

Project Head and Neck Cancer

Nils Mechtel, Tobias Hub, Niklas Urbanek, Pascal Poc

21 July 2019

General Introduction

Head and neck cancer is defined as a group of cancers, concerning the mouth, nose and throat (Figure 1). With over 90%, Squamous cell carcinoma is the most common type in HNC patients. With 5.5 million affected people in 2015 it is the seventh most frequent cancer and the ninth most frequent cause of death. 75% of all HNC cancer types were caused by tobacco or alcohol. Although the cure rate for HNC is really high, around 50 % of concerned patients remain suffering from an advanced disease.

In the century of next generation sequencing, the genome became an interesting target for scientists. Approaches that directly affect important oncogenes often fail due to the fact that these genes play an important role in healthy cells. The researchers in our paper (Jerby-Arnon, L., et al. (2014). “Predicting Cancer-Specific Vulnerability via Data-Driven Detection of Synthetic Lethality.” *Cell* 158(5): 1199-1209.) investigated synthetic lethality and synthetic dosage lethality that uses gene interactions to affect tumorous proliferation. Synthetic lethality describes a gene interaction in which single-gene defects are compatible with cell viability, but the combination of gene effects results in cell death. Synthetic dosage lethality occurs, when the overexpression of one gene is combined with the knockout of another gene.

Daisy consists of three statistical approaches (Figure 2), each processing different sub data from a cancer Dataset. The first strategy is “Survival of the fittest”, where just deleted SL-paired genes were selected, that are not in a surviving cancer culture. The second strategy is “functional examination” where SL genes were identified, by knocking out genes, with the additive, that the SL partners are inactive. If the tumor shows decreased proliferation, it accounts for the SL pair group. The third strategy is “pairwise gene co-expression” that is based on the fact that SL pairs are often closely related in biological pathways and therefore exhibit similar occurrence. If a gene pair fulfills every of the three criteria, it might be a potential SL pair according to DAISY. To check for SDL partners the criteria of inactive or active is replaced by under- or overactive, measured through expression numbers and copy number alterations.

Milestones

The strategy of our project consisted of 4 main milestones (Figure 3). At first we familiarized with the data and did the first descriptive statistics in milestone 1. Secondly, we identified potential genes “of interest” (SL and SDL candidates), according to DAISY-System. In our third milestone, the core task of our project was implemented. The identification of SL and SDL pairs was executed using functional examination, survival of the fittest and pairwise co-expression. Lastly, in our fourth milestone we tested logistic regression to predict mutations.

1. Data Cleanup & Descriptive Statistics

Loading data and defining HNC variables

```
# Load dataset
allDepMapData = readRDS("DepMap19Q1_allData.RDS")

# Creation of sub matrices
```

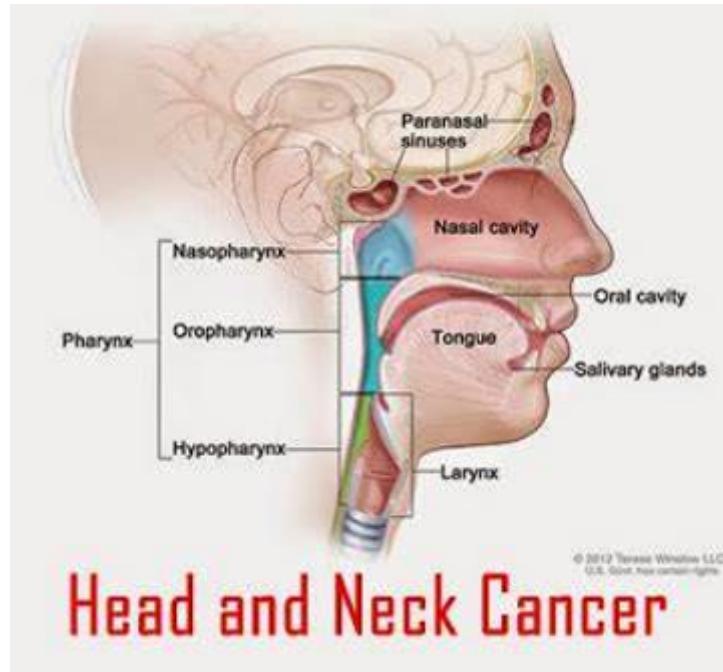


Figure 1: Most affected tissues and organs by head and neck cancer

```

expression = allDepMapData[["expression"]]
copynumber = allDepMapData[["copynumber"]]
mutation = allDepMapData[["mutation"]]
kd.ceres = allDepMapData[["kd.ceres"]]
kd.prob = allDepMapData[["kd.prob"]]
annotation = allDepMapData[["annotation"]]
rm(allDepMapData)

# Reducing the samples to head and neck cancer samples
annotation.HNC = annotation[which(annotation$Primary.Disease == "Head and Neck Cancer"), ]
ID = annotation.HNC$DepMap_ID

# Filtering of sub matrices by primary disease "Head and neck cancer"
expression.HNC = expression[ , which(colnames(expression) %in% ID)]
copynumber.HNC = copynumber[ , which(colnames(copynumber) %in% ID)]
kd.ceres.HNC = kd.ceres[ , which(colnames(kd.ceres) %in% ID)]
kd.prob.HNC = kd.prob[ , which(colnames(kd.prob) %in% ID)]
mutation.HNC = mutation[ID]

```

Expression analysis

Checking for NAs

```
sum(is.na(expression) == TRUE)
```

[1] 0

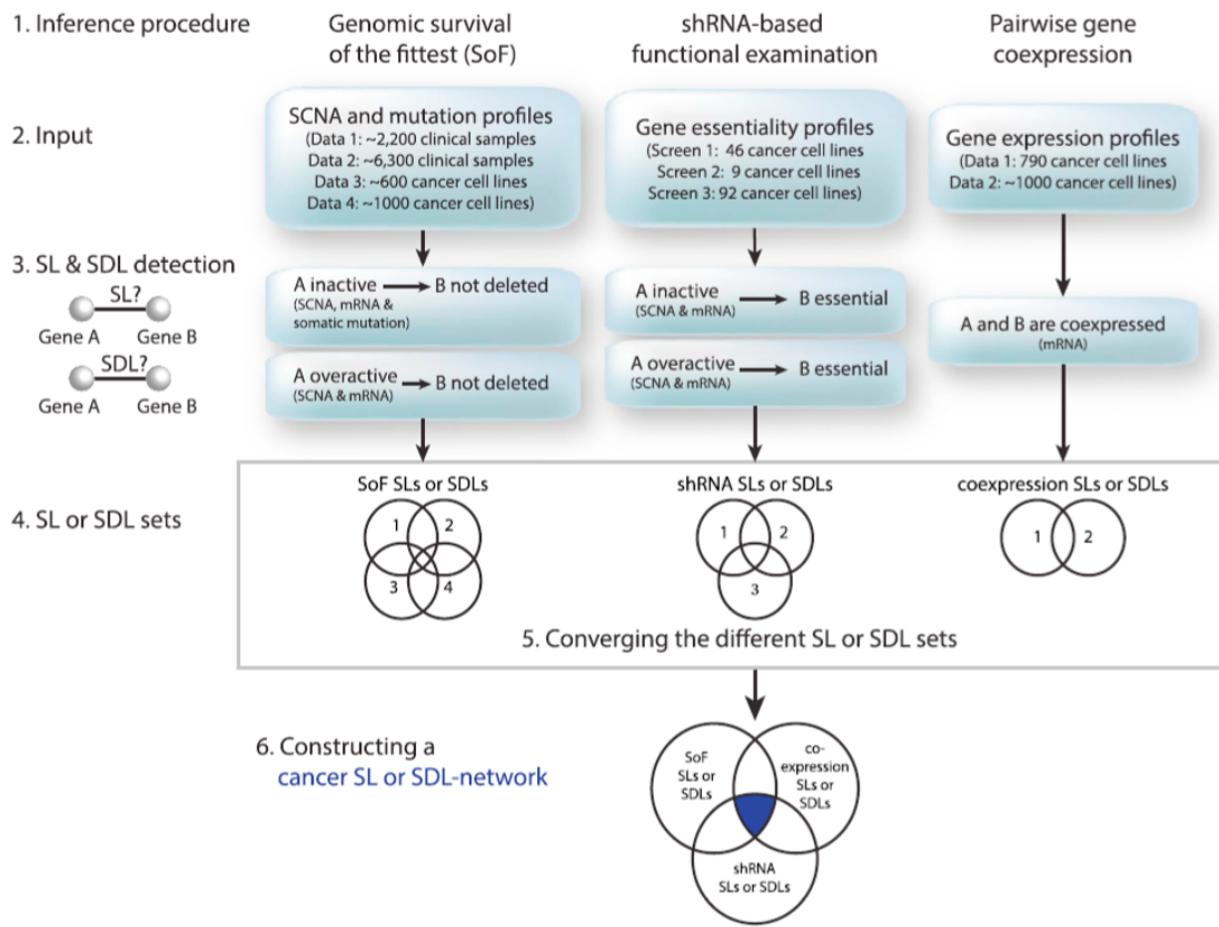


Figure 2: Daisy-Workflow

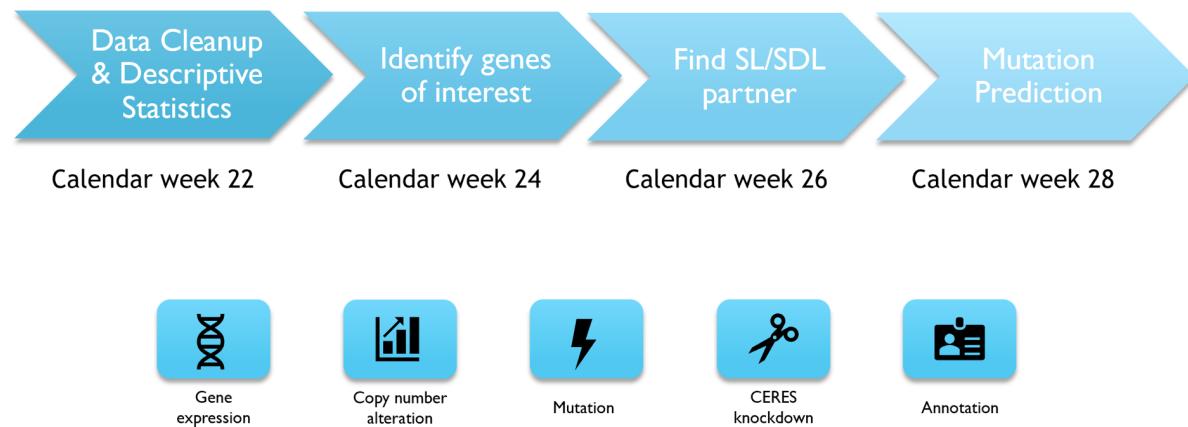


Figure 3: Milestones of our project

Result: There are no NAs in the expression data.

Examine expression values

Create a reference group of expression data

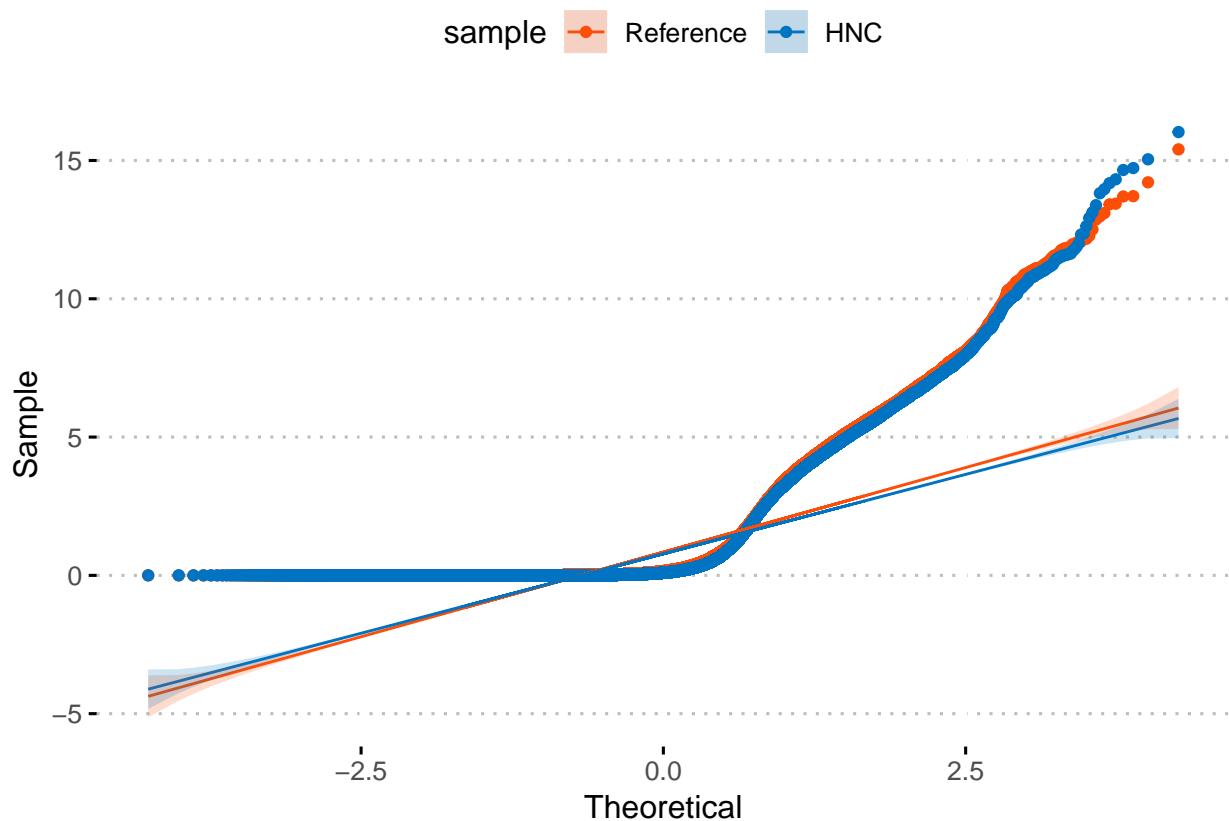
```
'%!in%' = function(x,y)!('%in%'(x,y)) # Define function "is not in"  
expression.reference = expression[ , which(colnames(expression) %!in% ID)]
```

and calculate their mean.

```
mean.exp.reference = apply(expression.reference, 1, mean)  
mean.exp.HNC = apply(expression.HNC, 1, mean)
```

Firstly, we want to check if the gene expressions of the HNC samples and the reference group are normally distributed.

```
HNC = data.frame(expression_values = mean.exp.HNC, sample = "HNC")  
Reference = data.frame(expression_values = mean.exp.reference, sample = "Reference")  
mean.expression = rbind(Reference, HNC)  
  
ggqqplot(mean.expression, x = "expression_values",  
         color = "sample",  
         palette = c("#FC4E07", "#0073C2FF"), # Red and blue  
         ggtheme = theme_pubclean())
```



Result: Gene expression is neither in HNC samples nor in reference samples normally distributed.

Then we compare HNC and reference group gene expression using a statistical test. A Wilcoxon rank sum test must be applied because the expression is not normally distributed.

```
wilcox.test(mean.exp.HNC, mean.exp.reference)
```

```
Wilcoxon rank sum test with continuity correction
```

```
data: mean.exp.HNC and mean.exp.reference
W = 1080700000, p-value < 2.2e-16
alternative hypothesis: true location shift is not equal to 0
```

Result: Mean gene expression differs between HNC and reference group samples significantly.

Next we check if the gene expression differs whether a gene has a deleterious mutation or not. AS an example, we compare the mean TP53 gene expression of all samples with and without deleterious TP53 mutation.

```
# Get the ID of samples whith and without deleterious mutation
TP53.mut.ID = lapply(1:length(mutation), function(a) {
  dat_picker = mutation[[a]]
  dat_picker = dat_picker[which(dat_picker$isDeleterious == TRUE),]
  out = ifelse("TP53" %in% dat_picker$Hugo_Symbol, a, NA) # Filter all IDs which have a deleterious mut
  return(out)
})

TP53.not_mut.ID = grep("NA", TP53.mut.ID) # ID of samples with no TP53 mutation
TP53.mut.ID = as.integer(TP53.mut.ID[is.na(TP53.mut.ID) == FALSE]) # ID of samples with TP53 mutation

# Create two vectors of expression values
exp.TP53.mut = as.numeric(expression["TP53", TP53.mut.ID]) # Expression of TP53 deleterious mutated samples
exp.TP53.not_mut = as.numeric(expression["TP53", TP53.not_mut.ID]) # Expression of TP53 not deleterious samples
```

Compare the expression values by applying Wilcoxon rank sum test.

```
wilcox.test(exp.TP53.mut , exp.TP53.not_mut)
```

```
Wilcoxon rank sum test with continuity correction
```

```
data: exp.TP53.mut and exp.TP53.not_mut
W = 8276.5, p-value < 2.2e-16
alternative hypothesis: true location shift is not equal to 0
```

Result: Gene expression of TP53 as an example gene varies significantly whether the samples has or has no deleterious TP53 mutation.

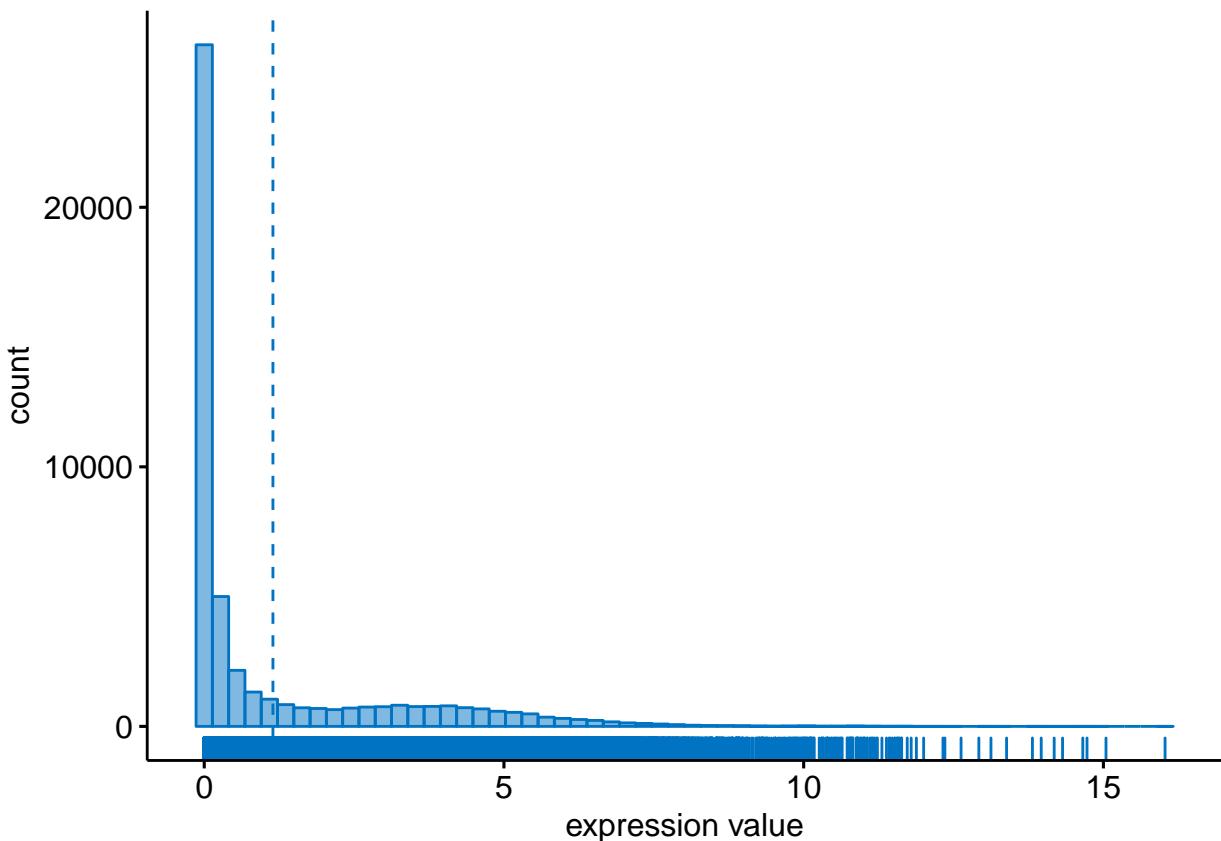
At last, we plot a histogram to check how the expression values are distributed.

```
gghistogram(as.data.frame(mean.exp.HNC),
            x = "mean.exp.HNC",
            xlab = "expression value",
```

```

bins = 60,
fill = "#0073C2FF",
color = "#0073C2FF",
add = "mean",
rug = TRUE)

```



Result: The most genes have expression values around 0.

Plot the 20 most under- and overexpressed genes of HNC samples.

For this we need to normalize the expression data from HNC samples

```

expr.HNC.norm = as.data.frame(apply(expression.HNC, 2, function(x) {
  x - mean.expr.reference
}))

```

and calculate the mean of the normalized expression values.

```
mean.expr.HNC.norm = as.data.frame(apply(expr.HNC.norm, 1, mean))
```

Then create a dataframe with needed information for the barplot

```

barplot.data = data.frame(Genes=rownames(expr.HNC.norm),
                           Values=mean.expr.HNC.norm)
names(barplot.data) = c("Genes", "Values")
barplot.data$Group = ifelse(barplot.data$Values>0, "overexpressed", "underexpressed")
barplot.data = barplot.data[order(barplot.data$Values),]
barplot.data = barplot.data[c(1:19, (nrow(barplot.data)-20):nrow(barplot.data)),]

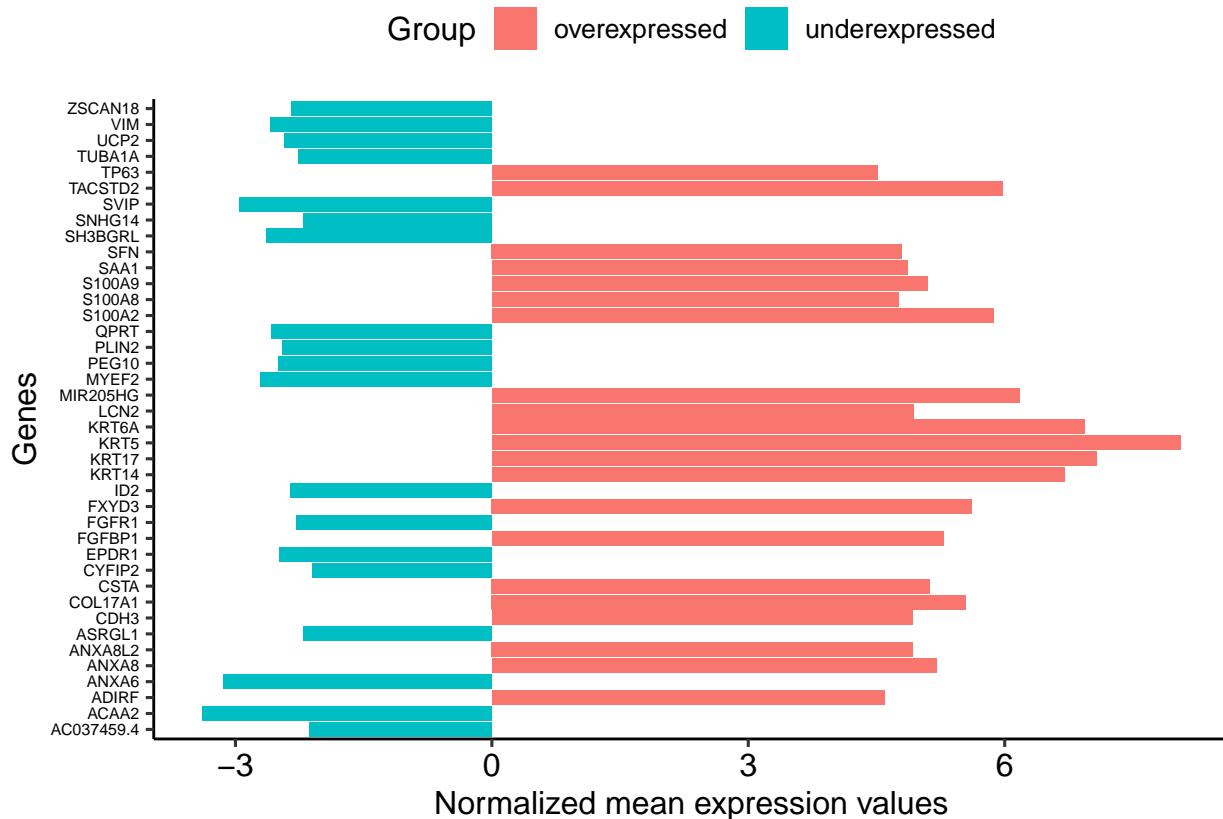
```

and plot this datafame.

```

ggplot(barplot.data,aes(x=Genes,y=Values,fill=Group)) +
  ylab("Normalized mean expression values") +
  geom_bar(stat="identity") +
  coord_flip() +
  theme(axis.text.y = element_text(size=6))

```



Cleanup of the enviroment

```

rm(HNC, Reference, mean.expression, TP53.mut.ID, TP53.not_mut.ID, exp.TP53.mut, exp.TP53.not_mut, mean.

```

Copynumber Alteration

Analysis of NA values

Examination of NA values in the copynumber.HNC data

```
# Sum up all NAs in each row
NA.rows = apply(copynumber.HNC, 1, function(x) {
  sum(is.na(x))
})
# Define all rows containing missing values
NA.rows = which(NA.rows > 0)
length(NA.rows)
```

```
[1] 107
```

Only 107 out of 23299 gens have NA values and are therefore deleted from the copynumber.HNC dataframe

```
copynumber.HNC = copynumber.HNC[-NA.rows,]
dim(copynumber.HNC)
```

```
[1] 23192    27
```

Creating a reference data and examination of its NA values

```
copynumber.reference = copynumber[, which(colnames(copynumber) %!in% ID)]
dim(copynumber.reference)
```

```
[1] 23299    517
```

```
NA.rows.ref = apply(copynumber.reference, 1, function(x) {
  sum(is.na(x))
})
NA.rows.ref = which(NA.rows.ref > 0)
length(NA.rows.ref)
```

```
[1] 107
```

```
summary(NA.rows == NA.rows.ref)
```

Mode	TRUE
logical	107

Genes with NA values are the same for the HNC samples and all the other samples. Therefore, they can be deleted in the reference data as well.

```
copynumber.reference = copynumber.reference[-NA.rows.ref,]
copynumber = copynumber[-NA.rows.ref,]
dim(copynumber.reference)
```

```
[1] 23192    517
```

```
dim(copynumber)
```

```
[1] 23192 544
```

Normalisation of the copynumber values

For the HNC normalisation we will use the mean CN value per gen of the reference data.

```
copynumber.ref.mean = apply(copynumber.reference, 1, mean)
copynumber.HNC.mean = apply(copynumber.HNC, 1, mean)
```

Creating a dataframe of all copynumber values. The values are normalized to the mean copynumbers of the reference data.

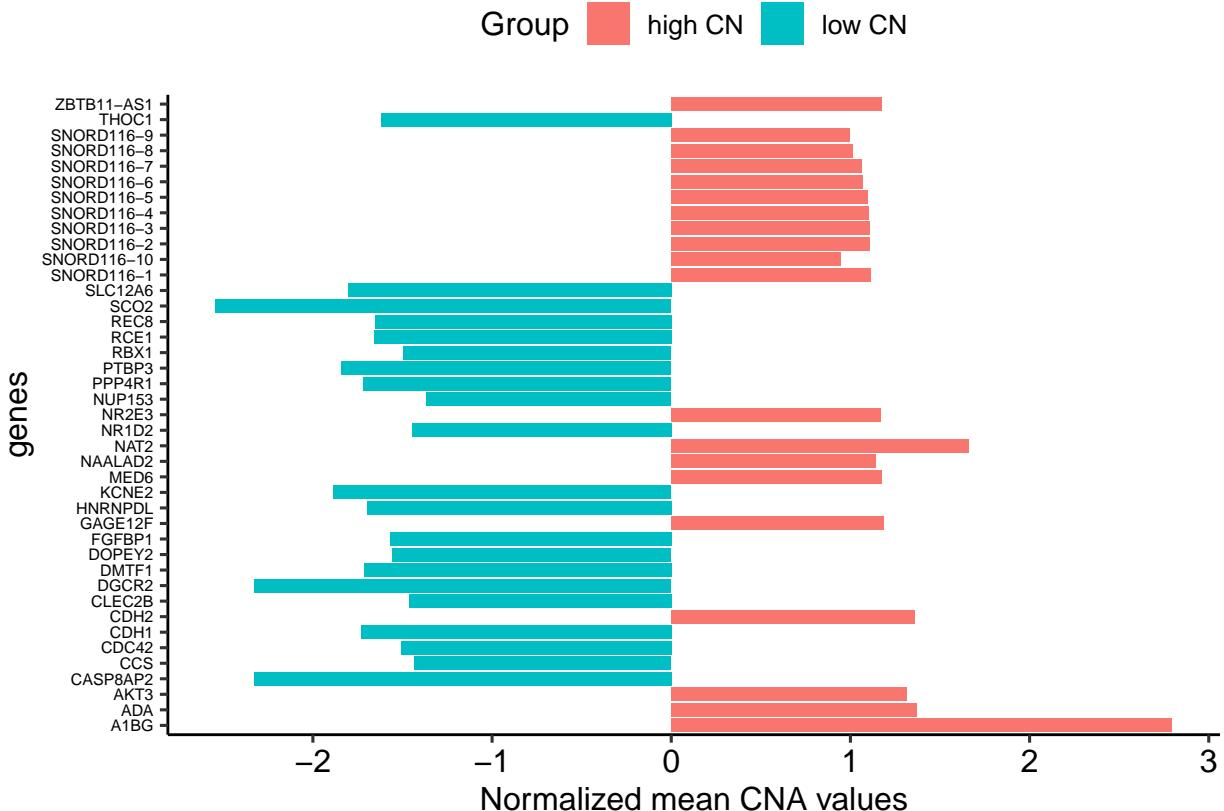
```
cna.HNC.norm = as.data.frame(apply(copynumber.HNC, 2, function(x) {
  x - copynumber.ref.mean
}))
```

Creating a dataframe of HNC copynumber values. The values are normalized to the mean copynumbers of the reference data. For further analysing the dataframe is ordered in a decreasing manner.

```
Values = copynumber.HNC.mean - copynumber.ref.mean
copynumber.HNC.norm = data.frame(values = sort(Values, decreasing = TRUE), genes = row.names(copynumber))
copynumber.HNC.norm$Group = ifelse(copynumber.HNC.norm$values > 0, "high CN", "low CN")
```

Plotting genes with the highest and lowest copynumbers.

```
ggplot(copynumber.HNC.norm[c(1:20, 23172:23192),], aes(x=genes, y=values, fill=Group)) +
  geom_bar(stat="identity") +
  ylab("Normalized mean CNA values") +
  coord_flip() +
  theme(axis.text.y = element_text(size=6))
```



Cleanup of the environment

```
rm(NA.rows, NA.rows.ref, copynumber.HNC.norm, copynumber.HNC.mean, copynumber.ref.mean, copynumber.ref...
```

Mutation

Find Mutations with the highest frequencies

Melt all mutation matrices together as data frame and find out most frequent mutations

```
all_mutations = do.call(rbind,mutation)
all_mutated_genes = table(all_mutations$Hugo_Symbol)
all_mutated_genes_df = as.data.frame(all_mutated_genes)
```

Order the found mutations by frequencies and extract 30 most frequent

```
ID_all_highest_frequencies = head(order(all_mutated_genes_df[["Freq"]], decreasing = TRUE), 30)
all_mutated_genes_df_highest_30 = all_mutated_genes_df[which(rownames(all_mutated_genes_df) %in% ID_all_highest_frequencies),]
ID_all_highest_30_frequencies = order(all_mutated_genes_df_highest_30[["Freq"]], decreasing = TRUE)
all_mutated_genes_highest_30_ordered = all_mutated_genes_df_highest_30[ID_all_highest_30_frequencies, ]
head(all_mutated_genes_highest_30_ordered)
```

```

  Vari Freq
17577   TTN 1567
10296  MUC16  774
17193  TP53  471
10213 MT-ND5  439
11210  OBSCN  368
10304  MUC4   361

```

Name the newly established data frame for the look

```

ID_all_highest_30_frequencies = order(all_mutated_genes_df_highest_30["Freq"], decreasing = TRUE)
all_mutated_genes_df_highest_30_ordered = all_mutated_genes_df_highest_30[ID_all_highest_30_frequencies]
rownames(all_mutated_genes_df_highest_30_ordered) = 1:30
colnames_ordered = c("Gene", "Freq")
colnames(all_mutated_genes_df_highest_30_ordered) = colnames_ordered

```

Melt only HNC sample mutation matrices together and find out most frequent mutations

```

HNC_mutations = do.call(rbind, mutation.HNC)
HNC_mutated_genes = table(HNC_mutations$Hugo_Symbol)
HNC_mutated_genes_df = as.data.frame(HNC_mutated_genes)

```

Order the found mutations by frequencies and name the data frame

```

ID_HNC_highest_frequencies = head(order(HNC_mutated_genes_df[["Freq"]], decreasing = TRUE), length(ID))
HNC_mutated_genes_df_highest_ordered = HNC_mutated_genes_df[ID_HNC_highest_frequencies, ]
rownames(HNC_mutated_genes_df_highest_ordered) = 1:length(ID)
colnames(HNC_mutated_genes_df_highest_ordered) = colnames_ordered

```

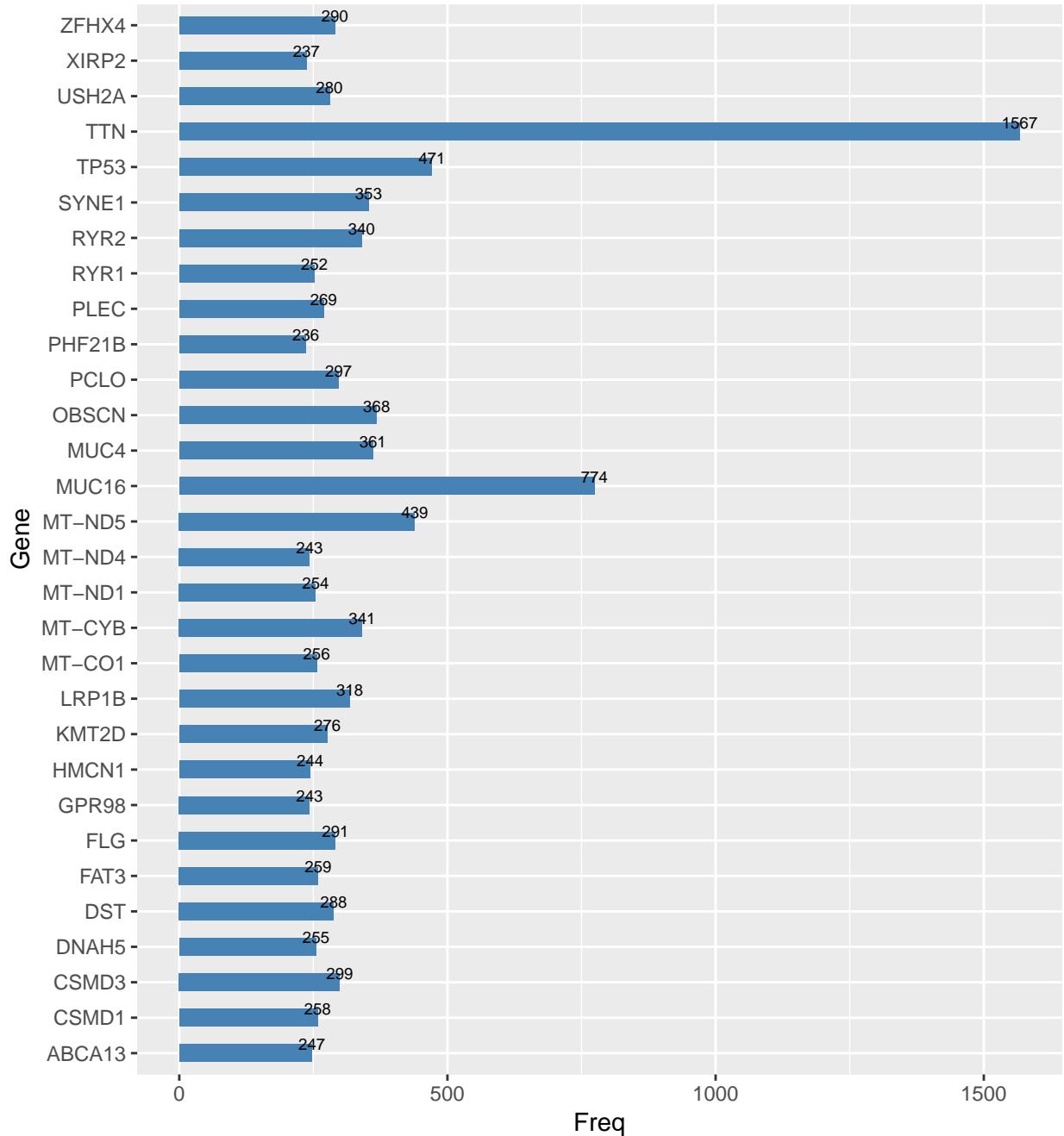
Plotting mutation frequencies of all samples and of HNC samples

Plot mutations in all samples

```

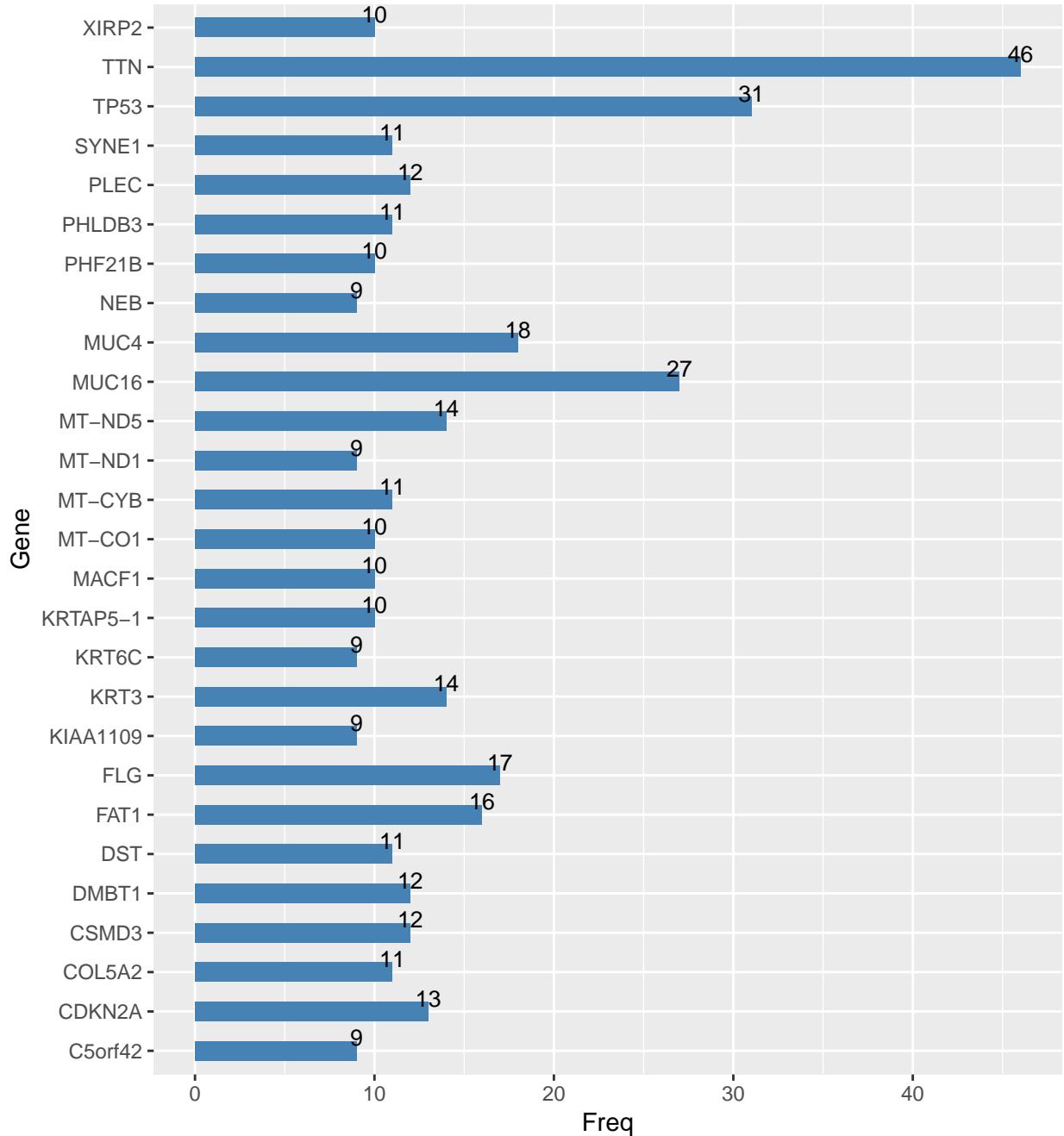
ggplot(data = all_mutated_genes_df_highest_30_ordered, aes(x=Gene, y=Freq)) +
  geom_bar(stat = "identity", width = 0.5, fill="steelblue") +
  geom_text(aes(label=Freq), vjust=-0.3, size=2.5) +
  theme_grey() + coord_flip()

```



Plot mutations in HNC samples

```
ggplot(data = HNC_mutated_genes_df_highest_ordered, aes(x=Gene, y=Freq)) +
  geom_bar(stat = "identity", width = 0.5, fill="steelblue") +
  geom_text(aes(label=Freq), vjust=-0.3, size=3.5) +
  theme_grey() +
  coord_flip()
```



Clean mutation from unnecessary data

Due to the needed conditions in the following “Daisy-tests” we now clean the mutation data and extract all “isDeleterious” mutations, since they are essential for the survival or death of a cell. In this way, we later are able to find our genes of interest for the SL more easily.

```
# Generating a function that searches all elements a of our mutation data set which are isDeleterious
'mutation.clean' = function(data){
  cleaned_data = lapply(1:length(data), function(a) { # Data will be mutation (length of 544) or mutation
    dat_picker = data[[a]] # Pick the element of the list on position 'a'
    ... })}
```

```

dat_picker = dat_picker[, c("Hugo_Symbol", "isDeleterious")] # Choose only the columns with gene names
dat_picker = dat_picker[which(dat_picker$isDeleterious == TRUE),] # Choose only deleterious mutations
return(dat_picker)
})
names(cleaned_data) = names(data) # Keep the names of the input data
return(cleaned_data)
}
# Apply the new function on our two mutation data sets in order to clean them
mutation = mutation.clean(mutation)
mutation.HNC = mutation.clean(mutation.HNC)
head(mutation.HNC[[1]])

```

	Hugo_Symbol	isDeleterious
1:	NBPF1	TRUE
2:	RBBP4	TRUE
3:	WARS2	TRUE
4:	IGHMBP2	TRUE
5:	KRT3	TRUE
6:	KRT3	TRUE

Cleanup of the environment

```
rm(all_mutations, all_mutated_genes, all_mutated_genes_df, ID_all_highest_30_frequencies, ID_all_highest_30_frequencies_df)
```

Knockdown

Overview of kd.ceres values in HNC patients

```
sum(is.na(kd.ceres.HNC) == TRUE) # Checking for NAs
```

```
[1] 0
```

```
range(kd.ceres.HNC) # Range of data
```

```
[1] -2.615780 2.575905
```

Examine knockdown values

```

# Definition of a compare group
kd.ceres.reference = kd.ceres[, which(colnames(kd.ceres) %!in% ID)]

# Defining mean of knockdown values from comparegroup and HNC patients
mean.kd.ceres.reference = apply(kd.ceres.reference, 1, mean)
mean.kd.ceres.HNC = apply(kd.ceres.HNC, 1, mean)

# Checking if knockdown values are normally distributed

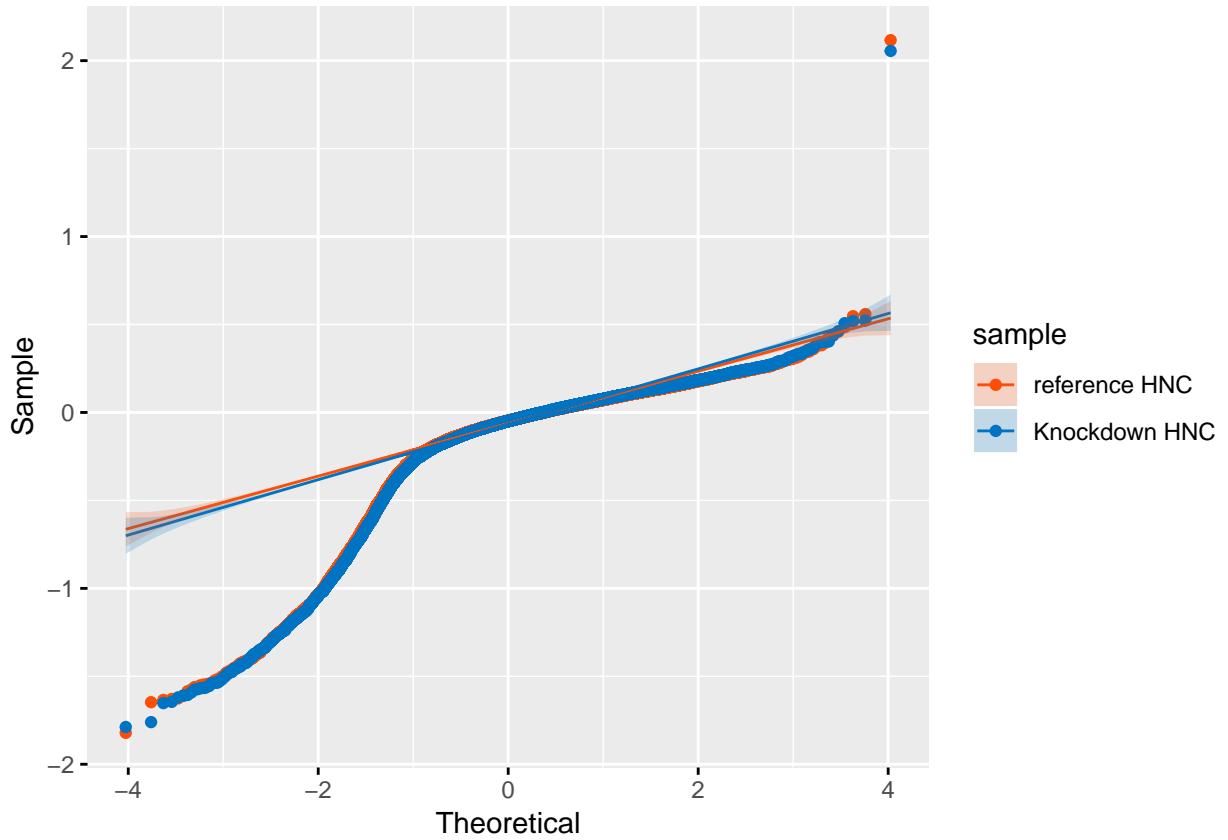
```

```

kd.ceres.HNC.dist = data.frame(kd_values = mean.kd.ceres.HNC, sample = "Knockdown HNC")
kd.ceres.reference.dist = data.frame(kd_values = mean.kd.ceres.reference, sample = "reference HNC")
mean.kd.ceres.dist = rbind(kd.ceres.reference.dist, kd.ceres.HNC.dist)

ggqqplot(mean.kd.ceres.dist, x = "kd_values",
          color = "sample",
          palette = c("#FC4E07", "#0073C2FF"),
          ggtheme = theme_grey())

```



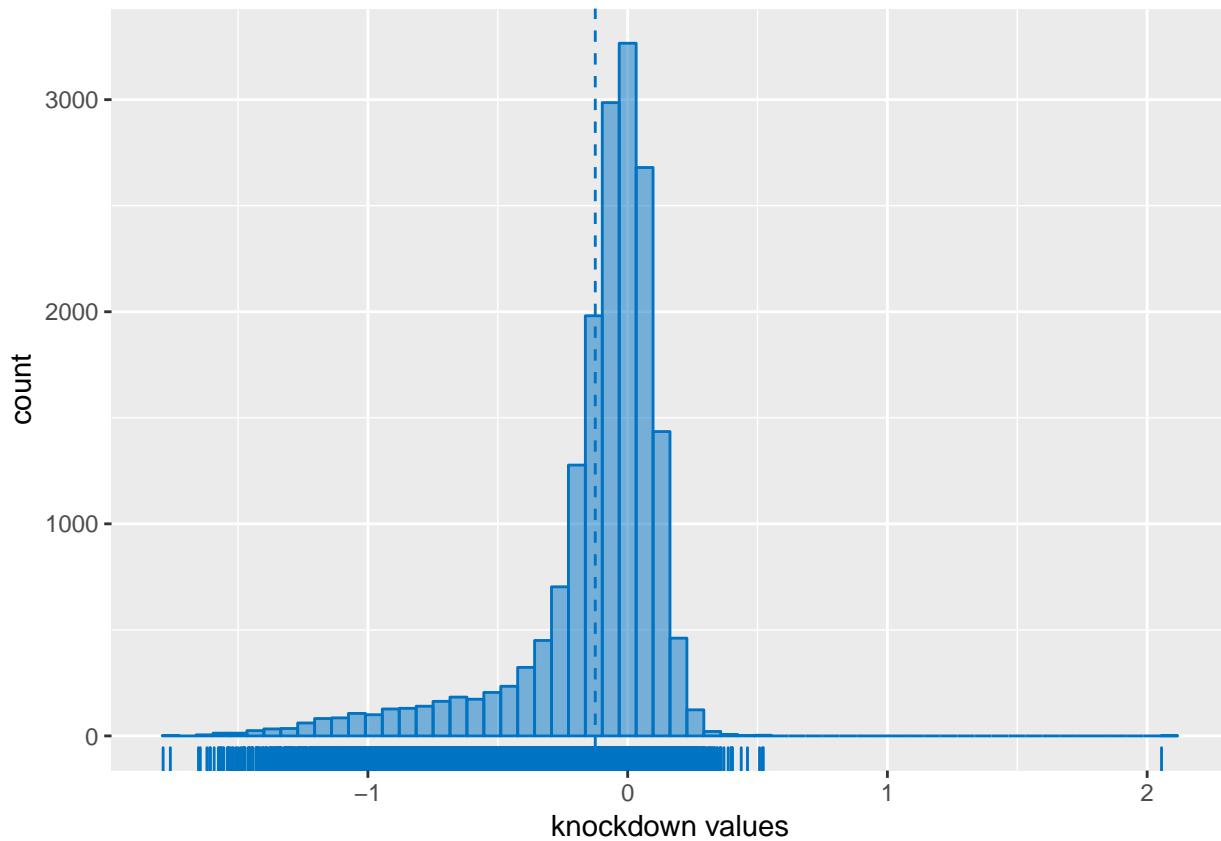
Result: kd.ceres values are not normal distributed

Checking the distribution for knockdown values

```

gghistogram(kd.ceres.HNC.dist, x = "kd_values", xlab = "knockdown values", bins = 60,
            fill = "#0073C2FF", color = "#0073C2FF",
            add = "mean", rug = TRUE)+theme_grey()

```



To check if there are more essential genes in HNC, the means of HNC patients with the reference group were compared.

```
# Are there genes that tend to be more essential in HNC?
esgene1 = kd.ceres.HNC[which(rowMeans(kd.ceres.HNC) < rowMeans(kd.ceres.reference)),]
# Sort essential genes
esgene1= esgene1[order(rowMeans(esgene1)),]
```

Example EGFR

Checking the summary of an example gene

```
# Transposition of dataframe esgene1
esgeneT = as.data.frame(t(esgene1))
summary(esgeneT$EGFR)
```

	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
EGFR	-0.9636	-0.5200	-0.4221	-0.4097	-0.2866	0.0189

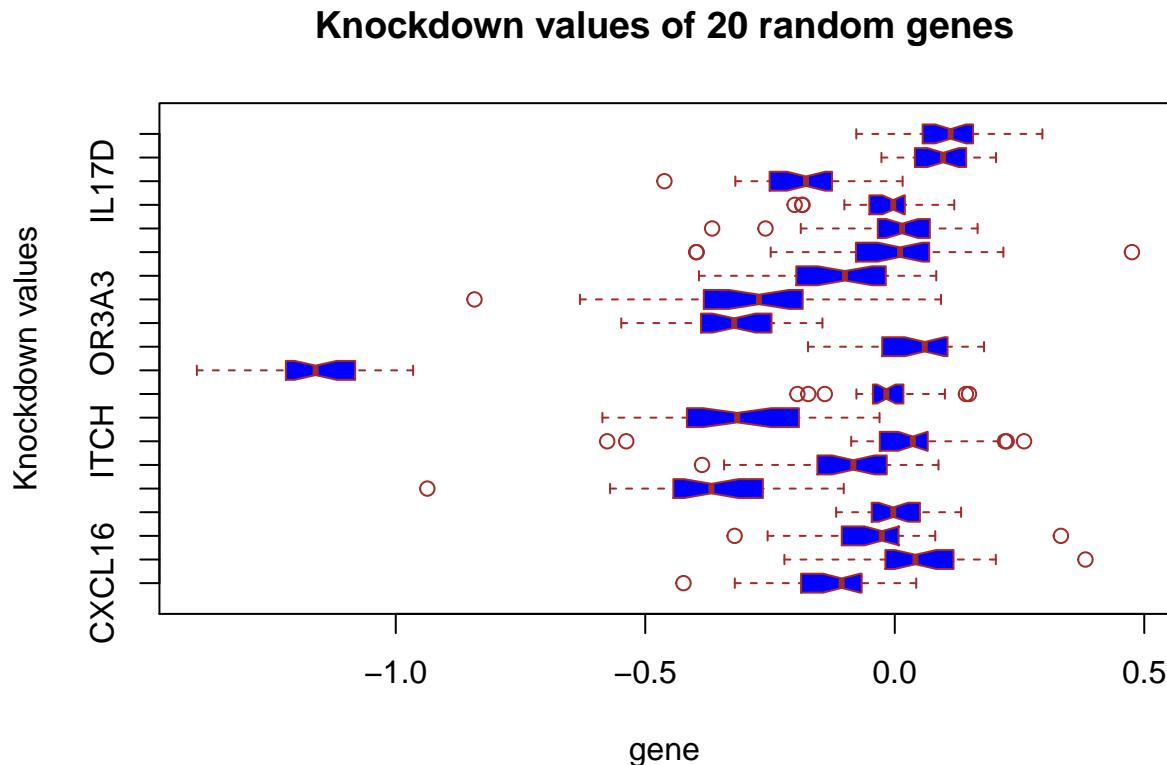
Boxplot of 20 random genes

```
# Boxplot of 20 of most significant genes according to knockdown analysis
boxplot(sample(esgeneT,20, replace = FALSE), xlab = "gene", horizontal = T, main="Knockdown values of 20 genes")
```

```

xlab="genes",
ylab="Knockdown values",
col="blue",
border="brown",
outline = TRUE,
notch = TRUE)
abline(col = c("blue", "red", "black", "orange"),
lty = 2)

```



Applying Wilcoxon rank sum test because knockdown values are not normally distributed

To check if the HNC knockdown values differ from the compare group, a wilcoxon test is executed.

```
wilcox.test(mean.kd.ceres.HNC, mean.kd.ceres.reference)
```

```

Wilcoxon rank sum test with continuity correction

data: mean.kd.ceres.HNC and mean.kd.ceres.reference
W = 155160000, p-value = 0.7421
alternative hypothesis: true location shift is not equal to 0

```

The test shows, that there isn't a significant difference between HNC patients and the reference group. Due to the fact, that knocking down of essential genes has the same effect in healthy cells, than in tumorous cells.

Cleanup of the environment

```
rm(kd.ceres.HNC.dist, kd.ceres.reference, kd.ceres.reference.dist, mean.kd.ceres.HNC, mean.kd.ceres.ref
```

2. Identify genes of interest

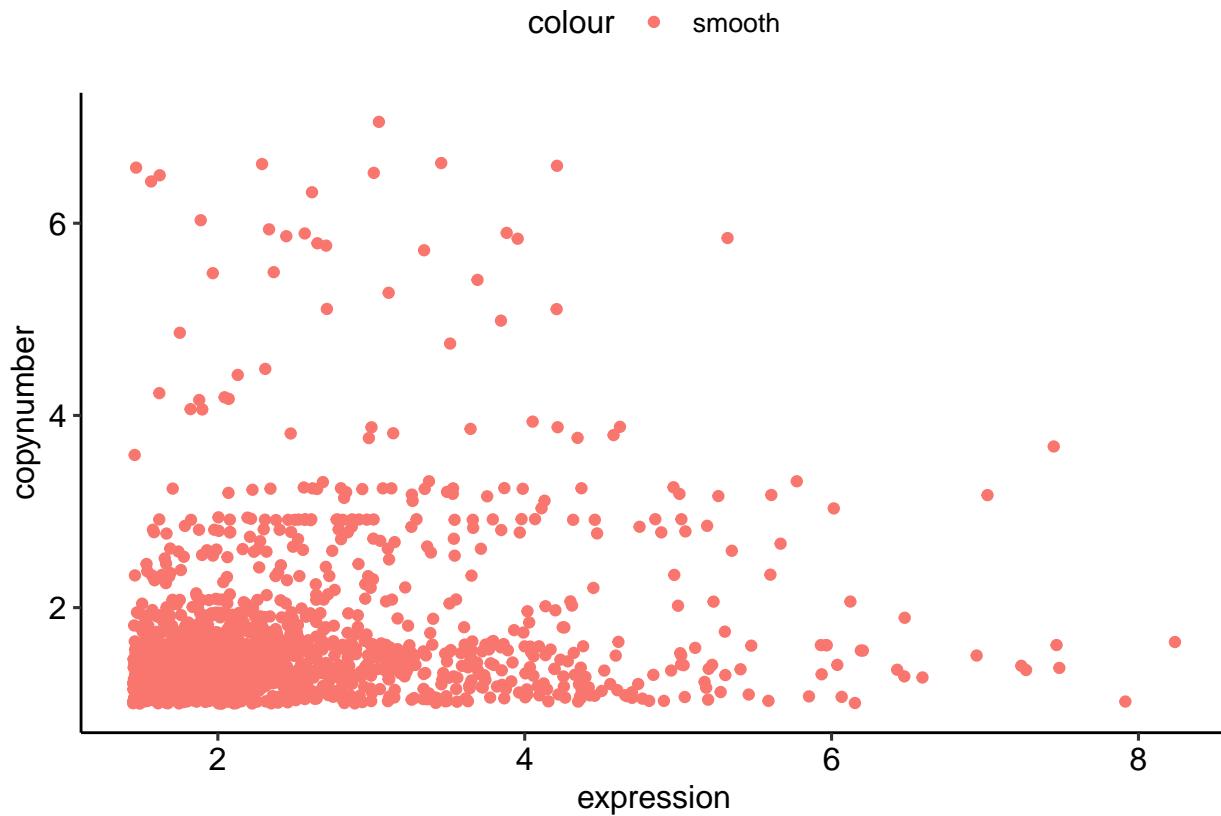
k-means clustering of CNA and expression data with normalized values(-reference) for SDL GOI

To find potential genes for SDL pair detection, clustering through k-means was executed.

```
km.genes = Reduce(intersect, list(rownames(expression.HNC), rownames(copynumber.HNC))) # Intersect set of genes

# Create data frame with expression and CNA values
km.expression = expr.HNC.norm[km.genes,]
km.copynumber = cna.HNC.norm[km.genes,]
km.data = lapply(1:length(ID), function(x) {
  out = data.frame(genes = km.genes, expression = km.expression[,x], copynumber = km.copynumber[,x])
  return (out)
})
# Combine rows for k mean clustering
comb.ex.cna = do.call(rbind, km.data)
comb.ex.cna = comb.ex.cna[which(comb.ex.cna$copynumber >= 1 & comb.ex.cna$expression >= quantile(comb.ex.cna$copynumber)),]

# Plotting of normalized expression values against CNA data for a short overview.
ggplot(comb.ex.cna[, 2:3],
       aes(x = expression, y = copynumber, color = "smooth")) +
  geom_point()
```



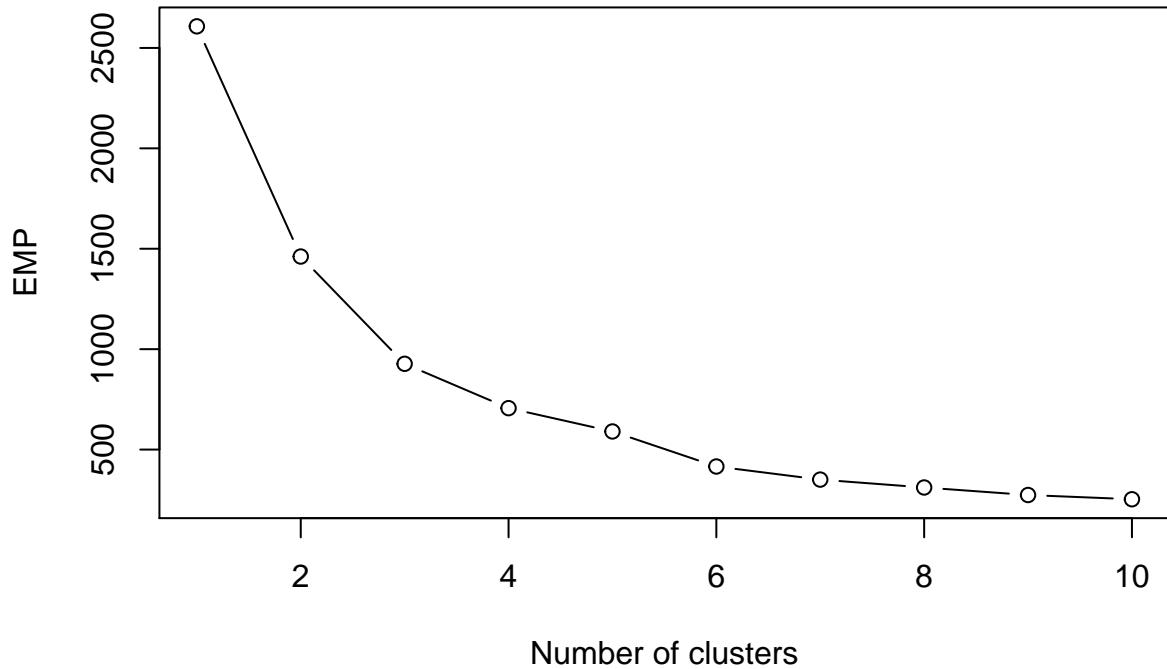
To check the perfect amount of clusters in k-means, an elbow test was implemented.

```
# Elbow method to find optimal amount of clusters
emp = vector() # Create an empty vector
for (i in 1:10) emp[i] = sum(kmeans(comb.ex.cna[2:3], i)$withinss) # Calculate the sum within squared v
emp # Look at the sum within squares for 10 clusters
```

```
[1] 2607.5505 1461.2845 927.0961 706.2404 590.1994 415.6683 350.4004
[8] 311.1543 273.6164 252.6120
```

```
# Plotting of Elbow method
plot(1:10,
      emp,
      type = 'b',
      main = paste('The Elbow Method'),
      xlab = 'Number of clusters',
      ylab = 'EMP')
```

The Elbow Method



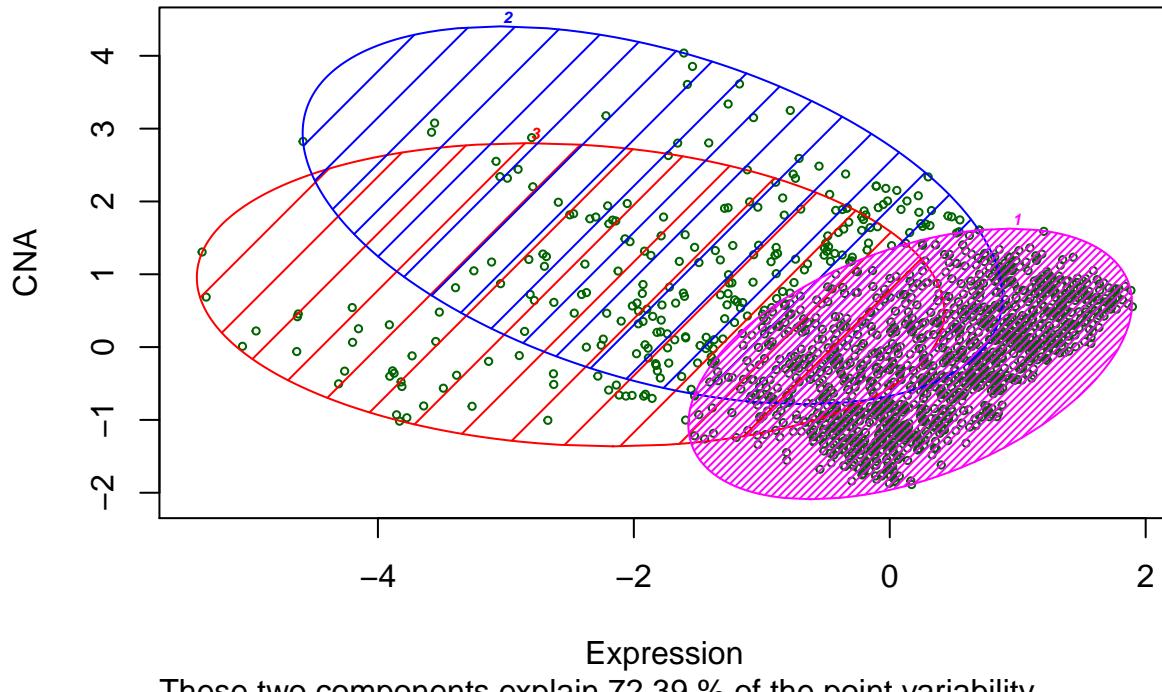
Result: The elbow test shows that 3 clusters are sufficient.

K-means were executed and plotted. Afterwards the potential SDL candidates were identified through higher expression values.

```
# Fitting K-Means to the dataset
kmeans = kmeans(x = comb.ex.cna[,2:3], centers = 3, iter.max = 1000, nstart = 1) # See elbow method (k = 3)
y_kmeans = kmeans$cluster

# Visualising the clusters
clusplot(comb.ex.cna,
          y_kmeans,
          lines = 0,
          shade = TRUE,
          color = TRUE,
          labels = 4,
          plotchar = FALSE,
          span = TRUE,
          stand = FALSE,
          cex = 0.5,
          main = paste('Clusters of Genes'),
          xlab = 'Expression',
          ylab = 'CNA')
```

Clusters of Genes



The Plot “Cluster of genes” features the 3 clusters of values from expression and CNA data. Due to little variance between the data pairs, the intersection set is comparatively big.

In order to define SDL candidates, the cluster with highest expression values was chosen and filtered again according to expression numbers.

```

comb.ex.cna = cbind(comb.ex.cna, y_kmeans)# Combine kmeans with genes
# Sort comb.ex.cna data decresing for selecting adequate number of cluster
comb.ex.cna = comb.ex.cna[order(comb.ex.cna$expression,decreasing =TRUE),]

# Filtering of the most expressed and altered genes
goi.sdl = comb.ex.cna[which(comb.ex.cna$y_kmeans == comb.ex.cna[1,4]),]

# Sort dataframe
goi.sdl = goi.sdl[order(goi.sdl$expression,decreasing=TRUE),]
kable(goi.sdl[1:5,])

```

	genes	expression	copynumber	y_kmeans
428012	DSG3	8.237872	1.641760	2
41915	FXYD3	7.914724	1.022479	2
33343	CST6	7.484452	1.372464	2
185157	F3	7.466245	1.610442	2
354894	FOXE1	7.447917	3.676721	2

```
goi.SDL = as.character(goi.sdl$genes[1:5])
rm(emp, i, km.genes, y_kmeans, kmeans, km.copynumber, km.data, km.expression, comb.ex.cna) # Clean envi
```

Investigate high frequencies of deleterious mutations SL GOI

Create a dataframe with data for the heatmap.

```
mutation.HNC.df = do.call(rbind, mutation.HNC) # All mutation data of HNC samples in one dataframe
heatmap.genes = as.data.frame(table(mutation.HNC.df$Hugo_Symbol)) # Dataframe with all mutated genes in HNC samples

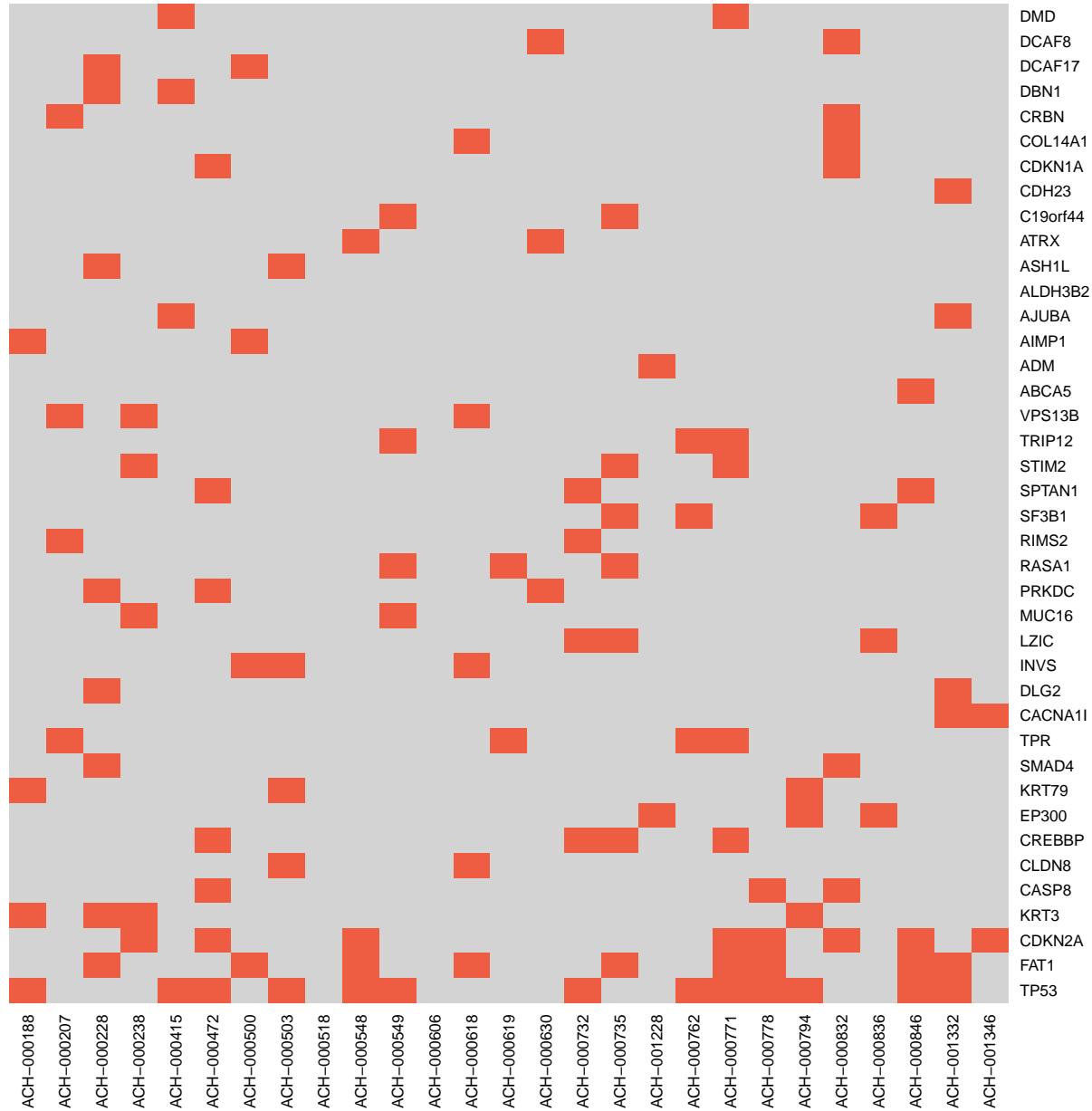
heatmap.data = as.data.frame(sapply(1:(length(ID)), function(a) {
  out = ifelse(heatmap.genes$Var1 %in% mutation.HNC[[a]]$Hugo_Symbol, 1, 0) # Per HNC sample: every gene is either mutated or not
  return(out)
}))

# Define row and column names
rownames(heatmap.data) = heatmap.genes$Var1
colnames(heatmap.data) = ID

# Order from high to low frequency
heatmap.data$sum = apply(heatmap.data, 1, sum)
heatmap.data = heatmap.data[order(-heatmap.data$sum), 1:27]

# Heatmap of the most frequently deleterious mutated genes in HNC samples
heatmap(data.matrix(heatmap.data)[1:40, ],
         Rowv=NA,
         Colv=NA,
         col = c("lightgrey", "tomato2"),
         scale="column",
         cexRow = 0.8,
         cexCol = 0.8,
         main = "Most frequently deleterious mutated genes in HNC samples")
```

Most frequently deleterious mutated genes in HNC samples



Result: TP53, FAT1, CDKN2A, KRT3 and CASP8 are the 5 most frequently deleterious mutated genes in our HNC samples

Table 1 | Genes with frequent and highly significant somatic mutations

Cellular process	Gene	Protein
Cell cycle	CDKN2A	p16 ^{INK4A}
	TP53	p53
	CCND1	G1-S-specific cyclin D1
Growth signals	EGFR	EGFR
	PIK3CA	Catalytic p110α subunit of class I PI3K
Survival	PTEN	PTEN
	FAT1	Protocadherin FAT1
WNT signalling	AJUBA	LIM domain-containing protein AJUBA
	NOTCH1	NOTCH1
	KMT2D	Histone-lysine N-methyltransferase KMT2D
Epigenetic regulation	NSD1	Histone-lysine N-methyltransferase NSD1

Data from REF. 3. Mutation data were taken from The Cancer Genome Atlas (TCGA). *Putative passenger mutation that requires further functional validation.

According to Leemans et al. (2018) these are frequent mutations in HNC:

Leemans, C. R. et al. (2018). The molecular landscape of head and neck cancer. Nat. Rev. Cancer 18, 269–282.

Compare our SL GOI with genes from literature.

```
# create dataframe with goi from literature
goi.lit = data.frame(Gene = c("CDKN2A", "TP53", "CCND1", "EGFR", "PIK3CA", "PTEN", "FAT1", "AJUBA", "NOTCH1"),
                      "Mutation frequency" = c(22, 72, 0.6, 4, 18, 3, 23, 7, 18, 16, 12),
                      "CNA frequency" = c(32, 1.4, 25, 11, 21, 4, 8, 1, 4, 0.4, 0.8))

heatmap_position = sapply(1:nrow(goi.lit), function(a) { # Search for the position of goi.lit in mutated genes
  position = which(rownames(heatmap.data) == goi.lit$Gene[a])
  if(length(position) == 0) {position = NA} # If gene is not part of the genes mutated in HNC samples it is NA
  return(position)
})

goi.lit$"Heatmap position" = heatmap_position # Add heatmap position to goi.lit
rownames(goi.lit) = goi.lit$Gene
goi.lit$Gene = NULL
goi.lit = goi.lit[order(goi.lit$"Heatmap position"),]
kable(goi.lit) # Show a table of goi.lit
```

	Mutation.frequency	CNA.frequency	Heatmap position
TP53	72.0	1.4	1
FAT1	23.0	8.0	2
CDKN2A	22.0	32.0	3
AJUBA	7.0	1.0	28
KMT2D	16.0	0.4	66
NOTCH1	18.0	4.0	82
NSD1	12.0	0.8	715
CCND1	0.6	25.0	NA
EGFR	4.0	11.0	NA
PIK3CA	18.0	21.0	NA
PTEN	3.0	4.0	NA

Because our most frequent mutated genes almost approximately match with frequent mutations from literature we choose the 5 genes with most frequent mutations in HNC samples.

```
goi.SL = rownames(heatmap.data)[1:5]
goi.SL

[1] "TP53"    "FAT1"     "CDKN2A"   "KRT3"     "CASP8"
```

Clean up of the environment

```
rm(mutation.HNC.df, heatmap.genes, heatmap.data, heatmap_position)
```

3. Find SL/SDL partner

SL/SDL partner search following the daisy model

To examine gene pairs A and B which fulfill the criteria of SoF and functional examination we perform a Wilcoxon rank sum test on copynumber or CERES knockdown data. We have 5 inactive genes (goi.SL) and 5 overactive genes (goi.SDL) which are defined as genes A. The Wilcoxon rank sum test now returns all genes B which pass the test in a significant manner ($p < 0.05$).

```
'Daisy.Wilcox' = function(input_genes, input_data, inactive_overactive, less_greater){
  Daisy_Wilcox = lapply(1:length(input_genes), function(m) {
    goi = input_genes[m] # Set 1 of 5 goi
    # Depending on SL or SDL partner search gene B needs to be inactive (SL) or overactive (SDL)
    if(inactive_overactive == 1) { # inactive_overactive == 1 chooses overactive
      mean.exp.goi = mean(t(expression[goi,]))
      exp.goi.norm = expression[goi,] - mean.exp.goi # Normalised expression values of goi
      mut_overa.ID = lapply(1:ncol(expression), function(a) {
        out = if(exp.goi.norm[goi, a] > 0 & copynumber[goi,a] > 0.3) {a} else {NA} # Filter all IDs which are overactive
        return(out)
      })
    } else { # inactive_overactive == 0 chooses inactive
      mut_overa.ID = lapply(1:length(mutation), function(a) {
        out = ifelse(goi %in% mutation[[a]]$Hugo_Symbol, a, NA) # Filter all IDs which have a mutation of goi
        return(out)
      })
    }
    not_mut_overa.ID = grep("NA", mut_overa.ID) # ID of samples which have NO inactivity/overactivity of goi
    mut_overa.ID = as.integer(mut_overa.ID[is.na(mut_overa.ID) == FALSE]) # ID of samples which HAVE inactivity/overactivity of goi

    p.value = sapply(1:nrow(input_data), function(b) {
      mut_overa.data = t(input_data[b, mut_overa.ID])
      not_mut_overa.data = t(input_data[b, not_mut_overa.ID])
      if (less_greater == 1) {
        p = wilcox.test(mut_overa.data, not_mut_overa.data, alternative = "greater")$p.value # One sided
      } else {
        p = wilcox.test(mut_overa.data, not_mut_overa.data, alternative = "less")$p.value # One sided (greater)
      }
    })
  })
}
```

```

    })

out = data.frame(genes = rownames(input_data), p_value = p.value)
out = out[which(out$p_value < 0.05),]
return(out)
})

names(Daisy_Wilcox) = input_genes
return(Daisy_Wilcox)
}

```

To examine gene pairs A and B which fulfill the criteria of gene co expression we performe a Spearman correlation test on the expression data. We have 5 inactive genes (goi.SL) and 5 overactive genes (goi.SDL) which are defined as genes A The correlation test now returns all genes B that correlate to gene A in a significant manner ($p<0.05$).

```

'Daisy.Spearman' = function(input_genes){
  Daisy_Spearman = lapply(1:length(input_genes), function(m) { # Build up a list with the 5 goi; lapply
    goi = input_genes[m]

    p.value = sapply(1:nrow(expression), function(a){ # Creation of a list with the length of the expression
      expression.goi = t(expression[goi,])           # Variable containing expressiondata of the goi (gene A)
      expression.GeneB = t(expression[a,])          # Variable containing all other genes (gene B)
      p = cor.test(expression.goi,expression.GeneB, alternative = "greater", method = "spearman", exact =
    })

    # Return a dataframe with the columns genes(HugoSymbols) and p_values (p.values)
    out = data.frame(genes = rownames(expression), p_value = p.value)
    out = out[which(out$p_value < 0.05),] # Filter for significant p.values
    out = out[which(out$genes != goi),]    # Select onl these wich are not the same gene as the goi (would
    return(out)
  })
  names(Daisy_Spearman) = input_genes
  return(Daisy_Spearman)
}

```

Both functions return a list, which contains the five genes of interest, each containing a dataframe with all genes examined as potential SL/SDL partners plus their calculated p-values.

SL-partter

SoF

```

SL.SoF = Daisy.Wilcox(goi.SL, copynumber, inactive_overactive = 0, less_greater = 1) # SoF, inactive, or active
head(SL.SoF[[1]]) # Example of SL SoF for first GOI (TP53)

```

	genes	p_value
10	NAALAD2	0.01270171
88	NAALADL1	0.01787355
105	MCTS2P	0.01801226
109	SNORD119	0.03415298
120	SNORD111B	0.04370297
148	MIR875	0.02447163

Functional examination

```
SL.functional.examination = Daisy.Wilcox(goi.SL, kd.ceres, inactive_overactive = 0, less_greater = 0) #  
head(SL.functional.examination[[1]]) # Example of SL functional examination for first GOI (TP53)
```

	genes	p_value
10	AADAC	0.008677123
21	AAR2	0.016419751
70	ABCG1	0.010374932
100	ABL2	0.042951952
149	ACN9	0.006751253
211	ACTR1B	0.010514096

Gene coexpression

```
SL.coexpression = Daisy.Spearman(goi.SL)  
head(SL.coexpression[[1]]) # Example of SL coexpression for first GOI (TP53)
```

	genes	p_value
4	SCYL3	7.196232e-06
5	C1orf112	2.516883e-04
10	NFYA	2.641665e-09
13	LAS1L	3.844908e-02
17	ANKIB1	1.345544e-02
24	CD99	8.535496e-03

SDL-partner

SoF

```
SDL.SoF = Daisy.Wilcox(goi(SDL, copynumber, inactive_overactive = 1, less_greater = 1) # SoF, overactive
```

Functional examination

```
SDL.functional.examination = Daisy.Wilcox(goi(SDL, kd.ceres, inactive_overactive = 1, less_greater = 0)
```

Gene coexpression

```
SDL.coexpression = Daisy.Spearman(goi(SDL))
```

Converging the different SL and SDL sets

Define function to converge SL/SDL sets.

```
'partner' = function(input_genes, SoF, functional.examination, coexpression){  
  partner = lapply(1:length(input_genes), function(a) {  
    dat_picker.SoF = SoF[[a]]  
    dat_picker.functional_examination = functional.examination[[a]]  
    dat_picker.coexpression = coexpression[[a]]  
  })
```

```

partnergenes = Reduce(intersect, list(dat_picker.SoF$genes, dat_picker.functional_examination$genes))
out = data.frame(genes = partnergenes, # Column with reduced genes
                 SoF = dat_picker.SoF[which(dat_picker.SoF$genes %in% partnergenes), "p_value"], # C
                 functional_examination = dat_picker.functional_examination[which(dat_picker.functiona
                 coexpression = dat_picker.coexpression[which(dat_picker.coexpression$genes %in% pa
         return(out)
     })
     names(partner) = input_genes
     return(partner)
}

```

Converge different sets.

```

SL.partner = partner(goi.SL, SL.SoF, SL.functional.examination, SL.coexpression)
SDL.partner = partner(goi(SDL, SDL.SoF, SDL.functional.examination, SDL.coexpression)
kable(data.frame("SL genes" = names(SL.partner),
                 "SL number of partner" = sapply(1:length(SL.partner), function(a){nrow(SL.partner[[a]])}),
                 "SDL genes" = names(SDL.partner),
                 "SDL number of partner" = sapply(1:length(SDL.partner), function(a){nrow(SDL.partner[[a]])}))
))

```

SL.genes	SL.number.of.partner	SDL.genes	SDL.number.of.partner
TP53	21	DSG3	96
FAT1	54	FXYD3	78
CDKN2A	60	CST6	232
KRT3	124	F3	78
CASP8	22	FOXE1	136

Plotting networks of the Interactions

Creation of a function for transforming given data into a format iGraph can work with and plotting of the network

```

'network' = function(X.partner, width, node.color, frame.color, node.size, Titel, label, GOI){ # (SL/SDL.partn

  color.vector = c("darkred", "darkgreen", "turquoise", "yellow", "orange") # Define colours
  # Transform data into a dataframe with 3 columns for each GOI
  edgelist = lapply(1:length(X.partner), function(a){
    dat_picker = X.partner[[a]]
    edge.list = data.frame(Gene.A = names(X.partner)[a], Gene.B = dat_picker$genes, colours = color.vec
  })
  edge.list = do.call(rbind, edgelist) # Combine all goi-dataframes to one.

  edge.list$colours = as.character(edge.list$colours) # After the lapply, column "colours" has class 'f

  net = graph_from_data_frame(edge.list, directed = T) # Transform the dataframe into an iGraph object
  layout1 = layout_with_kk(net) # Given layout

  # Plotting parameters are defined. vertex= Genes edges = interactions
  plot(net, layout = layout1, rescale = T,

```

```

    ylim = c(-0.9,1),
    xlim = c(-0.1,-0.1),
    edge.color = edge.list$colours,
    edge.width = width,
    edge.arrow.mode = 0,
    vertex.color = node.color,
    vertex.frame.color = frame.color,
    vertex.size = node.size,
    vertex.label.font = 2,
    vertex.label.color = "black",
    vertex.label = label,
    margin = 0.1)

# Add legend to the graph
legend (x = 1.2,
        y = 1, GOI,
        fill = c("darkred", "darkgreen", "turquoise", "yellow", "orange"))

# Add title to the graph
title(Titel,cex.main=3,col.main="black ")

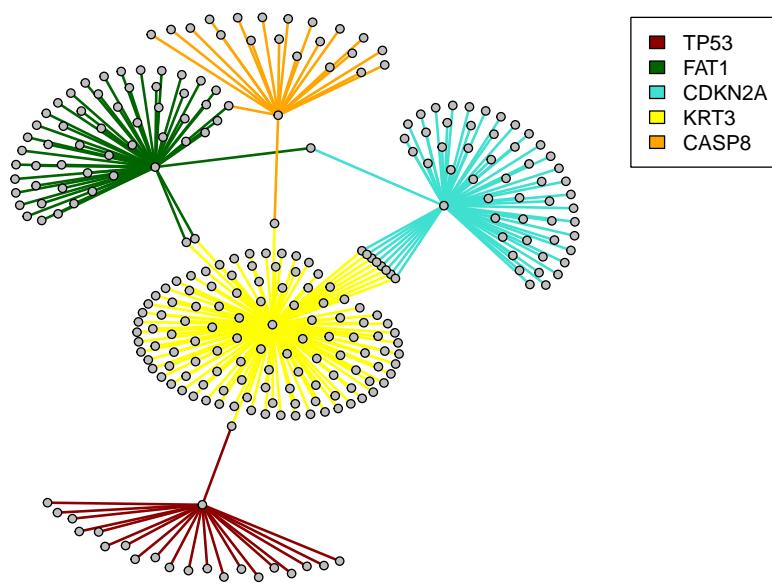
}

```

Creating a networkplot for the SL-partner

```
network(SL.partner,2,"grey","black",3,"Network of SL interaction", NA, goi.SL )
```

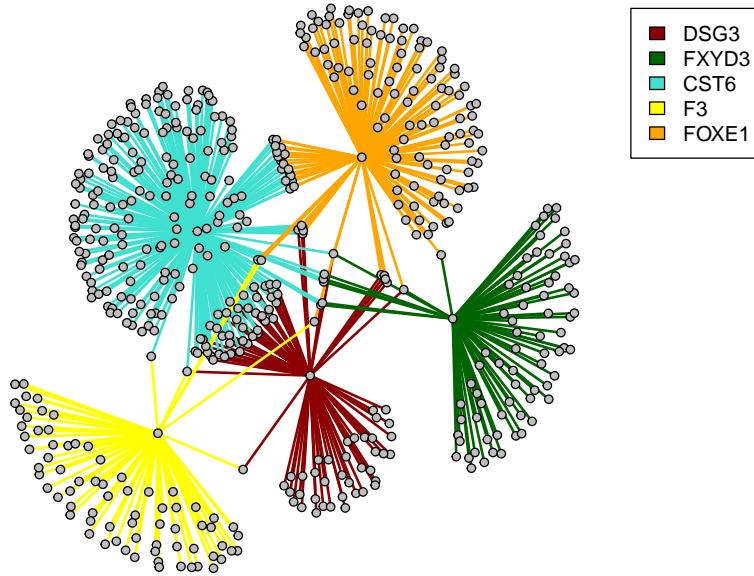
Network of SL interaction



Creating a network for the SDL-partner

```
network(SDL.partner,2,"grey","black",3,"Network of SL interaction", NA, goi.SDL )
```

Network of SL/SDL interaction



Because there are too many SL/SDL partner to plot a network we reduce SL/SDL partner to the most significant. To do so, we reduce the number of partner genes for better plotting without removing genes which are connected to more than one GOI.

```
'reduce.partner' = function(list){
  reduced_partner = lapply(1:length(list), function(a){ # Go through every element of SL/SDL partner
    dat_picker = list[[a]]

    if (nrow(dat_picker) > 20) { # (1) If length of dat_picker is larger than 20 reduce to the 20 most significant genes
      dat_picker$order = apply(dat_picker[,2:4], 1, mean) # Create an extra column with the mean p value
      ordered_data = dat_picker[order(dat_picker$order),][,1:4] # Order the partner genes after this mean

      gene_intersect = lapply(1:length(list), function(b){
        as.list(Reduce(intersect, list(dat_picker$genes), list[[b]]$genes)) # Search for genes that are in all partner genes
      })
      gene_intersect[a] = NULL # Remove intersect of the GOI partner genes with itself
      keep_genes = c(gene_intersect[[1]], gene_intersect[[2]], gene_intersect[[3]], gene_intersect[[4]])
      position = sapply(1:length(keep_genes), function(c){
        which(as.character(ordered_data$genes) == keep_genes[c])
      })
      position = position[which(position > 20)] # Partner genes that are already in the first 20 genes

      if(length(position) > 3) {position = position[1:3]} # If there are still more than 3 extra genes
      out = ordered_data[c(1:20, position),]
      return(out) # Return reduced data
    }
  })
}
```

```

    } else {
      return(ordered_data) # (2) If length of dat_picker is smaller than 20 return data unreduced
    }
  })
  names(reduced_partner) = names(list)
  return(reduced_partner)
}

```

Reduce SL.partner/SDL.partner to the most significant

```

SL.partner.reduced = reduce.partner(SL.partner)
SDL.partner.reduced = reduce.partner(SDL.partner)

```

```

kable(data.frame("SL genes" = names(SL.partner),
                 "SL partner" = sapply(1:length(SL.partner.reduced), function(a){nrow(SL.partner.reduced[[a]])}),
                 "SDL genes" = names(SDL.partner),
                 "SDL partner" = sapply(1:length(SDL.partner.reduced), function(a){nrow(SDL.partner.reduced[[a]])}))
))

```

SL.genes	SL.partner	SDL.genes	SDL.partner
TP53	20	DSG3	23
FAT1	21	FXYD3	23
CDKN2A	22	CST6	23
KRT3	23	F3	23
CASP8	20	FOXE1	23

Now the number of SL/SDL partner is much better to plot.

Plotting SL/SDL partner network with reduced number of partner

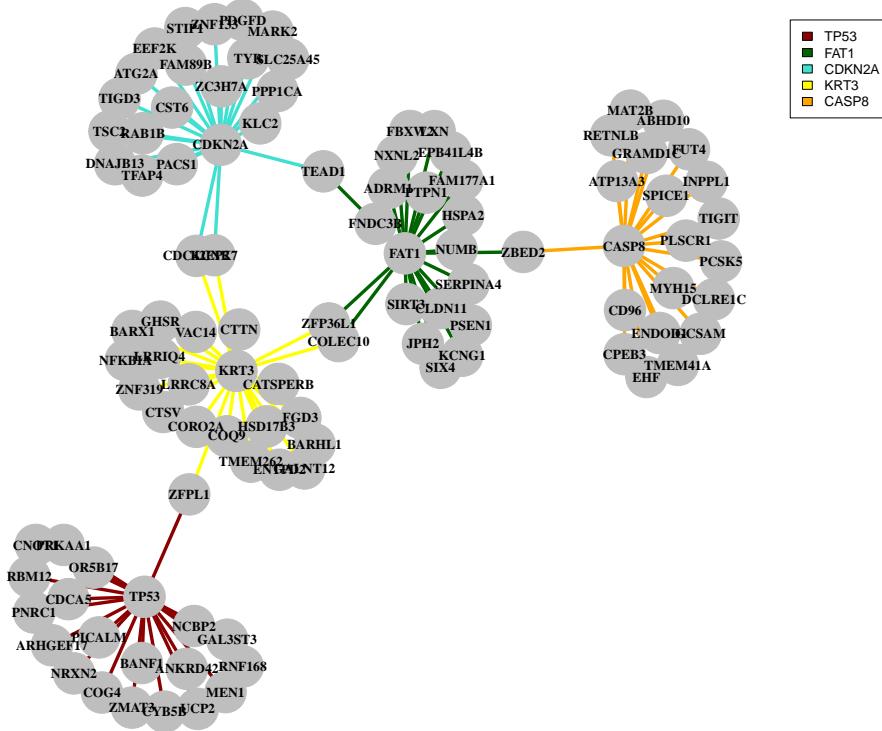
SL interaction plot with reduced partners

```

network(SL.partner.reduced,4,"grey","grey",12,"Network of SL interaction (reduced)", names(plot), goi.SL)

```

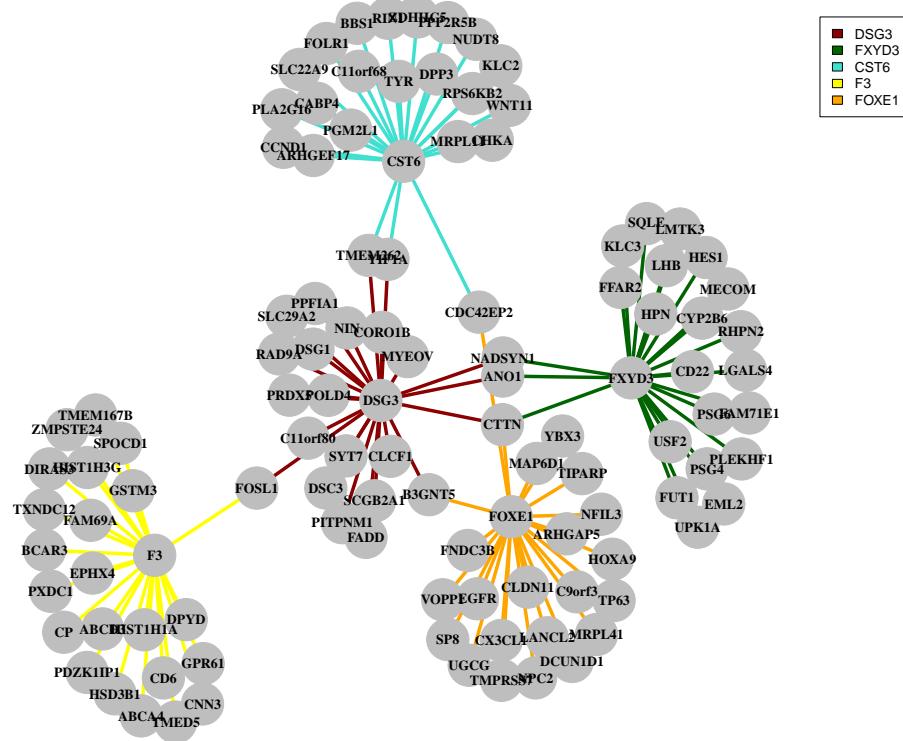
Network of SL interaction (reduced)



SDL interaction plot with reduced partners

```
network(SDL.partner.reduced,4,"grey","grey",12,"Network of SDL interaction (reduced)", names(plot),goi..
```

Network of SDL interaction (reduced)



4. Mutation prediction via logistical regression

Prepare data for regression model

```
# Reduce all genes to them of which we have expression, copynumber and knockdown data.
regression.genes = Reduce(intersect, list(rownames(expression), rownames(copynumber), rownames(kd.ceres)))

# Create a dataframe with expression, copynumber, knockdown and mutation data from a HNC sample.
regression.data = lapply(1:length(ID), function(a){

  regression.mutation = sapply(1:length(regression.genes), function(b){
    ifelse(regression.genes[b] %in% mutation.HNC[[a]]$Hugo_Symbol, TRUE, FALSE)
  })

  regression.data = data.frame("genes" = paste(regression.genes, ID[a], sep = "_"),
                               "expression" = expression.HNC[regression.genes, a],
                               "copynumber" = copynumber.HNC[regression.genes, a],
                               "knockdown" = kd.ceres.HNC[regression.genes, a],
                               "mutation" = regression.mutation)
})
```

```

})

# Bind these 27 dataframes to one dataframe
regression.data = do.call(rbind, regression.data)

rm(regression.genes)
dim(regression.data)

```

[1] 458190 5

```
head(regression.data)
```

	genes	expression	copynumber	kockdown	mutation
1	DPM1_ACH-000188	5.3593103	0.1574	-0.42737583	FALSE
2	SCYL3_ACH-000188	1.2570106	0.1441	-0.01577348	FALSE
3	C1orf112_ACH-000188	3.1093606	0.1441	-0.03149540	FALSE
4	FGR_ACH-000188	0.1634987	0.2019	-0.10972076	FALSE
5	CFH_ACH-000188	4.0285692	-0.0637	0.03189585	FALSE
6	FUCA2_ACH-000188	4.6536333	0.1872	0.02271554	FALSE

Reformat the data because strings can not be an input for a machine learning model

```
regression.data$mutation = factor(regression.data$mutation, levels = c("FALSE", "TRUE"))
head(regression.data)
```

	genes	expression	copynumber	kockdown	mutation
1	DPM1_ACH-000188	5.3593103	0.1574	-0.42737583	FALSE
2	SCYL3_ACH-000188	1.2570106	0.1441	-0.01577348	FALSE
3	C1orf112_ACH-000188	3.1093606	0.1441	-0.03149540	FALSE
4	FGR_ACH-000188	0.1634987	0.2019	-0.10972076	FALSE
5	CFH_ACH-000188	4.0285692	-0.0637	0.03189585	FALSE
6	FUCA2_ACH-000188	4.6536333	0.1872	0.02271554	FALSE

Split the data into train and test-data

```
inTrain = createDataPartition(y = regression.data$mutation, p = .75, list = FALSE)
train.set = regression.data[inTrain,] # Only get training data
rownames(train.set) = train.set$genes # Reformat the rownames
train.set = train.set[,2:ncol(train.set)] # Get rid of the gene names because they are now the rownames
head(train.set) # Check the data
```

	expression	copynumber	kockdown	mutation
DPM1_ACH-000188	5.3593103	0.1574	-0.42737583	FALSE
SCYL3_ACH-000188	1.2570106	0.1441	-0.01577348	FALSE
FGR_ACH-000188	0.1634987	0.2019	-0.10972076	FALSE
FUCA2_ACH-000188	4.6536333	0.1872	0.02271554	FALSE
GCLC_ACH-000188	4.1497471	0.1549	0.06840387	FALSE
NFYA_ACH-000188	3.1874511	0.1712	-0.13549254	FALSE

```

test.set = regression.data[-inTrain,]
rownames(test.set) = test.set$genes
test.set = test.set[,2:ncol(test.set)]
head(test.set)

```

	expression	copynumber	kockdown	mutation
C1orf112_ACH-000188	3.109361	0.1441	-0.03149540	FALSE
CFH_ACH-000188	4.028569	-0.0637	0.03189585	FALSE
SEMA3F_ACH-000188	4.303781	-0.5911	-0.40210314	FALSE
CYP51A1_ACH-000188	3.539779	-0.2043	0.11390942	FALSE
LASP1_ACH-000188	6.291309	-0.1920	-0.21685849	FALSE
CYP26B1_ACH-000188	2.922198	0.0749	0.04949324	FALSE

Train the model

```

lrfit = glm(mutation ~ ., data=train.set, family="binomial")
lrfit

```

Call: `glm(formula = mutation ~ ., family = "binomial", data = train.set)`

Coefficients:

(Intercept)	expression	copynumber	kockdown
-6.01316	0.02116	0.19787	0.11471

Degrees of Freedom: 343642 Total (i.e. Null); 343639 Residual
Null Deviance: 12250
Residual Deviance: 12240 AIC: 12250

```
summary(lrfit)
```

Call:
`glm(formula = mutation ~ ., family = "binomial", data = train.set)`

Deviance Residuals:

Min	1Q	Median	3Q	Max
-0.1190	-0.0735	-0.0712	-0.0695	3.5618

Coefficients:

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-6.01316	0.05072	-118.564	<2e-16 ***
expression	0.02116	0.01489	1.421	0.1555
copynumber	0.19787	0.08104	2.442	0.0146 *
kockdown	0.11471	0.12047	0.952	0.3410

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

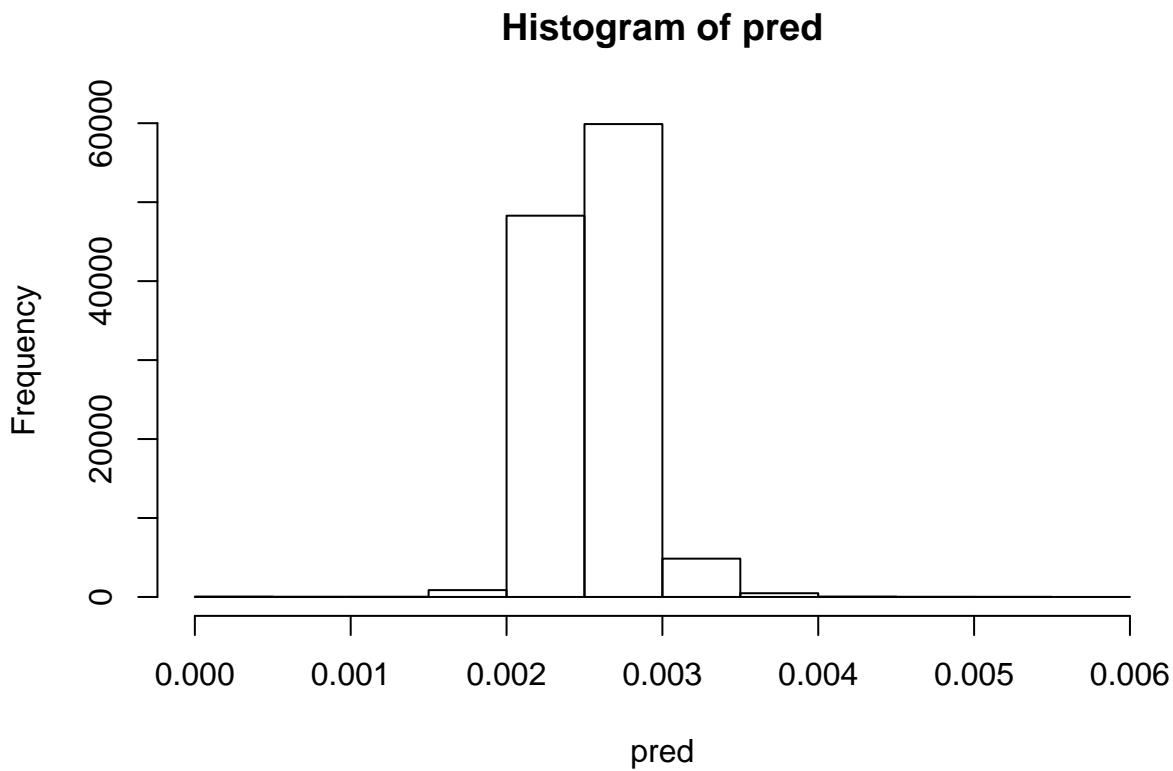
(Dispersion parameter for binomial family taken to be 1)

```
Null deviance: 12249 on 343642 degrees of freedom
Residual deviance: 12239 on 343639 degrees of freedom
AIC: 12247
```

Number of Fisher Scoring iterations: 9

Evaluate the precision and the recall of the model

```
pred = predict(lrfit,newdata=test.set,type="response")
hist(pred) # Look at the predictions
```



Devide prediction in tressholds

```
threshold_ls = seq(min(pred), max(pred), by=0.001) # Make a threshold list
threshold_list = lapply(seq_along(threshold_ls), function(a) table(pred>threshold_ls[a], test.set$mutu
```

Calculate the precision values

```
results_df = lapply(1:length(threshold_list), function(a) { # Make a df out of the list of lists
  threshold = threshold_list[[a]]
  threshold_value = threshold_ls[[a]]

  df_prec_recall = as.data.frame(list(threshold["FALSE", "FALSE"], threshold["FALSE", "TRUE"], threshold["
```

```

df_prec_recall$threshold = threshold_value
colnames(df_prec_recall) = c("TN", "FN", "FP", "TP", "Thresh")
return(df_prec_recall)
})

results_df = rbindlist(results_df) # Bind all the lists together
recall = apply(results_df, 1, function(x){ x[["TP"]]/(x[["TP"]]+x[["FN"]])}) # Calculate the recall
precision = apply(results_df, 1, function(x){ x[["TP"]]/(x[["TP"]]+x[["FP"]])}) # Calculate the precision
results_df$recall = recall # Bind recall to the data
results_df$precision = precision # Bind precision to the data
head(results_df) # Look at the data

```

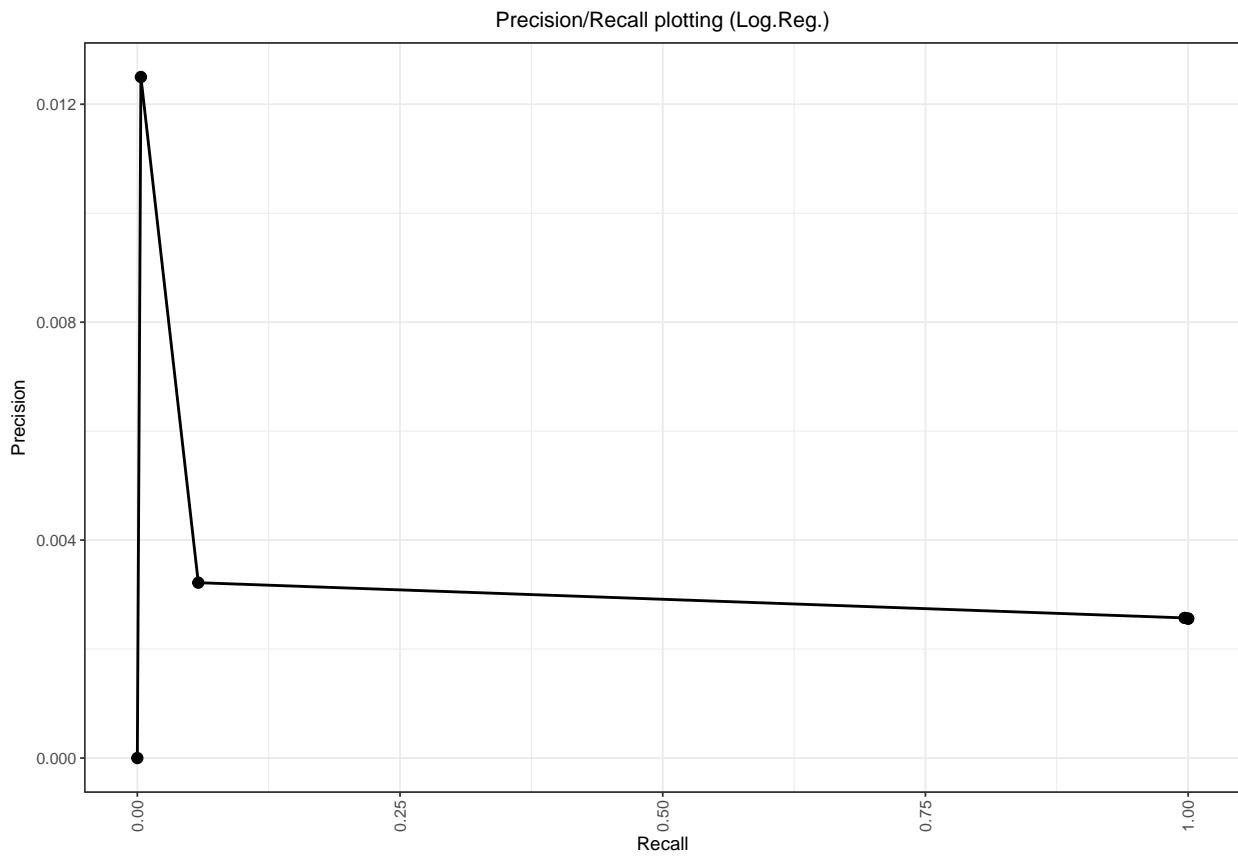
	TN	FN	FP	TP	Thresh	recall	precision
1:	1	0	114253	293	3.753460e-06	1.000000000	0.002557924
2:	69	0	114185	293	1.003753e-03	1.000000000	0.002559444
3:	1019	1	113235	292	2.003753e-03	0.996587031	0.002572075
4:	108989	276	5265	17	3.003753e-03	0.058020478	0.003218478
5:	114175	292	79	1	4.003753e-03	0.003412969	0.012500000
6:	114241	293	13	0	5.003753e-03	0.000000000	0.000000000

Plot the data

```

ggplot(results_df, aes(recall,precision)) +
  geom_point() +
  geom_line() +
  ggtitle("Precision/Recall plotting (Log.Reg.)") +
  ylab("Precision") +
  xlab("Recall") +
  theme_bw(base_size = 7) +
  theme(legend.position="bottom",
        legend.direction="horizontal",
        plot.title = element_text(hjust = 0.5),
        axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1),
        legend.title= element_blank(),
        strip.text.y = element_text(angle = 0))

```



Clean the enviroment

```
rm(precision, pred, recall, regression.genes, threshhold_ls, inTrain, lrfit, results_df, test.set, thre
```

References

Articles

- 1.) Jerby-Arnon, L., et al. (2014). "Predicting Cancer-Specific Vulnerability via Data-Driven Detection of Synthetic Lethality." *Cell* 158(5): 1199-1209.
- 2.) Ashworth, A., et al. (2011). "Genetic Interactions in Cancer Progression and Treatment." *Cell* 145(1): 30-38.
- 3.) O'Neil, N. J., et al. (2017). "Synthetic lethality and cancer." *Nature Reviews Genetics* 18: 613.

Figures

Figure 1 - <https://crukcambridgecentre.org.uk/patient-care/clinical-research/head-and-neck> Figure 2 - Jerby-Arnon, L., et al. (2014). "Predicting Cancer-Specific Vulnerability via Data-Driven Detection of Synthetic Lethality." *Cell* 158(5): 1199-1209.