Titer Analysis

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```
setwd("/Users/charlescostanzo/College/Su 2023/Chopra/African Green Monkeys Plague/")
# load in packages
library(tidyverse)
## -- Attaching core tidyverse packages ------ tidyverse 2.0.0 --
## v dplyr
           1.1.3
                        v readr
                                    2.1.4
## v forcats 1.0.0
                     v stringr
                                    1.5.0
## v ggplot2 3.4.3
                        v tibble
                                    3.2.1
## v lubridate 1.9.2
                        v tidyr
                                    1.3.0
## v purrr
              1.0.2
## -- Conflicts ----- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()
                    masks stats::lag()
## i Use the conflicted package (<a href="http://conflicted.r-lib.org/">http://conflicted.r-lib.org/</a>) to force all conflicts to become error
library(readxl)
library(scales)
##
## Attaching package: 'scales'
## The following object is masked from 'package:purrr':
##
       discard
##
## The following object is masked from 'package:readr':
##
##
       col_factor
library(rstatix)
##
## Attaching package: 'rstatix'
## The following object is masked from 'package:stats':
##
##
      filter
```

```
library(reshape)
##
## Attaching package: 'reshape'
## The following object is masked from 'package:lubridate':
##
##
      stamp
##
## The following object is masked from 'package:dplyr':
##
##
      rename
##
## The following objects are masked from 'package:tidyr':
##
##
      expand, smiths
library(dplyr)
library(ggpubr)
library(plyr)
## ------
## You have loaded plyr after dplyr - this is likely to cause problems.
## If you need functions from both plyr and dplyr, please load plyr first, then dplyr:
## library(plyr); library(dplyr)
##
## Attaching package: 'plyr'
## The following object is masked from 'package:ggpubr':
##
##
      mutate
##
## The following objects are masked from 'package:reshape':
##
##
      rename, round_any
##
## The following objects are masked from 'package:rstatix':
##
##
      desc, mutate
## The following objects are masked from 'package:dplyr':
##
##
      arrange, count, desc, failwith, id, mutate, rename, summarise,
##
      summarize
##
## The following object is masked from 'package:purrr':
##
##
      compact
library(datarium)
library(RColorBrewer)
library(afex)
```

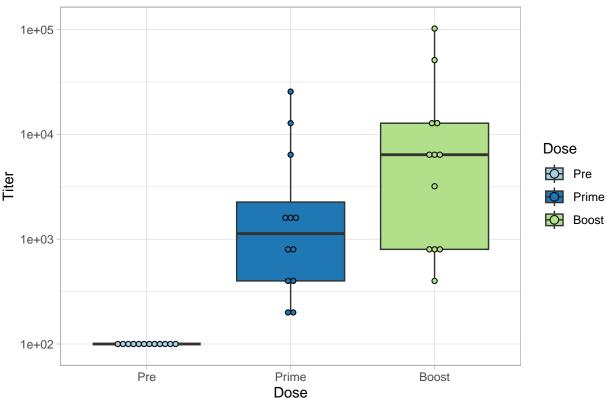
```
## Loading required package: lme4
## Loading required package: Matrix
##
## Attaching package: 'Matrix'
##
## The following object is masked from 'package:reshape':
##
##
       expand
##
## The following objects are masked from 'package:tidyr':
##
       expand, pack, unpack
##
## *******
## Welcome to afex. For support visit: http://afex.singmann.science/
## - Functions for ANOVAs: aov_car(), aov_ez(), and aov_4()
## - Methods for calculating p-values with mixed(): 'S', 'KR', 'LRT', and 'PB'
## - 'afex_aov' and 'mixed' objects can be passed to emmeans() for follow-up tests
## - Get and set global package options with: afex_options()
## - Set sum-to-zero contrasts globally: set sum contrasts()
## - For example analyses see: browseVignettes("afex")
## *******
##
## Attaching package: 'afex'
##
## The following object is masked from 'package:lme4':
##
##
       lmer
library(performance)
library(see)
# load in data
data <- read_excel("Data/primate.xlsx")</pre>
# create a new variable "id_treatment_sex" that combines "id",
# "treatment", and "sex" variables separated by a space
data2 <- data %>%
  mutate(id_treatment_sex = unite(data = data, col = "id_treatment_sex", c(id, treatment, sex), sep = "
# "make" time a factor variable with three levels
data2$time <- factor(data2$time, levels = c("Pre", "Prime", "Boost"))</pre>
# make "id_treatment_sex" a factor variable
data2$id_treatment_sex <- factor(data2$id_treatment_sex,</pre>
                                  levels = c("9501 PBS F1 Male",
                                             "9286 PBS F1 Male",
                                             "9035 F1 Male",
                                             "9050 F1 Male",
                                             "9330 F1 Male",
                                             "0303 PBS F1 Female",
                                             "0116 PBS F1 Female",
                                             "9022 F1 Female",
                                             "9051 F1 Female",
```

```
"9107 F1 Female",
                                             "9501 PBS LcrV Male",
                                             "9286 PBS LcrV Male",
                                             "9035 LcrV Male",
                                             "9050 LcrV Male",
                                             "9330 LcrV Male",
                                             "0303 PBS LcrV Female",
                                             "0116 PBS LcrV Female",
                                             "9022 LcrV Female",
                                             "9051 LcrV Female",
                                             "9107 LcrV Female"
                                 ))
\# filter out unvaccinated "control" monkeys (not sure if we can analyze those)
# convert columns sex, time, and treatment to factor
data2 <- data2 %>%
 filter(treatment == "F1" | treatment == "LcrV") %>%
 mutate(sex = as.factor(sex),
        time = as.factor(time),
        treatment = as.factor(treatment))
# create a new variable "log10_titer"
# that contains titer values on log base 10 scale
data2$log10_titer <- log(data2$titer, base = 10)</pre>
```

```
# Data Visualization
# generate a box-dotplot for titer by dose time
data2 %>%
 ggplot(aes(x = time, y = titer, fill = time)) +
 geom_boxplot() +
 scale_y_log10() +
 scale_fill_brewer(palette = "Paired",
              name = "Dose") +
 theme_light() +
 ylab("Titer") +
 xlab("Dose") +
 ggtitle("Titer by Dose Time") +
 geom_dotplot(binaxis = "y",
          stackdir = "center",
          stackgroups = FALSE,
          dotsize = 0.5,
          position = position_dodge())
```

Bin width defaults to 1/30 of the range of the data. Pick better value with ## 'binwidth'.

Titer by Dose Time



```
# check for outliers in titer column grouped by dose time
outlier_titer <- data2 %>%
 group_by(time) %>%
 identify_outliers(titer)
outlier_titer
## # A tibble: 4 x 10
##
   time name
                         id
                               treatment sex
                                               titer id_treatment_sex log10_titer
                   <chr> <fct> <fct> <fct> <dbl> <fct>
##
   <fct> <chr>
                                                                            <dbl>
## 1 Prime 9330 Vax Male~ 9330 LcrV
                                        Male 25600 9330 LcrV Male
                                                                            4.41
## 2 Prime 9050 Vax Male~ 9050 LcrV
                                        Male 12800 9050 LcrV Male
                                                                             4.11
                                        Male 102400 9330 LcrV Male
## 3 Boost 9330 Vax Male~ 9330 LcrV
                                                                             5.01
## 4 Boost 9050 Vax Male~ 9050 LcrV
                                        Male 51200 9050 LcrV Male
                                                                             4.71
## # i 2 more variables: is.outlier <lgl>, is.extreme <lgl>
# check for outliers in log10_titer column grouped by dose time
outlier_log10_titer <- data2 %>%
 filter(treatment == "F1") %>%
 group_by(time) %>%
 identify_outliers(log10_titer)
outlier_log10_titer
## [1] time
                                         id
                        name
                                                         treatment
## [5] sex
                        titer
                                         id_treatment_sex log10_titer
## [9] is.outlier
                        is.extreme
## <0 rows> (or 0-length row.names)
```

```
# Check Assumptions
#############
# Normality #
#############
# check for normality in F1 treatment group, excluding "Pre" dose rows
# (using Shapiro-Wilks test)
normality_f1 <- data2 %>%
 group_by(time) %>%
 filter(treatment == "F1") %>%
 filter(time != "Pre") %>%
 shapiro_test(log10_titer)
normality_f1
## # A tibble: 2 x 4
  time variable statistic
## <fct> <chr> <dbl> <dbl>
## 1 Prime log10_titer
                   0.853 0.167
# check for normality in LcrV treatment group, excluding "Pre" dose rows
normality_lcrv <- data2 %>%
 group_by(time) %>%
 filter(treatment == "LcrV") %>%
 filter(time != "Pre") %>%
 shapiro_test(log10_titer)
normality_lcrv
## # A tibble: 2 x 4
## time variable statistic
  <fct> <chr>
##
                 <dbl> <dbl>
## 1 Prime log10_titer 0.832 0.111
## 2 Boost log10 titer 0.863 0.201
# do we have to check for normality with both treatment (antiqen) groups together?
normality_both <- data2 %>%
 group_by(time) %>%
 filter(time != "Pre") %>%
 shapiro_test(log10_titer)
normality_both
## # A tibble: 2 x 4
   time variable statistic
##
## <fct> <chr> <dbl> <dbl>
## 1 Prime log10_titer 0.923 0.313
```

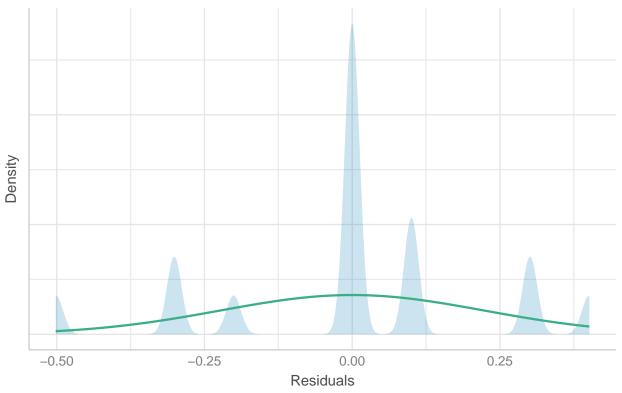
Contrasts set to contr.sum for the following variables: sex

```
## Warning: More than one observation per design cell, aggregating data using `fun_aggregate = mean`.
## To turn off this warning, pass `fun_aggregate = mean` explicitly.
is_norm <- check_normality(s1)

plot(is_norm)</pre>
```

Normality of Residuals

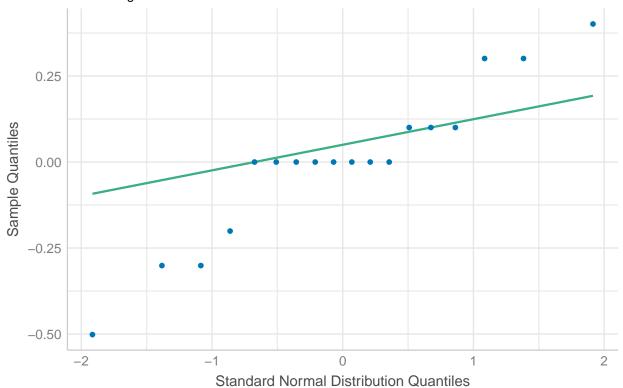
Distribution should be close to the normal curve



```
plot(is_norm, type = "qq")
```

For confidence bands, please install 'qqplotr'.

Normality of Residuals Dots should fall along the line



```
#############
# Sphericity #
#############
res_f1 <- anova_test(data = filter(data2, treatment == "F1"),</pre>
               dv = log10_titer,
               wid = id,
               between = sex,
               within = time)
res_f1
## ANOVA Table (type II tests)
##
## $ANOVA
## Effect DFn DFd F p p<.05 ges
      sex 1 4 2.571 0.184000 0.176
## 2 time 2 8 26.000 0.000316
                                    * 0.812
## 3 sex:time 2 8 2.571 0.137000
                                  0.300
## $'Mauchly's Test for Sphericity'
## Effect W p p<.05
## 1 time 0.735 0.63
## 2 sex:time 0.735 0.63
## $'Sphericity Corrections'
     Effect GGe DF[GG] p[GG] < .05 HFe DF[HF] p[HF] < .05
# sphericity is met for F1
res_lcrv <- anova_test(data = filter(data2, treatment == "LcrV"),</pre>
               dv = log10 titer,
               wid = id,
               between = sex,
               within = time)
res_lcrv
## ANOVA Table (type II tests)
##
## $ANOVA
## Effect DFn DFd F p p<.05 ges
## 1 sex 1 4 3.000 1.58e-01 0.324
## 2 time 2 8 120.667 1.06e-06 * 0.916
## 3 sex:time 2 8 2.889 1.14e-01
                                    0.206
## $'Mauchly's Test for Sphericity'
## Effect W p p<.05
## 1 time 0.062 0.015 *
## 2 sex:time 0.062 0.015
## $'Sphericity Corrections'
## Effect GGe DF[GG] p[GG] < .05 HFe DF[HF] p[HF]
## 1 time 0.516 1.03, 4.13 0.000323 * 0.532 1.06, 4.26 0.000265
```

```
## 2 sex:time 0.516 1.03, 4.13 0.163000
                                         0.532 1.06, 4.26 0.161000
## p[HF]<.05
## 1
## 2
# sphericity is met for LcrV
#############
# Equal Variance #
#############
library(afex)
library(performance) # for assumption checks
o1 <- aov_ez("id", "titer", filter(data2, treatment == "F1"),
            between = "sex",
            within = "time")
\mbox{\tt \#\#} Contrasts set to contr.sum for the following variables: sex
check_homogeneity(o1)
```

0K: There is not clear evidence for different variances across groups (Levene's Test, p = 0.175).

```
# ANOVA Testing - F1
# Two-Way
# Test effect of sex and time on log10_titer
two.way_f1 <- data2 %>%
 filter(treatment == "F1") %>%
 anova_test(dv = log10_titer,
         wid = id,
         between = sex,
         within = time,
         detailed = TRUE)
two.way_f1
## ANOVA Table (type II tests)
## $ANOVA
       Effect DFn DFd SSn SSd F p p<.05 ges
##
## 1 (Intercept) 1 4 118.760 0.282 1684.979 2.10e-06 * 0.993
        sex 1 4 0.181 0.282 2.571 1.84e-01
                                              0.176
## 3
        time 2 8 3.665 0.564 26.000 3.16e-04
                                             * 0.812
## 4 sex:time 2 8 0.362 0.564 2.571 1.37e-01
                                              0.300
## $'Mauchly's Test for Sphericity'
## Effect W p p<.05
     time 0.735 0.63
## 1
## 2 sex:time 0.735 0.63
##
## $'Sphericity Corrections'
## Effect GGe DF[GG] p[GG] <.05 HFe DF[HF] p[HF] <.05
## 1 time 0.79 1.58, 6.32 0.001 * 1.22 2.44, 9.76 0.000316
## 2 sex:time 0.79 1.58, 6.32 0.155
                                 1.22 2.44, 9.76 0.137000
\# Pairwise comparison between each time point combination within each sex
pwc_two.way_f1 <- data2 %>%
group_by(sex) %>%
 filter(treatment == "F1") %>%
 pairwise_t_test(
  log10_titer ~ time,
  paired = TRUE,
  p.adjust.method = "bonferroni"
pwc_two.way_f1
## # A tibble: 6 x 11
## sex .y. group1 group2 n1 n2 statistic df
                                               p p.adj p.adj.signif
## * <fct> <chr> <chr> <int> <int> <dbl> <dbl> <dbl> <dbl> <chr>
## 1 Fema~ log1~ Pre Prime
                             3 -3.46 2 0.074 0.223 ns
                        3
```

| ## | 2 | Fema~ | log1~ | Pre | Boost | 3 | 3 | -8 | 2 | 0.015 | 0.046 | * |
|----|---|---------------|--------|-------|--------|---|---|-------|---|-------|-------|----|
| ## | 3 | ${\tt Fema~}$ | log1~ | Prime | Boost | 3 | 3 | -2 | 2 | 0.184 | 0.552 | ns |
| ## | 4 | Male | log1~ | Pre | Prime | 3 | 3 | -3.46 | 2 | 0.074 | 0.223 | ns |
| ## | 5 | Male | log1~ | Pre | Boost | 3 | 3 | -5.29 | 2 | 0.034 | 0.102 | ns |
| ## | 6 | Male |] ng1~ | Prime | Boost. | 3 | 3 | -2.22 | 2 | 0.157 | 0.471 | ns |

```
#
#
# One-Way
#
#
# Test effect of time on log10_titer
one.way_f1 <- data2 %>%
 filter(treatment == "F1") %>%
 anova_test(dv = log10_titer,
         wid = id,
          within = time,
          detailed = TRUE)
one.way_f1
## ANOVA Table (type III tests)
## $ANOVA
       Effect DFn DFd SSn SSd F p p<.05 ges
## 1 (Intercept) 1 5 118.760 0.463 1282.049 3.20e-07 * 0.988
      time 2 10 3.665 0.926 19.783 3.34e-04
                                              * 0.725
##
## $'Mauchly's Test for Sphericity'
## Effect W p p<.05
## 1 time 0.578 0.335
##
## $'Sphericity Corrections'
## Effect GGe DF[GG] p[GG] <.05 HFe DF[HF] p[HF] <.05
## 1 time 0.703 1.41, 7.03 0.002 * 0.896 1.79, 8.96 0.000617
# Pairwise Comparison Between Time Points
pwc_one.way_f1 <- data2 %>%
 filter(treatment == "F1") %>%
 pairwise_t_test(
  log10_titer ~ time,
  paired = TRUE,
  p.adjust.method = "bonferroni"
 )
pwc_one.way_f1
## # A tibble: 3 x 10
            group1 group2 n1 n2 statistic df pp.adjp.adj.signif
## .y.
            <chr> <chr> <int> <int> <dbl> <dbl> <dbl> <dbl> <chr>
## * <chr>
                  Prime 6 6
                                   -5.48 5 0.003 0.008 **
## 1 log10_titer Pre
                         6 6 -5.97 5 0.002 0.006 **
## 2 log10_titer Pre
                  Boost
## 3 log10_titer Prime Boost 6 6 -2.33 5 0.067 0.201 ns
```

```
# ANOVA Testing - LcrV
# Two-Way
# Test effect of sex and time on log10_titer
two.way_lcrv <- data2 %>%
 filter(treatment == "LcrV") %>%
 anova_test(dv = log10_titer,
         wid = id,
         between = sex,
         within = time,
         detailed = TRUE)
two.way_lcrv
## ANOVA Table (type II tests)
## $ANOVA
##
       Effect DFn DFd SSn SSd F p p<.05 ges
## 1 (Intercept) 1 4 196.551 0.967 813.366 9.00e-06 * 0.992
        sex 1 4 0.725 0.967 3.000 1.58e-01
                                             0.324
## 3
        time 2 8 16.402 0.544 120.667 1.06e-06
                                             * 0.916
## 4 sex:time 2 8 0.393 0.544 2.889 1.14e-01
                                             0.206
## $'Mauchly's Test for Sphericity'
## Effect W p p<.05
     time 0.062 0.015
## 1
## 2 sex:time 0.062 0.015
##
## $'Sphericity Corrections'
## Effect GGe DF[GG] p[GG]<.05 HFe
                                           DF[HF]
## 1 time 0.516 1.03, 4.13 0.000323 * 0.532 1.06, 4.26 0.000265
## 2 sex:time 0.516 1.03, 4.13 0.163000
                                  0.532 1.06, 4.26 0.161000
## p[HF]<.05
## 1
## 2
# Pairwise comparison between each time point combination within each sex
pwc_two.way_lcrv <- data2 %>%
 group by(sex) %>%
 filter(treatment == "LcrV") %>%
 pairwise_t_test(
  log10_titer ~ time,
  paired = TRUE,
  p.adjust.method = "bonferroni"
pwc_two.way_lcrv
```

A tibble: 6 x 11

| ## | | sex | .у. | group1 | group2 | n1 | n2 | statistic | df | р | p.adj | <pre>p.adj.signif</pre> |
|----|---|-------------|-----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------------------|
| ## | * | <fct></fct> | <chr>></chr> | <chr></chr> | <chr></chr> | <int></int> | <int></int> | <dbl></dbl> | <dbl></dbl> | <dbl></dbl> | <dbl></dbl> | <chr></chr> |
| ## | 1 | Fema~ | log1~ | Pre | Prime | 3 | 3 | -7 | 2 | 0.02 | 0.059 | ns |
| ## | 2 | Fema~ | log1~ | Pre | Boost | 3 | 3 | -19.0 | 2 | 0.003 | 0.008 | ** |
| ## | 3 | Fema~ | log1~ | Prime | Boost | 3 | 3 | -5.00 | 2 | 0.038 | 0.113 | ns |
| ## | 4 | Male | log1~ | Pre | Prime | 3 | 3 | -5.27 | 2 | 0.034 | 0.103 | ns |
| ## | 5 | Male | log1~ | Pre | Boost | 3 | 3 | -9.83 | 2 | 0.01 | 0.031 | * |
| ## | 6 | Male | log1~ | Prime | Boost | 3 | 3 | -7.00 | 2 | 0.02 | 0.059 | ns |

```
#
#
# One-Way
#
# Test effect of time on log10_titer
one.way_lcrv <- data2 %>%
 filter(treatment == "LcrV") %>%
 anova_test(dv = log10_titer,
          wid = id,
          within = time,
          detailed = TRUE)
one.way_lcrv
## ANOVA Table (type III tests)
## $ANOVA
      Effect DFn DFd
                       SSn SSd
                                  F p p<.05 ges
## 1 (Intercept) 1 5 196.551 1.692 580.976 2.29e-06 * 0.987
      time 2 10 16.402 0.936 87.581 4.59e-07
##
## $'Mauchly's Test for Sphericity'
## Effect W p p<.05
## 1 time 0.337 0.114
##
## $'Sphericity Corrections'
                       p[GG] p[GG] < .05 HFe DF[HF] p[HF] < .05
## Effect GGe DF[GG]
## 1 time 0.601 1.2, 6.01 6.55e-05 * 0.687 1.37, 6.87 2.24e-05
# Pairwise Comparison Between Time Points
pwc_one.way_lcrv <- data2 %>%
 filter(treatment == "LcrV") %>%
 pairwise_t_test(
  log10_titer ~ time,
  paired = TRUE,
  p.adjust.method = "bonferroni"
pwc_one.way_lcrv
## # A tibble: 3 x 10
## .y. group1 group2 n1 n2 statistic df
                                                  p p.adj p.adj.signif
## * <chr>
          <chr> <chr> <int> <int> <dbl> <dbl> <dbl> <dbl> <chr>
## 1 log10_ti~ Pre
                        6 6
                                  -7.65 5 6.07e-4 2e-3 **
                 Prime
## 2 log10_ti~ Pre
                 Boost
                          6
                             6
                                   -11.2
                                          5 9.99e-5 3e-4 ***
                         6 6 -7.75
                                           5 5.73e-4 2e-3 **
## 3 log10 ti~ Prime Boost
# Should we use Kruskal-Wallis/Dunn's Test since the "Pre" group is not normal?
library(permuco)
```

```
mod <- aovperm(log10_titer ~ time*sex + Error(id/(time)),</pre>
                     data = filter(data2, treatment == "F1"), method = "Rd_kheradPajouh_renaud")
mod
##
## Resampling test using Rd_kheradPajouh_renaud to handle nuisance variables and 5000 permutations.
             SSn dfn SSd dfd MSEn
                                       MSEd
                                                 F parametric P(>F)
          ## sex
          3.6650 2 0.5639 8 1.8325 0.07048 26.000
                                                          0.000316
## time
## time:sex 0.3625 2 0.5639 8 0.1812 0.07048 2.571 0.137278
         resampled P(>F)
## sex
                  0.1794
## time
                   0.0004
## time:sex
                  0.1374
mod2 <- aovperm(log10_titer ~ time + sex + Error(id/(time)),</pre>
             data = filter(data2, treatment == "F1"), method = "Rd_kheradPajouh_renaud")
mod2
##
## Resampling test using Rd_kheradPajouh_renaud to handle nuisance variables and 5000 permutations.
          SSn dfn
                   SSd dfd MSEn
                                   MSEd
                                            F parametric P(>F)
## sex 0.1812 1 0.2819 4 0.1812 0.07048 2.571 0.1840740
## time 3.6650 2 0.9263 10 1.8325 0.09263 19.783
                                                    0.0003343
       resampled P(>F)
               0.1756
## SAY
               0.0004
## time
mod3 <- aovperm(log10_titer ~ time + Error(id/(time)),</pre>
              data = filter(data2, treatment == "F1"), method = "Rd_kheradPajouh_renaud")
mod3
##
## Resampling test using Rd_kheradPajouh_renaud to handle nuisance variables and 5000 permutations.
                  SSd dfd MSEn MSEd F parametric P(>F) resampled P(>F)
## SSn dfn
## time 3.665 2 0.9263 10 1.833 0.09263 19.78
                                               0.0003343
                                                                   0.0012
# Friedman
res.fried <- data2 %>%
 filter(treatment == "F1") %>% friedman_test(log10_titer ~ time |id)
res.fried
## # A tibble: 1 x 6
p method
                        11.6 2 0.00308 Friedman test
## 1 log10 titer 6
# Effect size - overall difference among group
data2 %>%
filter(treatment == "F1") %>%
friedman_effsize(log10_titer ~ time |id)
```

```
## # A tibble: 1 x 5
magnitude
## 1 log10_titer 6 0.964 Kendall W large
# pairwise comparisons
pwc <- data2 %>%
filter(treatment == "F1") %>%
# filter(time == "Pre" | time == "Prime") %>%
wilcox_test(log10_titer ~ time,
           paired = TRUE,
           p.adjust.method = "bonferroni")
pwc
## # A tibble: 3 x 9
         ## .y.
## * <chr>
## 1 log10_titer Pre Prime 6 6
                                       0 0.034 0.104 ns
## 2 log10_titer Pre Boost
                           6
                                6
                                         0 0.034 0.102 ns
## 3 log10_titer Prime Boost 6 6
                                         0 0.054 0.164 ns
# connect with mean, sd (numeric table)
# give p-values and adjusted p-values
# for LcrV
res.fried2 <- data2 %>%
 filter(treatment == "LcrV") %>% friedman_test(log10_titer ~ time |id)
res.fried2
## # A tibble: 1 x 6
p method
## 1 log10_titer 6
                      12 2 0.00248 Friedman test
# Effect size
data2 %>%
 filter(treatment == "LcrV") %>%
friedman_effsize(log10_titer ~ time |id)
## # A tibble: 1 x 5
## .y. n effsize method
## * <chr> <int> <dbl> <chr>
## .y.
                                magnitude
                                <ord>
## 1 log10_titer 6
                     1 Kendall W large
# pairwise comparisons
pwc2 <- data2 %>%
 filter(treatment == "LcrV") %>%
 wilcox_test(log10_titer ~ time,
           paired = TRUE,
           p.adjust.method = "bonferroni")
pwc2
```