RPPA analysis: R075 Octavio Mejia

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Contents

Introduction

The aim of this analysis is to profile protein expression after MDM4 knockdown and MDM4 knockdown + APR-246 treatment and identify deregulated proteins.

The samples and conditions are listed below:

Lysate.ID	Cell.Line.Tissue.type	Treatment	Time.point
OMH-7	breast orthotopic model	shMx + PBS	Endpoint
OMH-8	breast orthotopic model	shMx + PBS	Endpoint
OMH-9	breast orthotopic model	shMx + PBS	Endpoint
OMH-10	breast orthotopic model	shMx + PBS	Endpoint
OMH-11	breast orthotopic model	shMx + PBS	Early point
OMH-12	breast orthotopic model	shMx + PBS	Early point
OMH-13	breast orthotopic model	shMx + PBS	Early point
OMH-14	breast orthotopic model	shMx + APR-246	Endpoint
OMH-15	breast orthotopic model	shMx + APR-246	Endpoint
OMH-16	breast orthotopic model	shMx + APR-246	Endpoint
OMH-17	breast orthotopic model	shMx + APR-246	Endpoint
OMH-18	breast orthotopic model	shMx + APR-246	Early point
OMH-19	breast orthotopic model	shMx + APR-246	Early point
OMH-20	breast orthotopic model	shMx + APR-246	Early point
OMH-21	breast orthotopic model	shMDM4 + PBS	Endpoint
OMH-22	breast orthotopic model	shMDM4 + PBS	Endpoint
OMH-23	breast orthotopic model	shMDM4 + PBS	Endpoint
OMH-24	breast orthotopic model	shMDM4 + PBS	Endpoint
OMH-25	breast orthotopic model	shMDM4 + PBS	Early point
OMH-26	breast orthotopic model	shMDM4 + PBS	Early point
OMH-27	breast orthotopic model	shMDM4 + PBS	Early point
OMH-28	breast orthotopic model	shMDM4 + APR-246	Endpoint
OMH-29	breast orthotopic model	shMDM4 + APR-246	Endpoint
OMH-30	breast orthotopic model	shMDM4 + APR-246	Endpoint
OMH-31	breast orthotopic model	shMDM4 + APR-246	Endpoint
OMH-32	breast orthotopic model	shMDM4 + APR-246	Early point
OMH-33	breast orthotopic model	shMDM4 + APR-246	Early point
OMH-34	breast orthotopic model	shMDM4 + APR-246	Early point
OMH-35	MM468 (shMx)	Doxycycline	Day 4, 72 h
OMH-36	breast orthotopic model	shMDM4 + PBS	Endpoint
OMH-37	breast orthotopic model	shMDM4 + APR-246	Endpoint
OMH-38	MM468 (shMDM4)	Doxycycline	Day 4, 72 h
OMH-39	MM468 (shMx)	Doxycycline	Day 7, 144h
OMH-40	breast orthotopic model	shMx + APR-246	Endpoint
OMH-41	MM468 (shMDM4)	Doxycycline	Day 7, 144h
OMH-70	breast orthotopic model	shMDM4 + PBS	Endpoint
OMH-73	breast orthotopic model	shMDM4 + APR-246	Endpoint
OMH-74	breast orthotopic model	shMx + PBS	Endpoint
OMH-77	MM468 (shMx)	Doxycycline	Day 3, 48h
OMH-80	MM468 (shMDM4)	Doxycycline	Day 3, 48h
OMH-107	subcutaneous tumour	shMx + PBS	Endpoint
OMH-108	subcutaneous tumour	shMx + PBS	Endpoint
OMH-109	subcutaneous tumour	shMx + PBS	Endpoint
OMH-110	subcutaneous tumour	shMx + PBS	Endpoint
OMH-111	subcutaneous tumour	shMx + PBS	Endpoint
OMH-112	subcutaneous tumour	shMx + APR-246	Endpoint

Lysate.ID	Cell.Line.Tissue.type	Treatment	Time.point
OMH-113	subcutaneous tumour	shMx + APR-246	Endpoint
OMH-114	subcutaneous tumour	shMx + APR-246	Endpoint
OMH-115	subcutaneous tumour	shMx + APR-246	Endpoint
OMH-116	subcutaneous tumour	shMx + APR-246	Endpoint
OMH-117	subcutaneous tumour	shMx + APR-246	Endpoint
OMH-118	subcutaneous tumour	shMDM4 + PBS	Endpoint
OMH-119	subcutaneous tumour	shMDM4 + PBS	Endpoint
OMH-120	subcutaneous tumour	shMDM4 + PBS	Endpoint
OMH-121	subcutaneous tumour	shMDM4 + PBS	Endpoint
OMH-122	subcutaneous tumour	shMDM4 + PBS	Endpoint
OMH-123	subcutaneous tumour	shMDM4 + APR-246	Endpoint
OMH-124	subcutaneous tumour	shMDM4 + APR-246	Endpoint
OMH-125	subcutaneous tumour	shMDM4 + APR-246	Endpoint
OMH-126	subcutaneous tumour	shMDM4 + APR-246	Endpoint
OMH-127	subcutaneous tumour	shMDM4 + APR-246	Endpoint
OMH-128	subcutaneous tumour	shMx + PBS	Endpoint
OMH-129	subcutaneous tumour	shMx + PBS	Endpoint
OMH-130	subcutaneous tumour	shMx + PBS	Endpoint
OMH-131	subcutaneous tumour	shMx + PBS	Endpoint
OMH-132	subcutaneous tumour	shMx + PBS	Endpoint
OMH-133	subcutaneous tumour	shMDM4 + PBS	Endpoint
OMH-134	subcutaneous tumour	shMDM4 + PBS	Endpoint
OMH-135	subcutaneous tumour	shMDM4 + PBS	Endpoint
OMH-136	subcutaneous tumour	shMDM4 + PBS	Endpoint

Technical details

- Antibody Fox03a (Ab-8) did not perform well and was excluded from all analyses.

RFI boxplots

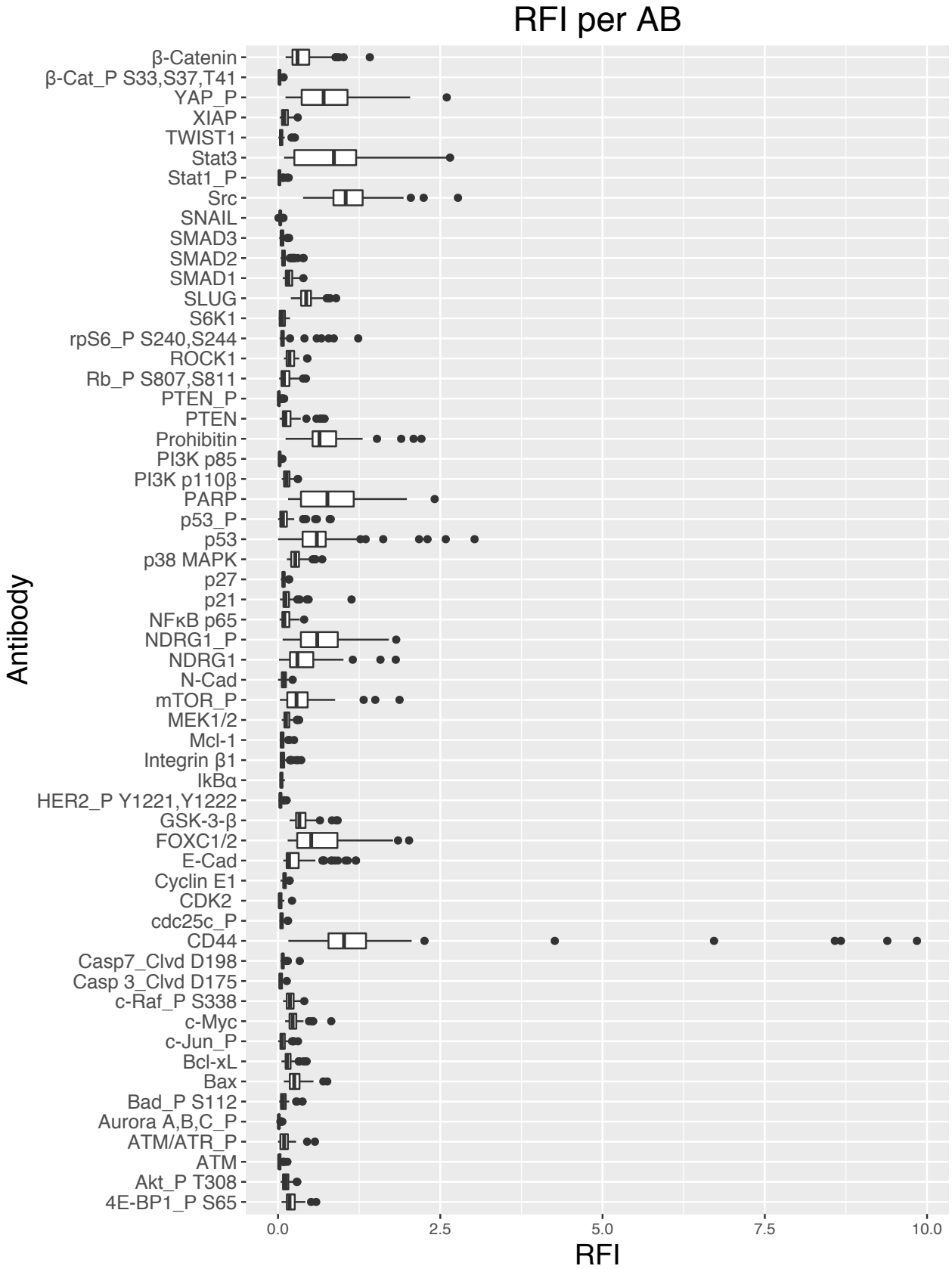
RFI per antibody

These boxplots show the spread of RFI (normalised to secondary) values across all samples, for each antibody.

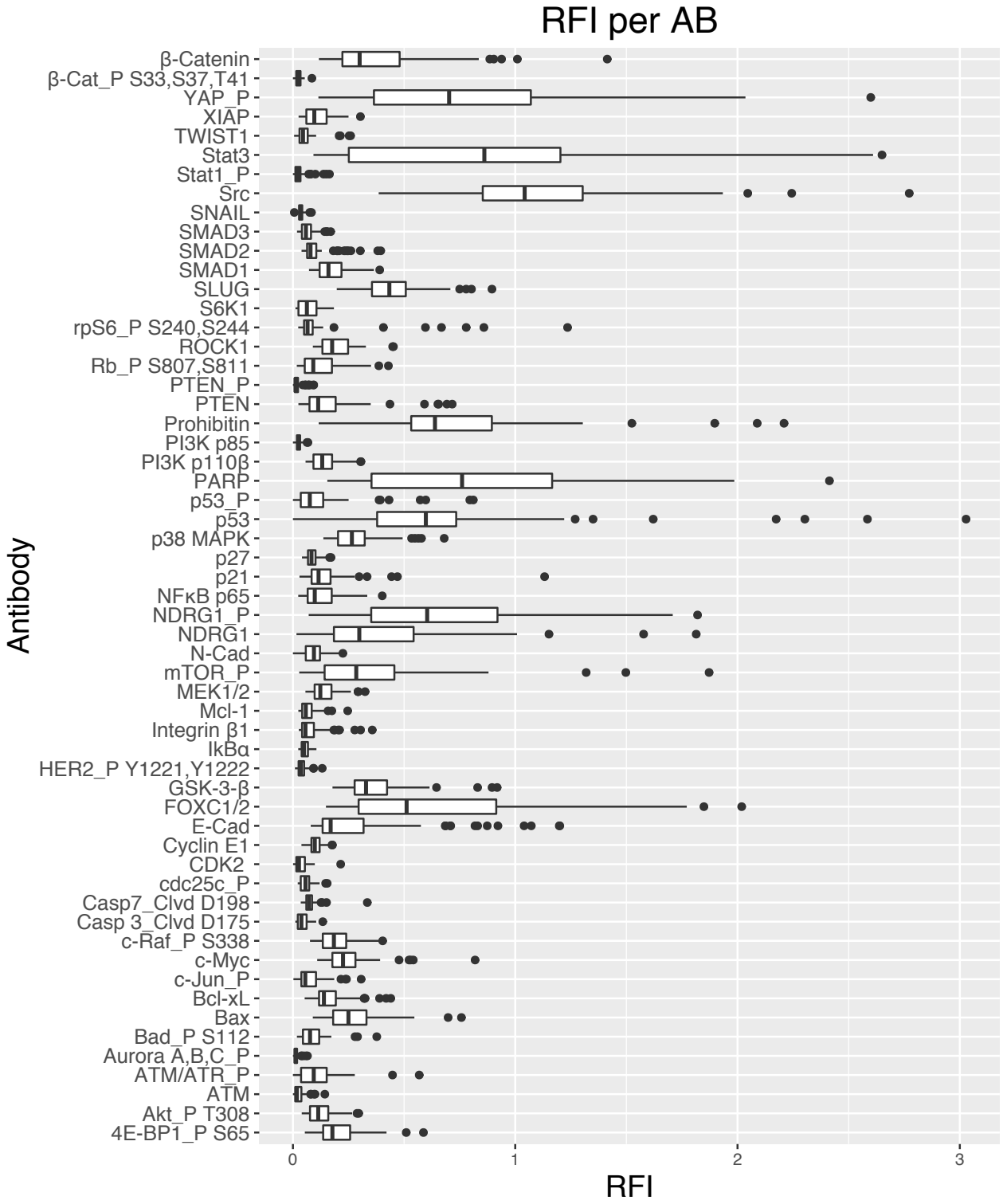
Each box is one antibody, and the spread of RFI values across all samples (including all technical replicates) is shown by the box and the 'whiskers'. The box represents the interquartile range, or the middle 50% of the data. The line in the box represents the median. The dots represent RFI (normalised to secondary) values that were more than 1.5 times the interquartile range from the ends of the box.

This plot shows you which proteins were expressed highly in all samples and which proteins were expressed lowly in all samples.

You can also see which proteins had a large range of expression values (i.e. were highly expressed in some samples but lowly expressed in others) by looking at how long the box and whiskers are. For example CD44 had quite a wide range of expression values while TWIST1 was expressed similarly in all samples.



Antibody CD44 was removed due to it's large RFI range and the boxplots re-made.



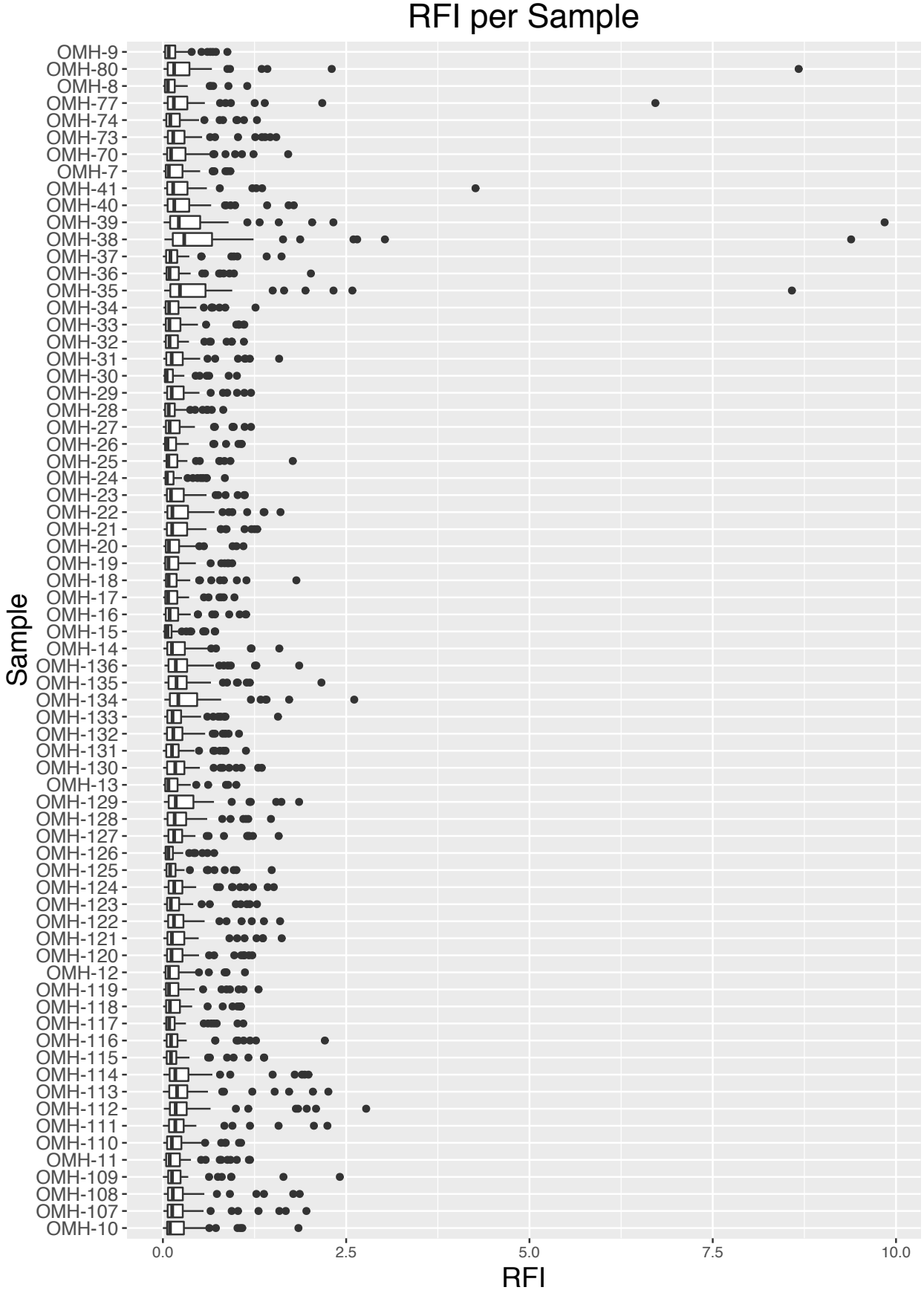
RFI per sample

These boxplots show the spread of RFI (normalised to secondary) values across all antibodies, for each sample.

Each box is one sample (both technical replicates for one sample are grouped together), and the spread of RFI values across all antibodies (for both technical replicates of that sample) is shown by the box and the 'whiskers'. The box represents the interquartile range, or the middle 50% of the data. The line in the box represents the median. The dots represent RFI (normalised to secondary) values that were more than 1.5 times the interquartile range from the ends of the box.

This plot shows you if some samples have high RFI (normalised to secondary) values for all antibodies or low RFI (normalised to secondary) values for all antibodies.

The spread of RFI (normalised to secondary) values for across antibodies appears to be quite similar for all samples but there does appear to be one or two very high RFI values for several samples.


RFI per Sample
Sample
OMH-9
OMH-80
OMH-8
OMH-77
OMH-74
OMH-73
OMH-70
OMH-7
OMH-41
OMH-40
OMH-39
OMH-38
OMH-37
OMH-36
OMH-35
OMH-34
OMH-33
OMH-32
OMH-31
OMH-30
OMH-29
OMH-28
OMH-27
OMH-26
OMH-25
OMH-24
OMH-23
OMH-22
OMH-21
OMH-20
OMH-19
OMH-18
OMH-17
OMH-16
OMH-15
OMH-14
OMH-136
OMH-135
OMH-134
OMH-133
OMH-132
OMH-131
OMH-130
OMH-13
OMH-129
OMH-128
OMH-127
OMH-126
OMH-125
OMH-124
OMH-123
OMH-122
OMH-121
OMH-120
OMH-12
OMH-119
OMH-118
OMH-117
OMH-116
OMH-115
OMH-114
OMH-113
OMH-112
OMH-111
OMH-110
OMH-11
OMH-109
OMH-108
OMH-107
OMH-10
0.0
2.5
5.0
7.5
10.0
RFI

RFI barplots

These plots can be found in the 'RFI_barplots' folder.

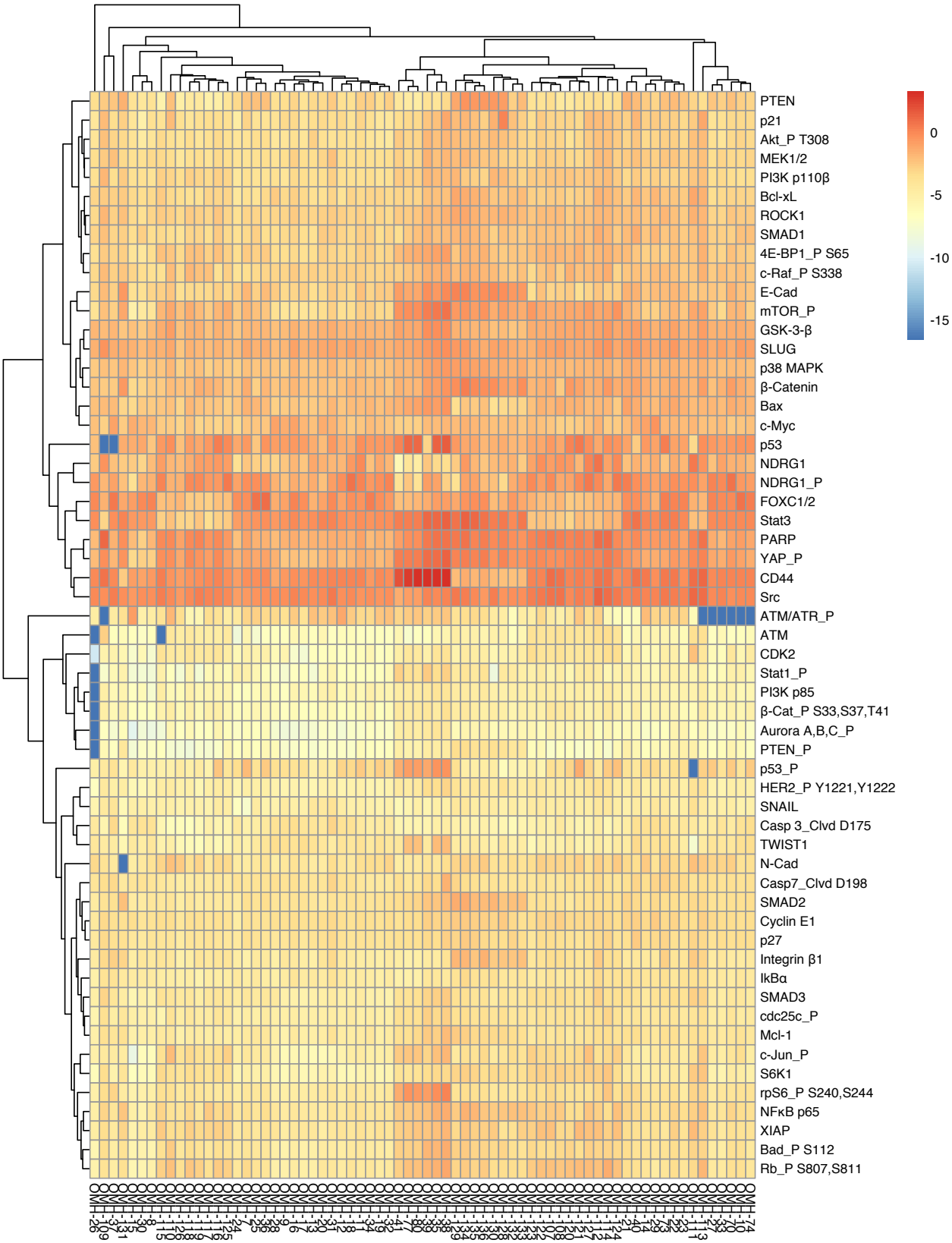
Bar plots were also made of:

- RFI value (normalised to secondary) per sample. Each plot is ONE antibody. The RFI (normalised to secondary) value is shown as a bar for all samples for that one antibody. This is repeated for all antibodies such that there is a separate bar plot for each antibody.
- RFI value (normalised to secondary) per antibody. Each plot is ONE sample. The RFI (normalised to secondary) value is shown as a bar for all antibodies for that one sample. This is repeated for all sample such that there is a separate bar plot for each sample.

Heatmap

Heatmap of logged RFI (normalised to secondary) values.

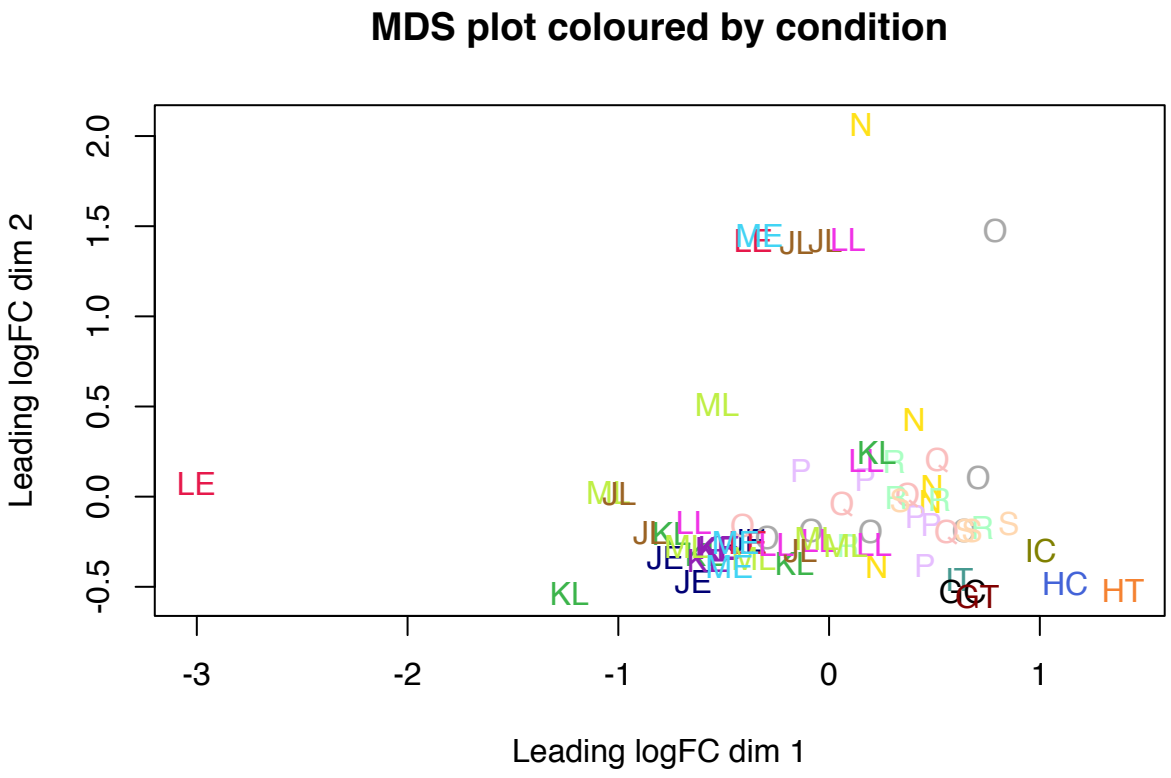
Both samples and antibodies are clustered using hierarchical clustering and this is shown on the row and column dendrograms.


PTEN
p21
Akt_P T308
MEK1/2
PI3K p110β
Bcl-xL
ROCK1
SMAD1
4E-BP1_P S65
c-Raf_P S338
E-Cad
mTOR_P
GSK-3-β
SLUG
p38 MAPK
β-Catenin
Bax
c-Myc
p53
NDRG1
NDRG1_P
FOXC1/2
Stat3
PARP
YAP_P
CD44
Src
ATM/ATR_P
ATM
CDK2
Stat1_P
PI3K p85
β-Cat_P S33,S37,T41
Aurora A,B,C_P
PTEN_P
p53_P
HER2_P Y1221,Y1222
SNAIL
Casp 3_Clvd D175
TWIST1
N-Cad
Casp7_Clvd D198
SMAD2
Cyclin E1
p27
Integrin β1
IkBα
SMAD3
cdc25c_P
Mcl-1
c-Jun_P
S6K1
rpS6_P S240,S244
NFκB p65
XIAP
Bad_P S112
Rb_P S807,S811
0
-5
-10
-15

MDS plot by condition

A multidimensional scaling (MDS) plot graphically represents relationships between objects. The distance between two samples approximates their similarity or dissimilarity.

In the MDS plot below, each letter code represents the sample, as per the submitted sample details table. Each code corresponds to the condition group the sample was in. MDS plot below is also coloured by condition of the sample.



Replicate consistency

The mean, standard deviation and coefficient of variation was calculated for all the samples in each condition group. The file named 'rep_consistency.tsv' contains these 3 calculations for each antibody of each condition group. Note that only condiiton groups which had greater than 1 sample in the group were included.

Removal of samples and anitbodies

Due to poor quality, the sample OMH-26 and the antibody ATM/ATR_P were removed for all further analyses.

Clustering

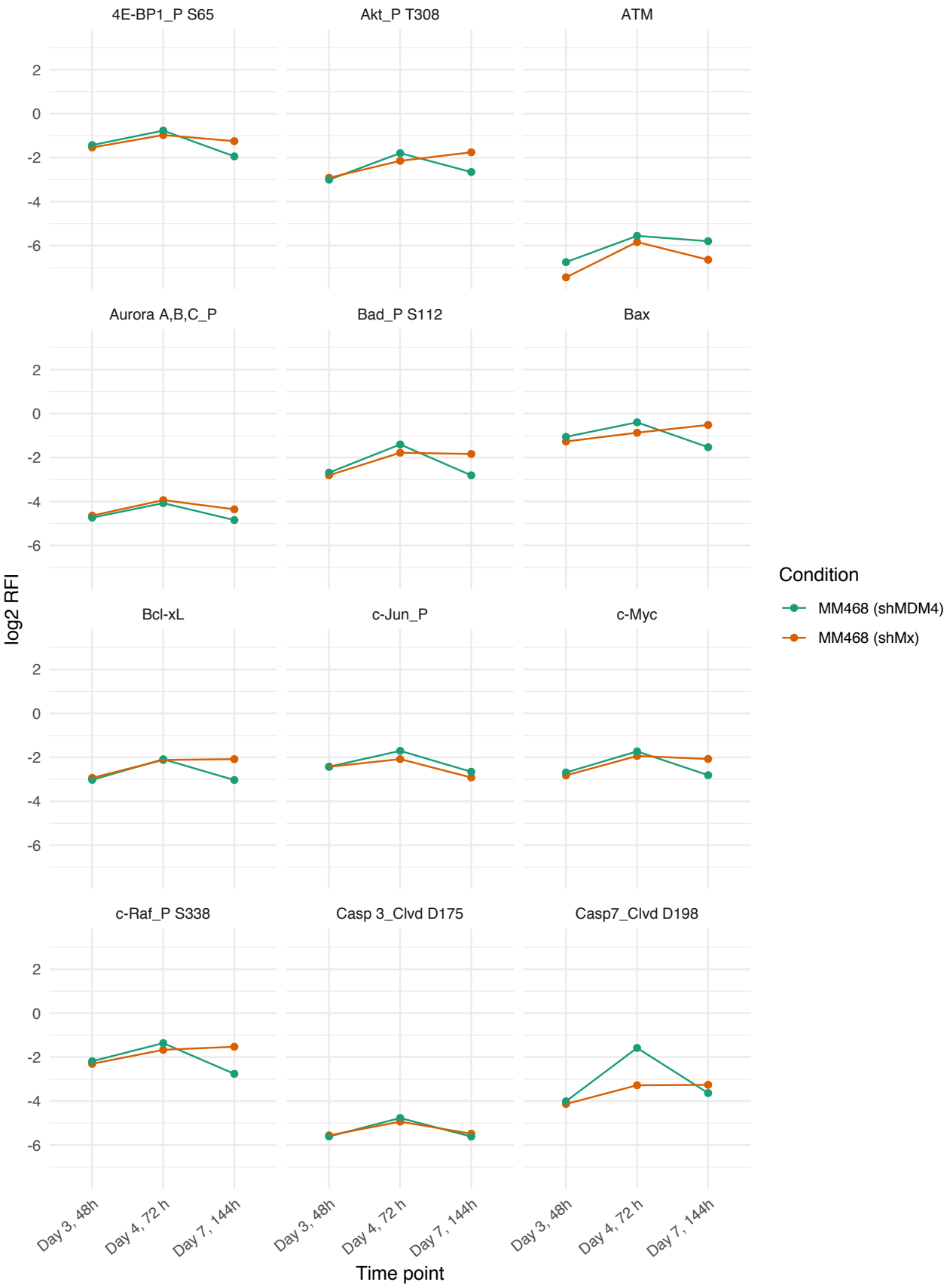
Hierarchical clustering was performed on z-score normalised RFI data to explore how well biological replicates correlated. This graph is saved in the file named 'replicate_clust.pdf'. Samples with the same condiitons are coloured the same.

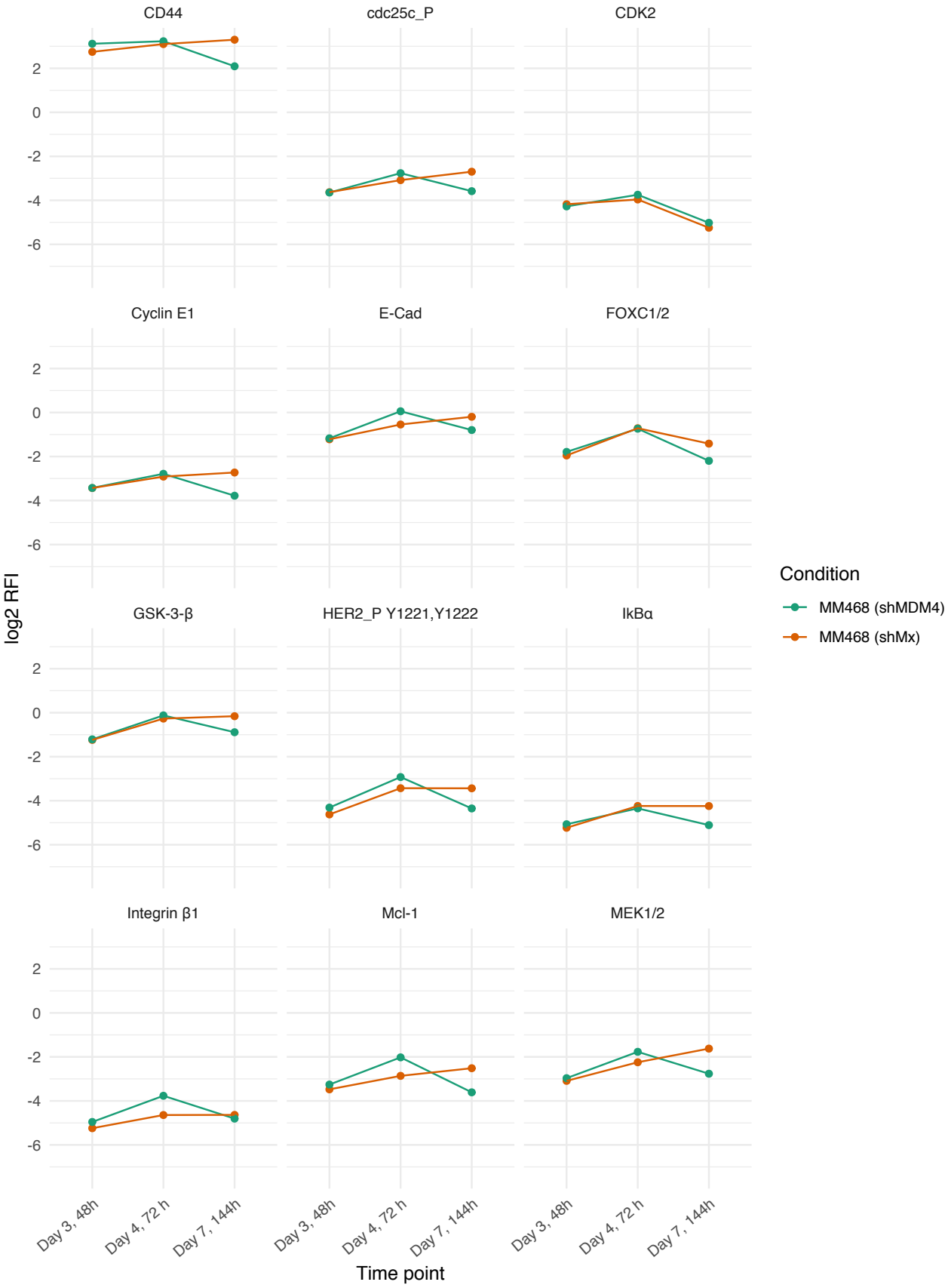
Heatmap by pathway

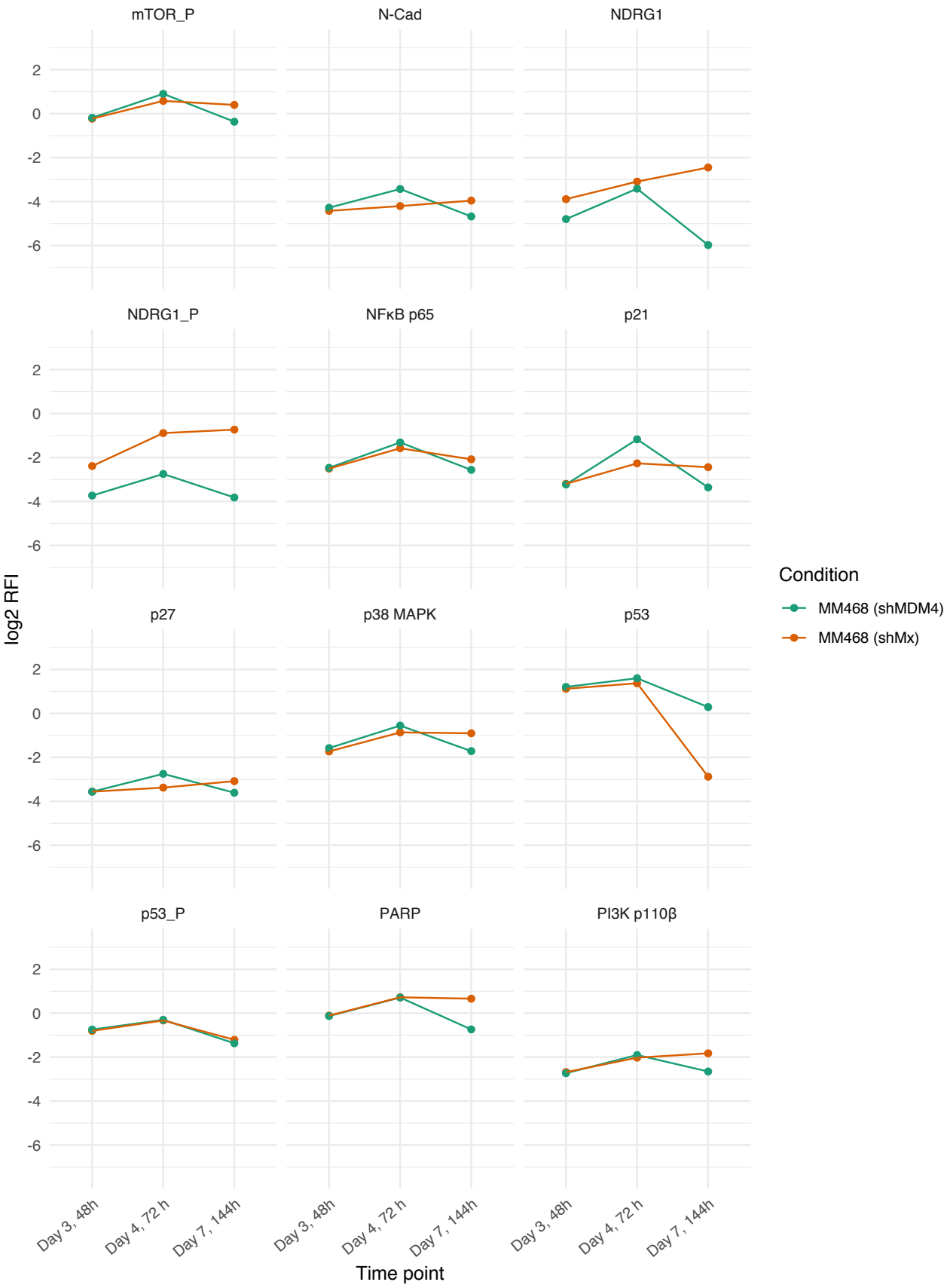
A heatmap for each pathway, which only includes the proteins involved in that pathway, is generated and can by found in the file "pathway_heatmaps.pdf". All samples are included in each heatmap but only proteins involved in that pathway are included in each heatmap.

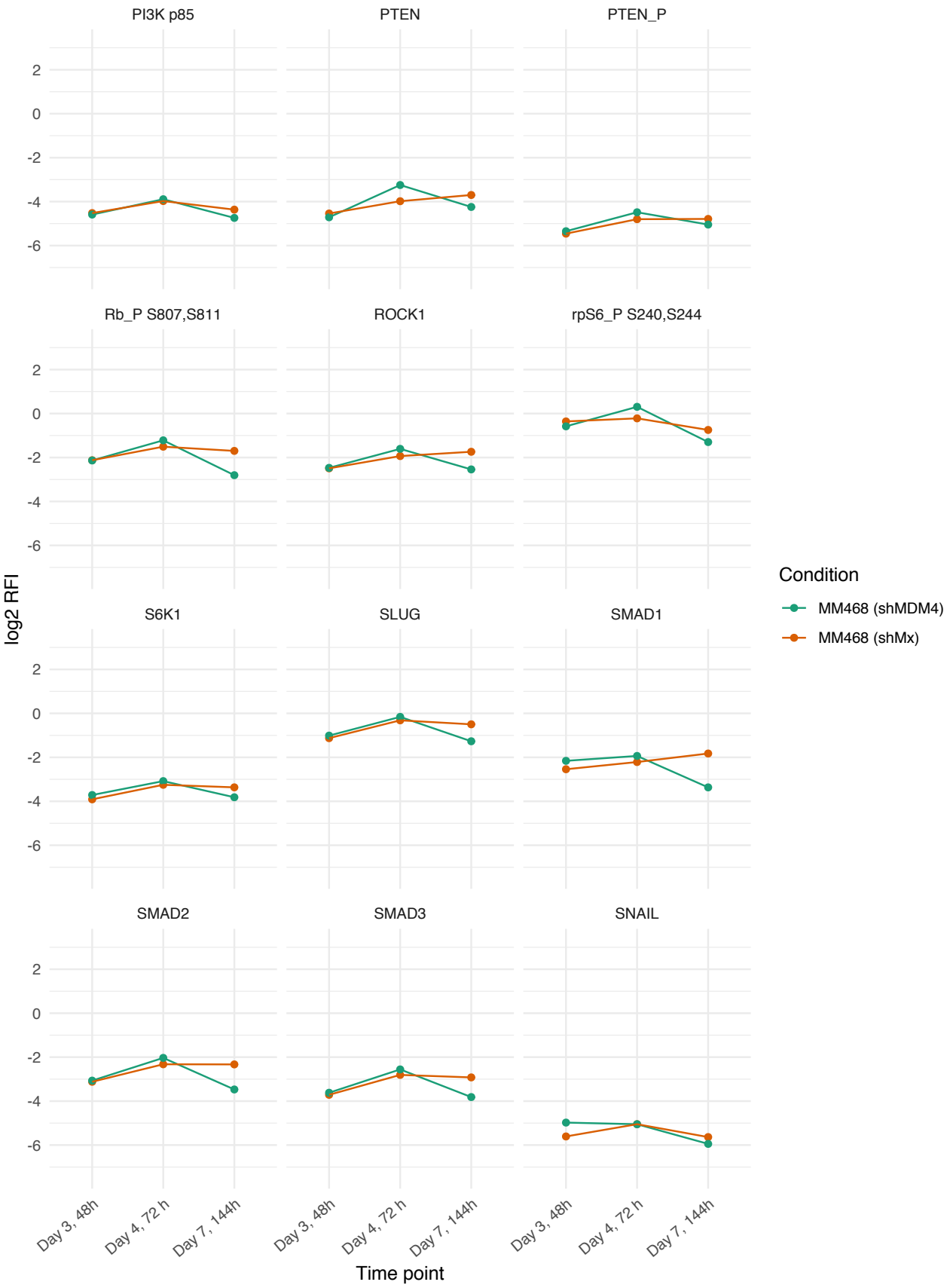
Breast Cancer Cell Line

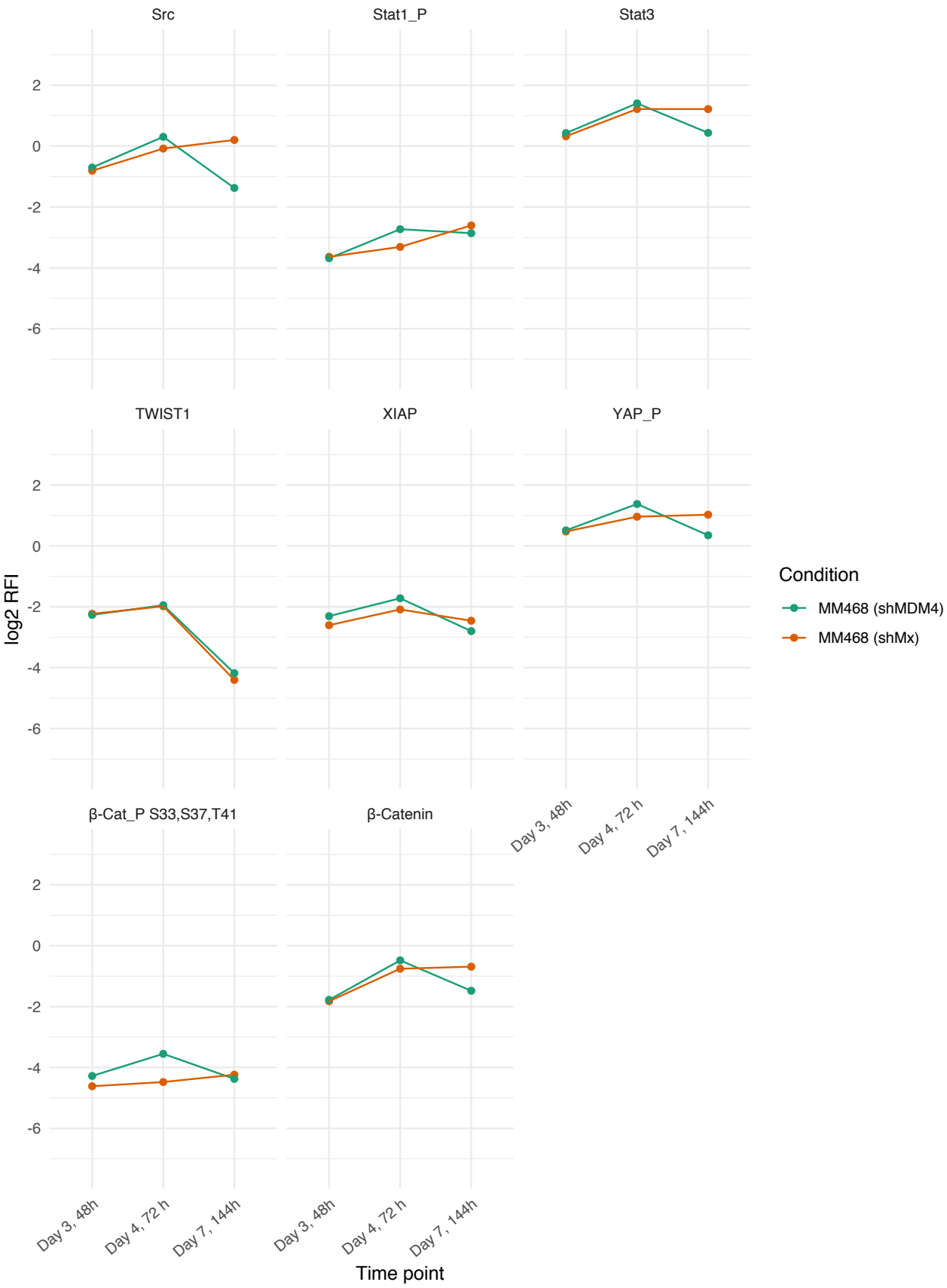
The log2 RFI value across the three time points (Day 3, 48h; Day 4, 72h; Day 7, 144h) for the two conditions shMDM4 vs shMx are plotted for each antibody. The RFI values were logged to imrpove visualisation of the lower RFI values (especially since the RFI values of CD44 were so high).


4E-BP1_P S65
Akt_P T308
ATM
Aurora A,B,C_P
Bad_P S112
Bax
Bcl-xL
c-Jun_P
c-Myc
c-Raf_P S338
Casp 3_Clvd D175
Casp7_Clvd D198
log2 RFI
2
0
-2
-4
-6
Day 3, 48h
Day 4, 72 h
Day 7, 144h
Time point
Condition
MM468 (shMDM4)
MM468 (shMx)


CD44
cdc25c_P
CDK2
Cyclin E1
E-Cad
FOXC1/2
GSK-3-β
HER2_P Y1221,Y1222
IkBα
Integrin β1
Mcl-1
MEK1/2
log2 RFI
2
0
-2
-4
-6
Day 3, 48h
Day 4, 72 h
Day 7, 144h
Time point
Condition
MM468 (shMDM4)
MM468 (shMx)


mTOR_P
N-Cad
NDRG1
NDRG1_P
NFκB p65
p21
p27
p38 MAPK
p53
p53_P
PARP
PI3K p110β
log2 RFI
2
0
-2
-4
-6
Day 3, 48h
Day 4, 72 h
Day 7, 144h
Time point
Condition
MM468 (shMDM4)
MM468 (shMx)


PI3K p85
PTEN
PTEN_P
Rb_P S807,S811
ROCK1
rpS6_P S240,S244
S6K1
SLUG
SMAD1
SMAD2
SMAD3
SNAIL
log2 RFI
2
0
-2
-4
-6
Day 3, 48h
Day 4, 72 h
Day 7, 144h
Time point
Condition
MM468 (shMDM4)
MM468 (shMx)


Src
Stat1_P
Stat3
TWIST1
XIAP
YAP_P
β-Cat_P S33,S37,T41
β-Catenin
log2 RFI
2
0
-2
-4
-6
Day 3, 48h
Day 4, 72 h
Day 7, 144h
Time point
Condition
MM468 (shMDM4)
MM468 (shMx)

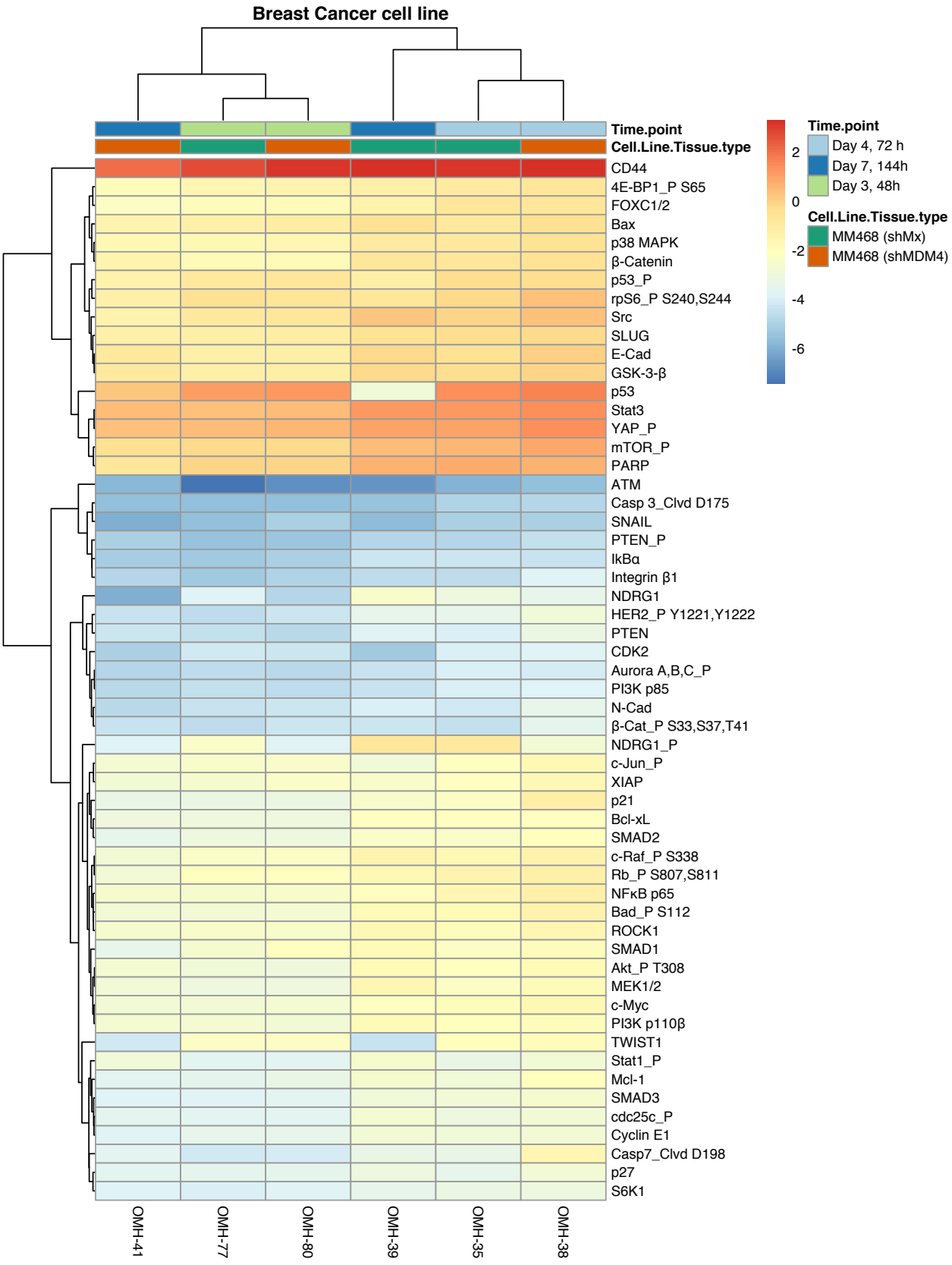
The difference between the RFI values between shMx and shMDM4 conditions, for each timepoint and antibody was calculated. e.g. for antibody p53, the normalised to secondary RFI value of the shMDM4 sample is divided by that of the shMx sample. A result of 0.5 means that the RFI value of shMDM4 is half that of the RFI value of shMx.

The timepoints and antibodies where the fold change was greater than 2 (i.e. double or half the RFI value in the shMDM4 condition), are listed below.

```
##     TimePoint Comparison   Antibody.Name Fold_Change
## 81      Day 7   IT vs IC           NDRG1  0.08661021
## 84      Day 7   IT vs IC         NDRG1_P  0.11764081
## 83      Day 4   HT vs HC         NDRG1_P  0.27531134
## 147     Day 7   IT vs IC             Src  0.33489518
## 135     Day 7   IT vs IC           SMAD1  0.34378555
## 105     Day 7   IT vs IC            PARP  0.37948306
## 82      Day 3   GT vs GC         NDRG1_P  0.39416491
## 30      Day 7   IT vs IC    c-Raf_P S338  0.42575131
## 39      Day 7   IT vs IC            CD44  0.43316508
## 72      Day 7   IT vs IC          MEK1/2  0.45328842
## 138     Day 7   IT vs IC           SMAD2  0.45368622
## 120     Day 7   IT vs IC  Rb_P S807,S811  0.46477122
## 69      Day 7   IT vs IC           Mcl-1  0.46809479
## 48      Day 7   IT vs IC       Cyclin E1  0.48135111
## 18      Day 7   IT vs IC             Bax  0.49521907
## 89      Day 4   HT vs HC             p21  2.13383139
## 35      Day 4   HT vs HC Casp7_Clvd D198  3.24554799
## 99      Day 7   IT vs IC             p53  8.98869864
```

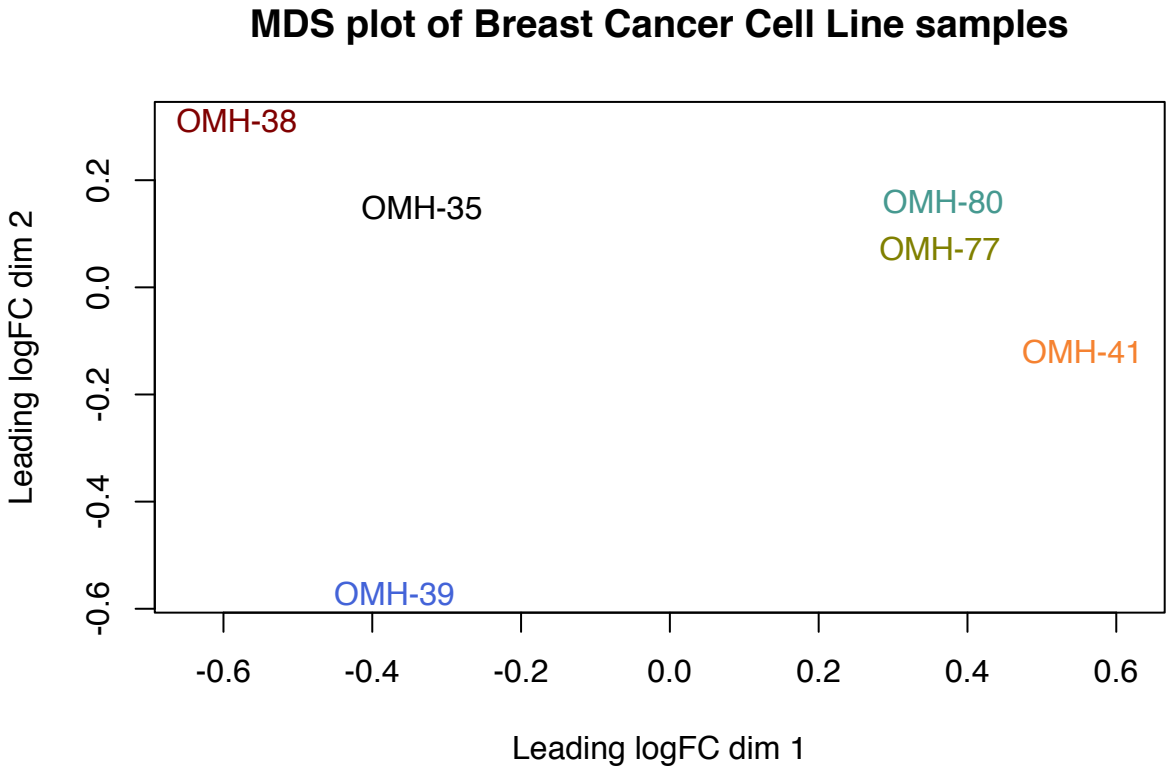
A heatmap of logged RFI (normalised to secondary) values for only the Breast Cancer Cell Line samples.

Both samples and antibodies are clustered using hierarchical clustering and this is shown on the row and column dendrograms.


Breast Cancer cell line
Time.point
Cell.Line.Tissue.type
CD44
4E-BP1_P S65
FOXC1/2
Bax
p38 MAPK
β-Catenin
p53_P
rpS6_P S240,S244
Src
SLUG
E-Cad
GSK-3-β
p53
Stat3
YAP_P
mTOR_P
PARP
ATM
Casp 3_Clvd D175
SNAIL
PTEN_P
IkBα
Integrin β1
NDRG1
HER2_P Y1221,Y1222
PTEN
CDK2
Aurora A,B,C_P
PI3K p85
N-Cad
β-Cat_P S33,S37,T41
NDRG1_P
c-Jun_P
XIAP
p21
Bcl-xL
SMAD2
c-Raf_P S338
Rb_P S807,S811
NFκB p65
Bad_P S112
ROCK1
SMAD1
Akt_P T308
MEK1/2
c-Myc
PI3K p110β
TWIST1
Stat1_P
Mcl-1
SMAD3
cdc25c_P
Cyclin E1
Casp7_Clvd D198
p27
S6K1
OMH-41
OMH-77
OMH-80
OMH-39
OMH-35
OMH-38
2
0
-2
-4
-6
Time.point
Day 4, 72 h
Day 7, 144h
Day 3, 48h
Cell.Line.Tissue.type
MM468 (shMx)
MM468 (shMDM4)

MDS plot

MDS plot of the six Breast Cancer Cell Line samples. Distance between samples is indicative of the degree of similarity or difference between samples.

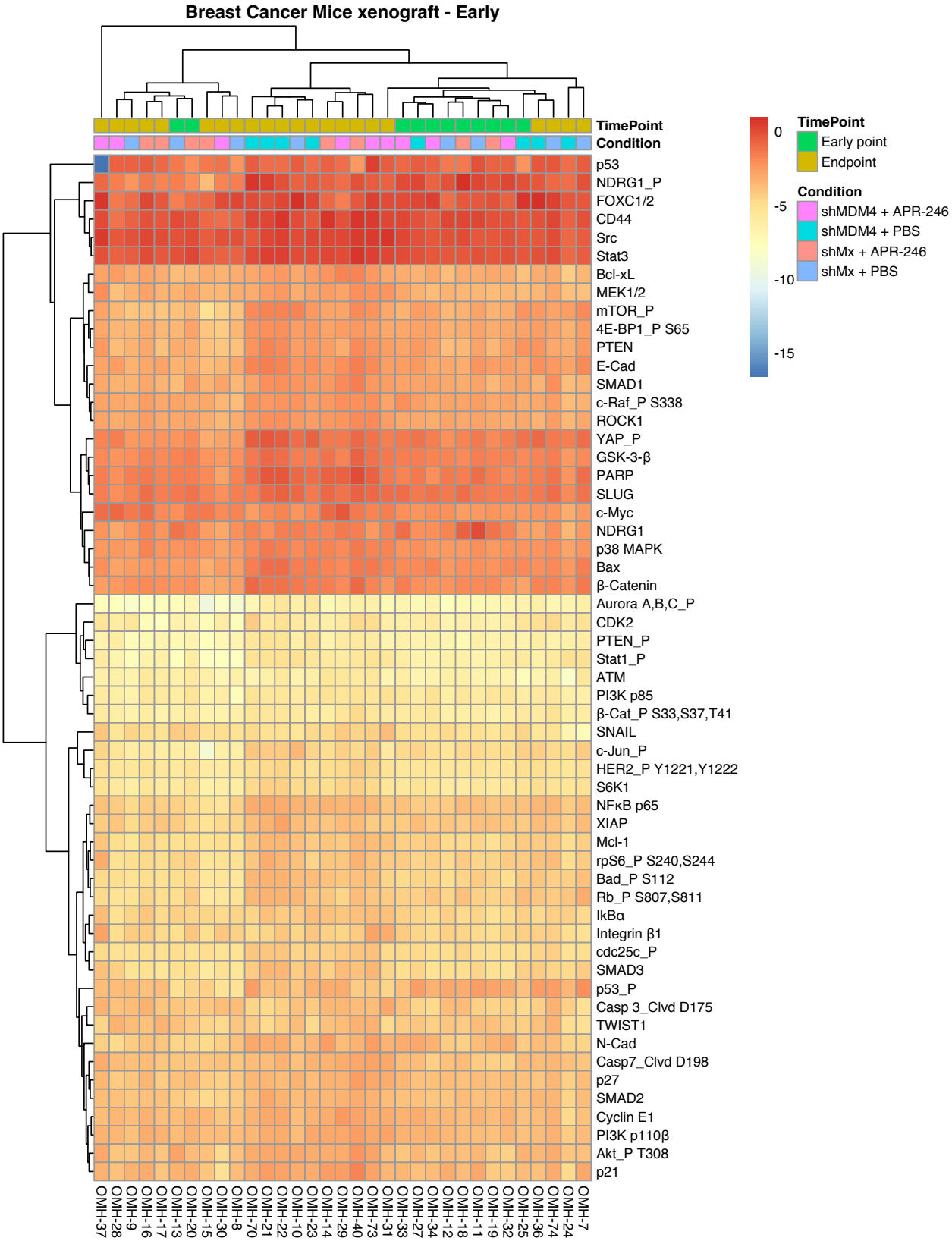


Breast Cancer Mice xenograft

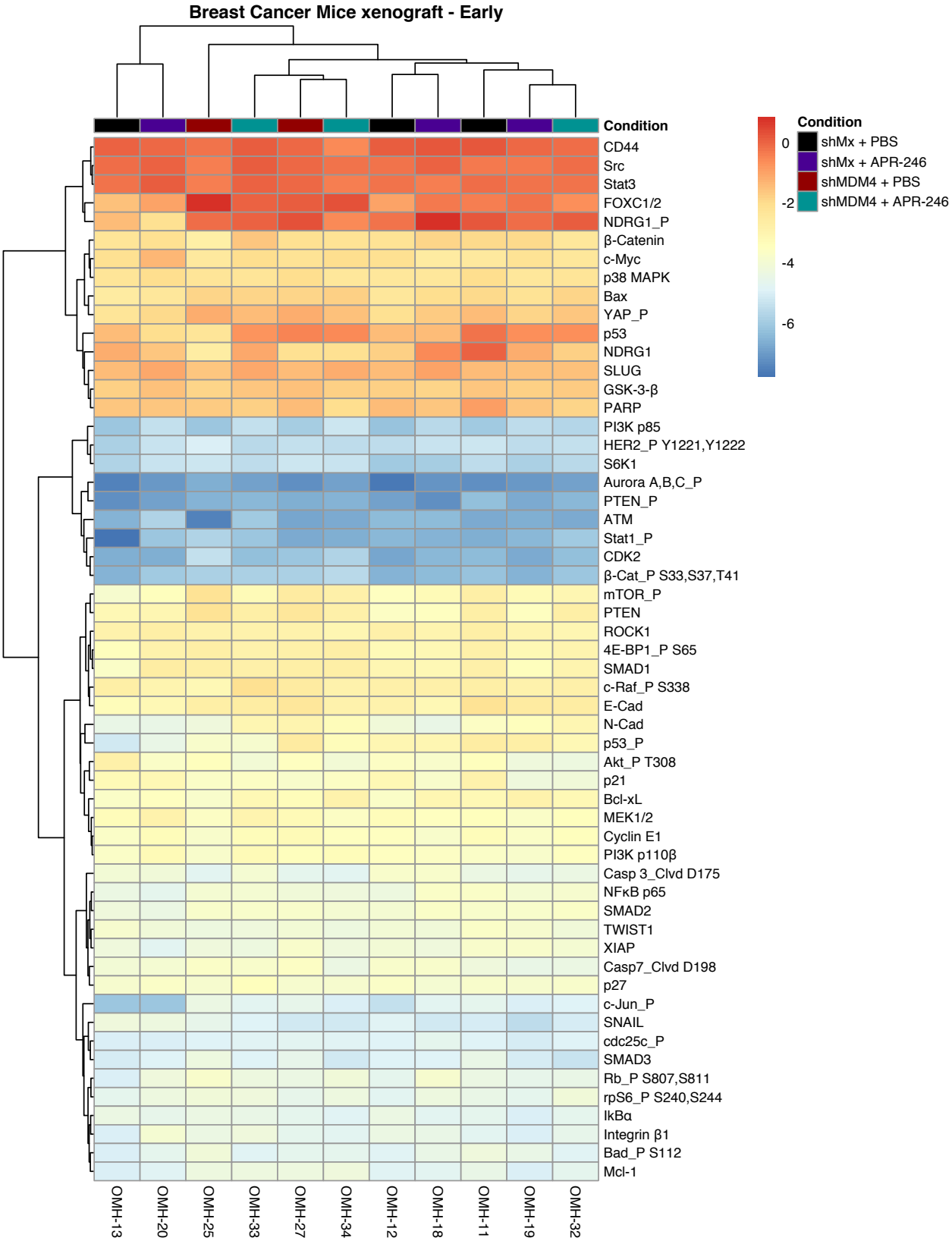
There are two time points (Early and End) and four conditions in these samples:

1. shMx + PBS
2. shMx + APR-246
3. shMDM4 + PBS
4. shMDM4 + APR-246

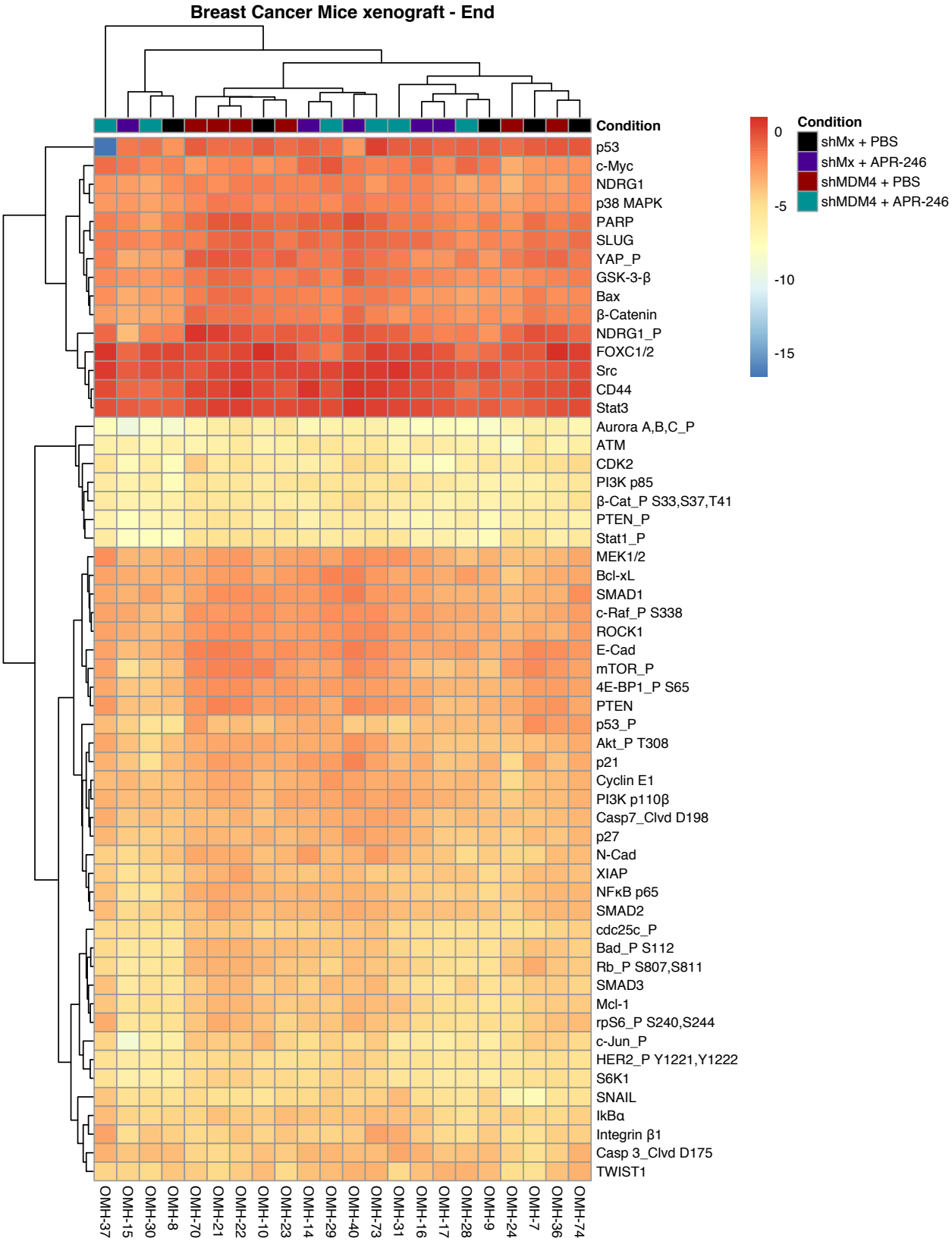
Heatmap with all samples. Both samples and antibodies are clustered using hierarchical clustering and this is shown on the row and column dendrograms.



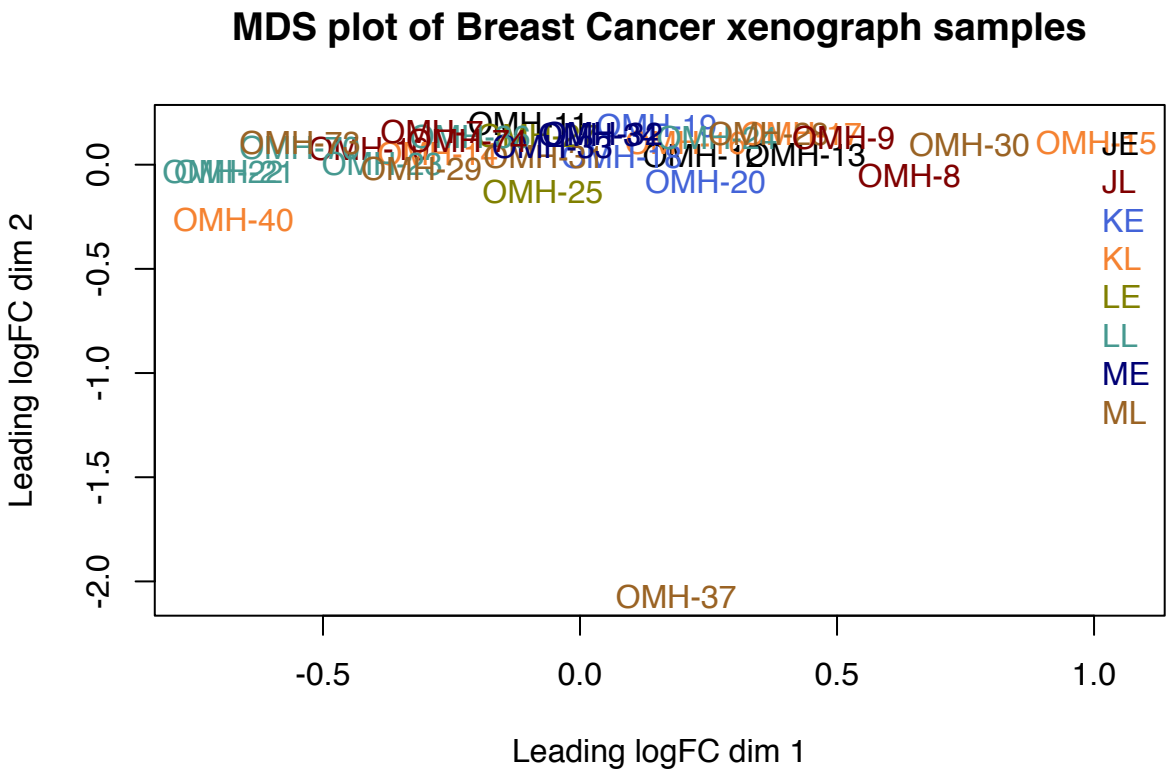
Heatmap with only early time point samples. Both samples and antibodies are clustered using hierarchical clustering and this is shown on the row and column dendrograms.



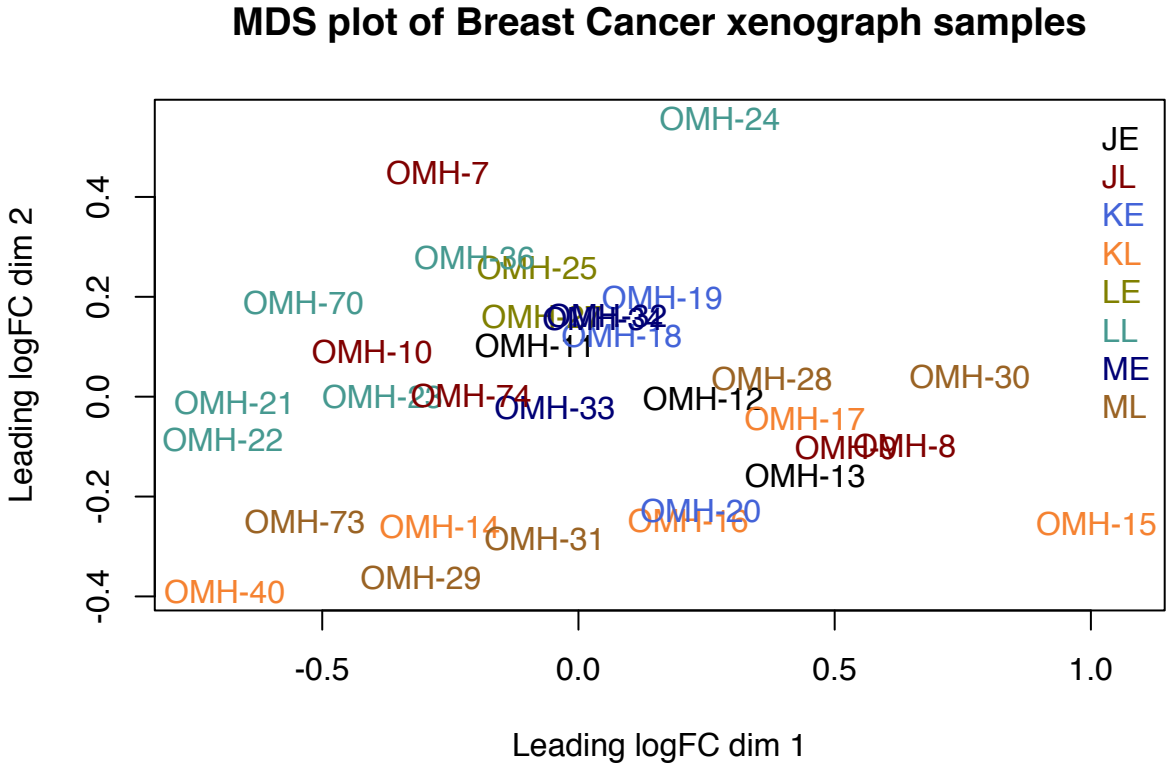
Heatmap with only end time point samples. Both samples and antibodies are clustered using hierarchical clustering and this is shown on the row and column dendrograms.


Breast Cancer Mice xenograft - End
Condition
p53
c-Myc
NDRG1
p38 MAPK
PARP
SLUG
YAP_P
GSK-3-β
Bax
β-Catenin
NDRG1_P
FOXC1/2
Src
CD44
Stat3
Aurora A,B,C_P
ATM
CDK2
PI3K p85
β-Cat_P S33,S37,T41
PTEN_P
Stat1_P
MEK1/2
Bcl-xL
SMAD1
c-Raf_P S338
ROCK1
E-Cad
mTOR_P
4E-BP1_P S65
PTEN
p53_P
Akt_P T308
p21
Cyclin E1
PI3K p110β
Casp7_Clvd D198
p27
N-Cad
XIAP
NFκB p65
SMAD2
cdc25c_P
Bad_P S112
Rb_P S807,S811
SMAD3
Mcl-1
rpS6_P S240,S244
c-Jun_P
HER2_P Y1221,Y1222
S6K1
SNAIL
IkBα
Integrin β1
Casp 3_Clvd D175
TWIST1
OMH-37
OMH-15
OMH-30
OMH-8
OMH-70
OMH-21
OMH-22
OMH-10
OMH-23
OMH-14
OMH-29
OMH-40
OMH-73
OMH-31
OMH-16
OMH-17
OMH-28
OMH-9
OMH-24
OMH-7
OMH-36
OMH-74
0
-5
-10
-15
Condition
shMx + PBS
shMx + APR-246
shMDM4 + PBS
shMDM4 + APR-246

MDS plot



Sample OMH-37 appears distinct from the other samples, most likely due to a very low RFI value in p53. Thus, the MDS is re-created without sample OMH-37.



Differential protein expression

Early point

To investigate differential protein expression, first an ANOVA test to look for differences between the four condition groups, was performed on all 58 antibodies for ONLY the early point samples.

The ten antibodies with the smallest p values are shown below. Both the raw p value and the Benjamini and Hochberg adjusted p values are shown.

```
##                          p.value adj.p.value
## PI3K p85            0.0008982254  0.05030062
## PTEN                0.0119252654  0.29823105
## Akt_P T308          0.0261043888  0.29823105
## Mcl-1               0.0283262109  0.29823105
## CDK2                0.0348409533  0.29823105
##  -Cat_P S33,S37,T41 0.0406274016  0.29823105
## Bax                 0.0423096077  0.29823105
## SMAD3               0.0493614253  0.29823105
## Aurora A,B,C_P      0.0503672111  0.29823105
## PARP                0.0575033645  0.29823105
```

After adjusting for multiple testing, there were no significant antibodies.

Late point

An ANOVA test to look for differences between the four condition groups, was performed on all 58 antibodies for ONLY the end point samples.

```
##                       p.value adj.p.value
## Casp 3_Clvd D175 0.002865525   0.1453179
## c-Myc            0.005189926   0.1453179
## PTEN_P           0.016138837   0.3012583
## YAP_P            0.041373359   0.3822891
## SMAD3            0.058861649   0.3822891
## Aurora A,B,C_P   0.069610274   0.3822891
## NDRG1_P          0.072465315   0.3822891
## SNAIL            0.074254512   0.3822891
## PI3K p85         0.078228137   0.3822891
## Casp7_Clvd D198  0.078943444   0.3822891
```

After adjusting for multiple testing, there were no significant antibodies.

shMx vs shMDM4

In this comparison the shMx + PBS and shMx + APR-246 groups are compared with the shMDM4 + PBS and shMDM4 + APR-246 groups, using a t-test and assuming equal variances. This is done for early then end point.

Early point:

```
##                     AB mean in group shMDM4 mean in group shMx      pvalue
## 5                   Bax           -1.8395624         -2.2634214 0.002910548
## 22                Mcl.1           -4.3420809         -4.7987841 0.001822869
## 37                 PTEN           -2.5736706         -3.2688444 0.003625829
## 55  .Cat_P.S33.S37.T41           -5.8486735         -6.3570265 0.002332147
```

```
## 14               CDK2.          -5.9705346         -6.6018172 0.005096135
## 54               YAP_P          -1.3687854         -1.8882136 0.011839525
## 3       Aurora.A.B.C_P          -6.8938151         -7.2704537 0.029455712
## 17             FOXC1.2           0.1335993         -0.7318202 0.026602081
## 24              mTOR_P          -2.7700426         -3.3486553 0.036962808
## 25               N.Cad          -3.3320819         -4.0824291 0.034250563
##     adj.p.val
## 5  0.05076160
## 22 0.05076160
## 37 0.05076160
## 55 0.05076160
## 14 0.05707671
## 54 0.11050223
## 3  0.15922440
## 17 0.15922440
## 24 0.15922440
## 25 0.15922440
```

Endpoint:

```
##                 AB mean in group shMDM4 mean in group shMx      pvalue
## 46           SMAD3            -4.078937          -4.812577 0.007828208
## 38          PTEN_P            -6.060844          -6.724167 0.019009359
## 2              ATM            -6.529149          -6.133102 0.143323847
## 3   Aurora.A.B.C_P            -6.756971          -7.436495 0.096694962
## 4       Bad_P.S112            -4.166431          -4.584557 0.185588104
## 7          c.Jun_P            -4.746196          -5.478981 0.147457964
## 11 Casp7_Clvd.D198            -3.380036          -3.690954 0.052197732
## 14           CDK2.            -5.567965          -6.234935 0.113604741
## 21     Integrin. 1           -4.045595          -4.496540 0.096862421
## 22           Mcl.1            -4.133726          -4.476637 0.174424573
##    adj.p.val
## 46 0.4383797
## 38 0.5322620
## 2  0.5911671
## 3  0.5911671
## 4  0.5911671
## 7  0.5911671
## 11 0.5911671
## 14 0.5911671
## 21 0.5911671
## 22 0.5911671
```

PBS vs APR-246

In this comparison the shMx + PBS and shMDM4 + PBS groups are compared with the shMx + APR-246 and shMDM4 + APR-246 groups, using a t-test and assuming equal variances. This is done for early then end point.

```
##                 AB mean in group APR-246 mean in group PBS       pvalue
## 36        PI3K.p85             -5.461016         -6.066802 2.086557e-05
## 1       Akt_P.T308             -3.994414         -3.352556 3.225682e-03
## 6           Bcl.xL             -3.118358         -3.519359 2.538624e-02
## 34            PARP             -1.727868         -1.398157 4.025187e-02
## 43            SLUG             -1.221238         -1.461135 4.656796e-02
```

```
## 46           SMAD3             -5.033802         -4.658261 3.931523e-02
## 20            IkB              -4.690256         -4.473356 5.739384e-02
## 2              ATM             -6.357849         -6.767831 1.298305e-01
## 3   Aurora.A.B.C_P             -6.984996         -7.236365 1.804561e-01
## 11 Casp7_Clvd.D198             -4.102311         -3.873682 2.225864e-01
##      adj.p.val
## 36 0.001168472
## 1  0.090319107
## 6  0.434634328
## 34 0.434634328
## 43 0.434634328
## 46 0.434634328
## 20 0.459150682
## 2  0.775616204
## 3  0.775616204
## 11 0.775616204
```

End point:

```
##                   AB mean in group APR-246 mean in group PBS       pvalue
## 8             c.Myc            -1.2596151        -2.1448546 0.0004105101
## 10 Casp.3_Clvd.D175            -3.5517966        -4.1922333 0.0020822715
## 3    Aurora.A.B.C_P            -7.4315202        -6.7001711 0.0708248022
## 4        Bad_P.S112            -4.6077950        -4.1051809 0.1062637666
## 6            Bcl.xL            -2.6287142        -3.0459940 0.0768203153
## 7           c.Jun_P            -5.5251530        -4.6334070 0.0725987020
## 11  Casp7_Clvd.D198            -3.3879732        -3.6547516 0.0987153485
## 14            CDK2.            -6.2100781        -5.5321888 0.1058670021
## 17          FOXC1.2            -0.3622312         0.1078102 0.1017238213
## 24           mTOR_P            -3.2855195        -2.4492327 0.0398359214
##     adj.p.val
## 8  0.02298857
## 10 0.05830360
## 3  0.27048959
## 4  0.27048959
## 6  0.27048959
## 7  0.27048959
## 11 0.27048959
## 14 0.27048959
## 17 0.27048959
## 24 0.27048959
```

in vitro vs in vivo

To explore differences between the breast cancer cell line (in vitro) and mice xenograft (in vivo) samples a heatmap is generated.

The *in vitro* samples were subjected two conditions:

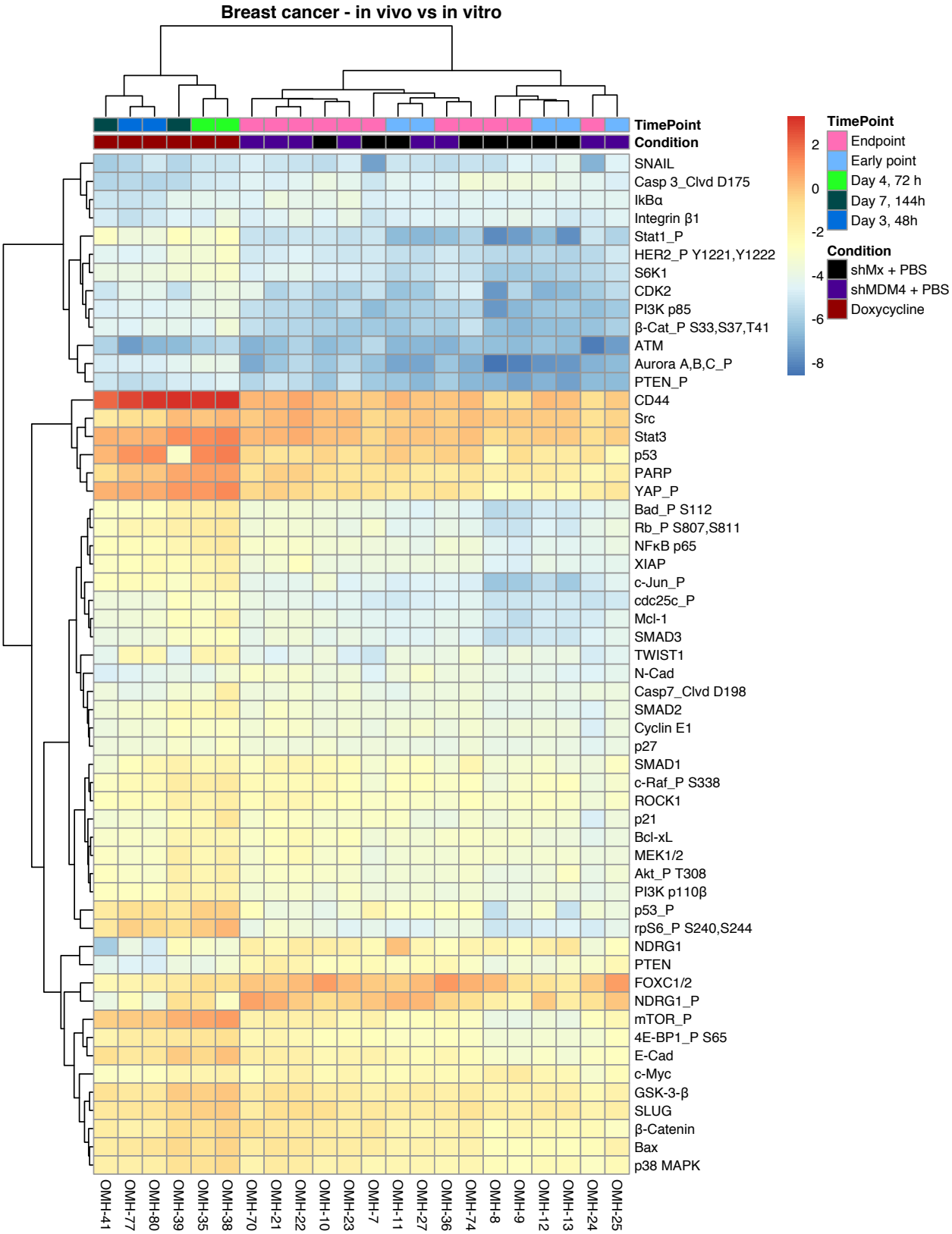
- shMx (control)
- shMDM4

The in vivo samples were subjected to four conditions, two of which correspond to the *in vivo* conditions:

- shMx + PBS
- shMDM4 + PBS

All the *in vitro* samples and the *in vivo* samples subjected to the two conditions (list above) were subset and a heatmap generated.

Both samples and antibodies are clustered using hierarchical clustering and this is shown on the row and column dendrograms.


Breast cancer - in vivo vs in vitro
TimePoint
Condition
SNAIL
Casp 3_Clvd D175
IkBα
Integrin β1
Stat1_P
HER2_P Y1221,Y1222
S6K1
CDK2
PI3K p85
β-Cat_P S33,S37,T41
ATM
Aurora A,B,C_P
PTEN_P
CD44
Src
Stat3
p53
PARP
YAP_P
Bad_P S112
Rb_P S807,S811
NFκB p65
XIAP
c-Jun_P
cdc25c_P
Mcl-1
SMAD3
TWIST1
N-Cad
Casp7_Clvd D198
SMAD2
Cyclin E1
p27
SMAD1
c-Raf_P S338
ROCK1
p21
Bcl-xL
MEK1/2
Akt_P T308
PI3K p110β
p53_P
rpS6_P S240,S244
NDRG1
PTEN
FOXC1/2
NDRG1_P
mTOR_P
4E-BP1_P S65
E-Cad
c-Myc
GSK-3-β
SLUG
β-Catenin
Bax
p38 MAPK
OMH-41
OMH-77
OMH-80
OMH-39
OMH-35
OMH-38
OMH-70
OMH-21
OMH-22
OMH-10
OMH-23
OMH-7
OMH-11
OMH-27
OMH-36
OMH-74
OMH-8
OMH-9
OMH-12
OMH-13
OMH-24
OMH-25
TimePoint
Endpoint
Early point
Day 4, 72 h
Day 7, 144h
Day 3, 48h
Condition
shMx + PBS
shMDM4 + PBS
Doxycycline
2
0
-2
-4
-6
-8