

Cancer

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This is part of the online course [Proteomics Data Analysis \(PDA\)](#)

1 Background

Eighteen Estrogen Receptor Positive Breast cancer tissues from from patients treated with tamoxifen upon recurrence have been assessed in a proteomics study. Nine patients had a good outcome (OR) and the other nine had a poor outcome (PD). The proteomes have been assessed using an LTQ-Orbitrap and the thermo output .RAW files were searched with MaxQuant (version 1.4.1.2) against the human proteome database (FASTA version 2012-09, human canonical proteome).

2 Data

We first import the data from peptide.txt file. This is the file containing your peptide-level intensities. For a MaxQuant search [6], this peptide.txt file can be found by default in the “path_to_raw_files/combined/txt/” folder from the MaxQuant output, with “path_to_raw_files” the folder where the raw files were saved.

We generate the object peptideFile with the path to the peptide.txt file. Using the `grepEcols` function, we find the columns that contain the expression data of the peptide in the peptide.txt file.

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

peptidesFile <- "https://raw.githubusercontent.com/statOmics/PDA22GTPB/data/quantification/cancer/peptide.txt"

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

Next, we read the data and store it in QFeatures object

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

The QFeatures object `pe` currently contains a single assay, named `peptideRaw`.

We extract the column names from the `peptideRaw` assay and see that this contains information about the prognosis.

```
colnames(pe[["peptideRaw"]])
```

```
## [1] "Intensity.OR.01" "Intensity.OR.04" "Intensity.OR.07" "Intensity.OR.09"
## [5] "Intensity.OR.10" "Intensity.OR.13" "Intensity.OR.20" "Intensity.OR.23"
## [9] "Intensity.OR.25" "Intensity.PD.02" "Intensity.PD.03" "Intensity.PD.04"
## [13] "Intensity.PD.06" "Intensity.PD.07" "Intensity.PD.08" "Intensity.PD.09"
## [17] "Intensity.PD.10" "Intensity.PD.11"
```

We rename the colnames by dropping the “Intensity.” from the name.

```
(newNames <- sub(
  pattern = "Intensity\\.\\.",
  replacement = "",
  colnames(pe[["peptideRaw"]]))
)
```

```
## [1] "OR.01" "OR.04" "OR.07" "OR.09" "OR.10" "OR.13" "OR.20" "OR.23" "OR.25"
## [10] "PD.02" "PD.03" "PD.04" "PD.06" "PD.07" "PD.08" "PD.09" "PD.10" "PD.11"
```

```
pe <- renameColname(pe,
                    i = "peptideRaw",
                    newNames)
pe <- renamePrimary(pe, newNames)
colnames(pe[["peptideRaw"]])
```

```
## [1] "OR.01" "OR.04" "OR.07" "OR.09" "OR.10" "OR.13" "OR.20" "OR.23" "OR.25"
## [10] "PD.02" "PD.03" "PD.04" "PD.06" "PD.07" "PD.08" "PD.09" "PD.10" "PD.11"
```

In the following code chunk, we add the prognosis of the patients that we can read in the raw file name to the colData.

```
colData(pe)$prognosis <-
  colnames(pe[["peptideRaw"]]) %>%
  substr(start = 1, stop = 2) %>%
  as.factor
colData(pe)$prognosis
```

```
## [1] OR OR OR OR OR OR OR OR OR OR PD PD PD PD PD PD PD PD PD PD
## Levels: OR PD
```

We calculate how many non zero intensities we have per peptide and this will be useful for filtering.

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

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

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

Look at the column names of the data to know the variables that you can use for filtering.

```
pe[["peptideRaw"]] %>% rowData %>% names
```

```
## [1] "Sequence"          "Proteins"          "Leading.razor.protein"
## [4] "Gene.names"        "Protein.names"     "Unique..Groups."
## [7] "Unique..Proteins." "Charges"           "PEP"
## [10] "Score"             "Slice.Average"     "Slice.Std..Dev."
## [13] "Slice.1"           "Unique.Slice.Average" "Unique.Slice.Std..Dev."
## [16] "Unique.Slice.1"     "Experiment.OR.01"   "Experiment.OR.04"
## [19] "Experiment.OR.07"   "Experiment.OR.09"   "Experiment.OR.10"
## [22] "Experiment.OR.13"   "Experiment.OR.20"   "Experiment.OR.23"
## [25] "Experiment.OR.25"   "Experiment.PD.02"   "Experiment.PD.03"
## [28] "Experiment.PD.04"   "Experiment.PD.06"   "Experiment.PD.07"
## [31] "Experiment.PD.08"   "Experiment.PD.09"   "Experiment.PD.10"
## [34] "Experiment.PD.11"   "Intensity"          "Reverse"
## [37] "Contaminant"        "id"                 "Protein.group.IDs"
## [40] "Mod..peptide.IDs"   "Evidence.IDs"       "MS.MS.IDs"
## [43] "Best.MS.MS"         "Oxidation..M..site.IDs" "nNonZero"
```

So we will filter on the “Reverse”, “Contaminant” and “nNonZero” column.

2.1 Data exploration

47% of all peptide intensities are missing and for some peptides we do not even measure a signal in any sample.

3 Preprocessing

This section performs preprocessing for the peptide data. This includes

- log transformation,
- filtering and
- summarisation of the data.

3.1 Log transform the data

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

3.2 Filtering

1. Handling overlapping protein groups

In our approach a peptide can map to multiple proteins, as long as there is none of these proteins present in a smaller subgroup.

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

2. Remove reverse sequences (decoys) and contaminants

We now remove the contaminants and peptides that map to decoy sequences.

```
pe <- filterFeatures(pe, ~Reverse != "+")  
pe <- filterFeatures(pe, ~Contaminant != "+")
```

3. Drop peptides that were only identified in one sample

We keep peptides that were observed at least twice.

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

```
## [1] 26696
```

We keep 26696 peptides upon filtering.

3.3 Normalize the data using median centering

We normalize the data by subtracting the sample median from every intensity for peptide p in a sample i :

$$y_{ip}^{\text{norm}} = y_{ip} - \hat{\mu}_i$$

with $\hat{\mu}_i$ the median intensity over all observed peptides in sample i .

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

3.4 Explore normalized data

Upon the normalisation the density curves are nicely registered

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

```
## Warning: Removed 188395 rows containing non-finite values (stat_density).
```



We can visualize our data using a Multi Dimensional Scaling plot, eg. as provided by the `limma` package.

```
pe[["peptideNorm"]] %>%  
  assay %>%  
  limma::plotMDS(col = as.numeric(colData(pe)$prognosis))
```



The first axis in the plot is showing the leading log fold changes (differences on the log scale) between the samples. We observe one outlying sample. In the second dimension we observe a separation according to prognosis.

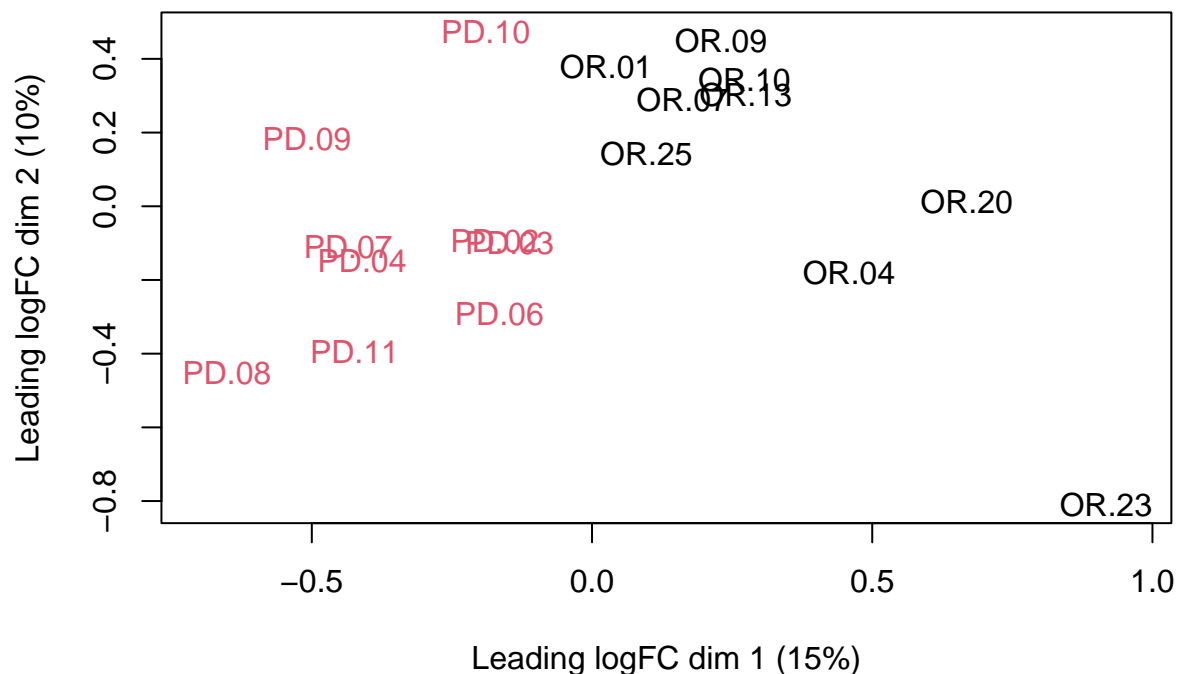
3.5 Summarization to protein level

- By default robust summarization is used: `fun = MsCoreUtils::robustSummary()`

```
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.
```

```
plotMDS(assay(pe[["protein"]]), col = as.numeric(colData(pe)$prognosis))
```



Note that the samples upon robust summarisation show a separation according to the prognosis.

4 Data Analysis

4.1 Estimation

We model the protein level expression values using `msqrob`. By default `msqrob2` estimates the model parameters using robust regression.

We will model the data with a different group mean. The group is incoded in the variable `prognosis` of the `colData`. We can specify this model by using a formula with the factor condition as its predictor: `formula = ~prognosis`.

Note, that a formula always starts with a symbol `~`.

```
pe <- msqrob(object = pe, i = "protein", formula = ~prognosis)
```

4.2 Inference

First, we extract the parameter names of the model by looking at the first model. The models are stored in the row data of the assay under the default name `msqrobModels`.

```
getCoef(rowData(pe[["protein"]])$msqrobModels[[1]])
```



```
## (Intercept) prognosisPD
## -1.1185468 0.4007461
```

We can also explore the design of the model that we specified using the the package `ExploreModelMatrix`

```
library(ExploreModelMatrix)
VisualizeDesign(colData(pe), ~prognosis)$plotlist
```

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



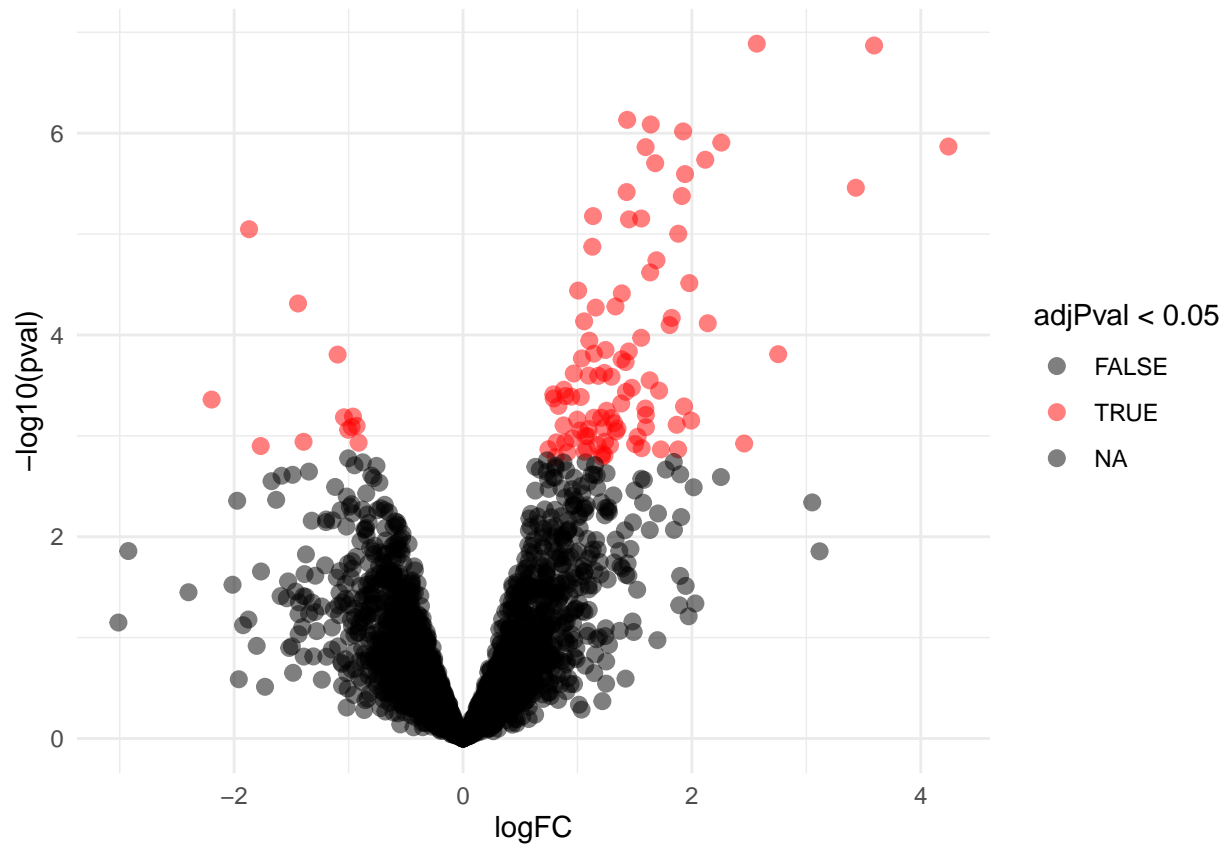
Spike-in condition A is the reference class. So the mean log₂ expression for samples from good prognosis (OR) is '(Intercept)'. The mean log₂ expression for samples from poor prognosis (PD) is '(Intercept)+prognosisPD'. Hence, the average log₂ fold change between prognosis PD and prognosis OR is modelled using the parameter 'conditionPD'. Thus, we assess the contrast 'conditionPD = 0' with our statistical test.

```
L <- makeContrast("prognosisPD=0", parameterNames = c("prognosisPD"))
pe <- hypothesisTest(object = pe, i = "protein", contrast = L)
```

4.3 Plots

4.3.1 Volcano-plot

```
volcano <- ggplot(rowData(pe[["protein"]])$prognosisPD,
  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()
volcano
```



Note, that 108 proteins are found to be differentially abundant.

4.3.2 Heatmap

Note, that we also order the sigNames according to statistical significance.

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



4.3.3 Detail plots

We make detail plots for the top 10 proteins to restrict the number of detail plots.

```
for (protName in sigNames)
#for (protName in orderProt[1:10])
{
  pePlot <- pe[protName, , c("peptideNorm","protein")]
  pePlotDf <- data.frame(longFormat(pePlot))
  pePlotDf$assay <- factor(pePlotDf$assay,
                          levels = c("peptideNorm", "protein"))
  pePlotDf$prognosis <- as.factor(colData(pePlot)[pePlotDf$colname, "prognosis"])

  # plotting
  p1 <- ggplot(data = pePlotDf,
               aes(x = colname, y = value, group = rowname)) +
    geom_line() +
    geom_point() +
    theme(axis.text.x = element_text(angle = 70, hjust = 1, vjust = 0.5)) +
    facet_grid(~assay) +
    ggtitle(protName)
  print(p1)

  # plotting 2
  p2 <- ggplot(pePlotDf, aes(x = colname, y = value, fill = prognosis)) +
```

```

geom_boxplot(outlier.shape = NA) +
geom_point(
  position = position_jitter(width = .1),
  aes(shape = rowname)) +
scale_shape_manual(values = 1:nrow(pePlotDf)) +
labs(title = protName, x = "sample", y = "peptide intensity (log2)") +
theme(axis.text.x = element_text(angle = 70, hjust = 1, vjust = 0.5)) +
facet_grid(~assay)
print(p2)
}

```



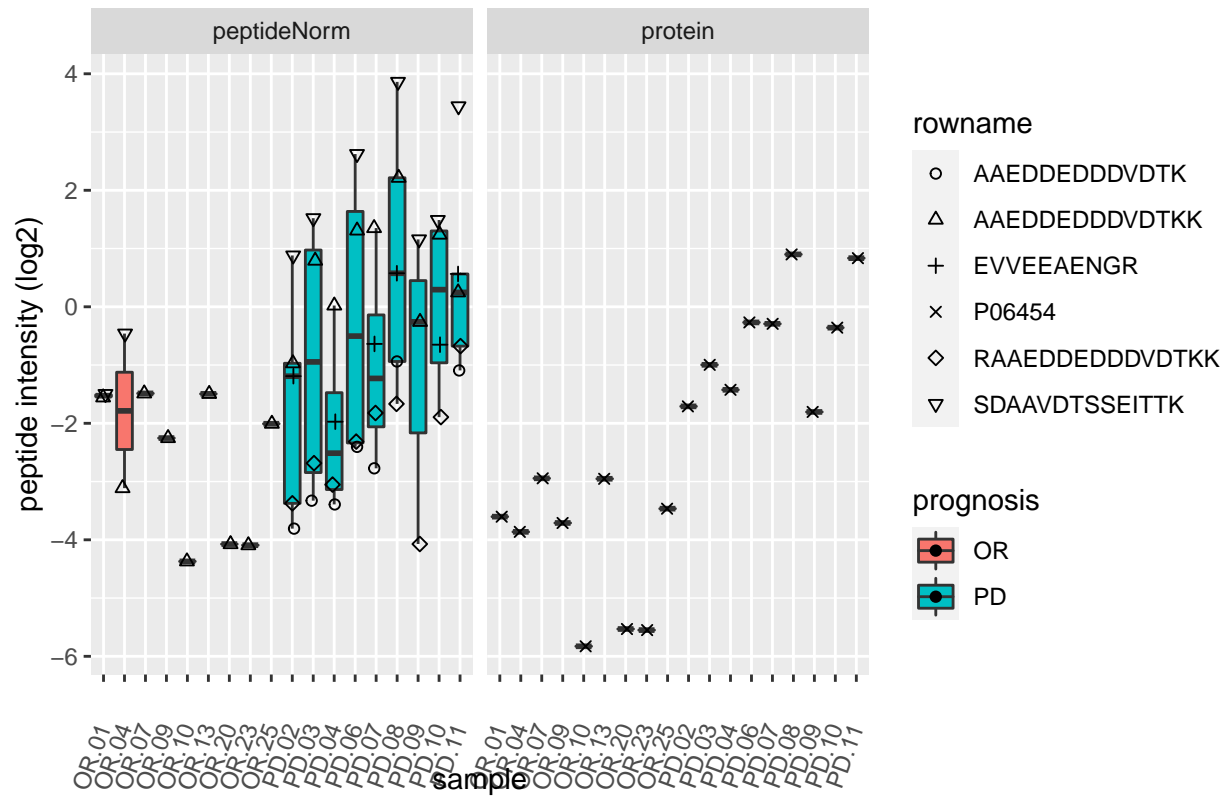
P61604



P06454



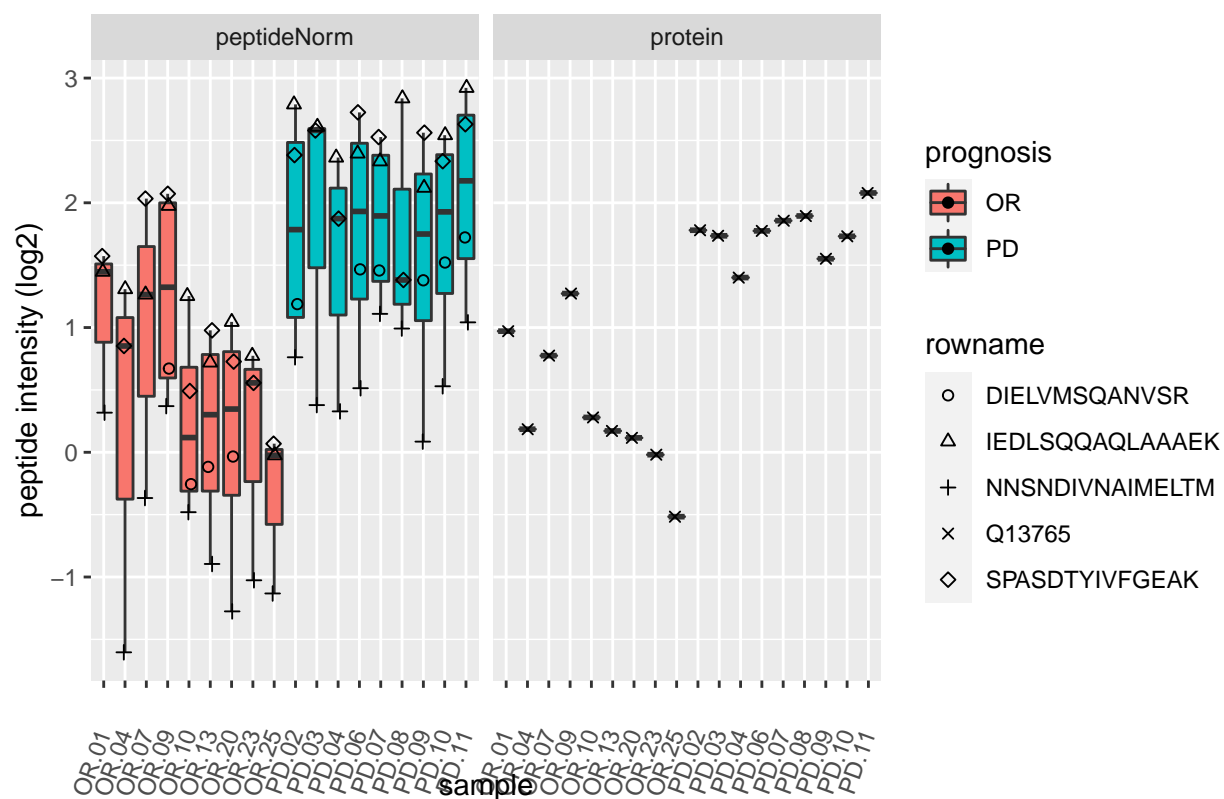
P06454



Q13765



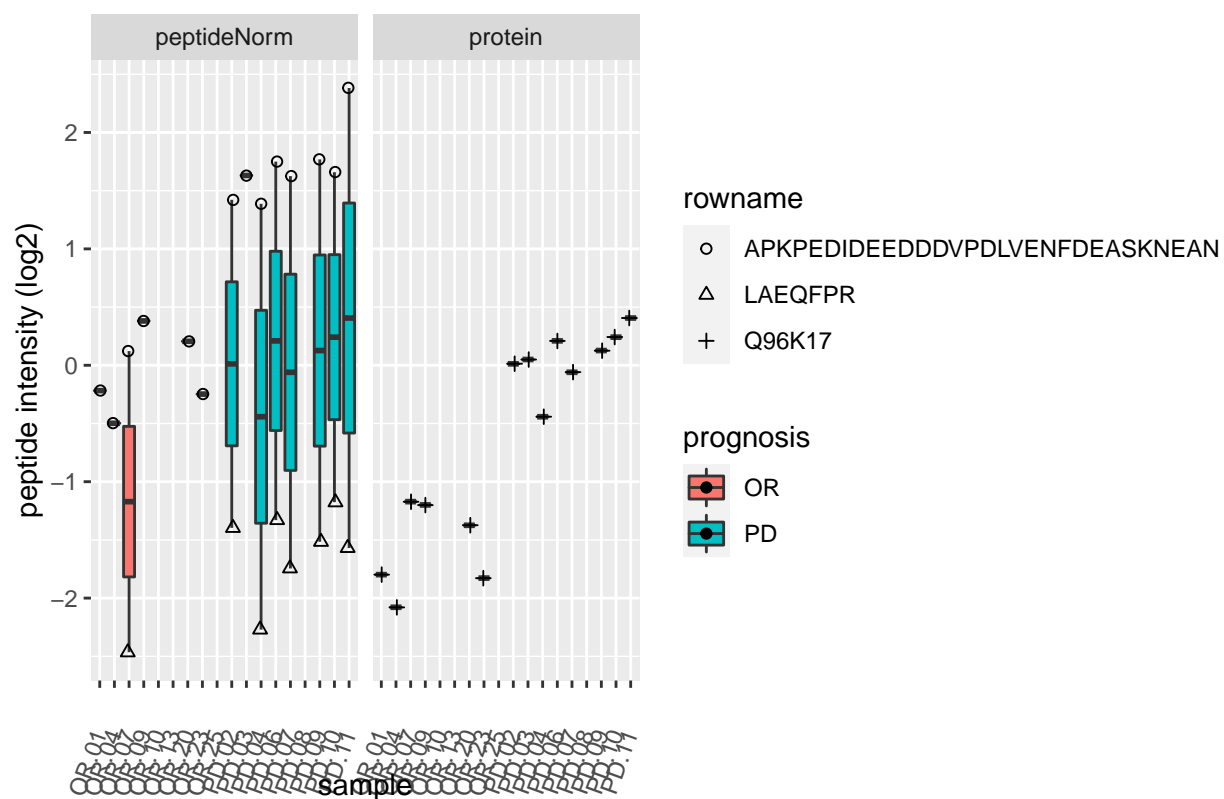
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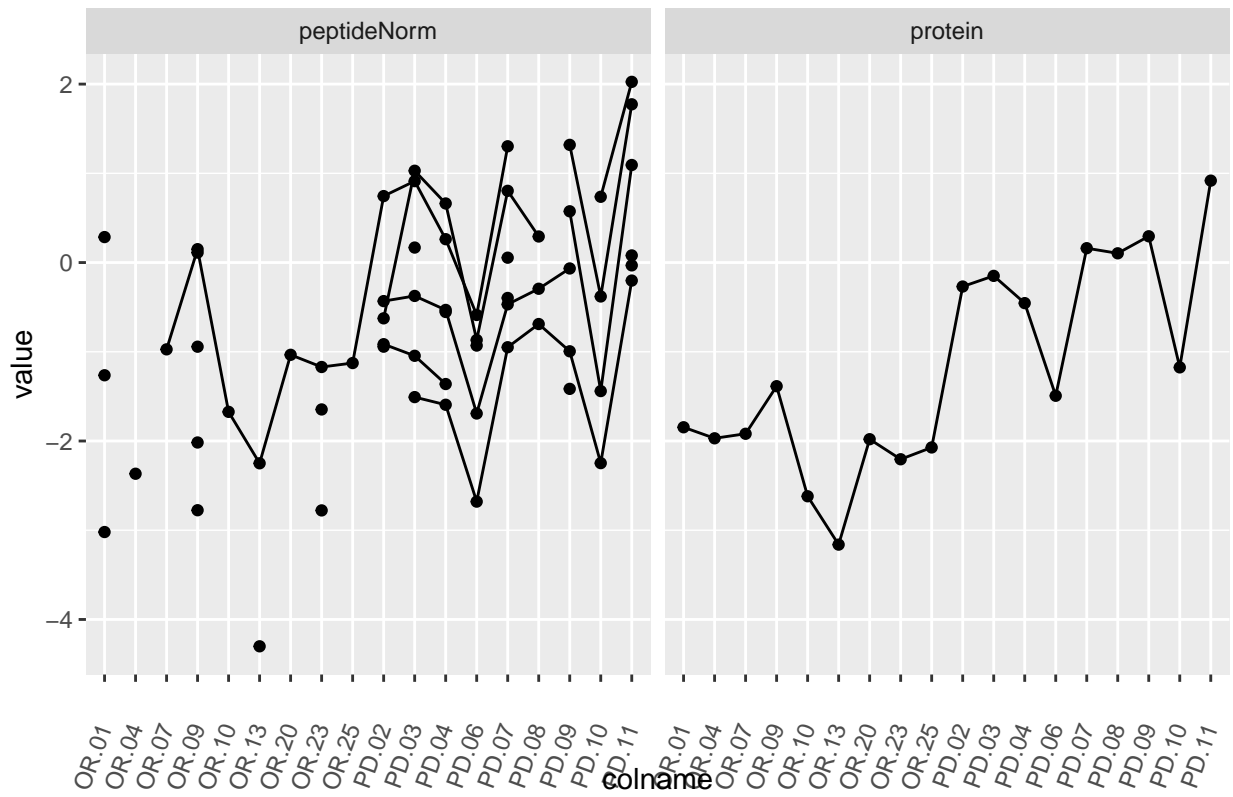
Q96K17

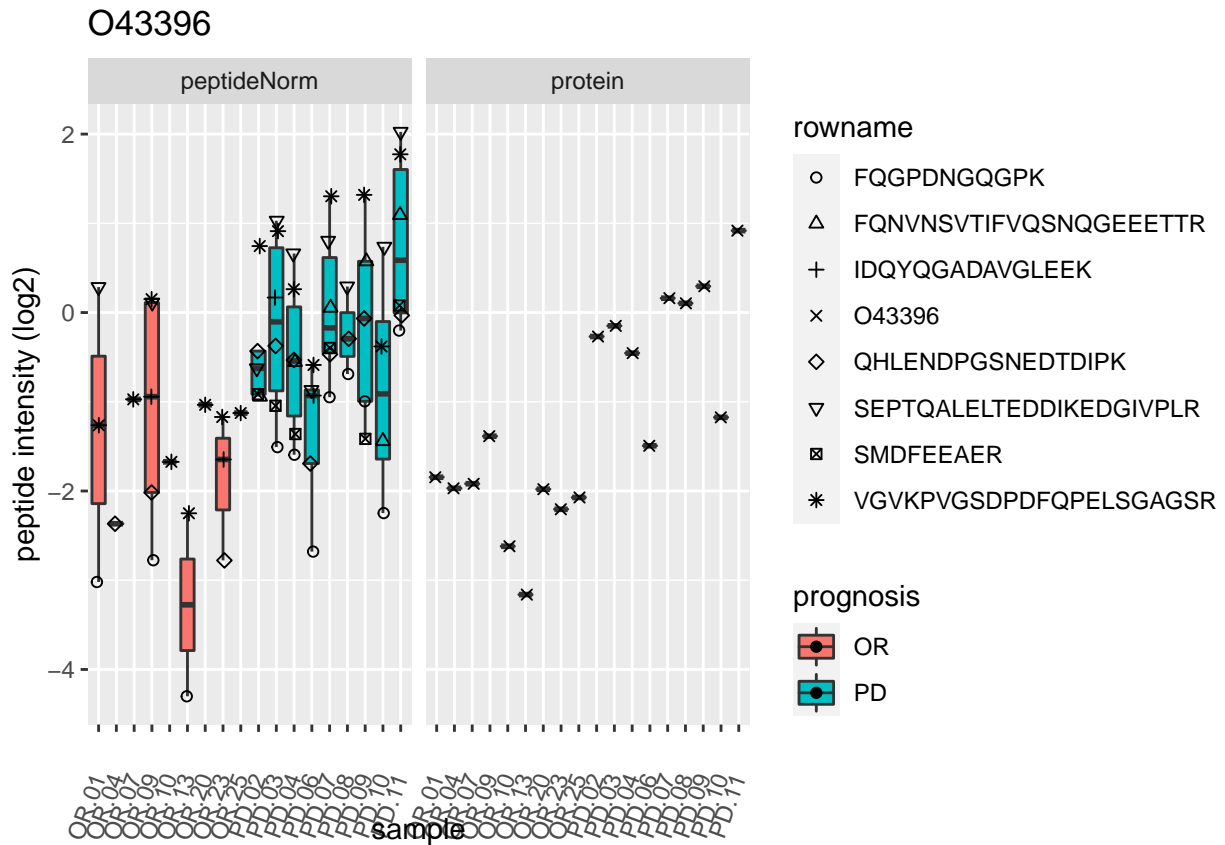


Q96K17



O43396

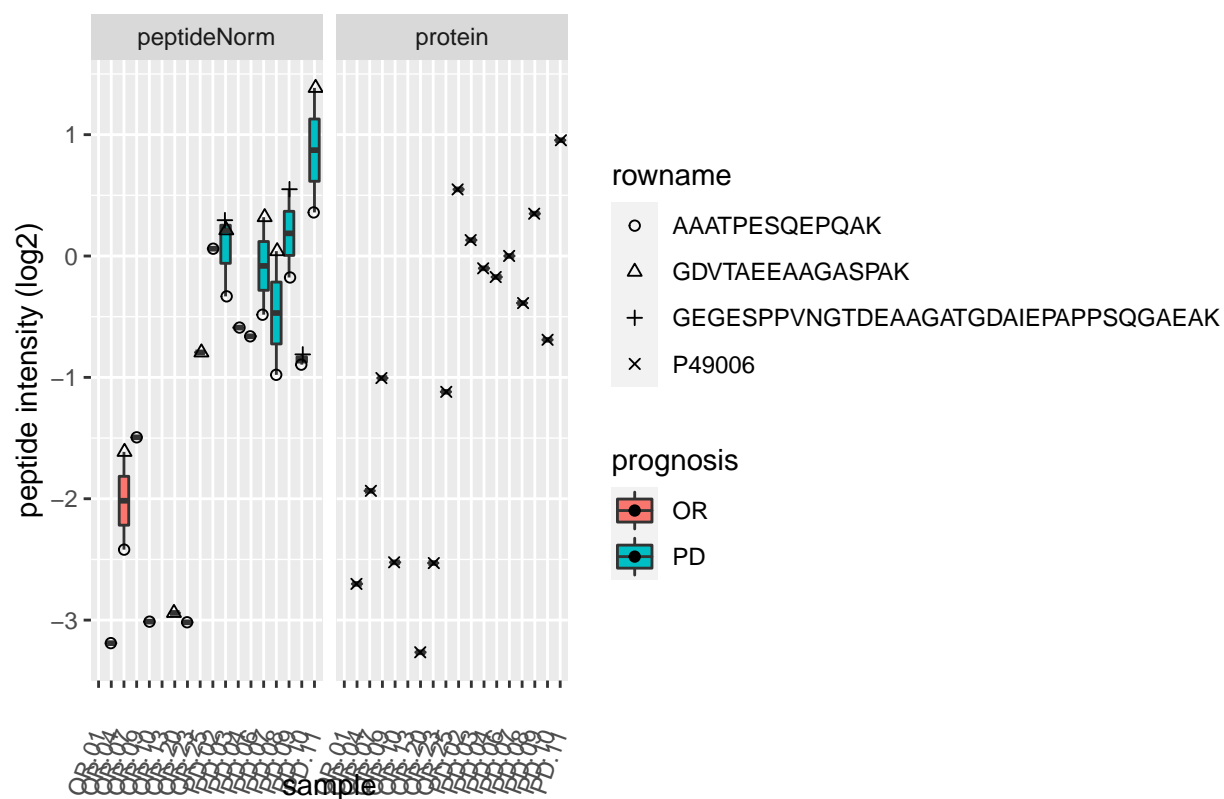




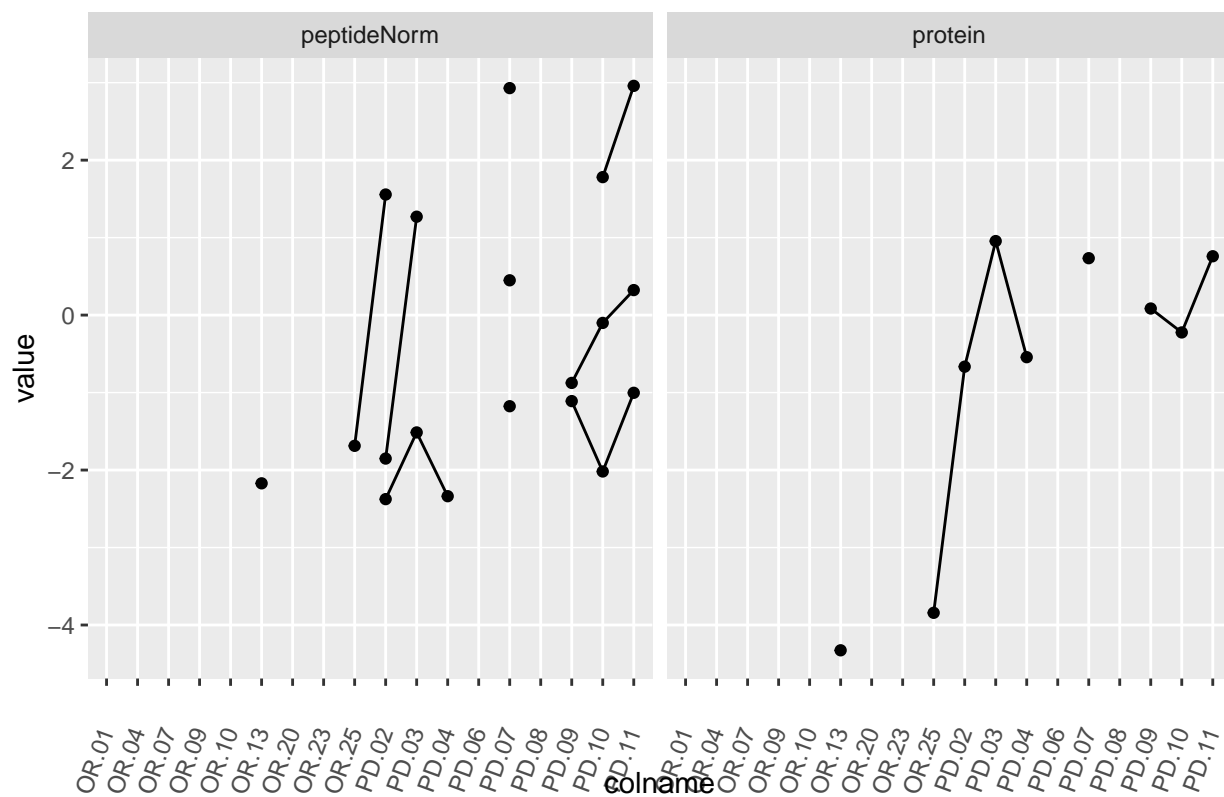
P49006



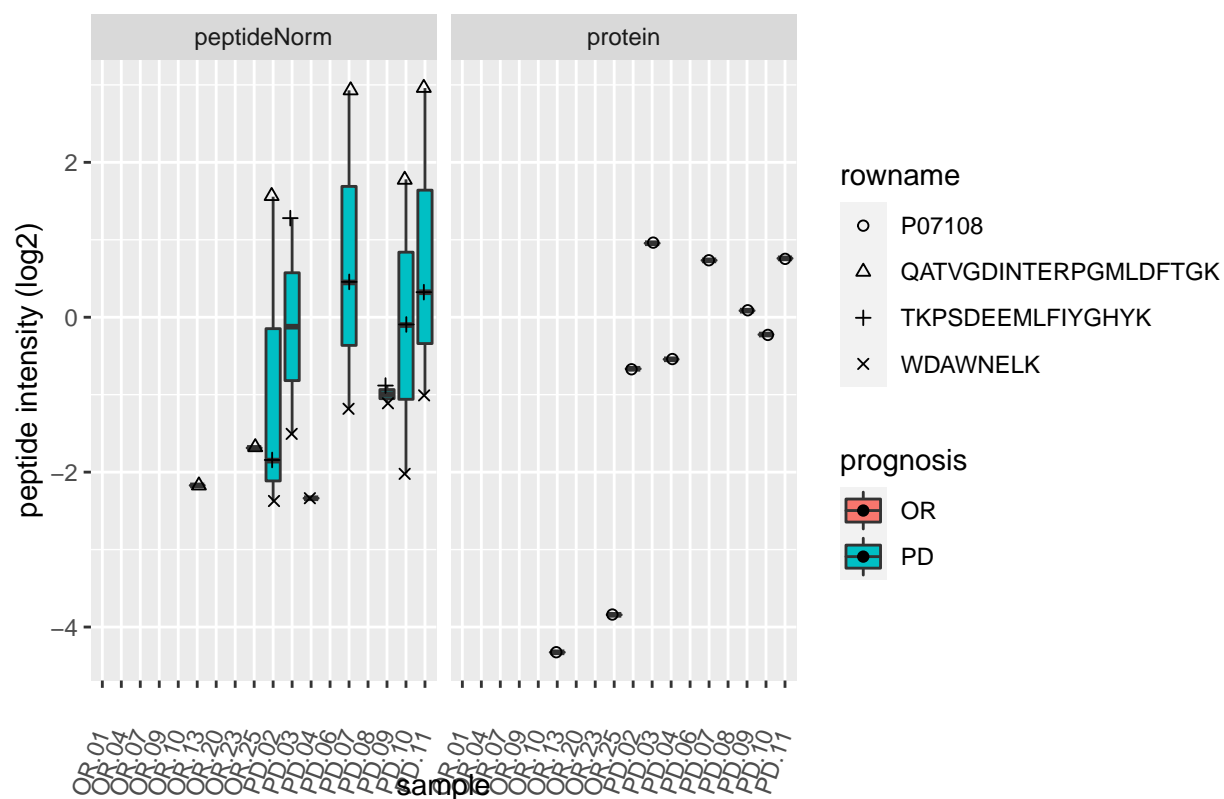
P49006



P07108

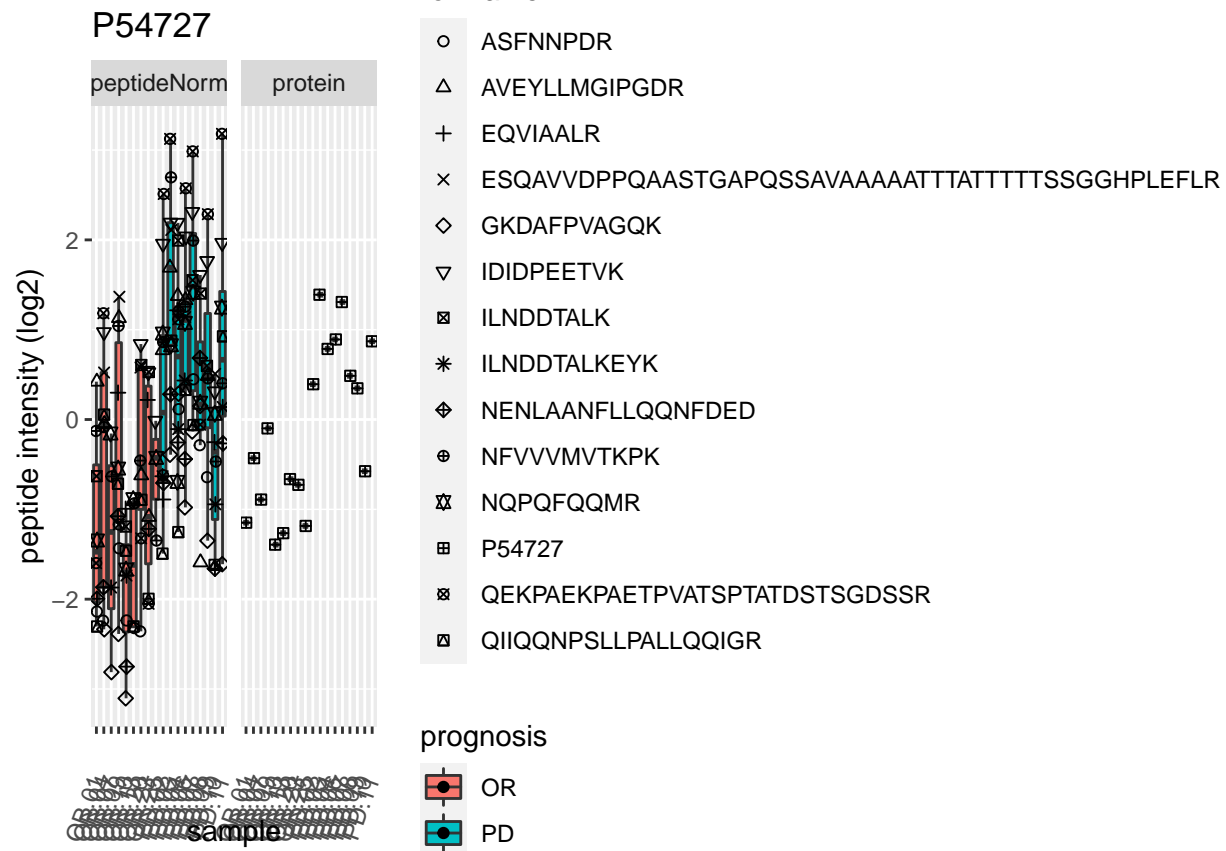


P07108



P54727

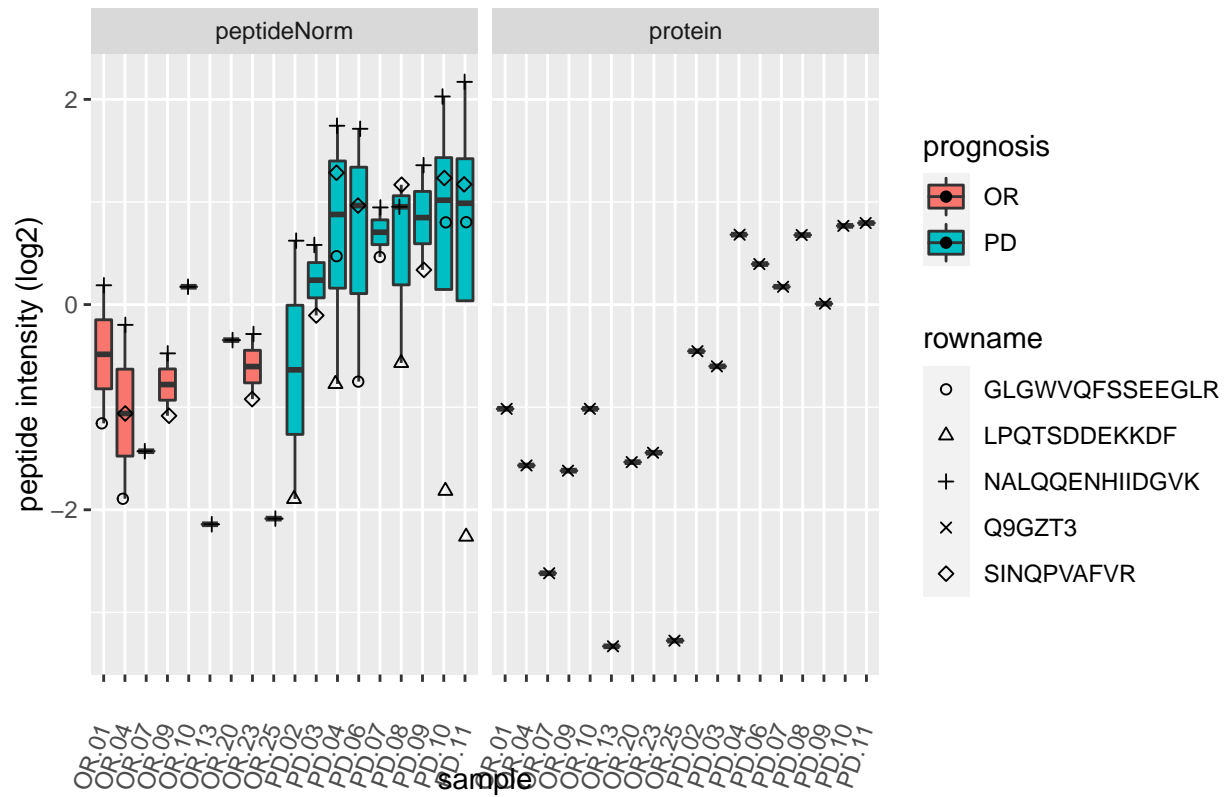




Q9GZT3



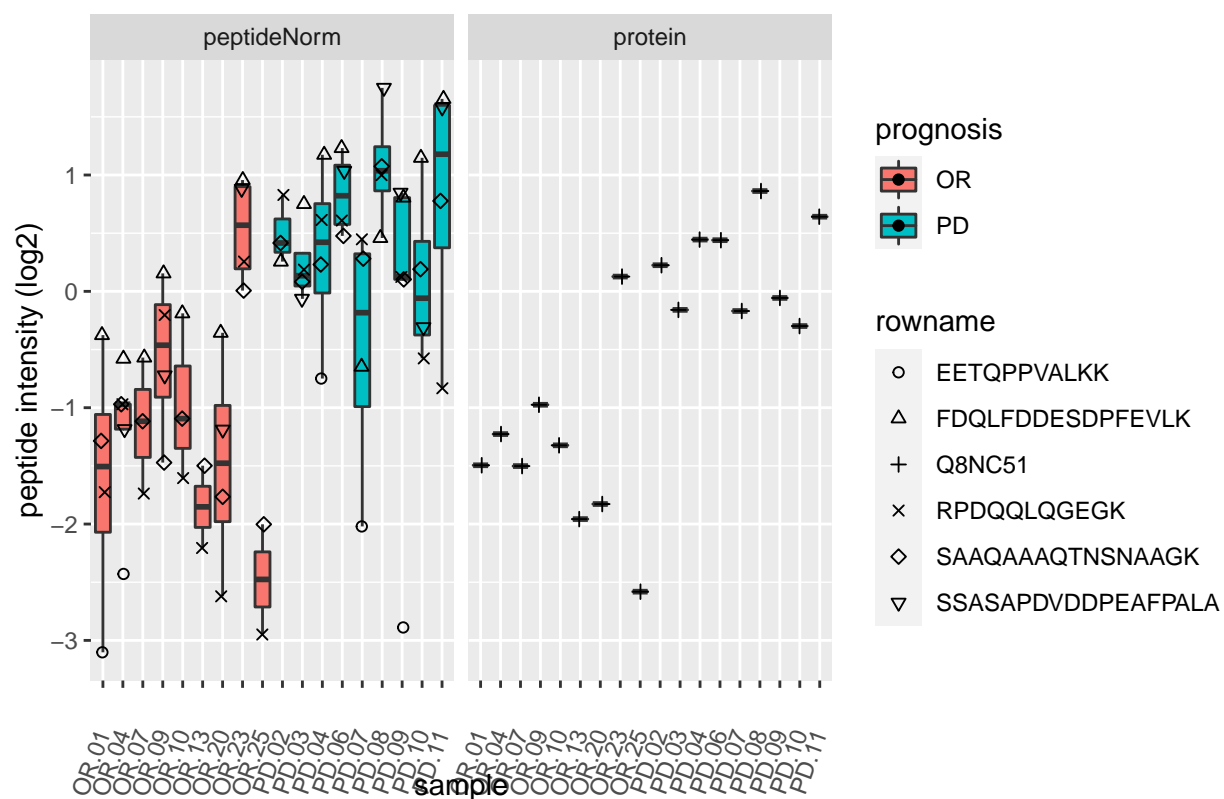
Q9GZT3



Q8NC51



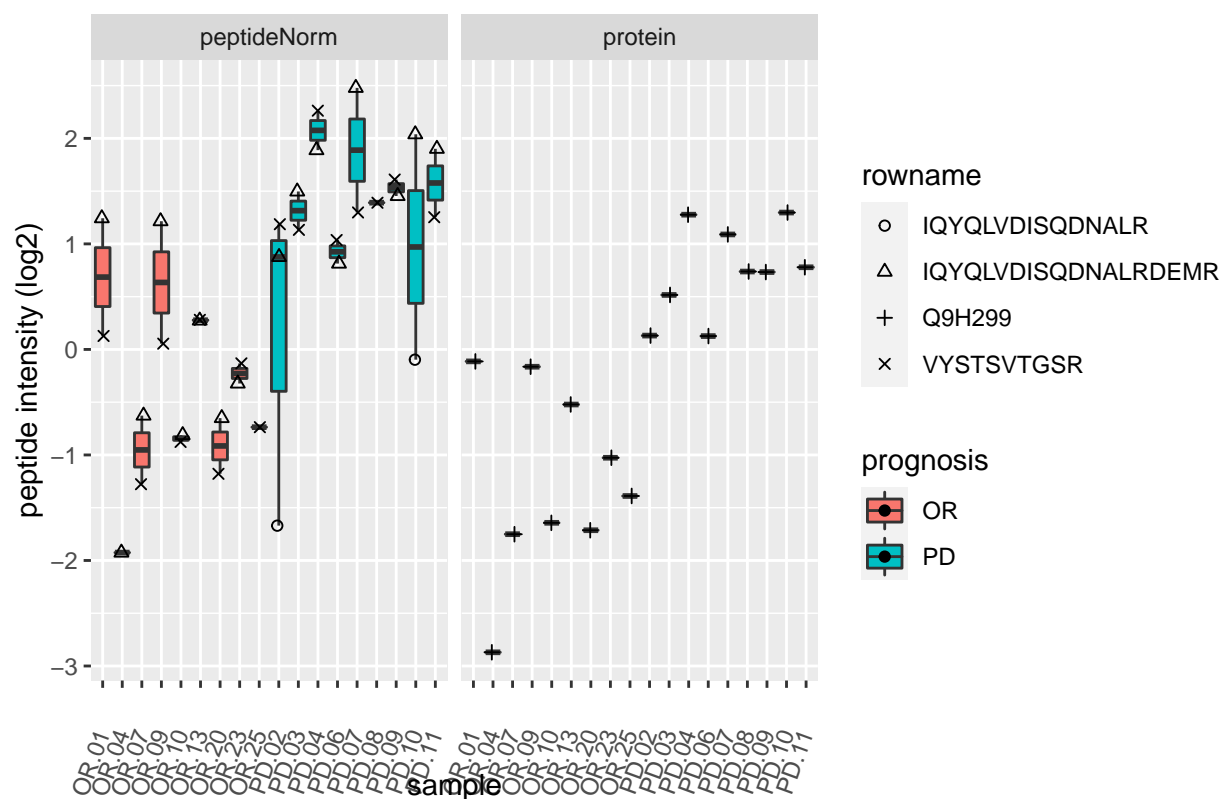
Q8NC51



Q9H299



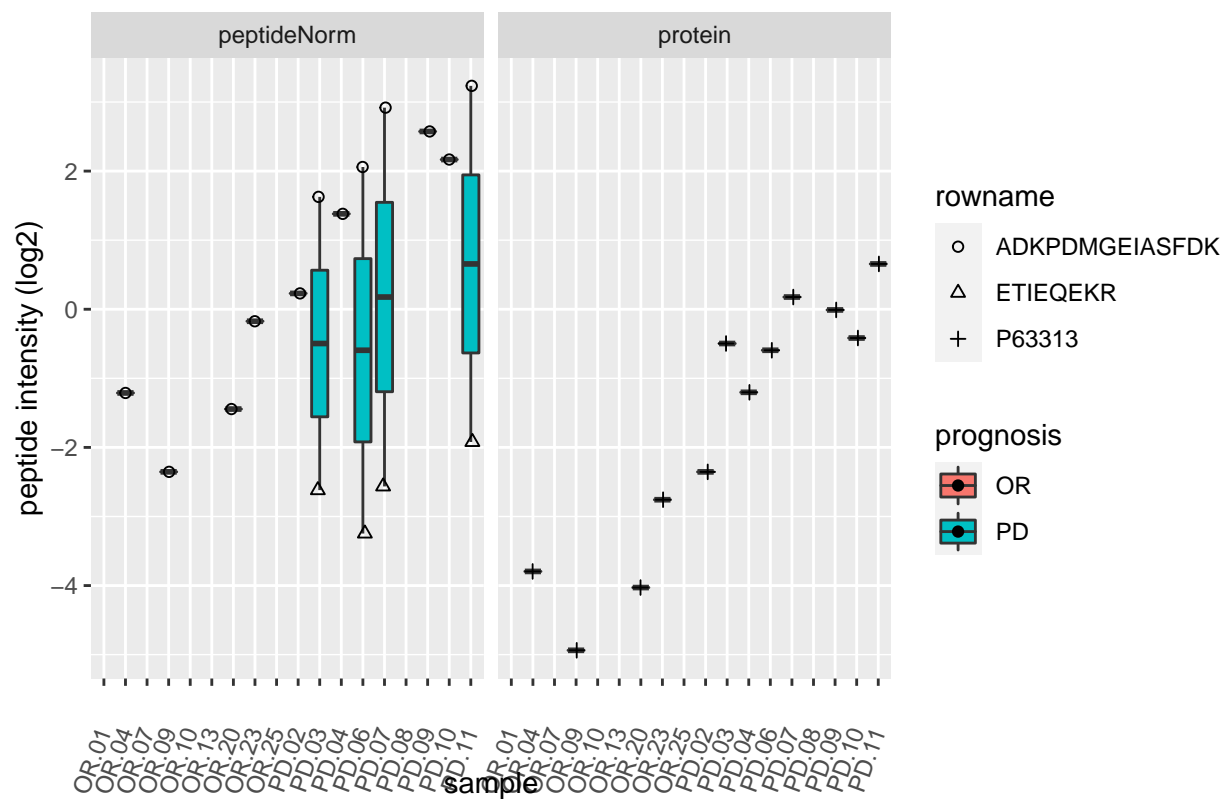
Q9H299



P63313



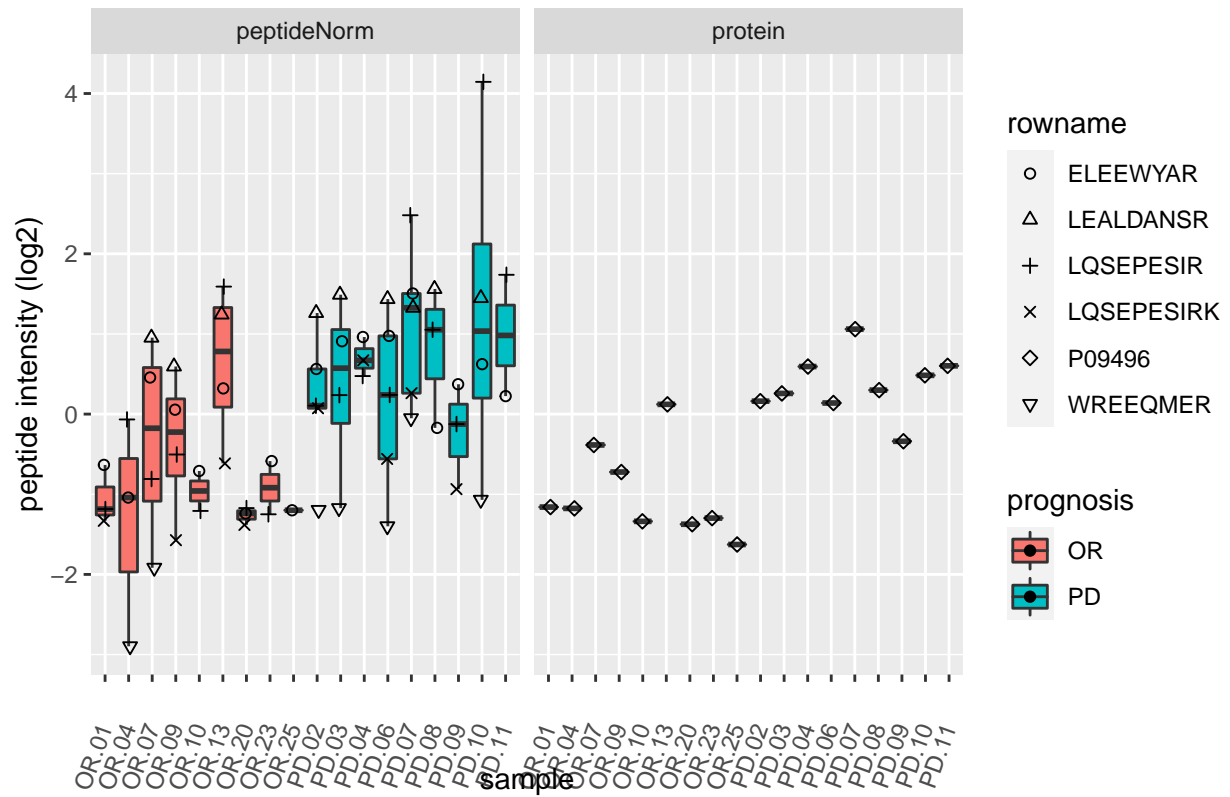
P63313



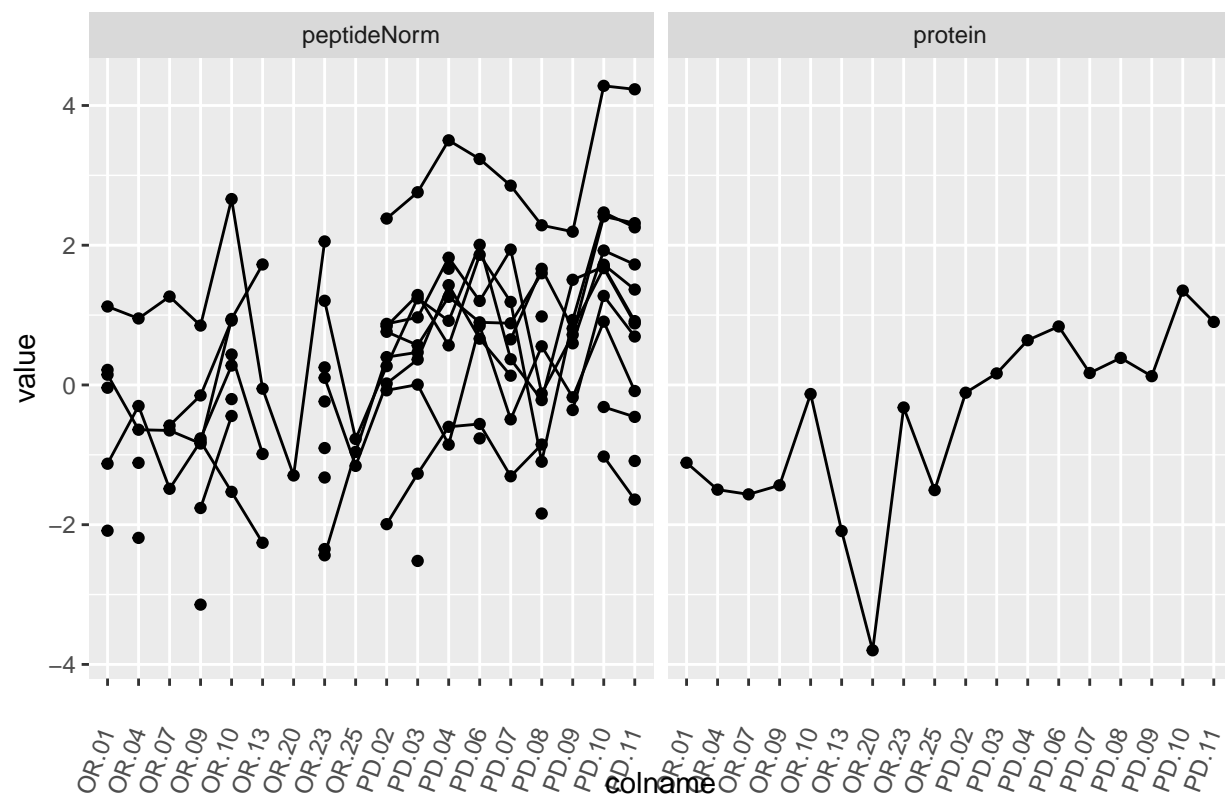
P09496

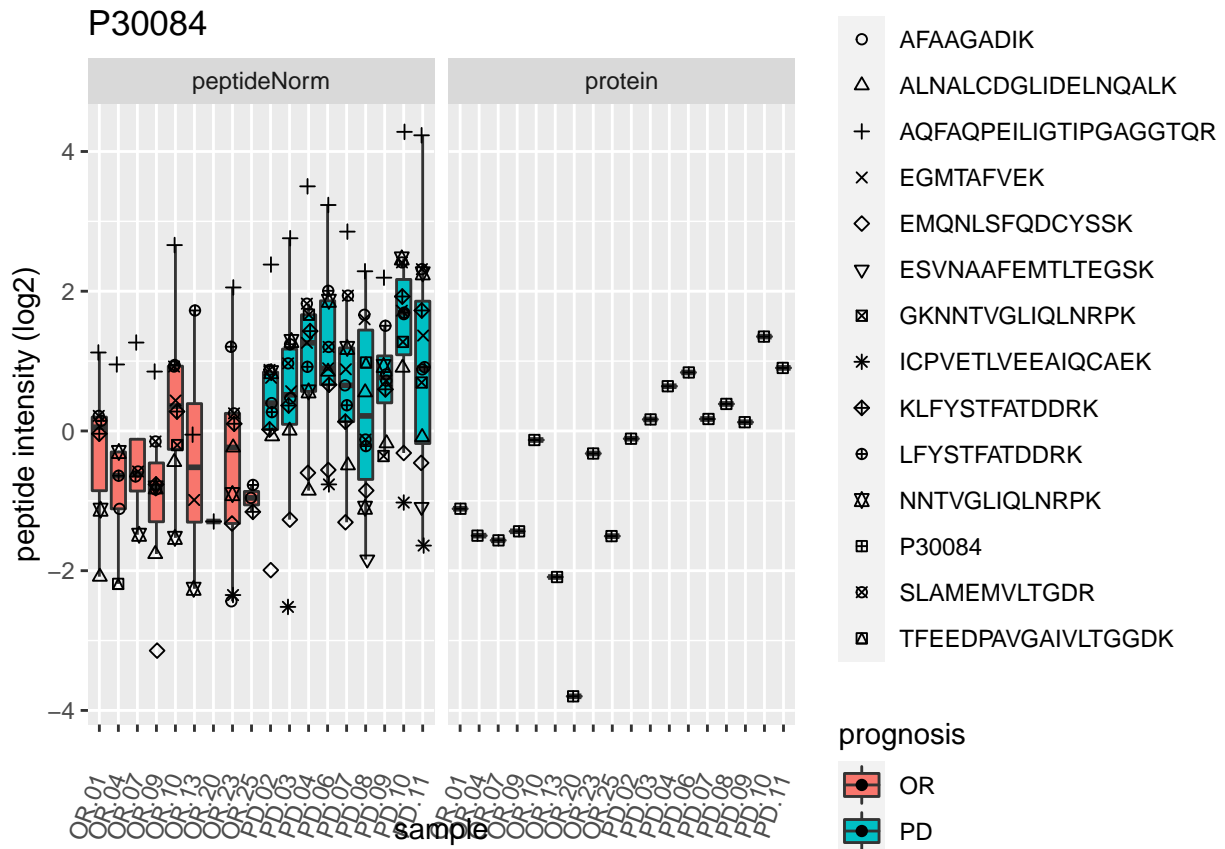


P09496



P30084

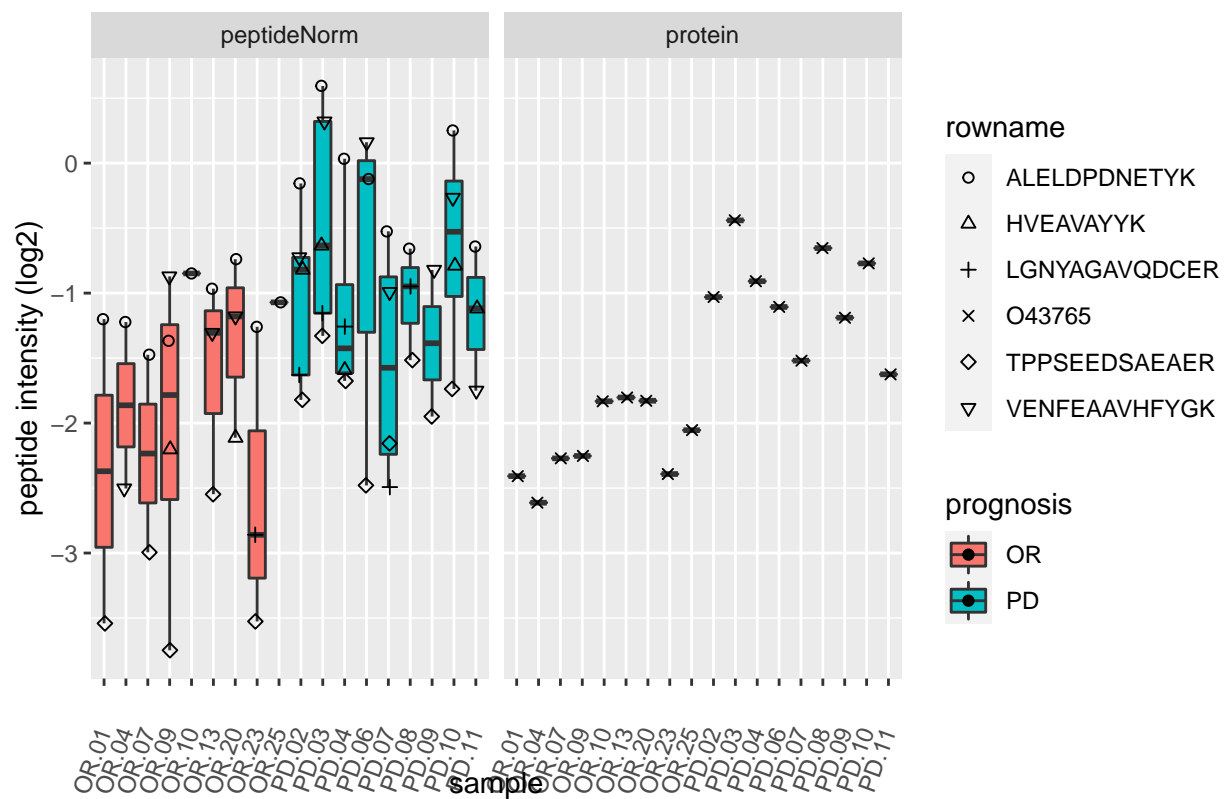




O43765

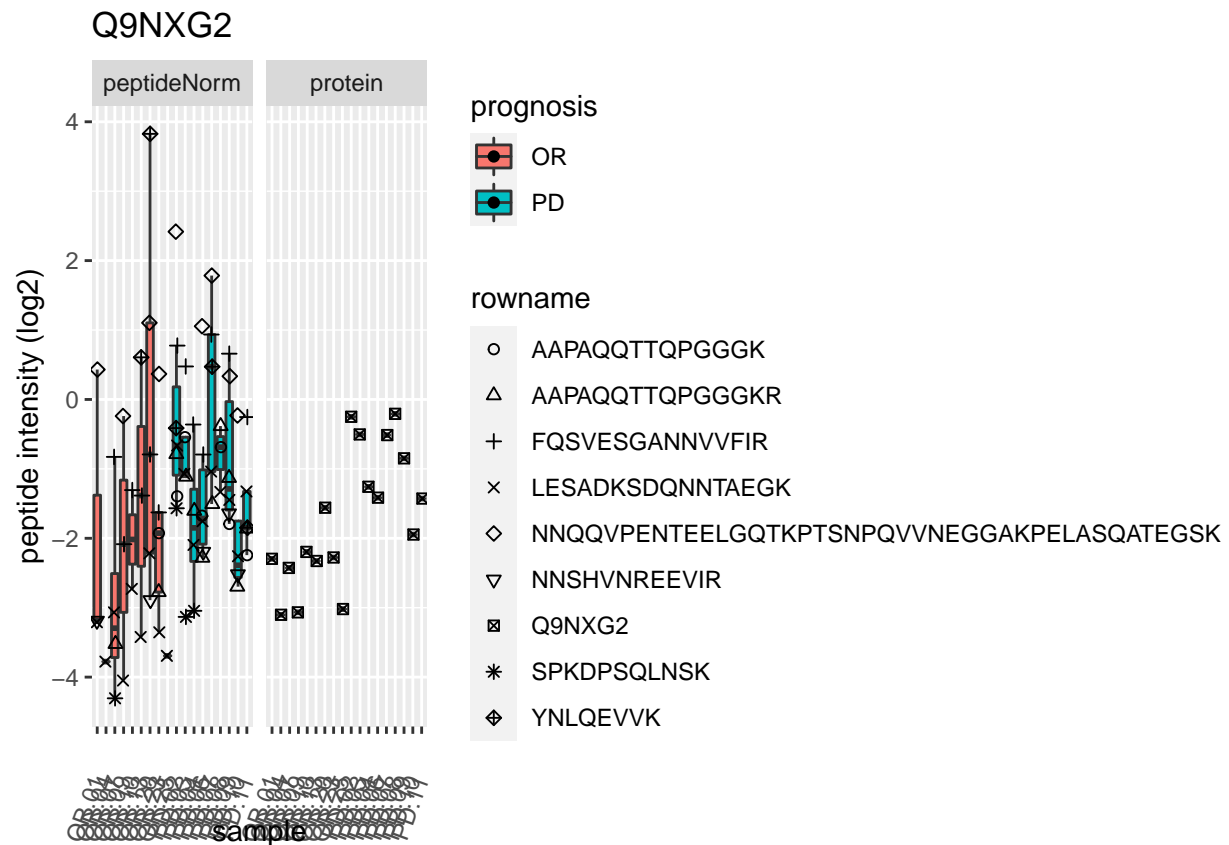


O43765



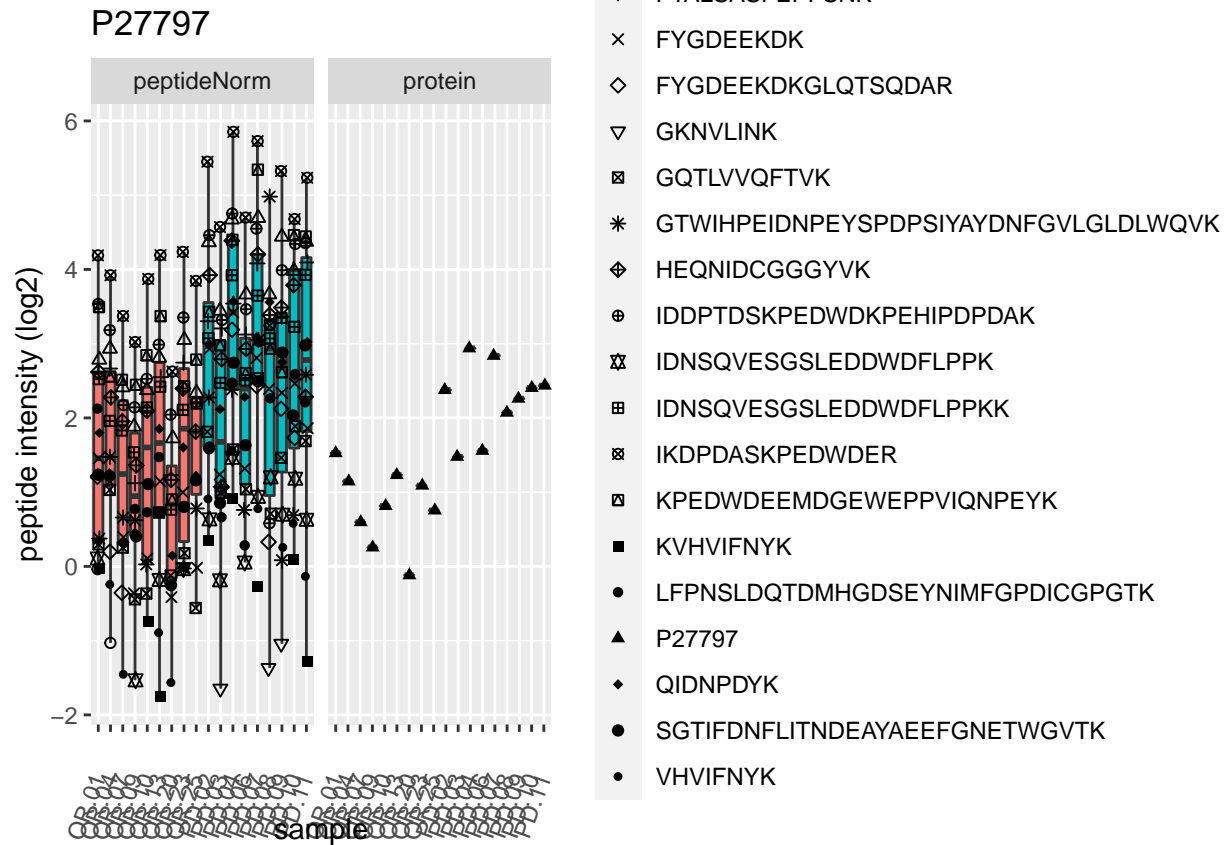
Q9NXG2



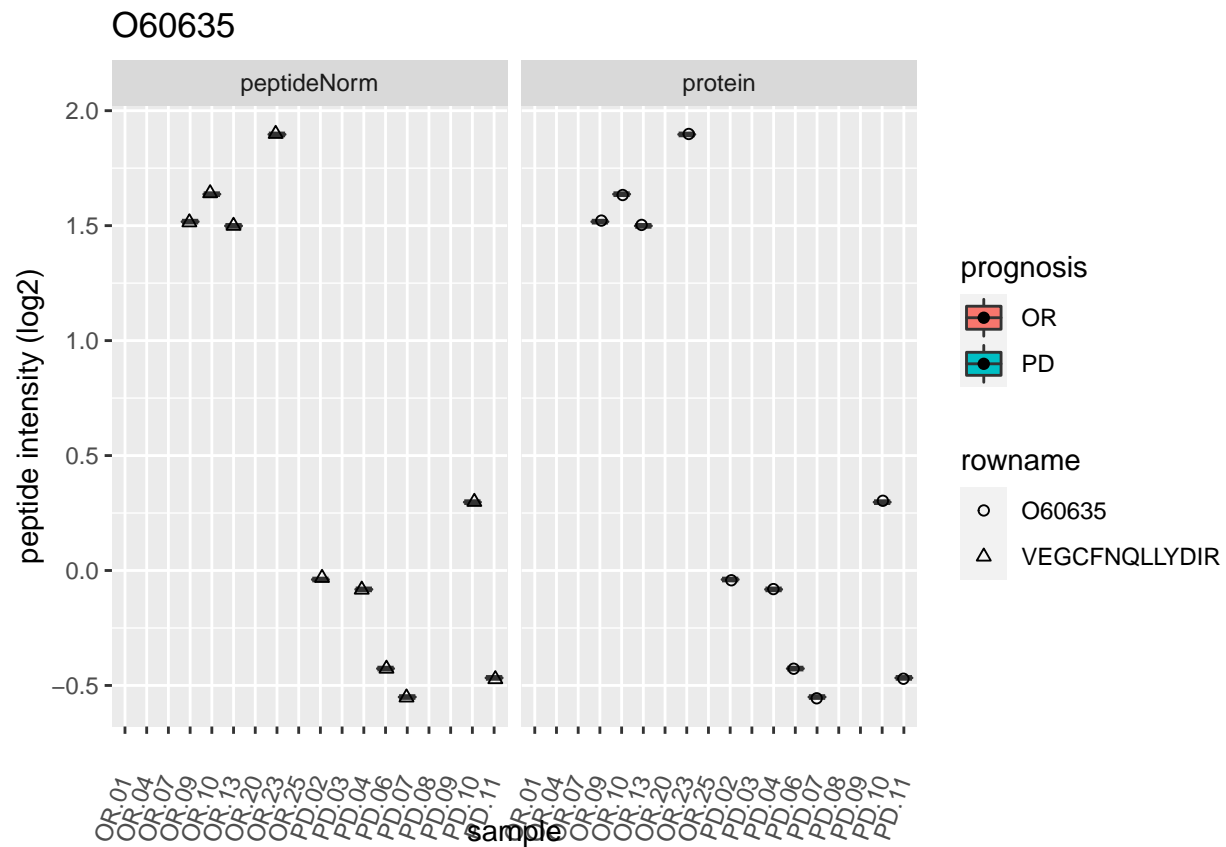


P27797





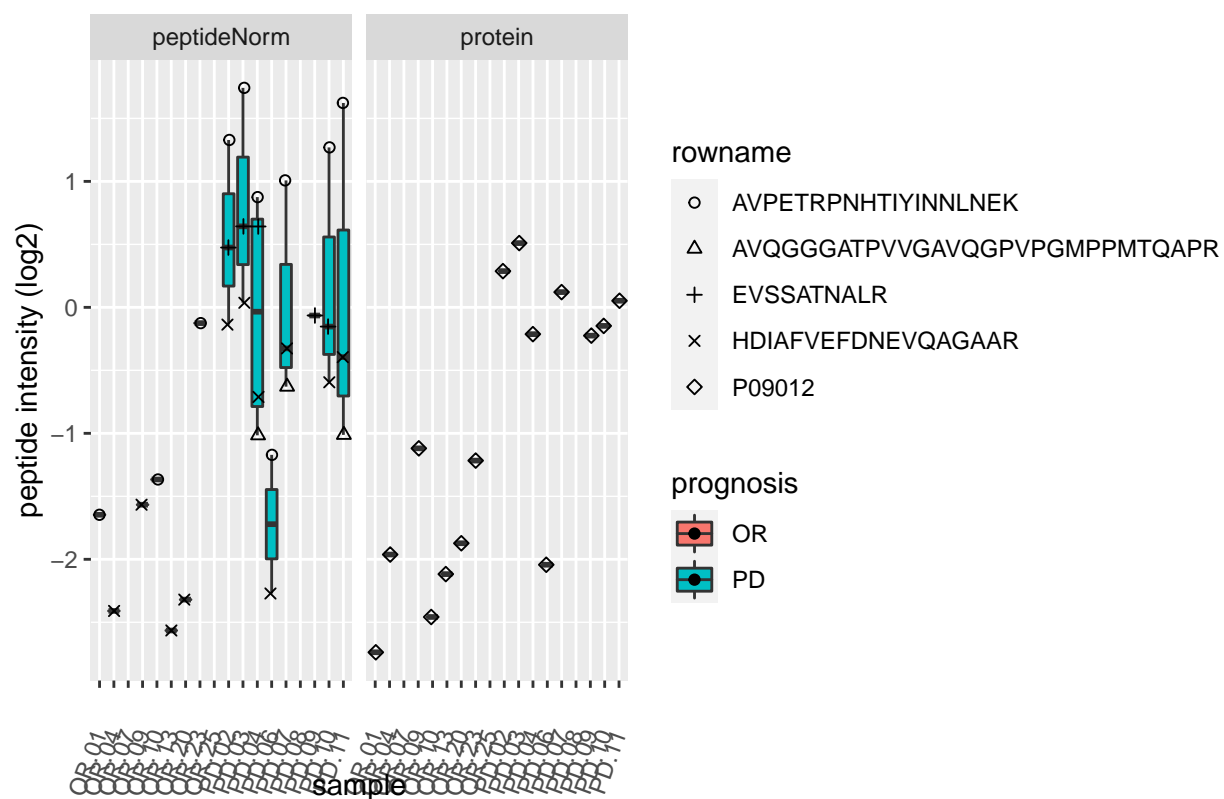




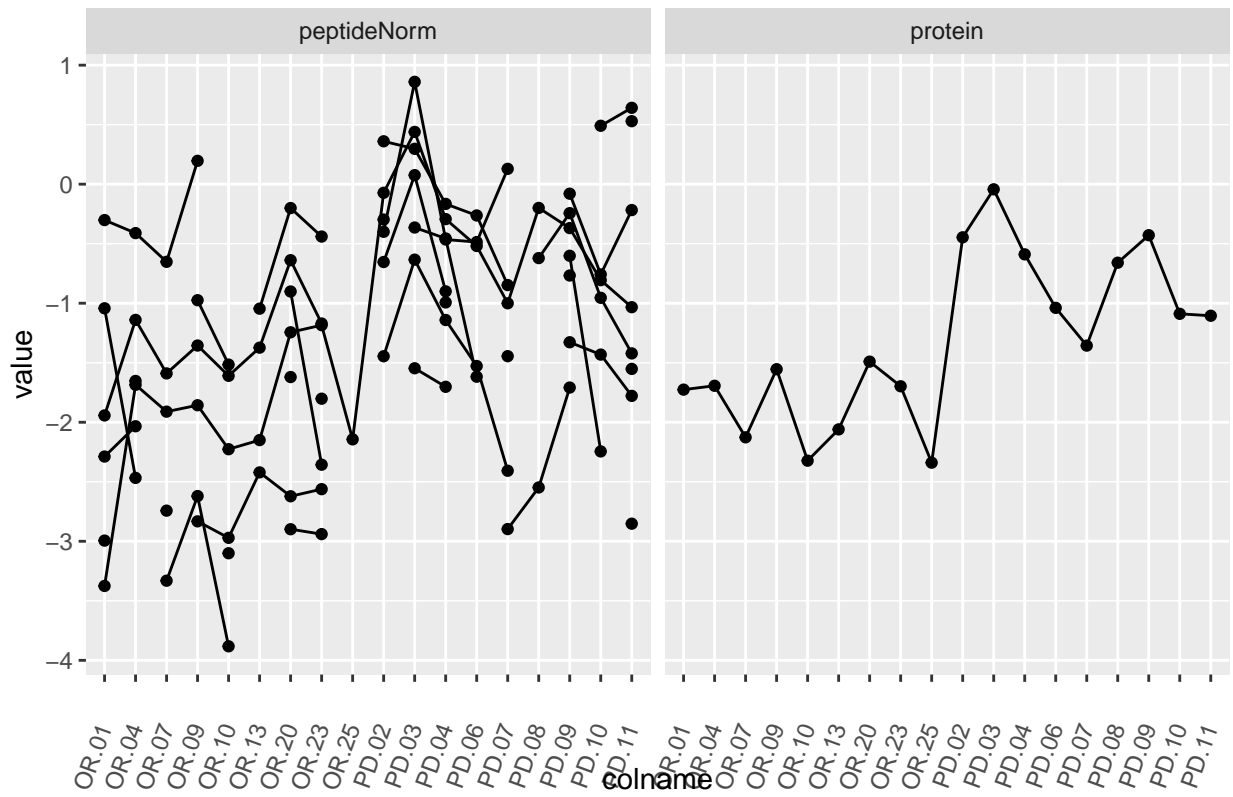
P09012

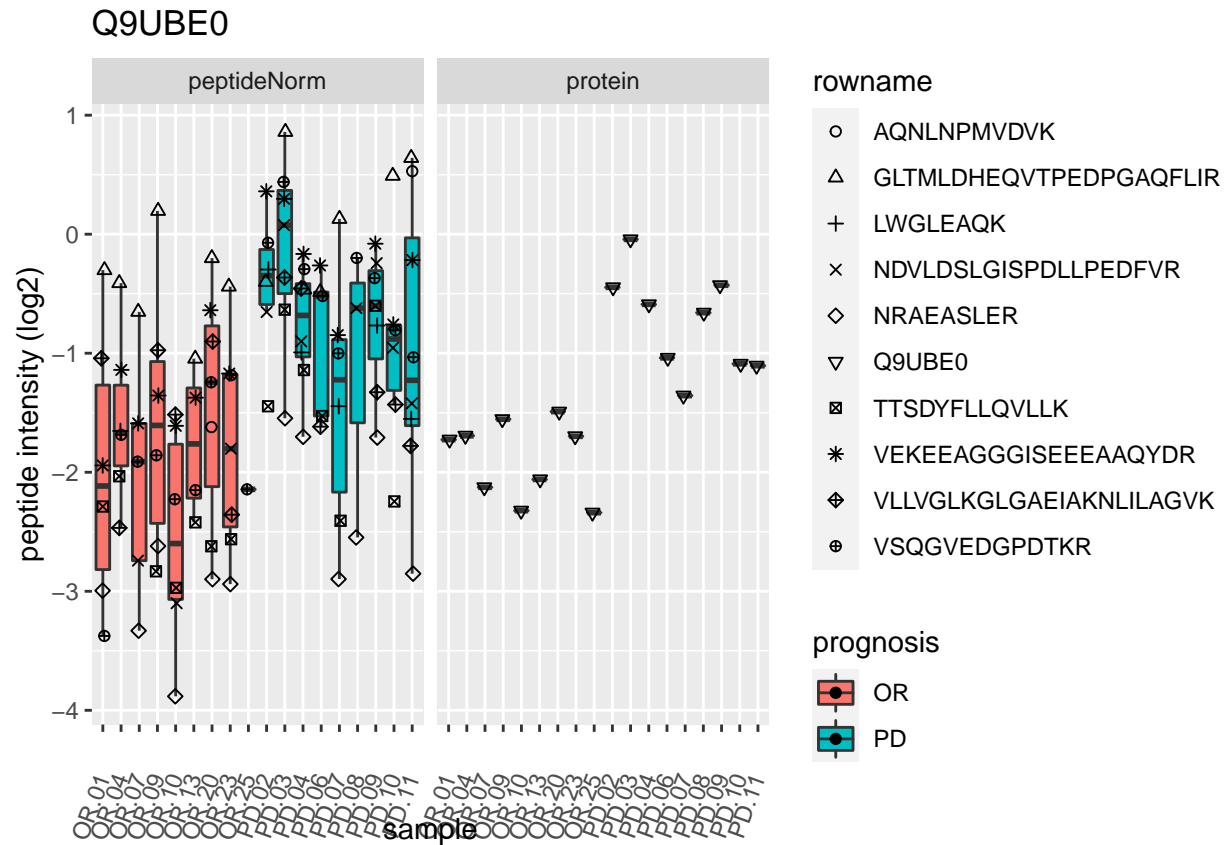


P09012



Q9UBE0

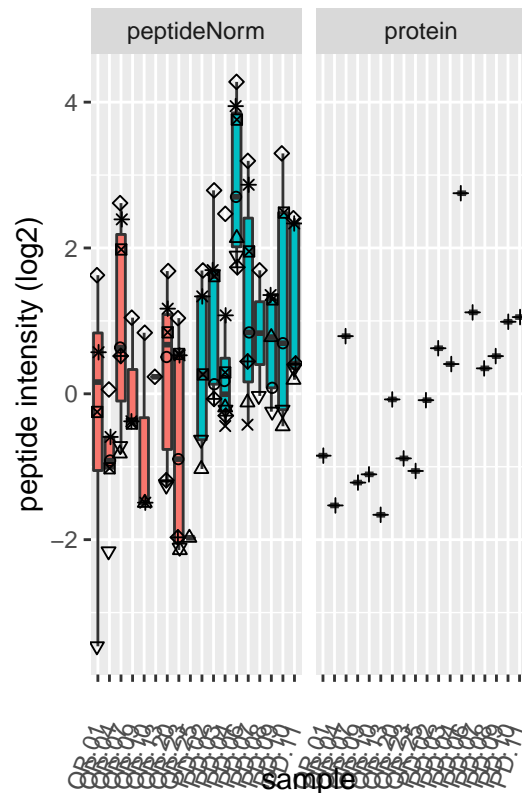




P55327



P55327



rowname

- ASAAFSSVGSVITK
- △ ELAKVEEEIQTLTSQVLAAG
- + P55327
- × SFEEKVENLK
- ◇ TDPVPEEGEDVAATISATETLSEEEQEELRR
- ▽ TSETLSQAGQK
- ⊠ VEEIQTLTSQVLAAG
- * VGGTKPAGGDFGEVLNSAANASATTEPLPEK
- ◆ VGGTKPAGGDFGEVLNSAANASATTEPLPEKTQESL

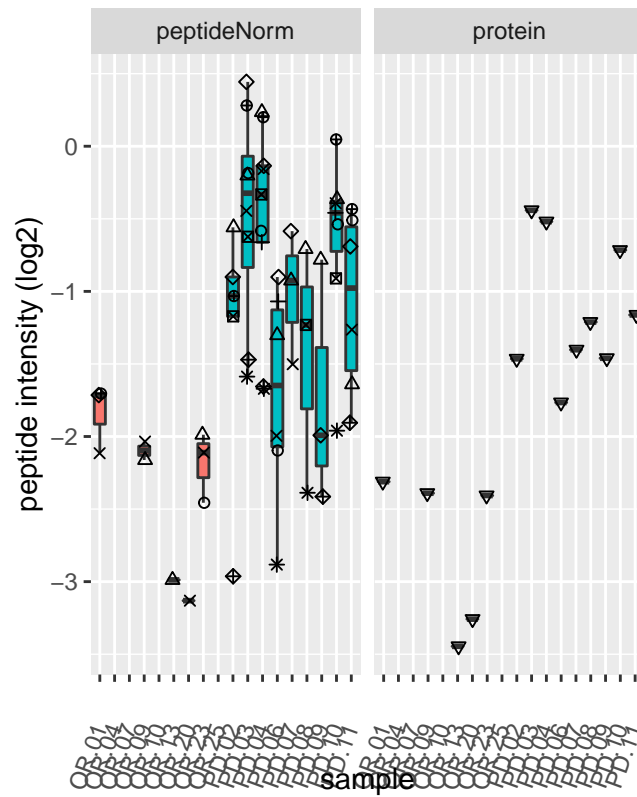
prognosis

- OR
- PD

P46108



P46108



rowname

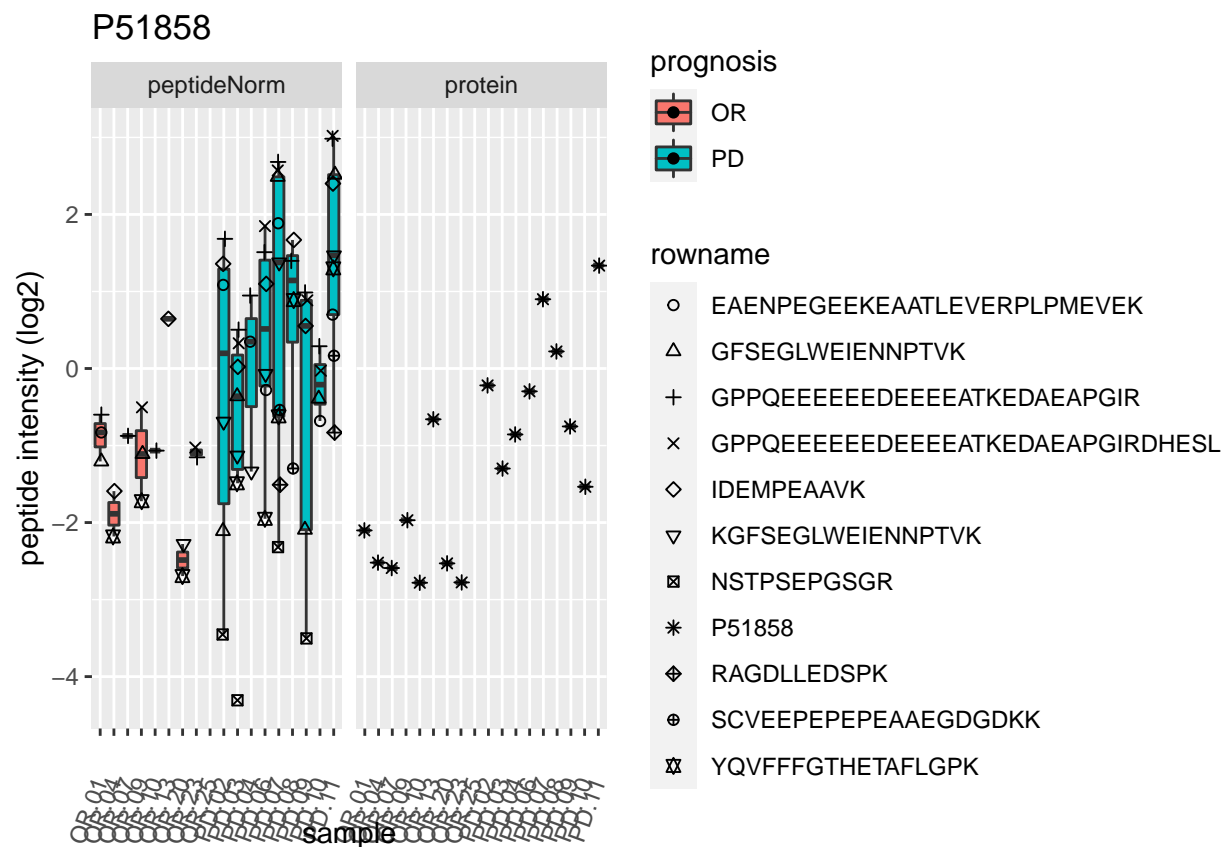
- ALFDFNGNDEEDLPFK
- △ DSSTSPGDYVLSVSENSR
- + HGVFLVR
- × IGDQEFDSLPALEFYK
- ◇ IHYLDTTTLIEPVSR
- ▽ P46108
- ⊠ QEAVALLQGQR
- * QGSGVILR
- ⊞ TALAELVGELVK
- ⊕ VSHYIINSSGPRPPVPPSPAQPPPGVSPSR

prognosis

- OR
- PD

P51858

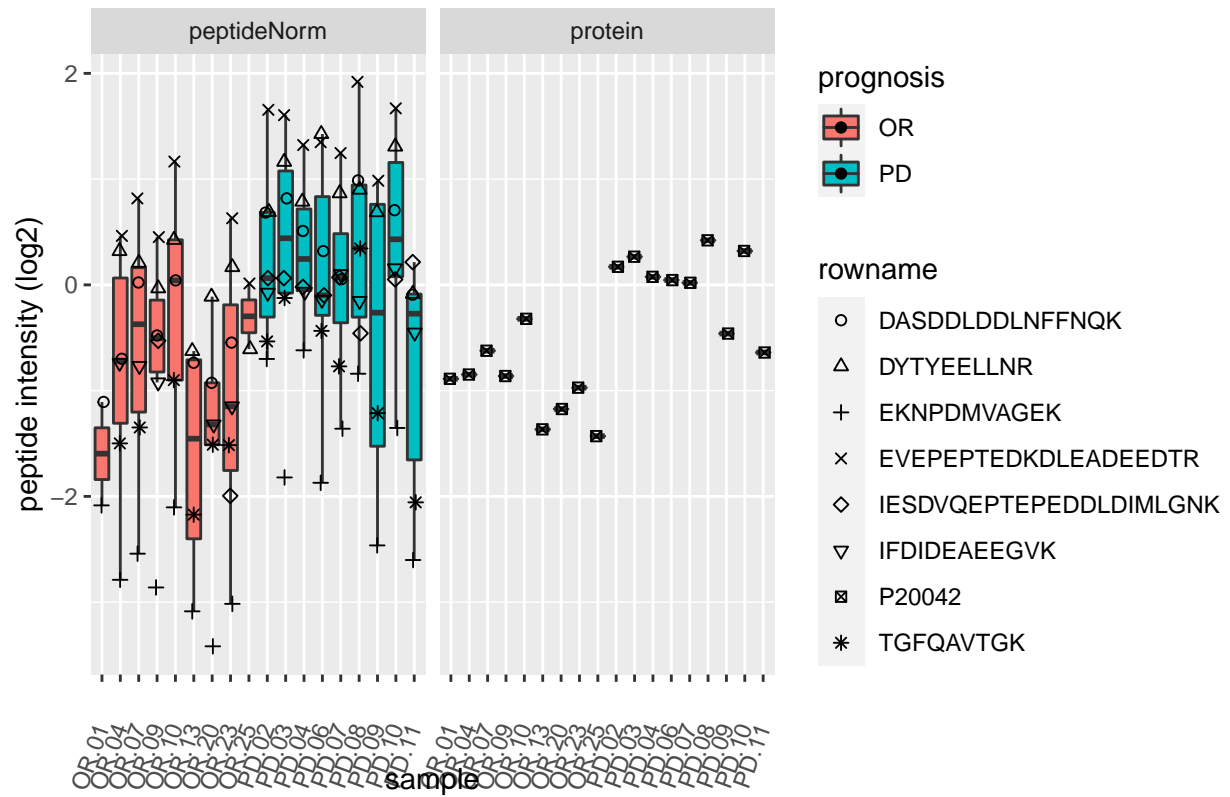




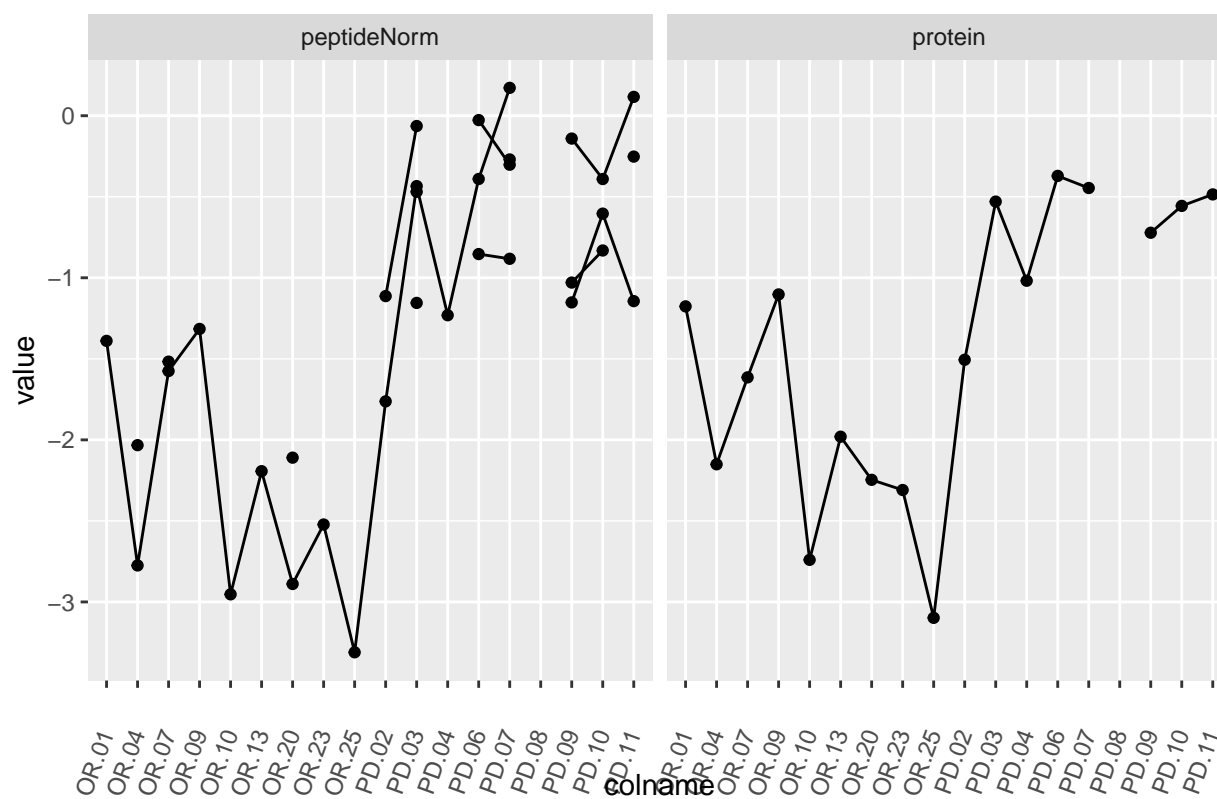
P20042



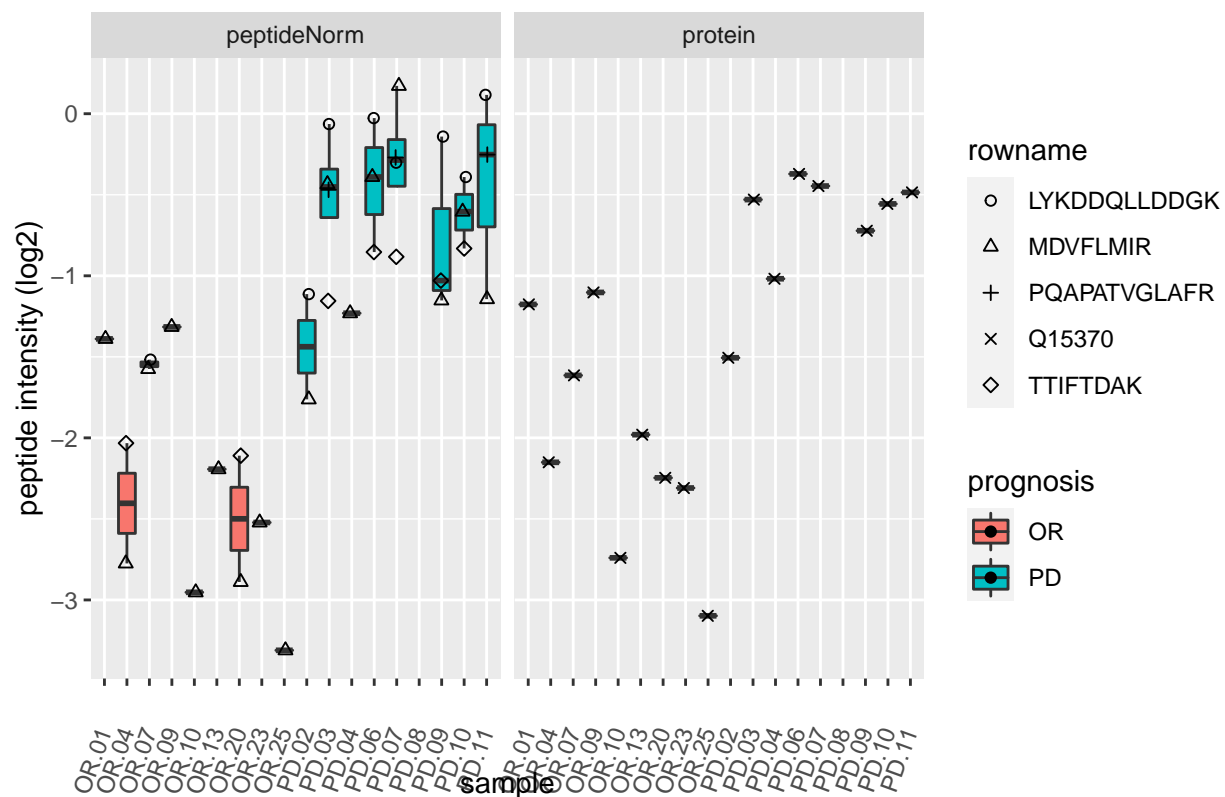
P20042



Q15370



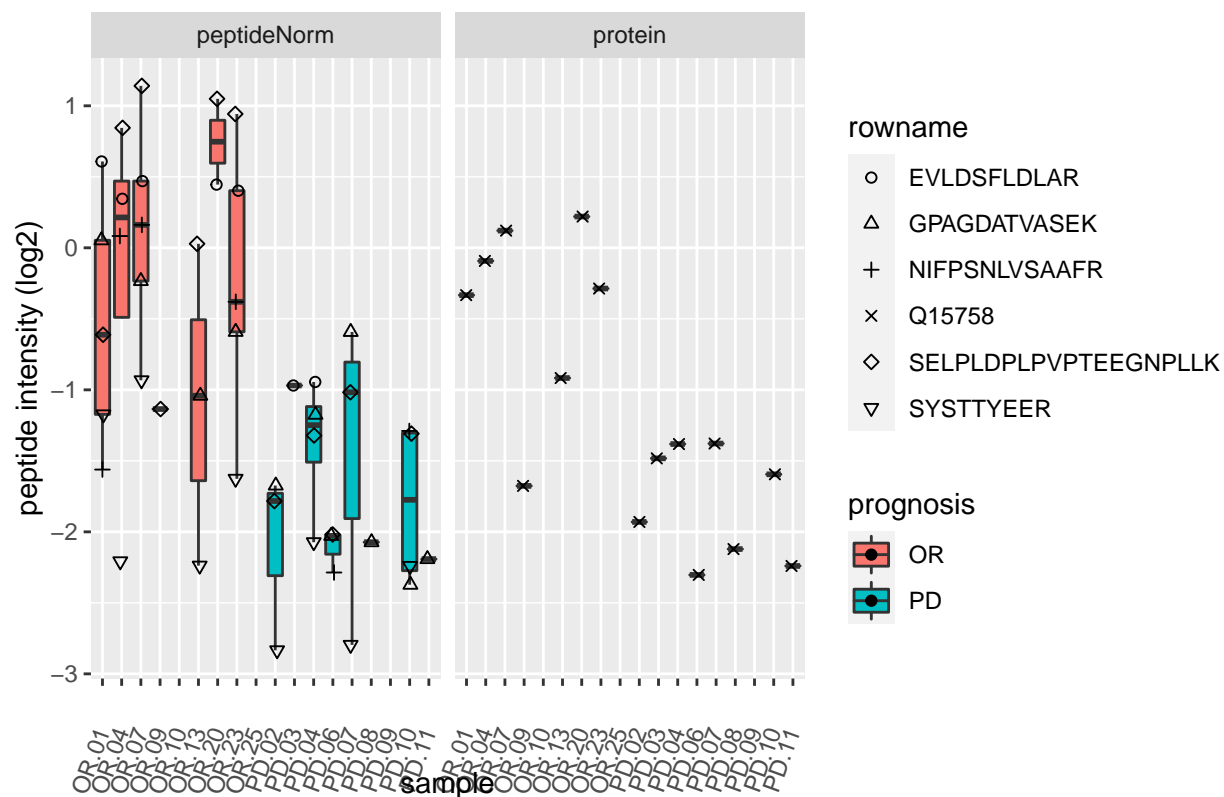
Q15370



Q15758



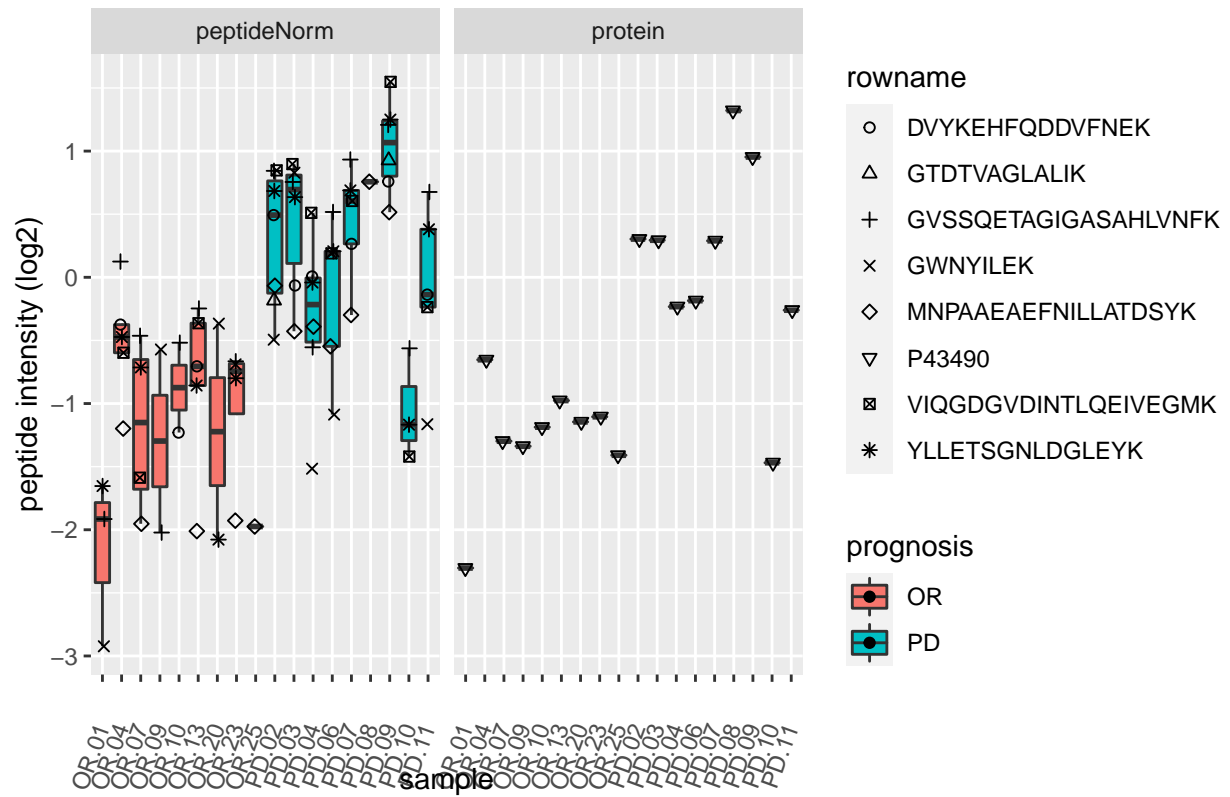
Q15758



P43490

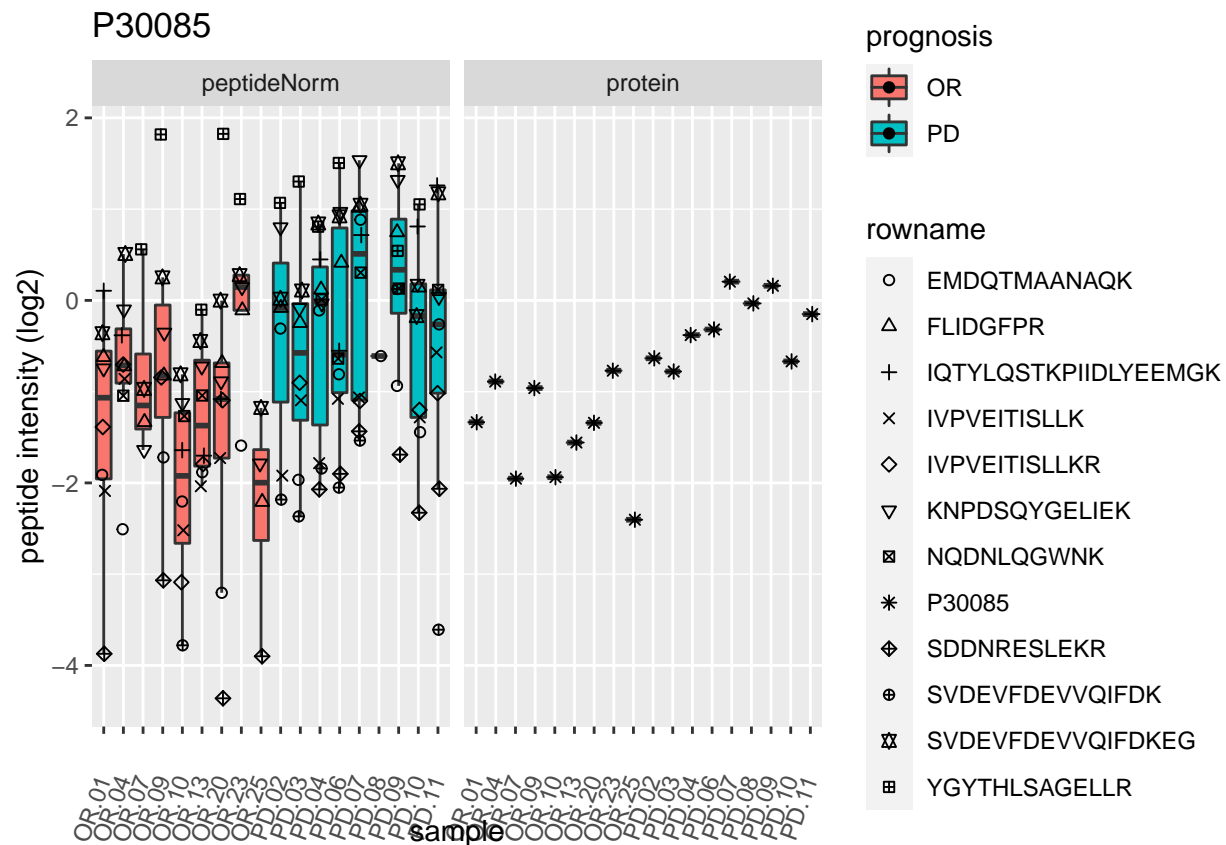


P43490



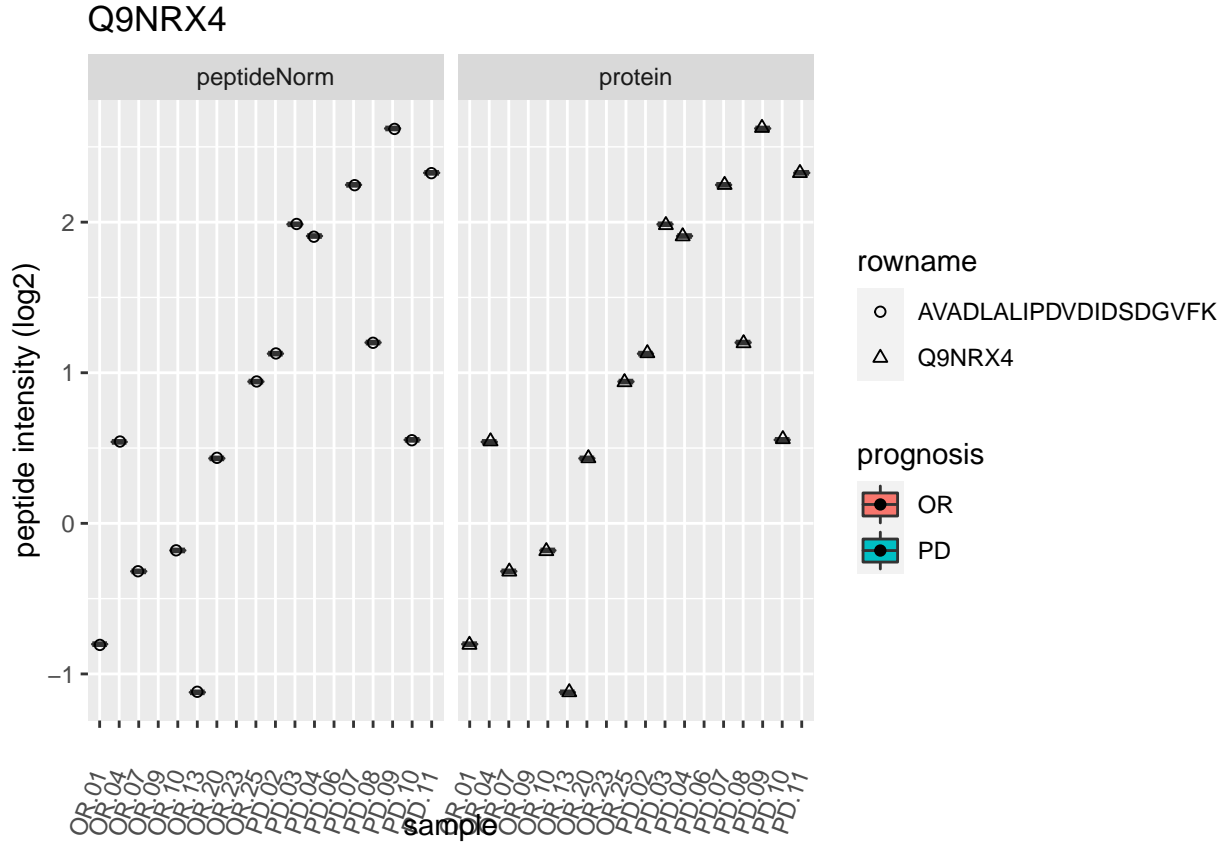
P30085





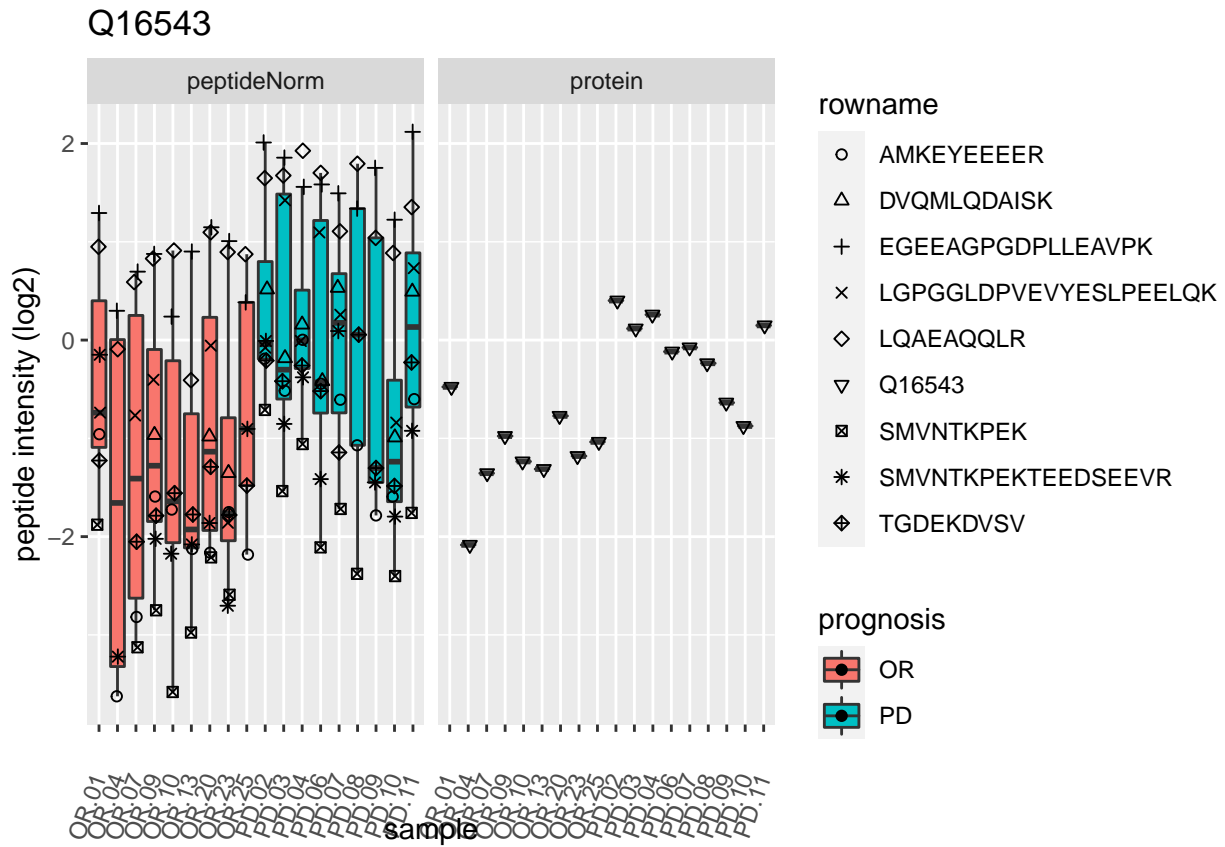
Q9NRX4





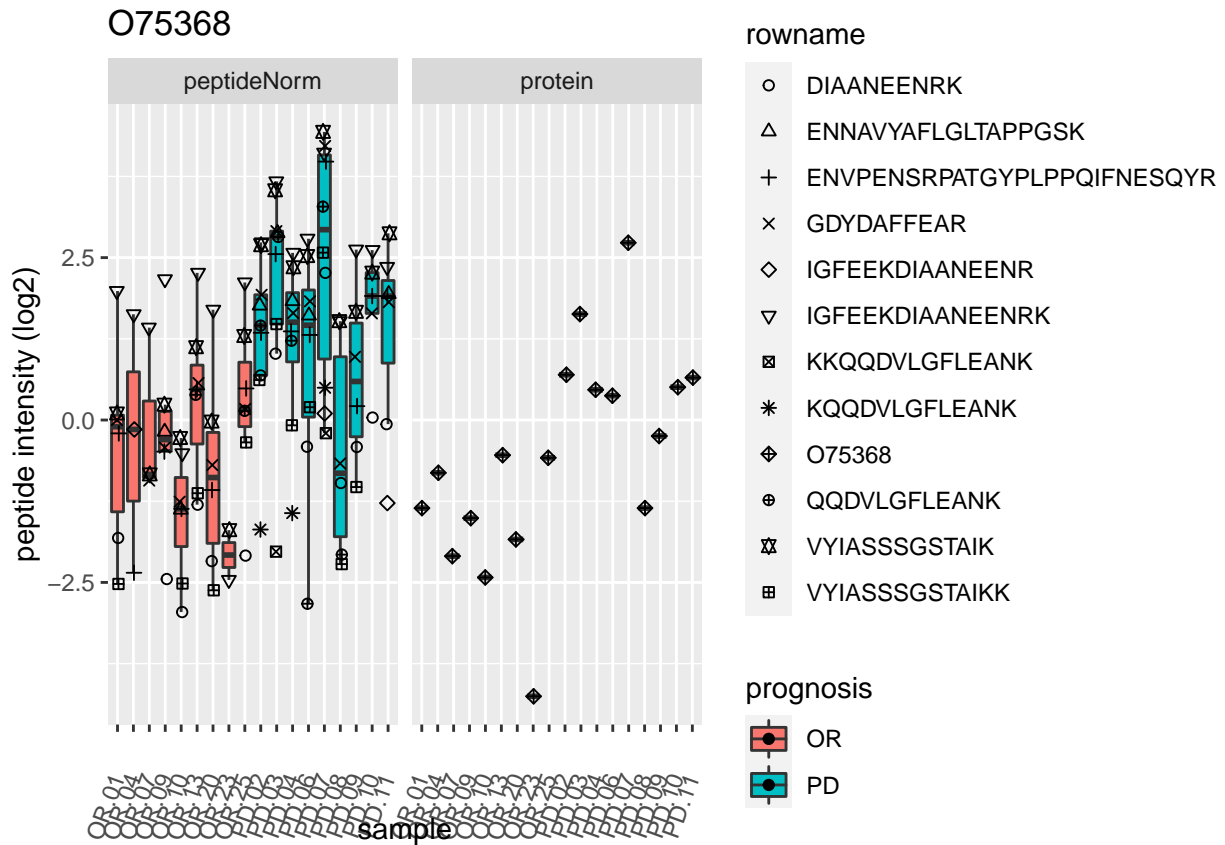
Q16543





O75368

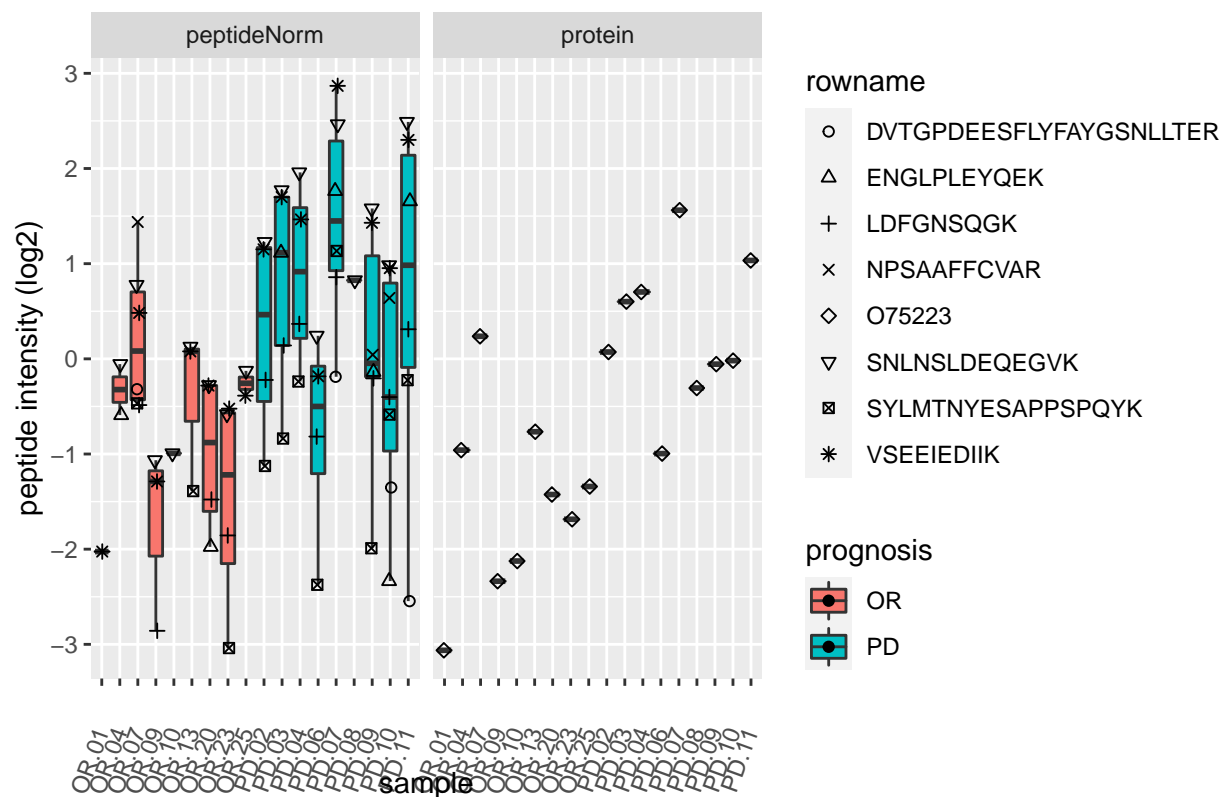




O75223



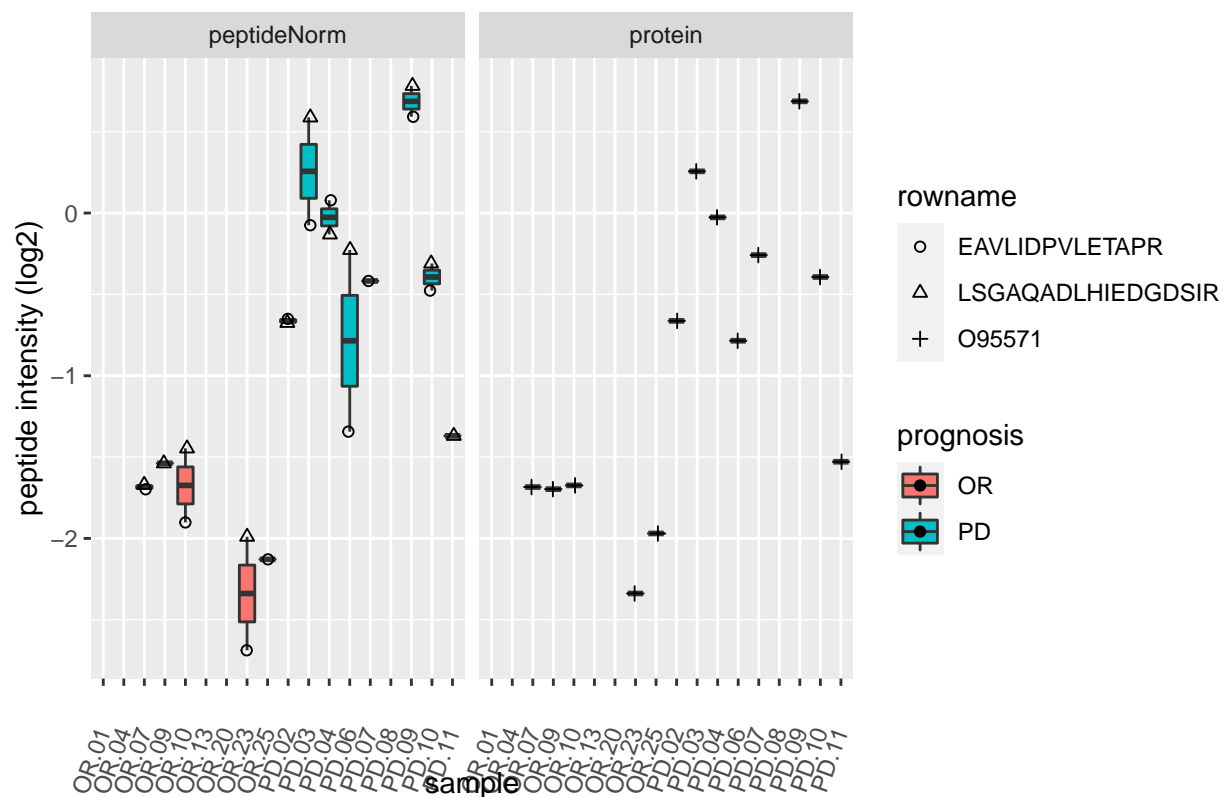
O75223



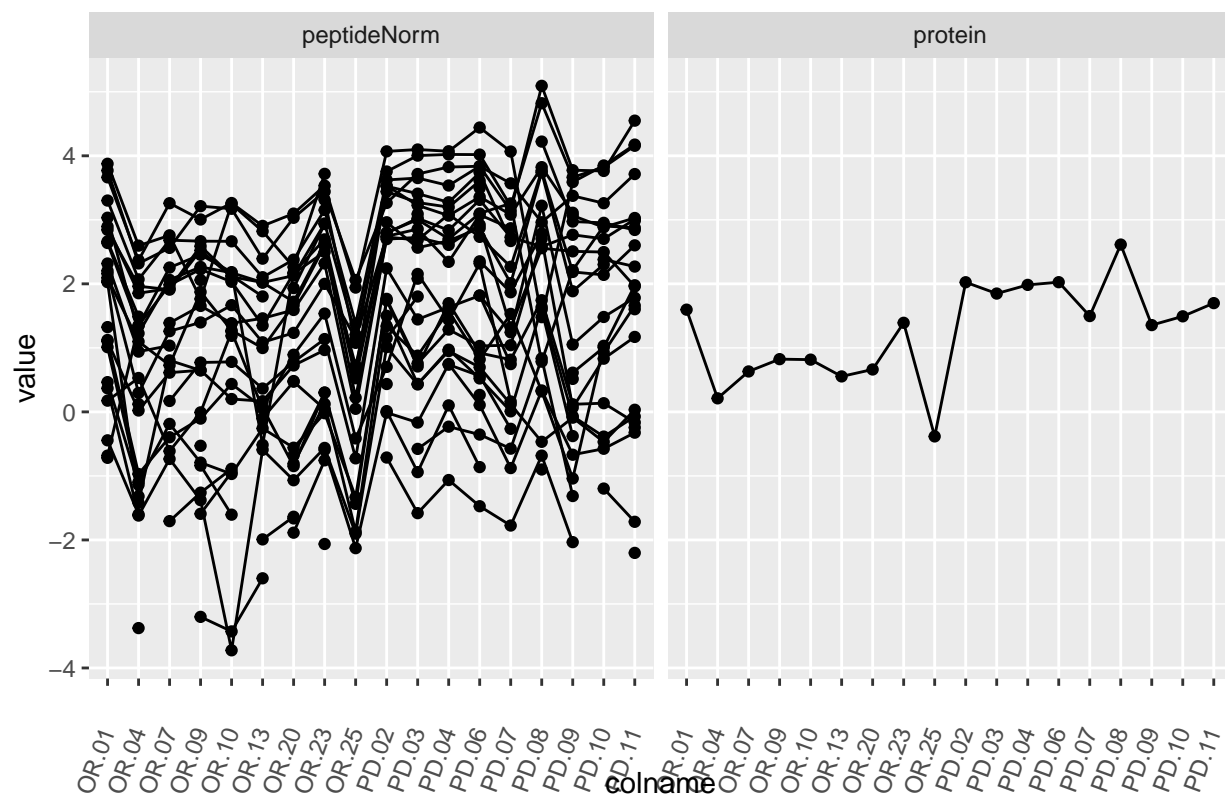
O95571

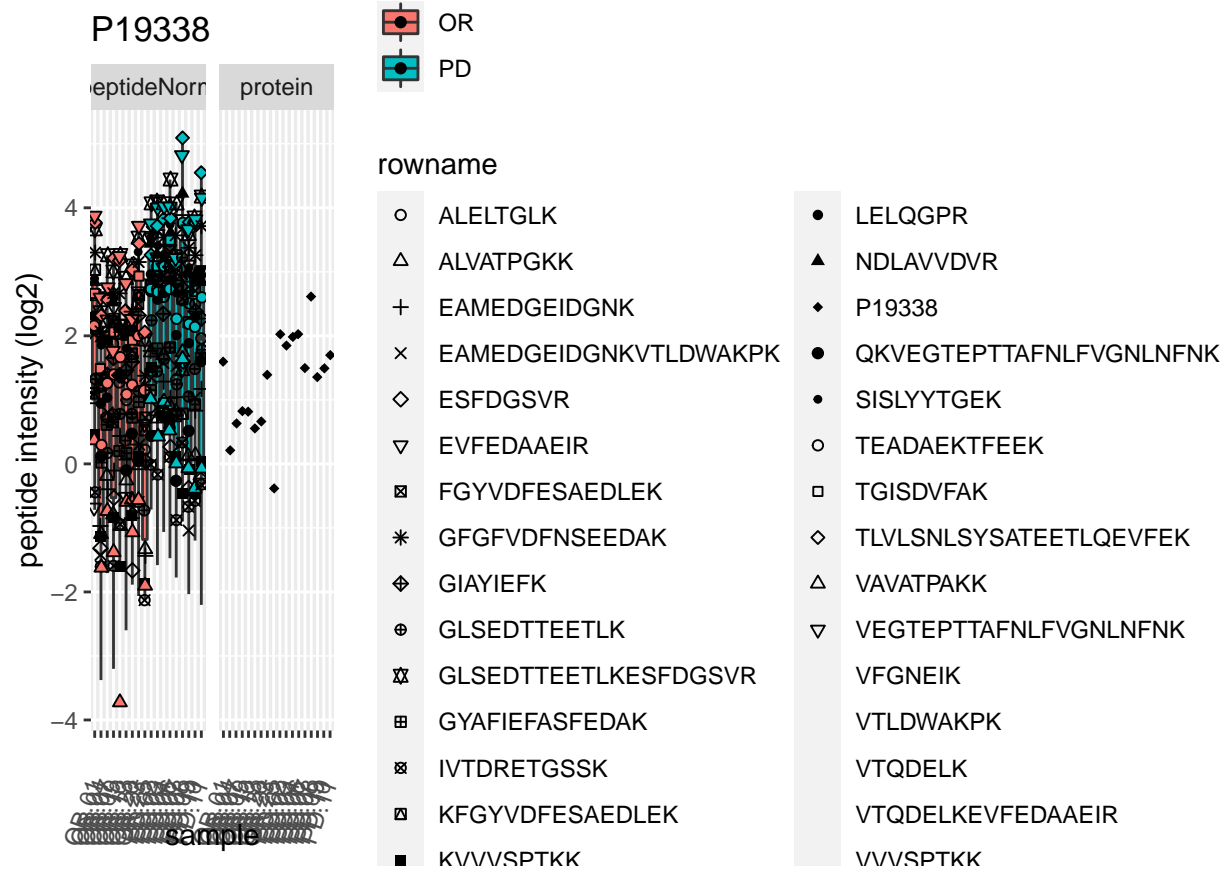


O95571



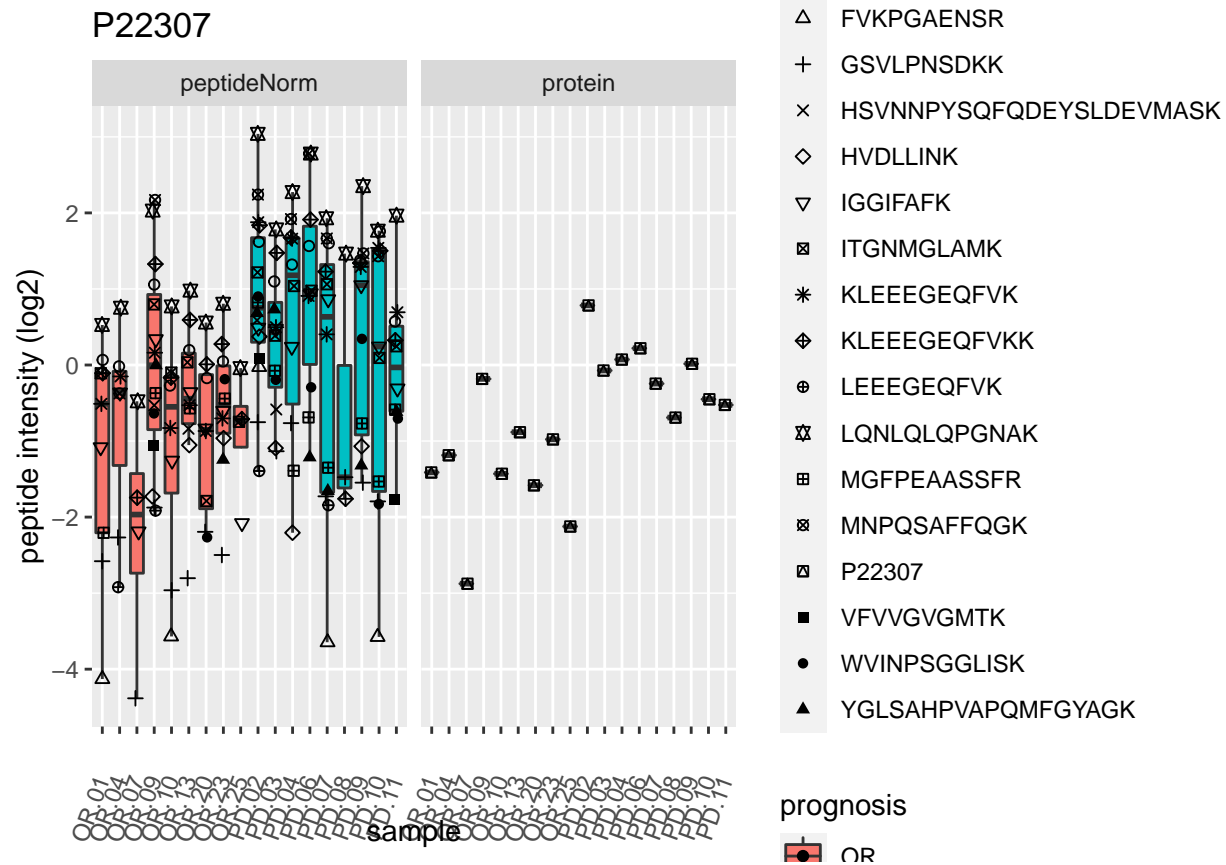
P19338





P22307

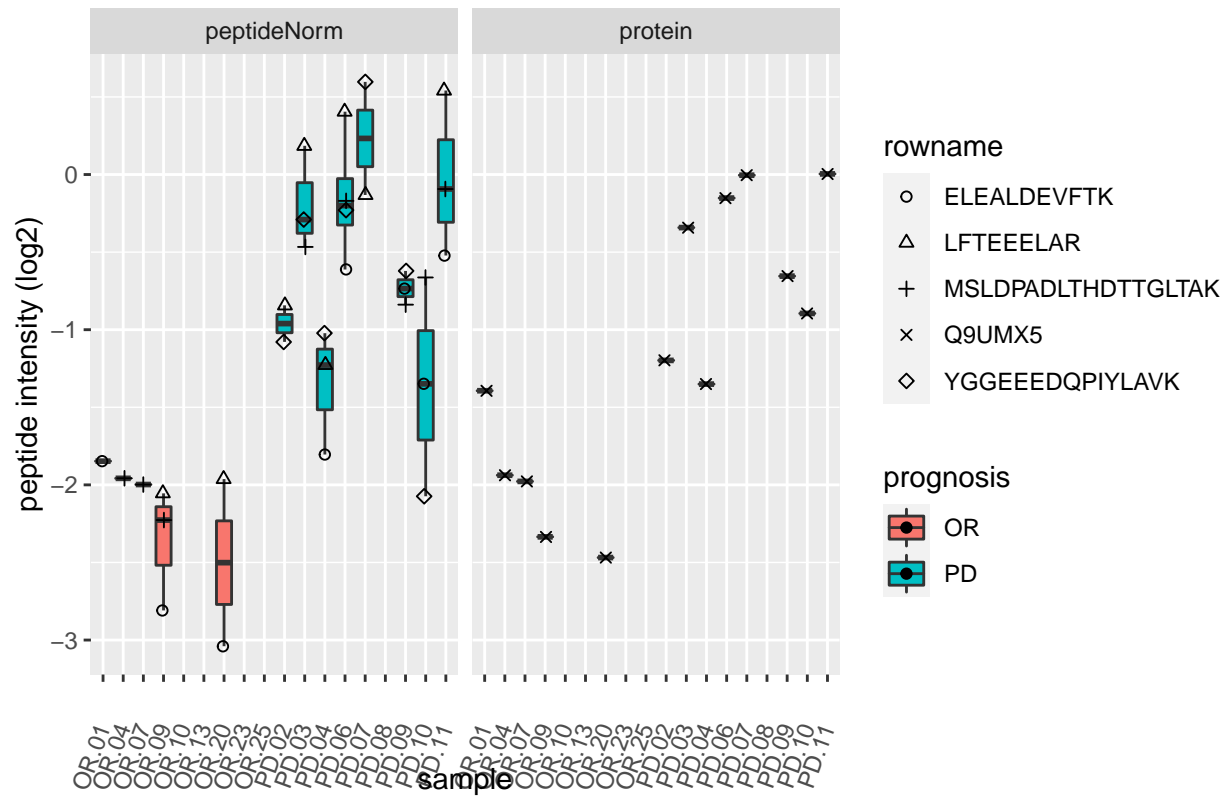




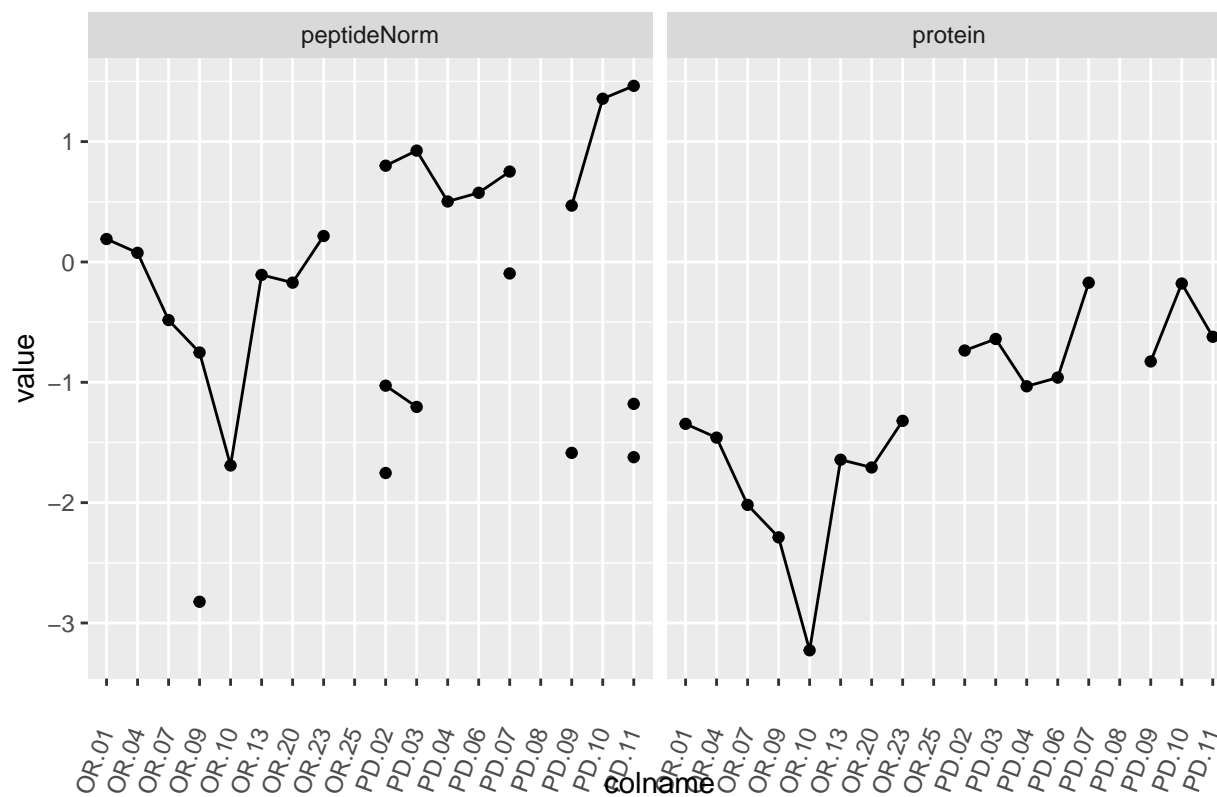
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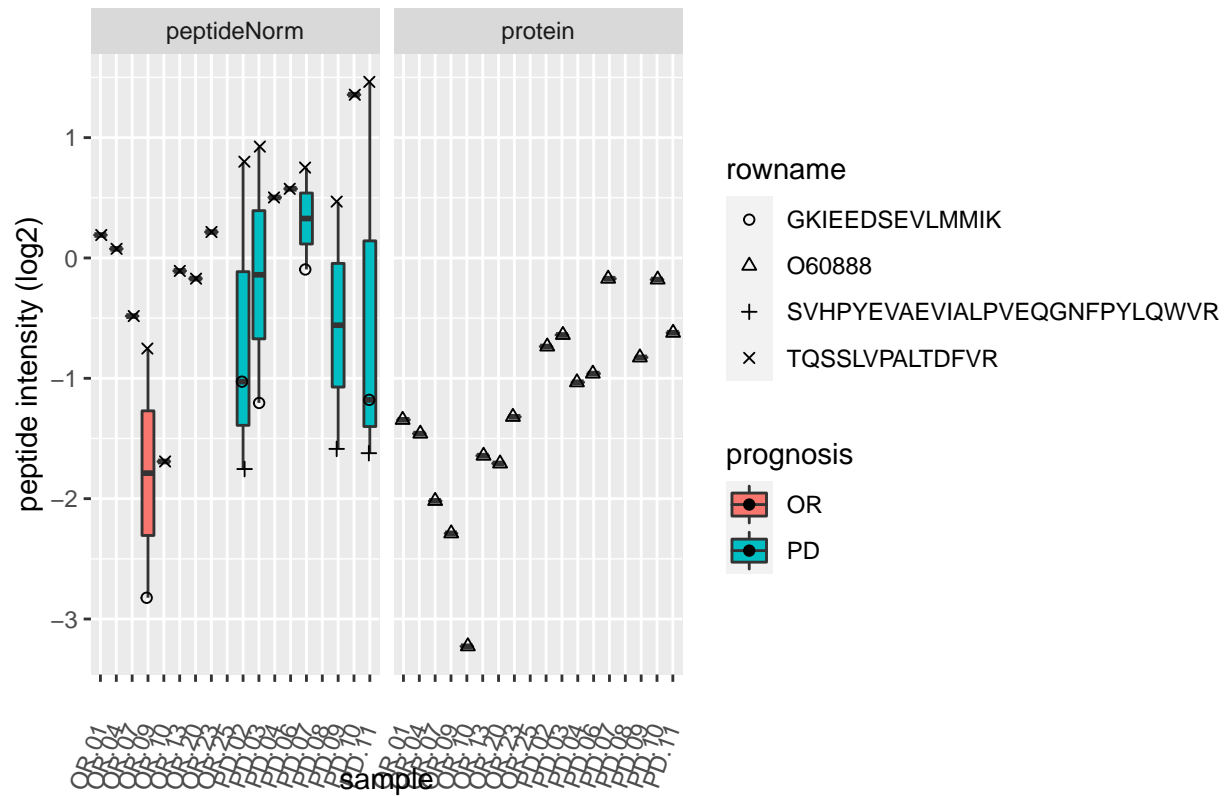
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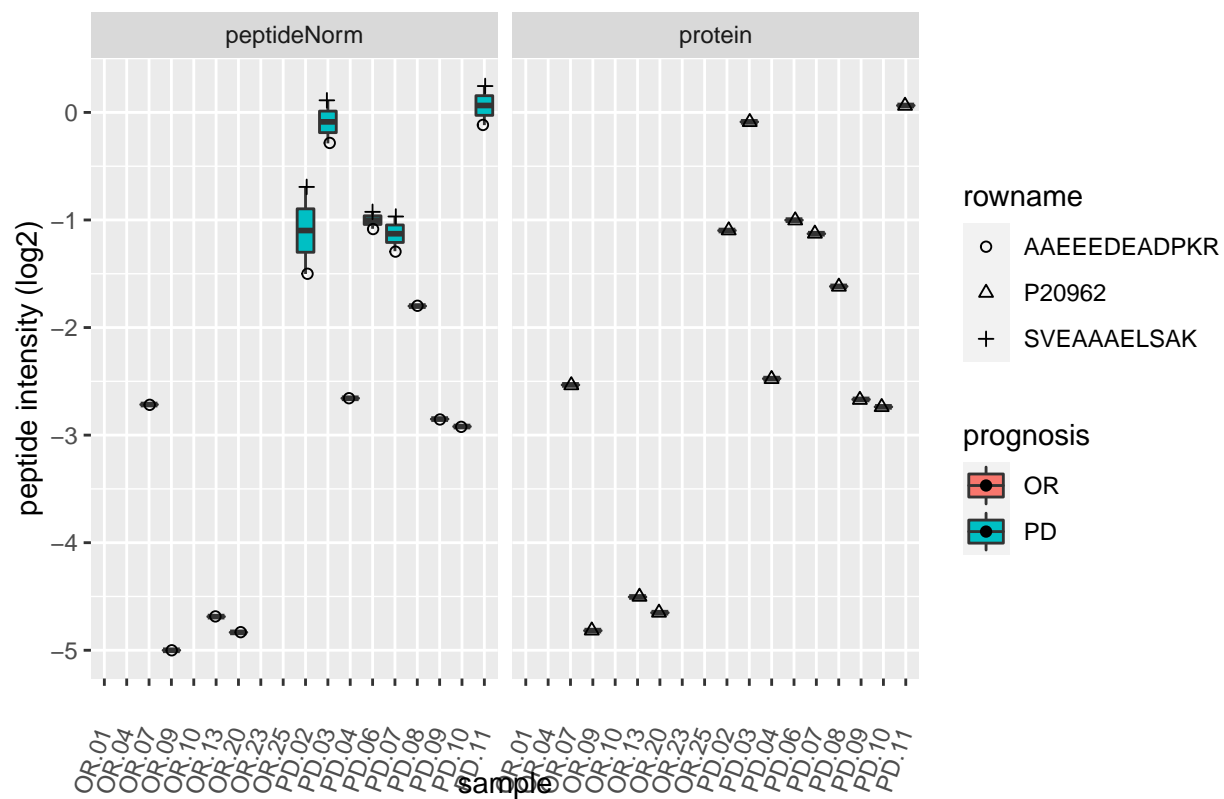
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P20962



P20962



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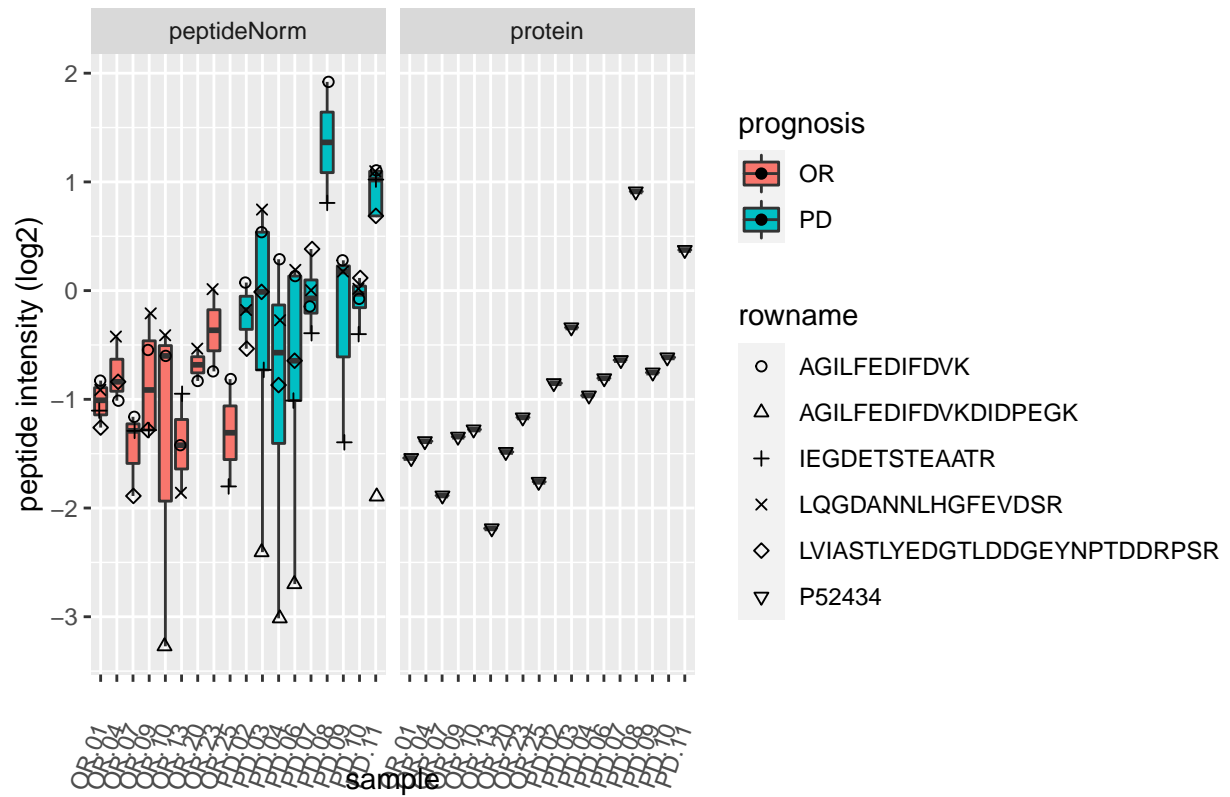


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	+ IQAELAVILK	=	LQQLGEAHQAETEVLR	O	QQEQAI
	· KHPSSPECLVSAQK	>	LSQLEEHLSQLQDNPPQEK	P	QQLSSL
-	KINQLSEENGDLSEFK	?	LTAQVASLTSELTTLNATIQQDQELAGLK	Q	QQNELA
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	/ KNSLISSLEEEVSILNR	A	LVMAESEK	S	RSQAG\
	0 KQQNQELQEQLR	B	MTMLLLYHSTMSSK	T	SAPASQ
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	2 LADDLSTLQEK	D	PSLSLGTITDEEMK	V	SLVEQH
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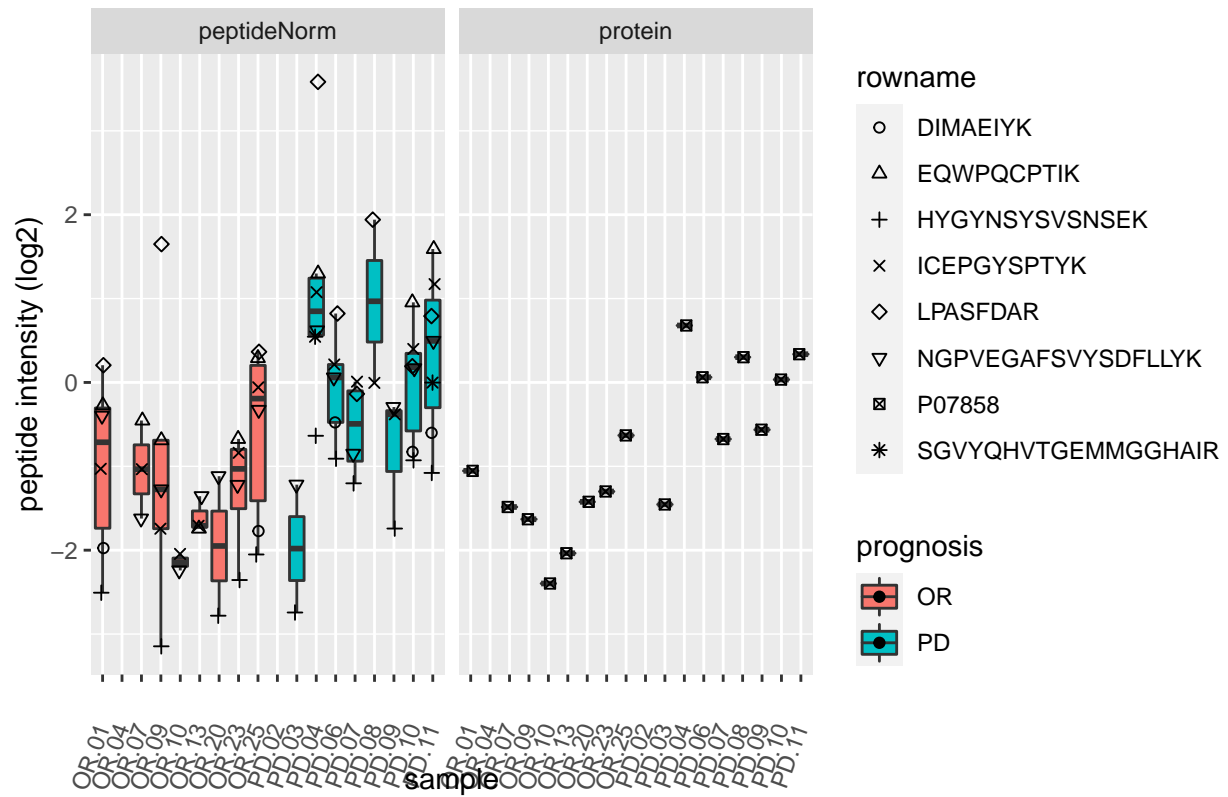
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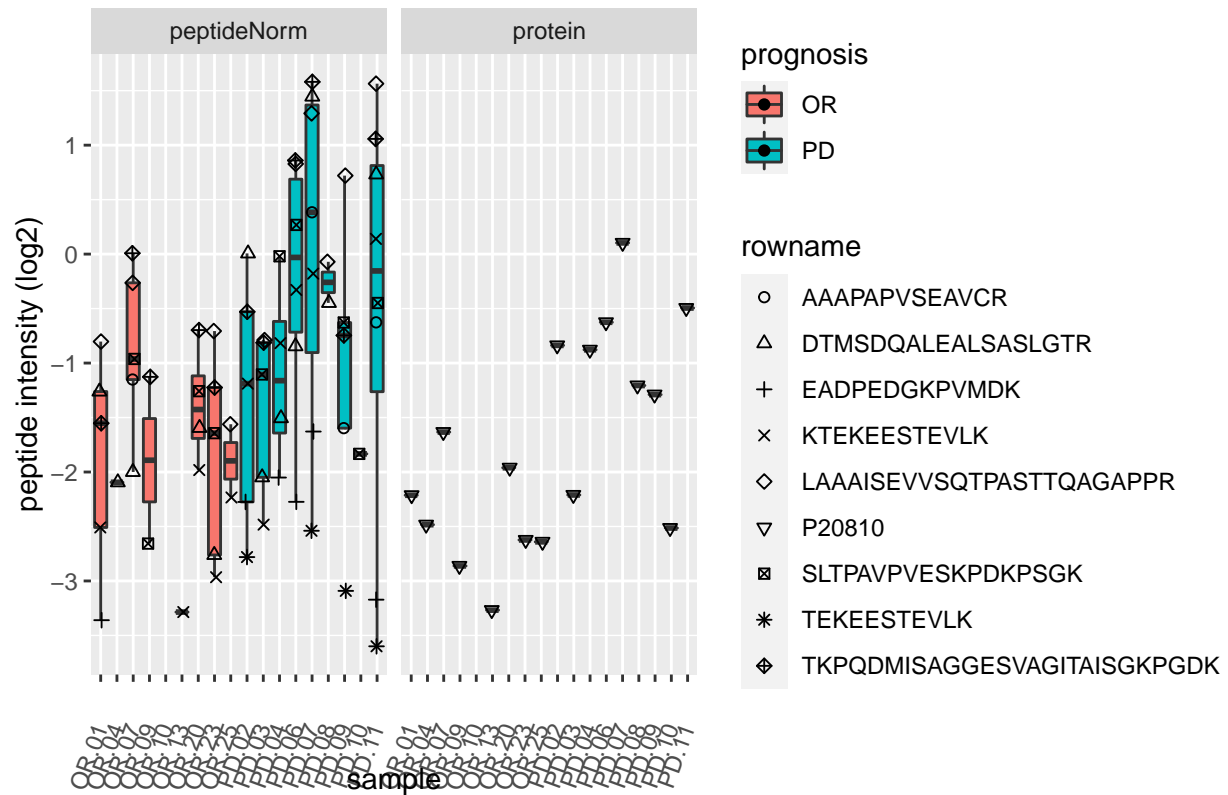
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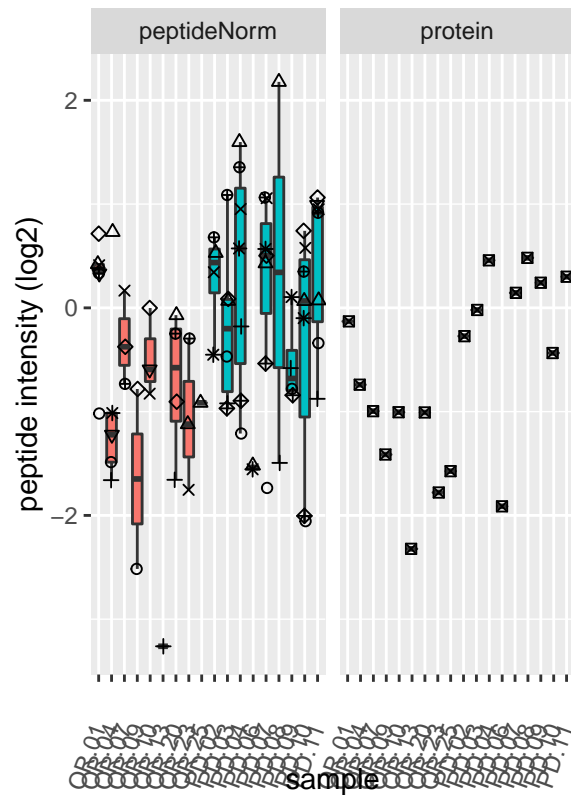
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P22061



P22061



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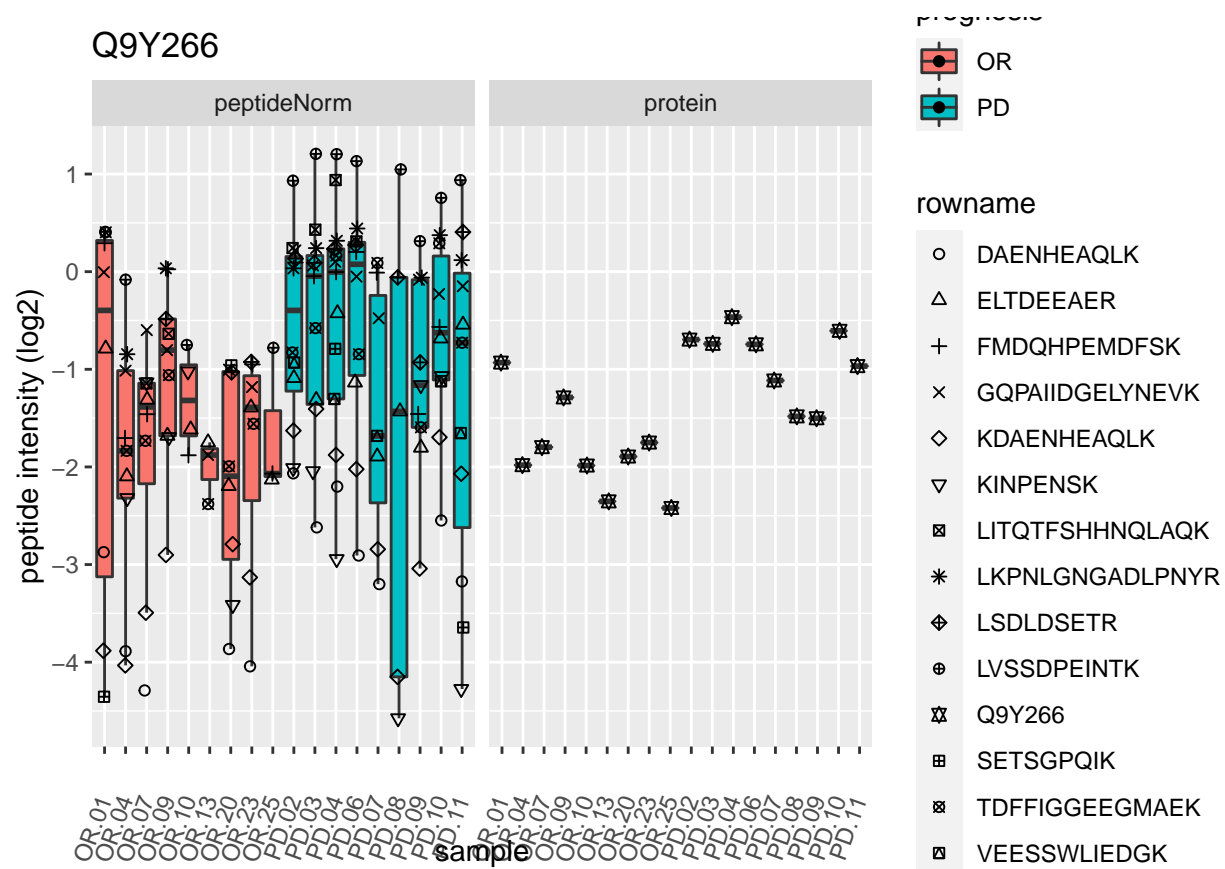
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prognosis

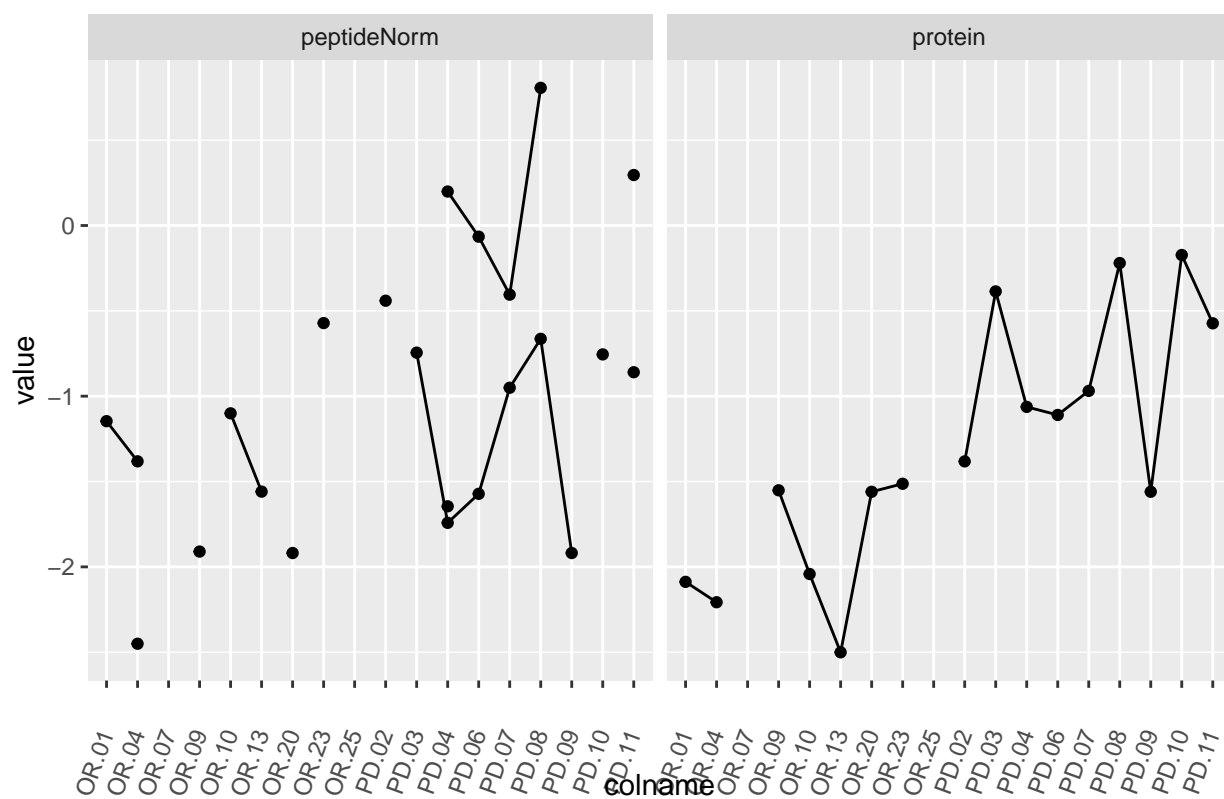
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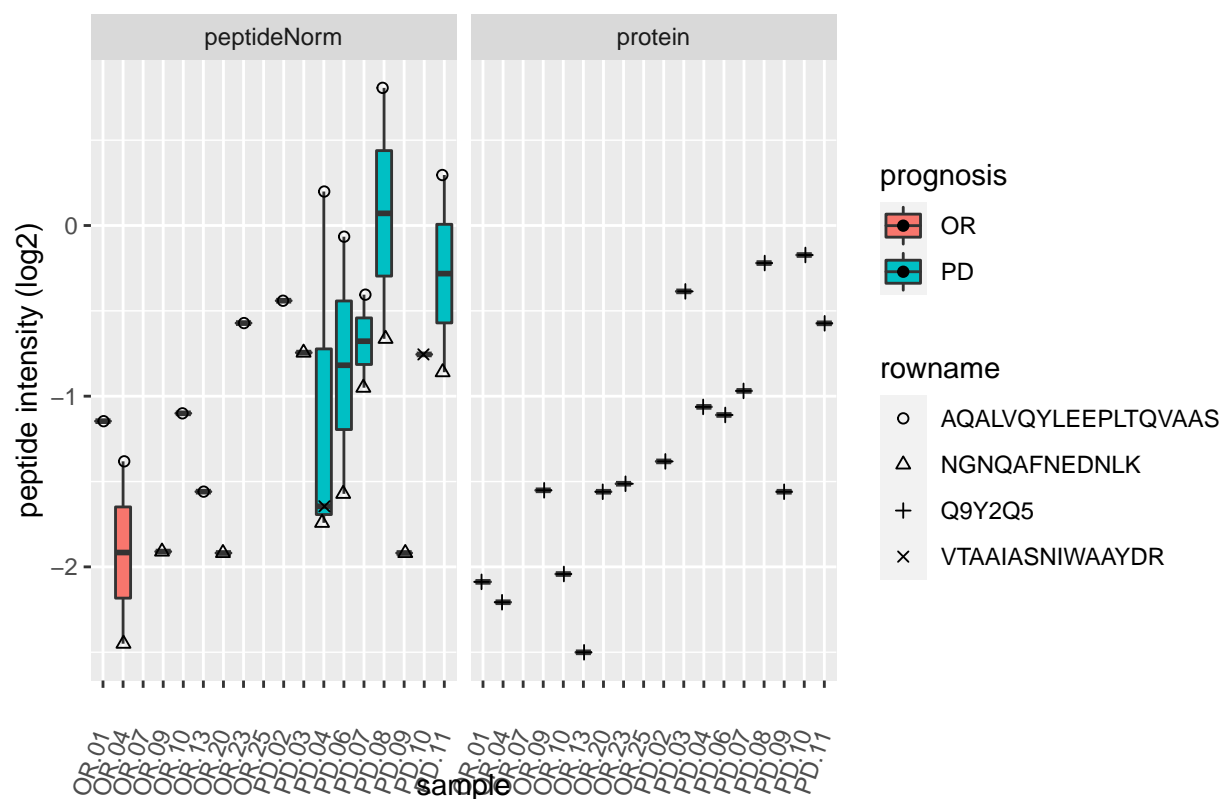




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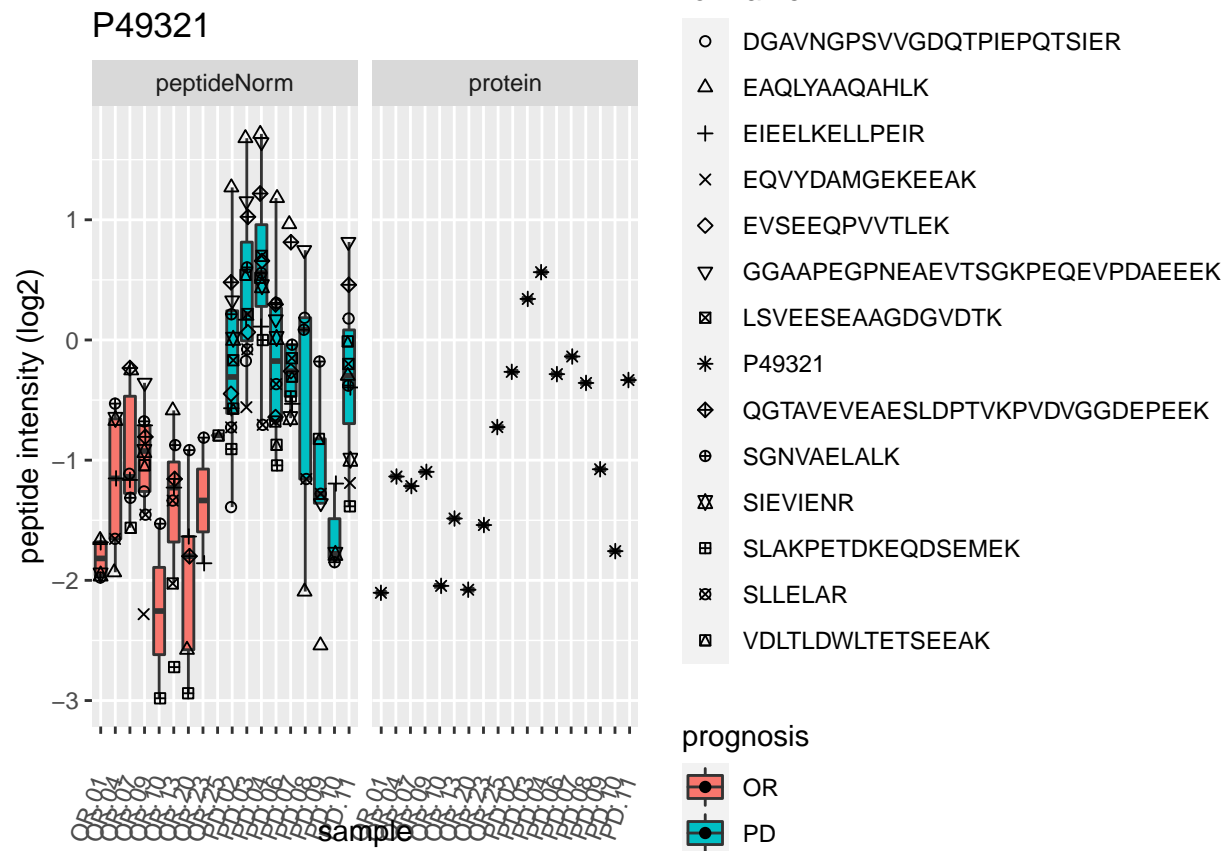


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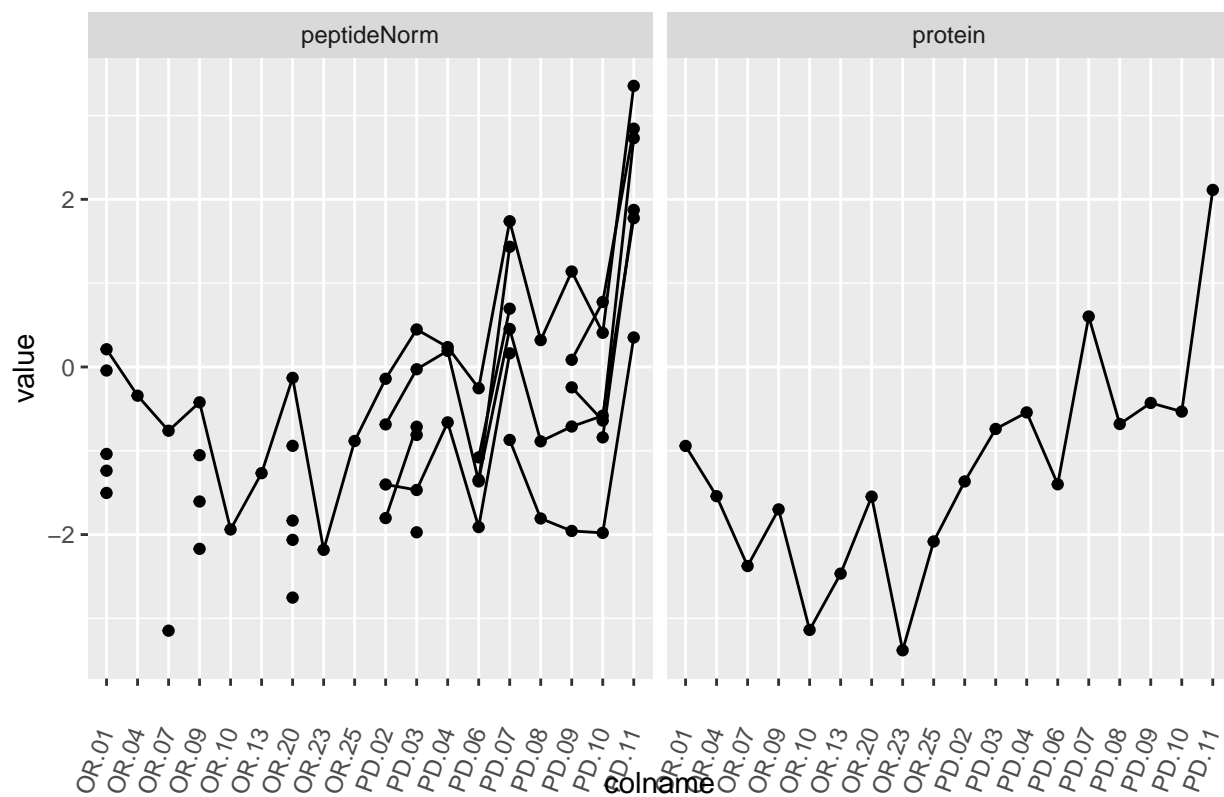




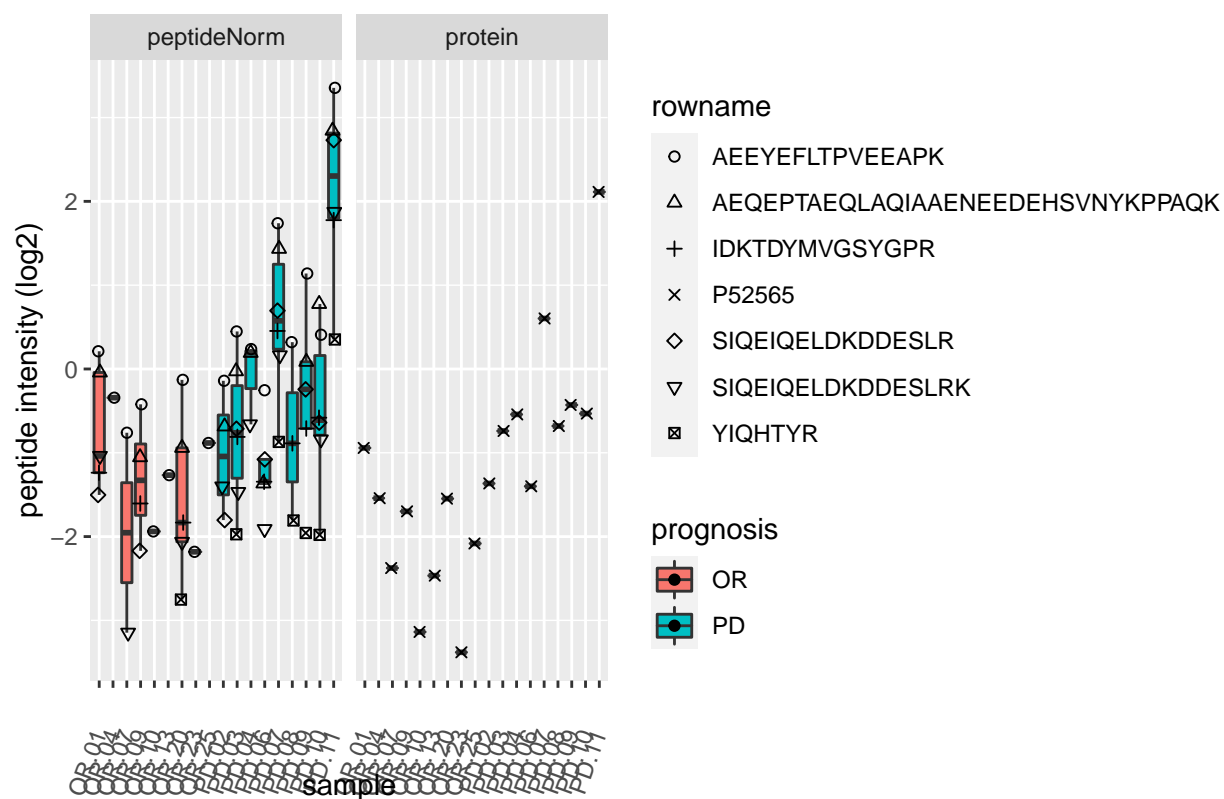
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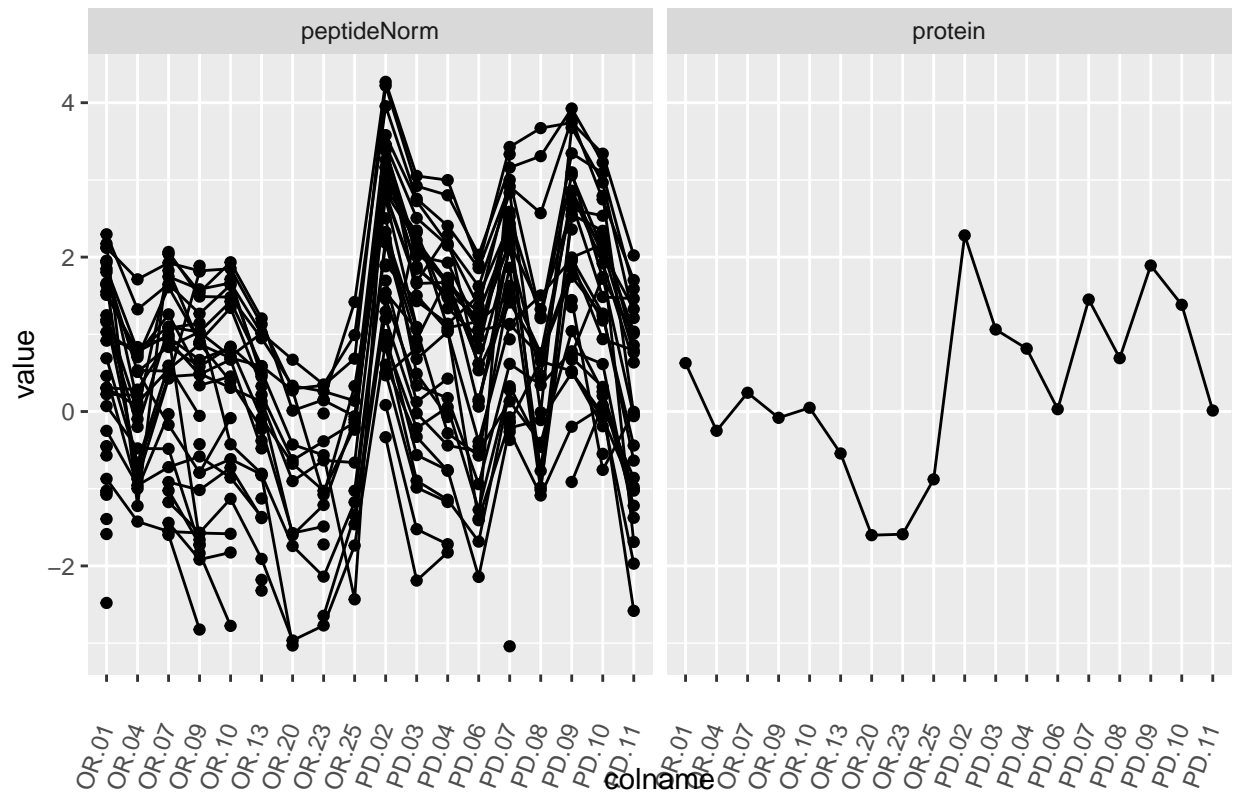
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P52565



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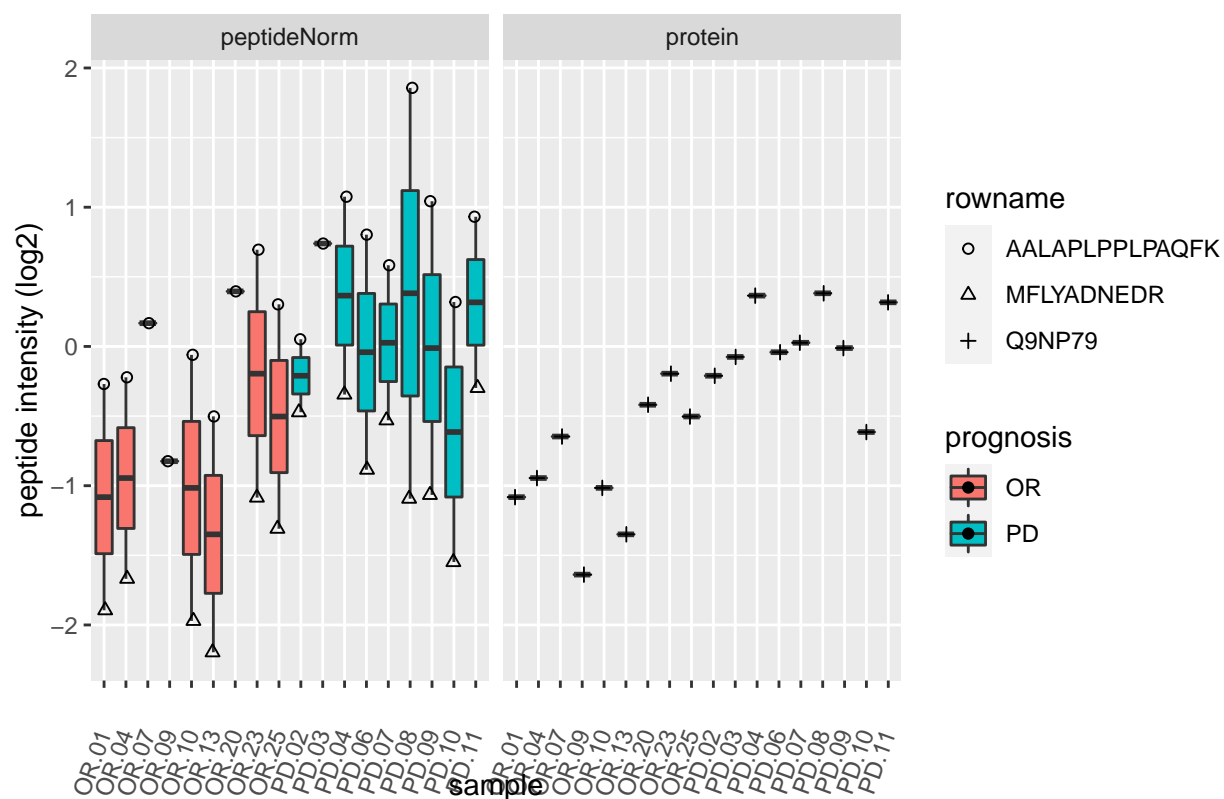


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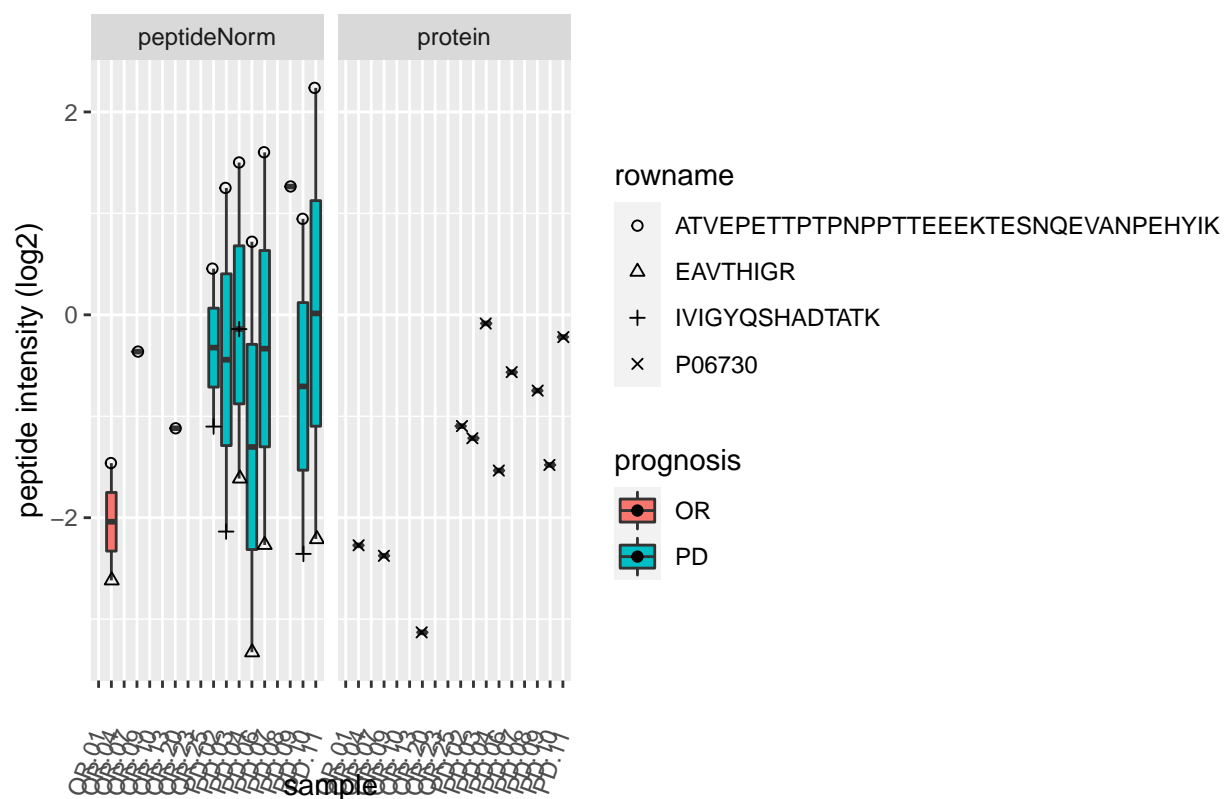
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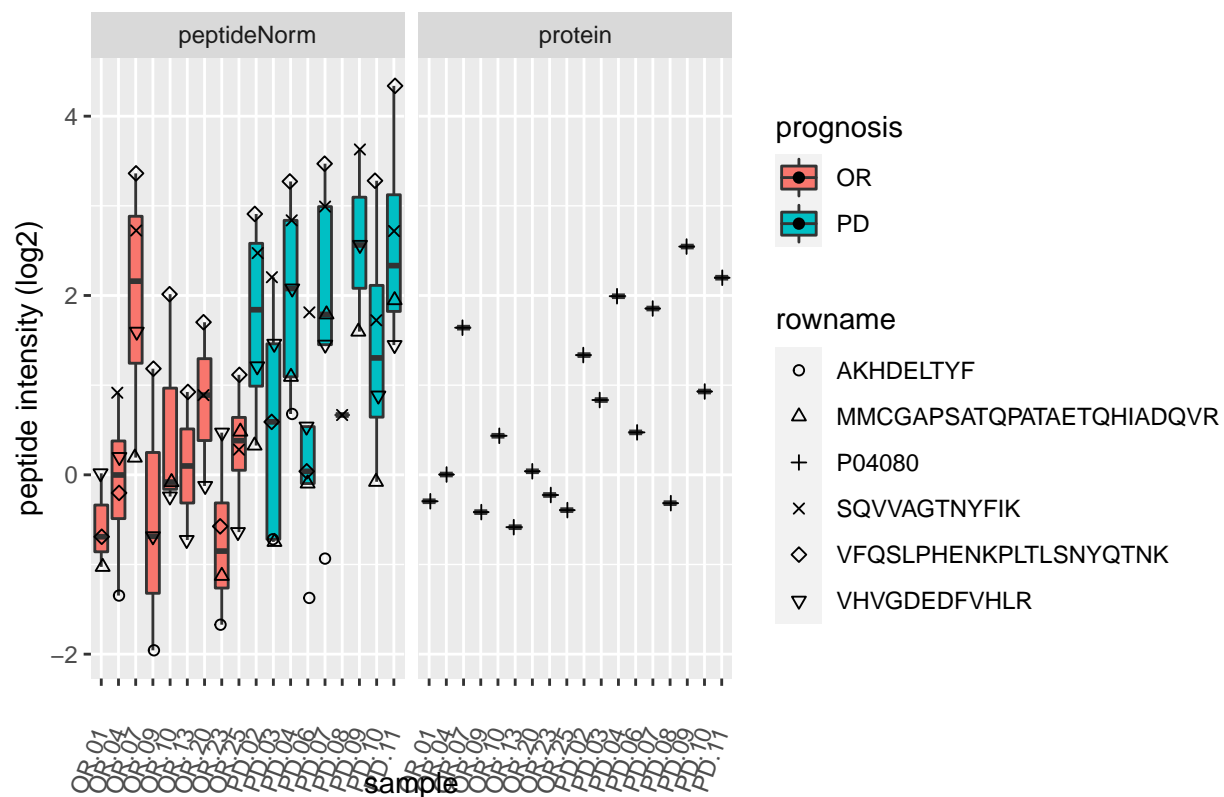
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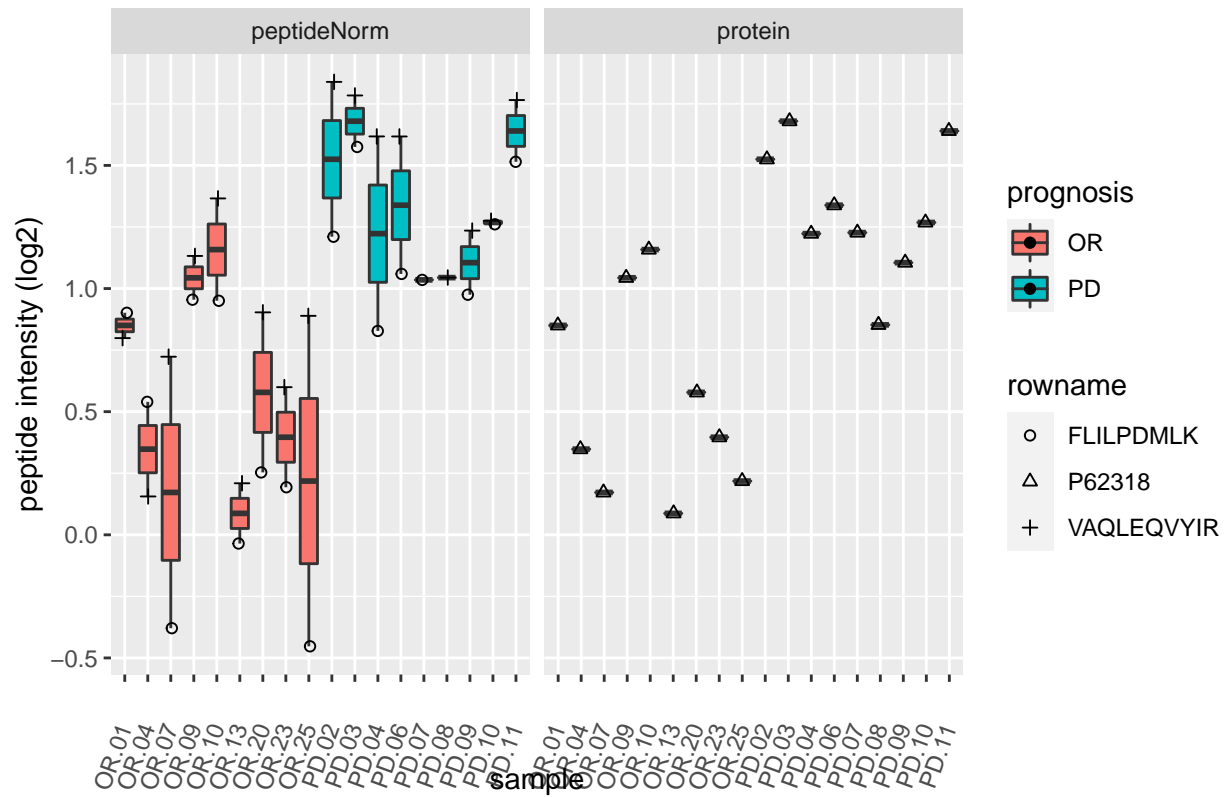
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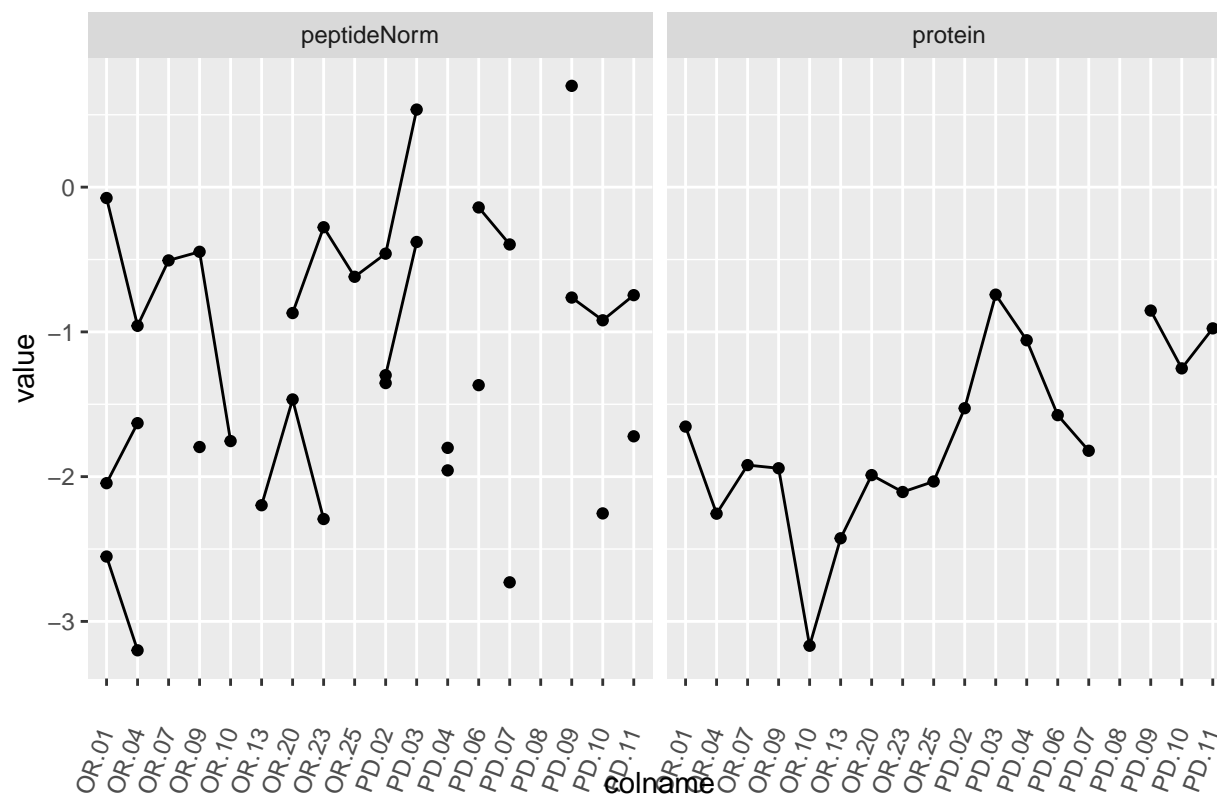
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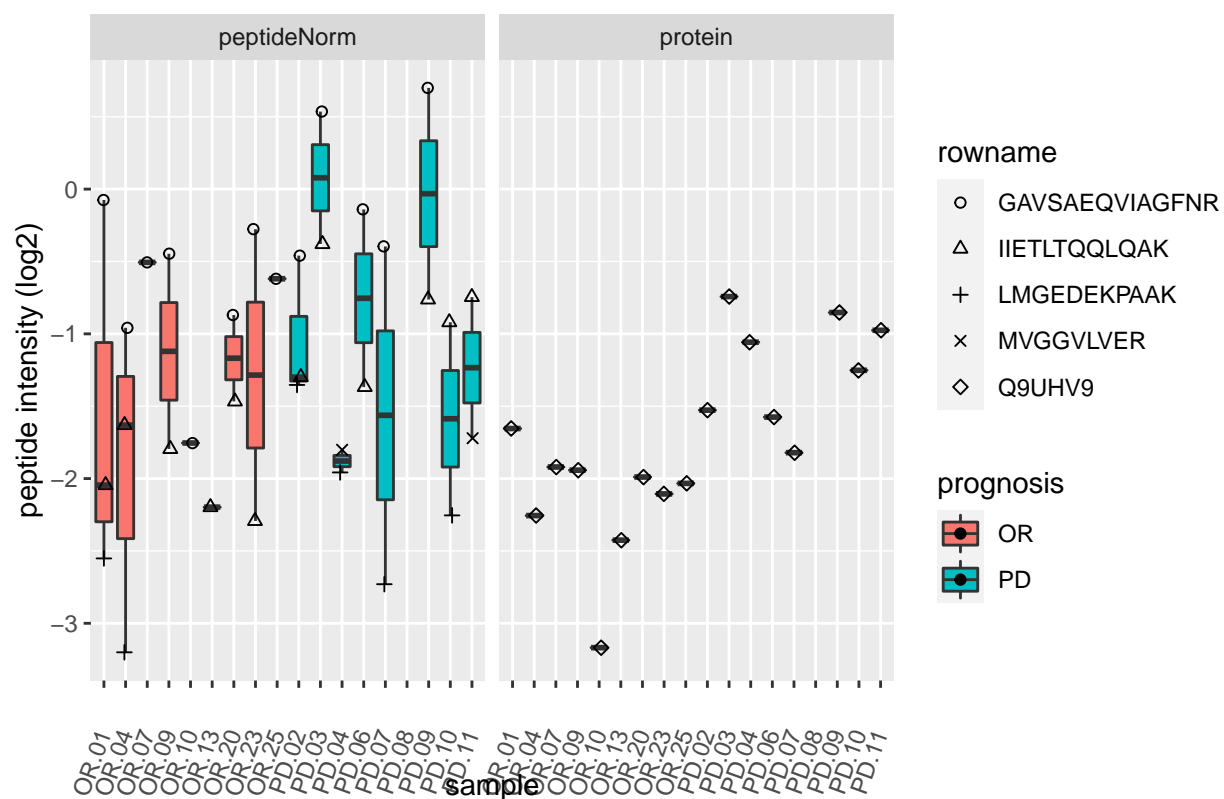
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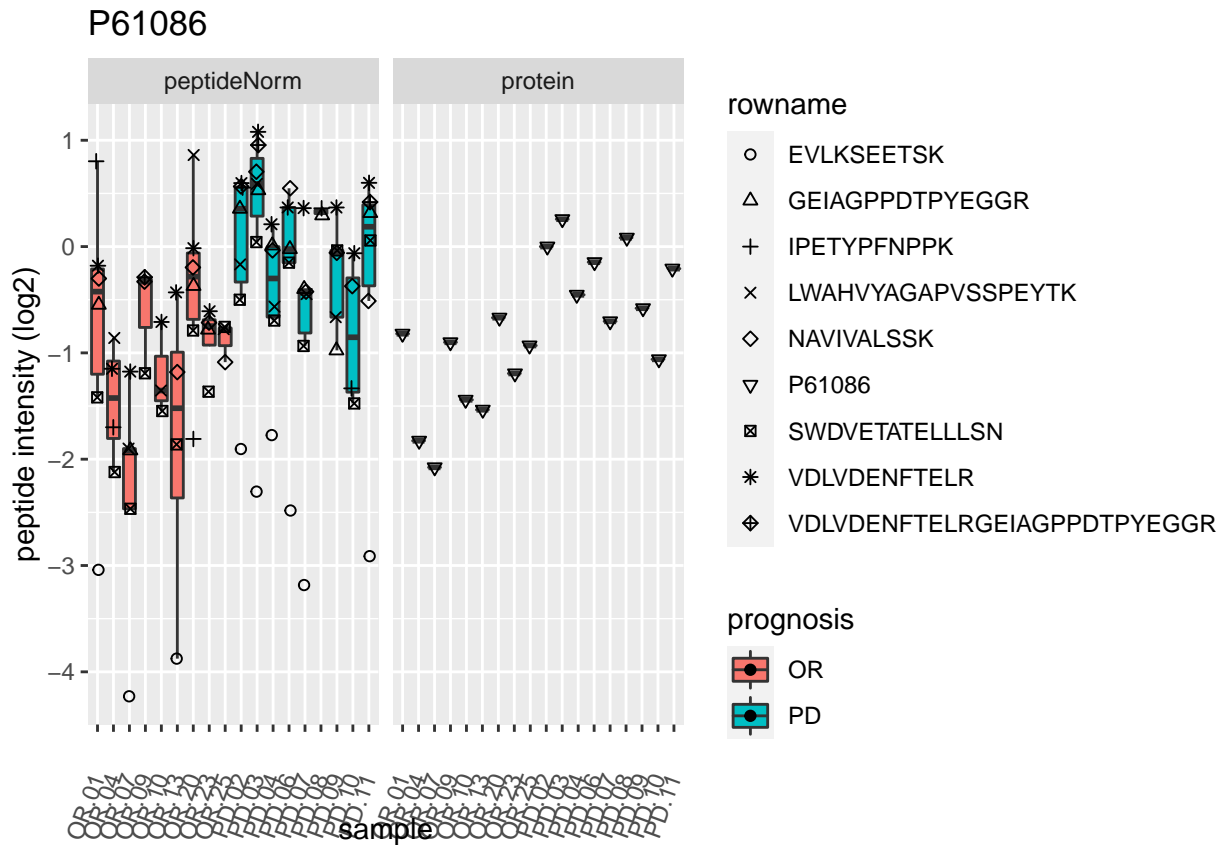


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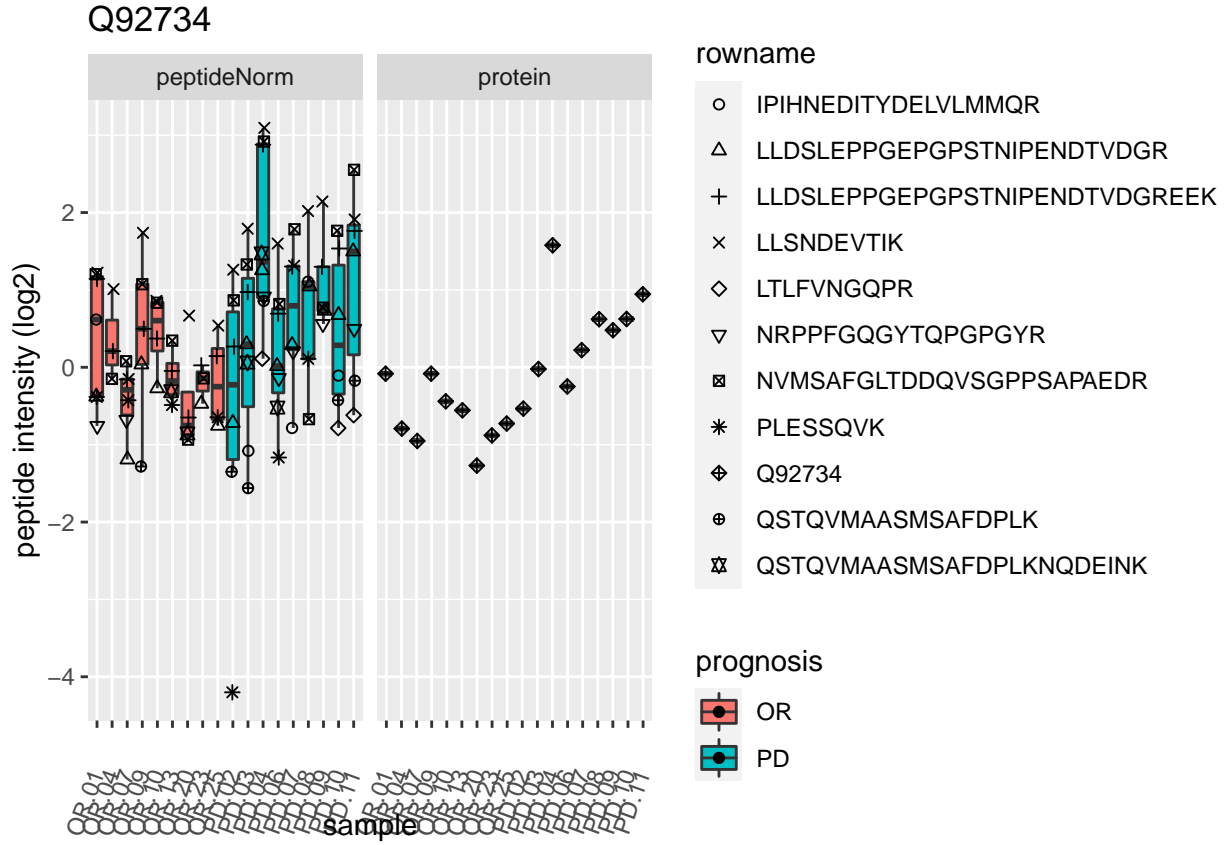
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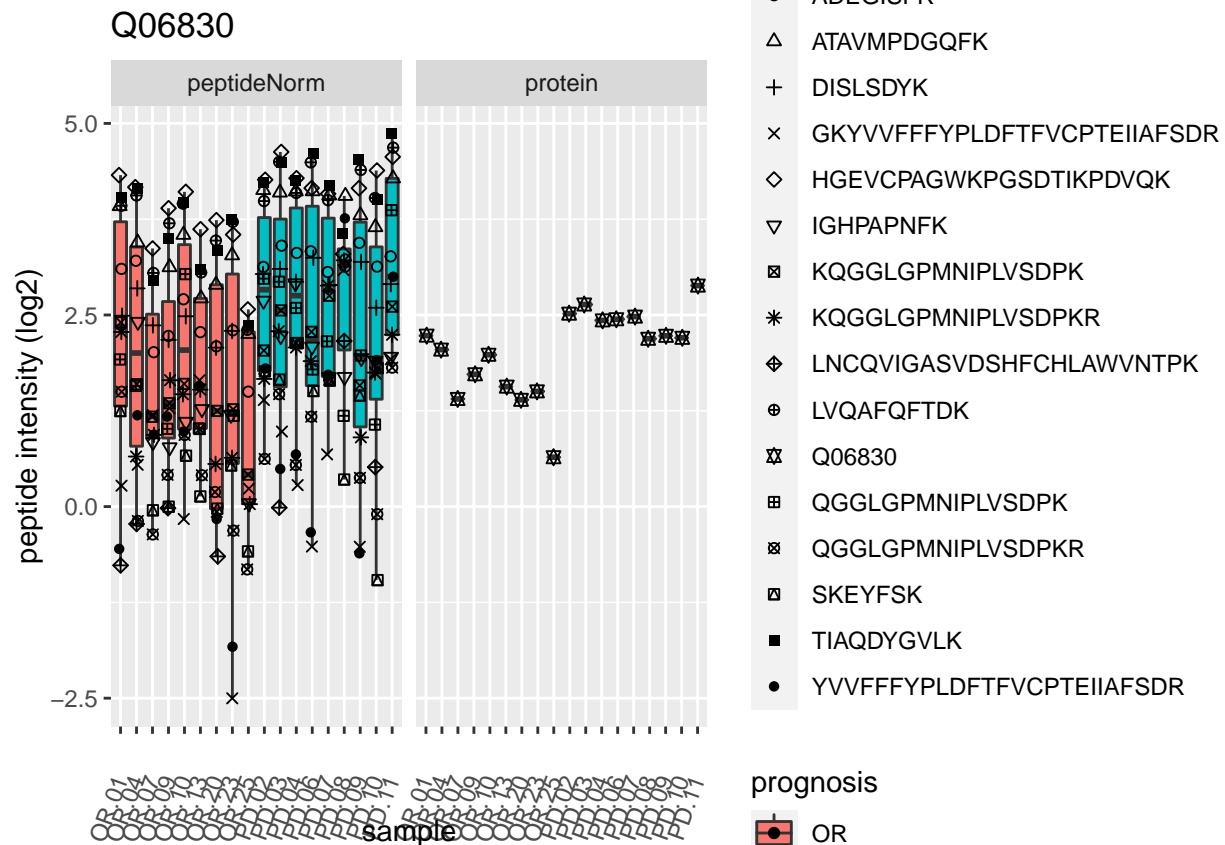
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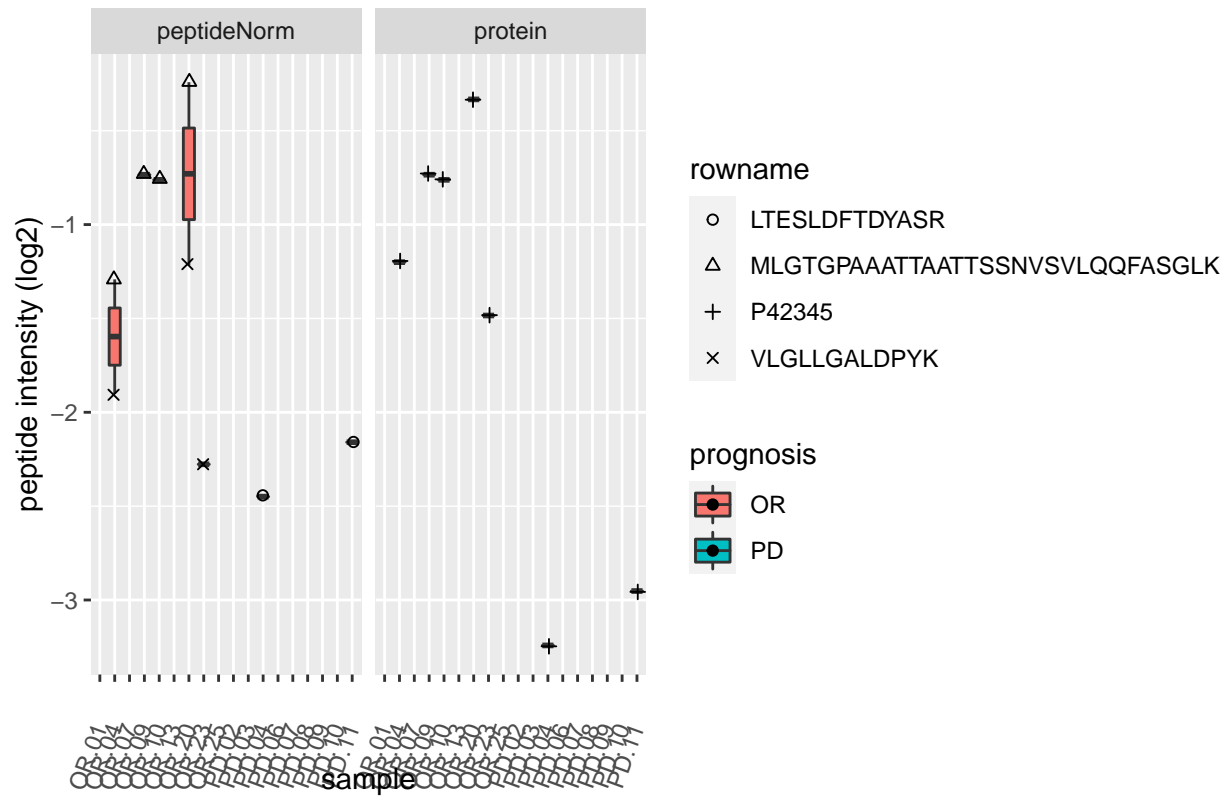




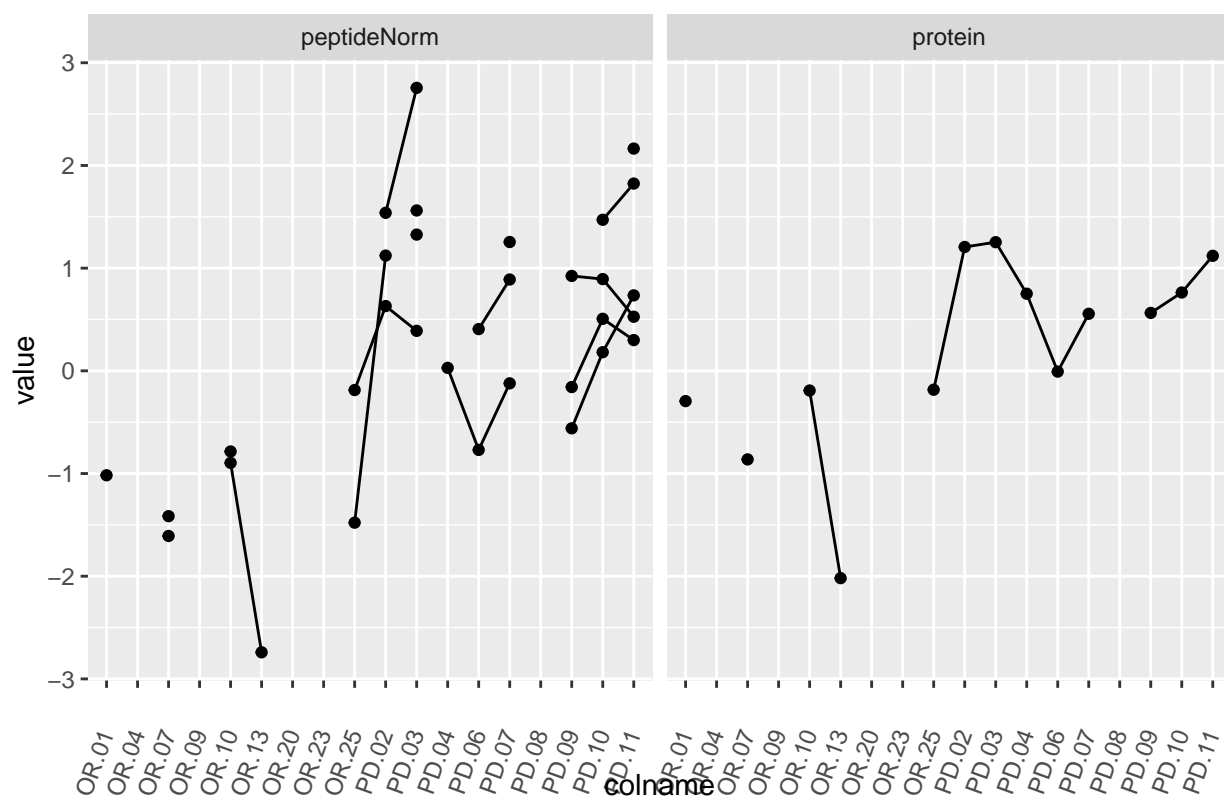
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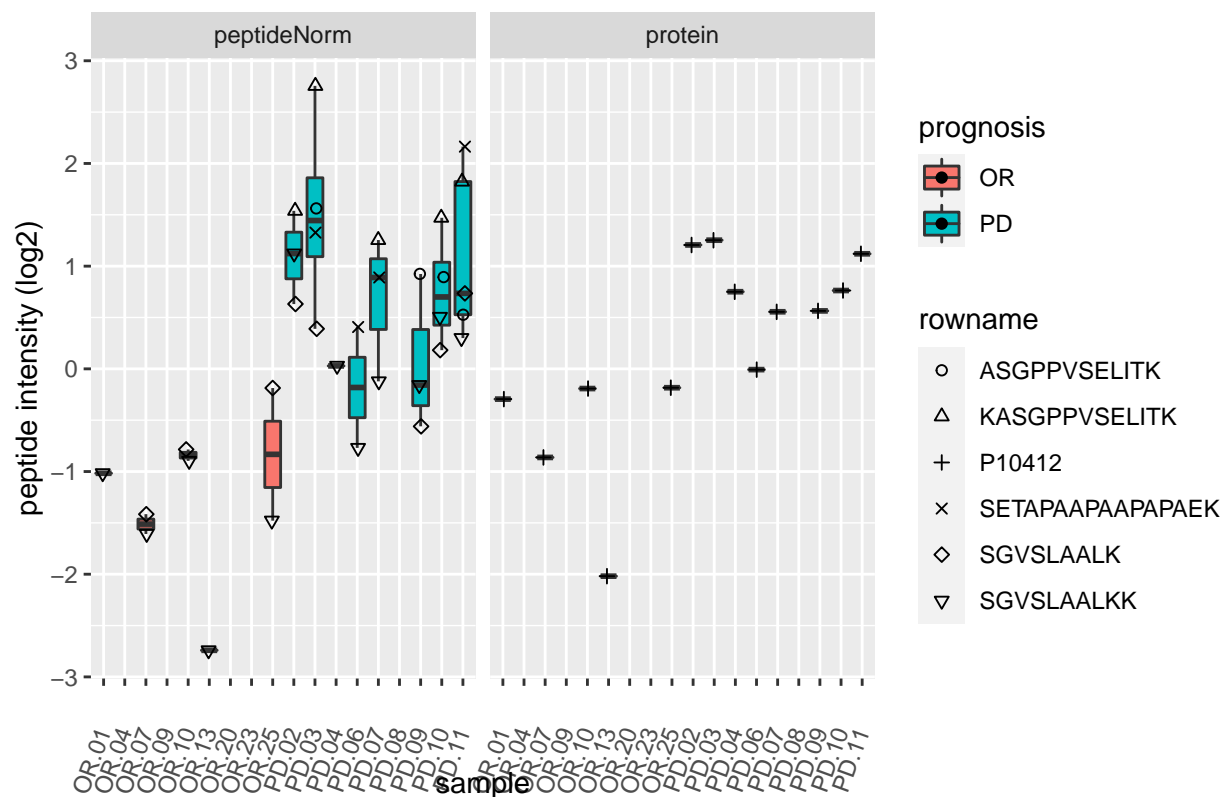
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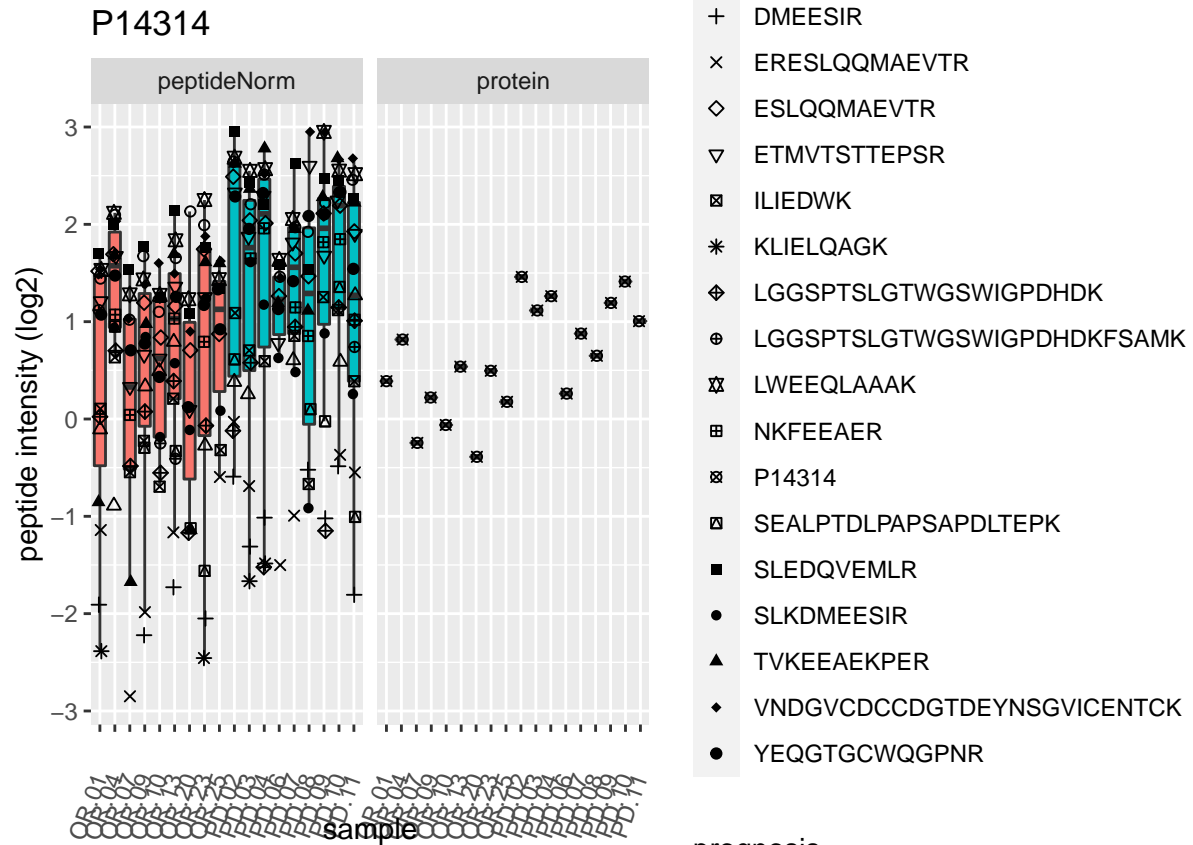


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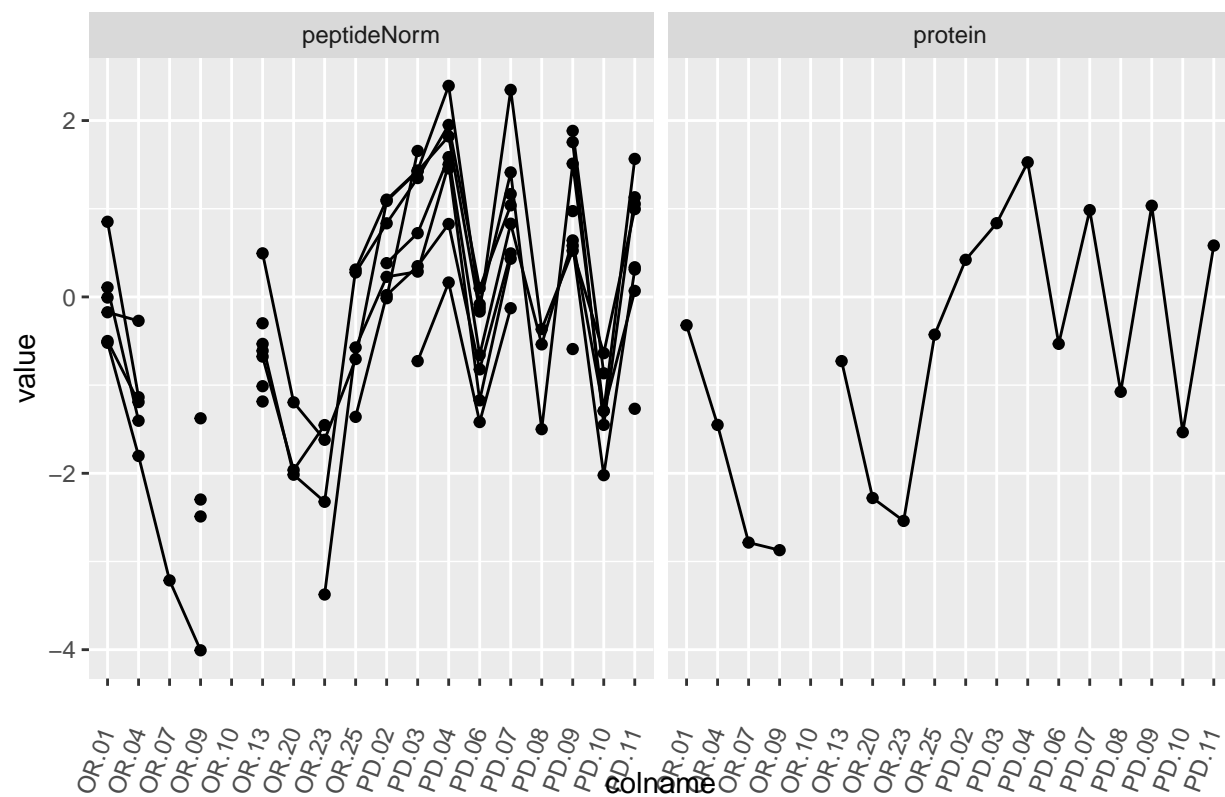


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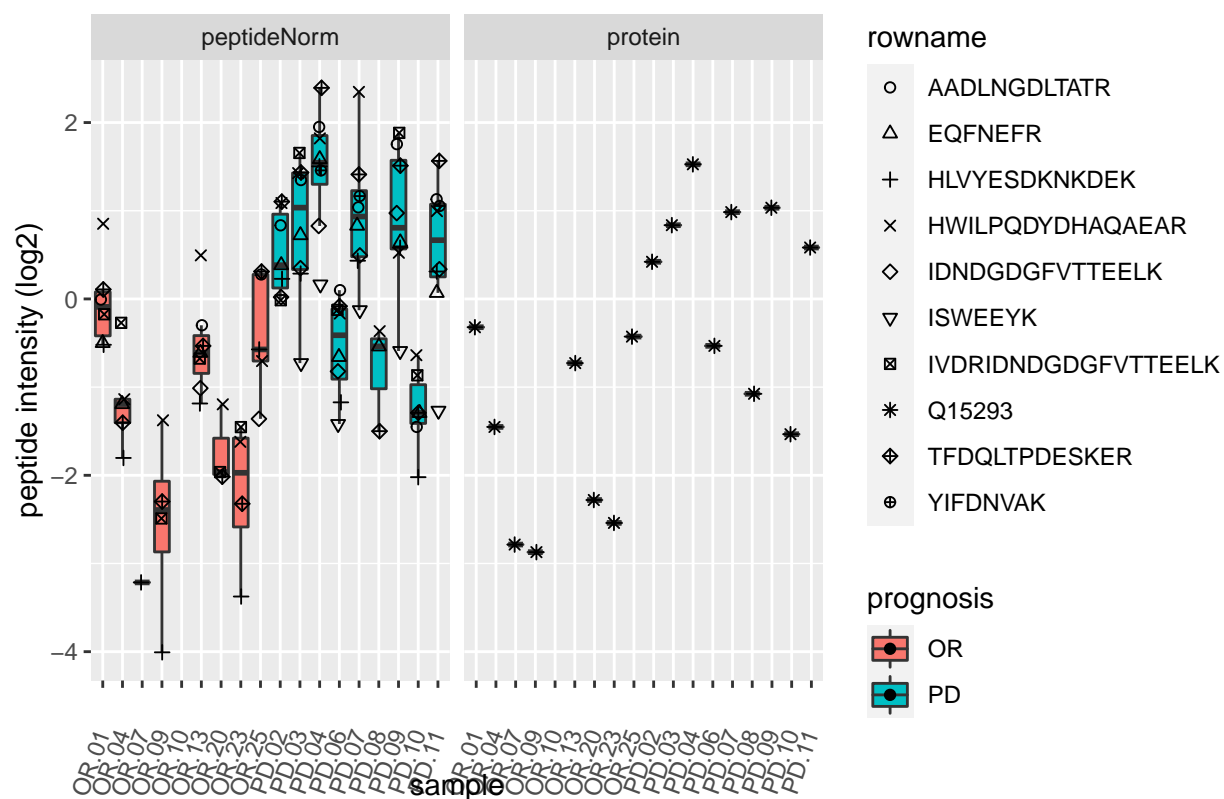




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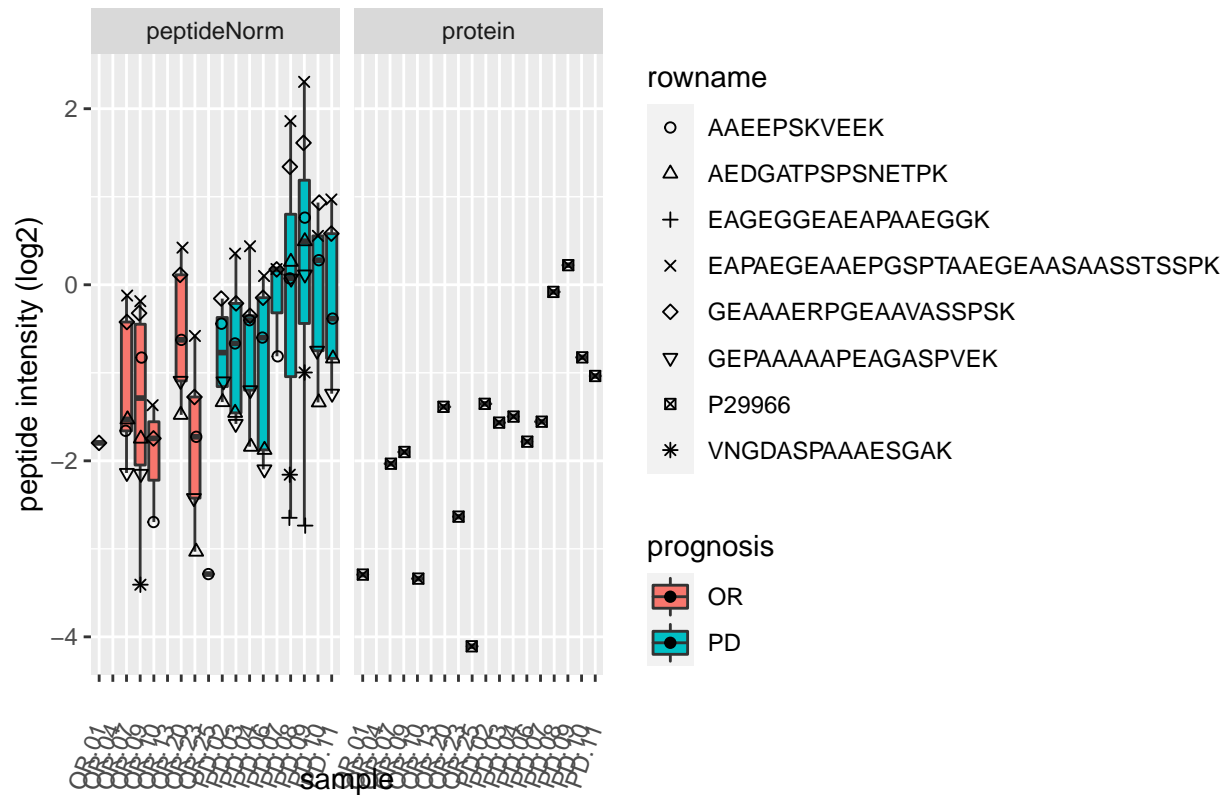
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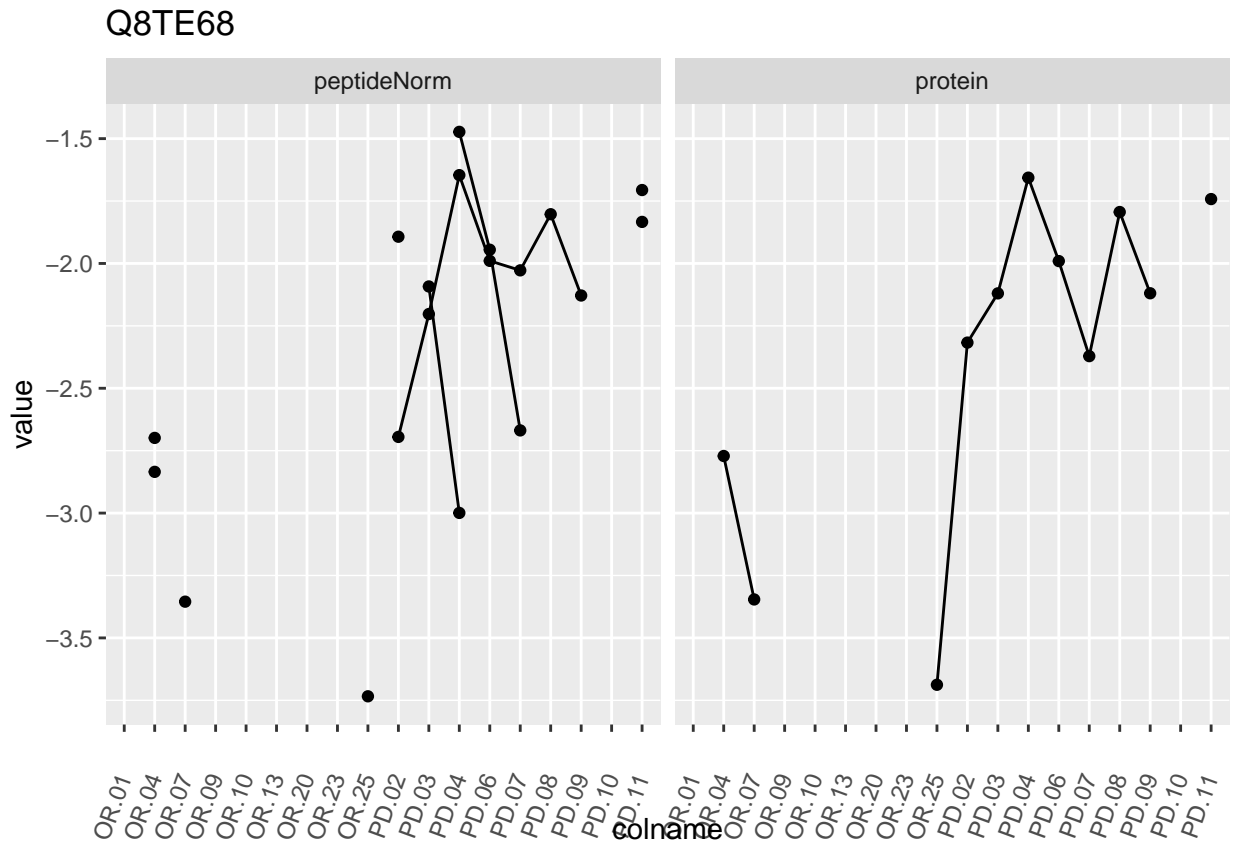


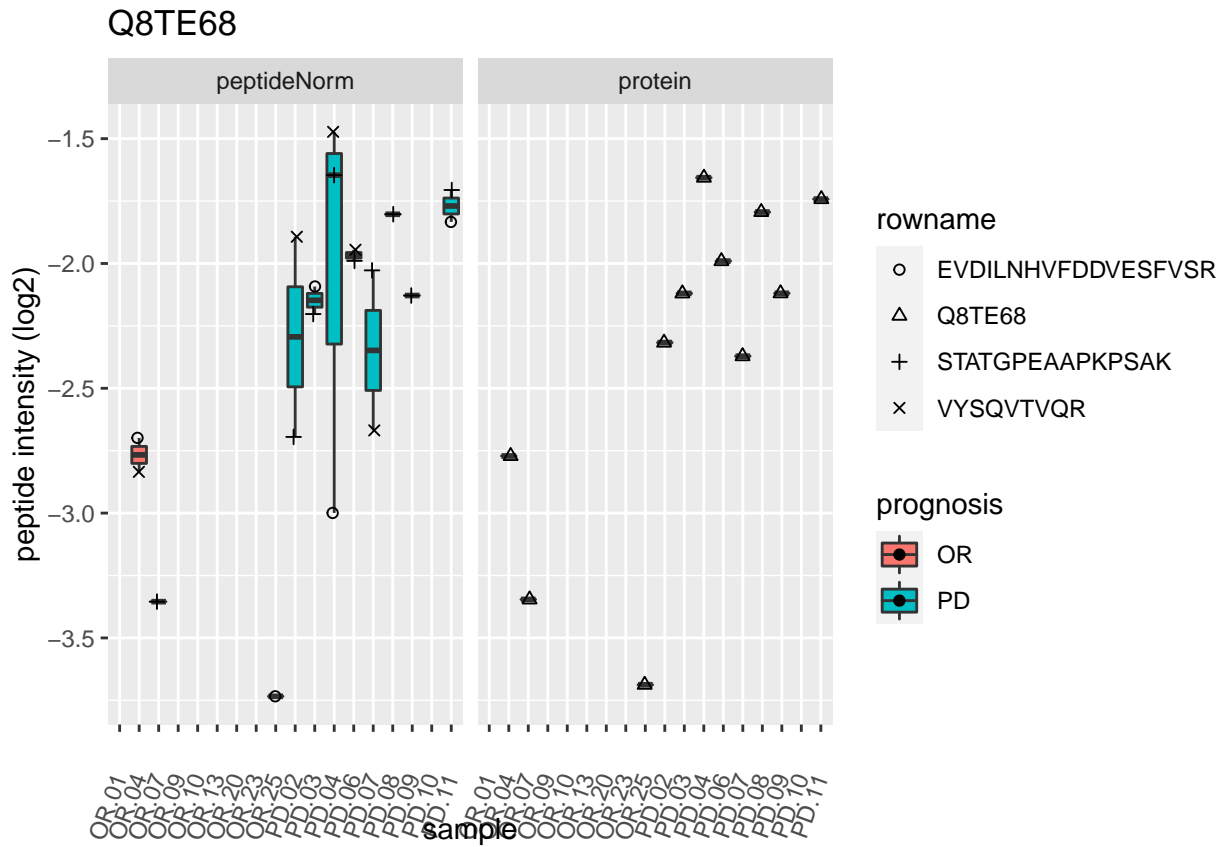
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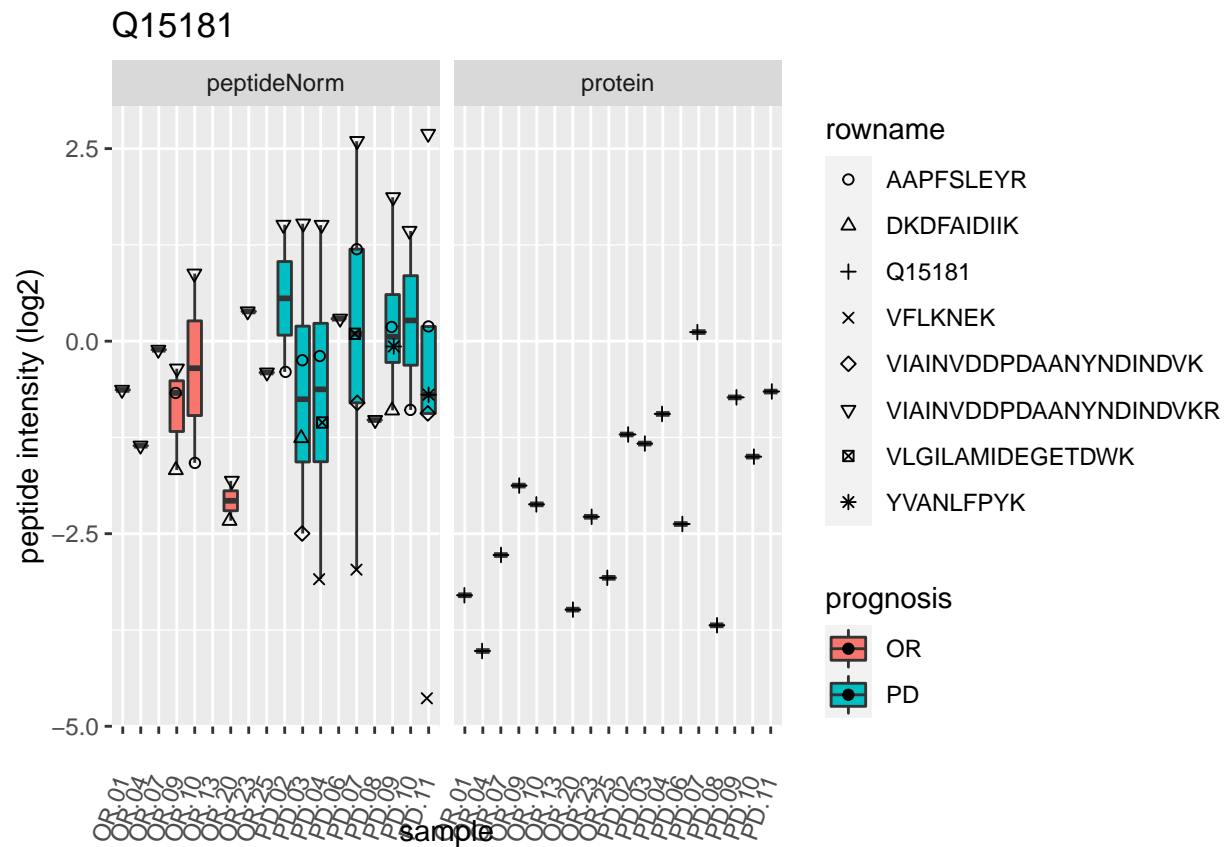






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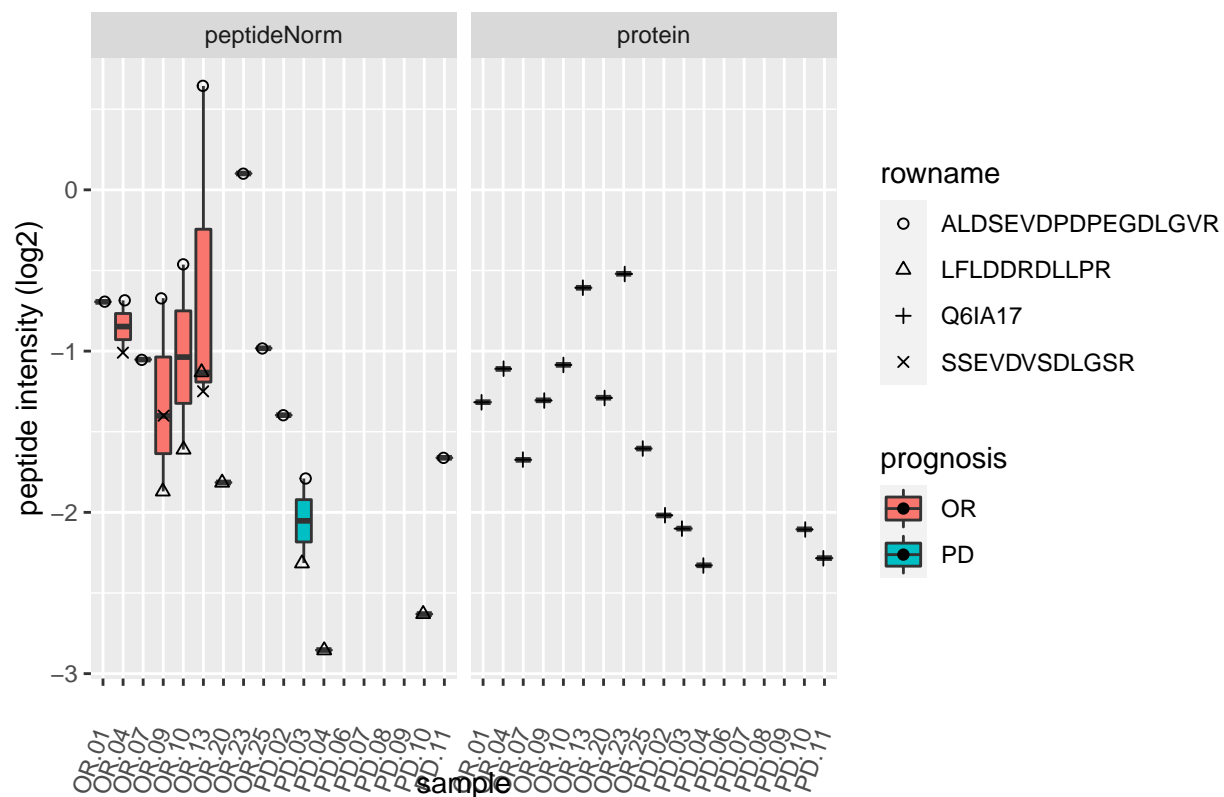




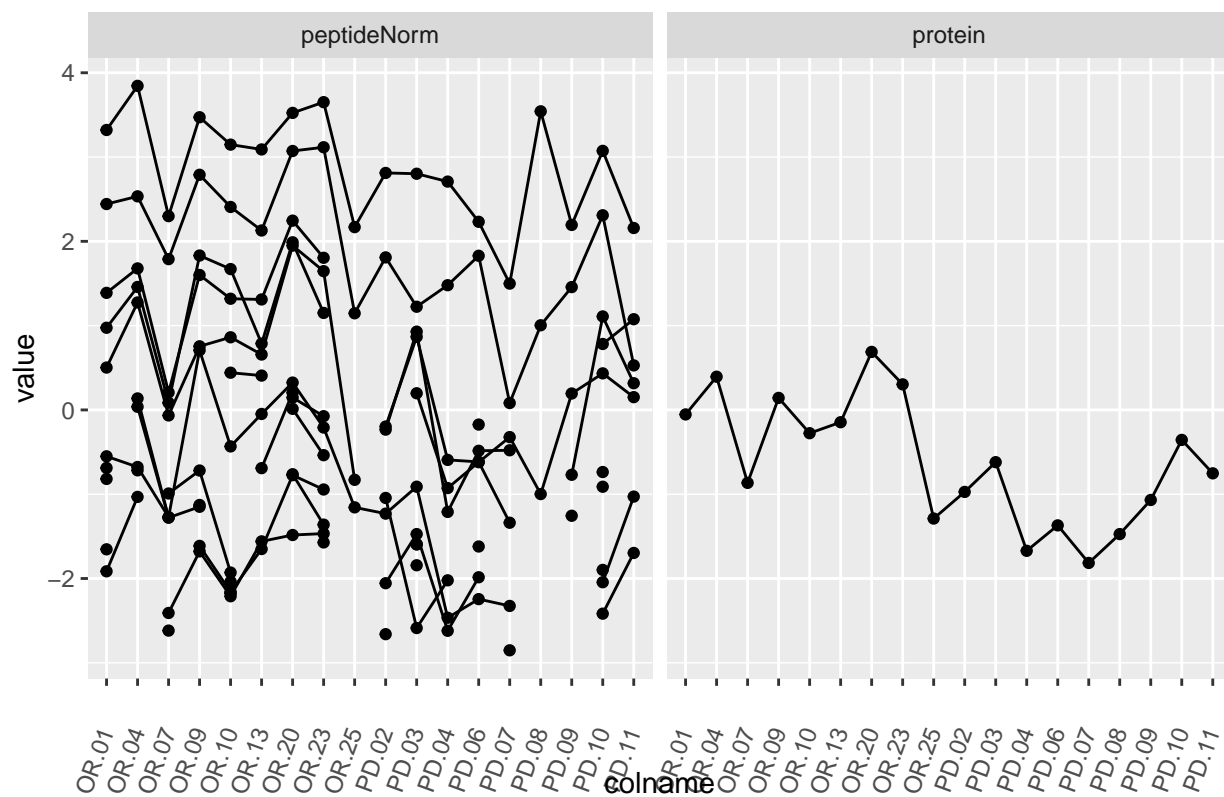
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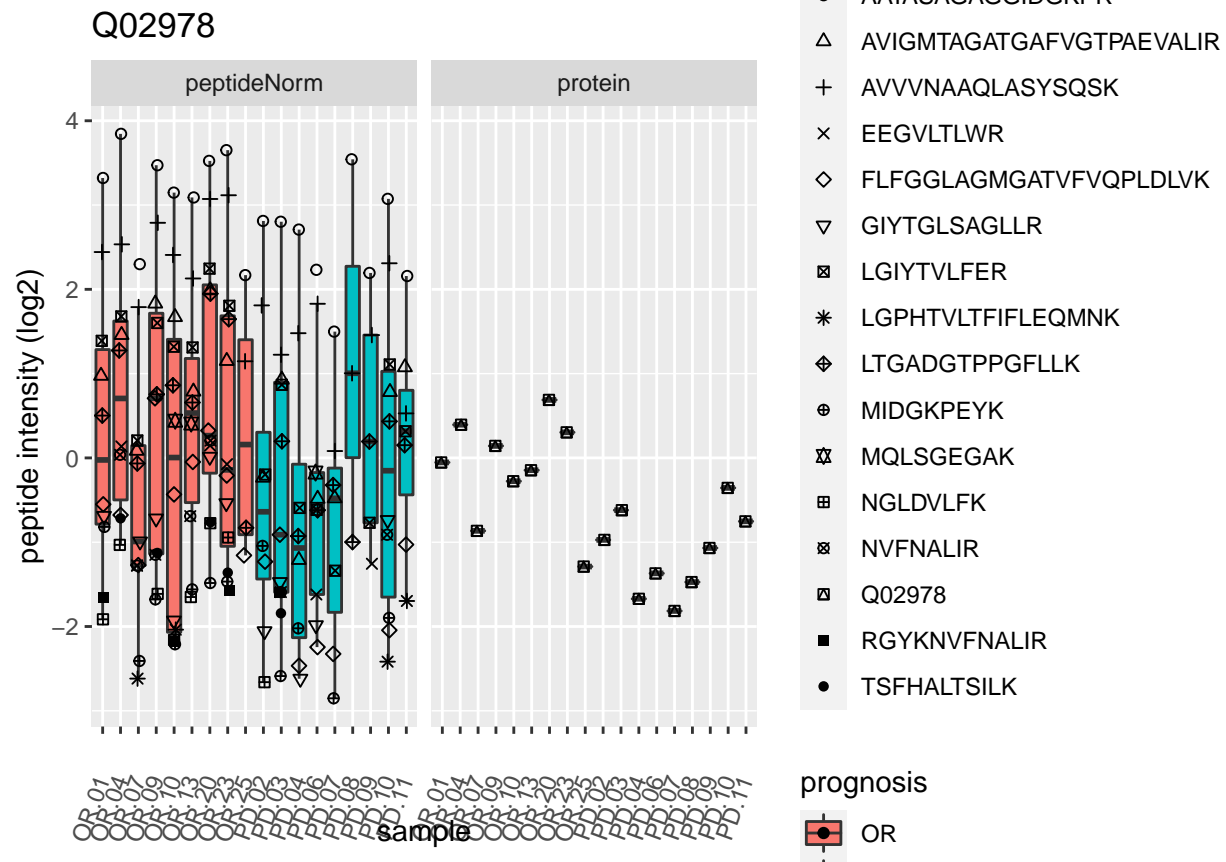


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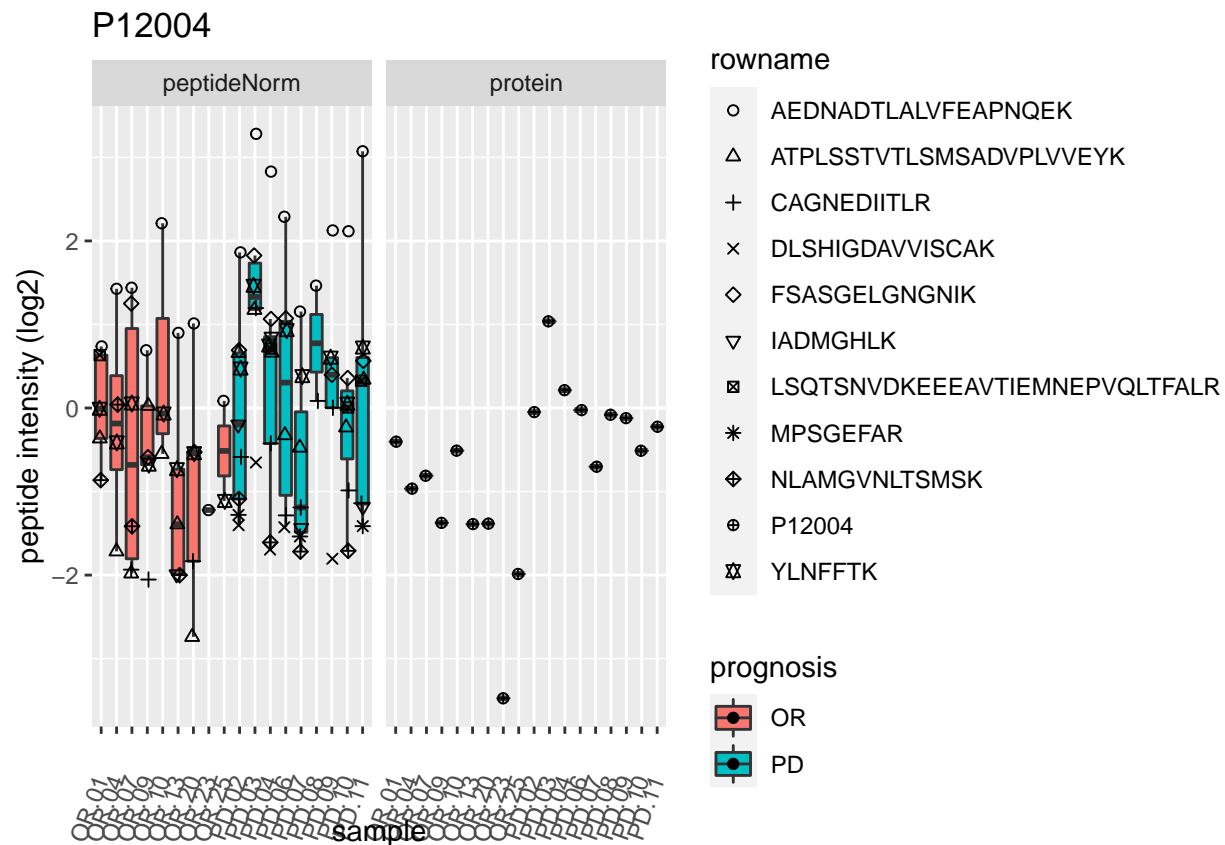
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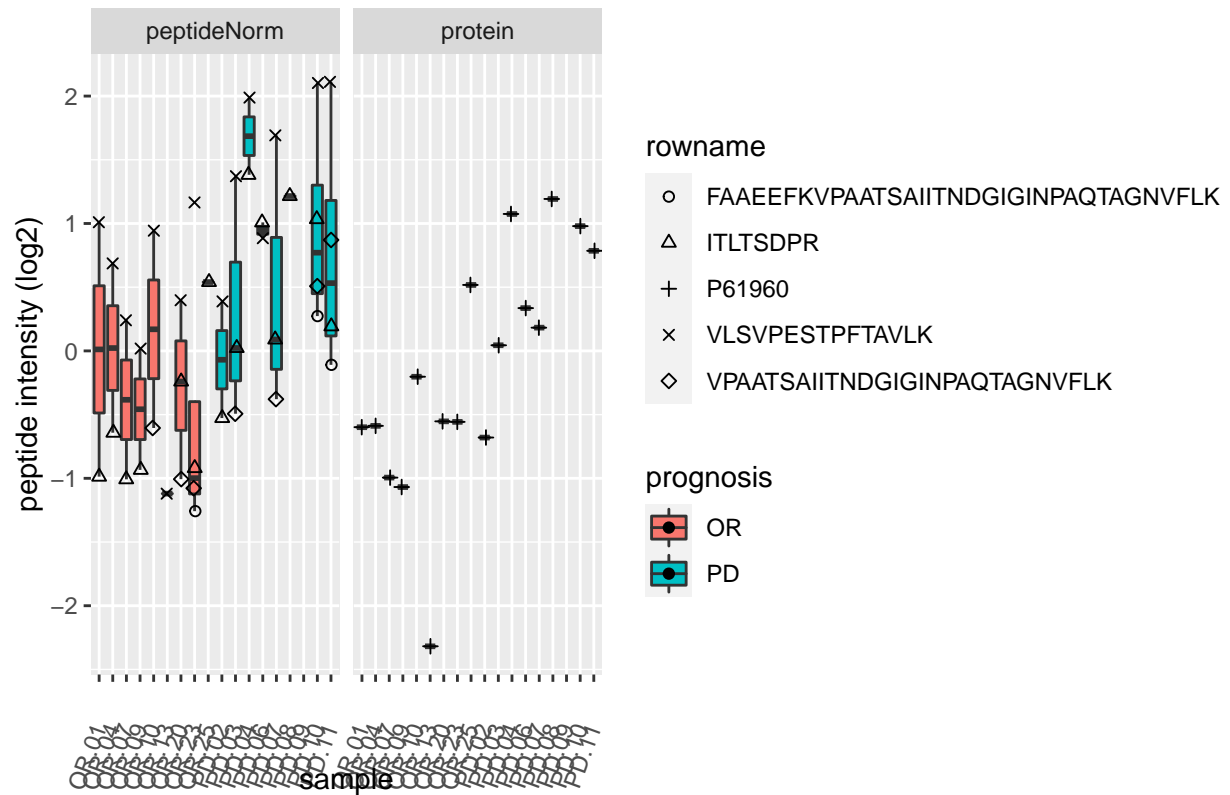




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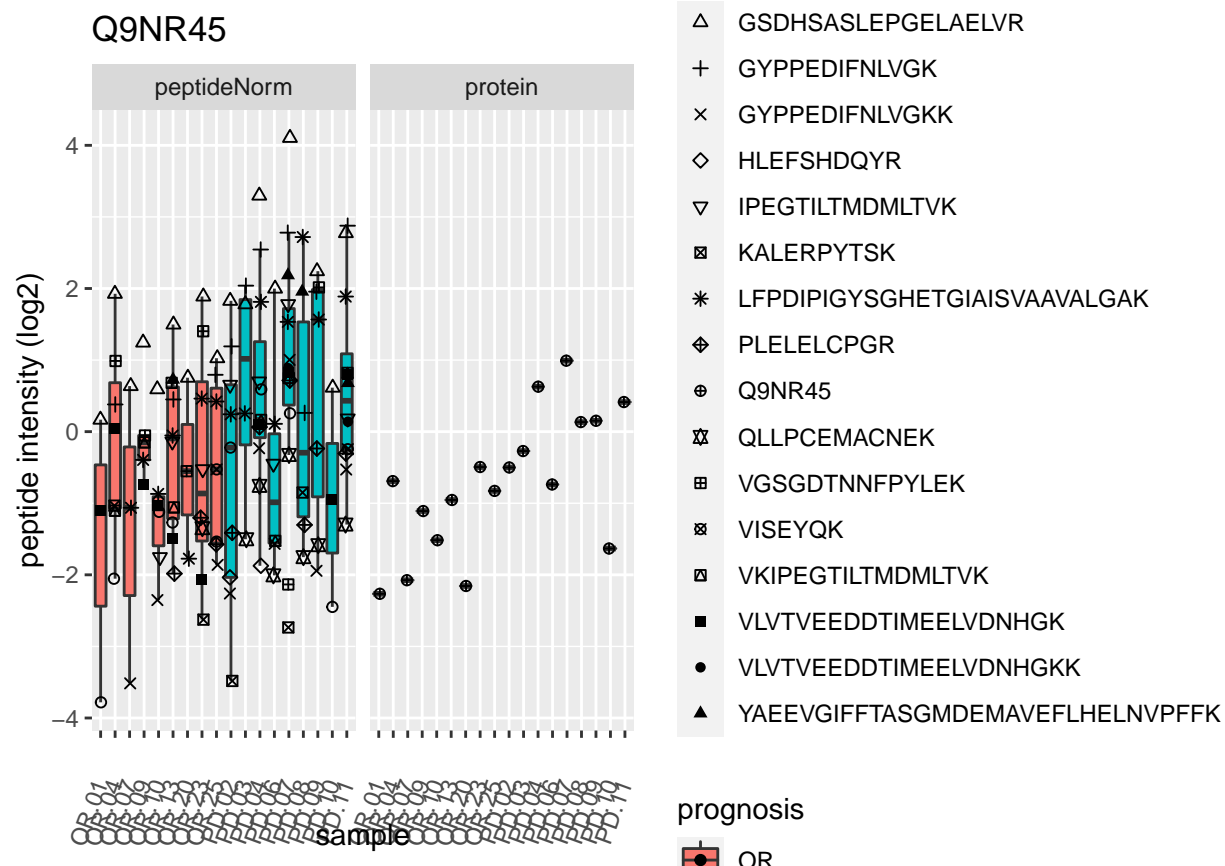


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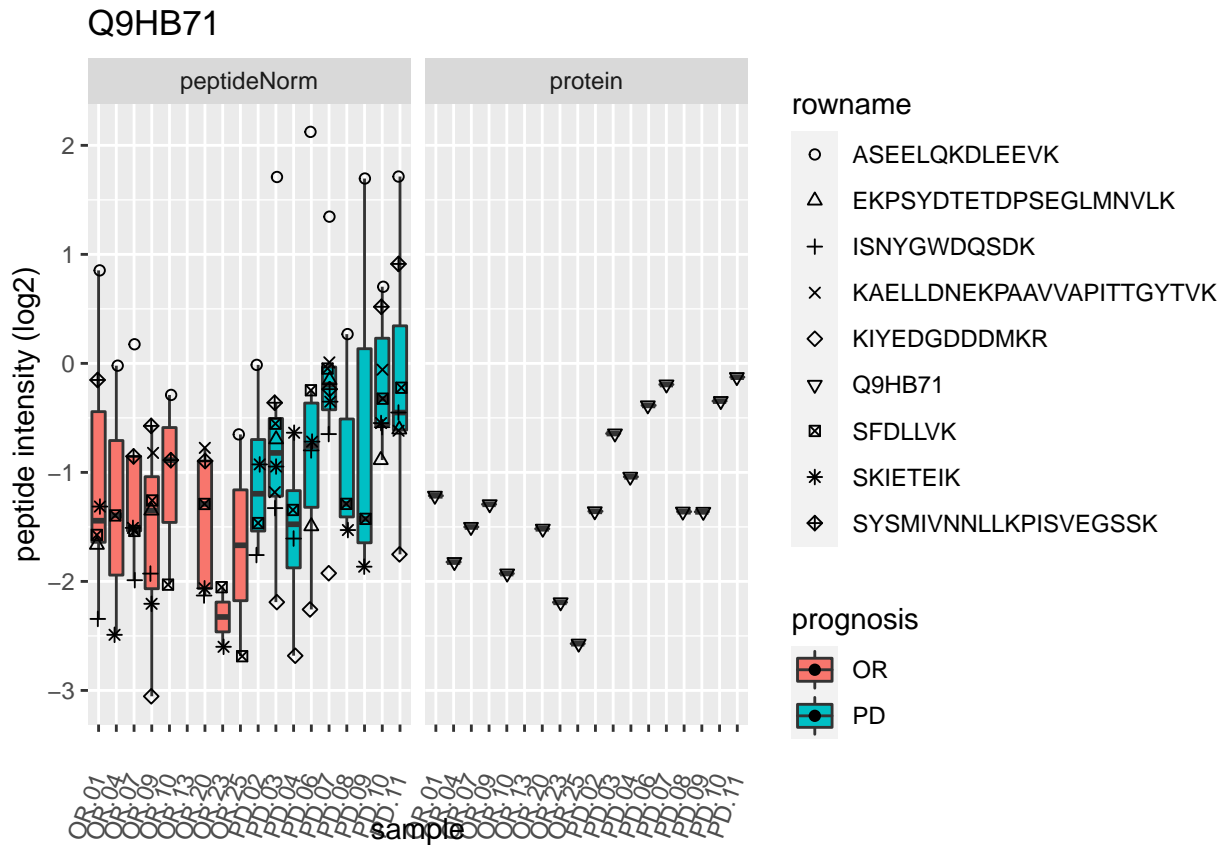
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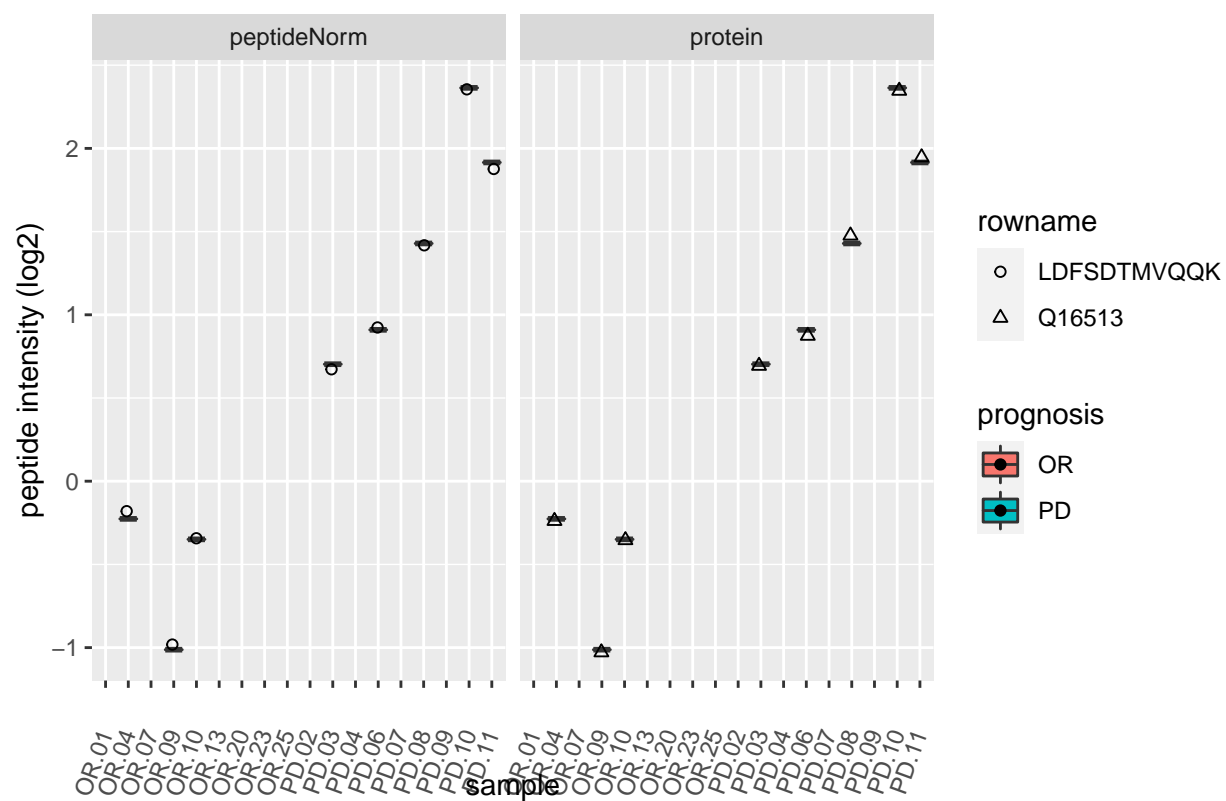




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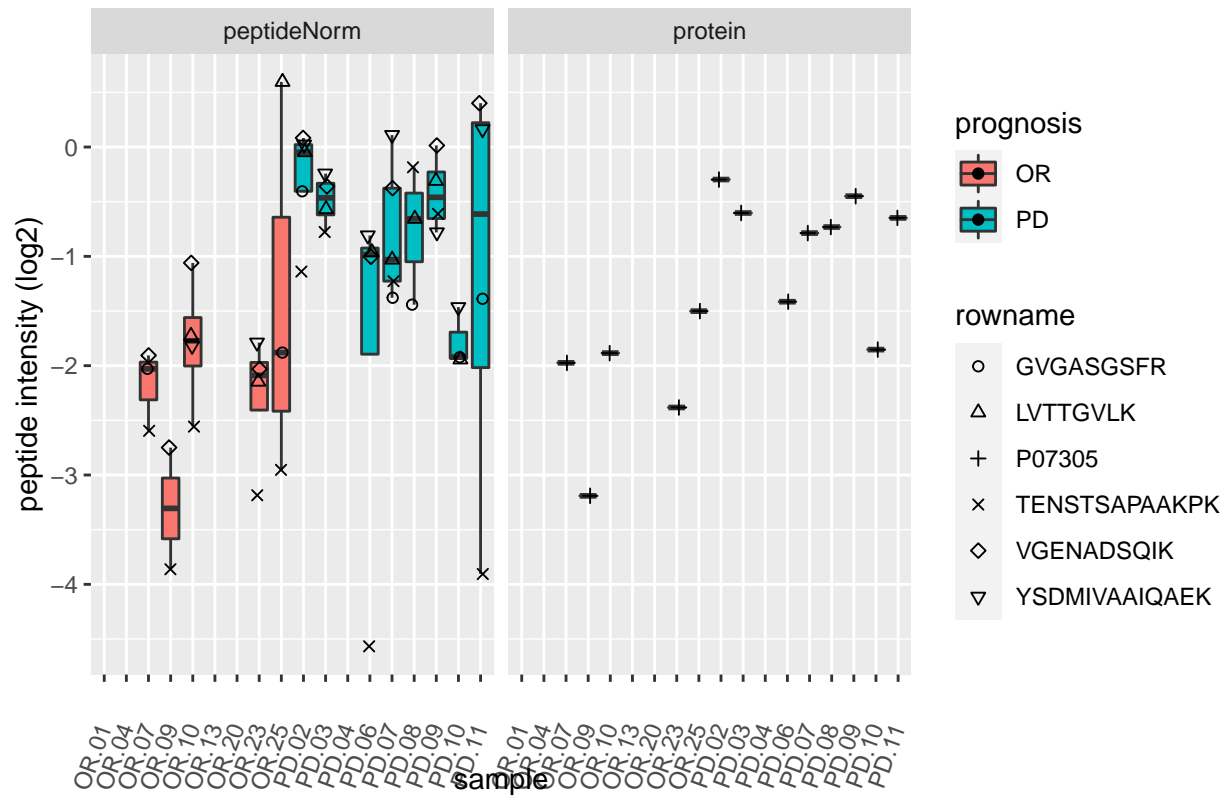
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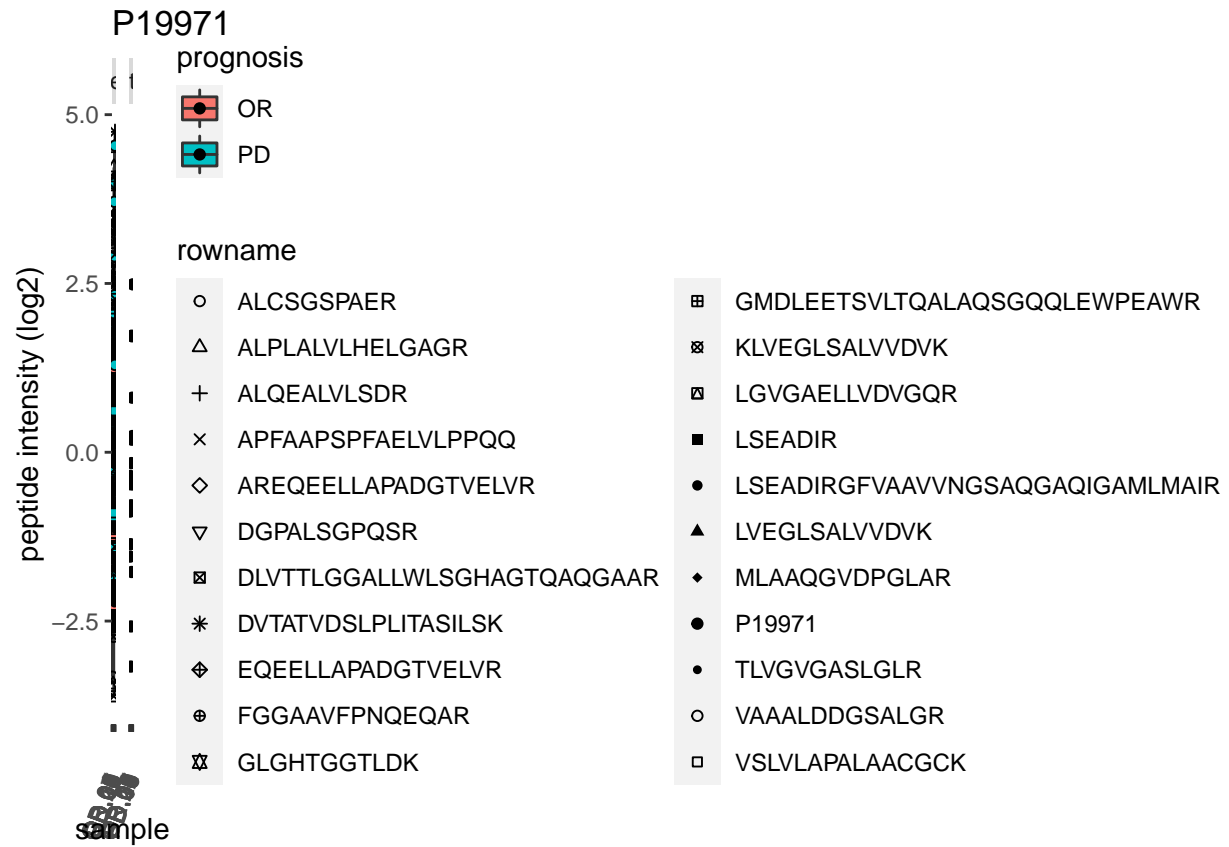


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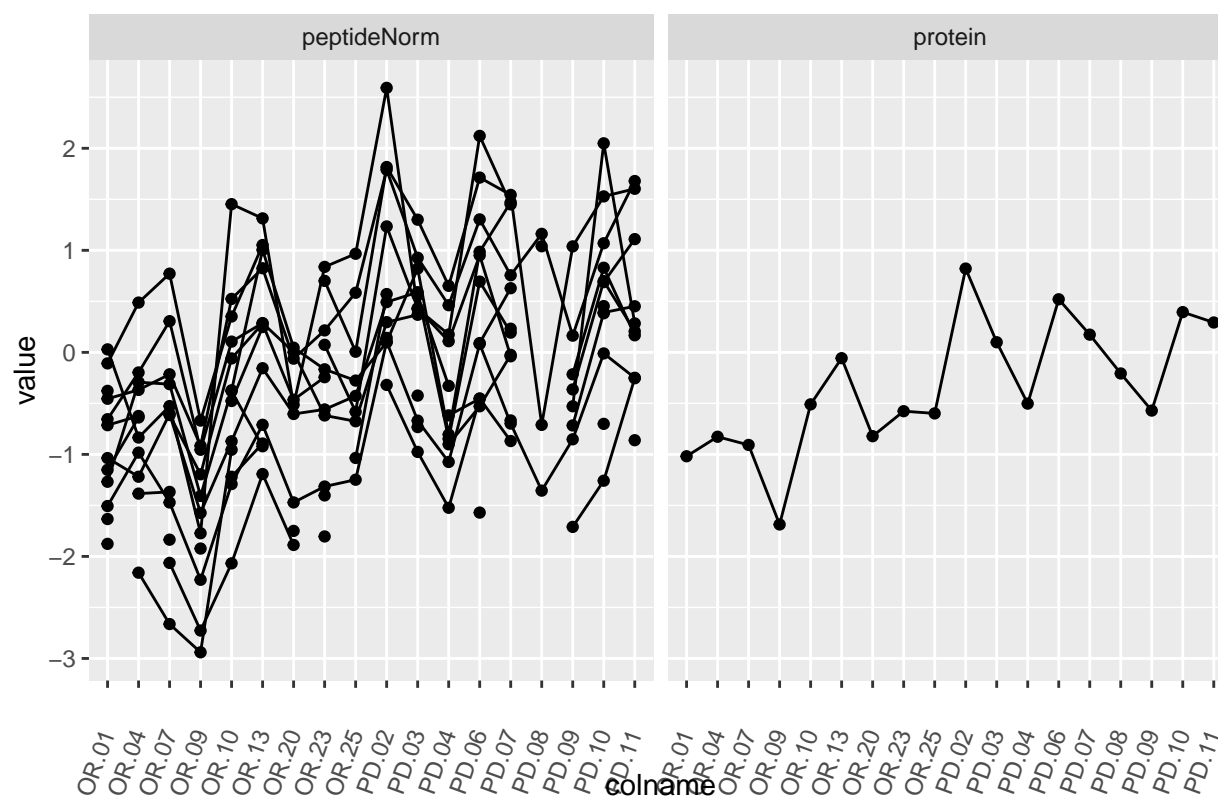


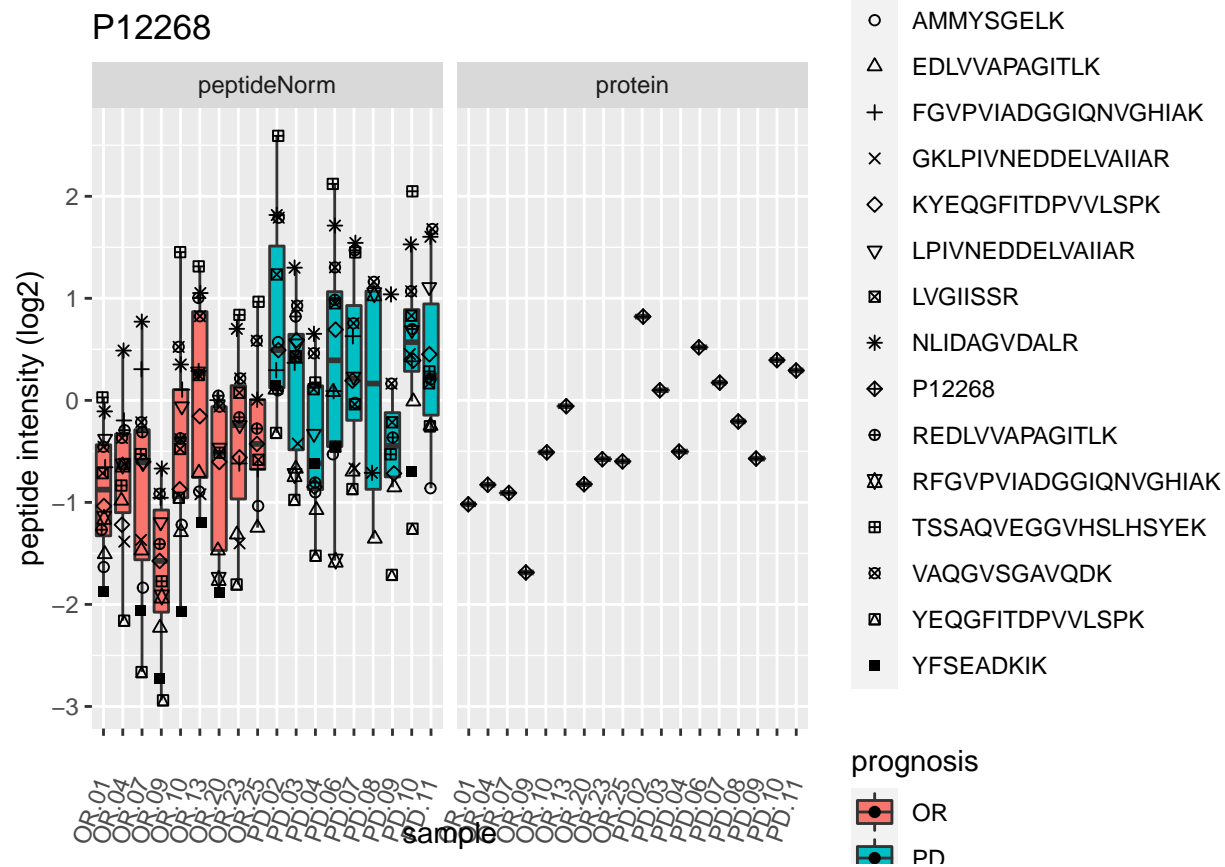
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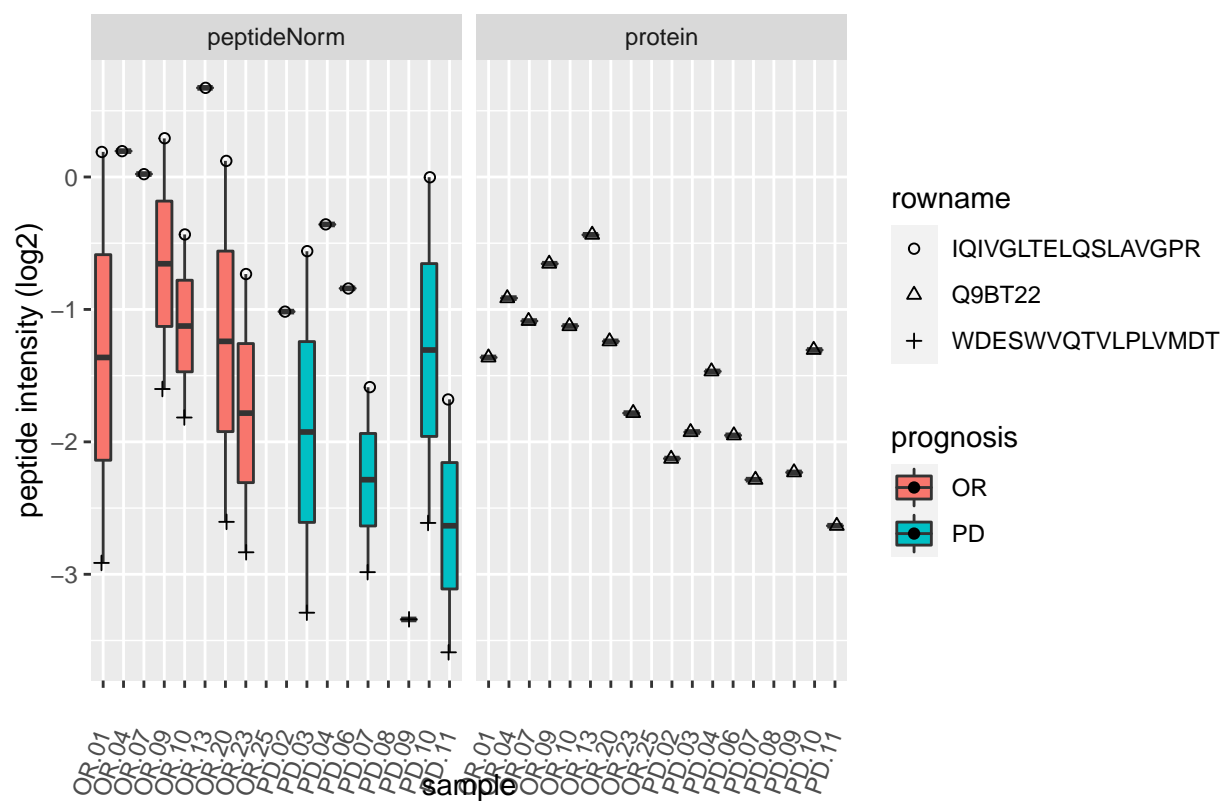




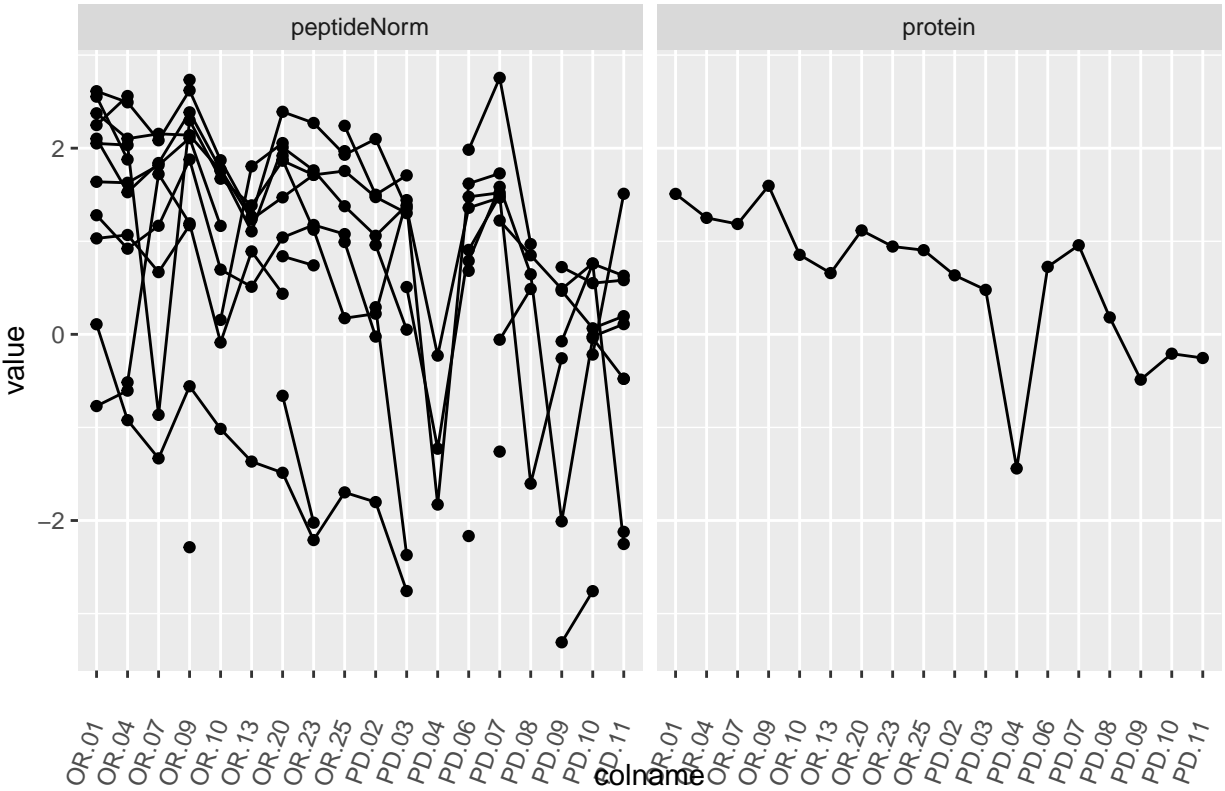
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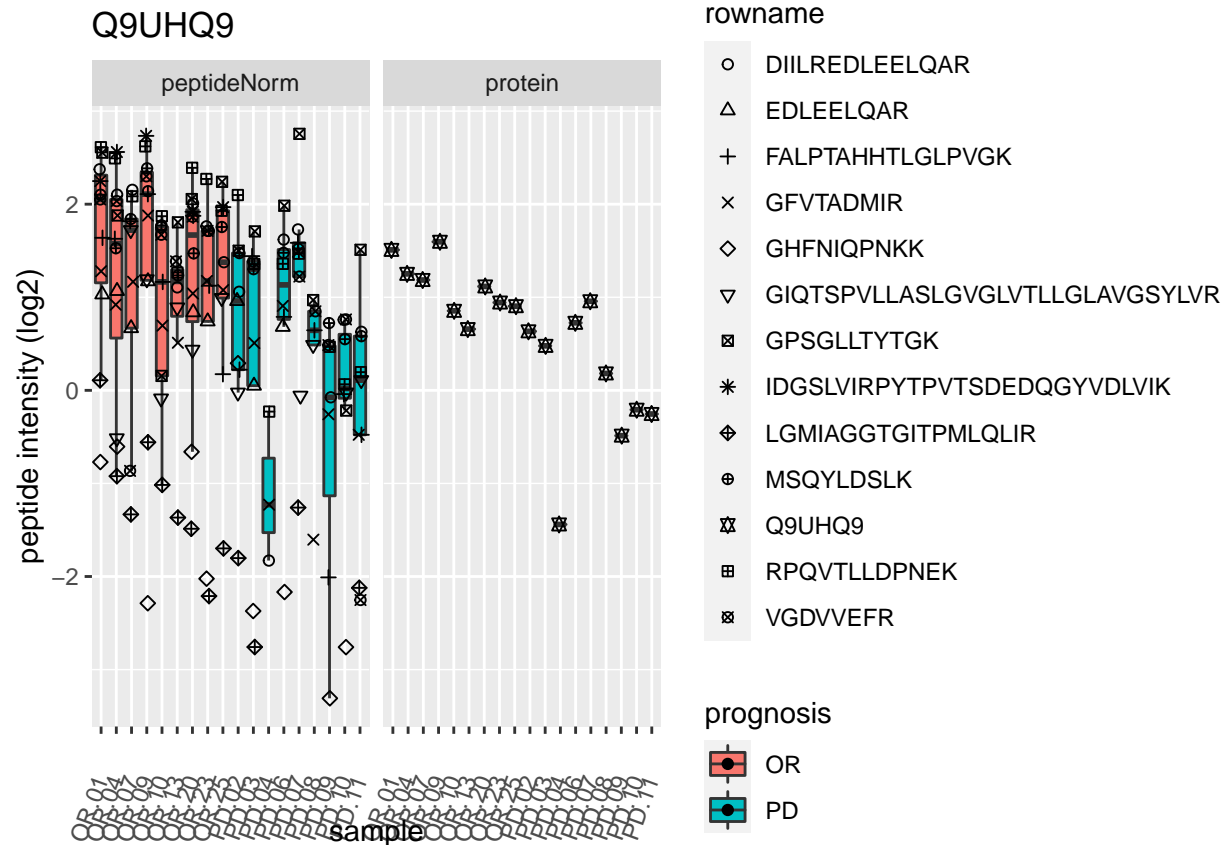


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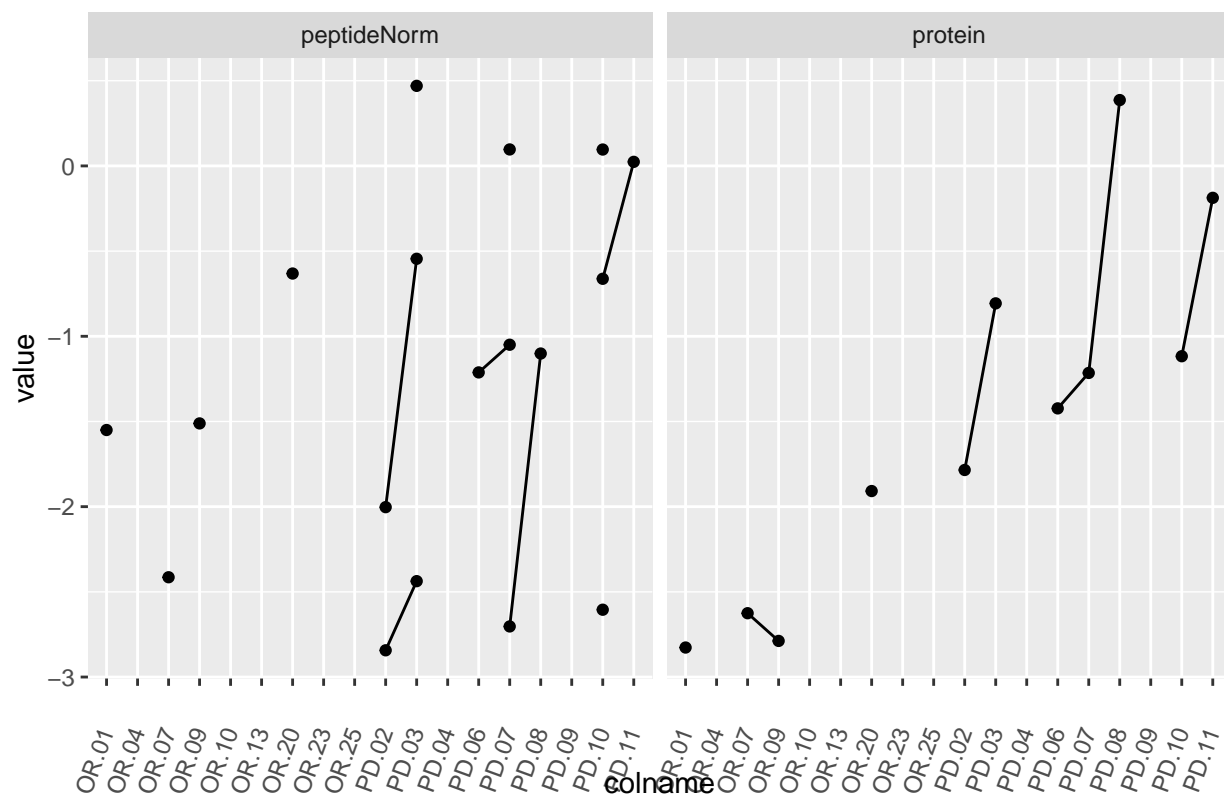


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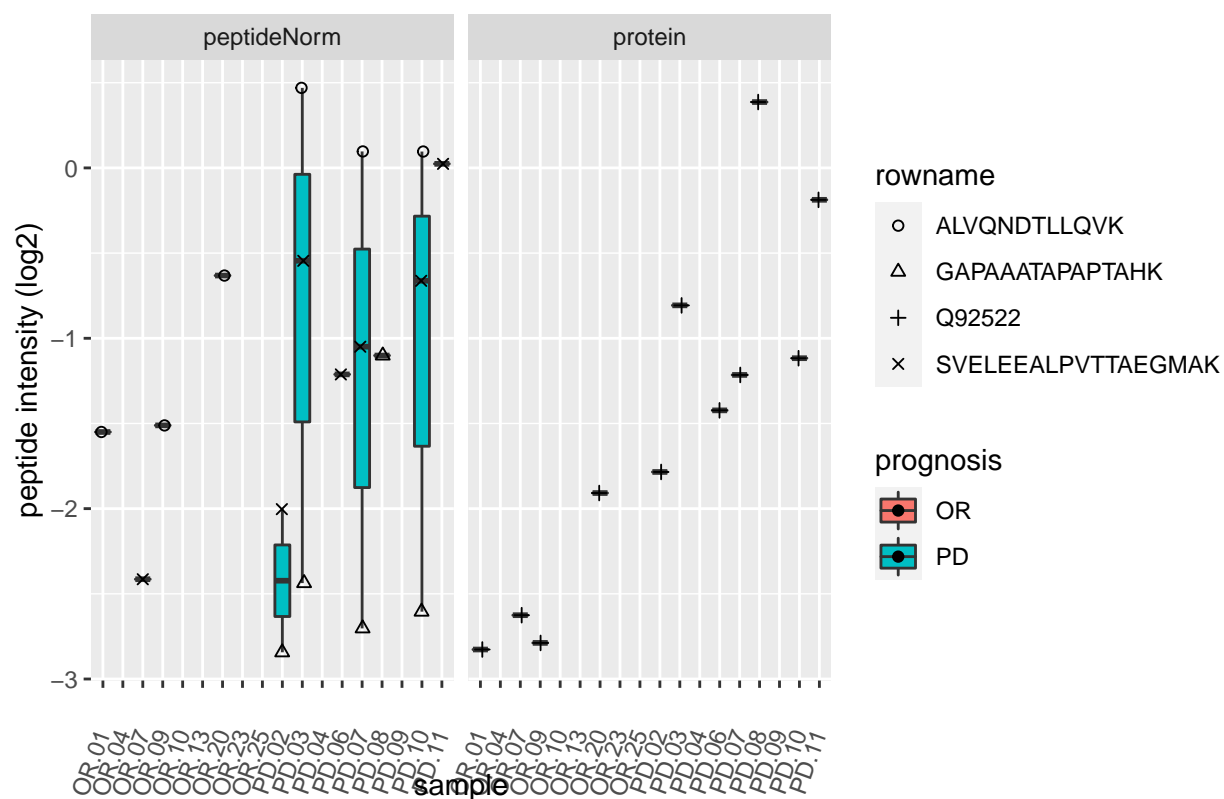




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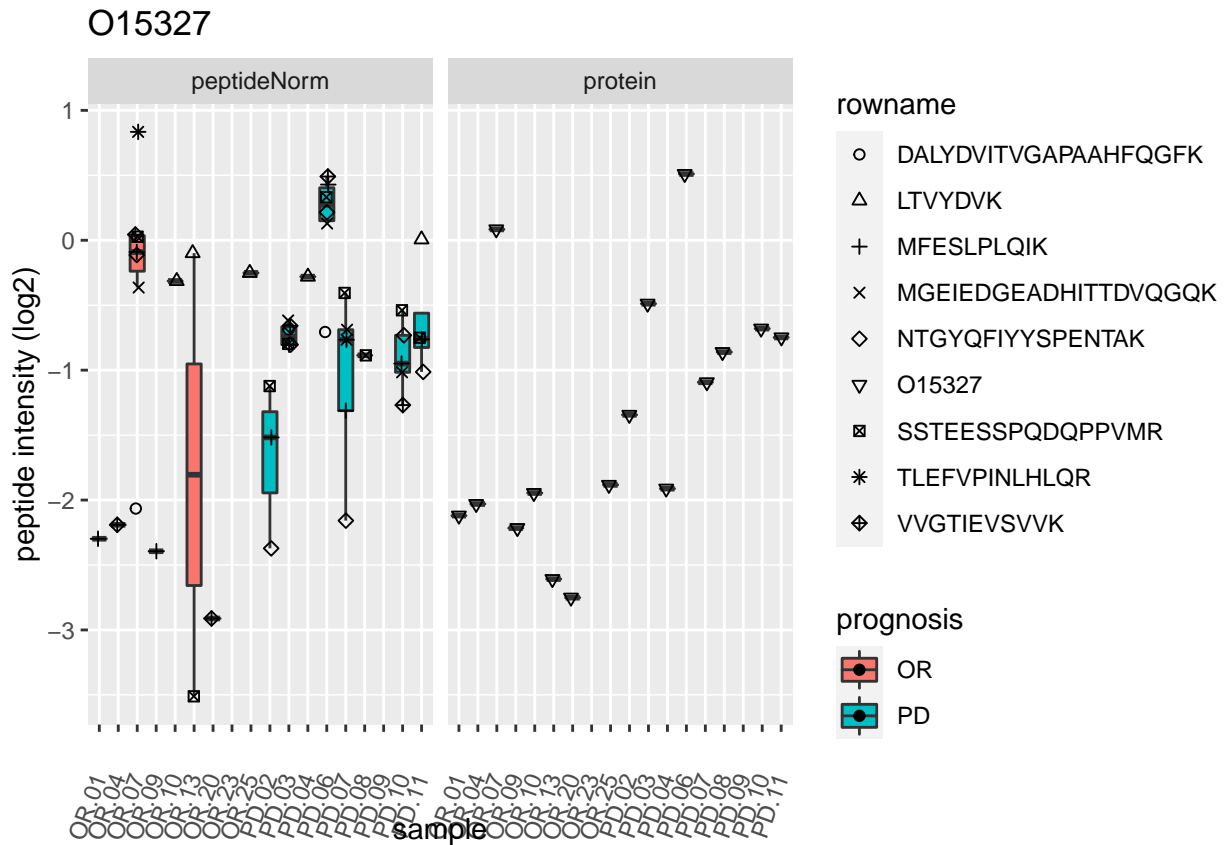


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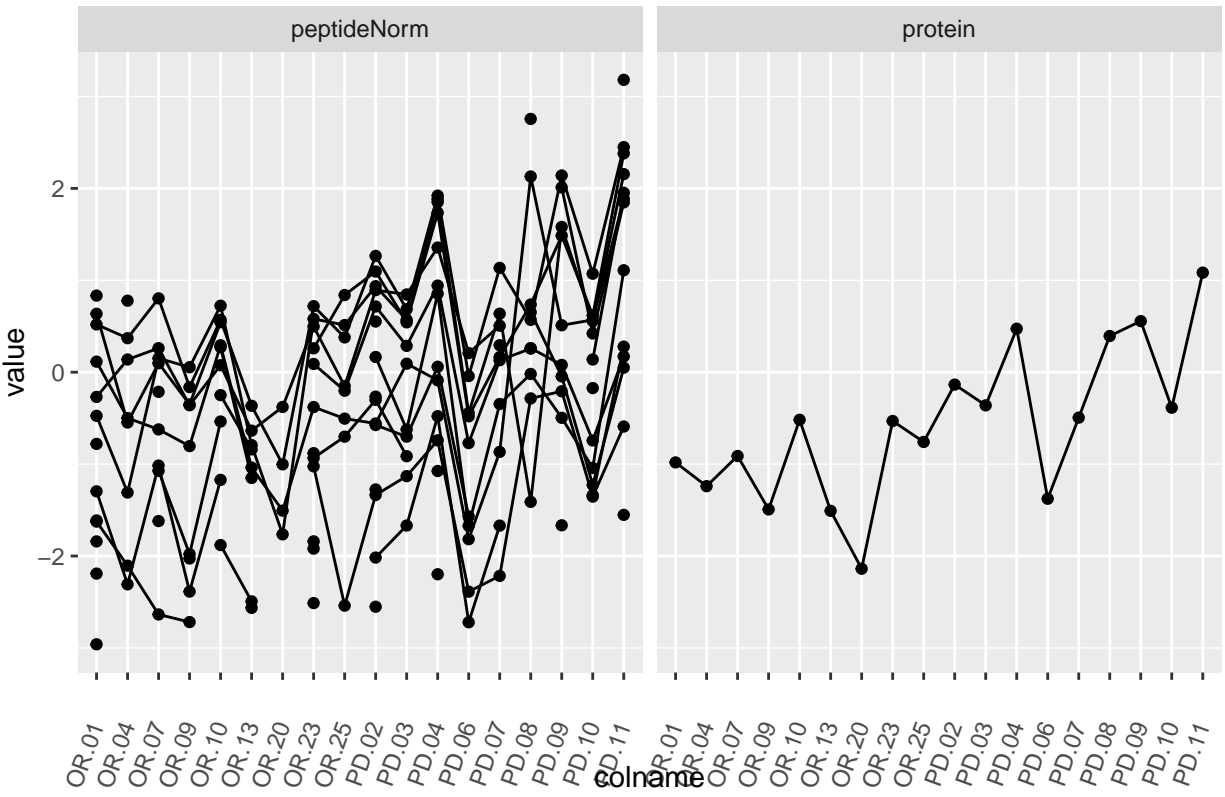
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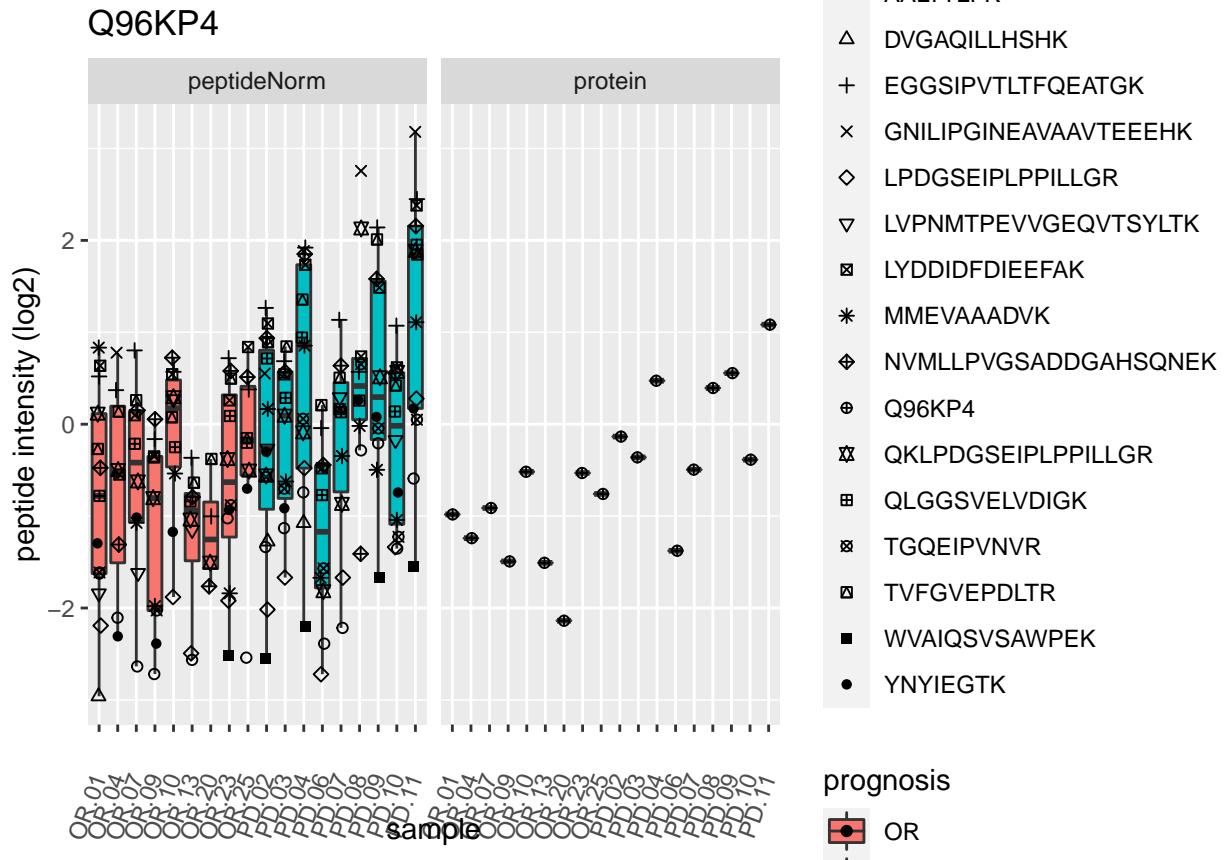
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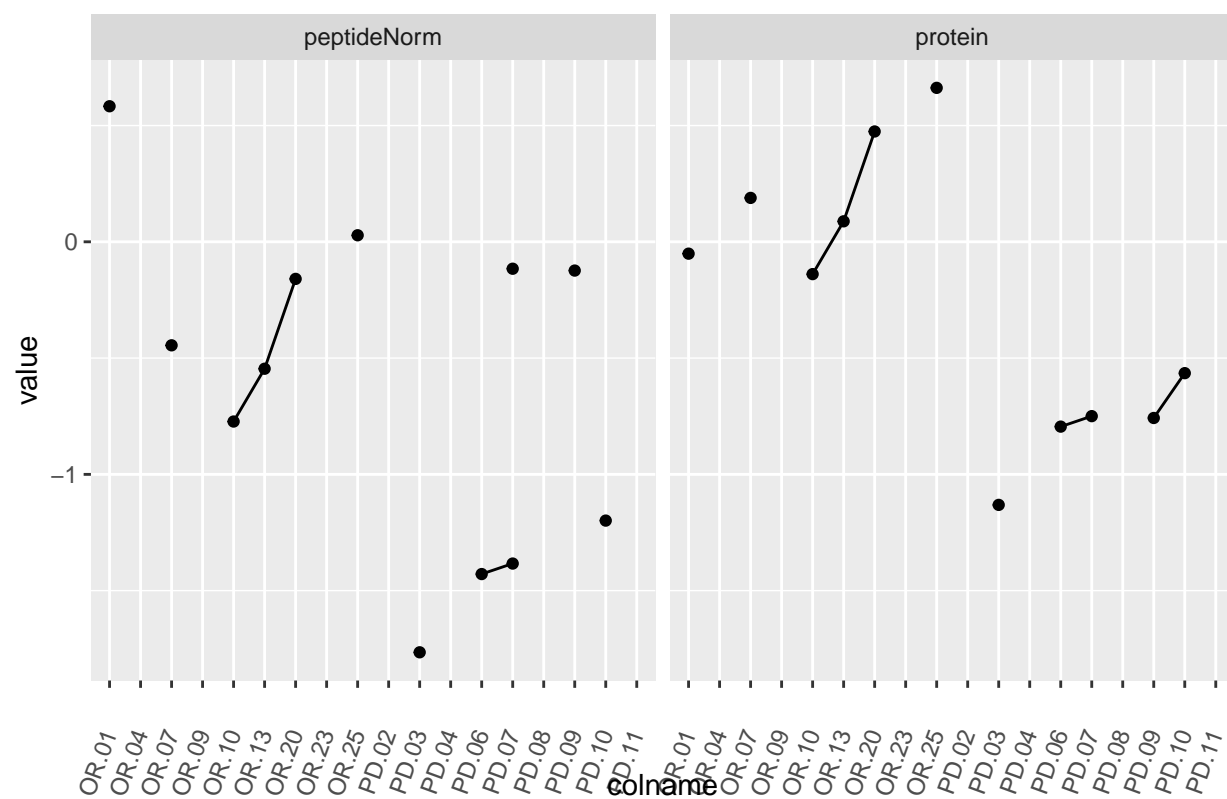
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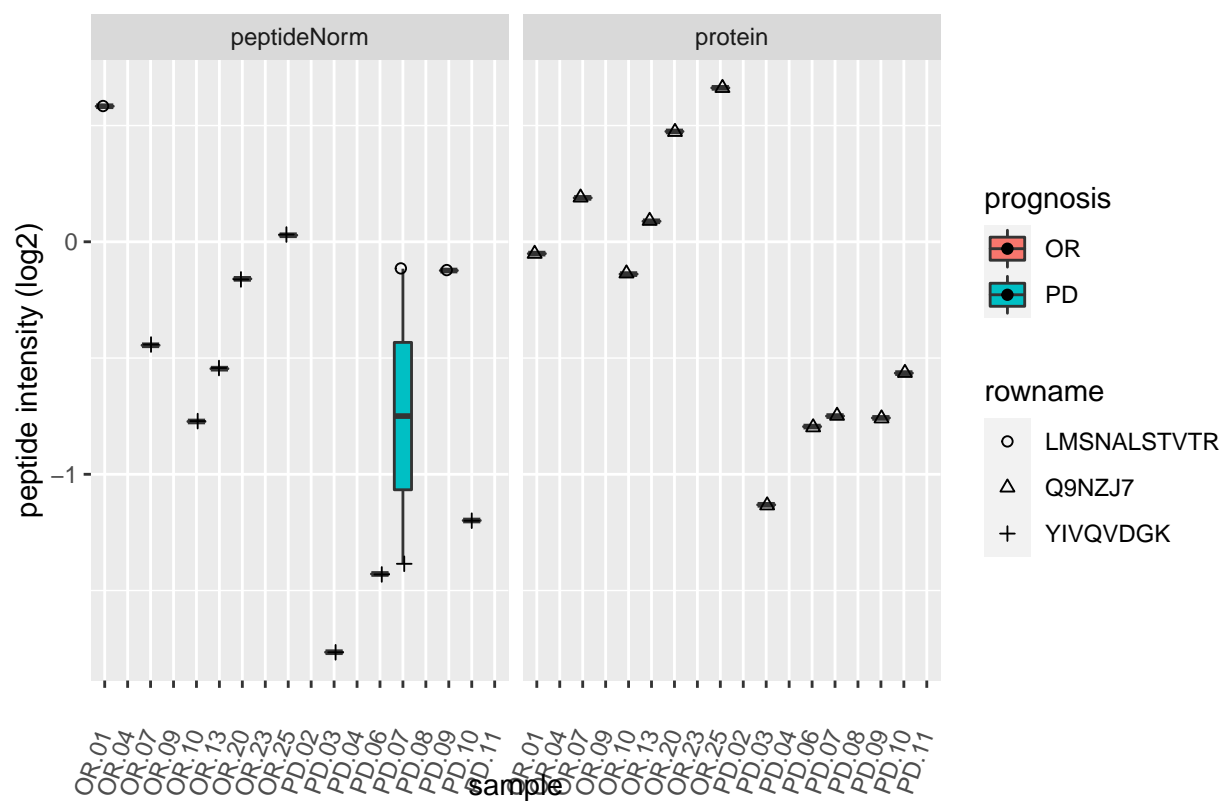




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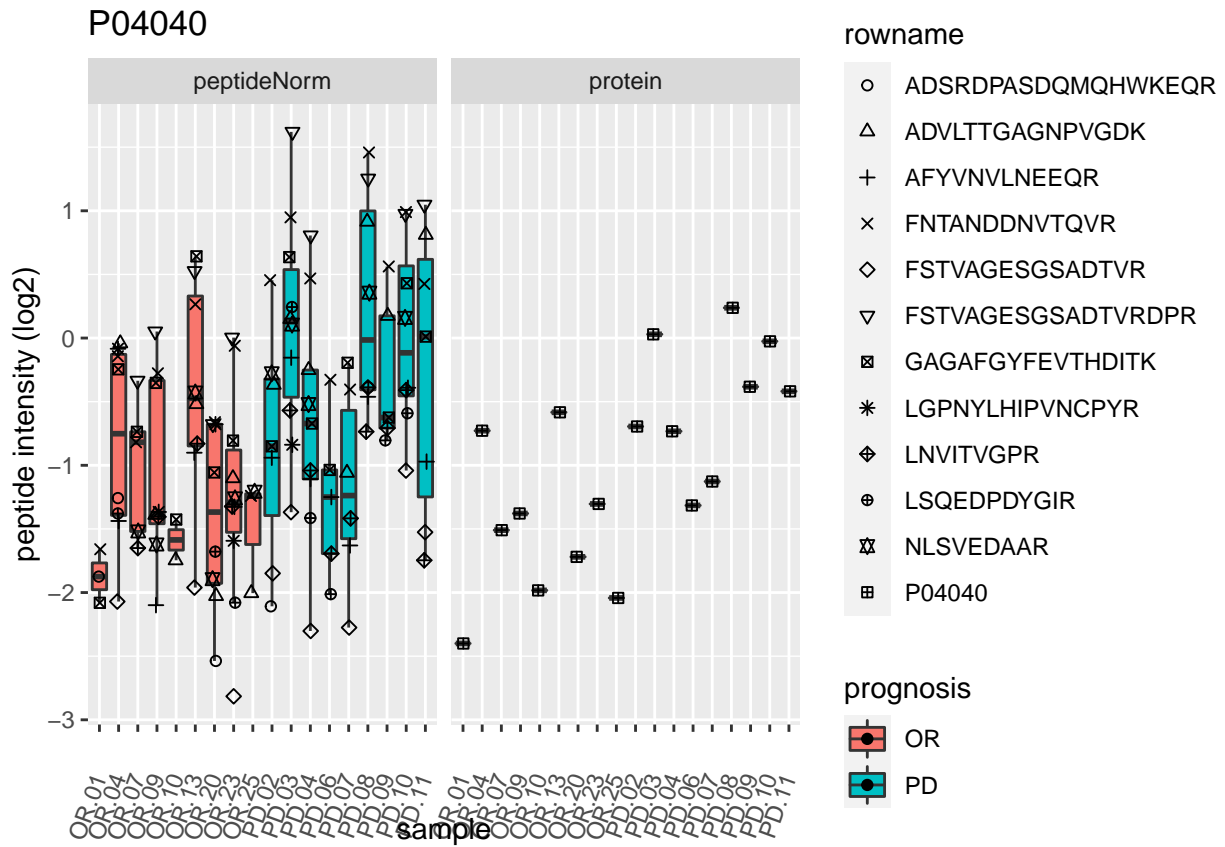


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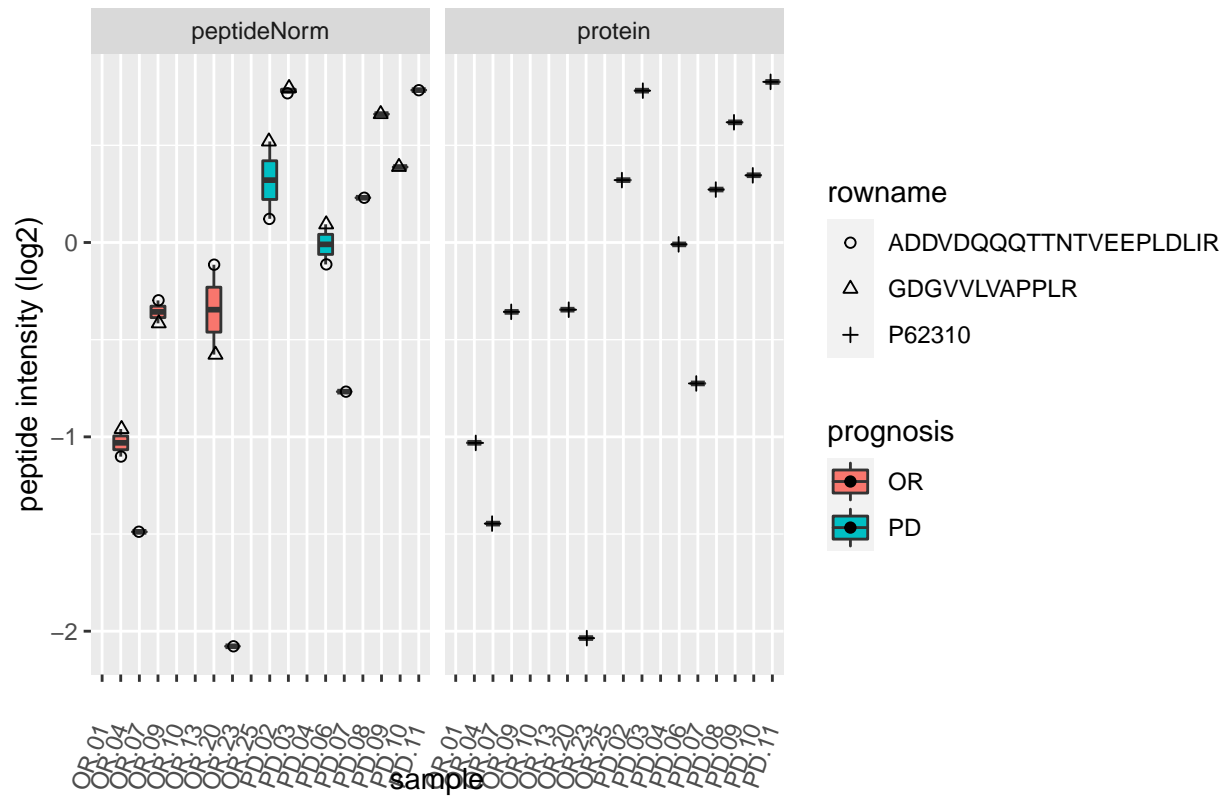




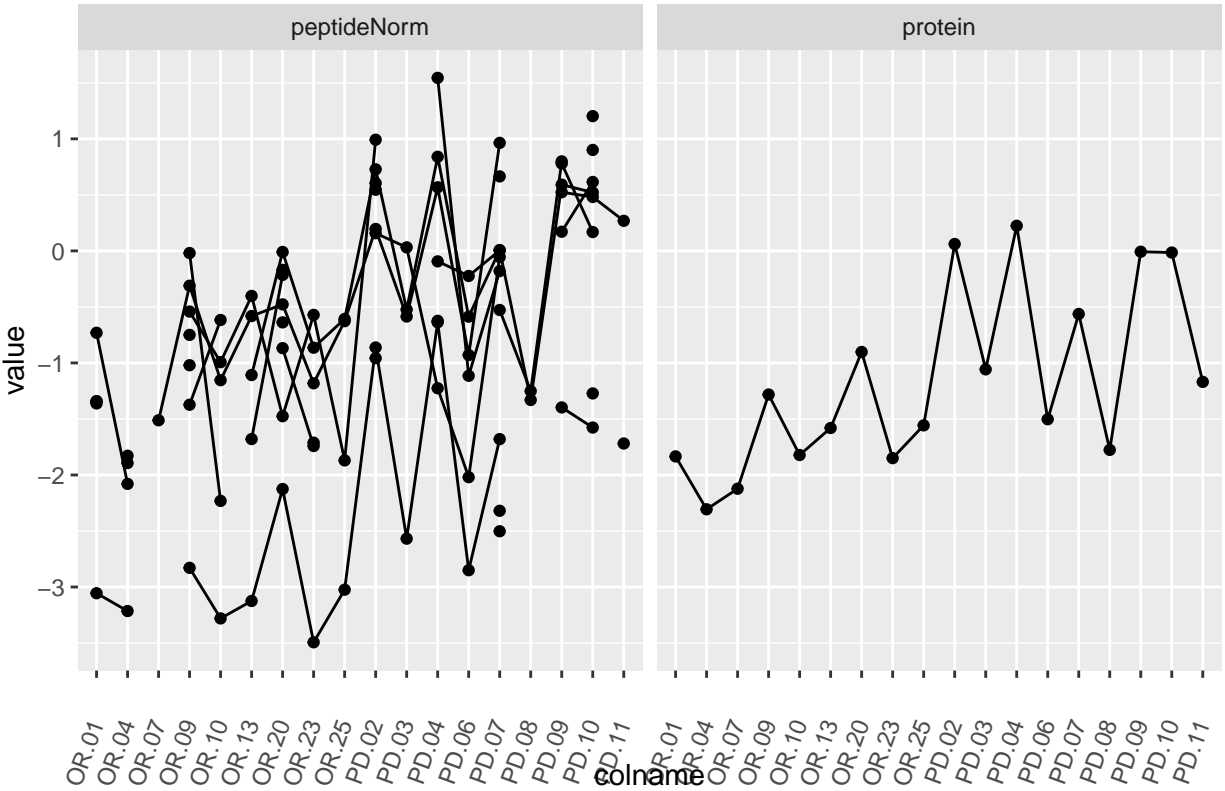
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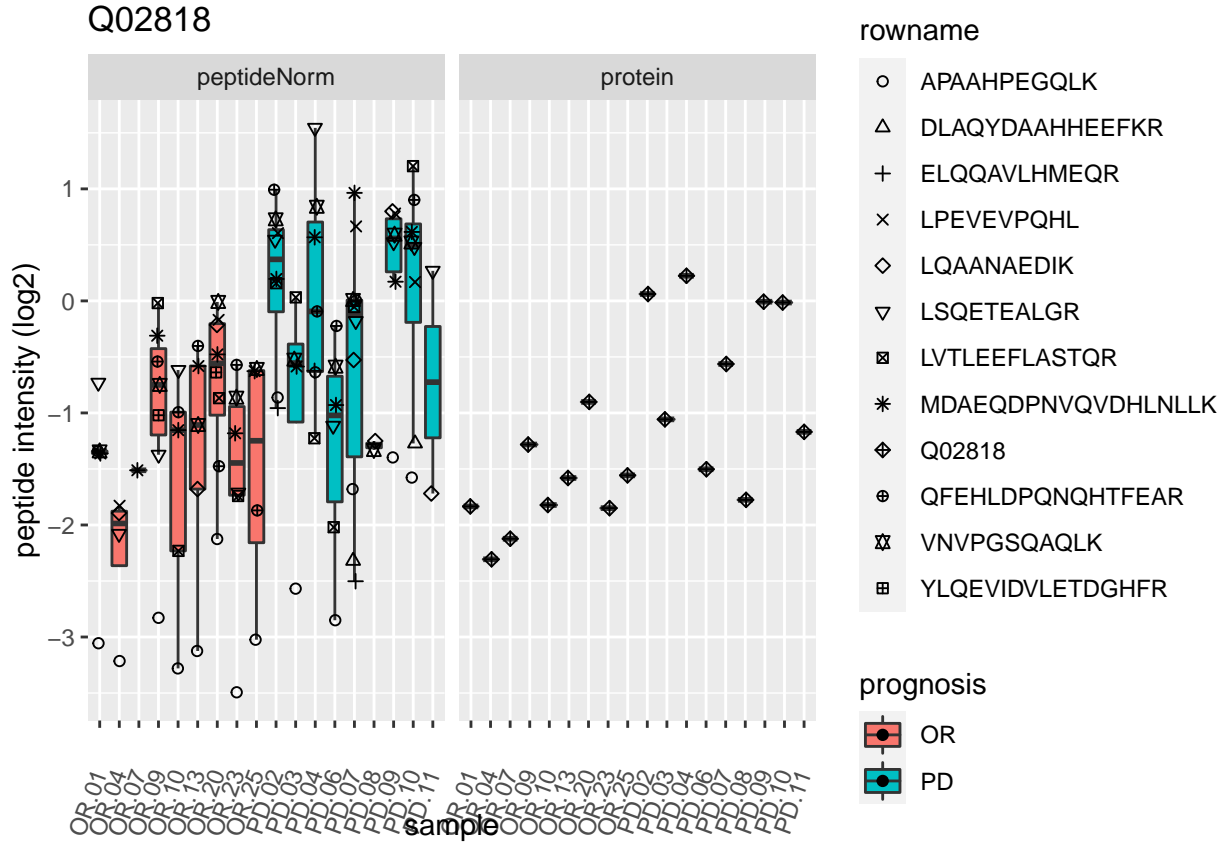


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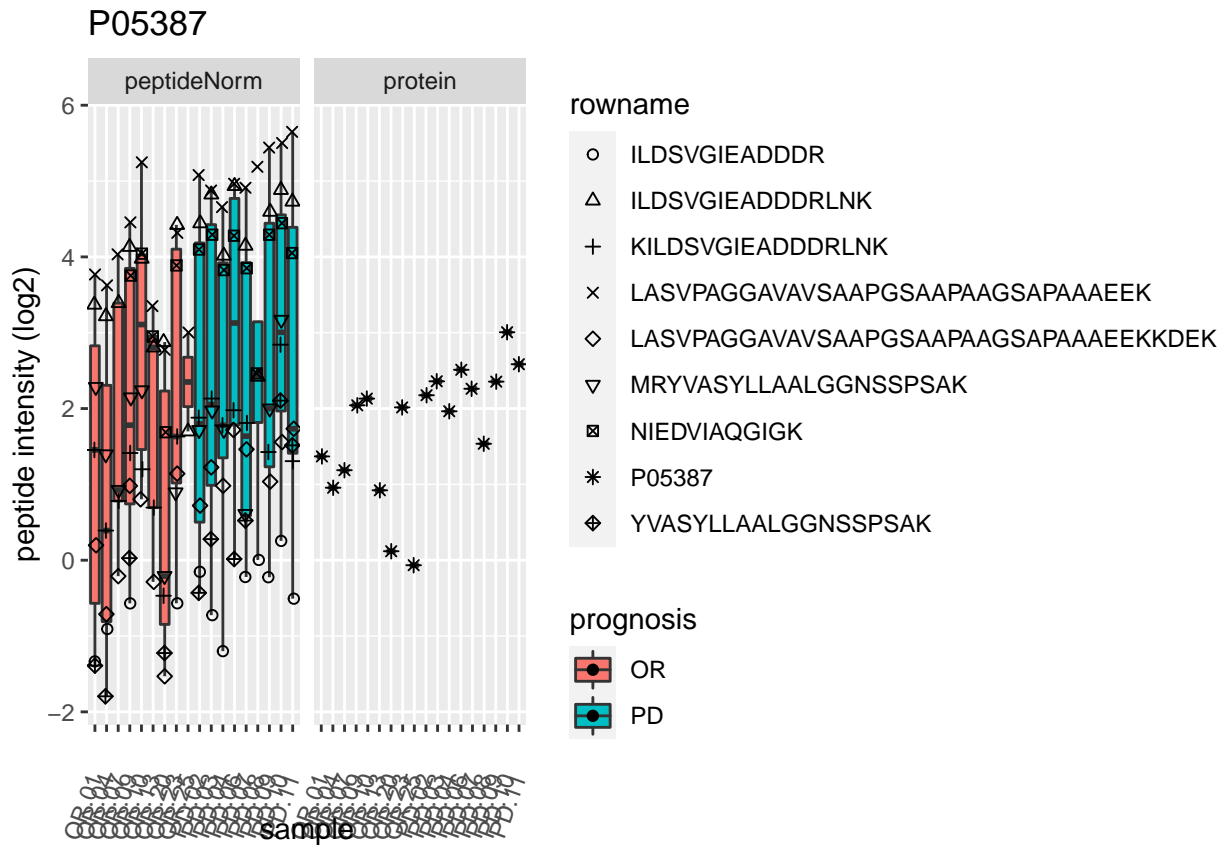
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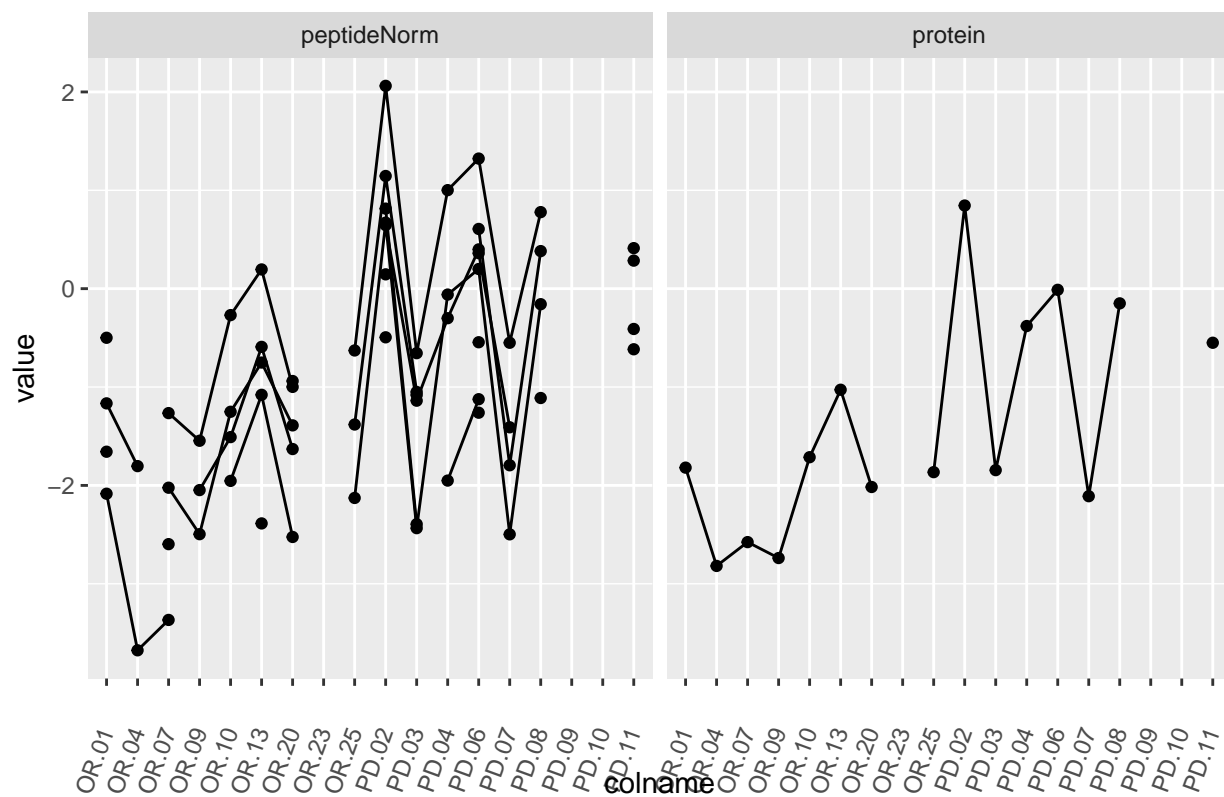


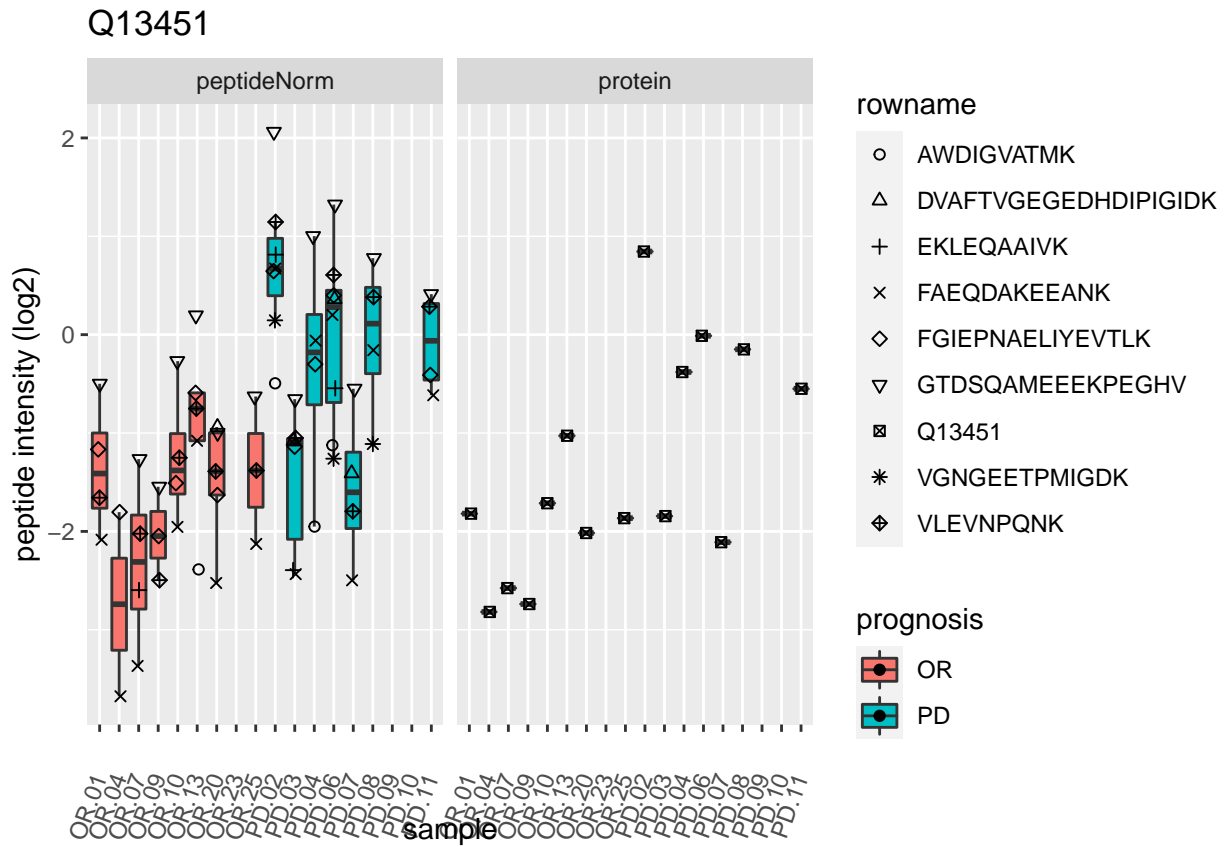
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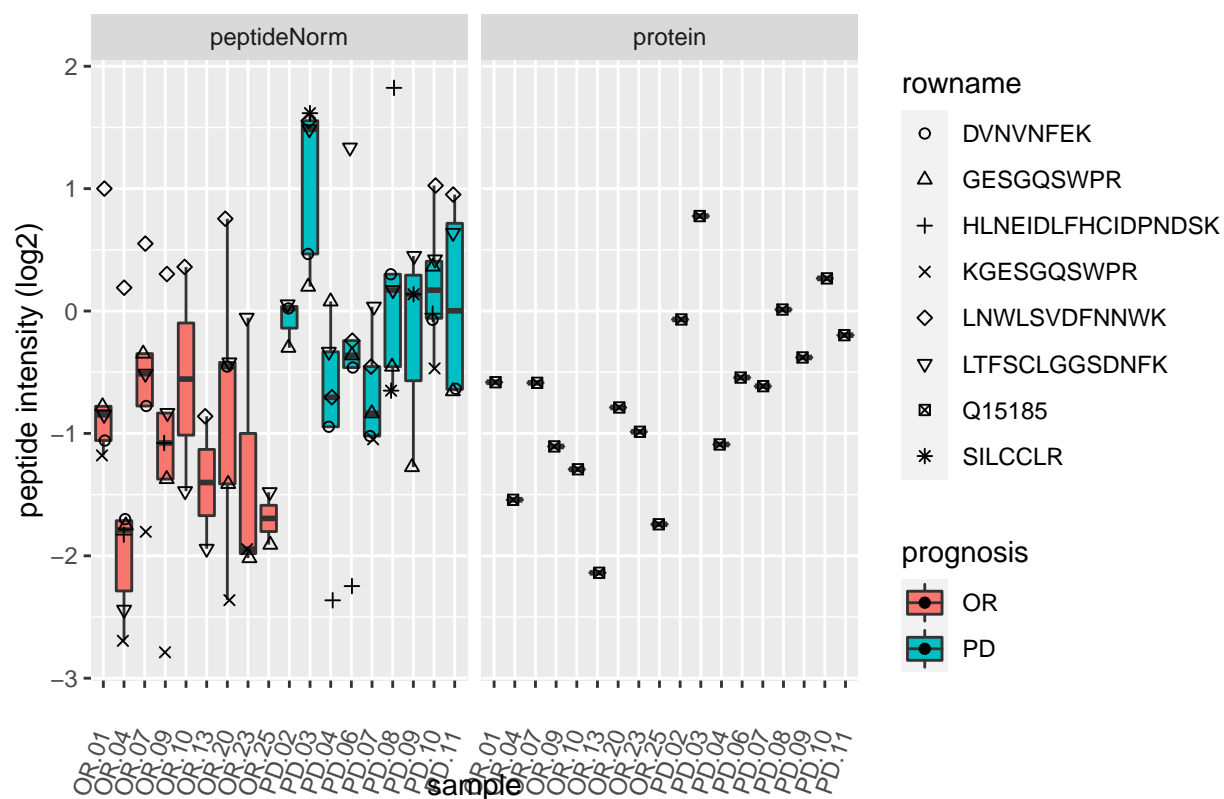




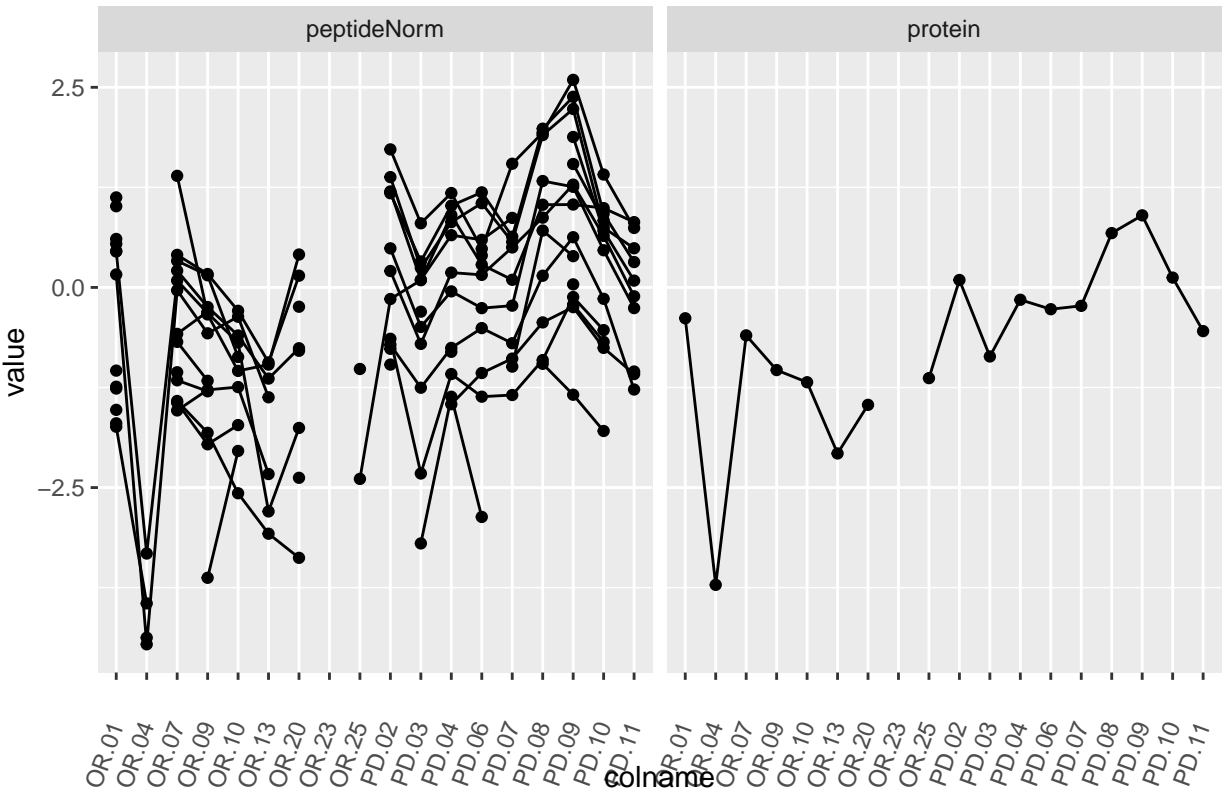
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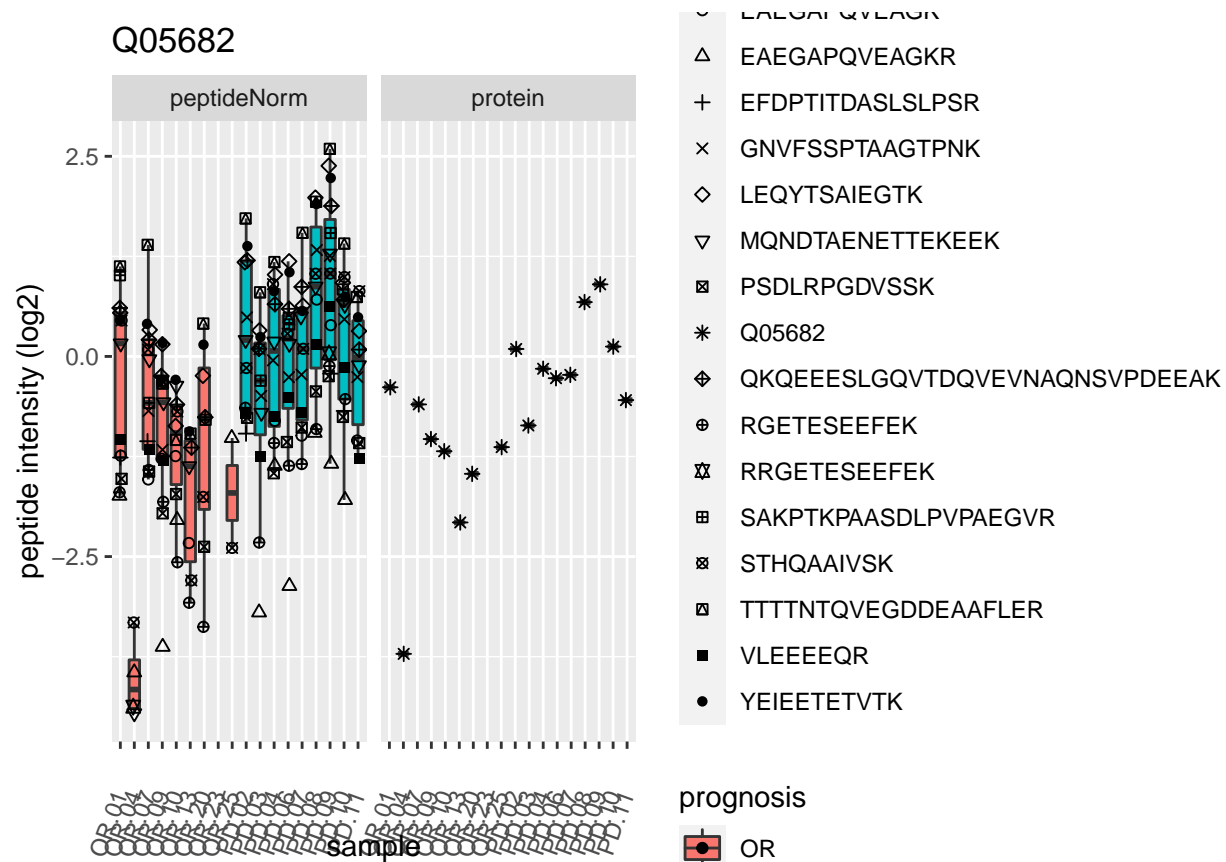


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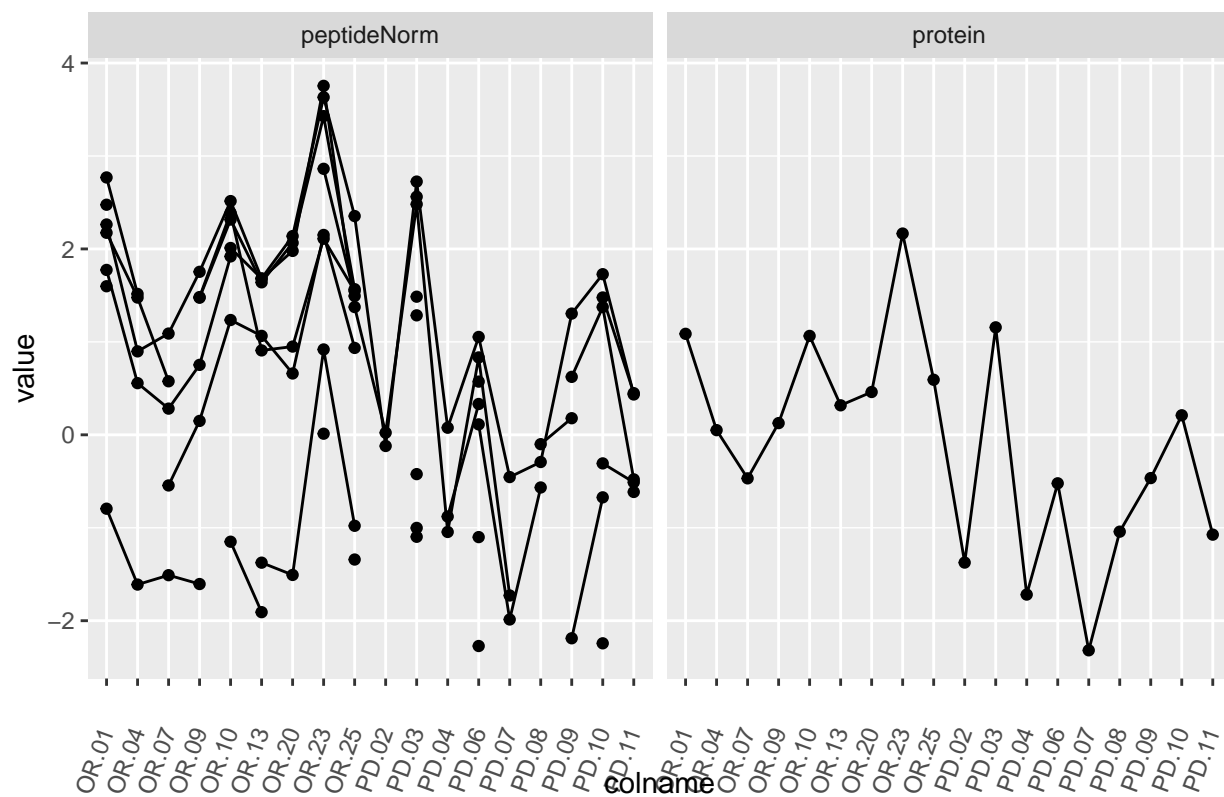


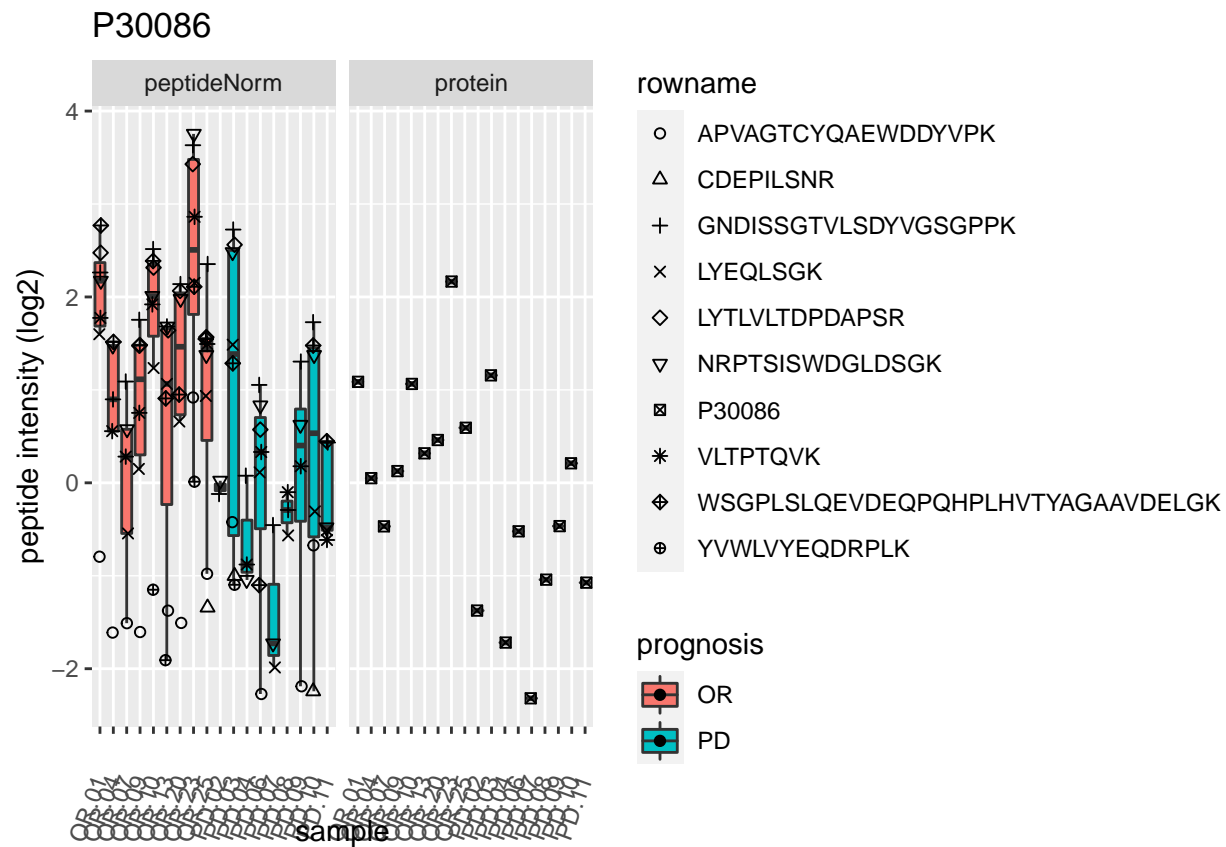
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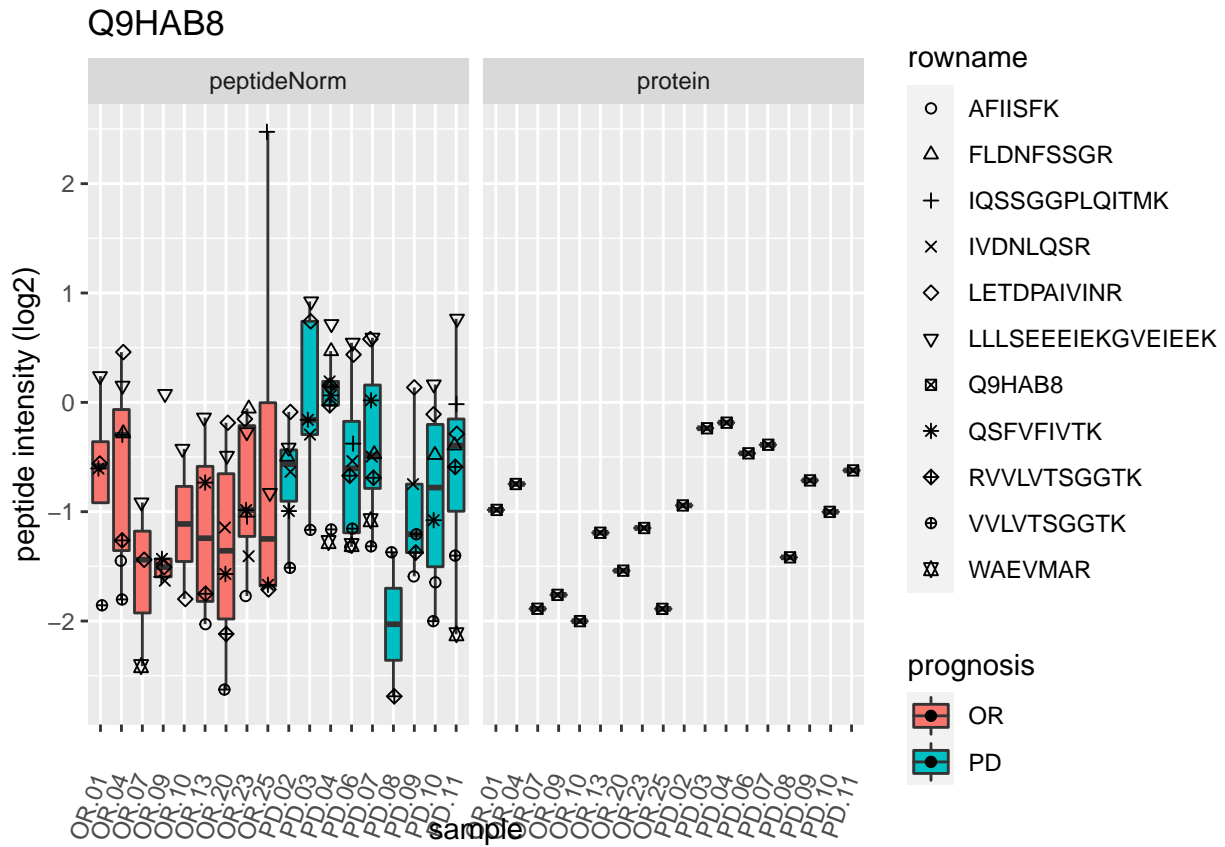
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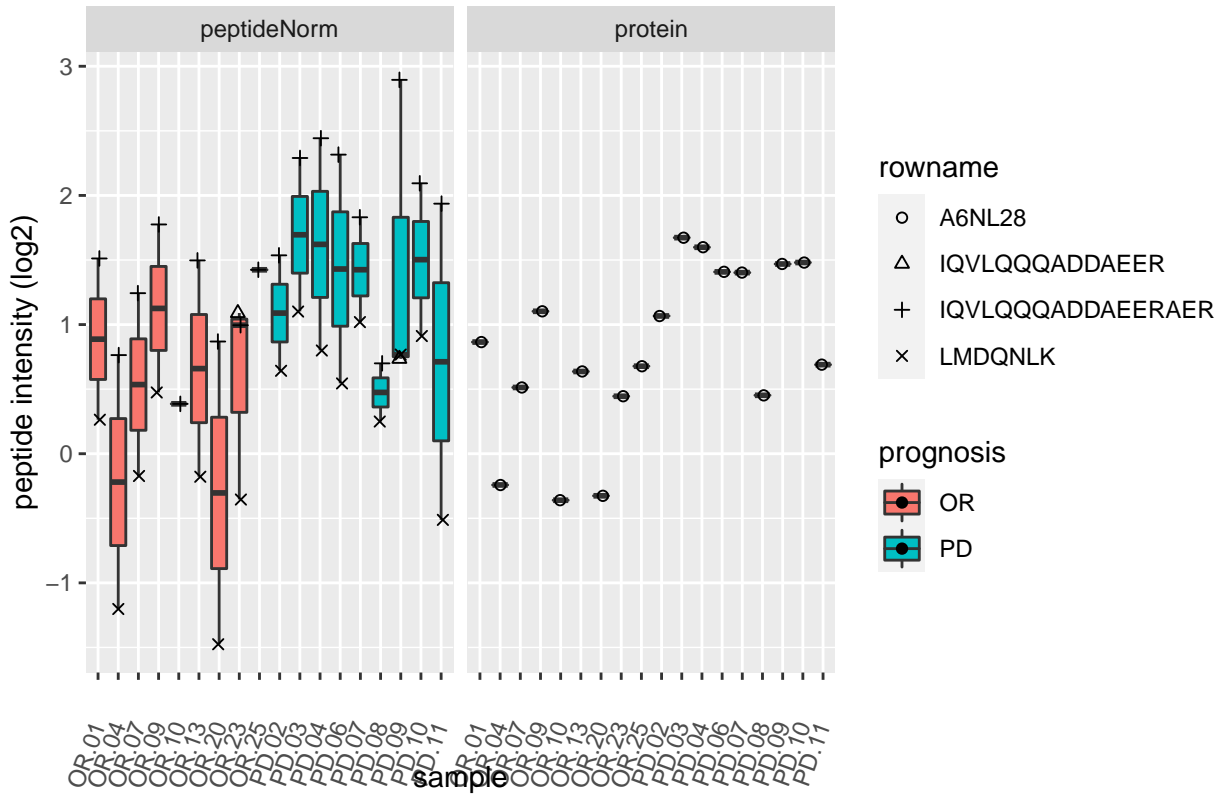




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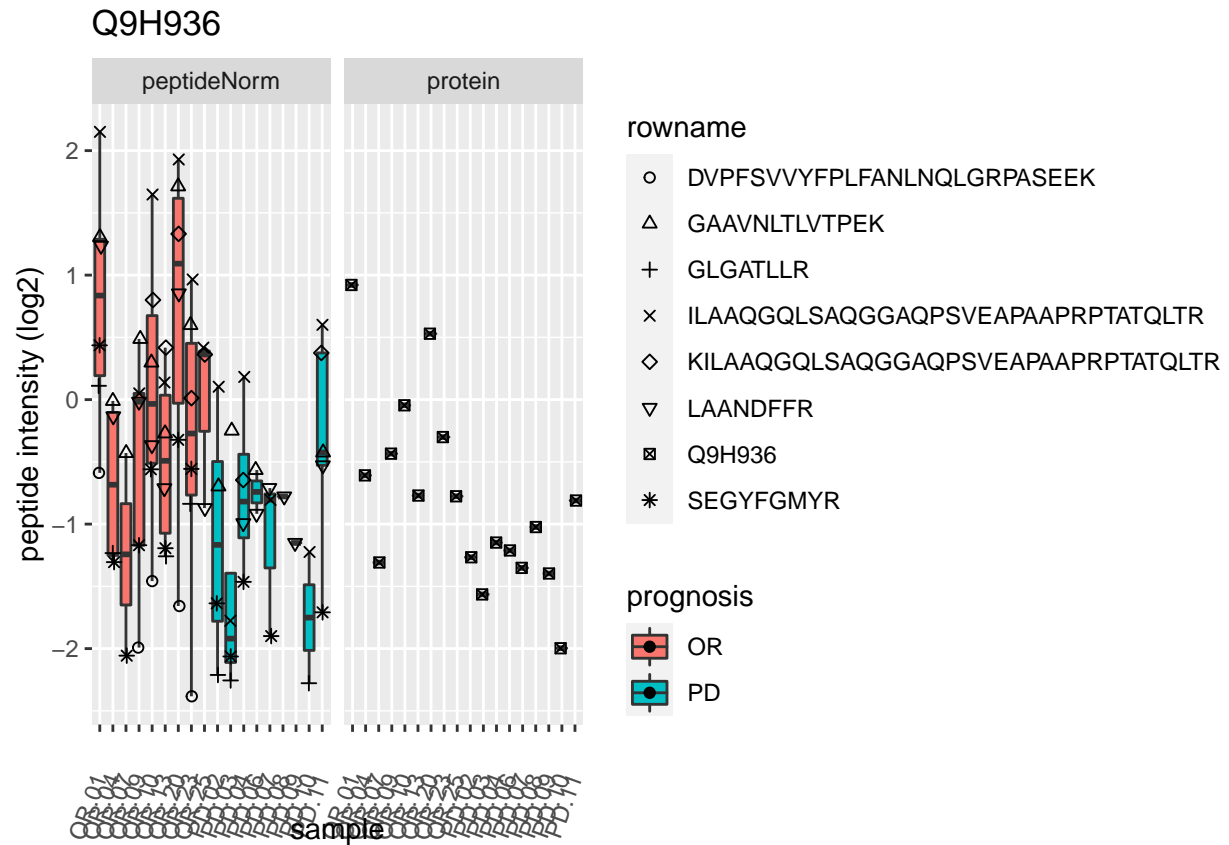


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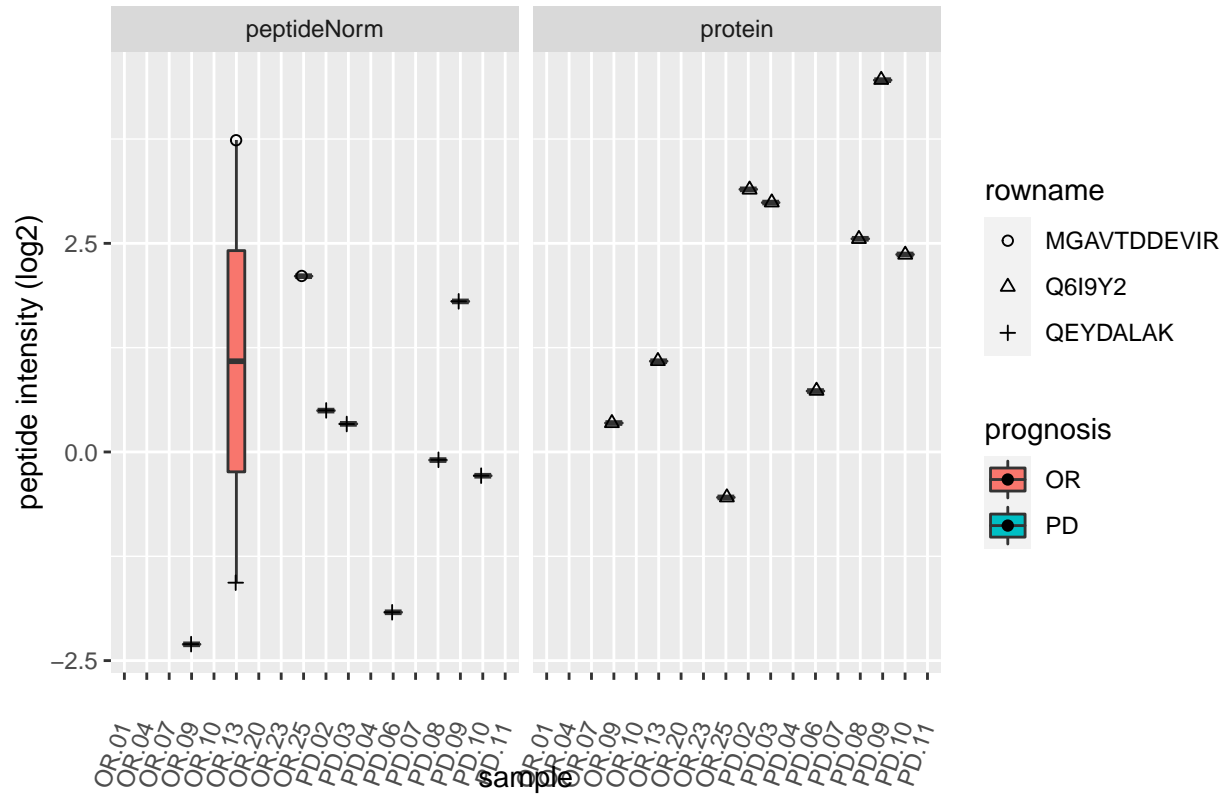




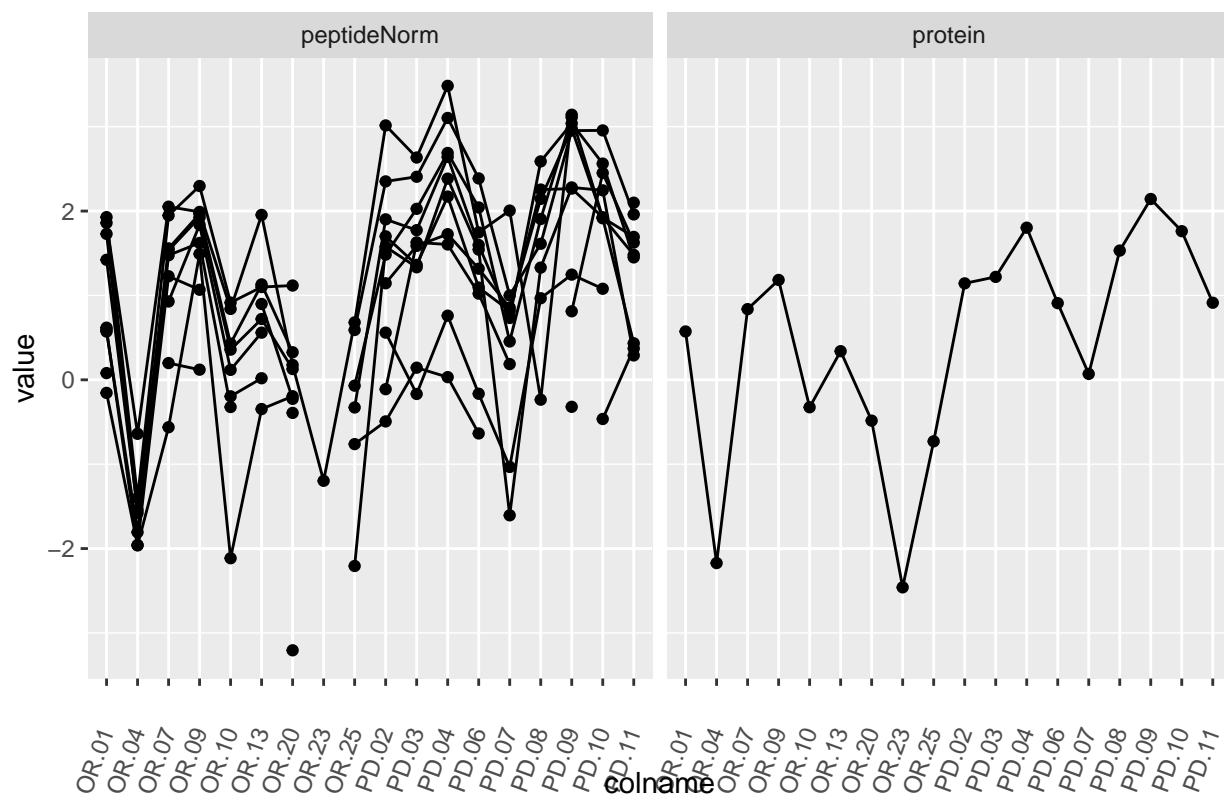
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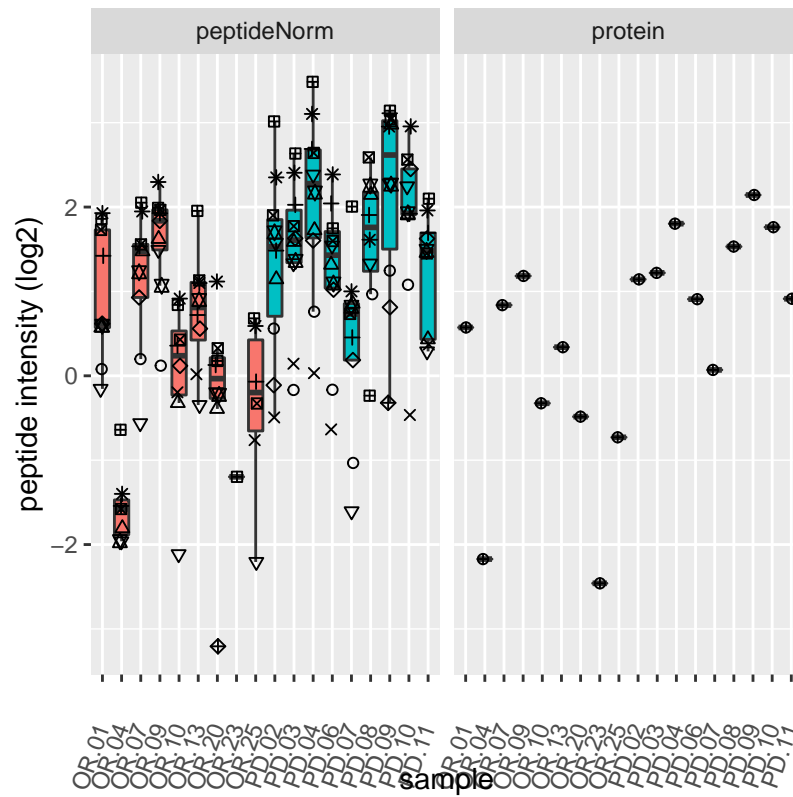
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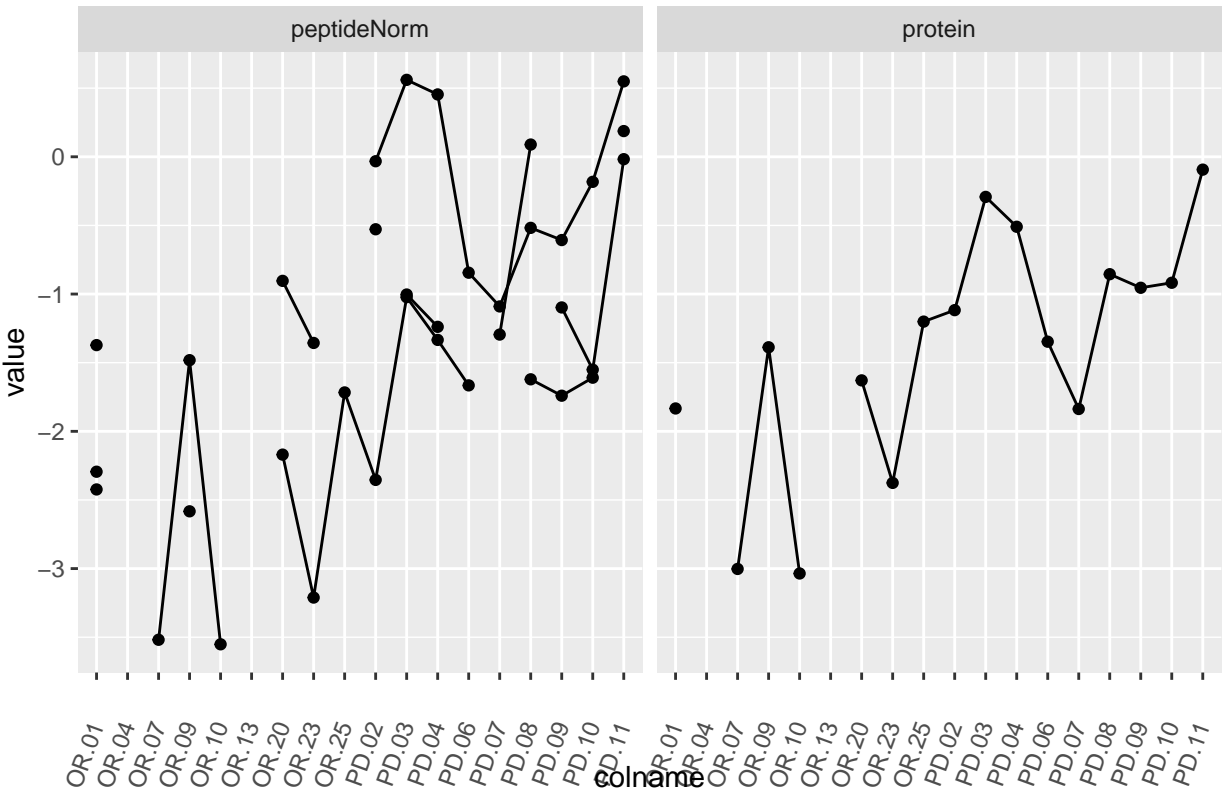
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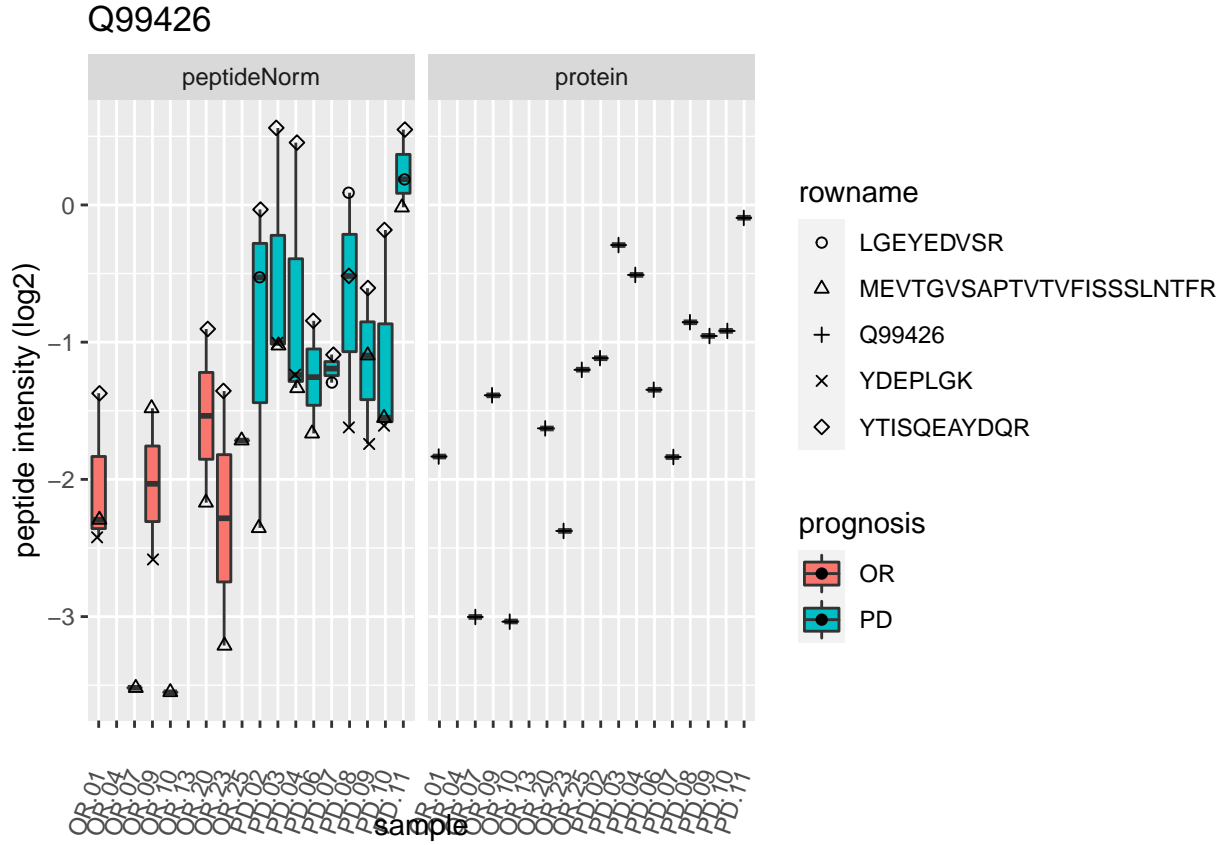
- AEGDVAALNR
- △ AGLNSLEAVK
- + AGLNSLEAVKR
- × CGDLEELKKNVTNNLK
- ◇ EENVGLHQTLDQTLNELNCI
- ▽ EKAEGDVAALNR
- ⊠ IQALQQQADEAEDR
- * KIQUALQQQADEAEDR
- ⊕ KLVILEGELERAEER
- ⊗ P67936
- ⊠ TIDDLLEEK
- ⊠ YSEKEDKYEEEEIK

prognosis

- OR
- PD

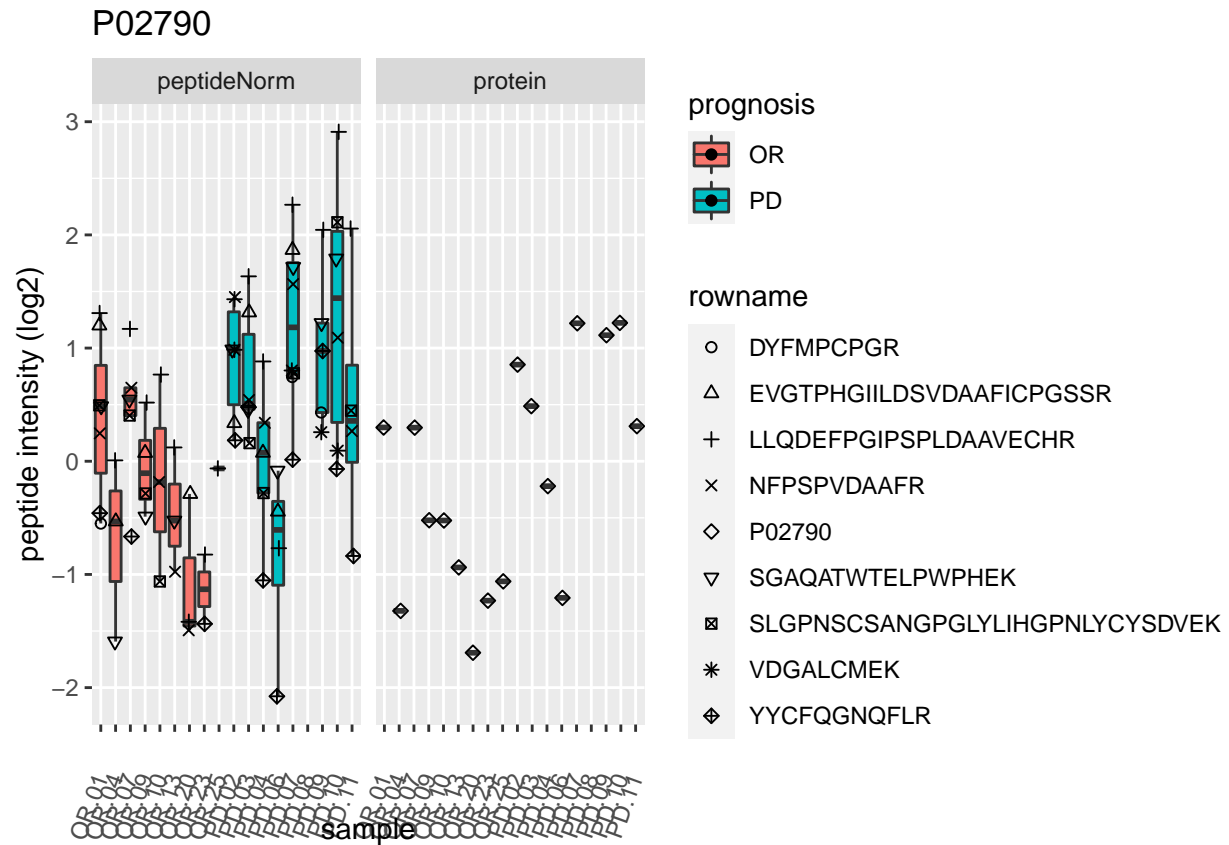
Q99426



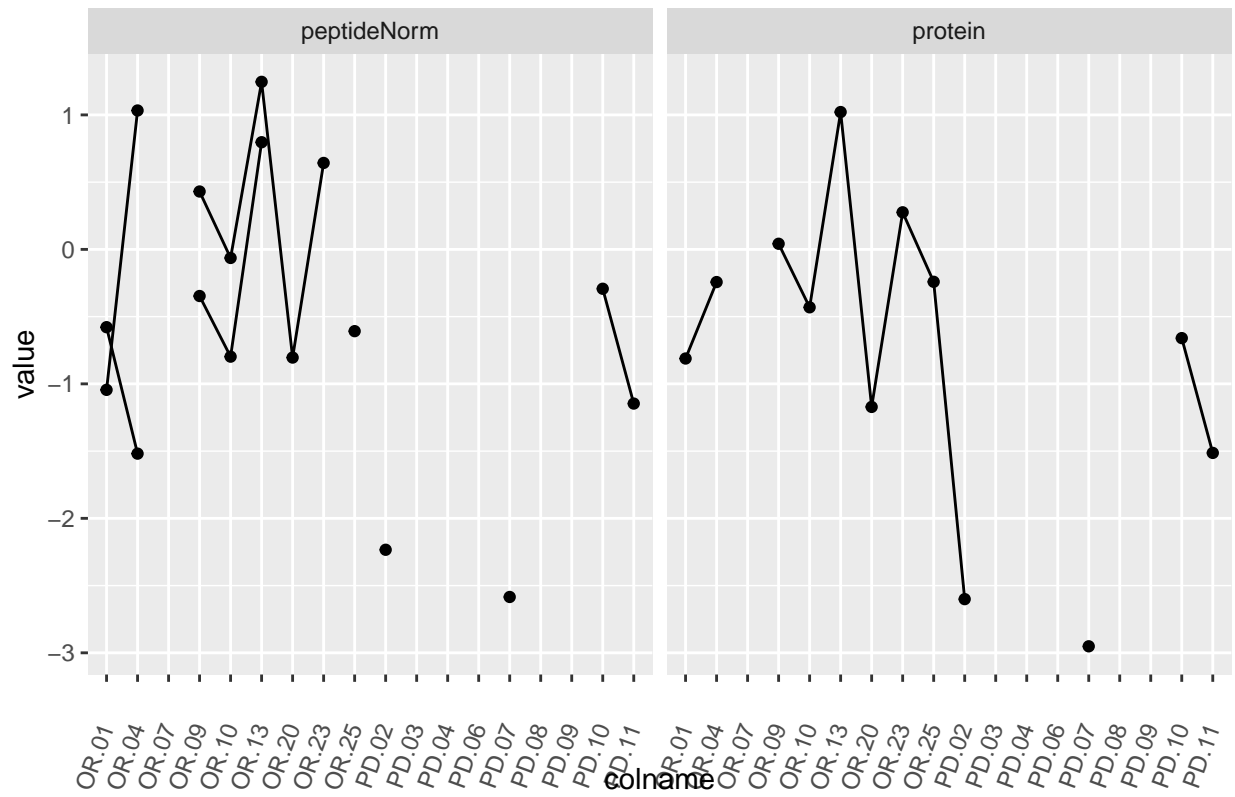


P02790

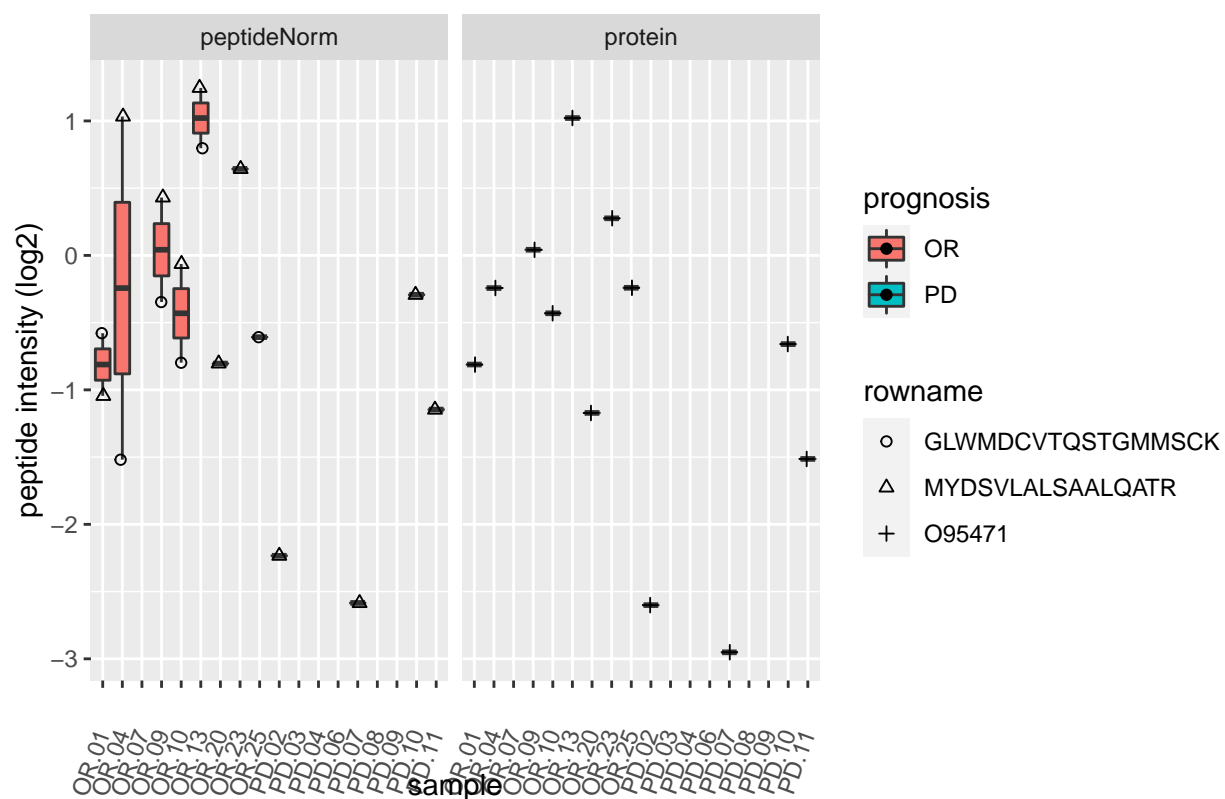




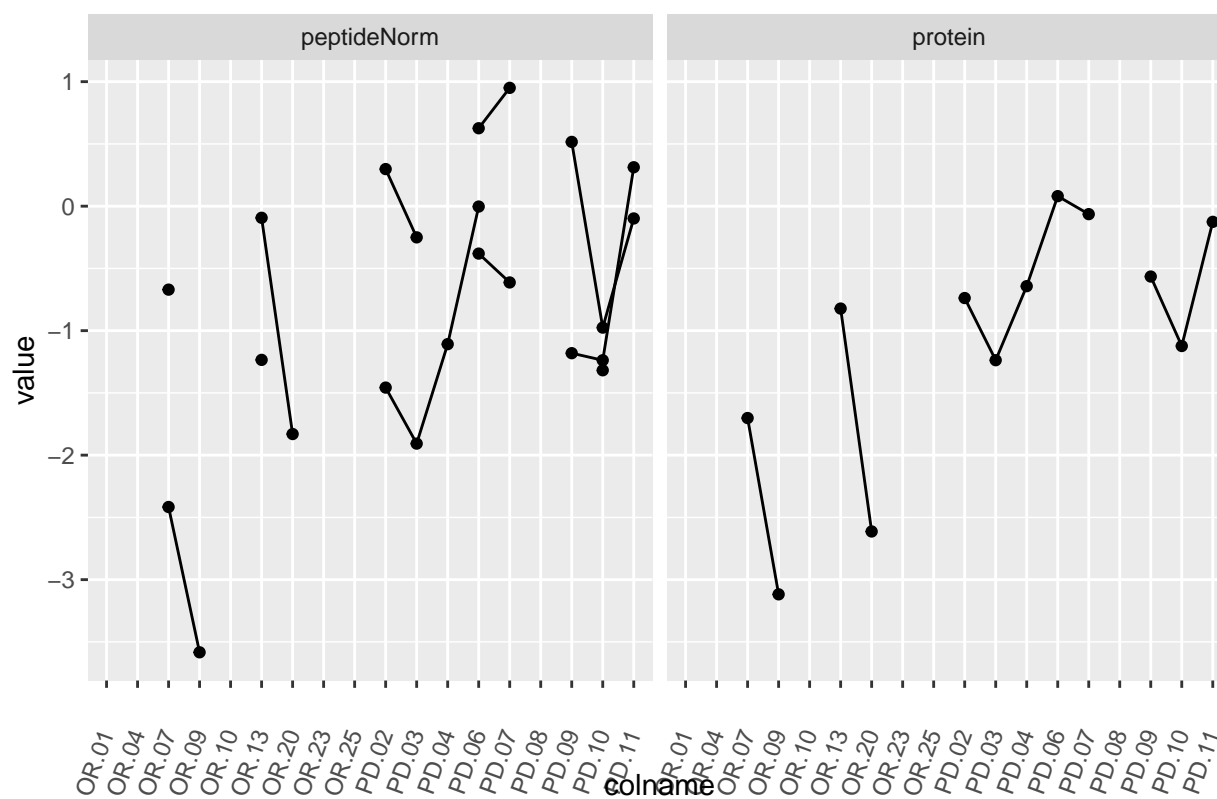
O95471

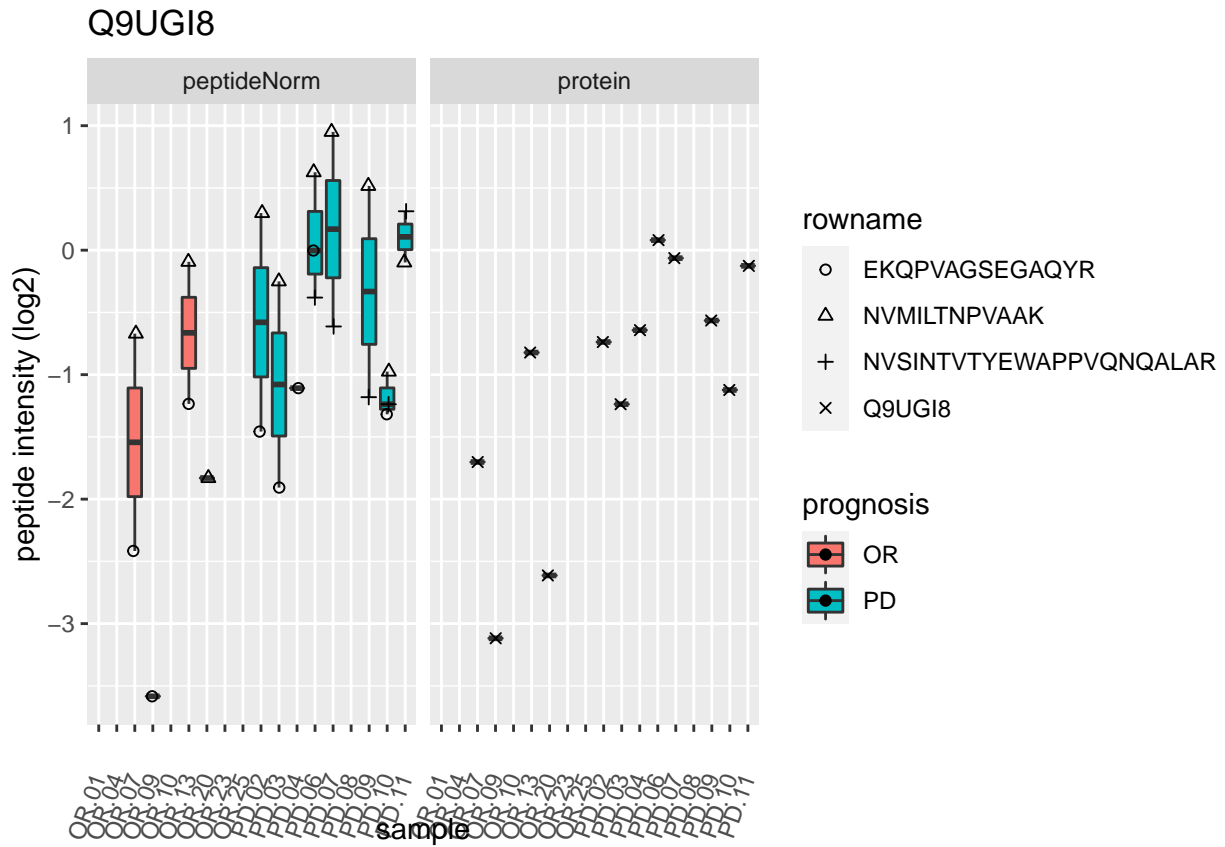


O95471



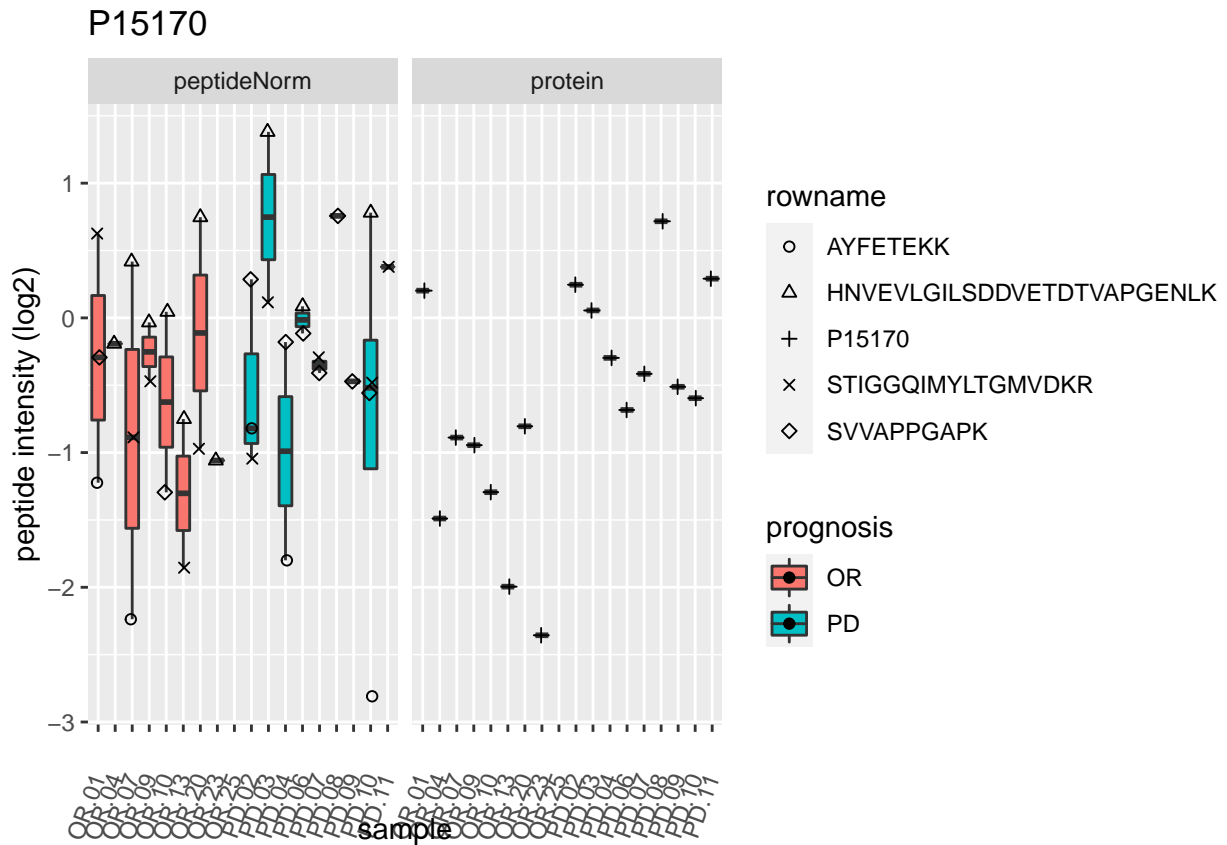
Q9UGI8





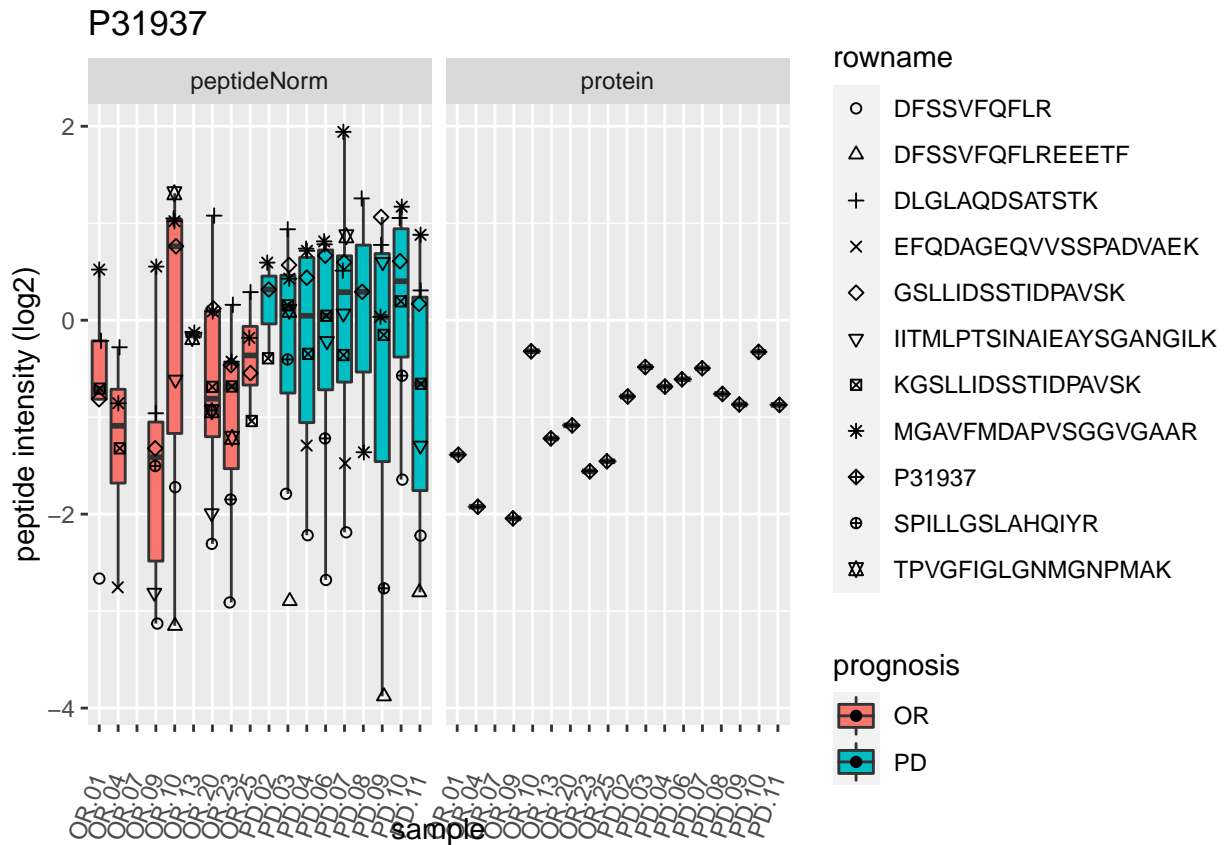
P15170



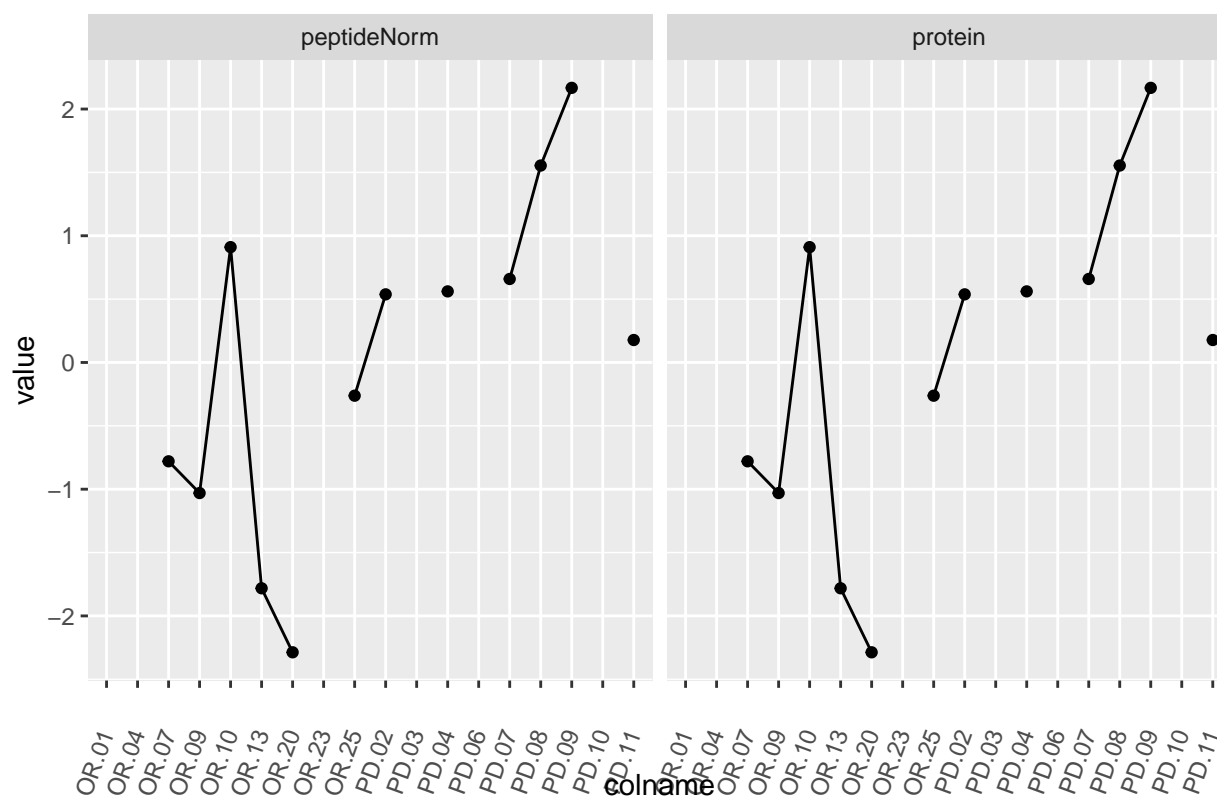


P31937

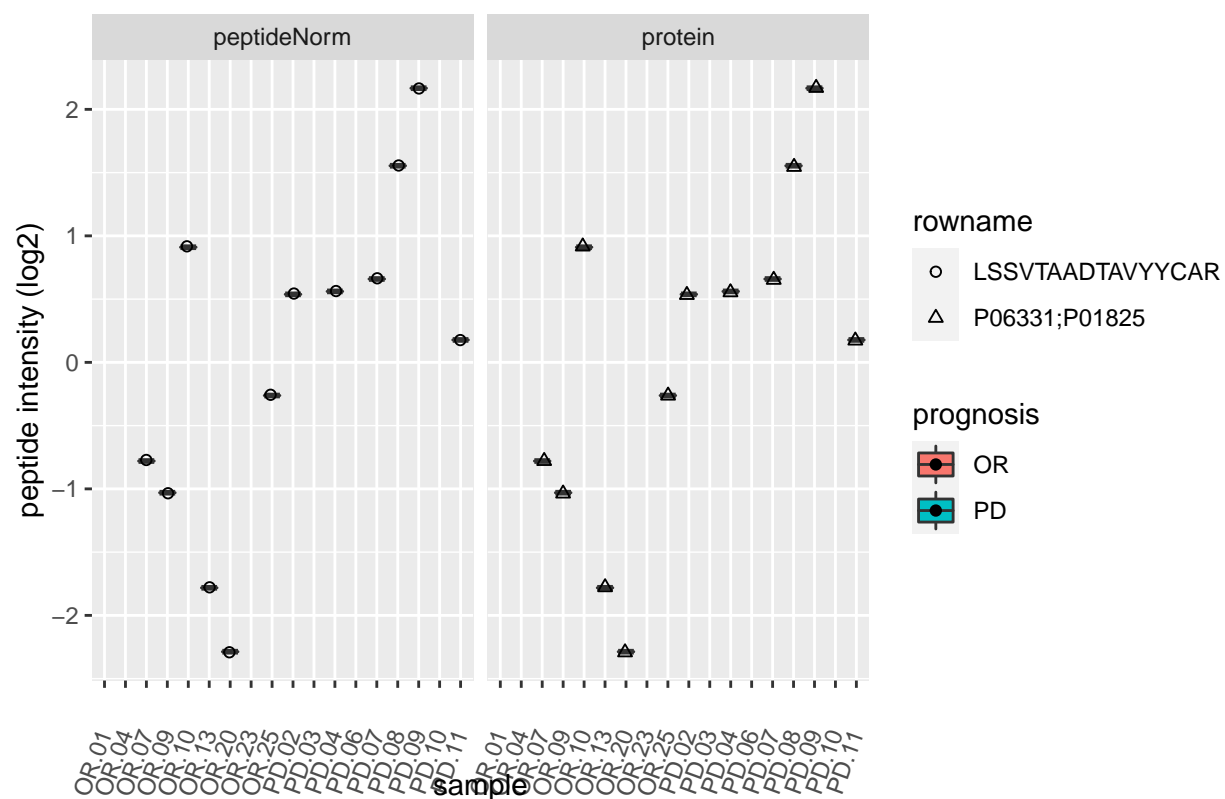




P06331;P01825



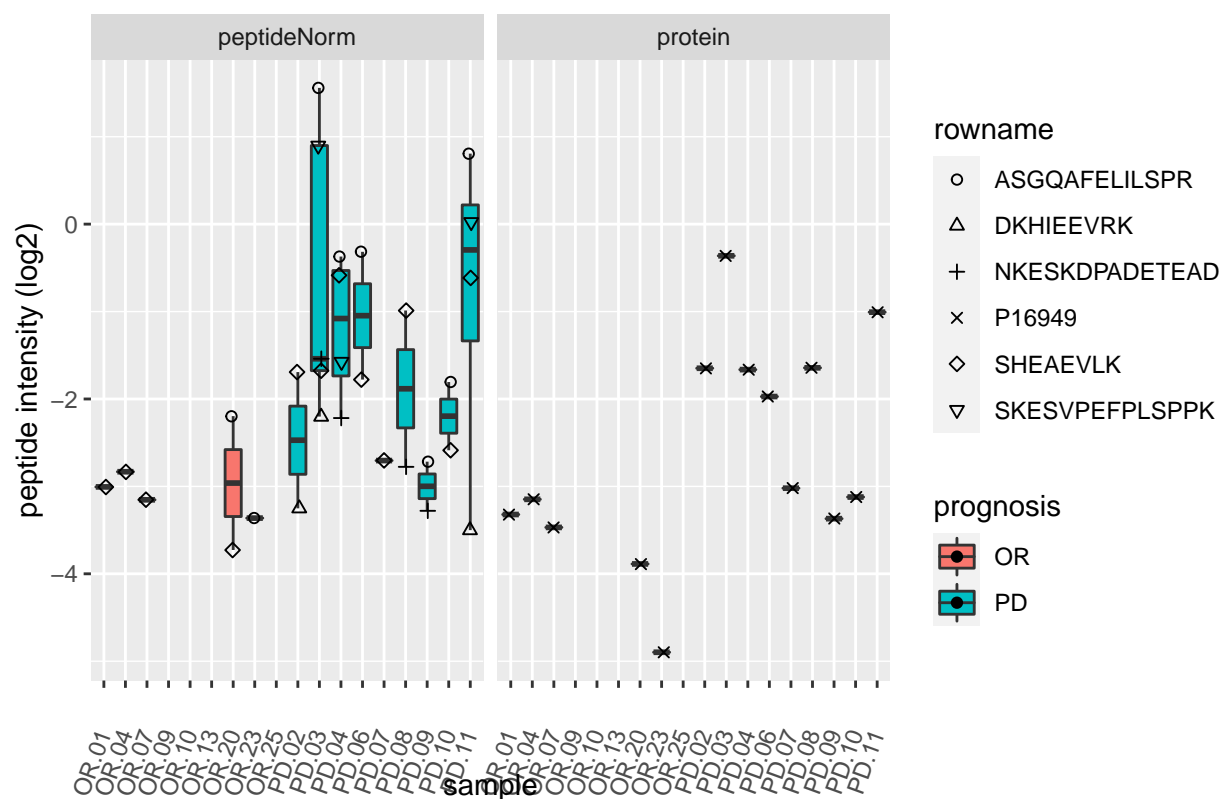
P06331;P01825



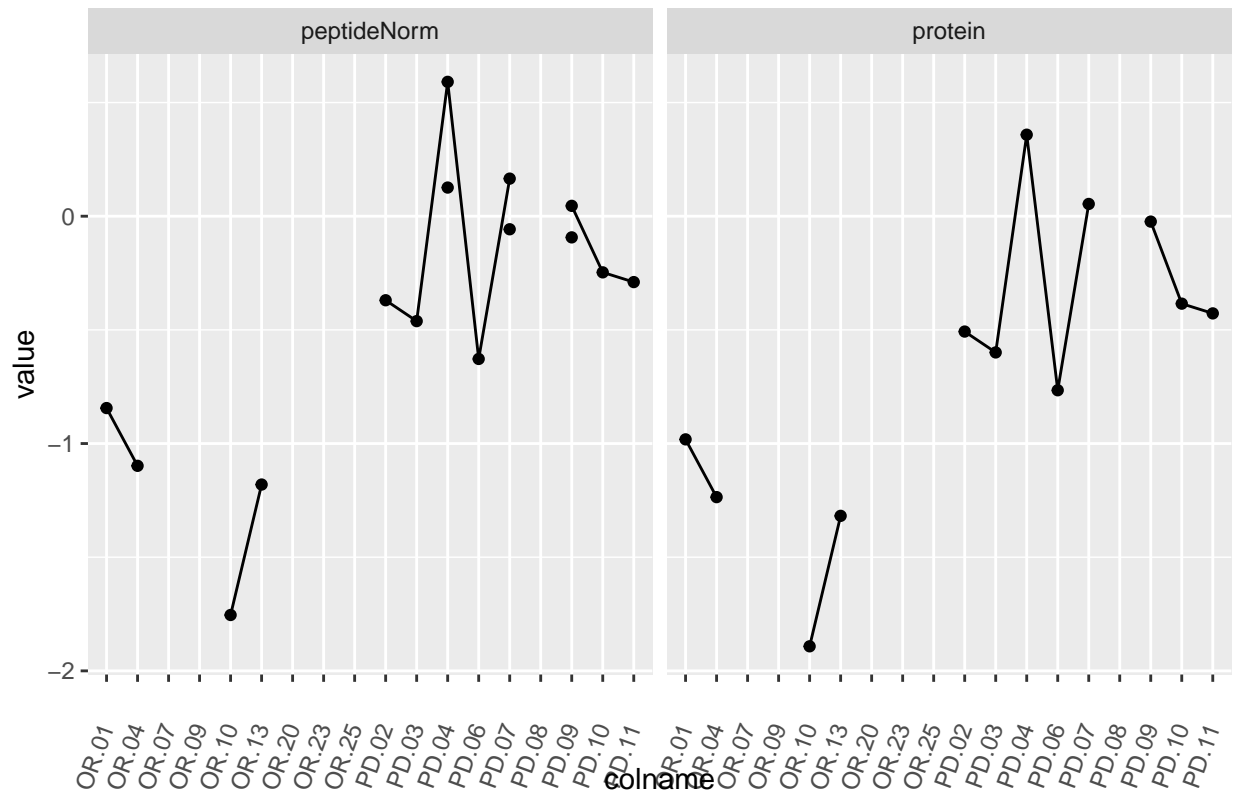
P16949



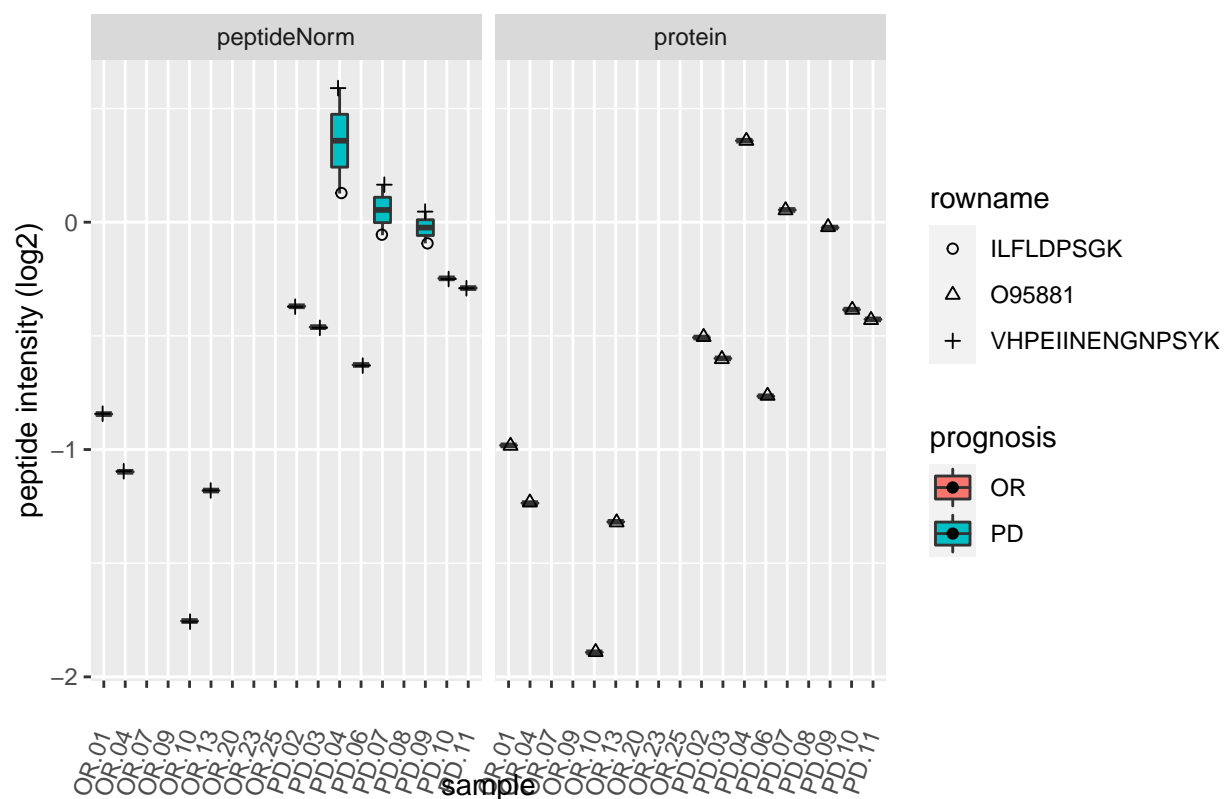
P16949



O95881



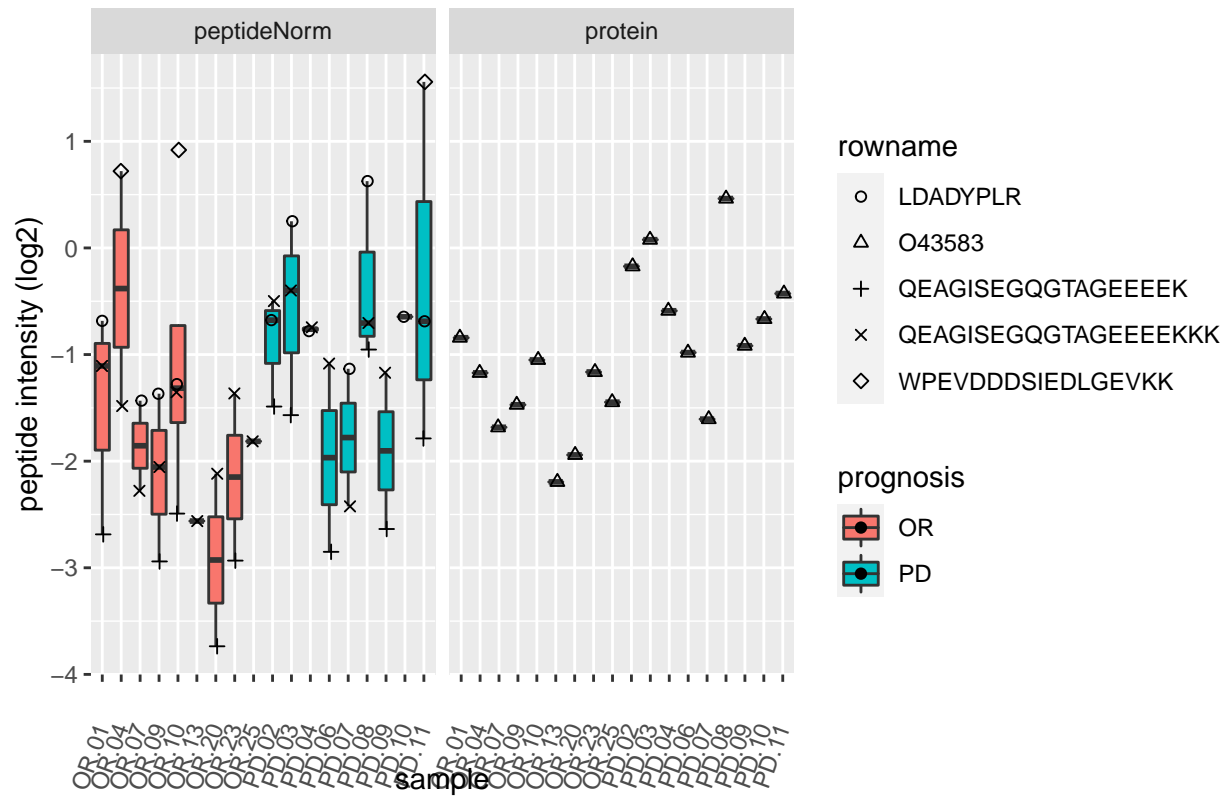
O95881



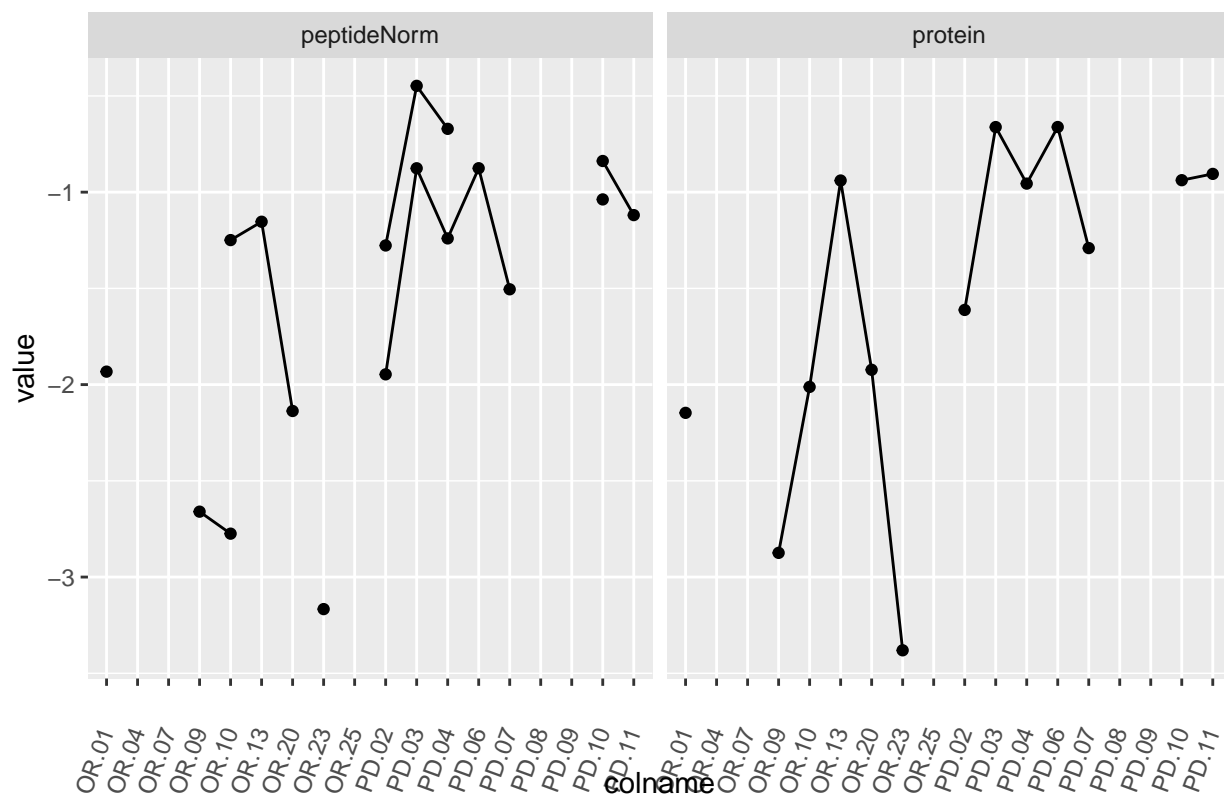
O43583



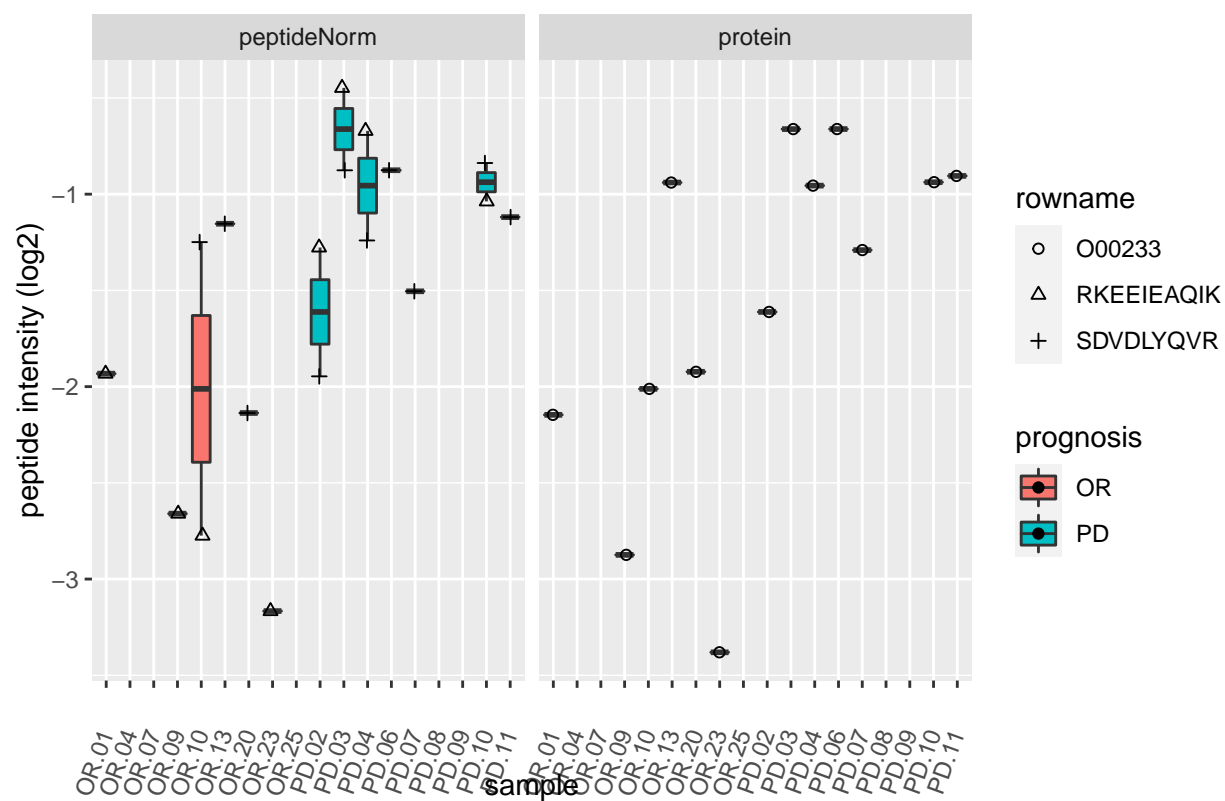
O43583



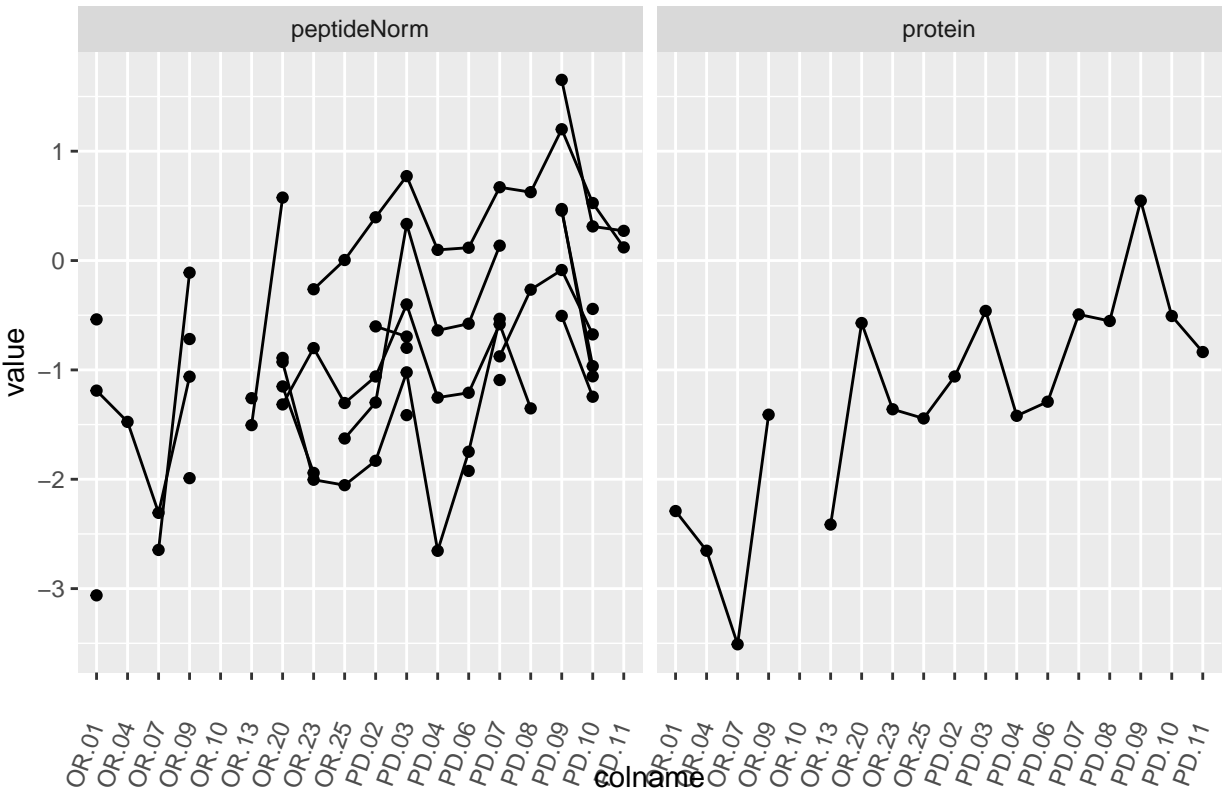
O00233



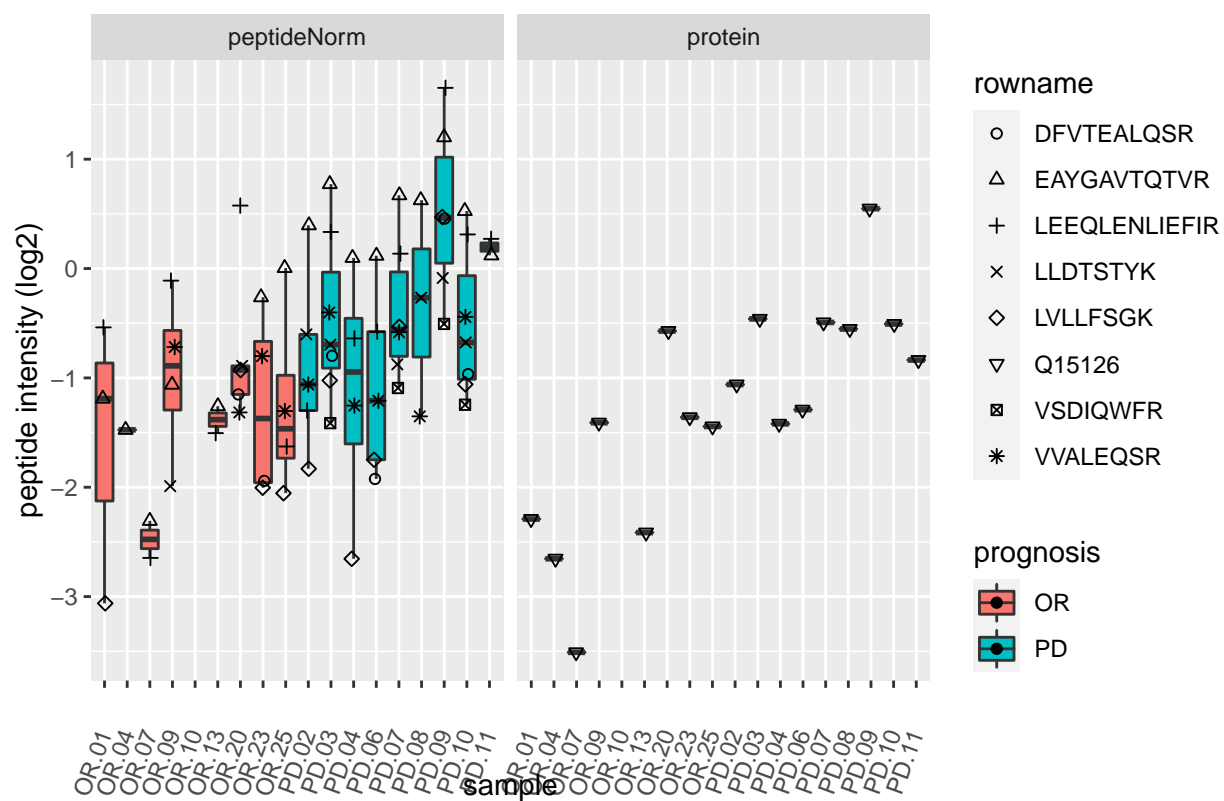
O00233



Q15126

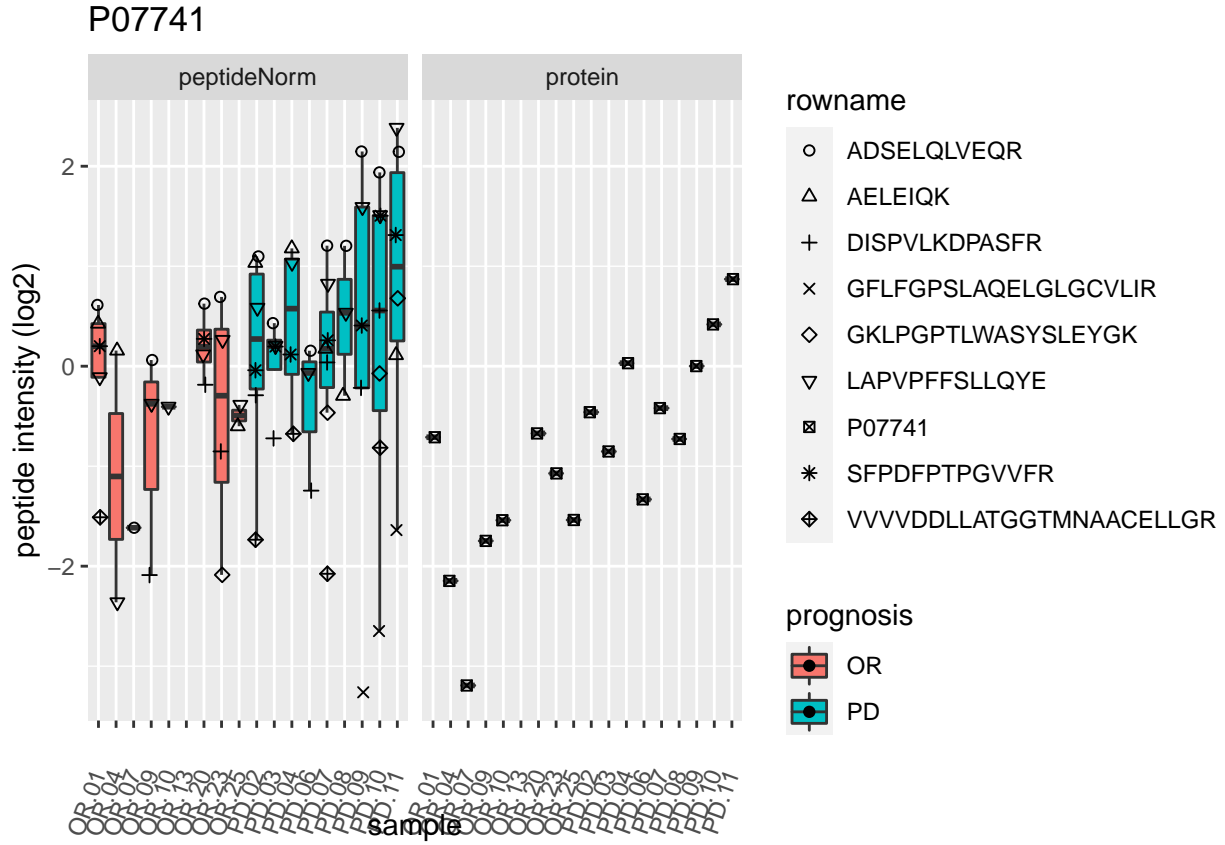


Q15126



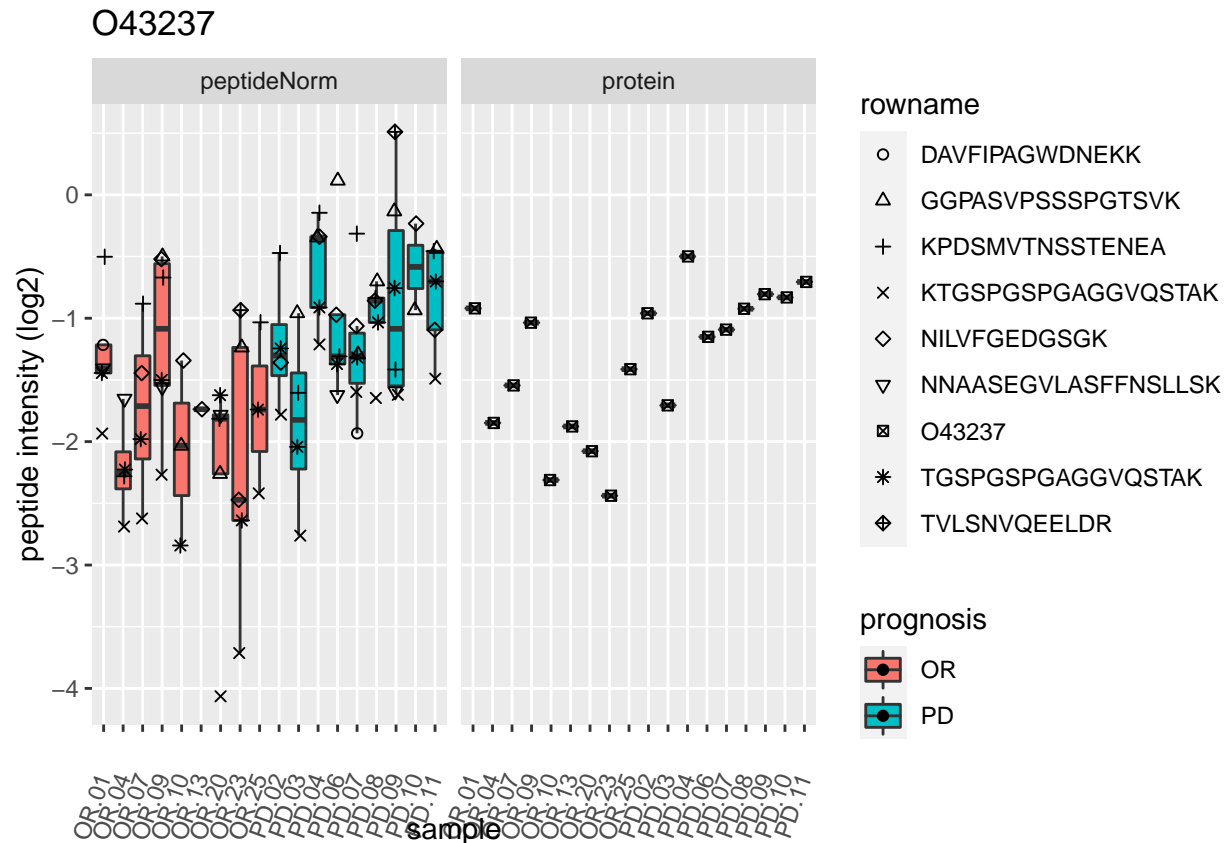
P07741





O43237





5 Session Info

With respect to reproducibility, it is highly recommended to include a session info in your script so that readers of your output can see your particular setup of R.

```
sessionInfo()
```

```
## R version 4.2.1 (2022-06-23)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 20.04.5 LTS
##
## Matrix products: default
## BLAS: /usr/lib/x86_64-linux-gnu/blas/libblas.so.3.9.0
## LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.9.0
##
## locale:
##  [1] LC_CTYPE=C.UTF-8      LC_NUMERIC=C           LC_TIME=C.UTF-8
##  [4] LC_COLLATE=C.UTF-8    LC_MONETARY=C.UTF-8    LC_MESSAGES=C.UTF-8
##  [7] LC_PAPER=C.UTF-8      LC_NAME=C              LC_ADDRESS=C
## [10] LC_TELEPHONE=C        LC_MEASUREMENT=C.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats4      stats      graphics  grDevices datasets  utils      methods
## [8] base
```

```
##
## other attached packages:
## [1] ExploreModelMatrix_1.8.0    plotly_4.10.0
## [3] msqrob2_1.4.0              QFeatures_1.6.0
## [5] MultiAssayExperiment_1.22.0 SummarizedExperiment_1.26.1
## [7] Biobase_2.56.0             GenomicRanges_1.48.0
## [9] GenomeInfoDb_1.32.2       IRanges_2.30.0
## [11] S4Vectors_0.34.0          BiocGenerics_0.42.0
## [13] MatrixGenerics_1.8.0      matrixStats_0.62.0
## [15] limma_3.52.1              forcats_0.5.1
## [17] stringr_1.4.1             dplyr_1.0.9
## [19] purrr_0.3.4               readr_2.1.2
## [21] tidyr_1.2.0               tibble_3.1.7
## [23] ggplot2_3.3.6             tidyverse_1.3.2
##
## loaded via a namespace (and not attached):
## [1] googledrive_2.0.0          minqa_1.2.4                colorspace_2.0-3
## [4] ellipsis_0.3.2            XVector_0.36.0            fs_1.5.2
## [7] clue_0.3-61              farver_2.1.0              DT_0.23
## [10] fansi_1.0.3              lubridate_1.8.0           xml2_1.3.3
## [13] codetools_0.2-18         splines_4.2.1             knitr_1.40.1
## [16] jsonlite_1.8.0           nloptr_2.0.3             broom_0.8.0
## [19] cluster_2.1.3            dbplyr_2.1.1             shinydashboard_0.7.2
## [22] shiny_1.7.1              BiocManager_1.30.18      compiler_4.2.1
## [25] httr_1.4.3              backports_1.4.1          assertthat_0.2.1
## [28] Matrix_1.4-1            fastmap_1.1.0            lazyeval_0.2.2
## [31] gargle_1.2.0             cli_3.3.0                later_1.3.0
## [34] htmltools_0.5.2         tools_4.2.1              igraph_1.3.2
## [37] gtable_0.3.0            glue_1.6.2               GenomeInfoDbData_1.2.8
## [40] Rcpp_1.0.8.3            cellranger_1.1.0         jquerylib_0.1.4
## [43] vctrs_0.4.1             nlme_3.1-157             rintrojs_0.3.0
## [46] xfun_0.33               lme4_1.1-29             rvest_1.0.2
## [49] mime_0.12              lifecycle_1.0.1         renv_0.15.4
## [52] googlesheets4_1.0.0     zlibbioc_1.42.0         MASS_7.3-57
## [55] scales_1.2.0            promises_1.2.0.1        hms_1.1.1
## [58] ProtGenerics_1.28.0     parallel_4.2.1          AnnotationFilter_1.20.0
## [61] yaml_2.3.5             sass_0.4.1              stringi_1.7.8
## [64] highr_0.9              boot_1.3-28             BiocParallel_1.30.2
## [67] rlang_1.0.2            pkgconfig_2.0.3         bitops_1.0-7
## [70] evaluate_0.16          lattice_0.20-45         htmlwidgets_1.5.4
## [73] labeling_0.4.2         cowplot_1.1.1          tidyselect_1.1.2
## [76] magrittr_2.0.3         R6_2.5.1               generics_0.1.2
## [79] DelayedArray_0.22.0    DBI_1.1.2              pillar_1.7.0
## [82] haven_2.5.0           withr_2.5.0            MsCoreUtils_1.8.0
## [85] RCurl_1.98-1.6        modelr_0.1.8           crayon_1.5.1
## [88] utf8_1.2.2            tzdb_0.3.0             rmarkdown_2.14
## [91] grid_4.2.1            readxl_1.4.0          data.table_1.14.2
## [94] reprex_2.0.1          digest_0.6.29         xtable_1.8-4
## [97] httpuv_1.6.5          munsell_0.5.0         viridisLite_0.4.0
## [100] bslib_0.3.1           shinyjs_2.1.0
```