

## 2. Heatmaps of lab and field, facs data

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### Import data

```
MICE <- read.csv("output_data/MICE.csv")
```

### Vectors for selecting genes

```
## vectors for selecting columns
facs_lab <- c("CD4", "Treg", "Div_Treg", "Treg17", "Th1", "Div_Th1", "Th17",
             "Div_Th17", "CD8", "Act_CD8", "Div_Act_CD8", "IFNy_CD4",
             "IFNy_CD8", "Treg_prop", "IL17A_CD4")

facs_field <- c("CD4", "Treg", "CD4", "Treg17", "Th1", "Th17", "CD8",
               "Act_CD8", "IFNy_CD4", "IL17A_CD4", "IFNy_CD8")
```

### Heatmap on lab FACS data

#### data cleaning

```
### Select the measurements from the mesenterial lymphnodes
### Prepare the annotation data frame for the heatmap
annotation_df <- MICE %>%
  filter(origin == "Lab") %>% # filter the lab data
  filter(Position == "mLN") %>%
  filter(infection == "challenge") %>%
  drop_na("CD4") %>%
  dplyr::select(c("Mouse_ID", "Parasite_challenge", "infection_history",
                 "mouse_strain", "max_WL"))

### Drop the columns that contain nas in the column CD4 of
#the facs columns

### Data tidying for the heatmap function

FACS <- MICE %>%
  dplyr::filter(origin == "Lab") %>% # filter the lab data
  filter(Position == "mLN") %>%
```

```

filter(infection == "challenge", dpi == dpi_max) %>%
drop_na("CD4") %>%
dplyr::select(c(Mouse_ID, all_of(facs_lab)))

# turn the data frame into a matrix and transpose it. We want to have each cell
# type as a row name
FACS <- t(as.matrix(FACS))

#switch the matrix back to a data frame format
FACS <- as.data.frame(FACS)

# turn the first row into column names
FACS %>%
  row_to_names(row_number = 1) -> FACS

# Now further prepare the data frame for plotting by removing the first row
## and convert the column to row names with the cells

heatmap_data <- FACS

# turn the columns to numeric other wise the heatmap function will not work
heatmap_data[] <- lapply(heatmap_data, function(x) as.numeric(as.character(x)))

annotation_df <- unique(annotation_df) %>%
  dplyr::filter(Mouse_ID %in% colnames(heatmap_data))

### Prepare the annotation columns for the heatmap
rownames(annotation_df) <- annotation_df$EH_ID

# Match the row names to the heatmap data frame
rownames(annotation_df) <- colnames(FACS)

#remove the unnecessary column
annotation_df <- annotation_df %>% dplyr::select(-Mouse_ID, )

heatmap_facs_LAB <- heatmap_data

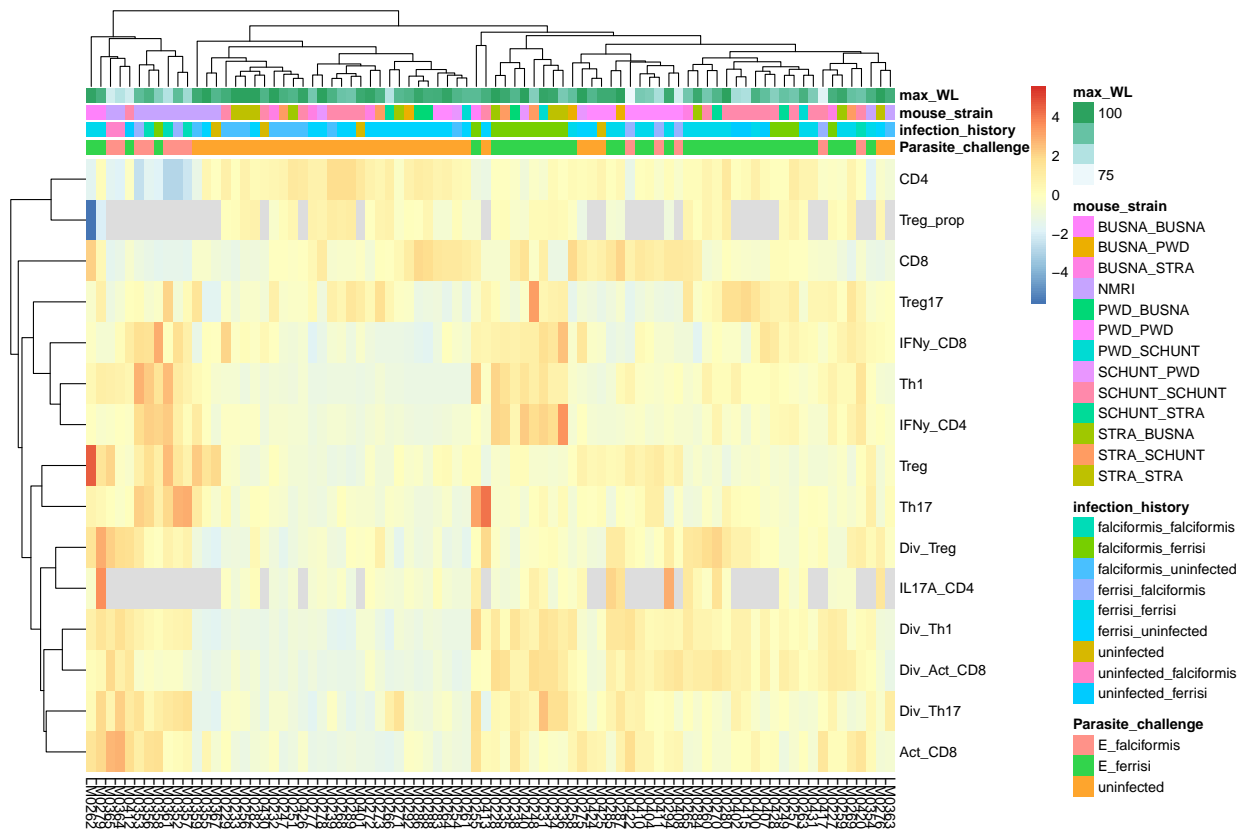
```

## Heatmap lab facs: Plot

```

heatmap_data %>%
  pheatmap(annotation_col = annotation_df, scale = "row")

```



## Field data

### data cleaning

```
### Select the measurements from the mesenterial lymphnodes
### Prepare the annotation data frame for the heatmap
annotation_df <- MICE %>%
  filter(origin == "Field") %>% # filter the lab data
  dplyr::select(c("Mouse_ID", "Sex", "HI", "delta_ct_cewe_MminusE")) %>%
  drop_na()

### Prepare the annotation columns for the heatmap
rownames(annotation_df) <- annotation_df$Mouse_ID

### Data tidying for the heatmap function

FACS <- MICE %>%
  dplyr::filter(origin == "Field") %>% # filter the lab data
  dplyr::select(c(Mouse_ID, all_of(facs_field)))

# turn the data frame into a matrix and transpose it. We want to have each cell
```

```

# type as a row name
FACS <- t(as.matrix(FACS))

#switch the matrix back to a data frame format
FACS <- as.data.frame(FACS)

# turn the first row into column names
FACS %>%
  row_to_names(row_number = 1) -> FACS

# Now further prepare the data frame for plotting by removing the first row
## and convert the column to row names with the cells
FACS -> heatmap_data

# turn the columns to numeric other wise the heatmap function will not work
heatmap_data[] <- lapply(heatmap_data, function(x) as.numeric(as.character(x)))

# remove columns with only NAs
heatmap_data <- Filter(function(x)!all(is.na(x)), heatmap_data)

#remove rows with only NAs
heatmap_data <- heatmap_data[, colSums(is.na(heatmap_data)) !=
  nrow(heatmap_data)]

annotation_df <- unique(annotation_df) %>%
  dplyr::filter(Mouse_ID %in% colnames(heatmap_data))

#select the row names from the annotation df
heatmap_data <- heatmap_data %>%
  dplyr::select(row.names(annotation_df))

# Match the row names to the heatmap data frame
rownames(annotation_df) <- colnames(heatmap_data)

annotation_df <- annotation_df %>%
  dplyr::select(-Mouse_ID)

heatmap_facs_FIELD <- heatmap_data

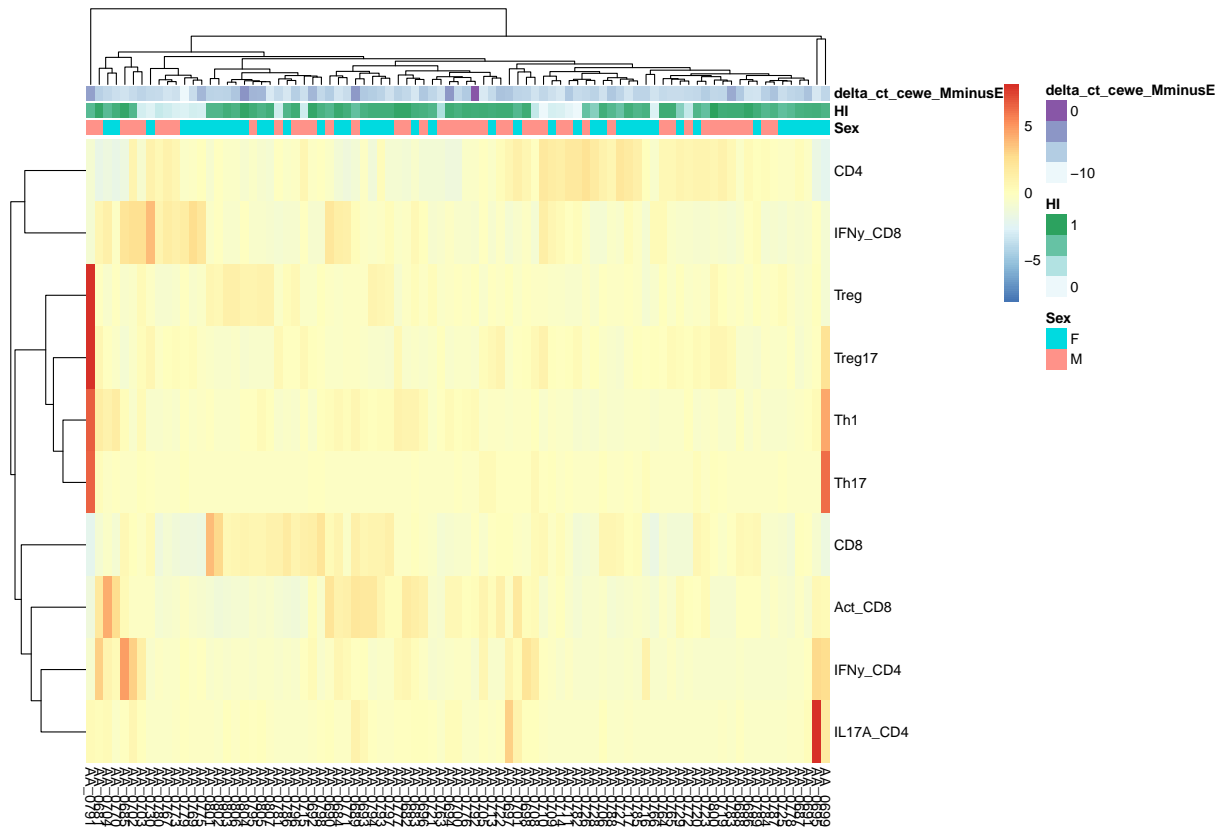
```

## Heatmap field facs: Plot

```

heatmap_data %>%
  pheatmap(annotation_col = annotation_df, scale = "row")

```



# Lab + Field Heatmap combination

```
# select the same cells for both
names_rows_field <- row.names(heatmap_facs_FIELD)

heatmap_facs_LAB <- heatmap_facs_LAB[row.names(heatmap_facs_FIELD), ]

heatmap_lab_field <- cbind(heatmap_facs_LAB, heatmap_facs_FIELD)

#Prepare the annotation data frame
annotation_df <- MICE %>%
  dplyr::select(origin, Mouse_ID)

annotation_df <- unique(annotation_df) %>%
  dplyr::filter(Mouse_ID %in% colnames(heatmap_lab_field))

### Prepare the annotation columns for the heatmap
rownames(annotation_df) <- annotation_df$Mouse_ID

annotation_df <- unique(annotation_df)

annotation_df <- as.data.frame(annotation_df)

# Match the row names to the heatmap data frame
rownames(annotation_df) <- colnames(heatmap_lab_field)
```

```
#remove the unnecessary column
annotation_df <- annotation_df %>% dplyr::select(-Mouse_ID, )
```

## Lab - Field data heatmap - plot

```
pheatmap(heatmap_lab_field, annotation_col = annotation_df, scale = "row")
```

