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# Copyright 2008, Wayne G. Shreffler. Free non-commercial use is welcome.
# This is a R analysis script for analysis of data set produced for the
manuscript, "Allergen-Specific Basophil Activation Associated with Clinical
Tolerance in Patients with Milk Allergy."
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#R is by the R Foundation for Statistical Computing and is freely available
at http://www.R-project.org. R Development Core Team (2006). R: A language
and environment for statistical computing. R Foundation for Statistical
Computing, Vienna, Austria. ISBN 3-900051-07-0, URL.
```

```
# THIS WILL RUN WITHOUT MODIFICATION ON OS X but will require editing of
pipe functions and substitution of quartz functions (e.g. with 'pdf') for
use on Windows OS.
```

```
# Direct questions or suggestions about this script to the author at
wayne.shreffler@mssm.edu
```

```
current_dir <- readLines(pipe("pwd"))
download.file("http://www.iisinai.org/shreffler/milk_baso_data.zip",
"milk_baso_data.zip")
pipe("unzip 'milk_baso_data.zip'", "w")
```

```
baso <- read.csv(paste(current_dir, "/data/data_sum.csv", sep=""),
header=TRUE)
```

```
# create table for comparison of groups accross stimulants using ech
visitID as unique -- note some duplicate patients at multiple time points
```

```
# use only wash_status 'no' data
baso_washless <- subset(baso, wash_status == "n")
# exclude non-responders
baso_washless <- subset(baso_washless, Responder==1)
```

```
tmp <- data.frame(ID=baso_washless$visit_ID, group=baso_washless$STATUS,
stim=c(array("E1", length(baso_washless$E1)), array("E2",
length(baso_washless$E2)),array("E3", length(baso_washless$E3)),
array("E4", length(baso_washless$E4)), array("E5", length(baso_washless
$E5)), array("E6", length(baso_washless$E6))), CD63=c(baso_washless$E1,
baso_washless$E2, baso_washless$E3, baso_washless$E4, baso_washless$E5,
baso_washless$E6), HMCD63=c(baso_washless$HM_E1, baso_washless$HM_E2,
baso_washless$HM_E3, baso_washless$HM_E4, baso_washless$HM_E5,
baso_washless$HM_E6))
```

```
# define factors
groupf <- tmp$group
stimf <- tmp$stim
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agg <- by(tmp$CD63, list(stimf, groupf), fivenum)

# plot figure 1A
quartz(,10,10);layout(matrix(c(1,2),2,1))
boxplot(agg, ylab="%CD63", cex.lab=1.4, names=array(c("E1", "E2", "E3",
"E4", "E5", "E6"),24), outline=FALSE); abline(v=c(6.5, 12.5, 18.5), lty=3)
mtext("Allergic", 3, line=1, at=3.5)
mtext("Control", 3, line=1, at=9.5)
mtext("HM tolerant", 3, line=1, at=15.5)
mtext("Outgrown", 3, line=1, at=21.5)
mtext("A", cex=4, at=-1)

# anova for HM tolerant versus Allergic
attach(subset(tmp, group == "HM tolerant" | group == "Allergic"))
model <- lm(CD63 ~ group + stim)
anova(model)
detach(subset(tmp, group == "HM tolerant" | group == "Allergic"))
# anova for HM tolerant versus Control
attach(subset(tmp, group == "HM tolerant" | group == "Control"))
model <- lm(CD63 ~ group + stim)
anova(model)
detach(subset(tmp, group == "HM tolerant" | group == "Control"))
# anova for HM tolerant versus Outgrown
attach(subset(tmp, group == "HM tolerant" | group == "Outgrown"))
model <- lm(CD63 ~ group + stim)
anova(model)
detach(subset(tmp, group == "HM tolerant" | group == "Outgrown"))

# analysis for anti-IgE, fMLP, and IL-3
tmp2 <- data.frame(ID=baso_washless$visit_ID, group=baso_washless$STATUS,
stim=c(array("anti_IgE", length(baso_washless$anti_IgE)), array("fMLP",
length(baso_washless$fMLP)), array("IL3", length(baso_washless$IL3))),
CD63=c(baso_washless$anti_IgE, baso_washless$fMLP, baso_washless$IL3))

groupf <- tmp2$group
stimf <- tmp2$stim
agg <- by(tmp2$CD63, list(stimf, groupf), fivenum)

# plot figure 1B
boxplot(agg, ylab="%CD63", cex.lab=1.4, names=array(c("IL-3", "anti-IgE",
"fMLP"),12), outline=FALSE); abline(v=c(3.5, 6.5, 9.5), lty=3)
mtext("Allergic", 3, line=1, at=2)
mtext("Control", 3, line=1, at=5)
mtext("HM tolerant", 3, line=1, at=8)
mtext("Outgrown", 3, line=1, at=11)

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mtext("B", cex=4, at=-0.5)

# rank sum test for differences between HM tolerant and Allergic responses
to controls
wilcox.test(subset(tmp2, stim == "fMLP" & group == "HM tolerant")$CD63,
subset(tmp2, stim == "fMLP" & group == "Allergic")$CD63)
wilcox.test(subset(tmp2, stim == "anti_IgE" & group == "HM tolerant")$CD63,
subset(tmp2, stim == "anti_IgE" & group == "Allergic")$CD63)
wilcox.test(subset(tmp2, stim == "IL3" & group == "HM tolerant")$CD63,
subset(tmp2, stim == "IL3" & group == "Allergic")$CD63)

# analysis for washing effect using subset of data with pre and post wash
data available
baso_wash <- subset(baso, wash_done == "y")
# exclude non-responders
baso_wash <- subset(baso_wash, Responder==1)
groupf <- factor(baso_wash$STATUS)
washf <- factor(baso_wash$wash_status)
by(baso_wash$E4, list(washf,groupf), length)
agg1 <- by(baso_wash$E1, list(washf,groupf), fivenum); agg2 <- by(baso_wash
$E2, list(washf,groupf), fivenum); agg3 <- by(baso_wash$E3,
list(washf,groupf), fivenum); agg4 <- by(baso_wash$E4, list(washf,groupf),
fivenum); agg5 <- by(baso_wash$E5, list(washf,groupf), fivenum); agg6 <-
by(baso_wash$E6, list(washf,groupf), fivenum)

wash_tmp <- subset(baso_wash, STATUS == "HM tolerant")
wilcox.test(subset(wash_tmp, wash_status == "y")$E6, subset(wash_tmp,
wash_status == "n")$E6, paired=TRUE, alternative = "greater")
wilcox.test(subset(wash_tmp, wash_status == "y")$E5, subset(wash_tmp,
wash_status == "n")$E5, paired=TRUE, alternative = "greater")
wilcox.test(subset(wash_tmp, wash_status == "y")$E4, subset(wash_tmp,
wash_status == "n")$E4, paired=TRUE, alternative = "greater")

# figure 2
quartz(,10,10); layout(matrix(c(1,2,3,4,5,6),3,2))
boxplot(agg1, ylab="%CD63", cex.axis=1.5, cex.lab=1.2, names=array(c("+",
"-"),8), outline=FALSE); abline(v=c(2.5, 4.5, 6.5), lty=3); mtext("E1",
3,0.5,cex=3,at=-.2); mtext("serum", 1 ,0.75, at=0);
mtext(c("Allergic","Control","HM tolerant","Outgrown"),
3,1,at=c(1.5,3.5,5.5,7.5))
boxplot(agg2, ylab="%CD63", cex.axis=1.5, cex.lab=1.2, names=array(c("+",
"-"),8), outline=FALSE); abline(v=c(2.5, 4.5, 6.5), lty=3); mtext("E2",
3,0.5,cex=3,at=-.2); mtext("serum", 1 ,0.75, at=0)
boxplot(agg3, ylab="%CD63", cex.axis=1.5, cex.lab=1.2, names=array(c("+",
"-"),8), outline=FALSE); abline(v=c(2.5, 4.5, 6.5), lty=3); mtext("E3",
3,0.5,cex=3,at=-.2); mtext("serum", 1 ,0.75, at=0)

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boxplot(agg4, ylab="%CD63", cex.axis=1.5, cex.lab=1.2, names=array(c("+",
"-"),8), outline=FALSE); abline(v=c(2.5, 4.5, 6.5), lty=3); mtext("E4",
3,0.5,cex=3,at=-.2); mtext("serum", 1 ,0.75, at=0)
mtext("***",3,-3,at=5.5,cex=2); mtext(c("Allergic","Control","HM
tolerant","Outgrown"),3,1,at=c(1.5,3.5,5.5,7.5))
boxplot(agg5, ylab="%CD63", cex.axis=1.5, cex.lab=1.2, names=array(c("+",
"-"),8), outline=FALSE); abline(v=c(2.5, 4.5, 6.5), lty=3); mtext("E5",
3,0.5,cex=3,at=-.2); mtext("serum", 1 ,0.75, at=0)
boxplot(agg6, ylab="%CD63", cex.axis=1.5, cex.lab=1.2, names=array(c("+",
"-"),8), outline=FALSE); abline(v=c(2.5, 4.5, 6.5), lty=3); mtext("E6",
3,0.5,cex=3,at=-.2); mtext("serum", 1 ,0.75, at=0)
mtext("***",3,-3,at=5.5,cex=2)

```

```

# wash effect on controls; figure E5
agg7 <- by(baso_wash$IL3, list(washf,groupf), fivenum); agg8 <-
by(baso_wash$anti_IgE, list(washf,groupf), fivenum); agg9 <- by(baso_wash
$fMLP, list(washf,groupf), fivenum)
quartz(,10,10); layout(matrix(c(1,2,3),3,1))
boxplot(agg7, ylab="%CD63", cex.axis=1.5, cex.lab=1.2, names=array(c("+",
"-"),8)); abline(v=c(2.5, 4.5, 6.5), lty=3); mtext("IL-3",
3,2,cex=2,at=0.1); mtext("serum", 1 ,0.75, at=0.5);
mtext(c("Allergic","Control","HM tolerant","Outgrown"),
3,1,at=c(1.5,3.5,5.5,7.5))
boxplot(agg8, ylab="%CD63", cex.axis=1.5, cex.lab=1.2, names=array(c("+",
"-"),8)); abline(v=c(2.5, 4.5, 6.5), lty=3); mtext("anti-IgE",
3,2,cex=2,at=0.4); mtext("serum", 1 ,0.75, at=0.5)
boxplot(agg9, ylab="%CD63", cex.axis=1.5, cex.lab=1.2, names=array(c("+",
"-"),8)); abline(v=c(2.5, 4.5, 6.5), lty=3); mtext("fMLP",
3,2,cex=2,at=0.2); mtext("serum", 1 ,0.75, at=0.5)

```

```

wilcox.test(subset(wash_tmp, wash_status == "y")$IL3, subset(wash_tmp,
wash_status == "n")$IL3, paired=TRUE, alternative = "greater")
wilcox.test(subset(wash_tmp, wash_status == "y")$anti_IgE, subset(wash_tmp,
wash_status == "n")$anti_IgE, paired=TRUE, alternative = "greater")
wilcox.test(subset(wash_tmp, wash_status == "y")$fMLP, subset(wash_tmp,
wash_status == "n")$fMLP, paired=TRUE, alternative = "greater")

```

```

# some additional data exploration comparing BAT to sIgE and PST; figure E3
quartz(,15,7); layout(matrix(c(1,2,3,4,5,6,7,8,9,10,11,12),2,6,
byrow=TRUE))
plot(baso_washless$E1,baso_washless$PST,type="n", ylab="mm wheal", xlab="%
CD63", cex=1.5)
points(subset(baso_washless, STATUS=="Allergic")$E1,subset(baso_washless,

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```
STATUS=="Allergic")$PST, col="red", pch=19)
points(subset(baso_washless, STATUS=="Control")$E1,subset(baso_washless,
STATUS=="Control")$PST, col="orange", pch=19)
points(subset(baso_washless, STATUS=="HM tolerant")
$E1,subset(baso_washless, STATUS=="HM tolerant")$PST, col="blue", pch=19)
points(subset(baso_washless, STATUS=="Outgrown")$E1,subset(baso_washless,
STATUS=="Outgrown")$PST, col="green", pch=19)
mtext("E1", side=1, at=0, cex=2, line =6)
```

```
plot(baso_washless$E2,baso_washless$PST,type="n", ylab="mm wheal", xlab="%
CD63", cex=1.5)
points(subset(baso_washless, STATUS=="Allergic")$E2,subset(baso_washless,
STATUS=="Allergic")$PST, col="red", pch=19)
points(subset(baso_washless, STATUS=="Control")$E2,subset(baso_washless,
STATUS=="Control")$PST, col="orange", pch=19)
points(subset(baso_washless, STATUS=="HM tolerant")
$E2,subset(baso_washless, STATUS=="HM tolerant")$PST, col="blue", pch=19)
points(subset(baso_washless, STATUS=="Outgrown")$E2,subset(baso_washless,
STATUS=="Outgrown")$PST, col="green", pch=19)
mtext("E2", side=1, at=0, cex=2, line =6)
```

```
plot(baso_washless$E3,baso_washless$PST,type="n", ylab="mm wheal", xlab="%
CD63", cex=1.5)
points(subset(baso_washless, STATUS=="Allergic")$E3,subset(baso_washless,
STATUS=="Allergic")$PST, col="red", pch=19)
points(subset(baso_washless, STATUS=="Control")$E3,subset(baso_washless,
STATUS=="Control")$PST, col="orange", pch=19)
points(subset(baso_washless, STATUS=="HM tolerant")
$E3,subset(baso_washless, STATUS=="HM tolerant")$PST, col="blue", pch=19)
points(subset(baso_washless, STATUS=="Outgrown")$E3,subset(baso_washless,
STATUS=="Outgrown")$PST, col="green", pch=19)
mtext("E3", side=1, at=0, cex=2, line =6)
```

```
plot(baso_washless$E4,baso_washless$PST,type="n", ylab="mm wheal", xlab="%
CD63", cex=1.5)
points(subset(baso_washless, STATUS=="Allergic")$E4,subset(baso_washless,
STATUS=="Allergic")$PST, col="red", pch=19)
points(subset(baso_washless, STATUS=="Control")$E4,subset(baso_washless,
STATUS=="Control")$PST, col="orange", pch=19)
points(subset(baso_washless, STATUS=="HM tolerant")
$E4,subset(baso_washless, STATUS=="HM tolerant")$PST, col="blue", pch=19)
points(subset(baso_washless, STATUS=="Outgrown")$E4,subset(baso_washless,
STATUS=="Outgrown")$PST, col="green", pch=19)
mtext("E4", side=1, at=0, cex=2, line =6)
```

```
plot(baso_washless$E5,baso_washless$PST,type="n", ylab="mm wheal", xlab="%
```

```

CD63", cex=1.5)
points(subset(baso_washless, STATUS=="Allergic")$E5,subset(baso_washless,
STATUS=="Allergic")$PST, col="red", pch=19)
points(subset(baso_washless, STATUS=="Control")$E5,subset(baso_washless,
STATUS=="Control")$PST, col="orange", pch=19)
points(subset(baso_washless, STATUS=="HM tolerant")
$E5,subset(baso_washless, STATUS=="HM tolerant")$PST, col="blue", pch=19)
points(subset(baso_washless, STATUS=="Outgrown")$E5,subset(baso_washless,
STATUS=="Outgrown")$PST, col="green", pch=19)
mtext("E5", side=1, at=0, cex=2, line =6)

plot(baso_washless$E6,baso_washless$PST,type="n", ylab="mm wheal", xlab="%
CD63", cex=1.5)
points(subset(baso_washless, STATUS=="Allergic")$E6,subset(baso_washless,
STATUS=="Allergic")$PST, col="red", pch=19)
points(subset(baso_washless, STATUS=="Control")$E6,subset(baso_washless,
STATUS=="Control")$PST, col="orange", pch=19)
points(subset(baso_washless, STATUS=="HM tolerant")
$E6,subset(baso_washless, STATUS=="HM tolerant")$PST, col="blue", pch=19)
points(subset(baso_washless, STATUS=="Outgrown")$E6,subset(baso_washless,
STATUS=="Outgrown")$PST, col="green", pch=19)
mtext("E6", side=1, at=0, cex=2, line =6)

plot(baso_washless$E1,baso_washless$sIgE,type="n", ylab="kU/L", xlab="%
CD63", cex=1.5)
points(subset(baso_washless, STATUS=="Allergic")$E1,subset(baso_washless,
STATUS=="Allergic")$sIgE, col="red", pch=19)
points(subset(baso_washless, STATUS=="Control")$E1,subset(baso_washless,
STATUS=="Control")$sIgE, col="orange", pch=19)
points(subset(baso_washless, STATUS=="HM tolerant")
$E1,subset(baso_washless, STATUS=="HM tolerant")$sIgE, col="blue", pch=19)
points(subset(baso_washless, STATUS=="Outgrown")$E1,subset(baso_washless,
STATUS=="Outgrown")$sIgE, col="green", pch=19)

plot(baso_washless$E2,baso_washless$sIgE,type="n", ylab="kU/L", xlab="%
CD63", cex=1.5)
points(subset(baso_washless, STATUS=="Allergic")$E2,subset(baso_washless,
STATUS=="Allergic")$sIgE, col="red", pch=19)
points(subset(baso_washless, STATUS=="Control")$E2,subset(baso_washless,
STATUS=="Control")$sIgE, col="orange", pch=19)
points(subset(baso_washless, STATUS=="HM tolerant")
$E2,subset(baso_washless, STATUS=="HM tolerant")$sIgE, col="blue", pch=19)
points(subset(baso_washless, STATUS=="Outgrown")$E2,subset(baso_washless,
STATUS=="Outgrown")$sIgE, col="green", pch=19)

plot(baso_washless$E3,baso_washless$sIgE,type="n", ylab="kU/L", xlab="%

```

```
CD63", cex=1.5)
points(subset(baso_washless, STATUS=="Allergic")$E3,subset(baso_washless,
STATUS=="Allergic")$sIgE, col="red", pch=19)
points(subset(baso_washless, STATUS=="Control")$E3,subset(baso_washless,
STATUS=="Control")$sIgE, col="orange", pch=19)
points(subset(baso_washless, STATUS=="HM tolerant")
$E3,subset(baso_washless, STATUS=="HM tolerant")$sIgE, col="blue", pch=19)
points(subset(baso_washless, STATUS=="Outgrown")$E3,subset(baso_washless,
STATUS=="Outgrown")$sIgE, col="green", pch=19)
```

```
plot(baso_washless$E4,baso_washless$sIgE,type="n", ylab="kU/L", xlab="%
CD63", cex=1.5)
points(subset(baso_washless, STATUS=="Allergic")$E4,subset(baso_washless,
STATUS=="Allergic")$sIgE, col="red", pch=19)
points(subset(baso_washless, STATUS=="Control")$E4,subset(baso_washless,
STATUS=="Control")$sIgE, col="orange", pch=19)
points(subset(baso_washless, STATUS=="HM tolerant")
$E4,subset(baso_washless, STATUS=="HM tolerant")$sIgE, col="blue", pch=19)
points(subset(baso_washless, STATUS=="Outgrown")$E4,subset(baso_washless,
STATUS=="Outgrown")$sIgE, col="green", pch=19)
```

```
plot(baso_washless$E5,baso_washless$sIgE,type="n", ylab="kU/L", xlab="%
CD63", cex=1.5)
points(subset(baso_washless, STATUS=="Allergic")$E5,subset(baso_washless,
STATUS=="Allergic")$sIgE, col="red", pch=19)
points(subset(baso_washless, STATUS=="Control")$E5,subset(baso_washless,
STATUS=="Control")$sIgE, col="orange", pch=19)
points(subset(baso_washless, STATUS=="HM tolerant")
$E5,subset(baso_washless, STATUS=="HM tolerant")$sIgE, col="blue", pch=19)
points(subset(baso_washless, STATUS=="Outgrown")$E5,subset(baso_washless,
STATUS=="Outgrown")$sIgE, col="green", pch=19)
```

```
plot(baso_washless$E6,baso_washless$sIgE,type="n", ylab="kU/L", xlab="%
CD63", cex=1.5)
points(subset(baso_washless, STATUS=="Allergic")$E6,subset(baso_washless,
STATUS=="Allergic")$sIgE, col="red", pch=19)
points(subset(baso_washless, STATUS=="Control")$E6,subset(baso_washless,
STATUS=="Control")$sIgE, col="orange", pch=19)
points(subset(baso_washless, STATUS=="HM tolerant")
$E6,subset(baso_washless, STATUS=="HM tolerant")$sIgE, col="blue", pch=19)
points(subset(baso_washless, STATUS=="Outgrown")$E6,subset(baso_washless,
STATUS=="Outgrown")$sIgE, col="green", pch=19)
```

```
# some additional plots, figure E2
quartz(,8,4); plot(baso_washless$PST ~ baso_washless$STATUS, ylab="PST mm
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```
wheal", xlab="")
```

```
#Figure 5
```

```
hm <- subset(baso_washless, STATUS == "HM tolerant")
quartz(,12,4); layout(matrix(c(1:3),1,3,byrow=FALSE))
plot(hm$E4 ~ hm$current_diet, cex.lab=1.3, cex.axis=1.5, ylab="%CD63
positive", xlab=""); mtext("E4",3,1,cex=2,at=0.3)
plot(hm$E5 ~ hm$current_diet, cex.lab=1.3, cex.axis=1.5, ylab="%CD63
positive", xlab=""); mtext("E5",3,1,cex=2,at=0.3)
plot(hm$E6 ~ hm$current_diet, cex.lab=1.3, cex.axis=1.5, ylab="%CD63
positive", xlab=""); mtext("E6",3,1,cex=2,at=0.3)
```

```
#Figure E7
```

```
quartz(,12,4); layout(matrix(c(1:3),1,3,byrow=FALSE))
plot(hm$IL3 ~ hm$current_diet, cex.lab=1.3, cex.axis=1.5, ylab="%CD63
positive", xlab=""); mtext("IL-3",3,1,cex=2,at=0.4)
plot(hm$anti_IgE ~ hm$current_diet, cex.lab=1.3, cex.axis=1.5, ylab="%CD63
positive", xlab=""); mtext("anti-IgE",3,1,cex=2,at=0.6)
plot(hm$fMLP ~ hm$current_diet, cex.lab=1.3, cex.axis=1.5, ylab="%CD63
positive", xlab=""); mtext("fMLP",3,1,cex=2,at=0.5)
```

```
#Figure E6
```

```
quartz(,16,4); layout(matrix(c(1:4),1,4,byrow=FALSE))
plot(hm$E6 ~ hm$current_diet, cex.lab=1.3, cex.axis=1.5, ylab="%CD63
positive", xlab=""); mtext("E6",3,1,cex=2,at=0.4)
plot(hm$PST ~ hm$current_diet, cex.lab=1.3, cex.axis=1.5, ylab="%CD63
positive", xlab=""); mtext("PST",3,1,cex=2,at=0.3)
plot(hm$sIgE ~ hm$current_diet, cex.lab=1.3, cex.axis=1.5, ylab="%CD63
positive", xlab=""); mtext("sIgE",3,1,cex=2,at=0.3)
plot(hm$casein_g4 ~ hm$current_diet, cex.lab=1.3, cex.axis=1.5, ylab="%CD63
positive", xlab=""); mtext("IgG4",3,1,cex=2,at=0.3)
```

```
# create before-after plot for wash data, figure 3
```

```
hm <- subset(baso_wash, STATUS == "HM tolerant")
```

```
quartz(,8,4); layout(matrix(c(1,2,3),1,3, byrow=TRUE))
plot(as.numeric(hm$wash_status), hm$E4, xlim=c(0,3), cex.lab=1.4, xaxt="n",
ylab="%CD63 positive", xlab="", mtext("E4",3,1,at=-0.2, cex=2));
mtext(c("+", "-"),1,1,at=c(1,2),cex=1.5); mtext("serum",1,1,at=0.1)
for (step in 1:length(subset(hm, wash_status == "y")$E4)) {
  lines(as.numeric(subset(hm, visit_ID == subset(hm, wash_status ==
"y")$visit_ID[step])$wash_status), subset(hm, visit_ID == subset(hm,
```

```

wash_status == "y")$visit_ID[step])$E4)}
wilcox.test(hm$E4 ~ hm$wash_status, paired=TRUE, alternative="less")

plot(as.numeric(hm$wash_status), hm$E5, xlim=c(0,3), cex.lab=1.4, xaxt="n",
ylab="%CD63 positive", xlab="", mtext("E5",3,1,at=-0.2, cex=2));
mtext(c("+", "-"),1,1,at=c(1,2),cex=1.5)
for (step in 1:length(subset(hm, wash_status == "y")$E5)) {
  lines(as.numeric(subset(hm, visit_ID == subset(hm, wash_status ==
"y")$visit_ID[step])$wash_status), subset(hm, visit_ID == subset(hm,
wash_status == "y")$visit_ID[step])$E5)}
wilcox.test(hm$E5 ~ hm$wash_status, paired=TRUE, alternative="less")

plot(as.numeric(hm$wash_status), hm$E6, xlim=c(0,3), cex.lab=1.4, xaxt="n",
ylab="%CD63 positive", xlab="", mtext("E6",3,1,at=-0.2, cex=2));
mtext(c("+", "-"),1,1,at=c(1,2),cex=1.5)
for (step in 1:length(subset(hm, wash_status == "y")$E6)) {
  lines(as.numeric(subset(hm, visit_ID == subset(hm, wash_status ==
"y")$visit_ID[step])$wash_status), subset(hm, visit_ID == subset(hm,
wash_status == "y")$visit_ID[step])$E6)}
wilcox.test(hm$E6 ~ hm$wash_status, paired=TRUE, alternative="less")

```

```
# dilution experiments
```

```
dose <- read.csv(paste(current_dir, "/data/dilution/dilution_data.csv",
sep=""), header=TRUE)
```

```
# separate plot for dilution, figure 4
```

```

dilute <- subset(dose, stim == "milk" | stim == "IgE")
dilute.E4 <- subset(dilute, Ag_dilution == 1e+04 | Ag_dilution == 1)
dilute.E4 <- subset(dilute.E4, wash == 0)
dilute.E4.IgE <- subset(dilute.E4, stim == "IgE")
dilute.E4.milk <- subset(dilute.E4, stim == "milk")

quartz(,12,4); layout(matrix(c(1,2,3),1,3))
plot(subset(dilute.E4, SampleID == "PP608")$DF, subset(dilute.E4, SampleID
== "PP608")$CD63, xlab="serum dilution factor", ylab="% CD63", cex=2,
cex.lab=1.3, xaxp=c(1,4,3)); legend("topleft", legend=c("IgE", "milk"),
lty=c(3,1), bty="n", pt.cex=2)
lines(subset(dilute.E4.IgE, SampleID == "PP608")$DF, subset(dilute.E4.IgE,
SampleID == "PP608")$CD63, lty=3)
lines(subset(dilute.E4.milk, SampleID == "PP608")$DF,
subset(dilute.E4.milk, SampleID == "PP608")$CD63)

```

```
plot(subset(dilute.E4, SampleID == "PP603")$DF, subset(dilute.E4, SampleID
```

```

== "PP603")$CD63, xlab="serum dilution factor", ylab="% CD63", cex=2,
cex.lab=1.3, xaxp=c(1,4,3)); legend("topleft", legend=c("IgE", "milk"),
lty=c(3,1), bty="n", pt.cex=2)
lines(subset(dilute.E4.IgE, SampleID == "PP603")$DF, subset(dilute.E4.IgE,
SampleID == "PP603")$CD63, lty=3)
lines(subset(dilute.E4.milk, SampleID == "PP603")$DF,
subset(dilute.E4.milk, SampleID == "PP603")$CD63)

```

```

plot(subset(dilute.E4, SampleID == "PP621")$DF, subset(dilute.E4, SampleID
== "PP621")$CD63, xlab="serum dilution factor", ylab="% CD63", cex=2,
cex.lab=1.3, xaxp=c(1,4,3)); legend("topleft", legend=c("IgE", "milk"),
lty=c(3,1), bty="n", pt.cex=2)
lines(subset(dilute.E4.IgE, SampleID == "PP621")$DF, subset(dilute.E4.IgE,
SampleID == "PP621")$CD63, lty=3)
lines(subset(dilute.E4.milk, SampleID == "PP621")$DF,
subset(dilute.E4.milk, SampleID == "PP621")$CD63)

```

```

# example plots of dose response +/- wash, figure E4
milk.dose <- subset(dose, stim == "milk")
milk.dose <- subset(milk.dose, DF == 1)
milk.dose.nowash <- subset(milk.dose, wash == 0)
milk.dose.wash <- subset(milk.dose, wash == 1)

```

```

quartz(,12,4); layout(matrix(c(1,2,3),1,3, byrow=TRUE))
plot(log(subset(milk.dose, SampleID == "PP608")$Ag_dilution, 10),
subset(milk.dose, SampleID == "PP608")$CD63, col=4, xlab="log Ag
dilutioin", ylab="% CD63", cex.lab=1.3, cex=2, cex.axis=1.5)
lines(log(subset(milk.dose.wash, SampleID == "PP608")$Ag_dilution, 10),
subset(milk.dose.wash, SampleID == "PP608")$CD63, col=4)
lines(log(subset(milk.dose.nowash, SampleID == "PP608")$Ag_dilution, 10),
subset(milk.dose.nowash, SampleID == "PP608")$CD63, col=4, lty=3)
plot(log(subset(milk.dose, SampleID == "PP603")$Ag_dilution, 10),
subset(milk.dose, SampleID == "PP603")$CD63, col=2, xlab="log Ag
dilutioin", ylab="% CD63", cex.lab=1.3, cex=2, cex.axis=1.5)
lines(log(subset(milk.dose.wash, SampleID == "PP603")$Ag_dilution, 10),
subset(milk.dose.wash, SampleID == "PP603")$CD63, col=2)
lines(log(subset(milk.dose.nowash, SampleID == "PP603")$Ag_dilution, 10),
subset(milk.dose.nowash, SampleID == "PP603")$CD63, col=2, lty=3)
plot(log(subset(milk.dose, SampleID == "PP621")$Ag_dilution, 10),
subset(milk.dose, SampleID == "PP621")$CD63, col=3, xlab="log Ag
dilutioin", ylab="% CD63", cex.lab=1.3, cex=2, cex.axis=1.5)
lines(log(subset(milk.dose.wash, SampleID == "PP621")$Ag_dilution, 10),
subset(milk.dose.wash, SampleID == "PP621")$CD63, col=3)
lines(log(subset(milk.dose.nowash, SampleID == "PP621")$Ag_dilution, 10),
subset(milk.dose.nowash, SampleID == "PP621")$CD63, col=3, lty=3)

```

```

#IgG depletion experiments
dep <- read.csv(paste(current_dir, "/data/IgG_depletion/
depletion_summary.csv", sep=""), header=TRUE)
quartz(,15,5); layout(matrix(c(1,2,3),1,3, byrow=TRUE))
plot(subset(dep, STIM == "IL3")$CD63 ~ subset(dep, STIM == "IL3")$IGG,
range=0, xlab="", cex.axis=1.5, ylab=""); mtext("IL-3",3,1, at=0.5, cex=2)
points(subset(dep, STIM == "IL3")$CD63 ~ subset(dep, STIM == "IL3")$IGG,
cex=2)
plot(subset(dep, STIM == "E4")$CD63 ~ subset(dep, STIM == "E4")$IGG,
range=0, xlab="", cex.axis=1.5, ylab=""); mtext("E4",3,1, at=0.5, cex=2)
points(subset(dep, STIM == "E4")$CD63 ~ subset(dep, STIM == "E4")$IGG,
cex=2)
plot(subset(dep, STIM == "a.IgE")$CD63 ~ subset(dep, STIM == "a.IgE")$IGG,
range=0, xlab="", cex.axis=1.5, ylab=""); mtext("anti-IgE",3,1, at=0.5,
cex=2)
points(subset(dep, STIM == "a.IgE")$CD63 ~ subset(dep, STIM == "a.IgE")
$IGG, cex=2)

dep_E4 <- subset(dep, STIM == "E4"); dep_IGE <- subset(dep, STIM ==
"a.IgE"); dep_IL3 <- subset(dep, STIM == "IL3")
wilcox.test(subset(dep_E4, IGG == "mock")$CD63, subset(dep_E4, IGG ==
"depleted")$CD63, paired=TRUE, alternative="less")
wilcox.test(subset(dep_IGE, IGG == "mock")$CD63, subset(dep_IGE, IGG ==
"depleted")$CD63, paired=TRUE, alternative="less")
wilcox.test(subset(dep_IL3, IGG == "mock")$CD63, subset(dep_IL3, IGG ==
"depleted")$CD63, paired=TRUE, alternative="less")

```