R Code: IHC Analysis of Protein Expression in GBM PDX (Sarkaria) and RNAseq Sept 3, 2019

Project with Graham Buchan

# Load required packages to the current workspace

> library(ggplot2)

> library(dplyr)

> library(tidyr)

> library(reshape)

# # # # # # EGR1 – GOOD vs. POOR # # # # # #

> egr1 <- read.csv("Dropbox/gladson/spreadsheets/2019\_08\_27\_\_\_ihc\_\_\_egr1\_\_graham\_buchan.csv", header = TRUE)

> head(egr1)

pdx\_id bev\_resp animal\_id field\_n neg wk plus\_one plus\_two sum rna\_tpm

1 g39 good 6919 1 0.20 0.70 0.10 0 1 18

2 g39 good 6919 2 0.20 0.70 0.10 0 1 18

3 g39 good 6919 3 0.10 0.75 0.15 0 1 18

4 g39 good 6919 4 0.10 0.80 0.10 0 1 18

5 g39 good 6919 5 0.15 0.70 0.15 0 1 18

6 g39 good 6919 6 0.10 0.80 0.10 0 1 18

> str(egr1)

'data.frame': 70 obs. of 10 variables:

$ pdx\_id : Factor w/ 4 levels "g108","g39","g59",..: 2 2 2 2 2 2 2 2 2 2 ...

$ bev\_resp : Factor w/ 2 levels "good","poor": 1 1 1 1 1 1 1 1 1 1 ...

$ animal\_id: int 6919 6919 6919 6919 6919 6919 6919 7244 7244 7244 ...

$ field\_n : int 1 2 3 4 5 6 7 1 2 3 ...

$ neg : num 0.2 0.2 0.1 0.1 0.15 0.1 0.2 0.1 0.15 0.15 ...

$ wk : num 0.7 0.7 0.75 0.8 0.7 0.8 0.75 0.75 0.75 0.7 ...

$ plus\_one : num 0.1 0.1 0.15 0.1 0.15 0.1 0.05 0.15 0.1 0.15 ...

$ plus\_two : num 0 0 0 0 0 0 0 0 0 0 ...

$ sum : int 1 1 1 1 1 1 1 1 1 1 ...

$ rna\_tpm : int 18 18 18 18 18 18 18 18 18 18 ...

# Add additional columns to the dataframe

> egr1 <- egr1 %>% mutate(positive = wk + plus\_one + plus\_two)

> egr1 <- egr1 %>% mutate(plus\_one\_two = plus\_one + plus\_two)

> egr1 <- egr1 %>% mutate(h\_score = (neg\*0) + (wk\*1) + (plus\_one\_two\*2))

# Summarize the data by pdx and animal

> egr1\_avg <- egr1 %>% group\_by(pdx\_id, animal\_id) %>% summarize(bev\_resp = first(bev\_resp), neg\_avg = mean(neg), wk\_avg = mean(wk), plus\_one\_two\_avg = mean(plus\_one\_two), rna\_tpm\_avg = mean(rna\_tpm), h\_avg = mean(h\_score))

# Plot the weighted h-score, averaged by animal and color-coded by PDX as a box and whisker plot

> egr1\_avg %>% ggplot(aes(x = bev\_resp, y = h\_avg)) + geom\_boxplot(outlier.shape = NA, alpha = 0.6) + geom\_jitter(aes(color = pdx\_id), width = 0.1, size = 3) + labs(title = "EGR1 Expression in GBM PDX by Bevacizumab Response", x = "Response to Bevacizumab", y = "IHC Staining Weighted H-Score", color = "PDX")

# Create a new dataframe that melts the average staining percentages for each staining intensity

# (neg, wk, plus\_one\_two) into one column (useful for creating pie charts)

> egr1\_reshape <- as.data.frame(egr1\_avg) %>% melt(id = c("pdx\_id", "animal\_id", "bev\_resp", "rna\_tpm\_avg"))

# Plot the pie chart to show the distribution of staining intensities (one pie chart per animal)

> egr1\_reshape %>% filter(variable != "h\_avg") %>% ggplot(aes(x = "", y = value, fill = factor(variable, level = c("neg\_avg", "wk\_avg", "plus\_one\_two\_avg"), labels = c("Neg", "Weak", "1+/2+")))) + geom\_bar(stat = "identity", width = 0.1, color = "black") + coord\_polar(theta = "y", start = 0) + facet\_wrap(~ factor(bev\_resp, level = c("poor", "good"), labels = c("Non-Responder", "Responder")) + pdx\_id + animal\_id, ncol = 5) + theme(axis.text = element\_blank(), panel.grid = element\_blank()) + labs(x = "", y = "", fill = "Staining Strength") + labs(title = "EGR1 Expression in GBM PDX by Bevacizumab Response using Immunohistochemical Staining", x = "", y = "", fill = "Staining Strength") + scale\_fill\_manual(values = c("white", "grey", "black"))



# Same pie chart as above but with brown color scheme (to match DAB substrate color)

> egr1\_reshape %>% filter(variable != "h\_avg") %>% ggplot(aes(x = "", y = value, fill = factor(variable, level = c("neg\_avg", "wk\_avg", "plus\_one\_two\_avg"), labels = c("Neg", "Weak", "1+/2+")))) + geom\_bar(stat = "identity", width = 10, color = "white") + coord\_polar(theta = "y", start = 0) + facet\_wrap(~ factor(bev\_resp, level = c("poor", "good"), labels = c("Non-Responder", "Responder")) + pdx\_id + animal\_id, ncol = 5) + theme(axis.text = element\_blank(), panel.grid = element\_blank()) + labs(title = "EGR1 Expression in GBM PDX by Bevacizumab Response using Immunohistochemical Staining", x = "", y = "", fill = "Staining Strength") + scale\_fill\_manual(values = c("#ffd9b3", "#ffa64d", "#994d00"))



# # # # # # EGR1 – BEV vs. PLACEBO # # # # # #

> egr1\_bev\_placebo <- read.csv("Dropbox/gladson/spreadsheets/2019\_08\_27\_\_\_ihc\_\_\_egr1\_\_graham\_buchan\_\_\_bev\_vs\_placebo.csv", header = TRUE)

> str(egr1\_bev\_placebo)

'data.frame': 140 obs. of 11 variables:

$ pdx\_id : Factor w/ 2 levels "g108","g64": 2 2 2 2 2 2 2 2 2 2 ...

$ bev\_resp : Factor w/ 1 level "poor": 1 1 1 1 1 1 1 1 1 1 ...

$ animal\_id : int 2632 2632 2632 2632 2632 2632 2632 2714 2714 2714 ...

$ staining\_type: Factor w/ 2 levels "cytoplasmic",..: 2 2 2 2 2 2 2 2 2 2 ...

$ field\_n : int 1 2 3 4 5 6 7 1 2 3 ...

$ treatment : Factor w/ 2 levels "bev","placebo": 2 2 2 2 2 2 2 2 2 2 ...

$ neg : num 0.25 0.3 0.2 0.15 0.2 0.25 0.25 0.55 0.6 0.5 ...

$ wk : num 0.6 0.5 0.6 0.7 0.7 0.65 0.65 0.45 0.4 0.4 ...

$ plus\_one : num 0.15 0.2 0.2 0.15 0.1 0.1 0.1 0 0 0.1 ...

$ plus\_two : num 0 0 0 0 0 0 0 0 0 0 ...

$ sum : int 1 1 1 1 1 1 1 1 1 1 ...

> head(egr1\_bev\_placebo)

pdx\_id bev\_resp animal\_id staining\_type field\_n treatment neg wk plus\_one plus\_two sum

1 g64 poor 2632 nuclear 1 placebo 0.25 0.60 0.15 0 1

2 g64 poor 2632 nuclear 2 placebo 0.30 0.50 0.20 0 1

3 g64 poor 2632 nuclear 3 placebo 0.20 0.60 0.20 0 1

4 g64 poor 2632 nuclear 4 placebo 0.15 0.70 0.15 0 1

5 g64 poor 2632 nuclear 5 placebo 0.20 0.70 0.10 0 1

6 g64 poor 2632 nuclear 6 placebo 0.25 0.65 0.10 0 1

# Add additional columns to the dataframe

> egr1\_bev\_placebo <- egr1\_bev\_placebo %>% mutate(positive = wk + plus\_one + plus\_two)

> egr1\_bev\_placebo <- egr1\_bev\_placebo %>% mutate(plus\_one\_two = plus\_one + plus\_two)

> egr1\_bev\_placebo <- egr1\_bev\_placebo %>% mutate(h\_score = (neg\*0) + (wk\*1) + (plus\_one\_two\*2))

# Summarize the data by pdx and animal in separate df for each nuclear and cytoplasmic staining

> egr1\_bev\_placebo\_avg\_nuc <- egr1\_bev\_placebo %>% filter(staining\_type == "nuclear") %>% group\_by(pdx\_id, animal\_id, treatment) %>% summarize(staining\_type = first(staining\_type), neg\_avg = mean(neg), wk\_avg = mean(wk), plus\_one\_two\_avg = mean(plus\_one\_two), h\_avg = mean(h\_score))

> egr1\_bev\_placebo\_avg\_cyto <- egr1\_bev\_placebo %>% filter(staining\_type == "cytoplasmic") %>% group\_by(pdx\_id, animal\_id, treatment) %>% summarize(staining\_type = first(staining\_type), neg\_avg = mean(neg), wk\_avg = mean(wk), plus\_one\_two\_avg = mean(plus\_one\_two), h\_avg = mean(h\_score))



# Plot the weighted h-score, averaged by animal and color-coded by bev response (nuclear)

> egr1\_bev\_placebo\_avg\_nuc %>% ggplot(aes(x = treatment, y = h\_avg)) + geom\_boxplot(outlier.shape = NA, alpha = 0.6) + geom\_jitter(aes(color = pdx\_id), width = 0.1, size = 3) + labs(title = "Effect of Bev on Nuclear EGR1 Expression in GBM PDX", x = "", y = "IHC Staining Weighted H-Score", color = "PDX")



# Plot the weighted h-score, averaged by animal and color-coded by bev response (nuclear)

> egr1\_bev\_placebo\_avg\_cyto %>% ggplot(aes(x = treatment, y = h\_avg)) + geom\_boxplot(outlier.shape = NA, alpha = 0.6) + geom\_jitter(aes(color = pdx\_id), width = 0.1, size = 3) + labs(title = "Effect of Bev on Cytoplasmic EGR1 Expression in GBM PDX", x = "", y = "IHC Staining Weighted H-Score", color = "PDX")

# Create a new dataframe that melts the average staining percentages for each staining intensity

> egr1\_bev\_placebo\_avg\_nuc\_reshape <- as.data.frame(egr1\_bev\_placebo\_avg\_nuc) %>% filter(staining\_type == "nuclear") %>% select(pdx\_id, animal\_id, treatment, neg\_avg, wk\_avg, plus\_one\_two\_avg, h\_avg) %>% melt(id = c("pdx\_id", "animal\_id", "treatment"))

# Plot the pie chart to show the distribution of staining intensities (one pie chart per animal)

> egr1\_bev\_placebo\_avg\_nuc\_reshape %>% filter(variable != "h\_avg") %>% ggplot(aes(x = "", y = value, fill = factor(variable, level = c("neg\_avg", "wk\_avg", "plus\_one\_two\_avg"), labels = c("Neg", "Weak", "1+/2+")))) + geom\_bar(stat = "identity", width = 0.1, color = "black") + coord\_polar(theta = "y", start = 0) + facet\_wrap(~ factor(treatment, level = c("placebo", "bev"), labels = c("Placebo", "Bevacizumab")) + pdx\_id + animal\_id, ncol = 5) + theme(axis.text = element\_blank(), panel.grid = element\_blank()) + labs(title = "Effect of Bev on Nuclear EGR1 Expression in GBM PDX", x = "", y = "", fill = "Staining Strength") + scale\_fill\_manual(values = c("white", "grey", "black"))



# Same pie chart as above but with brown color scheme (to match DAB substrate color)

> egr1\_bev\_placebo\_avg\_nuc\_reshape %>% filter(variable != "h\_avg") %>% ggplot(aes(x = "", y = value, fill = factor(variable, level = c("neg\_avg", "wk\_avg", "plus\_one\_two\_avg"), labels = c("Neg", "Weak", "1+/2+")))) + geom\_bar(stat = "identity", width = 0.1, color = "white") + coord\_polar(theta = "y", start = 0) + facet\_wrap(~ factor(treatment, level = c("placebo", "bev"), labels = c("Placebo", "Bevacizumab")) + pdx\_id + animal\_id, ncol = 5) + theme(axis.text = element\_blank(), panel.grid = element\_blank()) + labs(title = "Effect of Bev on Nuclear EGR1 Expression in GBM PDX", x = "", y = "", fill = "Staining Strength") + scale\_fill\_manual(values = c("#ffd9b3", "#ffa64d", "#994d00"))



# # # # # # ILF3 – GOOD vs. POOR # # # # # #

> ilf3 <- read.csv("Dropbox/gladson/spreadsheets/2019\_08\_27\_\_\_ihc\_\_\_ilf3\_\_graham\_buchan.csv", header = TRUE)

> head(ilf3)

pdx\_id bev\_resp animal\_id field\_n neg wk plus\_one plus\_two sum rna\_tpm

1 g39 good 6919 1 0.20 0.70 0.10 0 1 18

2 g39 good 6919 2 0.20 0.70 0.10 0 1 18

3 g39 good 6919 3 0.10 0.75 0.15 0 1 18

4 g39 good 6919 4 0.10 0.80 0.10 0 1 18

5 g39 good 6919 5 0.15 0.70 0.15 0 1 18

6 g39 good 6919 6 0.10 0.80 0.10 0 1 18

> str(ilf3)

'data.frame': 70 obs. of 10 variables:

$ pdx\_id : Factor w/ 4 levels "g108","g39","g59",..: 2 2 2 2 2 2 2 2 2 2 ...

$ bev\_resp : Factor w/ 2 levels "good","poor": 1 1 1 1 1 1 1 1 1 1 ...

$ animal\_id: int 6919 6919 6919 6919 6919 6919 6919 7244 7244 7244 ...

$ field\_n : int 1 2 3 4 5 6 7 1 2 3 ...

$ neg : num 0.2 0.2 0.1 0.1 0.15 0.1 0.2 0.1 0.15 0.15 ...

$ wk : num 0.7 0.7 0.75 0.8 0.7 0.8 0.75 0.75 0.75 0.7 ...

$ plus\_one : num 0.1 0.1 0.15 0.1 0.15 0.1 0.05 0.15 0.1 0.15 ...

$ plus\_two : num 0 0 0 0 0 0 0 0 0 0 ...

$ sum : int 1 1 1 1 1 1 1 1 1 1 ...

$ rna\_tpm : int 18 18 18 18 18 18 18 18 18 18 ...

# Add additional columns to the dataframe

> ilf3 <- ilf3 %>% mutate(positive = wk + plus\_one + plus\_two)

> ilf3 <- ilf3 %>% mutate(plus\_one\_two = plus\_one + plus\_two)

> ilf3 <- ilf3 %>% mutate(h\_score = (neg\*0) + (wk\*1) + (plus\_one\_two\*2))

# Summarize the data by pdx and animal

> ilf3\_avg <- ilf3 %>% group\_by(pdx\_id, animal\_id) %>% summarize(bev\_resp = first(bev\_resp), neg\_avg = mean(neg), wk\_avg = mean(wk), plus\_one\_two\_avg = mean(plus\_one\_two), rna\_tpm\_avg = mean(rna\_tpm), h\_avg = mean(h\_score))

# Plot the weighted h-score, averaged by animal and color-coded by PDX as a box and whisker plot

> ilf3\_avg %>% ggplot(aes(x = bev\_resp, y = h\_avg)) + geom\_boxplot(outlier.shape = NA, alpha = 0.6) + geom\_jitter(aes(color = pdx\_id), width = 0.1, size = 3) + labs(title = "ILF3 Expression in GBM PDX by Bevacizumab Response", x = "Response to Bevacizumab", y = "IHC Staining Weighted H-Score", color = "PDX")



# Create a new dataframe that melts the average staining percentages for each staining intensity

# (neg, wk, plus\_one\_two) into one column (useful for creating pie charts)

> ilf3\_reshape <- as.data.frame(egr1\_avg) %>% melt(id = c("pdx\_id", "animal\_id", "bev\_resp", "rna\_tpm\_avg"))

# Plot the pie chart to show the distribution of staining intensities (one pie chart per animal)

> ilf3\_reshape %>% filter(variable != "h\_avg") %>% ggplot(aes(x = "", y = value, fill = factor(variable, level = c("neg\_avg", "wk\_avg", "plus\_one\_two\_avg"), labels = c("Neg", "Weak", "1+/2+")))) + geom\_bar(stat = "identity", width = 0.1, color = "black") + coord\_polar(theta = "y", start = 0) + facet\_wrap(~ factor(bev\_resp, level = c("poor", "good"), labels = c("Non-Responder", "Responder")) + pdx\_id + animal\_id, ncol = 5) + theme(axis.text = element\_blank(), panel.grid = element\_blank()) + labs(x = "", y = "", fill = "Staining Strength") + labs(title = "ILF3 Expression in GBM PDX by Bevacizumab Response using Immunohistochemical Staining", x = "", y = "", fill = "Staining Strength") + scale\_fill\_manual(values = c("white", "grey", "black"))



# Same pie chart as above but with brown color scheme (to match DAB substrate color)

> ilf3\_reshape %>% filter(variable != "h\_avg") %>% ggplot(aes(x = "", y = value, fill = factor(variable, level = c("neg\_avg", "wk\_avg", "plus\_one\_two\_avg"), labels = c("Neg", "Weak", "1+/2+")))) + geom\_bar(stat = "identity", width = 10, color = "white") + coord\_polar(theta = "y", start = 0) + facet\_wrap(~ factor(bev\_resp, level = c("poor", "good"), labels = c("Non-Responder", "Responder")) + pdx\_id + animal\_id, ncol = 5) + theme(axis.text = element\_blank(), panel.grid = element\_blank()) + labs(title = "ILF3 Expression in GBM PDX by Bevacizumab Response using Immunohistochemical Staining", x = "", y = "", fill = "Staining Strength") + scale\_fill\_manual(values = c("#ffd9b3", "#ffa64d", "#994d00"))



# # # # # # ILF3 – BEV vs. PLACEBO # # # # # #

> ilf3\_bev\_placebo <- read.csv("Dropbox/gladson/spreadsheets/2019\_08\_27\_\_\_ihc\_\_\_ilf3\_\_graham\_buchan\_\_\_bev\_vs\_placebo.csv", header = TRUE)

> str(ilf3\_bev\_placebo)

'data.frame': 140 obs. of 11 variables:

$ pdx\_id : Factor w/ 2 levels "g108","g64": 2 2 2 2 2 2 2 2 2 2 ...

$ bev\_resp : Factor w/ 1 level "poor": 1 1 1 1 1 1 1 1 1 1 ...

$ animal\_id : int 2632 2632 2632 2632 2632 2632 2632 2714 2714 2714 ...

$ staining\_type: Factor w/ 2 levels "cytoplasmic",..: 2 2 2 2 2 2 2 2 2 2 ...

$ field\_n : int 1 2 3 4 5 6 7 1 2 3 ...

$ treatment : Factor w/ 2 levels "bev","placebo": 2 2 2 2 2 2 2 2 2 2 ...

$ neg : num 0.15 0.35 0.35 0.25 0.25 0.15 0.25 0.6 0.6 0.55 ...

$ wk : num 0.65 0.4 0.4 0.45 0.55 0.65 0.55 0.2 0.4 0.35 ...

$ plus\_one : num 0.2 0.25 0.25 0.3 0.2 0.2 0.2 0.2 0 0.1 ...

$ plus\_two : int 0 0 0 0 0 0 0 0 0 0 ...

$ sum : int 1 1 1 1 1 1 1 1 1 1 ...

> head(ilf3\_bev\_placebo)

pdx\_id bev\_resp animal\_id staining\_type field\_n treatment neg wk plus\_one plus\_two sum

1 g64 poor 2632 nuclear 1 placebo 0.15 0.65 0.20 0 1

2 g64 poor 2632 nuclear 2 placebo 0.35 0.40 0.25 0 1

3 g64 poor 2632 nuclear 3 placebo 0.35 0.40 0.25 0 1

4 g64 poor 2632 nuclear 4 placebo 0.25 0.45 0.30 0 1

5 g64 poor 2632 nuclear 5 placebo 0.25 0.55 0.20 0 1

6 g64 poor 2632 nuclear 6 placebo 0.15 0.65 0.20 0 1

# Add additional columns to the dataframe

> ilf3\_bev\_placebo <- ilf3\_bev\_placebo %>% mutate(positive = wk + plus\_one + plus\_two)

> ilf3\_bev\_placebo <- ilf3\_bev\_placebo %>% mutate(plus\_one\_two = plus\_one + plus\_two)

> ilf3\_bev\_placebo <- ilf3\_bev\_placebo %>% mutate(h\_score = (neg\*0) + (wk\*1) + (positive\*2))

# Summarize the data by pdx and animal in separate df for each nuclear and cytoplasmic staining

> ilf3\_bev\_placebo\_avg\_nuc <- ilf3\_bev\_placebo %>% filter(staining\_type == "nuclear") %>% group\_by(pdx\_id, animal\_id, treatment) %>% summarize(staining\_type = first(staining\_type), neg\_avg = mean(neg), wk\_avg = mean(wk), plus\_one\_two\_avg = mean(plus\_one\_two), h\_avg = mean(h\_score))

> ilf3\_bev\_placebo\_avg\_cyto <- ilf3\_bev\_placebo %>% filter(staining\_type == "cytoplasmic") %>% group\_by(pdx\_id, animal\_id, treatment) %>% summarize(staining\_type = first(staining\_type), neg\_avg = mean(neg), wk\_avg = mean(wk), plus\_one\_two\_avg = mean(plus\_one\_two), h\_avg = mean(h\_score))



# Plot the weighted h-score, averaged by animal and color-coded by bev response (nuclear)

> ilf3\_bev\_placebo\_avg\_nuc %>% ggplot(aes(x = treatment, y = h\_avg)) + geom\_boxplot(outlier.shape = NA, alpha = 0.6) + geom\_jitter(aes(color = pdx\_id), width = 0.1, size = 3) + labs(title = "Effect of Bev on Nuclear ILF3 Expression in GBM PDX", x = "", y = "IHC Staining Weighted H-Score", color = "PDX")



# Plot the weighted h-score, averaged by animal and color-coded by bev response (cytoplasmic)

> ilf3\_bev\_placebo\_avg\_cyto %>% ggplot(aes(x = treatment, y = h\_avg)) + geom\_boxplot(outlier.shape = NA, alpha = 0.6) + geom\_jitter(aes(color = pdx\_id), width = 0.1, size = 3) + labs(title = "Effect of Bev on Cytoplasmic ILF3 Expression in GBM PDX", x = "", y = "IHC Staining Weighted H-Score", color = "PDX")

# Create a new dataframe that melts the average staining percentages for each staining intensity

> ilf3\_bev\_placebo\_avg\_nuc\_reshape <- as.data.frame(ilf3\_bev\_placebo\_avg\_nuc) %>% filter(staining\_type == "nuclear") %>% select(pdx\_id, animal\_id, treatment, neg\_avg, wk\_avg, plus\_one\_two\_avg, h\_avg) %>% melt(id = c("pdx\_id", "animal\_id", "treatment"))

# Plot the pie chart to show the distribution of staining intensities (one pie chart per animal)

> ilf3\_bev\_placebo\_avg\_nuc\_reshape %>% filter(variable != "h\_avg") %>% ggplot(aes(x = "", y = value, fill = factor(variable, level = c("neg\_avg", "wk\_avg", "plus\_one\_two\_avg"), labels = c("Neg", "Weak", "1+/2+")))) + geom\_bar(stat = "identity", width = 0.1, color = "black") + coord\_polar(theta = "y", start = 0) + facet\_wrap(~ factor(treatment, level = c("placebo", "bev"), labels = c("Placebo", "Bevacizumab")) + pdx\_id + animal\_id, ncol = 5) + theme(axis.text = element\_blank(), panel.grid = element\_blank()) + labs(title = "Effect of Bev on Cytoplasmic ILF3 Expression in GBM PDX", x = "", y = "", fill = "Staining Strength") + scale\_fill\_manual(values = c("white", "grey", "black"))



# Same pie chart as above but with brown color scheme (to match DAB substrate color)

> ilf3\_bev\_placebo\_avg\_nuc\_reshape %>% filter(variable != "h\_avg") %>% ggplot(aes(x = "", y = value, fill = factor(variable, level = c("neg\_avg", "wk\_avg", "plus\_one\_two\_avg"), labels = c("Neg", "Weak", "1+/2+")))) + geom\_bar(stat = "identity", width = 0.1, color = "white") + coord\_polar(theta = "y", start = 0) + facet\_wrap(~ factor(treatment, level = c("placebo", "bev"), labels = c("Placebo", "Bevacizumab")) + pdx\_id + animal\_id, ncol = 5) + theme(axis.text = element\_blank(), panel.grid = element\_blank()) + labs(title = "Effect of Bev on Nuclear ILF3 Expression in GBM PDX", x = "", y = "", fill = "Staining Strength") + scale\_fill\_manual(values = c("#ffd9b3", "#ffa64d", "#994d00"))

