SHIME Metabolomics Processing

Contents

1 Load Packages

```
library(xlsx)#handling Excel files
library(openxlsx)#handling Excel files without Java deps
library(readxl) #faster handling of excel files

library(plyr)
library(dplyr) #data wrangling (mapvalues)
suppressPackageStartupMessages(library(dplyr))#data wrangling
library(tidyr)#tidy data
library(reshape2)#tidy data
suppressPackageStartupMessages(library(reshape2))#for melt function

library(vegan)#for ecological calculations
library(ropls) #for OPLS-DA

library(ggplot2)
library(ggbiplot)
library(ggplotify)#for adequate plotting
library(scales)#for percent formatting
library(gtools)
library(ggsignif)
library(ggpubr)
library(ggfortify)
library(ggrepel)
```

```
library(cowplot)
library(gplots)#

library(RColorBrewer)
library(viridis)

dpi = 300
```

2 Load Data

```
Targ.Data <- readxl::read_excel("20210215_PAR_CleanedUp_Compoundname_ForR.xlsx",
                                sheet = "Sheet1")
Targ.Data <- as.data.frame(Targ.Data)

Targ.Data$Treatment <- as.factor(Targ.Data$Treatment)

Targ.Metadata <- Targ.Data[1:6]
colnames(Targ.Data)[5] <- "Timepoint"
colnames(Targ.Metadata)[5] <- "Timepoint"
Targ.Metadata$Donor <- Targ.Metadata$SHIME - 1
Targ.PAR <- Targ.Data[7:124]

colnames(Targ.Data)[122] <- "vaccenic_acid"
colnames(Targ.Data)[17] <- "aminoisobutyric_acid"
colnames(Targ.Data)[7] <- "L_Histidine"
colnames(Targ.Data)[12] <- "L_Homoserine"
colnames(Targ.Data)[15] <- "L_Glutamine"
colnames(Targ.Data)[13] <- "D_Aspartic_acid"
colnames(Targ.Data)[26] <- "Pyroglutamic_acid"
colnames(Targ.Data)[52] <- "Gluconic_acid"
colnames(Targ.Data)[35] <- "Indoleacetic_acid"
colnames(Targ.Data)[11] <- "Asparagine"
```

3 Load Compound List

```
Compounds <- readxl::read_excel("20210215_DetectedCompounds.xlsx",
                                sheet = "Sheet1")
Compounds <- as.data.frame(Compounds)
```

4 Sort PAR data by category

```
Targ.PAR.t <- as.data.frame(t(Targ.PAR))
Compounds.PAR <- cbind(Compounds,Targ.PAR.t) #merge Compound names with PAR data
Compounds.PAR <- Compounds.PAR[-2] #erase KEGG-ID's

#Amino Acids
```

```
Compounds.PAR.AAs <- dplyr::filter(Compounds.PAR,Class == "Amino Acid")

Compounds.PAR.Alcoholes <- dplyr::filter(Compounds.PAR,Class == "Alcoholes")

Compounds.PAR.Carb <- dplyr::filter(Compounds.PAR,Class == "Carbohydrates")

Compounds.PAR.Polyoles <- dplyr::filter(Compounds.PAR,Class == "Polyoles")

Compounds.PAR.MonoAcids <- dplyr::filter(Compounds.PAR,Class == "Monocarboxylic acids")

Compounds.PAR.MultiAcids <- dplyr::filter(Compounds.PAR,Class == "Multicarboxylic acids")

Compounds.PAR.Ketones <- dplyr::filter(Compounds.PAR,Class == "Ketones")

Compounds.PAR.Phenoles <- dplyr::filter(Compounds.PAR,Class == "Phenoles")

Compounds.PAR.Phenoles_MA <- dplyr::filter(Compounds.PAR,Class ==
                                           "Phenoles/Monocarboxylic acids")

Compounds.PAR.Polyoles_MA <- dplyr::filter(Compounds.PAR,Class ==
                                           "Polyoles/Monocarboxylic acids")

Compounds.PAR.Ketoacids <- dplyr::filter(Compounds.PAR,Class == "Ketoacids")

Compounds.PAR.Ester<- dplyr::filter(Compounds.PAR,Class == "Ester")

Compounds.PAR.Esters<- dplyr::filter(Compounds.PAR,Class == "Esters")

Compounds.PAR.Ether<- dplyr::filter(Compounds.PAR,Class == "Ethers")

Compounds.PAR.Imidazole <- dplyr::filter(Compounds.PAR,Class == "Imidazole")

Compounds.PAR.Amines <- dplyr::filter(Compounds.PAR,Class == "Amines")

Compounds.PAR.Alkenes <- dplyr::filter(Compounds.PAR,Class == "Alkene")

Compounds.PAR.OtherN<- dplyr::filter(Compounds.PAR,Class == "Other N-compounds")

Compounds.PAR.Aldehydes <- dplyr::filter(Compounds.PAR,Class == "Aldehydes")

Compounds.PAR.HOAcids <- dplyr::filter(Compounds.PAR,Class == "Hydroxylic acids")

Compounds.PAR.disulfides <- dplyr::filter(Compounds.PAR,Class == "Disulfides")

Compounds.PAR.Phosphates <- dplyr::filter(Compounds.PAR,Class == "Phosphates")

Compounds.PAR.BA  <- dplyr::filter(Compounds.PAR,Class == "Bile acids")

NComp = nrow(Compounds.PAR.AAs) + nrow(Compounds.PAR.Carb) + nrow(Compounds.PAR.Polyoles)+
  nrow(Compounds.PAR.MonoAcids)+ nrow(Compounds.PAR.Ketones)+ nrow(Compounds.PAR.Phenoles)+
  nrow(Compounds.PAR.Phenoles_MA)+ nrow(Compounds.PAR.Polyoles_MA)+
  nrow(Compounds.PAR.Ketoacids)+ nrow(Compounds.PAR.Ester)+ nrow(Compounds.PAR.Imidazole)+
  nrow(Compounds.PAR.Amines)+ nrow(Compounds.PAR.Alkenes)+ nrow(Compounds.PAR.OtherN) +
```

```
  nrow(Compounds.PAR.Aldehydes) + nrow(Compounds.PAR.HOAcids) +
  nrow(Compounds.PAR.MultiAcids)+nrow(Compounds.PAR.Ether)+nrow(Compounds.PAR.Alcoholes)+
  nrow(Compounds.PAR.disulfides) + nrow(Compounds.PAR.Esters) + nrow(Compounds.PAR.BA)
```

5 Data Exploration: Boxplots

```
Targ.PAR.gather <- tidyr::gather(Targ.PAR,key = "Metabolite", value = "Conc")

Box_targ.data <- ggplot(Targ.PAR.gather, aes(x = Metabolite, y = Conc)) +
  geom_boxplot(fill = "darkgreen") +scale_y_log10()
Box_targ.data
```

6 Boxplots differential compounds

```
Targ.data.P80_0.05 <- dplyr::filter(Targ.Data, Treatment %in% c("Control - 0",
                                                                "P80 - 0,05"))

Box_targ.data_vacc_P0.05 <- ggplot(Targ.data.P80_0.05, aes(x=Treatment, y=vaccenic_acid,
                                                           fill = Emulsifier)) +
  geom_boxplot() + scale_y_log10() + ylab("Vaccenic acid (p = 0.00695)")
Box_targ.data_vacc_P0.05
```

```
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/P80_0.05_Obesity.tiff",
     width=6*dpi,height=6*dpi,res=dpi,compression="lzw")
Box_targ.data_vacc_P0.05
dev.off()

Box_targ.data_Asp_P0.05 <- ggplot(Targ.data.P80_0.05, aes(x=Treatment, y=Asparagine,
                                                          fill = Emulsifier)) +
  geom_boxplot() + scale_y_log10() + ylab("L-Asparagine (p = 0.0727)")+
  theme(legend.position = "none")
Box_targ.data_Asp_P0.05
```

```
Box_targ.data_ino_P0.05 <- ggplot(Targ.data.P80_0.05, aes(x=Treatment, y=inosine,
                                                          fill = Emulsifier)) +
  geom_boxplot() + scale_y_log10() + ylab("Inosine (p = 0.127)")
Box_targ.data_ino_P0.05
```

```
Gout_legend <- get_legend(Box_targ.data_ino_P0.05)
Box_targ.data_ino_P0.05 <-  Box_targ.data_ino_P0.05 + theme(legend.position = "none")

Goutfigure <- ggdraw() + draw_plot(Box_targ.data_Asp_P0.05,0,0,0.5,1) +
  draw_plot(Box_targ.data_ino_P0.05,0.5,0,0.5,1)

tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/P80_0.05_Gout.tiff",
     width=10*dpi,height=6*dpi,res=dpi,compression="lzw")
      # plot_grid(titledeseq,deseqgenusotuselect1,ncol=2,rel_widths=c(0.02,0.98))
plot_grid(Goutfigure,Gout_legend,ncol=1, rel_heights = c(0.90,0.1))
dev.off()
```

```
Box_targ.data_indo_P0.05 <- ggplot(Targ.data.P80_0.05,aes(x=Treatment, y=Indoleacetic_acid,
                                                          fill = Emulsifier)) +
  geom_boxplot() + scale_y_log10() +
      ylab("Indoleacetic acid (p = 0.0131)")+ theme(legend.position = "none")
Box_targ.data_indo_P0.05
```

```
Box_targ.data_Benz_P0.05 <- ggplot(Targ.data.P80_0.05,
                                   aes(x=Treatment, y=benzoic_acid, fill=Emulsifier))+
  geom_boxplot() + scale_y_log10() +
      ylab("Benzoic acid (p = 0.0798)")+ theme(legend.position = "none")
Box_targ.data_Benz_P0.05
```

```
Box_targ.data_Glyc_P0.05 <- ggplot(Targ.data.P80_0.05,
                                   aes(x=Treatment, y=glycerol, fill=Emulsifier)) +
  geom_boxplot() + scale_y_log10() +
      ylab("GLycerol (p = 0.177)")
Box_targ.data_Glyc_P0.05
```

```
Gout_legend <- get_legend(Box_targ.data_ino_P0.05)
Box_targ.data_Glyc_P0.05 <-  Box_targ.data_Glyc_P0.05 + theme(legend.position = "none")

Cryptofigure <- ggdraw() + draw_plot(Box_targ.data_indo_P0.05,0,0,0.33,1) +
  draw_plot(Box_targ.data_Benz_P0.05,0.33,0,0.33,1) +
        draw_plot(Box_targ.data_Glyc_P0.05,0.66,0,0.33,1)

tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/P80_0.05_Cryptosporium.tiff",
     width=16*dpi,height=7*dpi,res=dpi,compression="lzw")
      # plot_grid(titledeseq,deseqgenusotuselect1,ncol=2,rel_widths=c(0.02,0.98))
plot_grid(Cryptofigure,Gout_legend,ncol=1, rel_heights = c(0.90,0.1))
dev.off()


Box_targ.data_indo_P0.05 <- ggplot(Targ.data.P80_0.05,
                                   aes(x=Treatment, y=Indoleacetic_acid, fill=Emulsifier))+
  geom_boxplot() + scale_y_log10() + ylab("Indoleacetic acid (p = 0.131)")
Box_targ.data_indo_P0.05
```

```
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/P80_0.05_Enth_R_Arthritis.tiff",
     width=6*dpi,height=6*dpi,res=dpi,compression="lzw")
      # plot_grid(titledeseq,deseqgenusotuselect1,ncol=2,rel_widths=c(0.02,0.98))
Box_targ.data_indo_P0.05
dev.off()

#-------------------------------------------------------------------------------------------#

Targ.data.P80_0.5 <- dplyr::filter(Targ.Data, Treatment %in% c("Control - 0","P80 - 0,5"))

Box_targ.data_vacc_P0.5 <- ggplot(Targ.data.P80_0.5,
                                  aes(x=Treatment, y=vaccenic_acid, fill=Emulsifier)) +
  geom_boxplot() + scale_y_log10() +
      ylab("Vaccenic acid (p = 0.0368)")
Box_targ.data_vacc_P0.5
```

```
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/P80_0.5_Obesity.tiff",
     width=6*dpi,height=6*dpi,res=dpi,compression="lzw")
Box_targ.data_vacc_P0.5
dev.off()

Box_targ.data_indo_P0.5 <- ggplot(Targ.data.P80_0.5,aes(x=Treatment, y=Indoleacetic_acid,
                                                        fill = Emulsifier)) +
  geom_boxplot() + scale_y_log10() + ylab("Indoleacetic acid (p = 0.016)")
Box_targ.data_indo_P0.5
```

```
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/P80_0.5_Enth_R_Arthritis.tiff",
     width=6*dpi,height=6*dpi,res=dpi,compression="lzw")
      # plot_grid(titledeseq,deseqgenusotuselect1,ncol=2,rel_widths=c(0.02,0.98))
Box_targ.data_indo_P0.5
dev.off()

Box_targ.data_indo_P0.5 <- ggplot(Targ.data.P80_0.5,aes(x=Treatment, y=Indoleacetic_acid,
                                                        fill = Emulsifier)) +
  geom_boxplot() + scale_y_log10() +
      ylab("Indoleacetic acid (p = 0.016)")+ theme(legend.position = "none")
Box_targ.data_indo_P0.5
```

```
Box_targ.data_pyro_P0.5 <- ggplot(Targ.data.P80_0.5,aes(x=Treatment, y=Pyroglutamic_acid,
                                                        fill = Emulsifier)) +
  geom_boxplot() + scale_y_log10() + ylab("L-Pyroglutamic acid (p = 0.365)")+
  theme(legend.position = "none")
Box_targ.data_pyro_P0.5
```

```
Box_targ.data_Glyc_P0.5 <- ggplot(Targ.data.P80_0.5,
                                  aes(x = Treatment, y = glycerol, fill = Emulsifier)) +
  geom_boxplot() + scale_y_log10() + ylab("GLycerol (p = 0.565)")+
  theme(legend.position = "none")
Box_targ.data_Glyc_P0.5
```

```
Box_targ.data_Benz_P0.5 <- ggplot(Targ.data.P80_0.5, aes(x=Treatment, y=benzoic_acid,
                                                         fill = Emulsifier)) +
  geom_boxplot() + scale_y_log10() +
      ylab("Benzoic acid (p = 0.594)")+ theme(legend.position = "none")
Box_targ.data_Benz_P0.5
```

```
Box_targ.data_gluc_P0.5 <- ggplot(Targ.data.P80_0.5,
                                  aes(x=Treatment, y=Gluconic_acid, fill = Emulsifier)) +
  geom_boxplot() + scale_y_log10() +
      ylab("D-GLuconic acid (p = 0.737)")
Box_targ.data_gluc_P0.5
```

```
Cryptosporidium_legend <- get_legend(Box_targ.data_gluc_P0.5)
Box_targ.data_gluc_P0.5 <-  Box_targ.data_gluc_P0.5 + theme(legend.position = "none")

Cryptofigure <- ggdraw() + draw_plot(Box_targ.data_indo_P0.5,0,0.5,0.33,0.5) +
  draw_plot(Box_targ.data_pyro_P0.5,0.33,0.5,0.33,0.5) +
        draw_plot(Box_targ.data_Glyc_P0.5,0.66,0.5,0.33,0.5) +
  draw_plot(Box_targ.data_Benz_P0.5,0,0,0.33,0.5) +
        draw_plot(Box_targ.data_gluc_P0.5,0.33,0,0.33,0.5)
```

```
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/P80_0.5_Cryptosporium.tiff",
     width=15*dpi,height=10*dpi,res=dpi,compression="lzw")
      # plot_grid(titledeseq,deseqgenusotuselect1,ncol=2,rel_widths=c(0.02,0.98))
plot_grid(Cryptofigure,Cryptosporidium_legend,ncol=1, rel_heights = c(0.90,0.1))
dev.off()
#-------------------------------------------------------------------------------------#

Targ.data.RL_0.05 <- dplyr::filter(Targ.Data, Treatment %in% c("Control - 0","RL - 0,05"))

Box_targ.data_indo_R0.05 <- ggplot(Targ.data.RL_0.05,
                                   aes(x=Treatment, y=Indoleacetic_acid, fill = Emulsifier))+
  geom_boxplot() + scale_y_log10() + ylab("Indoleacetic acid (p = 0.01)")
Box_targ.data_indo_R0.05
```

```
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/RL_0.05_Enth_R_Arthritis.tiff",
     width=6*dpi,height=6*dpi,res=dpi,compression="lzw")
      # plot_grid(titledeseq,deseqgenusotuselect1,ncol=2,rel_widths=c(0.02,0.98))
Box_targ.data_indo_R0.05
dev.off()

Box_targ.data_vacc_R0.05 <- ggplot(Targ.data.RL_0.05,
                                   aes(x=Treatment, y=vaccenic_acid, fill = Emulsifier))+
  geom_boxplot() + scale_y_log10() + ylab("Vaccenic acid (p = 0.068)")
Box_targ.data_vacc_R0.05
```

```
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/RL_0.05_Obesity.tiff",
     width=6*dpi,height=6*dpi,res=dpi,compression="lzw")
      # plot_grid(titledeseq,deseqgenusotuselect1,ncol=2,rel_widths=c(0.02,0.98))
Box_targ.data_vacc_R0.05
dev.off()

Box_targ.data_Eth_R0.05 <- ggplot(Targ.data.RL_0.05,
                                  aes(x=Treatment, y=ethylbenzene, fill = Emulsifier))+
  geom_boxplot() + scale_y_log10() + ylab("Ethylbenzene (p = 0.0145)")+
  theme(legend.position = "none")
Box_targ.data_Eth_R0.05
```

```
Box_targ.data_Asp_R0.05 <- ggplot(Targ.data.RL_0.05,
                                  aes(x=Treatment, y=cyclohexanecarboxylic_acid,
                                  fill = Emulsifier)) +
  geom_boxplot() + scale_y_log10() + ylab("Cyclohexanecarboxolic acid (p = 0.411)") +
  theme(legend.position = "none")
Box_targ.data_Asp_R0.05
```

```
Box_targ.data_Val_R0.05 <- ggplot(Targ.data.RL_0.05,
                                  aes(x=Treatment, y=valeric_acid, fill = Emulsifier))+
  geom_boxplot() + scale_y_log10() + ylab("Valeric acid (p = 0.669)")
Box_targ.data_Val_R0.05
```

```
Diarhea_legend <- get_legend(Box_targ.data_Val_R0.05)
Box_targ.data_Val_R0.05 <-  Box_targ.data_Val_R0.05 + theme(legend.position = "none")

Diarheafigure <- ggdraw() + draw_plot(Box_targ.data_Eth_R0.05,0,0,0.33,1) +
  draw_plot(Box_targ.data_Asp_R0.05,0.33,0,0.33,1) +
```

```
  draw_plot(Box_targ.data_Val_R0.05,0.66,0,0.33,1)

tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/RL_0.05_Diarhea.tiff",
     width=16*dpi,height=7*dpi,res=dpi,compression="lzw")
      # plot_grid(titledeseq,deseqgenusotuselect1,ncol=2,rel_widths=c(0.02,0.98))
plot_grid(Diarheafigure,Diarhea_legend,ncol=1, rel_heights = c(0.90,0.1))
dev.off()

Box_targ.data_ino_R0.05 <- ggplot(Targ.data.RL_0.05,
                                  aes(x = Treatment, y = inosine, fill = Emulsifier)) +
  geom_boxplot() + scale_y_log10() +
      ylab("Inosine (p = 0.126)")+ theme(legend.position = "none")
Box_targ.data_ino_R0.05
```

```
Box_targ.data_Asp_R0.05 <- ggplot(Targ.data.RL_0.05,
                                  aes(x = Treatment, y = Asparagine, fill = Emulsifier))+
  geom_boxplot() + scale_y_log10() + ylab("Asparagine (p = 0.579)")
Box_targ.data_Asp_R0.05
```

```
Diarhea_legend <- get_legend(Box_targ.data_Asp_R0.05)
Box_targ.data_Asp_R0.05 <-  Box_targ.data_Asp_R0.05 + theme(legend.position = "none")

Goutfigure <- ggdraw() + draw_plot(Box_targ.data_ino_R0.05,0,0,0.5,1) +
  draw_plot(Box_targ.data_Asp_R0.05,0.5,0,0.5,1)

tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/RL_0.05_Gout.tiff",
     width=10*dpi,height=6*dpi,res=dpi,compression="lzw")
      # plot_grid(titledeseq,deseqgenusotuselect1,ncol=2,rel_widths=c(0.02,0.98))
plot_grid(Goutfigure,Diarhea_legend,ncol=1, rel_heights = c(0.90,0.1))
dev.off()


Targ.data.RL_0.5 <- dplyr::filter(Targ.Data, Treatment %in% c("Control - 0","RL - 0,5"))

Box_targ.data_vacc_R0.5 <- ggplot(Targ.data.RL_0.5,
                                  aes(x=Treatment, y=vaccenic_acid, fill=Emulsifier))+
  geom_boxplot() + scale_y_log10() +
      ylab("Vaccenic acid (p = 0.0348)")
Box_targ.data_vacc_R0.5
```

```
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/RL_0.5_Obesity.tiff",
     width=6*dpi,height=6*dpi,res=dpi,compression="lzw")
      # plot_grid(titledeseq,deseqgenusotuselect1,ncol=2,rel_widths=c(0.02,0.98))
Box_targ.data_vacc_R0.5
dev.off()

Box_targ.data_eth_R0.5 <- ggplot(Targ.data.RL_0.5,
                                 aes(x=Treatment, y=ethylbenzene, fill=Emulsifier)) +
  geom_boxplot() + scale_y_log10() + ylab("Ethylbenzene (p = 0.00134)")
Box_targ.data_eth_R0.5
```

```
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/RL_0.5_IBS.tiff",
     width=6*dpi,height=6*dpi,res=dpi,compression="lzw")
      # plot_grid(titledeseq,deseqgenusotuselect1,ncol=2,rel_widths=c(0.02,0.98))
```

```
Box_targ.data_eth_R0.5
dev.off()

Box_targ.data_pyro_R0.5 <- ggplot(Targ.data.RL_0.5,
                                  aes(x=Treatment, y=Pyroglutamic_acid, fill=Emulsifier)) +
  geom_boxplot() + scale_y_log10() + ylab("L-Pyroglutamic acid (p = 0.00381)")+
  theme(legend.position = "none")
Box_targ.data_pyro_R0.5
```

```
Box_targ.data_gluc_R0.5 <- ggplot(Targ.data.RL_0.5,
                                  aes(x=Treatment, y=Gluconic_acid, fill=Emulsifier)) +
  geom_boxplot() + scale_y_log10() + ylab("D-GLuconic acid (p = 0.0712)")+
  theme(legend.position = "none")
Box_targ.data_gluc_R0.5
```

```
Box_targ.data_indo_R0.5 <- ggplot(Targ.data.RL_0.5,
                                  aes(x=Treatment, y=Indoleacetic_acid, fill=Emulsifier))+
  geom_boxplot() + scale_y_log10() + ylab("3-indoleacetic acid (p = 0.0964)")
Box_targ.data_indo_R0.5
```

```
Diarhea_legend <- get_legend(Box_targ.data_indo_R0.5)
Box_targ.data_indo_R0.5 <-  Box_targ.data_indo_R0.5 + theme(legend.position = "none")

Cryptosporiumfigure <- ggdraw() + draw_plot(Box_targ.data_pyro_R0.5,0,0,0.33,1) +
  draw_plot(Box_targ.data_gluc_R0.5,0.33,0,0.33,1) +
  draw_plot(Box_targ.data_indo_R0.5,0.66,0,0.33,1)

tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/RL_0.5_Cryptosporidium.tiff",
     width=11*dpi,height=5*dpi,res=dpi,compression="lzw")
      # plot_grid(titledeseq,deseqgenusotuselect1,ncol=2,rel_widths=c(0.02,0.98))
plot_grid(Cryptosporiumfigure,Diarhea_legend,ncol=1, rel_heights = c(0.90,0.1))
dev.off()
```

7 Data Normalisation

```
#log-transformation
Targ.PAR.log <- log10(Targ.PAR+0.01)
Targ.PAR.log.gather <- tidyr::gather(Targ.PAR.log,key = "Metabolite", value = "Conc")
Box_targ.data.log <- ggplot(Targ.PAR.log.gather, aes(x = Metabolite, y = Conc)) +
  geom_boxplot(fill = "darkgreen")
Box_targ.data.log
```

```
# Pareto scaling
# Targ.PAR.log.pareto <- as.data.frame(t(scaling(as.data.frame(t(Targ.PAR.log)),
# type = "pareto")))
# Targ.PAR.log.pareto.gather <- tidyr::gather(Targ.PAR.log.pareto,key = "Metabolite",
# value = "Conc")
#
# Box_targ.data.log.pareto <- ggplot(Targ.PAR.log.pareto.gather, aes(x = Metabolite,
# y = Conc)) + geom_boxplot(fill = "darkgreen")
# Box_targ.data.log.pareto
```

```
Targ.PAR.colmeans <- colMeans(Targ.PAR.log)
Targ.PAR.colsds <- sapply(Targ.PAR.log[1:71,], sd)

Targ.PAR.log.pareto <- sweep(Targ.PAR.log,2,Targ.PAR.colmeans,"-")
Targ.PAR.log.pareto <- sweep(Targ.PAR.log.pareto,2,sqrt(Targ.PAR.colsds),"/")

Targ.PAR.log.pareto.gather <- tidyr::gather(Targ.PAR.log.pareto,key = "Metabolite",
                                            value = "Conc")

Box_targ.data.log.pareto <- ggplot(Targ.PAR.log.pareto.gather,
                                   aes(x = Metabolite, y = Conc)) +
  geom_boxplot(fill = "darkgreen")
Box_targ.data.log.pareto
```

8 PCA

```
#PCA raw data
PCA_PAR <- prcomp(Targ.PAR,center = TRUE)

plot(PCA_PAR)
```

```
biplot(PCA_PAR, Y=NULL, plot.axes = c(1,2), dir.axis1=1,dir.axis2=1, rn=NULL, main=NULL)
```

```
summary(PCA_PAR)

#plot using ggplot
PCA.PAR.data <- as.data.frame(PCA_PAR$x)[1:2]

PCA.PAR.merge <- cbind(PCA.PAR.data,Targ.Metadata)

PCA.PAR.plot1 <- ggplot(PCA.PAR.merge, aes(x = PC1, y = PC2, color = as.factor(Treatment),
                                           shape = as.factor(SHIME), size = 6)) +
  geom_point() + theme_bw() + labs(color = "Location",shape ="Donor")+
  xlab("PC1 (65,29%)") + ylab("PC2 (26,99%)")+
  geom_text(data = PCA.PAR.merge,
            aes(x= PC1, y = PC2,  label=Timepoint, hjust=1*(1-sign(PC1)),
                vjust=1*(1-sign(PC2)))) +
  # scale_color_viridis(option="A",discrete = TRUE)+
  # scale_color_manual(values = c("#754ba6","#faed75","mediumblue", "orange", "yellow",
  # Grey1", "Grey2", "Black"))+
  scale_color_manual(values = c("Black","#999999", "#d28eed", "#844da8" ,"#74dff7",
                                "#125087","#f5e2a2", "#e3d029", "#F781BF","#f2b385",
                                "#d97914"))+
  theme(axis.text.x = element_text(size = 10,family='sans',angle=0),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        # legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        # legend.position = "bottom",
```

```
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())
  # theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
PCA.PAR.plot1
```

```
#PCA log transformed data
PCA_PAR.log <- prcomp(Targ.PAR.log,center = TRUE)

plot(PCA_PAR.log)
```

```
biplot(PCA_PAR.log, Y=NULL,plot.axes = c(1,2),dir.axis1=1,dir.axis2=1,rn=NULL, main=NULL)
```

```
summary(PCA_PAR.log)

#plot using ggplot
PCA.PAR.log.data <- as.data.frame(PCA_PAR.log$x)[1:2]

PCA.PAR.log.merge <- cbind(PCA.PAR.log.data,Targ.Metadata)

PCA.PAR.log.plot1 <- ggplot(PCA.PAR.log.merge,
                            aes(x = PC1, y = PC2, color = as.factor(Treatment),
                                shape = as.factor(SHIME),size = 6)) +
  geom_point() + theme_bw() + labs(color = "Location",shape ="Donor")+
  xlab("PC1 (29,68%)") + ylab("PC2 (11,86%)")+
  # scale_color_viridis(option="A",discrete = TRUE)+
  # scale_color_manual(values = c("#754ba6","#faed75","mediumblue", "orange", "yellow",
  # "Grey1", "Grey2", "Black"))+
  scale_color_manual(values = c("Black","#999999", "#d28eed", "#844da8" ,"#74dff7",
                                "#125087","#f5e2a2", "#e3d029", "#F781BF","#f2b385",
                                "#d97914"))+
  theme(axis.text.x = element_text(size = 10,family='sans',angle=0),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        # legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        # legend.position = "bottom",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())
  # theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
PCA.PAR.log.plot1
```

```
#PCA log transformed and pareto scaled data
Nan <- unlist (lapply (Targ.PAR.log.pareto, function (x) which (is.na (x))))

Targ.PAR.log.pareto <- Targ.PAR.log.pareto[-c(15,51,87)]
PCA_PAR.log.pareto <- prcomp(Targ.PAR.log.pareto, center = TRUE)

# plot(PCA_PAR.log.pareto)
# biplot(PCA_PAR.log.pareto, Y=NULL, plot.axes = c(1,2), dir.axis1=1,
#        dir.axis2=1, rn=NULL, main=NULL)
```

```
# summary(PCA_PAR.log.pareto)

#plot using ggplot
PCA.PAR.log.pareto.data <- as.data.frame(PCA_PAR.log.pareto$x)[1:2]

PCA.PAR.log.pareto.merge <- cbind(PCA.PAR.log.pareto.data,Targ.Metadata)

PCA.PAR.log.pareto.plot1 <- ggplot(PCA.PAR.log.pareto.merge,
                                   aes(x = PC1, y = PC2, color = as.factor(Treatment),
                                       shape = as.factor(SHIME),size = 6)) +
  geom_point() + theme_bw() + labs(color = "Treatment",shape ="Donor",
                                   size = "Timepoint (Day)")+ xlab("PC1 (22.41%)") +
  ylab("PC2 (11.77%)")+ geom_text(data = PCA.PAR.log.pareto.merge,
                                  aes(x= PC1, y = PC2, label=Timepoint,
                                      hjust=1*(1-sign(PC1)),vjust=1*(1-sign(PC2)))) +
  # scale_color_viridis(option="A",discrete = TRUE)+
  # scale_color_manual(values = c("#754ba6","#faed75","mediumblue", "orange",
  # "yellow","Grey1", "Grey2", "Black"))+
  scale_color_manual(values = c("Black","#999999", "#d28eed", "#844da8" ,"#74dff7",
                                "#125087","#f5e2a2", "#e3d029", "#F781BF","#f2b385",
                                "#d97914"))+
  theme(axis.text.x = element_text(size = 10,family='sans',angle=0),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        # legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        # legend.position = "bottom",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())
  # theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
PCA.PAR.log.pareto.plot1
```

```
PCA.PAR.log.pareto.plot2 <- ggplot(PCA.PAR.log.pareto.merge,
                                   aes(x = PC1, y = PC2, color = as.factor (Timepoint))) +
  geom_point() + theme_bw() + labs(color = "Timepoint (Day)")+ xlab("PC1 (22.41%)") +
  ylab("PC2 (11.77%)")+
  # scale_color_viridis(option="A",discrete = TRUE)+
  # scale_color_manual(values = c("#754ba6","#faed75","mediumblue", "orange", "yellow",
  # "Grey1", "Grey2", "Black"))+
  scale_color_manual(values = c("Black","#999999", "#d28eed", "#844da8" ,"#74dff7",
                                "#125087","#f5e2a2", "#e3d029", "#F781BF","#f2b385",
                                "#d97914"))+
  stat_ellipse(level = 0.95)+
  theme(axis.text.x = element_text(size = 10,family='sans',angle=0),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        # legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        # legend.position = "bottom",
```

```
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())
  # theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
PCA.PAR.log.pareto.plot2
```

```
PCA.PAR.log.pareto.plot3 <- ggplot(PCA.PAR.log.pareto.merge,
                                   aes(x = PC1, y = PC2, color = as.factor (SHIME))) +
  geom_point(size = 3) + theme_bw() + labs(color = "Donor")+ xlab("PC1 (22.41%)") +
  ylab("PC2 (11.77%)")+
  # scale_color_viridis(option="A",discrete = TRUE)+
  # scale_color_manual(values = c("#754ba6","#faed75","mediumblue", "orange", "yellow",
  # "Grey1", "Grey2", "Black"))+
  scale_color_manual(values = c("#844da8" ,"#74dff7", "#125087","#f5e2a2", "#e3d029",
                                "#F781BF","#f2b385", "#d97914"))+
  stat_ellipse(level = 0.95)+
  theme(axis.text.x = element_text(size = 10,family='sans',angle=0),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        # legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        # legend.position = "bottom",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())
  # theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
PCA.PAR.log.pareto.plot3
```

```
PCA.PAR.log.pareto.plot4 <- ggplot(PCA.PAR.log.pareto.merge,
                                   aes(x = PC1, y = PC2, color = as.factor(Treatment))) +
  geom_point(size = 3) + theme_bw() + labs(color = "Treatment")+ xlab("PC1 (22.41%)") +
  ylab("PC2 (11.77%)")+
  # scale_color_viridis(option="A",discrete = TRUE)+
  # scale_color_manual(values = c("#754ba6","#faed75","mediumblue", "orange", "yellow",
  # "Grey1", "Grey2", "Black"))+
  scale_color_manual(values = c("Black","#999999", "#d28eed", "#844da8" ,"#74dff7",
                                "#125087","#f5e2a2", "#e3d029", "#F781BF","#f2b385",
                                "#d97914"))+
  stat_ellipse(level = 0.95)+
  theme(axis.text.x = element_text(size = 10,family='sans',angle=0),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        # legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        # legend.position = "bottom",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())
  # theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
PCA.PAR.log.pareto.plot4
```

```
dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA1.tiff",
     width=7*dpi,height=6*dpi,res=dpi,compression="lzw")
PCA.PAR.log.pareto.plot1
dev.off()

png("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA1.png",
    res = 300, width = 3000, height = 2500)
PCA.PAR.log.pareto.plot1
dev.off()


dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA2.tiff",
     width=7*dpi,height=6*dpi,res=dpi,compression="lzw")
PCA.PAR.log.pareto.plot2
dev.off()

png("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA2.png",
    res = 300, width = 3000, height = 2500)
PCA.PAR.log.pareto.plot2
dev.off()

dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA3.tiff",
     width=7*dpi,height=6*dpi,res=dpi,compression="lzw")
PCA.PAR.log.pareto.plot3
dev.off()

png("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA3.png",
    res = 300, width = 3000, height = 2500)
PCA.PAR.log.pareto.plot3
dev.off()


dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA4.tiff",
     width=7*dpi,height=6*dpi,res=dpi,compression="lzw")
PCA.PAR.log.pareto.plot4
dev.off()

png("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA4.png",
    res = 300, width = 3000, height = 2500)
PCA.PAR.log.pareto.plot4
dev.off()
```

9 PCA RL - P80

```
#PCA log transformed and pareto scaled data
Nan <- unlist (lapply (Targ.PAR.log.pareto, function (x) which (is.na (x))))
Targ.PAR.log.pareto <- Targ.PAR.log.pareto[-c(15,51,87)] #removal of Nan columns
```

```
Targ.PAR.log.pareto.meta <- cbind(Targ.PAR.log.pareto,Targ.Metadata)
Targ.PAR.log.pareto.meta.RL.P80 <- dplyr::filter(Targ.PAR.log.pareto.meta,
                                                 Emulsifier %in%
                                                   c("Fecal","Control","P80","RL"))
Targ.PAR.log.pareto.RL.P80 <-Targ.PAR.log.pareto.meta.RL.P80[1:111]


PCA_PAR.log.pareto.RL.P80  <- prcomp(Targ.PAR.log.pareto.RL.P80 , center = TRUE)

# plot(PCA_PAR.log.pareto.RL.P80 )
# biplot(PCA_PAR.log.pareto.RL.P80 , Y=NULL, plot.axes = c(1,2), dir.axis1=1,
#        dir.axis2=1, rn=NULL, main=NULL)
# summary(PCA_PAR.log.pareto.RL.P80)

#plot using ggplot
PCA.PAR.log.pareto.data.RL.P80  <- as.data.frame(PCA_PAR.log.pareto.RL.P80 $x)[1:2]
PCA.PAR.log.pareto.merge.RL.P80  <- cbind(PCA.PAR.log.pareto.data.RL.P80,
                                          Targ.PAR.log.pareto.meta.RL.P80[111:119])

PCA.PAR.log.pareto.RL.P80.plot1 <- ggplot(PCA.PAR.log.pareto.merge.RL.P80,
                                          aes(x=PC1, y=PC2, color = as.factor(Treatment),
                                              shape = as.factor(Donor),size = 6)) +
  geom_point() + theme_bw() +
  labs(color = "Treatment",shape ="Donor", size = "Timepoint (Day)")+ xlab("PC1 (20,16%)")+
  ylab("PC2 (14,22%)")+
  geom_text(data = PCA.PAR.log.pareto.merge.RL.P80,
            aes(x= PC1, y = PC2,label=Timepoint, hjust=1*(1-sign(PC1)),
               vjust=1*(1-sign(PC2)))) +
  # scale_color_viridis(option="A",discrete = TRUE)+
  #scale_color_manual(values = c("#754ba6","#faed75","mediumblue", "orange", "yellow",
  #                              "Grey1", "Grey2", "Black"))+
  scale_color_manual(values = c("Black","#d28eed", "#844da8" ,"#74dff7", "#125087",
                                "#f5e2a2", "#e3d029", "#F781BF","#f2b385", "#d97914"))+
  theme(axis.text.x = element_text(size = 10,family='sans',angle=0),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =16,family='sans',angle=0),
        axis.title.y = element_text(size=16,family='sans',angle=90),
        legend.text = element_text(size=14,family='sans',angle=0),
        legend.title = element_text(size = 16),
        # legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        # legend.position = "bottom",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())
  # theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
PCA.PAR.log.pareto.RL.P80.plot1
```

```
PCA.PAR.log.pareto.RL.P80.plot2 <- ggplot(PCA.PAR.log.pareto.merge.RL.P80,
                                          aes(x=PC1, y=PC2, color = as.factor (Timepoint)))+
  geom_point() + theme_bw() + labs(color = "Timepoint (Day)")+
  xlab("PC1 (20,16%)") + ylab("PC2 (14,22%)")+
  # scale_color_viridis(option="A",discrete = TRUE)+
```

```
  #scale_color_manual(values = c("#754ba6","#faed75","mediumblue", "orange", "yellow",
  #                               "Grey1", "Grey2", "Black"))+
  scale_color_manual(values = c("Black","#999999", "#d28eed", "#844da8" ,"#74dff7",
                                "#125087","#f5e2a2", "#e3d029", "#F781BF","#f2b385",
                                "#d97914"))+
  #stat_ellipse(level = 0.95)+
  theme(axis.text.x = element_text(size = 10,family='sans',angle=0),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        # legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        # legend.position = "bottom",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())
  # theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
PCA.PAR.log.pareto.RL.P80.plot2
```

```
PCA.PAR.log.pareto.RL.P80.plot3 <- ggplot(PCA.PAR.log.pareto.merge.RL.P80,
                                          aes(x=PC1, y=PC2, color = as.factor (Donor)))+
  geom_point(size = 3) + theme_bw() + labs(color = "Donor")+
  xlab("PC1 (20,16%)") + ylab("PC2 (14,22%)")+
  # scale_color_viridis(option="A",discrete = TRUE)+
  #scale_color_manual(values = c("#754ba6","#faed75","mediumblue", "orange", "yellow",
  #                               "Grey1", "Grey2", "Black"))+
  scale_color_manual(values = c("#844da8" ,"#74dff7", "#125087","#f5e2a2", "#e3d029",
                                "#F781BF","#f2b385", "#d97914"))+
  stat_ellipse(level = 0.95)+
  theme(axis.text.x = element_text(size = 10,family='sans',angle=0),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        # legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        # legend.position = "bottom",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())
  # theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
PCA.PAR.log.pareto.RL.P80.plot3
```

```
PCA.PAR.log.pareto.RL.P80.plot4 <- ggplot(PCA.PAR.log.pareto.merge.RL.P80,
                                          aes(x = PC1, y = PC2,
                                              color = as.factor(Treatment))) +
  geom_point(size = 3) + theme_bw() + labs(color = "Treatment")+
  xlab("PC1 (20,16%)") + ylab("PC2 (14,22%)")+
  # scale_color_viridis(option="A",discrete = TRUE)+
  #scale_color_manual(values = c("#754ba6","#faed75","mediumblue", "orange", "yellow",
  #                               "Grey1", "Grey2", "Black"))+
  scale_color_manual(values = c("Black","#999999", "#d28eed", "#844da8" ,"#74dff7",
                                "#125087","#f5e2a2", "#e3d029", "#F781BF","#f2b385",
```

```
                                "#d97914"))+
  stat_ellipse(level = 0.95)+
  theme(axis.text.x = element_text(size = 10,family='sans',angle=0),
        axis.text.y = element_text(size = 10,family='sans',angle=0),
        axis.title.x = element_text(size =12,family='sans',angle=0),
        axis.title.y = element_text(size=12,family='sans',angle=90),
        legend.text = element_text(size=10,family='sans',angle=0),
        # legend.title = element_text(size=10,family='sans',angle=0),
        strip.text = element_text(size=10,angle=0,family='sans'),
        # legend.position = "bottom",
        legend.key.size = unit(0.3,"cm"),
        panel.grid.major=element_blank(),
        panel.grid.minor=element_blank())
  # theme(axis.line.y=element_line(colour="black"),axis.line=element_line(colour="black"))
PCA.PAR.log.pareto.RL.P80.plot4
```

```
dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA1_RL_P80.tiff",
     width=7.5*dpi,height=6*dpi,res=dpi,compression="lzw")
PCA.PAR.log.pareto.RL.P80.plot1
dev.off()

png("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA1.png",
    res = 300, width = 3000, height = 2500)
PCA.PAR.log.pareto.RL.P80.plot1
dev.off()


dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA2_RL_P80.tiff",
     width=7*dpi,height=6*dpi,res=dpi,compression="lzw")
PCA.PAR.log.pareto.RL.P80.plot2
dev.off()

png("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA2.png",
    res = 300, width = 3000, height = 2500)
PCA.PAR.log.pareto.RL.P80.plot2
dev.off()

dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA3_RL_P80.tiff",
     width=7*dpi,height=6*dpi,res=dpi,compression="lzw")
PCA.PAR.log.pareto.RL.P80.plot3
dev.off()

png("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA3.png",
    res = 300, width = 3000, height = 2500)
PCA.PAR.log.pareto.RL.P80.plot3
dev.off()


dpi=300
tiff("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA4_RL_P80.tiff",
     width=7*dpi,height=6*dpi,res=dpi,compression="lzw")
```

```
PCA.PAR.log.pareto.RL.P80.plot4
dev.off()

png("/media/projects2/LisaM/SHIME_EM/Metabolomics/Figures/SHIME_TargMetab_PCA4.png",
    res = 300, width = 3000, height = 2500)
PCA.PAR.log.pareto.RL.P80.plot4
dev.off()
```