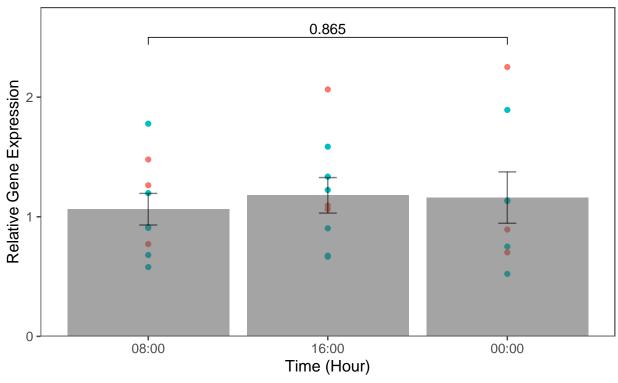
qpCR-Figures

C-T Berezin

10/30/2021

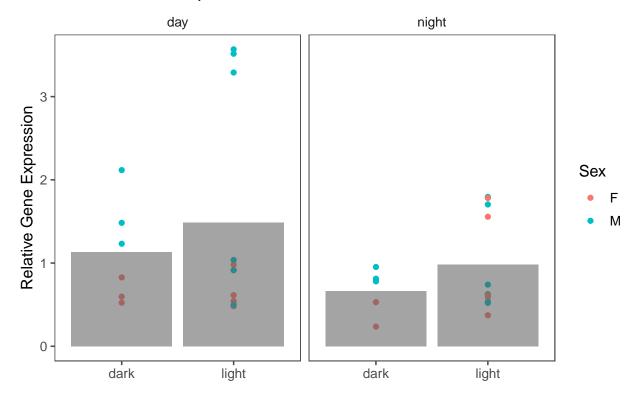
Is POMC under circadian regulation?

Figure 3
Expression of POMC mRNA



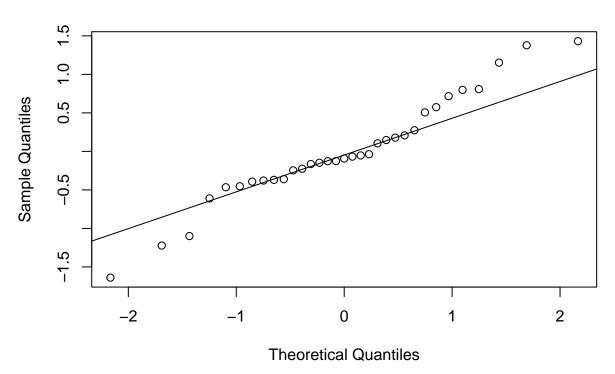
Is POMC expression light-driven?

POMC mRNA Expression



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

Normal Q-Q Plot



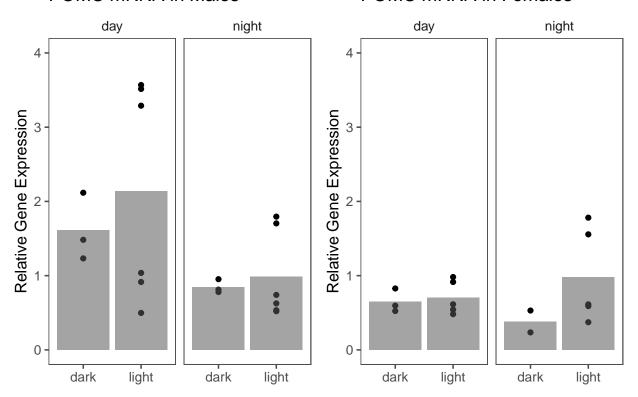
```
##
##
    Welch Two Sample t-test
##
## data: Relative.G.E. by Sex
## t = -2.6484, df = 23.38, p-value = 0.01425
## alternative hypothesis: true difference in means between group F and group M is not equal to 0
## 95 percent confidence interval:
   -1.2590046 -0.1553002
## sample estimates:
  mean in group F mean in group M
##
         0.7441239
                         1.4512763
## Analysis of Variance Table
##
## Response: Relative.G.E.
##
                  Df Sum Sq Mean Sq F value Pr(>F)
                     4.0914 4.0914 6.9085 0.01446 *
## Sex
                   1 0.7450
                             0.7450 1.2580 0.27270
## Light
## Time
                      2.1772
                              2.1772
                                     3.6763 0.06669
## Sex:Light
                      0.0008
                              0.0008
                                     0.0014 0.97091
                   1
## Sex:Time
                      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
  light-dark 0.3187344 -0.2665485 0.9040173 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:dark-F:dark
                    0.7211503 -0.56063890 2.0029395 0.4255743
## F:light-F:dark
                    0.3301872 -0.82923474 1.4896092 0.8612768
## M:light-F:dark
                    1.0303407 -0.09641495 2.1570963 0.0818368
## F:light-M:dark
                   -0.3909631 -1.48407659 0.7021504 0.7598424
                    0.3091904 -0.74921224 1.3675930 0.8521175
## M:light-M:dark
## M:light-F:light
                    0.7001534 -0.20620839 1.6065153 0.1728438
## $`Sex:Time`
##
                          diff
                                       lwr
                                                           p adj
                                                   upr
## 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
## light:day-dark:day
                           0.3295261 -0.7447932 1.4038454 0.8331289
## dark:night-dark:day
                          -0.5320491 -1.8138383 0.7497401 0.6678291
## light:night-dark:day
                          -0.1757383 -1.2500577 0.8985810 0.9690230
## dark:night-light:day
                          -0.8615752 -2.0032950 0.2801446 0.1885459
## light:night-light:day
                          -0.5052644 -1.4078732 0.3973443 0.4299273
## light:night-dark:night 0.3563108 -0.7854090 1.4980306 0.8259208
##
## $`Sex:Light:Time`
                                                                      p adj
##
                                        diff
                                                    lwr
                                                              upr
## M:dark:day-F:dark:day
                                0.961792839 -1.1118752 3.0354609 0.7842025
## F:light:day-F:dark:day
                                0.057760479 -1.7969846 1.9125056 1.0000000
## M:light:day-F:dark:day
                                1.488094717 -0.3077545 3.2839439 0.1583872
## 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-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:light:day-M:dark:day
                                -0.904032359 -2.7587774 0.9507127 0.7410782
```

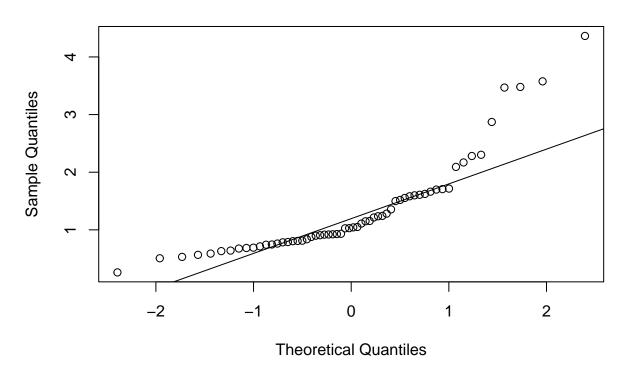
```
## M:light:day-M:dark:day
                                0.526301878 -1.2695473 2.3221511 0.9751004
## F:dark:night-M:dark:day
                               -1.227868002 -3.5462994 1.0905633 0.6580686
## M:dark:night-M:dark:day
                               -0.762773486 -2.8364415 1.3108946 0.9201432
## 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
## M:light:day-F:light:day
                                1.430334237 -0.1075391 2.9682076 0.0817383
## 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: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-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
## 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
## M:dark:night-F:dark:night
                                0.465094516 -1.8533368 2.7835259 0.9973289
## F:light:night-F:dark:night
                                0.599678572 -1.5251989 2.7245560 0.9797791
## M:light:night-F:dark:night
                                0.603965367 -1.4697027 2.6776334 0.9759316
## F:light:night-M:dark:night
                                0.134584056 -1.7201610 1.9893291 0.9999970
## M:light:night-M:dark:night
                                0.138870851 -1.6569783 1.9347201 0.9999953
## M:light:night-F:light:night
                                0.004286795 -1.5335866 1.5421602 1.0000000
```

POMC mRNA in Males

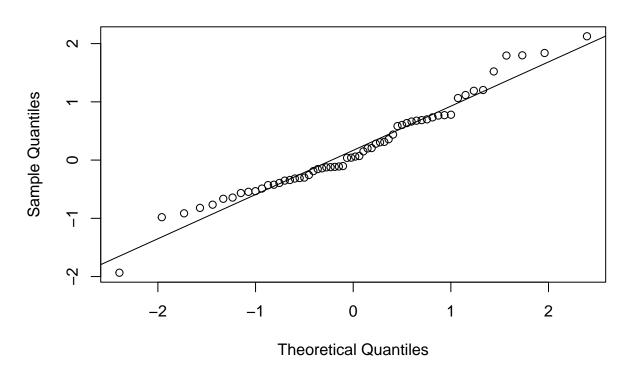
POMC mRNA in Females



Normal Q-Q Plot

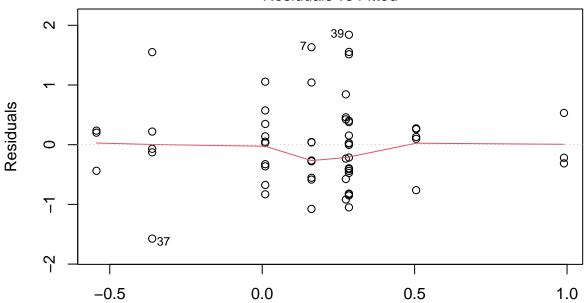


Normal Q-Q Plot



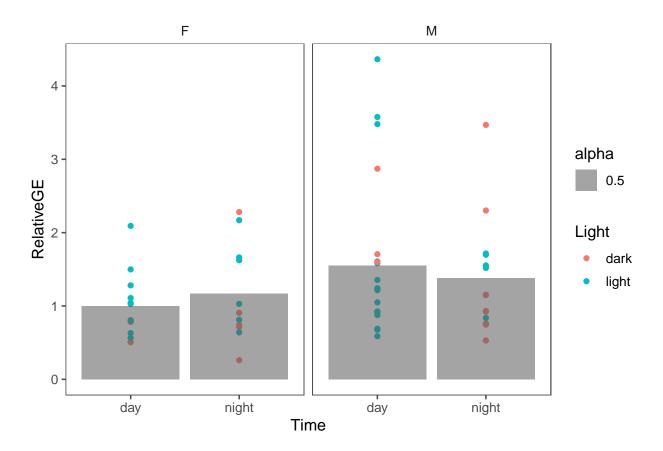
```
##
## Shapiro-Wilk normality test
##
## data: log2(pomc_all$RelativeGE)
## W = 0.97065, p-value = 0.157
```

Residuals vs Fitted



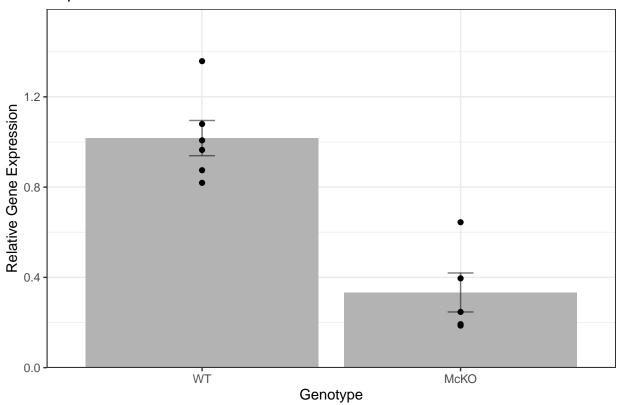
Fitted values Im(log2(RelativeGE) ~ Sex * Light * Time)

```
## 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
                     0.0643 0.06430 0.1115 0.73978
                  1
## Sex:Light
                    1.0107 1.01073 1.7529 0.19131
## Sex:Time
                  1
                     0.4649 0.46485 0.8062 0.37339
## Light:Time
                     0.9614 0.96137
                                    1.6673 0.20234
                  1
## Sex:Light:Time 1 0.6250 0.62495 1.0838 0.30266
## Residuals
                 52 29.9842 0.57662
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```



McKO validation

Expression of MOR mRNA



MOR mRNA in morphine vs saline treatment (retinas only)

Expression of MOR mRNA

