Project 1: Function to Summarize and Plot Gene Expression

2024-08-07

Import data

```
genes <- read.csv('/Users/isabellabaldacci/Desktop/QBS103/Project/QBS103_GSE157103_genes.csv',
                  sep = ',', header = TRUE)
metadata <- read.csv('/Users/isabellabaldacci/Desktop/QBS103/Project/QBS103_GSE157103_series_matrix.csv
                     header = TRUE)
```

Clean up metadata

```
## cleaning up metadata
# metadata were removed if they were not helpful or if they had too many unknowns
metadata_clean <- dplyr::select(metadata,
                                -c("X.Sample_submission_date","channel_count",
                                   "status","last_update_date","type",
                                   "channel_count","source_name_ch1",
                                   "organism_ch1","apacheii","ferritin.ng.ml.",
                                   "ddimer.mg.l_feu.","procalcitonin.ng.ml..",
                                   "lactate.mmol.l.","fibrinogen","sofa"))
```

Transpose Genes dataframe

```
#transposing the genes x samples matrix to samples x genes
rownames(genes) <- genes$X #assigning row names
genes_t <- as.data.frame(t(dplyr::select(genes, -c('X')))) #transpose as a dataframe
genes_t$participant_id <- rownames(genes_t) #assigning participant id column for later joining
#which(genes_t$participant_id == "COVID_06_.y_male_NonICU") #finding column with mismatch to metadata
genes_t$participant_id[6] <- "COVID_06_:y_male_NonICU" #reassinging value to match metadata
```

Combining metadata and genes dataframe

```
#combining the samples x genes matrix to the metadata
all_data <- dplyr::inner_join(genes_t, metadata_clean, by = 'participant_id')
```

Write function for plotting

```
plots <- function(df, genes_list, cont_cov, cat_cov) {
  #filter dataframe (received error message indicating to use "all of")
  df_filtered <- df %>% dplyr::select(participant_id,
                                      all_of(genes_list),
                                      all_of(cont_cov),
                                      all_of(cat_cov))

  #cast to long
  df_filtered_long <- df_filtered %>% tidyr::pivot_longer(cols = genes_list,
                                                          names_to = 'Gene',
                                                          values_to = 'Expression')

  for (gene in genes_list){ #for gene in genes list

    one_gene <- df_filtered_long %>% dplyr::filter(Gene == gene) #get dataframe for one gene

    #get data for plotting, need mean, sd, and the 75th quantile for positioning annotation box
    mean_expression <- round(mean(one_gene$Expression), 2)
    sd_expression <- round(sd(one_gene$Expression), 2)
    x_min_box <- quantile(one_gene$Expression, 3/4)
    n <- length(one_gene$Expression)

    #create labels
    hist_title <- paste('Distribution of ', gene, ' expression across samples')
    hist_x <- paste(gene, ' Expression')

    #plot histogram using theme parameters from previous assignment
    hist <- one_gene %>% ggplot(aes(Expression)) +
      geom_histogram(bins = 30, fill = 'slateblue1') +
      theme_minimal() +
      theme(plot.title = element_text(hjust = .4)) +
      labs(title = hist_title,
       x = hist_x,
       y = 'Frequency') +
      scale_color_manual(values = c('slateblue1')) +
      annotate('rect',
           xmin = x_min_box + 5,
           xmax = x_min_box + 25,
           ymin = 10,
           ymax = 14, fill = 'grey', alpha = .9)+
  annotate(geom = 'text', x = x_min_box + 15, y = 12, label = paste('N:', n,' samples',
                                                          '\n Mean Expression:', mean_expression,
                                                          '\nsd:', sd_expression), size = 2.5)
    #print to display the histogram
    print(hist)


    #filter the data to create the scatter plot
    #to avoid warnings of coerced NAs and to ensure cont_cov is numeric
    scatter_filtered <- one_gene %>% filter(one_gene[[cont_cov]] != ' unknown')
    scatter_filtered[cont_cov] <- as.numeric(scatter_filtered[[cont_cov]])
```

```
#assign the levels for crp
if (cont_cov == 'crp.mg.l.') {
  scatter_filtered$crp.level <- cut(scatter_filtered$crp.mg.l.,
                                    breaks = c(0, 3, 10, 100, 500000),
                                    labels = c('normal', 'moderate', 'high', 'severe'))
}

#set color palette and titles
colorPalette_scatter <- c('azure4', 'skyblue2', 'slateblue2', 'midnightblue')
title_scatter <- paste(toupper(cont_cov), ' vs ', gene, ' Expression')
x_scatter <- paste(toupper(cont_cov), '(mg/L)')
y_scatter <- paste(gene, ' Expression')

#create scatterplot with parameters from previous assignment
scatter <- scatter_filtered %>% ggplot(aes(x = .data[[cont_cov]],
                                           y = Expression,
                                           color = crp.level)) +
  geom_point(na.rm = TRUE) +
  theme(
    axis.text.x = element_text(angle=90),
    plot.title = element_text(hjust = .4),
    legend.position = c(.85,.75)) +
  labs(title = title_scatter, x = x_scatter, y = y_scatter)  +
  scale_color_manual(labels =
                       c('Normal (<3mg/L)', 'Moderate (3-10mg/L)',
                         'High (10 -100mg/L)', 'Severe (>100mg/L)'),
                     values = colorPalette_scatter)

#print scatterplot
print(scatter)


#creat titles for box plot
title_box <- paste(gene, ' Expression across ', cat_cov[1], ' and ', cat_cov[2])
x_lab_box <- cat_cov[1]
y_lab_box  <- paste(gene, ' Expression')
#get num_covid positive and negative for annotations
n_covid_pos <- length(dplyr::filter(
  one_gene, disease_status == 'disease state: COVID-19')$Expression)
n_covid_neg <- length(dplyr::filter(
  one_gene, disease_status != 'disease state: COVID-19')$Expression)
#set the y value for the annotations as the max of expression so that it will not cover the data
y_annot <- max(one_gene$Expression)

#generate box plot using same parameters as previous assignment
box <- one_gene %>% ggplot(aes_string(x = cat_cov[1], y = 'Expression', fill = cat_cov[2])) +
  geom_boxplot() +
  theme_minimal() +
  theme(legend.position = 'bottom') +
  labs(title = title_box,
       x = x_lab_box,
       y = y_lab_box) +
  scale_fill_manual(labels = c('no ventilator', 'ventilator'),
```

```
                        values = c('ghostwhite', 'mediumvioletred')) +
      scale_x_discrete(labels = c('COVID', 'NON-COVID')) +
      annotate(geom = 'text', x = 1, y = y_annot + 5,
               label = paste('Covid positive: N = ', n_covid_pos),
               color = 'black')+
      annotate(geom = 'text', x = 2, y = y_annot + 5,
               label = paste('Covid negative: N = ', n_covid_neg),
               color = 'black')
    print(box)

    #just an idea I had for putting it all together
    #all_plots <- ggpubr::ggarrange(hist, ggarrange(scatter, box, ncol = 2,
    #labels = c('B', 'C')), nrow = 2, labels = c('A'))
      #plotlist = list(box, scatter, hist), ncol = 3, nrow = 1, widths = c(5,5,5))
    #print(all_plots)
  }
  }
```

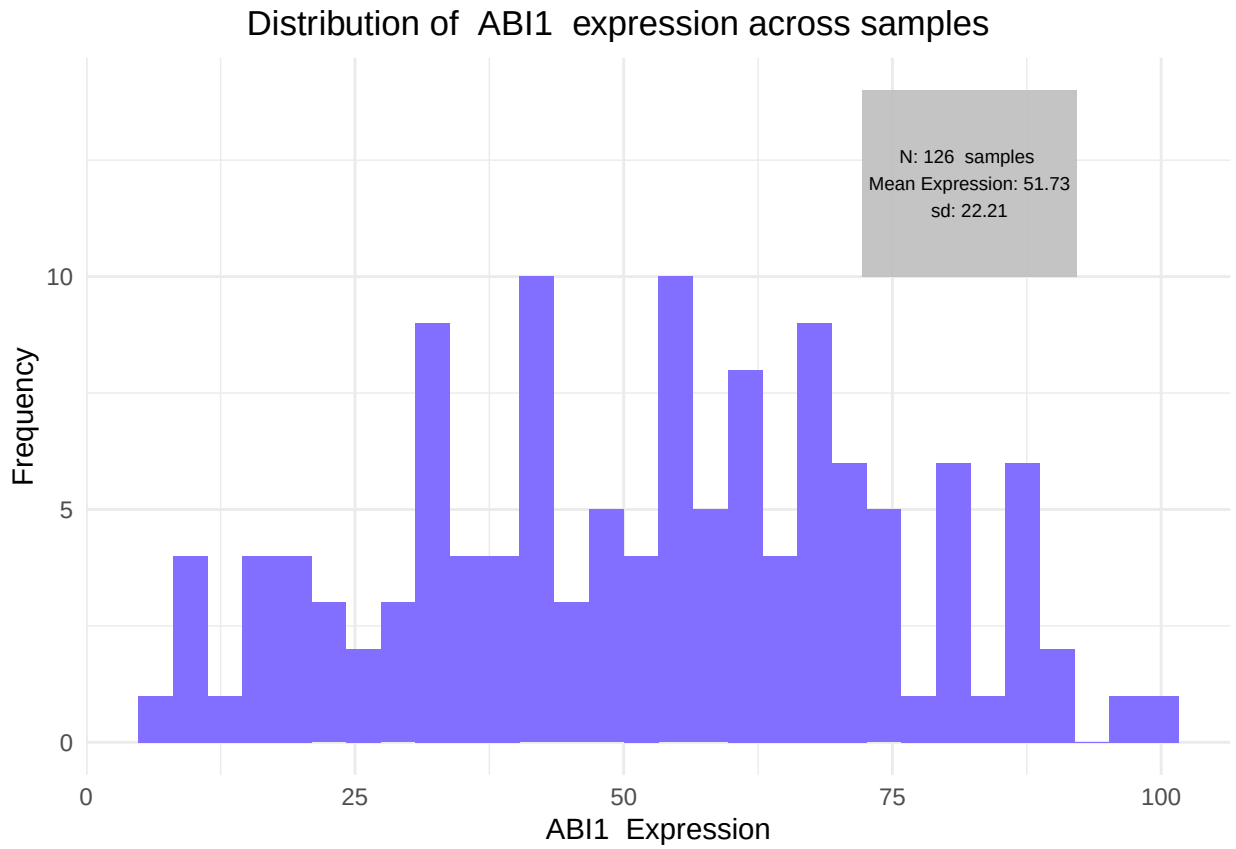
Observing expression patterns across three genes, one continuous covariate and two categorial covariates

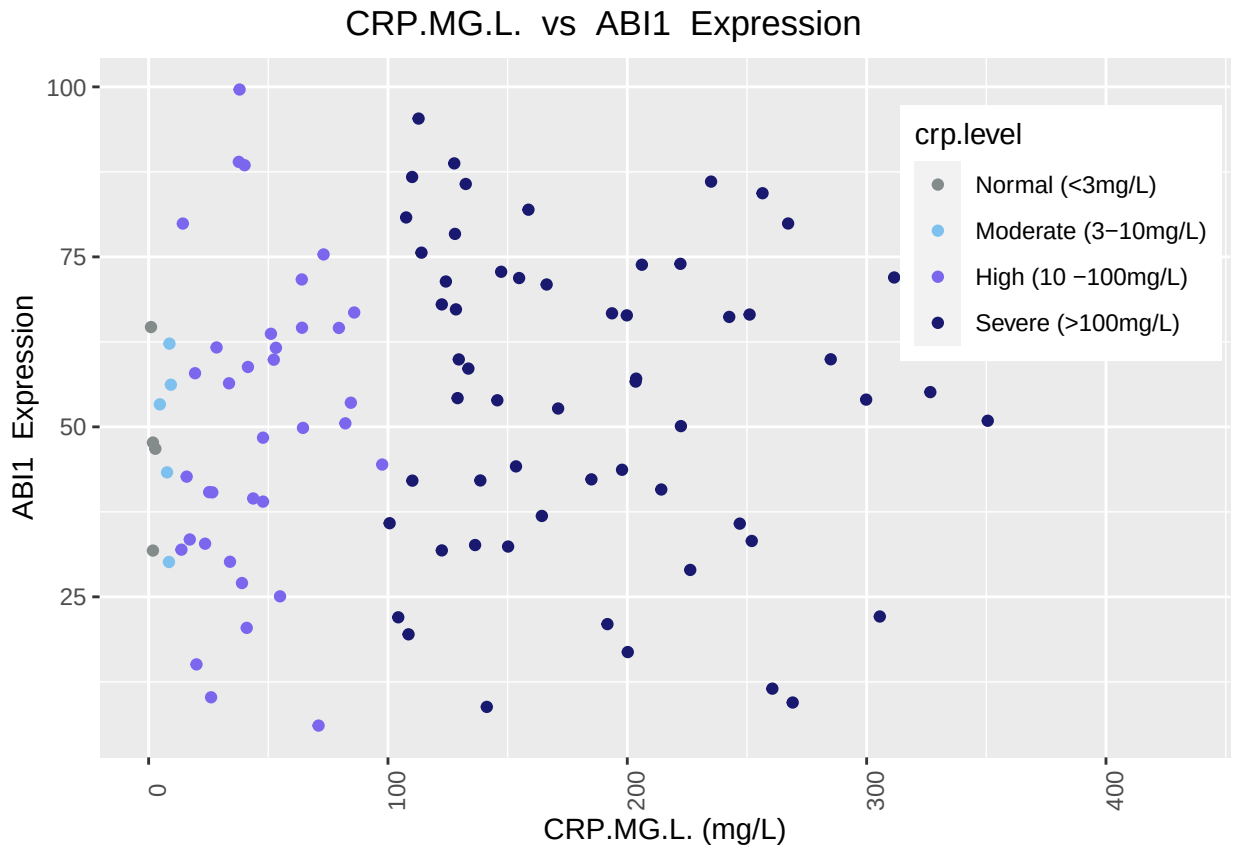
Chosen genes: ABI1, AAMP, ABHD17A

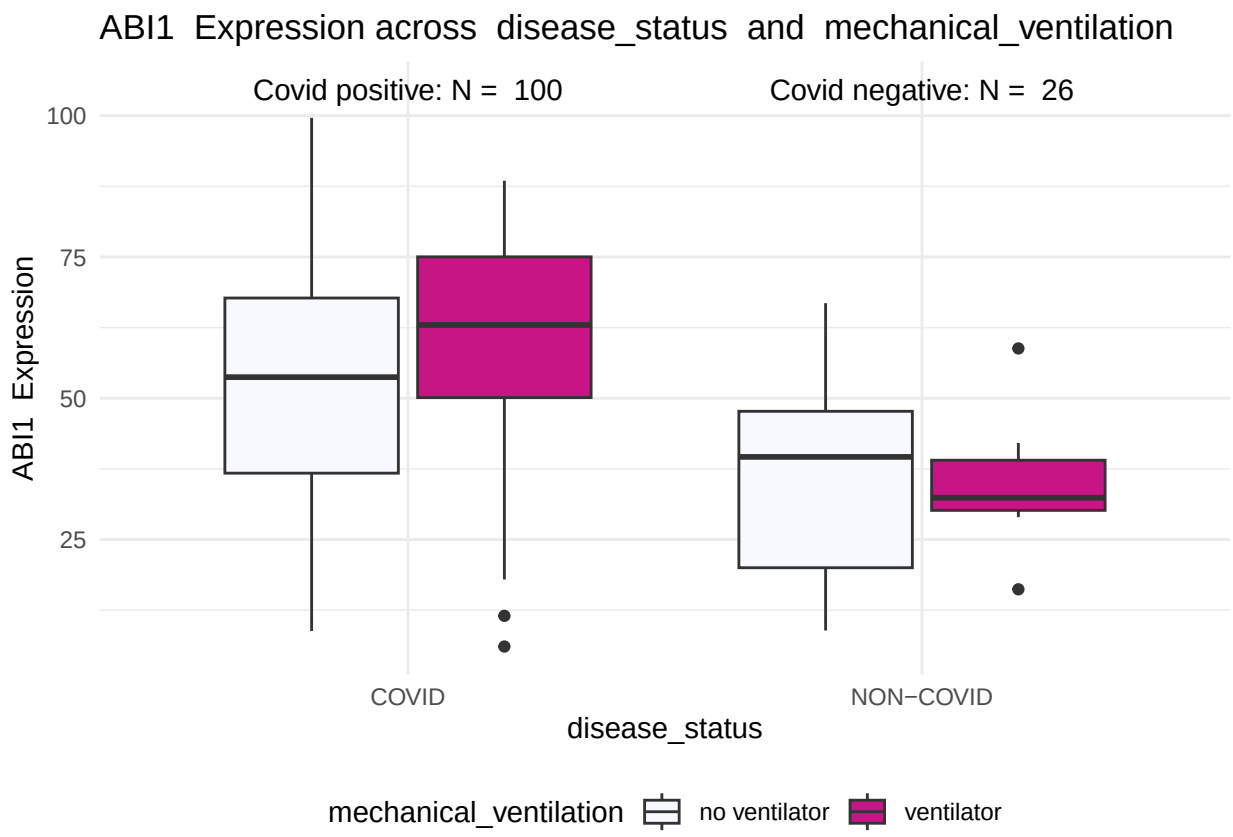
Continuous Covariate: Crp (mg/L)

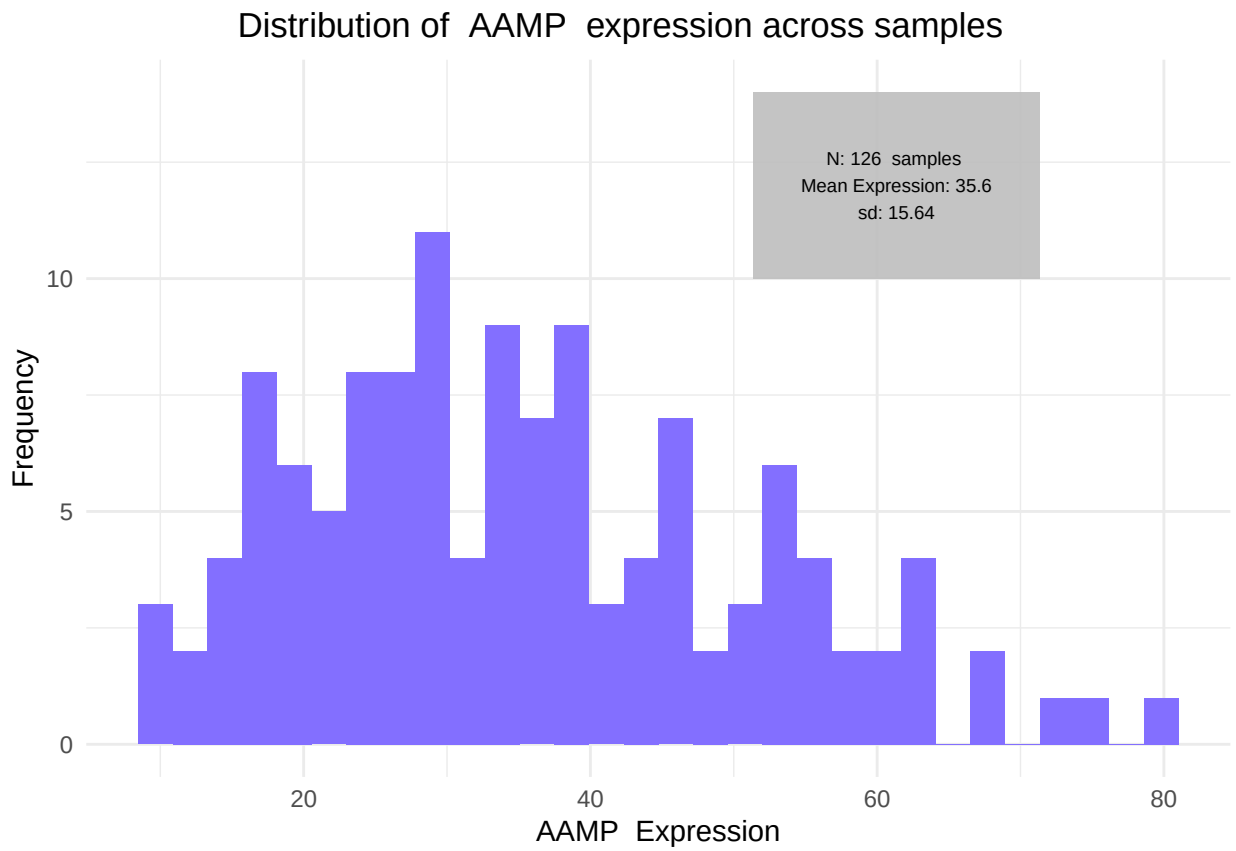
Categorial Covariates: Disease Status and Mechanical Ventilation

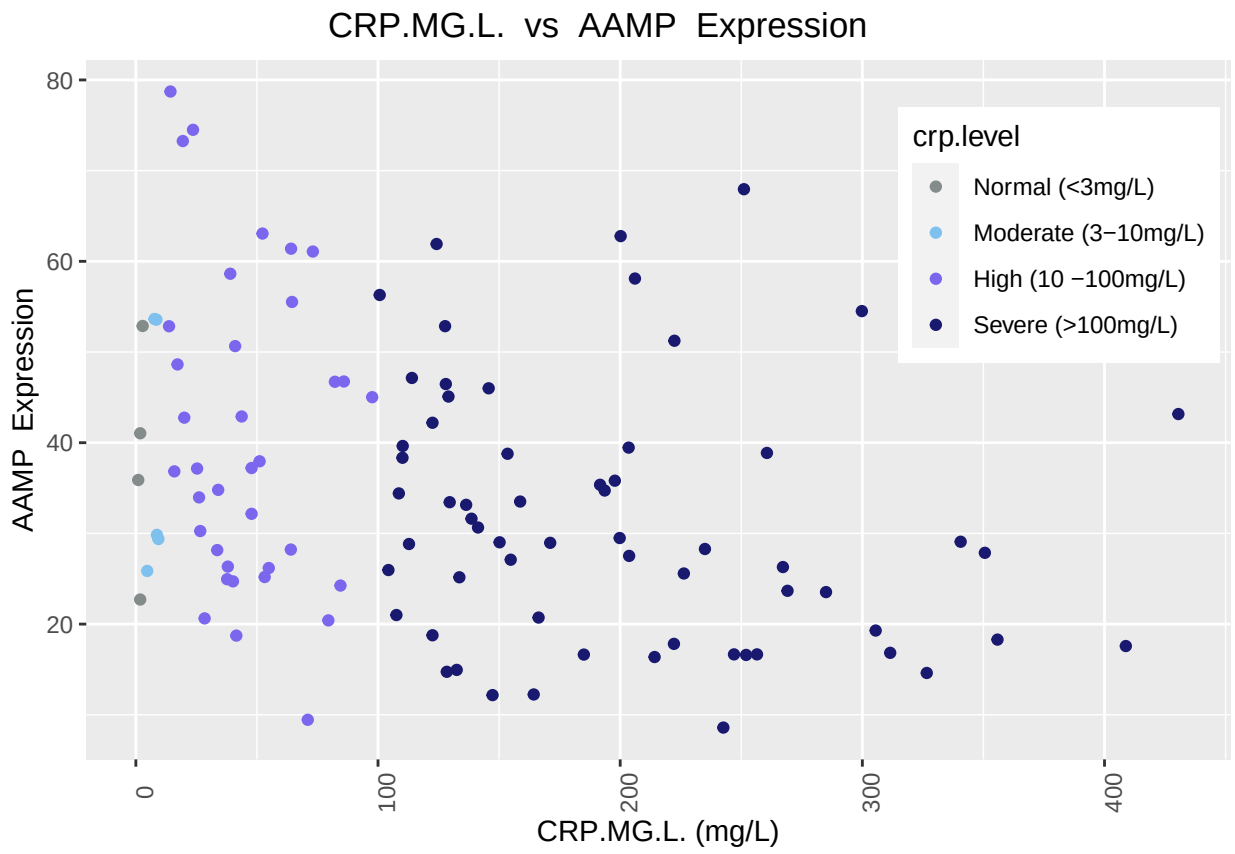
```
#a few warnings about deprecated methods, did not cause
#problems so decided to suppress for final output
suppressWarnings({ plots(all_data, c('ABI1', 'AAMP', 'ABHD17A'),
      c('crp.mg.l.'),
      c('disease_status', 'mechanical_ventilation')) } )
```

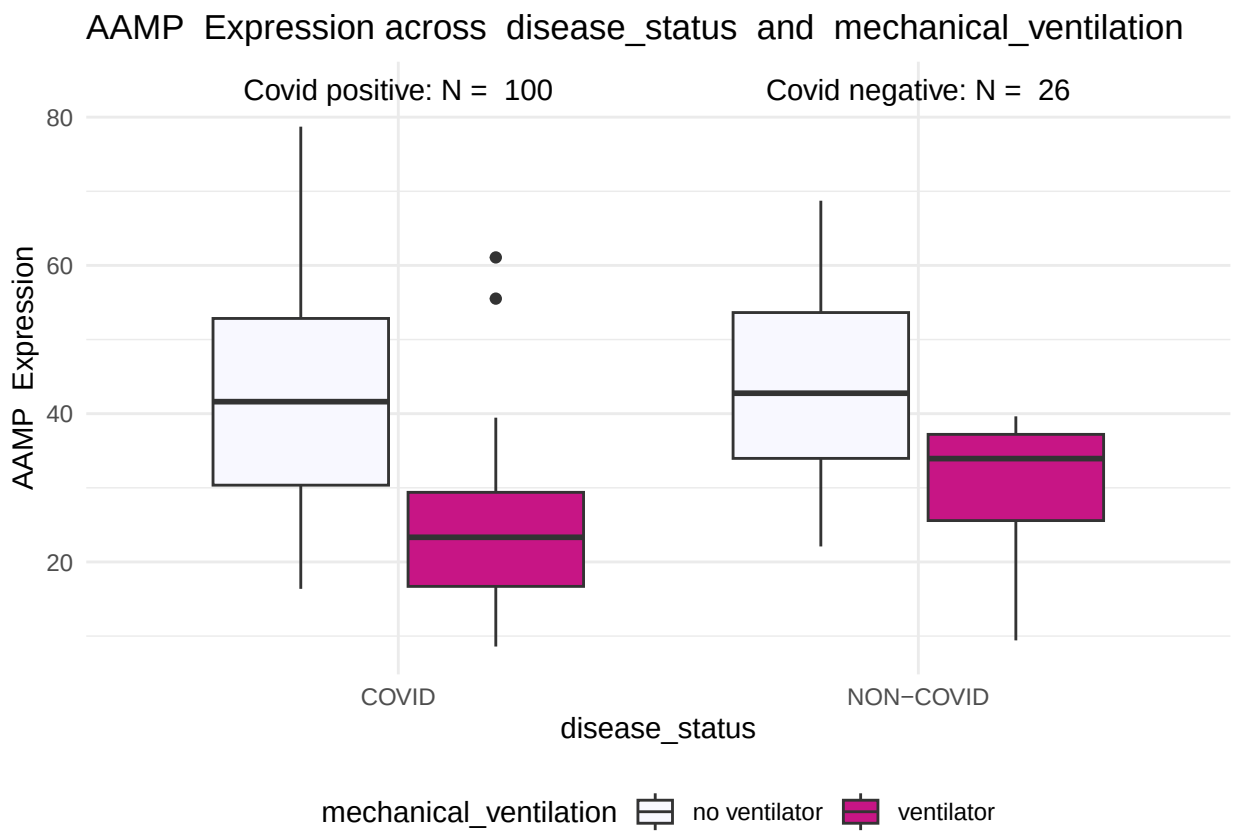

Distribution of ABI1 expression across samples
N: 126 samples
Mean Expression: 51.73
sd: 22.21
Frequency
0
5
10
0
25
50
75
100
ABI1 Expression

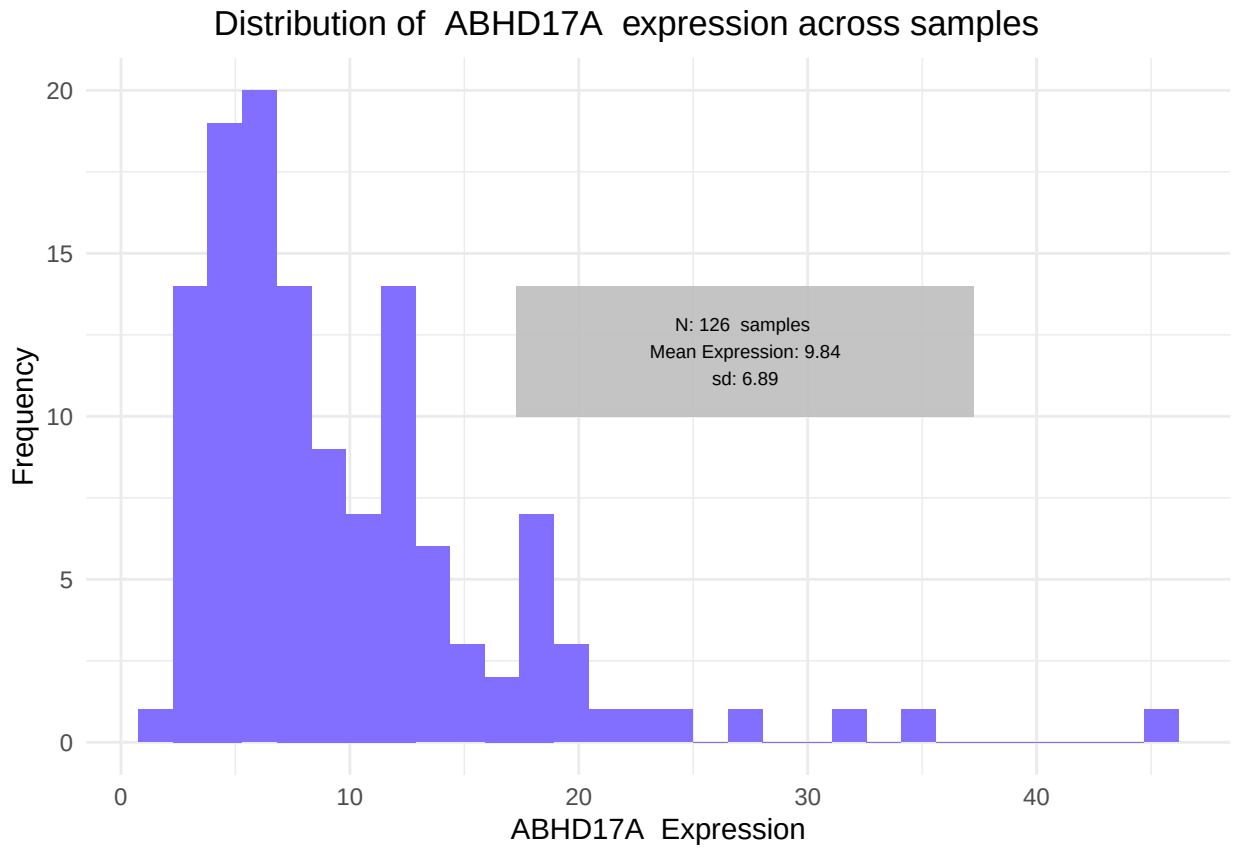

CRP.MG.L. vs ABI1 Expression
ABI1 Expression
100
75
50
25
0
100
200
300
400
CRP.MG.L. (mg/L)
crp.level
Normal (<3mg/L)
Moderate (3−10mg/L)
High (10 −100mg/L)
Severe (>100mg/L)

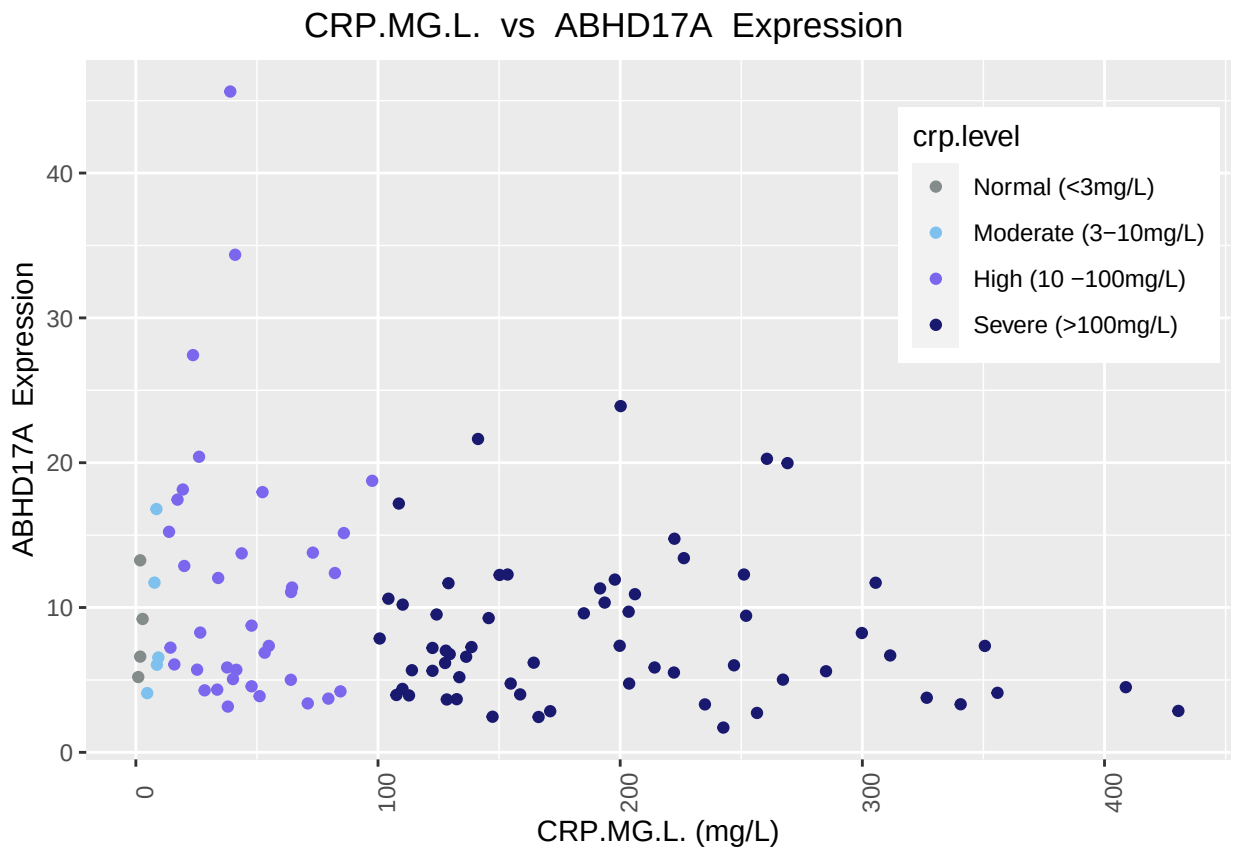

ABI1 Expression across disease_status and mechanical_ventilation
Covid positive: N = 100
Covid negative: N = 26
ABI1 Expression
100
75
50
25
COVID
NON−COVID
disease_status
mechanical_ventilation
no ventilator
ventilator

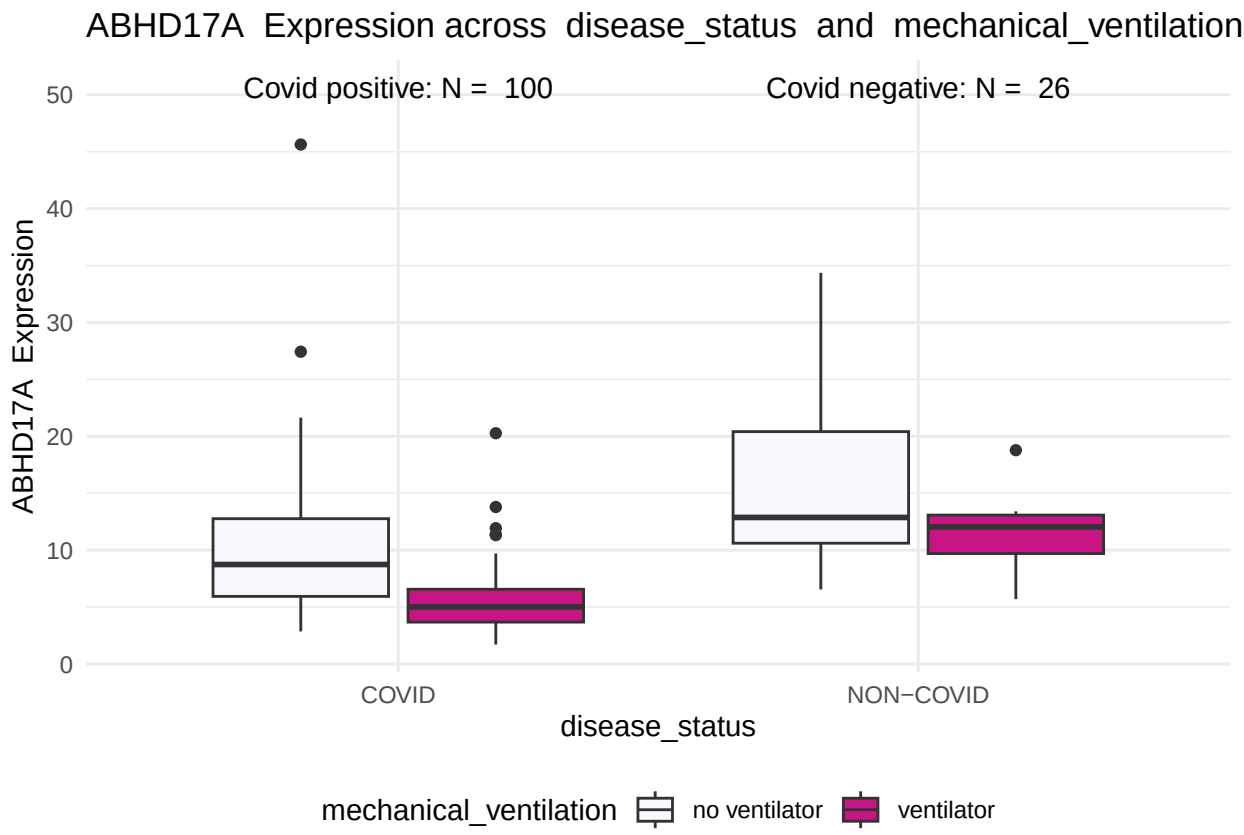

Distribution of AAMP expression across samples
N: 126 samples
Mean Expression: 35.6
sd: 15.64
Frequency
0
5
10
20
40
60
80
AAMP Expression


CRP.MG.L. vs AAMP Expression
AAMP Expression
80
60
40
20
0
100
200
300
400
CRP.MG.L. (mg/L)
crp.level
Normal (<3mg/L)
Moderate (3−10mg/L)
High (10 −100mg/L)
Severe (>100mg/L)


AAMP Expression across disease_status and mechanical_ventilation
Covid positive: N = 100
Covid negative: N = 26
AAMP Expression
80
60
40
20
COVID
NON−COVID
disease_status
mechanical_ventilation
no ventilator
ventilator


Distribution of ABHD17A expression across samples
Frequency
ABHD17A Expression
N: 126 samples
Mean Expression: 9.84
sd: 6.89
0
5
10
15
20
0
10
20
30
40


CRP.MG.L. vs ABHD17A Expression
crp.level
Normal (<3mg/L)
Moderate (3−10mg/L)
High (10 −100mg/L)
Severe (>100mg/L)
ABHD17A Expression
0
10
20
30
40
0
100
200
300
400
CRP.MG.L. (mg/L)


ABHD17A Expression across disease_status and mechanical_ventilation
Covid positive: N = 100
Covid negative: N = 26
ABHD17A Expression
50
40
30
20
10
0
COVID
NON−COVID
disease_status
mechanical_ventilation
no ventilator
ventilator