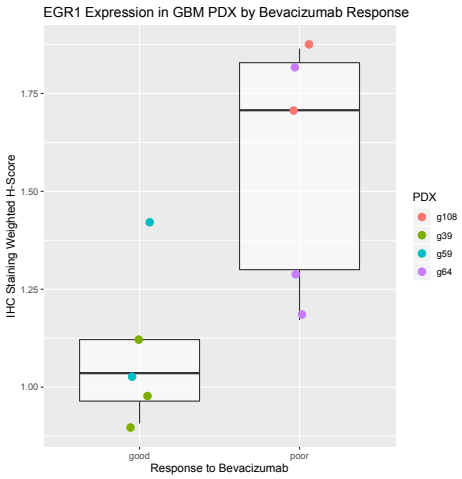
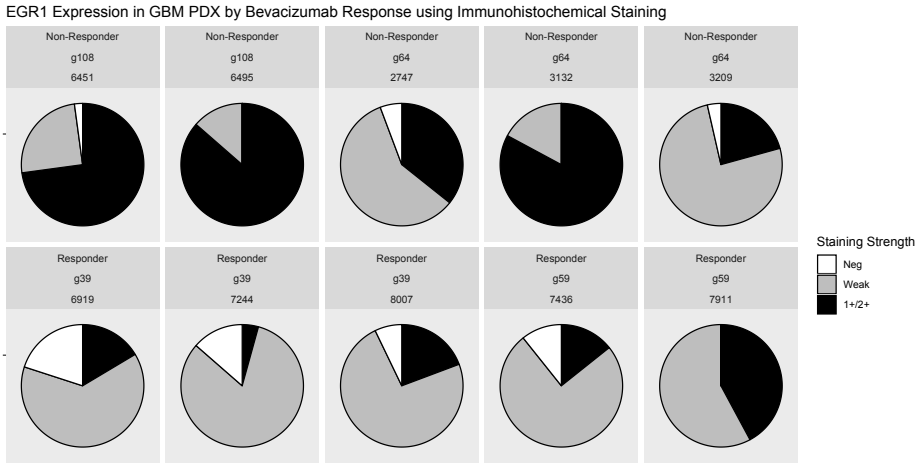
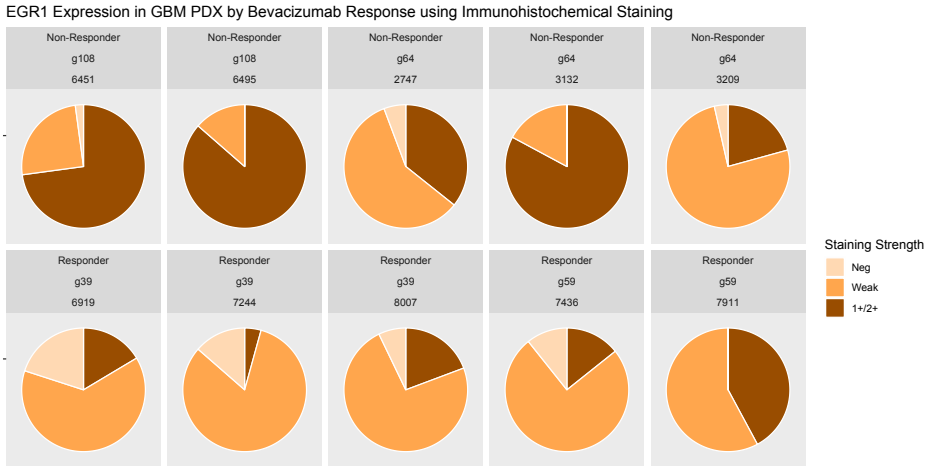
```
# Load required packages to the current workspace

> 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"))
```

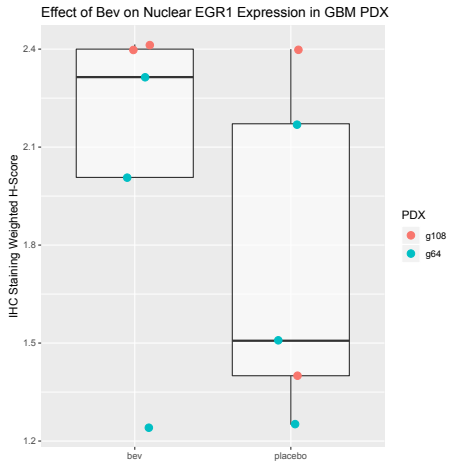



```
# Same pie chart as above but with brown color scheme (to match DAB substrate color)

> 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"))
```

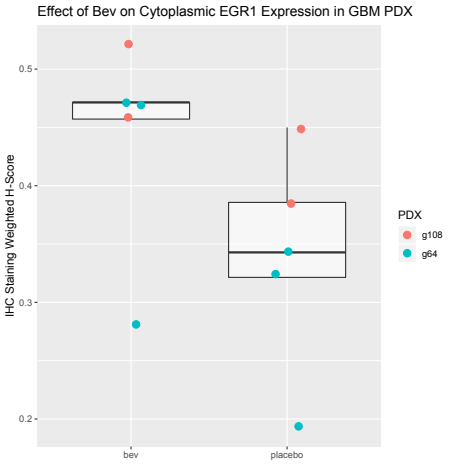


EGR1 – BEV vs. PLACEBO # # # # #

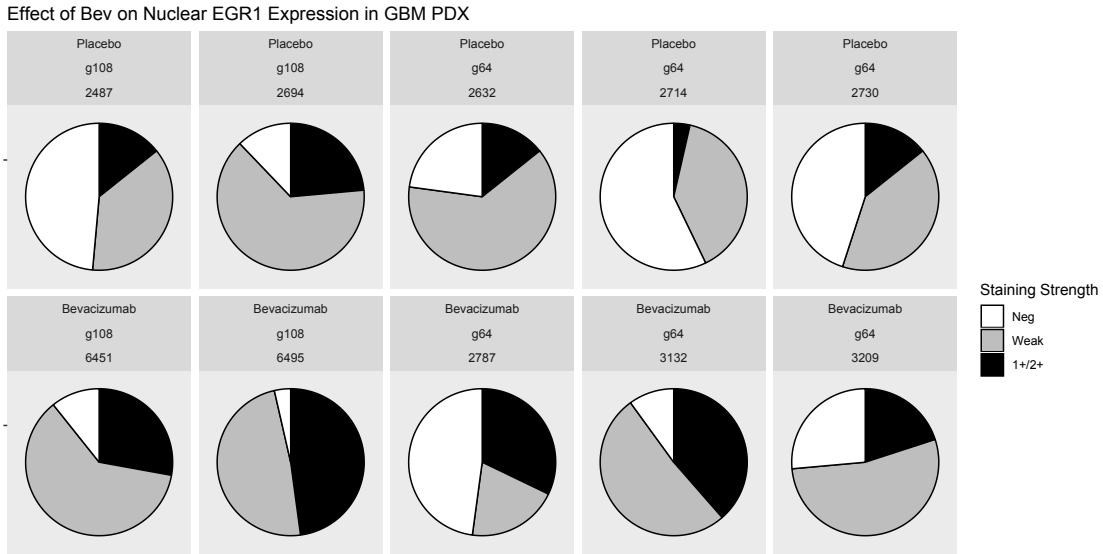
```
> egr1_bev_placebo <-
read.csv("Dropbox/gladson/spreadsheets/2019_08_27___ihc___egr1__graham_buchan___bev_vs_placebo.csv
", header = TRUE)

> 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")
```



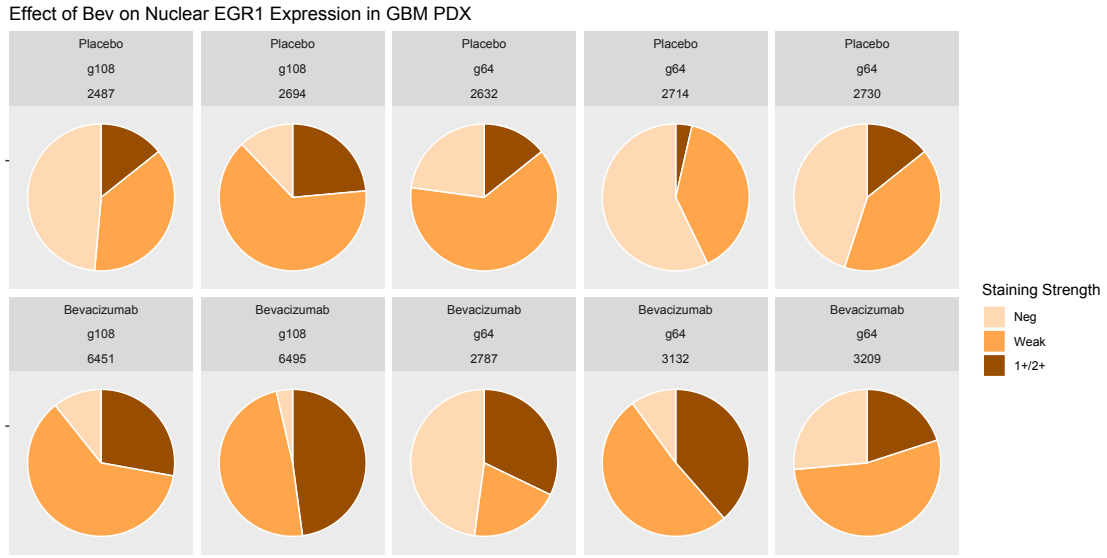
```
# Plot the weighted h-score, averaged by animal and color-coded by bev response (nuclear)

> 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"))
```



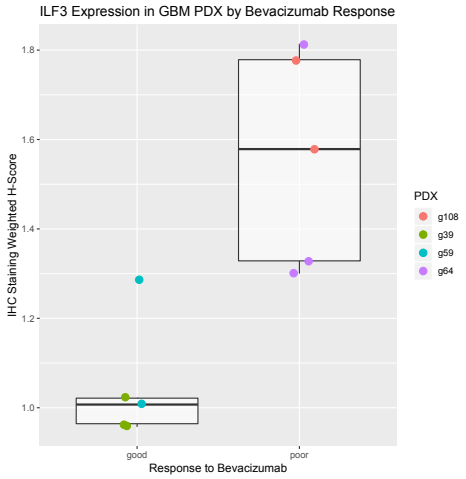
```
# Same pie chart as above but with brown color scheme (to match DAB substrate color)

> 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"))
```



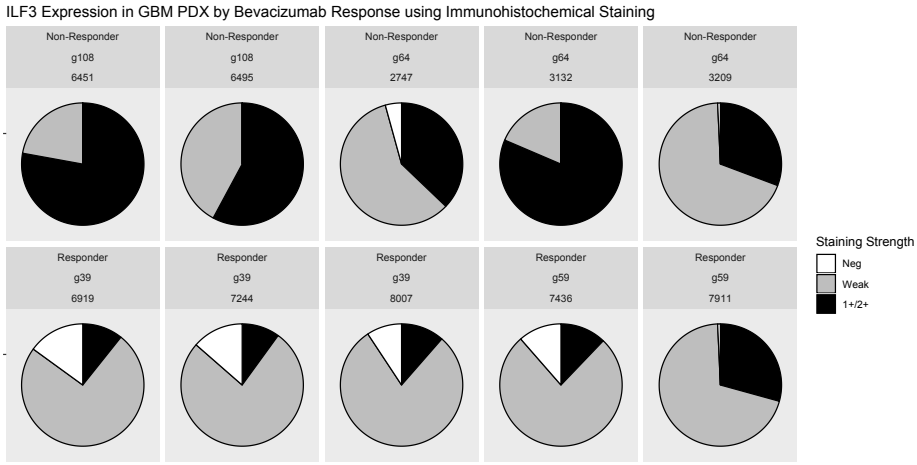
ILF3 – GOOD vs. POOR # # # # #

```
> 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 ...
```

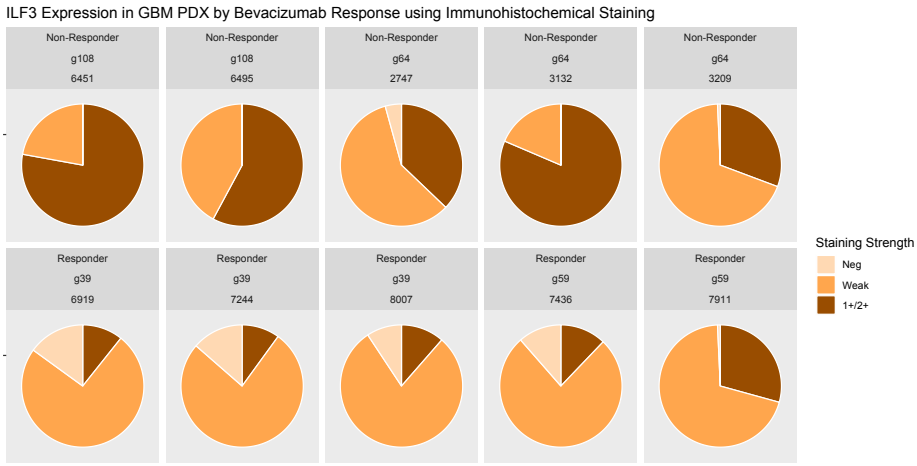
```
# Add additional columns to the dataframe

> 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"))
```

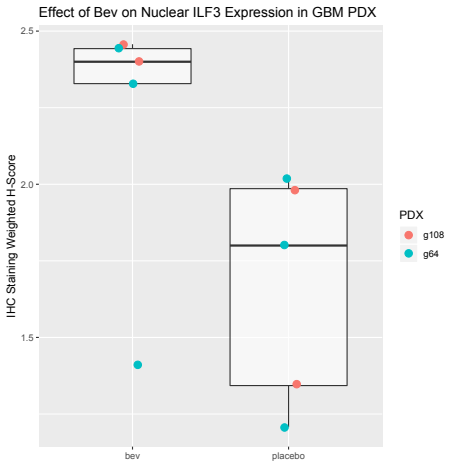



```
# Same pie chart as above but with brown color scheme (to match DAB substrate color)

> 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"))
```

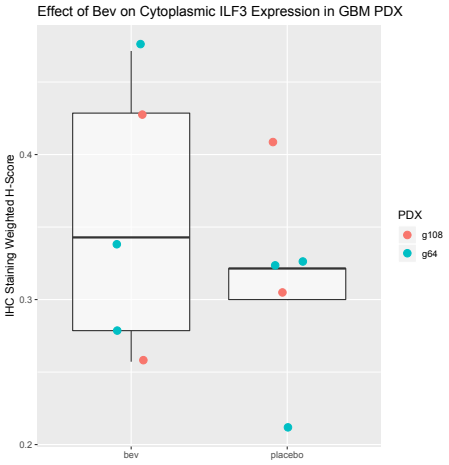


ILF3 – BEV vs. PLACEBO # # # # #

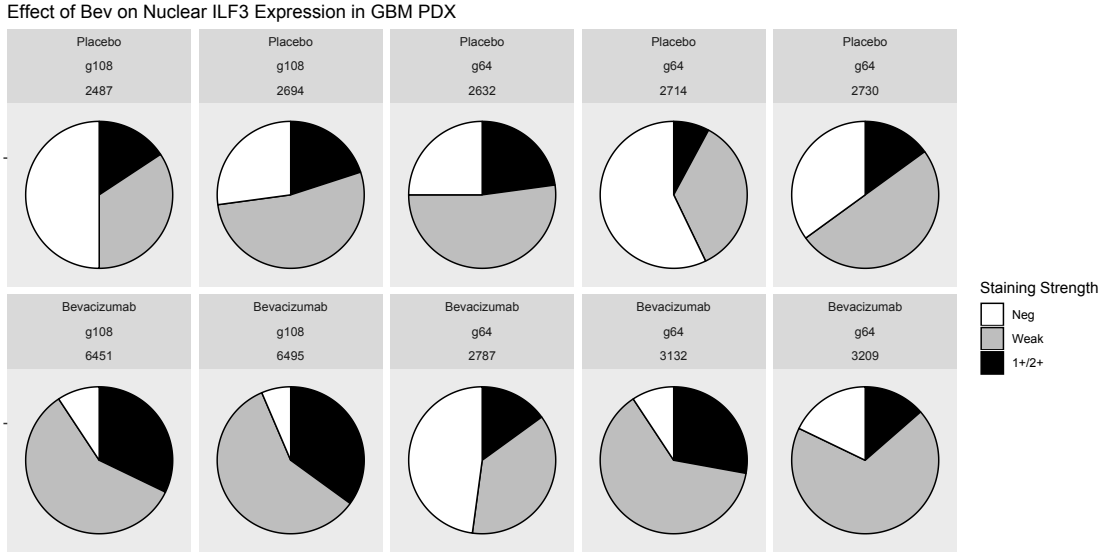
```
> ilf3_bev_placebo <-
read.csv("Dropbox/gladson/spreadsheets/2019_08_27___ihc___ilf3__graham_buchan___bev_vs_placebo.csv
", header = TRUE)

> 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"))
```