

EC_figure

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

#plot overall cluster map, genotypes merged
p1 <- DimPlot(object = full.dataset, reduction = "umap", label = TRUE, pt.size = 0.5,
               label.size = 4, repel = FALSE, cols = rev(hue_pal()(25))) +
  NoLegend() +
  theme(aspect.ratio=1.2) +
  #theme(axis.line = element_line(color="black", size = 0.7)) +
  theme(axis.title = element_text(size = 10))

#compare distribution of WT and Angpt1dNC cells
p2 <- DimPlot(object = full.dataset, reduction = "umap", label = FALSE, group.by = "genotype",
               pt.size = 0.5, label.size = 8) +
  NoLegend() +
  theme(aspect.ratio=1.2) +
  #theme(axis.line = element_line(color="black", size = 0.7)) +
  theme(axis.title = element_text(size = 10))

full.dataset$genotype <- factor(x = full.dataset$genotype, levels = c("WT", "KO"))

#Show number of Angpt1 expressing cells in WT and Angpt1dNC samples.
p3 <- VlnPlot(full.dataset, group.by = 'genotype', features = "Angpt1", assay = "RNA", pt.size = 0.5)
  NoLegend() +
  theme(aspect.ratio = 2.5) +
  #theme(axis.line = element_line(size = 0.7)) +
  theme(axis.ticks.x = element_blank()) +
  theme(title = element_blank())
  #theme(plot.margin = unit(c(0,0,0,0), "cm"))

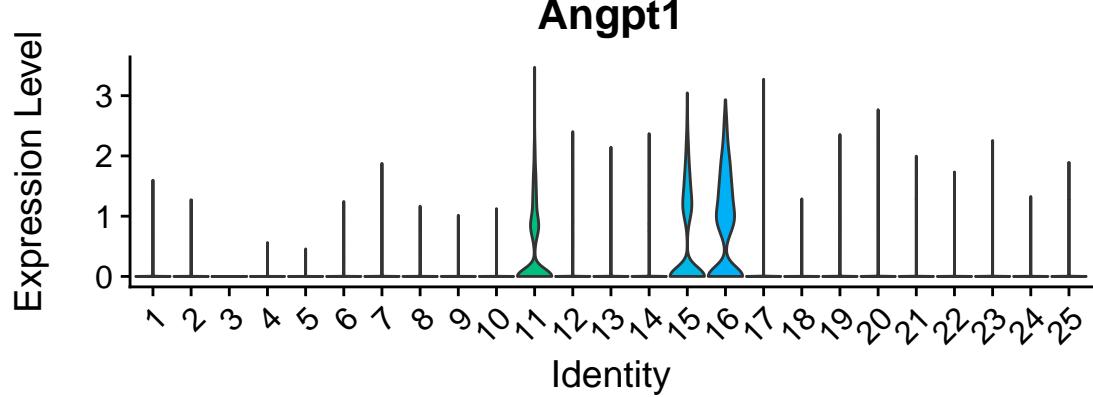
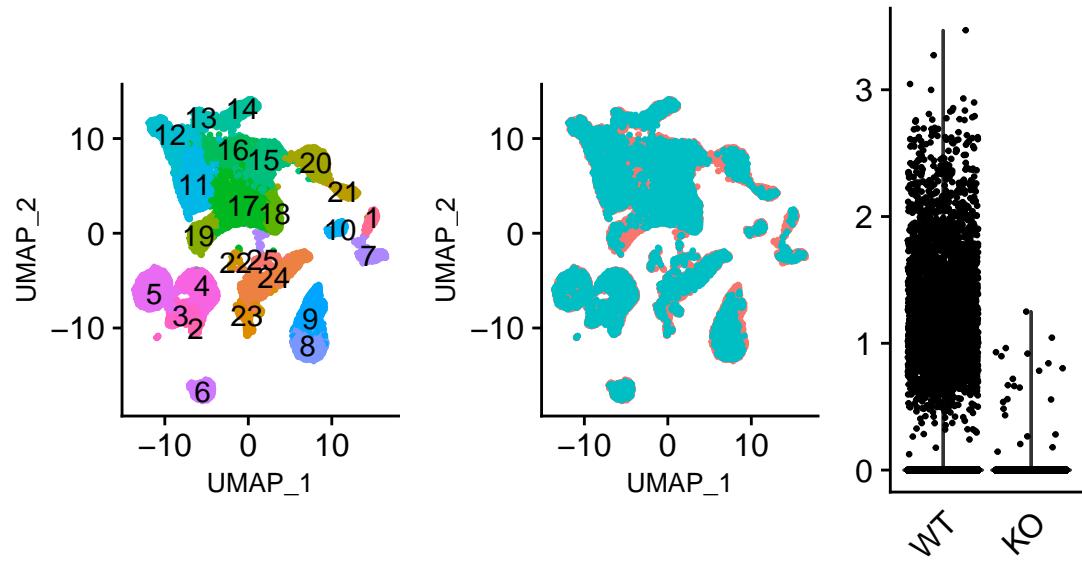
#plot the figure, including a vln plot showing Angpt1 expression by cluster in the WT dataset.

p4 <- VlnPlot(subset(full.dataset, subset = genotype == "WT"),
              features = "Angpt1", pt.size = 0, assay = "RNA") + NoLegend()
  #theme(aspect.ratio = 0.2)

#p4 + plot_layout(widths = 2.5)

top <- (p1 + p2 + p3) + plot_layout(widths = c(1,1,0.7), heights = c(1))

wrap_plots(top, p4, ncol = 1) + plot_layout(widths = 1, heights = c(4,1))
```

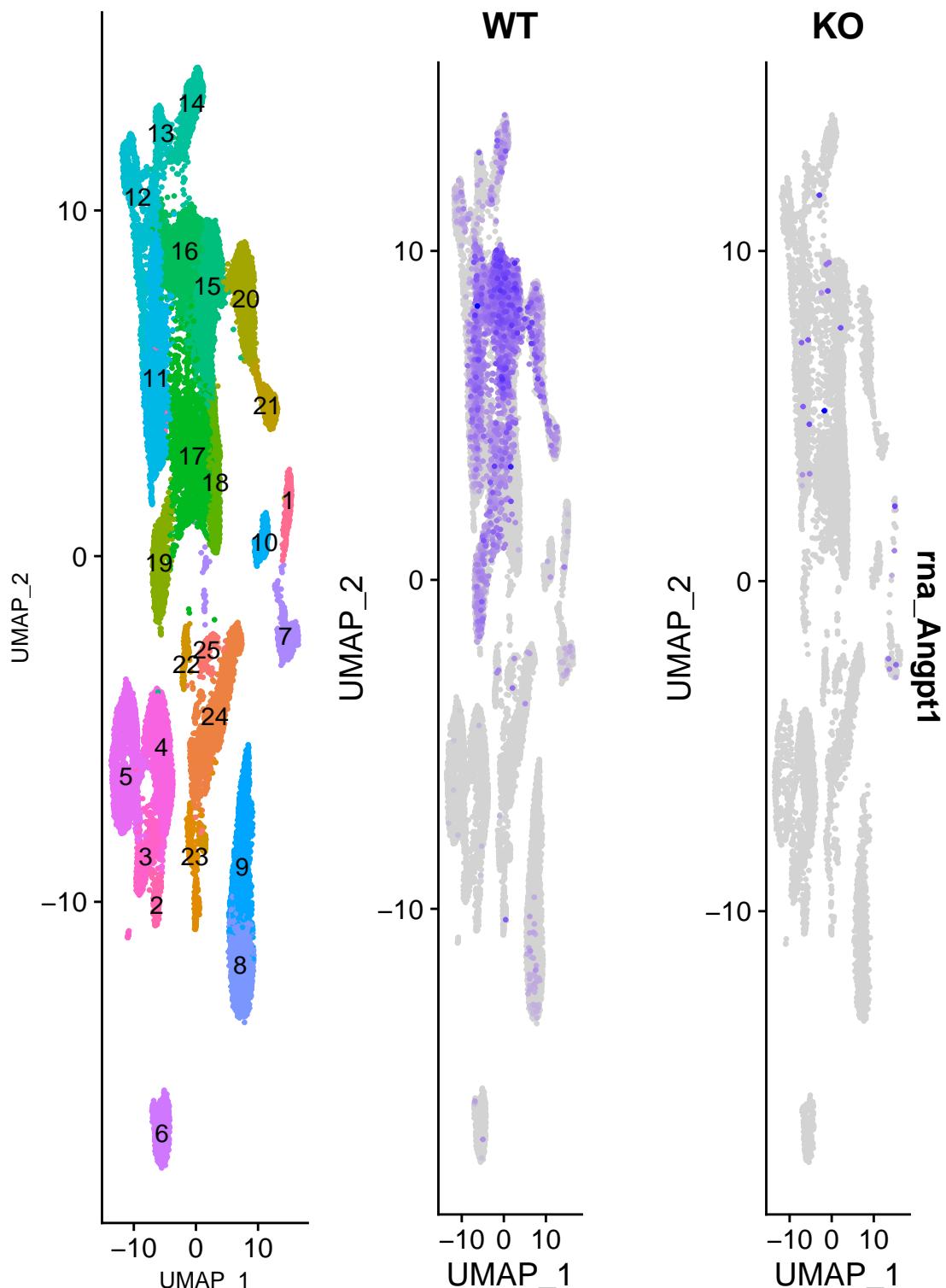


```
pdf(file = "../figures/full_figure_a-d.pdf", width = 5, height = 5)
wrap_plots(top, p4, ncol = 1) + plot_layout(widths = 1, heights = c(4,1))
dev.off()
```

```
p1 <- DimPlot(object = full.dataset, reduction = "umap", label = TRUE, pt.size = 0.5,
               label.size = 4, repel = FALSE, cols = rev(hue_pal()(25))) +
  NoLegend() +
  #theme(axis.line = element_line(color="black", size = 0.7)) +
  theme(axis.title = element_text(size = 10))

p2 <- FeaturePlot(full.dataset, features = "Angpt1", pt.size = 0.5,
                   split.by = "genotype", combine = T, order = T)

plot_grid(p1,p2, rel_widths = c(1,2))
```



```
selectedmarkers <- as.data.frame(read.csv("../diff_exp_by_cluster/FullClusterMarkers_10-2-20.csv",
                                         stringsAsFactors = FALSE))["Marker"]

rev.levels <- list()
for (x in seq_along(full.dataset@active.ident)) {
```

```

rev.levels[x] <- length(levels(full.dataset@active.ident)) - (x-1)
}

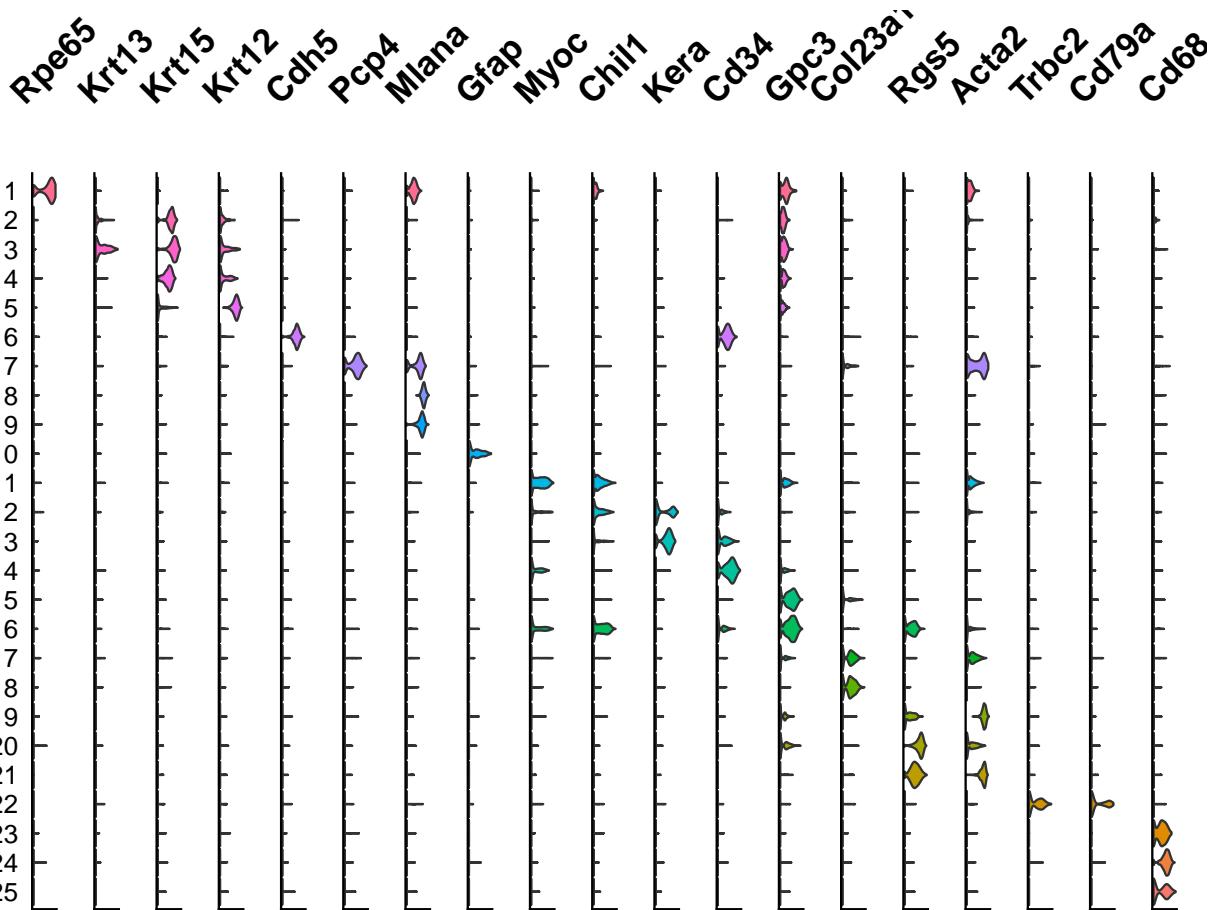
full.dataset.vln <- full.dataset
full.dataset.vln@active.ident <- factor(x = full.dataset.vln@active.ident, levels = rev.levels)

selectedmarkers <- unlist(selectedmarkers$Marker)
int.vln <- VlnPlot(full.dataset.vln, features = selectedmarkers, ncol = 1, pt.size = 0,
                     assay = "RNA", combine = FALSE)
p1 <- list()
for(i in seq_along(int.vln)) {
  p1[[i]] <- int.vln[[i]] + theme(axis.ticks.x = element_blank(), axis.text.x = element_blank(),
                                    axis.title = element_blank(), #title = element_blank(),
                                    axis.ticks.y = element_blank()) +
    NoLegend() + #theme(axis.text.y = element_blank()) +
    #theme(axis.line = element_line(size = 0.7)) +
    coord_flip() + theme(title = element_text(angle = 45))

  if(i != 1) {
    p1[[i]] <- p1[[i]] + theme(axis.text.y = element_blank())
  }
}

wrap_plots(p1, nrow = 1)

```



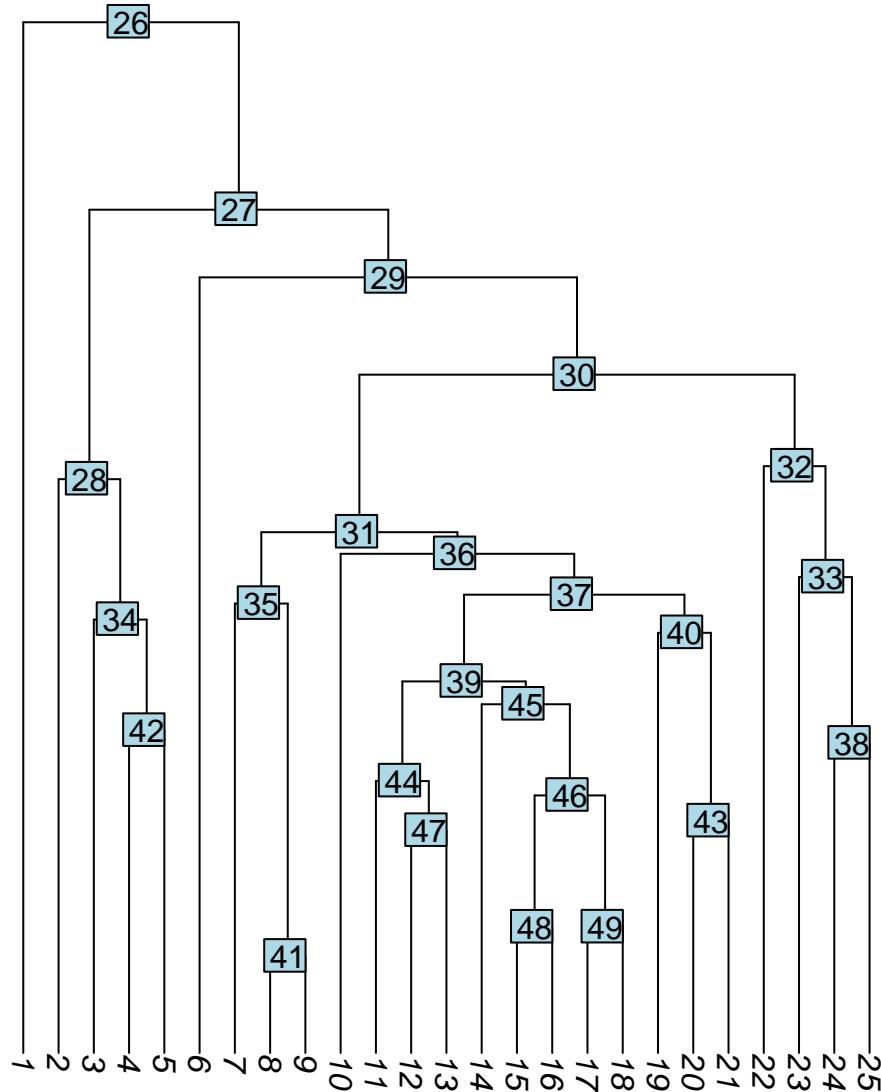
```

pdf(file = "../figures/full_figure_e.pdf", width = 5, height = 5)
wrap_plots(p1, nrow = 1)
dev.off()

rm(full.dataset.vln)

PlotClusterTree(full.dataset)

```



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

```

DefaultAssay(full.dataset) <- "RNA"
table(full.dataset@meta.data$orig.ident)
table(subset(full.dataset, subset = Angpt1 > 0)@meta.data$orig.ident)

```

Data from panel A was also used to generate Supplemental figure 6.

```

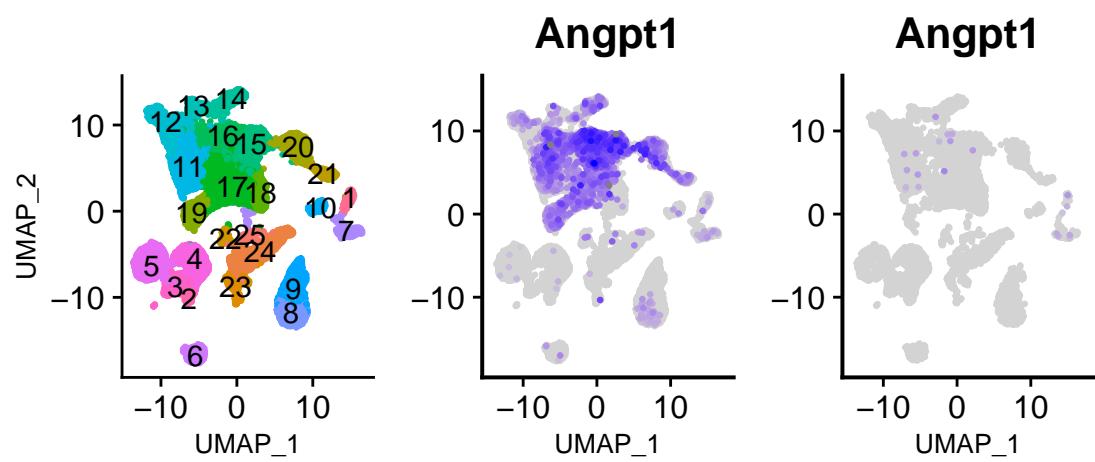
p1 <- DimPlot(object = full.dataset, reduction = "umap", label = TRUE, pt.size = 0.5,
               label.size = 4, repel = FALSE, cols = rev(hue_pal()(25))) +
  NoLegend() +
  theme(aspect.ratio=1.2) +
  theme(axis.title = element_text(size = 10))

p2 <- FeaturePlot(object = subset(full.dataset, subset = genotype == "WT"), features = "Angpt1",
                    reduction = "umap", label = F, pt.size = 0.5, label.size = 4, order = T) +
  coord_fixed() + theme(aspect.ratio=1.2) +
  theme(axis.line = element_line(color="black", size = 0.7)) +
  theme(axis.title = element_text(size = 10)) +
  theme(axis.title.y = element_blank()) +
  scale_color_gradientn( colours = c('lightgrey', 'blue'), limits = c(0, 3)) + NoLegend()

p3 <- FeaturePlot(object = subset(full.dataset, subset = genotype == "KO"), features = "Angpt1",
                    reduction = "umap", label = F, pt.size = 0.5, label.size = 4, order = T) +
  coord_fixed() + theme(aspect.ratio=1.2) +
  theme(axis.line = element_line(color="black", size = 0.7)) +
  theme(axis.title.x = element_text(size = 10)) +
  theme(axis.title.y = element_blank()) +
  scale_color_gradientn( colours = c('lightgrey', 'blue'), limits = c(0, 3)) + NoLegend()

p1 + p2 + p3

```



```
pdf(file = "../figures/Supp_figure_6.pdf", width = 7, height = 4)
plot(p1 + p2 + p3)
dev.off()
```