Supplementary text: Materials and Methods - Statistical analyses for JHM treatments (A case of insect female neoteny: E93 expression and links to the juvenile hormone in mealybugs)

Isabelle Vea

September 28, 2017

# Summary

You will find here all the analyses related to the statistics of hormonal treatments carried out in Vea et al. (submitted to Journal of Insect Biochemistry and Molecular Biology).

setwd("/Users/isabelle/Dropbox/\_Github\_reps/E93/")  
library(ggplot2)  
library(plyr)  
library(tidyr)

# Effect of pyriproxyfen treatment

## Data

#loading file JHM treatment  
#females  
dtf<-read.csv(file="E93-Pyr-N3D0.csv",header = TRUE)  
names(dtf)

## [1] "cDNA.." "Compound" "treatment.stage"  
## [4] "sex" "Day" "DayL"   
## [7] "N" "rpL32" "Kr.h1"   
## [10] "E93" "PkE93.1" "PkE93.2"   
## [13] "PkE93.3" "br.1" "br.2"   
## [16] "br.3"

head(dtf)

## cDNA.. Compound treatment.stage sex Day DayL N rpL32 Kr.h1  
## 1 203 Control N3D0 female 1 A 1 0.00281 4.22e-05  
## 2 204 Control N3D0 female 1 A 1 0.00405 6.70e-05  
## 3 205 Control N3D0 female 1 A 1 0.01160 9.58e-05  
## 4 206 Control N3D0 female 1 A 1 0.00519 7.69e-05  
## 5 207 Mimic N3D0 female 1 A 1 0.00281 9.39e-05  
## 6 209 Mimic N3D0 female 1 A 1 0.00161 3.51e-06  
## E93 PkE93.1 PkE93.2 PkE93.3 br.1 br.2 br.3  
## 1 9.75e-06 1.77e-06 3.33e-07 6.94e-08 1.29e-06 3.91e-05 6.71e-05  
## 2 1.81e-05 1.85e-06 3.36e-07 9.38e-08 1.01e-06 3.33e-05 2.80e-05  
## 3 3.81e-05 5.16e-06 1.08e-06 1.44e-07 5.26e-06 8.94e-05 1.29e-04  
## 4 2.78e-05 3.62e-06 5.92e-07 1.67e-07 4.03e-06 3.94e-05 9.86e-05  
## 5 4.46e-05 1.59e-05 1.08e-06 2.39e-08 2.19e-06 1.28e-05 8.69e-05  
## 6 4.14e-06 5.95e-07 1.22e-07 1.00e-07 6.68e-08 5.51e-06 4.13e-06

dtf<-gather(dtf,Gene,SDM,9:16)  
#dtf  
head(dtf)

## cDNA.. Compound treatment.stage sex Day DayL N rpL32 Gene SDM  
## 1 203 Control N3D0 female 1 A 1 0.00281 Kr.h1 4.22e-05  
## 2 204 Control N3D0 female 1 A 1 0.00405 Kr.h1 6.70e-05  
## 3 205 Control N3D0 female 1 A 1 0.01160 Kr.h1 9.58e-05  
## 4 206 Control N3D0 female 1 A 1 0.00519 Kr.h1 7.69e-05  
## 5 207 Mimic N3D0 female 1 A 1 0.00281 Kr.h1 9.39e-05  
## 6 209 Mimic N3D0 female 1 A 1 0.00161 Kr.h1 3.51e-06

#males  
dtm<-read.csv(file="E93-Pyr-male.csv",header = TRUE)  
names(dtm)

## [1] "Sample.ID" "treatment" "Day" "DayL" "Gene" "SDM.Gene"   
## [7] "SDM.rpL32"

head(dtm)

## Sample.ID treatment Day DayL Gene SDM.Gene SDM.rpL32  
## 1 299 Control 1 A Kr-h1 3.91e-05 0.00116  
## 2 300 Control 2 B Kr-h1 1.05e-05 0.00148  
## 3 301 Control 3 C Kr-h1 2.82e-06 0.00221  
## 4 302 Control 4 D Kr-h1 3.03e-06 0.00206  
## 5 303 Control 4 D Kr-h1 7.78e-06 0.00238  
## 6 304 Mimic 4 D Kr-h1 1.50e-04 0.00197

## Checking for data distribution

#female dataset subsetting to keep Kr-h1 and E93 1 and 2 subset from D4 to D8  
dtf<-subset(dtf, Gene=="Kr.h1" | Gene=="PkE93.1"| Gene=="PkE93.2"| Gene=="PkE93.3")  
dtf<-subset(dtf, Day == 4 |Day == 5 |Day == 6 |Day == 7 |Day == 8 )  
dtf$Gene

## [1] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [8] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [15] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [22] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [29] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [36] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [43] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [50] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [57] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [64] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [71] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [78] "PkE93.1" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [85] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [92] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [99] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [106] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [113] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.3" "PkE93.3"  
## [120] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [127] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [134] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [141] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [148] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [155] "PkE93.3" "PkE93.3"

dtf$Day

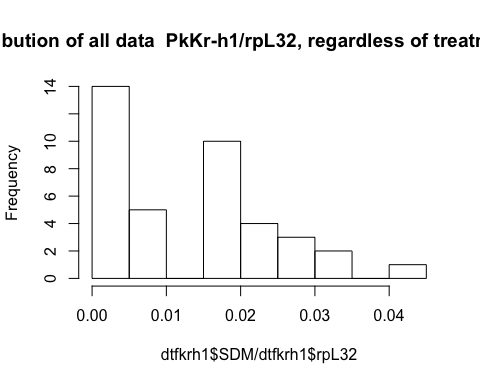
## [1] 4 4 4 4 4 4 5 5 5 5 5 5 6 6 6 6 6 6 7 7 7 7 7 7 8 8 8 8 8 8 8 8 8 8 8  
## [36] 8 8 8 8 4 4 4 4 4 4 5 5 5 5 5 5 6 6 6 6 6 6 7 7 7 7 7 7 8 8 8 8 8 8 8  
## [71] 8 8 8 8 8 8 8 8 4 4 4 4 4 4 5 5 5 5 5 5 6 6 6 6 6 6 7 7 7 7 7 7 8 8 8  
## [106] 8 8 8 8 8 8 8 8 8 8 8 8 4 4 4 4 4 4 5 5 5 5 5 5 6 6 6 6 6 6 7 7 7 7 7  
## [141] 7 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8

### Female dataset for PkKr-h1

#all female dataset Kr-h1  
dtfkrh1<-subset(dtf, Gene=="Kr.h1")  
dtfkrh1$Gene

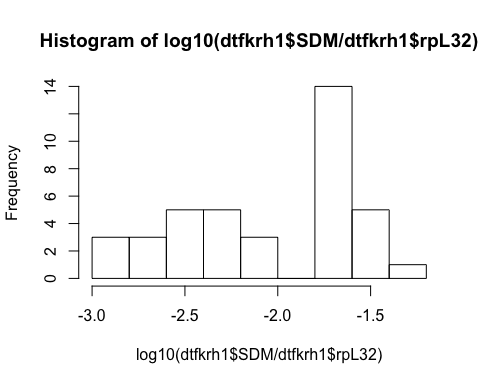
## [1] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"  
## [9] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"  
## [17] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"  
## [25] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"  
## [33] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"

hist(dtfkrh1$SDM/dtfkrh1$rpL32, main="Distribution of all data PkKr-h1/rpL32, regardless of treatment type")#not normal



The distribution of qPCR data collected for relative expression of PkKr-h1 is nor normal. We decided to transform the data to log10.

#log transformed  
hist(log10(dtfkrh1$SDM/dtfkrh1$rpL32)) #more or less normal



We also checked data distribution by treatment

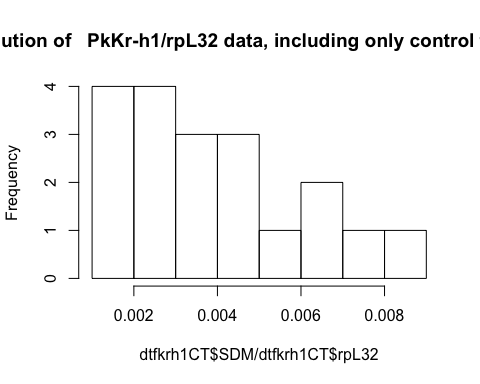
#by treatment  
dtfkrh1CT<-subset(dtfkrh1,Compound=="Control")  
dtfkrh1CT$Compound

## [1] Control Control Control Control Control Control Control Control  
## [9] Control Control Control Control Control Control Control Control  
## [17] Control Control Control  
## Levels: Control Mimic

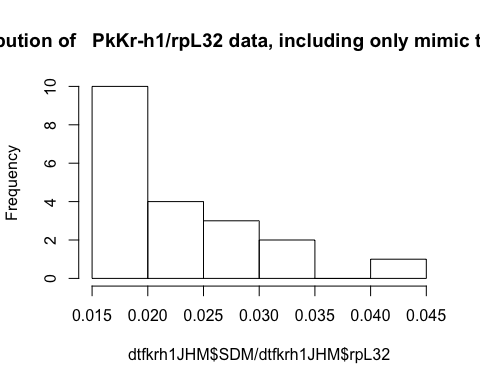
dtfkrh1JHM<-subset(dtfkrh1,Compound=="Mimic")  
dtfkrh1JHM$Compound

## [1] Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic  
## [12] Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic  
## Levels: Control Mimic

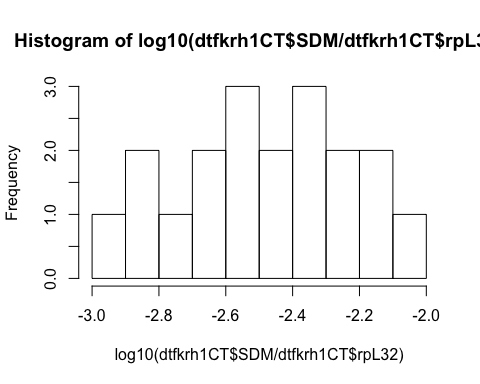
hist(dtfkrh1CT$SDM/dtfkrh1CT$rpL32, main="Distribution of PkKr-h1/rpL32 data, including only control treatments")



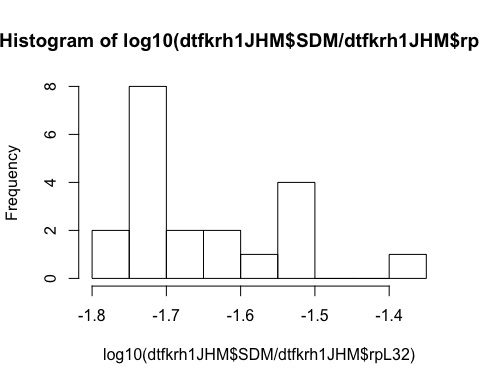
hist(dtfkrh1JHM$SDM/dtfkrh1JHM$rpL32, main="Distribution of PkKr-h1/rpL32 data, including only mimic treatments")



#log transformed  
hist(log10(dtfkrh1CT$SDM/dtfkrh1CT$rpL32)) #ok



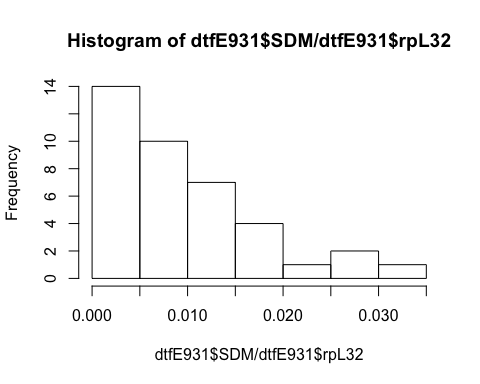
hist(log10(dtfkrh1JHM$SDM/dtfkrh1JHM$rpL32)) #skewed normal



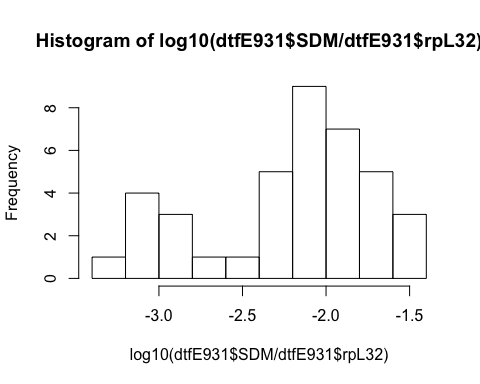
#all female dataset E93 1  
dtfE931<-subset(dtf, Gene=="PkE93.1")  
dtfE931$Gene

## [1] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [8] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [15] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [22] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [29] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [36] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"

hist(dtfE931$SDM/dtfE931$rpL32) #distribution not normal



#log transformed  
hist(log10(dtfE931$SDM/dtfE931$rpL32) )



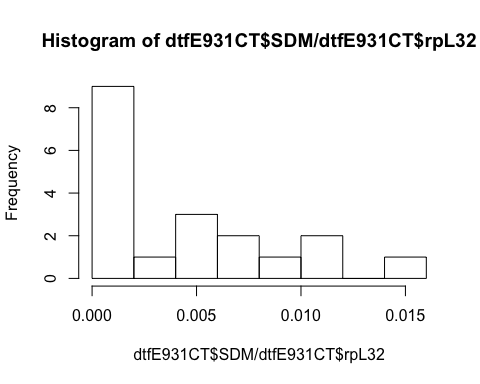
#by treatment  
dtfE931CT<-subset(dtfE931,Compound=="Control")  
dtfE931CT$Compound

## [1] Control Control Control Control Control Control Control Control  
## [9] Control Control Control Control Control Control Control Control  
## [17] Control Control Control  
## Levels: Control Mimic

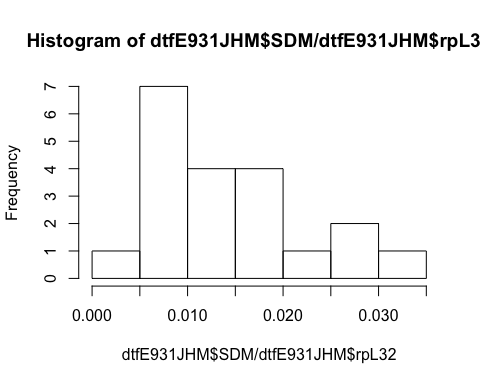
dtfE931JHM<-subset(dtfE931,Compound=="Mimic")  
dtfE931JHM$Compound

## [1] Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic  
## [12] Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic  
## Levels: Control Mimic

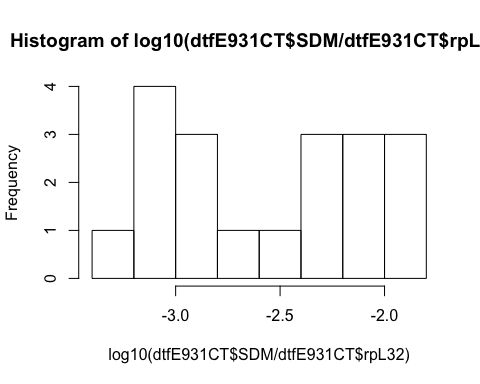
hist(dtfE931CT$SDM/dtfE931CT$rpL32) #not normal



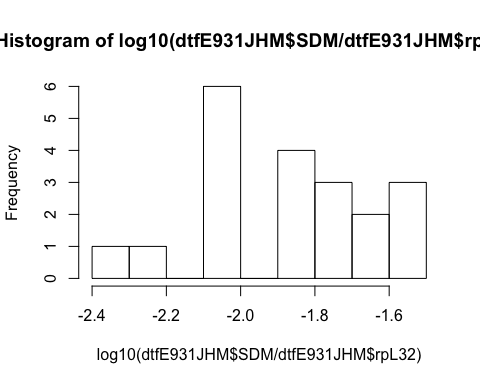
hist(dtfE931JHM$SDM/dtfE931JHM$rpL32)



#log transformed  
hist(log10(dtfE931CT$SDM/dtfE931CT$rpL32)) #two peaks



hist(log10(dtfE931JHM$SDM/dtfE931JHM$rpL32)) #weird!



#male dataset subset Krh1 and E93 1  
head(dtm)

## Sample.ID treatment Day DayL Gene SDM.Gene SDM.rpL32  
## 1 299 Control 1 A Kr-h1 3.91e-05 0.00116  
## 2 300 Control 2 B Kr-h1 1.05e-05 0.00148  
## 3 301 Control 3 C Kr-h1 2.82e-06 0.00221  
## 4 302 Control 4 D Kr-h1 3.03e-06 0.00206  
## 5 303 Control 4 D Kr-h1 7.78e-06 0.00238  
## 6 304 Mimic 4 D Kr-h1 1.50e-04 0.00197

#subsetting Kr-h1 and E93 1  
dtm<-subset(dtm, Gene=="Kr-h1" | Gene=="PkE93-1"| Gene=="PkE93-2"| Gene=="PkE93-3")  
dtm$Gene

## [1] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1   
## [9] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1   
## [17] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1   
## [25] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1   
## [33] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1   
## [41] Kr-h1 Kr-h1 Kr-h1 Kr-h1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [49] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [57] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [65] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [73] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [81] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [89] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## [97] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## [105] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## [113] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## [121] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## [129] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## [137] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## [145] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## [153] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## [161] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## [169] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## Levels: Kr-h1 PkE93-1 PkE93-2 PkE93-3

#subsetting from D3 to D6  
dtm<-subset(dtm, Day == 3| Day == 4| Day == 5| Day == 6)  
dtm$Day

## [1] 3 4 4 4 4 6 5 5 5 5 5 5 5 5 5 5 5 5 4 4 6 6 6 4 4 6 6 3 3 3 3 3 3 4 4  
## [36] 4 4 6 5 5 5 5 5 5 5 5 5 5 5 5 4 4 6 6 6 4 4 6 6 3 3 3 3 3 3 4 4 4 4 6  
## [71] 5 5 5 5 5 5 5 5 5 5 5 5 4 4 6 6 6 4 4 6 6 3 3 3 3 3 3 4 4 4 4 6 5 5 5  
## [106] 5 5 5 5 5 5 5 5 5 4 4 6 6 6 4 4 6 6 3 3 3 3 3

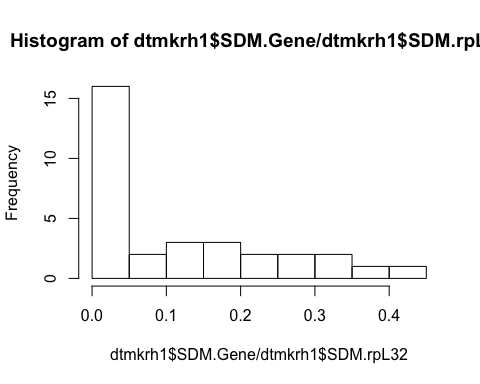
#all male krh1  
dtmkrh1<-subset(dtm, Gene=="Kr-h1")  
dtmkrh1$Gene

## [1] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1  
## [12] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1  
## [23] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1  
## Levels: Kr-h1 PkE93-1 PkE93-2 PkE93-3

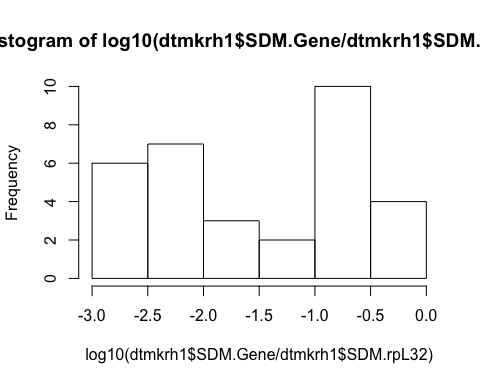
names(dtmkrh1)

## [1] "Sample.ID" "treatment" "Day" "DayL" "Gene" "SDM.Gene"   
## [7] "SDM.rpL32"

hist(dtmkrh1$SDM.Gene/dtmkrh1$SDM.rpL32) #not normally distributed



#log transformed  
hist(log10(dtmkrh1$SDM.Gene/dtmkrh1$SDM.rpL32)) #not normally distributed



#male Krh1 by treatment  
#by treatment  
dtmkrh1CT<-subset(dtmkrh1,treatment=="Control")  
dtmkrh1CT$treatment

## [1] Control Control Control Control Control Control Control Control  
## [9] Control Control Control Control Control Control Control Control  
## Levels: Control Mimic

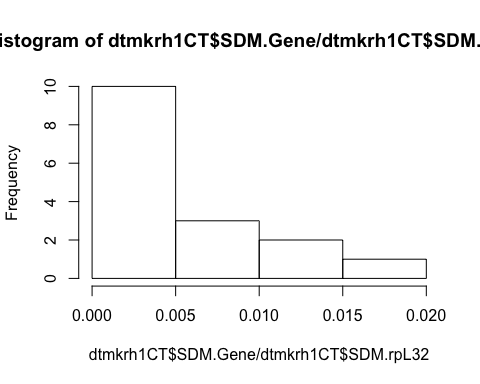
dtmkrh1JHM<-subset(dtmkrh1,treatment=="Mimic")  
dtmkrh1JHM$treatment

## [1] Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic Mimic  
## [12] Mimic Mimic Mimic Mimic Mimic  
## Levels: Control Mimic

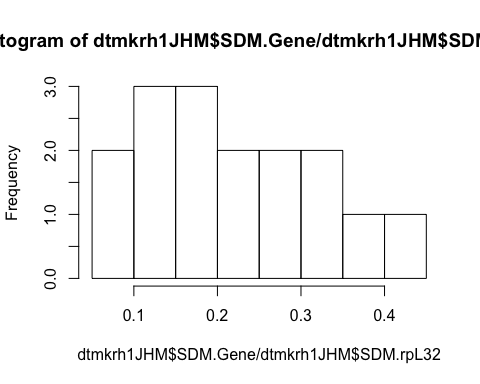
names(dtmkrh1CT)

## [1] "Sample.ID" "treatment" "Day" "DayL" "Gene" "SDM.Gene"   
## [7] "SDM.rpL32"

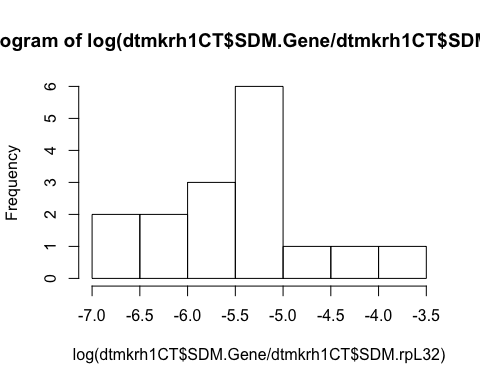
hist(dtmkrh1CT$SDM.Gene/dtmkrh1CT$SDM.rpL32)



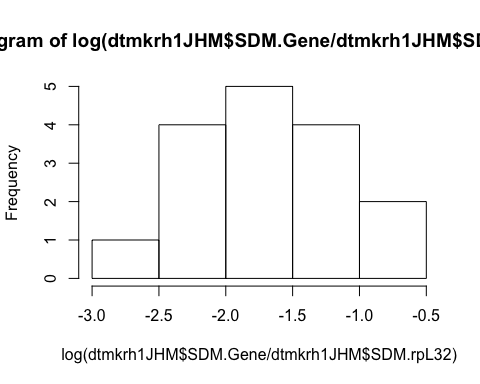
hist(dtmkrh1JHM$SDM.Gene/dtmkrh1JHM$SDM.rpL32)



#log transformed  
hist(log(dtmkrh1CT$SDM.Gene/dtmkrh1CT$SDM.rpL32)) #ok



hist(log(dtmkrh1JHM$SDM.Gene/dtmkrh1JHM$SDM.rpL32)) #ok

 We decided to use a log10 transformation of our absolute values normalized by the reference gene because the histograms did not show a normal distribution.

# statistical tests: general effect of JHM

## male on Kr-h1 expression by treatment and by day

par(mfrow=c(2,2))  
#dtm  
dtkrh1<-subset(dtm,Gene=="Kr-h1") #subsetting the dataset to have only Kr-h1  
dtkrh1$Gene

## [1] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1  
## [12] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1  
## [23] Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1 Kr-h1  
## Levels: Kr-h1 PkE93-1 PkE93-2 PkE93-3

is.numeric(dtkrh1$Day)

## [1] TRUE

dtkrh1$treatment

## [1] Control Control Control Mimic Mimic Control Control Control  
## [9] Control Control Control Control Mimic Mimic Mimic Mimic   
## [17] Mimic Mimic Control Control Mimic Mimic Mimic Mimic   
## [25] Mimic Control Control Control Control Mimic Mimic Mimic   
## Levels: Control Mimic

names(dtkrh1)

## [1] "Sample.ID" "treatment" "Day" "DayL" "Gene" "SDM.Gene"   
## [7] "SDM.rpL32"

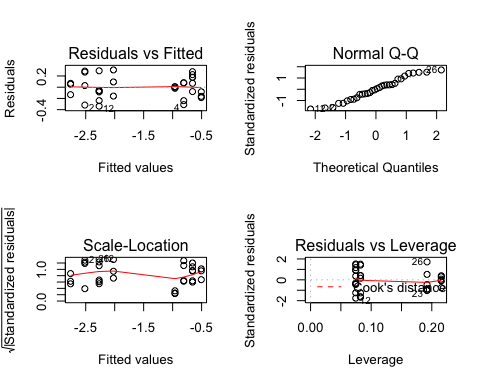
#fitting linear model on transformed data  
malelmkrh1<-lm(log10((dtkrh1$SDM.Gene/dtkrh1$SDM.rpL32))~dtkrh1$treatment+dtkrh1$Day+dtkrh1$treatment:dtkrh1$Day)  
summary.lm(malelmkrh1) #there is no effect of interaction between treatment type and day after treatment

##   
## Call:  
## lm(formula = log10((dtkrh1$SDM.Gene/dtkrh1$SDM.rpL32)) ~ dtkrh1$treatment +   
## dtkrh1$Day + dtkrh1$treatment:dtkrh1$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -0.33727 -0.13920 0.00086 0.11337 0.30757   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -3.50118 0.23334 -15.005 6.48e-15 \*\*\*  
## dtkrh1$treatmentMimic 2.08681 0.32999 6.324 7.69e-07 \*\*\*  
## dtkrh1$Day 0.24662 0.04996 4.936 3.30e-05 \*\*\*  
## dtkrh1$treatmentMimic:dtkrh1$Day -0.09507 0.07066 -1.346 0.189   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.1995 on 28 degrees of freedom  
## Multiple R-squared: 0.9542, Adjusted R-squared: 0.9493   
## F-statistic: 194.4 on 3 and 28 DF, p-value: < 2.2e-16

malelmkrh12<-lm(log10((dtkrh1$SDM.Gene/dtkrh1$SDM.rpL32))~dtkrh1$treatment+dtkrh1$Day)  
summary.lm(malelmkrh12) #effect of treatment and day

##   
## Call:  
## lm(formula = log10((dtkrh1$SDM.Gene/dtkrh1$SDM.rpL32)) ~ dtkrh1$treatment +   
## dtkrh1$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -0.34448 -0.16423 -0.00165 0.15343 0.37590   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -3.28429 0.17106 -19.199 < 2e-16 \*\*\*  
## dtkrh1$treatmentMimic 1.65304 0.07150 23.121 < 2e-16 \*\*\*  
## dtkrh1$Day 0.19909 0.03582 5.558 5.38e-06 \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.2022 on 29 degrees of freedom  
## Multiple R-squared: 0.9512, Adjusted R-squared: 0.9479   
## F-statistic: 282.7 on 2 and 29 DF, p-value: < 2.2e-16

plot(malelmkrh1)



The treatment has a significant effect on PkKr-h1 expression (p-value < 2e-16 if not considering the interaction between treatment and day after treatment) and the expression changes over time.

## male on E93-1 expression by treatment and by day

par(mfrow=c(2,2))  
dtE931<-subset(dtm,Gene=="PkE93-1")  
dtE931$Gene

## [1] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [9] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [17] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## [25] PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1 PkE93-1  
## Levels: Kr-h1 PkE93-1 PkE93-2 PkE93-3

dtE931$Day

## [1] 3 4 4 4 4 6 5 5 5 5 5 5 5 5 5 5 5 5 4 4 6 6 6 4 4 6 6 3 3 3 3 3

is.numeric(dtE931$Day)

## [1] TRUE

names(dtE931)

## [1] "Sample.ID" "treatment" "Day" "DayL" "Gene" "SDM.Gene"   
## [7] "SDM.rpL32"

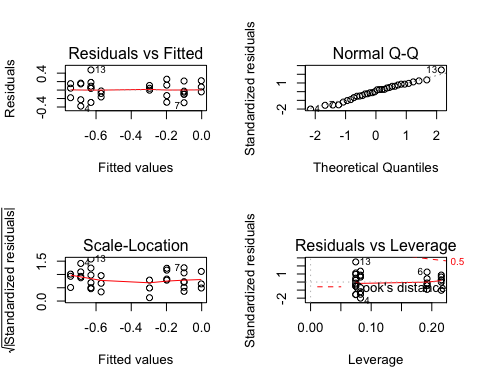
#transformed lm  
malelmE931<-lm(log10((dtE931$SDM.Gene/dtE931$SDM.rpL32))~dtE931$treatment+dtE931$Day+dtE931$treatment:dtE931$Day)  
summary.lm(malelmE931) # no significant interaction

##   
## Call:  
## lm(formula = log10((dtE931$SDM.Gene/dtE931$SDM.rpL32)) ~ dtE931$treatment +   
## dtE931$Day + dtE931$treatment:dtE931$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -0.37979 -0.10782 0.01913 0.12407 0.47623   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -0.59043 0.23181 -2.547 0.0166 \*  
## dtE931$treatmentMimic -0.32778 0.32783 -1.000 0.3259   
## dtE931$Day 0.09805 0.04963 1.976 0.0581 .  
## dtE931$treatmentMimic:dtE931$Day -0.04020 0.07019 -0.573 0.5714   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.1981 on 28 degrees of freedom  
## Multiple R-squared: 0.6763, Adjusted R-squared: 0.6416   
## F-statistic: 19.5 on 3 and 28 DF, p-value: 5.017e-07

#remove interaction  
malelmE9312<-lm(log10((dtE931$SDM.Gene/dtE931$SDM.rpL32))~dtE931$treatment+dtE931$Day)  
summary.lm(malelmE9312)

##   
## Call:  
## lm(formula = log10((dtE931$SDM.Gene/dtE931$SDM.rpL32)) ~ dtE931$treatment +   
## dtE931$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -0.3685 -0.1167 -0.0015 0.1184 0.4674   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -0.49873 0.16566 -3.011 0.00536 \*\*   
## dtE931$treatmentMimic -0.51118 0.06924 -7.383 3.91e-08 \*\*\*  
## dtE931$Day 0.07795 0.03469 2.247 0.03240 \*   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.1958 on 29 degrees of freedom  
## Multiple R-squared: 0.6725, Adjusted R-squared: 0.6499   
## F-statistic: 29.78 on 2 and 29 DF, p-value: 9.336e-08

plot(malelmE931)

 There is a significant effect of the treatment on E93 isoform 1 in males.

## male on E93-2 expression by treatment and by day

par(mfrow=c(2,2))  
dtE932<-subset(dtm,Gene=="PkE93-2")  
dtE932$Gene

## [1] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## [9] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## [17] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## [25] PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2 PkE93-2  
## Levels: Kr-h1 PkE93-1 PkE93-2 PkE93-3

dtE932$Day

## [1] 3 4 4 4 4 6 5 5 5 5 5 5 5 5 5 5 5 5 4 4 6 6 6 4 4 6 6 3 3 3 3 3

is.numeric(dtE932$Day)

## [1] TRUE

names(dtE932)

## [1] "Sample.ID" "treatment" "Day" "DayL" "Gene" "SDM.Gene"   
## [7] "SDM.rpL32"

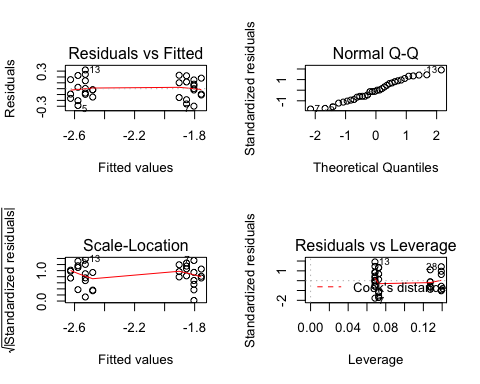
#transformed lm  
malelmE932<-lm(log10((dtE932$SDM.Gene/dtE932$SDM.rpL32))~dtE932$treatment+dtE932$Day+dtE932$treatment:dtE932$Day)  
summary.lm(malelmE932)

##   
## Call:  
## lm(formula = log10((dtE932$SDM.Gene/dtE932$SDM.rpL32)) ~ dtE932$treatment +   
## dtE932$Day + dtE932$treatment:dtE932$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -0.290985 -0.113743 -0.009838 0.135087 0.312932   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -1.97433 0.20590 -9.589 2.41e-10 \*\*\*  
## dtE932$treatmentMimic -0.87633 0.29118 -3.010 0.00549 \*\*   
## dtE932$Day 0.03243 0.04409 0.736 0.46802   
## dtE932$treatmentMimic:dtE932$Day 0.03353 0.06235 0.538 0.59493   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.176 on 28 degrees of freedom  
## Multiple R-squared: 0.8312, Adjusted R-squared: 0.8132   
## F-statistic: 45.97 on 3 and 28 DF, p-value: 6.048e-11

#remove interaction  
malelmE932<-lm(log10((dtE932$SDM.Gene/dtE932$SDM.rpL32))~dtE932$treatment+dtE932$Day)  
summary.lm(malelmE932)

##   
## Call:  
## lm(formula = log10((dtE932$SDM.Gene/dtE932$SDM.rpL32)) ~ dtE932$treatment +   
## dtE932$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -0.29832 -0.11649 -0.00932 0.13875 0.32027   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -2.05083 0.14704 -13.947 2.16e-14 \*\*\*  
## dtE932$treatmentMimic -0.72334 0.06146 -11.770 1.45e-12 \*\*\*  
## dtE932$Day 0.04920 0.03079 1.598 0.121   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.1738 on 29 degrees of freedom  
## Multiple R-squared: 0.8295, Adjusted R-squared: 0.8177   
## F-statistic: 70.54 on 2 and 29 DF, p-value: 7.25e-12

plot(malelmE932)

 There is a significant effect of treatment on E93 isoform 2 in males.

## male on E93-3 expression by treatment and by day

par(mfrow=c(2,2))  
dtE933<-subset(dtm,Gene=="PkE93-3")  
dtE933$Gene

## [1] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## [9] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## [17] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## [25] PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3 PkE93-3  
## Levels: Kr-h1 PkE93-1 PkE93-2 PkE93-3

dtE933$Day

## [1] 3 4 4 4 4 6 5 5 5 5 5 5 5 5 5 5 5 5 4 4 6 6 6 4 4 6 6 3 3 3 3 3

is.numeric(dtE933$Day)

## [1] TRUE

names(dtE933)

## [1] "Sample.ID" "treatment" "Day" "DayL" "Gene" "SDM.Gene"   
## [7] "SDM.rpL32"

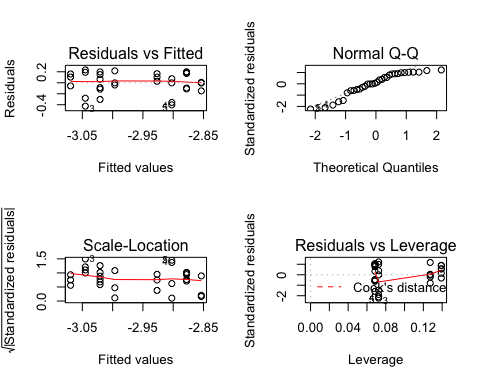
#transformed lm  
malelmE933<-lm(log10((dtE933$SDM.Gene/dtE933$SDM.rpL32))~dtE933$treatment+dtE933$Day+dtE933$treatment:dtE933$Day)  
summary.lm(malelmE933)

##   
## Call:  
## lm(formula = log10((dtE933$SDM.Gene/dtE933$SDM.rpL32)) ~ dtE933$treatment +   
## dtE933$Day + dtE933$treatment:dtE933$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -0.42411 -0.09895 0.01677 0.16604 0.23532   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -3.155343 0.235758 -13.384 1.09e-13 \*\*\*  
## dtE933$treatmentMimic 0.168377 0.333413 0.505 0.618   
## dtE933$Day 0.027168 0.050479 0.538 0.595   
## dtE933$treatmentMimic:dtE933$Day -0.005611 0.071389 -0.079 0.938   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.2015 on 28 degrees of freedom  
## Multiple R-squared: 0.1381, Adjusted R-squared: 0.04579   
## F-statistic: 1.496 on 3 and 28 DF, p-value: 0.2372

#remove interaction  
malelmE933<-lm(log10((dtE933$SDM.Gene/dtE933$SDM.rpL32))~dtE933$treatment+dtE933$Day)  
summary.lm(malelmE933)

##   
## Call:  
## lm(formula = log10((dtE933$SDM.Gene/dtE933$SDM.rpL32)) ~ dtE933$treatment +   
## dtE933$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -0.42569 -0.10018 0.01695 0.16650 0.23374   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -3.14254 0.16752 -18.759 <2e-16 \*\*\*  
## dtE933$treatmentMimic 0.14278 0.07002 2.039 0.0506 .   
## dtE933$Day 0.02436 0.03508 0.695 0.4929   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.198 on 29 degrees of freedom  
## Multiple R-squared: 0.1379, Adjusted R-squared: 0.07849   
## F-statistic: 2.32 on 2 and 29 DF, p-value: 0.1162

plot(malelmE933)

 There is no significant effect of the treatment on E93 isoform 3 in males.

## Female on Kr-h1 expression by treatment and by day

par(mfrow=c(2,2))  
dtfkrh1<-subset(dtf,Gene=="Kr.h1")  
dtfkrh1$Gene

## [1] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"  
## [9] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"  
## [17] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"  
## [25] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"  
## [33] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"

dtfkrh1$Day

## [1] 4 4 4 4 4 4 5 5 5 5 5 5 6 6 6 6 6 6 7 7 7 7 7 7 8 8 8 8 8 8 8 8 8 8 8  
## [36] 8 8 8 8

is.numeric(dtfkrh1$Day)

## [1] TRUE

names(dtfkrh1)

## [1] "cDNA.." "Compound" "treatment.stage"  
## [4] "sex" "Day" "DayL"   
## [7] "N" "rpL32" "Gene"   
## [10] "SDM"

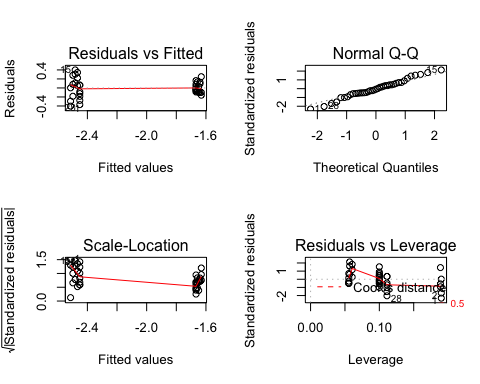
femalelmkrh1<-lm(log10((dtfkrh1$SDM/dtfkrh1$rpL32))~dtfkrh1$Compound+dtfkrh1$Day+dtfkrh1$Compound:dtfkrh1$Day)  
summary.lm(femalelmkrh1)

##   
## Call:  
## lm(formula = log10((dtfkrh1$SDM/dtfkrh1$rpL32)) ~ dtfkrh1$Compound +   
## dtfkrh1$Day + dtfkrh1$Compound:dtfkrh1$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -0.40368 -0.09917 -0.01173 0.10331 0.39999   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -2.57576 0.19531 -13.188 3.85e-15 \*\*\*  
## dtfkrh1$CompoundMimic 0.98013 0.27412 3.576 0.00104 \*\*   
## dtfkrh1$Day 0.01557 0.02962 0.526 0.60240   
## dtfkrh1$CompoundMimic:dtfkrh1$Day -0.02448 0.04134 -0.592 0.55749   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.1934 on 35 degrees of freedom  
## Multiple R-squared: 0.8345, Adjusted R-squared: 0.8203   
## F-statistic: 58.82 on 3 and 35 DF, p-value: 9.459e-14

#remove interaction  
femalelmkrh12<-lm(log10((dtfkrh1$SDM/dtfkrh1$rpL32))~dtfkrh1$Compound+dtfkrh1$Day)  
summary.lm(femalelmkrh12)

##   
## Call:  
## lm(formula = log10((dtfkrh1$SDM/dtfkrh1$rpL32)) ~ dtfkrh1$Compound +   
## dtfkrh1$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -0.43412 -0.08995 -0.02959 0.10959 0.39469   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -2.495036 0.138624 -17.999 < 2e-16 \*\*\*  
## dtfkrh1$CompoundMimic 0.821991 0.061422 13.383 1.53e-15 \*\*\*  
## dtfkrh1$Day 0.003001 0.020474 0.147 0.884   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.1917 on 36 degrees of freedom  
## Multiple R-squared: 0.8328, Adjusted R-squared: 0.8235   
## F-statistic: 89.67 on 2 and 36 DF, p-value: 1.04e-14

plot(femalelmkrh1)

 There is a significant effect of treatment on Kr-h1 in females.

## female on E93-1 expression by treatment and by day

par(mfrow=c(2,2))  
dtf$Gene

## [1] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [8] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [15] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [22] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [29] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [36] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [43] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [50] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [57] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [64] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [71] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [78] "PkE93.1" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [85] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [92] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [99] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [106] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [113] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.3" "PkE93.3"  
## [120] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [127] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [134] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [141] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [148] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [155] "PkE93.3" "PkE93.3"

dtfE931<-subset(dtf,Gene=="PkE93.1")  
dtfE931$Gene

## [1] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [8] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [15] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [22] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [29] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [36] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"

is.numeric(dtfE931$Day)

## [1] TRUE

names(dtfE931)

## [1] "cDNA.." "Compound" "treatment.stage"  
## [4] "sex" "Day" "DayL"   
## [7] "N" "rpL32" "Gene"   
## [10] "SDM"

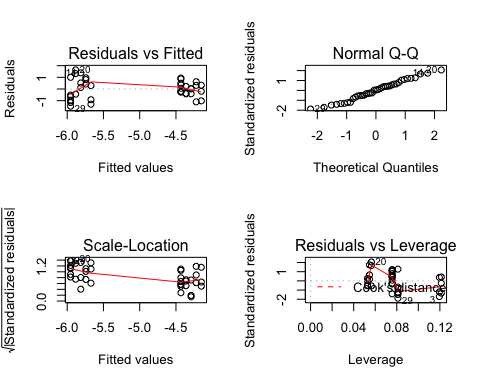
femalelmE931<-lm(log((dtfE931$SDM/dtfE931$rpL32))~dtfE931$Compound+dtfE931$Day+dtfE931$Compound:dtfE931$Day)  
summary.lm(femalelmE931) #no effect from interaction

##   
## Call:  
## lm(formula = log((dtfE931$SDM/dtfE931$rpL32)) ~ dtfE931$Compound +   
## dtfE931$Day + dtfE931$Compound:dtfE931$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.61143 -0.44584 0.07651 0.55483 1.70797   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -4.57996 0.81139 -5.645 2.27e-06 \*\*\*  
## dtfE931$CompoundMimic -0.06673 1.13876 -0.059 0.954   
## dtfE931$Day -0.19664 0.12306 -1.598 0.119   
## dtfE931$CompoundMimic:dtfE931$Day 0.24593 0.17173 1.432 0.161   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.8035 on 35 degrees of freedom  
## Multiple R-squared: 0.5167, Adjusted R-squared: 0.4753   
## F-statistic: 12.47 on 3 and 35 DF, p-value: 1.056e-05

femalelmE931<-lm(log((dtfE931$SDM/dtfE931$rpL32))~dtfE931$Compound+dtfE931$Day)  
summary.lm(femalelmE931)

##   
## Call:  
## lm(formula = log((dtfE931$SDM/dtfE931$rpL32)) ~ dtfE931$Compound +   
## dtfE931$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.47066 -0.48425 0.02964 0.50499 1.63485   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -5.39087 0.58956 -9.144 6.42e-11 \*\*\*  
## dtfE931$CompoundMimic 1.52186 0.26123 5.826 1.19e-06 \*\*\*  
## dtfE931$Day -0.07035 0.08708 -0.808 0.424   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.8151 on 36 degrees of freedom  
## Multiple R-squared: 0.4884, Adjusted R-squared: 0.46   
## F-statistic: 17.18 on 2 and 36 DF, p-value: 5.763e-06

plot(femalelmE931)

 There is a significant effect of the treatment on E93 isoform 1 when only looking at treatment.

## female on E93-2 expression by treatment and by day

par(mfrow=c(2,2))  
dtf$Gene

## [1] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [8] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [15] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [22] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [29] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [36] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [43] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [50] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [57] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [64] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [71] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [78] "PkE93.1" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [85] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [92] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [99] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [106] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [113] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.3" "PkE93.3"  
## [120] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [127] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [134] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [141] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [148] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [155] "PkE93.3" "PkE93.3"

dtfE932<-subset(dtf,Gene=="PkE93.2")  
dtfE932$Gene

## [1] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [8] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [15] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [22] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [29] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [36] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"

is.numeric(dtfE932$Day)

## [1] TRUE

names(dtfE932)

## [1] "cDNA.." "Compound" "treatment.stage"  
## [4] "sex" "Day" "DayL"   
## [7] "N" "rpL32" "Gene"   
## [10] "SDM"

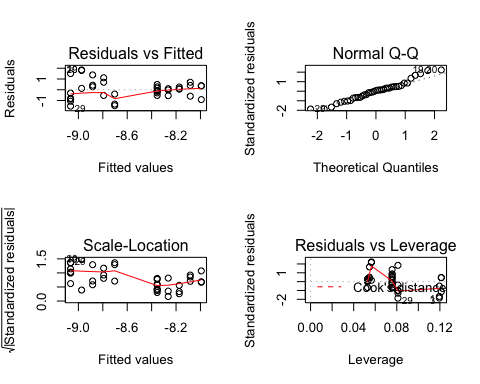
femalelmE932<-lm(log((dtfE932$SDM/dtfE932$rpL32))~dtfE932$Compound+dtfE932$Day+dtfE932$Compound:dtfE932$Day)  
summary.lm(femalelmE932)# no effect of interaction

##   
## Call:  
## lm(formula = log((dtfE932$SDM/dtfE932$rpL32)) ~ dtfE932$Compound +   
## dtfE932$Day + dtfE932$Compound:dtfE932$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.56560 -0.51661 0.04422 0.33988 1.82953   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -8.50603 0.87080 -9.768 1.56e-11 \*\*\*  
## dtfE932$CompoundMimic 1.03266 1.22214 0.845 0.404   
## dtfE932$Day -0.06469 0.13207 -0.490 0.627   
## dtfE932$CompoundMimic:dtfE932$Day -0.05001 0.18430 -0.271 0.788   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.8623 on 35 degrees of freedom  
## Multiple R-squared: 0.1765, Adjusted R-squared: 0.1059   
## F-statistic: 2.501 on 3 and 35 DF, p-value: 0.07543

femalelmE932<-lm(log((dtfE932$SDM/dtfE932$rpL32))~dtfE932$Compound+dtfE932$Day)  
summary.lm(femalelmE932)

##   
## Call:  
## lm(formula = log((dtfE932$SDM/dtfE932$rpL32)) ~ dtfE932$Compound +   
## dtfE932$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.52505 -0.54094 0.05639 0.38853 1.84440   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -8.34113 0.61562 -13.549 1.05e-15 \*\*\*  
## dtfE932$CompoundMimic 0.70962 0.27277 2.602 0.0134 \*   
## dtfE932$Day -0.09037 0.09092 -0.994 0.3269   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.8512 on 36 degrees of freedom  
## Multiple R-squared: 0.1748, Adjusted R-squared: 0.1289   
## F-statistic: 3.812 on 2 and 36 DF, p-value: 0.03149

plot(femalelmE932)

 There is no significant effect of treatment in E93 isoform 2 in females

## female on E93-3 expression by treatment and by day

par(mfrow=c(2,2))  
dtf$Gene

## [1] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [8] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [15] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [22] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [29] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1"   
## [36] "Kr.h1" "Kr.h1" "Kr.h1" "Kr.h1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [43] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [50] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [57] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [64] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [71] "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1" "PkE93.1"  
## [78] "PkE93.1" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [85] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [92] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [99] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [106] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2"  
## [113] "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.2" "PkE93.3" "PkE93.3"  
## [120] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [127] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [134] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [141] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [148] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [155] "PkE93.3" "PkE93.3"

dtfE933<-subset(dtf,Gene=="PkE93.3")  
dtfE933$Gene

## [1] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [8] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [15] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [22] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [29] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"  
## [36] "PkE93.3" "PkE93.3" "PkE93.3" "PkE93.3"

is.numeric(dtfE933$Day)

## [1] TRUE

names(dtfE933)

## [1] "cDNA.." "Compound" "treatment.stage"  
## [4] "sex" "Day" "DayL"   
## [7] "N" "rpL32" "Gene"   
## [10] "SDM"

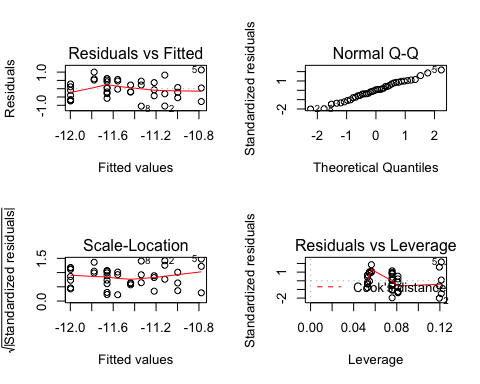
femalelmE933<-lm(log((dtfE933$SDM/dtfE933$rpL32))~dtfE933$Compound+dtfE933$Day+dtfE933$Compound:dtfE933$Day)  
summary.lm(femalelmE933)

##   
## Call:  
## lm(formula = log((dtfE933$SDM/dtfE933$rpL32)) ~ dtfE933$Compound +   
## dtfE933$Day + dtfE933$Compound:dtfE933$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.09318 -0.35854 -0.00049 0.40792 1.18010   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)  
## (Intercept) -10.10933 0.56711 -17.826 < 2e-16  
## dtfE933$CompoundMimic 0.08579 0.79592 0.108 0.91478  
## dtfE933$Day -0.24004 0.08601 -2.791 0.00846  
## dtfE933$CompoundMimic:dtfE933$Day 0.03942 0.12003 0.328 0.74454  
##   
## (Intercept) \*\*\*  
## dtfE933$CompoundMimic   
## dtfE933$Day \*\*   
## dtfE933$CompoundMimic:dtfE933$Day   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.5616 on 35 degrees of freedom  
## Multiple R-squared: 0.3238, Adjusted R-squared: 0.2658   
## F-statistic: 5.585 on 3 and 35 DF, p-value: 0.003075

femalelmE933<-lm(log((dtfE933$SDM/dtfE933$rpL32))~dtfE933$Compound+dtfE933$Day)  
summary.lm(femalelmE933)

##   
## Call:  
## lm(formula = log((dtfE933$SDM/dtfE933$rpL32)) ~ dtfE933$Compound +   
## dtfE933$Day)  
##   
## Residuals:  
## Min 1Q Median 3Q Max   
## -1.05082 -0.35002 0.02828 0.43669 1.13216   
##   
## Coefficients:  
## Estimate Std. Error t value Pr(>|t|)   
## (Intercept) -10.23931 0.40112 -25.527 < 2e-16 \*\*\*  
## dtfE933$CompoundMimic 0.34042 0.17773 1.915 0.063414 .   
## dtfE933$Day -0.21980 0.05924 -3.710 0.000696 \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## Residual standard error: 0.5546 on 36 degrees of freedom  
## Multiple R-squared: 0.3217, Adjusted R-squared: 0.284   
## F-statistic: 8.536 on 2 and 36 DF, p-value: 0.0009246

plot(femalelmE933)



Although E93 isoform 3 expression changes over time, there is no significant effect of the treatment.