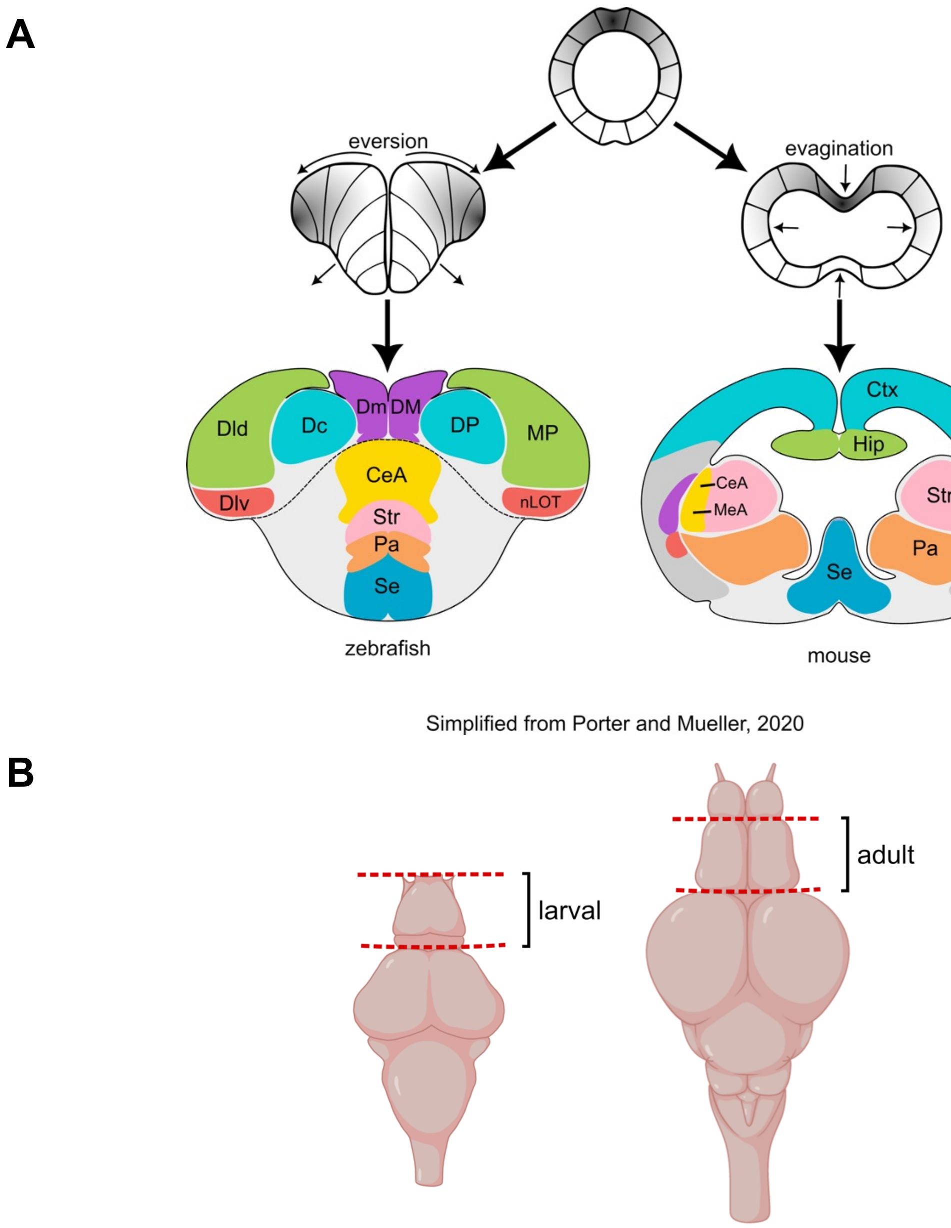
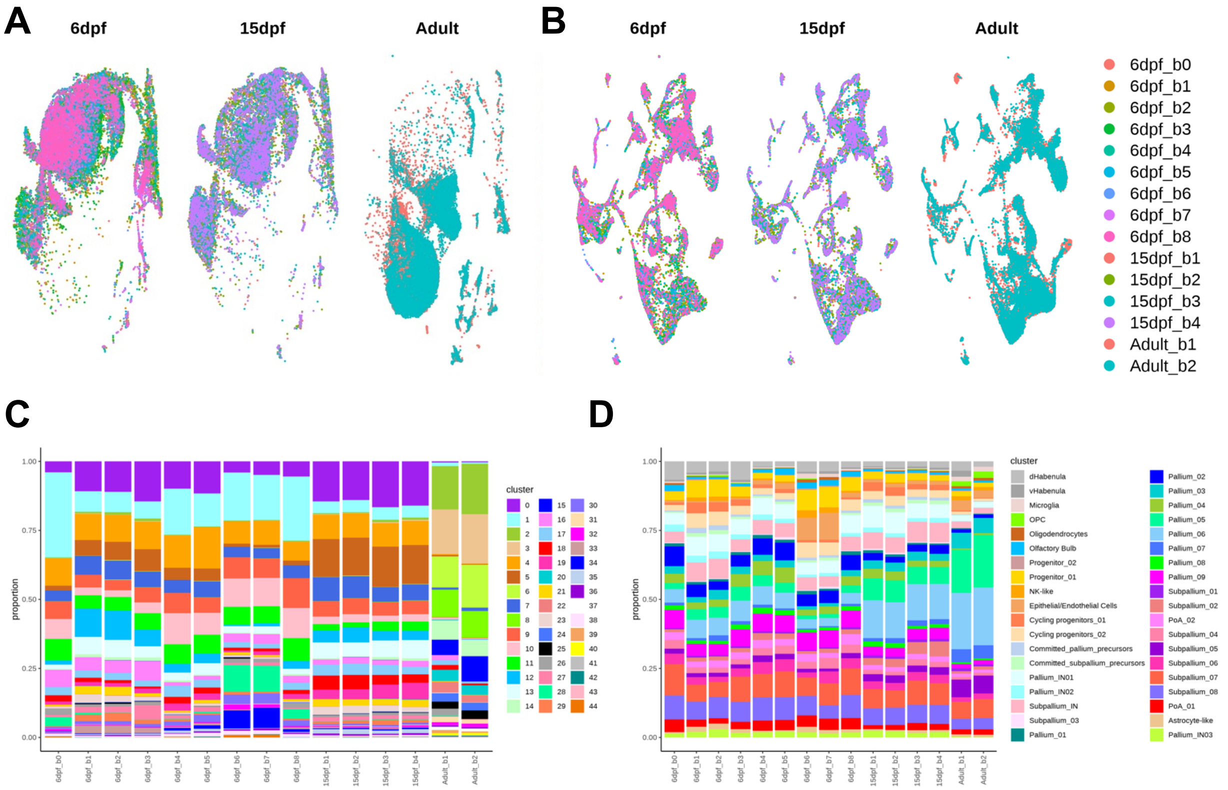
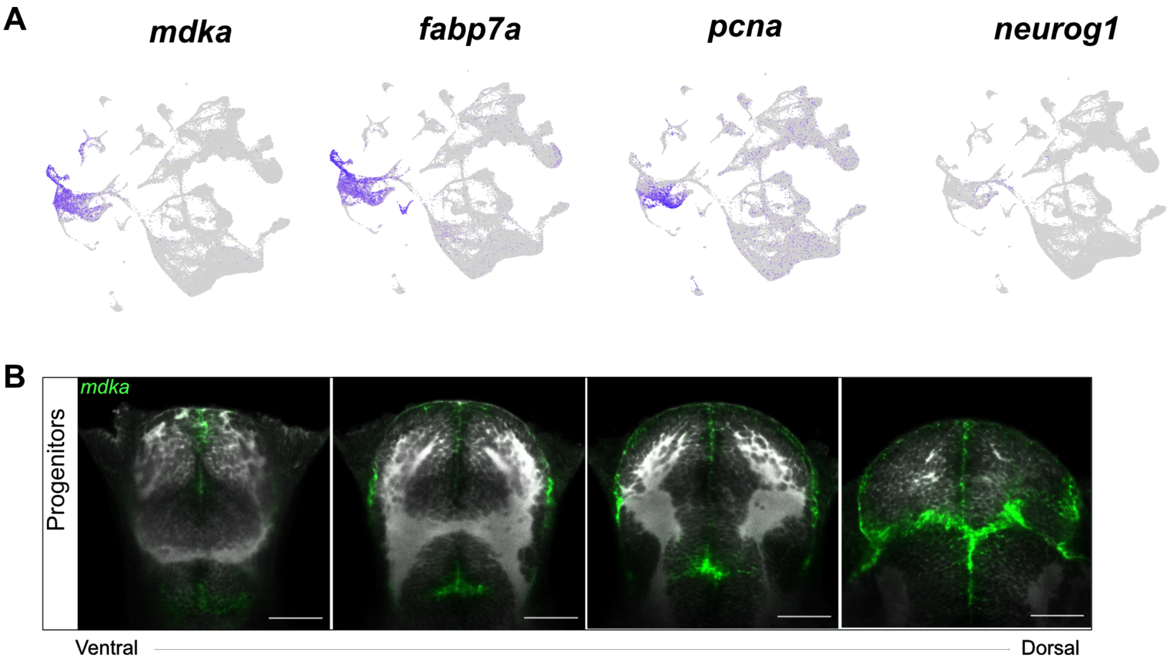


Simplified from Porter and Mueller, 2020

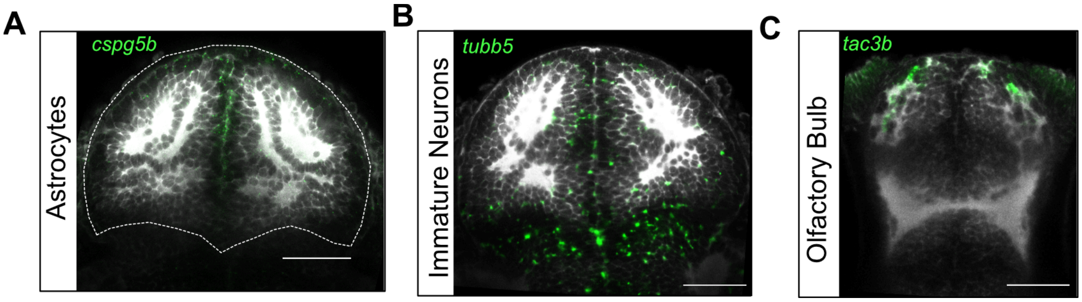
Supplemental Figure S1. The zebrafish telencephalon and its dissection. ***(A)*** Schematic of neural tube eversion (zebrafish) and evagination (mouse) to form the telencephalon. Simplified from Porter and Mueller (2020). Abbreviations: BLA, basolateral amygdala; CeA, central amygdala; Ctx, cortex; Dc, central zone of dorsal pallium (zebrafish); Dld, dorsal subdivision of lateral zone of dorsal pallium (zebrafish); Dlv, ventral subdivision of lateral zone of dorsal pallium (zebrafish); Dm, medial zone of dorsal pallium (zebrafish); DM, medial zone of dorsal pallium; DP, dorsal pallium; Hip, hippocampus; MeA, medial amygdala; MP, medial pallium; nLOT, nucleus of the lateral olfactory tract; Pa, pallidum; Se, septum; Str, striatum. Zebrafish pallium divisions are labelled according to Ganz et al. 2014 (Dld, Dlv, Dc, and Dm) and according to Porter and Mueller 2020 (MP, DP, DM). Posterior subdivision of dorsal pallium (Dp/IOP) not shown. ***(B)*** Diagram of telencephalon dissection boundaries (red dashed lines). The larval 6 dpf and 15 dpf samples included the olfactory and habenula regions, while we attempted to avoid these regions in the adult samples to enrich for cells from the pallium and subpallium



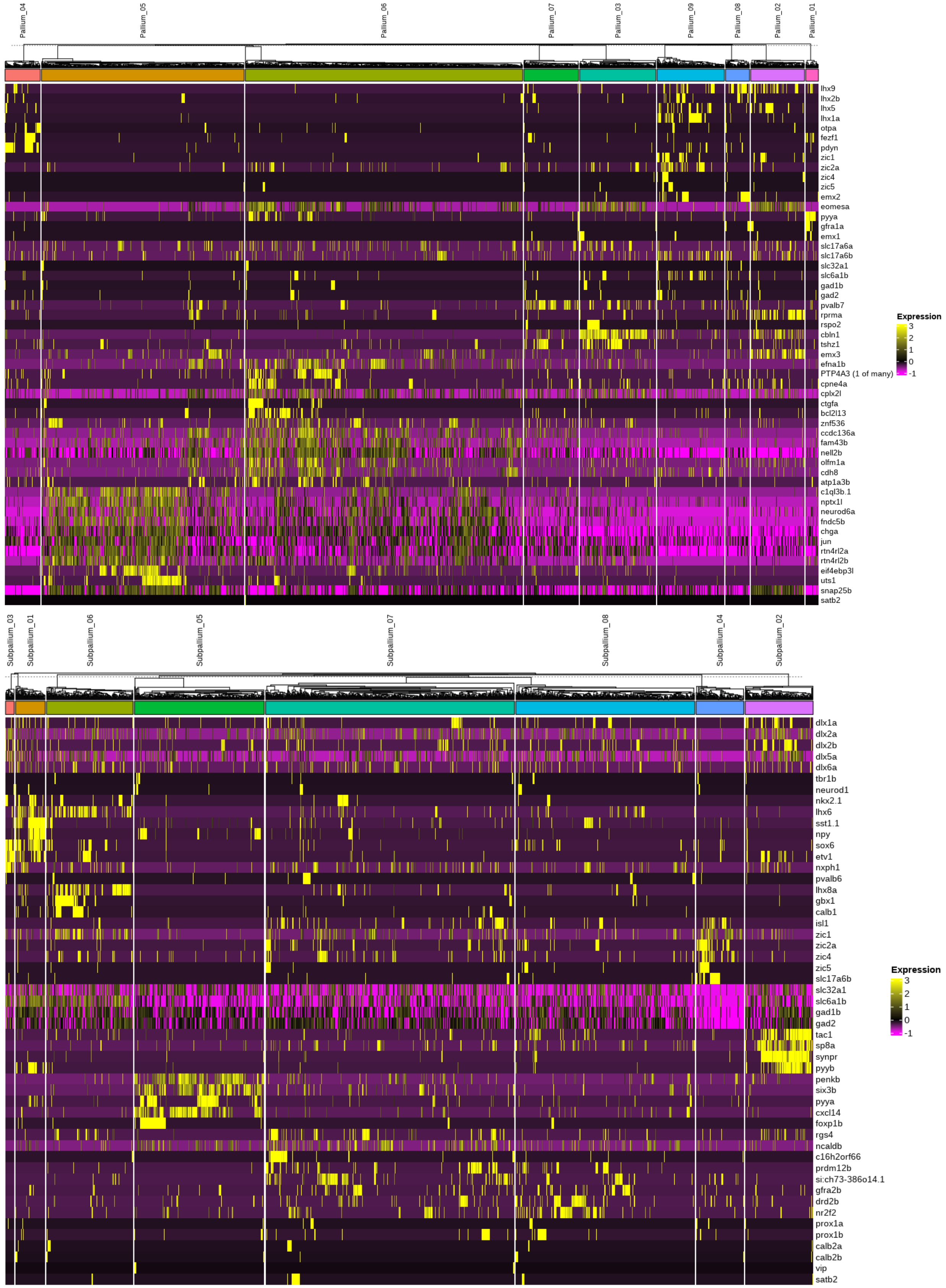
Supplemental Figure S2. Experimental batches for single-cell RNA-seq. ***(A)*** UMAP representation of non-integrated analysis across 6 dpf, 15 dpf, and adult zebrafish telencephalon, faceted by age, colored by batch. The adult cells do not overlap with cells from the two larval stages prior to integration. Minimal batch effects are observed within each age prior to integration. ***(B)*** UMAP representation of integrated analysis across 6 dpf, 15 dpf, and adult zebrafish telencephalon, faceted by age, colored by batch. The integration removed batch effects and age effects. ***(C)*** Proportion of cells contributing to each cluster in non-integrated analysis, split by batch, colored by cluster. Minimal batch effects are observed within each age prior to integration. The biggest differences are within the adult data, dpf6_b0, dpf6_b6, and dpf6_b7. ***(D)*** Proportion of cells contributing to each cluster in integrated analysis, split by batch, colored by cluster. The minimal batch effects are mostly gone, and the differences between ages seem consistent with biological expectations of differentiation.



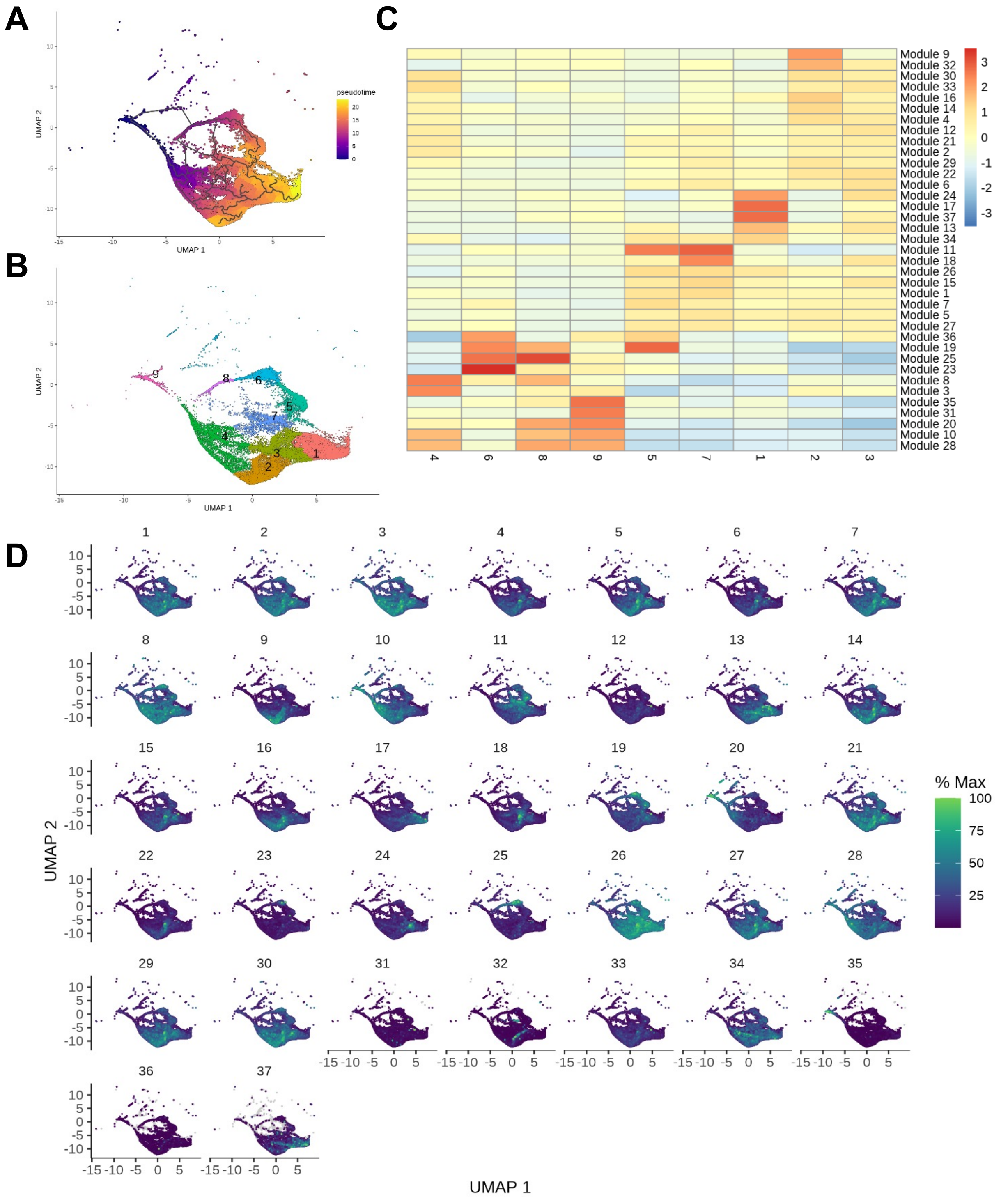
Supplemental Figure S3. Expression patterns of progenitor subtype-specific genes in the telencephalon in the integrated dataset. ***(A)*** Gene expression profiles of marker genes of the different progenitor clusters in the telencephalon. ***(B)*** *In situ* expression domain of *mdka* in larval telencephalon. RNA-FISH (green) was performed with a total-Erk (pale gray) co-stain for anatomy.



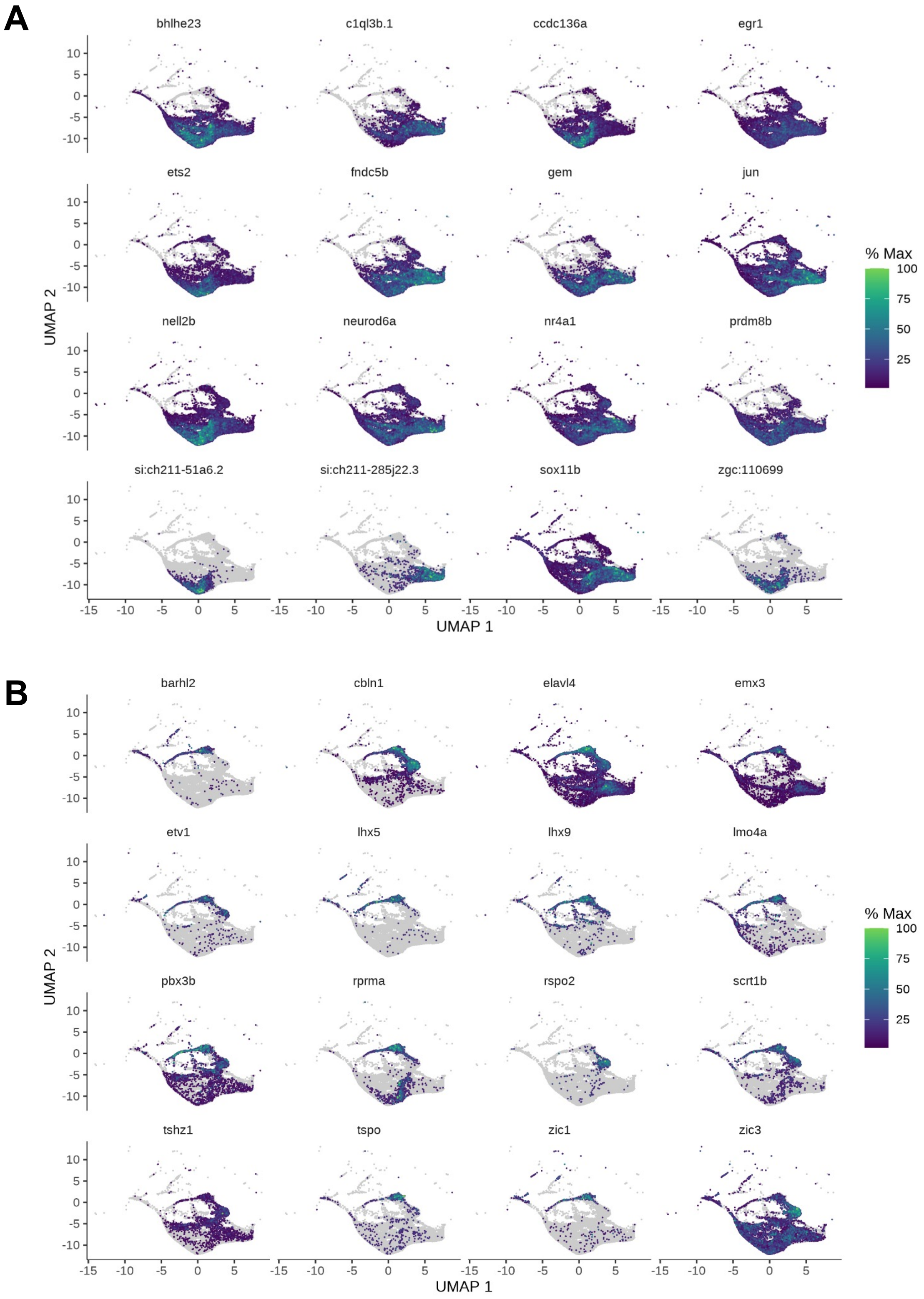
Supplemental Figure S4. Spatial localization of a marker gene for the astrocyte-like cluster, immature neurons, and the olfactory bulb. ***(A)*** *In situ* expression domain of *cspg5b*, a marker gene expressed in the putative telencephalic astrocyte cluster. ***(B)*** *In situ* expression domain of *tubb5,* a marker gene for immature neurons. ***(C)*** *In situ* expression domain of *tac3b* in the olfactory bulb.



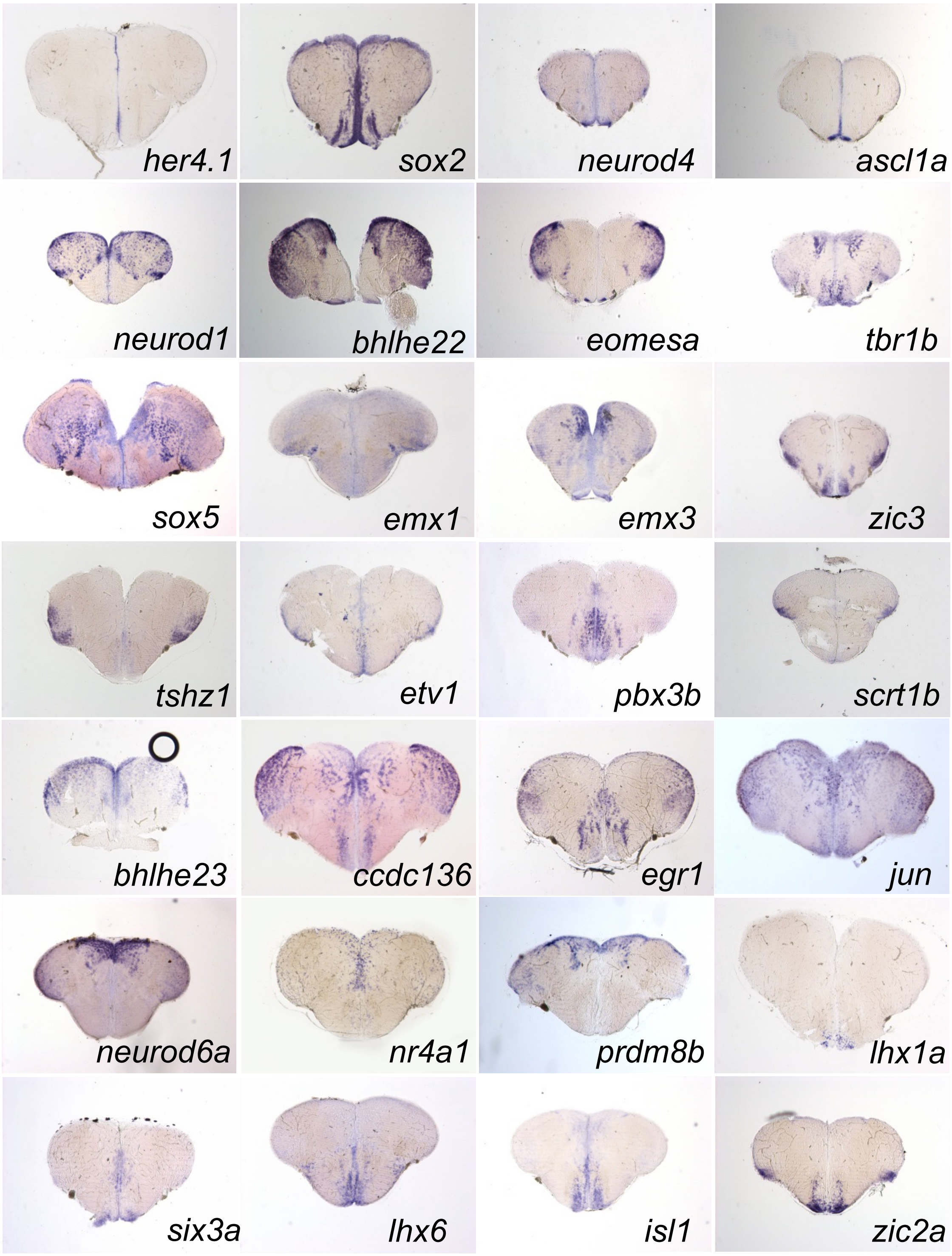
Supplemental Figure S5. Expression of pallium and subpallium marker genes.



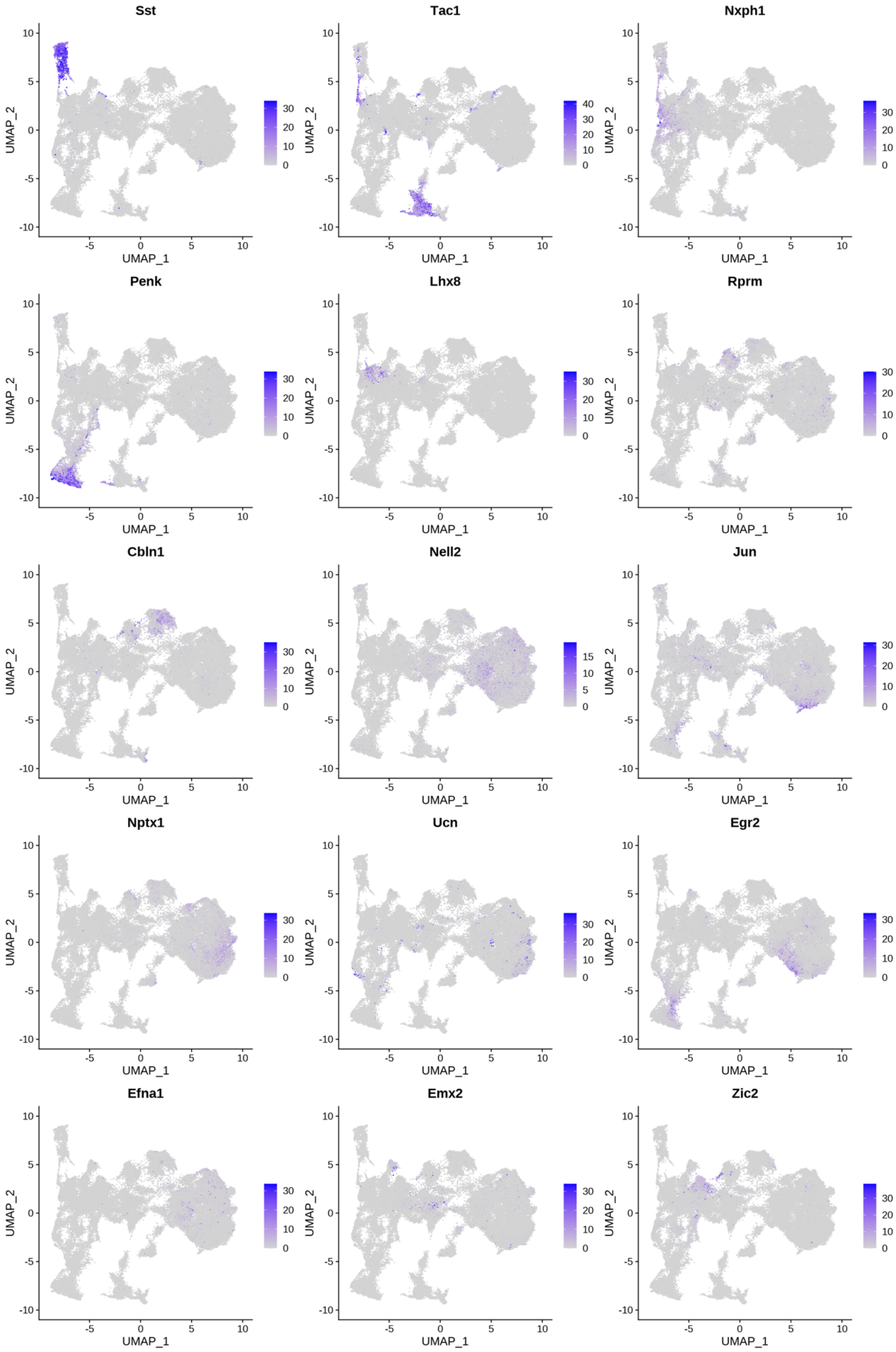
Supplemental Figure S6. Developmental trajectory of pallial neurons across pseudotime. ***(A)*** UMAP representation of Committed_pallium_precursors, Pallium_IN01, Pallium_IN02, Pallium_02, Pallium_03, Pallium_05, Pallium_06, and Pallium_07 reclustered by Monocle 3. ***(B)*** UMAP representation of cells colored by their position in pseudotime with root node in Committed_pallium_precursors. ***(C)*** Heatmap of 37 modules of co-regulated genes that are differentially expressed across pseudotime. ***(D)*** UMAP representation of gene expression in 37 modules.



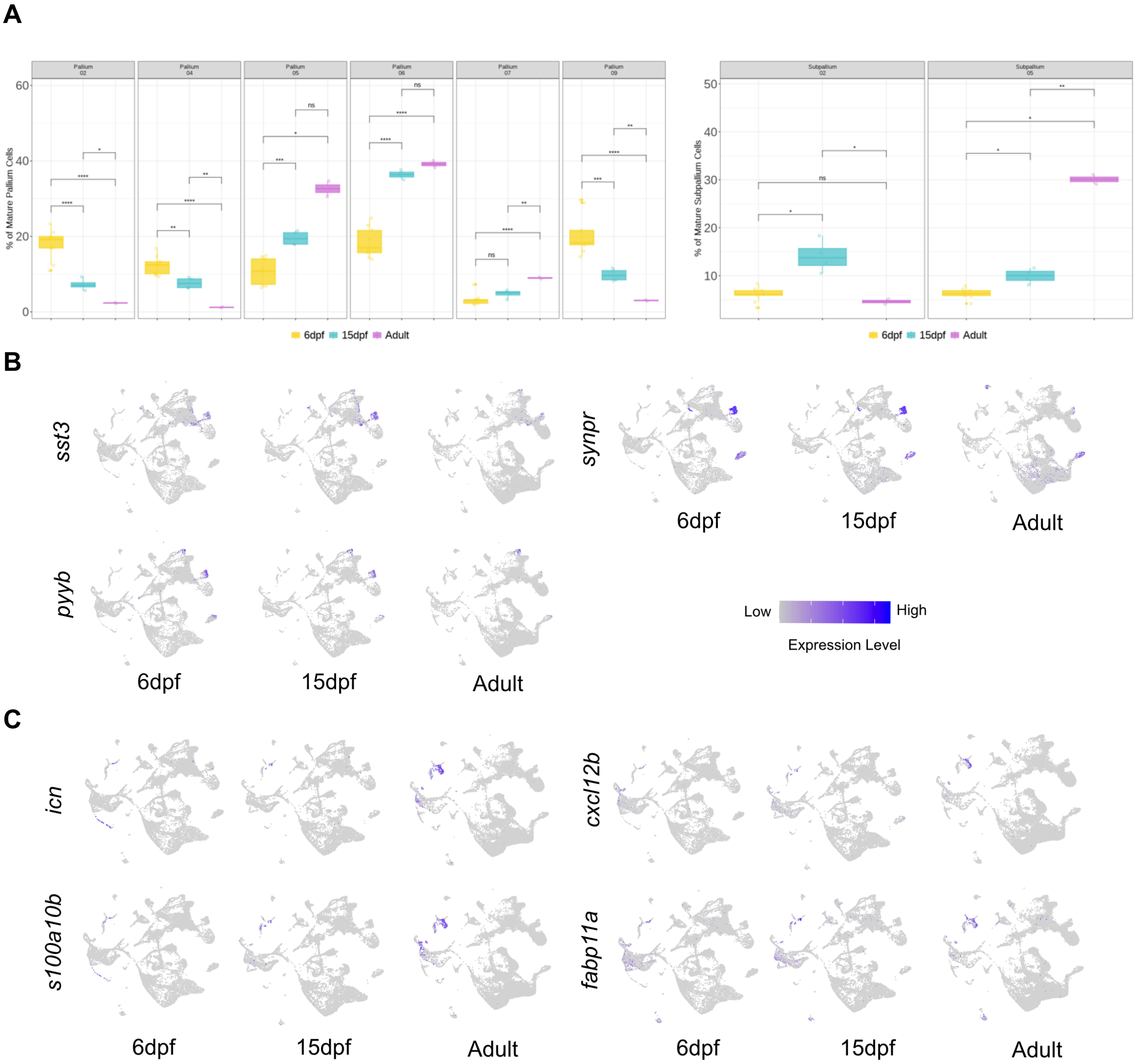
Supplemental Figure S7. Expression of multiple genes in select pseudotime modules. ***(A)*** UMAP representation of gene expression from representative genes in modules related to Pallium_05 and Pallium_06 (9, 13, 14, 16, 17). ***(B)*** UMAP representation of gene expression from representative genes in modules related to Pallium_02 and Pallium_03 (11, 19, 23, 25).



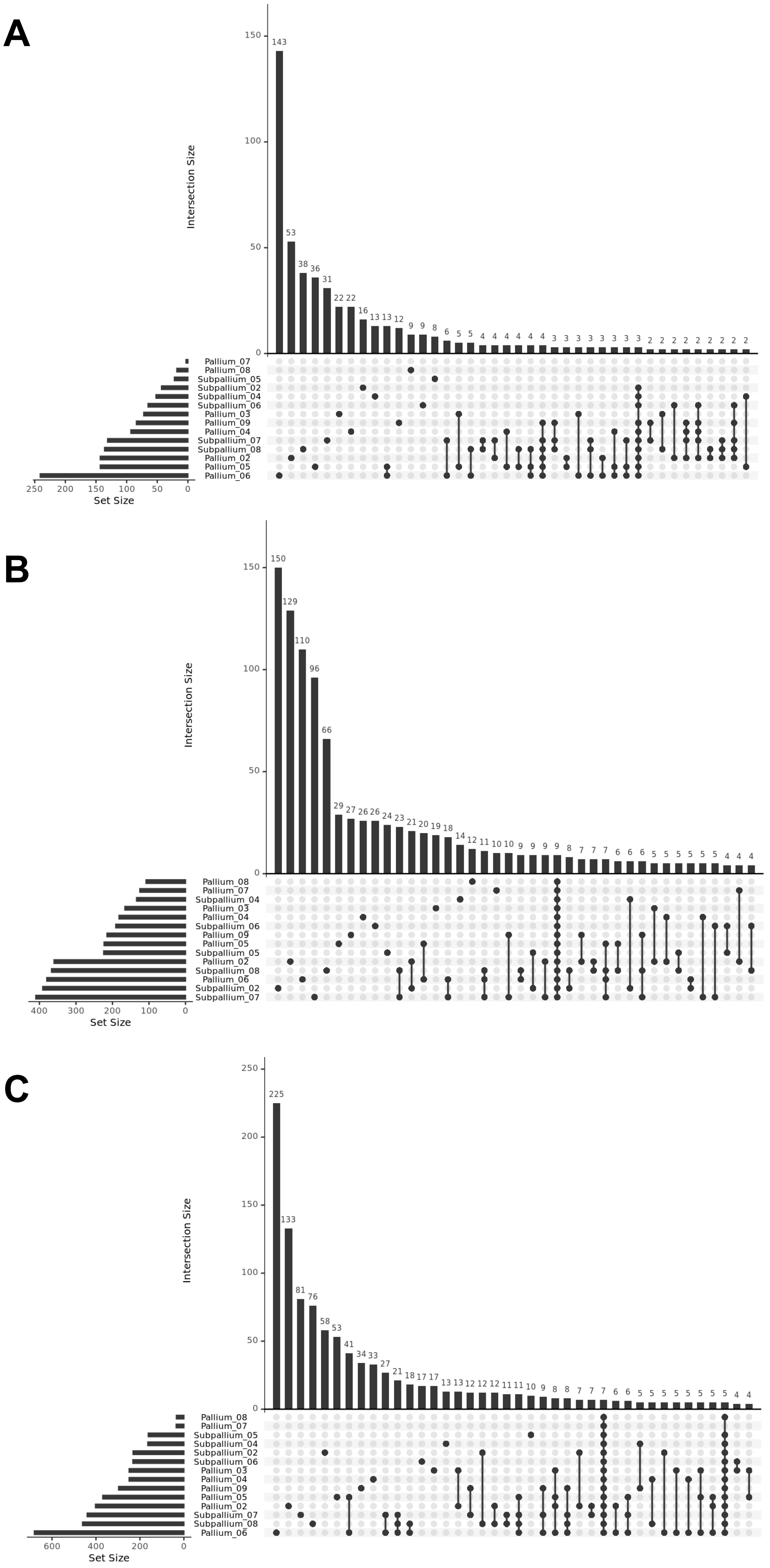
Supplemental Figure S8. Adult marker gene in situ from AGETAZ. Radial glia: *her4.1*, *sox2*. Committed pallium precursors: *neurod4*. Committed subpallium precursors: *ascl1a*. Broad and classical zebrafish pallium markers: *neurod1, bhlhe22, eomesa, tbr1b, sox5, emx1, emx3*. Markers of Pallium_02 and/or Pallium_03 and this pseudotime grouping (Fig. 7B): *zic3*, *tshz1*, *etv1, pbx3b, scrt1b*. Markers of Pallium_05 and Pallium_06 and this pseudotime grouping (Fig. 7A): *bhlhe23, ccdc136, egr1, jun, neurod6a, nr4a1, prmd8b*. Pallium_09: *lhx1a*. Subpallium_05: *six3a*. Subpallium_06: *lhx6*. Septum markers: *zic2a*, *isl1*.



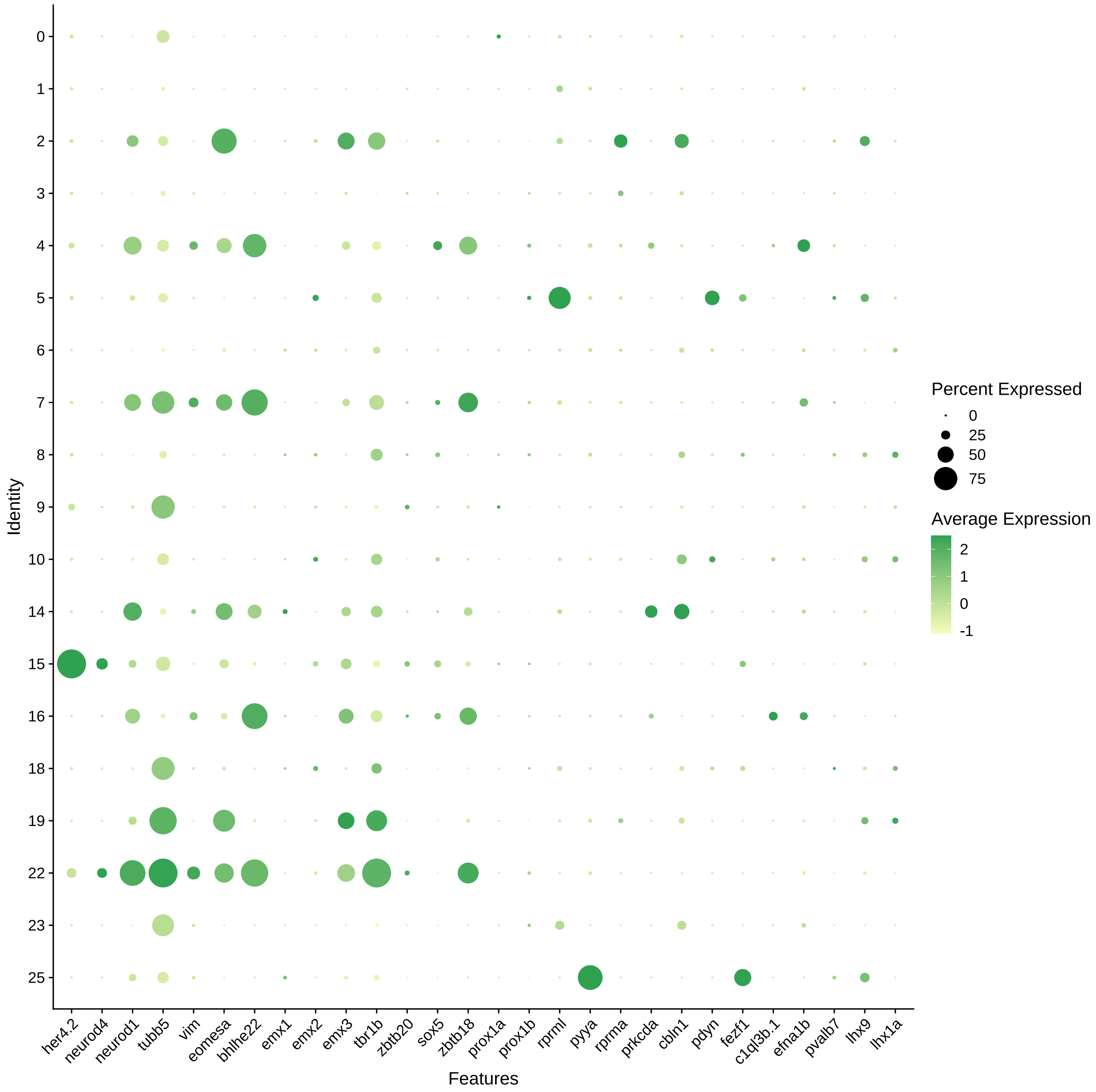
Supplemental Figure S9. Select feature plots from Harmony integration analysis.



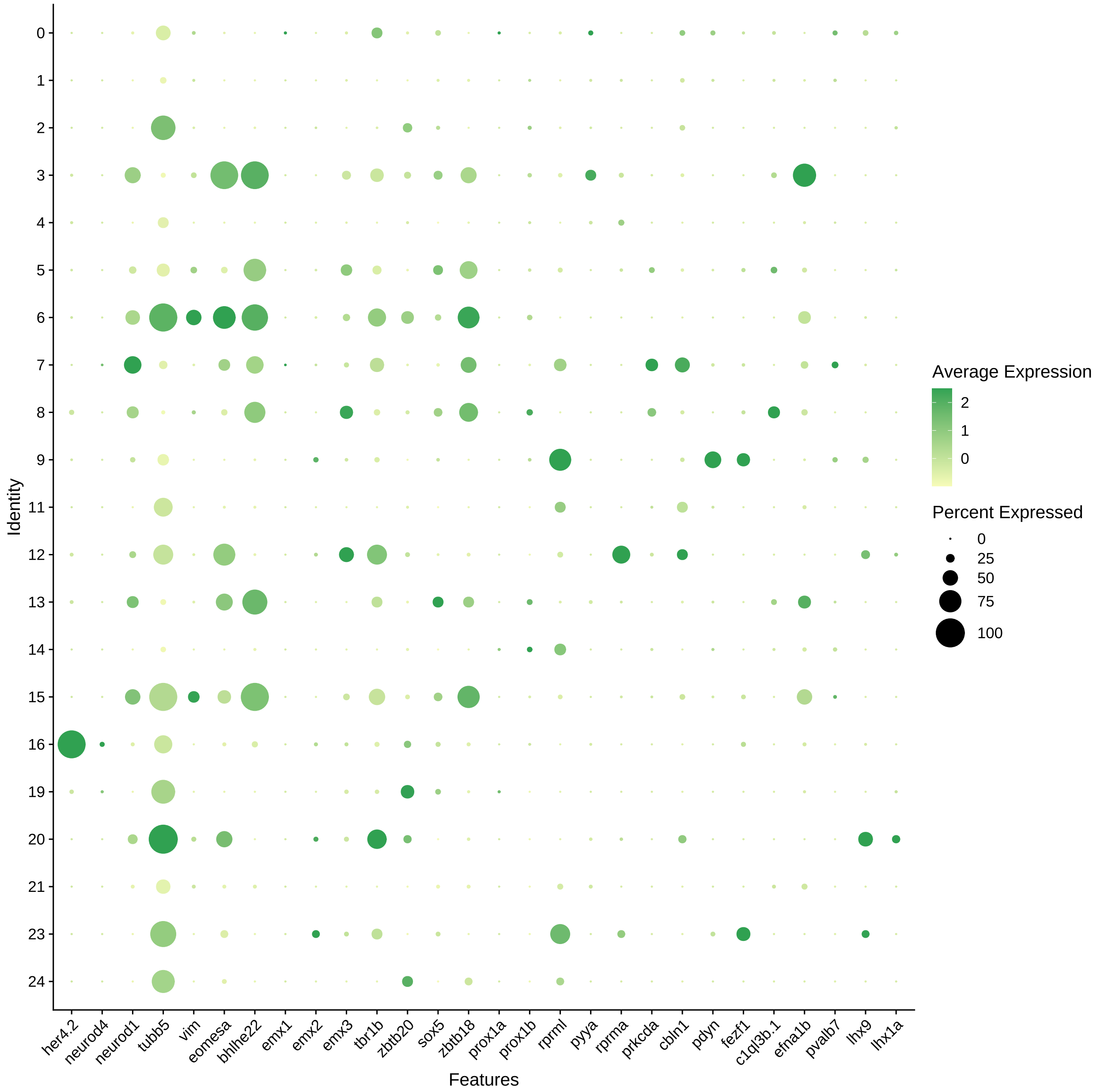
Supplemental Figure S10. Extended comparison of the larval telencephalon and adult telencephalon in the integrated data. ***(A)*** Cluster composition of cells in the pallium and the subpallium as a percentage of the total mature neurons in pallium and subpallium, respectively. Statistical test: unpaired two-tailed *t*-test with Benjamini & Hochberg correction for multiple comparisons (* < 0.05, ** < 0.01, *** < 0.001, **** < 1×10^{-4}). ***(B)*** Example marker genes from Subpallium_02 that are robustly expressed at 6 dpf and 15 dpf dataset but not in the adult dataset. ***(C)*** Gene expression profiles of endothelial cells that are specific to the adult telencephalon.



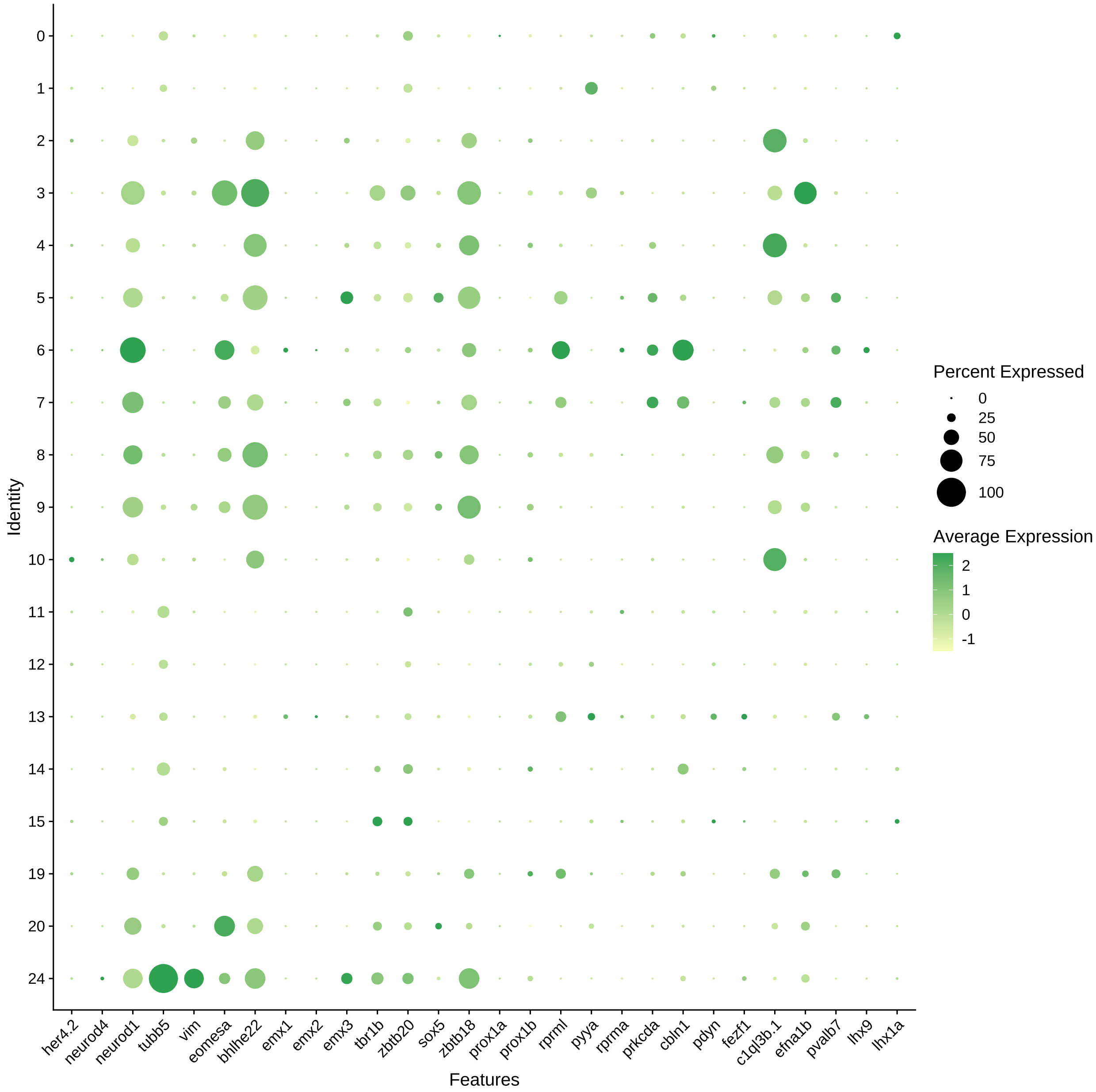
Supplemental Figure S11. Comparison of differentially expressed genes between ages. ***(A)*** UpSet plot showing the number of differentially expressed genes between a comparison of 15 dpf and 6 dpf animals in each mature pallium and subpallium cluster. ***(B)*** UpSet plot showing the number of differentially expressed genes between a comparison of adult and 15 dpf animals in each mature pallium and subpallium cluster. ***(C)*** UpSet plot showing the number of differentially expressed genes between a comparison of adult and 6 dpf animals in each mature pallium and subpallium cluster.



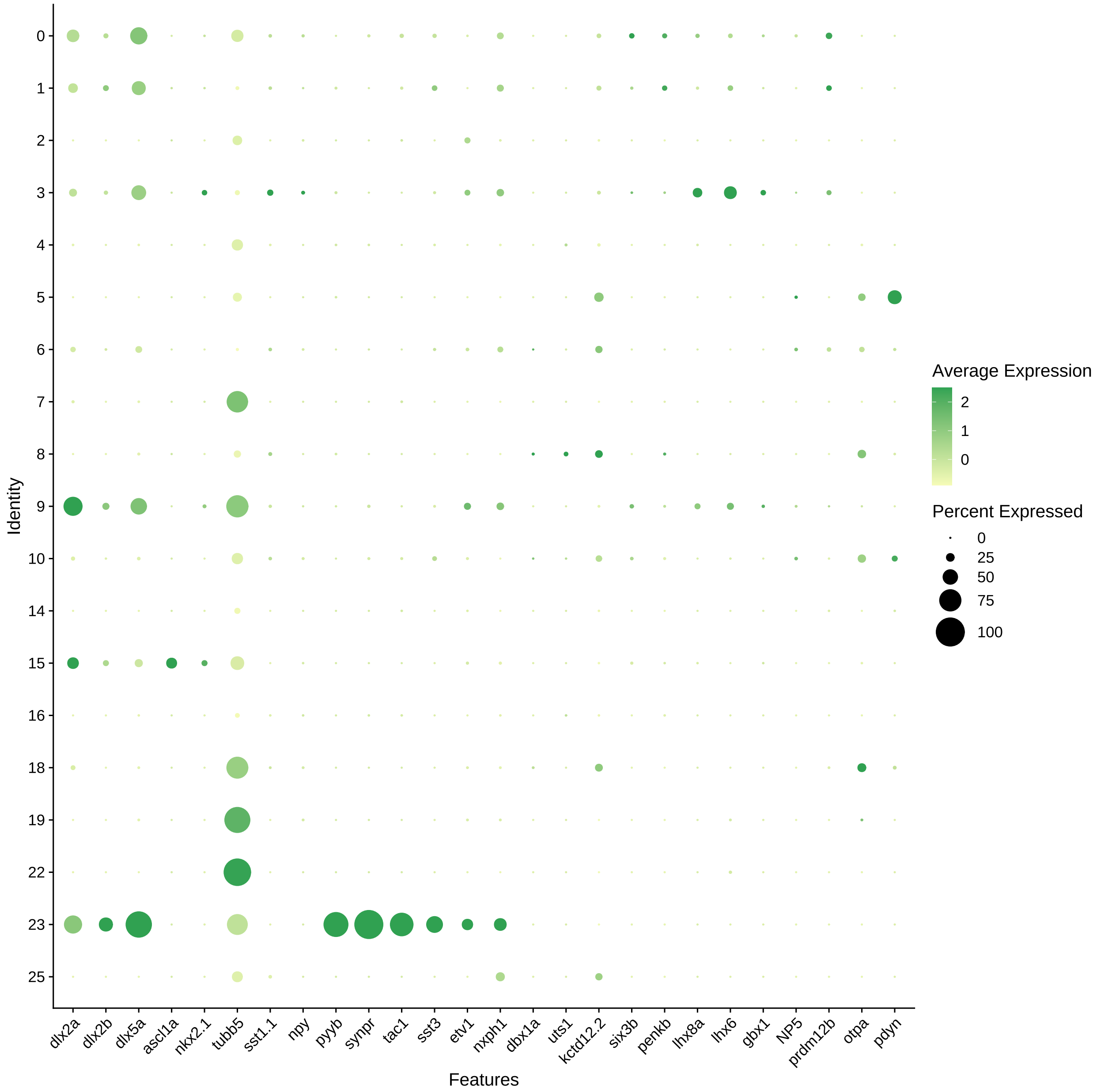
Supplemental Figure S12. Expression profiles of genes expressed in the pallium for neuronal clusters in 6 dpf data.



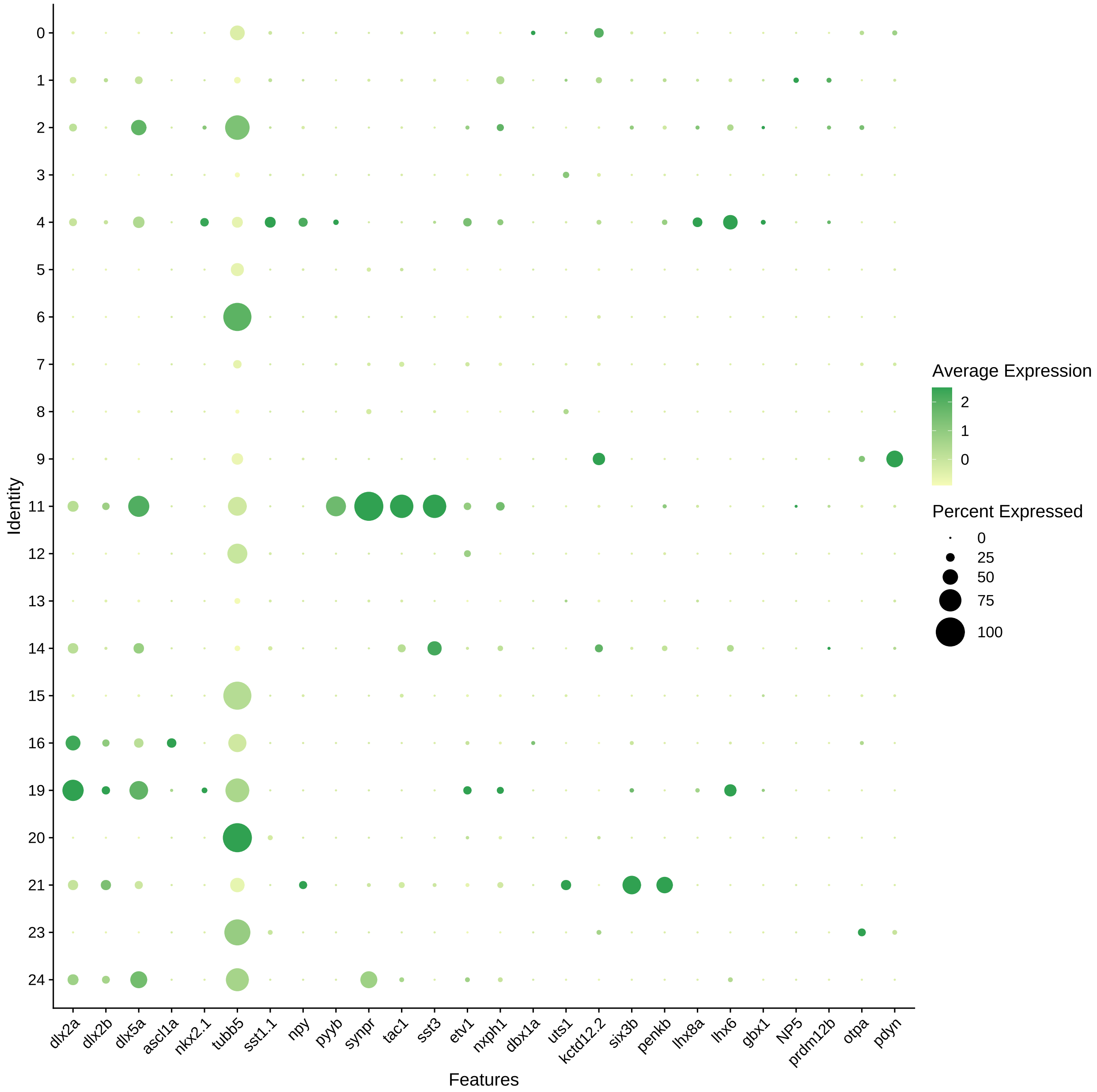
Supplemental Figure S13. Expression profiles of genes expressed in the pallium for neuronal clusters in 15 dpf data.



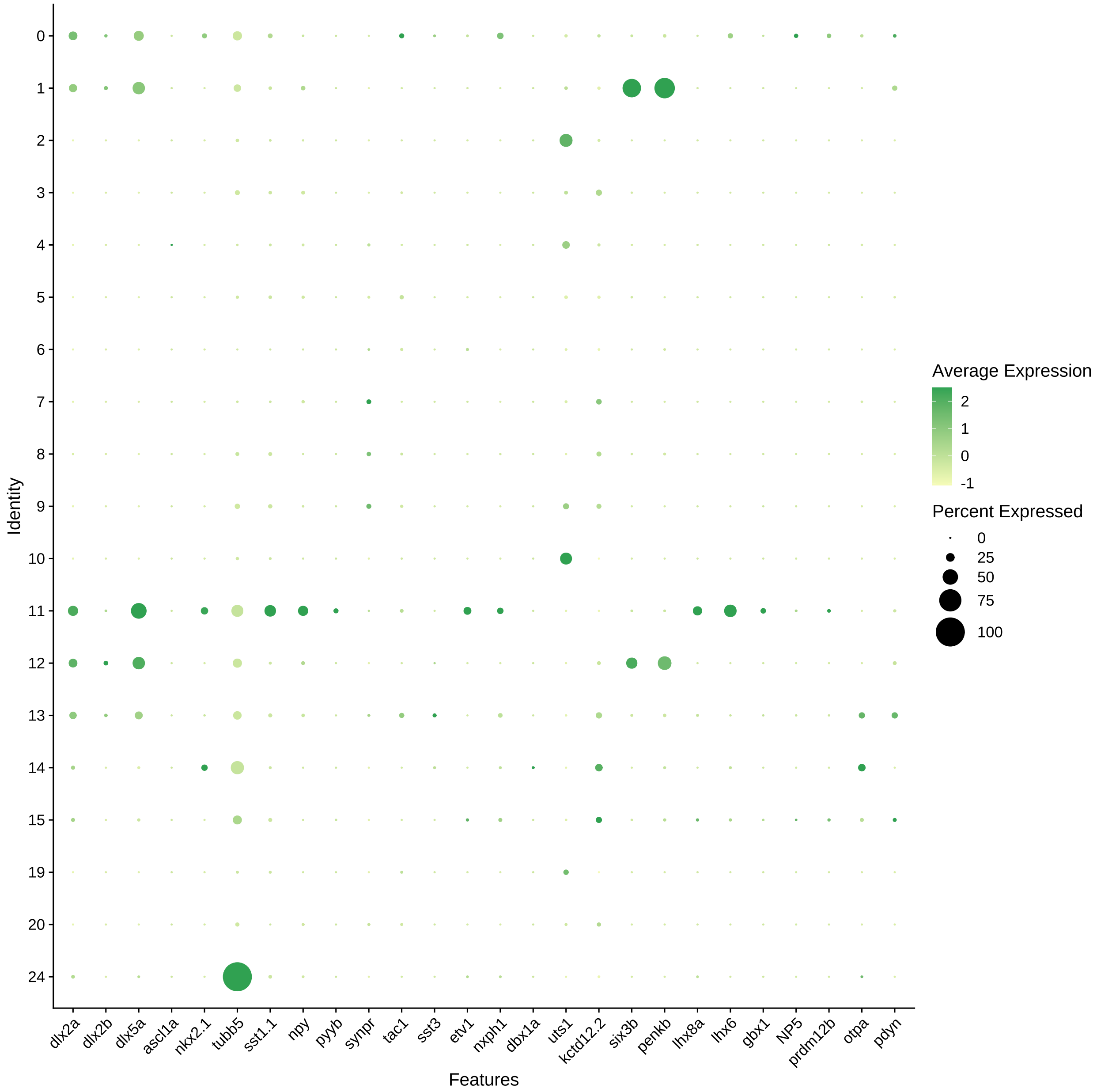
Supplemental Figure S14. Expression profiles of genes expressed in the pallium for neuronal clusters in adult data.



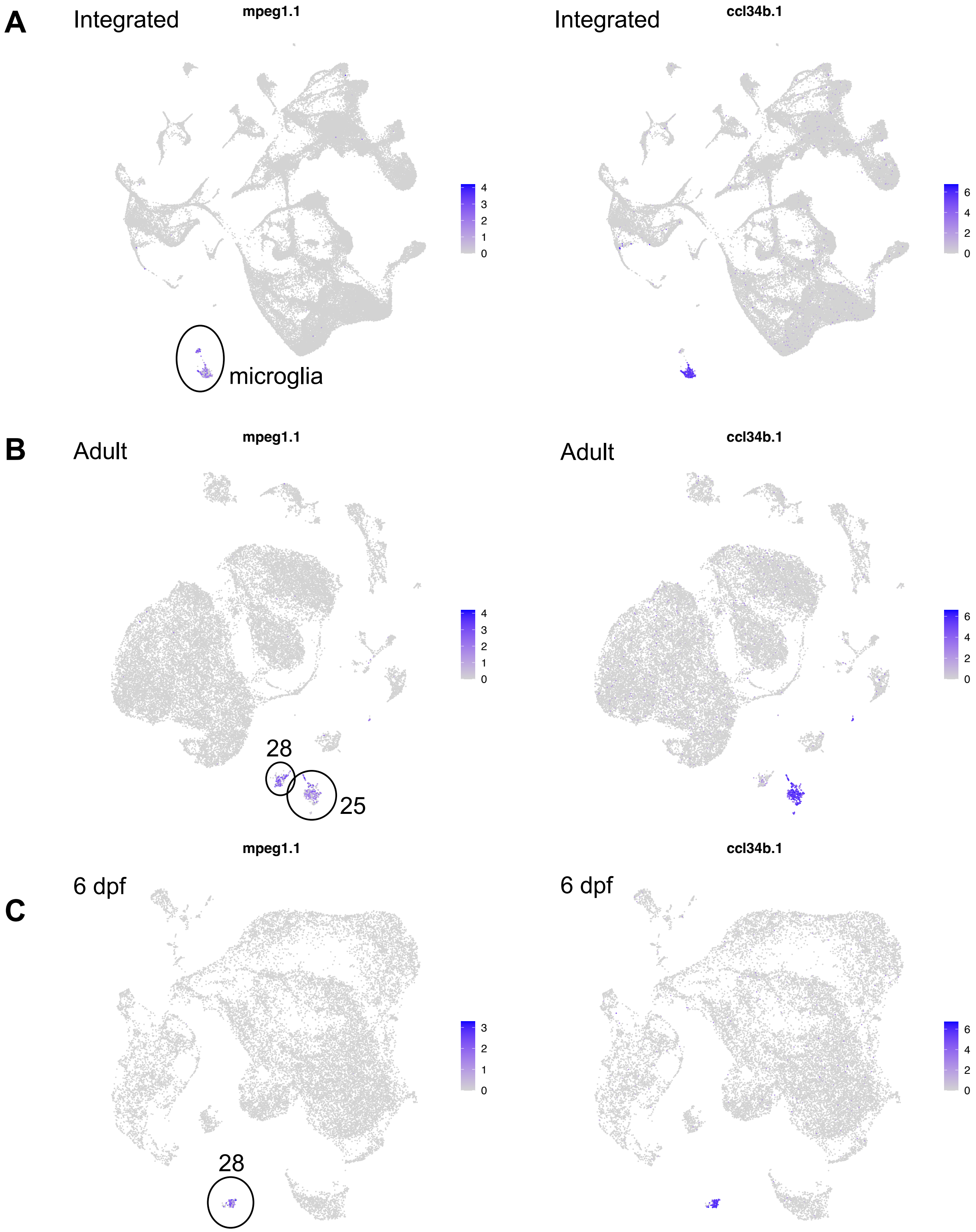
Supplemental Figure S15. Expression profiles of genes expressed in the subpallium for neuronal clusters in 6 dpf data.



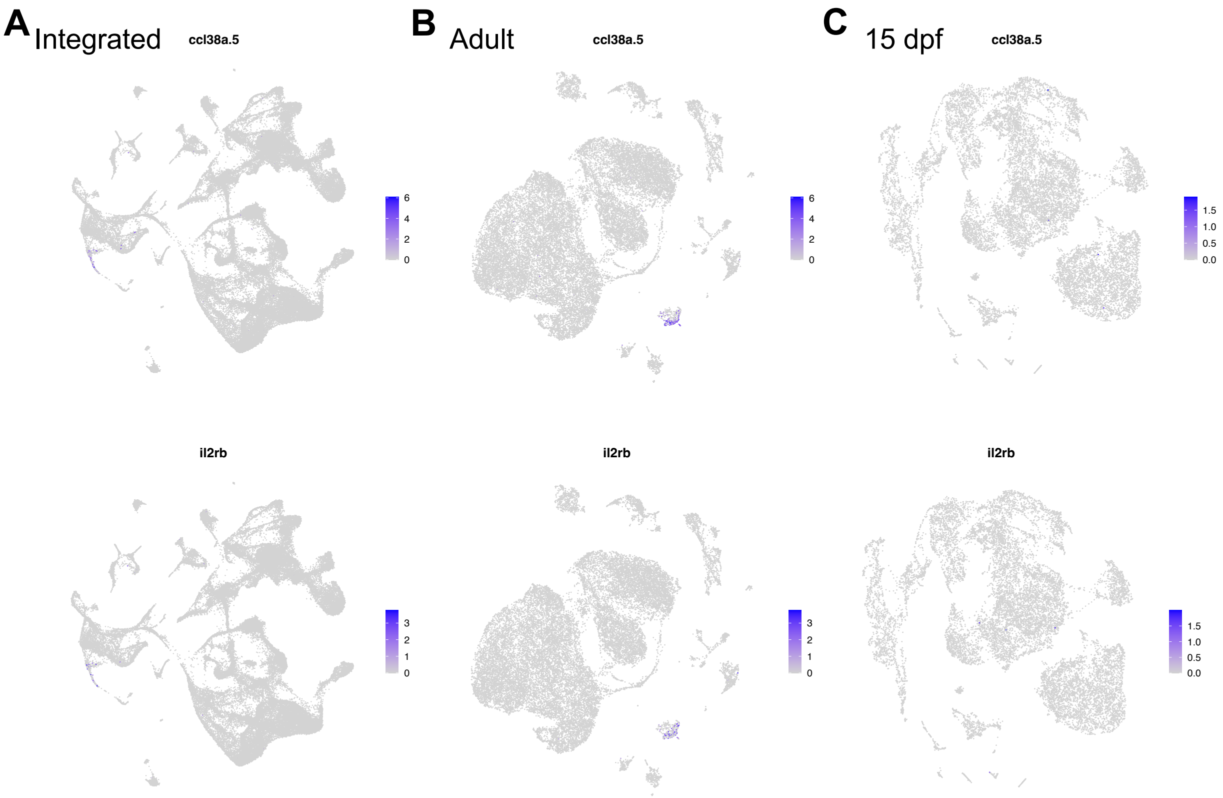
Supplemental Figure S16. Expression profiles of genes expressed in the subpallium for neuronal clusters in 15 dpf data.



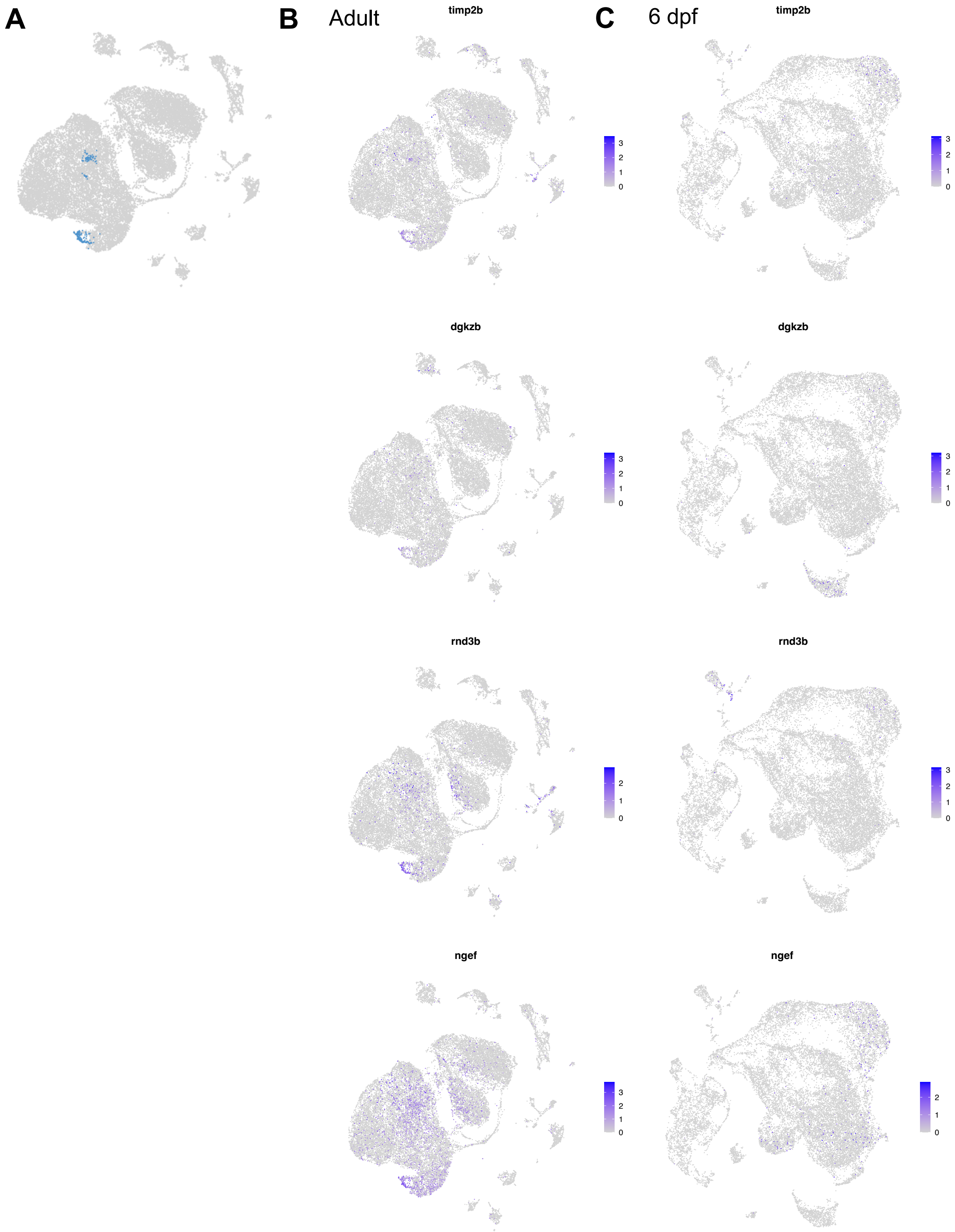
Supplemental Figure S17. Expression profiles of genes expressed in the subpallium for neuronal clusters in adult data.



Supplemental Figure S18. Markers of phagocytotic and regulatory microglia.



Supplemental Figure S19. Markers of NK-Like cells in the integrated, adult, and 15 dpf data.



Supplemental Figure S20. Markers of the adult pallium cluster 26.

Other Supplemental Materials for this manuscript

Supplemental Video S1. Registered map of telencephalon marker gene expression. The RNA-FISH data was registered into the same spatial coordinate space to compare the image stacks. The top two panels represent genes designated as belonging to the pallium (Fig. 3A), while the lower two panels represent genes designated as belonging to the subpallium (Fig. 3B). The cluster numbers corresponding to the main text figures are in parentheses following the gene name.

Supplemental Table S1. Markers for all integrated telencephalon clusters across all ages.

Supplemental Table S2. Primers for generating *in situ* probes.

Supplemental Table S3. Comparison of mouse and zebrafish marker genes.

Supplemental Table S4. Differentially enriched genes between 15 dpf and 6 dpf animals for every mature pallium and subpallium cluster.

Supplemental Table S5. Differentially enriched genes between adult and 15 dpf animals for every mature pallium and subpallium cluster.

Supplemental Table S6. Differentially enriched genes between adult and 6 dpf animals for every mature pallium and subpallium cluster.

Supplemental Table S7. Markers for all clusters in the 6 dpf data using a clustering resolution of 1.7.

Supplemental Table S8. Markers for all clusters in the 15 dpf data using a clustering resolution of 1.7.

Supplemental Table S9. Markers for all clusters in the adult data using a clustering resolution of 1.6.

Supplemental Table S10. Comparison of the clusters identified in the separated analysis for each age.

Supplemental Table S11. Markers for all clusters in the 15 dpf data aligned to Zv11 using STARsolo and using a clustering resolution of 1.7.