

RdatAI190410

Data from Agroinfiltration 190410, measured 5 dpi 190415. Strong hypersensitive response in 9-18, 9-19, 9-20 treatments. Treatments with fewest signs were sampled and measured.

```
# EC36309data1 <- read.table("190410Summary.csv", header=TRUE,
#                               sep=",") # fluorescence results AI190410 36309 + co-infiltrations
# attach(EC36309data1)
#
# boxplot(GFP~ID, data=EC36309data1, main="GFP",horizontal=TRUE, varwidth=TRUE, xlab="RFU", ylab="Treatments")
# boxplot(RFP~ID, data=EC36309data1, main="RFP", horizontal=TRUE, varwidth=TRUE, xlab="RFU", ylab="Treatments")
# boxplot(GFP.RFP~ID, data=EC36309data1, main="GFP to RFP", horizontal=TRUE, varwidth=TRUE, xlab="ratio", ylab="Treatments")
#
# sum(with(EC36309data1, ID=="9"))
# sum(with(EC36309data1, ID=="9.18"))
# sum(with(EC36309data1, ID=="9.19"))
# sum(with(EC36309data1, ID=="9.2"))
# sum(with(EC36309data1, ID=="9.25"))
# sum(with(EC36309data1, ID=="9.28"))
# summary(EC36309data1)
```

Data from 9-18, 9-19, 9-20 and 9-25 should not be trusted, hypersensitive response in most samples most likely skewed RFU data!!

Corrected data: 36309 w/ GFP signal lower than 3.5 RFU were deleted from dataset:

```
# EC36309data2 <- read.table("190410SummaryCorr.csv", header=TRUE,
#                               sep=",") # fluorescence results AI190410 36309 (co-)infiltrations corrected
# attach(EC36309data2)
#
# boxplot(GFP~ID, data=EC36309data2, main="GFP",horizontal=TRUE, varwidth=TRUE, xlab="RFU", ylab="Treatments")
# boxplot(RFP~ID, data=EC36309data2, main="RFP", horizontal=TRUE, varwidth=TRUE, xlab="RFU", ylab="Treatments")
# boxplot(GFP.RFP~ID, data=EC36309data2, main="GFP to RFP", horizontal=TRUE, varwidth=TRUE, xlab="ratio", ylab="Treatments")
#
# sum(with(EC36309data2, ID=="9"))
```

Do not use for data analysis!

Data from 7, 7-21, 7-22 co-infiltrations:

```
library(ggplot2)
library(reshape2)

#Combined data from AI190330 (n=6) and AI190410 (n=5)

EC36307_df <- read.table("190410-2.csv", header=TRUE,
                        sep=",")
attach(EC36307_df)

# changing the df to long format

EC36307_df_long <- melt(EC36307_df,
                        id.vars=c("ID"),
                        measure.vars=c("GFP", "RFP", "GFP.RFP" ),
```

```

        variable.name="Channel",
        value.name="RFU")

attach(EC36307_df_long)

## The following object is masked from EC36307_df:
##
##      ID
# taking out GFP/RFP ratios
EC36307_df_long_red <- EC36307_df_long[-c(67:99), ]
EC36307_df_long_red

```

```

##      ID Channel      RFU
## 1  T7-21      GFP    5.057
## 2  T7-21      GFP    1.356
## 3  T7-21      GFP    3.078
## 4  T7-21      GFP    3.530
## 5  T7-21      GFP    1.102
## 6  T7-21      GFP   17.228
## 7  T7-21      GFP   28.226
## 8  T7-21      GFP   25.809
## 9  T7-21      GFP   25.125
## 10 T7-21      GFP   12.576
## 11 T7-21      GFP   17.329
## 12 T7-22      GFP   27.255
## 13 T7-22      GFP   31.973
## 14 T7-22      GFP   29.393
## 15 T7-22      GFP   44.241
## 16 T7-22      GFP  125.598
## 17 T7-22      GFP  142.912
## 18 T7-22      GFP  163.303
## 19 T7-22      GFP  176.488
## 20 T7-22      GFP   95.409
## 21 T7-22      GFP  294.041
## 22 T7-22      GFP   50.330
## 23   T7      GFP  100.750
## 24   T7      GFP   57.142
## 25   T7      GFP   96.495
## 26   T7      GFP   78.805
## 27   T7      GFP  134.626
## 28   T7      GFP  958.945
## 29   T7      GFP  619.466
## 30   T7      GFP  440.280
## 31   T7      GFP 1589.265
## 32   T7      GFP 1075.704
## 33   T7      GFP  799.353
## 34 T7-21      RFP   12.968
## 35 T7-21      RFP    6.903
## 36 T7-21      RFP   10.138
## 37 T7-21      RFP   12.420
## 38 T7-21      RFP    3.581
## 39 T7-21      RFP   22.571
## 40 T7-21      RFP   17.927

```

```
## 41 T7-21 RFP 30.915
## 42 T7-21 RFP 34.951
## 43 T7-21 RFP 31.410
## 44 T7-21 RFP 19.209
## 45 T7-22 RFP 4.818
## 46 T7-22 RFP 4.884
## 47 T7-22 RFP 3.517
## 48 T7-22 RFP 4.866
## 49 T7-22 RFP 10.133
## 50 T7-22 RFP 6.241
## 51 T7-22 RFP 10.436
## 52 T7-22 RFP 10.193
## 53 T7-22 RFP 8.252
## 54 T7-22 RFP 12.871
## 55 T7-22 RFP 4.562
## 56 T7 RFP 33.938
## 57 T7 RFP 15.006
## 58 T7 RFP 25.411
## 59 T7 RFP 29.170
## 60 T7 RFP 44.675
## 61 T7 RFP 129.432
## 62 T7 RFP 158.553
## 63 T7 RFP 229.420
## 64 T7 RFP 638.882
## 65 T7 RFP 392.128
## 66 T7 RFP 237.850
```

```
# taking out GFP, RFP single values
EC36307_df_long_red2 <- EC36307_df_long[-c(1:66), ]
EC36307_df_long_red2
```

```
##      ID Channel  RFU
## 67 T7-21 GFP.RFP 0.390
## 68 T7-21 GFP.RFP 0.196
## 69 T7-21 GFP.RFP 0.304
## 70 T7-21 GFP.RFP 0.284
## 71 T7-21 GFP.RFP 0.308
## 72 T7-21 GFP.RFP 1.310
## 73 T7-21 GFP.RFP 0.635
## 74 T7-21 GFP.RFP 1.198
## 75 T7-21 GFP.RFP 1.391
## 76 T7-21 GFP.RFP 2.498
## 77 T7-21 GFP.RFP 1.108
## 78 T7-22 GFP.RFP 5.657
## 79 T7-22 GFP.RFP 6.546
## 80 T7-22 GFP.RFP 8.357
## 81 T7-22 GFP.RFP 9.092
## 82 T7-22 GFP.RFP 12.395
## 83 T7-22 GFP.RFP 22.899
## 84 T7-22 GFP.RFP 15.648
## 85 T7-22 GFP.RFP 17.315
## 86 T7-22 GFP.RFP 11.562
## 87 T7-22 GFP.RFP 22.845
## 88 T7-22 GFP.RFP 11.032
## 89 T7 GFP.RFP 2.969
```

```
## 90    T7 GFP.RFP 3.808
## 91    T7 GFP.RFP 3.797
## 92    T7 GFP.RFP 2.702
## 93    T7 GFP.RFP 3.013
## 94    T7 GFP.RFP 7.409
## 95    T7 GFP.RFP 3.907
## 96    T7 GFP.RFP 1.919
## 97    T7 GFP.RFP 2.488
## 98    T7 GFP.RFP 2.743
## 99    T7 GFP.RFP 3.361
```

```
# taking out treatment 7
EC36307mod_df <- read.table("190410-2mod.csv", header=TRUE,
                           sep=",")
attach(EC36307mod_df)
```

```
## The following object is masked from EC36307_df_long:
```

```
##
```

```
##      ID
```

```
## The following objects are masked from EC36307_df:
```

```
##
```

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

```
EC36307mod_df_long <- melt(EC36307mod_df,
                           id.vars=c("ID"),
                           measure.vars=c("GFP", "RFP", "GFP.RFP" ),
                           variable.name="Channel",
                           value.name="RFU")
attach(EC36307mod_df_long)
```

```
## The following object is masked from EC36307mod_df:
```

```
##
```

```
##      ID
```

```
## The following objects are masked from EC36307_df_long:
```

```
##
```

```
##      Channel, ID, RFU
```

```
## The following object is masked from EC36307_df:
```

```
##
```

```
##      ID
```

```
# taking out GFP/RFP ratios
```

```
EC36307mod_df_long_red <- EC36307mod_df_long[-c(45:66), ]
EC36307mod_df_long_red
```

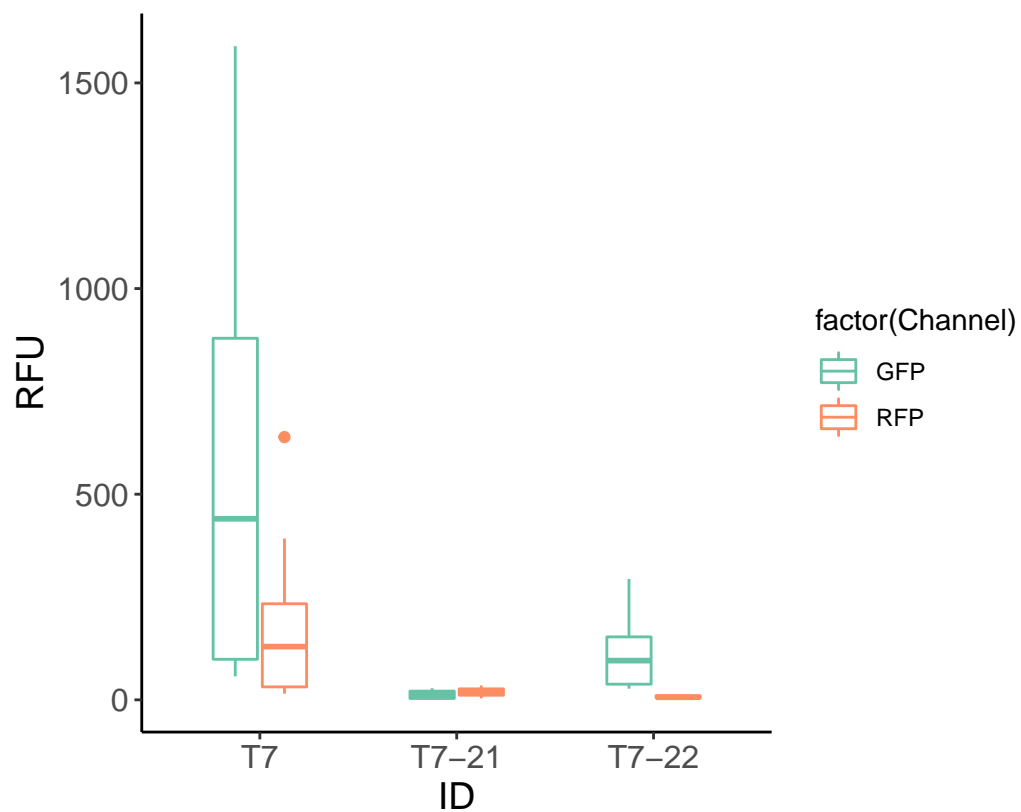
```
##      ID Channel      RFU
## 1  T7-21      GFP  5.057
## 2  T7-21      GFP  1.356
## 3  T7-21      GFP  3.078
## 4  T7-21      GFP  3.530
## 5  T7-21      GFP  1.102
## 6  T7-22      GFP 50.330
## 7  T7-21      GFP 17.228
## 8  T7-21      GFP 28.226
## 9  T7-21      GFP 25.809
## 10 T7-21      GFP 25.125
```

```
## 11 T7-21      GFP  12.576
## 12 T7-21      GFP  17.329
## 13 T7-22      GFP  27.255
## 14 T7-22      GFP  31.973
## 15 T7-22      GFP  29.393
## 16 T7-22      GFP  44.241
## 17 T7-22      GFP 125.598
## 18 T7-22      GFP 142.912
## 19 T7-22      GFP 163.303
## 20 T7-22      GFP 176.488
## 21 T7-22      GFP  95.409
## 22 T7-22      GFP 294.041
## 23 T7-21      RFP  12.968
## 24 T7-21      RFP   6.903
## 25 T7-21      RFP  10.138
## 26 T7-21      RFP  12.420
## 27 T7-21      RFP   3.581
## 28 T7-22      RFP   4.562
## 29 T7-21      RFP  22.571
## 30 T7-21      RFP  17.927
## 31 T7-21      RFP  30.915
## 32 T7-21      RFP  34.951
## 33 T7-21      RFP  31.410
## 34 T7-21      RFP  19.209
## 35 T7-22      RFP   4.818
## 36 T7-22      RFP   4.884
## 37 T7-22      RFP   3.517
## 38 T7-22      RFP   4.866
## 39 T7-22      RFP  10.133
## 40 T7-22      RFP   6.241
## 41 T7-22      RFP  10.436
## 42 T7-22      RFP  10.193
## 43 T7-22      RFP   8.252
## 44 T7-22      RFP  12.871
```

Boxplots:

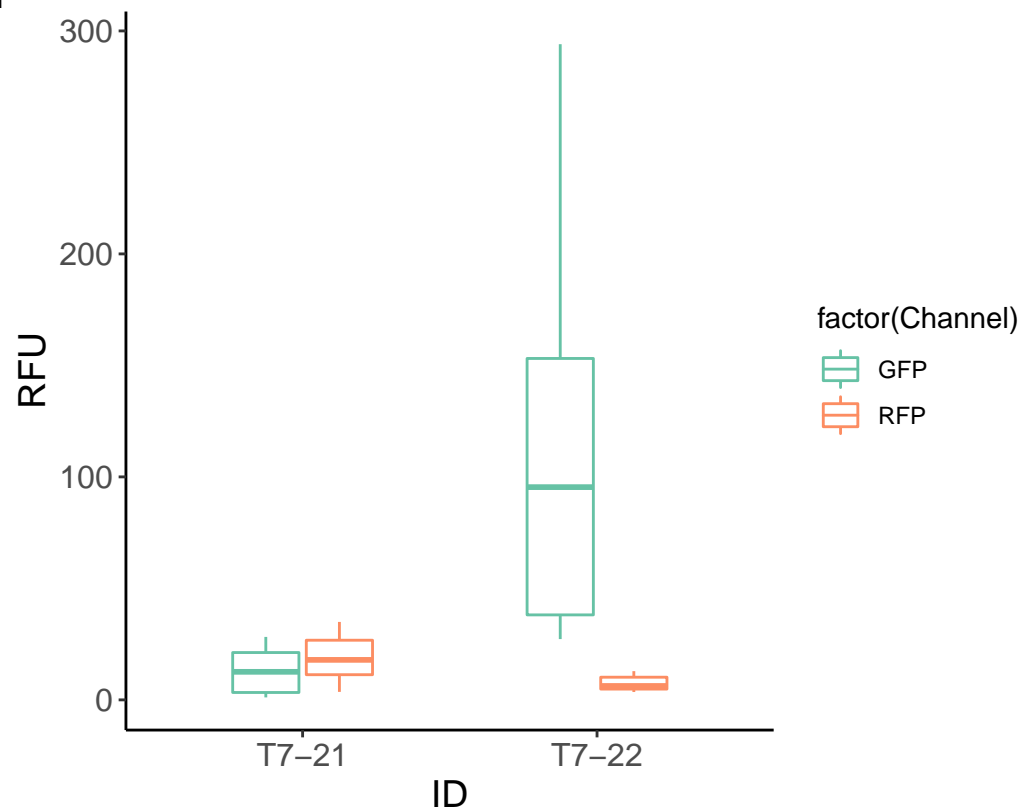
```
# GFP vs. RFP
ggplot(data=EC36307_df_long_red, aes(x=ID,y=RFU, colour=factor(Channel))) +
  geom_boxplot(position=position_dodge2(width=0.5), width=0.5) +
  labs(tag = "GFP vs. RFP") +
  scale_color_brewer(palette = "Set2") +
  theme_classic() +
#facet_wrap(~Channel) +
#coord_fixed(ratio = 0.0015, xlim = NULL, ylim = NULL) +
  theme(#axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 14),
        axis.title.y = element_text(size = 14),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12)
  )
```

GFP vs. RFP



```
#Plot of only 7-21, 7-22
ggplot(data=EC36307mod_df_long_red, aes(x=ID,y=RFU, colour=factor(Channel))) +
  geom_boxplot(position=position_dodge2(width=0.5), width=0.5) +
  labs(tag = "GFP vs. RFP") +
  scale_color_brewer(palette = "Set2") +
  theme_classic() +
  #facet_wrap(~Channel) +
  theme(#axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 14),
        axis.title.y = element_text(size = 14),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12)
  )
```

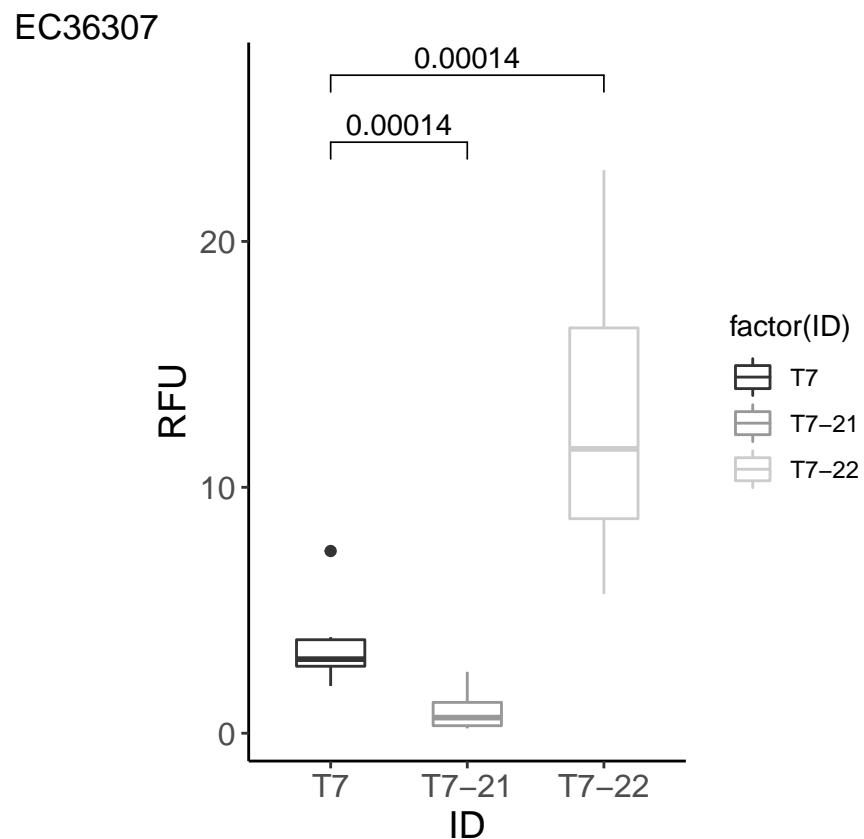
GFP vs. RFP



```
library(reshape2)
library(ggpubr)

## Loading required package: magrittr
my_comparisons <- list( c("T7", "T7-21"), c("T7", "T7-22"))

#GFP/RFP ratios
ggplot(data=EC36307_df_long_red2, aes(x=ID,y=RFU, colour=factor(ID))) +
  geom_boxplot(position=position_dodge(width=0.5), width=0.5) +
  labs(tag = "EC36307") +
  scale_color_grey() +
  theme_classic() +
  coord_fixed(ratio = 0.18, xlim = NULL, ylim = NULL) +
  #stat_compare_means(method = "t.test", label = "p.format") +
  theme(#axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 14),
        axis.title.y = element_text(size = 14),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12)
  ) +
  stat_compare_means(method = "wilcox.test", label = "p.format", comparisons = my_comparisons)
```



Paired boxplots:

```
# paired boxplots
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
```

```
t7_gr_df <- filter(EC36307_df_long_red, ID == "T7")
t7_gr_df
```

```
##   ID Channel    RFU
## 1  T7     GFP 100.750
## 2  T7     GFP  57.142
## 3  T7     GFP  96.495
## 4  T7     GFP  78.805
## 5  T7     GFP 134.626
## 6  T7     GFP 958.945
## 7  T7     GFP 619.466
## 8  T7     GFP 440.280
## 9  T7     GFP 1589.265
```



```
## 10 T7      GFP 1075.704
## 11 T7      GFP  799.353
## 12 T7      RFP   33.938
## 13 T7      RFP   15.006
## 14 T7      RFP   25.411
## 15 T7      RFP   29.170
## 16 T7      RFP   44.675
## 17 T7      RFP  129.432
## 18 T7      RFP  158.553
## 19 T7      RFP  229.420
## 20 T7      RFP  638.882
## 21 T7      RFP  392.128
## 22 T7      RFP  237.850
```

```
t721_gr_df <- filter(EC36307_df_long_red, ID == "T7-21")
t721_gr_df
```

```
##      ID Channel    RFU
## 1  T7-21      GFP   5.057
## 2  T7-21      GFP   1.356
## 3  T7-21      GFP   3.078
## 4  T7-21      GFP   3.530
## 5  T7-21      GFP   1.102
## 6  T7-21      GFP  17.228
## 7  T7-21      GFP  28.226
## 8  T7-21      GFP  25.809
## 9  T7-21      GFP  25.125
## 10 T7-21      GFP  12.576
## 11 T7-21      GFP  17.329
## 12 T7-21      RFP  12.968
## 13 T7-21      RFP   6.903
## 14 T7-21      RFP  10.138
## 15 T7-21      RFP  12.420
## 16 T7-21      RFP   3.581
## 17 T7-21      RFP  22.571
## 18 T7-21      RFP  17.927
## 19 T7-21      RFP  30.915
## 20 T7-21      RFP  34.951
## 21 T7-21      RFP  31.410
## 22 T7-21      RFP  19.209
```

```
t722_gr_df <- filter(EC36307_df_long_red, ID == "T7-22")
t722_gr_df
```

```
##      ID Channel    RFU
## 1  T7-22      GFP  27.255
## 2  T7-22      GFP  31.973
## 3  T7-22      GFP  29.393
## 4  T7-22      GFP  44.241
## 5  T7-22      GFP 125.598
## 6  T7-22      GFP 142.912
## 7  T7-22      GFP 163.303
## 8  T7-22      GFP 176.488
## 9  T7-22      GFP  95.409
## 10 T7-22      GFP 294.041
```

```
## 11 T7-22      GFP  50.330
## 12 T7-22      RFP   4.818
## 13 T7-22      RFP   4.884
## 14 T7-22      RFP   3.517
## 15 T7-22      RFP   4.866
## 16 T7-22      RFP  10.133
## 17 T7-22      RFP   6.241
## 18 T7-22      RFP  10.436
## 19 T7-22      RFP  10.193
## 20 T7-22      RFP   8.252
## 21 T7-22      RFP  12.871
## 22 T7-22      RFP   4.562
```

```
library(ggpubr)
```

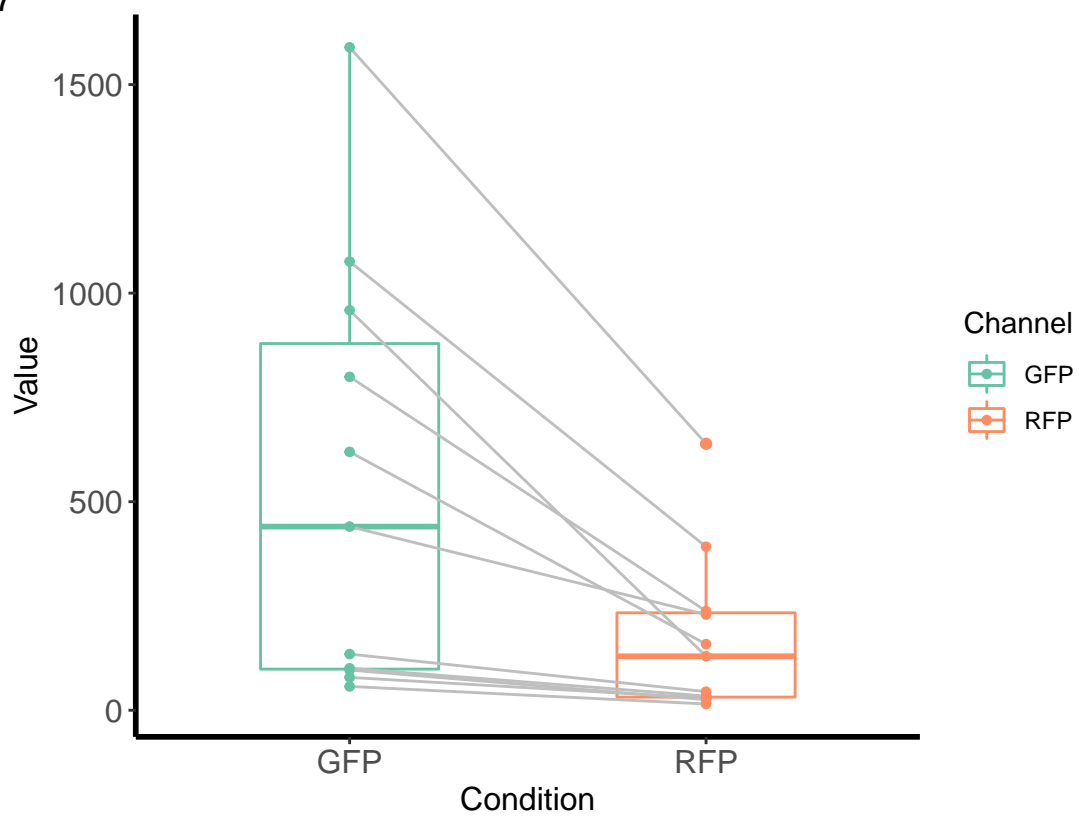
```
pt7 <- ggpaired(t7_gr_df, x = "Channel", y = "RFU", color = "Channel", width = 0.5, line.color = "gray",
  #geom_boxplot(position=position_dodge(width=0.4), width=0.4) +
  labs(tag = "EC36307") +
  scale_colour_brewer(palette = "Set2") +
  theme_classic() +
  #coord_fixed(ratio = 0.25, xlim = NULL, 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)
  ) #+
  #stat_compare_means(method = "t.test", label = "p.format", paired = TRUE)
```

```
pt721 <- ggpaired(t721_gr_df, x = "Channel", y = "RFU", color = "Channel", width = 0.5, line.color = "gray",
  #geom_boxplot(position=position_dodge(width=0.4), width=0.4) +
  labs(tag = "EC36307-21") +
  scale_colour_brewer(palette = "Set2") +
  theme_classic() +
  #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)
  )
```

```
pt722 <- ggpaired(t722_gr_df, x = "Channel", y = "RFU", color = "Channel", width = 0.5, line.color = "gray",
  #geom_boxplot(position=position_dodge(width=0.4), width=0.4) +
  labs(tag = "EC36307-22") +
  scale_colour_brewer(palette = "Set2") +
  theme_classic() +
  #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)
  )
```

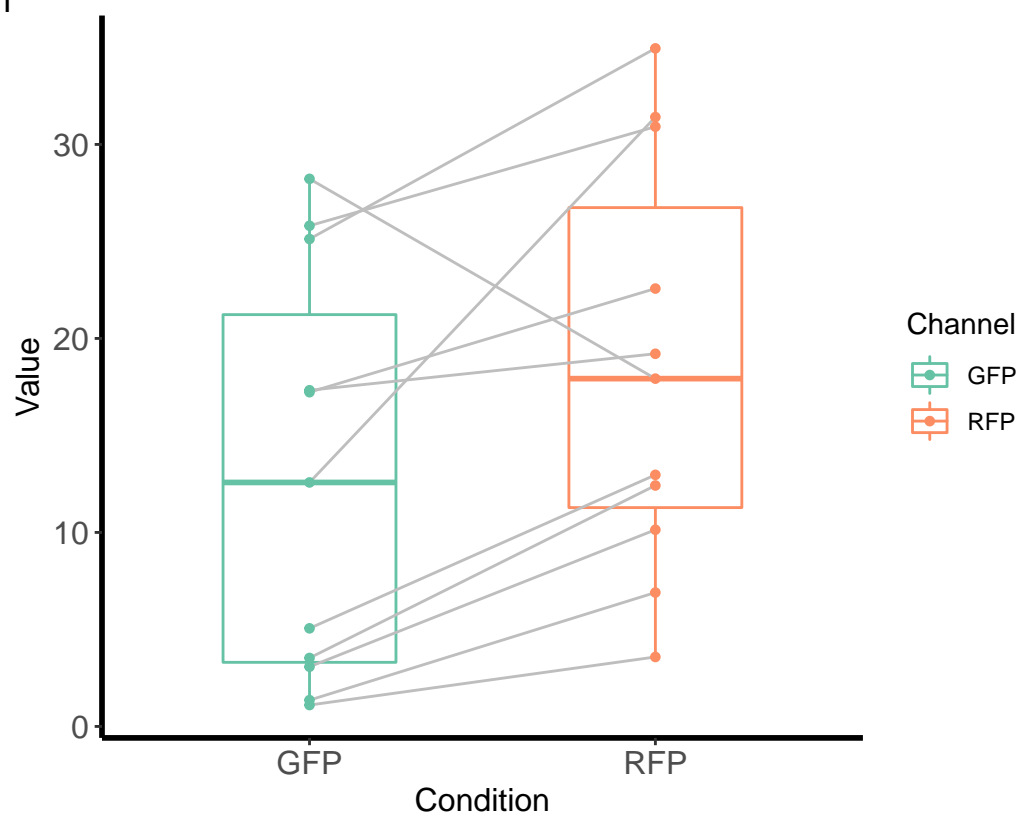
pt7

EC36307



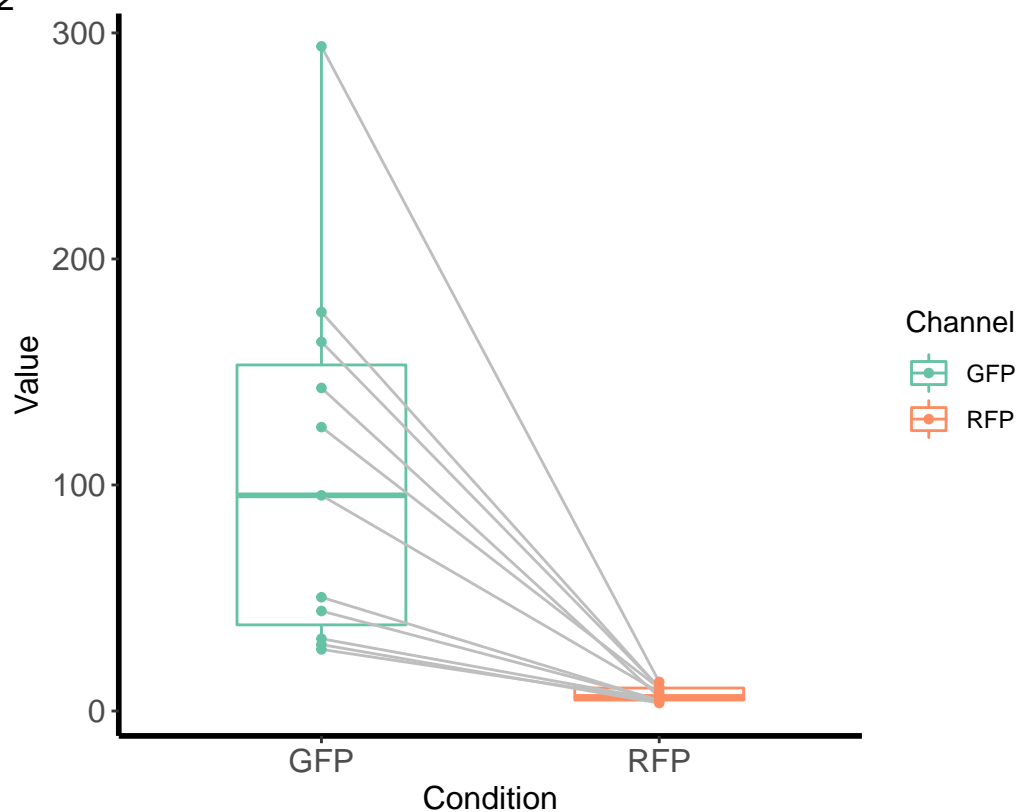
pt721

EC36307-21



pt722

EC36307-22



Statistical testing:

Comparison of Means:

Normality test: qq-plots and Shapiro-Wilk

Separating all three datasets

EC36307_df_1sam7 <- EC36307_df_long_red2[-c(1:22),] # 7

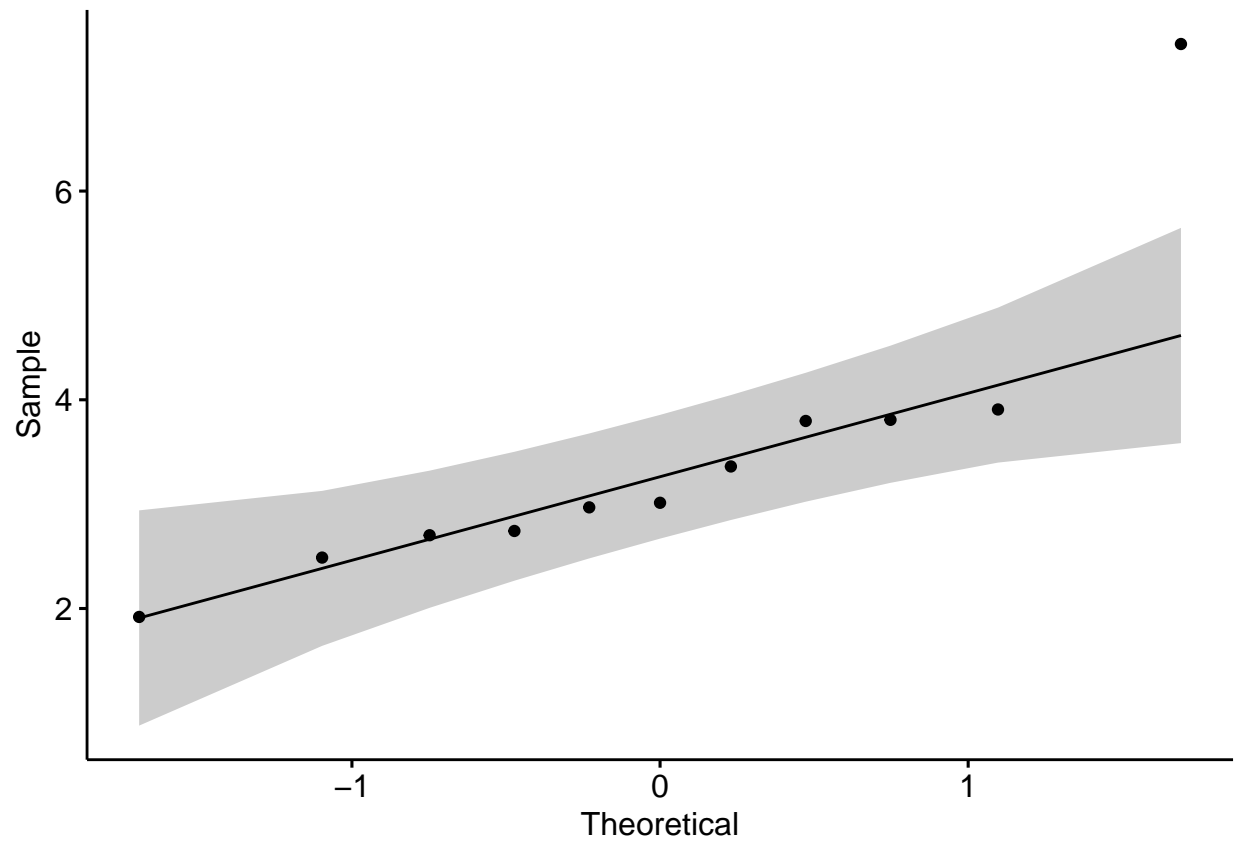
EC36307_df_1sam721 <- EC36307_df_long_red2[-c(12:33),] # 7-21

EC36307_df_1sam722 <- EC36307_df_long_red2[-c(1:11,23:33),] #7-22

library(ggpubr)

qq7 <- ggqqplot(data=EC36307_df_1sam7\$RFU)

qq7

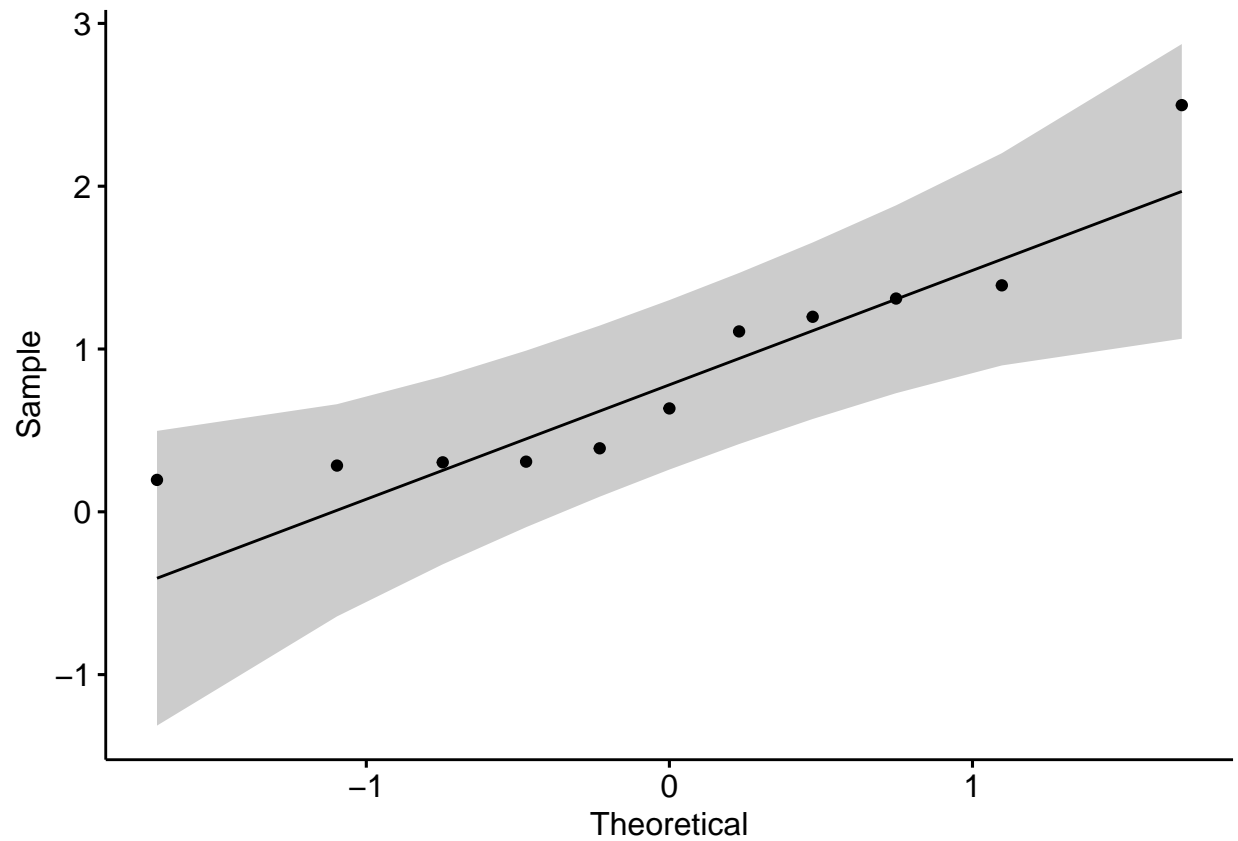


```
shapiro.test(EC36307_df_1sam7$RFU)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  EC36307_df_1sam7$RFU
## W = 0.7558, p-value = 0.002475
```

```
# ̳ does not appear to be normally distributed
```

```
qq721 <- ggqqplot(data=EC36307_df_1sam721$RFU)
qq721
```

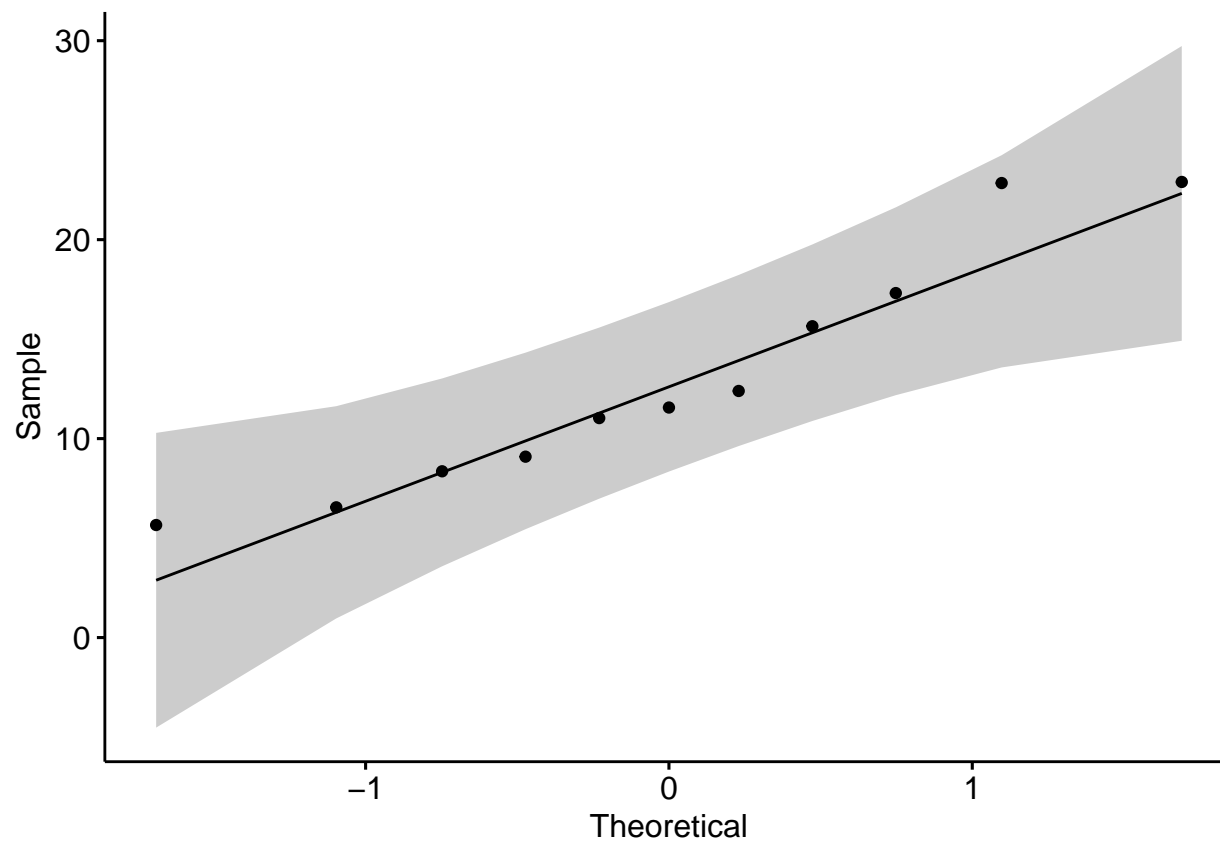


```
shapiro.test(EC36307_df_1sam721$RFU)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  EC36307_df_1sam721$RFU
## W = 0.84984, p-value = 0.04247
```

```
# 7-21 does not appear to be normally distributed
```

```
qq722 <- ggqqplot(data=EC36307_df_1sam722$RFU)
qq722
```



```
shapiro.test(EC36307_df_1sam722$RFU)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  EC36307_df_1sam722$RFU
## W = 0.91377, p-value = 0.2701
# 7-22 possibly normally distributed
```

```
## Testing for equal variances:
var(EC36307_df_1sam7$RFU)
```

```
## [1] 2.088761
```

```
var(EC36307_df_1sam721$RFU)
```

```
## [1] 0.4998904
```

```
var(EC36307_df_1sam722$RFU)
```

```
## [1] 35.92449
```

```
# reducing df to two sample-df:
```

```
EC36307_df_2sam1 <- EC36307_df_long_red2[-c(1:11), ] # 7, 7-22
EC36307_df_2sam1
```

```
##      ID Channel   RFU
## 78 T7-22 GFP.RFP  5.657
```



```
## 79 T7-22 GFP.RFP 6.546
## 80 T7-22 GFP.RFP 8.357
## 81 T7-22 GFP.RFP 9.092
## 82 T7-22 GFP.RFP 12.395
## 83 T7-22 GFP.RFP 22.899
## 84 T7-22 GFP.RFP 15.648
## 85 T7-22 GFP.RFP 17.315
## 86 T7-22 GFP.RFP 11.562
## 87 T7-22 GFP.RFP 22.845
## 88 T7-22 GFP.RFP 11.032
## 89 T7 GFP.RFP 2.969
## 90 T7 GFP.RFP 3.808
## 91 T7 GFP.RFP 3.797
## 92 T7 GFP.RFP 2.702
## 93 T7 GFP.RFP 3.013
## 94 T7 GFP.RFP 7.409
## 95 T7 GFP.RFP 3.907
## 96 T7 GFP.RFP 1.919
## 97 T7 GFP.RFP 2.488
## 98 T7 GFP.RFP 2.743
## 99 T7 GFP.RFP 3.361
```

```
EC36307_df_2sam2 <- EC36307_df_long_red2[-c(12:22), ] # 7, 7-21
EC36307_df_2sam2
```

```
## ID Channel RFU
## 67 T7-21 GFP.RFP 0.390
## 68 T7-21 GFP.RFP 0.196
## 69 T7-21 GFP.RFP 0.304
## 70 T7-21 GFP.RFP 0.284
## 71 T7-21 GFP.RFP 0.308
## 72 T7-21 GFP.RFP 1.310
## 73 T7-21 GFP.RFP 0.635
## 74 T7-21 GFP.RFP 1.198
## 75 T7-21 GFP.RFP 1.391
## 76 T7-21 GFP.RFP 2.498
## 77 T7-21 GFP.RFP 1.108
## 89 T7 GFP.RFP 2.969
## 90 T7 GFP.RFP 3.808
## 91 T7 GFP.RFP 3.797
## 92 T7 GFP.RFP 2.702
## 93 T7 GFP.RFP 3.013
## 94 T7 GFP.RFP 7.409
## 95 T7 GFP.RFP 3.907
## 96 T7 GFP.RFP 1.919
## 97 T7 GFP.RFP 2.488
## 98 T7 GFP.RFP 2.743
## 99 T7 GFP.RFP 3.361
```

```
# F-test for equal variances:
```

```
res.ftest1 <- var.test(RFU ~ ID, data = EC36307_df_2sam1)
res.ftest2 <- var.test(RFU ~ ID, data = EC36307_df_2sam2)
```

```
res.ftest1
```

```
##
```

```

## F test to compare two variances
##
## data: RFU by ID
## F = 0.058143, num df = 10, denom df = 10, p-value = 0.0001047
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
## 0.01564335 0.21610574
## sample estimates:
## ratio of variances
## 0.05814308
res.ftest2

##
## F test to compare two variances
##
## data: RFU by ID
## F = 4.1784, num df = 10, denom df = 10, p-value = 0.03376
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
## 1.124205 15.530381
## sample estimates:
## ratio of variances
## 4.178437

# -> p-values indicate unequal variances. Normality and equal variances not given. Use non-parametric t

## Wilcoxon rank-sum test:

wilcox.test(RFU ~ ID, data=EC36307_df_2sam1, alternative = c("two.sided"))

##
## Wilcoxon rank sum test
##
## data: RFU by ID
## W = 2, p-value = 1.134e-05
## alternative hypothesis: true location shift is not equal to 0
wilcox.test(RFU ~ ID, data=EC36307_df_2sam2, alternative = c("two.sided"))

##
## Wilcoxon rank sum test
##
## data: RFU by ID
## W = 119, p-value = 1.134e-05
## alternative hypothesis: true location shift is not equal to 0
# p-values of 1.134e-05 for both tests: populations are not the same. (There is a minimum p-value in th

Regression:
reg7 <- ggplot(EC36307_df, aes(x= RFP, y= GFP, shape = ID, colour=ID)) + #
  labs(x="RFP (RFU)", y="GFP (RFU)") +
  #scale_x_continuous(breaks = pretty(RFP, n = 5)) +
  #scale_y_continuous(breaks = pretty(GFP, n = 5)) +
  geom_point(size=2) +
  geom_smooth(method=lm, size=0.5) +

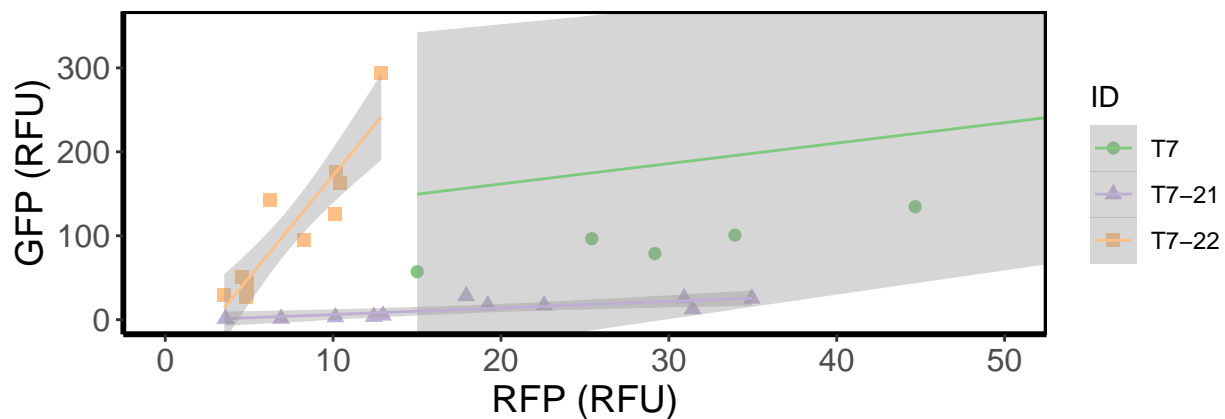
```

```

scale_color_brewer(type = 'div', palette = "Accent") +
#scale_color_grey() +
theme_classic() +
coord_fixed(ratio = 0.05, xlim = c(0:50), ylim = c(0:350), expand = TRUE, clip = "on") +
theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
      axis.title.x = element_text(size = 14),
      axis.title.y = element_text(size = 14),
      axis.text.x = element_text(size=12),
      axis.text.y = element_text(size=12),
      panel.border = element_rect(size = 1, colour = "black", fill = NA)
    ) +
guides(fill = "none")

```

reg7



```

reg8 <- ggplot(EC36307_df, aes(RFP, GFP, shape=ID, colour=ID)) + #
labs(x="RFP (RFU)", y="GFP (RFU)") +
#scale_x_continuous(breaks = pretty(RFP, n = 5)) +
#scale_y_continuous(breaks = pretty(GFP, n = 5)) +
geom_point( size=2) +
geom_smooth(method=lm, size=0.5) +
#scale_color_brewer(type = 'div', palette = "Spectral") +
scale_color_grey() +
theme_classic() +
guides(fill = "none") +
#coord_fixed(ratio = 0.05, xlim = c(0:50), ylim = c(0:350), expand = TRUE, clip = "on") +

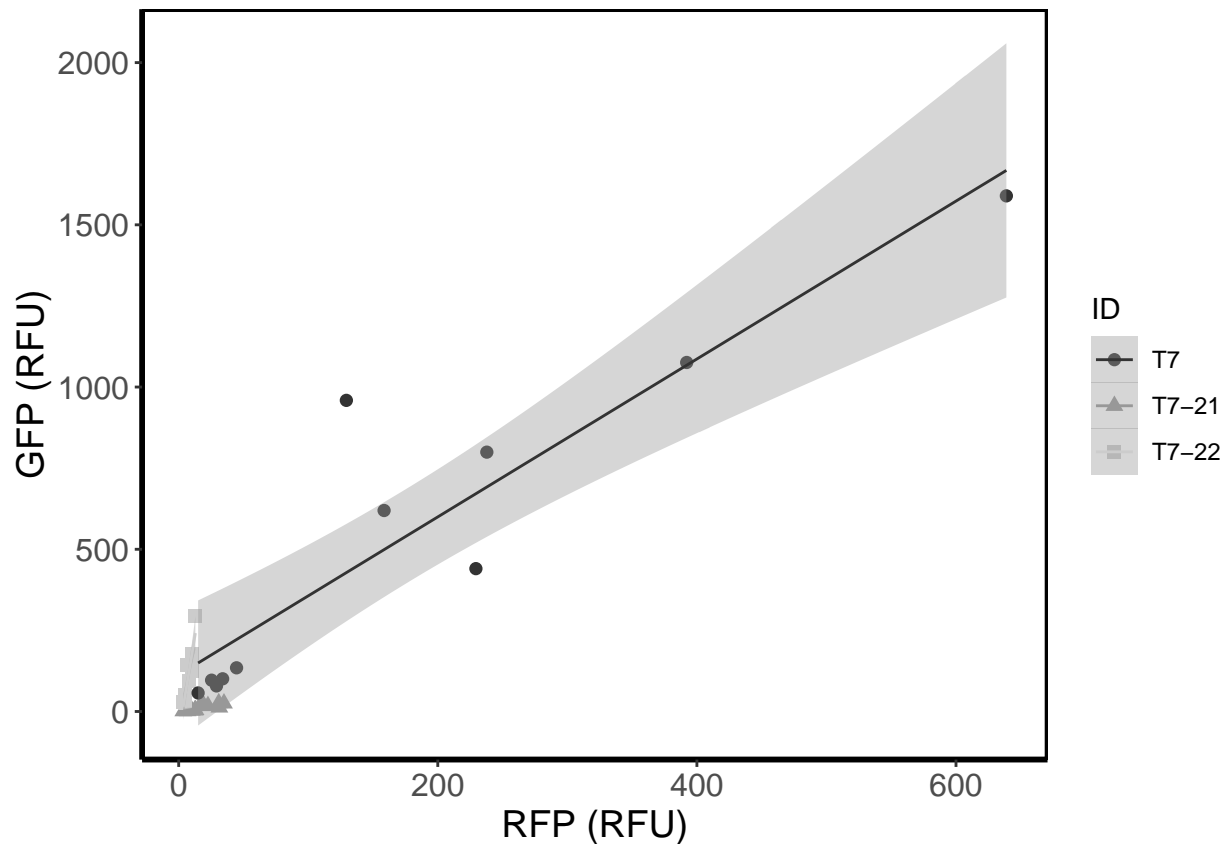
```

```

theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
      axis.title.x = element_text(size = 14),
      axis.title.y = element_text(size = 14),
      axis.text.x = element_text(size=12),
      axis.text.y = element_text(size=12),
      panel.border = element_rect(size = 1, colour = "black", fill = NA)
)

```

reg8



#plotting without extreme values of γ (- 6 samples):

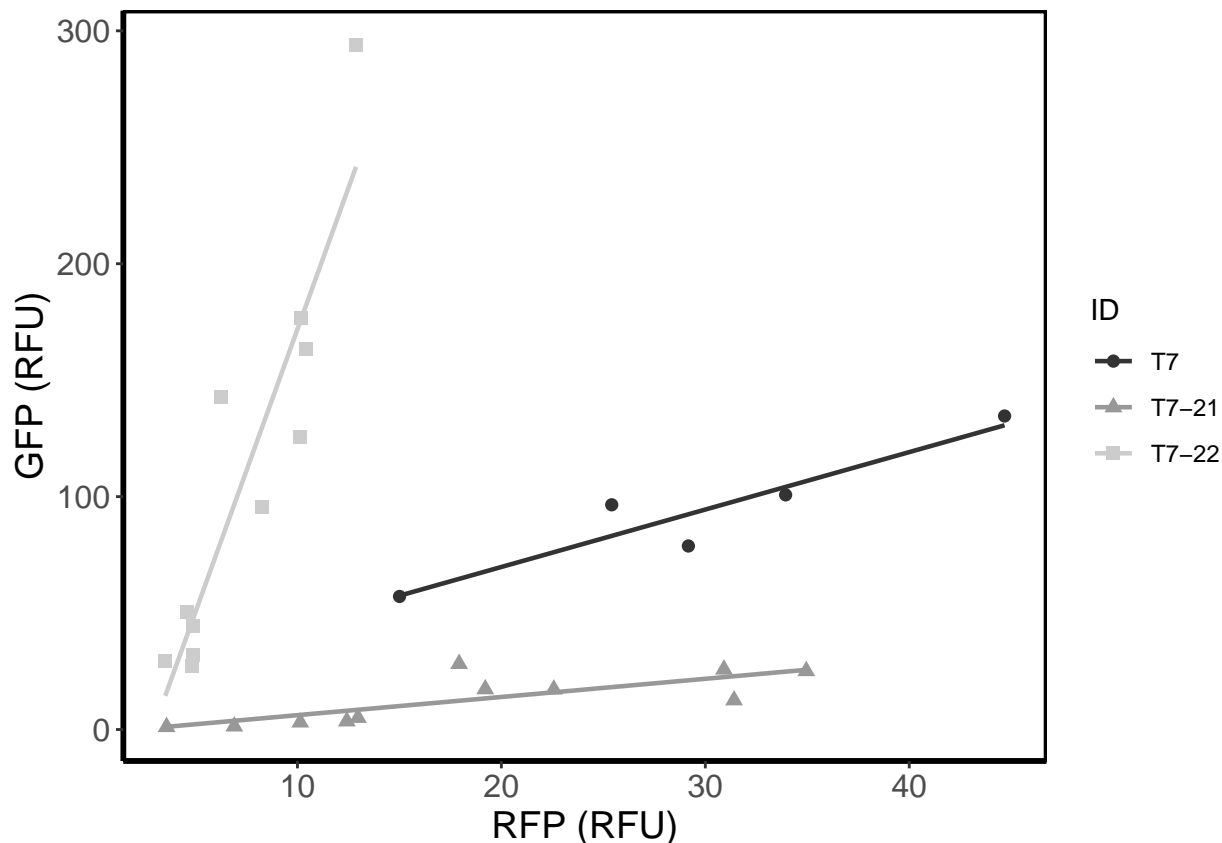
```
EC36307_df_low <- EC36307_df[-c(28:33), ]
```

```
EC36307_df_low # n = 5
```

##	ID	GFP	RFP	GFP.RFP
## 1	T7-21	5.057	12.968	0.390
## 2	T7-21	1.356	6.903	0.196
## 3	T7-21	3.078	10.138	0.304
## 4	T7-21	3.530	12.420	0.284
## 5	T7-21	1.102	3.581	0.308
## 6	T7-21	17.228	22.571	1.310
## 7	T7-21	28.226	17.927	0.635
## 8	T7-21	25.809	30.915	1.198
## 9	T7-21	25.125	34.951	1.391
## 10	T7-21	12.576	31.410	2.498
## 11	T7-21	17.329	19.209	1.108

```
## 12 T7-22 27.255 4.818 5.657
## 13 T7-22 31.973 4.884 6.546
## 14 T7-22 29.393 3.517 8.357
## 15 T7-22 44.241 4.866 9.092
## 16 T7-22 125.598 10.133 12.395
## 17 T7-22 142.912 6.241 22.899
## 18 T7-22 163.303 10.436 15.648
## 19 T7-22 176.488 10.193 17.315
## 20 T7-22 95.409 8.252 11.562
## 21 T7-22 294.041 12.871 22.845
## 22 T7-22 50.330 4.562 11.032
## 23 T7 100.750 33.938 2.969
## 24 T7 57.142 15.006 3.808
## 25 T7 96.495 25.411 3.797
## 26 T7 78.805 29.170 2.702
## 27 T7 134.626 44.675 3.013
```

```
ggplot(EC36307_df_low, aes(RFP, GFP, shape=ID, colour=ID)) + #
  labs(x="RFP (RFU)", y="GFP (RFU)") +
  #scale_x_continuous(breaks = pretty(RFP, n = 5)) +
  #scale_y_continuous(breaks = pretty(GFP, n = 5)) +
  geom_point( size=2) +
  geom_smooth(method=lm, size=0.8, se=FALSE, fullrange=FALSE) +
  #scale_color_brewer(
  scale_color_grey() +
  theme_classic() +
  guides(fill = "none") +
  #coord_fixed(ratio = 0.05)+#, xlim = c(0:50), ylim = c(0:350), expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 14),
        axis.title.y = element_text(size = 14),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12),
        panel.border = element_rect(size = 1, colour = "black", fill = NA)
  )
```



SLOPE COMPARISON

```
#####
```

```
# SLOPE COMPARISON
```

```
#####
```

```
# Scatterplot of GFP to RFP for 7 n = 5, (lowest 5 datapoints), 7-21 and 7-22
```

```
library(devtools)
```

```
source_gist("524eade46135f6348140")
```

```
## Sourcing https://gist.githubusercontent.com/kdauria/524eade46135f6348140/raw/676acaca9a0a144ef320ae2
```

```
## SHA-1 hash of file is c0b163b9fd2d7fe7bd5541e3266d8d36ff3b895d
```

```
ggplot(EC36307_df_low, aes(RFP, GFP, shape=ID, colour=ID)) + #
```

```
  labs(x="RFP (RFU)", y="GFP (RFU)") +
```

```
  #scale_x_continuous(breaks = pretty(RFP, n = 5)) +
```

```
  #scale_y_continuous(breaks = pretty(GFP, n = 5)) +
```

```
  geom_point( size=2) +
```

```
  stat_smooth_func(geom="text",method="lm",hjust=0,parse=TRUE) + #show equations in figure
```

```
  geom_smooth(method=lm, size=0.8, se=TRUE, alpha = 0.15, fullrange=FALSE) +
```

```
  #scale_color_brewer(
```

```
  scale_color_grey() +
```

```
  theme_classic() +
```

```
  guides(fill = "none") +
```

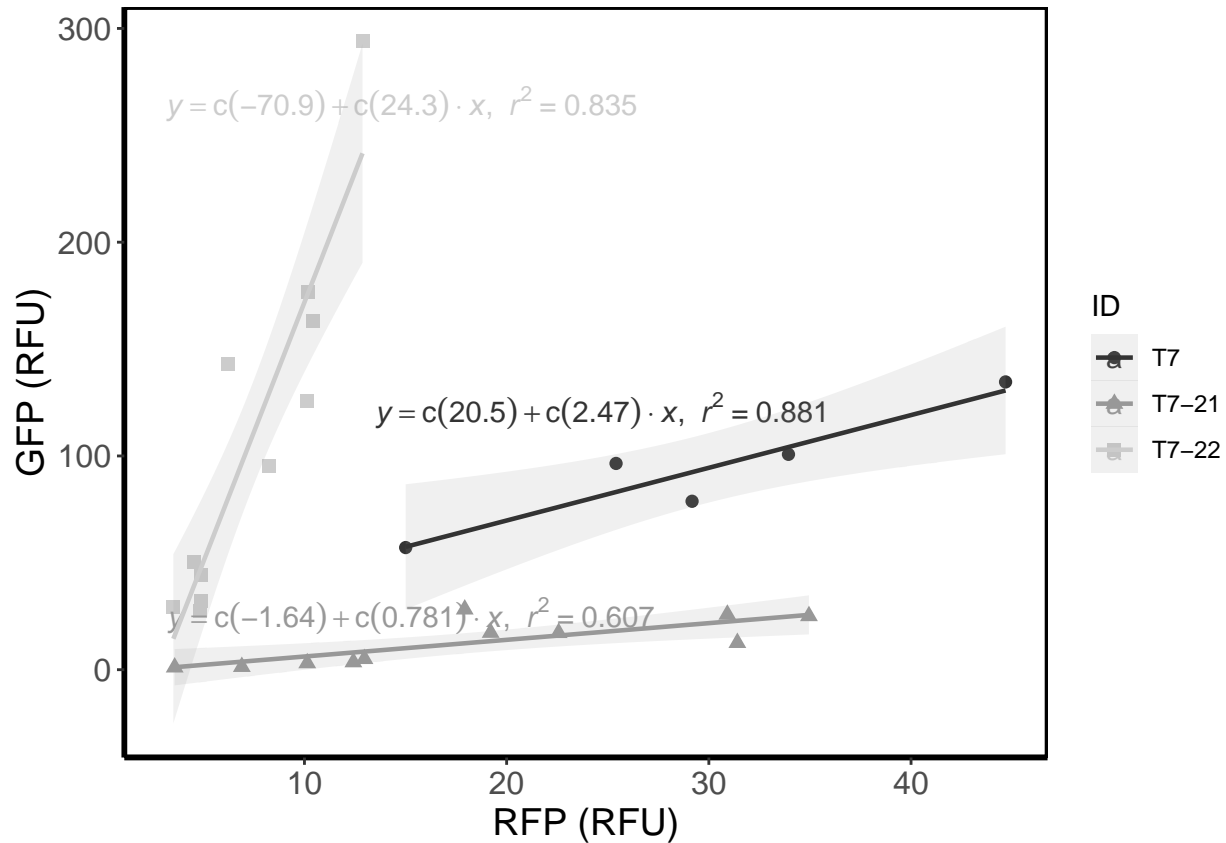
```
  #coord_fixed(ratio = 0.05)+#, xlim = c(0:50), ylim = c(0:350), expand = TRUE, clip = "on") +
```

```
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
```

```
        axis.title.x = element_text(size = 14),
```

```
        axis.title.y = element_text(size = 14),
```

```
axis.text.x= element_text(size=12),
axis.text.y = element_text(size=12),
panel.border = element_rect(size = 1, colour = "black", fill = NA)
)
```



```
# Comparing slopes, EC36307 only n = 5 (lowest five values)
library(lsmmeans)
```

```
## Loading required package: emmeans
```

```
##
```

```
## Attaching package: 'emmeans'
```

```
## The following object is masked from 'package:devtools':
```

```
##
```

```
## test
```

```
## The 'lsmmeans' package is now basically a front end for 'emmeans'.
```

```
## Users are encouraged to switch the rest of the way.
```

```
## See help('transition') for more information, including how to
```

```
## convert old 'lsmmeans' objects and scripts to work with 'emmeans'.
```

```
m.interaction <- lm(GFP ~ RFP*ID, data = EC36307_df_low)
```

```
m.interaction
```

```
##
```

```
## Call:
```

```
## lm(formula = GFP ~ RFP * ID, data = EC36307_df_low)
```

```
##
```

```
## Coefficients:
## (Intercept)          RFP          IDT7-21          IDT7-22  RFP:IDT7-21
##      20.469         2.466        -22.112        -91.395         -1.685
## RFP:IDT7-22
##      21.814
```

```
# ANOVA not possible due to non-normality and heteroscedasticity
```

```
# Obtain slopes
```

```
m.interaction$coefficients
```

```
## (Intercept)          RFP          IDT7-21          IDT7-22  RFP:IDT7-21  RFP:IDT7-22
##  20.468501     2.466096  -22.111687   -91.395362   -1.685325    21.813533
```

```
m.lst <- lstrends(m.interaction, "ID", var="RFP")
m.lst
```

```
## ID      RFP.trend      SE df lower.CL upper.CL
## T7         2.466  1.112 21     0.154     4.78
## T7-21      0.781  0.731 21    -0.739     2.30
## T7-22     24.280  2.440 21    19.206    29.35
##
## Confidence level used: 0.95
```

```
# Compare slopes
```

```
pairs(m.lst)
```

```
## contrast      estimate      SE df t.ratio p.value
## T7 - T7-21         1.69  1.33 21   1.267  0.4289
## T7 - T7-22        -21.81  2.68 21  -8.136 <.0001
## T7-21 - T7-22     -23.50  2.55 21  -9.226 <.0001
##
```

```
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
#####
```

```
##### SLOPE COMPARISON: All samples included
```

```
m.interaction2 <- lm(GFP ~ RFP*ID, data = EC36307_df) # prediction of GFP (dependent) signal through RF
```

```
# Obtain slopes
```

```
m.interaction2$coefficients
```

```
## (Intercept)          RFP          IDT7-21          IDT7-22  RFP:IDT7-21  RFP:IDT7-22
##  113.020507     2.433544 -114.663694 -183.947369   -1.652773    21.846085
```

```
m.lst2 <- lstrends(m.interaction2, "ID", var="RFP")
m.lst2
```

```
## ID      RFP.trend      SE df lower.CL upper.CL
## T7         2.434  0.203 27     2.02     2.85
## T7-21      0.781  3.763 27    -6.94     8.50
## T7-22     24.280 12.564 27    -1.50    50.06
##
## Confidence level used: 0.95
```

```
# Compare slopes
```

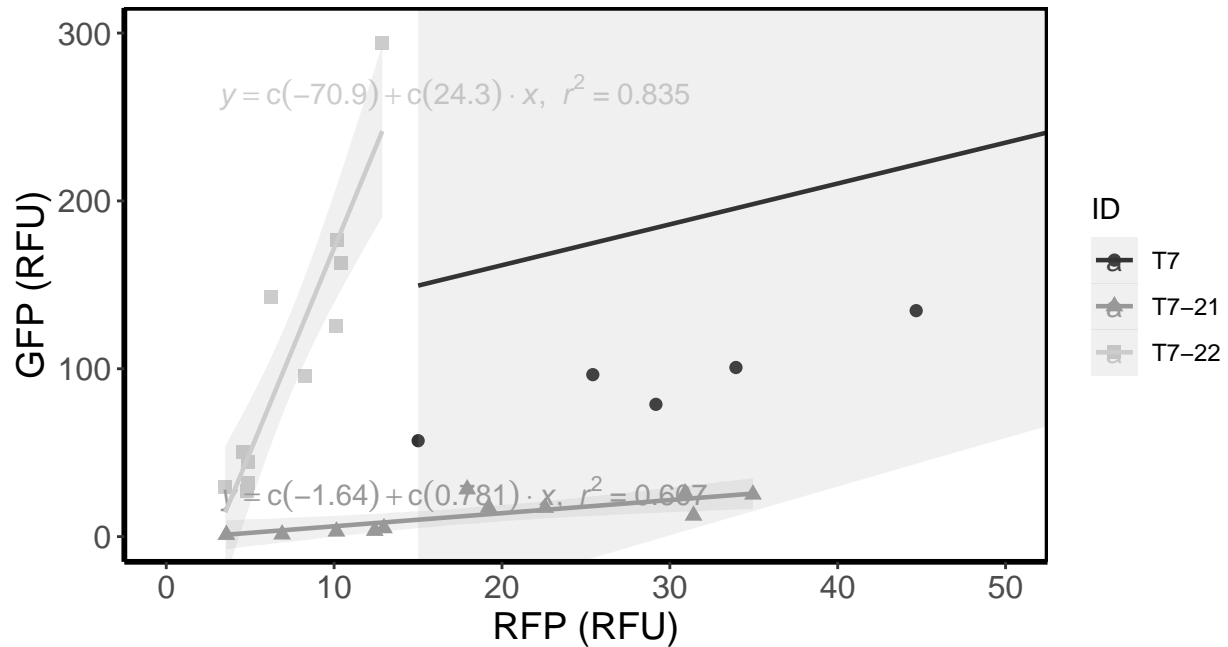
```
pairs(m.lst2)
```

```
## contrast      estimate      SE df t.ratio p.value
```



```
## T7 - T7-21          1.65  3.77 27  0.439  0.8998
## T7 - T7-22         -21.85 12.57 27 -1.739  0.2096
## T7-21 - T7-22      -23.50 13.12 27 -1.792  0.1914
##
## P value adjustment: tukey method for comparing a family of 3 estimates
# high p-values: including large datapoints of 7 made all interactions non-significant.

#visualize by scatterplot
ggplot(EC36307_df, aes(RFP, GFP, shape=ID, colour=ID)) + #
  labs(x="RFP (RFU)", y="GFP (RFU)") +
  #scale_x_continuous(breaks = pretty(RFP, n = 5)) +
  #scale_y_continuous(breaks = pretty(GFP, n = 5)) +
  geom_point( size=2) +
  stat_smooth_func(geom="text",method="lm",hjust=0,parse=TRUE) + #show equations in figure
  geom_smooth(method=lm, size=0.8, se=TRUE, alpha = 0.15, fullrange=FALSE) +
  #scale_color_brewer(
  scale_color_grey() +
  theme_classic() +
  guides(fill = "none") +
  coord_fixed(ratio = 0.1, xlim = c(0:50), ylim = c(0:300), expand = TRUE, clip = "on") +
  theme(axis.line = element_line(colour = "black", size = 1, linetype = "solid"),
        axis.title.x = element_text(size = 14),
        axis.title.y = element_text(size = 14),
        axis.text.x= element_text(size=12),
        axis.text.y = element_text(size=12),
        panel.border = element_rect(size = 1, colour = "black", fill = NA)
  )
```



Regression summary: p-values:

```
summary(m.interaction)
```

```
##
## Call:
## lm(formula = GFP ~ RFP * ID, data = EC36307_df_low)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -49.501  -7.414  -0.521   3.979  62.310
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    20.469     34.696   0.590  0.5615
## RFP             2.466      1.112   2.218  0.0377 *
## IDT7-21        -22.112     37.938  -0.583  0.5662
## IDT7-22        -91.395     39.729  -2.300  0.0318 *
## RFP:IDT7-21     -1.685      1.331  -1.267  0.2191
## RFP:IDT7-22     21.814      2.681   8.136 6.28e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 24.28 on 21 degrees of freedom
## Multiple R-squared:  0.9034, Adjusted R-squared:  0.8804
## F-statistic: 39.27 on 5 and 21 DF, p-value: 5.81e-10
```

```
summary(m.lst)
```

```
## ID      RFP.trend      SE df lower.CL upper.CL
## T7         2.466 1.112 21    0.154    4.78
## T7-21      0.781 0.731 21   -0.739    2.30
## T7-22     24.280 2.440 21   19.206   29.35
##
## Confidence level used: 0.95
```