Tumors that respond poorly to bevacizumab show upregulation of angiogenesis genes.

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```
knitr::opts_chunk$set(warning = FALSE, # turn off warnings
                      message = FALSE,
                      results = 'hide') # hide console output
knitr::opts_chunk$set(fig.width = 10, fig.height = 7) # set figure height and width
```

Loading packages and tools for bulk RNA-sequencing analysis

Load packages

```
for (package in c('BiocManager', 'tidyverse', 'matrixStats', 'cowplot', 'DT', 'plotly', 'gt', 'ggrepel'
  if (!require(package, character.only = T, quietly = T)) {
    install.packages(package,
      repos = "http://cran.us.r-project.org")
    library(package, character.only = T)
  }
}

bio_pkgs <- c("biomaRt", "tximport", "ensembldb", "EnsDb.Hsapiens.v86", "edgeR", "DESeq2", "limma", "ap
#BiocManager::install(bio_pkgs)
invisible(lapply(bio_pkgs, function (x) library(x, character.only = T)))
```

Load MART, design matrix, and counts

```
design <- read_tsv("input/prefilterstudydesign.txt")
sampleLabels <- design$sample
group <- factor(design$group)

gbmexpr <- read_csv("input/prefiltergbmexpr.csv")[2: 13] #pre-filtered
```

Preprocessing raw counts data

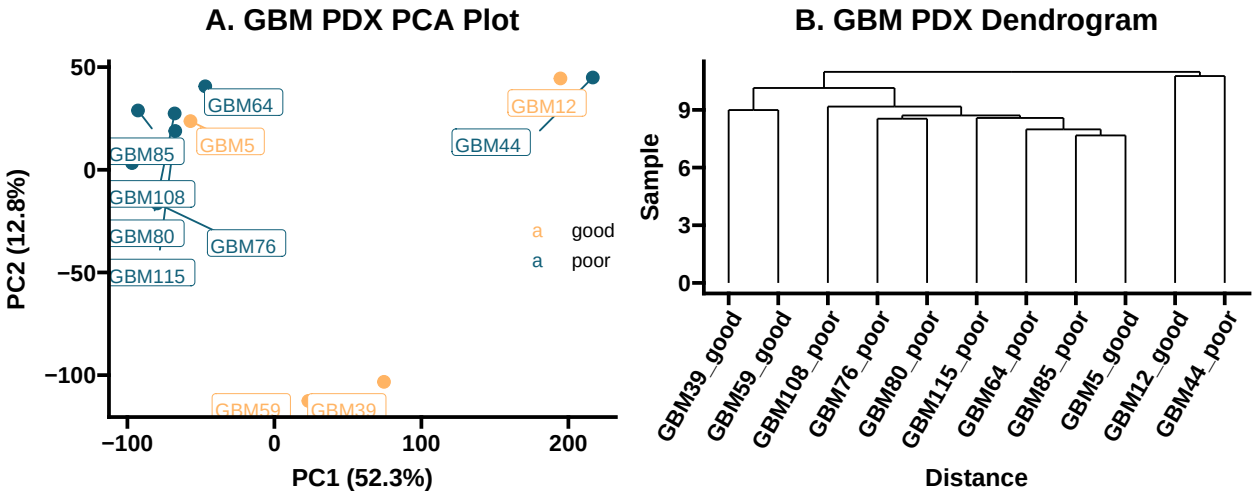
```
gbmexpr.matrix <- as.matrix(gbmexpr[, -1])
rownames(gbmexpr.matrix) <- unlist(gbmexpr[, 1])
myDGEList <- DGEList(gbmexpr.matrix)
myDGEList.filtered.norm <- calcNormFactors(myDGEList, method = "TMM") #normalize using TMM
log2.tpm.filtered.norm <- log2(gbmexpr.matrix + 1)
log2.tpm.filtered.norm.df <- as_tibble(log2.tpm.filtered.norm, rownames = "geneID")
```

Preprocessed PCA plot and clustering dendrogram

```
distance <- dist(t(log2.tpm.filtered.norm), method = "maximum")
clusters <- hclust(distance, method = "average")
den <- ggdendrogram(clusters) +
  labs(title = "B. GBM PDX Dendrogram") +
  xlab("Distance") +
  ylab("Sample") +
  theme_prism() +
  theme(axis.text.x = element_text(angle = 60, vjust = 1, hjust = 1))
##ggsave(path = "./plots/", filename = "dendrogram.png", plot = den, height = 5, width = 5)

sampleLabels <- substr(sampleLabels, 1, nchar(sampleLabels) - 5)
pca.res <- prcomp(t(log2.tpm.filtered.norm), scale. = F, retx = T)
pc.var <- pca.res$sdev ^ 2
pc.per <- round(pc.var / sum(pc.var) * 100, 1)
pca.res.df <- as_tibble(pca.res$x)
pca.plot <- ggplot(pca.res.df) +
  aes(x = PC1, y = PC2, label = sampleLabels, color = group) +
  geom_point(size = 3) +
  geom_label_repel(aes(label = sampleLabels), hjust = 0, vjust = 0) +
  scale_color_manual(values = c("#ffb464", "#126079")) +
  xlab(paste0("PC1 (", pc.per[1], "%", ")")) +
  ylab(paste0("PC2 (", pc.per[2], "%", ")")) +
  labs(title = "A. GBM PDX PCA Plot") +
  theme_prism() +
  theme(legend.position = c(0.9, 0.5))
##ggsave(path = "./plots/", filename = "prefilterpca.png", plot = pca.plot, height = 5, width = 7)

sf1 <- ggarrange(pca.plot, den, ncol = 2, nrow = 1)
sf1
```




```
#ggexport(sf1, filename = "./plots/supplementalfigure1.png", width = 1000, height = 500)
```

Dimensionality reduction analysis

Create counts matrix

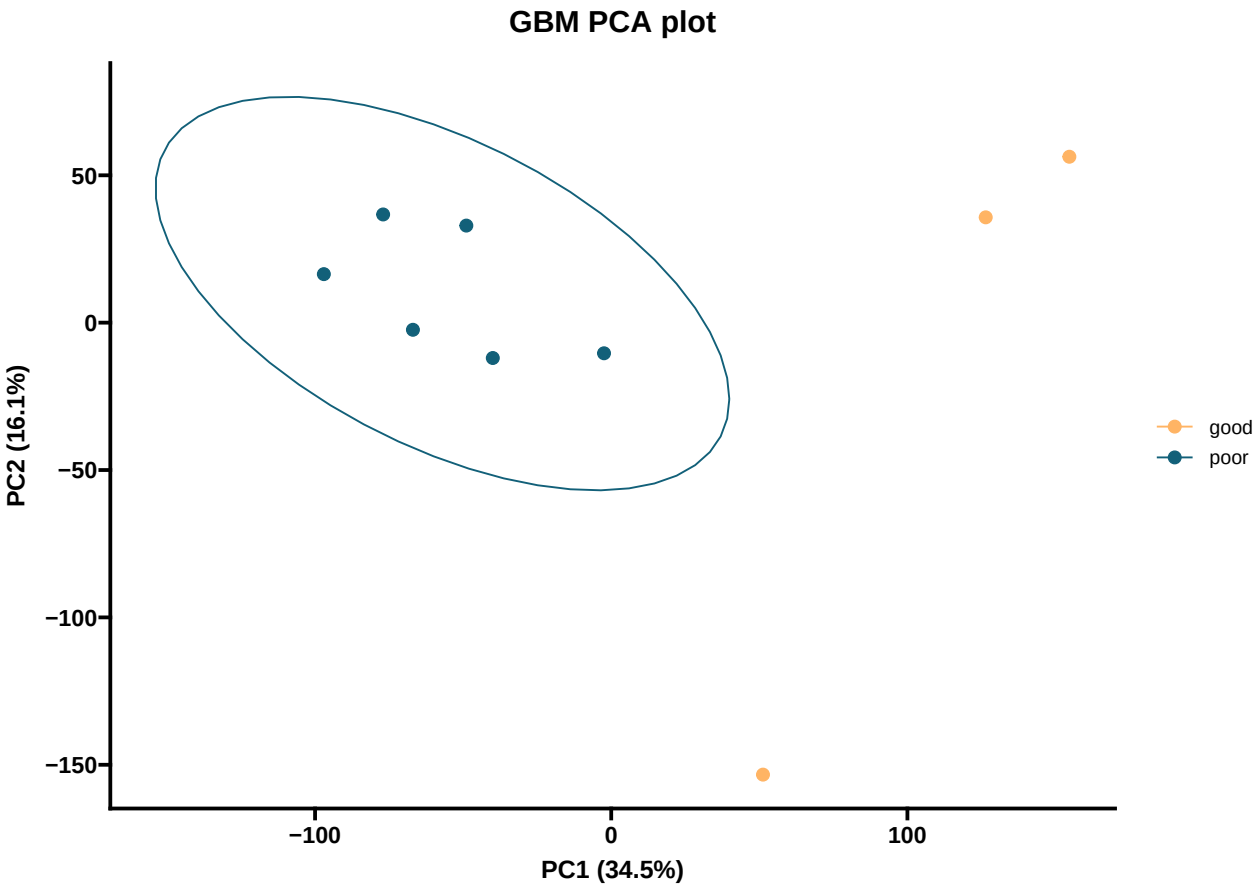
```
design <- read_tsv("./input/studydesign.txt")
sampleLabels <- design$sample
group <- factor(design$group)
mm <- model.matrix(~0 + group)

counts <- read.table(file = "./input/counts.tabular", header = TRUE, sep = "\t")
colnames(counts) <- c("geneID", sampleLabels)
counts$gene <- getSYMBOL(as.character(counts$geneID), data = 'org.Hs.eg')
counts <- counts %>% select(gene, everything())
counts <- counts[rowSums(counts <= 0) <= 3, ] %>% drop_na() #filter: at most 3 zeros
counts.matrix <- as.matrix(counts[3: 11])
rownames(counts.matrix) <- counts$gene
```

PCA

```
pca.res <- prcomp(t(log(counts.matrix + 1)), scale. = F, retx = T)
pc.var <- pca.res$sdev ^ 2
pc.per <- round(pc.var / sum(pc.var) * 100, 1)
pca.res.df <- as_tibble(pca.res$x)
pca.plot <- ggplot(pca.res.df) +
  aes(x = PC1, y = PC2, label = sampleLabels, color = group) +
  geom_point(size = 3) +
  #geom_text(aes(label = sampleLabels), hjust = 0, vjust = 0) +
  #stat_ellipse() +
  scale_color_manual(values = c("#ffb464", "#126079")) +
  xlab(paste0("PC1 (", pc.per[1], "%", ")")) +
```

```
  ylab(paste0("PC2 (", pc.per[2], "%", ")")) +
  labs(title = "GBM PCA plot") +
  # caption = paste0("produced on ", Sys.time())) +
  stat_ellipse() +
  theme_prism()
# #ggsave(path = "./plots/", filename = "postfilterpca.png", plot = pca.plot, height = 5, width = 7)
pca.plot
```



We choose not to perform UMAP due to limited sample size and use of PDX tumors.

Differential gene expression analysis

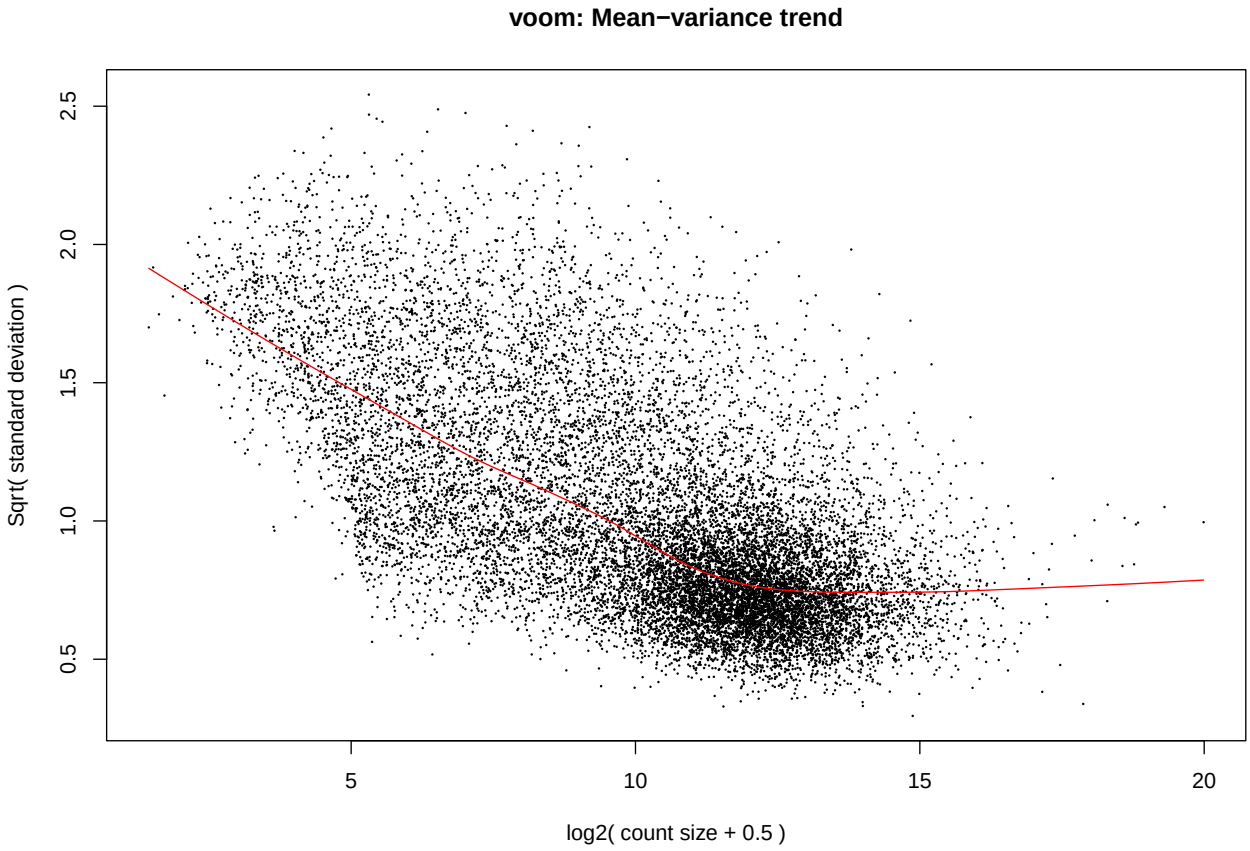
Processing counts data

```
mart = useEnsembl(biomart='ensembl', dataset = "hsapiens_gene_ensembl", mirror = "useast")
#mart <- useMart("ENSEMBL_MART_ENSEMBL") #coding genes for cleaning
#mart <- useDataset("hsapiens_gene_ensembl", mart)

coding_genes <- getBM(attributes = c("hgnc_symbol"),
  filters = c("biotype"),
  values = list(biotype = "protein_coding"),
  mart = mart)$hgnc_symbol
```

```
rownames(counts.matrix) <- counts$geneID
dds <- DESeqDataSetFromMatrix(countData = round(counts.matrix),
  colData = design,
  design = ~group)

keep <- rowSums(counts(dds)) >= 350 #determined via hyperparameter exploration
dds <- dds[keep, ]
dds <- DESeq(dds)
dds <- estimateSizeFactors(dds)
deseqvoom <- voom(counts(dds, normalized = TRUE), mm, plot = T)
```



Differential gene expression analysis

```
res <- results(dds)
res <- lfcShrink(dds, coef = "group_poor_vs_good", type = "ashr")
```

```
## using 'ashr' for LFC shrinkage. If used in published research, please cite:
##     Stephens, M. (2016) False discovery rates: a new deal. Biostatistics, 18:2.
##     https://doi.org/10.1093/biostatistics/kxw041
```

```
deseq <- as.data.frame(res) %>% drop_na() %>% arrange(padj) %>% arrange(desc(abs(log2FoldChange)))
deseq$gene <- getSYMBOL(rownames(deseq), data = 'org.Hs.eg')
deseq <- dplyr::filter(deseq, gene %in% coding_genes)
```

```r
deseq <- deseq %>%
  mutate(enrichment = case_when(
    (padj < 0.05) & (log2FoldChange > 1) ~"poor",
    (padj < 0.05) & (log2FoldChange <- 1) ~"good"))
deseq$enrichment[is.na(deseq$enrichment)] <- "none"
rownames(deseq) <- deseq$gene
#write_csv(deseq, "./tables/dge.csv")
head(deseq, 10)
```

```
##          baseMean log2FoldChange     lfcSE       pvalue         padj   gene
## MXRA5   1759.7796      10.873968 2.0639850 1.378951e-10 1.282932e-08  MXRA5
## FIGNL2   436.4693      10.618612 1.3501858 8.036464e-18 2.708289e-15 FIGNL2
## DPP10   2903.6767      10.550579 2.3641414 8.834061e-09 5.113303e-07  DPP10
## SHD     3326.0768      10.543546 1.0904000 1.100572e-24 1.192155e-21    SHD
## IGLON5  2932.8203      10.406657 1.5991788 1.909022e-13 3.272259e-11 IGLON5
## SYT13    925.2397      10.358431 1.9234684 1.523300e-10 1.408589e-08  SYT13
## NCAN   38401.3357      10.214752 0.6536549 2.376496e-57 3.603955e-53   NCAN
## SIX6     646.6990      10.041625 1.8530796 1.971098e-10 1.800705e-08   SIX6
## SCN3B   1313.9016       9.852600 1.3113978 3.510798e-16 9.859491e-14  SCN3B
## VGF    27367.6704       9.781803 1.4479717 8.682615e-14 1.586408e-11    VGF
##        enrichment
## MXRA5        poor
## FIGNL2       poor
## DPP10        poor
## SHD          poor
## IGLON5       poor
## SYT13        poor
## NCAN         poor
## SIX6         poor
## SCN3B        poor
## VGF          poor
```

```r
dgeplot <- ggplot(deseq) +
  aes(y = -log10(padj), x = log2FoldChange, colour = enrichment) +
  scale_color_manual(values = c("good" = "#126079", "none" = "grey", "poor" = "#ffb464")) +
  geom_point(size = 1.5, alpha = 0.25) +
  #facet_zoom(xlim = c(9, 11), zoom.size = 1) +
  geom_rect(mapping = aes(xmin = 9, xmax = 11, ymin = 2, ymax = 54), alpha = 0, color = 'black') +
  geom_text_repel(size = 4, data = head(deseq, 20), aes(label = gene), max.overlaps = Inf, colour = "bla
  #geom_text_repel(size = 3, data = subset(deseq, gene == 'EGR1'), aes(label = gene)) +
  geom_hline(yintercept = -log10(0.05), linetype = "longdash", colour = "black", size = .5) +
  geom_text(aes(-8, -log10(0.05), label = "p = 0.05", vjust = 1), colour = "black") +
  labs(title = "A. Differential RNA Expression") +
  #subtitle = "Positive logFC indicates upregulation in poor Bevacizumab responders") +
  xlab("Fold Change (log2)") +
  ylab("Significance (log10)") +
  theme_prism() +
  theme(legend.position = c(0.15, 0.8))
##ggsave(filename = "./plots/deseq.png", plot = dgeplot, height = 6, width = 6)
#dgeplot
```

Collagen Gene Set Analysis

```
collagen_genes <- c("COL1A1", "COL1A2", "COL2A1", "COL3A1", "COL4A1", "COL4A2", "COL4A3", "COL4A4", "COL

collagen_genes <- deseq %>% filter(gene %in% collagen_genes) %>% filter(enrichment != "none")
collagen_genes <- collagen_genes$gene

collagen <- counts %>%
  filter(gene %in% collagen_genes) %>%
  dplyr::select(-geneID)
rownames(collagen) <- collagen$gene
collagen <- collagen %>%
  dplyr::select(-gene)
collagen <- as.data.frame(t(collagen))
collagen$sample <- rownames(collagen)
rownames(collagen) <- NULL
collagen$group <- gsub("GBM[0-9]*_", "", collagen$sample)
collagen <- collagen %>% pivot_longer(cols = -c("sample", "group"),
  names_to = "gene",
  values_to = "expression")

collagen$log.expr <- log(collagen$expression + 1)
collagen$sample <- substr(collagen$sample,
  1,
  nchar(collagen$sample) - 5)

collagen_plot <- ggplot(data = collagen, mapping = aes(x = sample, y = gene, fill = log.expr)) +
  geom_tile() +
  scale_fill_gradient(high = "#ffb464", low = "#126079") +
  scale_colour_prism(palette = "colors") +
  xlab(label = "Patient Derived Xenograft") + # Add a nicer x-axis title
  ggtitle("D. Collagen Genes RNA Expression") +
  facet_grid(~group,
    switch = "x", scales = "free_x", space = "free_x") +
  #labs(color = "Your title here") +
  theme_prism() +
  theme(axis.title.y = element_blank(),
    axis.text.x = element_text(angle = 60, vjust = 0.7),
    legend.position = "bottom")
##ggsave(filename = "./plots/collagen.png", plot = collagen_plot, height = 6, width = 6)
#collagen_plot
```

Angiogenesis Gene Set Analysis

```
blood_vessel <- scan("./input/blood_vessel_geneset.txt", character(), quote = "")
blood_vessel <- deseq %>% filter((gene %in% blood_vessel) &
  (enrichment != "none") &
  (abs(log2FoldChange) > 4))

# angiogenesis heatmap
goi <- blood_vessel$gene
```

```
b_v_heatmap <- counts %>% filter(gene %in% goi) %>% dplyr::select(-geneID)
rownames(b_v_heatmap) <- b_v_heatmap$gene
b_v_heatmap <- b_v_heatmap %>% dplyr::select(-gene)
b_v_heatmap <- as.data.frame(t(b_v_heatmap))
b_v_heatmap$sample <- rownames(b_v_heatmap)
rownames(b_v_heatmap) <- NULL
b_v_heatmap$group <- gsub("GBM[0-9]*_", "", b_v_heatmap$sample)
b_v_heatmap <- b_v_heatmap %>% pivot_longer(cols = -c("sample", "group"),
  names_to = "gene",
  values_to = "expression")

b_v_heatmap$log.expr <- log(b_v_heatmap$expression + 1)
b_v_heatmap$sample <- substr(b_v_heatmap$sample, 1, nchar(b_v_heatmap$sample) - 5)

b_v_heatmap.plot <- ggplot(data = b_v_heatmap, mapping = aes(x = sample, y = gene, fill = log.expr)) +
  geom_tile() +
  scale_fill_gradient(high = "#ffb464", low = "#126079") +
  scale_colour_prism(palette = "colors") +
  xlab(label = "Patient Derived Xenograft") + # Add a nicer x - axis title
  ggtitle("C. Angiogenic Gene RNA Expression") +
  facet_grid(~group,
    switch = "x", scales = "free_x", space = "free_x") +
  #labs(color = "Your title here") +
  theme_prism() +
  theme(axis.title.y = element_blank(),
    axis.text.x = element_text(angle = 60, vjust = 0.7),
    legend.position = "bottom")
#b_v_heatmap.plot
##ggsave(filename = "./plots/angiogenesis.png",
```

Gene set enrichment anlaysis (GSEA)

GSEA data cleaning and KEGG pathway analysis

```
hs_gsea <- msigdbr(species = "Homo sapiens")
hs_gsea %>% dplyr::distinct(gs_cat, gs_subcat) %>% dplyr::arrange(gs_cat, gs_subcat)
hs_gsea_h <- msigdbr(species = "Homo sapiens",
    category = "H") %>%
  dplyr::select(gs_name, gene_symbol)
hs_gsea_kegg <- msigdbr(species = "Homo sapiens",
    category = "C2",
    subcategory = "CP:KEGG") %>%
  dplyr::select(gs_name, gene_symbol)

deseq.GSEA.select <- dplyr::select(deseq, gene, log2FoldChange, padj)
deseq.gsea <- abs(deseq.GSEA.select$log2FoldChange) / deseq.GSEA.select$log2FoldChange * -log10(deseq.GS
names(deseq.gsea) <- as.character(deseq.GSEA.select$gene)
deseq.gsea <- sort(deseq.gsea, decreasing = TRUE)
deseq.gsea.res <- GSEA(deseq.gsea, pvalueCutoff = 1, TERM2GENE = hs_gsea_kegg, verbose = FALSE)
deseq.GSEA.df <- as_tibble(deseq.gsea.res @result)
```

```
deseq.GSEA.df <- deseq.GSEA.df %>%
  mutate(phenotype = case_when(
    (NES > 0) & (p.adjust < 0.05) ~"poor",
    (NES < 0) & (p.adjust < 0.05) ~"good"))
deseq.GSEA.df$phenotype[is.na(deseq.GSEA.df$phenotype)] <- "none"
deseq.GSEA.df$Description <- gsub("_", " ", deseq.GSEA.df$Description)
#write_csv(deseq.GSEA.df, "./tables/gsea_kegg.csv")
```

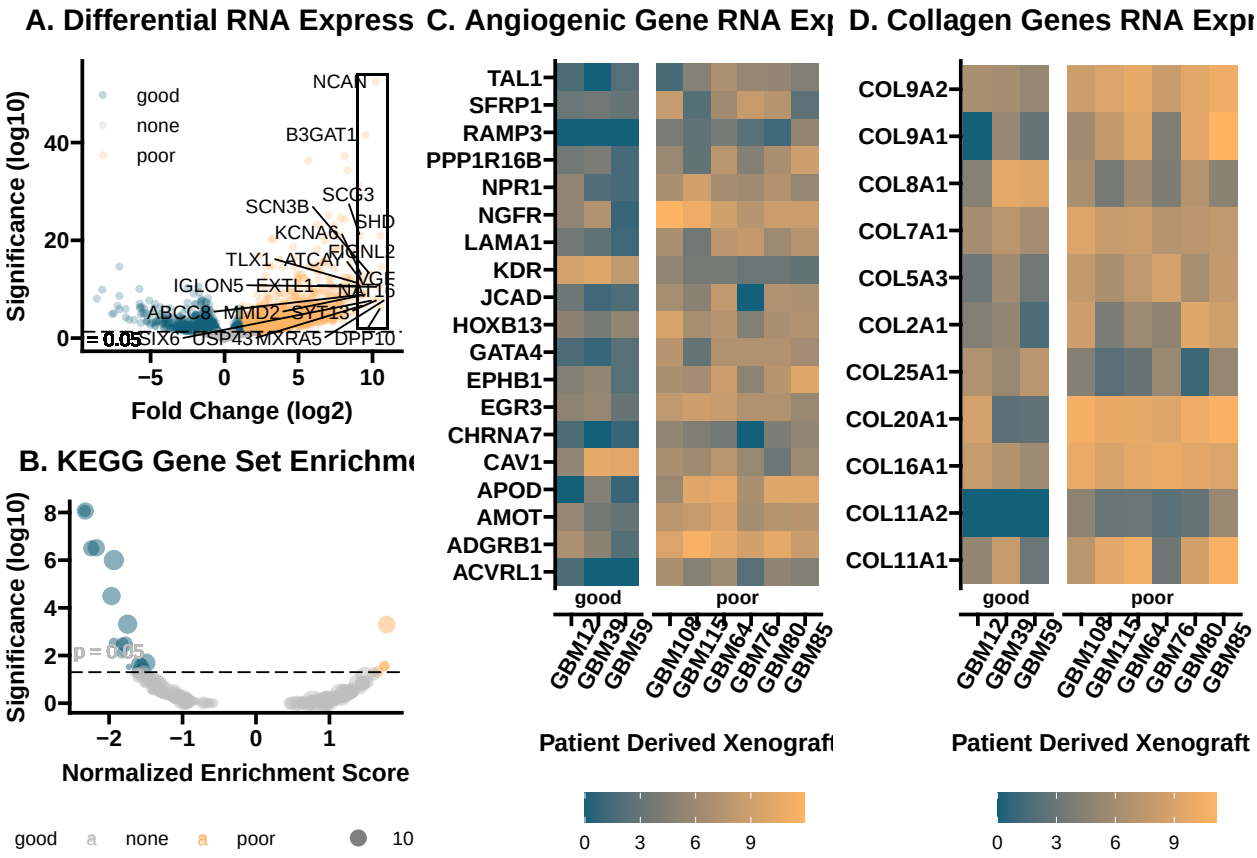
```
## # A tibble: 23 x 2
##    gs_cat gs_subcat
##    <chr>  <chr>
##  1 C1     ""
##  2 C2     "CGP"
##  3 C2     "CP"
##  4 C2     "CP:BIOCARTA"
##  5 C2     "CP:KEGG"
##  6 C2     "CP:PID"
##  7 C2     "CP:REACTOME"
##  8 C2     "CP:WIKIPATHWAYS"
##  9 C3     "MIR:MIRDB"
## 10 C3     "MIR:MIR_Legacy"
## # ... with 13 more rows
```

GSEA plot

```
kegg_gsea <- ggplot(deseq.GSEA.df, aes(x = NES, y = -log10(p.adjust), color = phenotype)) +
  geom_point(aes(size = setSize), alpha = 0.5) +
  scale_color_manual(values = c("good" = "#126079", "none" = "grey", "poor" = "#ffb464")) +
  geom_text(aes(-2, -log10(0.05), label = "p = 0.05", vjust = -1)) +
  geom_hline(yintercept = -log10(0.05), linetype = "longdash", size = .5) +
  #geom_text_repel(size = 4, data = (deseq.GSEA.df %>% dplyr::filter((NES > 0) & (p.adjust < 0.05)))[1,
  #  aes(label = Description)) +
  labs(title = "B. KEGG Gene Set Enrichment") +
  # subtitle = "Positive NES indicates upregulation of gene set in poor Bevacizumab responders") +
  ylab("Significance (log10)") +
  xlab("Normalized Enrichment Score") +
  theme_prism() +
  theme(legend.position = "bottom")
##ggsave(path = "./plots/", filename = "gsea_kegg.png", plot = kegg_gsea, width = 6, height = 6)
#kegg_gsea
```

Figure 1

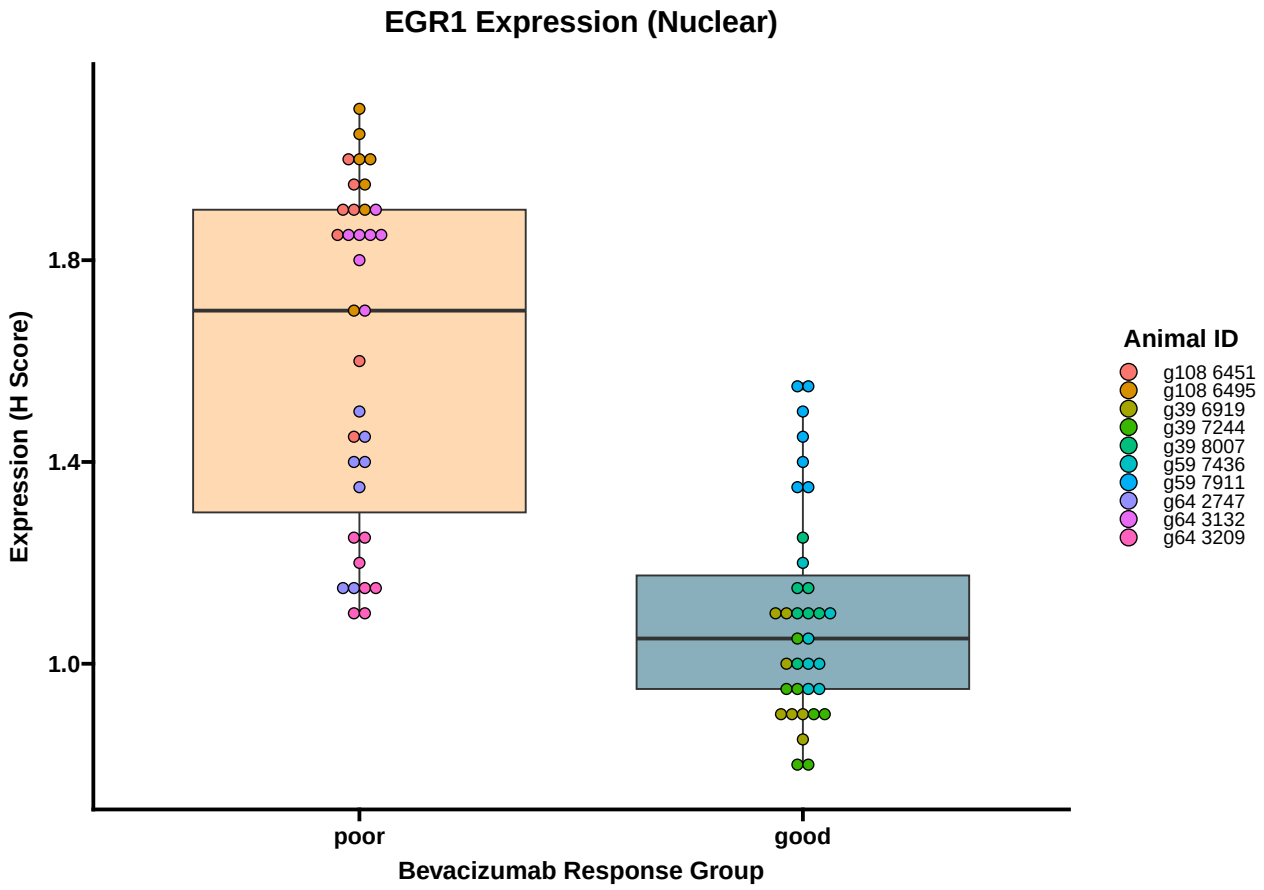
```
f1 <- ggarrange(ggarrange(dgeplot, kegg_gsea, ncol = 1, nrow = 2),
                b_v_heatmap.plot, collagen_plot, ncol = 3, nrow = 1)
f1
```



Immunohistochemical staining analysis

IHC-quantified protein experssion of EGR1 (good vs poor)

```
egr1.poor.good <- read.csv("./input/ihc/egr1poorgood.csv")
egr1.poor.good$fullname <- paste(egr1.poor.good$pdx_id, egr1.poor.good$animal_id)
ggplot(egr1.poor.good, aes(x = fct_rev(bev_resp), y = h_score)) +
  geom_boxplot(alpha = 0.5, fill = c("#ffb464", "#126079")) +
  geom_dotplot(aes(fill = factor(fullname)),
    binaxis = "y",
    stackdir = "center",
    dotsize = 0.5,
    binpositions = "all",
    stackgroups = TRUE) +
  theme_prism() +
  theme(
    legend.key.height = unit(10, "pt"),
    legend.title = element_text()) +
  guides(fill = guide_legend(title = "Animal ID")) +
  #stat_compare_means(label.x.npc = "left", label.y.npc = "bottom") +
  ggtitle("EGR1 Expression (Nuclear)") +
  xlab("Bevacizumab Response Group") + ylab("Expression (H Score)")
```



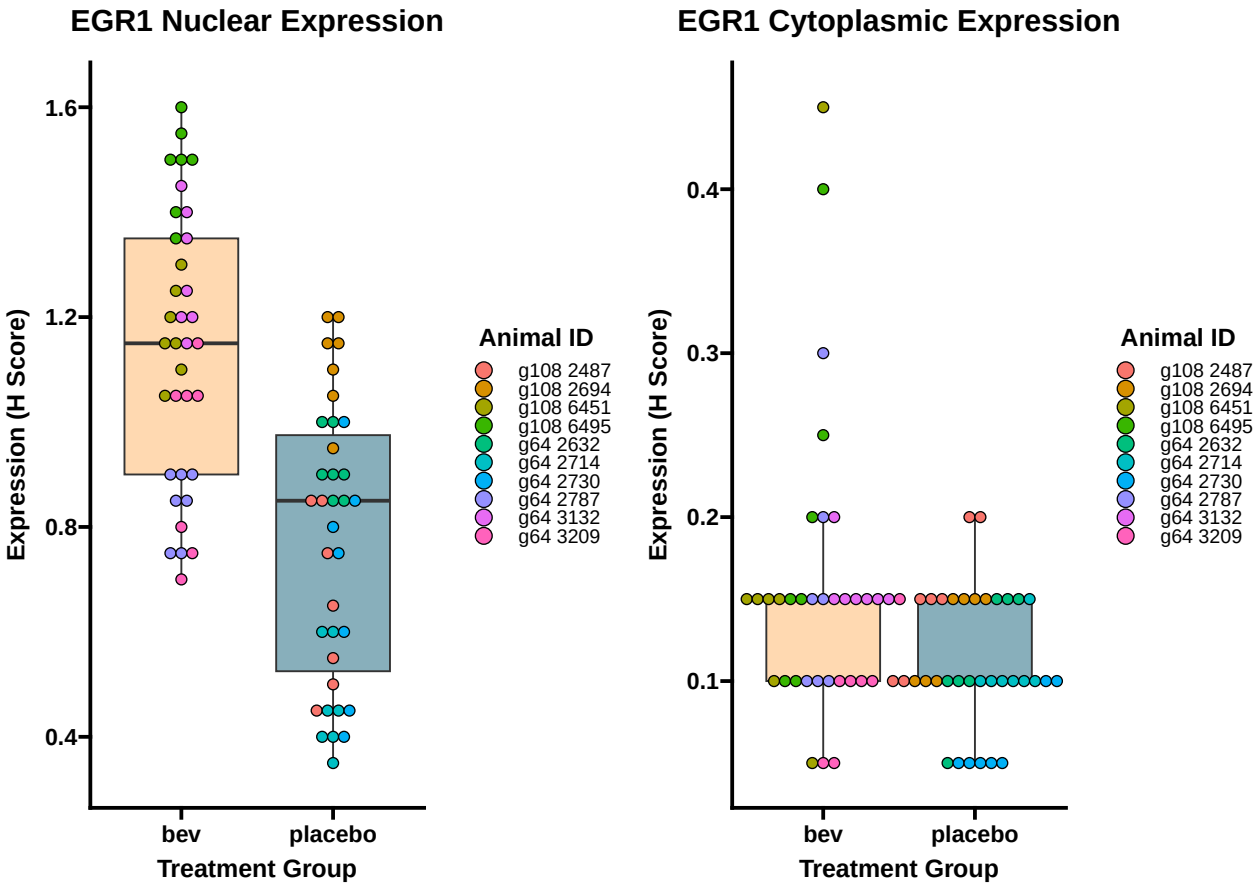

```
wilcox.test((egr1.poor.good %>% dplyr::filter(bev_resp == "good")) $h_score,
  (egr1.poor.good %>% dplyr::filter(bev_resp == "poor")) $h_score)
```

IHC-quantified protein experssion of EGR1 (poor vs placebo)

```
egr1.poor.placebo <- read.csv("./input/ihc/egr1poorplacebo.csv")
egr1.poor.placebo$fullname <- paste(egr1.poor.placebo$pdx_id, egr1.poor.placebo$animal_id)
poor.placebo.nuc <- ggplot(egr1.poor.placebo %>% dplyr::filter(staining_type == "nuclear"),
    aes(x = treatment, y = h_score)) +
  geom_boxplot(alpha = 0.5, fill = c("#ffb464", "#126079")) +
  geom_dotplot(aes(fill = factor(fullname)),
    binaxis = "y",
    stackdir = "center",
    dotsize = 0.5,
    binpositions = "all",
    stackgroups = TRUE) +
  theme_prism() +
  theme(
    legend.key.height = unit(10, "pt"),
    legend.title = element_text()) +
  guides(fill = guide_legend(title = "Animal ID")) +
  ggtitle("EGR1 Nuclear Expression") +
  xlab("Treatment Group") + ylab("Expression (H Score)")
```

```
poor.placebo.cyt <- ggplot(egr1.poor.placebo %>% dplyr::filter(staining_type == "cytoplasmic"),
    aes(x = treatment, y = h_score)) +
  geom_boxplot(alpha = 0.5, fill = c("#ffb464", "#126079")) +
  geom_dotplot(aes(fill = factor(fullname)),
    binaxis = "y",
    stackdir = "center",
    dotsize = 0.5,
    binpositions = "all",
    stackgroups = TRUE) +
  theme_prism() +
  theme(
    legend.key.height = unit(10, "pt"),
    legend.title = element_text()) +
  guides(fill = guide_legend(title = "Animal ID")) +
  #stat_compare_means(label.x.npc = "left", label.y.npc = "bottom") +
  ggtitle("EGR1 Cytoplasmic Expression") +
  xlab("Treatment Group") + ylab("Expression (H Score)")

sf2 <- ggarrange(poor.placebo.nuc, poor.placebo.cyt, nrow = 1, ncol = 2)
sf2
```




```
# Chi-squared
rawihc <- read_csv("./input/ihc/ihcraw.csv")
ihc <- rawihc %>%
  group_by(animal_id, bev_resp) %>%
```

```
  summarise_at(vars(neg, wk, plus_one), list(mean = mean))

#write.csv(ihc, "/input/ihc/ihc.csv")
#sortedihc <- read_csv("/input/ihc/ihc.csv")
sig <- ihc %>%
  rowwise() %>%
  mutate(
    test_stat = chisq.test(c(neg_mean, wk_mean, plus_one_mean))$statistic,
    p_val = chisq.test(c(neg_mean, wk_mean, plus_one_mean))$p.value
  )
#write.csv(sig, "ihc.csv")

ihc <- rawihc %>%
  group_by(bev_resp) %>%
  summarise_at(vars(neg, wk, plus_one), list(mean = mean))
chisq.test(c(0.103, 0.704, 0.187), c(0.0229, 0.38, 0.55))
```

Survival Analysis

Load packages

```
pkgs <- c("UCSCXenaTools", "dplyr", "survival", "survminer", "ggbreak", "ggprism", "svglite")
#BiocManager::install(pkgs)
invisible(lapply(pkgs, function (x) suppressMessages(library(x, character.only = T))))
```

Download TCGA glioblastoma dataset

```
gbm_cohort = XenaData %>%
  filter(XenaHostNames == "tcgaHub") %>%
  XenaScan("TCGA Glioblastoma") # microarray dataset, CNVA dataset DNA level

#download clinical data-- --
cli_query = gbm_cohort %>%
  filter(DataSubtype == "phenotype") %>% # select clinical dataset
  XenaGenerate() %>% # generate a XenaHub object
  XenaQuery() %>%
  XenaDownload()
cli = XenaPrepare(cli_query)

ge = gbm_cohort %>%
  filter(DataSubtype == "protein expression RPPA", Label == "RPPA (replicate-base normalization)")
# TODO: try AFFYmetrix

# download gene expression data
ge = gbm_cohort %>%
  filter(DataSubtype == "gene expression RNAseq", Label == "IlluminaHiSeq")
EGR1 = fetch_dense_values(host = ge$XenaHosts,
  dataset = ge$XenaDatasets,
```

```
  identifiers = "EGR1",
  use_probeMap = TRUE) %>% .[1, ]
EGR3 = fetch_dense_values(host = ge$XenaHosts,
  dataset = ge$XenaDatasets,
  identifiers = "EGR3",
  use_probeMap = TRUE) %>% .[1, ]
SOX10 = fetch_dense_values(host = ge$XenaHosts,
  dataset = ge$XenaDatasets,
  identifiers = "SOX10",
  use_probeMap = TRUE) %>% .[1, ]
RAMP3 = fetch_dense_values(host = ge$XenaHosts,
  dataset = ge$XenaDatasets,
  identifiers = "RAMP3",
  use_probeMap = TRUE) %>% .[1, ]
CHRNA7 = fetch_dense_values(host = ge$XenaHosts,
  dataset = ge$XenaDatasets,
  identifiers = "CHRNA7",
  use_probeMap = TRUE) %>% .[1, ]
```

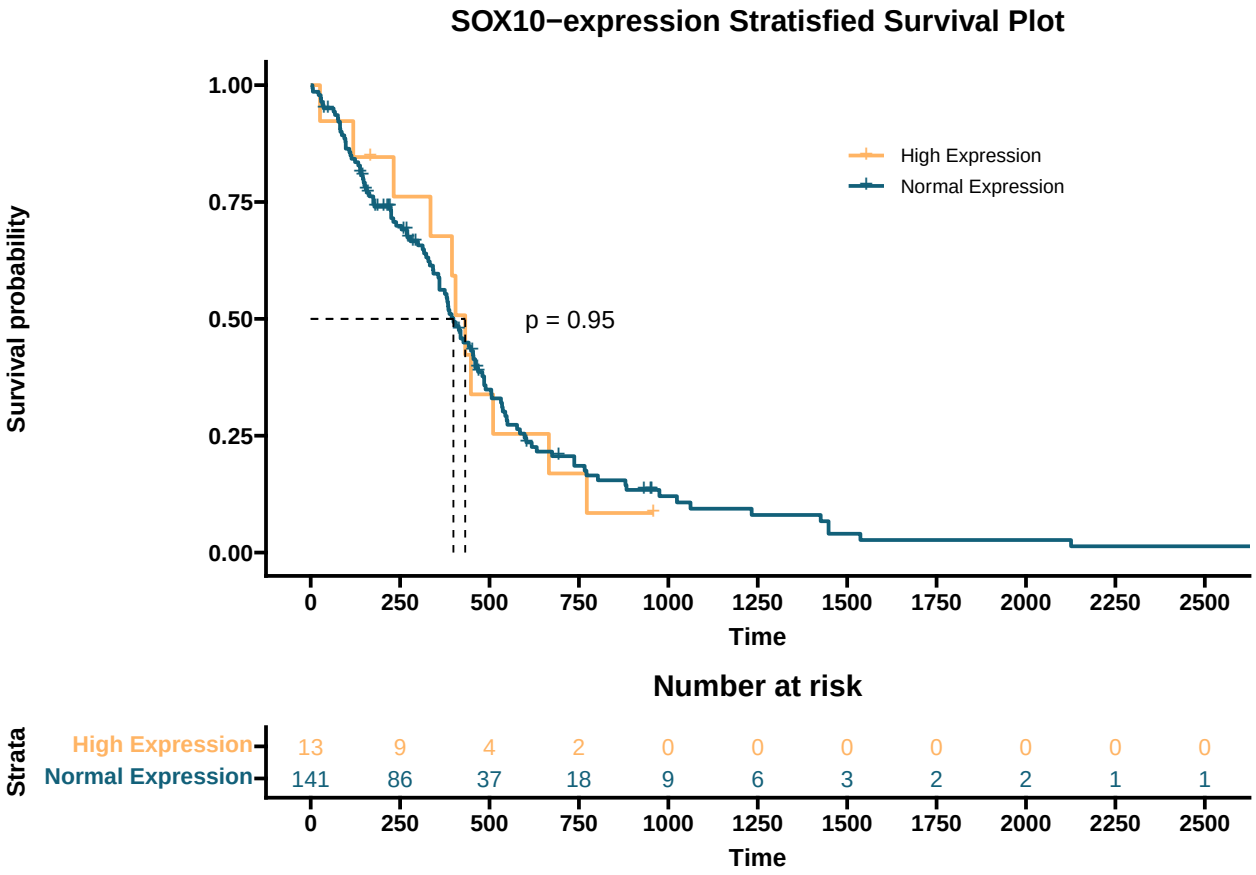
Survival stratified by SOX10 expression

```
#SOX10-- --
merged_SOX10 = tibble(sample = names(SOX10),
    SOX10_expression = as.numeric(SOX10)) %>%
  left_join(cli$GBM_survival.txt, by = "sample") %>%
  select(sample, SOX10_expression, OS.time, OS) %>%
  rename(time = OS.time,
    status = OS)
#ggplot(merged_SOX10, aes(x = SOX10_expression)) + geom_histogram(color="black", fill="white") + theme_

fit_SOX10 = coxph(Surv(time, status) ~ SOX10_expression, data = merged_SOX10)
fit_SOX10

merged_SOX10 = merged_SOX10 %>%
  mutate(group = case_when(
    SOX10_expression > quantile(SOX10_expression, 0.9) ~'High',
    (SOX10_expression < quantile(SOX10_expression, 0.9) &
      SOX10_expression > quantile(SOX10_expression, 0.1)) ~'Normal',
    SOX10_expression < quantile(SOX10_expression, 0.1) ~'Low',
    TRUE~NA_character_
  )) %>%
  mutate(z = (SOX10_expression - mean(SOX10_expression)) / sd(SOX10_expression)) %>%
  mutate(group = case_when(
    z > 1.5~'High',
    z < -1.5~'Low',
    (z < 1.5) & (z > -1.5) ~'Normal',
    TRUE~NA_character_
  ))
fit_SOX10 = survfit(Surv(time, status) ~group,
  data = merged_SOX10 %>% dplyr::filter(group != "Low"))
SOX10_plot <- ggsurvplot(fit_SOX10,
```

```
  pval = TRUE,
  pval.coord = c(600, 0.5),
  #xlim = c(0, 1000),
  palette = c("#ffb464", "#126079"),
  #conf.int = TRUE,
  #pval = TRUE,
  risk.table = TRUE,
  risk.table.col = "strata",
  legend.labs = c("High Expression", "Normal Expression"),
  surv.median.line = "hv",
  break.time.by = 250,
    ggtheme = theme_prism(),
    legend = c(0.7, 0.8),
    title = "SOX10-expression Stratisfied Survival Plot")
##ggsave("../plots/survival/SOX10survival.png", plot = print(SOX10_plot), height = 6, width = 6)
SOX10_plot
```



Survival stratified by EGR3 expression

```
#EGR3-- --
merged_EGR3 = tibble(sample = names(EGR3),
    EGR3_expression = as.numeric(EGR3)) %>%
  left_join(cli$GBM_survival.txt, by = "sample") %>%
```

```
  #filter(sample_type == "Primary Tumor") %>% # Keep only 'Primary Tumor'
  select(sample, EGR3_expression, OS.time, OS) %>%
  rename(time = OS.time,
    status = OS)

fit_EGR3 = coxph(Surv(time, status) ~EGR3_expression, data = merged_EGR3)
fit_EGR3

merged_EGR3 = merged_EGR3 %>%
  mutate(group = case_when(
    EGR3_expression > quantile(EGR3_expression, 0.9) ~'High',
    (EGR3_expression < quantile(EGR3_expression, 0.9) &
      EGR3_expression > quantile(EGR3_expression, 0.1)) ~'Normal',
    EGR3_expression < quantile(EGR3_expression, 0.1) ~'Low',
    TRUE~NA_character_
  )) %>%
  mutate(z = (EGR3_expression - mean(EGR3_expression)) / sd(EGR3_expression)) %>%
  mutate(group = case_when(
    z > 1.5~'High',
    z < -1.5~'Low',
    (z < 1.5) & (z > -1.5) ~'Normal',
    TRUE~NA_character_
  ))
fit_EGR3 = survfit(Surv(time, status) ~group,
  data = merged_EGR3 %>% dplyr::filter(group != "Low"))
EGR3_plot <- ggsurvplot(fit_EGR3,
  pval = TRUE,
  pval.coord = c(600, 0.5),
  #xlim = c(0, 1000),
  palette = c("#ffb464", "#126079"),
  #conf.int = TRUE,
  #pval = TRUE,
  risk.table = TRUE,
  risk.table.col = "strata",
  legend.labs = c("High Expression", "Normal Expression"),
  surv.median.line = "hv",
  break.time.by = 250,
    ggtheme = theme_prism(),
    legend = c(0.7, 0.8),
    title = "EGR3-expression Stratisfied Survival Plot")
##ggsave("../plots/survival/EGR3survival.png", plot = print(EGR3_plot), height = 6, width = 6)
```

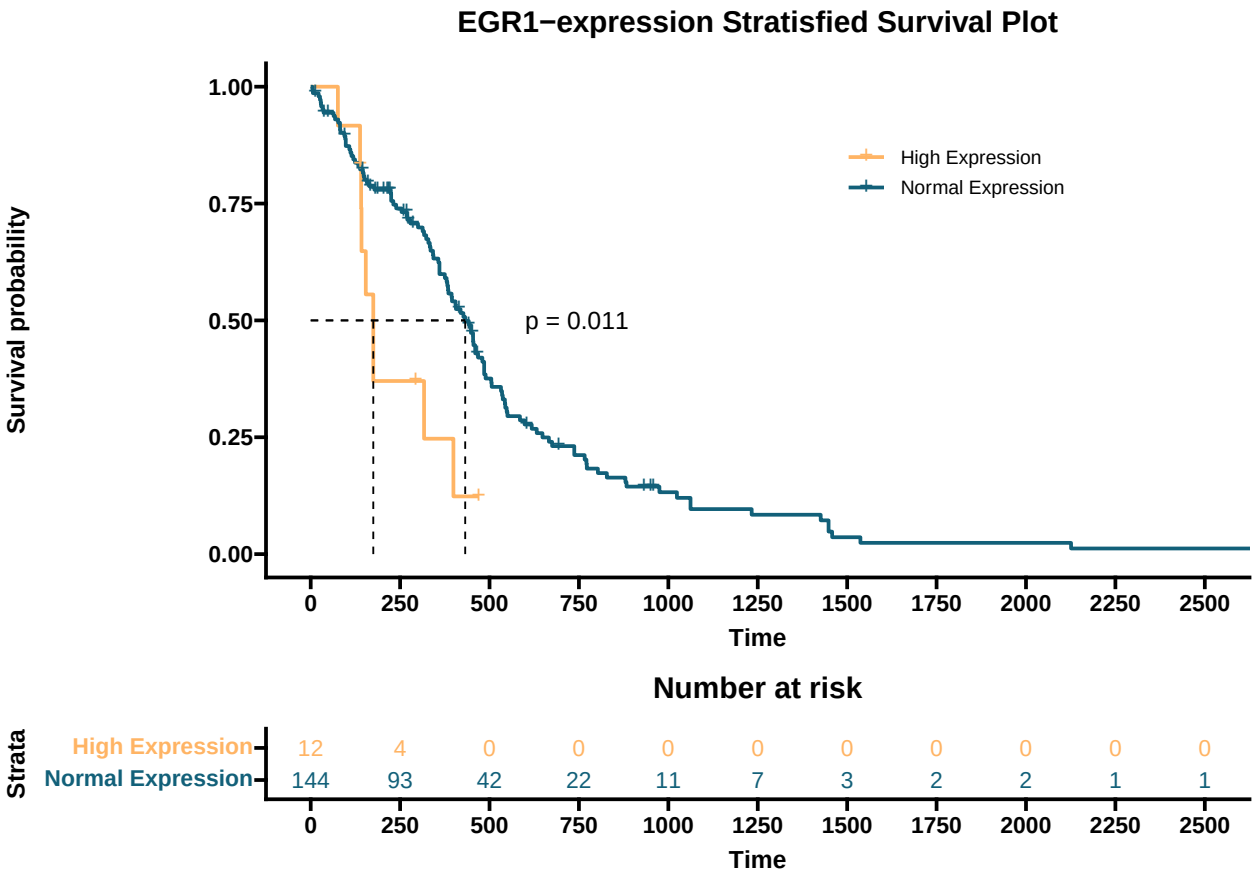
Survival stratified by EGR1 expression

```
#merge-- --
merged_EGR1 = tibble(sample = names(EGR1),
    EGR1_expression = as.numeric(EGR1)) %>%
  left_join(cli$GBM_survival.txt, by = "sample") %>%
  #filter(sample_type == "Primary Tumor") %>% # Keep only 'Primary Tumor'
  select(sample, EGR1_expression, OS.time, OS) %>%
  rename(time = OS.time,
```

```
    status = OS)

fit_EGR1 = coxph(Surv(time, status) ~EGR1_expression, data = merged_EGR1)
fit_EGR1

merged_EGR1 = merged_EGR1 %>%
  mutate(group = case_when(
    EGR1_expression > quantile(EGR1_expression, 0.9) ~'High',
    (EGR1_expression < quantile(EGR1_expression, 0.9) &
      EGR1_expression > quantile(EGR1_expression, 0.1)) ~'Normal',
    EGR1_expression < quantile(EGR1_expression, 0.1) ~'Low',
    TRUE~NA_character_
  )) %>%
  mutate(z = (EGR1_expression - mean(EGR1_expression)) / sd(EGR1_expression)) %>%
  mutate(group = case_when(
    z > 1.5~'High',
    z < -1.5~'Low',
    (z < 1.5) & (z > -1.5) ~'Normal',
    TRUE~NA_character_
  ))
fit_EGR1 = survfit(Surv(time, status) ~group,
  data = merged_EGR1 %>% dplyr::filter(group != "Low"))
EGR1_plot <- ggsurvplot(fit_EGR1,
  pval = TRUE,
  pval.coord = c(600, 0.5),
  #xlim = c(0, 1000),
  palette = c("#ffb464", "#126079"),
  #conf.int = TRUE,
  #pval = TRUE,
  risk.table = TRUE,
  risk.table.col = "strata",
  legend.labs = c("High Expression", "Normal Expression"),
  surv.median.line = "hv",
  break.time.by = 250,
    ggtheme = theme_prism(),
    legend = c(0.7, 0.8),
    title = "EGR1-expression Stratisfied Survival Plot")
##ggsave("../plots/survival/EGR1survival.svg", plot = print(EGR1_plot), height = 6, width = 6)
EGR1_plot
```



Survival stratified by RAMP3 expression

```
merged_RAMP3 = tibble(sample = names(RAMP3),
    RAMP3_expression = as.numeric(RAMP3)) %>%
  left_join(cli$GBM_survival.txt, by = "sample") %>%
  #filter(sample_type == "Primary Tumor") %>% # Keep only 'Primary Tumor'
select(sample, RAMP3_expression, OS.time, OS) %>%
  rename(time = OS.time,
    status = OS)

fit = coxph(Surv(time, status) ~RAMP3_expression, data = merged_RAMP3)
fit

merged_RAMP3 = merged_RAMP3 %>%
  mutate(group = case_when(
    RAMP3_expression > quantile(RAMP3_expression, 0.9) ~'High',
    (RAMP3_expression < quantile(RAMP3_expression, 0.9) &
      RAMP3_expression > quantile(RAMP3_expression, 0.1)) ~'Normal',
    RAMP3_expression < quantile(RAMP3_expression, 0.1) ~'Low',
    TRUE~NA_character_
  )) %>%
  mutate(z = (RAMP3_expression - mean(RAMP3_expression)) / sd(RAMP3_expression)) %>%
  mutate(group = case_when(
    z > 1.5~'High',
```

```
    z < -1.5~'Low',
    (z < 1.5) & (z > -1.5) ~'Normal',
    TRUE~NA_character_
  ))
fit_RAMP3 = survfit(Surv(time, status) ~group,
  data = merged_RAMP3 %>% dplyr::filter(group != "Low"))
RAMP3_plot <- ggsurvplot(fit_RAMP3,
  pval = TRUE,
  pval.coord = c(600, 0.5),
  #xlim = c(0, 1000),
  palette = c("#ffb464", "#126079"),
  #conf.int = TRUE,
  #pval = TRUE,
  risk.table = TRUE,
  risk.table.col = "strata",
  legend.labs = c("High Expression", "Normal Expression"),
  surv.median.line = "hv",
  break.time.by = 250,
    ggtheme = theme_prism(),
    legend = c(0.7, 0.8),
    title = "RAMP3-expression Stratisfied Survival Plot")
```

Survival stratified by CHRNA7 expression

```
merged_CHRNA7 = tibble(sample = names(CHRNA7),
    CHRNA7_expression = as.numeric(CHRNA7)) %>%
  left_join(cli$GBM_survival.txt, by = "sample") %>%
  #filter(sample_type == "Primary Tumor") %>% # Keep only 'Primary Tumor'
select(sample, CHRNA7_expression, OS.time, OS) %>%
  rename(time = OS.time,
    status = OS)

fit = coxph(Surv(time, status) ~CHRNA7_expression, data = merged_CHRNA7)
fit

merged_CHRNA7 = merged_CHRNA7 %>%
  mutate(group = case_when(
    CHRNA7_expression > quantile(CHRNA7_expression, 0.9) ~ 'High',
    (CHRNA7_expression < quantile(CHRNA7_expression, 0.9) &
      CHRNA7_expression > quantile(CHRNA7_expression, 0.1)) ~ 'Normal',
    CHRNA7_expression < quantile(CHRNA7_expression, 0.1) ~ 'Low',
    TRUE ~ NA_character_
  )) %>%
  mutate(z = (CHRNA7_expression - mean(CHRNA7_expression)) / sd(CHRNA7_expression)) %>%
  mutate(group = case_when(
    z > 1.5~'High',
    z < -1.5~'Low',
    (z < 1.5) & (z > -1.5) ~'Normal',
    TRUE~NA_character_
  ))
```

```
fit_CHRNA7 = survfit(Surv(time, status) ~group,
  data = merged_CHRNA7 %>% dplyr::filter(group != "Low"))
CHRNA7_plot <- ggsurvplot(fit_CHRNA7,
  pval = TRUE,
  pval.coord = c(600, 0.5),
  #xlim = c(0, 1000),
  palette = c("#ffb464", "#126079"),
  #conf.int = TRUE,
  #pval = TRUE,
  risk.table = TRUE,
  risk.table.col = "strata",
  legend.labs = c("High Expression", "Normal Expression"),
  surv.median.line = "hv",
  break.time.by = 250,
    ggtheme = theme_prism(),
    legend = c(0.7, 0.8),
    title = "CHRNA7-expression Stratisfied Survival Plot")
```

Survival stratified by EGR1 and SOX10 expression

```
# EGR1 and SOX10
EGR1_SOX10 <- merge(merged_EGR1, merged_SOX10, by = c("sample", "time", "status")) %>%
  select("sample", "time", "status", "z.x", "z.y") %>%
  mutate(group = case_when(
    (z.x > 1) & (z.y > 1) ~'High',
    (z.x < -1) & (z.y < -1) ~'Low',
    TRUE~'Normal'
  ))
EGR1_SOX10_corr <- ggplot(data = EGR1_SOX10, aes(x = z.x, y = z.y)) +
  geom_point() +
  theme_prism() +
  labs(title = "EGR1 and SOX10 expression correlation") +
  xlab("EGR1 expression") +
  ylab("SOX10 expression") +
  geom_smooth(method = "lm")
##ggsave("../plots/survival/EGR1.SOX10.corr.svg", plot = EGR1_SOX10_corr, height = 6, width = 6)

fit_EGR1_SOX10 = survfit(Surv(time, status) ~group,
  data = EGR1_SOX10 %>% dplyr::filter(group != "Low"))
EGR1_SOX10_plot <- ggsurvplot(fit_EGR1_SOX10,
  pval = TRUE,
  pval.coord = c(600, 0.5),
  #xlim = c(0, 1000),
  palette = c("#ffb464", "#126079"),
  #conf.int = TRUE,
  #pval = TRUE,
  risk.table = TRUE,
  risk.table.col = "strata",
  legend.labs = c("High Expression", "Normal Expression"),
  surv.median.line = "hv",
  break.time.by = 250,
```

```
    ggtheme = theme_prism(),
    legend = c(0.7, 0.8),
    title = "EGR1 and SOX10 expression Stratisfied Survival Plot")
```

Survival stratified by EGR1 and CHRNA7 expression

```
EGR1_CHRNA7 <- merge(merged_EGR1, merged_CHRNA7, by = c("sample", "time", "status")) %>%
  select("sample", "time", "status", "z.x", "z.y") %>%
  mutate(group = case_when(
    (z.x > 1) & (z.y > 1) ~'High',
    (z.x < -1) & (z.y < -1) ~'Low',
    TRUE~'Normal'
  ))
EGR1_CHRNA7_corr <- ggplot(data = EGR1_CHRNA7, aes(x = z.x, y = z.y)) +
  geom_point() +
  theme_prism() +
  labs(title = "EGR1 and CHRNA7 expression correlation") +
  xlab("EGR1 expression") +
  ylab("CHRNA7 expression") +
  geom_smooth(method = "lm")
##ggsave("../plots/survival/EGR1.CHRNA7.corr.svg", plot = EGR1_CHRNA7_corr, height = 6, width = 6)

fit_EGR1_CHRNA7 = survfit(Surv(time, status) ~group,
  data = EGR1_CHRNA7 %>% dplyr::filter(group != "Low"))
EGR1_CHRNA7_plot <- ggsurvplot(fit_EGR1_CHRNA7,
  pval = TRUE,
  pval.coord = c(600, 0.5),
  #xlim = c(0, 1000),
  palette = c("#ffb464", "#126079"),
  #conf.int = TRUE,
  #pval = TRUE,
  risk.table = TRUE,
  risk.table.col = "strata",
  legend.labs = c("High Expression", "Normal Expression"),
  surv.median.line = "hv",
  break.time.by = 250,
    ggtheme = theme_prism(),
    legend = c(0.7, 0.8),
    title = "EGR1 and CHRNA7 expression Stratisfied Survival Plot")
```

Survival stratified by EGR1 and RAMP3 expression

```
# EGR1 and RAMP3
EGR1_RAMP3 <- merge(merged_EGR1, merged_RAMP3, by = c("sample", "time", "status")) %>%
  select("sample", "time", "status", "z.x", "z.y") %>%
  mutate(group = case_when(
    (z.x > 1.5) & (z.y > 1.5) ~'High',
    (z.x < -1.5) & (z.y < -1.5) ~'Low',
    TRUE~'Normal'
```

```
  ))

EGR1_RAMP3_corr <- ggplot(data = EGR1_RAMP3, aes(x = z.x, y = z.y)) +
  geom_point() +
  theme_prism() +
  labs(title = "EGR1 and CHRNA7 expression correlation") +
  xlab("EGR1 expression") +
  ylab("CHRNA7 expression") +
  geom_smooth(method = "lm")
#ggsave("../plots/survival/EGR1.RAMP3.corr.svg", plot = EGR1_RAMP3_corr, height = 6, width = 6)

fit_EGR1_RAMP3 = survfit(Surv(time, status) ~group,
  data = EGR1_RAMP3 %>% dplyr::filter(group != "Low"))
EGR1_RAMP3_plot <- ggsurvplot(fit_EGR1_RAMP3,
  pval = TRUE,
  pval.coord = c(600, 0.5),
  #xlim = c(0, 1000),
  palette = c("#ffb464", "#126079"),
  #conf.int = TRUE,
  #pval = TRUE,
  risk.table = TRUE,
  risk.table.col = "strata",
  legend.labs = c("High Expression", "Normal Expression"),
  surv.median.line = "hv",
  break.time.by = 250,
    ggtheme = theme_prism(),
    legend = c(0.7, 0.8),
    title = "EGR1 and RAMP3 expression Stratisfied Survival Plot")
```