KET PRE VR5 SAC NORM means

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```
library(ggplot2)
library(car) # For levene.test() function

## Loading required package: carData
library(emmeans)
library(stringr)
library(MASS)
knitr::opts_chunk$set(fig.width=6, fig.height=4)
```

NORM intensities, grouped by rat

```
Sidak <- function(pvals)</pre>
  # takes a vector of p-values and corrects p-values according to
  # Sidaks method for multiple comparisons (1967)
  # Jonathan Ramos 3/12/2024
  adjusted <- c()
  j <- length(pvals)</pre>
  for (i in 1:j){
    adj_p <- 1-(1-pvals[i])^j</pre>
    adjusted <- c(adjusted, adj_p)</pre>
  return(adjusted)
}
eda_anova_1way <- function(fname)</pre>
  # doc
  df <- read.csv(fname, header=TRUE, sep=",")</pre>
  df$treat_factor <- as.factor(df$treat)</pre>
  df <- df[df$react == "VR5",c('norm_adj_mmbg','treat_factor','react')]</pre>
  ### check assumption of normality
  # quantitative assessment
  print(tapply(df$norm_adj_mmbg, df$treat_factor, shapiro.test))
```

```
# qualitative assessment
  g <- ggqqplot(df, x="norm_adj_mmbg", facet.by=c("treat_factor"))</pre>
  ### check assumption of equal variances
  # quantitative assessment
  print(leveneTest(y=df$norm_adj_mmbg, group=df$treat_factor, center='mean'))
  # qualitative assessment
  f <- ggplot(df, aes(x=treat_factor, y=norm_adj_mmbg)) + geom_boxplot(aes(fill=treat_factor), alpha=0.
    geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.7)+
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1))
  # run the ANOVA, display summary
  # since this is just a one way ANOVA with only 2 levels, this is essentially just a t-test
  # ANOVA is performed here for consistency
  df.lm <- lm(norm_adj_mmbg ~ treat_factor, data=df)</pre>
  df.III.aov <- car::Anova(df.lm, type = 3)</pre>
  #print(df.III.aov)
  # now let's actually just do the t-test
  print(t.test(df[df$treat_factor == "KET",]$norm_adj_mmbg, df[df$treat_factor == "SAL",]$norm_adj_mmbg
  # KS test
  print(kruskal.test(norm_adj_mmbg ~ treat_factor, data=df))
  # no post hoc is required for 1way ANOVA with 2 levels
  # return figure objects only
 return(list(g, f))
### this time with boxcox transformation first
eda_anova_1way_boxcox <- function(fname)</pre>
 # doc
 df <- read.csv(fname, header=TRUE, sep=",")</pre>
 df$treat_factor <- as.factor(df$treat)</pre>
  df <- df[df$react == "VR5",c('norm_adj_mmbg','treat_factor','react')]</pre>
  print(df$coloc_stain_type)
  norm.adj.mmbg <<- c(df$norm_adj_mmbg)</pre>
 norm.adj.mmbg <<- norm.adj.mmbg + 0.00000001</pre>
  # apply boxcox over range of lambdas
  b <- boxcox(lm(norm.adj.mmbg ~ 1))</pre>
  # Exact best lambda
  lambda.best <<- b$x[which.max(b$y)]</pre>
  print("best lamda")
  print(lambda.best)
```

```
boxcox.transformed <- c()</pre>
j <- length(norm.adj.mmbg)</pre>
for (i in 1:j){
  transformed.x <- (norm.adj.mmbg[i]^lambda.best-1)/lambda.best
  boxcox.transformed <- c(boxcox.transformed, transformed.x)</pre>
df$norm.adj.mmbg.boxcox <- boxcox.transformed</pre>
### check assumption of normality
# quantitative assessment
print(tapply(df$norm.adj.mmbg.boxcox, df$treat_factor, shapiro.test))
# qualitative assessment
g <- ggqqplot(df, x="norm.adj.mmbg.boxcox", facet.by=c("treat_factor"))</pre>
g
### check assumption of equal variances
# quantitative assessment
print(leveneTest(y=df$norm.adj.mmbg.boxcox, group=df$treat_factor, center='mean'))
# qualitative assessment
f <- ggplot(df, aes(x=treat_factor, y=norm.adj.mmbg.boxcox)) + geom_boxplot(aes(fill=treat_factor), a
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5, alpha=0.5)
theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1))
# run the ANOVA, display summary
\# since this is just a one way ANOVA with only 2 levels, this is essentially just a t-test
# ANOVA is performed here for consistency
df.lm <- lm(norm.adj.mmbg.boxcox ~ treat_factor, data=df)</pre>
df.III.aov <- car::Anova(df.lm, type = 3)</pre>
#print(df.III.aov)
# now let's actually just do the t-test
print(t.test(df[df$treat_factor == "KET",]$norm.adj.mmbg.boxcox, df[df$treat_factor == "SAL",]$norm.a
# KS test
print(kruskal.test(norm.adj.mmbg.boxcox ~ treat_factor, data=df))
# no post hoc is required for 1way ANOVA with 2 levels
# return figure objects only
return(list(g, f))
```

pulling out filenames

```
fnames <- list.files(path='NORM_means',pattern='NORM.csv', full.names=TRUE)
fnames

## [1] "NORM_means/KET-VR5_cFos_coloc_w_PV_NORM.csv"

## [2] "NORM_means/KET-VR5_cFos_coloc_w_WFA_NORM.csv"

## [3] "NORM_means/KET-VR5_PV_coloc_w_cFos_NORM.csv"

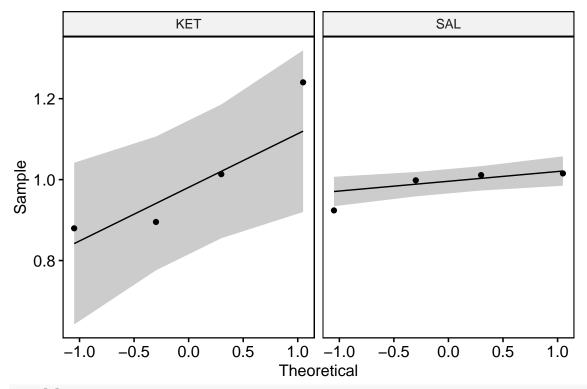
## [4] "NORM_means/KET-VR5_PV_coloc_w_WFA_NORM.csv"</pre>
```

```
## [5] "NORM_means/KET-VR5_single_cFos_NORM.csv"
```

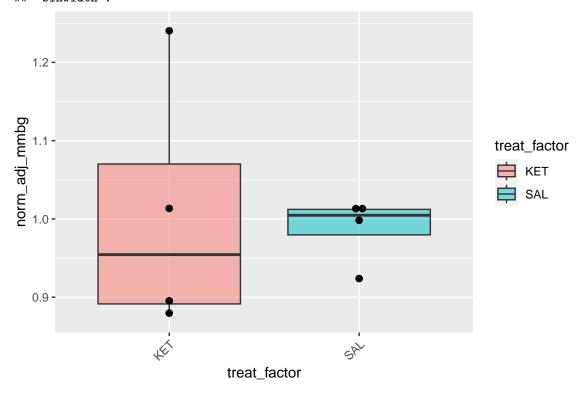
- ## [6] "NORM_means/KET-VR5_single_PV_NORM.csv"
- ## [7] "NORM_means/KET-VR5_single_WFA_NORM.csv"
- ## [8] "NORM_means/KET-VR5_triple_cFos_NORM.csv"
- ## [9] "NORM_means/KET-VR5_triple_PV_NORM.csv"
- ## [10] "NORM_means/KET-VR5_triple_WFA_NORM.csv"
- ## [11] "NORM_means/KET-VR5_WFA_coloc_w_cFos_NORM.csv"
- ## [12] "NORM_means/KET-VR5_WFA_coloc_w_PV_NORM.csv"

single PV

```
i <- str_which(fnames, "single_PV")</pre>
f <- fnames[i]</pre>
print(f)
## [1] "NORM_means/KET-VR5_single_PV_NORM.csv"
figs <- eda_anova_1way(f)
## $KET
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.86056, p-value = 0.2622
##
##
## $SAL
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.76813, p-value = 0.05627
##
##
## Levene's Test for Homogeneity of Variance (center = "mean")
         Df F value Pr(>F)
                3.4 0.1148
## group 1
##
          6
##
## Welch Two Sample t-test
## data: df[df$treat_factor == "KET", ]$norm_adj_mmbg and df[df$treat_factor == "SAL", ]$norm_adj_mmbg
## t = 0.23354, df = 3.3946, p-value = 0.8287
\#\# alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.2363387 0.2764882
## sample estimates:
## mean of x mean of y
## 1.0072263 0.9871515
##
##
## Kruskal-Wallis rank sum test
##
## data: norm_adj_mmbg by treat_factor
## Kruskal-Wallis chi-squared = 0.083333, df = 1, p-value = 0.7728
figs[1]
```

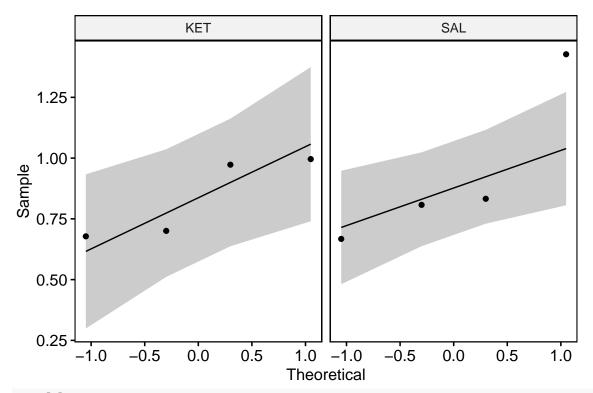


figs[2]

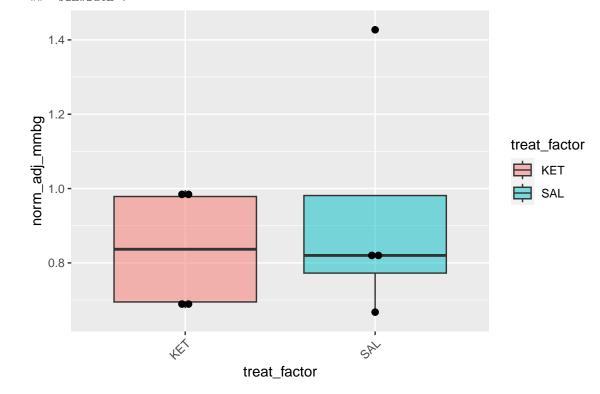


single WFA

```
i <- str_which(fnames, "single_WFA")</pre>
f <- fnames[i]</pre>
print(f)
## [1] "NORM_means/KET-VR5_single_WFA_NORM.csv"
figs <- eda_anova_1way(f)
## $KET
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.79416, p-value = 0.09215
##
##
## $SAL
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.81332, p-value = 0.1283
##
##
## Levene's Test for Homogeneity of Variance (center = "mean")
         Df F value Pr(>F)
## group 1 1.2109 0.3133
##
##
## Welch Two Sample t-test
## data: df[df$treat_factor == "KET", ]$norm_adj_mmbg and df[df$treat_factor == "SAL", ]$norm_adj_mmbg
## t = -0.51293, df = 4.4475, p-value = 0.6324
\#\# alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.6011072 0.4073495
## sample estimates:
## mean of x mean of y
## 0.8368427 0.9337216
##
##
## Kruskal-Wallis rank sum test
##
## data: norm_adj_mmbg by treat_factor
## Kruskal-Wallis chi-squared = 0, df = 1, p-value = 1
figs[1]
```

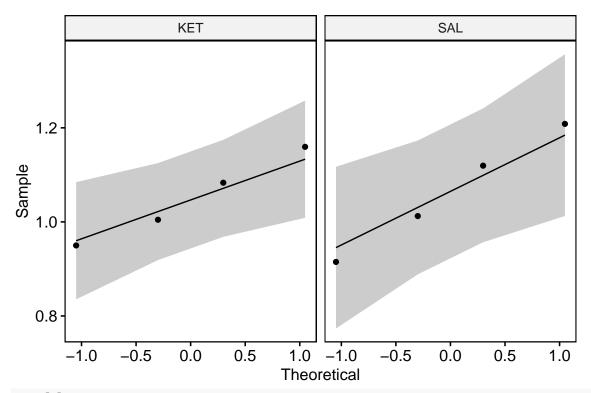


figs[2]



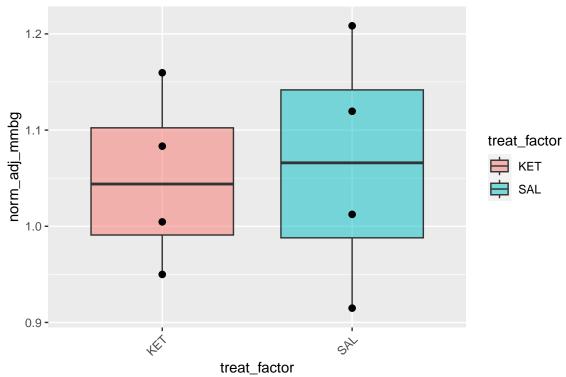
Single c-Fos

```
i <- str_which(fnames, "single_cFos")</pre>
f <- fnames[i]</pre>
print(f)
## [1] "NORM_means/KET-VR5_single_cFos_NORM.csv"
figs <- eda_anova_1way(f)
## $KET
##
  Shapiro-Wilk normality test
##
##
## data: X[[i]]
## W = 0.98066, p-value = 0.9059
##
##
## $SAL
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.987, p-value = 0.9416
##
##
## Levene's Test for Homogeneity of Variance (center = "mean")
         Df F value Pr(>F)
## group 1 0.7226 0.4279
##
##
## Welch Two Sample t-test
## data: df[df$treat_factor == "KET", ]$norm_adj_mmbg and df[df$treat_factor == "SAL", ]$norm_adj_mmbg
## t = -0.18416, df = 5.4443, p-value = 0.8606
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.2115231 0.1825922
## sample estimates:
## mean of x mean of y
## 1.049343 1.063809
##
##
## Kruskal-Wallis rank sum test
##
## data: norm_adj_mmbg by treat_factor
## Kruskal-Wallis chi-squared = 0.083333, df = 1, p-value = 0.7728
figs[1]
```



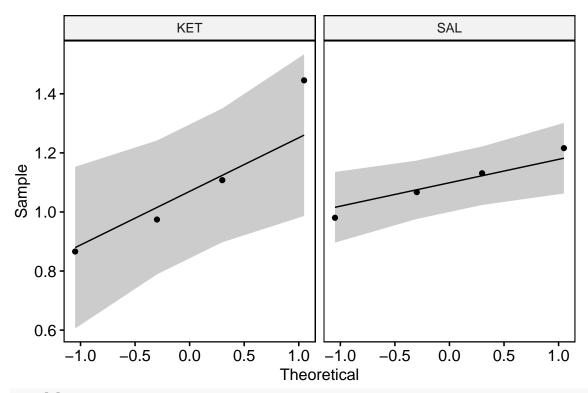
figs[2]

 $\mbox{\tt \#\#}$ Bin width defaults to 1/30 of the range of the data. Pick better value with $\mbox{\tt \#\#}$ `binwidth`.

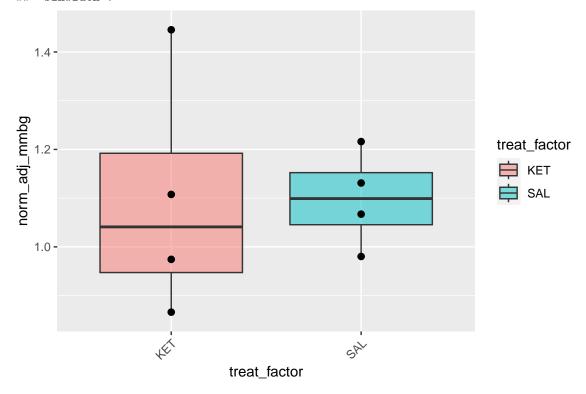


PV coloc w WFA

```
i <- str_which(fnames, "PV_coloc_w_WFA")</pre>
f <- fnames[i]</pre>
print(f)
## [1] "NORM_means/KET-VR5_PV_coloc_w_WFA_NORM.csv"
figs <- eda_anova_1way(f)
## $KET
##
##
   Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.93051, p-value = 0.5974
##
##
## $SAL
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.99923, p-value = 0.9978
##
##
## Levene's Test for Homogeneity of Variance (center = "mean")
         Df F value Pr(>F)
## group 1 1.8187 0.2261
##
##
## Welch Two Sample t-test
## data: df[df$treat_factor == "KET", ]$norm_adj_mmbg and df[df$treat_factor == "SAL", ]$norm_adj_mmbg
## t = -0.002015, df = 3.9199, p-value = 0.9985
\#\# alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.3790987 0.3785532
## sample estimates:
## mean of x mean of y
## 1.098372 1.098645
##
## Kruskal-Wallis rank sum test
##
## data: norm_adj_mmbg by treat_factor
## Kruskal-Wallis chi-squared = 0.33333, df = 1, p-value = 0.5637
figs[1]
```

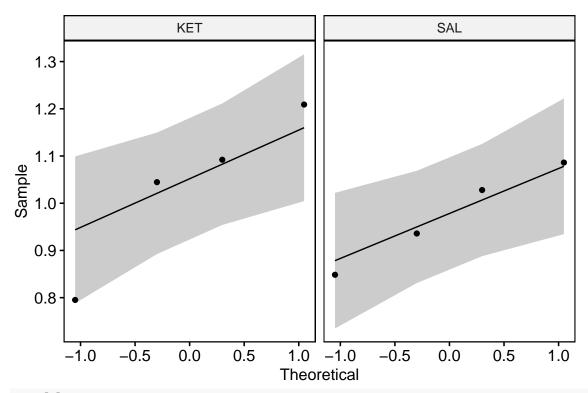


figs[2]

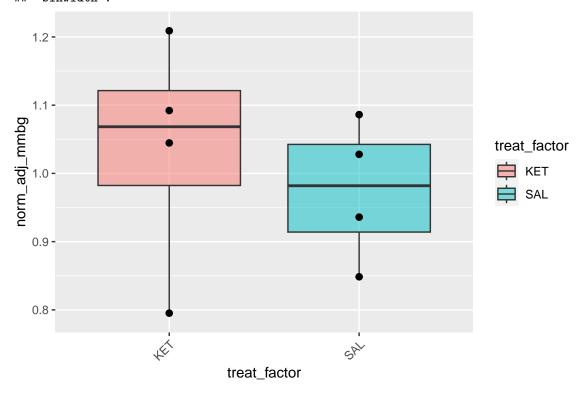


PV coloc w cFos

```
i <- str_which(fnames, "PV_coloc_w_cFos")</pre>
f <- fnames[i]</pre>
print(f)
## [1] "NORM_means/KET-VR5_PV_coloc_w_cFos_NORM.csv"
figs <- eda_anova_1way(f)
## $KET
##
   Shapiro-Wilk normality test
##
##
## data: X[[i]]
## W = 0.93739, p-value = 0.6385
##
##
## $SAL
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.97623, p-value = 0.8796
##
##
## Levene's Test for Homogeneity of Variance (center = "mean")
         Df F value Pr(>F)
## group 1 0.4345 0.5343
##
##
## Welch Two Sample t-test
## data: df[df$treat_factor == "KET", ]$norm_adj_mmbg and df[df$treat_factor == "SAL", ]$norm_adj_mmbg
## t = 0.59686, df = 4.907, p-value = 0.5771
\#\# alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.2020048 0.3232730
## sample estimates:
## mean of x mean of y
## 1.035257 0.974623
##
##
## Kruskal-Wallis rank sum test
##
## data: norm_adj_mmbg by treat_factor
## Kruskal-Wallis chi-squared = 0.75, df = 1, p-value = 0.3865
figs[1]
```

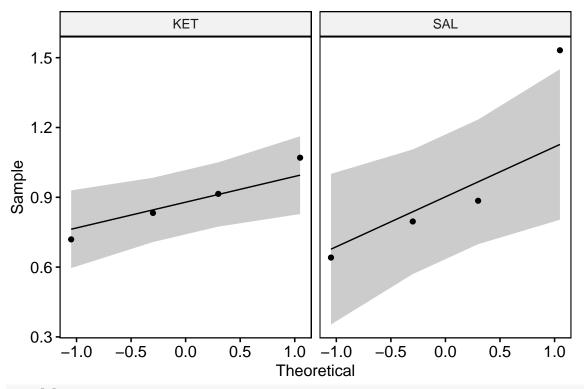


figs[2]

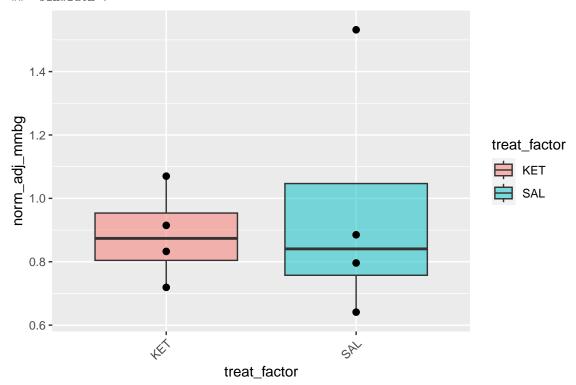


WFA coloc w PV

```
i <- str_which(fnames, "WFA_coloc_w_PV")</pre>
f <- fnames[i]
print(f)
## [1] "NORM_means/KET-VR5_WFA_coloc_w_PV_NORM.csv"
figs <- eda_anova_1way(f)
## $KET
##
   Shapiro-Wilk normality test
##
##
## data: X[[i]]
## W = 0.99326, p-value = 0.9735
##
##
## $SAL
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.85251, p-value = 0.2344
##
##
## Levene's Test for Homogeneity of Variance (center = "mean")
         Df F value Pr(>F)
## group 1 2.3698 0.1746
##
##
## Welch Two Sample t-test
## data: df[df$treat_factor == "KET", ]$norm_adj_mmbg and df[df$treat_factor == "SAL", ]$norm_adj_mmbg
## t = -0.37873, df = 3.8339, p-value = 0.7249
\#\# alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.6710372 0.5123601
## sample estimates:
## mean of x mean of y
## 0.8841805 0.9635191
##
##
## Kruskal-Wallis rank sum test
##
## data: norm_adj_mmbg by treat_factor
## Kruskal-Wallis chi-squared = 0.083333, df = 1, p-value = 0.7728
figs[1]
```

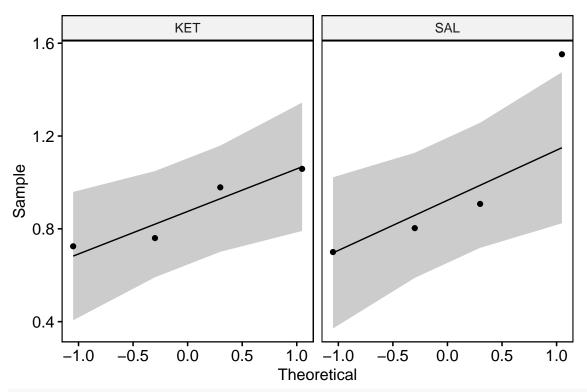


figs[2]

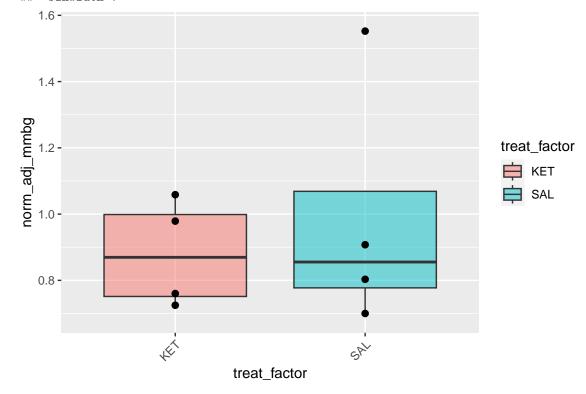


WFA coloc w cFos

```
i <- str_which(fnames, "WFA_coloc_w_cFos")</pre>
f <- fnames[i]</pre>
print(f)
## [1] "NORM_means/KET-VR5_WFA_coloc_w_cFos_NORM.csv"
figs <- eda_anova_1way(f)
## $KET
##
##
  Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.88176, p-value = 0.3462
##
##
## $SAL
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.82294, p-value = 0.1501
##
##
## Levene's Test for Homogeneity of Variance (center = "mean")
         Df F value Pr(>F)
## group 1 1.8735 0.2201
##
##
## Welch Two Sample t-test
## data: df[df$treat_factor == "KET", ]$norm_adj_mmbg and df[df$treat_factor == "SAL", ]$norm_adj_mmbg
## t = -0.528, df = 4.0522, p-value = 0.6251
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.6863533 0.4660778
## sample estimates:
## mean of x mean of y
## 0.8805976 0.9907353
##
##
## Kruskal-Wallis rank sum test
##
## data: norm_adj_mmbg by treat_factor
## Kruskal-Wallis chi-squared = 0, df = 1, p-value = 1
figs[1]
```

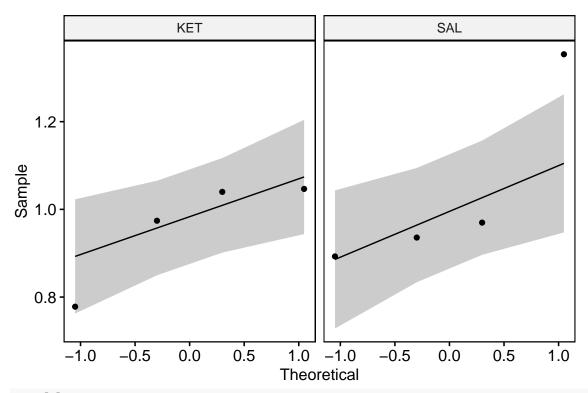


figs[2]

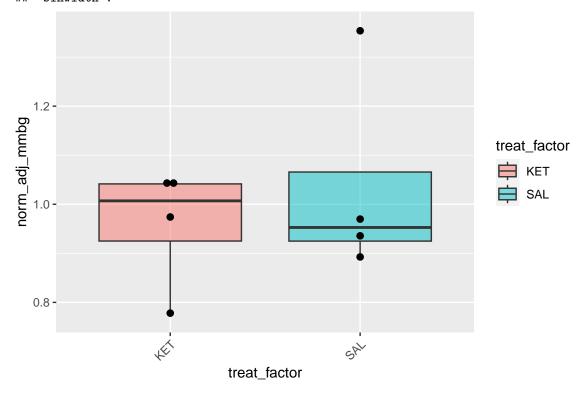


cFos coloc w PV

```
i <- str_which(fnames, "cFos_coloc_w_PV")</pre>
f <- fnames[i]
print(f)
## [1] "NORM_means/KET-VR5_cFos_coloc_w_PV_NORM.csv"
figs <- eda_anova_1way(f)
## $KET
##
   Shapiro-Wilk normality test
##
##
## data: X[[i]]
## W = 0.8102, p-value = 0.1218
##
##
## $SAL
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.76539, p-value = 0.05326
##
##
## Levene's Test for Homogeneity of Variance (center = "mean")
         Df F value Pr(>F)
## group 1 1.0707 0.3407
##
##
## Welch Two Sample t-test
## data: df[df$treat_factor == "KET", ]$norm_adj_mmbg and df[df$treat_factor == "SAL", ]$norm_adj_mmbg
## t = -0.6339, df = 4.8588, p-value = 0.5548
\#\# alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.3986195 0.2420183
## sample estimates:
## mean of x mean of y
## 0.9596533 1.0379539
##
##
## Kruskal-Wallis rank sum test
##
## data: norm_adj_mmbg by treat_factor
## Kruskal-Wallis chi-squared = 0.083333, df = 1, p-value = 0.7728
figs[1]
```

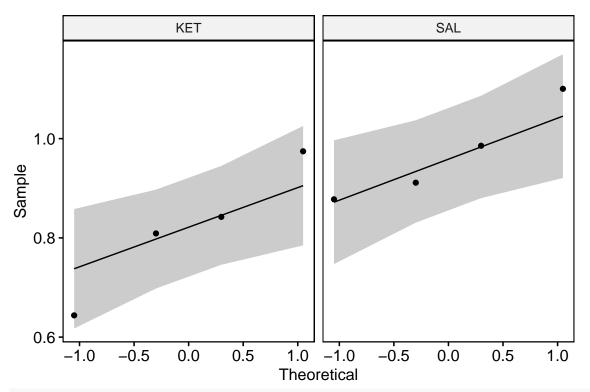


figs[2]

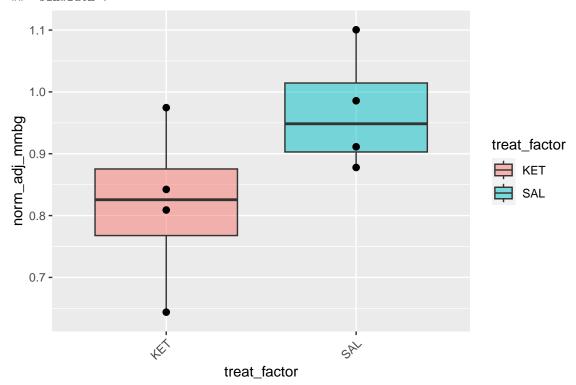


cFos coloc w WFA

```
i <- str_which(fnames, "cFos_coloc_w_WFA")</pre>
f <- fnames[i]</pre>
print(f)
## [1] "NORM_means/KET-VR5_cFos_coloc_w_WFA_NORM.csv"
figs <- eda_anova_1way(f)
## $KET
##
##
   Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.97656, p-value = 0.8816
##
##
## $SAL
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.93658, p-value = 0.6336
##
##
## Levene's Test for Homogeneity of Variance (center = "mean")
         Df F value Pr(>F)
## group 1 0.1136 0.7475
##
##
## Welch Two Sample t-test
## data: df[df$treat_factor == "KET", ]$norm_adj_mmbg and df[df$treat_factor == "SAL", ]$norm_adj_mmbg
## t = -1.8017, df = 5.4732, p-value = 0.1264
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.36186642 0.05910286
## sample estimates:
## mean of x mean of y
## 0.8174022 0.9687840
##
##
## Kruskal-Wallis rank sum test
##
## data: norm_adj_mmbg by treat_factor
## Kruskal-Wallis chi-squared = 3, df = 1, p-value = 0.08326
figs[1]
```

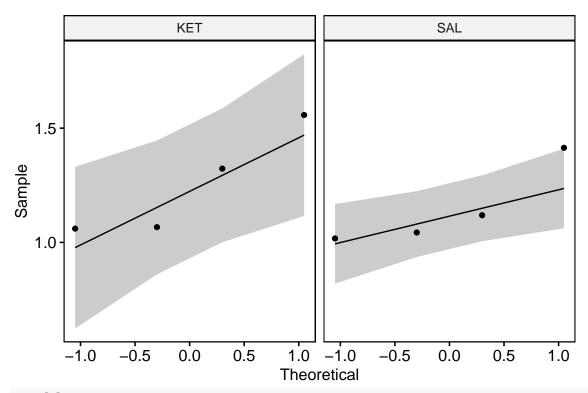


figs[2]

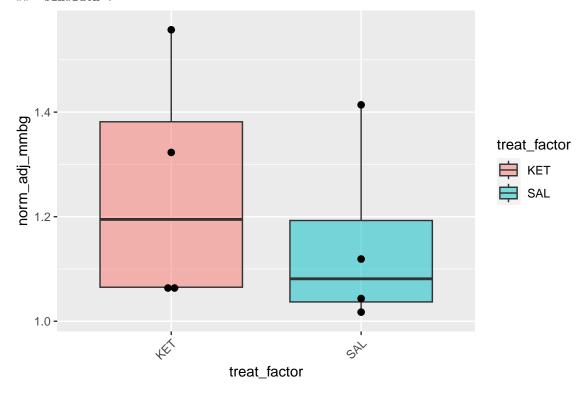


triple PV

```
i <- str_which(fnames, "triple_PV")</pre>
f <- fnames[i]</pre>
print(f)
## [1] "NORM_means/KET-VR5_triple_PV_NORM.csv"
figs <- eda_anova_1way(f)
## $KET
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.8716, p-value = 0.304
##
##
## $SAL
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.8164, p-value = 0.135
##
##
## Levene's Test for Homogeneity of Variance (center = "mean")
         Df F value Pr(>F)
## group 1 0.6551 0.4492
##
##
## Welch Two Sample t-test
## data: df[df$treat_factor == "KET", ]$norm_adj_mmbg and df[df$treat_factor == "SAL", ]$norm_adj_mmbg
## t = 0.69071, df = 5.6207, p-value = 0.5172
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.268984 0.475786
## sample estimates:
## mean of x mean of y
## 1.251871 1.148470
##
##
## Kruskal-Wallis rank sum test
##
## data: norm_adj_mmbg by treat_factor
## Kruskal-Wallis chi-squared = 0.75, df = 1, p-value = 0.3865
figs[1]
```

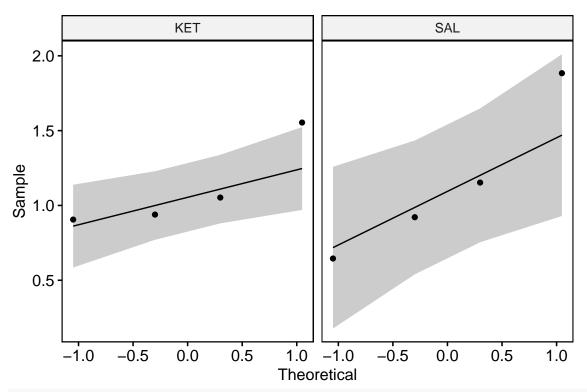


figs[2]

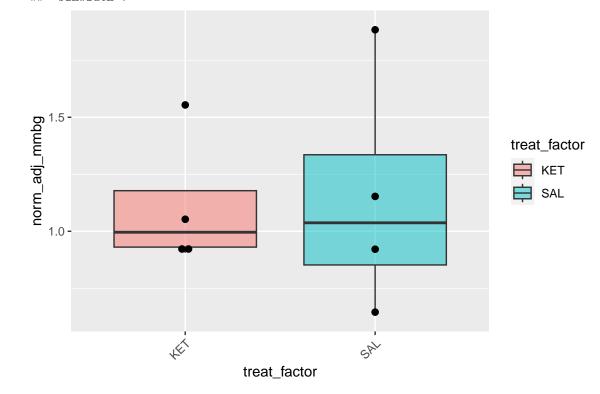


triple WFA

```
i <- str_which(fnames, "triple_WFA")</pre>
f <- fnames[i]</pre>
print(f)
## [1] "NORM_means/KET-VR5_triple_WFA_NORM.csv"
figs <- eda_anova_1way(f)
## $KET
##
  Shapiro-Wilk normality test
##
##
## data: X[[i]]
## W = 0.79507, p-value = 0.09367
##
##
## $SAL
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.93625, p-value = 0.6316
##
##
## Levene's Test for Homogeneity of Variance (center = "mean")
         Df F value Pr(>F)
## group 1 0.6778 0.4418
##
##
## Welch Two Sample t-test
## data: df[df$treat_factor == "KET", ]$norm_adj_mmbg and df[df$treat_factor == "SAL", ]$norm_adj_mmbg
## t = -0.12461, df = 4.7469, p-value = 0.9059
\#\# alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.8350187 0.7589848
## sample estimates:
## mean of x mean of y
## 1.112876 1.150893
##
##
## Kruskal-Wallis rank sum test
##
## data: norm_adj_mmbg by treat_factor
## Kruskal-Wallis chi-squared = 0, df = 1, p-value = 1
figs[1]
```

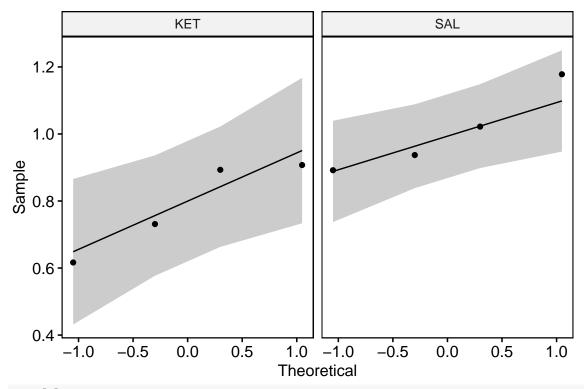


figs[2]



triple cFos

```
i <- str_which(fnames, "triple_cFos")</pre>
f <- fnames[i]
print(f)
## [1] "NORM_means/KET-VR5_triple_cFos_NORM.csv"
figs <- eda_anova_1way(f)
## $KET
##
  Shapiro-Wilk normality test
##
##
## data: X[[i]]
## W = 0.88815, p-value = 0.3746
##
##
## $SAL
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.93308, p-value = 0.6126
##
##
## Levene's Test for Homogeneity of Variance (center = "mean")
         Df F value Pr(>F)
## group 1 0.2505 0.6346
##
##
## Welch Two Sample t-test
## data: df[df$treat_factor == "KET", ]$norm_adj_mmbg and df[df$treat_factor == "SAL", ]$norm_adj_mmbg
## t = -2.3483, df = 5.9442, p-value = 0.05758
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.450226955 0.009766906
## sample estimates:
## mean of x mean of y
## 0.7870625 1.0072925
##
##
## Kruskal-Wallis rank sum test
##
## data: norm_adj_mmbg by treat_factor
## Kruskal-Wallis chi-squared = 3, df = 1, p-value = 0.08326
figs[1]
```



figs[2]

