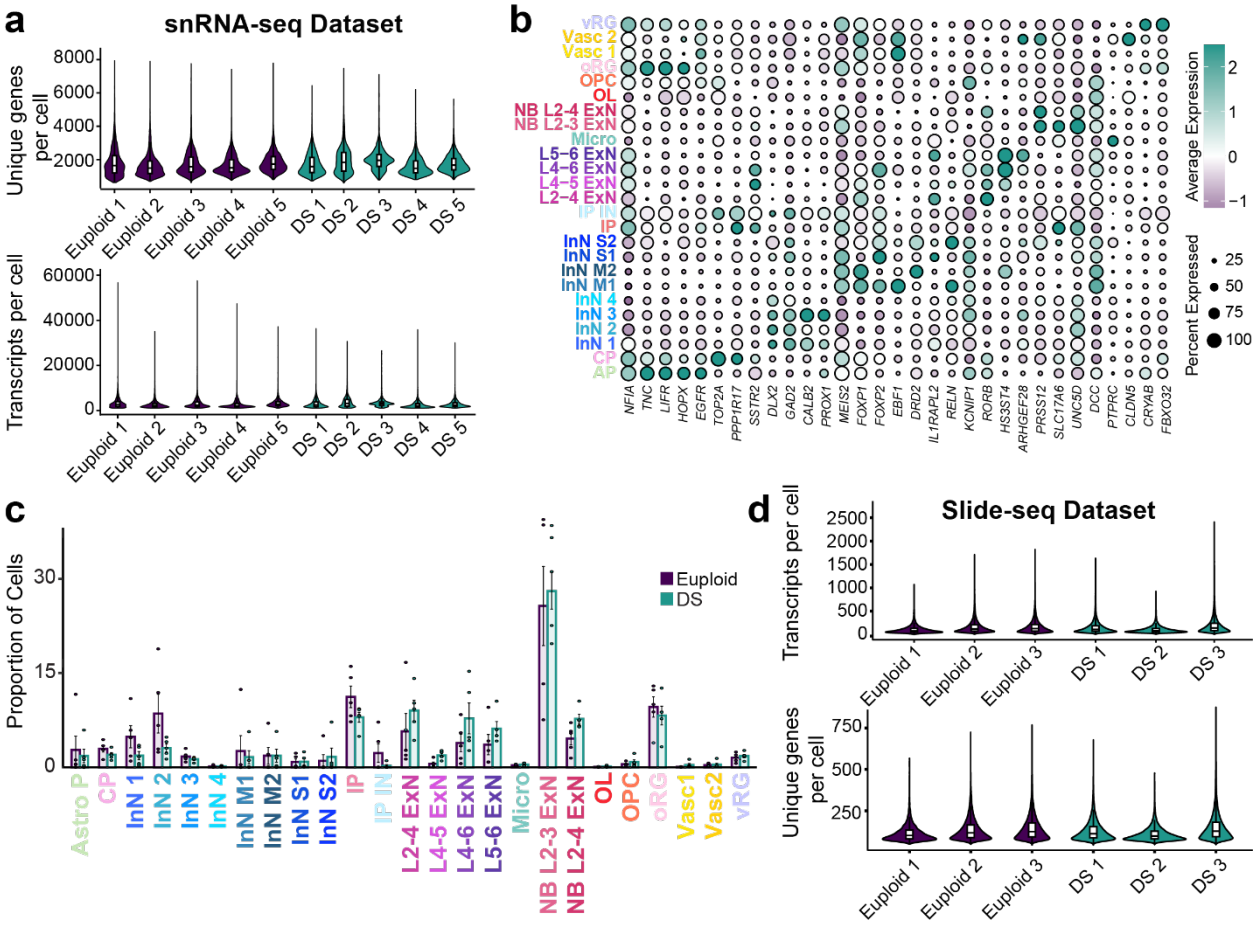
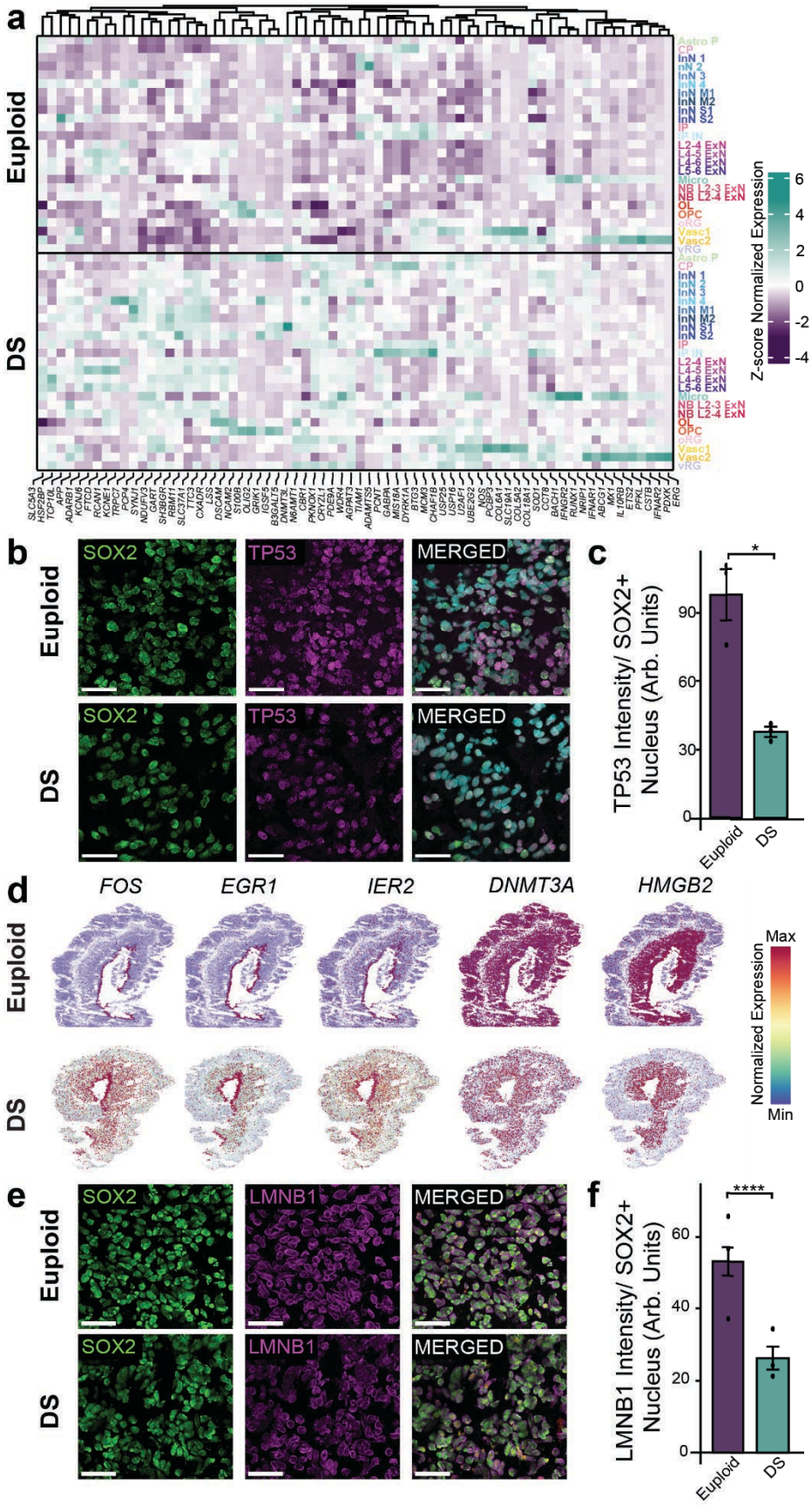
Supplementary Figures



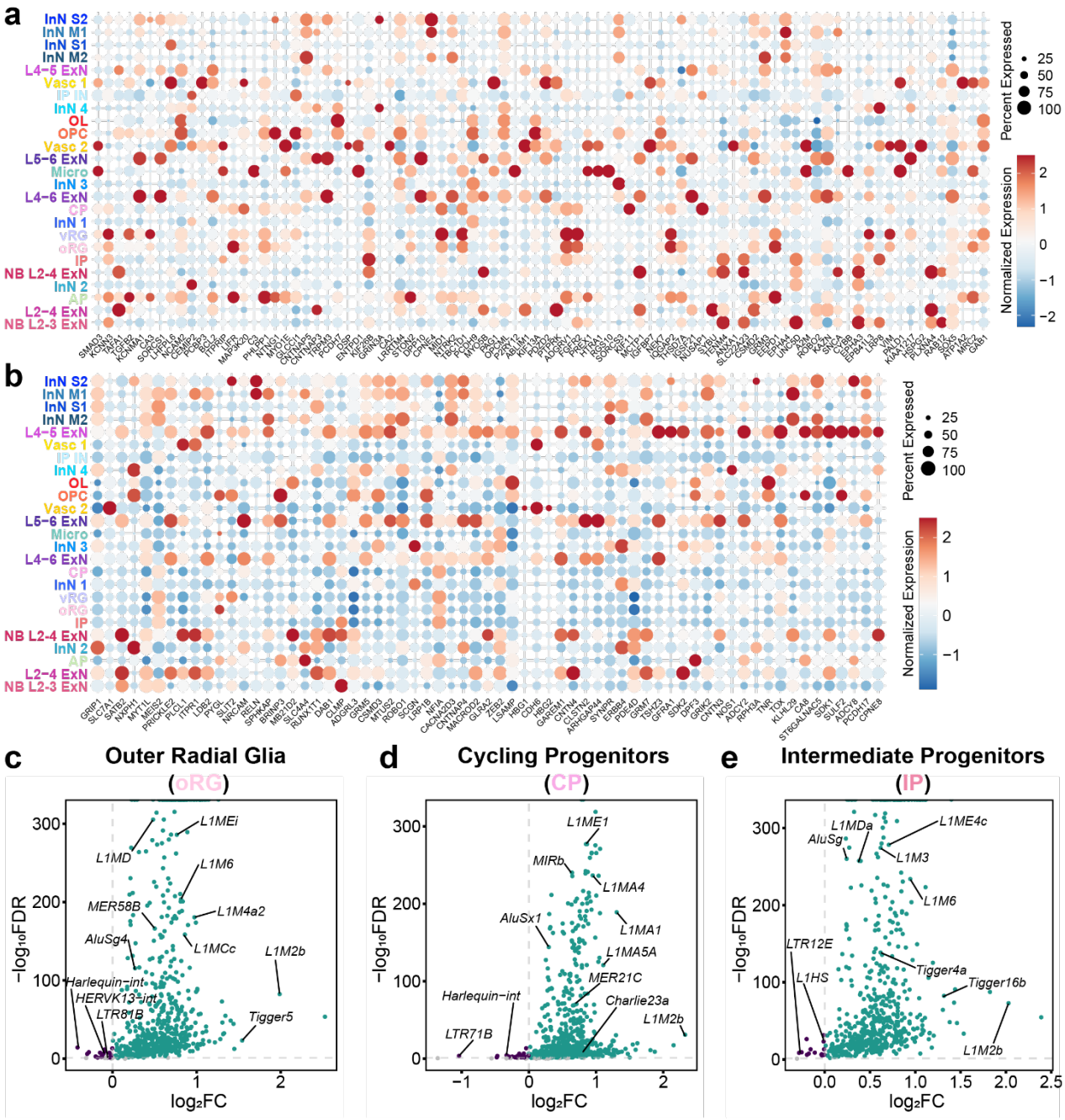
Supplementary Figure 1. Comprehensive transcriptomic and spatial profiling of the prenatal human Down syndrome brain.

a, Unique genes per cell and transcripts per cell detected in the prenatal human Down syndrome (DS; n = 5) and euploid (n = 5) brains from the single-nucleus (sn)RNA-seq dataset. **b,** Dotplot showing gene expression of canonical cell type markers used for cell subtype identification. Dot color represents normalized gene expression and dot diameter represents the proportion of nuclei expressing the gene. **c,** Barplot showing the proportion of cell subtypes in the snRNA-seq dataset, separated by condition (DS in teal, euploid in purple). Dots represent individual replicates and error bars represent standard errors. No significant differences in proportions were identified across cell subtypes in DS compared to euploid. **d,** Unique genes per cell and transcripts per cell detected in the prenatal human DS and euploid (n = 3 DS, n = 3 euploid) brains from the Slide-seq dataset.


a
Euploid
DS
Z-score Normalized Expression
6
4
2
0
-2
-4
b
SOX2
TP53
MERGED
Euploid
DS
c
TP53 Intensity/ SOX2+ Nucleus (Arb. Units)
*
90
60
30
0
Euploid
DS
d
FOS
EGR1
IER2
DNMT3A
HMGB2
Euploid
DS
Max
Normalized Expression
Min
e
SOX2
LMNB1
MERGED
Euploid
DS
f
LMNB1 Intensity/ SOX2+ Nucleus (Arb. Units)
****
60
40
20
0
Euploid
DS

Supplementary Figure 2. Differential gene expression analysis of the prenatal human Down syndrome brain.

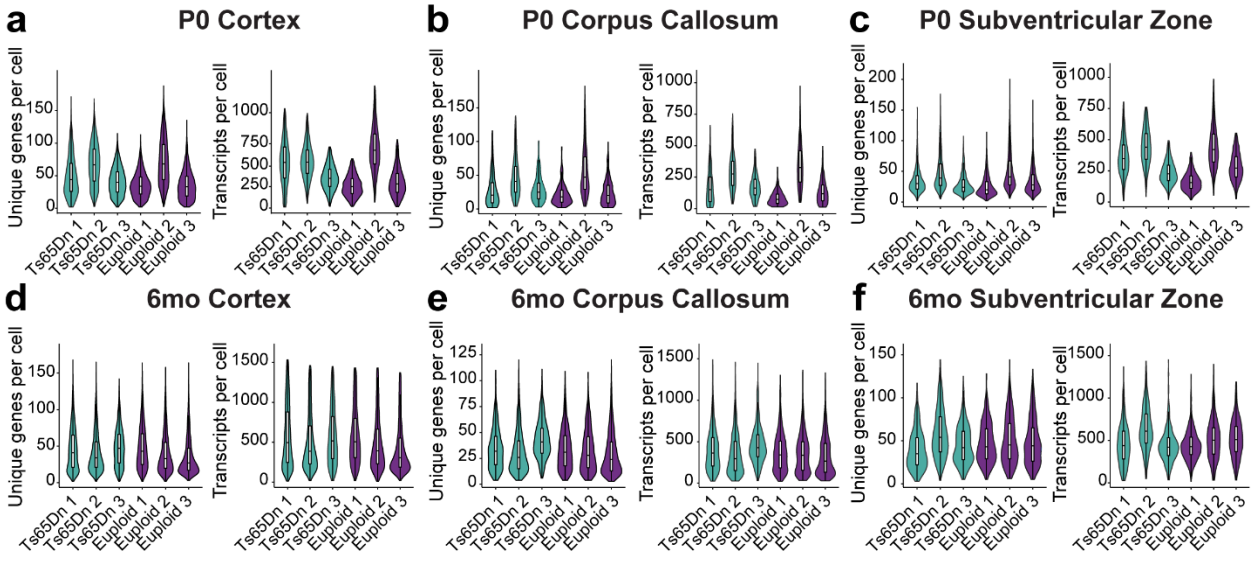
a, Hierarchically clustered heatmap of z-score normalized gene expression for genes located on human chromosome 21 across all cell subtypes and conditions identified in the snRNA-seq dataset. Each row represents a cell subtype, and each column a gene. Dendrograms reflect hierarchical clustering of genes based on expression similarity. Several genes of interest are indicated. **b,** Immunostaining of TP53 (pink) in $SOX2^+$ cells (green) and DAPI-labeled nuclei (cyan) located near the ventricular zone (VZ) in a representative prenatal human Down syndrome (DS) and euploid brain. Scale bar is 30 μm. **c,** Bar plots displaying TP53 intensity in $SOX2^+$ cells, measured in arbitrary units (arb. units). Each dot represents the average intensity for each prenatal human brain (n = 3 DS, n = 3 euploid). Bars indicate the average intensity per condition, with error bars representing the standard error of the mean. Statistical significance was assessed using a two-sided t-test; p-value = 0.006. **d,** Spatial plots of select immediate early (*FOS*, *EGR1*, *IER2*) and chromatin-associated genes (*DNMT3A*, *HMGB2*) in representative DS and euploid brains, with gene expression plotted from Slide-seq. Dot color represents normalized gene expression from the 10th to 90th percentiles. **e,** Immunostaining of LMNB1 (pink) in $SOX2^+$ cells (green) and DAPI-labeled nuclei (cyan) located near the ventricular zone (VZ) in a representative prenatal human Down syndrome (DS) and euploid brain. Scale bar is 30 μm. **f,** Bar plots displaying LMNB1 intensity in $SOX2^+$ cells, measured in arbitrary units (arb. units). Each dot represents the average intensity for each prenatal human brain (n = 3 DS, n = 3 euploid). Bars indicate the average intensity per condition, with error bars representing the standard error of the mean. Statistical significance was assessed using a two-sided t-test; p-value = 0.0001.



Supplementary Figure 3. Proteomic and transposable element profiling of the prenatal human DS brain.

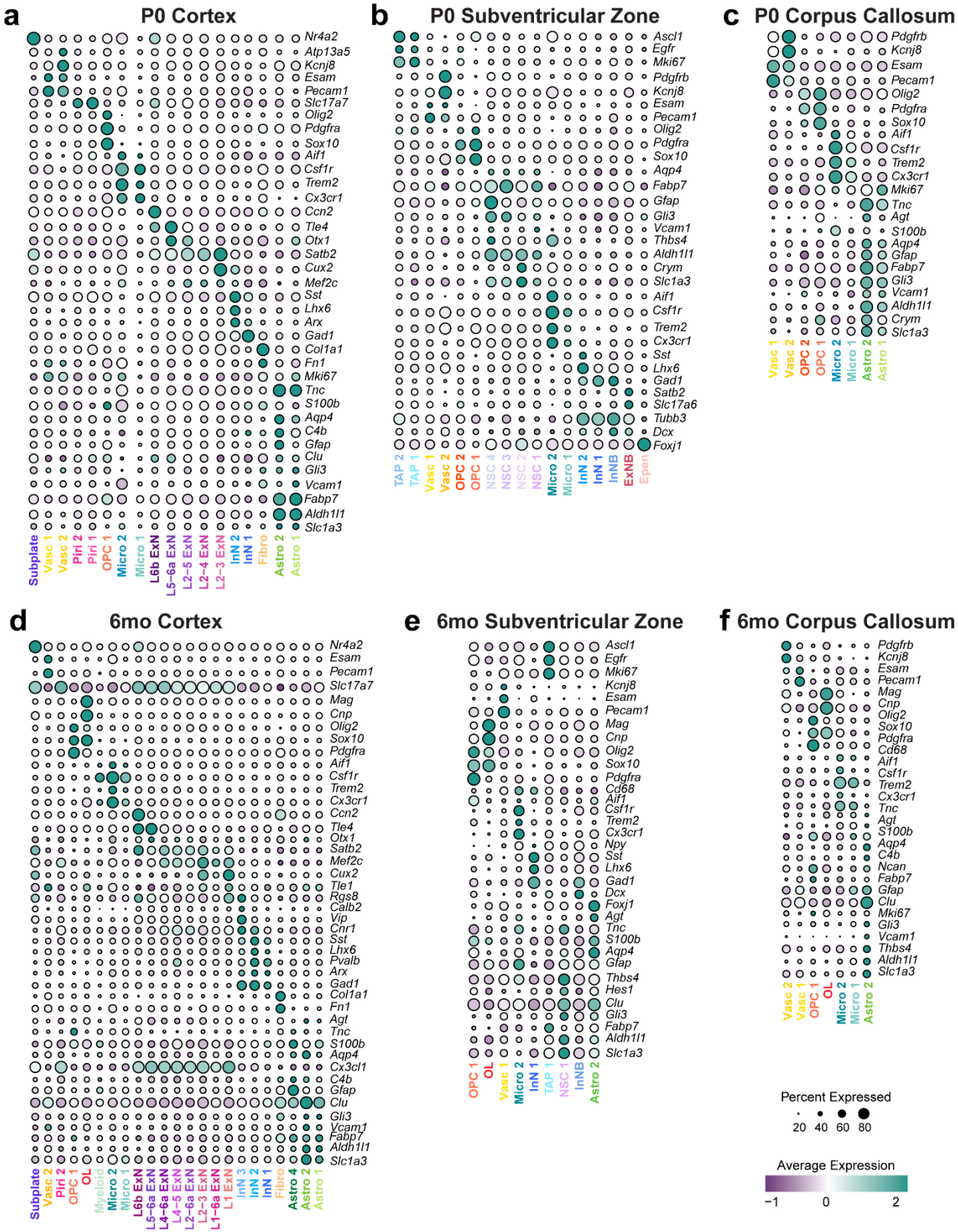
a-b, Dotplots of differentially **a,** upregulated ($log_2FC > 0$, FDR ≤ 0.05) and **b,** downregulated ($log_2FC < 0$, FDR ≤ 0.05) proteins mapped onto the prenatal human snRNA-seq dataset. Dots display the scaled RNA expression of corresponding genes across cell subtypes. Dot size reflects the percentage of cells expressing each gene, and dot color represents the average expression level. **c-e,** Volcano plots depicting differentially expressed transposable elements (TEs) in **c,** outer radial glia (oRG), **d,** cycling progenitors (CP) and **e,** intermediate progenitors (IP) from the prenatal

human snRNA-seq dataset. Each dot represents a TE and dots are colored according to enrichment: significantly upregulated ($log_2FC > 0$, FDR ≤ 0.05) in teal, significantly downregulated ($log_2FC < 0$, FDR ≤ 0.05) in purple, and non-significant genes in gray. Comparison is between DS and euploid samples. The horizontal line depicts FDR = 0.05, and the vertical line depicts $log_2FC = 0$.



Supplementary Figure 4. MERFISH quality metrics for postnatal (P0) and mature (6mo) Ts65Dn and control forebrain regions.

a-c, Unique genes per cell and transcripts per cell detected in the P0 trisomic (Ts65Dn; *n* = 3) and euploid (*n* = 3) **a,** cortex, **b,** corpus callosum, and **c,** subventricular zone from the MERFISH dataset. **f-g,** Unique genes per cell and transcripts per cell detected in the 6mo trisomic (Ts65Dn; *n* = 3) and euploid (*n* = 3) **d,** cortex, **e,** corpus callosum, and **f,** subventricular zone from the MERFISH dataset.


a
P0 Cortex
Nr4a2
Atp13a5
Kcnj8
Esam
Pecam1
Slc17a7
Olig2
Pdgfra
Sox10
Aif1
Csf1r
Trem2
Cx3cr1
Ccn2
Tle4
Otx1
Satb2
Cux2
Mef2c
Sst
Lhx6
Arx
Gad1
Col1a1
Fn1
Mki67
Tnc
S100b
Aqp4
C4b
Gfap
Clu
Gli3
Vcam1
Fabp7
Aldh1l1
Slc1a3
Subplate
Vasc 1
Vasc 2
Piri 2
Piri 1
OPC 1
Micro 2
Micro 1
L6b ExN
L5–6a ExN
L5–5 ExN
L2–4 ExN
L2–3 ExN
InN 2
InN 1
Fibro
Astro 2
Astro 1
b
P0 Subventricular Zone
Ascl1
Egfr
Mki67
Pdgfrb
Kcnj8
Esam
Pecam1
Olig2
Pdgfra
Sox10
Aqp4
Fabp7
Gfap
Gli3
Vcam1
Thbs4
Aldh1l1
Crym
Slc1a3
Aif1
Csf1r
Trem2
Cx3cr1
Sst
Lhx6
Gad1
Satb2
Slc17a6
Tubb3
Dcx
Foxj1
TAP 2
TAP 1
Vasc 1
Vasc 2
OPC 2
OPC 1
NSC 4
NSC 3
NSC 2
NSC 1
Micro 2
Micro 1
InN 2
InN 1
InNB
ExNB
Epen
c
P0 Corpus Callosum
Pdgfrb
Kcnj8
Esam
Pecam1
Olig2
Pdgfra
Sox10
Aif1
Csf1r
Trem2
Cx3cr1
Mki67
Tnc
Agt
S100b
Aqp4
Gfap
Fabp7
Gli3
Vcam1
Aldh1l1
Crym
Slc1a3
Vasc 1
Vasc 2
OPC 2
OPC 1
Micro 2
Micro 1
Astro 2
Astro 1
d
6mo Cortex
Nr4a2
Esam
Pecam1
Slc17a7
Mag
Cnp
Olig2
Sox10
Pdgfra
Aif1
Csf1r
Trem2
Cx3cr1
Ccn2
Tle4
Otx1
Satb2
Mef2c
Cux2
Tle1
Rgs8
Calb2
Vip
Cnr1
Sst
Lhx6
Pvalb
Arx
Gad1
Col1a1
Fn1
Agt
Tnc
S100b
Aqp4
Cx3cl1
C4b
Gfap
Clu
Gli3
Vcam1
Fabp7
Aldh1l1
Slc1a3
Subplate
Vasc 2
Piri 2
OPC 1
OL
Myeloid
Micro 2
Micro 1
L6b ExN
L5–6a ExN
L4–6a ExN
L4–5 ExN
L2–6a ExN
L2–3 ExN
L1–6a ExN
L1 ExN
InN 3
InN 2
InN 1
Fibro
Astro 4
Astro 2
Astro 1
e
6mo Subventricular Zone
Ascl1
Egfr
Mki67
Kcnj8
Esam
Pecam1
Mag
Cnp
Olig2
Sox10
Pdgfra
Cd68
Aif1
Csf1r
Trem2
Cx3cr1
Npy
Sst
Lhx6
Gad1
Dcx
Foxj1
Agt
Tnc
S100b
Aqp4
Gfap
Thbs4
Hes1
Clu
Gli3
Fabp7
Aldh1l1
Slc1a3
OPC 1
OL
Vasc 1
Micro 2
InN 1
TAP 1
NSC 1
InNB
Astro 2
f
6mo Corpus Callosum
Pdgfrb
Kcnj8
Esam
Pecam1
Mag
Cnp
Olig2
Sox10
Pdgfra
Cd68
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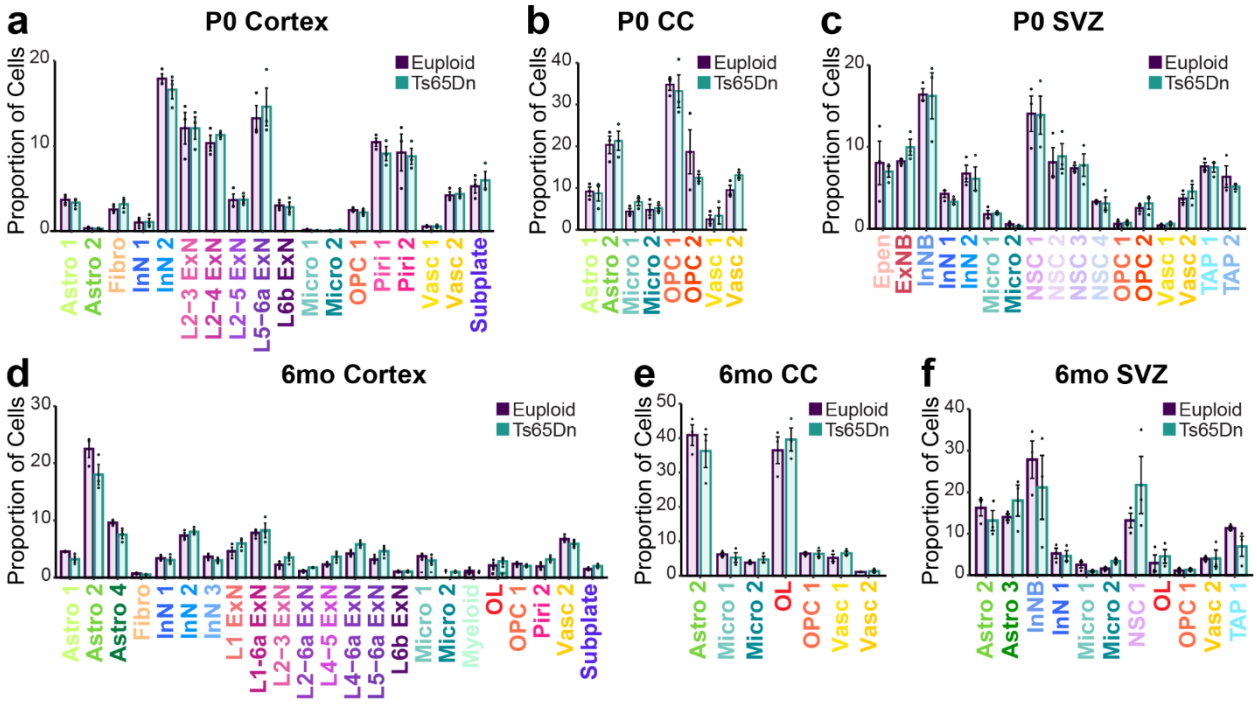
Agt
S100b
Aqp4
C4b
Ncan
Fabp7
Gfap
Clu
Mki67
Gli3
Vcam1
Thbs4
Aldh1l1
Slc1a3
Vasc 2
Vasc 1
OPC 1
OL
Micro 2
Micro 1
Astro 2
Percent Expressed
20 40 60 80
Average Expression
−1 0 2

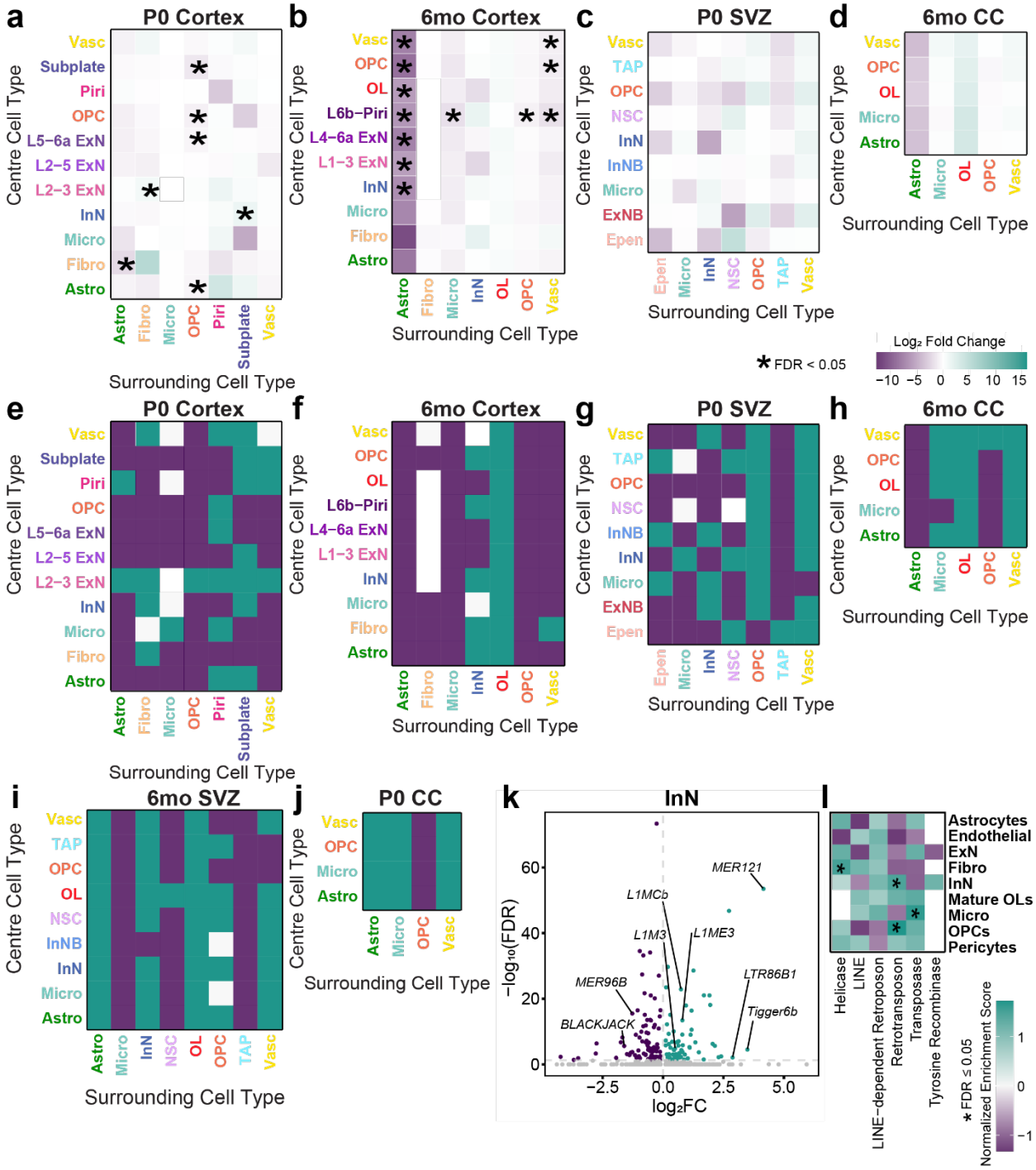
Supplementary Figure 5. MERFISH Cell Type Identification

a-f, Dotplot showing gene expression of canonical cell type markers used for cell subtype identification in the **a,** postnatal (P0) cortex, **b,** subventricular zone, **c,** corpus callosum, and **f,** 6mo cortex, **e,** subventricular zone, and **f,** corpus callosum. Dot color represents normalized gene expression and dot diameter represents the proportion of nuclei expressing the gene.



Supplementary Figure 6. Cellular compositional and communication changes in the postnatal and mature Ts65Dn mice.

a-f, Barplot showing the proportion of cell subtypes in the **a,** P0 cortex, **b,** corpus callosum (CC), **c,** subventricular zone (SVZ), and **d,** 6mo cortex, **e,** CC, and **f,** SVZ MERFISH datasets, separated by condition (Ts65Dn in teal, euploid in purple). Dots represent individual replicates and error bars represent standard errors. No significant differences in proportions were identified across cell subtypes in Ts65Dn compared to euploid.



Supplementary Figure 7. Cellular compositional changes in the postnatal and mature Ts65Dn mice.

a-d, Microenvironment analysis of the **a,** P0 cortex, **b,** 6mo cortex, **c,** P0 subventricular zone (SVZ), and **d,** 6mo corpus callosum (CC) from MERFISH, where a central cell type is selected, and the proportion of the top 100 nearest cells relative to this central cell (y-axis) is calculated in comparison to other cell types of interest (x-axis) and across conditions, with corrections made

using the Benjamini-Hochberg method. Teal indicates an increased abundance of query cells near the central cell in Ts65Dn compared to control, while purple indicates a decreased abundance of query cells near the central cell in Ts65Dn compared to control. **e-j,** Leave-one-subject-out (LOSO) analysis across brain region and time points: **e,** P0 cortex, **f,** 6mo cortex, **g,** P0 SVZ, **h,** 6mo CC, **i,** 6mo SVZ, **j,** P0 CC. In the LOSO approach, the microenvironmental analysis was iteratively performed while excluding one sample at a time. Mean effect sizes and standard deviations across iterations were used to assess the reproducibility of changes. Teal represents consistently upregulated interactions, purple indicates consistently downregulated interactions, and grey indicates variability in the direction of change across iterations. **k,** Volcano plot depicting differentially expressed transposable elements (TEs) in interneurons from the Ts65Dn snRNA-seq dataset. Each dot represents a TE and dots are colored according to enrichment: significantly upregulated ($log_2FC > 0$, FDR ≤ 0.05) in teal, significantly downregulated ($log_2FC < 0$, FDR ≤ 0.05) in purple, and non-significant genes in gray. Comparison is between Ts65Dn and euploid samples. The horizontal line depicts FDR = 0.05, and the vertical line depicts $log_2FC = 0$. **l,** Heatmap depicting the NES of TE subclasses in each cell subtype identified in the Ts65Dn snRNA-seq dataset. Color reflects NES values, with teal indicating positive enrichment and purple indicating negative enrichment in Ts65Dn vs euploid. Asterisks (*) denote an FDR ≤ 0.05.