Neuron, Volume *107*

## Supplemental Information

# Cell-Type Specificity of Genomic Imprinting in Cerebral Cortex

**Susanne Laukoter, Florian M. Pauler, Robert Beattie, Nicole Amberg, Andi H. Hansen, Carmen Streicher, Thomas Penz, Christoph Bock, and Simon Hippenmeyer**

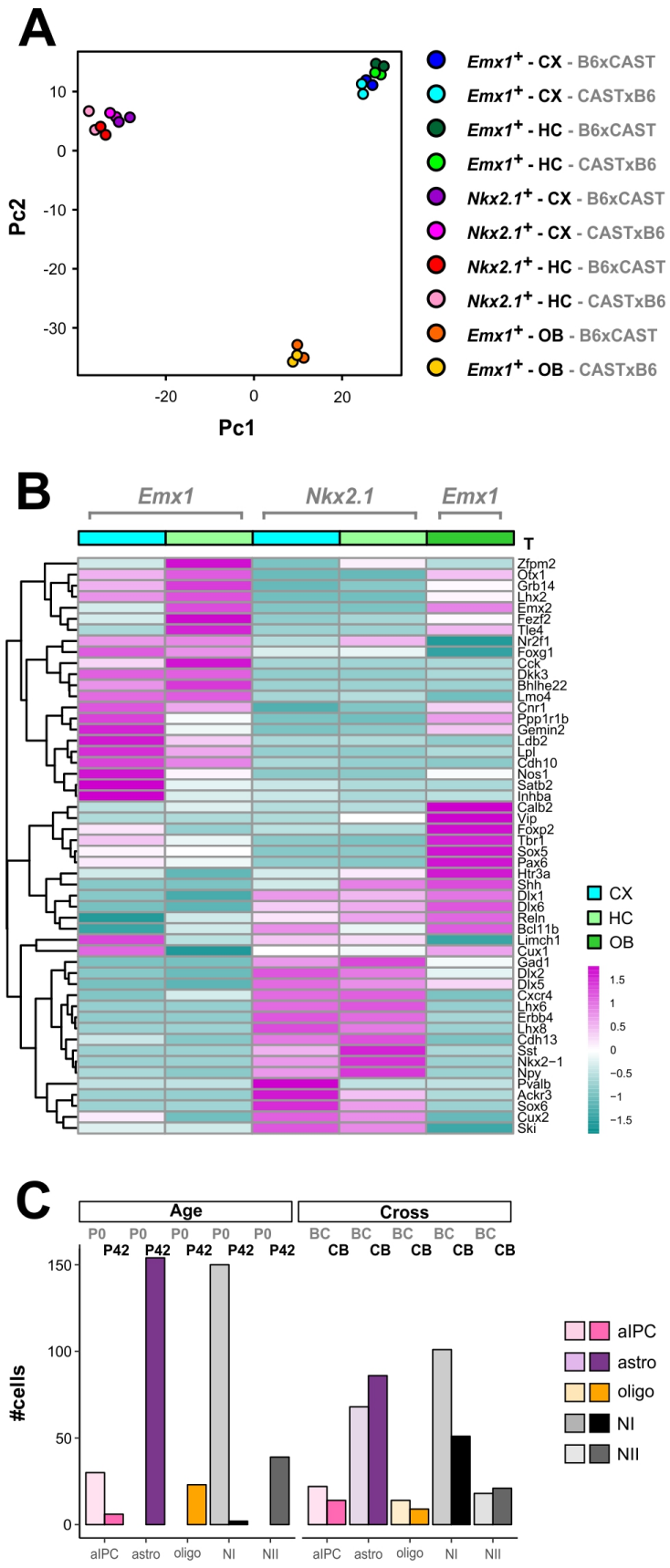
A
Pc2
Pc1
10
0
-10
-20
-30
-20
0
20
Emx1+ - CX - B6xCAST
Emx1+ - CX - CASTxB6
Emx1+ - HC - B6xCAST
Emx1+ - HC - CASTxB6
Nkx2.1+ - CX - B6xCAST
Nkx2.1+ - CX - CASTxB6
Nkx2.1+ - HC - B6xCAST
Nkx2.1+ - HC - CASTxB6
Emx1+ - OB - B6xCAST
Emx1+ - OB - CASTxB6
B
Emx1
Nkx2.1
Emx1
T
Zfpm2
Otx1
Grb14
Lhx2
Emx2
Fezf2
Tle4
Nr2f1
Foxg1
Cck
Dkk3
Bhlhe22
Lmo4
Cnr1
Ppp1r1b
Gemin2
Ldb2
Lpl
Cdh10
Nos1
Satb2
Inhba
Calb2
Vip
Foxp2
Tbr1
Sox5
Pax6
Htr3a
Shh
Dlx1
Dlx6
Reln
Bcl11b
Limch1
Cux1
Gad1
Dlx2
Dlx5
Cxcr4
Lhx6
Erbb4
Lhx8
Cdh13
Sst
Nkx2−1
Npy
Pvalb
Ackr3
Sox6
Cux2
Ski
CX
HC
OB
1.5
1
0.5
0
−0.5
−1
−1.5
C
Age
Cross
P0 P0 P0 P0 P0
P42 P42 P42 P42 P42
BC BC BC BC BC
CB CB CB CB CB
#cells
150
100
50
0
aIPC astro oligo NI NII
aIPC astro oligo NI NII
aIPC
astro
oligo
NI
NII

**Figure S1. Related to Figure 1. Isolation of Cell Type Specific Allelome using B6xCAST Hybrid Crosses. (A)** Visualization of sample distribution - *Emx1*$^{+}$ and *Nkx2.1*$^{+}$ cell types in neocortex (CX), hippocampus (HC) and olfactory bulb (OB) in B6/CAST F1 hybrids - using principal component (PC) analysis. Data points reflect individual biological samples from B6xCAST and CASTxB6 crosses. Note that in the nomenclature the maternal strain is indicated first (i.e. MATxPAT). **(B)** The heat map shows scaled expression of a representative set of marker genes defining individual cell types. Note that cell types (columns) were ordered arbitrarily and genes (rows) were clustered in an unbiased way. Colored bar above the heat map indicate cell types from distinct tissues (CX, blue; HC, light green; and OB, dark green). **(C)** Left: Numbers of single cells isolated from *Emx1*$^{+}$ lineage sorted from P0 (light color) and P42 (darker color) contributing to distinct cell types: astrocyte intermediate progenitors (aIPC), astrocytes (astro), oligodendrocytes (oligo), neurons I (NI) and neurons II (NII). Right: Numbers of single cells across distinct cell types from B6xCAST (BC, light color) and CASTxB6 (CB, darker color) crosses.

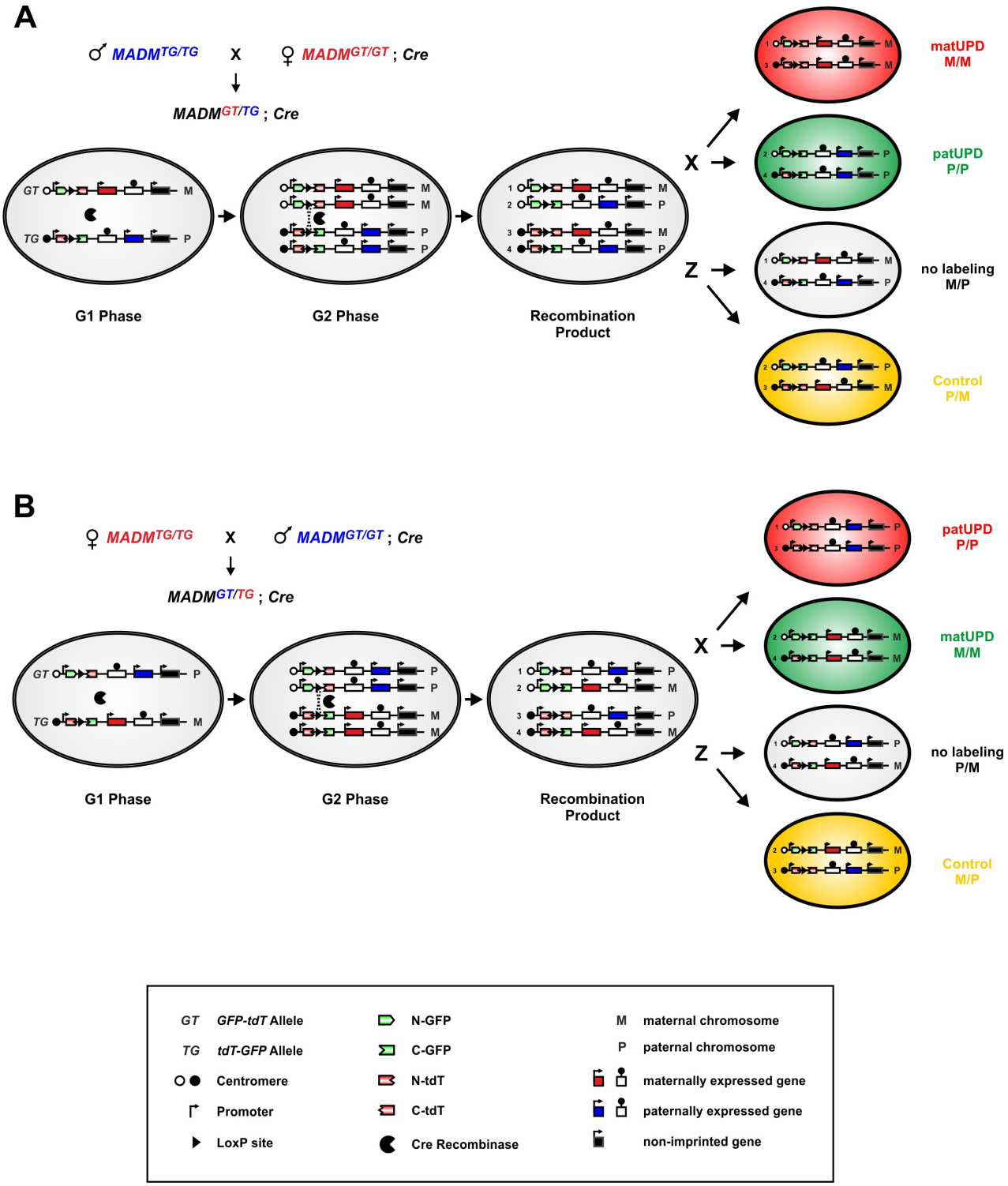


**Figure S2. Related to Figure 3. Experimental MADM Paradigm for Generating UPD with Single Cell Resolution. (A)** MADM utilizes Cre/LoxP-mediated interchromosomal recombination to reconstitute two reciprocal chimeric marker genes (GT/TG). Recombination in postmitotic cells or in G1 phase during cell division cycle results in the reconstitution of both (i.e. *tdT*, red; *GFP*, green) marker genes in the same cell, thus labeling the cell in yellow color but without altering the genotype (not indicated, see Hippenmeyer et al., 2013 for details). Recombination in G2 phase of the cell cycle with segregation of both recombinant chromosomes (including fully

reconstituted *tdT* and *GFP* marker genes) to the same daughter cell (Z-Segregation, lower branch) also does not alter the genotype and results in one yellow cell which serves as control. In contrast, recombination in G2 followed by X-Segregation (the two recombinant chromosomes end up in distinct daughter cells, upper branch) leads to green (*GFP*$^{+}$) and red (*tdT*$^{+}$) labeled cells, respectively, with near complete uniparental chromosome disomy (UPD). If the TG MADM cassette is inherited from the father and the GT MADM cassette from the mother as indicated, cells labeled in red show unimaternal chromosome disomy (MM; matUPD) and green cells unipaternal chromosome disomy (PP, patUPD). **(B)** MADM paradigm to generate UPD using reverse crossing scheme. Here, the TG MADM cassette is inherited from the mother and the GT MADM cassette from the father. Thus, matUPD are labeled in green (*GFP*$^{+}$) and patUPD are labeled in red (*tdT*$^{+}$). Symbols are detailed in the key.

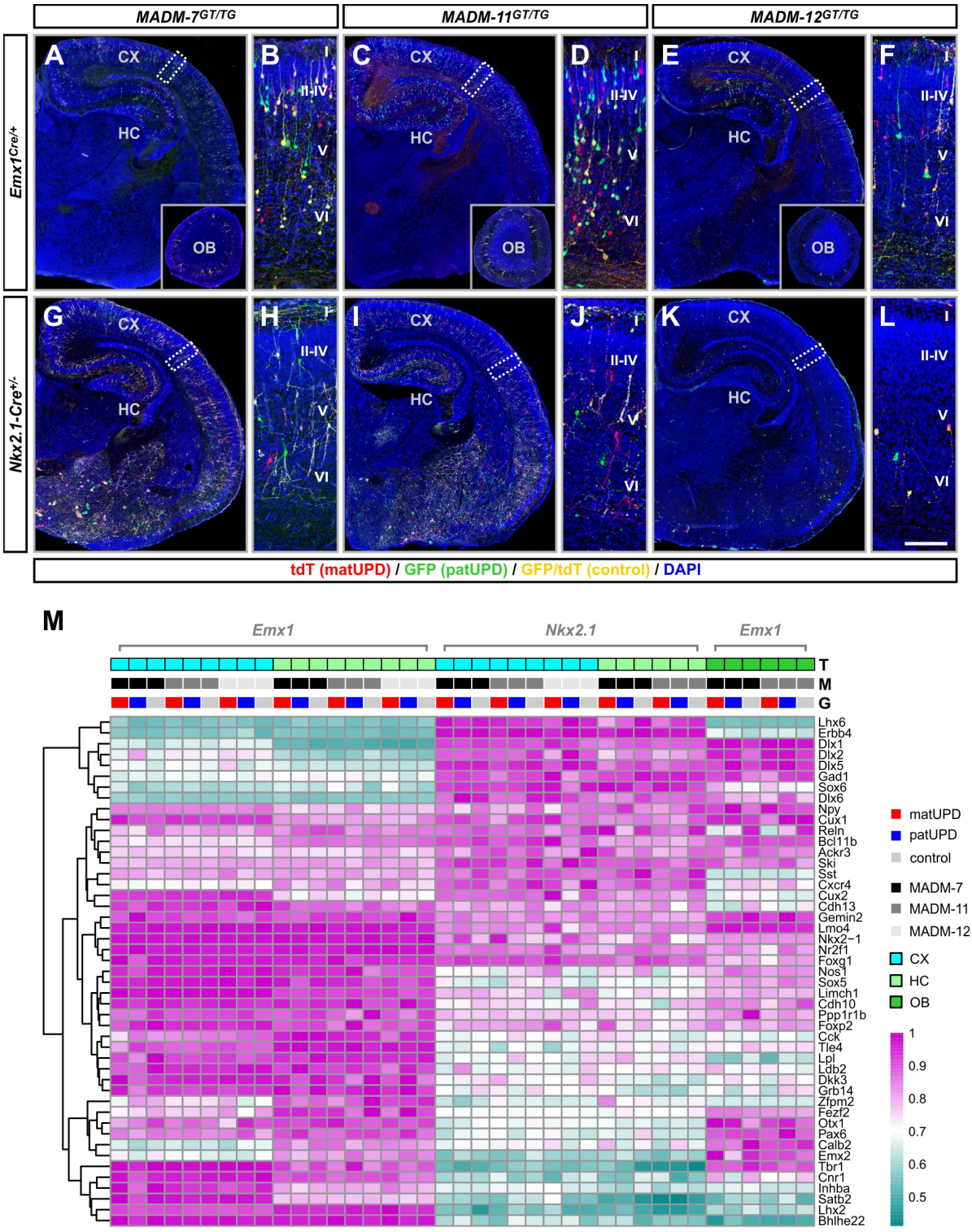


**Figure S3. Related to Figure 3. MADM-induced UPD in Distinct Cortical Cell Types does not Affect Cell Fate Specification. (A-L)** MADM-induced UPD of Chr. 7 (A, B, G, H), Chr. 11 (C, D, I, J), and Chr. 12 (E, F, K, L) at P0 in *Emx1*$^{+}$ (A-F) and *Nkx2.1*$^{+}$ (G-L) lineage in neocortex (CX), hippocampus (HC) and olfactory bulb (OB; insets in A, C, E). MatUPD is labeled in red (tdT$^{+}$), patUPD in green (GFP$^{+}$), yellow cells are GFP$^{+}$/tdT$^{+}$ and serve as control. Nuclei were stained using DAPI (blue). Scale bar, 500μm (A, C, E, G, I, K), 60μm (B, D, F, H,

J, L) and 600μm (OB insets in A, C, E). Cortical layers are indicated (roman numerals). **(M)** Heat map illustrating the relative expression of a representative set of marker genes defining individual cell types. Note that cell types (columns) were ordered arbitrarily and genes (rows) were clustered in an unbiased way. Colored bars above the heat map indicate genotype (G; matUPD in red, patUPD in blue, and control in light grey), MADM (M; MADM-7 in black, MADM-11 in grey and MADM-12 in light grey), and tissue (T; CX in blue, HC in light green, and OB in dark green).

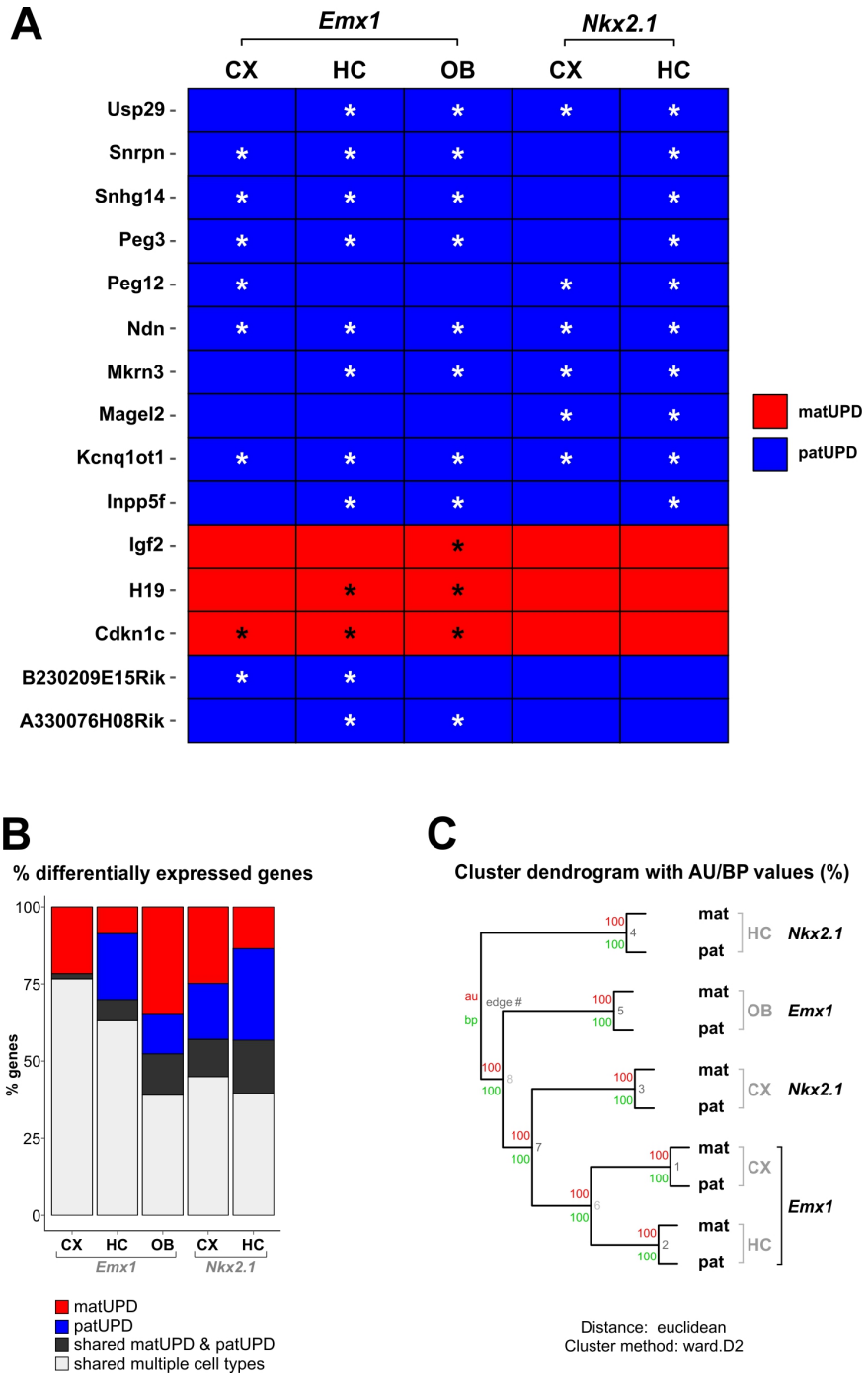


**Figure S4. Related to Figure 3. Transcriptional Changes in Genetically-defined Cell Types upon MADM-induced UPD of Chr. 7. (A)** Differential imprinted gene expression analysis in cells carrying MADM-induced matUPD and patUPD of Chr. 7. Heat map showing all expressed imprinted genes located on Chr. 7 in *Emx1*$^{+}$ and *Nkx2.1*$^{+}$ samples from neocortex (CX), hippocampus (HC), and olfactory bulb (OB). Genes with higher expression in matUPD cells ($log_2$ fold-change >0) are marked in red and genes with higher expression in patUPD cells ($log_2$ fold-change <0) in blue. Asterisks mark significant differential expression (padj<0.1) **(B)** Fraction of significantly DEGs (padj<0.05) unique for matUPD/control (red), unique for patUPD/control (blue) shared between matUPD/control and patUPD/control only within the same cell type (black) or shared in any other combination among analyzed samples (grey). **(C)** Cluster dendrogram based on score heat map shown in

Figure 3K. Significance of clustering analysis is shown as Approximately Unbiased (AU) / Approximately Unbiased (BP) values calculated by the R package pvclust. Note that matUPD and patUPD from the same cell type rather than matUPD or patUPDs from different cell types cluster together.

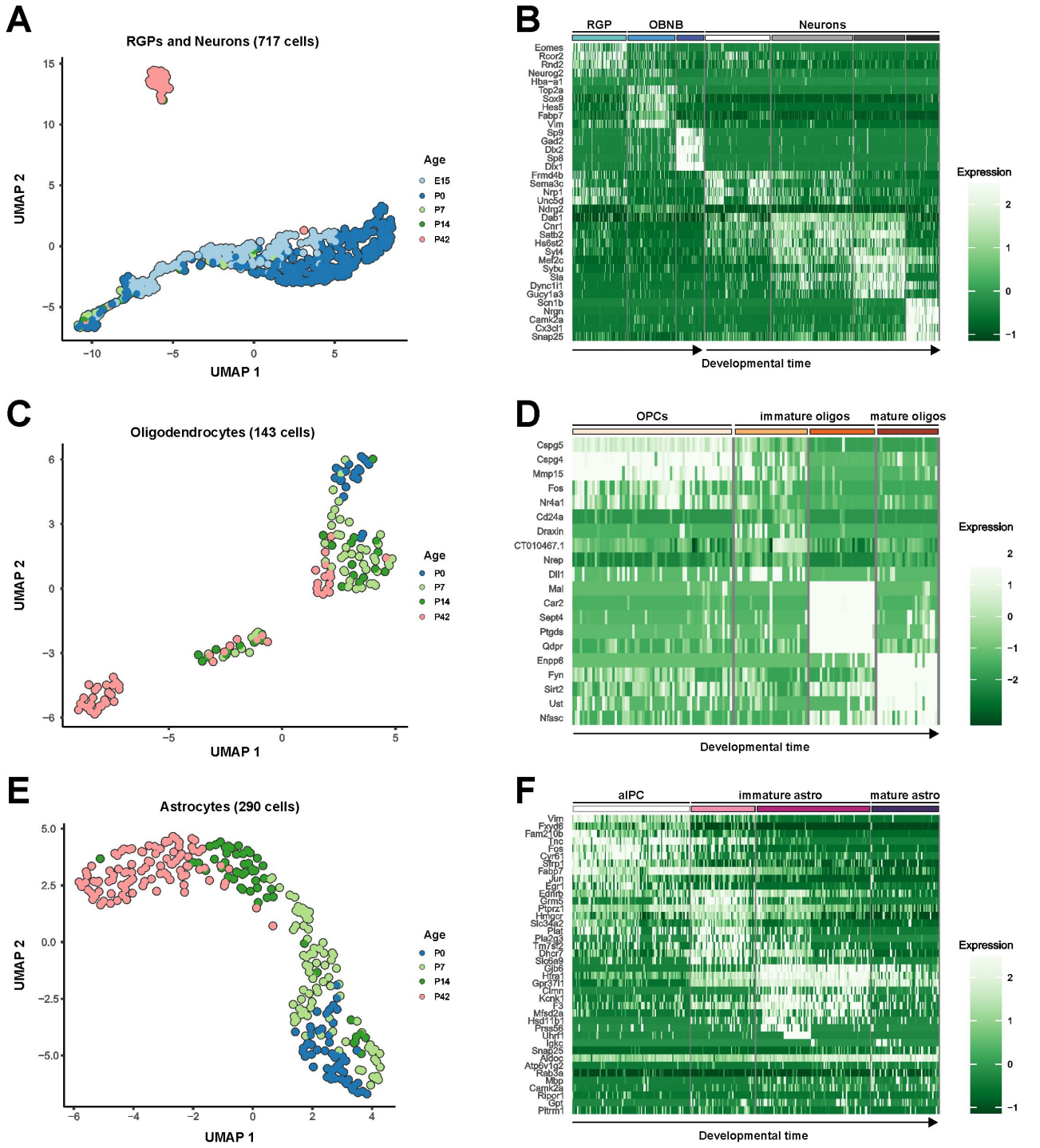


**Figure S5. Related to Figure 4. Gene Expression in Single matUPD and patUPD Cells in *Emx1*$^{+}$ Neuronal and Glial Cell Populations. (A)** Uniform Manifold Approximation and Projection (UMAP) of Radial glia progenitors (RGPs) and neurons. Single cells (dots, n=717) are colored according to the collection time points (E15: light blue; P0: dark blue; P7: light green; P14: dark green; P42: pink). **(B)** Heat map of marker genes for the classification of individual cell types in (A). Colored bars above heat map indicate different cell types, as in Figure 4E. RGPs (cyan) are precursor for both olfactory bulb neuroblasts (OBNB, 2 developmental states: light and dark blue) and neurons (4 developmental states white to dark grey). **(C)** UMAP of oligodendrocytes. Single cells (dots, n=143) are colored according to collection time point (P0: dark blue; P7: light green; P14: dark

green; P42: pink). **(D)** Heat map of marker genes for the classification of individual cell types in (C). Colored bars above heat map indicate different developmental states in the oligodendrocyte lineage, as in Figure 4I. Light orange to dark orange indicates development from oligodendrocyte precursor cell via two immature oligodendrocyte populations to mature myelinating oligodendrocytes. **(E)** UMAP of astrocytes. Single cells (dots, n=290) are colored according to collection time point (P0: dark blue; P7: light green; P14: dark green; P42: pink). **(F)** Heat map of marker genes for the classification of individual cell types in (E). Colored bars above heat map indicate different developmental states in the astrocyte lineage, as in Figure 4M. White, pink to purple indicates development from astrocyte intermediate progenitors (aIPCs) via two immature astrocyte populations to mature astrocytes.

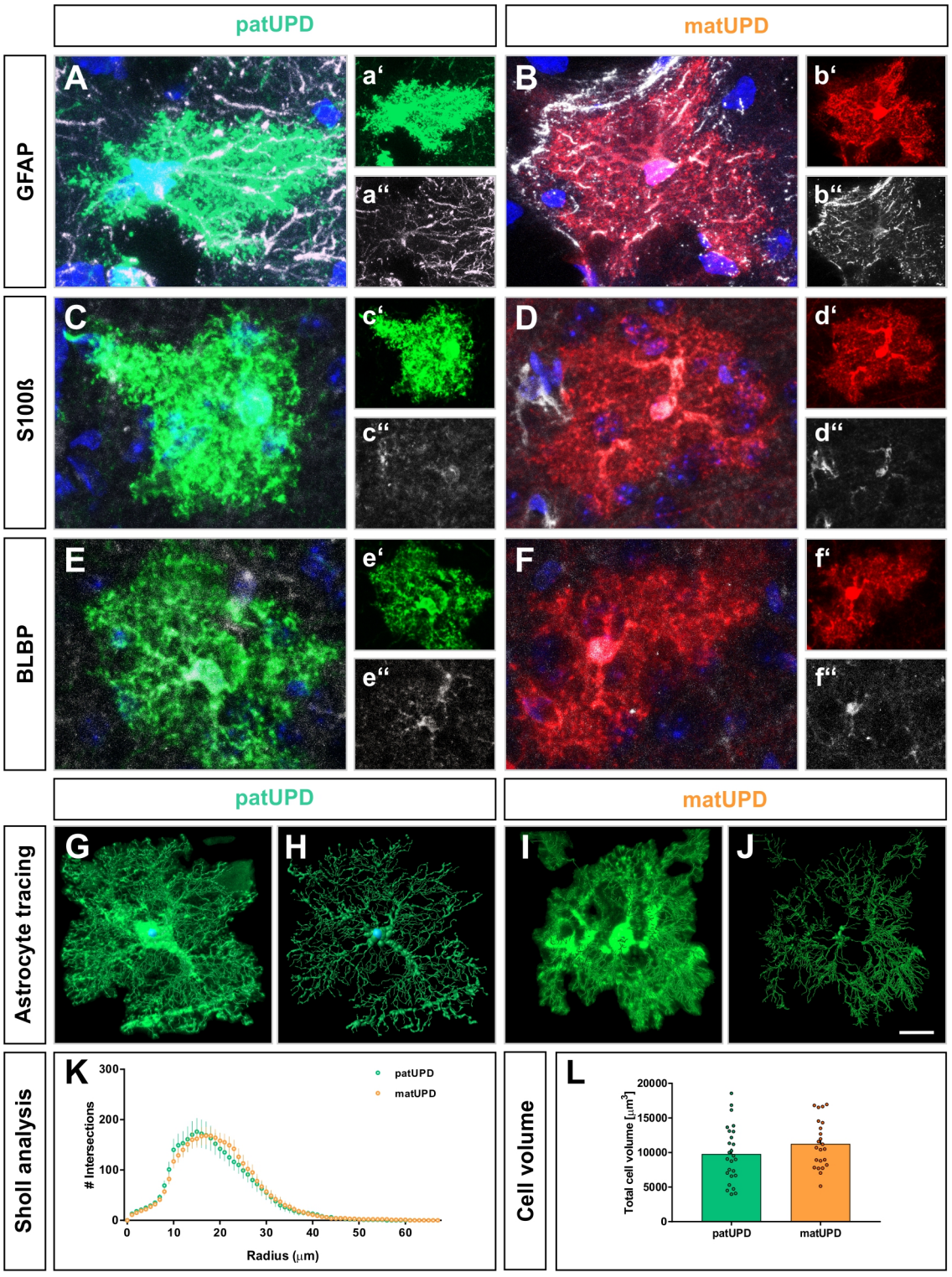


**Figure S6. Related to Figure 5. Analysis of Marker Expression and Cellular Morphology in Mature Astrocytes with Chr. 7 UPD. (A-F)** Expression of GFAP (white; A, a'', B, b''), S100β (white; C, c'', D, d'') and BLBP (white; E, e'', F, f'') in green GFP$^+$ (patUPD) and red tdT$^+$ (matUPD) MADM-labeled cortical astrocytes at P21. **(G-J)** Images of individual astrocytes (G, I) were processed using IMARIS software and Filament Trace was applied to assess astrocyte branching pattern (H, J). **(K)** Sholl analysis of matUPD and patUPD astrocytes

[n=45 (matUPD n=21; patUPD n=24)]. Note no significant difference between matUPD and patUPD. **(L)** Quantification of total cell volume [$\mu m^3$] of matUPD and patUPD astrocytes [N=49 (matUPD n=23; patUPD n=26)]. Note no significant difference between matUPD and patUPD. Scale bar: 10$\mu$m (A-J).

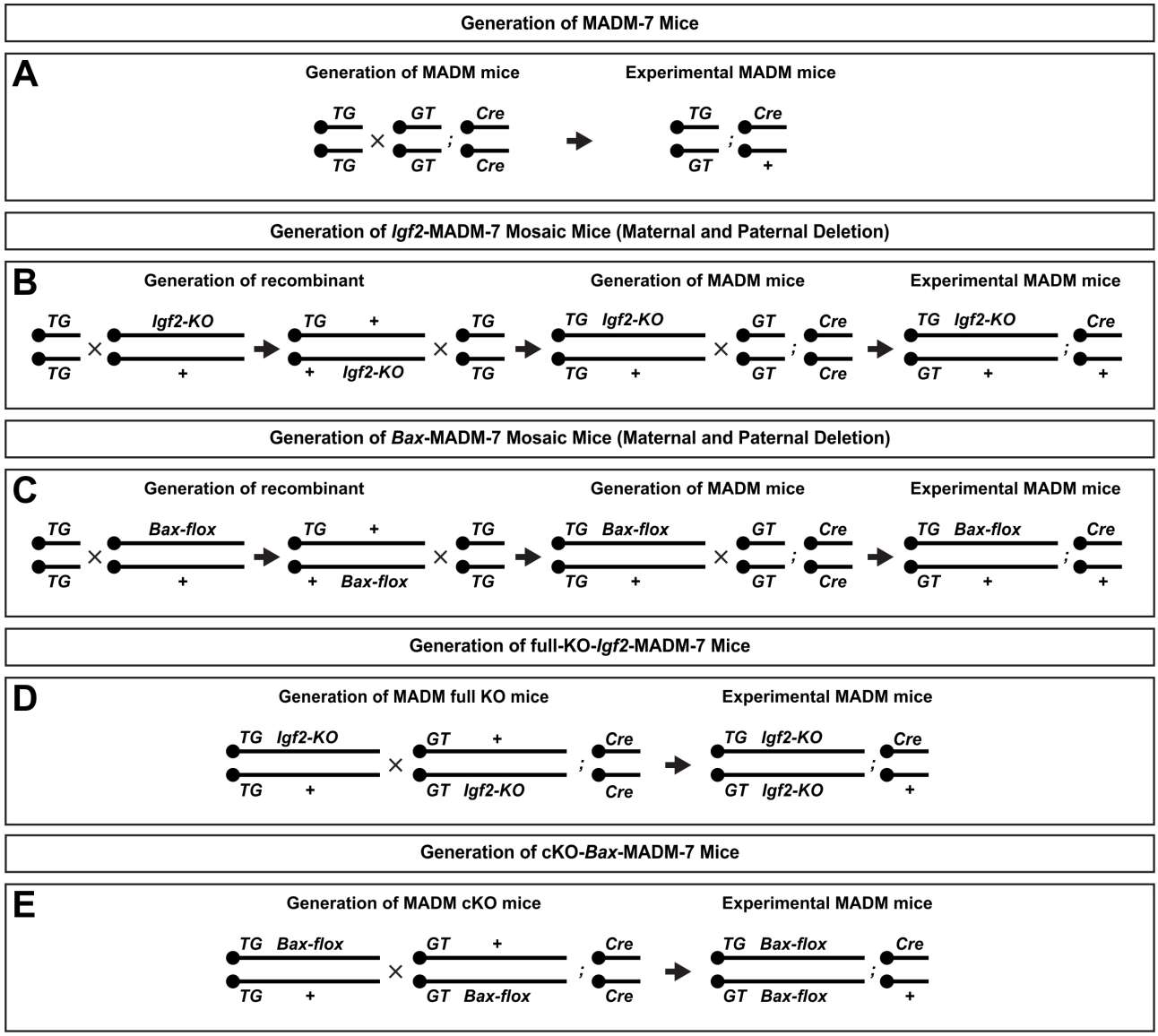


**Figure S7. Related to Figure 7. Breeding Schemes for the Generation of *Igf2* and *Bax* Genetic Mosaic, Full-KO or cKO Mice with Chr. 7 UPD. (A)** Breeding scheme for the generation of MADM-7 mice with Chr. 7 UPD. **(B)** Breeding scheme for the generation of *Igf2*-MADM-7 mosaic mice (maternal and paternal deletion). **(C)** Breeding scheme for the generation of *Bax*-MADM-7 mosaic mice (maternal and paternal deletion). **(D)** Breeding scheme for the generation of full-KO-*Igf2*-MADM-7 mice. **(E)** Breeding scheme for the generation of cKO-*Bax*-MADM-7 mice. Note that *Igf2* deletion (null allele) results in whole animal knock out, and *Bax* deletion (floxed allele) in conditional knockout. *Emx1*-Cre was used in all the breeding schemes.