Deconvolution Analysis with CIBERSORT

Cristiane Esteves, Mariana Boroni - Bioinformatics and Computational Biology Lab (LBBC/INCA-RJ)

```
#Load libPaths.
.libPaths(c("~/deconv_cibersort/deconv_cibersort/lib/","/home/manager/R/x86_64-pc-linux-gnu-library/4.2"))
#install.packages("ggdendro")
#pkgs <- c("survival", "survminer", "data.table", "dplyr", "ggplot2", "e1071", "parallel", "preprocessCore", "cor
rplot", "RColorBrewer", "parallel", "ggdendro")
#install.packages(pkgs)
```

```
#Load Packages
suppressPackageStartupMessages({
  library(tibble)
  library(dplyr)
  library(ggplot2)
  library(survival)
  library(survminer)
  library(e1071)
  library(parallel)
  library(preprocessCore)
  library(data.table)
  library(corrplot)
  library(RColorBrewer)
  library(readr)
})

#read script CIBERSORT and barplot function
source('CIBERSORT.R')
source('barplot_cibersort.R')
```

#Load signature matrix (LM22) and bulk RNA matrix (SKCM-Metastasis) LM22 is the signature genes file we used for Cibersort analyses (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4739640/ (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4739640/)). The file contains expression counts for 547 signature genes (547 rows) for 22 distinct human immune cells (22 columns).

```
lm22_signatures <- as.data.frame(fread("~/Data_Deconvolution/deconv_cibersort/data/lm22.txt"))
```

```
## Warning in fread("~/Data_Deconvolution/deconv_cibersort/data/lm22.txt"):
## Detected 22 column names but the data has 23 columns (i.e. invalid file). Added
## 1 extra default column name for the first column which is guessed to be row
## names or an index. Use setnames() afterwards if this guess is not correct, or
## fix the file write command that created the file to create a valid file.
```

```
print(head(lm22_signatures[,1:4]))
```

```
##       V1 B cells naive B cells memory Plasma cells
## 1  ABCB4     555.71345       10.74423     7.225819
## 2  ABCB9      15.60354       22.09479   653.392328
## 3  ACAP1     215.30595      321.62102    38.616872
## 4   ACHE      15.11795       16.64885    22.123737
## 5   ACP5     605.89738     1935.20148  1120.104684
## 6 ADAM28    1943.74270     1148.12014   324.780800
```

```
lm22_signatures <- tibble::column_to_rownames(lm22_signatures, "V1")

#Bulk TCGA-SKCM metastatic
skcm_bulk <- as.data.frame(fread("~/Data_Deconvolution/deconv_cibersort/data/bulk.txt"))
```

```
## Warning in fread("~/Data_Deconvolution/deconv_cibersort/data/bulk.txt"):
## Detected 366 column names but the data has 367 columns (i.e. invalid file).
## Added 1 extra default column name for the first column which is guessed to be
## row names or an index. Use setnames() afterwards if this guess is not correct,
## or fix the file write command that created the file to create a valid file.
```

```
skcm_bulk <- tibble::column_to_rownames(skcm_bulk, "V1")
print(head(skcm_bulk[,1:4]))
```

```
##        TCGA-EB-A5VV-06A-11R-A32P-07 TCGA-GN-A263-01A-11R-A18T-07
## ABCB4                       8.3243                       8.8439
## ABCB9                       1.1392                       0.6741
## ACAP1                     123.8629                       0.6941
## ACHE                       23.7050                       0.0790
## ACP5                       78.3980                      76.1972
## ADAM28                     43.5955                       0.5815
##        TCGA-HR-A2OG-06A-21R-A18U-07 TCGA-FS-A4F4-06A-12R-A266-07
## ABCB4                       1.8307                       1.3882
## ABCB9                       2.0190                       2.5264
## ACAP1                       6.3677                       2.7528
## ACHE                        1.1324                       1.1902
## ACP5                       66.2074                     132.7469
## ADAM28                      2.8839                       0.7676
```

#Deconvolution Analysis - CIBERSORT

i. perm = No. permutations; set to >=100 to calculate p-values (default = 0)
ii. QN = Quantile normalization of input mixture (default = TRUE) - (disabling is recommended for RNA-Seq data)
iii. absolute = Run CIBERSORT in absolute mode (default = FALSE)
- note that cell subsets will be scaled by their absolute levels and will not be represented as fractions (to derive the default output, normalize absolute levels such that they sum to 1 for each mixture sample)
- the sum of all cell subsets in each mixture sample will be added to the ouput (‘Absolute score’). If LM22 is used, this score will capture total immune content.

```
set.seed(42)
h1 <- Sys.time()
results.cibersort <- CIBERSORT(lm22_signatures, skcm_bulk, perm = 100, absolute = F, QN = F)
h2 <- Sys.time()
print(h2 - h1)
```

```
## Time difference of 31.70933 mins
```

```
results.sign = as.data.frame(results.cibersort)[which(as.data.frame(results.cibersort)$`P-value` <= 0.05),]
results.sign = results.sign[1:22]
```

```
saveRDS(results.sign, "~/Data_Deconvolution/deconv_cibersort/results_cibersort.rds")
```

Multivariate/survival analysis

```
############################ multivariate/survival analysis ##########
library(dplyr)
library(survival)
library(survminer)

dados_SKCM = readRDS("~/Data_Deconvolution/deconv_cibersort/data/Dados_SKCM.rds")
library(readr)
subtipos <- read_csv("data/subtipos.csv")
```

```
## New names:
## Rows: 7734 Columns: 11
## ── Column specification
## ──────────────────────────────────────────────────────── Delimiter: "," chr
## (9): pan.samplesID, cancer.type, Subtype_mRNA, Subtype_DNAmeth, Subtype_... dbl
## (2): ...1, Subtype_protein
## ℹ Use `spec()` to retrieve the full column specification for this data. ℹ
## Specify the column types or set `show_col_types = FALSE` to quiet this message.
## • `` -> `...1`
```

```
###### metastatic melanoma #####

#Identify the quartile of each sample in each cell type

rownames(results.sign) <- substr(rownames(results.sign),1,12)

results.sign1 <- results.sign
for (i in 1:length(colnames(results.sign))) {
  for (j in 1:5) {
    quant <- quantile(results.sign1[,i])
    results.sign[which(results.sign1[,i] > quant[j]),i] <- j
  }
}

results.sign$Mixture <- rownames(results.sign)

#Aggregate the cibersort result with clinical data

forest_data <- left_join(results.sign,dados_SKCM$survival_met[,c(1,8,16,17,2,5)], by= c("Mixture" = "bcr_patient_
barcode"))
forest_data <- left_join(forest_data,subtipos[,c(2,10)], by= c("Mixture" = "pan.samplesID"))

# Univariate Cox

surv_object <- Surv(time = forest_data$OS.time, event = forest_data$OS)
colnames(forest_data)[1:22] <- gsub(" ", "_", colnames(forest_data)[1:22])
colnames(forest_data)[9] <- "Treg"
colnames(forest_data)
```

```
##  [1] "B_cells_naive"                     "B_cells_memory"
##  [3] "Plasma_cells"                      "T_cells_CD8"
##  [5] "T_cells_CD4_naive"                 "T_cells_CD4_memory_resting"
##  [7] "T_cells_CD4_memory_activated"      "T_cells_follicular_helper"
##  [9] "Treg"                              "T_cells_gamma_delta"
## [11] "NK_cells_resting"                  "NK_cells_activated"
## [13] "Monocytes"                         "Macrophages_M0"
## [15] "Macrophages_M1"                    "Macrophages_M2"
## [17] "Dendritic_cells_resting"           "Dendritic_cells_activated"
## [19] "Mast_cells_resting"                "Mast_cells_activated"
## [21] "Eosinophils"                       "Neutrophils"
## [23] "Mixture"                           "Subtype_DNAmeth"
## [25] "OS"                                "OS.time"
## [27] "gender"                            "age_at_initial_pathologic_diagnosis"
## [29] "Subtype_other"
```

```
covariables <- colnames(forest_data)[c(1:22,27:29)]
univ_formulas <- sapply(covariables, function(x) as.formula(paste('surv_object ~', x)))

univ_models <- lapply(univ_formulas, function(x){coxph(x, data = forest_data)})

univ_results <- lapply(univ_models,
                       function(x){
                         x <- summary(x)
                         p.value<-signif(x$wald["pvalue"], digits=2)
                         wald.test<-signif(x$wald["test"], digits=2)
                         beta<-signif(x$coef[1], digits=2);#coeficient beta
                         HR <-signif(x$coef[2], digits=2);#exp(beta)
                         HR.confint.lower <- signif(x$conf.int[,"lower .95"], 2)
                         HR.confint.upper <- signif(x$conf.int[,"upper .95"],2)
                         HR <- paste0(HR, " (",
                                      HR.confint.lower, "-", HR.confint.upper, ")")
                         res<-c(beta, HR, wald.test, p.value)
                         names(res)<-c("beta", "HR (95% CI for HR)", "wald.test",
                                       "p.value")
                         return(res)
                         #return(exp(cbind(coef(x),confint(x))))
                       })

#res.bisque <- t(as.data.frame(univ_results, check.names = FALSE))
res.bisque = as.data.frame(t(do.call(cbind, univ_results)))
```

```
## Warning in (function (..., deparse.level = 1) : number of rows of result is not
## a multiple of vector length (arg 1)
```

```
res.bisque <- as.data.frame(res.bisque)
res.bisque$p.value <- as.character(res.bisque$p.value)
res.bisque$p.value <- as.numeric(res.bisque$p.value)
```

```
## Warning: NAs introduced by coercion
```

```
#Filter pval =< 0.05

res.bisque_filt <- res.bisque[which(res.bisque$p.value <= 0.05),]

res.bisque_filt
```

T_cells_CD8
T_cells_CD4_memory_activated
Macrophages_M1
Dendritic_cells_resting
age_at_initial_pathologic_diagnosis

5 rows | 1-1 of 8 columns

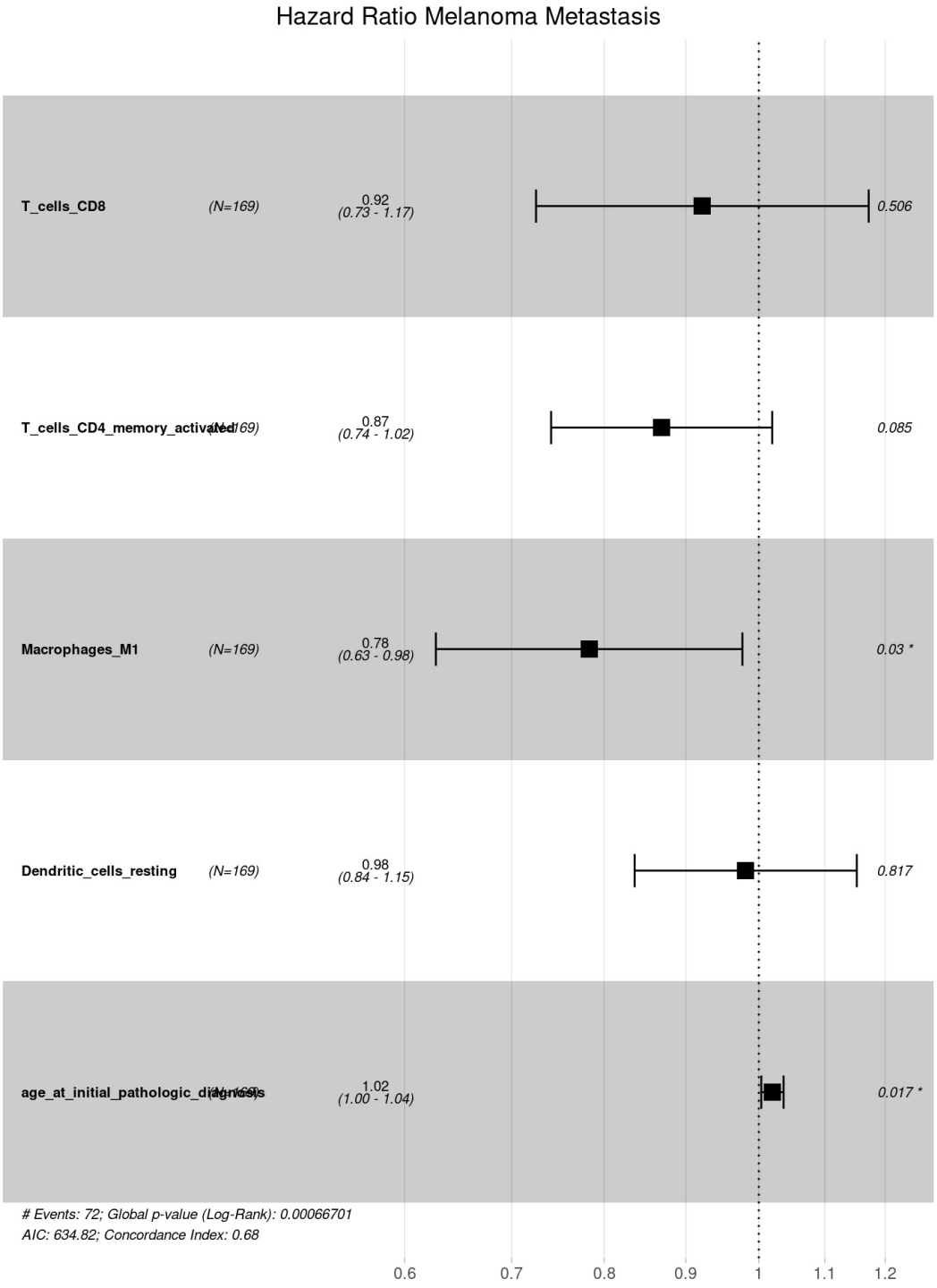
```
rownames(res.bisque_filt)
```

```
## [1] "T_cells_CD8"                         "T_cells_CD4_memory_activated"
## [3] "Macrophages_M1"                      "Dendritic_cells_resting"
## [5] "age_at_initial_pathologic_diagnosis"
```

```
#Multivariate Analysis

f1 <- as.formula(paste("Surv(forest_data$OS.time, event = forest_data$OS) ~ ",
                       paste(c(rownames(res.bisque_filt)), collapse= "+")))

fit.coxph <- coxph(f1, data = forest_data)
summary(fit.coxph)
```

```
## Call:
## coxph(formula = f1, data = forest_data)
##
##   n= 152, number of events= 72
##    (17 observations deleted due to missingness)
##
##                                        coef exp(coef)  se(coef)      z
## T_cells_CD8                         -0.081434  0.921794  0.122415 -0.665
## T_cells_CD4_memory_activated        -0.140161  0.869218  0.081358 -1.723
## Macrophages_M1                      -0.244781  0.782876  0.112806 -2.170
## Dendritic_cells_resting             -0.018913  0.981265  0.081754 -0.231
## age_at_initial_pathologic_diagnosis  0.019637  1.019831  0.008204  2.393
##                                     Pr(>|z|)
## T_cells_CD8                           0.5059
## T_cells_CD4_memory_activated          0.0849 .
## Macrophages_M1                        0.0300 *
## Dendritic_cells_resting               0.8170
## age_at_initial_pathologic_diagnosis   0.0167 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##                                     exp(coef) exp(-coef) lower .95 upper .95
## T_cells_CD8                            0.9218     1.0848    0.7252    1.1717
## T_cells_CD4_memory_activated           0.8692     1.1505    0.7411    1.0195
## Macrophages_M1                         0.7829     1.2773    0.6276    0.9766
## Dendritic_cells_resting                0.9813     1.0191    0.8360    1.1518
## age_at_initial_pathologic_diagnosis    1.0198     0.9806    1.0036    1.0364
##
## Concordance= 0.681  (se = 0.032 )
## Likelihood ratio test= 21.45  on 5 df,   p=7e-04
## Wald test            = 20.54  on 5 df,   p=0.001
## Score (logrank) test = 21.24  on 5 df,   p=7e-04
```

```
ggforest(fit.coxph, data = forest_data, main = "Hazard Ratio Melanoma Metastasis")
```



Barplot

```
names(forest_data)
```

```
##  [1] "B_cells_naive"                    "B_cells_memory"
##  [3] "Plasma_cells"                     "T_cells_CD8"
##  [5] "T_cells_CD4_naive"                "T_cells_CD4_memory_resting"
##  [7] "T_cells_CD4_memory_activated"     "T_cells_follicular_helper"
##  [9] "Treg"                             "T_cells_gamma_delta"
## [11] "NK_cells_resting"                 "NK_cells_activated"
## [13] "Monocytes"                        "Macrophages_M0"
## [15] "Macrophages_M1"                   "Macrophages_M2"
## [17] "Dendritic_cells_resting"          "Dendritic_cells_activated"
## [19] "Mast_cells_resting"               "Mast_cells_activated"
## [21] "Eosinophils"                      "Neutrophils"
## [23] "Mixture"                          "Subtype_DNAmeth"
## [25] "OS"                               "OS.time"
## [27] "gender"                           "age_at_initial_pathologic_diagnosis"
## [29] "Subtype_other"
```

```
#Filter for columns sample and Stage

data_barplot = forest_data[,c(23,29)]

data_barplot$Mixture <- make.names(data_barplot$Mixture, unique = T)
data_barplot$Mixture <- gsub("\\.", "-", data_barplot$Mixture)

rownames(data_barplot) = data_barplot$Mixture
data_barplot$Mixture = NULL


data_barplot$Subtype_other[which(is.na(data_barplot$Subtype_other))] <- "nan"
data_barplot$Subtype_other[which(data_barplot$Subtype_other == "-")] <- "nan"

res_cibersort = forest_data[, c("Mixture", colnames(forest_data)[1:22])]
res_cibersort$Mixture <- make.names(res_cibersort$Mixture, unique = T)
res_cibersort$Mixture <- gsub("\\.", "-", res_cibersort$Mixture)


#data_barplot$Mixture <- make.names(data_barplot$Mixture, unique = T)
#data_barplot$Mixture <- gsub("\\.", "-", data_barplot$Mixture)


plot.ciber.heat(ciber.obj = res_cibersort, ann_info = data_barplot, sample.column = 1)
```

```
## Loading required package: ggdendro
```

```
## Loading required package: gridExtra
```

```
##
## Attaching package: 'gridExtra'
```

```
## The following object is masked from 'package:dplyr':
##
##     combine
```

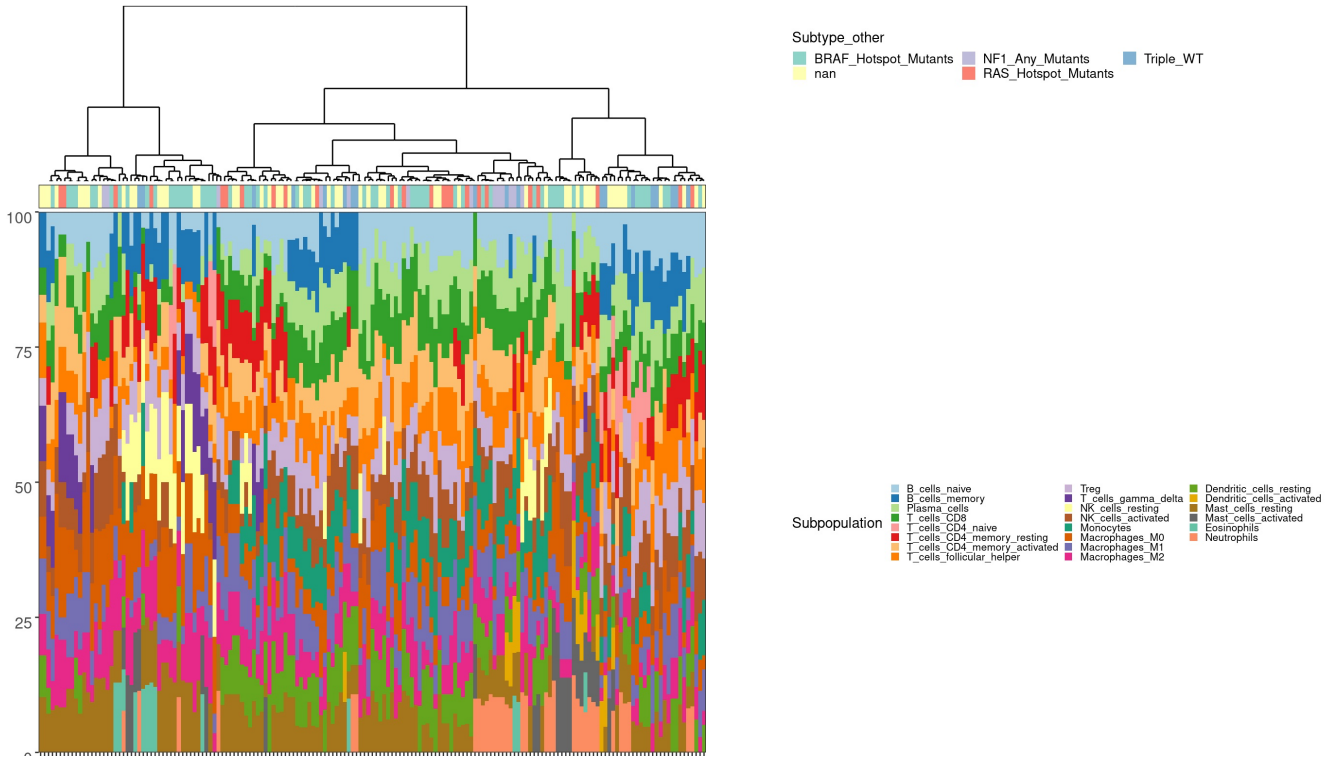
```
## Loading required package: grid
```

```
## Loading required package: cowplot
```

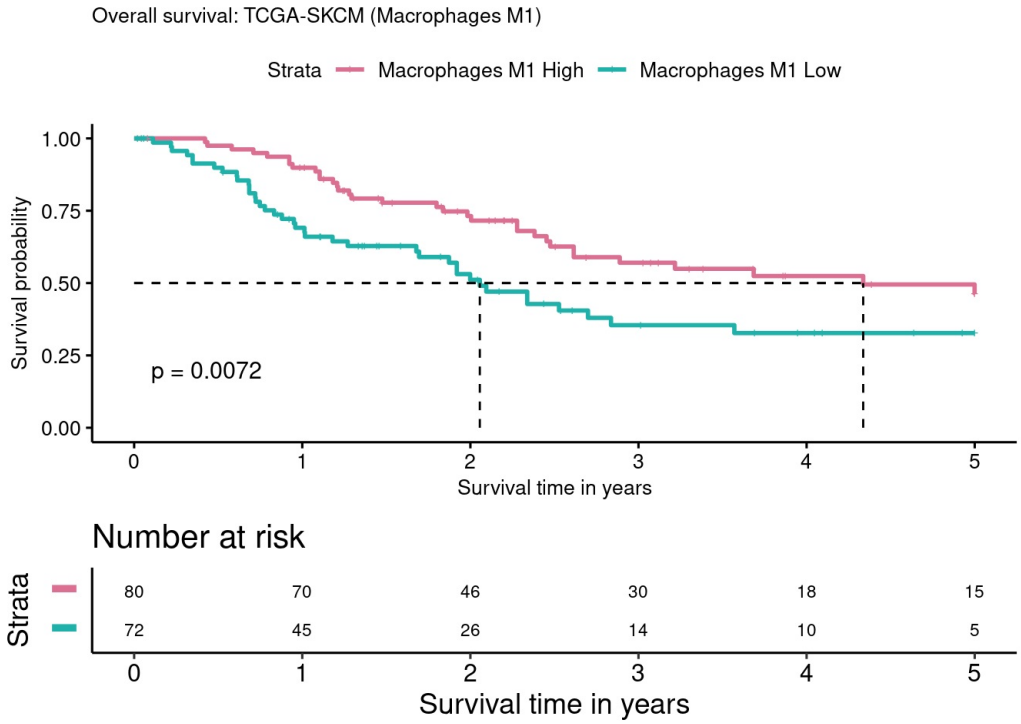
```
##
## Attaching package: 'cowplot'
```

```
## The following object is masked from 'package:ggpubr':
##
##     get_legend
```

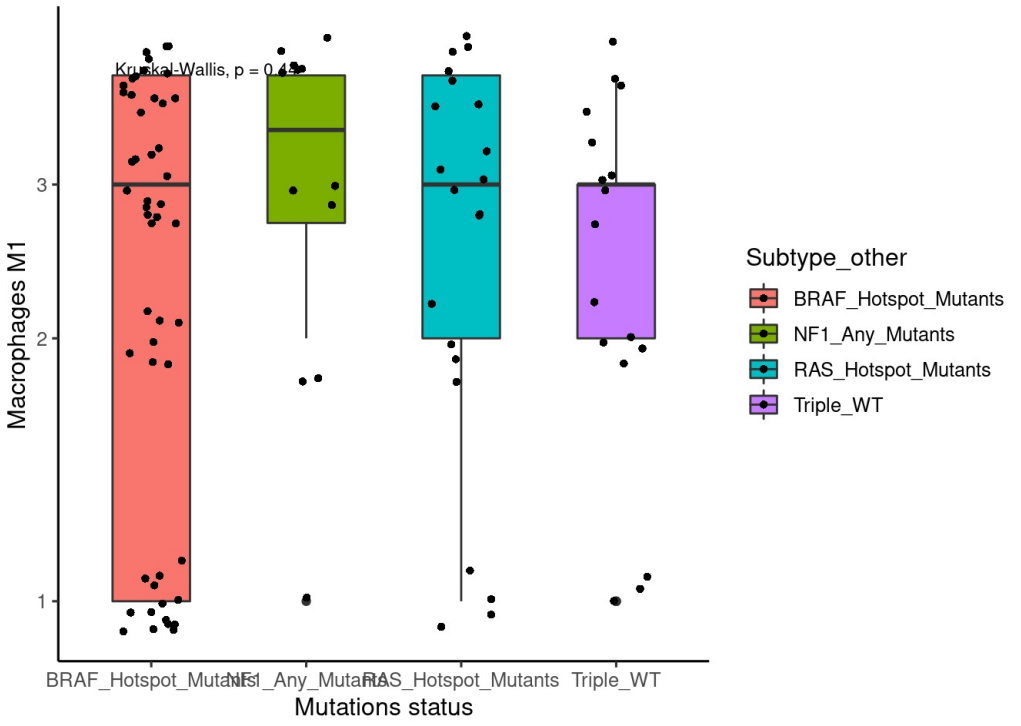
```
## Note: Using an external vector in selections is ambiguous.
## ℹ Use `all_of(sample.column)` instead of `sample.column` to silence this message.
## ℹ See <https://tidyselect.r-lib.org/reference/faq-external-vector.html>.
## This message is displayed once per session.
## Using Mixture as id variables
##
## Using Mixture as id variables
##
## Joining, by = "Mixture"
```



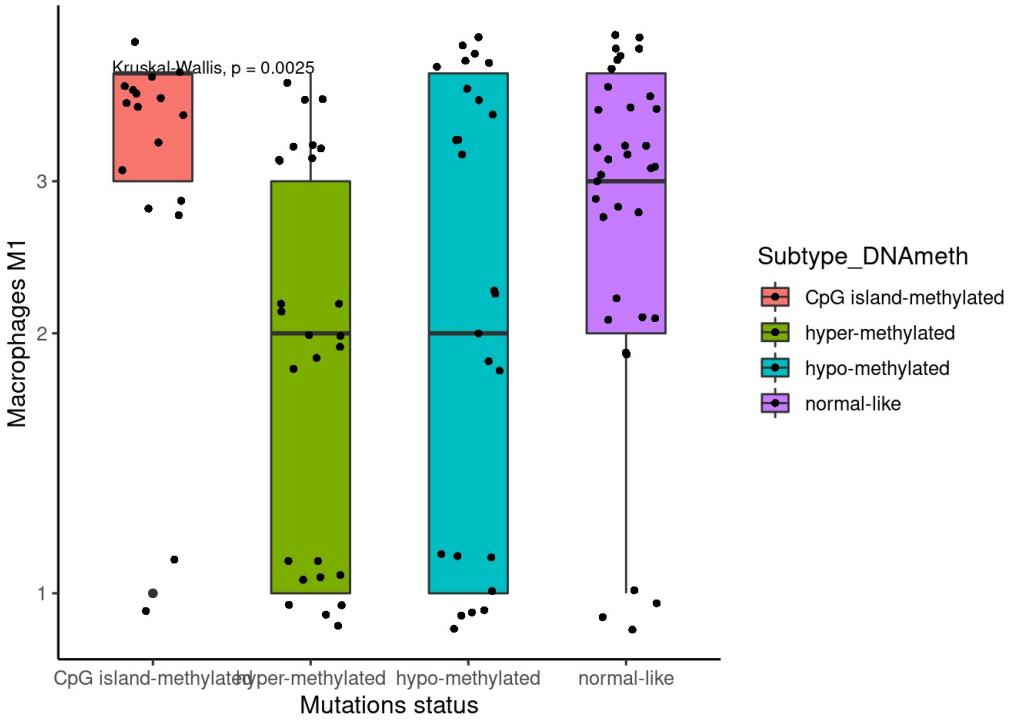

```
######## Levels expressions M1 macrophages


library(ggplot2)
library(survival)
library(survminer)


forest_data$Macrophages_M1_group = ifelse(forest_data$Macrophages_M1 >= mean(forest_data$Macrophages_M1), "High",
"Low")

fit <- survfit(Surv(OS.time, OS) ~ Macrophages_M1_group, data = forest_data)
ggsurvplot(fit, palette = c( "#DB7093","#20b2aa"), xlab = "Survival time in years",
           surv.median.line = c("hv"), cumcensor = F,  conf.int = F ,risk.table = TRUE, pval = T,
           title = 'Overall survival: TCGA-SKCM (Macrophages M1)', risk.table.y.text.col = T, # colour risk table
text annotations.
           risk.table.y.text = FALSE, font.main = c(10), font.legend = c(10), font.y = c(10),font.x = c(10), font
.caption = c(10),
           font.tickslab = c(10),legend.labs=c("Macrophages M1 High","Macrophages M1 Low"), fontsize = 3,risk.tab
le.height = 0.3, pval.size = 4, censor.size = 2,
           font.ytickslab = c(10))
```




```
### Correlating mutational profiles with M1 macrophage expression

ggplot(na.omit(forest_data[forest_data$Macrophages_M1 > 0 & !forest_data$Subtype_other == "-",]), aes(x=Subtype_o
ther, y=Macrophages_M1, fill=Subtype_other)) + stat_compare_means(size=3) +
  geom_boxplot( width=0.5)+  #scale_x_discrete(breaks=c("0","1"),labels=c("MUT","WT"))+
  labs(x="Mutations status",y="Macrophages M1") + scale_y_continuous(trans='log10') + geom_jitter(shape=16, posit
ion=position_jitter(0.2)) + theme_classic2()
```




```
# Correlating DNA meth profiles with M1 macrophage expression

ggplot(na.omit(forest_data[forest_data$Macrophages_M1 > 0,]), aes(x=Subtype_DNAmeth, y=Macrophages_M1, fill=Subty
pe_DNAmeth)) + stat_compare_means(size=3) +
  geom_boxplot( width=0.5)+  #scale_x_discrete(breaks=c("0","1"),labels=c("MUT","WT"))+
  labs(x="Mutations status",y="Macrophages M1") + scale_y_continuous(trans='log10') + geom_jitter(shape=16, posit
ion=position_jitter(0.2)) + theme_classic2()
```




```
# Correlation between lymphocytes and macrophages

corr.cells <-cor(results.sign[1:22])
#head(round(corr.cells,2))

library(corrplot)
library(RColorBrewer)

col <- colorRampPalette(c("blue","white","lightpink"))

corrplot(corr.cells[c(4:12),13:16], sig.level = 0.01,
         number.cex=0.70, cl.cex = 0.6,
         tl.cex = 0.65, tl.col = "black", method="color",addCoef.col = "black", addgrid.col = "black", is.corr=F,
mar=c(0,0,1,0))
```