KET_VR5 3way ANOVAs for Completeness: Checking Main Effect t-tests

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

```
## Loading required package: carData
```

```
library(emmeans)
library(stringr)
library(gplots)
```

```
##
## Attaching package: 'gplots'
```

```
## The following object is masked from 'package:stats':
##
##     lowess
```

```
require(gridExtra)
```

```
## Loading required package: gridExtra
```

```
library(knitr)
library(plyr)
```

```
##
## Attaching package: 'plyr'
```

```
## The following object is masked from 'package:ggpubr':
##
##     mutate
```

Some post hoc t-tests need new ANOVAs

I previously performed these ANOVAs in python but when I revisited these sets in R I skipped right to the post hoc. ANOVAs in python are terrible because the linear model is not properly specified (leading to differences in p values with R and Prism) and so really I should have repeated those 3way ANOVAs before performing the t-tests.

In general if there is an interaction effect:

- we do not interpret the main effects
- HOWEVER: there still may be a case to interpret the main effect in the presence of an interaction effect IF all data at one level of a particular factor are higher (or lower) than than all other data at a different level of that factor.

For example, if we were interested in PV intensity and found that there is a 2way interaction between react and the presence of cFos, if PV intensity in cFos cells is high across the board compared to non-cFos cells, then we can still interpret the main effect of cFos (with a t-test) and separately conclude that PV intensity is generally higher if cFos was present. In particular for this document, we are trying to determine whether or not the triangles are well separated (a long the y axis) from the circles. Unfortunately there is no definitive test to make the determination with and here we rely on qualitative analysis.

The 3way ANOVAs performed in this document are as follows:

- PV intensity: WFA+/- (reactivation by treatment by WFA) 3way
- PV intensity: Npas4+/- (reactivation by treatment by Npas4) 3way
- PV intensity: cFos+/- (reactivation by treatment by cFos) 3way
- WFA intensity: PV+/- (reactivation by treatment by PV) 3way
- WFA intensity: cFos+/- (reactivation by treatment by cFos) 3way
- WFA intensity: Npas4+/- (reactivation by treatment by Npas4) 3way
- Npas4 intensity: PV+/- (reactivation by treatment by PV) 3way
- Npas4 intensity: cFos+/- (reactivation by treatment by cFos) 3way
- Npas4 intensity: WFA+/- (reactivation by treatment by WFA) 3way
- cFos intensity: Npas4+/- (reactivation by treatment by Npas4) 3way
- cFos intensity: PV+/- (reactivation by treatment by PV) 3way
- cFos intensity: WFA+/- (reactivation by treatment by WFA) 3way

```
## Summarizes data.
## Gives count, mean, standard deviation, standard error of the mean, and confidence interval (default
##   data: a data frame.
##   measurevar: the name of a column that contains the variable to be summariezed
##   groupvars: a vector containing names of columns that contain grouping variables
##   na.rm: a boolean that indicates whether to ignore NA's
##   conf.interval: the percent range of the confidence interval (default is 95%)
summarySE <- function(data=NULL, measurevar, groupvars=NULL, na.rm=FALSE,
                      conf.interval=.95, .drop=TRUE) {

    # New version of length which can handle NA's: if na.rm==T, don't count them
    length2 <- function (x, na.rm=FALSE) {
        if (na.rm) sum(!is.na(x))
        else       length(x)
    }

    # This does the summary. For each group's data frame, return a vector with
    # N, mean, and sd
    datac <- ddply(data, groupvars, .drop=.drop,
      .fun = function(xx, col) {
        c(N    = length2(xx[[col]], na.rm=na.rm),
          mean = mean   (xx[[col]], na.rm=na.rm),
          sd   = sd     (xx[[col]], na.rm=na.rm)
        )
      },
      measurevar
    )
```

```
    # Rename the "mean" column
    datac <- rename(datac, c("mean" = measurevar))

    datac$se <- datac$sd / sqrt(datac$N)  # Calculate standard error of the mean

    # Confidence interval multiplier for standard error
    # Calculate t-statistic for confidence interval:
    # e.g., if conf.interval is .95, use .975 (above/below), and use df=N-1
    ciMult <- qt(conf.interval/2 + .5, datac$N-1)
    datac$ci <- datac$se * ciMult

    return(datac)
}
```

Single PV Norm Intensity

```
PV <- read.csv('NORM_single_PV.csv', header=TRUE, sep=',')
split <- str_split_fixed(PV$treatment, "_", 2)
PV$react <- split[,1]
PV$treat <- split[,2]

PV$react_treat_factor <- as.factor(PV$treatment)
PV$react_factor <- as.factor(PV$react)
PV$treat_factor <- as.factor(PV$treat)
PV$WFA_factor <- as.factor(PV$dummy_WFA)
PV$Npas4_factor <- as.factor(PV$dummy_Npas4)
PV$cFos_factor <- as.factor(PV$dummy_cFos)

str(PV)
```

```
## 'data.frame':    1266 obs. of  17 variables:
##  $ X                 : int  0 1 2 3 4 5 6 7 8 9 ...
##  $ stain_type        : chr  "PV" "PV" "PV" "PV" ...
##  $ intensity         : num  383 161 170 192 276 ...
##  $ treatment         : chr  "FR1_KET" "FR1_KET" "FR1_KET" "FR1_KET" ...
##  $ dummy_PV          : chr  "True" "True" "True" "True" ...
##  $ dummy_cFos        : chr  "False" "True" "False" "False" ...
##  $ dummy_Npas4       : chr  "True" "True" "True" "True" ...
##  $ dummy_WFA         : chr  "True" "False" "True" "True" ...
##  $ norm_intensity    : num  2.003 0.842 0.89 1.005 1.442 ...
##  $ react             : chr  "FR1" "FR1" "FR1" "FR1" ...
##  $ treat             : chr  "KET" "KET" "KET" "KET" ...
##  $ react_treat_factor: Factor w/ 4 levels "FR1_KET","FR1_SAL",..: 1 1 1 1 1 1 1 1 1 1 ...
##  $ react_factor      : Factor w/ 2 levels "FR1","VR5": 1 1 1 1 1 1 1 1 1 1 ...
##  $ treat_factor      : Factor w/ 2 levels "KET","SAL": 1 1 1 1 1 1 1 1 1 1 ...
##  $ WFA_factor        : Factor w/ 2 levels "False","True": 2 1 2 2 1 2 1 2 1 1 ...
##  $ Npas4_factor      : Factor w/ 2 levels "False","True": 2 2 2 2 1 2 2 2 1 2 ...
##  $ cFos_factor       : Factor w/ 2 levels "False","True": 1 2 1 1 1 1 2 2 2 2 ...
```

PV, WFA+/-

```
# WFA +/-
PV.WFA.lm <- lm(norm_intensity ~ react_factor*treat_factor*WFA_factor, contrasts = list(react_factor='c
PV.WFA.aov <- car::Anova(PV.WFA.lm, type=3)
print(PV.WFA.aov)
```

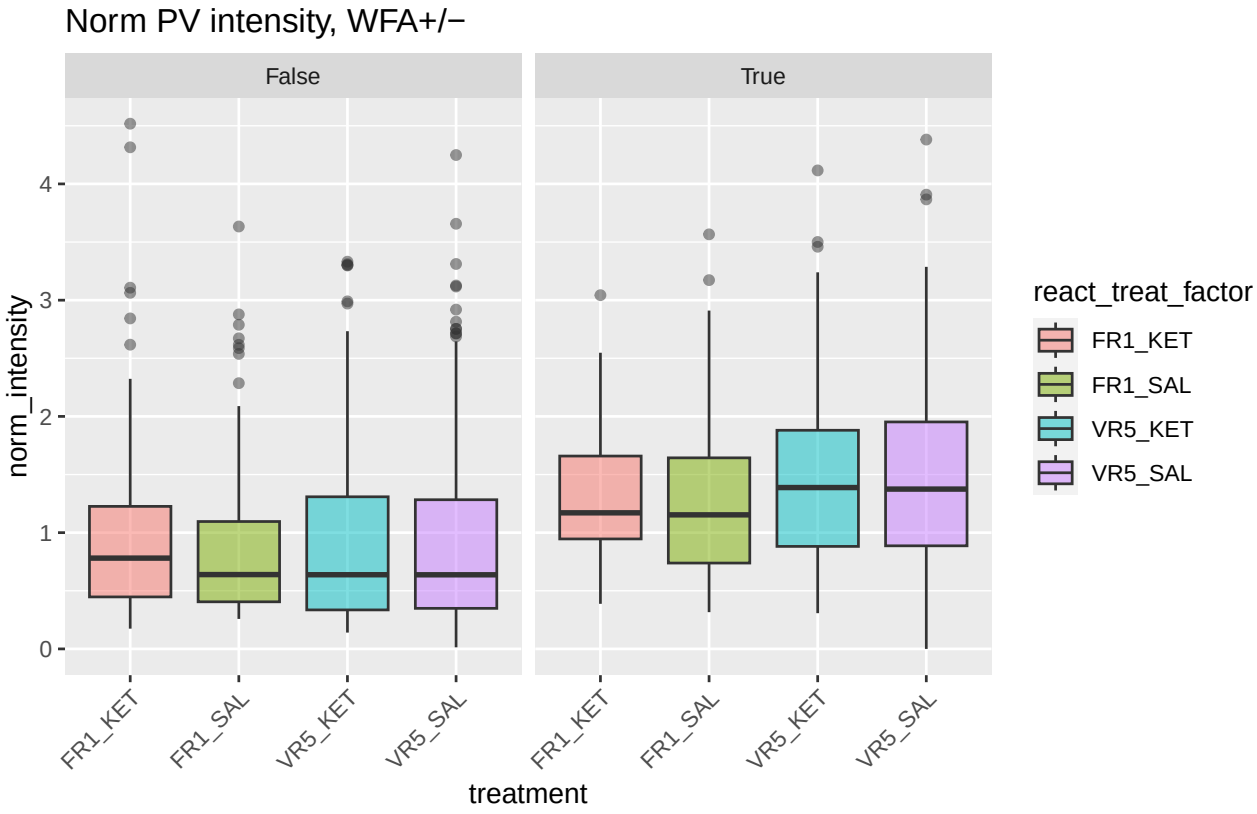
```
## Anova Table (Type III tests)
##
## Response: norm_intensity
##                                       Sum Sq   Df   F value    Pr(>F)
## (Intercept)                          1494.01    1 2920.8659 < 2.2e-16 ***
## react_factor                            3.47    1    6.7902  0.009274 **
## treat_factor                            0.25    1    0.4873  0.485251
## WFA_factor                             62.47    1  122.1272 < 2.2e-16 ***
## react_factor:treat_factor               0.99    1    1.9292  0.165089
## react_factor:WFA_factor                 1.47    1    2.8825  0.089797 .
## treat_factor:WFA_factor                 0.08    1    0.1487  0.699862
## react_factor:treat_factor:WFA_factor    0.00    1    0.0009  0.975527
## Residuals                             643.46 1258
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

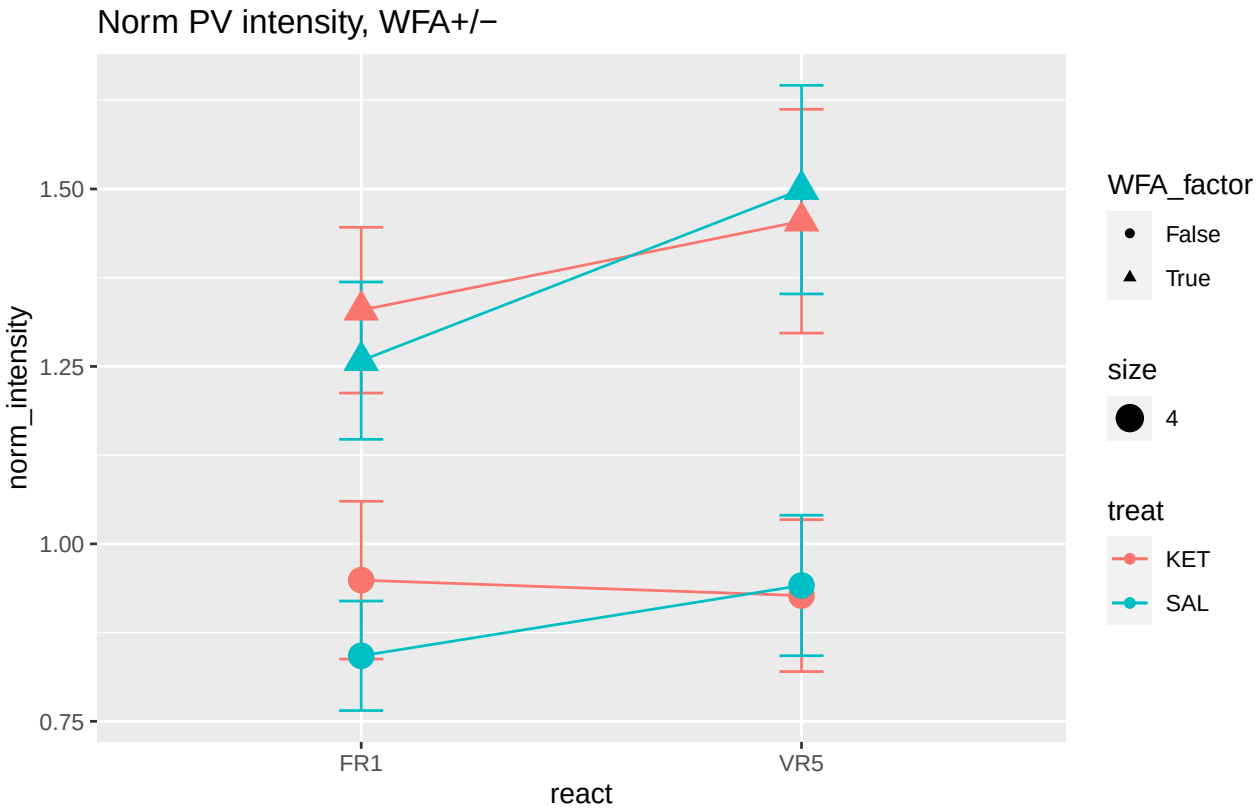
```
PV_with_WFA <- PV[PV$dummy_WFA == 'True', 'norm_intensity']
PV_without_WFA <- PV[PV$dummy_WFA == 'False', 'norm_intensity']
t.test(PV_with_WFA, PV_without_WFA)
```

```
##
##  Welch Two Sample t-test
##
## data:  PV_with_WFA and PV_without_WFA
## t = 11.088, df = 913.86, p-value < 2.2e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.3853413 0.5510806
## sample estimates:
## mean of x mean of y
## 1.3809838 0.9127729
```

```
# plot change in means: Can we still consider a main effect in the presence of an interaction?
f <- ggplot(PV, aes(x=treatment, y=norm_intensity)) + geom_boxplot(aes(fill=react_treat_factor), alpha=
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
  facet_wrap(~WFA_factor) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle('Norm PV intensity, WFA+/-')

PVc <- summarySE(PV, measurevar="norm_intensity", groupvars=c("WFA_factor","react", "treat"))
g <- ggplot(PVc, aes(x=react, y=norm_intensity, colour=treat, group=interaction(WFA_factor, treat))) +
  geom_errorbar(aes(ymin=norm_intensity-ci, ymax=norm_intensity+ci), width=.1) +
  geom_line() +
  geom_point(aes(shape=WFA_factor, size=4)) +
  ggtitle('Norm PV intensity, WFA+/-')
grid.arrange(f, g, nrow=2)
```


Norm PV intensity, WFA+/−
False
True
norm_intensity
0
1
2
3
4
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL
treatment
react_treat_factor
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL


Norm PV intensity, WFA+/−
norm_intensity
0.75
1.00
1.25
1.50
FR1
VR5
react
WFA_factor
False
True
size
4
treat
KET
SAL

PV, Npas4+/-

```
# Npas4 +/-
PV.Npas4.lm <- lm(norm_intensity ~ react_factor*treat_factor*Npas4_factor, contrasts = list(react_facto
PV.Npas4.aov <- car::Anova(PV.Npas4.lm, type=3)
print(PV.Npas4.aov)
```

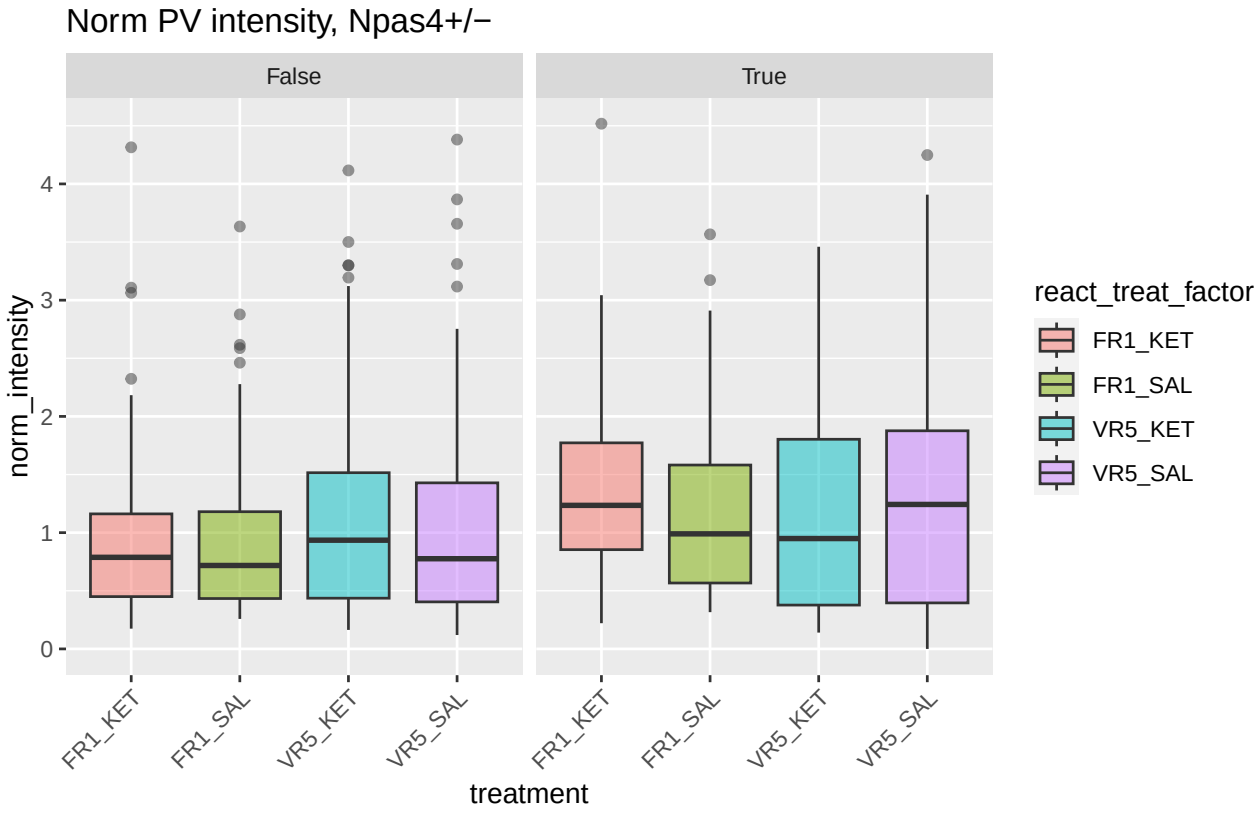
```
## Anova Table (Type III tests)
##
## Response: norm_intensity
##                                          Sum Sq   Df   F value    Pr(>F)
## (Intercept)                             1438.10    1 2635.2897 < 2.2e-16 ***
## react_factor                               1.43    1    2.6163   0.10602
## treat_factor                               0.62    1    1.1345   0.28703
## Npas4_factor                              19.30    1   35.3672 3.533e-09 ***
## react_factor:treat_factor                  1.39    1    2.5447   0.11092
## react_factor:Npas4_factor                  2.47    1    4.5228   0.03364 *
## treat_factor:Npas4_factor                  0.00    1    0.0056   0.94058
## react_factor:treat_factor:Npas4_factor     2.38    1    4.3603   0.03699 *
## Residuals                                686.50 1258
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

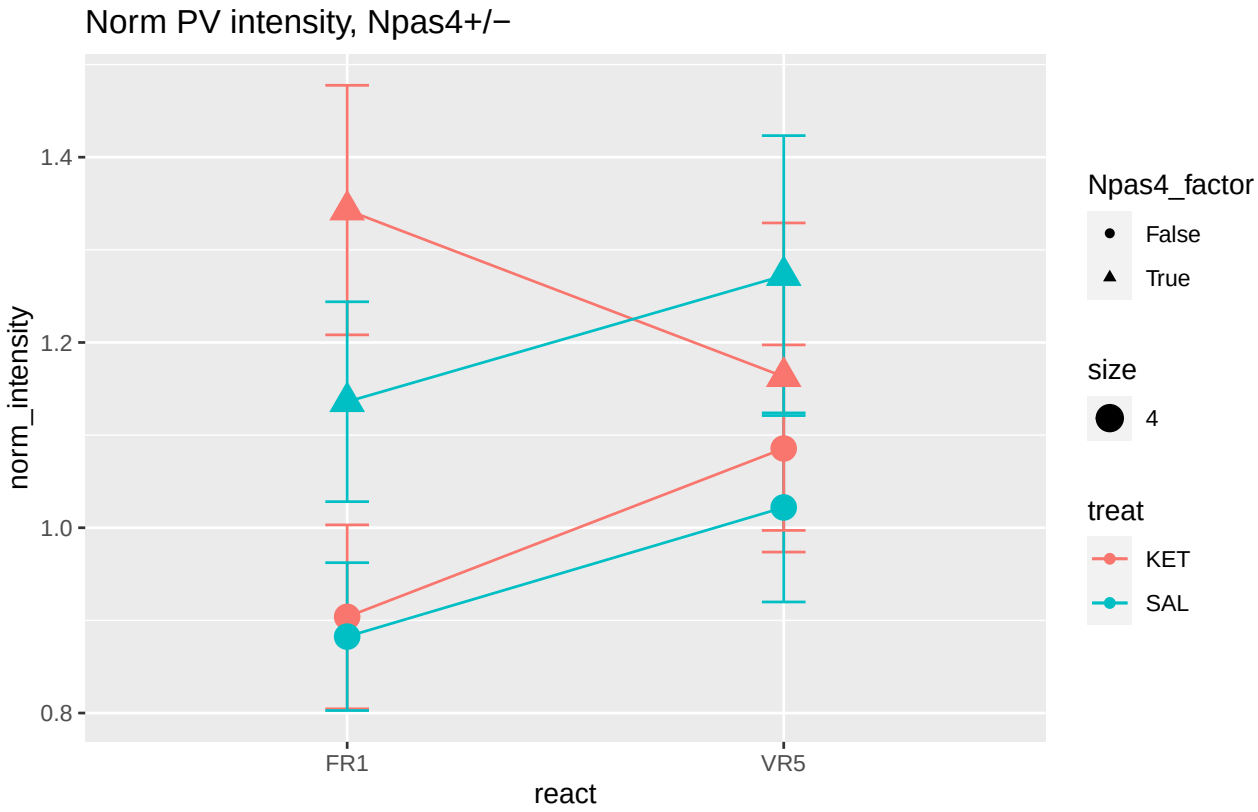
```
PV_with_Npas4 <- PV[PV$dummy_Npas4 == 'True', 'norm_intensity']
PV_without_Npas4 <- PV[PV$dummy_Npas4 == 'False', 'norm_intensity']
t.test(PV_with_Npas4, PV_without_Npas4)
```

```
##
##  Welch Two Sample t-test
##
## data:  PV_with_Npas4 and PV_without_Npas4
## t = 5.5642, df = 1017.1, p-value = 3.367e-08
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.1563086 0.3266201
## sample estimates:
## mean of x mean of y
## 1.2207436 0.9792793
```

```
# plot change in means: Can we still consider a main effect in the presence of an interaction?
f <- ggplot(PV, aes(x=treatment, y=norm_intensity)) + geom_boxplot(aes(fill=react_treat_factor), alpha=
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
  facet_wrap(~Npas4_factor) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle('Norm PV intensity, Npas4+/-')

PVc <- summarySE(PV, measurevar="norm_intensity", groupvars=c("Npas4_factor","react", "treat"))
g <- ggplot(PVc, aes(x=react, y=norm_intensity, colour=treat, group=interaction(Npas4_factor, treat))) +
  geom_errorbar(aes(ymin=norm_intensity-ci, ymax=norm_intensity+ci), width=.1) +
  geom_line() +
  geom_point(aes(shape=Npas4_factor, size=4)) +
  ggtitle('Norm PV intensity, Npas4+/-')
grid.arrange(f, g, nrow=2)
```


Norm PV intensity, Npas4+/−
False
True
norm_intensity
0
1
2
3
4
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL
treatment
react_treat_factor
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL


Norm PV intensity, Npas4+/−
norm_intensity
0.8
1.0
1.2
1.4
FR1
VR5
react
Npas4_factor
False
True
size
4
treat
KET
SAL

PV, cFos+/-

```
# cFos +/-
PV.cFos.lm <- lm(norm_intensity ~ react_factor*treat_factor*cFos_factor, contrasts = list(react_factor=
PV.cFos.aov <- car::Anova(PV.cFos.lm, type=3)
print(PV.cFos.aov)
```

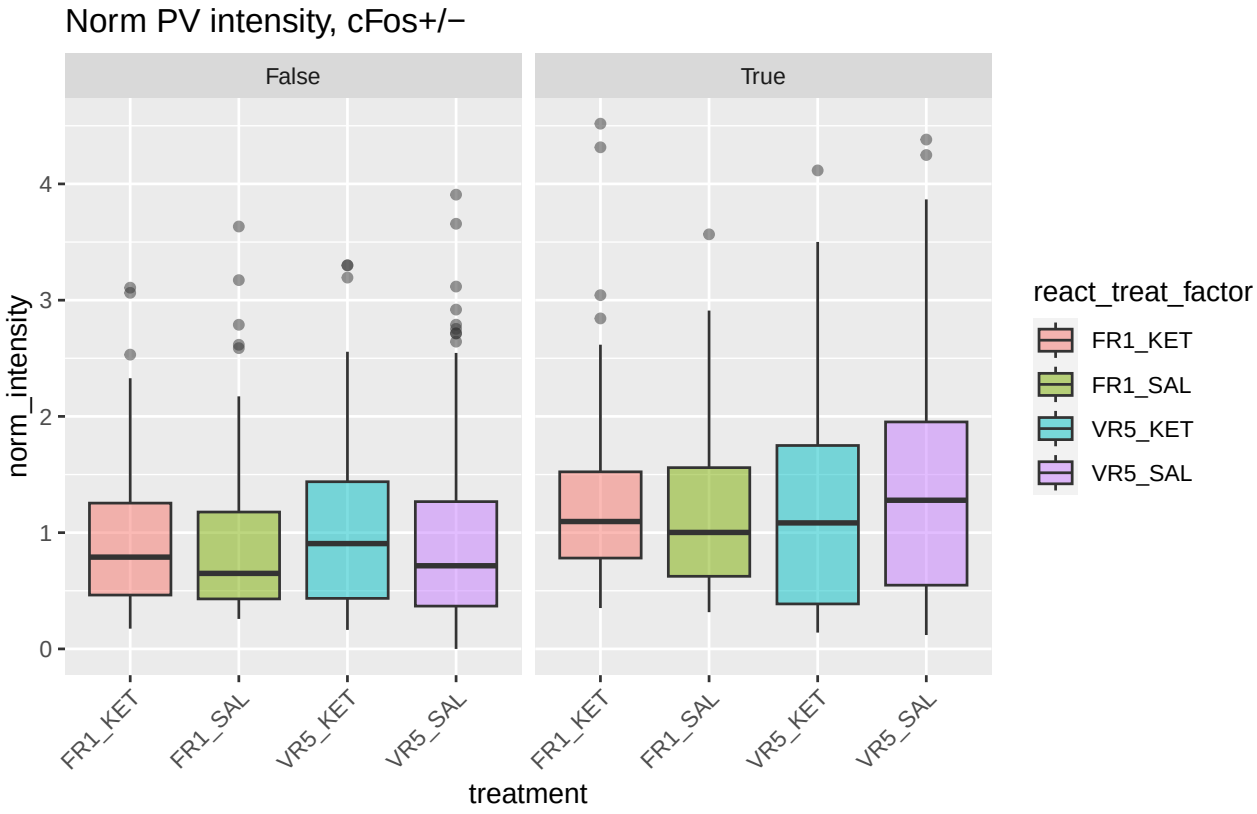
```
## Anova Table (Type III tests)
##
## Response: norm_intensity
##                                        Sum Sq   Df   F value    Pr(>F)
## (Intercept)                           1464.10    1 2707.7766 < 2.2e-16 ***
## react_factor                             2.27    1    4.1893   0.04089 *
## treat_factor                             0.33    1    0.6058   0.43653
## cFos_factor                             26.42    1   48.8625 4.443e-12 ***
## react_factor:treat_factor                0.93    1    1.7174   0.19027
## react_factor:cFos_factor                 0.15    1    0.2784   0.59787
## treat_factor:cFos_factor                 0.48    1    0.8796   0.34850
## react_factor:treat_factor:cFos_factor    0.73    1    1.3424   0.24684
## Residuals                              680.20 1258
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

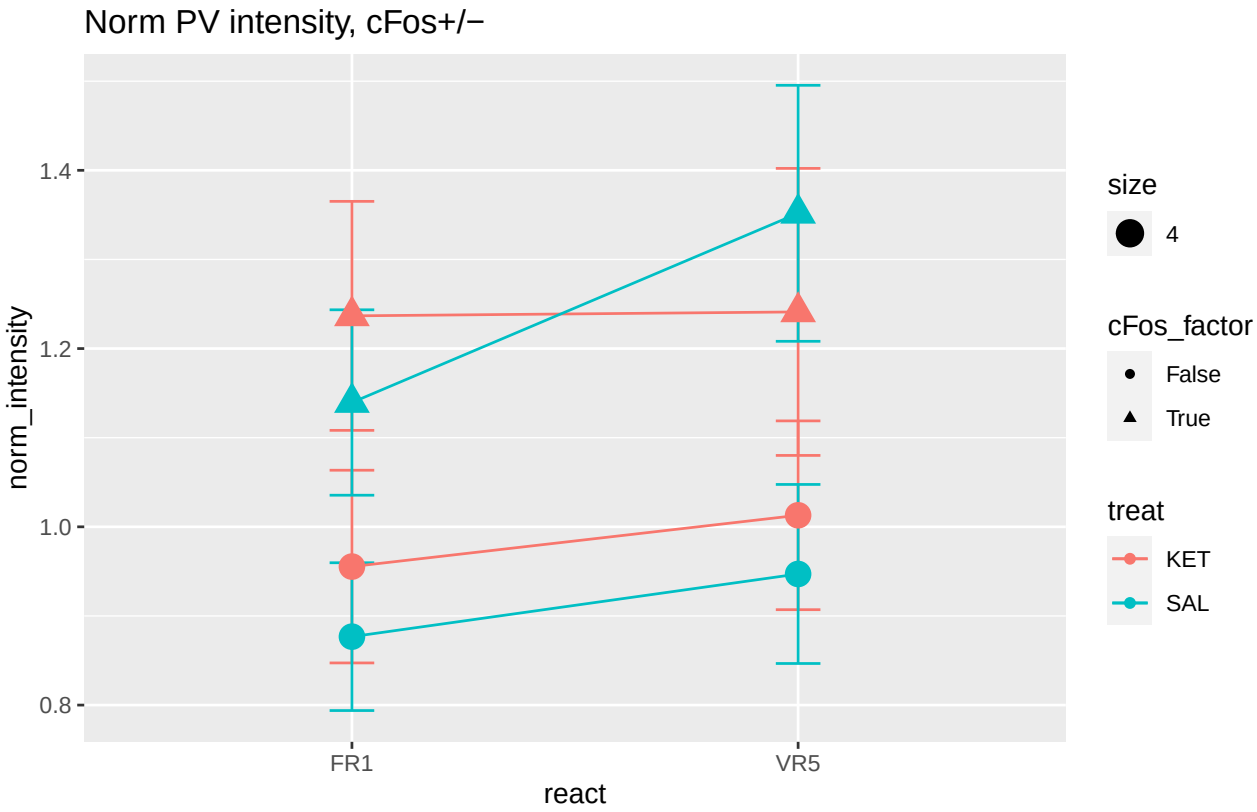
```
PV_with_cFos <- PV[PV$dummy_cFos == 'True', 'norm_intensity']
PV_without_cFos <- PV[PV$dummy_cFos == 'False', 'norm_intensity']
t.test(PV_with_cFos, PV_without_cFos)
```

```
##
##  Welch Two Sample t-test
##
## data:  PV_with_cFos and PV_without_cFos
## t = 6.9826, df = 1099.9, p-value = 5.005e-12
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.2127936 0.3791239
## sample estimates:
## mean of x mean of y
## 1.2406986 0.9447398
```

```
# plot change in means: Can we still consider a main effect in the presence of an interaction?
f <- ggplot(PV, aes(x=treatment, y=norm_intensity)) + geom_boxplot(aes(fill=react_treat_factor), alpha=
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
  facet_wrap(~cFos_factor) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle('Norm PV intensity, cFos+/-')

PVc <- summarySE(PV, measurevar="norm_intensity", groupvars=c("cFos_factor","react", "treat"))
g <- ggplot(PVc, aes(x=react, y=norm_intensity, colour=treat, group=interaction(cFos_factor, treat))) +
  geom_errorbar(aes(ymin=norm_intensity-ci, ymax=norm_intensity+ci), width=.1) +
  geom_line() +
  geom_point(aes(shape=cFos_factor, size=4)) +
  ggtitle('Norm PV intensity, cFos+/-')
grid.arrange(f, g, nrow=2)
```


Norm PV intensity, cFos+/−
False
True
norm_intensity
0
1
2
3
4
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL
treatment
react_treat_factor
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL


Norm PV intensity, cFos+/−
norm_intensity
0.8
1.0
1.2
1.4
FR1
VR5
react
size
4
cFos_factor
False
True
treat
KET
SAL

Single WFA Norm Intensity

```
WFA <- read.csv('NORM_single_WFA.csv', header=TRUE, sep=',')
split <- str_split_fixed(WFA$treatment, "_", 2)
WFA$react <- split[,1]
WFA$treat <- split[,2]

WFA$react_treat_factor <- as.factor(WFA$treatment)
WFA$react_factor <- as.factor(WFA$react)
WFA$treat_factor <- as.factor(WFA$treat)
WFA$PV_factor <- as.factor(WFA$dummy_PV)
WFA$Npas4_factor <- as.factor(WFA$dummy_Npas4)
WFA$cFos_factor <- as.factor(WFA$dummy_cFos)

str(WFA)
```

```
## 'data.frame':    903 obs. of  17 variables:
##  $ X                 : int  203 204 205 206 207 208 434 435 436 437 ...
##  $ stain_type        : chr  "WFA" "WFA" "WFA" "WFA" ...
##  $ intensity         : num  8.81 28.65 11.8 23.66 16.98 ...
##  $ treatment         : chr  "FR1_KET" "FR1_KET" "FR1_KET" "FR1_KET" ...
##  $ dummy_PV          : chr  "True" "True" "True" "True" ...
##  $ dummy_cFos        : chr  "True" "True" "False" "False" ...
##  $ dummy_Npas4       : chr  "True" "True" "True" "True" ...
##  $ dummy_WFA         : chr  "True" "True" "True" "True" ...
##  $ norm_intensity    : num  0.331 1.077 0.444 0.89 0.639 ...
##  $ react             : chr  "FR1" "FR1" "FR1" "FR1" ...
##  $ treat             : chr  "KET" "KET" "KET" "KET" ...
##  $ react_treat_factor: Factor w/ 4 levels "FR1_KET","FR1_SAL",..: 1 1 1 1 1 1 1 1 1 1 ...
##  $ react_factor      : Factor w/ 2 levels "FR1","VR5": 1 1 1 1 1 1 1 1 1 1 ...
##  $ treat_factor      : Factor w/ 2 levels "KET","SAL": 1 1 1 1 1 1 1 1 1 1 ...
##  $ PV_factor         : Factor w/ 2 levels "False","True": 2 2 2 2 2 2 2 2 1 2 ...
##  $ Npas4_factor      : Factor w/ 2 levels "False","True": 2 2 2 2 2 2 2 1 2 2 1 ...
##  $ cFos_factor       : Factor w/ 2 levels "False","True": 2 2 1 1 1 1 1 2 2 2 ...
```

WFA, PV+/-

```
# PV +/-
WFA.PV.lm <- lm(norm_intensity ~ react_factor*treat_factor*PV_factor, contrasts = list(react_factor='co
WFA.PV.aov <- car::Anova(WFA.PV.lm, type=3)
print(WFA.PV.aov)
```

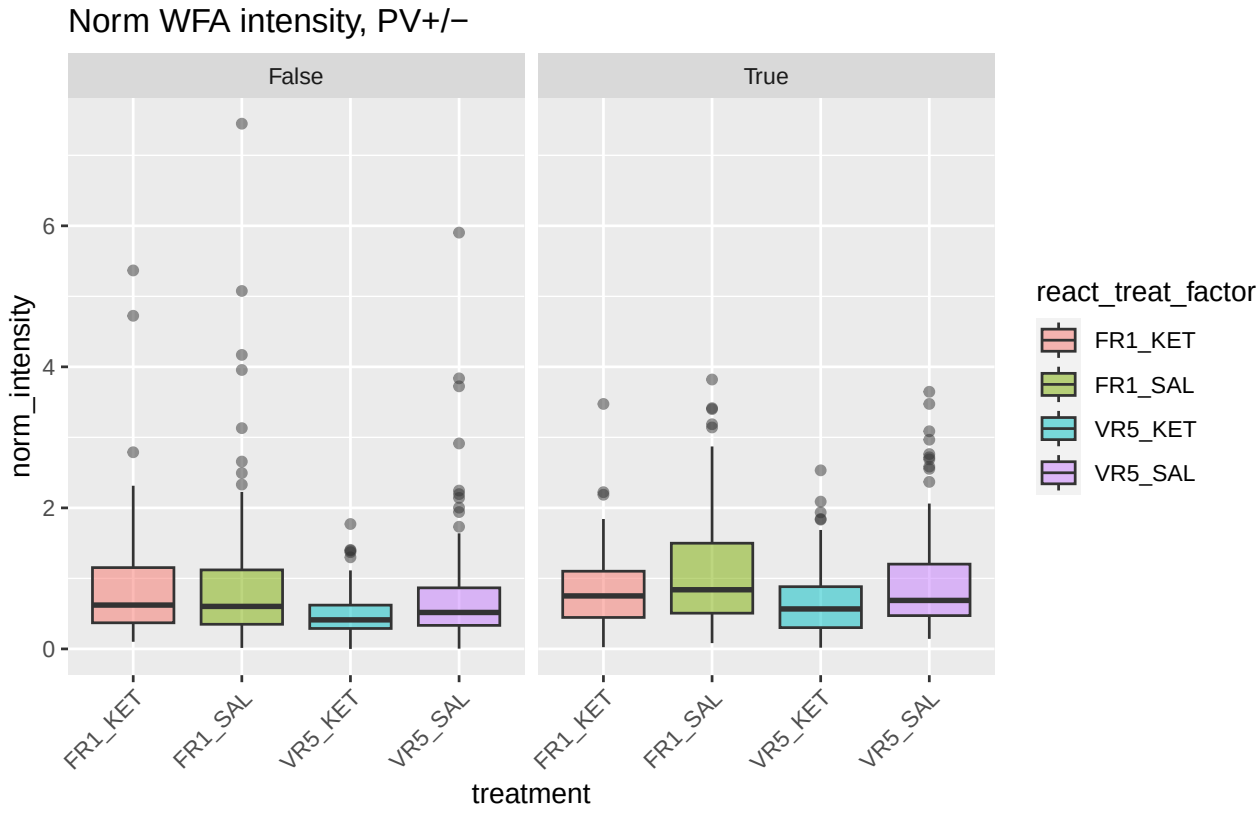
```
## Anova Table (Type III tests)
##
## Response: norm_intensity
##                                    Sum Sq  Df   F value    Pr(>F)
## (Intercept)                        596.03   1 1088.4142 < 2.2e-16 ***
## react_factor                        10.87   1   19.8433 9.469e-06 ***
## treat_factor                         7.19   1   13.1228 0.0003081 ***
## PV_factor                            4.15   1    7.5748 0.0060388 **
## react_factor:treat_factor            0.78   1    1.4334 0.2315332
## react_factor:PV_factor               0.75   1    1.3703 0.2420643
## treat_factor:PV_factor               0.81   1    1.4821 0.2237681
## react_factor:treat_factor:PV_factor  0.57   1    1.0339 0.3095101
## Residuals                          490.11 895
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

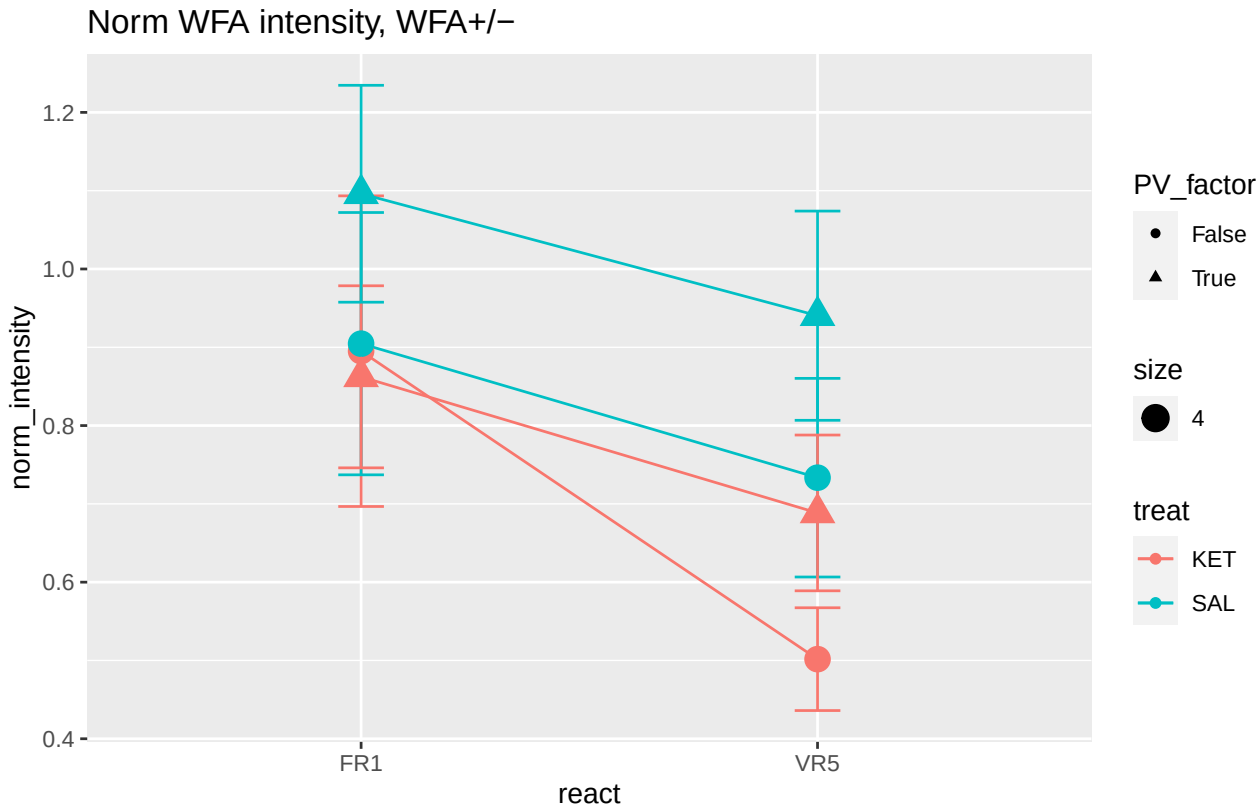
```
WFA_with_PV <- WFA[WFA$dummy_PV == 'True', 'norm_intensity']
WFA_without_PV <- WFA[WFA$dummy_PV == 'False', 'norm_intensity']
t.test(WFA_with_PV, WFA_without_PV)
```

```
##
##  Welch Two Sample t-test
##
## data:  WFA_with_PV and WFA_without_PV
## t = 3.0241, df = 885.85, p-value = 0.002566
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.05315352 0.24972367
## sample estimates:
## mean of x mean of y
## 0.9131588 0.7617202
```

```
# plot change in means: Can we still consider a main effect in the presence of an interaction?
f <- ggplot(WFA, aes(x=treatment, y=norm_intensity)) + geom_boxplot(aes(fill=react_treat_factor), alpha=
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
  facet_wrap(~PV_factor) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle('Norm WFA intensity, PV+/-')

WFAc <- summarySE(WFA, measurevar="norm_intensity", groupvars=c("PV_factor","react", "treat"))
g <- ggplot(WFAc, aes(x=react, y=norm_intensity, colour=treat, group=interaction(PV_factor, treat))) +
  geom_errorbar(aes(ymin=norm_intensity-ci, ymax=norm_intensity+ci), width=.1) +
  geom_line() +
  geom_point(aes(shape=PV_factor, size=4)) +
  ggtitle('Norm WFA intensity, WFA+/-')
grid.arrange(f, g, nrow=2)
```


Norm WFA intensity, PV+/−
False
True
norm_intensity
0
2
4
6
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL
treatment
react_treat_factor
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL


Norm WFA intensity, WFA+/−
norm_intensity
0.4
0.6
0.8
1.0
1.2
FR1
VR5
react
PV_factor
False
True
size
4
treat
KET
SAL

WFA, Npas4+/-

```
# Npas4 +/-
WFA.Npas4.lm <- lm(norm_intensity ~ react_factor*treat_factor*Npas4_factor, contrasts = list(react_facto
WFA.Npas4.aov <- car::Anova(WFA.Npas4.lm, type=3)
print(WFA.Npas4.aov)
```

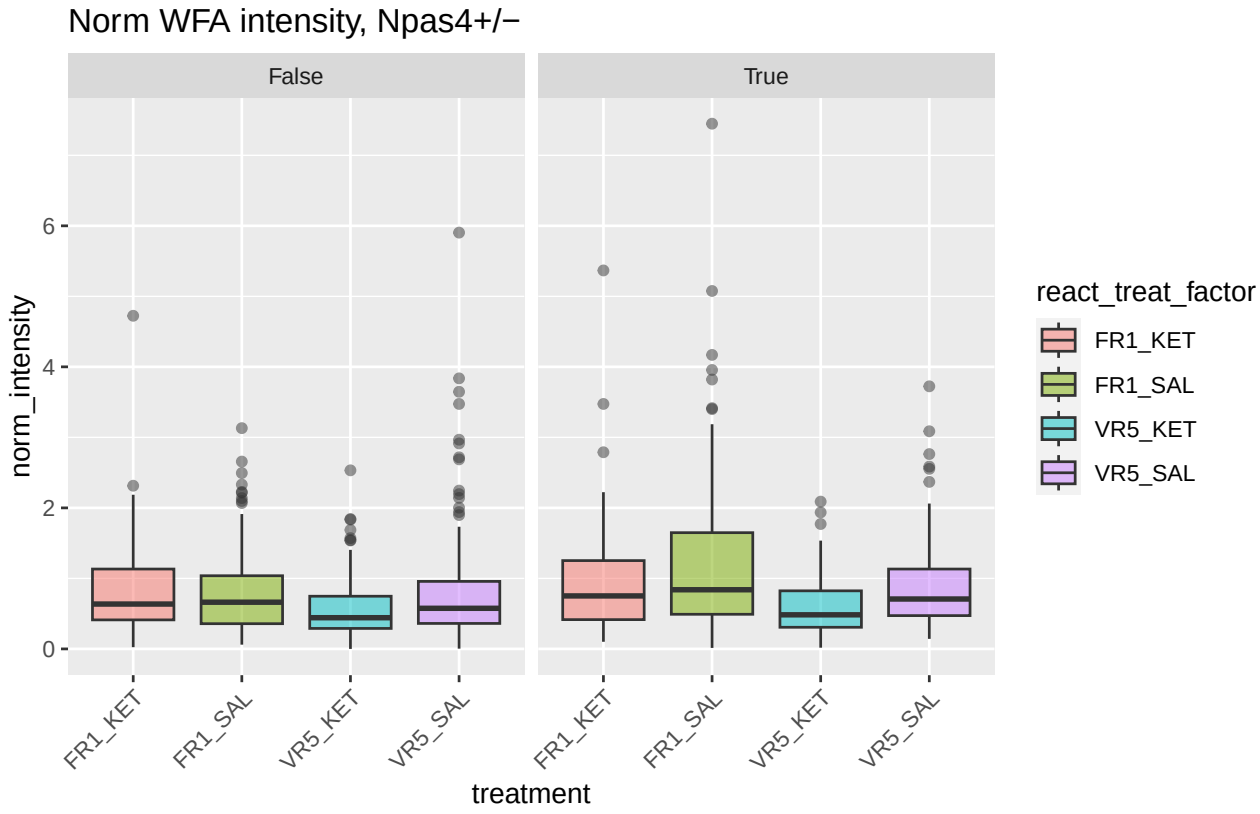
```
## Anova Table (Type III tests)
##
## Response: norm_intensity
##                                        Sum Sq  Df   F value    Pr(>F)
## (Intercept)                            558.10   1 1034.1373 < 2.2e-16 ***
## react_factor                             9.56   1   17.7071 2.836e-05 ***
## treat_factor                             7.35   1   13.6162 0.0002378 ***
## Npas4_factor                             6.63   1   12.2879 0.0004785 ***
## react_factor:treat_factor                0.56   1    1.0371 0.3087677
## react_factor:Npas4_factor                1.58   1    2.9214 0.0877561 .
## treat_factor:Npas4_factor                1.77   1    3.2710 0.0708518 .
## react_factor:treat_factor:Npas4_factor   0.86   1    1.6006 0.2061459
## Residuals                              483.02 895
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

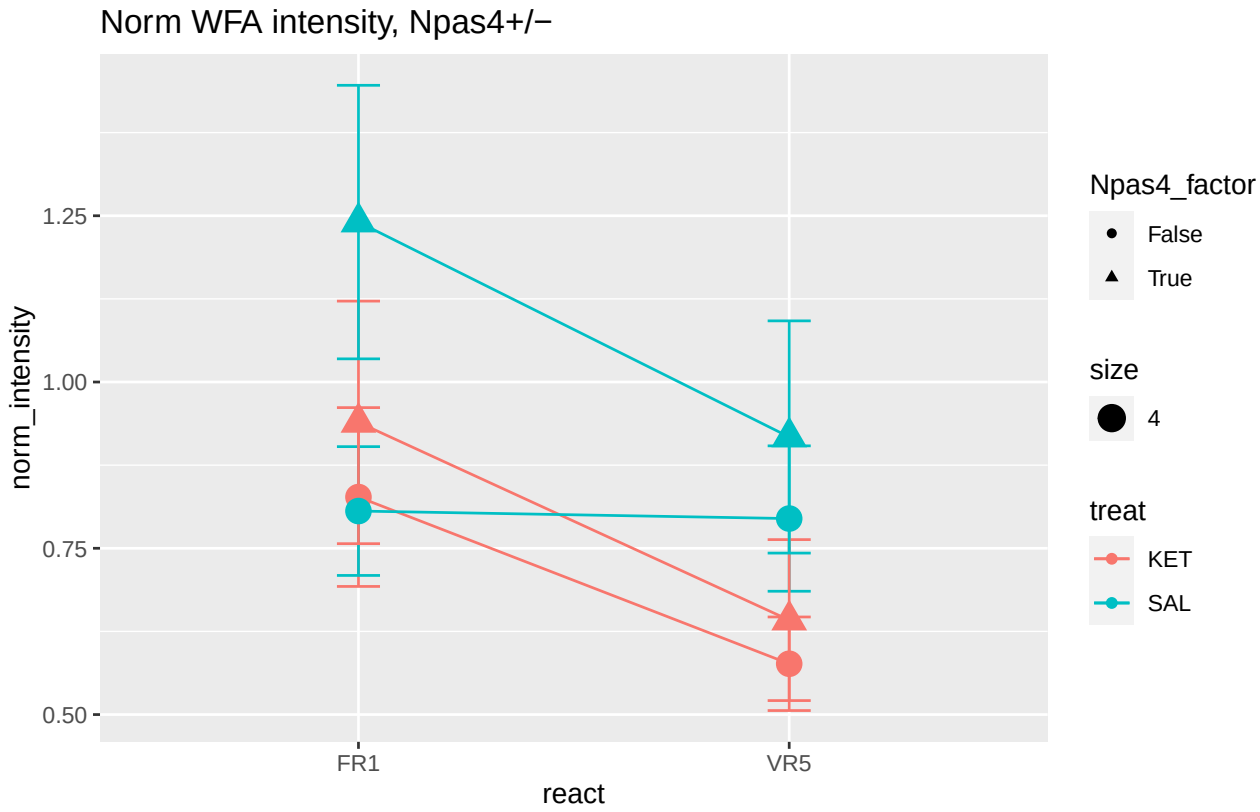
```
WFA_with_Npas4 <- WFA[WFA$dummy_Npas4 == 'True', 'norm_intensity']
WFA_without_Npas4 <- WFA[WFA$dummy_Npas4 == 'False', 'norm_intensity']
t.test(WFA_with_Npas4, WFA_without_Npas4)
```

```
##
##  Welch Two Sample t-test
##
## data:  WFA_with_Npas4 and WFA_without_Npas4
## t = 4.2297, df = 515.7, p-value = 2.769e-05
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.1285680 0.3515871
## sample estimates:
## mean of x mean of y
## 0.9894599 0.7493824
```

```
# plot change in means: Can we still consider a main effect in the presence of an interaction?
f <- ggplot(WFA, aes(x=treatment, y=norm_intensity)) + geom_boxplot(aes(fill=react_treat_factor), alpha=
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
  facet_wrap(~Npas4_factor) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle('Norm WFA intensity, Npas4+/-')

WFAc <- summarySE(WFA, measurevar="norm_intensity", groupvars=c("Npas4_factor","react", "treat"))
g <- ggplot(WFAc, aes(x=react, y=norm_intensity, colour=treat, group=interaction(Npas4_factor, treat)))
  geom_errorbar(aes(ymin=norm_intensity-ci, ymax=norm_intensity+ci), width=.1) +
  geom_line() +
  geom_point(aes(shape=Npas4_factor, size=4)) +
  ggtitle('Norm WFA intensity, Npas4+/-')
grid.arrange(f, g, nrow=2)
```


Norm WFA intensity, Npas4+/−
False
True
norm_intensity
0
2
4
6
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL
treatment
react_treat_factor
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL


Norm WFA intensity, Npas4+/−
norm_intensity
0.50
0.75
1.00
1.25
FR1
VR5
react
Npas4_factor
False
True
size
4
treat
KET
SAL

WFA, cFos+/-

```
# cFos +/-
WFA.cFos.lm <- lm(norm_intensity ~ react_factor*treat_factor*cFos_factor, contrasts = list(react_factor=
WFA.cFos.aov <- car::Anova(WFA.cFos.lm, type=3)
print(WFA.cFos.aov)
```

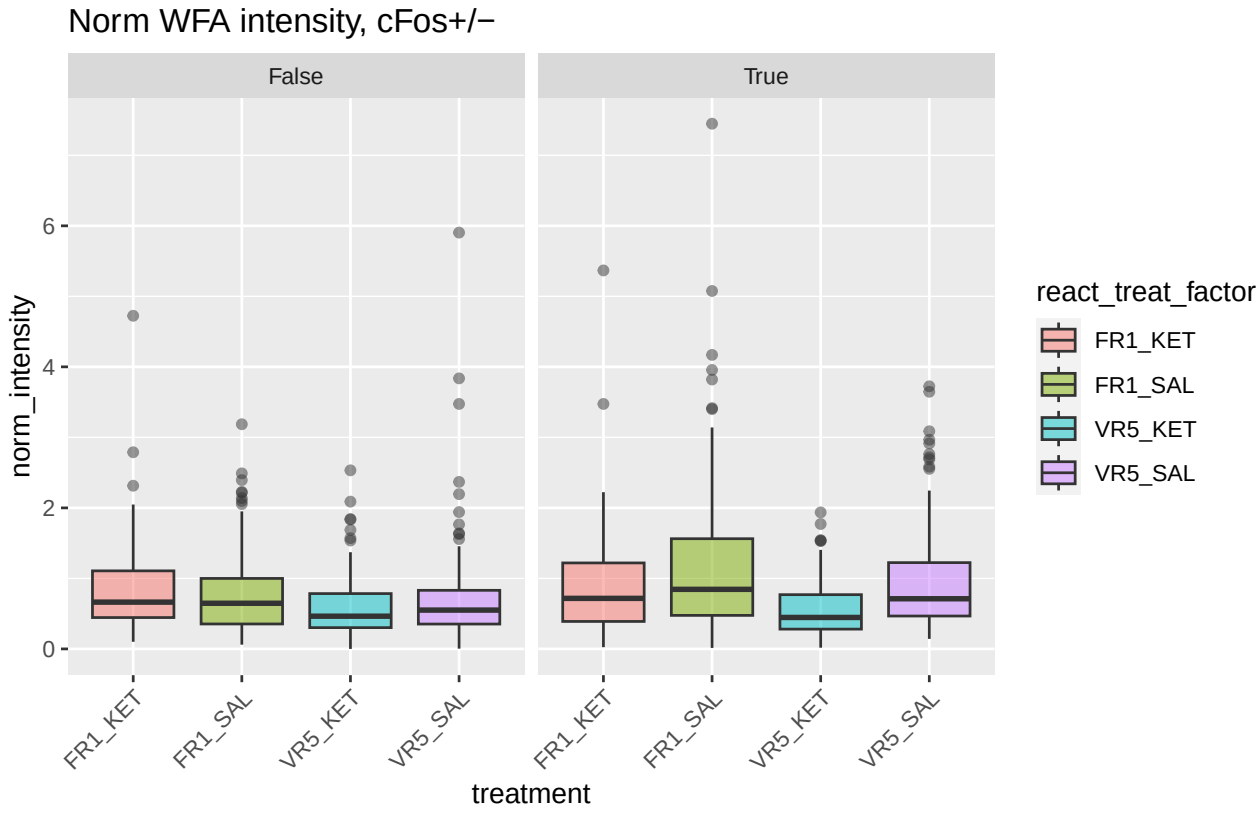
```
## Anova Table (Type III tests)
##
## Response: norm_intensity
##                                       Sum Sq  Df   F value    Pr(>F)
## (Intercept)                           594.85   1 1110.4897 < 2.2e-16 ***
## react_factor                            8.71   1   16.2597 5.993e-05 ***
## treat_factor                            8.30   1   15.4877 8.948e-05 ***
## cFos_factor                             6.92   1   12.9205 0.0003428 ***
## react_factor:treat_factor               1.39   1    2.5972 0.1074057
## react_factor:cFos_factor                0.21   1    0.3867 0.5341909
## treat_factor:cFos_factor                7.57   1   14.1348 0.0001812 ***
## react_factor:treat_factor:cFos_factor   0.01   1    0.0145 0.9041034
## Residuals                             479.42 895
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

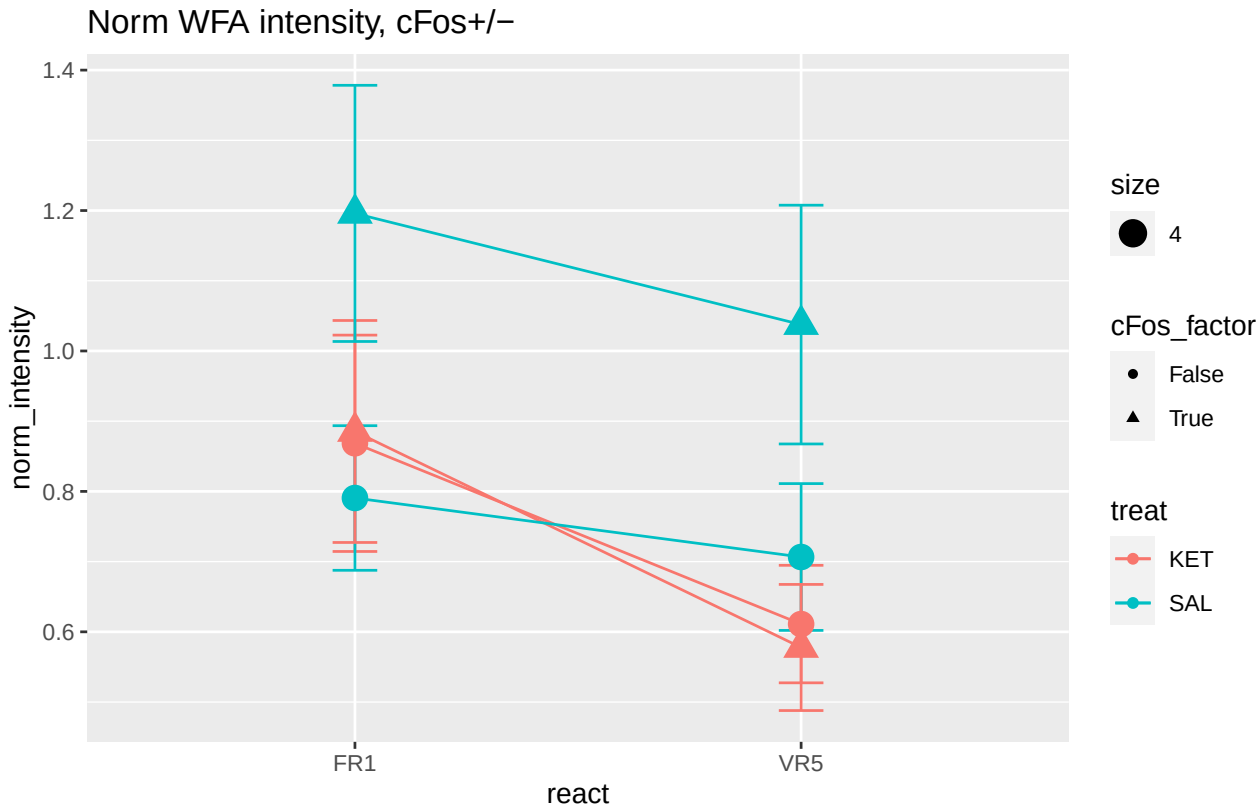
```
WFA_with_cFos <- WFA[WFA$dummy_cFos == 'True', 'norm_intensity']
WFA_without_cFos <- WFA[WFA$dummy_cFos == 'False', 'norm_intensity']
t.test(WFA_with_cFos, WFA_without_cFos)
```

```
##
##  Welch Two Sample t-test
##
## data:  WFA_with_cFos and WFA_without_cFos
## t = 4.3721, df = 721.22, p-value = 1.412e-05
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.1240019 0.3261341
## sample estimates:
## mean of x mean of y
## 0.9589824 0.7339144
```

```
# plot change in means: Can we still consider a main effect in the presence of an interaction?
f <- ggplot(WFA, aes(x=treatment, y=norm_intensity)) + geom_boxplot(aes(fill=react_treat_factor), alpha=
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
  facet_wrap(~cFos_factor) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle('Norm WFA intensity, cFos+/-')

WFAc <- summarySE(WFA, measurevar="norm_intensity", groupvars=c("cFos_factor","react", "treat"))
g <- ggplot(WFAc, aes(x=react, y=norm_intensity, colour=treat, group=interaction(cFos_factor, treat))) +
  geom_errorbar(aes(ymin=norm_intensity-ci, ymax=norm_intensity+ci), width=.1) +
  geom_line() +
  geom_point(aes(shape=cFos_factor, size=4)) +
  ggtitle('Norm WFA intensity, cFos+/-')
grid.arrange(f, g, nrow=2)
```


Norm WFA intensity, cFos+/−
False
True
norm_intensity
0
2
4
6
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL
treatment
react_treat_factor
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL


Norm WFA intensity, cFos+/−
norm_intensity
0.6
0.8
1.0
1.2
1.4
FR1
VR5
react
size
4
cFos_factor
False
True
treat
KET
SAL

Single cFos Norm Intensity

```
cFos <- read.csv('NORM_single_cFos.csv', header=TRUE, sep=',')
split <- str_split_fixed(cFos$treatment, "_", 2)
cFos$react <- split[,1]
cFos$treat <- split[,2]

cFos$react_treat_factor <- as.factor(cFos$treatment)
cFos$react_factor <- as.factor(cFos$react)
cFos$treat_factor <- as.factor(cFos$treat)
cFos$PV_factor <- as.factor(cFos$dummy_PV)
cFos$Npas4_factor <- as.factor(cFos$dummy_Npas4)
cFos$WFA_factor <- as.factor(cFos$dummy_WFA)

str(cFos)
```

```
## 'data.frame':    7999 obs. of  17 variables:
##  $ X                 : int  20 21 22 23 24 25 26 27 28 29 ...
##  $ stain_type        : chr  "cFos" "cFos" "cFos" "cFos" ...
##  $ intensity         : num  342 278 224 659 224 ...
##  $ treatment         : chr  "FR1_KET" "FR1_KET" "FR1_KET" "FR1_KET" ...
##  $ dummy_PV          : chr  "False" "False" "False" "False" ...
##  $ dummy_cFos        : chr  "True" "True" "True" "True" ...
##  $ dummy_Npas4       : chr  "True" "False" "True" "True" ...
##  $ dummy_WFA         : chr  "False" "False" "False" "False" ...
##  $ norm_intensity    : num  1.261 1.025 0.824 2.43 0.827 ...
##  $ react             : chr  "FR1" "FR1" "FR1" "FR1" ...
##  $ treat             : chr  "KET" "KET" "KET" "KET" ...
##  $ react_treat_factor: Factor w/ 4 levels "FR1_KET","FR1_SAL",..: 1 1 1 1 1 1 1 1 1 1 ...
##  $ react_factor      : Factor w/ 2 levels "FR1","VR5": 1 1 1 1 1 1 1 1 1 1 ...
##  $ treat_factor      : Factor w/ 2 levels "KET","SAL": 1 1 1 1 1 1 1 1 1 1 ...
##  $ PV_factor         : Factor w/ 2 levels "False","True": 1 1 1 1 2 1 1 1 1 1 ...
##  $ Npas4_factor      : Factor w/ 2 levels "False","True": 2 1 2 2 2 2 2 2 2 2 ...
##  $ WFA_factor        : Factor w/ 2 levels "False","True": 1 1 1 1 1 1 1 1 1 1 ...
```

cFos, PV+/-

```
# PV +/-
cFos.PV.lm <- lm(norm_intensity ~ react_factor*treat_factor*PV_factor, contrasts = list(react_factor='c
cFos.PV.aov <- car::Anova(cFos.PV.lm, type=3)
print(cFos.PV.aov)
```

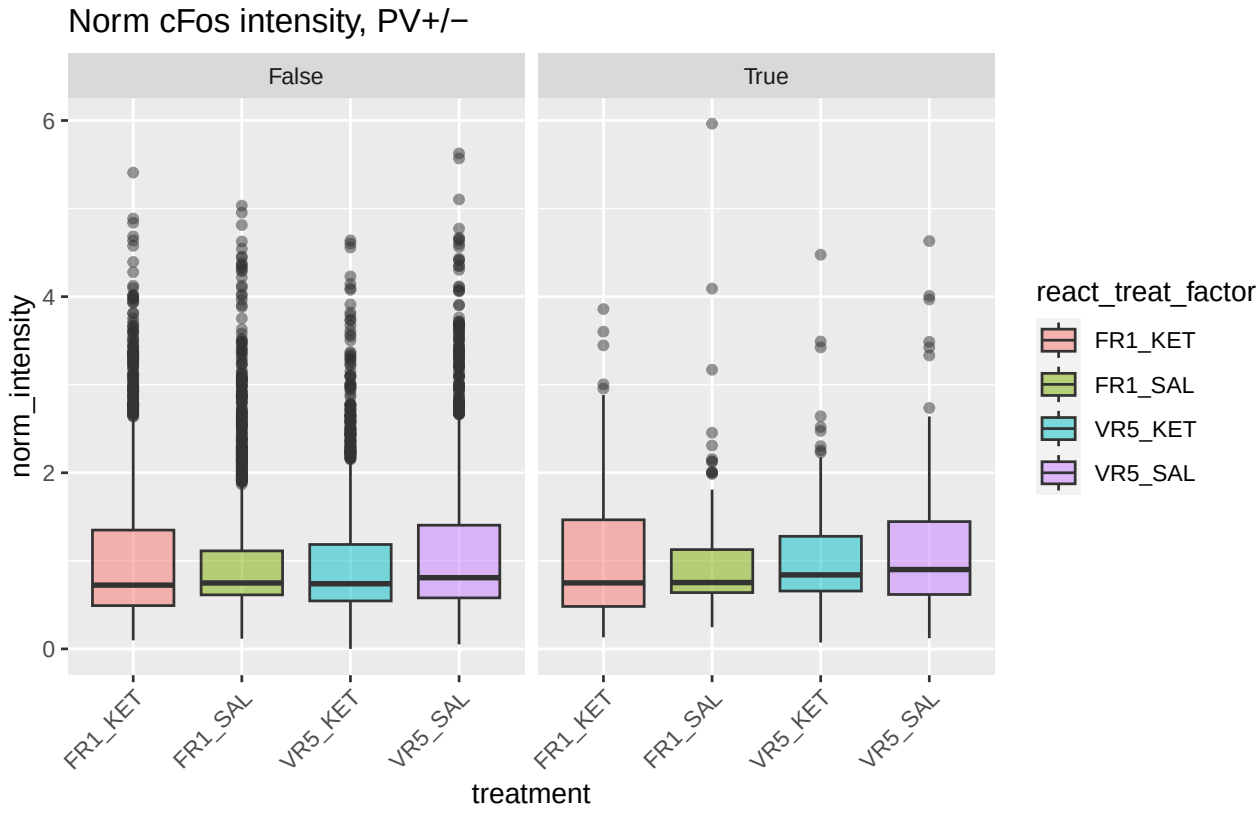
```
## Anova Table (Type III tests)
##
## Response: norm_intensity
##                                    Sum Sq   Df   F value  Pr(>F)
## (Intercept)                        2271.2    1 4068.6784 < 2e-16 ***
## react_factor                          0.9    1    1.6504 0.19895
## treat_factor                          0.2    1    0.3733 0.54122
## PV_factor                             0.3    1    0.5773 0.44739
## react_factor:treat_factor             3.2    1    5.7993 0.01605 *
## react_factor:PV_factor                0.4    1    0.7684 0.38075
## treat_factor:PV_factor                0.3    1    0.5275 0.46767
## react_factor:treat_factor:PV_factor   0.1    1    0.1097 0.74046
## Residuals                          4460.7 7991
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

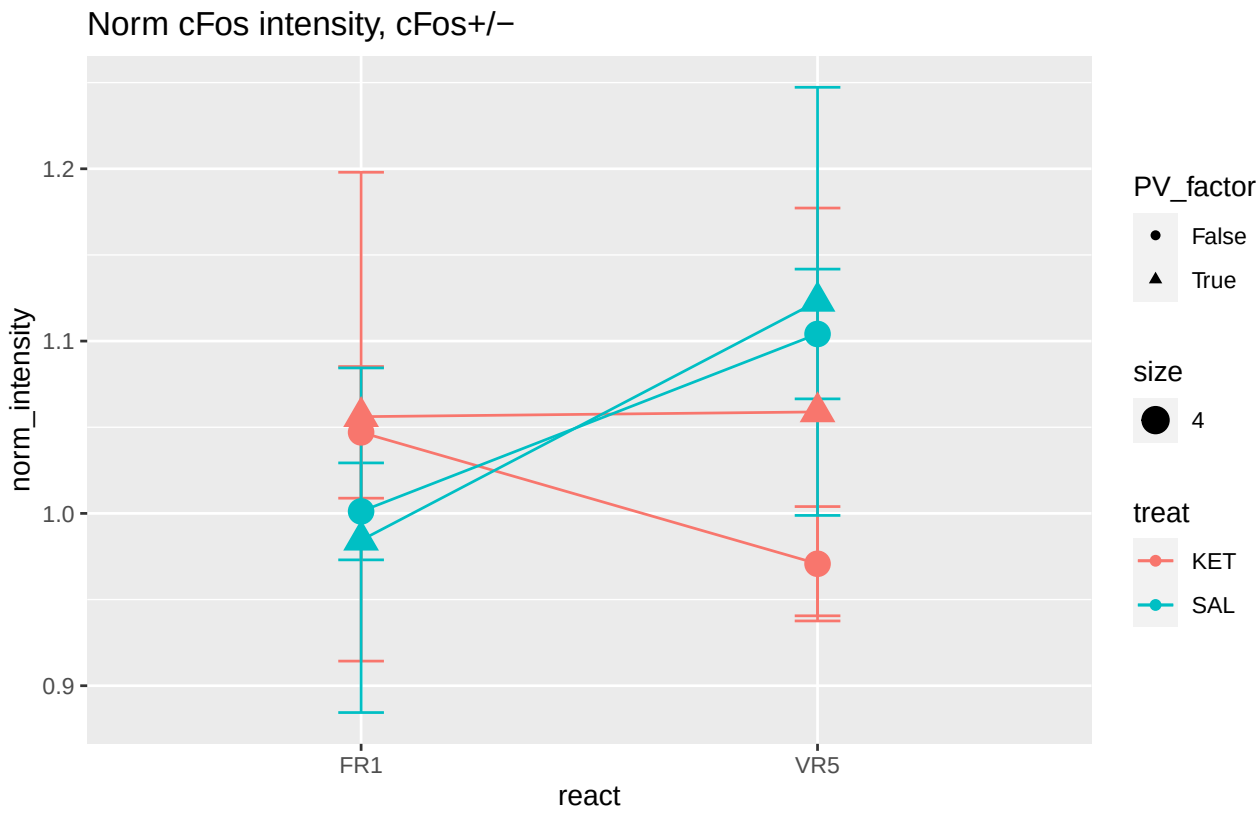
```
cFos_with_PV <- cFos[cFos$dummy_PV == 'True', 'norm_intensity']
cFos_without_PV <- cFos[cFos$dummy_PV == 'False', 'norm_intensity']
t.test(cFos_with_PV, cFos_without_PV)
```

```
##
##  Welch Two Sample t-test
##
## data:  cFos_with_PV and cFos_without_PV
## t = 0.75195, df = 668.55, p-value = 0.4523
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.03824046  0.08570774
## sample estimates:
## mean of x mean of y
##  1.054072  1.030338
```

```
# plot change in means: Can we still consider a main effect in the presence of an interaction?
f <- ggplot(cFos, aes(x=treatment, y=norm_intensity)) + geom_boxplot(aes(fill=react_treat_factor), alpha
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
  facet_wrap(~PV_factor) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle('Norm cFos intensity, PV+/-')

cFosc <- summarySE(cFos, measurevar="norm_intensity", groupvars=c("PV_factor","react", "treat"))
g <- ggplot(cFosc, aes(x=react, y=norm_intensity, colour=treat, group=interaction(PV_factor, treat))) +
  geom_errorbar(aes(ymin=norm_intensity-ci, ymax=norm_intensity+ci), width=.1) +
  geom_line() +
  geom_point(aes(shape=PV_factor, size=4)) +
  ggtitle('Norm cFos intensity, cFos+/-')
grid.arrange(f, g, nrow=2)
```


Norm cFos intensity, PV+/−
False
True
norm_intensity
6
4
2
0
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL
treatment
react_treat_factor
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL


Norm cFos intensity, cFos+/−
norm_intensity
1.2
1.1
1.0
0.9
FR1
VR5
react
PV_factor
False
True
size
4
treat
KET
SAL

cFos, Npas4+/-

```
# Npas4 +/-
cFos.Npas4.lm <- lm(norm_intensity ~ react_factor*treat_factor*Npas4_factor, contrasts = list(react_fac
cFos.Npas4.aov <- car::Anova(cFos.Npas4.lm, type=3)
print(cFos.Npas4.aov)
```

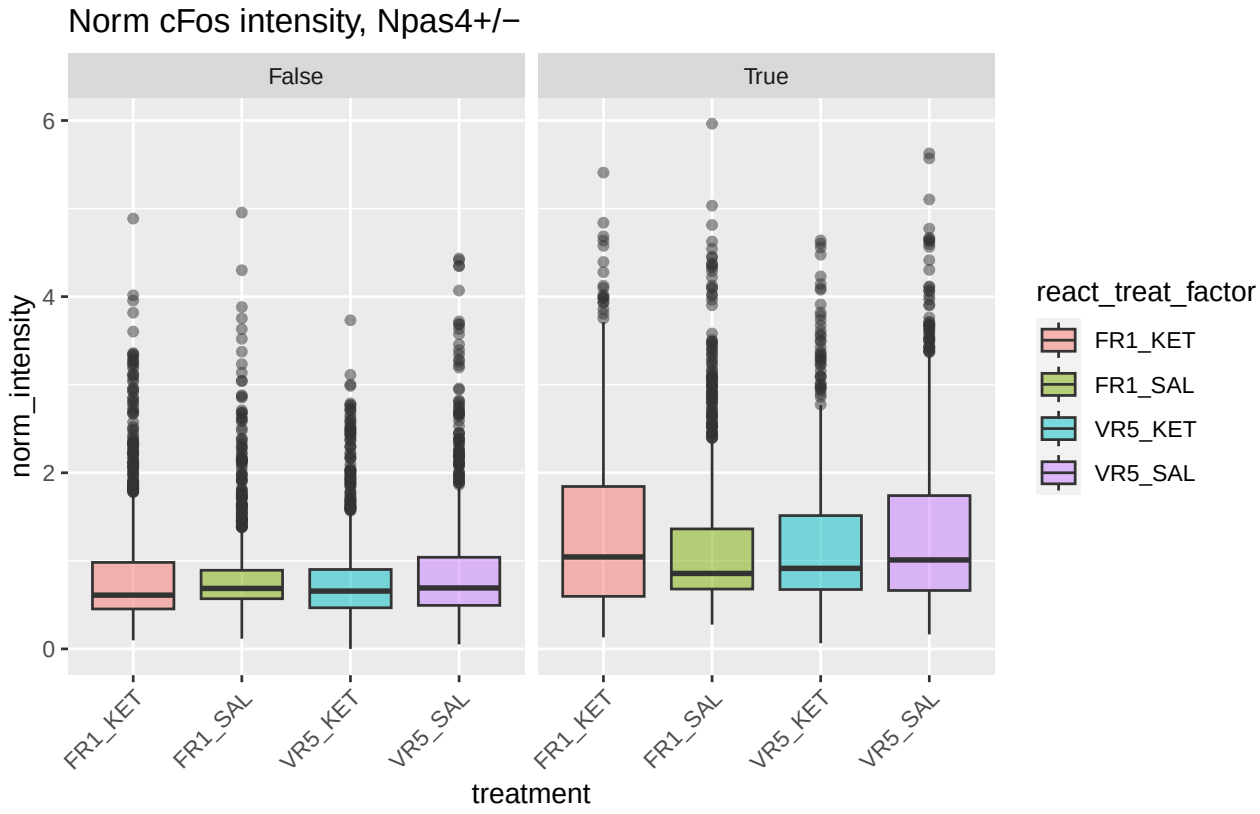
```
## Anova Table (Type III tests)
##
## Response: norm_intensity
##                                        Sum Sq   Df    F value    Pr(>F)
## (Intercept)                            8526.3    1 16527.7128 < 2.2e-16 ***
## react_factor                              0.1    1     0.1005 0.7512555
## treat_factor                              0.0    1     0.0304 0.8616226
## Npas4_factor                            335.7    1   650.7751 < 2.2e-16 ***
## react_factor:treat_factor                23.4    1    45.3088 1.799e-11 ***
## react_factor:Npas4_factor                 0.0    1     0.0930 0.7603499
## treat_factor:Npas4_factor                 3.8    1     7.3928 0.0065626 **
## react_factor:treat_factor:Npas4_factor    5.7    1    10.9864 0.0009219 ***
## Residuals                              4122.4 7991
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

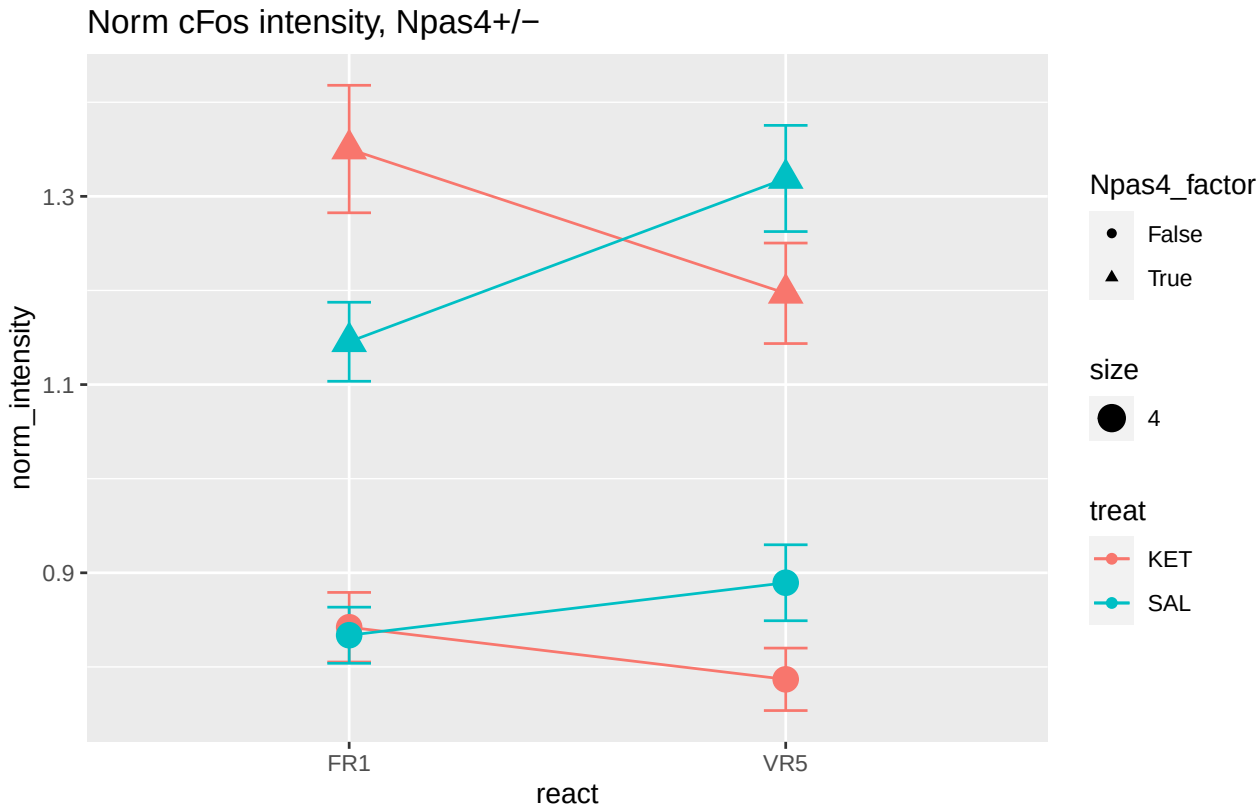
```
cFos_with_Npas4 <- cFos[cFos$dummy_Npas4 == 'True', 'norm_intensity']
cFos_without_Npas4 <- cFos[cFos$dummy_Npas4 == 'False', 'norm_intensity']
t.test(cFos_with_Npas4, cFos_without_Npas4)
```

```
##
##  Welch Two Sample t-test
##
## data:  cFos_with_Npas4 and cFos_without_Npas4
## t = 24.641, df = 6715.2, p-value < 2.2e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.3711868 0.4353503
## sample estimates:
## mean of x mean of y
## 1.2416101 0.8383416
```

```
# plot change in means: Can we still consider a main effect in the presence of an interaction?
f <- ggplot(cFos, aes(x=treatment, y=norm_intensity)) + geom_boxplot(aes(fill=react_treat_factor), alpha
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
  facet_wrap(~Npas4_factor) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle('Norm cFos intensity, Npas4+/-')

cFosc <- summarySE(cFos, measurevar="norm_intensity", groupvars=c("Npas4_factor","react", "treat"))
g <- ggplot(cFosc, aes(x=react, y=norm_intensity, colour=treat, group=interaction(Npas4_factor, treat))
  geom_errorbar(aes(ymin=norm_intensity-ci, ymax=norm_intensity+ci), width=.1) +
  geom_line() +
  geom_point(aes(shape=Npas4_factor, size=4)) +
  ggtitle('Norm cFos intensity, Npas4+/-')
grid.arrange(f, g, nrow=2)
```


Norm cFos intensity, Npas4+/−
False
True
norm_intensity
6
4
2
0
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL
treatment
react_treat_factor
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL


Norm cFos intensity, Npas4+/−
norm_intensity
1.3
1.1
0.9
FR1
VR5
react
Npas4_factor
False
True
size
4
treat
KET
SAL

cFos, WFA+/-

```
# WFA +/-
cFos.WFA.lm <- lm(norm_intensity ~ react_factor*treat_factor*WFA_factor, contrasts = list(react_factor=
cFos.WFA.aov <- car::Anova(cFos.WFA.lm, type=3)
print(cFos.WFA.aov)
```

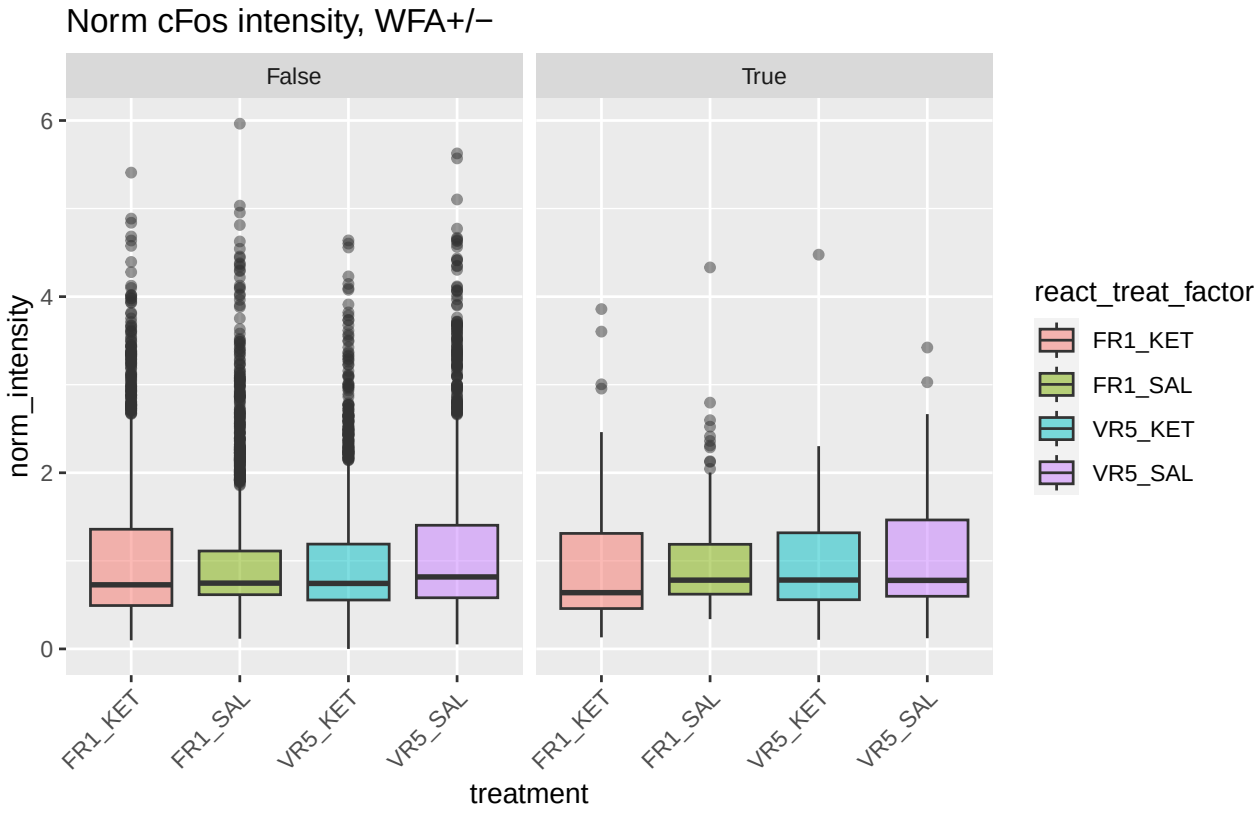
```
## Anova Table (Type III tests)
##
## Response: norm_intensity
##                                      Sum Sq   Df   F value  Pr(>F)
## (Intercept)                          1579.5    1 2829.2491 < 2e-16 ***
## react_factor                            0.1    1    0.1979 0.65647
## treat_factor                            0.8    1    1.3898 0.23847
## WFA_factor                              0.2    1    0.4235 0.51520
## react_factor:treat_factor               1.6    1    2.8472 0.09157 .
## react_factor:WFA_factor                 0.0    1    0.0003 0.98616
## treat_factor:WFA_factor                 0.0    1    0.0199 0.88778
## react_factor:treat_factor:WFA_factor    0.3    1    0.4478 0.50339
## Residuals                            4461.3 7991
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

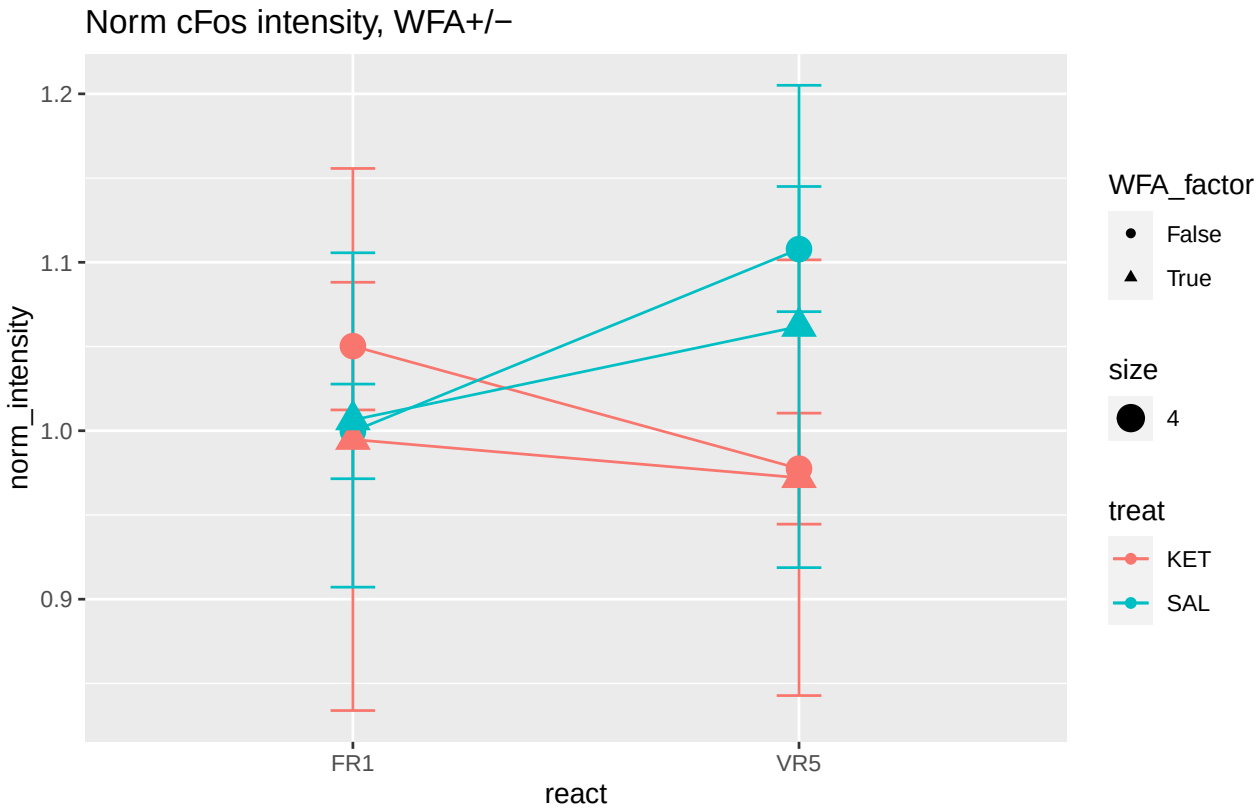
```
cFos_with_WFA <- cFos[cFos$dummy_WFA == 'True', 'norm_intensity']
cFos_without_WFA <- cFos[cFos$dummy_WFA == 'False', 'norm_intensity']
t.test(cFos_with_WFA, cFos_without_WFA)
```

```
##
##  Welch Two Sample t-test
##
## data:  cFos_with_WFA and cFos_without_WFA
## t = -0.71698, df = 471.93, p-value = 0.4737
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.09039544  0.04206424
## sample estimates:
## mean of x mean of y
##  1.009118  1.033283
```

```
# plot change in means: Can we still consider a main effect in the presence of an interaction?
f <- ggplot(cFos, aes(x=treatment, y=norm_intensity)) + geom_boxplot(aes(fill=react_treat_factor), alpha
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
  facet_wrap(~WFA_factor) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle('Norm cFos intensity, WFA+/-')

cFosc <- summarySE(cFos, measurevar="norm_intensity", groupvars=c("WFA_factor","react", "treat"))
g <- ggplot(cFosc, aes(x=react, y=norm_intensity, colour=treat, group=interaction(WFA_factor, treat))) +
  geom_errorbar(aes(ymin=norm_intensity-ci, ymax=norm_intensity+ci), width=.1) +
  geom_line() +
  geom_point(aes(shape=WFA_factor, size=4)) +
  ggtitle('Norm cFos intensity, WFA+/-')
grid.arrange(f, g, nrow=2)
```


Norm cFos intensity, WFA+/−
False
True
norm_intensity
6
4
2
0
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL
treatment
react_treat_factor
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL


Norm cFos intensity, WFA+/−
norm_intensity
1.2
1.1
1.0
0.9
FR1
VR5
react
WFA_factor
False
True
size
4
treat
KET
SAL

Single Npas4 Norm Intensity

```
Npas4 <- read.csv('NORM_single_Npas4.csv', header=TRUE, sep=',')
split <- str_split_fixed(Npas4$treatment, "_", 2)
Npas4$react <- split[,1]
Npas4$treat <- split[,2]

Npas4$react_treat_factor <- as.factor(Npas4$treatment)
Npas4$react_factor <- as.factor(Npas4$react)
Npas4$treat_factor <- as.factor(Npas4$treat)
Npas4$PV_factor <- as.factor(Npas4$dummy_PV)
Npas4$cFos_factor <- as.factor(Npas4$dummy_cFos)
Npas4$WFA_factor <- as.factor(Npas4$dummy_WFA)

str(Npas4)
```

```
## 'data.frame':    8464 obs. of  17 variables:
##  $ X                 : int  124 125 126 127 128 129 130 131 132 133 ...
##  $ stain_type        : chr  "Npas4" "Npas4" "Npas4" "Npas4" ...
##  $ intensity         : num  86.7 98.4 208.9 60.1 84.5 ...
##  $ treatment         : chr  "FR1_KET" "FR1_KET" "FR1_KET" "FR1_KET" ...
##  $ dummy_PV          : chr  "False" "False" "False" "False" ...
##  $ dummy_cFos        : chr  "False" "True" "True" "False" ...
##  $ dummy_Npas4       : chr  "True" "True" "True" "True" ...
##  $ dummy_WFA         : chr  "False" "False" "False" "False" ...
##  $ norm_intensity    : num  0.664 0.754 1.6 0.46 0.647 ...
##  $ react             : chr  "FR1" "FR1" "FR1" "FR1" ...
##  $ treat             : chr  "KET" "KET" "KET" "KET" ...
##  $ react_treat_factor: Factor w/ 4 levels "FR1_KET","FR1_SAL",..: 1 1 1 1 1 1 1 1 1 1 ...
##  $ react_factor      : Factor w/ 2 levels "FR1","VR5": 1 1 1 1 1 1 1 1 1 1 ...
##  $ treat_factor      : Factor w/ 2 levels "KET","SAL": 1 1 1 1 1 1 1 1 1 1 ...
##  $ PV_factor         : Factor w/ 2 levels "False","True": 1 1 1 1 1 1 1 2 1 1 ...
##  $ cFos_factor       : Factor w/ 2 levels "False","True": 1 2 2 1 2 2 1 2 2 2 ...
##  $ WFA_factor        : Factor w/ 2 levels "False","True": 1 1 1 1 1 1 1 2 1 1 ...
```

Npas4, PV+/-

```
# PV +/-
Npas4.PV.lm <- lm(norm_intensity ~ react_factor*treat_factor*PV_factor, contrasts = list(react_factor='
Npas4.PV.aov <- car::Anova(Npas4.PV.lm, type=3)
print(Npas4.PV.aov)
```

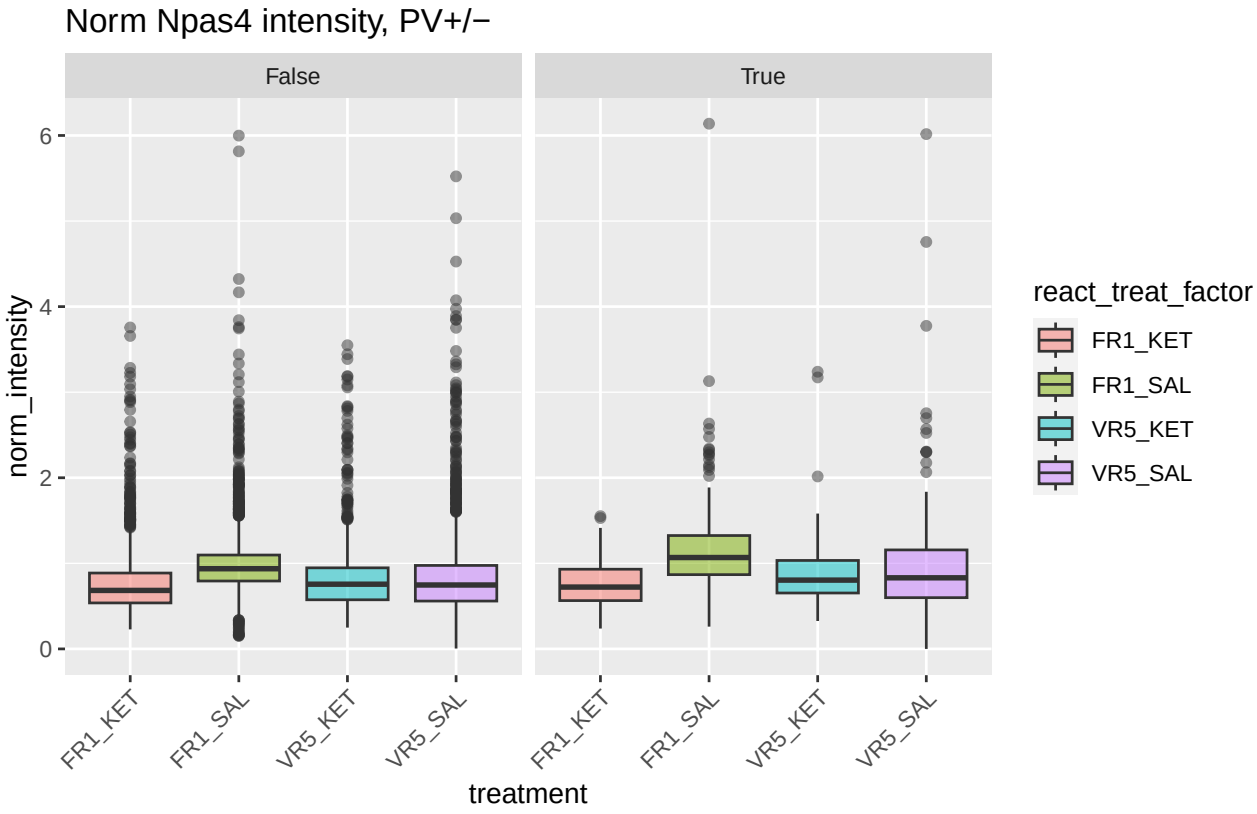
```
## Anova Table (Type III tests)
##
## Response: norm_intensity
##                                      Sum Sq   Df   F value    Pr(>F)
## (Intercept)                         1569.34    1 8712.6469 < 2.2e-16 ***
## react_factor                           0.83    1    4.6355   0.03135 *
## treat_factor                          19.22    1  106.6933 < 2.2e-16 ***
## PV_factor                              6.93    1   38.4745 5.808e-10 ***
## react_factor:treat_factor              6.48    1   35.9669 2.089e-09 ***
## react_factor:PV_factor                 0.09    1    0.5030   0.47821
## treat_factor:PV_factor                 2.76    1   15.3224 9.135e-05 ***
## react_factor:treat_factor:PV_factor    0.36    1    1.9730   0.16017
## Residuals                           1523.11 8456
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

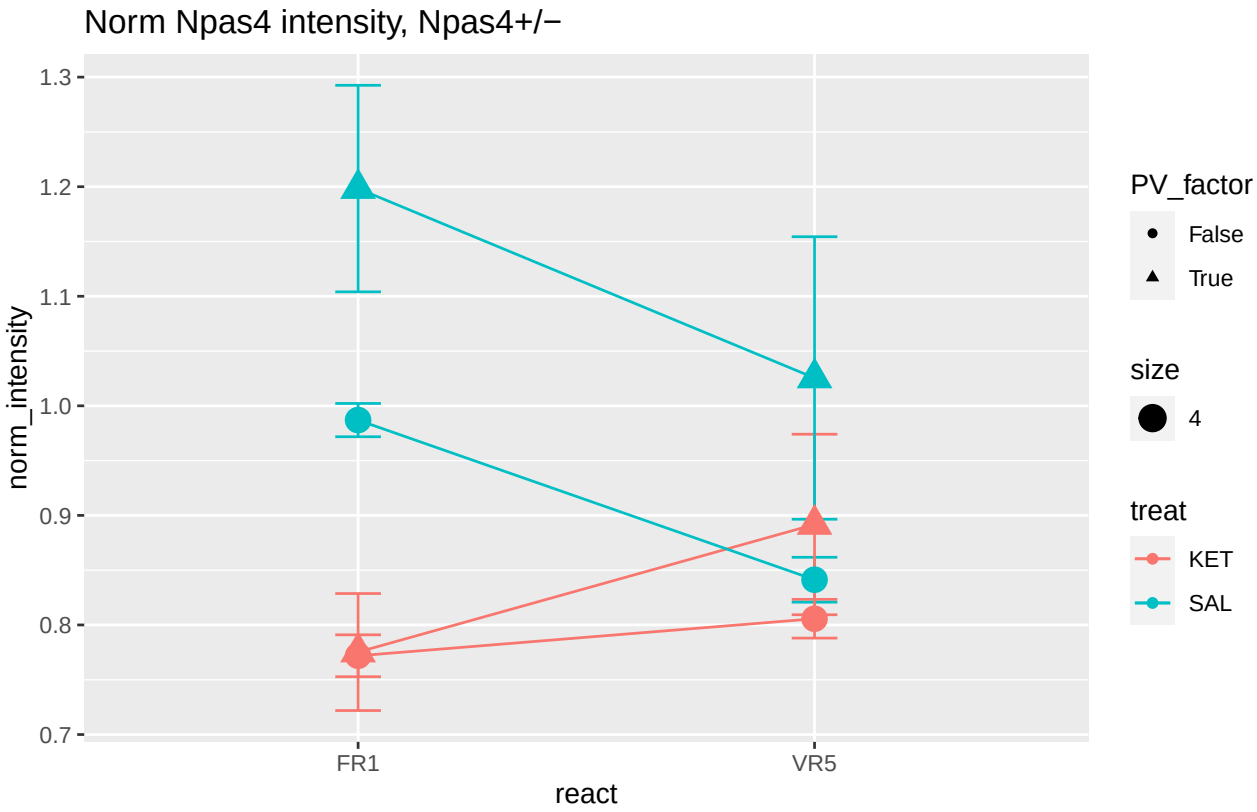
```
Npas4_with_PV <- Npas4[Npas4$dummy_PV == 'True', 'norm_intensity']
Npas4_without_PV <- Npas4[Npas4$dummy_PV == 'False', 'norm_intensity']
t.test(Npas4_with_PV, Npas4_without_PV)
```

```
##
##  Welch Two Sample t-test
##
## data:  Npas4_with_PV and Npas4_without_PV
## t = 5.08, df = 552.96, p-value = 5.17e-07
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.08328895 0.18830628
## sample estimates:
## mean of x mean of y
## 1.0015111 0.8657135
```

```
# plot change in means: Can we still consider a main effect in the presence of an interaction?
f <- ggplot(Npas4, aes(x=treatment, y=norm_intensity)) + geom_boxplot(aes(fill=react_treat_factor), alp
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
  facet_wrap(~PV_factor) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle('Norm Npas4 intensity, PV+/-')

Npas4c <- summarySE(Npas4, measurevar="norm_intensity", groupvars=c("PV_factor","react", "treat"))
g <- ggplot(Npas4c, aes(x=react, y=norm_intensity, colour=treat, group=interaction(PV_factor, treat))) +
  geom_errorbar(aes(ymin=norm_intensity-ci, ymax=norm_intensity+ci), width=.1) +
  geom_line() +
  geom_point(aes(shape=PV_factor, size=4)) +
  ggtitle('Norm Npas4 intensity, Npas4+/-')
grid.arrange(f, g, nrow=2)
```


Norm Npas4 intensity, PV+/−
False
True
norm_intensity
0
2
4
6
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL
treatment
react_treat_factor
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL


Norm Npas4 intensity, Npas4+/−
norm_intensity
0.7
0.8
0.9
1.0
1.1
1.2
1.3
FR1
VR5
react
PV_factor
False
True
size
4
treat
KET
SAL

Npas4, cFos+/-

```
# cFos +/-
Npas4.cFos.lm <- lm(norm_intensity ~ react_factor*treat_factor*cFos_factor, contrasts = list(react_facto
Npas4.cFos.aov <- car::Anova(Npas4.cFos.lm, type=3)
print(Npas4.cFos.aov)
```

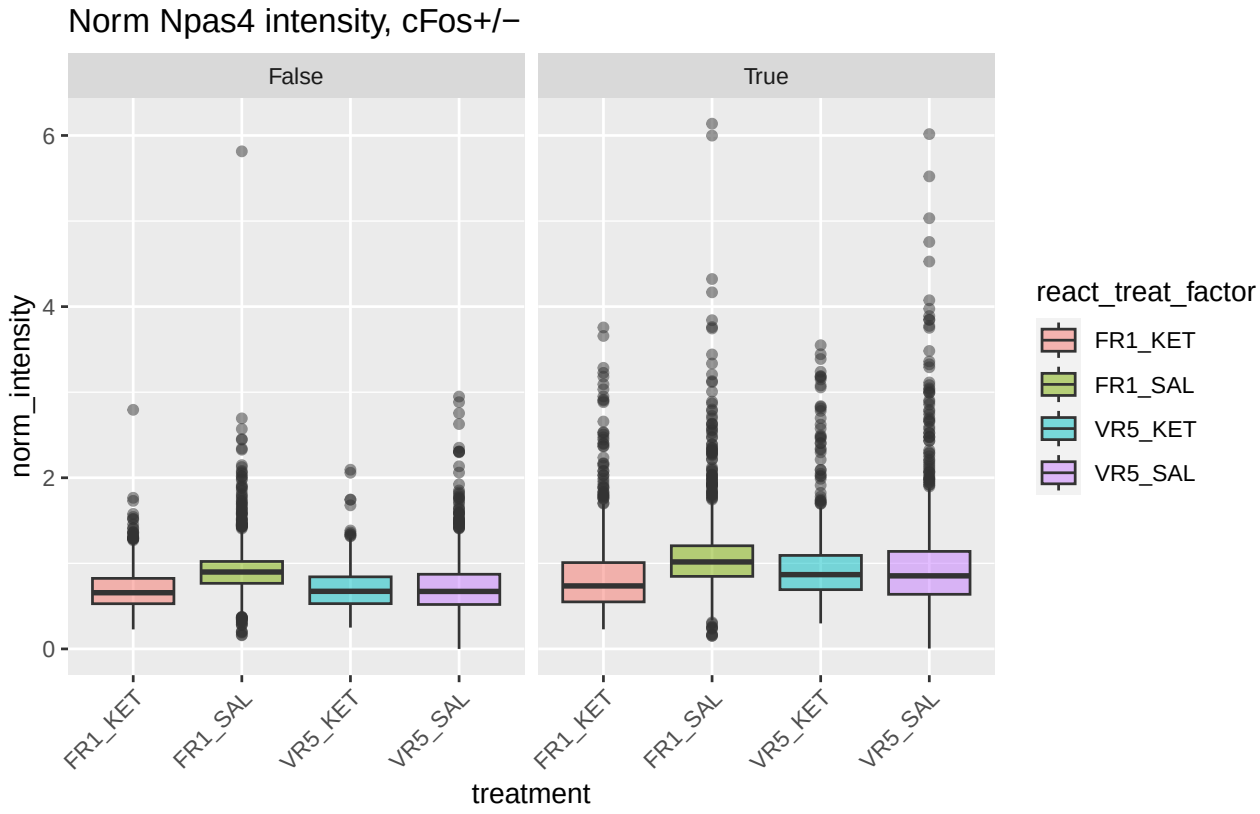
```
## Anova Table (Type III tests)
##
## Response: norm_intensity
##                                       Sum Sq   Df    F value    Pr(>F)
## (Intercept)                           6138.7    1 36218.0090 < 2.2e-16 ***
## react_factor                             4.2    1    24.6746 6.920e-07 ***
## treat_factor                            36.8    1   217.4035 < 2.2e-16 ***
## cFos_factor                             95.6    1   564.2636 < 2.2e-16 ***
## react_factor:treat_factor               15.9    1    93.6625 < 2.2e-16 ***
## react_factor:cFos_factor                 4.1    1    24.4701 7.693e-07 ***
## treat_factor:cFos_factor                 0.0    1     0.2386    0.6252
## react_factor:treat_factor:cFos_factor    0.0    1     0.1290    0.7195
## Residuals                             1433.2 8456
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

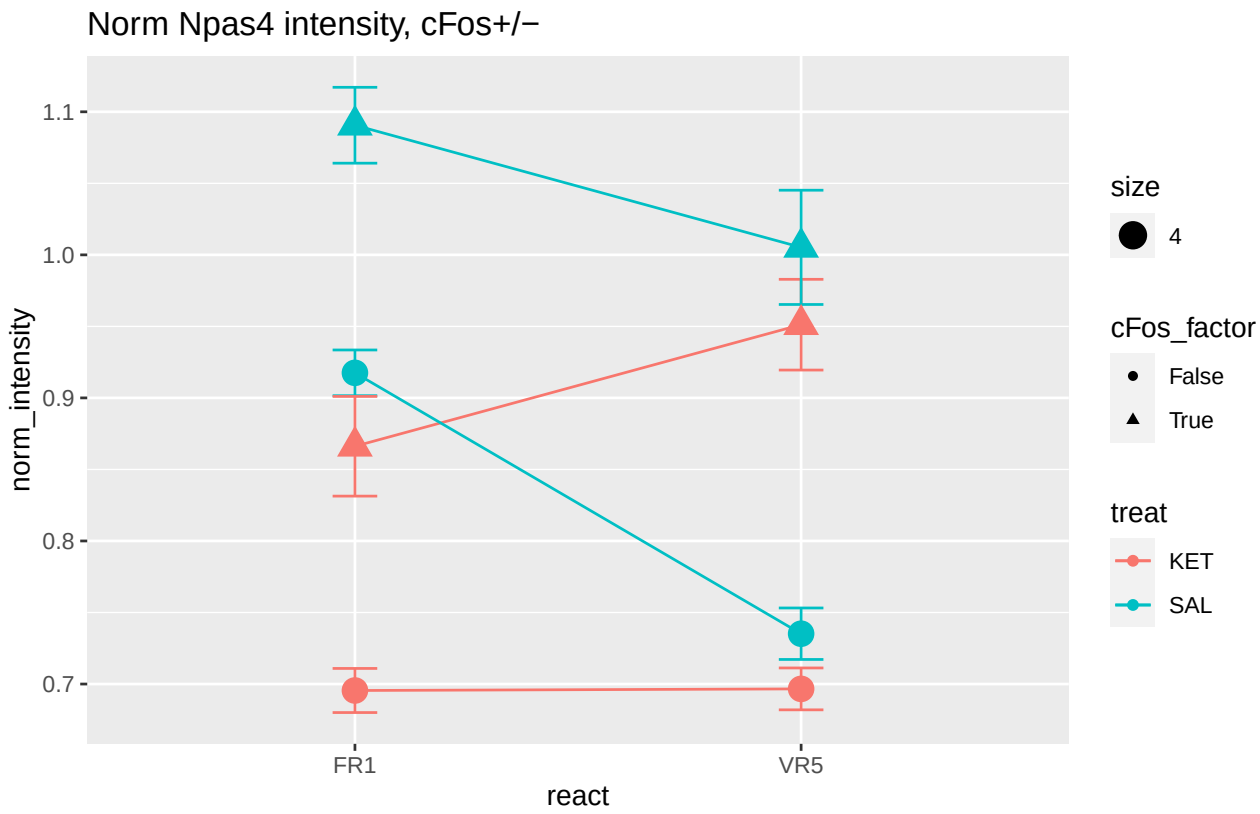
```
Npas4_with_cFos <- Npas4[Npas4$dummy_cFos == 'True', 'norm_intensity']
Npas4_without_cFos <- Npas4[Npas4$dummy_cFos == 'False', 'norm_intensity']
t.test(Npas4_with_cFos, Npas4_without_cFos)
```

```
##
##  Welch Two Sample t-test
##
## data:  Npas4_with_cFos and Npas4_without_cFos
## t = 22.857, df = 5830.7, p-value < 2.2e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.2014876 0.2392922
## sample estimates:
## mean of x mean of y
## 0.9944064 0.7740165
```

```
# plot change in means: Can we still consider a main effect in the presence of an interaction?
f <- ggplot(Npas4, aes(x=treatment, y=norm_intensity)) + geom_boxplot(aes(fill=react_treat_factor), alp
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
  facet_wrap(~cFos_factor) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle('Norm Npas4 intensity, cFos+/-')

Npas4c <- summarySE(Npas4, measurevar="norm_intensity", groupvars=c("cFos_factor","react", "treat"))
g <- ggplot(Npas4c, aes(x=react, y=norm_intensity, colour=treat, group=interaction(cFos_factor, treat))
  geom_errorbar(aes(ymin=norm_intensity-ci, ymax=norm_intensity+ci), width=.1) +
  geom_line() +
  geom_point(aes(shape=cFos_factor, size=4)) +
  ggtitle('Norm Npas4 intensity, cFos+/-')
grid.arrange(f, g, nrow=2)
```


Norm Npas4 intensity, cFos+/−
False
True
norm_intensity
6
4
2
0
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL
treatment
react_treat_factor
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL


Norm Npas4 intensity, cFos+/−
norm_intensity
1.1
1.0
0.9
0.8
0.7
FR1
VR5
react
size
4
cFos_factor
False
True
treat
KET
SAL

Npas4, WFA+/-

```
# WFA +/-
Npas4.WFA.lm <- lm(norm_intensity ~ react_factor*treat_factor*WFA_factor, contrasts = list(react_factor=
Npas4.WFA.aov <- car::Anova(Npas4.WFA.lm, type=3)
print(Npas4.WFA.aov)
```

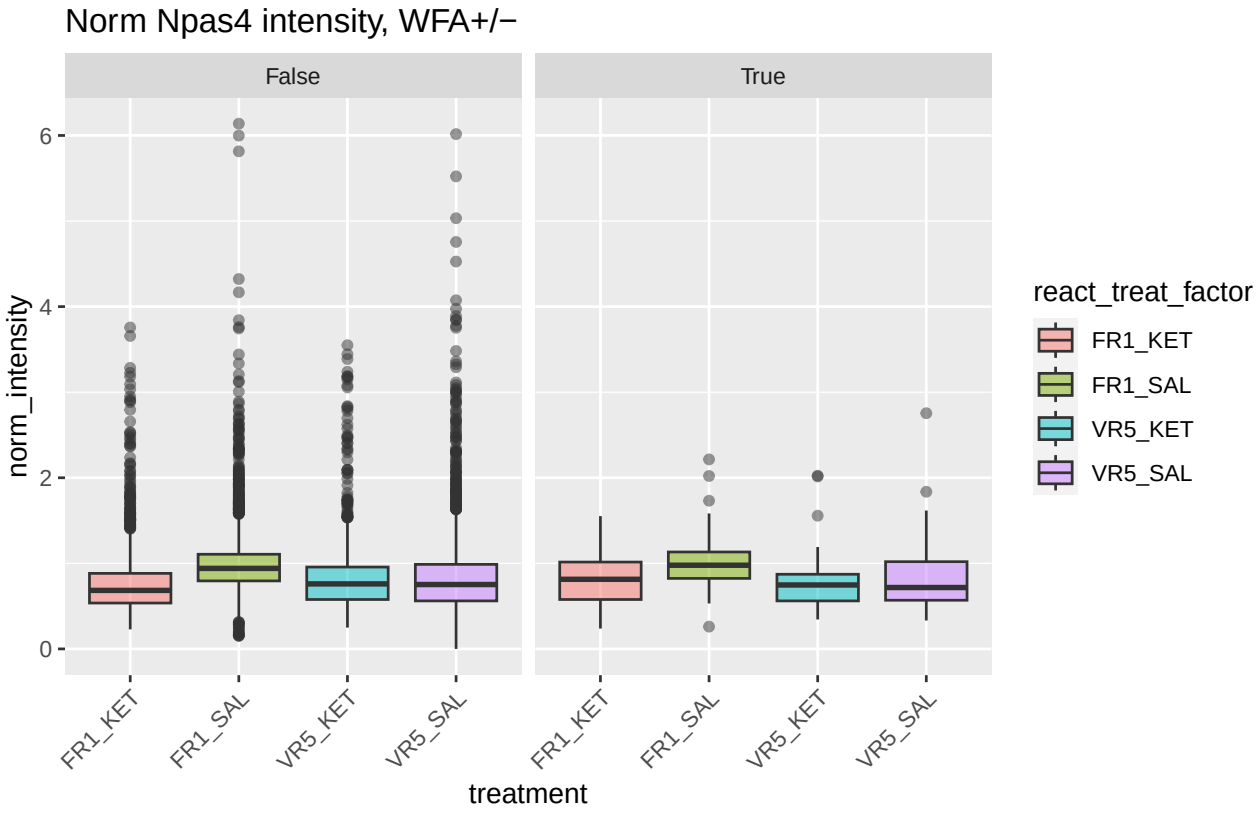
```
## Anova Table (Type III tests)
##
## Response: norm_intensity
##                                      Sum Sq   Df   F value    Pr(>F)
## (Intercept)                          876.41    1 4828.5344 < 2.2e-16 ***
## react_factor                           2.27    1   12.5242 0.0004039 ***
## treat_factor                           4.59    1   25.3000 5.008e-07 ***
## WFA_factor                             0.00    1    0.0017 0.9666438
## react_factor:treat_factor              2.16    1   11.8951 0.0005656 ***
## react_factor:WFA_factor                0.39    1    2.1266 0.1447994
## treat_factor:WFA_factor                0.04    1    0.2175 0.6409631
## react_factor:treat_factor:WFA_factor   0.02    1    0.1191 0.7300364
## Residuals                          1534.82 8456
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

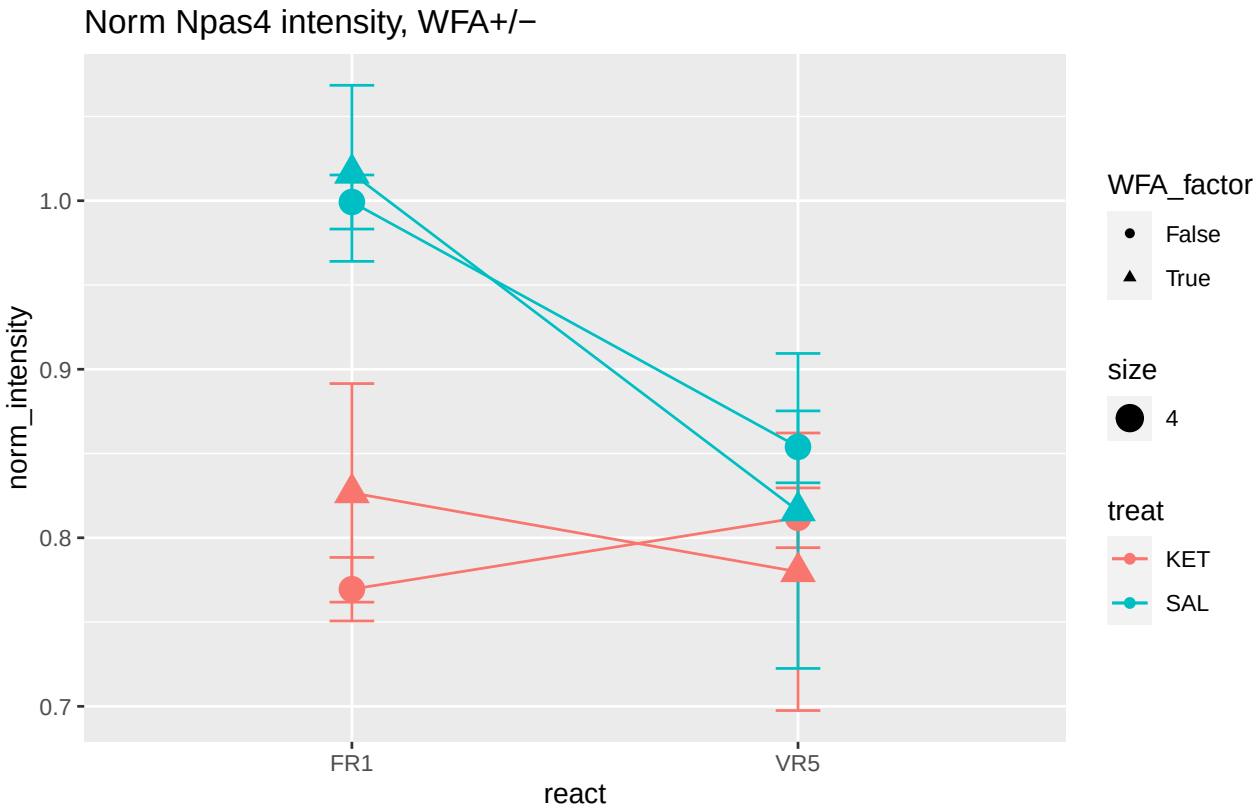
```
Npas4_with_WFA <- Npas4[Npas4$dummy_WFA == 'True', 'norm_intensity']
Npas4_without_WFA <- Npas4[Npas4$dummy_WFA == 'False', 'norm_intensity']
t.test(Npas4_with_WFA, Npas4_without_WFA)
```

```
##
##  Welch Two Sample t-test
##
## data:  Npas4_with_WFA and Npas4_without_WFA
## t = 0.61687, df = 375.64, p-value = 0.5377
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.02563051  0.04906345
## sample estimates:
## mean of x mean of y
## 0.8853175 0.8736010
```

```
# plot change in means: Can we still consider a main effect in the presence of an interaction?
f <- ggplot(Npas4, aes(x=treatment, y=norm_intensity)) + geom_boxplot(aes(fill=react_treat_factor), alp
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
  facet_wrap(~WFA_factor) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle('Norm Npas4 intensity, WFA+/-')

Npas4c <- summarySE(Npas4, measurevar="norm_intensity", groupvars=c("WFA_factor","react", "treat"))
g <- ggplot(Npas4c, aes(x=react, y=norm_intensity, colour=treat, group=interaction(WFA_factor, treat)))
  geom_errorbar(aes(ymin=norm_intensity-ci, ymax=norm_intensity+ci), width=.1) +
  geom_line() +
  geom_point(aes(shape=WFA_factor, size=4)) +
  ggtitle('Norm Npas4 intensity, WFA+/-')
grid.arrange(f, g, nrow=2)
```


Norm Npas4 intensity, WFA+/−
False
True
norm_intensity
0
2
4
6
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL
treatment
react_treat_factor
FR1_KET
FR1_SAL
VR5_KET
VR5_SAL


Norm Npas4 intensity, WFA+/−
norm_intensity
0.7
0.8
0.9
1.0
FR1
VR5
react
WFA_factor
False
True
size
4
treat
KET
SAL