RM_HW_W10_2

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Follow up to question 3

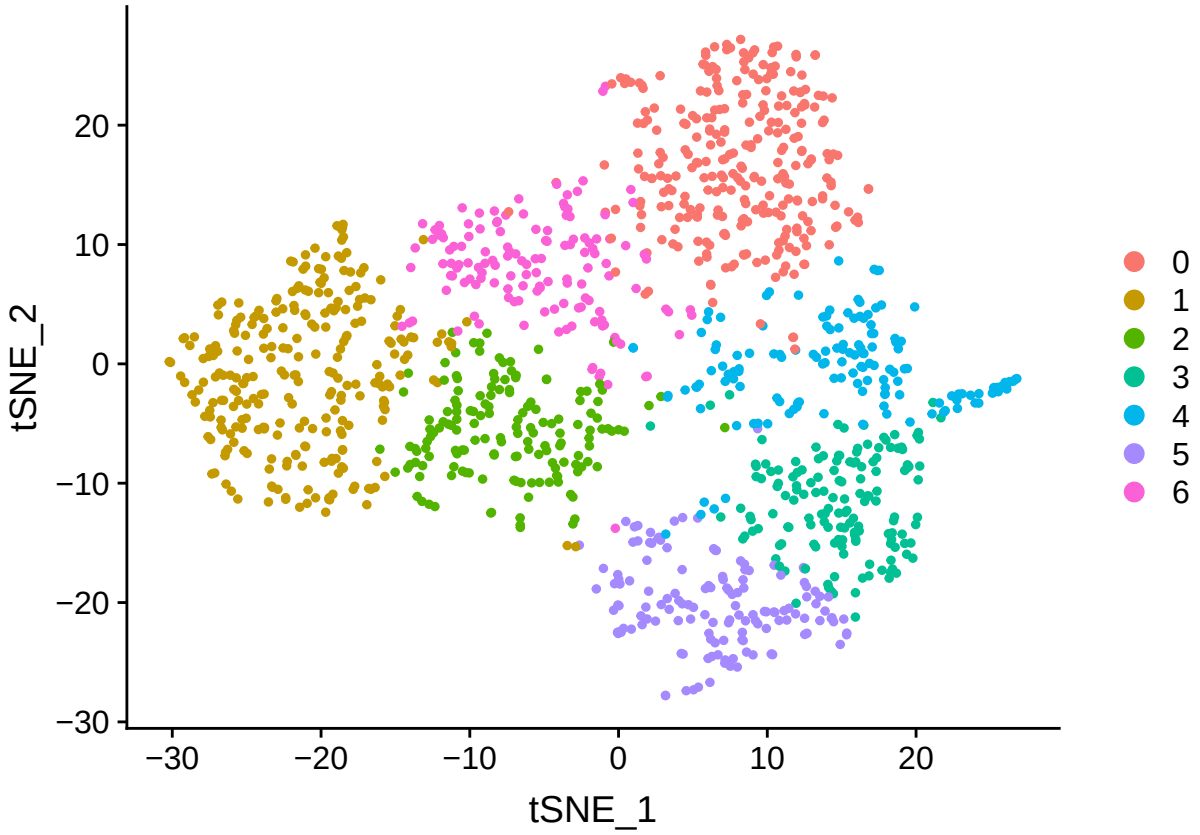
Seurat processing

some parts take a while so I am not knitting this part

```
# seurat processing
WT_seurat <- FindVariableFeatures(object = WT_seurat,
  selection.method = 'mean.var.plot', mean.cutoff = c(0.0125, 3),
  dispersion.cutoff = c(0.5, Inf))

length(x = VariableFeatures(object = WT_seurat))
VariableFeaturePlot(WT_seurat)

# regress out UMI and mitoDNA
WT_seurat <- ScaleData(object = WT_seurat,
features = rownames(x = WT_seurat),
  vars.to.regress = c("nCount_RNA", "fraction.mito"))


WT_seurat <- RunPCA(object = WT_seurat,
features = VariableFeatures(object = WT_seurat), verbose = FALSE)
ElbowPlot(object = WT_seurat, ndims=20)
WT_seurat<- FindNeighbors(object = WT_seurat, dims = 1:6)
WT_seurat <- FindClusters(object = WT_seurat, resolution = 1)
WT_seurat <- RunTSNE(object = WT_seurat, dims = 1:6)

saveRDS(WT_seurat,"/Users/rekhamurali/Desktop/lab/0327_WT_seurat_processed.rds")
```

view clusters

```
suppressPackageStartupMessages(library(Seurat))
WT_seurat <- readRDS("/Users/rekhamurali/Desktop/lab/0327_WT_seurat_processed.rds")
DimPlot(object = WT_seurat, reduction = 'tsne')
```



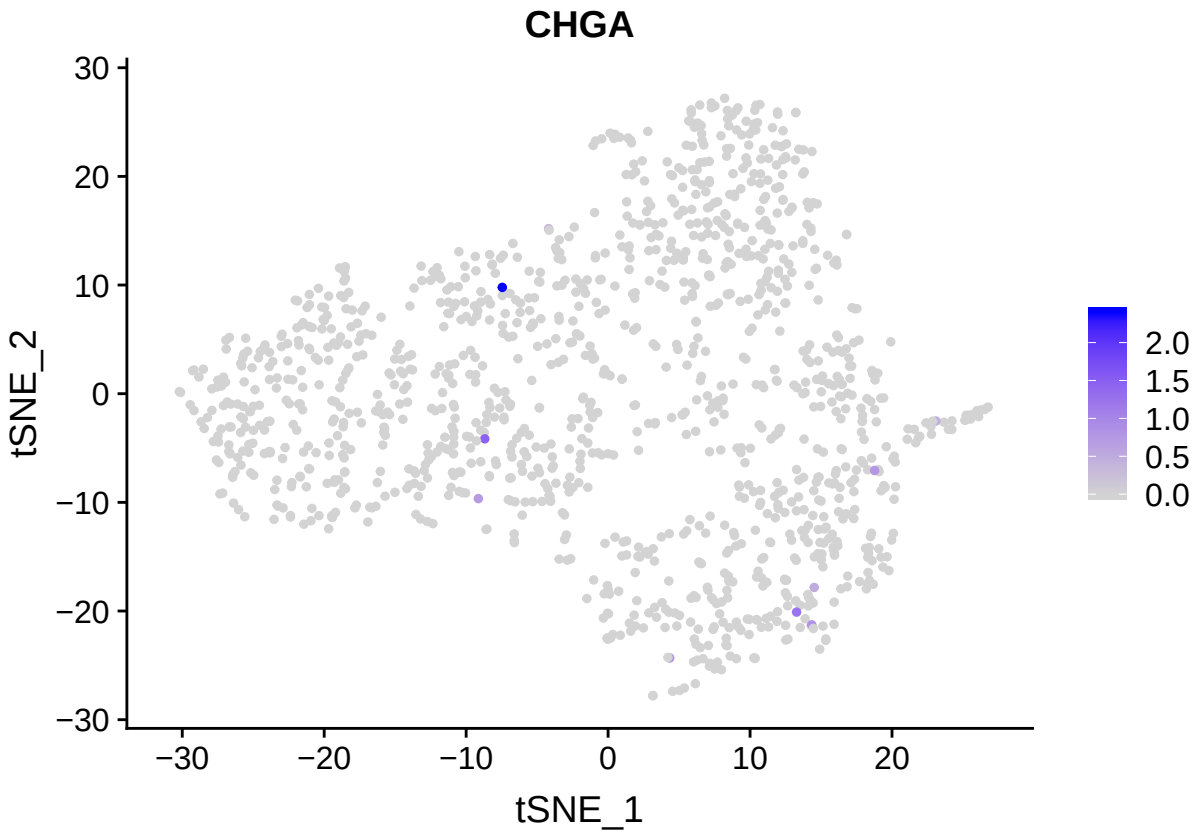
Explanation

I show 7 distinc clusters here but I also did not mess much with the parameters so this would need to be refined. Considering I might only be looking for one cell type this might not even make sense.

view expression of potential markers

validate first hypothesis

```
FeaturePlot(WT_seurat, reduction = "tsne", features = "CHGA")
```

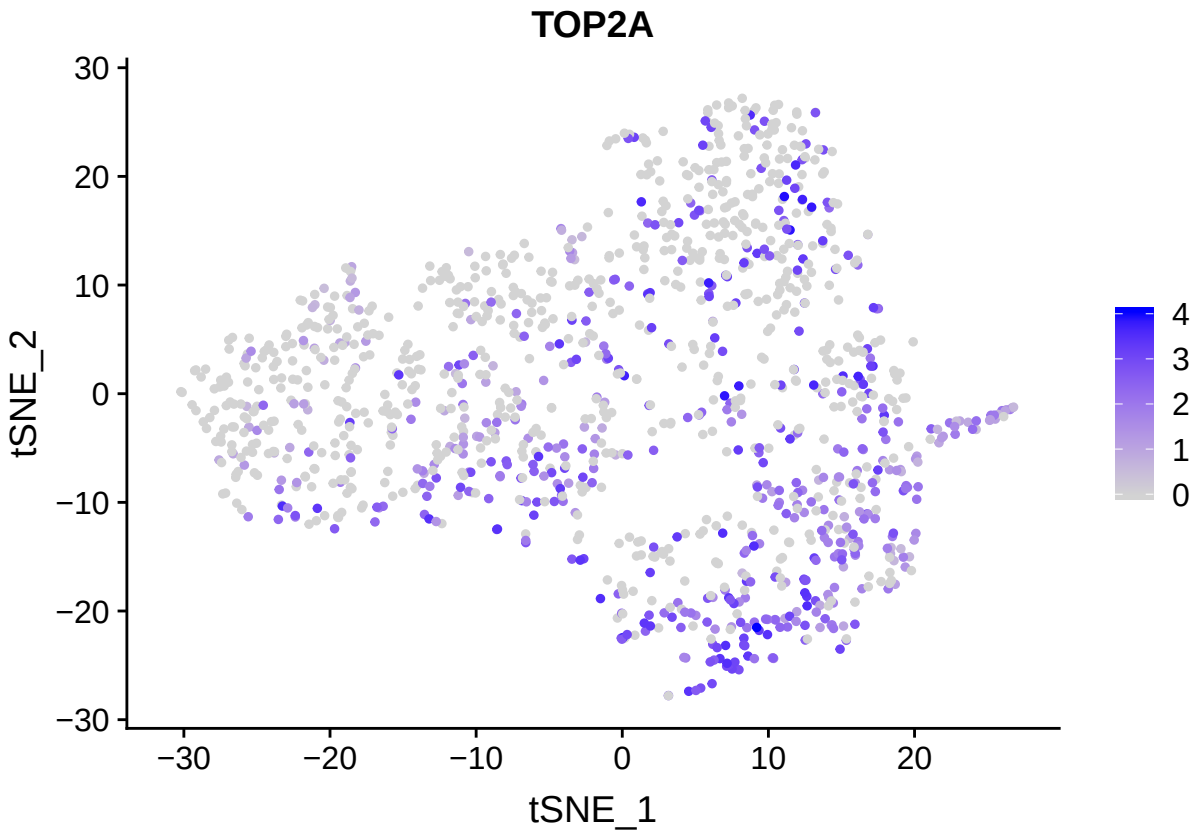


Explanation

This hypothesis was probably wrong, I should see CHGA in more cells if it were endocrine.

cell cycle gene

```
FeaturePlot(WT_seurat, reduction = "tsne", features = "TOP2A")
```



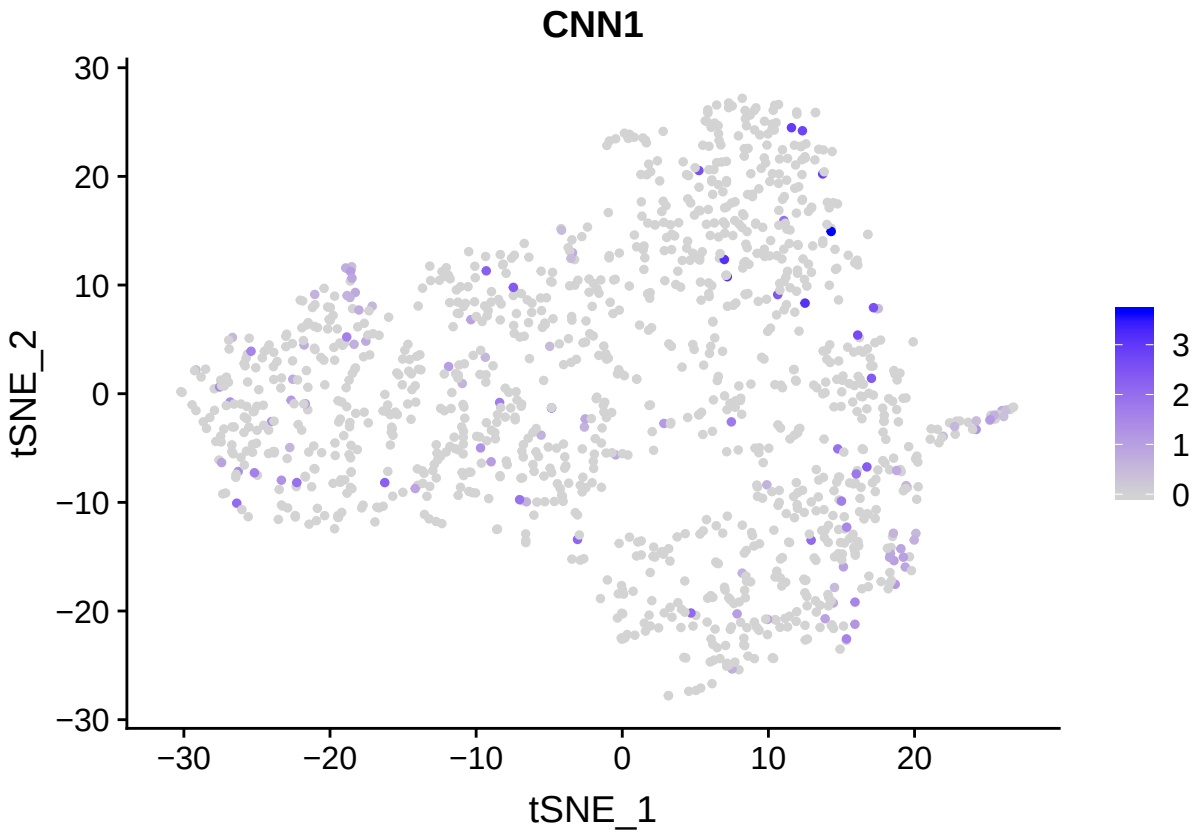
Explanation

Since it is a homoegenous population, I would expect the cells to cluster based on cell cycle. We do see pretty high expression of TOP2A so there are clearly a lof of clusters which are cycling.

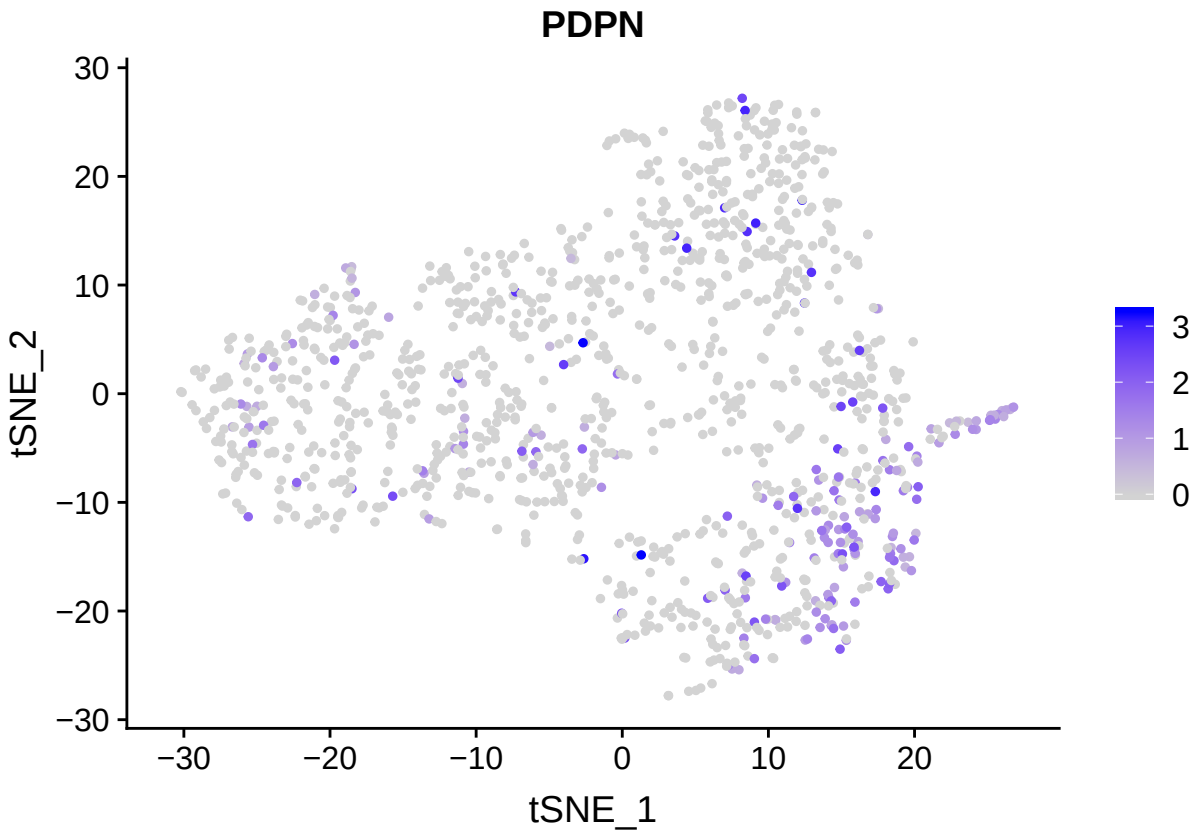
Test another hypothesis

These are some genes in a module defining salivary gland epithelial cells

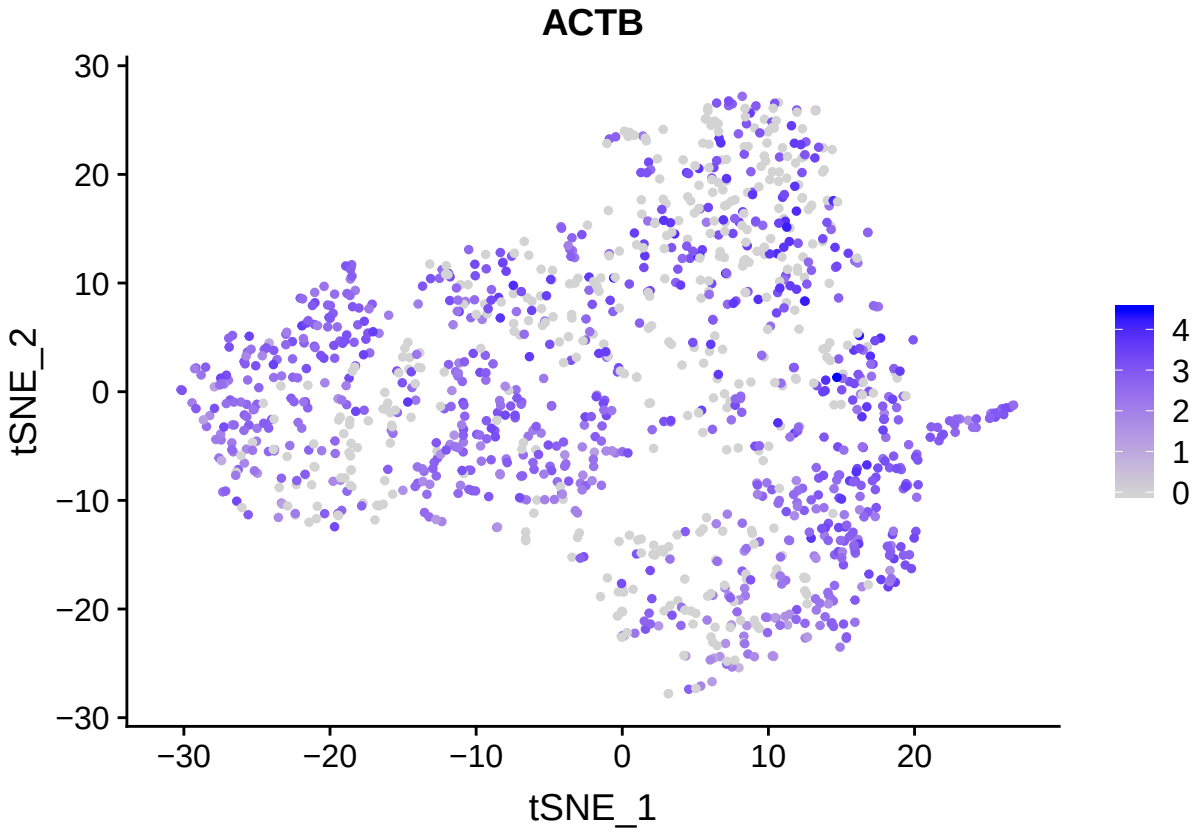
```
FeaturePlot(WT_seurat, reduction = "tsne", features = "CNN1")
```



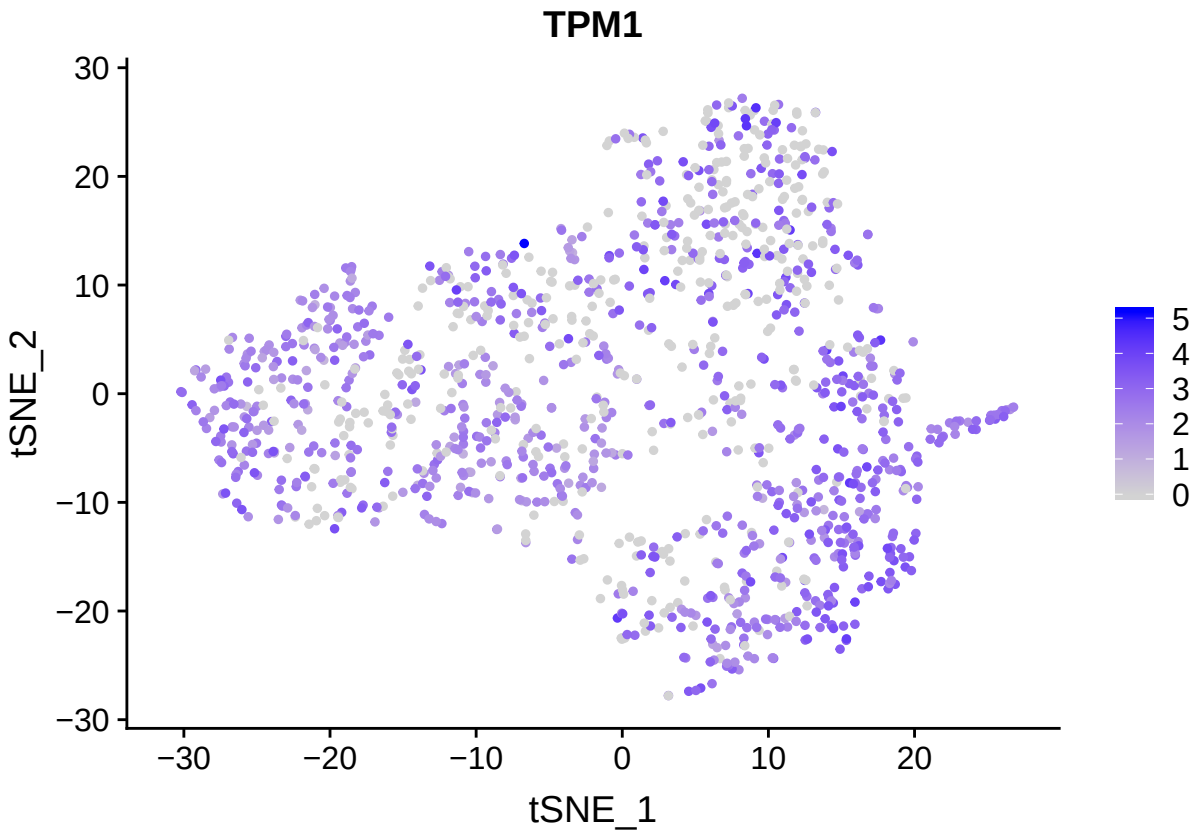

```
FeaturePlot(WT_seurat, reduction = "tsne", features = "PDPN")
```




```
FeaturePlot(WT_seurat, reduction = "tsne", features = "ACTB")
```




```
FeaturePlot(WT_seurat, reduction = "tsne", features = "TPM1")
```



explanation

Source:

http://biocc.hrbmu.edu.cn/CellMarker/search.jsp?species=Human&tissue=Salivary%20gland&cellname=Epithelial%20cell

You can see that I have expression of a lot of them, and while not all of them are tissue specific this could be a starting place. Some of the genes, such as CNN1 and TPM1 are expressed mostly in smooth muscle. TPM1 especially is highly expressed in muscle tissues. I don't have a concrete conclusion, but maybe this is a little more correct.