qpCR-Figures

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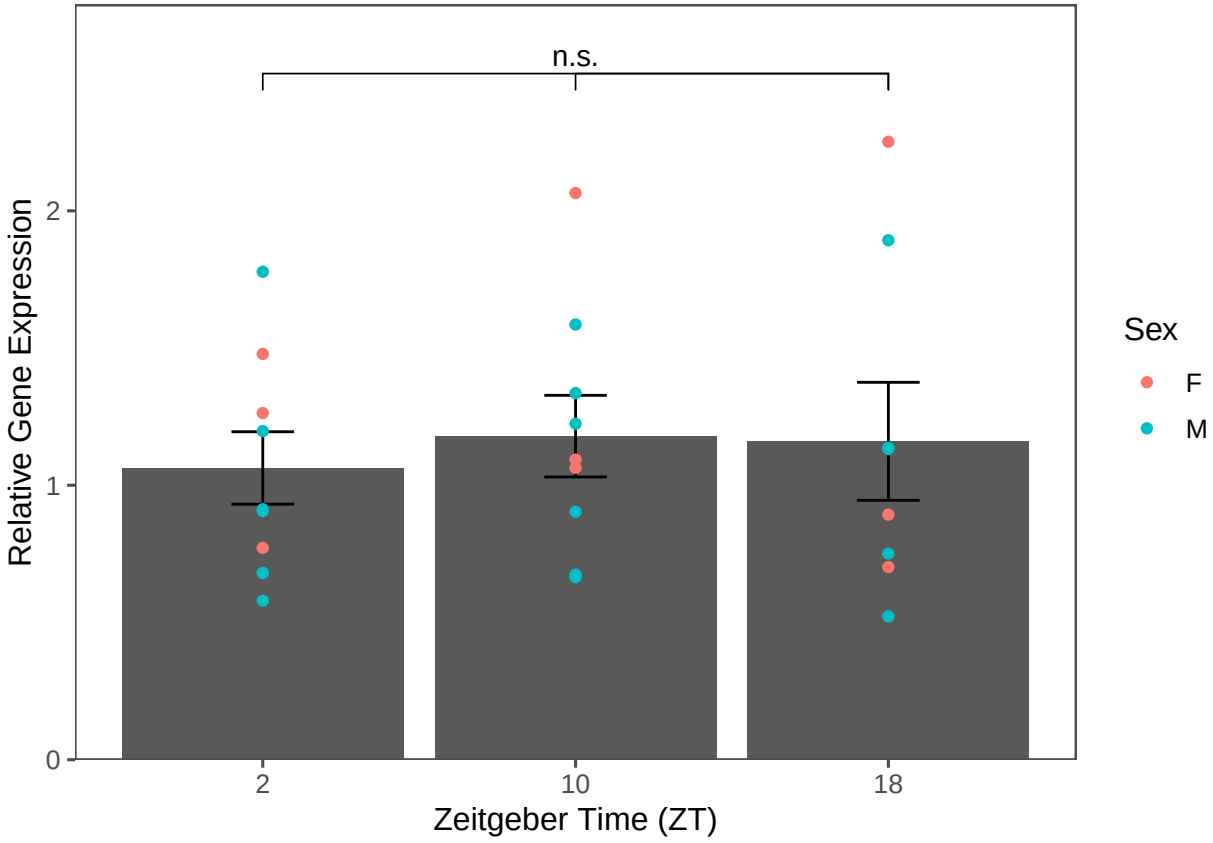
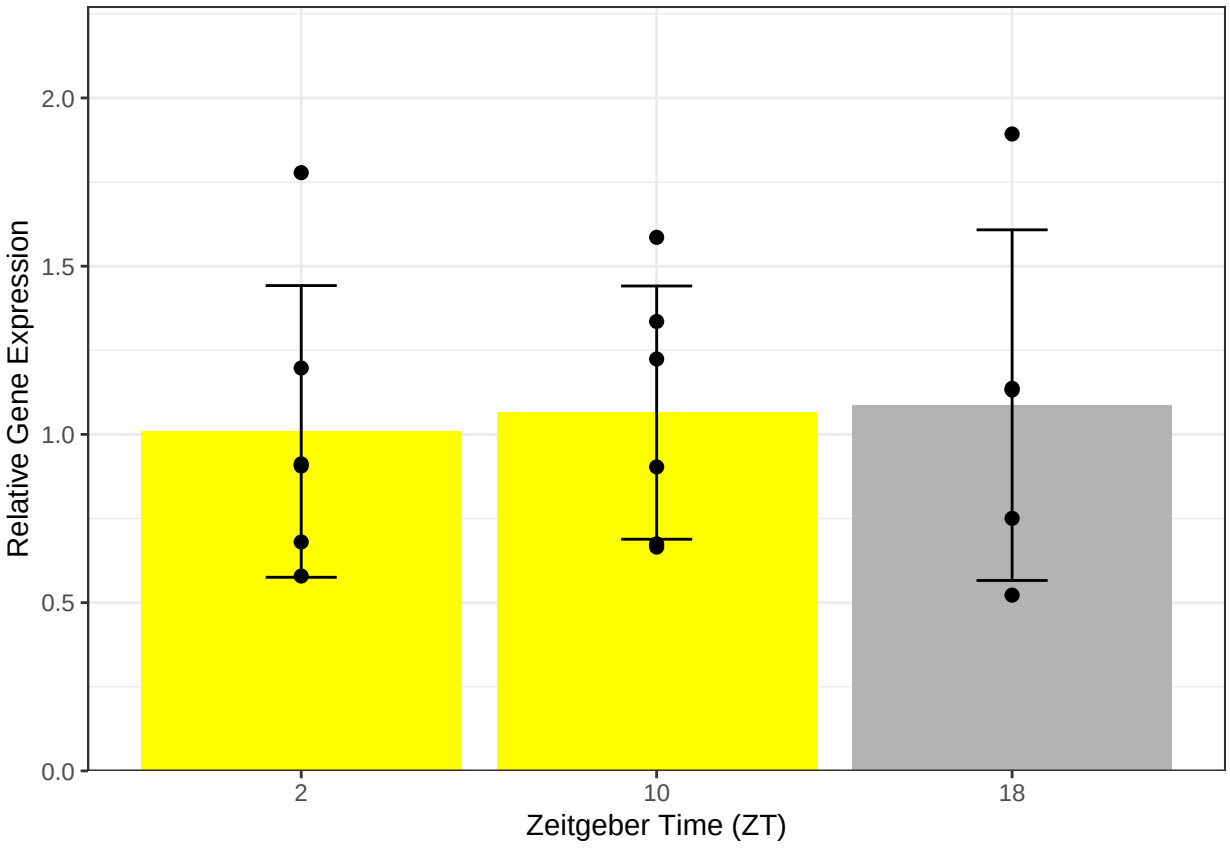
10/30/2021

Is POMC under circadian regulation?

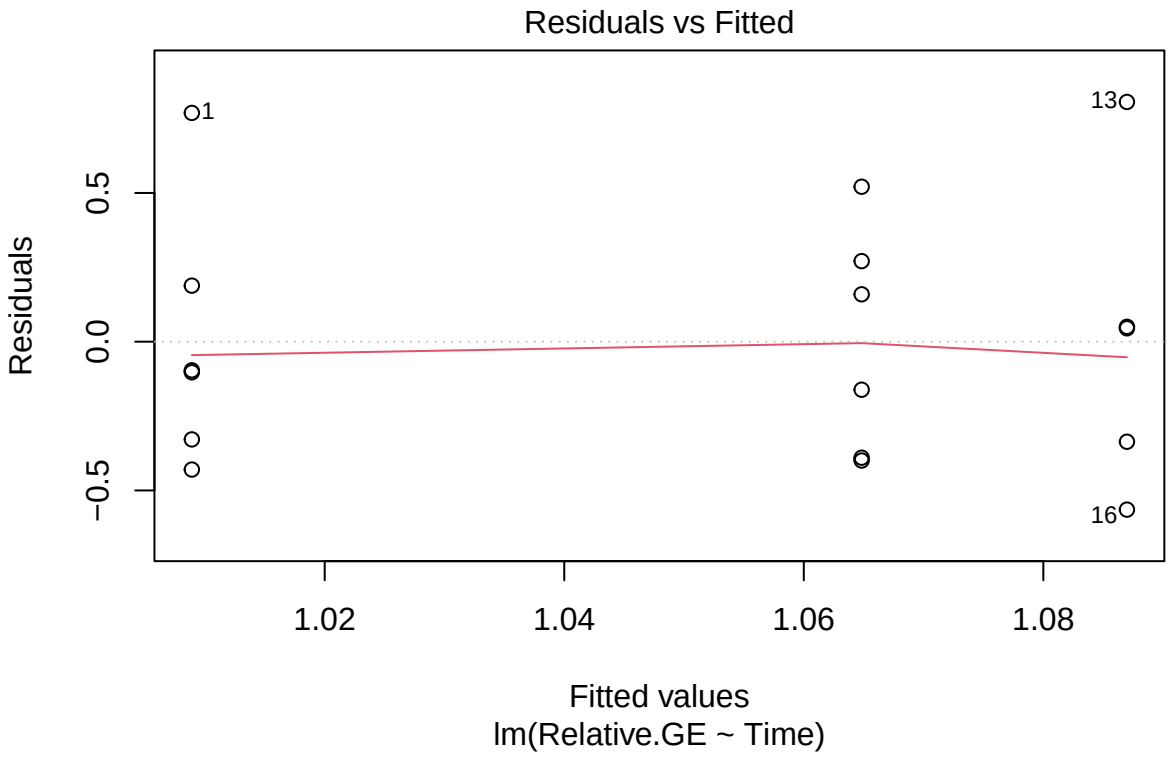


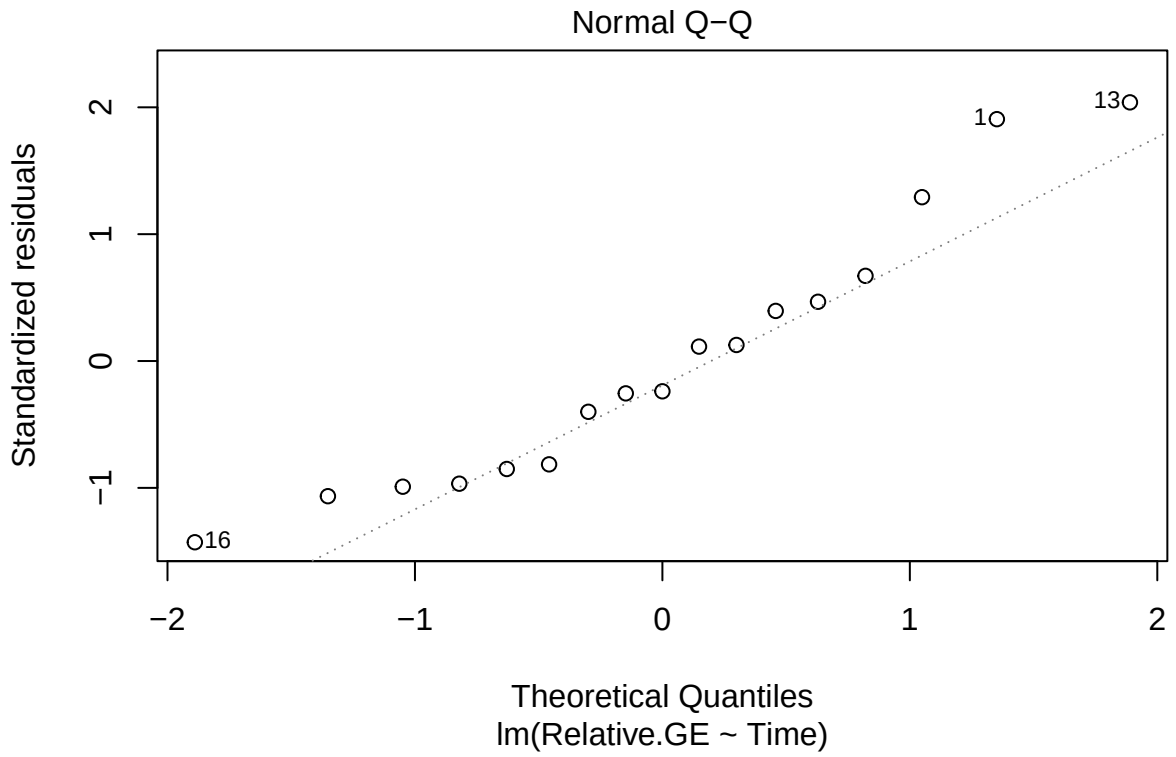

```
## # A tibble: 3 x 4
##   Time      n  mean    sd
##   <fct> <int> <dbl> <dbl>
## 1 2         6  1.01 0.433
## 2 10        6  1.06 0.376
## 3 18        5  1.09 0.521
```




```
##
##  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
```

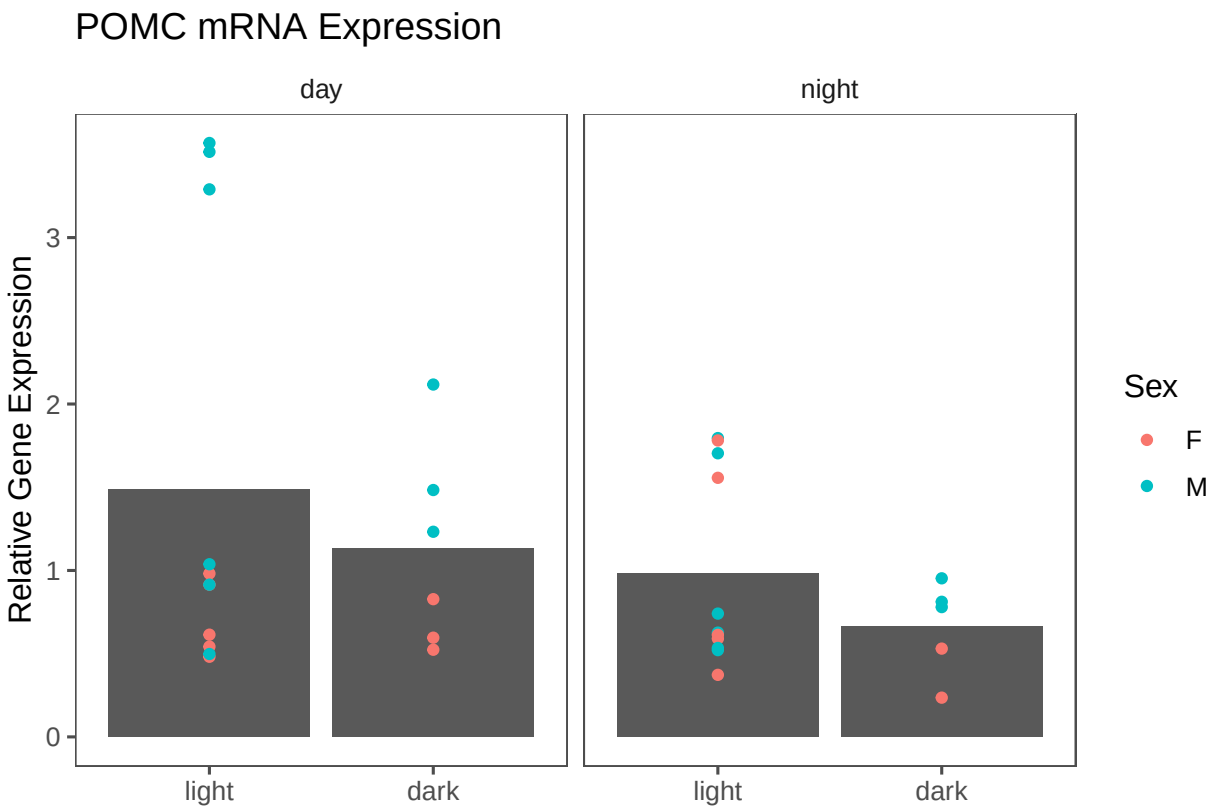
```
## [1] 0.009116243
```

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

```
##
##      Balanced one-way analysis of variance power calculation
##
##           groups = 3
```

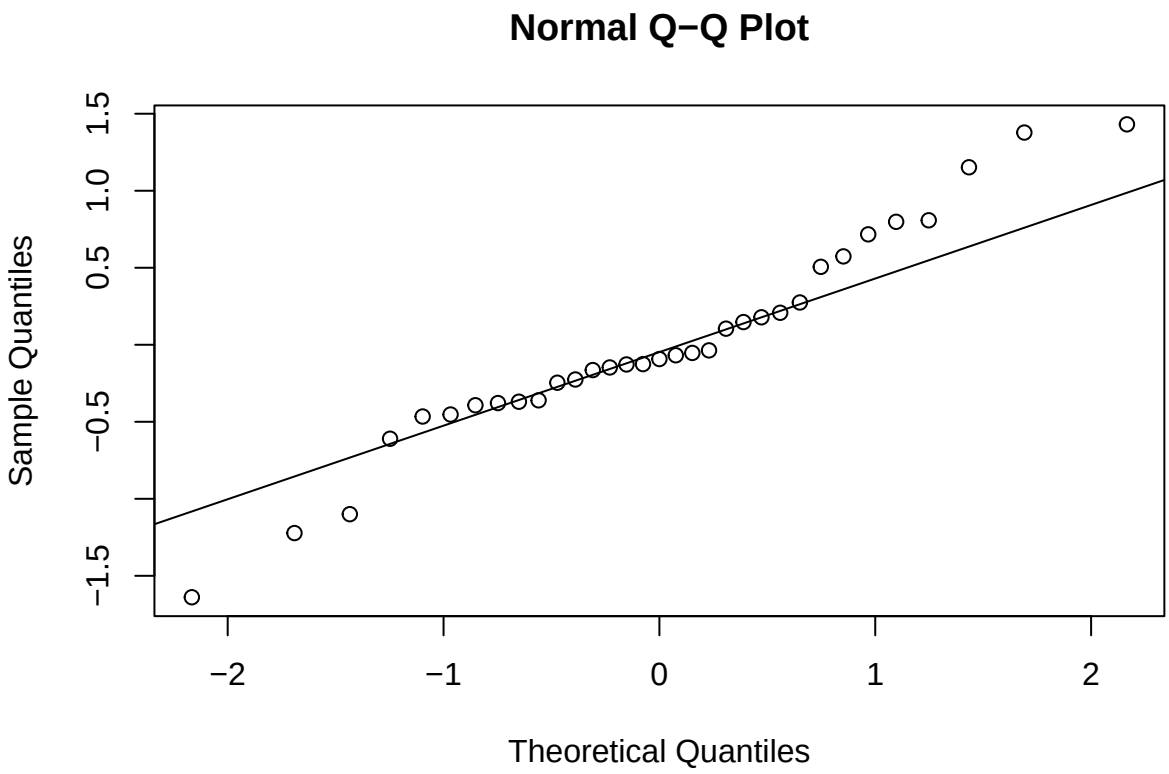
```
##               n = 5
##     between.var = 0.009116243
##      within.var = 0.1952332
##       sig.level = 0.05
##           power = 0.07865971
##
## NOTE: n is number in each group
```

Is POMC expression light-driven?




```
##
##  Shapiro-Wilk normality test
##
## data:  pomc_ld$Relative.G.E.
## W = 0.76124, p-value = 6.387e-06
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value    Pr(>F)
## group  7  7.2148 9.222e-05 ***
##       25
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

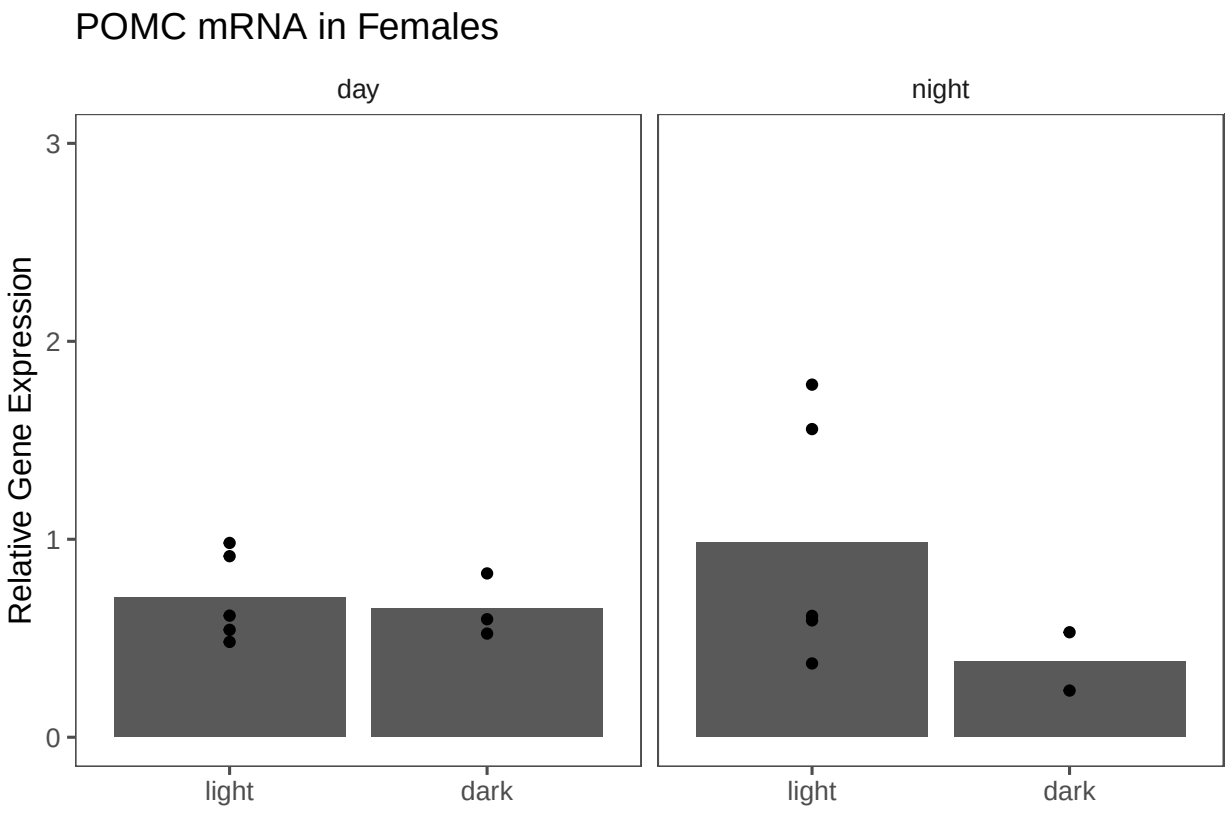


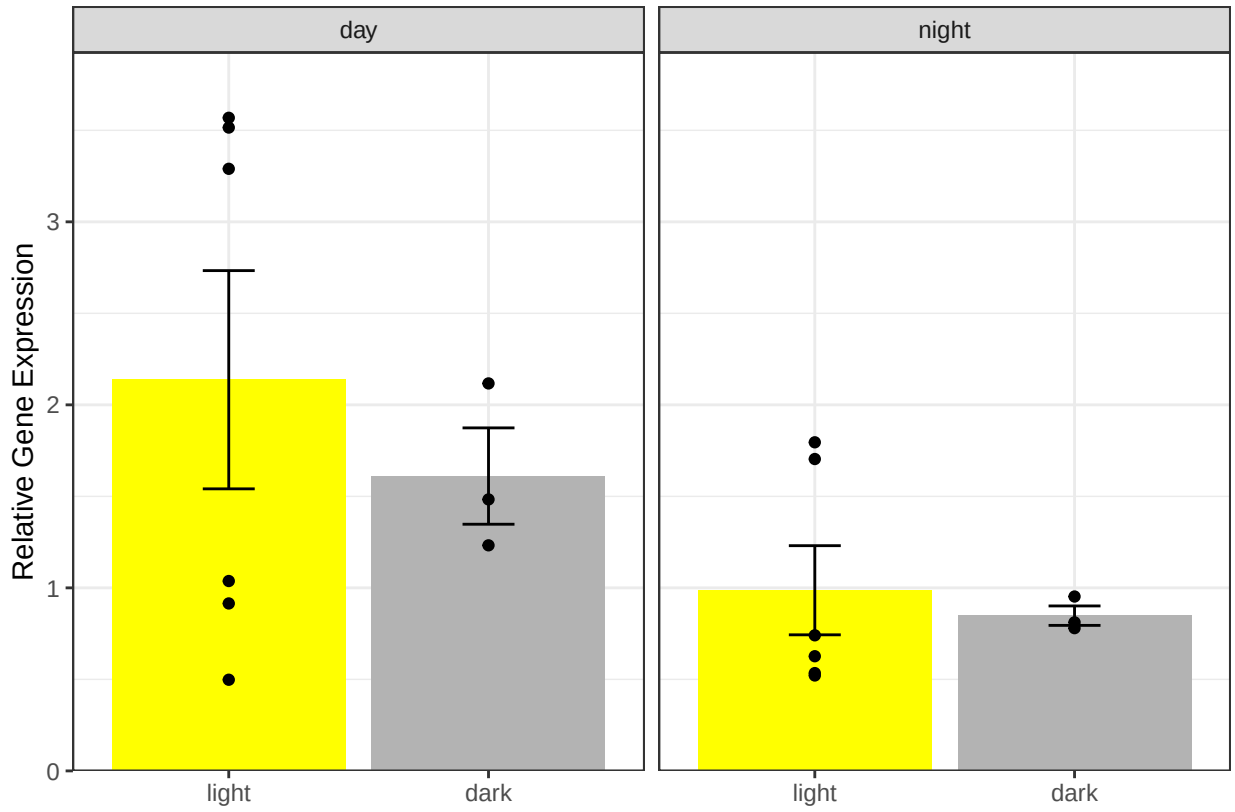

```
## Analysis of Variance Table
##
## Response: Relative.G.E.
##                Df  Sum Sq Mean Sq F value  Pr(>F)
## Sex             1  4.0914  4.0914  6.9085 0.01446 *
## Light           1  0.7450  0.7450  1.2580 0.27270
## Time            1  2.1772  2.1772  3.6763 0.06669 .
## Sex:Light       1  0.0008  0.0008  0.0014 0.97091
## Sex:Time        1  2.5523  2.5523  4.3096 0.04834 *
## Light:Time      1  0.0015  0.0015  0.0025 0.96038
## Sex:Light:Time  1  0.3867  0.3867  0.6530 0.42667
## Residuals      25 14.8058  0.5922
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = pomc_ld_lm)
##
## $Sex
##          diff      lwr      upr     p adj
## M-F 0.7071524 0.153048 1.261257 0.0144557
##
## $Light
##                  diff        lwr       upr     p adj
## dark-light -0.3187344 -0.9040173 0.2665485 0.2726958
```

```
##
## $Time
##                 diff       lwr        upr     p adj
## night-day -0.5131982 -1.065262 0.03886529 0.0670614
##
## $`Sex:Light`
##                        diff        lwr        upr     p adj
## M:light-F:light  0.7001534 -0.2062084 1.60651528 0.1728438
## F:dark-F:light  -0.3301872 -1.4896092 0.82923474 0.8612768
## M:dark-F:light   0.3909631 -0.7021504 1.48407659 0.7598424
## F:dark-M:light  -1.0303407 -2.1570963 0.09641495 0.0818368
## M:dark-M:light  -0.3091904 -1.3675930 0.74921224 0.8521175
## M:dark-F:dark    0.7211503 -0.5606389 2.00293950 0.4255743
##
## $`Sex:Time`
##                         diff        lwr         upr     p adj
## M:day-F:day      1.26278520  0.2342028  2.29136764 0.0120273
## F:night-F:day    0.09714678 -0.9984040  1.19269755 0.9947655
## M:night-F:day    0.24218992 -0.7863925  1.27077236 0.9153692
## F:night-M:day   -1.16563842 -2.2324080 -0.09886887 0.0284881
## M:night-M:day   -1.02059528 -2.0184668 -0.02272374 0.0436577
## M:night-F:night  0.14504315 -0.9217264  1.21181269 0.9817488
##
## $`Light:Time`
##                               diff       lwr       upr     p adj
## dark:day-light:day     -0.3295261 -1.403845 0.7447932 0.8331289
## light:night-light:day  -0.5052644 -1.407873 0.3973443 0.4299273
## dark:night-light:day   -0.8615752 -2.003295 0.2801446 0.1885459
## light:night-dark:day   -0.1757383 -1.250058 0.8985810 0.9690230
## dark:night-dark:day    -0.5320491 -1.813838 0.7497401 0.6678291
## dark:night-light:night -0.3563108 -1.498031 0.7854090 0.8259208
##
## $`Sex:Light:Time`
##                                      diff        lwr       upr     p adj
## M:light:day-F:light:day      1.430334237 -0.1075391 2.9682076 0.0817383
## F:dark:day-F:light:day      -0.057760479 -1.9125056 1.7969846 1.0000000
## M:dark:day-F:light:day       0.904032359 -0.9507127 2.7587774 0.7410782
## F:light:night-F:light:day    0.275842930 -1.3304134 1.8820993 0.9990020
## M:light:night-F:light:day    0.280129724 -1.2577437 1.8180031 0.9985453
## F:dark:night-F:light:day    -0.323835643 -2.4487131 1.8010418 0.9995401
## M:dark:night-F:light:day     0.141258874 -1.7134862 1.9960040 0.9999957
## F:dark:day-M:light:day      -1.488094717 -3.2839439 0.3077545 0.1583872
## M:dark:day-M:light:day      -0.526301878 -2.3221511 1.2695473 0.9751004
## F:light:night-M:light:day   -1.154491308 -2.6923647 0.3833821 0.2500589
## M:light:night-M:light:day   -1.150204513 -2.6165092 0.3161002 0.2064938
## F:dark:night-M:light:day    -1.754169880 -3.8278379 0.3194982 0.1421939
## M:dark:night-M:light:day    -1.289075364 -3.0849246 0.5067738 0.2984509
## M:dark:day-F:dark:day        0.961792839 -1.1118752 3.0354609 0.7842025
## F:light:night-F:dark:day     0.333603409 -1.5211417 2.1883485 0.9986579
## M:light:night-F:dark:day     0.337890204 -1.4579590 2.1337394 0.9982137
## F:dark:night-F:dark:day     -0.266075163 -2.5845065 2.0523562 0.9999303
## M:dark:night-F:dark:day      0.199019353 -1.8746487 2.2726874 0.9999792
## F:light:night-M:dark:day    -0.628189430 -2.4829345 1.2265556 0.9466955
## M:light:night-M:dark:day    -0.623902635 -2.4197518 1.1719466 0.9394779
```

```
## F:dark:night-M:dark:day     -1.227868002 -3.5462994 1.0905633 0.6580686
## M:dark:night-M:dark:day     -0.762773486 -2.8364415 1.3108946 0.9201432
## M:light:night-F:light:night  0.004286795 -1.5335866 1.5421602 1.0000000
## F:dark:night-F:light:night  -0.599678572 -2.7245560 1.5251989 0.9797791
## M:dark:night-F:light:night  -0.134584056 -1.9893291 1.7201610 0.9999970
## F:dark:night-M:light:night  -0.603965367 -2.6776334 1.4697027 0.9759316
## M:dark:night-M:light:night  -0.138870851 -1.9347201 1.6569783 0.9999953
## M:dark:night-F:dark:night    0.465094516 -1.8533368 2.7835259 0.9973289
```

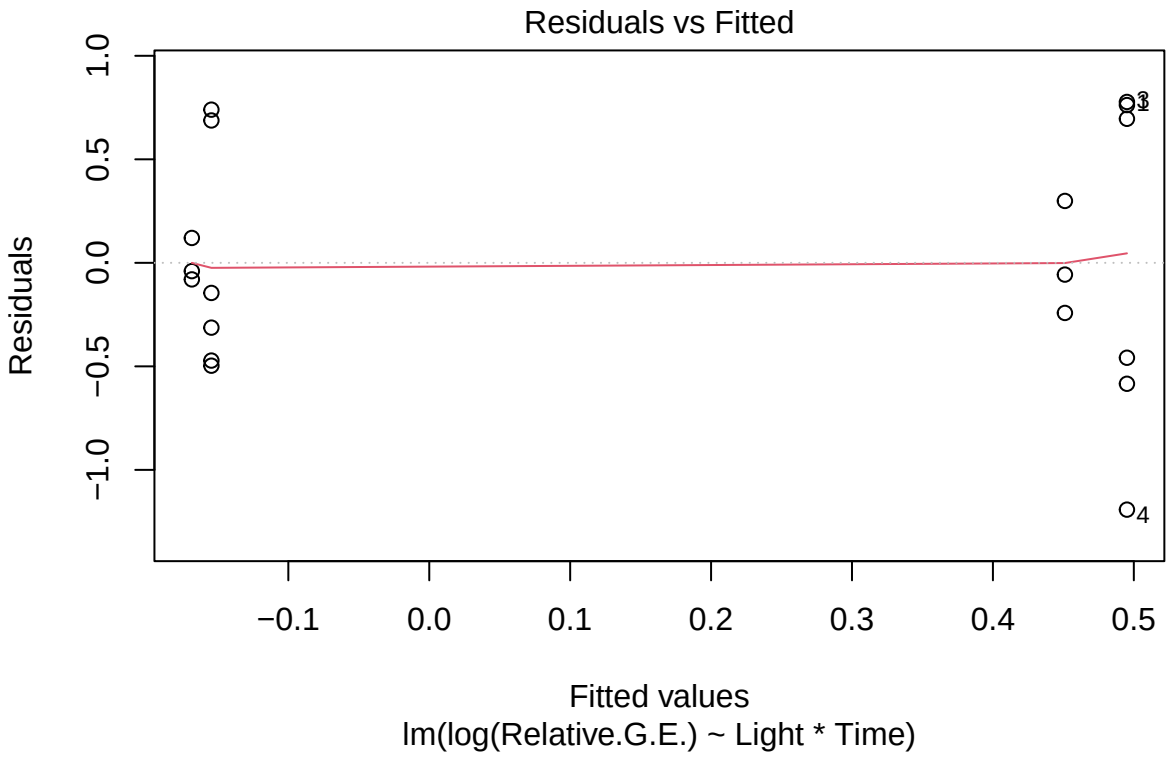


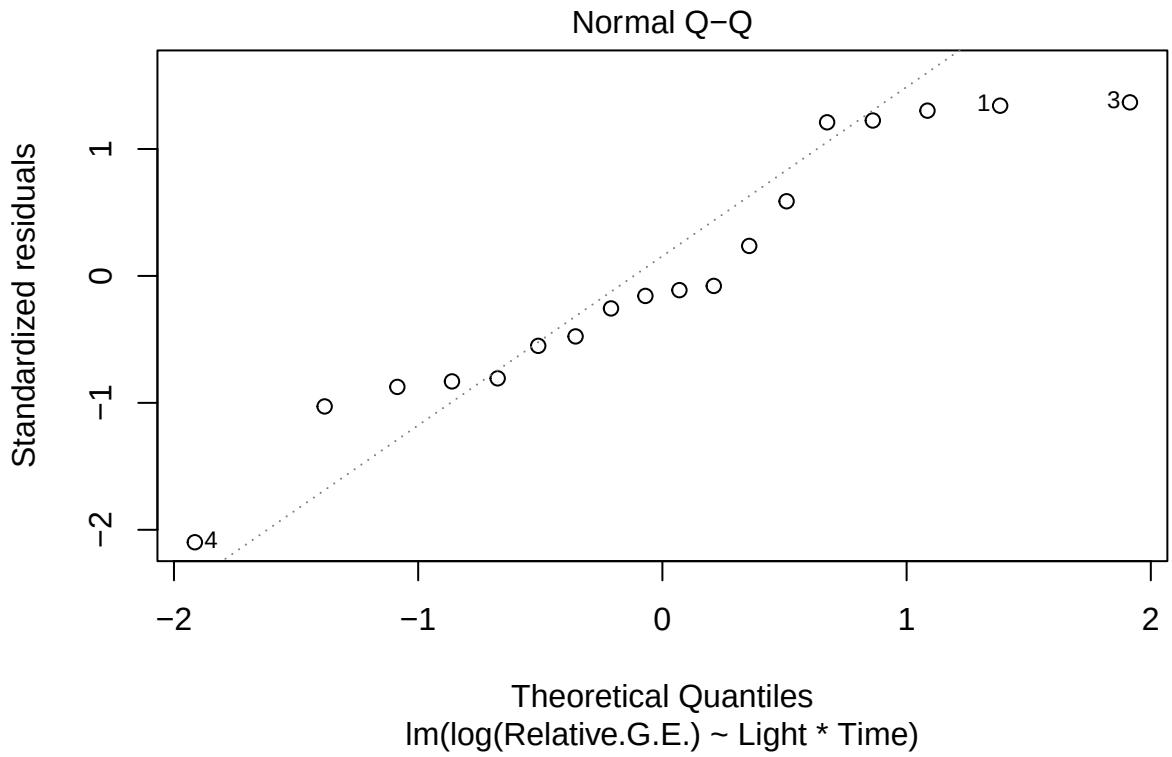



```
##
##  Shapiro-Wilk normality test
##
## data:  pomc_ld_m$Relative.G.E.
## W = 0.80779, p-value = 0.001961
```

```
##
##  Shapiro-Wilk normality test
##
## data:  log(pomc_ld_m$Relative.G.E.)
## W = 0.92574, p-value = 0.1633
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value  Pr(>F)
## group  3  4.2211 0.02538 *
##       14
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```


Residuals vs Fitted
Residuals
1.0
0.5
0.0
−0.5
−1.0
−0.1
0.0
0.1
0.2
0.3
0.4
0.5
Fitted values
lm(log(Relative.G.E.) ~ Light * Time)



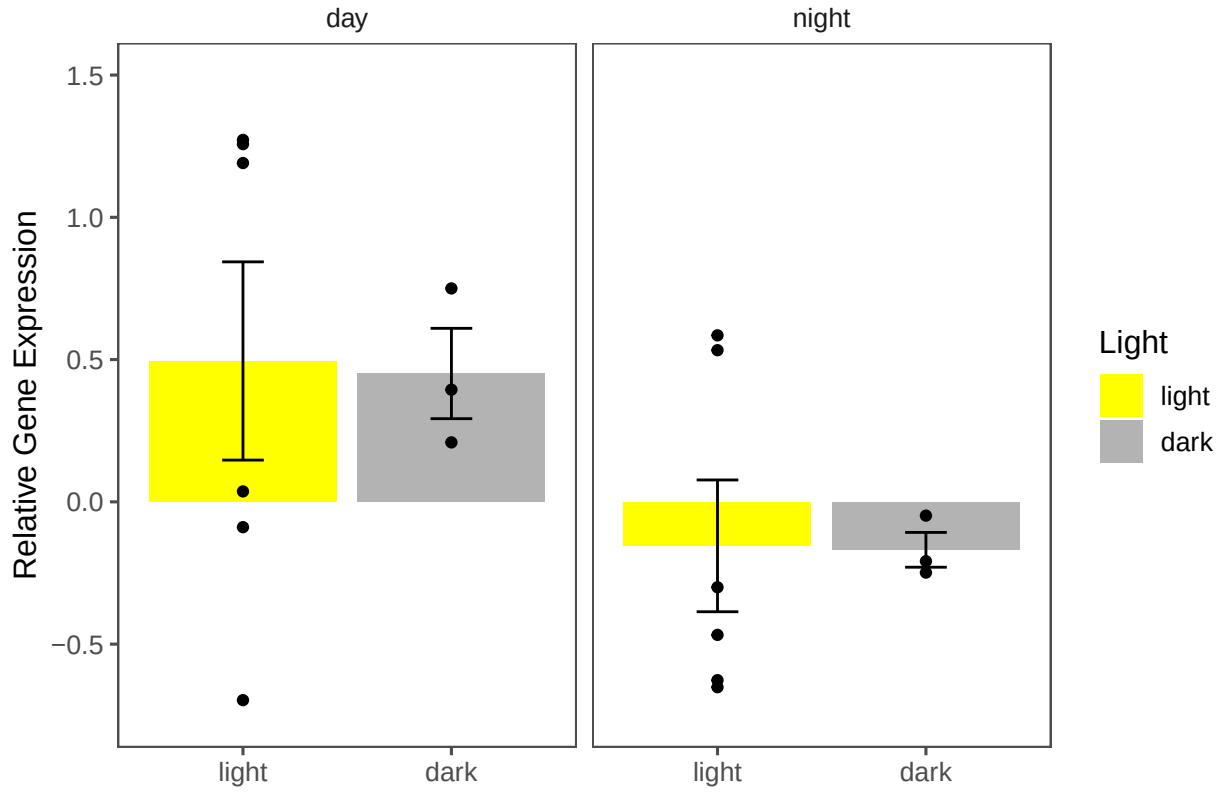

```
## Analysis of Variance Table
##
## Response: log(Relative.G.E.)
##            Df Sum Sq Mean Sq F value  Pr(>F)
## Light       1 0.0034 0.00335  0.0087 0.92719
## Time        1 1.8414 1.84137  4.7538 0.04679 *
## Light:Time  1 0.0009 0.00091  0.0023 0.96204
## Residuals  14 5.4229 0.38735
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##  contrast                estimate    SE df t.ratio p.value
##  light day - dark day       0.0440 0.440 14   0.100  0.9996
##  light day - light night    0.6497 0.359 14   1.808  0.3102
##  light day - dark night     0.6636 0.440 14   1.508  0.4589
##  dark day - light night     0.6057 0.440 14   1.376  0.5332
##  dark day - dark night      0.6196 0.508 14   1.219  0.6254
##  light night - dark night   0.0139 0.440 14   0.032  1.0000
##
## Results are given on the log (not the response) scale.
## P value adjustment: tukey method for comparing a family of 4 estimates
```

```
## Time = day:
##  contrast     estimate   SE df t.ratio p.value
##  light - dark   0.0440 0.44 14   0.100  0.9217
##
## Time = night:
```

```
##  contrast     estimate   SE df t.ratio p.value
##  light - dark   0.0139 0.44 14   0.032  0.9753
##
## Results are given on the log (not the response) scale.
```

```
## Light = light:
##  contrast    estimate    SE df t.ratio p.value
##  day - night     0.65 0.359 14   1.808  0.0921
##
## Light = dark:
##  contrast    estimate    SE df t.ratio p.value
##  day - night     0.62 0.508 14   1.219  0.2429
##
## Results are given on the log (not the response) scale.
```



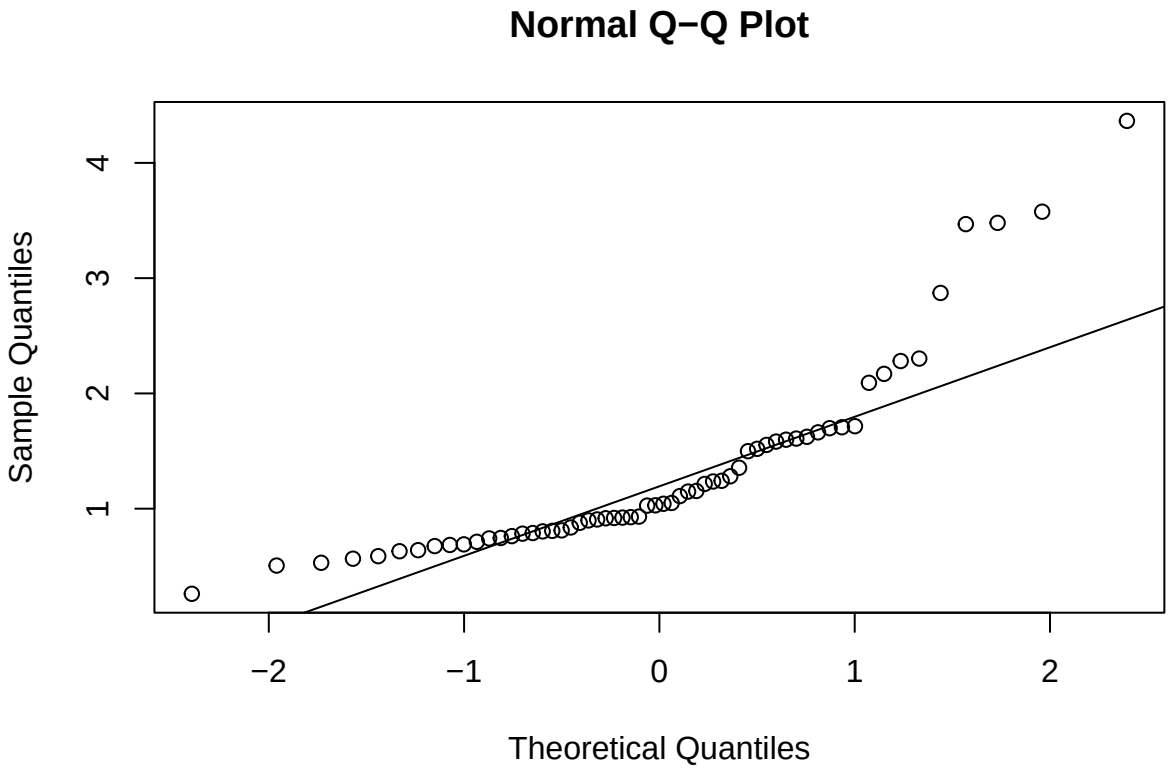

```
## # A tibble: 4 x 5
## # Groups:   Light [2]
##   Light Time      n  mean log_mean
##   <fct> <fct> <int> <dbl>    <dbl>
## 1 light day       6 2.14     0.495
## 2 light night     6 0.987   -0.155
## 3 dark  day       3 1.61     0.451
## 4 dark  night     3 0.848   -0.168
```

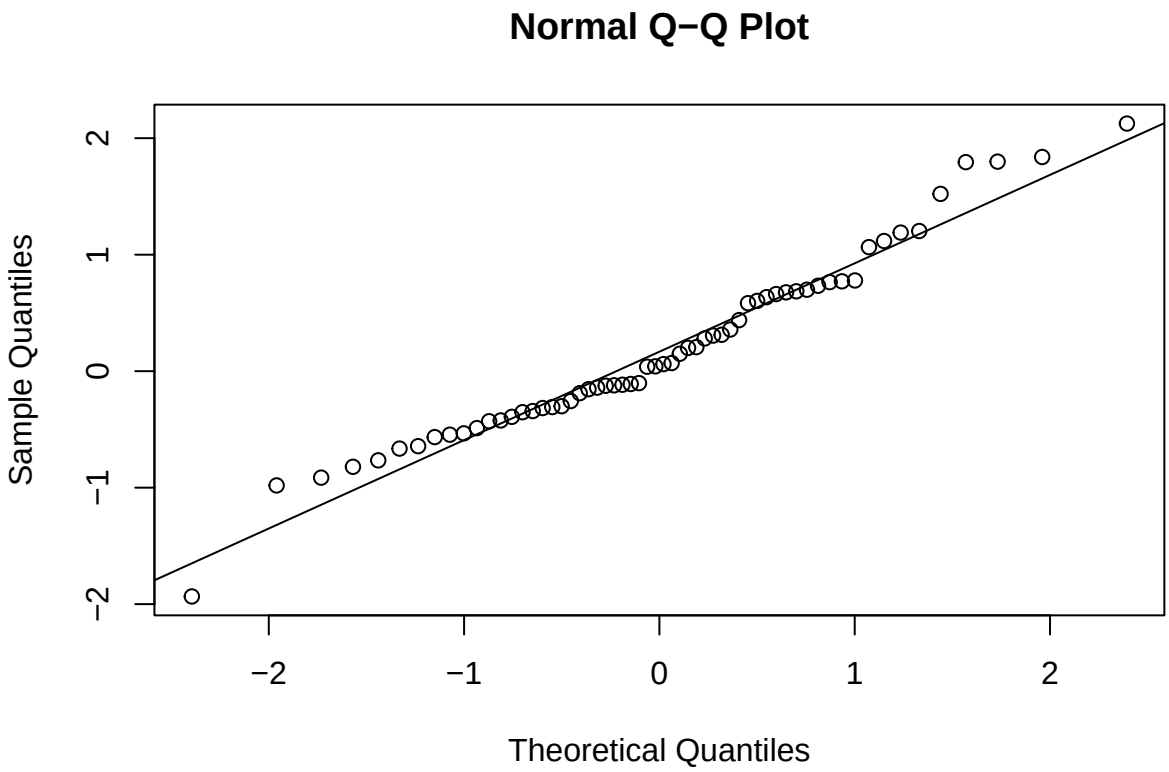
```
## [1] 1.411641
```

```
## [1] 0.3873501
```

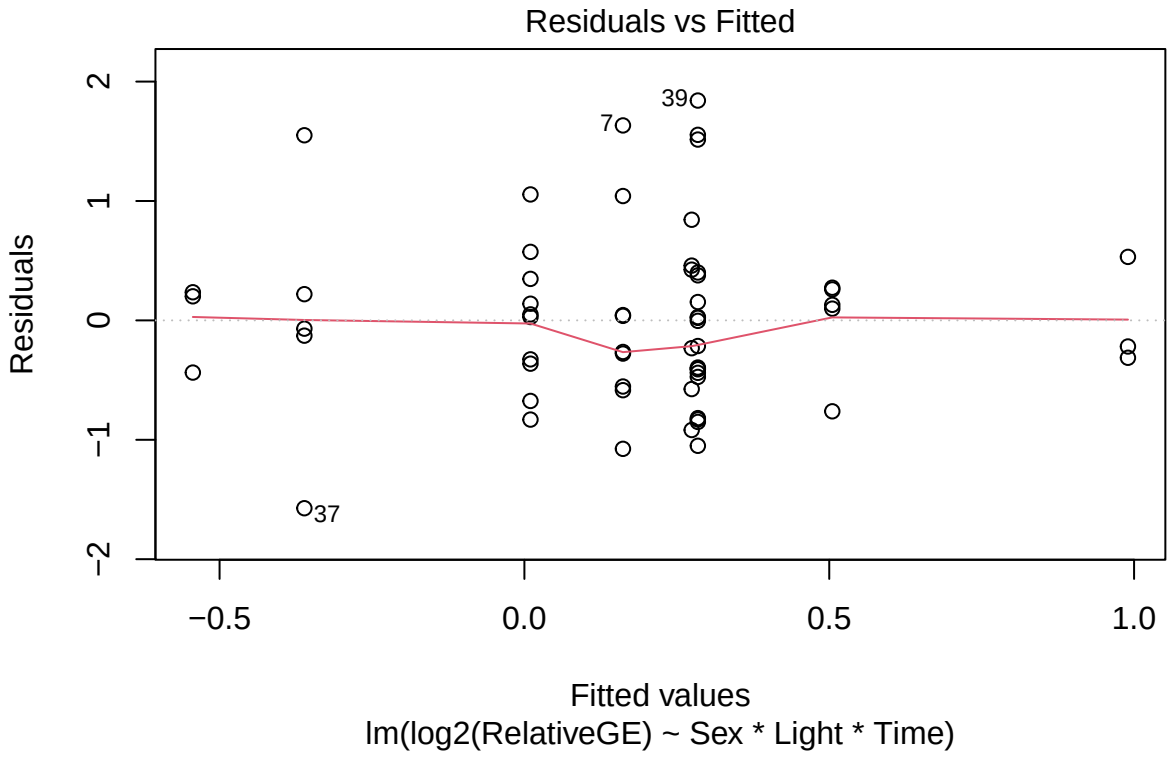
```
##
##      Balanced one-way analysis of variance power calculation
##
##          groups = 4
##               n = 3
##     between.var = 1.411641
##      within.var = 0.3873501
##       sig.level = 0.05
##           power = 0.9709752
##
## NOTE: n is number in each group
```

POMC circadian + light-dark

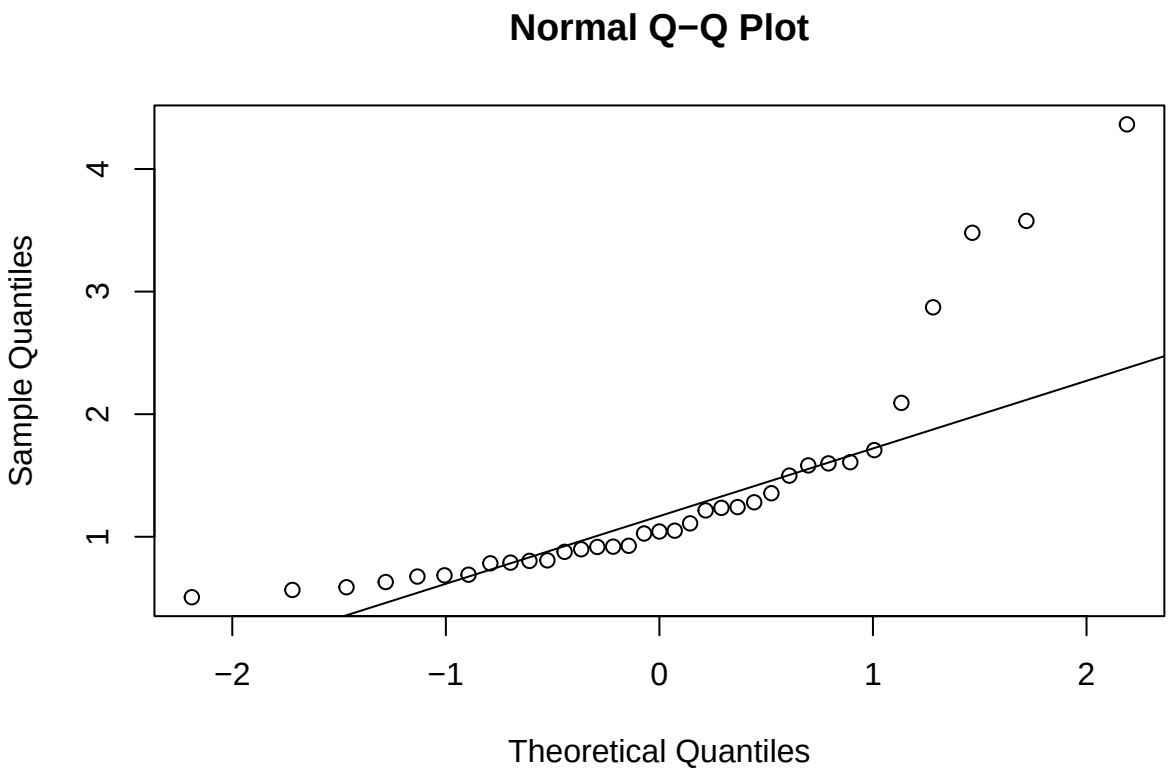


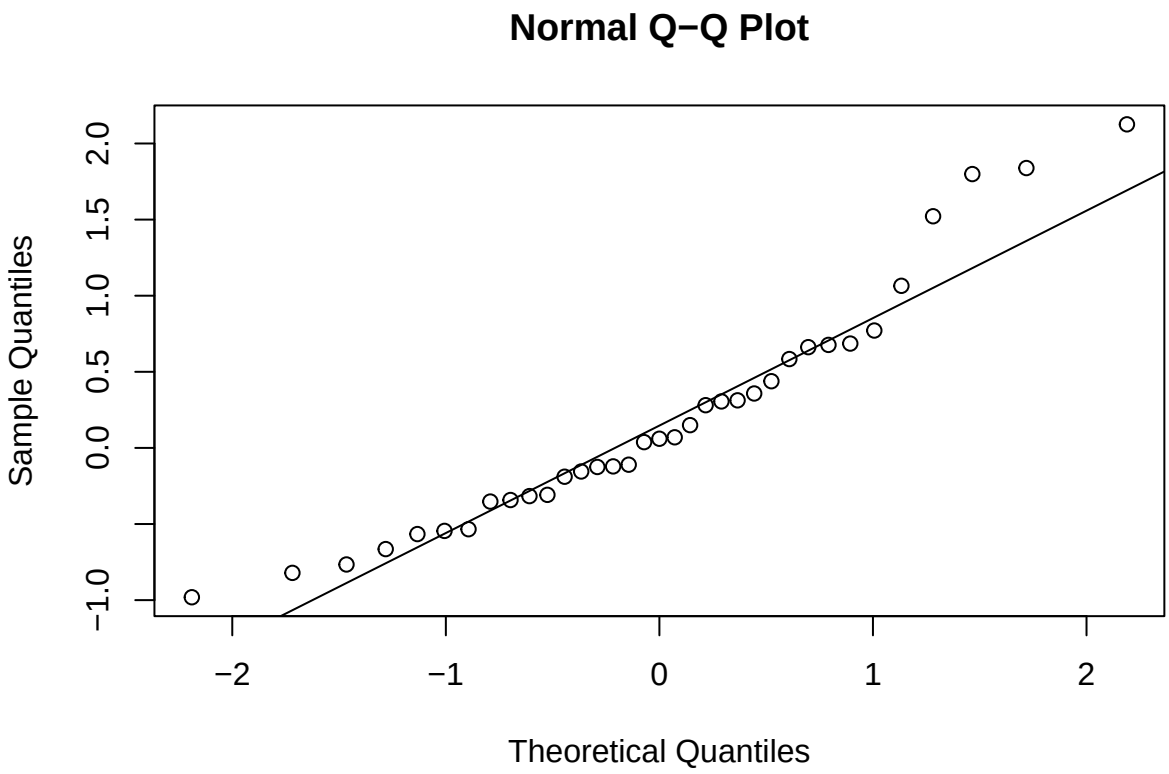


```
##
##  Shapiro-Wilk normality test
##
## data:  log2(pomc_all$RelativeGE)
## W = 0.97065, p-value = 0.157
```



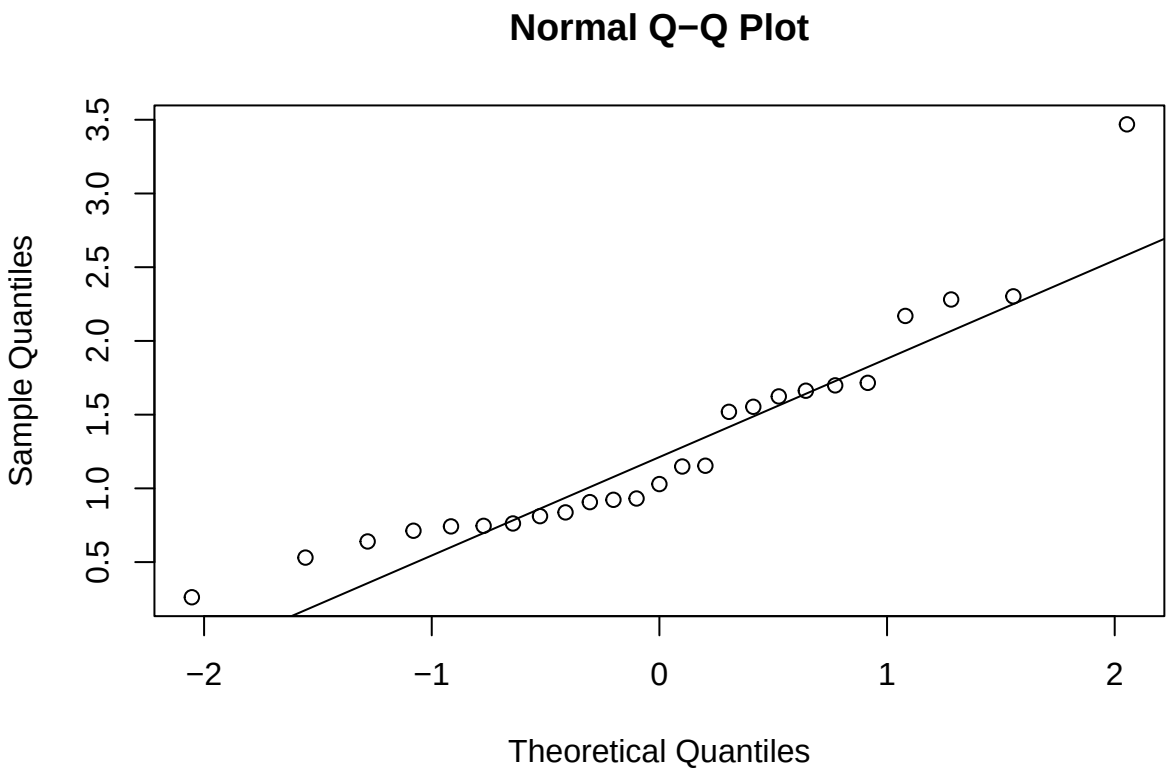

```
## Analysis of Variance Table
##
## Response: log2(RelativeGE)
##                Df  Sum Sq Mean Sq F value  Pr(>F)
## Sex             1  2.4635 2.46353  4.2724 0.04373 *
## Light           1  0.4941 0.49406  0.8568 0.35890
## Time            1  0.0643 0.06430  0.1115 0.73978
## Sex:Light       1  1.0107 1.01073  1.7529 0.19131
## Sex:Time        1  0.4649 0.46485  0.8062 0.37339
## Light:Time      1  0.9614 0.96137  1.6673 0.20234
## Sex:Light:Time  1  0.6250 0.62495  1.0838 0.30266
## Residuals      52 29.9842 0.57662
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

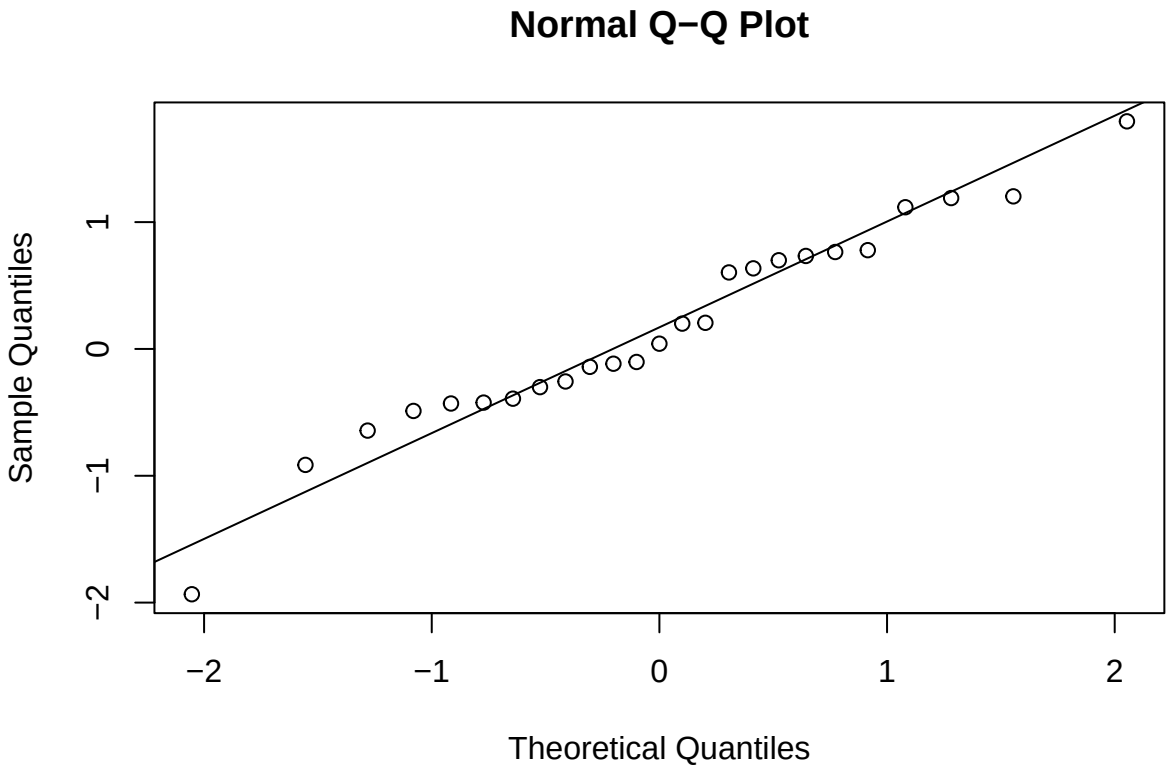

Normal Q−Q Plot
Sample Quantiles
Theoretical Quantiles
1
2
3
4
−2
−1
0
1
2




```
##
##  Shapiro-Wilk normality test
##
## data:  log2(day_pomc_all$RelativeGE)
## W = 0.93297, p-value = 0.03432
```

```
## Analysis of Variance Table
##
## Response: log2(RelativeGE)
##           Df Sum Sq Mean Sq F value Pr(>F)
## Hour       2  0.599 0.29948   0.487 0.6189
## Residuals 32 19.678 0.61495
```

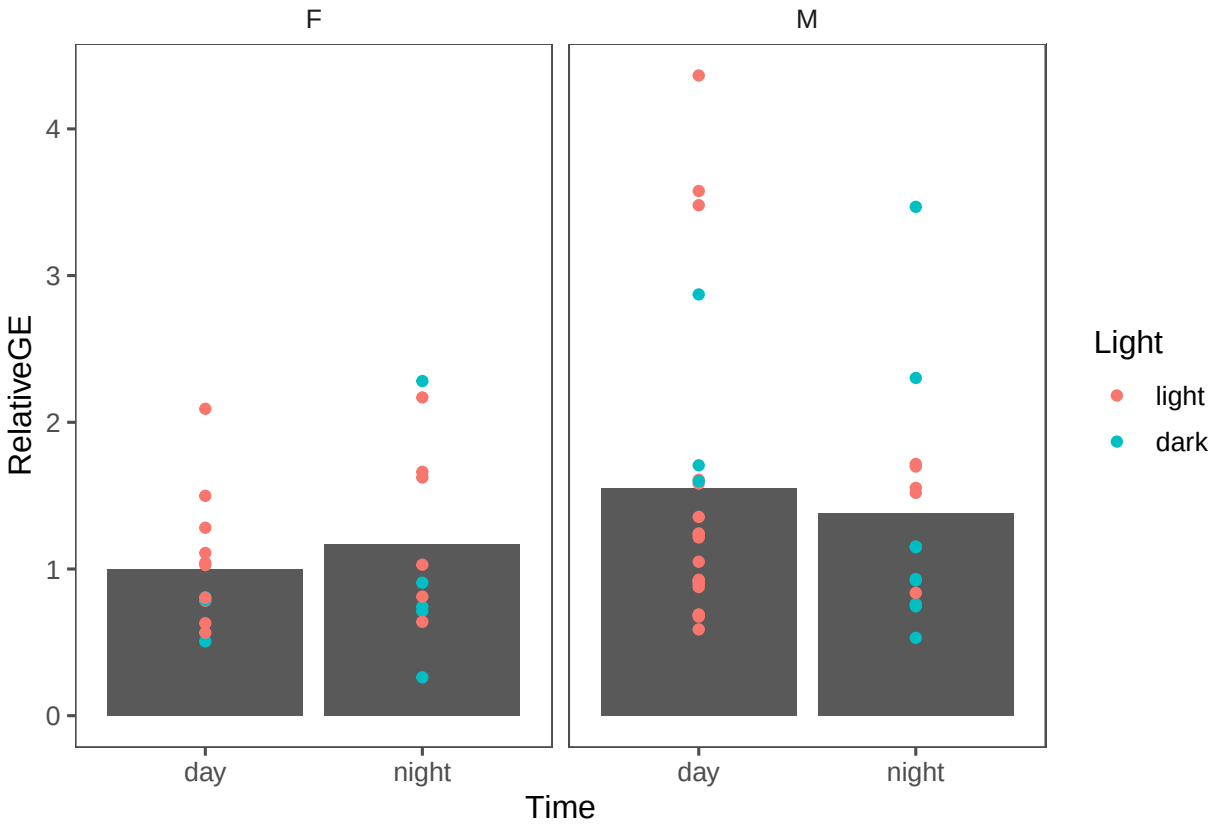

Normal Q−Q Plot
Sample Quantiles
0.5
1.0
1.5
2.0
2.5
3.0
3.5
−2
−1
0
1
2
Theoretical Quantiles

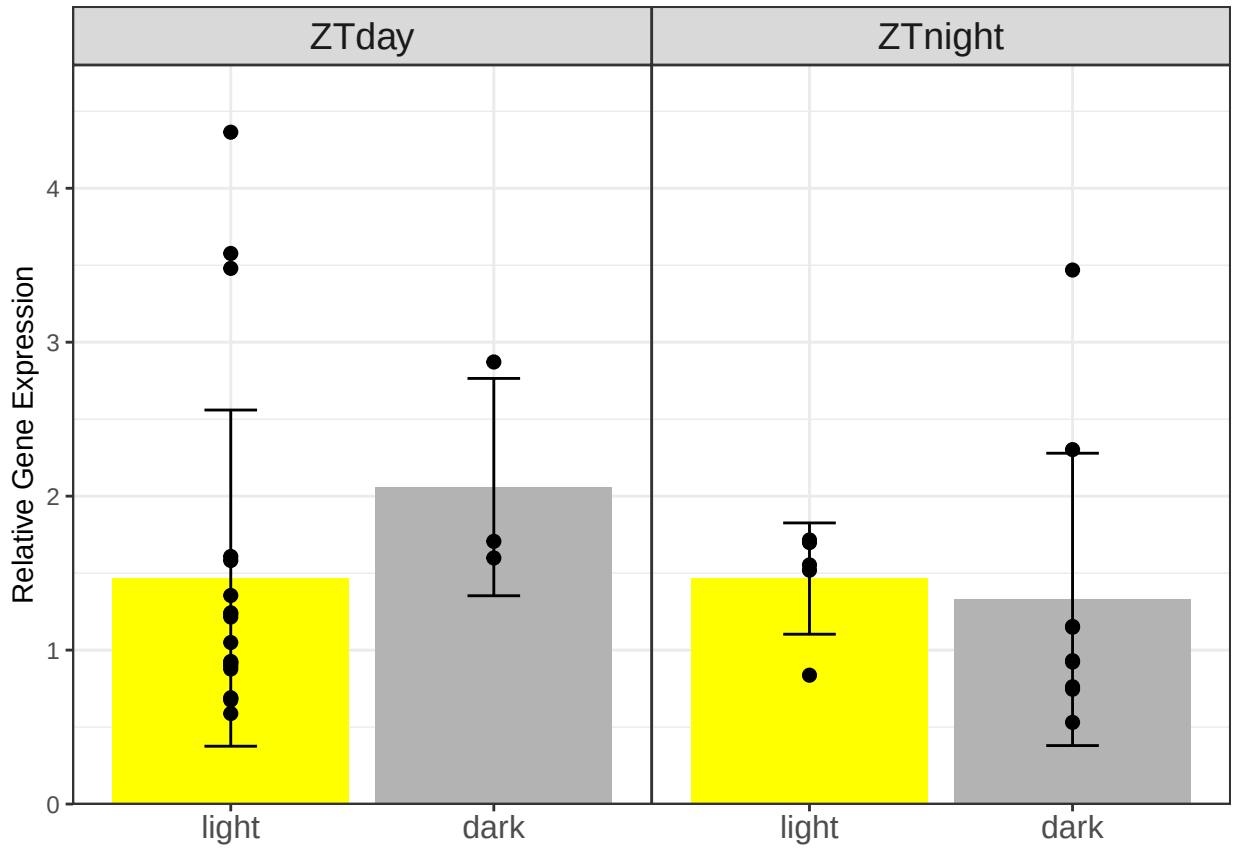



```
##
##  Shapiro-Wilk normality test
##
## data:  log2(night_pomc_all$RelativeGE)
## W = 0.96923, p-value = 0.6256
```

```
## Analysis of Variance Table
##
## Response: log2(RelativeGE)
##           Df  Sum Sq Mean Sq F value Pr(>F)
## Hour       1  0.2848 0.28478  0.4231 0.5218
## Residuals 23 15.4792 0.67301
```

```
##
##  Welch Two Sample t-test
##
## data:  RelativeGE by Hour
## t = 0.83152, df = 10.598, p-value = 0.424
## alternative hypothesis: true difference in means between group 12am and group 1am is not equal to 0
## 95 percent confidence interval:
##  -0.4895652  1.0796662
## sample estimates:
## mean in group 12am  mean in group 1am
##           1.474012           1.178962
```

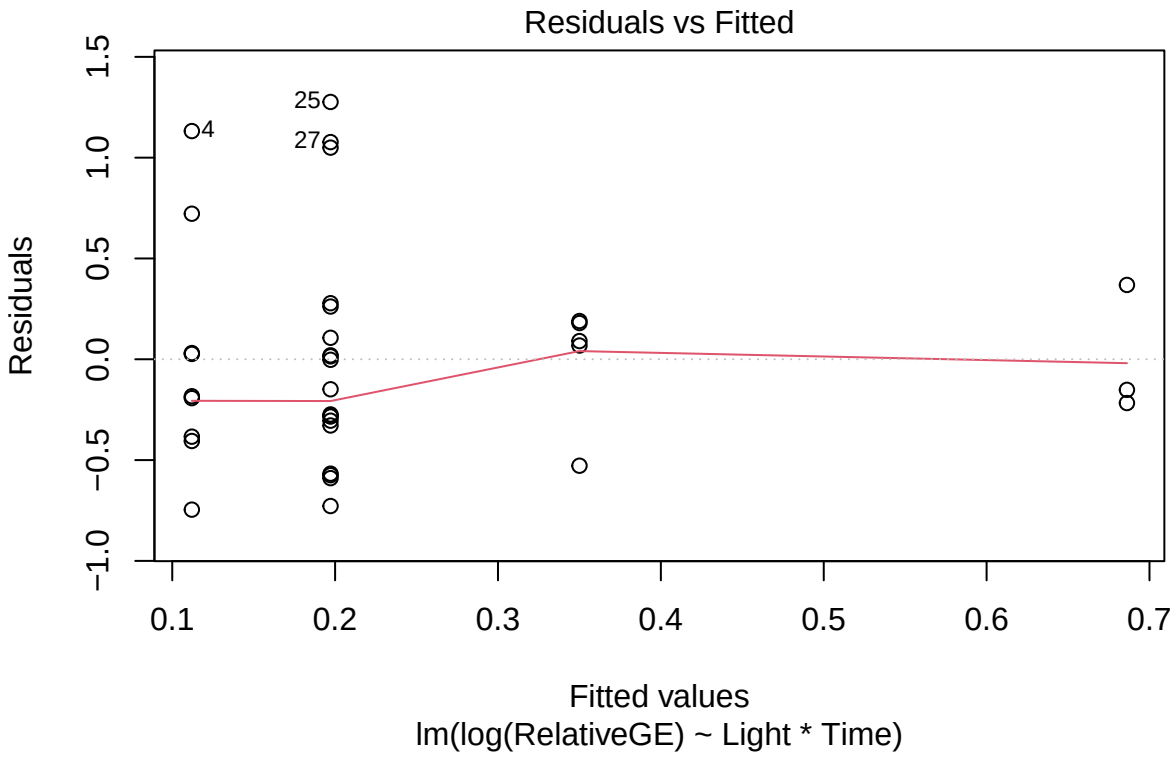

F
M
RelativeGE
4
3
2
1
0
day
night
day
night
Time
Light
light
dark

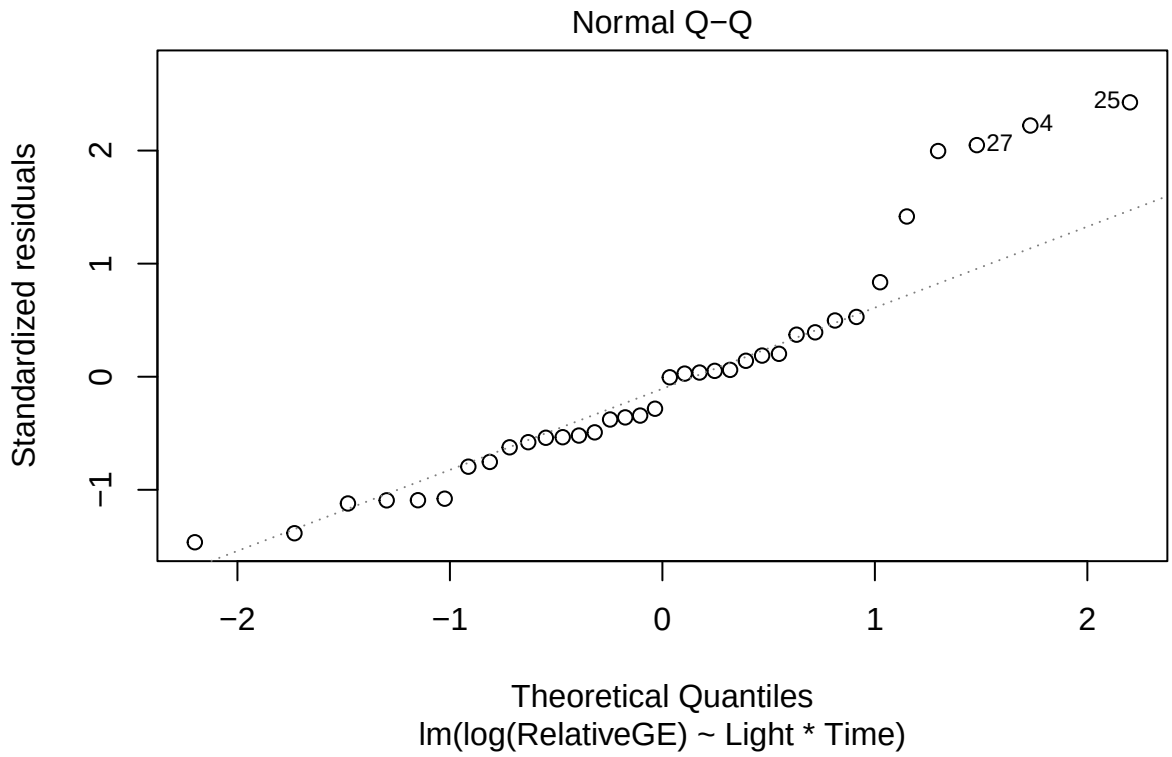



```
##
##  Shapiro-Wilk normality test
##
## data:  m_pomc_all$RelativeGE
## W = 0.78974, p-value = 1.025e-05
```

```
##
##  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  3  0.7557 0.5273
##       32
```


Residuals vs Fitted
Residuals
Fitted values
lm(log(RelativeGE) ~ Light * Time)
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



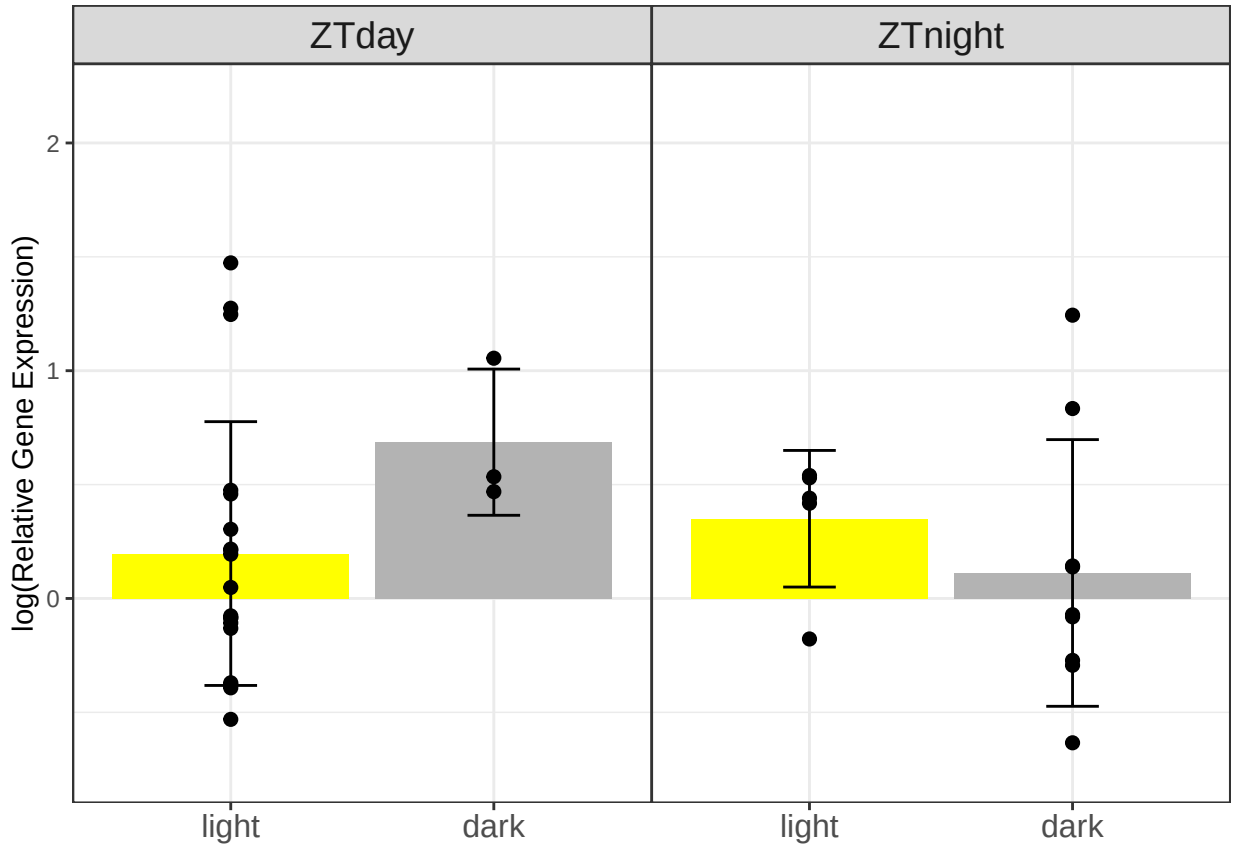

```
## Analysis of Variance Table
##
## Response: log(RelativeGE)
##            Df Sum Sq Mean Sq F value Pr(>F)
## Light       1 0.0056 0.00563  0.0193 0.8904
## Time        1 0.0760 0.07598  0.2602 0.6135
## Light:Time  1 0.7582 0.75816  2.5964 0.1169
## Residuals  32 9.3443 0.29201
```

```
##  contrast                     estimate    SE df t.ratio p.value
##  light ZTday - dark ZTday      -0.4890 0.336 32  -1.457  0.4748
##  light ZTday - light ZTnight   -0.1528 0.272 32  -0.563  0.9424
##  light ZTday - dark ZTnight     0.0852 0.219 32   0.389  0.9796
##  dark ZTday - light ZTnight     0.3361 0.395 32   0.852  0.8293
##  dark ZTday - dark ZTnight      0.5741 0.360 32   1.594  0.3964
##  light ZTnight - dark ZTnight   0.2380 0.301 32   0.790  0.8586
##
## Results are given on the log (not the response) scale.
## P value adjustment: tukey method for comparing a family of 4 estimates
```

```
## Time = ZTday:
##  contrast     estimate    SE df t.ratio p.value
##  light - dark   -0.489 0.336 32  -1.457  0.1550
##
## Time = ZTnight:
##  contrast     estimate    SE df t.ratio p.value
##  light - dark    0.238 0.301 32   0.790  0.4355
```

```
##
## Results are given on the log (not the response) scale.
```

```
## Light = light:
##  contrast        estimate    SE df t.ratio p.value
##  ZTday - ZTnight   -0.153 0.272 32  -0.563  0.5776
##
## Light = dark:
##  contrast        estimate    SE df t.ratio p.value
##  ZTday - ZTnight    0.574 0.360 32   1.594  0.1208
##
## Results are given on the log (not the response) scale.
```




```
## # A tibble: 4 x 5
## # Groups:   Light [2]
##   Light Time        n  mean log_mean
##   <fct> <fct>   <int> <dbl>    <dbl>
## 1 light ZTday      19  1.47    0.197
## 2 light ZTnight     5  1.46    0.350
## 3 dark  ZTday       3  2.06    0.686
## 4 dark  ZTnight     9  1.33    0.112
```

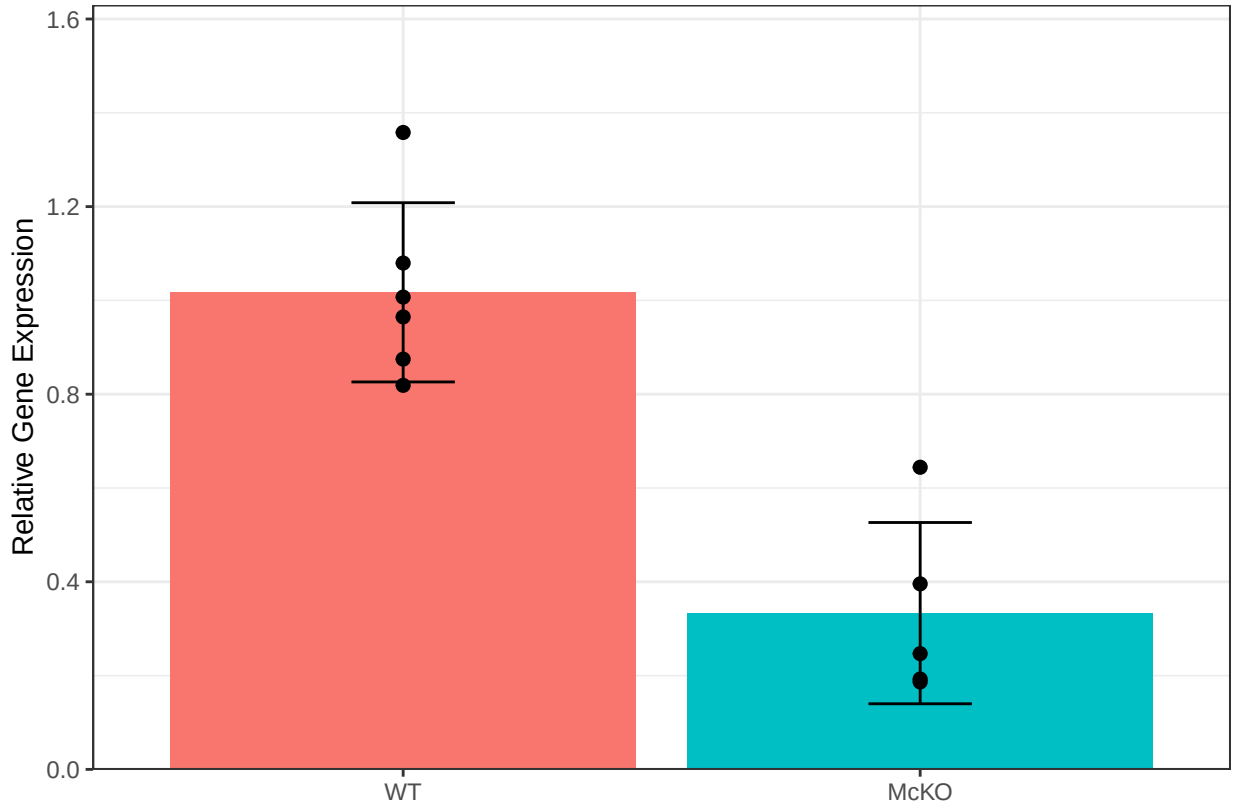
```
## [1] NaN
```

```
## [1] 0.2920105
```

```
##
##      Balanced one-way analysis of variance power calculation
```

```
##
##          groups = 4
##               n = 3
##     between.var = 0.0006157598
##      within.var = 0.2920105
##       sig.level = 0.05
##           power = 0.05072033
##
## NOTE: n is number in each group
```

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
```

MOR mRNA in morphine vs saline treatment (retinas only)