

# Liver E13.5 Clustering Report

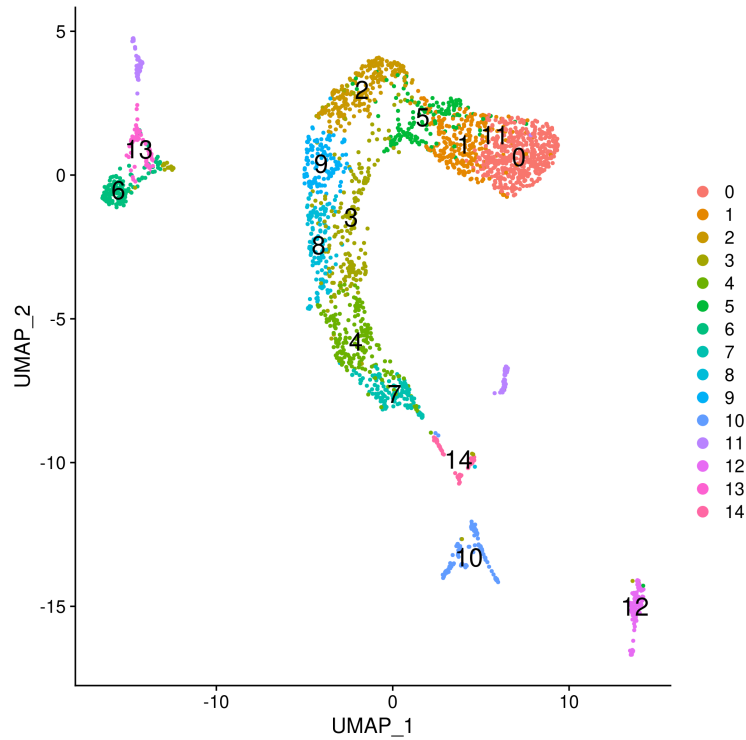
Xun Zhao

*Date: August 3, 2019*

## 1 Seurat Codes and Parameters

## 2 Clustering and Annotation

### 2.1 Clustering



**Figure 1:** Unlabeled Clusters

**Figure 1** is clustering result using 0.8 resolution. RDS file is available in `/p200/liujiang_group/yinyao/Dataset/Seurat/Liver.rds`. After extracting markers, some of these clusters can be annotated, but others need further clustering, like C11 (cluster 11, purple), which consists of two parts away from each other.

## 2.2 Annotation

*Afp*, *Alb* are reported as the markers of hepatocyte or hepatoblast(Gordillo et al., 2015; Chaudhari et al., 2016; Su et al., 2017; Han et al., 2018). Moreover, besides *Afp* and *Alb*, hepatocyte also expresses *Hnf4 $\alpha$*  and *Prox1*(Gordillo et al., 2015). Therefore, C6 and C13 are annotated as hepatocyte and hepatoblast, respectively, for their high expression of *Afp* and *Alb*, and for expression of *Hnf4 $\alpha$*  and *Prox1* in C13 (Figure 2).

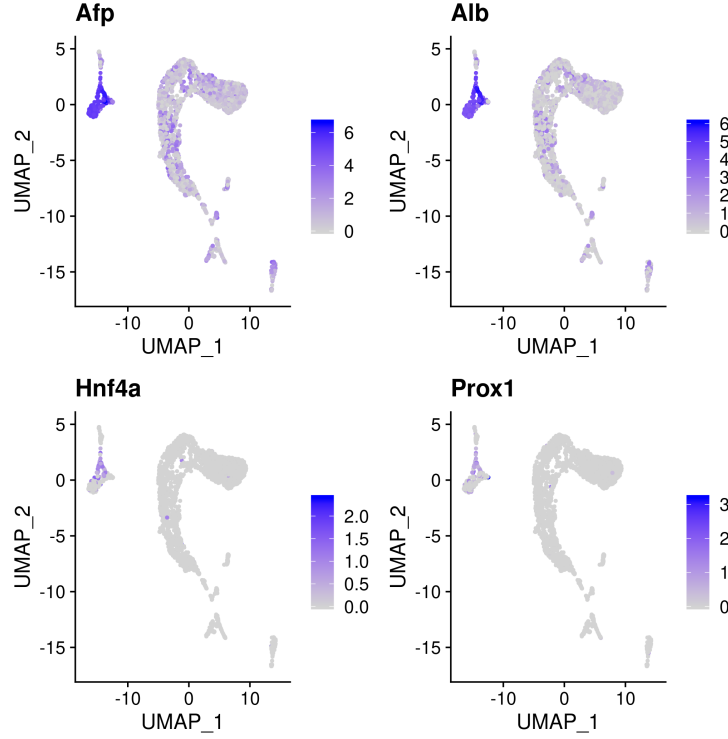


Figure 2: Markers of C6/C13

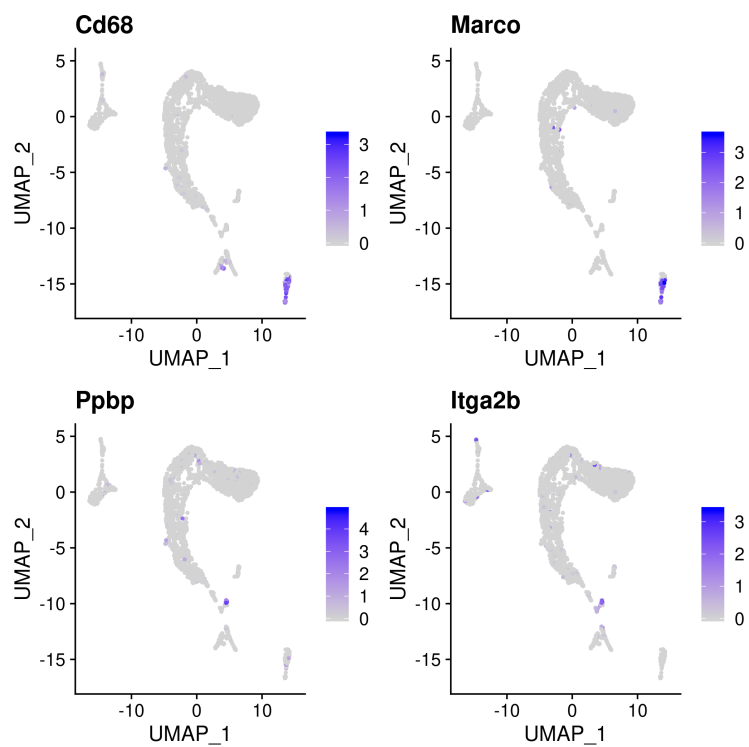
*Cd68*, *Marco* are reported as the markers of macrophage(Su et al., 2017; Han et al., 2018), so C12 is annotated as macrophage. *Ppbp*, *Itga2b* are reported as the markers of megakaryocyte(Su et al., 2017), so C14 is annotated as megakaryocyte (Figure 3).

In the main body of Figure 1 (C0-5, C7-9), *Hba-a2*, *Hba-a1*, *Hbb-bs*, *Hbb-bt*, the markers of erythroid(Han et al., 2018), express higher in C0, C1, C5 (Figure 4). *Klf1*, *Alad*, *Blvrb*, *Gata1*, the markers of erythroid progenitor(Han et al., 2018), express higher in C2, C7, C8, C9 (Figure 5). Both erythroid and erythroid progenitor markers are not significant in C3, so C3 is not annotated yet.

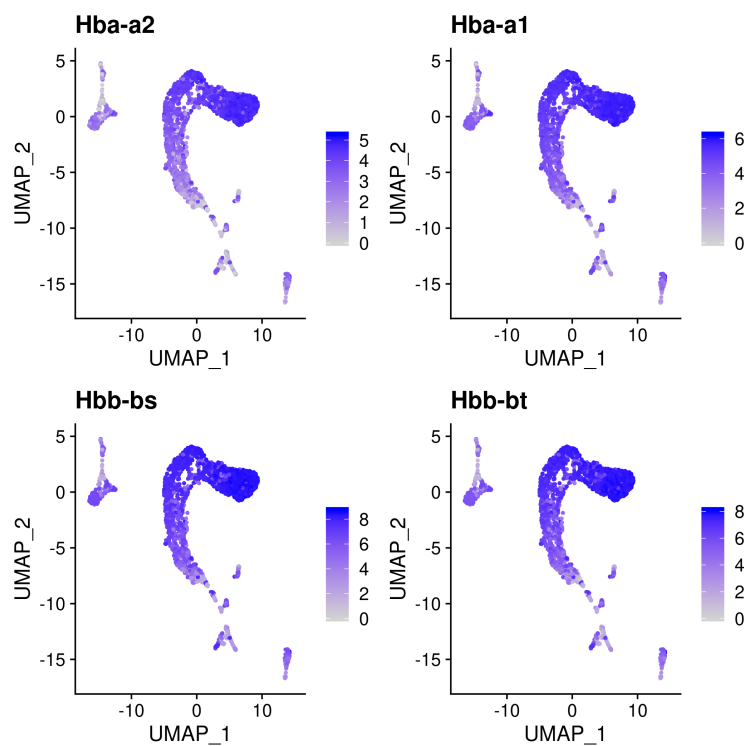
However, in C10, there are two kinds of cell markers, stem cells' and neutrophils'. Similarly, in C11, there are endothelial cells' and mesenchymal cells'. So they need further clustering.

## 2.3 Subclustering

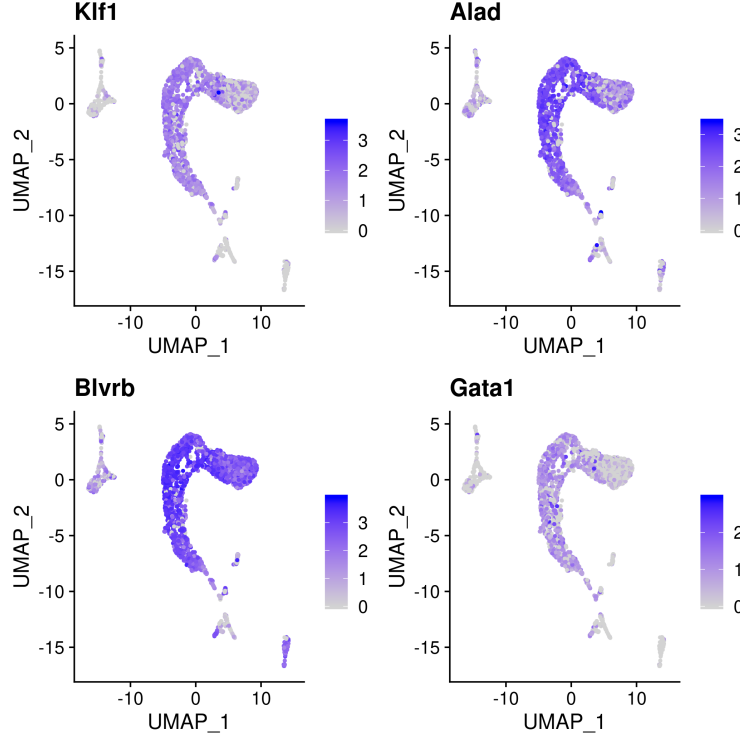
In order to simply divide one cluster into fewer parts, the resolution used in this part is 0.6.



**Figure 3:** Markers of C12/C14



**Figure 4:** Markers of Erythroid



**Figure 5:** Markers of Erythroid Progenitor

**Cluster 10** C10 is divided into 3 parts (**Figure 6**), saved as `/p200/liujiang_group/yinyao/Dataset/Seurat/liver10.rds`. Stem cell marker *Cd34*, *Cmtm7* (Han et al., 2018) are highly expressed in subcluster 0 of C10. Neutrophil marker *S100a9*, *S100a8* (Han et al., 2018) are highly expressed in subcluster 2 of C10 (**Figure 7**, **Figure 8**). The annotation of subcluster 1 is not defined yet.

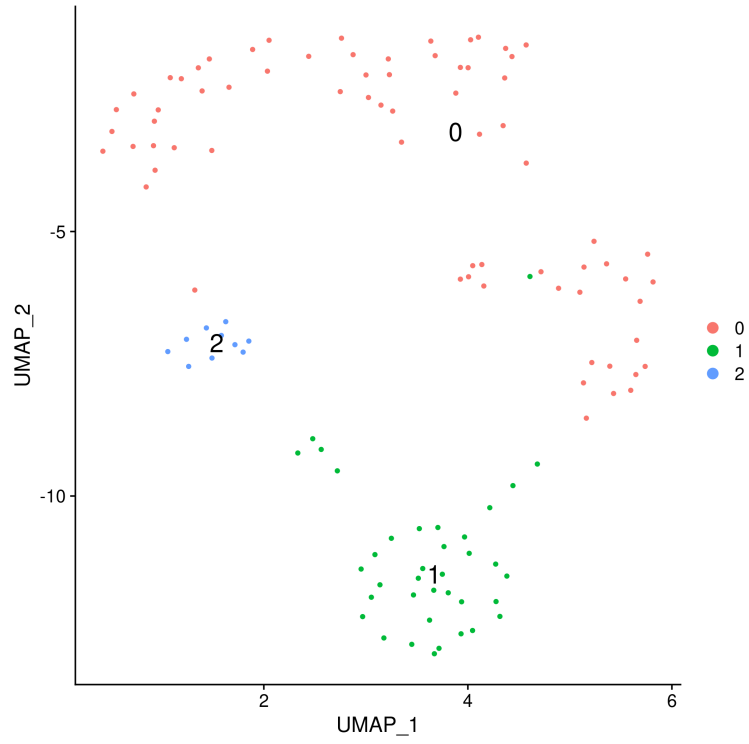
**Cluster 11** C11 is divided into 2 parts as expected (**Figure 9**), one of which is annotated as endothelial cell and the other is annotated as mesenchymal cell, due to the endothelial marker *Lyve1*, *Kdr* (Gordillo et al., 2015) in subcluster 0, and the mesenchymal marker *Pdgfra*, *Col1a2* (Han et al., 2018) in subcluster 1 (**Figure 10**, **Figure 11**). The `.rds` file is saved at `/p200/liujiang_group/yinyao/Dataset/Seurat/liver11.rds`

## 2.4 Final Annotation

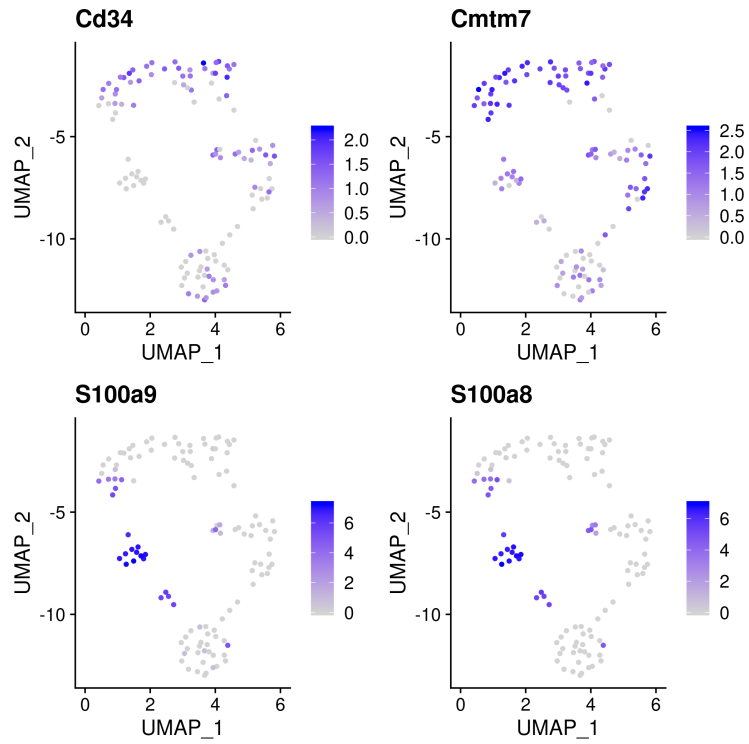
Except cell type of C3 and subcluster 1 of C10 is not defined yet, their labels maintain 3 and 10 in **Figure 12**. And the final `.rds` file is available at `/p200/liujiang_group/yinyao/Dataset/Seurat/liver_labeled.rds`.

## References

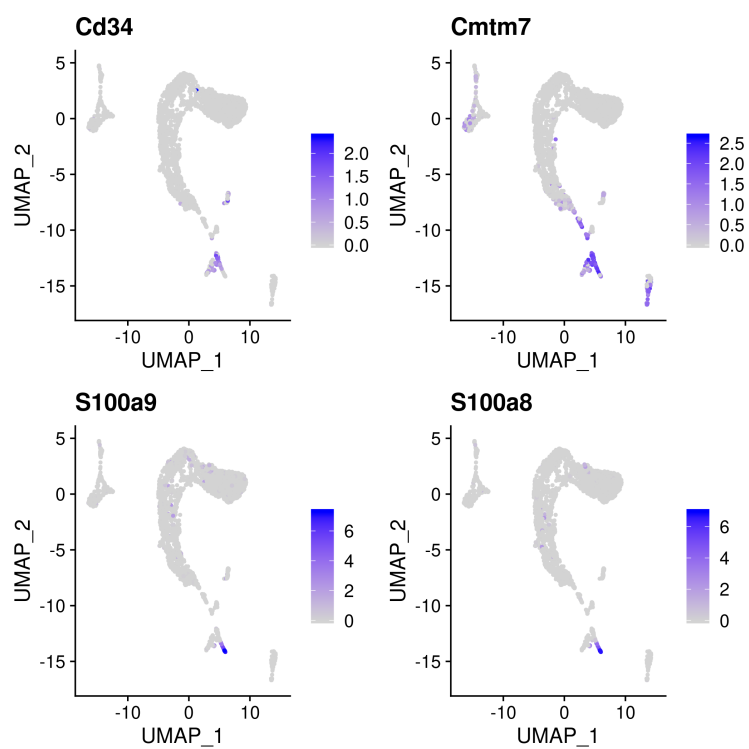
- Chaudhari, P., Tian, L., Deshmukh, A., and Jang, Y.-Y. (2016). Expression kinetics of hepatic progenitor markers in cellular models of human liver development recapitulating hepatocyte and biliary cell fate commitment. *Experimental Biology and Medicine*, 241(15):1653–1662.
- Gordillo, M., Evans, T., and Gouon-Evans, V. (2015). Orchestrating liver development. *Development*, 142(12):2094–2108.



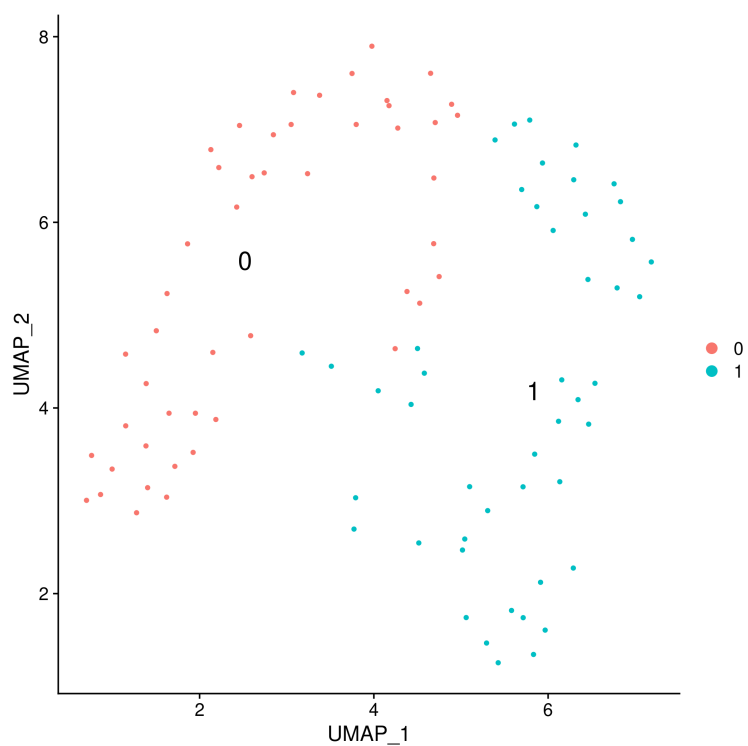
**Figure 6:** Subclusters of C10



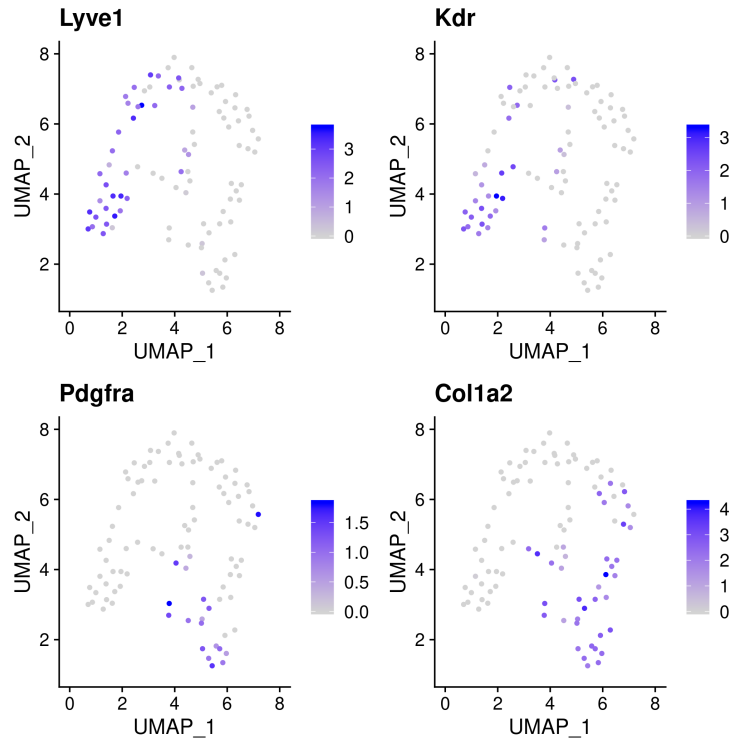
**Figure 7:** Stem and Neutrophil Markers in C10



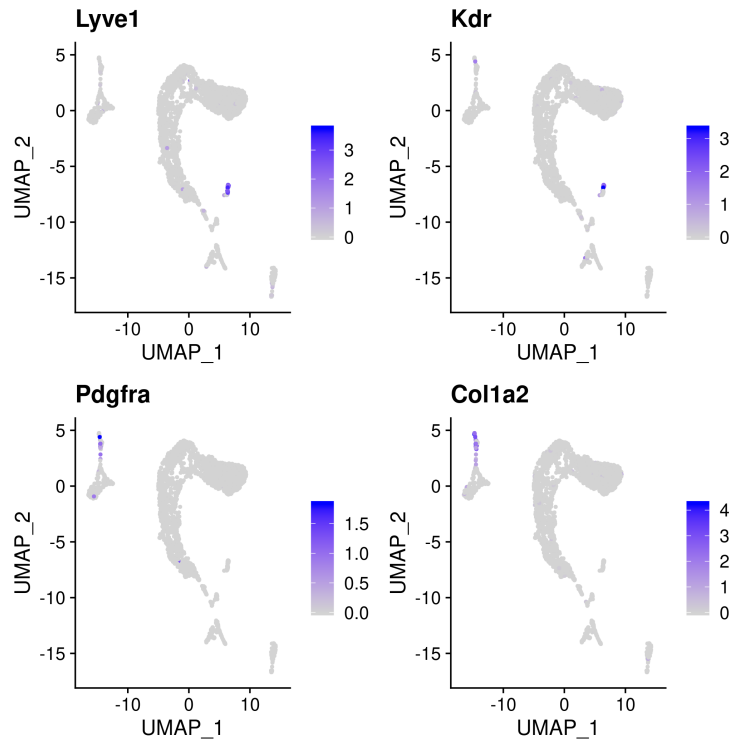
**Figure 8:** Stem and Neutrophil Markers in Whole Data



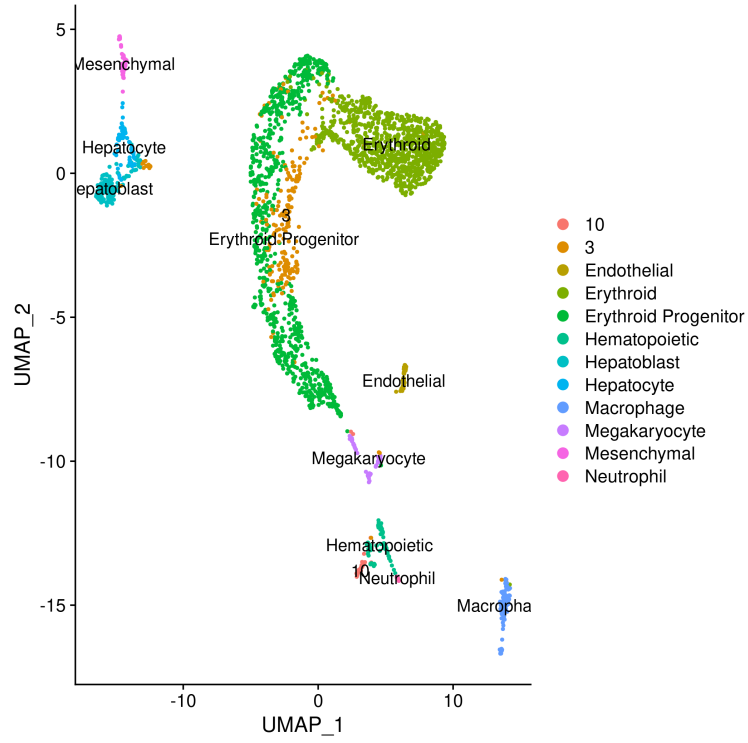
**Figure 9:** Subclusters of C11



**Figure 10:** Endothelial and Mesenchymal Markers in C11



**Figure 11:** Endothelial and Mesenchymal Markers in Whole Data



**Figure 12:** Final Annotation

Han, X., Wang, R., Zhou, Y., Fei, L., Sun, H., Lai, S., Saadatpour, A., Zhou, Z., Chen, H., Ye, F., Huang, D., Xu, Y., Huang, W., Jiang, M., Jiang, X., Mao, J., Chen, Y., Lu, C., Xie, J., Fang, Q., Wang, Y., Yue, R., Li, T., Huang, H., Orkin, S. H., Yuan, G.-C., Chen, M., and Guo, G. (2018). Mapping the Mouse Cell Atlas by Microwell-Seq. *Cell*, 172(5):1091–1107.e17.

Su, X., Shi, Y., Zou, X., Lu, Z.-N., Xie, G., Yang, J. Y. H., Wu, C.-C., Cui, X.-F., He, K.-Y., Luo, Q., Qu, Y.-L., Wang, N., Wang, L., and Han, Z.-G. (2017). Single-cell RNA-Seq analysis reveals dynamic trajectories during mouse liver development. *BMC Genomics*, 18(1):1–14.