

# Effect of Rapamycin on Expression of Differentiation Markers

May 17, 2014

This uses the input file qRT-PCR combined data.csv. This is located in  
/Users/davebridges/Documents/Source/DrosophilaMuscleFunction/CellCulture.  
It was most recently run on Sat May 17 06:54:34 2014.

First we did an ANOVA looking at all of the genes, treatments and experi-  
ments 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

[1] "mar7 plate 2"

```

              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

```

```
[1] "Mar7 plate1"
```

```

              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

```

```
[1] "dose response "
```

```

              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

```

```
[1] "20-Feb"
```

```

              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

```
[1] "feb28 plate 2"
```

```

Shapiro-Wilk normality test

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

[1] "log transformed"

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

[1] "ANOVA"

      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

[1] "post hoc T-test"

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

[1] "mar7 plate 2"

Shapiro-Wilk normality test

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

[1] "log transformed"

Shapiro-Wilk normality test

data: residuals(aov(log(value) ~ gene * treatment, data = subset(data.of.interest,
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
```

[1] "ANOVA"

```
      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

[1] "post hoc T-test"

```
      pval
Actb  0.0077264194
Cdkn1a 0.0119573375
Mef2c  0.0003566990
Myf5   0.0006424166
Myod1  0.2006887579
Myog   0.0194950170
```

[1] "Mar7 plate1"

Shapiro-Wilk normality test

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

Exp == "M

[1] "no need for log transform"

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

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

[1] "ANOVA"

```
      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

```
[1] "post hoc T-test"
```

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

```
[1] "20-Feb"
```

```
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
```

```
[1] "log transformed"
```

```
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
```

```
[1] "ANOVA"
```

```
      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
```

```
[1] "post hoc T-test"
```

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

```

[1] "dose response "

      Shapiro-Wilk normality test

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

[1] "log transformed"

      Shapiro-Wilk normality test

data: residuals(aov(log(value) ~ gene * treatment, data = subset(data.of.interest, Exp
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

Since we did not meet the assumption for equal variance we had to run a Kruskal-
Wallis test instead of a normal ANOVA. We were not able to run a T-test since
there are 3 groups in this experiment

[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

```

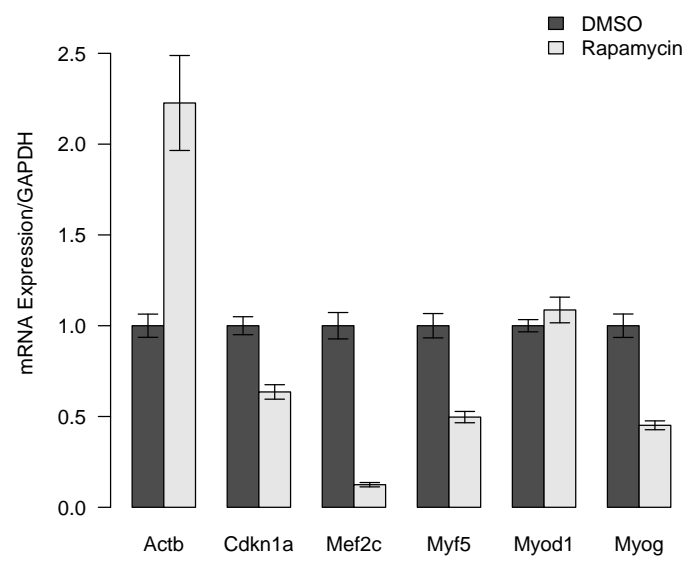


Figure 1: Combined Data from All Experiments

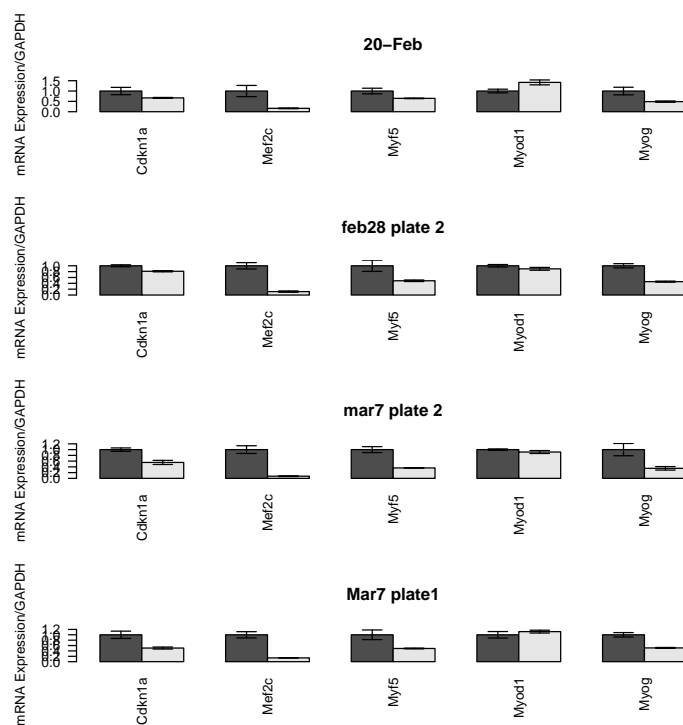


Figure 2: Data for Each Experiment



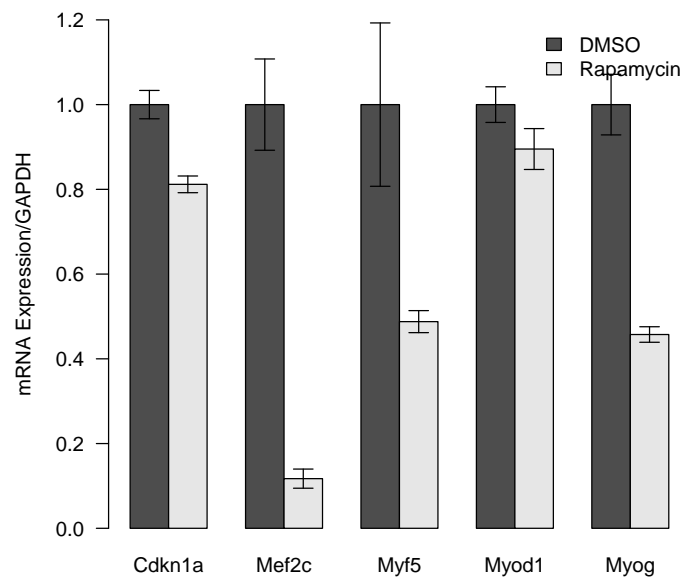


Figure 3: Feb 28 Data