------------------------------------------------------
#>  setting  value
#>  version  R version 4.3.1 (2023-06-16 ucrt)
#>  os       Windows 11 x64 (build 22621)
#>  system   x86_64, mingw32
#>  ui       RTerm
#>  language (EN)
#>  collate  English_United States.utf8
#>  ctype    English_United States.utf8
#>  tz       America/New_York
#>  date     2023-11-16
#>  pandoc   3.1.1 @ C:/Program Files/RStudio/resources/app/bin/quarto/bin/tools/ (via rmarkd
#>
#> - Packages -------------------------------------------------------------------
#>  package      * version date (UTC) lib source
#>  backports      1.4.1   2021-12-13 [1] CRAN (R 4.3.0)
#>  bit            4.0.5   2022-11-15 [1] CRAN (R 4.3.1)
#>  bit64          4.0.5   2020-08-30 [1] CRAN (R 4.3.1)
#>  broom          1.0.5   2023-06-09 [1] CRAN (R 4.3.1)
#>  cachem         1.0.8   2023-05-01 [1] CRAN (R 4.3.1)
#>  callr          3.7.3   2022-11-02 [1] CRAN (R 4.3.1)
#>  cli            3.6.1   2023-03-23 [1] CRAN (R 4.3.1)
#>  codetools      0.2-19  2023-02-01 [2] CRAN (R 4.3.1)
#>  colorspace     2.1-0   2023-01-23 [1] CRAN (R 4.3.1)
#>  crayon         1.5.2   2022-09-29 [1] CRAN (R 4.3.1)
#>  devtools       2.4.5   2022-10-11 [1] CRAN (R 4.3.1)
#>  digest         0.6.33  2023-07-07 [1] CRAN (R 4.3.1)
#>  dplyr        * 1.1.3   2023-09-03 [1] CRAN (R 4.3.1)
#>  ellipsis       0.3.2   2021-04-29 [1] CRAN (R 4.3.1)
#>  EnvStats       2.8.1   2023-08-22 [1] CRAN (R 4.3.1)
#>  evaluate       0.22    2023-09-29 [1] CRAN (R 4.3.1)
#>  fansi          1.0.5   2023-10-08 [1] CRAN (R 4.3.1)
#>  farver         2.1.1   2022-07-06 [1] CRAN (R 4.3.1)
#>  fastmap        1.1.1   2023-02-24 [1] CRAN (R 4.3.1)
#>  forcats      * 1.0.0   2023-01-29 [1] CRAN (R 4.3.1)
#>  fs             1.6.3   2023-07-20 [1] CRAN (R 4.3.1)
#>  furrr        * 0.3.1   2022-08-15 [1] CRAN (R 4.3.1)
#>  future       * 1.33.0  2023-07-01 [1] CRAN (R 4.3.1)
#>  generics       0.1.3   2022-07-05 [1] CRAN (R 4.3.1)
#>  ggplot2      * 3.4.4   2023-10-12 [1] CRAN (R 4.3.1)
#>  globals        0.16.2  2022-11-21 [1] CRAN (R 4.3.0)
#>  glue           1.6.2   2022-02-24 [1] CRAN (R 4.3.1)
#>  gt           * 0.10.0  2023-10-07 [1] CRAN (R 4.3.1)
#>  gtable         0.3.4   2023-08-21 [1] CRAN (R 4.3.1)
#>  hms            1.1.3   2023-03-21 [1] CRAN (R 4.3.1)
#>  htmltools      0.5.6.1 2023-10-06 [1] CRAN (R 4.3.1)
#>  htmlwidgets    1.6.2   2023-03-17 [1] CRAN (R 4.3.1)
#>  httpuv         1.6.11  2023-05-11 [1] CRAN (R 4.3.1)
#>  igraph         1.5.1   2023-08-10 [1] CRAN (R 4.3.1)
#>  jsonlite       1.8.7   2023-06-29 [1] CRAN (R 4.3.1)
#>  knitr        * 1.44    2023-09-11 [1] CRAN (R 4.3.1)
#>  KRSA         * 1.0.0   2023-08-09 [1] Github (CogDisResLab/KRSA@0bbeca5)
#>  labeling       0.4.3   2023-08-29 [1] CRAN (R 4.3.1)
#>  later          1.3.1   2023-05-02 [1] CRAN (R 4.3.1)
#>  lattice        0.21-8  2023-04-05 [2] CRAN (R 4.3.1)
#>  lifecycle      1.0.3   2022-10-07 [1] CRAN (R 4.3.1)
#>  listenv        0.9.0   2022-12-16 [1] CRAN (R 4.3.1)
#>  lubridate    * 1.9.3   2023-09-27 [1] CRAN (R 4.3.1)
#>  magrittr       2.0.3   2022-03-30 [1] CRAN (R 4.3.1)
#>  Matrix         1.6-1.1 2023-09-18 [1] CRAN (R 4.3.1)
#>  memoise        2.0.1   2021-11-26 [1] CRAN (R 4.3.1)
#>  mgcv           1.9-0   2023-07-11 [1] CRAN (R 4.3.1)
#>  mime           0.12    2021-09-28 [1] CRAN (R 4.3.0)
#>  miniUI         0.1.1.1 2018-05-18 [1] CRAN (R 4.3.1)
#>  munsell        0.5.0   2018-06-12 [1] CRAN (R 4.3.1)
#>  nlme           3.1-162 2023-01-31 [2] CRAN (R 4.3.1)
#>  parallelly     1.36.0  2023-05-26 [1] CRAN (R 4.3.0)
#>  pheatmap       1.0.12  2019-01-04 [1] CRAN (R 4.3.1)
#>  pillar         1.9.0   2023-03-22 [1] CRAN (R 4.3.1)
#>  pkgbuild       1.4.2   2023-06-26 [1] CRAN (R 4.3.1)
#>  pkgconfig      2.0.3   2019-09-22 [1] CRAN (R 4.3.1)
#>  pkgload        1.3.3   2023-09-22 [1] CRAN (R 4.3.1)
#>  prettyunits    1.2.0   2023-09-24 [1] CRAN (R 4.3.1)
#>  processx       3.8.2   2023-06-30 [1] CRAN (R 4.3.1)
#>  profvis        0.3.8   2023-05-02 [1] CRAN (R 4.3.1)
#>  promises       1.2.1   2023-08-10 [1] CRAN (R 4.3.1)
#>  ps             1.7.5   2023-04-18 [1] CRAN (R 4.3.1)
#>  purrr        * 1.0.2   2023-08-10 [1] CRAN (R 4.3.1)
#>  R6              2.5.1   2021-08-19 [1] CRAN (R 4.3.1)
#>  RColorBrewer    1.1-3   2022-04-03 [1] CRAN (R 4.3.0)
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#>  remotes         2.4.2.1 2023-07-18 [1] CRAN (R 4.3.1)
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#>  urlchecker      1.0.1   2021-11-30 [1] CRAN (R 4.3.1)
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#>
#>  [1] C:/Users/marzi/AppData/Local/R/win-library/4.3
#>  [2] C:/Program Files/R/R-4.3.1/library
#>
#> ──────────────────────────────────────────────────────────────────────────────
```