

# Quantification of Effects of Rapamycin on MyoD Levels

*Dave Bridges*

*December 1, 2014*

C2C12 Cells were grown to confluence and then treated for 4h with 500 nM Rapamycin, 1 uM LYS6K2, or 10 uM MG132. These data include experiments done on 2014-11-26 and 2014-11-29. These data are located in /Volumes/bridges\_lab/Bridges/Cell Culture/C2C12 Differentiation/MyoD Levels During Differentiation/2014-11-29 and was most recently updated on Thu Dec 11 08:27:55 2014.

```
myod_data_file <- 'MyoD Quantification.txt'
myod_data_file_2 <- 'MyoD Quantification-2.txt'
myod_data <- read.csv(myod_data_file)

akt_data_file <- 'Akt Quantification.txt'
akt_data_file_2 <- 'Akt Quantification-2.txt'
akt_data <- rbind(read.csv(akt_data_file), read.csv(akt_data_file_2))

s6_data_file <- 'S6 Quantification.txt'
s6_data_file_2 <- 'S6 Quantification-2.txt'
s6_data <- rbind(read.csv(s6_data_file), read.csv(s6_data_file_2))
```

The input files are: Akt Quantification.txt and MyoD Quantification.txt.

## MyoD Quantification

```
myod_data <- myod_data[grepl("Lane", myod_data$Lane.Name),]
myod_data <- myod_data[myod_data$Channel == 800,]
myod_data$Lane.Number <- as.integer(sapply(strsplit(as.character(myod_data$Lane.Name), " "), "[", 2))
myod_data$Protein <- rep(c('MyoD'),12)

library(reshape2)
myod_lane_summary <- dcast(myod_data, Scan + Lane.Number ~ Protein, value.var='I.I..K.Counts.')
myod_lane_summary$Treatment <- as.factor(rep(c("DMSO",
      "Rapamycin",
      "HS + DMSO",
      "HS + Rapamycin",
      "HS + Rapamycin + MG132",
      "HS + LYS6K2"),2))
myod_lane_summary$Treatment <- factor(myod_lane_summary$Treatment,
      levels(myod_lane_summary$Treatment)[c(2,4,3,5,1,6)])

myod_lane_summary$MyoD_Norm <- c(
  myod_lane_summary[c(1:6), 'MyoD']/myod_lane_summary[myod_lane_summary$Treatment=='HS + DMSO', 'MyoD']
  myod_lane_summary[c(7:12), 'MyoD']/myod_lane_summary[myod_lane_summary$Treatment=='HS + DMSO', 'MyoD'])

library(dplyr)
se <- function(x) sd(x)/sqrt(length(x))
myod_summary <-
```

```

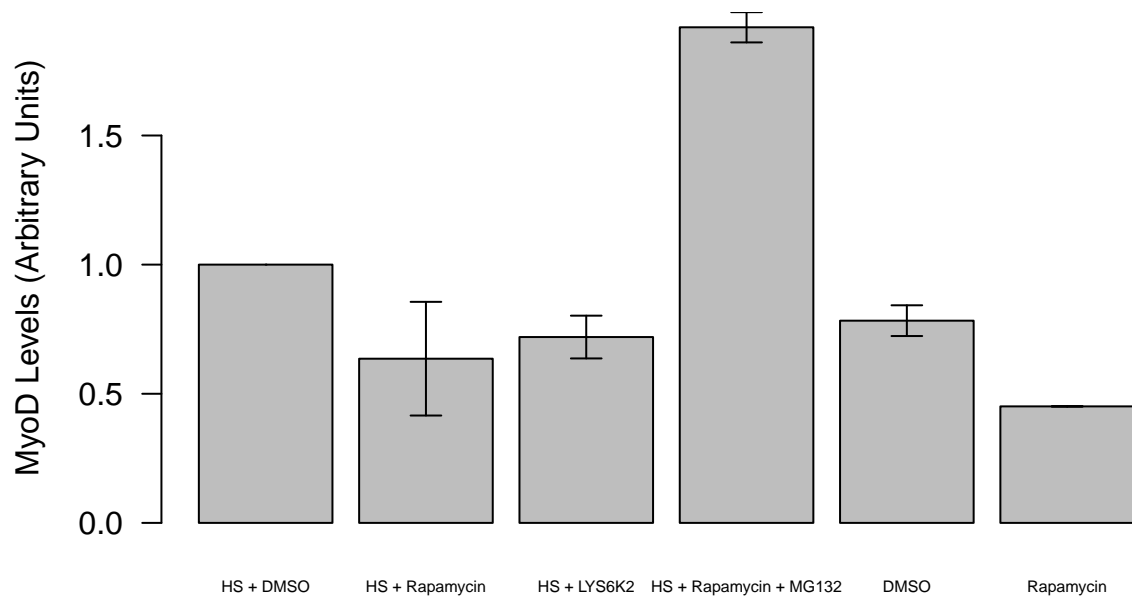
myod_lane_summary %>%
  group_by(Treatment) %>%
  summarize(mean=mean(MyoD_Norm),
            se = se(MyoD_Norm),
            sd = sd(MyoD_Norm),
            n = length(MyoD_Norm))

ymax = max(myod_summary$mean + myod_summary$se)
plot <- with(myod_summary, barplot(mean, names.arg=Treatment,
                                  las=1, ylim=c(0,ymax),cex.names=0.5,
                                  ylab="MyoD Levels (Arbitrary Units)"))

superpose.eb <- function (x, y, ebl, ebu = ebl, length = 0.08, ...)
  arrows(x, y + ebu, x, y - ebl, angle = 90, code = 3,
        length = length, ...)

superpose.eb(plot, myod_summary$mean, myod_summary$se)

```



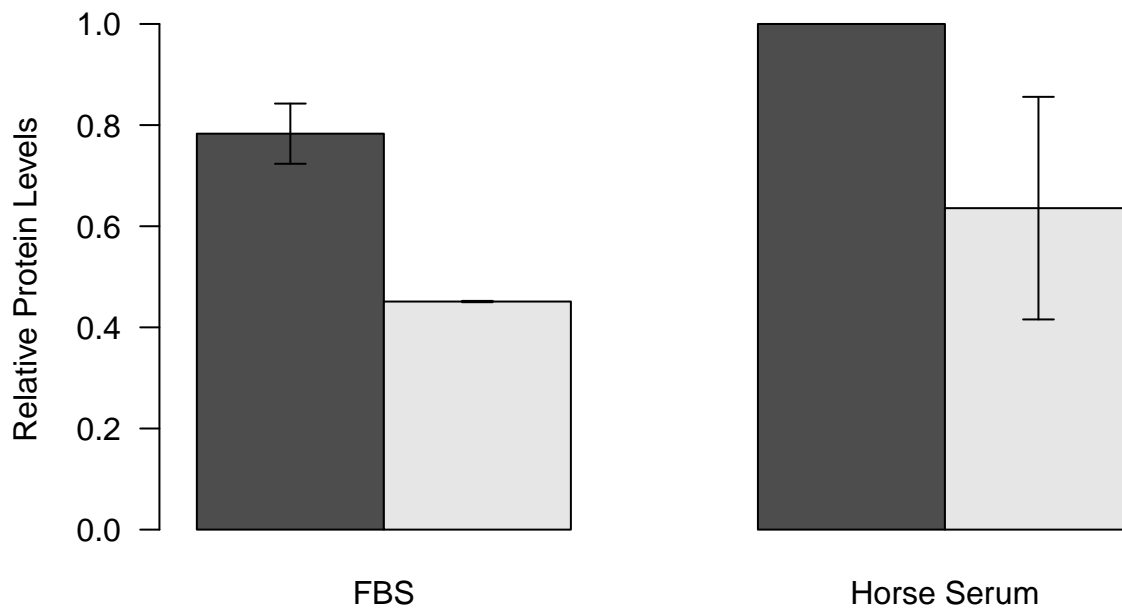
```

myod_summary$Serum <- c(rep("Horse Serum",4), rep("FBS",2))
myod_summary$Drug <- c("DMSO","Rapamycin","LYS6K","Rapamycin + MG132", "DMSO", "Rapamycin")
plot <- barplot(as.matrix(dcast(subset(myod_summary, Drug %in% c("DMSO","Rapamycin")),
                               Drug~Serum, value.var='mean')[2:3]),
               beside=T, las=1, ylab="Relative Protein Levels")

superpose.eb(plot,
             as.matrix(dcast(subset(myod_summary, Drug %in% c("DMSO","Rapamycin")),

```

```
Drug~Serum, value.var='mean')[2:3]),
as.matrix(dcast(subset(myod_summary, Drug %in% c("DMSO", "Rapamycin")),
Drug~Serum, value.var='se')[2:3]))
```



Based on these data, Rapamycin treatment reduced MyoD levels in the presence of Horse Serum by 36.4243043% +/- 22.0096815%. The p-value by one-way t-test is 0.1730156.

In the absence of horse serum, there was a 57.6058284% reduction. The p-value from the two-way t-test of that comparison is 0.1391155.

## pS6/S6 Quantification

```
#for the first experiment
s6_data <- s6_data[grepl("Lane", s6_data$Lane.Name),]
s6_data <- s6_data[s6_data$Name == "Band 1",]
s6_data$Lane.Number <- as.integer(sapply(strsplit(as.character(s6_data$Lane.Name), " "), "[", 2))
s6_data <- s6_data[order(s6_data$Lane.Number, s6_data$Channel),]
for (row.name in rownames(s6_data)) {
  if (s6_data[row.name, 'Channel'] == '700')
    s6_data[row.name, 'Protein'] <- 'pS6'
  else
    s6_data[row.name, 'Protein'] <- 'S6'
}
```

```

s6_lane_summary <- dcast(s6_data, Scan + Lane.Number ~ Protein, value.var='I.I..K.Counts.', mean)
s6_lane_summary$Ratio <- s6_lane_summary$pS6/s6_lane_summary$S6
s6_lane_summary$Treatment <- as.factor(rep(c("DMSO",
      "Rapamycin",
      "HS + DMSO",
      "HS + Rapamycin",
      "HS + Rapamycin + MG132",
      "HS + LYS6K2"),3))

s6_lane_summary$Treatment <- factor(s6_lane_summary$Treatment,
      levels(s6_lane_summary$Treatment)[c(2,4,3,5,1,6)])
s6_lane_summary$Ratio.norm <- c(
  s6_lane_summary[c(1:6), 'Ratio']/s6_lane_summary[s6_lane_summary$Treatment=='DMSO', 'Ratio'][1],
  s6_lane_summary[c(7:12), 'Ratio']/s6_lane_summary[s6_lane_summary$Treatment=='DMSO', 'Ratio'][2],
  s6_lane_summary[c(13:18), 'Ratio']/s6_lane_summary[s6_lane_summary$Treatment=='DMSO', 'Ratio'][3])

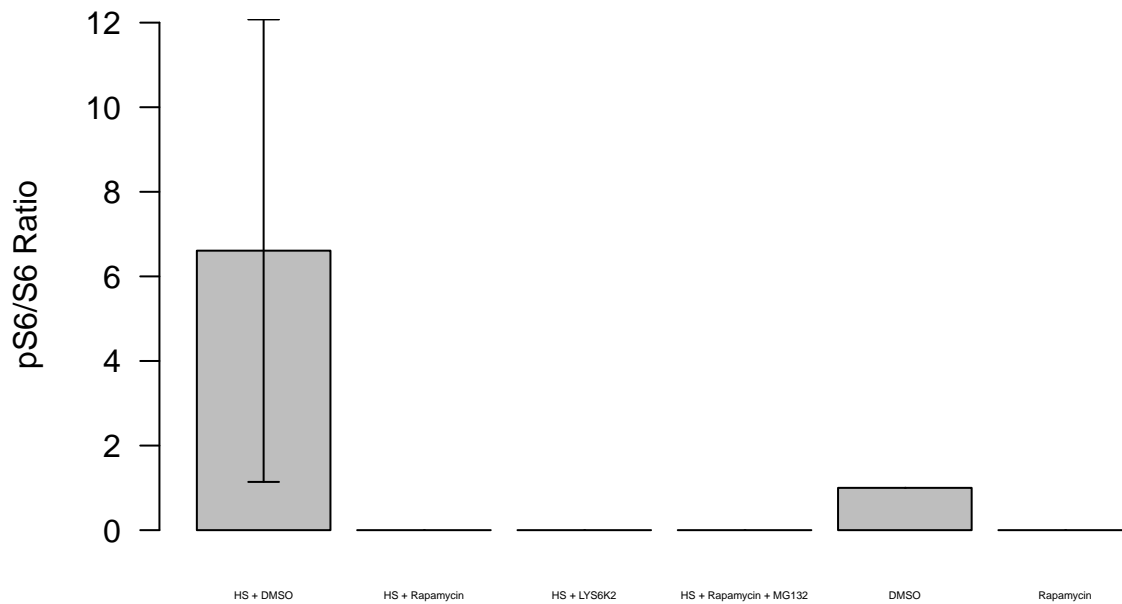
s6_lane_summary[is.na(s6_lane_summary$Ratio.norm), 'Ratio.norm'] <- 0

s6_summary <-
  s6_lane_summary %>%
  group_by(Treatment) %>%
  summarize(mean=mean(Ratio.norm),
    se = se(Ratio.norm),
    sd = sd(Ratio.norm),
    n = length(Ratio.norm))

ymax = max(s6_summary$mean + s6_summary$se)
plot <- with(s6_summary, barplot(mean, names.arg=Treatment,
  las=1, ylim=c(0,ymax), cex.names=0.3,
  ylab="pS6/S6 Ratio"))

superpose.eb(plot, s6_summary$mean, s6_summary$se)

```



The addition of horse serum caused an increase in S6 phosphorylation ratio of 6.6082765 fold ( $\pm 5.4673737$  fold). The one way t-test for this effect had a p-value of 0.

## pAkt/Akt Quantification

```
akt_data <- akt_data[grepl("Lane", akt_data$Lane.Name),]
akt_data$Lane.Number <- as.integer(sapply(strsplit(as.character(akt_data$Lane.Name), " "), "[", 2))

for (row.name in rownames(akt_data)) {
  if (akt_data[row.name, 'Channel'] == '700')
    akt_data[row.name, 'Protein'] <- 'pAkt'
  else
    akt_data[row.name, 'Protein'] <- 'Akt'
}
akt_data <- akt_data[akt_data$Lane.Name != 'Lane 8',]

akt_lane_summary <- dcast(akt_data, Scan + Lane.Number ~ Protein, value.var='I.I..K.Counts.')
akt_lane_summary$Ratio <- akt_lane_summary$pAkt/akt_lane_summary$Akt
akt_lane_summary$Treatment <- as.factor(rep(c("DMSO",
      "Rapamycin",
      "HS + DMSO",
      "HS + Rapamycin",
      "HS + Rapamycin + MG132",
      "HS + LYS6K2"), 3))
```

```

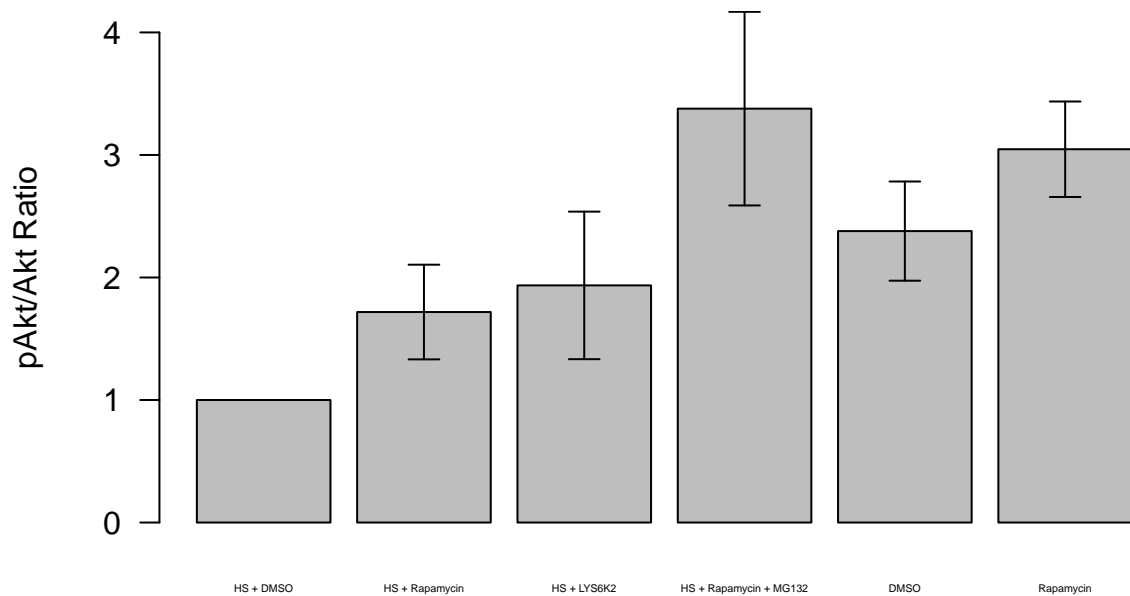
akt_lane_summary$Treatment <- factor(akt_lane_summary$Treatment,
                                     levels(akt_lane_summary$Treatment)[c(2,4,3,5,1,6)])
akt_lane_summary$Ratio.norm <- c(
  akt_lane_summary[c(1:6), 'Ratio']/akt_lane_summary[akt_lane_summary$Treatment=='HS + DMSO', 'Ratio'],
  akt_lane_summary[c(7:12), 'Ratio']/akt_lane_summary[akt_lane_summary$Treatment=='HS + DMSO', 'Ratio'],
  akt_lane_summary[c(13:18), 'Ratio']/akt_lane_summary[akt_lane_summary$Treatment=='HS + DMSO', 'Ratio'])

akt_summary <-
  akt_lane_summary %>%
  group_by(Treatment) %>%
  summarize(mean=mean(Ratio.norm),
            se = se(Ratio.norm),
            sd = sd(Ratio.norm),
            n = length(Ratio.norm))

ymax = max(akt_summary$mean + akt_summary$se)
plot <- with(akt_summary, barplot(mean, names.arg=Treatment,
                                las=1, ylim=c(0,ymax), cex.names=0.3,
                                ylab="pAkt/Akt Ratio"))

superpose.eb(plot, akt_summary$mean, akt_summary$se)

```



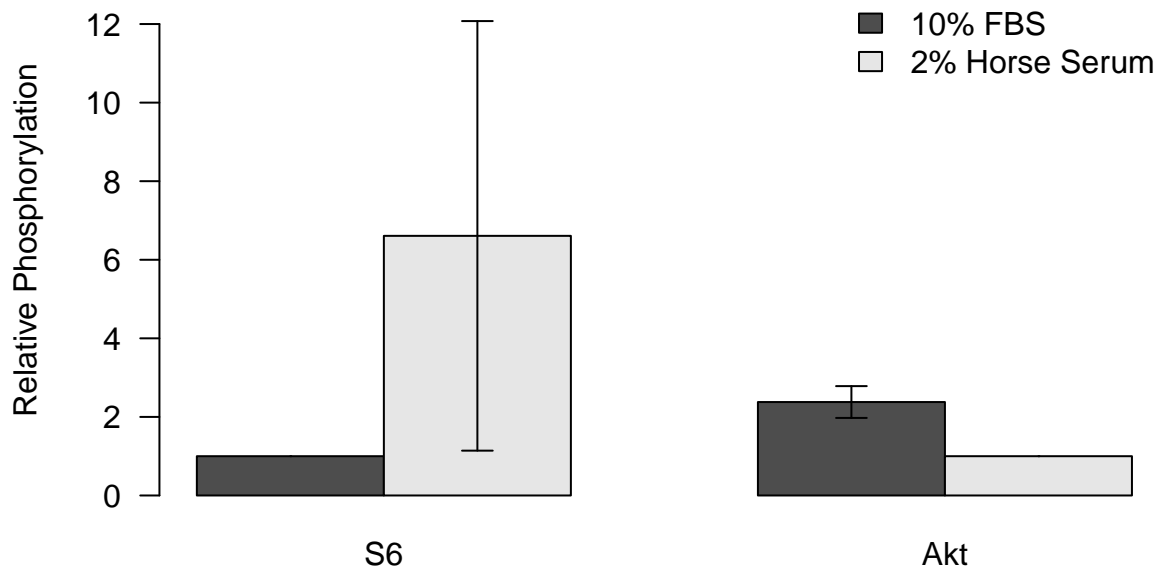
The addition of horse serum caused an increase in Akt phosphorylation ratio of 2.3786261 fold ( $\pm$  0.4047908 fold). The one way t-test for this effect had a p-value of 0.

## General Effects of Horse Serum

```
plot <- barplot(as.matrix(cbind(
  s6_summary[grepl("DMSO", s6_summary$Treatment), 'mean'][c(2:1),],
  akt_summary[grepl("DMSO", akt_summary$Treatment), 'mean',][c(2:1),])),
  beside=TRUE, las=1, ylim=c(0,13),
  names.arg=c('S6', 'Akt'), ylab='Relative Phosphorylation')
legend("topright", c('10% FBS', '2% Horse Serum'), bty='n', fill=grey.colors(2))
superpose.eb(plot,
  as.matrix(cbind(
    s6_summary[grepl("DMSO", s6_summary$Treatment), 'se'][c(2:1),],
    akt_summary[grepl("DMSO", akt_summary$Treatment), 'se',][c(2:1),])),
  as.matrix(cbind(
    s6_summary[grepl("DMSO", s6_summary$Treatment), 'se'][c(2:1),],
    akt_summary[grepl("DMSO", akt_summary$Treatment), 'se',][c(2:1),])))
```

```
## Warning in arrows(x, y + ebu, x, y - ebl, angle = 90, code = 3, length =
## length, : zero-length arrow is of indeterminate angle and so skipped
```

```
## Warning in arrows(x, y + ebu, x, y - ebl, angle = 90, code = 3, length =
## length, : zero-length arrow is of indeterminate angle and so skipped
```



## Combined Figure

```
par(mfrow=c(1,2))
plot <- barplot(as.matrix(dcast(subset(myod_summary, Drug %in% c("DMSO", "Rapamycin")),
                               Drug~Serum, value.var='mean')[2:3]),
               beside=T, las=1, ylab="Relative Protein Levels", main="MyoD")
legend("topleft", c('DMSO', 'Rapamycin'), bty='n', fill=grey.colors(2))
superpose.eb(plot,
             as.matrix(dcast(subset(myod_summary, Drug %in% c("DMSO", "Rapamycin")),
                               Drug~Serum, value.var='mean')[2:3]),
             as.matrix(dcast(subset(myod_summary, Drug %in% c("DMSO", "Rapamycin")),
                               Drug~Serum, value.var='se')[2:3]))
```

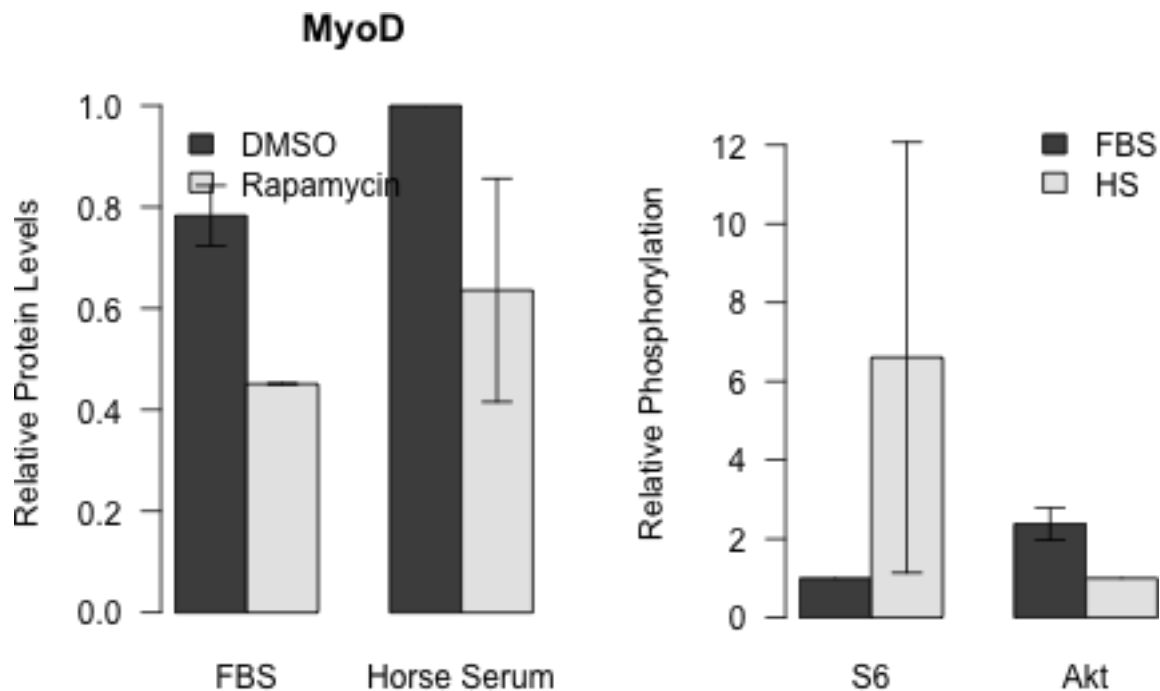
```
## Warning in arrows(x, y + ebu, x, y - ebl, angle = 90, code = 3, length =
## length, : zero-length arrow is of indeterminate angle and so skipped
```

```
plot <- barplot(as.matrix(cbind(
  s6_summary[grepl("DMSO", s6_summary$Treatment), 'mean'][c(2:1),],
  akt_summary[grepl("DMSO", akt_summary$Treatment), 'mean',][c(2:1),])),
               beside=TRUE, las=1, ylim=c(0,13),
               names.arg=c('S6', 'Akt'), ylab='Relative Phosphorylation')
legend("topright", c('FBS', 'HS'), bty='n', fill=grey.colors(2))
superpose.eb(plot,
             as.matrix(cbind(
  s6_summary[grepl("DMSO", s6_summary$Treatment), 'mean'][c(2:1),],
  akt_summary[grepl("DMSO", akt_summary$Treatment), 'mean',][c(2:1),])),
             as.matrix(cbind(
  s6_summary[grepl("DMSO", s6_summary$Treatment), 'se'][c(2:1),],
  akt_summary[grepl("DMSO", akt_summary$Treatment), 'se',][c(2:1),]))
```

```
## Warning in arrows(x, y + ebu, x, y - ebl, angle = 90, code = 3, length =
## length, : zero-length arrow is of indeterminate angle and so skipped
```

```
## Warning in arrows(x, y + ebu, x, y - ebl, angle = 90, code = 3, length =
## length, : zero-length arrow is of indeterminate angle and so skipped
```





## Session Information

```
sessionInfo()
```

```
## R version 3.1.1 (2014-07-10)
## Platform: x86_64-apple-darwin13.1.0 (64-bit)
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods   base
##
## other attached packages:
## [1] dplyr_0.3.0.2  reshape2_1.4.1
##
## loaded via a namespace (and not attached):
## [1] assertthat_0.1 DBI_0.3.1      digest_0.6.5   evaluate_0.5.5
## [5] formatR_1.0     htmltools_0.2.6 knitr_1.8       lazyeval_0.1.9
## [9] magrittr_1.5    parallel_3.1.1 plyr_1.8.1     Rcpp_0.11.3
## [13] rmarkdown_0.3.10 stringr_0.6.2  tools_3.1.1    yaml_2.1.13
```