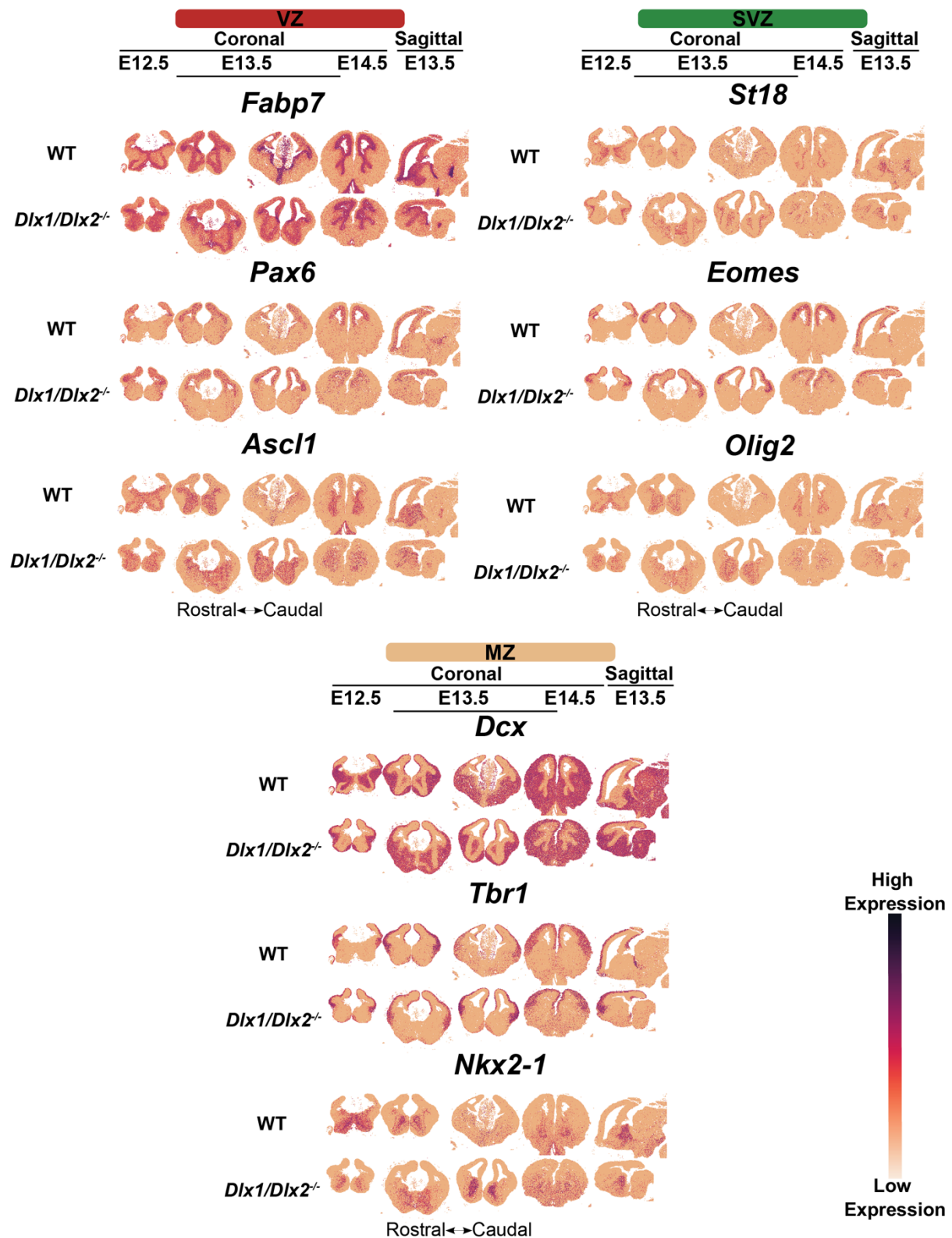


The DLX/Notch axis is necessary for spatiotemporal regulation of neural cell fate

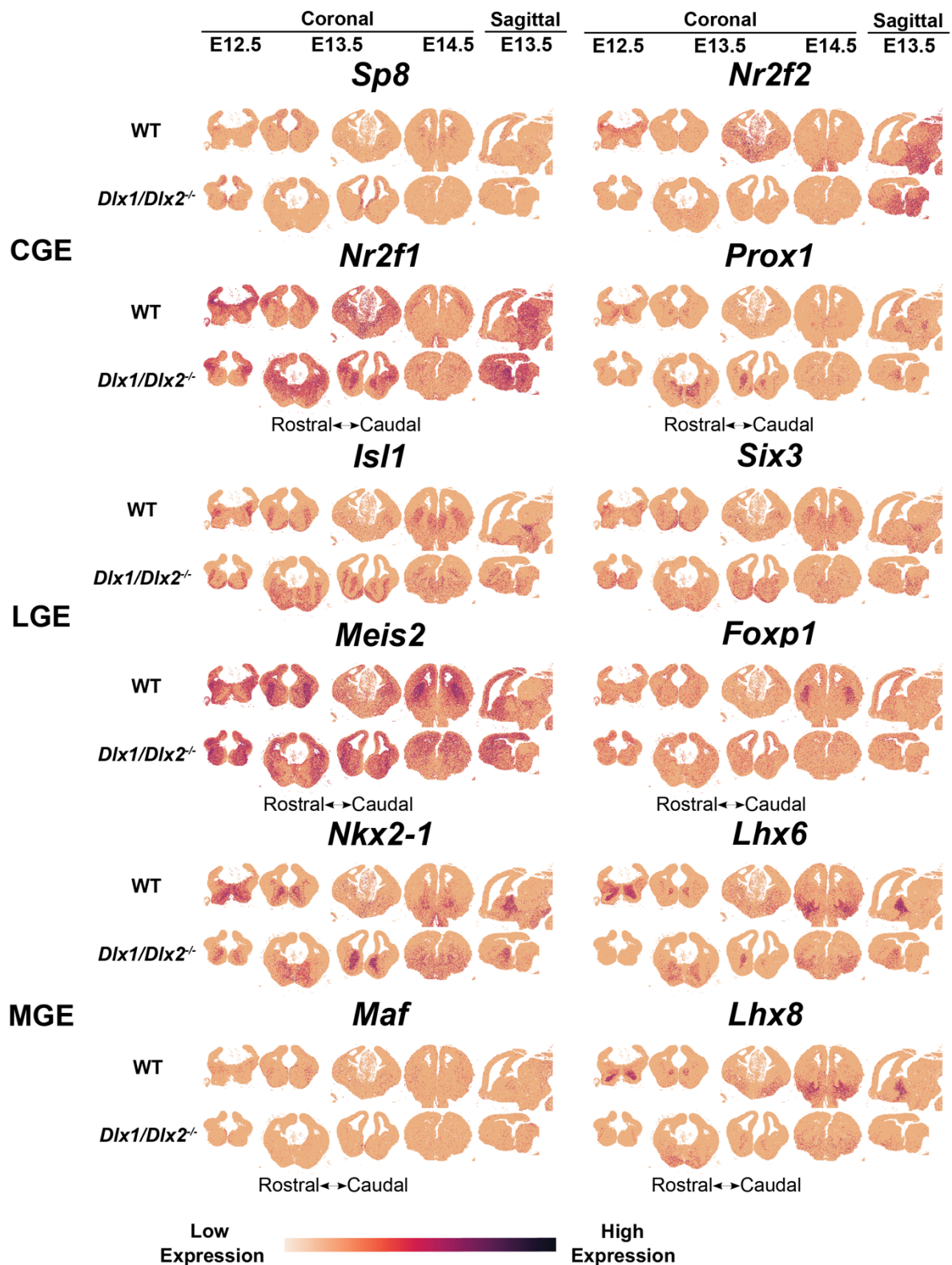
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Supplementary Figures: 14

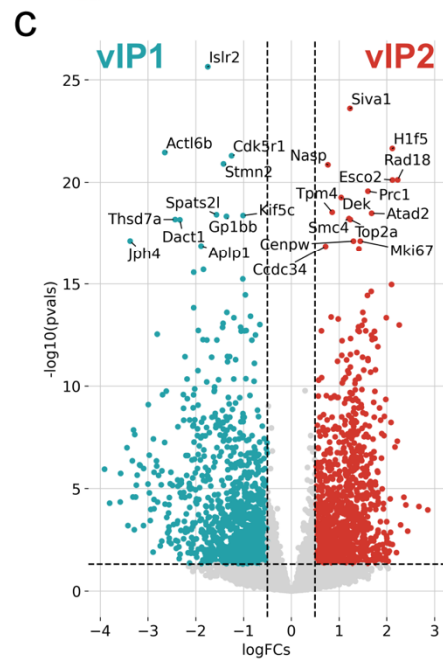
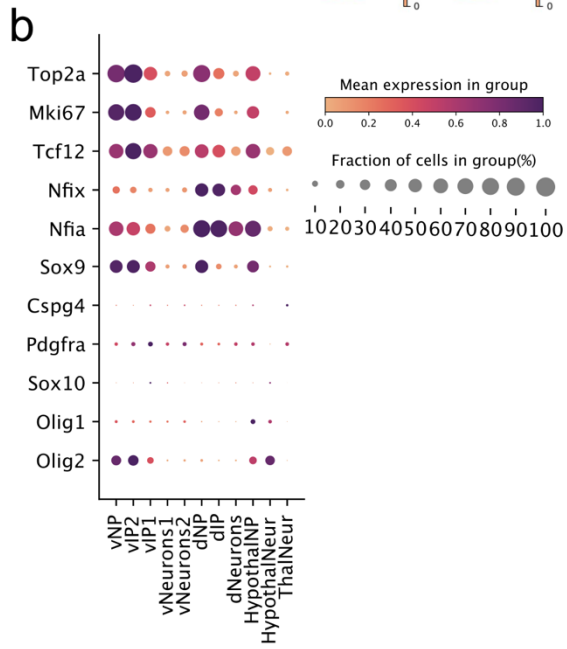
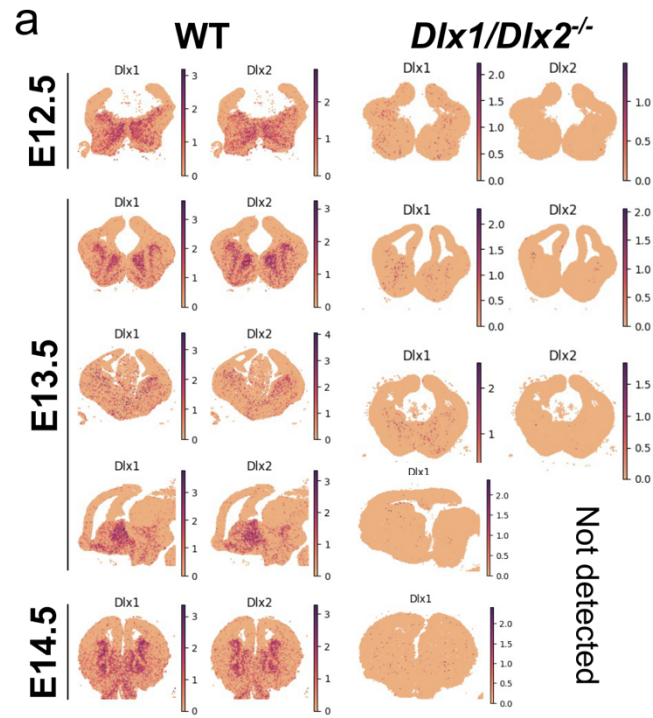
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Supplementary Figure 1. Spatial transcriptomics captured the laminal specific gene expression in WT and *Dlx1/Dlx2*^{-/-} forebrain. Expression plots of E12.5-E14.5 WT and *Dlx1/Dlx2*^{-/-} forebrain sections showing the expression of markers of VZ (*Fabp7*, *Pax6*, *Ascl1*), SVZ (*St18*, *Eomes*, *Olig2*), and MZ (*Dcx*, *Tbr1*, *Nkx2-1*). (E13.5 n=2, E12.5/E14.5 n=1).



Supplementary Figure 2. GE subregion specific marker gene expression in WT and *Dlx1/Dlx2*^{-/-} forebrain. Expression plots of E12.5-E14.5 WT and *Dlx1/Dlx2*^{-/-} forebrain sections showing the expression of markers of CGE (*Sp8*, *Nr2f1*, *Nr2f2*, *Prox1*), LGE (*Isl1*, *Six3*, *Meis2*, *Foxp1*), and MGE (*Nkx2-1*, *Lhx6*, *Lhx8*, *Maf*). (E13.5 n=2, E12.5/E14.5 n=1).

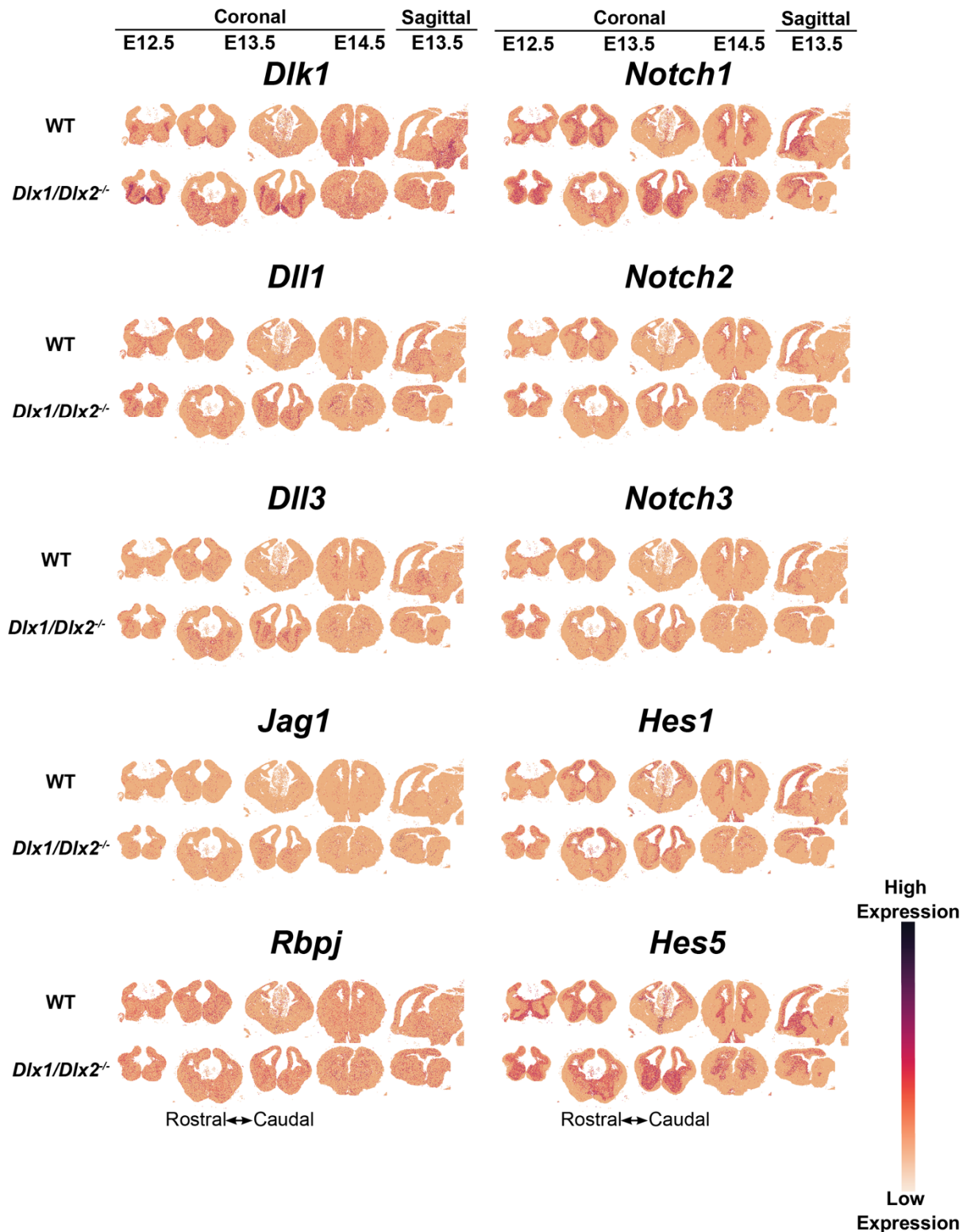


Supplementary Figure 3. Loss of Spatial transcriptomics confirmed *Dlx1/Dlx2*^{-/-} and confirmation of glial gene expression and vIPs differential expression.

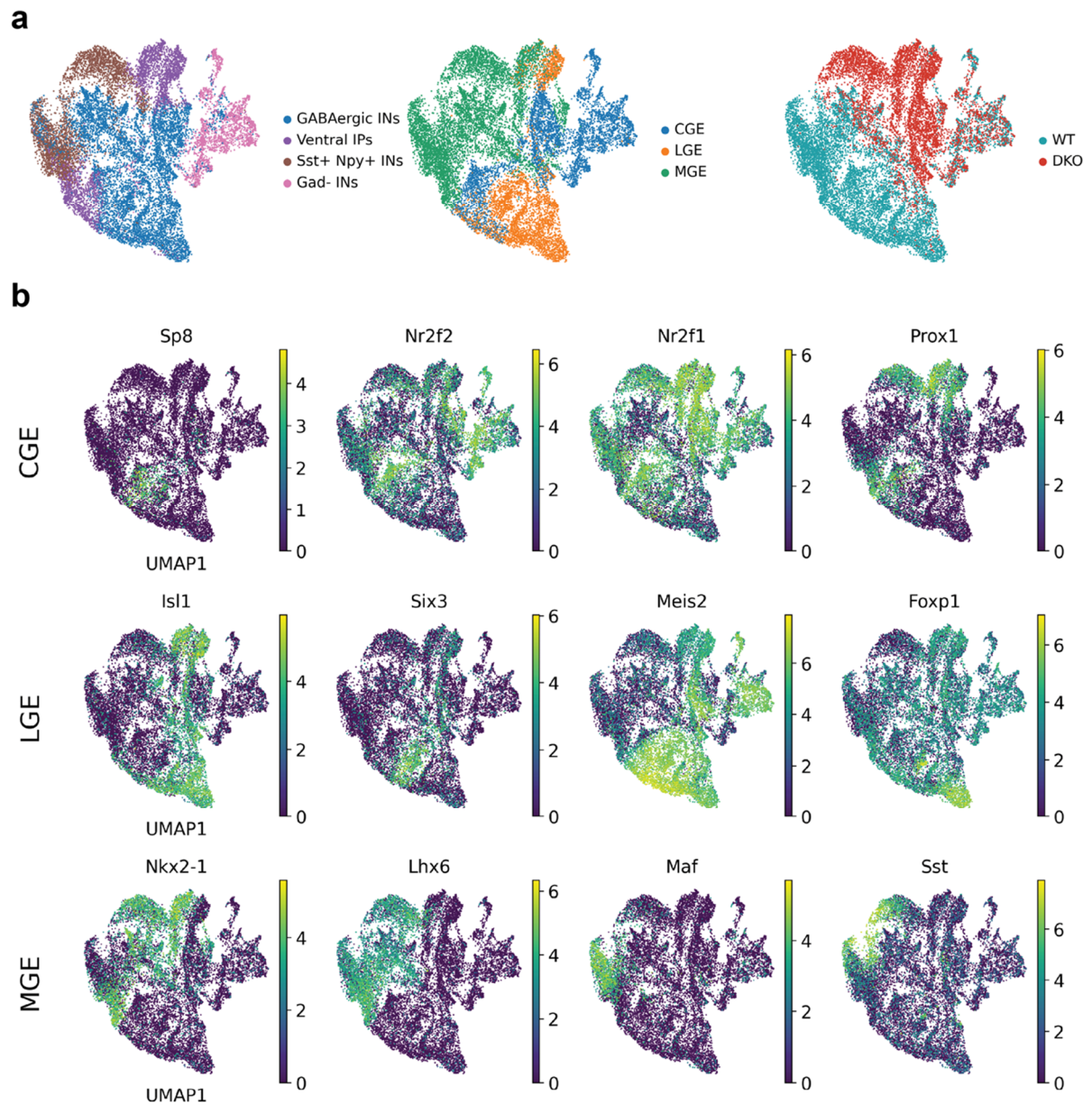
(a) Expression of *Dlx1* and *Dlx2* in WT (left) or *Dlx1/Dlx2*^{-/-} sections (right), showing little to no expression detected in the *Dlx1/Dlx2*^{-/-} forebrain. No sequences were mapped to *Dlx2* in the E13.5 sagittal and E14.5 coronal *Dlx1/Dlx2*^{-/-} samples. The *Dlx1* and *Dlx2* transcripts detected in the *Dlx1/Dlx2*^{-/-} forebrain reflect the remaining sequences in exon 1 of *Dlx1* and *Dlx2* given the knockout of exons 2 and 3 and intergenic enhancers of *Dlx1* and *Dlx2*.

(b) Expression of proliferative markers (*Top2a*, *Mki67*), glial promoting factors (*Tcf12*, *Nfix*, *Nfia*, *Sox9*), and OPC markers (*Cspg4*, *Pdgfra*, *Sox10*, *Olig1*, *Olig2*) in all sections. vNP: ventral neural progenitors; vIP: ventral intermediate progenitors; vNeurons: ventral neurons; dNP: dorsal neural progenitors; dIP: dorsal intermediate progenitors; dNeurons: dorsal neurons; HypothalNP: hypothalamic neural progenitors; HypothalNeur: hypothalamic neurons; ThalNeur: thalamic neurons.

(c) Volcano plot showing the differentially expressed genes in vIP1 (blue) and vIP2 (red) of both WT and *Dlx1/Dlx2*^{-/-} GE. Labelled are the 25 most significantly differentially expressed genes.

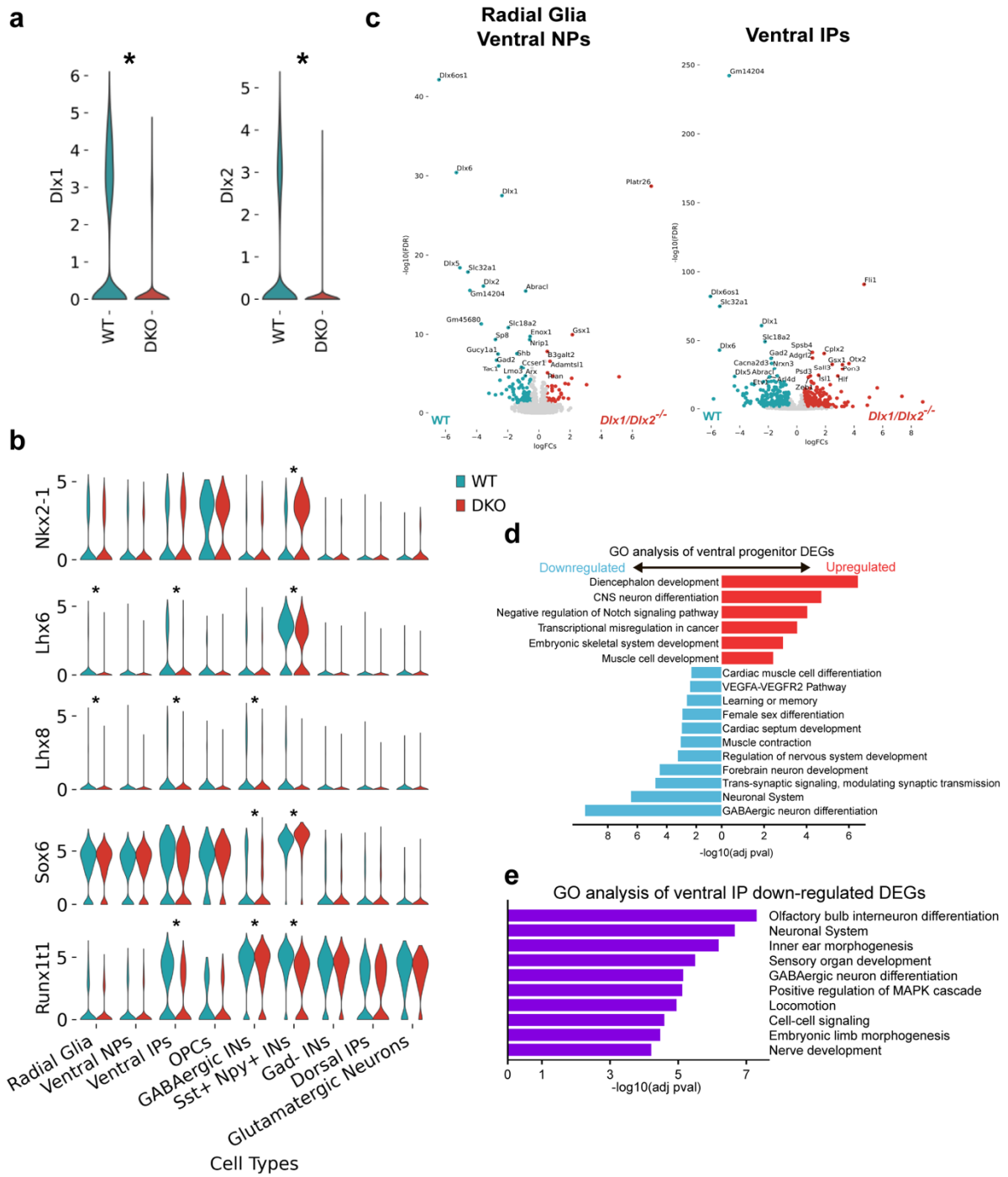


Supplementary Figure 4. Differential expression of Notch signalling genes in WT and *Dlx1/Dlx2*^{-/-} GE. Expression plots of E12.5-E14.5 WT and *Dlx1/Dlx2*^{-/-} forebrain sections showing the expression of *Dlk1*, *Dll1*, *Dll3*, *Jag1*, *Rbpj*, *Notch1*, *Notch2*, *Notch3*, *Hes1*, and *Hes5*. (E13.5 n=2, E12.5/E14.5 n=1).



Supplementary Figure 5. GE subregion resolution in WT and *Dlx1/Dlx2*^{-/-} snMultiome data set.

- (a)** UMAPs of the ventral IPs and IN population from snMultiome WT and *Dlx1/Dlx2*^{-/-} GE coloured by: (left) cell types, (middle) GE subregions, and (right) genotype.
- (b)** UMAPs showing GE subregion marker expression profiles in ventral IPs and IN population WT and *Dlx1/Dlx2*^{-/-} GE.



Supplementary Figure 6. Differential gene expression in WT vs *Dlx1/Dlx2*^{-/-} ventral progenitors (radial glia and ventral NPs) and ventral IPs.

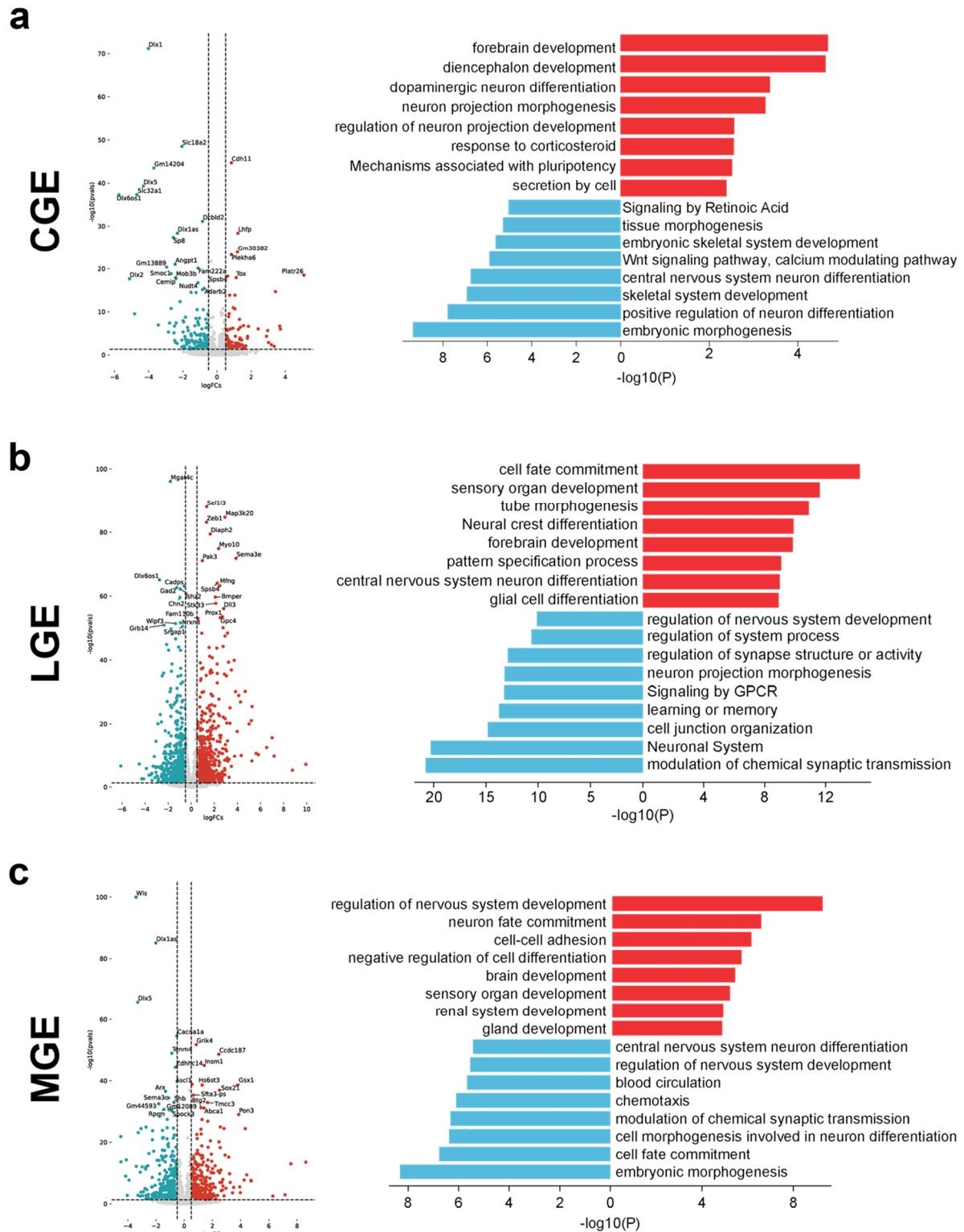
(a) Violin plots showing expression of *Dlx1* and *Dlx2*. * Denotes FDR<0.05.

(b) Violin plots of *Sst*⁺ *Npy*⁺ INs promoting TFs *Nkx2-1*, *Lhx6*, *Lhx8*, *Sox6*, and *Runx1t1*. * Denotes FDR<0.05.

(c) Volcano plots showing the differentially expressed genes in the radial glia and ventral NPs (left) and ventral IPs (right).

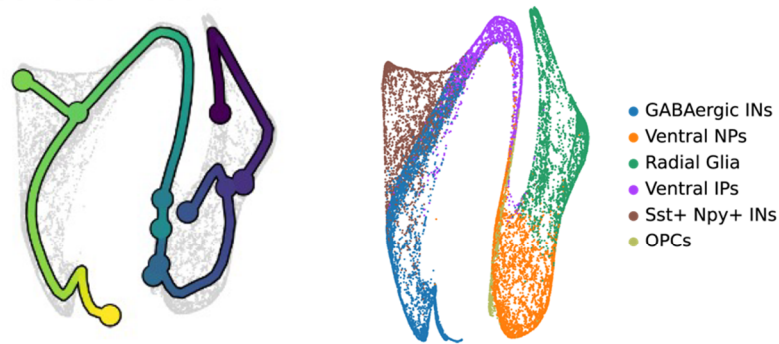
(d) GO analysis of upregulated and downregulated genes against all genes expressed in the ventral progenitors, including radial glia and ventral NPs in *Dlx1/Dlx2*^{-/-} GE.

(e) GO analysis of downregulated genes against all genes expressed in the *Dlx1/Dlx2*^{-/-} ventral IPs.

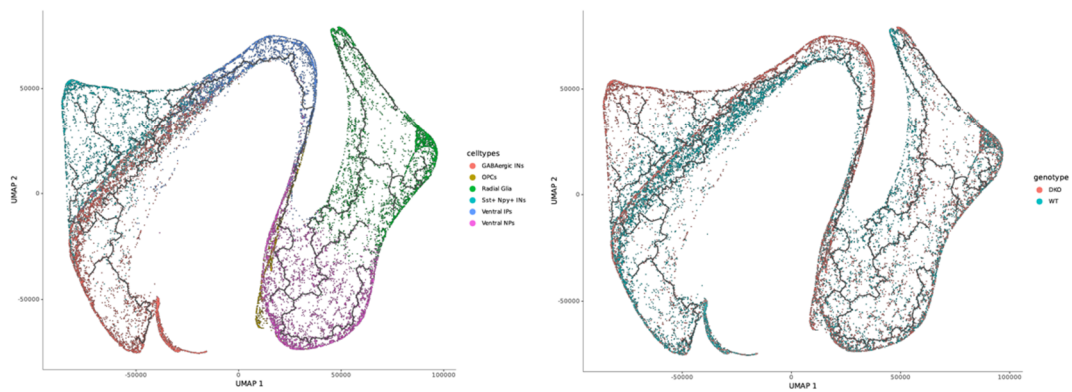


Supplementary Figure 7. Differential expression analysis of GE subregions. (left) Volcano plots showing the differential expression analysis comparing *Dlx1/Dlx2*^{-/-} vs WT (a) CGE, (b) LGE, and (c) MGE. (right) Gene ontology analyses of the differentially expressed genes from *Dlx1/Dlx2*^{-/-} vs WT (a) CGE, (b) LGE, and (c) MGE. Enrichment analysis performed against all genes expressed in respective GE subregions.

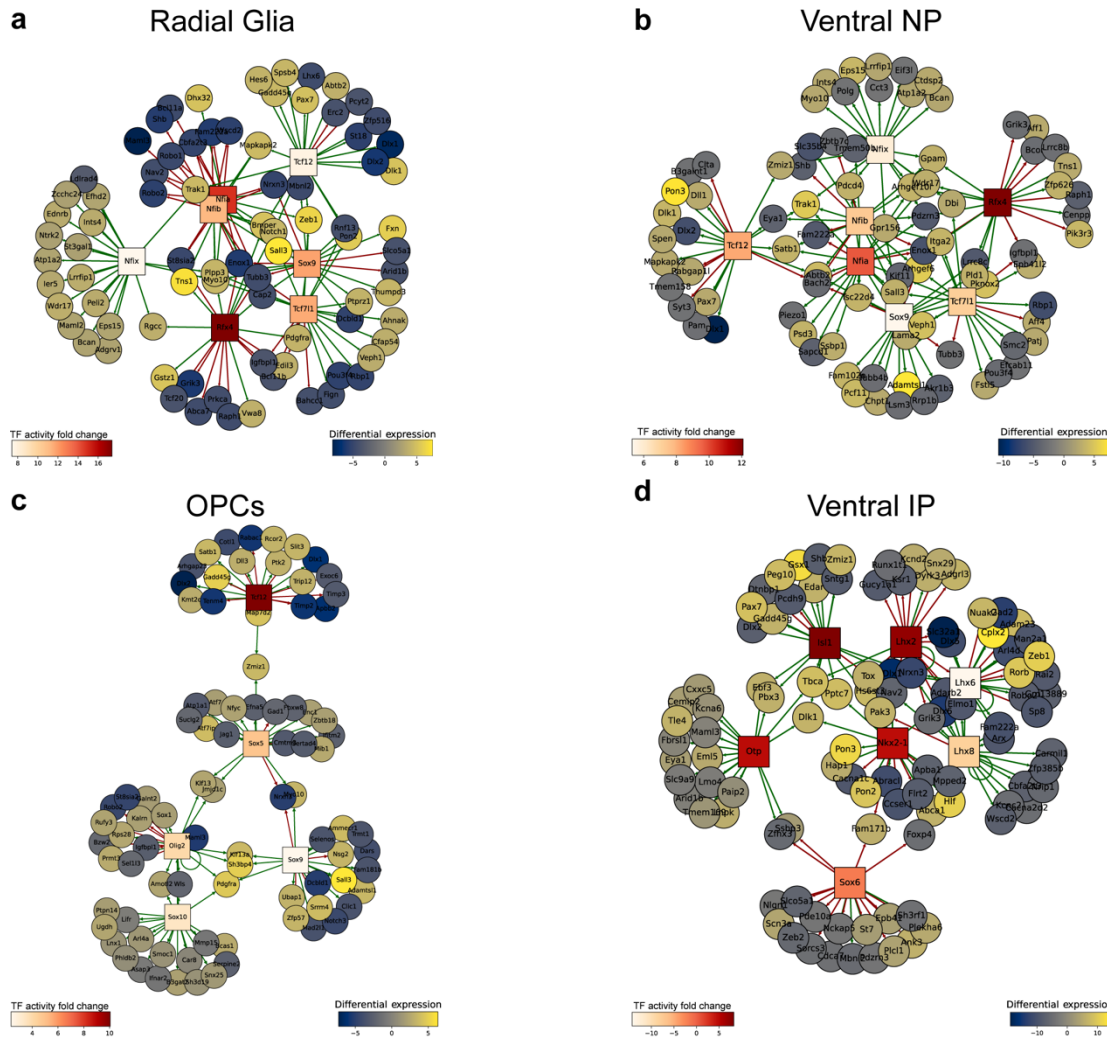
a
scFates result



b
Monocle3 result



Supplementary Figure 8. Trajectory analyses results comparison. (a) scFates and (b) Monocle3 results.

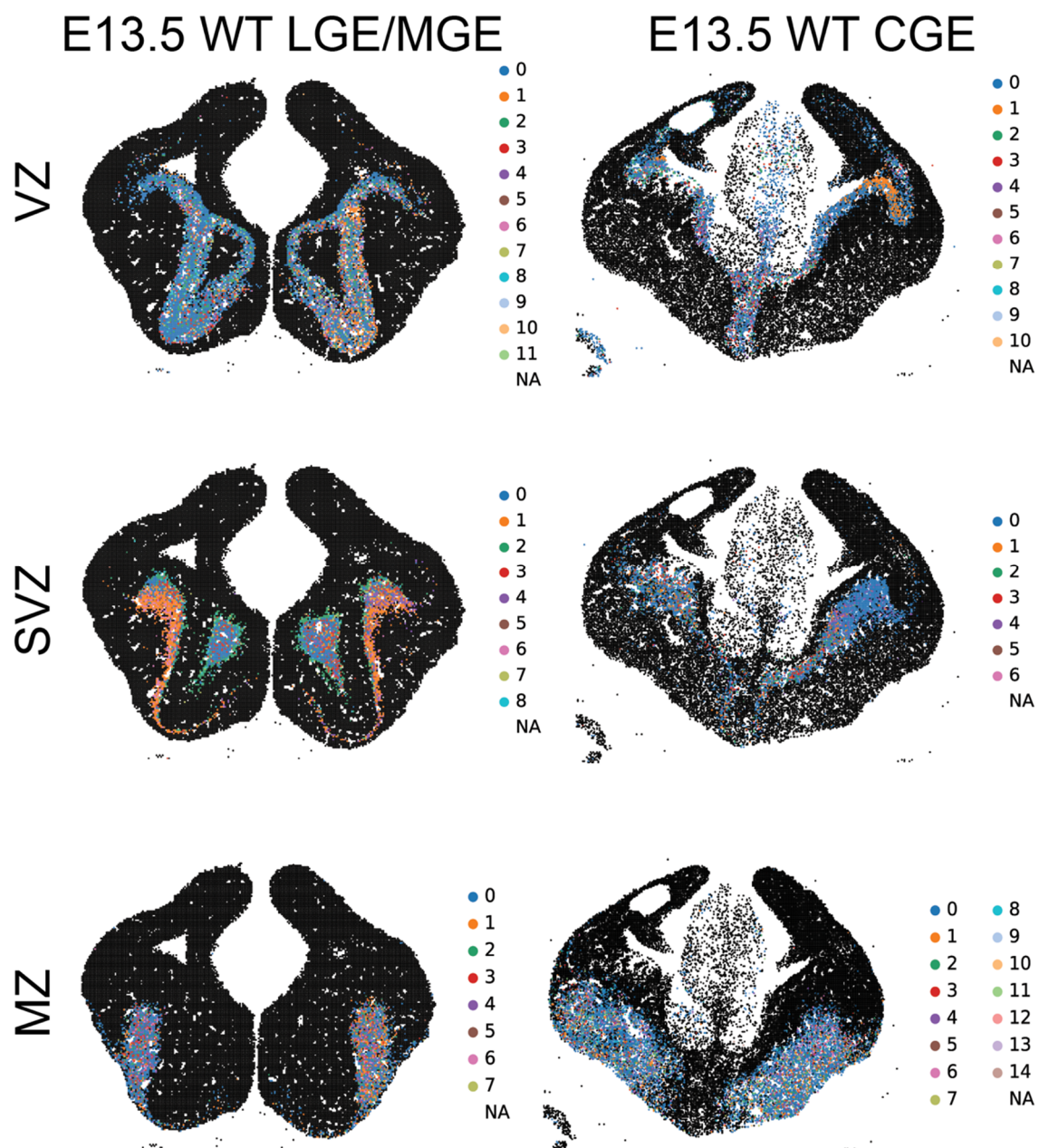


Supplementary Figure 9. eRegulon networks and differential TF activities in WT and *Dlx1/Dlx2*^{-/-} GE.

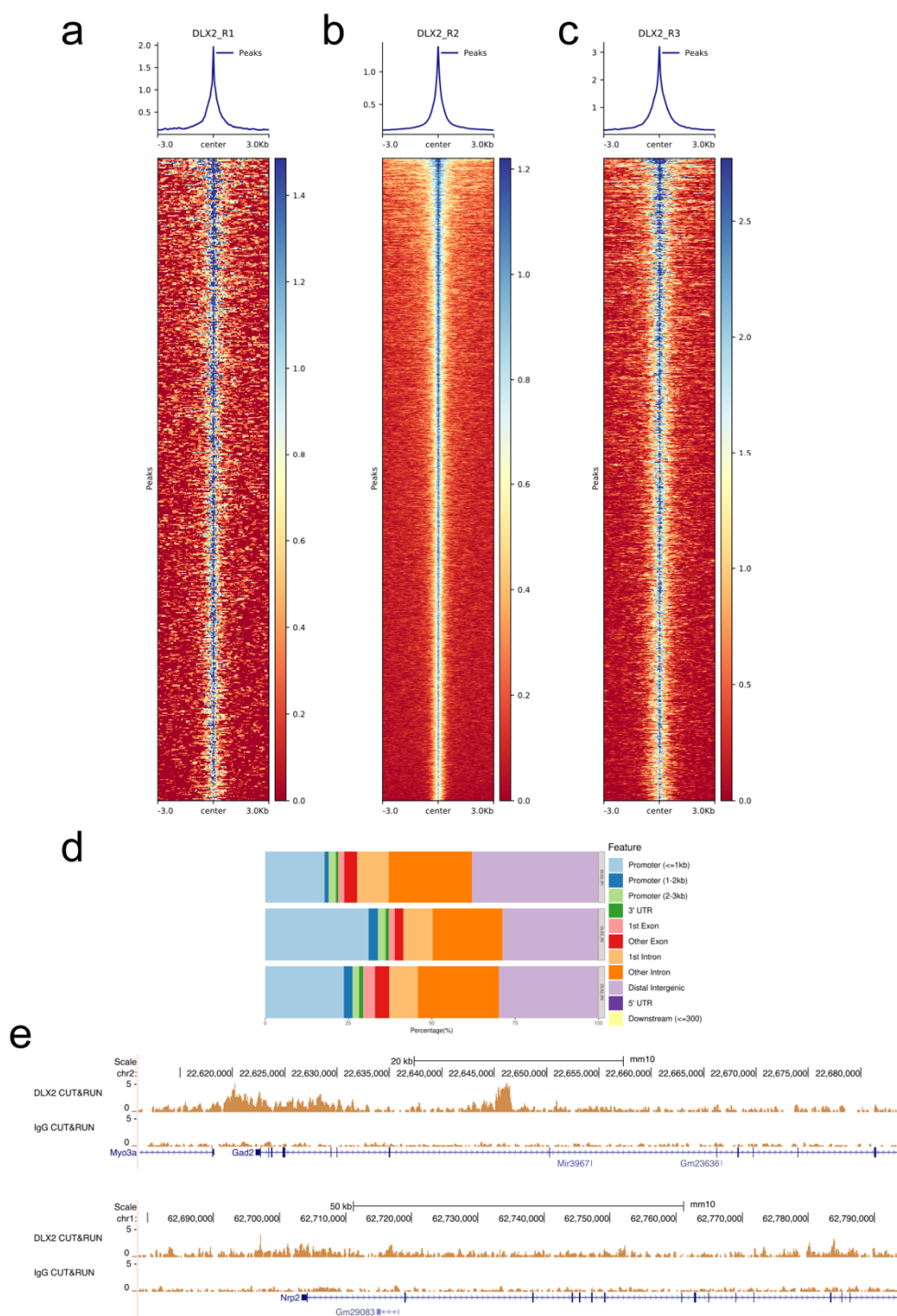
(a-b) Gene regulatory networks of differentially upregulated glial promoting eRegulons in (a) radial glia and (b) ventral NPs, including RFX4, NFIA, NFIB, NFIX, SOX9, TCF12, and TCF7L1. eRegulon (squares) colours represents the TF activity fold change, and gene target (circles) colours represents the expression fold change in WT vs *Dlx1/Dlx2*^{-/-} ventral progenitors.

(c) Gene regulatory networks of differentially upregulated glial promoting eRegulons in OPCs, including TCF12, SOX5, OLIG2, SOX9, and SOX10. eRegulon (squares) colours represents the TF activity fold change, and gene target (circles) colours represents the expression fold change in WT vs *Dlx1/Dlx2*^{-/-} ventral progenitors.

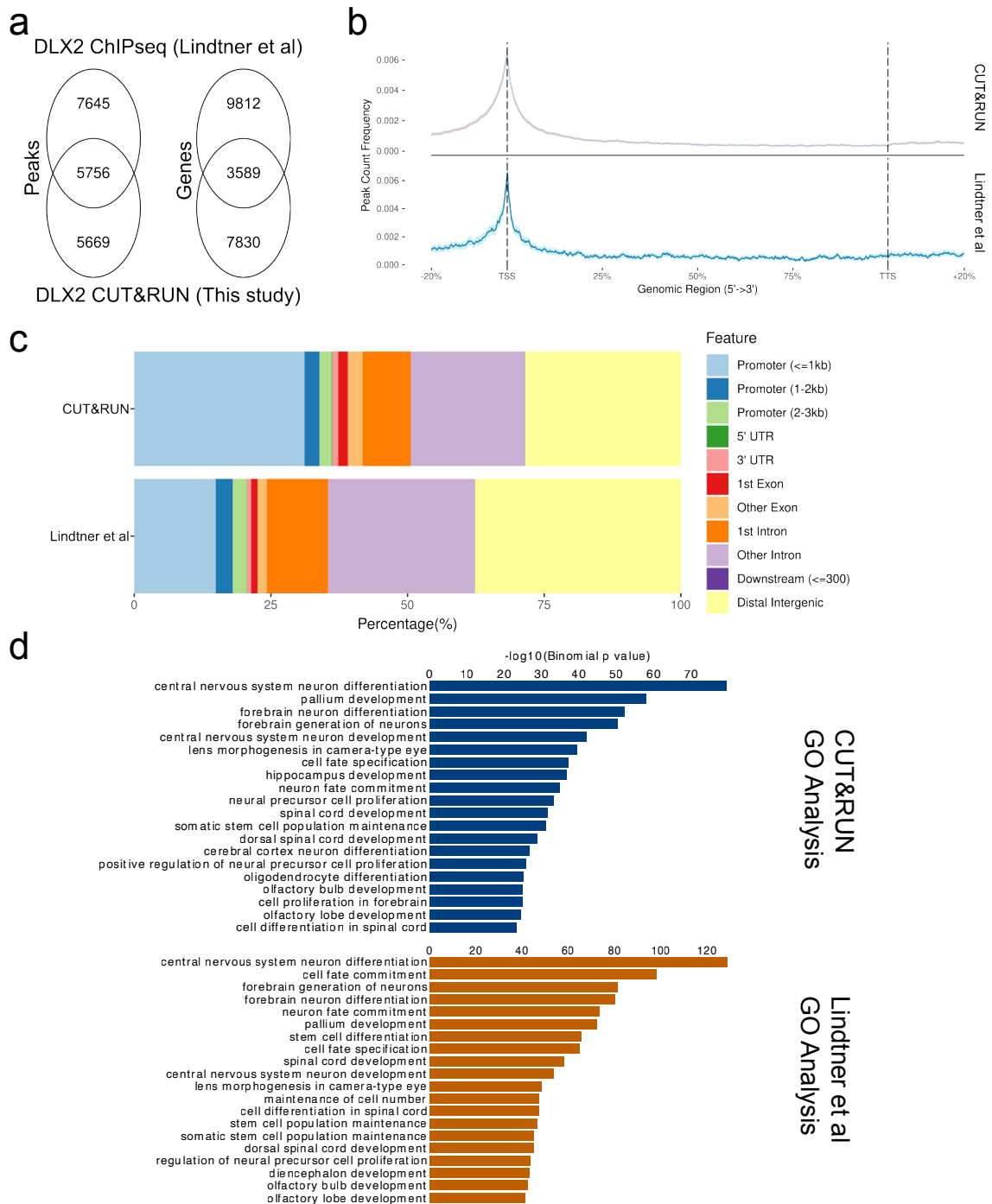
(d) Gene regulatory networks of differentially upregulated glial promoting eRegulons in ventral IP, including ISL1, LHX2, OTP, SOX6, NKX2-1, LHX6, LHX8. eRegulon (squares) colours represents the TF activity fold change, and gene target (circles) colours represents the expression fold change in WT vs *Dlx1/Dlx2*^{-/-} ventral progenitors.



Supplementary Figure 10. Sub-laminal spatial patterns were present in E13.5 WT LGE and MGE.



Supplementary Figure 11. Validation of DLX2 CUT&RUN using WT E13.5 mouse GE.
(a-c) DLX2 CUT&RUN replicates peak heatmap (n=3).
(d) Genomic annotation of DLX2 CUT&RUN replicates (n=3).
(e) Consensus DLX2 CUT&RUN signal at the *Gad2* (top) and *Nrp2* (bottom) loci confirming that DLX2 occupies the promoter regions of *Gad2* and *Nrp2* genes.



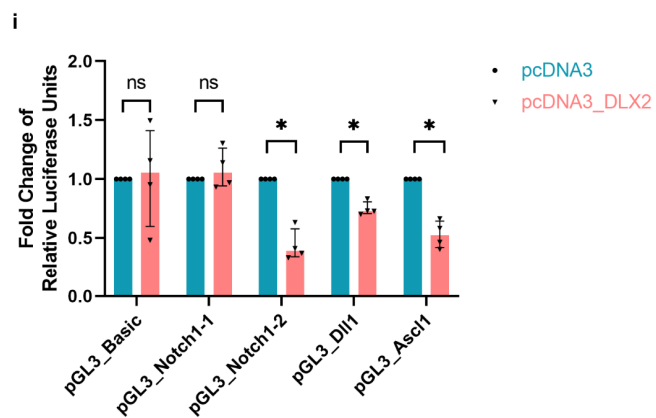
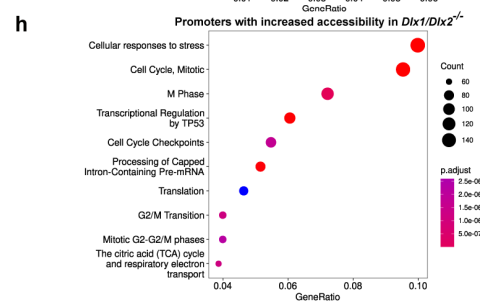
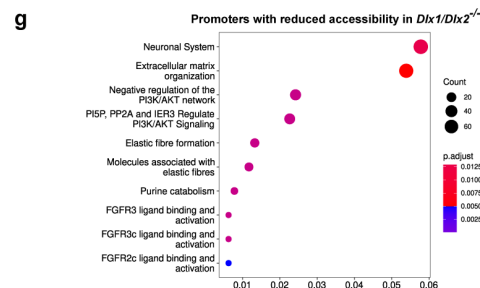
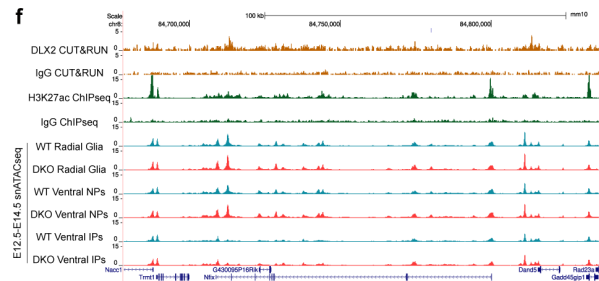
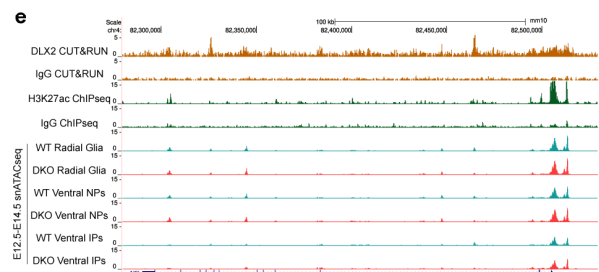
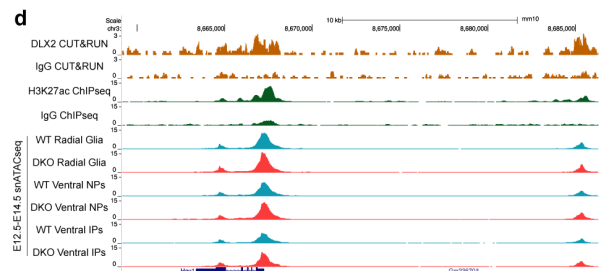
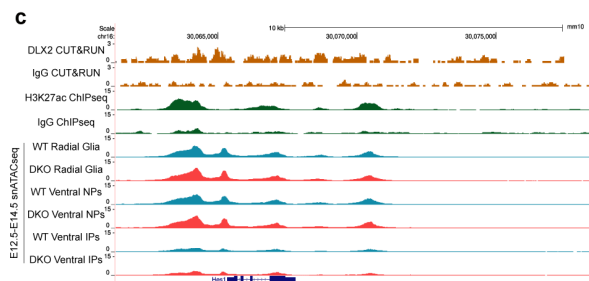
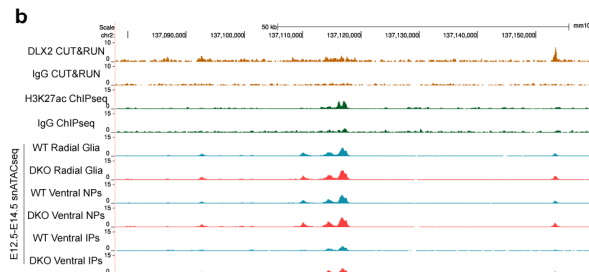
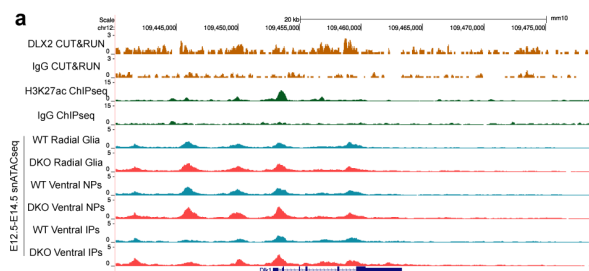
Supplementary Figure 12. Comparison of DLX2 CUT&RUN (this study) and DLX2 ChIPseq (Lindtner et al 2019).

(a) Venn diagram showing the peaks (left) and genes (right) that were common or unique in the DLX2 CUT&RUN and DLX2 ChIPseq (Lindtner et al 2019).

(b) Genomic location and relative frequency of the peaks from DLX2 CUT&RUN and ChIPseq around transcription start sites (TSS) and transcription termination site (TTS).

(c) Comparison of the genomic annotation of DLX2 CUT&RUN and DLX2 ChIPseq.

(d) GO analysis of DLX2 CUT&RUN and DLX2 ChIPseq.

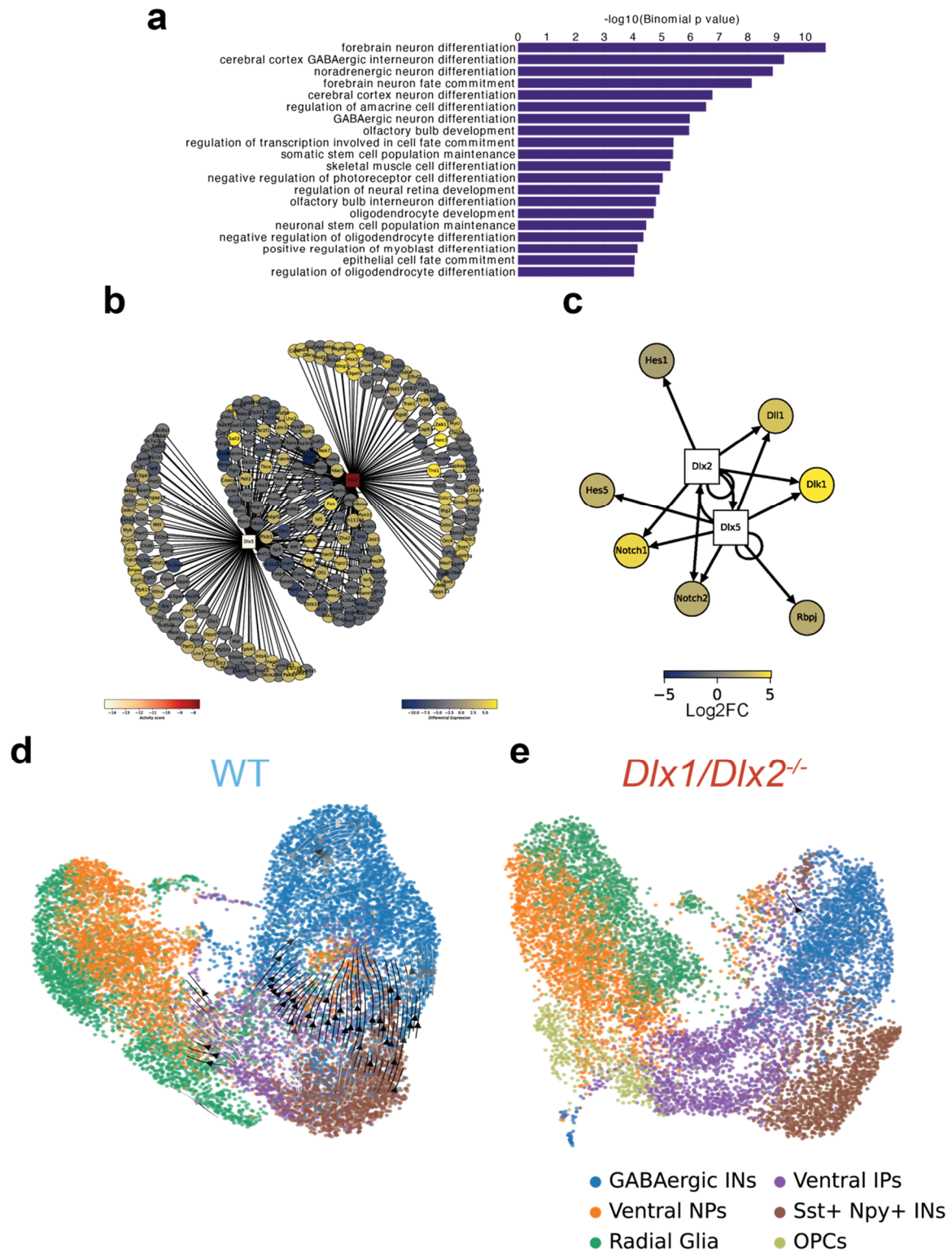


Supplementary Figure 13. Genomic tracks of Notch signalling genes and glial promoting TFs regulated by DLX2.

(a-f) DLX2 CUT&RUN and H3K27ac ChIPseq of E13.5 WT GE and snATACseq of E12.5-E14.5 WT and *Dlx1/Dlx2*^{-/-} GE coverage at (A) *Dlk1*, (B) *Jag1*, (C) *Hes1*, (D) *Hey1*, (E) *Nfib*, and (F) *Nfix* loci. CUT&RUN tracks shown in orange, ChIPseq tracks shown in green, WT snATACseq tracks shown in red and *Dlx1/Dlx2*^{-/-} snATACseq tracks shown in blue.

(g-h) GO of promoter regions with reduced (g) or increased (h) accessibility in *Dlx1/Dlx2*^{-/-} GE

(i) Luciferase assay results showing DLX2 represses reporter gene expression when co-expressed with Notch1, Dll1, and Ascl1 promoter regions. Shown are mean ± interquartile range, n=3 independent experiments, *p-value <0.005 (pGL_Notch1_2: 0.004, pGL3_Dll1: 0.003, pGL3_Asc1: 0.004). Statistical significance determined by two-sided paired t-test.



Supplementary Figure 14. Both DLX5 and DLX2 promote INs differentiation and shares transcriptional gene targets.

- (a) Gene ontology analysis of DLX5 ChIPseq of E13.5 WT mouse GE (Lindtner et al., 2019).
- (b) Gene network map of unique and common DLX2 and DLX5 target genes identified using CUT&RUN and ChIPseq analysis (Lindtner et al., 2019), respectively.
- (c) Network analysis of DLX2 and DLX5 regulation of Notch signalling pathway genes.
- (d) DLX5 perturbation simulation of WT snMultiome data results in showing a shift of ventral IPs to progenitors and *Sst*⁺ *Npy*⁺ INs into GABAergic INs depicted by the black arrows.
- (e) DLX5 perturbation simulation of *Dlx1/Dlx2*^{-/-} snMultiome data results in showing a minimal shift in GABAergic INs depicted by the black arrows.