morphine_regression

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```r
regression <- read.csv("../data/morphine__sex_diff_transporters.csv", fileEncoding = 'UTF-8-BOM')

regression$conc_wt <- (regression$raw_conc/regression$tissue_weight)

str(regression)
```

```
## 'data.frame':     48 obs. of  11 variables:
##  $ animal       : chr  "1M" "2M" "4M" "5M" ...
##  $ raw_conc     : num  56.6 22.1 49.5 87.4 36.6 ...
##  $ tissue_weight: num  20.1 10.3 15.1 31.4 14.7 14.3 20.8 15.7 43.7 14.6 ...
##  $ body_wt      : num  27.2 30.1 25.8 26.1 27.3 26.2 23.7 27.2 21.3 22.9 ...
##  $ dose         : num  20.6 18.6 21.7 20 19.1 19.9 22 19.2 20.4 19 ...
##  $ pgp          : num  2.43 2.7 2.92 2.77 2.46 ...
##  $ bcrp         : num  1.037 1.249 1.151 0.986 1.205 ...
##  $ stage        : chr  "male" "male" "male" "male" ...
##  $ group        : chr  "male" "male" "male" "male" ...
##  $ tissue       : chr  "brain" "brain" "brain" "brain" ...
##  $ conc_wt      : num  2.82 2.14 3.28 2.78 2.49 ...
```

```r
regression <- regression %>% mutate(stage = as.factor(stage),
                                    group = as.factor(group),
                                    tissue = factor(as.factor(tissue), c("retina", "brain"))
                                    )
head(regression)
```

```
##   animal raw_conc tissue_weight body_wt dose       pgp       bcrp stage group
## 1     1M  56.5895          20.1    27.2 20.6 2.429027 1.0366970  male  male
## 2     2M  22.0798          10.3    30.1 18.6 2.699340 1.2487060  male  male
## 3     4M  49.5114          15.1    25.8 21.7 2.923597 1.1505545  male  male
## 4     5M  87.4161          31.4    26.1 20.0 2.770560 0.9860451  male  male
## 5     6M  36.5960          14.7    27.3 19.1 2.464103 1.2046594  male  male
## 6     7M  41.6730          14.3    26.2 19.9 2.381759 1.1407836  male  male
##   tissue  conc_wt
## 1  brain 2.815398
## 2  brain 2.143670
## 3  brain 3.278901
## 4  brain 2.783952
## 5  brain 2.489524
## 6  brain 2.914196
```

```r
pgp_plot <- ggplot(regression, aes(pgp, conc_wt)) +
  geom_point(col="black") +
  geom_smooth(method = "lm", se = T, aes(fill=tissue, col=tissue)) +
  xlim(0,5) +
```

```r
  theme_bw(20) +
  ylab("[Morphine] per mg tissue (ng/mg)") +
  scale_fill_manual(values=c("dodgerblue", "goldenrod1"),
                    labels=c("retina" = "Retina", "brain" = "Brain")) +
  scale_color_manual(values=c("dodgerblue", "goldenrod1"),
                    labels=c("retina" = "Retina", "brain" = "Brain"))

bcrp_plot <- ggplot(regression, aes(bcrp, conc_wt)) +
  geom_point(col="black") +
  geom_smooth(method = "lm", se = T, aes(fill=tissue, col=tissue)) +
  xlim(0,5) +
  ylab("") +
  theme_bw(20) %+replace%
  theme(axis.text.y=element_blank()) +
  scale_fill_manual(values=c("dodgerblue", "goldenrod1"),
                    labels=c("retina" = "Retina", "brain" = "Brain")) +
  scale_color_manual(values=c("dodgerblue", "goldenrod1"),
                    labels=c("retina" = "Retina", "brain" = "Brain"))

combined_correlation_plots <- ggarrange(pgp_plot, bcrp_plot, ncol=2, common.legend = TRUE, legend="botto
```

```
## `geom_smooth()` using formula 'y ~ x'
## `geom_smooth()` using formula 'y ~ x'
## `geom_smooth()` using formula 'y ~ x'
```

```r
combined_correlation_plots
```
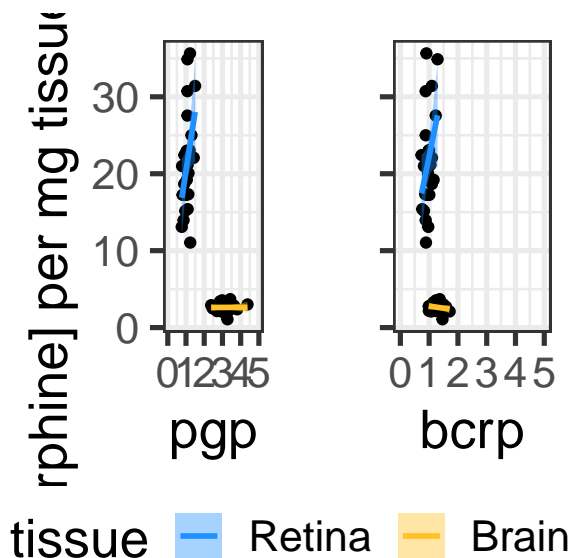


```r
#ggsave("../figures/combined_correlation_plots.svg", plot=combined_correlation_plots, width=10, height
```

```r
regression_brain <- subset(regression, tissue == "brain")
head(regression_brain)
```

```
##   animal raw_conc tissue_weight body_wt dose      pgp      bcrp stage group
## 1     1M  56.5895          20.1    27.2 20.6 2.429027 1.0366970  male  male
## 2     2M  22.0798          10.3    30.1 18.6 2.699340 1.2487060  male  male
## 3     4M  49.5114          15.1    25.8 21.7 2.923597 1.1505545  male  male
```

```
## 4     5M  87.4161           31.4    26.1 20.0 2.770560 0.9860451  male  male
## 5     6M  36.5960           14.7    27.3 19.1 2.464103 1.2046594  male  male
## 6     7M  41.6730           14.3    26.2 19.9 2.381759 1.1407836  male  male
##   tissue  conc_wt
## 1  brain 2.815398
## 2  brain 2.143670
## 3  brain 3.278901
## 4  brain 2.783952
## 5  brain 2.489524
## 6  brain 2.914196
```
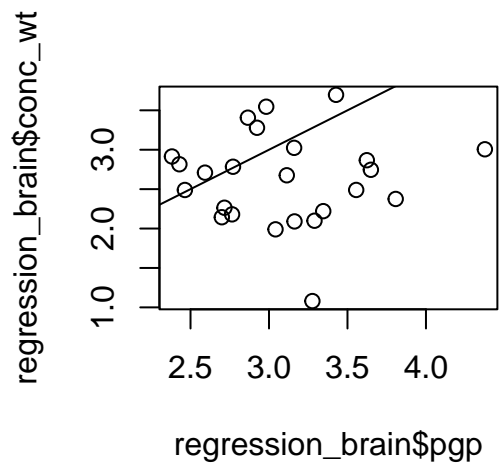
```r
shapiro.test(regression_brain$conc_wt)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  regression_brain$conc_wt
## W = 0.9661, p-value = 0.5723
```

```r
shapiro.test(regression_brain$pgp)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  regression_brain$pgp
## W = 0.96357, p-value = 0.5143
```

```r
plot(regression_brain$pgp, regression_brain$conc_wt)
abline(0,1)
```



```r
plot(regression_brain$pgp, log(regression_brain$conc_wt))
abline(-2,1)
```
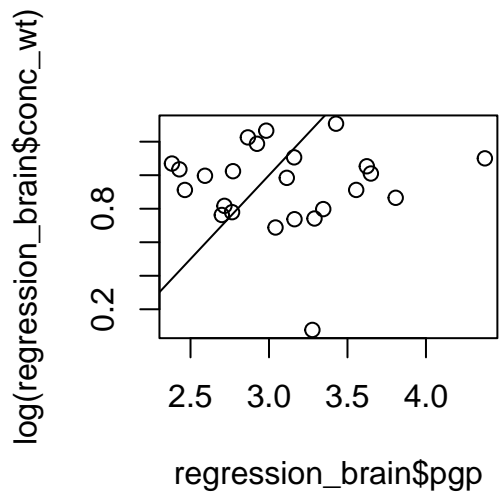
```
plot(log2(regression_brain$pgp), log(regression_brain$conc_wt))
abline(-1,1)
```
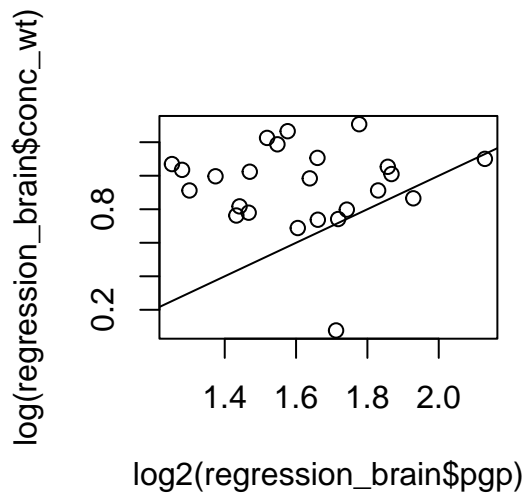


```
cor.test(regression_brain$pgp, regression_brain$conc_wt, method = "pearson")
```

```
##
##  Pearson's product-moment correlation
##
## data:  regression_brain$pgp and regression_brain$conc_wt
## t = 0.05577, df = 22, p-value = 0.956
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  -0.3933942  0.4133038
## sample estimates:
##        cor
## 0.01188931
```
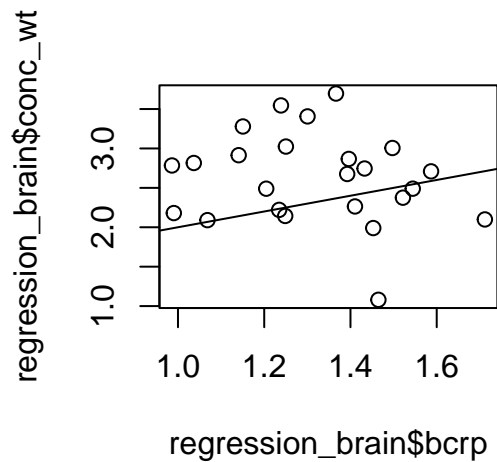
```
cor.test(regression_brain$pgp, regression_brain$conc_wt, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  regression_brain$pgp and regression_brain$conc_wt
## S = 2306, p-value = 0.9919
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##          rho
## -0.002608696
```
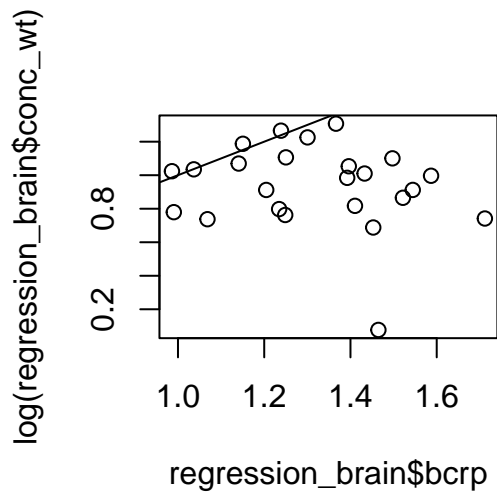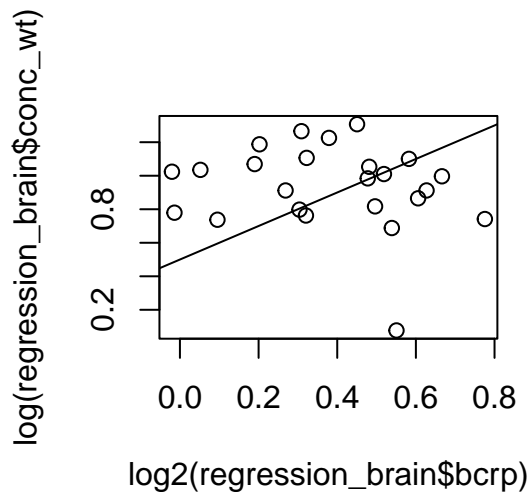
```
shapiro.test(regression_brain$bcrp)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  regression_brain$bcrp
## W = 0.9744, p-value = 0.7749
```

```
plot(regression_brain$bcrp, regression_brain$conc_wt)
abline(1,1)
```



```
plot(regression_brain$bcrp, log(regression_brain$conc_wt))
abline(0,1)
```

```
plot(log2(regression_brain$bcrp), log(regression_brain$conc_wt))
abline(0.5,1)
```



```
cor.test(regression_brain$bcrp, regression_brain$conc_wt, method = "pearson")
```

```
##
##  Pearson's product-moment correlation
##
## data:  regression_brain$bcrp and regression_brain$conc_wt
## t = -0.86512, df = 22, p-value = 0.3963
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  -0.5449099  0.2395396
## sample estimates:
##        cor
## -0.1813841
```

```r
cor.test(regression_brain$bcrp, regression_brain$conc_wt, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  regression_brain$bcrp and regression_brain$conc_wt
## S = 2736, p-value = 0.3733
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##        rho
## -0.1895652
```

```r
regression_retina <- subset(regression, tissue == "retina")
head(regression_retina)
```

```
##    animal raw_conc tissue_weight body_wt  dose       pgp      bcrp stage group
## 25     1M 104.7333           3.8    27.2 20.55 1.0782765 1.2298197  male  male
## 26     2M  86.2347           5.0    30.1 18.57 1.0045893 0.9196512  male  male
## 27     4M  88.3202           4.0    25.8 21.67 1.4228127 1.0984593  male  male
## 28     5M  99.2028           4.3    26.1 20.00 1.1925447 0.9961628  male  male
## 29     6M  97.7141           7.0    27.3 19.12 0.8683951 0.8810145  male  male
## 30     7M  99.8877           5.2    26.2 19.92 1.0440075 1.1553400  male  male
##     tissue  conc_wt
## 25  retina 27.56139
## 26  retina 17.24694
## 27  retina 22.08005
## 28  retina 23.07042
## 29  retina 13.95916
## 30  retina 19.20917
```
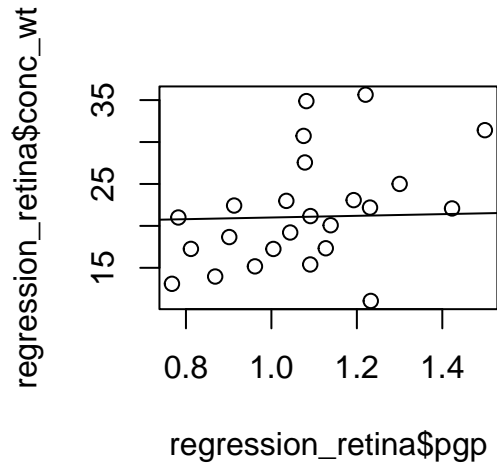
```r
shapiro.test(regression_retina$conc_wt)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  regression_retina$conc_wt
## W = 0.94676, p-value = 0.2303
```
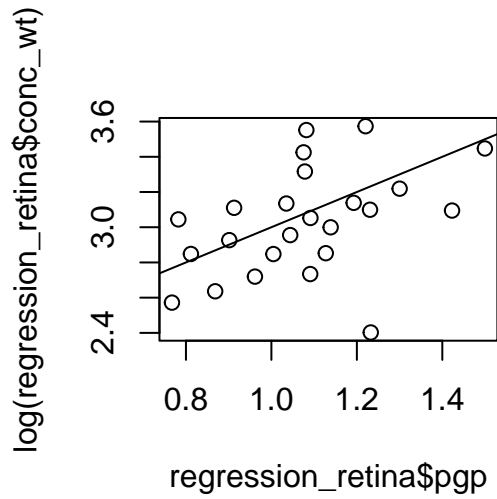
```r
shapiro.test(regression_retina$pgp)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  regression_retina$pgp
## W = 0.973, p-value = 0.7409
```
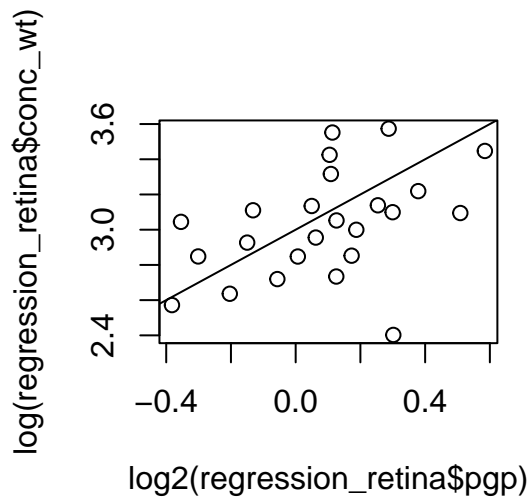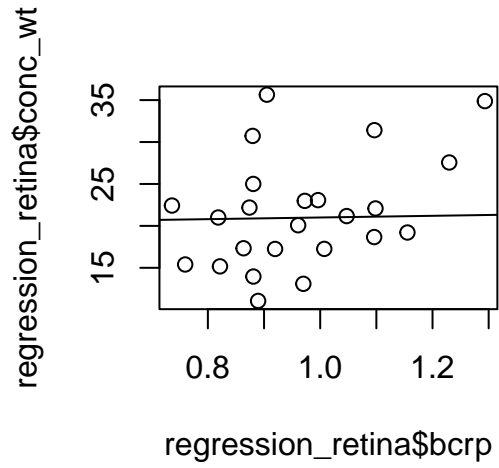
```r
plot(regression_retina$pgp, regression_retina$conc_wt)
abline(20,1)
```

```
plot(regression_retina$pgp, log(regression_retina$conc_wt))
abline(2,1)
```



```
plot(log2(regression_retina$pgp), log(regression_retina$conc_wt))
abline(3,1)
```

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```
cor.test(regression_retina$pgp, regression_retina$conc_wt, method = "pearson")
```

```
##
##  Pearson's product-moment correlation
##
## data:  regression_retina$pgp and regression_retina$conc_wt
## t = 2.2598, df = 22, p-value = 0.03408
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.03714565 0.71265643
## sample estimates:
##       cor
## 0.4340385
```

```
cor.test(regression_retina$pgp, regression_retina$conc_wt, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  regression_retina$pgp and regression_retina$conc_wt
## S = 1304, p-value = 0.03568
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## 0.4330435
```
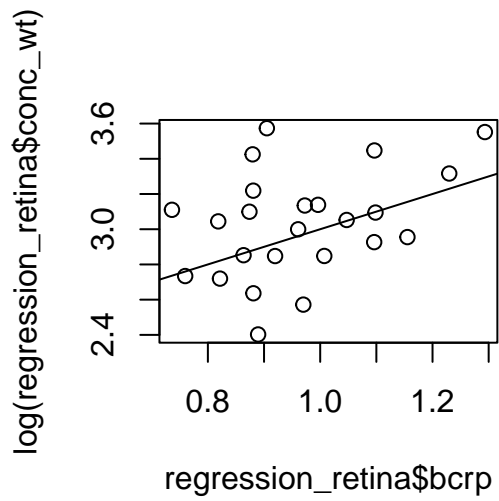
```
shapiro.test(regression_retina$bcrp)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  regression_retina$bcrp
## W = 0.9579, p-value = 0.3978
```
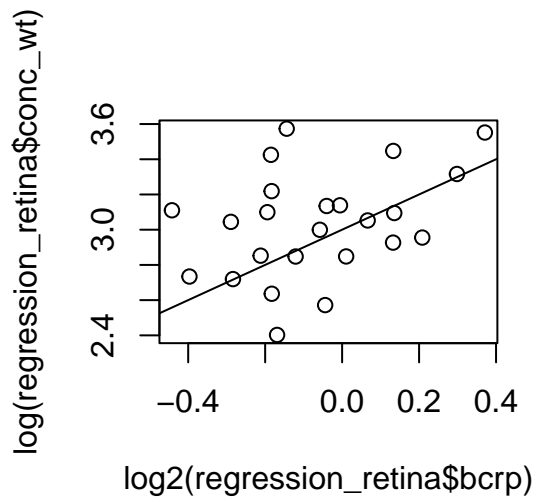
```
plot(regression_retina$bcrp, regression_retina$conc_wt)
abline(20,1)
```

```
plot(regression_retina$bcrp, log(regression_retina$conc_wt))
abline(2,1)
```



```
plot(log2(regression_retina$bcrp), log(regression_retina$conc_wt))
abline(3,1)
```
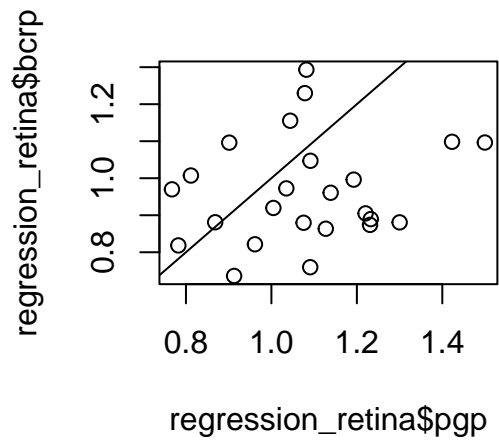
```
cor.test(regression_retina$bcrp, regression_retina$conc_wt, method = "pearson")
```

```
##
##  Pearson's product-moment correlation
##
## data:  regression_retina$bcrp and regression_retina$conc_wt
## t = 2.0013, df = 22, p-value = 0.05785
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  -0.01300906  0.68707216
## sample estimates:
##       cor
## 0.3924471
```
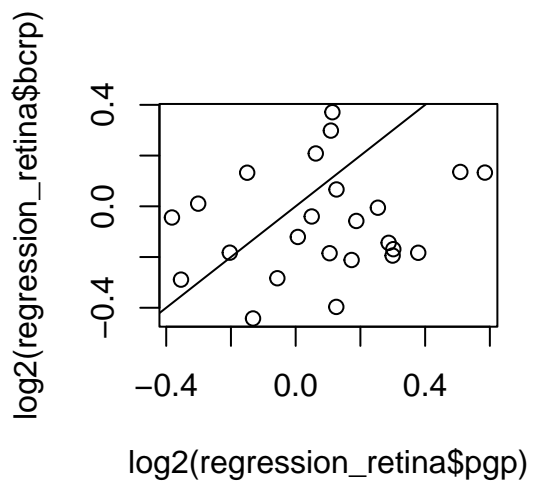
```
cor.test(regression_retina$bcrp, regression_retina$conc_wt, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  regression_retina$bcrp and regression_retina$conc_wt
## S = 1632, p-value = 0.1682
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## 0.2904348
```
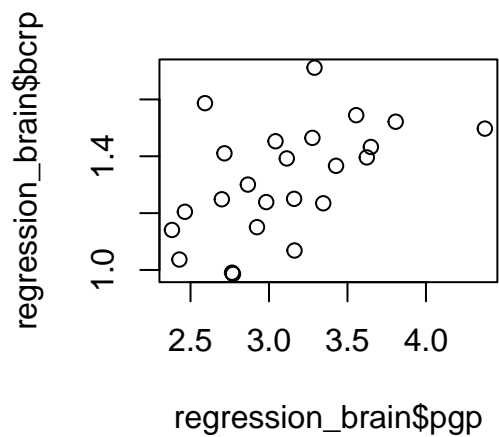
```
plot(regression_retina$pgp, regression_retina$bcrp)
abline(0,1)
```
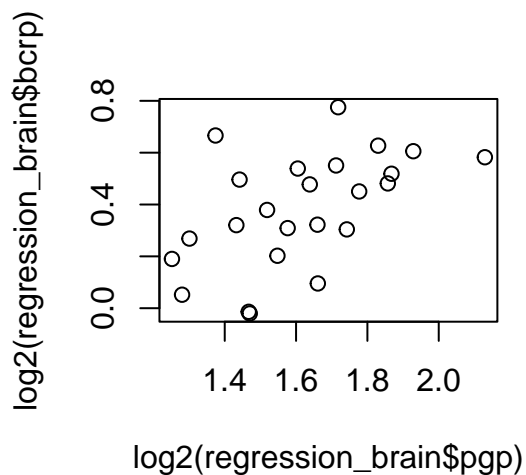
```
plot(log2(regression_retina$pgp), log2(regression_retina$bcrp))
abline(0,1)
```



```
plot(regression_brain$pgp, regression_brain$bcrp)
abline(0,1)
```

```
plot(log2(regression_brain$pgp), log2(regression_brain$bcrp))
abline(0,1)
```



```
cor.test(regression_retina$pgp, regression_retina$bcrp, method = "pearson")
```

```
##
##  Pearson's product-moment correlation
##
## data:  regression_retina$pgp and regression_retina$bcrp
## t = 0.95938, df = 22, p-value = 0.3478
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  -0.2208580  0.5586306
## sample estimates:
##       cor
## 0.2003923
```

```
cor.test(regression_retina$pgp, regression_retina$bcrp, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  regression_retina$pgp and regression_retina$bcrp
## S = 1966, p-value = 0.4967
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## 0.1452174
```

```
cor.test(regression_brain$pgp, regression_brain$bcrp, method = "pearson")
```

```
##
##  Pearson's product-moment correlation
##
## data:  regression_brain$pgp and regression_brain$bcrp
## t = 2.9218, df = 22, p-value = 0.0079
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.1593194 0.7682678
## sample estimates:
##       cor
## 0.5287349
```

```
cor.test(regression_brain$pgp, regression_brain$bcrp, method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  regression_brain$pgp and regression_brain$bcrp
## S = 1068, p-value = 0.007797
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## 0.5356522
```
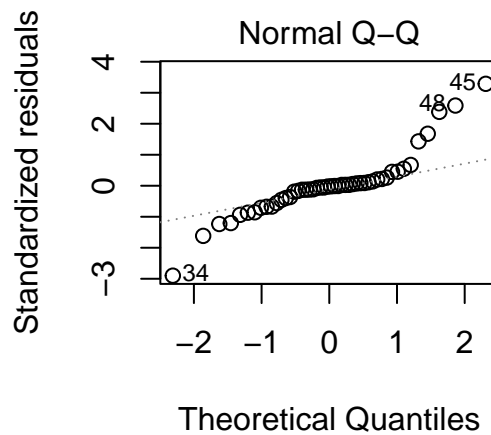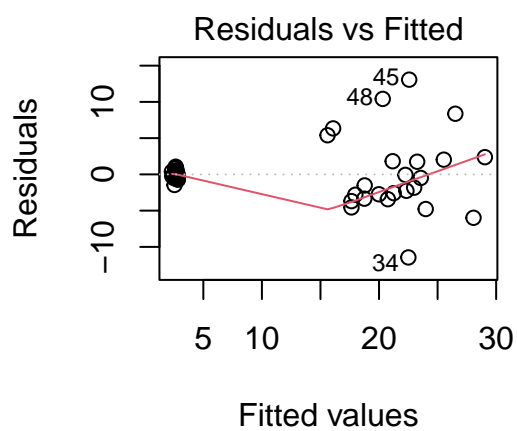
```
gene_plot <- ggplot(regression, aes(pgp, bcrp, col=tissue)) +
  geom_point() +
  geom_smooth(method = "lm", se = T) +
  xlim(0,5) +
  theme_bw()

gene_plot
```

```
## `geom_smooth()` using formula 'y ~ x'
```
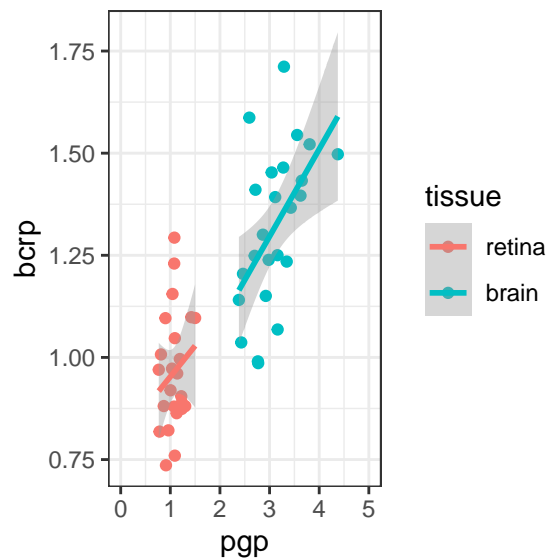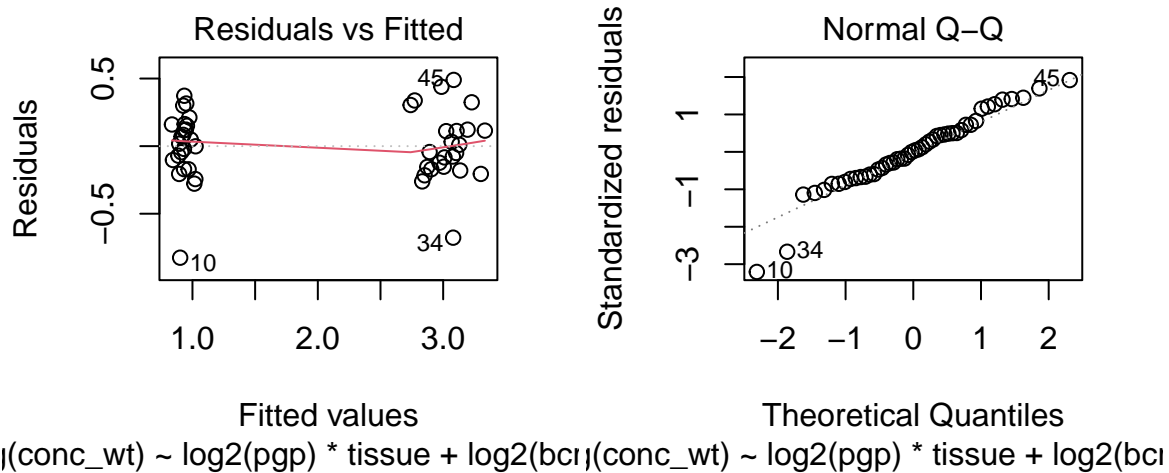
```
lm_ex1 <- lm(conc_wt ~ pgp*tissue + bcrp*tissue, data = regression)
plot(lm_ex1, which=c(1,2))
```



```
lm_ex <- lm(log(conc_wt) ~ log2(pgp)*tissue + log2(bcrp)*tissue, data = regression)
plot(lm_ex, which=c(1,2))
```

Residuals vs Fitted / Normal Q–Q

Fitted values   Theoretical Quantiles

ɪ(conc_wt) ~ log2(pgp) * tissue + log2(bcɪɪ(conc_wt) ~ log2(pgp) * tissue + log2(bcɪ

```
summary(lm_ex)$coefficient
```

```
##                          Estimate Std. Error    t value      Pr(>|t|)
## (Intercept)             3.0193972 0.06176213 48.8875210 1.186804e-38
## log2(pgp)               0.4385311 0.22416220  1.9563116 5.709991e-02
## tissuebrain            -2.1538742 0.43938139 -4.9020608 1.462185e-05
## log2(bcrp)              0.4272982 0.27311844  1.5645161 1.252008e-01
## log2(pgp):tissuebrain  -0.3335960 0.37326691 -0.8937197 3.765647e-01
## tissuebrain:log2(bcrp) -0.6872715 0.40615462 -1.6921424 9.802618e-02
```

```
pgp_subset <- regression %>% select(-bcrp)
bcrp_subset <- regression %>% select(-pgp)

#untransformed
#lm_pgp <- lm(conc_wt ~ pgp * tissue, data=pgp_subset)
#plot(lm_pgp, which=c(1,2))

#concentration log transformed
lm_pgp_log <- lm(log(conc_wt) ~ pgp * tissue, data=pgp_subset)
plot(lm_pgp_log, which=c(1,2))
```
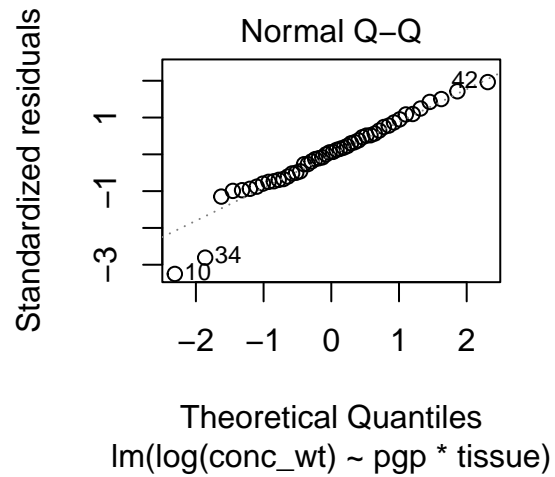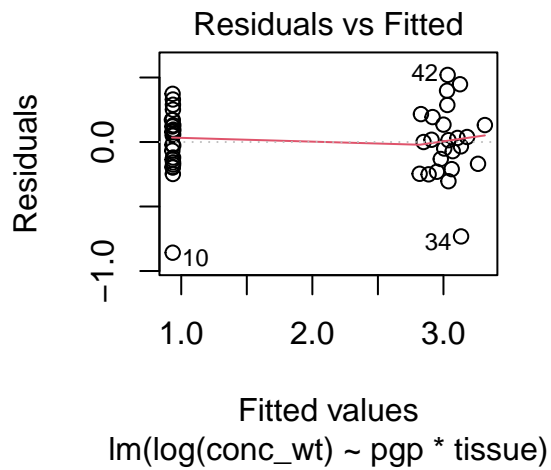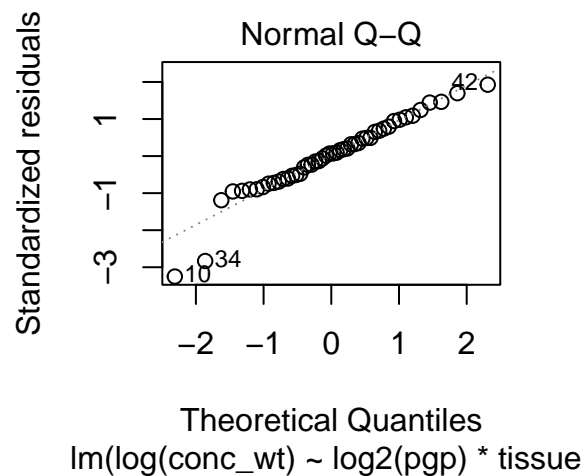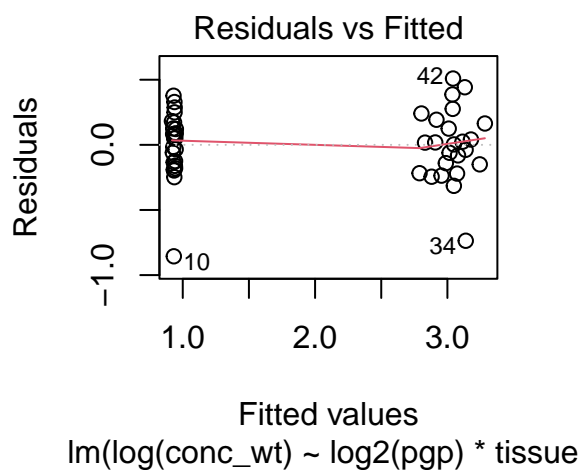
Residuals vs Fitted — lm(log(conc_wt) ~ pgp * tissue)

Normal Q–Q — lm(log(conc_wt) ~ pgp * tissue)

```
#pgp log transformed
#lm_pgp_log1 <- lm(conc_wt ~ log2(pgp) * tissue, data=pgp_subset)
#plot(lm_pgp_loglog, which=c(1,2))

#both transformed
lm_pgp_loglog <- lm(log(conc_wt) ~ log2(pgp) * tissue, data=pgp_subset)
plot(lm_pgp_loglog, which=c(1,2))
```


Residuals vs Fitted — lm(log(conc_wt) ~ log2(pgp) * tissue)

Normal Q–Q — lm(log(conc_wt) ~ log2(pgp) * tissue)

```
summary(lm_pgp_log)
```

```
##
## Call:
## lm(formula = log(conc_wt) ~ pgp * tissue, data = pgp_subset)
##
## Residuals:
##      Min      1Q   Median      3Q      Max
## -0.85801 -0.16073  0.01583  0.14377  0.52006
```

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```
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)       2.2967     0.3281   7.000 1.14e-08 ***
## pgp               0.6796     0.3000   2.265  0.02850 *
## tissuebrain      -1.3453     0.4906  -2.742  0.00879 **
## pgp:tissuebrain  -0.6846     0.3218  -2.128  0.03901 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2705 on 44 degrees of freedom
## Multiple R-squared:  0.9427, Adjusted R-squared:  0.9388
## F-statistic: 241.4 on 3 and 44 DF,  p-value: < 2.2e-16
```

```
anova(lm_pgp_log)
```

```
## Analysis of Variance Table
##
## Response: log(conc_wt)
##           Df Sum Sq Mean Sq  F value    Pr(>F)
## pgp        1 45.719  45.719 624.9245 < 2.2e-16 ***
## tissue     1  6.921   6.921  94.6050  1.56e-12 ***
## pgp:tissue 1  0.331   0.331   4.5267   0.03901 *
## Residuals 44  3.219   0.073
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans::emmeans(lm_pgp_log, pairwise ~ tissue)$contrasts
```

```
## NOTE: Results may be misleading due to involvement in interactions

##  contrast       estimate    SE df t.ratio p.value
##  retina - brain     2.78 0.335 44   8.294  <.0001
##
## Results are given on the log (not the response) scale.
```