

# Effect of Rapamycin on Expression of Differentiation Markers

May 9, 2014

This uses the input file qRT-PCR combined data.csv. This is located in /Users/innocenceharvey/DrosophilaMuscleFunction/CellCulture. It was most recently run on Fri May 9 16:35:49 2014.

First we did an ANOVA looking at all of the genes, treatments and experiments together. These results are shown below:

|                    | Df  | Sum Sq | Mean Sq | F value | Pr(>F)       |
|--------------------|-----|--------|---------|---------|--------------|
| gene               | 5   | 18.380 | 3.676   | 39.433  | < 2e-16 ***  |
| treatment          | 3   | 2.688  | 0.896   | 9.610   | 1.18e-05 *** |
| Exp                | 4   | 1.722  | 0.431   | 4.619   | 0.001794 **  |
| gene:treatment     | 13  | 15.703 | 1.208   | 12.957  | < 2e-16 ***  |
| gene:Exp           | 18  | 2.556  | 0.142   | 1.523   | 0.096578 .   |
| treatment:Exp      | 4   | 2.038  | 0.510   | 5.466   | 0.000492 *** |
| gene:treatment:Exp | 16  | 1.967  | 0.123   | 1.319   | 0.199912     |
| Residuals          | 104 | 9.695  | 0.093   |         |              |

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Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Since we saw an interaction between the experiment and gene and treatment terms we separated out the experiments and did 2-way ANOVA analyses on each. These results are below:

[1] "feb28 plate 2"

|                | Df | Sum Sq | Mean Sq | F value | Pr(>F)       |
|----------------|----|--------|---------|---------|--------------|
| gene           | 5  | 4.600  | 0.9200  | 12.622  | 4.47e-06 *** |
| treatment      | 1  | 0.195  | 0.1952  | 2.678   | 0.115        |
| gene:treatment | 5  | 4.600  | 0.9200  | 12.622  | 4.47e-06 *** |
| Residuals      | 24 | 1.749  | 0.0729  |         |              |

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Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

|      | Df | Sum Sq | Mean Sq | F value | Pr(>F)       |
|------|----|--------|---------|---------|--------------|
| gene | 5  | 3.270  | 0.6540  | 17.50   | 2.58e-07 *** |

```

treatment      1  0.832  0.8320   22.26 8.50e-05 ***
gene:treatment  5  3.270  0.6540   17.50 2.58e-07 ***
Residuals      24  0.897  0.0374
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

              Df Sum Sq Mean Sq F value    Pr(>F)
gene           5  2.4501   0.4900   18.89 1.27e-07 ***
treatment      1  0.5681   0.5681   21.91 9.36e-05 ***
gene:treatment  5  2.4501   0.4900   18.89 1.27e-07 ***
Residuals     24  0.6225   0.0259
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

              Df Sum Sq Mean Sq F value    Pr(>F)
gene           3  2.5749   0.8583   53.585 1.22e-05 ***
treatment      3  1.8075   0.6025   37.616 4.60e-05 ***
gene:treatment  9  1.3712   0.1524    9.512  0.0021 **
Residuals      8  0.1281   0.0160
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

              Df Sum Sq Mean Sq F value    Pr(>F)
gene           5  7.559   1.5118    5.761 0.00125 **
treatment      1  0.023   0.0226    0.086 0.77158
gene:treatment  5  7.559   1.5118    5.761 0.00125 **
Residuals     24  6.298   0.2624
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

## Testing ANOVA Assumptions

We next tested our ANOVA analyses for normality and equal variance by first looking at a Shapiro-Wilk test and then potentially a log-transformed Shapiro-Wilk test, followed by a Levene's Test. Based on these results we did Student's T-Tests for each gene

Shapiro-Wilk normality test

```
data: residuals(aov(value ~ gene * treatment, data = subset(data.of.interest,
W = 0.8009, p-value = 1.699e-05
```

Shapiro-Wilk normality test

```
data: residuals(aov(log(value) ~ gene * treatment, data = subset(data.of.interest,
W = 0.9764, p-value = 0.6229
```

Levene's Test for Homogeneity of Variance (center = median)

|       |    |         |        |
|-------|----|---------|--------|
|       | Df | F value | Pr(>F) |
| group | 11 | 0.5941  | 0.815  |
|       | 24 |         |        |

|                |    |        |         |         |              |
|----------------|----|--------|---------|---------|--------------|
|                | Df | Sum Sq | Mean Sq | F value | Pr(>F)       |
| gene           | 5  | 7.425  | 1.4851  | 36.32   | 1.96e-10 *** |
| treatment      | 1  | 2.445  | 2.4449  | 59.80   | 5.73e-08 *** |
| gene:treatment | 5  | 7.404  | 1.4807  | 36.22   | 2.02e-10 *** |
| Residuals      | 24 | 0.981  | 0.0409  |         |              |

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

|        |              |
|--------|--------------|
|        | pval         |
| Actb   | 0.0220569009 |
| Cdkn1a | 0.0072971453 |
| Mef2c  | 0.0009335880 |
| Myf5   | 0.0223318648 |
| Myod1  | 0.1805853201 |
| Myog   | 0.0007129232 |

Shapiro-Wilk normality test

data: residuals(aov(value ~ gene \* treatment, data = subset(data.of.interest, Exp == "m  
W = 0.9278, p-value = 0.0215

Shapiro-Wilk normality test

data: residuals(aov(log(value) ~ gene \* treatment, data = subset(data.of.interest, Exp  
W = 0.9761, p-value = 0.6135

Levene's Test for Homogeneity of Variance (center = median)

|       |    |         |        |
|-------|----|---------|--------|
|       | Df | F value | Pr(>F) |
| group | 11 | 0.7292  | 0.701  |
|       | 24 |         |        |

|                |    |        |         |         |              |
|----------------|----|--------|---------|---------|--------------|
|                | Df | Sum Sq | Mean Sq | F value | Pr(>F)       |
| gene           | 5  | 9.163  | 1.833   | 41.05   | 5.40e-11 *** |
| treatment      | 1  | 5.497  | 5.497   | 123.13  | 6.21e-11 *** |
| gene:treatment | 5  | 8.883  | 1.777   | 39.79   | 7.50e-11 *** |
| Residuals      | 24 | 1.071  | 0.045   |         |              |

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

|        |              |
|--------|--------------|
|        | pval         |
| Actb   | 0.0077264194 |
| Cdkn1a | 0.0119573375 |

```

Mef2c 0.0003566990
Myf5 0.0006424166
Myod1 0.2006887579
Myog 0.0194950170

```

Shapiro-Wilk normality test

```

data: residuals(aov(value ~ gene * treatment, data = subset(data.of.interest, Exp == "M
W = 0.9762, p-value = 0.6176

```

Levene's Test for Homogeneity of Variance (center = median)

```

      Df F value Pr(>F)
group 11  0.7787 0.6578
      24

```

```

      Df Sum Sq Mean Sq F value    Pr(>F)
gene      5  2.4501   0.4900    18.89 1.27e-07 ***
treatment 1  0.5681   0.5681    21.91 9.36e-05 ***
gene:treatment 5  2.4501   0.4900    18.89 1.27e-07 ***
Residuals 24  0.6225   0.0259
---

```

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

```

      pval
Actb 0.003494267
Cdkn1a 0.024727887
Mef2c 0.001769465
Myf5 0.048016215
Myod1 0.418954729
Myog 0.004332754

```

Shapiro-Wilk normality test

```

data: residuals(aov(value ~ gene * treatment, data = subset(data.of.interest, Exp == "2
W = 0.7907, p-value = 1.071e-05

```

Shapiro-Wilk normality test

```

data: residuals(aov(log(value) ~ gene * treatment, data = subset(data.of.interest, Exp
W = 0.9749, p-value = 0.5724

```

Levene's Test for Homogeneity of Variance (center = median)

```

      Df F value Pr(>F)
group 11  0.6446 0.7737
      24

```

```

      Df Sum Sq Mean Sq F value    Pr(>F)
gene      5  7.013   1.4027    16.95 3.43e-07 ***

```

```

treatment      1  0.840  0.8403  10.16  0.00396 **
gene:treatment  5  6.745  1.3490  16.30  4.88e-07 ***
Residuals      24  1.986  0.0827

```

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

```

          pval
Actb    0.047362045
Cdkn1a  0.098430095
Mef2c   0.004045253
Myf5    0.035140962
Myod1   0.044237560
Myog    0.031918406

```

Shapiro-Wilk normality test

```

data: residuals(aov(value ~ gene * treatment, data = subset(data.of.interest,
W = 0.7866, p-value = 0.0001792

```

Shapiro-Wilk normality test

```

data: residuals(aov(log(value) ~ gene * treatment, data = subset(data.of.interest,
W = 0.9458, p-value = 0.2191

```

Levene's Test for Homogeneity of Variance (center = median)

```

      Df    F value    Pr(>F)
group 15 3.1203e+30 < 2.2e-16 ***
      8

```

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

[1] 0.3116156

```

          pval-low  pval-high
Actb    0.133498028 0.781207337
Cdkn1a          NA          NA
Mef2c   0.006423015 0.004777345
Myf5          NA          NA
Myod1   0.030032472 0.104138664
Myog    0.013727306 0.034779776

```

```

> library(plyr)
> complete.experiments <- c('20-Feb','feb28 plate 2','mar7 plate 2', 'Mar7 plate1')
> summary.data <- ddply(subset(data.of.interest, Exp %in% complete.experiments), .(gene, Rap),
+                       mean = mean(value),
+                       sd = sd(value),
+                       se = sd(value)/sqrt(length(value)),
+                       n = length(value))
>

```

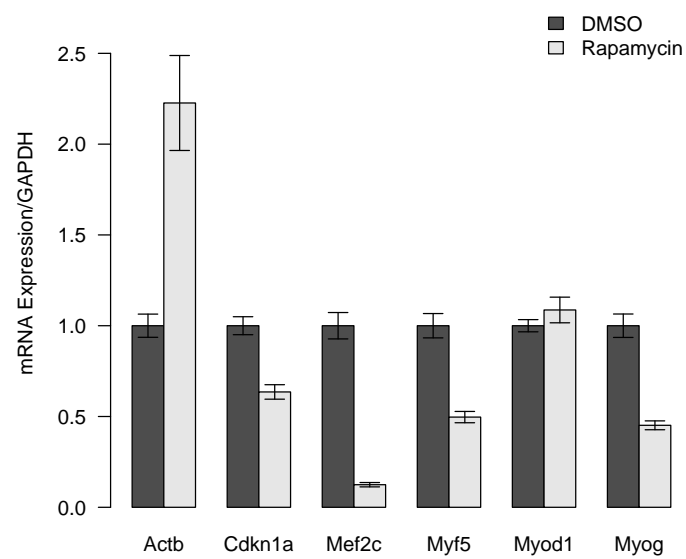


Figure 1: Combined Data from All Experiments

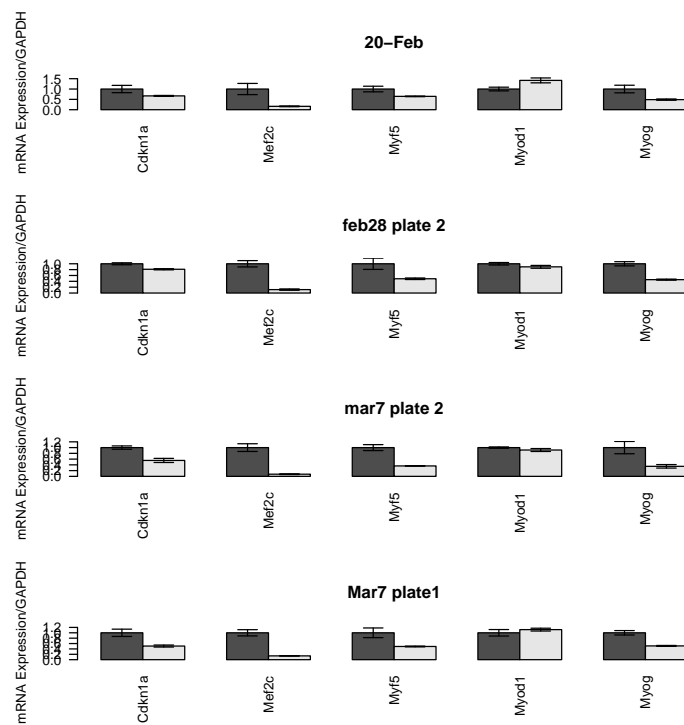


Figure 2: Data for Each Experiment

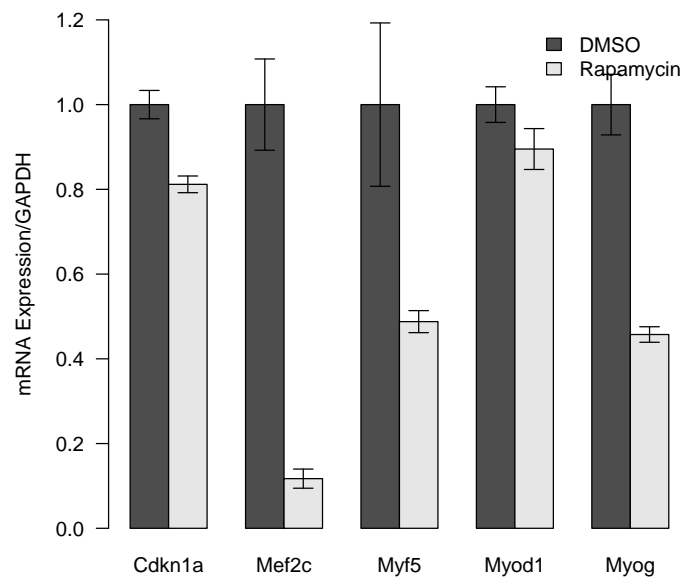


Figure 3: Feb 28 Data