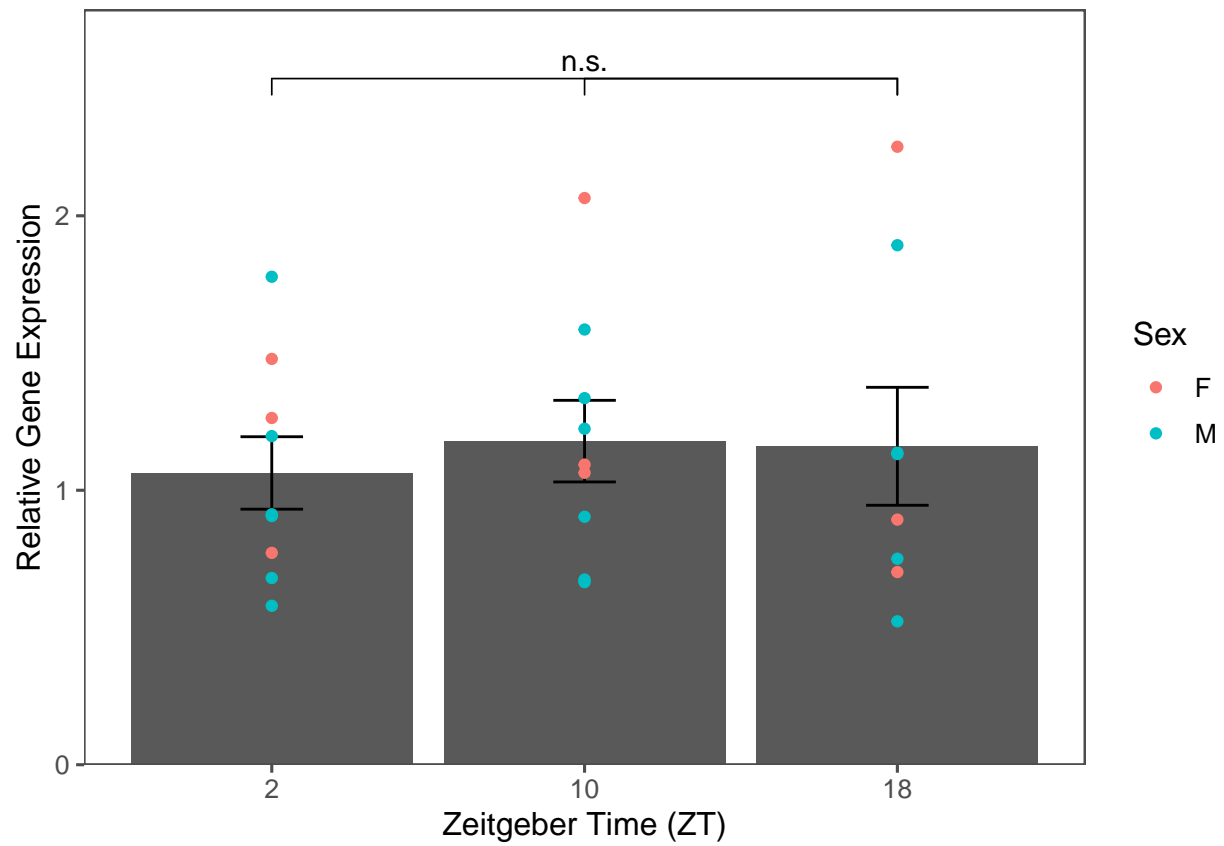


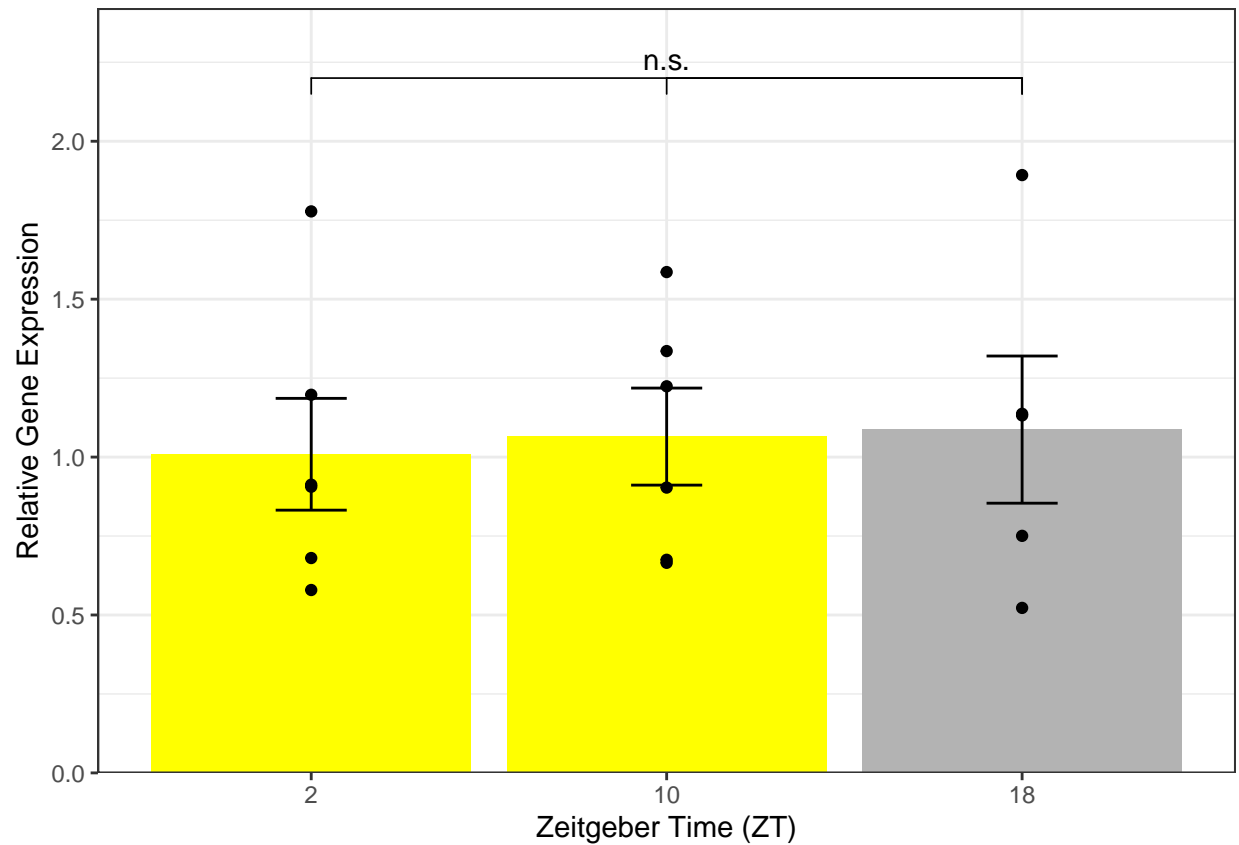
qpCR-Figures

C-T Berezin

10/30/2021

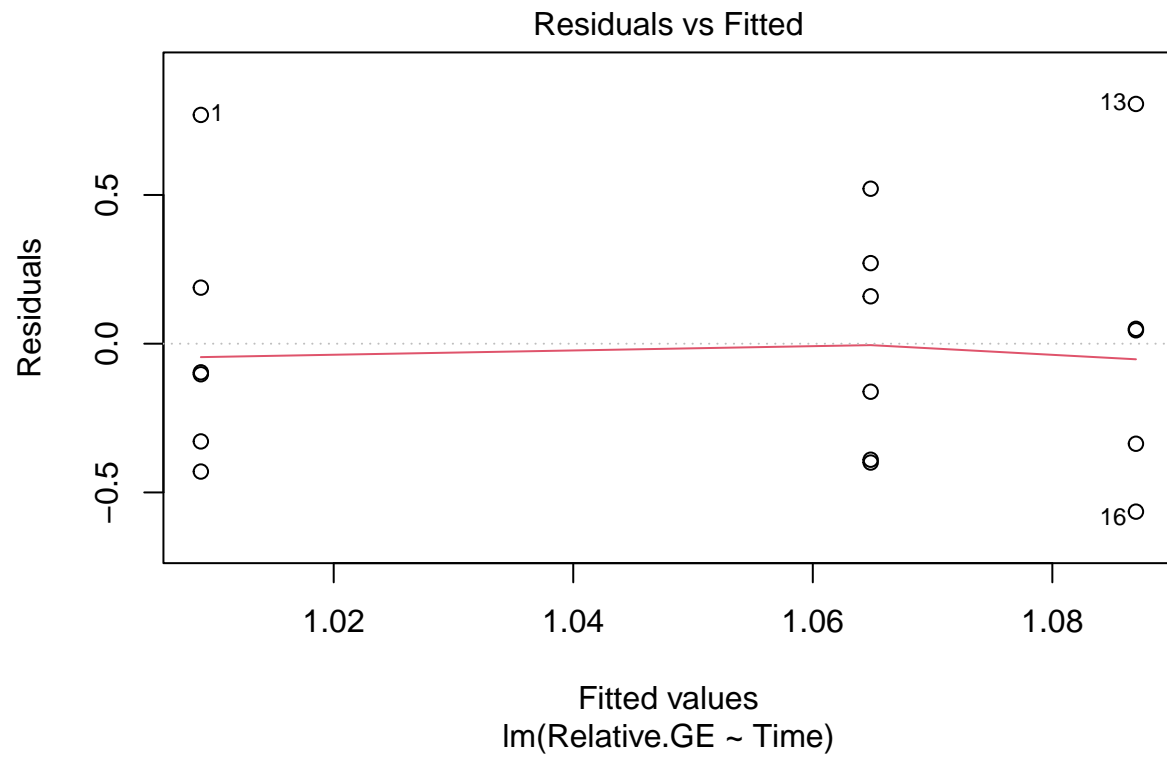
Is POMC under circadian regulation?

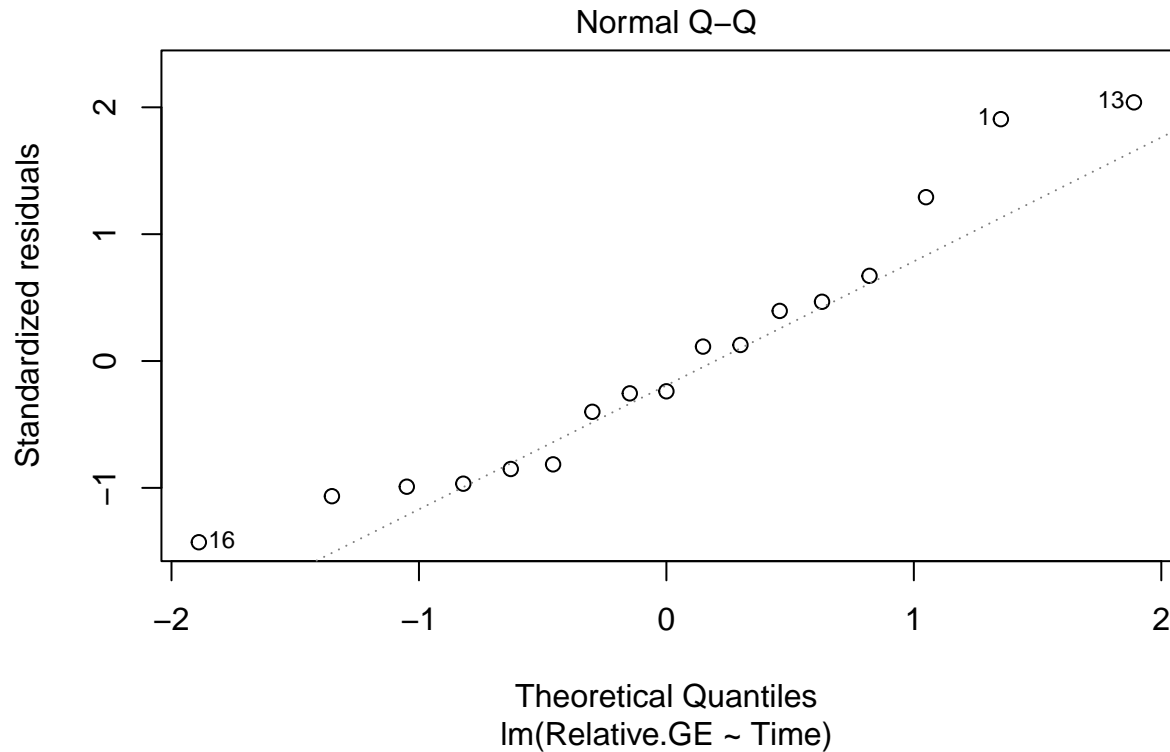




```
##
## Shapiro-Wilk normality test
##
## data: pomc_circ_males$Relative.GE
## W = 0.92555, p-value = 0.1832

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





```
## 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 3
##   Time      n mean
##   <fct> <int> <dbl>
## 1 2         6  1.01
## 2 10        6  1.06
## 3 18        5  1.09

## [1] 0.009116243

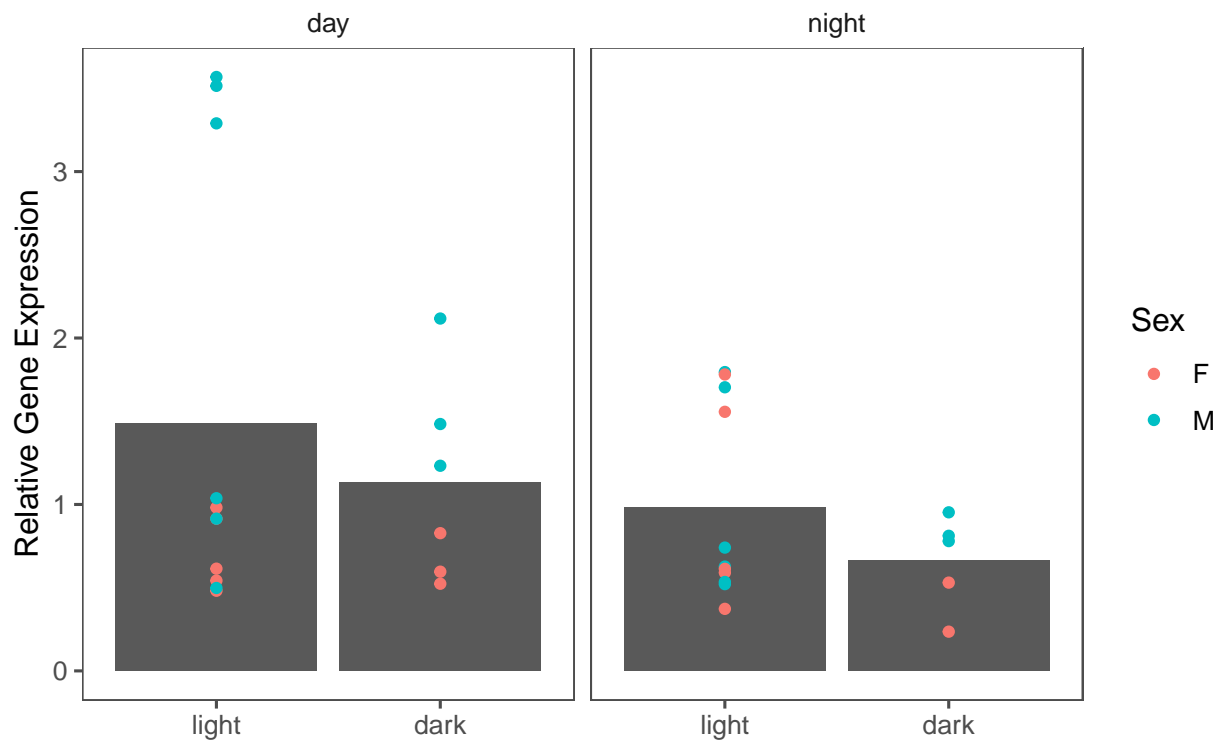
## [1] 0.1952332

##
##      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
```

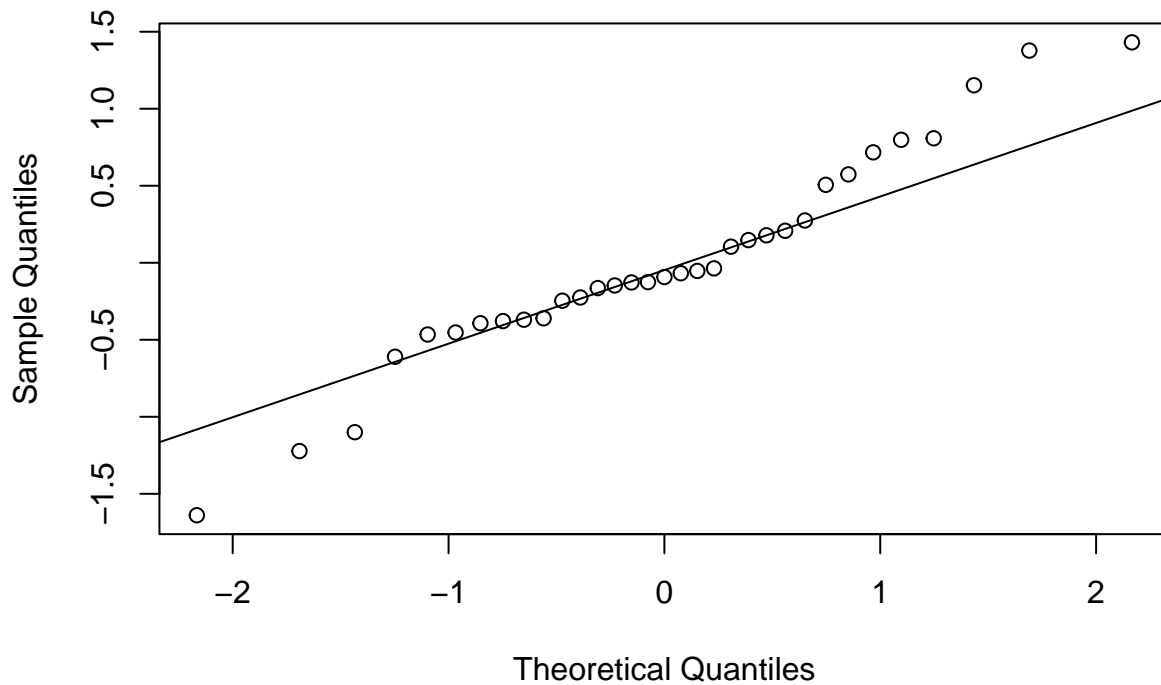
Is POMC expression light-driven?

POMC mRNA Expression



```
##
## 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
```

Normal Q-Q Plot



```
## 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

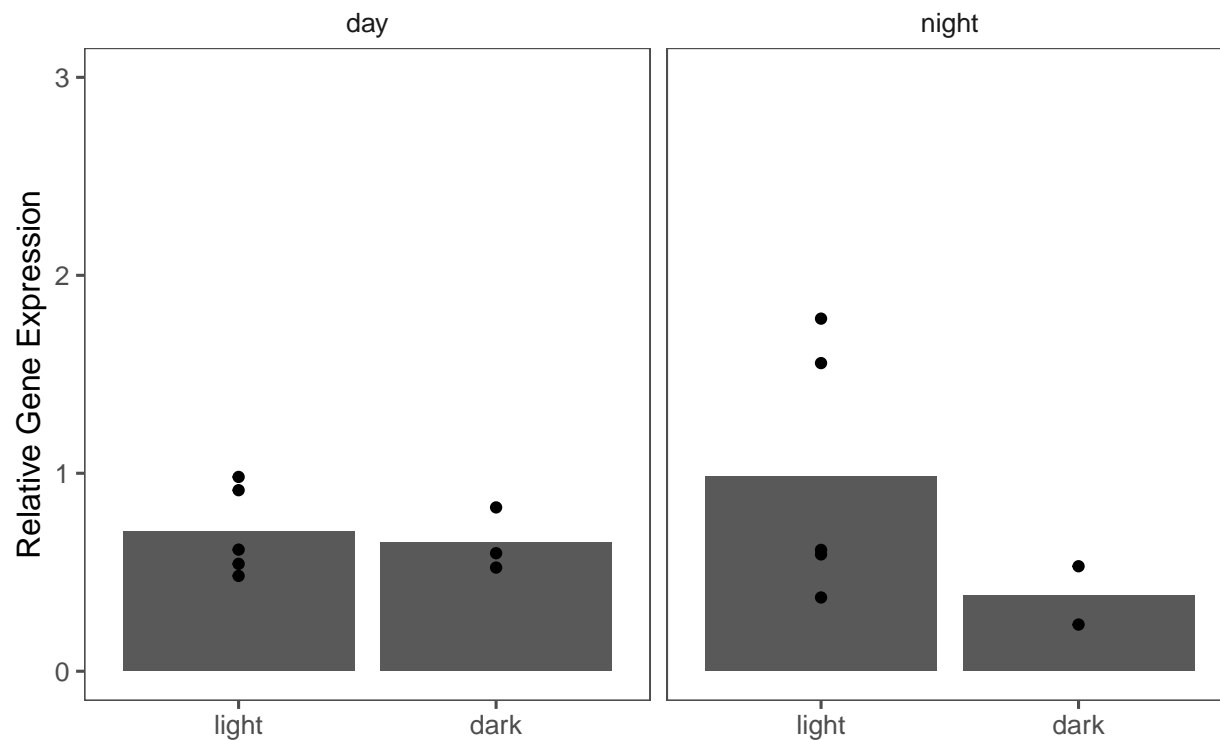
```

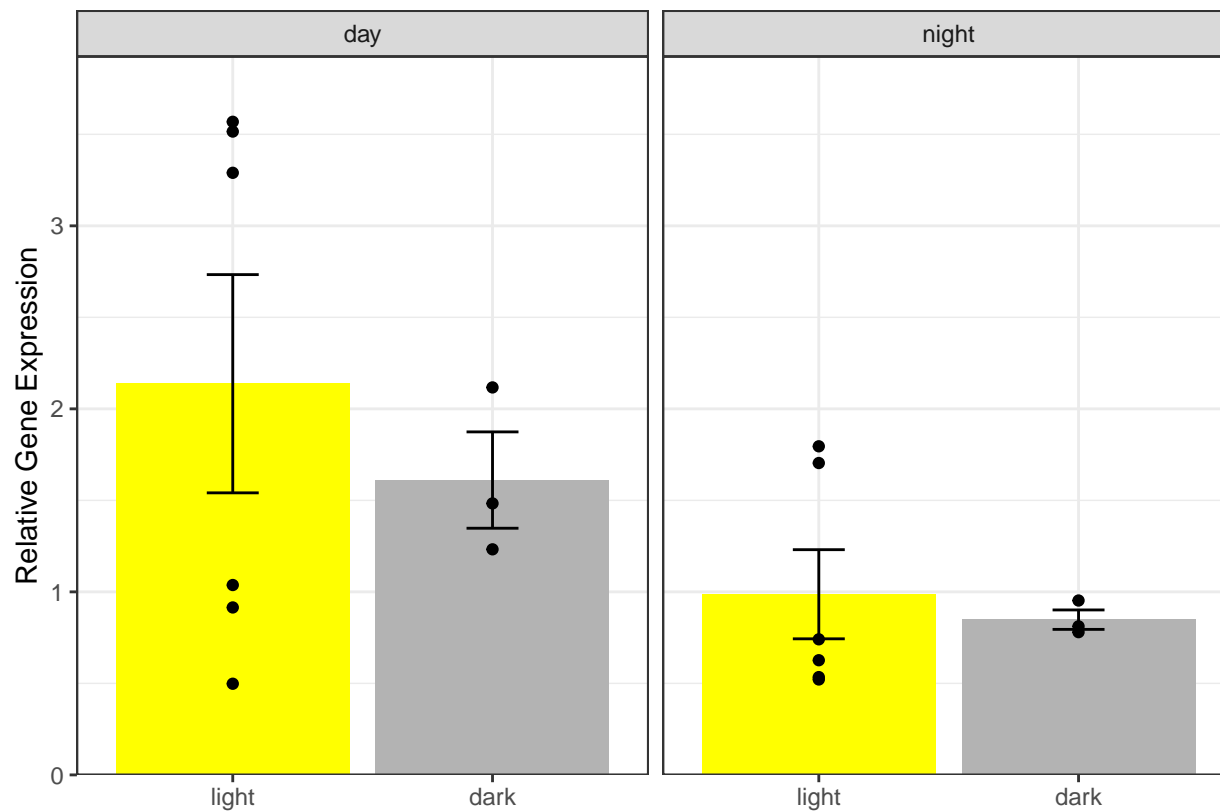
```

## 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

```

POMC mRNA in Females

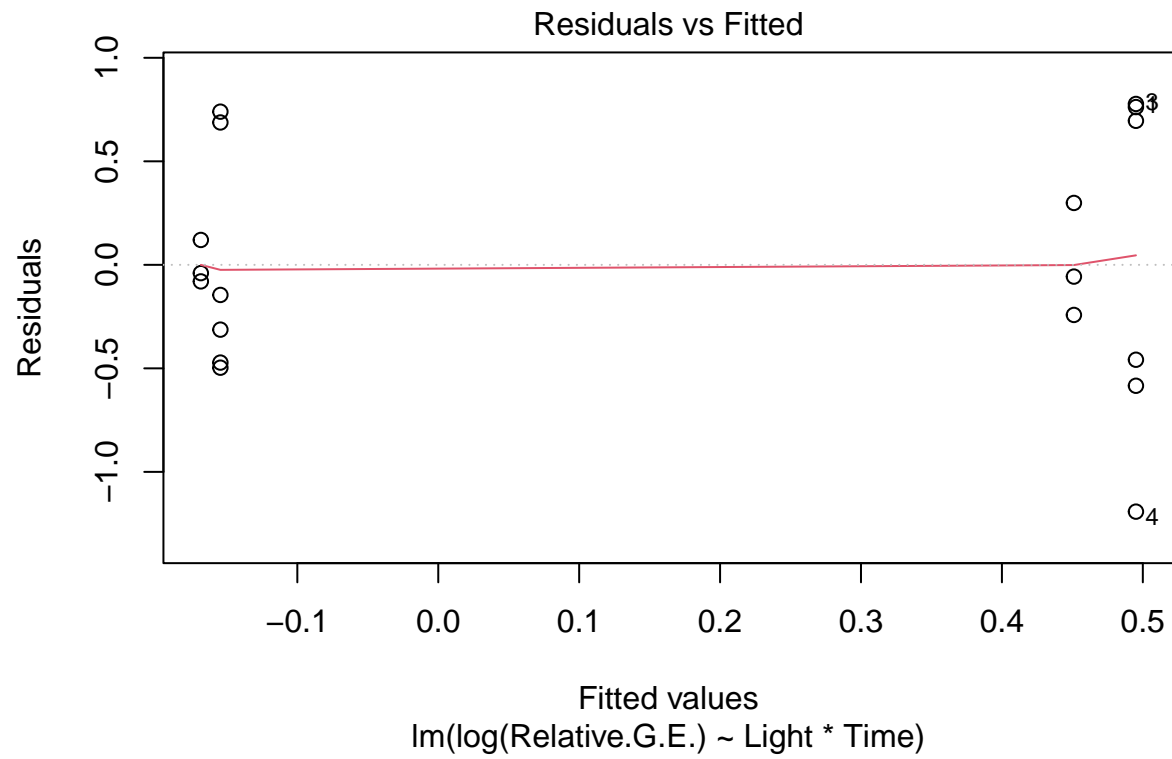


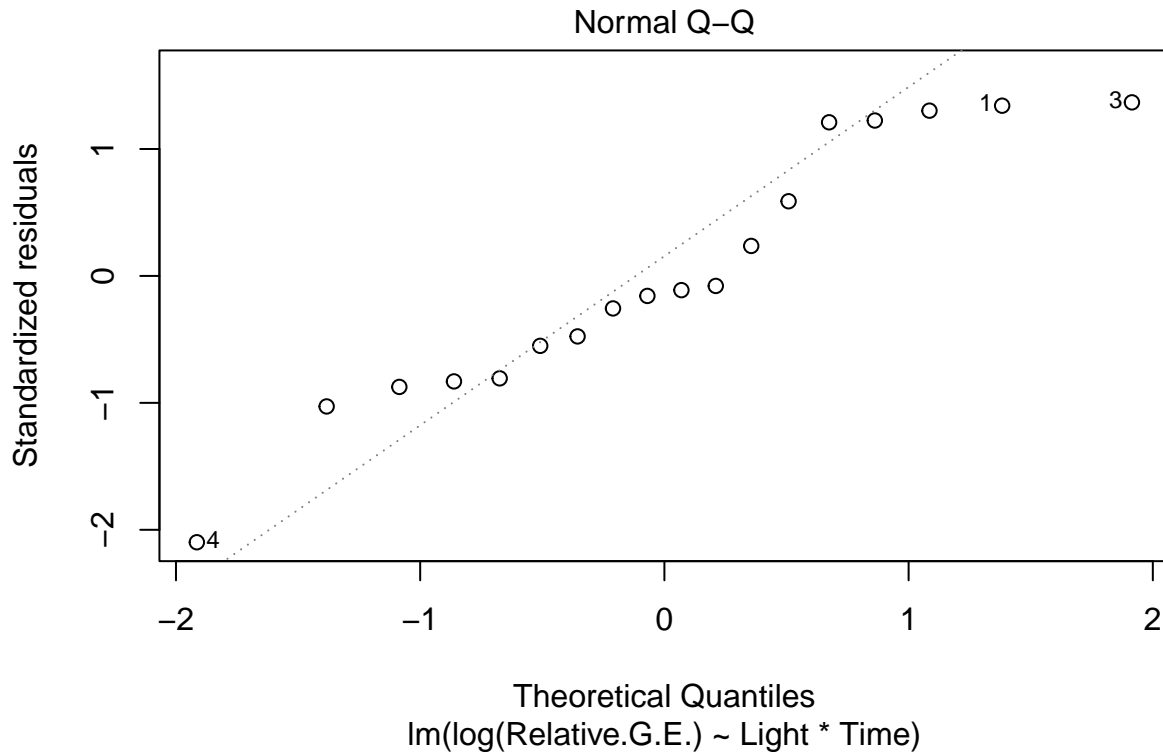


```
##
## Shapiro-Wilk normality test
##
## data:  pomc_ld_m$Relative.G.E.
## W = 0.80779, p-value = 0.001961

##
## 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
```





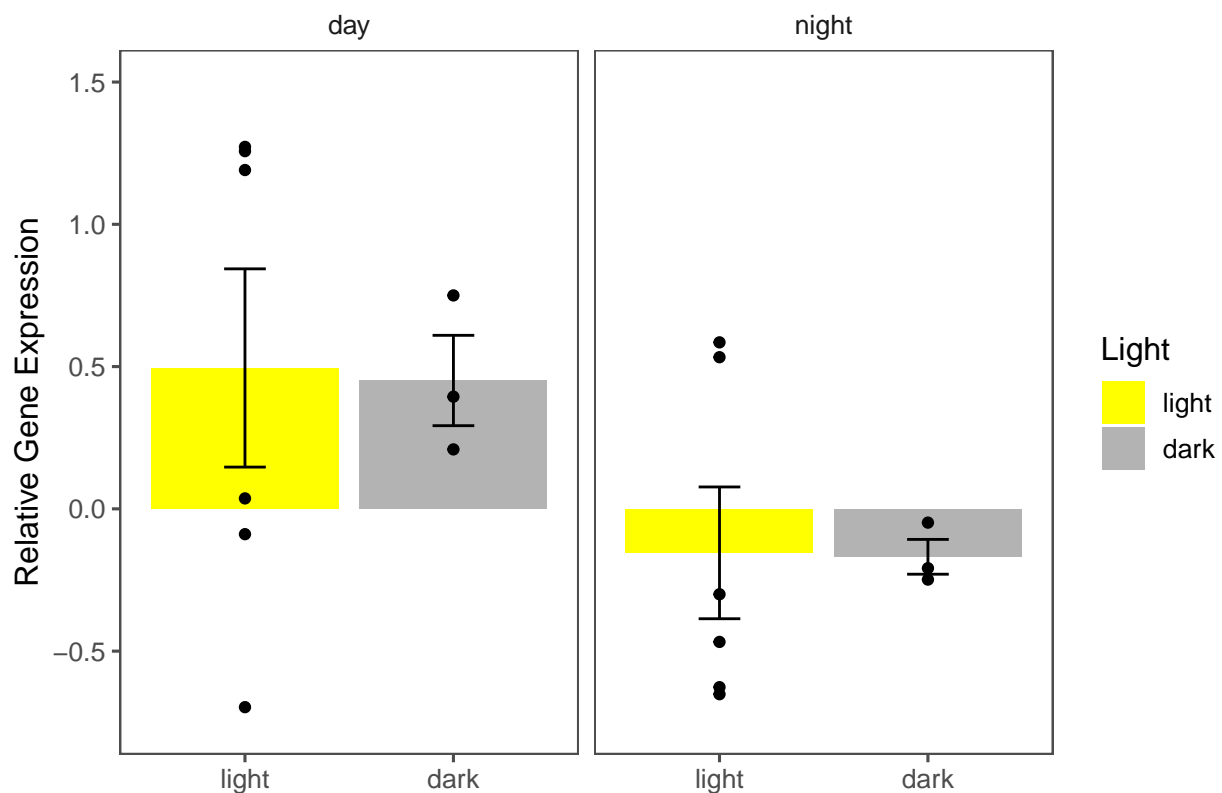
```
## Analysis of Variance Table
##
## Response: log(Relative.G.E.)
##           Df Sum Sq Mean Sq F value Pr(>F)
## Light      1  0.0034  0.00335   0.0087 0.92719
## Time       1  1.8414  1.84137   4.7538 0.04679 *
## Light:Time  1  0.0009  0.00091   0.0023 0.96204
## Residuals 14  5.4229  0.38735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



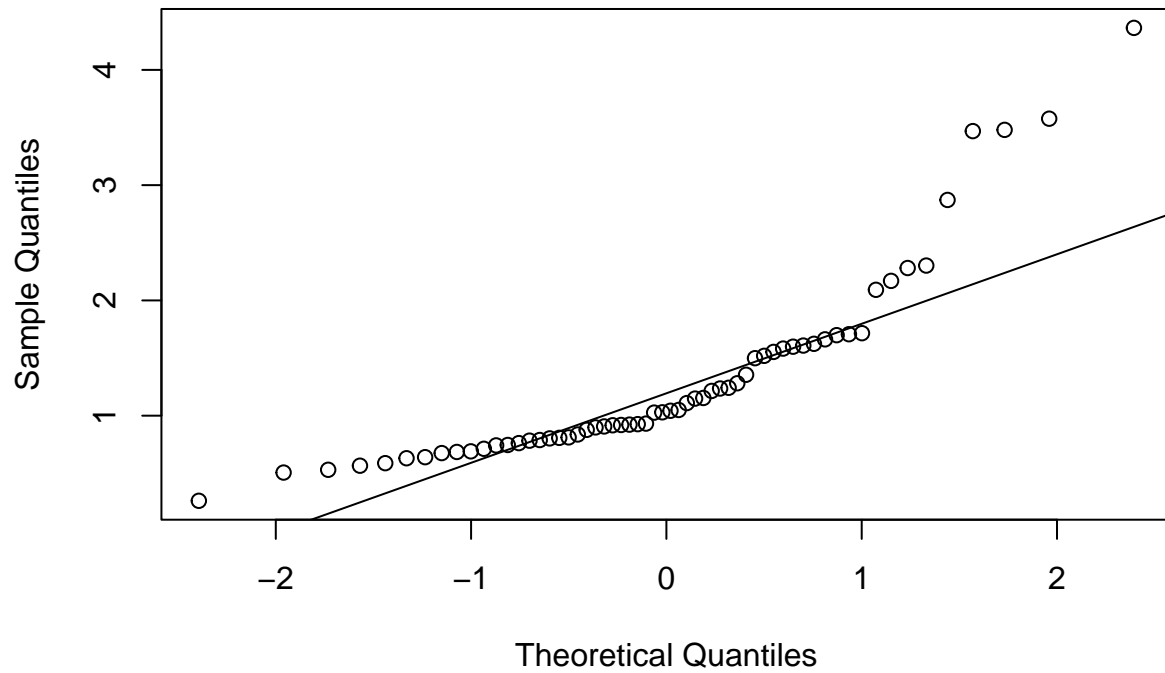
```
## # A tibble: 4 x 5
## # Groups:   Light [2]
##   Light Time      n mean log_mean
##   <fct> <fct> <int> <dbl>    <dbl>
## 1 light day      6 2.14      0.495
## 2 light night    6 0.987    -0.155
## 3 dark  day      3 1.61      0.451
## 4 dark  night    3 0.848    -0.168

## [1] 1.411641
## [1] 0.3873501
```

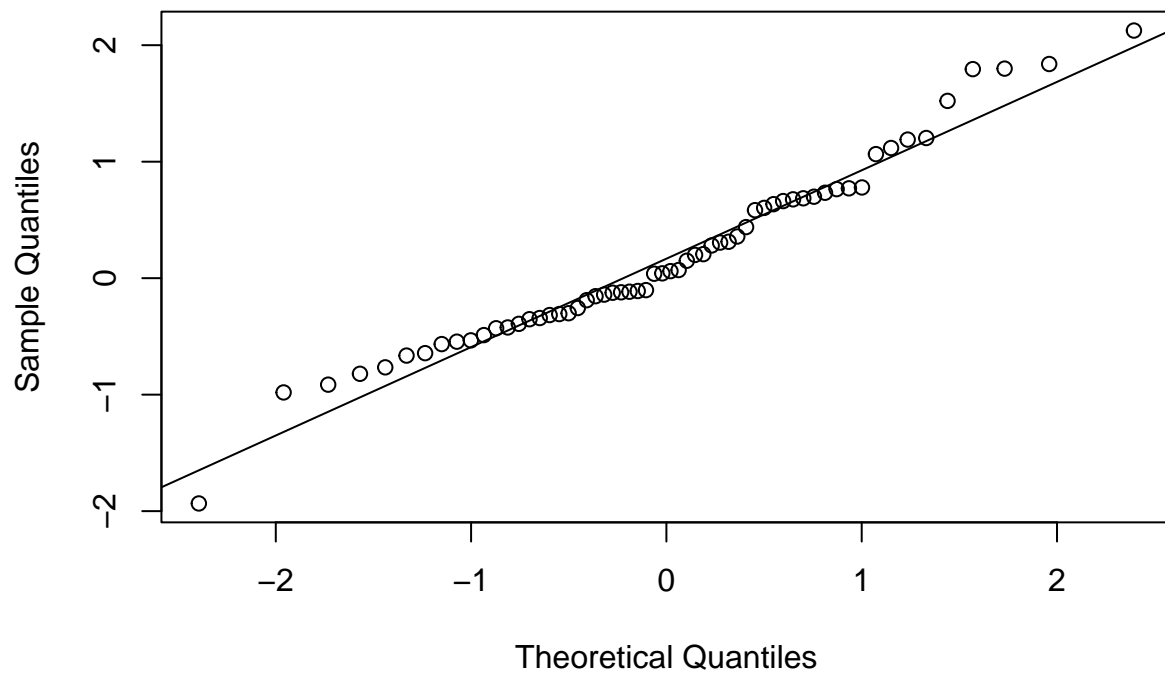
```
##
##      Balanced one-way analysis of variance power calculation
##
##      groups = 4
##      n = 3
##      between.var = 1.411641
##      within.var = 0.3873501
##      sig.level = 0.05
##      power = 0.9709752
##
## NOTE: n is number in each group
```

POMC circadian + light-dark

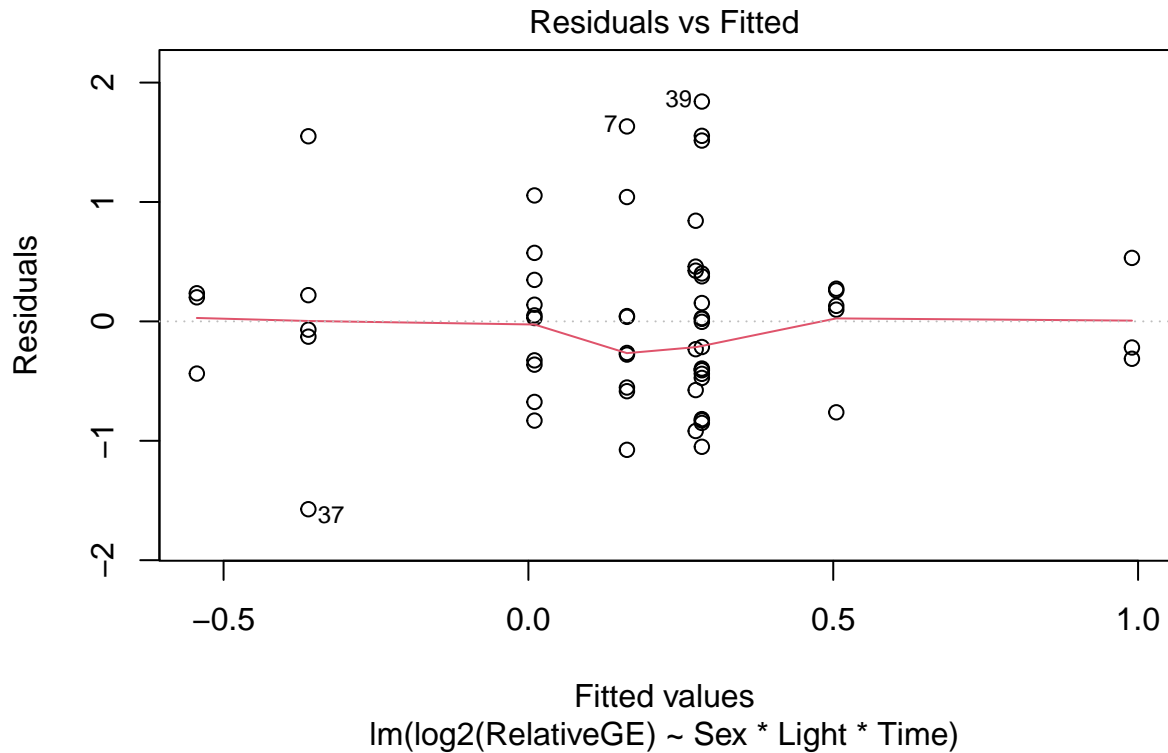
Normal Q-Q Plot



Normal Q-Q Plot



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

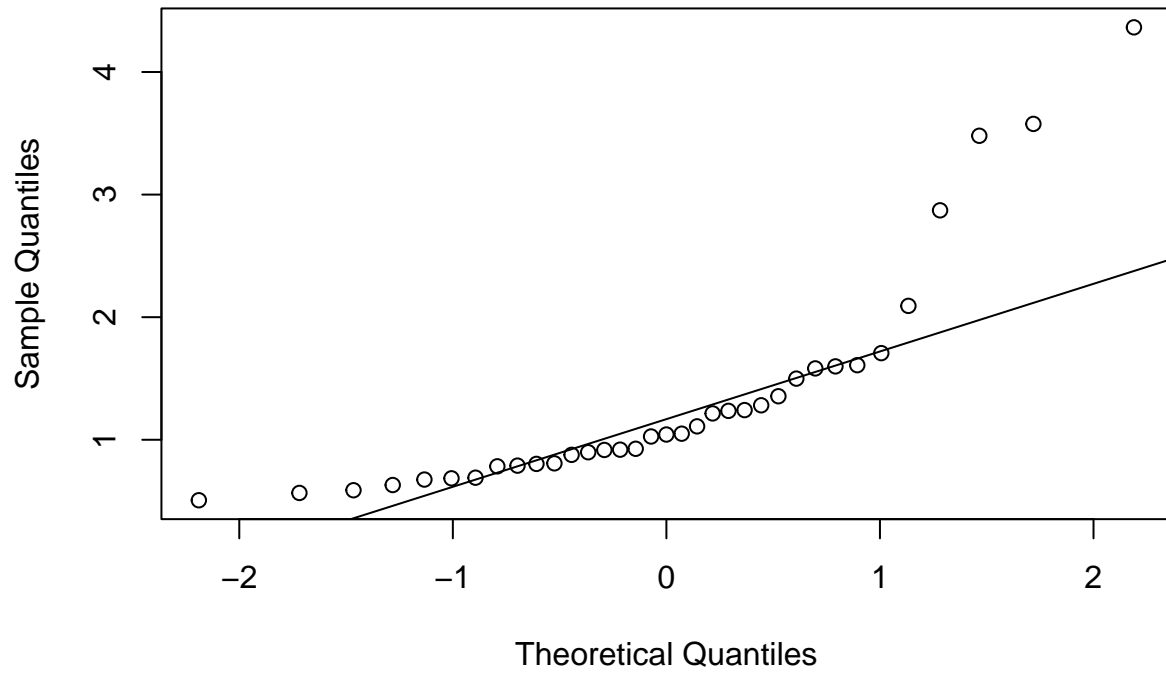


```
## Analysis of Variance Table
##
## Response: log2(RelativeGE)
##
```

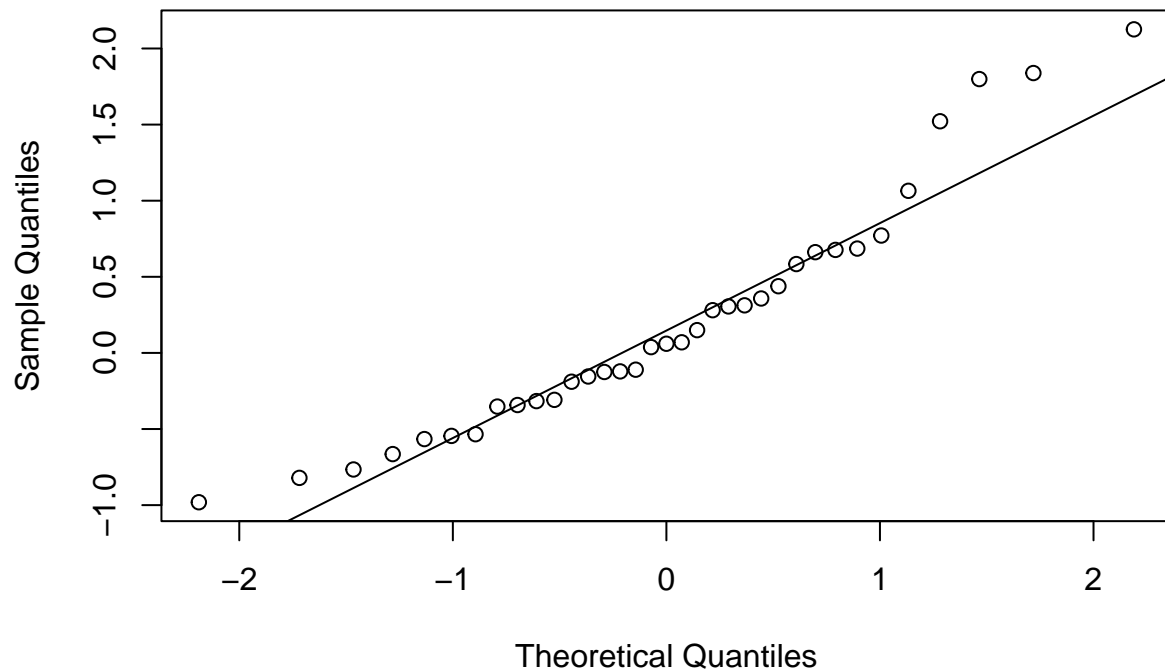
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
## Sex	1	2.4635	2.46353	4.2724	0.04373 *
## Light	1	0.4941	0.49406	0.8568	0.35890
## Time	1	0.0643	0.06430	0.1115	0.73978
## Sex:Light	1	1.0107	1.01073	1.7529	0.19131
## Sex:Time	1	0.4649	0.46485	0.8062	0.37339
## Light:Time	1	0.9614	0.96137	1.6673	0.20234
## Sex:Light:Time	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



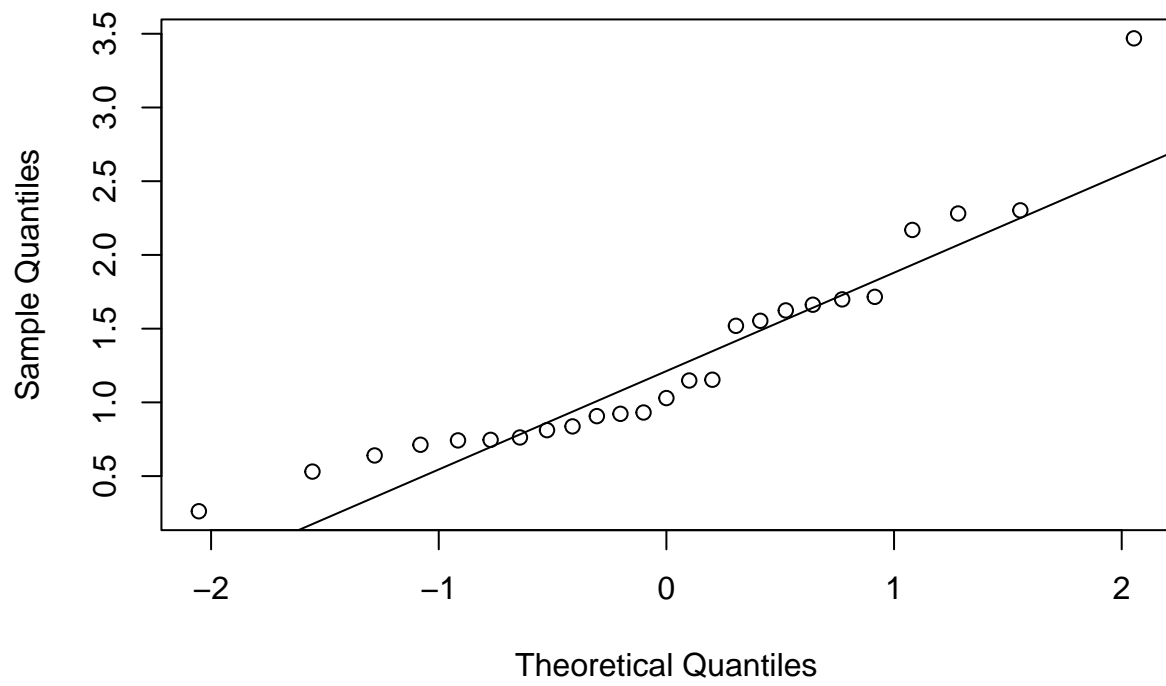
Normal Q-Q Plot



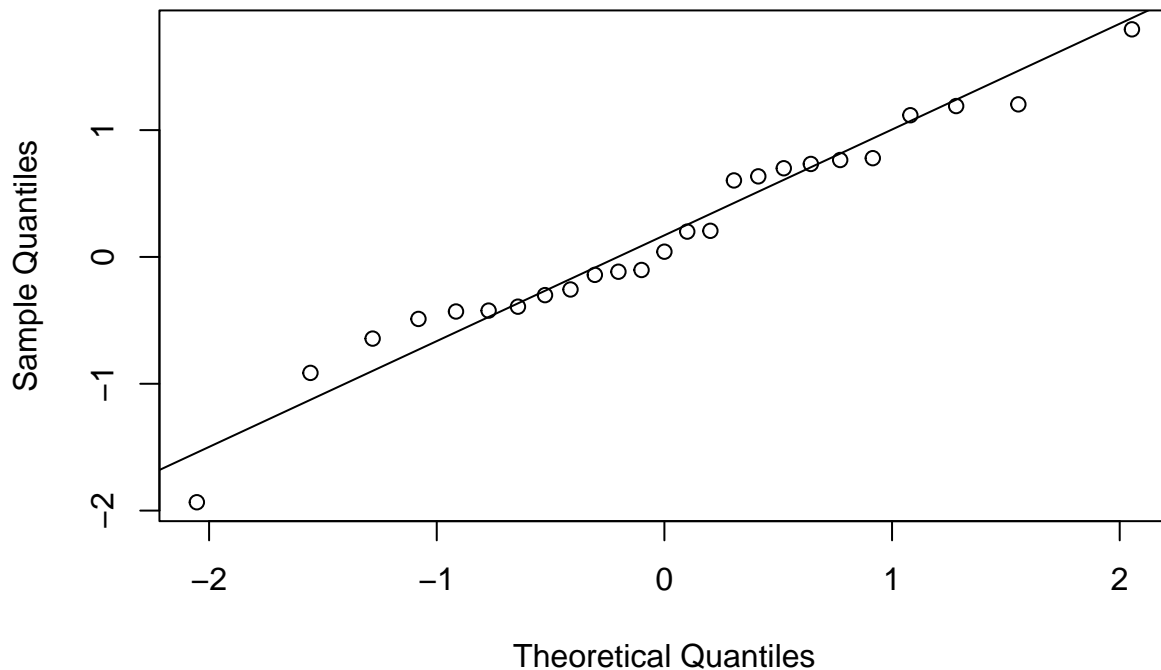
```
##
##  Shapiro-Wilk normality test
##
## data:  log2(day_pomc_all$RelativeGE)
## W = 0.93297, p-value = 0.03432

## 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



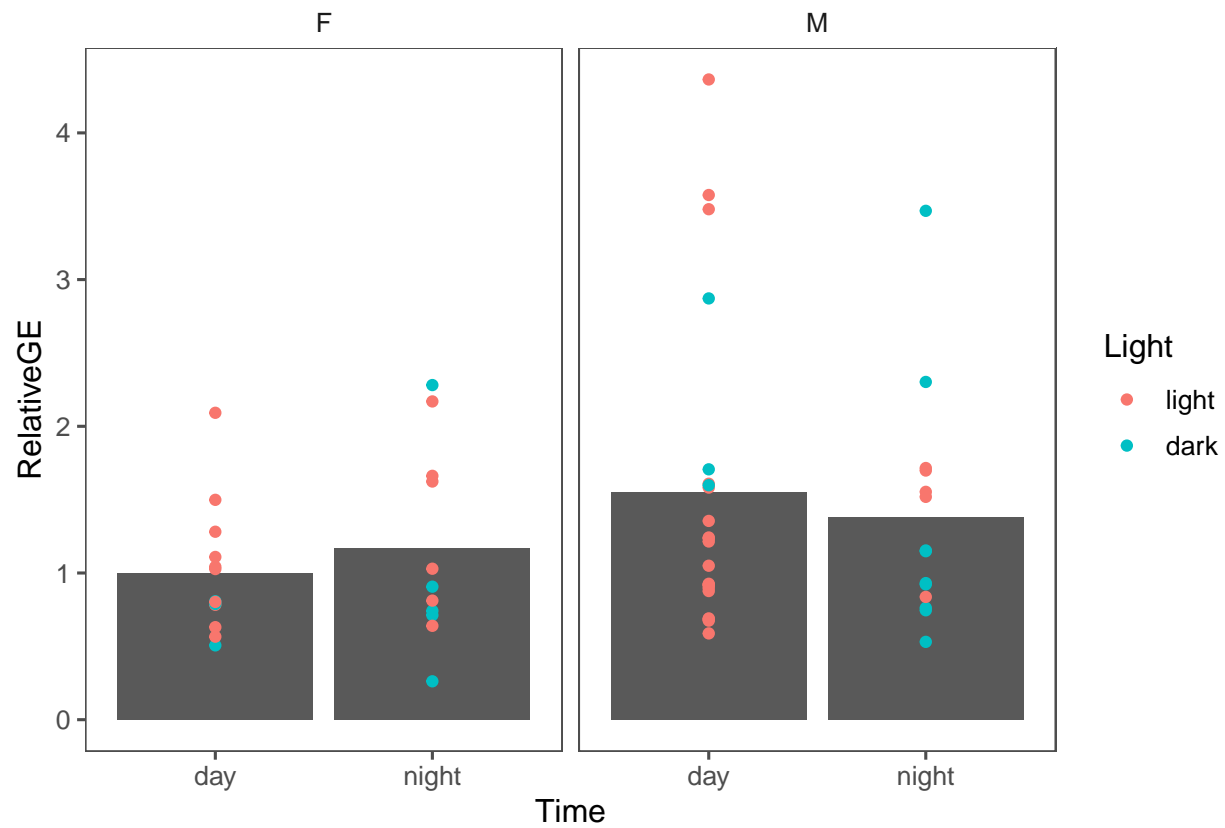
Normal Q-Q Plot



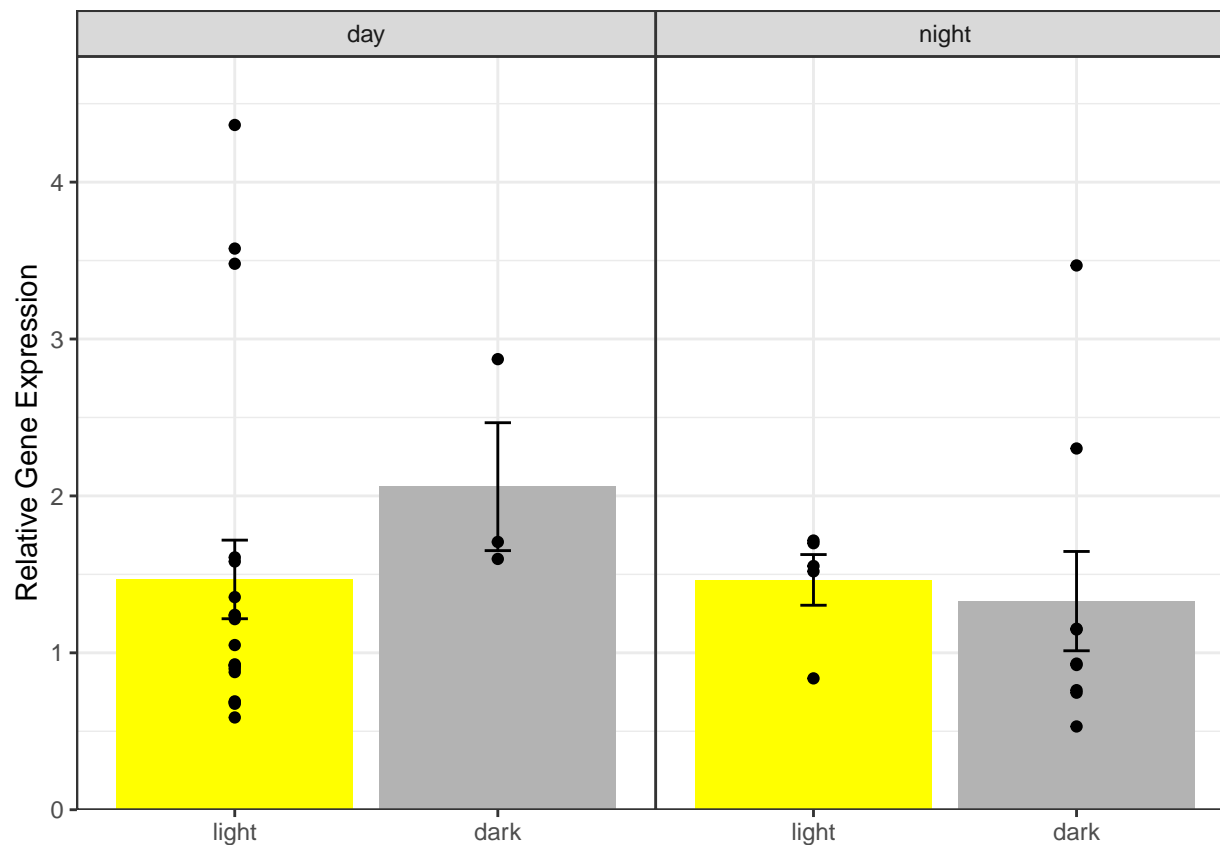
```
##
##  Shapiro-Wilk normality test
##
## data:  log2(night_pomc_all$RelativeGE)
## W = 0.96923, p-value = 0.6256

## 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
```



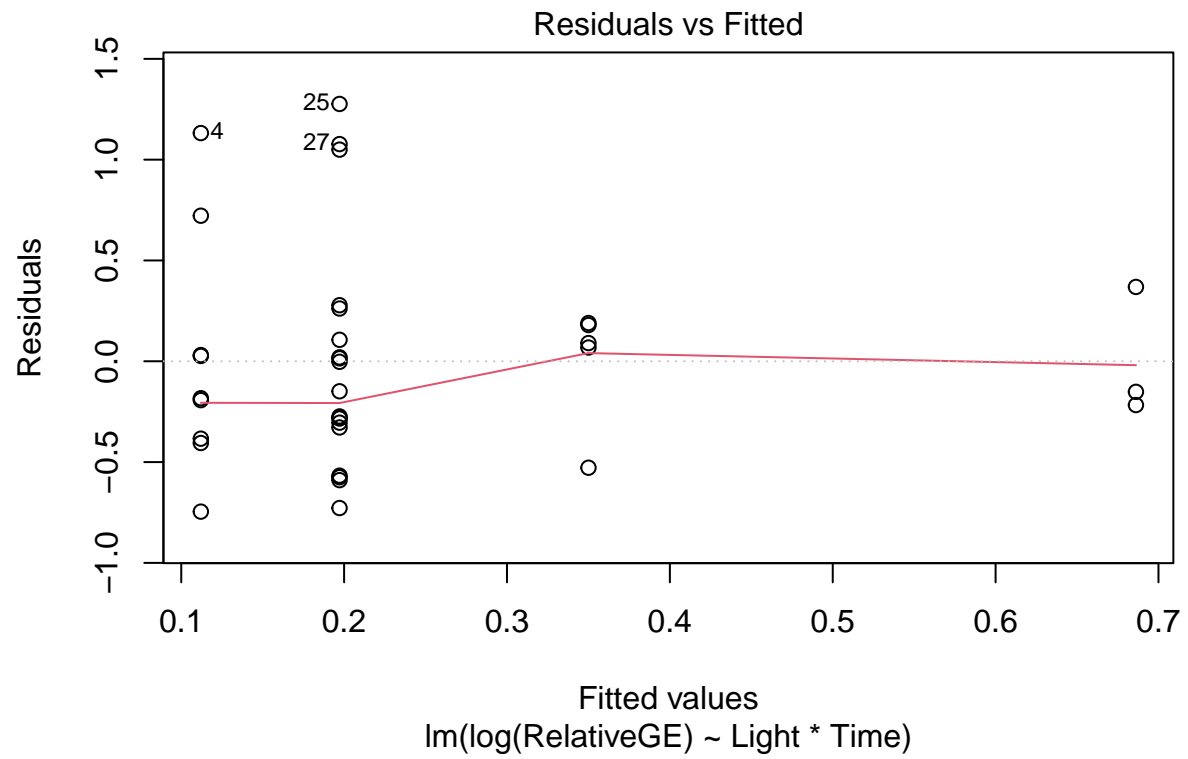
```
## # A tibble: 4 x 4
## # Groups:   Time [2]
##   Time Light    n mean
##   <fct> <fct> <int> <dbl>
## 1 day   light    19  1.47
## 2 day   dark     3  2.06
## 3 night light     5  1.46
## 4 night dark     9  1.33
```

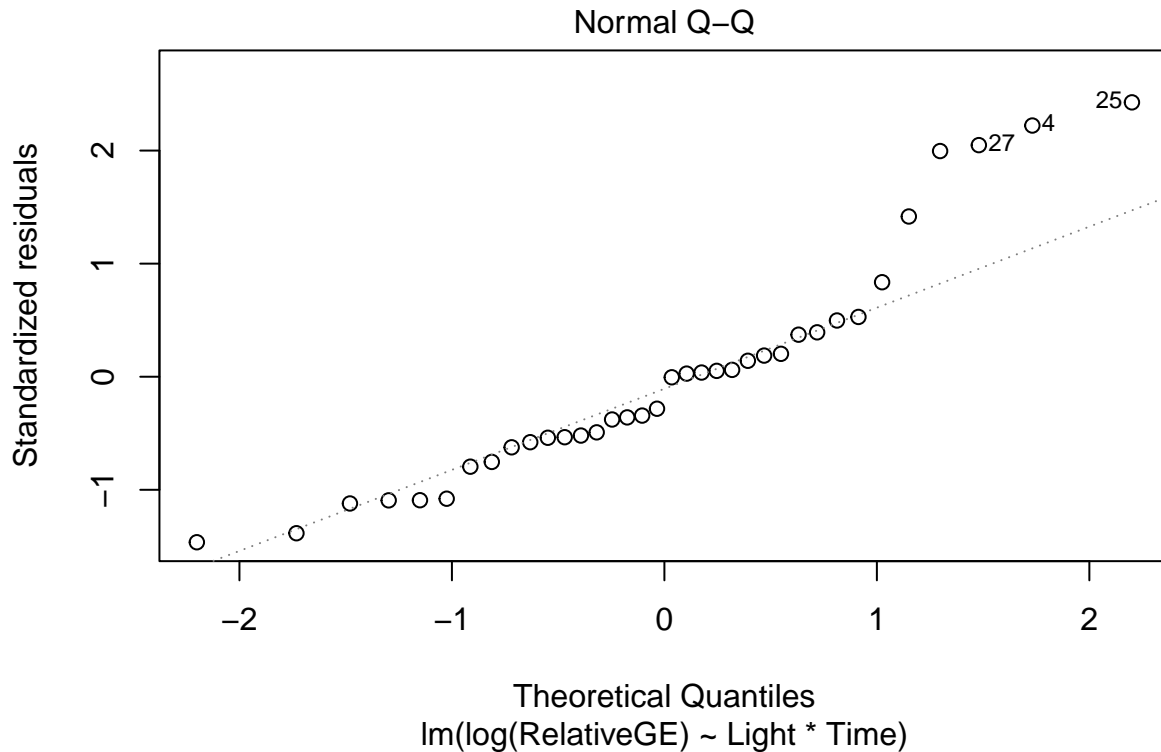


```
##
## Shapiro-Wilk normality test
##
## data: m_pomc_all$RelativeGE
## W = 0.78974, p-value = 1.025e-05

##
## 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
```



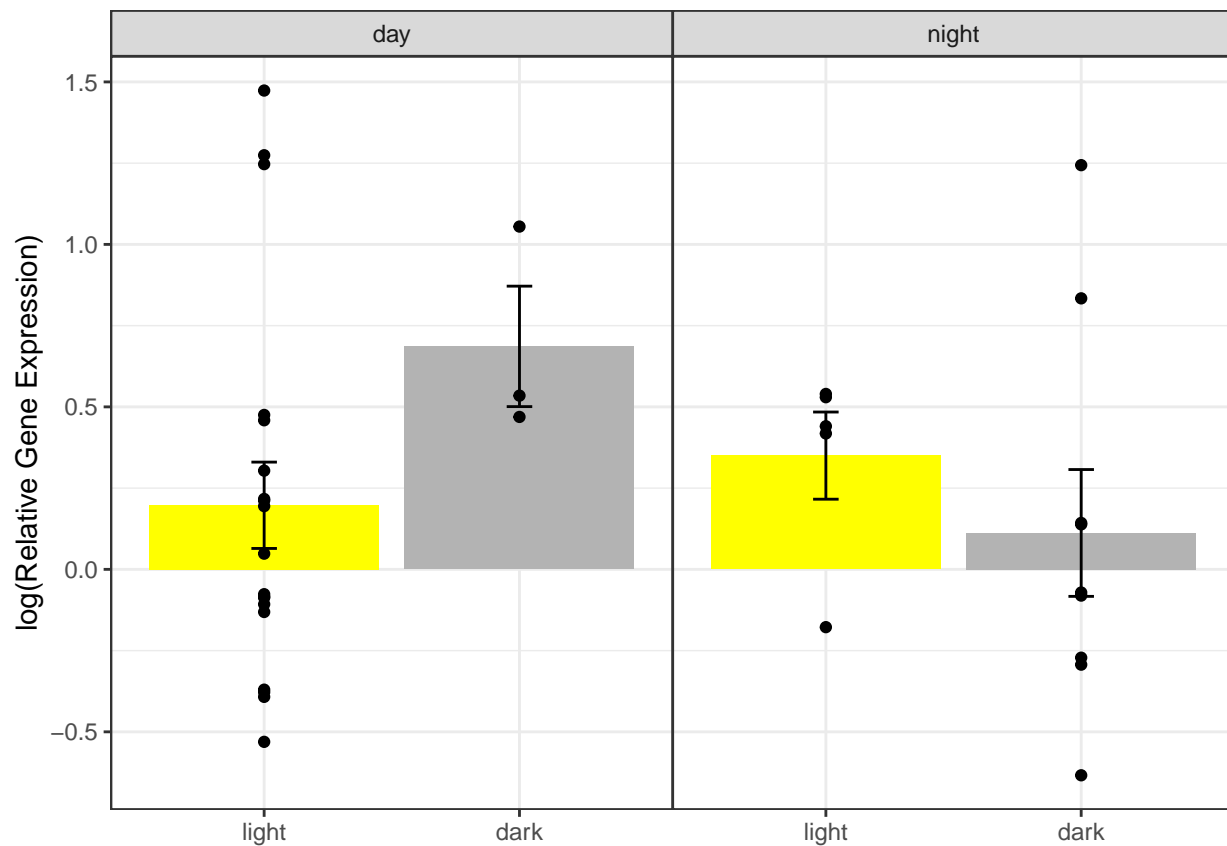


```
## Analysis of Variance Table
##
## Response: log(RelativeGE)
##           Df Sum Sq Mean Sq F value Pr(>F)
## Light       1  0.0056  0.00563   0.0193 0.8904
## Time        1  0.0760  0.07598   0.2602 0.6135
## Light:Time   1  0.7582  0.75816   2.5964 0.1169
## Residuals  32  9.3443  0.29201

## contrast      estimate    SE df t.ratio p.value
## light day - dark day    -0.4890 0.336 32  -1.457  0.4748
## light day - light night  -0.1528 0.272 32  -0.563  0.9424
## light day - dark night    0.0852 0.219 32   0.389  0.9796
## dark day - light night    0.3361 0.395 32   0.852  0.8293
## dark day - dark night    0.5741 0.360 32   1.594  0.3964
## light night - dark night  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 = day:
## contrast      estimate    SE df t.ratio p.value
## light - dark   -0.489 0.336 32  -1.457  0.1550
##
## Time = night:
## 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
## day - night   -0.153 0.272 32  -0.563  0.5776
##
## Light = dark:
## contrast      estimate      SE df t.ratio p.value
## day - night    0.574 0.360 32   1.594  0.1208
##
## Results are given on the log (not the response) scale.
```

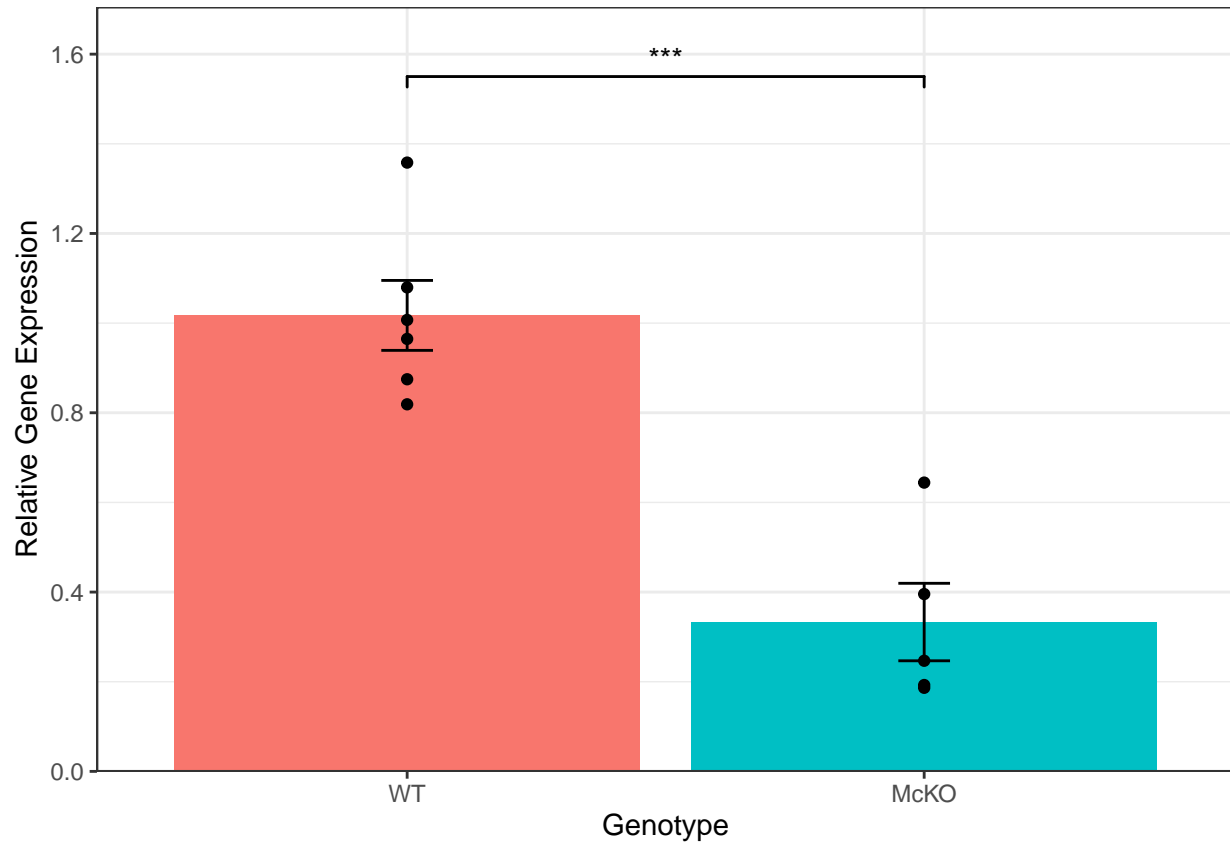


```
## # A tibble: 4 x 5
## # Groups:   Light [2]
##   Light Time      n mean log_mean
##   <fct> <fct> <int> <dbl>    <dbl>
## 1 light day      19  1.47    0.197
## 2 light night     5  1.46    0.350
## 3 dark  day       3  2.06    0.686
## 4 dark  night      9  1.33    0.112
## [1] 0.0006157598
## [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 2
##   Genotype `mean(Relative.GE)`
##   <fct>      <dbl>
## 1 WT          1.02
## 2 McKO         0.333
```

MOR mRNA in morphine vs saline treatment (retinas only)

