qpCR Transporter Figures

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```r
library(tidyverse)
library(ggplot2)
library(ggthemes)
library(forcats)
library(ggpubr)
#library(writexl)
library(emmeans)
library(lme4)
library(car)
library(lmerTest)
library(svglite)
```

```r
transporters <- read.csv("../data/07222022-transporter-gene-study.csv", fileEncoding = 'UTF-8-BOM')

transporters <- transporters %>% filter(Sample != "calib")

transporters <- transporters %>% mutate(Tissue = str_sub(Sample, start=-3L, end=-1L),
                                        Treatment = str_extract(Sample, "[:alpha:]+(?=[:digit:])"),
                                        Sample = str_extract(Sample, "[:alnum:]+(?=-)"))

transporters <- transporters %>% mutate(Treatment = factor(Treatment, c("S", "M", "Flo", "Fhi", "C")),
                                        Tissue = factor(Tissue, c("ret", "hyp")))

transporters_long <- transporters %>% pivot_longer(cols=c("Pgp.RGE", "Bcrp.RGE"), names_to = "gene", va

transporters_long <- transporters_long %>% mutate(gene = str_extract(gene, "[:alpha:]+(?=.)"))
transporters_long <- transporters_long %>% mutate(gene = factor(as.factor(gene), c("Pgp", "Bcrp")))

transporter_lm <- lmer(rge ~ gene * Tissue * Treatment + (1|Sample), data=transporters_long)
plot(transporter_lm, type=c("p","smooth"), col.line=1)
```
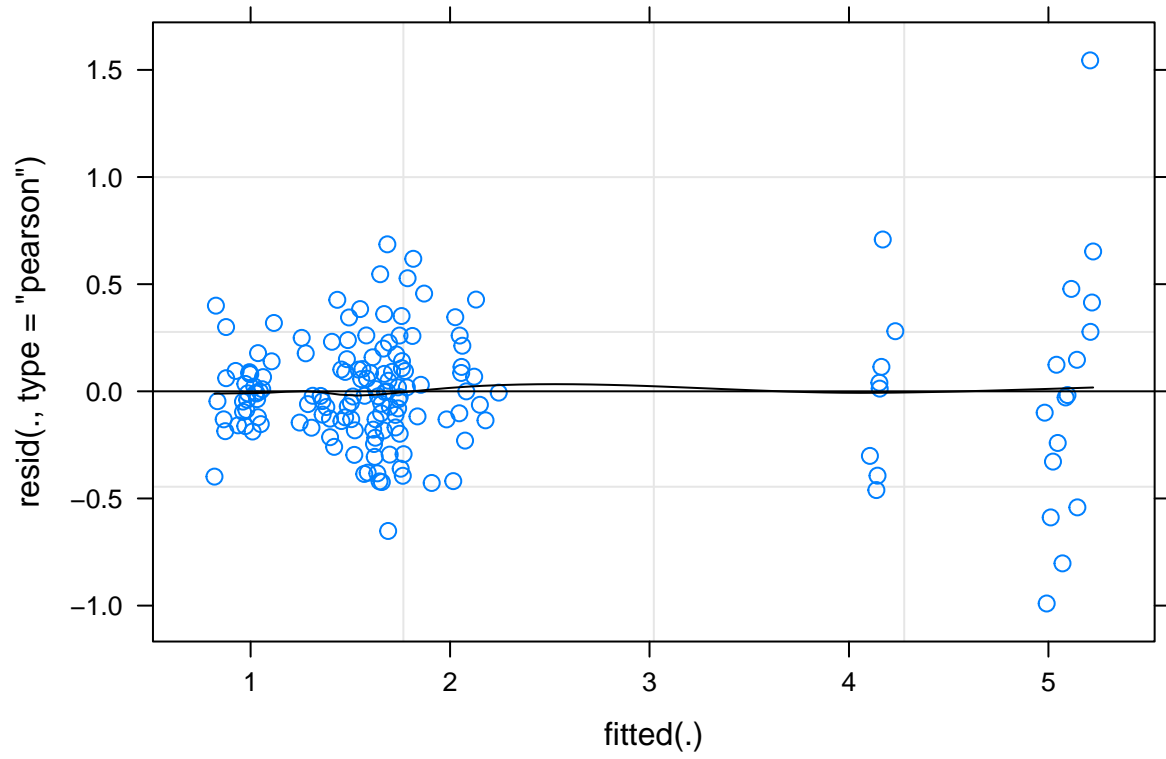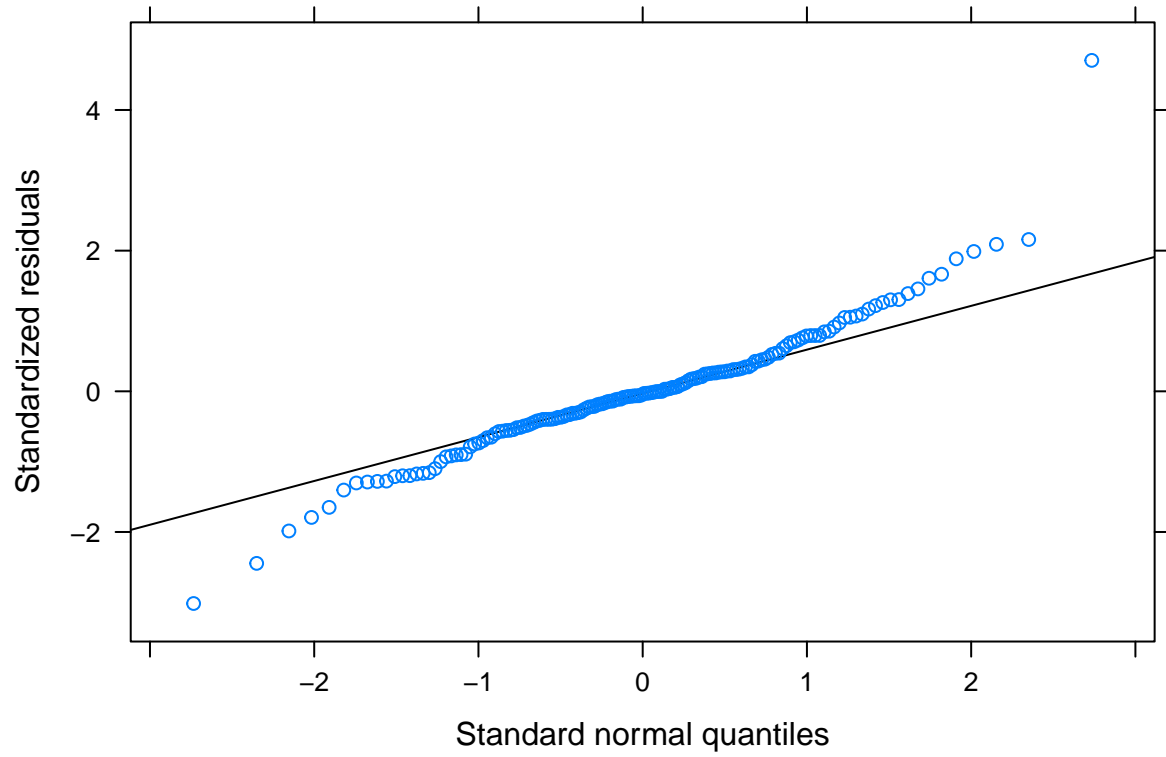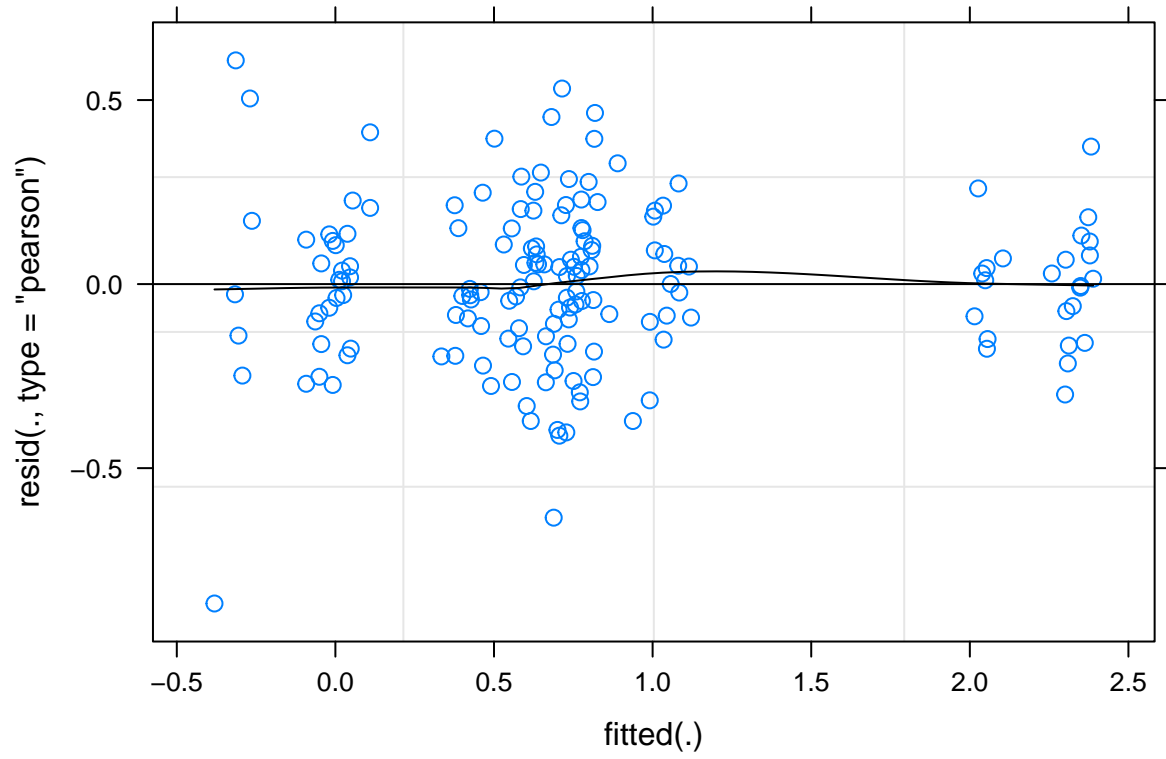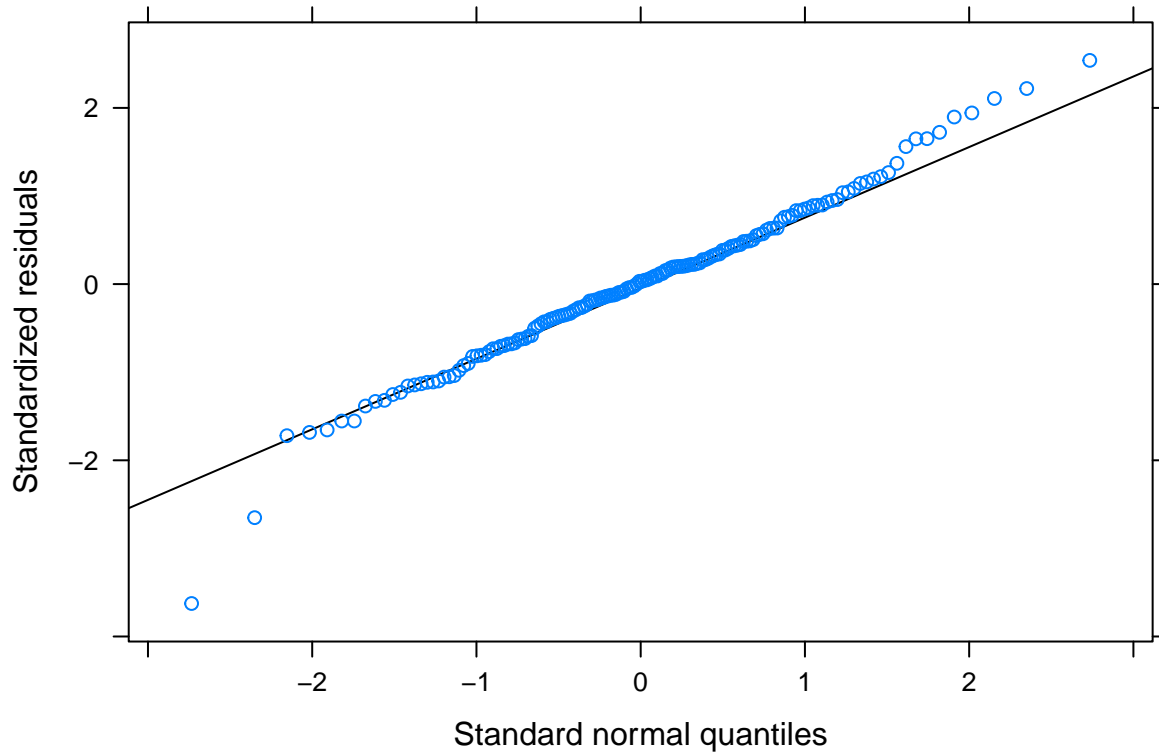
```
lattice::qqmath(transporter_lm)
```

```
transporter_log2_lm <- lmer(log2(rge) ~ gene * Tissue * Treatment + (1|Sample), data=transporters_long)
plot(transporter_log2_lm, type=c("p","smooth"), col.line=1)
```

```
lattice::qqmath(transporter_log2_lm)
```

```
shapiro.test(transporters_long$rge)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  transporters_long$rge
## W = 0.75086, p-value = 3.492e-15
```

```
shapiro.test(log2(transporters_long$rge))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  log2(transporters_long$rge)
## W = 0.92972, p-value = 4.647e-07
```

```
anova(transporter_log2_lm)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                 Sum Sq Mean Sq NumDF DenDF  F value    Pr(>F)
## gene            6.9904  6.9904     1   105 122.0416 < 2.2e-16 ***
## Tissue         29.0690 29.0690     1   105 507.4983 < 2.2e-16 ***
## Treatment      17.8233  4.4558     4    35  77.7915 < 2.2e-16 ***
## gene:Tissue     5.8013  5.8013     1   105 101.2816 < 2.2e-16 ***
```

```
## gene:Treatment          5.6966  1.4241    4   105  24.8634 1.699e-14 ***
## Tissue:Treatment        1.1712  0.2928    4   105   5.1118 0.0008386 ***
## gene:Tissue:Treatment   1.6600  0.4150    4   105   7.2453 3.424e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans::emmeans(transporter_log2_lm, pairwise ~ Treatment | Tissue, by="gene")$contrasts
```

```
## Tissue = ret, gene = Pgp:
##  contrast   estimate     SE  df t.ratio p.value
##  S - M       -0.6263 0.122 136  -5.124  <.0001
##  S - Flo     -0.7575 0.140 136  -5.398  <.0001
##  S - Fhi     -0.7569 0.113 136  -6.694  <.0001
##  S - C        0.3061 0.127 136   2.415  0.1176
##  M - Flo     -0.1312 0.143 136  -0.915  0.8909
##  M - Fhi     -0.1306 0.117 136  -1.117  0.7974
##  M - C        0.9324 0.130 136   7.162  <.0001
##  Flo - Fhi    0.0006 0.136 136   0.004  1.0000
##  Flo - C      1.0636 0.147 136   7.221  <.0001
##  Fhi - C      1.0630 0.122 136   8.740  <.0001
##
## Tissue = hyp, gene = Pgp:
##  contrast   estimate     SE  df t.ratio p.value
##  S - M       -1.2693 0.122 136 -10.383  <.0001
##  S - Flo     -1.5800 0.140 136 -11.260  <.0001
##  S - Fhi     -1.5491 0.113 136 -13.700  <.0001
##  S - C        0.0809 0.127 136   0.638  0.9685
##  M - Flo     -0.3108 0.143 136  -2.167  0.1986
##  M - Fhi     -0.2798 0.117 136  -2.394  0.1232
##  M - C        1.3502 0.130 136  10.370  <.0001
##  Flo - Fhi    0.0310 0.136 136   0.228  0.9994
##  Flo - C      1.6610 0.147 136  11.276  <.0001
##  Fhi - C      1.6300 0.122 136  13.401  <.0001
##
## Tissue = ret, gene = Bcrp:
##  contrast   estimate     SE  df t.ratio p.value
##  S - M       -0.5798 0.122 136  -4.743  0.0001
##  S - Flo     -0.6456 0.140 136  -4.601  0.0001
##  S - Fhi     -0.4050 0.113 136  -3.582  0.0043
##  S - C       -0.0115 0.127 136  -0.091  1.0000
##  M - Flo     -0.0658 0.143 136  -0.459  0.9908
##  M - Fhi      0.1748 0.117 136   1.496  0.5671
##  M - C        0.5683 0.130 136   4.365  0.0002
##  Flo - Fhi    0.2406 0.136 136   1.773  0.3934
##  Flo - C      0.6340 0.147 136   4.304  0.0003
##  Fhi - C      0.3934 0.122 136   3.235  0.0131
##
## Tissue = hyp, gene = Bcrp:
##  contrast   estimate     SE  df t.ratio p.value
##  S - M       -0.2989 0.122 136  -2.445  0.1097
##  S - Flo     -0.4793 0.140 136  -3.416  0.0074
##  S - Fhi     -0.5519 0.113 136  -4.881  <.0001
##  S - C       -0.2040 0.127 136  -1.609  0.4941
##  M - Flo     -0.1804 0.143 136  -1.258  0.7174
```

```
## M - Fhi     -0.2530 0.117 136  -2.164  0.1996
## M - C        0.0949 0.130 136   0.729  0.9495
## Flo - Fhi   -0.0726 0.136 136  -0.535  0.9835
## Flo - C      0.2752 0.147 136   1.869  0.3393
## Fhi - C      0.3479 0.122 136   2.860  0.0387
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
emmeans::emmeans(transporter_log2_lm, pairwise ~ Tissue | Treatment, by="gene")$contrasts
```

```
## Treatment = S, gene = Pgp:
##  contrast  estimate    SE  df t.ratio p.value
##  ret - hyp   -0.780 0.113 105  -6.916  <.0001
##
## Treatment = M, gene = Pgp:
##  contrast  estimate    SE  df t.ratio p.value
##  ret - hyp   -1.423 0.120 105 -11.893  <.0001
##
## Treatment = Flo, gene = Pgp:
##  contrast  estimate    SE  df t.ratio p.value
##  ret - hyp   -1.603 0.151 105 -10.589  <.0001
##
## Treatment = Fhi, gene = Pgp:
##  contrast  estimate    SE  df t.ratio p.value
##  ret - hyp   -1.572 0.102 105 -15.408  <.0001
##
## Treatment = C, gene = Pgp:
##  contrast  estimate    SE  df t.ratio p.value
##  ret - hyp   -1.005 0.128 105  -7.859  <.0001
##
## Treatment = S, gene = Bcrp:
##  contrast  estimate    SE  df t.ratio p.value
##  ret - hyp   -0.510 0.113 105  -4.519  <.0001
##
## Treatment = M, gene = Bcrp:
##  contrast  estimate    SE  df t.ratio p.value
##  ret - hyp   -0.229 0.120 105  -1.913  0.0584
##
## Treatment = Flo, gene = Bcrp:
##  contrast  estimate    SE  df t.ratio p.value
##  ret - hyp   -0.344 0.151 105  -2.270  0.0253
##
## Treatment = Fhi, gene = Bcrp:
##  contrast  estimate    SE  df t.ratio p.value
##  ret - hyp   -0.657 0.102 105  -6.436  <.0001
##
## Treatment = C, gene = Bcrp:
##  contrast  estimate    SE  df t.ratio p.value
##  ret - hyp   -0.702 0.128 105  -5.490  <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
```

```
emmeans::emmeans(transporter_log2_lm, pairwise ~ gene | Treatment, by="Tissue")$contrasts
```

```
## Treatment = S, Tissue = ret:
##  contrast    estimate    SE  df t.ratio p.value
##  Pgp - Bcrp   0.0000 0.113 105   0.000  1.0000
##
## Treatment = M, Tissue = ret:
##  contrast    estimate    SE  df t.ratio p.value
##  Pgp - Bcrp   0.0465 0.120 105   0.389  0.6983
##
## Treatment = Flo, Tissue = ret:
##  contrast    estimate    SE  df t.ratio p.value
##  Pgp - Bcrp   0.1119 0.151 105   0.739  0.4614
##
## Treatment = Fhi, Tissue = ret:
##  contrast    estimate    SE  df t.ratio p.value
##  Pgp - Bcrp   0.3519 0.102 105   3.448  0.0008
##
## Treatment = C, Tissue = ret:
##  contrast    estimate    SE  df t.ratio p.value
##  Pgp - Bcrp  -0.3177 0.128 105  -2.483  0.0146
##
## Treatment = S, Tissue = hyp:
##  contrast    estimate    SE  df t.ratio p.value
##  Pgp - Bcrp   0.2704 0.113 105   2.397  0.0183
##
## Treatment = M, Tissue = hyp:
##  contrast    estimate    SE  df t.ratio p.value
##  Pgp - Bcrp   1.2408 0.120 105  10.369  <.0001
##
## Treatment = Flo, Tissue = hyp:
##  contrast    estimate    SE  df t.ratio p.value
##  Pgp - Bcrp   1.3712 0.151 105   9.059  <.0001
##
## Treatment = Fhi, Tissue = hyp:
##  contrast    estimate    SE  df t.ratio p.value
##  Pgp - Bcrp   1.2676 0.102 105  12.421  <.0001
##
## Treatment = C, Tissue = hyp:
##  contrast    estimate    SE  df t.ratio p.value
##  Pgp - Bcrp  -0.0146 0.128 105  -0.114  0.9095
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
```

```
trans_sumstats <- transporters_long %>% group_by(Treatment, Tissue, gene) %>% summarise(
  n = n(),
  mean = mean(rge),
  sd = sd(rge),
  log2_mean = mean(log2(rge)),
  log2_sd = sd(log2(rge))
)
```

```
## `summarise()` has grouped output by 'Treatment', 'Tissue'. You can override using the `.groups` argu
```

```
trans_sumstats
```

```
## # A tibble: 20 x 8
## # Groups:   Treatment, Tissue [10]
##    Treatment Tissue gene      n  mean     sd log2_mean log2_sd
##    <fct>     <fct>  <fct> <int> <dbl>  <dbl>     <dbl>   <dbl>
##  1 S         ret    Pgp       9 1.02   0.204 -1.11e-11   0.280
##  2 S         ret    Bcrp      9 1.01   0.117 -4.39e-11   0.163
##  3 S         hyp    Pgp       9 1.77   0.448  7.80e- 1   0.385
##  4 S         hyp    Bcrp      9 1.44   0.244  5.10e- 1   0.242
##  5 M         ret    Pgp       8 1.57   0.340  6.26e- 1   0.305
##  6 M         ret    Bcrp      8 1.51   0.212  5.80e- 1   0.202
##  7 M         hyp    Pgp       8 4.16   0.411  2.05e+ 0   0.141
##  8 M         hyp    Bcrp      8 1.76   0.199  8.09e- 1   0.163
##  9 Flo       ret    Pgp       5 1.70   0.220  7.57e- 1   0.190
## 10 Flo       ret    Bcrp      5 1.58   0.234  6.46e- 1   0.209
## 11 Flo       hyp    Pgp       5 5.17   0.698  2.36e+ 0   0.201
## 12 Flo       hyp    Bcrp      5 2.01   0.347  9.89e- 1   0.268
## 13 Fhi       ret    Pgp      11 1.71   0.289  7.57e- 1   0.242
## 14 Fhi       ret    Bcrp     11 1.33   0.157  4.05e- 1   0.168
## 15 Fhi       hyp    Pgp      11 5.07   0.705  2.33e+ 0   0.193
## 16 Fhi       hyp    Bcrp     11 2.10   0.257  1.06e+ 0   0.182
## 17 C         ret    Pgp       7 0.853  0.284 -3.06e- 1   0.527
## 18 C         ret    Bcrp      7 1.01   0.109  1.15e- 2   0.150
## 19 C         hyp    Pgp       7 1.65   0.285  6.99e- 1   0.258
## 20 C         hyp    Bcrp      7 1.67   0.375  7.14e- 1   0.308
```
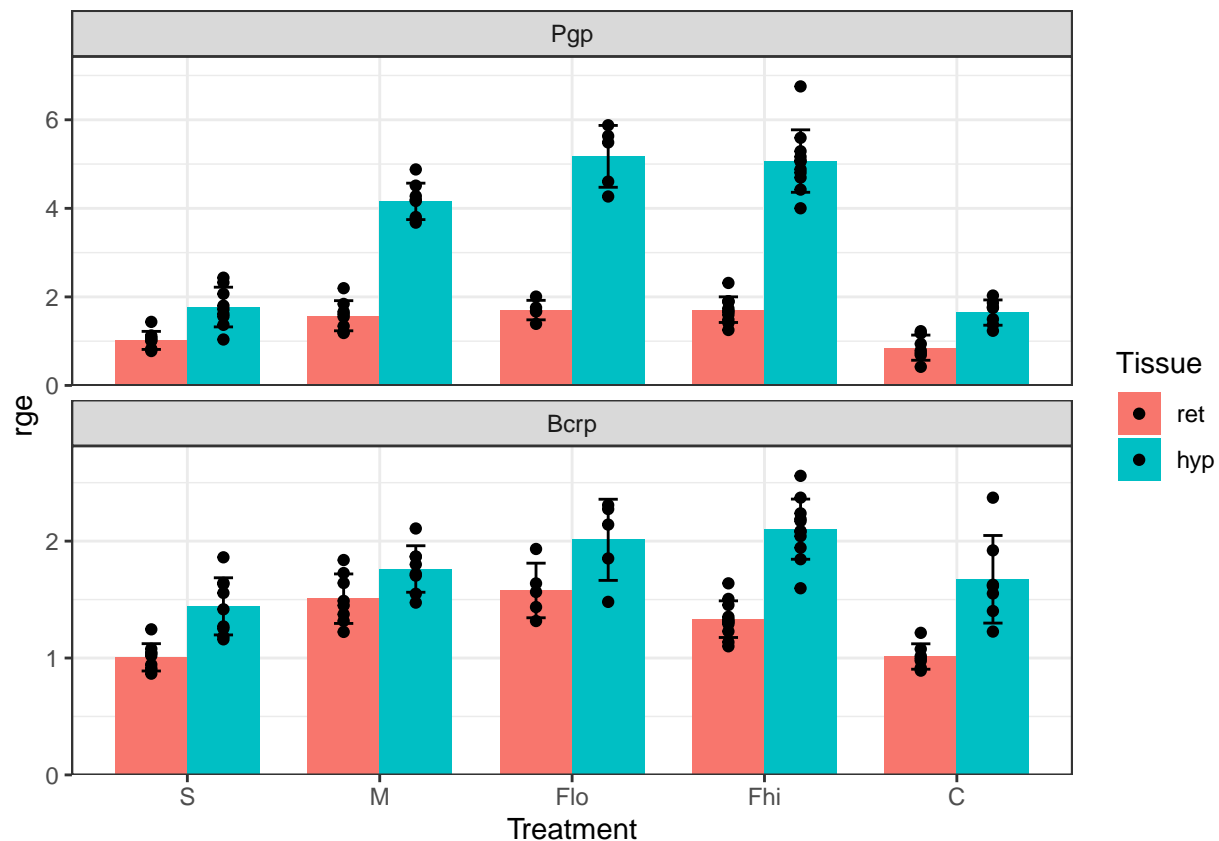
Comparing hyp vs ret within each treatment/gene

```
trans_hyp_ret_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
               fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=mean-sd, ymax=mean+sd, fill=Tissue),
                width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
             stat="identity", position=position_dodge(width=0.75)) +
  facet_wrap(~gene, scales="free_y", ncol=1) +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw()
```

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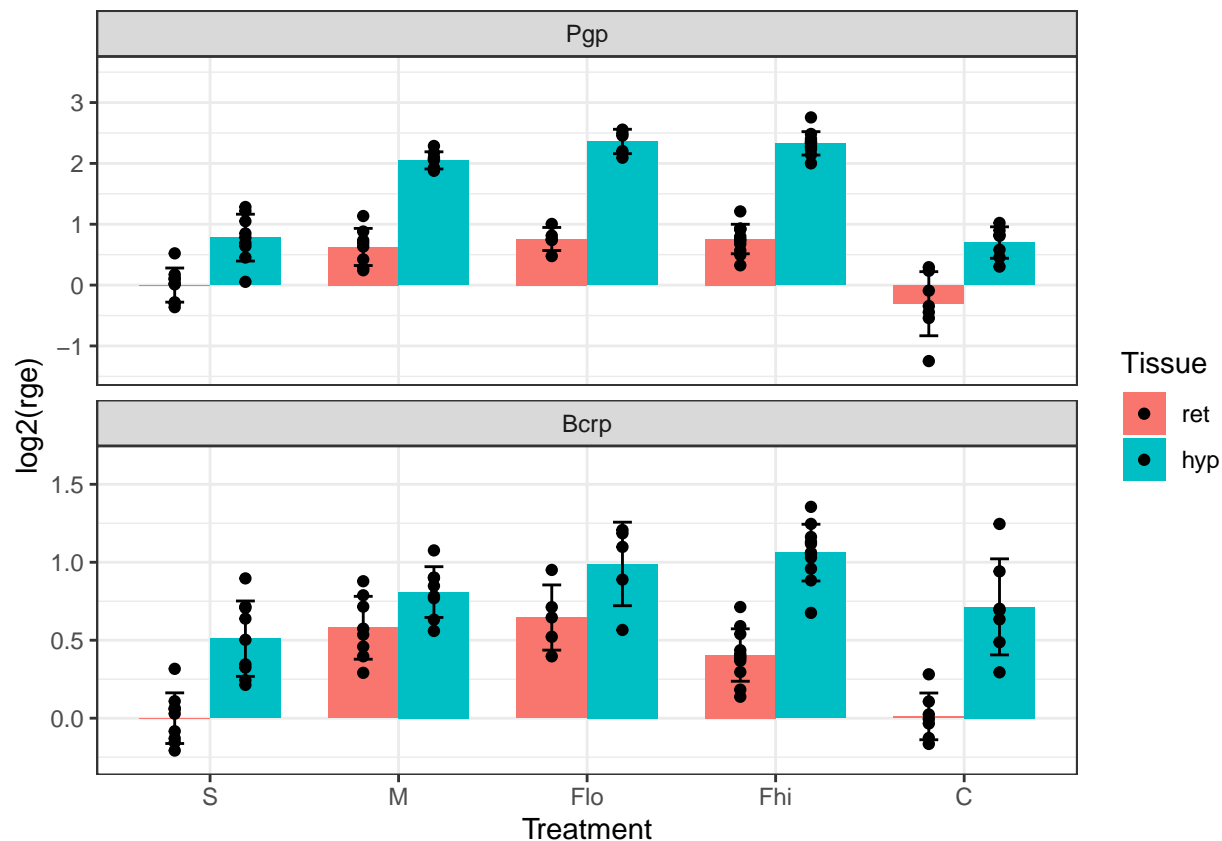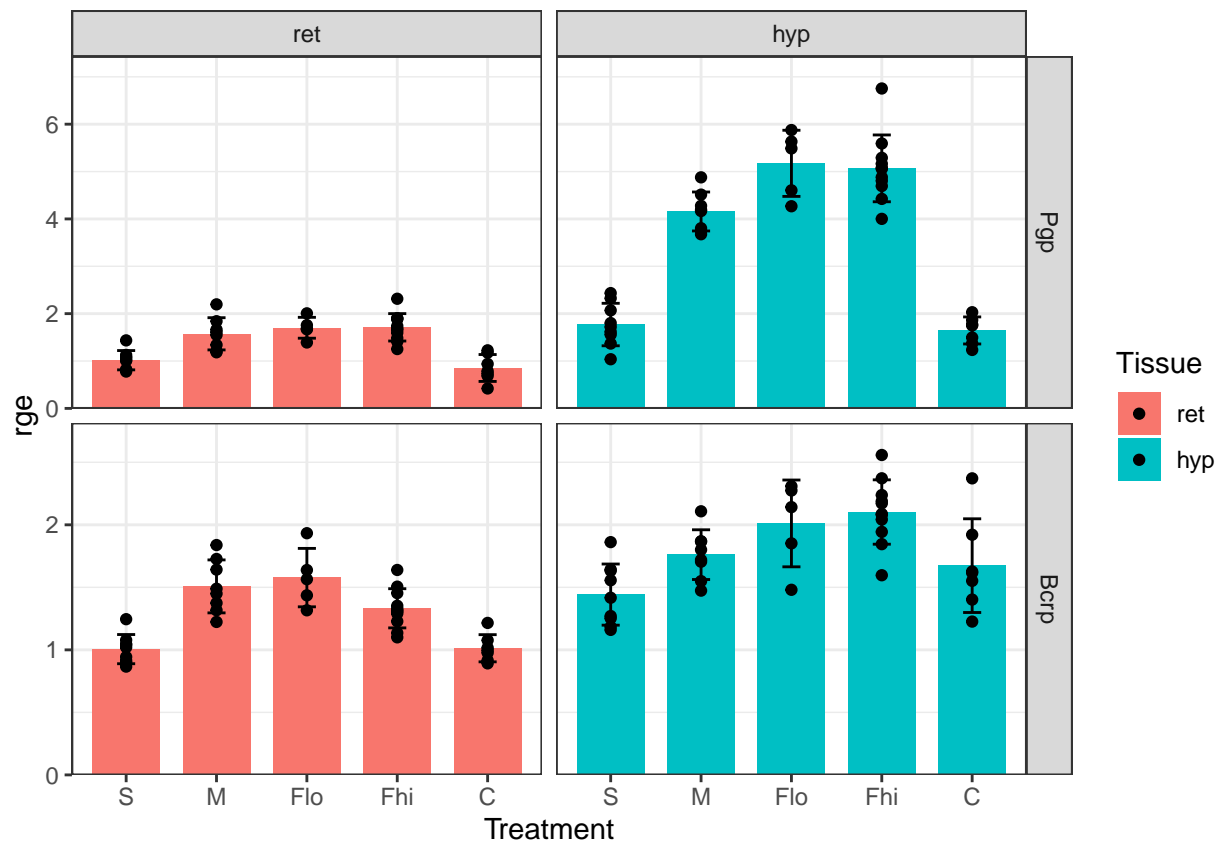
```
trans_hyp_ret_plot
```

```
#ggsave(filename="../figures/trans_hyp_ret.png", plot=trans_hyp_ret_plot, height=4, width=6)
#ggsave(filename="../figures/trans_hyp_ret.svg", plot=trans_hyp_ret_plot, height=4, width=6)
```

```
trans_hyp_ret_log_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Treatment, y=log2(rge), fill=Tissue),
               fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=log2_mean-log2_sd,
                                         ymax=log2_mean+log2_sd, fill=Tissue),
                width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Treatment, y=log2(rge), fill=Tissue),
             stat="identity", position=position_dodge(width=0.75)) +
  facet_wrap(~gene, scales="free_y",ncol=1) +
  scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw()
```

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

```
trans_hyp_ret_log_plot
```

Comparing treatments within each tissue/gene

```
trans_trt_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
               fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=mean-sd, ymax=mean+sd, fill=Tissue),
                width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
             stat="identity", position=position_dodge(width=0.75)) +
  facet_grid(gene~Tissue, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw()
```

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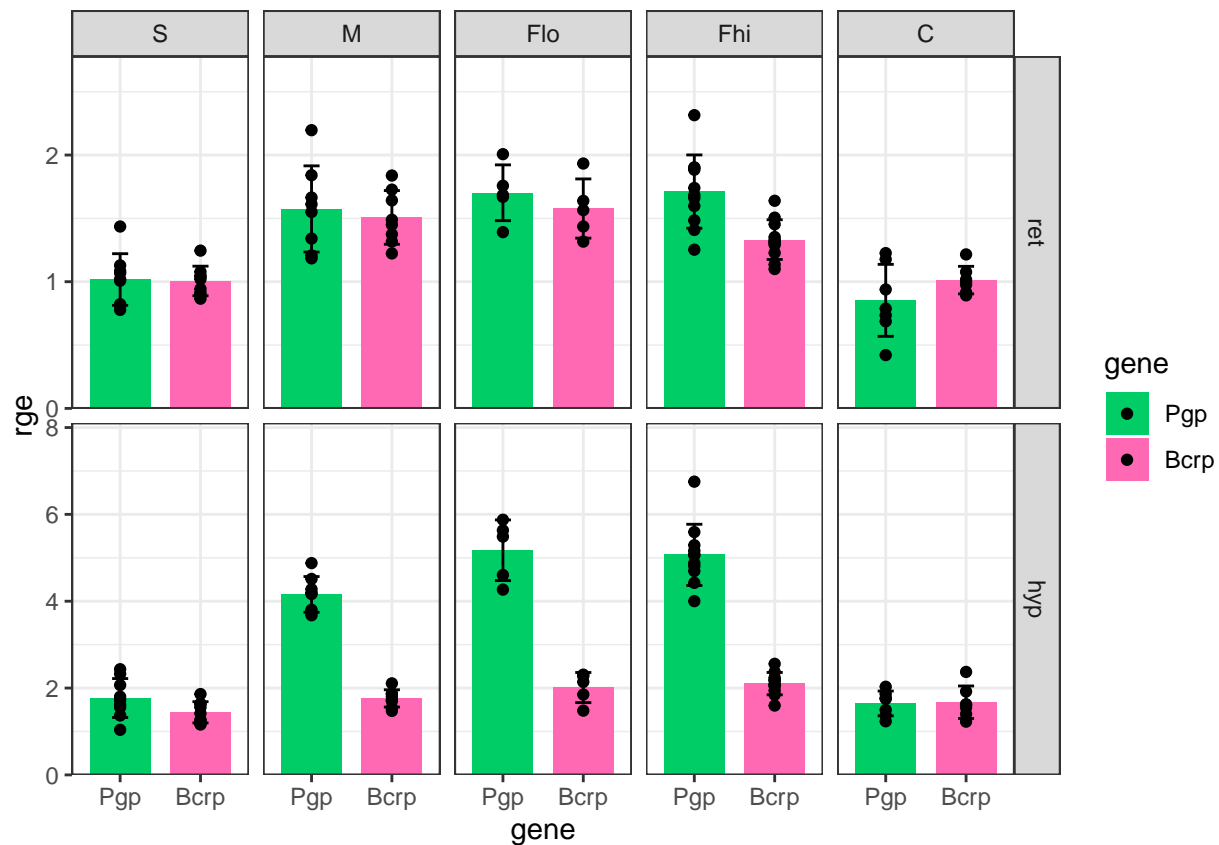
```
trans_trt_plot
```

```
#ggsave(filename="../figures/trans_trt.png", plot=trans_trt_plot, height=4, width=6)
#ggsave(filename="../figures/trans_trt.svg", plot=trans_trt_plot, height=4, width=6)


trans_trt_log_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Treatment, y=log2(rge), fill=Tissue),
               fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=log2_mean-log2_sd,
                                         ymax=log2_mean+log2_sd, fill=Tissue),
                width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Treatment, y=log2(rge), fill=Tissue),
             stat="identity", position=position_dodge(width=0.75)) +
  facet_grid(gene~Tissue, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw()
```

```
## Warning: Ignoring unknown aesthetics: fill
```
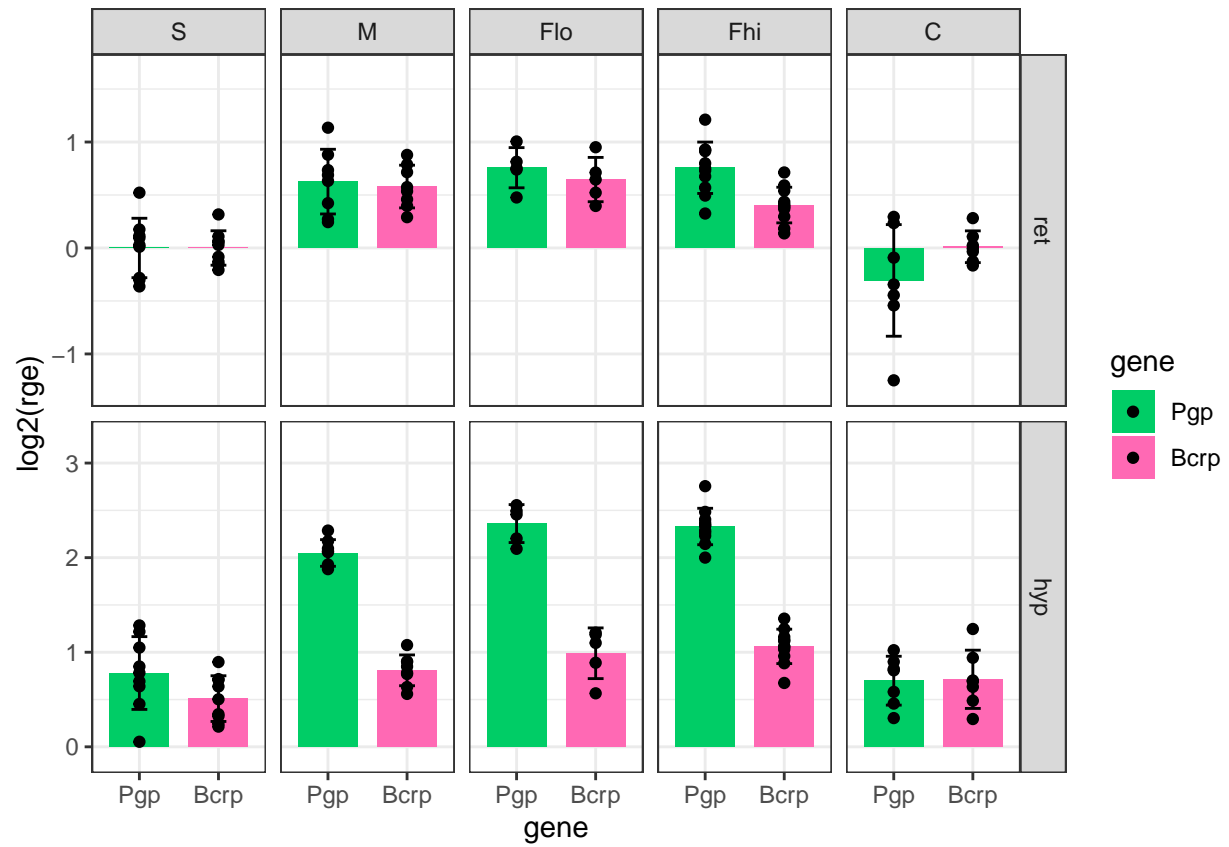
```
trans_trt_log_plot
```
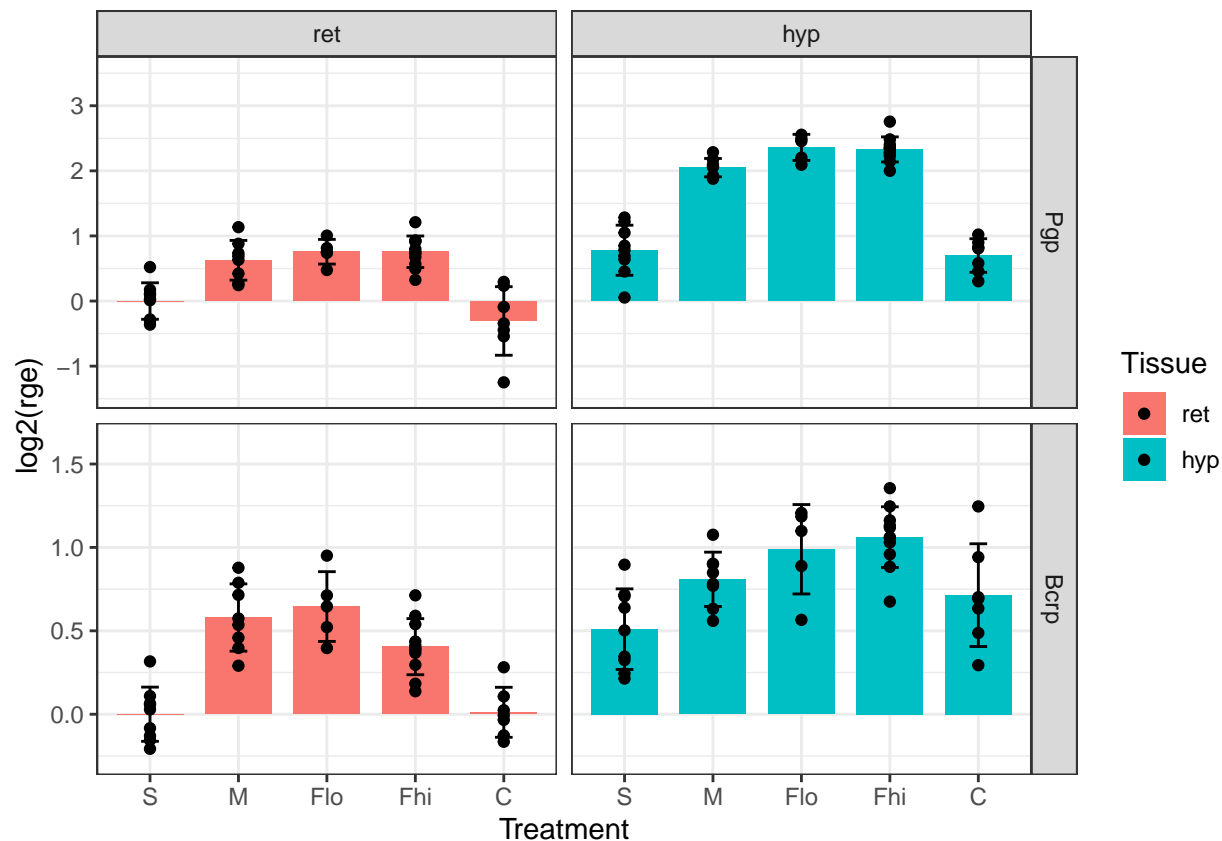
```
#ggsave(filename="../figures/trans_gene_to_gene.png", plot=trans_g2g_plot, height=4, width=6)
#ggsave(filename="../figures/trans_gene_to_gene.svg", plot=trans_g2g_plot, height=4, width=6)
```

```
trans_g2g_log_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=gene, y=log2(rge), fill=gene),
               fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=gene, ymin=log2_mean-log2_sd,
                                         ymax=log2_mean+log2_sd, fill=gene),
                width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=gene, y=log2(rge), fill=gene),
             position=position_dodge(width=0.75)) +
  facet_grid(Tissue~Treatment, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  scale_fill_manual(values=c("springgreen3", "hotpink")) +
  theme_bw()
```

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

```
trans_g2g_log_plot
```

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```
#ggsave(filename="../figures/trans_g2g_log.png", plot=trans_g2g_log_plot, height=4, width=6)
#ggsave(filename="../figures/trans_g2g_log.svg", plot=trans_g2g_log_plot, height=4, width=6)
```