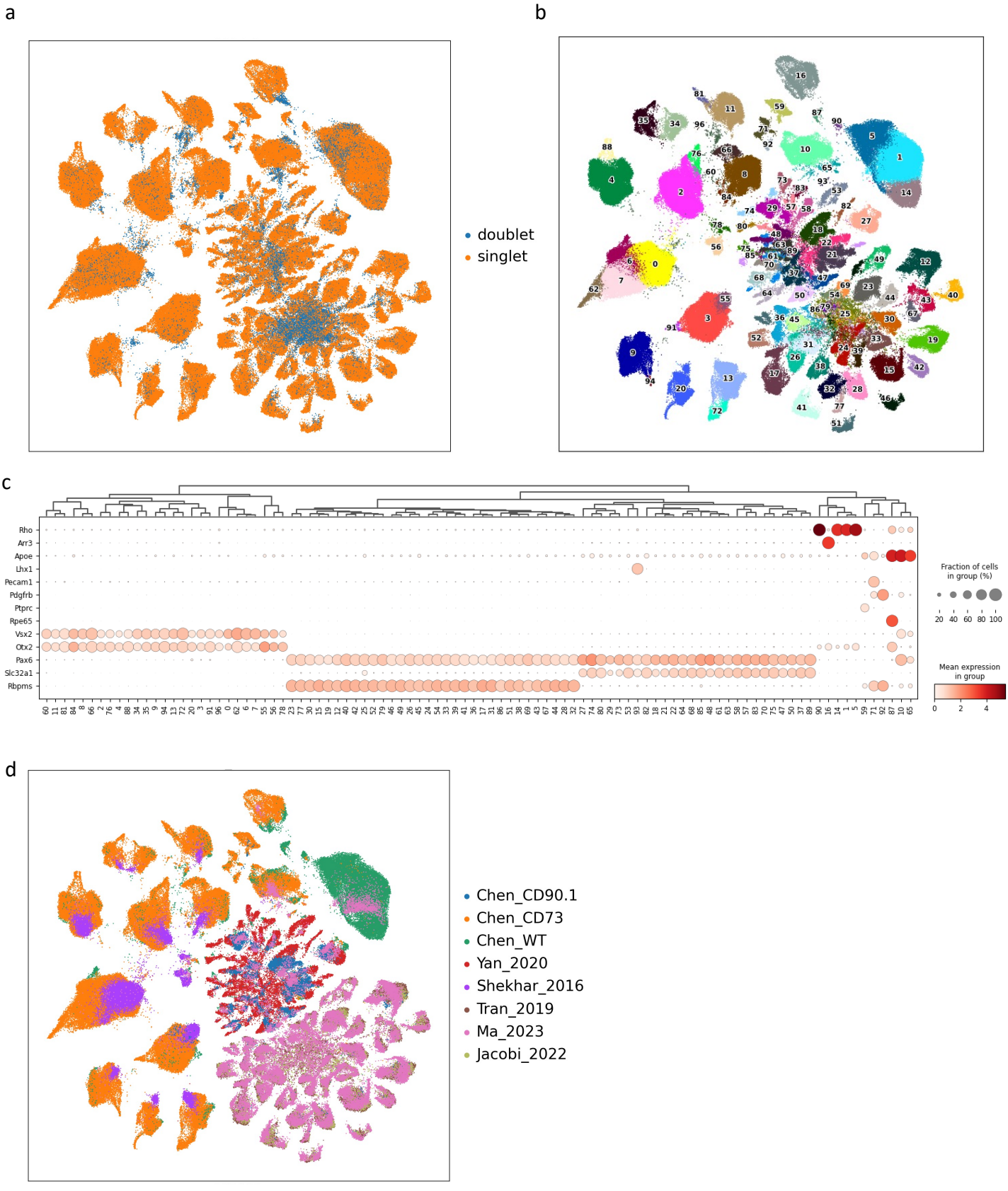
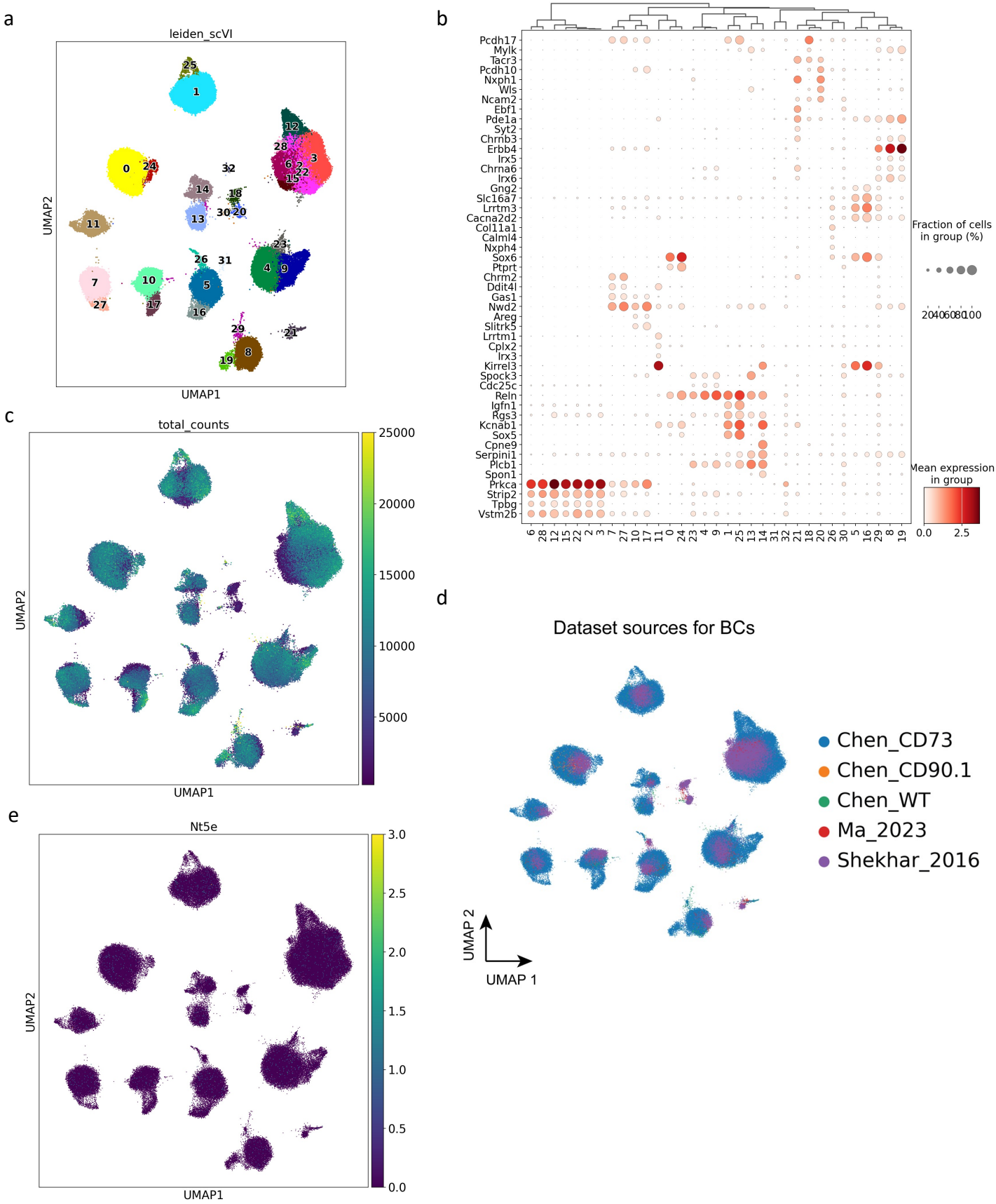


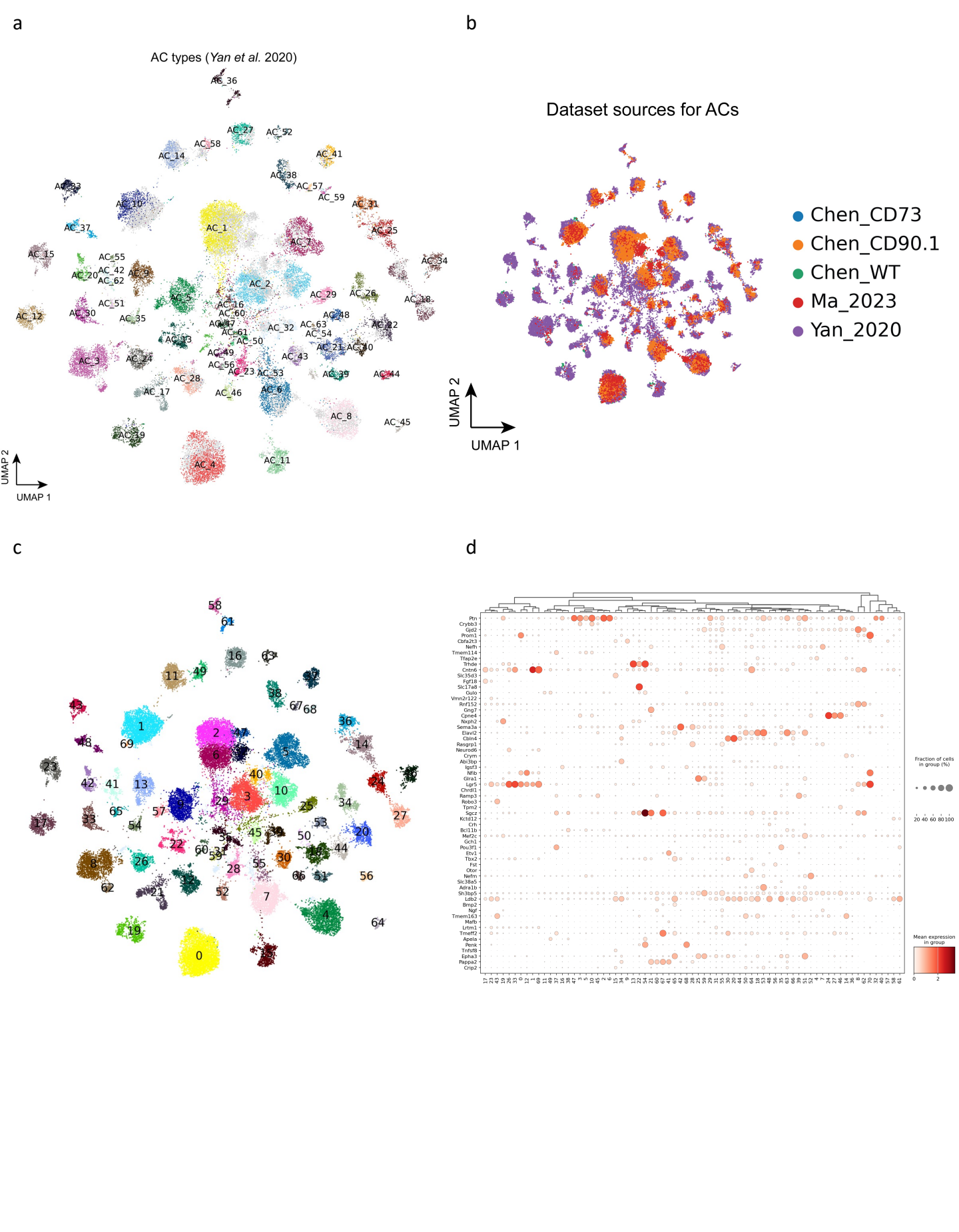
Supplementary Figure 1. Cell type annotation of major classes

(a) UMAP plot of the integrated data colored by doublet status using SOLO algorithm. Doublets are labeled in blue, while singlets are labeled in orange. (**b)** UMAP plot of the integrated data colored by each Leiden cluster. The Leiden cluster ID is placed on top of each cell cluster. **(c)** Dot plot of expression patterns of canonical markers of major classes in each Leiden cluster. Marker gene expression allows the annotation of 11 major classes. **(d)** UMAP plot of the integrated data colored by data sources. An even distribution of cells across clusters is shown by the expected data source.



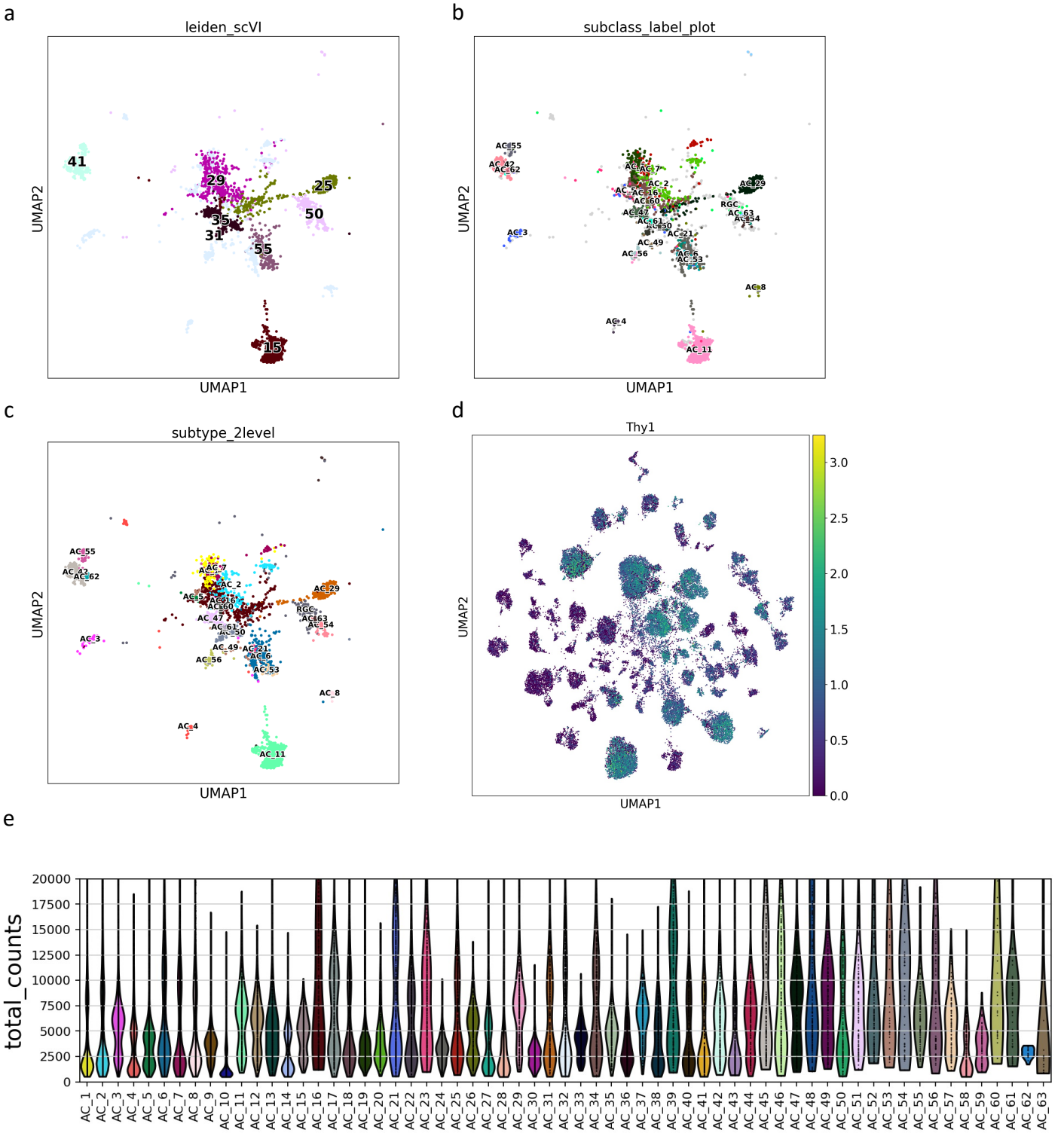
Supplementary Figure 2. Cell type annotation of bipolar cells

(a) UMAP visualization of BCs colored by cell clusters. **(b)** Dot plot of BC type marker gene expression in each cluster. **(c)** UMAP plot of BCs colored by the total UMI counts. **(d)** UMAP plot of BCs colored by data sources. **(e)** UMAP plot of *Nt5e* (CD73) expression in BCs.



Supplementary Figure 3. Cell type annotation of amacrine cells

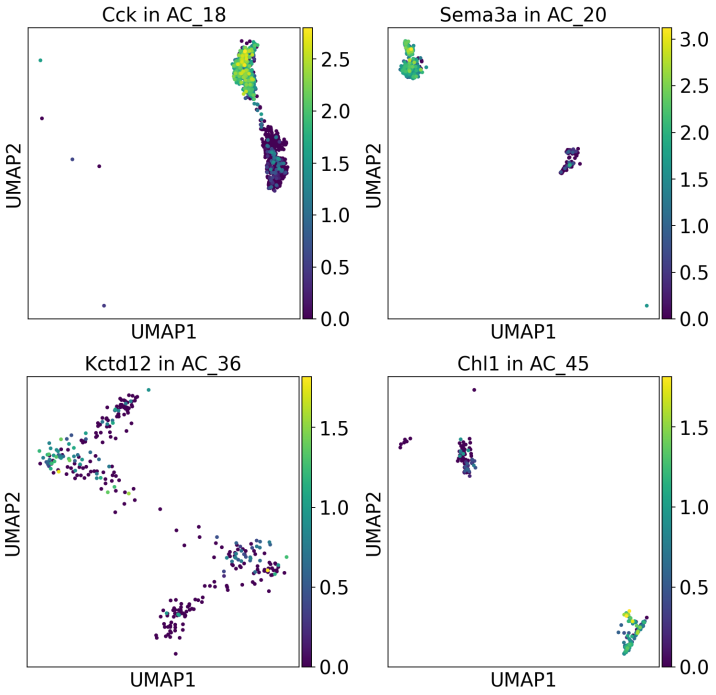
(a) UMAP visualization of ACs colored by public cell type labels from *Yan et al.* 2020. The newly discovered cells without public labeling are colored in gray. **(b)** UMAP plot of ACs colored by data sources. **(c)** UMAP visualization of ACs colored by 71 cell clusters. **(d)** Dot plot of AC type marker gene expression in 71 clusters.



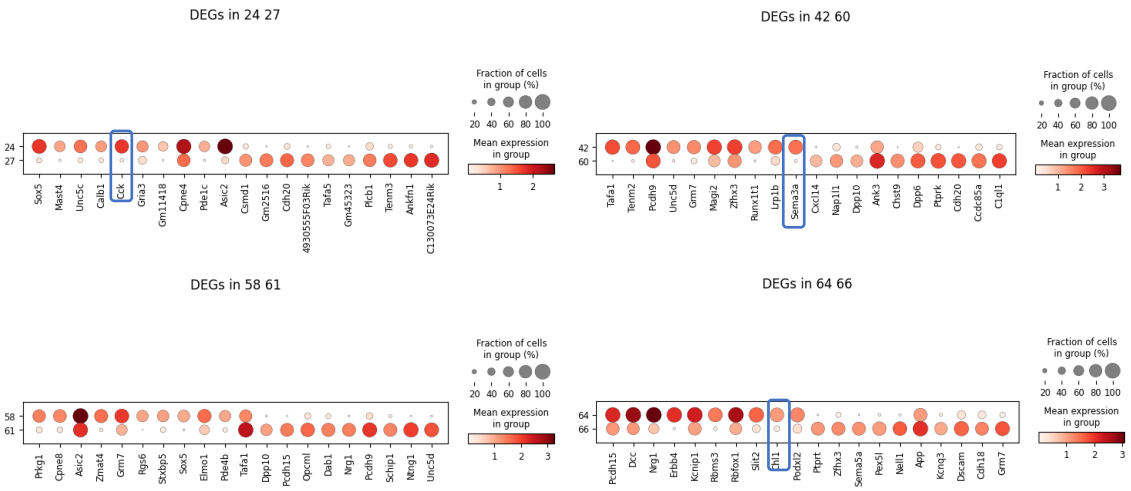
Supplementary Figure 4. Annotation of over-clustered amacrine cells

(a) UMAP visualization of the 8 cell clusters in ACs that contain more than one type, based on *Yan et al.* 2020. The 8 clusters are C15, C25, C29, C31, C35, C41, C50, C55. **(b)** UMAP visualization of the 8 clusters colored by public cell type labels from *Yan et al.* 2020. **(c)** UMAP visualization of the 8 clusters colored by AC types using the two-level annotation approach in this study. **(d)** UMAP plot of *Thy1* (CD90) expression in ACs. **(e)** Violin plot showing the UMI counts of the annotated AC types.

a

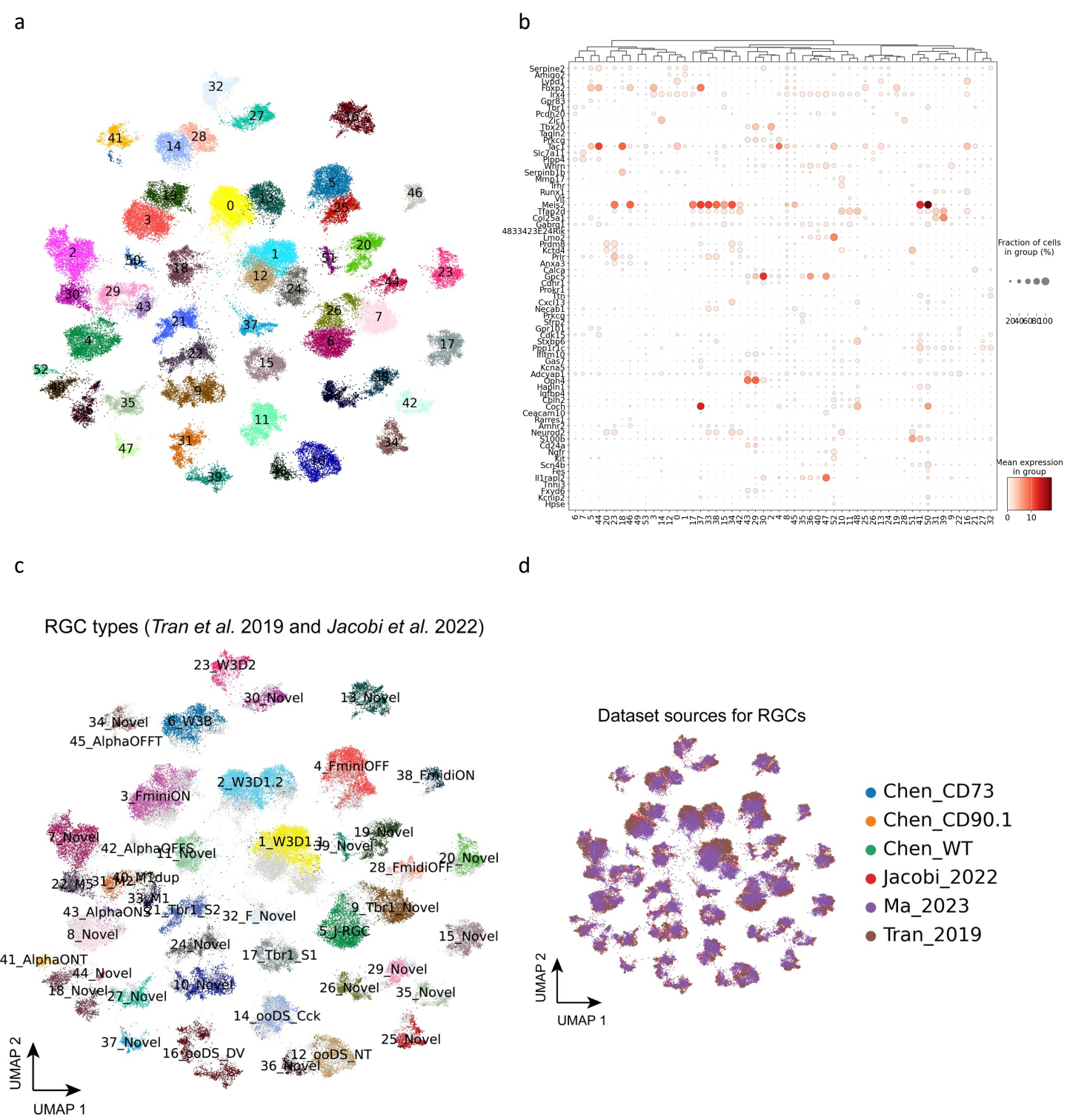


b



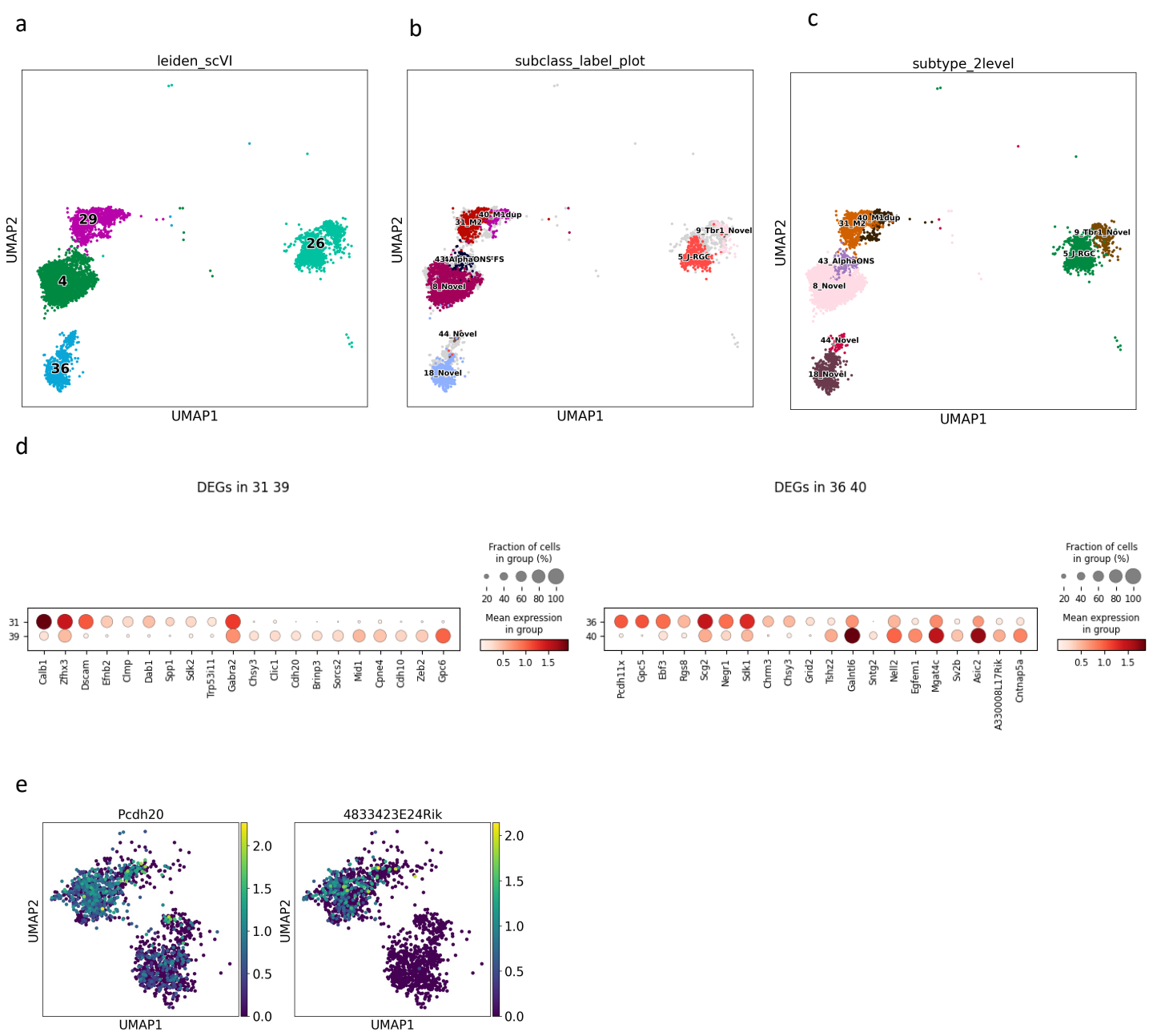
Supplementary Figure 5. Annotation of under-clustered amacrine cells

(a) Feature plot showing the marker gene expression of previously under-clustered AC types. **(b)** Dot plot showing the top 10 differentially expressed genes between split clusters for previously under-clustered AC types.

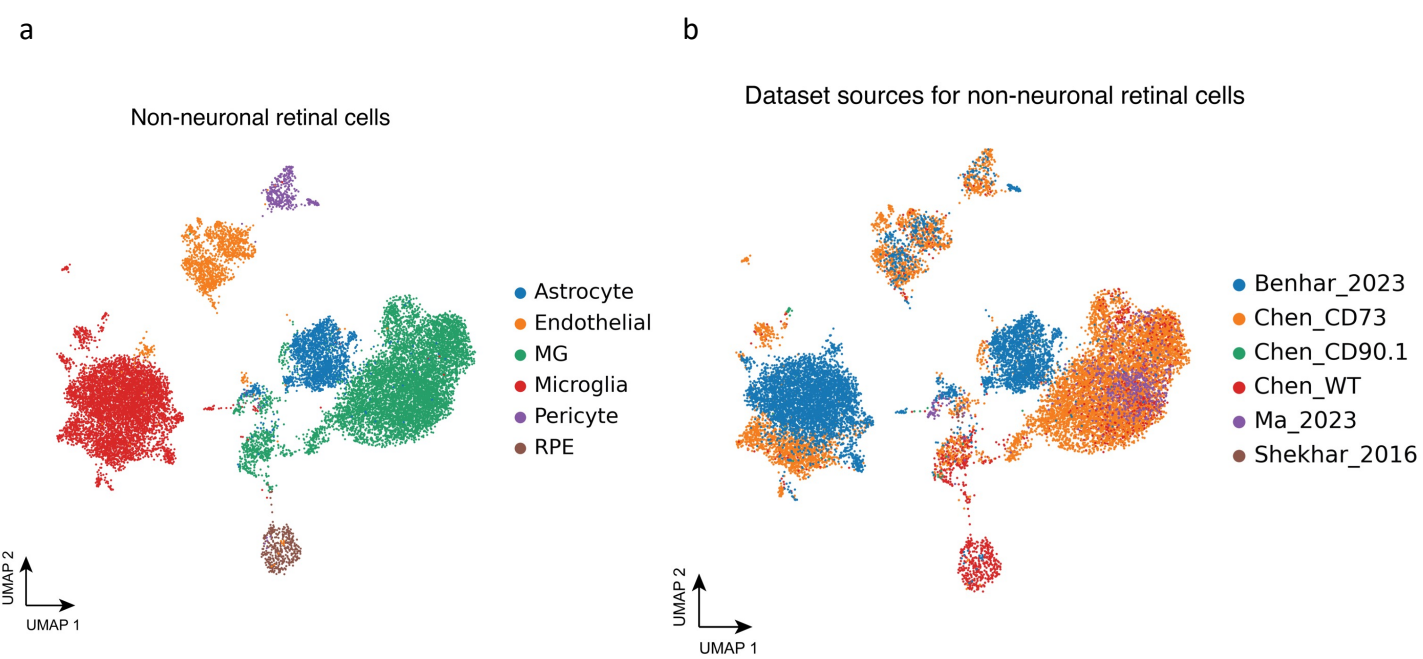


Supplementary Figure 6. Cell type annotation of retinal ganglion cells

(a) UMAP visualization of RGCs colored by cluster numbers. **(b)** Dot plot of RGC type marker gene expression in each cluster. **(c)** UMAP visualization of RGCs colored by public cell type labels from *Tran et al.* 2019 and *Jacobi et al.* 2022. The newly discovered cells without public labeling are colored in gray. **(d)** UMAP plot of RGCs colored by data sources.



Supplementary Figure 7. Annotation of over- and under-clustered retinal ganglion cells
(a) UMAP plot of the 4 clusters in RGCs that contain more than one type, based on *Tran et al.* 2019 and *Jacobi et al.* 2022. **(b)** UMAP plot of the 4 clusters colored by public cell type labels from *Tran et al.* 2019 and *Jacobi et al.* 2022. **(c)** UMAP visualization of the 4 clusters colored by RGC types using the two-level annotation approach in this study. **(d)** Dot plot showing the top 10 differentially expressed genes between split clusters for previously under-clustered RGC types. **(e)** Feature plot showing the marker gene expression of 18_Novel type. Both markers are enriched in one cluster, but not the other.



Supplementary Figure 8. Non-neuronal retinal cells
(a) UMAP visualization of non-neuronal retinal cells colored by major classes. **(b)** UMAP plot of non-neuronal retinal cells colored by dataset sources.