Integration, QC, Filtering, and Normalization

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

```
# map GSM ids to info
files <- list(
  "GSM4446535" = "week8_001",
  "GSM4446536" = "week9_063",
  "GSM4446537" = "week6_088",
  "GSM4446538" = "week14_123",
  "GSM4446539" = "week12_124",
  "GSM4446540" = "week8_125",
  "GSM4446541" = "week9_005",
  "GSM4446542" = "week11_006",
  "GSM4446543" = "week9_007",
  "GSM4734601" = "week8_016",
  "GSM4734602" = "week9_031_paraganglia",
  "GSM4734603" = "week12_035",
  "GSM4734604" = "week12_036_extraadrenal"
)
```

```
# List all H5 files in the raw data directory
h5_files <- list.files("data/raw/GSE147821_RAW", pattern = ".h5$", full.names = TRUE)
h5_files
```

1. 'data/raw/GSE147821_RAW/GSM4446535_10X_19_001.raw_feature_bc_matrix.h5'
2. 'data/raw/GSE147821_RAW/GSM4446536_10X_19_063.raw_feature_bc_matrix.h5'
3. 'data/raw/GSE147821_RAW/GSM4446537_10X_19_088.raw_feature_bc_matrix.h5'
4. 'data/raw/GSE147821_RAW/GSM4446538_10X_19_123.raw_feature_bc_matrix.h5'
5. 'data/raw/GSE147821_RAW/GSM4446539_10X_19_124.raw_feature_bc_matrix.h5'
6. 'data/raw/GSE147821_RAW/GSM4446540_10X_19_125.raw_feature_bc_matrix.h5'
7. 'data/raw/GSE147821_RAW/GSM4446541_10X_20_005.raw_feature_bc_matrix.h5'
8. 'data/raw/GSE147821_RAW/GSM4446542_10X_20_006.raw_feature_bc_matrix.h5'
9. 'data/raw/GSE147821_RAW/GSM4446543_10X_20_007.raw_feature_bc_matrix.h5'
10. 'data/raw/GSE147821_RAW/GSM4734601_10X_20_016.raw_feature_bc_matrix.h5'
11. 'data/raw/GSE147821_RAW/GSM4734602_10X_20_031.raw_feature_bc_matrix.h5'
12. 'data/raw/GSE147821_RAW/GSM4734603_10X_20_035.raw_feature_bc_matrix.h5'
13. 'data/raw/GSE147821_RAW/GSM4734604_10X_20_036.raw_feature_bc_matrix.h5'

```
names(h5_files) <- sub("_10X_.*", "", basename(h5_files))
h5_files
```

GSM4446535 'data/raw/GSE147821_RAW/GSM4446535_10X_19_001.raw_feature_bc_matrix.h5'GSM4446536
'data/raw/GSE147821_RAW/GSM4446536_10X_19_063.raw_feature_bc_matrix.h5'GSM4446537
'data/raw/GSE147821_RAW/GSM4446537_10X_19_088.raw_feature_bc_matrix.h5'GSM4446538
'data/raw/GSE147821_RAW/GSM4446538_10X_19_123.raw_feature_bc_matrix.h5'GSM4446539
'data/raw/GSE147821_RAW/GSM4446539_10X_19_124.raw_feature_bc_matrix.h5'GSM4446540
'data/raw/GSE147821_RAW/GSM4446540_10X_19_125.raw_feature_bc_matrix.h5'GSM4446541
'data/raw/GSE147821_RAW/GSM4446541_10X_20_005.raw_feature_bc_matrix.h5'GSM4446542
'data/raw/GSE147821_RAW/GSM4446542_10X_20_006.raw_feature_bc_matrix.h5'GSM4446543
'data/raw/GSE147821_RAW/GSM4446543_10X_20_007.raw_feature_bc_matrix.h5'GSM4734601
'data/raw/GSE147821_RAW/GSM4734601_10X_20_016.raw_feature_bc_matrix.h5'GSM4734602
'data/raw/GSE147821_RAW/GSM4734602_10X_20_031.raw_feature_bc_matrix.h5'GSM4734603
'data/raw/GSE147821_RAW/GSM4734603_10X_20_035.raw_feature_bc_matrix.h5'GSM4734604
'data/raw/GSE147821_RAW/GSM4734604_10X_20_036.raw_feature_bc_matrix.h5'

Load each sample into a list of Seurat objects and apply filtering

```
# Load each sample into a list of Seurat objects
# 3. Process each sample
seurat_list <- lapply(names(files), function(gsm_id) {

  # Get sample info
  sample_name <- files[[gsm_id]]
  week <- as.numeric(sub("week(\\d+).*", "\\1", sample_name))
  sample_id <- sub(".*_(\\d+).*", "\\1", sample_name)
  # Read data
  counts <- Read10X_h5(h5_files[[gsm_id]])
  # Create Seurat object
  seurat_obj <- CreateSeuratObject(
    counts = counts,
    project = sample_name,
    min.cells = 3,
    min.features = 200
  )
  seurat_obj <- subset(seurat_obj, downsample = ncol(seurat_obj)/4)  # Keep 3rd of the cells
  # Add comprehensive metadata
  seurat_obj$orig.ident <- sample_name
  seurat_obj$sample <- sample_name
  seurat_obj$week <- week
  seurat_obj$sample_id <- sample_id
  seurat_obj$gsm_id <- gsm_id
  seurat_obj$condition <- ifelse(
    grepl("paraganglia|extraadrenal", sample_name),
    "special", "regular"
  )
  # Calculate mitochondrial percentage
  seurat_obj[["percent.mt"]] <- PercentageFeatureSet(
    seurat_obj,
```

```
    pattern = "^MT-"
  )


  return(seurat_obj)
})
```

```
names(seurat_list) <- sapply(seurat_list, function(x) unique(x$sample))
```

QC and More Filtering

These genes are not to be filtered out

```
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))
```

```
alist = list()
for (i in 1:length(seurat_list)) {
  setdiff(markers_unique,rownames(seurat_list[[i]]))
  alist <- union(alist,setdiff(markers_unique,rownames(seurat_list[[i]])))
}
to_remove <- unique(alist)

genes_to_conserve <- setdiff(markers_unique,to_remove)
genes_to_conserve
```

1. 'SOX10'
2. 'PLP1'
3. 'FOXD3'
4. 'ELAVL3'
5. 'ELAVL4'
6. 'PHOX2B'
7. 'TH'
8. 'STMN2'
9. 'NR5A1'
10. 'MITF'

11. ‘PRRX1’
12. ‘PECAM1’
13. ‘KDR’
14. ‘GATA4’
15. ‘HAND2’
16. ‘SPINK2’
17. ‘FCGR1A’
18. ‘CD163’
19. ‘HBA2’
20. ‘HBB’

```
# QC
seurat_list <- lapply(seurat_list, function(x) {
  x[["percent.mt"]] <- PercentageFeatureSet(x, pattern = "^MT-")
  x <- subset(x, subset = nFeature_RNA > 500 & nFeature_RNA < 6000 & percent.mt < 15)
  x <- NormalizeData(x)
  x <- FindVariableFeatures(x, selection.method = "vst", nfeatures = 2000)

  # Add them to the variable features
  VariableFeatures(x) <- union(VariableFeatures(x), genes_to_conserve)
  return(x)
})
```

```
Normalizing layer: counts

Finding variable features for layer counts

Normalizing layer: counts

Finding variable features for layer counts

Normalizing layer: counts

Finding variable features for layer counts

Normalizing layer: counts

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```

```
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Normalizing layer: counts

Finding variable features for layer counts

Normalizing layer: counts

Finding variable features for layer counts
```

Cell Cycle Information

```r
# Add cell cycle correction
s_genes <- cc.genes$s.genes
g2m_genes <- cc.genes$g2m.genes
seurat_list <- lapply(seurat_list, function(x) {
  x <- CellCycleScoring(
    x,
    s.features = s_genes,
    g2m.features = g2m_genes,
    set.ident = FALSE
  )
  x$CC.Difference <- x$S.Score - x$G2M.Score  # for regression
  return(x)
})
```

```
Warning message:
"The following features are not present in the object: MLF1IP, not searching for symbol synonyms"
Warning message:
"The following features are not present in the object: FAM64A, HN1, not searching for symbol synonyr
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Warning message:
"The following features are not present in the object: MLF1IP, not searching for symbol synonyms"
```

```
Warning message:
"The following features are not present in the object: FAM64A, HN1, not searching for symbol synonym
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Warning message:
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```

```r
features <- SelectIntegrationFeatures(object.list = seurat_list)
```

```r
length(features)
```

2000

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

1. 'SOX10'
2. 'PLP1'
3. 'FOXD3'
4. 'ELAVL3'
5. 'ELAVL4'
6. 'PHOX2B'
7. 'TH'
8. 'STMN2'
9. 'NR5A1'
10. 'MITF'
11. 'PAX2'
12. 'PRRX1'
13. 'PECAM1'
14. 'KDR'
15. 'GATA4'
16. 'HAND2'
17. 'HNF4A'
18. 'AHSG'
19. 'SPINK2'
20. 'AZU1'
21. 'FCGR1A'
22. 'CD163'
23. 'HBA2'
24. 'HBB'

Perform Integration

```
# Select integration anchors
anchors <- FindIntegrationAnchors(
  object.list = seurat_list,
  dims = 1:30,
  anchor.features = union(features, genes_to_conserve),
  normalization.method = "LogNormalize"
)
```

```
rm(seurat_list) # to open memory space
```

```
anchors
```

```
An AnchorSet object containing 374062 anchors between 13 Seurat objects
 This can be used as input to IntegrateData.
```

```
# Integrate data
integrated <- IntegrateData(
  anchorset = anchors,
  dims = 1:30,
```

```
  new.assay.name = "integrated"
)
```

```
# Switch to integrated assay for downstream analysis
DefaultAssay(integrated) <- "integrated"
```

Scale data

Scaling and regression for cell cycle

```
integrated <- ScaleData(integrated, vars.to.regress = "CC.Difference",  verbose = FALSE)
```

Run PCA

```
integrated <- RunPCA(integrated)
```

```
PC_ 1
Positive:  HLA-E, EGFL7, IFITM3, IGFBP4, KDR, TMSB4X, FLT1, ELK3, RAMP2, PLVAP
       CD93, CYBA, CAVIN2, CALCRL, CDH5, ARHGAP29, TGFBR2, ANXA2, PLPP3, TFPI
       ETS1, EMCN, PECAM1, PRCP, ADGRF5, TMEM88, CLDN5, ESAM, CD109, NEAT1
Negative:  NRCAM, DLK1, CDH2, FDXR, STAR, CADM1, APOA1, PEG3, KCNK3, NR5A1
       DHCR24, ALDH1A2, PEG10, NOV, APOE, ASB4, PEBP1, MC2R, PPIF, DPP10
       RALYL, INHA, MCF2, CYP11A1, COL15A1, SNCG, CACNB2, MGARP, TBX3, SLC16A9
PC_ 2
Positive:  COL1A1, COL3A1, COL1A2, PLAC9, COL5A1, COL12A1, GPC3, COL5A2, ISLR, CDH11
       CXCL12, PCOLCE, FBN1, COL6A3, VIM, CALD1, OGN, FZD1, PCDH7, DCN
       COL16A1, COL6A1, PDE5A, LRRC17, POSTN, PDGFRA, PRRX1, CD248, SULT1E1, TSHZ2
Negative:  LAPTM5, ARHGAP18, HLA-B, MAN1A1, CD74, TYROBP, C1QC, MEF2C, SRGN, FYB1
       LYVE1, CSF1R, DAB2, MRC1, C1QB, CD163, STAB1, C1QA, FCER1G, CD36
       MS4A6A, HCST, PLD4, MS4A7, PTPRE, DOCK8, VSIG4, CD83, GYPC, CYBB
PC_ 3
Positive:  NOSTRIN, HSPG2, TIMP3, SQLE, SPARC, GNG11, TSPAN13, MGST2, CALCRL, PLPP3
       HPGD, KDR, FLT1, SH3BP5, PRCP, CAVIN2, TMEM47, CDH5, BTNL9, PLVAP
       MMRN2, GJA1, TMEM88, CLDN5, F8, ROBO4, TM4SF18, CLEC14A, SNCG, PEG10
Negative:  TYROBP, C1QA, C1QB, C1QC, FYB1, CSF1R, CYBB, CD163, PLD4, MS4A7
       FCGR1A, AIF1, VSIG4, HCST, FGD2, RUNX1, MS4A4A, MPEG1, NCKAP1L, RGS1
       ADAP2, FCGR3A, TFEC, PTPRC, CD68, FCER1G, FOLR2, CCR1, TYMP, CD53
PC_ 4
Positive:  CTSC, PLAGL1, C7, COL1A2, APOE, NRK, COL14A1, DCN, VCAN, NR2F1
       COL3A1, TPM2, FBN2, RARRES2, DAB2, CXCL12, COL1A1, GPC6, ZEB2, SPARC
       AXL, CDH11, NPR3, TGFBI, HLA-DRB1, FSTL1, COL5A1, LRRC17, COL12A1, COL11A1
Negative:  STMN2, DBH, EML5, HAND2-AS1, PCSK1N, RGS5, HAND2, CHGB, MIAT, PHOX2B
       EEF1A2, ELAVL4, GATA2, PHOX2A, CHGA, ISL1, CHRNA3, ELAVL3, CD24, TFAP2B
       TUBB2B, STMN4, LINC00682, GAL, GATA3, DPP6, VSTM2L, SCN3B, SCG2, SLC18A1
PC_ 5
Positive:  HMGB1, STMN1, TUBB, HMGN2, H2AFZ, TMSB4X, JUN, KLF6, HSPA5, CALM2
       TUBA1B, MEG3, FOS, DUT, CST3, NAMPT, TMPO, HELLS, HSPD1, CCND1
```

```
       SMC4, VMP1, PEBP1, MEF2C, MEG8, IRF1, IER2, TOP2A, SLC8A1, CYBA
Negative:  SLC4A1, ALAS2, HBG1, HBA2, HBA1, AHSP, HBG2, HEMGN, HBM, GYPA
       GYPB, SPTA1, TENT5C, HMBS, SELENBP1, SLC25A37, EPB42, HBB, TRIM58, SNCA
       KLF1, BPGM, MYL4, ANK1, TMEM56, TMCC2, NFE2, BLVRB, HBQ1, RBM38
```

Run UMAP

```r
integrated <- RunUMAP(integrated, dims = 1:50)
```

```
19:30:46 UMAP embedding parameters a = 0.9922 b = 1.112

19:30:46 Read 19803 rows and found 50 numeric columns

19:30:46 Using Annoy for neighbor search, n_neighbors = 30

19:30:46 Building Annoy index with metric = cosine, n_trees = 50

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

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19:30:47 Writing NN index file to temp file /var/folders/wl/jrkngsm57b944tj7rtjg12000000gn/T//RtmpMI

19:30:47 Searching Annoy index using 1 thread, search_k = 3000

19:30:49 Annoy recall = 100%

19:30:50 Commencing smooth kNN distance calibration using 1 thread
 with target n_neighbors = 30

19:30:50 Initializing from normalized Laplacian + noise (using RSpectra)

19:30:51 Commencing optimization for 200 epochs, with 912616 positive edges

19:30:51 Using rng type: pcg

19:30:54 Optimization finished
```
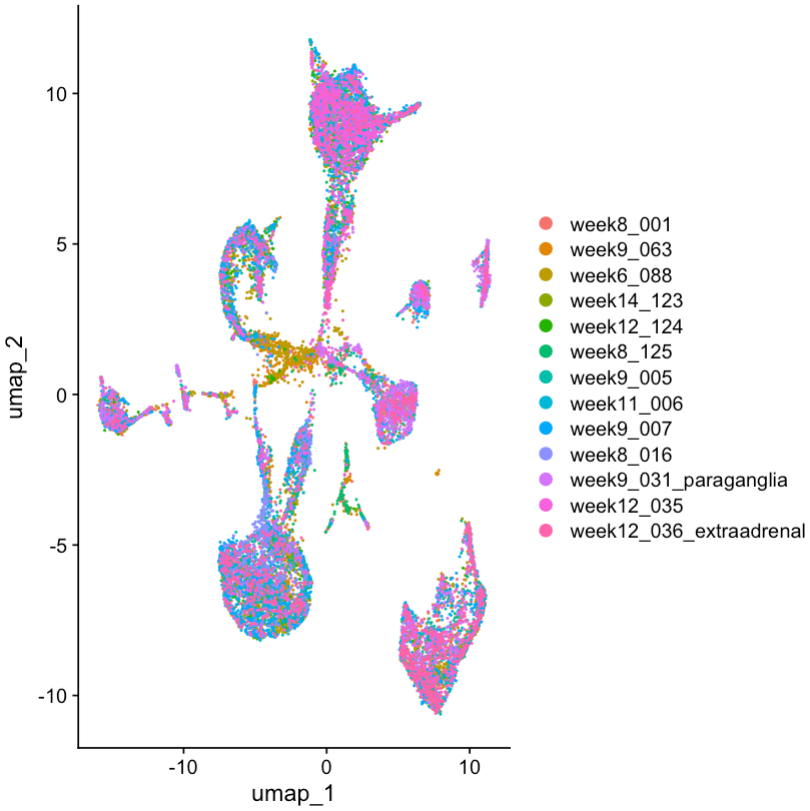

```
integrated
```

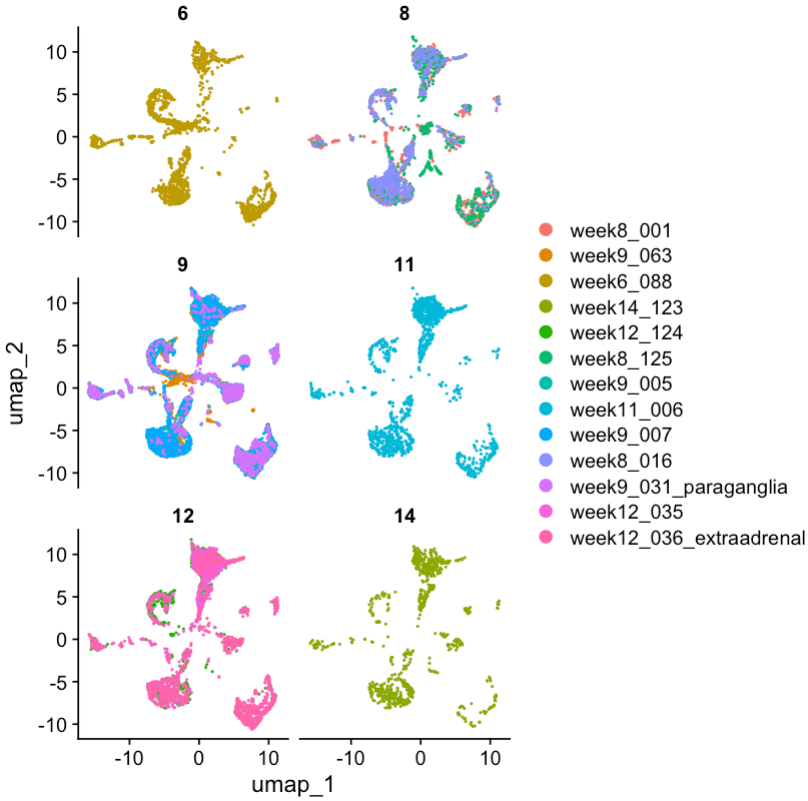
```
An object of class Seurat
28181 features across 19803 samples within 2 assays
Active assay: integrated (2000 features, 2000 variable features)
 2 layers present: data, scale.data
 1 other assay present: RNA
 2 dimensional reductions calculated: pca, umap
```

Umap Plot

```
umapfig <- DimPlot(integrated, raster.dpi = c(600,400))
umapfig
```




```
DimPlot(integrated, raster.dpi = c(600,400),split.by = "week", ncol = 2)
```




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

```
library(ggplot2)
ggsave('plots/umap_after_integration.pdf', width = 10, height = 7)
```

Notes: Integration Succesful

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)
```

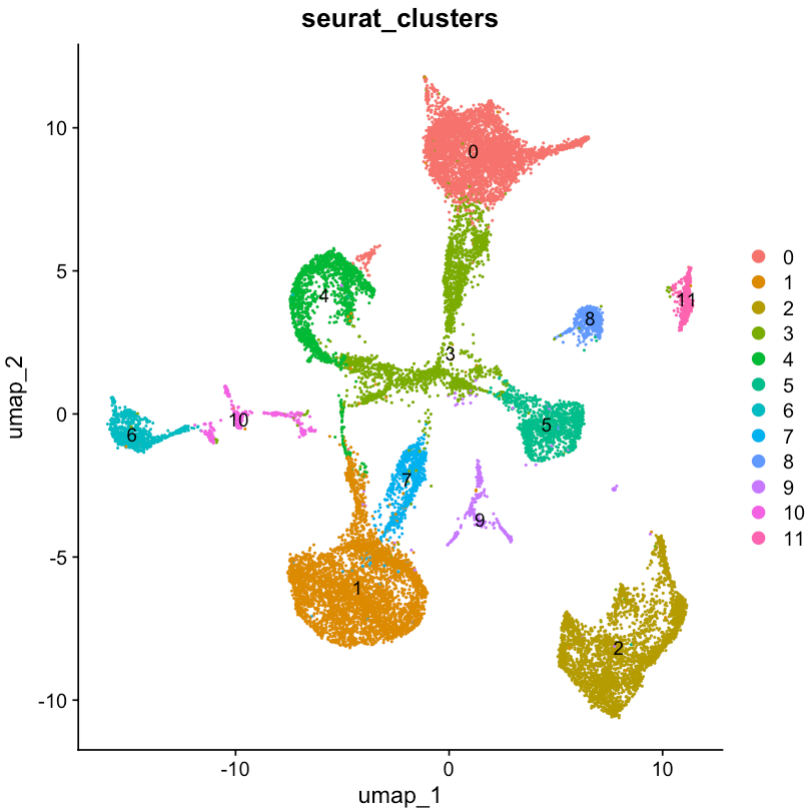
```
Computing nearest neighbor graph

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.ident	nCount_RNA	nFeature_RNA	sample	week	sample_id	gsm_id	condition	percent.mt	S.Score	G2M.Score	Phase	CC.Difference	integrated_snn_res...	seurat_clusters
	<chr>	<dbl>	<int>	<chr>	<dbl>	<chr>	<chr>	<chr>	<dbl>	<dbl>	<dbl>	<chr>	<dbl>	<fct>	<fct>
AAAC[illegible]-1_1	[illegible]	[illegible]	[illegible]	week8_001	8	001	GSM4446535	regular	[illegible]	[illegible]	[illegible]	G1	[illegible]	[illegible]	1
AAAC[illegible]-1_1	[illegible]	[illegible]	[illegible]	week8_001	8	001	GSM4446535	regular	[illegible]	[illegible]	[illegible]	G1	[illegible]	[illegible]	8
AAAG[illegible]-1_1	[illegible]	[illegible]	[illegible]	week8_001	8	001	GSM4446535	regular	[illegible]	[illegible]	[illegible]	G1	[illegible]	[illegible]	0
AAAG[illegible]-1_1	[illegible]	[illegible]	[illegible]	week8_001	8	001	GSM4446535	regular	[illegible]	[illegible]	[illegible]	G1	[illegible]	[illegible]	0
AAAG[illegible]-1_1	[illegible]	[illegible]	[illegible]	week8_001	8	001	GSM4446535	regular	[illegible]	[illegible]	[illegible]	G1	[illegible]	[illegible]	8
AAAG[illegible]-1_1	[illegible]	[illegible]	[illegible]	week8_001	8	001	GSM4446535	regular	[illegible]	[illegible]	[illegible]	G1	[illegible]	[illegible]	10

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



Cluster annotation

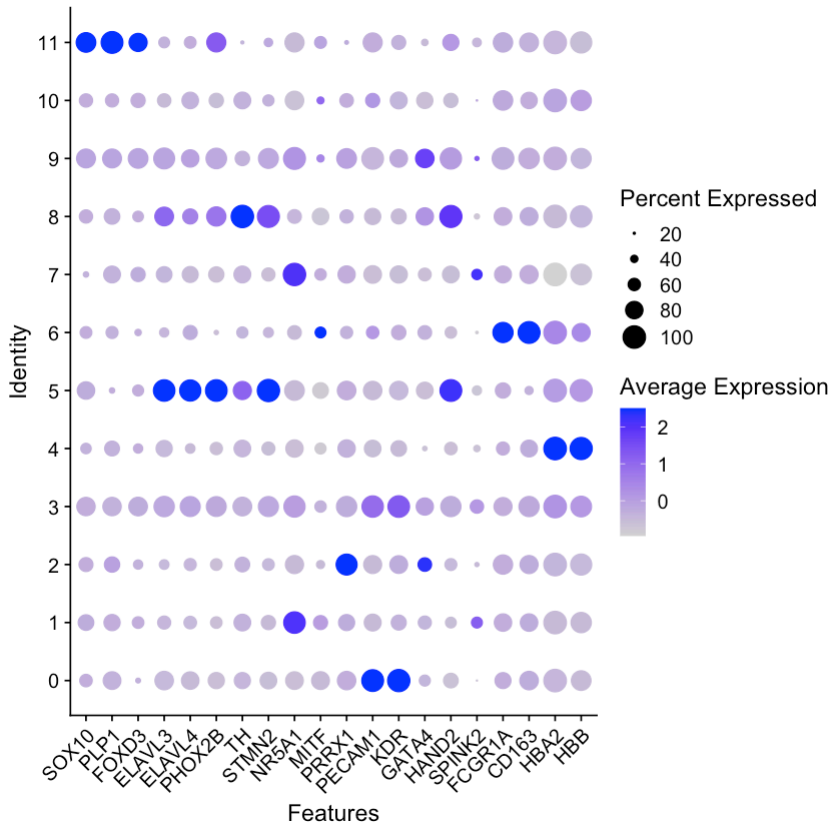
From the https://www.nature.com/articles/s41588-021-00818-x

```
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

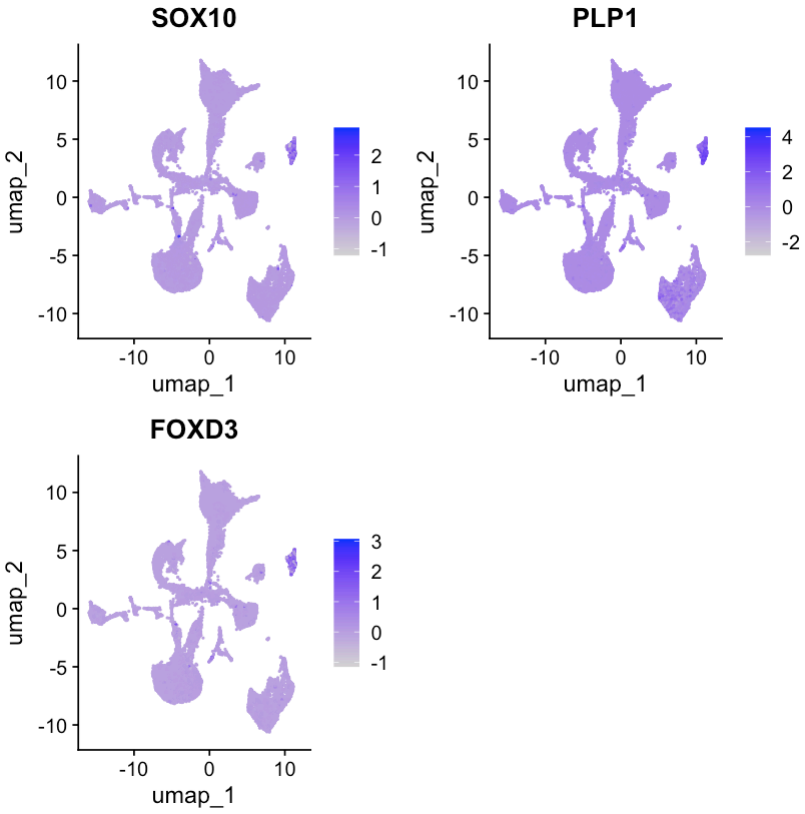
```
DotPlot(integrated, features = unique(unlist(cell_type_markers))) + RotatedAxis()
```



in depth analysis for manual annotation

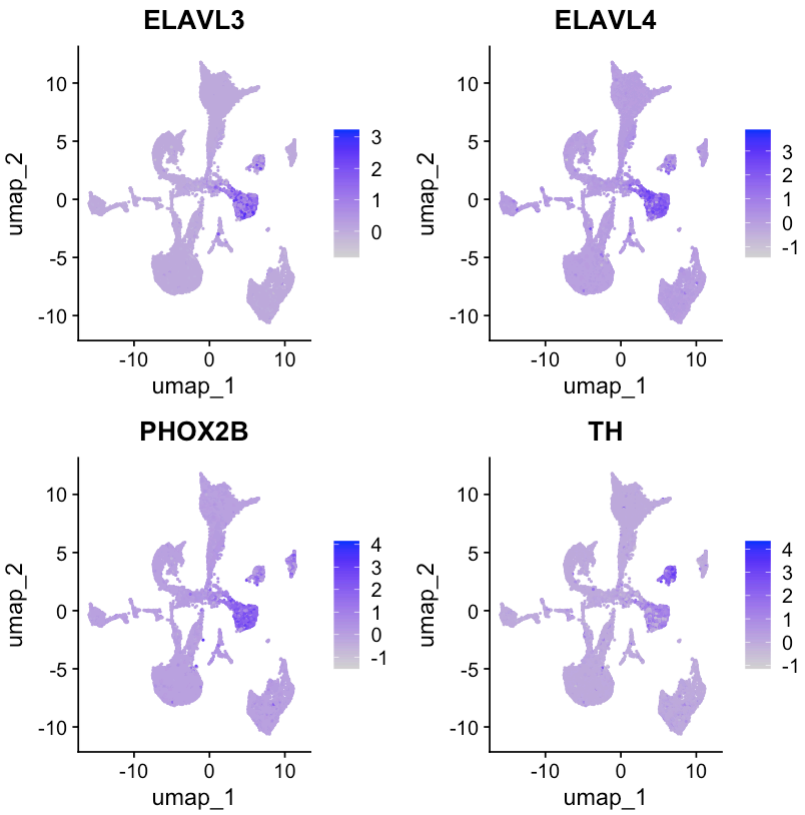
SCPs

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

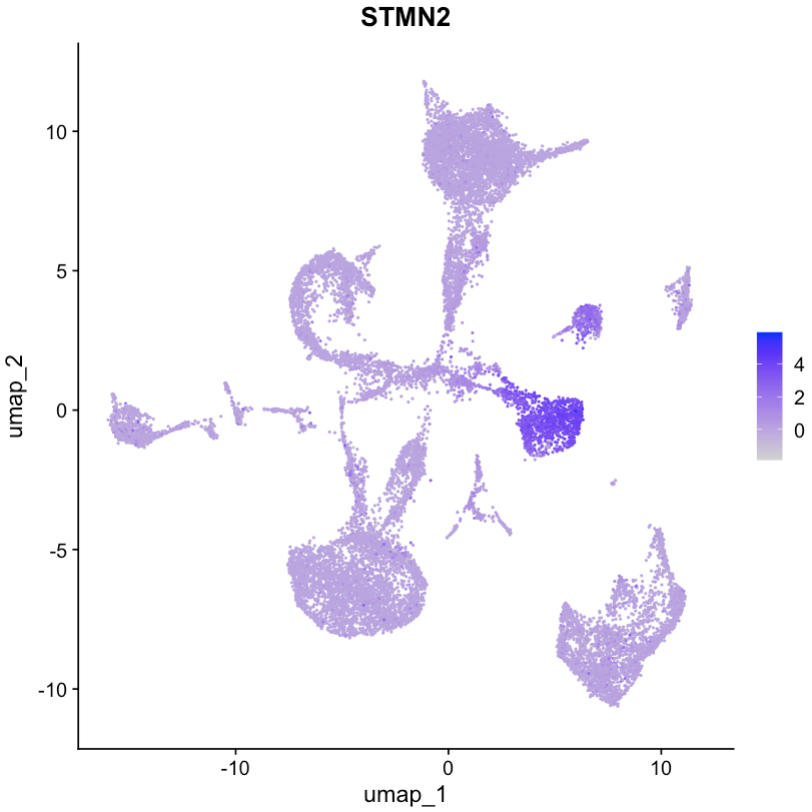


chromaffin cells

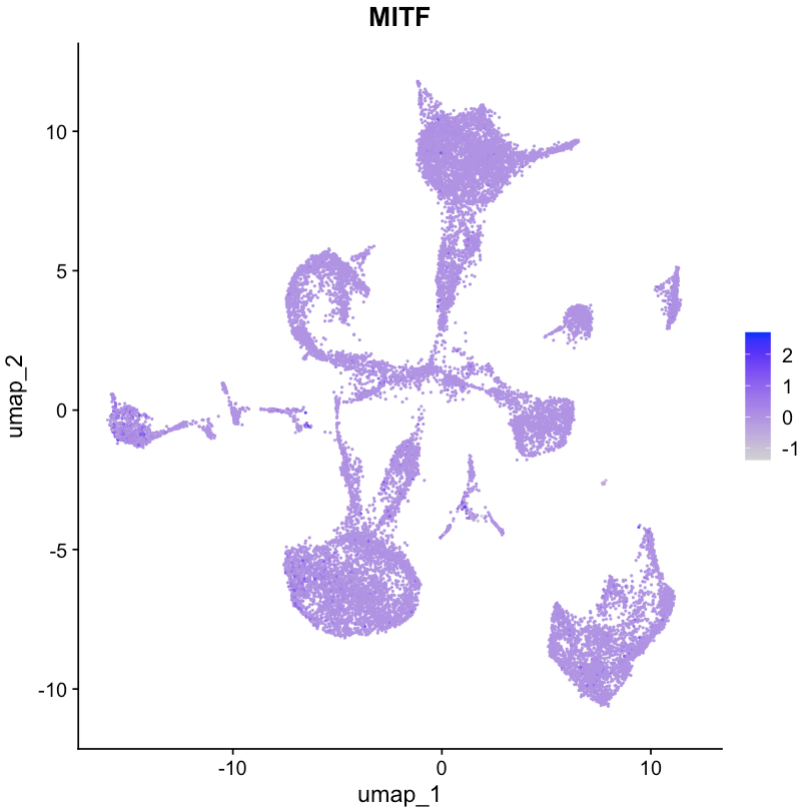
```
FeaturePlot(integrated, c("ELAVL3", "ELAVL4", "PHOX2B", "TH"), ncol=2, raster.dpi = c(800,100))
```



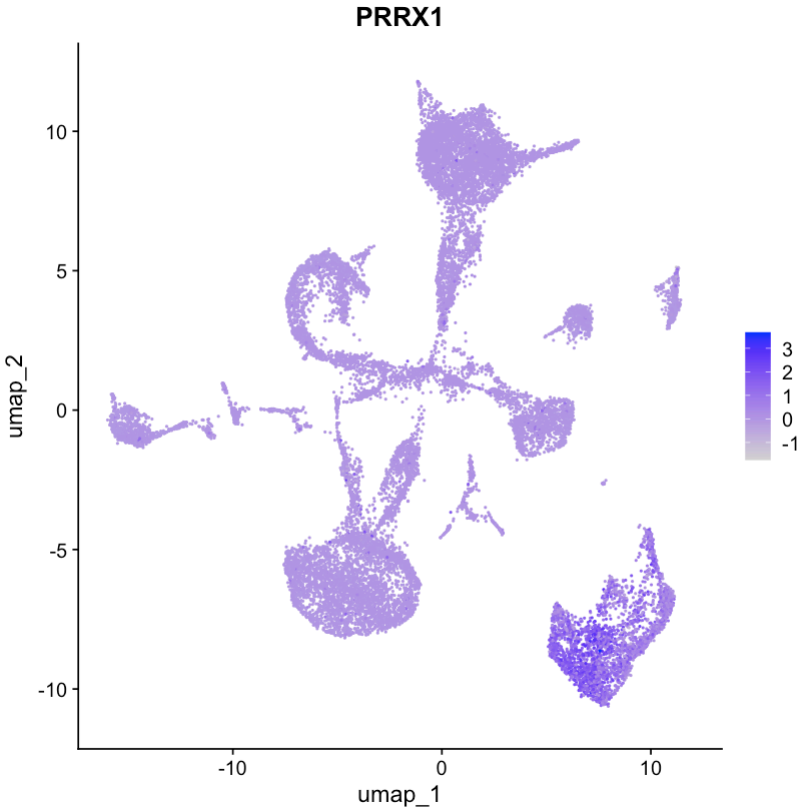
```
FeaturePlot(integrated, c("STMN2"), ncol=1, raster.dpi = c(800,100))
```



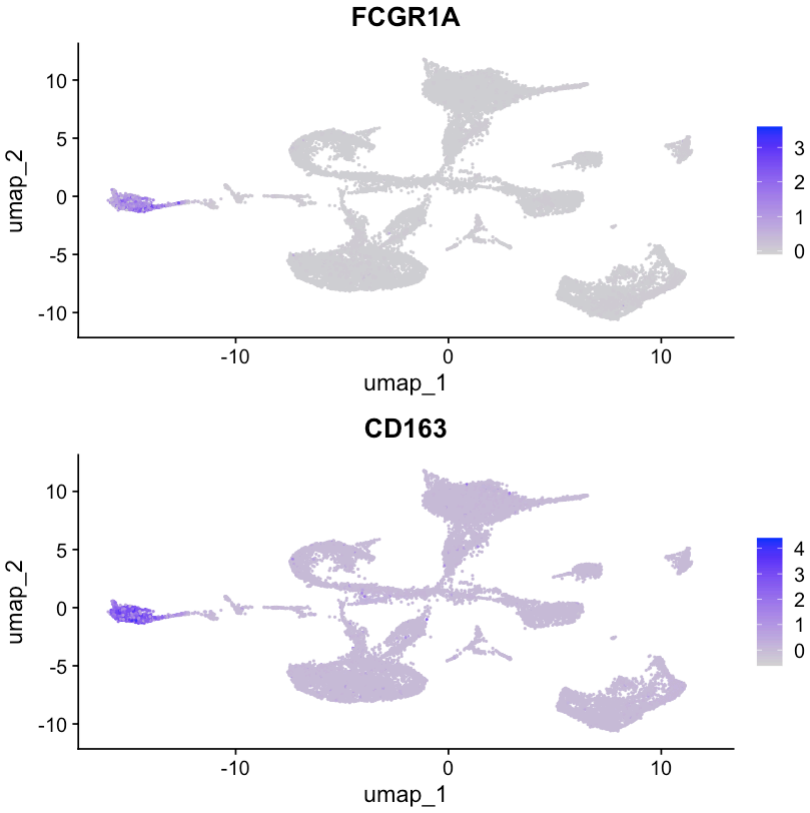

```
FeaturePlot(integrated, c("MITF"), ncol=1, raster.dpi = c(800,100))
```



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




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

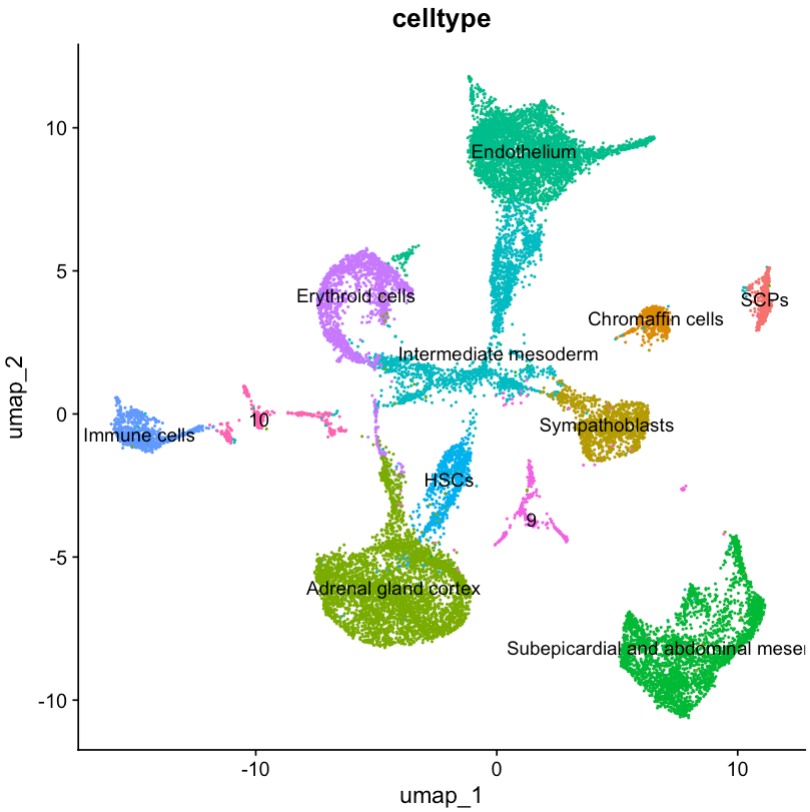


```
cluster <- list(
  "11" = "SCPs", #= c("SOX10", "PLP1", "FOXD3"),
  "8" = "Chromaffin cells", #= c("ELAVL3", "ELAVL4", "PHOX2B", "TH"),
  "5"= "Sympathoblasts", #= c("STMN2"),
  "1" = "Adrenal gland cortex", #= c("NR5A1"),
  # "6" = "Melanocytes", #= c("MITF"),
  #"Kidney" = c("PAX2"),
  "2" = "Subepicardial 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))
```

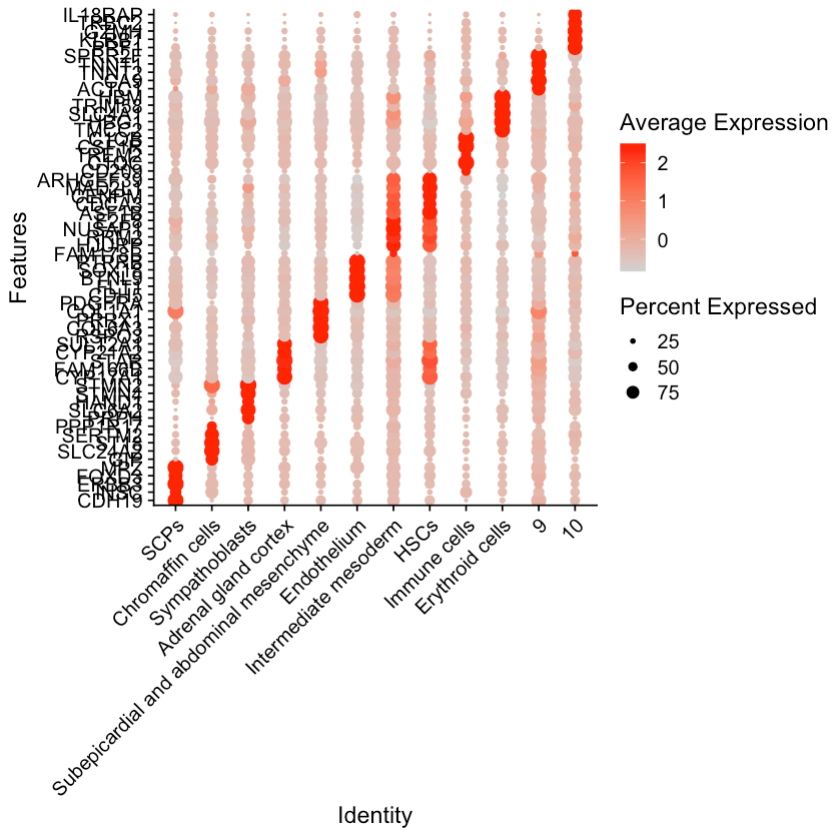
```
head(top5)
```

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

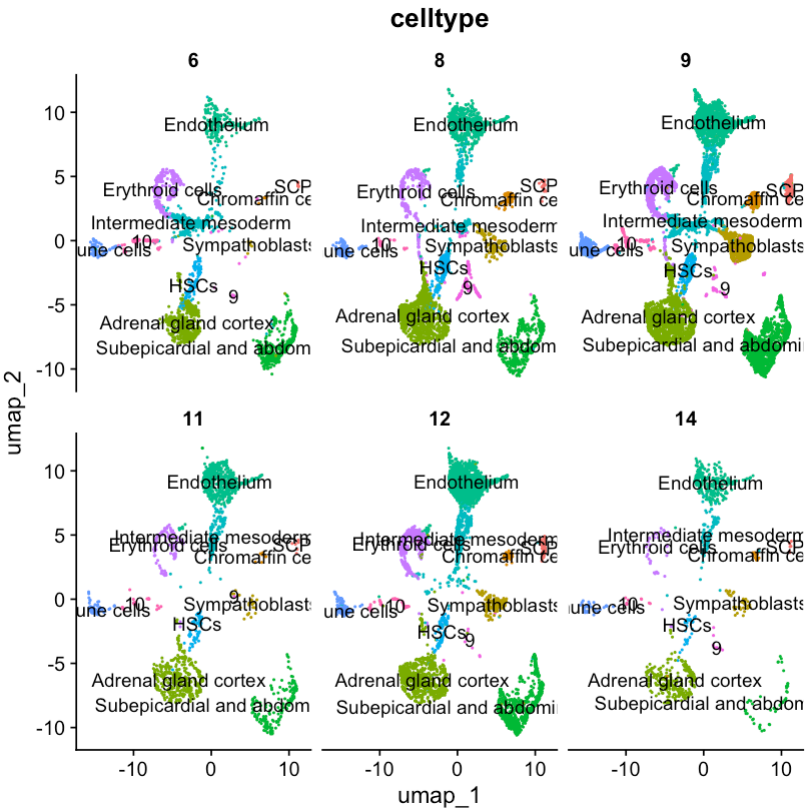
p_val <dbl>	avg_log2FC <dbl>	pct.1 <dbl>	pct.2 <dbl>	p_val_adj <dbl>	cluster <fct>	gene <chr>
1.815618e-113	8.241624	0.735	0.382	3.631235e-110	Chromaffin cells	GIP

```
# 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")
```

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//Rtmpa
```

```
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)
```

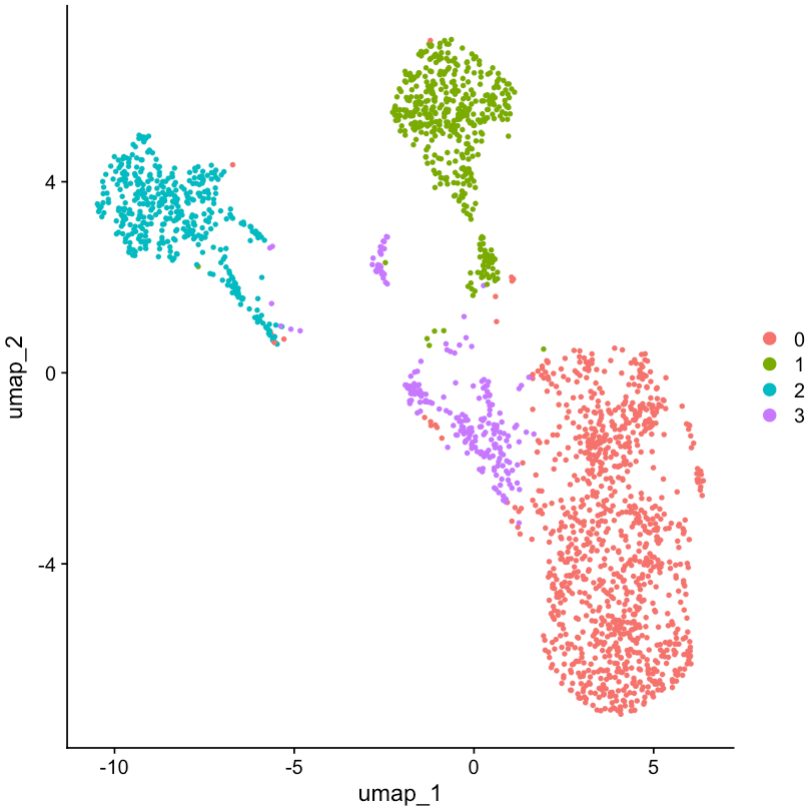
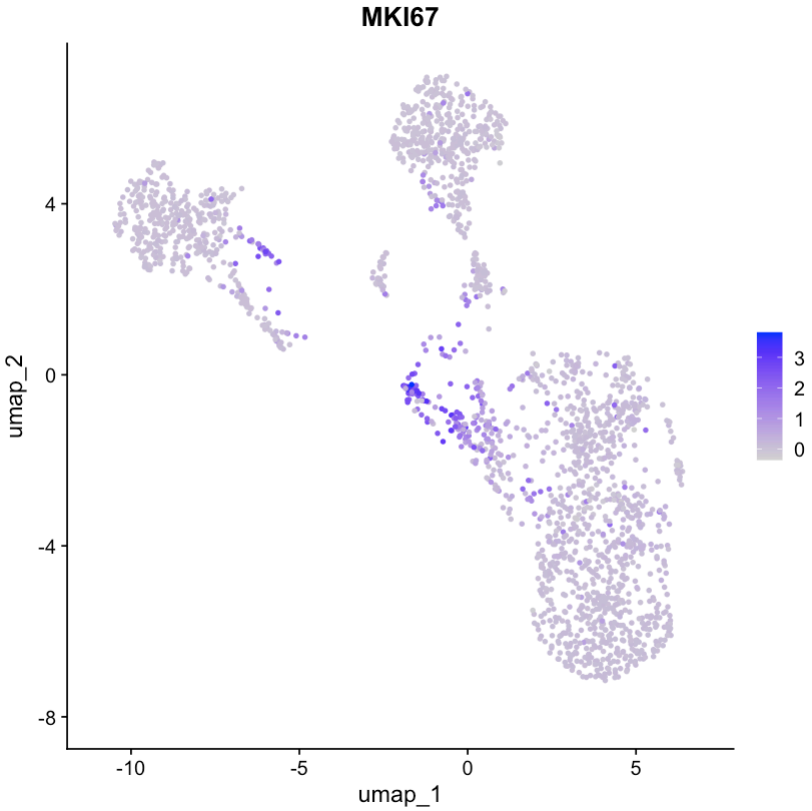


Fig 2 Markers from the paper

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

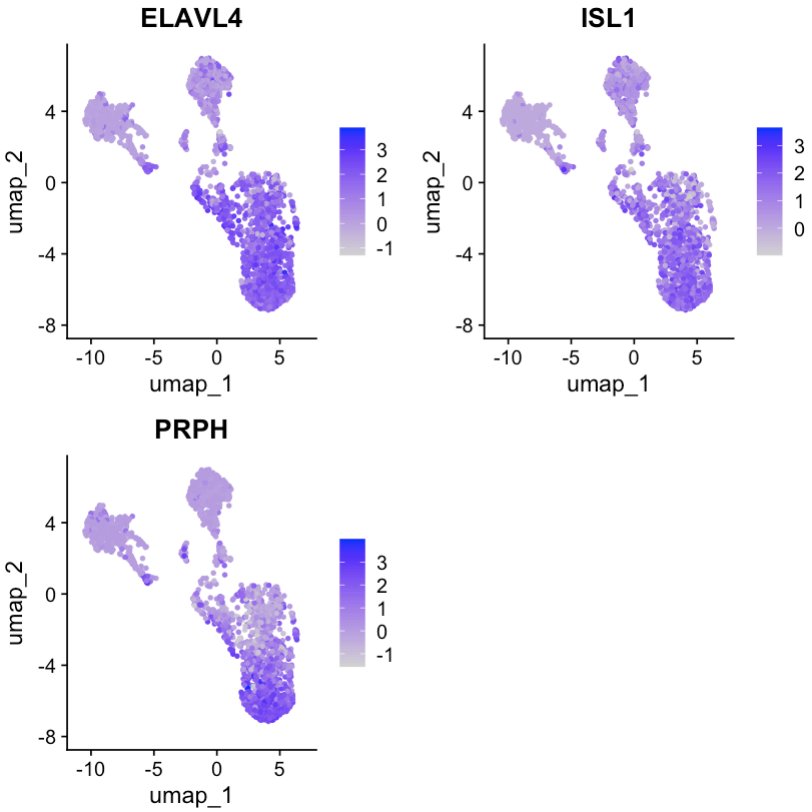
Markers for Prol. Sympathoblasts

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



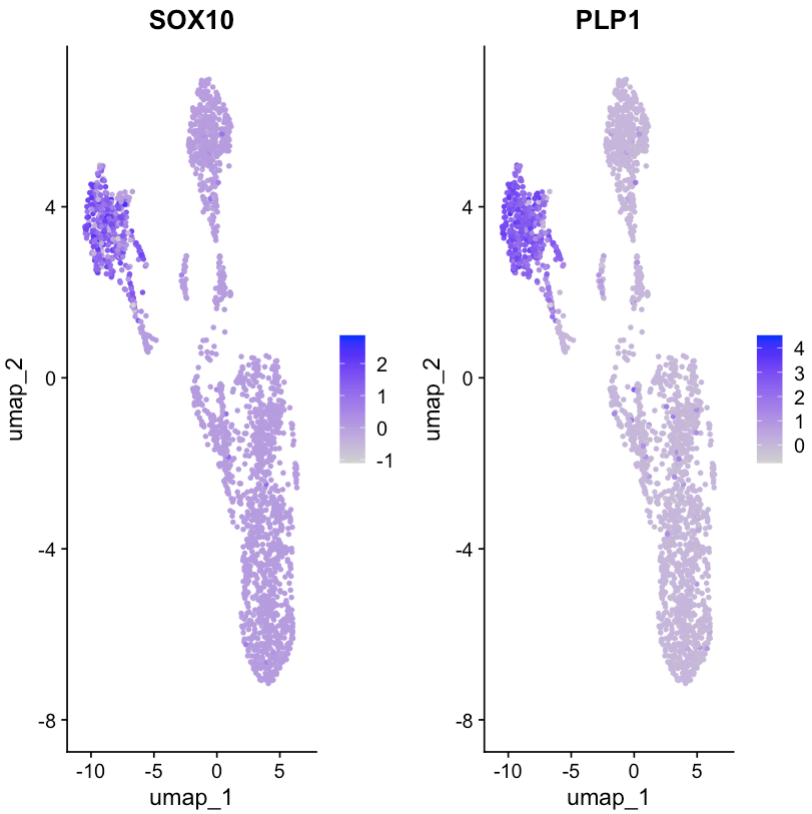
Markers for Sympathoblasts

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



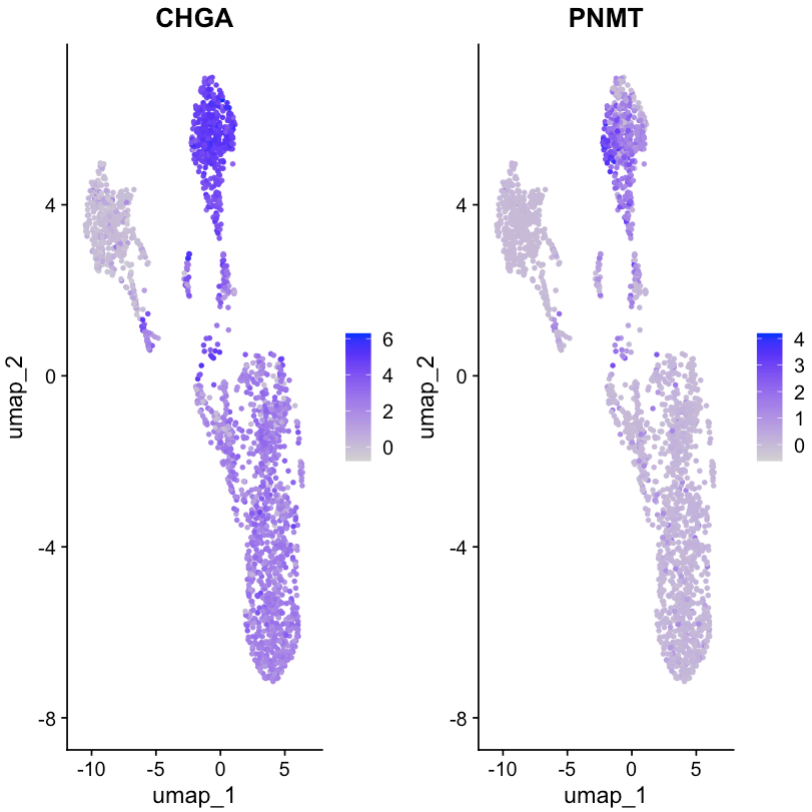
Markers for SCPs

```
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)
```

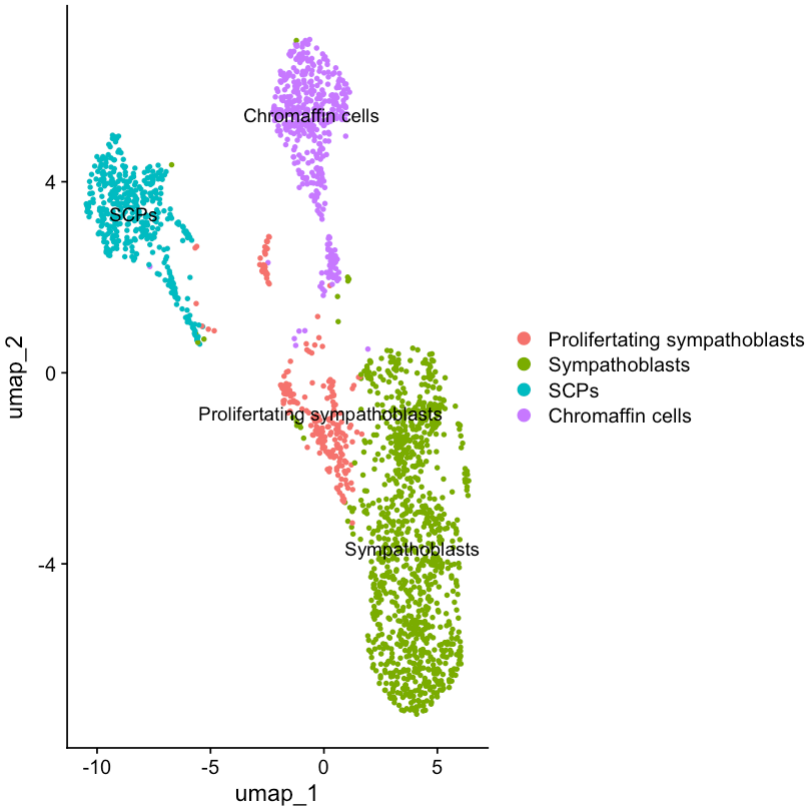
```
unique(seu_subset$celltype)
```

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")
```

Trajectory analysis

```
import scanpy as sc
import cellestial as cl
from lets_plot import *

LetsPlot.setup_html()
```

```
Unable to display output for mime type(s): text/html
```

```
adata = sc.read("data/processed/AMC_subset_annotated.h5ad")
```

```
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/anndata/compat/__init__.py:371: FutureWa

This is where adjacency matrices should go now.
  warn(
```

```
adata.obs["celltype"] = adata.obs["celltype"].astype(str).astype("category")
```

Note seurat object to h5ad did not retain celltype info

```
adata.obs["celltype"].unique()
```

```
['3', '1', '0', '2']
Categories (4, object): ['0', '1', '2', '3']
```

Plot with Cellestial

```
cl.umap(adata, "celltype", size=2, axis_type="arrow", legend_ondata="True")
```

```
<lets_plot.plot.core.PlotSpec at 0x35c640b00>
```

Assingn cell types

```
cluster = {
    "0": "Proliferating sympathoblasts",  # = "MKI67",
    "1": "Sympathoblasts",  # = c("ELAVL4", "ISL1", "PRPH"),
    "2": "SCPs",  # = c("SOX10","PLP1"),
    "3": "Chromaffin cells",  # = c("CHGA","PNMT")
}
adata.obs["cell_type"] = adata.obs["celltype"].map(cluster)
adata.obs["cell_type"].unique()
```

```
['Chromaffin cells', 'Sympathoblasts', 'Proliferating sympathoblasts', 'SCPs']
Categories (4, object): ['Proliferating sympathoblasts', 'Sympathoblasts', 'SCPs', 'Chromaffin cells
```

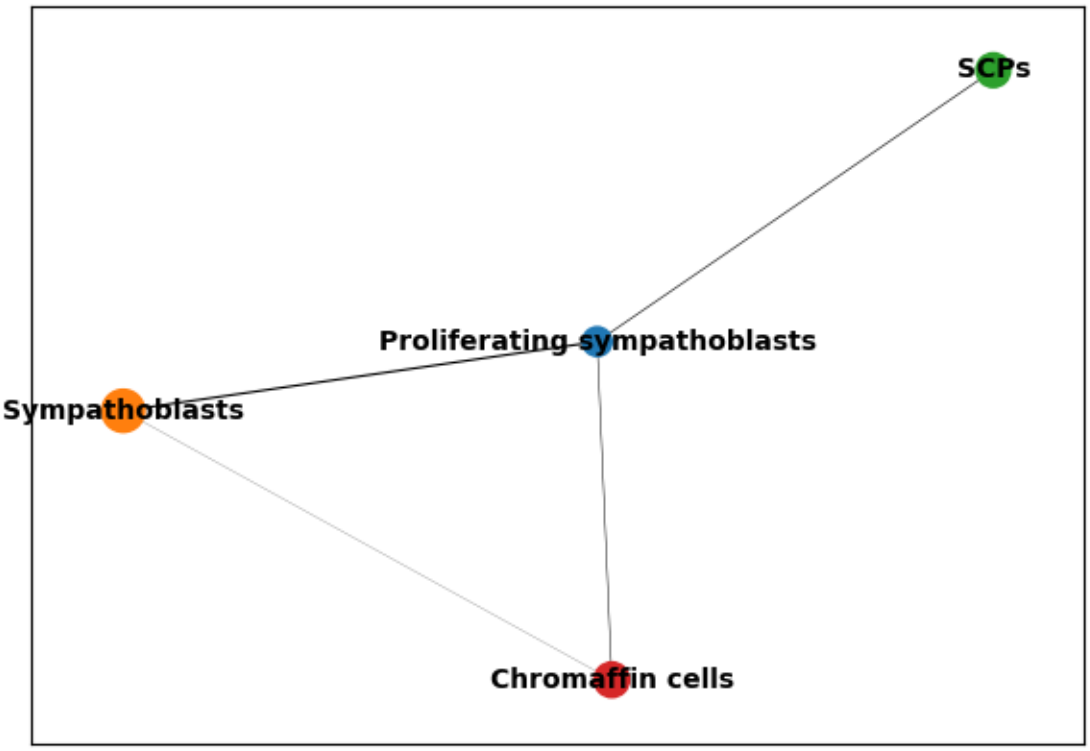
```
cl.umaps(
    adata,
    ["celltype", "cell_type"],
    size=2,
    axis_type="arrow",
    legend_ondata="True",
    ncol=2,
)
```

```
<lets_plot.plot.subplots.SupPlotsSpec at 0x3517f1a90>
```

Overall Dataset

PAGA

```
sc.tl.paga(adata, groups="cell_type")
sc.pl.paga(adata, color="cell_type")
```



Re calculate Neigbors and UMAP

```
sc.pp.neighbors(adata, n_neighbors=15, n_pcs=40)
```

```
sc.tl.umap(adata, init_pos="paga")
umap_all =cl.umap(
    adata, key="cell_type", legend_ondata=True, axis_type="arrow", ondata_size=8, size=3
) + ggtitle("All AMC cells")
umap_all
```

```
<lets_plot.plot.core.PlotSpec at 0x30dfff140>
```

```
print(adata.obs["cell_type"].unique())
```

```
['Chromaffin cells', 'Sympathoblasts', 'Proliferating sympathoblasts', 'SCPs']
Categories (4, object): ['Proliferating sympathoblasts', 'Sympathoblasts', 'SCPs', 'Chromaffin cells
```

Run DPT

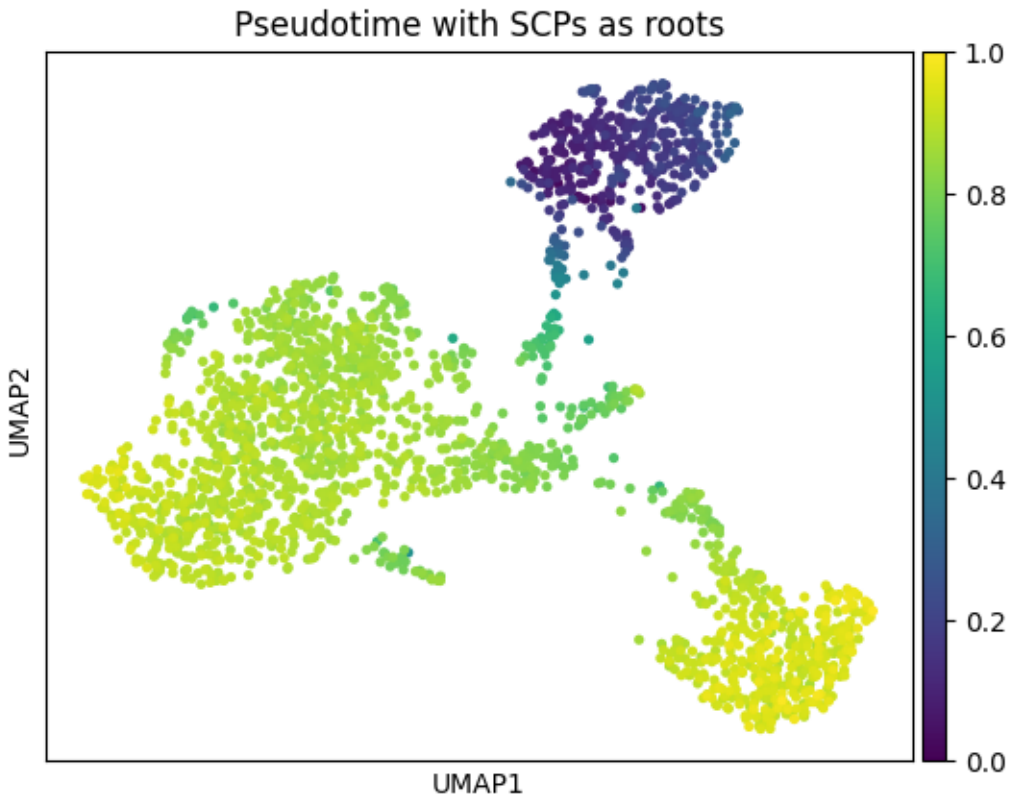
```
root_cell = adata.obs[adata.obs["cell_type"] == "SCPs"].index[0]
adata.uns["iroot"] = adata.obs.index.get_loc(root_cell)
sc.tl.dpt(adata)
```

```
WARNING: Trying to run `tl.dpt` without prior call of `tl.diffmap`. Falling back to `tl.diffmap` wit
```

```
umap_all_dpt =(
    cl.umap(
        adata,
        key="dpt_pseudotime",
        size=3,
        axis_type="arrow",
        add_tooltips=["cell_type"],
    )
    + scale_color_viridis()
    + ggtitle("All AMC cells")
    + ggsize(600, 500)
)
umap_all_dpt
```

```
<lets_plot.plot.core.PlotSpec at 0x30dfff2f0>
```

```
sc.pl.umap(adata, color="dpt_pseudotime", title="Pseudotime with SCPs as roots")
```




```
(
    cl.umap(
        adata,
        key="dpt_pseudotime",
        size=2,
        axis_type="arrow",
        add_tooltips=["cell_type"],
    )
```

```
    + scale_color_viridis()
)
```

```
<lets_plot.plot.core.PlotSpec at 0x35ca15d90>
```

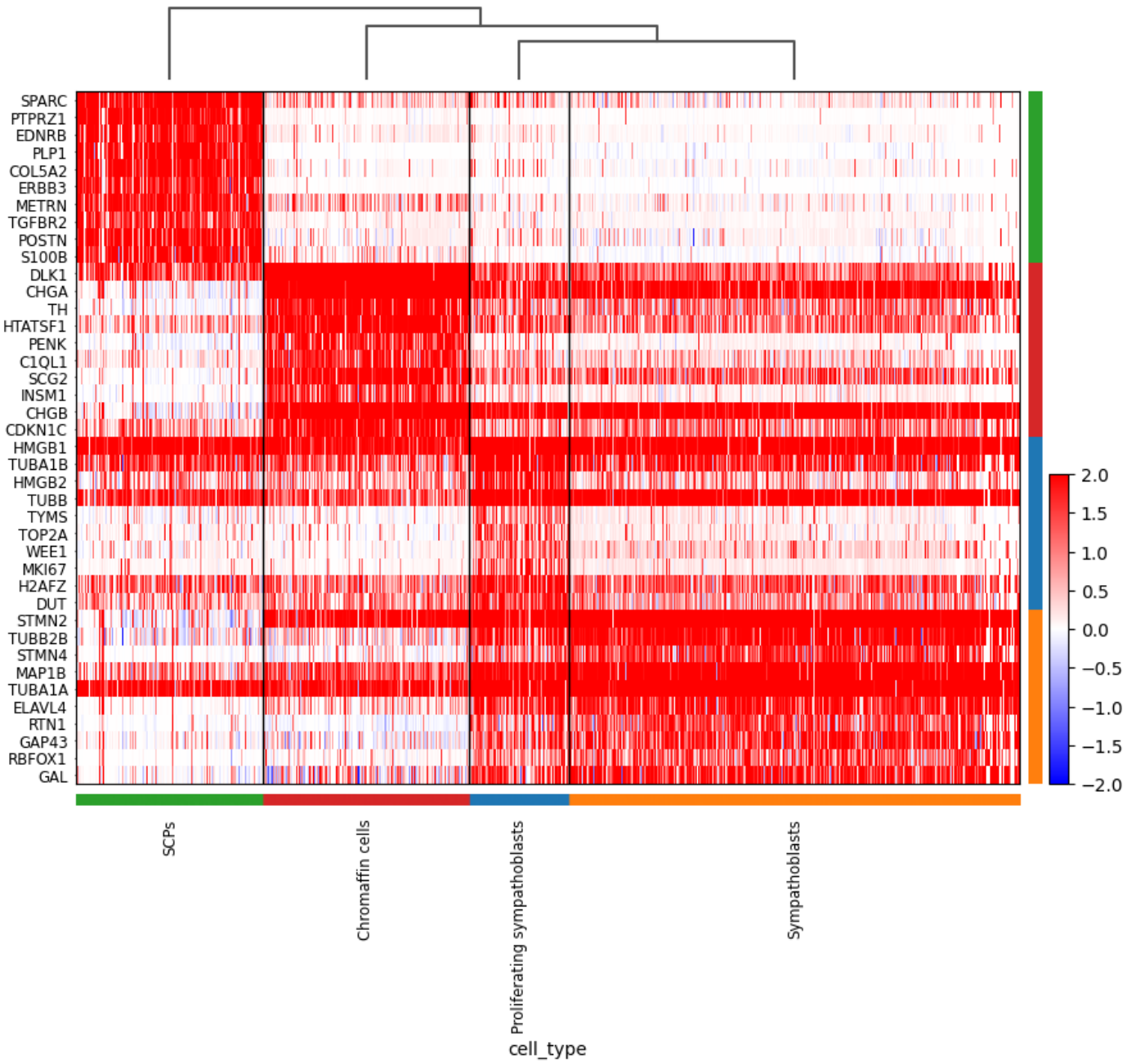
Heatmap

```
sc.tl.rank_genes_groups(adata, 'cell_type', method='t-test')
```

```
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_rank_genes_groups.py:484:
  self.stats[group_name, "logfoldchanges"] = np.log2(
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_rank_genes_groups.py:484:
  self.stats[group_name, "logfoldchanges"] = np.log2(
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_rank_genes_groups.py:484:
  self.stats[group_name, "logfoldchanges"] = np.log2(
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_rank_genes_groups.py:484:
  self.stats[group_name, "logfoldchanges"] = np.log2(
```

```
sc.pl.rank_genes_groups_heatmap(
    adata,
    n_genes=10,  # show top 10 per group
    groupby='cell_type',
    show_gene_labels=True,
    cmap='bwr',
    swap_axes=True,
    vmin=-2, vmax=2  #
)
```

```
WARNING: dendrogram data not found (using key=dendrogram_cell_type). Running `sc.tl.dendrogram` wit
```



Subsets

1. SCPs to Sympathoblasts
2. SCPs to Chromaffin Cells
3. Chromaffin Cells to Sympathoblasts

subset1

```
subset1 = ["Proliferating sympathoblasts", "Sympathoblasts", "SCPs"]
adata1 = adata[adata.obs["cell_type"].isin(subset1)]
```

subset2

```
subset2 = ["Chromaffin cells", "SCPs","Proliferating sympathoblasts"]
adata2 = adata[adata.obs["cell_type"].isin(subset2)]
```

subset3

```
subset3 = ["Chromaffin cells", "Proliferating sympathoblasts", "Sympathoblasts"]
adata3 = adata[adata.obs["cell_type"].isin(subset3)]
```

SCPs to sympathoblasts

```
adata1.obs["cell_type"].unique()
```

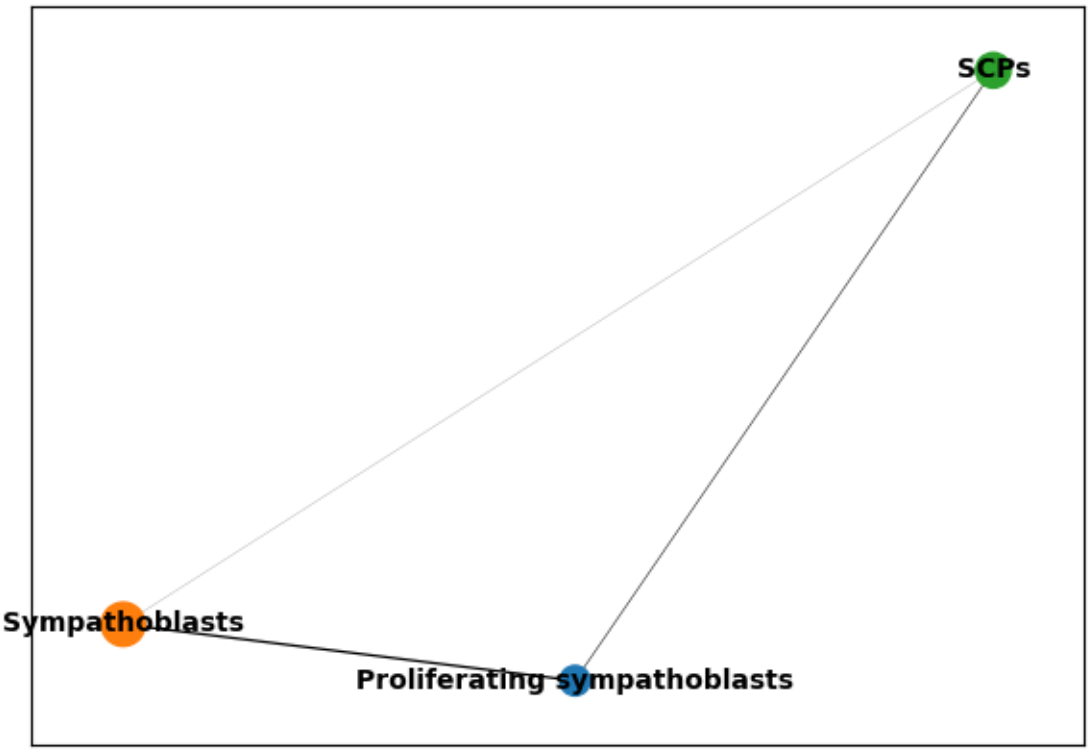
```
['Sympathoblasts', 'Proliferating sympathoblasts', 'SCPs']
Categories (3, object): ['Proliferating sympathoblasts', 'Sympathoblasts', 'SCPs']
```

PAGA workflow

```
sc.tl.paga(adata1, groups="cell_type")
```

```
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_paga.py:139: ImplicitModi
  adata.uns[groups + "_sizes"] = np.array(paga.ns)
```

```
sc.pl.paga(adata1, color='cell_type')
```



```
sc.pp.neighbors(adata1, n_neighbors=15, n_pcs=40)
```

```
sc.tl.umap(adata1, init_pos="paga")
```

```
umap1 =(
    cl.umap(
        adata1,
        key="cell_type",
        legend_ondata=True,
        axis_type="arrow",
        ondata_size=8,
        size=3,
    )
    + ggtitle("SCPs and sympathoblasts")
    + ggsize(600, 500)
)
umap1
```

```
<lets_plot.plot.core.PlotSpec at 0x30dfa6cc0>
```

Run DPT

```
root_cell = adata1.obs[adata1.obs["cell_type"] == "SCPs"].index[0]
adata1.uns["iroot"] = adata1.obs.index.get_loc(root_cell)
sc.tl.dpt(adata1)
```

```
umap1_dpt =(
    cl.umap(
        adata1,
        key="dpt_pseudotime",
        size=3,
        axis_type="arrow",
        add_tooltips=["cell_type"],
    )
    + scale_color_viridis()
    + ggtitle("SCPs and sympathoblasts ")
    + ggsize(600, 500)
)
umap1_dpt
```

```
<lets_plot.plot.core.PlotSpec at 0x30dfff0b0>
```

Find changing genes along the trajectory

```
sc.tl.rank_genes_groups(adata1, 'cell_type', method='t-test')
```

```
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_rank_genes_groups.py:484:
  self.stats[group_name, "logfoldchanges"] = np.log2(
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_rank_genes_groups.py:484:
  self.stats[group_name, "logfoldchanges"] = np.log2(
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_rank_genes_groups.py:484:
  self.stats[group_name, "logfoldchanges"] = np.log2(
```

```
top_genes = adata1.uns['rank_genes_groups']['names']
top_genes[:10]
```

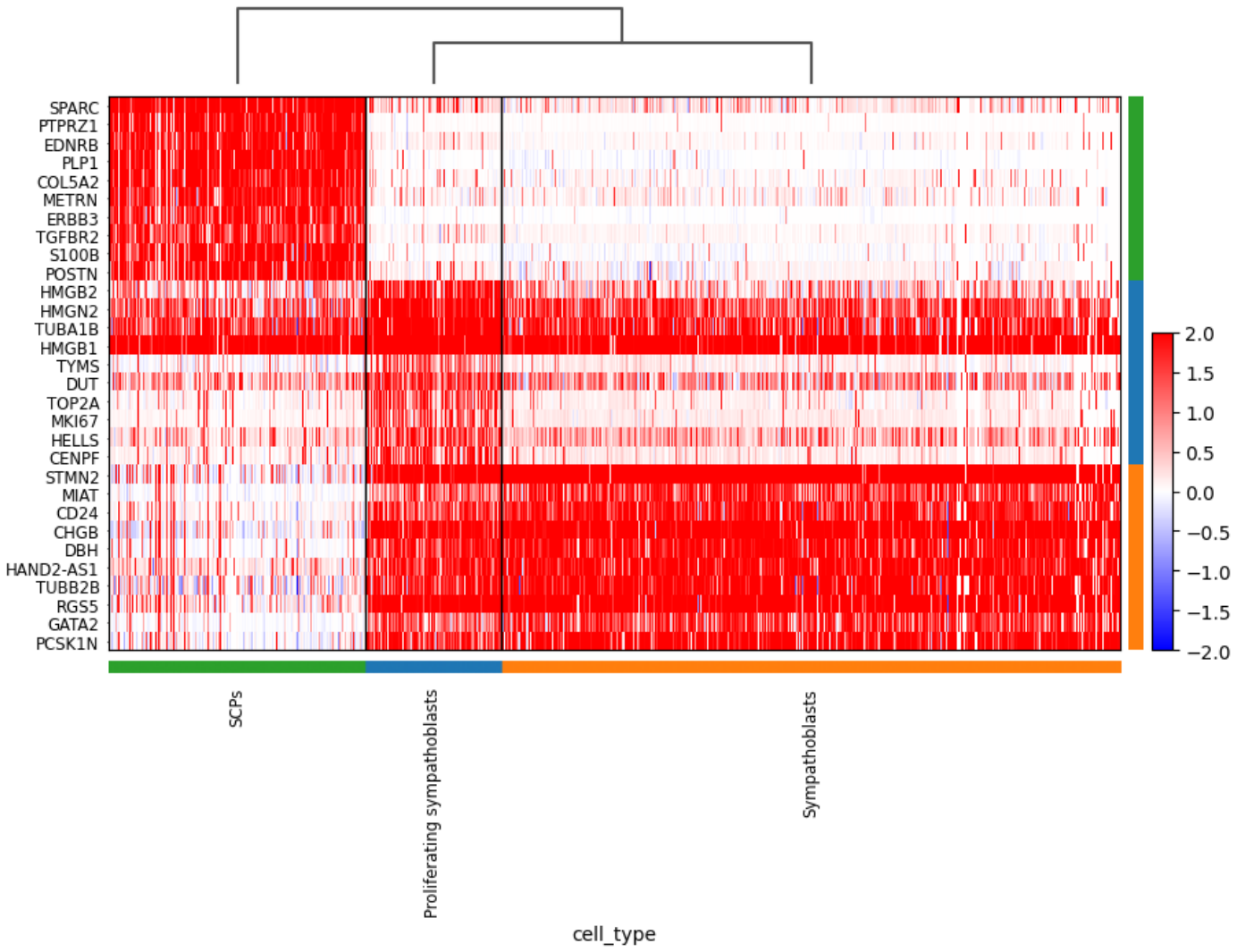
```
rec.array([('HMGB2', 'STMN2', 'SPARC'), ('HMGN2', 'MIAT', 'PTPRZ1'),
           ('TUBA1B', 'CD24', 'EDNRB'), ('HMGB1', 'CHGB', 'PLP1'),
           ('TYMS', 'DBH', 'COL5A2'), ('DUT', 'HAND2-AS1', 'METRN'),
           ('TOP2A', 'TUBB2B', 'ERBB3'), ('MKI67', 'RGS5', 'TGFBR2'),
           ('HELLS', 'GATA2', 'S100B'), ('CENPF', 'PCSK1N', 'POSTN')],
          dtype=[('Proliferating sympathoblasts', 'O'), ('Sympathoblasts', 'O'), ('SCPs', 'O')])
```

Heatmap of genes chaning along the trajectory

```
sc.tl.dendrogram(adata1, groupby="cell_type")
```

```
heatmap1 = sc.pl.rank_genes_groups_heatmap(
    adata1,
    n_genes=10,
    groupby='cell_type',
    show_gene_labels=True,
    cmap='bwr',
    swap_axes=True,
    save='_scps_and_sympathoblasts.pdf',
    vmin=-2, vmax=2)
heatmap1
```

```
WARNING: saving figure to file figures/heatmap_scps_and_sympathoblasts.pdf
```



SCPs to Chromaffin Cells

```
adata2.obs["cell_type"].unique()
```

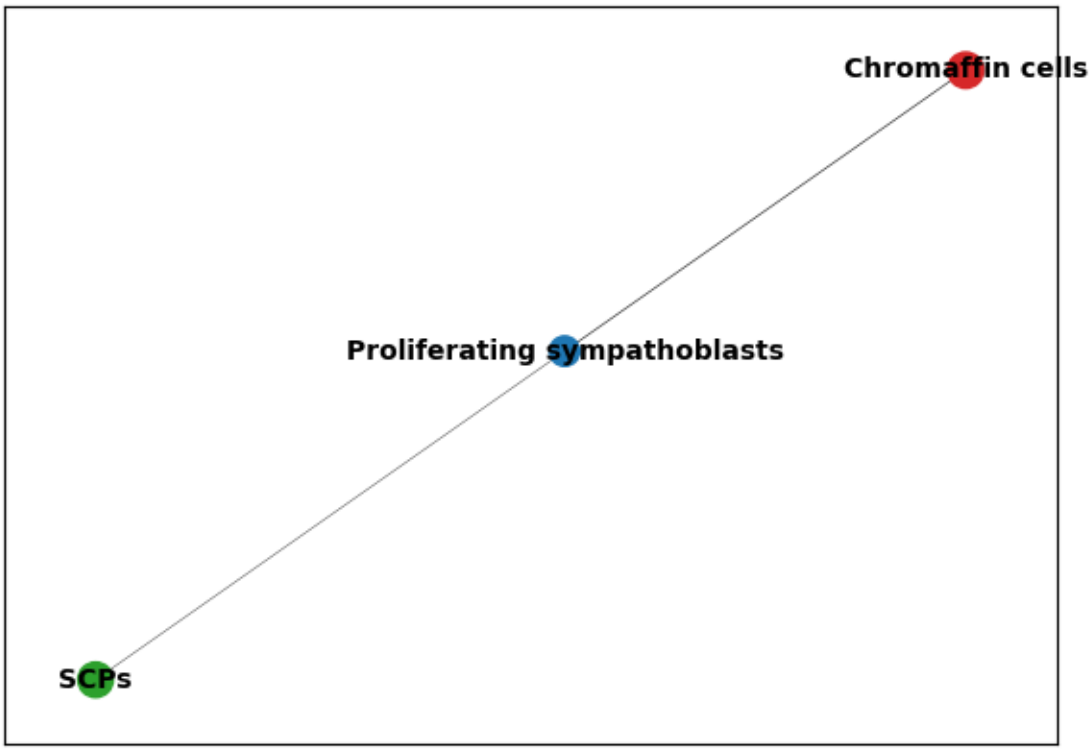
```
['Chromaffin cells', 'Proliferating sympathoblasts', 'SCPs']
Categories (3, object): ['Proliferating sympathoblasts', 'SCPs', 'Chromaffin cells']
```

PAGA

```
sc.tl.paga(adata2, groups="cell_type")
```

```
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_paga.py:139: ImplicitModi
  adata.uns[groups + "_sizes"] = np.array(paga.ns)
```

```
sc.pl.paga(adata2, color='cell_type')
```




```
sc.pp.neighbors(adata2, n_neighbors=15, n_pcs=40)
```

```
sc.tl.umap(adata2, init_pos="paga")
```

```
umap2 =(
    cl.umap(
        adata2,
        key="cell_type",
        legend_ondata=True,
        axis_type="arrow",
        ondata_size=8,
        size=3,
    )
    + ggtitle("SCPs and Chromaffin Cells")
    + ggsize(600, 500)
)
umap2
```

```
<lets_plot.plot.core.PlotSpec at 0x366175be0>
```

Run DPT

```
root_cell = adata2.obs[adata2.obs["cell_type"] == "SCPs"].index[0]
adata2.uns["iroot"] = adata2.obs.index.get_loc(root_cell)
sc.tl.dpt(adata2)
```

```
umap2_dpt =(
    cl.umap(
        adata2,
        key="dpt_pseudotime",
        size=3,
        axis_type="arrow",
        add_tooltips=["cell_type"],
    )
    + scale_color_viridis()
    + ggtitle("SCPs and Chromaffin Cells")
    + ggsize(600, 500)
)
umap2_dpt
```

```
<lets_plot.plot.core.PlotSpec at 0x334f2cc20>
```

Find changing genes along the trajectory

```
sc.tl.rank_genes_groups(adata2, 'cell_type', method='t-test')
```

```
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_rank_genes_groups.py:484:
  self.stats[group_name, "logfoldchanges"] = np.log2(
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_rank_genes_groups.py:484:
  self.stats[group_name, "logfoldchanges"] = np.log2(
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_rank_genes_groups.py:484:
  self.stats[group_name, "logfoldchanges"] = np.log2(
```

```
top_genes = adata2.uns['rank_genes_groups']['names']
top_genes[:10]
```

```
rec.array([('TUBB', 'PTPRZ1', 'CHGA'), ('MAP1B', 'PLP1', 'DLK1'),
           ('STMN1', 'SPARC', 'TH'), ('TUBB2B', 'COL5A2', 'CHGB'),
           ('STMN2', 'EDNRB', 'SCG2'), ('HMGB1', 'ANXA2', 'HTATSF1'),
           ('ELAVL4', 'ERBB3', 'RGS4'), ('BASP1', 'TGFBR2', 'RAMP1'),
           ('KIF21A', 'MPZ', 'SLC18A1'), ('TUBA1B', 'OLFML2A', 'PENK')],
          dtype=[('Proliferating sympathoblasts', 'O'), ('SCPs', 'O'), ('Chromaffin cells', 'O')])
```

Heatmap of genes chaning along the trajectory

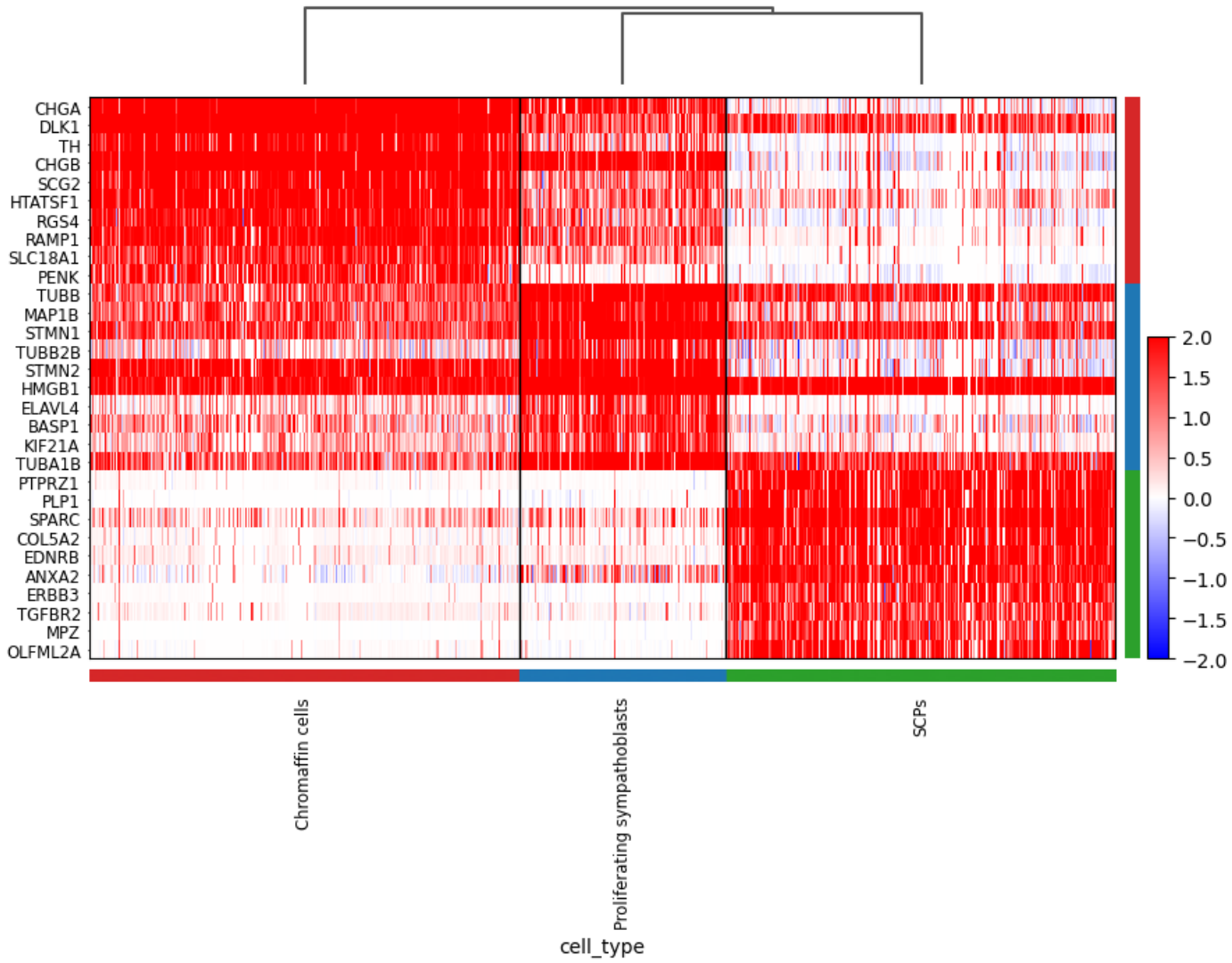
```
sc.tl.dendrogram(adata2, groupby="cell_type")
```

```
heatmap2 = sc.pl.rank_genes_groups_heatmap(
    adata2,
    n_genes=10,
    groupby='cell_type',
    show_gene_labels=True,
    cmap='bwr',
```

```
    swap_axes=True,
    save='_scps_and_chromaffin.pdf',
    vmin=-2, vmax=2)

heatmap2
```

```
WARNING: saving figure to file figures/heatmap_scps_and_chromaffin.pdf
```



Chromaffin Cells to Sympathoblasts

```
adata3.obs["cell_type"].unique()
```

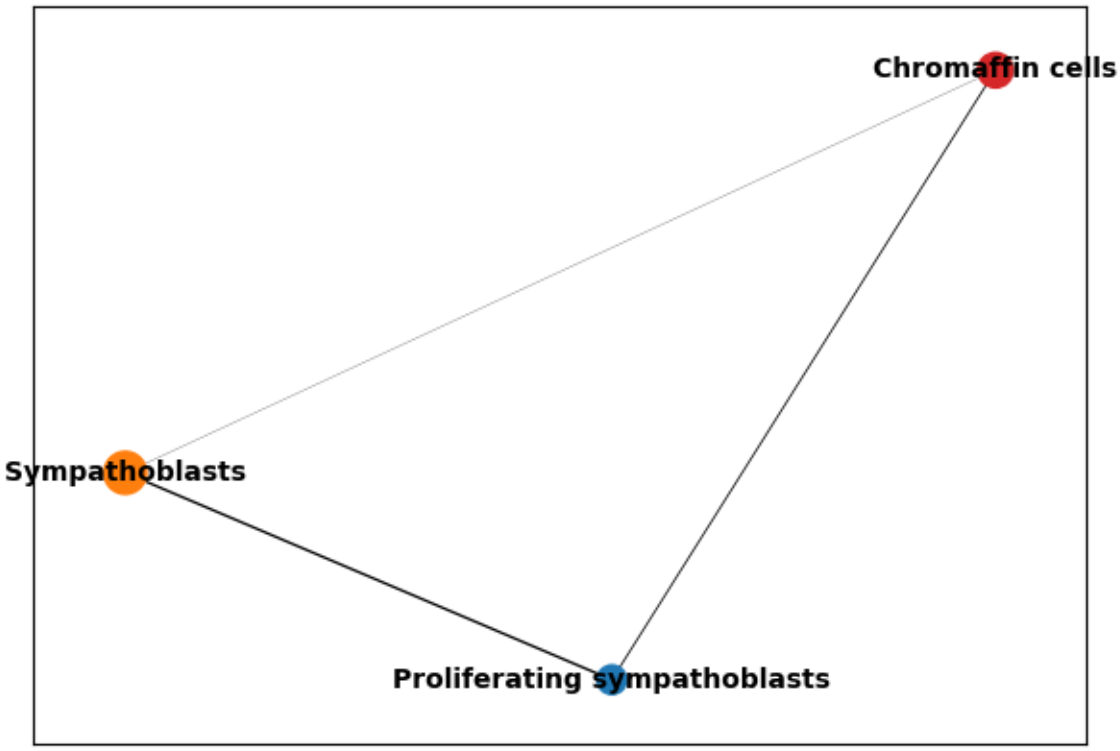
```
['Chromaffin cells', 'Sympathoblasts', 'Proliferating sympathoblasts']
Categories (3, object): ['Proliferating sympathoblasts', 'Sympathoblasts', 'Chromaffin cells']
```

PAGA

```
sc.tl.paga(adata3, groups="cell_type")
```

```
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_paga.py:139: ImplicitModi
  adata.uns[groups + "_sizes"] = np.array(paga.ns)
```

```
sc.pl.paga(adata3, color="cell_type")
```




```
sc.pp.neighbors(adata3, n_neighbors=15, n_pcs=40)
```

```
sc.tl.umap(adata3, init_pos="paga")
```

```
umap3 = (
    cl.umap(
        adata3,
        key="cell_type",
        legend_ondata=True,
        axis_type="arrow",
        ondata_size=8,
        size=3,
    )
    + ggtitle("Chromaffin Cells and Sympathoblasts")
    + ggsize(600, 500)
)
```

Run DPT

```
root_cell = adata3.obs[adata3.obs["cell_type"] == "Sympathoblasts"].index[0]
adata3.uns["iroot"] = adata3.obs.index.get_loc(root_cell)
sc.tl.dpt(adata3)
```

```
umap3_dpt = (
    cl.umap(
        adata3,
        key="dpt_pseudotime",
        size=3,
        axis_type="arrow",
        add_tooltips=["cell_type"],
    )
    + scale_color_viridis()
    + ggtitle("Chromaffin Cells and Sympathoblasts")
    + ggsize(600, 500)
)
umap3_dpt
```

```
<lets_plot.plot.core.PlotSpec at 0x335ef0200>
```

Find changing genes along the trajectory

```
sc.tl.rank_genes_groups(adata3, 'cell_type', method='t-test')
```

```
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_rank_genes_groups.py:484:
  self.stats[group_name, "logfoldchanges"] = np.log2(
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_rank_genes_groups.py:484:
  self.stats[group_name, "logfoldchanges"] = np.log2(
/Users/zaf4/dev/CCRItask/.venv/lib/python3.13/site-packages/scanpy/tools/_rank_genes_groups.py:484:
  self.stats[group_name, "logfoldchanges"] = np.log2(
```

```
top_genes = adata3.uns['rank_genes_groups']['names']
top_genes[:10]
```

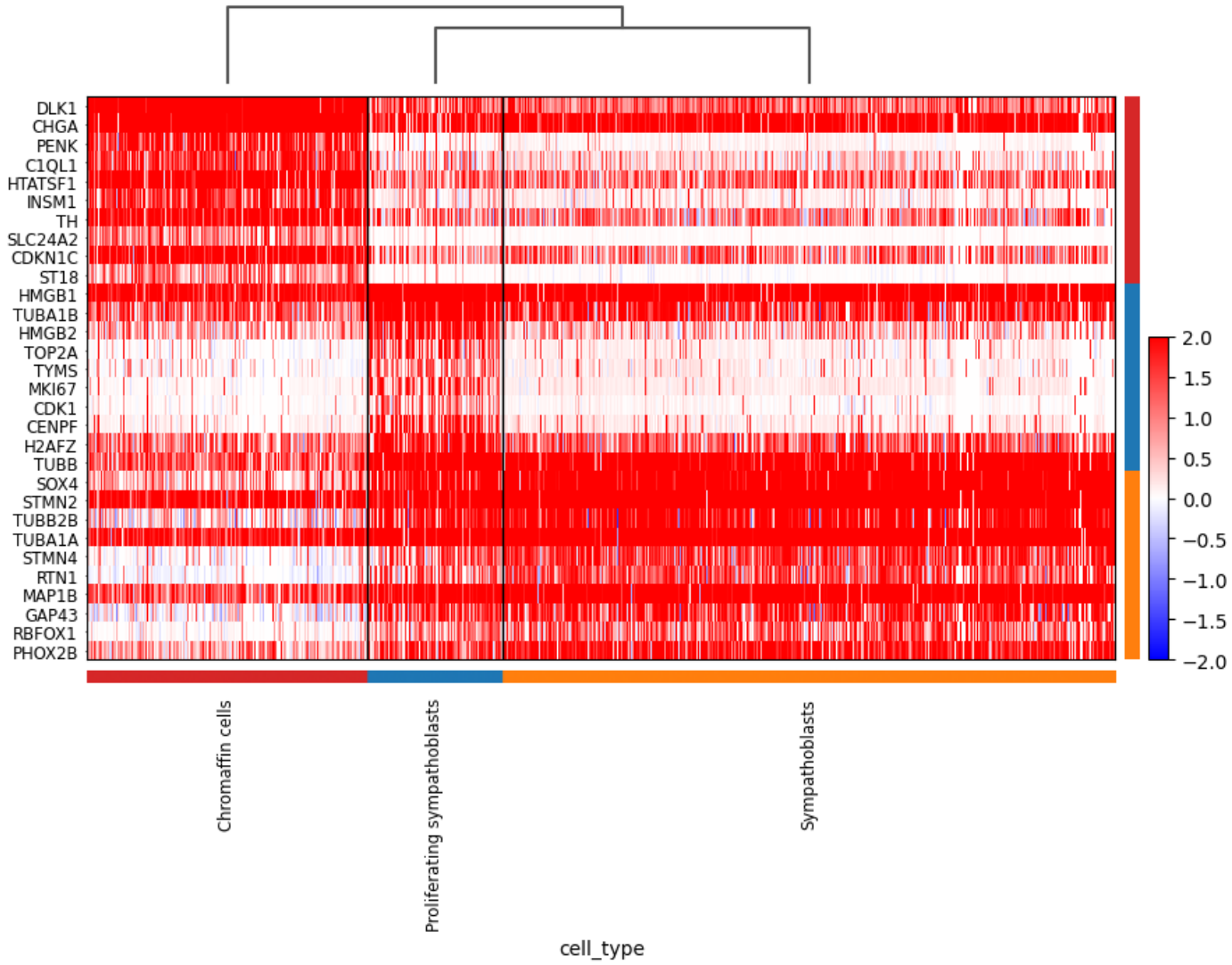
```
rec.array([('HMGB1', 'SOX4', 'DLK1'), ('TUBA1B', 'STMN2', 'CHGA'),
           ('HMGB2', 'TUBB2B', 'PENK'), ('TOP2A', 'TUBA1A', 'C1QL1'),
           ('TYMS', 'STMN4', 'HTATSF1'), ('MKI67', 'RTN1', 'INSM1'),
           ('CDK1', 'MAP1B', 'TH'), ('CENPF', 'GAP43', 'SLC24A2'),
           ('H2AFZ', 'RBFOX1', 'CDKN1C'), ('TUBB', 'PHOX2B', 'ST18')],
          dtype=[('Proliferating sympathoblasts', 'O'), ('Sympathoblasts', 'O'), ('Chromaffin cells
```

Heatmap of genes chaning along the trajectory

```
sc.tl.dendrogram(adata3, groupby="cell_type")
```

```
heatmap3 = sc.pl.rank_genes_groups_heatmap(
    adata3,
    n_genes=10,
    groupby='cell_type',
    show_gene_labels=True,
    cmap='bwr',
    swap_axes=True,
    save='_chromaffin_and_sympathoblasts.pdf',
    vmin=-2, vmax=2,return_fig=True)
heatmap3
```

```
WARNING: saving figure to file figures/heatmap_chromaffin_and_sympathoblasts.pdf
```




```
grid = gggrid([umap_all, umap_all_dpt, umap1, umap1_dpt, umap2, umap2_dpt, umap3, umap3_dpt], ncol=2
grid
```

```
<lets_plot.plot.subplots.SupPlotsSpec at 0x3480a7000>
```

```
ggsave(grid, filename='plots/umap_pseudotime.svg', path='.')
```

```
'/Users/zaf4/dev/CCRItask/plots/umap_pseudotime.svg'
```