

Bulk RNA-seq: Sen High UT vs Sen Low UT

Supplementary Figures S1 H,I

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Fig. S1 H. Volcano plot of differential gene expression analysis (DGE) results between Sen High (n = 3) vs Sen Low (n= 3) untreated samples.

```
#DGE analysis model design ~condition
dimicco <- readRDS("20240529_131347_DGE.rds")

folder <- '05-DGE_UT'

dge_HIGH <- dimicco$deseq$dge_HIGH$`UT_HIGH-UT_LOW`

dg <- dge_HIGH[dge_HIGH$FDR <= 0.05,]
dg <- dg[!is.na(dg),]
dg <- dg %>% drop_na()
gg <- rownames(dg)
dim(dg[dg$logFC > 0, ])

## [1] 290    5

dim(dg[dg$logFC < 0, ])

## [1] 281    5

sample_info <- dimicco$sample_info
sample_info$condition <- factor(sample_info$condition, levels=c('UT_LOW', 'UT_HIGH'))

plotVolcano <- function(dge_HIGH, adj.pval.thr, logfc.thr, titlex) {
  data <- data.frame(gene = row.names(dge_HIGH), pval = -log10(dge_HIGH$FDR), lfc
= dge_HIGH$logFC)
  data <- na.omit(data)
  data <- mutate(data, color = case_when(data$lfc > logfc.thr & data$pval > -log10
(adj.pval.thr) ~ "Overexpressed",
                                         data$lfc < -logfc.thr & data$pval > -log10
(adj.pval.thr) ~ "Underexpressed",
                                         abs(data$lfc) < logfc.thr | data$pval < -
log10(adj.pval.thr) ~ "NonSignificant"))
  data <- data[order(data$pval, decreasing = TRUE),]
  high1 <- head(subset(data, color == "Overexpressed"), 20)
  low1 <- head(subset(data, color == "Underexpressed"), 20)
  tt <- data.frame(rbind( high1, low1))
  vol <- ggplot(data, aes(x = lfc, y = pval, color = color)) +
    theme(legend.position = "right") +
    xlab("log2 Fold Change") +
    ylab(expression(-log[10]("adjusted p-value"))) +
    geom_hline(yintercept = -log10(adj.pval.thr), colour = "darkgray") +
    geom_point(size = 2) + # alpha = 0.8, na.rm = T) +
```

```

scale_color_manual(name = "Expression",
                    values = c(Overexpressed = "#CD4F39",
                               Underexpressed = "#008B00",
                               NonSignificant = "black")) +
geom_text_repel(data=tt,aes(x = lfc, y = pval,label=gene),show.legend= FALSE,
                box.padding = 0.1,max.overlaps =Inf , force = 8, size = 4)+
theme_classic() +
theme(axis.text.x = element_text(colour="black",size=16),
      axis.text.y = element_text(colour="black",size=16),
      axis.title = element_text(colour="black",size=18),
      legend.position = "bottom",
      legend.key.size = unit(1, 'cm'), #change legend key size
      legend.key.height = unit(1, 'cm'), #change legend key height
      legend.key.width = unit(1, 'cm'), #change legend key width
      legend.text = element_text(size=8),
      #legend.justification = 0.05,
      panel.grid.major.x = element_line(colour = "grey80", linetype = "dashed"
"),
      panel.grid.minor.y = element_blank(),
      panel.grid.major.y = element_line(colour = "grey80", linetype = "dashed"
"),
      panel.grid.minor.x = element_blank()) +
guides(colour = guide_legend(override.aes = list(size=8))) #+
#coord_cartesian(x=c(-10,10))+
#ggtitle(titlex)+theme(plot.title = element_text(size = 16, face = "bold"))
return(vol)
}

adj.pval.thr = 0.05
logfc.thr = 0
titlex = 'SenHigh UT vs SenLow UT'

ds <- c('SenHigh UT vs SenLow UT' = 'DESeq-UT_HIGH-UT_LOW.txt')
x <- list()
xx <- list()
folder = 'results_UT_samples'
for (name in names(ds)) {
  dt <- read.table(ds[[name]])
  x[[name]] <- plotVolcano(dt, 0.05, 0,name)
  plot(x[[name]])
}

```

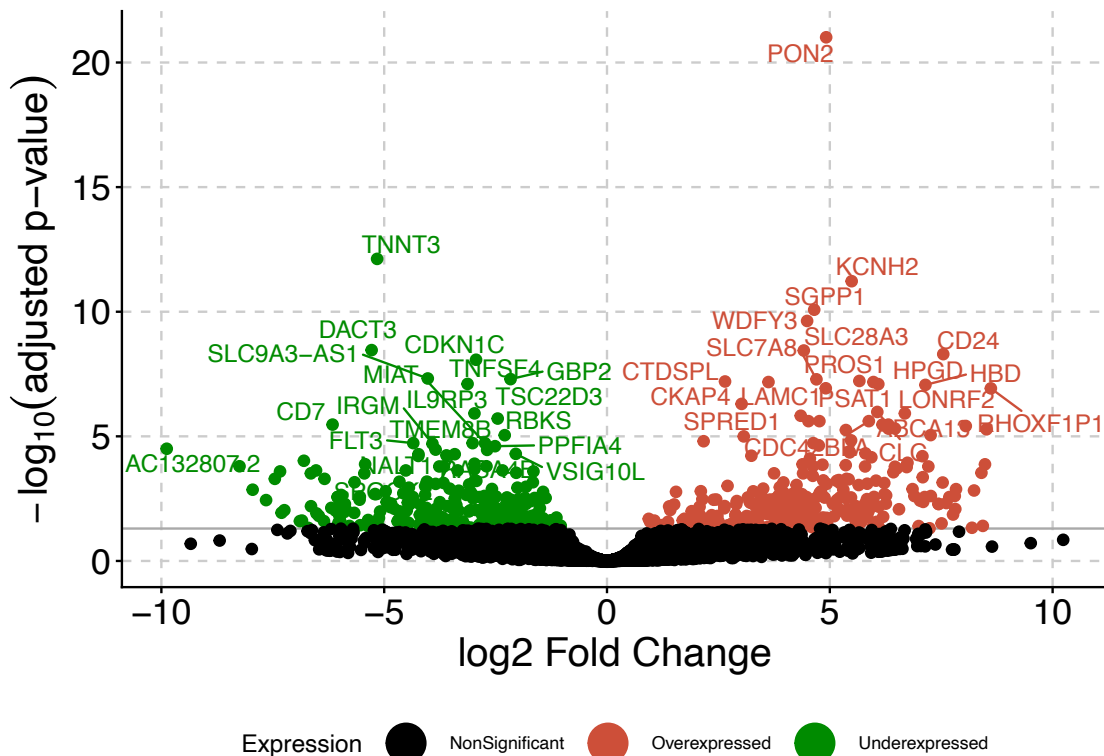


Fig. S1 I. Gene set enrichment analysis (GSEA) results showing the normalized enriched score (NES) of significant Hallmark categories from MSigDB v7.2. Genes are ranked by decreasing Log2 fold change obtained from the contrast Sen High UT vs Sen Low UT (GSEA function and parameters nPerm = 10000, pvalueCutoff = 0.05).

```
wdir= 'results_UT_samples'

listx <- c("gsea-h.all.v7.2.symbols.txt")
nameslist <- c('gsea-h.all.v7.2.symbols.txt'='HALLMARK_v7.2')

folder <- 'post-analysis'
hallmark_ds <- c('UT_HIGH-UT_LOW')

hallmark.full <- data.frame()
for (ds in hallmark_ds) {

  for (val in listx) {
    ff <- paste(folder, ds, 'tables', paste0(ds, '-', val), sep = "/")

    ds.t <- read.table(ff, header = T, sep = "\t", quote="")
    if (nrow(ds.t) != 0) {
      ds.t.filt <- ds.t[,c("Description", "setSize", "enrichmentScore", "NES", "pvalue", "p.adjust", "qvalue", "core_enrichment"), drop = FALSE]
      ds.t.filt$ID <- ds.t.filt$Description
    }
  }
}
```



```
ggtitle('Sen High vs Sen Low - (UT)') # for hallmark  
plot(my_plot)
```

Sen High vs Sen Low – (UT)

type ■ negative ■ positive

