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Stats analyses of Yuki’s double-crossed mutants data

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> library(gsignal)

> library(ggplot2)

> library(gghighlight)

> library(ggsignif)

> library(gridExtra)

> library(ggpubr)

> library(plotrix)

> library(tidyverse)

> library(lubridate)

> library(lme4)

> library(lmerTest)

>

> #####

> # Functions

> #####

> checkTtests<-function(dT,p) {

+ # go through each freq and do t-tests on every combination in case the overall p-value is significant

+ # and you want to look freq by freq

+ xSteps=unique(dT$select2)

+ count1=0

+ count2=0

+ for (x in xSteps) {

+ # print(x)

+ dTsub<-filter(dT,select2==x)

+ wt<-dTsub$select3[dTsub$select1==genotypes[1]]

+ vGlut<-dTsub$select3[dTsub$select1==genotypes[3]]

+ double<-dTsub$select3[dTsub$select1==genotypes[4]]

+ if (sum(!is.nan(vGlut))>3&sum(!is.nan(double))>3){

+ tFit1<-t.test(vGlut,double,paired = FALSE,na.rm=TRUE)

+ if (tFit1$p.value<0.05){

+ print(sprintf('x axis with a significant p-value vGLut vs double: %3.1f',x))

+ print(tFit1)

+ if (sum(!is.nan(wt))>3) {

+ tFit2<-t.test(wt,double,paired = FALSE,na.rm=TRUE)

+ print('control:wt vs double')

+ print(tFit2)

+ }

+ count1=count1+1

+ }

+ }

+ }

+ print(sprintf('Number of x axis steps with a significant p-value: %2.0f',count1))

+ }

>

> #####

> # Load data and clean it

> #####

> v1 <- tibble(read.csv('v1.txt'))

> v2 <- tibble(read.csv('v2.txt'))

> v3 <- tibble(read.csv('v3.txt'))

> v4 <- tibble(read.csv('v4.txt'))

>

> genotypes = c('WT','Alpha9KO','VGLUT3KO','VGLUT3KOAlpha9KO')

>

> v1$experiment<-factor(v1$experiment)

> v1$genotype<-factor(v1$genotype,levels=genotypes)

> v1$freq<-v1$freq/1000

> v1$phi<-v1$phi\*2\*pi

> v2$experiment<-factor(v2$experiment)

> v2$genotype<-factor(v2$genotype,levels=genotypes)

> v2$freq<-v2$freq/1000

> v3$experiment<-factor(v3$experiment)

> v3$genotype<-factor(v3$genotype,levels=genotypes)

> v4$experiment<-factor(v4$experiment)

>

> ###

> # create table that fits the organization of the Linear Mixed-Effects Example.R function

> # and then call it to analyze the data statistically

> ###

> print('')

[1] ""

> print('')

[1] ""

> print('')

[1] ""

> degree=3

>

> # gain vs genotype

> dTable<-rename(v2,id=experiment, cohort=genotype)

> dTable<-filter(dTable,freq<13)

> dTable<-filter(dTable,freq>8)

> filename='Yuki gain vs genotype.pdf'

> fit<-lmer(gain ~ cohort \* stats::poly(freq,degree) + (1|id), data=dTable)

> fit\_anova<-anova(fit)

> #print(fit\_anova)

> print(sprintf('p-value: %5.5f',last(fit\_anova$`Pr(>F)`)))

[1] "p-value: 0.01977"

> if (last(fit\_anova$`Pr(>F)`)<0.05) {

+ dT=select(dTable,select=c(cohort,freq,gain))

+ p=FALSE

+ checkTtests(dT,p)

+ }

[1] "x axis with a significant p-value vGLut vs double: 9.0"

Welch Two Sample t-test

data: vGlut and double

t = 2.283, df = 14.744, p-value = 0.03771

alternative hypothesis: true difference in means is not equal to 0

95 percent confidence interval:

0.5279586 15.7202958

sample estimates:

mean of x mean of y

24.39111 16.26699

[1] "control:wt vs double"

Welch Two Sample t-test

data: wt and double

t = 0.16128, df = 13.075, p-value = 0.8743

alternative hypothesis: true difference in means is not equal to 0

95 percent confidence interval:

-6.412032 7.447304

sample estimates:

mean of x mean of y

16.78462 16.26699

[1] "x axis with a significant p-value vGLut vs double: 9.5"

Welch Two Sample t-test

data: vGlut and double

t = 2.4179, df = 11.739, p-value = 0.03286

alternative hypothesis: true difference in means is not equal to 0

95 percent confidence interval:

0.728738 14.352121

sample estimates:

mean of x mean of y

27.71905 20.17862

[1] "control:wt vs double"

Welch Two Sample t-test

data: wt and double

t = -0.0042477, df = 13.378, p-value = 0.9967

alternative hypothesis: true difference in means is not equal to 0

95 percent confidence interval:

-7.134915 7.106832

sample estimates:

mean of x mean of y

20.16458 20.17862

[1] "Number of x axis steps with a significant p-value: 2"

>

> # BF and Q10dB vs genotype

> dTable<-rename(v3,id=experiment, cohort=genotype)

> filename='Yuki BF vs genotype.pdf'

> fit<-lmer(bf ~ cohort \* stats::poly(level,degree) + (1|id), data=dTable)

> fit\_anova<-anova(fit)

> #print(fit\_anova)

> print(sprintf('p-value: %5.5f',last(fit\_anova$`Pr(>F)`)))

[1] "p-value: 0.82159"

> if (last(fit\_anova$`Pr(>F)`)<0.05) {

+ dT=select(dTable,select=c(cohort,level,bf))

+ p=FALSE

+ checkTtests(dT,p)

+ }

>

> filename='Yuki Q10db vs genotype.pdf'

> fit<-lmer(q ~ cohort \* stats::poly(level,degree) + (1|id), data=dTable)

> fit\_anova<-anova(fit)

> #print(fit\_anova)

> print(sprintf('p-value: %5.5f',last(fit\_anova$`Pr(>F)`)))

[1] "p-value: 0.85521"

> if (last(fit\_anova$`Pr(>F)`)<0.05) {

+ dT=select(dTable,select=c(cohort,level,q))

+ p=FALSE

+ checkTtests(dT,p)

+ }

>

> # calculate anova for figure parts D,E,F

> filename='Yuki maxGain vs genotype.pdf'

> dTab\_controls<-filter(v4,(genotype==genotypes[1])|(genotype==genotypes[2])|(genotype==genotypes[4]))

> fit<-aov(maxGain~genotype, data=v4)

> #print(summary(fit))

> x<-summary(fit)

> print(sprintf('p-value: %5.5f',first(x[[1]]$`Pr(>F)`)))

[1] "p-value: 0.01127"

> if (first(x[[1]]$`Pr(>F)`)<0.05) {

+ vGlut<-v4$maxGain[v4$genotype==genotypes[3]]

+ double<-v4$maxGain[v4$genotype==genotypes[4]]

+

+ tFit1<-t.test(vGlut,double,paired = FALSE,na.rm=TRUE)

+ print(tFit1)

+ tFit2<-aov(maxGain~genotype, data=dTab\_controls)

+ print(summary(tFit2))

+ }

Welch Two Sample t-test

data: vGlut and double

t = 2.4755, df = 14.667, p-value = 0.02603

alternative hypothesis: true difference in means is not equal to 0

95 percent confidence interval:

1.435699 19.482031

sample estimates:

mean of x mean of y

53.58632 43.12745

Df Sum Sq Mean Sq F value Pr(>F)

genotype 2 100.2 50.11 0.721 0.497

Residuals 24 1668.8 69.53

>

> filename='Yuki sensCF vs genotype.pdf'

> fit<-aov(sensCF~genotype, data=v4)

> #print(summary(fit))

> x<-summary(fit)

> print(sprintf('p-value: %5.5f',first(x[[1]]$`Pr(>F)`)))

[1] "p-value: 0.00132"

> if (first(x[[1]]$`Pr(>F)`)<0.05) {

+ vGlut<-v4$sensCF[v4$genotype==genotypes[3]]

+ double<-v4$sensCF[v4$genotype==genotypes[4]]

+

+ tFit1<-t.test(vGlut,double,paired = FALSE,na.rm=TRUE)

+ print(tFit1)

+ tFit2<-aov(sensCF~genotype, data=dTab\_controls)

+ print(summary(tFit2))

+ }

Welch Two Sample t-test

data: vGlut and double

t = 3.4225, df = 14.716, p-value = 0.003871

alternative hypothesis: true difference in means is not equal to 0

95 percent confidence interval:

4.419217 19.076435

sample estimates:

mean of x mean of y

85.92526 74.17743

Df Sum Sq Mean Sq F value Pr(>F)

genotype 2 23.2 11.60 0.31 0.736

Residuals 24 897.8 37.41

>

> filename='Yuki sens5k vs genotype.pdf'

> fit<-aov(sens5k~genotype, data=v4)

> #print(summary(fit))

> x<-summary(fit)

> print(sprintf('p-value: %5.5f',first(x[[1]]$`Pr(>F)`)))

[1] "p-value: 0.60528"

> if (first(x[[1]]$`Pr(>F)`)<0.05) {

+ vGlut<-v4$sens5k[v4$genotype==genotypes[3]]

+ double<-v4$sens5k[v4$genotype==genotypes[4]]

+

+ tFit1<-t.test(vGlut,double,paired = FALSE,na.rm=TRUE)

+ print(tFit1)

+ tFit2<-aov(sens5k~genotype, data=dTab\_controls)

+ print(summary(tFit2))

+ }

>

>

>

>

>

> # tuning curves vs genotype

> degree=3

> dTab<-rename(v1,id=experiment, cohort=genotype,phase=phi)

> dTab<-filter(dTab,freq<10)

> dTab<-filter(dTab,freq>4)

> dTab<-filter(dTab,level>5)

> dTable<-filter(dTab,(cohort==genotypes[3])|(cohort==genotypes[4]))

> filename='YukiTC Vglut3 vs doubles.pdf'

> compareTuningCurves(dTable,filename,degree)

[1] "Mag:Number of frequencies with a significant p-value: 3"

[1] "Phase:Number of frequencies with a significant p-value: 1"

[1] "Magnitude data cohort comparison p-value: 0.00001"

[1] "Phase data cohort comparison p-value: 0.06419"

[1] ""

Warning messages:

1: Some predictor variables are on very different scales: consider rescaling

2: Some predictor variables are on very different scales: consider rescaling

3: Removed 4 rows containing missing values (`geom\_point()`).

4: Removed 10 rows containing missing values (`geom\_line()`).

5: Removed 19 rows containing missing values (`geom\_point()`).

6: Some predictor variables are on very different scales: consider rescaling

7: Some predictor variables are on very different scales: consider rescaling

8: Removed 9 rows containing missing values (`geom\_point()`).

>

> dTable<-filter(dTab,(cohort==genotypes[1])|(cohort==genotypes[2])|(cohort==genotypes[4]))

> filename='YukiTC wt vs alpha9 vs doubles.pdf'

> compareTuningCurves(dTable,filename,degree)

[1] "Mag:Number of frequencies with a significant p-value: 1"

[1] "Phase:Number of frequencies with a significant p-value: 7"

[1] "Magnitude data cohort comparison p-value: 0.37195"

[1] "Phase data cohort comparison p-value: 0.00006"

[1] ""

Warning messages:

1: Some predictor variables are on very different scales: consider rescaling

2: Some predictor variables are on very different scales: consider rescaling

3: Removed 9 rows containing missing values (`geom\_point()`).

4: Removed 16 rows containing missing values (`geom\_line()`).

5: Removed 35 rows containing missing values (`geom\_point()`).

6: Some predictor variables are on very different scales: consider rescaling

7: Some predictor variables are on very different scales: consider rescaling

8: Removed 6 rows containing missing values (`geom\_point()`).

>

> # dTable<-filter(dTab,(cohort==genotypes[1])|(cohort==genotypes[4]))

> # filename='YukiTC wt vs doubles.pdf'

> # compareTuningCurves(dTable,filename,degree)

> #

> # dTable<-filter(dTab,(cohort==genotypes[2])|(cohort==genotypes[4]))

> # filename='YukiTC alpha9 vs doubles.pdf'

> # compareTuningCurves(dTable,filename,degree)