Group 02 - Skin Cancer

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24.07.2019

Preparations

1.Load following packages:

```
library(ggplot2)
library(relaimpo)
library(factoextra)
library(gridExtra)
library(reshape2)
library(data.table)
library(cluster)
library(rstudioapi)
library(pheatmap)
library(caret)
library(tidyverse)
library(dendextend)
library(factoextra)
library(devtools)
library(ggfortify)
library(rstudioapi)
library(data.table)
library(ggplot2)
library(scales)
library(stats)
library(caTools)
```

3. Setting the sys-path and loading the data:

```
root.dir = dirname(rstudioapi::getSourceEditorContext()$path)
data = readRDS(paste0(root.dir, "/DepMap19Q1_allData.RDS"))
```

2. Loading the dataset:

```
data = readRDS("C:/Users/LeoTh/Documents/GitHub/project-01-group-
02/DepMap19Q1_allData.RDS")
```

Part 1: Data Cleanup

1.1 Extracting and splitting our data

The mutation data is different from the other matrices, so we define a new matrix only containing the mutation data

```
mut <- data$mutation
```

Additionally to the mutation matrix we want another matrix only containing all data exept the mutation data.

```
'%!in%' <- function(x,y)!('%in%'(x,y))  #We define an operator that will only pick the data that is NOT defined in the list; so the data that needs to be excluded
dt_new <- lapply(which(names(data) %!in% "mutation"), function(a) data[[a]]) #extracting the non-mutation data
names(dt_new) <- names(data)[which(names(data) %!in% "mutation")] #rename the data with the original names
```

Defining which samples we will take out of the original dataset

```
sample_case = c("Skin Cancer")
```

We look at the annotation matrix and search only for the Primary diseases which match the previous defined sample_case. We are looking at the column which tells us to which cancer type this cell line belongs and only taking the cell lines for which the sample case ist true. We are getting a vector of all the cell line names we want to look at

```
samples = data$annotation$DepMap_ID[which(data$annotation$Primary.Disease == sample_case)]
```

We extract all cell lines we defined in the previous step out of our data (except the mutation matrix)

```
processed_data <- lapply(1:length(dt_new), function(a) { #picking the data for our sample
  dat_picker <- dt_new[[a]] #picking one file at each iteration
  if(names(dt_new[a])== "annotation"){ # treating the annotations differnetly because the cell line names are in a colum and are not the columnames like in the other matrices
    output <- dat_picker[which(dat_picker[,1] %in% samples),]
  } else {
  output <- dat_picker[,which(colnames(dat_picker) %in% samples)]# only taking the skin cancer cell lines
  output <- output[complete.cases(output),] # only taking rows without NAs
  output <- output[order(rownames(output)),] # reordering the Genes acording to their name
  }
  return(output)
})
names(processed_data) <- names(dt_new) # rename the objects according to the original data
rm(dt_new,sample_case) # remove objects we don`t need anymore
```

Now we extract our cellines from the mutation data

```
ids = which(names(mut) %in% samples)
allDepMap_mutation_SkinCancer = lapply(ids, function(a) {
```

```
  mut[[a]]})
rm(mut, ids, data) #tidying
```

Losing the mutations which are not deleterious meaning not interesting to us

```
allDepMap_mutation_SkinCancer = lapply(1:34, function(a) {

allDepMap_mutation_SkinCancer[[a]][which(allDepMap_mutation_SkinCancer[[a]][,
"isDeleterious"]== TRUE), ]
    })
names(allDepMap_mutation_SkinCancer) <- samples
```

losing all the genes which are not in every data frame First we need to pick all Gene names we have out of our data

```
Genenames <-
unique(c(rownames(processed_data[[1]]),rownames(processed_data[[2]]),rownames
(processed_data[[3]]),rownames(processed_data[[4]])))
```

Then we pick these genes which are in all 4 dataframes we need for further analysis

```
i <- 1
out <- vector("character", length(seq_along(1:16970)))
for (x in seq_along(Genenames)) {
  if(Genenames[x] %in% rownames(processed_data$expression) & Genenames[x]
%in% rownames(processed_data$copynumber) & Genenames[x] %in%
rownames(processed_data$kd.ceres) & Genenames[x] %in%
rownames(processed_data$kd.prob))
  {out[i] <- Genenames[x]
  i <- i+1
  }
}

allDepMap_annotation_SkinCancer <- processed_data$annotation # saving the
annotation object in a seperate dataframe
# because it doesnt contain any information about the genes

processed_data <- lapply(processed_data[1:4], function(a) {
  a <- a[which(rownames(a) %in% out),]
  return(a)
})

processed_data$mutation <- allDepMap_mutation_SkinCancer
processed_data$annotation <- allDepMap_annotation_SkinCancer
rm(i,out, Genenames,x, allDepMap_annotation_SkinCancer, samples,
allDepMap_mutation_SkinCancer)
```

Part 2: Data vizualisation

2.1 Preparing our data for plotting

2.1.1 Extracting our data for plotting

We will not need all our data for plotting so we have to prepare our data for the following plots.

```
generalPlottingData <- lapply(1:(length(processed_data)-2), function(a) { #
we will not need annotation
  dtPicker <- processed_data[[a]]
  out <- melt(dtPicker) #bind the data togehter that we have samples and
values as columns
  out$Gene <- rep(rownames(dtPicker), ncol(dtPicker)) #add the genes;
probably this might be useful in a later stage
  out$Case <- names(processed_data)[1:(length(processed_data)-1)][a] #add a
labelling column
  colnames(out) <- c("Sample", "Value", "Gene", "Case") #rename the columns
  return(out)
})
```

```
## No id variables; using all as measure variables
## No id variables; using all as measure variables
## No id variables; using all as measure variables
## No id variables; using all as measure variables
```

```
names(generalPlottingData) <-
names(processed_data)[1:(length(processed_data)-2)] #rename the data
```

2.1.2 Plotting Data - Driver Mutations

Producing a vector which encompases every gene which at least mutated once

```
singleGenes <-
as.vector(unique(as.data.frame(rbindlist(lapply(seq_along(processed_data$muta
tion), function(a) {
  out <-
as.data.frame(as.vector(unique(processed_data$mutation[[a]]$Hugo_Symbol))))}))
))[,1])
```

Creating a dataframe which contains how often every gene is mutated

```
geneCounts <- sapply(seq_along(singleGenes), function(a) {
  genePicker <- singleGenes[a] #pick one gene
  sumGene <- lapply(seq_along(processed_data$mutation), function(b) {
    mutPicker <- processed_data$mutation[[b]] #pick one of the 34 mutation
lists
    out <- as.data.frame(length(which(mutPicker$Hugo_Symbol == genePicker)))
#look how often an entry is in the mutation list
    return(out)
  })
```

```
  geneCount <- colSums(as.data.frame(rbindlist(sumGene))) #sum it up to get
the total count for each gene
  return(geneCount)
})
names(geneCounts) <- singleGenes #rename
geneCounts <- as.data.frame(geneCounts) #make a nice dataframe
colnames(geneCounts) <- c("Value")
geneCounts <- geneCounts[order(-geneCounts$Value), , drop = FALSE] #sort the
data frame
head(geneCounts)
```

```
##         Value
## TTN        13
## TP53        9
## HMCN1       8
## TMTC2       7
## RYR2        7
## CACNA1I     7
```

Extacting the data for the top 10 which will be our driver mutations in our further investigation

```
dataTopDriverGenes <- lapply(1:(length(processed_data)-2), function(a) {
#picking the data for our sample
  dat_picker <- processed_data[[a]] #pick one file at each iteration
  output <- dat_picker[which(rownames(dat_picker) %in%
rownames(geneCounts)[1:10]),] # compare the rownames of the picked data with
the names of the 10 most mutated genes
  return(output)
})
names(dataTopDriverGenes) <- names(processed_data)[1:4]

rm(singleGenes)
```

2.1.3 Extracting the drivermutations for every Celline

Putting all mutation data in one Matrix

```
OneMatrix <- data.frame()
for (i in c(1:34)) {
  OneMatrix <-
rbind(OneMatrix,processed_data$mutation[[i]][,Hugo_Symbol:DepMap_ID])
}
```

Extracting just the column of the Gene name and the cell line

```
CelllinesMutations <- OneMatrix[which(OneMatrix$Hugo_Symbol %in%
rownames(geneCounts)[1:10] ),]
CelllinesMutations <- cbind(CelllinesMutations$Hugo_Symbol,
CelllinesMutations$DepMap_ID)
```

Extracting the drivermuations for every cell line out of the dataframe and putting it into another dataframe so it can be used for the plotting

```
Genes <- c("COL11A1,TMTC2,TTN", " HMCN1", "COL11A1,HMCN1,SLC510",
"HMCN1,TMTC2", "COL11A1,TP53,TTN","none","ZNF292","RYR2","HMCN"
,"none2","none3", "TP53, TTN","HMCN1", "TTN,ZNF292","TMTC2,TP53,NEB","TP53",
"TMTC2,NEB","none4","TMTC2,TTN,ZNF292",
"none5","CACNA1I","HMCN1,TP53,ZNF292","none6","none7","HMCN1,TMTC2,ZNF292","R
YR2,TMTC2,NEB","RYR2,NEB,TTN,CACNA1I","HMCM1,TP53","TTN","COL11A1,SLC5A10","C
OL11A1,CACNA1I","TTN,CACNA1I","RYR2,CACNA1I,ZNF292","TP53,TTN,CACNA1I" )
Zelllines <- c(colnames(processed_data$expression))
zellinesMutations <- as.data.frame(cbind(Zelllines, Genes))

rm(OneMatrix, Genes, ZelllinesMutations, Zelllines,i)

## Warning in rm(OneMatrix, Genes, ZelllinesMutations, Zelllines, i): Objekt
## 'ZelllinesMutations' nicht gefunden
```

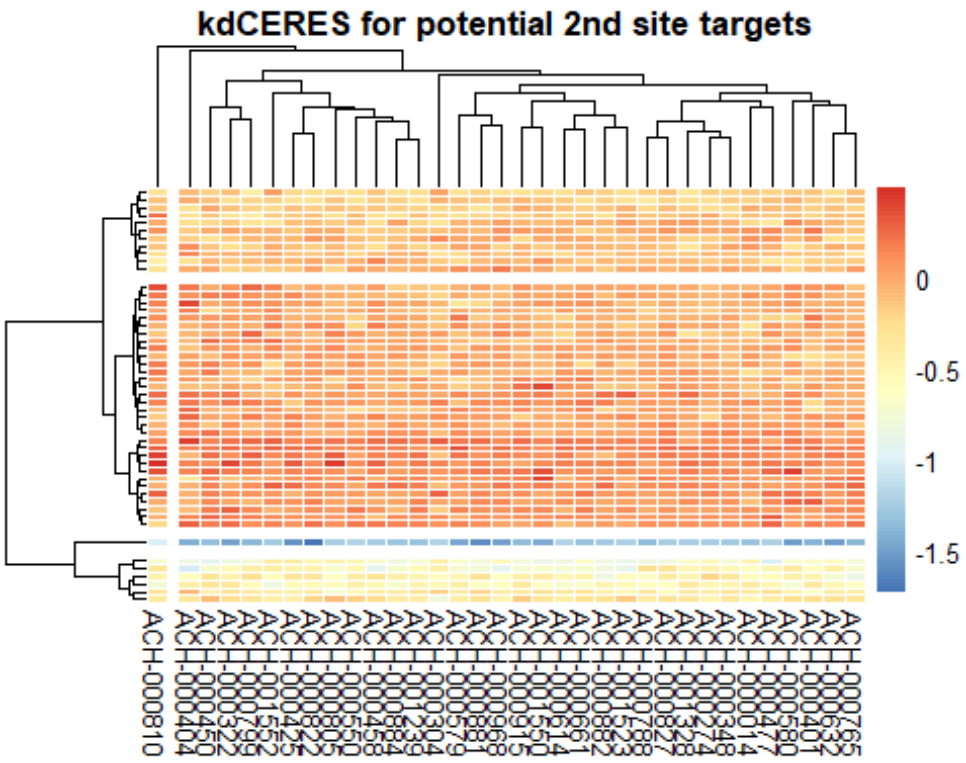
The explanation for the previous extraction will be outlined in the following visualization part

2.2 Visualizing our data

2.2.1 Heatmap with the knock down data

We start with a heatmap of our knock down data (the kd.ceres matrix) This matrix consist of gene knockdown scores. The impact of the knocked out gene on the cell survival is reflected by that score. The impact can be a reduction or an increase in proliferation. It could also mean that there is no change in cell proliferation at all. Smaller values refer to higher importance.

```
pheatmap(as.matrix(processed_data$kd.ceres[1:50,]), clustering_method =
"ward.D2",border_color = "white", fontsize = 10,
         main = paste0("kdCERES for potential 2nd site targets"),
         show_rownames = F, show_colnames = T,
         cutree_rows = 4,
         cutree_cols = 2,
         fontsize_row=10)
```

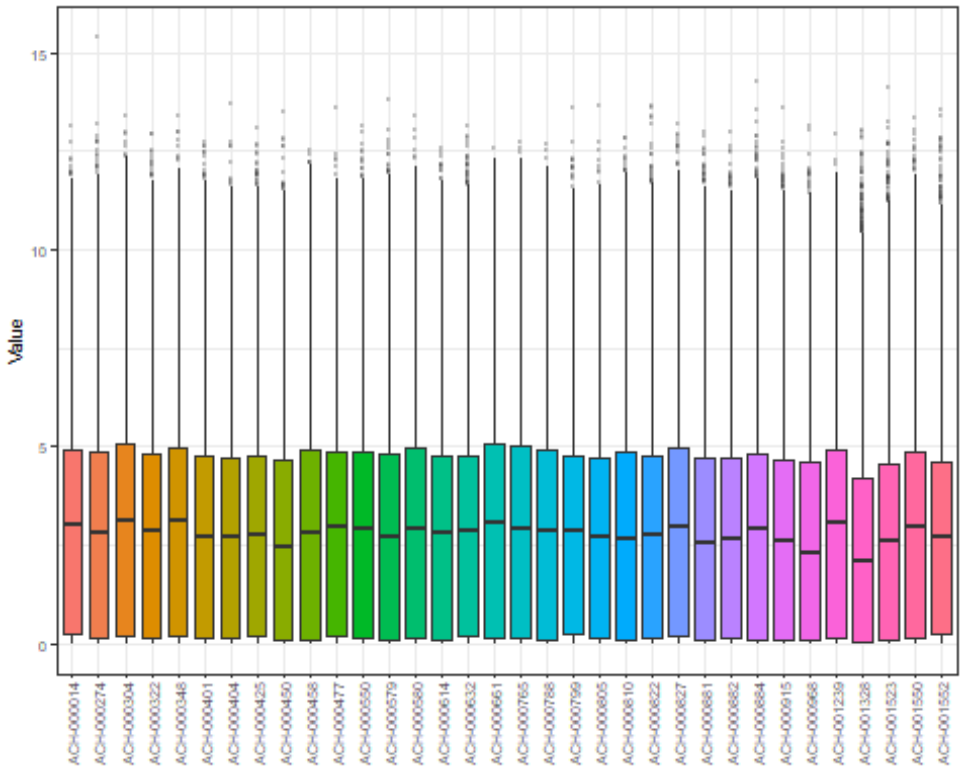


We can see that there are clear differences between the knockdown data depending on the knocked out gene in a specific cell. The cell lines behave differently when the sme gene is knocked out.

2.2.1 Distribution of the expression values between the different cell lines

We create a boxplot with the expression matrix to see how the expression of the genes is distributed over the different cell lines.

```
ggplot(data = generalPlottingData$expression, aes(x=Sample, y=Value)) +
  geom_boxplot(aes(fill = Sample), outlier.size = 0.1, outlier.alpha = 0.2) +
#reconstruct the outliers a bit (so reduce them in size; because we are
interested in the boxplots and not the outliers)
  theme_bw(base_size = 7) + #format the size of the theme nicely
  theme(legend.position= "none", #define the legend position (here no leghend
will be needed)
        legend.direction="horizontal", #define the legend direction if one is
there
        plot.title = element_text(hjust = 0.5), #make the title of the plot
into the middle
        axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1), #define
the orientation of the text on the x-axis
        legend.title= element_blank(), #no title of the legend should be
plotted
        axis.title.x = element_blank(), #no title of the x-axis is relevant;
because that would be samples and that is cleare due to the naming
        strip.text.y = element_text(angle = 90)) #define the orientation of
the text of the y-axis
```



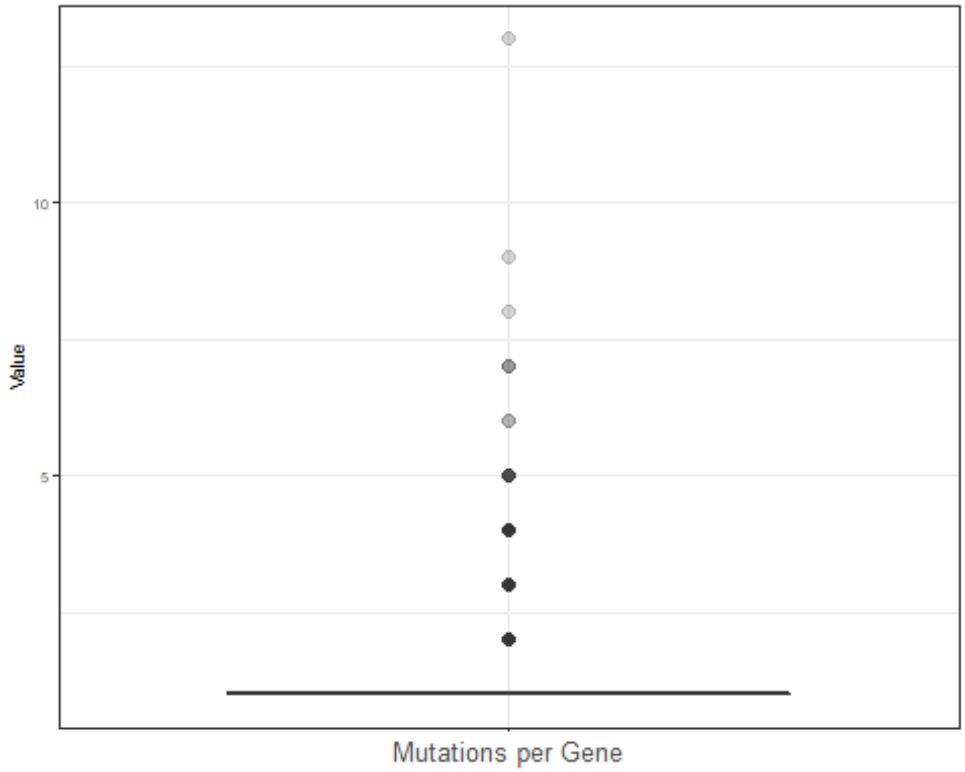
Many genes are distributed between the 25 and 75 quantile. But there are also some outliers which are of special interest for us in the following data analysis. For now we can say that the data is differnetly distributed between the celllines.based on different mutations in the different cell lines.

2.2.1 Plotting how often a gene is mutated over all Cell

This plot should show us the mutation rate of a gene

```
geneCounts <- cbind(geneCounts, "Mutations per Gene")

ggplot(data = geneCounts, aes(x="Mutations per Gene", y=Value)) +
  geom_boxplot(aes(fill = "Mutations per Gene"), outlier.size = 2,
outlier.alpha = 0.2) + #reconstruct the outliers a bit (so reduce them in
size; because we are interested in the boxplots and not the outliers)
  theme_bw(base_size = 7) + #format the size of the theme nicely
  theme(legend.position= "none", #define the legend position (here no leghend
will be needed)
        legend.direction="horizontal", #define the legend direction if one is
there
        plot.title = element_text(hjust = 0.5), #make the title of the plot
into the middle
        axis.text.x = element_text(angle = 0, vjust = 0.5, hjust= 0.5, size =
10), #define the orientation of the text on the x-axis
        legend.title= element_blank(), #no title of the legend should be
plotted
        axis.title.x = element_blank(), #no title of the x-axis is relevant;
because that would be samples and that is cleare due to the naming
```

```
        strip.text.y = element_text(angle = 90)) #define the orientation of
the text of the y-axis
```

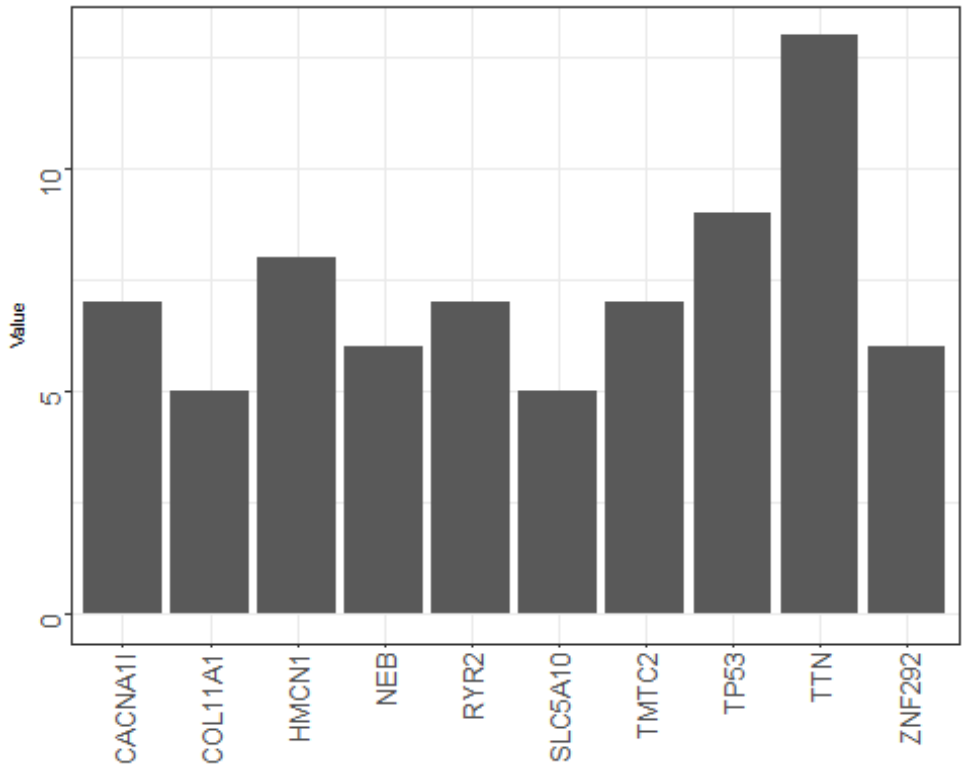


So we have seen that the expression values show a different distribution for the different cell lines. Different expression values can arise from gene mutaitons of specific genes So the question is if there are mutations occuring more often than others. We suspect that these may be one of the reasons for the differing expression values. So as we can see: yes there are Mutations which occure significantly more often

Now we want to see which Mutations are the top 10 mutated Genes These 10 Genes will be our driver genes of which we want to identify interactions with other genes.

```
plotData <- geneCounts[1:10, ,drop = FALSE]

plotData$Gene <- rownames(plotData)

ggplot(data = plotData) +
  (geom_bar(mapping = aes(x = Gene, y = Value), stat = "identity")) +
  theme_bw(base_size = 7) + #format the size of the theme nicely
  theme(legend.position= "none", #define the legend position (here no legend
will be needed)
        legend.direction="horizontal", #define the legend direction if one is
there
        plot.title = element_text(hjust = 0.5), #make the title of the plot
into the middle
        axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1, size =
10), #define the orientation of the text on the x-axis
        axis.text.y = element_text(angle = 90, vjust = 0.5, hjust=1, size =
```

```
10), #define the orientation of the text on the x-axis
        legend.title= element_blank(), #no title of the legend should be
plotted
        axis.title.x = element_blank(), #no title of the x-axis is relevant;
because that would be samples and that is cleare due to the naming
        strip.text.y = element_text(angle = 90)) #define the orientation of
the text of the y-axis
```




```
rm(plotData)
```

3. Dimensionality reduction

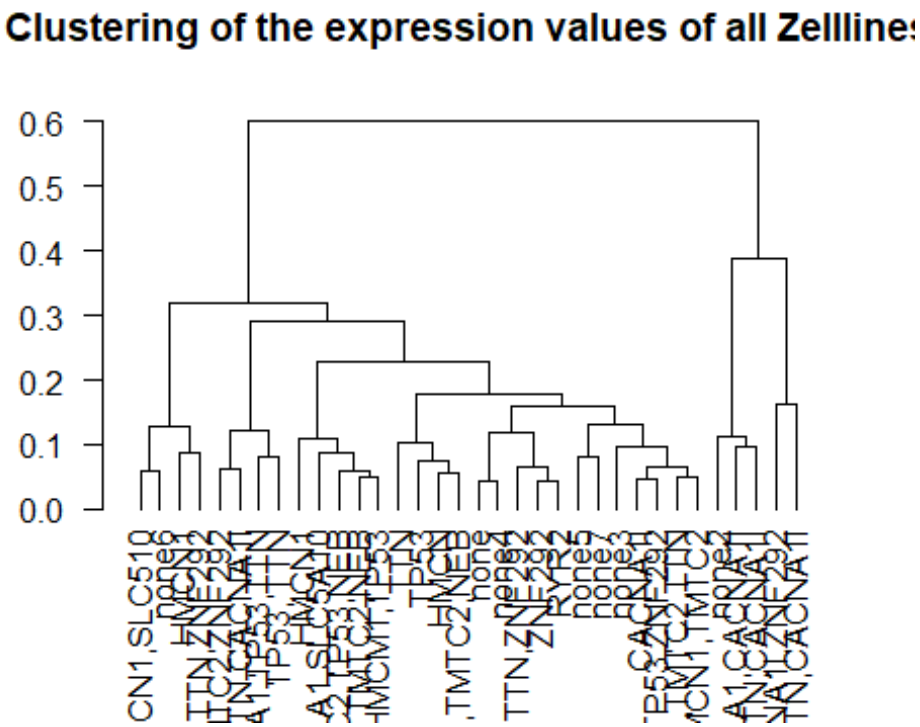
General questions: * can we group the different Driver mutations together so that we can see in which other genes the Cell lines with a specific driver mutation differentiate * through that we could gain insight which other genes are our secound targets

3.1 Hierachical clustering

Creating a hierachical cluster with our driver mutations

```
drivergene <- 3
# determines which of the driver mutations will be seen in the cluster at the
x axis
dataset <- processed_data$expression # determines which dataset we use

colnames(dataset)[which(colnames(dataset) %in%
```

```
unique(zellinesMutations[which(zellinesMutations[,1] ==
rownames(geneCounts)[drivergene]),2]))] <- rownames(geneCounts)[drivergene]
colnames(dataset) <- zellinesMutations$Genes

cor.mat = cor(dataset[1:50,], method = "spearman")
cor.dist = as.dist(1 - cor.mat)
cor.hc = hclust(cor.dist, method = "ward.D2")
cor.hc = as.dendrogram(cor.hc)
plot(cor.hc, las = 2, cex.lab = 2, main = "Clustering of the expression
values of all Zelllines")
```




```
rm(drivergene, realcelllinenames, dataset, cor.hc, cor.mat, cor.dist)

## Warning in rm(drivergene, realcelllinenames, dataset, cor.hc, cor.mat,
## cor.dist): Objekt 'realcelllinenames' nicht gefunden
```

3.2 K-means

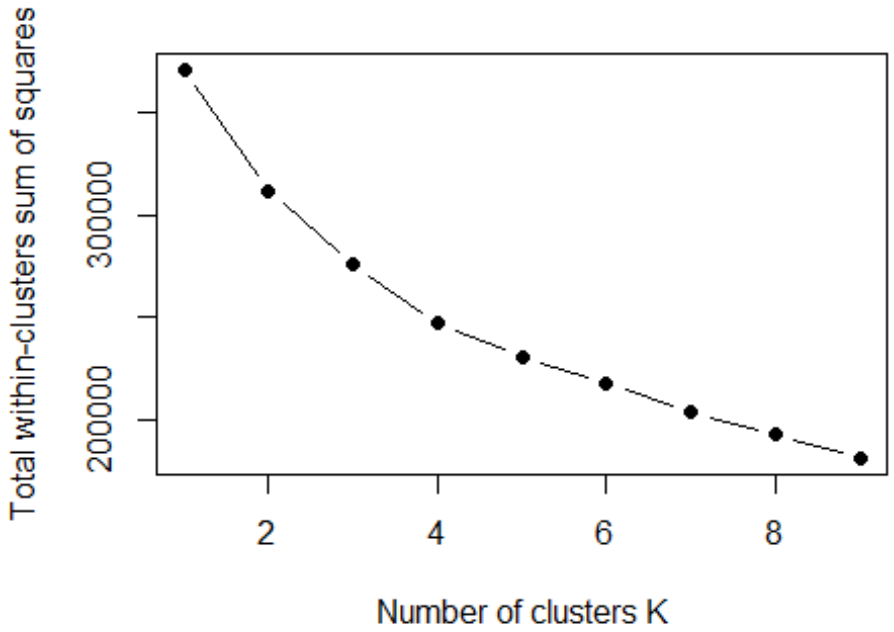
Performing a k-means

```
dataset <- t(processed_data$expression[-
which(rownames(processed_data$expression) %in% rownames(geneCounts)[1:10]),])
# determines which dataset we use
# we are trying to cluster the cell lines with the same drivermutations in
the same cluster according to the
# expression data without the expression of the Drivermutaitons
# because we want to see what is driving the differences betweent the cell
```

```
lines except for the Drivermutation expression values

rownames(dataset) <- zellinesMutations$Genes

dataset <- dataset[,-which(apply(dataset, 2, function(x) {
  var(x)
}) == 0)]
```

For choosing the best number centers for the clusters we try the kink method

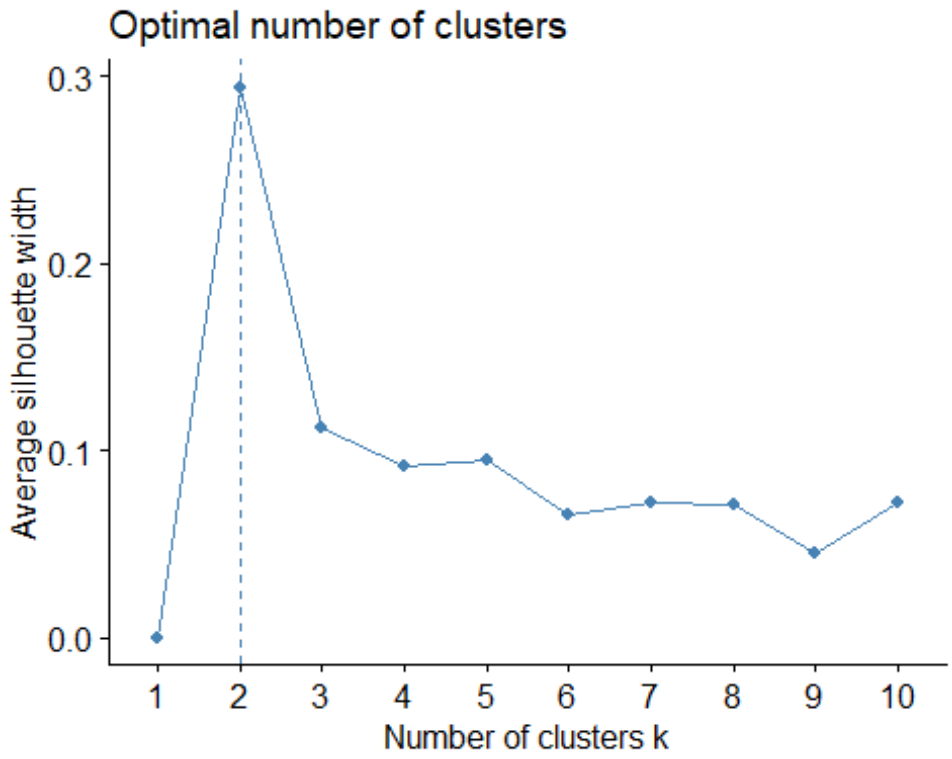
```
wss = sapply(1:9, function(k) {
  kmeans(x = dataset, centers = k)$tot.withinss
})
plot(1:9, wss, type = "b", pch = 19, xlab = "Number of clusters K", ylab =
"Total within-clusters sum of squares")
```



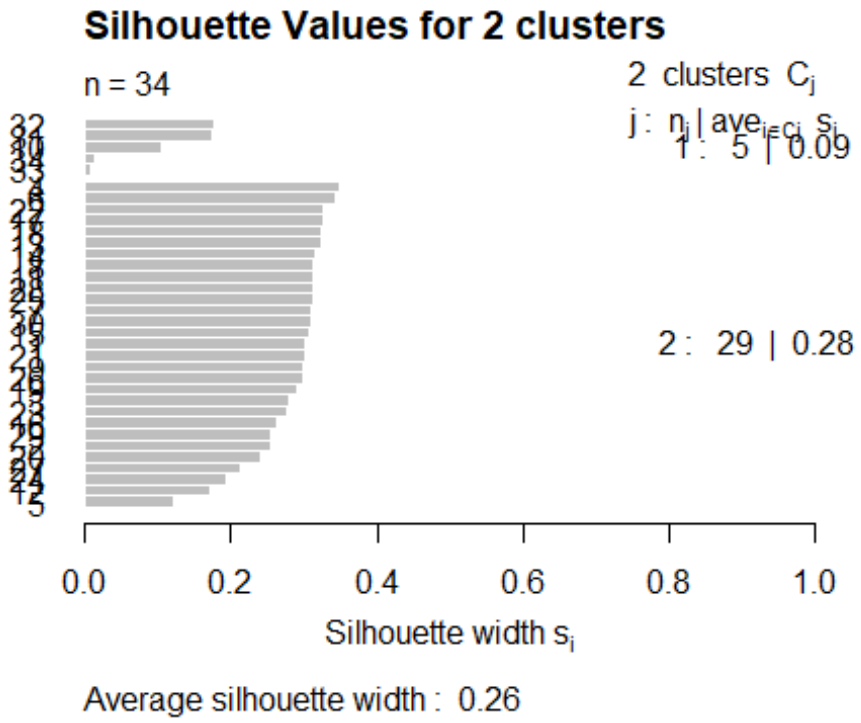
But theres no kink in this curve so we need to use other methods to tell us how much centers to choose

Now we try the silouette method

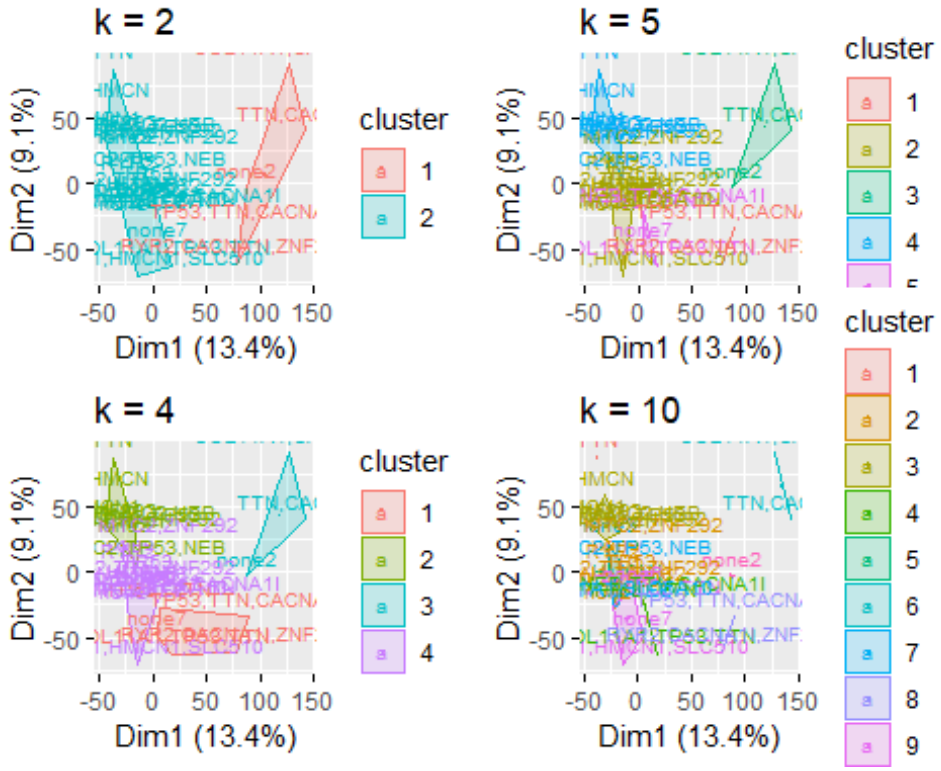
```
fviz_nbclust(dataset, kmeans, method = "silhouette")
```



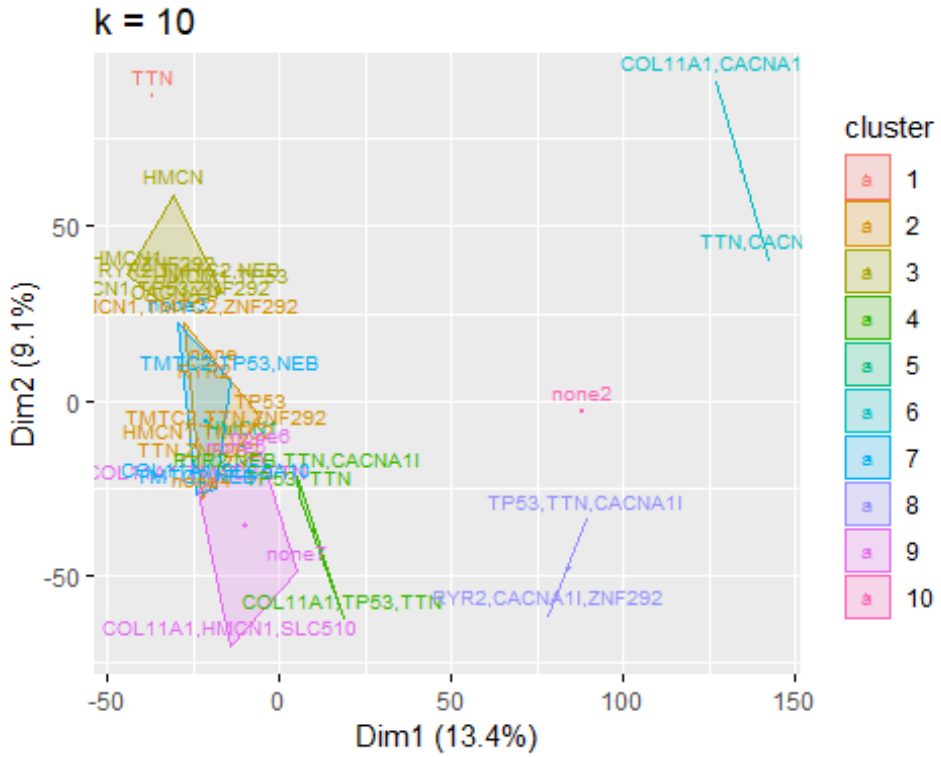

```r
# the clustering with two centers seems to be the best by far according to
the

# were taking a look at the silhouette values for the clustering with two
centers and
km = kmeans(x =dataset, centers = 2, nstart = 100)
plot(silhouette(km$cluster,dist(dataset)), main = "Silhouette Values for 2
clusters")
```




```
km2 <- kmeans(dataset, centers = 2, nstart = 100)
km3 <- kmeans(dataset, centers = 5, nstart = 100)
km4 <- kmeans(dataset, centers = 4, nstart = 100)
km5 <- kmeans(dataset, centers = 10, nstart = 100)

p1 <- fviz_cluster(km2,geom = "text", labelsize = 8, data = dataset) +
ggtitle("k = 2")
p2 <- fviz_cluster(km3, geom = "text", labelsize = 8, data = dataset) +
ggtitle("k = 5")
p3 <- fviz_cluster(km4, geom = "text", labelsize = 8, data = dataset) +
ggtitle("k = 4")
p4 <- fviz_cluster(km5, geom = "text", labelsize = 8, data = dataset) +
ggtitle("k = 10")

grid.arrange(p1, p2, p3, p4, nrow = 2)
```

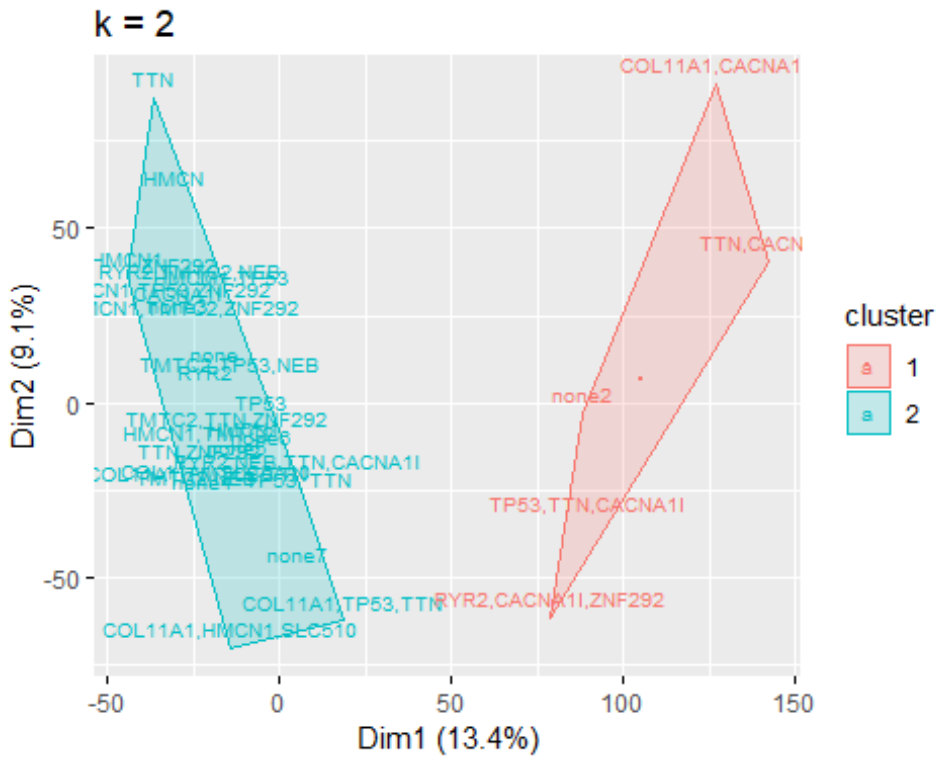



```
plot(p4) # clearly the clustering with 10 centers does not conclude in clusters with the same Drivermutations
```




```
# the reason for that may be that most of our cell lines have more than one Driver mutation
```

```
plot(p1)
```




```
rm(km,km2,km3,km4,km5,p1,p2,p3,p4, dataset,wss)
```

The clustering with two centers seems to be the best one. Our next step in the pca will be to see which of the genes drive the differentation of the celllines in this plot because they will be the most variable and thus most interesting ones.

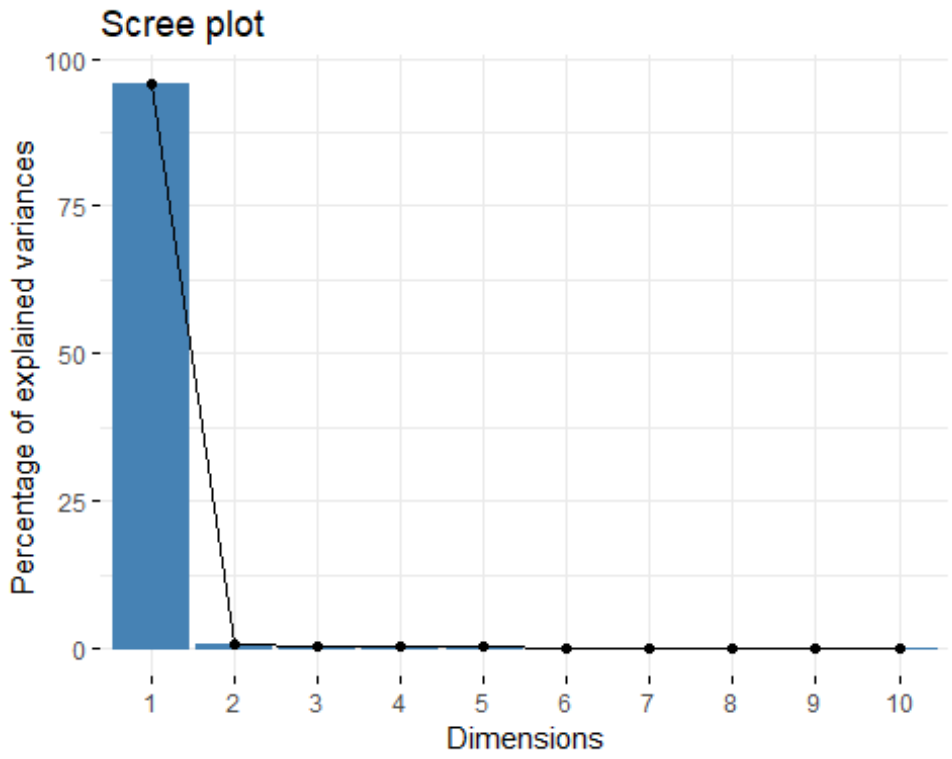
3.23 PCA

Now after we saw how the data clustered together we want to see what is driving the differences. We are looking at the first two Principal Components

```
#drivergene <- 4 # determines which of the Drivermutations will be seen in
the cluster at the x axis
dataset <- processed_data$expression # determines which dataset we use


#colnames(dataset)[which(colnames(dataset) %in%
unique(ZelllinesMutations[which(ZelllinesMutations[,1] ==
topDriverGenes[drivergene]),2]))] <- topDriverGenes[drivergene]
colnames(dataset)<- zellinesMutations$Genes

pca = prcomp(t(dataset), center = F, scale. = F)
summary(pca)
```

```
## Importance of components:
##                             PC1      PC2      PC3      PC4      PC5
## Standard deviation     495.3869 46.27533 35.11722 26.07913 24.64502
## Proportion of Variance   0.9573  0.00835  0.00481  0.00265  0.00237
## Cumulative Proportion    0.9573  0.96567  0.97048  0.97313  0.97550
##                             PC6      PC7      PC8      PC9     PC10
## Standard deviation     21.01126 20.73196 19.77350 19.31929 17.76823
## Proportion of Variance  0.00172  0.00168  0.00153  0.00146  0.00123
## Cumulative Proportion   0.97723  0.97890  0.98043  0.98188  0.98312
##                            PC11    PC12     PC13     PC14     PC15
## Standard deviation     17.71421 16.8245 16.18356 15.71160 15.43367
## Proportion of Variance  0.00122  0.0011  0.00102  0.00096  0.00093
## Cumulative Proportion   0.98434  0.9854  0.98647  0.98743  0.98836
##                            PC16     PC17     PC18     PC19     PC20
## Standard deviation     15.26658 14.96113 14.46341 13.90066 13.62804
## Proportion of Variance  0.00091  0.00087  0.00082  0.00075  0.00072
## Cumulative Proportion   0.98927  0.99014  0.99096  0.99171  0.99243
##                            PC21     PC22     PC23     PC24     PC25
## Standard deviation     13.48726 13.20117 13.08648 12.66417 12.34321
## Proportion of Variance  0.00071  0.00068  0.00067  0.00063  0.00059
## Cumulative Proportion   0.99314  0.99382  0.99449  0.99512  0.99571
##                            PC26     PC27     PC28     PC29     PC30
## Standard deviation     11.96792 11.74491 11.66818 11.40272 11.21416
## Proportion of Variance  0.00056  0.00054  0.00053  0.00051  0.00049
## Cumulative Proportion   0.99627  0.99681  0.99734  0.99785  0.99834
##                            PC31     PC32     PC33    PC34
## Standard deviation     10.83176 10.65546 10.26134 9.49512
## Proportion of Variance  0.00046  0.00044  0.00041 0.00035
## Cumulative Proportion   0.99879  0.99924  0.99965 1.00000
```

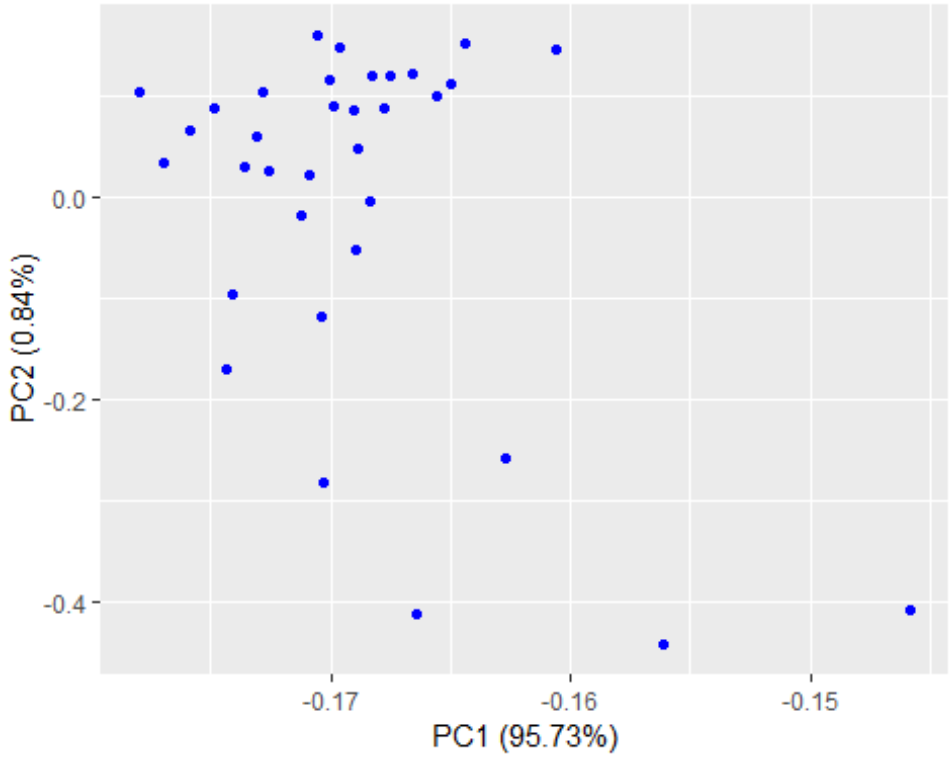
```r
#zum Anzeigen von labels (Zelllinien)


fviz_eig(pca)
```



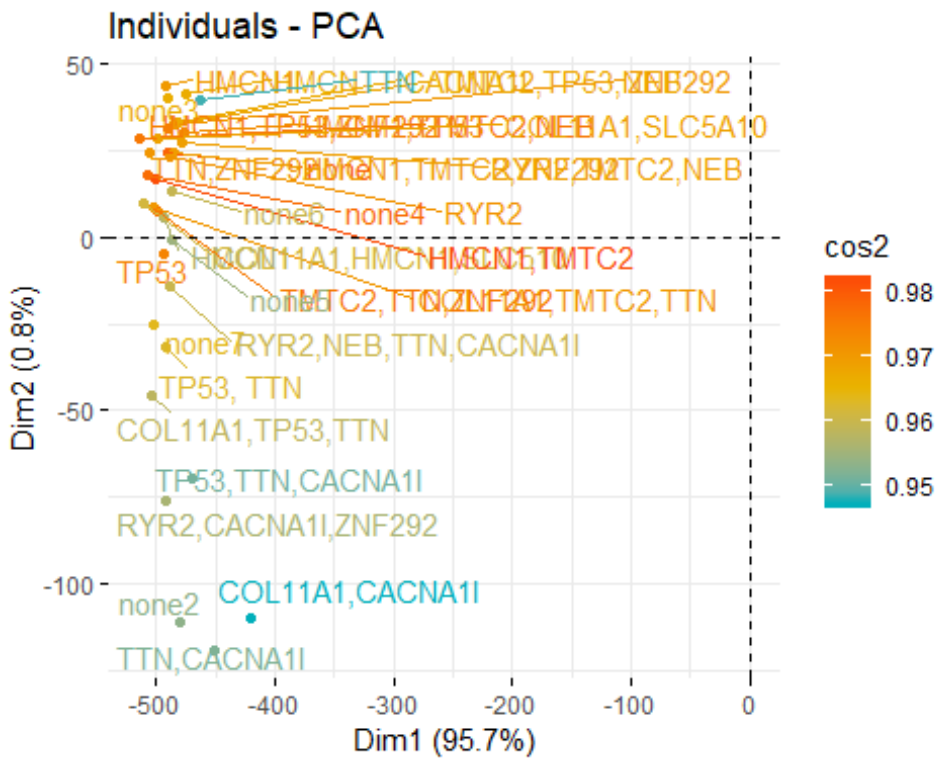

```
str(pca)
```

```
## List of 5
##  $ sdev    : num [1:34] 495.4 46.3 35.1 26.1 24.6 ...
##  $ rotation: num [1:16970, 1:34] -9.20e-03 -3.19e-05 -7.79e-03 -1.45e-04 -
2.30e-03 ...
##   ..- attr(*, "dimnames")=List of 2
##   .. ..$ : chr [1:16970] "A1BG" "A1CF" "A2M" "A2ML1" ...
##   .. ..$ : chr [1:34] "PC1" "PC2" "PC3" "PC4" ...
##  $ center  : logi FALSE
##  $ scale   : logi FALSE
##  $ x       : num [1:34, 1:34] -502 -494 -511 -500 -504 ...
##   ..- attr(*, "dimnames")=List of 2
##   .. ..$ : chr [1:34] "COL11A1,TMTC2,TTN" " HMCN1" "COL11A1,HMCN1,SLC510"
"HMCN1,TMTC2" ...
##   .. ..$ : chr [1:34] "PC1" "PC2" "PC3" "PC4" ...
##  - attr(*, "class")= chr "prcomp"
```

```
autoplot(pca, colour = 'blue')
```




```r
fviz_pca_ind(pca,
             col.ind = "cos2", # Color by the quality of representation
             gradient.cols = c("#00AFBB", "#E7B800", "#FC4E07"),
             repel = TRUE     # Avoid text overlapping
)
```



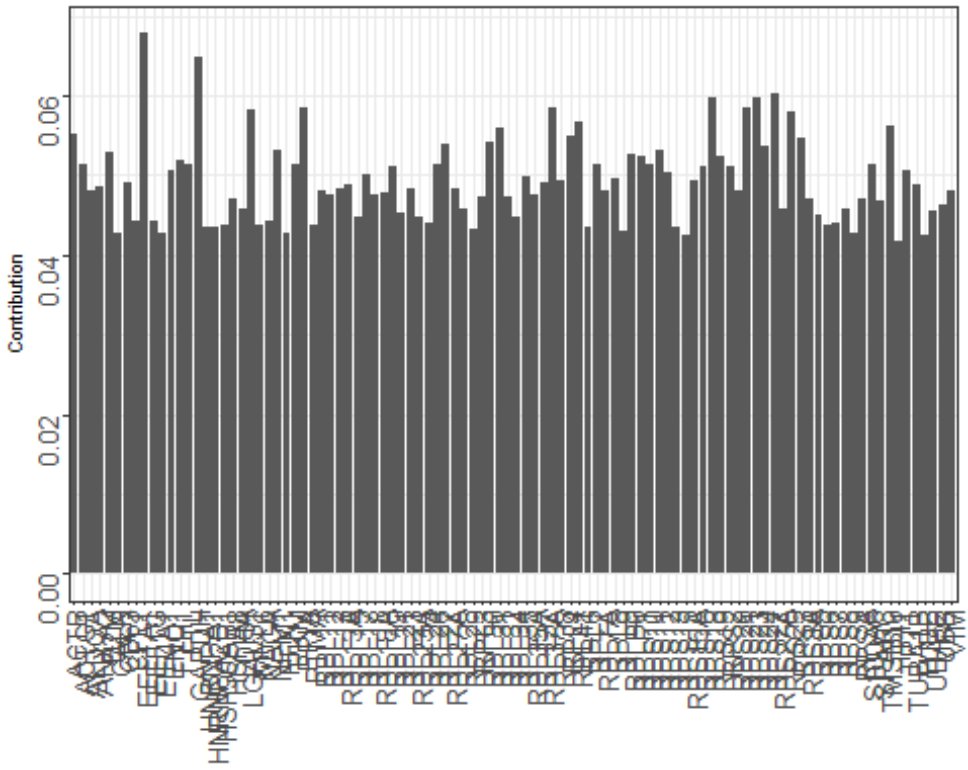
Again we see two clusters. The first principal component contains the most information about the data.

```
var_coord_func <- function(loadings, comp.sdev){
  loadings*comp.sdev
}

loadings <- pca$rotation
sdev <- pca$sdev
var.coord <- t(apply(loadings, 1, var_coord_func, sdev))

var.cos2 <- var.coord^2
comp.cos2 <- apply(var.cos2, 2, sum)
contrib <- function(var.cos2, comp.cos2){var.cos2*100/comp.cos2}

var.contrib <- t(apply(var.cos2,1, contrib, comp.cos2))
head(var.contrib[, 1:4])
```

```
##                  PC1          PC2          PC3          PC4
## A1BG   8.458140e-03 4.042140e-02 2.820464e-03 1.626858e-03
## A1CF   1.017925e-07 9.118405e-08 6.046034e-06 1.241073e-05
## A2M    6.063349e-03 4.405875e-02 8.758703e-02 1.918378e-02
## A2ML1  2.113426e-06 8.293155e-04 5.108695e-04 6.804066e-04
## A4GALT 5.277506e-04 5.244157e-02 2.770271e-03 6.278979e-03
## A4GNT  3.632784e-06 1.020123e-08 5.599425e-05 1.554282e-05
```

```
top100var.contrib <- var.contrib[,1]
top100var.contrib <- as.data.frame(top100var.contrib[order(-
top100var.contrib)])
top100var.contrib$Genes <- rownames(top100var.contrib)
```

```
top100var.contrib <- top100var.contrib[1:100,]
colnames(top100var.contrib)[1] <- "Contribution"


ggplot(data = top100var.contrib) +
  (geom_bar(mapping = aes(x = Genes, y = Contribution), stat = "identity")) +
  theme_bw(base_size = 7) + #format the size of the theme nicely
  theme(legend.position= "none", #define the legend position (here no leghend
will be needed)
        legend.direction="horizontal", #define the legend direction if one is
there
        plot.title = element_text(hjust = 0.5), #make the title of the plot
into the middle
        axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1, size =
10), #define the orientation of the text on the x-axis
        axis.text.y = element_text(angle = 90, vjust = 0.5, hjust=1, size =
10), #define the orientation of the text on the x-axis
        legend.title= element_blank(), #no title of the legend should be
plotted
        axis.title.x = element_blank(), #no title of the x-axis is relevant;
because that would be samples and that is cleare due to the naming
        strip.text.y = element_text(angle = 90)) #define the orientation of
the text of the y-axis
```



These are the Components which are contributing the most to our variation in the data. Maybe we will find some of these in our result of the p-test.

```
rm( drivergene, realcelllinenames, dataset, loadings, pca, realcelllinenames,
var.contrib, var.coord, var.cos2, comp.cos2, sdev)
```

```
## Warning in rm(drivergene, realcelllinenames, dataset, loadings, pca,
## realcelllinenames, : Objekt 'drivergene' nicht gefunden
```

```
## Warning in rm(drivergene, realcelllinenames, dataset, loadings, pca,
## realcelllinenames, : Objekt 'realcelllinenames' nicht gefunden
```

```
## Warning in rm(drivergene, realcelllinenames, dataset, loadings, pca,
## realcelllinenames, : Objekt 'realcelllinenames' nicht gefunden
```

4. Statistical test

We want to perform a p-test and compare the p-values.

```
driverGenes <- rownames(geneCounts)[1:10] #only use the TOP 10 driver genes
ttestgenes <- rownames(processed_data$kd.ceres)

potSecondSites <- lapply(seq_along(driverGenes), function(a) {
  genePicker <- driverGenes[a] #pick one driver gene
  print(paste0("I am doing driver mut: ", a))
  output <- sapply(seq_along(rownames(processed_data$kd.ceres)), function(b)
{ #the kdCERES matrix is of interest take its' rownames as refrence
    secondSitePicker <- rownames(processed_data$kd.ceres)[b] #pick a
potetnial 2nd site target
    if (secondSitePicker != genePicker) {
      drMUT <-
processed_data$kd.ceres[which(rownames(processed_data$kd.ceres) ==
genePicker),] #pick the driver mut data
      sndMUT <-
as.vector(processed_data$kd.ceres[which(rownames(processed_data$kd.ceres) ==
secondSitePicker),]) #pick the 2nd site data
      cor.val <- cor.test(unlist(drMUT, use.names=FALSE) , unlist(sndMUT,
use.names=FALSE), method = "spearman") #make a spearman correlation
      return(cor.val$p.value) #return the p-values
    } else {
      return(1)
    }
  })
  names(output) <- rownames(processed_data$kd.ceres) #rename all
  output <- as.data.frame(output) #get a nice dataframe
  return(output)
})
```

```
## [1] "I am doing driver mut: 1"
## [1] "I am doing driver mut: 2"
## [1] "I am doing driver mut: 3"
## [1] "I am doing driver mut: 4"
## [1] "I am doing driver mut: 5"
## [1] "I am doing driver mut: 6"
```

```
## [1] "I am doing driver mut: 7"
## [1] "I am doing driver mut: 8"
## [1] "I am doing driver mut: 9"
## [1] "I am doing driver mut: 10"
```

```
names(potSecondSites) <- driverGenes #rename the list of lists
lapply(potSecondSites, head) #Look at the nice data
```

```
## $TTN
##            output
## A1BG   0.70023480
## A1CF   0.39115670
## A2M    0.34286907
## A2ML1  0.11397865
## A4GALT 0.19132453
## A4GNT  0.01504808
##
## $TP53
##            output
## A1BG   0.28160340
## A1CF   0.70023480
## A2M    0.39697321
## A2ML1  0.64097590
## A4GALT 0.09015868
## A4GNT  0.60183071
##
## $HMCN1
##           output
## A1BG   0.4227534
## A1CF   0.8657359
## A2M    0.6534159
## A2ML1  0.7917565
## A4GALT 0.8725280
## A4GNT  0.3437615
##
## $TMTC2
##            output
## A1BG   0.45154526
## A1CF   0.43701759
## A2M    0.95872743
## A2ML1  0.75867863
## A4GALT 0.01716129
## A4GNT  0.62127398
##
## $RYR2
##             output
## A1BG   0.879329218
## A1CF   0.669727445
## A2M    0.002884766
## A2ML1  0.213302043
## A4GALT 0.088108676
## A4GNT  0.304196025
```

```
##
## $CACNA1I
##            output
## A1BG   0.93400823
## A1CF   0.09259686
## A2M    0.14278128
## A2ML1  0.61030460
## A4GALT 0.10401228
## A4GNT  0.53711418
##
## $ZNF292
##            output
## A1BG   0.27458108
## A1CF   0.75736391
## A2M    0.57435603
## A2ML1  0.07565286
## A4GALT 0.38922902
## A4GNT  0.08058396
##
## $NEB
##            output
## A1BG   0.42275339
## A1CF   0.07595393
## A2M    0.36190890
## A2ML1  0.33314716
## A4GALT 0.04869324
## A4GNT  0.05084604
##
## $COL11A1
##            output
## A1BG   0.07535272
## A1CF   0.49777121
## A2M    0.80910520
## A2ML1  0.26539892
## A4GALT 0.18653536
## A4GNT  0.68237842
##
## $SLC5A10
##             output
## A1BG   0.991742072
## A1CF   0.467398656
## A2M    0.834622511
## A2ML1  0.453641927
## A4GALT 0.916192275
## A4GNT  0.007613915
```

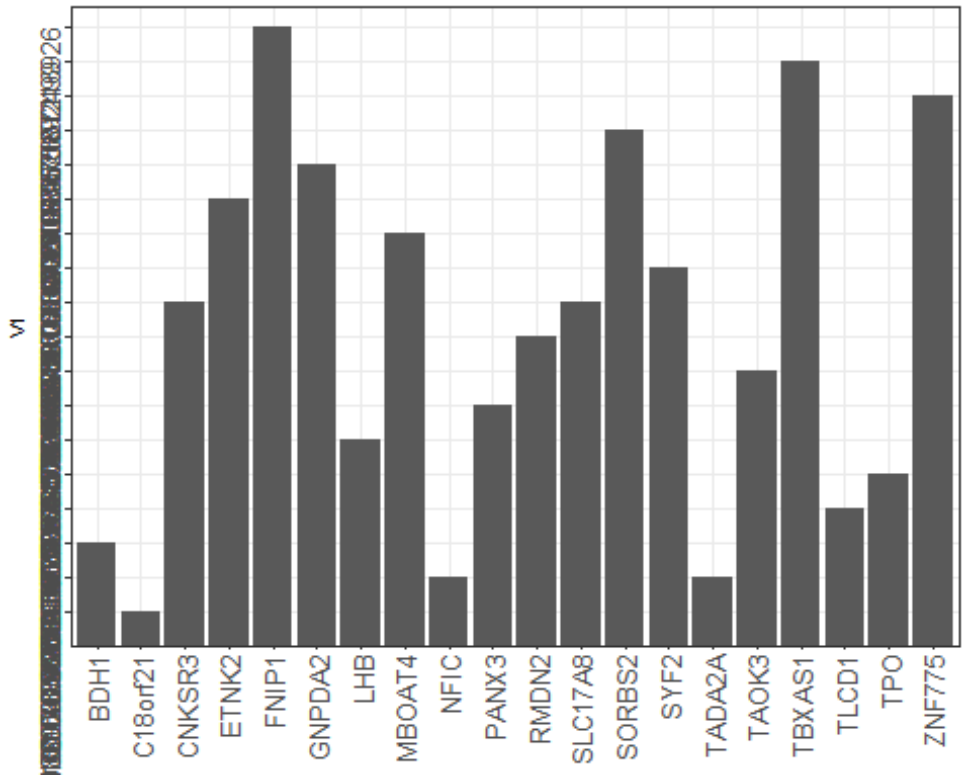
Now that we got all those p-values we want to order the data according to their p-values. So we can see the smallest ones which are the most important ones.

```
potSecondSites <- lapply(potSecondSites, function(a){
  a <- as.data.frame(cbind(a$output, rownames(a)))
```

```
  a <- a[order(a[1]), ]
})
```

Selecting the 20 Genes out of every DriverGene List with the lowest p score

```
potSecondSitestop20 <- lapply(seq_along(potSecondSites), function (a){
  output <- potSecondSites[[a]][1:20,]
  return(output)
})
names(potSecondSitestop20) <- driverGenes

ggplot(data = potSecondSitestop20$TTN) +
  (geom_bar(mapping = aes(x = V2, y = V1), stat = "identity")) +
  theme_bw(base_size = 7) + #format the size of the theme nicely
  theme(legend.position= "none", #define the legend position (here no leghend
will be needed)
        legend.direction="horizontal", #define the legend direction if one is
there
        plot.title = element_text(hjust = 0.5), #make the title of the plot
into the middle
        axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1, size =
10), #define the orientation of the text on the x-axis
        axis.text.y = element_text(angle = 90, vjust = 0.5, hjust=1, size =
10), #define the orientation of the text on the x-axis
        legend.title= element_blank(), #no title of the legend should be
plotted
        axis.title.x = element_blank(), #no title of the x-axis is relevant;
because that would be samples and that is cleare due to the naming
        strip.text.y = element_text(angle = 90)) #define the orientation of
the text of the y-axis
```




```
rm(potSecondSites, ttestgenes)
```

4. Multiple linear regression analysis

Predicting the expression of our driver genes with all the data

First creating the dataframe for the multiple linear regression with all the dataframes as columns, the rows are every gene in every cell line

Doing the multiple linear regression. Then comparing the predicted values of our model with the real values of the test_data by spearman correlaiton We perform this with every driver gene.

```
a <- generalPlottingData$expression[,1:3]
a <-a[,c(1,3,2)]
copynumber <- generalPlottingData$copynumber[,2]
kd.ceres <- generalPlottingData$kd.ceres[,2]
kd.prob <- generalPlottingData$kd.prob[,2]

RegData <- cbind(a,copynumber,kd.ceres,kd.prob)

# doing the multiple linear Regression
# then comparint the predicted values of our model with the real values
# of the test_data by spearman correlaiton
# doing this for every Driver Gene
```

```r
Regressionanalysis <-lapply(1:10, function(x){
  RegData <- cbind(a,copynumber,kd.ceres,kd.prob)
  Driverexpression <- c()
  for (i in 1:34) {
    a <- 16970*i
    c <- (16970* (i-1))+1
    b <- colnames(processed_data$expression)[i]
    Driverexpression[c:a] <-
processed_data$expression[rownames(geneCounts)[x],b]
  }
  print(paste0("I am doing driver mut: ", rownames(geneCounts)[x]))
  RegData <- cbind(RegData,Driverexpression)
  RegData <-as.data.frame(RegData)
  colnames(RegData) <- as.vector(colnames(RegData))
  set.seed(123) #initialize the random numbers
  split = sample.split(RegData, SplitRatio = 0.8) #split the dataset into 4/5
Training and 1/5 Testing dataset
  training_set = subset(RegData, split == TRUE) #use the labels to get the
training data
  test_set = subset(RegData, split == FALSE)
  rm(RegData)
  # Fitting Multiple Linear Regression to the Training set
  regressor = lm(Driverexpression ~ Value + copynumber + kd.ceres + kd.prob ,
data = training_set) #predict profit based on ALL (=.) the input variables
for one company
  # Predicting the Test set results
  y_pred = predict(regressor, newdata = test_set, se.fit = TRUE) #predict the
expression based on your testing data
  test_set$Prediction = y_pred$fit #add your predictions to the dataset
  #Now compare the Predictions (last column) with the real values of the
startups (2nd last column)
  Results <- cor.test(test_set$Driverexpression, test_set$Prediction, method
= "spearman", exact=FALSE)
  return(Results)
})
```

```
## [1] "I am doing driver mut: TTN"
## [1] "I am doing driver mut: TP53"
## [1] "I am doing driver mut: HMCN1"
## [1] "I am doing driver mut: TMTC2"
## [1] "I am doing driver mut: RYR2"
## [1] "I am doing driver mut: CACNA1I"
## [1] "I am doing driver mut: ZNF292"
## [1] "I am doing driver mut: NEB"
## [1] "I am doing driver mut: COL11A1"
## [1] "I am doing driver mut: SLC5A10"
```

```r
names(Regressionanalysis) <- rownames(geneCounts)[1:10]
Regressionanalysis <- as.vector(Regressionanalysis)
rm(RegData,kd.ceres,kd.prob,copynumber,a)
```

```r
ResultsRegression <- melt(lapply(1:length(Regressionanalysis), function(x){
  return(Regressionanalysis[[x]][3])
}))
ResultsRegression <-
cbind(ResultsRegression,melt(lapply(1:length(Regressionanalysis),
function(x){
  return(Regressionanalysis[[x]][1])
})))

ResultsRegression$L2 <- rownames(geneCounts)[1:10]
ResultsRegression <- ResultsRegression [,c(2,1,4)]
colnames(ResultsRegression) <- c("DriverGene", "pvalue", "Svalue" )

print(ResultsRegression)
```

```
##    DriverGene       pvalue       Svalue
## 1         TTN 5.509211e-16 7.317806e+14
## 2        TP53 4.997652e-09 7.359220e+14
## 3       HMCN1 5.739113e-37 7.233205e+14
## 4       TMTC2 1.486016e-36 7.234577e+14
## 5        RYR2 1.671884e-30 7.255677e+14
## 6     CACNA1I 7.949257e-20 7.299161e+14
## 7      ZNF292 9.481412e-12 7.341441e+14
## 8         NEB 5.286592e-14 7.328378e+14
## 9     COL11A1 1.548789e-77 7.124150e+14
## 10    SLC5A10 4.727922e-25 7.276653e+14
```

```r
# with these low p-values we can say with confidence that our Model is able
to reproduce and predict
# the Expressionvalues of our drivergenes

# using just our top 20 out of the statistical testing we hoped to see that
the p values would not increase that much
# this would verify our these that these genes are the essential components
which drive the differnet expression of the Driver Gene
# as you can see below this ist not the case and the p values are very much
increased

Regressionanalysistop20 <-lapply(1:10, function(x){
  a <-
generalPlottingData$expression[which(generalPlottingData$expression[,3] %in%
as.character(potSecondSitestop20[[x]][,2])),1:3]
  a <-a[,c(1,3,2)]
  copynumber <-
generalPlottingData$copynumber[which(generalPlottingData$copynumber[,3] %in%
as.character(potSecondSitestop20[[x]][,2])),2]
  kd.ceres <-
generalPlottingData$kd.ceres[which(generalPlottingData$kd.ceres[,3] %in%
as.character(potSecondSitestop20[[x]][,2])),2]
  kd.prob <-
```

```
generalPlottingData$kd.prob[which(generalPlottingData$kd.prob[,3] %in%
as.character(potSecondSitestop20[[x]][,2])),2]
  RegData <- cbind(a,copynumber,kd.ceres,kd.prob)
  h <-
length(generalPlottingData$expression[which(generalPlottingData$copynumber[,3
] %in% as.character(potSecondSitestop20[[x]][,2])),2])
  Driverexpression <- c()
  for (i in 1:34) {
    a <- h*i
    c <- (h* (i-1))+1
    b <- colnames(processed_data$expression)[i]
    Driverexpression[c:a] <-
processed_data$expression[rownames(geneCounts)[x],b]
  }
  print(paste0("I am doing driver mut: ", rownames(geneCounts)[x]))
  RegData <- cbind(RegData,Driverexpression)
  RegData <-as.data.frame(RegData)
  colnames(RegData) <- as.vector(colnames(RegData))
  set.seed(123) #initialize the random numbers
  split = sample.split(RegData, SplitRatio = 0.8) #split the dataset into 4/5
Training and 1/5 Testing dataset
  training_set = subset(RegData, split == TRUE) #use the labels to get the
training data
  test_set = subset(RegData, split == FALSE)
  rm(RegData)
  # Fitting Multiple Linear Regression to the Training set
  regressor = lm(Driverexpression ~ Value + copynumber + kd.ceres + kd.prob ,
data = training_set) #predict profit based on ALL (=.) the input variables
for one company
  # Predicting the Test set results
  y_pred = predict(regressor, newdata = test_set, se.fit = TRUE) #predict the
expression based on your testing data
  test_set$Prediction = y_pred$fit #add your predictions to the dataset
  #Now compare the Predictions (last column) with the real values of the
startups (2nd last column)
  Results <- cor.test(test_set$Driverexpression, test_set$Prediction, method
= "spearman", exact=FALSE)
  return(Results)
})
```

```
## [1] "I am doing driver mut: TTN"
```

```
## Warning in data.frame(..., check.names = FALSE): row names were found from
## a short variable and have been discarded
```

```
## [1] "I am doing driver mut: TP53"
```

```
## Warning in data.frame(..., check.names = FALSE): row names were found from
## a short variable and have been discarded
```

```
## [1] "I am doing driver mut: HMCN1"
```

```
## Warning in data.frame(..., check.names = FALSE): row names were found from
## a short variable and have been discarded

## [1] "I am doing driver mut: TMTC2"

## Warning in data.frame(..., check.names = FALSE): row names were found from
## a short variable and have been discarded

## [1] "I am doing driver mut: RYR2"

## Warning in data.frame(..., check.names = FALSE): row names were found from
## a short variable and have been discarded

## [1] "I am doing driver mut: CACNA1I"

## Warning in data.frame(..., check.names = FALSE): row names were found from
## a short variable and have been discarded

## [1] "I am doing driver mut: ZNF292"

## Warning in data.frame(..., check.names = FALSE): row names were found from
## a short variable and have been discarded

## [1] "I am doing driver mut: NEB"

## Warning in data.frame(..., check.names = FALSE): row names were found from
## a short variable and have been discarded

## [1] "I am doing driver mut: COL11A1"

## Warning in data.frame(..., check.names = FALSE): row names were found from
## a short variable and have been discarded

## [1] "I am doing driver mut: SLC5A10"

## Warning in data.frame(..., check.names = FALSE): row names were found from
## a short variable and have been discarded

names(Regressionanalysistop20) <- rownames(geneCounts)[1:10]
Regressionanalysistop20 <- as.vector(Regressionanalysistop20)

ResultsRegressiontop20 <- melt(lapply(1:length(Regressionanalysistop20),
function(x){
  return(Regressionanalysistop20[[x]][3])
}))
ResultsRegressiontop20 <-
cbind(ResultsRegressiontop20,melt(lapply(1:length(Regressionanalysistop20),
function(x){
  return(Regressionanalysistop20[[x]][1])
})))

ResultsRegressiontop20$L2 <- rownames(geneCounts)[1:10]
ResultsRegressiontop20 <- ResultsRegressiontop20 [,c(2,1,4)]
colnames(ResultsRegressiontop20) <- c("DriverGene", "pvalue", "Svalue" )
```

```r
print(ResultsRegressiontop20)
```

```
##    DriverGene     pvalue      Svalue
## 1         TTN 0.09746992 49026556057
## 2        TP53 0.04382470 49238451647
## 3       HMCN1 0.88944564 48128985972
## 4       TMTC2 0.12381370 48956640123
## 5        RYR2 0.10252586 49012065541
## 6     CACNA1I 0.42022190 48523361801
## 7      ZNF292 0.07410218 49102647128
## 8         NEB 0.79788512 48198209845
## 9     COL11A1 0.80113984 48195718948
## 10    SLC5A10 0.20397571 48797830898
```

```r
# so with this result we can not define confidently the secound targets
```

RESULTS

```r
Resultspresentation <- lapply(1:length(potSecondSitestop20), function(x){
  return(potSecondSitestop20[[x]][2])
})

names(Resultspresentation) <- rownames(geneCounts)[1:10]
print(Resultspresentation)
```

```
## $TTN
##             V2
## 1619  C18orf21
## 9311      NFIC
## 14283   TADA2A
## 1308      BDH1
## 14708    TLCD1
## 15183      TPO
## 7750       LHB
## 10332    PANX3
## 14321    TAOK3
## 12208    RMDN2
## 2967    CNKSR3
## 13171  SLC17A8
## 14213     SYF2
## 8296    MBOAT4
## 4571     ETNK2
## 5693    GNPDA2
## 13698   SORBS2
## 16853   ZNF775
## 14435   TBXAS1
## 5161     FNIP1
##
## $TP53
##             V2
## 2499    CDKN1A
```

```
## 11816    RAD50
## 14298     TAF4
## 14764    TMCC1
## 4371      ELP5
## 2900    CLNS1A
## 3317    CSNK1E
## 15153  TP53BP1
## 1038      ATE1
## 5012     FEM1B
## 10303    PAGR1
## 12378    RPL23
## 16904   ZNF862
## 11548    PSME3
## 11231  PPP1R42
## 4149    DYNLT1
## 11199 PPP1R12A
## 9363     NIPBL
## 16084    WDR83
## 14889  TMEM207
##
## $HMCN1
##           V2
## 3855    DLEC1
## 7868      LPA
## 14241    SYT1
## 2696   CHI3L2
## 1086  ATP13A4
## 15195   TPRX1
## 3970     DNM1
## 997     ASGR1
## 5454     GCKR
## 321       ADO
## 2893     CLMN
## 564      AMTN
## 12134  RHBDD2
## 14043   STBD1
## 5219     FPGT
## 8695  MRGPRX1
## 289     ADCY2
## 11622  PTPN13
## 16185    XPO4
## 6637    IFNW1
##
## $TMTC2
##            V2
## 6823     INPP1
## 6415    HPCAL4
## 6838     INSL4
## 10807     PIM1
## 1630  C19orf44
## 13439   SLC6A5
```

```
## 12357     RPF2
## 13018     SHBG
## 14524    TENM4
## 9418      NME1
## 12112    RGS13
## 11239  PPP2R2A
## 13478  SLCO2A1
## 12557    RWDD3
## 4841    FAM78A
## 15160   TP53RK
## 7610     LAMC1
## 14552    TEX33
## 5893     GRIK4
## 16316   ZC3H7A
##
## $RYR2
##            V2
## 5170      FOS
## 10974   PLSCR1
## 4126     DUSP7
## 13270  SLC26A8
## 4693   FAM118B
## 13662    SNX21
## 12493     RRP8
## 6148     HERC4
## 2600   CEP57L1
## 15432     TSPO
## 13161  SLC16A7
## 6609    IFITM2
## 10984   PLXNA2
## 4233   EFCAB14
## 8288      MBIP
## 16664   ZNF501
## 12256    RNF14
## 13123  SLC10A1
## 4750  FAM177A1
## 173      ACSF3
##
## $CACNA1I
##             V2
## 13544     SMDT1
## 8832       MSX2
## 7726    LGALS12
## 9657      NUCB1
## 7536  KRTAP21-3
## 4033       DPRX
## 3564       DAOA
## 10200    OSBPL3
## 10618      PENK
## 4608    EXOC3L4
## 11025    PNPLA5
```

```
## 12802     SEH1L
## 760    APOBEC3F
## 9013      MYO1H
## 15604     UBA52
## 12857    SERHL2
## 1598   C16orf97
## 3660       DDR1
## 39         ABCA7
## 15108    TNRC6B
##
## $ZNF292
##             V2
## 7630    LARP4B
## 10984   PLXNA2
## 13213  SLC24A1
## 13373  SLC39A9
## 10203   OSBPL7
## 15231 TRAPPC10
## 12266   RNF152
## 1824   C6orf99
## 5586      GLB1
## 8021      LSM5
## 10214     OSMR
## 14875  TMEM190
## 15768   UNC93A
## 10717    PHF5A
## 3094      COPA
## 11354   PRKCSH
## 16138    WNT5A
## 8275       MAX
## 2703      CHL1
## 4649     F2RL2
##
## $NEB
##             V2
## 3318   CSNK1G1
## 3931   DNAJC10
## 7082    KCNAB3
## 12359 RPGRIP1L
## 2675    CHCHD5
## 15311   TRIM61
## 9390    NKX6-1
## 11828    RAD9B
## 16674   ZNF514
## 3429     CWC25
## 2332      CD22
## 13376  SLC40A1
## 8895  MTRNR2L2
## 4583     EVA1A
## 5809    GPR183
## 14900  TMEM219
```

```
## 1213     BAALC
## 11643    PTPRH
## 15120   TOM1L2
## 8621     MOCOS
##
## $COL11A1
##             V2
## 3713  DEFB108B
## 5233       FRK
## 10256      OXT
## 16705   ZNF560
## 2882     CLIC6
## 9029     MYOM1
## 14943   TMEM37
## 930      ARMS2
## 11982     RBP1
## 1922    CACNG7
## 7372    KLHL36
## 4932    FBXO11
## 15437   TSPYL6
## 1078    ATP10D
## 8008    LRTOMT
## 14339  TAS2R13
## 1898    CACHD1
## 5332    GABBR2
## 15616  UBASH3B
## 11202 PPP1R13B
##
## $SLC5A10
##              V2
## 8177     MAP2K4
## 13850     SPNS2
## 16525    ZNF256
## 6648      IFT52
## 13815     SPEM1
## 7249  KIAA1324L
## 4226      EEF2K
## 8987       MYL4
## 10888   PLAC8L1
## 14539     TEX10
## 16738    ZNF593
## 15072   TNFSF12
## 13018      SHBG
## 3795     DHRS11
## 11358     PRKD3
## 11415   PRPSAP2
## 10410     PCBP4
## 13153  SLC16A11
## 14303     TAF6L
## 4107     DUSP10
```

```r
print(top100var.contrib[1:20,2])
```

```
##  [1] "EEF1A1" "GAPDH"  "RPS27"  "RPS23"  "RPS18"  "RPL37A" "PPIA"
##  [8] "RPS21"  "LGALS1" "RPS29"  "RPL41"  "TMSB10" "RPL31"  "ACTB"
## [15] "RPL4"   "RPS3"   "RPL30"  "RPL27"  "RPS24"  "RPS11"
```

```r
which(top100var.contrib[1:20,2] %in%
as.character(melt(Resultspresentation)[,1]))
```

```
## Using V2 as id variables
## Using V2 as id variables
## Using V2 as id variables
## Using V2 as id variables
## Using V2 as id variables
## Using V2 as id variables
## Using V2 as id variables
## Using V2 as id variables
## Using V2 as id variables
## Using V2 as id variables
```

```
## integer(0)
```

```r
# the 2nd targets from the pca and the regression are not the same like we
have wished (due to the data or mistakes in the skript)
# although the kmeans and the pca doesnt redrocude the same 2nd side targets
we present as
# our results the 20top 2nd site genes from our p-test
# this regressionmodel shows a really low p-value at which grounds we
conclude that following gens
# should be taken in account as targets for drug development in skin cancer
```

Predicting the expression of the driver mutations with the expression of the other genes

```r
# we also wanted to do another Regression model on just the expression data
with the so that the coeffizients of the
# analysis would tell us how improtant each gene is for the model, but this
regression does not work and we doesnt know why
# maybe you can take a look and help us
#dataset <- t(processed_data$expression)

#dataset <- as.data.frame(dataset)



#set.seed(123) #initialize the random numbers
#split = sample.split(dataset, SplitRatio = 0.5) #split the dataset into 4/5
Training and 1/5 Testing dataset
#training_set = subset(dataset, split == TRUE) #use the labels to get the
training data
#test_set = subset(dataset, split == FALSE)
#rm(dataset)
```

```
# Fitting Multiple Linear Regression to the Training set
# regressor = lm(TP53 ~ ., data = training_set) #predict profit based on ALL
(=.) the input variables for one company

# Predicting the Test set results
# y_pred = predict(regressor, newdata = test_set, se.fit = TRUE) #predict the
expression based on your testing data
# test_set$Prediction = y_pred$fit #add your predictions to the dataset
#Now compare the Predictions (last column) with the real values of the
startups (2nd last column)
# Results <- cor.test(test_set$Driverexpression, test_set$Prediction, method
= "spearman", exact=FALSE)
```

```
sessionInfo() #finally done:)
```

```
## R version 3.5.1 (2018-07-02)
## Platform: i386-w64-mingw32/i386 (32-bit)
## Running under: Windows 10 x64 (build 17763)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=German_Germany.1252  LC_CTYPE=German_Germany.1252
## [3] LC_MONETARY=German_Germany.1252 LC_NUMERIC=C
## [5] LC_TIME=German_Germany.1252
##
## attached base packages:
## [1] grid      stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] caTools_1.17.1.2  scales_1.0.0      ggfortify_0.4.7
##  [4] usethis_1.5.0     devtools_2.0.2    dendextend_1.12.0
##  [7] forcats_0.3.0     stringr_1.3.1     dplyr_0.7.7
## [10] purrr_0.2.5       readr_1.1.1       tidyr_0.8.2
## [13] tibble_1.4.2      tidyverse_1.2.1   caret_6.0-84
## [16] lattice_0.20-35   pheatmap_1.0.12   rstudioapi_0.8
## [19] cluster_2.0.7-1   data.table_1.12.2 reshape2_1.4.3
## [22] gridExtra_2.3     factoextra_1.0.5  relaimpo_2.2-3
## [25] mitools_2.4       survey_3.36       survival_2.42-3
## [28] Matrix_1.2-14     boot_1.3-20       MASS_7.3-50
## [31] ggplot2_3.1.0
##
```

```
## loaded via a namespace (and not attached):
##  [1] stringi_1.2.4      evaluate_0.13      memoise_1.1.0
##  [4] processx_3.3.1     haven_1.1.2        callr_3.2.0
##  [7] bitops_1.0-6       ps_1.2.0           cli_1.1.0
## [10] prodlim_2018.04.18 DBI_1.0.0          desc_1.2.0
## [13] bindr_0.1.1        nlme_3.1-137       ggrepel_0.8.1
## [16] rprojroot_1.3-2    tools_3.5.1        magrittr_1.5
## [19] Rcpp_0.12.19       xml2_1.2.0         pkgload_1.0.2
## [22] readxl_1.1.0       httr_1.3.1         rmarkdown_1.12
## [25] assertthat_0.2.0   sessioninfo_1.1.1  R6_2.3.0
## [28] fs_1.2.6           nnet_7.3-12        timeDate_3043.102
## [31] munsell_0.5.0      cellranger_1.1.0   digest_0.6.18
## [34] codetools_0.2-15   splines_3.5.1      generics_0.0.2
## [37] colorspace_1.3-2   stats4_3.5.1       pkgconfig_2.0.2
## [40] pillar_1.3.0       gower_0.2.1        bindrcpp_0.2.2
## [43] iterators_1.0.10   plyr_1.8.4         gtable_0.2.0
## [46] xfun_0.6           tidyselect_0.2.5   rvest_0.3.2
## [49] knitr_1.22         viridisLite_0.3.0  pkgbuild_1.0.3
## [52] rlang_0.3.0.1      broom_0.5.0        glue_1.3.0
## [55] backports_1.1.2    prettyunits_1.0.2  RColorBrewer_1.1-2
## [58] ipred_0.9-9        lubridate_1.7.4    modelr_0.1.2
## [61] lava_1.6.5         hms_0.4.2          recipes_0.1.5
## [64] remotes_2.1.0      labeling_0.3       class_7.3-14
## [67] htmltools_0.3.6    yaml_2.2.0         lazyeval_0.2.1
## [70] ggpubr_0.2         ModelMetrics_1.2.2 crayon_1.3.4
## [73] withr_2.1.2        corpcor_1.6.9      viridis_0.5.1
## [76] jsonlite_1.5       rpart_4.1-13       foreach_1.4.4
## [79] compiler_3.5.1
```

finally done :)