

Expression Analysis of Breast Cancer Cell-lines (E-GEOD-18494, GSE47533 and GSE41491)

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HIF1a, !VHL & !O2
 p53, !Mdm2
 Mdm2, p53 & !VHL
 VHL, HIF1a & !p53
 p300, ((p53 & HIF1a) & !VHL) | (!p53 & HIF1a) & VHL
 BIM, !MCL_1 & !BCLXL & !BCL2
 BAD, p53
 BID, (!HIF1a & (p53 & VHL)) | (!MCL_1 & !BCLXL & !BCL2)
 BIK, !MCL_1 & !BCLXL & !BCL2
 MCL_1, HIF1a
 BCLXL, HIF1a & !(p53 & VHL) & (!(Casp3 & !BAD) | (!Casp3 & BCL2))
 BCL2, HIF1a & !(p53 & VHL) & ((MCL_1 & !BIM & !BIK & !BAD) | (!BIM & !BIK & BCLXL & !BAD))
 IAPs, !DIABLO
 BAX, (BIM & !BCLXL) | (BIK & !BCLXL & !BCL2) | (BID & !BCLXL & !BCL2) | (BIM & BID) | (BIM & BIK) | (BIM & !BCL2) | (!MCL_1 & BIM)
 BAK, (!MCL_1 & BIM & !BCLXL) | (BID & !BCL2) | (BID & !BCLXL) | (!MCL_1 & BID) | (!MCL_1 & BIK & !BCLXL) | (BIM & BID) | (BIK & BID)
 DIABLO, BAX | BAK
 Cyto_C, BAX | BAK
 Casp9, Casp3 | (!IAPs & Cyto_C)
 Casp3, !IAPs & Casp9
 BCLXL ?
 No VHL in GSE41491

```
# Selected genes from HIF Axis
hif.symbols <- c("HIF1A", "TP53", "MDM2", "VHL", "EP300", "TMBIM1", "TMBIM4", "TMBIM6", "BAD", "BIK", "MCL1")

hif.probes <- anno.EGEOD18494$probes[anno.EGEOD18494$symbol %in% hif.symbols]

# Select the probes and genes
# EGEOD18494
expr.EGEOD18494.hif <- as.data.frame(expr.EGEOD18494) %>%
  rownames_to_column('probes') %>%
  filter(probes %in% hif.probes) %>%
  merge(anno.EGEOD18494[anno.EGEOD18494$symbol %in% hif.symbols, c("probes", "symbol")], by = "probes") %>%
  mutate(., symbol=ifelse(symbol %in% c("TMBIM1", "TMBIM4", "TMBIM6"), "BIM", symbol)) %>%
  mutate(., symbol=ifelse(symbol %in% c("BIRC2", "BIRC3", "BIRC5", "BIRC6", "BIRC7"), "IAPs", symbol)) %>%
  group_by(symbol) %>%
  summarise_at(vars(-probes), funs(mean(., na.rm=TRUE))) %>%
  column_to_rownames(var = "symbol") %>%
  dplyr::select(c(data.EGEOD18494$codes[data.EGEOD18494$cell_line == "MDA-MB231 breast cancer"])))
```

```
## Warning: `funs()` is deprecated as of dplyr 0.8.0.
## Please use a list of either functions or lambdas:
##
##   # Simple named list:
##   list(mean = mean, median = median)
##
##   # Auto named with `tibble::lst()`:
##   tibble::lst(mean, median)
##
##   # Using lambdas
##   list(~ mean(., trim = .2), ~ median(., na.rm = TRUE))
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_warnings()` to see where this warning was generated.
```

```
hif.probes <- anno.GSE47533$probes[anno.GSE47533$symbol %in% hif.symbols]
```

```
# GSE47533
```

```
expr.GSE47533.hif <- as.data.frame(expr.GSE47533) %>%
  rownames_to_column('probes') %>%
  filter(probes %in% hif.probes) %>%
  merge(anno.GSE47533[anno.GSE47533$symbol %in% hif.symbols, c("probes", "symbol")], by = "probes") %>%
  mutate(., symbol=ifelse(symbol %in% c("TMBIM1", "TMBIM4", "TMBIM6"), "BIM", symbol)) %>%
  mutate(., symbol=ifelse(symbol %in% c("BIRC2", "BIRC3", "BIRC5", "BIRC6", "BIRC7"), "IAPs", symbol)) %>%
  group_by(symbol) %>%
  summarise_at(vars(-probes), funs(mean(., na.rm=TRUE))) %>%
  column_to_rownames(var = "symbol")
```

```
hif.probes <- anno.GSE41491$probes[anno.GSE41491$symbol %in% hif.symbols]
```

```
# GSE41491
```

```
expr.GSE41491.hif <- as.data.frame(expr.GSE41491) %>%
  rownames_to_column('probes') %>%
  filter(probes %in% hif.probes) %>%
  merge(anno.GSE41491[anno.GSE41491$symbol %in% hif.symbols, c("probes", "symbol")], by = "probes") %>%
  mutate(., symbol=ifelse(symbol %in% c("TMBIM1", "TMBIM4", "TMBIM6"), "BIM", symbol)) %>%
  mutate(., symbol=ifelse(symbol %in% c("BIRC2", "BIRC3", "BIRC5", "BIRC6", "BIRC7"), "IAPs", symbol)) %>%
  group_by(symbol) %>%
  summarise_at(vars(-probes), funs(mean(., na.rm=TRUE))) %>%
  column_to_rownames(var = "symbol")
```

```
write.table(expr.GSE47533.hif, "expr.GSE47533.txt", sep = "\t")
write.table(expr.EGEOD18494.hif, "expr.EGEOD18494.txt", sep = "\t")
write.table(expr.GSE41491.hif, "expr.GSE41491.txt", sep = "\t")
```

```
expr.EGEOD18494.tdm <- tdm_transform(ref_file = "expr.GSE47533.txt", file = "expr.EGEOD18494.txt")
```

```
##
## Attaching package: 'data.table'
```

```
## The following objects are masked from 'package:dplyr':
##
##   between, first, last
```

```

## The following object is masked from 'package:purrr':
##
##      transpose

##
## Attaching package: 'scales'

## The following object is masked from 'package:purrr':
##
##      discard

## The following object is masked from 'package:readr':
##
##      col_factor

expr.GSE41491.tdm <- tdm_transform(ref_file = "expr.GSE47533.txt", file = "expr.GSE41491.txt")

symbols <- expr.EGEOD18494.tdm$gene
expr.EGEOD18494.tdm$gene <- NULL

expr.EGEOD18494.tdm <- as.data.frame(matrix(as.numeric(unlist(expr.EGEOD18494.tdm)),
                                           nrow = dim(expr.EGEOD18494.tdm)[1],
                                           ncol = dim(expr.EGEOD18494.tdm)[2]))
colnames(expr.EGEOD18494.tdm) <- colnames(expr.EGEOD18494.hif)

rownames(expr.EGEOD18494.tdm) <- symbols

row_medians_assayData <-
  Biobase::rowMedians(as.matrix(expr.GSE47533))

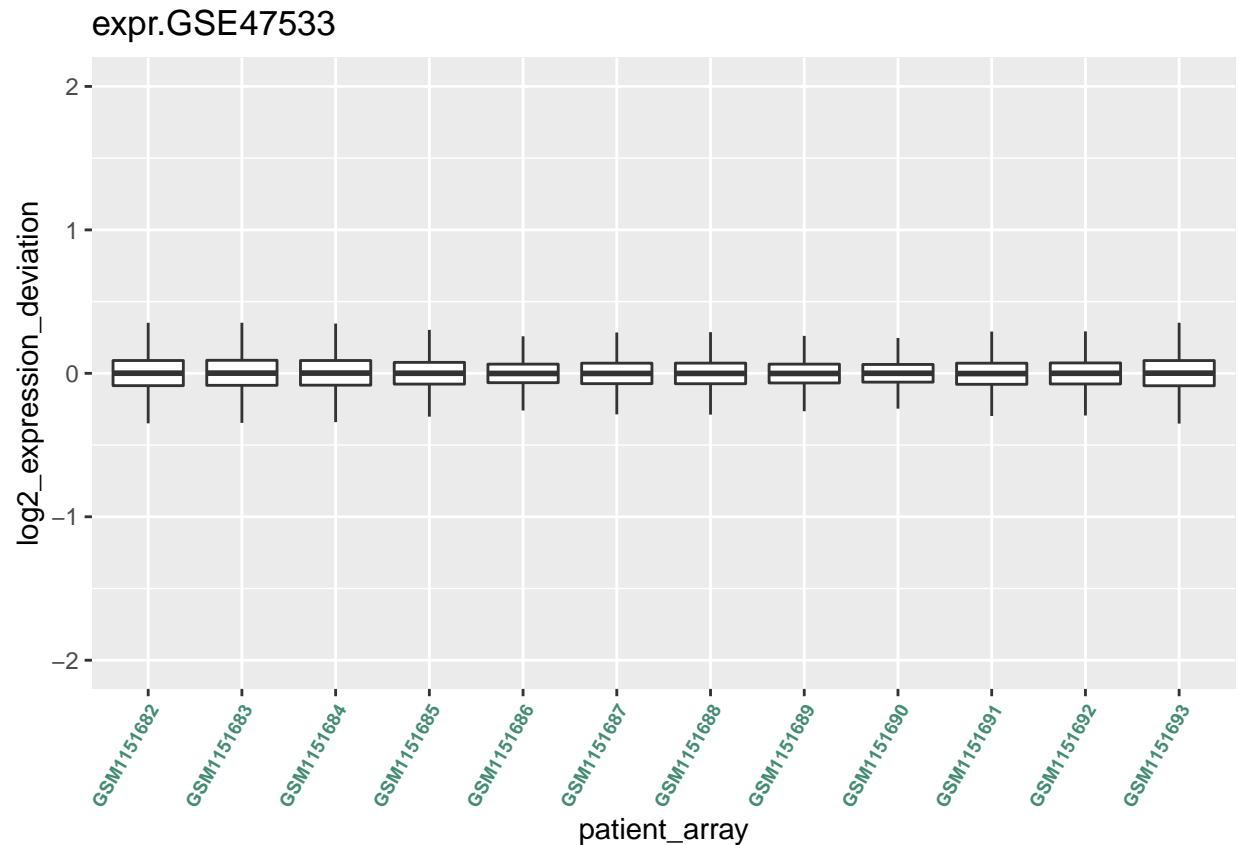
RLE_data <- sweep(expr.GSE47533, 1, row_medians_assayData)

RLE_data <- as.data.frame(RLE_data)
RLE_data_gathered <-
  tidyr::gather(RLE_data, patient_array, log2_expression_deviation)

ggplot2::ggplot(RLE_data_gathered, aes(patient_array,
                                       log2_expression_deviation)) +
  geom_boxplot(outlier.shape = NA) +
  ylim(c(-2, 2)) +
  ggtitle("expr.GSE47533") +
  theme(axis.text.x = element_text(colour = "aquamarine4",
                                   angle = 60, size = 6.5, hjust = 1 ,
                                   face = "bold"))

## Warning: Removed 142 rows containing non-finite values (stat_boxplot).

```

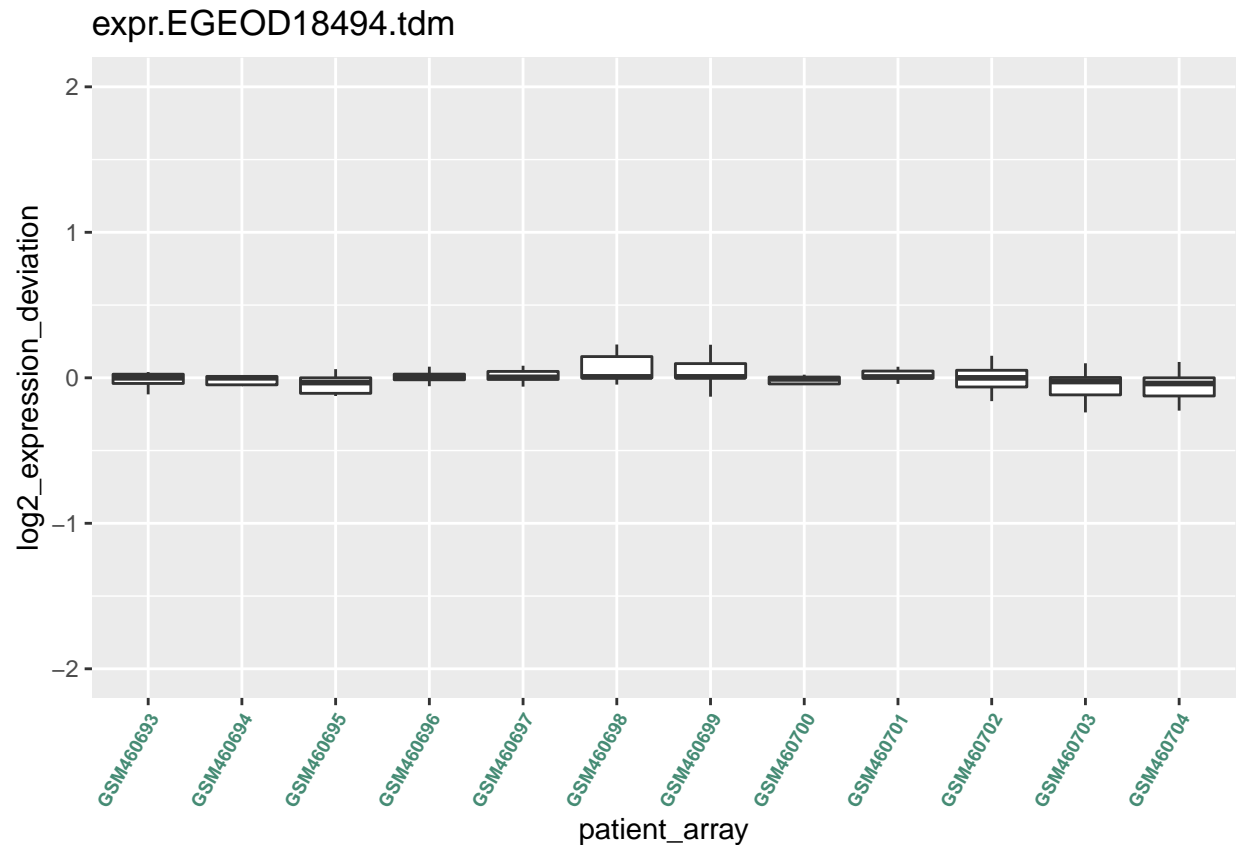


```
row_medians_assayData <-
  Biobase::rowMedians(as.matrix(expr.EGEO18494.tdm))

RLE_data <- sweep(expr.EGEO18494.tdm, 1, row_medians_assayData)

RLE_data <- as.data.frame(RLE_data)
RLE_data_gathered <-
  tidyr::gather(RLE_data, patient_array, log2_expression_deviation)

ggplot2::ggplot(RLE_data_gathered, aes(patient_array,
                                       log2_expression_deviation)) +
  geom_boxplot(outlier.shape = NA) +
  ylim(c(-2, 2)) +
  ggtitle("expr.EGEO18494.tdm") +
  theme(axis.text.x = element_text(colour = "aquamarine4",
                                    angle = 60, size = 6.5, hjust = 1 ,
                                    face = "bold"))
```

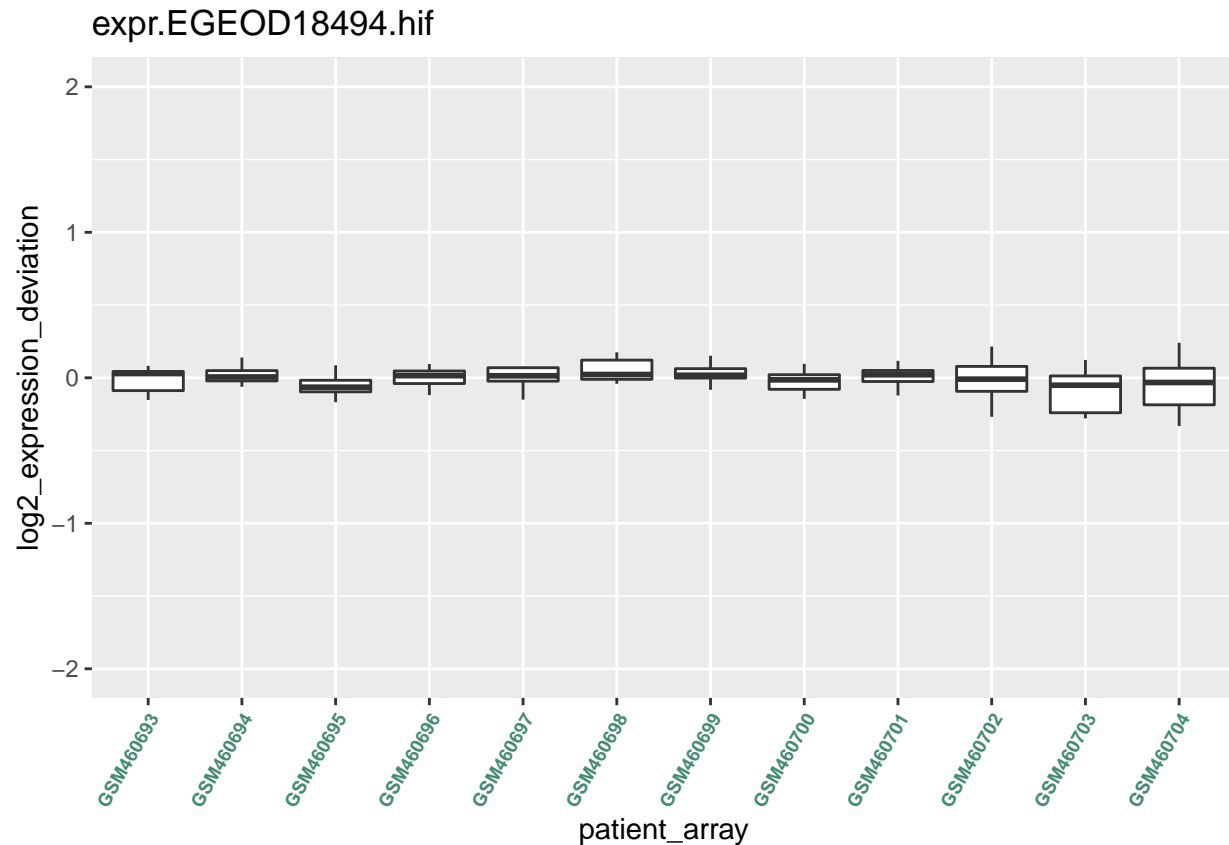


```
row_medians_assayData <-
  Biobase::rowMedians(as.matrix(expr.EGEOD18494.hif))

RLE_data <- sweep(expr.EGEOD18494.hif, 1, row_medians_assayData)

RLE_data <- as.data.frame(RLE_data)
RLE_data_gathered <-
  tidyr::gather(RLE_data, patient_array, log2_expression_deviation)

ggplot2::ggplot(RLE_data_gathered, aes(patient_array,
                                       log2_expression_deviation)) +
  geom_boxplot(outlier.shape = NA) +
  ylim(c(-2, 2)) +
  ggtitle("expr.EGEOD18494.hif") +
  theme(axis.text.x = element_text(colour = "aquamarine4",
                                    angle = 60, size = 6.5, hjust = 1 ,
                                    face = "bold"))
```



```
rm(RLE_data, RLE_data_gathered, row_medians_assayData)
```

```
hif.probes <- anno.GSE47533$probes[anno.GSE47533$symbol %in% hif.symbols]
```

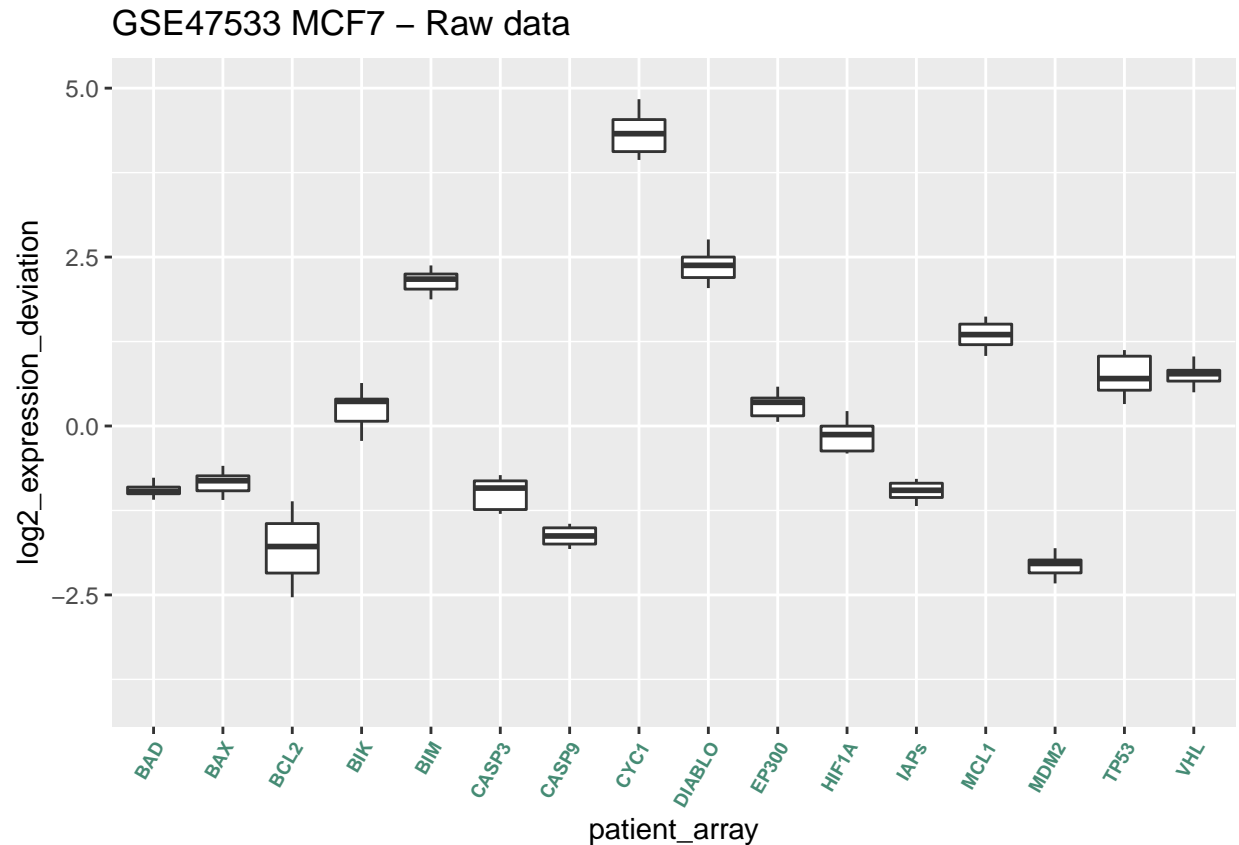
```
row_medians_assayData <-  
  Biobase::rowMedians(as.matrix(t(expr.GSE47533.hif)))
```

```
RLE_data <- sweep(t(expr.GSE47533.hif), 1, row_medians_assayData)
```

```
RLE_data <- as.data.frame(RLE_data)
```

```
RLE_data_gathered <-  
  tidyr::gather(RLE_data, patient_array, log2_expression_deviation)
```

```
ggplot2::ggplot(RLE_data_gathered, aes(patient_array,  
                                       log2_expression_deviation)) +  
  geom_boxplot(outlier.shape = NA) +  
  ylim(c(-4, 5)) +  
  ggtitle("GSE47533 MCF7 - Raw data") +  
  theme(axis.text.x = element_text(colour = "aquamarine4",  
                                    angle = 60, size = 6.5, hjust = 1 ,  
                                    face = "bold"))
```

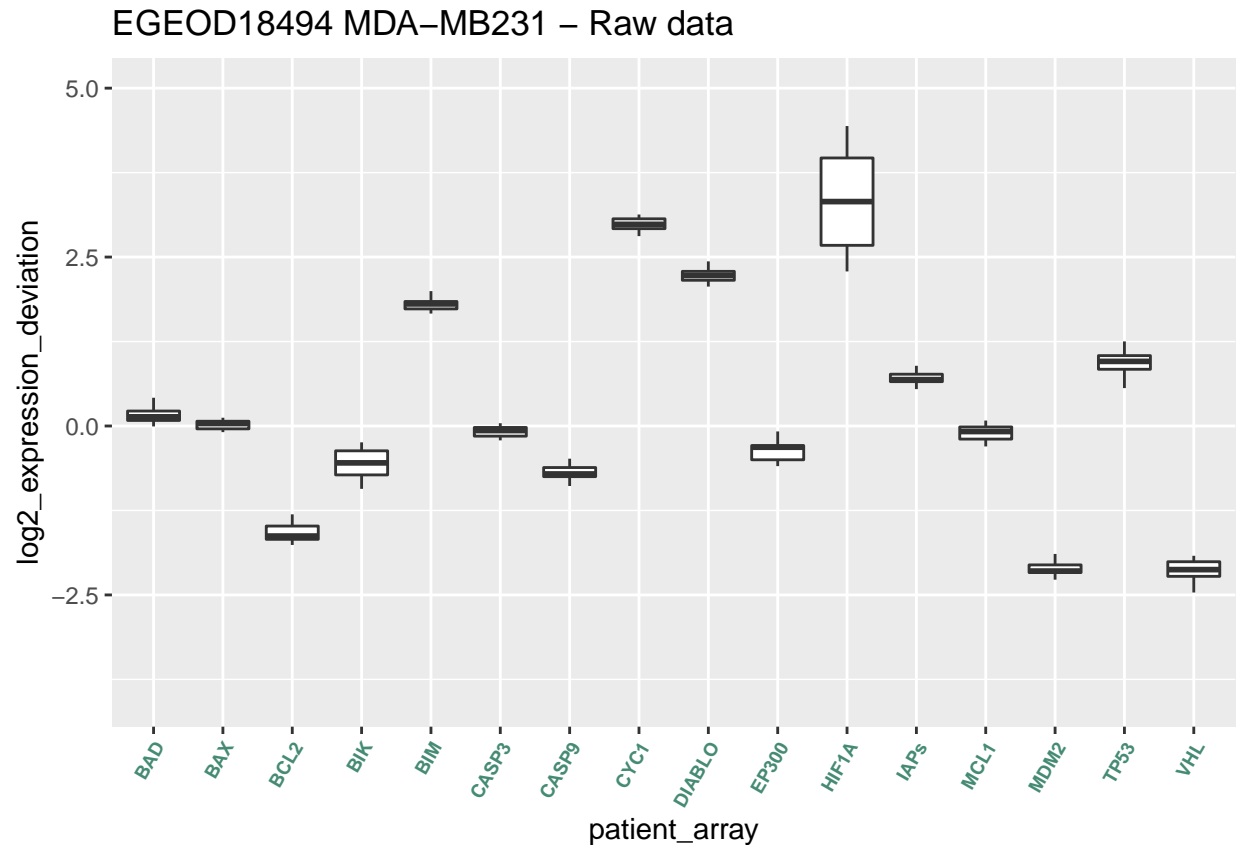


```
row_medians_assayData <-
  Biobase::rowMedians(as.matrix(t(expr.EGEOD18494.hif)))

RLE_data <- sweep(t(expr.EGEOD18494.hif), 1, row_medians_assayData)

RLE_data <- as.data.frame(RLE_data)
RLE_data_gathered <-
  tidyr::gather(RLE_data, patient_array, log2_expression_deviation)

ggplot2::ggplot(RLE_data_gathered, aes(patient_array,
                                       log2_expression_deviation)) +
  geom_boxplot(outlier.shape = NA) +
  ggtitle("EGEOD18494 MDA-MB231 - Raw data") +
  ylim(c(-4, 5)) +
  theme(axis.text.x = element_text(colour = "aquamarine4",
                                    angle = 60, size = 6.5, hjust = 1 ,
                                    face = "bold"))
```

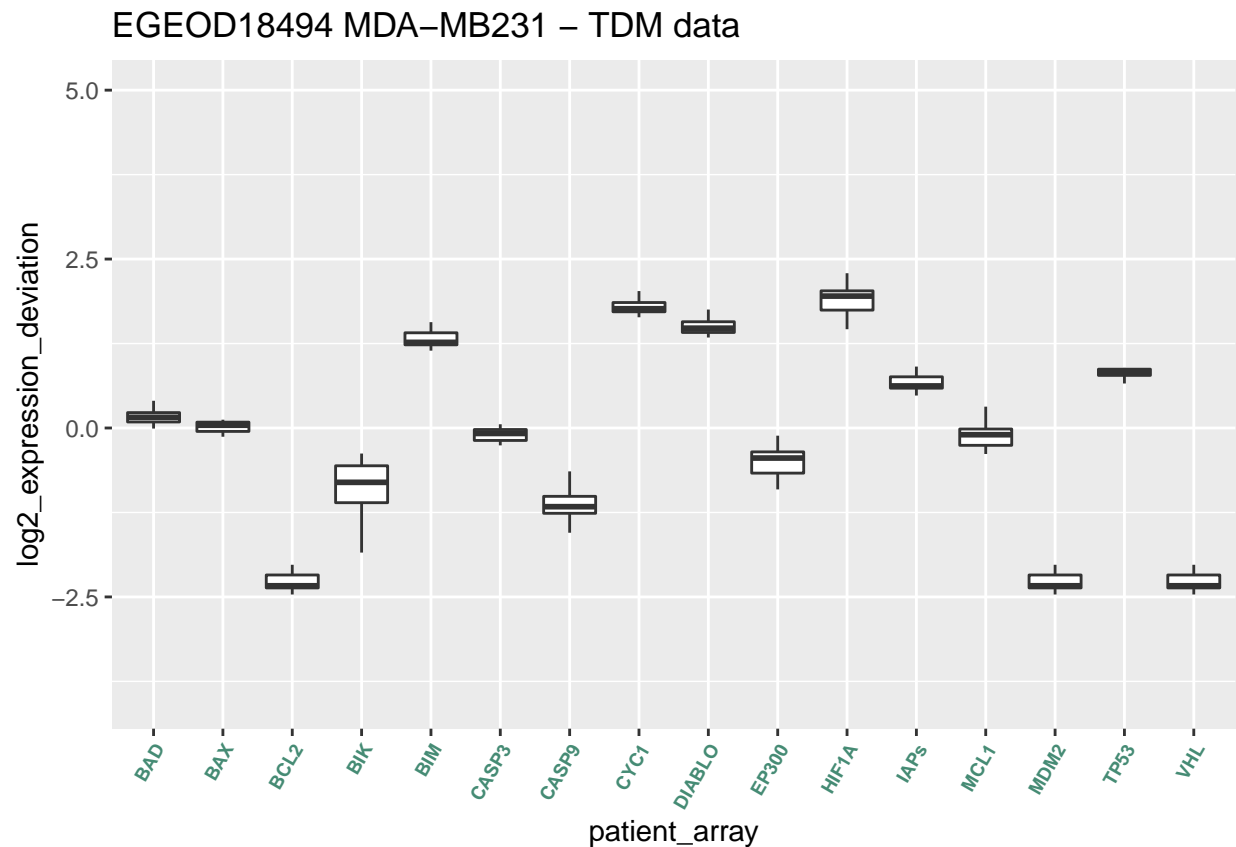


```
row_medians_assayData <-
  Biobase::rowMedians(as.matrix(t(expr.EGEOD18494.tdm)))

RLE_data <- sweep(t(expr.EGEOD18494.tdm), 1, row_medians_assayData)

RLE_data <- as.data.frame(RLE_data)
RLE_data_gathered <-
  tidyr::gather(RLE_data, patient_array, log2_expression_deviation)

ggplot2::ggplot(RLE_data_gathered, aes(patient_array,
                                       log2_expression_deviation)) +
  geom_boxplot(outlier.shape = NA) +
  ylim(c(-4, 5)) +
  ggtitle("EGEOD18494 MDA-MB231 - TDM data") +
  theme(axis.text.x = element_text(colour = "aquamarine4",
                                    angle = 60, size = 6.5, hjust = 1 ,
                                    face = "bold"))
```

```
rm(RLE_data, RLE_data_gathered, row_medians_assayData)
```

```
require(BiTrinA)
```

```
## Loading required package: BiTrinA
```

```
## Loading required package: diptest
```

```
expr.GSE47533.hif.bin <- binarizeMatrix(expr.GSE47533.hif,
  method = c("BASCA"),
  adjustment = "none")

expr.GSE47533.hif.bin$symbol <- row.names(expr.GSE47533.hif.bin)

expr.GSE47533.hif.bin <- expr.GSE47533.hif.bin[, c(as.character(data.GSE47533$codes), c("threshold", "p

names(expr.GSE47533.hif.bin) <- c(paste0(substr(data.GSE47533$condition,1,4), ".", data.GSE47533$time, "

head(expr.GSE47533.hif.bin) %>%
  knitr::kable(.)
```

	Norm.0	Norm.1	Norm.2	Hypo.16h	Hypo.16h	Hypo.16h	Hypo.32h	Hypo.32h	Hypo.32h	Hypo.32h	Hypo.48h	Hypo.48h	Hypo.48h	log2value	symbol
BAD	0	0	0	0	0	0	0	0	0	1	1	1	1	7.925009	BAD
BAX	0	1	1	0	0	0	1	0	0	1	1	1	1	7.978194	BAX
BCL2	1	1	1	0	0	0	0	0	0	0	0	0	0	7.272830	BCL2
BIK	0	0	0	1	1	1	1	1	1	1	1	1	1	8.755742	BIK
BIM	1	1	0	1	0	0	1	0	1	1	0	1	1	10.921169	BIM
CASP3	1	1	1	0	0	1	1	0	0	0	0	0	0	7.799389	CASP3

```

expr.GSE47533.hif.mean <- expr.GSE47533.hif.bin %>%
  mutate(Norm = rowMeans(dplyr::select(., starts_with("Norm"))),
         Hypo.16h = rowMeans(dplyr::select(., starts_with("Hypo.16h"))),
         Hypo.32h = rowMeans(dplyr::select(., starts_with("Hypo.32h"))),
         Hypo.48h = rowMeans(dplyr::select(., starts_with("Hypo.48h")))) %>%
  dplyr::select(., -ends_with(c(".1", ".2", ".3")))

expr.GSE47533.hif.pivot <- expr.GSE47533.hif.mean %>%
  group_by(symbol) %>%
  pivot_longer(cols = starts_with(c("Norm", "Hypo")), names_to = "codes", values_to = "value")

expr.GSE47533.hif.pivot$codes <- factor(expr.GSE47533.hif.pivot$codes, levels = c("Norm", "Hypo.16h", "Hypo.32h", "Hypo.48h"))

expr.GSE47533.hif.pivot$time <- as.numeric(expr.GSE47533.hif.pivot$codes)

# hif.symbols <- c("HIF1A", "TP53", "MDM2", "VHL", "EP300", "TMBIM1", "TMBIM4", "TMBIM6", "BAD", "BIK", "MCL1", "BAX", "BCL2", "CASP3")

p.MCF7 <- ggplot(aes(x = factor(time), y = value, group = symbol, color="red"),
  data = expr.GSE47533.hif.pivot[expr.GSE47533.hif.pivot$symbol %in% c("HIF1A", "TP53", "MDM2"),])
  geom_point() +
  geom_line() +
  scale_x_discrete(breaks = c(1, 2, 3, 4),
    labels = c("Normoxia", "Hypoxia: 16h", "Hypoxia: 32h", "Hypoxia: 48h")) +
  xlab("Conditions") + ylab("Gene Expression") +
  ggtitle("GSE47533 MCF7 - Raw data") +
  theme(legend.position = "none", axis.text.x=element_text(color = "black", size=7, angle=30, vjust=.8),
    #geom_line(aes(linetype=Symbol, color=Symbol)) +
    facet_wrap(~ symbol))
p.MCF7

```

```
# expr.EGEOD18494.hif.bin <- binarizeMatrix(expr.EGEOD18494.tdm,  
# method = c("BASCA"),  
# tau = 0.15,  
# #sigma = 0.9,  
# adjustment = "none")  
  
expr.EGEOD18494.hif.bin <- binarizeMatrix(expr.EGEOD18494.hif)  
  
#expr.EGEOD18494.hif.bin <- expr.EGEOD18494.tdm  
  
expr.EGEOD18494.hif.bin$symbol <- row.names(expr.EGEOD18494.hif.bin)  
  
row <- data.EGEOD18494$cell_line == "MDA-MB231 breast cancer"  
expr.EGEOD18494.hif.bin <- expr.EGEOD18494.hif.bin[, c(as.character(data.EGEOD18494$codes[row]), c("thr  
names(expr.EGEOD18494.hif.bin) <- c(paste0(substr(data.EGEOD18494$condition[row],1,4),".", data.EGEOD18  
#names(expr.EGEOD18494.hif.bin) <- c(paste0(substr(data.EGEOD18494$condition[row],1,4),".", data.EGEOD1  
# head(expr.EGEOD18494.hif.bin) %>%  
# knitr::kable(.)
```

```

expr.EGEOD18494.hif.mean <- expr.EGEOD18494.hif.bin %>%
  mutate(norm = rowMeans(dplyr::select(., starts_with("norm"))),
         hypo.4h = rowMeans(dplyr::select(., starts_with("hypo.4h"))),
         hypo.8h = rowMeans(dplyr::select(., starts_with("hypo.8h"))),
         hypo.12h = rowMeans(dplyr::select(., starts_with("hypo.12h")))) %>%
  dplyr::select(., -ends_with(c(".1", ".2", ".3")))

expr.EGEOD18494.hif.pivot <- expr.EGEOD18494.hif.mean %>%
  group_by(symbol) %>%
  pivot_longer(cols = starts_with(c("Norm", "Hypo")), names_to = "codes", values_to = "value")

expr.EGEOD18494.hif.pivot$codes <- factor(expr.EGEOD18494.hif.pivot$codes, levels = c("norm", "hypo.4h", "hypo.8h", "hypo.12h"))

expr.EGEOD18494.hif.pivot$time <- as.numeric(expr.EGEOD18494.hif.pivot$codes)

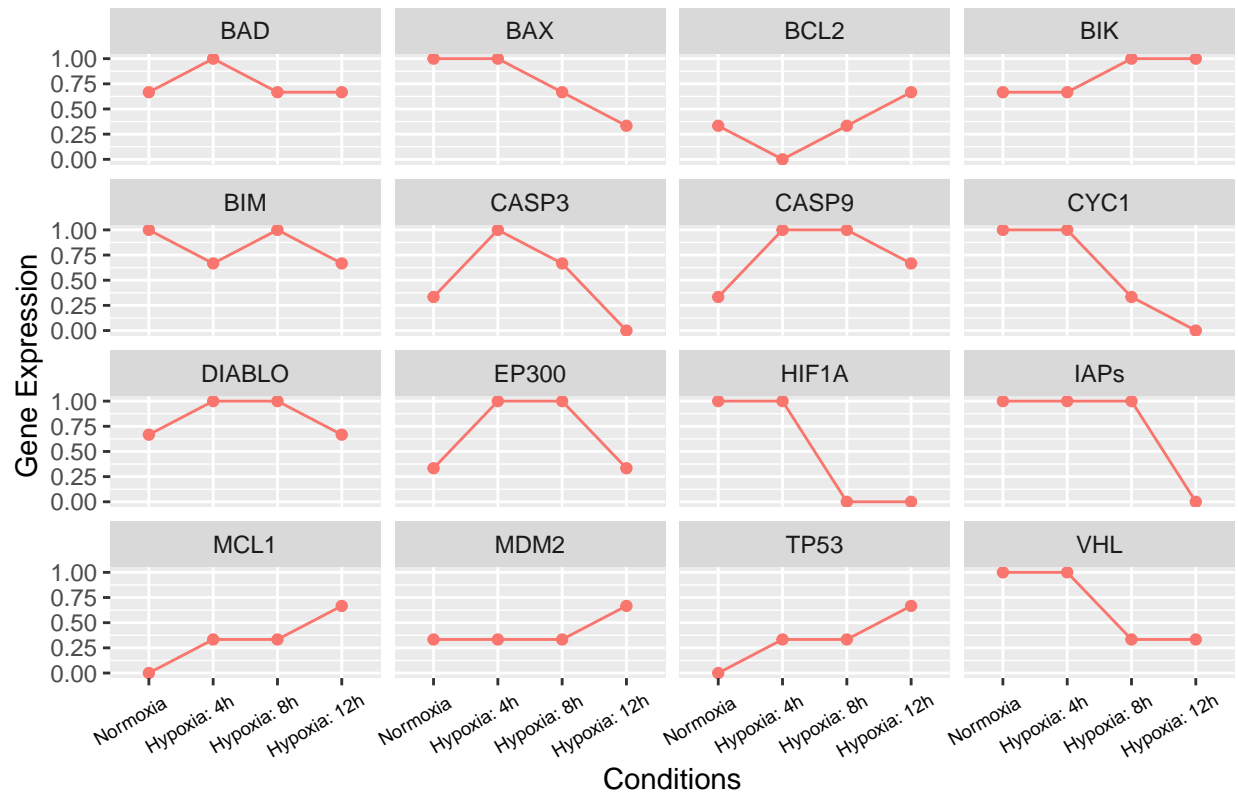
# hif.symbols <- c("HIF1A", "TP53", "MDM2", "VHL", "EP300", "TMBIM1", "TMBIM4", "TMBIM6", "BAD", "BIK", "MCL1", "FOXO3", "FOXO1", "FOXO2", "FOXO4", "FOXO6", "FOXO7", "FOXO8", "FOXO9", "FOXO10", "FOXO11", "FOXO12", "FOXO13", "FOXO14", "FOXO15", "FOXO16", "FOXO17", "FOXO18", "FOXO19", "FOXO20", "FOXO21", "FOXO22", "FOXO23", "FOXO24", "FOXO25", "FOXO26", "FOXO27", "FOXO28", "FOXO29", "FOXO30", "FOXO31", "FOXO32", "FOXO33", "FOXO34", "FOXO35", "FOXO36", "FOXO37", "FOXO38", "FOXO39", "FOXO40", "FOXO41", "FOXO42", "FOXO43", "FOXO44", "FOXO45", "FOXO46", "FOXO47", "FOXO48", "FOXO49", "FOXO50", "FOXO51", "FOXO52", "FOXO53", "FOXO54", "FOXO55", "FOXO56", "FOXO57", "FOXO58", "FOXO59", "FOXO60", "FOXO61", "FOXO62", "FOXO63", "FOXO64", "FOXO65", "FOXO66", "FOXO67", "FOXO68", "FOXO69", "FOXO70", "FOXO71", "FOXO72", "FOXO73", "FOXO74", "FOXO75", "FOXO76", "FOXO77", "FOXO78", "FOXO79", "FOXO80", "FOXO81", "FOXO82", "FOXO83", "FOXO84", "FOXO85", "FOXO86", "FOXO87", "FOXO88", "FOXO89", "FOXO90", "FOXO91", "FOXO92", "FOXO93", "FOXO94", "FOXO95", "FOXO96", "FOXO97", "FOXO98", "FOXO99", "FOXO100")

p.MDA <- ggplot(aes(x = factor(time), y = value, group = symbol, color="red"),
               data = expr.EGEOD18494.hif.pivot[expr.EGEOD18494.hif.pivot$symbol %in% c("HIF1A", "TP53", "MDM2", "VHL", "EP300", "TMBIM1", "TMBIM4", "TMBIM6", "BAD", "BIK", "MCL1", "FOXO3", "FOXO1", "FOXO2", "FOXO4", "FOXO6", "FOXO7", "FOXO8", "FOXO9", "FOXO10", "FOXO11", "FOXO12", "FOXO13", "FOXO14", "FOXO15", "FOXO16", "FOXO17", "FOXO18", "FOXO19", "FOXO20", "FOXO21", "FOXO22", "FOXO23", "FOXO24", "FOXO25", "FOXO26", "FOXO27", "FOXO28", "FOXO29", "FOXO30", "FOXO31", "FOXO32", "FOXO33", "FOXO34", "FOXO35", "FOXO36", "FOXO37", "FOXO38", "FOXO39", "FOXO40", "FOXO41", "FOXO42", "FOXO43", "FOXO44", "FOXO45", "FOXO46", "FOXO47", "FOXO48", "FOXO49", "FOXO50", "FOXO51", "FOXO52", "FOXO53", "FOXO54", "FOXO55", "FOXO56", "FOXO57", "FOXO58", "FOXO59", "FOXO60", "FOXO61", "FOXO62", "FOXO63", "FOXO64", "FOXO65", "FOXO66", "FOXO67", "FOXO68", "FOXO69", "FOXO70", "FOXO71", "FOXO72", "FOXO73", "FOXO74", "FOXO75", "FOXO76", "FOXO77", "FOXO78", "FOXO79", "FOXO80", "FOXO81", "FOXO82", "FOXO83", "FOXO84", "FOXO85", "FOXO86", "FOXO87", "FOXO88", "FOXO89", "FOXO90", "FOXO91", "FOXO92", "FOXO93", "FOXO94", "FOXO95", "FOXO96", "FOXO97", "FOXO98", "FOXO99", "FOXO100")],
               geom_point() +
               geom_line() +
               scale_x_discrete(breaks = c(1, 2, 3, 4),
                               labels = c("Normoxia", "Hypoxia: 4h", "Hypoxia: 8h", "Hypoxia: 12h")) +
               xlab("Conditions") + ylab("Gene Expression") +
               ggtitle("EGEOD18494 MDA-MB231 - Raw data") +
               theme(legend.position = "none", axis.text.x=element_text(color = "black", size=7, angle=30, vjust=.8),
                     #geom_line(aes(linetype=Symbol, color=Symbol)) +
                     facet_wrap(~ symbol))

p.MDA

```

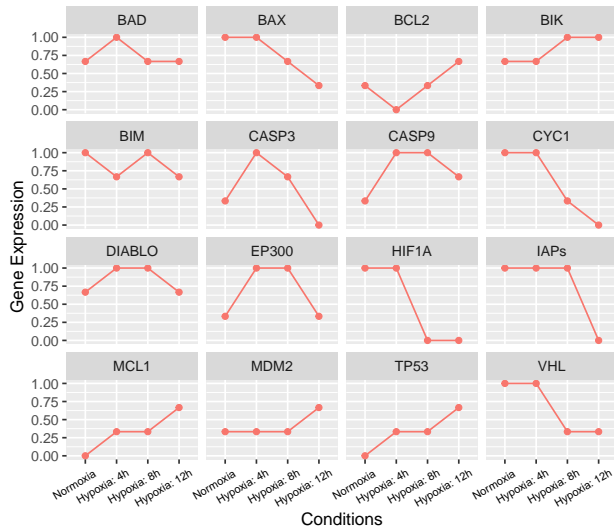
EGEOD18494 MDA-MB231 – Raw data



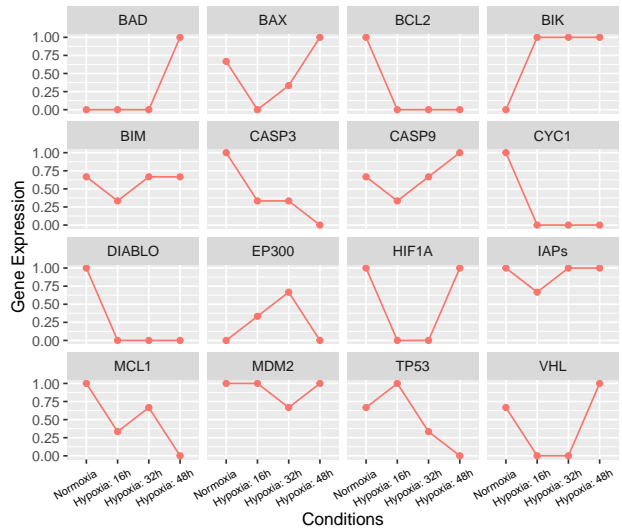
```
library(cowplot)
```

```
plot_grid(p.MDA, p.MCF7, labels = c('A', 'B'))
```

A EGEOD18494 MDA-MB231 – Raw data



B GSE47533 MCF7 – Raw data



Heatmaps - EGEOD18494

Multivariate Shapiro-Wilk normality test

From the output, the p-value > 0.05 implying that the distribution of the data are not significantly different from normal distribution. In other words, we can assume the normality.

```
library(rstatix)
```

```
##  
## Attaching package: 'rstatix'  
  
## The following object is masked from 'package:stats':  
##  
## filter
```

```
rstatix::mshapiro_test(expr.EGEOD18494.hif) %>%  
knitr::kable(.)
```

statistic	p.value
0.3598048	2e-07

```
library("pheatmap")  
library("ComplexHeatmap")
```

```
## =====  
## ComplexHeatmap version 2.2.0  
## Bioconductor page: http://bioconductor.org/packages/ComplexHeatmap/  
## Github page: https://github.com/jokergoo/ComplexHeatmap  
## Documentation: http://jokergoo.github.io/ComplexHeatmap-reference  
##  
## If you use it in published research, please cite:  
## Gu, Z. Complex heatmaps reveal patterns and correlations in multidimensional  
## genomic data. Bioinformatics 2016.  
## =====
```

```
data.EGEOD18494$time <- factor(data.EGEOD18494$time, levels = c("control", "4h", "8h", "12h"))  
  
row <- data.EGEOD18494$cell_line == "MDA-MB231 breast cancer"  
  
annotation_for_heatmap <- droplevels(data.frame(time = data.EGEOD18494$time[row], condition = data.EGEOD18494$condition[row]))  
  
row.names(annotation_for_heatmap) <- paste0(substr(data.EGEOD18494$condition[row],1,4),".", data.EGEOD18494$time[row])  
  
dists <- as.matrix(dist(t(expr.EGEOD18494.hif), method = "manhattan"))  
  
rownames(dists) <- c(paste0(substr(data.EGEOD18494$condition[row],1,4),".", data.EGEOD18494$time[row]),  
colnames(dists) <- c(paste0(substr(data.EGEOD18494$condition[row],1,4),".", data.EGEOD18494$time[row]),
```

```

hmcol <- rev(colorRampPalette(RColorBrewer::brewer.pal(9, "YlOrRd"))(255))

diag(dists) <- NA

ann_colors <- list(
  time = RColorBrewer::brewer.pal(length(levels(data.EGEOD18494$time)), "Set2"),
  condition = c("red", "blue")
)

ann_colors

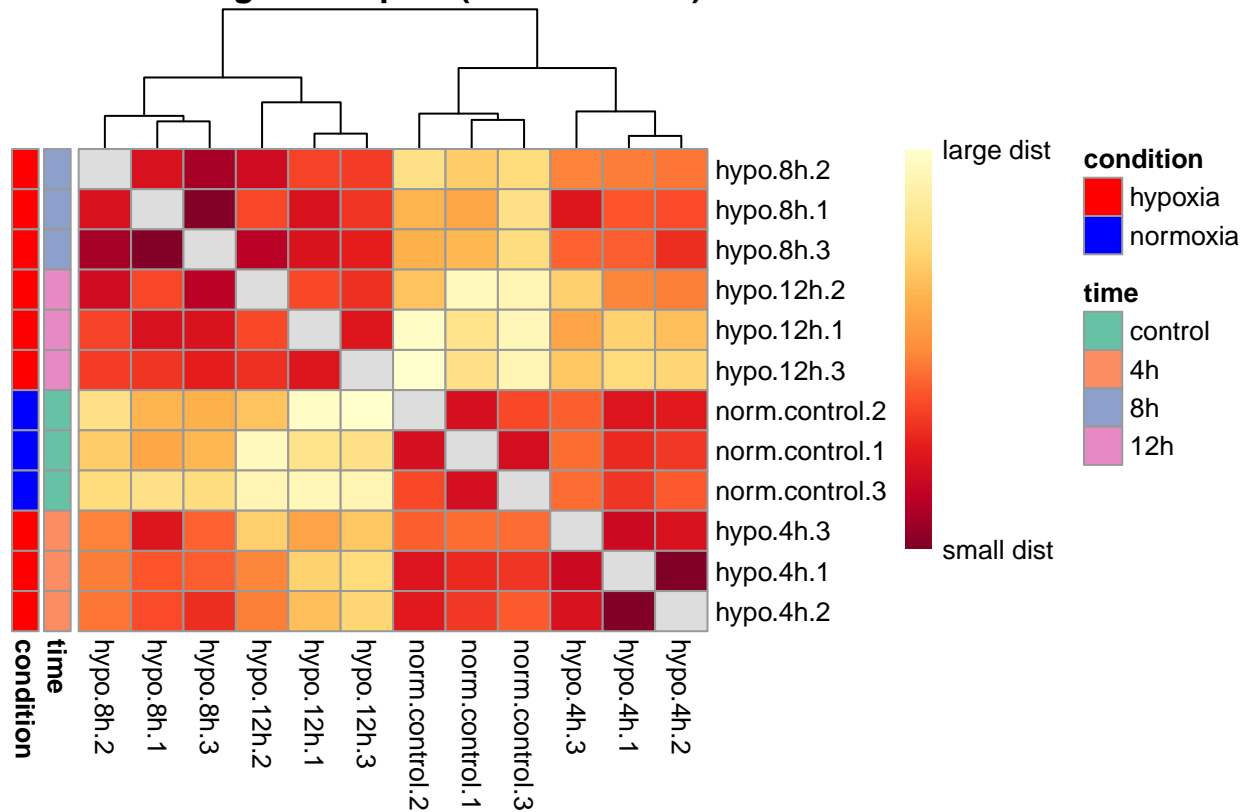
## $time
## [1] "#66C2A5" "#FC8D62" "#8DA0CB" "#E78AC3"
##
## $condition
## [1] "red" "blue"

names(ann_colors$time) <- levels(data.EGEOD18494$time)
names(ann_colors$condition) <- levels(data.EGEOD18494$condition)

pheatmap(dists, col = (hmcol),
  annotation_row = annotation_for_heatmap,
  annotation_colors = ann_colors,
  legend = TRUE,
  treeheight_row = 0,
  legend_breaks = c(min(dists, na.rm = TRUE),
                     max(dists, na.rm = TRUE)),
  legend_labels = (c("small dist", "large dist")),
  main = "Clustering of Samples (EGEOD18494)")

```

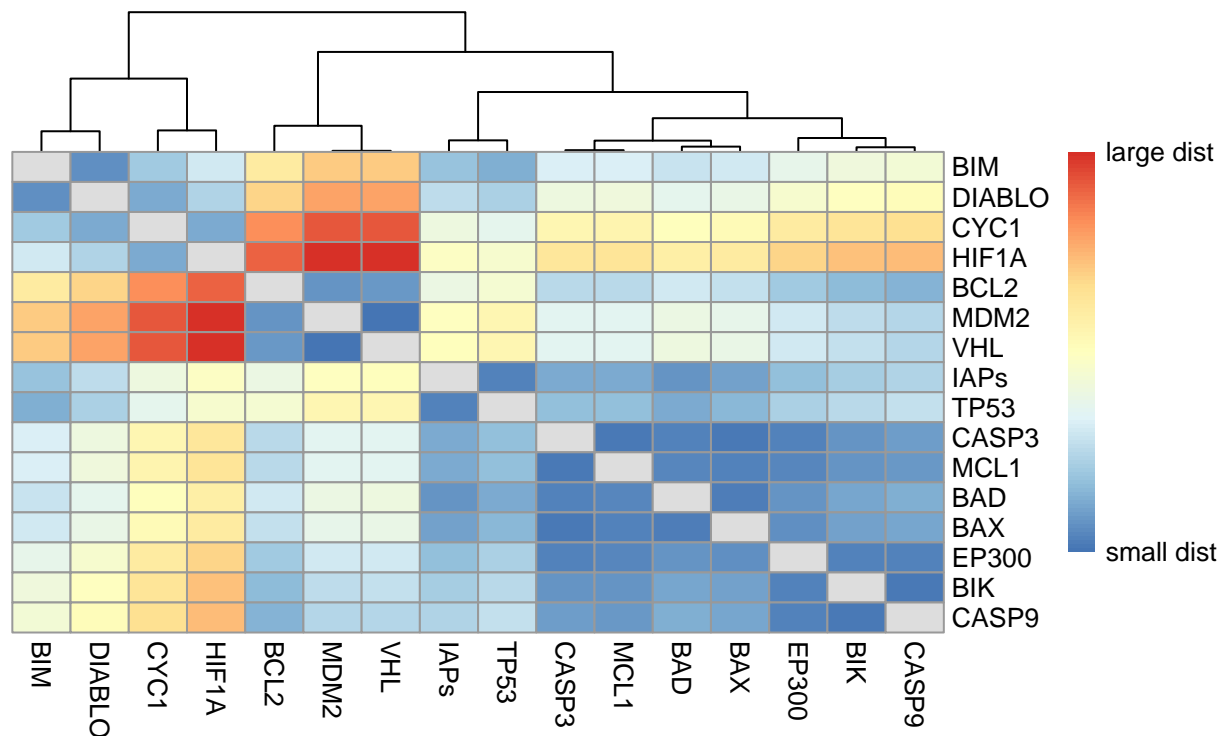
Clustering of Samples (EGEOD18494)



```
dists <- as.matrix(dist(expr.EGEOD18494.hif, method = "euclidean"))
rownames(dists) <- rownames(expr.EGEOD18494.hif)
colnames(dists) <- rownames(expr.EGEOD18494.hif)
diag(dists) <- NA

pheatmap(dists, #row = (hmcov),
          #annotation_col = annotation_for_heatmap,
          #annotation_colors = ann_colors,
          legend = TRUE,
          treeheight_row = 0,
          legend_breaks = c(min(dists, na.rm = TRUE),
                             max(dists, na.rm = TRUE)),
          legend_labels = (c("small dist", "large dist")),
          main = "Clustering of Gene Expression \n Euclidian Distance (EGEOD18494)")
```


Clustering of Gene Expression Euclidian Distance (EGEOD18494)



```
#-----

expr.row <- (colnames(expr.EGEOD18494.hif) %in% data.EGEOD18494$codes[data.EGEOD18494$cell_line == "MDA-MB231 breast cancer"])
dists <- as.matrix(dist(expr.EGEOD18494.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.EGEOD18494.hif[expr.row])
colnames(dists) <- rownames(expr.EGEOD18494.hif[expr.row])
diag(dists) <- NA

p1 <- pheatmap(dists,
  legend = TRUE,
  treeheight_row = 0,
  legend_breaks = c(min(dists, na.rm = TRUE),
    max(dists, na.rm = TRUE)),
  legend_labels = (c("small dist", "large dist")),
  main = "Clustering of Gene Expression on Hypoxia \n Euclidian Distance (EGEOD18494)",
  silent=T)

#-----

row <- data.EGEOD18494$cell_line == "MDA-MB231 breast cancer" & data.EGEOD18494$condition == "normoxia"
annotation_for_heatmap <- droplevels(data.frame(time = data.EGEOD18494$time[row], condition = data.EGEOD18494$condition[row]))
expr.row <- (colnames(expr.EGEOD18494.hif) %in% data.EGEOD18494$codes[data.EGEOD18494$cell_line == "MDA-MB231 breast cancer"])
row.names(annotation_for_heatmap) <- colnames(expr.EGEOD18494.hif[expr.row])
```

```

dists <- as.matrix(dist(expr.EGEOD18494.hif[expr.row], method = "euclidean"))

rownames(dists) <- rownames(expr.EGEOD18494.hif[expr.row])

hmcol <- rev(colorRampPalette(RColorBrewer::brewer.pal(9, "YlOrRd"))(255))
colnames(dists) <- rownames(expr.EGEOD18494.hif[expr.row])
diag(dists) <- NA

ann_colors <- list(
  time = RColorBrewer::brewer.pal(length(levels(data.EGEOD18494$time)), "Set2"),
  condition = c("#EF8A62", "#67A9CF")
)

ann_colors

## $time
## [1] "#66C2A5" "#FC8D62" "#8DA0CB" "#E78AC3"
##
## $condition
## [1] "#EF8A62" "#67A9CF"

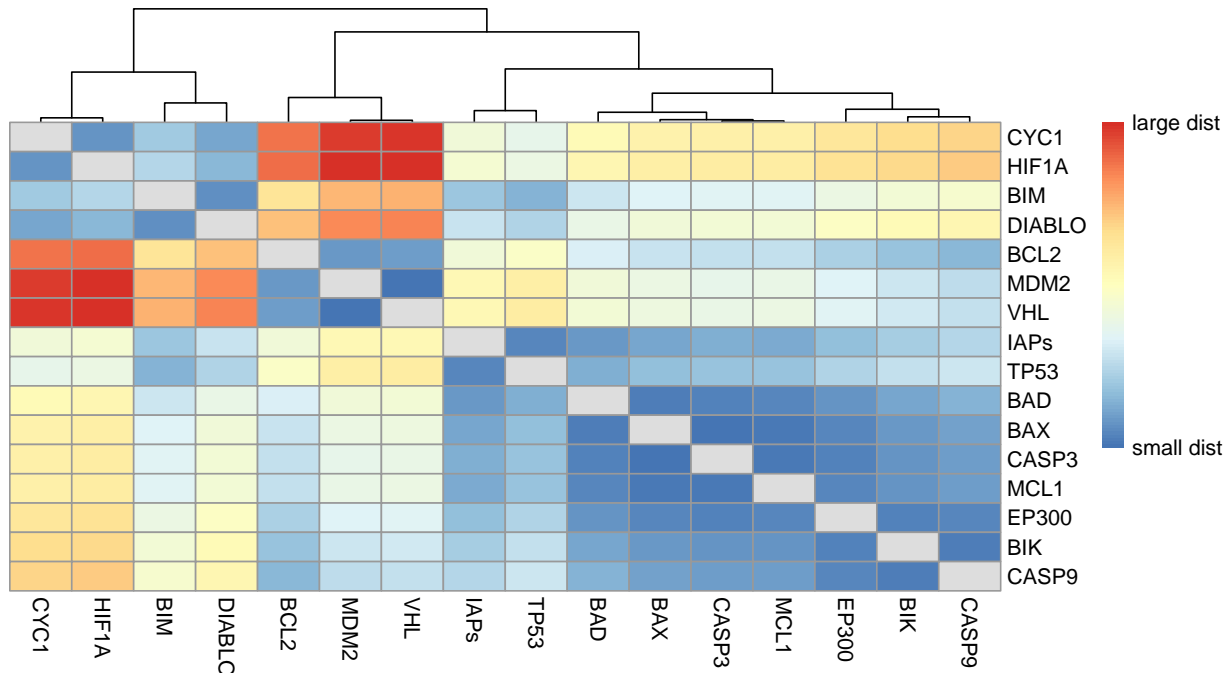
names(ann_colors$time) <- levels(data.EGEOD18494$time)
names(ann_colors$condition) <- levels(data.EGEOD18494$condition)

p2 <- pheatmap(dists, #row = (hmcol),
  #annotation_col = annotation_for_heatmap,
  #annotation_colors = ann_colors,
  legend = TRUE,
  treeheight_row = 0,
  legend_breaks = c(min(dists, na.rm = TRUE),
    max(dists, na.rm = TRUE)),
  legend_labels = (c("small dist", "large dist")),
  main = "Clustering of Gene Expression on Normoxia \n Euclidian Distance (EGEOD18494)",
  silent=T)

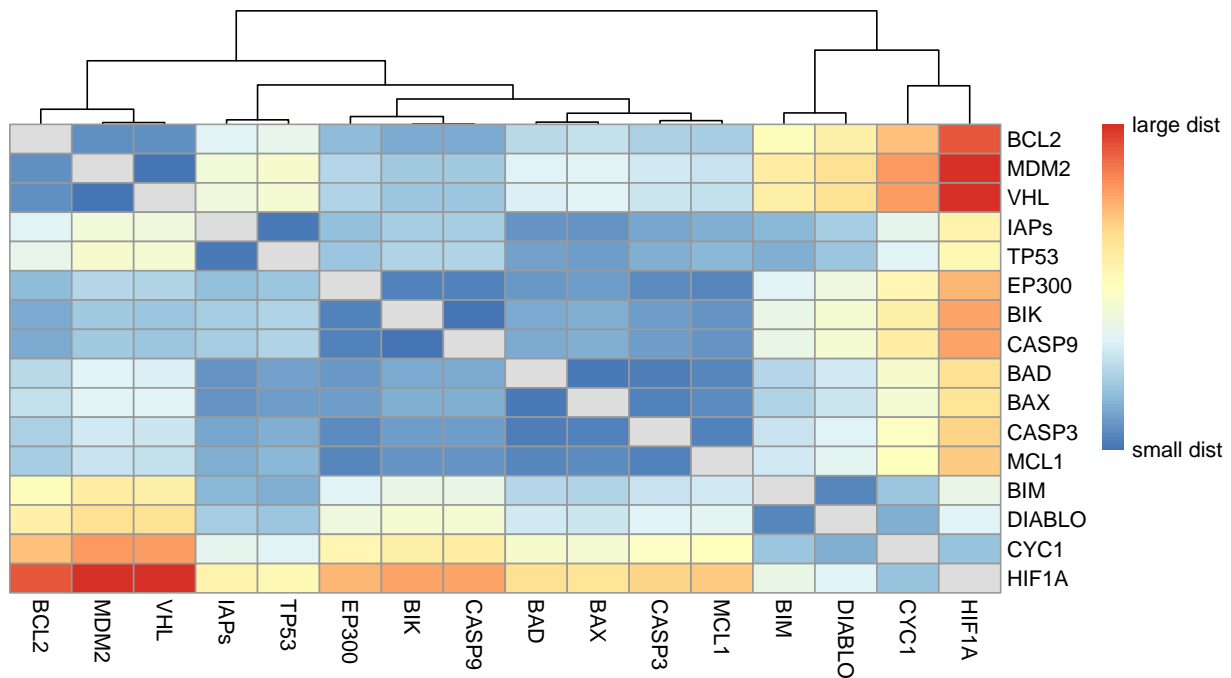
gridExtra::grid.arrange(grobs=list(p1$gtable, p2$gtable),
  nrow = 2 , labels=c('A', 'B'))

```

Clustering of Gene Expression on Hypoxia Euclidian Distance (EGEOD18494)



Clustering of Gene Expression on Normoxia Euclidian Distance (EGEOD18494)



```
data.EGEOD18494$time <- factor(data.EGEOD18494$time, levels = c("control", "4h", "8h", "12h 4h"))

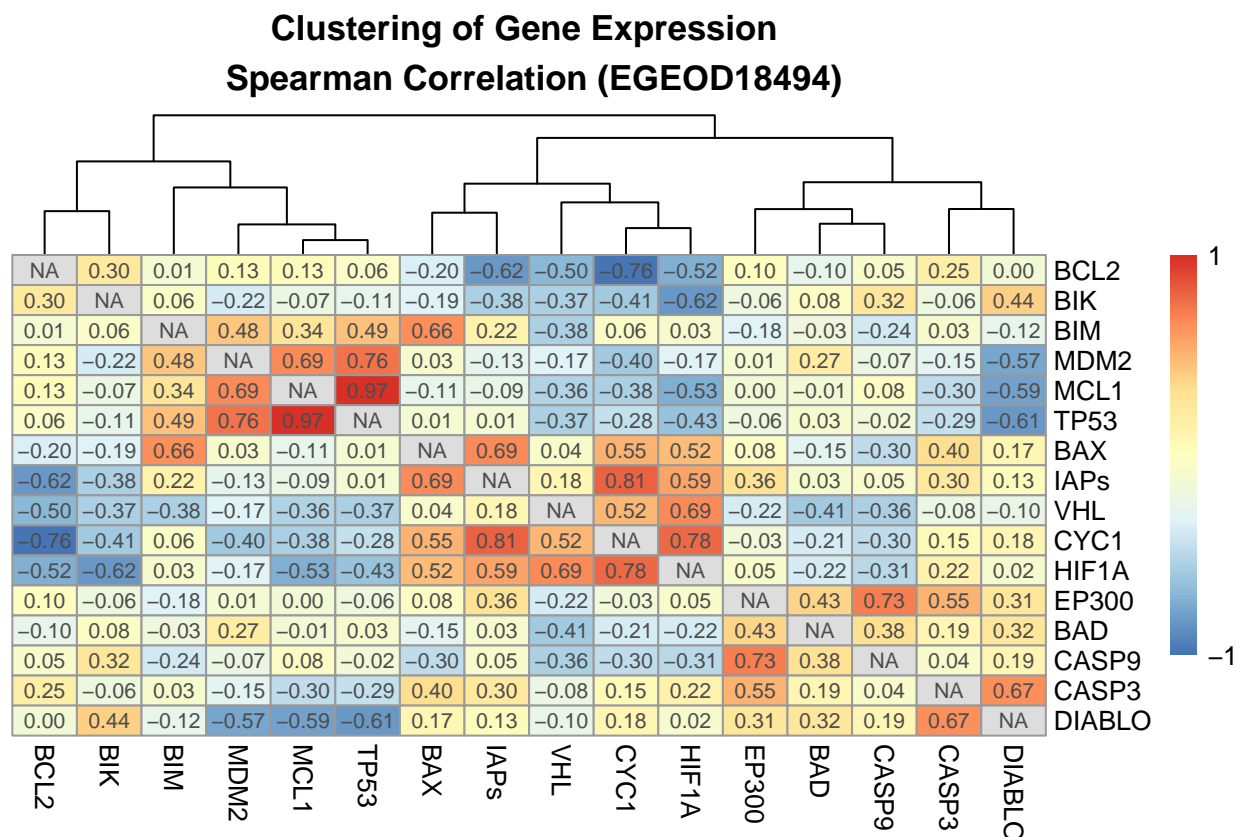
dists <- cor(t(expr.EGEOD18494.hif), use = "pairwise.complete.obs", method = "spearman")
rownames(dists) <- rownames(expr.EGEOD18494.hif)
```

```

hmccl <- rev(colorRampPalette(RColorBrewer::brewer.pal(9, "YlOrRd"))(255))
colnames(dists) <- rownames(expr.EGEOD18494.hif)
diag(dists) <- NA

pheatmap(dists, #row = (hmccl),
          #annotation_col = annotation_for_heatmap,
          #annotation_colors = ann_colors,
          legend = TRUE,
          display_numbers = T,
          treeheight_row = 0,
          legend_breaks = c(min(dists, na.rm = TRUE),
                             max(dists, na.rm = TRUE)),
          legend_labels = (c("-1", "1")),
          main = "Clustering of Gene Expression \n Spearman Correlation (EGEOD18494)")

```



```

dists <- cor(t(expr.EGEOD18494.hif), use = "pairwise.complete.obs", method = "pearson")
rownames(dists) <- rownames(expr.EGEOD18494.hif)
hmccl <- rev(colorRampPalette(RColorBrewer::brewer.pal(9, "YlOrRd"))(255))
colnames(dists) <- rownames(expr.EGEOD18494.hif)
diag(dists) <- NA

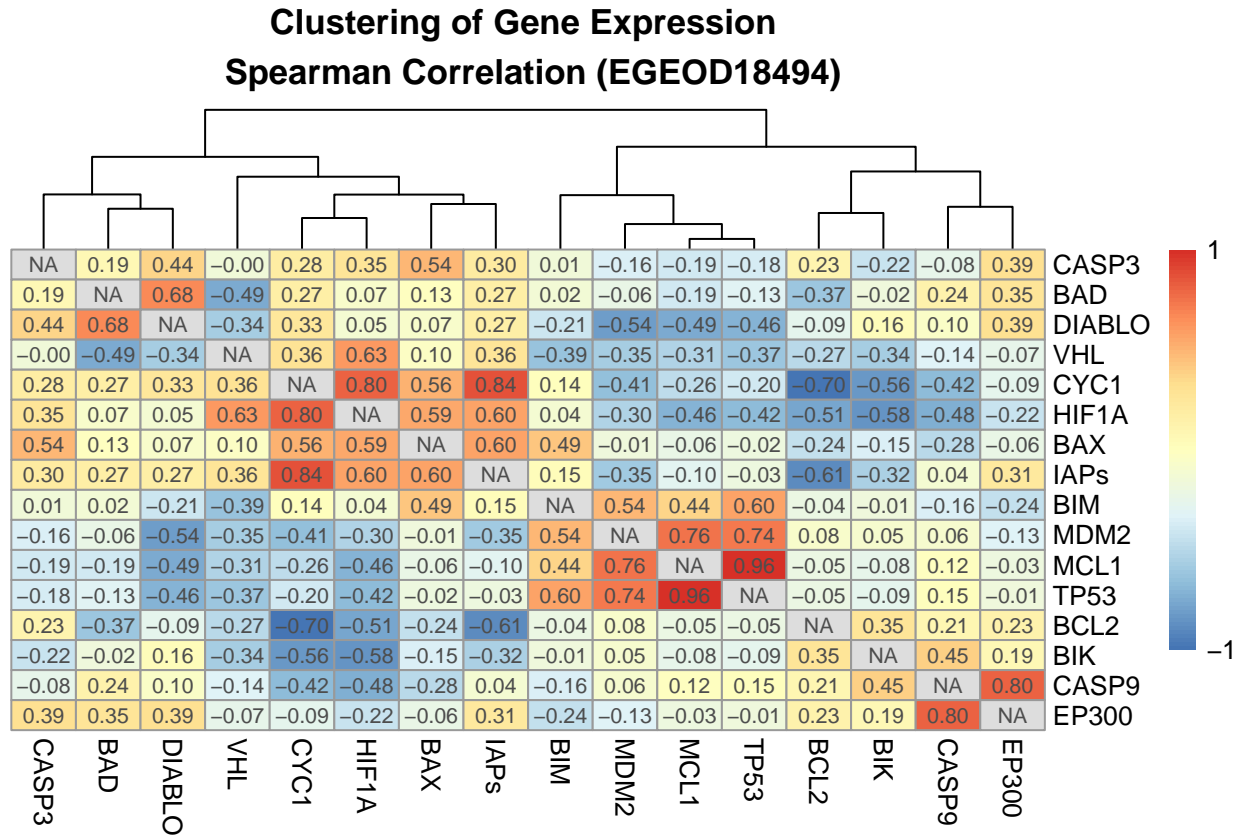
pheatmap(dists, #row = (hmccl),
          #annotation_col = annotation_for_heatmap,
          #annotation_colors = ann_colors,
          legend = TRUE,

```

```

display_numbers = T,
treeheight_row = 0,
legend_breaks = c(min(dists, na.rm = TRUE),
                    max(dists, na.rm = TRUE)),
legend_labels = (c("-1", "1")),
main = "Clustering of Gene Expression \n Spearman Correlation (EGEOD18494)"

```



```
# Heatmaps - GSE47533
```

```
## Multivariate Shapiro-Wilk normality test
```

From the output, the p-value > 0.05 implying that the distribution of the data are not significantly different from normality.

```

```r
library(rstatix)

rstatix::mshapiro_test(expr.GSE47533.hif) %>%
 knitr::kable(.)

```

statistic	p.value
0.3834331	3e-07

```

library("pheatmap")
library("ComplexHeatmap")

data.GSE47533$time <- factor(data.GSE47533$time, levels = c("0", "16h", "32h", "48h"))

annotation_for_heatmap <- droplevels(data.frame(time = data.GSE47533$time, condition = data.GSE47533$condition))

row.names(annotation_for_heatmap) <- paste0(substr(data.GSE47533$condition,1,4),".", data.GSE47533$time)

dists <- as.matrix(dist(t(expr.GSE47533.hif), method = "manhattan"))

rownames(dists) <- c(paste0(substr(data.GSE47533$condition,1,4),".", data.GSE47533$time, ".", data.GSE47533$condition),
colnames(dists) <- c(paste0(substr(data.GSE47533$condition,1,4),".", data.GSE47533$time, ".", data.GSE47533$condition))

hmcol <- rev(colorRampPalette(RColorBrewer::brewer.pal(9, "YlOrRd"))(255))

diag(dists) <- NA

ann_colors <- list(
 time = RColorBrewer::brewer.pal(length(levels(data.GSE47533$time)), "Set2"),
 condition = c("red", "blue")
)

ann_colors

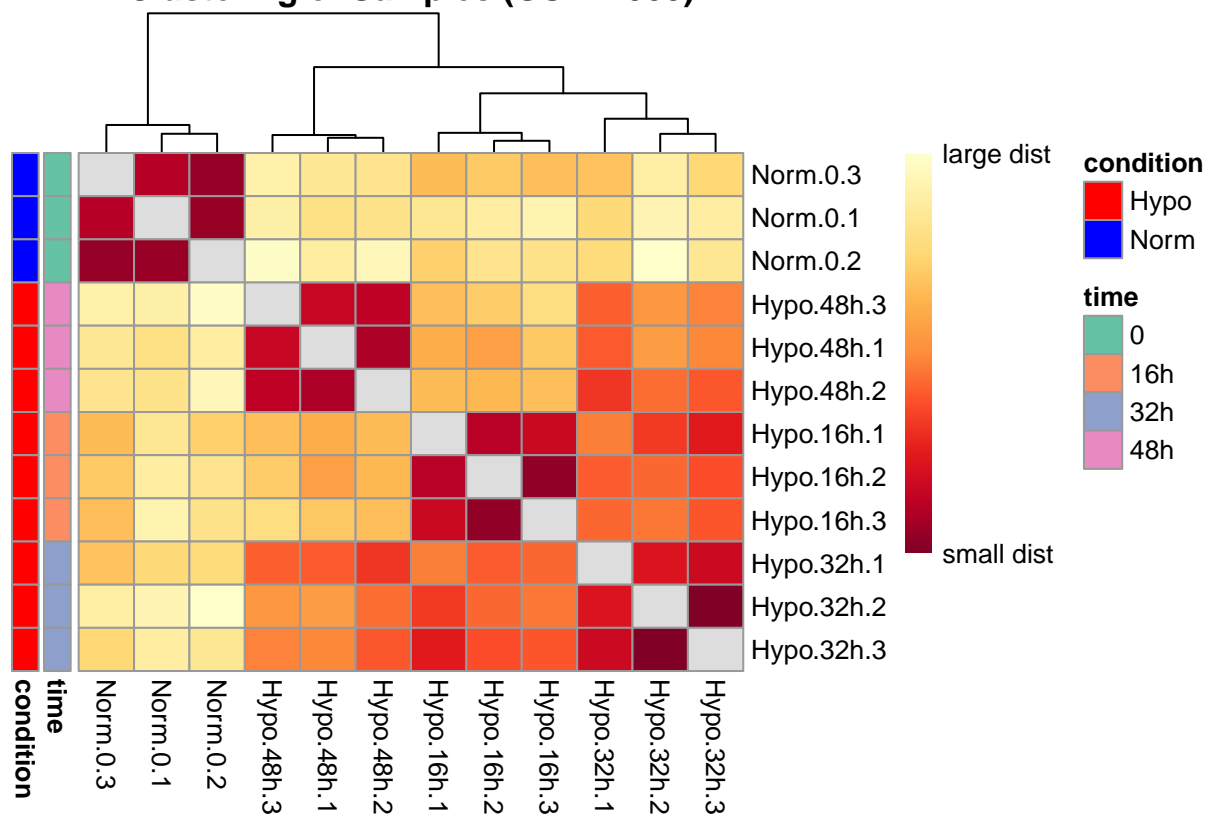
$time
[1] "#66C2A5" "#FC8D62" "#8DA0CB" "#E78AC3"
##
$condition
[1] "red" "blue"

names(ann_colors$time) <- levels(data.GSE47533$time)
names(ann_colors$condition) <- levels(data.GSE47533$condition)

pheatmap(dists, col = (hmcol),
 annotation_row = annotation_for_heatmap,
 annotation_colors = ann_colors,
 legend = TRUE,
 treeheight_row = 0,
 legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
 legend_labels = (c("small dist", "large dist")),
 main = "Clustering of Samples (GSE47533)")

```

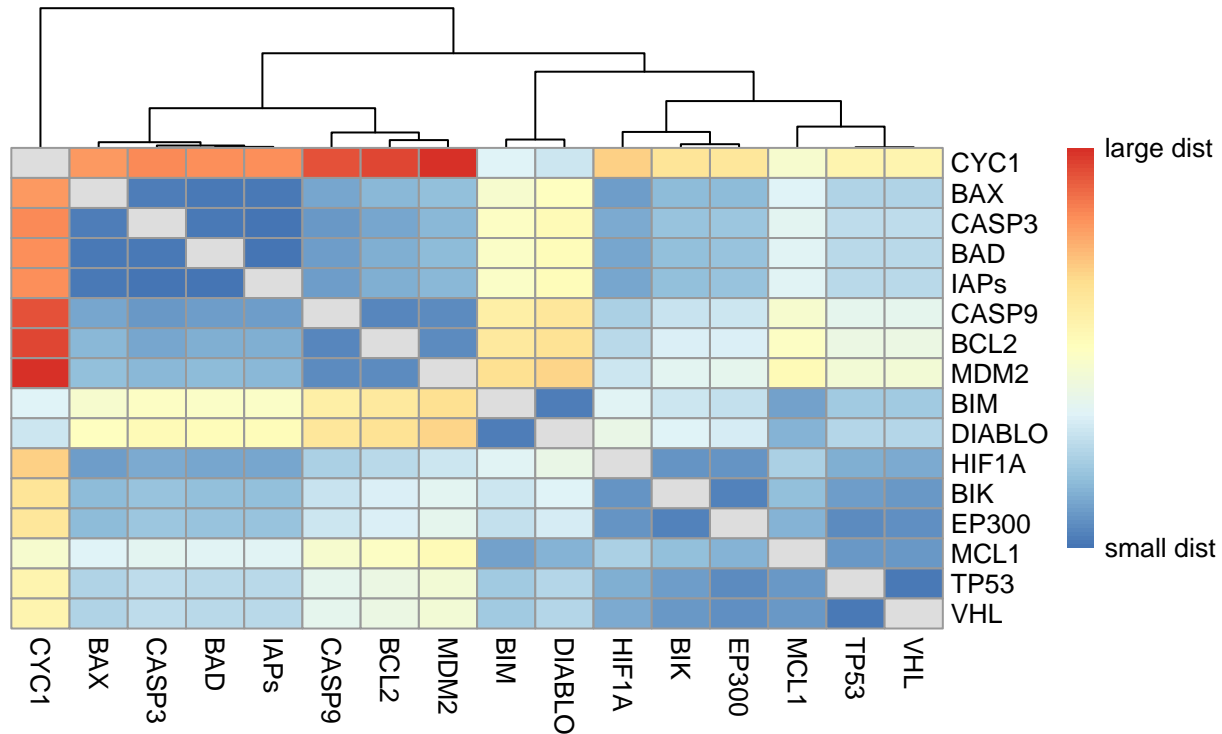
## Clustering of Samples (GSE47533)



```
dists <- as.matrix(dist(expr.GSE47533.hif, method = "euclidean"))
rownames(dists) <- rownames(expr.GSE47533.hif)
colnames(dists) <- rownames(expr.GSE47533.hif)
diag(dists) <- NA

pheatmap(dists,
 legend = TRUE,
 treeheight_row = 0,
 legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
 legend_labels = (c("small dist", "large dist")),
 main = "Clustering of Gene Expression \n Euclidian Distance (GSE47533)")
```

## Clustering of Gene Expression Euclidian Distance (GSE47533)



```
#-----
expr.row <- (colnames(expr.GSE47533.hif) %in% data.GSE47533$codes[data.GSE47533$condition == "Hypo"])
dists <- as.matrix(dist(expr.GSE47533.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.GSE47533.hif[expr.row])
colnames(dists) <- rownames(expr.GSE47533.hif[expr.row])
diag(dists) <- NA

p1 <- pheatmap(dists,
 legend = TRUE,
 treeheight_row = 0,
 legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
 legend_labels = (c("small dist", "large dist")),
 main = "Clustering of Gene Expression on Hypoxia \n Euclidian Distance (GSE47533)",
 silent=T)

#-----

expr.row <- (colnames(expr.GSE47533.hif) %in% data.GSE47533$codes[data.GSE47533$condition == "Norm"])
dists <- as.matrix(dist(expr.GSE47533.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.GSE47533.hif[expr.row])
colnames(dists) <- rownames(expr.GSE47533.hif[expr.row])
diag(dists) <- NA
```



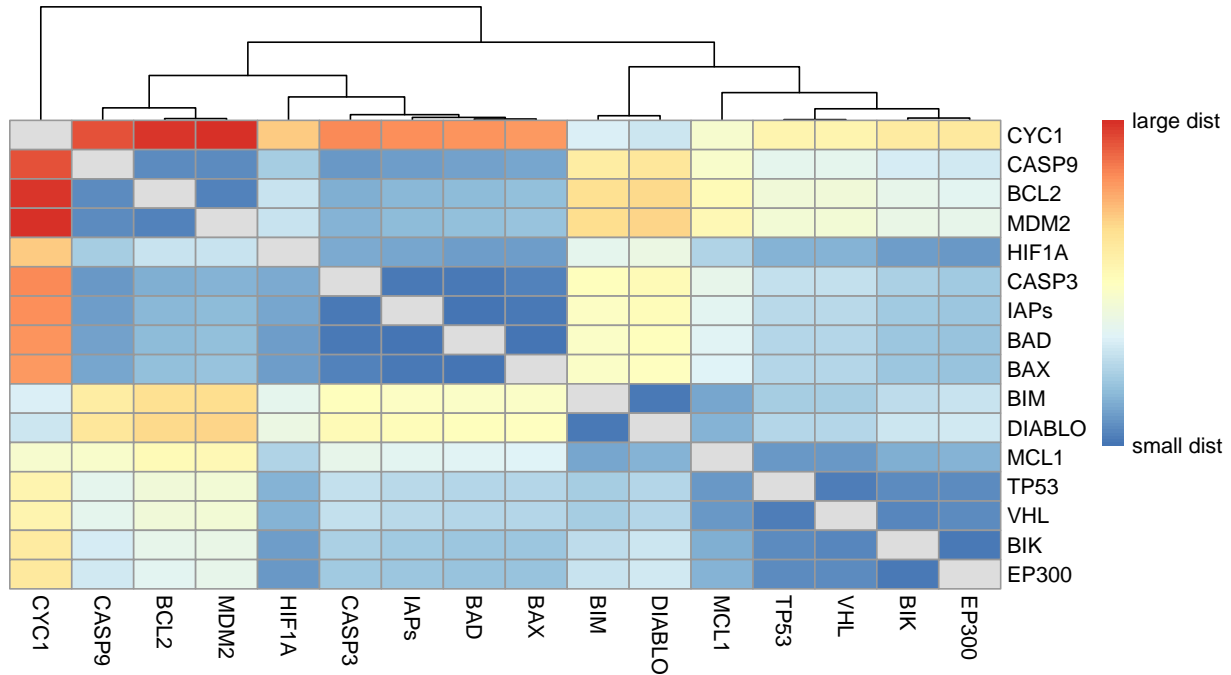
```

p2 <- pheatmap(dists,
 legend = TRUE,
 treeheight_row = 0,
 legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
 legend_labels = (c("small dist", "large dist")),
 main = "Clustering of Gene Expression on Normoxia \n Euclidian Distance (GSE47533)",
 silent=T)

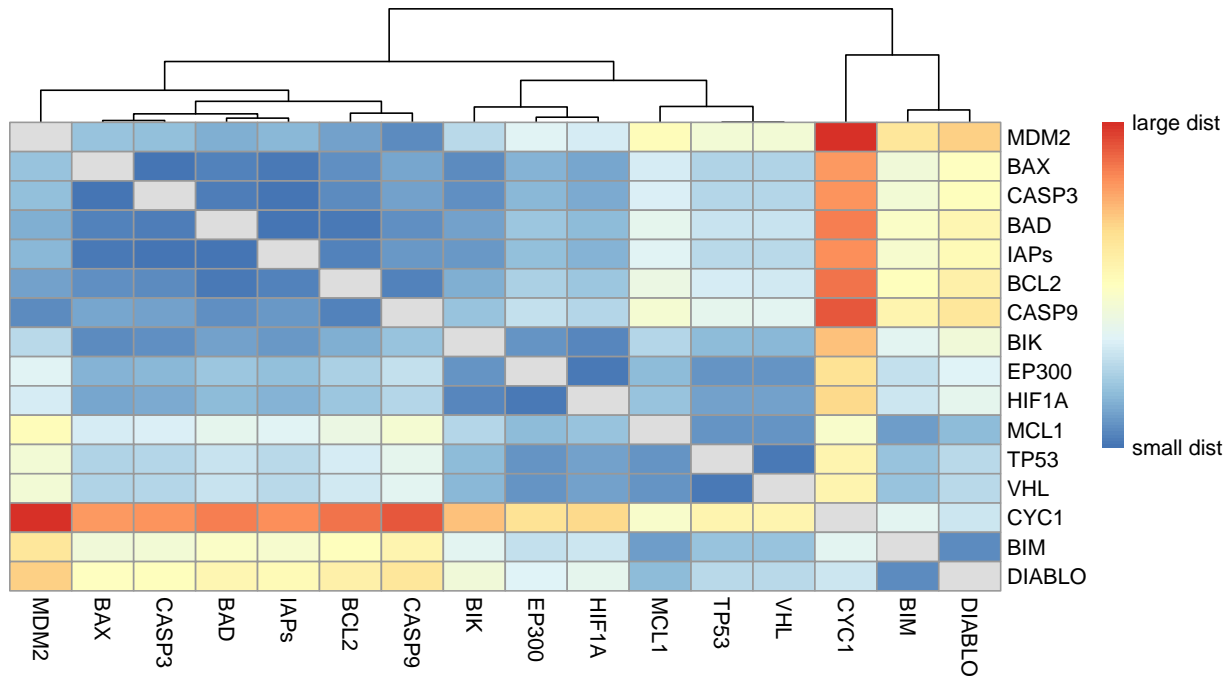
gridExtra::grid.arrange(grobs=list(p1$gtable, p2$gtable),
 nrow = 2 , labels=c('A', 'B'))

```

### Clustering of Gene Expression on Hypoxia Euclidian Distance (GSE47533)

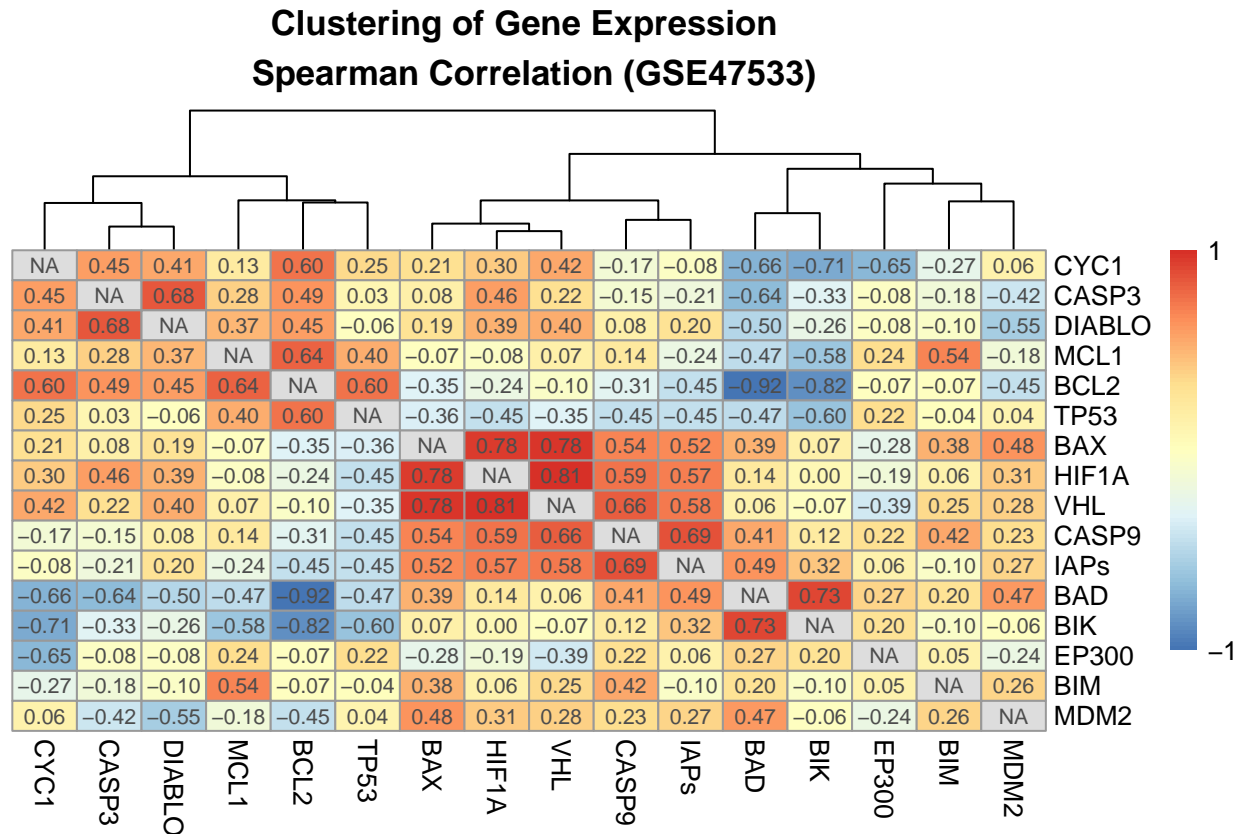


### Clustering of Gene Expression on Normoxia Euclidian Distance (GSE47533)



```
dists <- cor(t(expr.GSE47533.hif), use = "pairwise.complete.obs", method = "spearman")
rownames(dists) <- rownames(expr.GSE47533.hif)
colnames(dists) <- rownames(expr.GSE47533.hif)
diag(dists) <- NA
```

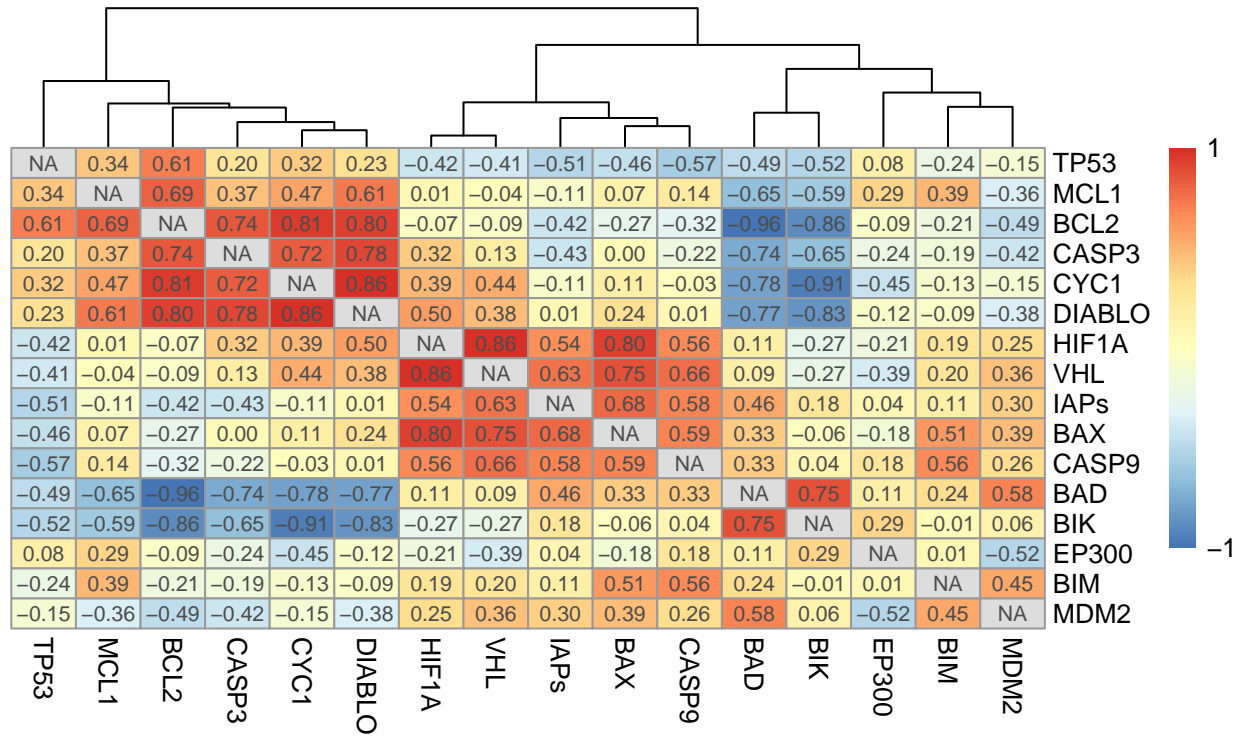
```
pheatmap(dists,
 legend = TRUE,
 display_numbers = T,
 treeheight_row = 0,
 legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
 legend_labels = (c("-1", "1")),
 main = "Clustering of Gene Expression \n Spearman Correlation (GSE47533)")
```



```
dists <- cor(t(expr.GSE47533.hif), use = "pairwise.complete.obs", method = "pearson")
rownames(dists) <- rownames(expr.GSE47533.hif)
colnames(dists) <- rownames(expr.GSE47533.hif)
diag(dists) <- NA

pheatmap(dists,
 legend = TRUE,
 display_numbers = T,
 treeheight_row = 0,
 legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
 legend_labels = (c("-1", "1")),
 main = "Clustering of Gene Expression \n Pearson Correlation (GSE47533)")
```

## Clustering of Gene Expression Pearson Correlation (GSE47533)



## Heatmaps - All datasets Breast Cell-lines (E-GEOD-18494, GSE47533, and GSE41491)

- E-GEOD-18494 2012 / MDA-MB231 / breast / 4h, 8h, 12h / microarray
- GSE41491 2012 / MCF7 / breast / 1h, 2h, 4h, 8h, 12h, 16h, 24h / microarray
- GSE47534 2014 / MCF7 / breast / normoxia, 16h, 32h, 48h / mRNA

```
Imput the mean of all VHL values
mean.vhl <- mean(unlist(expr.GSE47533.hif["VHL",], expr.EGEO18494.hif["VHL",]))
expr.GSE41491.hif["VHL",] <- rep(mean.vhl, 24)

expr.all.hif <- cbind(expr.GSE47533.hif, expr.EGEO18494.hif, expr.GSE41491.hif)

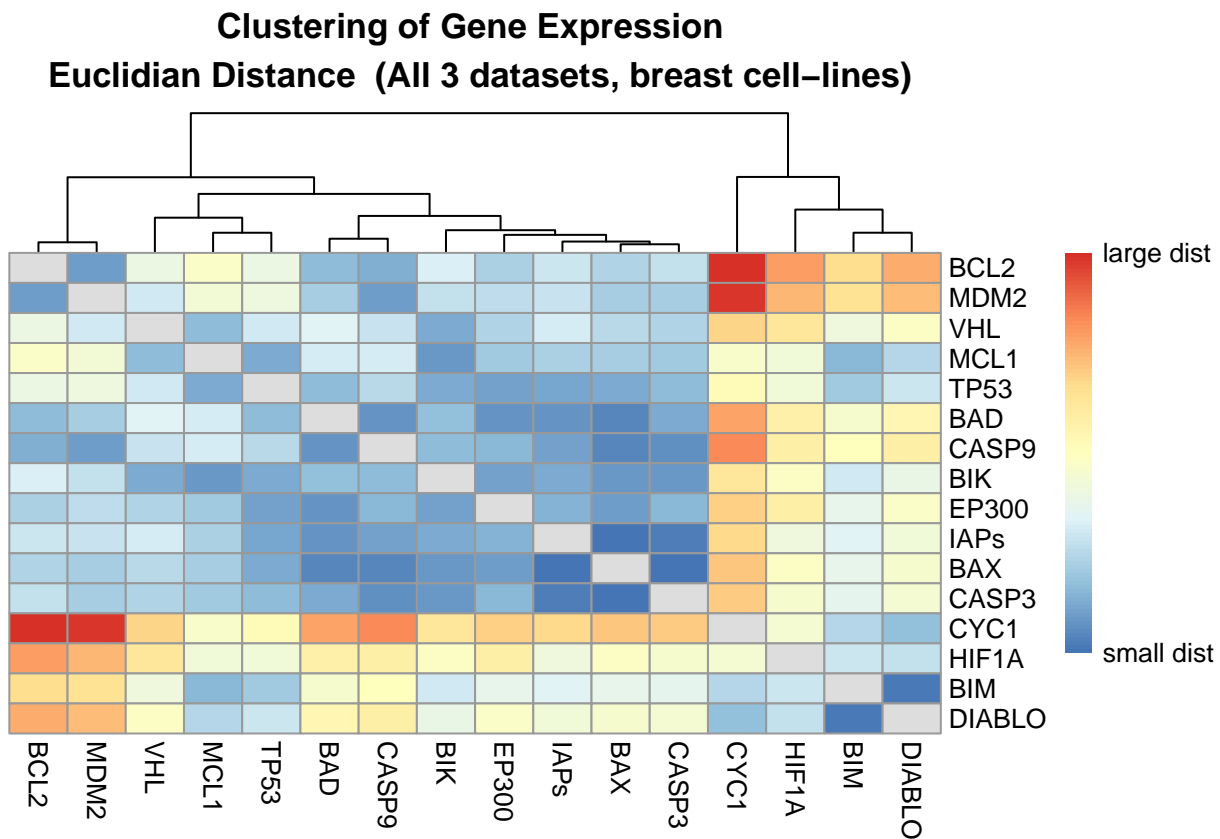
col_brca <- union(data.GSE47533$codes[data.GSE47533$cell_line == "MCF7"],
 union(data.EGEO18494$codes[data.EGEO18494$cell_line == "MDA-MB231 breast cancer"],
 data.GSE41491$codes[data.GSE41491$cell_line == "MCF7"]))

expr.all.hif <- expr.all.hif[, (colnames(expr.all.hif) %in% col_brca)]

dists <- as.matrix(dist(expr.all.hif, method = "euclidean"))
rownames(dists) <- rownames(expr.all.hif)
```

```
colnames(dists) <- rownames(expr.all.hif)
diag(dists) <- NA

pheatmap(dists,
 legend = TRUE,
 treeheight_row = 0,
 legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
 legend_labels = (c("small dist", "large dist")),
 main = "Clustering of Gene Expression \n Euclidian Distance (All 3 datasets, breast cell-lines)
```



```
#-----

col_hypo <- union(data.GSE47533$codes[data.GSE47533$condition == "Hypo"],
 union(data.EGEOD18494$codes[data.EGEOD18494$condition == "hypoxia"],
 data.GSE41491$codes[data.GSE41491$condition == "hy"]))

expr.row <- (colnames(expr.all.hif) %in% col_hypo)
dists <- as.matrix(dist(expr.all.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.all.hif[expr.row])
colnames(dists) <- rownames(expr.all.hif[expr.row])
diag(dists) <- NA

p1 <- pheatmap(dists,
```

```

legend = TRUE,
treeheight_row = 0,
legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
legend_labels = (c("small dist", "large dist")),
main = "Clustering of Gene Expression on Hypoxia \n Euclidian Distance (All 3 datasets, break
silent=T)

#-----

col_norm <- union(data.GSE47533$codes[data.GSE47533$condition == "Norm"],
 union(data.EGEO18494$codes[data.EGEO18494$condition == "normoxia"],
 data.GSE41491$codes[data.GSE41491$condition == "no"]))

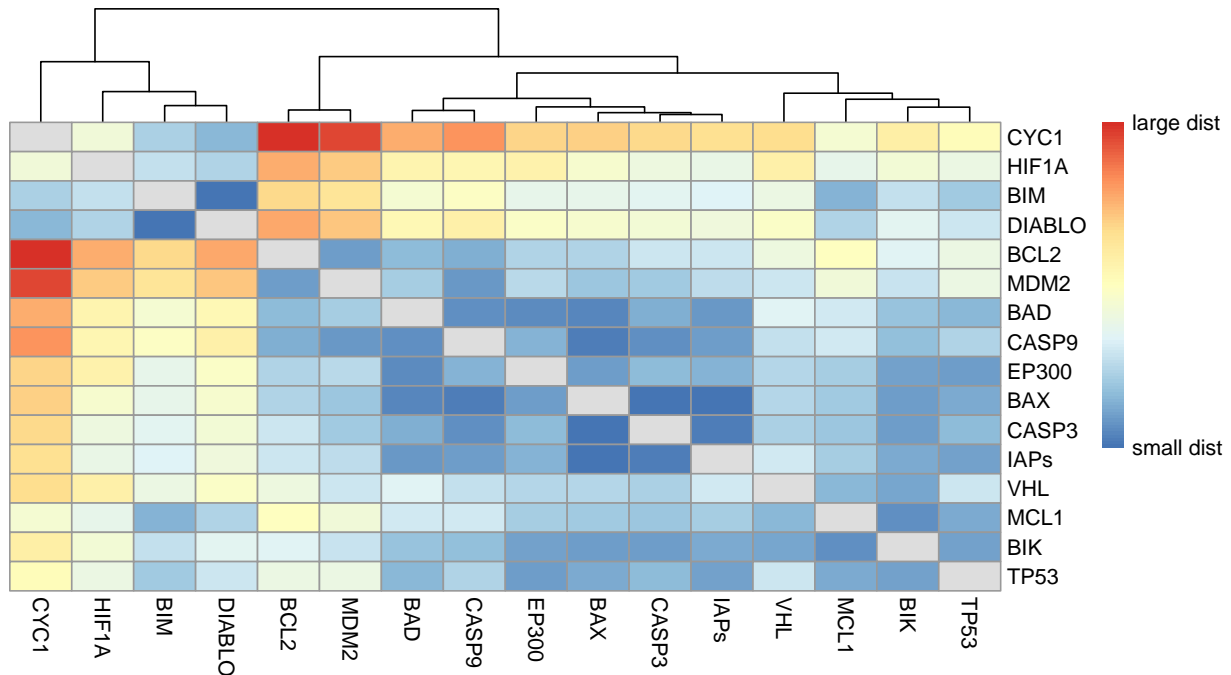
expr.row <- (colnames(expr.all.hif) %in% col_norm)
dists <- as.matrix(dist(expr.all.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.all.hif[expr.row])
colnames(dists) <- rownames(expr.all.hif[expr.row])
diag(dists) <- NA

p2 <- pheatmap(dists,
 legend = TRUE,
 treeheight_row = 0,
 legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
 legend_labels = (c("small dist", "large dist")),
 main = "Clustering of Gene Expression on Normoxia \n Euclidian Distance (All 3 datasets, break
 silent=T)

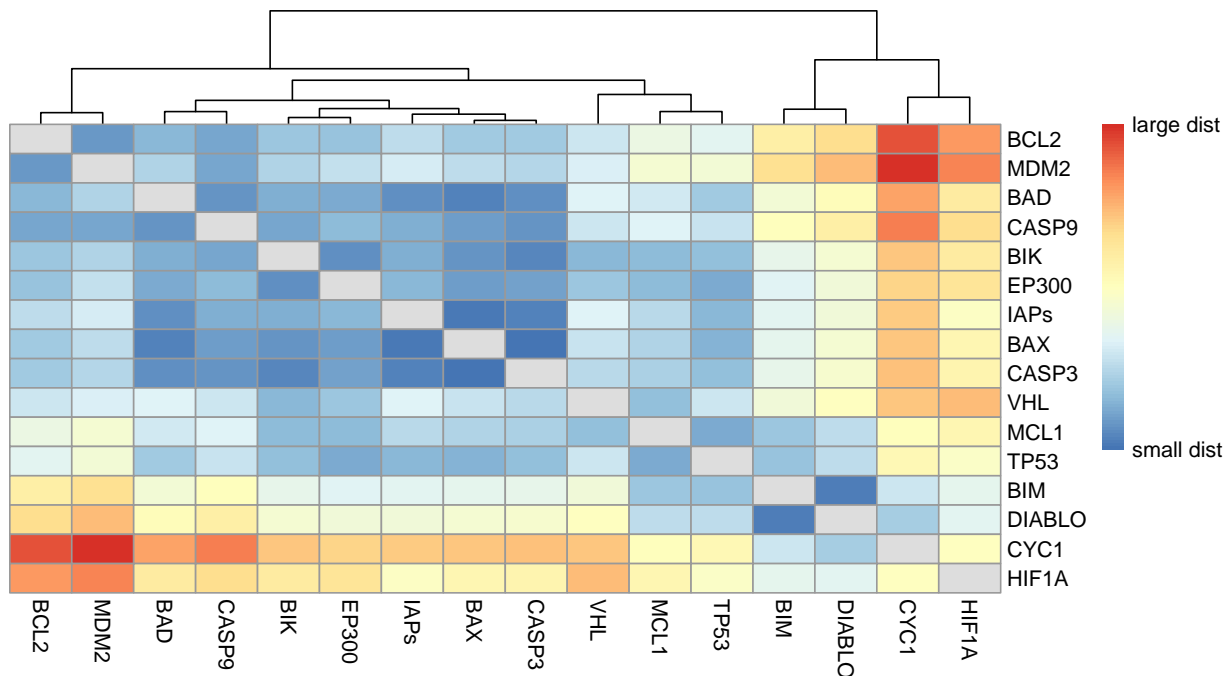
gridExtra::grid.arrange(grobs=list(p1$gtable, p2$gtable),
 nrow = 2 , labels=c('A', 'B'))

```

### Clustering of Gene Expression on Hypoxia Euclidian Distance (All 3 datasets, breast cell-lines)

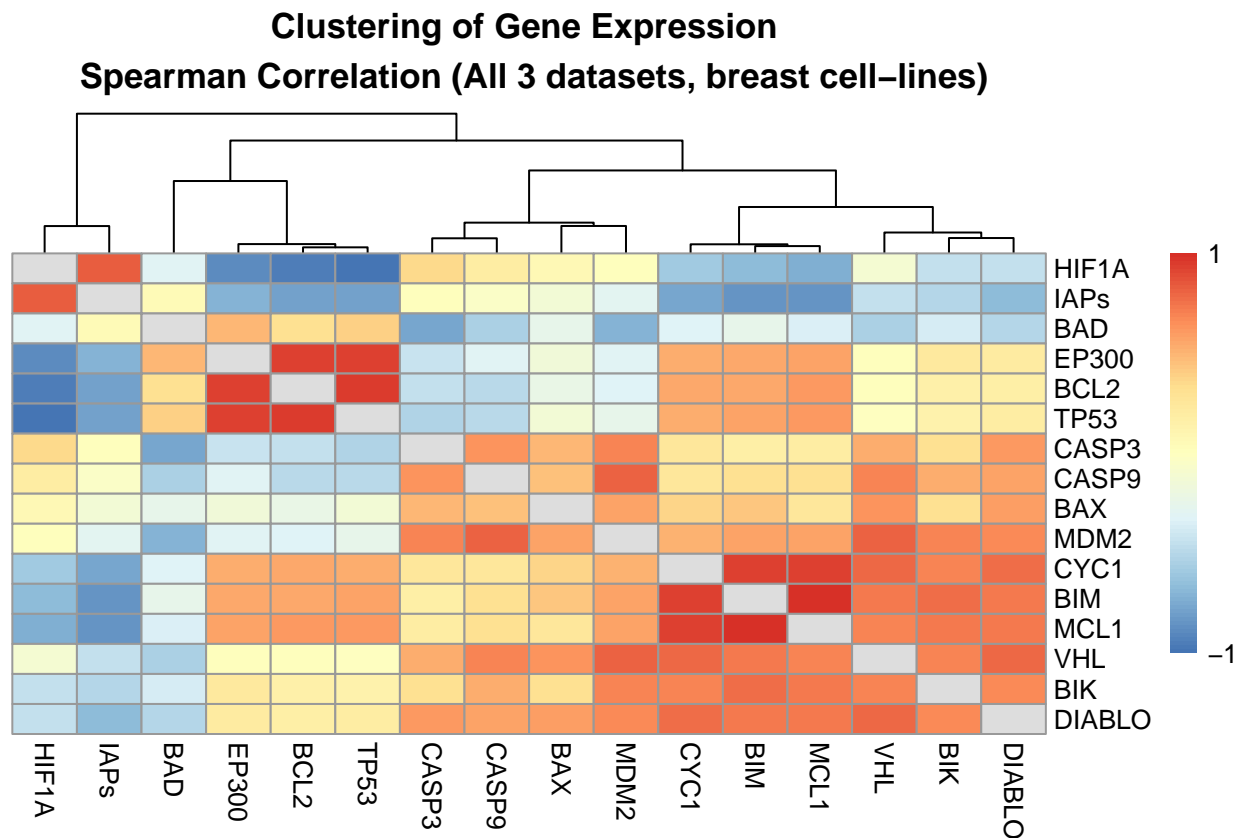


### Clustering of Gene Expression on Normoxia Euclidian Distance (All 3 datasets, breast cell-lines)



```
dists <- cor(t(expr.all.hif), use = "pairwise.complete.obs", method = "spearman")
rownames(dists) <- rownames(expr.all.hif)
colnames(dists) <- rownames(expr.all.hif)
diag(dists) <- NA
```

```
pheatmap(dists,
 legend = TRUE,
 treeheight_row = 0,
 legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
 legend_labels = (c("-1", "1")),
 main = "Clustering of Gene Expression \n Spearman Correlation (All 3 datasets, breast cell-lines)
```



## Heatmaps - All datasets All Cell-lines (E-GEOD-18494, GSE47533, and GSE41491)

- E-GEOD-18494 2012 / HepG2, U87, MDA-MB231 / hepatoma, glioma, breast / 4h, 8h, 12h / microarray
- GSE41491 2012 / DU145, HT29, MCF7 / prostate, colon, breast / 1h, 2h, 4h, 8h, 12h, 16h, 24h / microarray
- GSE47534 2014 / MCF7 / breast / normoxia, 16h, 32h, 48h / mRNA

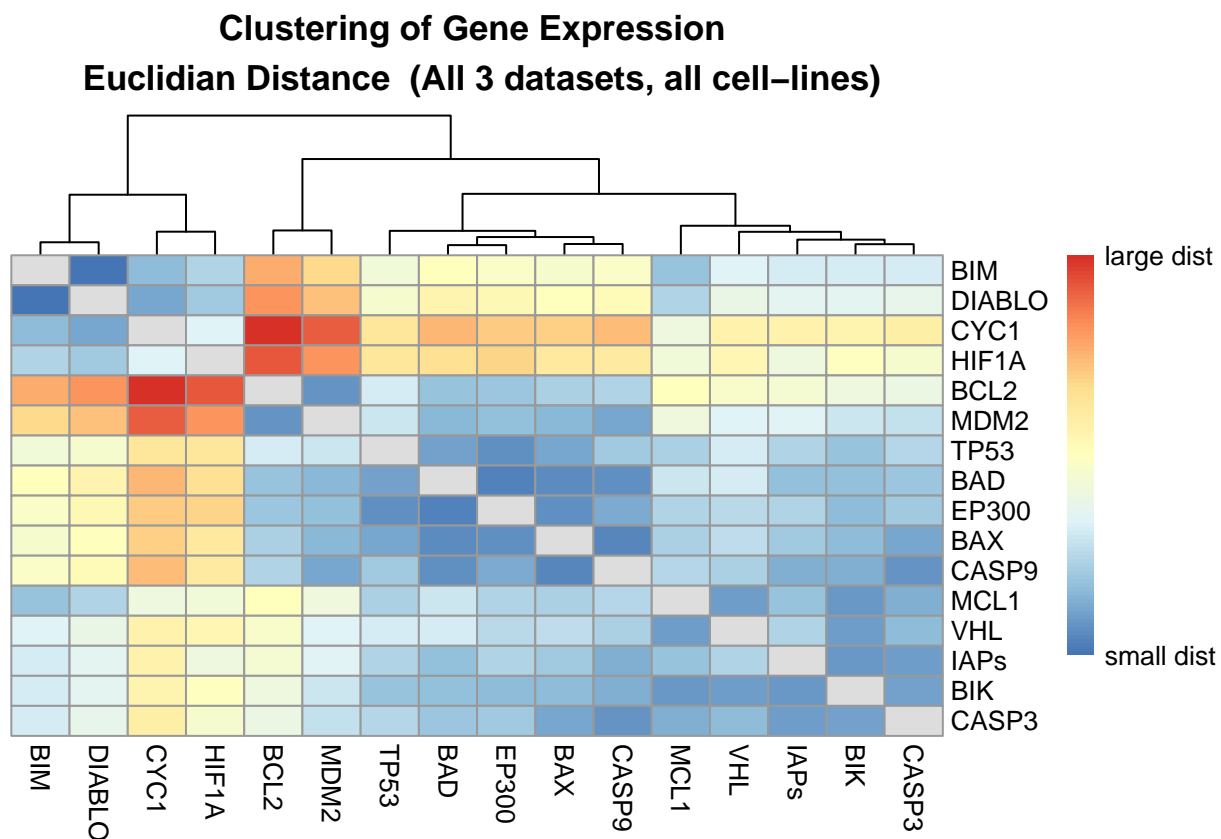
```
Imput the mean of all VHL values
mean.vhl <- mean(unlist(expr.GSE47533.hif["VHL",], expr.EGEOD18494.hif["VHL",]))
expr.GSE41491.hif["VHL",] <- rep(mean.vhl, 24)
```



```
expr.all.hif <- cbind(expr.GSE47533.hif, expr.EGEO18494.hif, expr.GSE41491.hif)
```

```
dists <- as.matrix(dist(expr.all.hif, method = "euclidean"))
rownames(dists) <- rownames(expr.all.hif)
colnames(dists) <- rownames(expr.all.hif)
diag(dists) <- NA
```

```
pheatmap(dists,
 legend = TRUE,
 treeheight_row = 0,
 legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
 legend_labels = (c("small dist", "large dist")),
 main = "Clustering of Gene Expression \n Euclidian Distance (All 3 datasets, all cell-lines)")
```



```
#-----
col_hypo <- union(data.GSE47533$codes[data.GSE47533$condition == "Hypo"],
 union(data.EGEO18494$codes[data.EGEO18494$condition == "hypoxia"],
 data.GSE41491$codes[data.GSE41491$condition == "hy"]))

expr.row <- (colnames(expr.all.hif) %in% col_hypo)
dists <- as.matrix(dist(expr.all.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.all.hif[expr.row])
```

```

colnames(dists) <- rownames(expr.all.hif[expr.row])
diag(dists) <- NA

p1 <- pheatmap(dists,
 legend = TRUE,
 treeheight_row = 0,
 legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
 legend_labels = (c("small dist", "large dist")),
 main = "Clustering of Gene Expression on Hypoxia \n Euclidian Distance (All 3 datasets, all c
 silent=T)

#-----

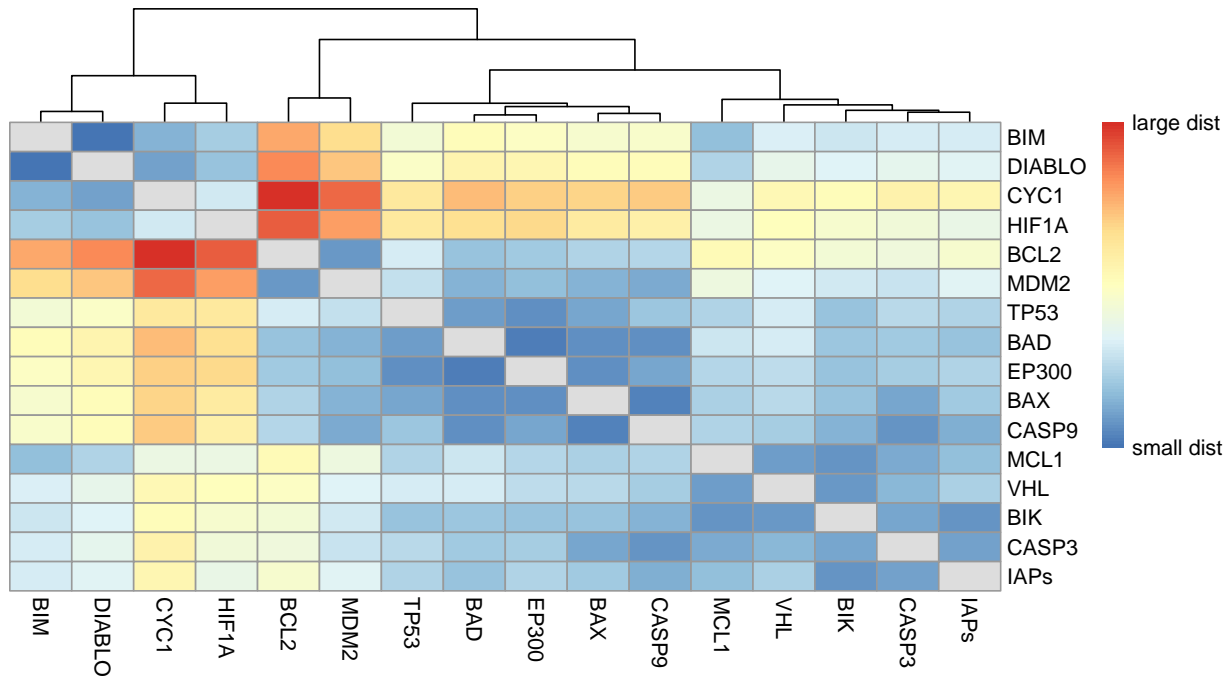
expr.row <- (colnames(expr.all.hif) %in% data.GSE47533$codes[data.GSE47533$condition == "Norm"])
dists <- as.matrix(dist(expr.all.hif[expr.row], method = "euclidean"))
rownames(dists) <- rownames(expr.all.hif[expr.row])
colnames(dists) <- rownames(expr.all.hif[expr.row])
diag(dists) <- NA

p2 <- pheatmap(dists,
 legend = TRUE,
 treeheight_row = 0,
 legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
 legend_labels = (c("small dist", "large dist")),
 main = "Clustering of Gene Expression on Normoxia \n Euclidian Distance (All 3 datasets, all c
 silent=T)

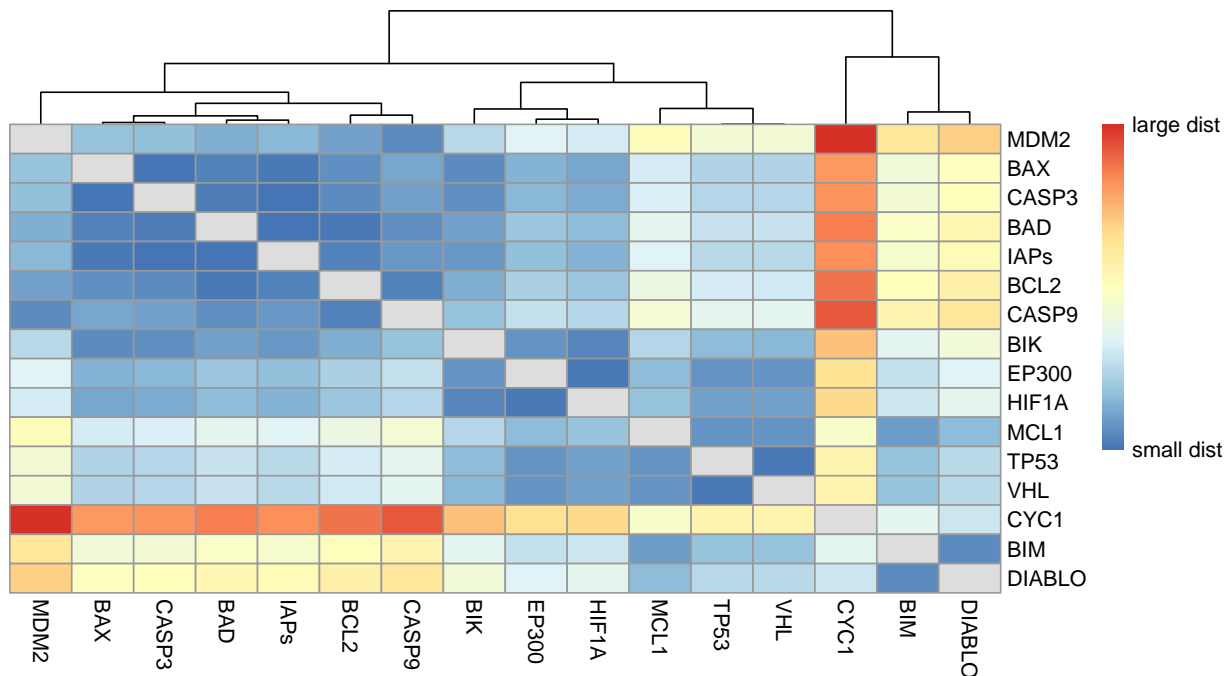
gridExtra::grid.arrange(grobs=list(p1$gtable, p2$gtable),
 nrow = 2 , labels=c('A', 'B'))

```

### Clustering of Gene Expression on Hypoxia Euclidian Distance (All 3 datasets, all cell-lines)



### Clustering of Gene Expression on Normoxia Euclidian Distance (All 3 datasets, all cell-lines)



```
dists <- cor(t(expr.all.hif), use = "pairwise.complete.obs", method = "spearman")
rownames(dists) <- rownames(expr.all.hif)
colnames(dists) <- rownames(expr.all.hif)
diag(dists) <- NA
```

```
pheatmap(dists,
 legend = TRUE,
 treeheight_row = 0,
 legend_breaks = c(min(dists, na.rm = TRUE),
 max(dists, na.rm = TRUE)),
 legend_labels = (c("-1", "1")),
 main = "Clustering of Gene Expression \n Spearman Correlation (All 3 datasets, all cell-lines)
```

