

DE plot

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```
suppressMessages(library(readxl))  
suppressMessages(library(tidyr))  
suppressMessages(library(ggplot2))  
suppressMessages(library(dplyr))  
suppressMessages(library(EnhancedVolcano))  
suppressMessages(library(pheatmap))
```

loading function to save pheatmap as pdf

```
source("save_pheatmap_pdf.R")
```

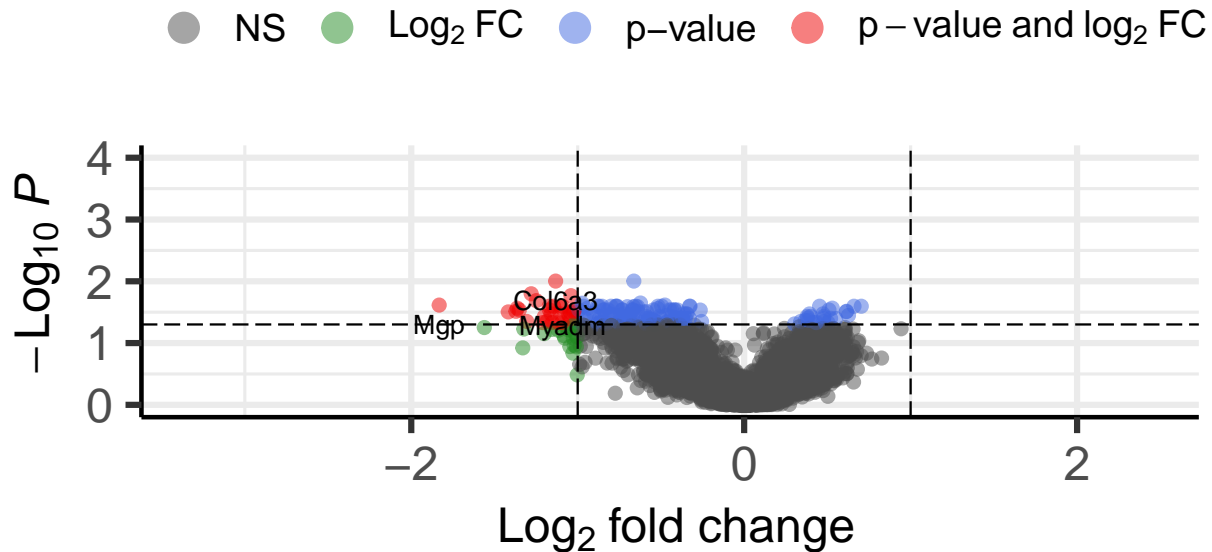
Loading perilesional vs intralesional

making volcano plot

```
DE_perivsintra$padj <- as.numeric(DE_perivsintra$`Adjusted pvalue`)  
  
EnhancedVolcano(DE_perivsintra, DE_perivsintra$`Target name`, x = "Log2", y = "padj", title= "DE-perile
```

DE-perilesional vs intralesional

EnhancedVolcano



total = 19962 variables

```
#ggsave("volcanoplot/Volcano_perivsintra.pdf", width = 15, height = 10)
```

Gene for Heatmap (in red in the volcanoplot)

```
gene_significant <- subset(DE_perivsintra, (Log2 > 1 | Log2 < -1) & padj <= 0.05)
gene_significant <- gene_significant$`Target name`
```

load the annotation file from Q3 - publications

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

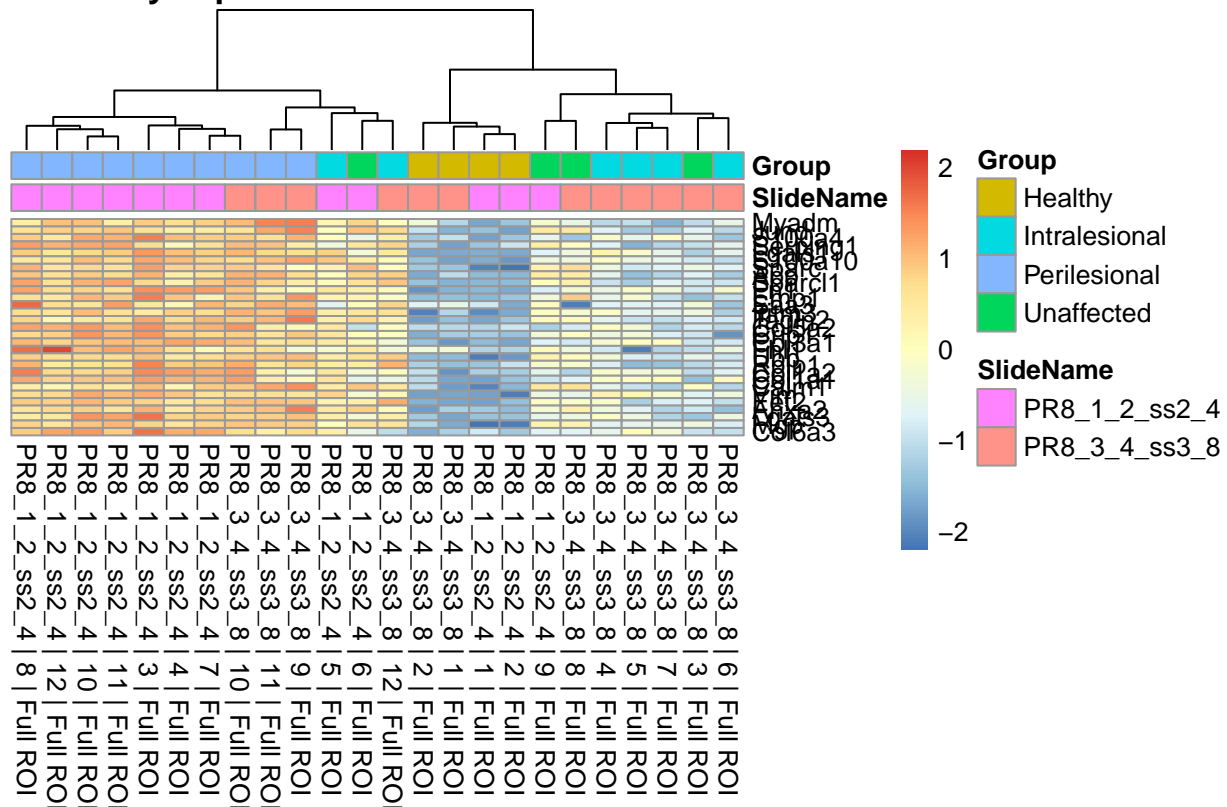
Load the normalised count matrix and making the heatmap

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

rld <- log2(Q3_publication)

df <- as.data.frame(colData[,c("SlideName", "Group")])
Heatmap_perivsintra <- pheatmap(rld[gene_significant,], cluster_rows=FALSE, show_rownames=T,
                                cluster_cols=T, annotation_col=df, scale = "row", main = "Genes differentially expressed in Perivascular space")
```

Differentially expressed in Perilesional over intralesional



```
#save_pheatmap_pdf(Heatmap_perivsintra, "Heatmap/Heatmap_perivsintra.pdf", width = 10, height = 10)
```

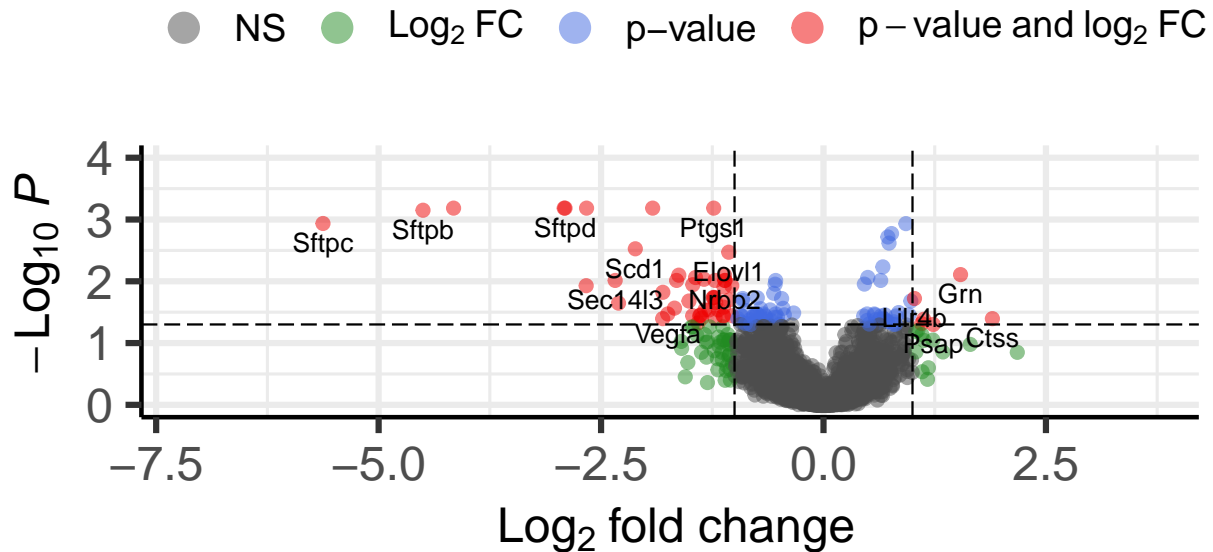
Loading unaffected vs intralesional

```
DE_UnaffectedvsIntra <- read_excel("DE-Unaffected-Intra.xlsx", sheet = "VolcanoPlot", range = "C7:H1996")
DE_UnaffectedvsIntra$padj <- as.numeric(DE_UnaffectedvsIntra$`Adjusted pvalue`)

EnhancedVolcano(DE_UnaffectedvsIntra, DE_UnaffectedvsIntra$`Target name`, x = "Log2", y = "padj", title = "DE_UnaffectedvsIntra")
```

DE-unaffected vs intralesionnal

EnhancedVolcano



total = 19962 variables

```
#ggsave("volcanoplot/Volcano_UnaffectedvsIntra.pdf", width = 15, height = 10)
```

Heatmap !! je ne prends pas tous les gènes significatif

```
gene_significant <- subset(DE_UnaffectedvsIntra, (Log2 > 1 | Log2 < -1) & padj <= 0.05)
gene_significant_pos <- gene_significant[gene_significant$Log2 >= 1,]
gene_significant_neg <- gene_significant[gene_significant$Log2 <= -1,]
gene_significant_neg <- gene_significant_neg[order(gene_significant_neg$Log2),]
gene_toplot <- c(gene_significant_neg$`Target name`[1:20], gene_significant_pos$`Target name`)
```

```
Heatmap_UnaffectedvsIntra <- pheatmap(rld[gene_toplot,], cluster_rows=FALSE, show_rownames=T,
  cluster_cols=T, annotation_col=df, scale = "row", main = "Genes differentially expressed in un")
```

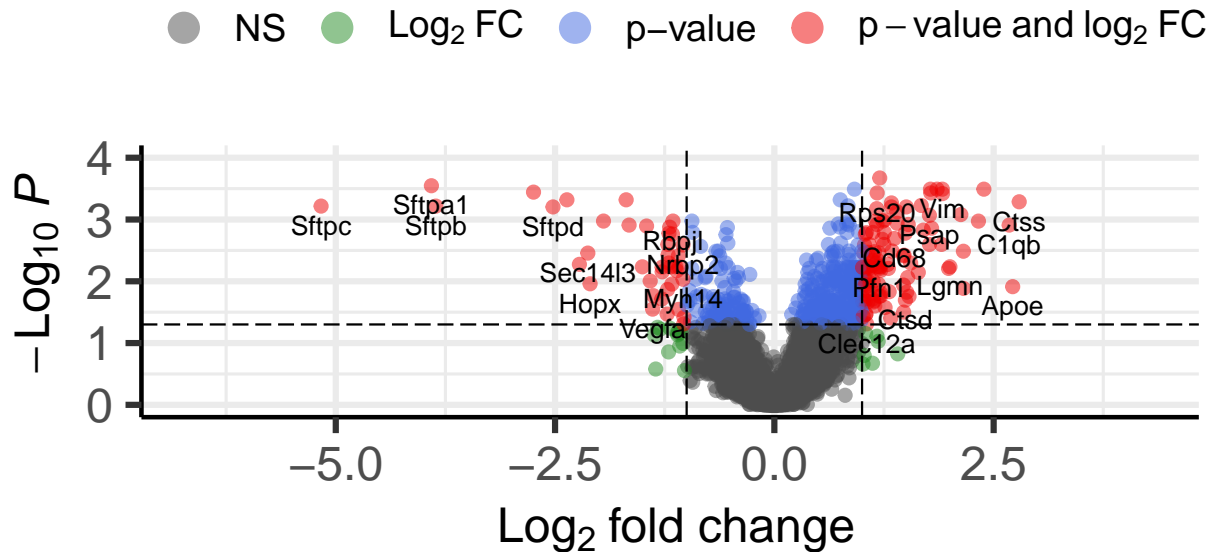
[illegible]

DE unaffected vs perilesional

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DE-unaffected vs perilesional

EnhancedVolcano



total = 19962 variables

```
#ggsave("volcanoplot/Volcano_UnaffectedvsPeri.pdf", width = 15, height = 10)
```

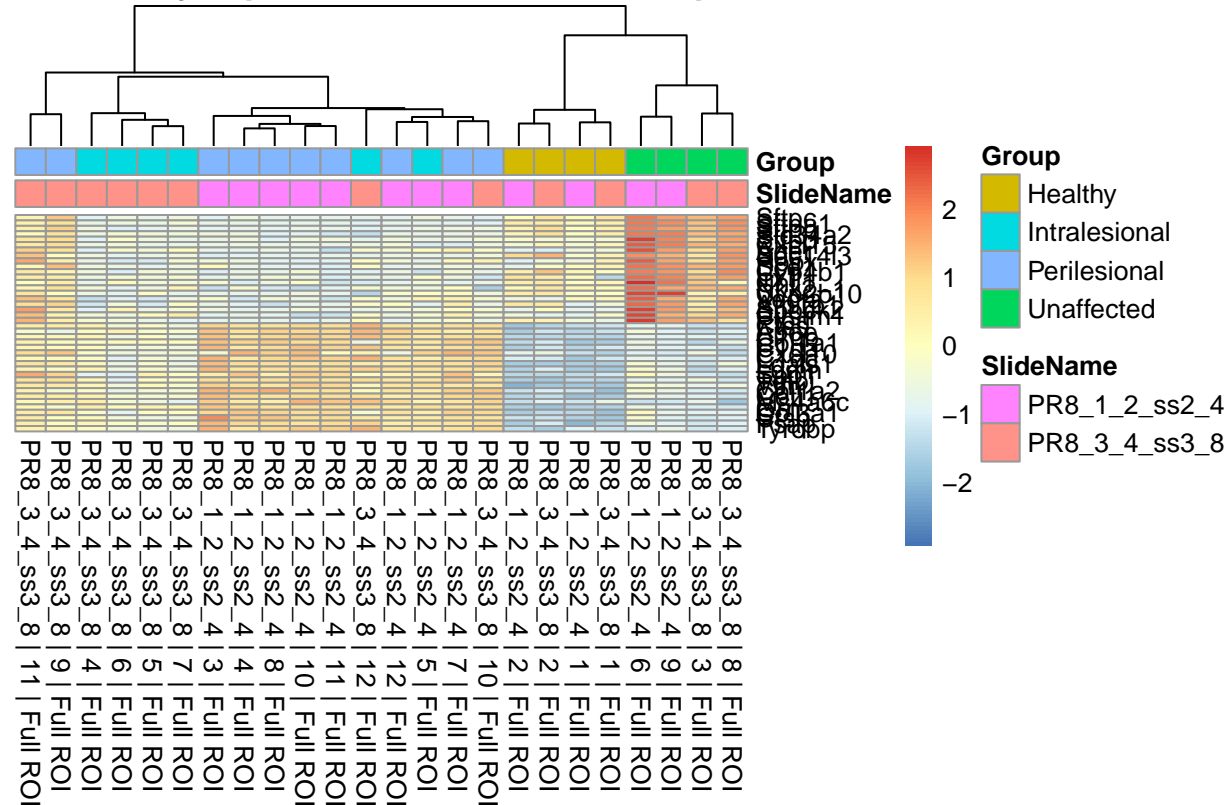
Heatmap !! je ne prends pas tous les gènes significatif

```
gene_significant <- subset(DE_UnaffectedvsPeri, (Log2 > 1 | Log2 < -1) & padj <= 0.05)
gene_significant_pos <- gene_significant[gene_significant$Log2 >= 1,]
gene_significant_pos <- gene_significant_pos[order(gene_significant_pos$Log2, decreasing = T),]
gene_significant_neg <- gene_significant[gene_significant$Log2 <= -1,]
gene_significant_neg <- gene_significant_neg[order(gene_significant_neg$Log2),]

gene_toplot <- c(gene_significant_neg$`Target name`[1:20], gene_significant_pos$`Target name`[1:20])

Heatmap_UnaffectedvsPeri <- pheatmap(rld[gene_toplot,], cluster_rows=FALSE, show_rownames=T,
  cluster_cols=T, annotation_col=df, scale = "row", main = "Genes differentially expressed in un")
```

differentially expressed in unaffected over perilesional



`#save_pheatmap_pdf(Heatmap_UnaffectedvsPeri, "Heatmap/Heatmap_UnaffectedvsPeri.pdf", width = 10, height`