

# TM\_figure

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12/2/2020

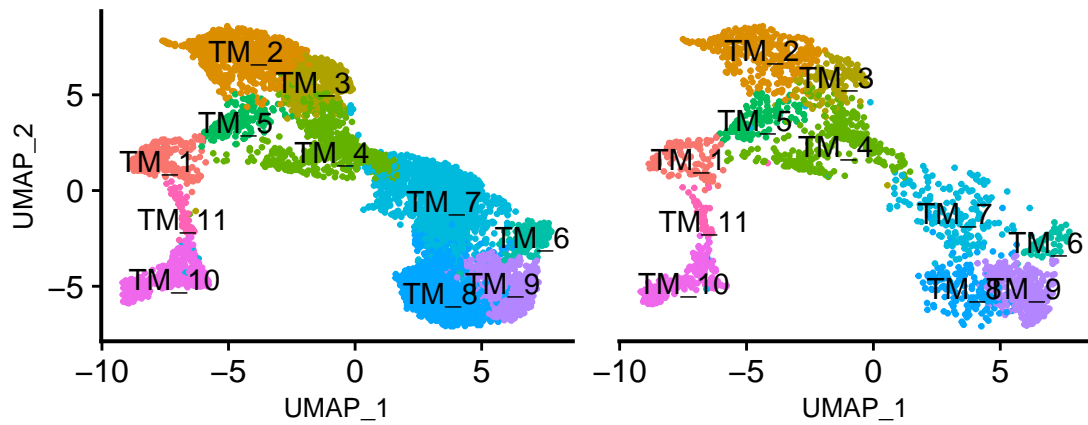
```
TM.dataset <- readRDS("../data/6wk_TM_subset_10-7-20.rds.gz")
full.dataset <- readRDS("../data/full_dataset_10-2-20.rds.gz")

TM.dataset@active.ident <- factor(TM.dataset@active.ident,
                                  levels = c("TM_1", "TM_2", "TM_3", "TM_4", "TM_5", "TM_6",
                                              "TM_7", "TM_8", "TM_9", "TM_10", "TM_11"))

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

panel.a.fig.ko <- DimPlot(object = subset(TM.dataset, subset = genotype == "KO"),
                          reduction = "umap", label = TRUE, pt.size = 0.5, label.size = 4) +
  NoLegend() + coord_fixed() + theme(aspect.ratio=0.7) +
  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())

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



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

keep(TM.dataset, full.dataset, sure = T)
```

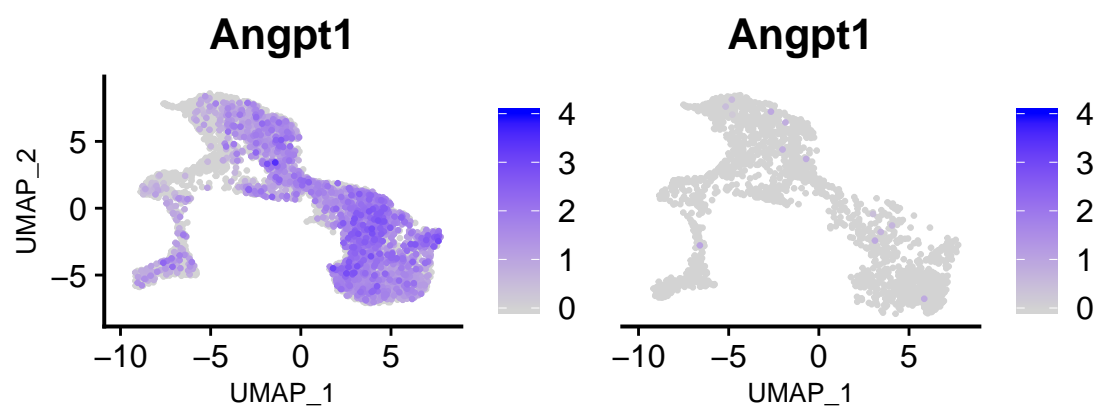
```

DefaultAssay(TM.dataset) <- "RNA"
panel.b.fig.wt <- FeaturePlot(object = subset(TM.dataset, subset = genotype == "WT"), features = "Angi",
                             reduction = "umap", label = F, pt.size = 0.5, label.size = 4, order = T) +
  coord_fixed() + theme(aspect.ratio=0.7) +
  theme(axis.line = element_line(color="black", size = 0.7)) +
  theme(axis.title = element_text(size = 10)) +
  scale_color_gradientn( colours = c('lightgrey', 'blue'), limits = c(0, 4))

panel.b.fig.ko <- FeaturePlot(object = subset(TM.dataset, subset = genotype == "KO"), features = "Angi",
                             reduction = "umap", label = F, pt.size = 0.5, label.size = 4, order = T) +
  coord_fixed() + theme(aspect.ratio=0.7) +
  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()) +
  scale_color_gradientn( colours = c('lightgrey', 'blue'), limits = c(0, 4))

panel.b.fig.wt + panel.b.fig.ko

```



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

keep(TM.dataset, full.dataset, sure = T)
```

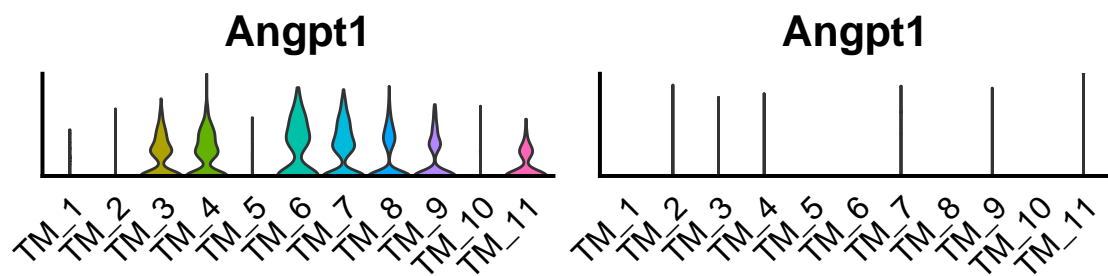
```

DefaultAssay(TM.dataset) <- "RNA"
panel.c.fig.wt <- VlnPlot(object = subset(TM.dataset, subset = genotype == "WT"), features = "Angpt1",
  label = F, pt.size = 0, label.size = 4) + NoLegend() +
  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=0.2) +
  theme(axis.title.y = element_blank())

panel.c.fig.ko <- VlnPlot(object = subset(TM.dataset, subset = genotype == "KO"), features = "Angpt1",
  label = F, pt.size = 0, label.size = 4) + NoLegend() +
  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=0.2) +
  theme(axis.title.y = element_blank())

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

```



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

keep(TM.dataset, full.dataset, sure = T)
```

```

total.cells <- table(full.dataset@meta.data$genotype)

sample.names <- c("WT", "KO")

#get total number of cells per TM cluster and calculate each cluster as % of total cells per genotype
cells.per.cluster <- table(TM.dataset@active.ident, TM.dataset@meta.data$genotype)
WT <- cells.per.cluster[, "WT"] / total.cells[["WT"]] * 100
KO <- cells.per.cluster[, "KO"] / total.cells[["KO"]] * 100
diff <- KO/WT * 100
cells.per.cluster.pct <- data.frame(WT, KO, diff, row.names = rownames(cells.per.cluster))

#add the total number of corneal/limbal epithelial cells
cells.total.epi <- table(full.dataset@active.ident, full.dataset@meta.data$genotype)[3:5,]
WT <- sum(cells.total.epi[, "WT"]) / total.cells[["WT"]] * 100
KO <- sum(cells.total.epi[, "KO"]) / total.cells[["KO"]] * 100
diff <- KO/WT * 100
epi.df <- data.frame(WT, KO, diff, row.names = "Total epi")

#merge the two frames
cells.per.cluster.pct <- rbind(epi.df, cells.per.cluster.pct)

cells.per.cluster.pct %>%
kbl(caption = "Cells per cluster (percent of all cells in dataset)") %>%
kable_minimal(full_width = FALSE, position = "left")

write.csv(cells.per.cluster.pct, file = "../figures/TM_cell_number_pcts.csv")

keep(TM.dataset, full.dataset, sure = T)

```

```

plot.list.e <- VlnPlot(TM.dataset, assay = "RNA", features = c( "Kera", "Cdh2", "Atp1b1", "Cldn10", "I
pt.size = 0, combine = F)

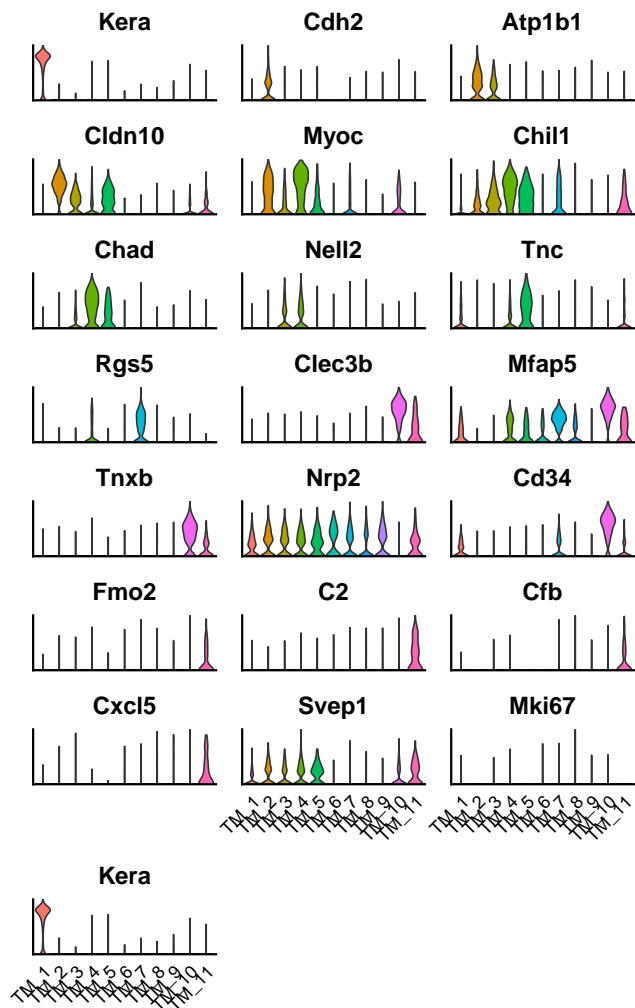
for(i in seq_along(plot.list.e)) {
  plot.list.e[[i]] <- plot.list.e[[i]] +
    theme(axis.ticks= element_blank(), axis.text.y = element_blank(),
          axis.title.x = element_blank()) +
    theme(title = element_text(family = "sans", face = "italic")) +
    theme(axis.line = element_line(size = 0.7))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

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

```

```
}  
  
wrap_plots(plot.list.e, ncol = 3)
```

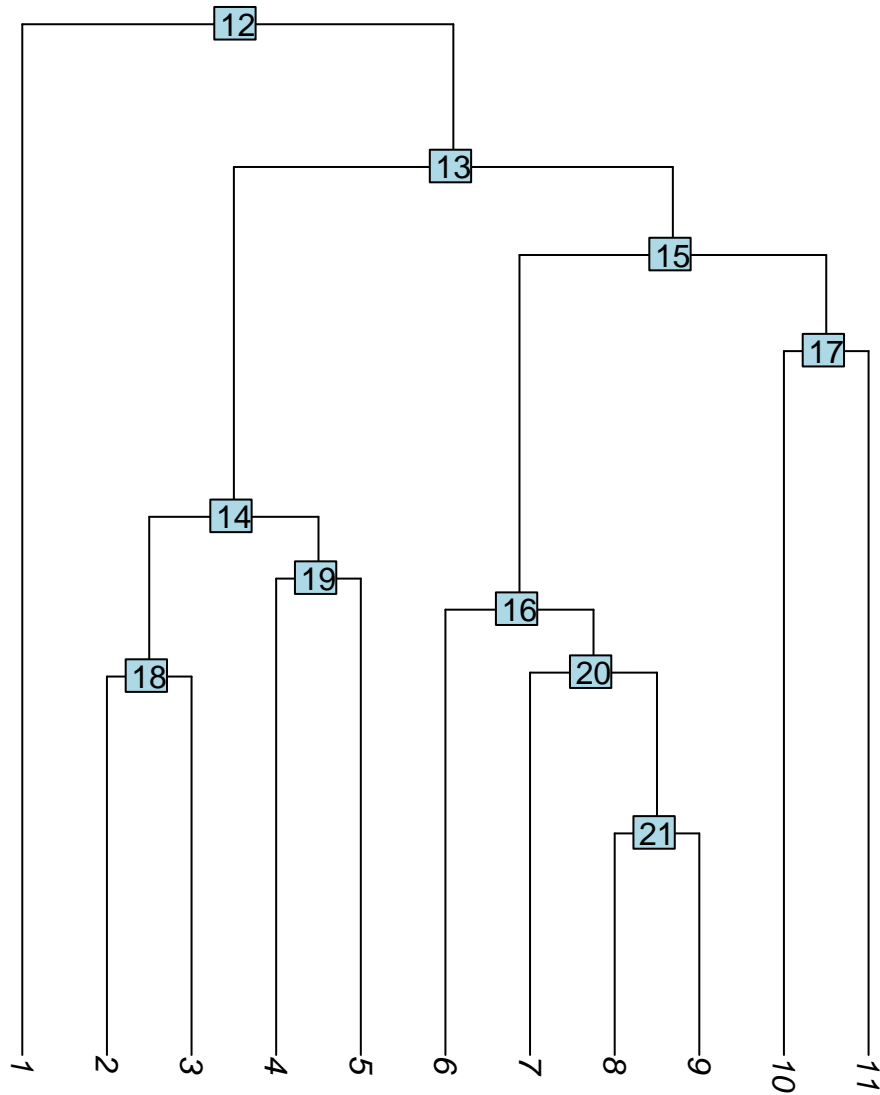




```
pdf(file = "../figures/TM_figure_e_v2.pdf", width = 5, height = 5)
plot(wrap_plots(plot.list.e))
dev.off()
```

```
keep(TM.dataset, full.dataset, sure = T)
```

```
PlotClusterTree(TM.dataset)
```



```
pdf(file = "../figures/TM_figure_I_.pdf", width = 5, height = 5)
PlotClusterTree(TM.dataset)
dev.off()
```