

EC_figure

Ben Thomson

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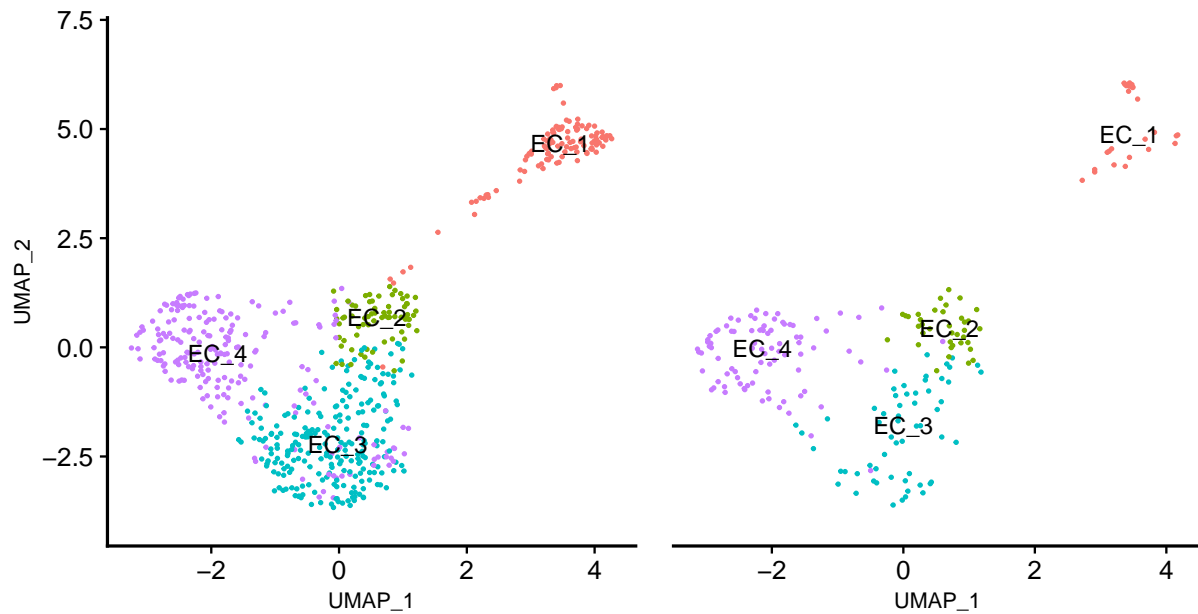
```
ec.dataset <- readRDS("../data/6wk_endos_10-1-20.rds.gz")  
lec.dataset <- readRDS("../data/6wk_LECs_10-1-20.rds.gz")
```

```
panel.a <- RenameIdents(ec.dataset, SC = "EC_1", LEC = "EC_1")
```

```
panel.a.fig.wt <- DimPlot(object = subset(panel.a, subset = genotype ==  
  "WT"), reduction = "umap", label = TRUE, pt.size = 0.5, label.size = 4) +  
  NoLegend() + coord_fixed() + theme(aspect.ratio = 1) + theme(axis.line = element_line(color = "black"  
  size = 0.7)) + theme(axis.title = element_text(size = 10)) +  
  ylim(c(-4, 7))
```

```
panel.a.fig.ko <- DimPlot(object = subset(panel.a, subset = genotype ==  
  "KO"), reduction = "umap", label = TRUE, pt.size = 0.5, label.size = 4) +  
  NoLegend() + coord_fixed() + theme(aspect.ratio = 1) + theme(axis.line = element_line(color = "black"  
  size = 0.7)) + theme(axis.line.y = element_blank(), axis.ticks.y = element_blank(),  
  axis.text.y = element_blank()) + theme(axis.title.x = element_text(size = 10)) +  
  theme(axis.title.y = element_blank()) + ylim(c(-4, 7))
```

```
panel.a.fig.wt + panel.a.fig.ko
```



```
pdf(file = "../figures/EC_figure_a.pdf", width = 5, height = 2)
plot(panel.a.fig.wt + panel.a.fig.ko)
dev.off()
```

```
## pdf
## 2
```

```
panel.b <- RenameIdents(ec.dataset, SC = "EC_1", LEC = "EC_1")

plot.list.b <- VlnPlot(panel.b, assay = "RNA", features = c("Cc121a",
  "Vwf", "Kdr", "Plvap", "Prox1", "Ihh", "Ptprb", "Tek", "Sox17"),
  pt.size = 0, combine = F)

for (i in seq_along(plot.list.b)) {
  plot.list.b[[i]] <- plot.list.b[[i]] + theme(axis.ticks = element_blank(),
    axis.text.y = element_blank(), axis.title.x = element_blank()) +
    theme(title = element_text(family = "sans", face = "italic")) +
    theme(axis.line = element_line(size = 0.7)) + NoLegend() +
    scale_y_continuous(expand = c(0, 0)) + theme(aspect.ratio = 0.3) +
    theme(axis.title.y = element_blank())

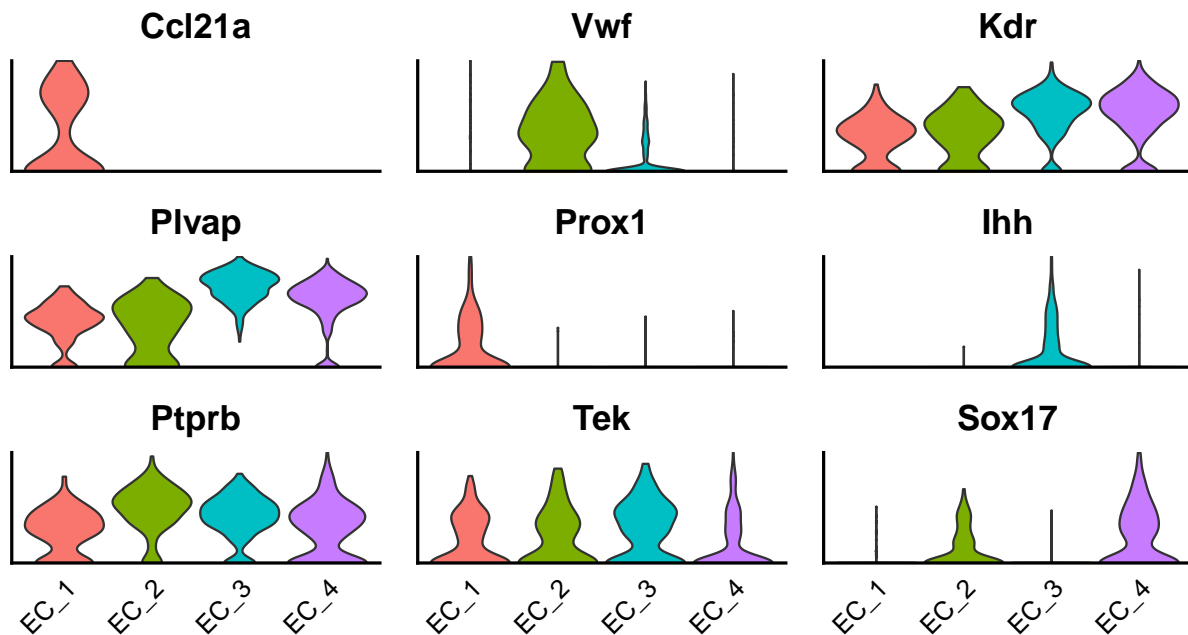
  if (i > (length(plot.list.b) - 3)) {
    plot.list.b[[i]] <- plot.list.b[[i]] + theme(axis.text.x = element_text())
  }
}
```

```

} else {
  plot.list.b[[i]] <- plot.list.b[[i]] + theme(axis.text.x = element_blank())
}
}

wrap_plots(plot.list.b)

```



```

pdf(file = "../figures/EC_figure_b.pdf", width = 5, height = 5)
plot(wrap_plots(plot.list.b))
dev.off()

```

```

## pdf
## 2

```

```

panel.c <- lec.dataset

```

```

panel.c.fig.wt <- DimPlot(object = subset(panel.c, subset = genotype ==
  "WT"), reduction = "umap", label = TRUE, pt.size = 0.5, label.size = 4) +
  NoLegend() + coord_fixed() + theme(aspect.ratio = 1) + theme(axis.line = element_line(color = "black"
  size = 0.7)) + theme(axis.title = element_text(size = 10)) +
  xlim(c(-6, 3)) + ylim(c(-5, 5))

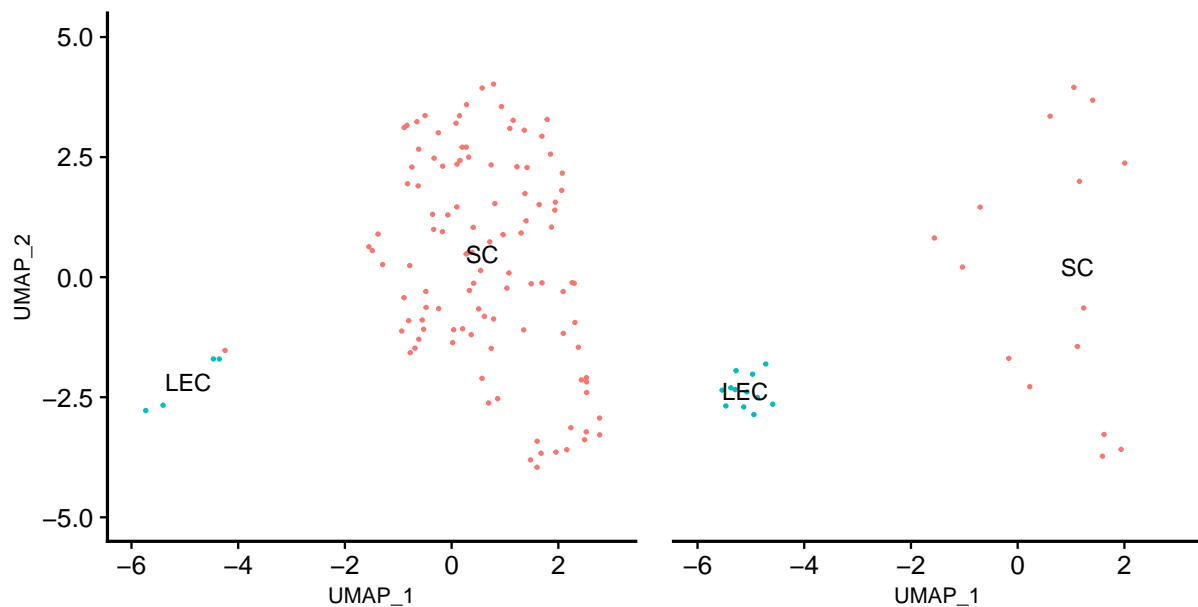
```

```

panel.c.fig.ko <- DimPlot(object = subset(panel.c, subset = genotype ==
  "KO"), reduction = "umap", label = TRUE, pt.size = 0.5, label.size = 4) +
  NoLegend() + coord_fixed() + theme(aspect.ratio = 1) + theme(axis.line = element_line(color = "black"
  size = 0.7)) + theme(axis.line.y = element_blank(), axis.ticks.y = element_blank(),
  axis.text.y = element_blank()) + theme(axis.title.x = element_text(size = 10)) +
  theme(axis.title.y = element_blank()) + xlim(c(-6, 3)) +
  ylim(c(-5, 5))

panel.c.fig.wt + panel.c.fig.ko

```



```

pdf(file = "../figures/EC_figure_c.pdf", width = 5, height = 2)
plot(panel.c.fig.wt + panel.c.fig.ko)
dev.off()

```

```

## pdf
## 2

```

```

panel.d <- RenameIdents(ec.dataset, EC_2 = "BEC", EC_3 = "BEC",
  EC_4 = "BEC")
panel.d@active.ident <- factor(x = panel.d@active.ident, levels = c("BEC",
  "LEC", "SC"))

```

```

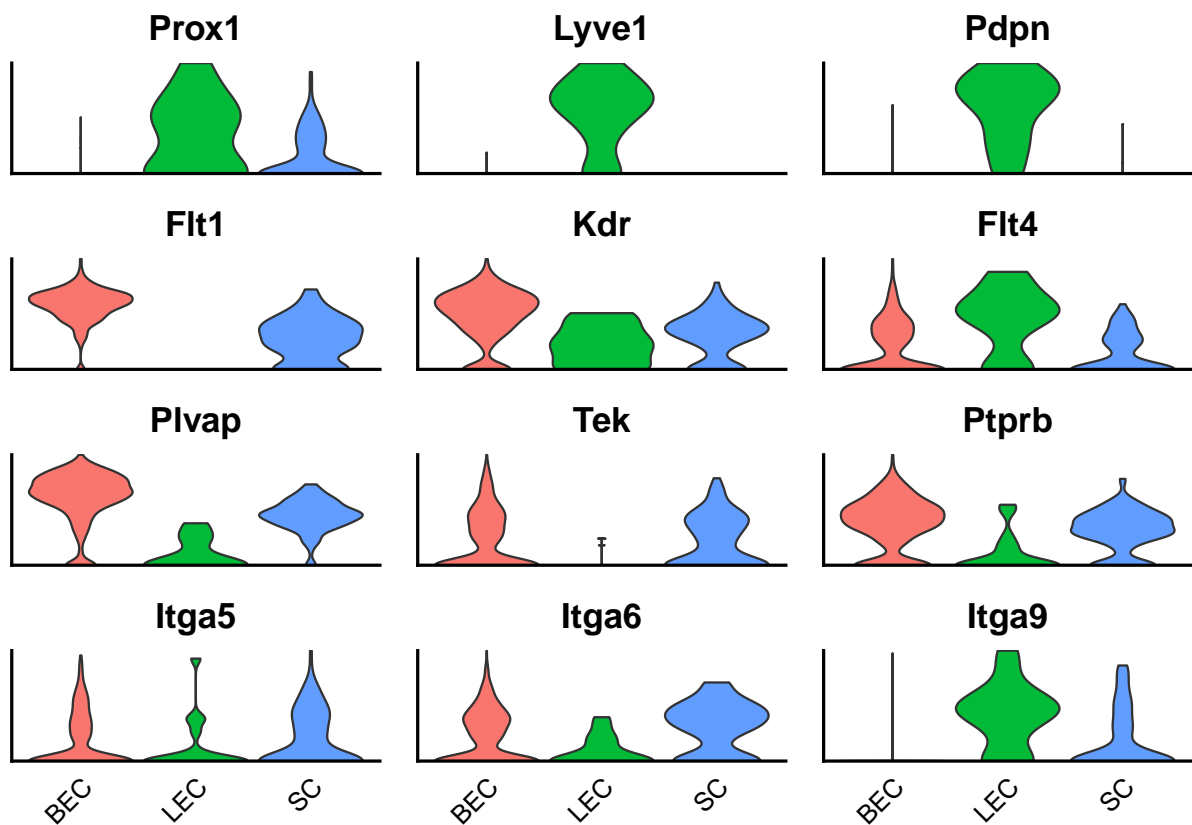
plot.list.d <- VlnPlot(panel.d, assay = "RNA", features = c("Prox1",
"Lyve1", "Pdpn", "Flt1", "Kdr", "Flt4", "Plvap", "Tek", "Ptprb",
"Itga5", "Itga6", "Itga9"), pt.size = 0, combine = FALSE)

for (i in seq_along(plot.list.d)) {
  plot.list.d[[i]] <- plot.list.d[[i]] + theme(axis.ticks = element_blank(),
axis.text.y = element_blank(), axis.title.x = element_blank()) +
  theme(title = element_text(family = "sans", face = "italic")) +
  theme(axis.line = element_line(size = 0.7)) + NoLegend() +
  scale_y_continuous(expand = c(0, 0)) + theme(aspect.ratio = 0.3) +
  theme(axis.title.y = element_blank())

  if (i > (length(plot.list.d) - 3)) {
    plot.list.d[[i]] <- plot.list.d[[i]] + theme(axis.text.x = element_text())
  } else {
    plot.list.d[[i]] <- plot.list.d[[i]] + theme(axis.text.x = element_blank())
  }
}

wrap_plots(plot.list.d, ncol = 3)

```



```
pdf(file = "../figures/EC_figure_d.pdf", width = 5, height = 6)
plot(wrap_plots(plot.list.d, ncol = 3))
dev.off()
```

```
## pdf
## 2
```

```
boot.data <- FetchData(subset(ec.dataset, subset = genotype ==
  "WT"), vars = "ident")$ident
ko.data <- FetchData(subset(ec.dataset, subset = genotype ==
  "KO"), vars = "ident")$ident

pct.gt <- function(data, value) {
  total <- length(data)
  selected <- length(data[data == value])
  return(selected/total)
}

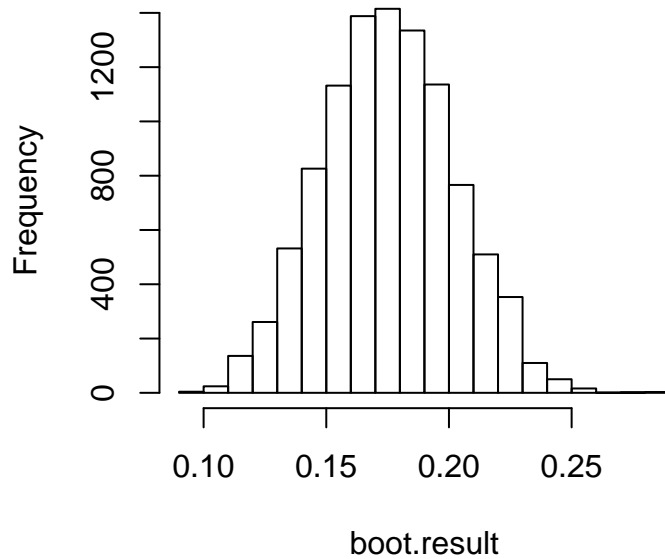
nboots <- 10000
boot.result <- numeric(nboots)
set.seed(42)

# number of KO endothelial cells
sample.size <- length(ko.data)

for (i in 1:nboots) {
  boot.sample <- sample(boot.data, sample.size, replace = TRUE)
  boot.result[i] <- pct.gt(boot.sample, "SC")
}

hist(boot.result)
```

Histogram of boot.result



```
quantile(boot.result, c(0.025, 0.975))
```

```
##      2.5%      97.5%
## 0.1244019 0.2296651
```

```
mean(boot.result)
```

```
## [1] 0.1751062
```

```
bar.data <- data.frame(genotype = c("WT", "KO"), percent.sc = c(mean(boot.result),
  pct.gt(ko.data, "SC")), bot = c(quantile(boot.result, 0.025),
  0), top = c(quantile(boot.result, 0.975), 0))
```

```
bar.data$genotype <- factor(bar.data$genotype, levels = c("WT",
  "KO"))
```

```
p1 <- ggplot(data = bar.data, aes(x = genotype, y = percent.sc,
  fill = genotype)) + theme(axis.line = element_line(size = 0.7)) +
  theme(axis.ticks.y = element_line(size = 0.7), axis.ticks.x = element_blank()) +
  theme(aspect.ratio = 2.5)
```

```
p1 <- p1 + geom_bar(stat = "identity", color = "black", position = position_dodge(width = 0.8),
  width = 0.8)
```

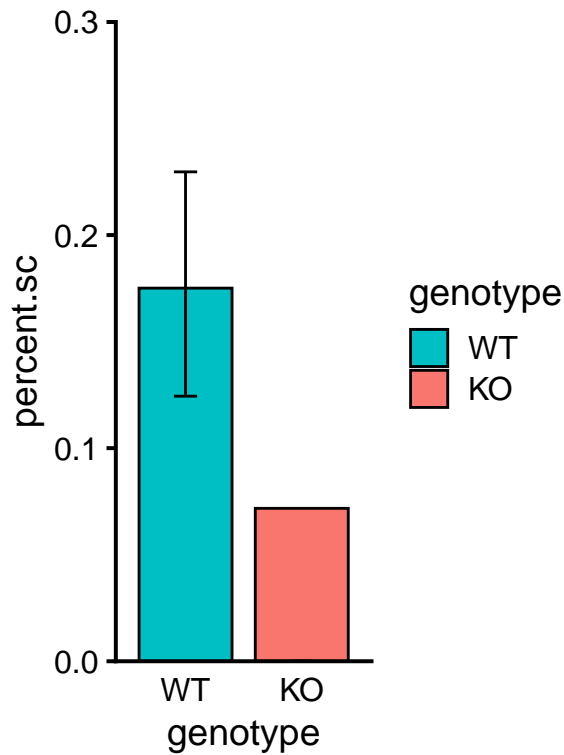
```
p1 <- p1 + geom_errorbar(aes(ymin = bot, ymax = top), width = 0.2,
  position = position_dodge(0.9))
```

```
p1 <- p1 + scale_fill_manual(values = c("#00bfc4", "#f8766d"))
```

```
p1 <- p1 + scale_y_continuous(expand = c(0, 0), limits = c(0,
```

```
0.3), breaks = c(0, 0.1, 0.2, 0.3))
```

```
plot(p1)
```



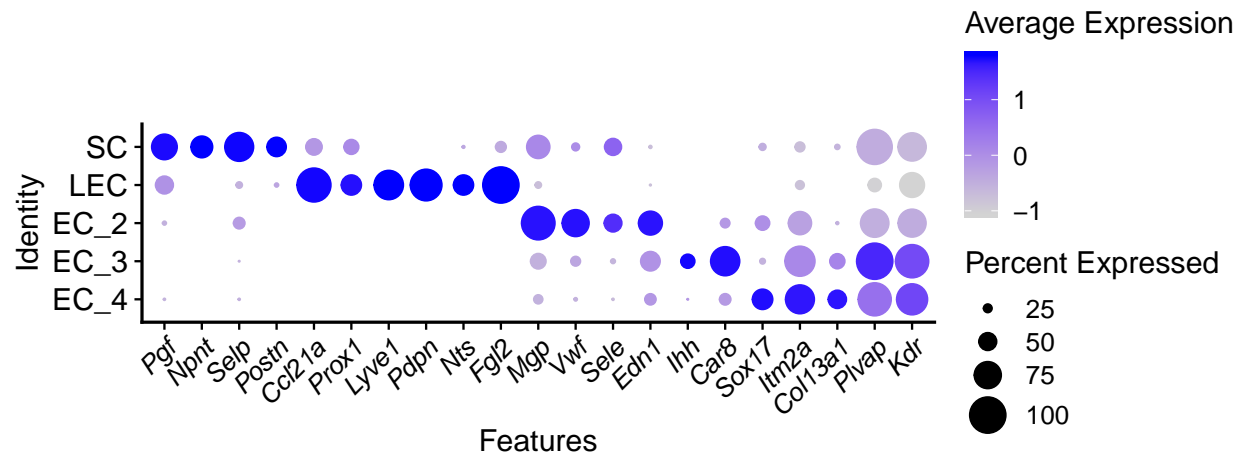
```
pdf(file = "../figures/EC_figure_e.pdf", width = 4, height = 4)
plot(p1)
dev.off()
```

```
## pdf
## 2
```

```
panel.g <- ec.dataset
panel.g@active.ident <- factor(x = panel.g@active.ident, levels = c("EC_4",
  "EC_3", "EC_2", "LEC", "SC"))
genelist <- read.csv("../figures/genes_for_ec_heatmap.csv", stringsAsFactors = F,
  fileEncoding = "UTF-8-BOM")$gene

dot <- DotPlot(panel.g, features = genelist, assay = "RNA", dot.scale = 7.5,
  dot.min = 0.05) + theme(axis.line = element_line(size = 0.7)) +
  theme(aspect.ratio = 0.25) + theme(axis.text.x = element_text(angle = 45,
  face = "italic", vjust = 1, hjust = 1)) + theme(axis.text.y = element_text(size = 14))

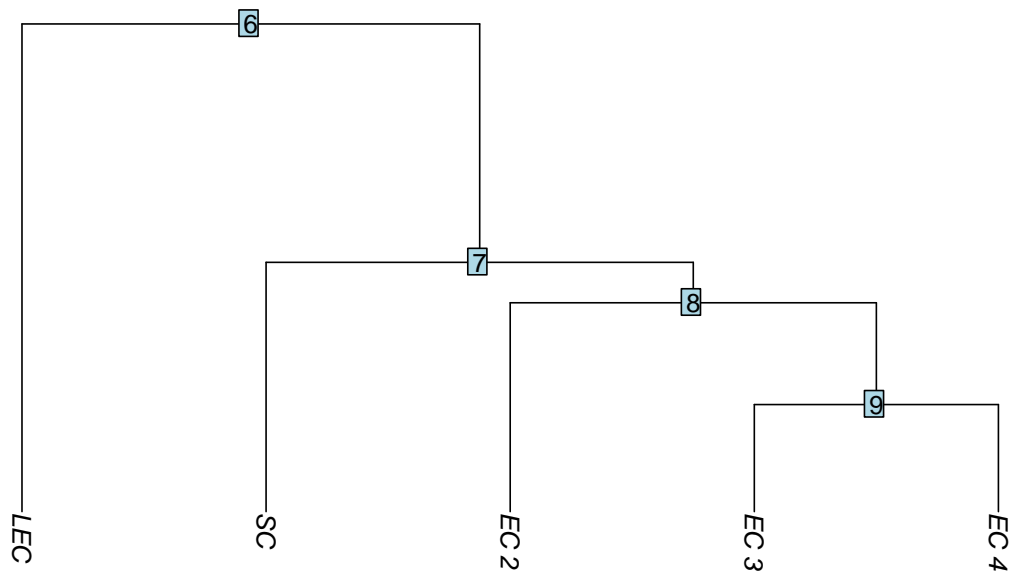
plot(dot)
```

```
pdf(file = "../figures/EC_figure_g.pdf", width = 8, height = 4)
plot(dot)
dev.off()
```

```
## pdf
## 2
```

```
panel.f <- BuildClusterTree(ec.dataset, assay = "RNA", reorder = T)
PlotClusterTree(panel.f)
```

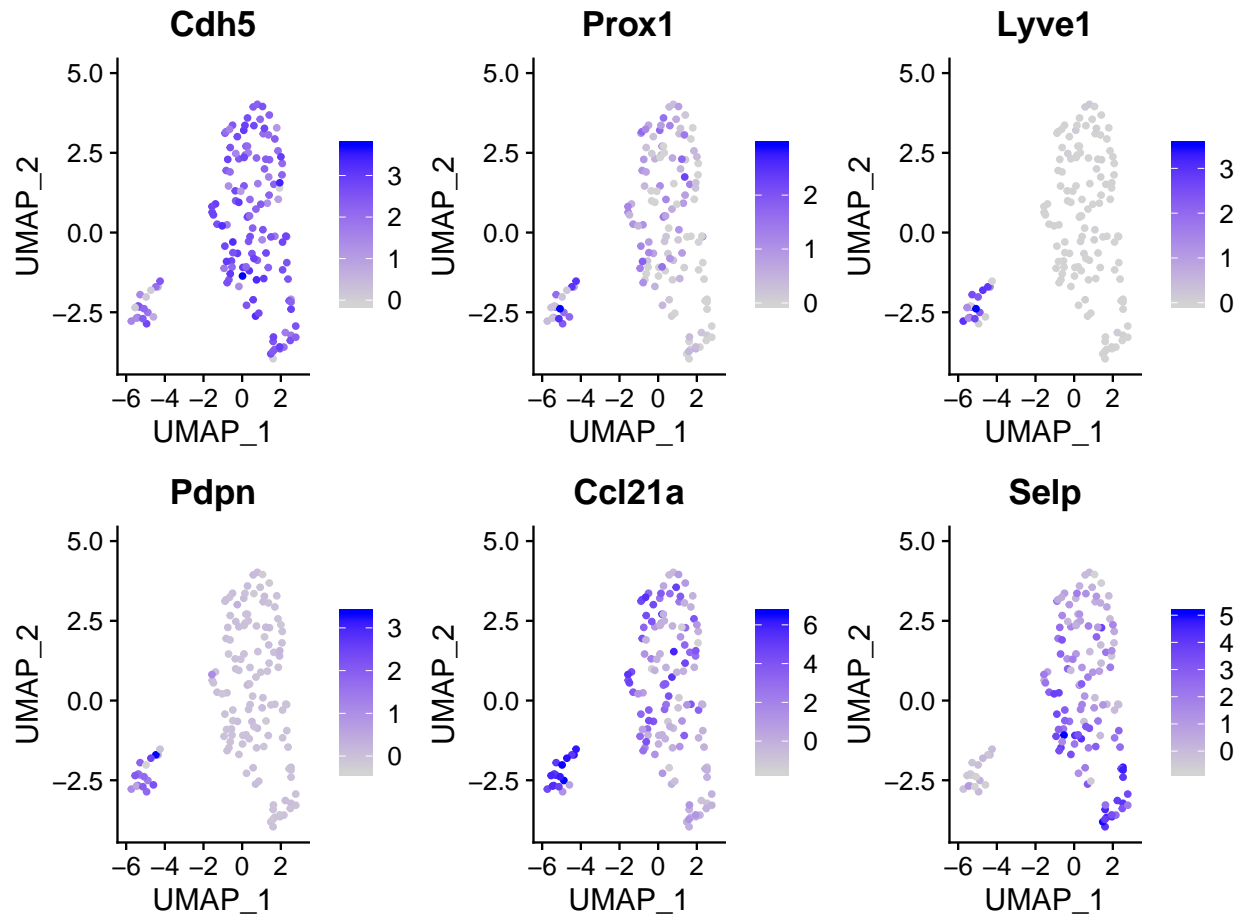


```
pdf(file = "../figures/EC_figure_f.pdf", width = 4, height = 4)
PlotClusterTree(panel.f)
dev.off()
```

```
## pdf
## 2
```

The data from Panel C was also used to generate Supplemental figure 7.

```
supp.fig.7 <- FeaturePlot(panel.c, features = c("Cdh5", "Prox1",
  "Lyve1", "Pdpn", "Ccl21a", "Selp"), ncol = 3)
plot(supp.fig.7)
```



```
pdf(file = "../figures/Supp_Fig_7.pdf", width = 7, height = 4)
plot(supp.fig.7)
dev.off()
```

```
## pdf
## 2
```