

P259: Publication figures and tables

Dendritic cells (pDC)

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```
#Create directories of outputs
dir.create("publication/fig/", showWarnings = FALSE, recursive = TRUE)
dir.create("publication/table/", showWarnings = FALSE, recursive = TRUE)

#Load packages used across all data
library(tidyverse)
library(limma)
#Set seed
set.seed(4389)
```

Tables

RNA-seq library and patient metadata

```
attach("data_clean/P259_pDC_clean.RData")

dat.pDC.voom$targets %>%
  select(libID, everything()) %>%
  arrange(libID) %>%
  write_csv(., "publication/table/Tables.metadata.csv")
```

Patient summary demographics

```
#Age
dat.pDC.voom$targets %>%
  distinct(experiment, donorID, Age) %>%
  group_by(experiment) %>%
  summarise(.groups = "keep",
            n = n(),
            mean.age = mean(Age, na.rm=TRUE),
            sd.age = sd(Age, na.rm=TRUE)/sqrt(n))
```

```
## # A tibble: 2 x 4
## # Groups:   experiment [2]
##   experiment      n mean.age sd.age
##   <chr>         <int>   <dbl> <dbl>
## 1 P259_1         4      28   2.83
## 2 P259_2         7     12.9  0.738
```

```
#Sex
dat.pDC.voom$targets %>%
  distinct(experiment, donorID, Sex) %>%
  count(experiment, Sex) %>%
  group_by(experiment) %>%
  mutate(pct = n/sum(n))
```

```
## # A tibble: 5 x 4
## # Groups:   experiment [2]
##   experiment Sex      n  pct
##   <chr>      <chr> <int> <dbl>
## 1 P259_1    Female     1 0.25
## 2 P259_1    Male       1 0.25
## 3 P259_1    <NA>       2 0.5
## 4 P259_2    Female     3 0.429
## 5 P259_2    Male       4 0.571
```

RNA-seq normalized log2 counts

```
#attach("data_clean/P259_pDC_clean.RData")

as.data.frame(dat.pDC.voom$E) %>%
  rownames_to_column("geneName") %>%
#Save
write_csv(., "publication/table/TableS.norm.log2.counts.csv")
```

All contrast model results

```
#Get all model results
read_csv("results/gene_level/P259.1_gene_pval.csv") %>%
  #Add experiment variable
  mutate(experiment = "P259_1") %>%
  bind_rows(read_csv("results/gene_level/P259.2_gene_pval.csv")) %>%
  #Fill in experiment variable
  mutate(experiment = ifelse(is.na(experiment), "P259_2", experiment)) %>%
  #Keep only contrasts model
```

```

filter(model == "contrasts") %>%
#Reorder and rename variables
select(experiment, model, geneName, hgnc_symbol, group,
       AveExpr:group) %>%
rename(contrast = group) %>%
arrange(experiment, geneName, contrast) %>%
#Save
write_csv(., "publication/table/TableS.models.csv")

```

GSEA results

```

read_csv("results/GSEA_FoldChange/h_GSEA.result.csv") %>%
  rename(contrast = group) %>%
  #Add experiment variable
  mutate(experiment = ifelse(grepl(".1", contrast), "P259_1", "P259_2")) %>%
  #Set gene set variable
  mutate(gene.set = "HALLMARK",
         pathway = gsub("HALLMARK_", "", pathway)) %>%
  #Format contrast to match model results
  mutate(contrast = gsub(".1", "", contrast),
         contrast = gsub(".2", "", contrast),
         contrast = gsub("[.]n", " - n", contrast),
         contrast = gsub("[.]A", " - A", contrast),
         contrast = gsub("[.]E", " - E", contrast)) %>%
  #Reorder variables
  select(experiment, gene.set, pathway, contrast, everything()) %>%
  arrange(experiment, pathway, contrast) %>%
#Save
write_csv(., "publication/table/TableS.GSEA.csv")

```

Figures

PCA

```
library(cowplot)
```

Calculate PCA for P259.1 and P259.2

```

#attach("data_clean/P259_pDC_clean.RData")

PCA1 <- as.data.frame(dat.pDC.voom_1$E) %>%
  t() %>%
  prcomp(scale. = TRUE)

#Extract axes labels
PC1.label1 <- paste("PC1 (",
                    round(summary(PCA1)$importance[2,1]*100, digits=1),
                    "%)", sep="")
PC2.label1 <- paste("PC2 (",
                    round(summary(PCA1)$importance[2,2]*100, digits=1),
                    "%)", sep="")

```

```

PCA2 <- as.data.frame(dat.pDC.voom_2$E) %>%
  t() %>%
  prcomp(scale. = TRUE)

#Extract axes labels
PC1.label2 <- paste("PC1 (",
                    round(summary(PCA2)$importance[2,1]*100, digits=1),
                    "%)", sep="")
PC2.label2 <- paste("PC2 (",
                    round(summary(PCA2)$importance[2,2]*100, digits=1),
                    "%)", sep="")

```

Combine experiments and metadata.

```

# Extract PC values
PCA.dat <- as.data.frame(PCA1$x) %>%
  rownames_to_column("libID") %>%
  bind_rows(rownames_to_column(as.data.frame(PCA2$x), "libID")) %>%
  # Select PCs
  dplyr::select(libID, PC1, PC2) %>%
  # Merge with metadata
  left_join(dat.pDC.voom$targets, by="libID")

```

Plot PCAs

```

#Define ggplot colors
group.cols <- c("none:none"="#dadaeb",
                "none:AntiIL5"="#9e9ac8",
                "none:EOS.supp"="#54278f",
                "HRV:none"="#c7e9c0",
                "HRV:AntiIL5"="#74c476",
                "HRV:EOS.supp"="#006d2c",
                "flu:none"="#fdae6b",
                "flu:AntiIL5"="#e6550d")

PCA1 <- PCA.dat %>%
  filter(experiment=="P259_1") %>%

  ggplot(aes(PC1, PC2, color=virus:IL5)) +
  geom_point(size=5) +
  #Beautify
  theme_classic(base_size = 16) +
  labs(x=PC1.label1, y=PC2.label1) +
  coord_fixed(ratio=1) +
  scale_color_manual(values = group.cols,
                    labels = c("media", "+ EOS sup",
                              "+ RV", "+ RV + EOS sup"),
                    name="") +
  theme(legend.position = "bottom") +
  guides(color=guide_legend(nrow=2))

PCA2 <- PCA.dat %>%
  filter(experiment=="P259_2") %>%

  ggplot(aes(PC1, PC2, color=virus:IL5)) +

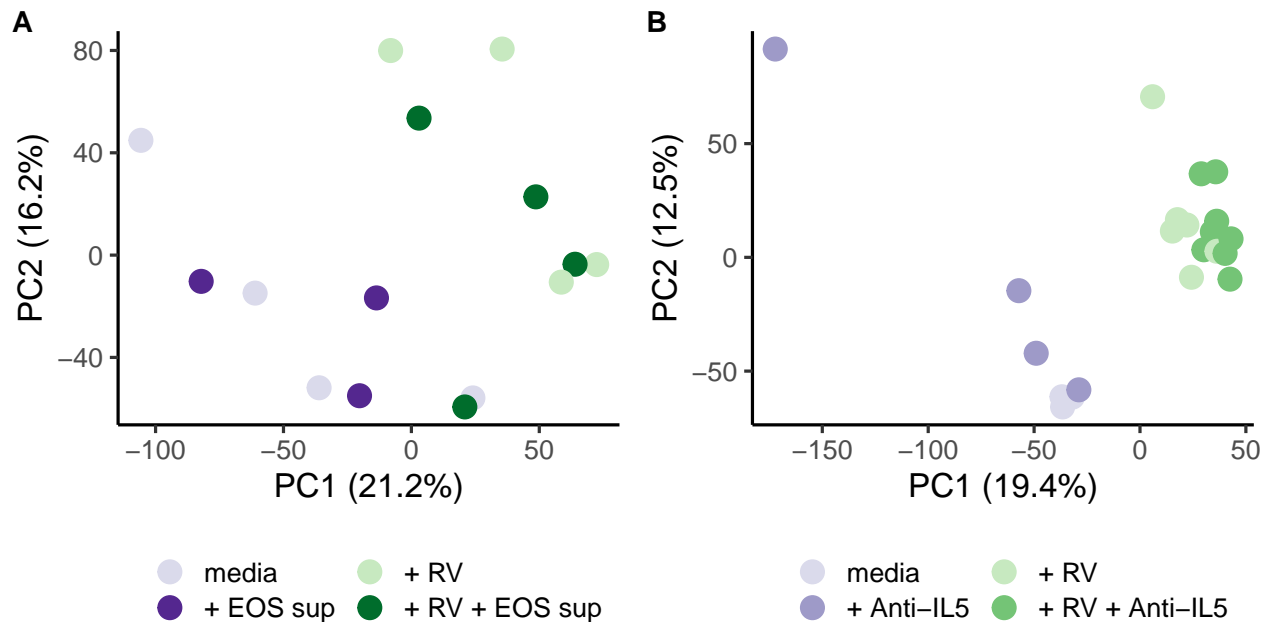
```

```

geom_point(size=5) +
#Beautify
theme_classic(base_size = 16) +
labs(x=PC1.label2, y=PC2.label2) +
coord_fixed(ratio=1) +
scale_color_manual(values = group.cols,
                    labels = c("media", "+ Anti-IL5",
                              "+ RV", "+ RV + Anti-IL5"),
                    name="") +
theme(legend.position = "bottom") +
guides(color=guide_legend(nrow=2))

plot_grid(PCA1,PCA2, align = "hv", labels = c("A","B"))

```



```

#Save
ggsave("publication/fig/PCA.pdf",
       plot_grid(PCA1,PCA2, align = "hv", labels = c("A","B")),
       width=10, height=5)

```

Contrast model venn

```
library(venn)
```

Genes significant for contrasts at FDR < 0.2

```

#Load pvalue results
pval_1 <- read_csv("results/gene_level/P259.1_gene_pval.csv") %>%
  filter(model == "contrasts")
pval_2 <- read_csv("results/gene_level/P259.2_gene_pval.csv") %>%
  filter(model == "contrasts")

#Set FDR cutoff
fdr.list <- c(0.2)

```

```

#Plot venns
for(fdr.cutoff in fdr.list){
  #P259.1
  venn.list_1 <- list()
  ##Signif for virus
  venn.list_1[["RV-infected\nvs media"]] <- pval_1 %>%
    filter(group %in% c("none_HRV - none_none",
                        "EOS.supp_HRV - EOS.supp_none") &
            adj.P.Val <= fdr.cutoff) %>%
    distinct(geneName) %>%
    select(geneName) %>% unlist(use.names = FALSE)

  ##Signif for treatment
  venn.list_1[["- RV\nEOS supernatant\nvs none"]] <- pval_1 %>%
    filter(group == "EOS.supp_none - none_none" &
            adj.P.Val <= fdr.cutoff) %>%
    distinct(geneName) %>%
    select(geneName) %>% unlist(use.names = FALSE)

  venn.list_1[["+ RV\nEOS supernatant\nvs none"]] <- pval_1 %>%
    filter(group == "EOS.supp_HRV - none_HRV" &
            adj.P.Val <= fdr.cutoff) %>%
    distinct(geneName) %>%
    select(geneName) %>% unlist(use.names = FALSE)

  #P259.2
  venn.list_2 <- list()
  ##Signif for virus
  venn.list_2[["RV-infected\nvs media"]] <- pval_2 %>%
    filter(group %in% c("none_HRV - none_none",
                        "AntiIL5_HRV - AntiIL5_none") &
            adj.P.Val <= fdr.cutoff) %>%
    distinct(geneName) %>%
    select(geneName) %>% unlist(use.names = FALSE)

  ##Signif for treatment
  venn.list_2[["- RV\nAnti-IL5 therapy\nvs none"]] <- pval_2 %>%
    filter(group == "AntiIL5_none - none_none" &
            adj.P.Val <= fdr.cutoff) %>%
    distinct(geneName) %>%
    select(geneName) %>% unlist(use.names = FALSE)

  venn.list_2[["+ RV\nAnti-IL5 therapy\nvs none"]] <- pval_2 %>%
    filter(group == "AntiIL5_HRV - none_HRV" &
            adj.P.Val <= fdr.cutoff) %>%
    distinct(geneName) %>%
    select(geneName) %>% unlist(use.names = FALSE)

  #Save
  pdf(paste("publication/fig/contrast.venn_", fdr.cutoff, ".pdf", sep=""),
      height=5, width=12)
  par(mfrow = c(1,2))
  venn(ilab=FALSE, zcolor = "style", ilcs=1.5, sncs=1.5, box=FALSE,

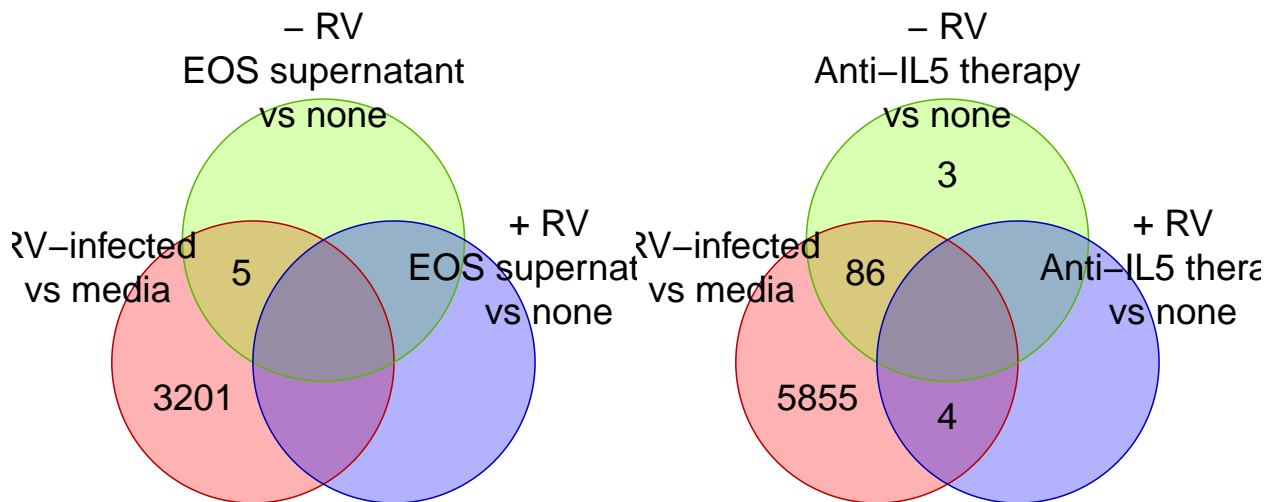
```

```

x=venn.list_1)
venn(ilab=FALSE, zcolor = "style", ilcs=1.5, sncs=1.5, box=FALSE,
x=venn.list_2)
dev.off()

#Print to Rmd
par(mfrow = c(1,2))
venn(ilab=FALSE, zcolor = "style", ilcs=1.5, sncs=1.5, box=FALSE,
x=venn.list_1)
venn(ilab=FALSE, zcolor = "style", ilcs=1.5, sncs=1.5, box=FALSE,
x=venn.list_2)
}

```



Hallmark GSEA

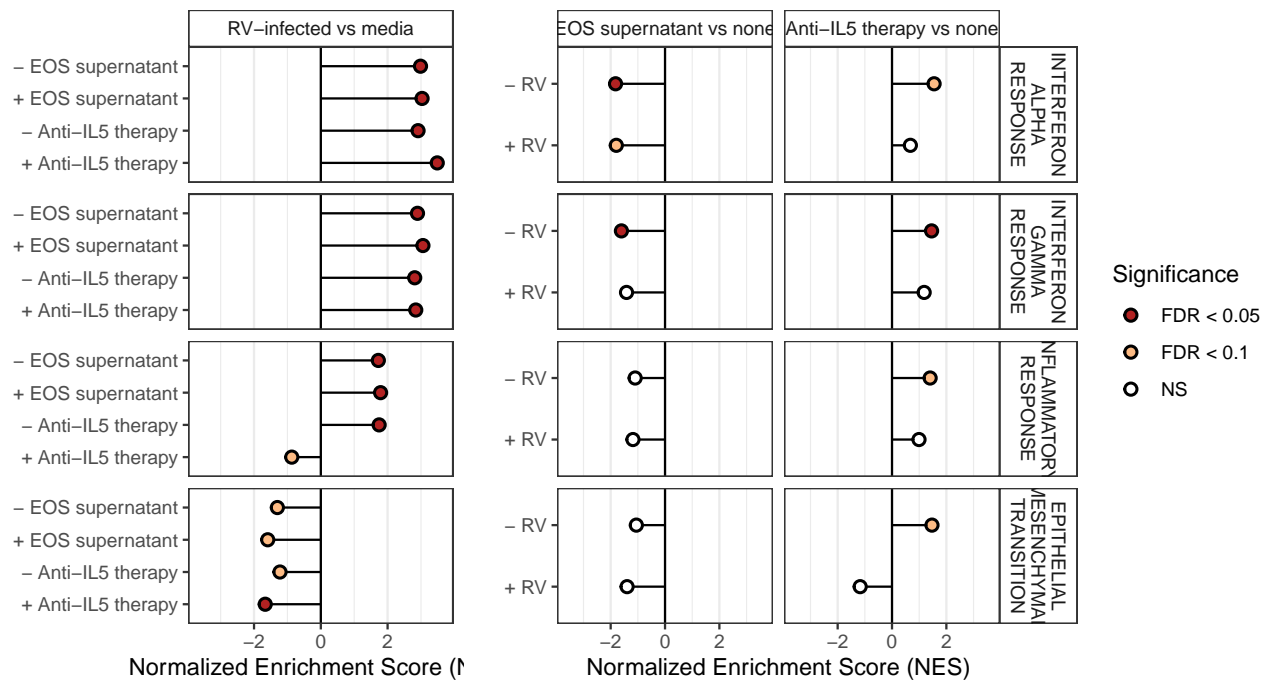
Enrichment score

```

#Load plotting function
source("scripts/GSEA.score.plot.R")

#Create plot
gsea.plot(gsea = read_csv("results/GSEA_FoldChange/h_GSEA.result.csv"),
fdr.cut = 0.1, prefix = "H", print.plot = TRUE,
save.plot = TRUE, height = 5, width = 11,
outdir="publication/fig/")

```



Mean fold change

```
library(cowplot)
library(ggrepel)

#List genes in each Hallmark term
myGO <- fgsea::gmtPathways("data_clean/Broad_gmt/h.all.v7.1.symbols.gmt")
GO.df <- plyr::ldply(myGO, rbind) %>%
  rename(term = `id`) %>%
  pivot_longer(-term, values_to="hgnc_symbol")

#Expression data
#attach("data_clean/P259_pDC_clean.RData")

#### Format data ####
#Select genes of interest and format
## P259.2
h.combo.plot_2 <- as.data.frame(dat.pDC.voom_2$E) %>%
  rownames_to_column("geneName") %>%
  #Get HGNC symbols
  left_join(dat.pDC.voom_2$genes) %>%
  select(hgnc_symbol, everything()) %>%
  #Filter to genes in terms
  inner_join(GO.df) %>%
  select(term, hgnc_symbol, geneName, everything(),
    -c(`Previous symbols`:gene_biotype), -name) %>%
  #long format
  pivot_longer(-c(term:geneName), names_to = "libID",
    values_to = "expression") %>%
  #Add metadata
  mutate(experiment = "P259.2") %>%
  left_join(select(dat.pDC.voom_2$targets, donorID, libID,
```



```

IL5, virus.detail))

## P259.1
h.combo.plot_1 <- as.data.frame(dat.pDC.voom_1$E) %>%
  rownames_to_column("geneName") %>%
  #Get HGNC symbols
  left_join(dat.pDC.voom_1$genes) %>%
  select(hgnc_symbol, everything()) %>%
  #Filter to genes in terms
  inner_join(GO.df) %>%
  select(term, hgnc_symbol, geneName, everything(),
    -c(`Previous symbols`:gene_biotype), -name) %>%
  #long format
  pivot_longer(-c(term:geneName), names_to = "libID",
    values_to = "expression") %>%
  #Add metadata
  mutate(experiment = "P259.1") %>%
  left_join(select(dat.pDC.voom_1$targets, donorID,
    libID, IL5, virus.detail))

#Combine data and calculate fold change
h.combo.plot <- bind_rows(h.combo.plot_1, h.combo.plot_2) %>%
  mutate(group = paste(virus.detail, IL5, sep=".")) %>%
  select(-IL5, -virus.detail, -libID) %>%
  #Averages
  group_by(term, hgnc_symbol, experiment, group) %>%
  summarise(meanE = mean(expression, na.rm=TRUE)) %>%
  #Fold change
  pivot_wider(names_from = group, values_from = meanE) %>%
  rowwise() %>%
  #Simplify group names
  mutate(
    #Virus in untreated
    virus.untreat_old = oldHRV.none-none.none,
    virus.untreat_new = newHRV.none-none.none,
    #Virus in treated
    virus.treat_old1 = oldHRV.EOS.supp-none.EOS.supp,
    virus.treat_old2 = oldHRV.AntiIL5-none.AntiIL5,
    virus.treat_new = newHRV.AntiIL5-none.AntiIL5,
    #Treatment in media
    treat.media_1 = none.EOS.supp-none.none,
    treat.media_2 = none.AntiIL5-none.none,
    #Treatment in virus
    treat.virus_old1 = oldHRV.EOS.supp-oldHRV.none,
    treat.virus_old2 = oldHRV.AntiIL5-oldHRV.none,
    treat.virus_new = newHRV.AntiIL5-newHRV.none) %>%
  ungroup() %>%

  #long format
  select(term:experiment, virus.untreat_old:treat.virus_new) %>%
  pivot_longer(virus.untreat_old:treat.virus_new) %>%
  drop_na(value) %>%

```

```

#Average old and new HRV values
separate(name, into=c("group"), sep="_") %>%
group_by(term, hgnc_symbol, experiment, group) %>%
summarise(value = mean(value, na.rm=TRUE)) %>%
ungroup() %>%
#Beautify for plotting
mutate(term = gsub("HALLMARK_", "", term),
       term = gsub("_", " ", term)) %>%
mutate(col.group = ifelse(value < 0, "down", "up")) %>%
mutate(group = factor(group, levels=c("treat.media", "treat.virus",
                                       "virus.untreat", "virus.treat")))

#Set jitter
pos <- position_jitter(width = 0.3, seed = 589, height = 0)

#### List genes to label ####
#Set max number of terms
no.genes <- 5
#Set gene sets of interest
term.OI.ls <- c("INTERFERON ALPHA RESPONSE", "INTERFERON GAMMA RESPONSE",
               "INFLAMMATORY RESPONSE",
               "EPITHELIAL MESENCHYMAL TRANSITION")

#Find genes with largest absolute fold change
to.label <- list()

for(term.OI in term.OI.ls){
  #If this term, find genes that go down with virus
  if(term.OI == "EPITHELIAL MESENCHYMAL TRANSITION"){
    genes.v <- h.combo.plot %>%
      filter(term == term.OI &
             group %in% c("virus.treat", "virus.untreat")) %>%
      group_by(term, hgnc_symbol) %>%
      filter(max(value, na.rm=TRUE) < 0) %>%
      summarise(max.fc = max(abs(value), na.rm=TRUE)) %>%
      ungroup() %>%
      slice_max(max.fc, n=no.genes) %>%
      distinct(hgnc_symbol)
  } else{
    #All other terms, find genes that go up with virus
    genes.v <- h.combo.plot %>%
      filter(term == term.OI &
             group %in% c("virus.treat", "virus.untreat")) %>%
      group_by(term, hgnc_symbol) %>%
      filter(min(value, na.rm=TRUE) > 0) %>%
      summarise(max.fc = max(value, na.rm=TRUE)) %>%
      ungroup() %>%
      slice_max(max.fc, n=no.genes) %>%
      distinct(hgnc_symbol)
  }
}

#Save genes to list object
to.label[[paste("v", term.OI, sep=".")] ] <- genes.v

```

```

#Find genes that go down with EOS supp
down.eos <- h.combo.plot %>%
  filter(term == term.OI & group %in% c("treat.media","treat.virus") &
    experiment == "P259.1") %>%
  group_by(term, hgnc_symbol) %>%
  filter(max(value, na.rm=TRUE)<0) %>%
  ungroup() %>%
  distinct(hgnc_symbol) %>% unlist(use.names = FALSE)

#Find genes that go up with AntiIL5
up.aIL5 <- h.combo.plot %>%
  filter(term == term.OI & group %in% c("treat.media","treat.virus") &
    experiment == "P259.2") %>%
  group_by(term, hgnc_symbol) %>%
  filter(min(value, na.rm=TRUE)>0) %>%
  ungroup() %>%
  distinct(hgnc_symbol) %>% unlist(use.names = FALSE)

#Concatenate to genes that go up/down with virus and down with EOS and
# up with AntiIL5
## i.e. follow the directions in the enrichment score plot
genes.t <- h.combo.plot %>%
  filter(term == term.OI &
    group %in% c("virus.treat","virus.untreat")) %>%
  filter(hgnc_symbol %in% intersect(down.eos,up.aIL5)) %>%

  group_by(term, hgnc_symbol) %>%
  filter(max(abs(value), na.rm=TRUE)>0) %>%
  summarise(max.fc = max(abs(value), na.rm=TRUE)) %>%
  ungroup() %>%
  slice_max(max.fc,n=no.genes) %>%
  distinct(hgnc_symbol)

#Save to list object
to.label[[paste("t",term.OI,sep=".")] <- genes.t
}

#Convert genes to label to df
to.label.df <- data.table::rbindlist(to.label, idcol="name") %>%
  separate(name, into=c("vt","term"), sep=".") %>%
  mutate(group1 = ifelse(vt == "t", "treat.media", "virus.untreat"),
    group2 = ifelse(vt == "t", "treat.virus", "virus.treat")) %>%
  pivot_longer(group1:group2, values_to="group") %>%
  dplyr::select(-name, -vt) %>%
  mutate(to.label = "y")

#add genes to label info to other plot data
h.combo.plot.lab <- h.combo.plot %>%
  full_join(to.label.df, by = c("term", "hgnc_symbol", "group"))

### FC plot data ###
FC.plot.dat <- h.combo.plot.lab %>%
  #Beautify labels

```

```

mutate(group = paste(experiment, group, sep="_")) %>%
mutate(group1 = recode_factor(factor(group),
  "P259.2_virus.treat"="+ Anti-IL5 therapy",
  "P259.2_virus.untreat"="- Anti-IL5 therapy",
  "P259.1_virus.treat"="+ EOS supernatant",
  "P259.1_virus.untreat"="- EOS supernatant",

  "P259.1_treat.virus"="+ RV",
  "P259.2_treat.virus"="+ RV",
  "P259.1_treat.media"="- RV",
  "P259.2_treat.media"="- RV"),
group2 = recode_factor(factor(group),
  "P259.1_virus.untreat"="RV-infected vs media",
  "P259.2_virus.untreat"="RV-infected vs media",
  "P259.1_virus.treat"="RV-infected vs media",
  "P259.2_virus.treat"="RV-infected vs media",

  "P259.1_treat.virus"="EOS supernatant vs none",
  "P259.2_treat.virus"="Anti-IL5 therapy vs none",
  "P259.1_treat.media"="EOS supernatant vs none",
  "P259.2_treat.media"="Anti-IL5 therapy vs none")) %>%

#Reorder terms
mutate(term.ord = recode_factor(factor(term),
  "INTERFERON ALPHA RESPONSE"="INTERFERON\nALPHA\nRESPONSE",
  "INTERFERON GAMMA RESPONSE"="INTERFERON\nGAMMA\nRESPONSE",
  "INFLAMMATORY RESPONSE"="INFLAMMATORY\nRESPONSE",
  "EPITHELIAL MESENCHYMAL TRANSITION"="EPITHELIAL\nMESENCHYMAL\nTRANSITION"))

#### FC plot: VIRUS ####
dat.v <- FC.plot.dat %>%
  #Reorder terms
  filter(group2 == "RV-infected vs media" &
    term.ord %in% c("INTERFERON\nALPHA\nRESPONSE",
      "INTERFERON\nGAMMA\nRESPONSE",
      "INFLAMMATORY\nRESPONSE",
      "EPITHELIAL\nMESENCHYMAL\nTRANSITION"))

plot.v <- dat.v %>%
  ggplot(aes(x = group1, y=value)) +
  geom_violin() +
  #Add non-labeled points
  geom_jitter(data=filter(dat.v, is.na(to.label)),
    aes(color=col.group), position = pos) +
  #Add labeled points
  geom_point(data=filter(dat.v, !is.na(to.label)),
    aes(color=col.group), ) +
  #Add labels left
  geom_text_repel(data=filter(dat.v, !is.na(to.label)),
    aes(label=hgnc_symbol), direction="y",
    hjust=1, nudge_x=-0.4,
    show.legend = FALSE, size=3) +
  #Add line at 0
  #geom_hline(yintercept = 0, size=0.5) +

```

```

#Add mean lines
stat_summary(fun="mean", geom="crossbar")+
facet_grid(term.ord~group2, scales="free") +
coord_flip() +
#Beautify
theme_bw() +
labs(x="", y="Gene mean log2 fold change") +
scale_color_manual(values=c("down"="#6baed6", "up"="#ef3b2c")) +
theme(strip.text.y = element_blank()+
theme(legend.position = "none",
      panel.grid.major.y = element_blank(),
      panel.grid.minor.y = element_blank(),
      strip.background =element_rect(fill="white"))

#plot.v

#### FC plot: TREATMENT ####
dat.t <- FC.plot.dat %>%
  filter(group2 != "RV-infected vs media" &
         term.ord %in% c("INTERFERON\NALPHA\NRESPONSE",
                        "INTERFERON\NGAMMA\NRESPONSE",
                        "INFLAMMATORY\NRESPONSE",
                        "EPITHELIAL\NMESENCHYMAL\NTRANSITION"))

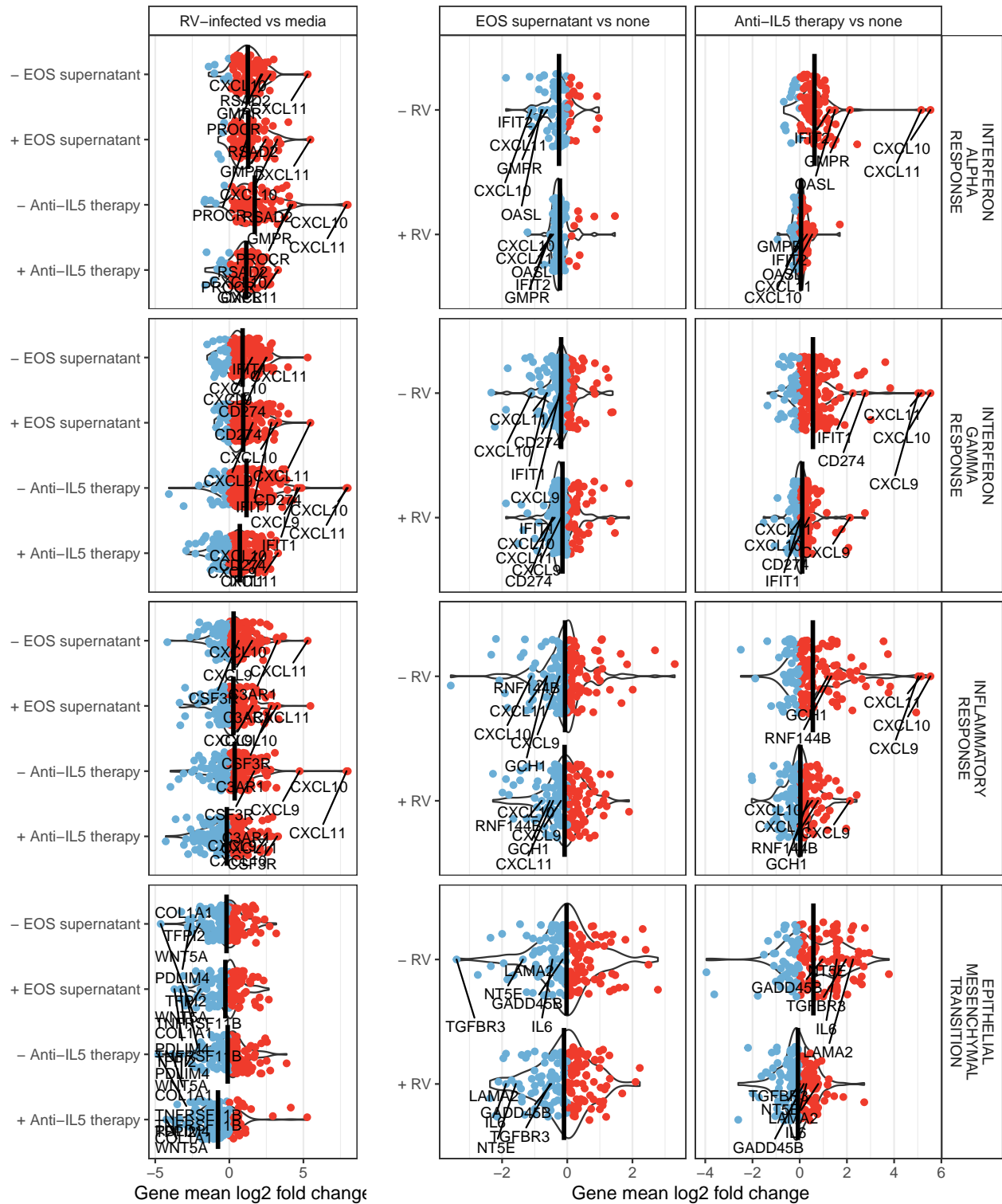
plot.t <- dat.t %>%
  ggplot(aes(x = group1, y=value)) +
  geom_violin() +
  #Add non-labeled points
  geom_jitter(data=filter(dat.t, is.na(to.label)),
             aes(color=col.group), position = pos) +
  #Add labeled points
  geom_point(data=filter(dat.t, !is.na(to.label)),
            aes(color=col.group), ) +
  #Add labels left
  geom_text_repel(data=filter(dat.t, !is.na(to.label)),
                 aes(label=hgnc_symbol), direction="y",
                 hjust=1, nudge_x=-0.4,
                 show.legend = FALSE, size=3) +
  #Add line at 0
  #geom_hline(yintercept = 0, size=0.5) +
  #Add mean lines
  stat_summary(fun="mean", geom="crossbar")+
  facet_grid(term.ord~group2, scales="free") +
  coord_flip() +
  #Beautify
  theme_bw() +
  labs(x="", y="Gene mean log2 fold change") +
  scale_color_manual(values=c("down"="#6baed6", "up"="#ef3b2c")) +
  theme(legend.position = "none",
        panel.grid.major.y = element_blank(),
        panel.grid.minor.y = element_blank(),
        strip.background =element_rect(fill="white"))

#plot.t

```

Combine FC plots

```
fc.plot.all <- plot_grid(plot.v, plot.t, ncol=2, rel_widths = c(0.6,1))
fc.plot.all
```



```
ggsave("publication/fig/H_GSEA_FoldChange.pdf", fc.plot.all,
width = 20, height = 15)
```

Genes of interest

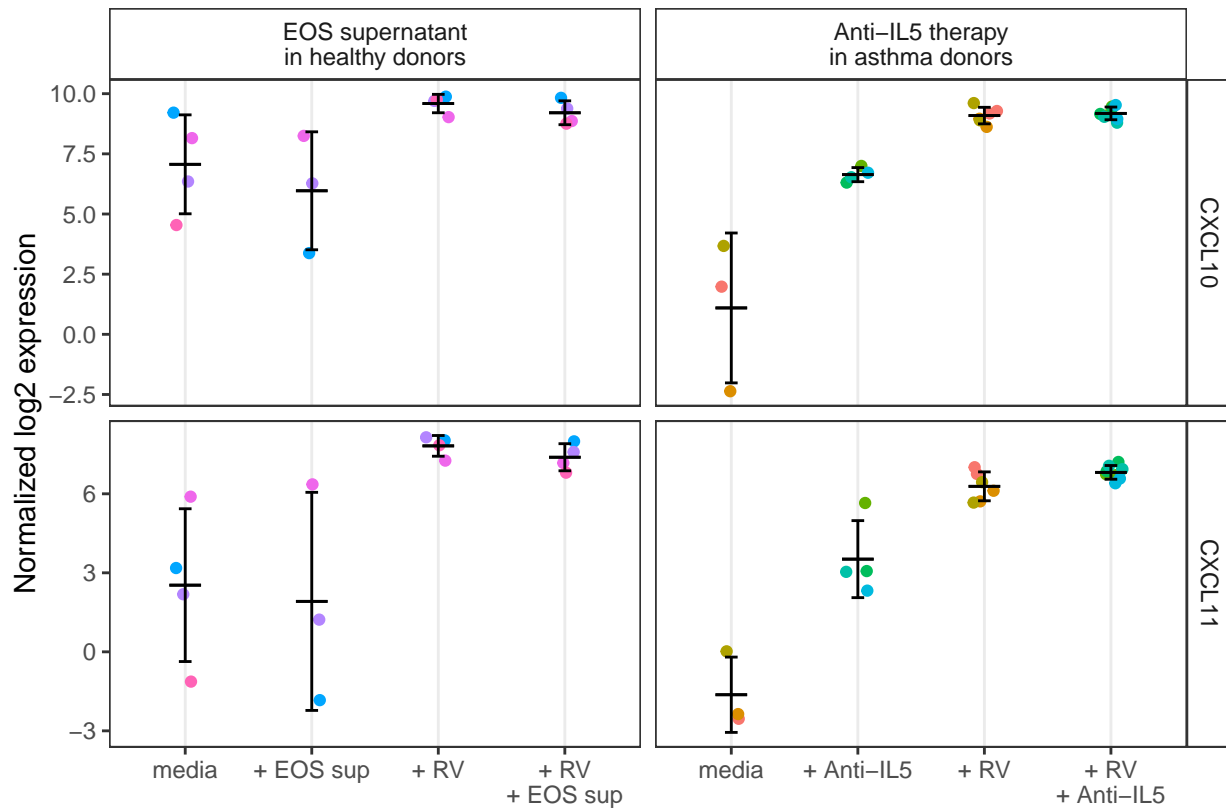
```
# List all genes to plot
GOI <- c("CXCL10", "CXCL11")

#Get results for genes of interest
dat.GOI <- bind_rows(h.combo.plot_1, h.combo.plot_2) %>%
  filter(hgnc_symbol %in% GOI) %>%
  dplyr::select(-term) %>% distinct()

plot.GOI <- dat.GOI %>%
  #Beautify labels
  mutate(x.lab=paste(virus.detail,IL5, sep="_"),
         x.lab=gsub("oldH|newH", "", x.lab),
         x.lab=recode_factor(factor(x.lab),
                             "none_none"="media",
                             "none_EOS.sup"="+ EOS sup",
                             "none_AntiIL5"="+ Anti-IL5",
                             "RV_none"="+ RV",
                             "RV_EOS.sup"="+ RV\n+ EOS sup",
                             "RV_AntiIL5"="+ RV\n+ Anti-IL5")) %>%
  mutate(ex.lab = recode_factor(factor(experiment),
                                   "P259.1"="EOS supernatant\nin healthy donors",
                                   "P259.2"="Anti-IL5 therapy\nin asthma donors")) %>%
  arrange(x.lab) %>%

  #Plot
  ggplot(aes(x=x.lab, y=expression, color=donorID)) +
  geom_jitter(width=0.1, height=0) +
  #Add mean and error bars
  stat_summary(fun.data=mean_sdl,
              fun.args = list(mult=1),
              geom="errorbar", color="black", width=0.1) +
  stat_summary(fun=mean, geom="errorbar",
              aes(ymax=..y.., ymin=..y..),
              color="black", width=0.25) +
  #facet experiments and genes
  facet_grid(hgnc_symbol~ex.lab, scales="free") +
  #Beautify
  theme_bw() +
  labs(x="", y="Normalized log2 expression") +
  theme(legend.position = "none",
        panel.grid.major.y = element_blank(),
        panel.grid.minor.y = element_blank(),
        strip.background =element_rect(fill="white"))

plot.GOI
```



```
ggsave("publication/fig/G0I.pdf", plot.G0I,
       height=6, width=6)
```

R session

```
sessionInfo()
```

```
## R version 4.0.0 (2020-04-24)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Catalina 10.15.5
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRblas.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods   base
##
## other attached packages:
## [1] ggrepel_0.8.2  venn_1.9      cowplot_1.0.0  limma_3.44.3
## [5] forcats_0.5.0  stringr_1.4.0 dplyr_1.0.0    purrr_0.3.4
## [9] readr_1.3.1    tidyr_1.1.0   tibble_3.0.3   ggplot2_3.3.2
## [13] tidyverse_1.3.0
##
```



```
## loaded via a namespace (and not attached):
## [1] fs_1.4.2          lubridate_1.7.9    RColorBrewer_1.1-2
## [4] httr_1.4.2        tools_4.0.0        backports_1.1.8
## [7] utf8_1.1.4        R6_2.4.1           rpart_4.1-15
## [10] Hmisc_4.4-0       DBI_1.1.0          colorspace_1.4-1
## [13] nnet_7.3-14       withr_2.2.0        tidyselect_1.1.0
## [16] gridExtra_2.3     compiler_4.0.0     cli_2.0.2
## [19] rvest_0.3.6       htmlTable_2.0.1    xml2_1.3.2
## [22] labeling_0.3      scales_1.1.1       checkmate_2.0.0
## [25] digest_0.6.25     foreign_0.8-80     rmarkdown_2.3
## [28] base64enc_0.1-3   jpeg_0.1-8.1       pkgconfig_2.0.3
## [31] htmltools_0.5.0   dbplyr_1.4.4       htmlwidgets_1.5.1
## [34] rlang_0.4.7       readxl_1.3.1       rstudioapi_0.11
## [37] farver_2.0.3      generics_0.0.2     jsonlite_1.7.0
## [40] BiocParallel_1.22.0 acepack_1.4.1      magrittr_1.5
## [43] Formula_1.2-3     Matrix_1.2-18      Rcpp_1.0.5
## [46] munsell_0.5.0     fansi_0.4.1        lifecycle_0.2.0
## [49] stringi_1.4.6     yaml_2.2.1         plyr_1.8.6
## [52] grid_4.0.0        blob_1.2.1         parallel_4.0.0
## [55] crayon_1.3.4      lattice_0.20-41    haven_2.3.1
## [58] splines_4.0.0     hms_0.5.3          knitr_1.29
## [61] pillar_1.4.6      fgsea_1.14.0       admisc_0.8
## [64] fastmatch_1.1-0   reprex_0.3.0       glue_1.4.1
## [67] evaluate_0.14     latticeExtra_0.6-29 data.table_1.13.0
## [70] modelr_0.1.8      vctrs_0.3.2        png_0.1-7
## [73] cellranger_1.1.0  gtable_0.3.0       assertthat_0.2.1
## [76] xfun_0.16         broom_0.7.0        survival_3.2-3
## [79] cluster_2.1.0     ellipsis_0.3.1
```
