

Normal tissues CTA expression

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This script generates plots to see the expression of CTAs in normal tissues from GTEx project. This allow us to visualize the most tumor specific CTAs. We added EGFR to compare with an housekeeping genes.

Load libraries

```
library(ComplexHeatmap)
library(dplyr)
library(ggplot2)
library(colorRamp2)
library(plotly)
```

Functions

```
# Compute scatter plot df
compute_scatter_data <- function(df_int_cta) {
  # Compute mean
  df_mean_int_all <- as.data.frame(rowMeans(df_int_cta))
  colnames(df_mean_int_all) <- "Intensity"
  df_mean_int_all$SYMBOL <- rownames(df_mean_int_all)

  # Merge data
  df_scatter <- merge(df_mean_int_all, df_mean_tissue, by = "SYMBOL")
  df_scatter <- merge(df_scatter, df_clust_cta_conv, by = "SYMBOL",
    all.x = TRUE)
  df_scatter <- merge(df_scatter, df_clust_cta_all, by = "SYMBOL",
    all.x = TRUE)

  # Clean and rename
  rownames(df_scatter) <- df_scatter$SYMBOL
  df_scatter <- df_scatter[, -which(colnames(df_scatter) ==
    "SYMBOL")]
  colnames(df_scatter) <- c("Intensity", "Mean_expression_tissues",
    "Cluster_conv", "Cluster_all")

  return(df_scatter)
}

# Plot function
create_plot <- function(df_scatter, cluster_col, nudge_x, nudge_y) {
  ggplot(df_scatter, aes(x = Mean_expression_tissues, y = Intensity,
    color = {
      {
        cluster_col
      }
    }
  )) + geom_point(size = 1) + scale_color_manual(values = c(`1` = "#3498DB",
    `2` = "#E74C3C", `3` = "#2ECC71"), na.value = "#dadada") +
  geom_label(data = df_scatter[rownames(df_scatter) %in%
    housekp_genes, ], aes(label = rownames(df_scatter)[rownames(df_scatter) %in%
```

```
housekp_genes]), nudge_x = nudge_x, nudge_y = nudge_y,
size = 3, color = "black") + theme_minimal()
}
```

Load data

We use expression matrix generated by script 7, the list of CTA that impact survival probabilities in conventional chondrosarcomas and the intensities data from (E-MTAB-7462).

```
# Load mean expression of CTA in each tissue
df_expr <- read.table("../results/matrix_expr_cta_tissues_gtex.tsv",
  sep = "\t", header = TRUE, row.names = 1)

# Read list of CTA that impact survival probabilities
l_CTA_conv <- read.table("../data/CTA_signif_coxph_conv_indiv.txt",
  sep = "\t", header = FALSE)$V1

# Load intensities data
df_int <- read.table("../results/whole_gene_int_CTA_sign_imm_clean.tsv",
  sep = "\t", row.names = 1, header = TRUE)

# List of housekeeping genes
housekp_genes <- c("EGFR", "ERBB2", "FOLH1", "SSTR1", "SSTR2",
  "SSTR3", "SSTR4", "SSTR5")

# Metadata Read the metadata and select individuals that
# have survival data
df_metadata <- read.table("../results/metadata.tsv", sep = "\t",
  header = TRUE, check.names = FALSE, dec = ",")
df_metadata_surv <- df_metadata[, c("Patient", "OS.delay", "OS.event")]
df_metadata_surv <- na.omit(df_metadata_surv)

# Conventional chondrosarcoma
df_metadata_conv <- df_metadata[df_metadata$Histology != "N/A" &
  df_metadata$Histology != "benign" & df_metadata$Histology !=
  "dedifferentiated", ]
df_metadata_surv_conv <- df_metadata_conv[, c("Patient", "OS.delay",
  "OS.event")]
df_metadata_surv_conv <- na.omit(df_metadata_surv_conv)
```

I. Expression of all the CTAs in normal tissues

This part show that these genes are CTAs are expressed in the testis.

```
# Log10 to scale the data
df_log10 <- log10(t(df_expr) + 1)
df_log10_all_cta <- df_log10[, !colnames(df_log10) %in% housekp_genes]
df_log10_all_cta <- t(df_log10_all_cta)

# CTA annotation
```

```

l_cta_validated <- read.table("../data/CTA_validated_list.txt",
  sep = "\t", header = FALSE)$V1

df_cta_validated <- df_log10_all_cta[, colnames(df_log10_all_cta) %in%
  l_cta_validated]
df_cta_putatives <- df_log10_all_cta[, !colnames(df_log10_all_cta) %in%
  l_cta_validated]

# Create heatmaps
colors = colorRamp2(c(min(df_log10_all_cta), max(df_log10_all_cta)),
  c("black", "red"))
ht_validated <- Heatmap(as.matrix(df_cta_validated), cluster_rows = FALSE,
  cluster_columns = FALSE, col = colors, border = NA, show_column_names = TRUE,
  show_row_names = TRUE, column_title = "Validated CTAs", column_names_gp = gpar(fontsize = 1),
  row_names_gp = gpar(fontsize = 10), heatmap_legend_param = list(title = "Expression"),
  rect_gp = gpar(col = NA))

ht_putatives <- Heatmap(as.matrix(df_cta_putatives), cluster_rows = FALSE,
  cluster_columns = FALSE, col = colors, show_column_names = TRUE,
  show_row_names = TRUE, column_title = "Putatives CTAs", column_names_gp = gpar(fontsize = 1),
  row_names_gp = gpar(fontsize = 10), rect_gp = gpar(col = NA))
# png('../results/figures/heatmaps/heatmap_cta_normal_tissues.png',
# width = 3000, height = 1800, res = 300)
draw(ht_validated + ht_putatives, merge_legend = TRUE)

# dev.off()

```

So, this heatmap shows that genes are generally expressed in the testis and less expressed in other tissues.

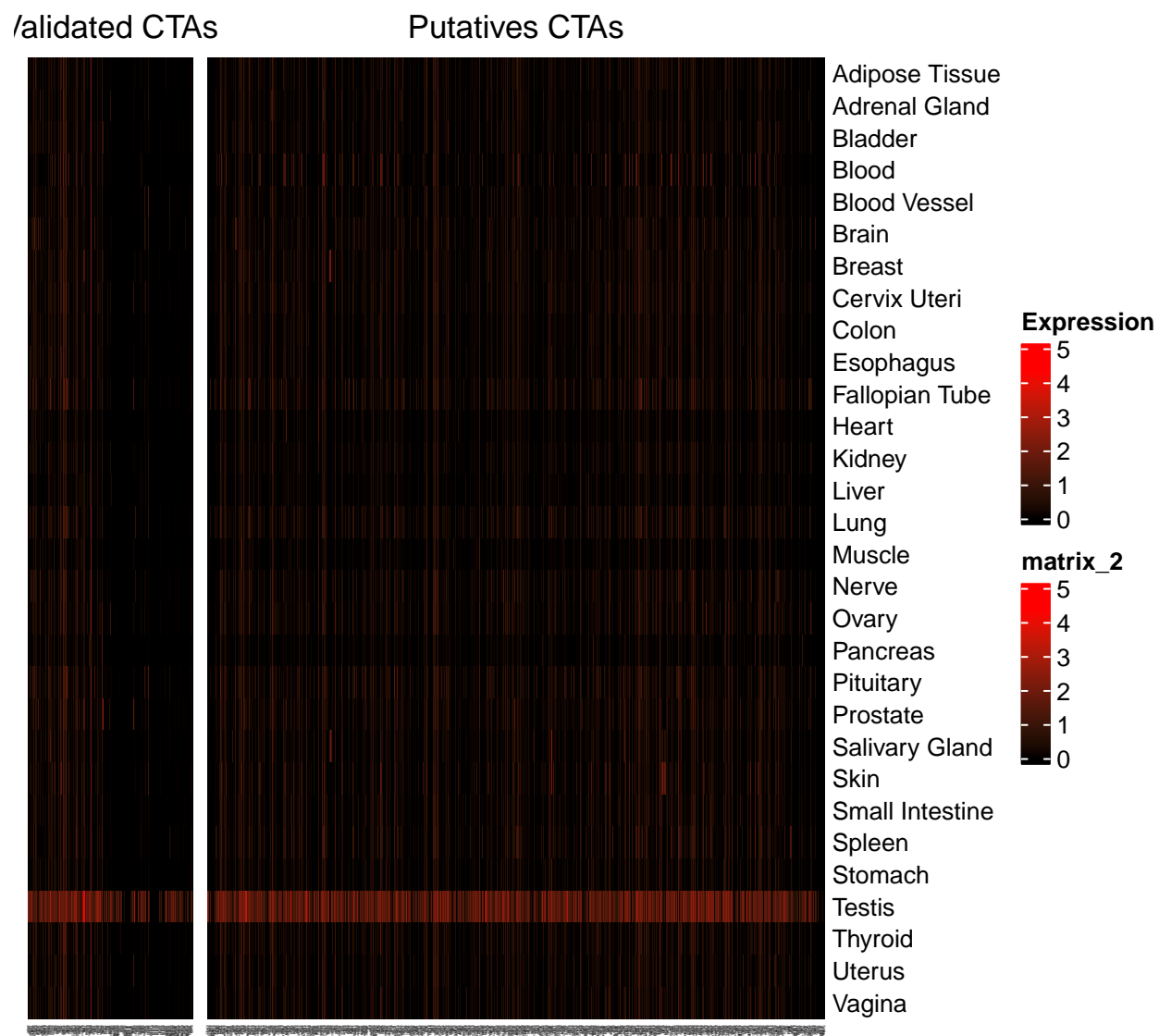


Figure 1: Heatmap of CTAs expression in normal tissues

II. Expression of CTAs that impact survival probabilities in conventional chondrosarcoma in normal tissues

This section shows the expression of CTAs that impact survival probabilities in conventional chondrosarcoma in normal tissues. This permit to see if these specific CTAs are expressed in normal tissues.

```
# Select CTA
df_log10_conv_CTA <- df_log10[rownames(df_log10) %in% 1_CTA_conv,
]

# Df to have genes and their correspondent color
df_clust_cta_conv <- read.table("../results/clusters_indiv/clusters_cta_signif_conv_indiv.tsv",
  sep = "\t", header = TRUE)
rownames(df_clust_cta_conv) <- df_clust_cta_conv$SYMBOL
df_clust_cta_conv <- df_clust_cta_conv[rownames(df_clust_cta_conv) %in%
  rownames(df_log10_conv_CTA), ]
df_clust_cta_conv$Cluster <- as.character(df_clust_cta_conv$Cluster)

# Colors of clusters
clust_colors <- c(`1` = "#3498DB", `2` = "#E74C3C", `3` = "#2ECC71")

# Heatmap
# pdf('../results/figures/heatmaps/heatmap_expr_cta_conv_gtex.pdf')
Heatmap(as.matrix(df_log10_conv_CTA), cluster_rows = TRUE, cluster_columns = TRUE,
  cluster_column_slices = TRUE, clustering_distance_columns = "euclidean",
  clustering_method_columns = "complete", show_column_dend = TRUE,
  col = colors, column_gap = unit(0, "mm"), row_gap = unit(0,
  "mm"), show_column_names = TRUE, show_row_names = TRUE,
  column_names_gp = gpar(fontsize = 7), row_names_gp = gpar(fontsize = 3),
  right_annotation = rowAnnotation(Cluster = df_clust_cta_conv$Cluster,
  col = list(Cluster = clust_colors)), heatmap_legend_param = list(title = "Expression Level"),
  rect_gp = gpar(col = "transparent", lwd = 0), )

# dev.off()
```

We see that there is some groups that are not really expressed in normal tissues so they could be good biomarkers in chondrosarcomas.

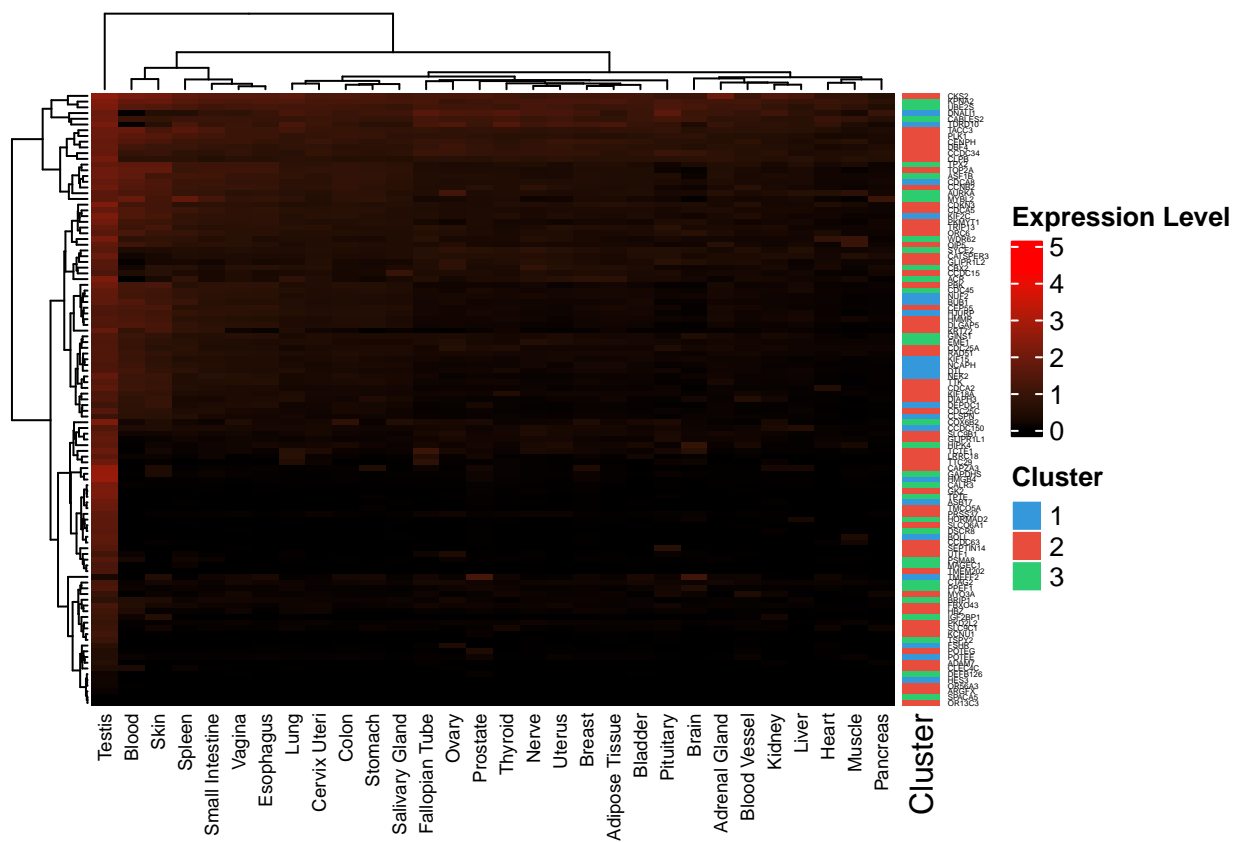


Figure 2: Heatmap of CTAs expression that impact survival in normal tissues

III. Scatter plot of CTAs intensities and their expression in normal tissues.

In this part, we want to see the less expressed CTAs in normal tissues and the more expressed CTAs in chondrosarcomas.

1) n = 82

a- Conventional survival CTAs list

```
# add cluster info
df_clust_cta_all <- read.table("../results/clusters_indiv/clusters_cta_signif_coxph_all_indiv.tsv",
  sep = "\t", header = TRUE)
rownames(df_clust_cta_all) <- df_clust_cta_all$SYMBOL
df_clust_cta_all <- df_clust_cta_all[rownames(df_clust_cta_all) %in%
  rownames(df_log10), ]
df_clust_cta_all$Cluster <- as.character(df_clust_cta_all$Cluster)
colnames(df_clust_cta_all) <- c("Cluster_all", "SYMBOL")

# Select CTA intensities
df_int_cta <- df_int %>%
  filter(CTA != "NA") %>%
  select(-Signature, -CTA)
df_housekp_genes <- df_int[rownames(df_int) %in% housekp_genes,
  ]
df_int_cta <- rbind(df_int_cta, df_housekp_genes[, -c(1, 2)])

# Compute mean for each CTA in all tissues excluding Testis
df <- as.data.frame(t(df_expr[!rownames(df_expr) == "Testis",
  ]))
df_mean_tissue <- as.data.frame(rowMeans(df))
df_mean_tissue$SYMBOL <- rownames(df_mean_tissue)

# Compute mean for each CTA for all patients
df_scatter_82 <- compute_scatter_data(df_int_cta[, colnames(df_int_cta) %in%
  df_metadata_surv$Patient])

# Generate plot
p <- create_plot(df_scatter_82, Cluster_conv, 0.8, -0.3)
p + scale_x_log10() + labs(title = "Scatter plot of tumor specificity of CTAs (n = 82)",
  x = "Normal tissues expression (Log10 TPM mean)", y = "Chondrosarcoma CTA expression (intensities)"
  color = "Clustering gene\nimpacting survival\n(conventional CHS)")
```

i) Log10 scale

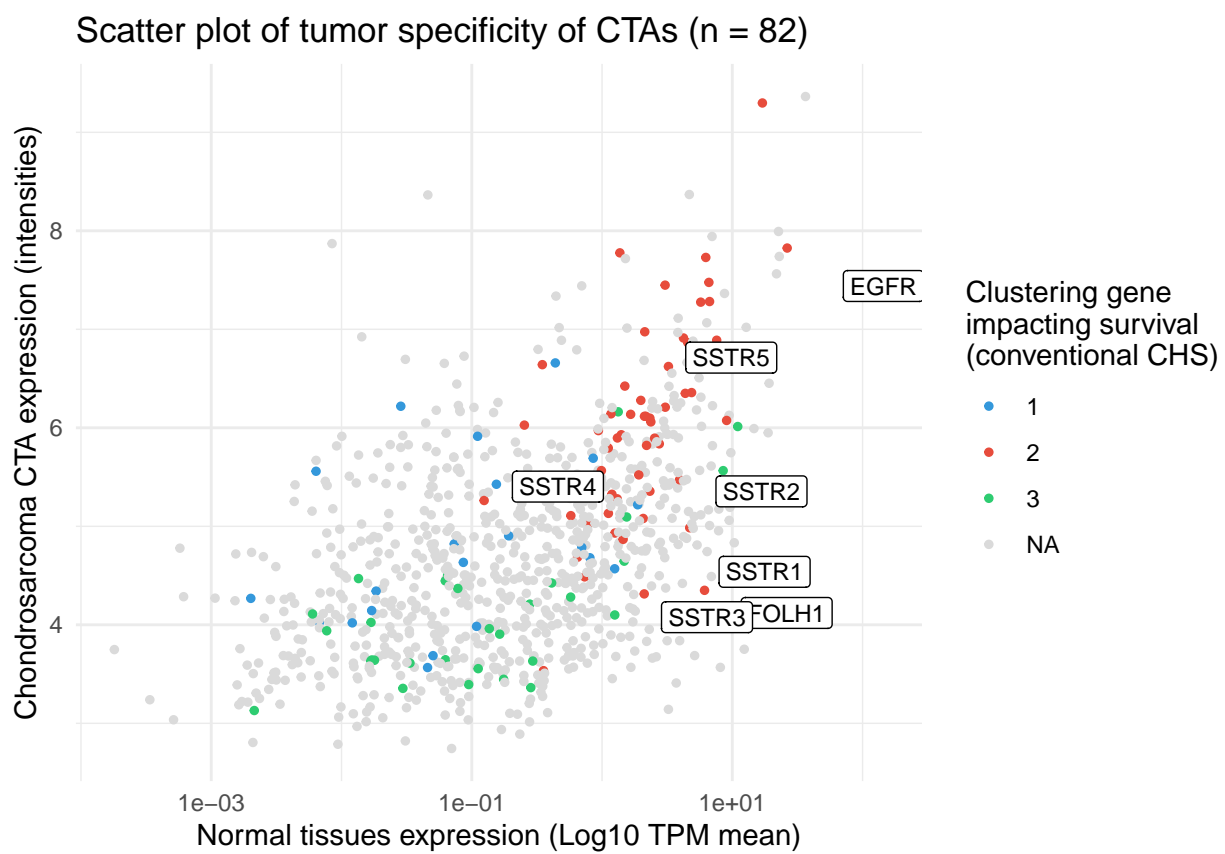


Figure 3: Scatter plot of CTAs intensities and their expression in normal tissues with clustering colors from n = 63 (log10 scale) with n = 82

```
# Generate plot
p <- create_plot(df_scatter_82, Cluster_conv, 4, -0.5)
p + labs(title = "Scatter plot of tumor specificity of CTAs (n = 82)",
  x = "Normal tissues expression (TPM mean)", y = "Chondrosarcoma CTA expression (intensities)",
  color = "Clustering gene\nimpacting survival\n(Conventional CHS)")
```

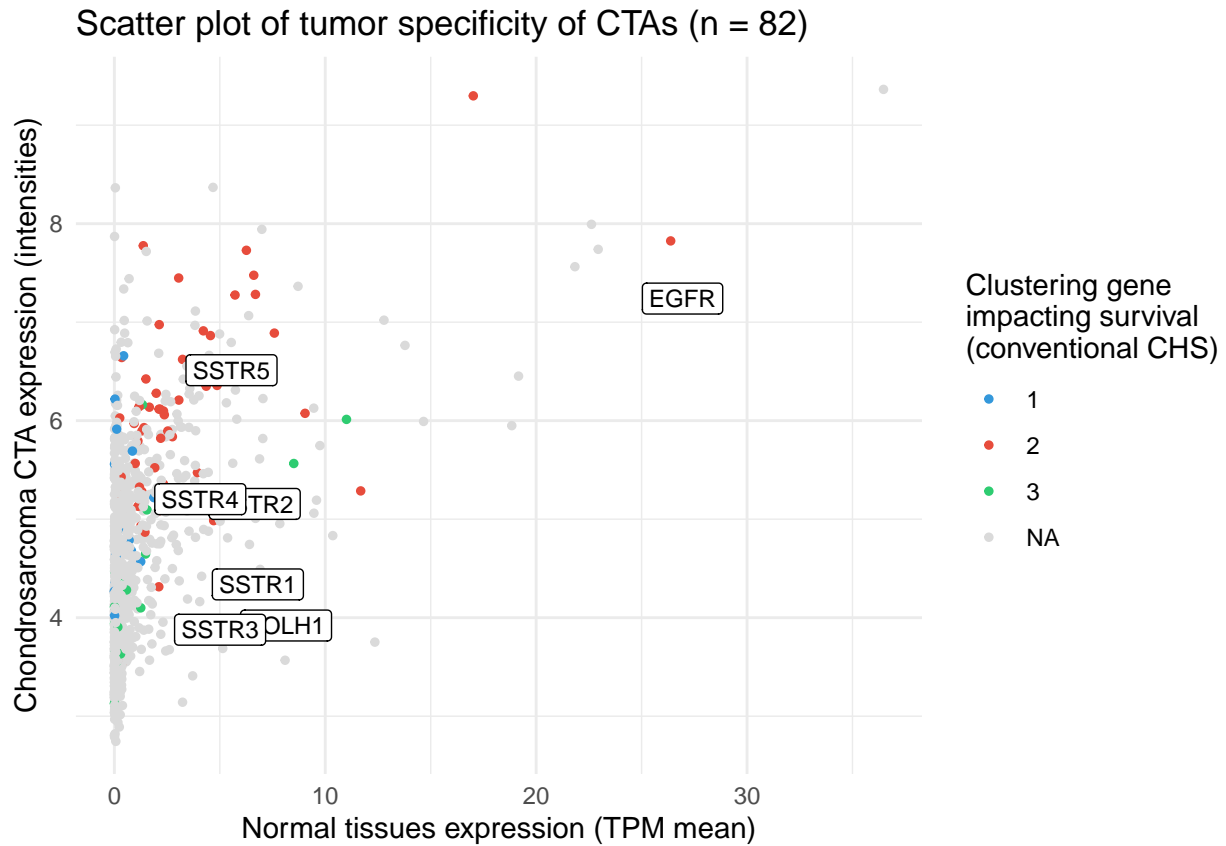


Figure 4: Scatter plot of CTAs intensities and their expression in normal tissues with clustering colors from n = 63 (linear scale) with n = 82

ii) Linear scale

b- N = 82 survival CTAs list

```
# Generate plot
p <- create_plot(df_scatter_82, Cluster_all, 0.8, -0.3)
p + scale_x_log10() + labs(title = "Scatter plot of tumor specificity of CTAs (n = 82)",
  x = "Normal tissues expression (Log10 TPM mean)", y = "Chondrosarcoma CTA expression (intensities)"
  color = "Clustering gene\nimpacting survival\n(conventional CHS)")
```

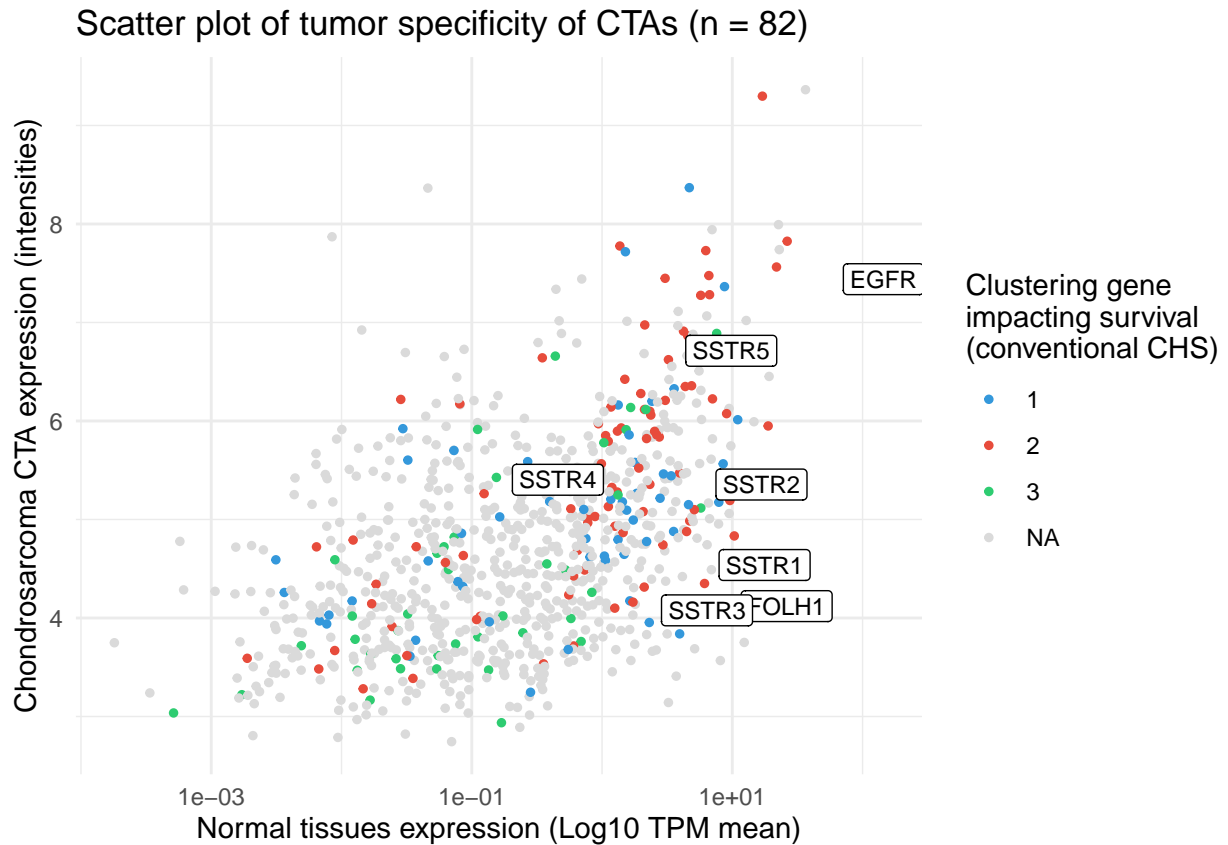


Figure 5: Scatter plot of CTAs intensities and their expression in normal tissues with clustering colors from n = 82 (log10 scale) with n = 82

i) Log10 scale

```
# Generate plot
p <- create_plot(df_scatter_82, Cluster_all, 4, -0.5)
p + labs(title = "Scatter plot of tumor specificity of CTAs (n = 82)",
  x = "Normal tissues expression (TPM mean)", y = "Chondrosarcoma CTA expression (intensities)",
  color = "Clustering gene\nimpacting survival\n(conventional CHS)")
```

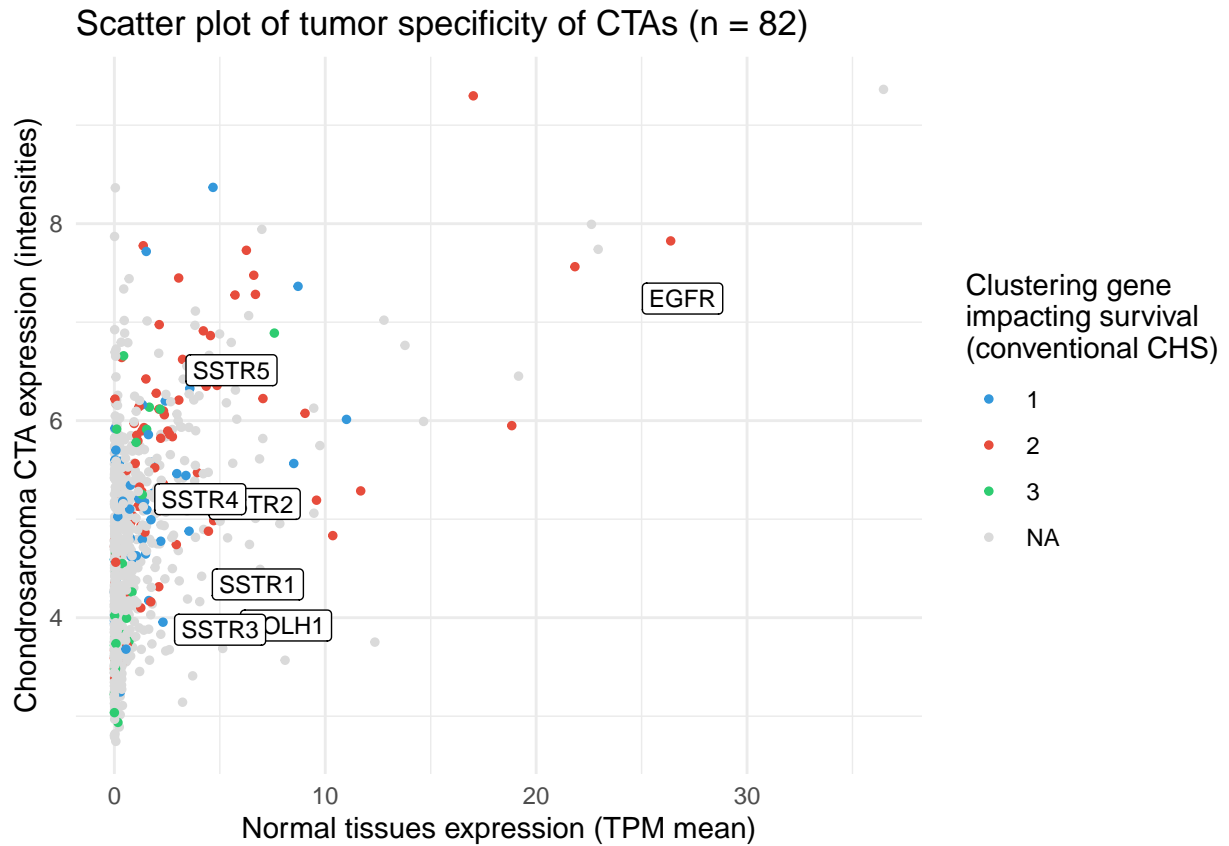


Figure 6: Scatter plot of CTAs intensities and their expression in normal tissues with clustering colors from n = 82 (linear scale) with n = 82

ii) **Linear scale**

2) $n = 63$

a- Conventional survival CTAs list

```
# Compute mean for each CTA for all patients
df_scatter_63 <- compute_scatter_data(df_int_cta[, colnames(df_int_cta) %in%
  df_metadata_surv_conv$Patient])

# Generate plot
p <- create_plot(df_scatter_63, Cluster_conv, 0.8, -0.3)
p + scale_x_log10() + labs(title = "Scatter plot of tumor specificity of CTAs (n = 63)",
  x = "Normal tissues expression (Log10 TPM mean)", y = "Chondrosarcoma CTA expression (intensities)"
  color = "Clustering gene\nimpacting survival\n(conventional CHS)")
```

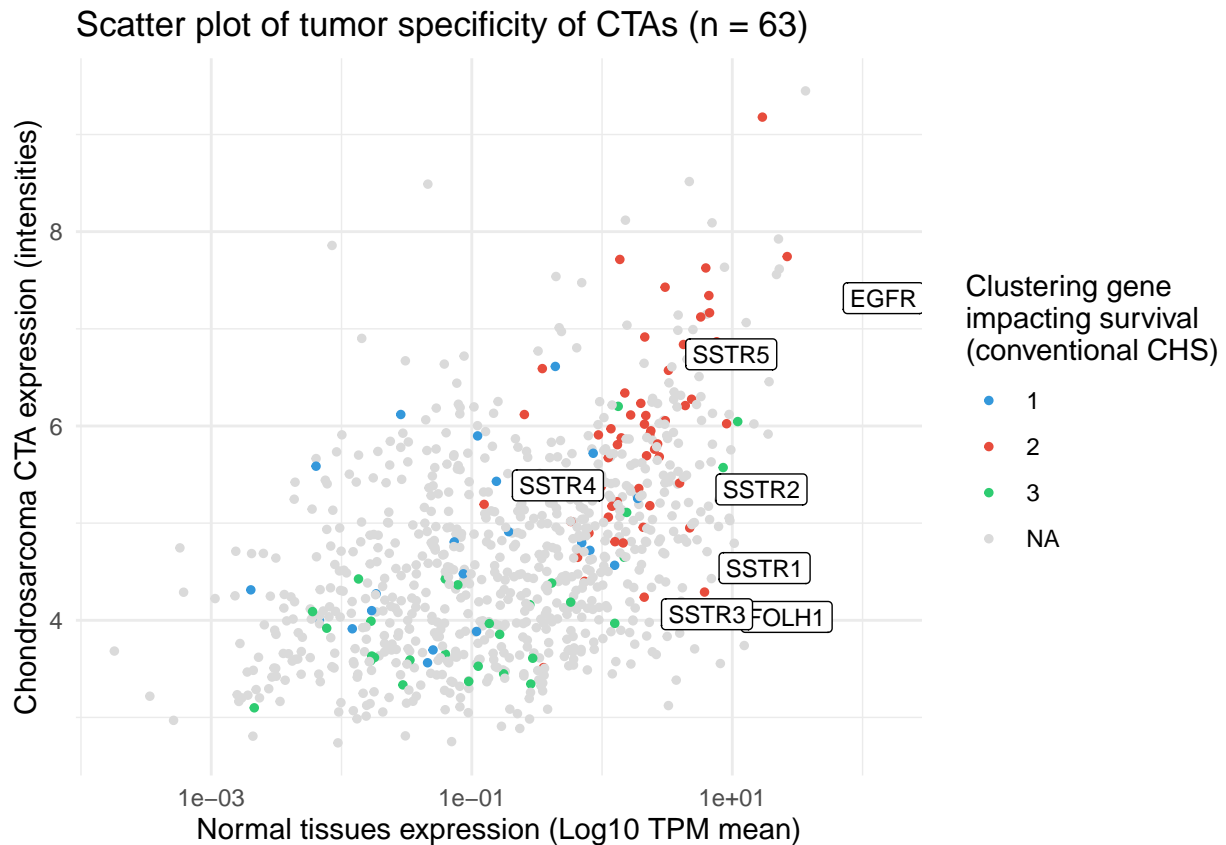


Figure 7: Scatter plot of CTAs intensities and their expression in normal tissues with clustering colors from $n = 63$ (log10 scale) with $n = 63$

i) Log10 scale

```
# Generate plot
p <- create_plot(df_scatter_63, Cluster_conv, 4, -0.5)
```

```
p + labs(title = "Scatter plot of tumor specificity of CTAs (n = 63)",
  x = "Normal tissues expression (TPM mean)", y = "Chondrosarcoma CTA expression (intensities)",
  color = "Clustering gene\nimpacting survival\n(conventional CHS)")
```

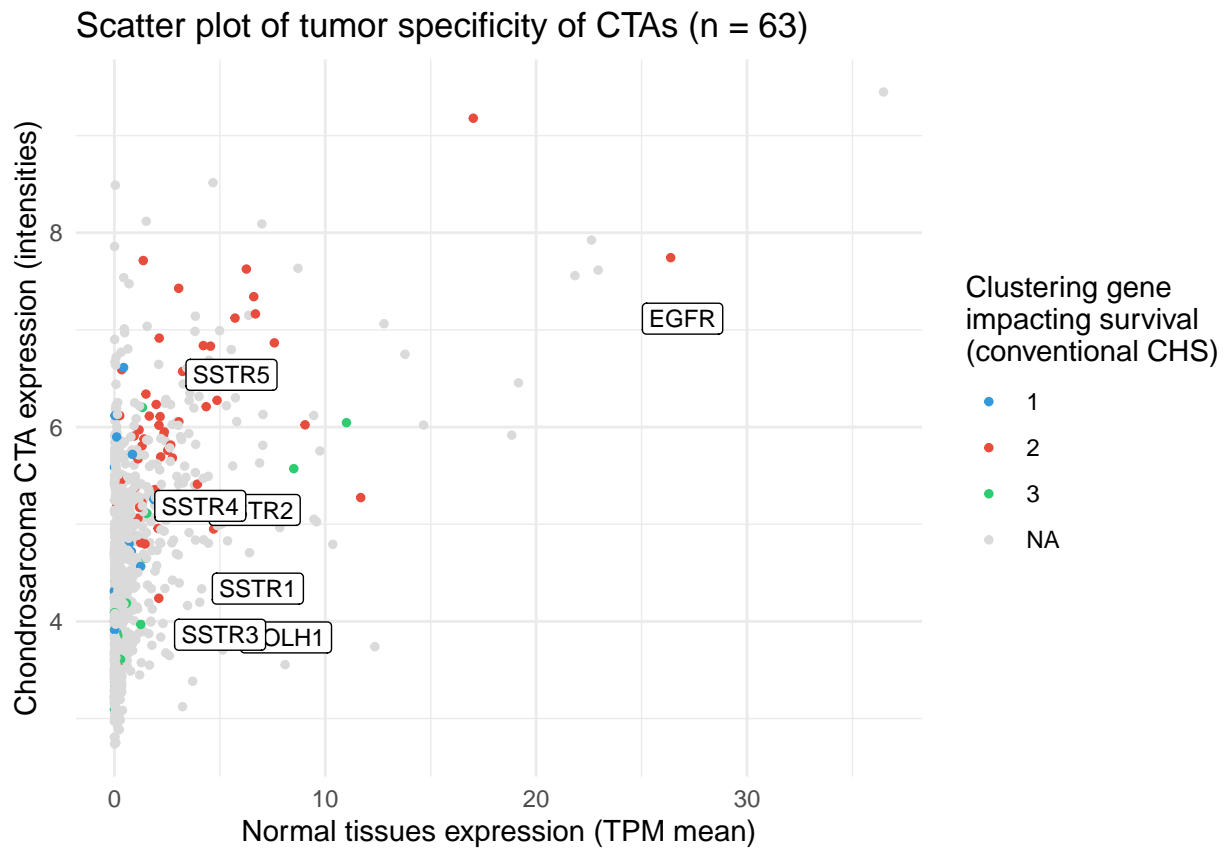


Figure 8: Scatter plot of CTAs intensities and their expression in normal tissues with clustering colors from n = 63 (linear scale) with n = 63

ii) Linear scale

b- N = 82 survival CTAs list

```
# Generate plot
p <- create_plot(df_scatter_63, Cluster_all, 0.8, -0.3)
p + scale_x_log10() + labs(title = "Scatter plot of tumor specificity of CTAs (n = 63)",
  x = "Normal tissues expression (Log10 TPM mean)", y = "Chondrosarcoma CTA expression (intensities)"
  color = "Clustering gene\nimpacting survival\n(conventional CHS)")
```

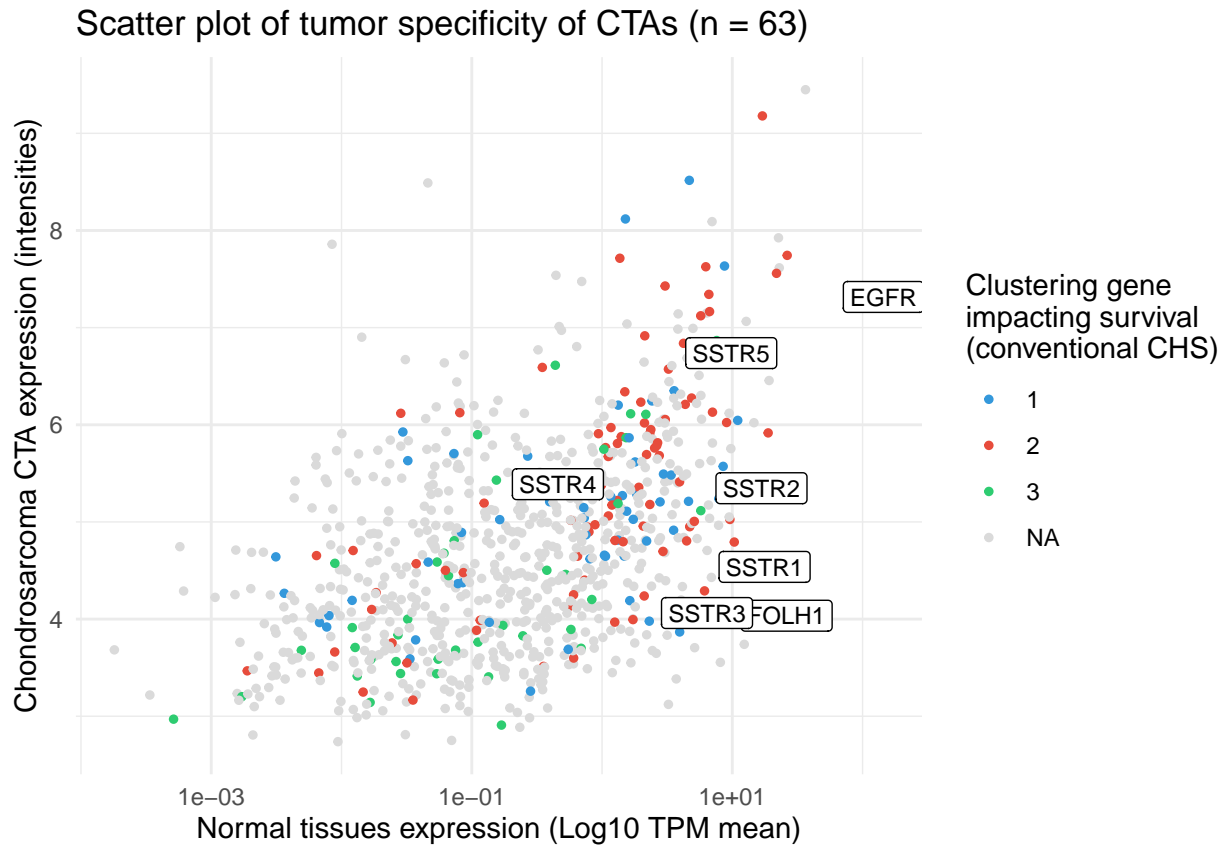


Figure 9: Scatter plot of CTAs intensities and their expression in normal tissues with clustering colors from n = 82 (log10 scale) with n = 63

i) Log10 scale

```
# Generate plot
p <- create_plot(df_scatter_63, Cluster_all, 4, -0.5)
p + labs(title = "Scatter plot of tumor specificity of CTAs (n = 63)",
  x = "Normal tissues expression (TPM mean)", y = "Chondrosarcoma CTA expression (intensities)",
  color = "Clustering gene\nimpacting survival\n(conventional CHS)")
```

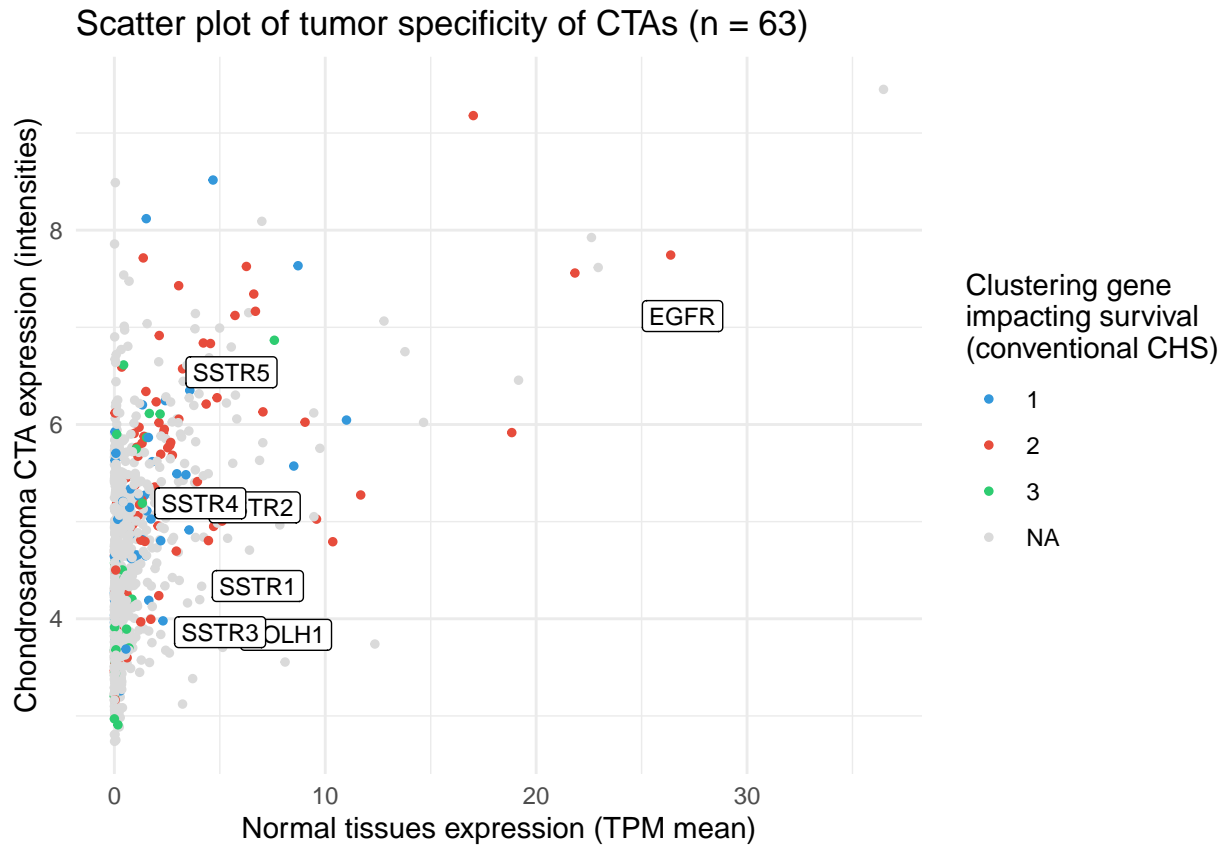


Figure 10: Scatter plot of CTAs intensities and their expression in normal tissues with clustering colors from n = 82 (linear scale) with n = 63

ii) Linear scale

IV. Select interested CTAs thanks thresholds

In the literature, PSMA is a known marker and used to treat prostate cancer, so the expression of PSMA gene (FOLH1) is used as a threshold with the median expression in chondrosarcoma

```
# FOLH1 expression
folh1_expr <- df_scatter_63[rownames(df_scatter_63) %in% "FOLH1",
  2]

# Select
selected_cta <- df_scatter_63[df_scatter_63$Intensity > median(df_scatter_63$Intensity) &
  df_scatter_63$Mean_expression_tissues < df_scatter_63["FOLH1",
    "Mean_expression_tissues"], ]

selected_cta <- selected_cta[!rownames(selected_cta) %in% housekp_genes,
  ]

# Save write.table(rownames(selected_cta), file =
# '../results/selected_cta_scatter.txt', quote = FALSE,
# row.names = FALSE, col.names = FALSE)
```

V. Generate plot for report

```
# Replace
df_scatter_63$Cluster_conv <- ifelse(df_scatter_63$Cluster_conv ==
  "1", "a", ifelse(df_scatter_63$Cluster_conv == "2", "b",
    ifelse(df_scatter_63$Cluster_conv == "3", "c", df_scatter_63$Cluster_conv)))

df_scatter_63 <- df_scatter_63[!rownames(df_scatter_63) %in%
  housekp_genes, ]

save(df_scatter_63, file = "../results/df_scatter_63.RData")

# Generate plot
ggplot(df_scatter_63, aes(x = Mean_expression_tissues, y = Intensity,
  color = Cluster_conv)) + geom_point(size = 1) + geom_vline(xintercept = folh1_expr,
  linetype = "dashed", color = "black") + geom_hline(yintercept = median(df_scatter_63$Intensity),
  linetype = "dashed", color = "black") + annotate("text",
  x = folh1_expr - 3.1, y = max(df_scatter_63$Intensity), label = "FOLH1 expression",
  size = 2.5) + annotate("text", x = max(df_scatter_63$Mean_expression_tissues) -
  2.6, y = median(df_scatter_63$Intensity) + 0.4, label = "Median\nexpression",
  size = 2.5) + scale_color_manual(values = c(a = "#dadada",
  b = "#dadada", c = "#dadada"), na.value = "#dadada") + theme_minimal() +
  scale_x_log10() + labs(title = "Scatter plot of tumor specificity of CTAs (n = 63)",
  x = "Normal tissues expression (Log10 TPM mean)", y = "Chondrosarcoma CTA expression (intensities)"
  color = "Clustering gene\nimpacting survival\n(conventional CHS)")
```

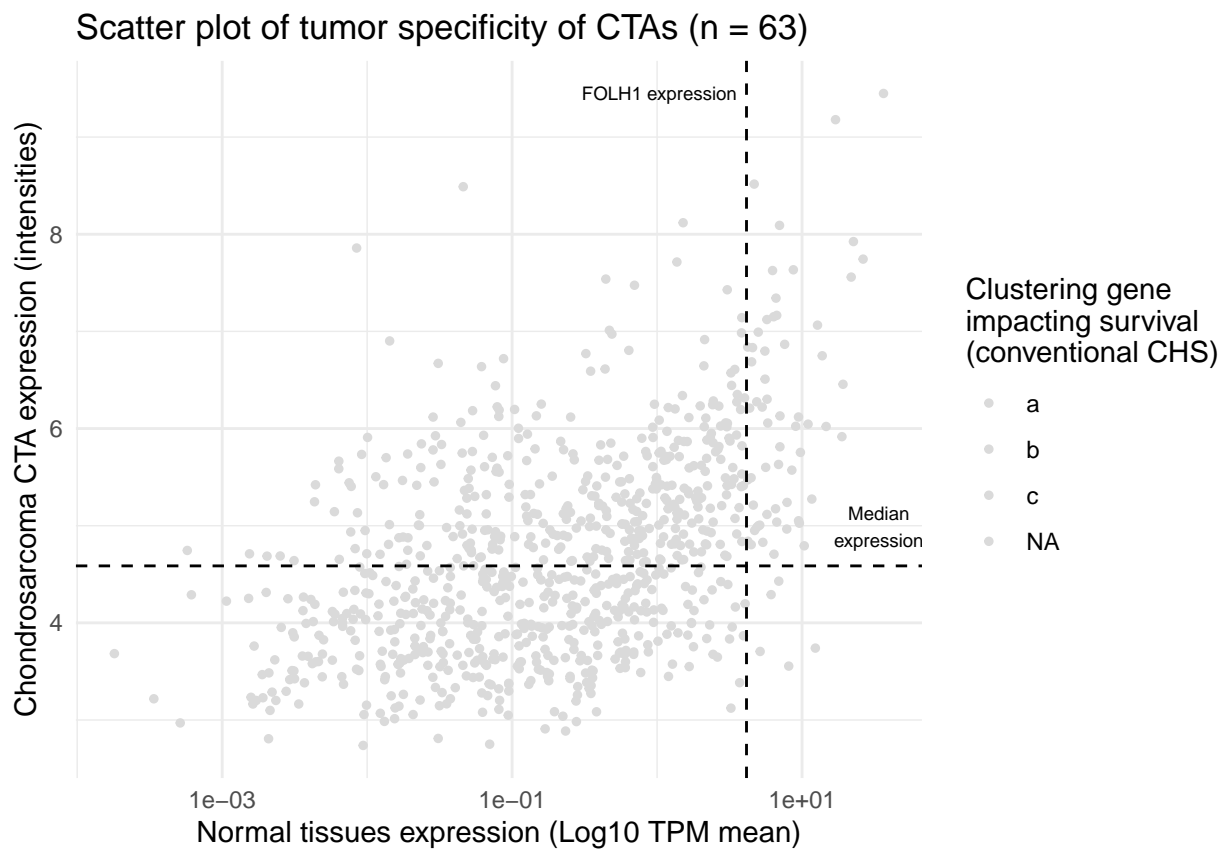


Figure 11: Scatter plot of CTAs intensities and their expression in normal tissues (n = 63)

```

# Adding data on pearson correlation
df_hot_cold <- read.table("../results/clusters_indiv/clusters_cta_pearson_63.tsv",
  header = T, sep = "\t")
colnames(df_hot_cold) <- c("Associated_immunophenotype", "SYMBOL")
df_scatter_63$SYMBOL <- rownames(df_scatter_63)
df_scatter_anno <- merge(df_scatter_63, df_hot_cold, by = "SYMBOL")

# Adding HR data
df_hr <- read.table("../results/results_coxph_cta_conv_indiv.tsv",
  header = T, sep = "\t")
df_hr <- df_hr %>%
  select(Variable, HR, Pvalue) %>%
  filter(Pvalue < 0.05) %>%
  rename(SYMBOL = Variable)
df_scatter_anno <- merge(df_scatter_anno, df_hr, by = "SYMBOL",
  all.x = T)

# Add HR value to add point size
df_scatter_anno$HR <- ifelse(is.na(df_scatter_anno$HR), 0.1,
  df_scatter_anno$HR)

# Generate plot
ggplot(df_scatter_anno, aes(x = Mean_expression_tissues, y = Intensity,
  color = Cluster_conv, size = HR)) + geom_point(alpha = 0.8) +
  geom_vline(xintercept = 4.139, linetype = "dashed", color = "black") +
  geom_hline(yintercept = median(df_scatter_63$Intensity),
    linetype = "dashed", color = "black") + annotate("text",
  x = folh1_expr - 3.1, y = max(df_scatter_63$Intensity), label = "FOLH1 expression",
  size = 2.5) + annotate("text", x = max(df_scatter_63$Mean_expression_tissues) -
  2.6, y = median(df_scatter_63$Intensity) + 0.4, label = "Median\nexpression",
  size = 2.5) + scale_size_continuous(range = c(1, 3)) + scale_color_manual(values = c(a = "#3498DB",
  b = "#E74C3C", c = "#2ECC71"), na.value = "#dadada") + theme_minimal() +
  scale_x_log10() + labs(title = "Scatter plot of tumor specificity of CTAs (n = 63)",
  x = "Normal tissues expression (Log10 TPM mean)", y = "Chondrosarcoma CTA expression (intensities)"
  color = "Clustering gene\nimpacting survival\n(conventional CHS)",
  size = "Significant HR")

```

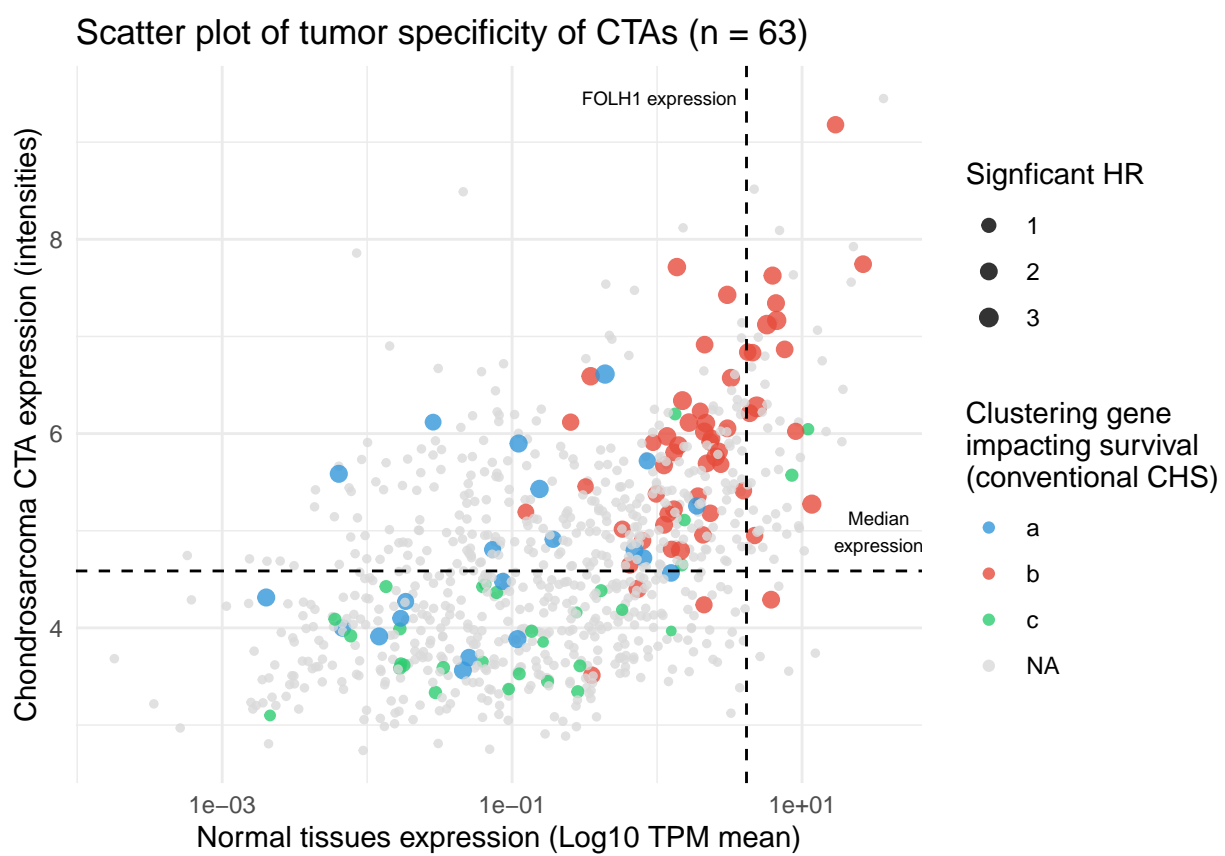


Figure 12: Scatter plot of CTAs intensities and their expression in normal tissues with clustering colors, size by HR (significant), and shape by associated immunophenotype (n = 63)

```
# Generate plot
ggplot(df_scatter_anno, aes(x = Mean_expression_tissues, y = Intensity,
  color = Cluster_conv, size = HR, shape = Associated_immunophenotype)) +
  geom_point(alpha = 0.8) + geom_vline(xintercept = 4.139,
  linetype = "dashed", color = "black") + geom_hline(yintercept = median(df_scatter_63$Intensity),
  linetype = "dashed", color = "black") + annotate("text",
  x = folh1_expr - 3.1, y = max(df_scatter_63$Intensity), label = "FOLH1 expression",
  size = 2.5) + annotate("text", x = max(df_scatter_63$Mean_expression_tissues) -
  2.6, y = median(df_scatter_63$Intensity) + 0.4, label = "Median\nexpression",
  size = 2.5) + scale_shape_manual(values = c(COLD = 15, HOT = 16)) +
  scale_size_continuous(range = c(1, 3)) + scale_color_manual(values = c(a = "#3498DB",
  b = "#E74C3C", c = "#2ECC71", na.value = "#dadada")) + theme_minimal() +
  scale_x_log10() + labs(title = "Scatter plot of tumor specificity of CTAs (n = 63)",
  x = "Normal tissues expression (Log10 TPM mean)", y = "Chondrosarcoma CTA expression (intensities)",
  color = "Clustering gene\nimpacting survival\n(conventional CHS)",
  shape = "Associated Immunophenotype", size = "Significant HR")
```

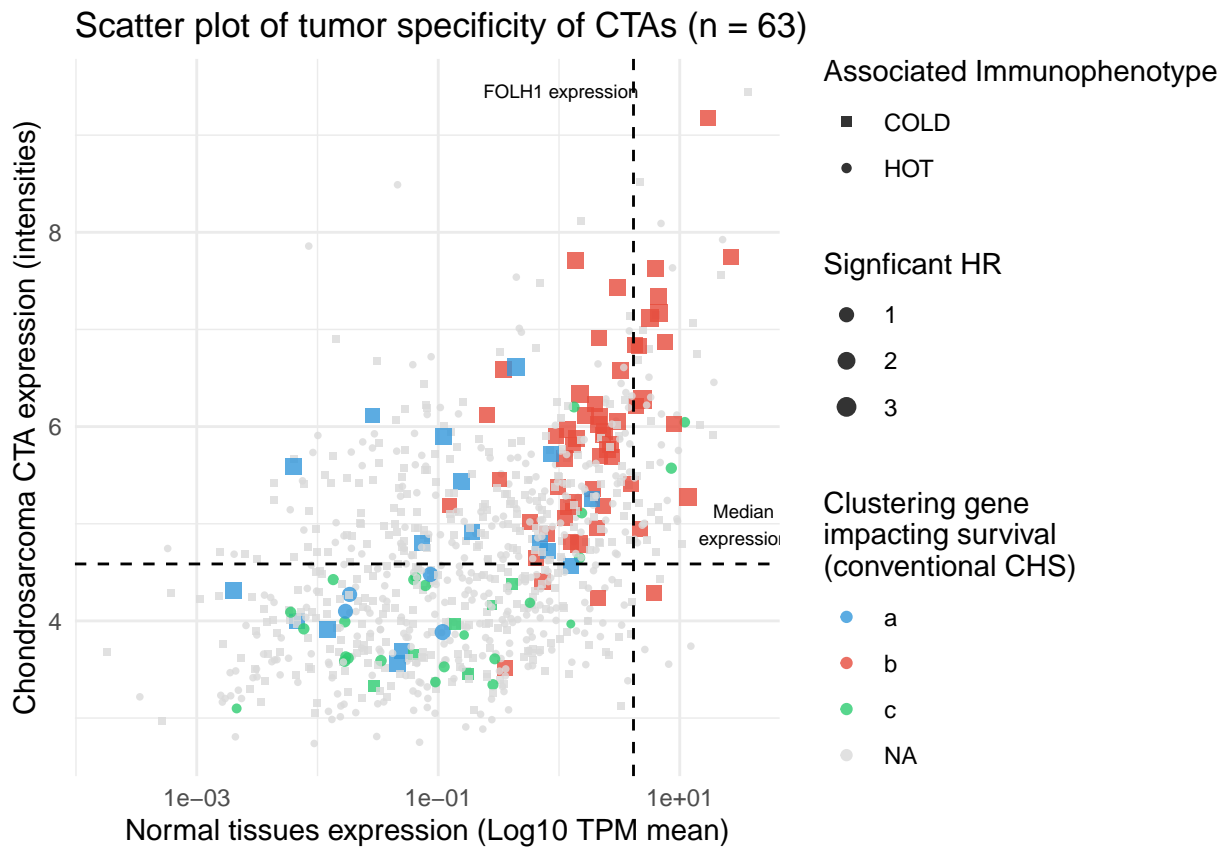


Figure 13: Scatter plot of CTAs intensities and their expression in normal tissues with clustering colors, size by HR (significant), and shape by associated immunophenotype (n = 63)

```

p <- ggplot(df_scatter_anno, aes(x = Mean_expression_tissues,
  y = Intensity, color = Cluster_conv, size = HR, shape = Associated_immunophenotype,
  text = paste0("Gene: ", SYMBOL, "<br>Mean expression: ",
    round(Mean_expression_tissues, 3), "<br>Intensity: ",
    round(Intensity, 3), "<br>HR: ", round(HR, 3), "<br>Cluster: ",
    Cluster_conv, "<br>Associated Immunophenotype: ", Associated_immunophenotype))) +
  geom_point(alpha = 0.8) + geom_vline(xintercept = 4.139,
  linetype = "dashed", color = "black") + geom_hline(yintercept = median(df_scatter_63$Intensity),
  linetype = "dashed", color = "black") + scale_shape_manual(values = c(COLD = 15,
  HOT = 16)) + scale_size_continuous(range = c(1, 3)) + scale_color_manual(values = c(a = "#3498DB",
  b = "#E74C3C", c = "#2ECC71"), na.value = "#dadada") + theme_minimal() +
  scale_x_log10() + labs(title = "Scatter plot of tumor specificity of CTAs (n = 63)",
  x = "Normal tissues expression (Log10 TPM mean)", y = "Chondrosarcoma CTA expression (intensities)"
  color = "Clustering gene\nimpacting survival\n(conventional CHS)",
  shape = "Associated Immunophenotype", size = "Significant HR")

# ggplotly(p, tooltip = 'text')

```

This last plot is an hmtl output because it's an interactive plot. It is available in the html notebook.