CD8 T

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```
library(ggplot2)
library(tidyverse)
library(Seurat)
library(Matrix)
library(SingleCellExperiment)
library(DropletUtils)
library(reshape2)
library(ComplexHeatmap)
library(circlize)
```

Single-cell RNA-seq of sorted CD8+ T cells from B16 melanoma tumors

Processed and filtered scRNA-seq data from NCBI GEO accession GSE116390. Filtered dataset contains 3574 cells filtered based on expression of CD8 T cell markers (Cd8a, Cd8b1, Cd2, not Cd4)

Read sparse matrix

```
b16_matrix <- Matrix::readMM("input/B16_data/matrix.mtx")
b16_genes <- read.table("input/B16_data/genes.tsv", sep = "\t")
```

Starting with looking at specific genes of interest

```
#genes_of_interest <-c("Tcf7", "Atf4", "Ddit3", "Ifng", "Slc2a1", "Mapk14", "Mapk11", "Mapk12", "Mapk13
genes_of_interest <-c("Tcf7", "Atf4", "Ddit3", "Ifng", "Slc2a1")
```

Filter out genes of interest from the B16 dataset

```
b16_indices <- b16_genes %>% mutate(i = 1:n()) %>% filter(V1 %in% genes_of_interest) %>% pull(i)
b16_gene_expression <- b16_matrix %>% as.matrix() %>% t() %>% as_tibble() %>%
  select(b16_indices)
```

```
## Warning: The `x` argument of `as_tibble.matrix()` must have unique column names if `.name_repair` is
## Using compatibility `.name_repair`.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was generated.
```

```
names(b16_gene_expression) <- genes_of_interest
```

LogNormalize gene_expressions, remove rows where all values are 0

```
b16_gene_expression <- b16_gene_expression %>% LogNormalize() %>% as_tibble()
b16_gene_expression <- b16_gene_expression %>% filter_all(any_vars(. != 0))
```

Distribution of expression for each TF

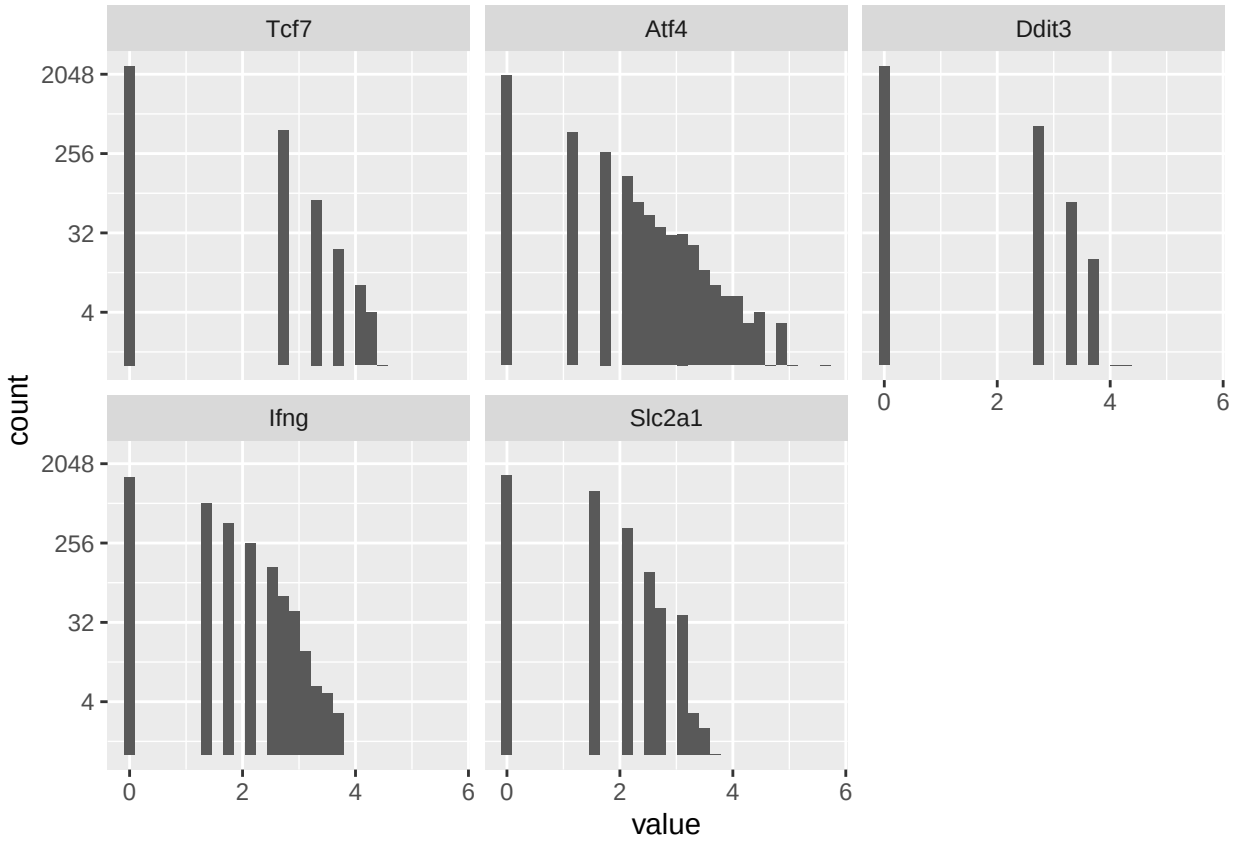
```
b16_gene_expression %>% melt() %>% ggplot(aes(value)) + geom_histogram() + facet_wrap(~ variable) + scal
```

```
## No id variables; using all as measure variables
```

```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```

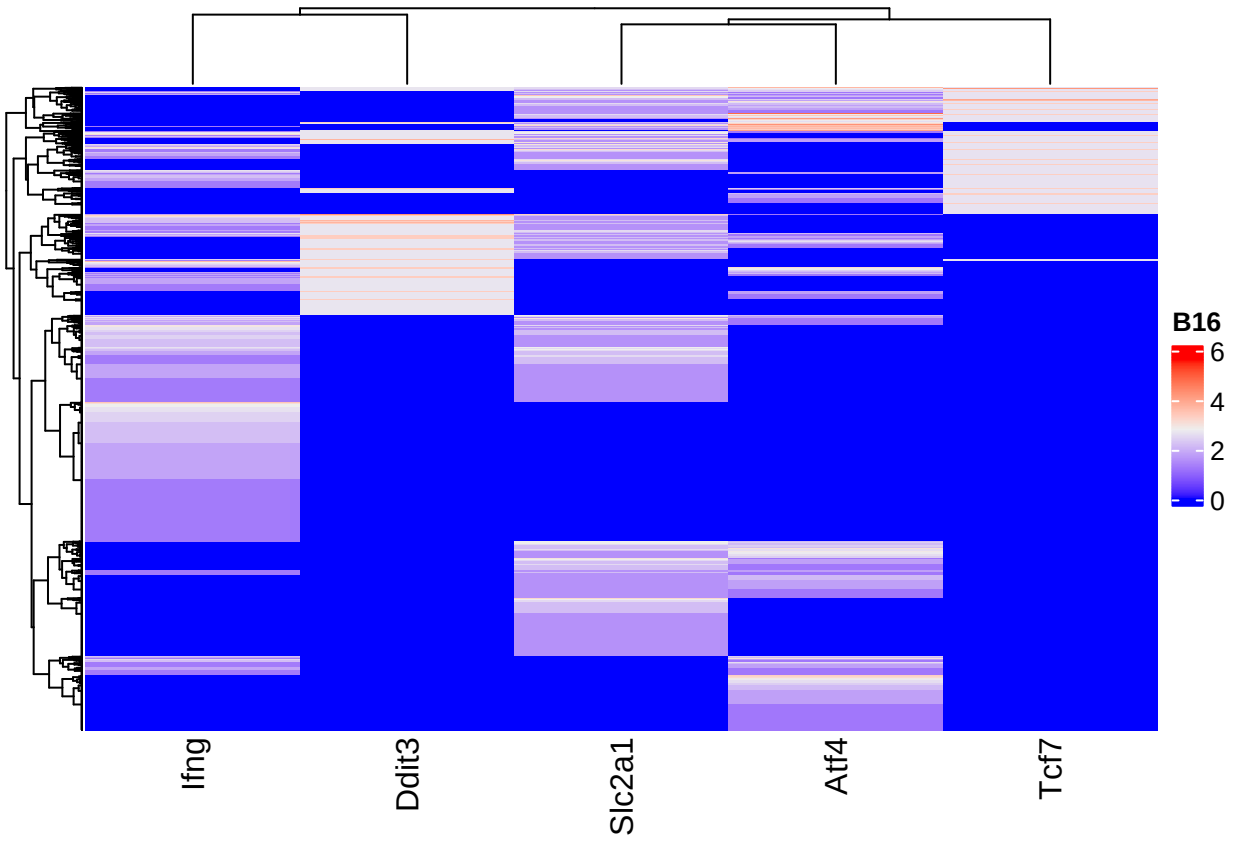
```
## Warning: Transformation introduced infinite values in continuous y-axis
```

```
## Warning: Removed 97 rows containing missing values (geom_bar).
```



Heatmap (default settings)

```
#f1 <- colorRamp2(c(0, 0.1, 1, 4, 10), c("grey", "blue", "#EEEEEE", "red", "black"))
f1 <- colorRamp2(seq(min(b16_gene_expression), max(b16_gene_expression), length = 3), c("blue", "#EEEEE
h1 <- Heatmap(as.matrix(b16_gene_expression), name = "B16", col = f1)
draw(h1)
```



Single cell RNA-seq profiling of T cells isolated from MC38 mouse tumors

Processed scRNA-seq data from EMBL-EBI accession E-MTAB-7919

Read sparse matrix

```
mc38_matrix <- Matrix::readMM("input/MC38_data/matrix.mtx")
mc38_genes <- read.table("input/MC38_data/genes.tsv", sep = "\t")
```

Filter out genes of interest

```
mc38_indices <- mc38_genes %>%
  mutate(i = 1:n()) %>%
  filter(V2 %in% genes_of_interest) %>%
  pull(i)
mc38_gene_expression <- mc38_matrix %>%
  as.matrix() %>%
  t() %>%
  as_tibble() %>%
  select(mc38_indices)
names(mc38_gene_expression) <- genes_of_interest
```

LogNormalize gene_expressions, remove rows where all values are 0

```
mc38_gene_expression <- mc38_gene_expression %>% LogNormalize() %>% as_tibble()
mc38_gene_expression <- mc38_gene_expression %>% filter_all(any_vars(. != 0))
```

Distribution of expression for each TF

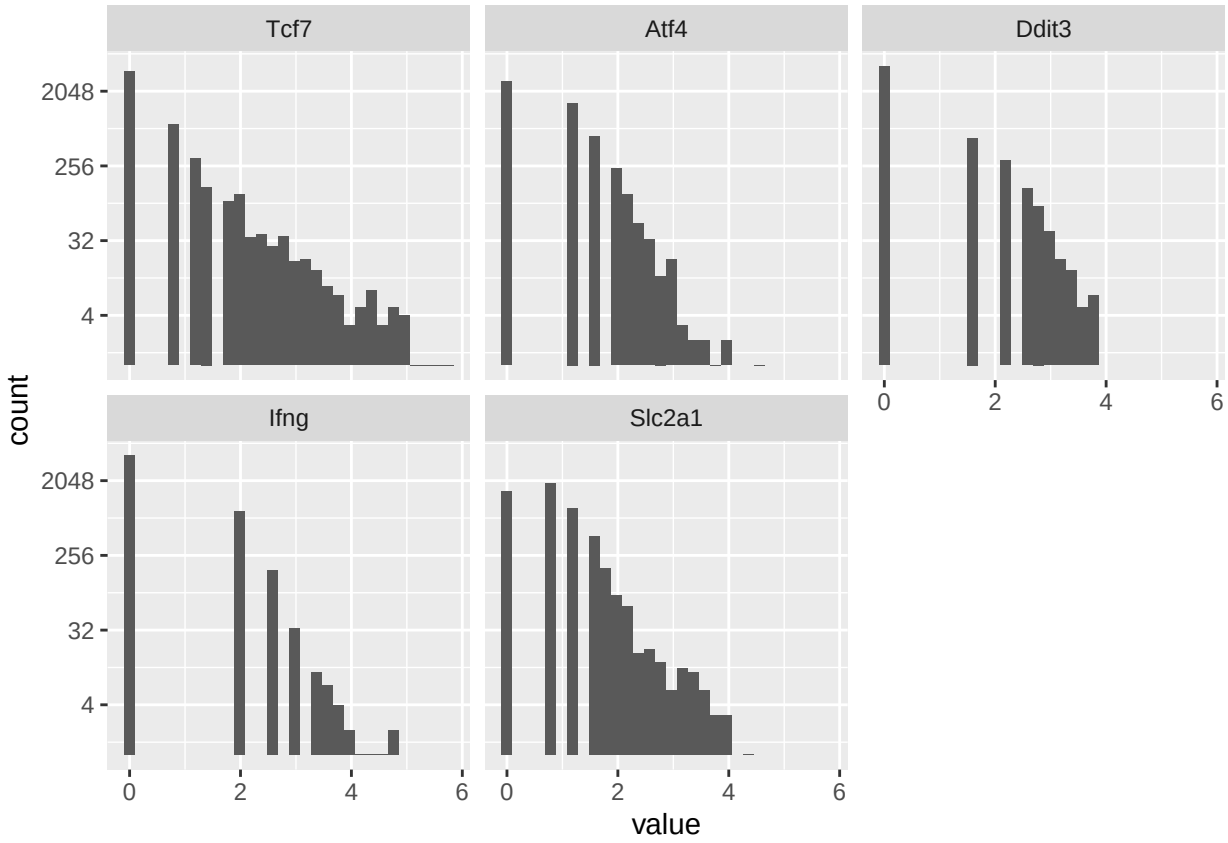
```
mc38_gene_expression %>% melt() %>% ggplot(aes(value)) + geom_histogram() + facet_wrap(~ variable) + sca
```

```
## No id variables; using all as measure variables
```

```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```

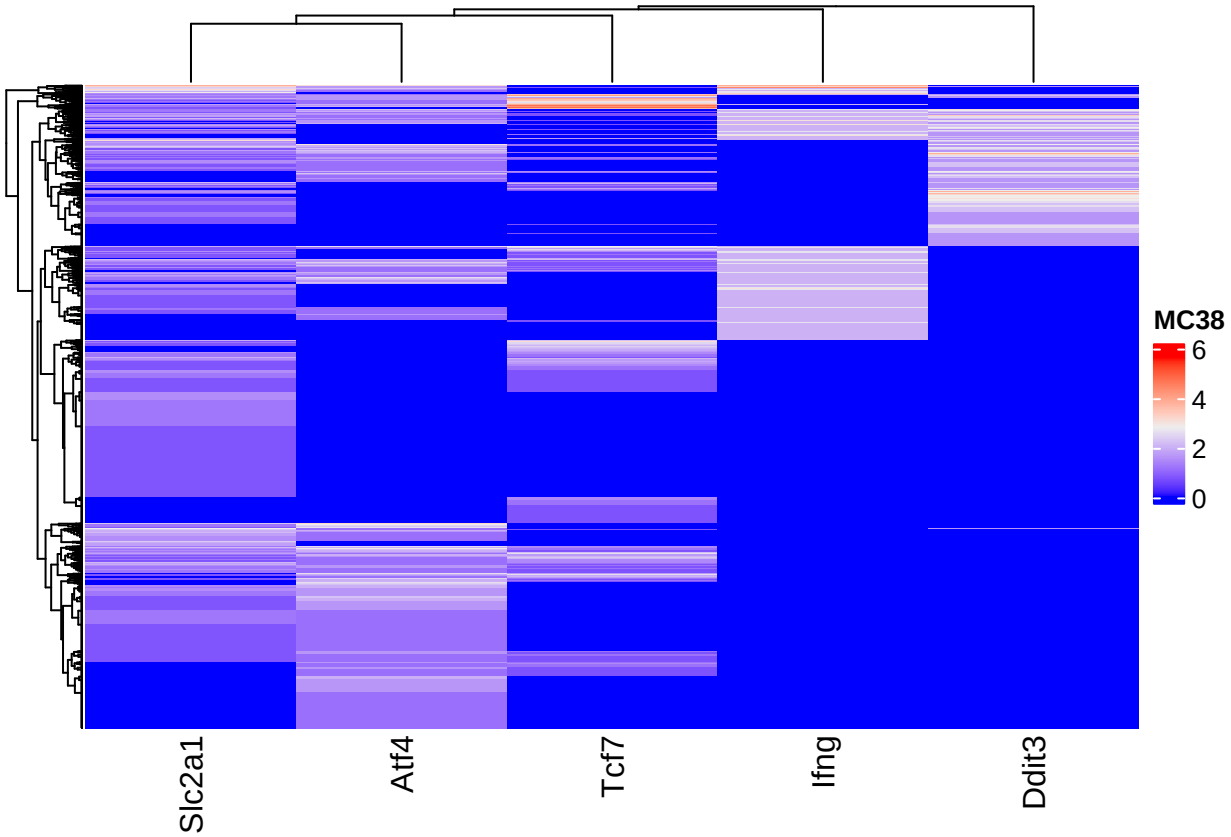
```
## Warning: Transformation introduced infinite values in continuous y-axis
```

```
## Warning: Removed 71 rows containing missing values (geom_bar).
```



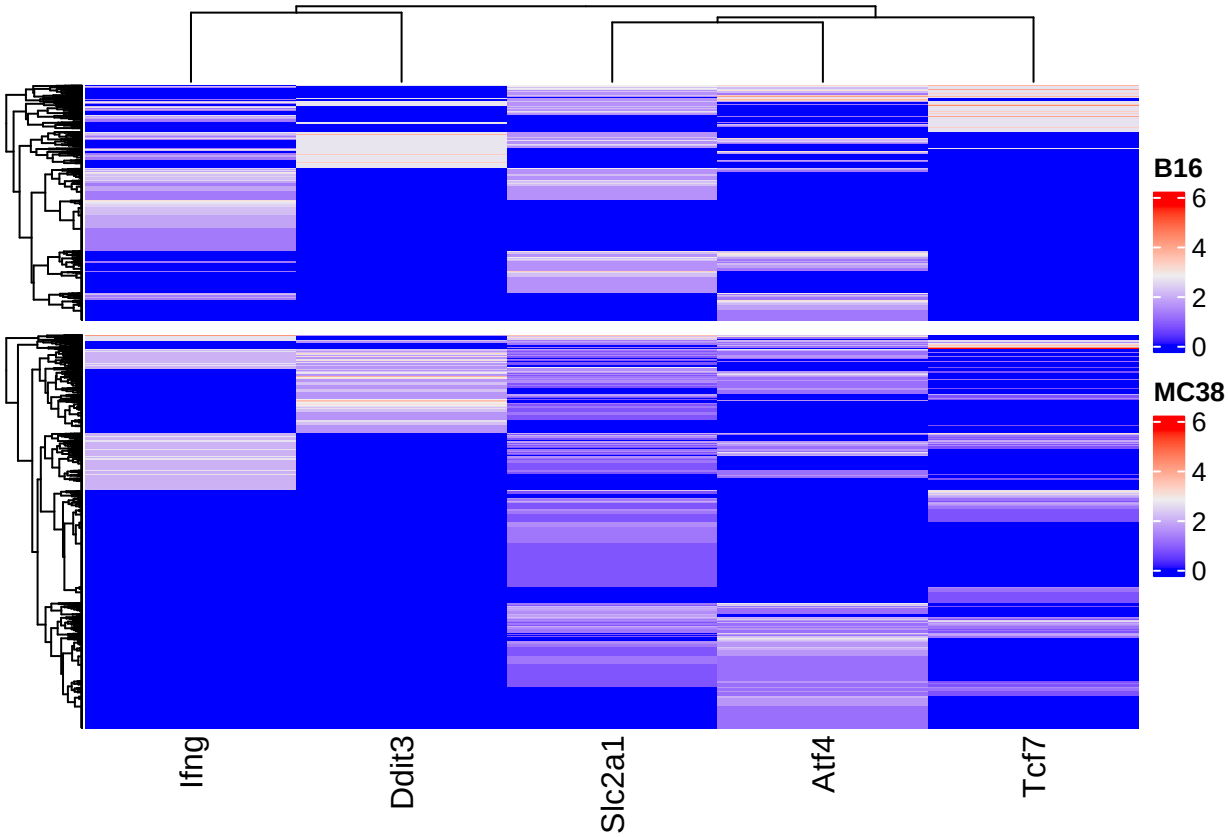
Heatmap using default settings

```
h2 <- Heatmap(as.matrix(mc38_gene_expression), name = "MC38", col = f1)
draw(h2)
```



Draw heatmaps from the two studies together

```
draw(h1 %v% h2)
```


B16
6
4
2
0
MC38
6
4
2
0
Ifng
Ddit3
Slc2a1
Atf4
Tcf7