

```

# Input Parameters
tissue <- "Plasma"
filepath <- "/Users/usa/Desktop/Zaganjor lab lipidomics results/lipidomics-analysis/"

# Load packages
require(tidyverse)
require(lipidr)
require(viridis)

# Read in data
tissue_data <- read.csv(paste0(filepath, tissue, ".csv"))

# Remove extraneous col
tissue_data$lipid_id <- NULL

# Remove "bad" entries (eg. 13-Docosenamide)
tissue_data <- subset(tissue_data, grepl(":", lipids))

#remove NAs
tissue_data <- na.omit(tissue_data)

# Convert Lipid Names to Systematic Nomenclature
temp <- tissue_data$lipids
fix = sub(";(O\\d*)", "(\\1)", temp)
fix = sub(" O-", "O ", fix)
fix = sub(" P-", "P ", fix)
fix = sub("-FA", "/", fix)
fix = sub(";(.*)$", "(\\1)", fix)
fix <- sub("([\\^\\])\\|", "\\1(m)|", fix)
fix <- sub("(.*\\(m\\))\\|(.*)$", "\\1|\\2(m2)", fix)
fix <- sub("PE-Cer", "PECer", fix)
fix <- sub("PI-Cer", "PICer", fix)
fix <- sub("LPE-N ", "LPEN ", fix)
fix <- sub("LPE-N ", "LPEN ", fix)

tissue_data$converted_lipid_name <- fix

all_match <- lipidr::annotate_lipids(tissue_data[[ncol(tissue_data)]])
bad_match <- all_match %>% filter(not_matched)
good_match <- subset(all_match, not_matched == "FALSE")

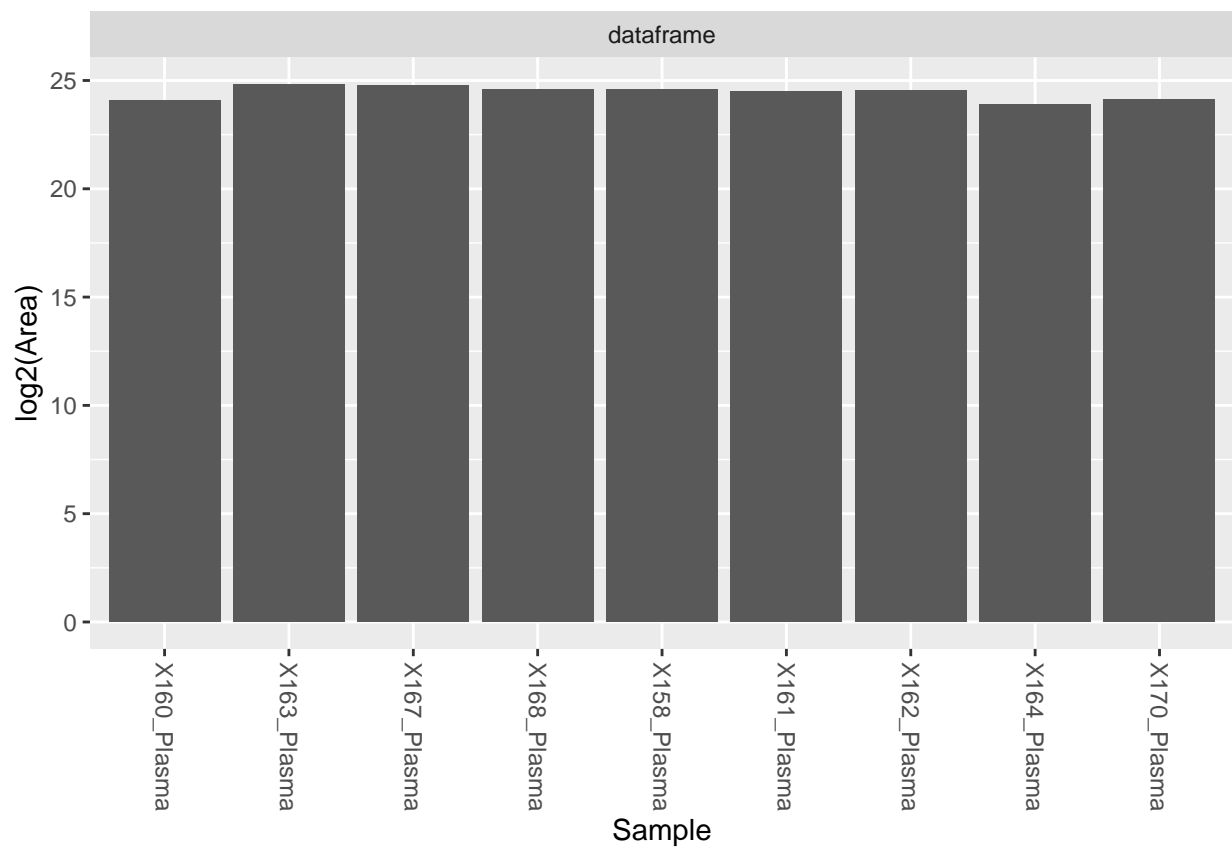
tissue_data %>%
  select(lipids, converted_lipid_name) %>%
  write_csv(paste0(filepath, "Results/", tissue, "_name_converted.csv"))

# Begin lipidr analysis - setup lipidr object
tissue_data$lipids <- tissue_data$converted_lipid_name
tissue_data$converted_lipid_name <- NULL

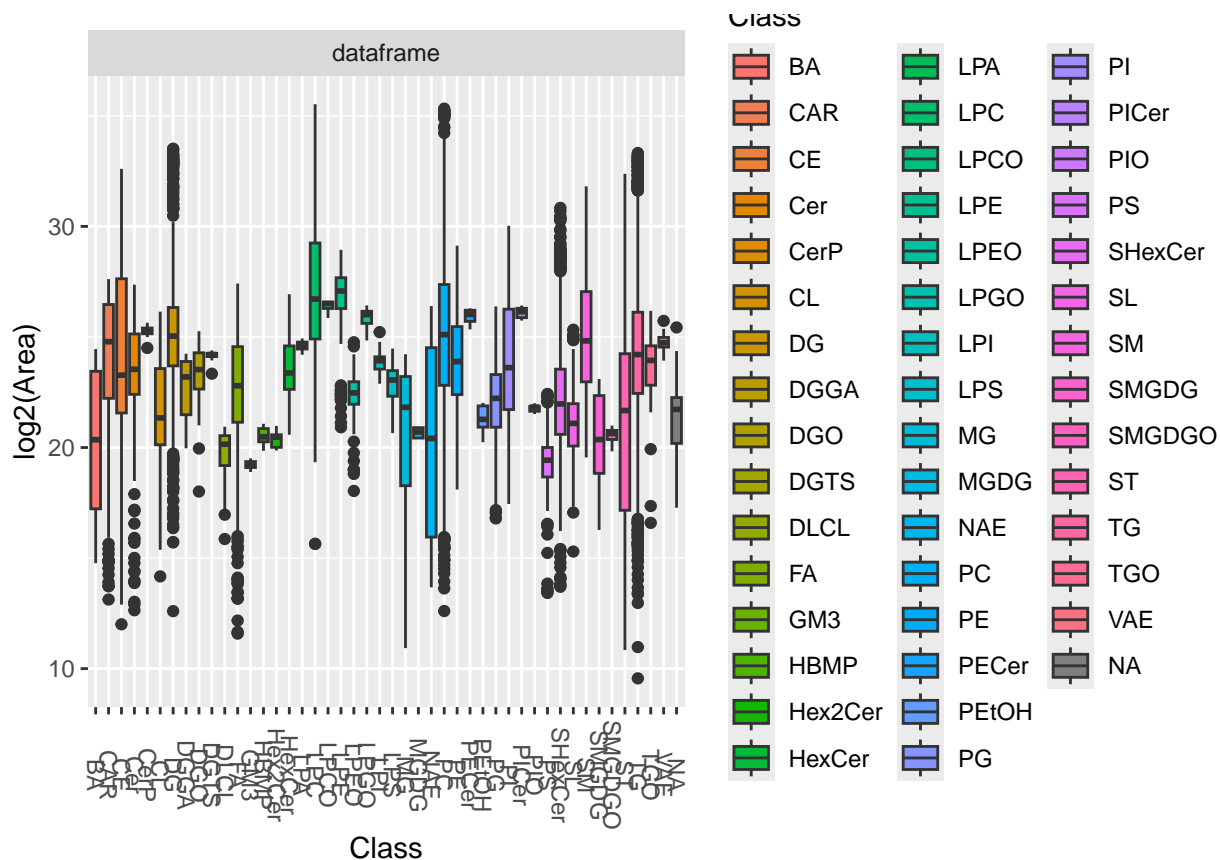
d <- as_lipidomics_experiment(tissue_data)
meta <- read.csv(paste0(filepath, tissue, "_meta.csv"))
d <- add_sample_annotation(d, meta)

# QC
plot_samples(d, type = "tic", log = TRUE)

```

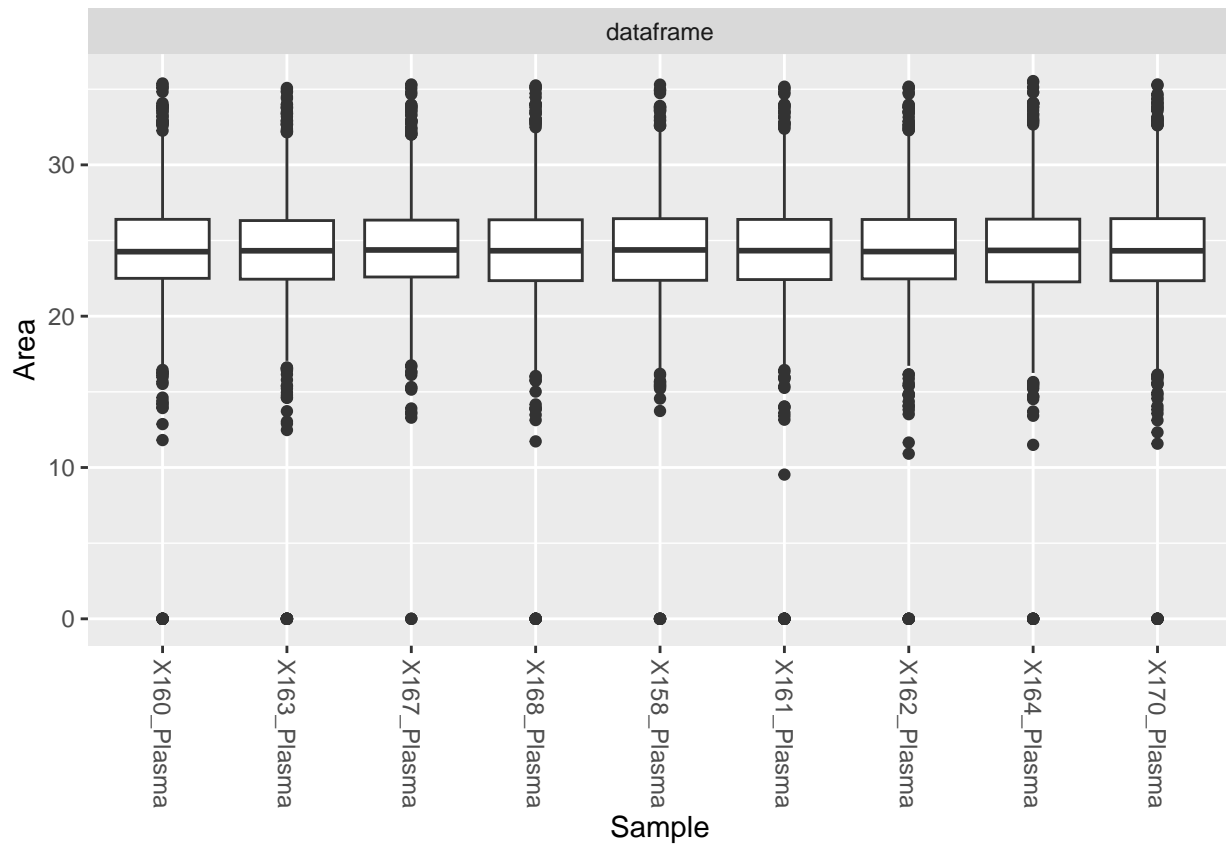


```
ggsave(paste0(filepath, "Plots/", tissue, "_samples.png"))  
plot_lipidclass(d, "boxplot")
```



```
ggsave(paste0(filepath, "Plots/", tissue, "_classes.png"))
```

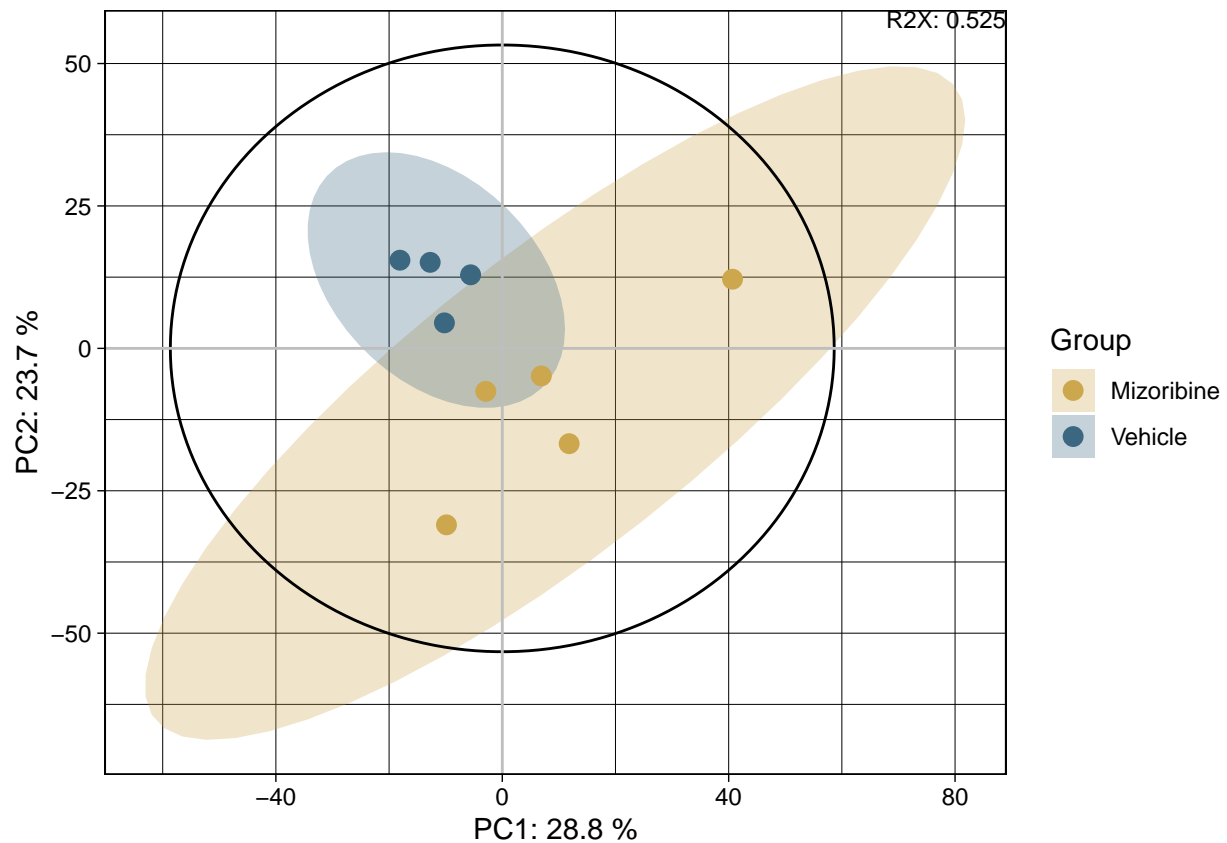
```
# Resolve Duplicate Lipids (from multiple transitions) by selecting maximum, Normalize with PQN method,
d_summarized <- summarize_transitions(d, method = "max")
d_normalized <- normalize_pqn(d_summarized, measure = "Area", exclude = "blank", log = TRUE)
plot_samples(d_normalized, "boxplot")
```



```
ggsave(paste0(filepath, "Plots/", tissue, "_normalization.png"))
```

```
# PCA
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```
mvareresults <- mva(d_normalized, measure="Area", method="PCA")
pca_obj <- plot_mva(mvareresults, color_by="Treatment", components = c(1,2))
pca_obj + theme_linedraw() + scale_color_manual(values = c("Mizoribine" = "#CCA74E", "Vehicle" = "#3C
```



```
ggsave(paste0(filepath, "Plots/", tissue, "_pca.png"))

# DE - make comparisons
de_results <- de_analysis(data=d_normalized, group_col = "Treatment", Mizoribine - Vehicle, measure=
significant_molecules(de_results, p.cutoff = 0.05, logFC.cutoff = 0)

## named list()

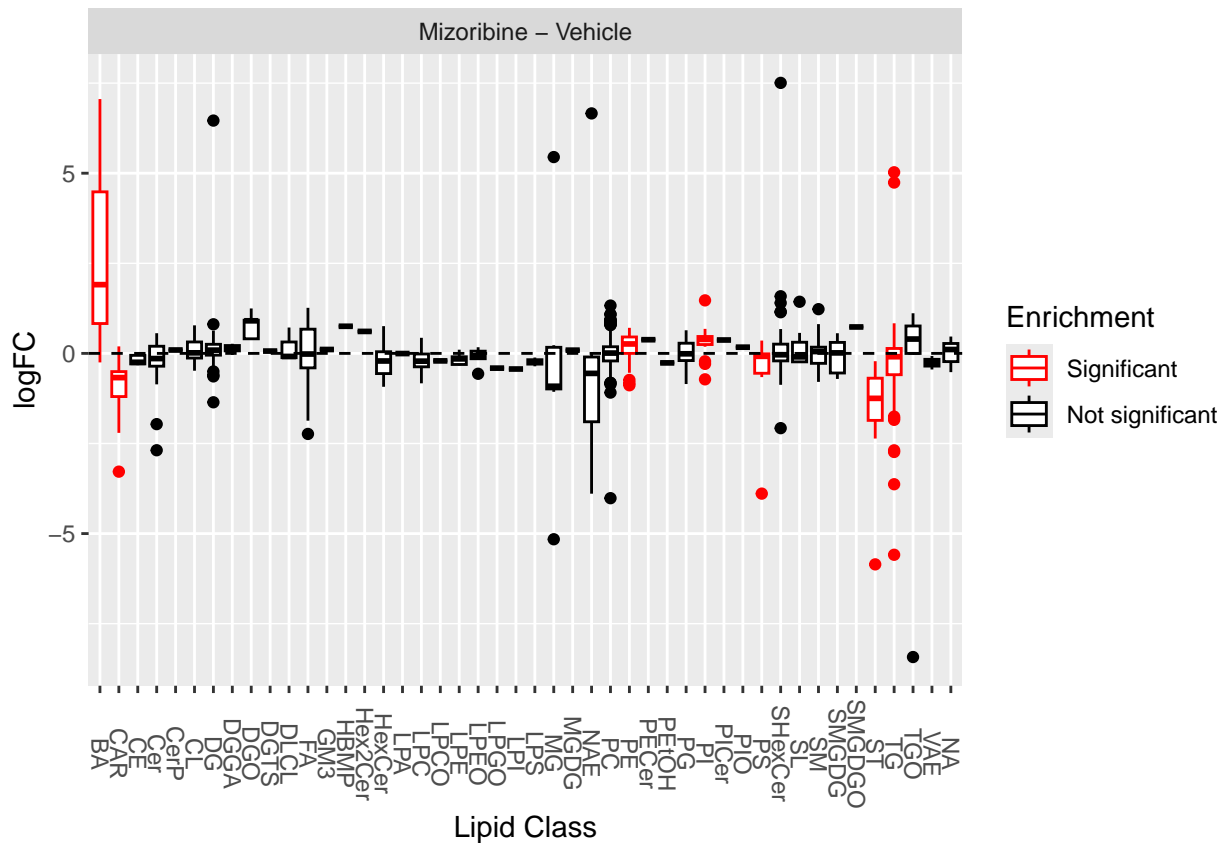
write_csv(de_results, paste0(filepath, "Results/", tissue, "_de.csv"))

# LSEA
enrich_results <- lsea(de_results, rank.by = "logFC")
sig_lipidsets <- significant_lipidsets(enrich_results)

write_csv(enrich_results, paste0(filepath, "Results/", tissue, "_lsea.csv"))

# Visualizations

# Significant Lipid Classes (Boxplot)
plot_enrichment(de_results, sig_lipidsets, annotation="class")
```



```
ggsave(paste0(filepath, "Plots/", tissue, "_enrichment_boxplot.png"))
```

```
## Saving 6.5 x 4.5 in image
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```
# Volcano plot with TGs highlighted
```

```
de_results_volcano <- de_results %>%
```

```
  mutate(colorcode = ifelse(P.Value < 0.05, ifelse(Class=="TG", "Significant Triglycerides", "All Other Significant Lipids"), "Not Significant")
```

```
p_volcano <- ggplot(de_results_volcano, aes(x = logFC, y = -log10(P.Value))) +
```

```
  geom_point(aes(color = colorcode)) +
```

```
  scale_color_manual(values = c("Not Significant" = "grey", "All Other Significant Lipids" = "#3C6780", "Significant Triglycerides" = "#E41A1C")) +
```

```
  theme_linedraw() +
```

```
  xlab("Log2(Fold Change)") +
```

```
  ylab("-Log10(p value)") +
```

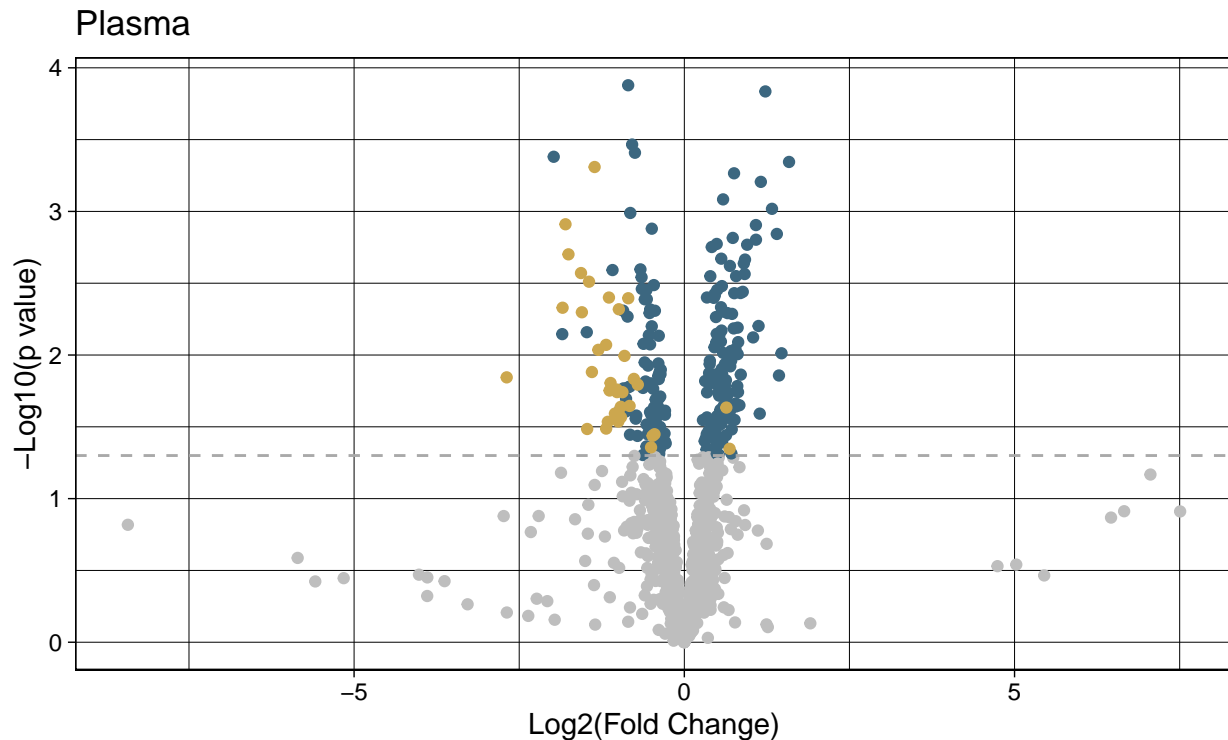
```
  labs(color = "Legend") +
```

```
  ggtitle(tissue) +
```

```
  theme(legend.position = "bottom") +
```

```
  geom_hline(yintercept = 1.3, linetype = "dashed", color = "darkgrey")
```

```
p_volcano
```



Legend ● All Other Significant Lipids ● Not Significant ● Significant Triglycerides

```
ggsave(paste0(filepath, "Plots/", tissue, "_TG_volcano.png"))
```

```
## Saving 6.5 x 4.5 in image
```

```
# Significant Lipid Classes (Barplot)
enrich_results$type <- sub("_.*", "", enrich_results$set)
enrich_results$class <- sub("^[_]*_", "", enrich_results$set)

lsea_tissue_data <- enrich_results %>%
  filter(type == "Class") %>%
  mutate(abs_NES = abs(NES))

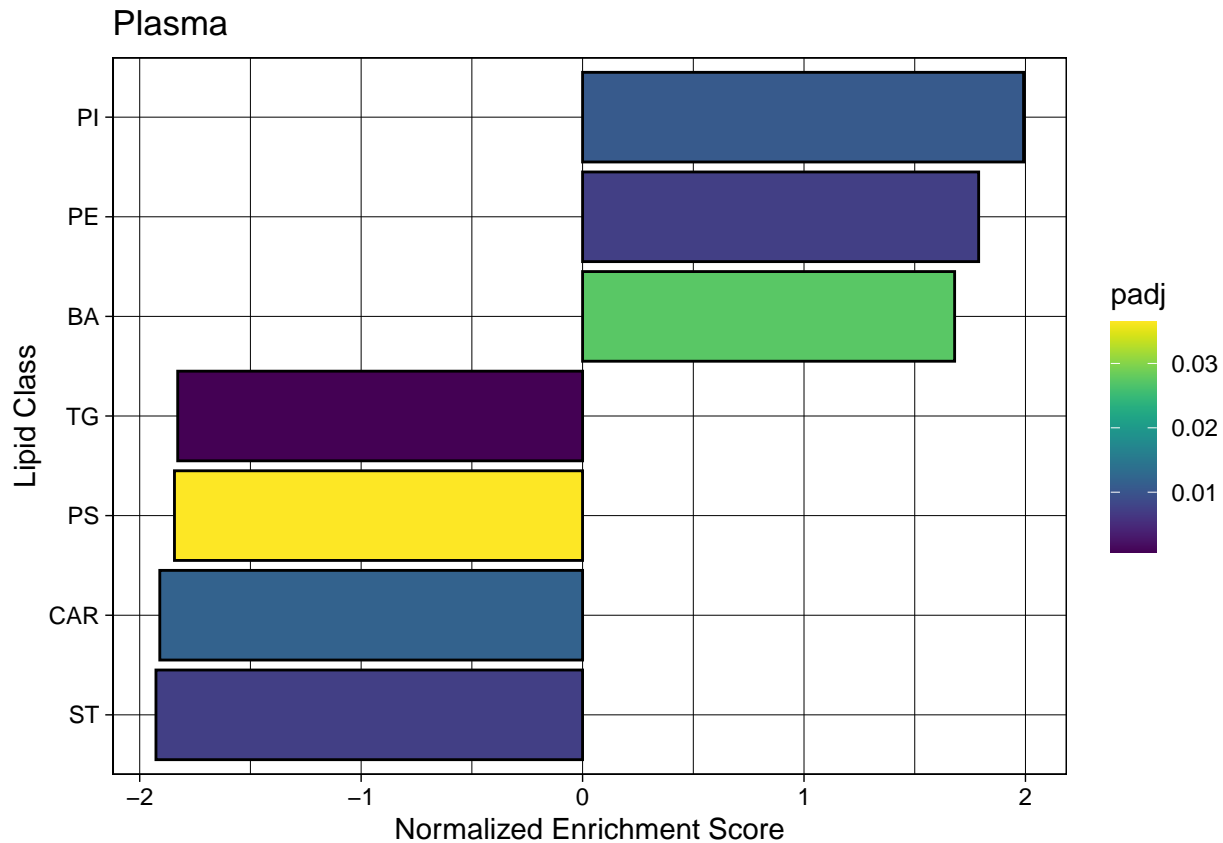
df_sig <- lsea_tissue_data %>% filter(padj < 0.05) %>%
  arrange(desc(NES))

p_enrich <- ggplot(df_sig, aes(x = NES, y = reorder(class, NES), fill = padj)) +
  geom_bar(stat = "identity", color = "black") +
  scale_fill_continuous() + # Adjust the scale for continuous color
labs(x = "Normalized Enrichment Score", y = "Lipid Class", title = tissue) +
  theme_linedraw() +
  scale_fill_viridis()
```

```
## Scale for fill is already present.
```

```
## Adding another scale for fill, which will replace the existing scale.
```

```
p_enrich
```



```
ggsave(paste0(filepath, "Plots/", tissue, "_enrichment_barplot_sig.png"))
```

```
## Saving 6.5 x 4.5 in image
```

```
# TG Heatmat
```

```
de_results_tg <- de_results %>%  
  filter(Class == "TG")
```

```
p_grid <- ggplot(de_results_tg, aes(total_cs, total_cl, fill = logFC)) + geom_tile() +  
  facet_wrap(~Class) +  
  xlab("Total chain unsaturation") + ylab("Total chain length") +  
  scale_fill_gradient2(midpoint = 0) +  
  xlim(1, 18) + ylim(26, 76) + theme_linedraw() +  
  scale_fill_gradient2(low = "blue", mid = "grey", high = "red") +  
  scale_x_continuous(breaks = seq(1, 18, by = 2)) +  
  scale_y_continuous(breaks = seq(26, 76, by = 2))
```

```
## Scale for fill is already present.
```

```
## Adding another scale for fill, which will replace the existing scale.
```

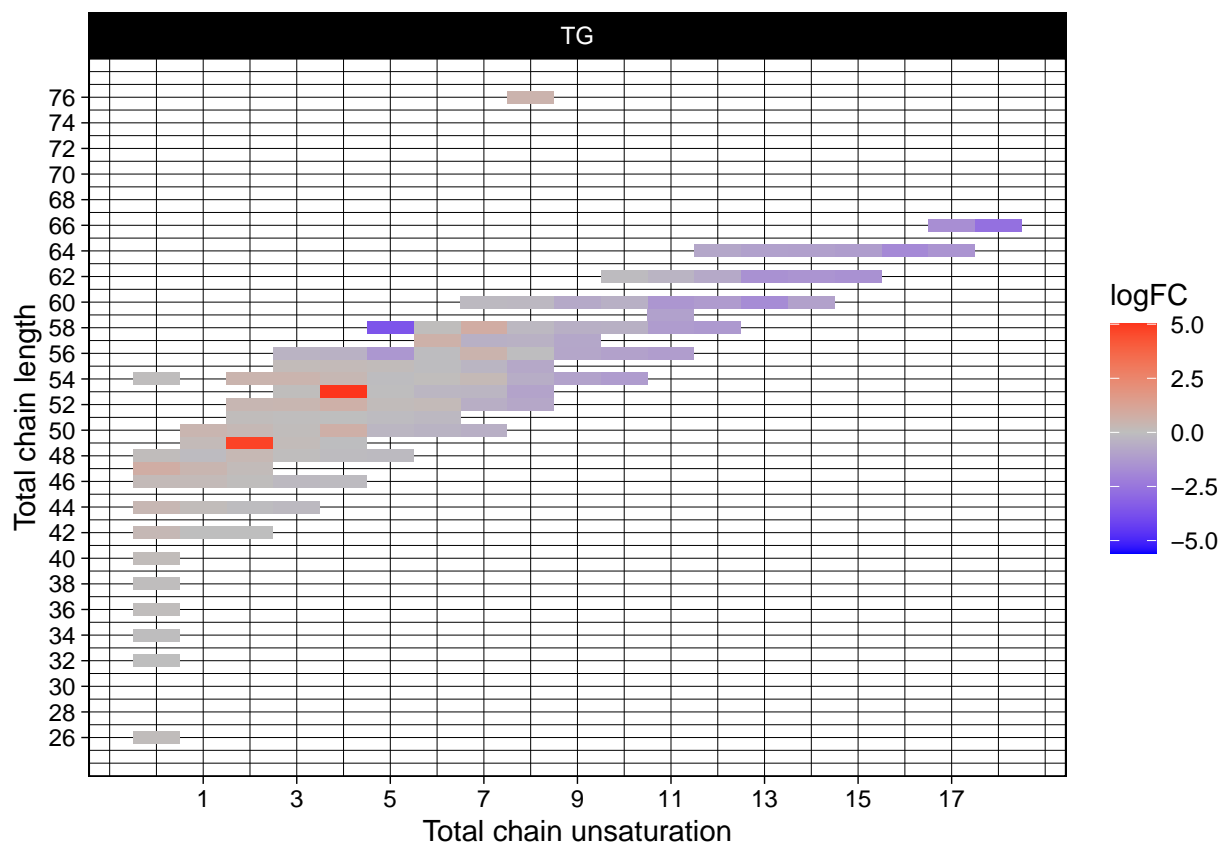
```
## Scale for x is already present.
```

```
## Adding another scale for x, which will replace the existing scale.
```

```
## Scale for y is already present.
```

```
## Adding another scale for y, which will replace the existing scale.
```

```
p_grid
```

```
ggsave(paste0(filepath, "Plots/", tissue, "_TG_map.png"))
```

```
## Saving 6.5 x 4.5 in image
```