SUPPLEMENTARY DATA

Supplementary Figure S1

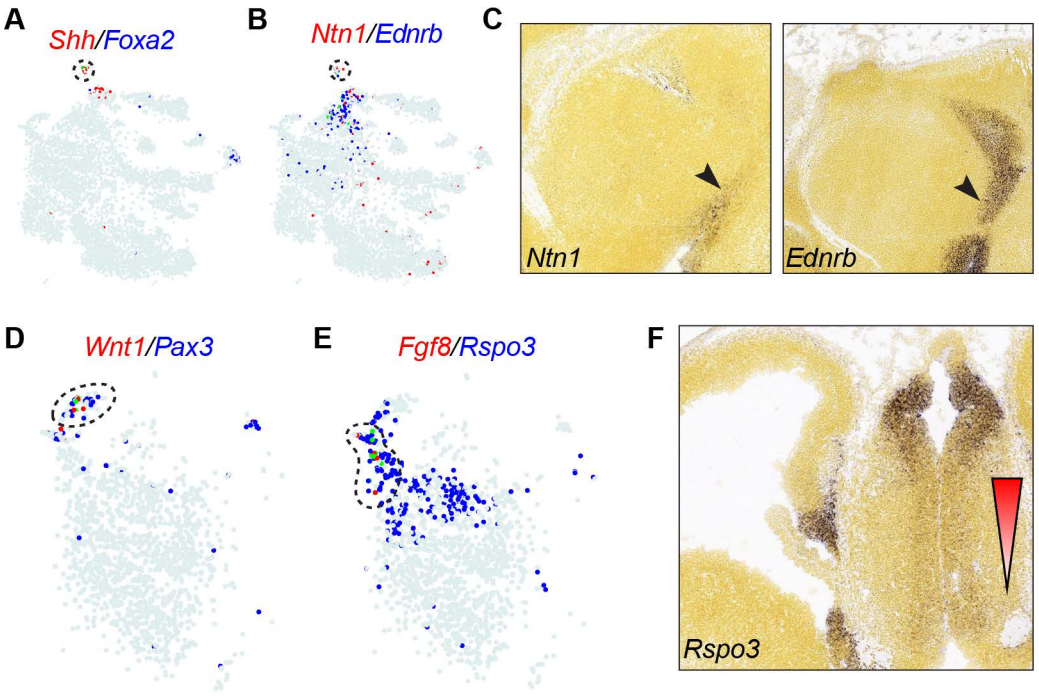
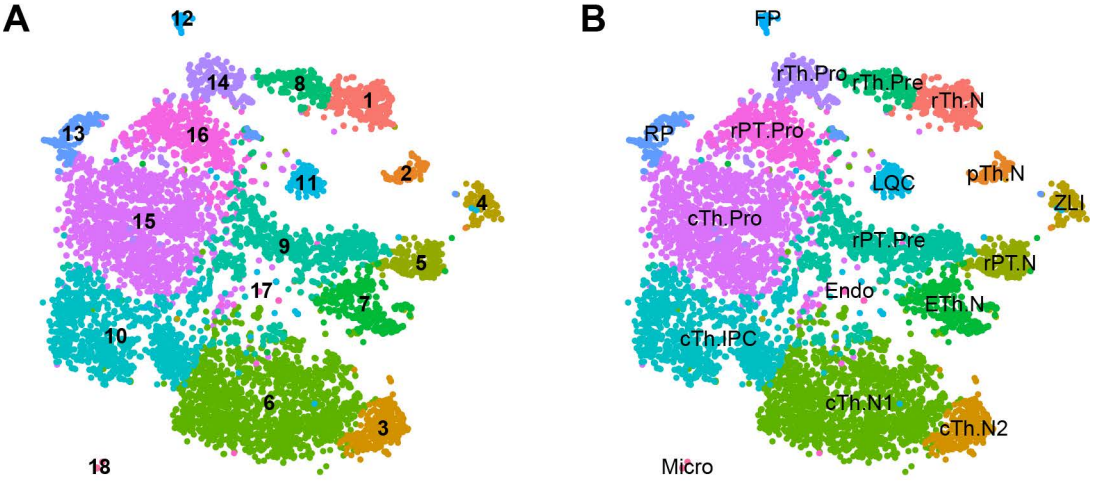


Figure S1. Assignments of cell groups corresponding to the basal plate and the roof plate. (**A** and **B**) t-SNE plotting of the expression of markers for cluster 12 (circled by dashed lines). (**C**) *In situ* hybridization on sagittal sections of E13.5 mouse brain. (**D** and **E**) t-SNE showing expression of *Fgf8* and *Wnt1* in two distinct subgroups of cells (circled by dashed lines) within cluster 13. (**F**) *In situ* hybridization on coronal sections of E13.5 mouse brain. The triangle indicates the decreasing gradient of *Rspo3* expression in the dorsal-to-ventral direction. Images in C and F are from Allen Developing Mouse Brain Atlas.

Supplementary Figure S2



C

Cell group	Cluster ID	Cell num	Cell ratio	Description
rTh.N	1	288	3.91	rostral thalamic neurons
pTh.N	2	114	1.55	prethalamic neurons
cTh.N2	3	262	3.56	caudal thalamus neuron II
ZLI	4	136	1.85	zona limitans intrathalamica
rPT.N	5	235	3.19	rostral pretectal neurons
cTh.N1	6	1,539	20.90	caudal thalamus neuron I
ETh.N	7	314	4.26	epithalamic neurons
rTh.Pre	8	205	2.78	rostral thalamic neuronal precursor
rPT.pre	9	666	9.04	rostral pretectal neuronal precursor
cTh.IPC	10	1,005	13.65	caudal thalamic intermediate progenitor
LQC	11	157	2.13	low quality cell
FP	12	50	0.68	floor/basal plate
RP	13	160	2.17	roof plate
rTh.Pro	14	230	3.12	rostral thalamic neural progenitor
cTh.Pro	15	1,455	19.76	caudal thalamic neural progenitor
rPT.Pro	16	503	6.83	rostral pretecal neural progenitor
Endo	17	27	0.37	endothelium
Micro	18	19	0.26	migroglia

Figure S2. Summary of cell clustering. (**A** and **B**) t-SNE plotting of cell clusters with the original identification number (A) and cell identity annotation (B). (**C**) Tabulation of the number and percentage of cells in each cluster.

Supplementary Figure S3

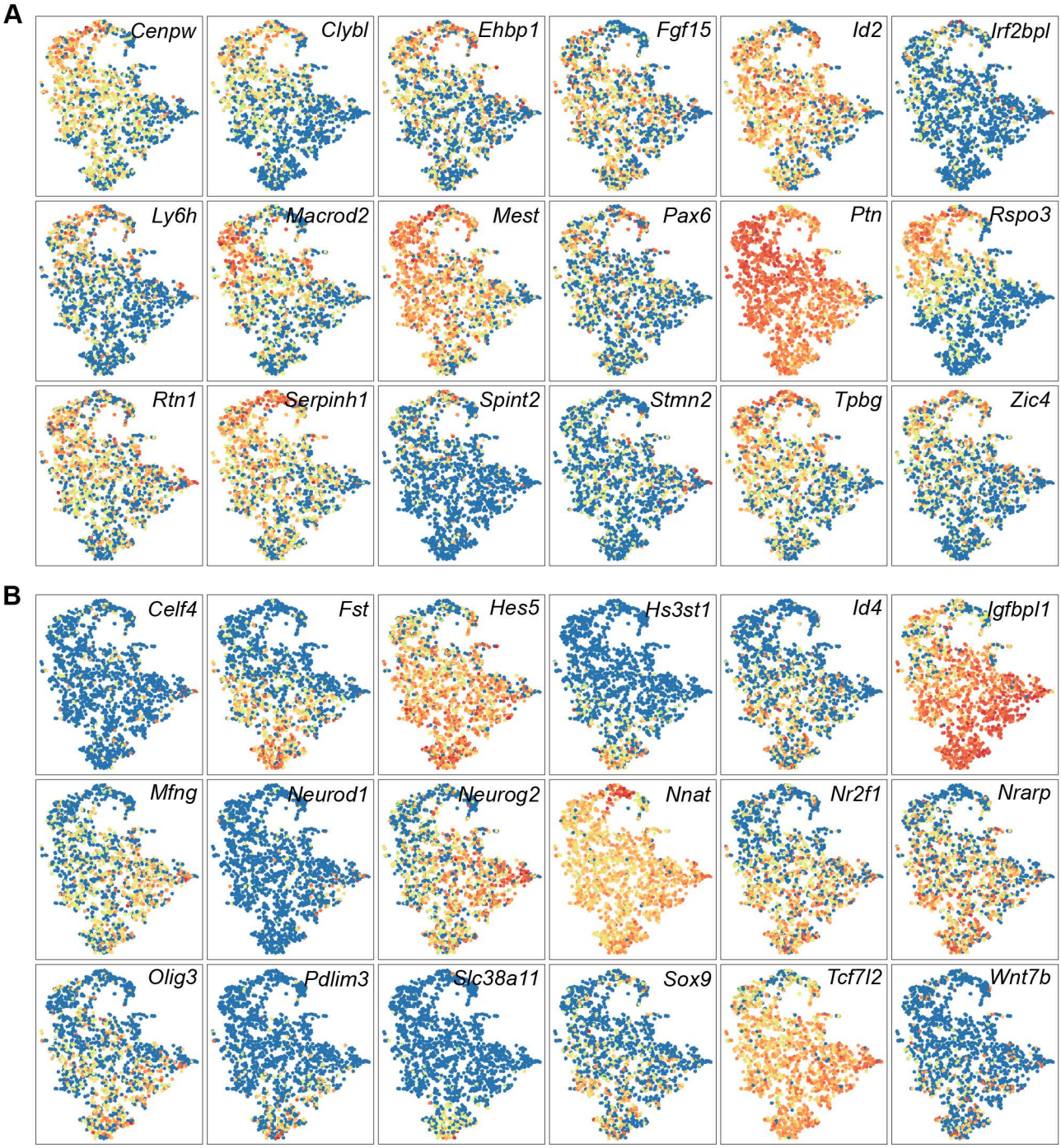


Figure S3. Trendsceek analysis of spatial expression patterns with scRNAseq data. (**A** and **B**) rendsceek-identified significant genes that exhibit dorsal^{high}-ventral^{low} (A) or dorsal^{low}-ventral^{high} (B) gradients in the p2 ventricular zone.

Supplementary Figure S4

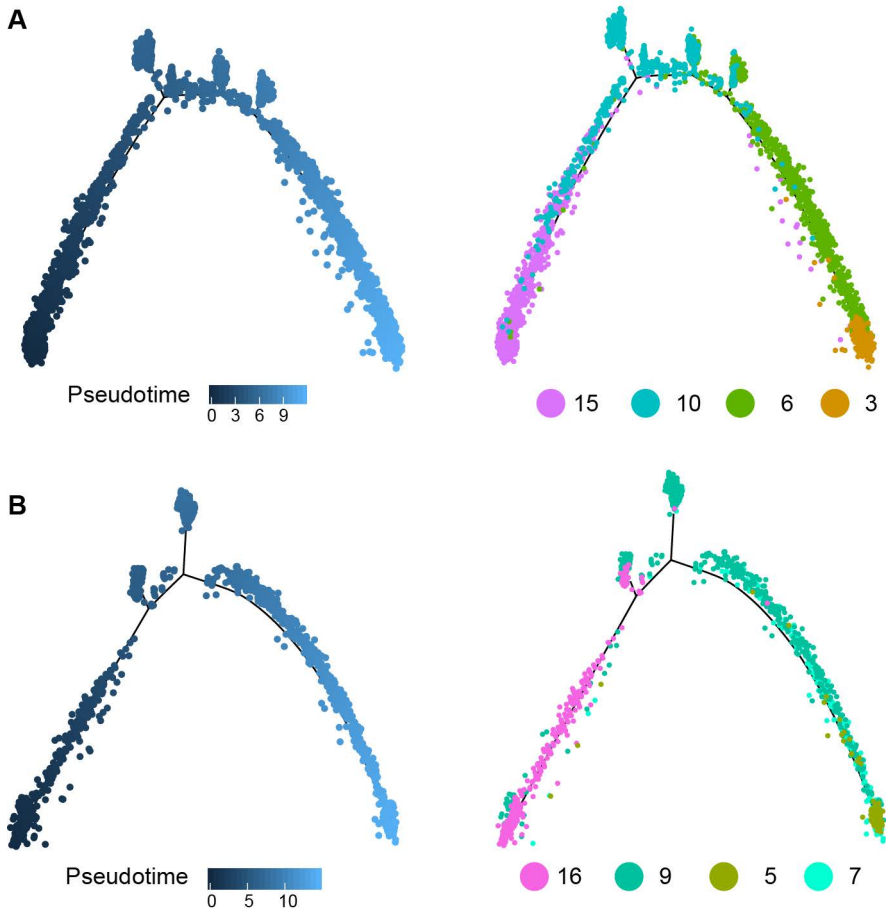


Figure S4. Distribution of Seurat-identified cell clusters in Monocle-inferred trajectories.
(**A** and **B**) Developmental trajectories in the caudal thalamus (A) and rostral pretectum (B).

Supplementary Figure S5

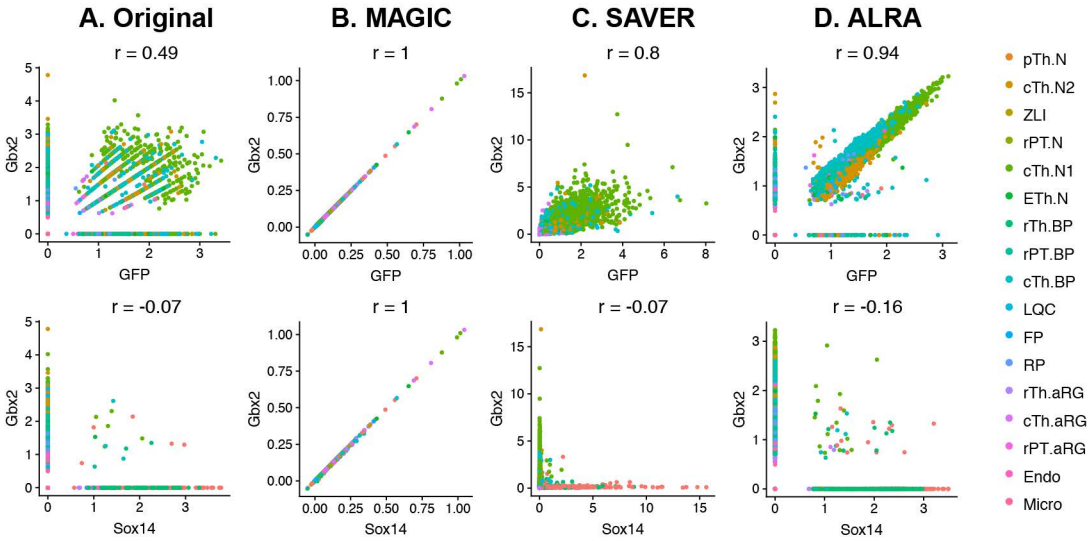


Figure S5. Comparison of gene expression recovery methods. (**A-D**) Scatter plot showing the expression of *Gbx2* and *GFP* (top row) or *Gbx2* and *Sox14* (bottom) across all cells, which are colored according to their cluster identification. Plots in columns show original (A), MAGIC (B), SAVER (C), and ALRA (D) processed data. Pearson correlation (r) between the two genes is displayed above the plot.

Supplementary Figure S6

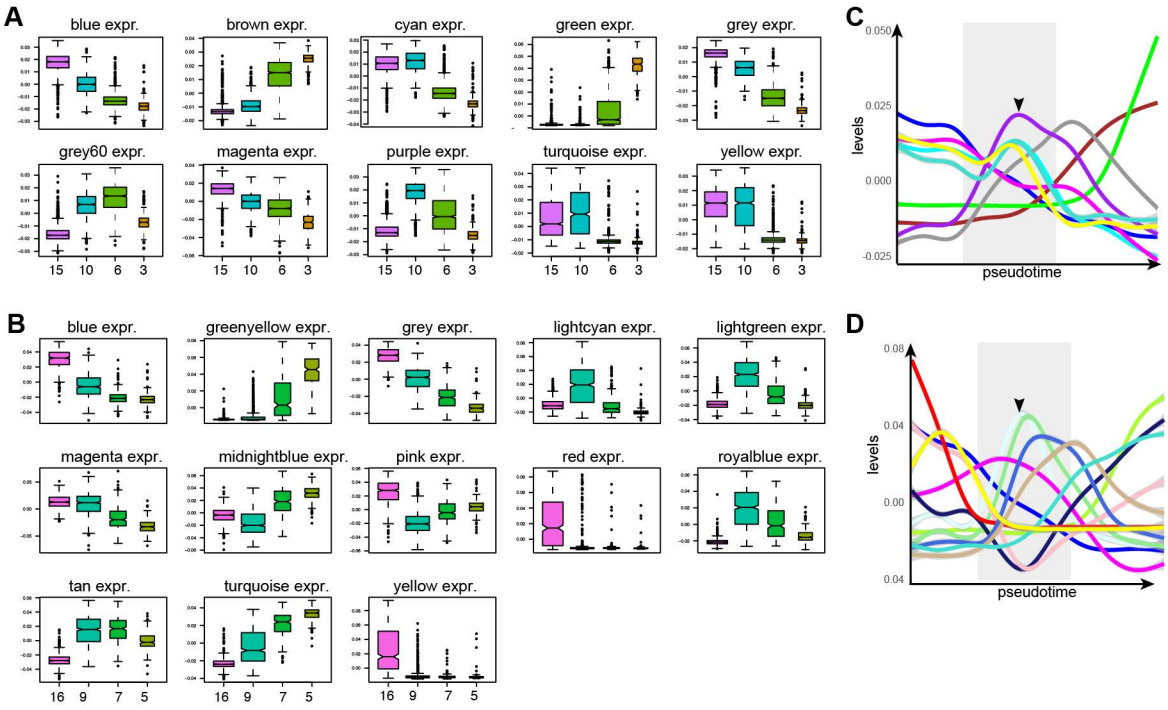


Figure S6. Distributions of module eigengene values. (**A** and **B**) Distribution of eigengene in different cell groups. All modules show significant difference in the distribution among cell groups ($p < 0.001$, Kruksal-Wallis test). The purple module is specifically increased in caudal thalamic IPCs (cluster 10; A), whereas the lightcyan module is specifically increased in cluster 9 (B). (**C** and **D**) Eigengene changes of each module in cells arranged according to pseudotime in caudal thalamus (C) and rostral pretectum (D). Note that the grey60 and purple modules have similar eigengene profile in C, whereas lightcyan and lightgreen have similar profile in D, suggesting they are related. The arrowheads indicate the peak of purple and lightcyan module eigengene.

Supplementary Figure S7

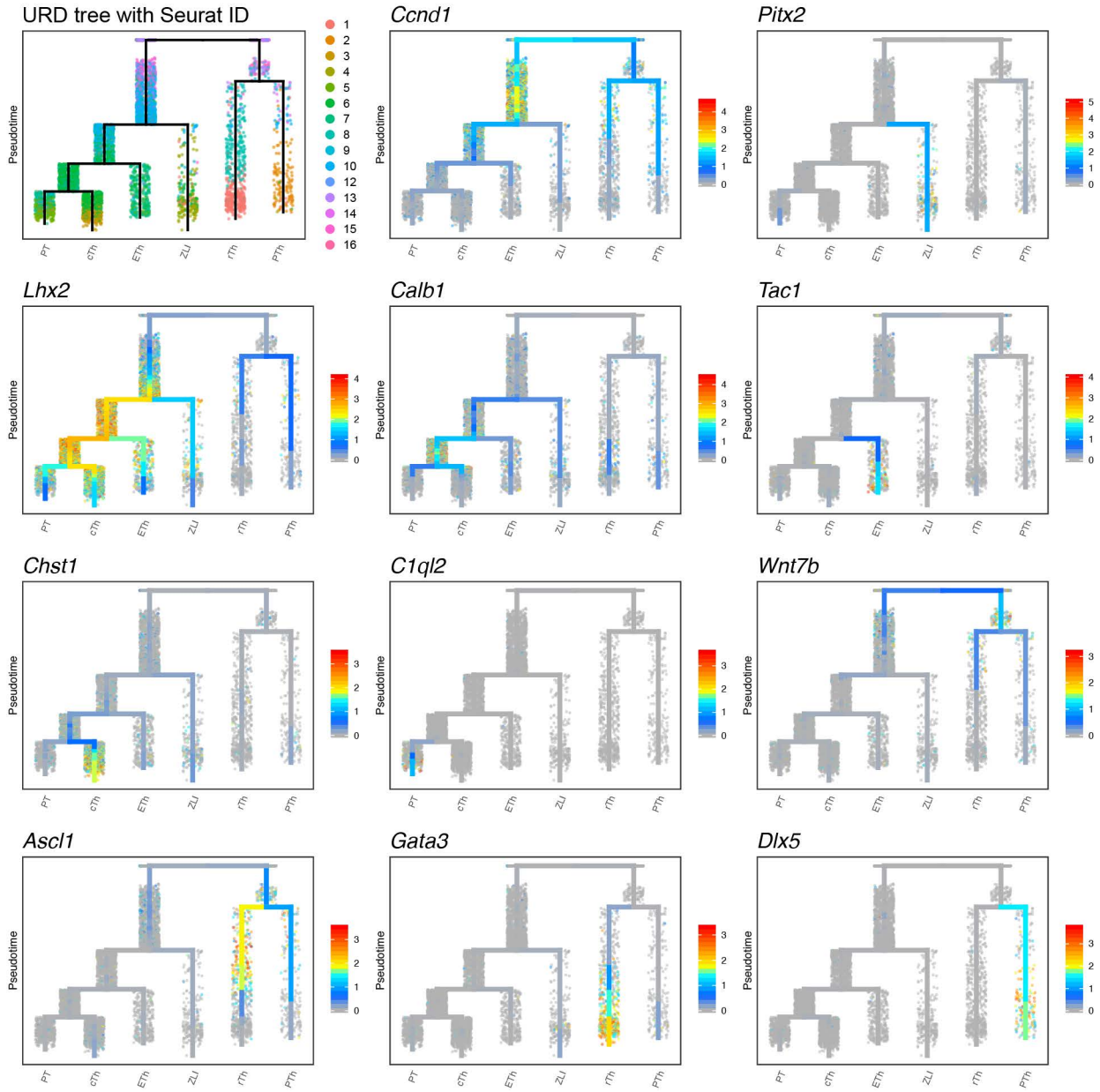


Figure S7. Expression of marker genes in the URD-inferred trajectory tree. Variable expression of markers highlighting distinct segment(s) of the trajectory as expected.

Supplementary Figure S8

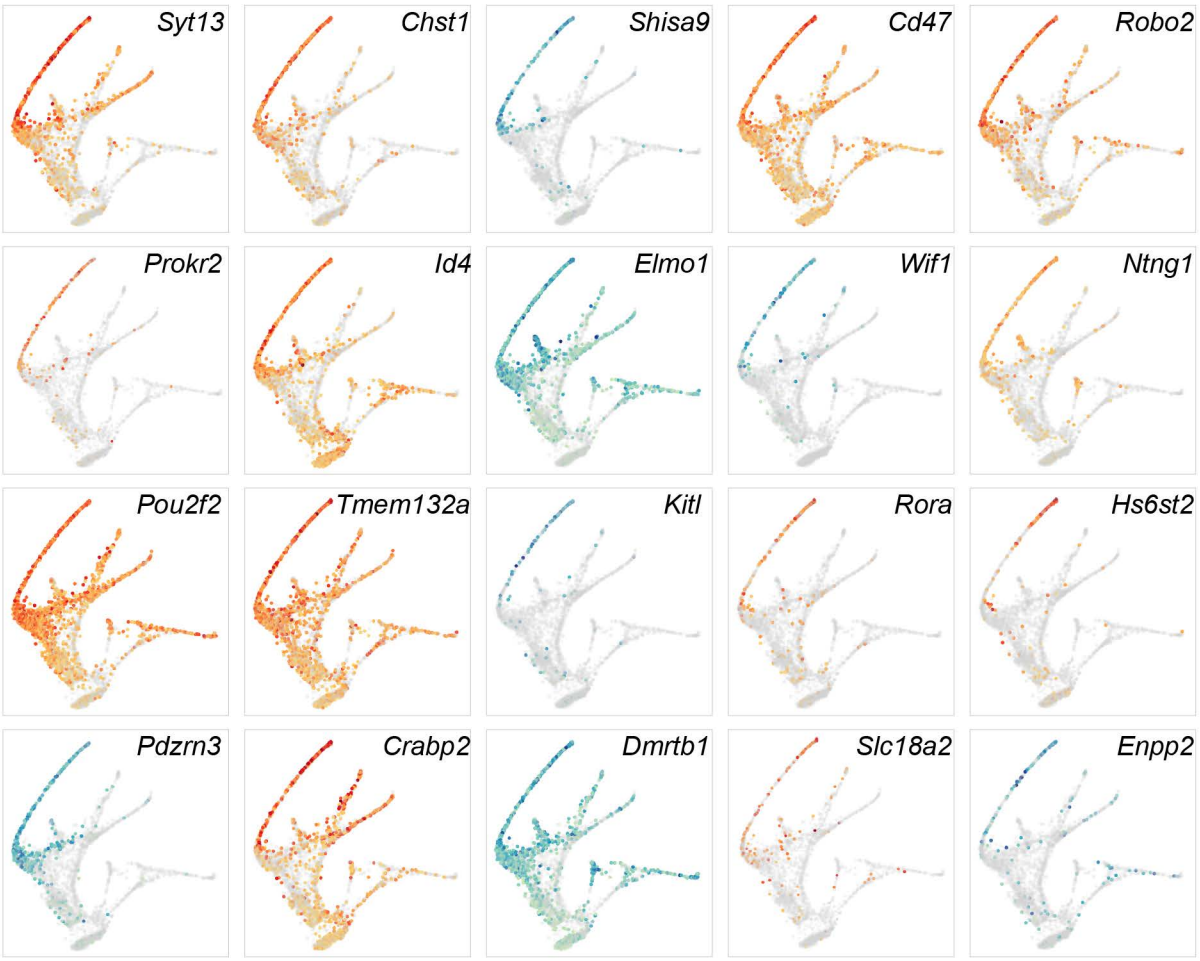


Figure S8. Expression of thalamus-specific genes overlaid on the trajectory tree. Note the gradual increase and the enrichment of gene expression along the trajectory corresponding to the caudal thalamus. Genes that are previously identified as thalamus-specific genes (Mallika et al., 2015; Suzuki-Hirano et al., 2011) are plotted in orange, whereas new markers are plotted in blue/green.

Supplementary Figure S9

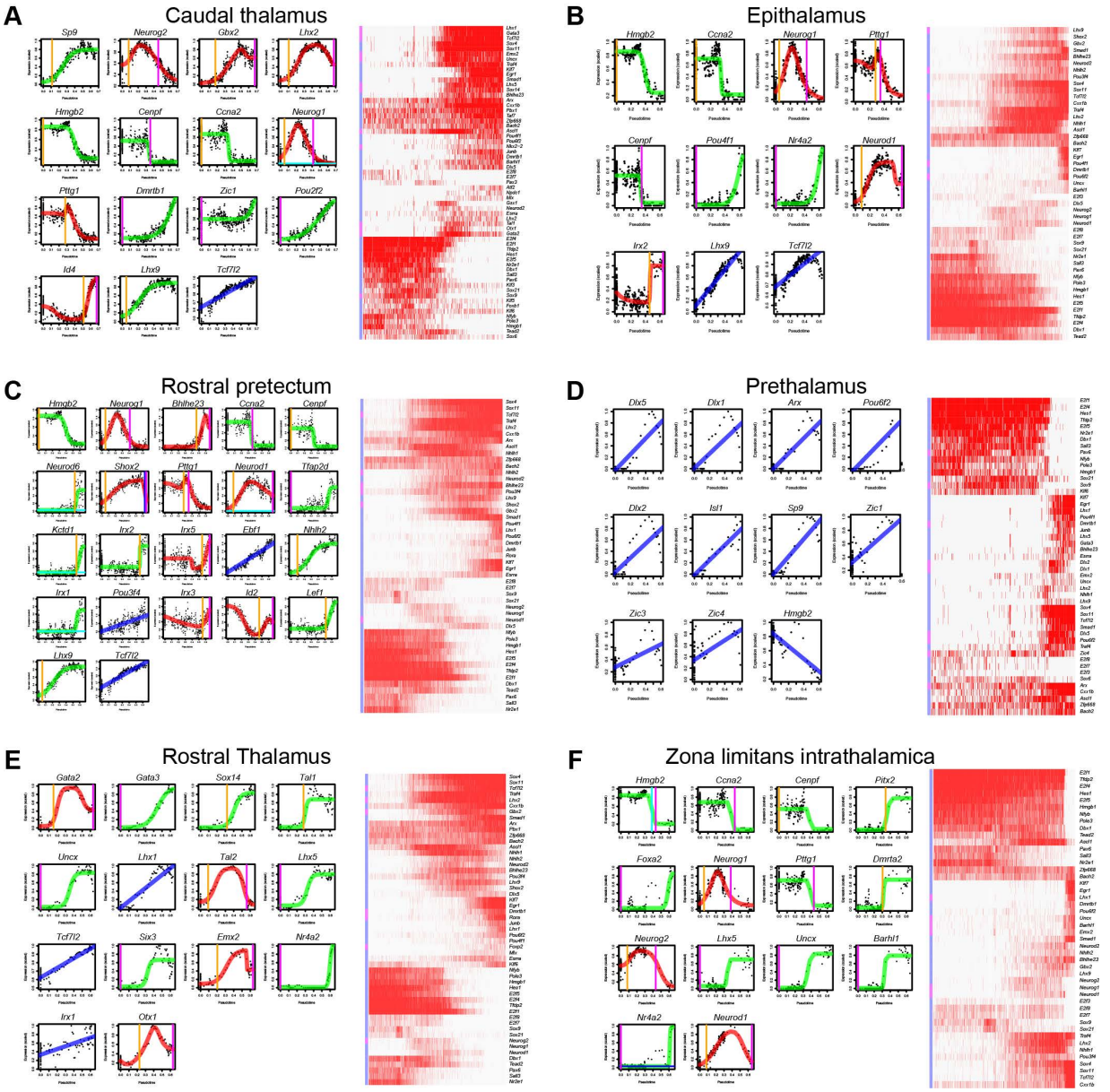


Figure S9. Temporal dynamics of key transcription factors and the associated regulons in different diencephalic lineages. (A-F) Temporal expression of transcription factors (left), and binary regulon activities (right) in six lineages of the diencephalon. In the x axis, cells are arranged according to pseudotime (increases from left to right). Each dot represents the moving-window average of expression levels; lines show impulse response fitting: blue for genes that change at a constant rate, green for a single sigmoid impulse, and red for a convex or concave impulse; vertical lines indicate the onset (orange) and offset (magenta) of regulon activation. The transcription factors that are significantly changed during lineage specification are indicated (in red) by the color bar on the left side of the heatmap.

Table S1. **Excel file of mark genes of each cluster**. (**A**) Differentially expressed genes identified by Seurat (first spreadsheet). (**B**) Top 20 markers for each cluster (second spreadsheet). The known markers are shown in blue. (**C**) Marker genes for neural progenitor cells (Pro), neuron precursors (Pre), and postmitotic neurons (N) (third spreadsheet). Column A: gene symbol; B: transcription factor; column C: description of gene name; column D: unadjusted p value; Column E: adjusted p value based on bonferroni correction using all genes in the dataset; Column F: log2 fold-change of the average expression between the two groups (positive values indicate that the gene is more highly expressed in the first group); Column G: The percentage of cells where the gene is detected in the testing cluster; Column H: the percentage of cells where the gene is detected in the rest of the dataset; Column I: difference of the percentage of cells where the gene is detected; Column J: cluster identification; Column K: annotation of the cell cluster.

Click here to Download Table S1

Table S2. **Excel file of thalamic aRG versus IPC analysis**. (**A**) Differentially expressed genes identified by Seurat (first spreadsheet). (**B**) Functional and pathway enrichment of thalamic apical radial glial feature genes (second spreadsheet). (**C**) IPC-specific genes are enriched for Foxo pathway (third spreadsheet).

Click here to Download Table S2

Table S3. **Excel file of module membership of genes**. (**A** and **B**) Module membership (kME) vales of genes in the caudal thalamus (first spreadsheet) and rostral pretectum (second spreadsheet). The module whose module eigengene is closely corrected with IPCs is highlighted in yellow.

Click here to Download Table S3

Table S4. **Excel table of cascade genes of six diencephalic lineages**. (**A**) Cascade genes identified by URD's *aucprTestAlongTree* function (first spreadsheet). (**B**) Lineage specific markers (second spreadsheet).

Click here to Download Table S4

Computer codes

Click here to Download zip file containing computer codes associated with the manuscript.