

# qpCR-Figures

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```
library(tidyverse)

## -- Attaching packages ----- tidyverse 1.3.1 --
## v ggplot2 3.3.5      v purrr 0.3.4
## v tibble 3.1.6       v dplyr 1.0.7
## v tidyr 1.1.4        v stringr 1.4.0
## v readr 2.1.0        v forcats 0.5.1

## -- Conflicts ----- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()     masks stats::lag()

library(ggplot2)
library(ggthemes)
library(forcats)
library(ggpubr)
library(writexl)
```

## Is POMC under circadian regulation?

```
pomc_circ <- read.csv("../data/08202021-POMC-final.csv", fileEncoding = 'UTF-8-BOM')
pomc_circ <- pomc_circ %>% mutate(Time = as.factor(Time)) %>%
  mutate(Time = fct_relevel(Time, c("8am", "4pm", "12am"))) %>%
  mutate(Time = fct_recode(Time,
                           "08:00" = "8am",
                           "16:00" = "4pm",
                           "00:00" = "12am")) %>%
  filter(Relative.GE < 3) #remove mouse with high relative GE (data not normal)

#p-values from SigmaPlot
pomc_circ_stats <- tibble::tribble(
  ~group1, ~group2, ~group3, ~p.adj,
  "08:00", "16:00", "00:00", "0.865"
)

pomc_circ_plot <- ggplot(pomc_circ, aes(x=Time, y=Relative.GE)) +
  geom_point() +
  stat_summary(fun = 'mean', geom="bar", aes(alpha=0.5), fill="darkorange") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1, alpha=0.5) +
  theme_few() +
  theme(legend.position = "none") +
```

```

labs(x="Time (Hour)",
     y="Relative Gene Expression",
     title = "Figure 3",
     subtitle = "Expression of POMC mRNA") +
geom_bracket(xmin="08:00", xmax="00:00",
             label=pomc_circ_stats$p.adj,
             data=pomc_circ_stats,
             y.position=2.5) +
scale_y_continuous(expand = expansion(mult = c(0, 0.1)))

ggsave(filename="../figures/pomc_circ.png", plot=pomc_circ_plot, height=3, width=3)

```

### Is POMC expression light-driven?

```

pomc_ld <- read.csv("../data/01142022-POMC-light-dark.csv", fileEncoding = 'UTF-8-BOM')
pomc_ld <- pomc_ld %>% mutate(Time = as.factor(Time.of.Day),
                             Light = as.factor(Light.Condition)) %>%
  select(-Time.of.Day, -Light.Condition)

pomc_ld_plot <- ggplot(pomc_ld, aes(x=Light, y=Relative.G.E.)) +
  geom_point() +
  theme_few() +
  facet_wrap(~Time) +
  labs(x="",
       y="Relative Gene Expression",
       title = "POMC mRNA Expression")

ggsave(filename="../figures/pomc_ld_facet_by_time.png", plot=pomc_ld_plot, height=3, width=3)

```

### McKO validation

```

mcko <- read.csv("../data/08162021-McKO-totalret-final.csv", fileEncoding = 'UTF-8-BOM')
mcko <- mcko %>%
  mutate(Genotype = fct_relevel(Genotype, c("WT", "McKO")))

#p-values from SigmaPlot
mcko_stats <- tibble::tribble(
  ~group1, ~group2, ~p.adj,
  "WT", "McKO", "<0.001"
)

mcko_plot <- mcko %>%
  ggplot(aes(x=Genotype, y=Relative.GE)) +
  geom_point() +
  stat_summary(fun = 'mean', geom="bar", aes(alpha=0.5), fill="darkorange") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1, alpha=0.5) +
  theme_few() +
  theme(legend.position = "none") +
  labs(y="Relative Gene Expression",

```

```

    title="Expression of MOR mRNA") +
  stat_pvalue_manual(mcko_stats,
    y.position = 1.5, step.increase = 0.1,
    label = "p.adj") +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1)))
ggsave(filename="../figures/mcko_fig_bars_orange.png", plot=mcko_plot, height=4, width=3)

```

### MOR mRNA in morphine vs saline treatment (retinas only)

```

treatment <- c("saline", "saline", "saline", "saline",
              "morphine", "morphine", "morphine", "morphine")
sample_num <- c("1", "2", "3", "4",
               "1", "2", "3", "4")
rge <- c(1.025,
        1.054,
        1.12,
        0.822,
        0.677,
        1.054,
        0.938,
        1.057)
mor <- tibble(treatment, sample_num, rge)
mor <- mor %>%
  mutate(treatment = fct_relevel(treatment, c("saline", "morphine")))

#p-value from SigmaPlot
mor_stats <- tibble::tribble(
  ~group1, ~group2, ~p.adj,
  "saline", "morphine", "0.528"
)

mor_plot <- mor %>% ggplot(aes(x=treatment, y=rge)) +
  stat_summary(fun = 'mean', geom="bar", fill="grey40") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1, alpha=0.5) +
  geom_point() +
  labs(title = "Expression of MOR mRNA",
       x="",
       y="Relative Gene Expression") +
  theme_few() +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  stat_pvalue_manual(mor_stats,
    y.position = 1.25, step.increase = 0.1,
    label = "p.adj")

ggsave(filename="../figures/mor_morphine_qpcr.png", plot=mor_plot, height=4, width=3)

```