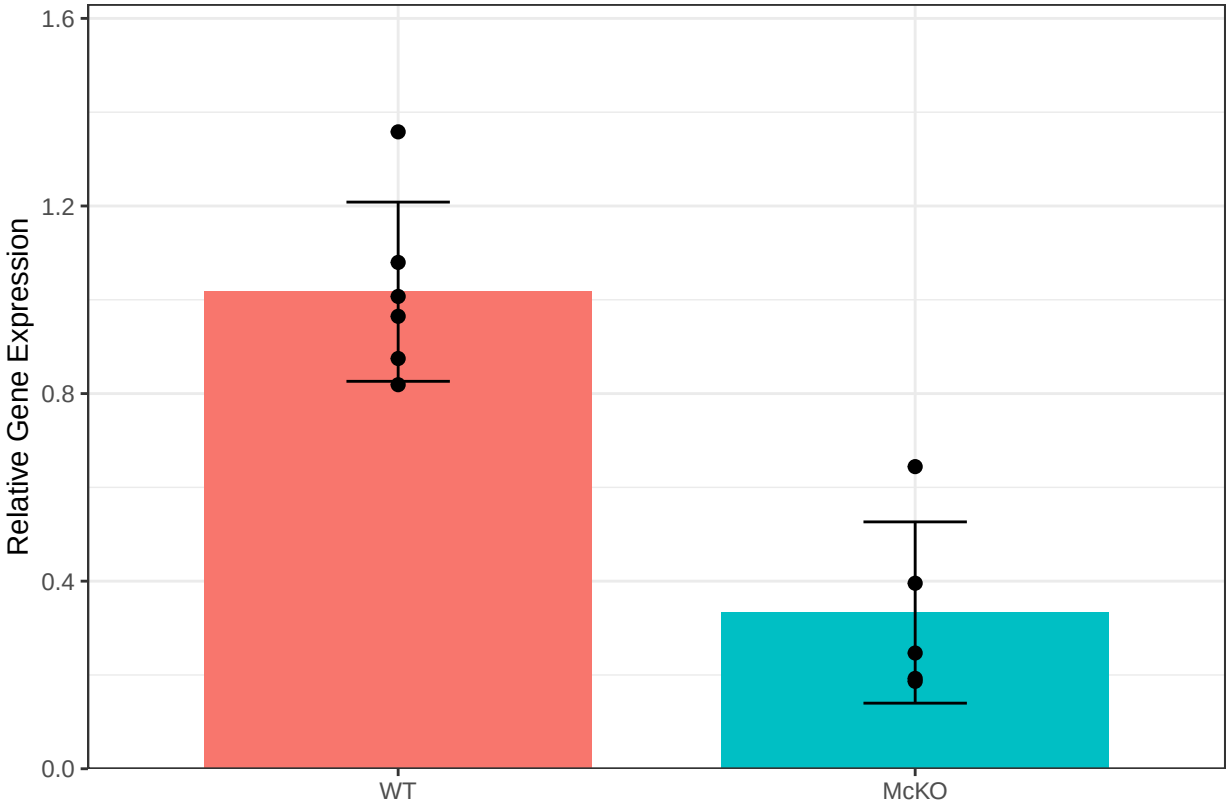
qpCR-Figures

C-T Berezin

10/30/2021

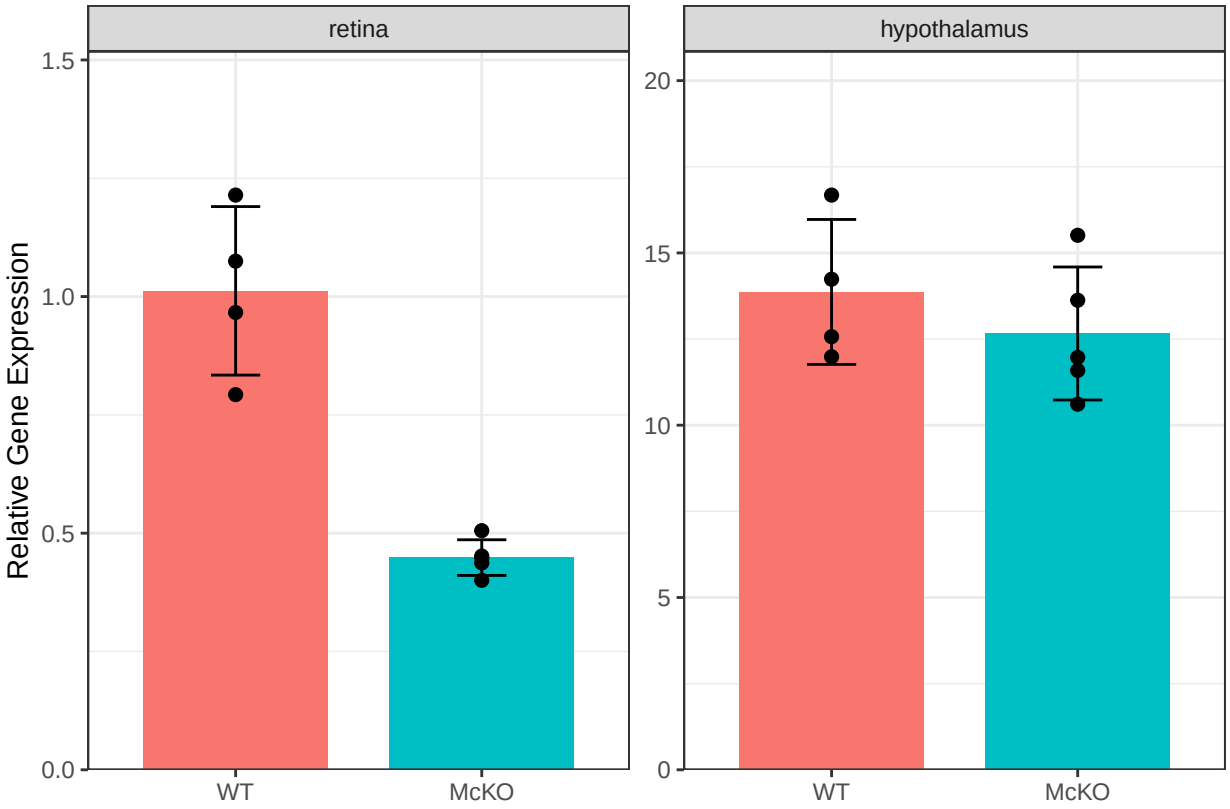
McKO validation

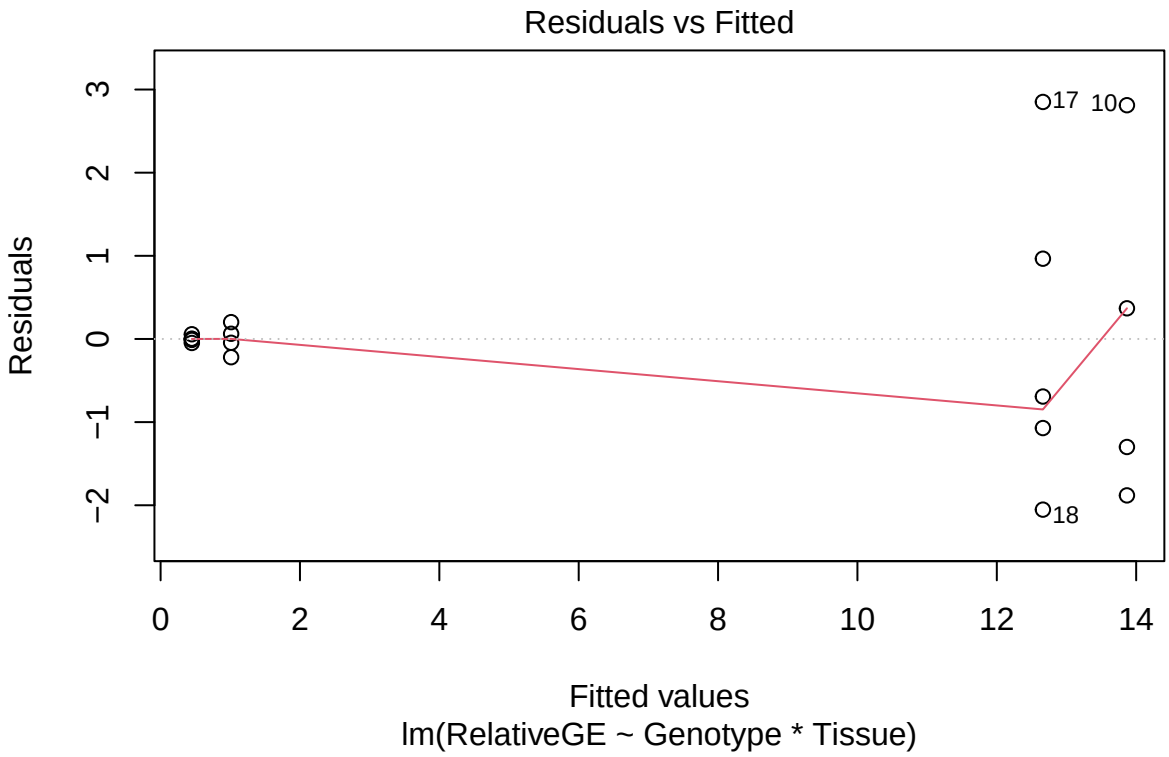


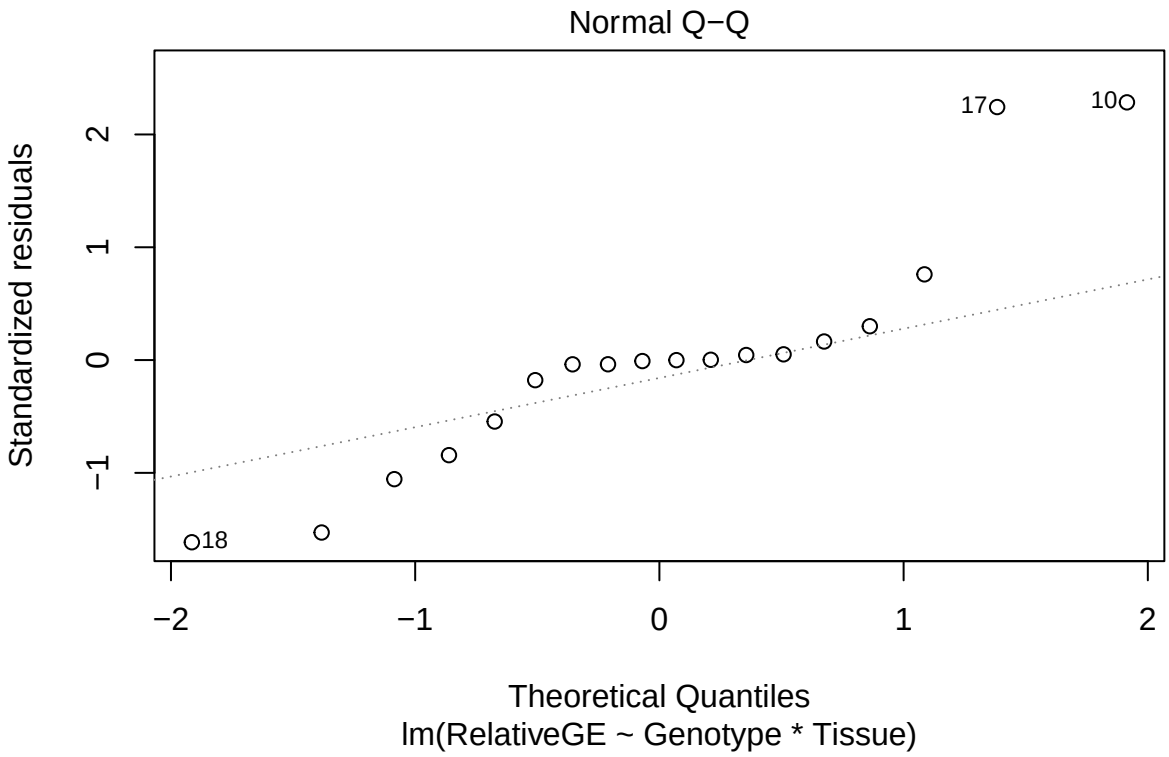

```
## # A tibble: 2 x 4
##   Genotype     n  mean    sd
##   <fct>    <int> <dbl> <dbl>
## 1 WT           6 1.02  0.191
## 2 McKO         5 0.333 0.193

##
##  Welch Two Sample t-test
##
## data:  mcko$Relative.GE by mcko$Genotype
## t = 5.8755, df = 8.604, p-value = 0.0002812
```

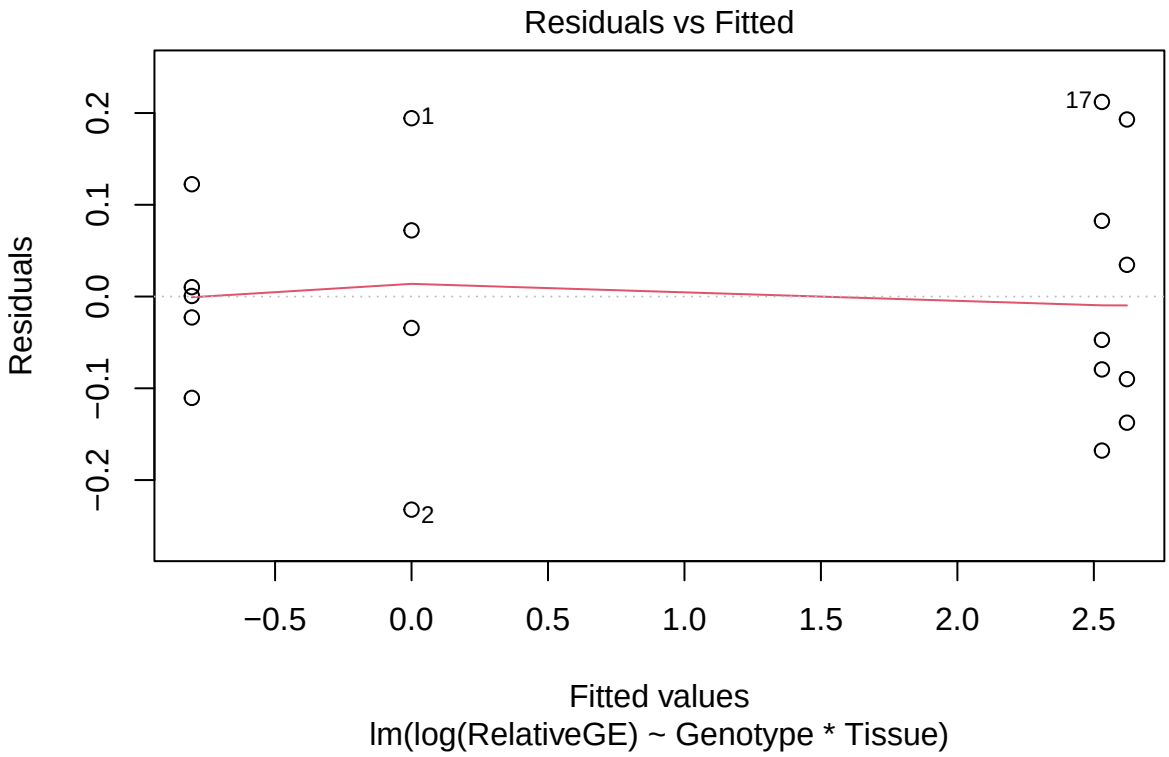
```
## alternative hypothesis: true difference in means between group WT and group McKO is not equal to 0
## 95 percent confidence interval:
##  0.4188365 0.9493181
## sample estimates:
##   mean in group WT mean in group McKO
##          1.0172214          0.3331441
```

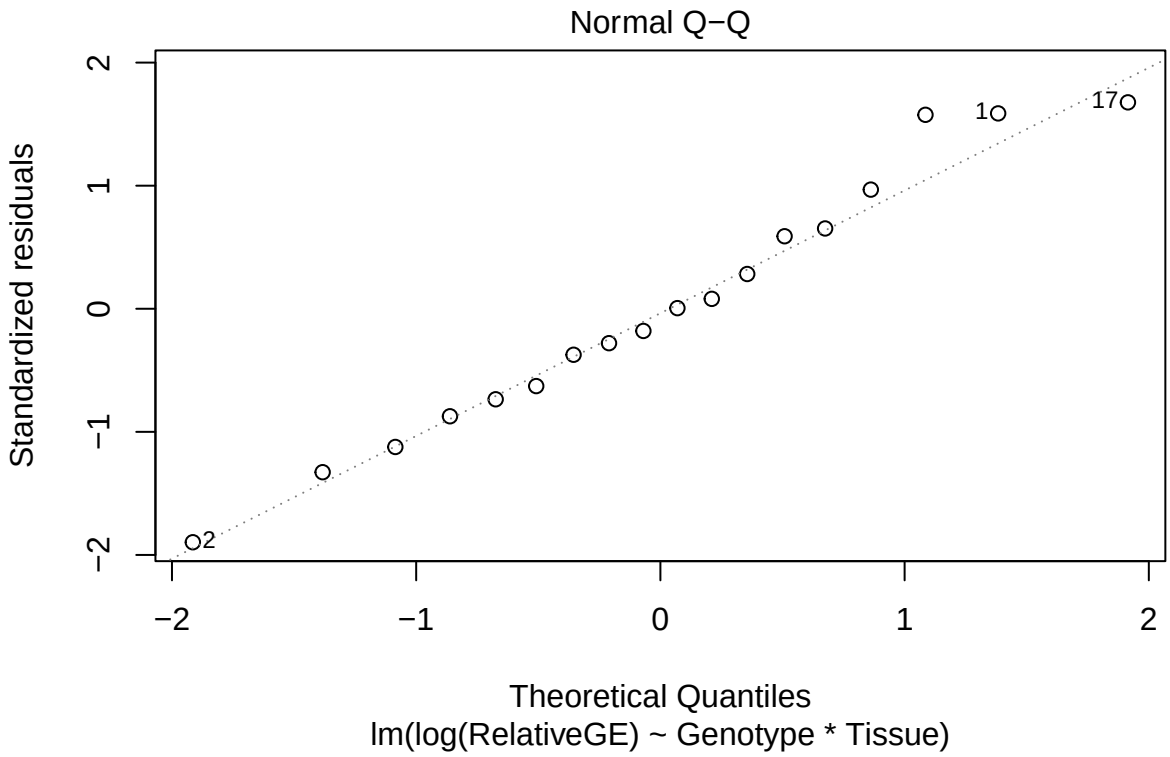
McKO validation, retina & hypothalamus



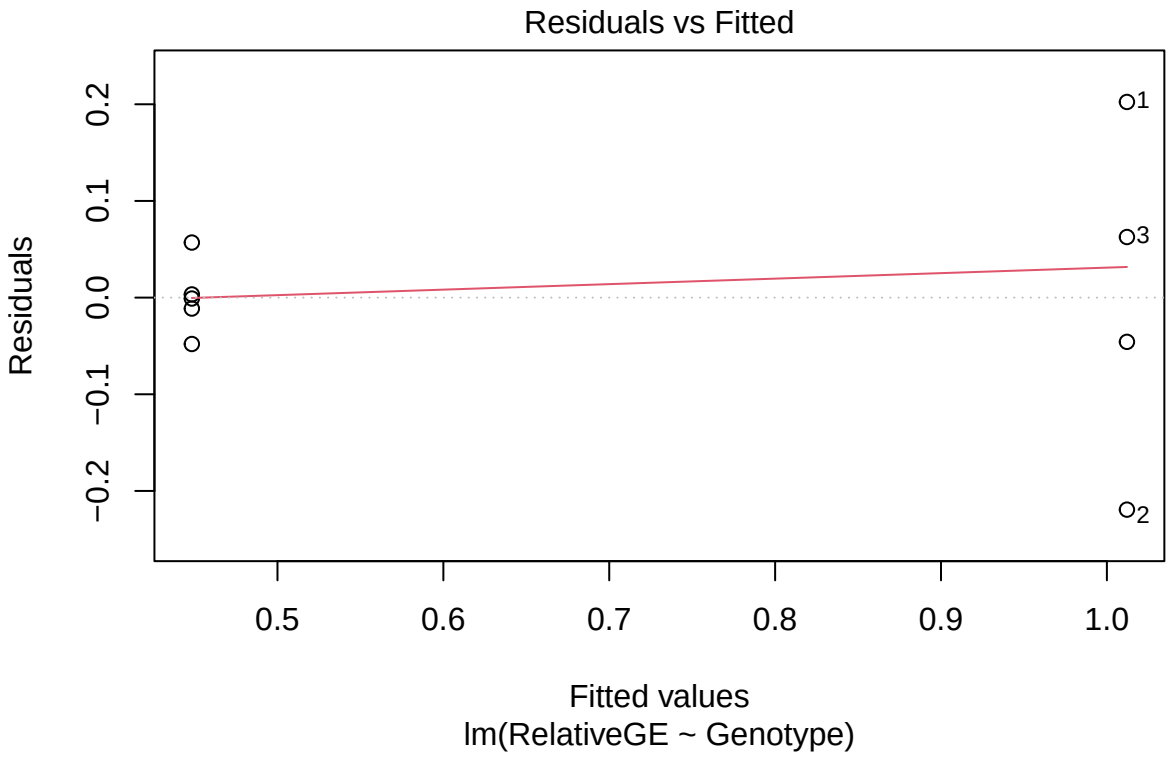

Residuals vs Fitted
Residuals
Fitted values
lm(RelativeGE ~ Genotype * Tissue)
17
10
18
3
2
1
0
−1
−2
0
2
4
6
8
10
12
14

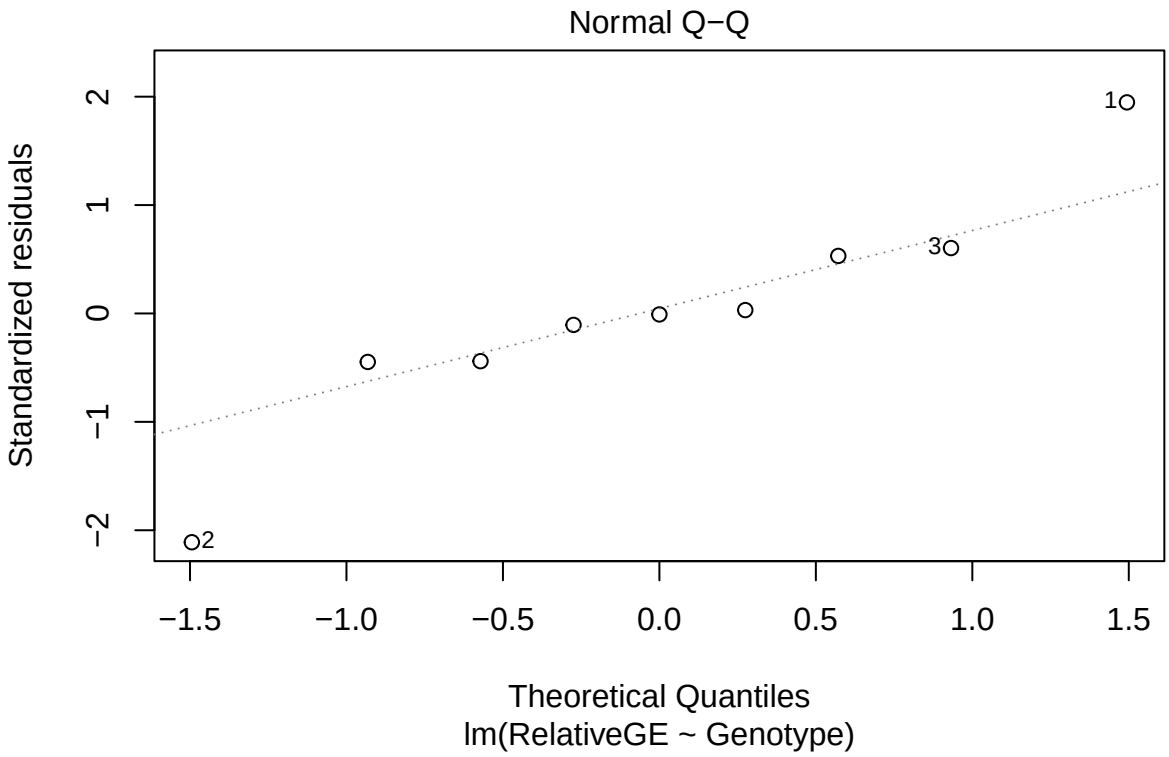

Normal Q−Q
Standardized residuals
Theoretical Quantiles
lm(RelativeGE ~ Genotype * Tissue)
17
10
18
−2
−1
0
1
2

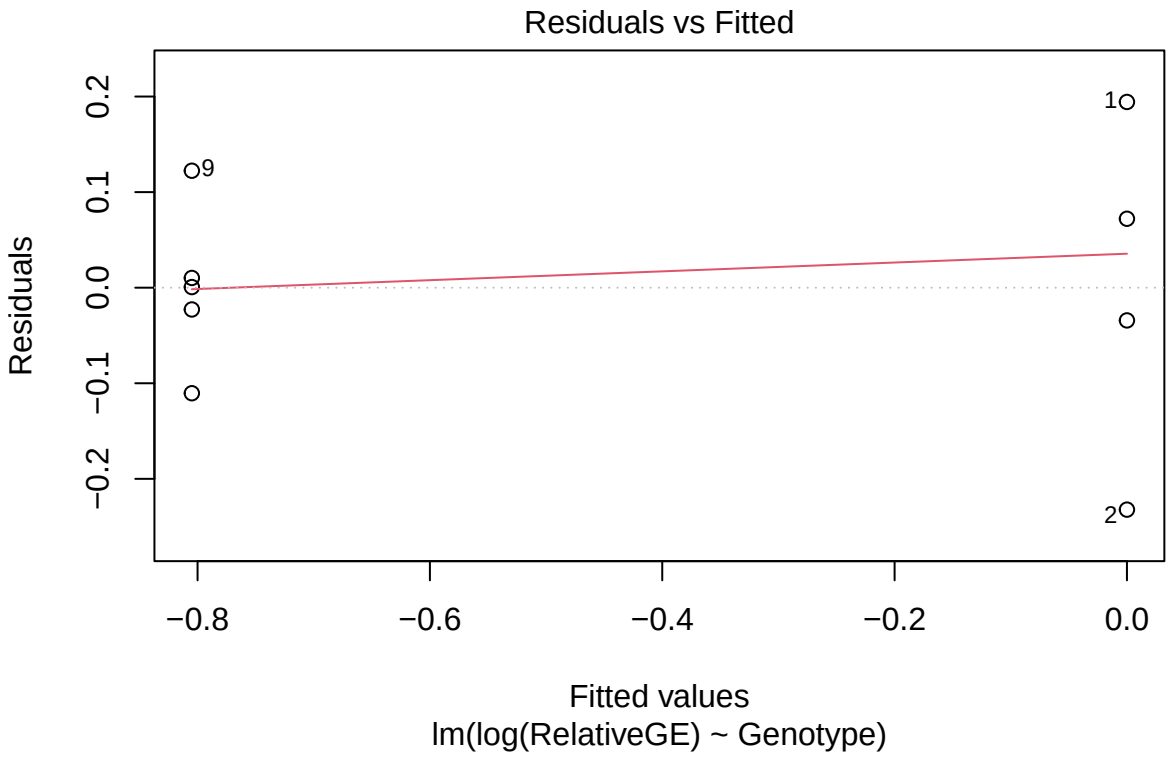

Residuals vs Fitted
Residuals
0.2
0.1
0.0
−0.1
−0.2
1
2
17
−0.5
0.0
0.5
1.0
1.5
2.0
2.5
Fitted values
lm(log(RelativeGE) ~ Genotype * Tissue)

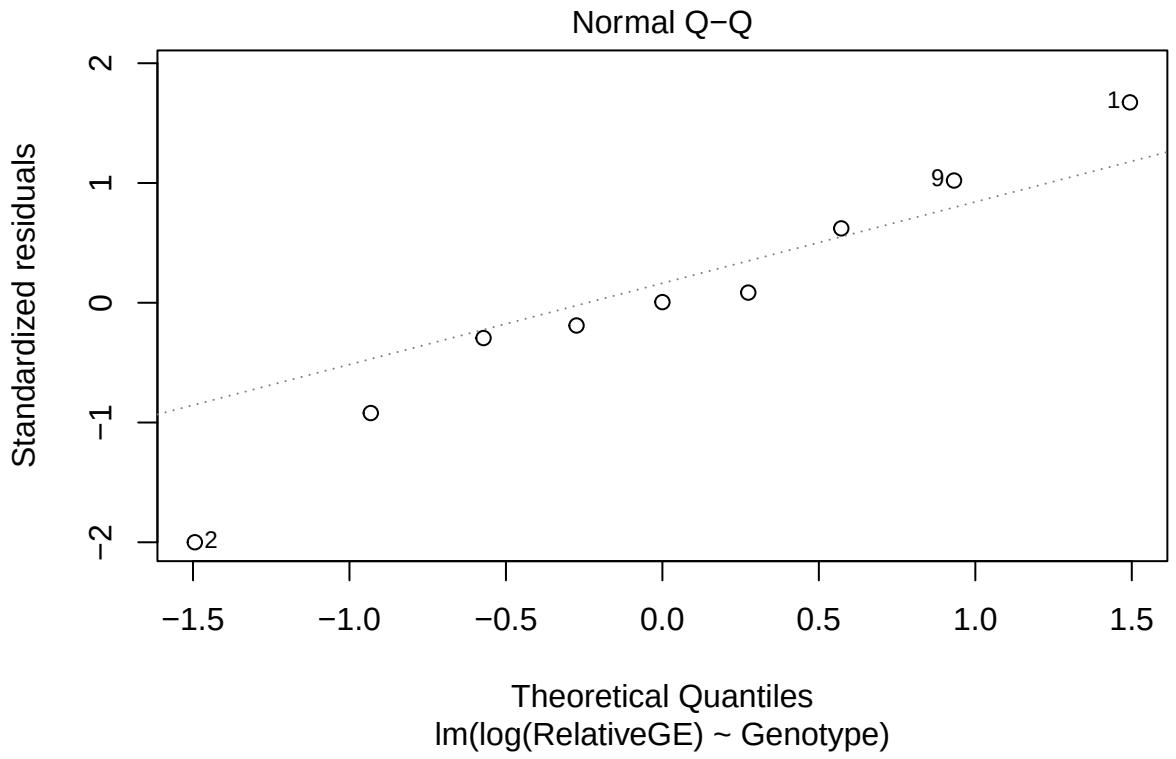



```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  0.7707 0.5294
##       14

##
##  Shapiro-Wilk normality test
##
## data:  log(mor$RelativeGE)
## W = 0.77584, p-value = 0.0007061
```


Residuals vs Fitted
Residuals
0.2
0.1
0.0
−0.1
−0.2
1
3
2
0.5
0.6
0.7
0.8
0.9
1.0
Fitted values
lm(RelativeGE ~ Genotype)

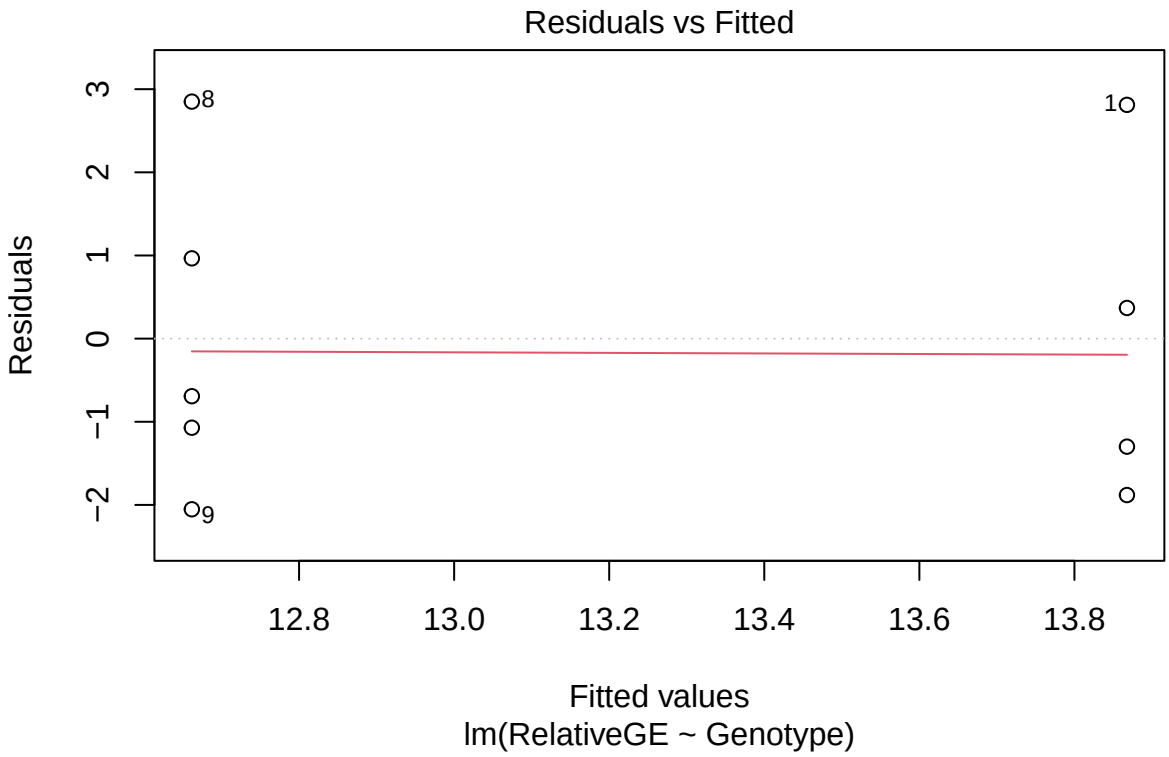

Normal Q−Q
Standardized residuals
2
1
0
−1
−2
1
3
2
−1.5
−1.0
−0.5
0.0
0.5
1.0
1.5
Theoretical Quantiles
lm(RelativeGE ~ Genotype)

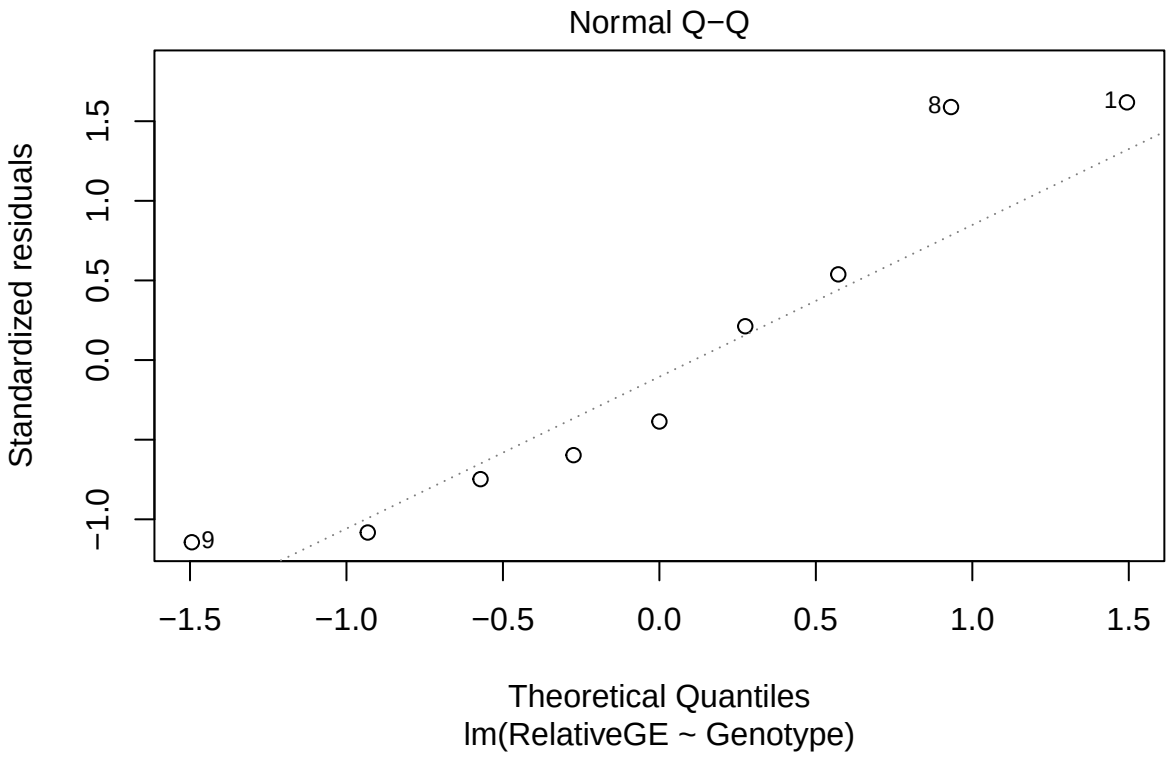

Residuals vs Fitted
Residuals
0.2
0.1
0.0
−0.1
−0.2
9
1
2
−0.8
−0.6
−0.4
−0.2
0.0
Fitted values
lm(log(RelativeGE) ~ Genotype)

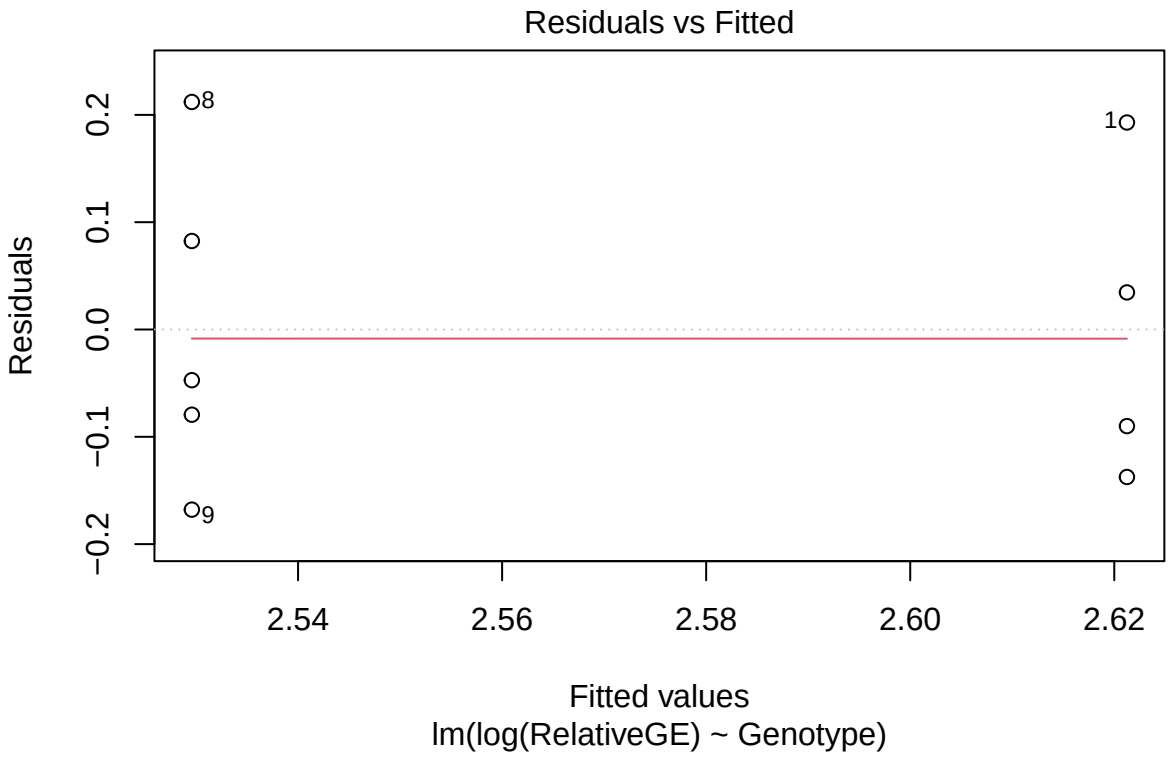


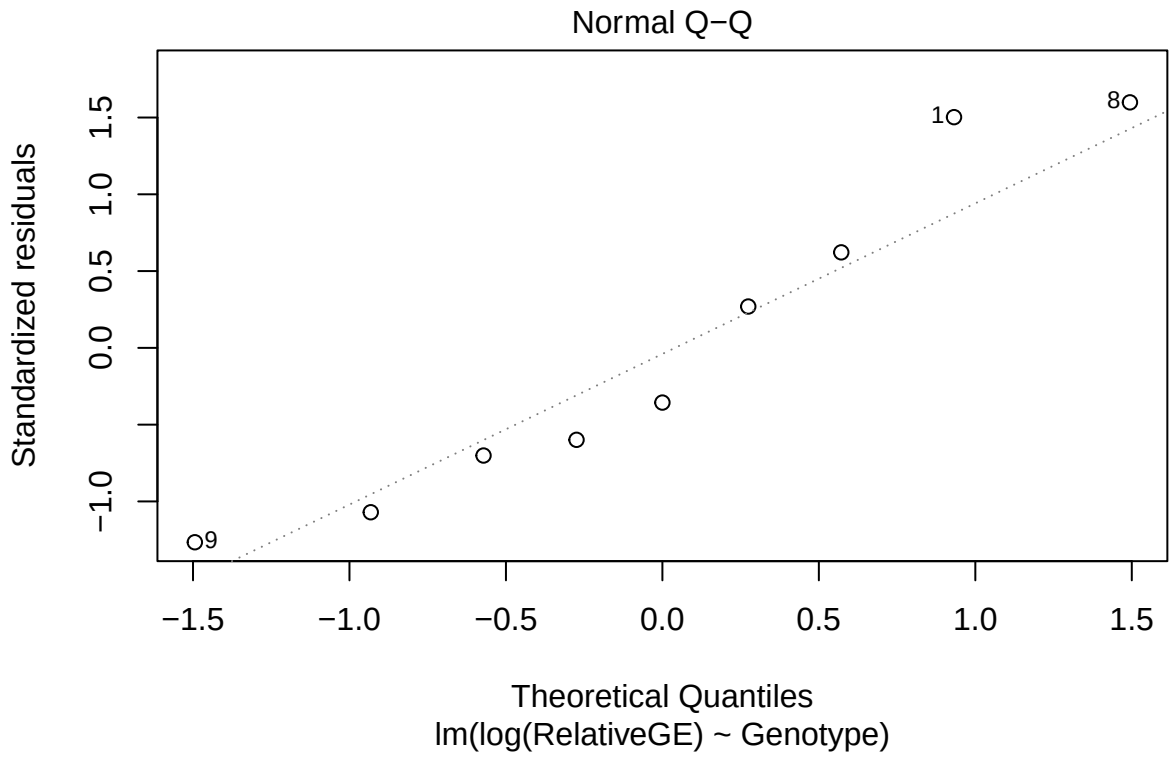

```
##
##  Shapiro-Wilk normality test
##
## data:  mor_retina$RelativeGE
## W = 0.83593, p-value = 0.05203

##
##  Shapiro-Wilk normality test
##
## data:  log(mor_retina$RelativeGE)
## W = 0.84636, p-value = 0.06794

## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value  Pr(>F)
## group  1  6.5685 0.03739 *
##        7
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  1  2.3645  0.168
##        7

##
```

```
##  Welch Two Sample t-test
##
## data:  log(mor_retina$RelativeGE) by mor_retina$Genotype
## t = 8.2319, df = 4.0202, p-value = 0.001162
## alternative hypothesis: true difference in means between group WT and group McKO is not equal to 0
## 95 percent confidence interval:
##  0.5339792 1.0758771
## sample estimates:
##   mean in group WT mean in group McKO
##      -6.252367e-11      -8.049282e-01
```




Normal Q−Q
Standardized residuals
−1.0
0.0
0.5
1.0
1.5
9
8
1
−1.5
−1.0
−0.5
0.0
0.5
1.0
1.5
Theoretical Quantiles
lm(RelativeGE ~ Genotype)

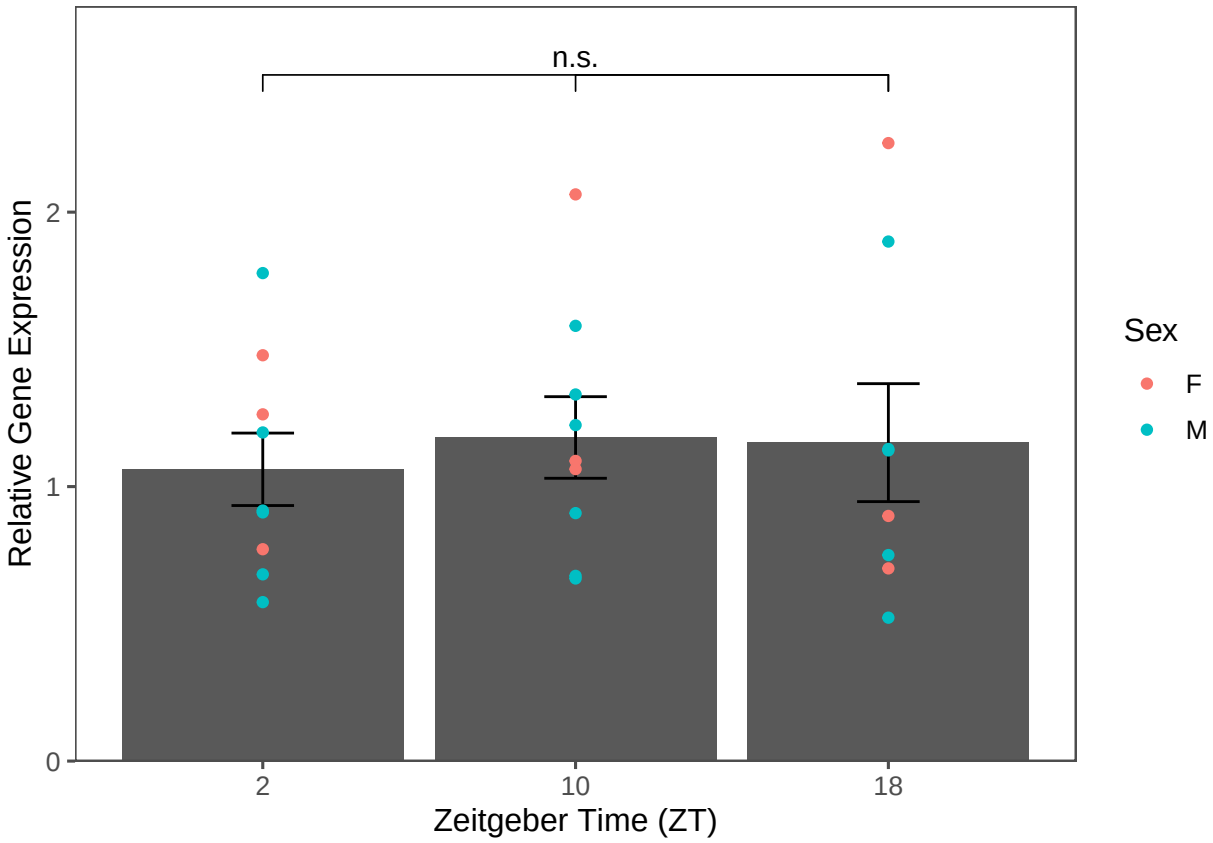

Residuals vs Fitted
Residuals
0.2
0.1
0.0
−0.1
−0.2
8
1
9
2.54
2.56
2.58
2.60
2.62
Fitted values
lm(log(RelativeGE) ~ Genotype)



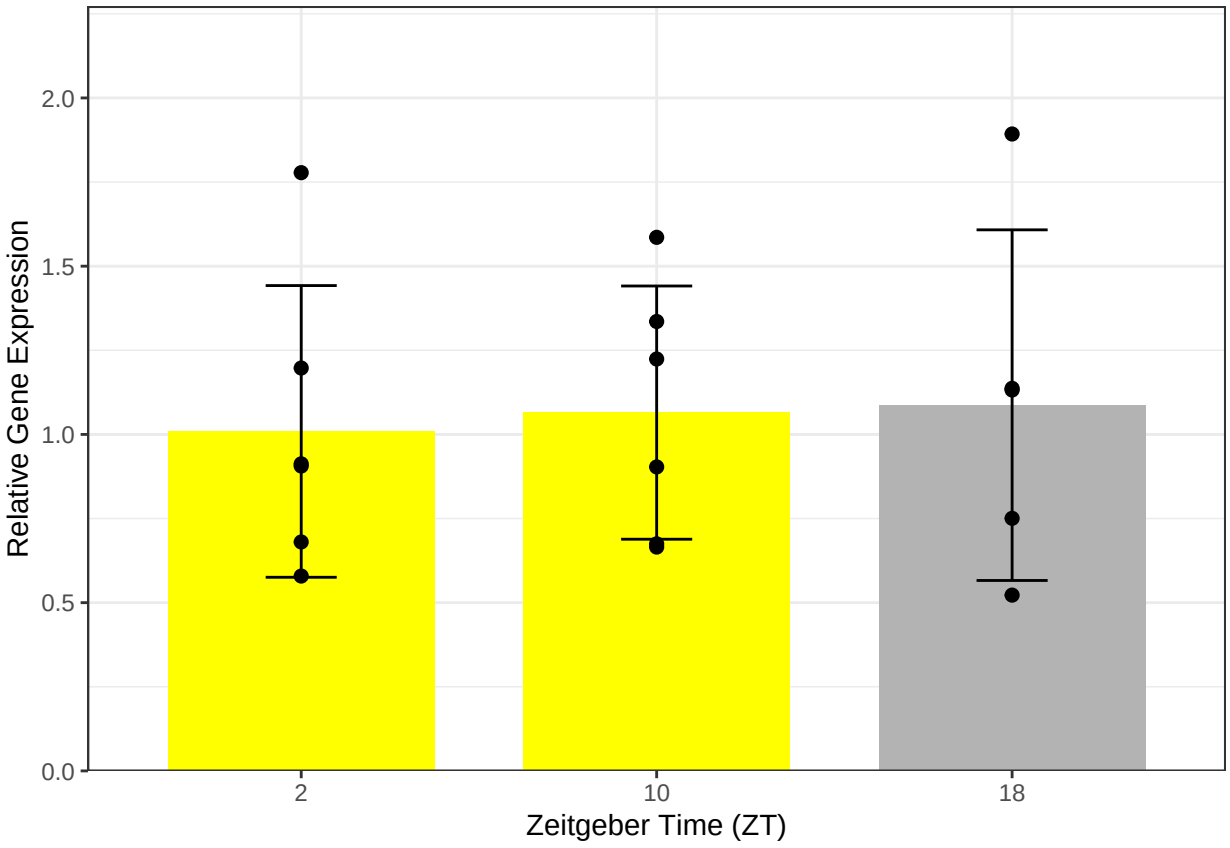

```
##
##  Shapiro-Wilk normality test
##
## data:  mor_hyp$RelativeGE
## W = 0.94614, p-value = 0.6478

## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  1  0.0544 0.8222
##        7

## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  1  0.0078  0.932
##        7


##
##  Welch Two Sample t-test
##
## data:  mor_hyp$RelativeGE by mor_hyp$Genotype
## t = 0.88662, df = 6.2698, p-value = 0.408
## alternative hypothesis: true difference in means between group WT and group McKO is not equal to 0
## 95 percent confidence interval:
##  -2.088038  4.500149
## sample estimates:
```

```
##   mean in group WT mean in group McKO
##           13.86774            12.66169
```

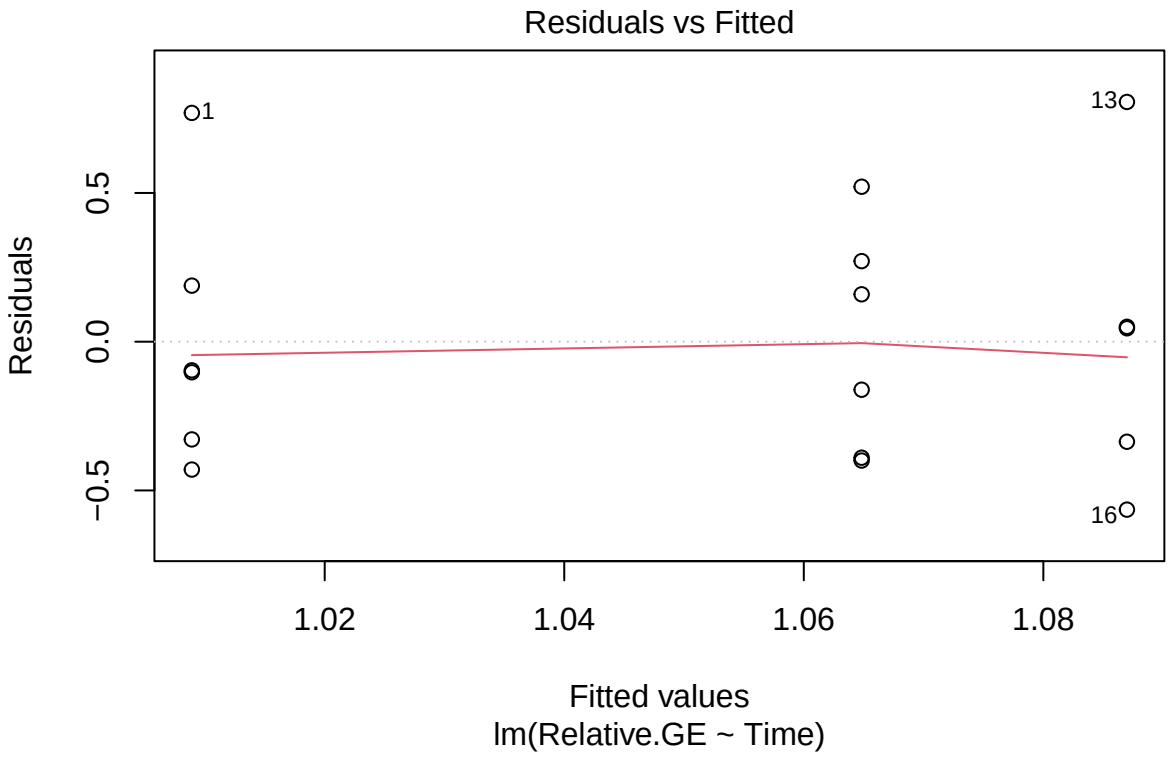
Is POMC under circadian regulation?

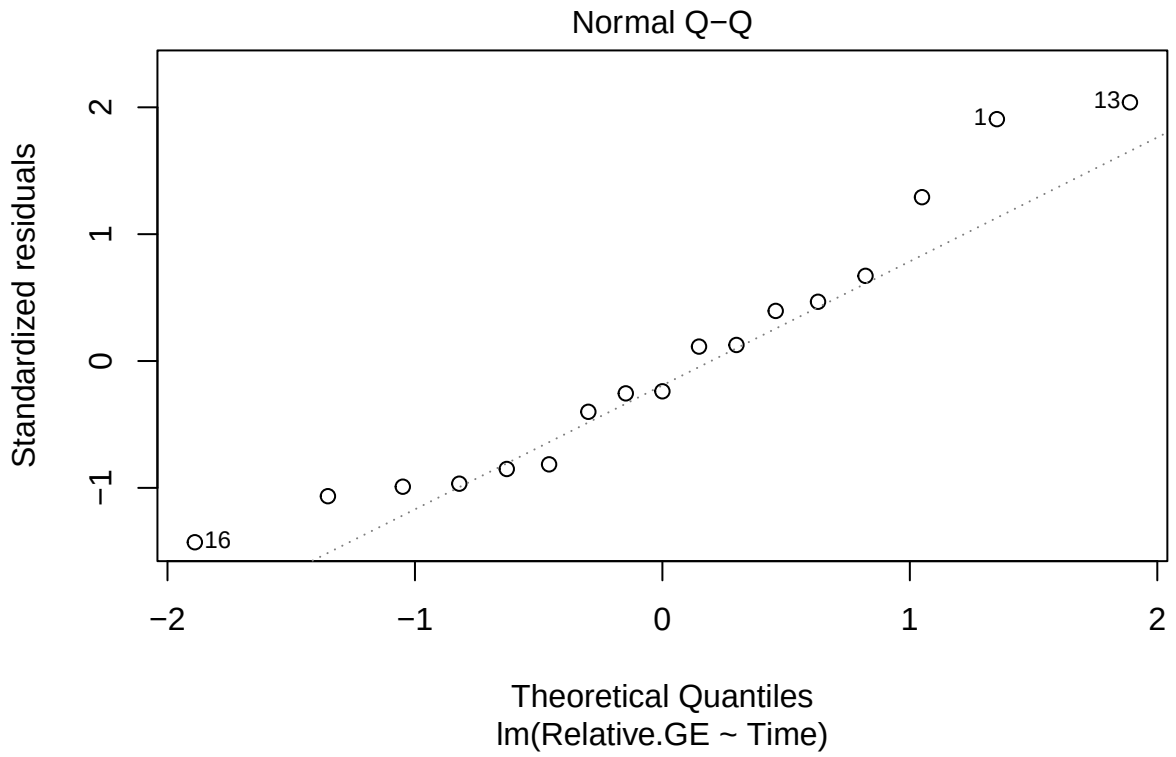



```
## # A tibble: 3 x 4
##   Time      n  mean    sd
##   <fct> <int> <dbl> <dbl>
## 1 2         6  1.01 0.433
## 2 10        6  1.06 0.376
## 3 18        5  1.09 0.521
```




```
##
##  Shapiro-Wilk normality test
##
## data:  pomc_circ_males$Relative.GE
## W = 0.92555, p-value = 0.1832

## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  2  0.0726 0.9304
##       14
```


Residuals vs Fitted
Residuals
0.5
0.0
−0.5
1
13
16
1.02
1.04
1.06
1.08
Fitted values
lm(Relative.GE ~ Time)




```
## Analysis of Variance Table
##
## Response: Relative.GE
##           Df  Sum Sq  Mean Sq F value Pr(>F)
## Time       2 0.01824 0.009122  0.0467 0.9545
## Residuals 14 2.73326 0.195233
```

```
##  contrast estimate    SE df t.ratio p.value
##  2 - 10    -0.0559 0.255 14  -0.219  0.9739
##  2 - 18    -0.0781 0.268 14  -0.292  0.9543
##  10 - 18   -0.0221 0.268 14  -0.083  0.9962
##
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
## # A tibble: 3 x 4
##   Time      n  mean    sd
##   <fct> <int> <dbl> <dbl>
## 1 2         6  1.01 0.433
## 2 10        6  1.06 0.376
## 3 18        5  1.09 0.521
```

```
## [1] 0.009116243
```

```
## [1] 0.1952332
```

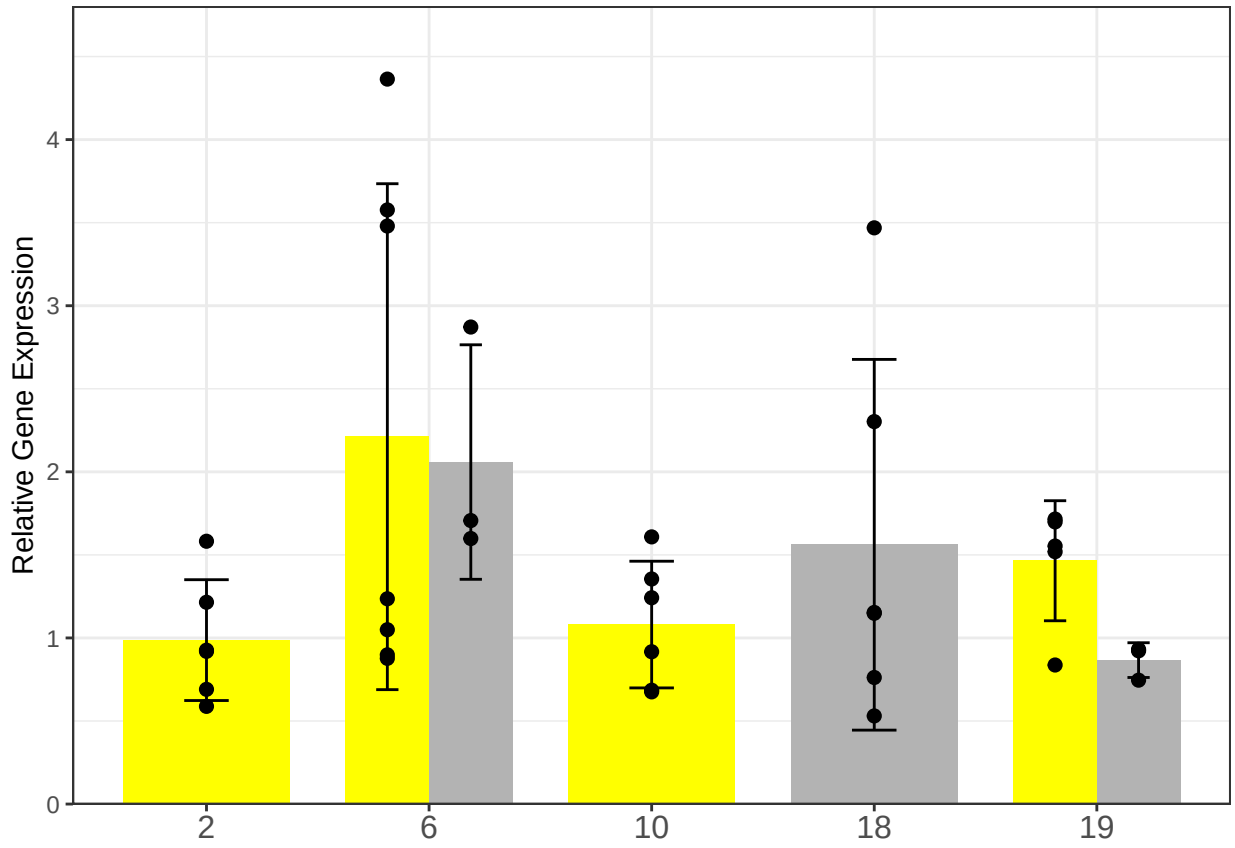
```
##
##      Balanced one-way analysis of variance power calculation
##
##          groups = 3
##               n = 5
##     between.var = 0.009116243
##      within.var = 0.1952332
##       sig.level = 0.05
##           power = 0.07865971
##
## NOTE: n is number in each group
```

POMC circadian + light-dark analysis

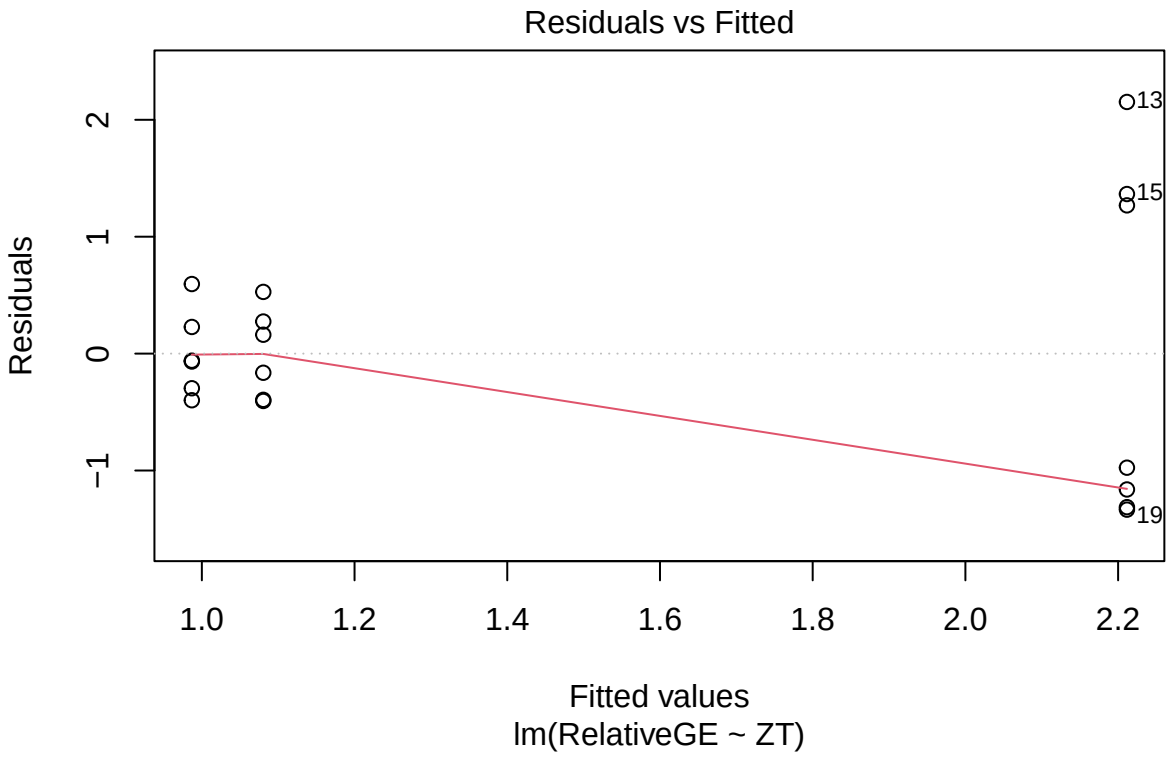
```
##   Sample RelativeGE Sex Hour  Time Light ZT
## 1 12am-1  2.3023761   M 12am night  dark 18
## 2 12am-5  1.1535702   M 12am night  dark 18
## 3 12am-6  1.1484090   M 12am night  dark 18
## 4 12am-7  3.4688319   M 12am night  dark 18
## 5 12am-8  0.5306166   M 12am night  dark 18
## 6 12am-9  0.7619939   M 12am night  dark 18
```

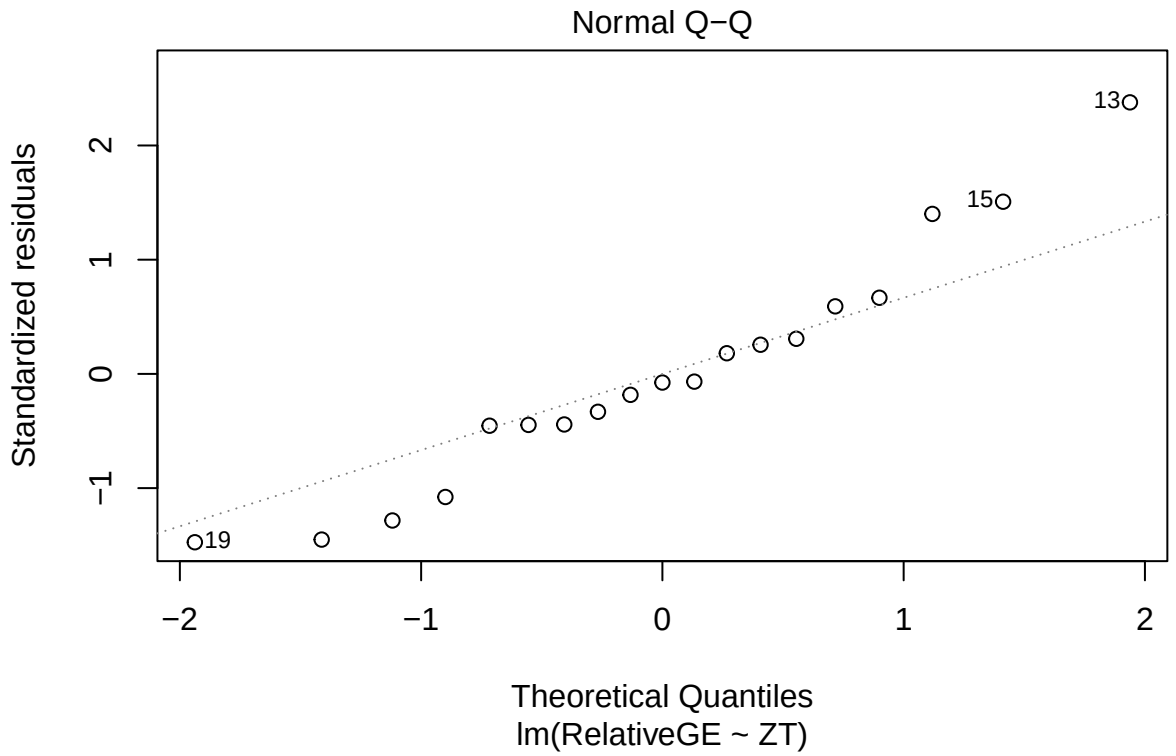
```
## # A tibble: 7 x 7
## # Groups:   ZT [5]
##   ZT    Light     n  mean    sd log_mean log_sd
##   <fct> <fct> <int> <dbl> <dbl>    <dbl>  <dbl>
## 1 2     light     6 0.987 0.364  -0.0680  0.361
## 2 6     light     7 2.21  1.52    0.574   0.721
## 3 6     dark      3 2.06  0.706   0.686   0.321
## 4 10    light     6 1.08  0.381   0.0231  0.365
## 5 18    dark      6 1.56  1.12    0.242   0.694
## 6 19    light     5 1.46  0.361   0.350   0.300
## 7 19    dark      3 0.867 0.105  -0.148   0.126
```

```
## Warning: Ignoring unknown aesthetics: fill
```

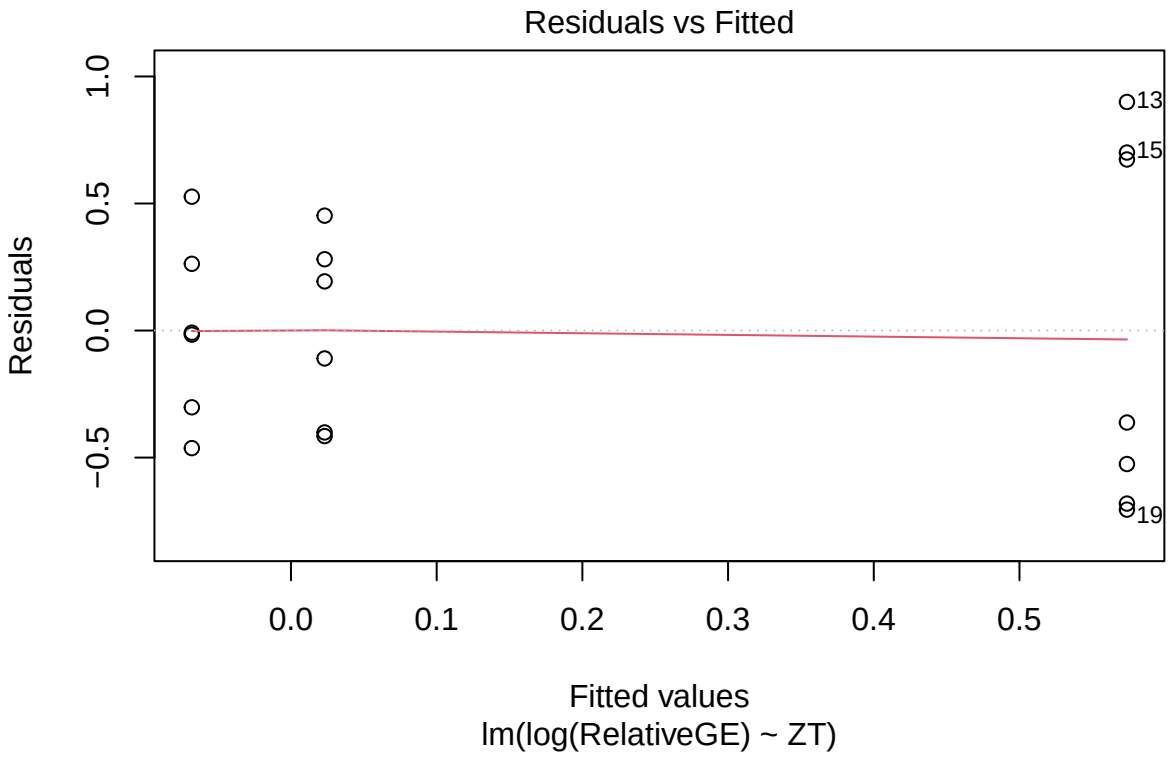


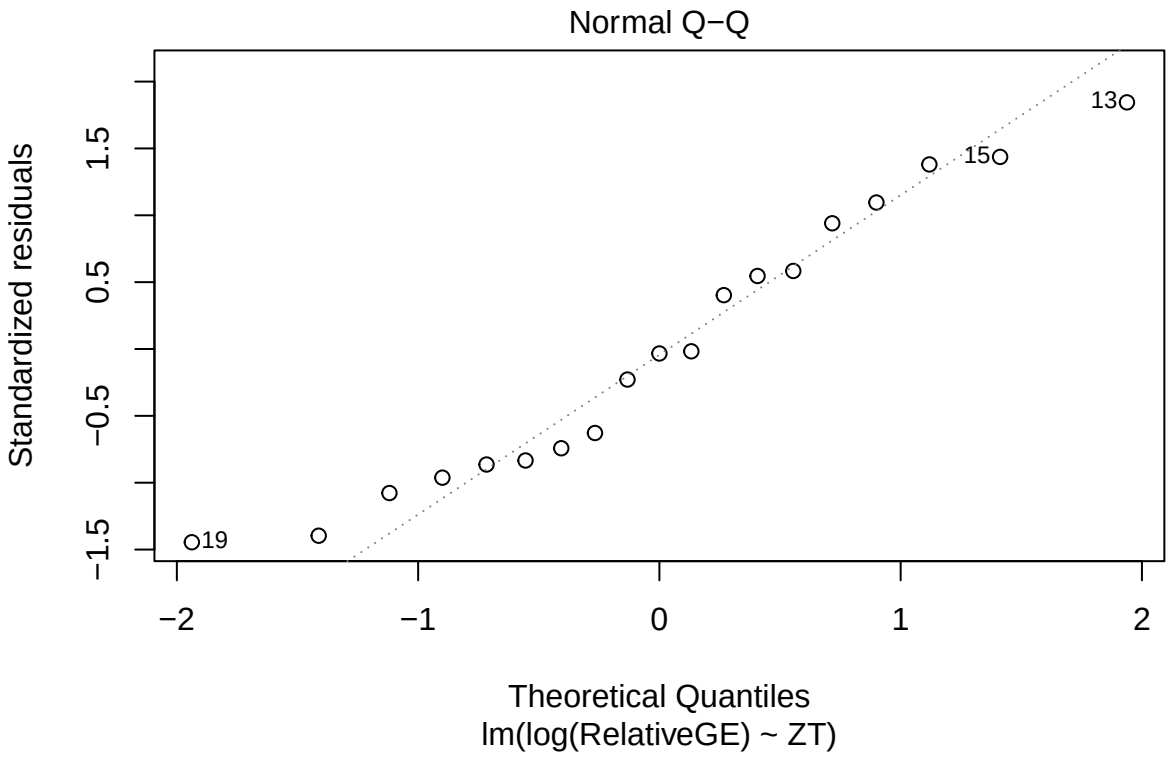

```
##
##  Shapiro-Wilk normality test
##
## data:  m_pomc_day$RelativeGE
## W = 0.70478, p-value = 6.342e-05

## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value  Pr(>F)
## group  2  3.0081 0.07781 .
##       16
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```


Residuals vs Fitted
Residuals
2
1
0
−1
13
15
19
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Fitted values
lm(RelativeGE ~ ZT)



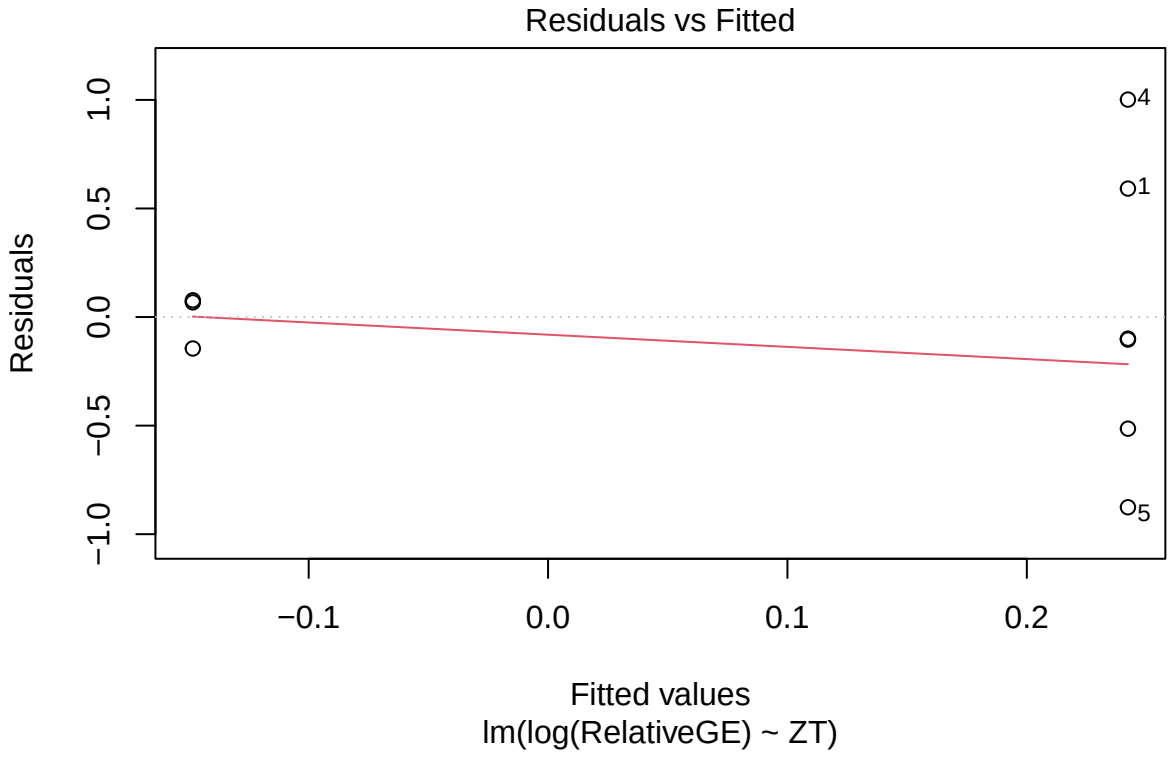

```
##
##  Shapiro-Wilk normality test
##
## data:  log(m_pomc_day$RelativeGE)
## W = 0.87315, p-value = 0.01635

## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  2  1.8683 0.1865
##       16
```

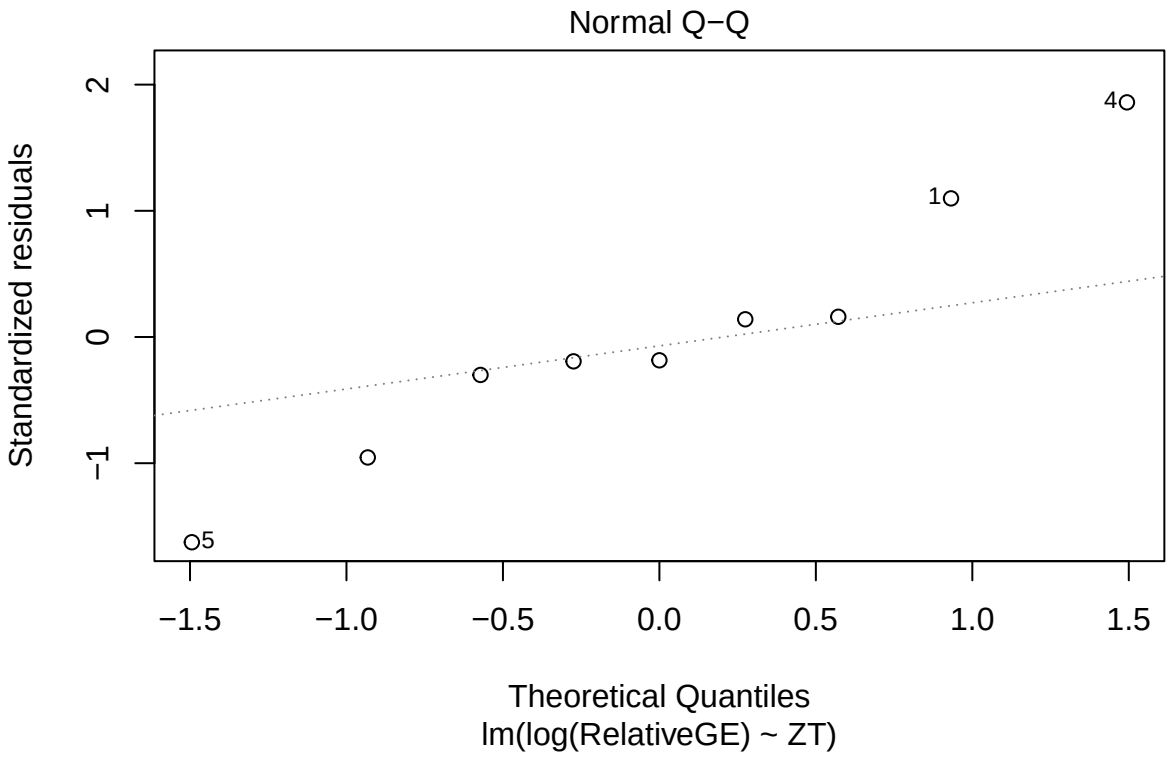

Residuals vs Fitted
Residuals
1.0
0.5
0.0
−0.5
13
15
19
0.0
0.1
0.2
0.3
0.4
0.5
Fitted values
lm(log(RelativeGE) ~ ZT)



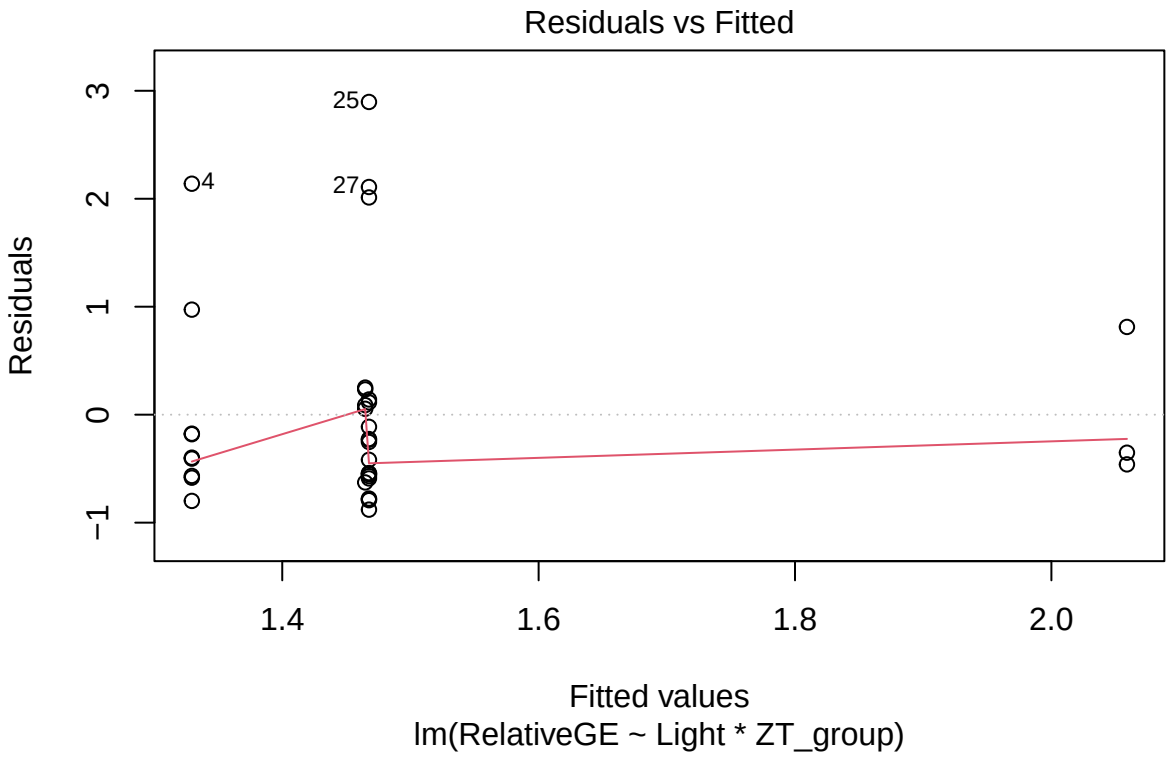

```
## Analysis of Variance Table
##
## Response: log(RelativeGE)
##           Df Sum Sq Mean Sq F value  Pr(>F)
## ZT         2 1.5965 0.79826  2.8768 0.08565 .
## Residuals 16 4.4398 0.27749
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

##  contrast estimate    SE df t.ratio p.value
##  2 - 6     -0.6418 0.293 16  -2.190  0.1035
##  2 - 10    -0.0911 0.304 16  -0.300  0.9519
##  6 - 10     0.5507 0.293 16   1.879  0.1769
##
## Results are given on the log (not the response) scale.
## P value adjustment: tukey method for comparing a family of 3 estimates

##
##  Shapiro-Wilk normality test
##
## data:  log(m_pomc_night$RelativeGE)
## W = 0.90321, p-value = 0.2712

## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
```

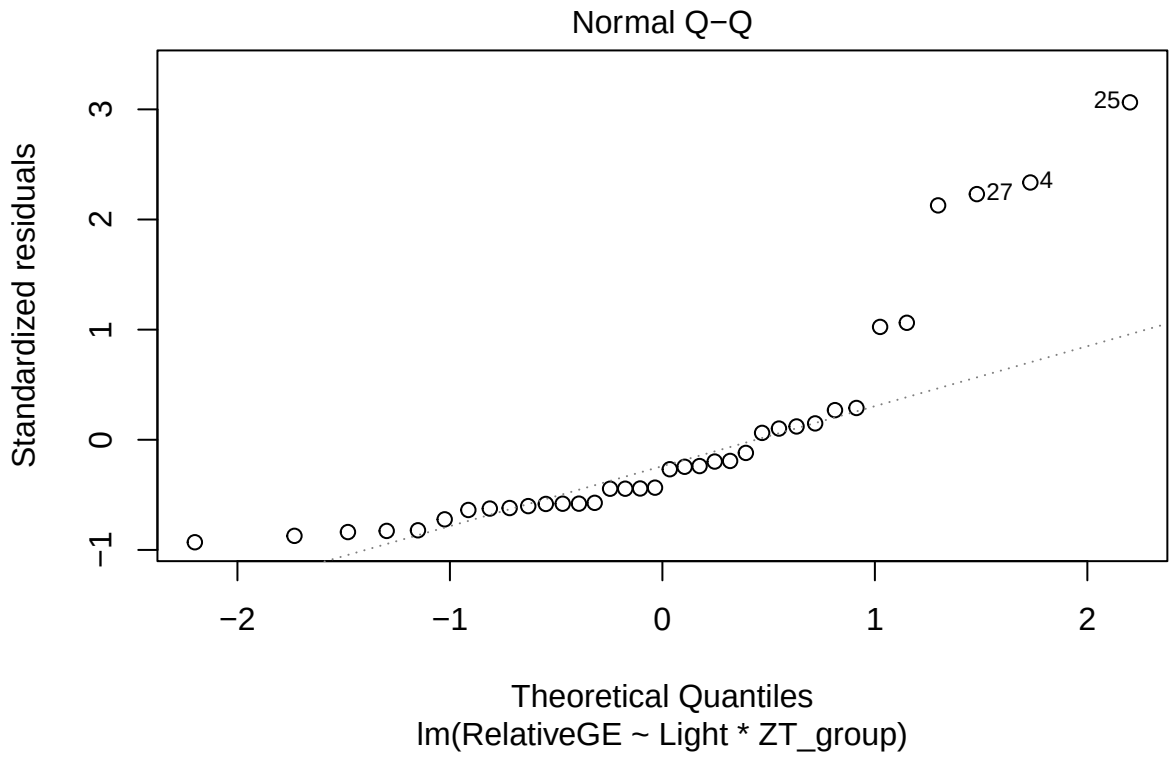
```
## group  1  2.4951 0.1582
##        7
```



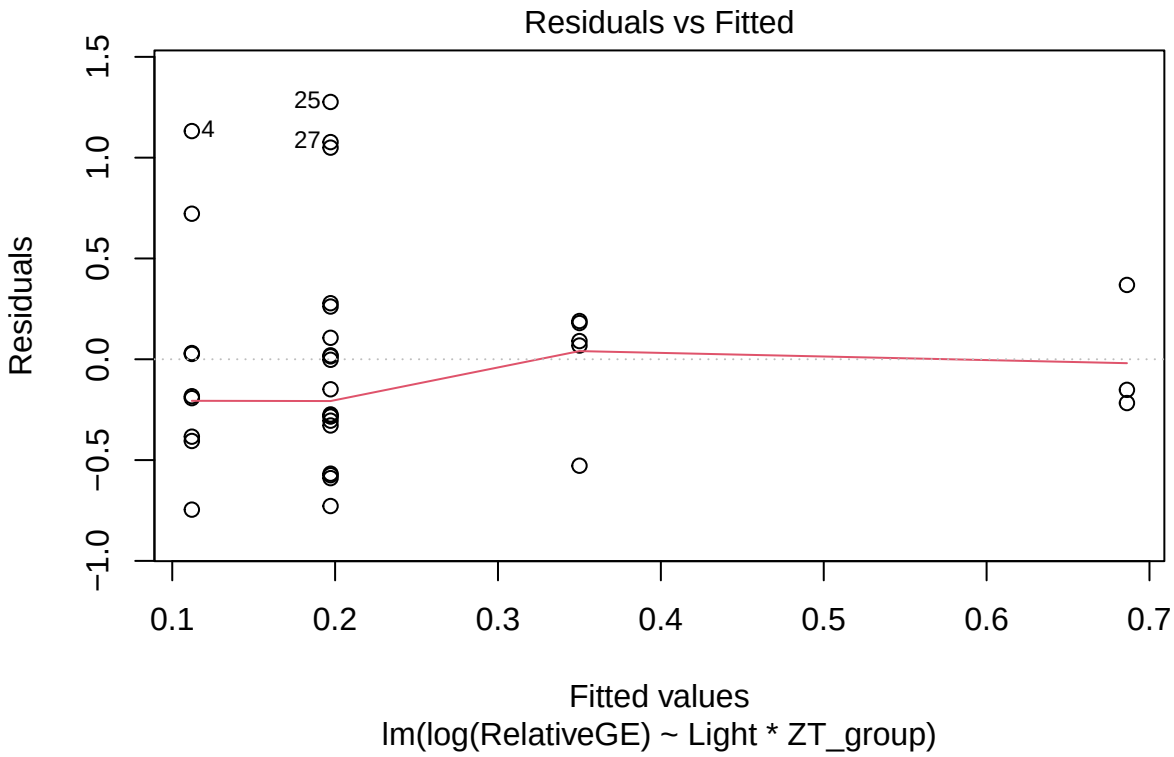


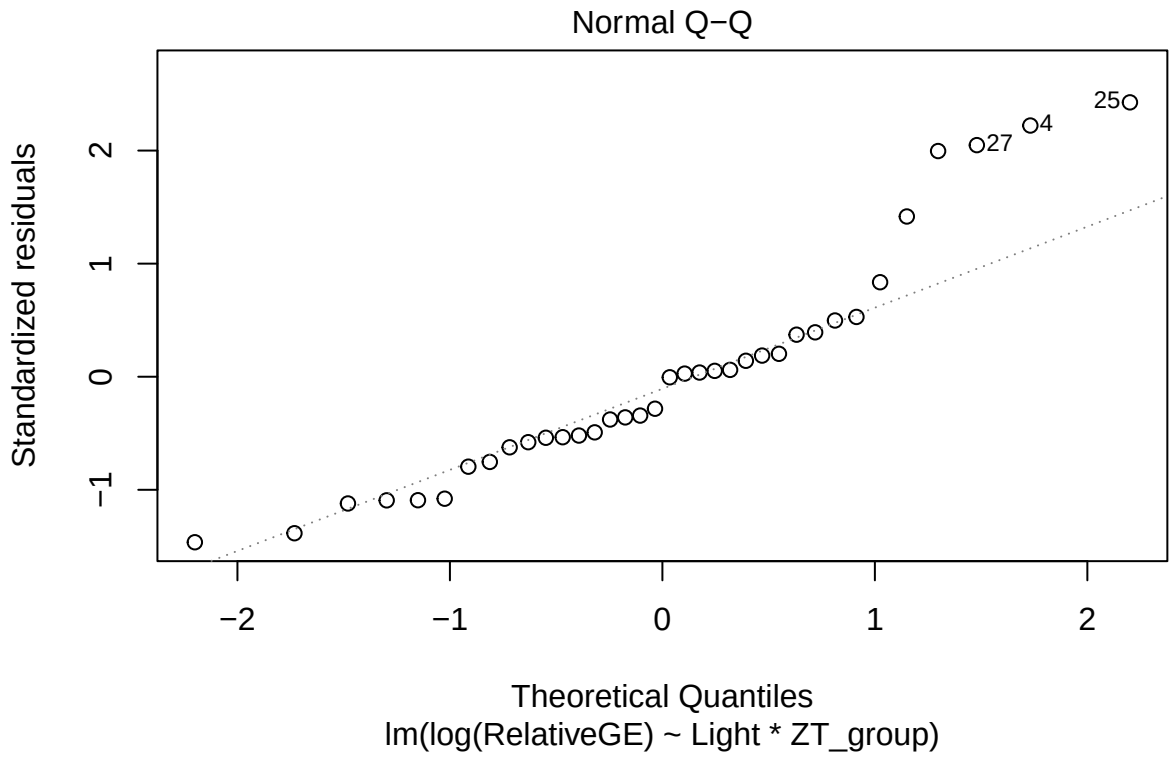

```
## Analysis of Variance Table
##
## Response: log(RelativeGE)
##           Df  Sum Sq Mean Sq F value Pr(>F)
## ZT         1 0.30519 0.30519  0.8766 0.3803
## Residuals  7 2.43701 0.34814

##  contrast estimate    SE df t.ratio p.value
##  18 - 19     0.391 0.417  7   0.936  0.3803
##
## Results are given on the log (not the response) scale.

##
##  Shapiro-Wilk normality test
##
## data:  m_pomc_all$RelativeGE
## W = 0.78974, p-value = 1.025e-05

## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  1  0.3413  0.563
##       34
```


Residuals vs Fitted
Residuals
Fitted values
lm(RelativeGE ~ Light * ZT_group)
25
27
4
3
2
1
0
−1
1.4
1.6
1.8
2.0




```
##  [1] dark  dark  dark  dark  dark  dark  light light light light light light
## [13] light light light light light light dark  dark  dark  dark  dark  dark
## [25] light light light light light light light light light light light light
## Levels: light dark

##
##  Shapiro-Wilk normality test
##
## data:  log(m_pomc_all$RelativeGE)
## W = 0.94191, p-value = 0.05829

## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  1  0.1345 0.7161
##       34

## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  1  0.2034 0.6548
##       34
```

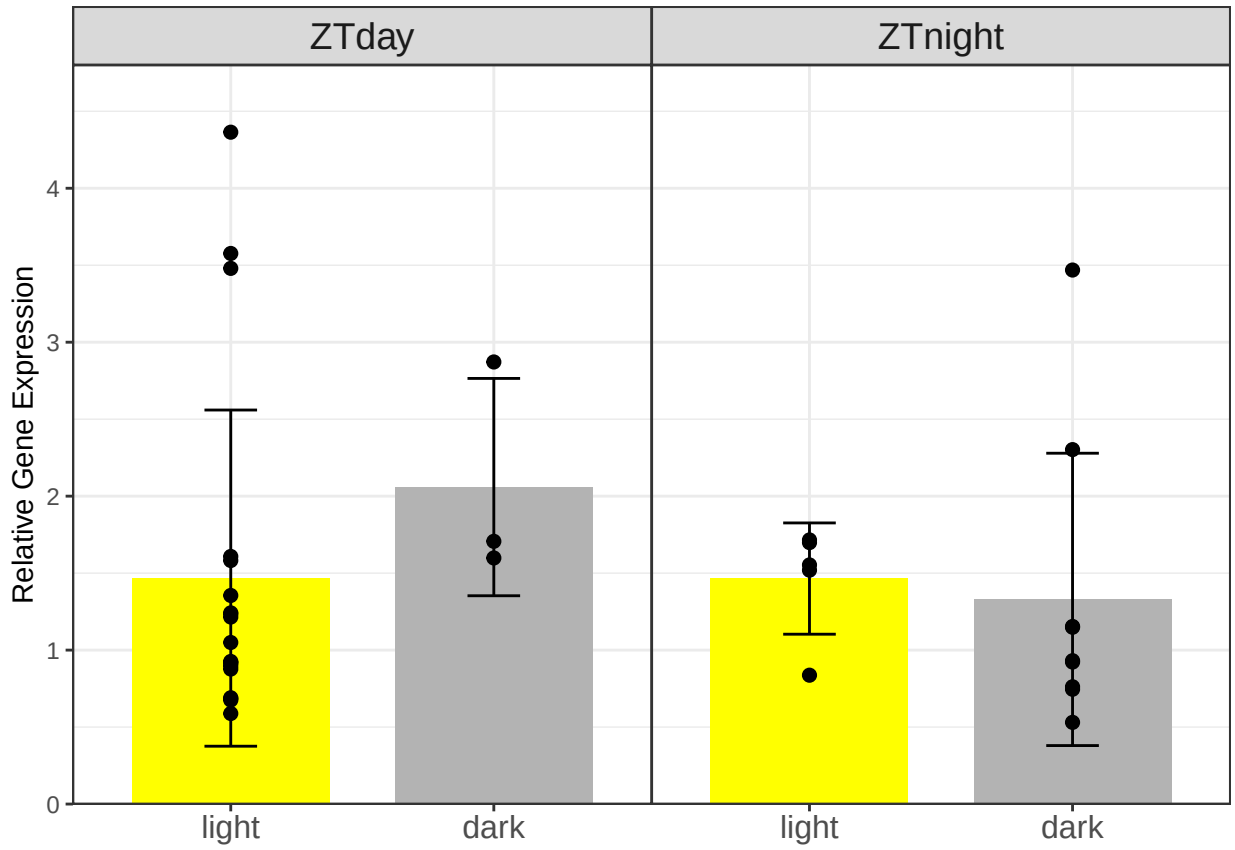

Residuals vs Fitted
Residuals
Fitted values
lm(log(RelativeGE) ~ Light * ZT_group)
25
27
4
1.5
1.0
0.5
0.0
−0.5
−1.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7



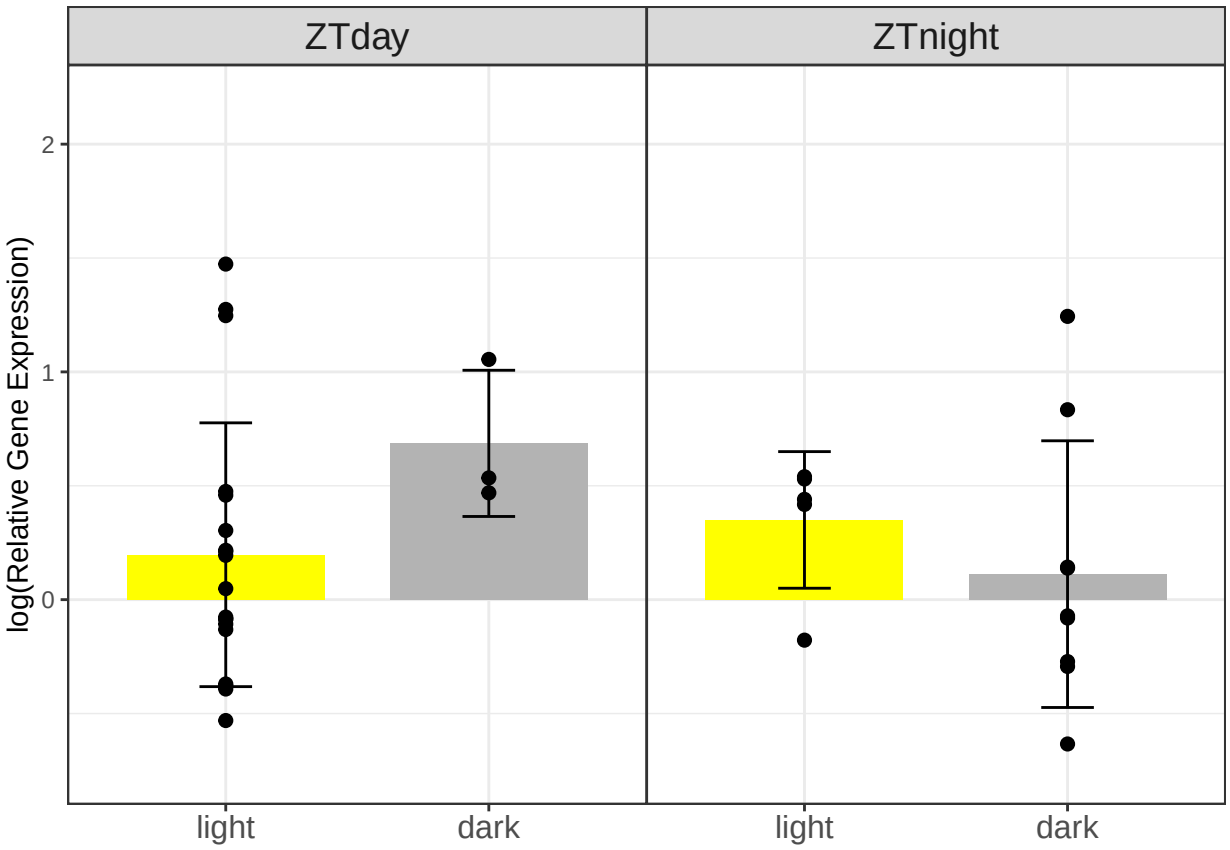

```
## Analysis of Variance Table
##
## Response: log(RelativeGE)
##                Df Sum Sq Mean Sq F value Pr(>F)
## Light           1 0.0056 0.00563  0.0193 0.8904
## ZT_group        1 0.0760 0.07598  0.2602 0.6135
## Light:ZT_group  1 0.7582 0.75816  2.5964 0.1169
## Residuals      32 9.3443 0.29201
```

```
## # A tibble: 4 x 7
## # Groups:   ZT_group [2]
##   ZT_group Light     n  mean    sd log_mean log_sd
##   <fct>    <fct> <int> <dbl> <dbl>    <dbl>  <dbl>
## 1 ZTday    light    19  1.47 1.09     0.197  0.579
## 2 ZTday    dark      3  2.06 0.706    0.686  0.321
## 3 ZTnight  light     5  1.46 0.361    0.350  0.300
## 4 ZTnight  dark      9  1.33 0.949    0.112  0.585
```

```
## Warning: Ignoring unknown aesthetics: fill
```




```
## Warning: Ignoring unknown aesthetics: fill
```


ZTday
ZTnight
log(Relative Gene Expression)
2
1
0
light
dark
light
dark