

ANM_Lipid_Saturation

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```
#Load libraries
library(tidyverse)
```

```
## Warning: pakke 'ggplot2' blev bygget under R version 4.3.1
```

```
## Warning: pakke 'purrr' blev bygget under R version 4.3.1
```

```
## Warning: pakke 'dplyr' blev bygget under R version 4.3.1
```

```
## -- Attaching core tidyverse packages ----- tidyverse 2.0.0 --
```

```
## v dplyr      1.1.3      v readr      2.1.4
```

```
## v forcats   1.0.0      v stringr   1.5.0
```

```
## v ggplot2   3.4.3      v tibble    3.2.1
```

```
## v lubridate 1.9.2      v tidyr     1.3.0
```

```
## v purrr     1.0.2
```

```
## -- Conflicts ----- tidyverse_conflicts() --
```

```
## x dplyr::filter() masks stats::filter()
```

```
## x dplyr::lag()     masks stats::lag()
```

```
## i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to become errors
```

```
library(here)
```

```
## here() starts at H:/Desktop/ANM_PRS/ANM_Data_Analysis
```

```
library(vroom)
```

```
## Warning: pakke 'vroom' blev bygget under R version 4.3.1
```

```
##
```

```
## Vedhæfter pakke: 'vroom'
```

```
##
```

```
## De følgende objekter er maskerede fra 'package:readr':
```

```
##
```

```
## as.col_spec, col_character, col_date, col_datetime, col_double,
```

```
## col_factor, col_guess, col_integer, col_logical, col_number,
```

```
## col_skip, col_time, cols, cols_condense, cols_only, date_names,
```

```
## date_names_lang, date_names_langs, default_locale, fwf_cols,
```

```
## fwf_empty, fwf_positions, fwf_widths, locale, output_column,
```

```
## problems, spec
```

```

#Set color palette
color_palette <- c("#11A1B7", "#FF660C", "#OCA61E", "#FE3C1A",
                  "#9966CC", "#4DDDF2C", "#FE5387", "#85D0AB",
                  "#18548A", "#FCBB0B", "#FD908F", "#DF56BD", "#F0E4AD")

color_palette2 <- c("Cer" = "#935116", "DG" = "#b7950b", "LacCer" = "#ec407a",
                  "LPC" = "#48c9b0", "PA" = "#ec7063", "PC" = "#2874a6",
                  "PE" = "#7d3c98", "PI" = "#f8c471", "SM" = "#1e8449",
                  "TG" = "#C71B42", "Not significant" = "#808080")

#Load in linear regression data
reg_data <- vroom(here("data/sup_table_lipid_regression_v1.6.csv"))

## Rows: 8576 Columns: 7
## -- Column specification -----
## Delimiter: "\t"
## chr (3): Model, Lipid, Outcome
## dbl (4): Estimate, StdError, Pval, PvalFDR
##
## i Use 'spec()' to retrieve the full column specification for this data.
## i Specify the column types or set 'show_col_types = FALSE' to quiet this message.

library(ggrepel)

#Volcano plot of regression data
volcano_regression_A <- tibble(reg_data) %>%
  filter(Outcome == "AD Female not Adjusted for APOE" |
         Outcome == "AD Male not Adjusted for APOE") %>%
  mutate(Outcome = gsub("AD Female not Adjusted for APOE", "Female", Outcome)) %>%
  mutate(Outcome = gsub("AD Male not Adjusted for APOE", "Male", Outcome)) %>%
  mutate(tmp_lipid = Lipid) %>%
  mutate(Lipid = gsub("_A", "", Lipid)) %>%
  mutate(Lipid = gsub("_B", "", Lipid)) %>%
  separate(tmp_lipid, sep = "\\(", c("Family", "Chain1")) %>%
  mutate(Family = ifelse(PvalFDR > 0.05, "Not significant", Family)) %>%
  ggplot(aes(x = Estimate, y = -log10(PvalFDR), color = Family)) +
  geom_rect(aes(xmin = -Inf, xmax = Inf, ymin = -log10(0.05), ymax = -Inf),
            fill="grey", alpha=0.05, show.legend = FALSE) +
  geom_point(alpha = 1) +
  scale_color_manual(values = color_palette2,
                    limits = names(color_palette2)[-11]) +
  geom_hline(yintercept = -log10(0.05), linetype = "dashed", colour = "grey") +
  geom_text_repel(aes(label = Lipid), size = 3,
                 max.overlaps = 8,
                 show.legend = FALSE) +
  scale_y_continuous(limits = c(0, NA), labels = function(i) round(10^-i, 2)) +
  ylab(label = "P-value") +
  xlab(label = "Estimate") +
  labs(color = "Lipid\nFamily") +
  facet_grid(.~Outcome) +
  theme_bw() +
  theme(strip.background = element_blank(),
        strip.text = element_text(face = "bold", size = 12),

```



```

library(ggpubr)
library(grid)

#Load data
data <- vroom(here("data/ANM_Lipid_Preprocessed_v4.csv"))

## Rows: 841 Columns: 293
## -- Column specification -----
## Delimiter: "\t"
## chr (8): ID, Site, Date, Status, Sex, DOB, Accommodation, Marital_Status
## dbl (285): Visit, Order, Label, Age, Fulltime_Education_Years, apoe, e4_p, e...
##
## i Use 'spec()' to retrieve the full column specification for this data.
## i Specify the column types or set 'show_col_types = FALSE' to quiet this message.

colnames(data) <- colnames(data) %>%
  gsub("\\(", "", .) %>%
  gsub("\\)", "", .) %>%
  gsub("\\:", "", .) %>%
  gsub("\\-", "", .) %>%
  gsub("\\_", "", .) %>%
  gsub("\\/", "", .)

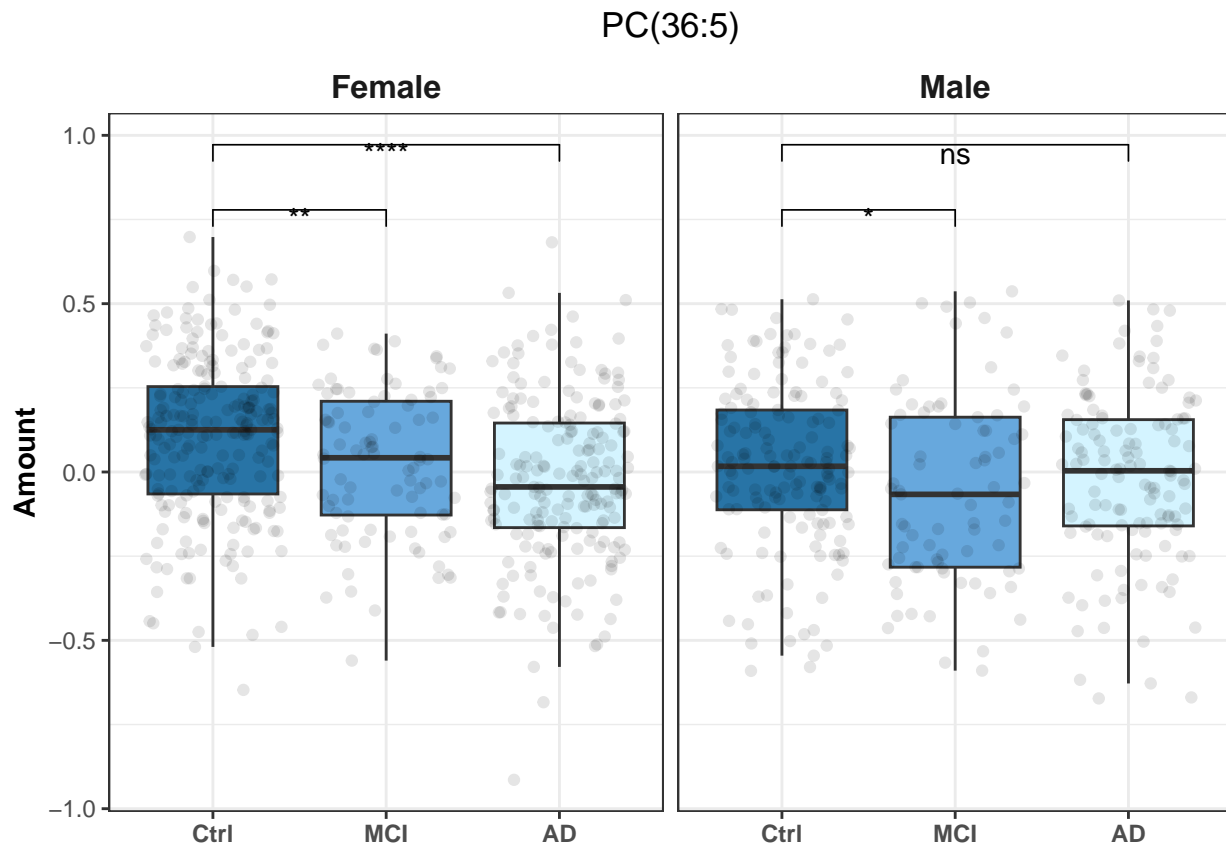
my_comparisons <- list( c("Ctrl", "MCI"), c("Ctrl", "AD"))

#Boxplot of PC(36:5)
Box_PC <- data %>%
  mutate(Status = factor(Status, levels = c("CTL", "MCI", "ADC"),
    labels = c("Ctrl", "MCI", "AD"))) %>%
  ggplot(aes(x = Status, y = PC365, fill = Status))+
  geom_boxplot(outlier.shape = NA)+
  geom_jitter(alpha = 0.1)+
  scale_fill_manual(values = c("#2874a6",
    "#67a8dd",
    "#d4f4ff"))+

  facet_grid(. ~ Sex)+
  labs(title = "PC(36:5)")+
  ylab("Amount")+
  stat_compare_means(comparisons = my_comparisons,
    method = "t.test",
    label = "p.signif",
    na.rm = TRUE,
    vjust = 1.25)+

  theme_bw()+
  theme(axis.title.x = element_blank(),
    #axis.title.y = element_blank(),
    legend.position = "none",
    strip.background = element_blank(),
    strip.text = element_text(face = "bold", size = 12),
    axis.text.x = element_text(face = "bold"),
    axis.title.y = element_text(face = "bold"),
    plot.title = element_text(hjust = 0.5))

```



```
#Boxplot of PE(40:7)
Box_PE <- data %>%
  mutate(Status = factor(Status, levels = c("CTL", "MCI", "ADC"),
    labels = c("Ctrl", "MCI", "AD"))) %>%

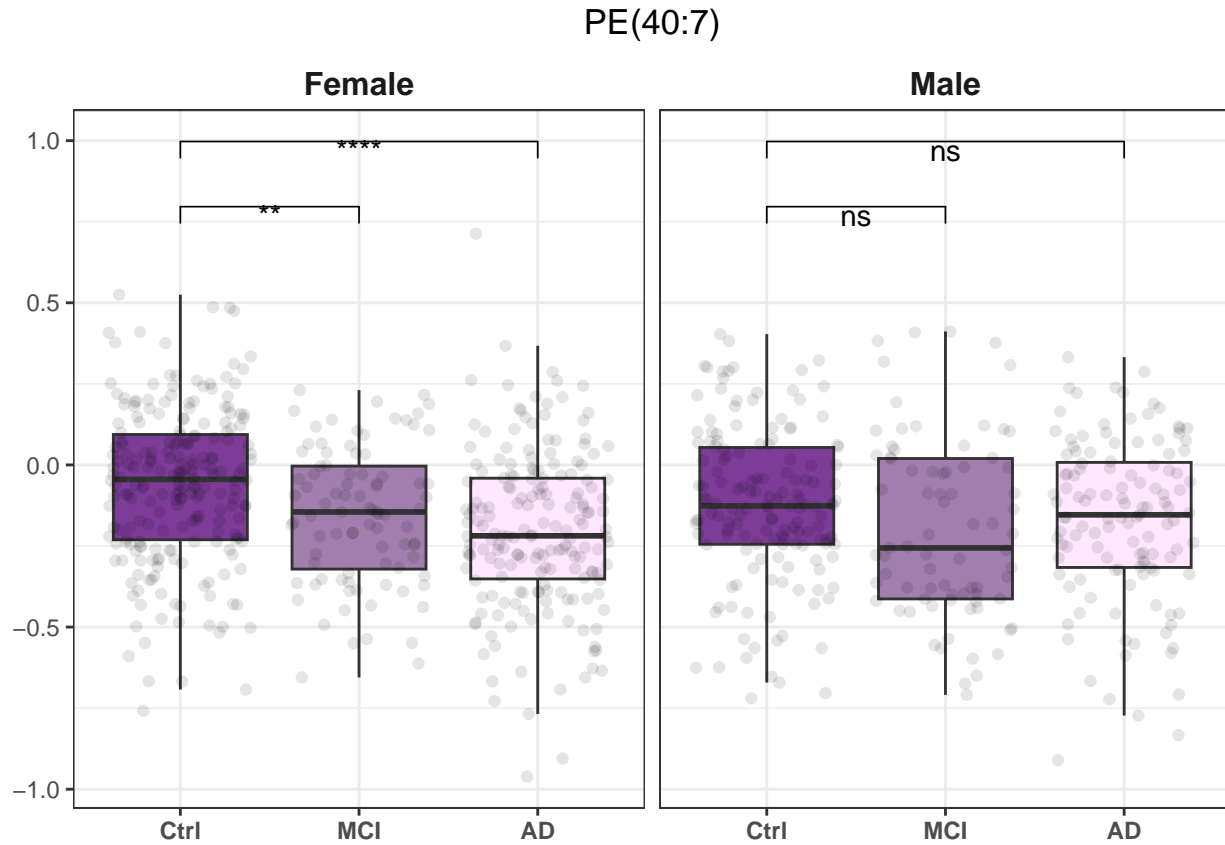
  ggplot(aes(x = Status, y = PE385, fill = Status))+
  geom_boxplot(outlier.shape = NA)+
  geom_jitter(alpha = 0.1)+
  scale_fill_manual(values = c("#7d3c98",
    "#a17ead",
    "#ffe8ff"))+

  facet_grid(. ~ Sex)+
  labs(title = "PE(40:7)")+
  stat_compare_means(comparisons = my_comparisons,
    method = "t.test",
    label = "p.signif",
    na.rm = TRUE,
    vjust = 1.25)+

  theme_bw()+
  theme(axis.title.x = element_blank(),
    axis.title.y = element_blank(),
    legend.position = "none",
    strip.background = element_blank(),
    strip.text = element_text(face = "bold", size = 12),
```

```
axis.text.x = element_text(face = "bold"),
plot.title = element_text(hjust = 0.5))
```

Box_PE



```
#Boxplot of TG(58:10)
Box_TG <- data %>%
  mutate(Status = factor(Status, levels = c("CTL", "MCI", "ADC"),
    labels = c("Ctrl", "MCI", "AD"))) %>%

  ggplot(aes(x = Status, y = TG5810, fill = Status))+
  geom_boxplot(outlier.shape = NA)+
  geom_jitter(alpha = 0.1)+
  scale_fill_manual(values = c("#c71b42",
    "#ff6678",
    "#ffa7b4"))+

  facet_grid(. ~ Sex)+
  labs(title = "TG(58:10)")+
  stat_compare_means(comparisons = my_comparisons,
    method = "t.test",
    label = "p.signif",
    na.rm = TRUE,
    vjust = 1.25)+

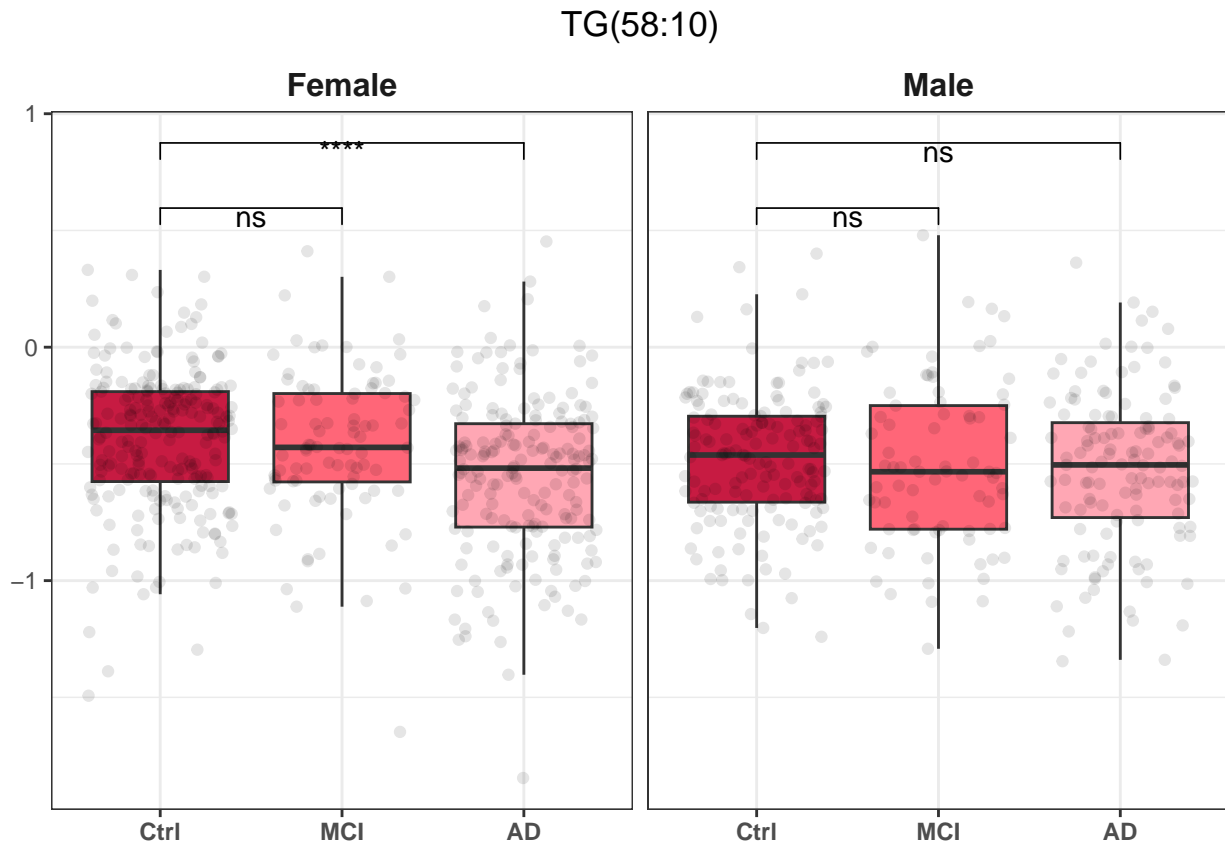
  theme_bw()+
  theme(axis.title.x = element_blank(),
    axis.title.y = element_blank(),
```

```

legend.position = "none",
strip.background = element_blank(),
strip.text = element_text(face = "bold", size = 12),
axis.text.x = element_text(face = "bold"),
plot.title = element_text(hjust = 0.5))

```

Box_TG



```
library(ggh4x)
```

```
## Warning: pakke 'ggh4x' blev bygget under R version 4.3.1
```

```

#Individual strip colors
tmp_strip <- strip_themed(background_x = elem_list_rect(fill = color_palette2[c("PC", "PE", "TG"))))

#Individual scales
tmp_scale <- list(
  scale_x_continuous(breaks = 0:10,
                     limits = c(0, 10)), #PC
  scale_x_continuous(breaks = 0:7,
                     limits = c(0, 7)), #PE
  scale_x_continuous(breaks = 0:11,
                     limits = c(0, 11))) #TG

```

```

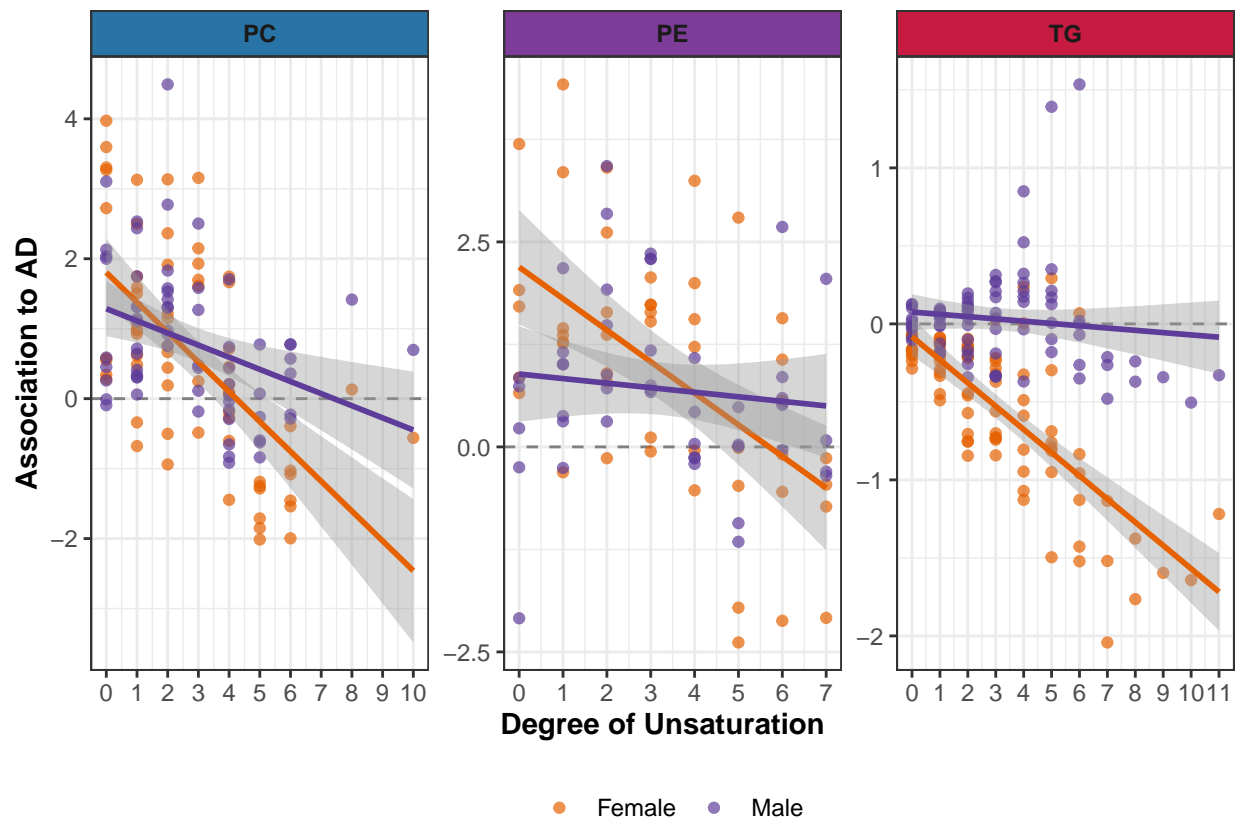
#subset and plot
Unsaturation_plot_B <- tibble(reg_data) %>%
  mutate(lipid_family = str_extract(Lipid, ".*(?=\\(\\))") %>%
    mutate(double_bonds = str_extract(Lipid, "\\d+\\(\\)") %>%
      mutate(double_bonds = as.numeric(str_extract(double_bonds, "\\d+")))) %>%
    mutate(Lipid = gsub("_A", "", Lipid)) %>%
    mutate(Lipid = gsub("_B", "", Lipid)) %>%
    filter(Outcome == "AD Female not Adjusted for APOE" |
      Outcome == "AD Male not Adjusted for APOE") %>%
    mutate(Outcome = gsub("AD Female not Adjusted for APOE", "Female", Outcome)) %>%
    mutate(Outcome = gsub("AD Male not Adjusted for APOE", "Male", Outcome)) %>%
    filter(lipid_family == "TG" |
      lipid_family == "PC" |
      lipid_family == "PE") %>%
    ggplot(aes(x = double_bonds, y = Estimate, color = Outcome))+
    geom_hline(yintercept = 0, colour = "#808080", linetype = "dashed")+
    geom_point(alpha = 0.7)+
    geom_smooth(method = lm, show.legend = FALSE)+
    scale_color_manual(values = c("#e66101", "#5e3c99"))+
    xlab("Degree of Unsaturation")+
    ylab("Association to AD")+
    facet_wrap2(~lipid_family, strip = tmp_strip, scales = "free")+
    faceted_pos_scales(x = tmp_scale)+
    theme_bw()+
    theme(legend.title = element_blank(),
      strip.text.x = element_text(face = "bold"),
      axis.title = element_text(face = "bold"),
      legend.position = "bottom")

# #Save figure
# pdf(here("figures/Unsaturation_plot_v1.0.pdf"), width = 8, height = 4)

Unsaturation_plot_B

```

```
## 'geom_smooth()' using formula = 'y ~ x'
```

```
# dev.off()

rm(tmp_strip, tmp_scale)

library(patchwork)

#Load Mediation plot
Mediation_plot <- readRDS(here("data/Mediation_plot_v1.4.rds"))

# # #Load dag plot
# Dag_plot <- readRDS(here("data/dag_plot_v1.0.rds"))

#Merge plots for Figure 3
# Figure3 <- (volcano_regression_A / Mediation_plot)+
#   plot_layout(heights = c(5, 1))

Figure3 <- (volcano_regression_A | ((Box_PC | Box_PE | Box_TG) /
  Unsaturation_plot_B))/Mediation_plot +
  plot_layout(heights = c(6, 1))

Figure3 <- Figure3 + plot_annotation(tag_levels = 'A')

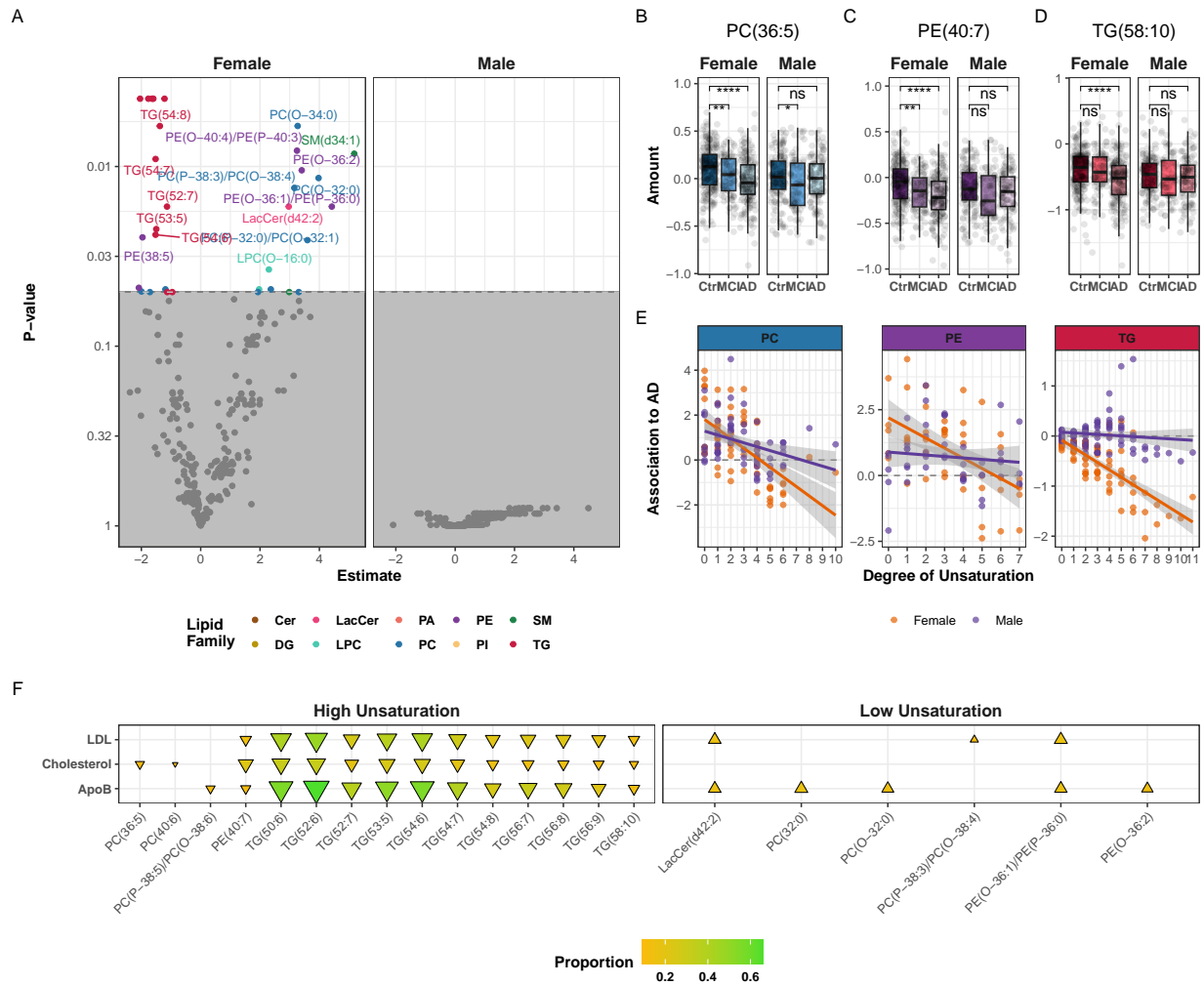
# #Save figure
# pdf(here("figures/Figue_3_unsaturated_plot_v1.6.pdf"),
#   width = 12, height = 10)
```

Figure3

```
## 'geom_smooth()' using formula = 'y ~ x'
```

```
## Warning: ggrepel: 252 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```

```
## Warning: ggrepel: 268 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```



```
# dev.off()
```

```
#rm(fig4_A_volcano, fig4_B_venn, fig4_C_barplot, Figure4)
```