Viz of surface and meiotic markers

```
setwd("/Users/virginiahowick/Documents/Tryps")
library(slingshot)
library(SingleCellExperiment)
```

```
## Warning: package 'BiocGenerics' was built under R version 4.0.5
```

```
## Warning: package 'GenomeInfoDb' was built under R version 4.0.5
```

```
library(scater)
library(viridis)
library(pheatmap)
library(reshape2)
library(RColorBrewer)
library(colorspace)
library(tidyverse)
```

```
sling.sg <- readRDS("/Users/virginiahowick/Documents/Tryps/1738sling.sg_jan2021.rds")
markergenes <- read.csv("/Users/virginiahowick/Documents/Tryps/WendyMarkers_4.csv", header=T)
```

```
exp <- as.data.frame(logcounts(sling.sg))
dim(exp)
```

```
## [1] 9225  181
```

```
exp$feature_symbol <- rownames(exp)
subexp <- exp[exp$feature_symbol %in% markergenes$gene_id, ]
subexp <- subexp[match(markergenes$gene_id, subexp$feature_symbol), ]

#remove the feature_symbol column you just added
subexp2 <- subexp[, 1:181]
```

```
cd <- as.data.frame(colData(sling.sg))
cd <- cd[order(cd$slingPseudotime_1), ]
subppt <- cd["slingPseudotime_1"]
subppt <- rownames(subppt)

subexp3 <- subexp2[, subppt]
```

```
markergenes2 <- markergenes[match(rownames(subexp2), markergenes$gene_id), ]
rownames(markergenes2) <- markergenes2$gene_id
group <- markergenes2["group"]

markergenes2$anno <- paste(markergenes2$gene_id, markergenes2$gene_name, sep="::")
```

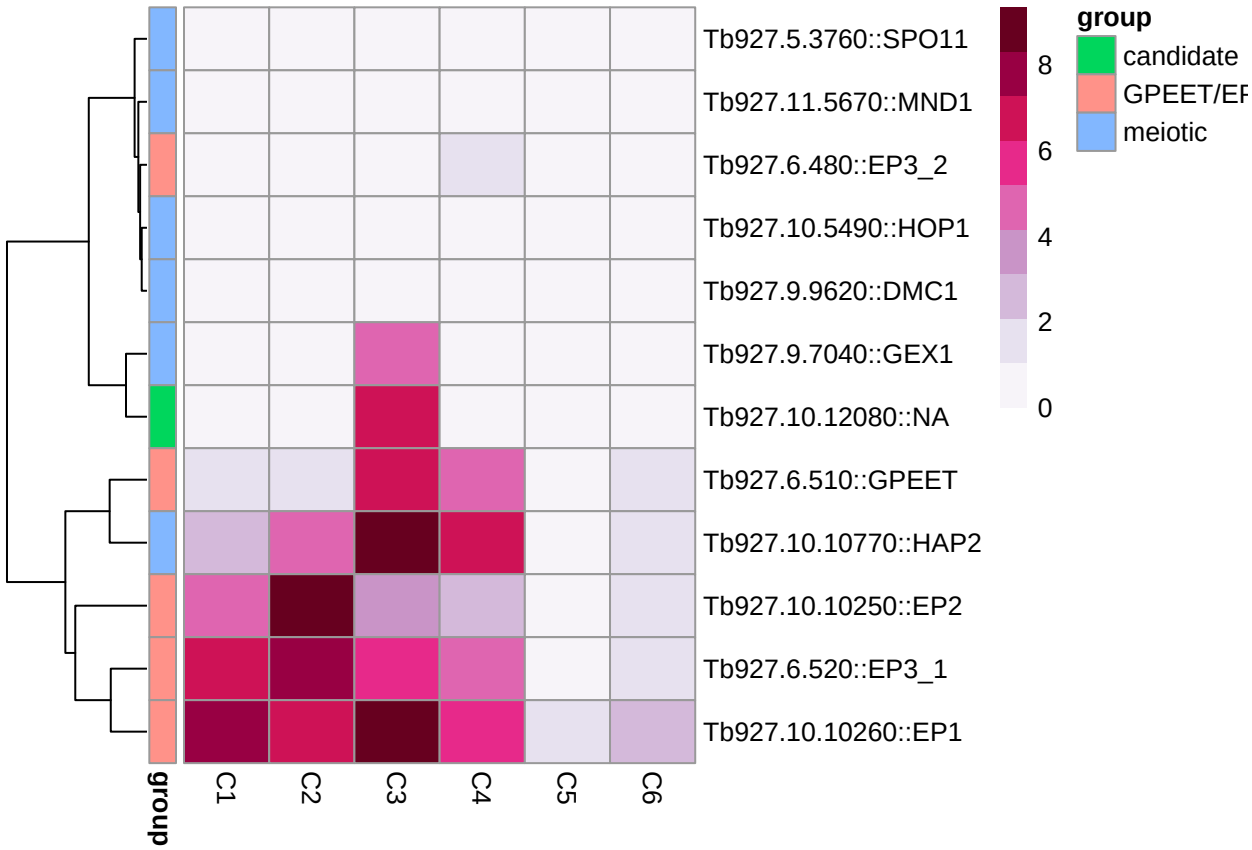
```
test <- which(group$group != dplyr::lag(group$group))
test2 <- test-1
```

```
attachment <- cd["attachment"]
time <- cd["time"]
combo <- cbind(attachment, time)
```

```
all <- readRDS("/Users/virginiahowick/Documents/Tryps/for_github/sc_data/howick_tryps_sce.rds")

all_lc <- as.data.frame(logcounts(all))

all_lc$feature_symbol <- rownames(all_lc)
subexp <- all_lc[all_lc$feature_symbol %in% markergenes$gene_id, ]
subexp <- subexp[match(markergenes$gene_id, subexp$feature_symbol), ]

#remove the feature_symbol column you just added
subexp2 <- subexp[, 1:388]

tall <- as.data.frame(t(subexp2))
tall[1:5, 1:5]
```

```
##      Tb927.6.510 Tb927.6.480 Tb927.6.520 Tb927.10.10250 Tb927.10.10260
## PV_2    0.000000           0    0.000000      11.016918       6.008534
## PV_3    0.000000           0    4.616115       9.482884       6.778707
## PV_5    0.000000           0    6.348798       9.021091       6.780272
## PV_6    2.890746           0    5.709630      10.052388       7.107334
## MG_8    0.000000           0    6.785191       8.917995       9.166881
```

```
tall$cluster <- as.factor(all$cluster_name)


clustmean <- aggregate(tall[, 1:33], by = list(as.factor(as.character(tall$cluster))),
    mean)
rownames(clustmean) <- clustmean$Group.1

clustmean2 <- clustmean[, 2:34]

tcm2 <- as.data.frame(t(clustmean2))


barps <- markergenes[markergenes$group=="BARP", ]
nob <- tcm2[!(rownames(tcm2) %in% barps$gene_id), ]

markergenes3 <- markergenes2[!(rownames(markergenes2) %in% barps$gene_id), ]
group2 <- markergenes3["group"]

rownames(nob) <- markergenes3$anno
group3 <- group2
rownames(group3) <- rownames(nob)
pheatmap(nob, show_colnames = TRUE, show_rownames = TRUE, cluster_cols = FALSE, annotation_row = group3
    color = brewer.pal(9, "PuRd"))
```

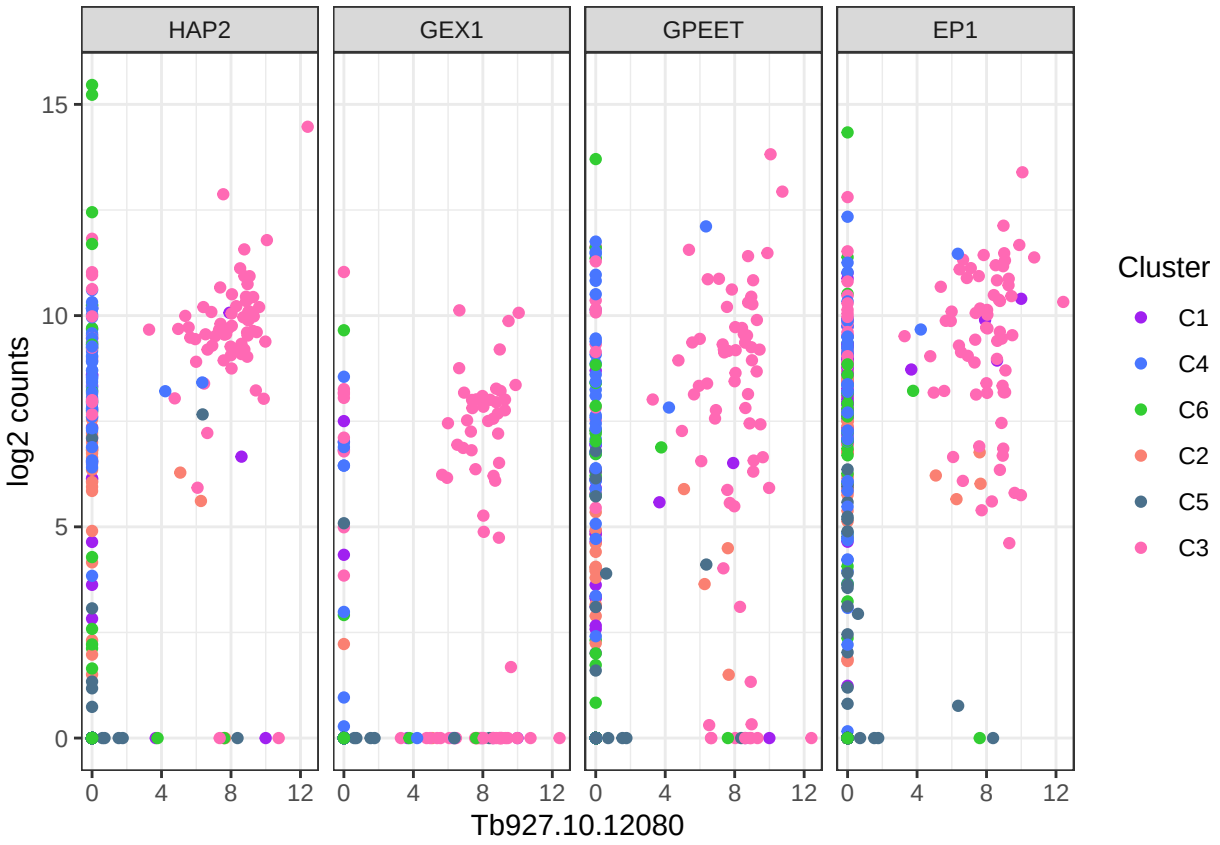



```
subexp4 <- subexp3[!(rownames(subexp3) %in% barps$gene_id), ]

rownames(subexp4) <- rownames(nob)
group4 <- group2
rownames(group4) <- rownames(nob)
```

fig 6 A

```
impmarks <- c("Tb927.10.12080","Tb927.6.510", "Tb927.10.10260", "Tb927.9.7040", "Tb927.10.10770")

all_lc <- as.data.frame(logcounts(all))

subexp <- all_lc[rownames(all_lc) %in% impmarks, ]

tsub <- as.data.frame(t(subexp))
tsub$cluster <- all$cluster_name
tsub2 <- tsub %>%
  pivot_longer(!(c(Tb927.10.12080, cluster)), names_to = "other_gene", values_to = "exp")

hap2 <- tsub2[tsub2$other_gene=="Tb927.10.10770", ]
p <- ggplot(hap2, aes(Tb927.10.12080, exp)) + geom_point(aes(colour=cluster))


clustcol <- c(`C1` = "purple", `C4` = "royalblue1", `C6` = "limegreen", `C2` = "salmon",
    `C5` = "skyblue4", `C3` = "hotpink")
```

```
tsub2$anno <- markergenes3[match(tsub2$other_gene, markergenes3[, 1]), 2]

tsub2$anno <- ordered(tsub2$anno, levels=c("HAP2", "GEX1", "GPEET", "EP1"))
ggplot(tsub2, aes(Tb927.10.12080, exp)) + geom_point(aes(colour=cluster)) + facet_grid(~anno) + scale_c
```




```
yb <- tcm2[rownames(tcm2) %in% barps$gene_id, ]
ybmean <- as.data.frame(colMeans(yb))

tcm2 <- as.data.frame(t(clustmean2))

barps <- markergenes[markergenes$group=="BARP", ]
nob <- tcm2[!(rownames(tcm2) %in% barps$gene_id), ]
```

Fig 5A

```
subexp5 <- subexp4
rownames(subexp5) <- rownames(nob)
rownames(group2) <- rownames(nob)
test <- which(group4$group != dplyr::lag(group4$group))
test2 <- test-1

attach_col <- c(attached = "#0E3F5C", free = "#D1FBD4")
time_col <- c(day40 = "dodgerblue", day21 = "darkmagenta", day24 = "darkolivegreen1")
group_col <- c("GPEET/EP"= "mediumpurple1", "meiotic"="salmon", "candidate"="deepskyblue")
```

```
barp_col <- sequential_hcl(10, palette = "Mint")
ann_c <- list(time = time_col, attachment = attach_col, group4=group_col, ybmean=barp_col)

pheatmap(subexp5, cluster_cols=FALSE, cluster_rows=FALSE ,color=inferno(10), annotation_names_row = TRU
```

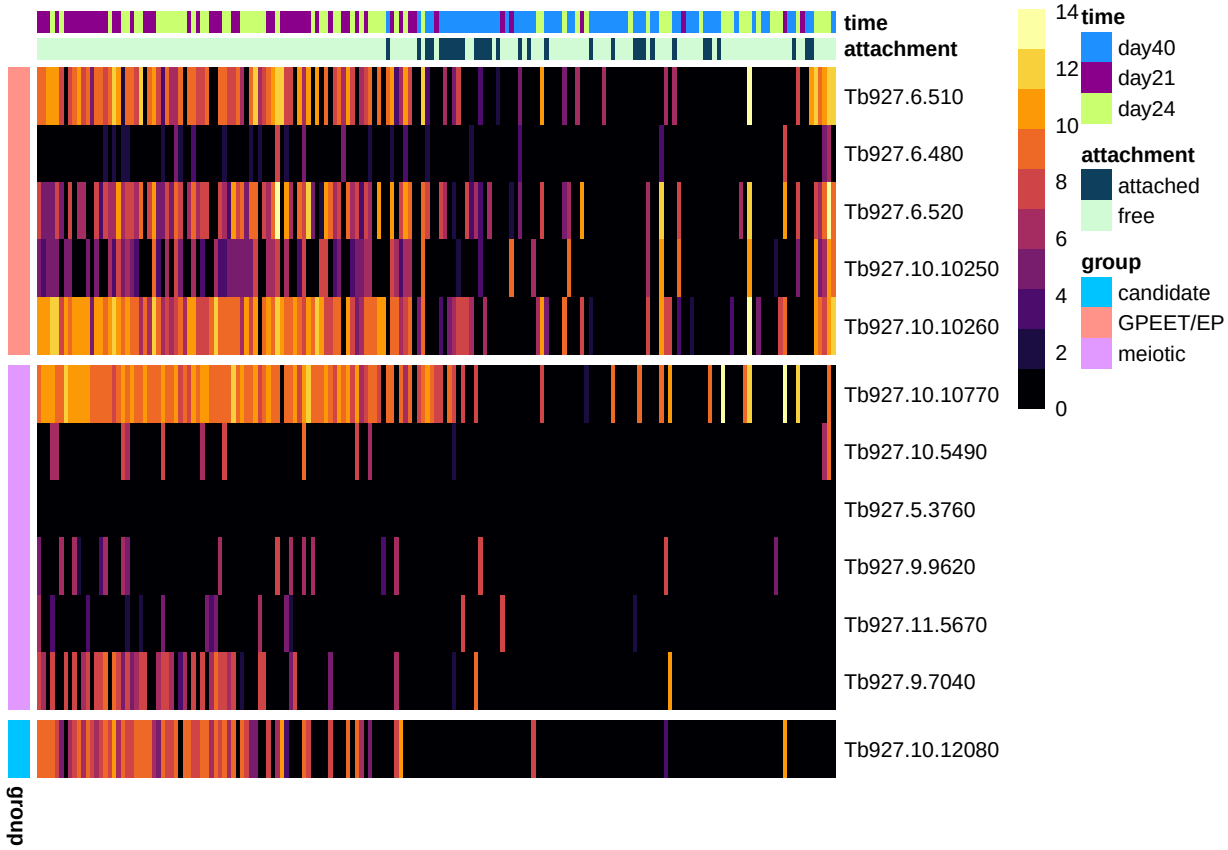
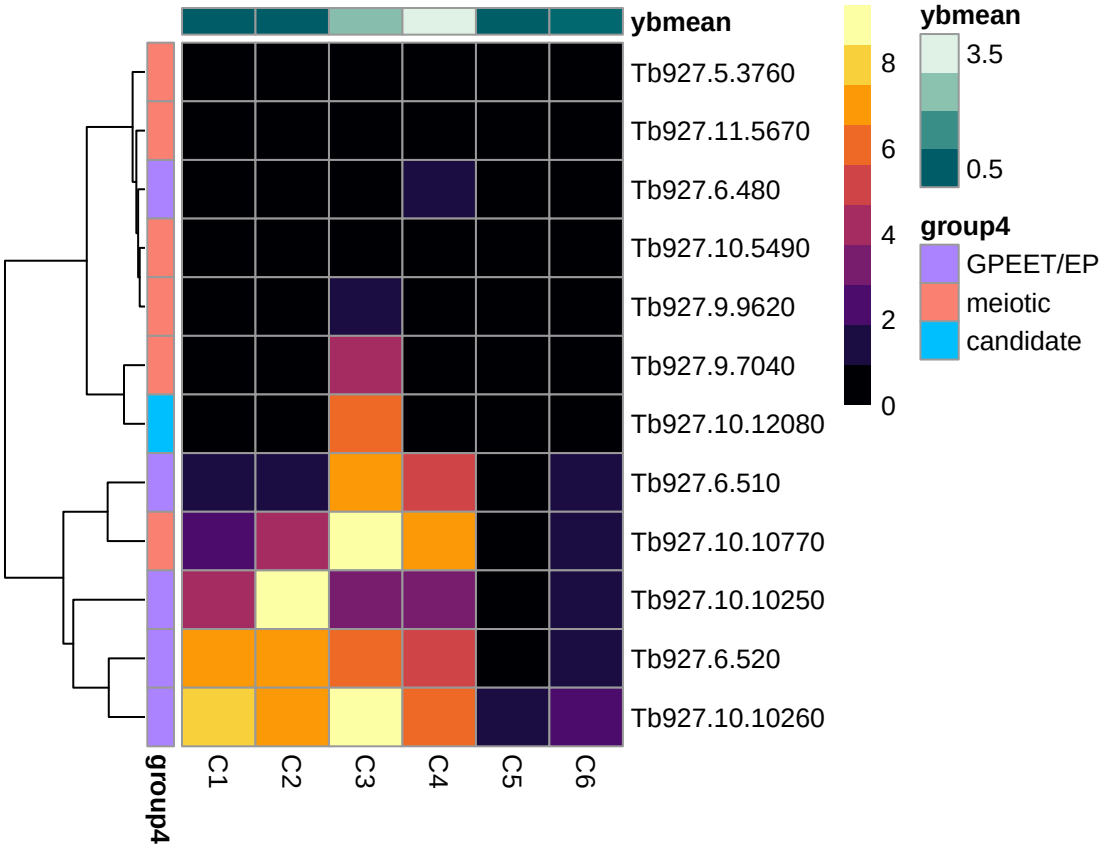


Fig 5B

```
colnames(group4) <- "group4"
colnames(ybmean) <- "ybmean"


pheatmap(nob, show_colnames = TRUE, show_rownames = TRUE, cluster_cols = FALSE, annotation_row = group4
    color = inferno(10), annotation_colors=ann_c)
```




```
hcl_palettes(plot = TRUE)
```


Qualitative
Pastel 1
Dark 2
Dark 3
Set 2
Set 3
Warm
Cold
Harmonic
Dynamic
Sequential (single−hu
Grays
Light Grays
Blues 2
Blues 3
Purples 2
Purples 3
Reds 2
Reds 3
Greens 2
Greens 3
Oslo
Sequential (multi−hu
Purple−Blu
Red−Purpl
Red−Blue
Purple−Ora
Blue−Yellow
Green−Yell
Red−Yellow
Heat
Heat 2
Terrain
Terrain 2
Viridis
Plasma
Inferno
Rocket
Dark Mint
Mint
BluGrn
Teal
TealGrn
Emrld
BluYl
ag_GrnYl
Peach
PinkYl
Burg
BurgYl
RedOr
OrYel
Purp
PurpOr
Sunset
Magenta
SunsetDark
ag_Sunset
BrwnYl
YlOrRd
YlOrBr
OrRd
Oranges
YlGn
YlGnBu
Reds
RdPu
PuRd
Purples
PuBuGn
PuBu
Greens
BuGn
GnBu
BuPu
Blues
Lajolla
Turku
Hawaii
Batlow
Diverging
Blue−Red
Blue−Red 2
Blue−Red 3
Red−Green
Purple−Gre
Purple−Bro
Green−Bro
Blue−Yellow
Blue−Yellow
Green−Ora
Cyan−Mag
Tropic
Broc
Cork
Vik
Berlin
Lisbon
Tofino