

PRE VR5-KET Npas4/cFos+/- vs cFos/Npas4+/- Cell Counts

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```
library(ggplot2)
library(ggpubr)
library(car)

## Loading required package: carData

library(emmeans)
library(stringr)
library(rstatix)

##
## Attaching package: 'rstatix'

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

ANOVAs for Cell Counts

I had previously looked for differences in mean cell counts between PV/cFos+/Npas4- and PV/Npas4+/cFos- cells to potentially disambiguate two distinct populations of PV cells. From my last R document I concluded the following:

“In general we do not see any differences in the mean cell counts of cFos+/Npas4- vs Npas4+/cFos- however, once we consider whether there was a PNN, we can see that in Npas4+/cFos- there is a difference between PNN+ vs PNN- populations (but NOT in cFos+/Npas4-), in particular VR5_KET mean cell counts are down compared to VR5_SAL, and FR1_SAL mean cell counts are down compared to VR5_SAL. However, since there are so few of these stain type combinations observed per image (we are looking at a difference on the order of 1, maybe 2, cells per image), and only 5-7 rats per treatment, I would likely not consider that this data set powered up enough to adequately address this particular question.”

Here I aim to repeat this set of analyses with out restricting the counts to PV cells only.

The ANOVAs presented in this document are as follows:

- cFos+: Npas4- vs Npas4+ (reactivation by treatment by Npas4) 3way
- Npas4: cFos- vs cFos+ (reactivation by treatment by cFos) 3way
- cFos+/Npas4- vs Npas4+/cFos- (reactivation by treatment by “ensemble”) 3way
- Npas4+/cFos-: WFA- vs WFA+ (reactivation by treatment by WFA) 3way
- cFos+/Npas4-: WFA- vs WFA+ (reactivation by treatment by WFA) 3way

Overall, I found no interactions worth following up, and so we are unable to conclude that there are two distinct populations of cFos vs Npas4 cells. The shape of the box plots appears to be somewhat compelling but again, there are only 5-7 ns per treatment combination and so unless these data were extremely tight, I

would not consider that this experiment was powered up enough to adequately address this question, even looking beyond just PV cells.

```
##### reusing some function definitions for convenience
Sidak <- function(pvals)
  # 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)

    for (i in 1:j){
      adj_p <- 1-(1-pvals[i])^j
      adjusted <- c(adjusted, adj_p)
    }
    return(adjusted)
  }

eda_anova <- function(df, qual=TRUE, quant=TRUE)
  # takes a filename, loads data from csv; data 4 columns:
  # react_treat, react, treat, and norm_int (response var)
  # react_treat is just react and treat in one string separated by "_"
  # builds factor cols for categorical cols (norm_int is numeric, all others are categorical)
  # then performs the following tasks:
  # checks assumptions of normality with qqplot and shapiro wilk tests
  # checks assumptions of equal variances with box plot and levene test
  # performs 2way ANOVA (2 by 2, react by treat)
  # performs post hoc pairwise comparisons (emmeans of levels of react by treat
  # and emmeans of levels of treat by react)
  # prints out all statistical test results and returns plot objects
  # for the two plots: the qqplots and the box plots
  #
  # Jonathan Ramos 3/12/2024
  {
    ### check assumption of normality
    # quantitative assessment
    if (quant) {
      print(tapply(df$mean_cell_n, df$react_treat_factor, shapiro.test))
    }

    # qualitative assessment
    if (qual) {
      g <- ggqqplot(df, x="mean_cell_n", facet.by=c("treat_factor", "react_factor"))
    }

    ### check assumption of equal variances
    # quantitative assessment
    if (quant) {
      print(leveneTest(y = df$mean_cell_n, group=df$react_treat_factor, center='mean'))
    }
  }
}
```

```

# qualitative assessment
if (qual) {
  f <- ggplot(df, aes(x=treat_factor, y=mean_cell_n)) + geom_boxplot(aes(fill=treat_factor), alpha=0.5) +
    #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
    facet_wrap(~react_factor) +
    theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1))
}

# run the ANOVA, display summary
df.lm <- lm(mean_cell_n ~ treat_factor + react_factor + treat_factor*react_factor, contrasts=list(treat_factor = "contrasts"))
df.III.aov <- car::Anova(df.lm, type = 3)
print(df.III.aov)

# post hoc pairwise comparisons
emm <- emmeans(df.lm, ~ treat_factor * react_factor)
p1 <- pairs(emm, simple="treat_factor", adjust="tukey")
p2 <- pairs(emm, simple="react_factor", adjust="tukey")

# add col to summary dataframe containing sidak adjusted p-values
adjusted_p.value1 <- Sidak(summary(p1, adjust="tukey")$p.value)
s1 <- summary(p1)
s1['adjusted_p.value'] <- adjusted_p.value1

adjusted_p.value2 <- Sidak(summary(p2, adjust="tukey")$p.value)
s2 <- summary(p2)
s2['adjusted_p.value'] <- adjusted_p.value2

# display results
print(s1)
print(s2)

if (qual) {
  return(list(g, f))
}
}

```

cFos: Npas4- vs Npas4+

No 3way interaction to follow up on here.

```

# loading in data
cFos.Npas4m <- read.csv('cFos_Npas4m_COUNTS.csv')
cFos.Npas4p <- read.csv('cFos_Npas4p_COUNTS.csv')

# concat dataframes
cFos.Npas4 <- rbind(cFos.Npas4m, cFos.Npas4p)

# build dummy cols
cFos.Npas4$react_factor <- as.factor(cFos.Npas4$react)
cFos.Npas4$treat_factor <- as.factor(cFos.Npas4$treat)
cFos.Npas4$Npas4_factor <- as.factor(cFos.Npas4$Npas4)
str(cFos.Npas4)

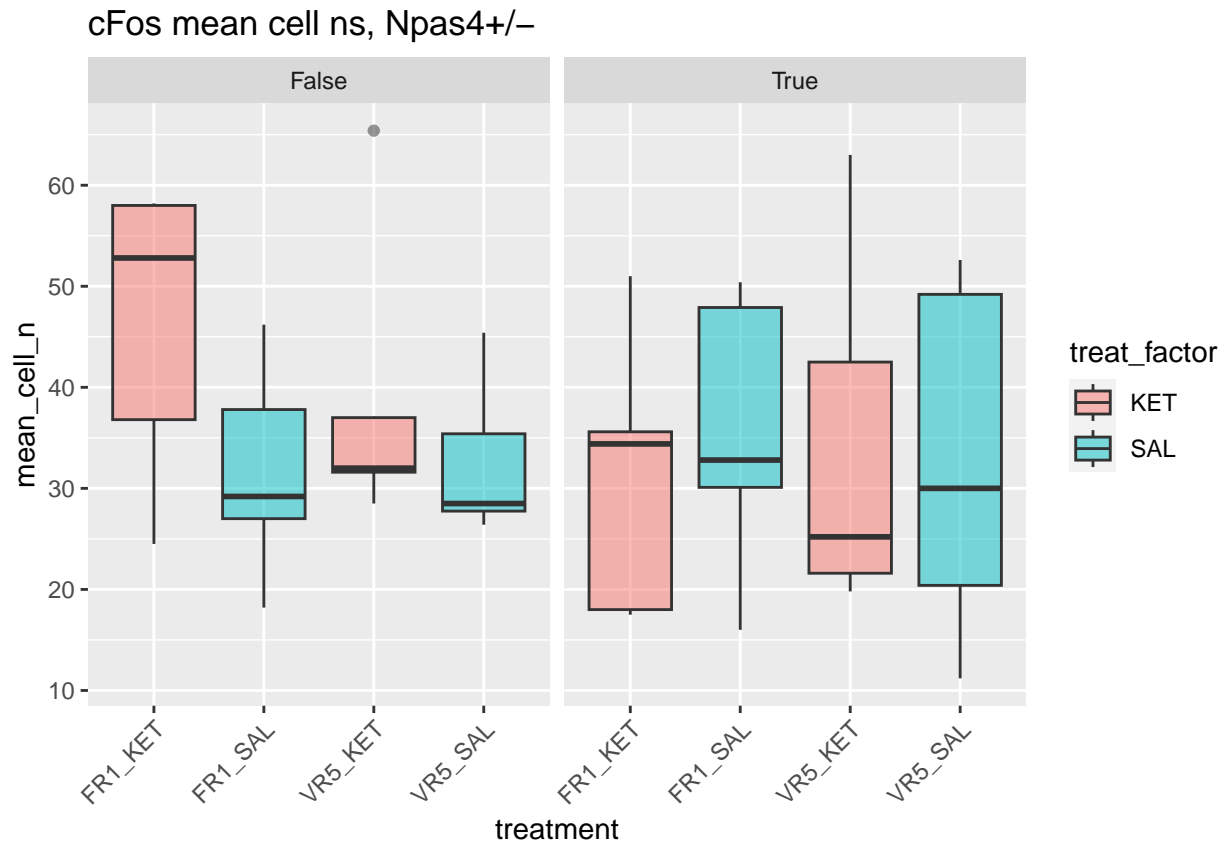
```

```
## 'data.frame': 46 obs. of 14 variables:
## $ X : int 0 1 2 3 4 5 6 7 8 9 ...
## $ rat_n : chr "KET-10-12" "KET-9-1" "PE-12-1" "PE-12-2" ...
## $ treatment : chr "FR1_KET" "FR1_KET" "FR1_KET" "FR1_KET" ...
## $ stain_type : chr "cFos" "cFos" "cFos" "cFos" ...
## $ cell_count_sums: int 291 98 290 264 184 135 231 135 91 146 ...
## $ image_n : int 5 4 5 5 5 5 5 5 5 5 ...
## $ mean_cell_n : num 58.2 24.5 58 52.8 36.8 27 46.2 27 18.2 29.2 ...
## $ react : chr "FR1" "FR1" "FR1" "FR1" ...
## $ treat : chr "KET" "KET" "KET" "KET" ...
## $ cFos : chr "True" "True" "True" "True" ...
## $ Npas4 : chr "False" "False" "False" "False" ...
## $ 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 2 2 2 2 2 ...
## $ Npas4_factor : Factor w/ 2 levels "False","True": 1 1 1 1 1 1 1 1 1 1 ...

# 3way ANOVA
cFos.Npas4.lm <- lm(mean_cell_n ~ react_factor*treat_factor*Npas4_factor, contrasts = list(react_factor=
cFos.Npas4.aov <- car::Anova(cFos.Npas4.lm, type=3)
print(cFos.Npas4.aov)

## Anova Table (Type III tests)
##
## Response: mean_cell_n
##
## Sum Sq Df F value Pr(>F)
## (Intercept) 56887 1 294.6544 <2e-16 ***
## react_factor 37 1 0.1936 0.6624
## treat_factor 209 1 1.0845 0.3043
## Npas4_factor 143 1 0.7397 0.3952
## react_factor:treat_factor 0 1 0.0023 0.9618
## react_factor:Npas4_factor 27 1 0.1377 0.7126
## treat_factor:Npas4_factor 414 1 2.1455 0.1512
## react_factor:treat_factor:Npas4_factor 146 1 0.7588 0.3892
## Residuals 7336 38
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

f <- ggplot(cFos.Npas4, aes(x=treatment, y=mean_cell_n)) + geom_boxplot(aes(fill=treat_factor), alpha=0
#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('cFos mean cell ns, Npas4+/-')
f
```



```
# just to be sure we didn't find anything
# eda_anova(cFos.Npas4[cFos.Npas4$Npas4_factor == 'False'], qual=FALSE, quant=FALSE)

# just to be sure we didn't find anything
# eda_anova(cFos.Npas4[cFos.Npas4$Npas4_factor == 'True'], qual=FALSE, quant=FALSE)
```

Npas4: cFos- vs cFos+

No 3way interaction to follow up on here.

```
# loading in data
Npas4.cFosm <- read.csv('Npas4_cFosm_COUNTS.csv')
Npas4.cFosp <- read.csv('Npas4_cFosp_COUNTS.csv')

# concat dataframes
Npas4.cFos <- rbind(Npas4.cFosm, Npas4.cFosp)

# build dummy cols
Npas4.cFos$react_factor <- as.factor(Npas4.cFos$react)
Npas4.cFos$treat_factor <- as.factor(Npas4.cFos$treat)
Npas4.cFos$cFos_factor <- as.factor(Npas4.cFos$cFos)
str(Npas4.cFos)

## 'data.frame':   46 obs. of  14 variables:
##  $ X          : int  0 1 2 3 4 5 6 7 8 9 ...
##  $ rat_n      : chr  "KET-10-12" "KET-9-1" "PE-12-1" "PE-12-2" ...
##  $ treatment  : chr  "FR1_KET" "FR1_KET" "FR1_KET" "FR1_KET" ...
##  $ stain_type : chr  "Npas4" "Npas4" "Npas4" "Npas4" ...
```

```
## $ cell_count_sums: int 148 98 206 252 236 217 243 242 144 208 ...
## $ image_n : int 5 4 5 5 5 5 5 5 5 ...
## $ mean_cell_n : num 29.6 24.5 41.2 50.4 47.2 43.4 48.6 48.4 28.8 41.6 ...
## $ react : chr "FR1" "FR1" "FR1" "FR1" ...
## $ treat : chr "KET" "KET" "KET" "KET" ...
## $ Npas4 : chr "True" "True" "True" "True" ...
## $ cFos : chr "False" "False" "False" "False" ...
## $ 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 2 2 2 2 ...
## $ cFos_factor : Factor w/ 2 levels "False","True": 1 1 1 1 1 1 1 1 1 1 ...
```

```
# 3way ANOVA
```

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

```
## Anova Table (Type III tests)
```

```
##
```

```
## Response: mean_cell_n
```

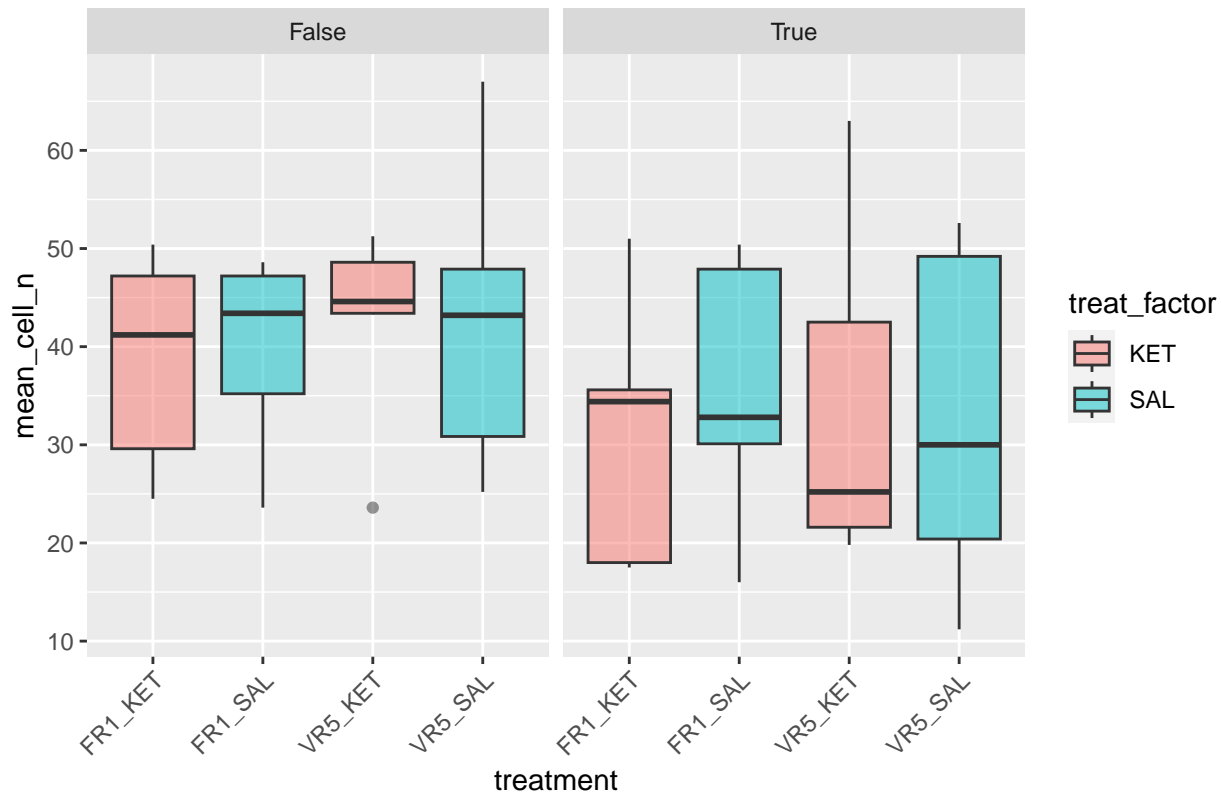
	Sum Sq	Df	F value	Pr(>F)
## (Intercept)	62722	1	319.5720	< 2e-16 ***
## react_factor	22	1	0.1106	0.74134
## treat_factor	19	1	0.0955	0.75897
## cFos_factor	570	1	2.9061	0.09641 .
## react_factor:treat_factor	46	1	0.2362	0.62975
## react_factor:cFos_factor	32	1	0.1607	0.69076
## treat_factor:cFos_factor	2	1	0.0123	0.91232
## react_factor:treat_factor:cFos_factor	21	1	0.1090	0.74309
## Residuals	7458	38		

```
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
f <- ggplot(Npas4.cFos, aes(x=treatment, y=mean_cell_n)) + geom_boxplot(aes(fill=treat_factor), alpha=0
#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('Npas4 mean cell ns, cFos+/-')
f
```

Npas4 mean cell ns, cFos+/-



```
# just to be sure we didn't find anything
# eda_anova(Npas4.cFos[Npas4.cFos$cFos_factor == 'False'], qual=FALSE, quant=FALSE)

# just to be sure we didn't find anything
# eda_anova(Npas4.cFos[Npas4.cFos$cFos_factor == 'True'], qual=FALSE, quant=FALSE)
```

Testing directly cFos+/-/Npas4- vs Npas4+/cFos-

As expected, this ANOVA doesn't come out.

```
cFos.Npas4m$ensemble <- 'Npas4'
Npas4.cFosm$ensemble <- 'cFos'

cFos.Npas4m$react_factor <- as.factor(cFos.Npas4m$react)
cFos.Npas4m$treat_factor <- as.factor(cFos.Npas4m$treat)
Npas4.cFosm$react_factor <- as.factor(Npas4.cFosm$react)
Npas4.cFosm$treat_factor <- as.factor(Npas4.cFosm$treat)

cFos.Npas4m.ensemble <- cFos.Npas4m[c('mean_cell_n', 'react_factor', 'treat_factor', 'ensemble')]
Npas4.cFosm.ensemble <- Npas4.cFosm[c('mean_cell_n', 'react_factor', 'treat_factor', 'ensemble')]

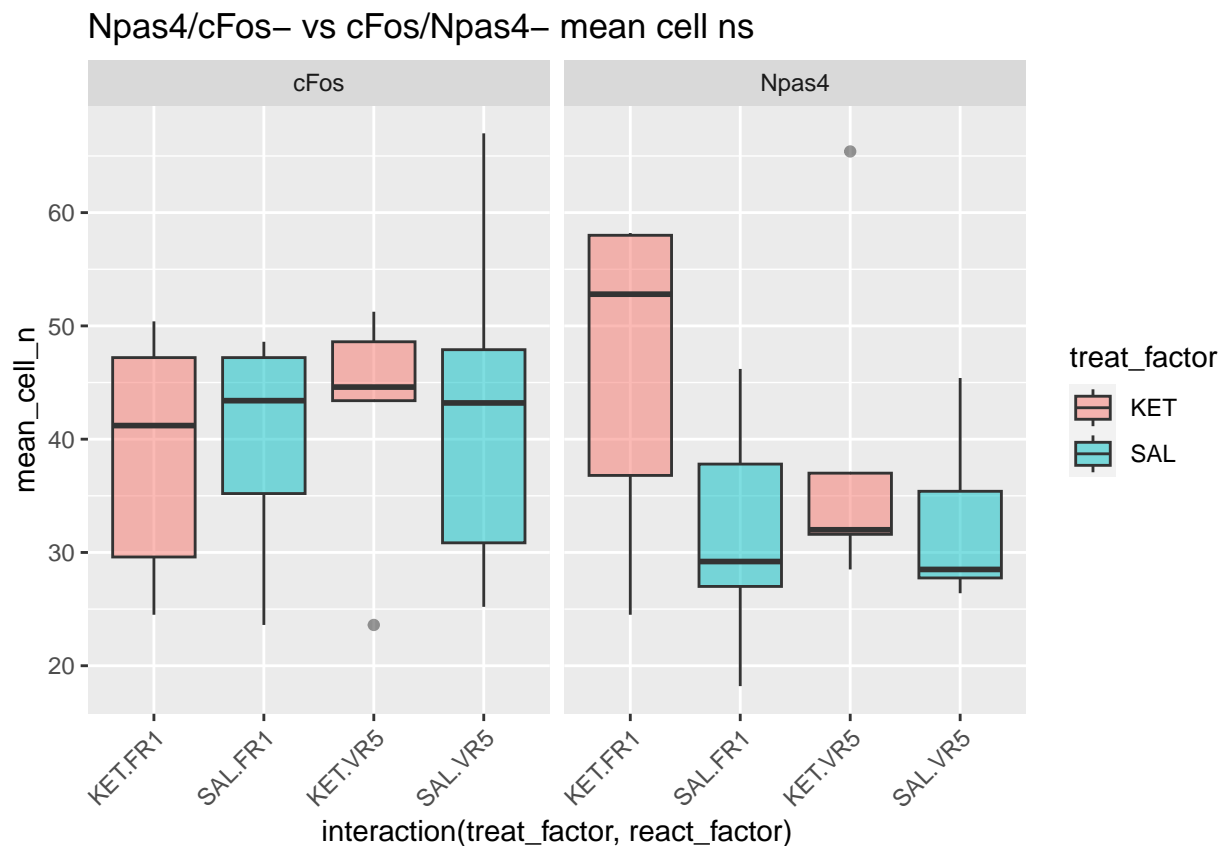
ensemble <- rbind(cFos.Npas4m.ensemble, Npas4.cFosm.ensemble)
ensemble$ensemble_factor <- as.factor(ensemble$ensemble)

# 3way ANOVA: reactivation x treatment x ensemble (2 x 2 x 2) in PV cells
ensemble.lm <- lm(mean_cell_n ~ treat_factor*react_factor*ensemble_factor, contrasts = list(treat_factor = c(1, -1),
react_factor = c(1, -1), ensemble_factor = c(1, -1)))
ensemble.aov <- car::Anova(ensemble.lm, type=3)
```

```
print(ensemble.aov)
```

```
## Anova Table (Type III tests)
##
## Response: mean_cell_n
##
## Sum Sq Df F value Pr(>F)
## (Intercept) 68850 1 488.8421 <2e-16 ***
## treat_factor 257 1 1.8228 0.1850
## react_factor 0 1 0.0018 0.9668
## ensemble_factor 142 1 1.0110 0.3210
## treat_factor:react_factor 28 1 0.1990 0.6580
## treat_factor:ensemble_factor 353 1 2.5093 0.1215
## react_factor:ensemble_factor 116 1 0.8239 0.3698
## treat_factor:react_factor:ensemble_factor 56 1 0.3970 0.5324
## Residuals 5352 38
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
f <- ggplot(ensemble, aes(x=interaction(treat_factor, react_factor), y=mean_cell_n)) + geom_boxplot(aes(
  #geom_dotplot(binaxis = "y", stackdir = "center", dotsize=0.5) +
  facet_wrap(~ensemble_factor) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  ggtitle('Npas4/cFos- vs cFos/Npas4- mean cell ns')
f
```



```
eda_anova(cFos.Npas4m.ensemble, qual=FALSE, quant=FALSE)
```

```
## Anova Table (Type III tests)
```



```
##
## Response: mean_cell_n
##               Sum Sq Df  F value    Pr(>F)
## (Intercept)    31365.2  1 227.8794 4.921e-12 ***
## treat_factor      606.3  1   4.4050  0.04943 *
## react_factor      63.5  1   0.4614  0.50518
## treat_factor:react_factor    81.6  1   0.5926  0.45087
## Residuals      2615.2 19
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## react_factor = FR1:
## contrast estimate SE df t.ratio p.value adjusted_p.value
## KET - SAL      14.17 6.87 19   2.063  0.0530          0.103
##
## react_factor = VR5:
## contrast estimate SE df t.ratio p.value adjusted_p.value
## KET - SAL       6.57 7.10 19   0.924  0.3669          0.599
##
## treat_factor = KET:
## contrast estimate SE df t.ratio p.value adjusted_p.value
## FR1 - VR5       7.160 7.42 19   0.965  0.3467          0.573
##
## treat_factor = SAL:
## contrast estimate SE df t.ratio p.value adjusted_p.value
## FR1 - VR5      -0.448 6.53 19  -0.069  0.9460          0.997
eda_anova(Npas4.cFosm.ensemble, qual=FALSE, quant=FALSE)
```

```
## Anova Table (Type III tests)
##
## Response: mean_cell_n
##               Sum Sq Df  F value    Pr(>F)
## (Intercept)    37627  1 261.2154 1.471e-12 ***
## treat_factor      4  1   0.0268  0.8718
## react_factor     53  1   0.3664  0.5521
## treat_factor:react_factor    2  1   0.0165  0.8990
## Residuals      2737 19
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## react_factor = FR1:
## contrast estimate SE df t.ratio p.value adjusted_p.value
## KET - SAL     -1.477 7.03 19  -0.210  0.8358          0.973
##
## react_factor = VR5:
## contrast estimate SE df t.ratio p.value adjusted_p.value
## KET - SAL     -0.177 7.27 19  -0.024  0.9809          1.000
##
## treat_factor = KET:
## contrast estimate SE df t.ratio p.value adjusted_p.value
## FR1 - VR5     -3.71 7.59 19  -0.489  0.6306          0.864
##
## treat_factor = SAL:
## contrast estimate SE df t.ratio p.value adjusted_p.value
## FR1 - VR5     -2.41 6.68 19  -0.361  0.7222          0.923
```

Do PNNs matter? cFos+/Npas4- vs Npas4+/cFos- mean cell ns in WFA+/- cells

```
cFos.Npas4m.WFam <- read.csv('cFos_Npas4m_WFam_COUNTS.csv')
cFos.Npas4m.WFap <- read.csv('cFos_Npas4m_WFap_COUNTS.csv')
cFos.Npas4p.WFam <- read.csv('cFos_Npas4p_WFam_COUNTS.csv')
cFos.Npas4p.WFap <- read.csv('cFos_Npas4p_WFap_COUNTS.csv')

Npas4.cFosm.WFam <- read.csv('Npas4_cFosm_WFam_COUNTS.csv')
Npas4.cFosm.WFap <- read.csv('Npas4_cFosm_WFap_COUNTS.csv')
Npas4.cFosp.WFam <- read.csv('Npas4_cFosp_WFam_COUNTS.csv')
Npas4.cFosp.WFap <- read.csv('Npas4_cFosp_WFap_COUNTS.csv')
```

Npas4+/cFos- with or without PNNs

only a main effect of WFA here, which makes sense since the number of Npas4 cells without PNNs vastly outnumber the number of Npas4 cells with PNNs.

```
# slice
Npas4.cFosm.WFam <- Npas4.cFosm.WFam[c('mean_cell_n', 'treatment', 'react', 'treat', 'WFA')]
Npas4.cFosm.WFap <- Npas4.cFosm.WFap[c('mean_cell_n', 'treatment', 'react', 'treat', 'WFA')]

# concat
Npas4.cFosm.WFA <- rbind(Npas4.cFosm.WFam, Npas4.cFosm.WFap)
```

```
# build dummy cols
Npas4.cFosm.WFA$react_factor <- as.factor(Npas4.cFosm.WFA$react)
Npas4.cFosm.WFA$treat_factor <- as.factor(Npas4.cFosm.WFA$treat)
Npas4.cFosm.WFA$treatment_factor <- as.factor(Npas4.cFosm.WFA$treatment)
Npas4.cFosm.WFA$WFA_factor <- as.factor(Npas4.cFosm.WFA$WFA)
str(Npas4.cFosm.WFA)
```

```
## 'data.frame': 45 obs. of 9 variables:
## $ mean_cell_n : num 28.4 23.2 40.4 49.8 46.2 ...
## $ treatment : chr "FR1_KET" "FR1_KET" "FR1_KET" "FR1_KET" ...
## $ react : chr "FR1" "FR1" "FR1" "FR1" ...
## $ treat : chr "KET" "KET" "KET" "KET" ...
## $ WFA : chr "False" "False" "False" "False" ...
## $ 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 2 2 2 2 2 ...
## $ treatment_factor: Factor w/ 4 levels "FR1_KET","FR1_SAL",...: 1 1 1 1 1 2 2 2 2 2 ...
## $ WFA_factor : Factor w/ 2 levels "False","True": 1 1 1 1 1 1 1 1 1 1 ...
```

```
# 3way ANOVA: reactivation x treatment x WFA (2 x 2 x 2) in Npas4+/cFos-
```

```
Npas4.cFosm.WFA.lm <- lm(mean_cell_n ~ treat_factor*react_factor*WFA_factor, contrasts = list(treat_factor = c(1, -1), react_factor = c(1, -1), WFA_factor = c(1, -1)))
Npas4.cFosm.WFA.aov <- car::Anova(Npas4.cFosm.WFA.lm, type=3)
print(Npas4.cFosm.WFA.aov)
```

```
## Anova Table (Type III tests)
```

```
##
```

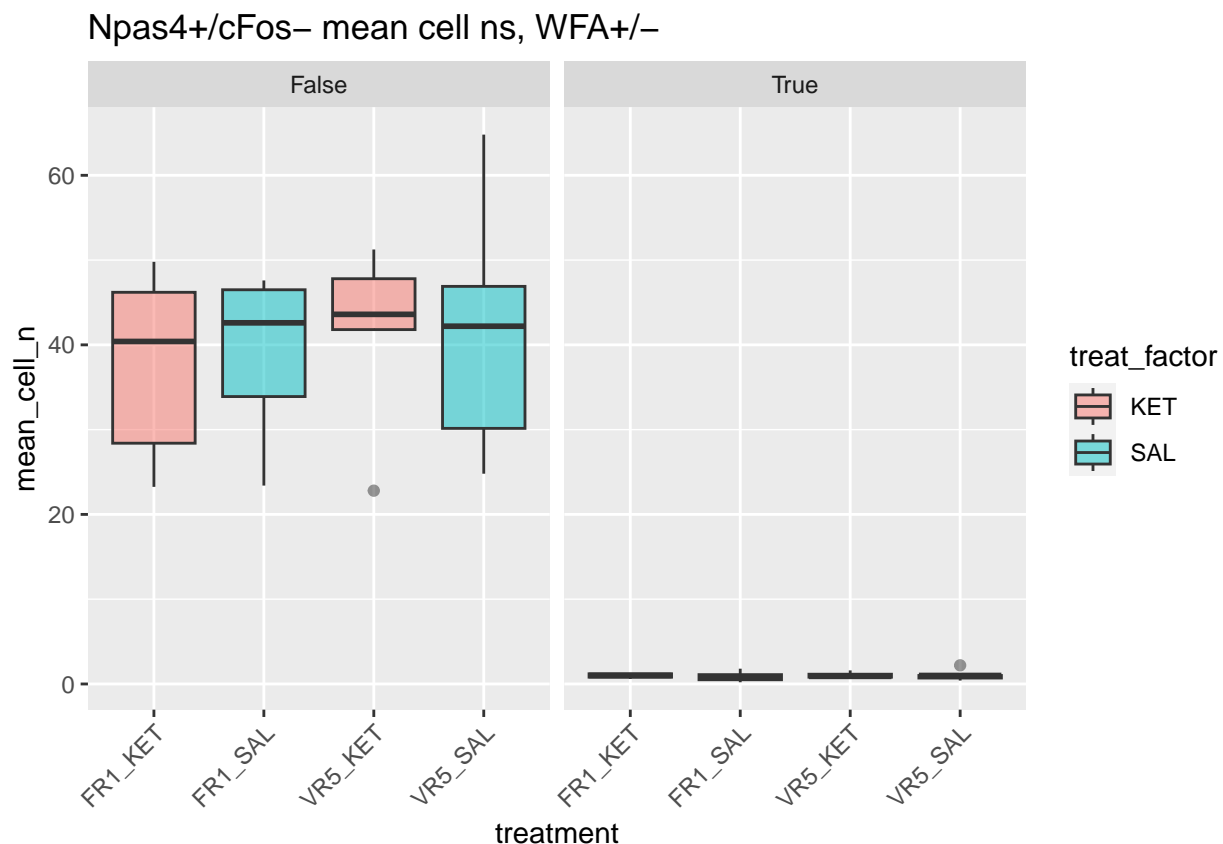
```
## Response: mean_cell_n
```

```
##
```

	Sum Sq	Df	F value	Pr(>F)
## (Intercept)	18220.1	1	250.7655	<2e-16 ***
## treat_factor	1.4	1	0.0195	0.8896

```
## react_factor                27.3  1  0.3753 0.5439
## WFA_factor                  16519.7  1 227.3633 <2e-16 ***
## treat_factor:react_factor    1.6  1  0.0214 0.8846
## treat_factor:WFA_factor      2.0  1  0.0272 0.8700
## react_factor:WFA_factor      23.0  1  0.3170 0.5768
## treat_factor:react_factor:WFA_factor  2.0  1  0.0272 0.8700
## Residuals                    2688.3 37
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
f <- ggplot(Npas4.cFosm.WFA, aes(x=treatment, y=mean_cell_n)) + geom_boxplot(aes(fill=treat_factor), al
#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('Npas4+/cFos- mean cell ns, WFA+/-')
f
```



```
Npas4.cFosm.WFap <- Npas4.cFosm.WFA[Npas4.cFosm.WFA$WFA_factor == 'True',]
Npas4.cFosm.WFam <- Npas4.cFosm.WFA[Npas4.cFosm.WFA$WFA_factor == 'False',]
```

```
eda_anova(Npas4.cFosm.WFap, quant=FALSE, qual=TRUE)
```

```
## Anova Table (Type III tests)
```

```
##
```

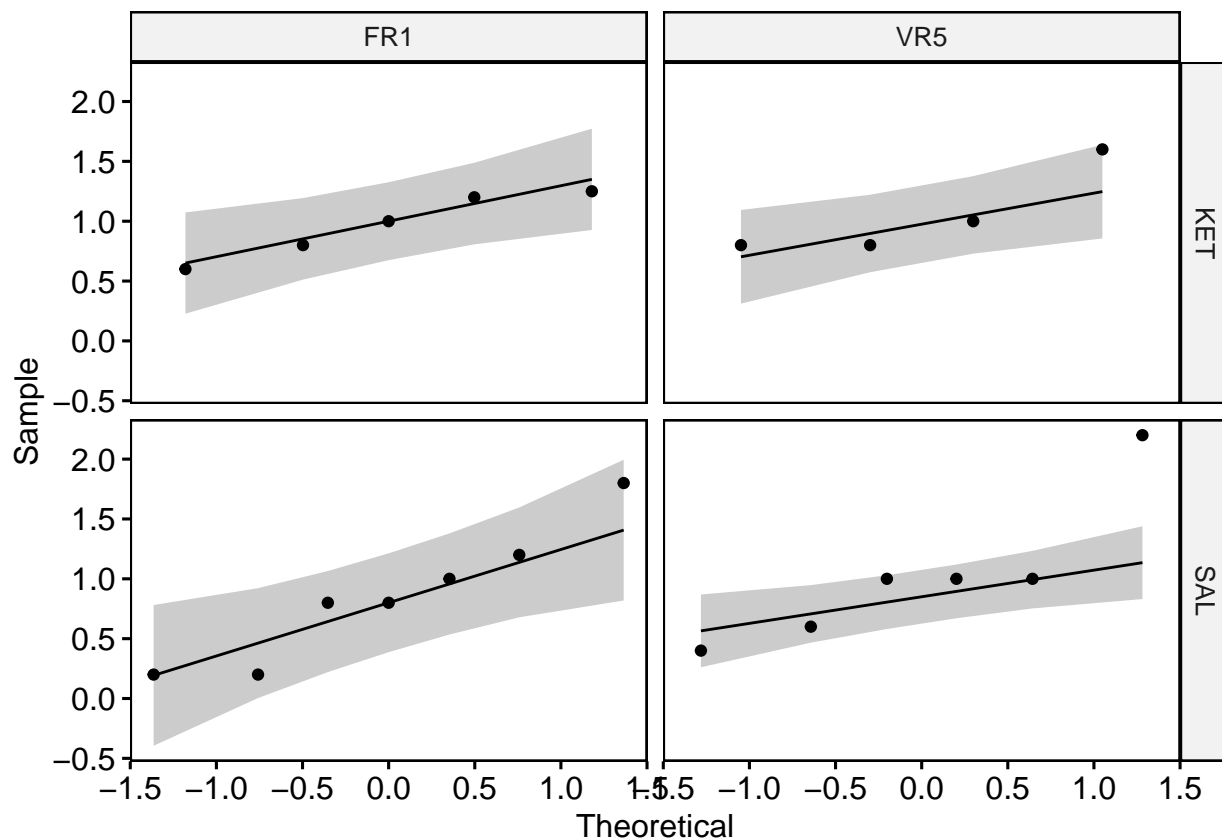
```
## Response: mean_cell_n
```

```
##
```

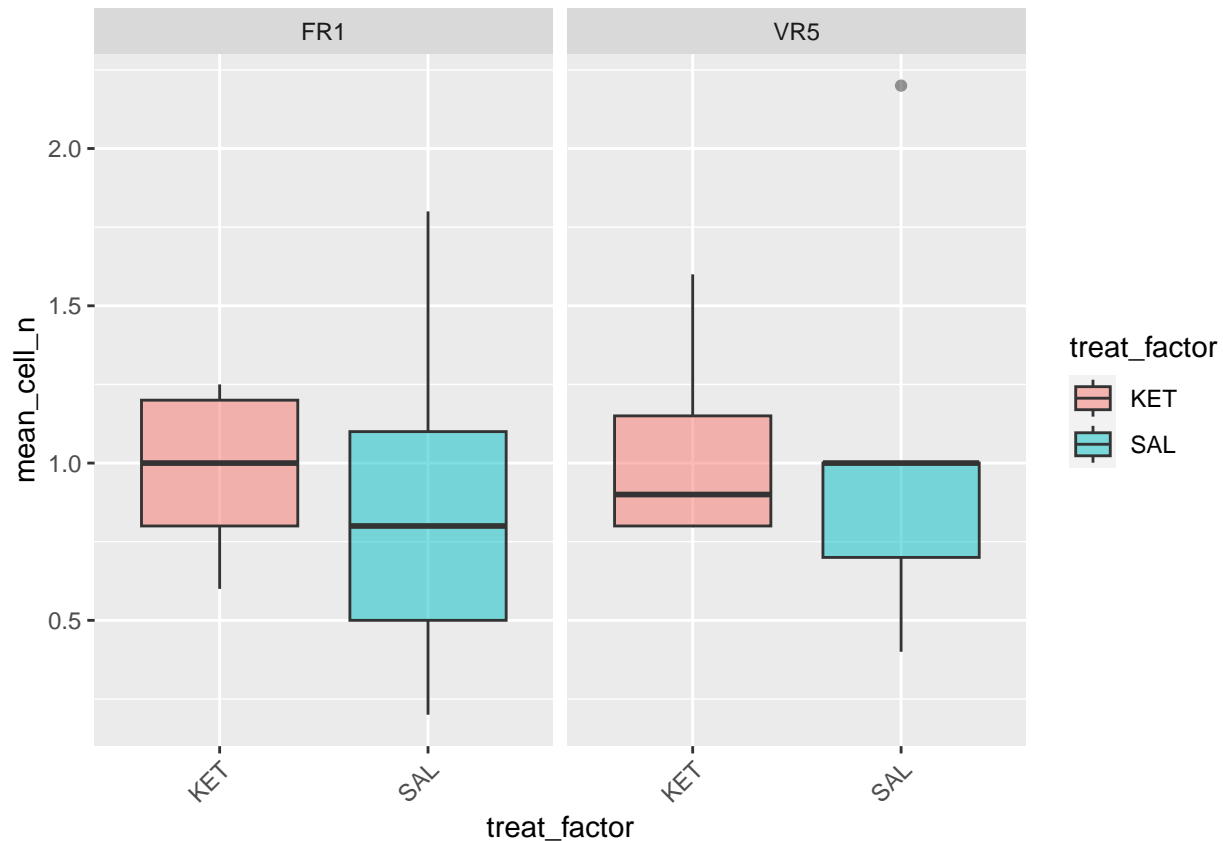
```
## (Intercept)                Sum Sq Df F value    Pr(>F)
```

```
## treat_factor                0.0221  1  0.0868    0.7716
```

```
## react_factor          0.0864  1  0.3397    0.5672
## treat_factor:react_factor 0.0122  1  0.0479    0.8292
## Residuals              4.5785 18
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## react_factor = FR1:
## contrast estimate      SE df t.ratio p.value adjusted_p.value
## KET - SAL    0.1129 0.295 18   0.382  0.7068          0.914
##
## react_factor = VR5:
## contrast estimate      SE df t.ratio p.value adjusted_p.value
## KET - SAL    0.0167 0.326 18   0.051  0.9597          0.998
##
## treat_factor = KET:
## contrast estimate      SE df t.ratio p.value adjusted_p.value
## FR1 - VR5   -0.080 0.338 18  -0.236  0.8157          0.966
##
## treat_factor = SAL:
## contrast estimate      SE df t.ratio p.value adjusted_p.value
## FR1 - VR5   -0.176 0.281 18  -0.628  0.5379          0.786
##
## [[1]]
```



```
##
## [[2]]
```

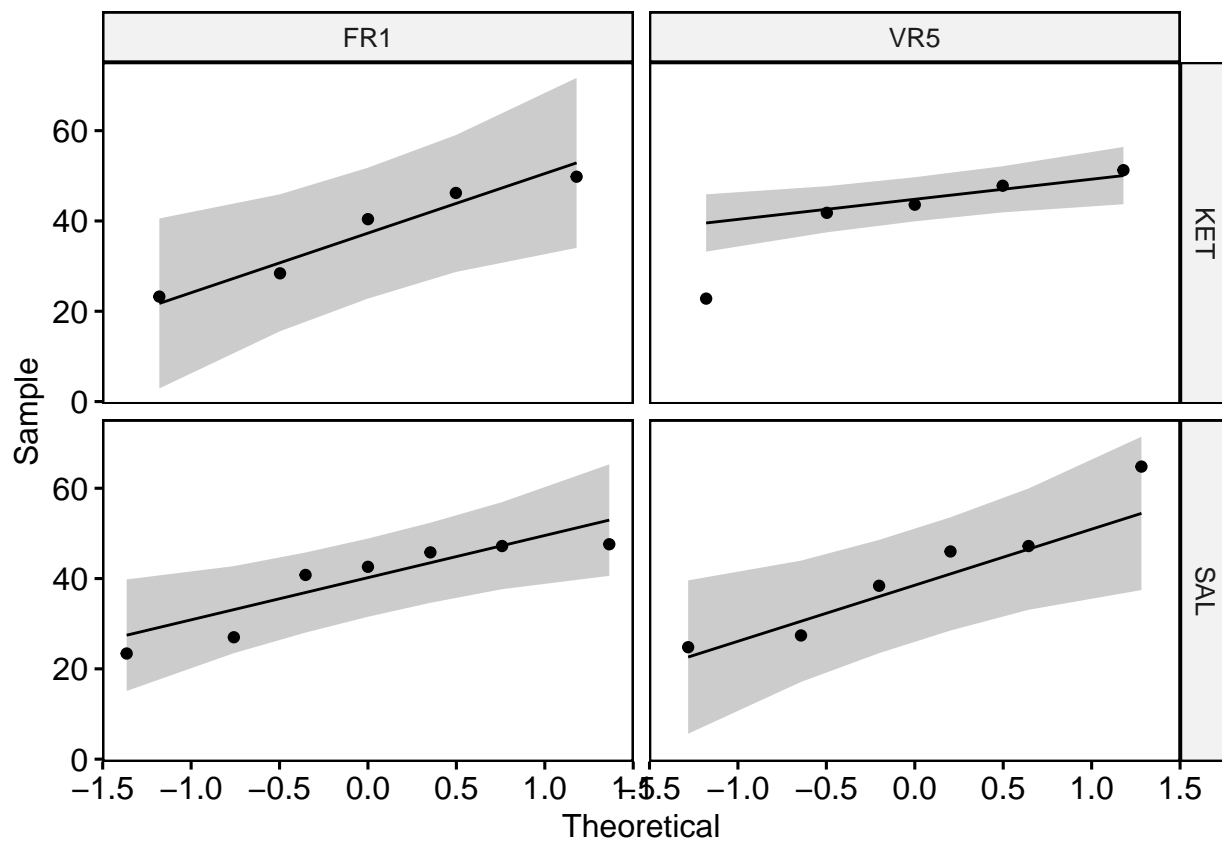


```
eda_anova(Npas4.cFosm.WFam, quant=FALSE, qual=TRUE)
```

```
## Anova Table (Type III tests)
##
## Response: mean_cell_n
##
##               Sum Sq Df F value    Pr(>F)
## (Intercept)      35942  1 254.4577 1.856e-12 ***
## treat_factor         3  1   0.0247   0.8768
## react_factor        52  1   0.3680   0.5513
## treat_factor:react_factor  4  1   0.0258   0.8742
## Residuals        2684 19
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## react_factor = FR1:
## contrast estimate SE df t.ratio p.value adjusted_p.value
## KET - SAL  -1.5900 6.96 19  -0.228  0.8217          0.968
##
## react_factor = VR5:
## contrast estimate SE df t.ratio p.value adjusted_p.value
## KET - SAL   0.0167 7.20 19   0.002  0.9982          1.000
##
## treat_factor = KET:
## contrast estimate SE df t.ratio p.value adjusted_p.value
## FR1 - VR5    -3.84 7.52 19  -0.511  0.6153          0.852
##
## treat_factor = SAL:
## contrast estimate SE df t.ratio p.value adjusted_p.value
```

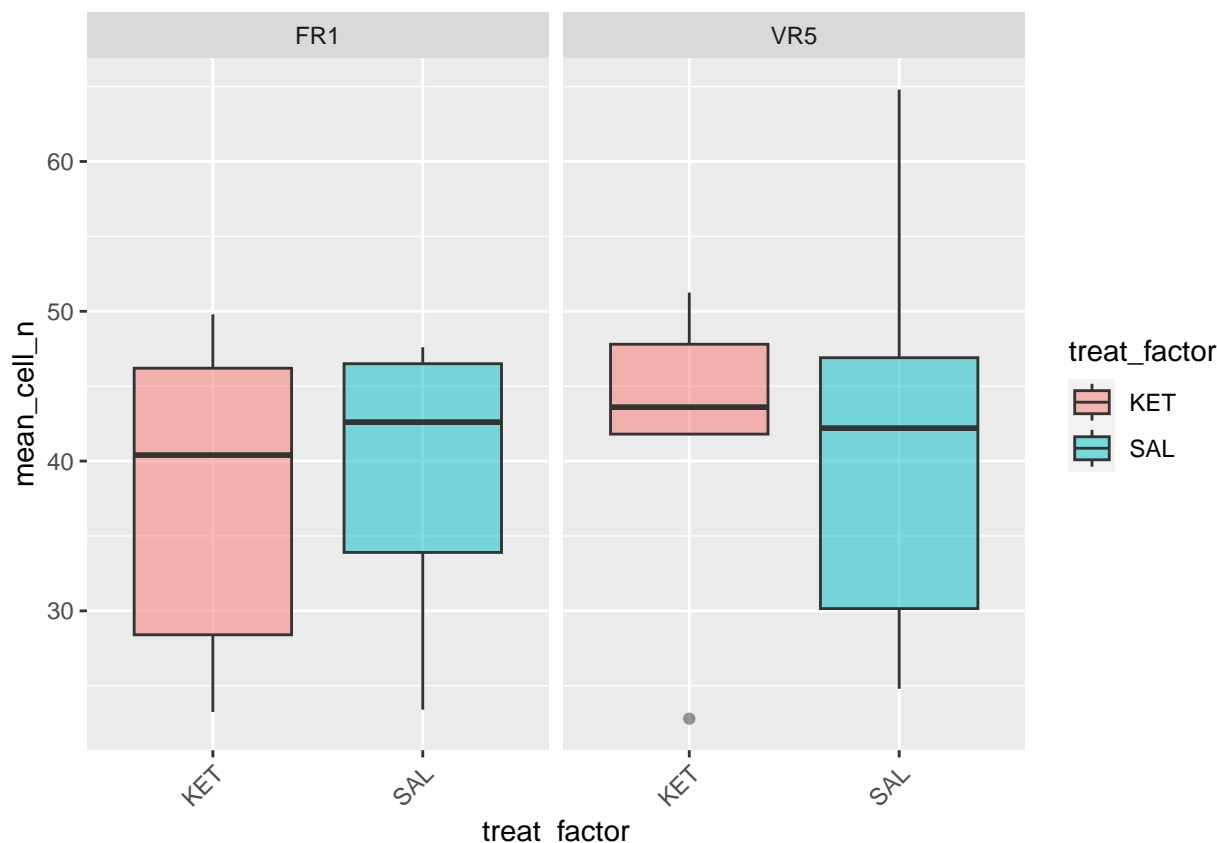
```
## FR1 - VR5 -2.23 6.61 19 -0.338 0.7392 0.932
```

```
## [[1]]
```



```
##
```

```
## [[2]]
```



cFos+/Npas4- with or without PNNs

Again, we see a main effect of WFA since the number of cFos cells without PNNs vastly outnumbers the number of cFos cells with PNNs. No other interesting interactions to report here.

```
# slice
cFos.Npas4m.WFam <- cFos.Npas4m.WFam[c('mean_cell_n', 'treatment', 'react', 'treat', 'WFA')]
cFos.Npas4m.WFap <- cFos.Npas4m.WFap[c('mean_cell_n', 'treatment', 'react', 'treat', 'WFA')]

# concat
cFos.Npas4m.WFA <- rbind(cFos.Npas4m.WFam, cFos.Npas4m.WFap)
```

```
# build dummy cols
cFos.Npas4m.WFA$react_factor <- as.factor(cFos.Npas4m.WFA$react)
cFos.Npas4m.WFA$treat_factor <- as.factor(cFos.Npas4m.WFA$treat)
cFos.Npas4m.WFA$treatment_factor <- as.factor(cFos.Npas4m.WFA$treatment)
cFos.Npas4m.WFA$WFA_factor <- as.factor(cFos.Npas4m.WFA$WFA)
str(cFos.Npas4m.WFA)
```

```
## 'data.frame':   46 obs. of  9 variables:
## $ mean_cell_n   : num  56.8 24 56.2 50.4 35.6 25.4 45 25.8 17.4 27.4 ...
## $ treatment     : chr   "FR1_KET" "FR1_KET" "FR1_KET" "FR1_KET" ...
## $ react         : chr   "FR1" "FR1" "FR1" "FR1" ...
## $ treat         : chr   "KET" "KET" "KET" "KET" ...
## $ WFA           : chr   "False" "False" "False" "False" ...
## $ 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 2 2 2 2 2 ...
```

```
## $ treatment_factor: Factor w/ 4 levels "FR1_KET","FR1_SAL",...: 1 1 1 1 1 2 2 2 2 2 ...
## $ WFA_factor      : Factor w/ 2 levels "False","True": 1 1 1 1 1 1 1 1 1 1 ...

# 3way ANOVA: reactivation x treatment x WFA (2 x 2 x 2) in cFos+/Npas4-
cFos.Npas4m.WFA.lm <- lm(mean_cell_n ~ treat_factor*react_factor*WFA_factor, contrasts = list(treat_fac
cFos.Npas4m.WFA.aov <- car::Anova(cFos.Npas4m.WFA.lm, type=3)
print(cFos.Npas4m.WFA.aov)

## Anova Table (Type III tests)
##
## Response: mean_cell_n
##
##               Sum Sq Df F value  Pr(>F)
## (Intercept)    15682.6  1 236.0135 < 2e-16 ***
## treat_factor      303.2  1   4.5622 0.03919 *
## react_factor      31.8  1   0.4778 0.49361
## WFA_factor     12973.9  1 195.2493 < 2e-16 ***
## treat_factor:react_factor    40.8  1   0.6138 0.43822
## treat_factor:WFA_factor     290.8  1   4.3765 0.04317 *
## react_factor:WFA_factor     54.1  1   0.8149 0.37237
## treat_factor:react_factor:WFA_factor    42.8  1   0.6443 0.42716
## Residuals          2525.0 38
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

f <- ggplot(cFos.Npas4m.WFA, aes(x=treatment, y=mean_cell_n)) + geom_boxplot(aes(fill=treat_factor), al
#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('cFos+/Npas4- mean cell ns, WFA+/-')
f
```