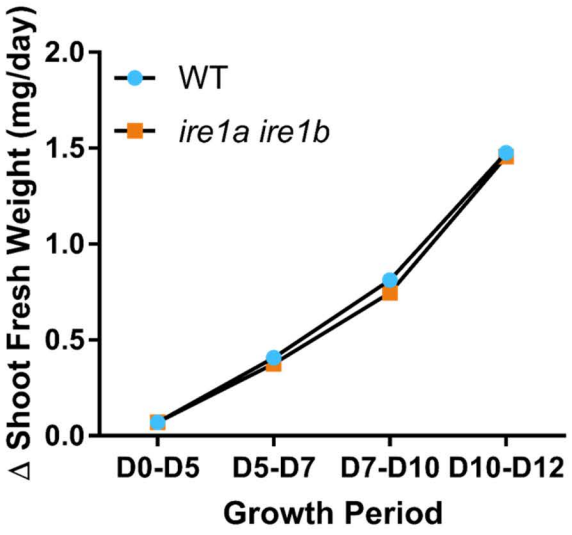
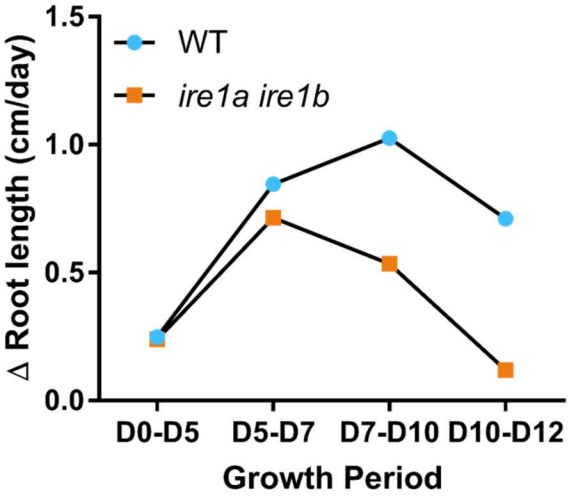
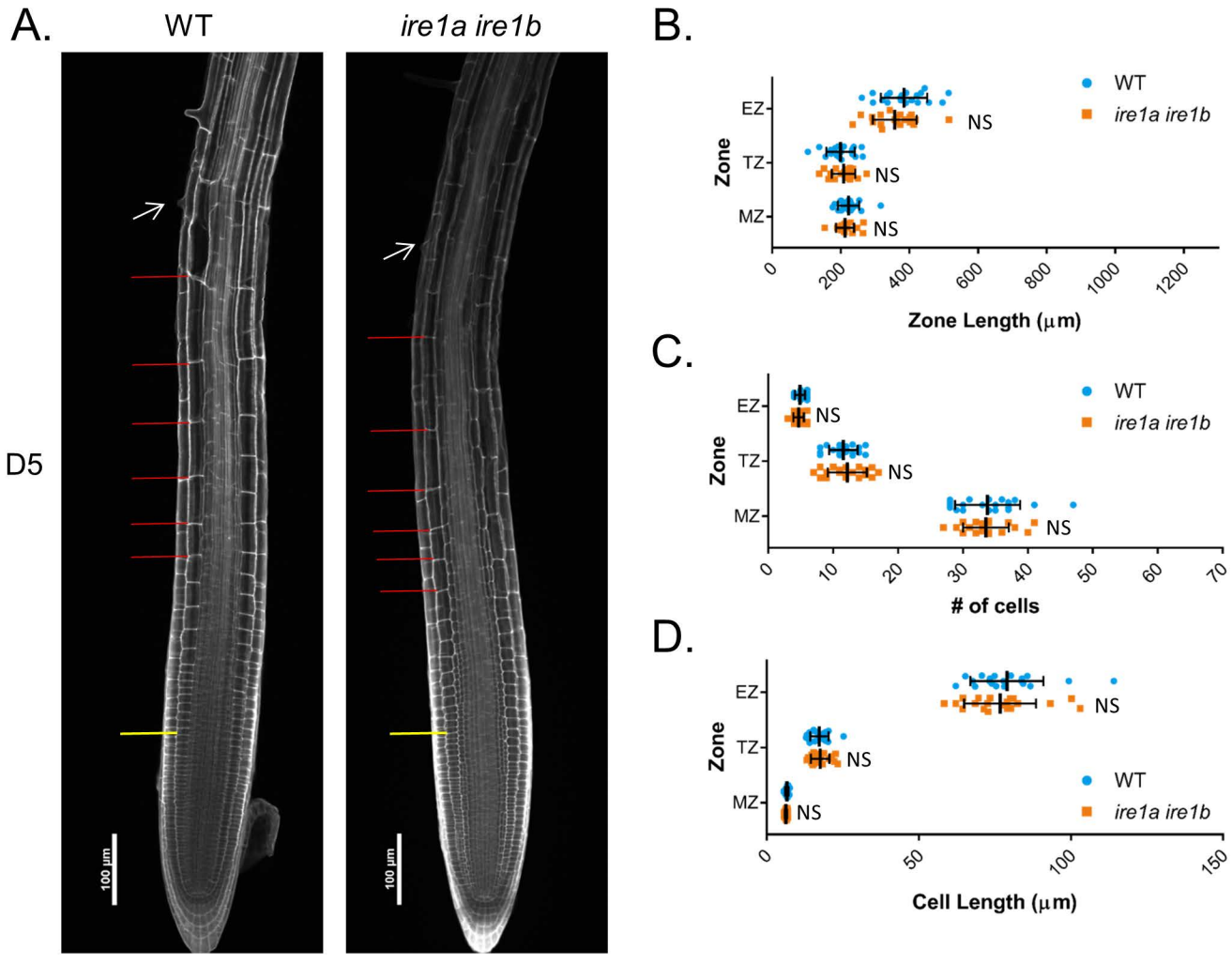
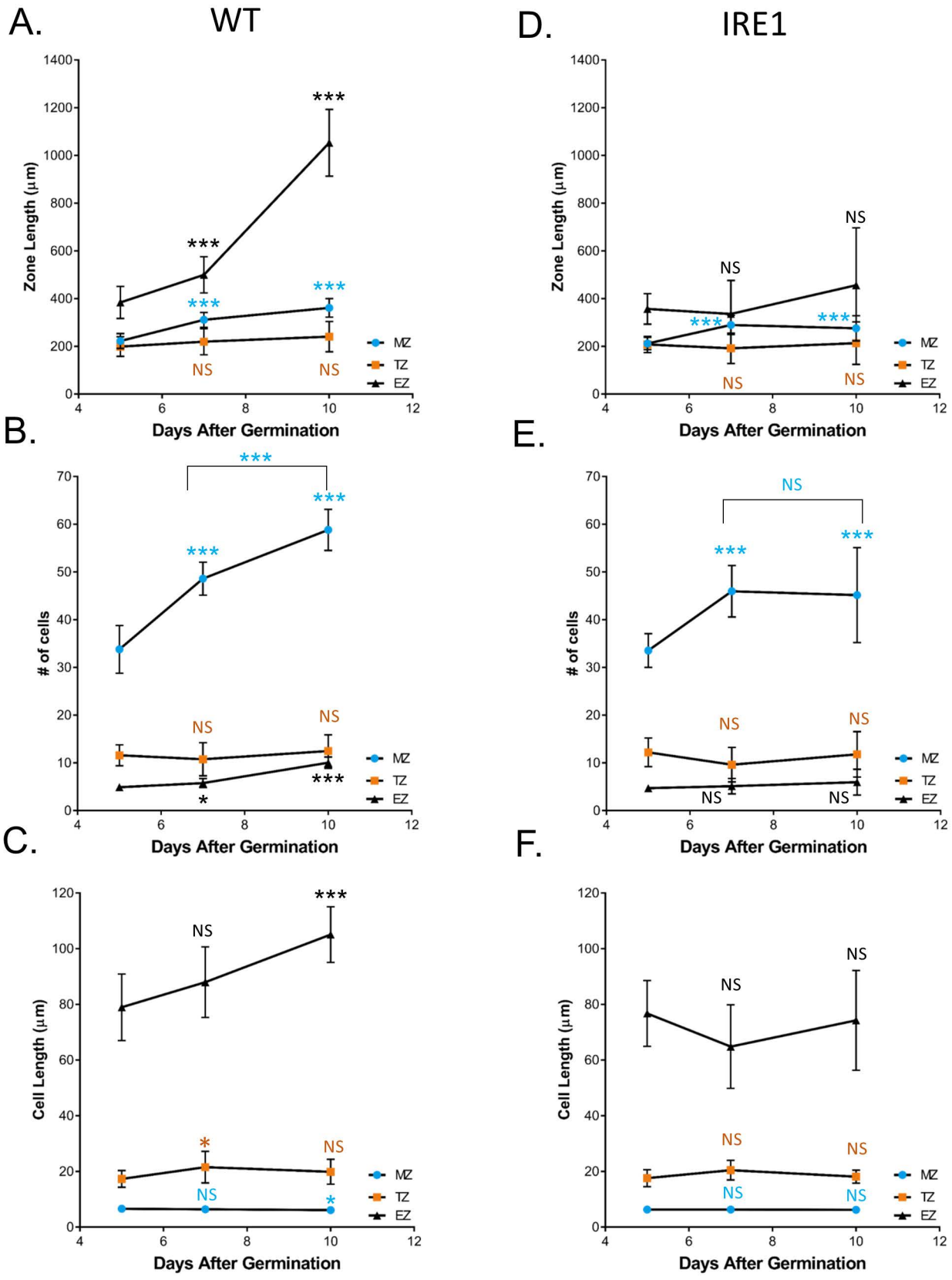
A.


Δ Shoot Fresh Weight (mg/day)
2.0
1.5
1.0
0.5
0.0
WT
ire1a ire1b
D0-D5
D5-D7
D7-D10
D10-D12
Growth Period

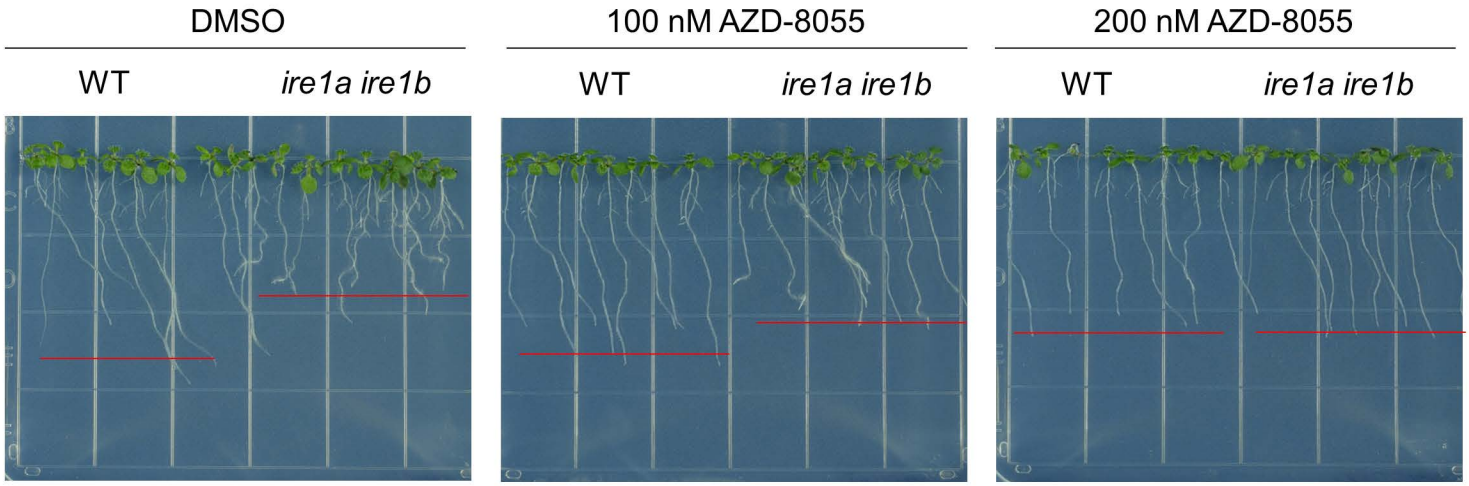
B.


Δ Root length (cm/day)
1.5
1.0
0.5
0.0
WT
ire1a ire1b
D0-D5
D5-D7
D7-D10
D10-D12
Growth Period

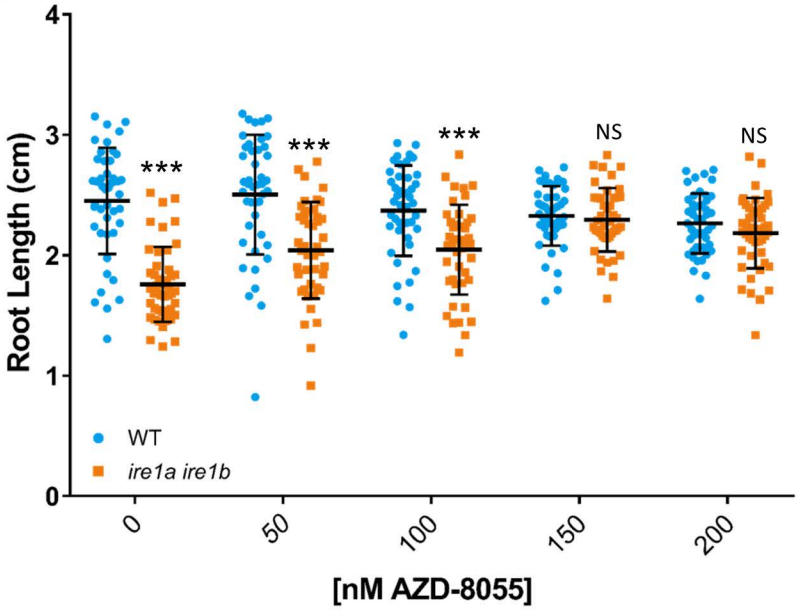

A.
WT
ire1a ire1b
D5
100 µm
100 µm
B.
WT
ire1a ire1b
EZ
TZ
MZ
Zone
NS
NS
NS
0
200
400
600
800
1000
1200
Zone Length (µm)
C.
WT
ire1a ire1b
EZ
TZ
MZ
Zone
NS
NS
NS
0
10
20
30
40
50
60
70
# of cells
D.
WT
ire1a ire1b
EZ
TZ
MZ
Zone
NS
NS
NS
0
50
100
150
Cell Length (µm)

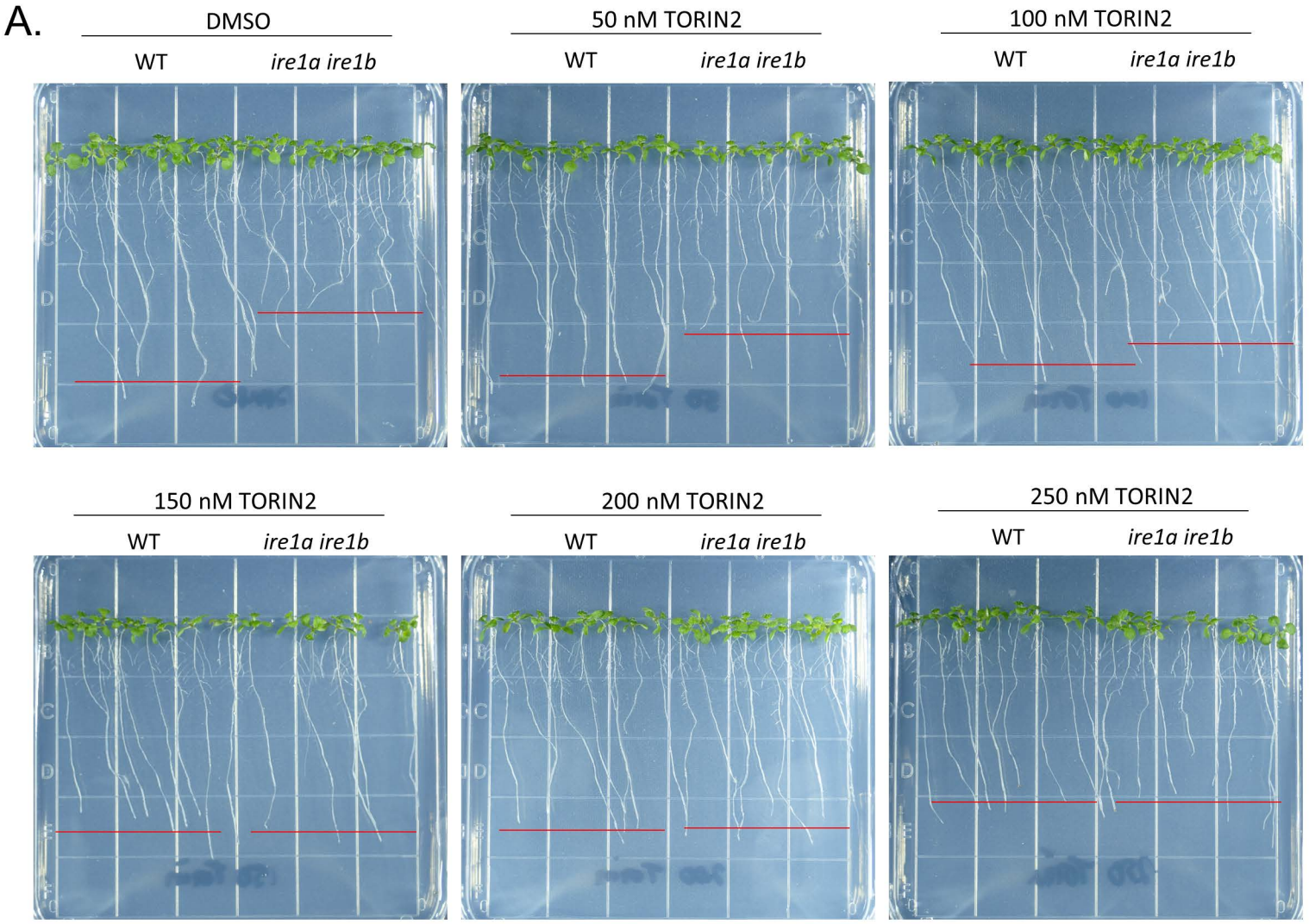

A.
WT
D.
IRE1
B.
E.
C.
F.
Zone Length (µm)
# of cells
Cell Length (µm)
Days After Germination
MZ
TZ
EZ
***
NS
*

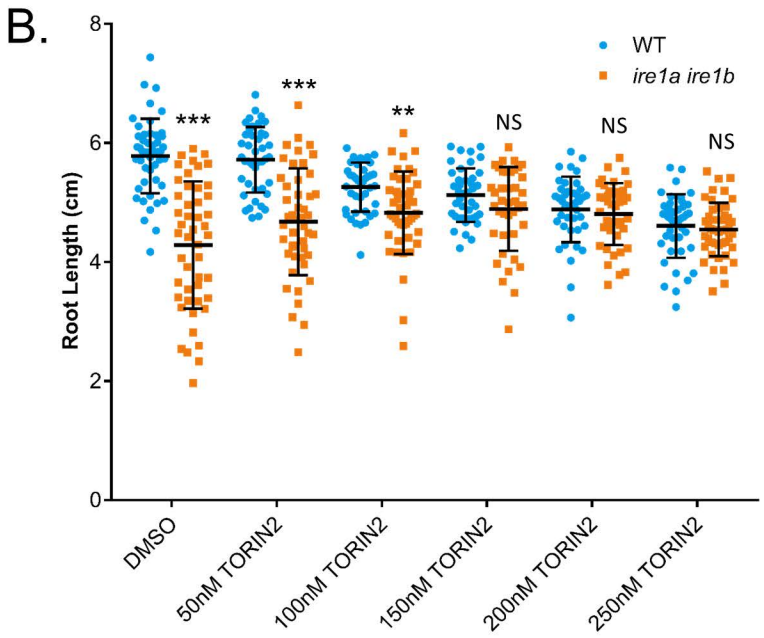
A.

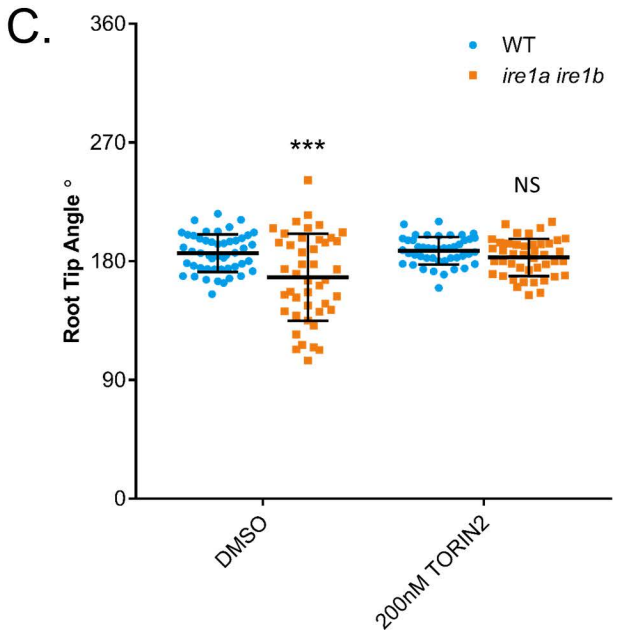

DMSO
100 nM AZD-8055
200 nM AZD-8055
WT
ire1a ire1b
WT
ire1a ire1b
WT
ire1a ire1b

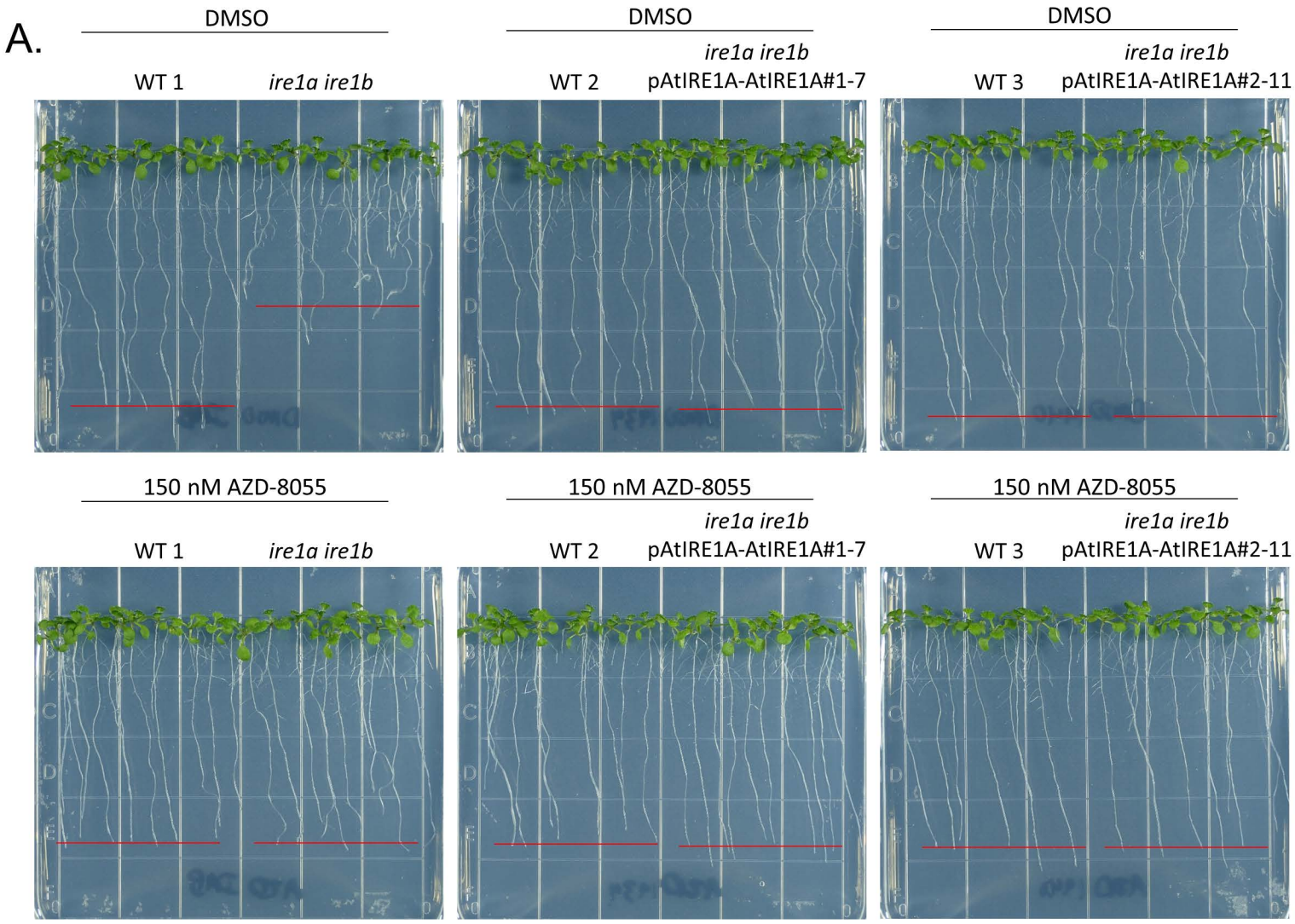
B.

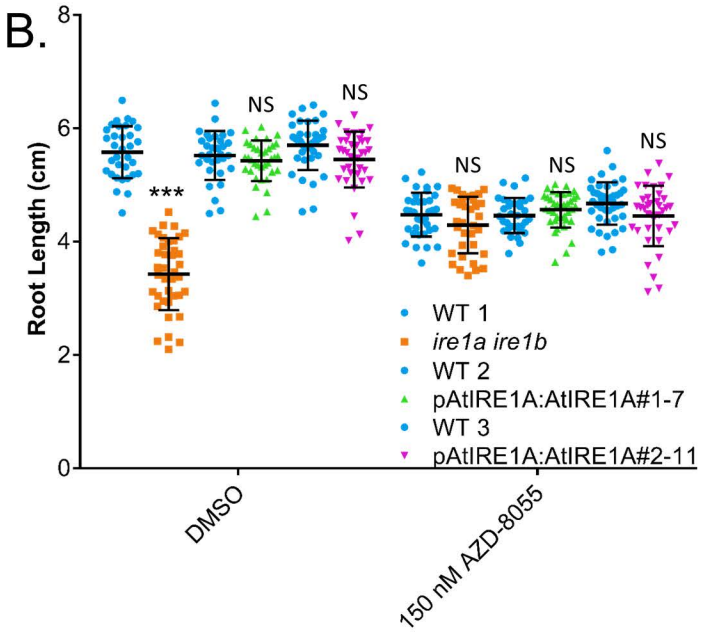

Root Length (cm)
0
1
2
3
4
***
***
***
NS
NS
WT
ire1a ire1b
0
50
100
150
200
[nM AZD-8055]

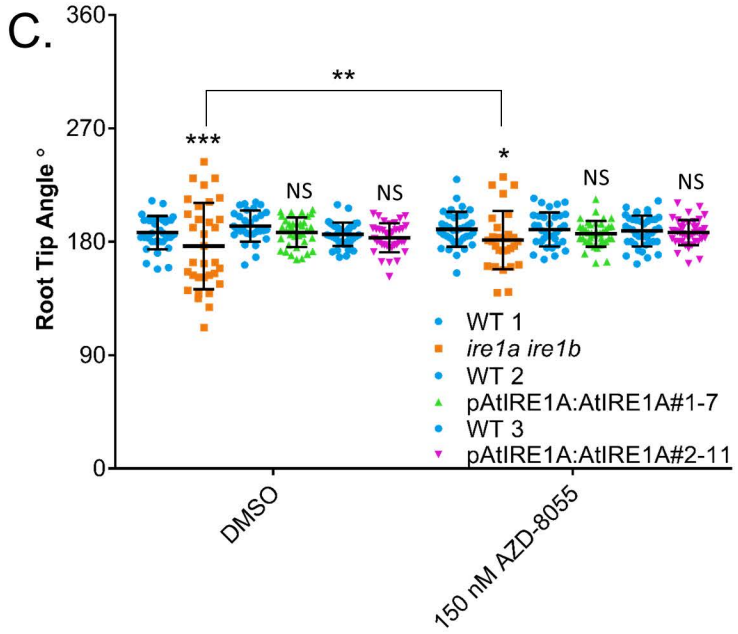

A.
DMSO
WT
ire1a ire1b
50 nM TORIN2
WT
ire1a ire1b
100 nM TORIN2
WT
ire1a ire1b
150 nM TORIN2
WT
ire1a ire1b
200 nM TORIN2
WT
ire1a ire1b
250 nM TORIN2
WT
ire1a ire1b

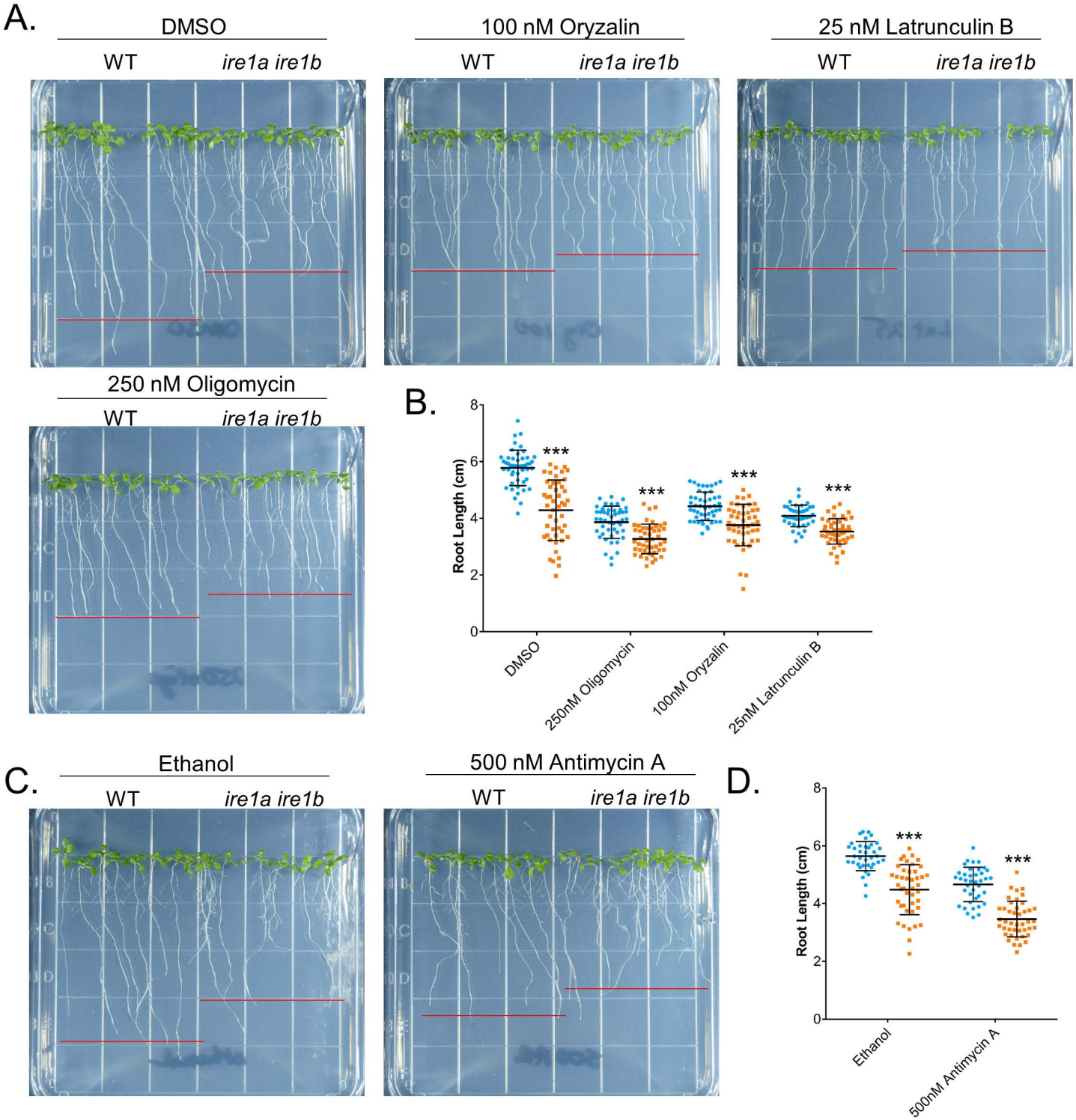

B.
Root Length (cm)
0
2
4
6
8
WT
ire1a ire1b
***
***
**
NS
NS
NS
DMSO
50nM TORIN2
100nM TORIN2
150nM TORIN2
200nM TORIN2
250nM TORIN2

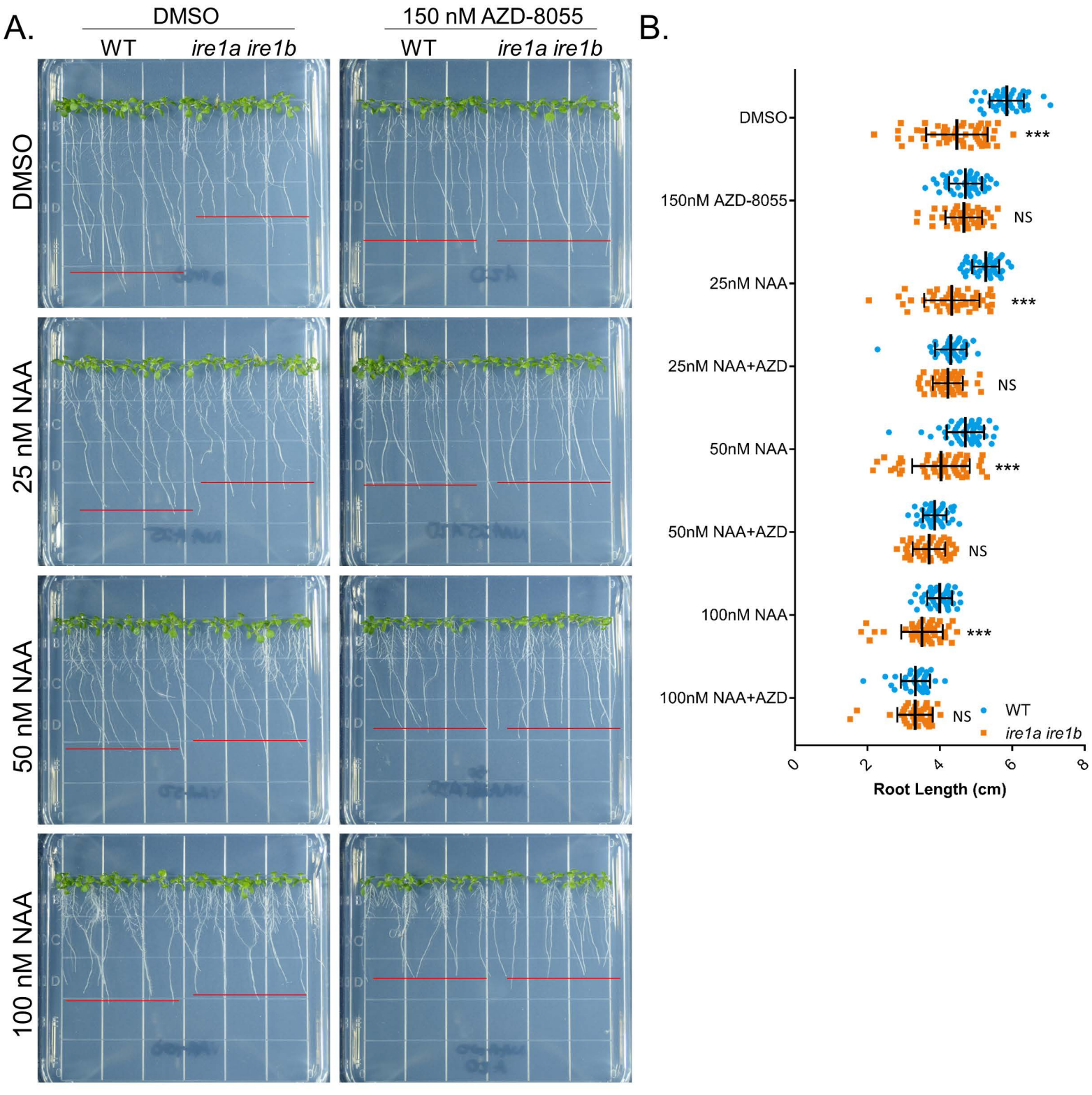

C.
Root Tip Angle °
0
90
180
270
360
WT
ire1a ire1b
***
NS
DMSO
200nM TORIN2

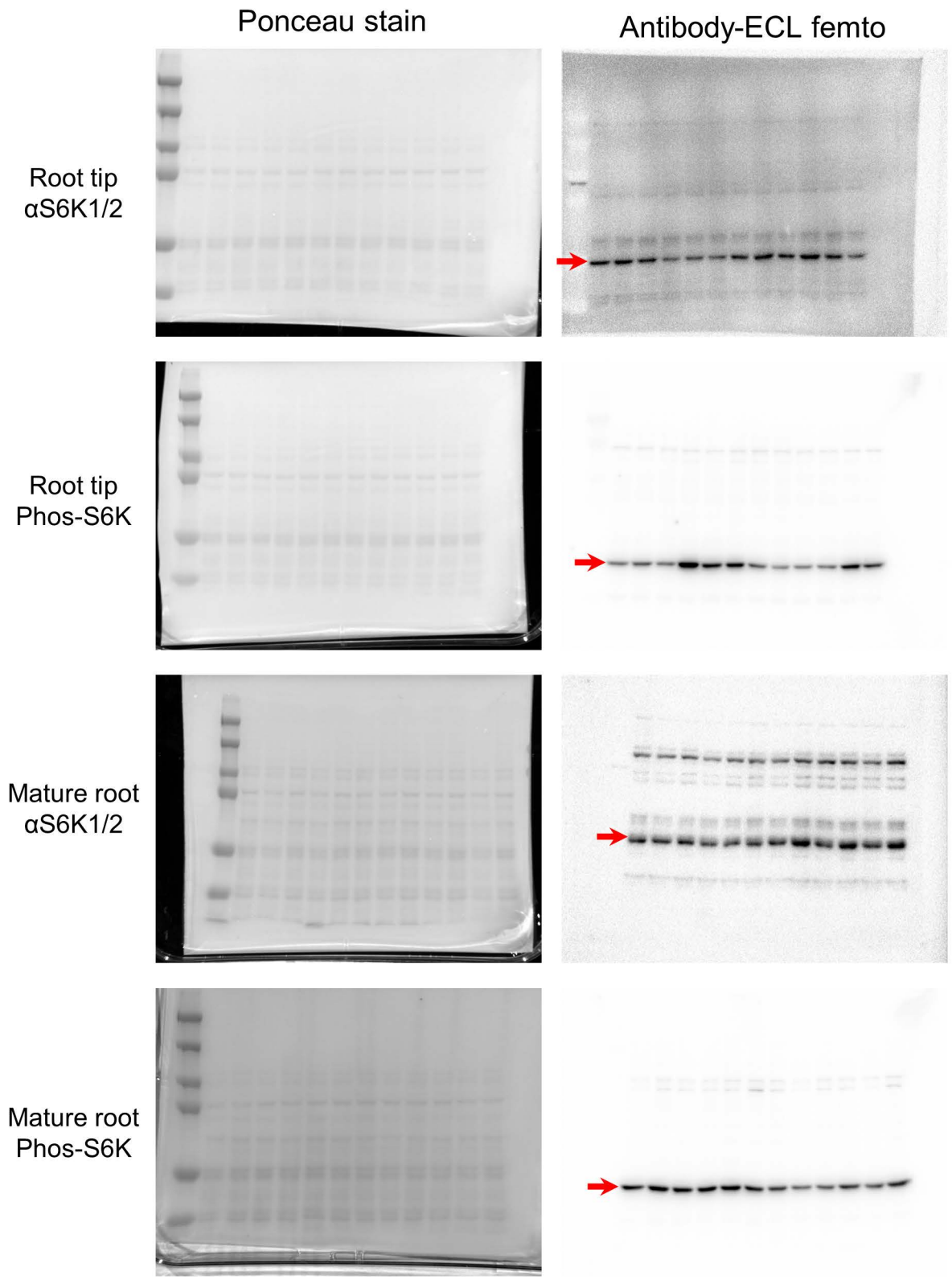

A.
DMSO
DMSO
DMSO
WT 1
ire1a ire1b
WT 2
ire1a ire1b
pAtIRE1A-AtIRE1A#1-7
WT 3
ire1a ire1b
pAtIRE1A-AtIRE1A#2-11
150 nM AZD-8055
150 nM AZD-8055
150 nM AZD-8055
WT 1
ire1a ire1b
WT 2
ire1a ire1b
pAtIRE1A-AtIRE1A#1-7
WT 3
ire1a ire1b
pAtIRE1A-AtIRE1A#2-11

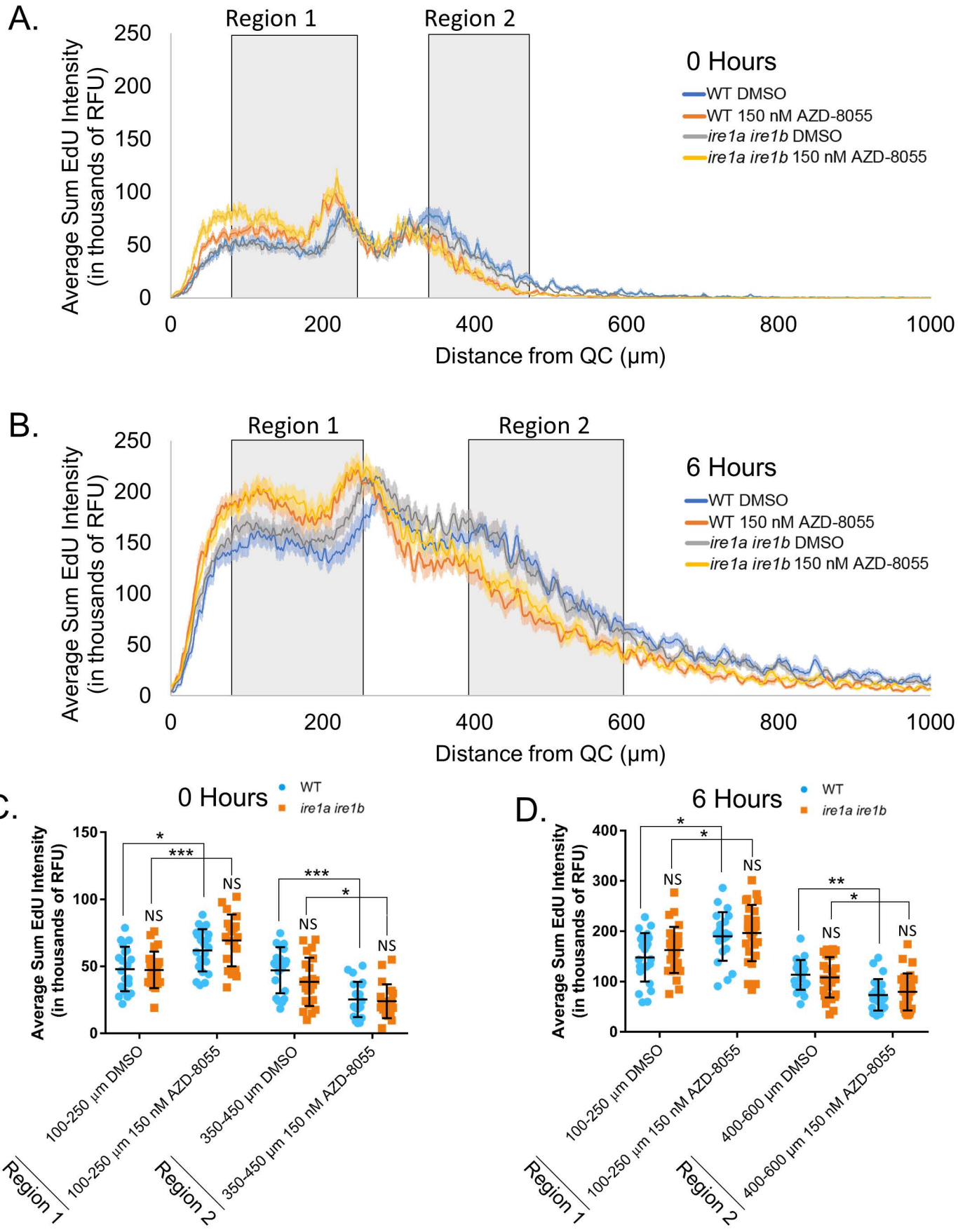

B.
Root Length (cm)
0
2
4
6
8
***
NS
NS
NS
NS
NS
DMSO
150 nM AZD-8055
WT 1
ire1a ire1b
WT 2
pAtIRE1A:AtIRE1A#1-7
WT 3
pAtIRE1A:AtIRE1A#2-11

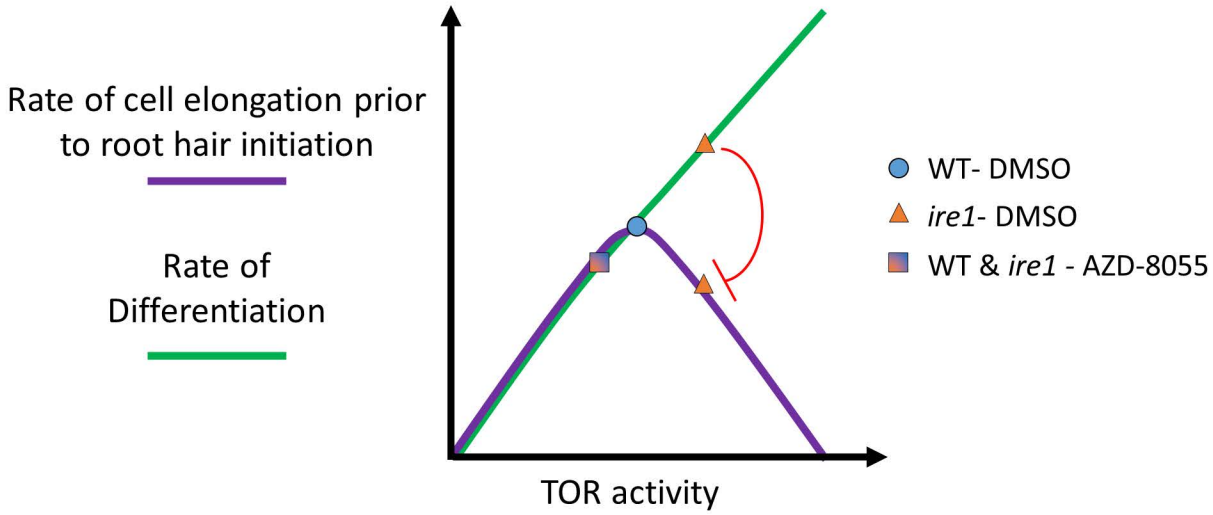

C.
Root Tip Angle °
0
90
180
270
360
**
***
*
NS
NS
NS
NS
DMSO
150 nM AZD-8055
WT 1
ire1a ire1b
WT 2
pAtIRE1A:AtIRE1A#1-7
WT 3
pAtIRE1A:AtIRE1A#2-11


A.
DMSO
WT
ire1a ire1b
100 nM Oryzalin
WT
ire1a ire1b
25 nM Latrunculin B
WT
ire1a ire1b
250 nM Oligomycin
WT
ire1a ire1b
B.
Root Length (cm)
0
2
4
6
8
***
***
***
***
DMSO
250nM Oligomycin
100nM Oryzalin
25nM Latrunculin B
C.
Ethanol
WT
ire1a ire1b
500 nM Antimycin A
WT
ire1a ire1b
D.
Root Length (cm)
0
2
4
6
8
***
***
Ethanol
500nM Antimycin A


A.
DMSO
150 nM AZD-8055
WT
ire1a ire1b
WT
ire1a ire1b
DMSO
25 nM NAA
50 nM NAA
100 nM NAA
B.
DMSO
150nM AZD-8055
25nM NAA
25nM NAA+AZD
50nM NAA
50nM NAA+AZD
100nM NAA
100nM NAA+AZD
***
NS
***
NS
***
NS
***
NS
WT
ire1a ire1b
0
2
4
6
8
Root Length (cm)


Ponceau stain
Antibody-ECL femto
Root tip
αS6K1/2
Root tip
Phos-S6K
Mature root
αS6K1/2
Mature root
Phos-S6K


A.
Region 1
Region 2
Average Sum EdU Intensity (in thousands of RFU)
250
200
150
100
50
0
0
200
400
600
800
1000
Distance from QC (μm)
0 Hours
WT DMSO
WT 150 nM AZD-8055
ire1a ire1b DMSO
ire1a ire1b 150 nM AZD-8055
B.
Region 1
Region 2
Average Sum EdU Intensity (in thousands of RFU)
250
200
150
100
50
0
0
200
400
600
800
1000
Distance from QC (μm)
6 Hours
WT DMSO
WT 150 nM AZD-8055
ire1a ire1b DMSO
ire1a ire1b 150 nM AZD-8055
C.
0 Hours
WT
ire1a ire1b
Average Sum EdU Intensity (in thousands of RFU)
150
100
50
0
*
***
NS
NS
***
*
NS
NS
100-250 μm DMSO
100-250 μm 150 nM AZD-8055
350-450 μm DMSO
350-450 μm 150 nM AZD-8055
Region 1
Region 2
D.
6 Hours
WT
ire1a ire1b
Average Sum EdU Intensity (in thousands of RFU)
400
300
200
100
0
*
*
NS
NS
NS
**
*
NS
NS
100-250 μm DMSO
100-250 μm 150 nM AZD-8055
400-600 μm DMSO
400-600 μm 150 nM AZD-8055
Region 1
Region 2


Rate of cell elongation prior to root hair initiation
Rate of Differentiation
TOR activity
WT- DMSO
ire1- DMSO
WT & ire1 - AZD-8055

Shoot Fresh Weight

Three way ANOVA with log transformation (total number of shoot groups measured* = 96)

Variable	Sum Sq	Df	F value	Pr(>F)
Genotype	0.0004	1	0.0578	0.810669
AZD	0.0186	1	2.4961	0.117888
Day	13.4755	2	904.5419	< 2.2e-16
Genotype:AZD	0.0058	1	0.7823	0.378956
Genotype:Day	0.0028	2	0.1859	0.830704
AZD:Day	0.0083	2	0.5549	0.576235
Genotype:AZD:Day	0.0022	2	0.15	0.860925
Residuals	0.6257	84		

*8-10 shoots of a single genotype were weighed as a group. To calculate an individual measurement, the sum

Root Length

Three way wANOVA (total number of roots measured = 899)

Variable	Sum Sq	Df	F value	Pr(>F)
Genotype	30.8	1	18.747	1.66E-05
AZD	122	1	74.315	< 2.2e-16
Day	6527.7	2	1988.251	< 2.2e-16
Genotype:AZD	23.4	1	14.229	0.000173
Genotype:Day	488	2	148.646	< 2.2e-16
AZD:Day	65.2	2	19.863	3.64E-09
Genotype:AZD:Day	220.8	2	67.253	< 2.2e-16
Residuals	1456.1	887		

Root Tip Angle

Three way wANOVA (total number of roots measured = 843)

Variable	Sum Sq	Df	F value	Pr(>F)
Genotype	0.4	1	0.2271	0.63382
AZD	0	1	0.0022	0.96238
Day	15.2	2	4.1923	0.01543
Genotype:AZD	0.6	1	0.3229	0.57002
Genotype:Day	16.1	2	4.439	0.01209
AZD:Day	3.6	2	0.9862	0.37344
Genotype:AZD:Day	6.8	2	1.8714	0.15456
Residuals	1508	831		

This experiment was performed 2 times with similar results. The results from 1 representative experiment are

weight was divided by the number of seedlings in that group.

```
suppressMessages(library(readxl))
suppressMessages(library(tidyverse))
suppressMessages(library(ggpubr))
suppressMessages(library(car))
suppressMessages(library(performance))
suppressMessages(library(lmtest))
suppressMessages(library(MASS))
suppressMessages(library(WRS2))
suppressMessages(library(rstatix))
suppressMessages(library(emmeans))

# Replace the XXXXXX with your file path and make sure the variables label (i.e. gen azd and day) in the parenthesis matches the variables at the top of your columns in the spreadsheet
data2 <- read_excel("E:/XXXXXX.xlsx", sheet = "Sheet1")
data2$v1=as.factor(data2$gen)
data2$v2=as.factor(data2$azd)
data2$v3=as.factor(data2$day)
data2$v1=relevel(as.factor(data2$gen), ref='wt')
data2$v2=relevel(as.factor(data2$azd), ref='dmso')
data2$v3=relevel(as.factor(data2$day), ref='7')

# Check if data and its variables are correct
str(data2)
print(head(data2))

# Interaction means plot
ggboxplot(data2, x = "v2", y = "datax", color = "v1", facet.by = "v3",
      add = c("mean_se"),
      ylab = "Dependent Variable", xlab = "Experimental",
      palette = c("#000099", "#CC0000"))

####
data2$logdatax = log(data2$datax)

# Interaction means plot
ggboxplot(data2, x = "v2", y = "logdatax", color = "v1", facet.by = "v3",
        add = c("mean_se"),
        ylab = "Dependent Variable", xlab = "Treatment",
        palette = c("#000099", "#CC0000"))

# ANOVA analysis

# 3-way ANOVA using the untransformed data
M11 <- aov(datax ~ v1*v2*v3,data2)
summary(M11)
Anova(M11, type = "III")

# The residuals versus fittted plot, QQ residual plot, assumption checks for M11_ pass or fail?
plot(M11,which=1:3)
x
x
x
leveneTest(datax ~ v1*v2*v3,data2)
```

```
bptest(M11)
shapiro.test(x = residuals(M11))

# 3-way ANOVA using the log transformed data
M12 <- aov(logdatax ~ v1*v2*v3,data2)
summary(M12)
Anova(M12, type = "III")

# The residuals versus fittted plot, QQ residual plot, assumption checks for M12_ pass or fail?
plot(M12,which=1:3)
x
x
x
leveneTest(logdatax ~ v1*v2*v3,data2)
bptest(M12)
shapiro.test(x = residuals(M12))


# box cox transformation use the graph to determine the box cox transformation value
boxcox(M11)

# 3-way ANOVA using the boxcox transformed data
M13 <- aov((( datax ^ 0.2) - 1) / 0.2) ~ v1*v2*v3,data2)
summary(M13)
Anova(M13, type = "III")

# The residuals versus fittted plot, QQ residual plot, assumption checks for M13_ pass or fail?
plot(M13,which=1:3)
x
x
x
leveneTest(logdatax ~ v1*v2*v3,data2)
bptest(M13)
shapiro.test(x = residuals(M13))

# Weighted Least Squares
data2$logdatax = log(data2$datax)
attach(data2)
plot(x=data2$v1:data2$v3,y=data2$datax)
model.1 <- lm(datax ~ v1*v2*v3,data=data2)
summary(model.1)
Anova(model.1,type = "III")
plot(fitted(model.1), residuals(model.1), col= v1)
plot(v3, residuals(model.1))
plot(v3, abs(residuals(model.1)))
wts <- 1/fitted(lm(abs(residuals(model.1)) ~ v1:v2:v3))^2
model.2 <- lm(datax ~ v1*v2*v3,data=data2,weights = wts)
summary(model.2)
plot(fitted(model.2), rstandard(model.2), col=v3)
Anova(model.2,type = "III")

#pairwise comparison for a statistically significant interaction between variables
pwc1<-data2 %>%
  group_by(v2, v3) %>%
```

```
  emmeans_test(datax ~ v1, p.adjust.method = "bonferroni", model = model.2)
pwc1

pwc2<-data2 %>%
  group_by(v1, v3) %>%
  emmeans_test(datax ~ v2, p.adjust.method = "bonferroni", model = model.2)
pwc2

detach(data2)
```