main analysis

2022-09-21

setup rmarkdown knit profile

```
library(tidyverse)
```

```
## -- Attaching packages --------------------------------------- tidyverse 1.3.2 --
## v ggplot2 3.3.6     v purrr   0.3.5
## v tibble  3.2.1     v dplyr   1.1.2
## v tidyr   1.2.1     v stringr 1.4.1
## v readr   2.1.3     v forcats 0.5.2
## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
# Some function Reuqires the in-house package "protools"
if (!require("protools", quietly = TRUE))
   devtools::install_github("https://github.com/FDUguchunhui/protools")
library('protools')
```

Import data

```
# import gastric primary cell nsaf data that filtered with Spectral count (SpC) >=2
gastric_nsaf <- as.matrix(read.csv('gastric-primary-cell/gastric_primary_cell_nsaf_2.csv', row.names = 1
# # import gastric primary cell SpC data
gastric_count <- read_csv('gastric-primary-cell/gastric_primary_cell_count_2.csv', show_col_types = FALS
```

```
## New names:
## * `` -> `...1`
```

```
# rename the index column as "accession"
colnames(gastric_count)[1] <- 'accession'
# import information for mapping between accession, gene symbol, and ensembl ID
omics_metadata <- read_csv('support-data/omics_metadata.csv', show_col_types = FALSE)
# load missing protein information
missing_protein_df <- readxl::read_xlsx('support-data/PE2-5.xlsx')
```

Create regular protein set and missing protein set

create a character vector of missing protein accession only use P2-4 proteins from nextprot the regular protein is not the just a complementary of missing protein, need to filter P2-5 proteins

```
# missing_proteins is a character vector of MP gene symbols
# similar P2_5_proteins are protein gene symbols under P2-5 category in nextprot
# P2_5_proteins will be used for negative filtering to get regular proteins
missing_proteins_gene_symnol <- missing_protein_df %>% filter(PE != 'Uncertain') %>% select(`gene name(s
P2_5_proteins_gene_symbol <- missing_protein_df  %>% select(`gene name(s)`) %>% pull()

# missing_proteins is a character vector of MP accession ID
# similar P2_5_proteins are protein accesion ID under P2-5 category in nextprot
missing_proteins_accession <- missing_protein_df %>% filter(PE != 'Uncertain') %>% select(Accession) %>%
P2_5_proteins_accession <- missing_protein_df %>% select(Accession) %>% pull()
```

check the number of SpC for each protein for both missing and regular proteins

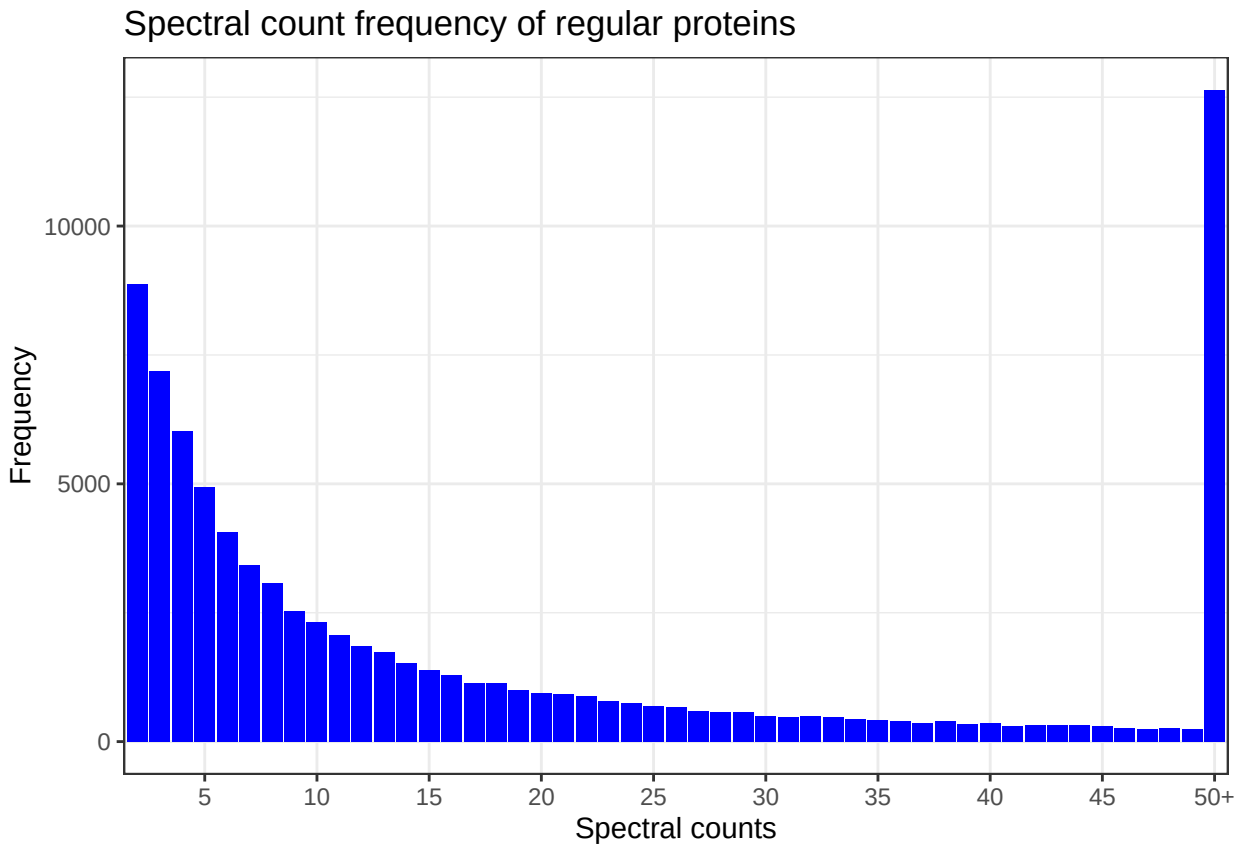
```
# IP0981_1701 IP0982_1701 IP0993_1701 IP0995_1701 IP0999_1701 IP7100_1701 IP7103_1701 IP7105_1701 sampl
# rename sample to Sample1-8
# colnames(gastric_count) <- c('accession', paste('Sample', 1:8, sep = ' '))

# create long format used for barplot
MP_counts <- gastric_count %>% filter(accession %in% missing_proteins_accession)
MP_counts_long <- MP_counts %>% pivot_longer(names_to = 'IPAS', values_to = 'count', cols = c(-accessio
MP_counts_long$type <- 'Missing'

RP_counts <-  gastric_count %>% filter(!(accession %in% P2_5_proteins_accession))
RP_counts_long <- RP_counts %>% pivot_longer(names_to = 'IPAS', values_to = 'count', cols = c(-accessio
RP_counts_long$type <- 'Regular'

all_counts_long <- rbind(MP_counts_long, RP_counts_long)
```

Figure S4 The peptide-spectral-matching (PSM) count distribution for regular proteins (Left blue) and missing proteins (Right red)

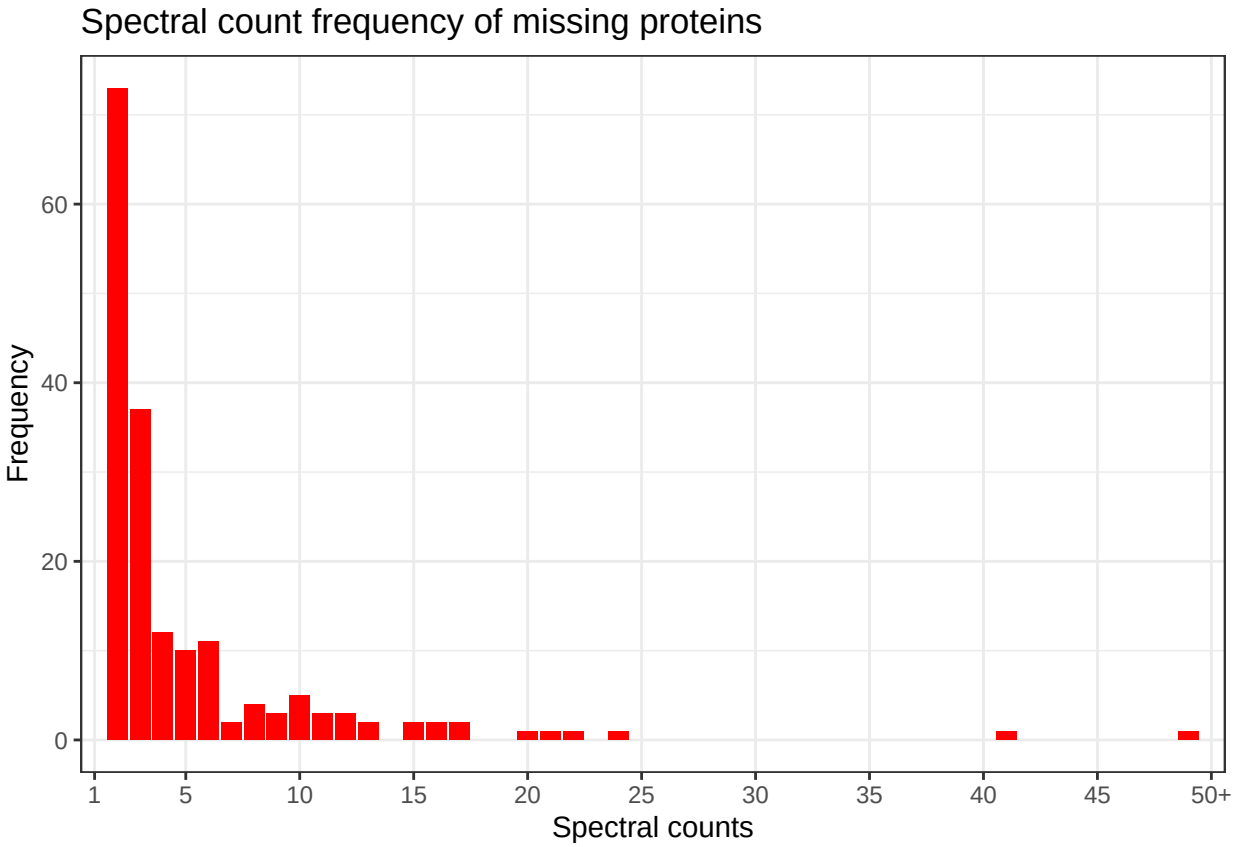
Frequency of Spectral count for regular proteins

```
# collapse SpC >= 50 into one single category "50+"
RP_barplot_data <-  RP_counts_long %>% mutate(count_discrete=ifelse(count >= 50, '50+', count))
RP_barplot_data$count_discrete <- factor(RP_barplot_data$count_discrete, levels=c(as.character(1:49), '5
RP_barplot_data %>%
  ggplot(aes(x=count_discrete)) +
  geom_bar(position = 'identity', fill='blue') +
theme_bw() +
  xlab('Spectral counts') +
  ylab('Frequency') +
```

```
  ggtitle('Spectral count frequency of regular proteins') +
  scale_x_discrete(breaks=c('1', seq(5, 50, 5), '50+'))
```




```
MP_barplot_data <-  MP_counts_long %>% mutate(count_discrete=ifelse(count >= 50, '50+', count))
MP_barplot_data$count_discrete <- factor(MP_barplot_data$count_discrete, levels=c(as.character(1:49), '
MP_barplot_data %>%
  ggplot(aes(x=count_discrete)) +
  geom_bar(position = 'identity', fill='red') +
theme_bw() +
  xlab('Spectral counts') +
  ylab('Frequency') +
  ggtitle('Spectral count frequency of missing proteins') +
  scale_x_discrete(breaks=c('1', seq(5, 50, 5), '50+'), drop=FALSE)
```



check the number of SpC for missing proteins

```
MP_counts_long %>% filter(count >= 2) %>% summary()
```

```
##   accession             IPAS              count           type
##  Length:177         Length:177         Min.   : 2.000   Length:177
##  Class :character   Class :character   1st Qu.: 2.000   Class :character
##  Mode  :character   Mode  :character   Median : 3.000   Mode  :character
##                                        Mean   : 5.153
##                                        3rd Qu.: 6.000
##                                        Max.   :49.000
```

Match mRNA expression to corresponding protein

The oirginal proteomics data is in accession level and the original transcription data is in gene level.

For example: There are two proteins coded by gene CTAGE1: Q96RT6 and Q9HC47

and we have the following expression NSAF Q96RT6: 3 NSAF Q9HC47: 0 TPM CTAGE1: 3

Then after match mRNA expression, each protein will have a column indicating its protein expression in NSAF and another column indicating its corresponding gene expression in TPM

Q96RT6: NSAF: 3 TPM: 3 Q9HC47: NSAF: 0 TPM: 3

create a dictionary used for mapping ensembl_gene_id to gene symbol

When a same ensembl_gene_id can be map to multiple gene symbol (not common) use only the first one (ordered by alphabeta)

```
dict_accession_to_gene_symbol <- omics_metadata %>% dplyr::select(accession_id, gene_symbol) %>% unique
```

make long-format gastric NSAF data

```
gastric_nsaf_df <- as_tibble(gastric_nsaf, rownames='accession')
gastric_nsaf_df_long <- gastric_nsaf_df %>% pivot_longer(names_to = 'IPAS', values_to = 'NSAF', cols = 
# type variable show whether a protein is a regular protein (RP, non-missing), missing protein (MP), or
gastric_nsaf_df_long <- gastric_nsaf_df_long %>% mutate(type=if_else(accession %in% missing_proteins_ac
                                                        if_else(!(accession %in% P2_5_proteins_accession)
```

get the correpsongding gene symbol for each protein

```
gastric_nsaf_df_long$gene_symbol <- mapping(gastric_nsaf_df_long$accession, dict_accession_to_gene_symb
rm(dict_accession_to_gene_symbol)
```

load transcription data

```
count_TPM_gene_symbol <- read.csv("RNA-data/processed_RNA_data.csv", row.names = 1)
```

make long-format of RNA expression data

```
count_TPM_gene_symbol_df <- as_tibble(count_TPM_gene_symbol,  rownames='gene_symbol')
count_TPM_gene_symbol_df_long <- count_TPM_gene_symbol_df %>% pivot_longer(names_to = 'IPAS', values_to
```

join the RNA expression to protein data

```
dat_combined_long <- left_join(gastric_nsaf_df_long, count_TPM_gene_symbol_df_long, by=c('IPAS', 'gene_
dat_combined_long$NSAF <- ifelse(is.na(dat_combined_long$NSAF), 0, dat_combined_long$NSAF)
dat_combined_long$TPM <- ifelse(is.na(dat_combined_long$TPM), 0, dat_combined_long$TPM)
```

Unique Missing protein accession

```
(MP_unique_accession <- dat_combined_long %>% filter(NSAF > 0, TPM > 0, type=='MP') %>% select(accessio
```

```
##  [1] "A0A096LP55" "A0A0B4J273" "P58512"     "P60606"     "Q6ZVZ8"
##  [6] "Q86UF2"     "Q8IXR9"     "Q8IZJ4"     "Q8N431"     "U3KPV4"
## [11] "A4D2H0"     "A6NFK2"     "A6NHG4"     "A6NK53"     "F2Z3F1"
## [16] "F8VTS6"     "P0DJD0"     "P0DKX4"     "P0DN37"     "Q52LC2"
## [21] "Q5T035"     "Q5T2Q4"     "Q5VX52"     "Q6IEE8"     "Q6T423"
## [26] "Q6VEQ5"     "Q7RTU9"     "Q8N4K4"     "Q8N5U1"     "Q8N9H9"
## [31] "Q8NEX6"     "Q8NH16"     "Q96KF2"     "Q9H3Y0"     "Q9UIL4"
## [36] "Q9Y4R7"     "A0A1B0GVH4" "Q6PB30"     "Q7RTY5"     "Q8N9P6"
## [41] "Q96A28"     "Q96NG8"     "Q9Y6U7"     "A6NJJ6"     "A6PVI3"
## [46] "A9Z1Z3"     "E5RHQ5"     "O75678"     "Q3B8N5"     "Q8N8V8"
## [51] "A6NFN9"     "A8MTL9"     "Q6JVE9"     "Q7Z572"     "Q7Z5M5"
## [56] "A0A087WT02" "A6NH13"     "C9J202"     "Q5RIA9"     "Q8IZJ6"
## [61] "Q9NUZ1"     "A0A075B6T7" "A4QPB2"     "E5RQL4"     "Q86V71"
## [66] "Q96KX1"     "Q9H158"
```

```
length(MP_unique_accession)
```

```
## [1] 67
```

combine and match RNA and proteomics data into long format

Each row is a protein identified by gene symbol and sample ID primary key: (accession, IPAS) in term of SQL TPM is the RNA expression of the gene that coding those proteins NSAF is the combined protein expressions for proteomics translated from that gene

```
combined_long <- dat_combined_long  %>% filter(!(TPM == 0 & NSAF == 0))
```

Create a helper function for calculate the number of proteins-at-gene-level has non-zero RNA expression

```
#' @title calculate proportion of protein products supported with RNA products
#' @description It product the proportion of each condition based on different settings
#' @param tbl a tibble object, see details in "dplyr"
#' @param missing_protein whether to only include missing proteins
#' @param group_by_IPAS whether to stratified by IPAS samples
#' @param TPM_cutoff float, the cutoff used for RNA TPM value to consider a RNA product to be existing
#' @param NSAF_cutoff float, the cutoff used for RNA TPM value to consider a protein product to be exis
#' @param cases a vector of integer, used to decide whether cases to included for calculation
summarize_by_case <- function(tbl, protein_type, remove_IPAS=NULL, group_by_IPAS=FALSE, TPM_cutoff = 0,

  tbl <- tbl %>% filter(type==protein_type)


  if (!is.null(remove_IPAS)) {
    tbl <- tbl %>% filter(!(IPAS %in% remove_IPAS))
  }

  tbl <- tbl %>% rowwise() %>%
    mutate(case=if_else(TPM > TPM_cutoff & NSAF > NSAF_cutoff, 1,
                        if_else(TPM <= TPM_cutoff & NSAF > NSAF_cutoff, 2,
```

```
                                if_else(TPM > TPM_cutoff & NSAF <= NSAF_cutoff, 3, 4)
                        )
    )
    ) %>%
    filter(case %in% cases) %>%
    group_by(IPAS, case) %>%
    summarise(count=n(), ) %>%
    ungroup(IPAS, case)

  if (group_by_IPAS) {
    tbl <- tbl %>% group_by(IPAS)
  }

  tbl <- tbl %>%
    group_by(case, .add=TRUE) %>%
    summarise(count= sum(count)) %>%
    mutate(countT=sum(count)) %>%
    mutate(per=paste0(round(100*count/countT,2),'%'))

  return(tbl)
}
```

the proportion of PE2-5 in the missing protein dataset

```
table(missing_protein_df$PE)
```

```
##
## Evidence at transcript level       Inferred from homology
##                         1135                          195
##                    Predicted                    Uncertain
##                           13                          609
```

check missing proteins with corresponding RNA expression

```
# check the protein and RNA expression for the detected missing proteins
full_table_detected_MP <- combined_long %>% filter(type=='MP') %>% filter(NSAF > 0) %>% arrange(accessi

write_csv(full_table_detected_MP, 'detected_177_MP_products_with_RNA.csv')
```

association analysis

Table 2 The proportions of protein products with mRNA products for missing proteins and regular proteins

Table 2 missing protein part

```
# the coverage rate of RNA product in protein product for missing protein
(a <- summarize_by_case(combined_long, protein_type = 'MP', group_by_IPAS = TRUE, cases = c(1, 2)))
```

```
## `summarise()` has grouped output by 'IPAS'. You can override using the
## `.groups` argument.
## `summarise()` has grouped output by 'IPAS'. You can override using the
## `.groups` argument.
```

```
## # A tibble: 16 x 5
## # Groups:   IPAS [8]
##    IPAS         case count countT per
##    <chr>       <dbl> <int>  <int> <chr>
##  1 IP0981_1701     1     4      9 44.44%
##  2 IP0981_1701     2     5      9 55.56%
##  3 IP0982_1701     1    27     44 61.36%
##  4 IP0982_1701     2    17     44 38.64%
##  5 IP0993_1701     1    13     28 46.43%
##  6 IP0993_1701     2    15     28 53.57%
##  7 IP0995_1701     1     9     23 39.13%
##  8 IP0995_1701     2    14     23 60.87%
##  9 IP0999_1701     1    11     18 61.11%
## 10 IP0999_1701     2     7     18 38.89%
## 11 IP7100_1701     1     9     16 56.25%
## 12 IP7100_1701     2     7     16 43.75%
## 13 IP7103_1701     1     3     15 20%
## 14 IP7103_1701     2    12     15 80%
## 15 IP7105_1701     1    12     24 50%
## 16 IP7105_1701     2    12     24 50%
```

```
# write_csv(a, file='output/sample_MP_stats.csv')

## overall without removing the outlier
(b <- summarize_by_case(combined_long, protein_type = 'MP', group_by_IPAS = FALSE, cases = c(1, 2)))
```

```
## `summarise()` has grouped output by 'IPAS'. You can override using the
## `.groups` argument.
```

```
## # A tibble: 2 x 4
##    case count countT per
##   <dbl> <int>  <int> <chr>
## 1     1    88    177 49.72%
## 2     2    89    177 50.28%
```

```r
## overall with removing the outlier
(c <- summarize_by_case(combined_long, protein_type = 'MP', group_by_IPAS = FALSE, cases = c(1, 2), rem
```

```
## `summarise()` has grouped output by 'IPAS'. You can override using the
## `.groups` argument.
```

```
## # A tibble: 2 x 4
##    case count countT per
##   <dbl> <int>  <int> <chr>
## 1     1    81    153 52.94%
## 2     2    72    153 47.06%
```

histogram

show the number of detected missing protein and proportion of them having corresponding mRNA expression

```r
b$IPAS = 'Combined'
c$IPAS = 'Combined w.o. outlier'
dat_hist <- rbind(a, b, c)


dat_hist$case <- factor(dat_hist$case, levels = c('1', '2'), labels = c('RNA (+)', 'RNA (-)'))
dat_hist$case <- relevel(dat_hist$case, 'RNA (+)')

dat_hist$IPAS <- factor(dat_hist$IPAS, levels = c("IP0981_1701", "IP0982_1701", "IP0993_1701", "IP0995_
                                                  "IP7100_1701", "IP7103_1701", "IP7105_1701", "Combine


ggplot(data=dat_hist, aes(x=IPAS, y=count, group=case, fill=case)) +
  labs(title="Coverage rate for prodein product with RNA product in missing proteins", subtitle = '',
       x = "IPAS", y = 'count') +
  geom_bar( stat = 'identity',  position=position_dodge()) +
  geom_text(aes(label=per), color="black",size=3, vjust=-0.5, position = position_dodge(1)) +
  theme(
       axis.text.x = element_text(angle = 45, hjust = 1))
```

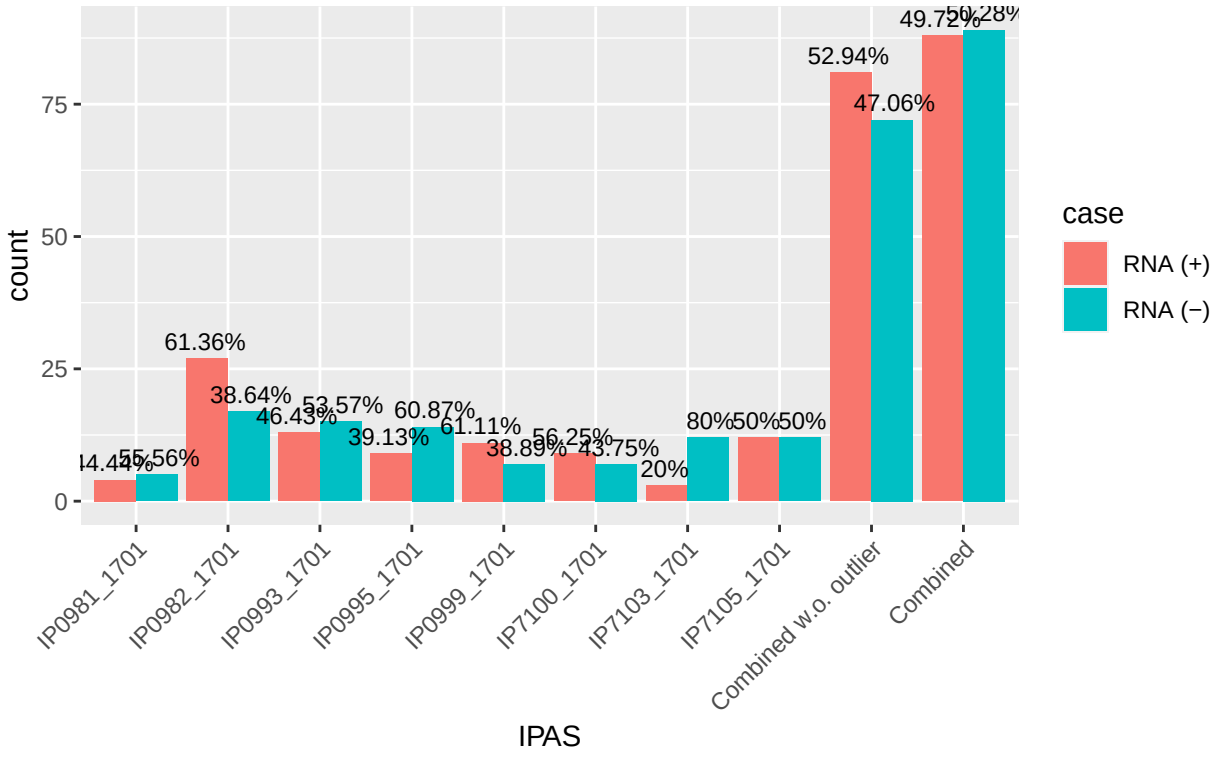


Table 2 non-missing proteins part

The execution of the following chucks of code could be slow for the size of non-missing proteins Code is split into several chuck otherwise the computation time required is long.

```
# the coverage rate of RNA product in protein product for non-missing protein
(a_RP <- summarize_by_case(combined_long, protein_type = 'RP', group_by_IPAS = TRUE, cases = c(1, 2)))
```

```
## `summarise()` has grouped output by 'IPAS'. You can override using the
## `.groups` argument.
## `summarise()` has grouped output by 'IPAS'. You can override using the
## `.groups` argument.
```

```
## # A tibble: 16 x 5
## # Groups:   IPAS [8]
##    IPAS          case count countT per
##    <chr>        <dbl> <int>  <int> <chr>
##  1 IP0981_1701     1  5063   5593 90.52%
##  2 IP0981_1701     2   530   5593 9.48%
##  3 IP0982_1701     1 12642  13864 91.19%
##  4 IP0982_1701     2  1222  13864 8.81%
##  5 IP0993_1701     1 13113  14225 92.18%
##  6 IP0993_1701     2  1112  14225 7.82%
##  7 IP0995_1701     1  9667  10436 92.63%
##  8 IP0995_1701     2   769  10436 7.37%
```

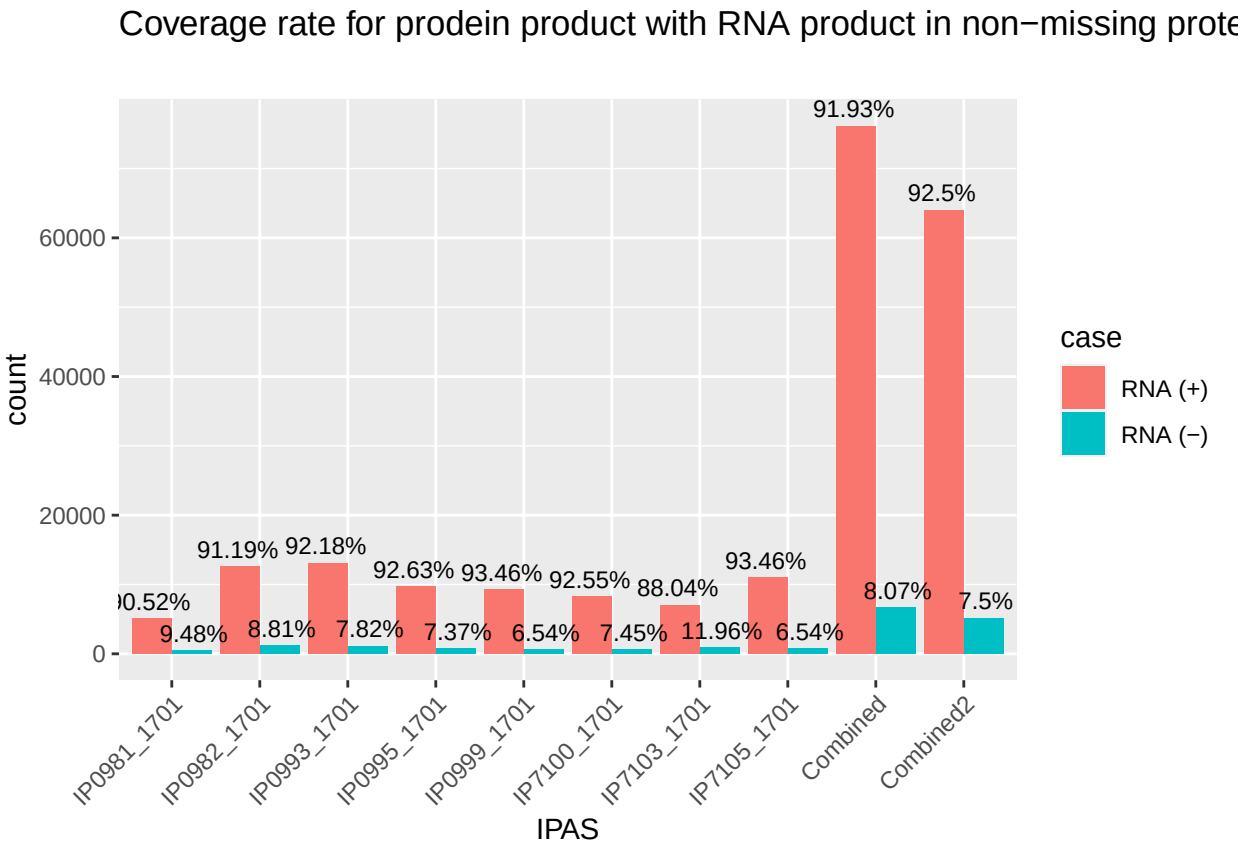
```
##  9 IP0999_1701     1  9267   9915 93.46%
## 10 IP0999_1701     2   648   9915 6.54%
## 11 IP7100_1701     1  8273   8939 92.55%
## 12 IP7100_1701     2   666   8939 7.45%
## 13 IP7103_1701     1  7058   8017 88.04%
## 14 IP7103_1701     2   959   8017 11.96%
## 15 IP7105_1701     1 11064  11838 93.46%
## 16 IP7105_1701     2   774  11838 6.54%
```

```
## overall without removing the outlier
b_RP <- a_RP %>% group_by(case) %>%
    summarise(count= sum(count)) %>%
    mutate(countT=sum(count)) %>%
    mutate(per=paste0(round(100*count/countT,2),'%'))
```

```
## overall after removing the outlier
c_RP <- a_RP %>% filter(!IPAS %in% c('IP0981_1701', 'IP7103_1701')) %>%
  group_by(case) %>%
    summarise(count= sum(count)) %>%
    mutate(countT=sum(count)) %>%
    mutate(per=paste0(round(100*count/countT,2),'%'))
```

barplot for non-missing proteins

```
b_RP$IPAS = 'Combined'
c_RP$IPAS = 'Combined2'
dat_hist <- rbind(a_RP, b_RP, c_RP)

dat_hist$case <- factor(dat_hist$case, levels = c('1', '2'), labels = c('RNA (+)', 'RNA (-)'))
dat_hist$case <- relevel(dat_hist$case, 'RNA (+)')

dat_hist$IPAS <- factor(dat_hist$IPAS, levels = c("IP0981_1701", "IP0982_1701", "IP0993_1701", "IP0995_
                                                  "IP7100_1701", "IP7103_1701", "IP7105_1701", "Combine

ggplot(data=dat_hist, aes(x=IPAS, y=count, group=case, fill=case)) +
  labs(title="Coverage rate for prodein product with RNA product in non-missing proteins", subtitle = '
        x = "IPAS", y = 'count') +
  geom_bar( stat = 'identity',  position=position_dodge()) +
  geom_text(aes(label=per), color="black",size=3, vjust=-0.5, position = position_dodge(1)) +
  theme(
        axis.text.x = element_text(angle = 45, hjust = 1))
```



Data preparation for Figure 3: The scatter plot of protein-mRNA-product matched pairs.

generate data that can be used for plot the scatter plot

Four datasets were created: 1) glmm_dat_missing: paired RNA/protein expressions for missing protein 2) glmm_dat_missing_filtered: pair RNA/protein expressions for missing protein and only keep proteins with both valid (>0) RNA and protein expressions 3) glmm_dat_non_missing: paired RNA/protein expressions for regular protein 4) glmm_dat_non_missing_filtered: air RNA/protein expressions for regular proteins and only keep proteins with both valid (>0) RNA and protein expressions

`All TPM/NSAF values were log-transformed in all four datasets`

```
glmm_dat_missing <- dat_combined_long %>% filter(type=='MP') %>% mutate(TPM = log(TPM + 1), NSAF = log(
glmm_dat_missing_filtered <- glmm_dat_missing %>% filter(TPM > 0 & NSAF > 0)


glmm_dat_non_missing <-dat_combined_long %>% filter(type=='RP') %>% mutate(TPM = log(TPM + 1), NSAF = lo
glmm_dat_non_missing_filtered <- glmm_dat_non_missing %>% filter(TPM > 0 & NSAF > 0)
```

Generalization linear mixed model using RNA expression as random effects in different samples

check whether the correlation relationship is different in different samples

```
library(lme4)
```

```
## Loading required package: Matrix
```

```
##
## Attaching package: 'Matrix'
```

```
## The following objects are masked from 'package:tidyr':
##
##     expand, pack, unpack
```

```
glmm_model <- lmer(NSAF ~ TPM + (0 + TPM | IPAS), data = glmm_dat_missing_filtered)
glm_model <- lm(NSAF ~ TPM, data = glmm_dat_missing_filtered)
summary(glmm_model)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: NSAF ~ TPM + (0 + TPM | IPAS)
##    Data: glmm_dat_missing_filtered
##
## REML criterion at convergence: 240.6
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.09524 -0.69004  0.01369  0.61513  2.74569
##
## Random effects:
##  Groups   Name Variance Std.Dev.
##  IPAS     TPM  0.009444 0.09718
##  Residual      0.834885 0.91372
## Number of obs: 88, groups:  IPAS, 8
##
## Fixed effects:
##             Estimate Std. Error t value
## (Intercept)  4.22987    0.12801  33.043
## TPM          0.19079    0.07764   2.458
##
## Correlation of Fixed Effects:
##     (Intr)
## TPM -0.567
```

No significant evidence for support using RNA expression as random effects

```
anova(glmm_model, glm_model)
```

```
## refitting model(s) with ML (instead of REML)
```

```
## Data: glmm_dat_missing_filtered
## Models:
## glm_model: NSAF ~ TPM
## glmm_model: NSAF ~ TPM + (0 + TPM | IPAS)
##            npar    AIC    BIC  logLik deviance Chisq Df Pr(>Chisq)
## glm_model     3 240.46 247.89 -117.23   234.46
## glmm_model    4 242.46 252.37 -117.23   234.46     0  1          1
```

So, a simple linear regression can be used

```
model.lm <- lm(NSAF ~ TPM, data = glmm_dat_missing_filtered)
summary(model.lm)
```

```
##
## Call:
## lm(formula = NSAF ~ TPM, data = glmm_dat_missing_filtered)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.93447 -0.64185 -0.00102  0.56857  2.53510
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  4.23100    0.12980  32.597  < 2e-16 ***
## TPM          0.17953    0.06804   2.639  0.00988 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.9275 on 86 degrees of freedom
## Multiple R-squared:  0.0749, Adjusted R-squared:  0.06414
## F-statistic: 6.962 on 1 and 86 DF,  p-value: 0.00988
```

The scatterplot with group-wise linear regression line validates the result

```
ggplot(data      = glmm_dat_missing_filtered,
       aes(x     = TPM,
           y     = NSAF,
           col   = IPAS,
           group = IPAS))+ #to add the colours for different classes
  geom_point(size     = 1.2,
             alpha    = .8,
             position = "jitter")+ #to add some random noise for plotting purposes
  theme_minimal() +
  geom_smooth(method = lm,
              se     = FALSE,
              size   = .5,
              alpha  = .8)+ # to add regression line
  labs(title    = "Normalized spectral abundance factor (NASF) vs. Transcripts per million (TPM)",
       subtitle = "missing proteins on gene level in gastric cancer primary cell samples",
       xlab = 'Log2(TPM) + 1',
       ylab = 'Log2(NSAF) + 1',
  )
```

```
## `geom_smooth()` using formula 'y ~ x'
```

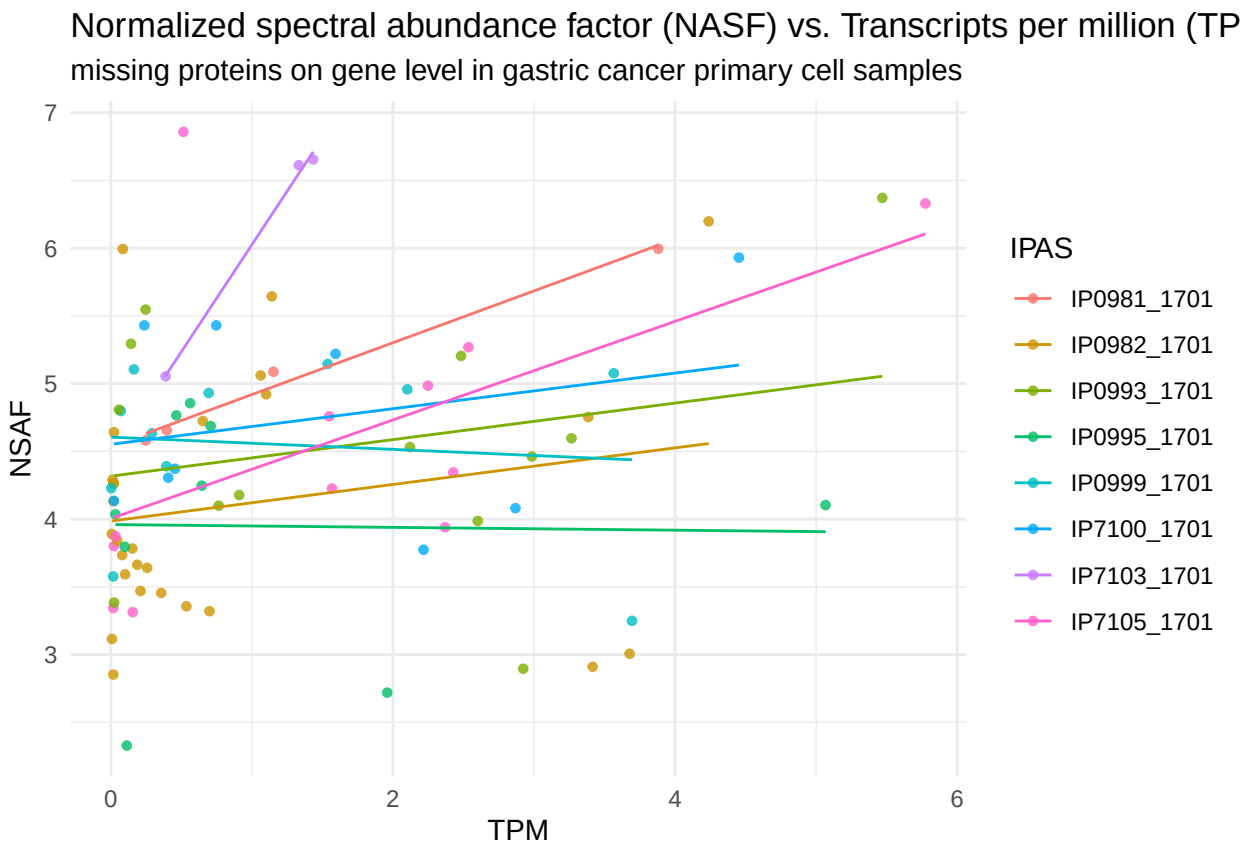
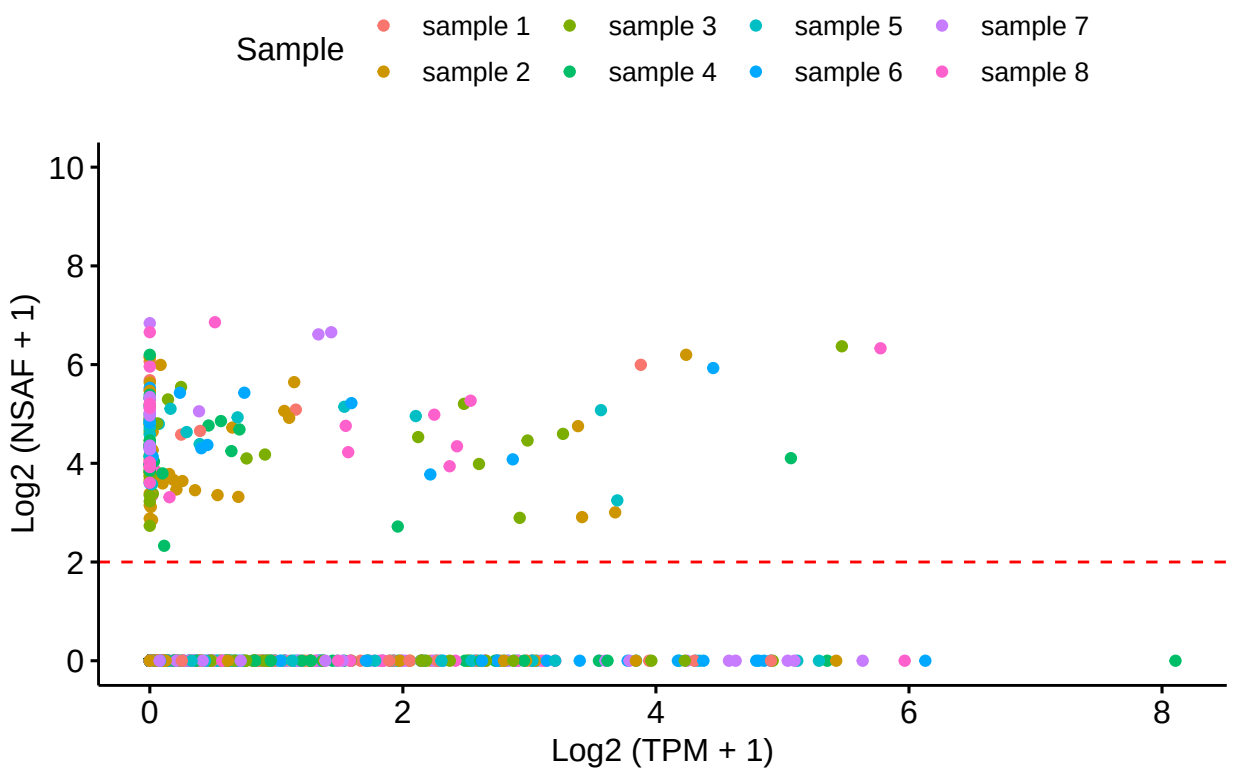


Figure 3 with spearman correlation for missing proteins

The scatter plot of protein-mRNA-product matched pairs. (a): All protein-mRNA pairs for missing proteins. (b): protein-mRNA pairs with TPM > 0 and NSAF > 0. (c): All protein-mRNA pairs for regular proteins. (d): All protein-mRNA pairs for regular proteins with TPM > 0 and NSAF > 0. Blue line: Simple linear regression. Spearman correlations and corresponding p-value were calculated for (b) and (d).

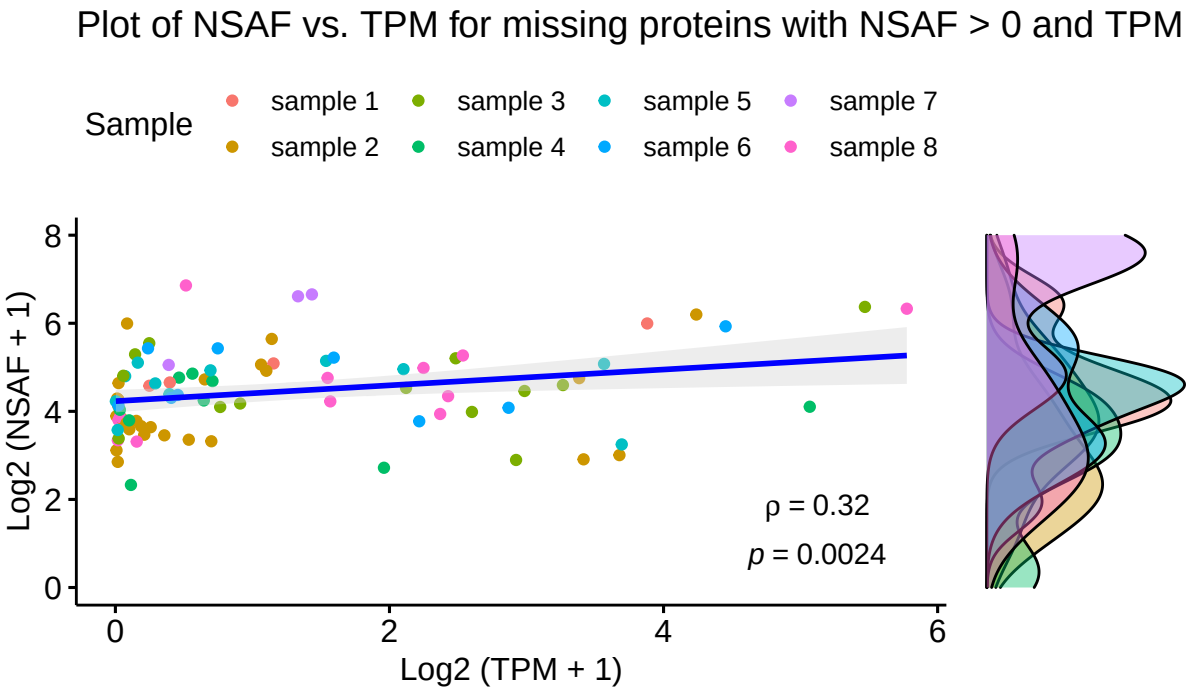
```
par(mfrow=c(2, 2))
ggpubr::ggscatter(glmm_dat_missing, x = "TPM", y = "NSAF",
   color = 'IPAS', size = 1.5, # Points color, shape and size
   title='Plot of NSAF vs. TPM for missing proteins',
   xlab = 'Log2 (TPM + 1)',
   ylab = 'Log2 (NSAF + 1)',
   ) +
  scale_color_discrete(labels=paste('sample', 1:8)) +
  scale_y_continuous(name='Log2 (NSAF + 1)', breaks=c(0, 2, 4, 6, 8, 10), limits = c(0, 10)) +
  # geom_vline(xintercept=0.1, linetype=2, color='red', size=0.5) +
  geom_hline(yintercept=2, linetype=2, color='red', size=0.5) +
  theme(legend.text = element_text(size=10)) +
  labs(color='Sample')
```



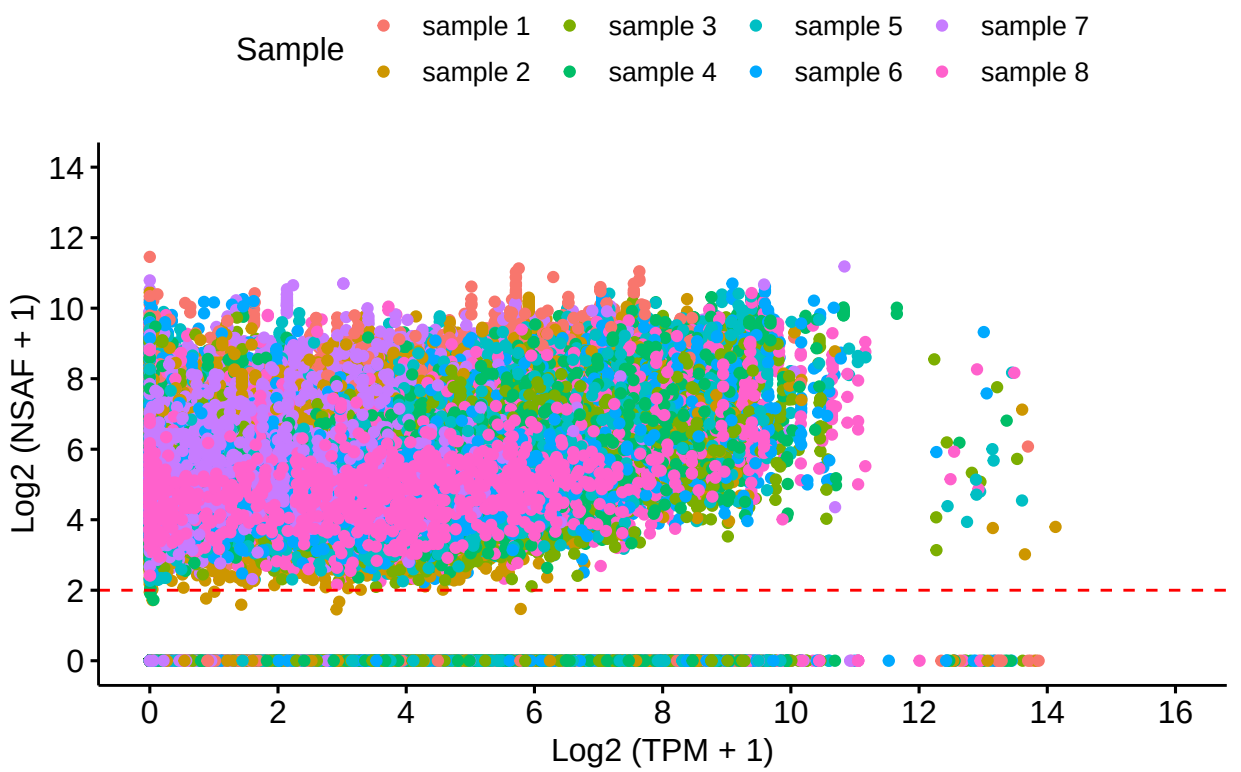

```
# plot with spearman correlation for missing proteins
plt <- ggpubr::ggscatter(glmm_dat_missing_filtered, x = "TPM", y = "NSAF",
   color = 'IPAS', size = 1.5, # Points color, shape and size
   add = "reg.line",  # Add regressin line
   add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
   conf.int = TRUE, # Add confidence interval
   cor.coef = TRUE, # Add correlation coefficient. see ?stat_cor
   cor.coeff.args = list(method = "spearman", cor.coef.name = "rho", label.x.npc=0.8, label.sep = "\n",
                         label.y.npc = 0.2),
   title='Plot of NSAF vs. TPM for missing proteins with NSAF > 0 and TPM > 0',
   # subtitle='missing proteins on gene level in gastric cancer primary cell sample',
   xlab = 'Log2 (TPM + 1)',
   ylab = 'Log2 (NSAF + 1)',
   ) +
   scale_color_discrete(labels=paste('sample', 1:8)) +
   scale_y_continuous(name='Log2 (NSAF + 1)', breaks=c(0, 2, 4, 6, 8, 10), limits = c(0, 8)) +
   theme(legend.text = element_text(size=10)) +
   labs(color='Sample')

dens1 <- ggplot(glmm_dat_missing_filtered, aes(x = TPM, fill = IPAS)) +
  geom_density(alpha = 0.4) +
  theme_void() +
  theme(legend.position = "none")

dens2 <- ggplot(glmm_dat_missing_filtered, aes(x = NSAF, fill = IPAS)) +
  geom_density(alpha = 0.4) +
  theme_void() +
```

```
  theme(legend.position = "none") +
  coord_flip()

dens1 + patchwork::plot_spacer() + plt + dens2 +  patchwork::plot_layout(ncol = 2, nrow = 2, widths = c
```

```
## `geom_smooth()` using formula 'y ~ x'
```




```
# plot with spearman correlation for non-missing proteins
ggpubr::ggscatter(glmm_dat_non_missing, x = "TPM", y = "NSAF",
   color = 'IPAS', size = 1.5, # Points color, shape and size
   title='Plot of NSAF vs. TPM for regular proteins',
   xlab = 'Log2 (TPM + 1)',
   ylab = 'Log2 (NSAF + 1)') +
  scale_color_discrete(labels=paste('sample', 1:8)) +
   scale_x_continuous(name='Log2 (TPM + 1)', breaks=seq(0, 16, 2), limits = c(0, 16)) +
   scale_y_continuous(name='Log2 (NSAF + 1)', breaks=seq(0, 14, 2), limits = c(0, 14)) +
   theme(legend.text = element_text(size=10)) +
  # geom_vline(xintercept=0.1, linetype=2, color='red', size=0.5) +
  geom_hline(yintercept=2, linetype=2, color='red', size=0.5) +
  labs(color='Sample')
```




```
plt <- ggpubr::ggscatter(glmm_dat_non_missing_filtered, x = "TPM", y = "NSAF",
   color = 'IPAS', size = 1.5, # Points color, shape and size
   add = "reg.line",  # Add regressin line
   add.params = list(color = "blue", fill = "lightgray"), # Customize reg. line
   conf.int = TRUE, # Add confidence interval
   cor.coef = TRUE, # Add correlation coefficient. see ?stat_cor
   cor.coeff.args = list(method = "spearman", cor.coef.name = "rho",  label.x.npc=0.85, label.sep = "\n
                         label.y.npc = 0.2),
   title='Plot of NSAF vs. TPM for regular proteins with NSAF > 0 and TPM > 0',
   # subtitle='missing proteins on gene level in gastric cancer primary cell sample',
   xlab = 'Log2 (TPM + 1)',
   ylab = 'Log2 (NSAF + 1)') +
  scale_color_discrete(labels=paste('sample', 1:8)) +
   scale_x_continuous(name='Log2 (TPM + 1)', breaks=seq(0, 16, 2), limits = c(0, 16)) +
   scale_y_continuous(name='Log2 (NSAF + 1)', breaks=seq(0, 14, 2), limits = c(0, 14)) +
    theme(legend.text = element_text(size=10)) +
  labs(color='Sample')

dens1 <- ggplot(glmm_dat_non_missing_filtered, aes(x = TPM, fill = IPAS)) +
  geom_density(alpha = 0.4) +
  theme_void() +
  theme(legend.position = "none")

dens2 <- ggplot(glmm_dat_non_missing_filtered, aes(x = NSAF, fill = IPAS)) +
  geom_density(alpha = 0.4) +
  theme_void() +
  theme(legend.position = "none") +
```

```
  coord_flip()

dens1 + patchwork::plot_spacer() + plt + dens2 +  patchwork::plot_layout(ncol = 2, nrow = 2, widths = c
```

```
## `geom_smooth()` using formula 'y ~ x'
```

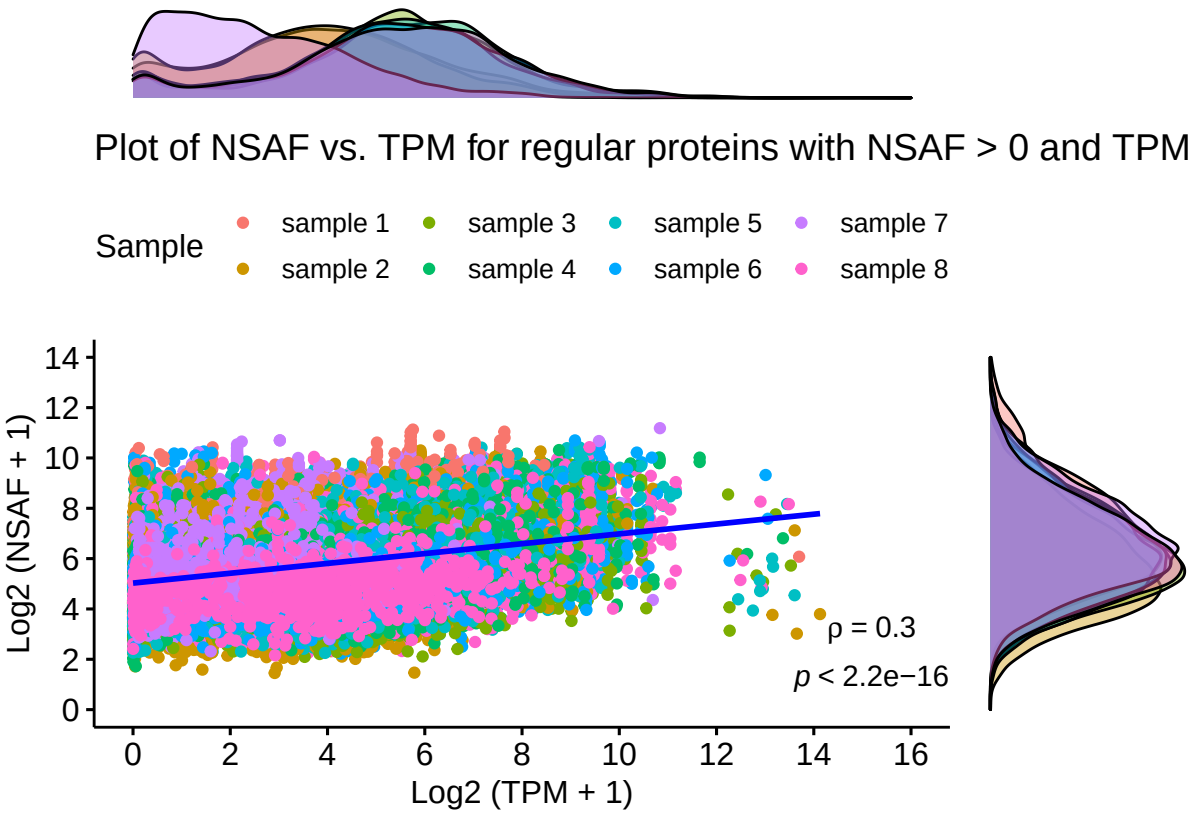


Figure 2: Heatmap for missing protein

Extract the 85 identified unique missing proteins Criterion: has both valid protein expression and RNA expression i.e. NASF > 0 & TPM > 0

```
# the number should be 85 if using two-SpC-rule
identified_MPs <- combined_long  %>% filter(NSAF > 0, TPM > 0, type=='MP') %>% arrange(accession) %>% g
# the details of the 85 identified missing proteins
identified_MPs
```

```
##  [1] "A0A075B6T7" "A0A087WT02" "A0A096LP55" "A0A0B4J273" "A0A1B0GVH4"
##  [6] "A4D2H0"     "A4QPB2"     "A6NFK2"     "A6NFN9"     "A6NH13"
## [11] "A6NHG4"     "A6NJJ6"     "A6NK53"     "A6PVI3"     "A8MTL9"
## [16] "A9Z1Z3"     "C9J202"     "E5RHQ5"     "E5RQL4"     "F2Z3F1"
## [21] "F8VTS6"     "O75678"     "P0DJD0"     "P0DKX4"     "P0DN37"
## [26] "P58512"     "P60606"     "Q3B8N5"     "Q52LC2"     "Q5RIA9"
## [31] "Q5T035"     "Q5T2Q4"     "Q5VX52"     "Q6IEE8"     "Q6JVE9"
## [36] "Q6PB30"     "Q6T423"     "Q6VEQ5"     "Q6ZVZ8"     "Q7RTU9"
```

```
## [41] "Q7RTY5"     "Q7Z572"     "Q7Z5M5"     "Q86UF2"     "Q86V71"
## [46] "Q8IXR9"     "Q8IZJ4"     "Q8IZJ6"     "Q8N431"     "Q8N4K4"
## [51] "Q8N5U1"     "Q8N8V8"     "Q8N9H9"     "Q8N9P6"     "Q8NEX6"
## [56] "Q8NH16"     "Q96A28"     "Q96KF2"     "Q96KX1"     "Q96NG8"
## [61] "Q9H158"     "Q9H3Y0"     "Q9NUZ1"     "Q9UIL4"     "Q9Y4R7"
## [66] "Q9Y6U7"     "U3KPV4"
```

```
identified_MPs_gene_symol <- combined_long  %>% filter(NSAF > 0, TPM > 0, type=='MP') %>% select(gene_sy
```

the PE (protein evidence) distribution of the identified missing proteins

```
missing_protein_df %>% filter(`Accession` %in% identified_MPs) %>% select(PE) %>% pull() %>% table()
```

```
## .
## Evidence at transcript level                   Predicted
##                           66                           1
```

Figure 2A)

Heatmap representations for quantified missing proteins in the eight gastric cancer primary cell samples. Color represents the level of protein expression, and values were log (NSAF +1) transformed

```
# double check clustering
library(pheatmap)
heatmap_dat <- log(gastric_nsaf[identified_MPs, ] + 1)
colnames(heatmap_dat) =paste('sample', 1:8)
pheatmap(heatmap_dat,
         show_rownames = T,
         angle_col=45,
         cluster_cols = T,
         cluster_rows = T)
```

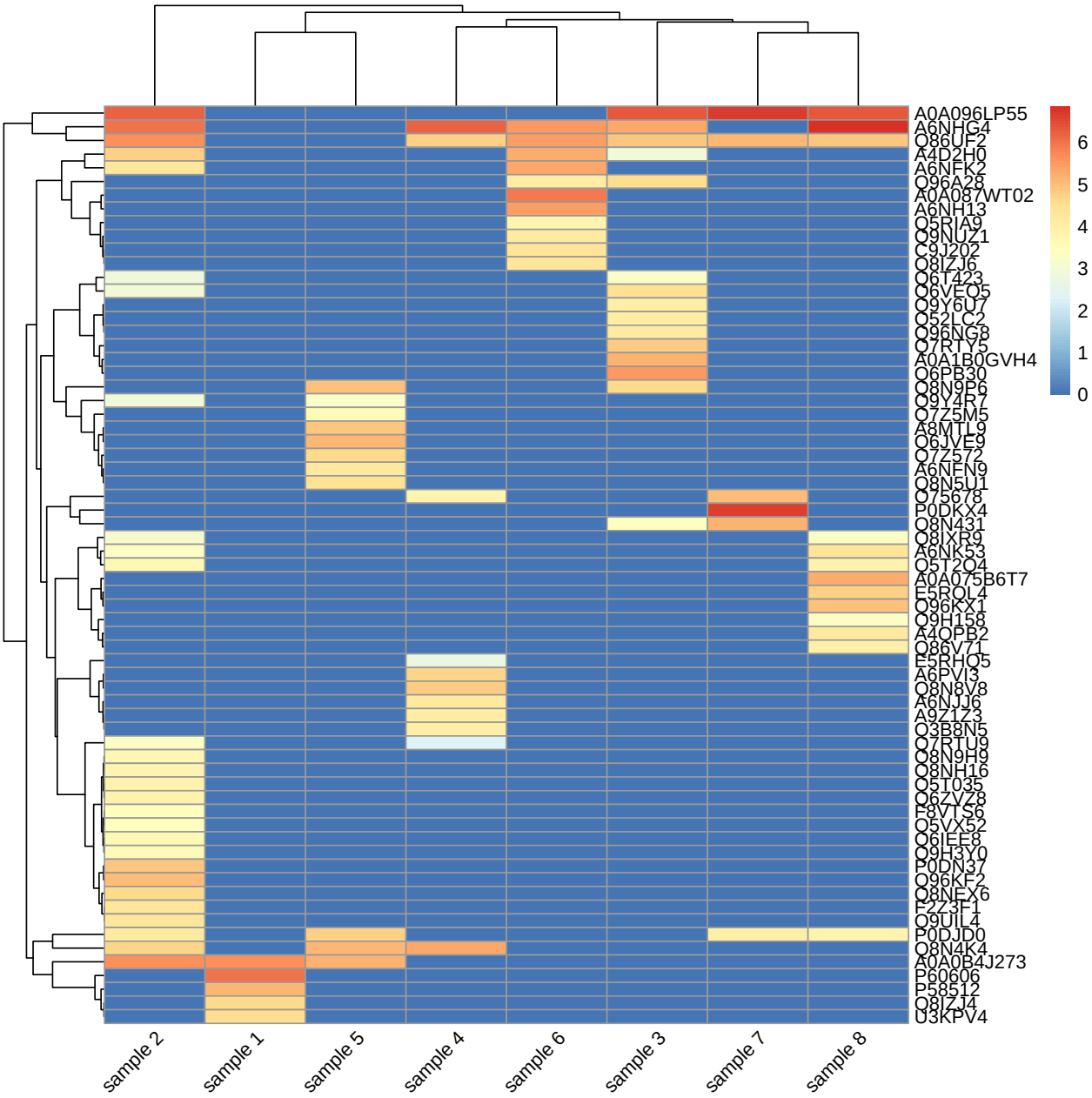
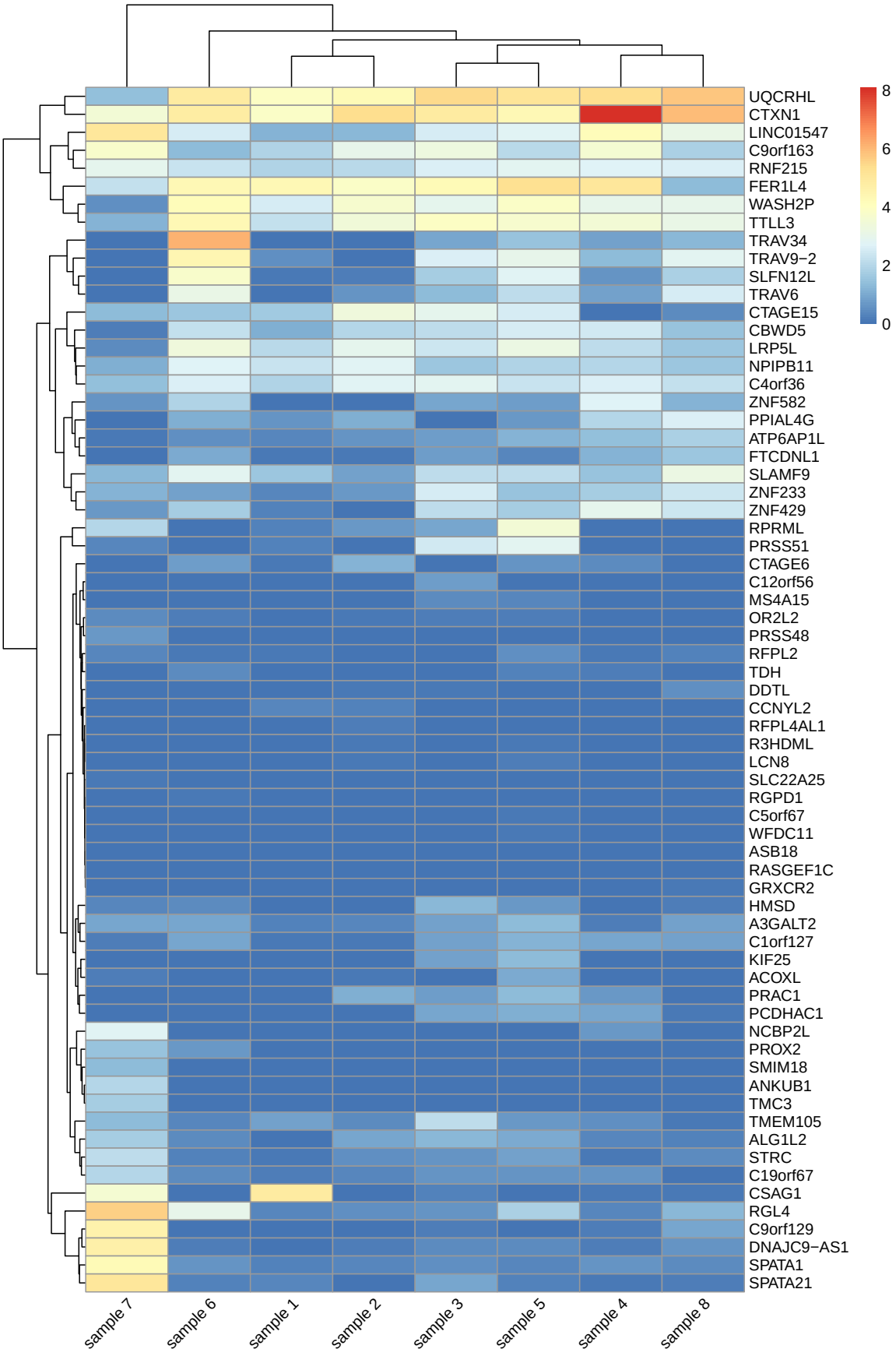


Figure 2 additional

Heatmap representations for quantified missing proteins in the eight gastric cancer primary cell samples. Color represents the level of mRNA expression, and values were log (TPM +1) transformed

```
heatmap_dat_TPM <- log(count_TPM_gene_symbol[identified_MPs_gene_symol, ] + 1)
colnames(heatmap_dat_TPM) =paste('sample', 1:8)
pheatmap(heatmap_dat_TPM,
         show_rownames = T,
         angle_col=45,
         cluster_cols = T,
         cluster_rows = T)
```


UQCRHL
CTXN1
LINC01547
C9orf163
RNF215
FER1L4
WASH2P
TTLL3
TRAV34
TRAV9−2
SLFN12L
TRAV6
CTAGE15
CBWD5
LRP5L
NPIPB11
C4orf36
ZNF582
PPIAL4G
ATP6AP1L
FTCDNL1
SLAMF9
ZNF233
ZNF429
RPRML
PRSS51
CTAGE6
C12orf56
MS4A15
OR2L2
PRSS48
RFPL2
TDH
DDTL
CCNYL2
RFPL4AL1
R3HDML
LCN8
SLC22A25
RGPD1
C5orf67
WFDC11
ASB18
RASGEF1C
GRXCR2
HMSD
A3GALT2
C1orf127
KIF25
ACOXL
PRAC1
PCDHAC1
NCBP2L
PROX2
SMIM18
ANKUB1
TMC3
TMEM105
ALG1L2
STRC
C19orf67
CSAG1
RGL4
C9orf129
DNAJC9−AS1
SPATA1
SPATA21
sample 7
sample 6
sample 1
sample 2
sample 3
sample 5
sample 4
sample 8
8
6
4
2
0

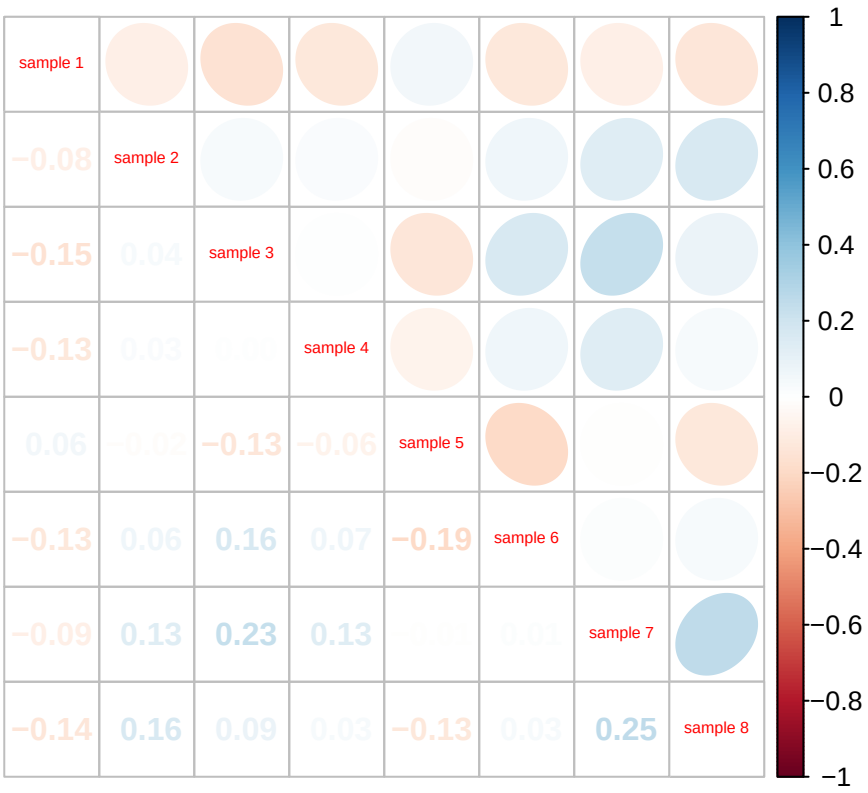
Checking Sample heterogeneity

Check whether RNA expression, protein expression, and protein-RNA-expression relationship were similar between samples ## correlation plot for pairwise correlation of missing protein expression

```
library(corrplot)
```

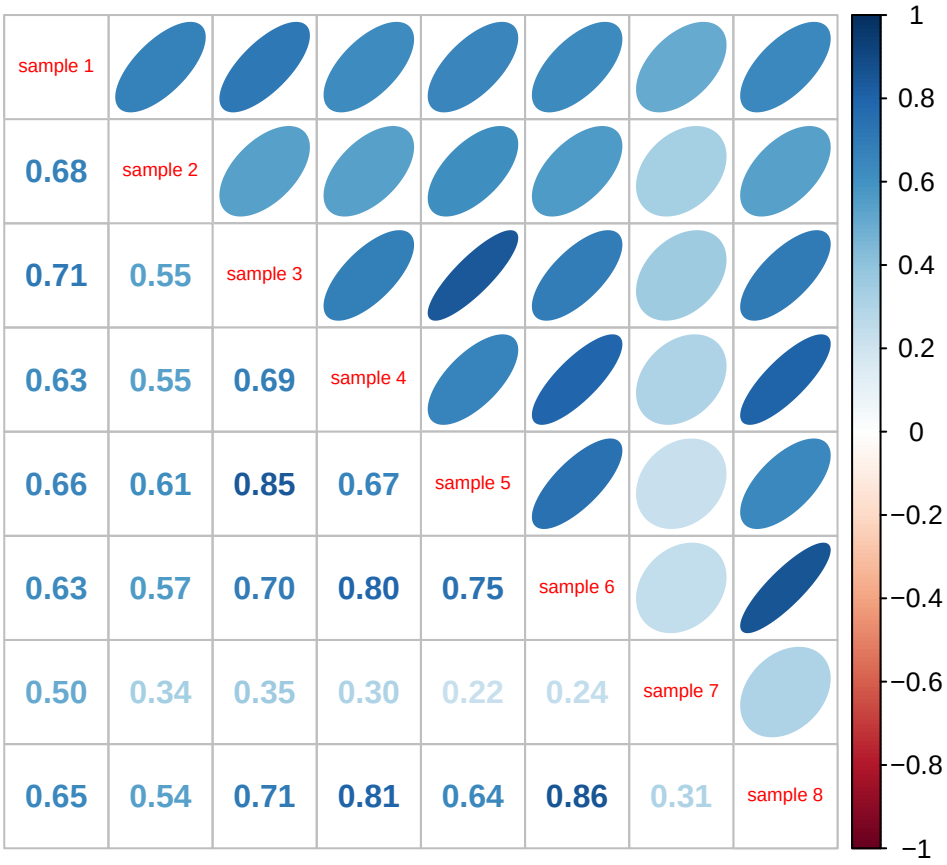
```
## corrplot 0.92 loaded
```

```
corr <- cor(heatmap_dat, method = "spearman")
corrplot.mixed(corr, lower = 'number', upper='ellipse', tl.cex=0.5,
               na.label.col='black',
               mar=c(0,0,2,0))
```



correlation plot for pairwise correlation of missing protein gene expression

```
corr <- cor(heatmap_dat_TPM, method = "spearman")
corrplot.mixed(corr, lower = 'number', upper='ellipse', tl.cex=0.6, na.label.col='black')
```


sample 1
0.68
sample 2
0.71
0.55
sample 3
0.63
0.55
0.69
sample 4
0.66
0.61
0.85
0.67
sample 5
0.63
0.57
0.70
0.80
0.75
sample 6
0.50
0.34
0.35
0.30
0.22
0.24
sample 7
0.65
0.54
0.71
0.81
0.64
0.86
0.31
sample 8
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1