Kinase Array Report (STK)

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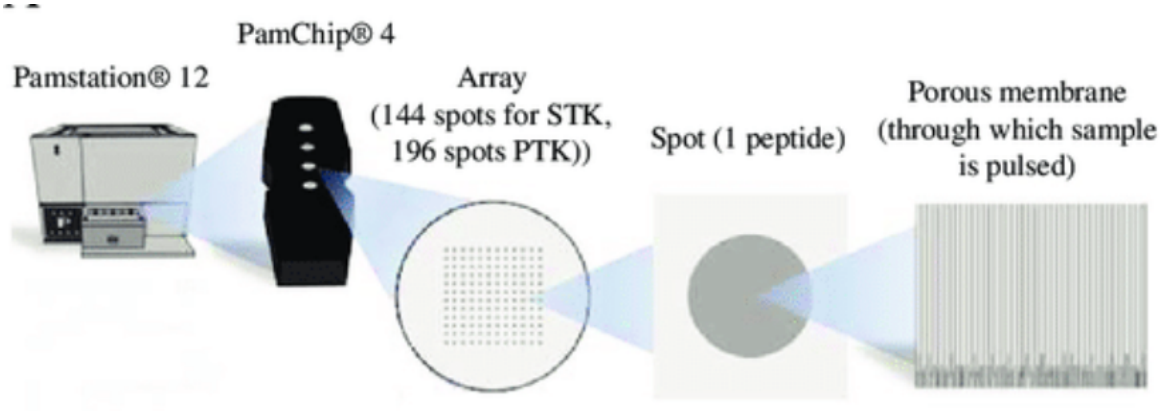
{The Department of Neurosciences at the University of Toledo Medical Center}

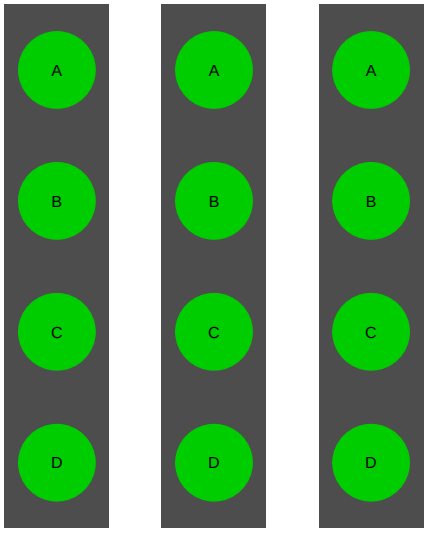
Introduction

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 1, Pair 1, Chip 1,2, & 3

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.

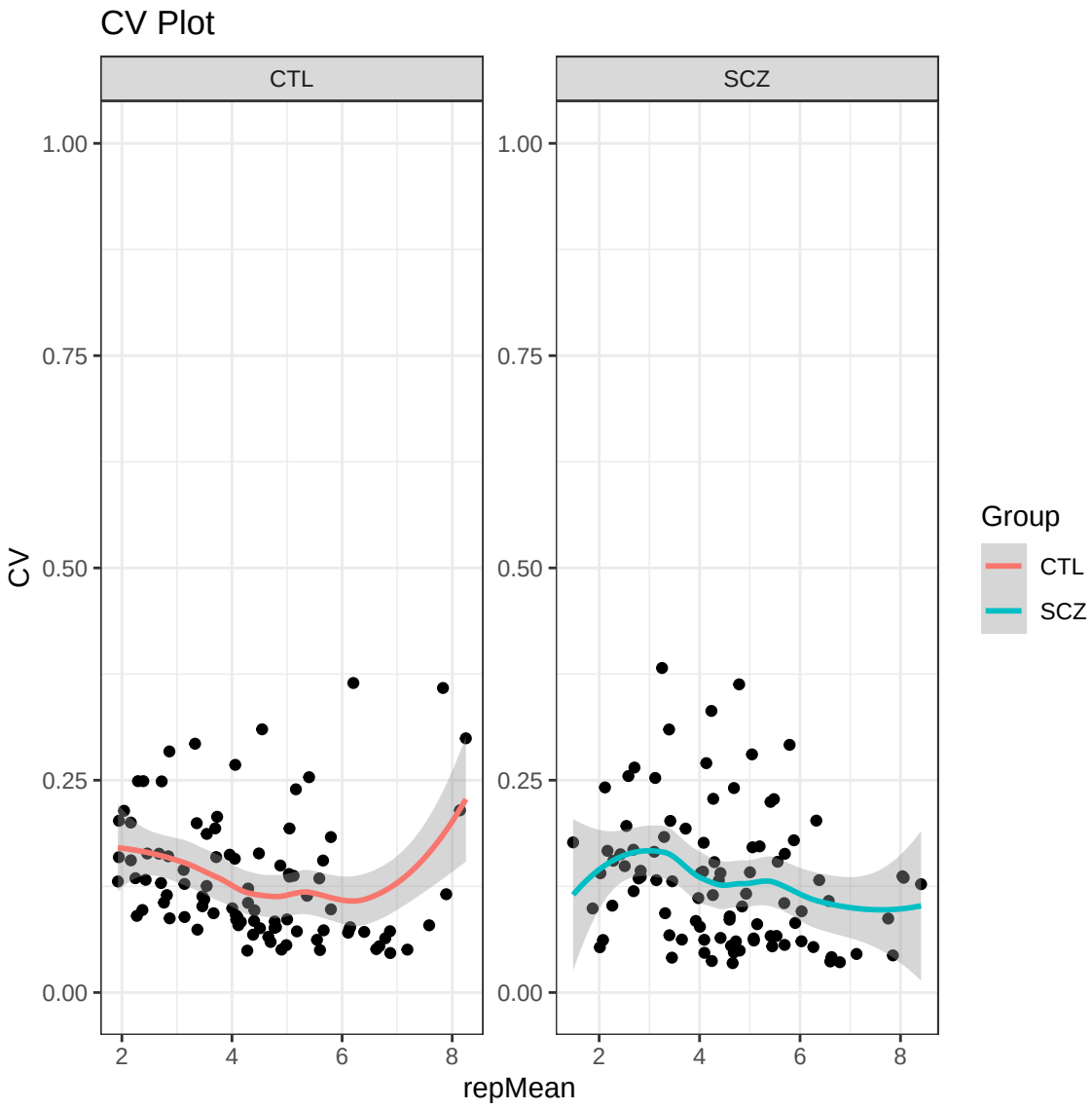


Results

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.


CV Plot
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SCZ
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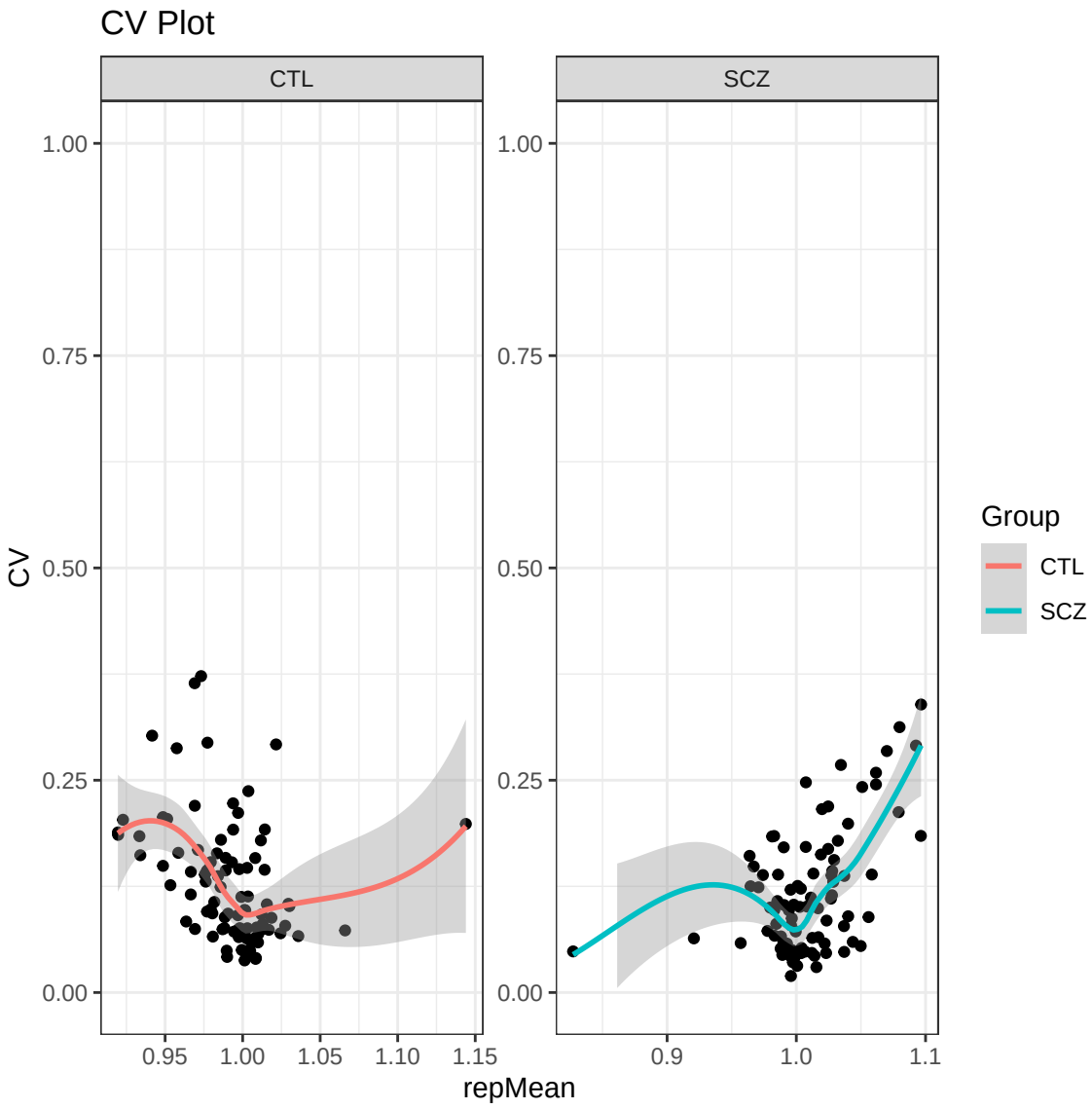
0.75
0.50
0.25
0.00
CV
2
4
6
8
repMean
Group
CTL
SCZ


CV Plot
CTL
SCZ
CV
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0.75
0.50
0.25
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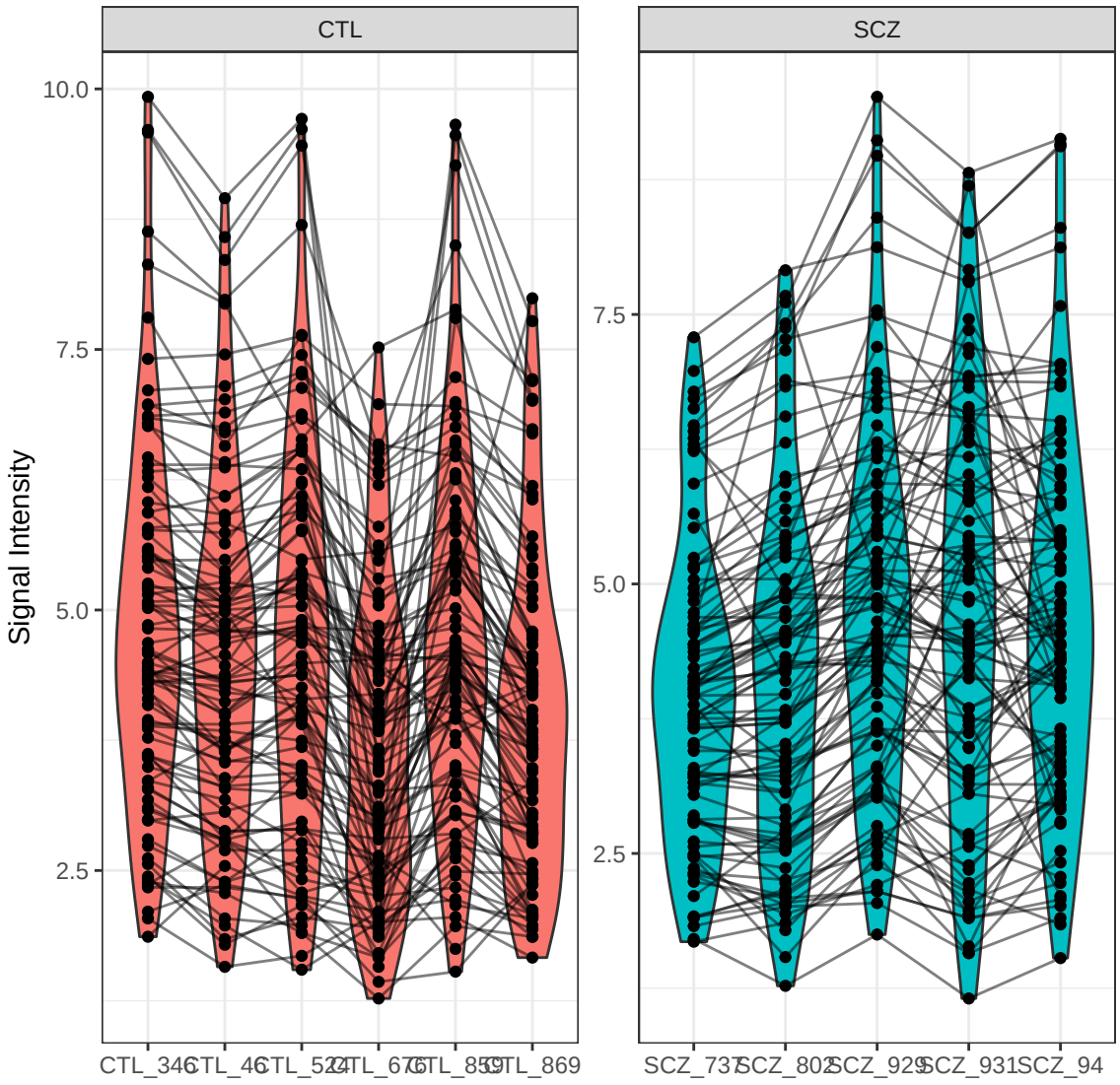
1.0
1.1
repMean
Group
CTL
SCZ


CTL
SCZ
Signal Intensity
10.0
7.5
5.0
2.5
7.5
5.0
2.5
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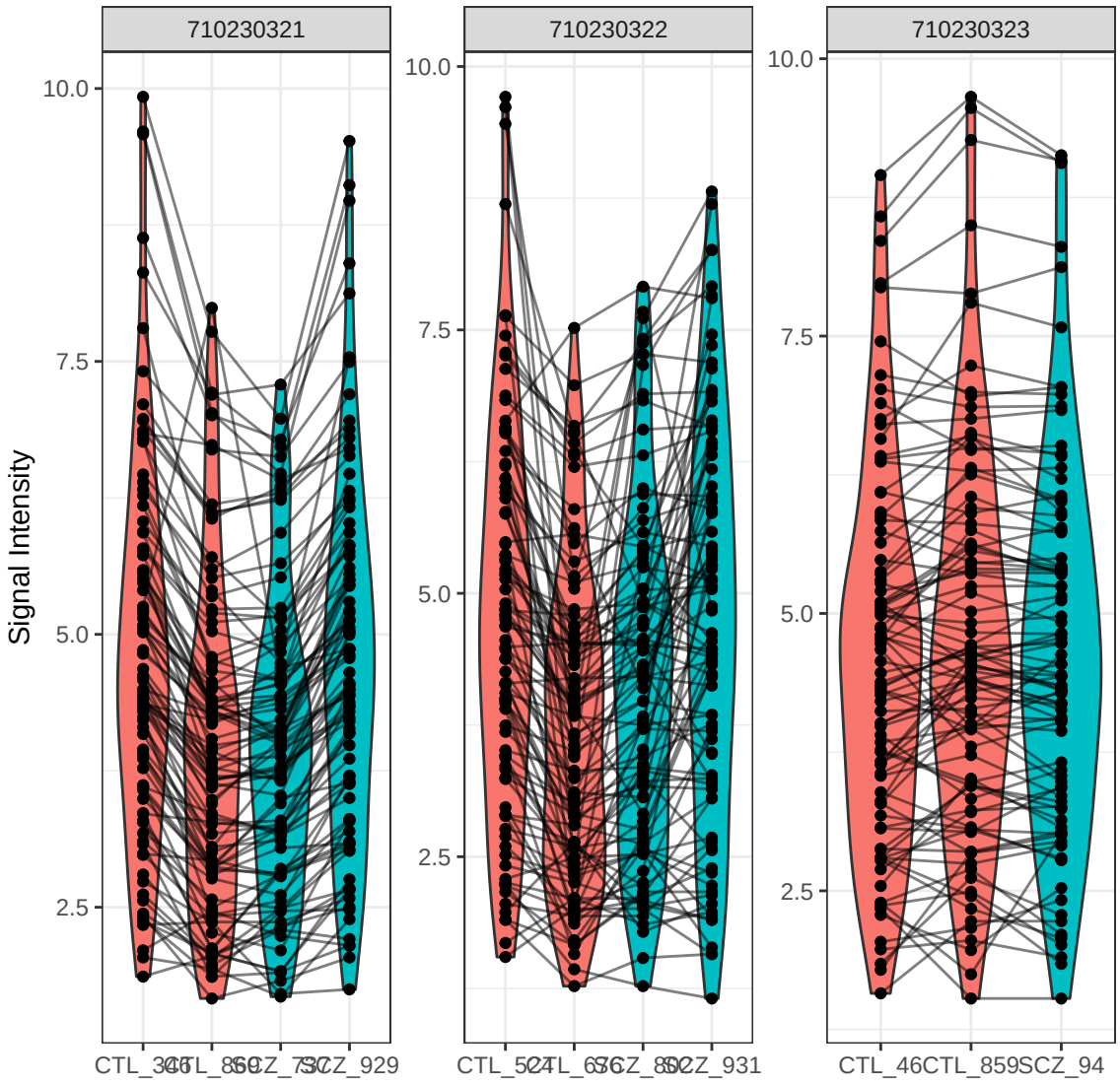
CTL_46
CTL_524
CTL_676
CTL_859
CTL_869
SCZ_737
SCZ_802
SCZ_929
SCZ_931
SCZ_94


710230321
710230322
710230323
Signal Intensity
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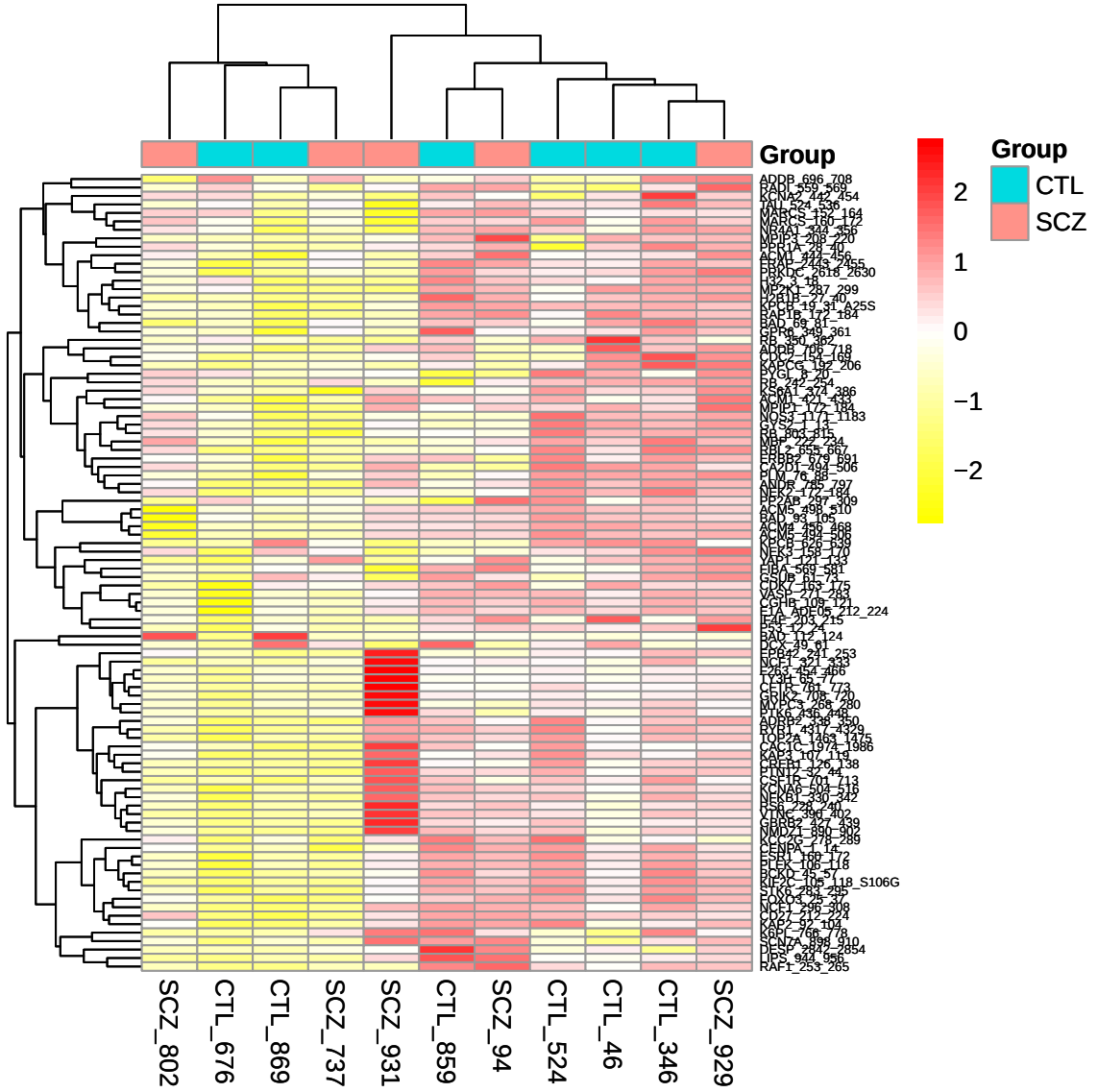
7.5
5.0
2.5
CTL_46
CTL_859
SCZ_94
CTL_931


Group
CTL
SCZ
2
1
0
−1
−2
SCZ_802
CTL_676
CTL_869
SCZ_737
SCZ_931
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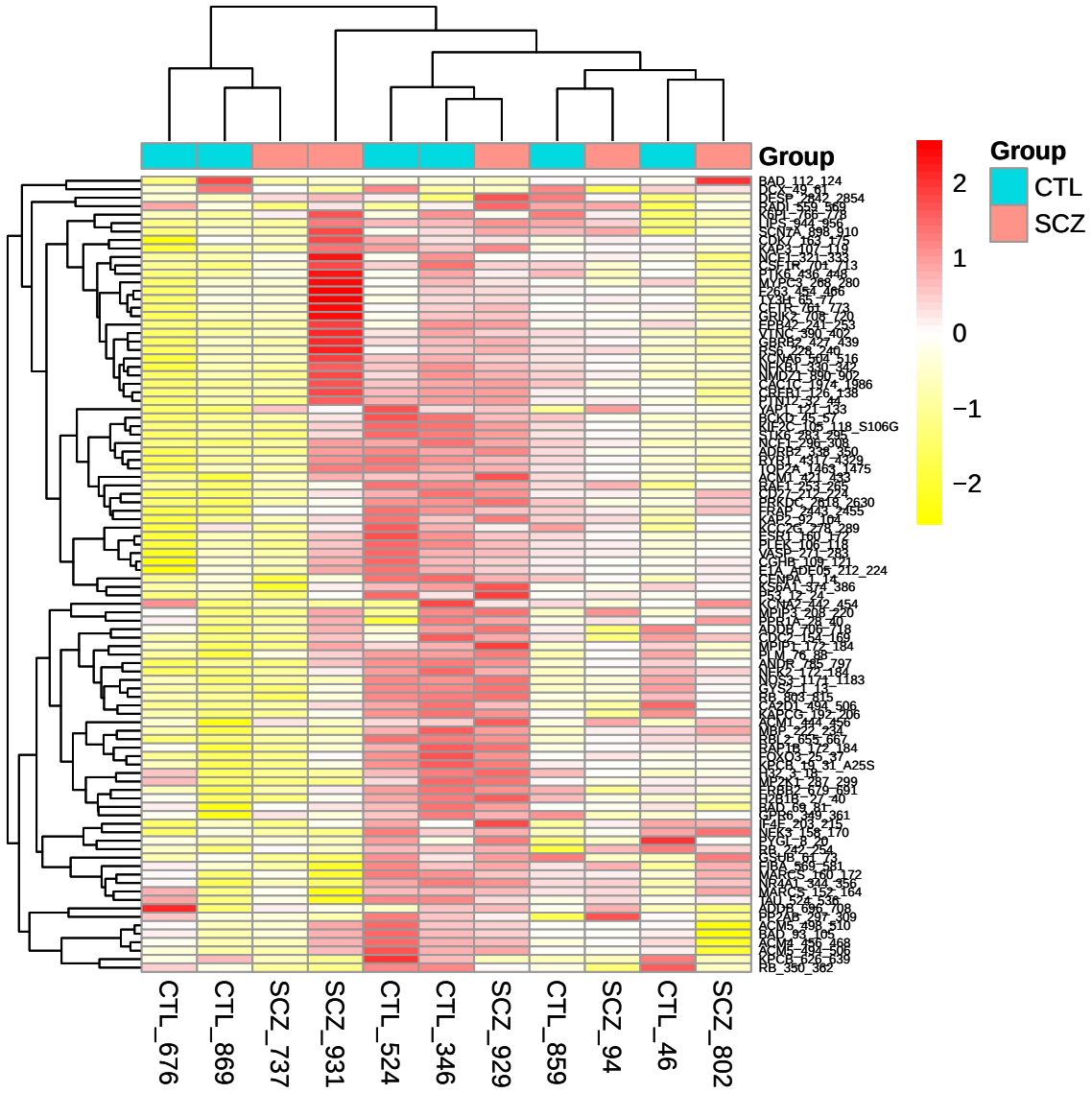
SCZ_94
CTL_524
CTL_46
CTL_346
SCZ_929


Group
CTL
SCZ
2
1
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CTL_859
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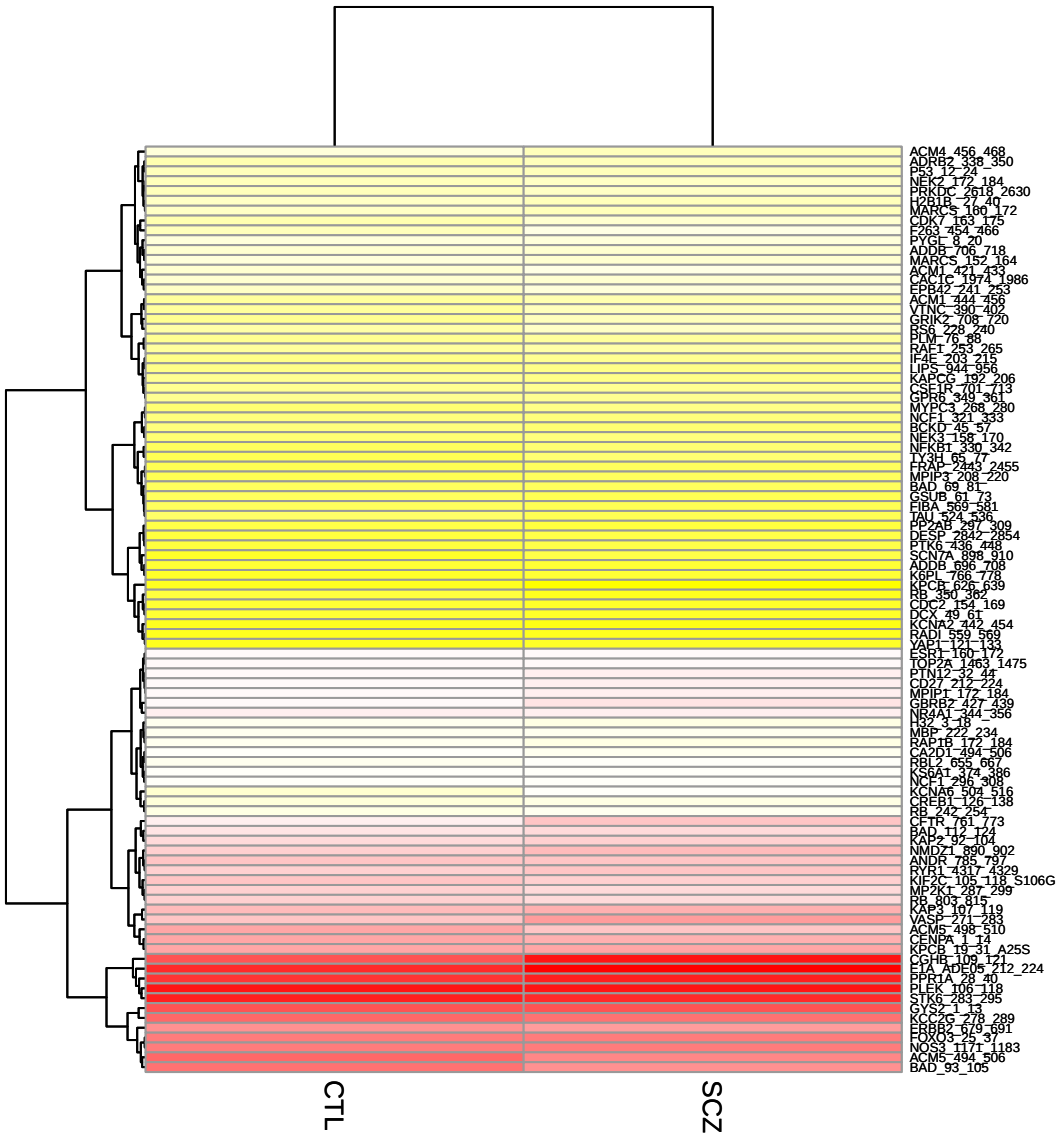
CTL_46
SCZ_802



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

- Control vs. Schizophrenia (Pair 1.2)

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:

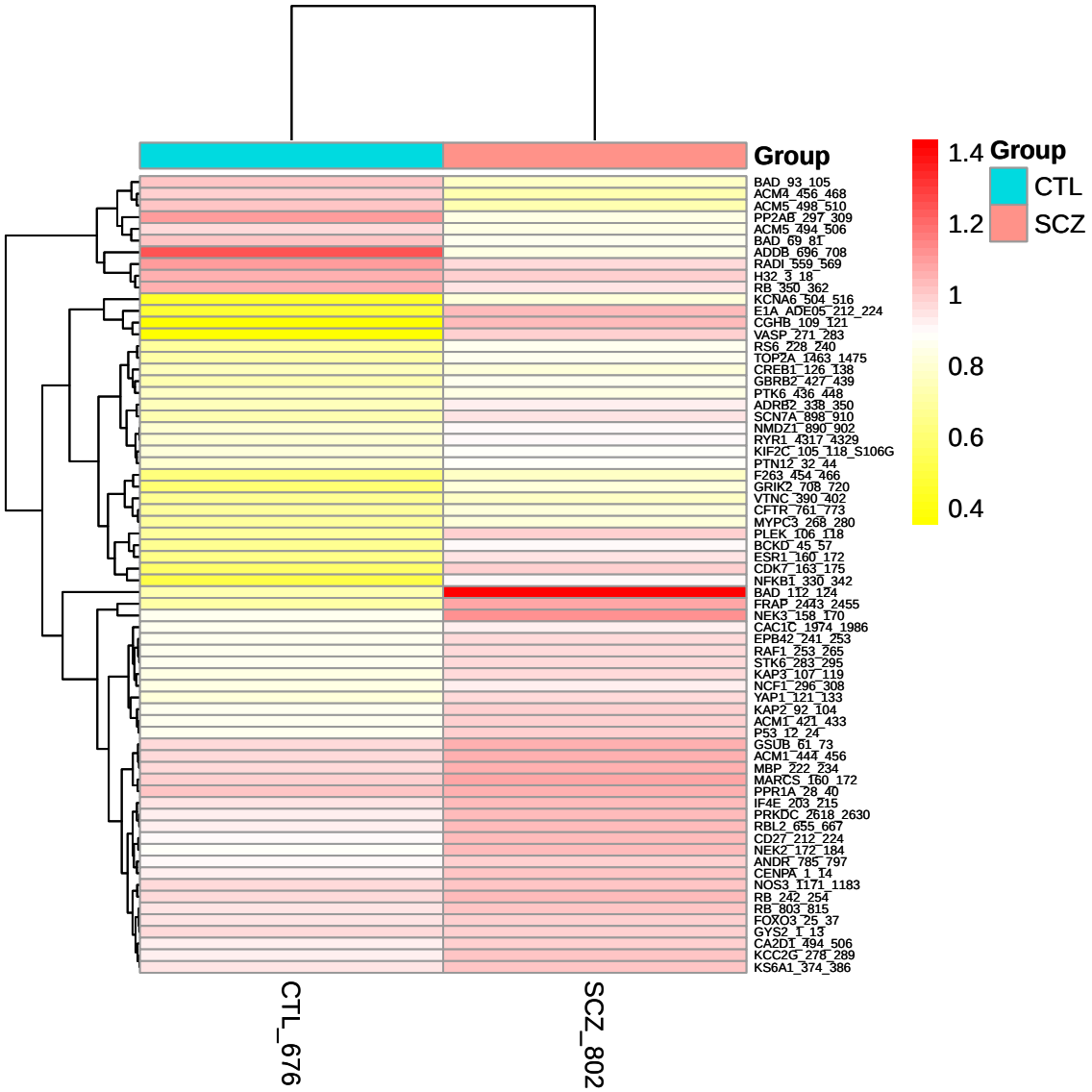
- The *Medain_ SigmBg* at max exposure *200ms* must be above a certain value

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

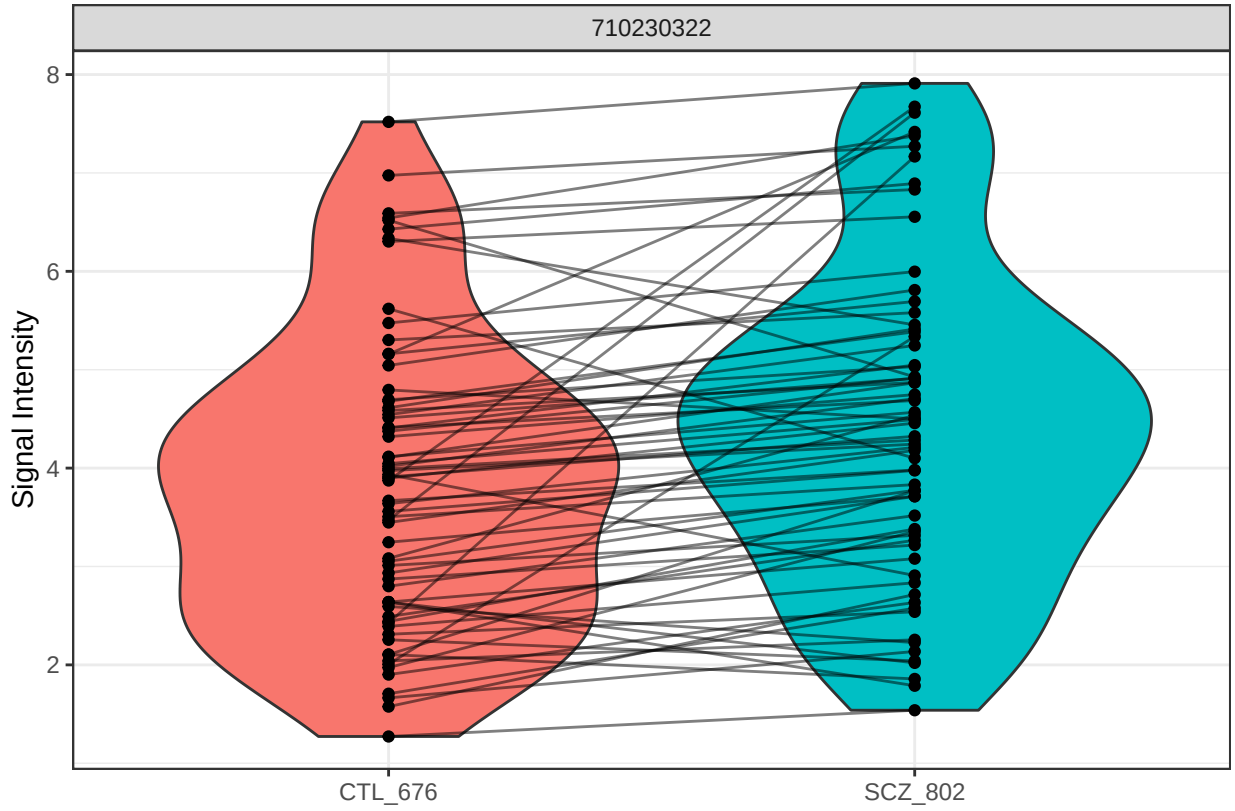
These *Filtering Parameters* (fold change threshold, QC criteria) can be modified to adjust the stringency of the analysis. The *Filtering Parameters* that are used for this analysis:

- 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)

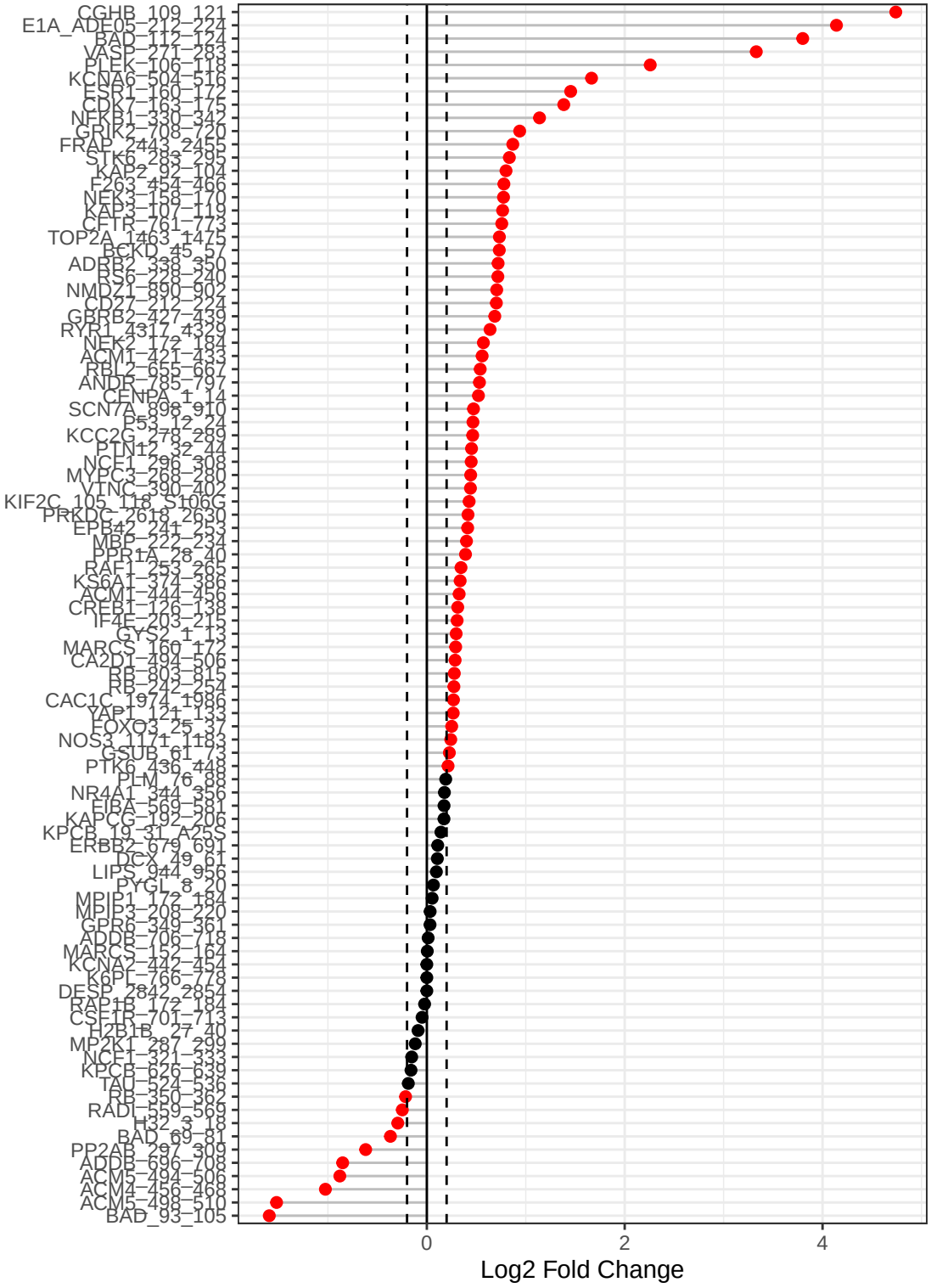
Schizophrenia vs. Control (Pair 1.2) After applying the *Filtering Parameters* for this group comparison, only *68*/141 peptides carried forward in the analysis (i.e. *68 hits*). Below are some figures to visualize the differences between these samples for considering these *hits*.



Run1 Comp2



Run1 Comp2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BAD_112_124
VASP_271_283
PLEK_106_118
KCNA6_504_516
ESR1_160_172
CDK7_163_175
NFKB1_330_342
GRIK2_708_720
FRAP_2443_2455
STK6_283_295
KAP2_92_104
F263_454_466
NEK3_158_170
KAP3_107_119
CFTR_761_773
TOP2A_1463_1475
BCKD_45_57
ADRB2_338_350
RS6_228_240
NMDZ1_890_902
CD27_212_224
GRB2_427_439
GRIK1_437_449
RYR1_4317_4329
NEK2_172_184
ACM1_421_433
RBL2_655_667
ANDR_785_797
CENPA_1_14
SCN7A_898_910
P53_12_24
KCC2G_278_290
PTN12_32_44
NCF1_296_308
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KIF2C_105_118_S106G
PRKDC_2618_2630
EPB42_241_253
MBP_222_234
PPP1A_28_40
RAF1_253_265
KS6A1_374_386
ACM1_444_456
CREB1_126_138
IF4E_203_215
GYS2_1_13
MARCS_160_172
CA2D1_494_506
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RB_903_915
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FOXO3_25_37
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NCF1_321_333
KPCB_626_639
TAU_524_536
RB_350_362
RADI_559_569
H32_3_16
BAD_69_81
PP2AB_297_309
ADDB_696_708
ACM5_494_506
ACM4_456_468
ACM5_498_510
BAD_93_105
0
2
4
Log2 Fold Change

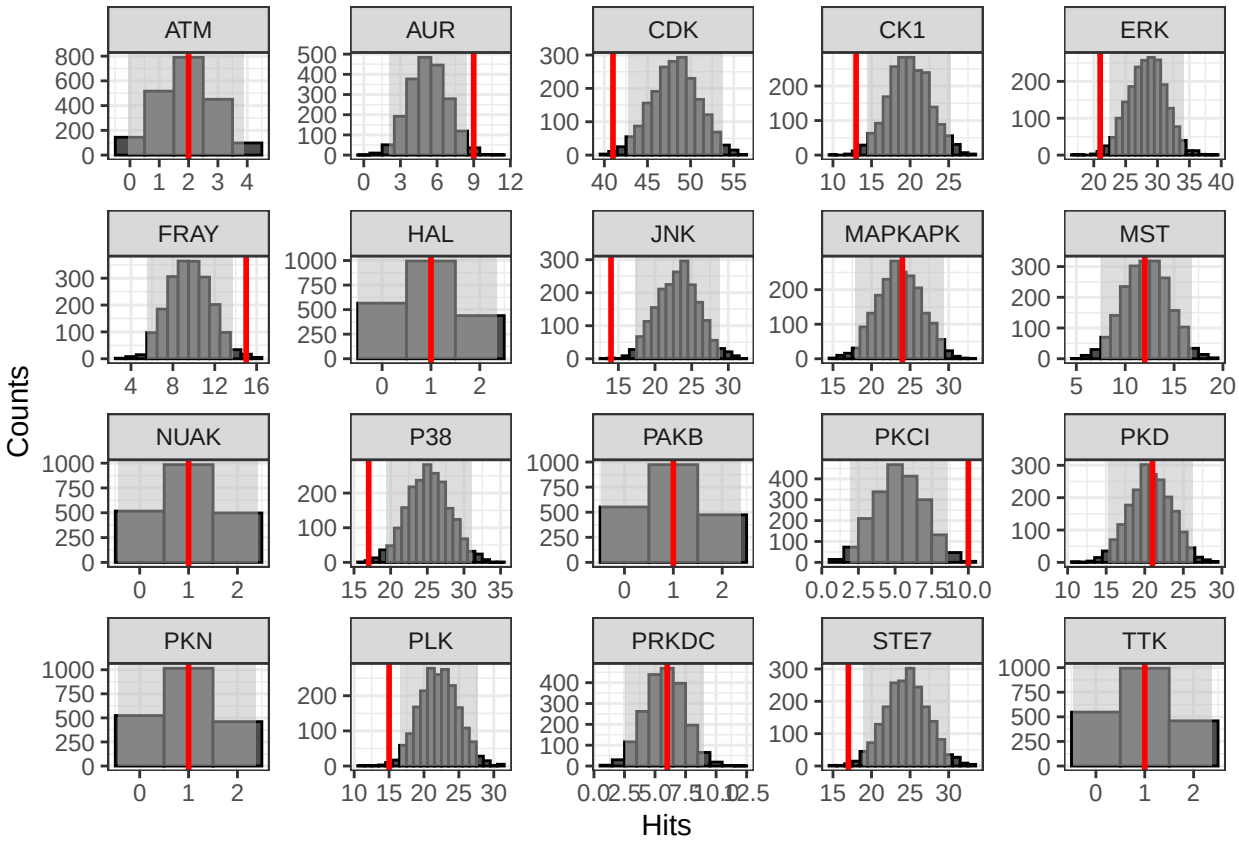
Run1 Comp2


Linear Model Fit of Signal Intensity Relative to Exposure Time at Post−wash Cycle of
ACM1_421_433
ACM1_444_456
ACM4_456_468
ACM5_494_506
ACM5_498_510
ADDB_696_708
ADRB2_338_350
ANDR_785_797
BAD_112_124
BAD_69_81
BAD_93_105
BCKD_45_57
CA2D1_494_506
CAC1C_1974_1986
CD27_212_224
CDK7_163_175
CENPA_1_14
CFTR_761_773
CGHB_109_121
CREB1_126_138
1A_ADE05_212_22
EPB42_241_253
ESR1_160_172
F263_454_466
FOXO3_25_37
FRAP_2443_2455
GBRB2_427_439
GRIK2_708_720
GSUB_61_73
GYS2_1_13
H32_3_18
IF4E_203_215
KAP2_92_104
KAP3_107_119
KCC2G_278_289
KCNA6_504_516
F2C_105_118_S106
KS6A1_374_386
MARCS_160_172
MBP_222_234
MYPC3_268_280
NCF1_296_308
NEK2_172_184
NEK3_158_170
NFKB1_330_342
NMDZ1_890_902
NOS3_1171_1183
P53_12_24
PLEK_106_118
PP2AB_297_309
PPR1A_28_40
PRKDC_2618_2630
PTK6_436_448
PTN12_32_44
RADI_559_569
RAF1_253_265
RB_242_254
RB_350_362
RB_803_815
RBL2_655_667
RS6_228_240
RYR1_4317_4329
SCN7A_898_910
STK6_283_295
TOP2A_1463_1475
VASP_271_283
VTNC_390_402
YAP1_121_133
Signal
ExposureTime
factor(Group)
CTL
SCZ

Run1 Comp2

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
JNK	14	23.125	2.871	-3.178
PKCI	10	5.295	1.668	2.821
P38	17	25.164	2.907	-2.809
STE7	17	24.561	2.804	-2.696
FRAY	15	9.598	2.048	2.637
CDK	41	48.198	2.746	-2.621
PLK	15	22.118	2.786	-2.555
ERK	21	28.379	2.894	-2.549
CK1	13	19.879	2.711	-2.537
AUR	9	5.287	1.579	2.353
MLK	13	8.690	2.015	2.139
MSN	4	1.938	0.986	2.091
NMO	2	5.288	1.598	-2.058
STKR	1	3.390	1.279	-1.869
BARK2	3	1.476	0.841	1.812
AKT	24	19.237	2.709	1.758
PKA	34	28.856	2.937	1.751
PKCD	9	6.230	1.731	1.600
MARK	1	2.924	1.203	-1.599
PAKA	20	15.976	2.595	1.550
IRAK	4	6.643	1.767	-1.496
DYRK	22	26.160	2.896	-1.437
VRK1	4	2.433	1.093	1.434
NAK	0	0.962	0.705	-1.365
IKK	24	27.945	2.933	-1.345



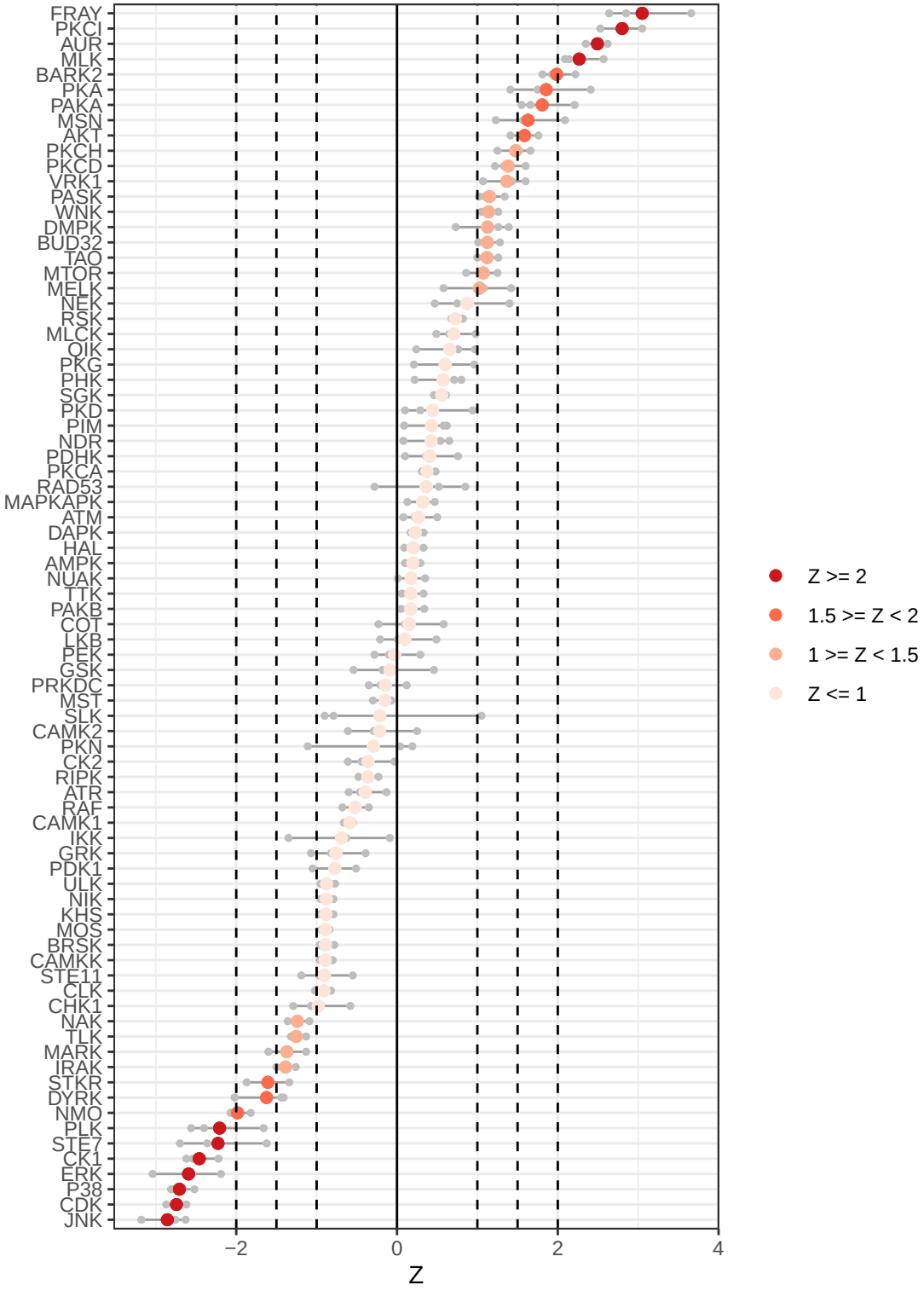
	meanLFC		
Kinase	0.2	0.25	0.3
JNK	-3.18	-2.63	-2.76
PKCI	2.82	2.53	3.05
P38	-2.81	-2.52	-2.79
STE7	-2.70	-2.36	-1.62
FRAY	2.64	2.85	3.66
CDK	-2.62	-2.87	-2.74
PLK	-2.56	-2.40	-1.66
ERK	-2.55	-2.19	-3.04
CK1	-2.54	-2.62	-2.22
AUR	2.35	2.62	2.51
MLK	2.14	2.57	2.09
MSN	2.09	1.23	1.57
NMO	-2.06	-1.82	-2.07
STKR	-1.87	-1.61	-1.34
BARK2	1.81	1.93	2.22
AKT	1.76	1.59	1.41
PKA	1.75	1.41	2.41
PKCD	1.60	1.33	1.22

MARK	-1.60	-1.38	-1.13
PAKA	1.55	1.66	2.21
IRAK	-1.50	-1.26	-1.40
DYRK	-1.44	-1.41	-2.02
VRK1	1.43	1.60	1.07
NAK	-1.36	-1.27	-1.09
IKK	-1.35	-0.63	-0.09
TLK	-1.32	-1.31	-1.13
CHK1	-1.29	-1.07	-0.58
DMPK	1.26	0.73	1.39
PKCH	1.25	1.52	1.66
STE11	-1.19	-0.97	-0.55
MELK	1.08	0.58	1.42
GRK	-1.07	-0.82	-0.39
WNK	1.05	1.11	1.26
SLK	1.05	-0.90	-0.79
PASK	1.02	1.09	1.34
CLK	-1.02	-0.89	-0.82
BUD32	1.01	1.08	1.28
TAO	1.00	1.10	1.26
QIK	0.97	0.76	0.24
CAMKK	-0.96	-0.92	-0.80
KHS	-0.96	-0.91	-0.79
BRSK	-0.96	-0.93	-0.78
ULK	-0.95	-0.91	-0.77
NIK	-0.95	-0.90	-0.79
MOS	-0.94	-0.89	-0.84
MTOR	0.86	1.25	1.11
RAD53	0.85	0.52	-0.28
PHK	0.80	0.22	0.71
RSK	0.68	0.68	0.82
RAF	-0.68	-0.35	-0.53
PKG	0.63	0.21	0.96
SGK	0.61	0.60	0.46
CAMK2	-0.61	-0.29	0.25
CK2	-0.61	-0.44	-0.03
COT	0.58	-0.23	0.10
PIM	0.58	0.09	0.62
CAMK1	-0.55	-0.66	-0.55
NDR	0.54	0.65	0.08
GSK	-0.54	-0.18	0.46
PDK1	-0.51	-1.05	-0.76
MLCK	0.49	0.65	0.98
RIPK	-0.48	-0.38	-0.23
NEK	0.47	0.75	1.40

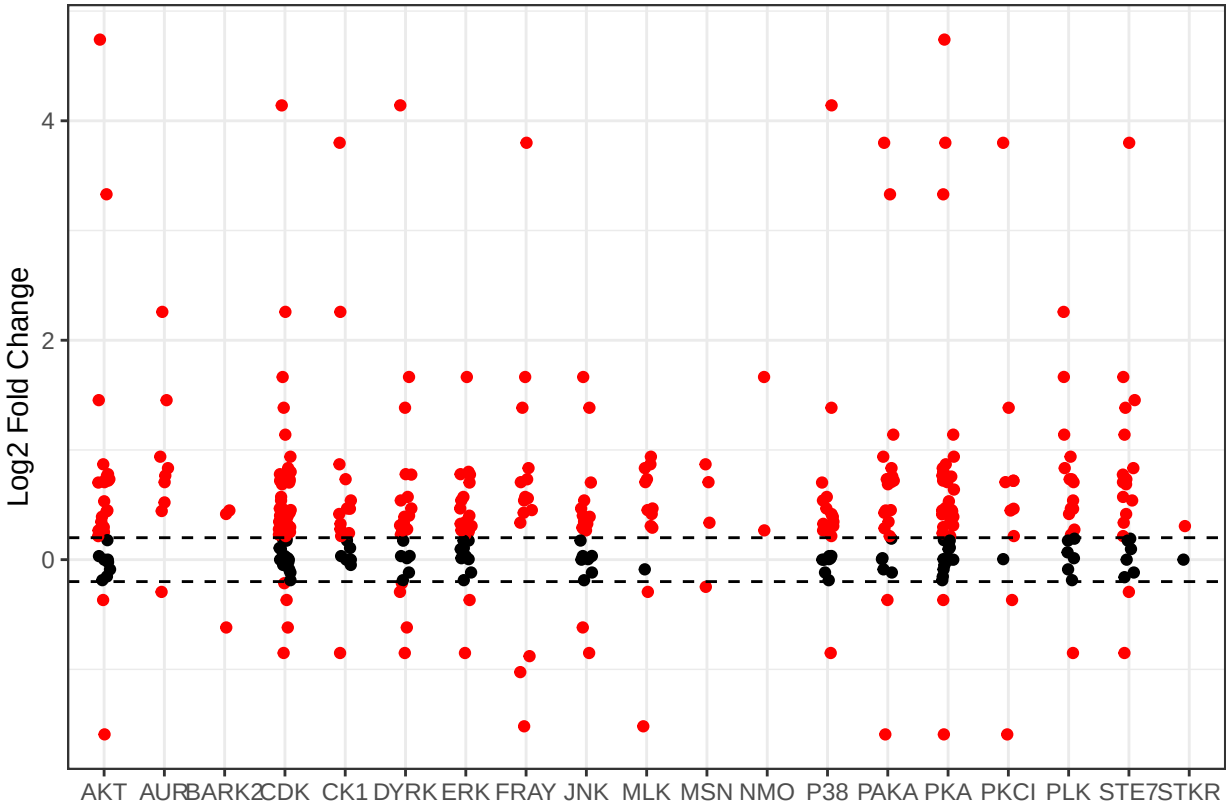
PDHK	0.36	0.10	0.76
PKCA	0.33	0.48	0.31
PEK	-0.28	-0.10	0.29
LKB	-0.21	0.01	0.49
AMPK	0.20	0.10	0.29
DAPK	0.18	0.17	0.33
ATR	-0.13	-0.46	-0.60
MAPKAPK	0.13	0.36	0.47
PRKDC	0.12	-0.20	-0.35
PKD	0.10	0.29	0.94
HAL	0.09	0.18	0.33
ATM	0.08	0.22	0.50
MST	-0.07	-0.08	-0.30
TTK	0.06	0.13	0.33
PAKB	0.05	0.13	0.34
PKN	0.04	0.19	-1.11
NUAK	0.01	0.17	0.35

Method	NumberOfPeptides
meanLFC.0.2	68
meanLFC.0.25	63
meanLFC.0.3	54

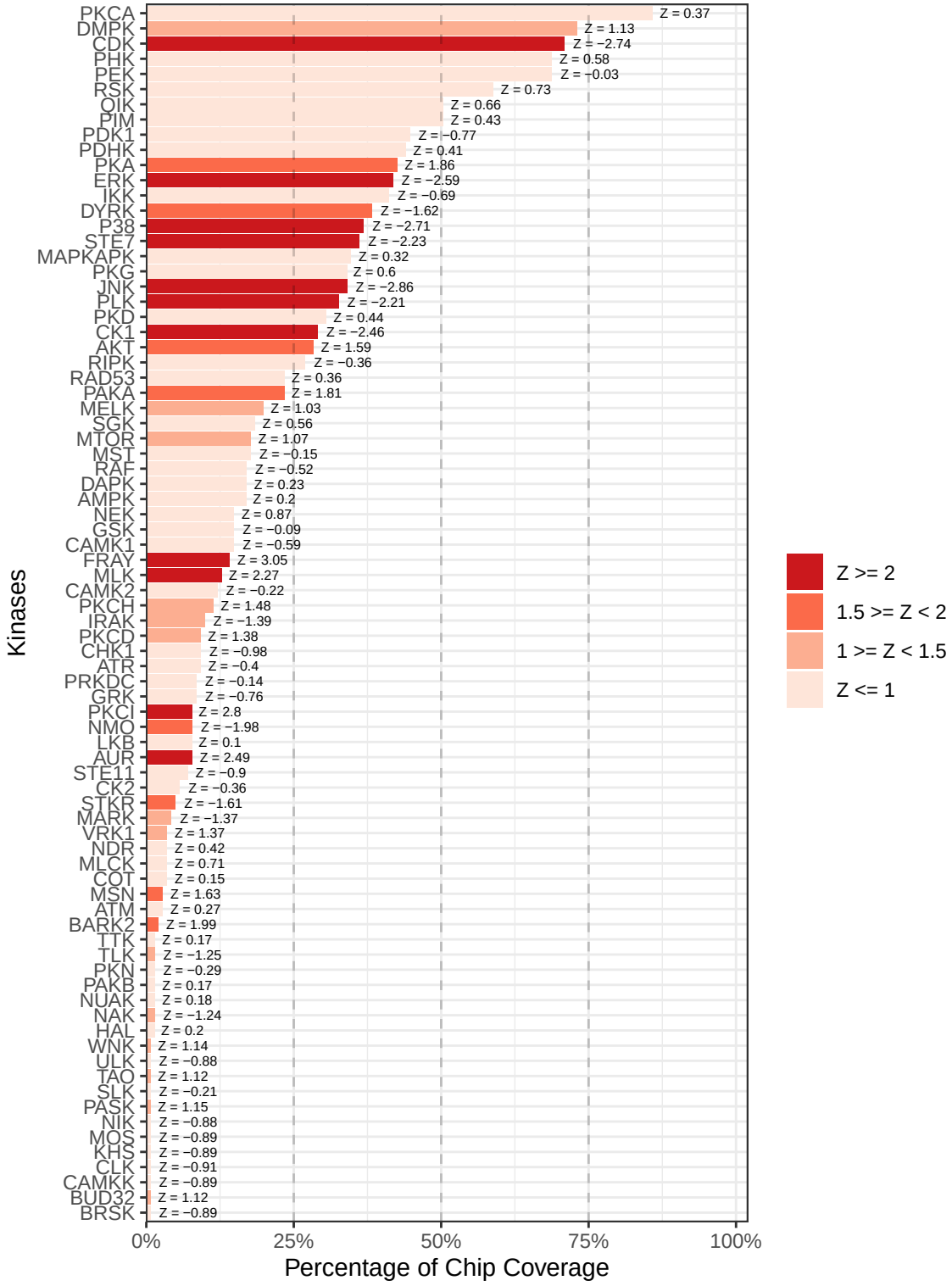
Run1 Comp2


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

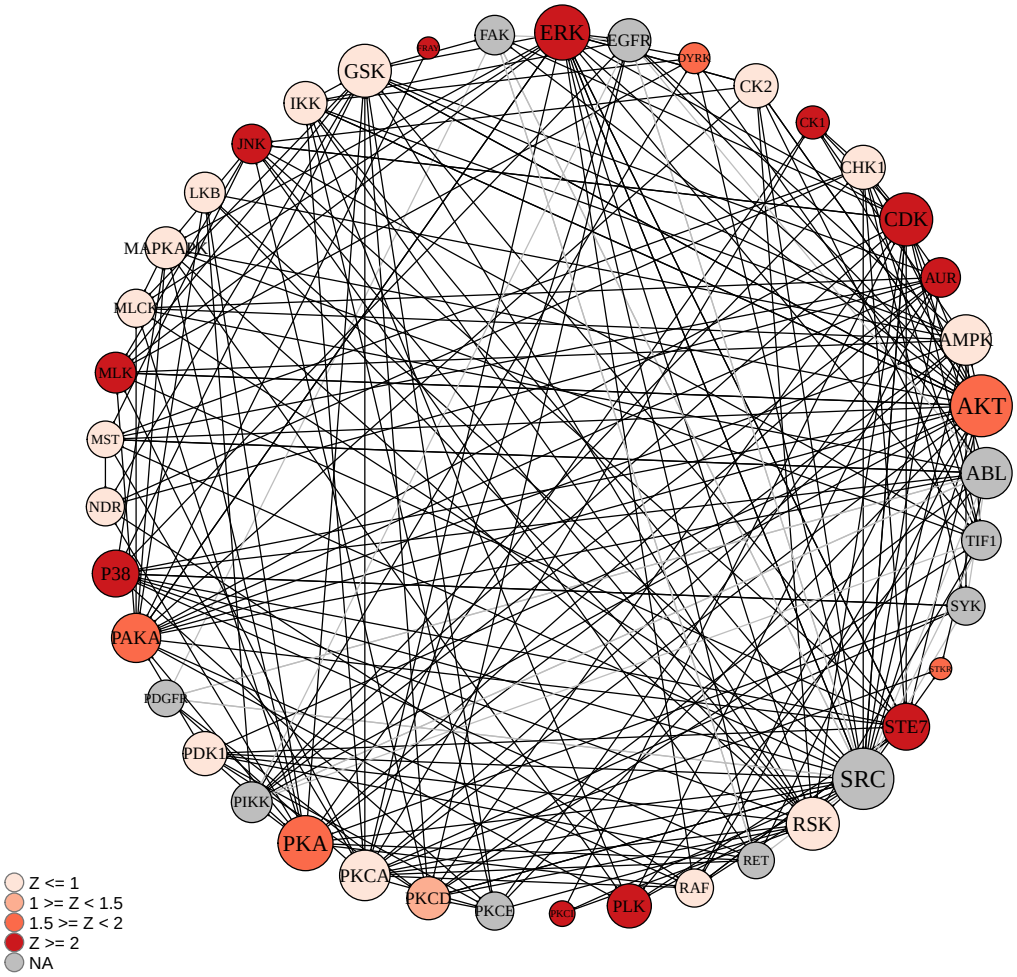
Run1 Comp2


Log2 Fold Change
4
2
0
AKT
AUR
BARK
CDK
CK1
DYRK
ERK
FRAY
JNK
MLK
MSN
NMO
P38
PAKA
PKA
PKCI
PLK
STE7
STKR

Run1 Comp2


PKCA Z = 0.37
DMPK Z = 1.13
CDK Z = −2.74
PHK Z = 0.58
PEK Z = −0.03
RSK Z = 0.73
OIK Z = 0.66
PIM Z = 0.43
PDK1 Z = −0.77
PDHK Z = 0.41
PKA Z = 1.86
ERK Z = −2.59
IKK Z = −0.69
DYRK Z = −1.62
P38 Z = −2.71
STE7 Z = −2.23
MAPKAPK Z = 0.32
PKG Z = 0.6
JNK Z = −2.86
PLK Z = −2.21
PKD Z = 0.44
CK1 Z = −2.46
AKT Z = 1.59
RIPK Z = −0.36
RAD53 Z = 0.36
PAKA Z = 1.81
MELK Z = 1.03
SGK Z = 0.56
MTOR Z = 1.07
MST Z = −0.15
RAF Z = −0.52
DAPK Z = 0.23
AMPK Z = 0.2
NEK Z = 0.87
GSK Z = −0.09
CAMK1 Z = −0.59
FRAY Z = 3.05
MLK Z = 2.27
CAMK2 Z = −0.22
PKCH Z = 1.48
IRAK Z = −1.39
PKCD Z = 1.38
CHK1 Z = −0.98
ATR Z = −0.4
PRKDC Z = −0.14
GRK Z = −0.76
PKCI Z = 2.8
NMO Z = −1.98
LKB Z = 0.1
AUR Z = 2.49
STE11 Z = −0.9
CK2 Z = −0.36
STKR Z = −1.61
MARK Z = −1.37
VRK1 Z = 1.37
NDR Z = 0.42
MLCK Z = 0.71
COT Z = 0.15
MSN Z = 1.63
ATM Z = 0.27
BARK2 Z = 1.99
TTK Z = 0.17
TLK Z = −1.25
PKN Z = −0.29
PAKB Z = 0.17
NUAK Z = 0.18
NAK Z = −1.24
HAL Z = 0.2
WNK Z = 1.14
ULK Z = −0.88
TAO Z = 1.12
SLK Z = −0.21
PASK Z = 1.15
NIK Z = −0.88
MOS Z = −0.89
KHS Z = −0.89
CLK Z = −0.91
CAMKK Z = −0.89
BUD32 Z = 1.12
BRSK Z = −0.89
Kinases
0%
25%
50%
75%
100%
Percentage of Chip Coverage
Z >= 2
1.5 >= Z < 2
1 >= Z < 1.5
Z <= 1

Run1 Comp2




```
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"
```