4.Gene\_expressions\_analysis - Analyzing differences between field and lab

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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()
library(optimx)
```

#### 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>
```

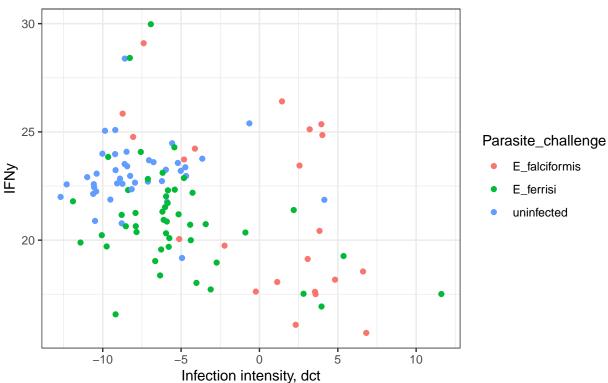
#### Selecting genes

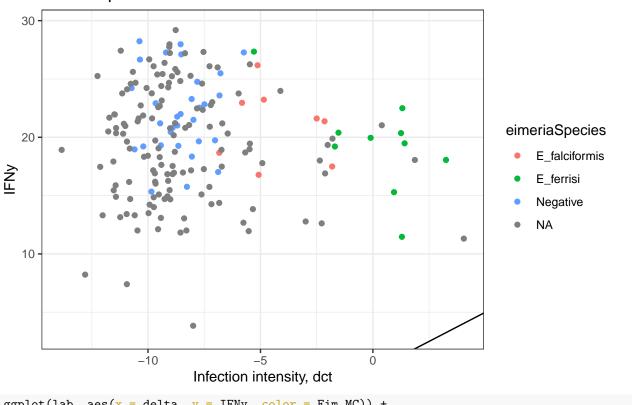
```
"IDO1", "IRGM1", "MPO", "MUC2", "MUC5AC", "MYD88", "NCR1", "PRF1", "RETNLB", "SOCS1", "TICAM1", "TNF")
```

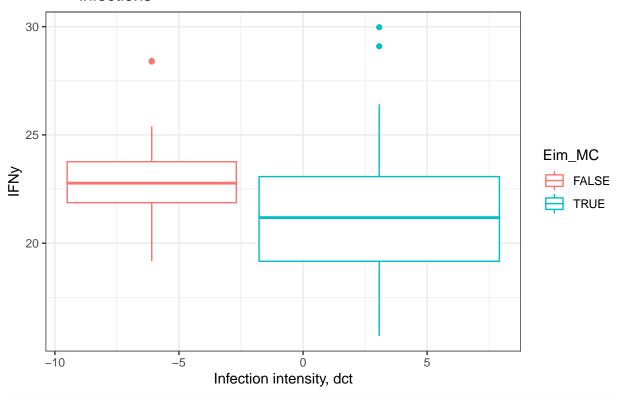
#### 1. IFNy

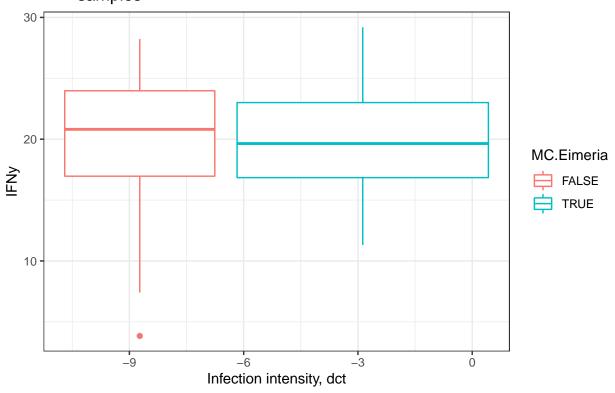
## Warning: Removed 6 rows containing missing values (geom\_point).

#### Immune gene expression against infection intensity in lab infections

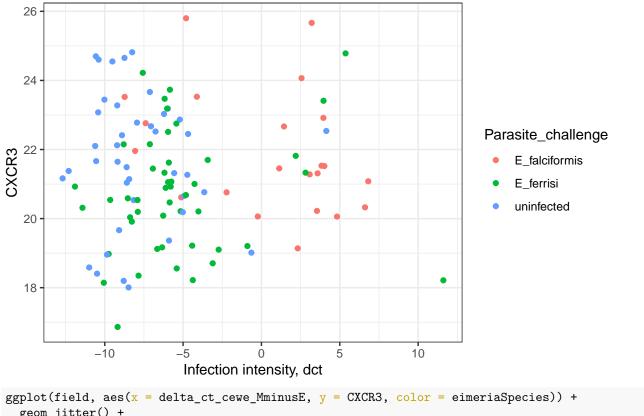


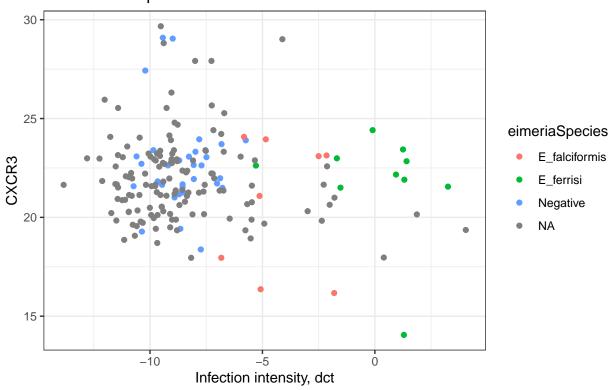




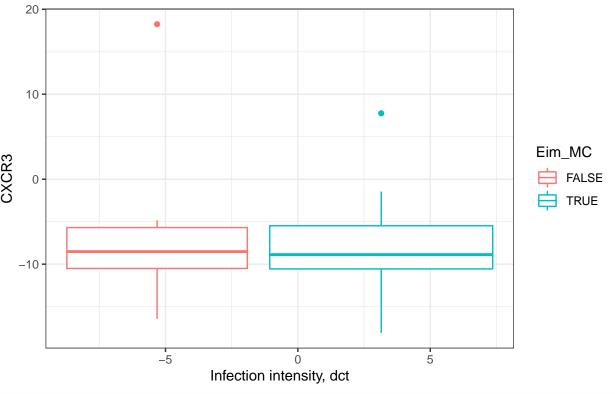


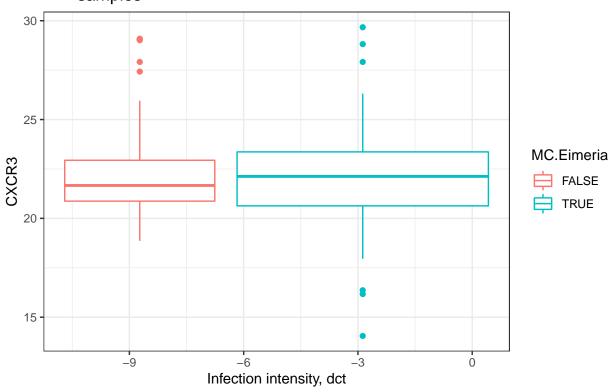
#### 2. CXCR3



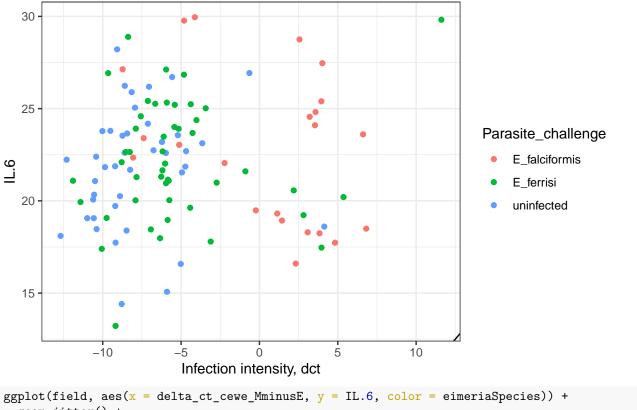


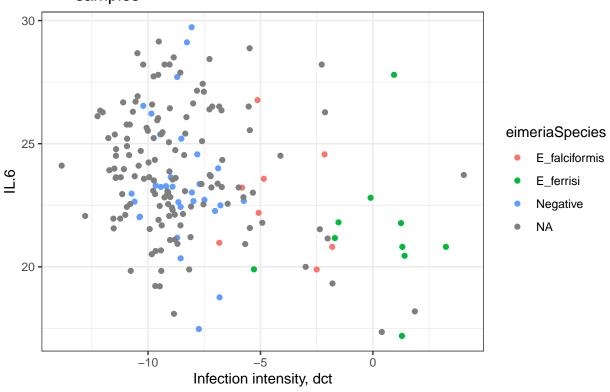
- ## Warning: Removed 6 rows containing missing values (stat\_boxplot).
- ## Warning: Removed 49 rows containing non-finite values (stat\_boxplot).



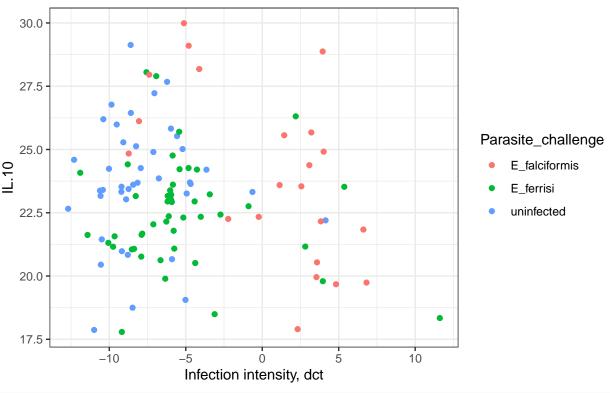


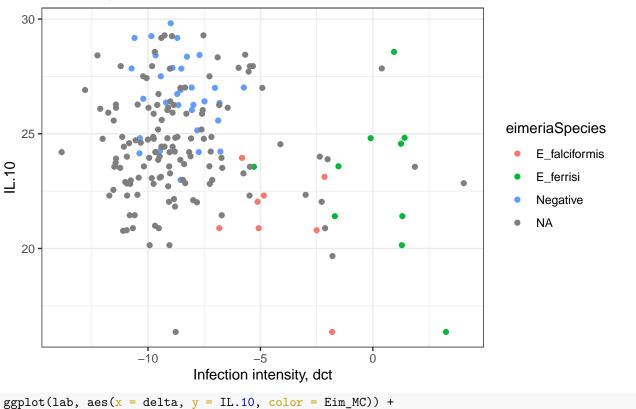
#### 3. IL.6

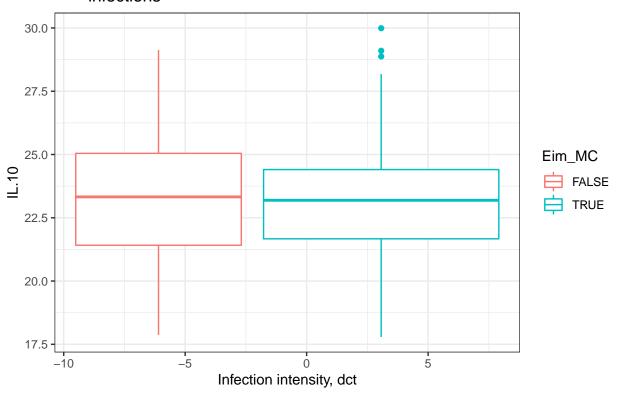


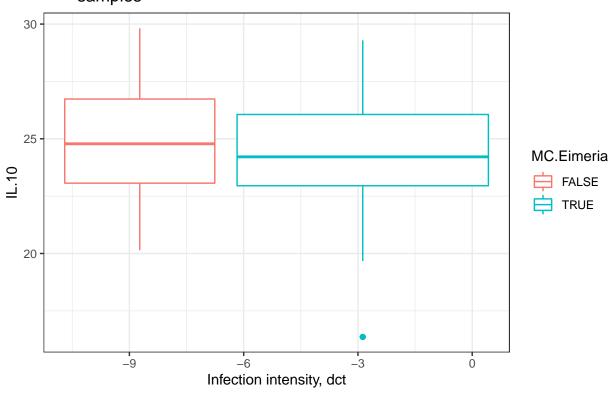


#### 4. IL.10

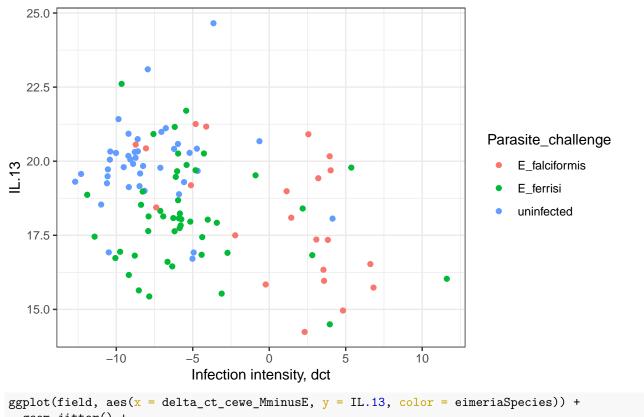


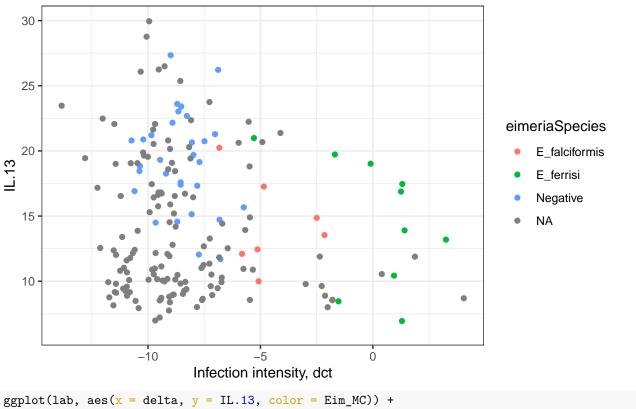


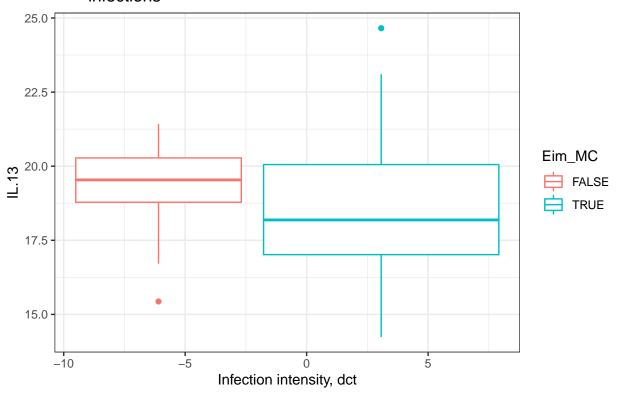


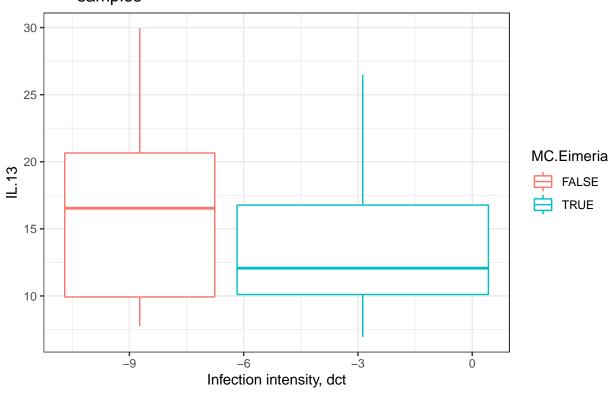


#### 5. IL.13

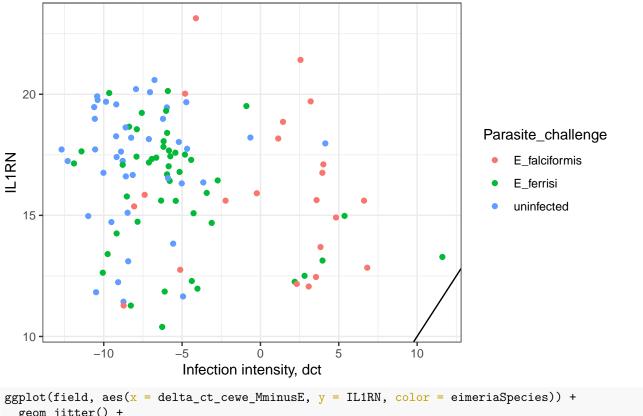


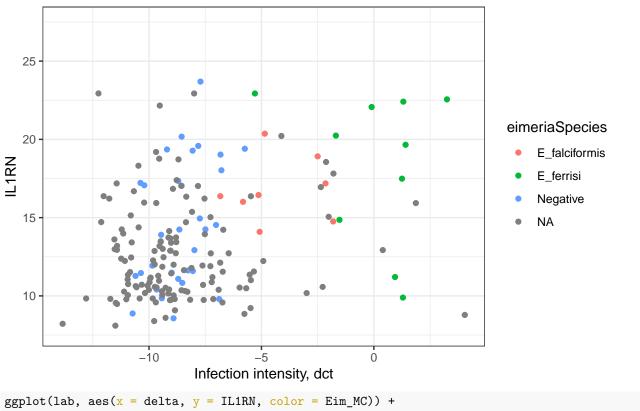


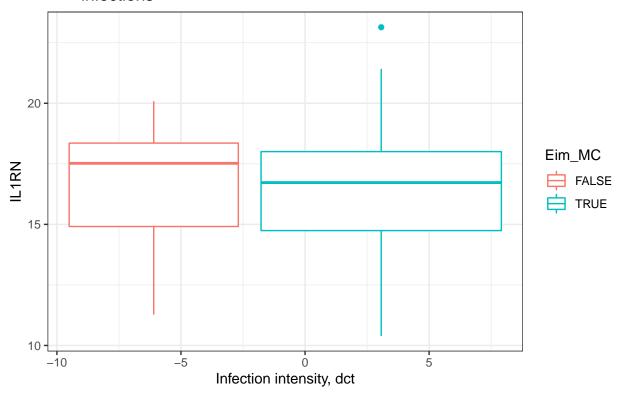


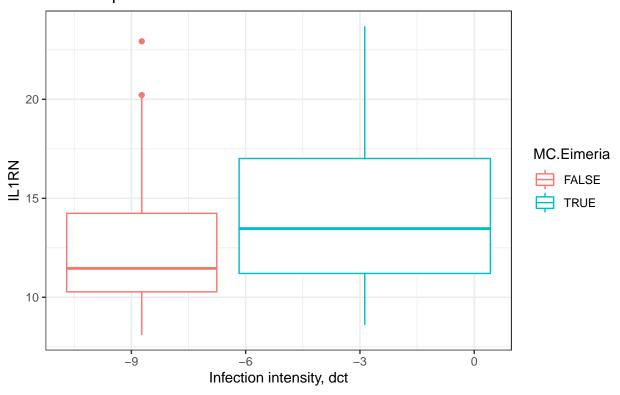


#### 6. IL1RN

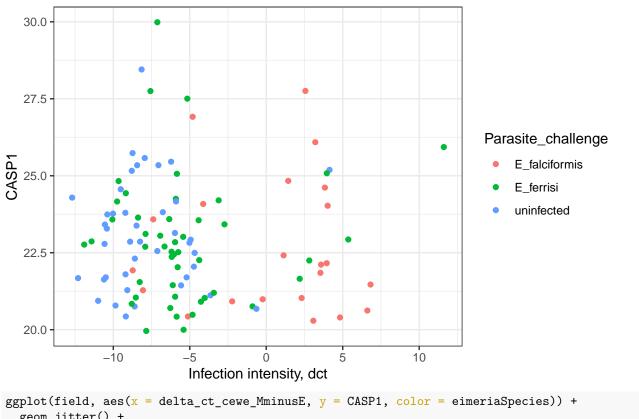


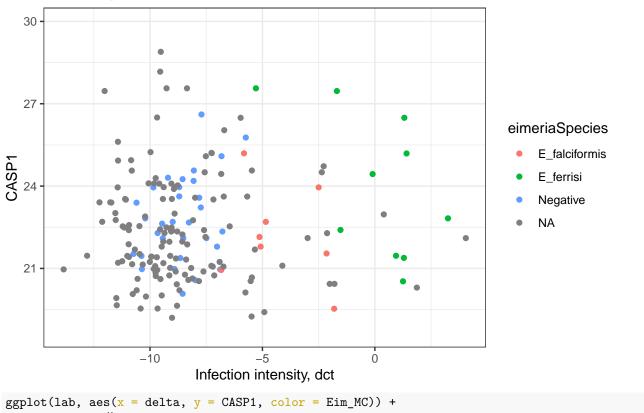


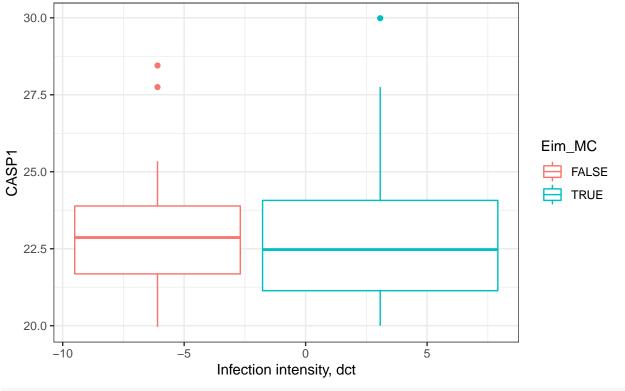


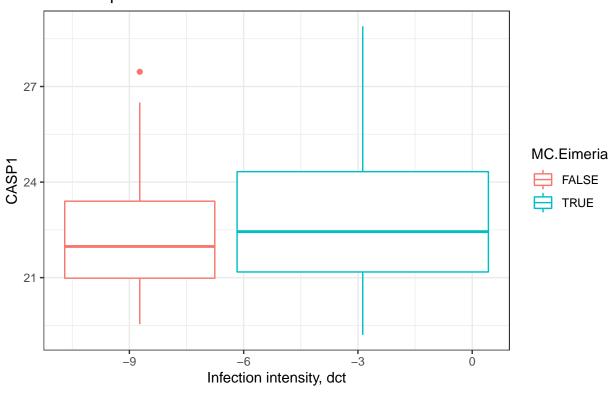


#### **7.** CASP1

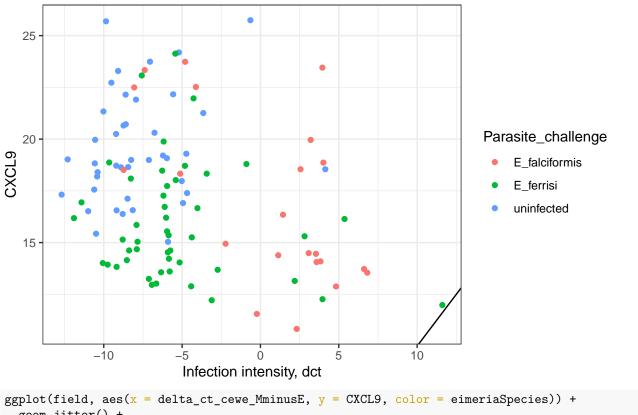


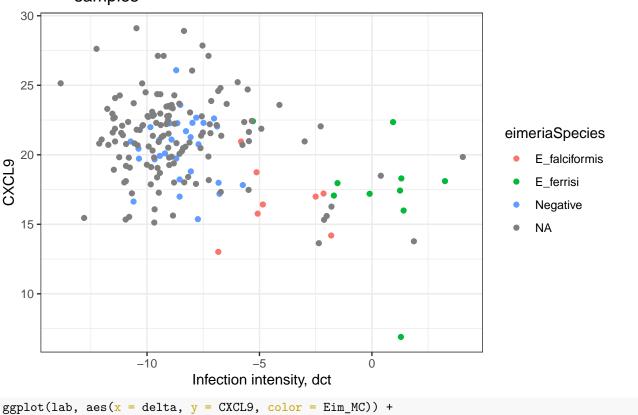


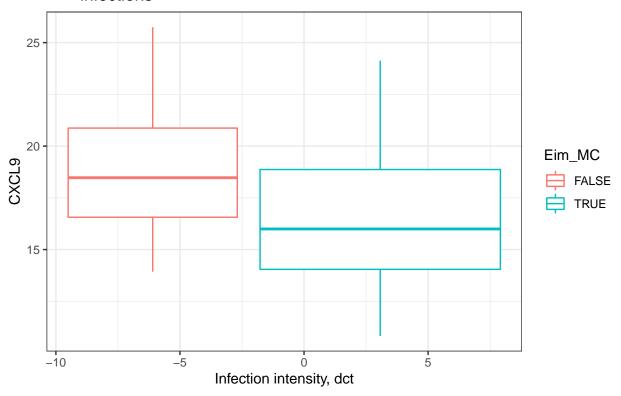


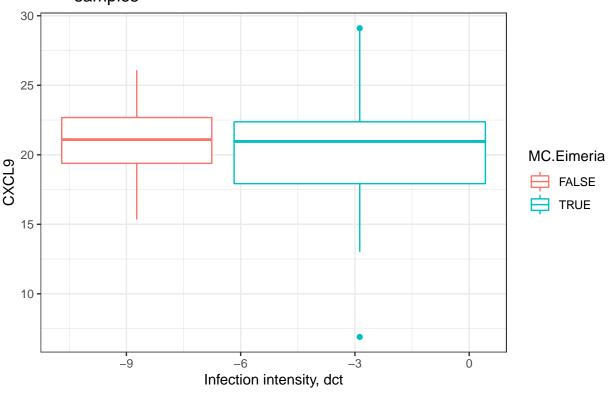


#### 8. CXCL9

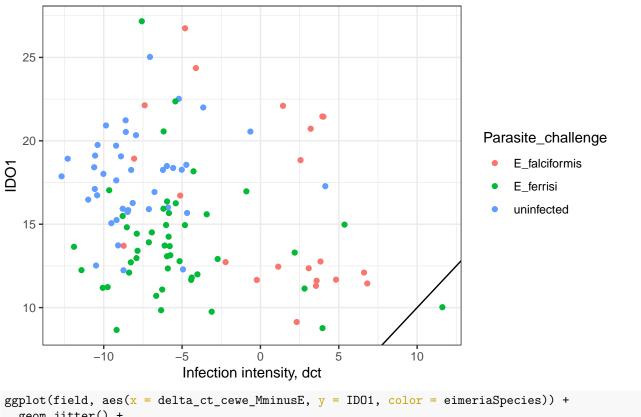


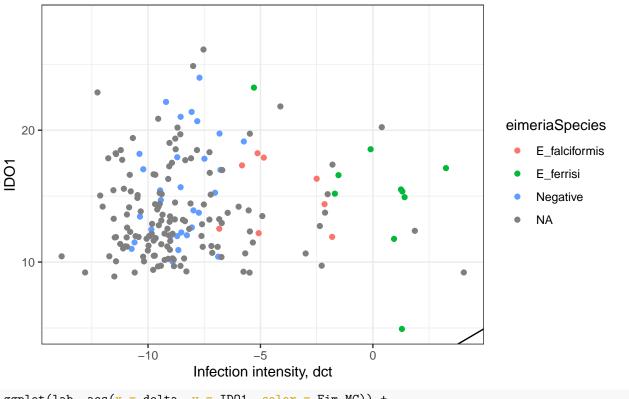


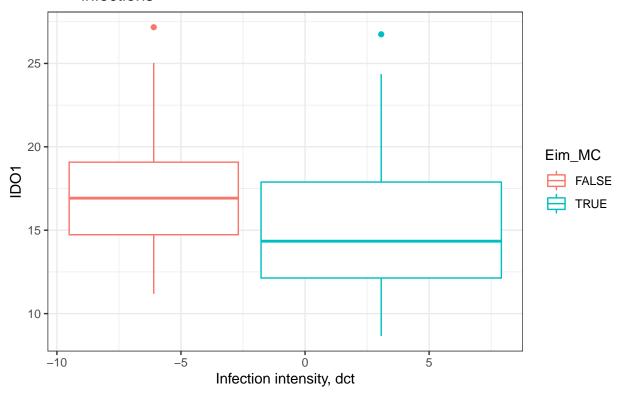


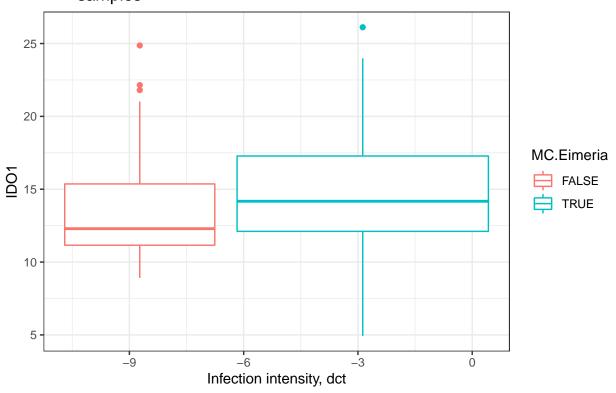


#### 9. IDO1

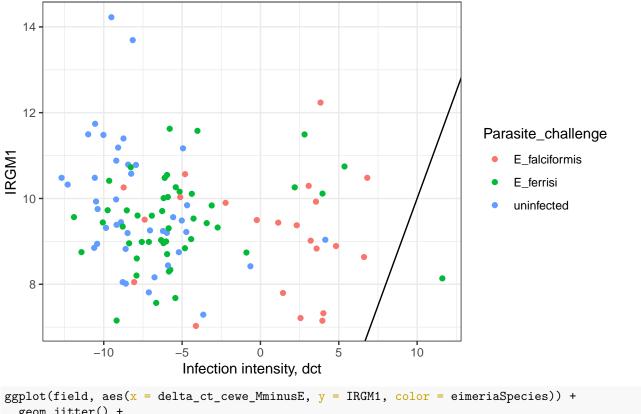


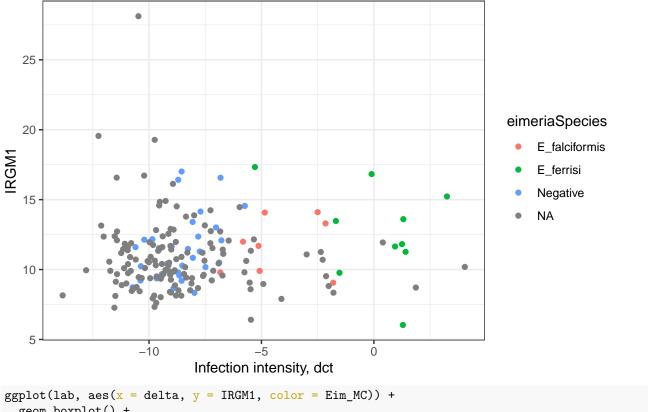


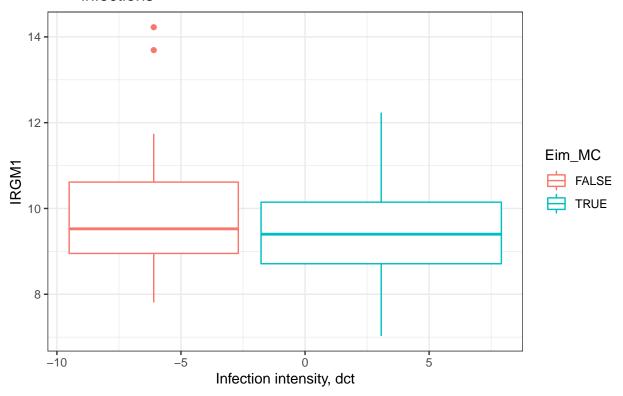


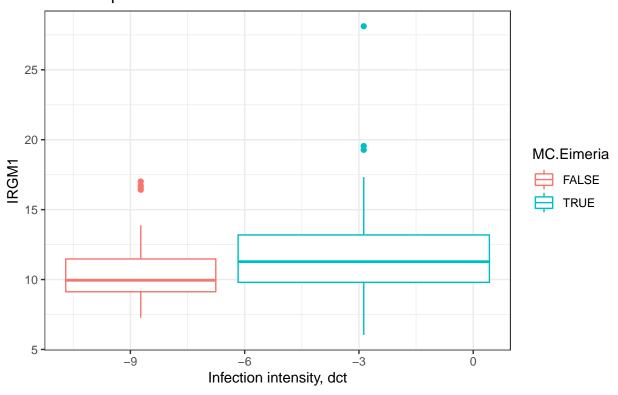


#### 10. IRGM1

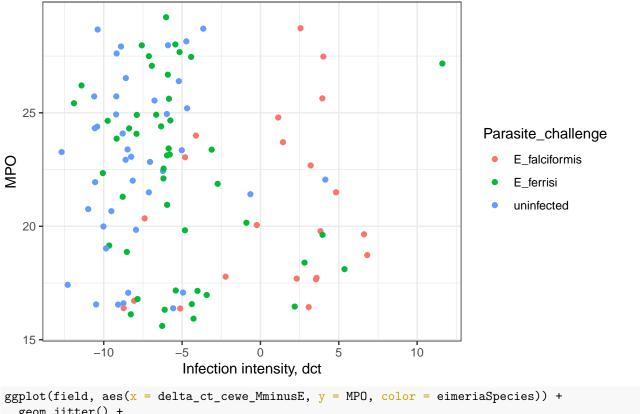


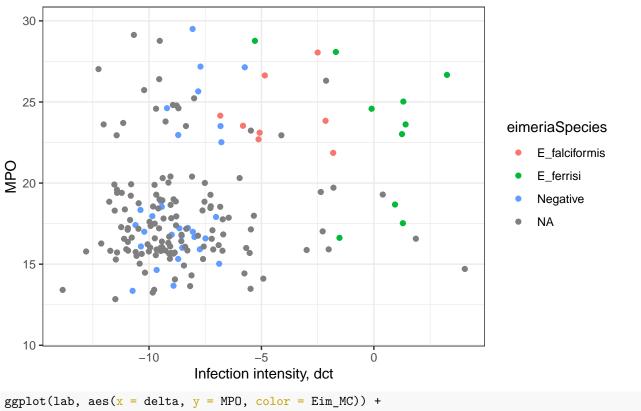


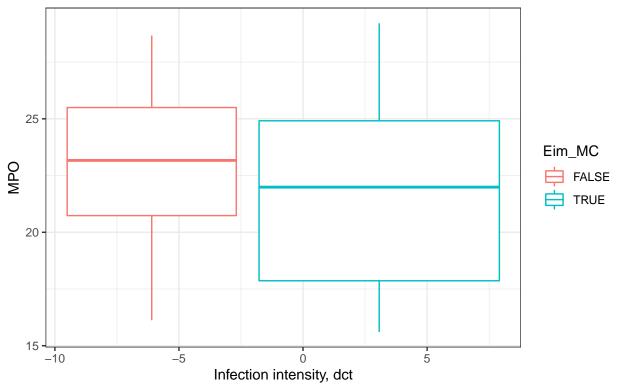


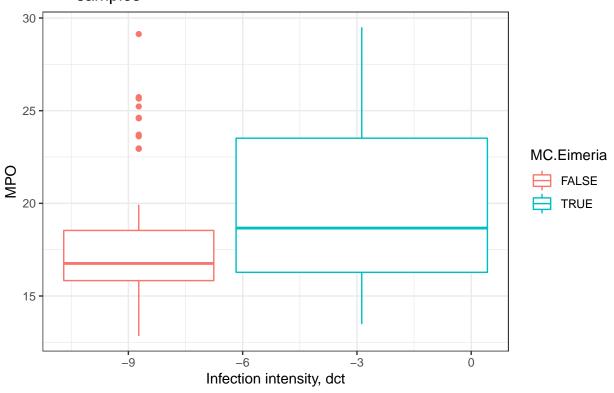


#### 11. MPO

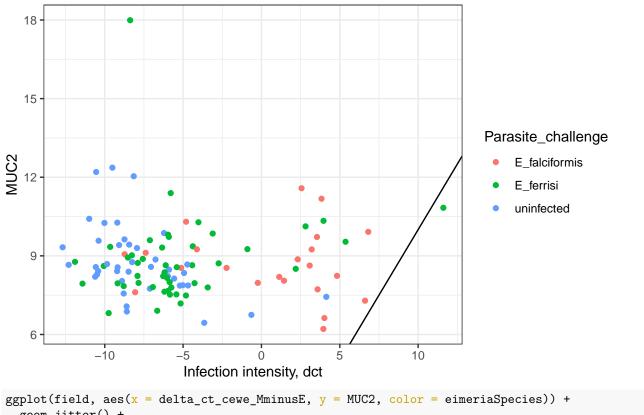


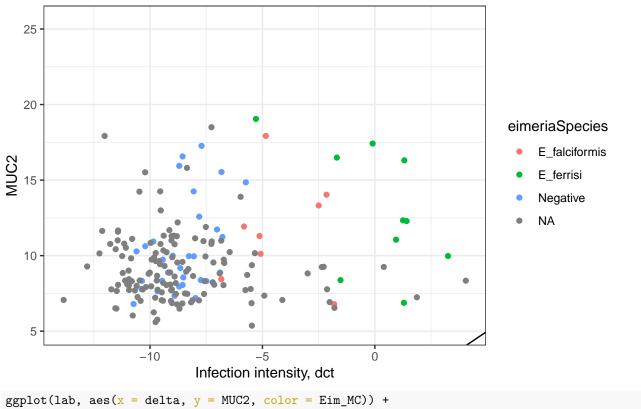


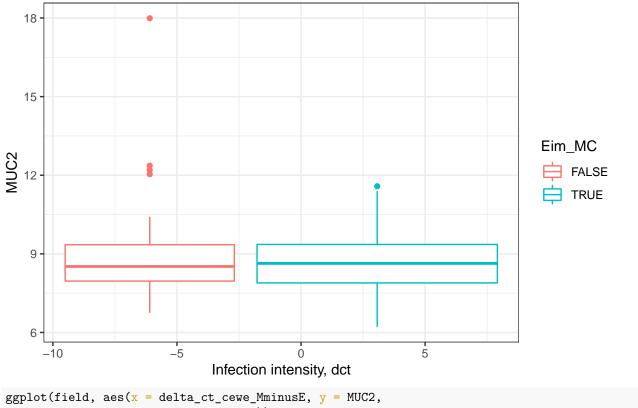


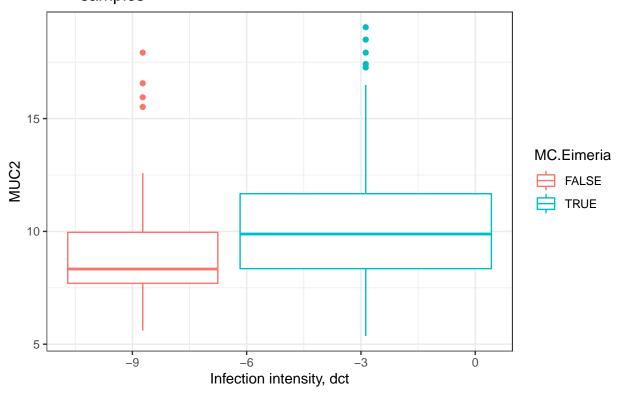


#### 12. MUC2

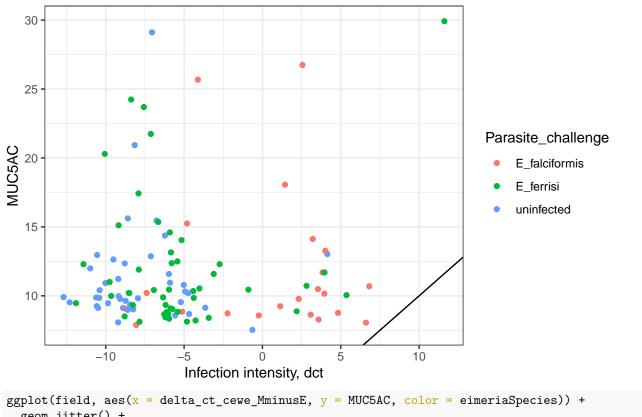


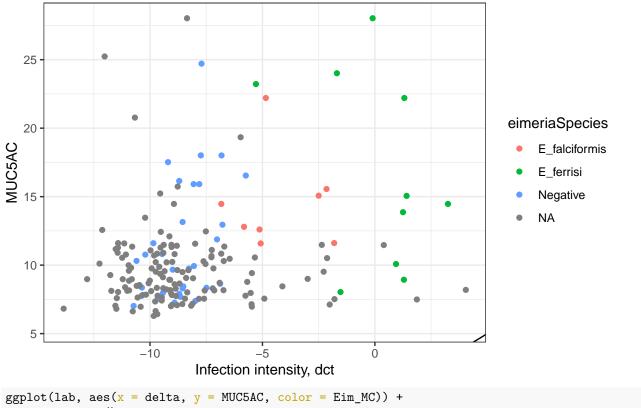


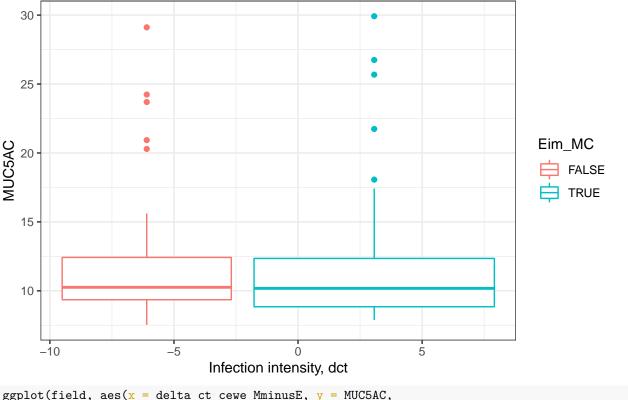


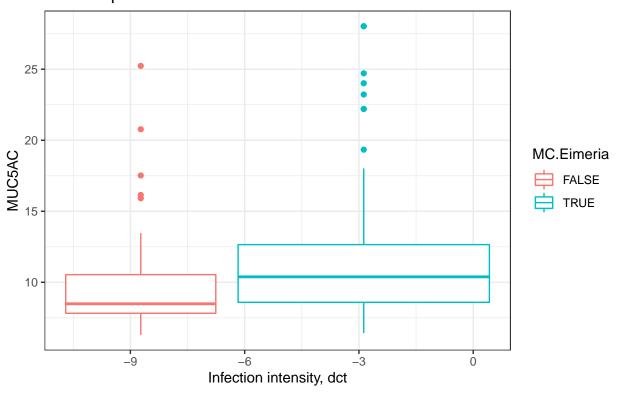


#### 13. MUC5AC

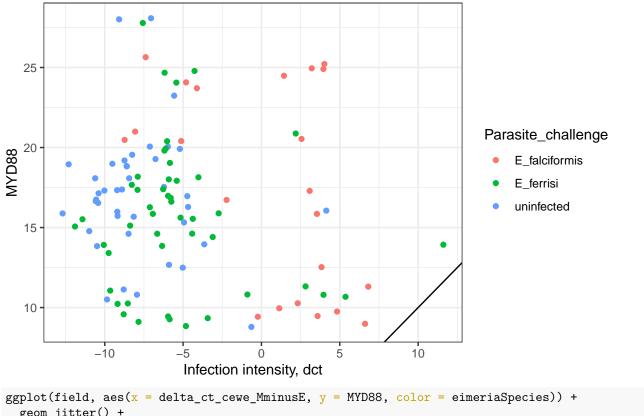


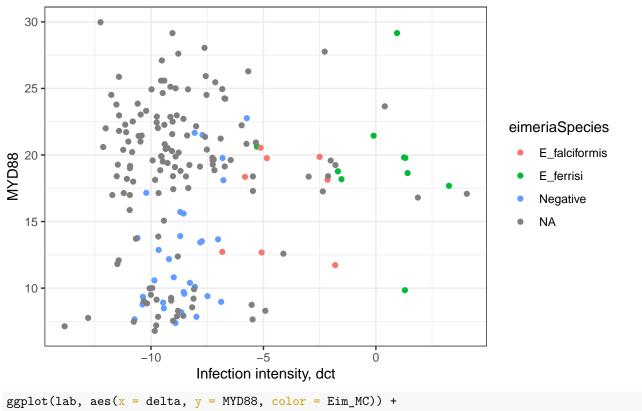


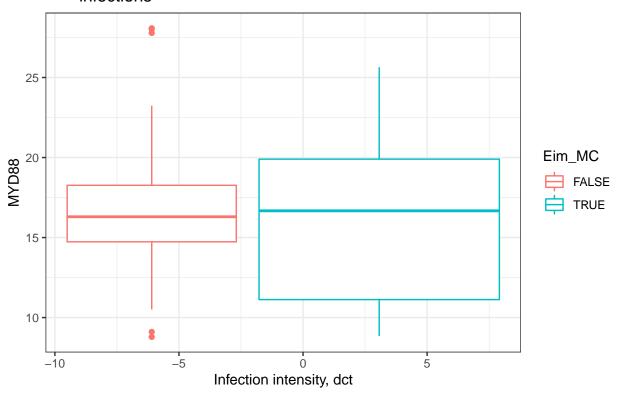


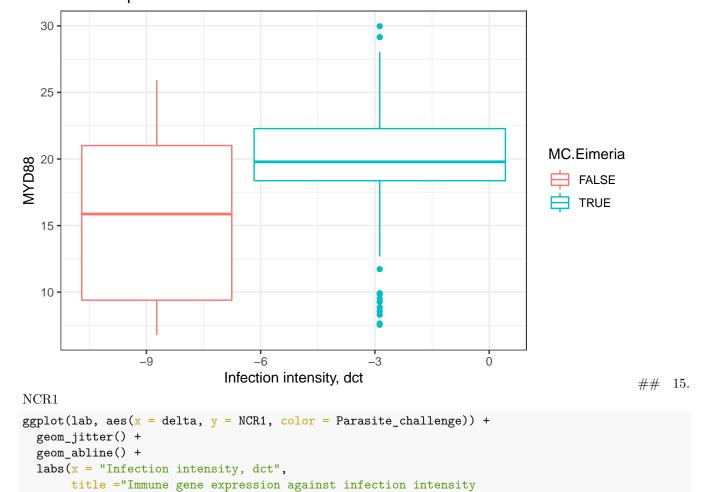


#### 14. MYD88





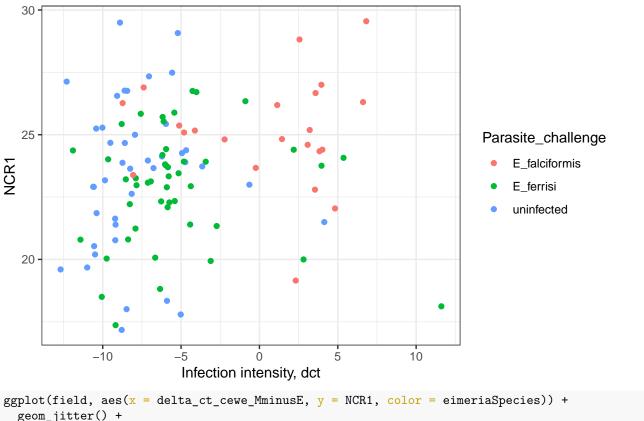


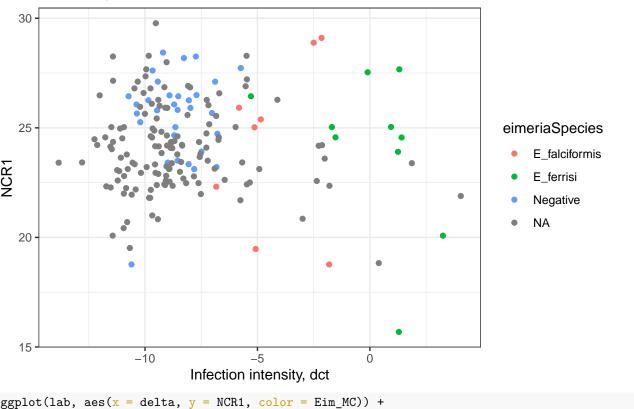


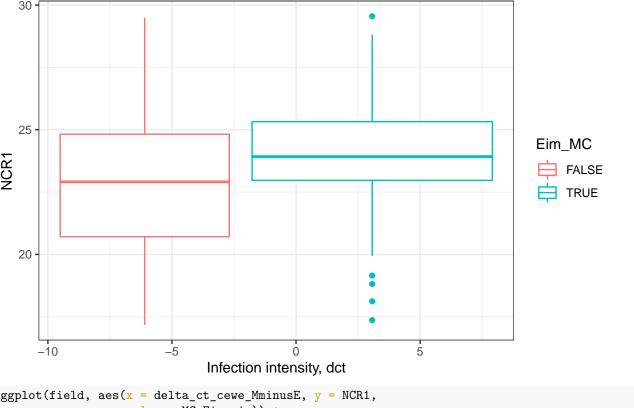
## Warning: Removed 6 rows containing missing values (geom\_point).

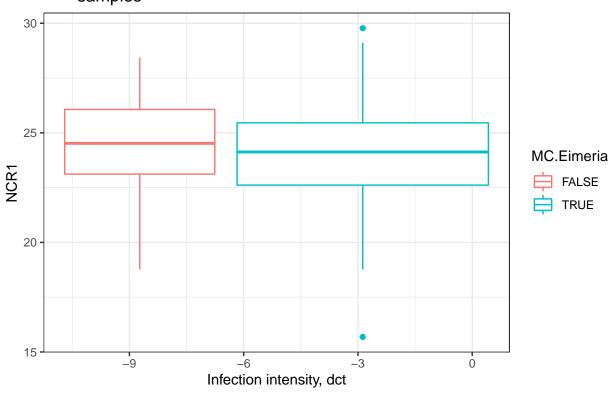
in lab infections") +

theme\_bw()

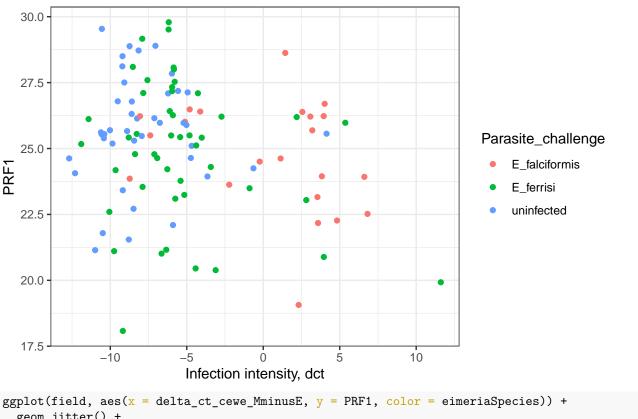


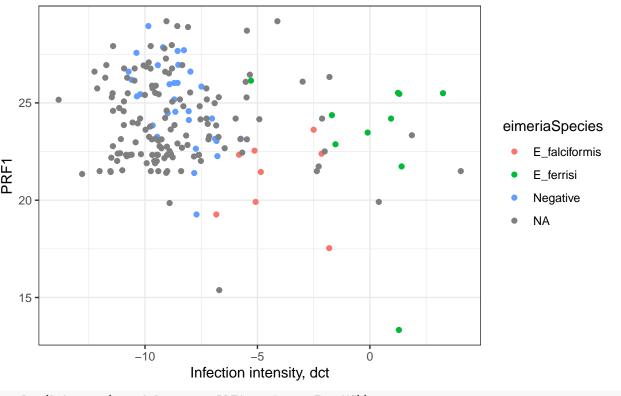


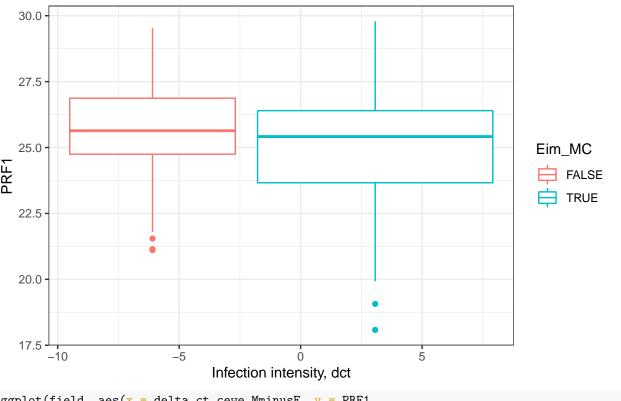


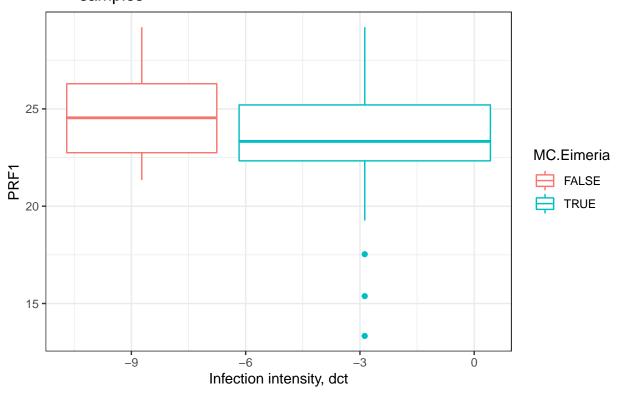


#### 16. PRF1

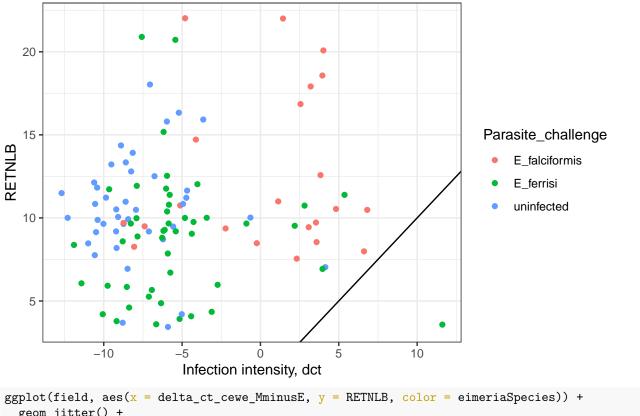


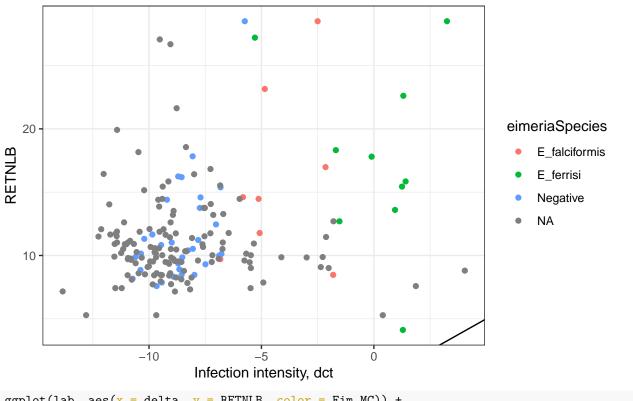


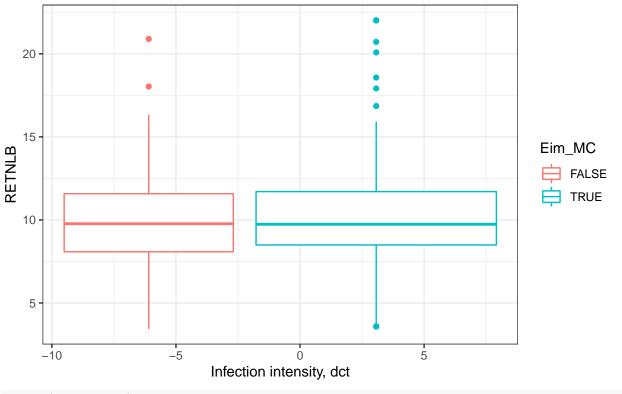


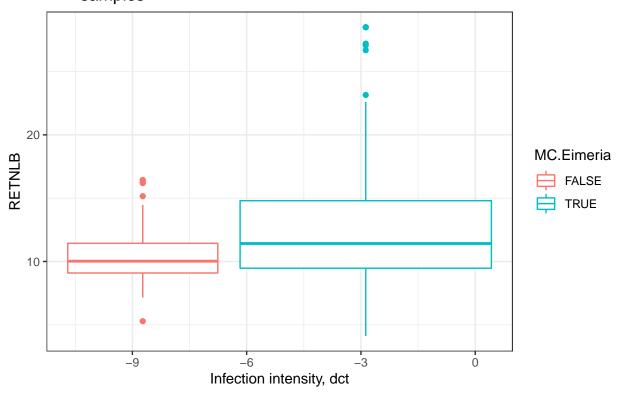


#### 17. RETNLB

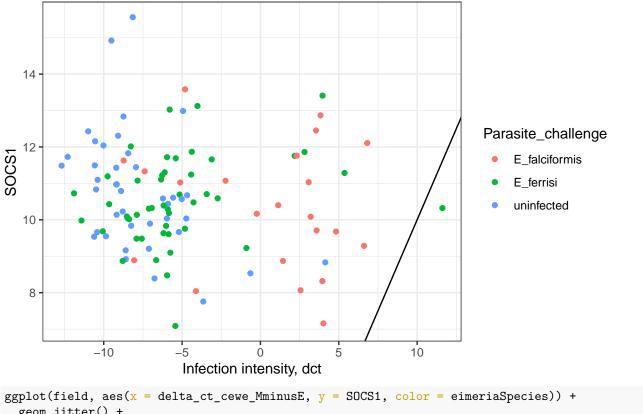


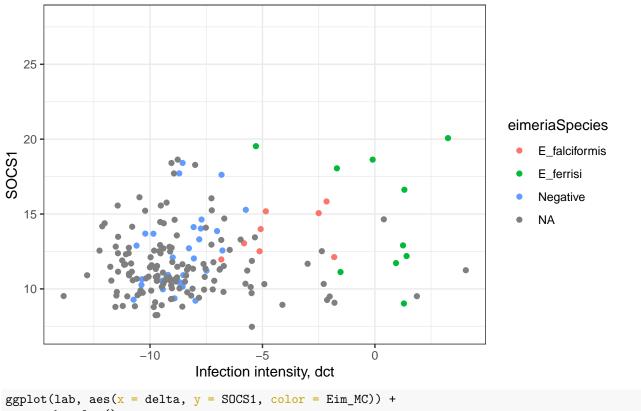


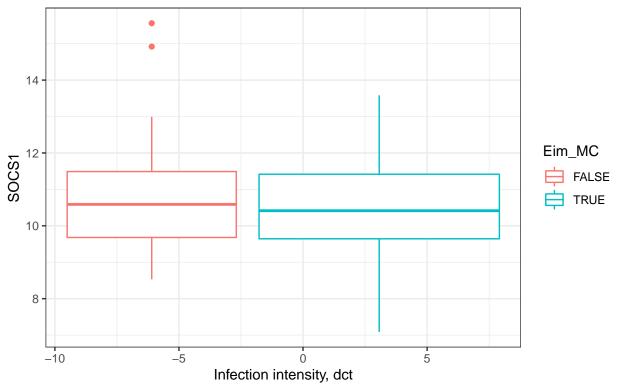


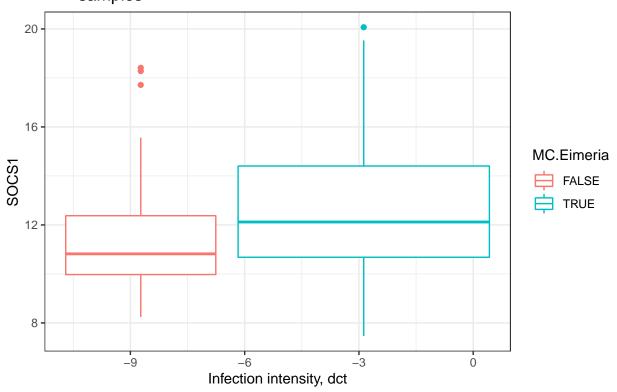


#### 18. SOCS1

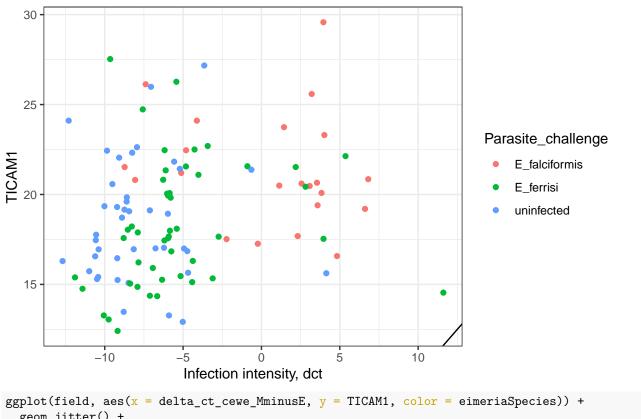




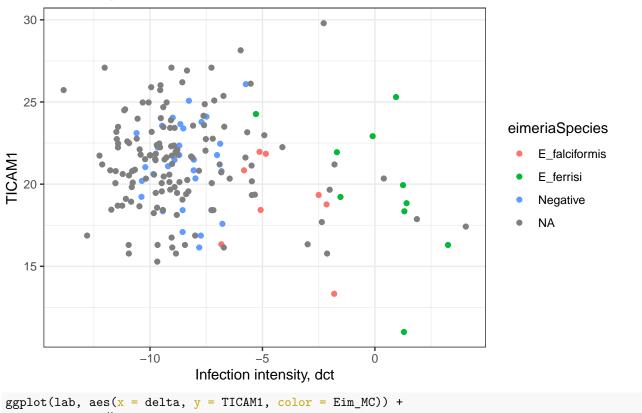


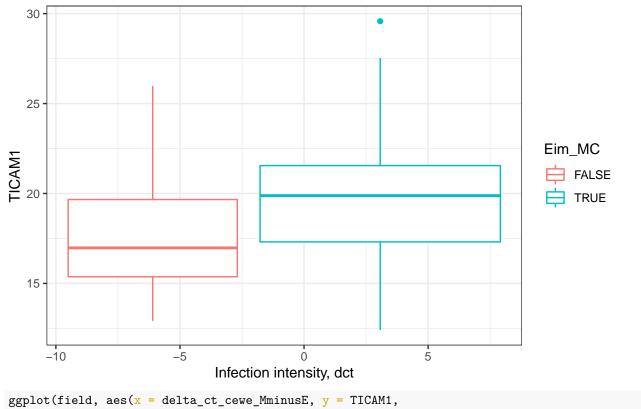


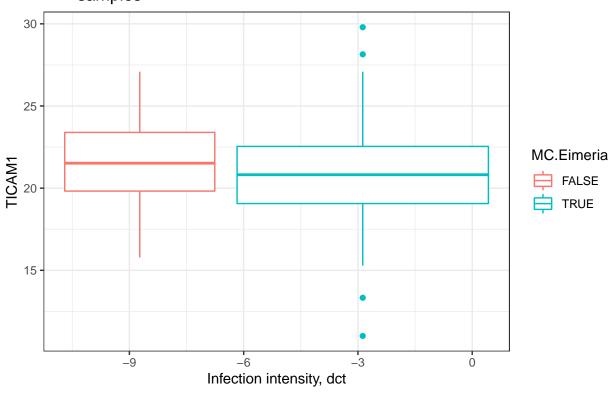
#### 19. TICAM1



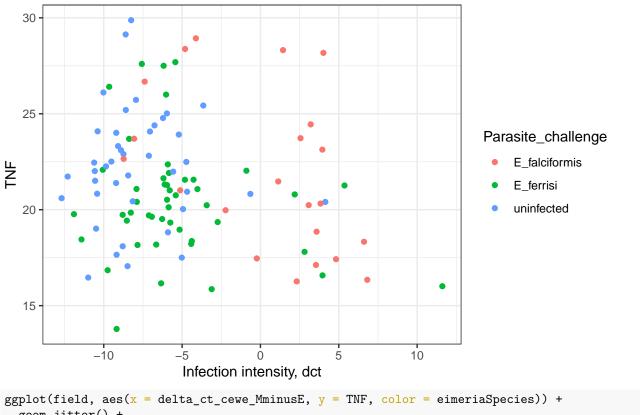
```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = TICAM1, color = eimeriaSpecies)) +
   geom_jitter() +
   geom_abline() +
   labs(x = "Infection intensity, dct",
        title ="Immune gene expression against infection intensity in field
        samples") +
   theme_bw()
```

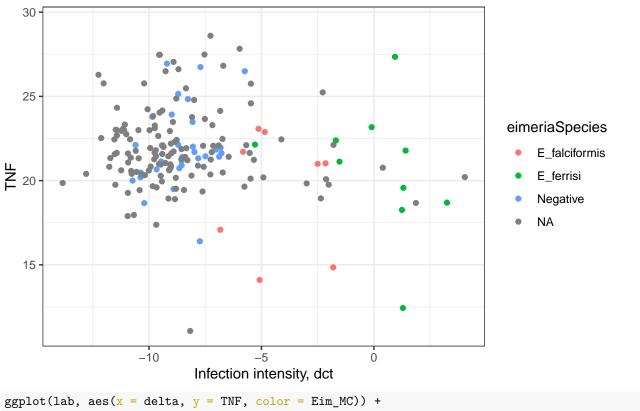


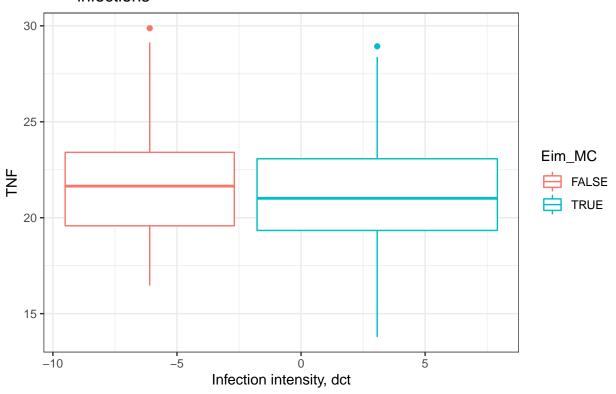


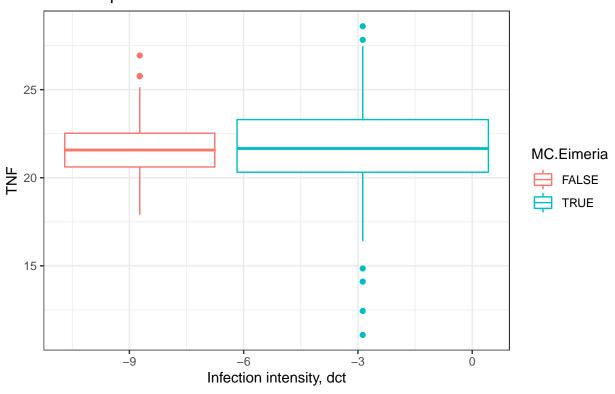


#### 20. TNF









#### 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
                                   0.8443114
                                                  3.898003e-06
                                                                       0.01489448
##
         lab.uni lab.inf field.uni field.inf lab.uni field uni lab.inf field.inf
## CXCR3
               0.7240836
                                     0.834139
                                                   6.110718e-23
                                                                      8.025602e-31
##
        lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IL.6
              0.4569897
                                  0.05500413
                                                  0.0009836655
                                                                        0.1259247
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IL.10
               0.6664722
                                   0.07224988
                                                   0.0005858587
                                                                        0.01284744
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IL.13
              0.01136685
                                  0.003696082
                                                   1.797684e-07
                                                                      4.358343e-16
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IL1RN
               0.7694255
                                 0.0005816463
                                                   3.000582e-11
                                                                      0.0003306339
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## CASP1
               0.9965703
                                      0.01341
                                                     0.03392336
                                                                         0.9314781
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
                                                                      2.325014e-10
            3.645686e-05
                                    0.2722695
                                                    0.004333807
##
        lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## ID01
             0.01723246
                                  0.02809745
                                                  1.480942e-06
                                                                        0.5102659
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
  IRGM1
                                 0.0003797562
                                                     0.07525817
##
              0.03189147
                                                                      2.273495e-10
##
       lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## MPO
                                                 7.643367e-10
             0.6247867
                               4.431343e-05
                                                                     0.003084519
        lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
##
## MUC2
              0.4783091
                                0.0004607668
                                                     0.8243095
                                                                     3.134409e-06
##
          lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## MUC5AC
                0.8906956
                                  0.0002337175
                                                     0.005060887
                                                                           0.871851
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## MYD88
               0.6477168
                                 1.336611e-06
                                                       0.723573
                                                                      5.178099e-05
##
        lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
                                   0.2811749
## NCR1
              0.1069354
                                                   0.004499826
                                                                        0.5085016
        lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## PRF1
              0.1370779
                                 0.002647012
                                                   0.009069733
                                                                     0.0004315407
          lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## RETNLB
                0.6017666
                                  6.188518e-05
                                                       0.4800438
                                                                       0.0009378679
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## SOCS1
               0.2327638
                                  0.000177738
                                                      0.1015706
                                                                      1.028948e-09
```

```
##
         lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## TICAM1
              0.04802866
                                  0.1746463 1.082312e-06 0.0307961
      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
##
## TNF
            0.4766501
                                                 0.9639207
                               0.7745215
                                                                  0.3303136
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)
Which genes are significant to predict weight loss?
Lab_wl <- lm(formula = max_WL ~ IFNy + CXCR3 + IL.6 + IL.10 + IL.13 + IL1RN +
              CXCR3 + CASP1 + CXCL9 + IDO1 + IRGM1 + MPO + MUC2 + MUC5AC +
              MYD88 + NCR1 + PRF1 + RETNLB + SOCS1 + TICAM1 + TNF, data = lab)
summary(Lab_wl)
##
## Call:
## lm(formula = max_WL ~ IFNy + CXCR3 + IL.6 + IL.10 + IL.13 + IL1RN +
      CXCR3 + CASP1 + CXCL9 + IDO1 + IRGM1 + MPO + MUC2 + MUC5AC +
      MYD88 + NCR1 + PRF1 + RETNLB + SOCS1 + TICAM1 + TNF, data = lab)
##
##
## Residuals:
      Min
               1Q Median
                              ЗQ
## -5.6176 -2.4799 0.1734 1.8098 9.5301
##
## Coefficients:
##
               Estimate Std. Error t value Pr(>|t|)
## (Intercept) -3.000006 15.405379 -0.195
                                           0.8466
## IFNy
             0.322858   0.428596   0.753   0.4557
## CXCR3
              -0.092789 0.140074 -0.662 0.5115
## IL.6
              -0.037201 0.319540 -0.116
                                            0.9079
## IL.10
             -0.074093 0.412013 -0.180 0.8582
## IL.13
              1.304887 0.948274 1.376 0.1765
              -0.797456   0.571481   -1.395   0.1706
## IL1RN
## CASP1
              -0.616740 0.499440 -1.235
                                            0.2241
## CXCL9
              -0.241405 0.574569 -0.420 0.6766
              -0.878372   0.464389   -1.891   0.0658
## ID01
## IRGM1
              0.175299 1.221502 0.144 0.8866
## MPO
              0.371152  0.359642  1.032  0.3083
## MUC2
              -0.074745 0.671834 -0.111 0.9120
## MUC5AC
              -0.179853 0.316862 -0.568
                                           0.5735
## MYD88
              -0.547750 0.695541 -0.788
                                            0.4356
## NCR1
              -0.227017 0.452027 -0.502
                                            0.6183
## PRF1
              0.510498 0.381384 1.339
                                            0.1883
                         0.350031 -0.010
## RETNLB
              -0.003365
                                            0.9924
## SOCS1
              -0.174075
                         1.442321 -0.121
                                            0.9045
## TICAM1
              0.861394
                         0.749231 1.150
                                            0.2571
## TNF
               0.221457
                         0.475055 0.466
                                            0.6436
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 4.214 on 40 degrees of freedom
```

```
(55 observations deleted due to missingness)
## Multiple R-squared: 0.4005, Adjusted R-squared: 0.1008
## F-statistic: 1.336 on 20 and 40 DF, p-value: 0.213
AIC(Lab_wl)
## [1] 366.853
Lab_MC <- lm(formula = Eim_MC ~ IFNy + CXCR3 + IL.6 + IL.10 + IL.13 + IL1RN +
              CXCR3 + CASP1 + CXCL9 + IDO1 + IRGM1 + MPO + MUC2 + MUC5AC +
              MYD88 + NCR1 + PRF1 + RETNLB + SOCS1 + TICAM1 + TNF, data = lab)
summary(Lab_MC)
##
## Call:
## lm(formula = Eim_MC ~ IFNy + CXCR3 + IL.6 + IL.10 + IL.13 + IL1RN +
##
       CXCR3 + CASP1 + CXCL9 + IDO1 + IRGM1 + MPO + MUC2 + MUC5AC +
##
      MYD88 + NCR1 + PRF1 + RETNLB + SOCS1 + TICAM1 + TNF, data = lab)
##
## Residuals:
##
       Min
                 1Q
                      Median
                                   3Q
                                           Max
## -0.96381 -0.21804 0.03771 0.22030 0.87795
##
## Coefficients:
##
                Estimate Std. Error t value Pr(>|t|)
## (Intercept) -0.5822570 1.5120281 -0.385
              -0.0009029 0.0420665 -0.021
## IFNy
                                              0.9830
## CXCR3
              -0.0103727 0.0137482 -0.754
                                              0.4550
## IL.6
              0.0081549 0.0313627
                                     0.260
                                             0.7962
## IL.10
              0.0193551 0.0404388
                                     0.479
                                              0.6348
## IL.13
              0.0725771 0.0930725
                                      0.780
                                              0.4401
## IL1RN
              0.0319357 0.0560905
                                      0.569
                                              0.5723
## CASP1
              0.0332448 0.0490198
                                      0.678
                                             0.5016
## CXCL9
              -0.0792809 0.0563935 -1.406
                                              0.1675
## ID01
              -0.0899176 0.0455795 -1.973
                                              0.0555
## IRGM1
              -0.1607594 0.1198897 -1.341
                                              0.1875
## MPO
              -0.0072732 0.0352986 -0.206
                                              0.8378
## MUC2
              -0.0175636  0.0659401  -0.266
                                              0.7913
## MUC5AC
              -0.0113556 0.0310998 -0.365
                                              0.7169
## MYD88
              0.0886323 0.0682669
                                     1.298
                                              0.2016
## NCR1
              -0.0240597 0.0443661 -0.542
                                              0.5906
## PRF1
               0.0210632 0.0374326
                                     0.563
                                              0.5768
## RETNLB
               0.0075148 0.0343553
                                      0.219
                                              0.8280
## SOCS1
              0.0706436 0.1415629
                                     0.499
                                              0.6205
## TICAM1
               0.0674897 0.0735366
                                              0.3642
                                      0.918
## TNF
              -0.0476275  0.0466263  -1.021
                                              0.3132
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 0.4136 on 40 degrees of freedom
## (55 observations deleted due to missingness)
## Multiple R-squared: 0.5502, Adjusted R-squared: 0.3253
## F-statistic: 2.447 on 20 and 40 DF, p-value: 0.007899
AIC(Lab_MC)
```

## [1] 83.65866

#### 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
- 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

 $https://docs.google.com/spreadsheets/d/1JWjsyzlQxWlSttZ5KwxYSsZapjZCgMCO1OcF0Yz9\_ZQ/edit?usp = sharing$ 

#### It is time to apply the package of Alice Balard et al. on our predictions!

Let's see if we indeed have differences across the hybrid index with our predicted weight loss.

#### Install the package

```
##
## * checking for file '/tmp/RtmpdMPgGf/remotes33bb7d41b7f8ce/alicebalard-parasiteLoad-1b43216/DESCRIPTION
## * preparing 'parasiteLoad':
## * checking DESCRIPTION meta-information ... OK
## * checking for LF line-endings in source and make files and shell scripts
## * checking for empty or unneeded directories
## * building 'parasiteLoad_0.1.0.tar.gz'
```