

RRdatAI190418 AI190506

```
library(ggplot2)
library(reshape2)
library(ggpubr)

## Loading required package: magrittr

library(dplyr)

##
## Attaching package: 'dplyr'
##
## The following objects are masked from 'package:stats':
##
##   filter, lag
##
## The following objects are masked from 'package:base':
##
##   intersect, setdiff, setequal, union

library(RColorBrewer)
library(plotly)

##
## Attaching package: 'plotly'
##
## The following object is masked from 'package:ggplot2':
##
##   last_plot
##
## The following object is masked from 'package:stats':
##
##   filter
##
## The following object is masked from 'package:graphics':
##
##   layout

library(rstatix)

##
## Attaching package: 'rstatix'
##
## The following object is masked from 'package:stats':
##
##   filter

library(RATest)

## Loading required package: gridExtra

##
## Attaching package: 'gridExtra'
##
## The following object is masked from 'package:dplyr':
##
##   combine
```

```

AIsumm_df <- read.table("AIsumm.csv", header=TRUE,
                        sep=",") # merged fluorecence data of CRISPR assay 190418 and 190506
attach(AIsumm_df)

# changing the df to long format

AIsumm_df_long <- melt(AIsumm_df,
                      id.vars=c("ID"),
                      measure.vars=c("GFP", "RFP", "GFP.RFP" ),
                      variable.name="Channel",
                      value.name="RFU")

```

Boxplots:

```

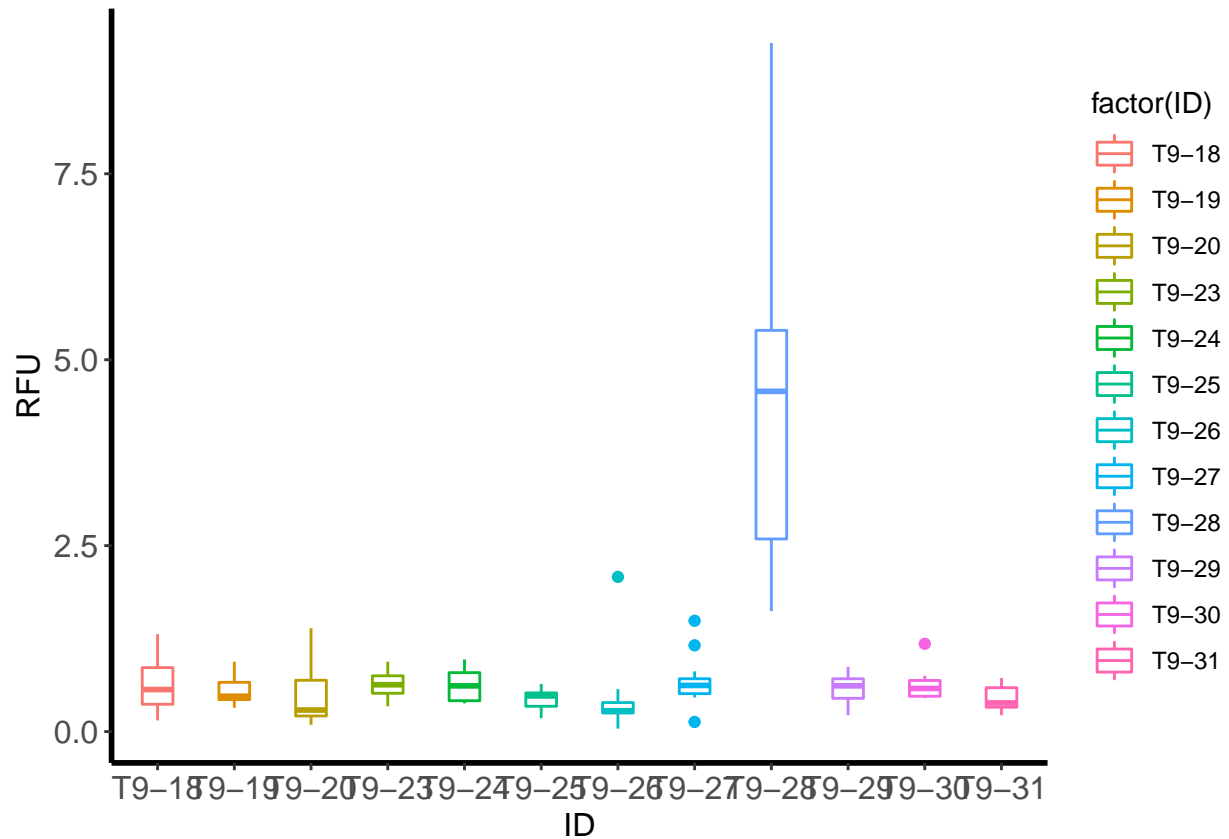
# Looking only at GFP/RFP ratios

AIsumm_df_long_red2 <- AIsumm_df_long[-c(1:282), ]

pall <- ggplot(data=AIsumm_df_long_red2, aes(x=ID,y=RFU, colour=factor(ID))) +
  geom_boxplot(position=position_dodge2(width=0.4), width=0.4) +
  #scale_y_continuous(breaks = 1) +
  #scale_color_grey() +
  theme_classic() +
  #coord_fixed(ratio = 3, xlim = NULL, ylim = c(0,1), expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12)
  )

pall

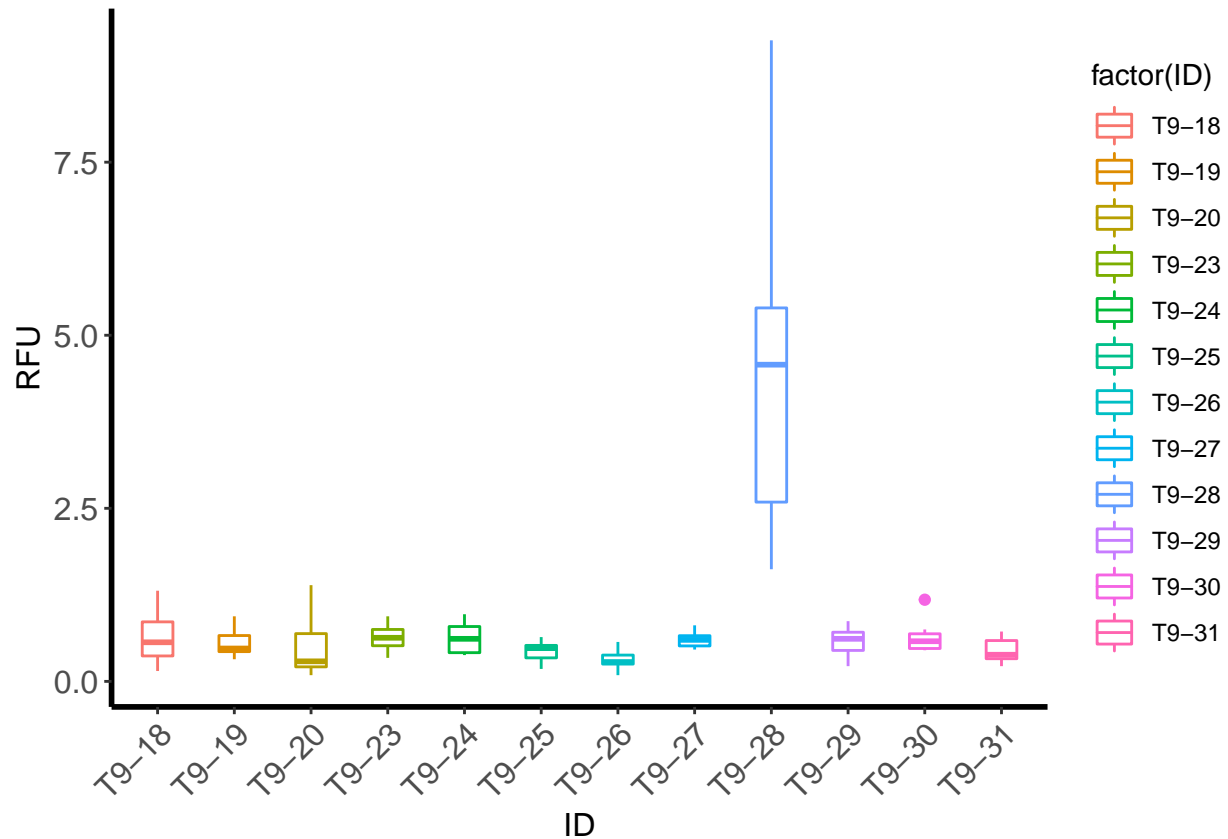
```



```
# removing outliers in 9-26 (1) and 9-27 (3)
AIsumm_df_long_red2_mod <- AIsumm_df_long_red2[-c(105,116,124,126,127), ]

pall_mod <- ggplot(data=AIsumm_df_long_red2_mod, aes(x=ID,y=RFU, colour=factor(ID))) +
  geom_boxplot(position=position_dodge2(width=0.4), width=0.4) +
  #scale_y_continuous(breaks = 1) +
  #scale_color_grey() +
  #colorRampPalette(brewer.pal(n_palette, "palette_name"))(n_plot),
  theme_classic() +
  #coord_fixed(ratio = 3, xlim = NULL, ylim = c(0,1), expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x= element_text(size=12, angle = 45, hjust = 1),
        axis.text.y = element_text(size=12)
  )

pall_mod
```



Separating data according to Cas-type:

```
AIsumm1_df <- read.table("AIsumm1.csv", header=TRUE,
                        sep=",") # merged fluorecence data of CRISPR assay 190418 and 190506
AIsumm2_df <- read.table("AIsumm2.csv", header=TRUE,
                        sep=",") # merged fluorecence data of CRISPR assay 190418 and 190506
AIsumm3_df <- read.table("AIsumm3.csv", header=TRUE,
                        sep=",") # merged fluorecence data of CRISPR assay 190418 and 190506
```

```
attach(AIsumm1_df)
```

```
## The following objects are masked from AIsumm_df:
```

```
##
```

```
## GFP, GFP.RFP, ID, RFP
```

```
attach(AIsumm2_df)
```

```
## The following objects are masked from AIsumm1_df:
```

```
##
```

```
## GFP, GFP.RFP, ID, RFP
```

```
##
```

```
## The following objects are masked from AIsumm_df:
```

```
##
```

```
## GFP, GFP.RFP, ID, RFP
```

```
attach(AIsumm3_df)
```

```
## The following objects are masked from AIsumm2_df:
```

```

##
##      GFP, GFP.RFP, ID, RFP
## The following objects are masked from AIsumm1_df:
##
##      GFP, GFP.RFP, ID, RFP
## The following objects are masked from AIsumm_df:
##
##      GFP, GFP.RFP, ID, RFP
AIsumm1_df_long <- melt(AIsumm1_df,
                        id.vars=c("ID"),
                        measure.vars=c("GFP", "RFP", "GFP.RFP" ),
                        variable.name="Channel",
                        value.name="RFU")

AIsumm2_df_long <- melt(AIsumm2_df,
                        id.vars=c("ID"),
                        measure.vars=c("GFP", "RFP", "GFP.RFP" ),
                        variable.name="Channel",
                        value.name="RFU")

AIsumm3_df_long <- melt(AIsumm3_df,
                        id.vars=c("ID"),
                        measure.vars=c("GFP", "RFP", "GFP.RFP" ),
                        variable.name="Channel",
                        value.name="RFU")

AIsumm1_df_long_red <- AIsumm1_df_long[-c(87:129), ]
AIsumm1_df_long_red2 <- AIsumm1_df_long[-c(1:86), ]

AIsumm2_df_long_red <- AIsumm2_df_long[-c(77:114), ]
AIsumm2_df_long_red2 <- AIsumm2_df_long[-c(1:76), ]

AIsumm3_df_mod <- AIsumm3_df[-c(24, 35, 43, 45, 46), ]
AIsumm3_df_long_red <- AIsumm3_df_long[-c(121:180), ]
AIsumm3_df_long_red2 <- AIsumm3_df_long[-c(1:120), ]

#removing outliers from 9-26 (2) and 9-27 (3)
AIsumm3_df_long_red2_mod <- AIsumm3_df_long_red2[-c(24, 35, 43, 45, 46), ]

# summarize GFP or RFP between groups
group_by(AIsumm1_df, ID) %>%
  summarise(
    count = n(),
    #medianG = median(GFP, na.rm = TRUE),
    meanG = mean(GFP, na.rm = TRUE),
    sdevG = sd(GFP, na.rm = TRUE),
    #medianR = median(RFP, na.rm = TRUE),
    meanR = mean(RFP, na.rm = TRUE),
    sdevR = sd(RFP, na.rm = TRUE)
    #IQR = IQR(GFP, na.rm = TRUE)
  )

```

```
## # A tibble: 4 x 6
##   ID      count meanG sdevG meanR sdevR
##   <fct> <int> <dbl> <dbl> <dbl> <dbl>
## 1 T9-18     10  2.06 1.28   3.28 0.820
## 2 T9-19      8  1.79 0.578  3.37 0.837
## 3 T9-20     13  1.47 1.13   3.22 0.611
## 4 T9-29     12  2.81 1.53   4.85 2.08
```

```
group_by(AIsumm2_df, ID) %>%
  summarise(
    count = n(),
    #medianG = median(GFP, na.rm = TRUE),
    meanG = mean(GFP, na.rm = TRUE),
    sdevG = sd(GFP, na.rm = TRUE),
    #medianR = median(RFP, na.rm = TRUE),
    meanR = mean(RFP, na.rm = TRUE),
    sdevR = sd(RFP, na.rm = TRUE)
    #IQR = IQR(GFP, na.rm = TRUE)
  )
```

```
## # A tibble: 4 x 6
##   ID      count meanG sdevG meanR sdevR
##   <fct> <int> <dbl> <dbl> <dbl> <dbl>
## 1 T9-23     11  2.14 0.826  3.40 0.943
## 2 T9-24      8  2.39 0.767  3.92 0.875
## 3 T9-25      9  1.50 0.389  3.71 0.801
## 4 T9-30     10  2.87 0.864  4.75 1.49
```

```
group_by(AIsumm3_df, ID) %>%
  summarise(
    count = n(),
    #medianG = median(GFP, na.rm = TRUE),
    meanG = mean(GFP, na.rm = TRUE),
    sdevG = sd(GFP, na.rm = TRUE),
    #medianR = median(RFP, na.rm = TRUE),
    meanR = mean(RFP, na.rm = TRUE),
    sdevR = sd(RFP, na.rm = TRUE)
    #IQR = IQR(GFP, na.rm = TRUE)
  )
```

```
## # A tibble: 4 x 6
##   ID      count meanG sdevG meanR sdevR
##   <fct> <int> <dbl> <dbl> <dbl> <dbl>
## 1 T9-26     21  2.49 2.70   6.22 2.75
## 2 T9-27     13  3.36 2.99   4.54 1.71
## 3 T9-28     12 19.4  8.46   4.61 1.87
## 4 T9-31     14  2.28 0.877  5.41 2.18
```

```
# summarize GFP/RFP ratios between groups
group_by(AIsumm1_df_long_red2, ID) %>%
  summarise(
    count = n(),
    mean = mean(RFU, na.rm = TRUE),
    sdev = sd(RFU, na.rm = TRUE)
  )
```

```
## # A tibble: 4 x 4
##   ID      count mean  sdev
##   <fct> <int> <dbl> <dbl>
## 1 T9-18     10 0.643 0.389
## 2 T9-19      8 0.555 0.216
## 3 T9-20     13 0.465 0.372
## 4 T9-29     12 0.578 0.190

group_by(AIsumm2_df_long_red2, ID) %>%
  summarise(
    count = n(),
    mean = mean(RFU, na.rm = TRUE),
    sdev = sd(RFU, na.rm = TRUE)
  )
```

```
## # A tibble: 4 x 4
##   ID      count mean  sdev
##   <fct> <int> <dbl> <dbl>
## 1 T9-23     11 0.640 0.176
## 2 T9-24      8 0.631 0.241
## 3 T9-25      9 0.427 0.145
## 4 T9-30     10 0.632 0.219

group_by(AIsumm3_df_long_red2_mod, ID) %>%
  summarise(
    count = n(),
    mean = mean(RFU, na.rm = TRUE),
    sdev = sd(RFU, na.rm = TRUE)
  )
```

```
## # A tibble: 4 x 4
##   ID      count mean  sdev
##   <fct> <int> <dbl> <dbl>
## 1 T9-26     19 0.308 0.112
## 2 T9-27     10 0.597 0.111
## 3 T9-28     12 4.57  2.23
## 4 T9-31     14 0.448 0.154
```

Boxplots GFP vs. RFP

```
p1 <- ggplot(data=AIsumm1_df_long_red, aes(x=ID,y=RFU, colour=factor(Channel))) +
  geom_boxplot(position=position_dodge2(width=0.4), width=0.4) +
  labs(tag = "GFP vs. RFP") +
  scale_color_brewer(palette = "Set2") +
  theme_classic() +
  coord_cartesian(ylim = c(0, 8)) +
  #facet_wrap(~Channel) +
  #coord_fixed(ratio = 0.05, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12)
  )
```

```

p2 <- ggplot(data=AISumm2_df_long_red, aes(x=ID,y=RFU, colour=factor(Channel))) +
  geom_boxplot(position=position_dodge2(width=0.4), width=0.4) +
  labs(tag = "GFP vs. RFP") +
  scale_color_brewer(palette = "Set2") +
  theme_classic() +
  coord_cartesian(ylim = c(0, 8)) +
  #facet_wrap(~Channel) +
  #coord_fixed(ratio = 0.05, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12)
  )

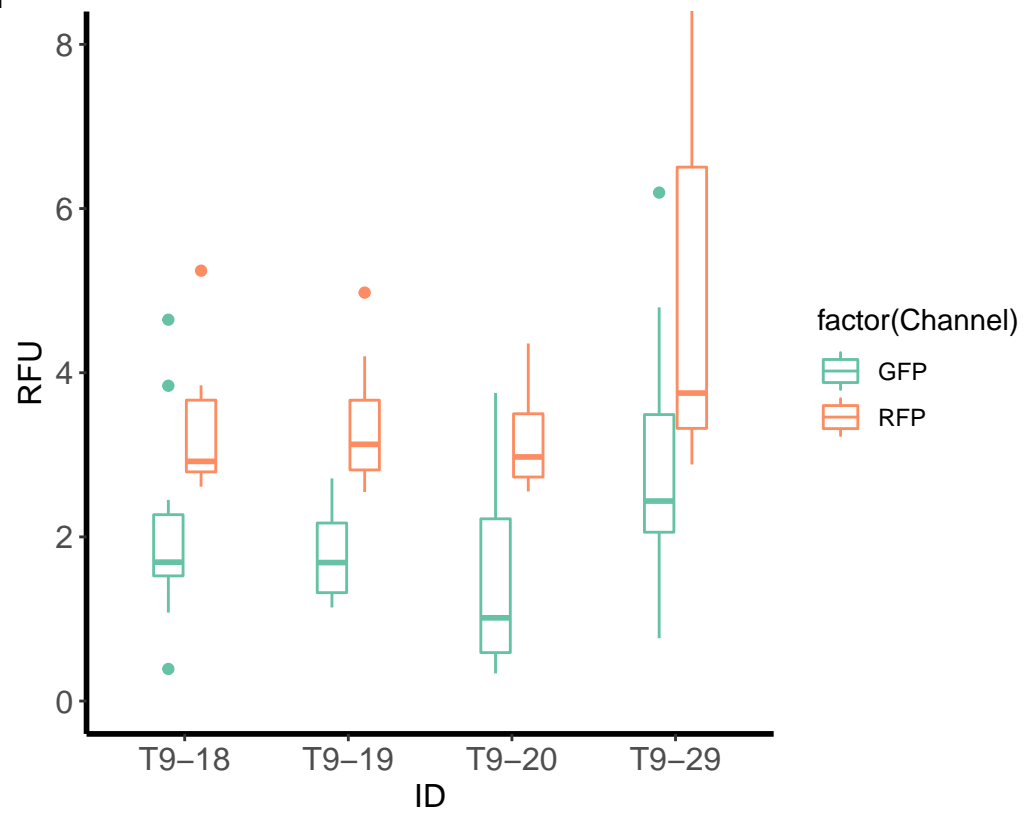
p3 <- ggplot(data=AISumm3_df_long_red, aes(x=ID,y=RFU, colour=factor(Channel))) +
  geom_boxplot(position=position_dodge2(width=0.4), width=0.4) +
  labs(tag = "GFP vs. RFP") +
  scale_color_brewer(palette = "Set2") +
  theme_classic() +
  #facet_wrap(~Channel) +
  #coord_fixed(ratio = 0.05, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12)
  )

p3zoom <- ggplot(data=AISumm3_df_long_red, aes(x=ID,y=RFU, colour=factor(Channel))) +
  geom_boxplot(position=position_dodge2(width=0.4), width=0.4) +
  labs(tag = "GFP vs. RFP") +
  scale_color_brewer(palette = "Set2") +
  theme_classic() +
  coord_cartesian(ylim = c(0, 8)) +
  #facet_wrap(~Channel) +
  #coord_fixed(ratio = 0.05, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12)
  )

```

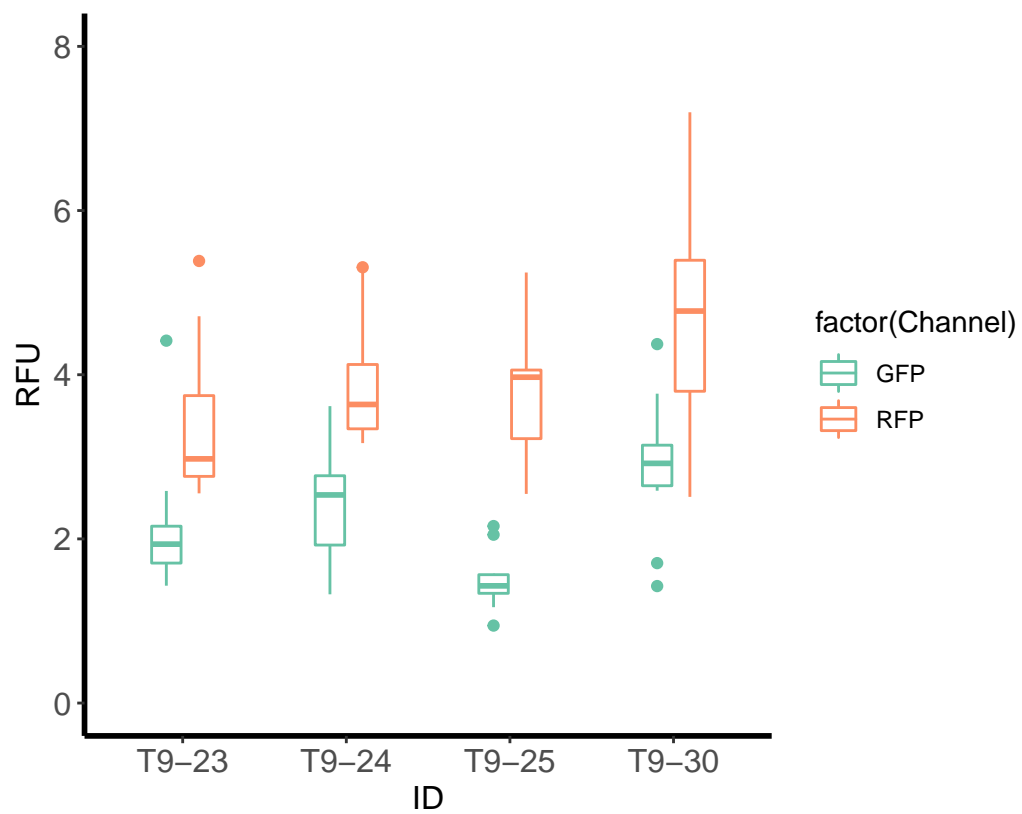
p1

GFP vs. RFP



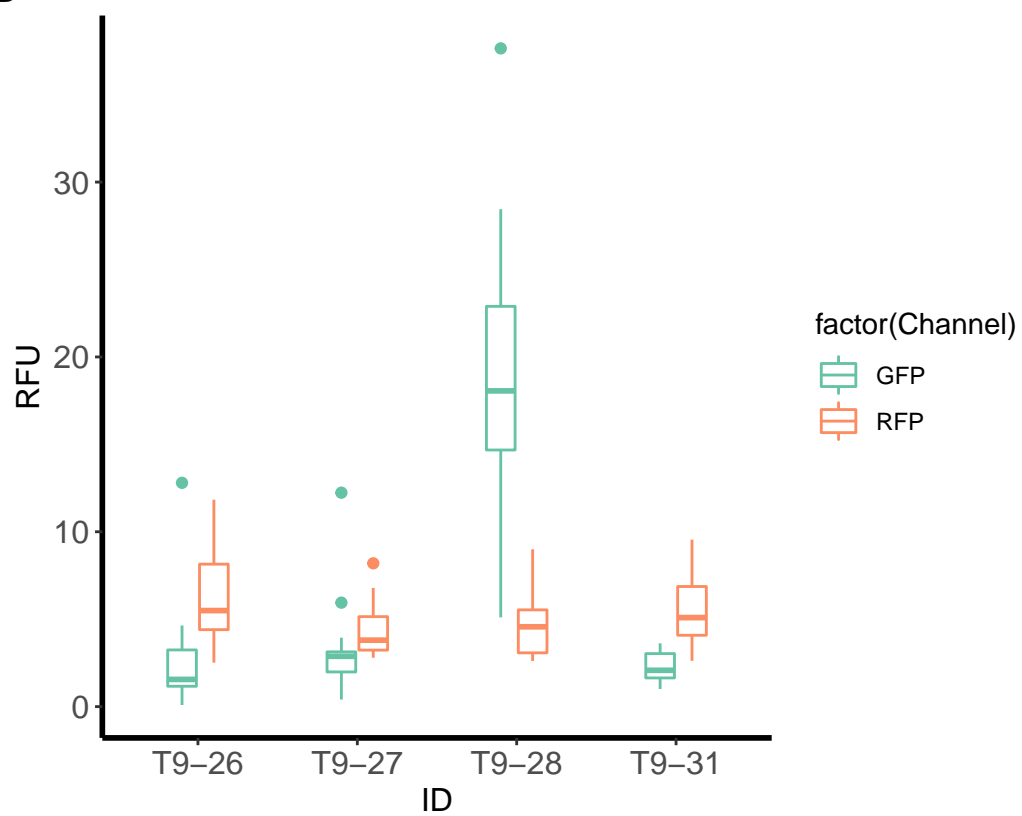
p2

GFP vs. RFP



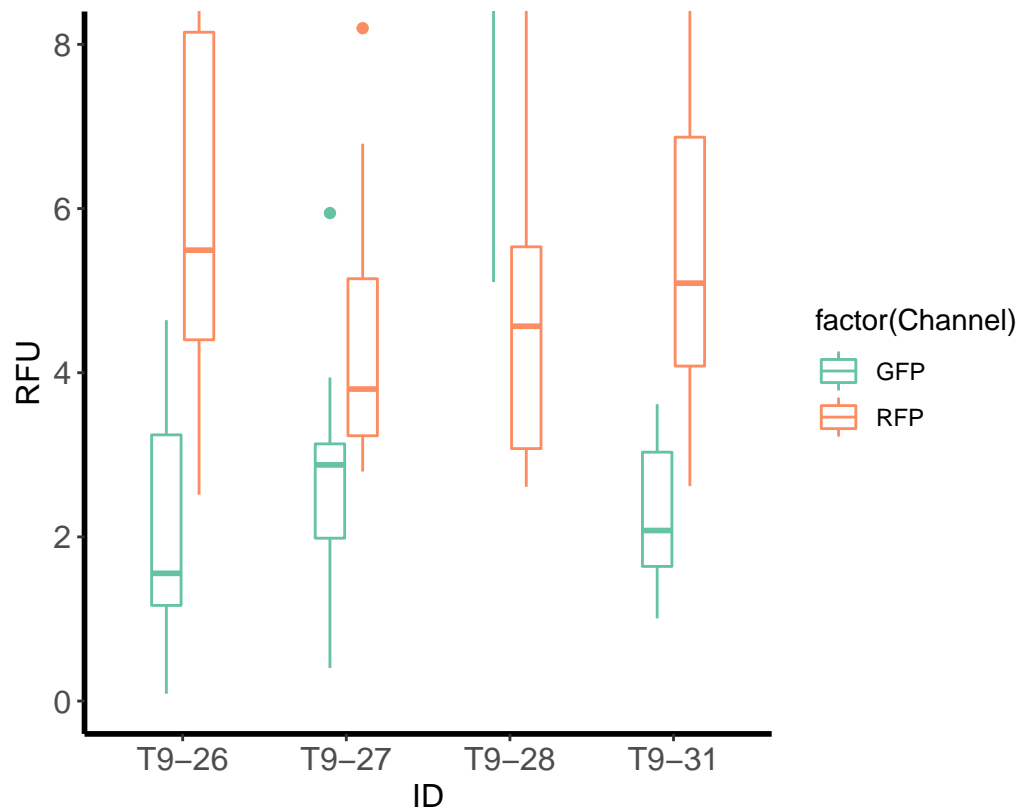
p3

GFP vs. RFP



p3zoom

GFP vs. RFP



Boxplots ratios:

```
p4 <- ggplot(data=AISumm1_df_long_red2, aes(x=ID,y=RFU)) +
  geom_boxplot(position=position_dodge2(width=0.4), width=0.4) +
  #geom_dotplot(binaxis='y', stackdir='center', dotsize=0.4, colour='grey', fill='grey') +
  labs(tag = "GFP vs. RFP") +
  scale_color_grey() +
  theme_classic() +
  coord_cartesian(ylim = c(0, 1.5)) +
  #facet_wrap(~Channel) +
  #coord_fixed(ratio = 3.55, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12)
  )

p5 <- ggplot(data=AISumm2_df_long_red2, aes(x=ID,y=RFU)) +
  geom_boxplot(position=position_dodge2(width=0.4), width=0.4) +
  #geom_dotplot(binaxis='y', stackdir='center', dotsize=0.5, colour='grey', fill='grey') +
  labs(tag = "GFP vs. RFP") +
  scale_color_grey() +
  theme_classic() +
  coord_cartesian(ylim = c(0, 1.5)) +
  #facet_wrap(~Channel) +
  #coord_fixed(ratio = 0.55, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on") +
```

```

    theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
          axis.title.x = element_text(size = 12),
          axis.title.y = element_text(size = 12),
          axis.text.x = element_text(size=12),
          axis.text.y = element_text(size=12)
    )

p6 <- ggplot(data=AISumm3_df_long_red2_mod, aes(x=ID,y=RFU)) +
  geom_boxplot(position=position_dodge2(width=0.3), width=0.3) +
  #geom_dotplot(binaxis='y', stackdir='center', dotsize=0.5, colour='grey', fill='grey') +
  labs(tag = "GFP vs. RFP") +
  scale_color_grey() +
  theme_classic() +
  #facet_wrap(~Channel) +
  #coord_fixed(ratio = 0.55, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x = element_text(size=12),
        axis.text.y = element_text(size=12)
  )

p6zoom <- ggplot(data=AISumm3_df_long_red2_mod, aes(x=ID,y=RFU)) +
  geom_boxplot(position=position_dodge2(width=0.4), width=0.4) +
  labs(tag = "GFP vs. RFP") +
  scale_color_grey() +
  theme_classic() +
  coord_cartesian(ylim = c(0, 1.5)) +
  #facet_wrap(~Channel) +
  #coord_fixed(ratio = 0.55, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 12),
        axis.title.y = element_text(size = 12),
        axis.text.x = element_text(size=12),
        axis.text.y = element_text(size=12)
  )

comparisons1 <- list(c("T9-29", "T9-18"), c("T9-29", "T9-19"), c("T9-29", "T9-20"))
comparisons2 <- list(c("T9-30", "T9-23"), c("T9-30", "T9-24"), c("T9-30", "T9-25"))
comparisons3 <- list(c("T9-31", "T9-26"), c("T9-31", "T9-27"), c("T9-31", "T9-28"))

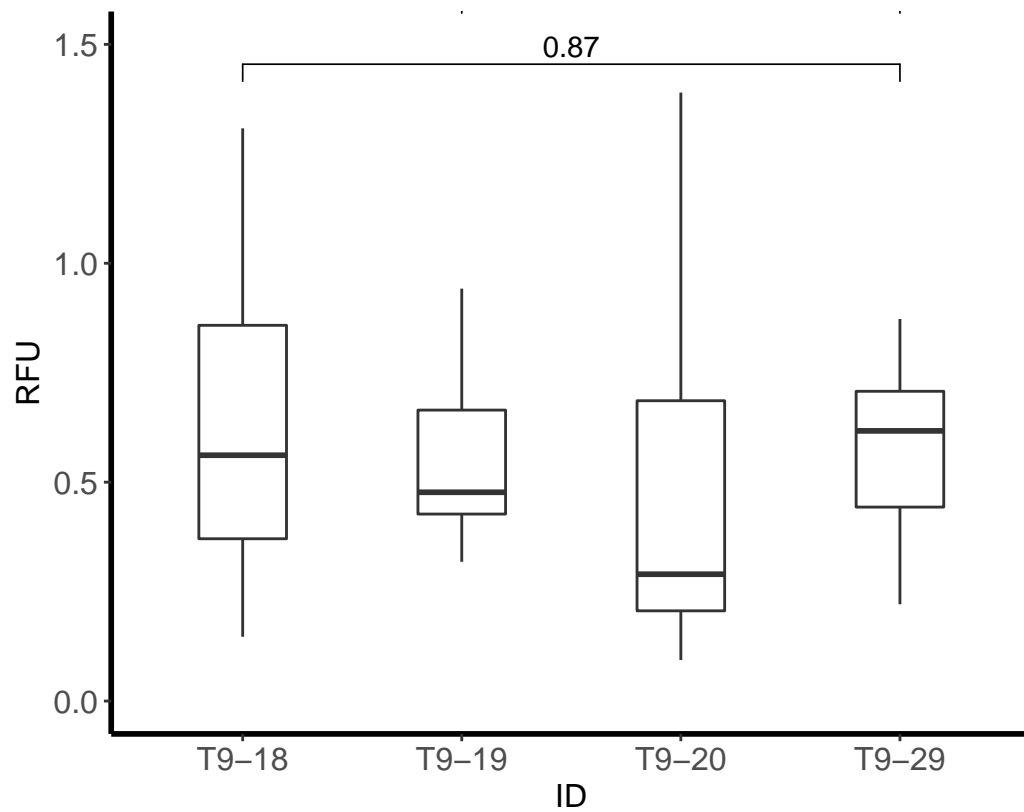
p4p <- p4 + stat_compare_means(method = "wilcox.test", label = "p.format", comparisons = comparisons1)
p5p <- p5 + stat_compare_means(method = "wilcox.test", label = "p.format", comparisons = comparisons2)
p6p <- p6 + stat_compare_means(method = "wilcox.test", label = "p.format", comparisons = comparisons3)

pctrl <- pall_mod + stat_compare_means(method = "wilcox.test", label = "p.format", comparisons = compar

p4p

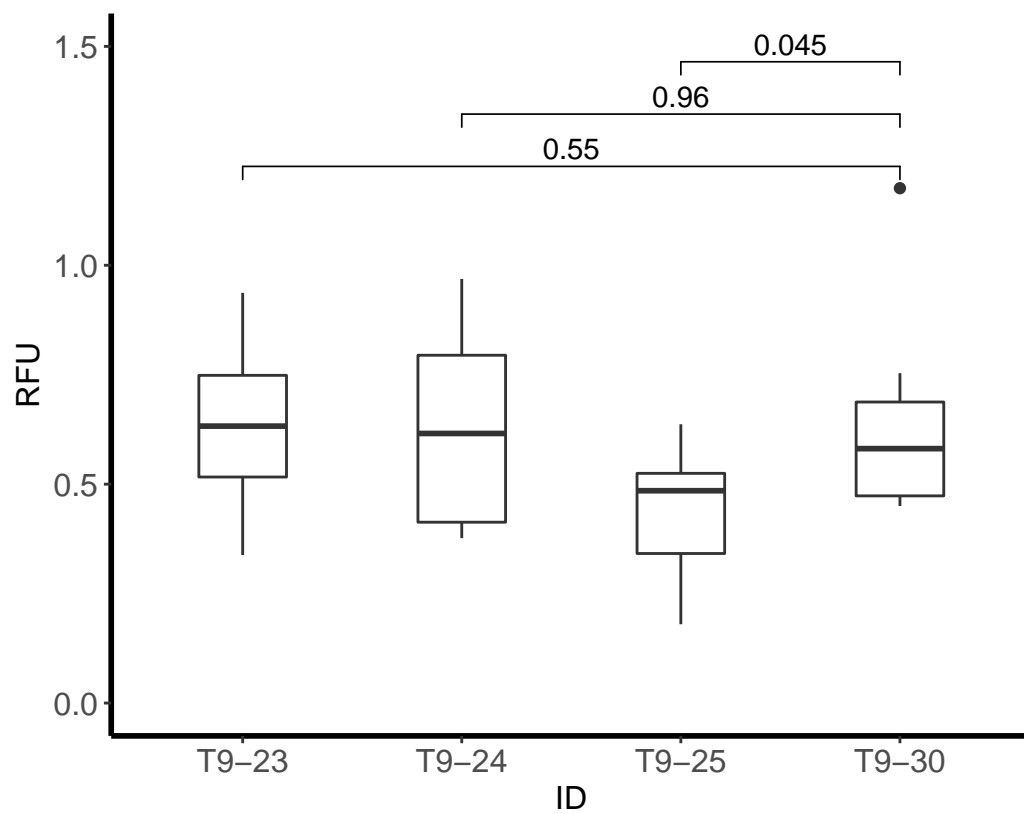
```

GFP vs. RFP

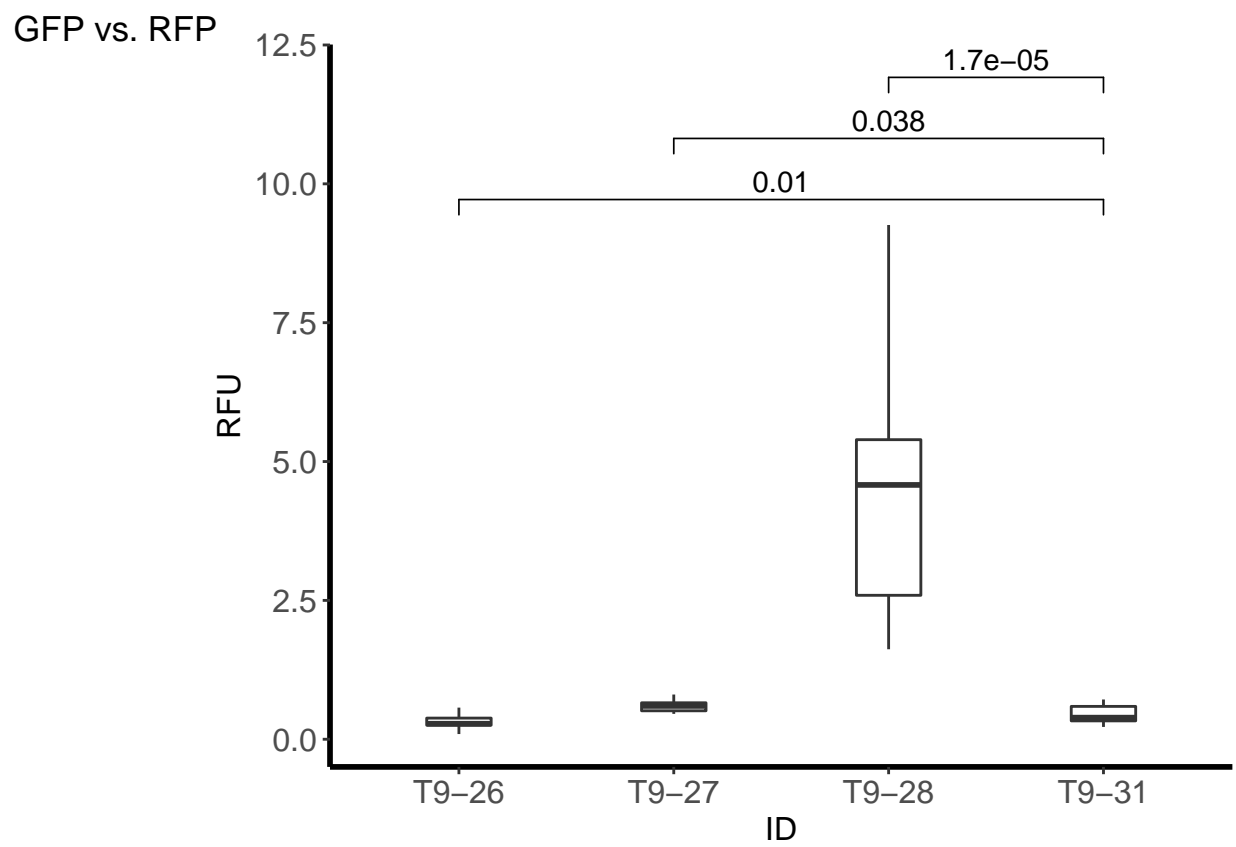


p5p

GFP vs. RFP

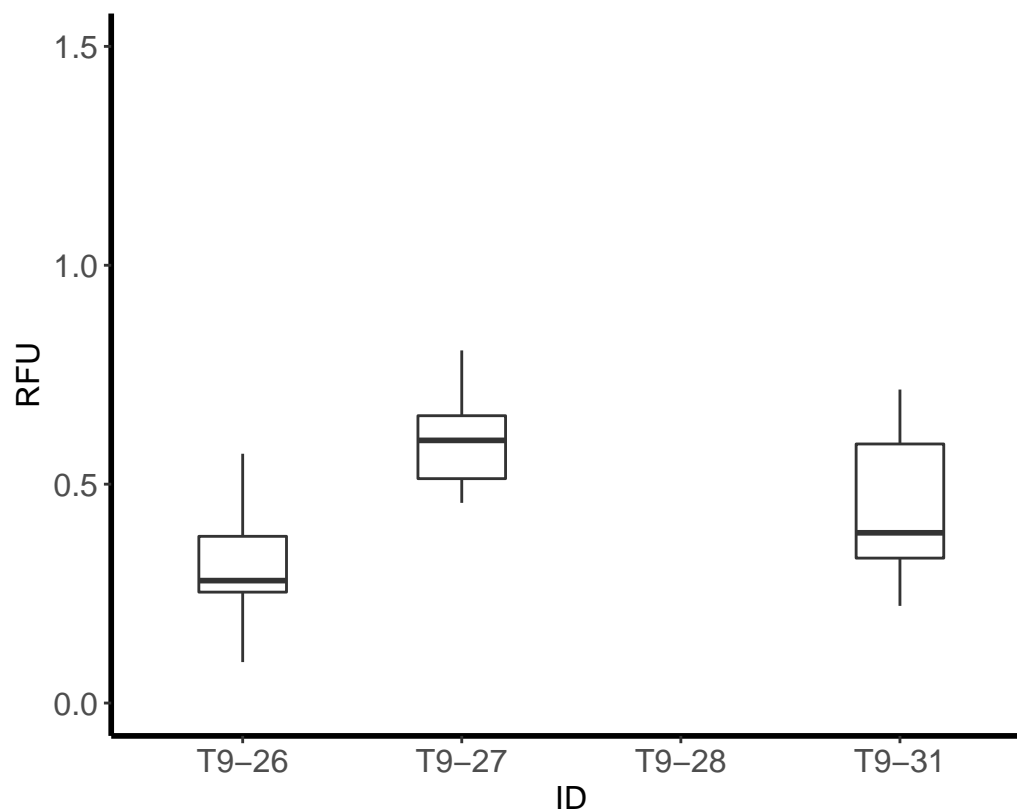


p6p



p6zoom

GFP vs. RFP



Splitting dataframes:

```
library('dplyr')
```

```
##### Only ratios
```

```
summary(AIsumm1_df_long_red2)
```

```
##      ID      Channel      RFU
## T9-18:10 GFP      : 0  Min.   :0.0937
## T9-19: 8 RFP      : 0  1st Qu.:0.3308
## T9-20:13 GFP.RFP:43  Median :0.5351
## T9-29:12      Mean   :0.5546
##      3rd Qu.:0.7050
##      Max.    :1.3899
```

```
t918_df <- filter(AIsumm1_df_long_red2, ID == "T9-18")
```

```
t919_df <- filter(AIsumm1_df_long_red2, ID == "T9-19")
```

```
t920_df <- filter(AIsumm1_df_long_red2, ID == "T9-20")
```

```
t929_df <- filter(AIsumm1_df_long_red2, ID == "T9-29")
```

```
summary(AIsumm2_df_long_red2)
```

```
##      ID      Channel      RFU
## T9-23:11 GFP      : 0  Min.   :0.1800
## T9-24: 8 RFP      : 0  1st Qu.:0.4527
## T9-25: 9 GFP.RFP:38  Median :0.5303
```

```
## T9-30:10          Mean    :0.5856
##                  3rd Qu.:0.7143
##                  Max.    :1.1760
```

```
t923_df <- filter(AIsumm2_df_long_red2, ID == "T9-23")
t924_df <- filter(AIsumm2_df_long_red2, ID == "T9-24")
t925_df <- filter(AIsumm2_df_long_red2, ID == "T9-25")
t930_df <- filter(AIsumm2_df_long_red2, ID == "T9-30")
```

```
summary(AIsumm3_df_long_red2_mod)
```

```
##      ID      Channel      RFU
## T9-26:19 GFP      : 0  Min.   :0.0936
## T9-27:10 RFP      : 0  1st Qu.:0.3165
## T9-28:12 GFP.RFP:55  Median :0.4730
## T9-31:14          Mean   :1.3271
##          3rd Qu.:0.7143
##          Max.    :9.2588
```

```
t926_df <- filter(AIsumm3_df_long_red2, ID == "T9-26")
t927_df <- filter(AIsumm3_df_long_red2, ID == "T9-27")
t928_df <- filter(AIsumm3_df_long_red2, ID == "T9-28")
t931_df <- filter(AIsumm3_df_long_red2, ID == "T9-31")
```

```
#Controls
```

```
tctrl_df <- rbind(t929_df, t930_df, t931_df)
summary(tctrl_df)
```

```
##      ID      Channel      RFU
## T9-31 :14 GFP      : 0  Min.   :0.2211
## T9-29 :12 RFP      : 0  1st Qu.:0.3926
## T9-30 :10 GFP.RFP:36  Median :0.5423
## T9-18 : 0          Mean   :0.5428
## T9-19 : 0          3rd Qu.:0.6532
## T9-20 : 0          Max.    :1.1760
## (Other): 0
```

Summary stats within

```
summary(t918_df)
```

```
##      ID      Channel      RFU
## T9-18:10 GFP      : 0  Min.   :0.1469
## T9-19: 0 RFP      : 0  1st Qu.:0.3708
## T9-20: 0 GFP.RFP:10  Median :0.5615
## T9-29: 0          Mean   :0.6426
##          3rd Qu.:0.8583
##          Max.    :1.3082
```

Statistic analysis:

```
### Normality tests:
```

```
qq918 <- ggqqplot(data=t918_df$RFU) + labs(tag = "crispr-18")
qq919 <- ggqqplot(data=t919_df$RFU) + labs(tag = "crispr-19")
qq920 <- ggqqplot(data=t920_df$RFU) + labs(tag = "crispr-20")
qq929 <- ggqqplot(data=t929_df$RFU) + labs(tag = "crispr-ctrl")

qq923 <- ggqqplot(data=t923_df$RFU) + labs(tag = "crisprI-23")
```

```

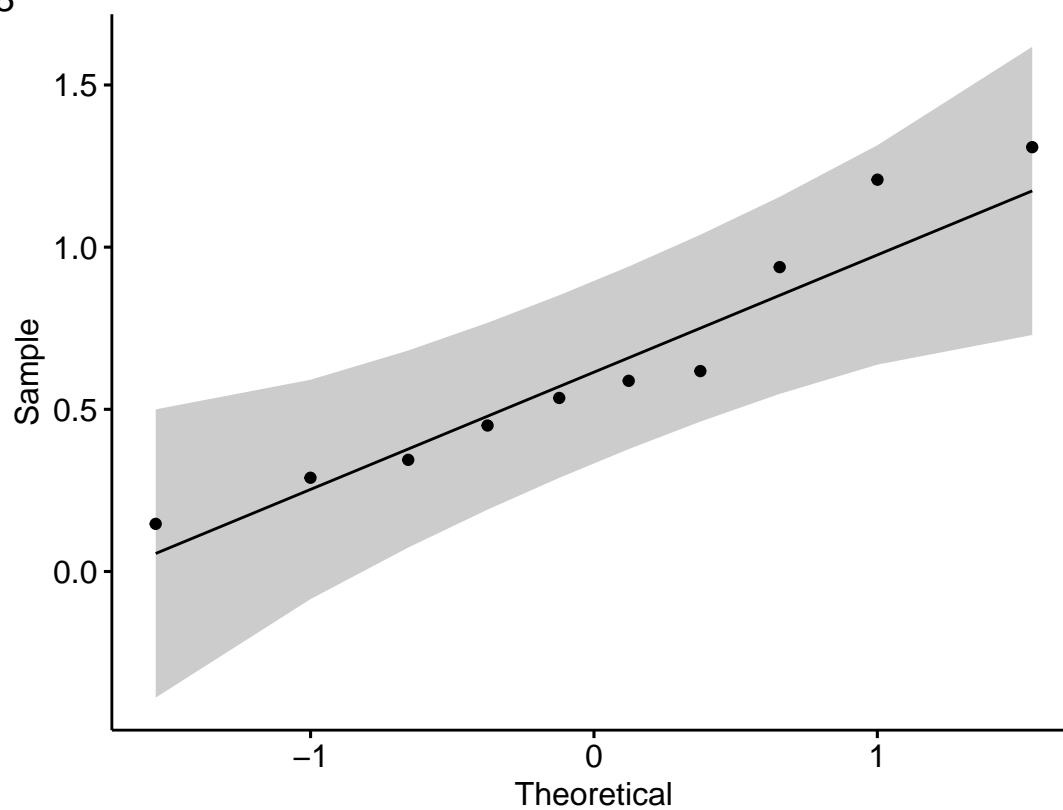
qq924 <- ggqqplot(data=t924_df$RFU) + labs(tag = "crisprI-24")
qq925 <- ggqqplot(data=t925_df$RFU) + labs(tag = "crisprI-25")
qq930 <- ggqqplot(data=t930_df$RFU) + labs(tag = "crisprI-ctrl")

qq926 <- ggqqplot(data=t926_df$RFU) + labs(tag = "crisprA-26")
qq927 <- ggqqplot(data=t927_df$RFU) + labs(tag = "crisprA-27")
qq928 <- ggqqplot(data=t928_df$RFU) + labs(tag = "crisprA-28")
qq931 <- ggqqplot(data=t931_df$RFU) + labs(tag = "crisprA-ctrl")

```

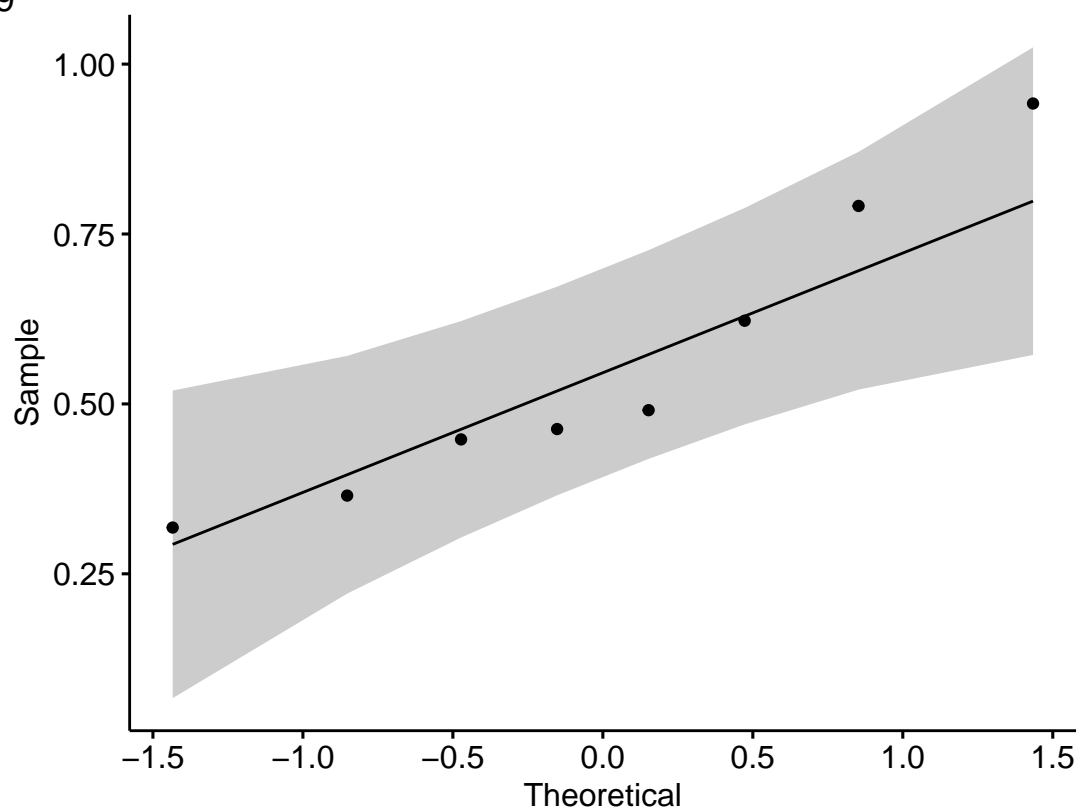
qq918

crispr-18



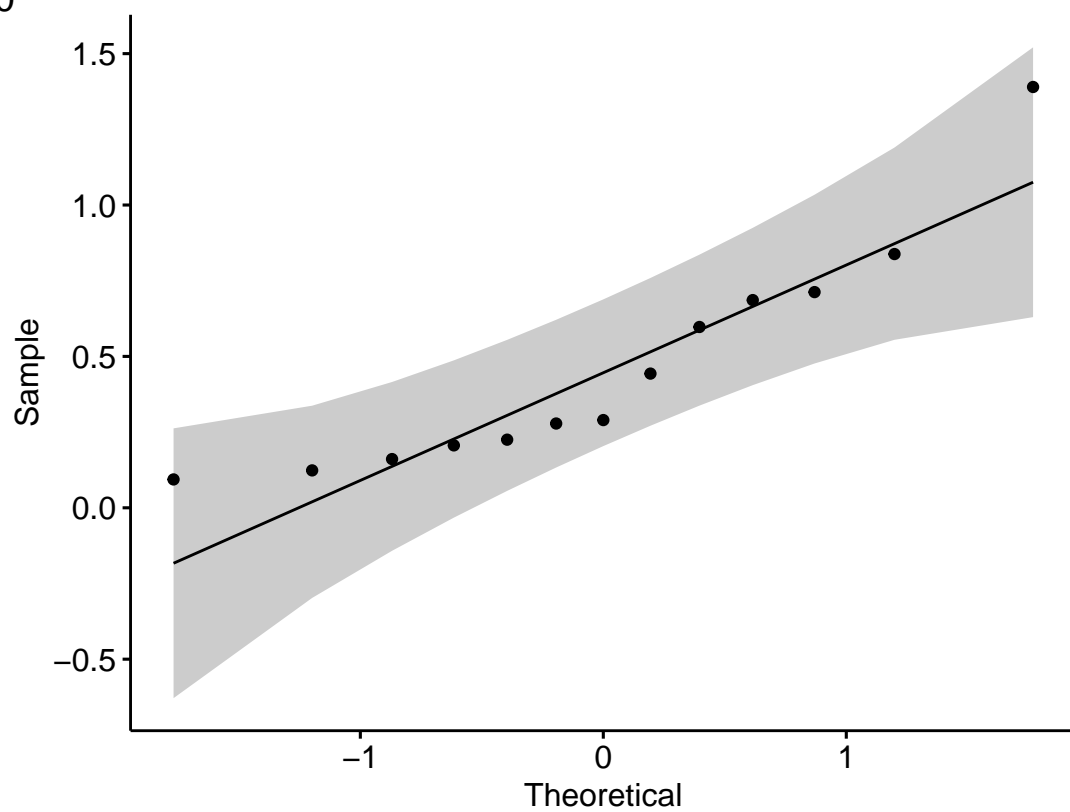
qq919

crispr-19



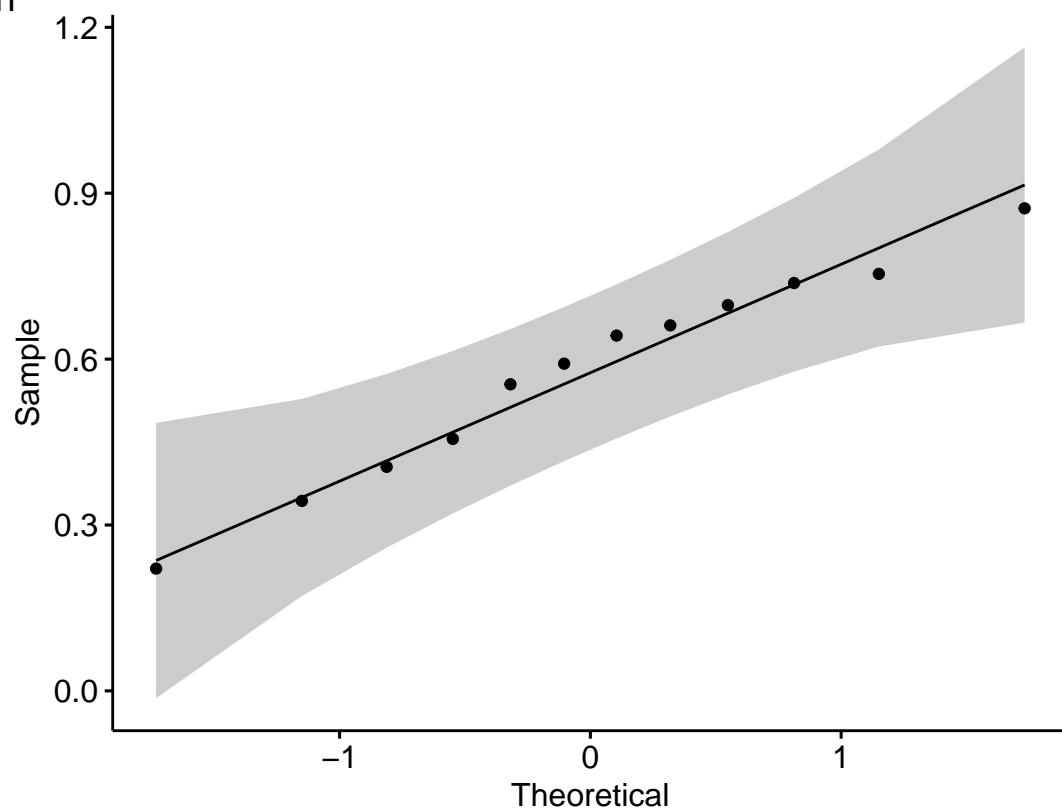
qq920

crispr-20



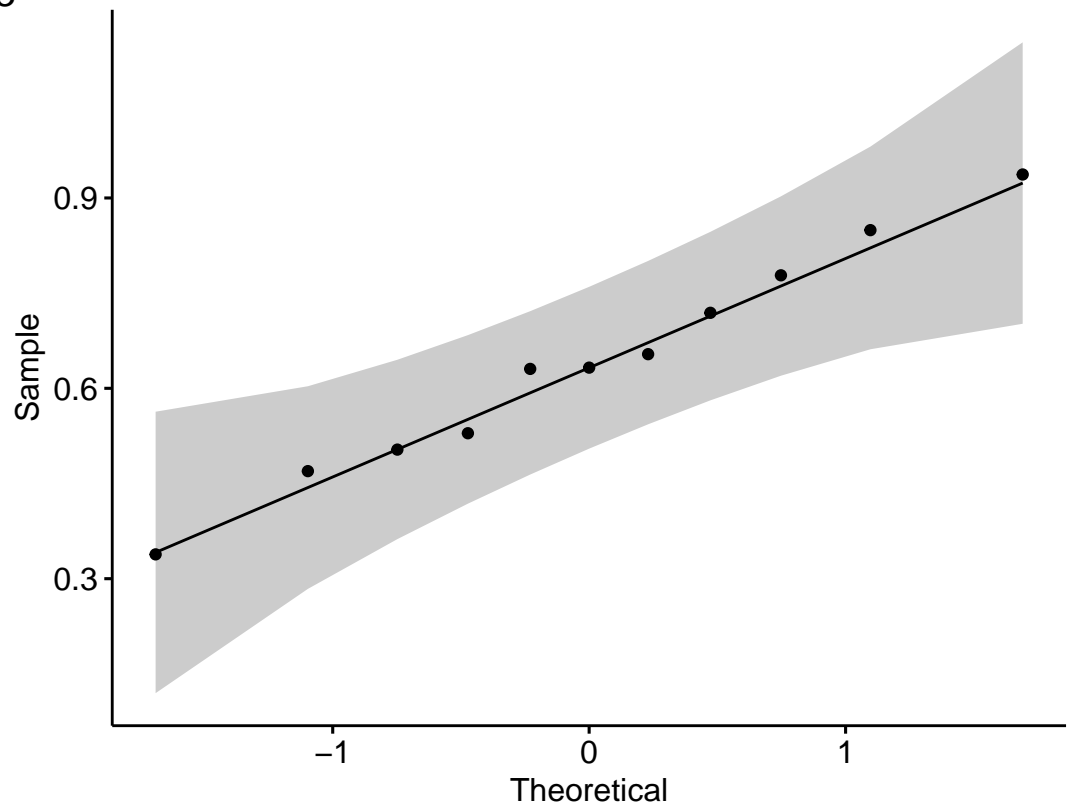
qq929

crispr-ctrl



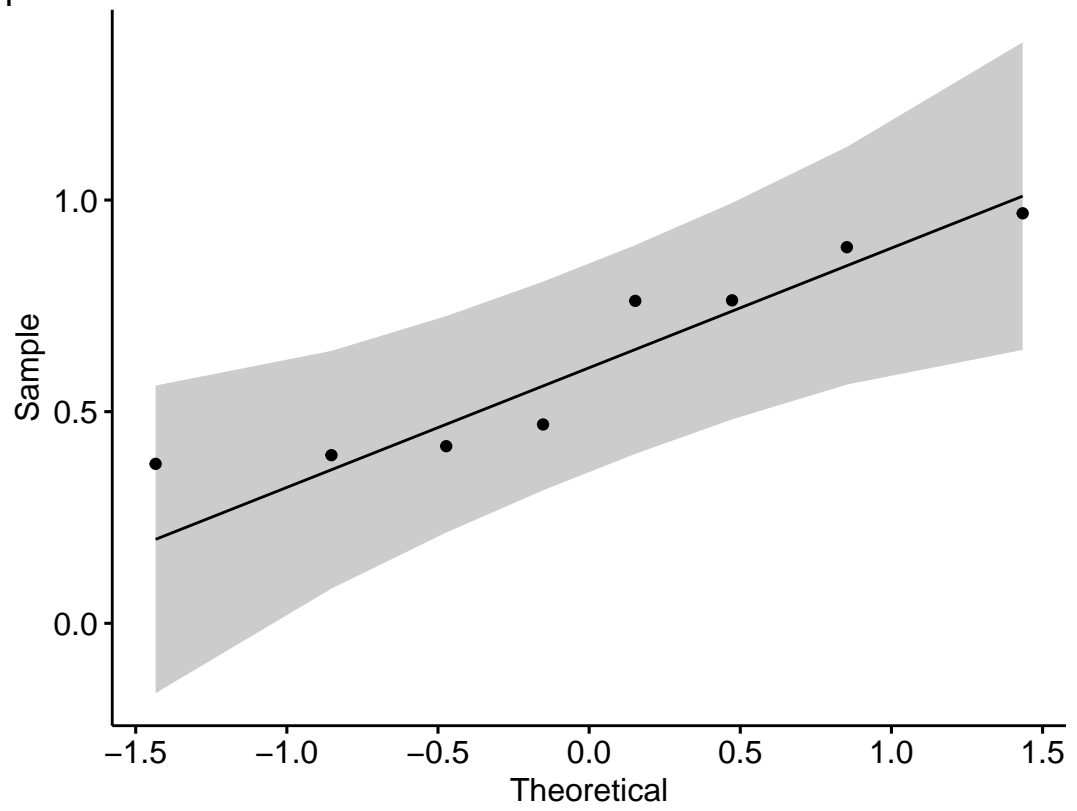
qq923

crisprl-23



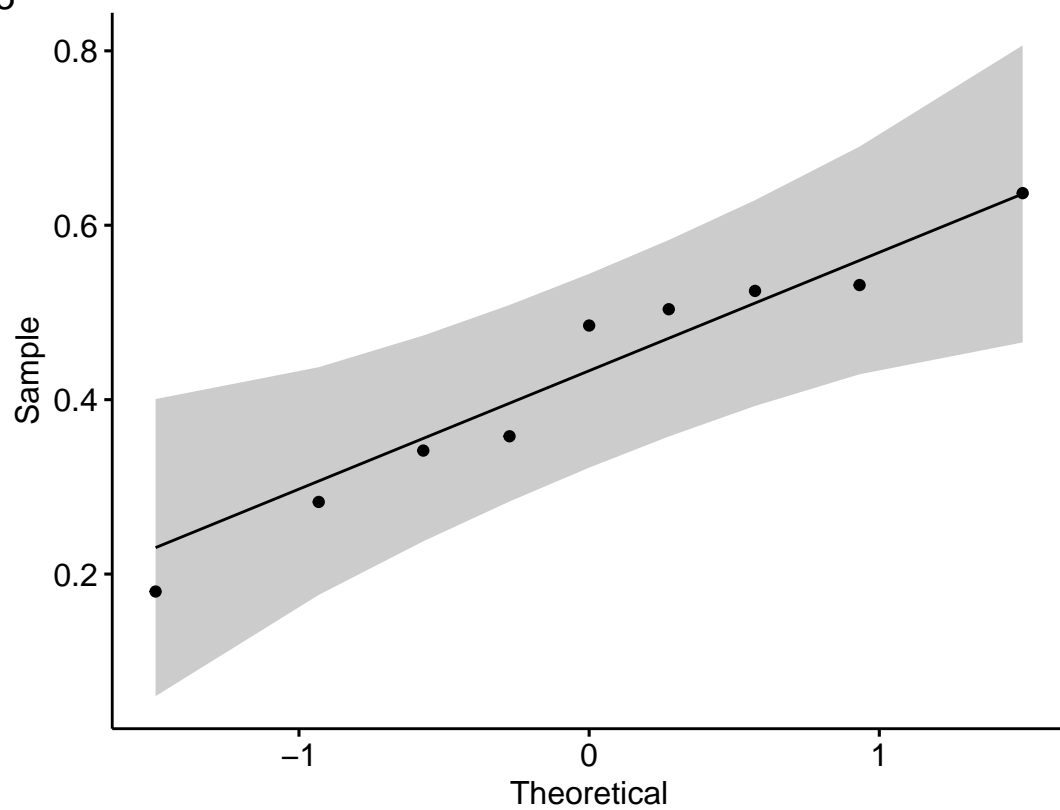
qq924

crisprl-24



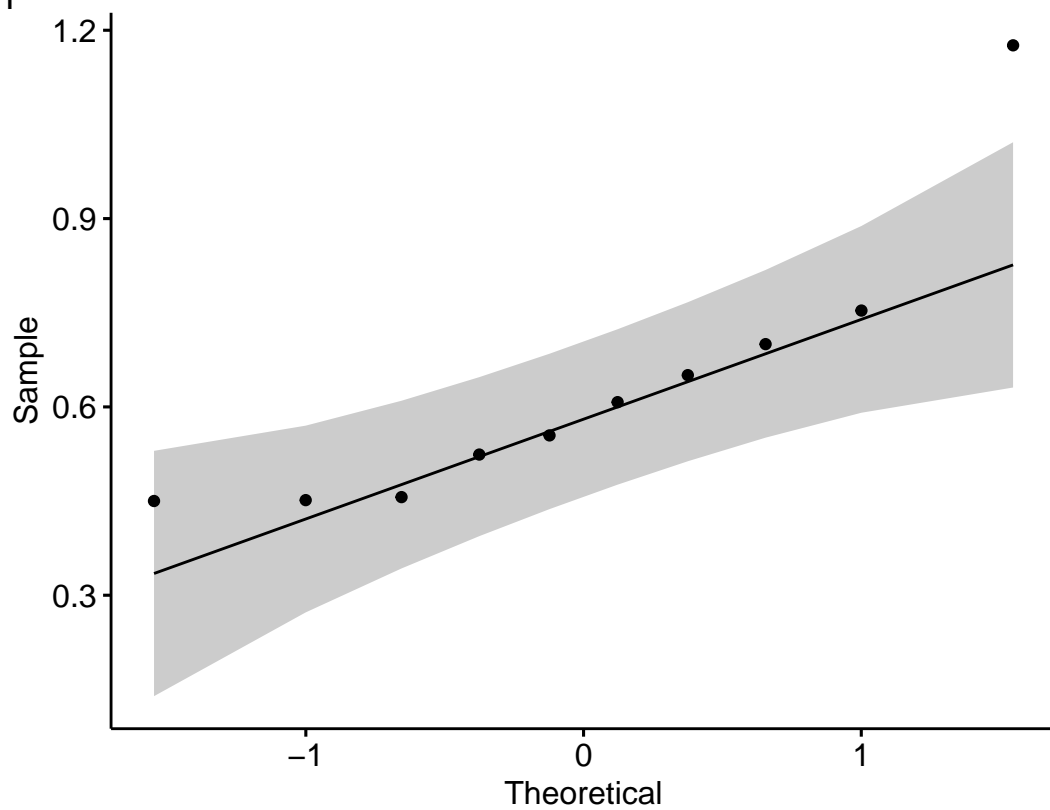
qq925

crisprl-25



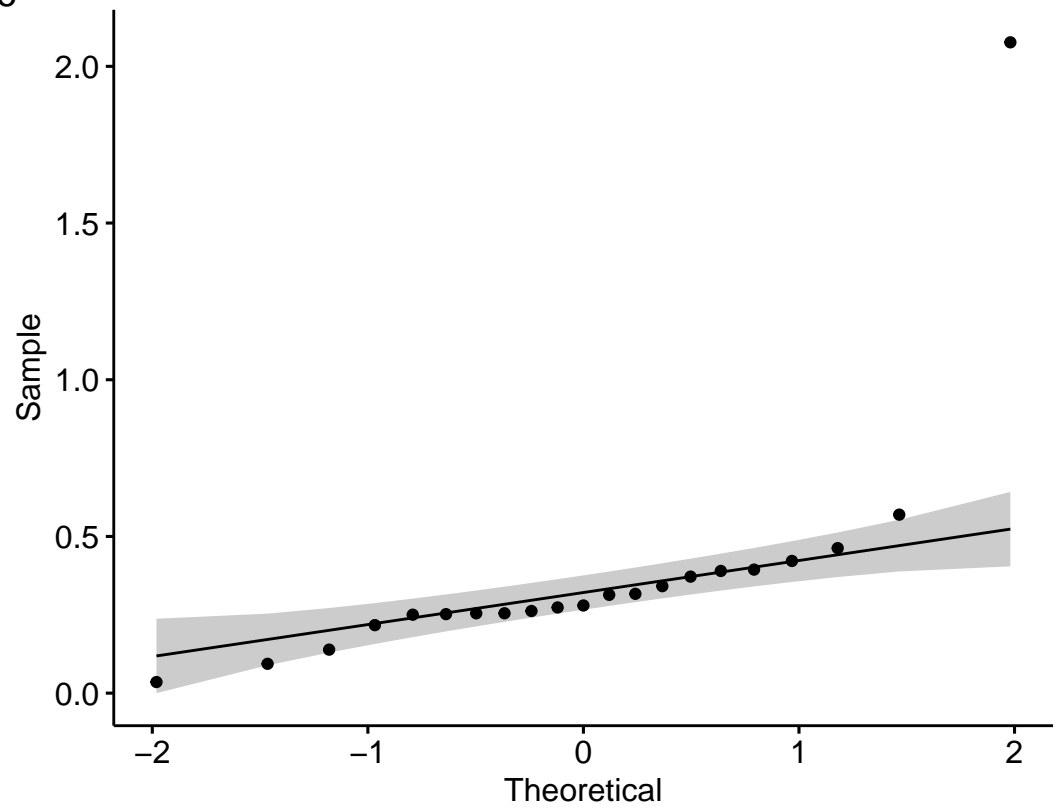
qq930

crisprl-ctrl



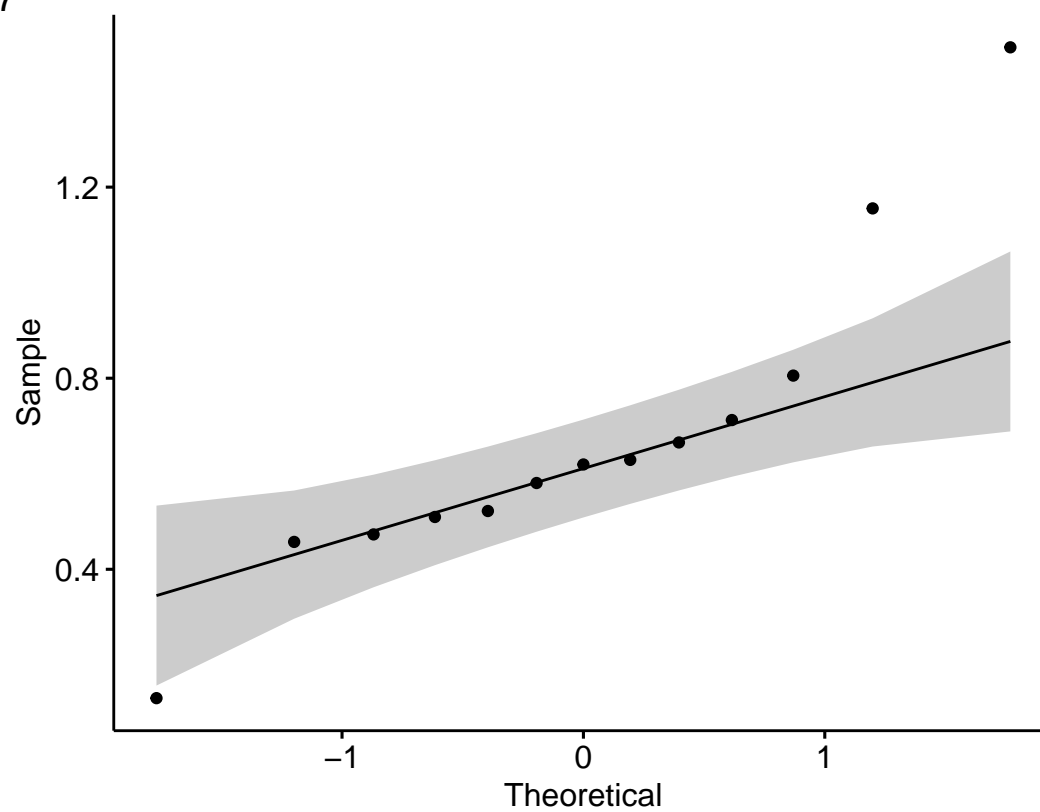
qq926

crisprA-26



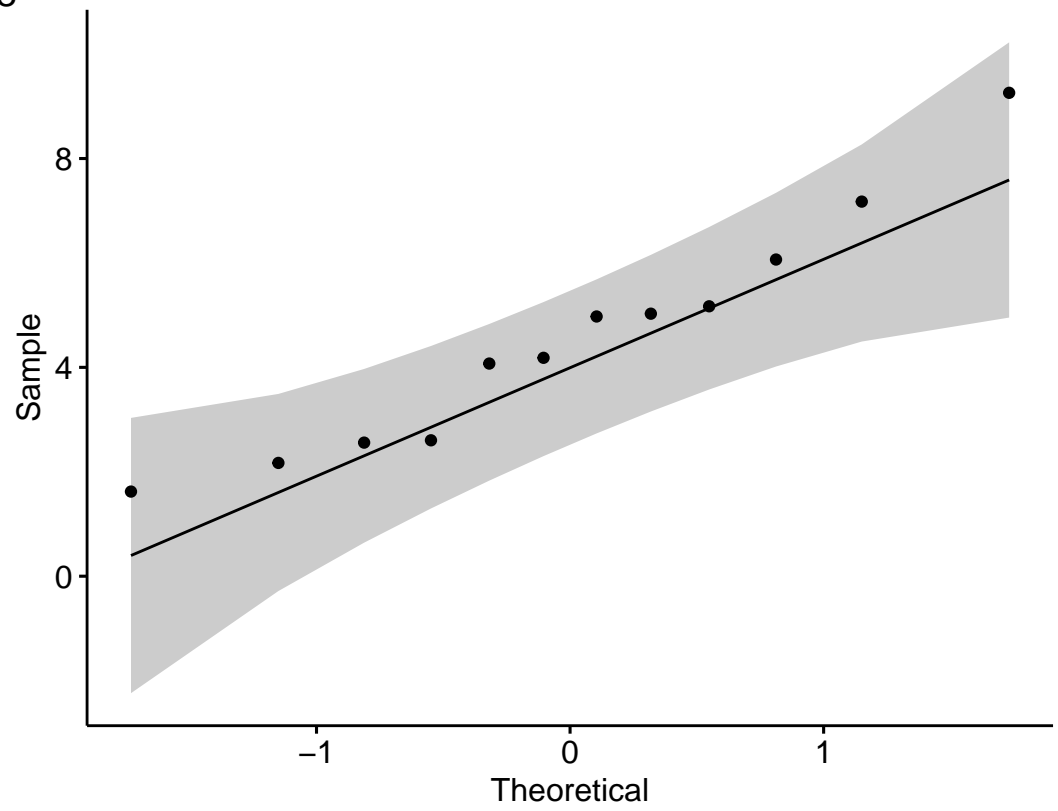
qq927

crisprA-27



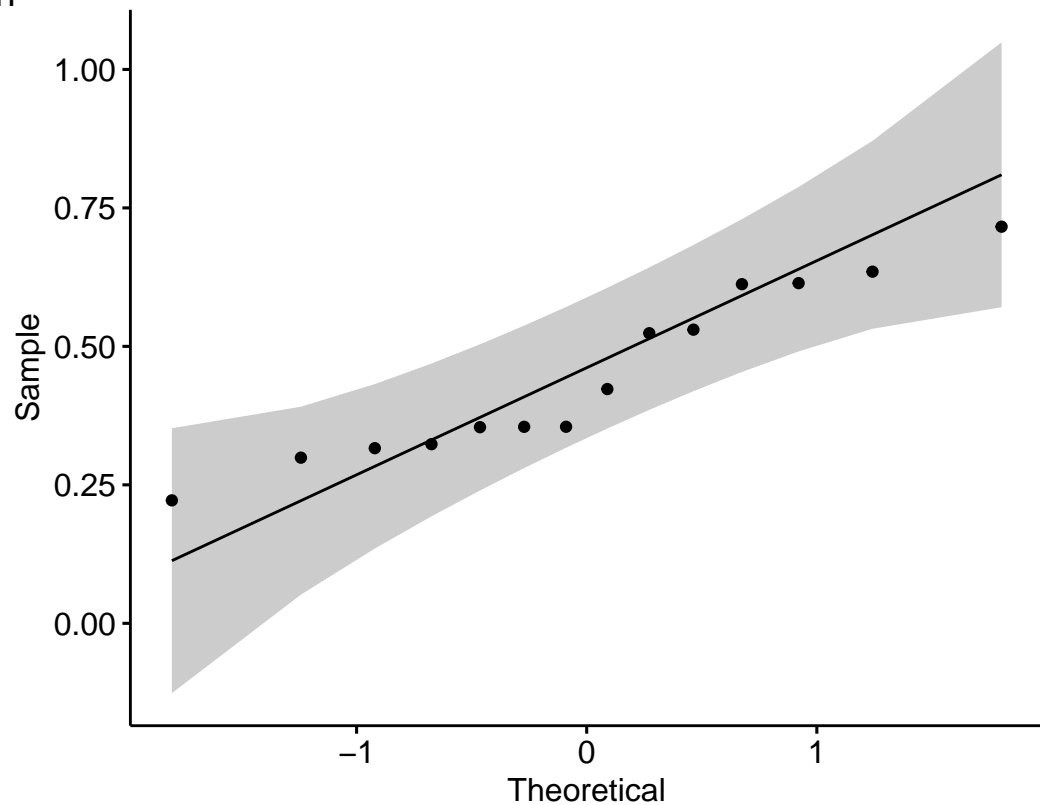
qq928

crisprA-28



qq931

crisprA-ctrl



```
# follow for the most part noramlity criteria
```

```
## Shapiro-wilk test
```

```
shapiro.test(t918_df$RFU) # p-value = 0.346
```

```
##
```

```
## Shapiro-Wilk normality test
```

```
##
```

```
## data: t918_df$RFU
```

```
## W = 0.91867, p-value = 0.346
```

```
shapiro.test(t919_df$RFU) # p-value = 0.3411
```

```
##
```

```
## Shapiro-Wilk normality test
```

```
##
```

```
## data: t919_df$RFU
```

```
## W = 0.90814, p-value = 0.3411
```

```
shapiro.test(t920_df$RFU) # p-value = 0.04243 ..tests not normal
```

```
##
```

```
## Shapiro-Wilk normality test
```

```
##
```

```
## data: t920_df$RFU
```

```
## W = 0.86318, p-value = 0.04243
```

```

shapiro.test(t929_df$RFU) # p-value = 0.9347

##
## Shapiro-Wilk normality test
##
## data:  t929_df$RFU
## W = 0.97245, p-value = 0.9347
shapiro.test(t923_df$RFU) # p-value = 0.9936

##
## Shapiro-Wilk normality test
##
## data:  t923_df$RFU
## W = 0.98741, p-value = 0.9936
shapiro.test(t924_df$RFU) # p-value = 0.1198

##
## Shapiro-Wilk normality test
##
## data:  t924_df$RFU
## W = 0.85991, p-value = 0.1198
shapiro.test(t925_df$RFU) # p-value = 0.7255

##
## Shapiro-Wilk normality test
##
## data:  t925_df$RFU
## W = 0.95323, p-value = 0.7255
shapiro.test(t930_df$RFU) # p-value = 0.01332    ..tests not normal

##
## Shapiro-Wilk normality test
##
## data:  t930_df$RFU
## W = 0.79698, p-value = 0.01332
shapiro.test(t926_df$RFU) # p-value = 2.379e-07    ..tests not normal

##
## Shapiro-Wilk normality test
##
## data:  t926_df$RFU
## W = 0.50735, p-value = 2.379e-07
shapiro.test(t927_df$RFU) # p-value = 0.06551    ..tests not normal

##
## Shapiro-Wilk normality test
##
## data:  t927_df$RFU
## W = 0.87728, p-value = 0.06551
shapiro.test(t928_df$RFU) # p-value = 0.6075

##

```

```

## Shapiro-Wilk normality test
##
## data:  t928_df$RFU
## W = 0.94797, p-value = 0.6075
shapiro.test(t931_df$RFU) # p-value = 0.237

##
## Shapiro-Wilk normality test
##
## data:  t931_df$RFU
## W = 0.92227, p-value = 0.237

# Calculating and adding adjusted p-values to the boxplots
#Source: https://github.com/kassambara/ggpubr/issues/65#issuecomment-407211245
#stat_pvalue_manual function: https://www.rdocumentation.org/packages/ggpubr/versions/0.2/topics/stat\_pvalue\_manual

# Pairwise wilcoxon rank sum test between groups
stat.test1 <- AIsomm1_df_long_red2 %>%
  group_by("ID") %>%
  wilcox_test(RFU ~ ID) %>%
  adjust_pvalue(method = 'holm') %>%
  mutate(y.position = 2) #, p = signif(p, digits = 4),
  #p.adj = signif(p.adj, digits = 6))

#adding adjusted p-value to boxplot
p4adjust <- p4p + stat_pvalue_manual(stat.test1, label = "p = {p.adj}")

## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position

stat.test2 <- AIsomm2_df_long_red2 %>%
  group_by("ID") %>%
  wilcox_test(RFU ~ ID) %>%
  adjust_pvalue(method = 'holm') %>%
  mutate(y.position = 2) #, p = signif(p, digits = 4),
  #p.adj = signif(p.adj, digits = 6))

p5adjust <- p5p + stat_pvalue_manual(stat.test2, label = "p = {p.adj}")

## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position

stat.test3 <- AIsomm3_df_long_red2_mod %>%
  group_by("ID") %>%
  wilcox_test(RFU ~ ID) %>%
  adjust_pvalue(method = 'holm') %>%
  mutate(y.position = 7.5) #, p = signif(p, digits = 4),
  #p.adj = signif(p.adj, digits = 6))

p6adjust <- p6p + stat_pvalue_manual(stat.test3, label = "p = {p.adj}")

## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position

# comparing means of controls 9-29, 9-30, 9-31
stat.testCtrl <- tctrl_df %>%
  group_by("ID") %>%
  kruskal_test(RFU ~ ID) %>%
  #adjust_pvalue(method = 'holm') %>%

```



```
mutate(y.position = 7.5)#, p = signif(p, digits = 4),
#p.adj = signif(p.adj, digits = 6))

# Output: comparing means within CRISPR-groups (wilcoxon)
stat.test1

## # A tibble: 6 x 9
##   `ID` .y. group1 group2 statistic      p p.adj p.adj.signif y.position
##   <chr> <chr> <chr> <chr>      <dbl> <dbl> <dbl> <chr>      <dbl>
## 1 ID RFU T9-18 T9-19      42 0.897      1 ns          2
## 2 ID RFU T9-18 T9-20      84 0.257      1 ns          2
## 3 ID RFU T9-18 T9-29      57 0.872      1 ns          2
## 4 ID RFU T9-19 T9-20      70 0.21       1 ns          2
## 5 ID RFU T9-19 T9-29      43 0.734      1 ns          2
## 6 ID RFU T9-20 T9-29      52 0.168      1 ns          2

stat.test2

## # A tibble: 6 x 9
##   `ID` .y. group1 group2 statistic      p p.adj p.adj.signif y.position
##   <chr> <chr> <chr> <chr>      <dbl> <dbl> <dbl> <chr>      <dbl>
## 1 ID RFU T9-23 T9-24      46 0.904      1 ns          2
## 2 ID RFU T9-23 T9-25      79 0.0251 0.151 ns          2
## 3 ID RFU T9-23 T9-30      64 0.557      1 ns          2
## 4 ID RFU T9-24 T9-25      52 0.139 0.556 ns          2
## 5 ID RFU T9-24 T9-30      39 0.965      1 ns          2
## 6 ID RFU T9-25 T9-30      20 0.0435 0.217 ns          2

stat.test3

## # A tibble: 6 x 9
##   `ID` .y. group1 group2 statistic      p p.adj p.adj.signif
##   <chr> <chr> <chr> <chr>      <dbl> <dbl> <dbl> <chr>
## 1 ID RFU T9-26 T9-27      5 1.90e-6 7.60e-6 ****
## 2 ID RFU T9-26 T9-28      0 1.42e-8 8.52e-8 ****
## 3 ID RFU T9-26 T9-31      62 8.85e-3 1.77e-2 *
## 4 ID RFU T9-27 T9-28      0 3.09e-6 9.27e-6 ****
## 5 ID RFU T9-27 T9-31     106 3.58e-2 3.58e-2 *
## 6 ID RFU T9-28 T9-31     168 2.07e-7 1.03e-6 ****
## # ... with 1 more variable: y.position <dbl>

# Output: comparing means of controls 9-29, 9-30, 9-31
stat.testCtrl

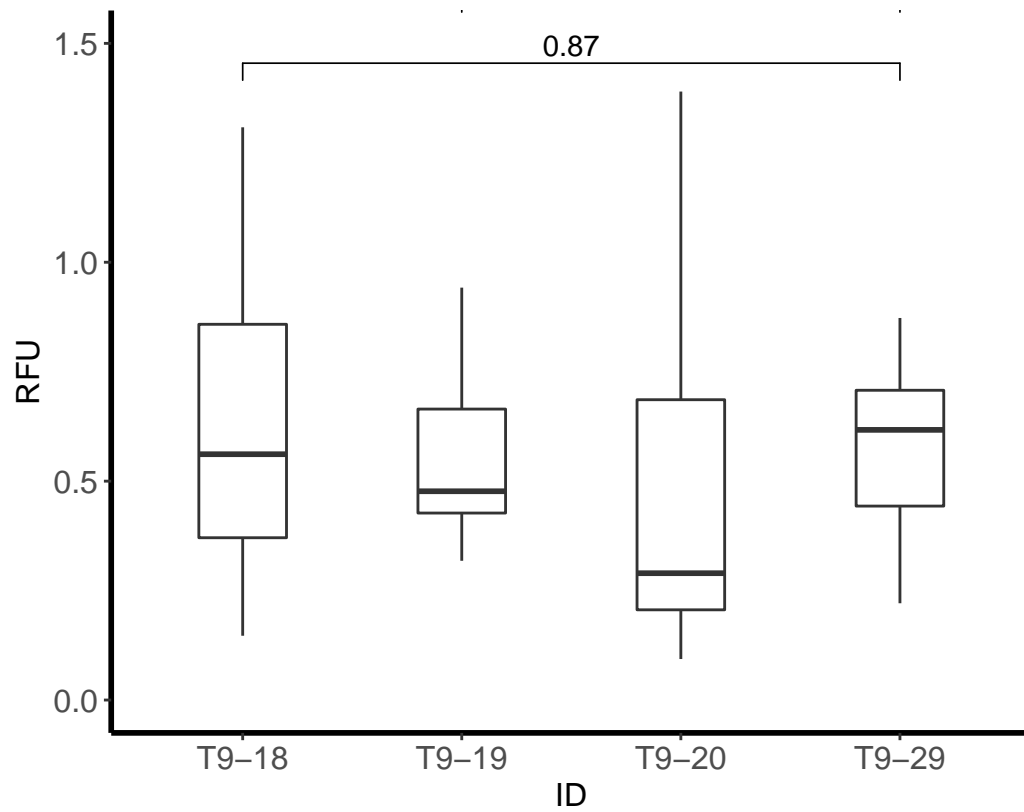
## # A tibble: 1 x 7
##   `ID` .y. statistic df      p method y.position
##   <chr> <chr>      <dbl> <int> <dbl> <chr>      <dbl>
## 1 ID RFU      5.69      2 0.0582 Kruskal-Wallis      7.5

kruskal.test(RFU ~ ID, data = tctrl_df)

##
## Kruskal-Wallis rank sum test
##
## data: RFU by ID
## Kruskal-Wallis chi-squared = 5.6882, df = 2, p-value = 0.05819
```

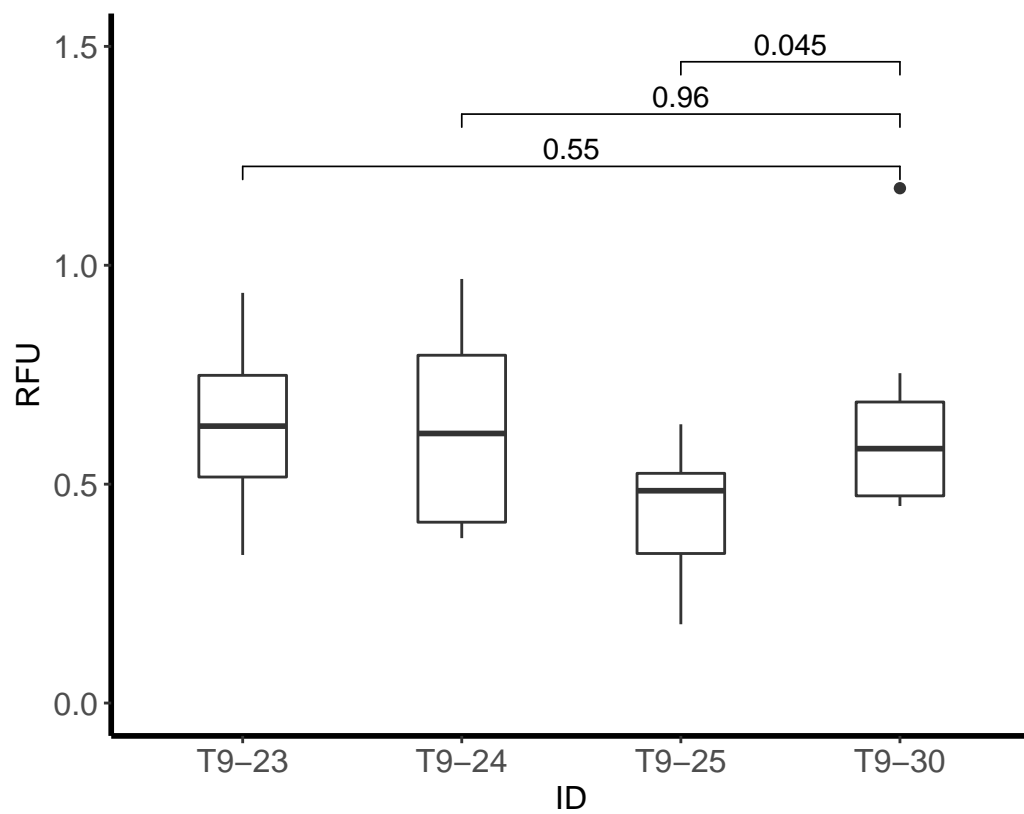
p4adjust

GFP vs. RFP



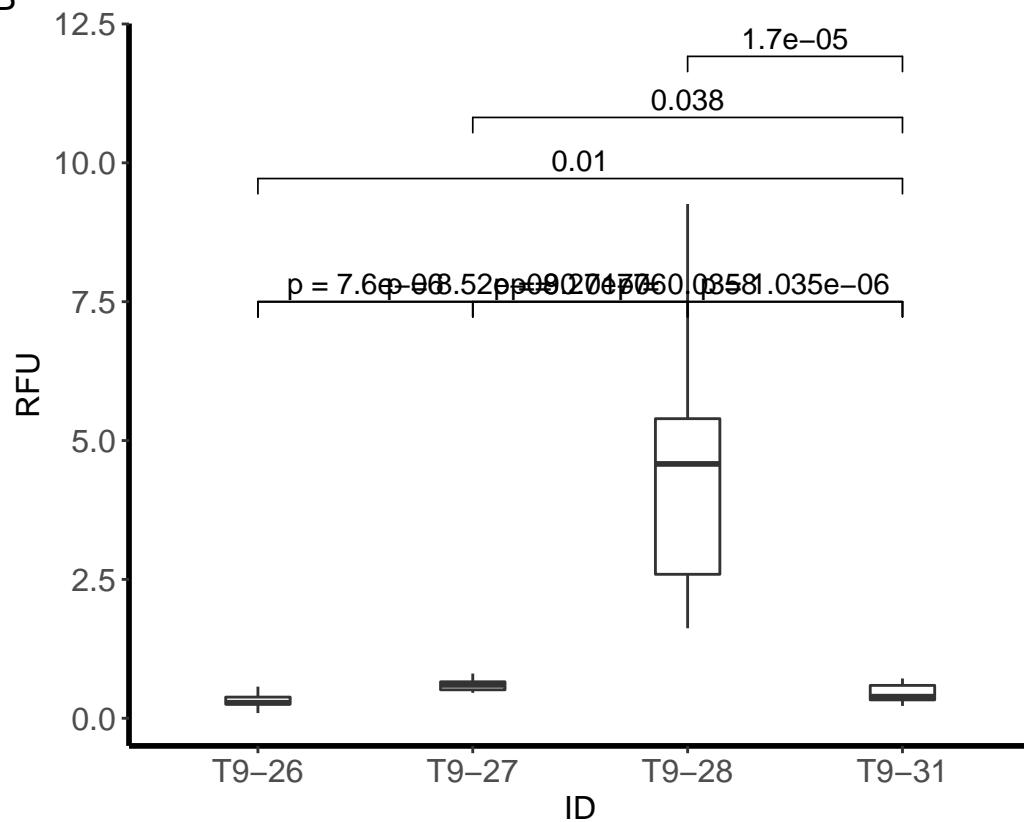
p5adjust

GFP vs. RFP



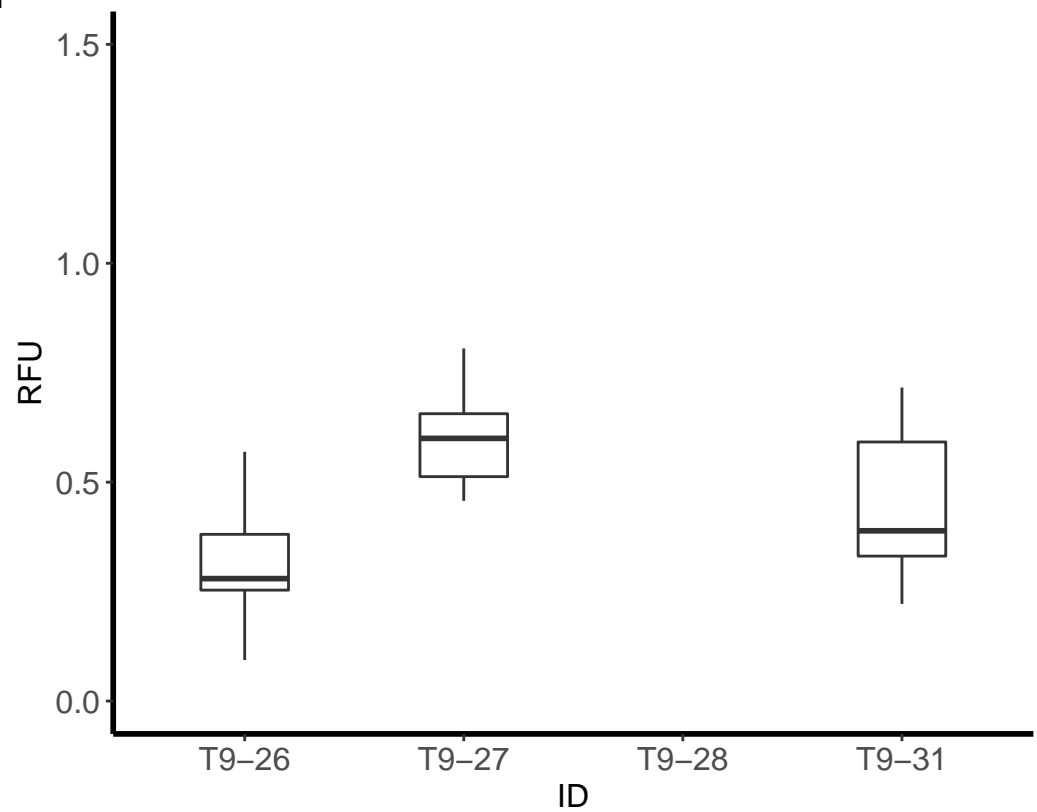
p6adjust

GFP vs. RFP



p6zoom

GFP vs. RFP



```
# RA test von Mau
#install.packages("RATest")

#male<-rnorm(50,1,1)
#female<-rnorm(50,1,2)
#dta<-data.frame(group=c(rep(1,50),rep(2,50)),outcome=c(male,female))
#rpt.var<-RPT(dta$outcome~dta$group,test="variances")
#summary(rpt.var)
#rpt.var$pvalue

#rpt.ben <-
```