4.Gene_expressions_analysis - Analyzing differences between field and lab gene expression data

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Aims:

- Compare immune gene expression between lab and field data
- How do eimeria infections affect the expression of various immune genes?
- Does the expression vary between lab and field data?

load libraries

```
library(ggplot2)
library(tidyverse)
## -- Attaching packages -
                                                 ----- tidyverse 1.3.1 --
## v tibble 3.1.8
                    v dplyr
                            1.0.9
## v tidyr 1.2.0
                    v stringr 1.4.0
## v readr
         2.1.2
                    v forcats 0.5.1
## v purrr
          0.3.4
## -- Conflicts ------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()
                  masks stats::lag()
```

Import data:

Here, we have the experimental / field data, inlcuding imputed data

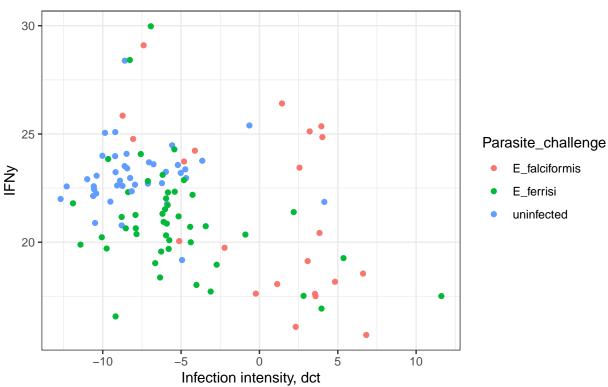
```
lab <- read.csv("output_data/gene_expression/data_products/lab_imputed_gene_expression.csv")
field <- read.csv("output_data/gene_expression/data_products/field_imputed_gene_expression.csv")</pre>
```

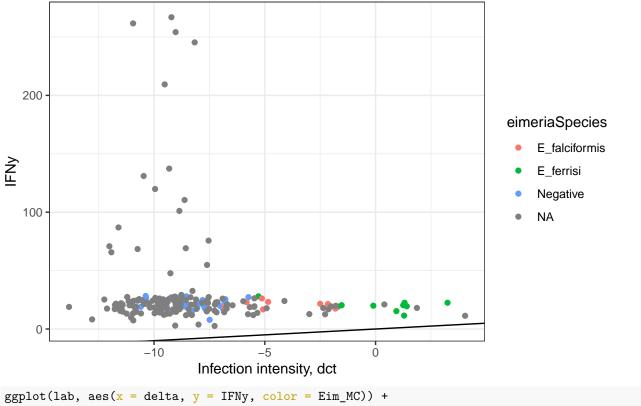
Selecting genes

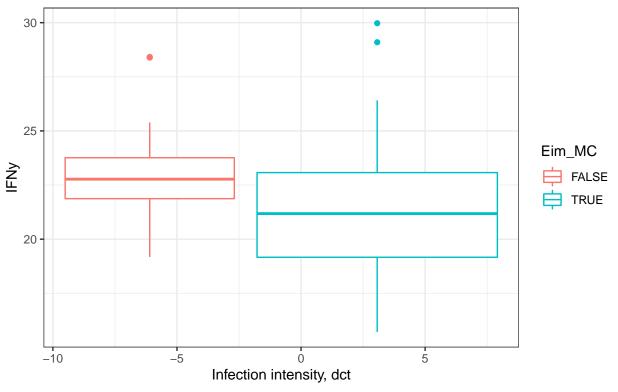
1. IFNy

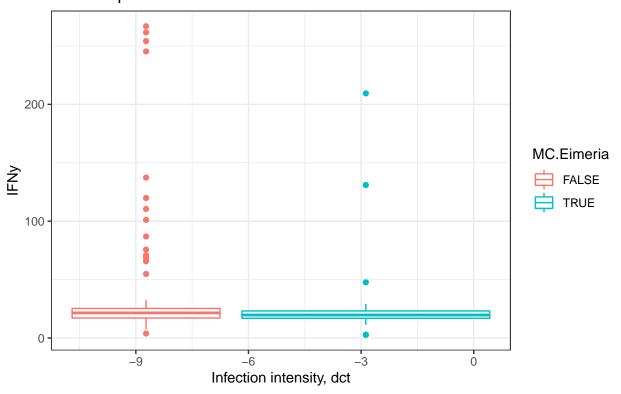
Warning: Removed 6 rows containing missing values (geom_point).

Immune gene expression against infection intensity in lab infections

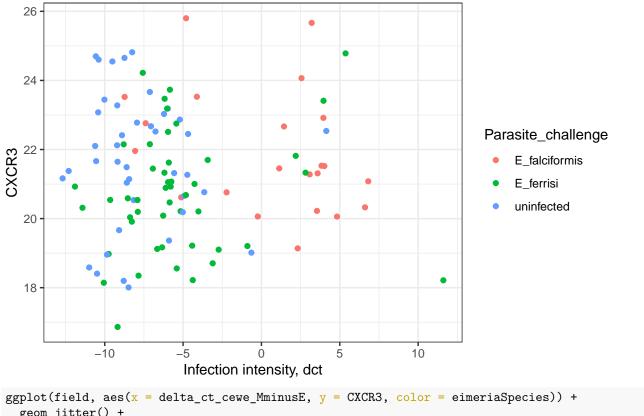


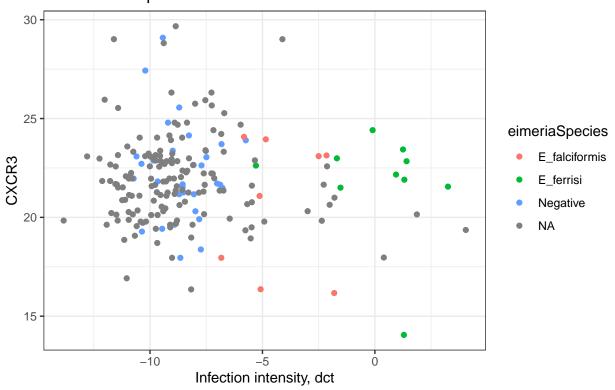




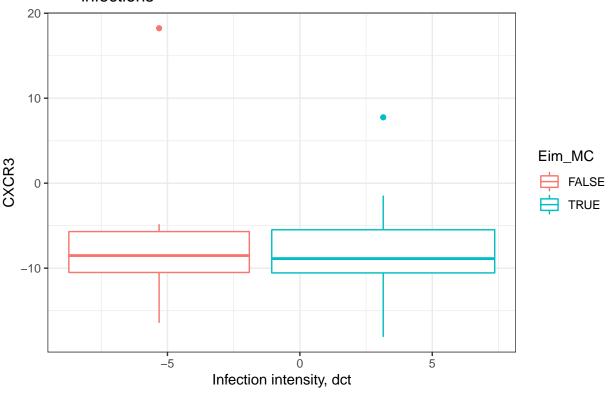


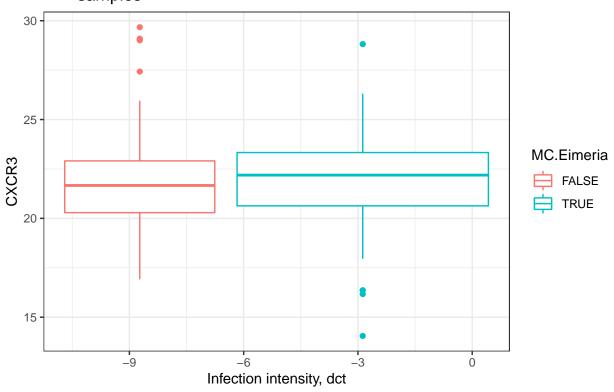
2. CXCR3



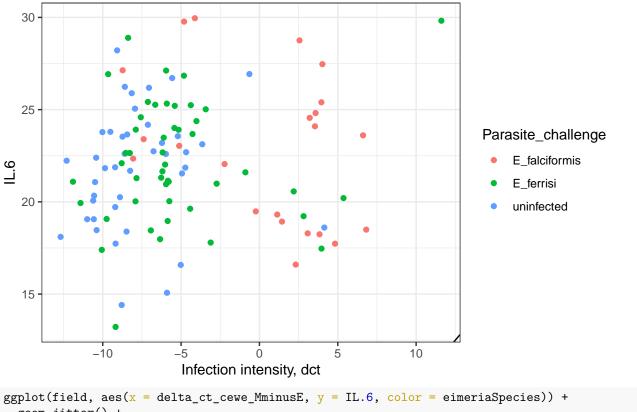


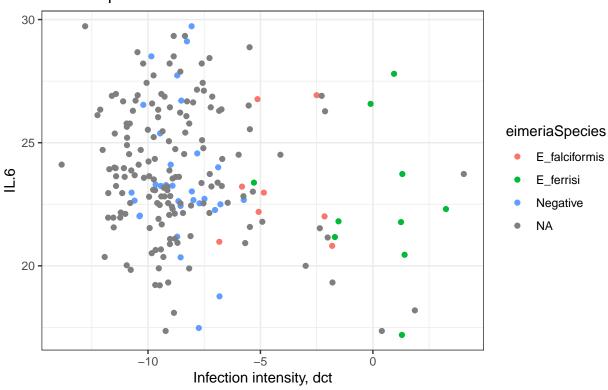
- ## Warning: Removed 6 rows containing missing values (stat_boxplot).
- ## Warning: Removed 49 rows containing non-finite values (stat_boxplot).



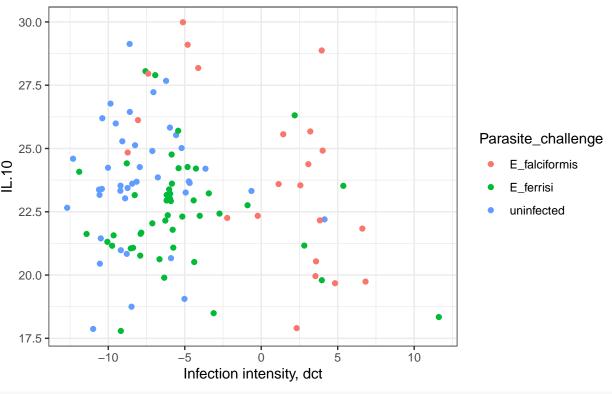


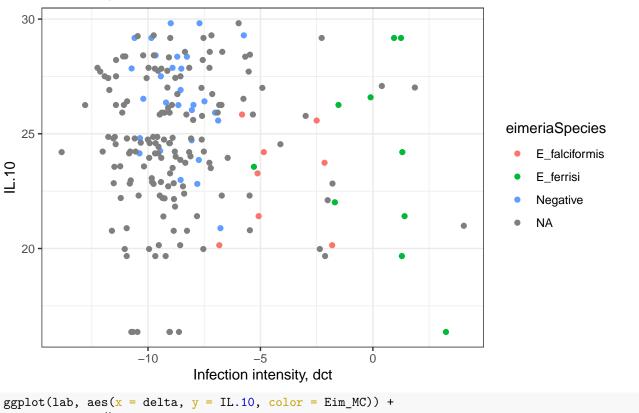
3. IL.6

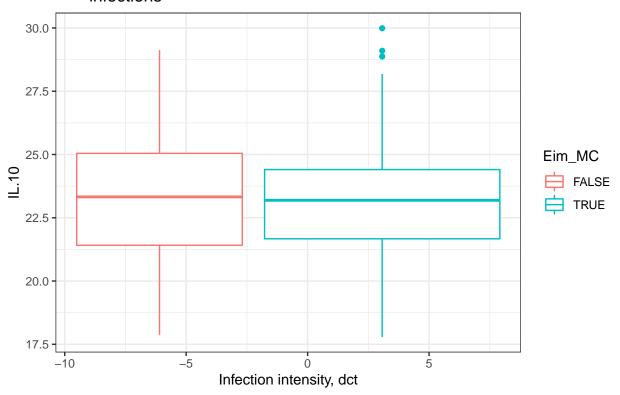


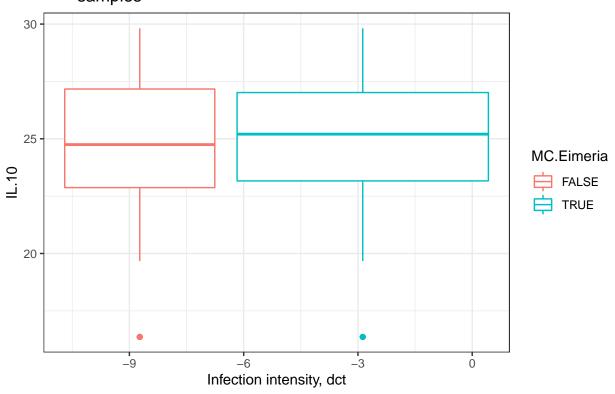


4. IL.10

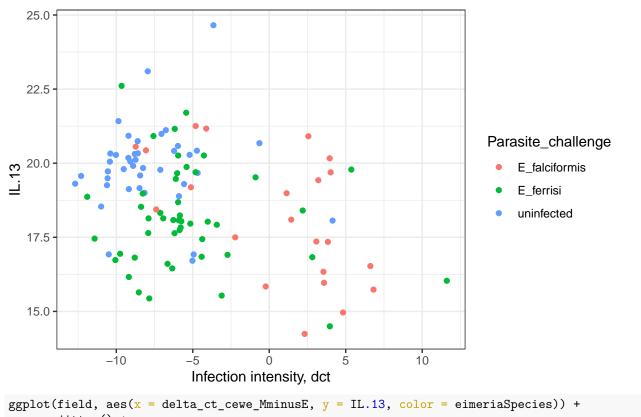


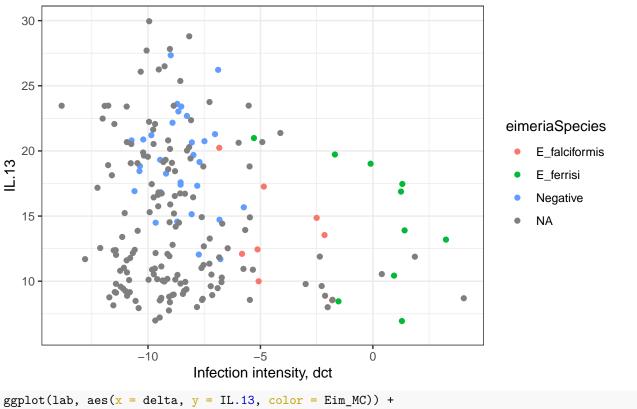


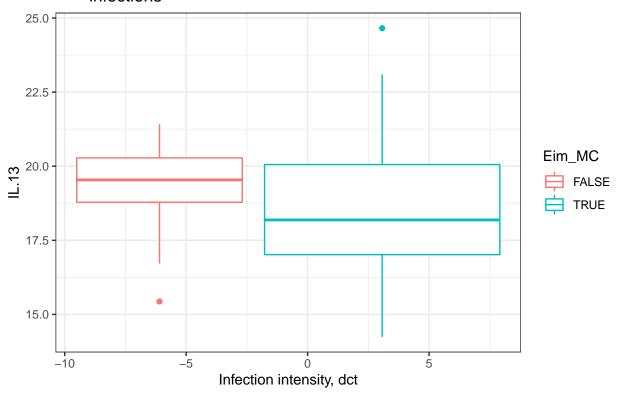


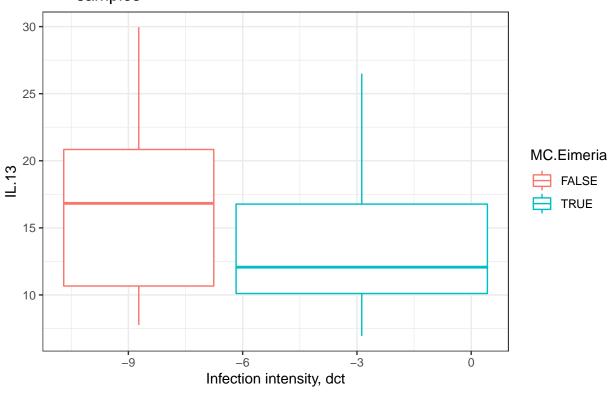


5. IL.13

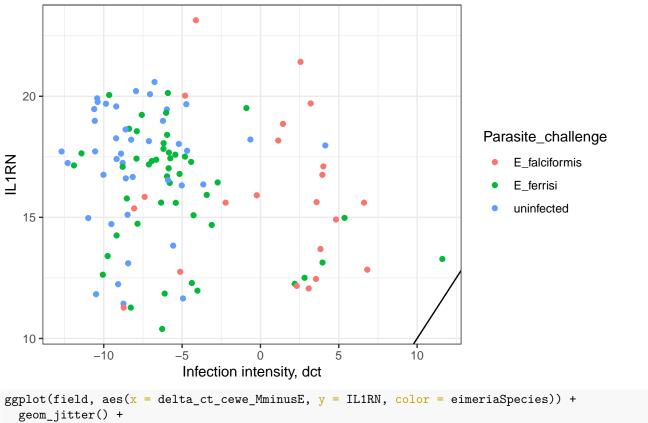


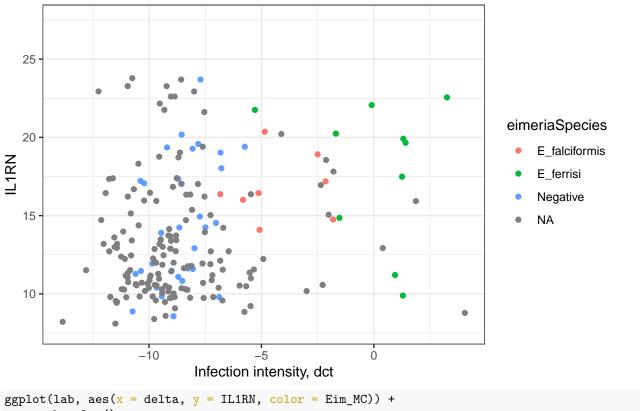


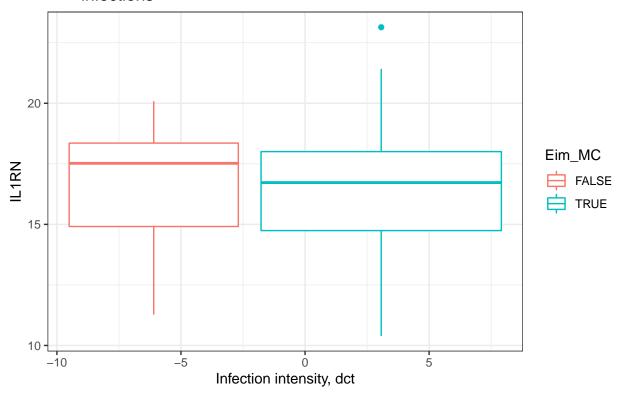


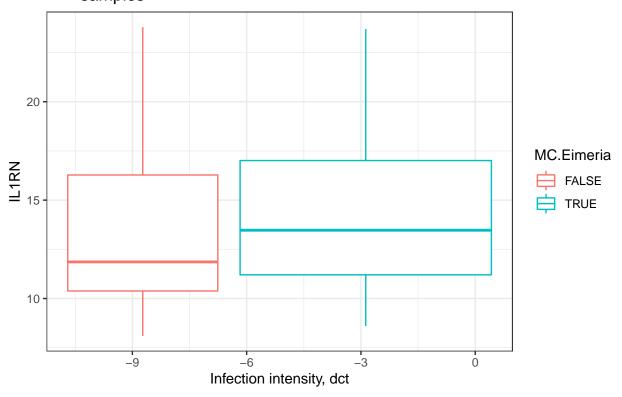


6. IL1RN

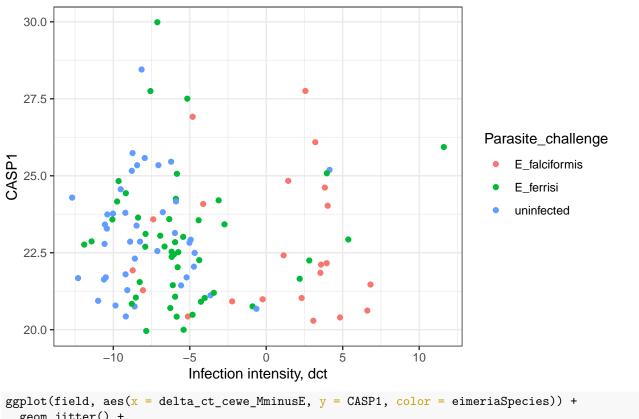


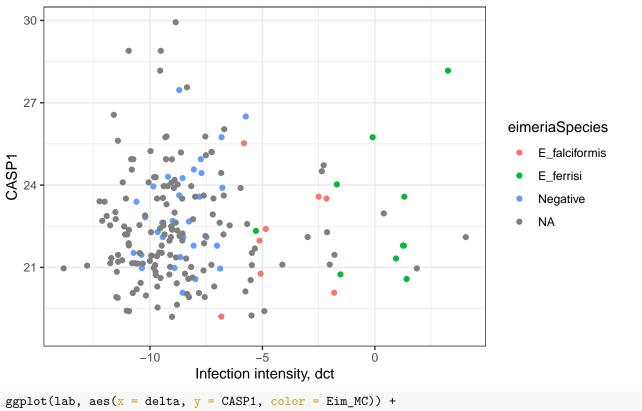


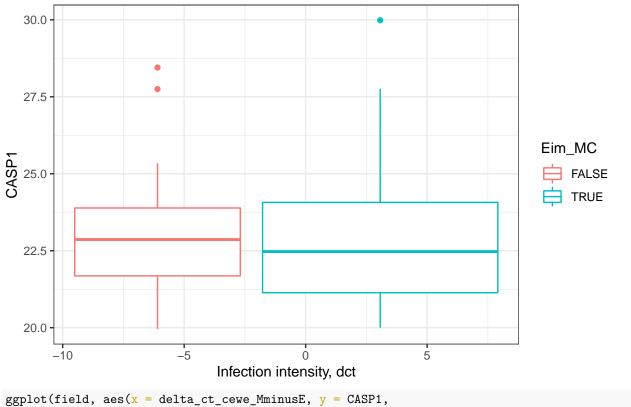


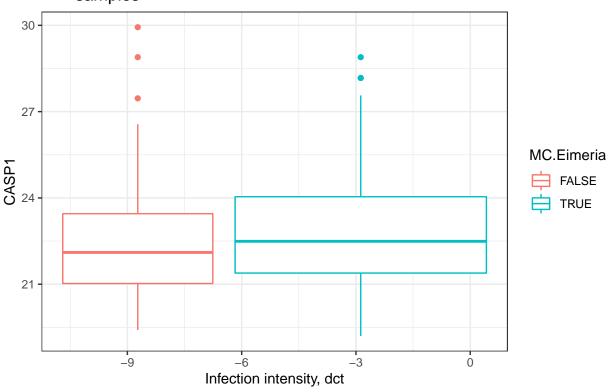


7. CASP1

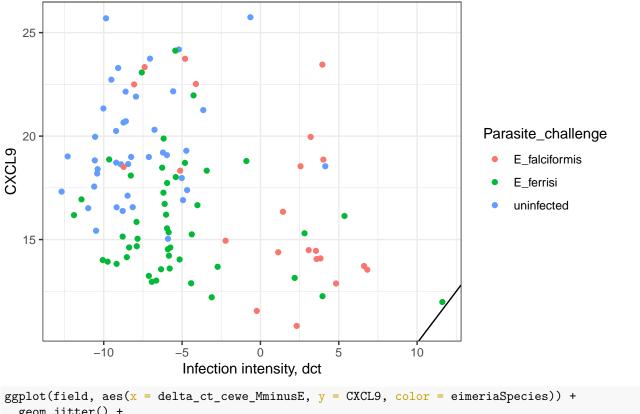


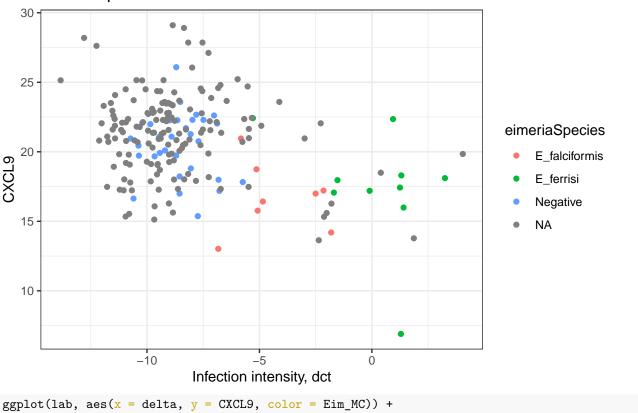


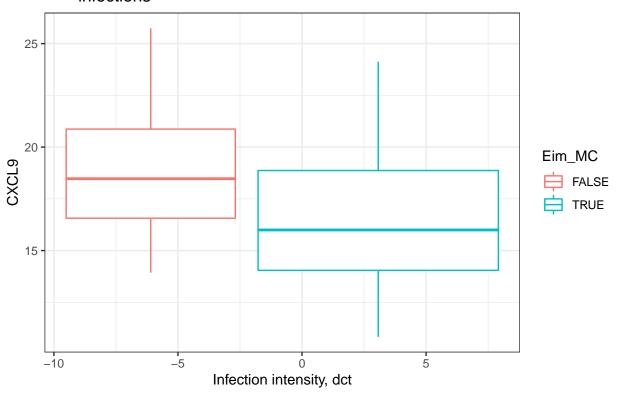


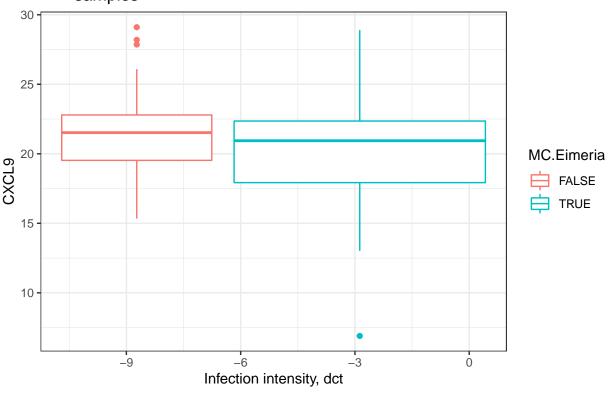


8. CXCL9

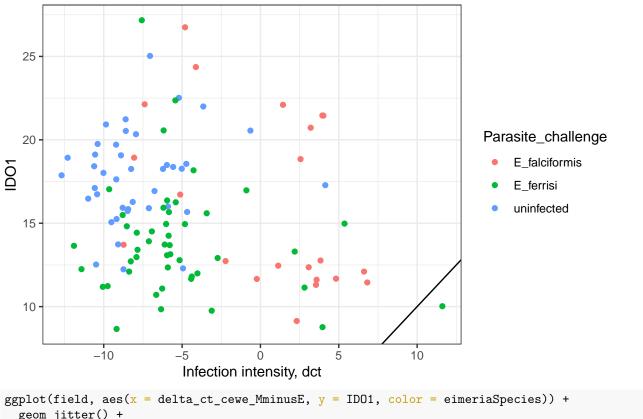


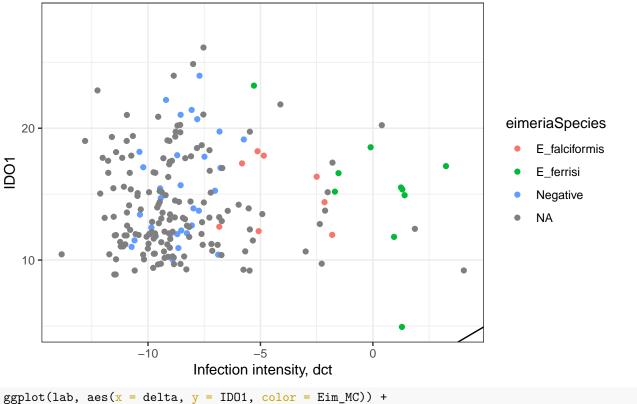


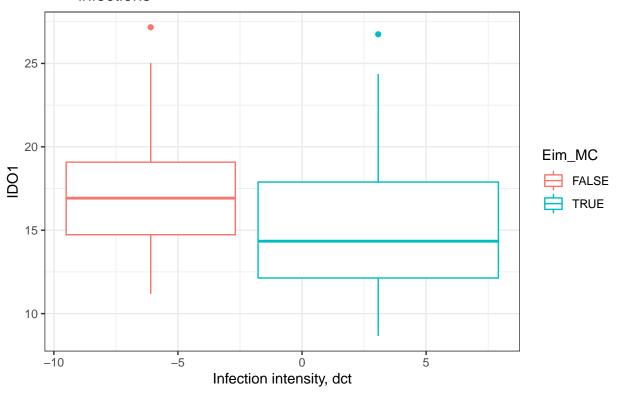


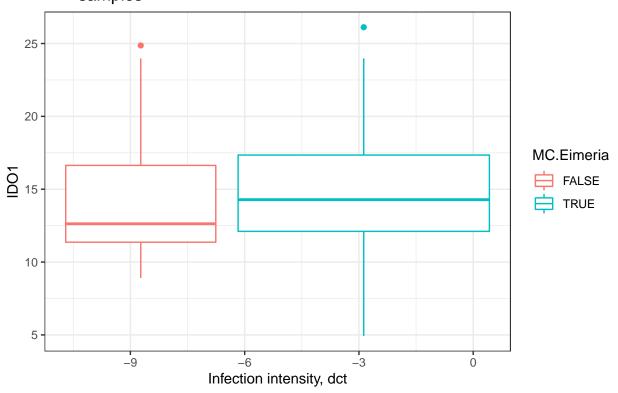


9. IDO1

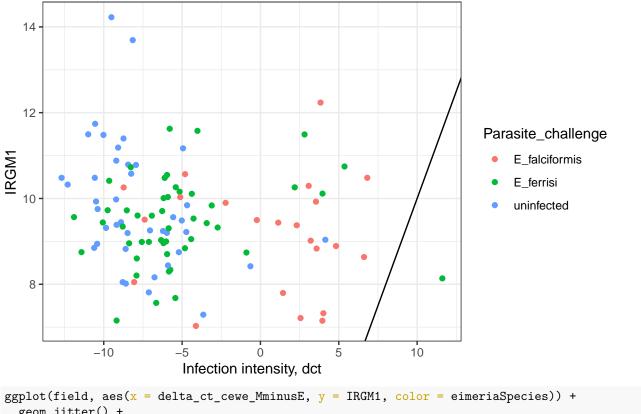


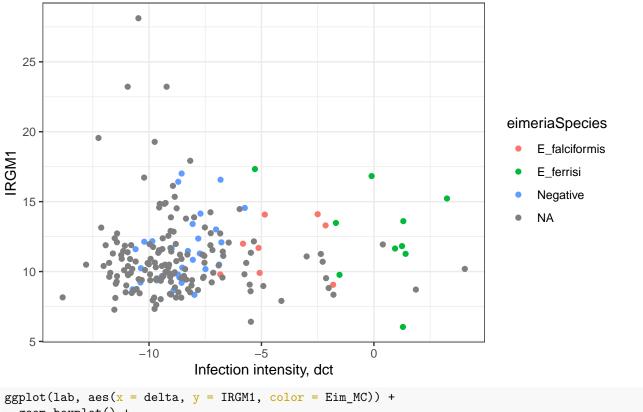


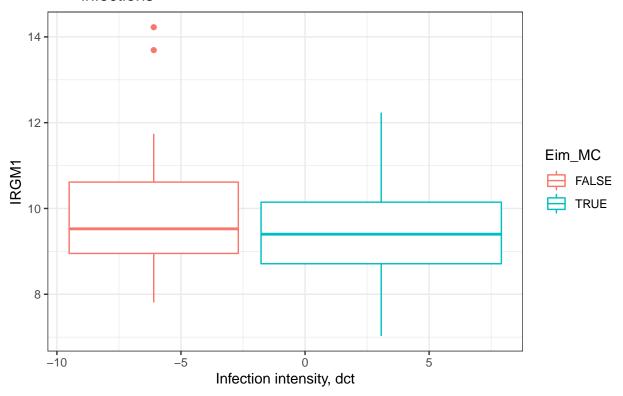


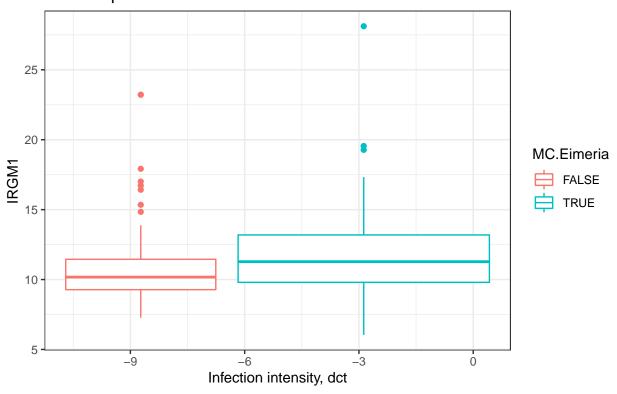


10. IRGM1

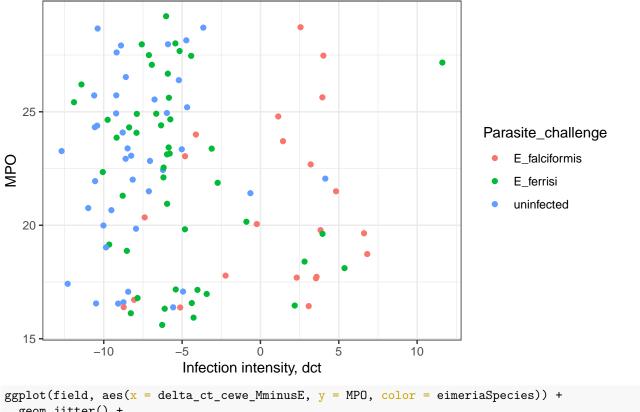


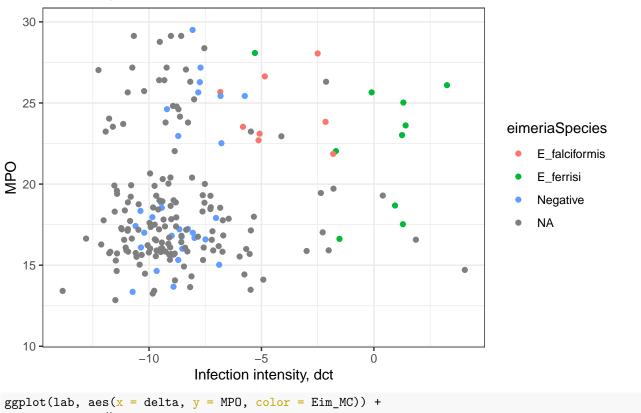


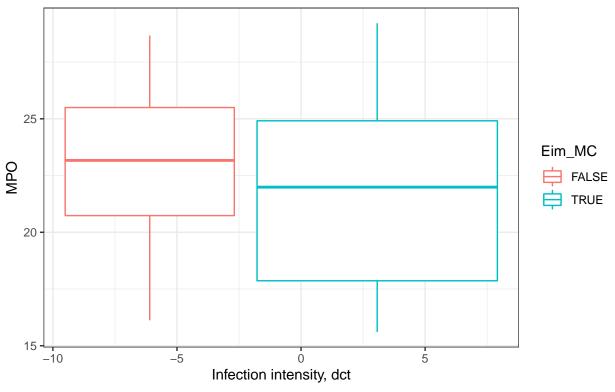


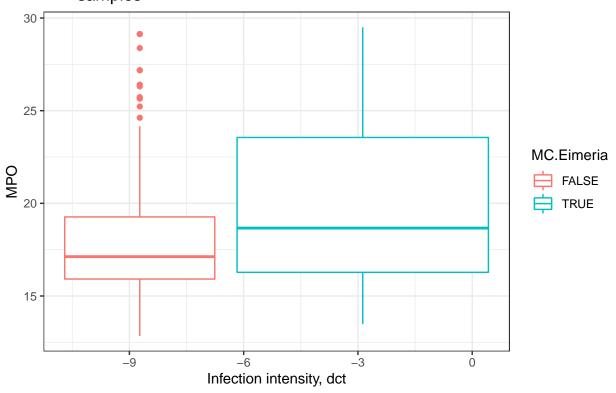


11. MPO

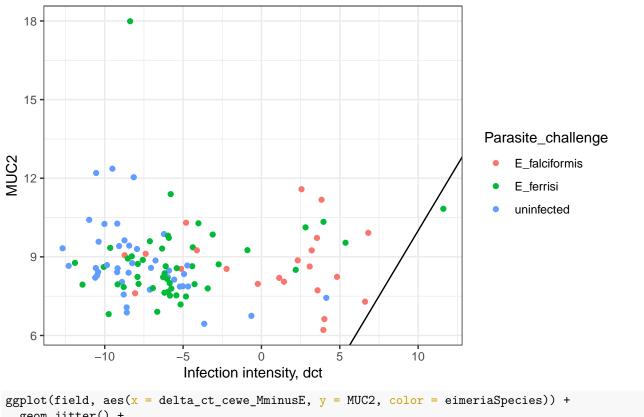


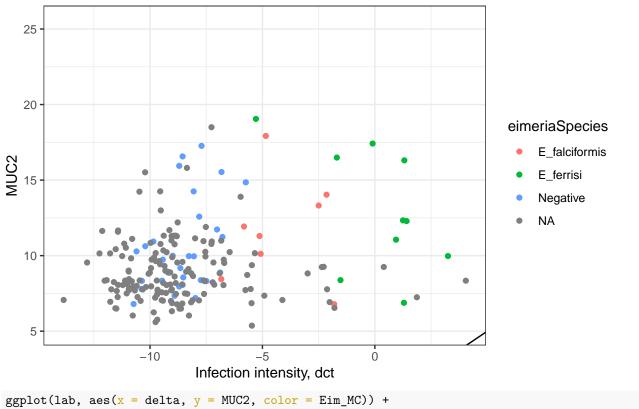


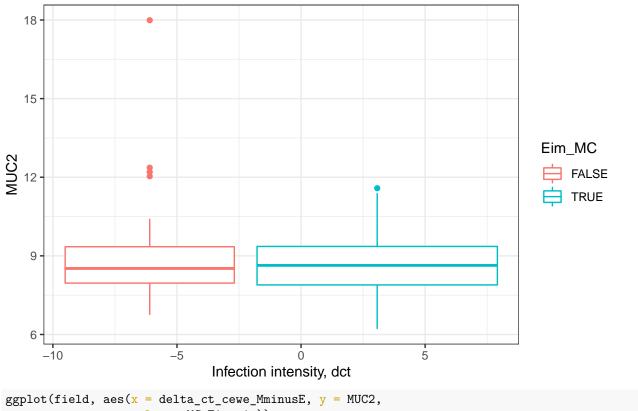


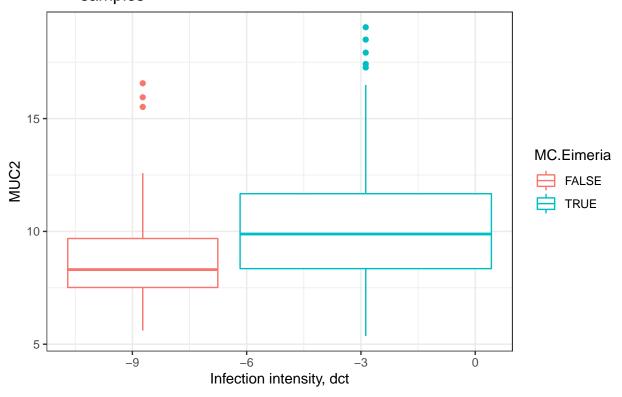


12. MUC2

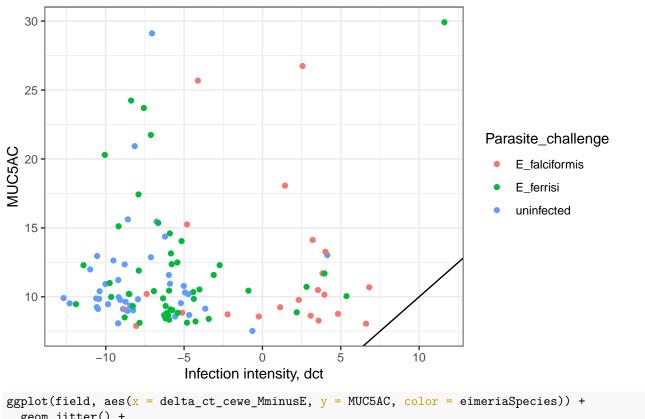


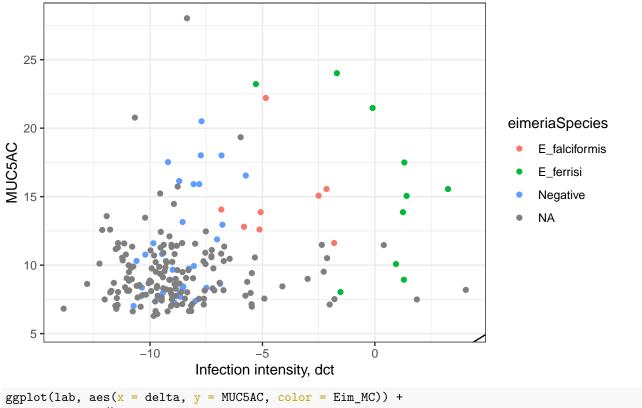


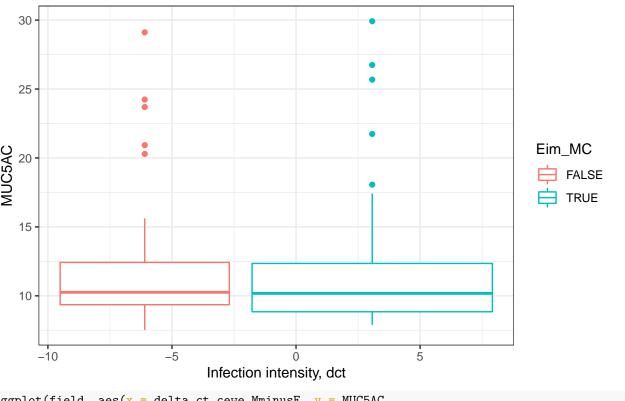


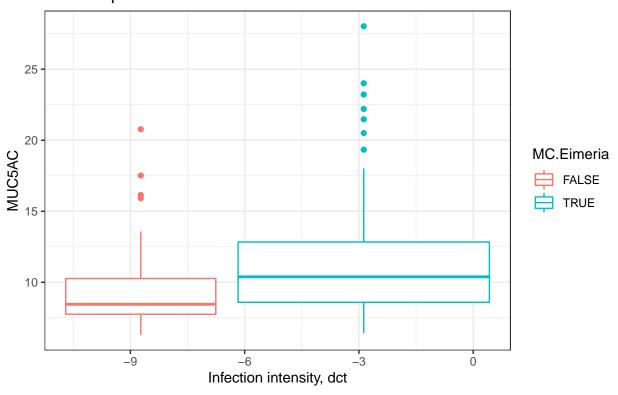


13. MUC5AC

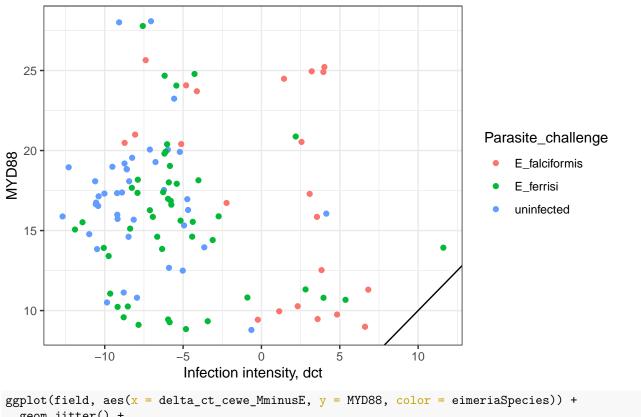


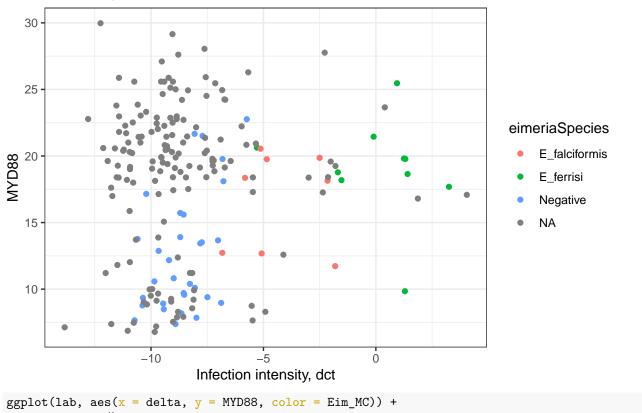


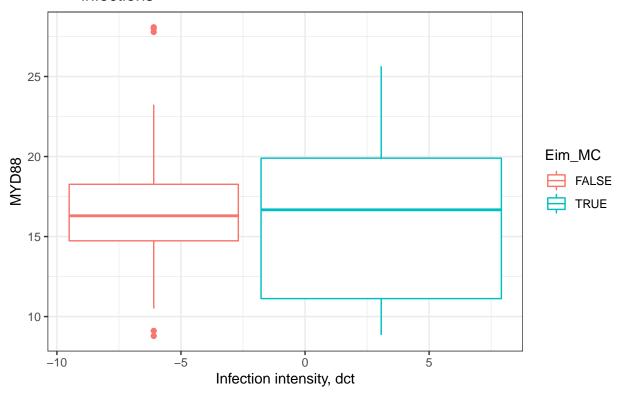


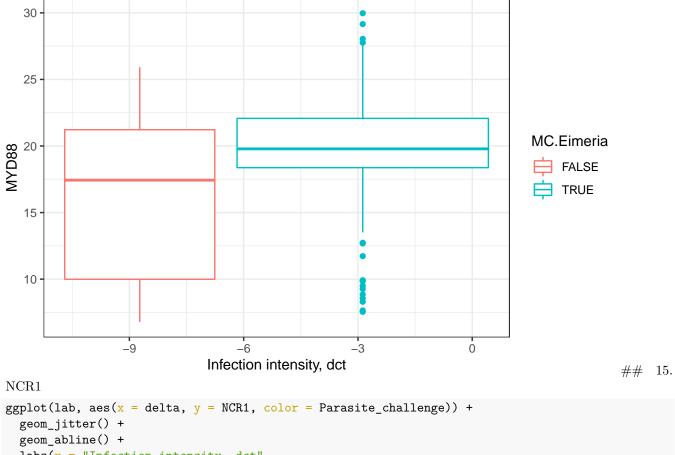


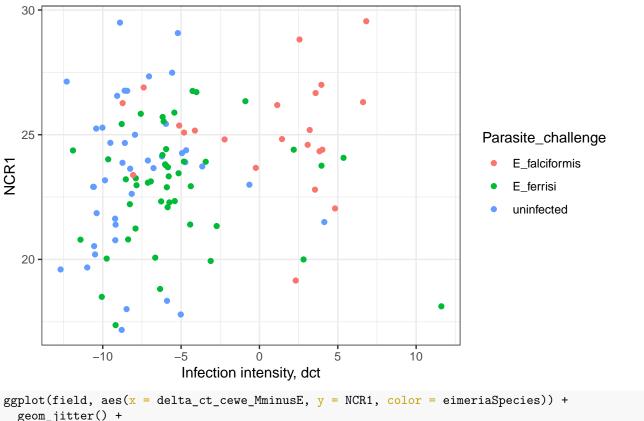
14. MYD88

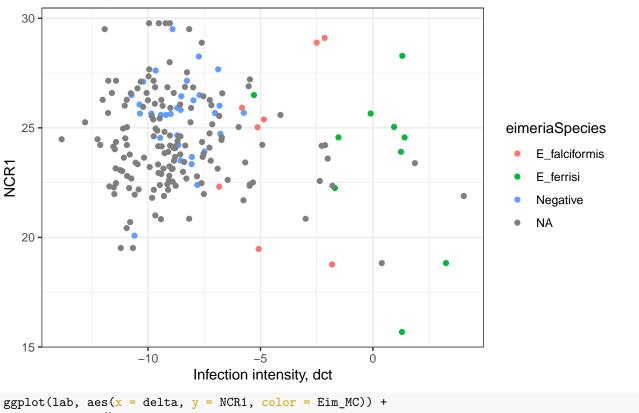


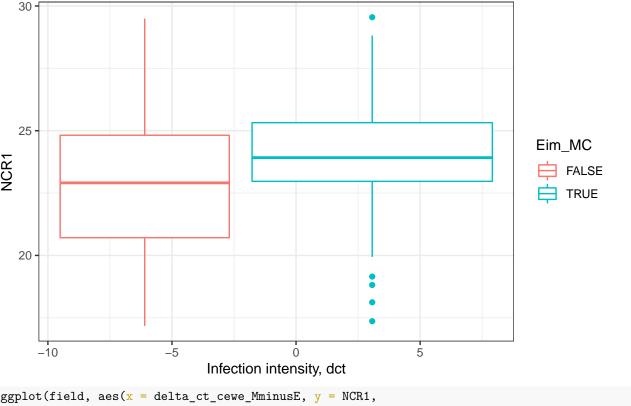


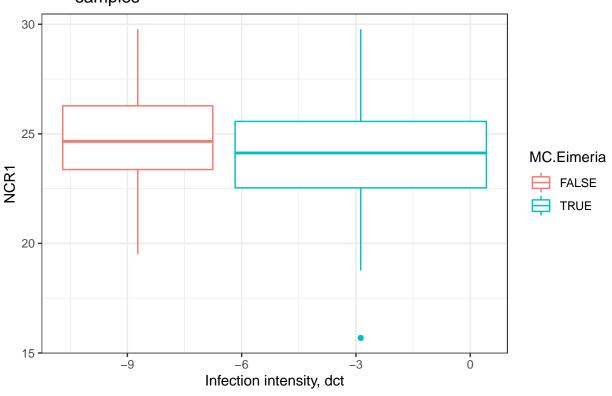




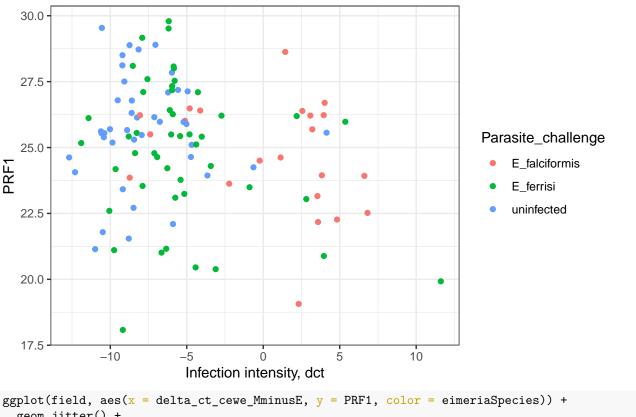


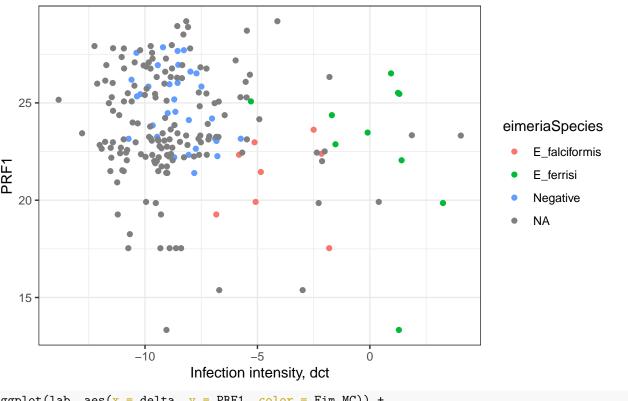


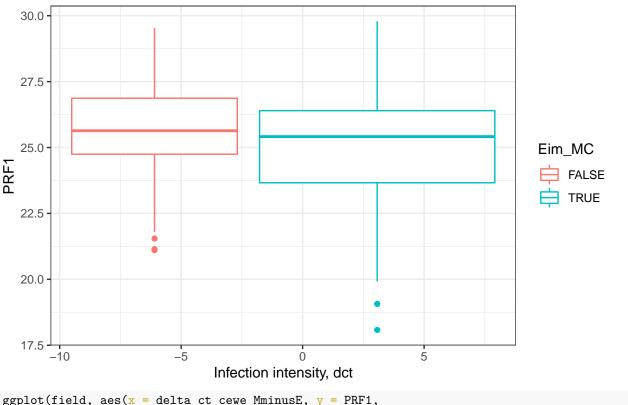


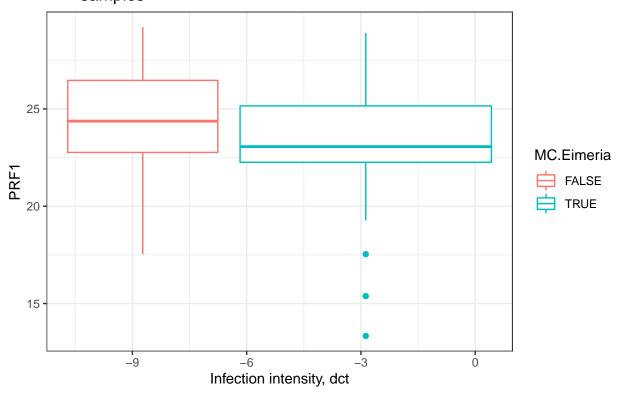


16. PRF1

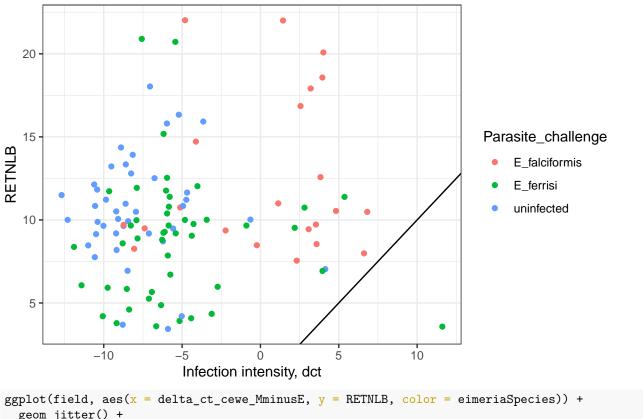


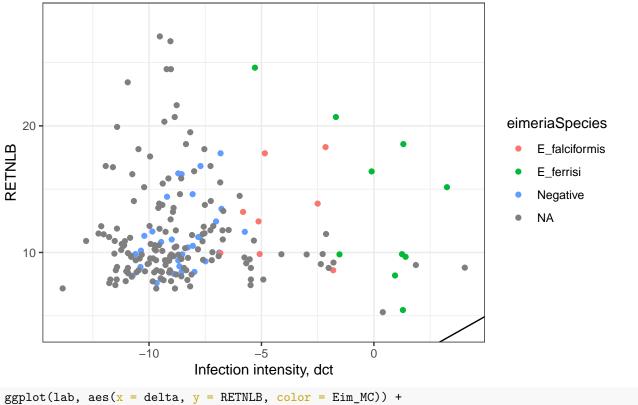


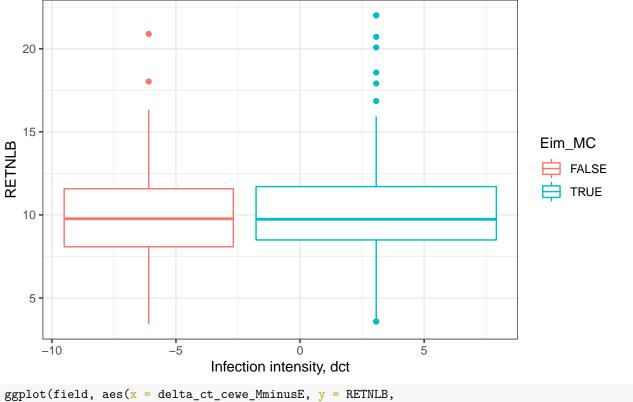


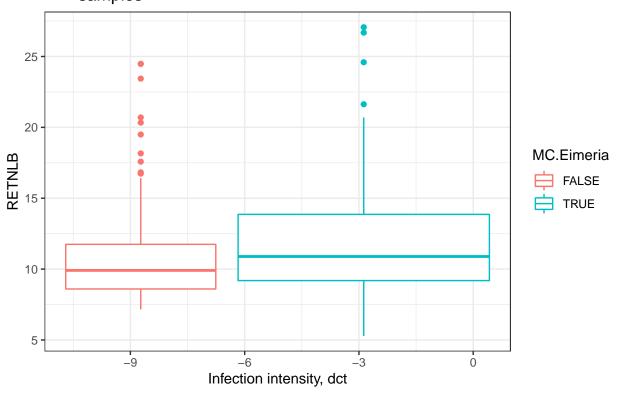


17. RETNLB

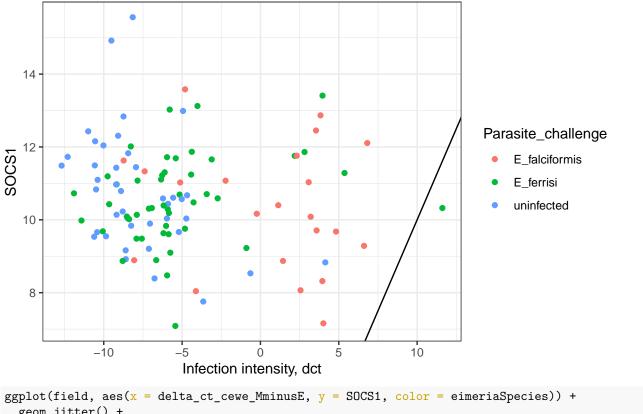


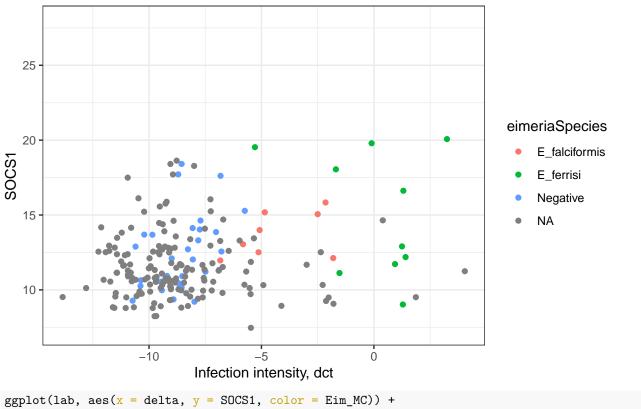


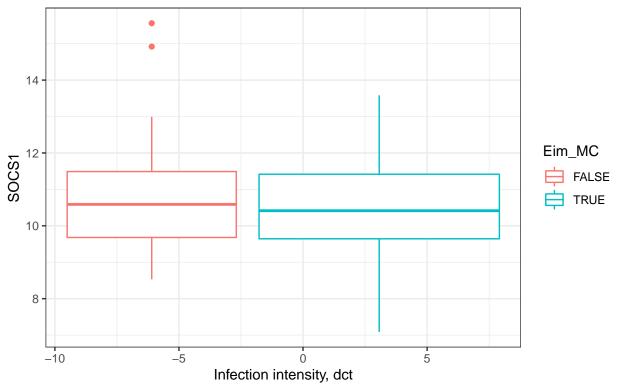


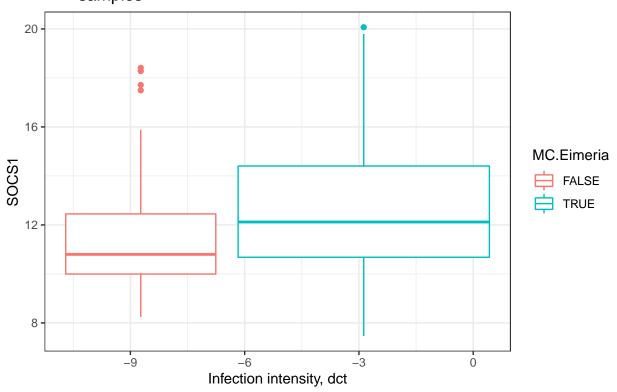


18. SOCS1

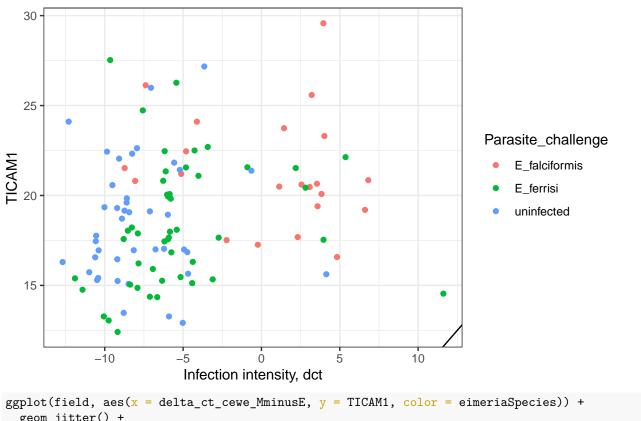


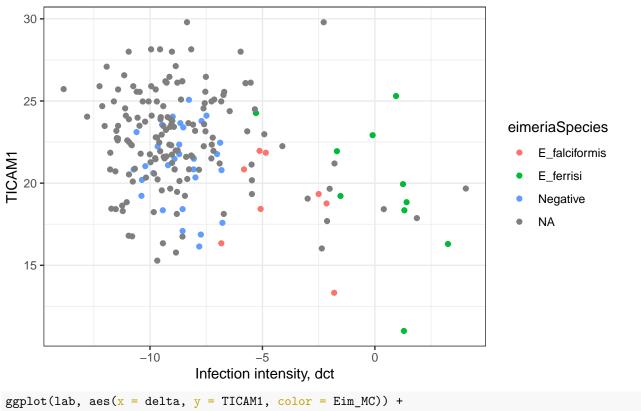


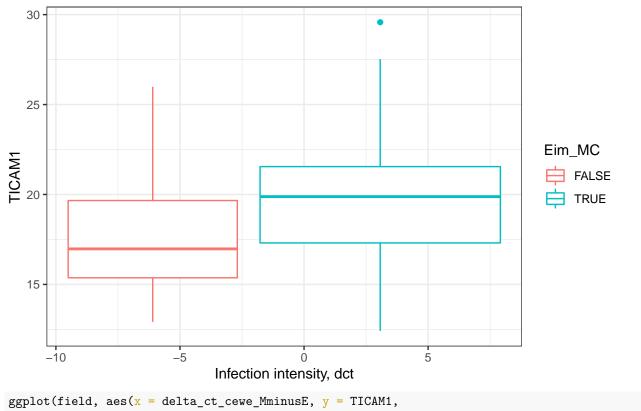


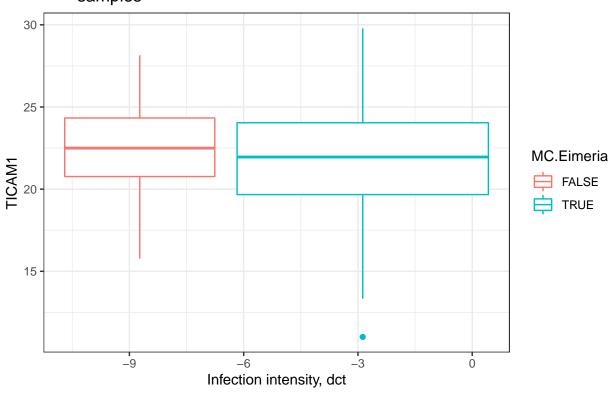


19. TICAM1

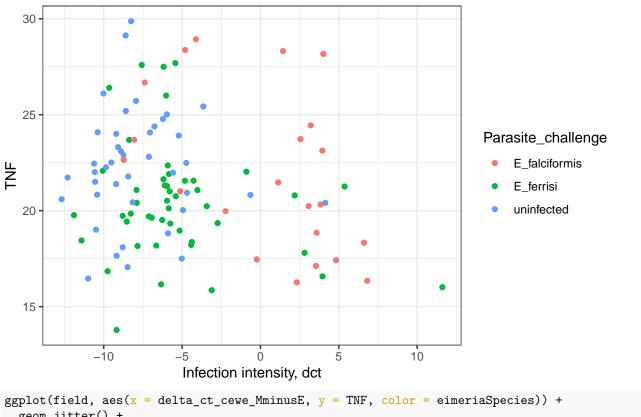


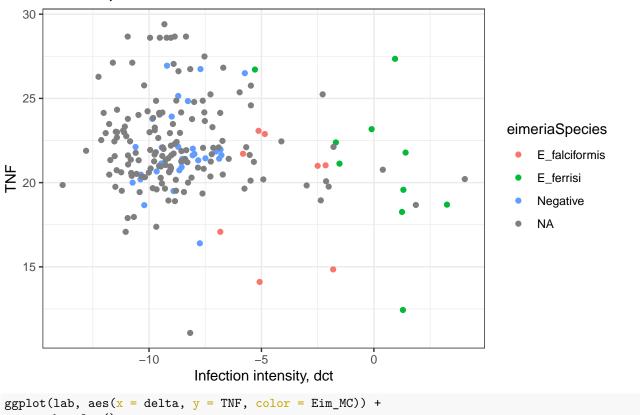


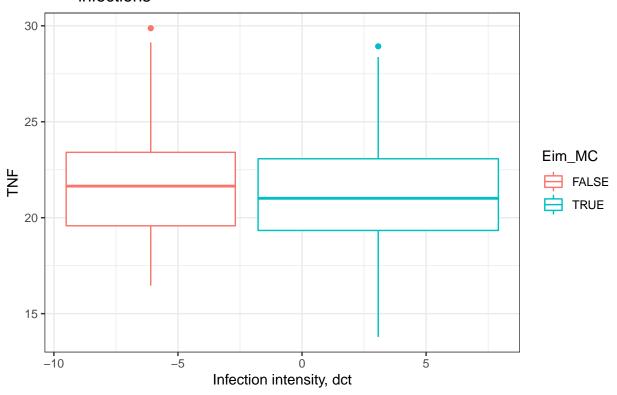


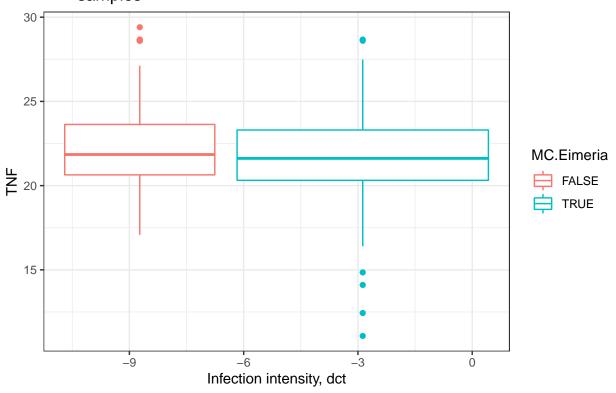


20. TNF









Compare the differences in uninfected and infected data and field / lab

Write a function which does a t test to quantify the differences of gene expression between uninfected and infected mice in the lab and field infections and also compares the uninfected lab and field and the infected lab and field

Function to print the p values for each t tet

```
eimeria_t_test_pvalue <- function(x) {</pre>
 # select the uninfected (delta ct under -5 mice)
  lab_uni <- lab %>%
    dplyr::filter(Eim_MC == "FALSE")
# select the infected lab mice
  lab_inf <- lab %>%
    dplyr::filter(Eim_MC == "TRUE")
# select the uninfected field mice (delta ct under -5 mice)
  field uni <- field %>%
    dplyr::filter(MC.Eimeria == "FALSE")
# select the infected lab mice
  field_inf <- field %>%
    dplyr::filter(MC.Eimeria == "TRUE")
lab.uni_lab.inf <- t.test(lab_uni %>% dplyr::select(all_of(x)),
                          lab_inf %>% dplyr::select(all_of(x)))$p.value
field.uni_field.inf <- t.test(field_uni %>% dplyr::select(all_of(x)),
```

```
field_inf %>% dplyr::select(all_of(x)))$p.value
lab.uni_field_uni <- t.test(lab_uni %>% dplyr::select(all_of(x)),
                             field_uni %>% dplyr::select(all_of(x)))$p.value
lab.inf_field.inf <- t.test(lab_inf %>% dplyr::select(all_of(x)),
                             field_inf %>% dplyr::select(all_of(x)))$p.value
A <- data.frame(lab.uni_lab.inf,field.uni_field.inf,lab.uni_field_uni,
                   lab.inf_field.inf)
rownames(A) <- toString(x)
print(A)
P <- lapply(intersect(Genes_field, Genes_lab), eimeria_t_test_pvalue)
        lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IFNy
           0.0008701234
                                  0.02306666
                                                   0.009114009
                                                                        0.4665444
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## CXCR3
               0.7240836
                                    0.9875716
                                                   6.892882e-23
                                                                      1.609715e-30
##
        lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IL.6
              0.4569897
                                  0.06787592
                                                  0.0008034704
                                                                       0.08853236
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IL.10
               0.6664722
                                    0.6846399
                                                    0.007287372
                                                                      0.0004755641
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IL.13
              0.01136685
                                 8.790546e-05
                                                   3.030428e-06
                                                                      4.358343e-16
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IL1RN
               0.7694255
                                   0.08933739
                                                   2.494644e-07
                                                                      0.0002133729
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## CASP1
               0.9965703
                                   0.05714562
                                                     0.08776055
                                                                         0.9422483
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
   CXCL9
            3.645686e-05
                                   0.03663115
                                                    0.001032312
                                                                      5.396998e-10
##
        lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## ID01
             0.01723246
                                   0.1606432
                                                  3.080484e-05
                                                                         0.601396
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
  IRGM1
##
              0.03189147
                                  0.008218303
                                                      0.01225175
                                                                      2.273495e-10
##
       lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## MPO
                                                 3.373386e-07
             0.6247867
                                 0.01078007
                                                                     0.004021206
##
        lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## MUC2
              0.4783091
                                 1.14159e-05
                                                     0.5692909
                                                                     3.134409e-06
##
          lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## MUC5AC
                0.8906956
                                  9.076836e-06
                                                     0.001266663
                                                                          0.9759575
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## MYD88
               0.6477168
                                 1.543588e-05
                                                      0.7718497
                                                                      6.623496e-05
##
        lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## NCR1
              0.1069354
                                  0.01499511
                                                  0.0004910738
                                                                        0.6735697
        lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## PRF1
              0.1370779
                                 0.004534779
                                                   0.001882949
                                                                     5.780491e-05
          lab.uni lab.inf field.uni field.inf lab.uni field uni lab.inf field.inf
## RETNLB
                0.6017666
                                    0.08135791
                                                      0.09105424
                                                                         0.01363267
         lab.uni lab.inf field.uni field.inf lab.uni field uni lab.inf field.inf
## SOCS1
               0.2327638
                                 0.0001628878
                                                     0.07303862
                                                                      1.128447e-09
```

```
lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
##
## TICAM1
               0.04802866
                                    0.2056721
                                                    6.600607e-10
                                                                       8.18763e-05
       lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
##
## TNF
             0.4766501
                                 0.1809073
                                                    0.2633122
                                                                       0.394101
t.test.p.value <- reduce(P, rbind)</pre>
t.test.p.value <- tibble::rownames to column(t.test.p.value, "Genes")
write.csv(t.test.p.value,
          "output_data/gene_expression/data_products/t.test.p.values_gene_exp.csv",
          row.names = FALSE)
```

Results

- 1. Significant difference between means of lab uni and lab inf:
- IFNy
- IL.13
- PRF1
- TICAM1
- 2. Significant difference between means of field uni and field inf:
- IFNy
- IL.6
- IL.13
- IL1RN
- CXCL9
- MPO
- MUC5AC
- PRF1
- TICAM1
- TNF
- 3. Significant difference between means of lab uni and field uni: IFNy CXCR3 IL.6 IL.10 IL.13 IL1RN CXCL9 IDO1 IRGM1 MPO MUC2 MUC5AC NCR1 PRF1 RETNLB SOCS1 TICAM1
- 4. No Significant difference between means of lab uni and field uni:
- CASP1
- MYD88 -TNF
- 5. Significant difference between means of lab inf and field inf: IFNy CXCR3 IL.13 IRGM1 MUC2 MYD88 PRF1 SOCS1
- 6. No Significant difference between means of lab inf and field inf: IL.6 IL.10 IL1RN CASP1 CXCL9 IDO1 MPO MUC5AC NCR1 RETNLB TICAM1 TNF

 $https://docs.google.com/spreadsheets/d/1JWjsyzlQxWlSttZ5KwxYSsZapjZCgMCO1OcF0Yz9_ZQ/edit?usp = sharing$