UMAP, clustering, and annotation

```
library(Seurat)
library(ggplot2)
library(dplyr)
library(patchwork)
library(ggplot2)
setwd("~/dev/CCRItask")
```

```
Loading required package: SeuratObject
```

```
Loading required package: sp
```

```

Attaching package: 'SeuratObject'
```

```

The following objects are masked from 'package:base':

    intersect, t
```

```


Attaching package: 'dplyr'
```

```

The following objects are masked from 'package:stats':

    filter, lag
```

```

The following objects are masked from 'package:base':

    intersect, setdiff, setequal, union
```

Load the integrated data

```
integrated <- readRDS("data/processed/integrated.rds")
```

Perfrom Clustering

```r
integrated <- FindNeighbors(integrated, dims = 1:30)
integrated <- FindClusters(integrated, resolution = 0.1)
```

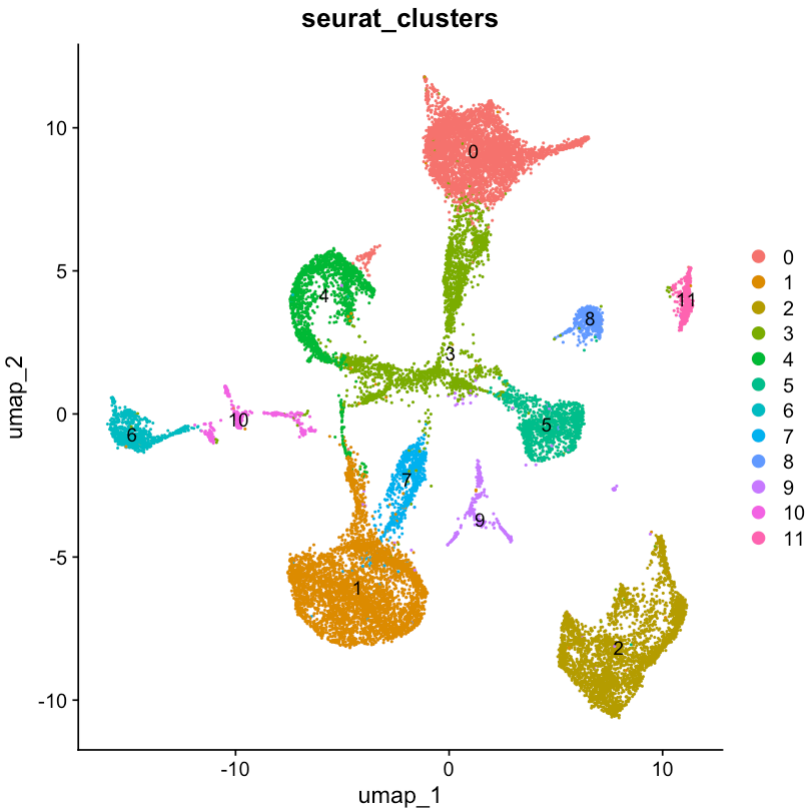
```
Computing nearest neighbor graph

Computing SNN


Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 19803
Number of edges: 805203

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.9772
Number of communities: 12
Elapsed time: 2 seconds
```

```r
head(integrated[[]])
```

A data.frame: 6 x 15

	orig.identnCount_nFeatureAsample_RNA	week	sample	gsm_id	conditionpercent.mt	S.Score	G2M.Score	Phase	CC.Difference	integrated_snn_res.0.1	seurat_clusters
	<chr> <dbl> <int> <chr>	<dbl>	<chr>	<chr>	<chr> <dbl>	<dbl>	<dbl>	<chr>	<dbl>	<fct>	<fct>
AAACGAAGTCGCAACC-1_1 (overprinted: week8_1083 4507)	week8_8	001	001	GSM4446535 regular 9.716130	-0.01013	0.3154301	G1	0.1441617	32	1
AAACGCTGTCATTCCA-1_1 (overprinted: week8_5589 2236)	week8_8	001	001	GSM4446535 regular 3.954196	-0.084168	0.4183235	G1	0.0991585	05	8
AAAGAACAGAAGCGGT-1_1 (overprinted: week8_3090 1568)	week8_8	001	001	GSM4446535 regular 1.818182	-0.094712	0.0751724	G1	0.0804600	40	0
AAAGGATAGCGTTGTG-1_1 (overprinted: week8_6259 2271)	week8_8	001	001	GSM4446535 regular 4.649305	-0.0464329	0.2688638	G1	0.2224307	9	0
AAAGGATTCTGGCATG-1_1 (overprinted: week8_4952 2206)	week8_8	001	001	GSM4446535 regular 4.886914	-0.1312636	0.2573361	G1	0.1260782	53	8
AAAGGGCCAGGAATAC-1_1 (overprinted: week8_5899 2103)	week8_8	001	001	GSM4446535 regular 3.492117	-0.0414277	0.1686783	G1	0.1272501	61	10

```r
DimPlot(integrated,reduction = "umap", group.by = "seurat_clusters", label = TRUE)
```



Cluster annotation

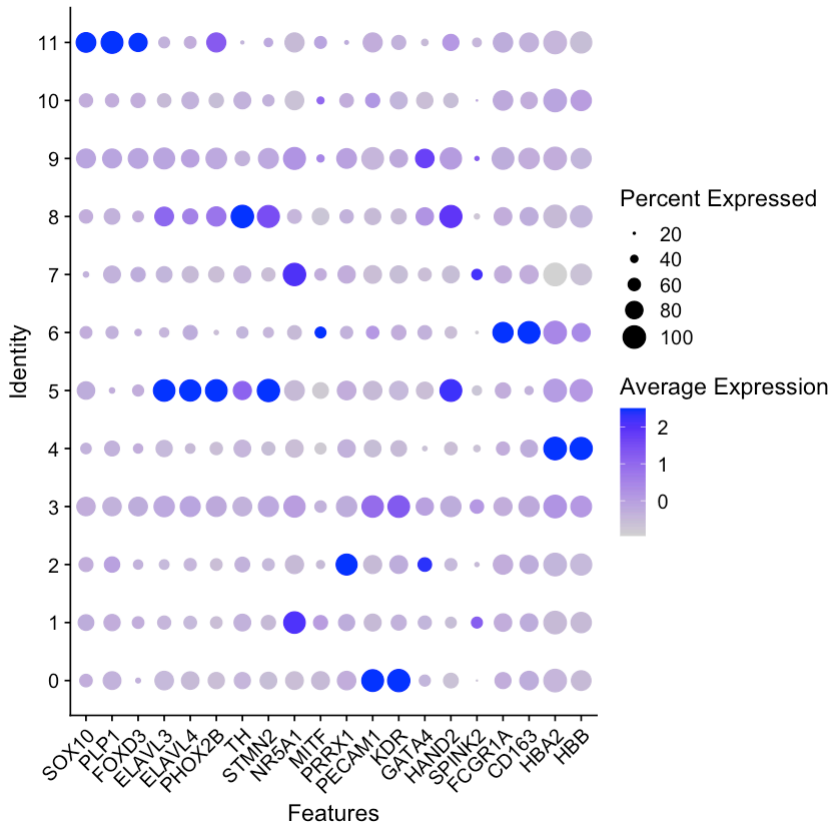
From the https://www.nature.com/articles/s41588-021-00818-x

```
cell_type_markers <- list(
  "SCPs" = c("SOX10", "PLP1", "FOXD3"),
  "Chromaffin cells" = c("ELAVL3", "ELAVL4", "PHOX2B", "TH"),
  "Sympathoblasts" = c("STMN2"),
  "Adrenal gland cortex" = c("NR5A1"),
  "Melanocytes" = c("MITF"),
  #"Kidney" = c("PAX2"),
  "Subepicardial and abdominal mesenchyme" = c("PRRX1"),
  "Endothelium" = c("PECAM1", "KDR"),
  "Intermediate mesoderm" = c("GATA4", "HAND2"),
  #"Liver" = c("HNF4A", "AHSG"),
  "HSCs" = c("SPINK2"), # AZU1
  "Immune cells" = c("FCGR1A", "CD163"),
  "Erythroid cells" = c("HBA2", "HBB")
)

markers_unique  = unique(unlist(cell_type_markers))
```

```
length(unique(unlist(cell_type_markers)))
```

Dotplot to map clusters to cell types

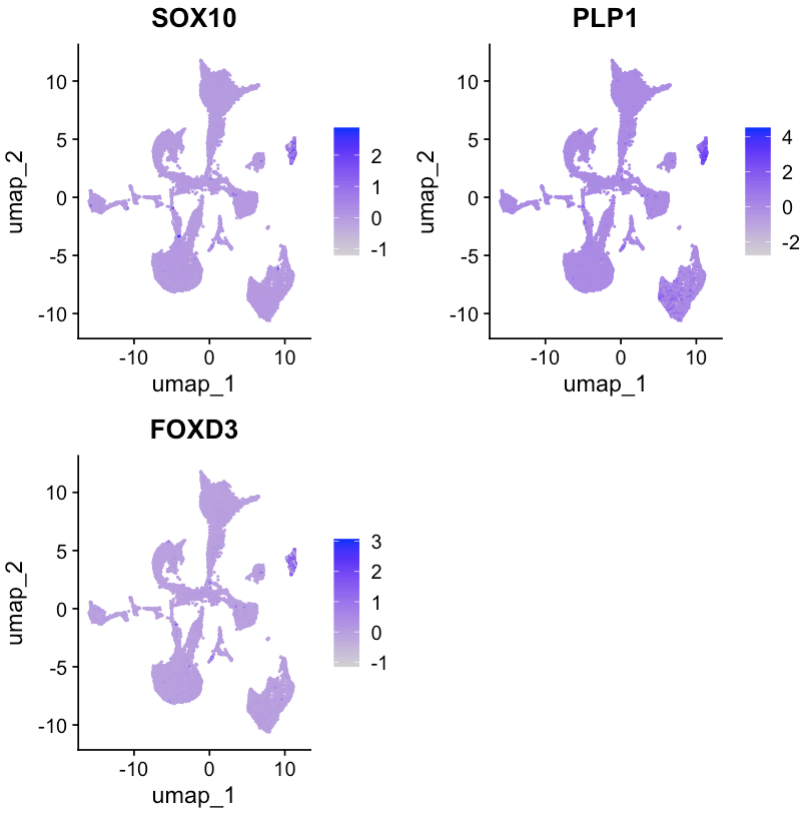
```
DotPlot(integrated, features = unique(unlist(cell_type_markers))) + RotatedAxis()
```



in depth analysis for manual annotation

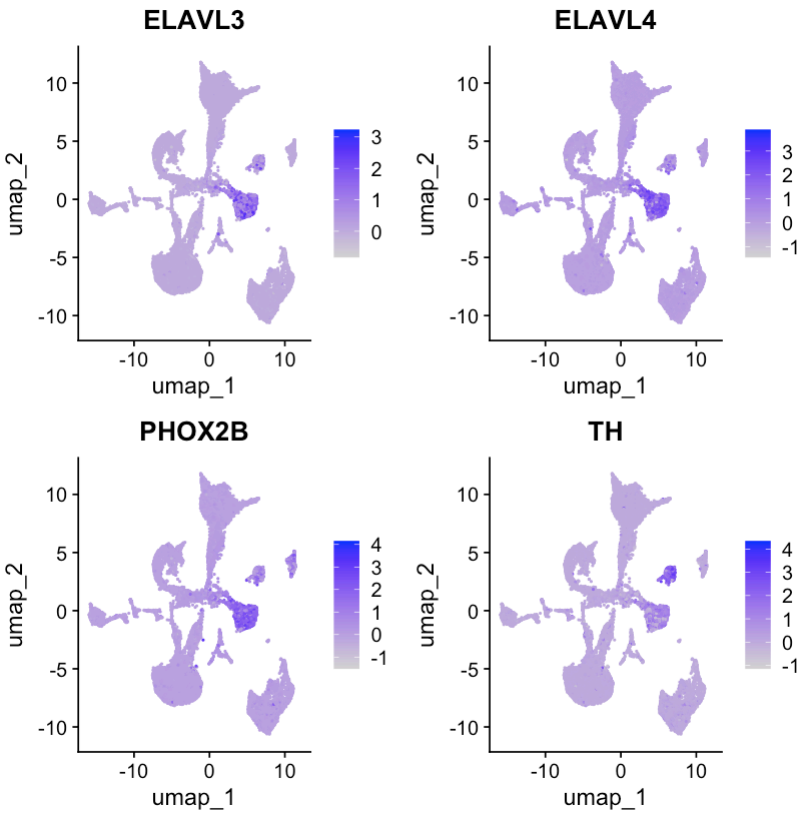
SCPs

```
FeaturePlot(integrated, c("SOX10", "PLP1", "FOXD3"), ncol=2, raster.dpi = c(800,100))
```

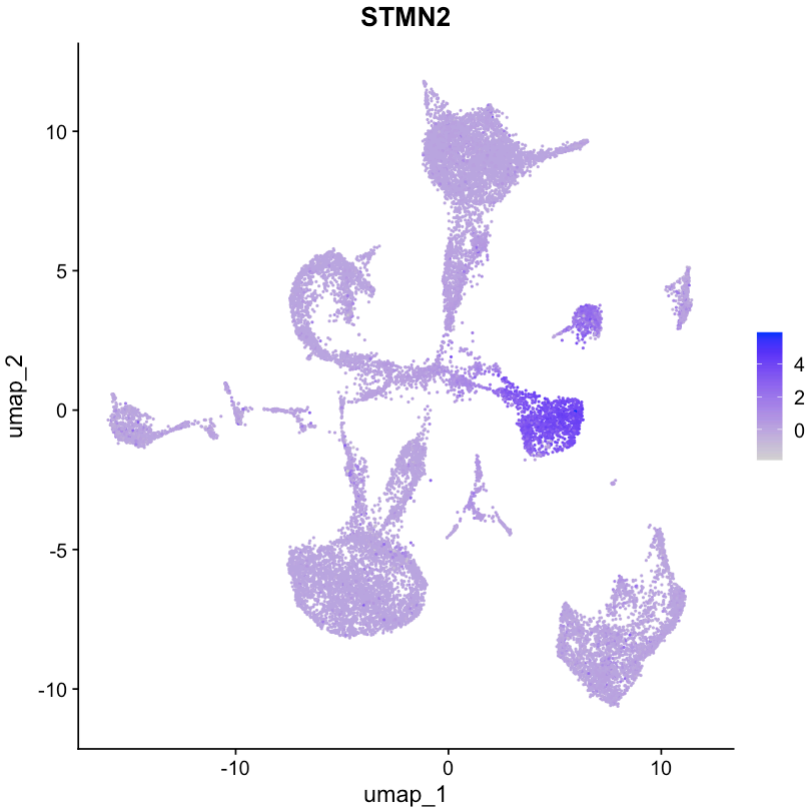


chromaffin cells

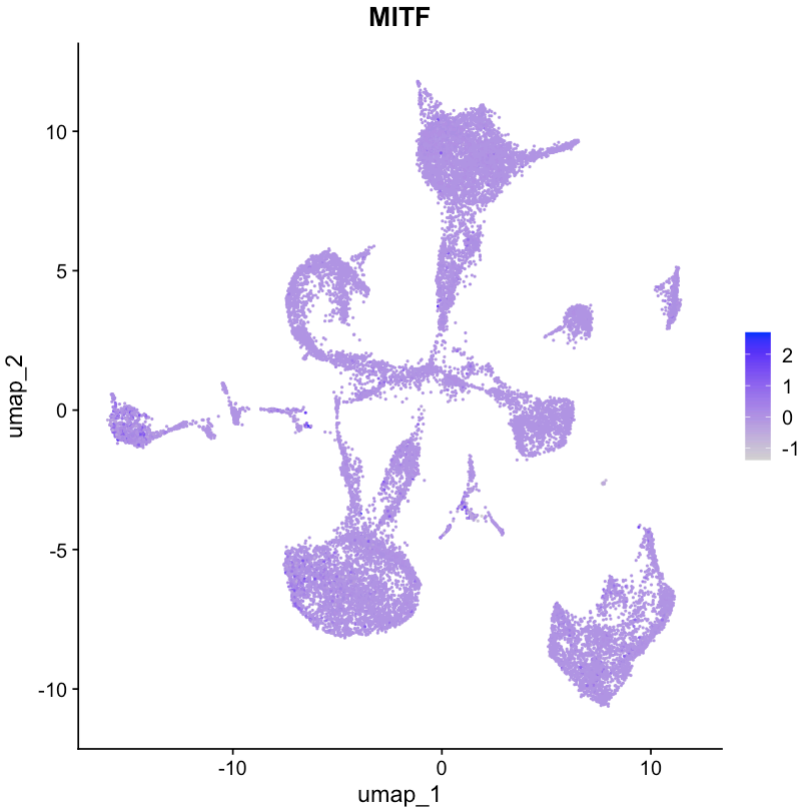
```
FeaturePlot(integrated, c("ELAVL3", "ELAVL4", "PHOX2B", "TH"), ncol=2, raster.dpi = c(800,100))
```



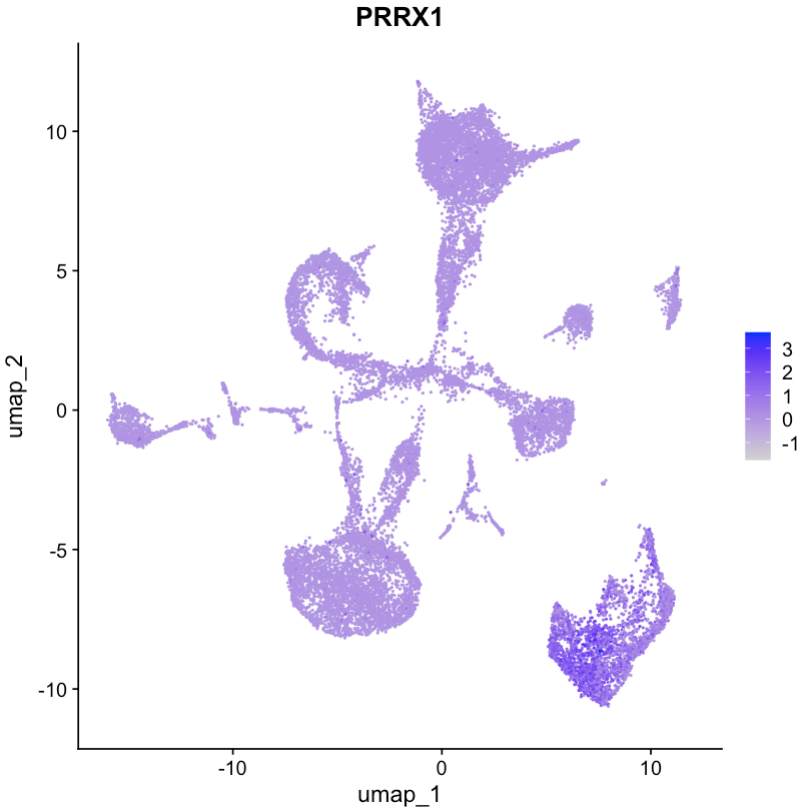
```
FeaturePlot(integrated, c("STMN2"), ncol=1, raster.dpi = c(800,100))
```



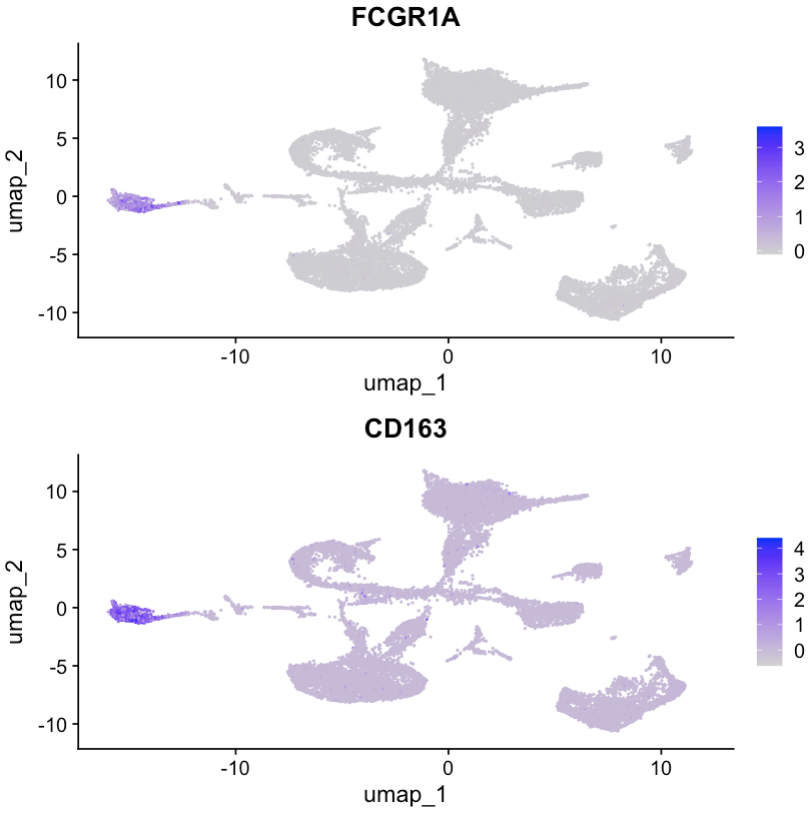

```
FeaturePlot(integrated, c("MITF"), ncol=1, raster.dpi = c(800,100))
```



```
FeaturePlot(integrated, c("PRRX1"), ncol=1, raster.dpi = c(800,100))
```




```
FeaturePlot(integrated, c("FCGR1A", "CD163"), ncol=1, raster.dpi = c(800,100))
```

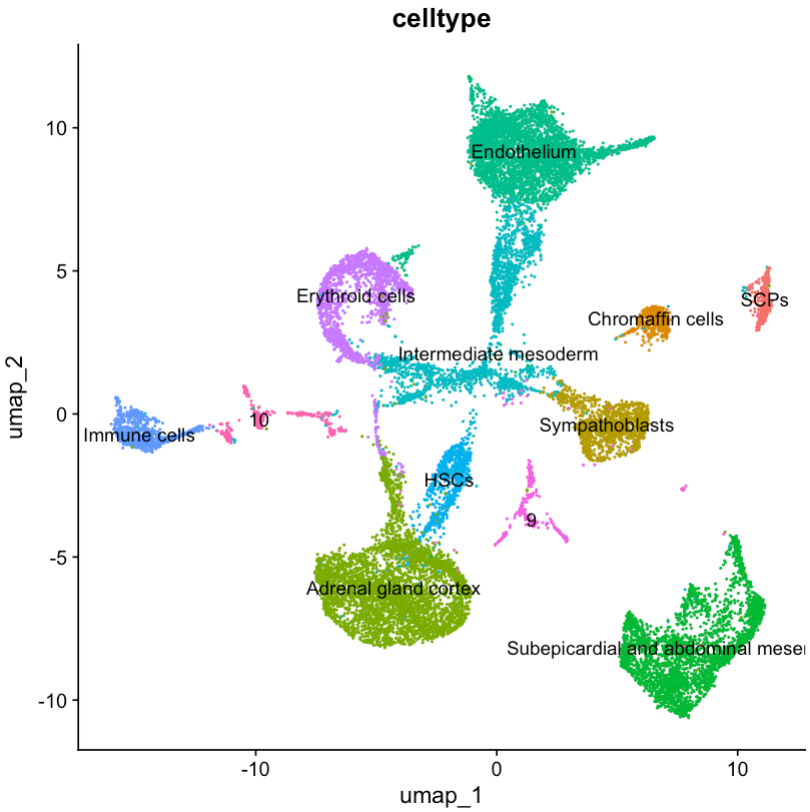


```
cluster <- list(
  "11" = "SCPs", #= c("SOX10", "PLP1", "FOXD3"),
  "8" = "Chromaffin cells", #= c("ELAVL3", "ELAVL4", "PHOX2B", "TH"),
  "5"= "Sympathoblasts", #= c("STMN2"),
  "1" = "Adrenal gland cortex", #= c("NR5A1"),
  # "6" = "Melanocytes", #= c("MITF"),
  #"Kidney" = c("PAX2"),
  "2" = "Subepicardial and abdominal mesenchyme",# = c("PRRX1"),
  "0" = "Endothelium", #= c("PECAM1", "KDR"),
  "3" = "Intermediate mesoderm", #c("GATA4", "HAND2"),
  #"Liver" = c("HNF4A", "AHSG"),
  "7"= "HSCs", #c("SPINK2"), # AZU1
  "6" = "Immune cells", #= #c("FCGR1A", "CD163"),
  "4" = "Erythroid cells" #= #c("HBA2", "HBB")
)
```

Mapping clusters to cell types

```
# Convert list to a character vector for easier handling
cluster <- unlist(cluster)
# Rename the identities
integrated <- RenameIdents(integrated, cluster)
# Save the renamed cluster identities
integrated$celltype <- Idents(integrated)
```

```
umap_ann <- DimPlot(integrated, group.by = "celltype", label = TRUE) + NoLegend()
umap_ann
```




```
ggsave("plots/umap_annotated.pdf", umap_ann, width = 10, height = 10)
```

Automatic cluster annotation

did not work properly

```
# Score each cell for each cell type based on marker genes
integrated <- AddModuleScore(
  integrated,
  features = cell_type_markers,
  name = names(cell_type_markers),
  ctrl = 20,
  replace = TRUE
)
```

```
install.packages('devtools')
devtools::install_github('immunogenomics/presto')
```

```
The downloaded binary packages are in
    /var/folders/wl/jrkngsm57b944tj7rtjg12000000gn/T//RtmpJlmekZ/downloaded_packages

Using GitHub PAT from the git credential store.

Skipping install of 'presto' from a github remote, the SHA1 (7636b3d0) has not changed since last in
  Use `force = TRUE` to force installation
```

Dot Plot for top5 markers for each cluster

```
# Find all markers for each cluster
markers <- FindAllMarkers(object = integrated,
                          only.pos = TRUE,    # Only consider positive markers
                          min.pct = 0.25,     # Minimum detection fraction
                          logfc.threshold = 0.25)  # Minimum log fold change
```

```
Calculating cluster SCPs

Warning message in mean.fxn(object[features, cells.1, drop = FALSE]):
"NaNs produced"
Calculating cluster Chromaffin cells

Warning message in mean.fxn(object[features, cells.1, drop = FALSE]):
"NaNs produced"
Calculating cluster Sympathoblasts

Warning message in mean.fxn(object[features, cells.1, drop = FALSE]):
"NaNs produced"
Calculating cluster Adrenal gland cortex

Warning message in mean.fxn(object[features, cells.1, drop = FALSE]):
"NaNs produced"
Calculating cluster Subepicardial and abdominal mesenchyme

Warning message in mean.fxn(object[features, cells.1, drop = FALSE]):
"NaNs produced"
Calculating cluster Endothelium

Warning message in mean.fxn(object[features, cells.1, drop = FALSE]):
"NaNs produced"
Calculating cluster Intermediate mesoderm

Warning message in mean.fxn(object[features, cells.1, drop = FALSE]):
"NaNs produced"
Calculating cluster HSCs

Warning message in mean.fxn(object[features, cells.1, drop = FALSE]):
"NaNs produced"
Calculating cluster Immune cells

Warning message in mean.fxn(object[features, cells.1, drop = FALSE]):
"NaNs produced"
Calculating cluster Erythroid cells

Warning message in mean.fxn(object[features, cells.1, drop = FALSE]):
"NaNs produced"
Calculating cluster 9
```

```
Warning message in mean.fxn(object[features, cells.1, drop = FALSE]):
"NaNs produced"
Calculating cluster 10

Warning message in mean.fxn(object[features, cells.1, drop = FALSE]):
"NaNs produced"
Warning message in mean.fxn(object[features, cells.2, drop = FALSE]):
"NaNs produced"
```

```
head(markers)
```

A data.frame: 6 x 7

	p_val <dbl>	avg_log2FC <dbl>	pct.1 <dbl>	pct.2 <dbl>	p_val_adj <dbl>	cluster <fct>	gene <chr>
PTPRZ1	1.063855e-243	6.412992	0.990	0.783	2.127709e-240	SCPs	PTPRZ1
ERBB3	1.696545e-233	8.156112	0.972	0.566	3.393090e-230	SCPs	ERBB3
DST	5.987636e-231	3.781085	1.000	0.891	1.197527e-227	SCPs	DST
PLP1	7.904205e-229	7.297576	0.970	0.727	1.580841e-225	SCPs	PLP1
TRPM3	3.513331e-227	7.403232	0.970	0.622	7.026663e-224	SCPs	TRPM3
MPZ	1.720136e-225	8.064614	0.965	0.670	3.440272e-222	SCPs	MPZ

```
# Get top 5 markers per cluster
top5 <- markers %>%
  group_by(cluster) %>%
  top_n(n = 5, wt = avg_log2FC)%>%
  arrange(cluster, desc(avg_log2FC))
```

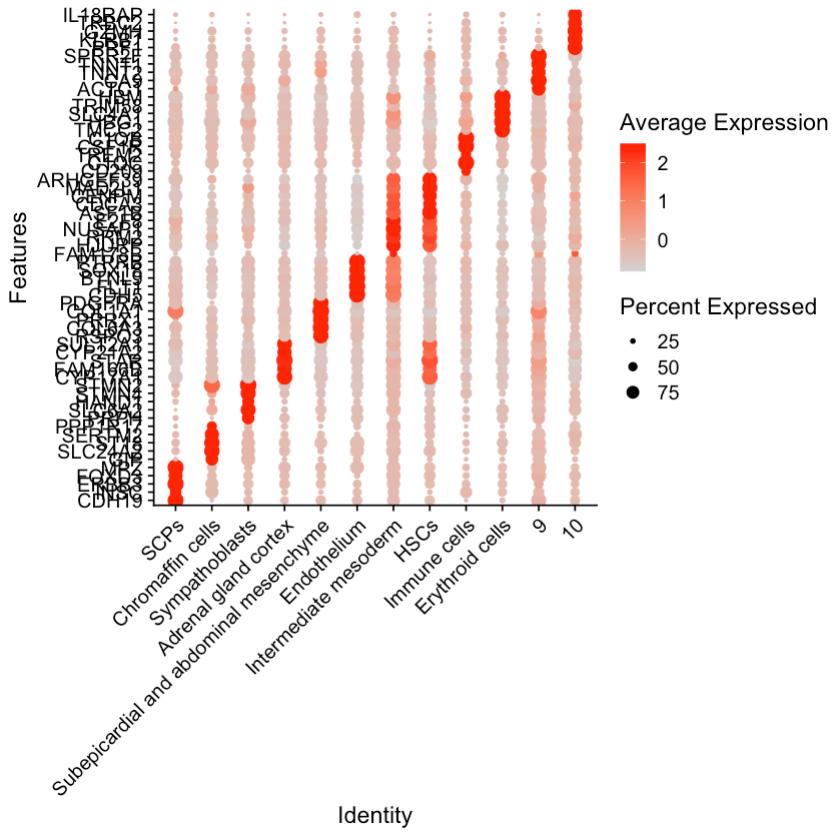
```
head(top5)
```

A grouped_df: 6 x 7

p_val <dbl>	avg_log2FC <dbl>	pct.1 <dbl>	pct.2 <dbl>	p_val_adj <dbl>	cluster <fct>	gene <chr>
1.150230e-221	8.924888	0.952	0.516	2.300459e-218	SCPs	CDH19
5.514697e-88	8.242869	0.656	0.437	1.102939e-84	SCPs	INSC
1.696545e-233	8.156112	0.972	0.566	3.393090e-230	SCPs	ERBB3
6.326074e-136	8.108642	0.824	0.519	1.265215e-132	SCPs	FOXD3
1.720136e-225	8.064614	0.965	0.670	3.440272e-222	SCPs	MPZ

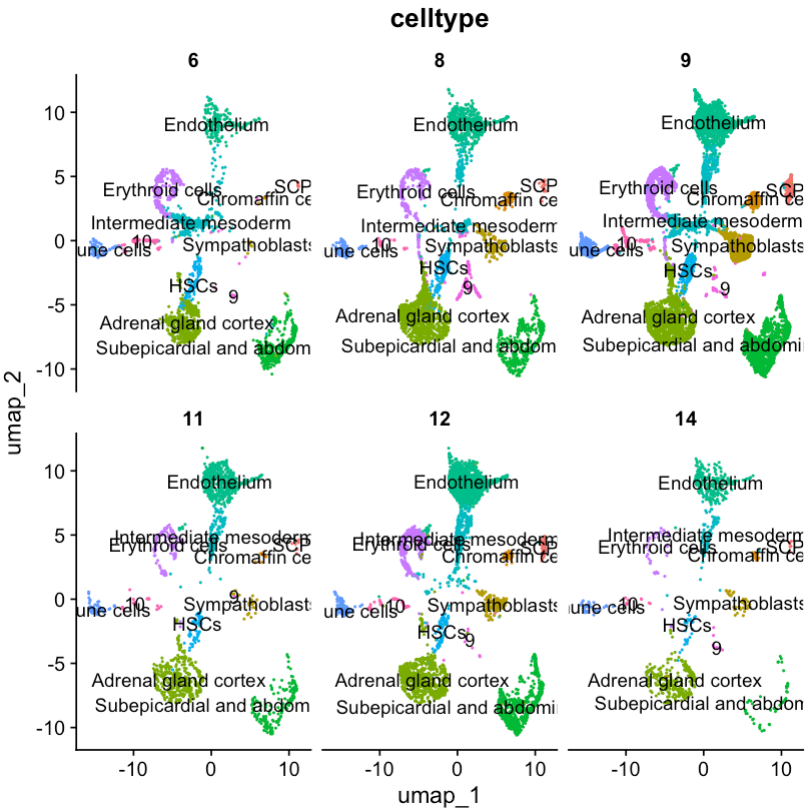
p_val <dbl>	avg_log2FC <dbl>	pct.1 <dbl>	pct.2 <dbl>	p_val_adj <dbl>	cluster <fct>	gene <chr>
1.815618e-113	8.241624	0.735	0.382	3.631235e-110	Chromaffin cells	GIP

```
# Create dot plot
dp <- DotPlot(object = integrated,
        features = unique(top5$gene),  # unique genes top5
        cols = c("lightgrey", "red"), # to match the paper
        dot.scale = 4) +

  coord_flip()+
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
dp
```




```
ggsave("plots/dotplot_top5_perCluster.pdf", dp, width = 8, height = 16)
```

```
umap_ann_week <- DimPlot(integrated, group.by = "celltype", label = TRUE, split.by = "week", ncol=3)
ggsave("plots/umap_ann_week.png", width = 10, height = 10)
umap_ann_week
```




```
saveRDS(integrated, "data/processed/integrated_annotated.rds")
```