Adreanal Medulla Reclustering

```
library(Seurat)
library(ggplot2)
```

```
Loading required package: SeuratObject

Loading required package: sp


Attaching package: 'SeuratObject'


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

    intersect, t
```

Load the data

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

Subset AMC (Schwann cell precursors (SCPs), Chromaffin cells, Sympathoblasts)

```
unique(seu$celltype)
```

1. Adrenal gland cortex
2. Chromaffin cells
3. Endothelium
4. 10
5. Subepicardial and abdominal mesenchyme
6. Intermediate mesoderm
7. Sympathoblasts
8. Erythroid cells
9. HSCs
10. 9
11. Immune cells
12. SCPs

Levels: 1. 'SCPs' 2. 'Chromaffin cells' 3. 'Sympathoblasts' 4. 'Adrenal gland cortex' 5. 'Subepicardial and abdominal mesenchyme' 6. 'Endothelium' 7. 'Intermediate mesoderm' 8. 'HSCs' 9. 'Immune cells' 10. 'Erythroid cells' 11. '9' 12. '10'

```
seu_subset <- subset(seu, subset = celltype %in% c("SCPs", "Chromaffin cells", "Sympathoblasts"))
```

```
unique(seu_subset$celltype)
```

1. Chromaffin cells
2. Sympathoblasts
3. SCPs

Levels: 1. 'SCPs' 2. 'Chromaffin cells' 3. 'Sympathoblasts' 4. 'Adrenal gland cortex' 5. 'Subepicardial and abdominal mesenchyme' 6. 'Endothelium' 7. 'Intermediate mesoderm' 8. 'HSCs' 9. 'Immune cells' 10. 'Erythroid cells' 11. '9' 12. '10'

Recluster

```
seu_subset <- FindVariableFeatures(seu_subset)
```

```
Warning message in FindVariableFeatures.Assay(object = object[[assay]], selection.method = selectio
"selection.method set to 'vst' but count slot is empty; will use data slot instead"
Warning message in eval(predvars, data, env):
"NaNs produced"
Warning message in hvf.info$variance.expected[not.const] <- 10^fit$fitted:
"number of items to replace is not a multiple of replacement length"
```

```
seu_subset <- ScaleData(seu_subset)
seu_subset <- RunPCA(seu_subset)
seu_subset <- FindNeighbors(seu_subset, dims = 1:30)
```

```
Centering and scaling data matrix

PC_ 1
Positive:  STMN2, RGS5, CHGB, DBH, PCSK1N, EEF1A2, HAND2-AS1, EML5, CD24, BEX1
       GATA3, HAND2, SYT1, MIAT, MAP1B, GATA2, CHGA, CNTN1, BASP1, PHOX2A
       TUBB2B, ELAVL4, ISL1, CHRNA3, DPP6, SEZ6L2, RAMP1, RGS4, KIF21A, ELAVL3
Negative:  PLP1, PTPRZ1, EDNRB, COL5A2, ERBB3, OLFML2A, MPZ, SPARC, S100B, CDH19
       VCAN, TGFBR2, POSTN, MOXD1, TRPM3, NR2F2, ABCA8, GPM6B, TTYH1, PLAT
       METRN, NID1, HSPG2, COL2A1, SOX10, LMO4, COL1A1, LGI4, GAS7, PLEKHA4
PC_ 2
Positive:  TUBB, SOX4, STMN1, TUBA1A, RTN1, GAP43, TUBB2B, TMSB15A, STMN4, ELAVL4
       SOX11, TUBA1B, SLC6A2, HMGB1, SYNE2, SMC4, TMSB4X, ASPM, SLC38A1, MLLT11
       KIF21A, PLXNA4, RBFOX1, VIM, PHOX2B, TOP2A, PRC1, MKI67, JPT1, TMPO
Negative:  DLK1, PENK, SLC24A2, ST18, INSM1, CCSER1, PNMT, VWA5B2, NDUFA4L2, CHGA
       ADM, ARC, SERTM2, C1QL1, CDKN1C, SCARB1, F10, TH, CCND2, HTATSF1
       PCSK2, VEGFA, JUNB, ROBO2, DGKK, SCG2, CARTPT, HIST1H2AC, SMIM1, RALYL
PC_ 3
Positive:  CENPF, MKI67, TOP2A, NUSAP1, CENPE, CDK1, ASPM, NUF2, GTSE1, TTK
```

```
       RRM2, UBE2C, KIF23, CCNB1, TPX2, PBK, CCNA2, PIMREG, HMGB2, CDCA8
       NCAPG, AURKB, PTTG1, CDKN3, PLK1, FOXM1, DLGAP5, BUB1, KIF11, BIRC5
Negative:  SOX4, PRPH, STMN4, MAOA, TUBA1A, GAP43, CCND1, GAL, TUBB2B, NEFL
       RBFOX1, RTN1, NPY, C4orf48, PLPPR3, PLXNA4, UCHL1, MAPT, DPYSL3, PHOX2B
       ARHGDIG, SCN9A, SCN3B, TUBB2A, ANXA2, TBX2, STMN2, MAP1B, ANK2, HBG2
PC_ 4
Positive:  TMEM88, PLVAP, CD93, CD34, EMCN, F8, ROBO4, TIE1, FLT1, SOX18
       CD109, RASGRP3, CAVIN2, A2M, CDH5, ADGRF5, TFPI, CALCRL, VAMP5, BTNL9
       LMO2, HLA-E, TM4SF18, KDR, NOSTRIN, PECAM1, LRRC32, ICAM2, LYVE1, PCDH12
Negative:  NUF2, MKI67, CENPF, KIFC1, GAS2L3, ASPM, PTTG1, DST, SLITRK2, TROAP
       DLGAP5, MXD3, KIF2C, FLRT3, KIF4A, UBE2C, RTKN2, ZEB2, AURKB, MNS1
       EGFLAM, FOXM1, NCAPG, PLP1, TPX2, BIRC5, GTSE1, COL25A1, ERBB3, S100B
PC_ 5
Positive:  NR5A1, MGARP, CYP11A1, FDXR, ASB4, SIGLEC11, STAR, APOE, TCEA3, ALDH1A2
       GIPC2, MRAP, SNCG, CCDC141, MT3, MC2R, NRK, MCF2, ZG16B, AC020571.1
       FDX1, DHCR24, SULT2A1, GRB14, RBM47, NOV, TNNI3, INHA, CYP17A1, MAP3K15
Negative:  PLVAP, FLT1, CALCRL, CAVIN2, F8, PECAM1, CD93, TM4SF18, CLDN5, BTNL9
       CDH5, CD34, ICAM2, SOX18, TMEM88, TIE1, TEK, ROBO4, PROCR, KDR
       EMCN, ADGRF5, PCAT19, FGF23, CETP, RASGRP3, HLA-E, IRX3, EHD3, CEACAM1

Computing nearest neighbor graph

Computing SNN
```

```
seu_subset <- FindClusters(seu_subset, resolution = 0.1)
seu_subset <- RunUMAP(seu_subset, dims = 1:40)
```

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

Number of nodes: 2118
Number of edges: 94548

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.9551
Number of communities: 4
Elapsed time: 0 seconds


20:32:52 UMAP embedding parameters a = 0.9922 b = 1.112

20:32:52 Read 2118 rows and found 40 numeric columns

20:32:52 Using Annoy for neighbor search, n_neighbors = 30

20:32:52 Building Annoy index with metric = cosine, n_trees = 50

0%   10   20   30   40   50   60   70   80   90   100%

[----|----|----|----|----|----|----|----|----|----|

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20:32:53 Writing NN index file to temp file /var/folders/wl/jrkngsm57b944tj7rtjg12000000gn/T//Rtmpal
```

```
20:32:53 Searching Annoy index using 1 thread, search_k = 3000

20:32:53 Annoy recall = 100%

20:32:53 Commencing smooth kNN distance calibration using 1 thread
 with target n_neighbors = 30

20:32:53 Initializing from normalized Laplacian + noise (using RSpectra)

20:32:53 Commencing optimization for 500 epochs, with 93720 positive edges

20:32:53 Using rng type: pcg

20:32:54 Optimization finished
```

```
DimPlot(seu_subset)
```

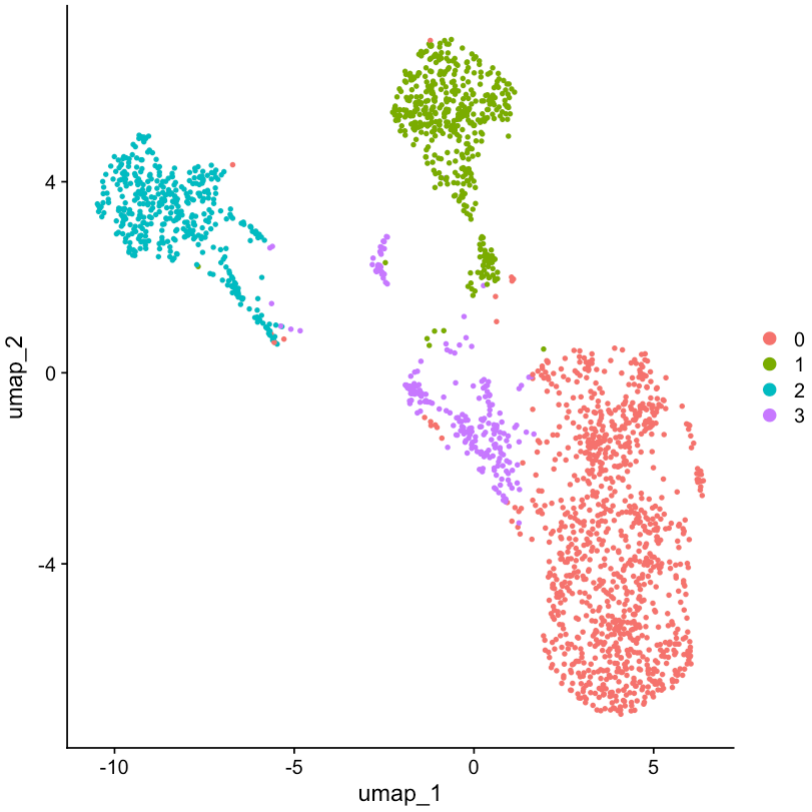
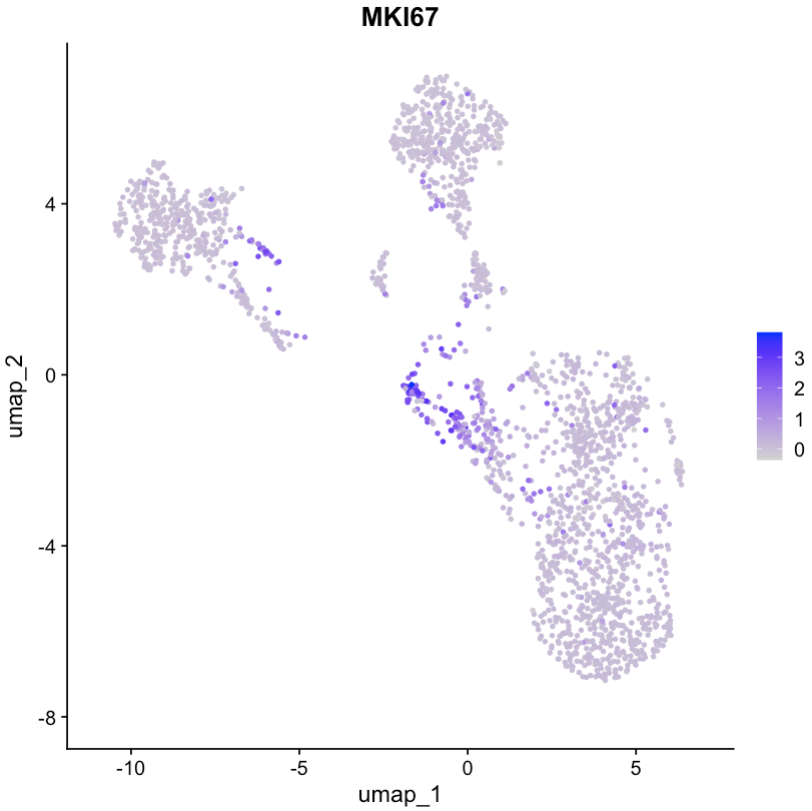


Fig 2 Markers from the paper

```
markers <- list(
    "Prolifertating sympathoblasts" = "MKI67",
    "Sympathoblasts" = c("ELAVL4", "ISL1", "PRPH"),
    "SCPs" = c("SOX10","PLP1"),
    "Chromaffin cells" = c("CHGA","PNMT")
)
```

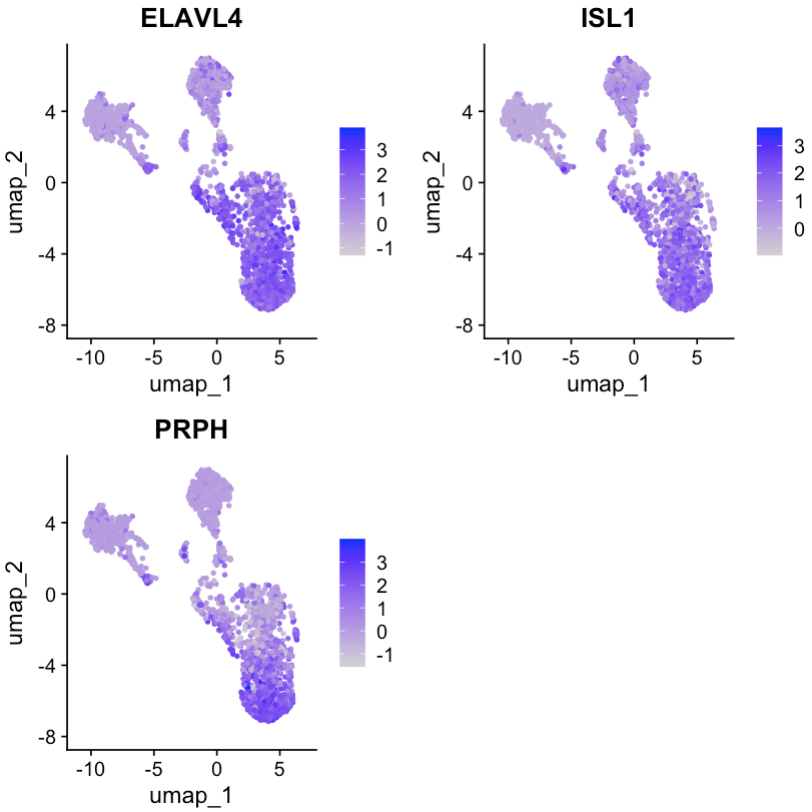
Markers for Prol. Sympathoblasts

```
FeaturePlot(seu_subset,markers[[1]])
# cluster 3
```



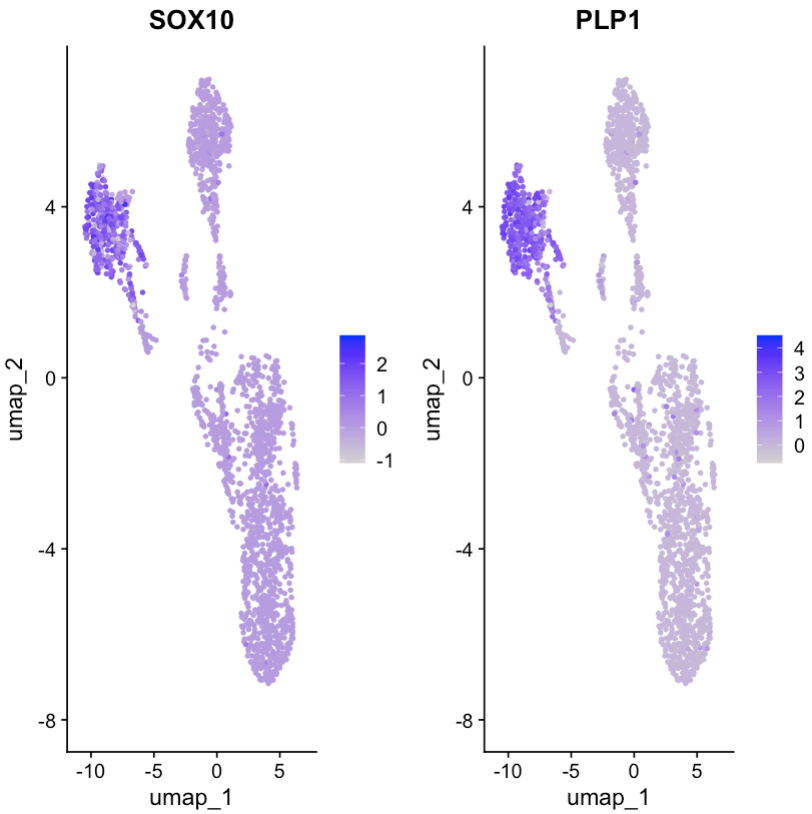
Markers for Sympathoblasts

```
FeaturePlot(seu_subset,markers[[2]])
# cluster 0
```



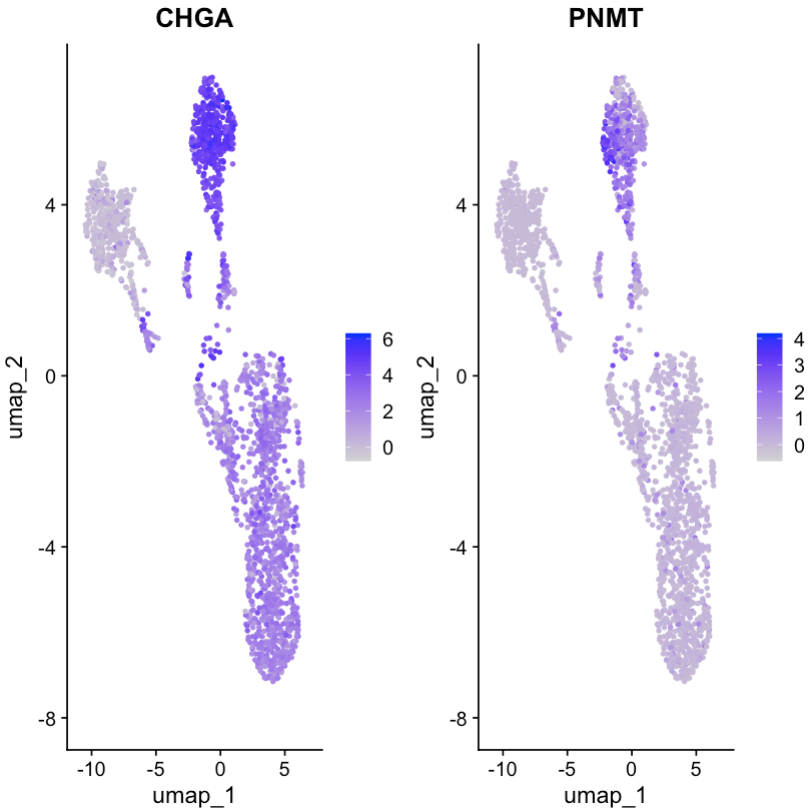
Markers for SCPs

```
FeaturePlot(seu_subset,markers[[3]], ncol=2)
# cluster 2
```



Chromaffin cells

```
FeaturePlot(seu_subset,markers[[4]], ncol=2)
# cluster 1
```




```
cluster <- list(
   "3" = "Prolifertating sympathoblasts",# = "MKI67",
   "0" = "Sympathoblasts",# = c("ELAVL4", "ISL1", "PRPH"),
   "2" = "SCPs",# = c("SOX10","PLP1"),
   "1" = "Chromaffin cells"# = c("CHGA","PNMT")
)
```

```
cluster <- unlist(cluster)
seu_subset <- RenameIdents(seu_subset, cluster)
seu_subset$celltype <- Idents(seu_subset)
```

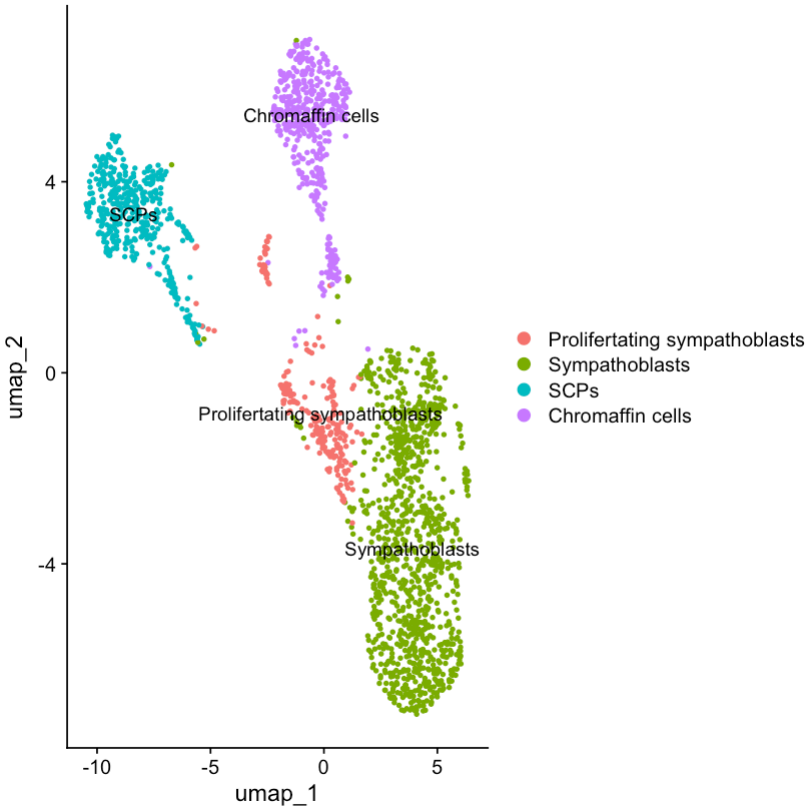
```
unique(seu_subset$celltype)
```

1. Chromaffin cells
2. Sympathoblasts
3. Prolifertating sympathoblasts
4. SCPs

Levels: 1. 'Prolifertating sympathoblasts' 2. 'Sympathoblasts' 3. 'SCPs' 4. 'Chromaffin cells'

UMAP with Annotations

```
umap_ann_subset =DimPlot(seu_subset, label=TRUE)
umap_ann_subset
```




```
ggsave("plots/AMC_subset_UMAP_annotated.pdf",umap_ann_subset, width = 10, height = 8)
```

```
saveRDS(seu_subset,"data/processed/AMC_subset_annotated.rds")
```