

Plot from Differential expression

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Loading packages

```
suppressMessages(library(readxl))
suppressMessages(library(tidyverse))
suppressMessages(library(ggplot2))
suppressMessages(library(dplyr))
suppressMessages(library(EnhancedVolcano))
suppressMessages(library(ComplexHeatmap))
suppressMessages(library(formatR))
```

load the annotation file from Q3 - publications

```
colData <- read_excel("../Data_Thomas/Q3 - publication - V2.xlsx", sheet =
"SegmentProperties")
colData <- as.data.frame(colData)
rownames(colData) <- colData$SegmentDisplayName

# Annotation table for Heatmap
df <- as.data.frame(colData[,c("SlideName","Group")])
```

Loading the normalised count matrix

```
Q3_publication <- read_excel("../Data_Thomas/Q3 - publication - V2.xlsx", sheet =
"TargetCountMatrix")
Q3_publication <- as.data.frame(Q3_publication)
rownames(Q3_publication) <- Q3_publication$TargetName
Q3_publication$TargetName <- NULL

rld <- log2(Q3_publication)
```

DE perilesional vs intraleisional

```
DE_perivsintra <- read_excel("Data/DE_Intra-vs-Peri-V2.xlsx", sheet = "VolcanoPlot",
range = "C7:H19969")

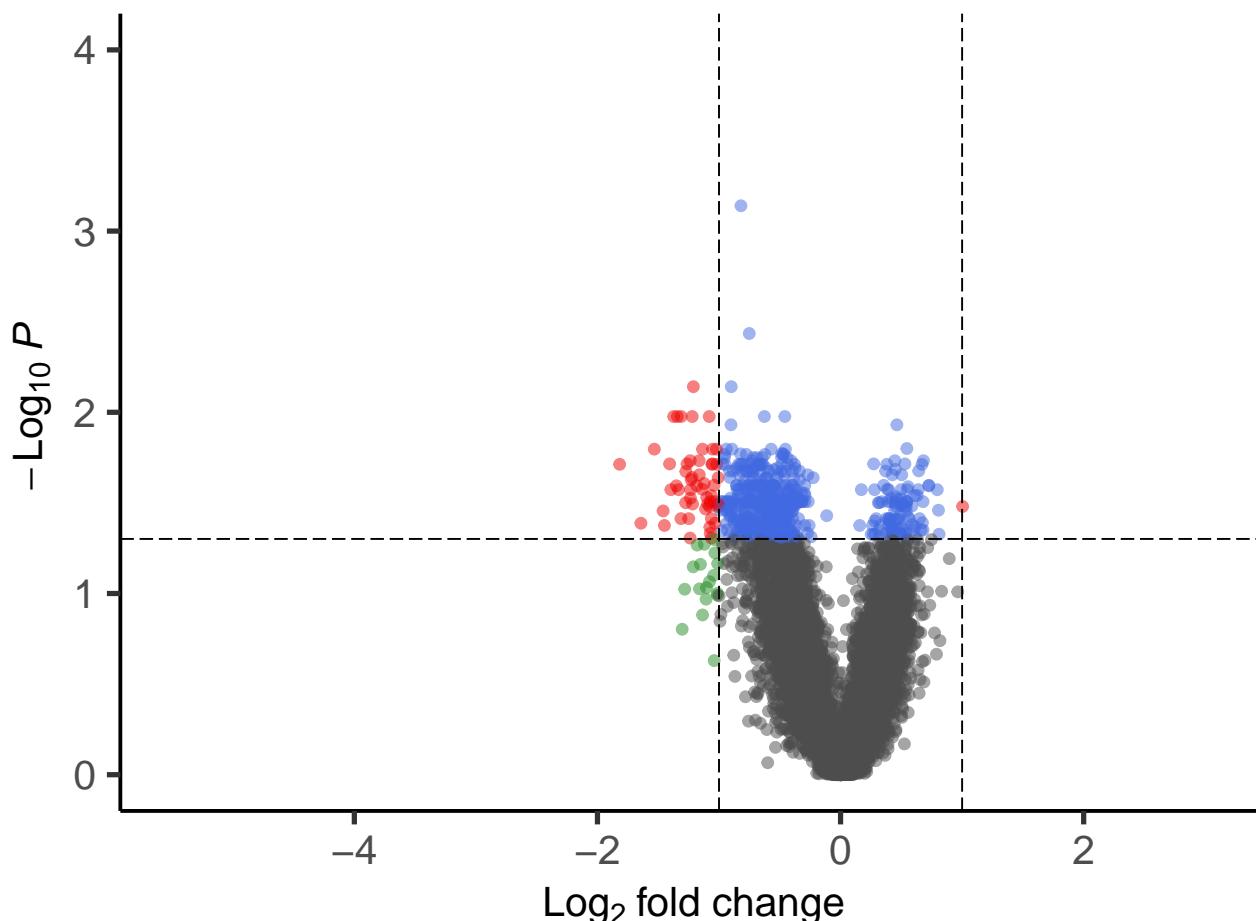
DE_perivsintra$padj <- as.numeric(DE_perivsintra$`Adjusted pvalue`)
```

```
EnhancedVolcano(DE_perivsintra, DE_perivsintra$`Target name`, x = "Log2", y = "padj",
title= "DE-perilesional vs intralesional", FCcutoff = 1, pCutoff = 0.05, labSize = 0.1,
ylim = c(0,4), xlim = c(-5.5,3), gridlines.major= F, gridlines.minor = F)
```

DE–perilesional vs intralesional

EnhancedVolcano

● NS ● Log₂ FC ● p-value ● p – value and log₂ FC



total = 19962 variables

```
#ggsave("volcanoplot/Volcano_perivsintraV2.pdf", width = 10, height = 10)
```

DE extralesional vs intralesionnal

Unaffected correspond to extralesional

```

DE_UnaffectedvsIntra <- read_excel("Data/DE-Intra-vs-Unaffected-V2.xlsx", sheet =
"VolcanoPlot", range = "C7:H19969")
DE_UnaffectedvsIntra$padj <- as.numeric(DE_UnaffectedvsIntra$`Adjusted pvalue`)

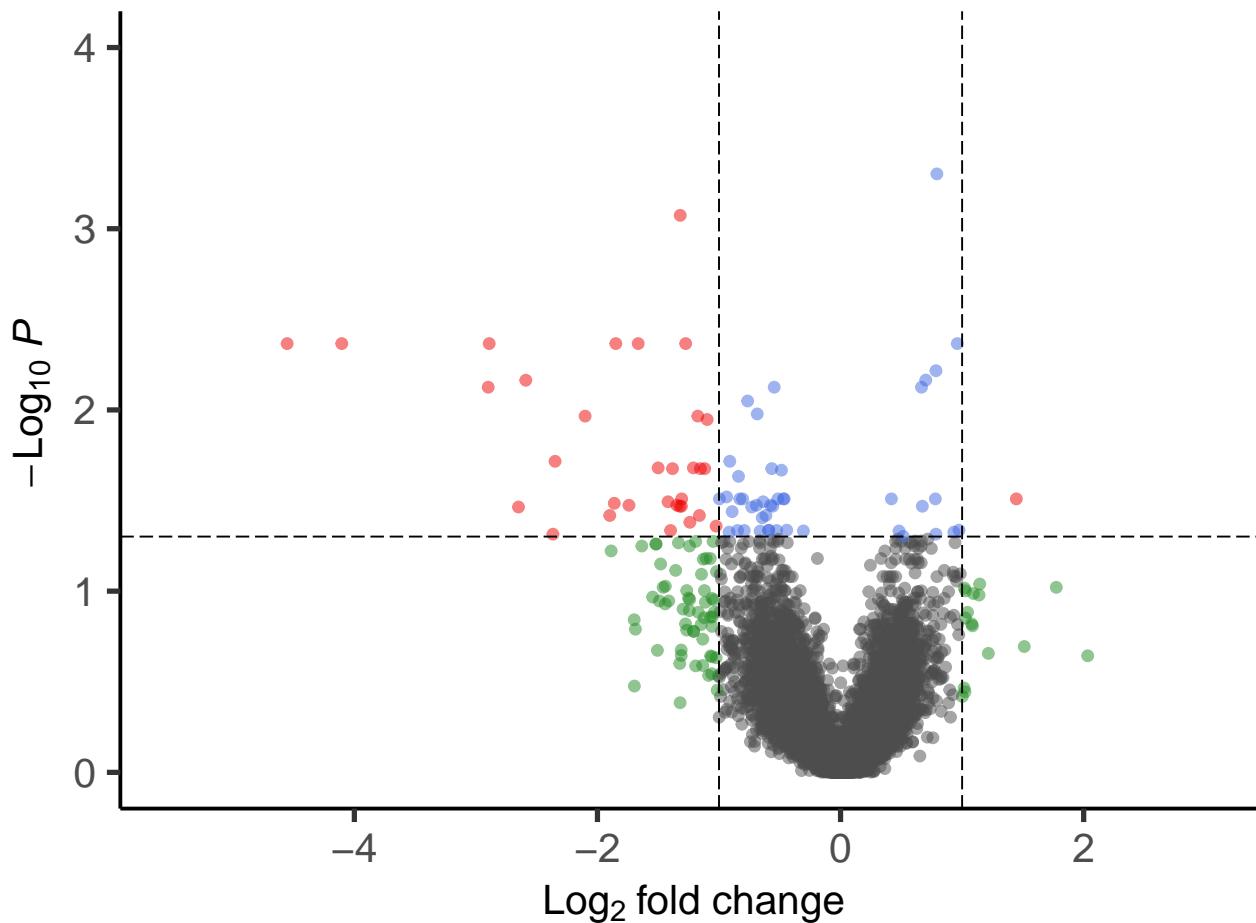
EnhancedVolcano(DE_UnaffectedvsIntra, DE_UnaffectedvsIntra$`Target name`, x = "Log2",
"padj", title= "DE-extraleisional vs intraleisionnal", FCCcutoff = 1, pCutoff = 0.05,
labSize = 0.1, ylim = c(0,4), xlim = c(-5.5,3), gridlines.major= F, gridlines.minor = F)

```

DE-extraleisional vs intraleisionnal

EnhancedVolcano

● NS ● Log₂ FC ● p-value ● p – value and log₂ FC



total = 19962 variables

```
#ggsave("volcanoplot/Volcano_UnaffectedvsIntraV2.pdf", width = 10, height = 10)
```

DE extralesional vs perilesional

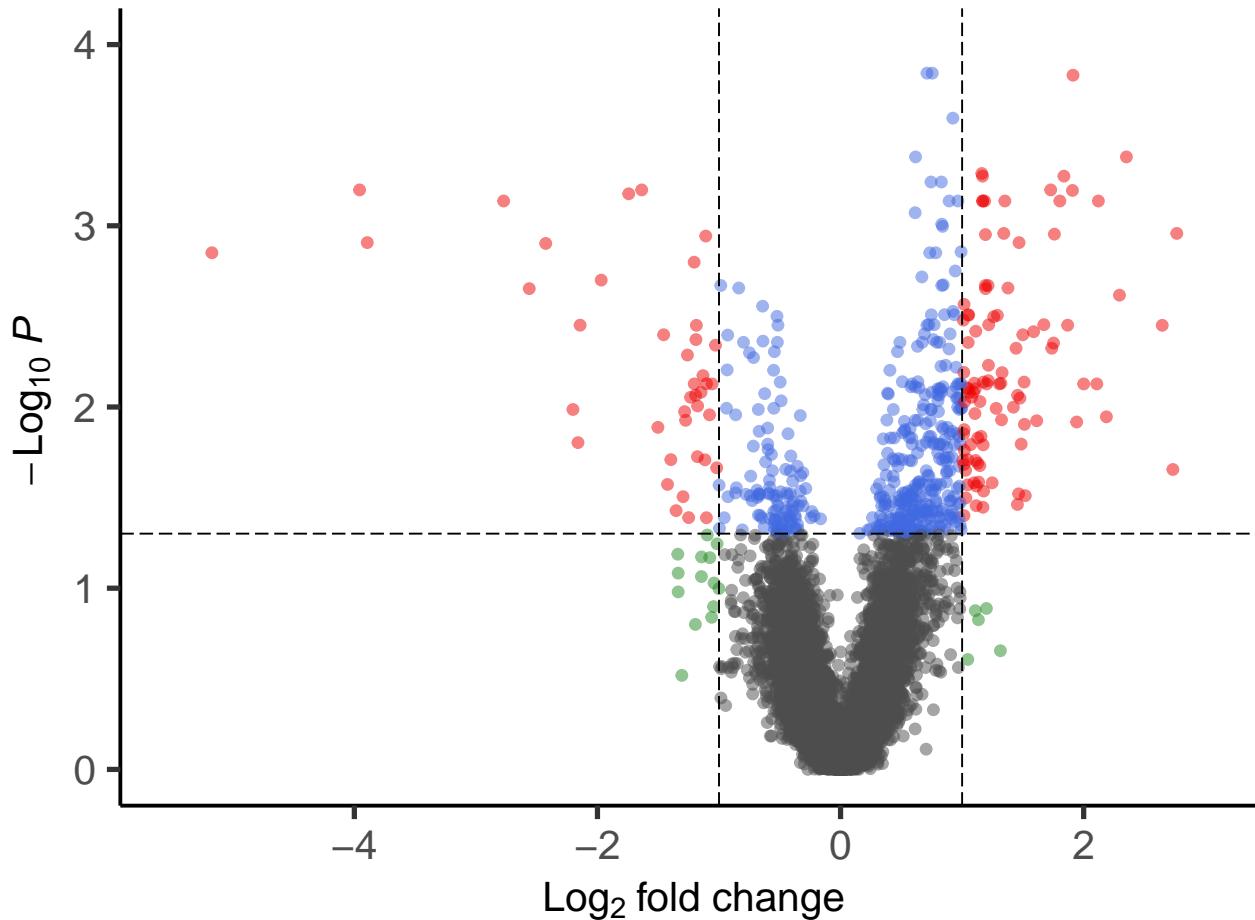
```
# Loading results from DE
DE_UnaffectedvsPeri <- read_excel("Data/DE-Peri-vs-Unaffected-V2.xlsx", sheet =
"VolcanoPlot", range = "C7:H19969")
DE_UnaffectedvsPeri$padj <- as.numeric(DE_UnaffectedvsPeri$`Adjusted pvalue`)

EnhancedVolcano(DE_UnaffectedvsPeri, DE_UnaffectedvsPeri$`Target name`, x = "Log2", y =
"padj", title= "DE-extraleisional vs perilesional", FCcutoff = 1, pCutoff = 0.05, labSize
= 0.1, ylim = c(0,4), xlim = c(-5.5,3), gridlines.major= F, gridlines.minor = F)
```

DE-extraleisional vs perilesional

EnhancedVolcano

● NS ● Log₂ FC ● p-value ● p-value and log₂ FC



```
#ggsave("volcanoplot/Volcano_UnaffectedvsPeri.pdf", width = 10, height = 10)
```

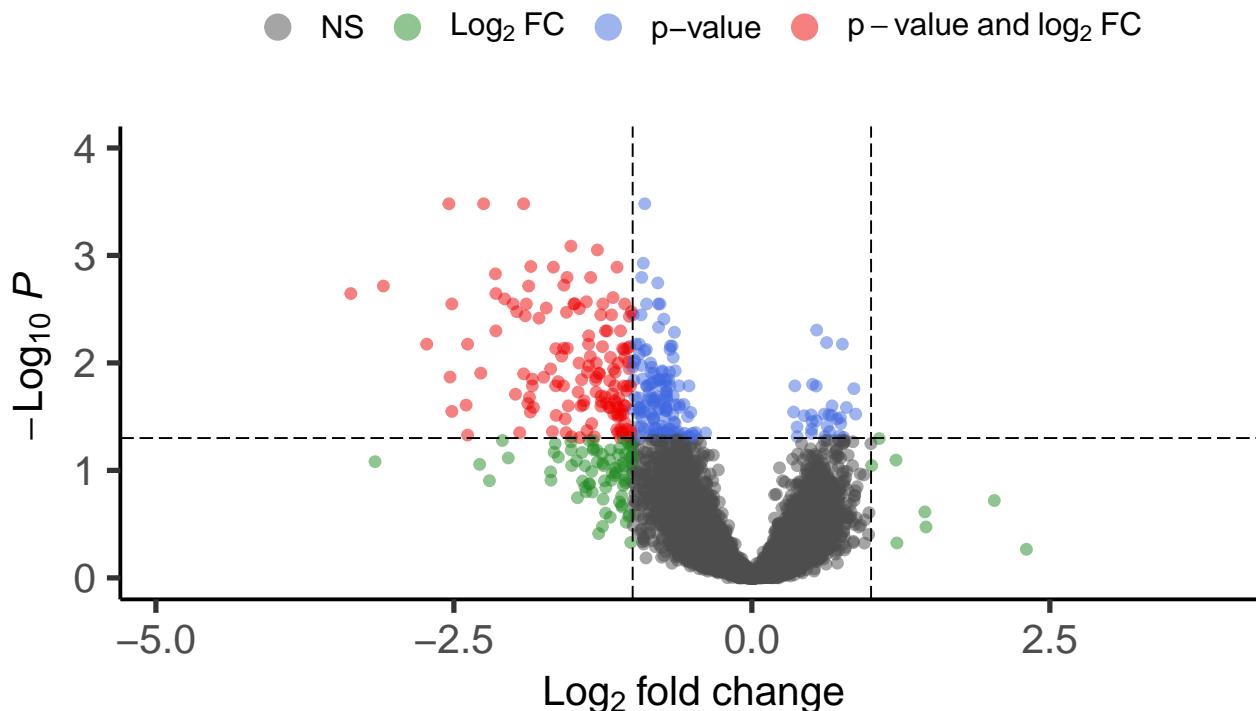
DE unaffected vs Healthy Control

```
DE_UnaffectedvsHealthy <- read_excel("Data/DE-Unaffected-vs-Healthy-V2.xlsx", sheet = "VolcanoPlot", range = "C7:H19969")
DE_UnaffectedvsHealthy$padj <- as.numeric(DE_UnaffectedvsHealthy$`Adjusted pvalue`)

EnhancedVolcano(DE_UnaffectedvsHealthy, DE_UnaffectedvsHealthy$`Target name`, x = "Log2", y = "padj", title= "DE-unaffected vs Control", FCcutoff = 1, pCutoff = 0.05, labSize = 0.1, ylim = c(0,4), gridlines.major= F, gridlines.minor = F)
```

DE-unaffected vs Control

EnhancedVolcano



total = 19962 variables

```
#ggsave("volcanoplot/Volcano_UnaffectedvsHealthy.pdf", width = 15, height = 15)
```

DE Perilesional vs Healthy Control

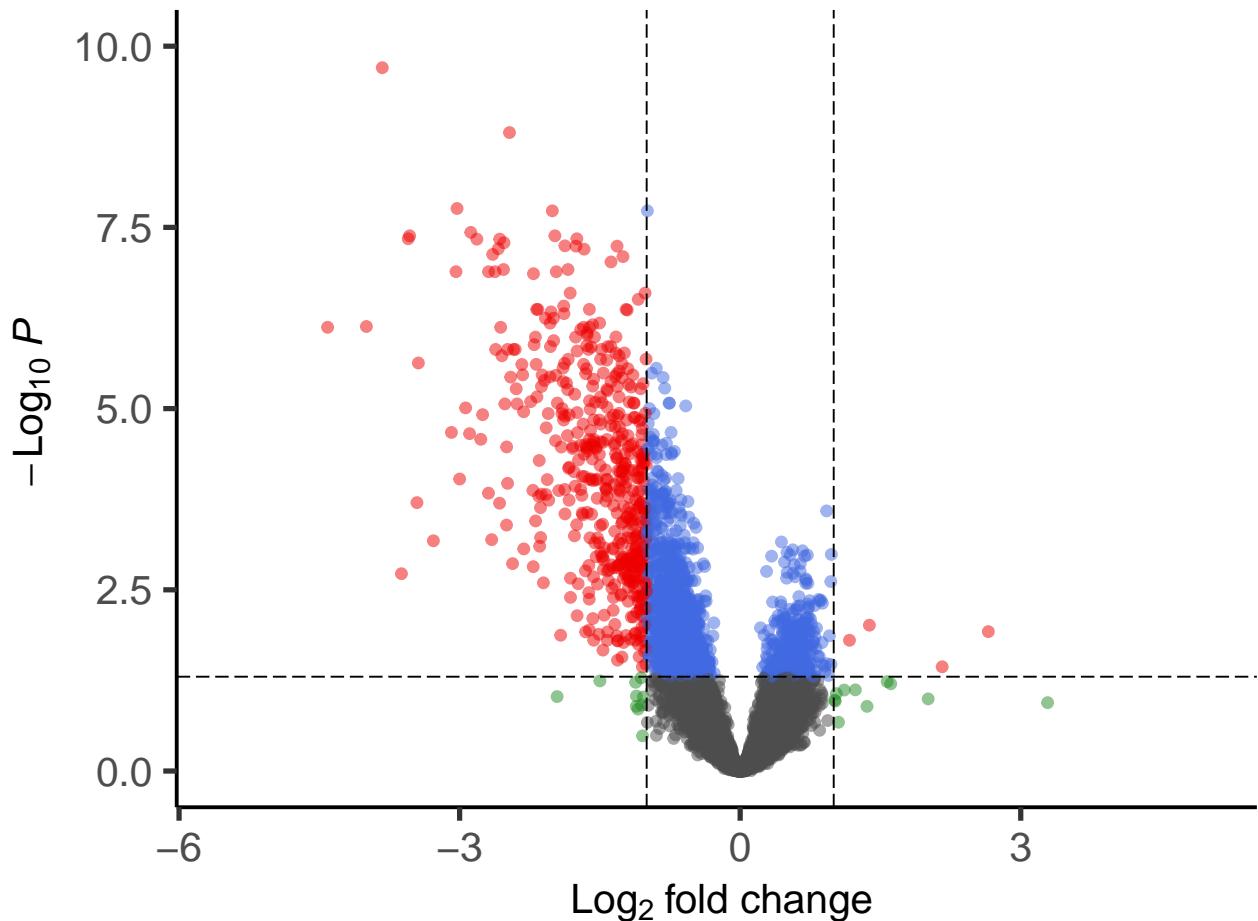
```
DE_HealthyvsPeri <- read_excel("Data/DE_PerivsHealthy-V2.xlsx", sheet = "VolcanoPlot", range = "C7:H19969")
DE_HealthyvsPeri$padj <- as.numeric(DE_HealthyvsPeri$`Adjusted pvalue`)

EnhancedVolcano(DE_HealthyvsPeri, DE_HealthyvsPeri$`Target name`, x = "Log2", y = "padj", title= "DE- Peri vs Control", FCcutoff = 1, pCutoff = 0.05, labSize = 0.1, ylim = c(0,10), xlim = c(-5.5,5), gridlines.major= F, gridlines.minor = F)
```

DE- Peri vs Control

EnhancedVolcano

● NS ● Log₂ FC ● p-value ● p – value and log₂ FC



```
#ggsave("volcanoplot/Volcano_DE_HealthyvsPeriV2.pdf", width = 10, height = 10)
```

Heatmap of 522 genes upregulated in perilesional areas

```
DE_522genes <- read_excel("Data/522-genes-UP-peri-Heatmap.xlsx", sheet = "Dendrogram", range = "C5:AA532", col_names = F)
DE_522genes <- DE_522genes[-c(3,4),]
sample_name <- paste(DE_522genes[1,], DE_522genes[2,],DE_522genes[3,], sep=" | ")

colnames(DE_522genes) <- c("Target Name", sample_name[-1])
DE_522genes <- DE_522genes[-c(1,2,3,4),]
DE_522genes <- as.data.frame(DE_522genes)
```

```

rownames(DE_522genes) <- DE_522genes[,1]
DE_522genes <- DE_522genes[,-1]

# convert data to numeric matrix
DE522genes_num <- apply(DE_522genes, 2, as.numeric)
rownames(DE522genes_num) <- rownames(DE_522genes)
colnames(DE522genes_num) <- colnames(DE_522genes)

color_df <- list(Group =
  c("Healthy" = "gray",
    "Intralesional" = "yellow",
    "Perilesional" = "orange",
    "Unaffected" = "white"))

rownames(df) <- gsub(" ", "", rownames(df))
df <- df[c(15, 24, 6, 11, 10, 14, 7, 8, 16, 4, 2, 3, 13, 17, 1, 5, 19, 18, 21, 20, 22, 9, 12, 23),]

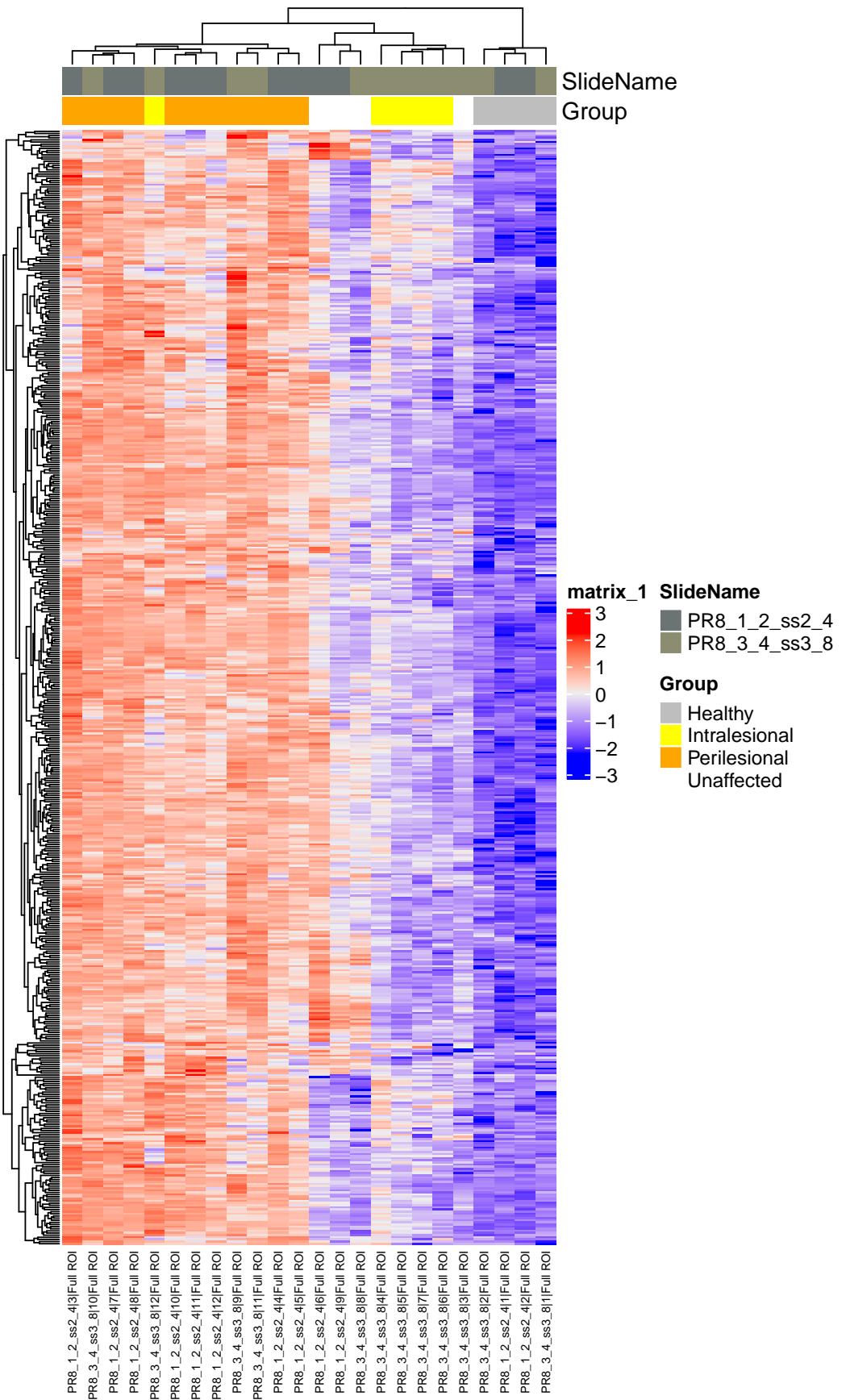
modified_vector <- substring(rownames(df), 1, nchar(rownames(df)) - 3) # Extract all but last 3 characters
rownames(df) <- paste(modified_vector, substring(rownames(df), nchar(rownames(df)) - 2),
sep = " ") # Concatenate with last 3 characters separated by a space

# verify that the 2 vector are the same
setequal(rownames(df), colnames(DE522genes_num))

Heatmap_522genes <- Heatmap(t(scale(t(DE522genes_num))), cluster_columns = T,
  top_annotation = HeatmapAnnotation(df = df, col = color_df),
  cluster_rows = T,
  show_row_names = F,
  row_names_gp = gpar(fontsize = 2),
  column_names_gp = gpar(fontsize = 6))

Heatmap_522genes

```



```

#tidyHeatmap::save_pdf(Heatmap_522genes, "522-genes-UP-peri-Heatmap.pdf", width = 15,
height = 50, units = "cm")

sessionInfo()

## R version 4.3.1 (2023-06-16)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 22.04.3 LTS
##
## Matrix products: default
## BLAS:    /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3
## LAPACK:  /usr/lib/x86_64-linux-gnu/openblas-pthread/libopenblas-r0.3.20.so; LAPACK version 3.10.0
##
## locale:
## [1] LC_CTYPE=en_US.UTF-8          LC_NUMERIC=C
## [3] LC_TIME=fr_BE.UTF-8          LC_COLLATE=en_US.UTF-8
## [5] LC_MONETARY=fr_BE.UTF-8      LC_MESSAGES=en_US.UTF-8
## [7] LC_PAPER=fr_BE.UTF-8         LC_NAME=C
## [9] LC_ADDRESS=C                  LC_TELEPHONE=C
## [11] LC_MEASUREMENT=fr_BE.UTF-8   LC_IDENTIFICATION=C
##
## time zone: Europe/Brussels
## tzcode source: system (glibc)
##
## attached base packages:
## [1] grid      stats     graphics grDevices utils     datasets methods
## [8] base
##
## other attached packages:
## [1] formatR_1.14           ComplexHeatmap_2.16.0 EnhancedVolcano_1.18.0
## [4] ggrepel_0.9.3          dplyr_1.1.2            ggplot2_3.4.2
## [7] tidyverse_1.3.0         readxl_1.4.2
##
## loaded via a namespace (and not attached):
## [1] utf8_1.2.3              generics_0.1.3        shape_1.4.6
## [4] rematch_1.0.1            digest_0.6.33        magrittr_2.0.3
## [7] RColorBrewer_1.1-3       evaluate_0.21        iterators_1.0.14
## [10] circlize_0.4.15          fastmap_1.1.1        cellranger_1.1.0
## [13] foreach_1.5.2            doParallel_1.0.17    GlobalOptions_0.1.2
## [16] purrrr_1.0.1             fansi_1.0.4          scales_1.2.1
## [19] codetools_0.2-19          cli_3.6.1            rlang_1.1.1
## [22] crayon_1.5.2             munsell_0.5.0        withr_2.5.0
## [25] yaml_2.3.7               tools_4.3.1          parallel_4.3.1
## [28] colorspace_2.1-0          BiocGenerics_0.46.0  GetoptLong_1.0.5
## [31] vctrs_0.6.3              R6_2.5.1              png_0.1-8
## [34] magick_2.7.5              stats4_4.3.1          matrixStats_1.0.0
## [37] lifecycle_1.0.3            S4Vectors_0.38.1     IRanges_2.34.0
## [40] clue_0.3-64               cluster_2.1.4        pkgconfig_2.0.3
## [43] pillar_1.9.0              gtable_0.3.3          glue_1.6.2
## [46] Rcpp_1.0.11                highr_0.10            xfun_0.39
## [49] tibble_3.2.1               tidyselect_1.2.0     rstudioapi_0.14
## [52] knitr_1.43                 farver_2.1.1          rjson_0.2.21
## [55] htmltools_0.5.5            labeling_0.4.2        rmarkdown_2.23

```

```
## [58] compiler_4.3.1
```