

Liver E13.5 Clustering Report

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1 Seurat Codes and Parameters

2 Clustering and Annotation

2.1 Clustering

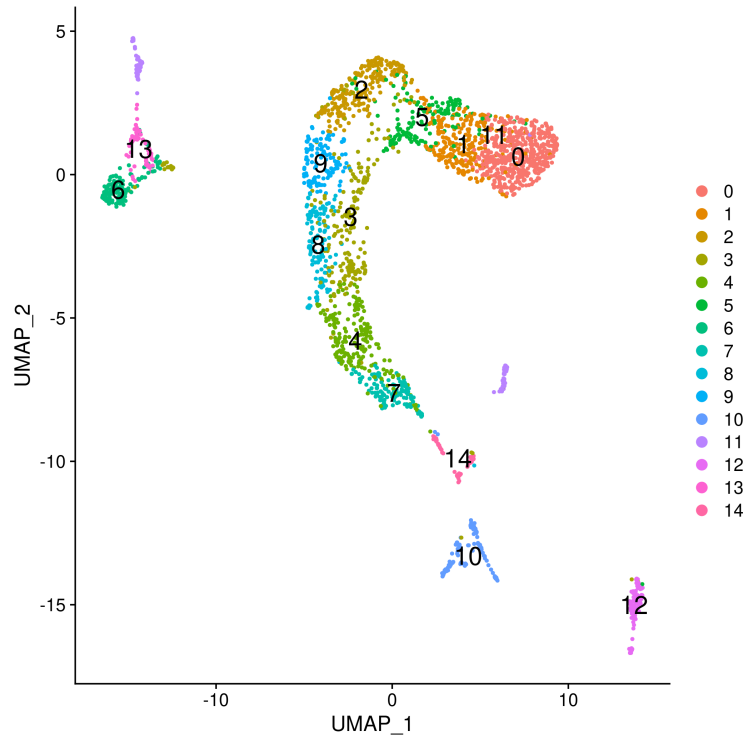


Figure 1: Unlabeled Clusters

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 α* 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 α* and *Prox1* in C13 (**Figure 2**).

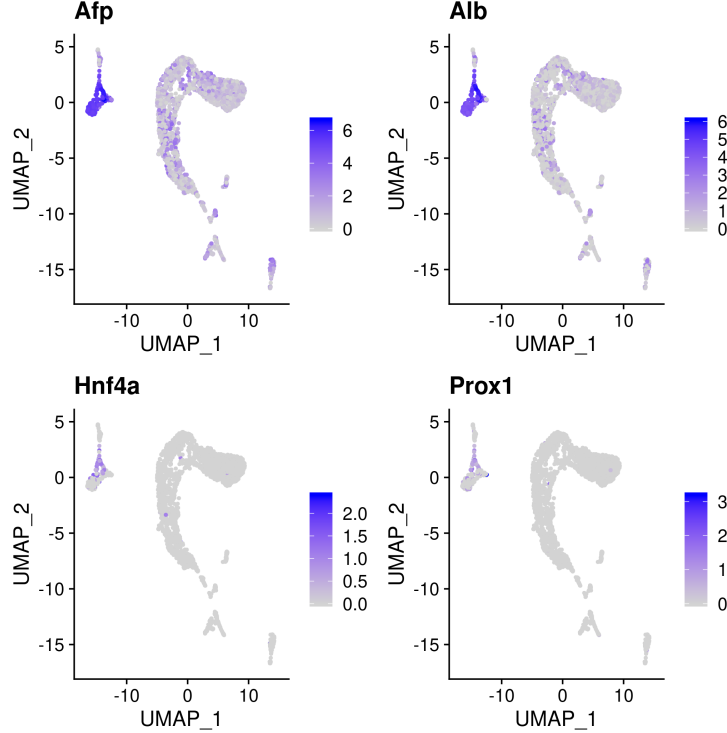


Figure 2: Markers of C6/C13

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.

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.

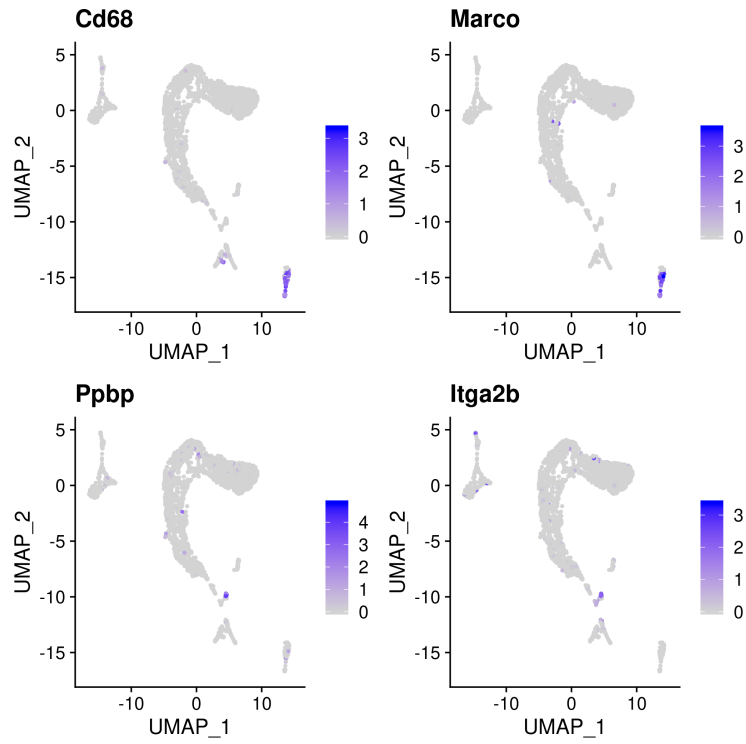


Figure 3: Markers of C12/C14

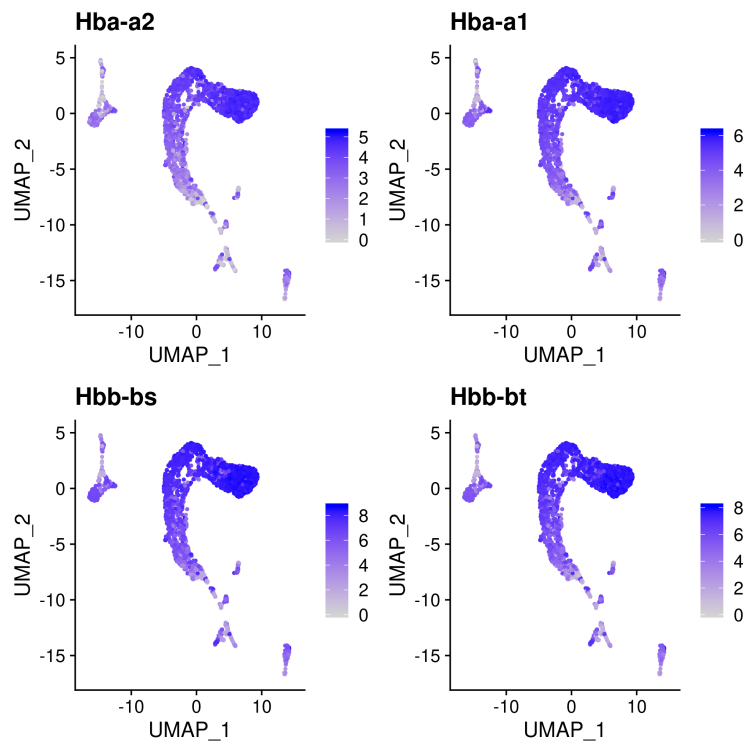


Figure 4: Markers of Erythroid

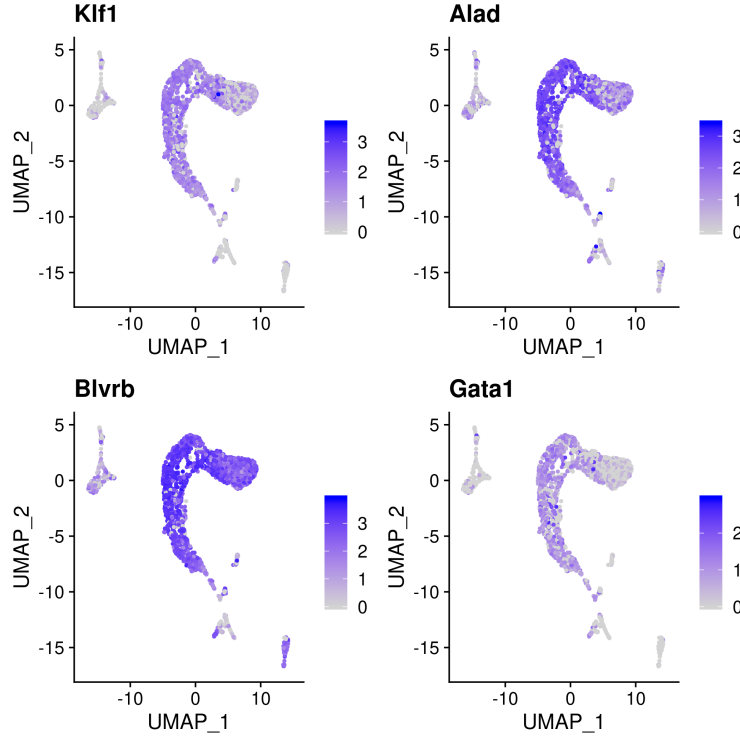


Figure 5: Markers of Erythroid Progenitor

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.
- 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.

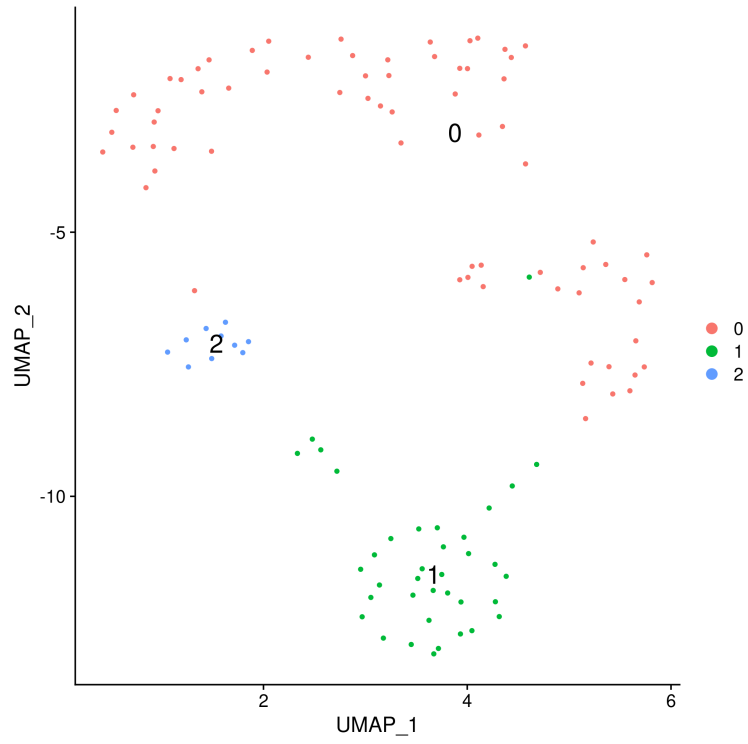


Figure 6: Subclusters of C10

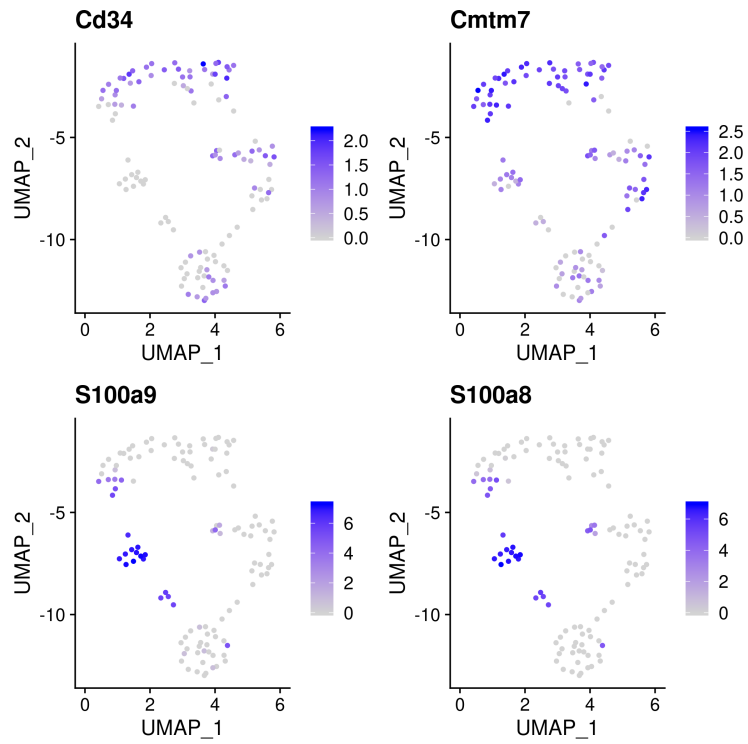


Figure 7: Stem and Neutrophil Markers in C10

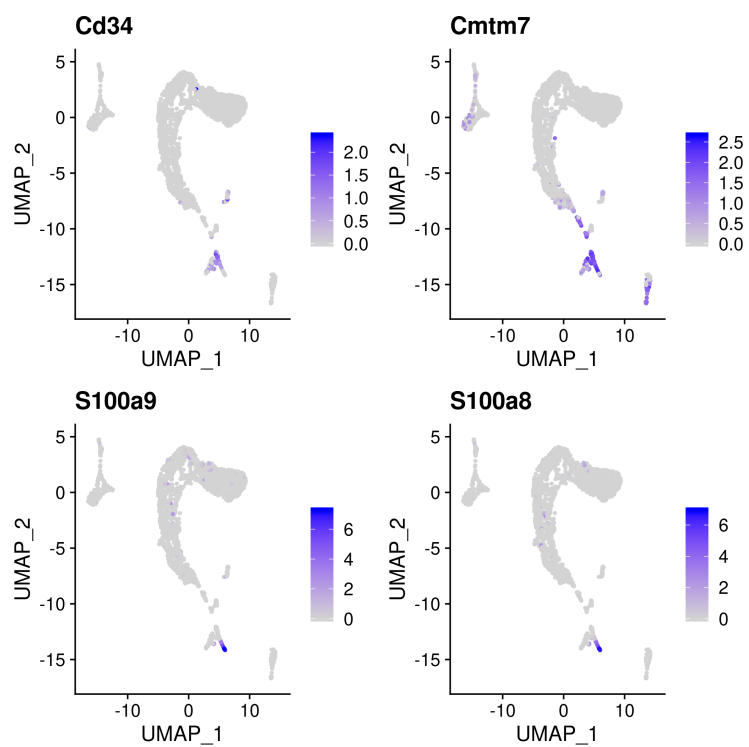


Figure 8: Stem and Neutrophil Markers in Whole Data

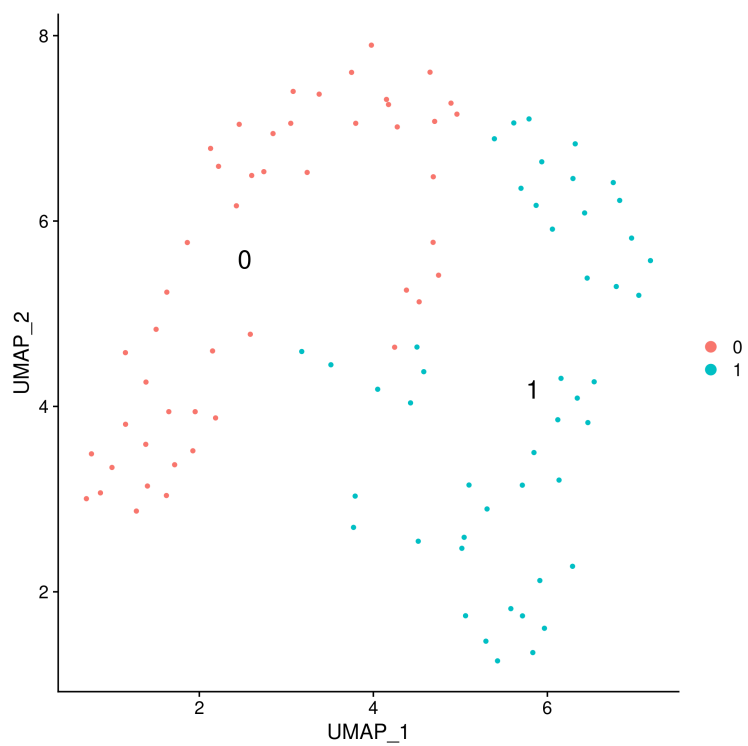


Figure 9: Subclusters of C11

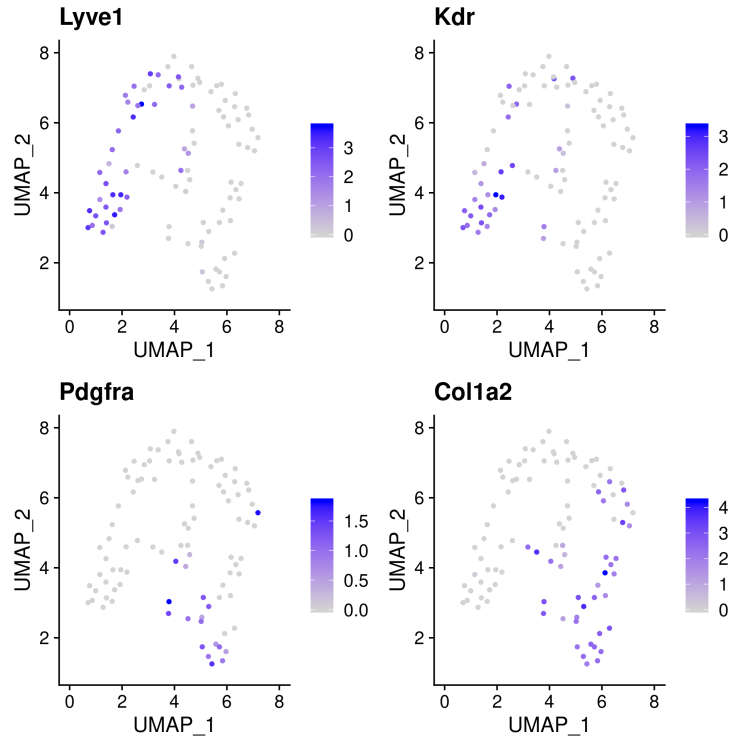


Figure 10: Endothelial and Mesenchymal Markers in C11

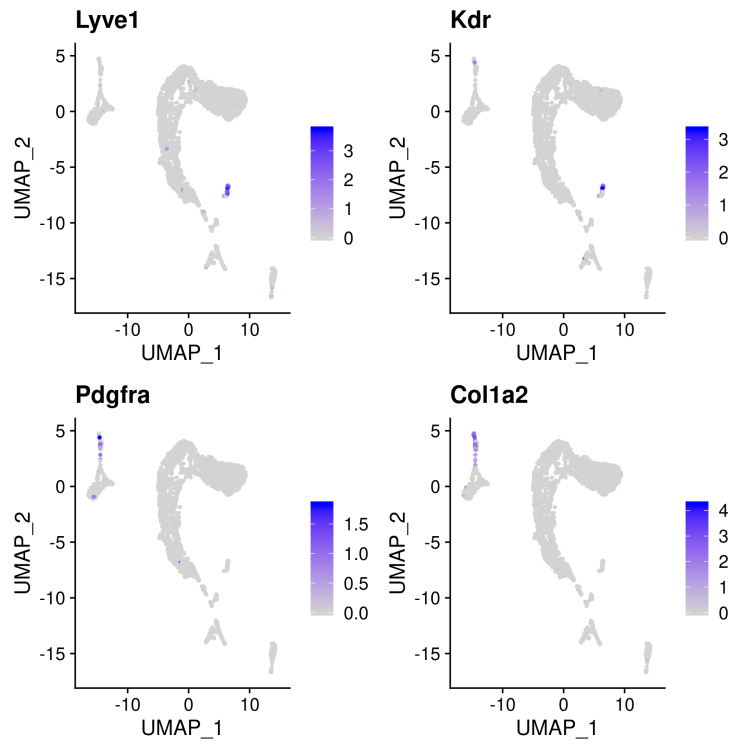


Figure 11: Endothelial and Mesenchymal Markers in Whole Data

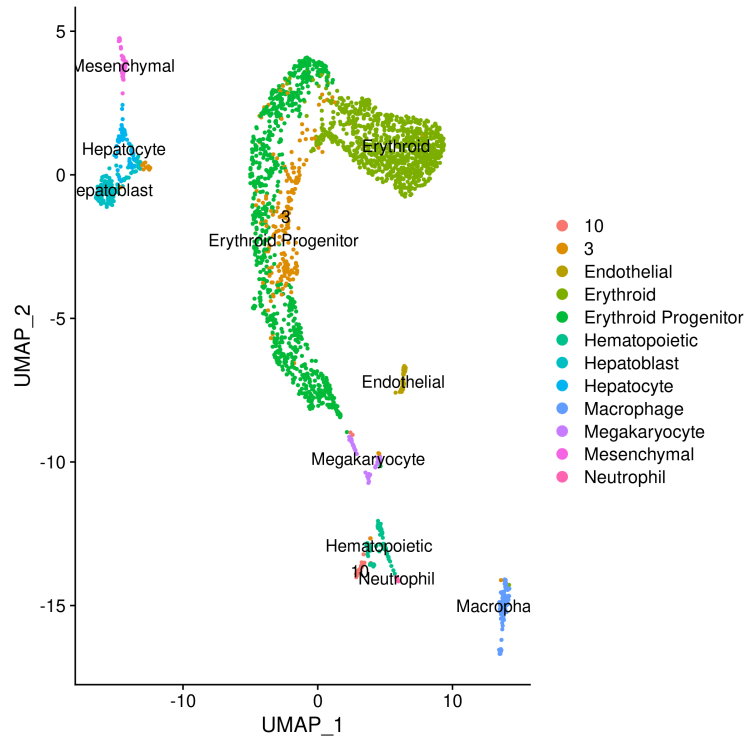


Figure 12: Final Annotation

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.