

Statistical Methods for Quantitative MS-based Proteomics: Part II. Differential Abundance Analysis

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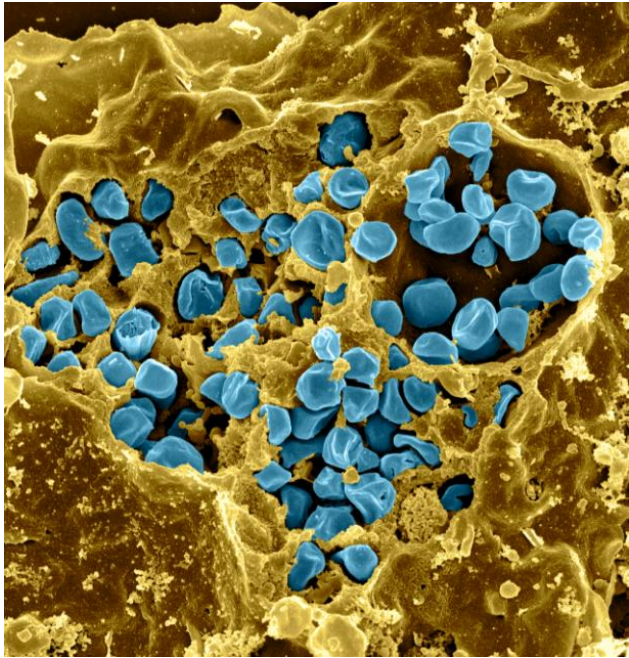
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Outline

- Francisella tularensis Example
- Hypothesis testing
- Multiple testing
- Moderated statistics
- Experimental design

Note, that the R-code is included for learners who are aiming to develop R/markdown scripts to automate their quantitative proteomics data analyses. According to the target audience of the course we either work with a graphical user interface (GUI) in a R/shiny App msqrob2gui (e.g. Proteomics Bioinformatics course of the EBI and the Proteomics Data Analysis course at the Gulbenkian institute) or with R/markdowns scripts (e.g. Bioinformatics Summer School at UCLouvain or the Statistical Genomics Course at Ghent University).

1 *Francisella tularensis* experiment



- Pathogen: causes tularemia
- Metabolic adaptation key for intracellular life cycle of pathogenic microorganisms.
- Upon entry into host cells quick phagosomal escape and active multiplication in cytosolic compartment.
- *Francisella* is auxotroph for several amino acids, including arginine.
- Inactivation of arginine transporter delayed bacterial phagosomal escape and intracellular multiplication.
- Experiment to assess difference in proteome using 3 WT vs 3 ArgP KO mutants

1.1 Import the data in R

[Click to see code](#)

1. Load libraries

```
library(tidyverse)
library(limma)
library(QFeatures)
library(msqrob2)
library(plotly)
library(ggplot2)
```

2. We use a peptides.txt file from MS-data quantified with maxquant that contains MS1 intensities summarized at the peptide level.

```
peptidesFile <- "https://raw.githubusercontent.com/statOmics/PDA20/data/quantification/francisella/pept."
```

3. Maxquant stores the intensity data for the different samples in columns that start with Intensity. We can retrieve the column names with the intensity data with the code below:

```
ecols <- grep("Intensity\\.", names(read.delim(peptidesFile)))
```

4. Read the data and store it in QFeatures object

```
pe <- readQFeatures(
  table = peptidesFile,
  fnames = 1,
  ecol = ecols,
  name = "peptideRaw", sep="\t")
```

5. Update data with information on design

```
colData(pe)$genotype <- pe[[1]] %>%
  colnames %>%
  substr(12,13) %>%
  as.factor %>%
  relevel("WT")
pe %>% colData
```

```
## DataFrame with 6 rows and 1 column
##               genotype
##               <factor>
## Intensity.1WT_20_2h_n3_3      WT
## Intensity.1WT_20_2h_n4_3      WT
## Intensity.1WT_20_2h_n5_3      WT
## Intensity.3D8_20_2h_n3_3      D8
## Intensity.3D8_20_2h_n4_3      D8
## Intensity.3D8_20_2h_n5_3      D8
```

1.2 Preprocessing

Click to see code to log-transform the data

1. Log transform

- Calculate number of non zero intensities for each peptide

```
rowData(pe[["peptideRaw"]])$nNonZero <- rowSums(assay(pe[["peptideRaw"]]) > 0)
```

- Peptides with zero intensities are missing peptides and should be represented with a NA value rather than 0.

```
pe <- zeroIsNA(pe, "peptideRaw") # convert 0 to NA
```

- Logtransform data with base 2

```
pe <- logTransform(pe, base = 2, i = "peptideRaw", name = "peptideLog")
```

2. Filtering

- Handling overlapping protein groups

```
pe[["peptideLog"]] <-  
  pe[["peptideLog"]][rowData(pe[["peptideLog"]])$Proteins  
  %in% smallestUniqueGroups(rowData(pe[["peptideLog"]])$Proteins),]
```

- Remove reverse sequences (decoys) and contaminants. Note that this is indicated by the column names Reverse and depending on the version of maxQuant with Potential.contaminants or Contaminants.

```
pe[["peptideLog"]] <- pe[["peptideLog"]][rowData(pe[["peptideLog"]])$Reverse != "+", ]  
pe[["peptideLog"]] <- pe[["peptideLog"]][rowData(pe[["peptideLog"]])$  
  Contaminant != "+", ]
```

- Drop peptides that were only identified in one sample

```
pe[["peptideLog"]] <- pe[["peptideLog"]][rowData(pe[["peptideLog"]])$nNonZero >= 2, ]  
nrow(pe[["peptideLog"]])
```

```
## [1] 6525
```

We keep 6525 peptides upon filtering.

3. Normalization by median centering

```
pe <- normalize(pe,  
  i = "peptideLog",  
  name = "peptideNorm",  
  method = "center.median")
```

4. Summarization. We use the standard summarisation in aggregateFeatures, which is a robust summarisation method.

```
pe <- aggregateFeatures(pe,  
  i = "peptideNorm",  
  fcol = "Proteins",  
  na.rm = TRUE,  
  name = "protein")
```

```
## Your quantitative and row data contain missing values. Please read the  
## relevant section(s) in the aggregateFeatures manual page regarding the  
## effects of missing values on data aggregation.
```

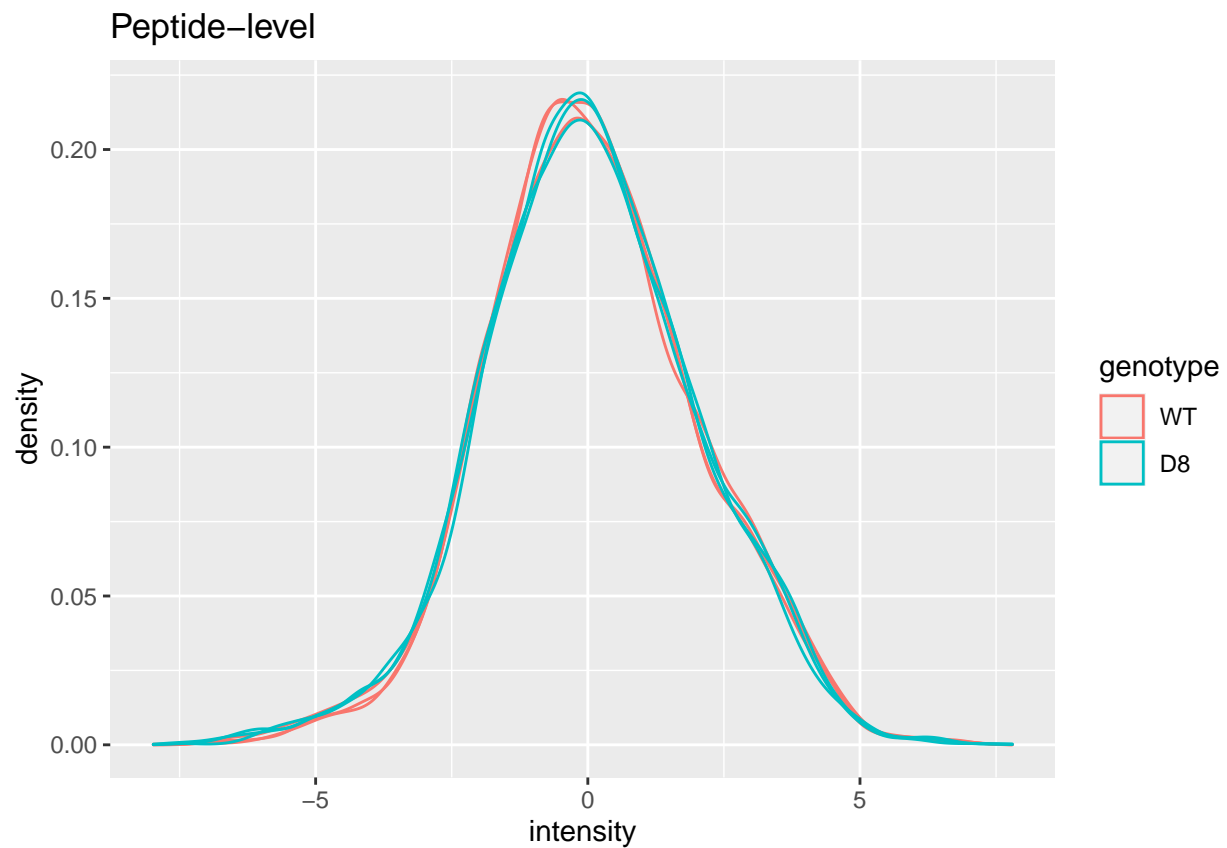
Plot of preprocessed data

```

pe[["peptideNorm"]] %>%
  assay %>%
  as.data.frame() %>%
  gather(sample, intensity) %>%
  mutate(genotype = colData(pe)[sample,"genotype"]) %>%
  ggplot(aes(x = intensity,group = sample,color = genotype)) +
    geom_density() +
    ggtitle("Peptide-level")

```

Warning: Removed 7561 rows containing non-finite values (stat_density).

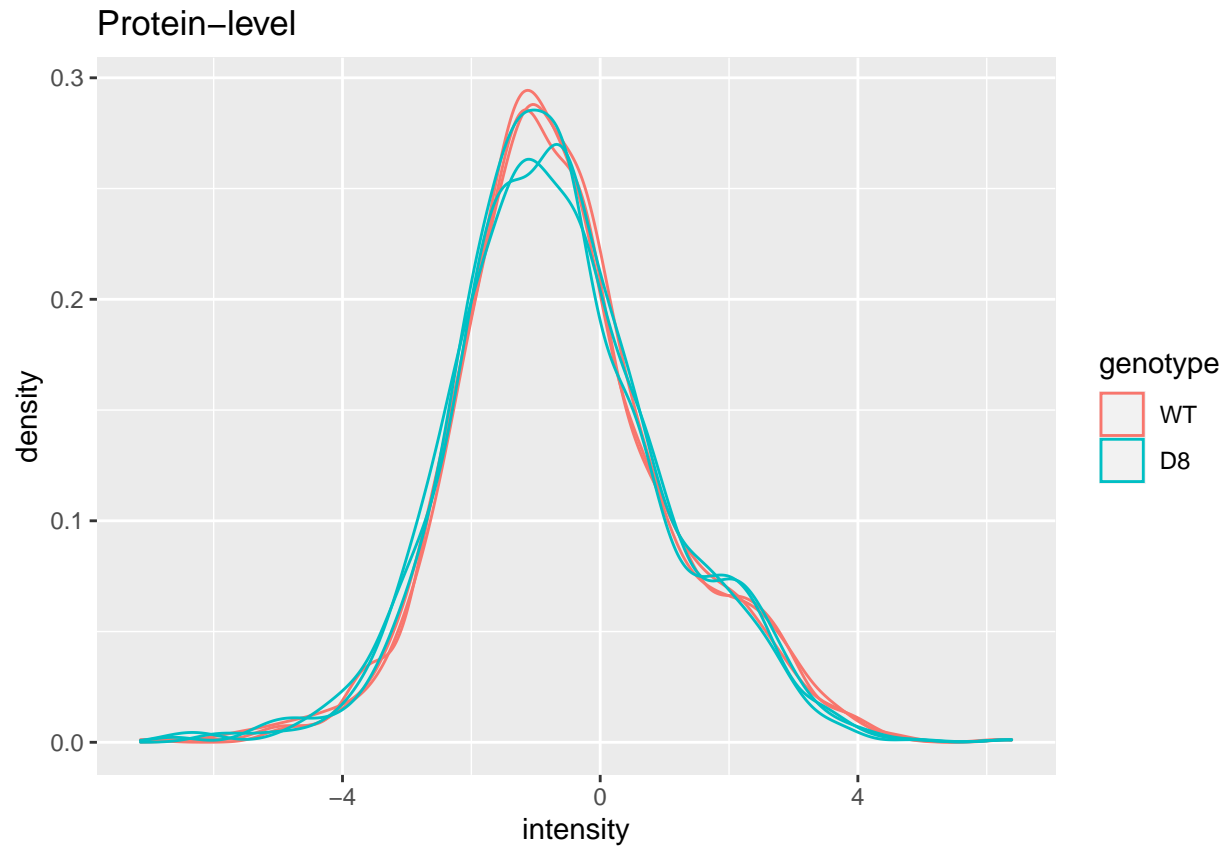


```

pe[["protein"]] %>%
  assay %>%
  as.data.frame() %>%
  gather(sample, intensity) %>%
  mutate(genotype = colData(pe)[sample,"genotype"]) %>%
  ggplot(aes(x = intensity,group = sample,color = genotype)) +
    geom_density() +
    ggtitle("Protein-level")

```

Warning: Removed 428 rows containing non-finite values (stat_density).



1.3 Summarized data structure

1.3.1 Design

```
pe %>%
  colData %>%
  knitr::kable()
```

	genotype
Intensity.1WT_20_2h_n3_3	WT
Intensity.1WT_20_2h_n4_3	WT
Intensity.1WT_20_2h_n5_3	WT
Intensity.3D8_20_2h_n3_3	D8
Intensity.3D8_20_2h_n4_3	D8
Intensity.3D8_20_2h_n5_3	D8

- WT vs KO
- 3 vs 3 repeats

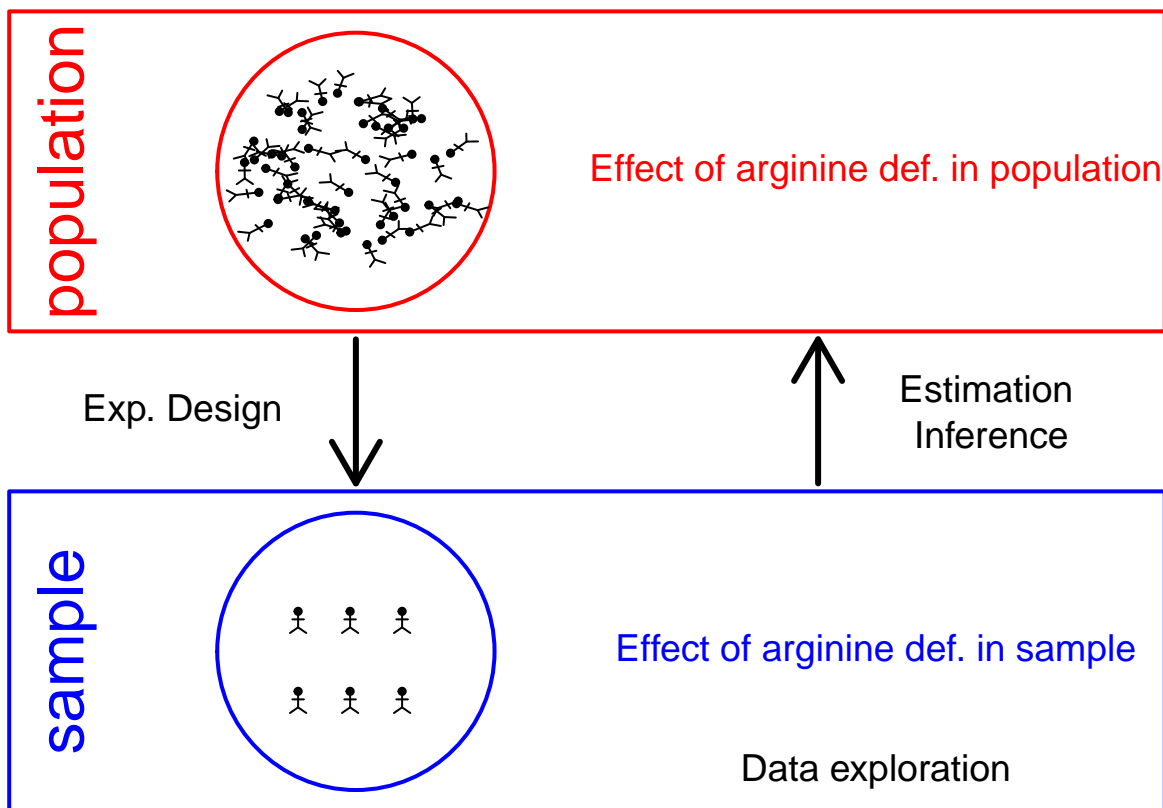
1.3.2 Summarized intensity matrix

```
pe[["protein"]] %>% assay() %>% head() %>% knitr::kable()
```

	Intensity.1WT_20_2h_n5_3	Intensity.1WT_20_2h_n5_3	Intensity.1WT_20_2h_n5_3	Intensity.1WT_20_2h_n5_3	Intensity.1WT_20_2h_n5_3	Intensity.1WT_20_2h_n5_3
WP_003013027	48775	-0.0856247	0.1595370	-0.2809009	0.0035526	0.0567110
WP_003013860	NA	NA	-0.2512039	NA	NA	-0.4865646
WP_003013908	51118	-0.8161658	-0.7557906	-0.4591476	-0.5449424	-0.4962482
WP_003014068	95386	0.8522239	1.1344852	0.5459176	0.9187714	0.5974741
WP_003014077	30863	-1.0430741	-0.8091715	-1.1743951	-1.1924725	-1.2565893
WP_003014089	51672	-0.3361704	-0.2151930	-0.3855747	-0.2802011	-0.5801771

- 1115 proteins

1.3.3 Hypothesis testing: a single protein



1.3.3.1 T-test

$$\log_2 \text{FC} = \bar{y}_{p1} - \bar{y}_{p2}$$

$$T_g = \frac{\log_2 \text{FC}}{\text{se}_{\log_2 \text{FC}}}$$

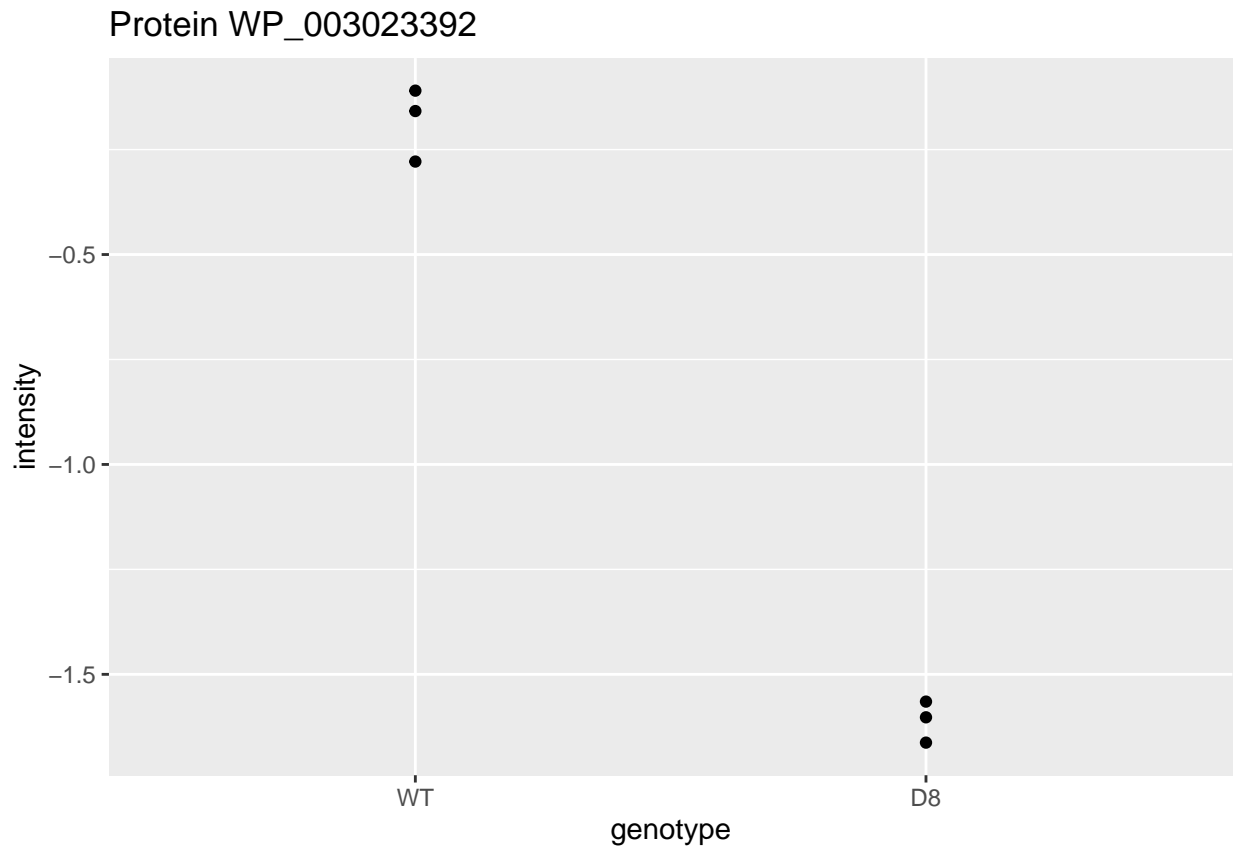
$$T_g = \frac{\widehat{\text{signal}}}{\widehat{\text{Noise}}}$$

If we can assume equal variance in both treatment groups:

$$\text{se}_{\log_2 \text{FC}} = \text{SD} \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

```
WP_003023392 <- data.frame(
  intensity = assay(pe[["protein"]][["WP_003023392"],]) %>% c(),
  genotype = colData(pe)[,1])

WP_003023392 %>%
  ggplot(aes(x=genotype,y=intensity)) +
  geom_point() +
  ggtitle("Protein WP_003023392")
```



$$t = \frac{\log_2 \widehat{\text{FC}}}{\text{se}_{\log_2 \widehat{\text{FC}}}} = \frac{-1.43}{0.0577} = -24.7$$

- Is $t = -24.7$ indicating that there is an effect?
- How likely is it to observe $t = -24.7$ when there is no effect of the argP KO on the protein expression?

1.3.3.2 Null hypothesis (H_0) and alternative hypothesis (H_1)

- With data we can never prove a hypothesis (falsification principle of Popper)
- With data we can only reject a hypothesis
- In general we start from *alternative hypothesis* H_1 : we want to show an effect of the KO on a protein

H_1 : On average the protein abundance in WT is different from that in KO

- But, we will assess this by falsifying the opposite:
 H_0 : On average the protein abundance in WT is equal to that in KO

```
t.test(intensity ~ genotype, data = WP_003023392, var.equal=TRUE)
```

```
##
## Two Sample t-test
##
## data: intensity by genotype
## t = 24.747, df = 4, p-value = 1.582e-05
## alternative hypothesis: true difference in means between group WT and group D8 is not equal to 0
## 95 percent confidence interval:
##  1.267666 1.588058
## sample estimates:
## mean in group WT mean in group D8
##      -0.1821147      -1.6099769
```

- How likely is it to observe an equal or more extreme effect than the one observed in the sample when the null hypothesis is true?
- When we make assumptions about the distribution of our test statistic we can quantify this probability: *p-value*. The p-value will only be calculated correctly if the underlying assumptions hold!
- When we repeat the experiment, the probability to observe a fold change for this gene that is more extreme than a 2.69 fold ($\log_2 FC = -1.43$) down or up regulation by random change (if H_0 is true) is `round(summary(lmHlp)$coef[2,4]*1e6,0)` out of 1 000 000.
- If the p-value is below a significance threshold α we reject the null hypothesis. *We control the probability on a false positive result at the α -level (type I error)*
- Note, that the p-values are uniform under the null hypothesis, i.e. when H_0 is true all p-values are equally likely.

1.4 Multiple hypothesis testing

- Consider testing DA for all $m = 1115$ proteins simultaneously
- What if we assess each individual test at level α ? \rightarrow Probability to have a false positive among all m simultaneous test $\gg \alpha = 0.05$
- Suppose that 800 proteins are non-DA, then we could expect to discover on average $800 \times 0.05 = 40$ false positive proteins. Hence, we are bound to call false positive proteins each time we run the experiment.

1.4.1 Multiple testing

When we want to infer on differential abundance of m multiple proteins we have to address the multiple testing issue.

The table shows the results of m hypothesis tests in a single experiment.

	accept H_{0i}	reject H_{0i}	Total
null	TN	FP	m_0
non-null	FN	TP	m_1
Total	NR	R	m

- TN : number of true negative: random and unobserved
- FP : number of false positives: random and unobserved
- FN : number of false negatives: random and unobserved
- TP : number of true positives: random and unobserved
- NR : number of acceptances (negative results): random and observed
- R : number of rejections (positive results): random and observed
- m_0 and m_1 : fixed and unobserved
- m : fixed and observed

1.4.1.1 Family-wise error rate The family-wise error rate (FWER) addresses the multiple testing issue by no longer controlling the individual type I error for each protein, instead it controls:

$$\text{FWER} = P[FP \geq 1].$$

The Bonferroni method is widely used to control the type I error:

- assess each test at

$$\alpha_{\text{adj}} = \frac{\alpha}{m}$$

- or use adjusted p-values and compare them to α :

$$p_{\text{adj}} = \min(p \times m, 1)$$

Problem, the method is very conservative!

1.4.1.2 False discovery rate

- FDR: Expected proportion of false positives on the total number of positives you return.
- An FDR of 1% means that on average we expect 1% false positive proteins in the list of proteins that are called significant.
- Defined by Benjamini and Hochberg in their seminal paper Benjamini, Y. and Hochberg, Y. (1995). "Controlling the false discovery rate: a practical and powerful approach to multiple testing". Journal of the Royal Statistical Society Series B, 57 (1): 289–300.

The **False Discovery Proportion (FDP)** is the fraction of false positives that are returned, i.e.

$$\text{FDP} = \frac{FP}{R}$$

However, this quantity cannot be observed because in practice we only know R but we do not know FP .

Therefore, Benjamini and Hochberg, 1995, defined The **False Discovery Rate (FDR)** as

$$\text{FDR} = \mathbb{E} \left[\frac{FP}{R} \right] = \mathbb{E}[\text{FDP}]$$

the expected FDP.

- Controlling the FDR allows for more discoveries (i.e. longer lists with significant results), while the fraction of false discoveries among the significant results is well controlled on average. As a consequence, more of the true positive hypotheses will be detected.

The Benjamini and Hochberg (1995) procedure for controlling the FDR at α :

1. Let $p_{(1)} \leq \dots \leq p_{(m)}$ denote the ordered p -values.
2. Let $k = \max\{i : p_{(i)} \leq i\alpha/m\}$, i.e. k is the largest integer so that $p_{(k)} \leq k\alpha/m$.
3. If such a k exists, reject the k null hypotheses associated with $p_{(1)}, \dots, p_{(k)}$. Otherwise none of the null hypotheses is rejected.

The adjusted p -value (also known as the q -value in FDR literature):

$$q_{(i)} = \tilde{p}_{(i)} = \min \left[\min_{j=i, \dots, m} (mp_{(j)}/j), 1 \right].$$

Click to see code

```
ttestMx <- function(y,group) {
  test <- try(t.test(y[group], y[!group], var.equal=TRUE), silent=TRUE)
  if(is(test, "try-error")) {
    return(c(log2FC=NA, se=NA, tstat=NA, p=NA))
  } else {
    return(c(log2FC= (test$estimate*%*%c(1,-1)), se=test$stderr, tstat=test$statistic, pval=test$p.value))
  }
}

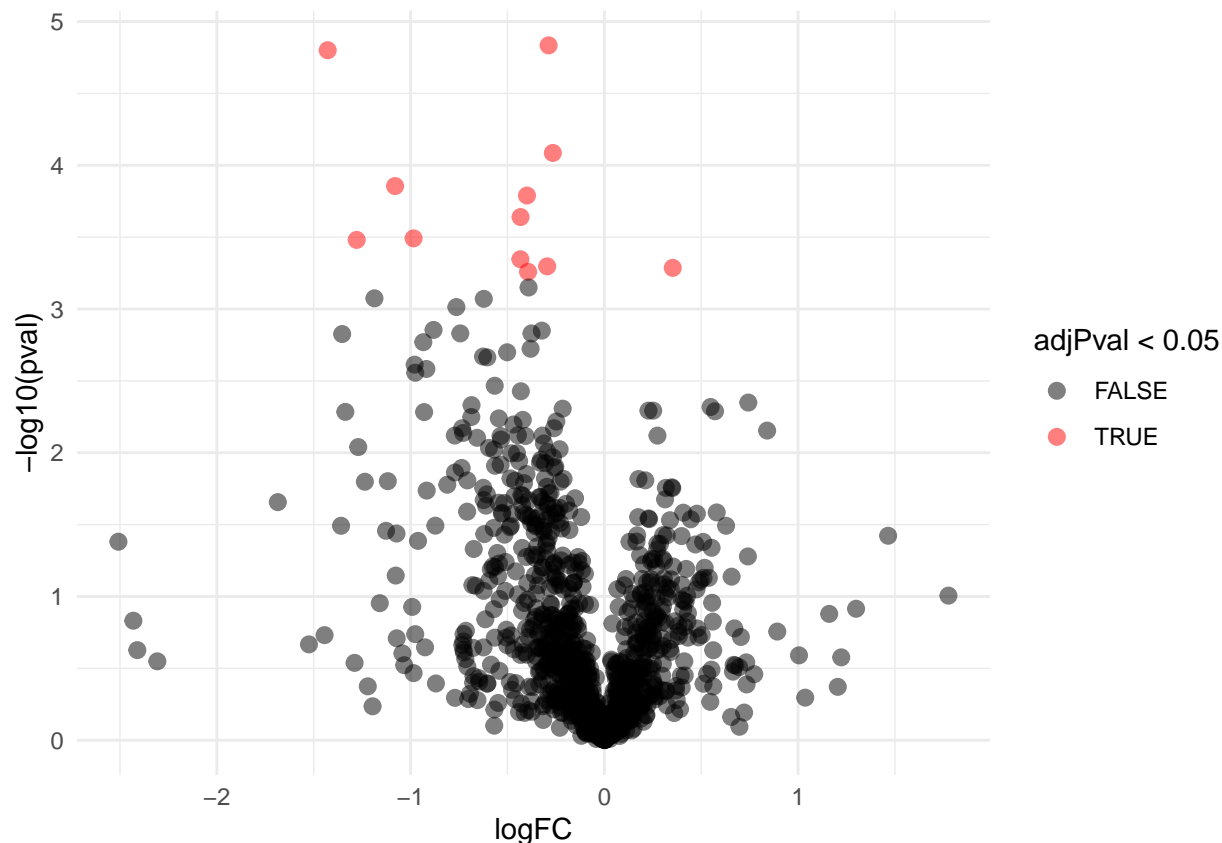
res <- apply(
  assay(pe[["protein"]]),
  1,
  ttestMx,
  group = colData(pe)$genotype=="D8") %>%
  t
colnames(res) <- c("logFC", "se", "tstat", "pval")
res <- res %>% as.data.frame %>% na.exclude %>% arrange(pval)
res$adjPval <- p.adjust(res$pval, "fdr")
alpha <- 0.05
res$adjAlphaForm <- paste0(1:nrow(res), " x ", alpha, "/", nrow(res))
res$adjAlpha <- alpha * (1:nrow(res))/nrow(res)
res$"pval < adjAlpha" <- res$pval < res$adjAlpha
res$"adjPval < alpha" <- res$adjPval < alpha
```

	logFC	pval	adjPval	adjAlphaForm	adjAlpha	pval < adjAlpha	adjPval < alpha
WP_003038940	-	0.0000146	0.0084347	1 x	0.0000469	TRUE	TRUE
	0.2876290			0.05/1066			
WP_003023392	-	0.0000158	0.0084347	2 x	0.0000938	TRUE	TRUE
	1.4278622			0.05/1066			
WP_003039212	-	0.0000820	0.0291520	3 x	0.0001407	TRUE	TRUE
	0.2658247			0.05/1066			
WP_003026016	-	0.0001395	0.0346124	4 x	0.0001876	TRUE	TRUE
	1.0800305			0.05/1066			
WP_003039615	-	0.0001623	0.0346124	5 x	0.0002345	TRUE	TRUE
	0.3992190			0.05/1066			
WP_011733588	-	0.0002291	0.0407034	6 x	0.0002814	TRUE	TRUE
	0.4323262			0.05/1066			
WP_003014552	-	0.0003224	0.0440266	7 x	0.0003283	TRUE	TRUE
	0.9843865			0.05/1066			
WP_003040849	-	0.0003304	0.0440266	8 x	0.0003752	TRUE	TRUE
	1.2780743			0.05/1066			
WP_003038430	-	0.0004505	0.0489078	9 x	0.0004221	FALSE	TRUE
	0.4331987			0.05/1066			
WP_003033975	-	0.0005047	0.0489078	10 x	0.0004690	FALSE	TRUE
	0.2949061			0.05/1066			
WP_011733643	-	0.0005171	0.0489078	11 x	0.0005159	FALSE	TRUE
	0.3531405			0.05/1066			
WP_011733723	-	0.0005506	0.0489078	12 x	0.0005629	TRUE	TRUE
	0.3935768			0.05/1066			
WP_003038679	-	0.0007083	0.0580821	13 x	0.0006098	FALSE	FALSE
	0.3909725			0.05/1066			
WP_003033719	-	0.0008426	0.0603810	14 x	0.0006567	FALSE	FALSE
	1.1865453			0.05/1066			

Click to see code

```
volcanoT <- res %>%
  ggplot(aes(x = logFC, y = -log10(pval), color = adjPval < 0.05)) +
  geom_point(cex = 2.5) +
  scale_color_manual(values = alpha(c("black", "red"), 0.5)) +
  theme_minimal()
```

volcanoT



1.4.1.3 Intuition of BH-FDR procedure Consider $m = 10,000$ tests

- Suppose that a researcher rejects all null hypotheses for which $p < 0.001$.
- If we use $p < 0.001$, we expect $0.001 \times m_0$ tests to return false positives. A conservative estimate of the number of false positives that we can expect can be obtained by considering that the null hypotheses are true for all features, $m_0 = m = 10000$. We then would expect $0.001 \times 10,000 = 10$ false positives ($FP = 10$).
- Suppose that the researcher found 200 genes with $p < 0.001$ ($R = 200$).
- The proportion of false positive results (FDP = false positive proportion) among the list of $R = 200$ genes can then be estimated as

$$\widehat{FDP} = \frac{FP}{R} = \frac{10}{200} = \frac{0.001 \times 10000}{200} = 0.05.$$

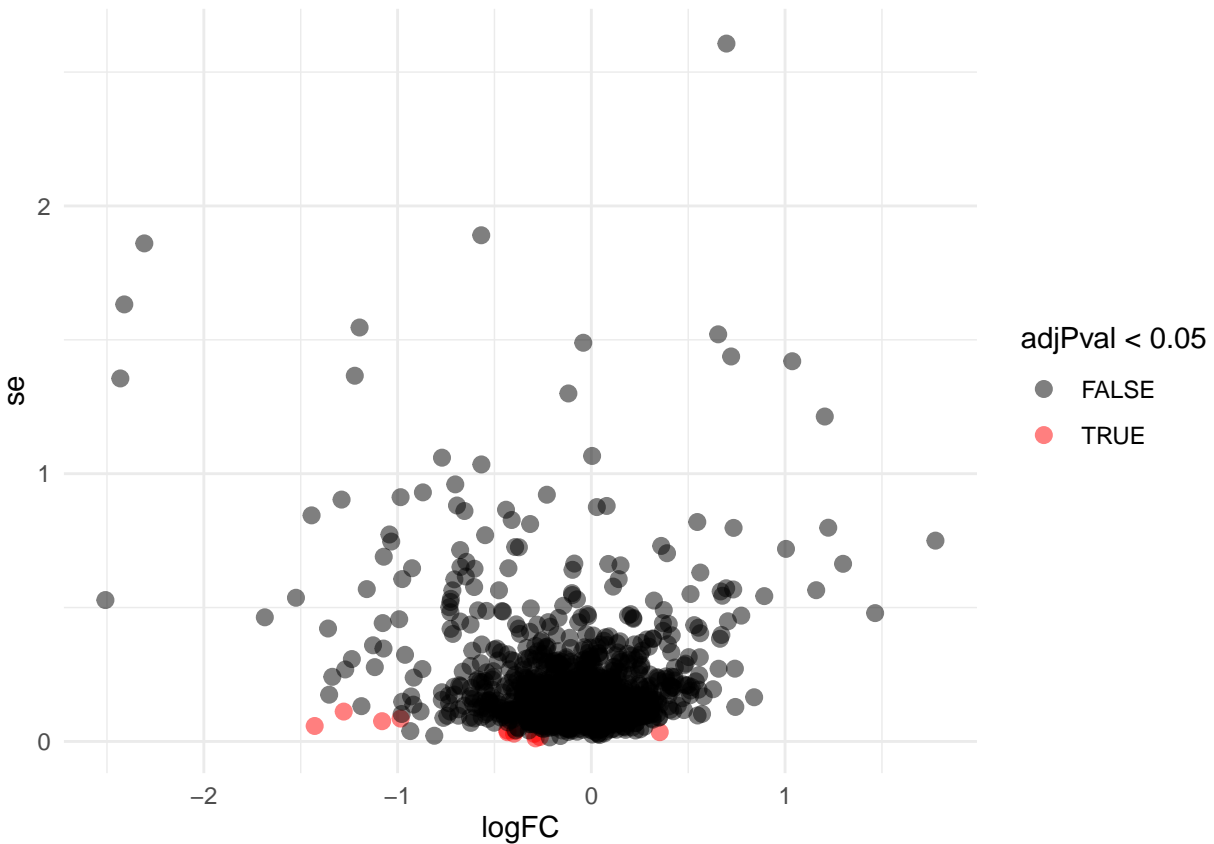
Recall that the B&H (1995) procedure involves finding the largest integer k so that $p_{(k)} \leq k\alpha/m$, or, equivalently, $p_{(k)}m/k \leq \alpha$.

In this example: $k = 200$, $p_{(k)} = 0.001$, $m = 10,000$ and $\alpha = 0.05$.

1.5 Moderated Statistics

Problems with ordinary t-test

```
res %>%
  ggplot(aes(x = logFC, y = se, color = adjPval < 0.05)) +
  geom_point(cex = 2.5) +
  scale_color_manual(values = alpha(c("black", "red"), 0.5)) +
  theme_minimal()
```



A general class of moderated test statistics is given by

$$T_g^{mod} = \frac{\bar{Y}_{g1} - \bar{Y}_{g2}}{C \tilde{S}_g},$$

where \tilde{S}_g is a moderated standard deviation estimate.

- C is a constant depending on the design e.g. $\sqrt{1/n_1 + 1/n_2}$ for a t-test and of another form for linear models.
- $\tilde{S}_g = S_g + S_0$: add small positive constant to denominator of t-statistic.
- This can be adopted in Perseus.

Click to see code

```
simI<-sapply(res$se/sqrt(1/3+1/3),function(n,mean,sd) rnorm(n,mean,sd),n=6,mean=0) %>% t
resSim <- apply(
  simI,
  1,
  ttestMx,
```

```

    group = colData(pe)$genotype=="D8") %>%
  t
colnames(resSim) <- c("logFC","se","tstat","pval")
resSim <- as.data.frame(resSim)
tstatSimPlot <- resSim %>%
  ggplot(aes(x=tstat)) +
    geom_histogram(aes(y=..density.., fill=..count..),bins=30) +
    stat_function(fun=dt,
      color="red",
      args=list(df=4)) +
  ylim(0,.6) +
  ggtitle("t-statistic")

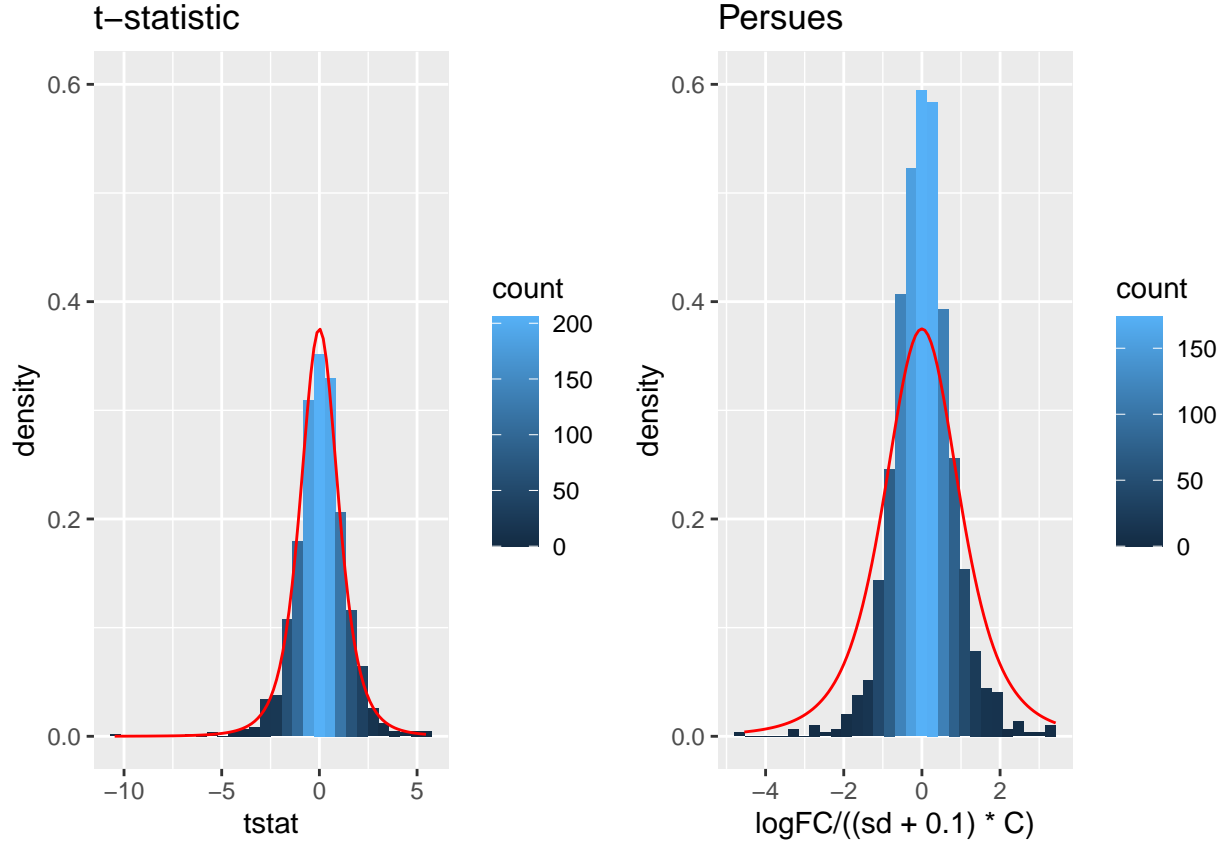
resSim$C <- sqrt(1/3+1/3)
resSim$sd <- resSim$se/resSim$C
tstatSimPerseus <- resSim %>%
  ggplot(aes(x=logFC/((sd+.1)*C))) +
    geom_histogram(aes(y=..density.., fill=..count..),bins=30) +
    stat_function(fun=dt,
      color="red",
      args=list(df=4)) +
  ylim(0,.6) +
  ggtitle("Persues")

```

```

gridExtra::grid.arrange(tstatSimPlot,tstatSimPerseus,nrow=1)

```



- The choice of S_0 in Perseus is ad hoc and the t-statistic is no-longer t-distributed. - Permutation test, but is difficult for more complex designs. - Allows for Data Dredging because user can choose S_0

1.5.1 Empirical Bayes

A general class of moderated test statistics is given by

$$T_g^{mod} = \frac{\bar{Y}_{g1} - \bar{Y}_{g2}}{C \tilde{S}_g},$$

where \tilde{S}_g is a moderated standard deviation estimate.

- **empirical Bayes** theory provides formal framework for borrowing strength across proteins,
- Implemented in popular bioconductor package **limma** and **msqrob2**

$$\tilde{S}_g = \sqrt{\frac{d_g S_g^2 + d_0 S_0^2}{d_g + d_0}},$$

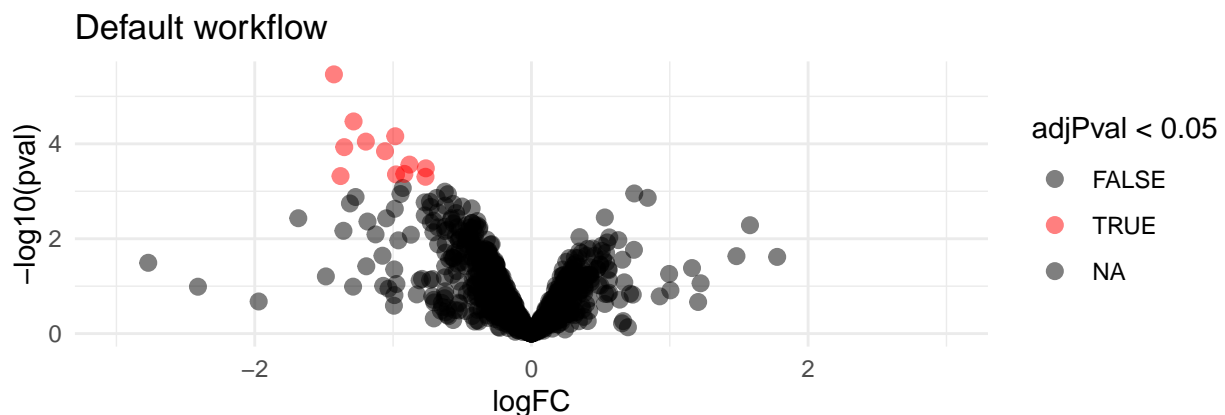
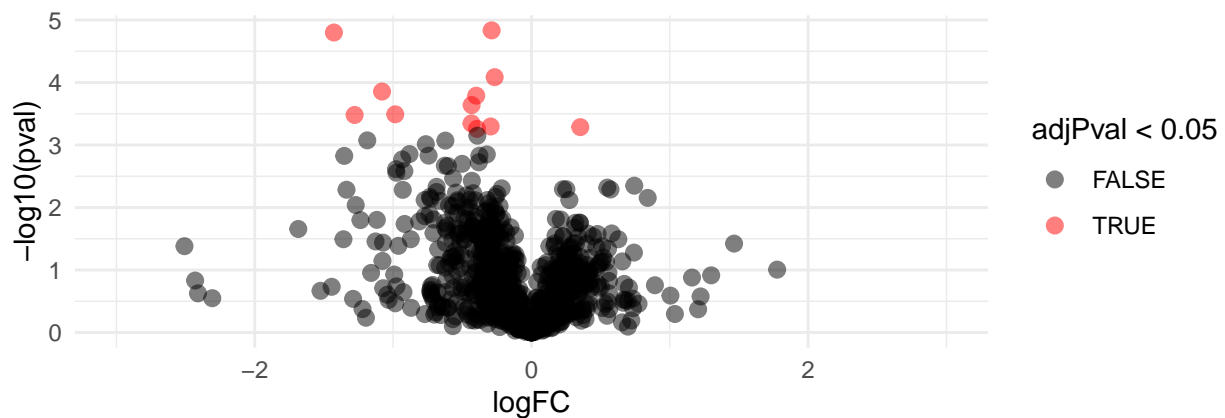
- S_0^2 : common variance (over all proteins)
- Moderated t-statistic is t-distributed with $d_0 + d_g$ degrees of freedom.
- Note that the degrees of freedom increase by borrowing strength across proteins!


```
pe <- msqrob(object = pe, i = "protein", formula = ~genotype)
L <- makeContrast("genotypeD8 = 0", parameterNames = c("genotypeD8"))
pe <- hypothesisTest(object = pe, i = "protein", contrast = L)
```

```
volcano <- ggplot(
  rowData(pe[["protein"]])$genotypeD8,
  aes(x = logFC, y = -log10(pval), color = adjPval < 0.05)
) +
  geom_point(cex = 2.5) +
  scale_color_manual(values = alpha(c("black", "red"), 0.5)) +
  theme_minimal() +
  ggtitle("Default workflow")
```

```
gridExtra::grid.arrange(volcanoT + xlim(-3,3)
,volcano + xlim(-3,3)
,nrow=2)
```

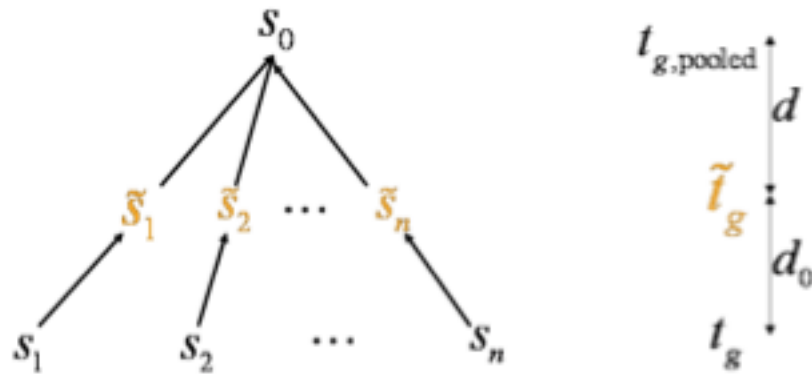
Warning: Removed 109 rows containing missing values (geom_point).



- The volcano plot opens up when using the EB variance estimator
- Borrowing strength to estimate the variance using empirical Bayes solves the issue of returning proteins with a low fold change as significant due to a low variance.

1.5.2 Shrinkage of the variance and moderated t-statistics

Shrinkage of Standard Deviations



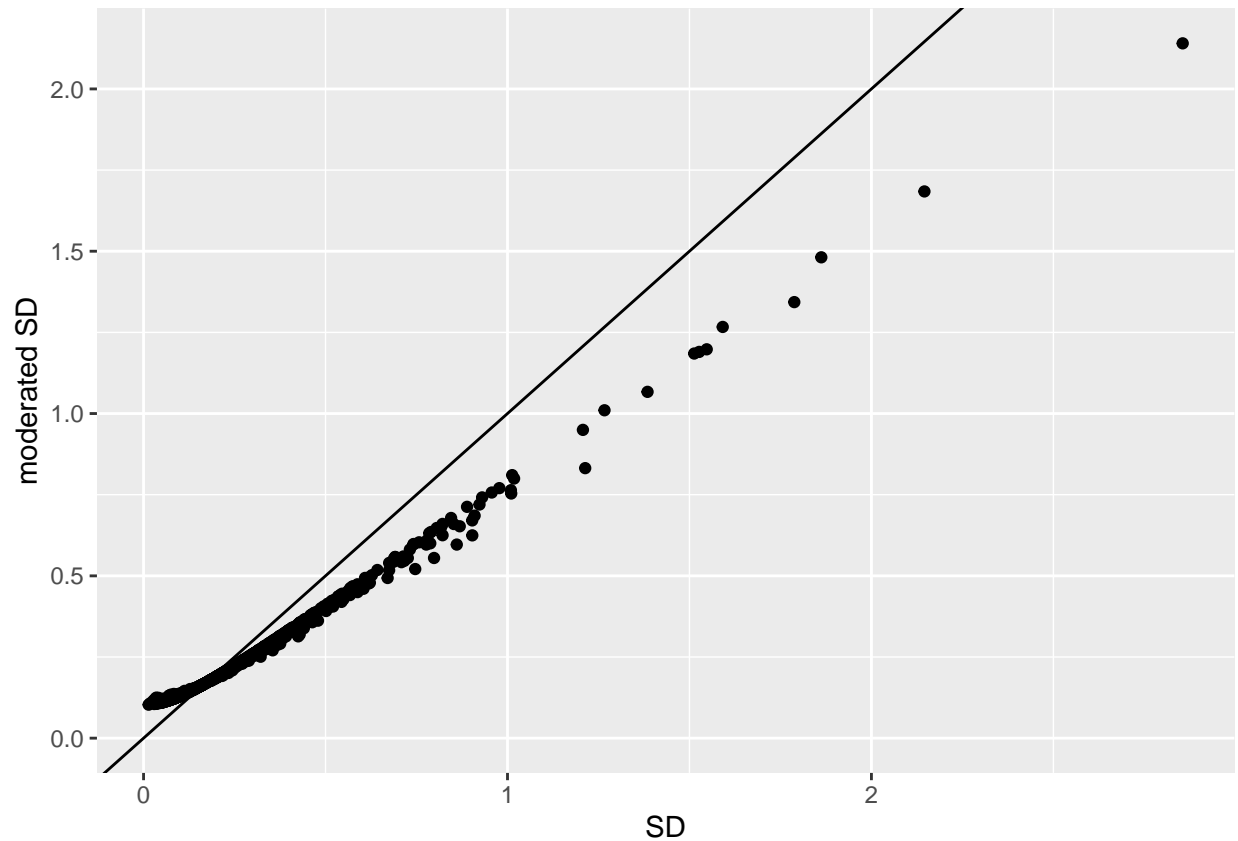
The data decides whether \tilde{t}_g
should be closer to $t_{g,pooled}$ or to t_g

Figure

courtesy to Rafael Irizarry

```
qplot(
  supply(rowData(pe[["protein"]])$msqrobModels, getSigma),
  supply(rowData(pe[["protein"]])$msqrobModels, getSigmaPosterior)) +
  xlab("SD") +
  ylab("moderated SD") +
  geom_abline(intercept = 0, slope = 1) +
  geom_hline(yintercept = )
```

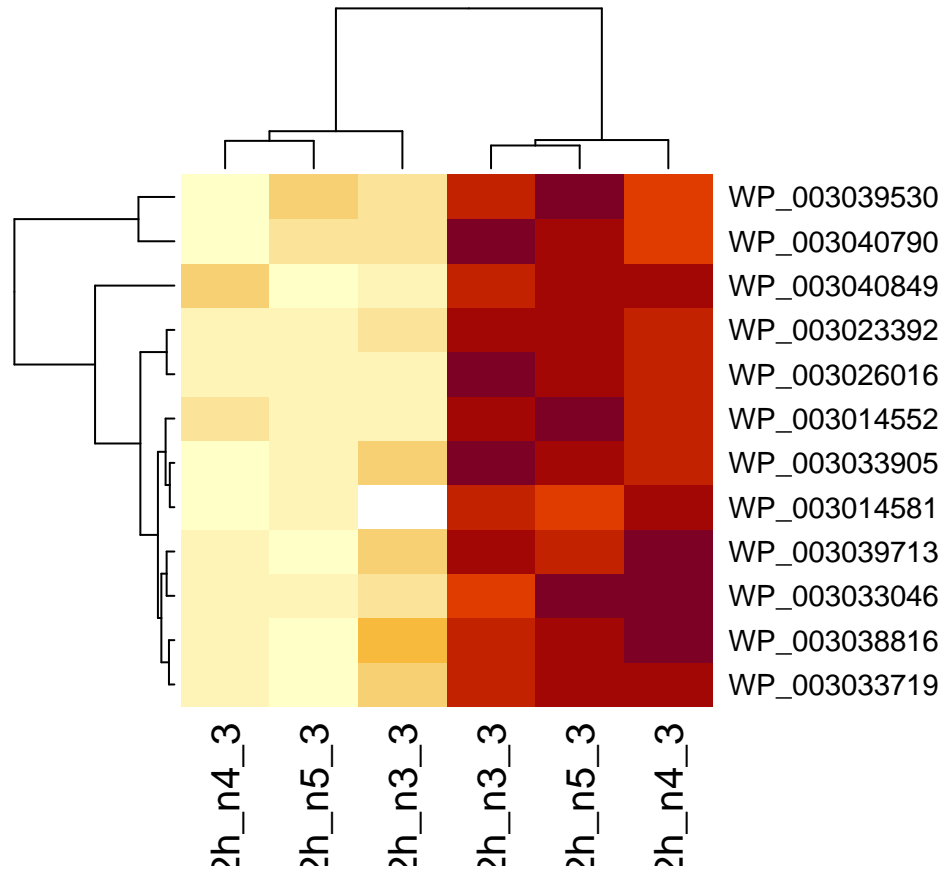
Warning: Removed 109 rows containing missing values (geom_point).



- Small variances are shrunk towards the common variance resulting in large EB variance estimates
- Large variances are shrunk towards the common variance resulting in smaller EB variance estimates
- Pooled degrees of freedom of the EB variance estimator are larger because information is borrowed across proteins to estimate the variance

1.6 Plots

```
sigNames <- rowData(pe[["protein"]])$genotypeD8 %>%
  rownames_to_column("protein") %>%
  filter(adjPval < 0.05) %>%
  pull(protein)
heatmap(assay(pe[["protein"]])[sigNames, ])
```



```
for (protName in sigNames)
{
  pePlot <- pe[protName, , c("peptideNorm", "protein")]
  pePlotDf <- data.frame(longFormat(pePlot))
  pePlotDf$assay <- factor(pePlotDf$assay,
    levels = c("peptideNorm", "protein")
  )
  pePlotDf$genotype <- as.factor(colData(pePlot)[pePlotDf$colname, "genotype"])

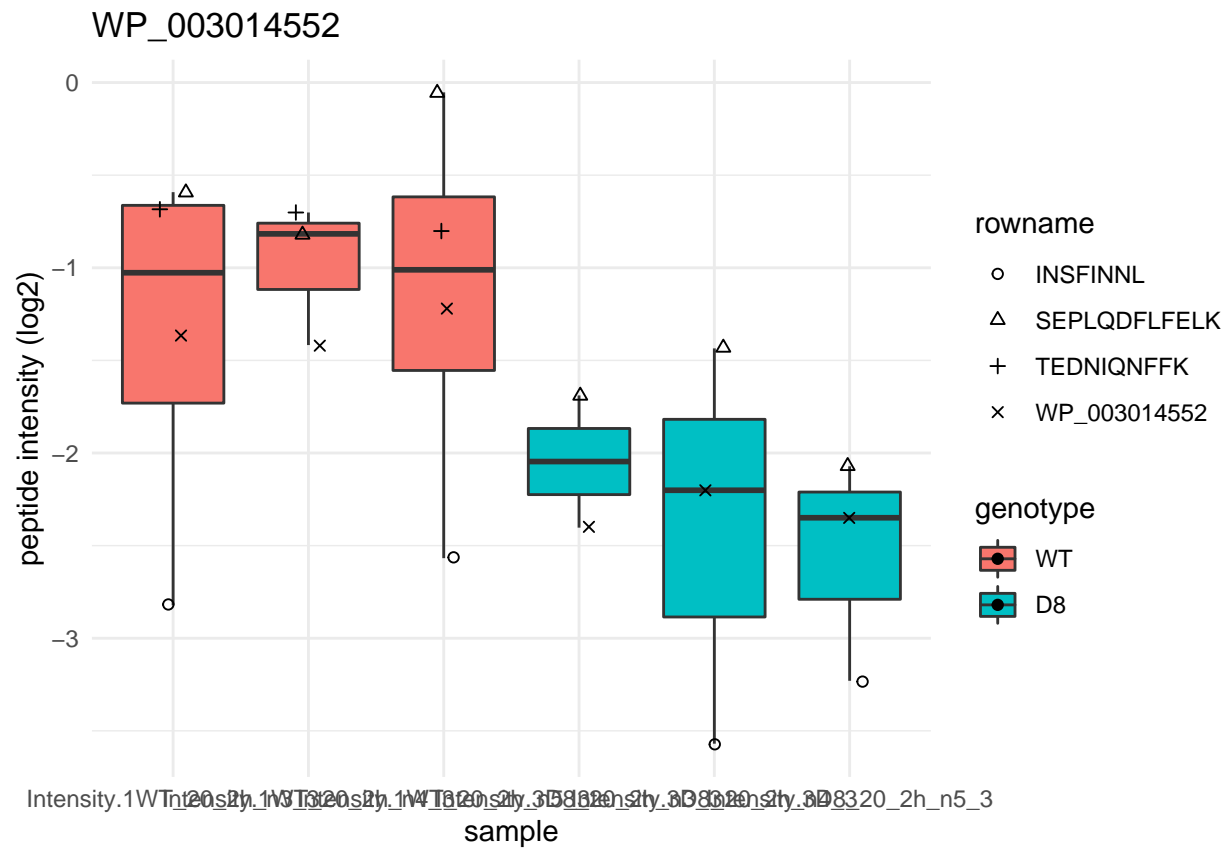
  # plotting
  p1 <- ggplot(
    data = pePlotDf,
    aes(x = colname, y = value, group = rowname)
  ) +
    geom_line() +
    geom_point() +
    theme_minimal() +
    facet_grid(~assay) +
    ggtitle(protName)
  print(p1)

  # plotting 2
  p2 <- ggplot(pePlotDf, aes(x = colname, y = value, fill = genotype)) +
    geom_boxplot(outlier.shape = NA) +
    geom_point(
      position = position_jitter(width = .1),

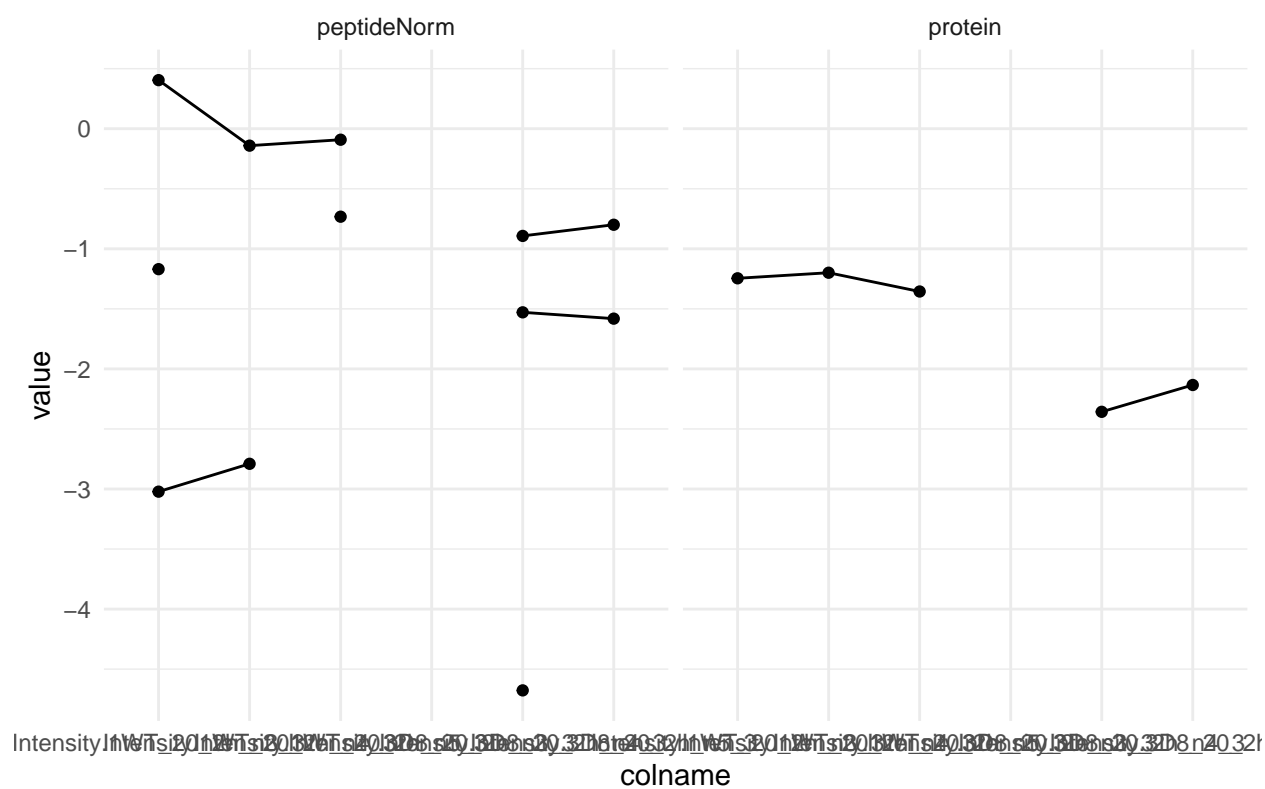
```

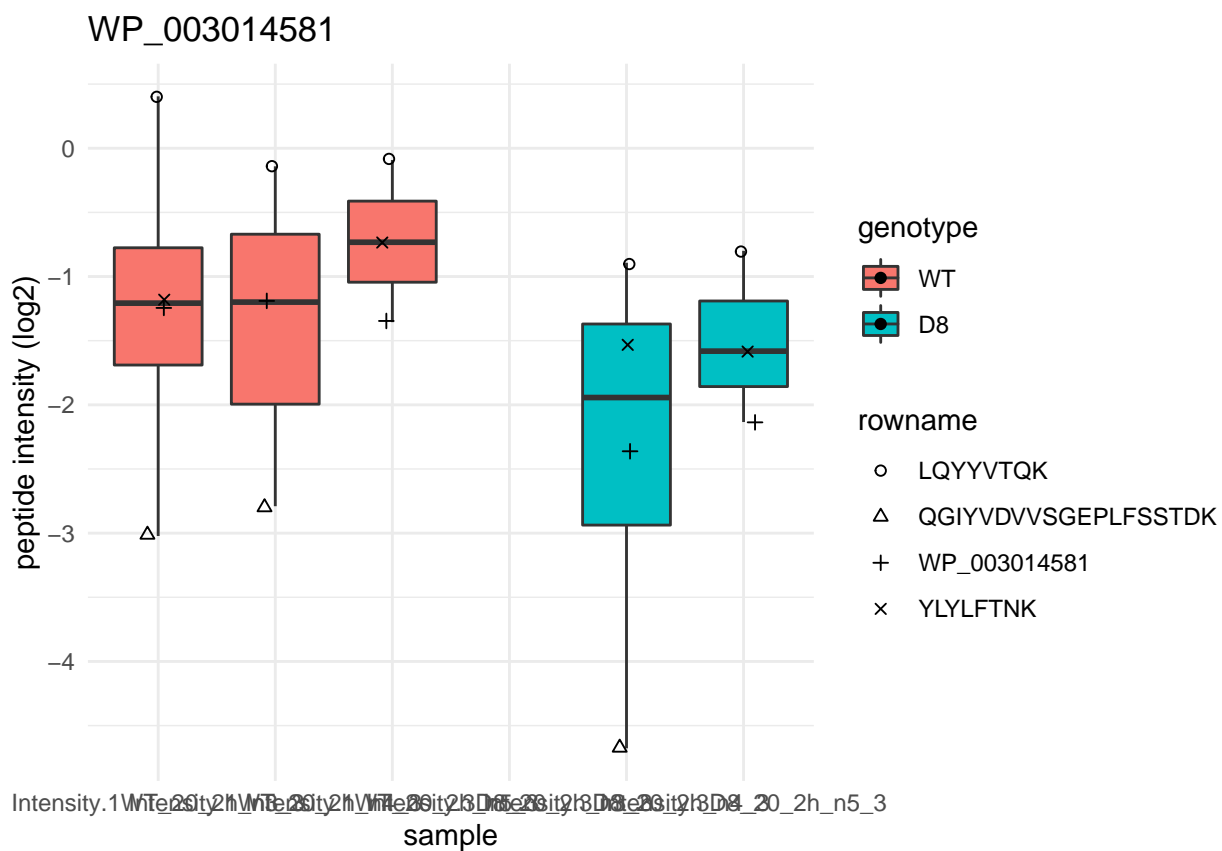
}



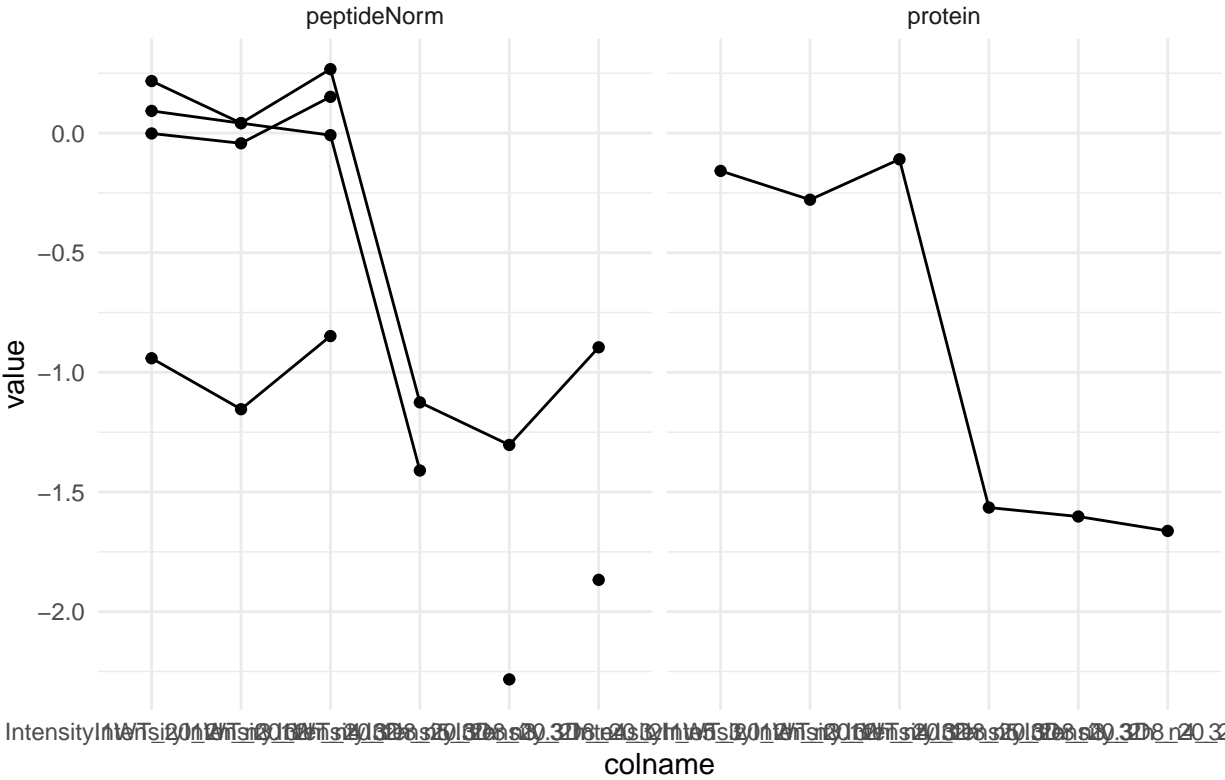


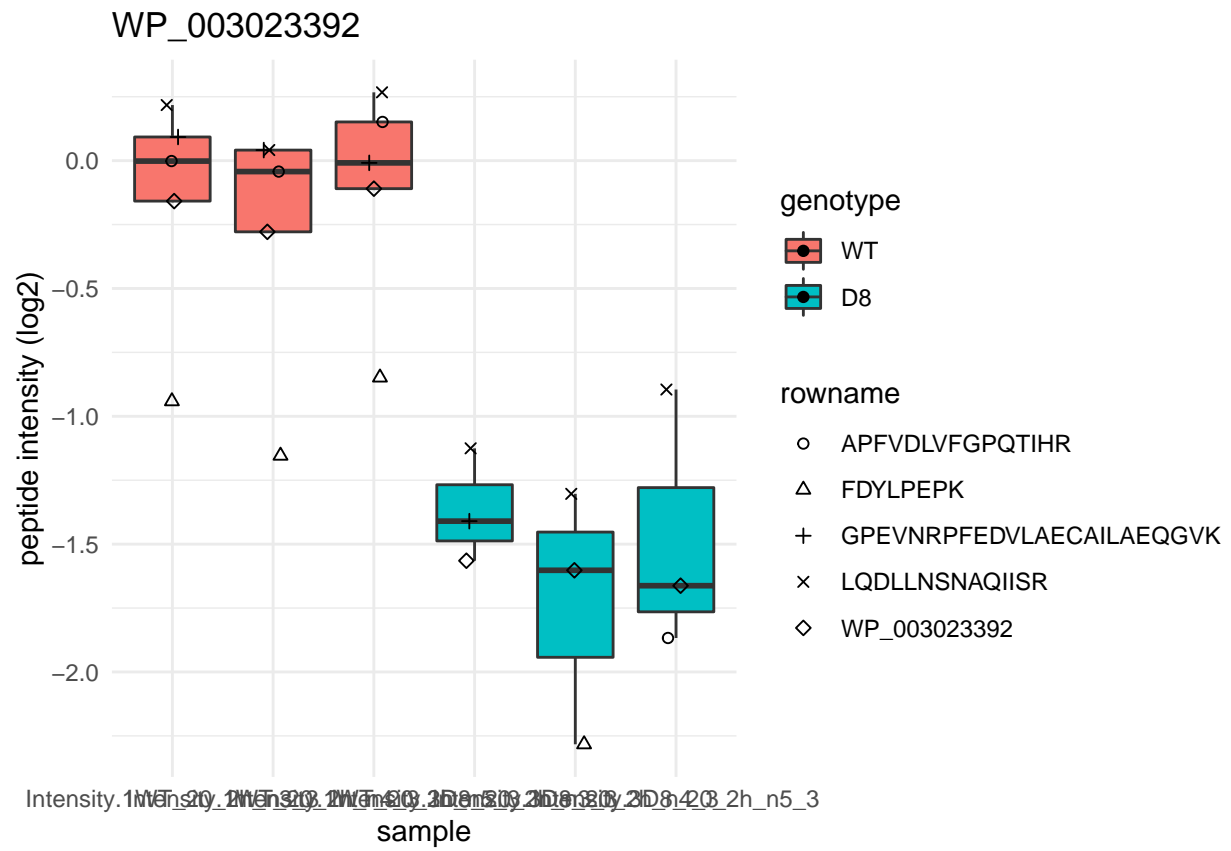
WP_003014581



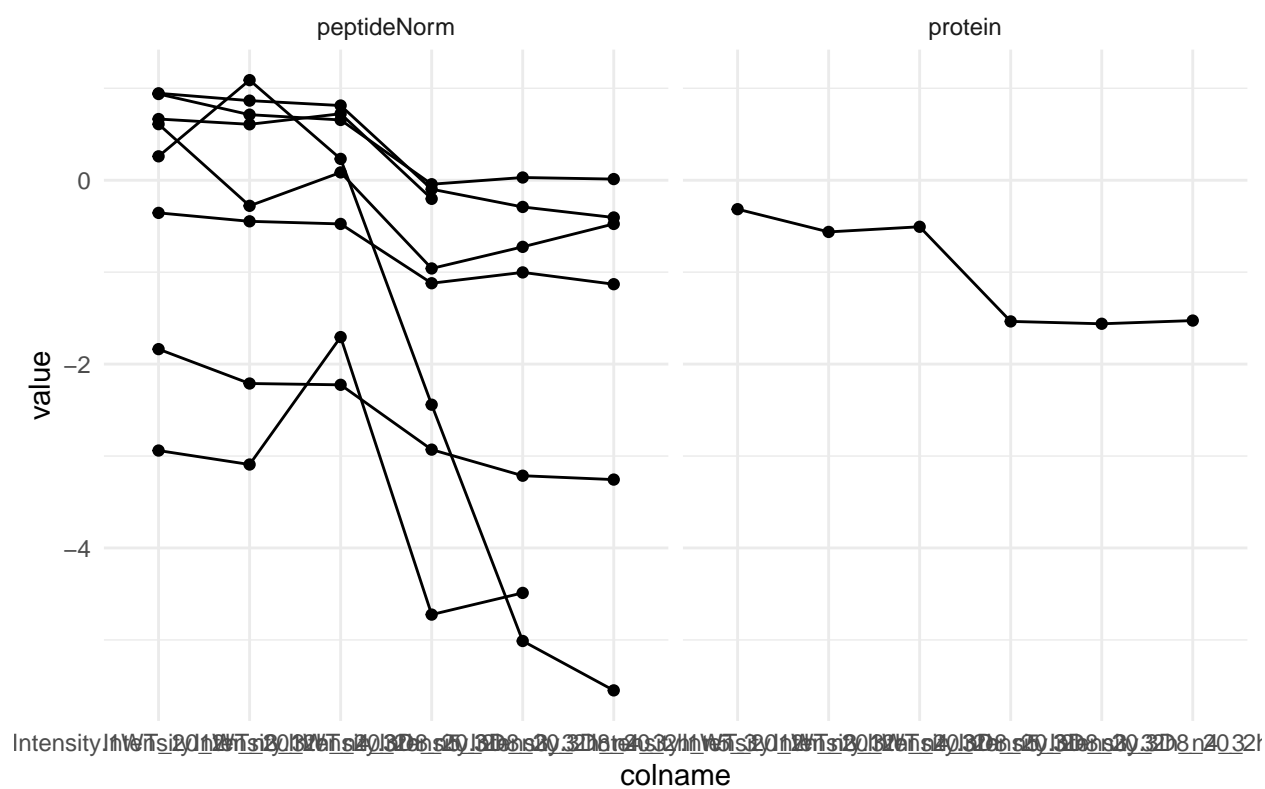


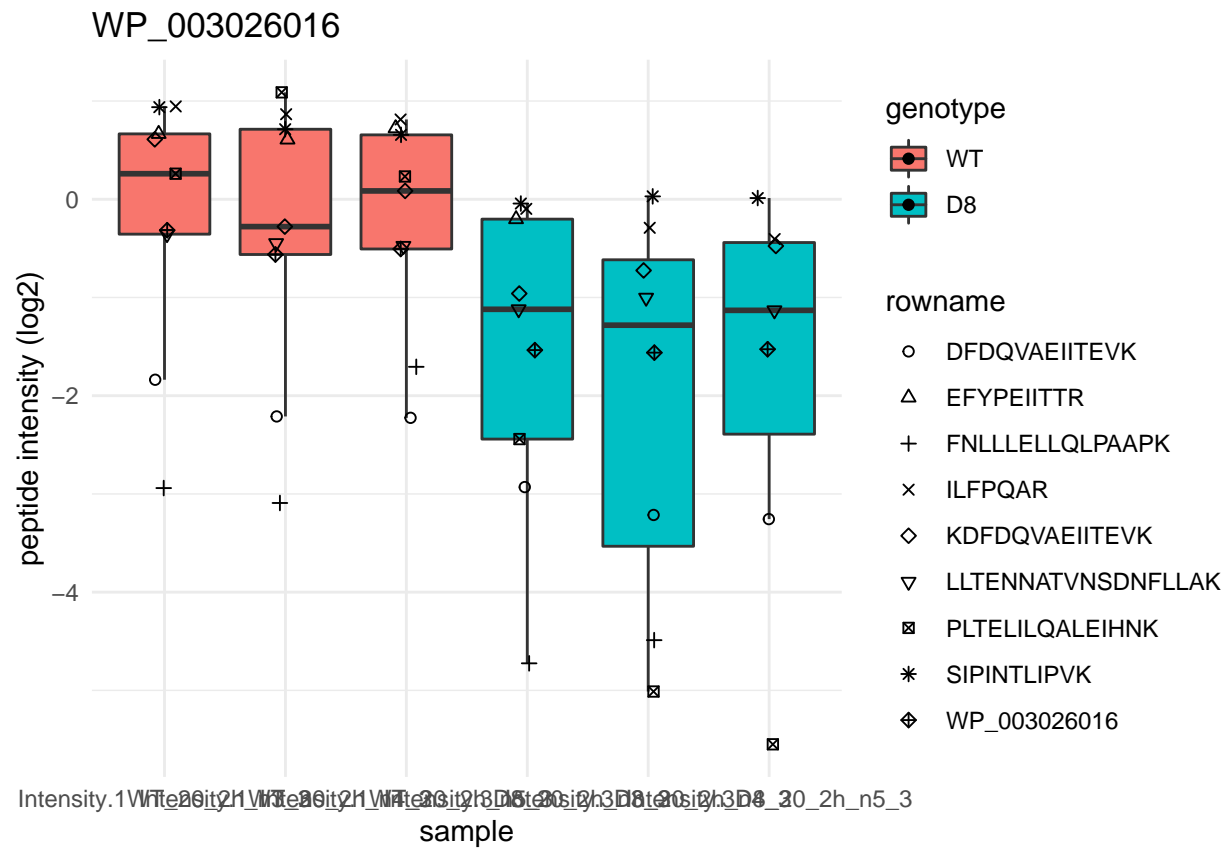
WP_003023392

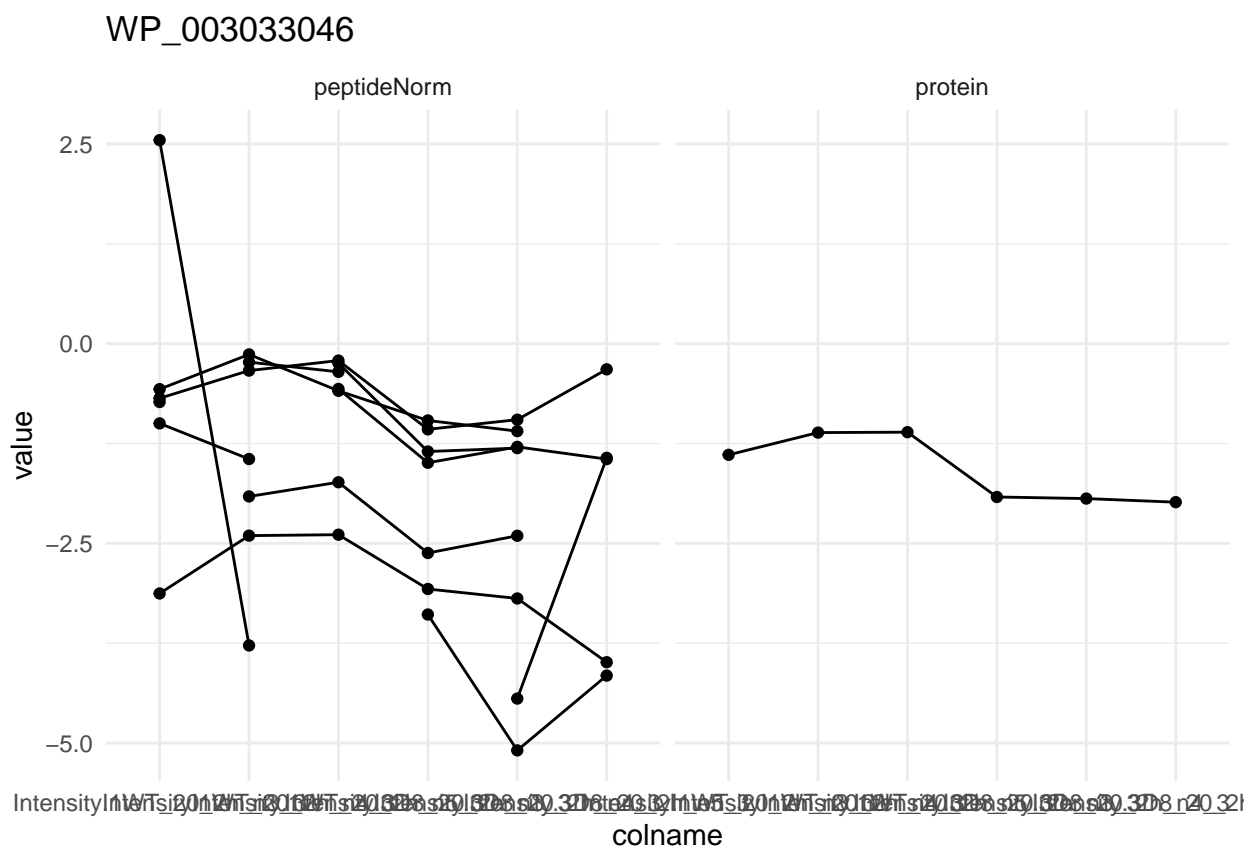


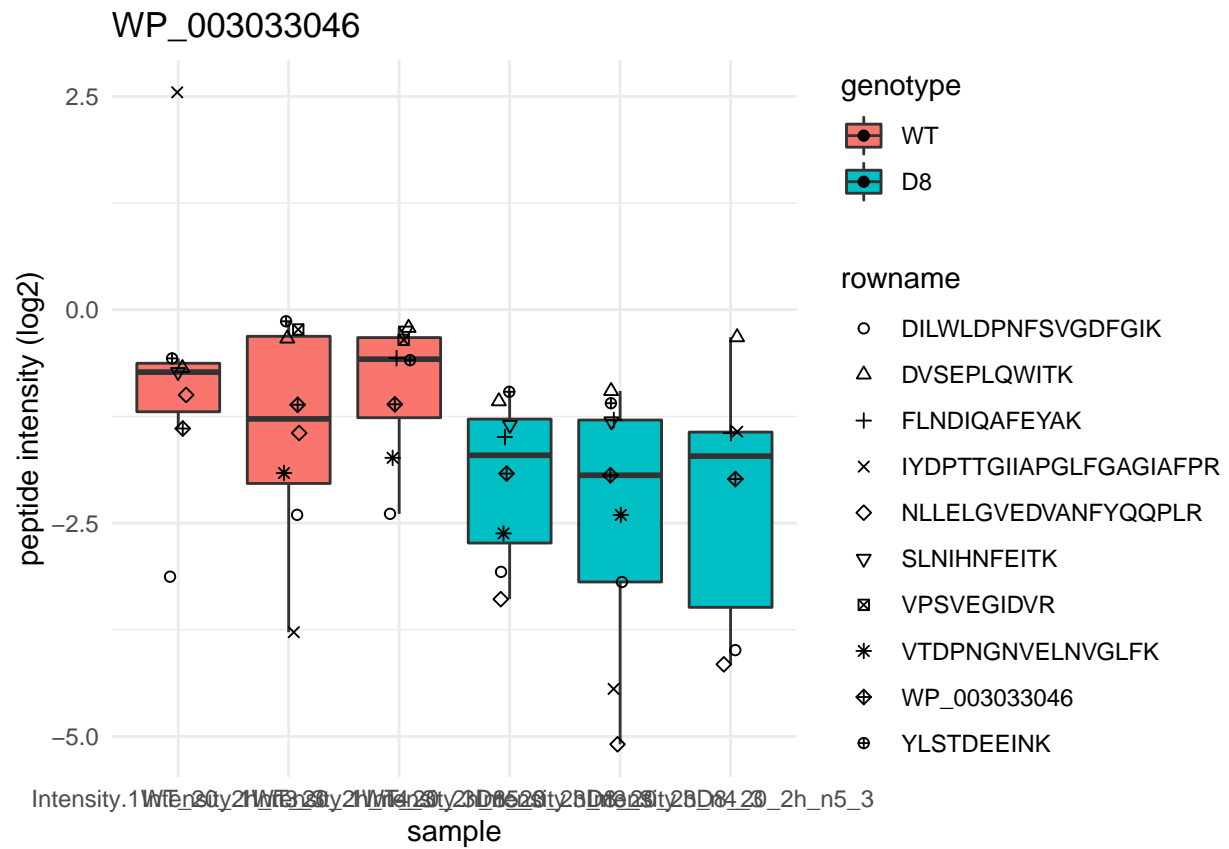


WP_003026016

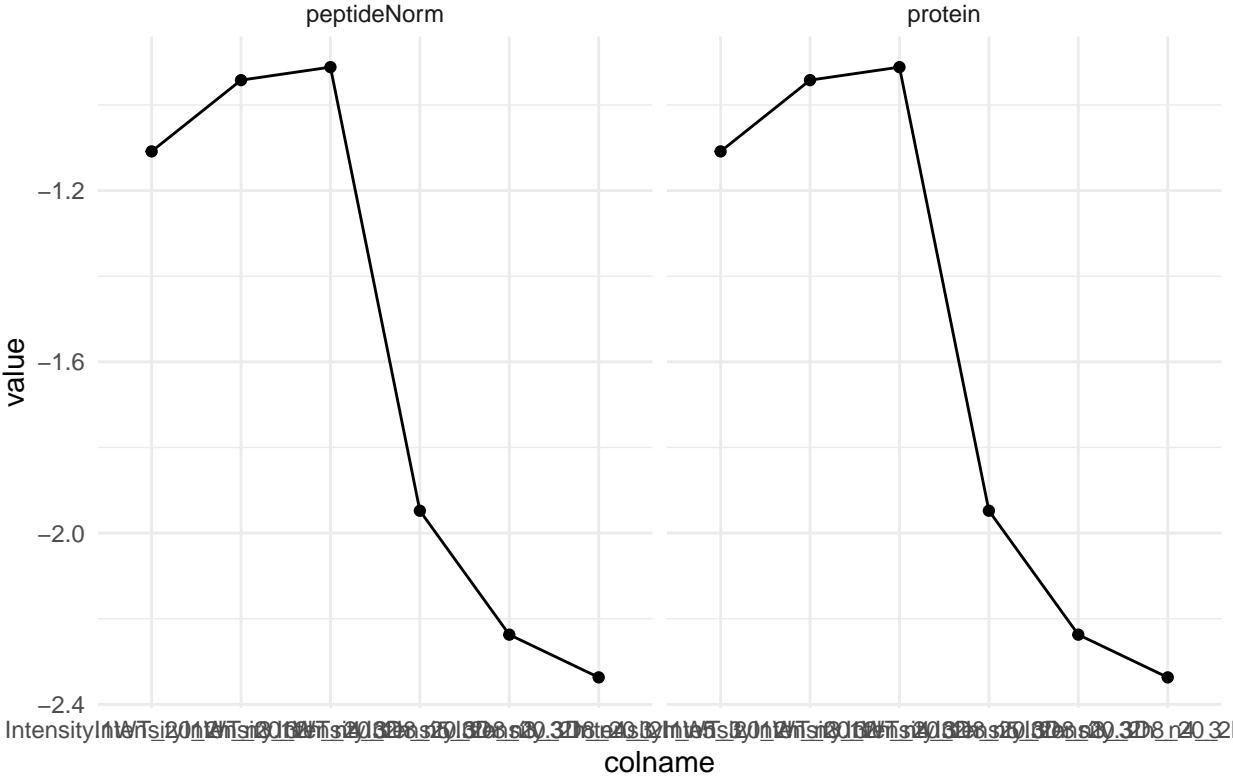




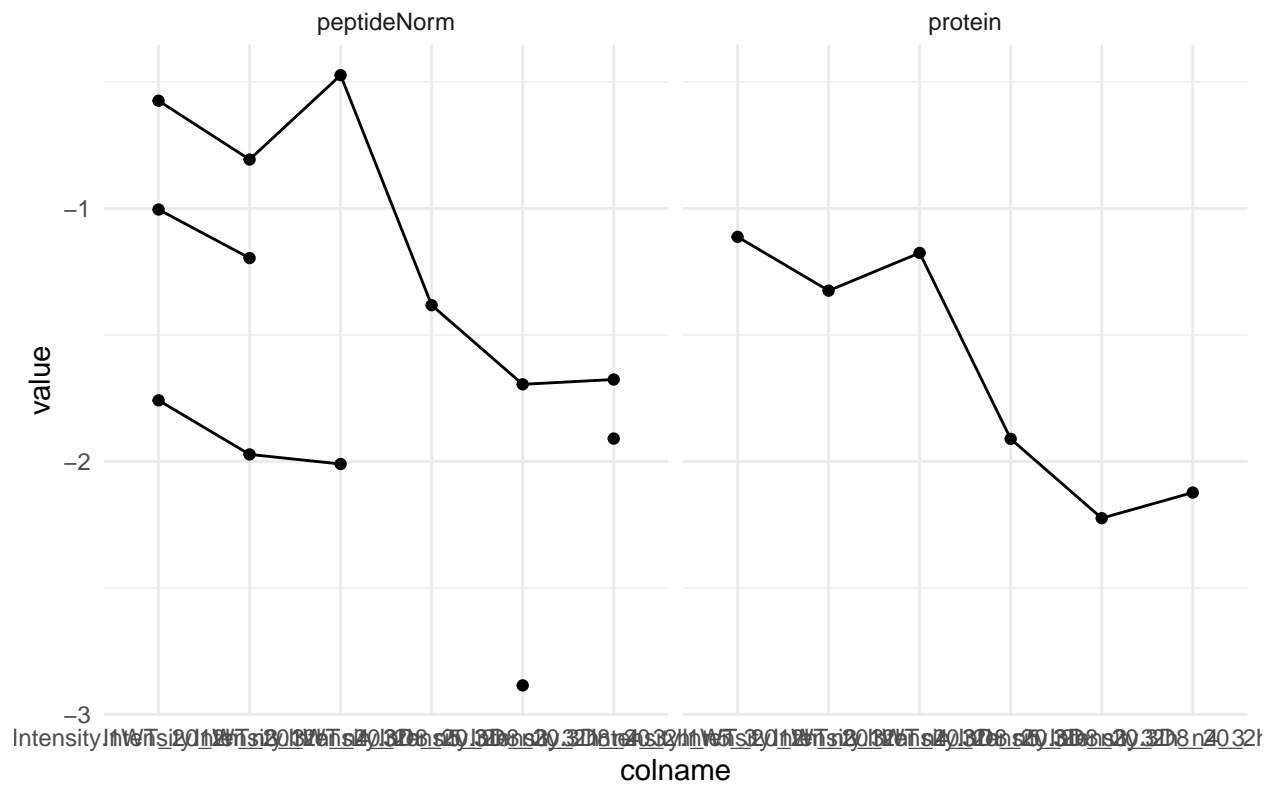


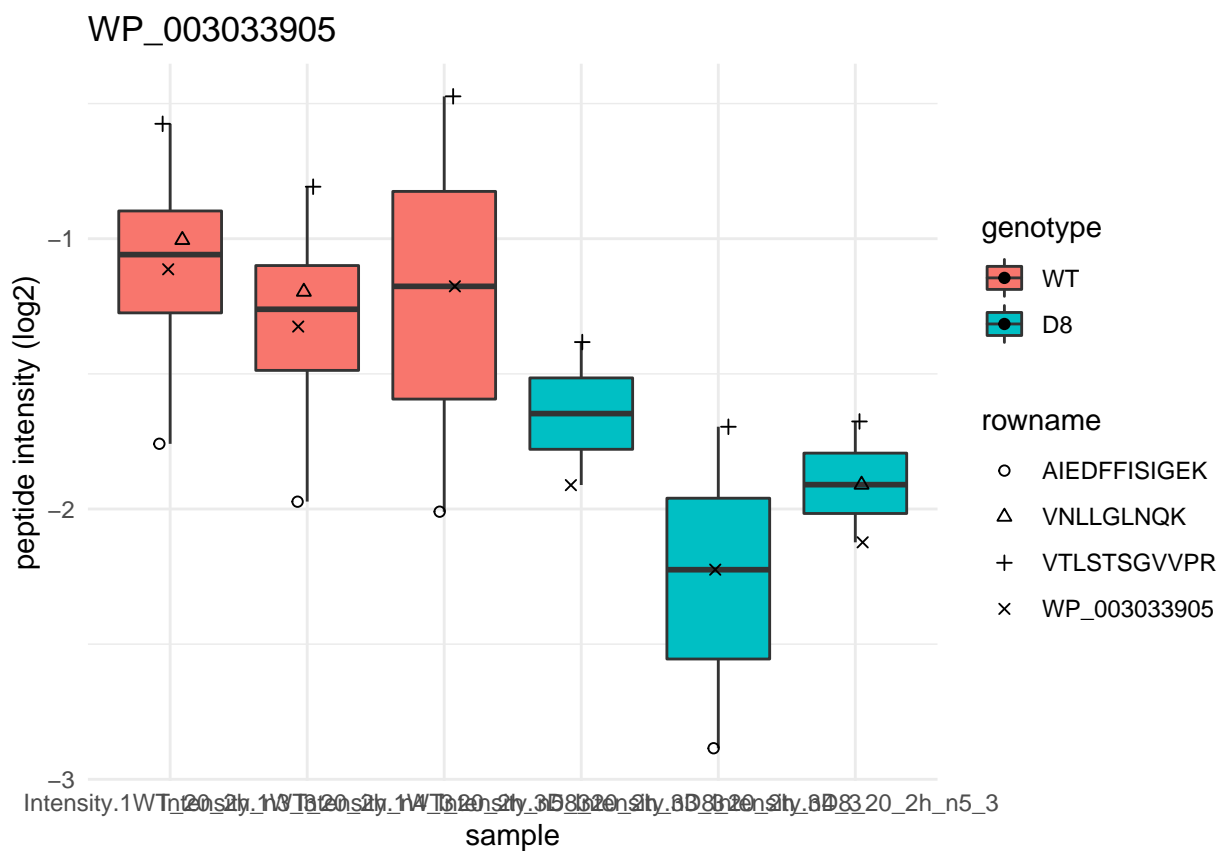


WP 003033719

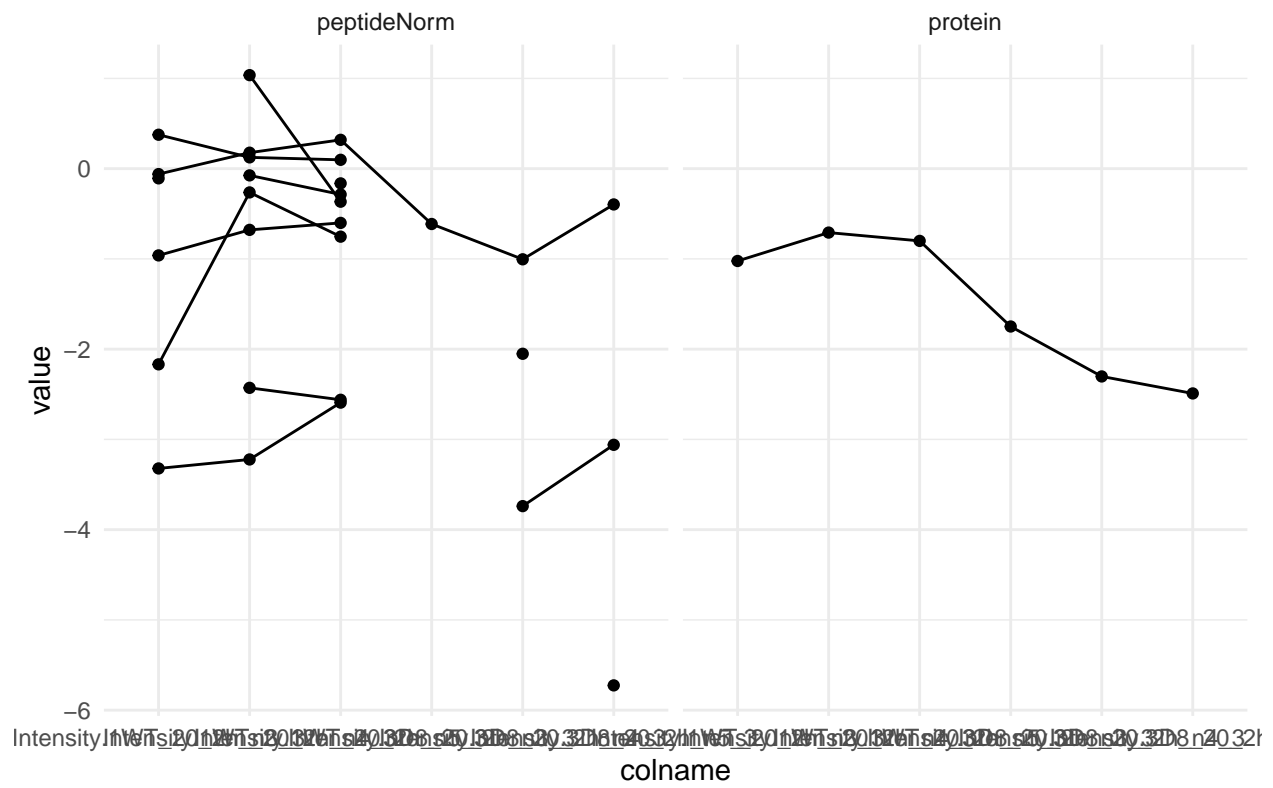


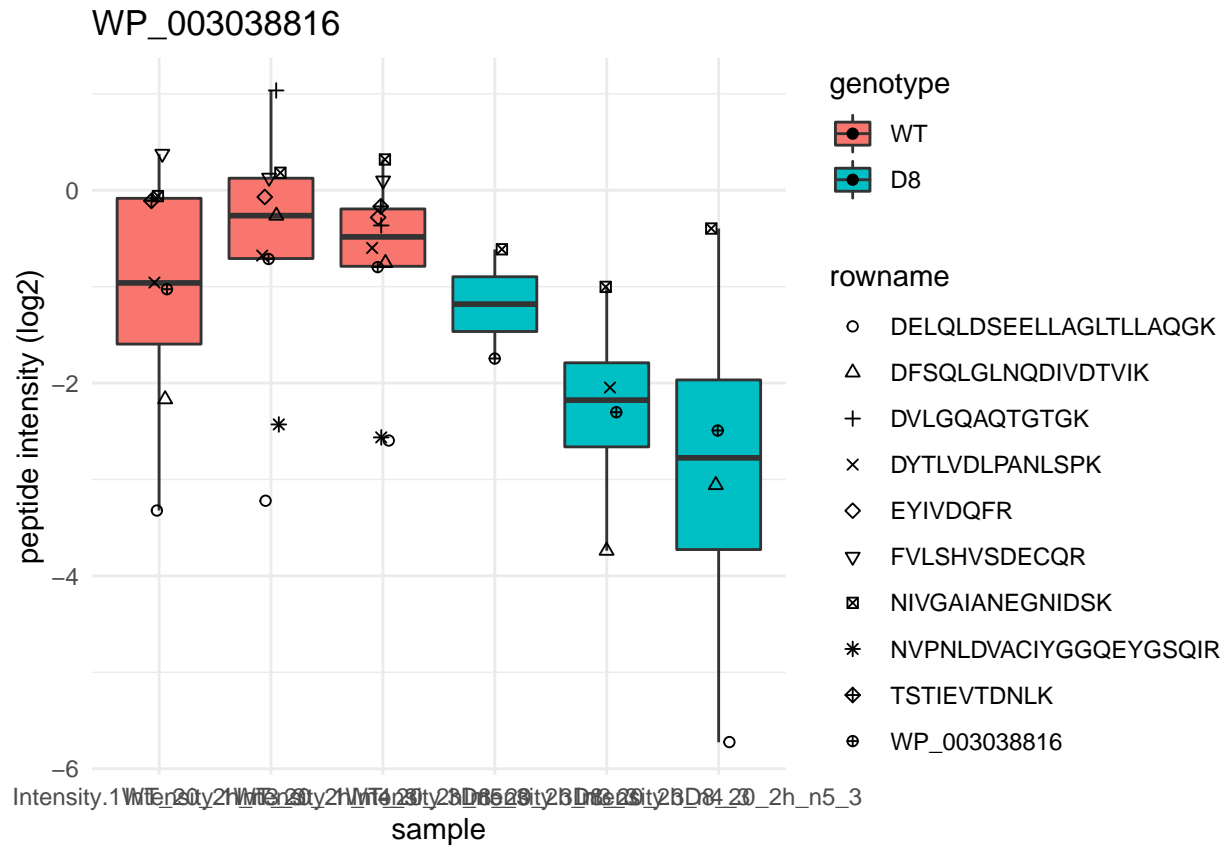
WP_003033905



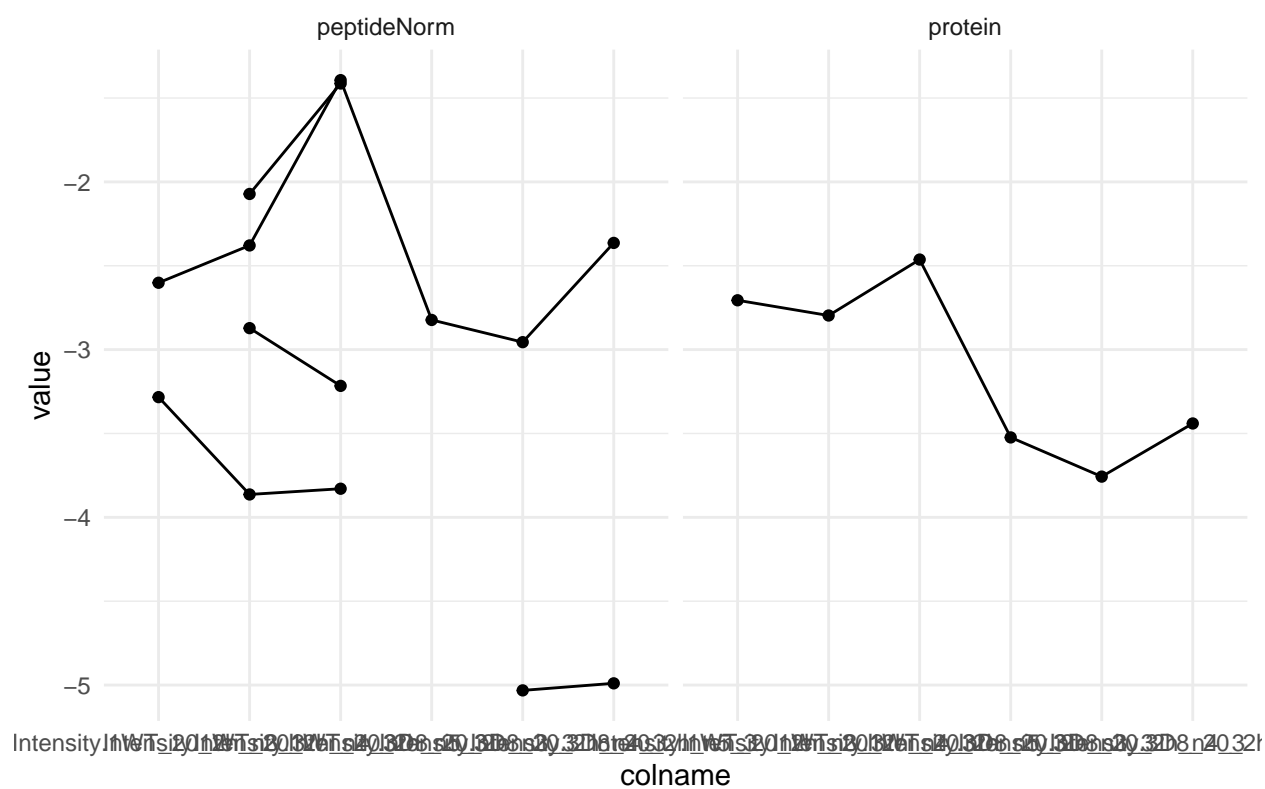


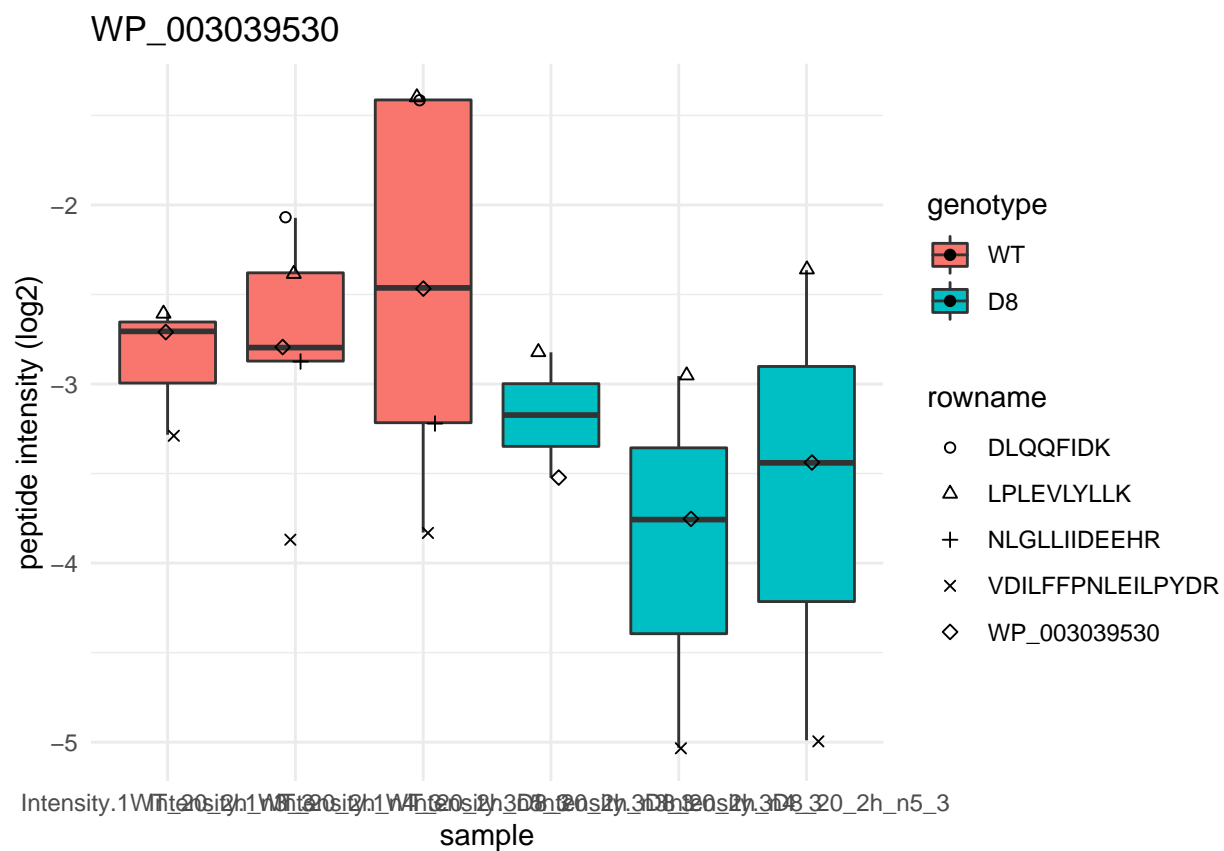
WP_003038816



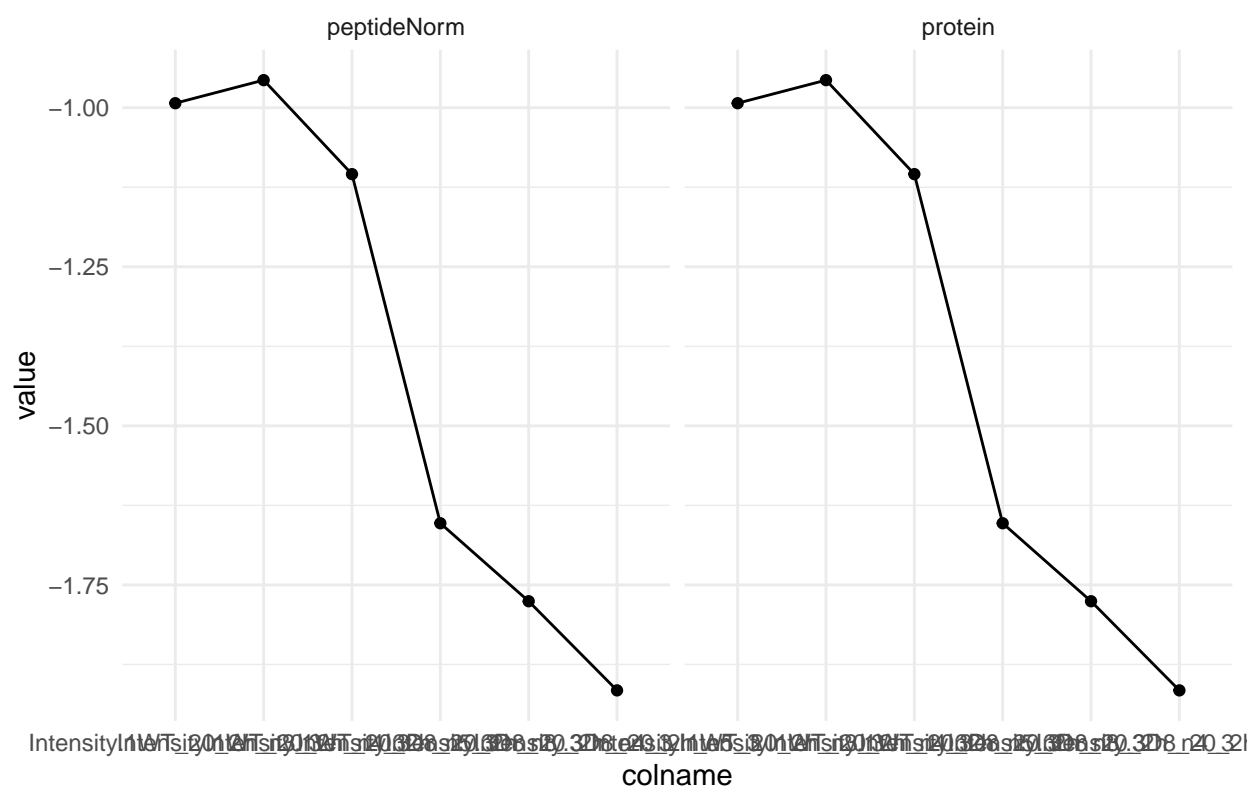


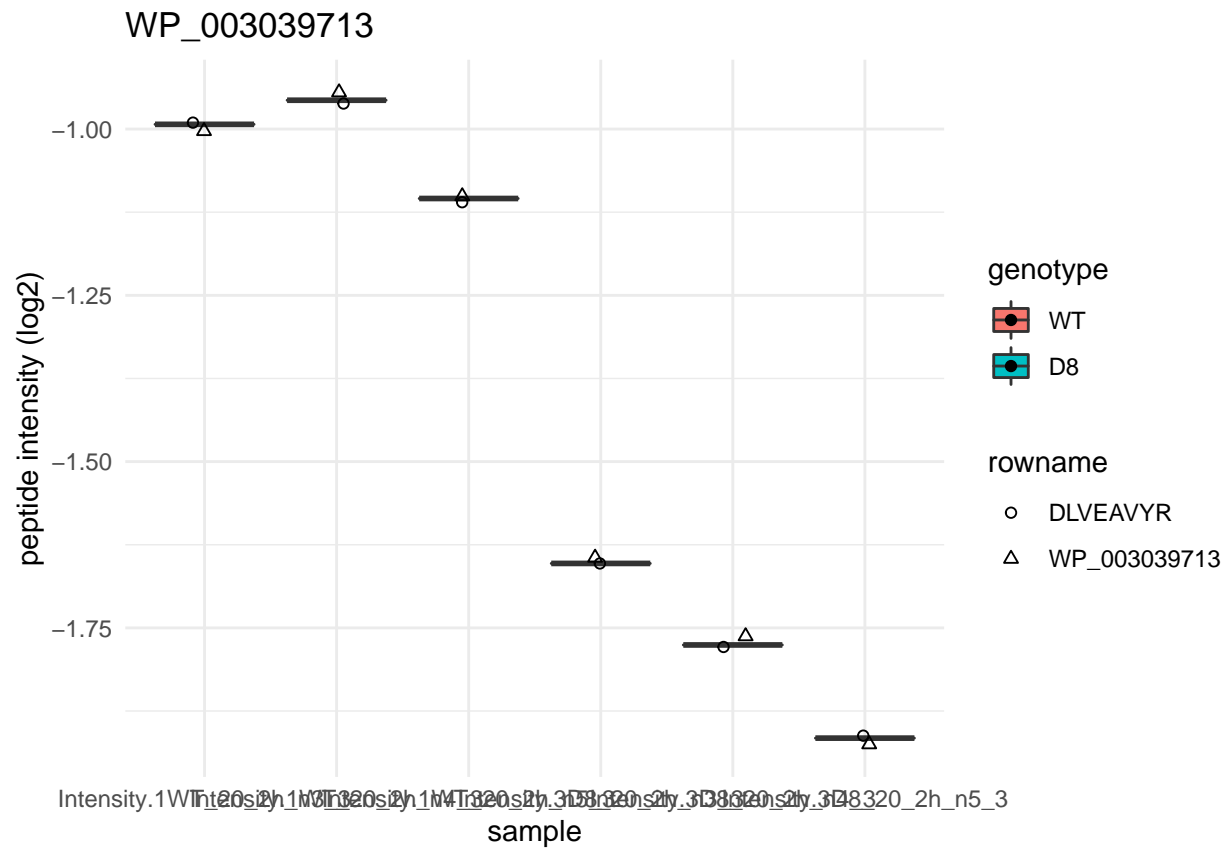
WP 003039530



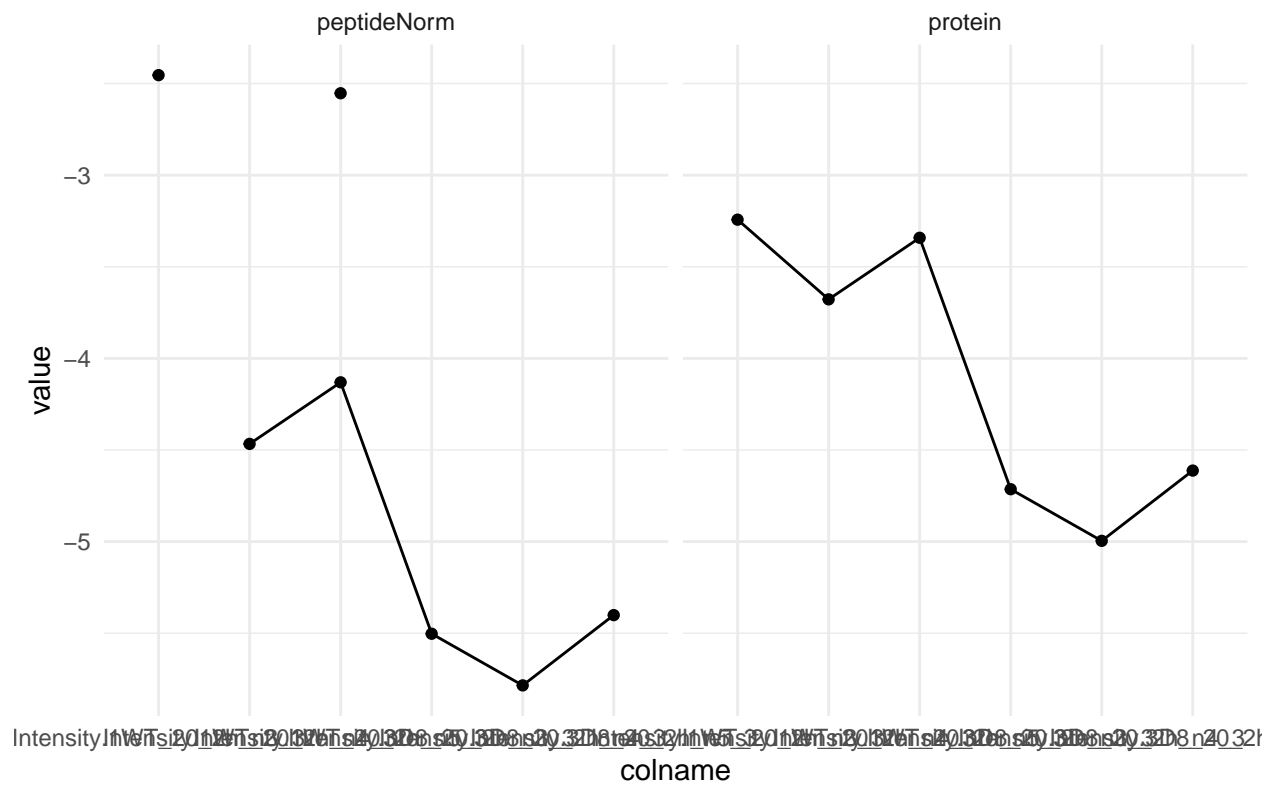


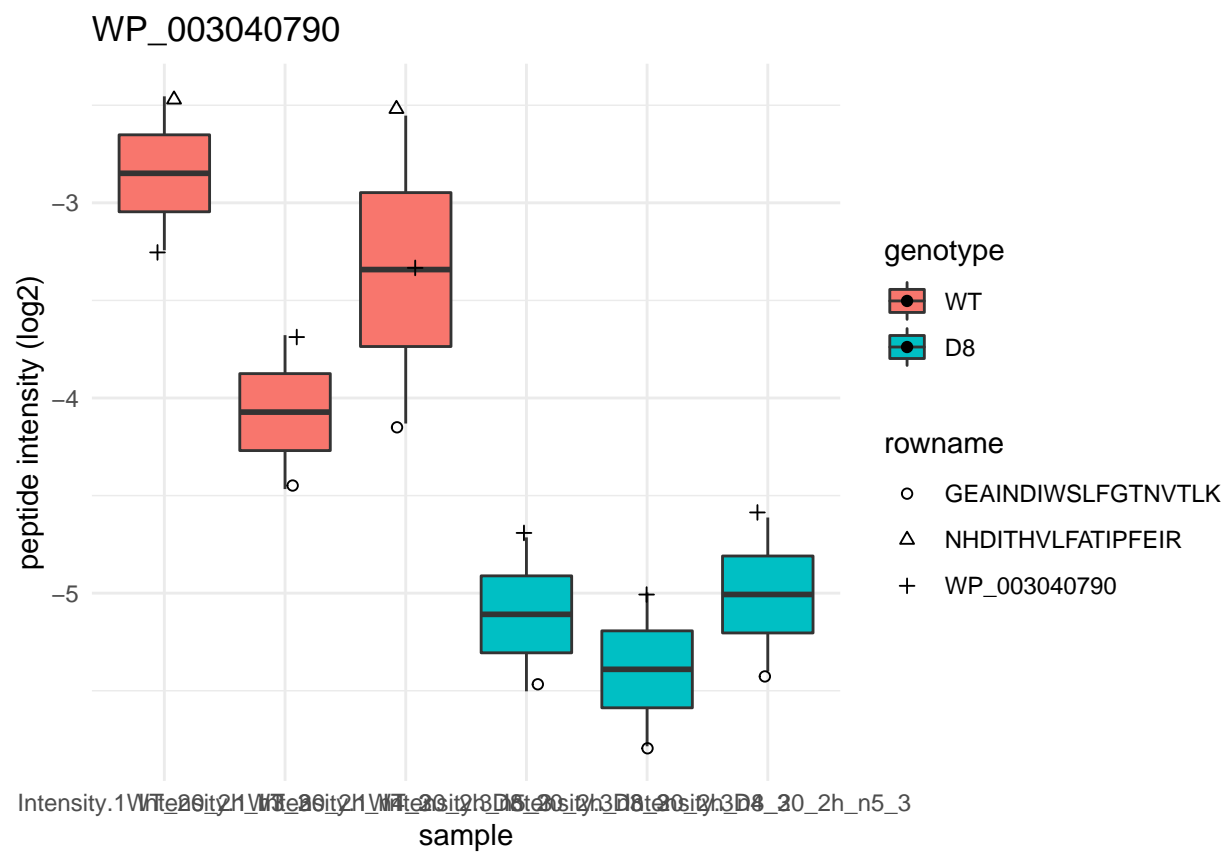
WP_003039713



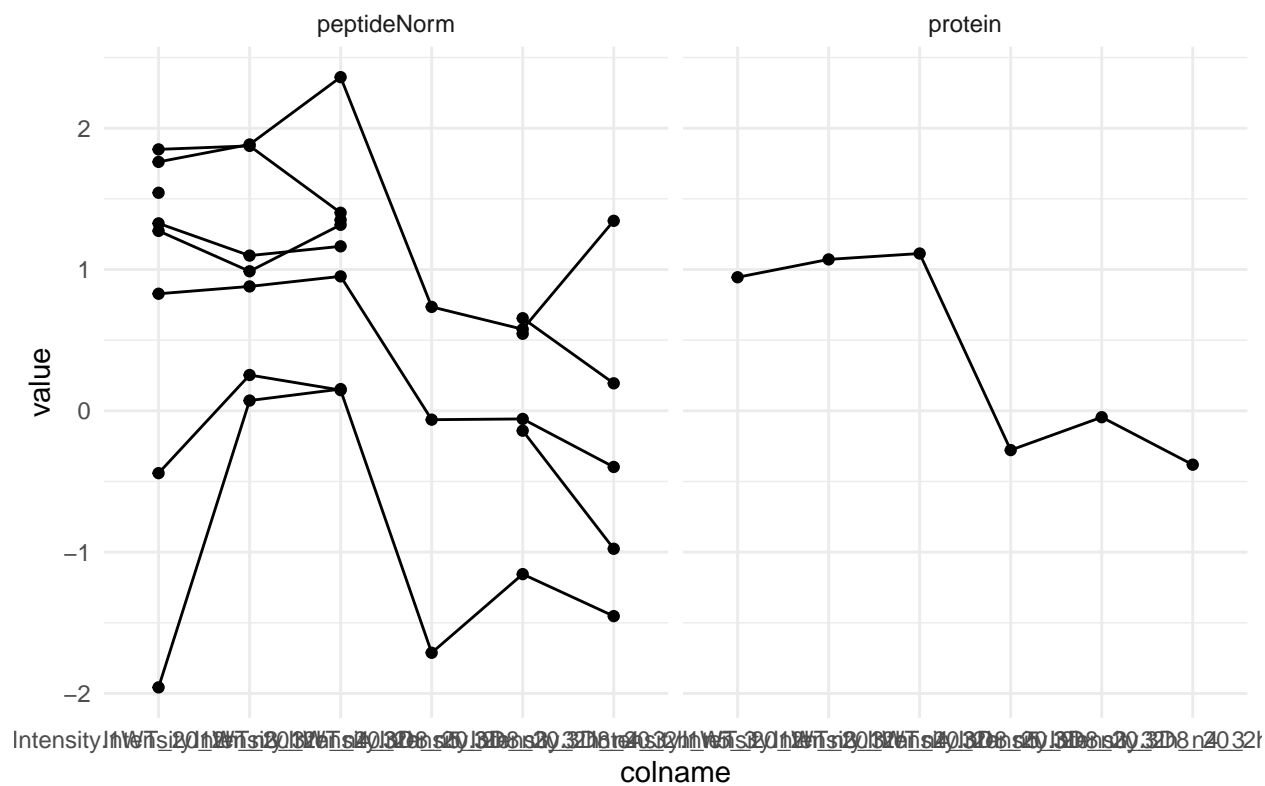


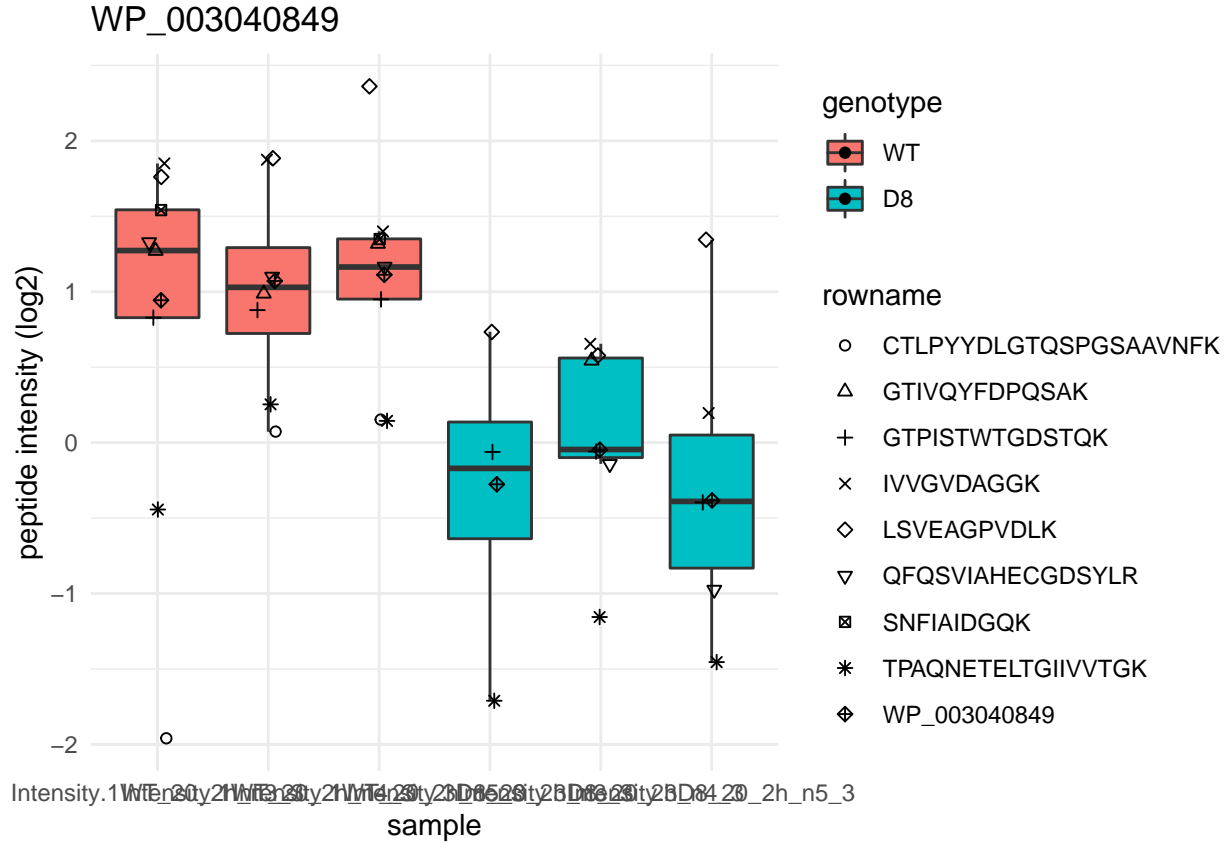
WP_003040790





WP_003040849





2 Experimental Design

2.1 Sample size

$$\log_2 \text{FC} = \bar{y}_{p1} - \bar{y}_{p2}$$

$$T_g = \frac{\log_2 \text{FC}}{\text{se}_{\log_2 \text{FC}}}$$

$$T_g = \frac{\widehat{\text{signal}}}{\widehat{\text{Noise}}}$$

If we can assume equal variance in both treatment groups:

$$\text{se}_{\log_2 \text{FC}} = \text{SD} \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

→ if number of bio-repeats increases we have a higher power!

- cfr. Study of tamoxifen treated Estrogen Receptor (ER) positive breast cancer patients

2.2 Blocking

$$\sigma^2 = \sigma_{bio}^2 + \sigma_{lab}^2 + \sigma_{extraction}^2 + \sigma_{run}^2 + \dots$$

- Biological: fluctuations in protein level between mice, fluctuations in protein level between cells, ...
- Technical: cage effect, lab effect, week effect, plasma extraction, MS-run, ...

2.3 Nature methods: Points of significance - Blocking

<https://www.nature.com/articles/nmeth.3005.pdf>

2.4 Mouse example

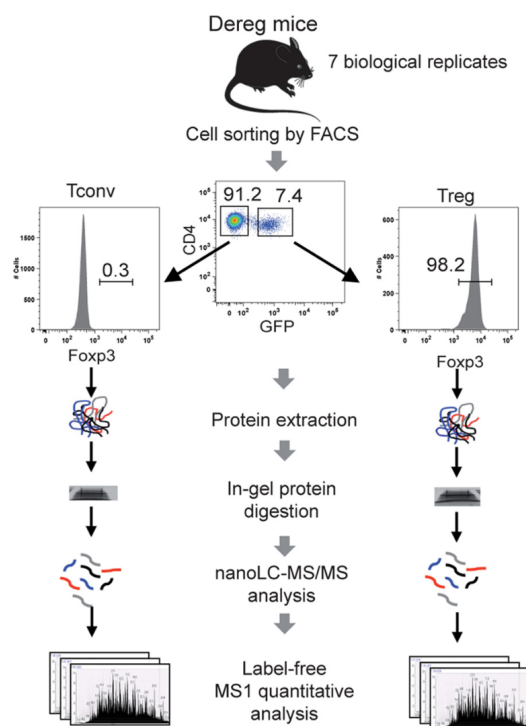


FIG. 1. **Label-free quantitative analysis of conventional and regulatory T cell proteomes.** General analytical workflow based on cell sorting by flow cytometry using the DEREK mouse model and parallel proteomic analysis of Tconv and Treg cell populations by nanoLC-MS/MS and label-free relative quantification.

Duguet et al. (2017) MCP 16(8):1416-1432. doi:

10.1074/mcp.m116.062745

- All treatments of interest are present within block!
- We can estimate the effect of the treatment within block!
- We can isolate the between block variability from the analysis using linear model:

$$y \sim \text{type} + \text{mouse}$$

- Not possible with Perseus!

2.4.1 Assess the impact of blocking in the tutorial session!

- Completely randomized design with only one cell type per mouse (Treg and Tconv)



- Randomized complete block design assessing Treg and Tconv on each mouse