

Chapter 4 - Plotting

```
library(Seurat)
library(dplyr)
library(magrittr)
library(tidyr)
library(ggplot2)
library(ggpubr)
library(stringr)
library(scales)
library(viridis)

setwd("~/Desktop/YAP1/")

## Merged Sample Level -----
-----
meta <- read.delim("data/merged_visium_xenium2.meta", row.names = 1)
%>%
dplyr::arrange(YAP1_5SA)
meta$YAP1_5SA.colour <- viridis::inferno(n = nrow(meta))

plot_df <- meta %>% dplyr::select(sample, Stiffness, YAP1_5SA.hetero)
%>% distinct()
ggplot(plot_df, aes(x = Stiffness, y = YAP1_5SA.hetero)) +
geom_boxplot() + stat_compare_means(vjust = 3, method = "t.test") +
theme_bw() +
labs(title = "Cancer Cell Heterogeneity", subtitle = "Heterogeneity
by YAP1-5SA") + xlab("Fibroblast Stiffness") + ylab("YAP1-5SA")

plot_df <- meta %>% dplyr::select(sample, Stiffness, YAP1_5SA) %>%
dplyr::group_by(sample, Stiffness) %>%
summarise(mean_YAP1_5SA = mean(YAP1_5SA, na.rm = TRUE), .groups =
'drop')
ggplot(plot_df, aes(x = Stiffness, y = mean_YAP1_5SA)) +
geom_boxplot() + stat_compare_means(vjust = 3, method = "t.test") +
theme_bw() +
labs(title = "YAP1-5SA Activation Score") + xlab("Fibroblast
Stiffness") + ylab("YAP1-5SA")
```

```

## Each Sample Level -----
-----
GSM_cohort <- plot_df[grepl("GSM", plot_df$sample), ]

high_stiffness <- GSM_cohort$sample[GSM_cohort$Stiffness ==
"high_stiff"]
low_stiffness <- GSM_cohort$sample[GSM_cohort$Stiffness ==
"low_stiff"]

i <- low_stiffness[[6]]

for(i in c(high_stiffness, low_stiffness)){
setwd(file.path("/Users/polly_hung/Desktop/YAP1/data/spatial/GSE21195
6", i))
spatial <- readRDS("output/labelled2.rds")
## Use Viridis from all the samples, the normalized version
submeta <- spatial@meta.data
viridis <- meta %>% dplyr::filter(sample == i) %>%
dplyr::select(sample, YAP1_5SA.colour)
rownames(viridis) <- gsub(i, "", rownames(viridis))
rownames(viridis) <- gsub("_", "", rownames(viridis))
viridis$sample <- NULL
viridis <- viridis[rownames(submeta), ]
submeta <- cbind(submeta, viridis)
## colour non-cancer cells
submeta <- submeta %>% dplyr::mutate(viridis = case_when(cell.types
== "Fibroblast" ~ "#A7CF5D",
cell.types == "Ovarian.cancer.cell" ~ viridis,
TRUE ~ "darkgrey"))
## Add Spatial Coordinates
submeta <- submeta[rownames(spatial@images$slice1@coordinates), ]
submeta$imagerow <- spatial@images$slice1@coordinates$imagerow
submeta$imagecol <- spatial@images$slice1@coordinates$imagecol
## Plot
n = table(submeta$cell.types)[["Ovarian.cancer.cell"]]
ggplot(submeta, aes(x = imagerow, y = imagecol, color = viridis)) +
geom_point(size = 1.5) +
scale_color_identity() + theme_void() + coord_flip()+
scale_x_reverse()
ggsave("plots/overlayed-YAP1-5SA.png", width = 5, height = 5, units =
"in", dpi = 600)

```

```
## save the plot
write.table(submeta, "output/plot_setting.meta", sep = "\t", quote =
F, row.names = T, col.names = T)
}
```

```
## Create a Colour Bar?
merged <- readRDS("~/Desktop/YAP1/data/merged_visium_xenium2.rds")
meta <- meta[rownames(merged@meta.data), ]
merged@meta.data <- meta
```

```
FeaturePlot(merged, feature = "YAP1_5SA", ) +
scale_color_viridis(option = "inferno", direction = 1)
```

```
## YAP1-5SA score and fibroblast percentage
# GSM <- meta %>% dplyr::filter(grepl("GSM", sample))
GSM <- meta %>%
dplyr::filter(cell.types == "Ovarian.cancer.cell") %>%
dplyr::group_by(sample) %>% summarise(mean(YAP1_5SA))
```

```
fibro <- as.data.frame.matrix(table(merged$sample,
merged$cell.types))
fibro$All.Cells <- rowSums(fibro)
fibro$Fibroblast.Pct <- 100*(fibro$Fibroblast / fibro$All.Cells)
fibro <- fibro[GSM$sample, ]
```

```
plot_df <- data.frame(sample = GSM$sample,
YAP1_5SA_mean = GSM$`mean(YAP1_5SA)`,
fibroblast_pct = fibro$Fibroblast.Pct,
group = ifelse(grepl("GSM", GSM$sample), "visium", "xenium"))
```

```
ggplot(plot_df, aes(x = fibroblast_pct, y = YAP1_5SA_mean, colour =
group)) +
geom_point() +
geom_text(aes(label = plot_df$sample), vjust = -1, size = 3) +
geom_smooth(method = "lm", se = FALSE, colour = "#5F8B4C") +
xlim(-10, 120) + ylim(-0.14, 0.12) +
theme_bw() +
labs(title = "YAP1-5SA score in Cancer Cells Positively Correlates
with Increased Fibroblast Percentage in Tissue Section") +
xlab("Percent of Fibroblast in Tissue Section") +
```

```

ylab("Mean YAP1-5SA score in Cancer Cells")

## Each Sample Level -----
-----
GSM_cohort <- list.dirs("data/spatial/GSE211956", recursive = F)

for(i in GSM_cohort){
  setwd(file.path("/Users/polly_hung/Desktop/YAP1/", i))
  submeta <- read.delim("output/plot_setting.meta", row.names = 1)
  ## Plot
  n = table(submeta$cell.types)[["Ovarian.cancer.cell"]]
  ggplot(submeta, aes(x = imagerow, y = imagecol, color = viridis)) +
  geom_point(size =1.5) +
  scale_color_identity() + theme_void() + coord_flip()+
  scale_x_reverse()
  ggsave("plots/overlayed-YAP1-5SA.png", width = 5, height = 5, units =
  "in", dpi = 600)
}

```