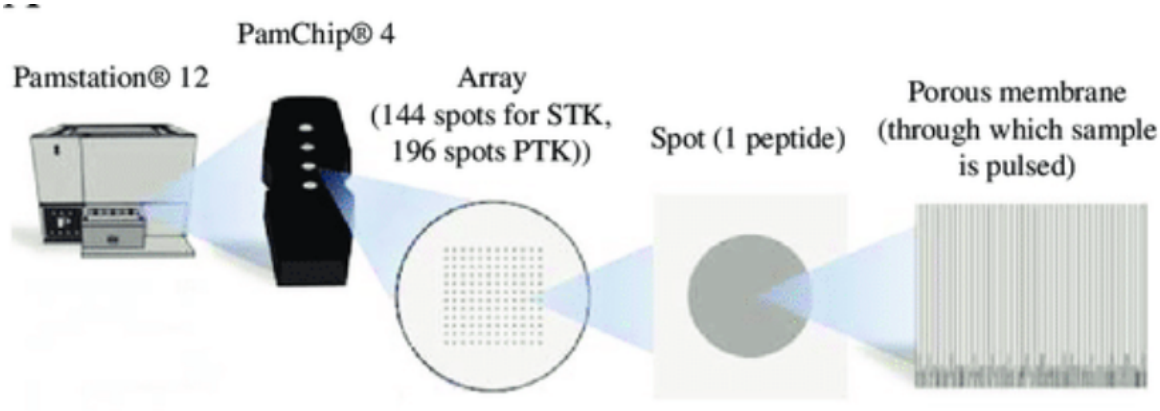
Kinase Array Report (STK)

{The Cognitive Disorders Research Laboratory (CDRL)}

{The Department of Neurosciences at the University of Toledo Medical Center}

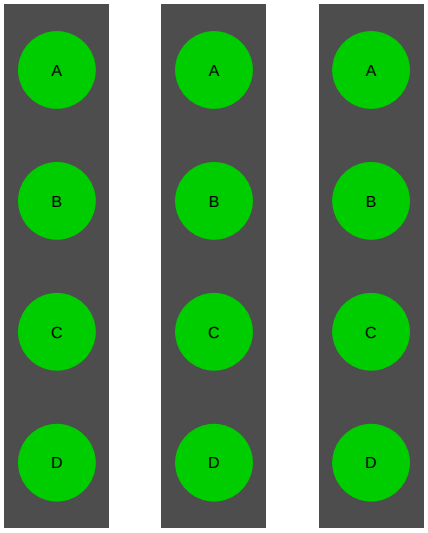
Introduction

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


PamChip® 4
Pamstation® 12
Array
(144 spots for STK,
196 spots PTK))
Spot (1 peptide)
Porous membrane
(through which sample
is pulsed)

Samples Info Schizophrenia vs. Control

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.

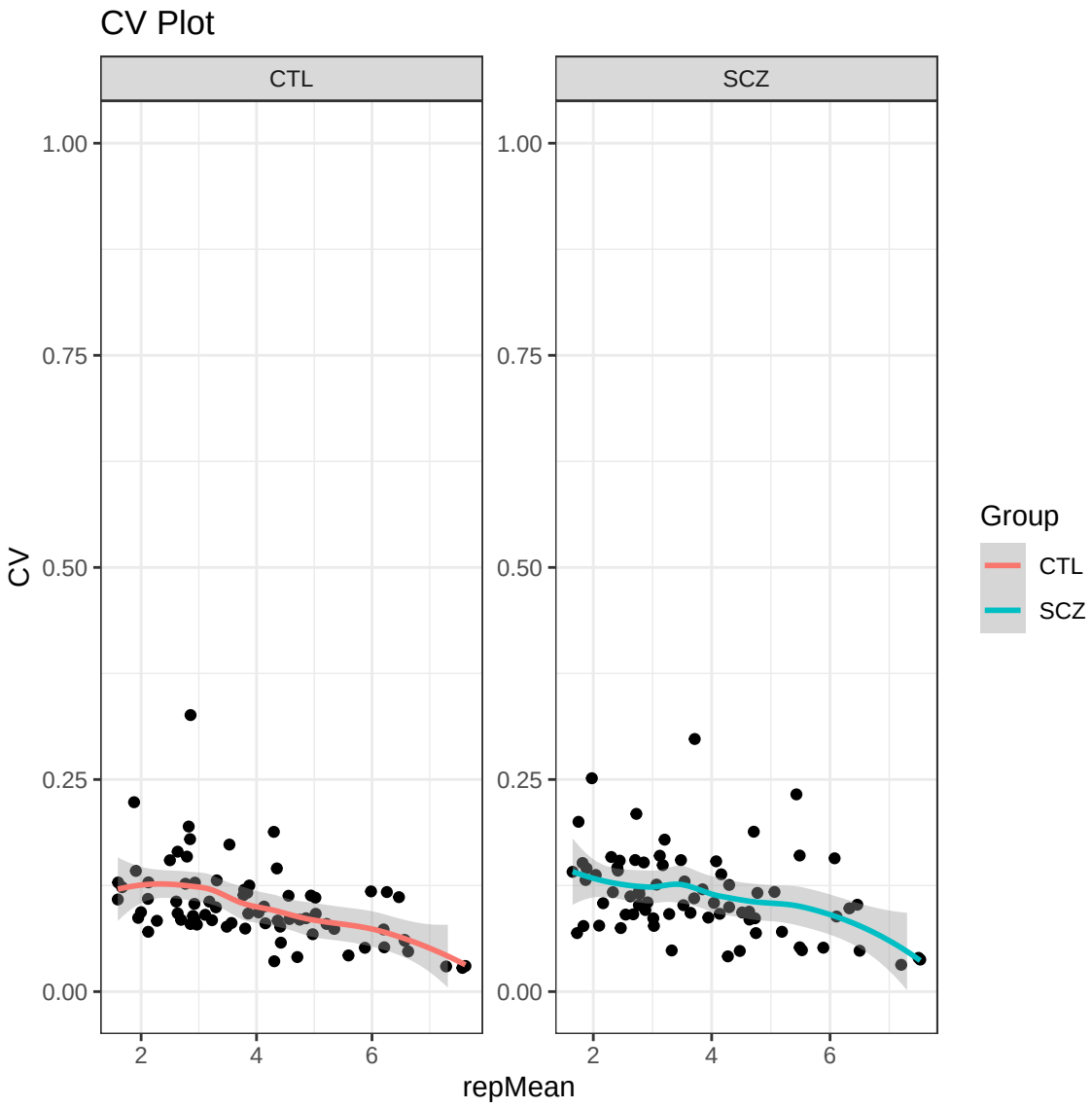


Results

Image Anlaysis 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).

Data Tidying and Modeling The raw data is then transformed to be tidy for an easier analysis, modeling, and visualizing. In order to combine the values from different exposure times into a single value, a simple linear regression model of the *Medain_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*).

Global Signal Intensity For a global signal intensity across all samples/groups, a heatmap is constructed based on the *Slope_Transformed* values. This heatmap represents all the peptides present on the chip except the positive/internal controls and peptides that failed to pass QC. The heatmap is 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.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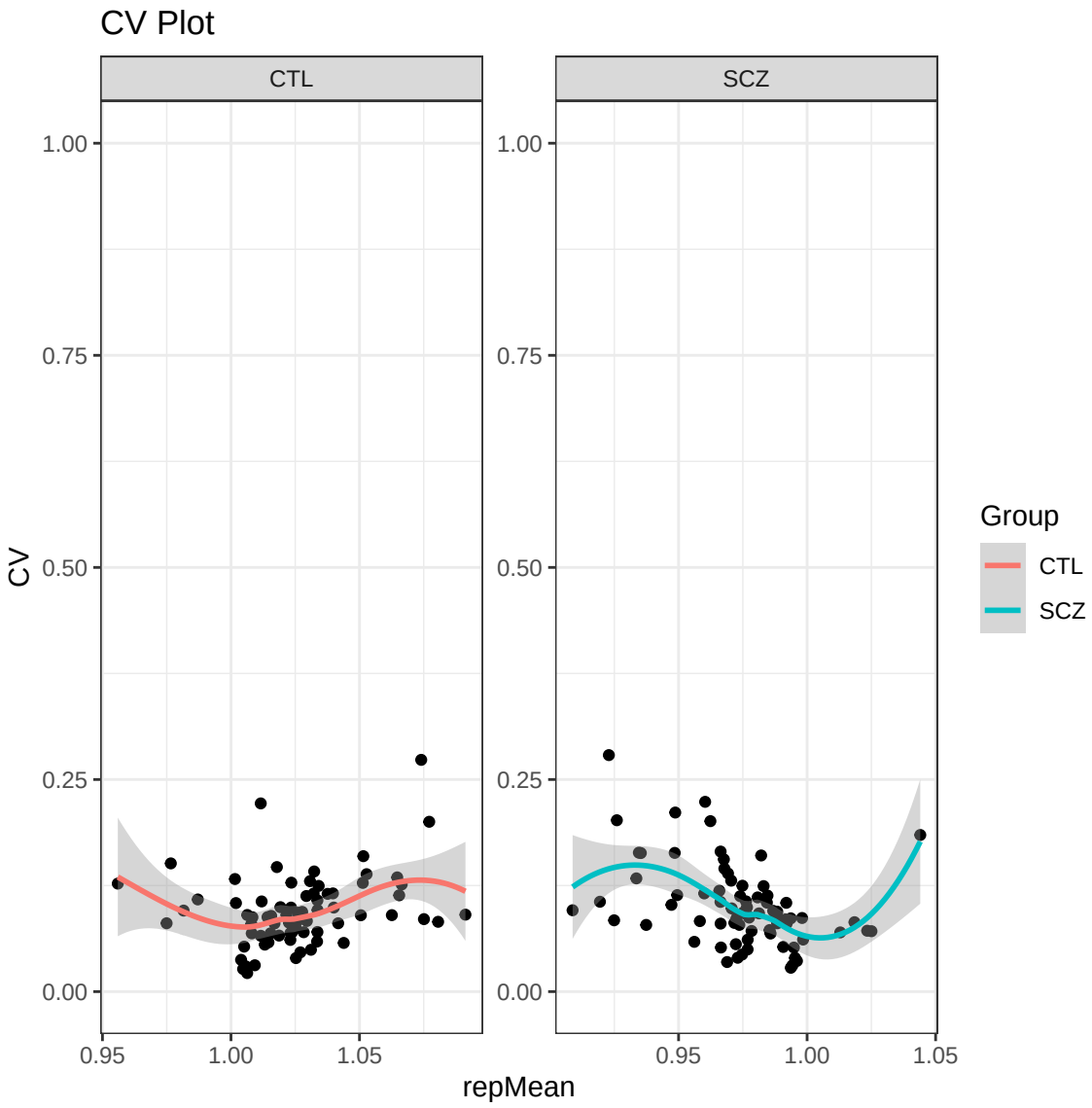
0.50
0.25
0.00
CV
2
4
6
repMean
Group
CTL
SCZ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CV
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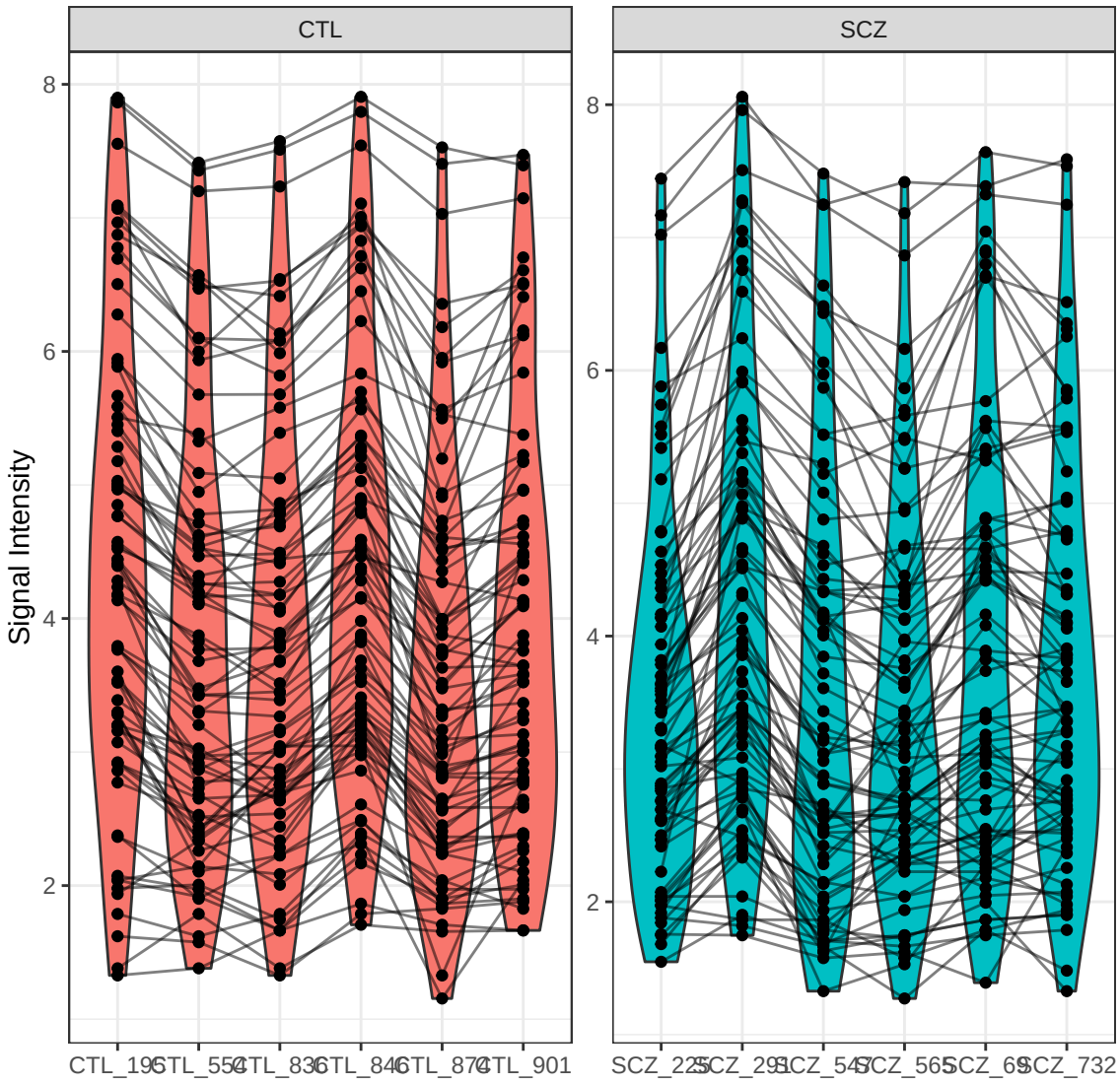
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4
2
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CTL_836
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CTL_874
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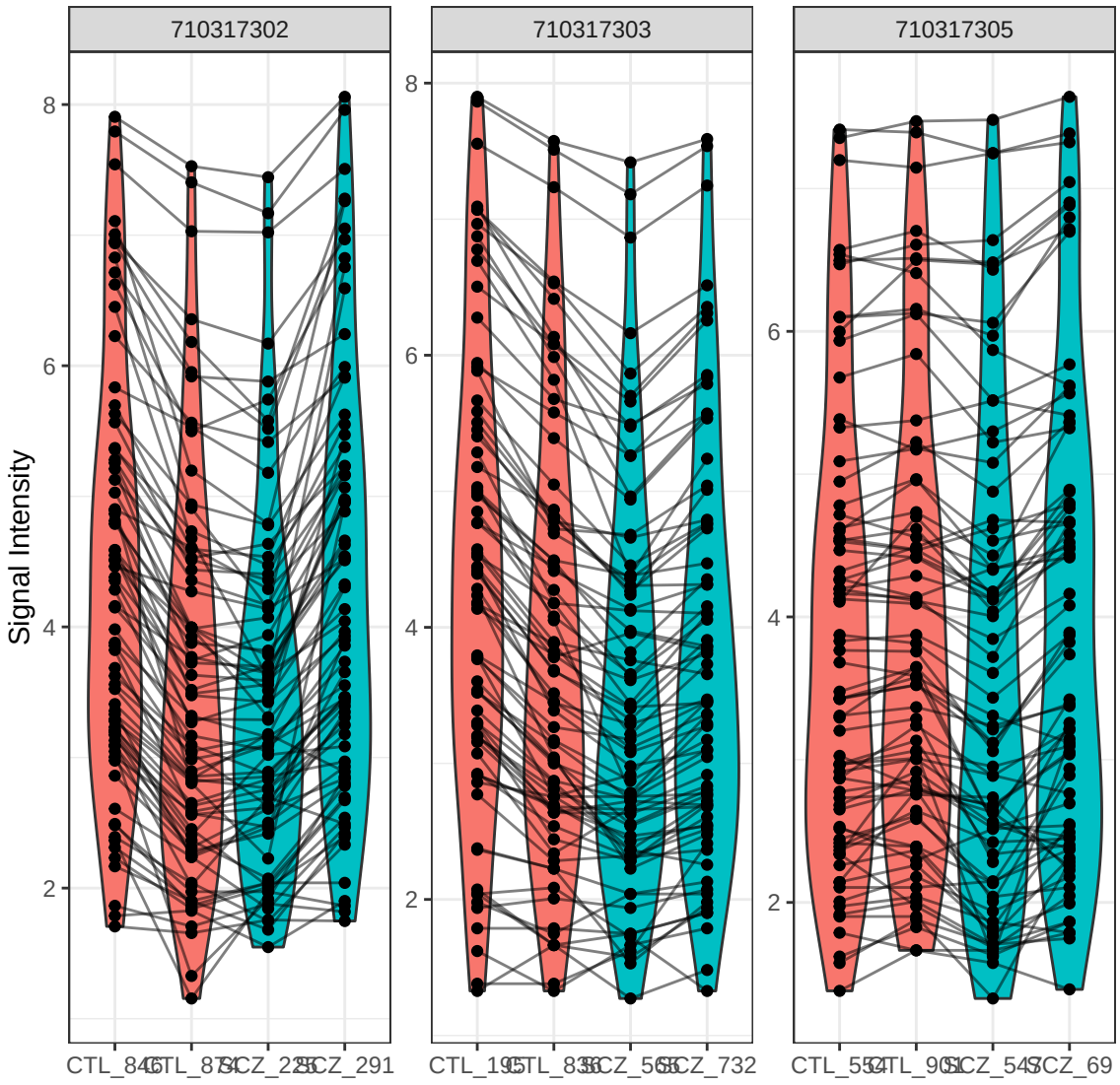
SCZ_293
SCZ_543
SCZ_565
SCZ_69
SCZ_732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710317305
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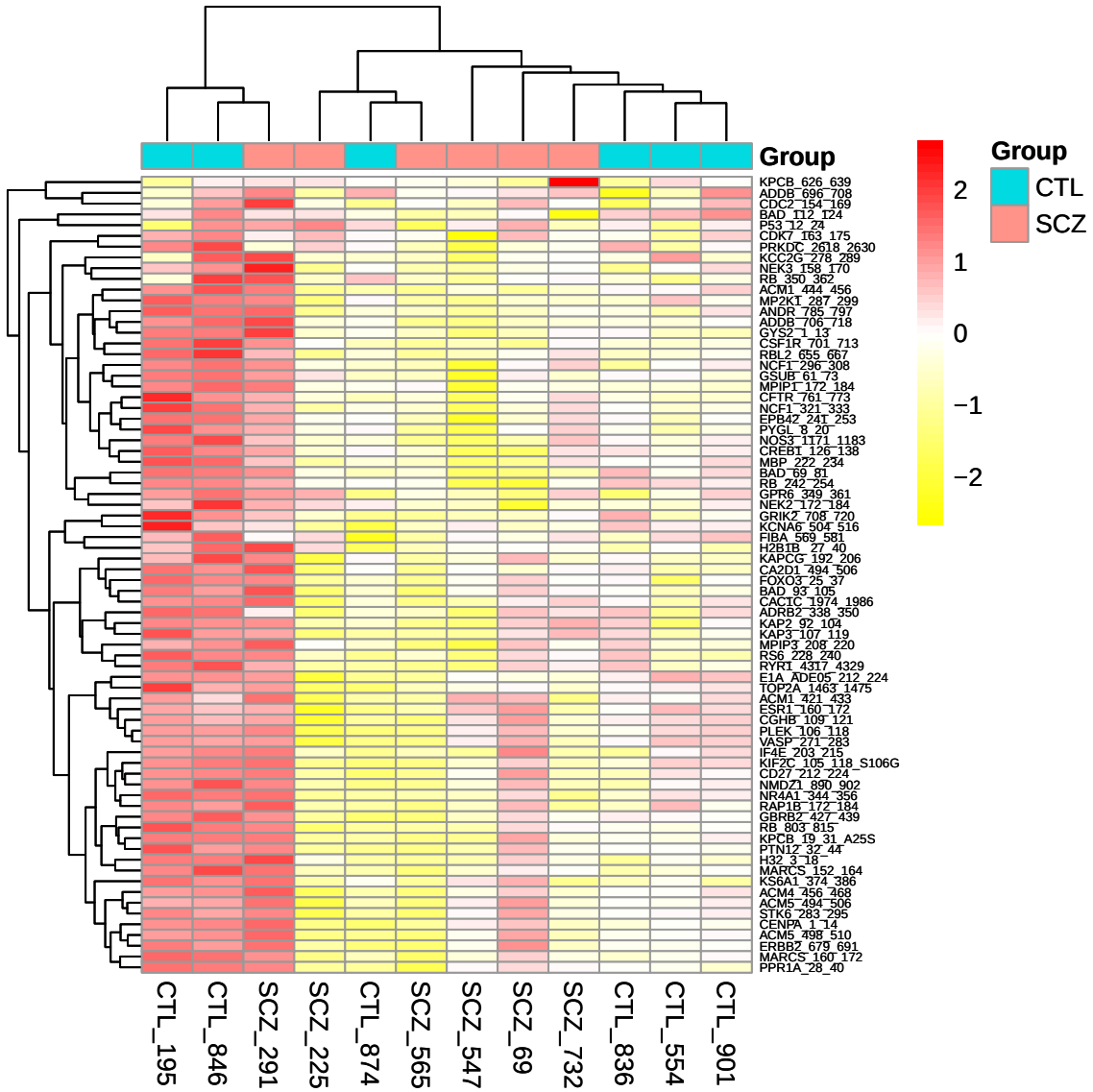
SCZ_543
SCZ_69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BAD_112_124
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PRKDC_2618_2630
KCC2G_278_289
NEK3_158_170
RB_350_362
ACM1_444_456
MP2K1_287_299
ANDR_785_797
ADDB_706_718
GYS2_1_13
CSF1R_701_713
RBL2_655_667
NCF1_296_308
GSUB_61_73
MPIP1_172_184
CFTR_761_773
NCF1_321_333
EPB42_241_253
PYGL_8_20
NOS3_1171_1183
CREB1_126_138
MBP_222_234
BAD_69_81
RB_242_254
GPR6_349_361
NEK2_172_184
GRIK2_708_720
KCNA6_504_516
FIBA_569_581
H2B1B_27_40
KAPCG_192_206
CA2D1_494_506
FOXO3_25_37
BAD_93_105
CACTC_1974_1986
ADRB2_338_350
KAP2_92_104
KAP3_107_119
MPIP3_208_220
RS6_228_240
RYR1_4317_4329
E1A_ADE05_212_224
TOP2A_1463_1475
ACM1_421_433
ESR1_160_172
CGHB_109_121
PLEK_106_118
VASP_271_283
IF4E_203_215
KIF2C_105_118_S106G
CD27_212_224
NMDZ1_890_902
NR4A1_344_356
RAP1B_172_184
GBRB2_427_439
RB_803_815
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PTN12_32_44
H32_3_18
MARCS_152_164
KS6A1_374_386
ACM4_456_468
ACM5_494_506
STK6_283_295
CENPA_1_14
ACM5_498_510
ERBB2_679_691
MARCS_160_172
PPR1A_28_40
CTL_195
CTL_846
SCZ_291
SCZ_225
CTL_874
SCZ_565
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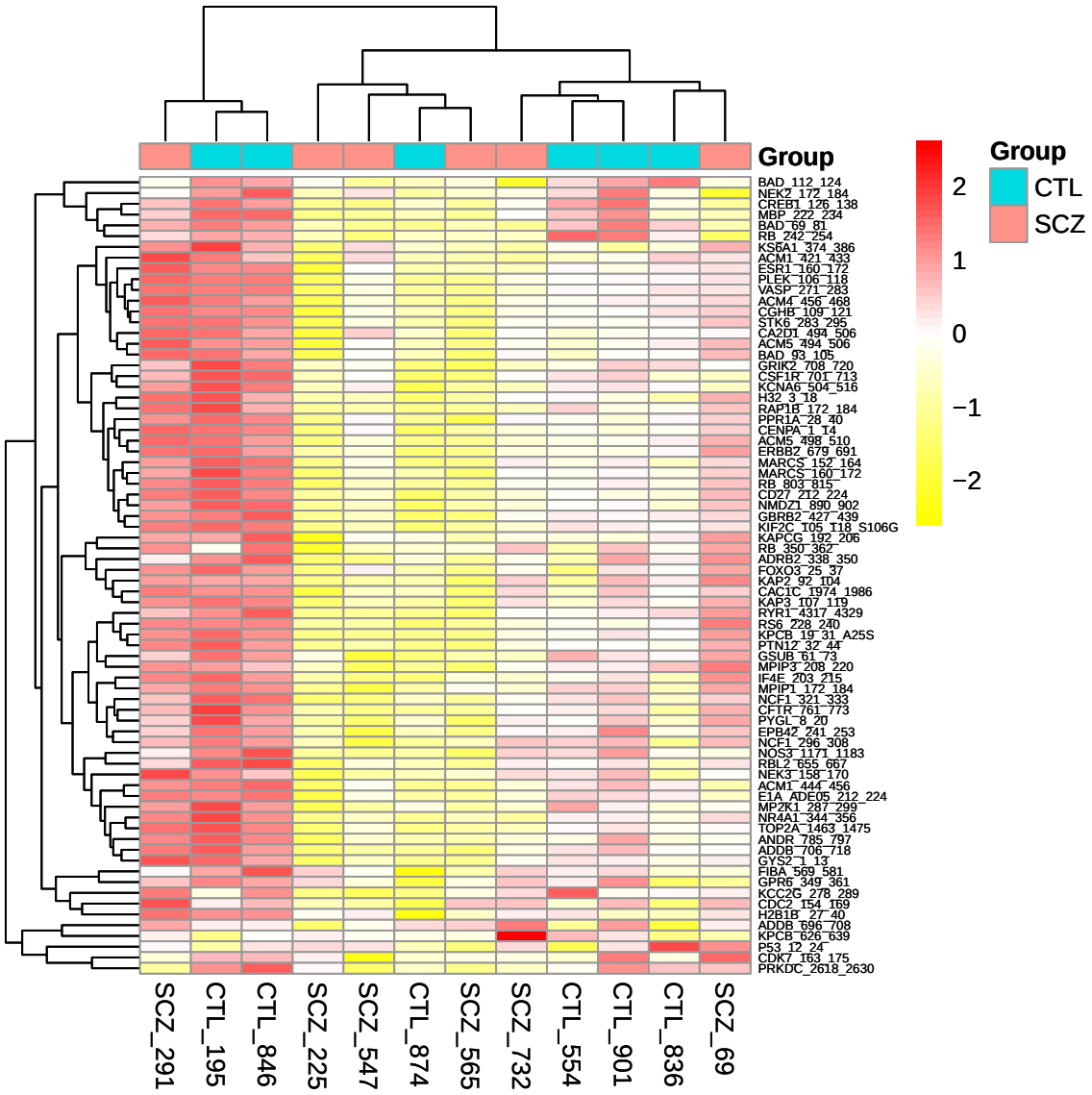
CTL_836
CTL_554
CTL_901
2
1
0
−1
−2
Group
CTL
SCZ


Group
BAD_112_124
NEK2_172_184
CREB1_126_138
MBP_222_234
BAD_69_81
RB_242_254
KS6A1_374_386
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ESR1_160_172
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CGHB_109_121
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CA2D1_494_506
ACM5_494_506
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CSF1R_701_713
KCNA6_504_516
H32_3_18
RAP1B_172_184
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CENPA_1_14
ACM5_498_510
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MARCS_152_164
MARCS_160_172
RB_803_815
CD27_212_224
NMDZ1_890_902
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KIF2C_105_118_S106G
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MPIP3_208_220
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MPIP1_172_184
NCF1_321_333
CFTR_761_773
PYGL_8_20
EPB42_241_253
NCF1_296_308
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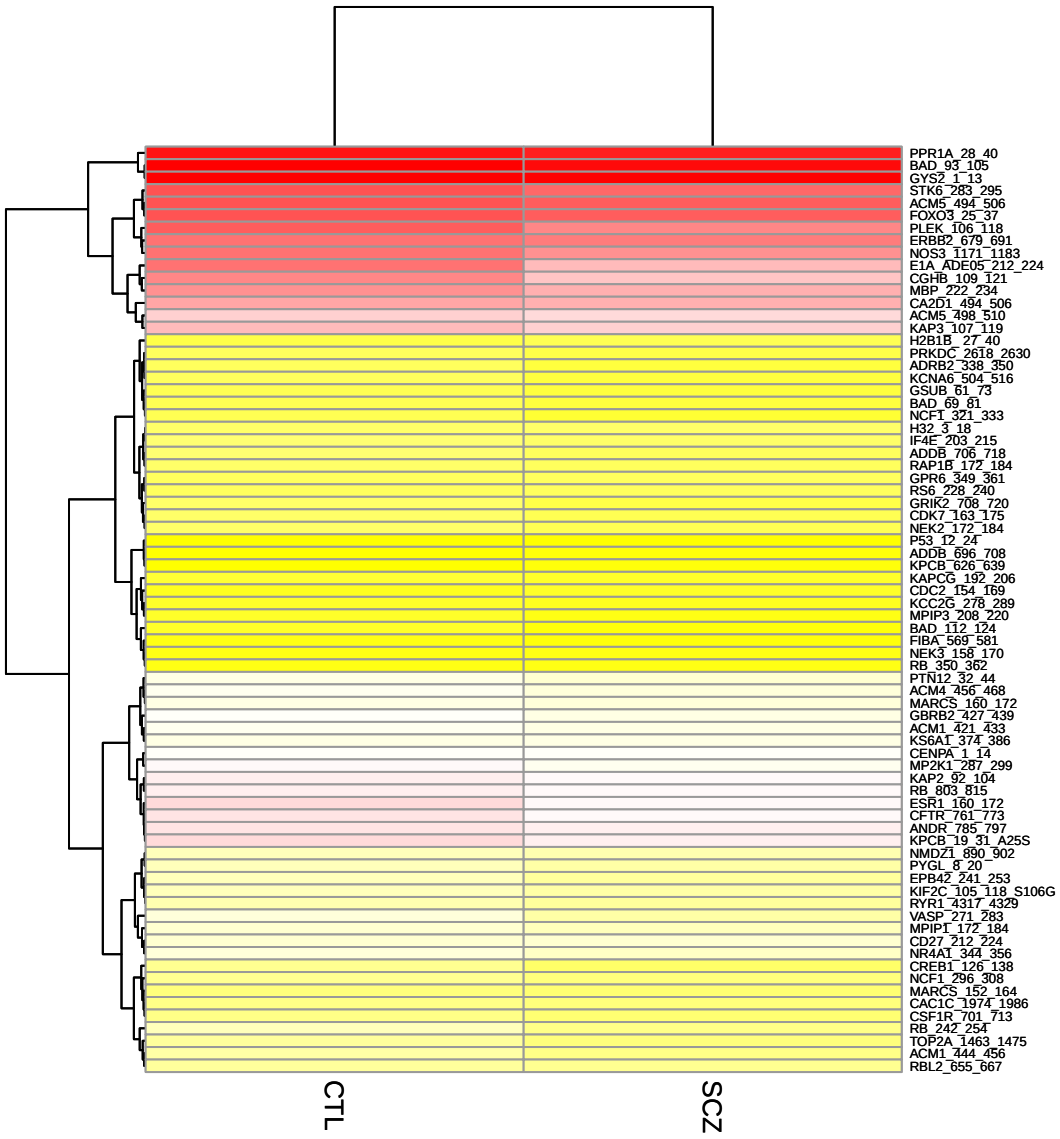
CTL_195
CTL_846
SCZ_225
SCZ_547
CTL_874
SCZ_565
SCZ_732
CTL_554
CTL_901
CTL_836
SCZ_69
Group
CTL
SCZ
2
1
0
−1
−2



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

- Control vs. Schizophrenia (Pair 2.4)

The *Slope_ Transforemed* ratio between each group, paired by chip, is calculated to represent the final peptide signal to be used to calculate 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:

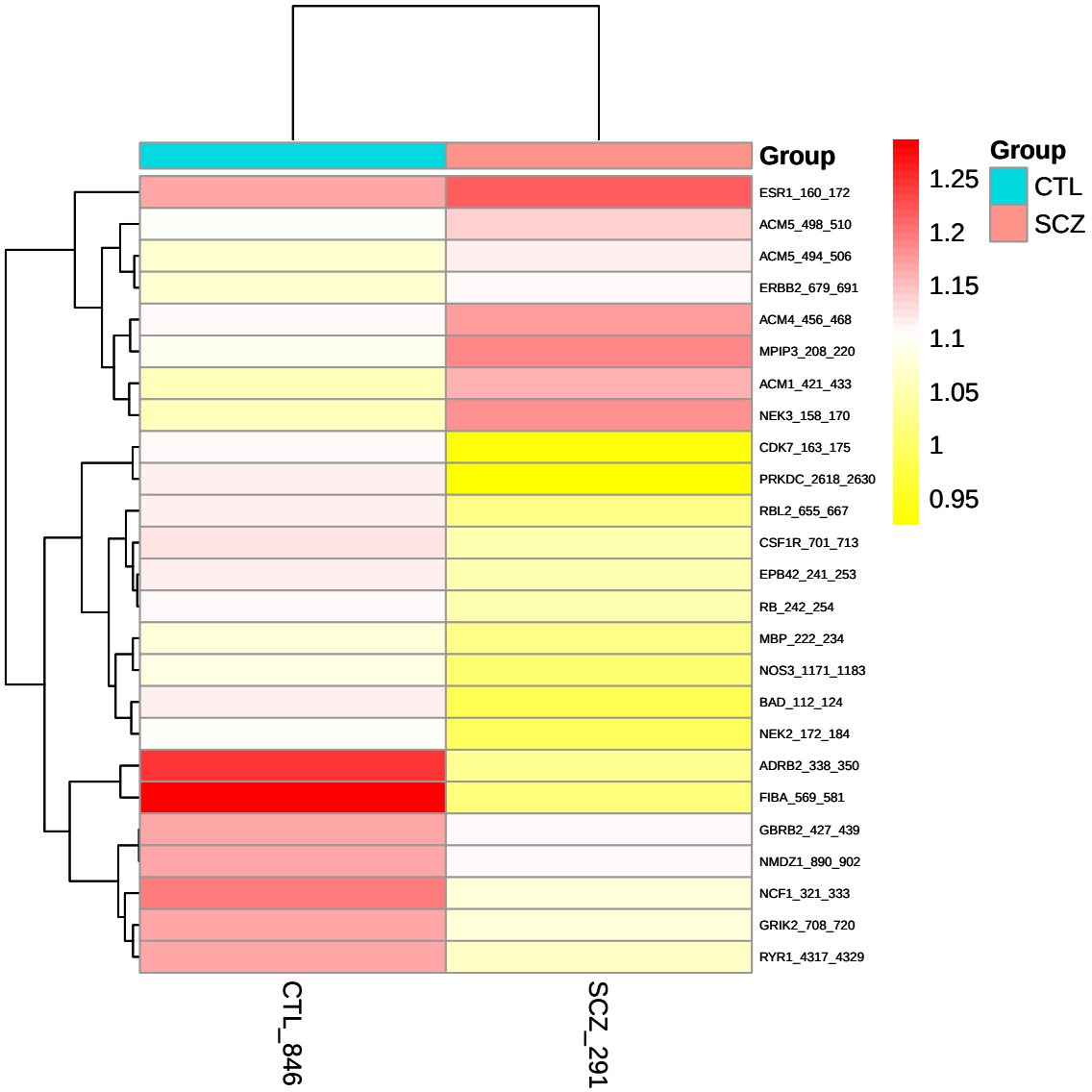
- The *Medain_ SigmBg* at max exposure *200ms* must be above a certain value

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

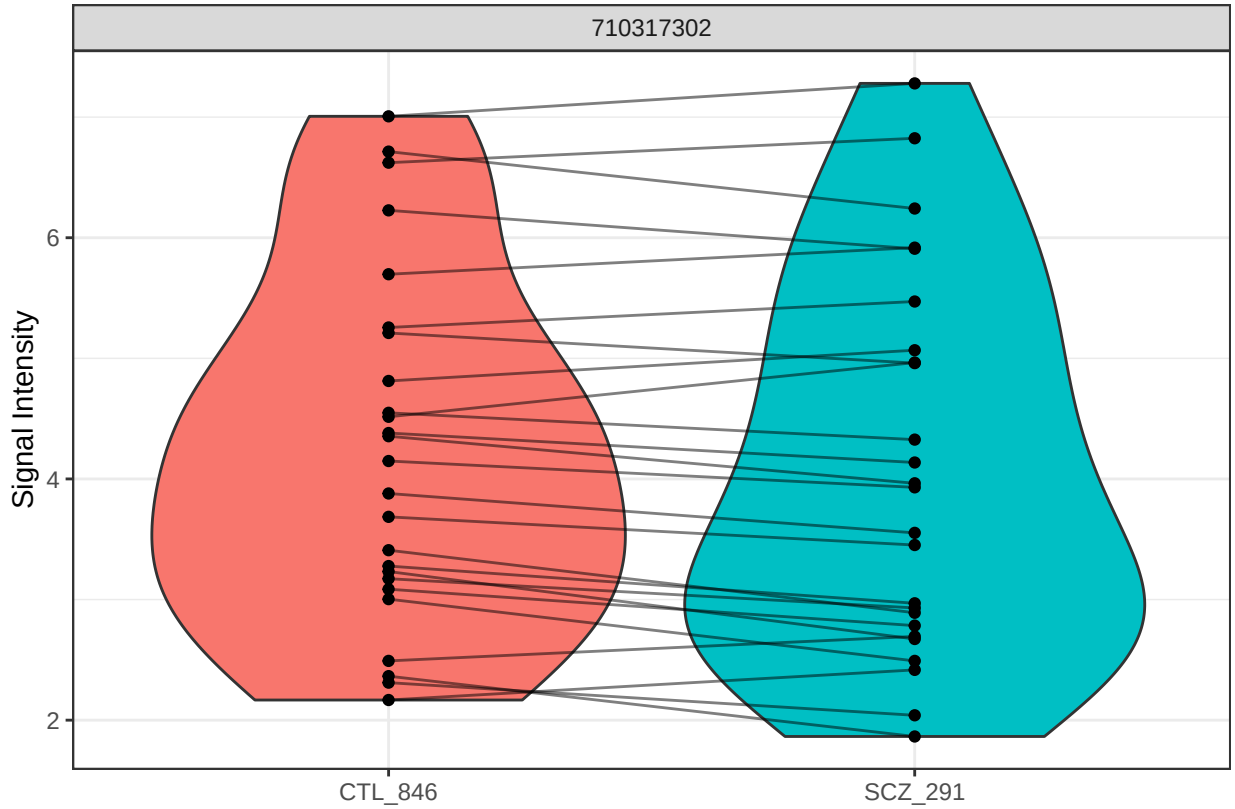
These *Filtering Parameters* (fold change threshold, QC criteria) can be modified to adjust the stringency of the analysis. The *Filtering Parameters* that are used for this analysis:

- The *Medain_SigmBg* at max exposure *200ms* must be equal or above 5
- R^2 of the linear model fit must be above or equal 0.9
- Log fold change (LFC) cutoffs at (0.2,0.3)

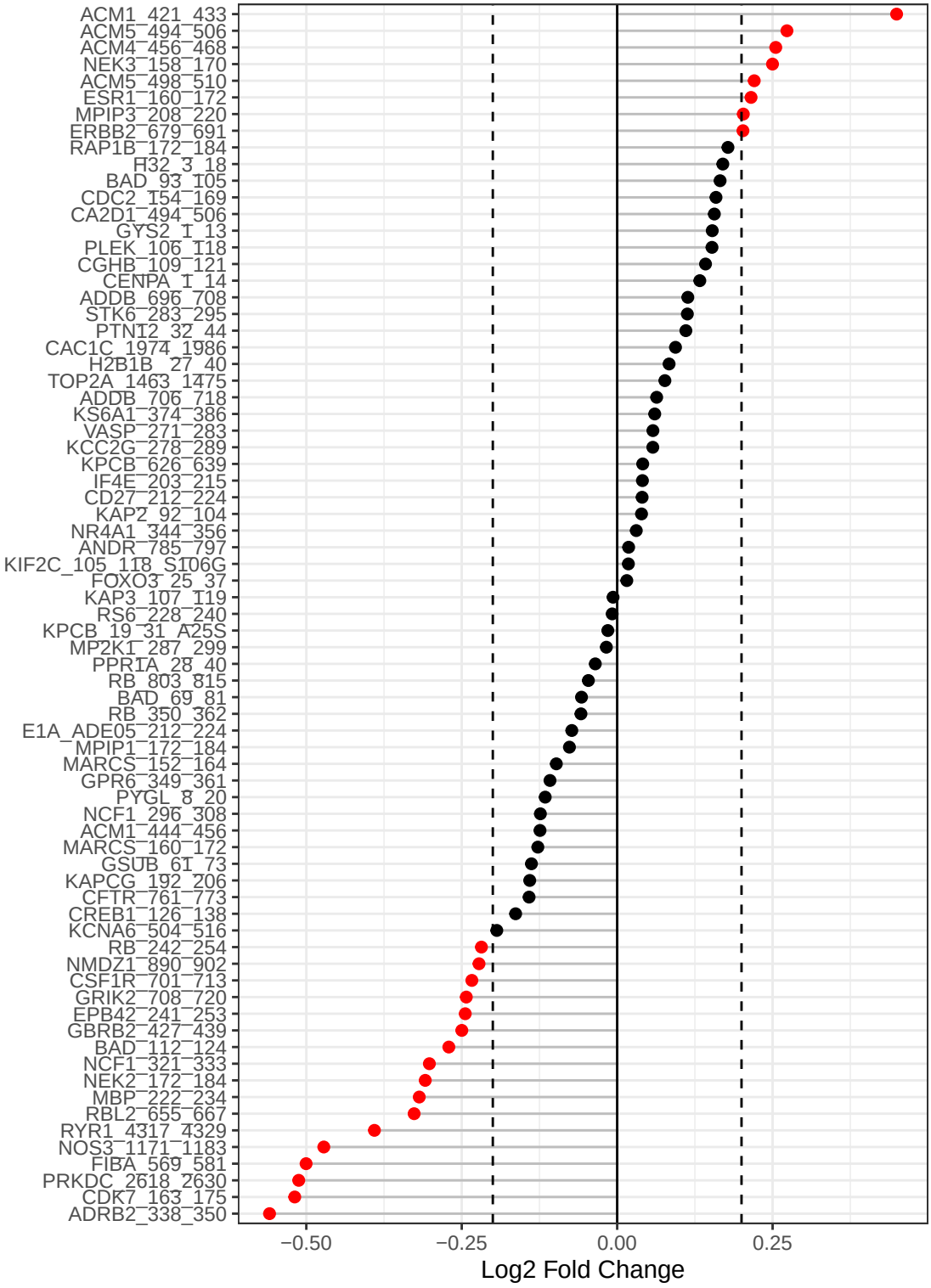
Schizophrenia vs. Control (Pair 2.4) After applying the *Filtering Parameters* for this group comparison, only *25*/141 peptides carried forward in the analysis (i.e. *25 hits*). Below are some figures to visualize the differences between these samples for considering these *hits*.



Run2 Comp4



Run2 Comp4


ACM1_421_433
ACM5_494_506
ACM4_456_468
NEK3_158_170
ACM5_498_510
ESR1_160_172
MPIP3_208_220
ERBB2_679_691
RAP1B_172_184
H32_3_18
BAD_93_105
CDC2_154_169
CA2D1_494_506
GYS2_1_13
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ADDB_696_708
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RS6_228_240
KPCB_19_31_A25S
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RB_803_815
BAD_69_81
RB_350_362
E1A_ADE05_212_224
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GPR6_349_361
PYGL_8_20
NCF1_296_308
ACM1_444_456
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GSUB_61_73
KAPCG_192_206
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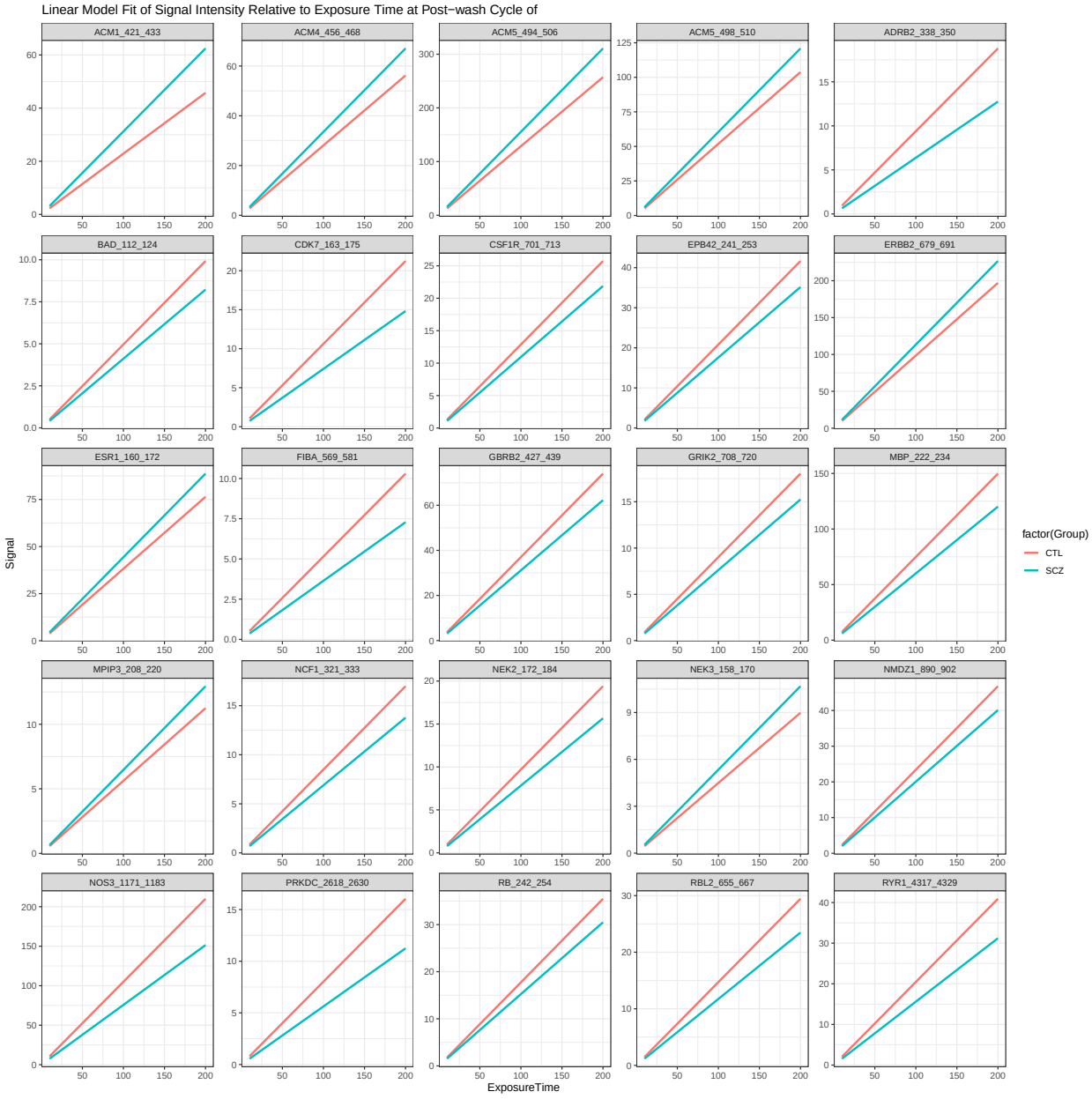
RYR1_4317_4329
NOS3_1171_1183
FIBA_569_581
PRKDC_2618_2630
CDK7_163_175
ADRB2_338_350
−0.50
−0.25
0.00
0.25
Log2 Fold Change

Run2 Comp4


Linear Model Fit of Signal Intensity Relative to Exposure Time at Post−wash Cycle of
ACM1_421_433
ACM4_456_468
ACM5_494_506
ACM5_498_510
ADRB2_338_350
BAD_112_124
CDK7_163_175
CSF1R_701_713
EPB42_241_253
ERBB2_679_691
ESR1_160_172
FIBA_569_581
GBRB2_427_439
GRIK2_708_720
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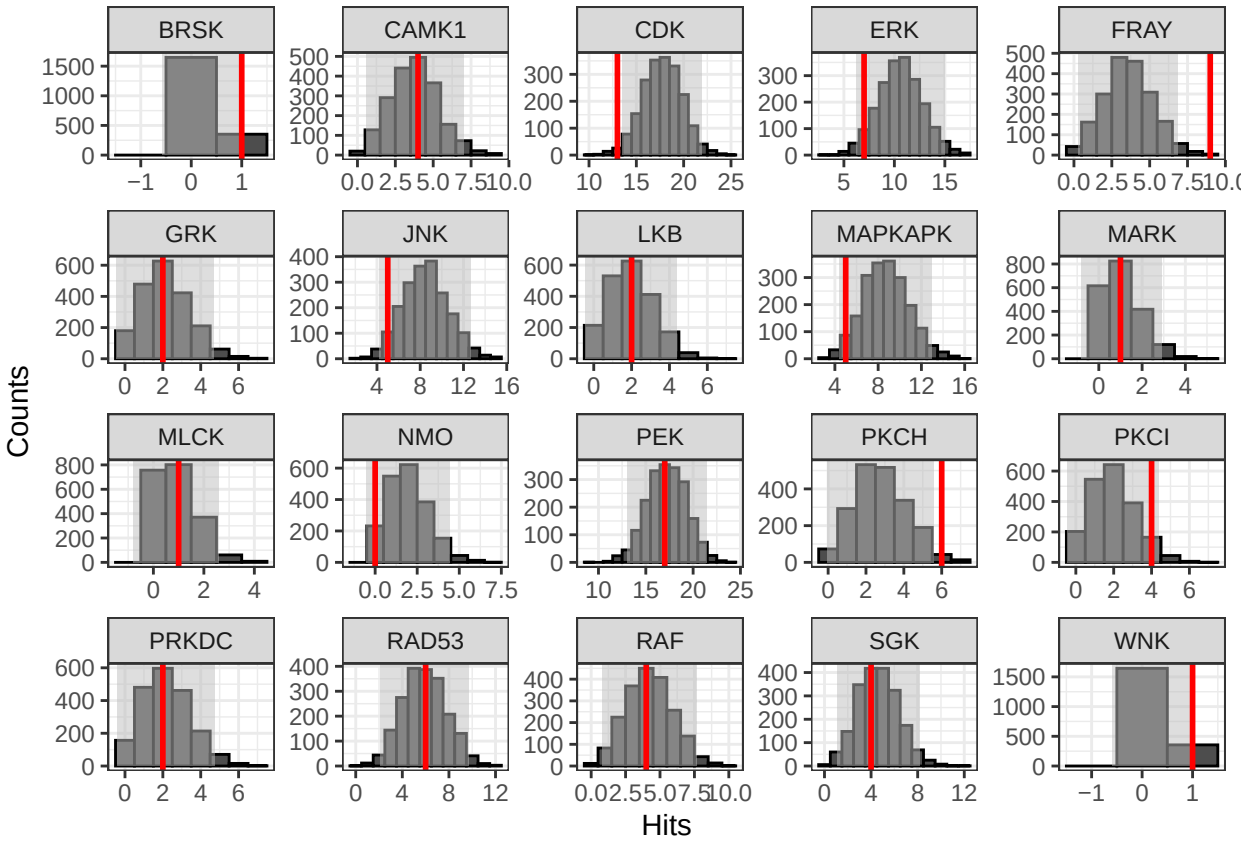
NCF1_321_333
NEK2_172_184
NEK3_158_170
NMDZ1_890_902
NOS3_1171_1183
PRKDC_2618_2630
RB_242_254
RBL2_655_667
RYR1_4317_4329
Signal
ExposureTime
factor(Group)
CTL
SCZ

Run2 Comp4

Upstream Kinase Anlaysis 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. This is done by using Kinome Random Sampling Analyzer (*KRSA*). *KRSA* is a software tool developed by the lab to perform the random sampling analysis. 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	Observed	SamplingAvg	SD	Z
FRAY	9	3.587	1.639	3.303
PKCH	6	2.783	1.410	2.281
CDK	13	17.733	2.104	-2.250
BRSK	1	0.176	0.381	2.163
WNK	1	0.178	0.383	2.148
MAPKAPK	5	8.720	2.138	-1.740
PKCI	4	1.968	1.215	1.673
JNK	5	8.478	2.121	-1.640
ERK	7	10.606	2.202	-1.638
NMO	0	1.935	1.245	-1.554
P38	6	9.237	2.212	-1.464
IKK	7	10.306	2.285	-1.447
DAPK	2	4.231	1.678	-1.330
STKR	0	1.273	1.006	-1.265
PKD	5	7.636	2.124	-1.241
DYRK	7	9.661	2.173	-1.224
RSK	12	14.723	2.296	-1.186
HAL	1	0.364	0.547	1.163
MELK	7	4.987	1.812	1.111
VRK1	0	0.911	0.839	-1.086
STE11	3	1.732	1.168	1.086
COT	0	0.905	0.838	-1.080
AKT	5	7.180	2.045	-1.066
PHK	15	17.174	2.162	-1.006
NDR	0	0.842	0.843	-0.998



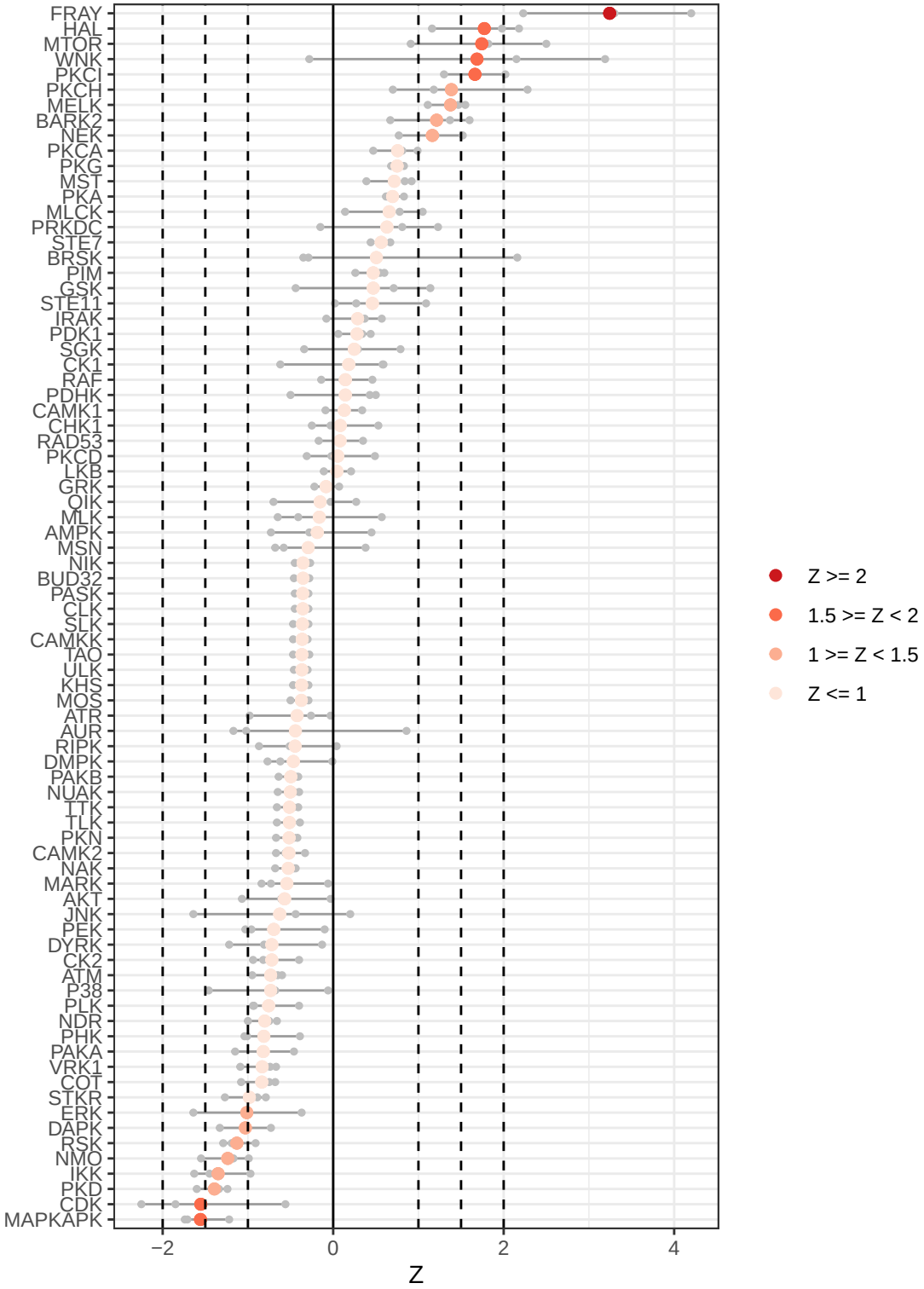
	meanLFC		
Kinase	0.2	0.25	0.3
FRAY	3.30	4.20	2.23
PKCH	2.28	1.18	0.70
CDK	-2.25	-1.85	-0.56
BRSK	2.16	-0.35	-0.29
WNK	2.15	3.19	-0.28
MAPKAPK	-1.74	-1.71	-1.22
PKCI	1.67	2.02	1.30
JNK	-1.64	-0.44	0.20
ERK	-1.64	-1.03	-0.37
NMO	-1.55	-1.17	-0.99
P38	-1.46	-0.68	-0.06
IKK	-1.45	-1.63	-0.97
DAPK	-1.33	-1.03	-0.73
STKR	-1.27	-0.89	-0.79
PKD	-1.24	-1.34	-1.60
DYRK	-1.22	-0.81	-0.13
RSK	-1.19	-1.29	-0.91
HAL	1.16	1.98	2.18

MELK	1.11	1.55	1.47
VRK1	-1.09	-0.74	-0.67
STE11	1.09	0.02	0.27
COT	-1.08	-0.75	-0.68
AKT	-1.07	-0.61	-0.03
PHK	-1.01	-1.04	-0.39
NDR	-1.00	-0.75	-0.66
PKCA	0.99	0.81	0.47
ATR	-0.98	-0.26	-0.03
ATM	-0.95	-0.65	-0.60
PLK	-0.94	-0.93	-0.40
MST	0.92	0.39	0.84
MTOR	0.91	1.82	2.50
RIPK	-0.87	-0.51	0.04
AUR	0.86	-1.17	-1.02
NEK	0.77	1.52	1.20
QIK	-0.70	-0.03	0.27
NAK	-0.68	-0.46	-0.44
PKG	0.68	0.74	0.83
BARK2	0.67	1.37	1.60
PKN	-0.67	-0.46	-0.42
CAMK2	-0.67	-0.56	-0.33
TTK	-0.66	-0.47	-0.41
TLK	-0.66	-0.49	-0.39
NUAK	-0.65	-0.46	-0.40
PAKB	-0.64	-0.44	-0.41
PKA	0.62	0.64	0.83
CK1	-0.62	0.58	0.59
DMPK	-0.62	-0.77	-0.01
PIM	0.60	0.55	0.26
MLK	0.57	-0.65	-0.41
CHK1	0.53	-0.25	-0.03
MOS	-0.50	-0.33	-0.29
PKCD	0.49	-0.31	-0.02
CAMKK	-0.47	-0.32	-0.30
SLK	-0.47	-0.32	-0.29
KHS	-0.47	-0.35	-0.29
TAO	-0.47	-0.35	-0.28
BUD32	-0.46	-0.32	-0.28
ULK	-0.46	-0.34	-0.30
PAKA	-0.46	-0.85	-1.15
CLK	-0.45	-0.33	-0.29
PASK	-0.45	-0.33	-0.29
AMPK	0.45	-0.28	-0.73
NIK	-0.45	-0.34	-0.27

GSK	-0.44	0.71	1.14
STE7	0.44	0.58	0.67
PDHK	0.43	0.50	-0.50
CK2	-0.40	-0.94	-0.82
MSN	0.38	-0.68	-0.58
IRAK	0.37	0.57	-0.08
PDK1	0.34	0.44	0.06
SGK	-0.34	0.29	0.79
PRKDC	-0.15	0.81	1.23
CAMK1	0.14	-0.09	0.34
MLCK	0.14	0.78	1.05
RAF	-0.14	0.46	0.11
GRK	-0.10	-0.22	0.07
PEK	-0.10	-0.96	-1.03
RAD53	0.06	-0.17	0.35
MARK	-0.06	-0.84	-0.73
LKB	0.03	-0.11	0.21

Method	NumberOfPeptides
meanLFC.0.2	25
meanLFC.0.25	14
meanLFC.0.3	11

Run2 Comp4


FRAY
HAL
MTOR
WNK
PKCI
PKCH
MELK
BARK2
NEK
PKCA
PKG
MST
PKA
MLCK
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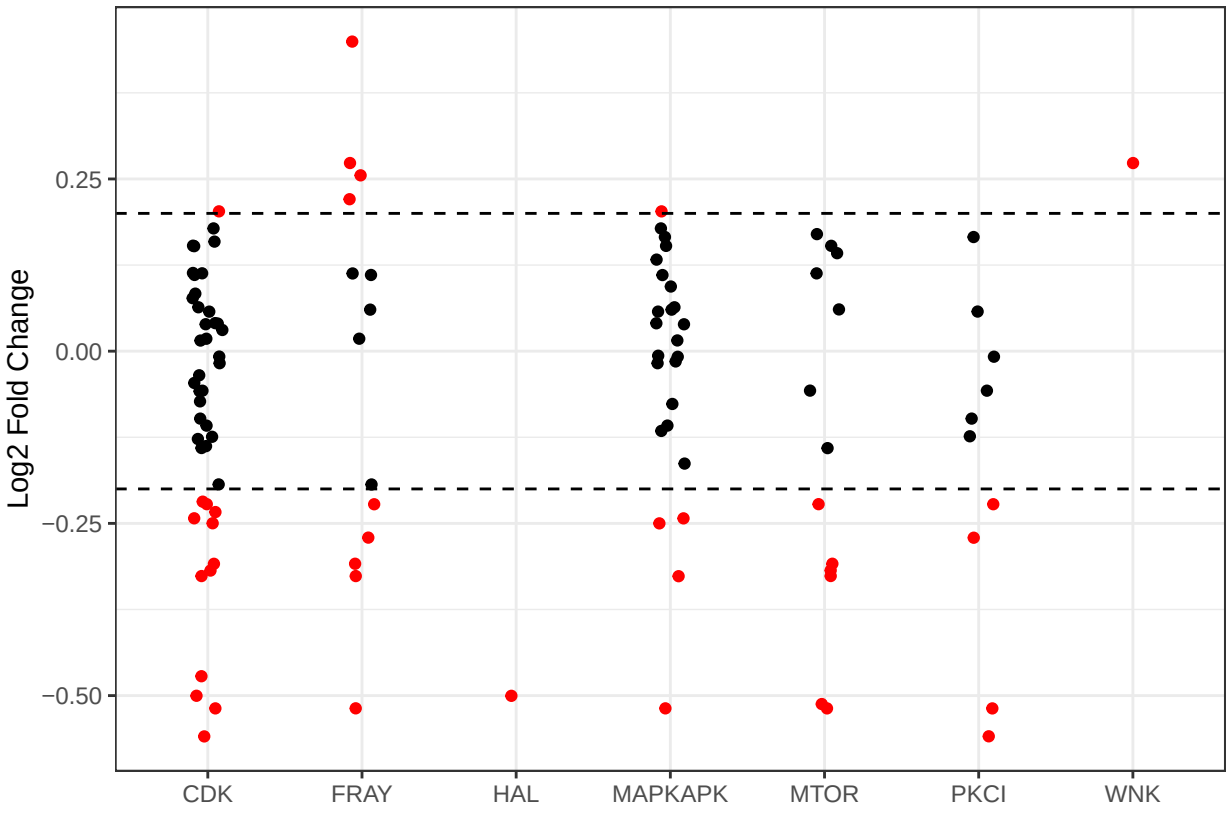
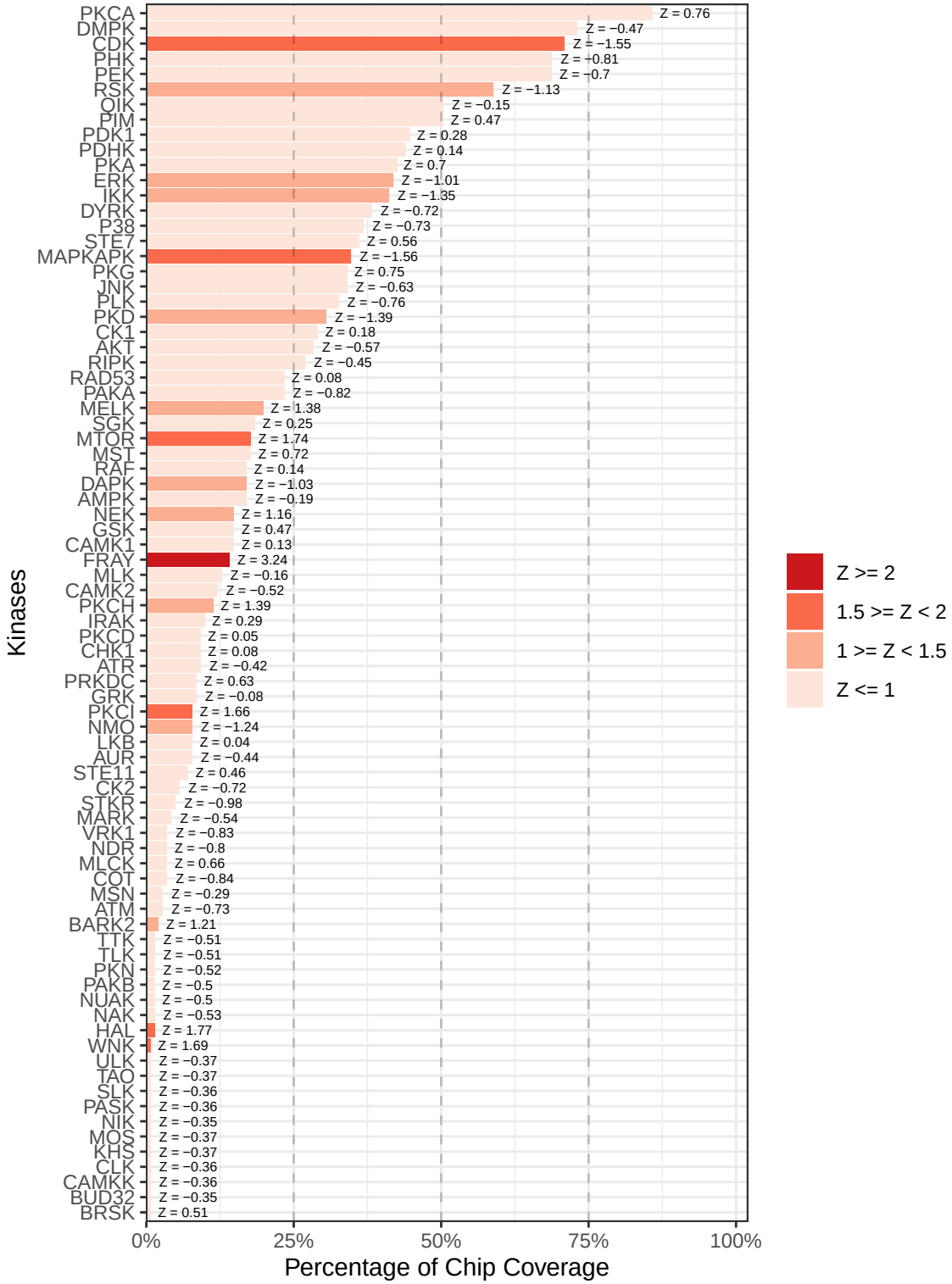
STE7
BRSK
PIM
GSK
STE11
IRAK
PDK1
SGK
CK1
RAF
PDHK
CAMK1
CHK1
RAD53
PKCD
LKB
GRK
QIK
MLK
AMPK
MSN
NIK
BUD32
PASK
CLK
SLK
CAMKK
TAO
ULK
KHS
MOS
ATR
AUR
RIPK
DMPK
PAKB
NUAK
ITK
TLK
PKN
CAMK2
NAK
MARK
AKT
JNK
PEK
DYRK
CK2
ATM
P38
PLK
NDR
PHK
PAKA
VRK1
COT
STKR
ERK
DAPK
RSK
NMO
IKK
PKD
CDK
MAPKAPK
−2
0
2
4
Z
Z >= 2
1.5 >= Z < 2
1 >= Z < 1.5
Z <= 1

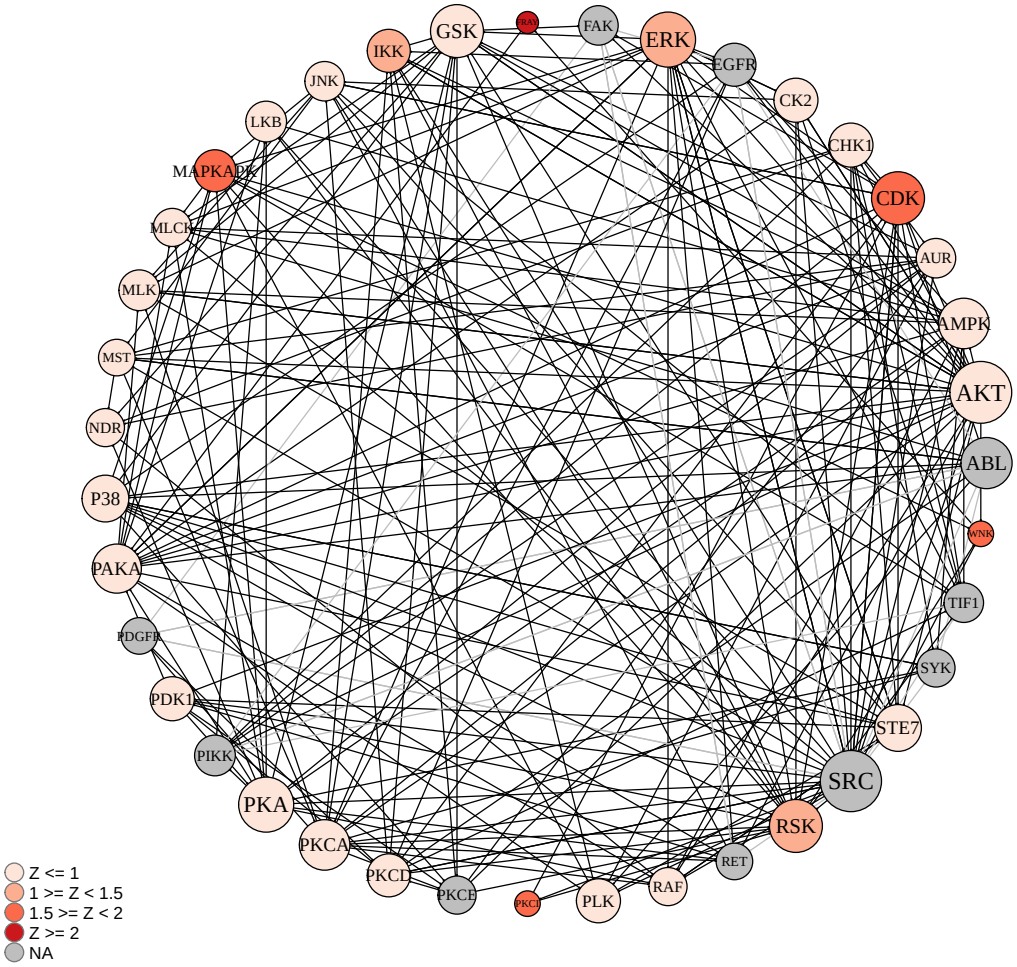
Run2 Comp4


Log2 Fold Change
0.25
0.00
−0.25
−0.50
CDK
FRAY
HAL
MAPKAPK
MTOR
PKCI
WNK

Run2 Comp4


PKCA Z = 0.76
DMPK Z = −0.47
CDK Z = −1.55
PHK Z = +0.81
PEK Z = −0.7
RSK Z = −1.13
OIK Z = −0.15
PIM Z = 0.47
PDK1 Z = 0.28
PDHK Z = 0.14
PKA Z = 0.7
ERK Z = −1.01
IKK Z = −1.35
DYRK Z = −0.72
P38 Z = −0.73
STE7 Z = 0.56
MAPKAPK Z = −1.56
PKG Z = 0.75
JNK Z = −0.63
PLK Z = −0.76
PKD Z = −1.39
CK1 Z = 0.18
AKT Z = −0.57
RIPK Z = −0.45
RAD53 Z = 0.08
PAKA Z = −0.82
MELK Z = 1.38
SGK Z = 0.25
MTOR Z = 1.74
MST Z = 0.72
RAF Z = 0.14
DAPK Z = −1.03
AMPK Z = −0.19
NEK Z = 1.16
GSK Z = 0.47
CAMK1 Z = 0.13
FRAY Z = 3.24
MLK Z = −0.16
CAMK2 Z = −0.52
PKCH Z = 1.39
IRAK Z = 0.29
PKCD Z = 0.05
CHK1 Z = 0.08
ATR Z = −0.42
PRKDC Z = 0.63
GRK Z = −0.08
PKCI Z = 1.66
NMO Z = −1.24
LKB Z = 0.04
AUR Z = −0.44
STE11 Z = 0.46
CK2 Z = −0.72
STKR Z = −0.98
MARK Z = −0.54
VRK1 Z = −0.83
NDR Z = −0.8
MLCK Z = 0.66
COT Z = −0.84
MSN Z = −0.29
ATM Z = −0.73
BARK2 Z = 1.21
TTK Z = −0.51
TLK Z = −0.51
PKN Z = −0.52
PAKB Z = −0.5
NUAK Z = −0.5
NAK Z = −0.53
HAL Z = 1.77
WNK Z = 1.69
ULK Z = −0.37
TAO Z = −0.37
SLK Z = −0.36
PASK Z = −0.36
NIK Z = −0.35
MOS Z = −0.37
KHS Z = −0.37
CLK Z = −0.36
CAMKK Z = −0.36
BUD32 Z = −0.35
BRSK Z = 0.51
Kinases
0%
25%
50%
75%
100%
Percentage of Chip Coverage
Z >= 2
1.5 >= Z < 2
1 >= Z < 1.5
Z <= 1

Run2 Comp4




```
installed.packages()[names(sessionInfo()$otherPkgs), "Version"]
```

```
##     furrr    future     knitr        gt      KRSA   forcats   stringr     dplyr
##   "0.2.2"  "1.21.0"    "1.30"   "0.2.2"   "0.9.4"   "0.5.0"   "1.4.0"   "1.0.3"
##     purrr     readr     tidyr    tibble   ggplot2 tidyverse
##   "0.3.4"   "1.4.0"   "1.1.2"   "3.0.5"   "3.3.3"   "1.3.0"
```