Supplementary Information

Conserved transcriptional regulation by BRN1/2 in mammalian neural progenitors drive neural specification and neocortical expansion

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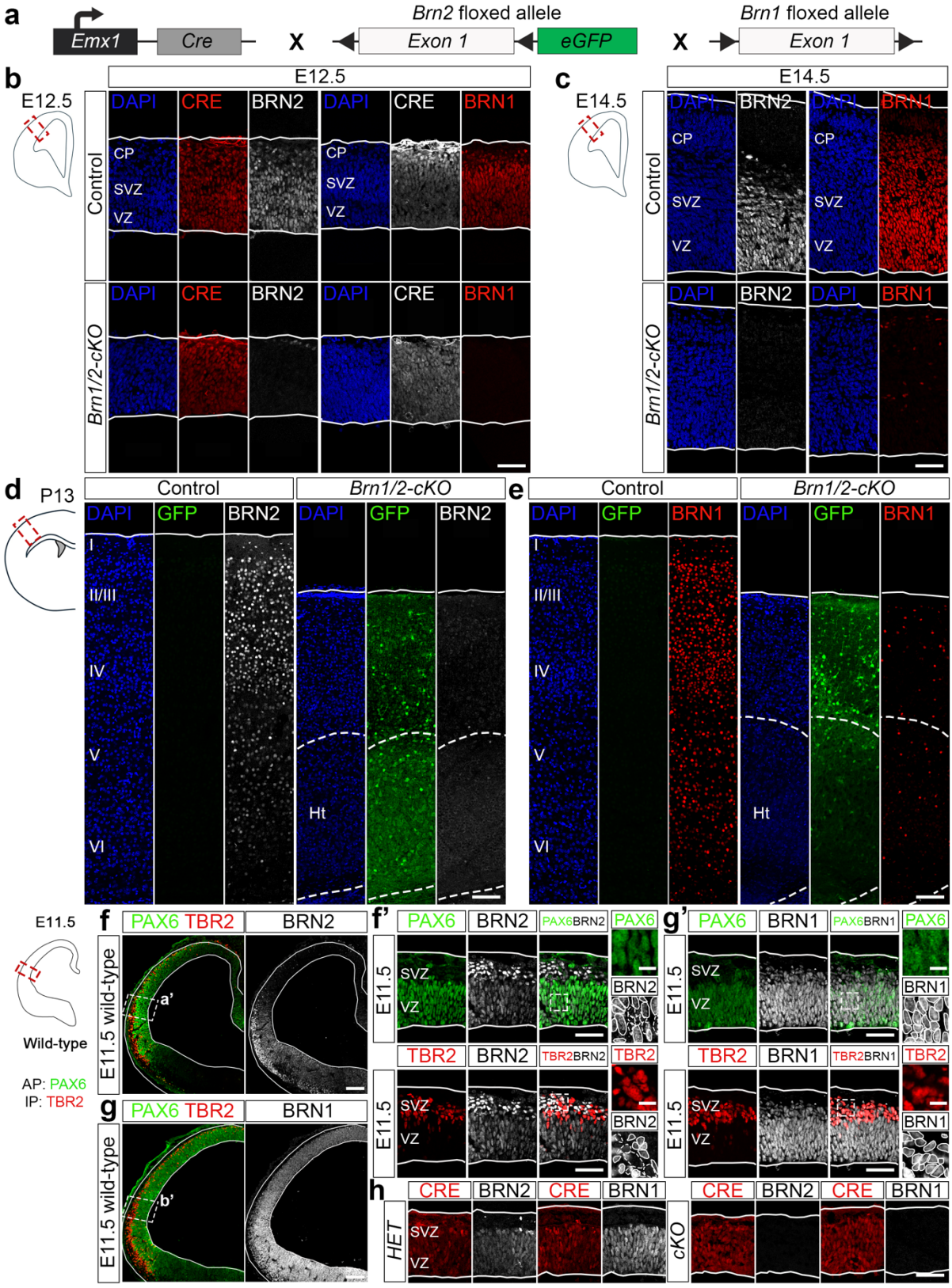
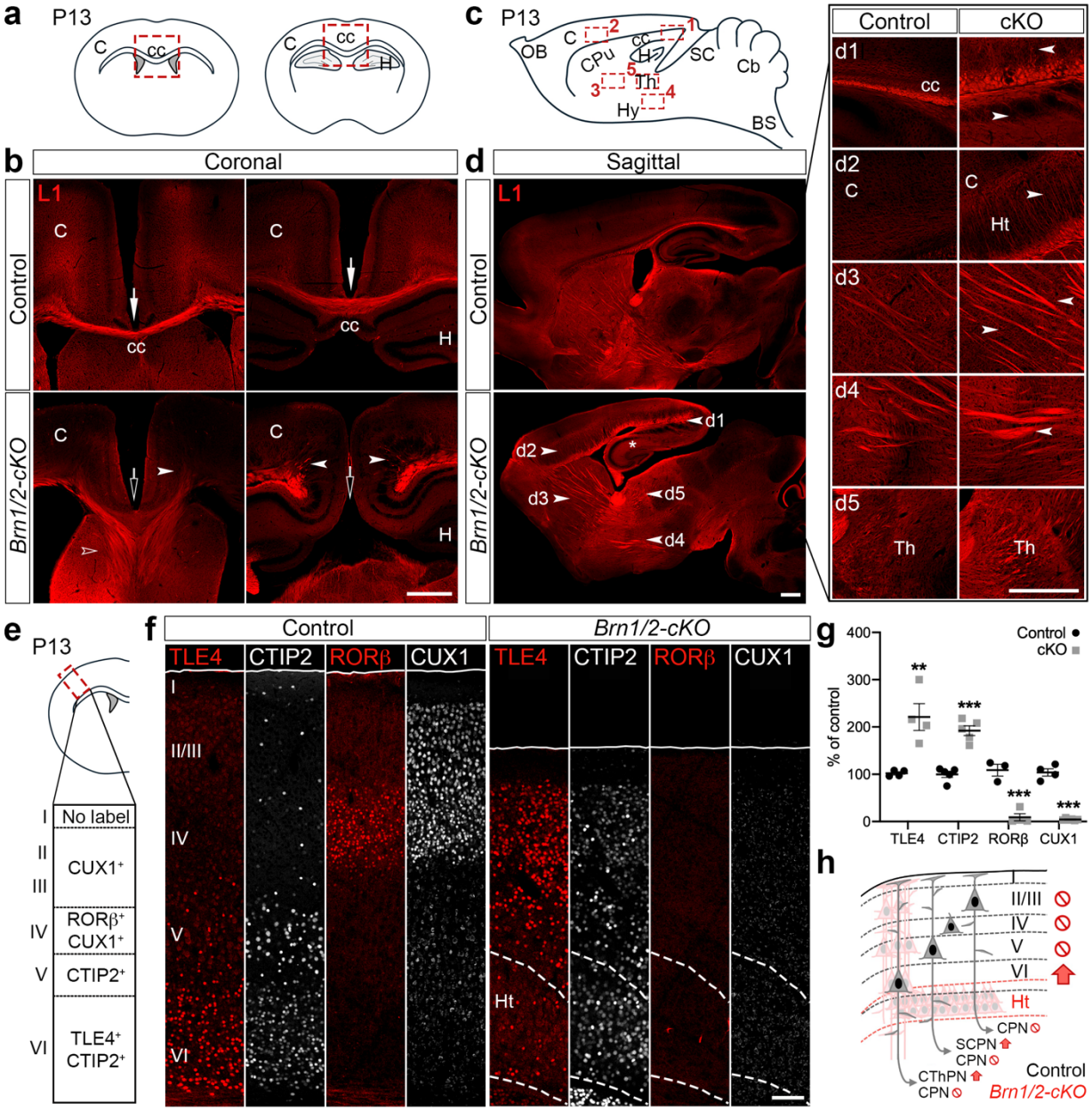
Included in this file are:

Supplementary Figure 1



Supplementary Fig. 1 BRN1 and BRN2 expression. **a,** Diagram of experimental strategy. *Emx1-Cre* mice were crossed with *Brn1*$^{fl/fl}$;*Brn2*$^{fl/fl}$ mice to inactivate *Brn1* and *Brn2* in the dorsal telencephalon. **b, c,** DAPI staining (blue) and BRN2 (grey), CRE (E12.5, red), BRN1 (red) and CRE (E12.5, grey) immunolabeling in cortical sections of control and *Brn1/2-cKO* mice at E12.5 (b) and E14.5 (c). Low and top lines represent the limits of the ventricular zone (VZ) and cortical plate (CP), respectively. Scale bar: 50 μm. **d, e,** DAPI staining (blue) and GFP (green), BRN2 (d, grey) and BRN1 (e, red) immunolabeling in cortical sections of control and *Brn1/2-cKO* mice at P13. BRN1 is also expressed in interneurons and therefore those cells, that are not recombined in *Emx1-Cre* mice, remain positive for BRN1 in the cortex of *Brn1/2-cKO* mice at P13 (e). Neocortical cell layers I-VI are indicated. Lines represent the limits of the cortical plate; Dashed lines outline the cortical heterotopia (Ht). Scale bar: 100 μm. **f, g,** Overview of E11.5 wild-type brains labeled for DAPI (blue), PAX6 (green), TBR2 (red), BRN2 (f, grey) and BRN1 (g, grey). Meningeal and ventricular border indicated by white lines. Scale bar: 100 μm. **f', g',** Higher magnification view for TBR2 (red), PAX6 (green), BRN2 (f', grey) or BRN1 (g', grey) immunolabeling in the squared area highlighted in f or g, respectively. Low and top lines represent the limits of the VZ and CP, respectively. Boxed area shown at higher magnification on the rightmost panels. TBR2^{+} and PAX6^{+} cells expressing or lacking BRN1/2 expression are outlined by lines or dashed lines, respectively. Scale bars: 50 μm (lower magnification), 10 μm (higher magnification). **h,** CRE (red), BRN2 and BRN1 (grey) immunolabeling in cortical sections of control heterozygous and *Brn1/2-cKO* mice at E11.5. SVZ, Subventricular Zone; AP, Apical Progenitors; BP, Basal Progenitors. Low and top lines represent the limits of the VZ and CP, respectively. Scale bars: 50 μm. Source data are provided as a Source Data file.

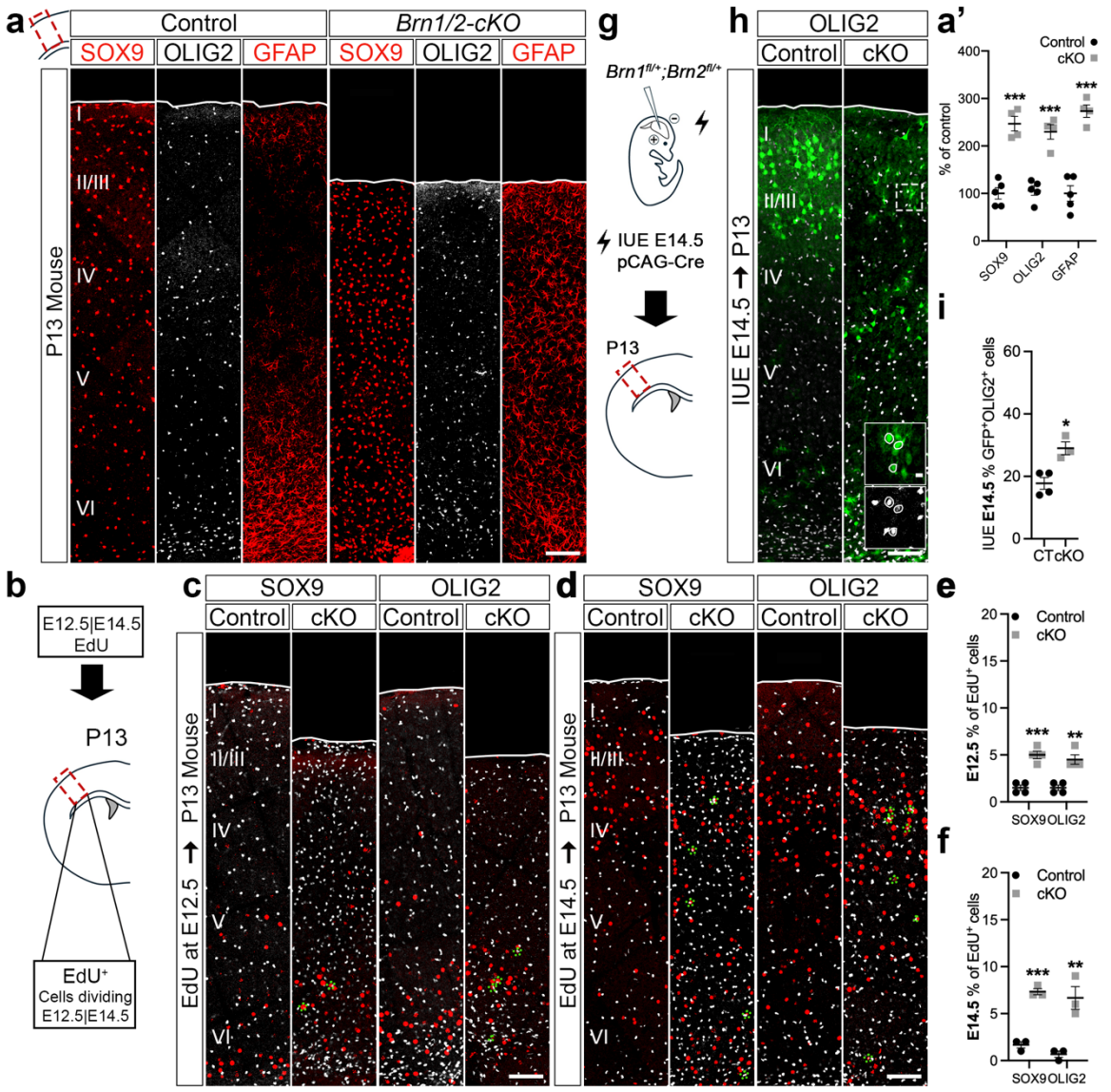
Supplementary Figure 2



Supplementary Fig. 2 BRN1/2 are essential for proper neuronal specification and circuit development in the neocortex. a, Schematic of coronal brain sections at two histological levels highlighting the corpus callosum (cc). C, cortex; H, hippocampus. **b,** Cc of control and *Brn1/2-cKO* mice at different histological levels analyzed by L1 immunolabeling (red) at P13. Open arrows indicate absent cc in *Brn1/2-cKO* (cKO) mice. Open arrowhead shows the abnormal ventral

misrouting of $L1^+$ projections in *Brn1/2-cKO* mice. Arrowheads indicate misrouting and abnormal defasciculation of $L1^+$ neurons in *Brn1/2-cKO* mice. **c,** Schematic of a midsagittal brain section highlighting the cc (1), cortex (C, 2), subcortical projections (3), hypothalamus (Hy, 4) and thalamus (Th, 5). OB, olfactory bulb; CPu, Caudate putamen; SC, superior colliculus; Cb, cerebellum; BS, brain stem. **d,** L1 immunolabeling of control and *Brn1/2-cKO* neuronal projections in midsagittal P13 brain sections. Arrowheads show morphological changes in the *Brn1/2-cKO* brains. Numbers indicate areas shown in panel d1-d5. d1, absent cc; d2, cortical neuronal heterotopia (Ht); d3, more $L1^+$ neuronal fibers projecting to subcortical areas; d4, more disorganized $L1^+$ neuronal fibers projecting to subcortical areas; d5, more $L1^+$ neuronal fibers targeting the thalamus; *disorganized hippocampus as previously described[22]. **e,** Schematic of a coronal brain section highlighting the somatosensory cortex analyzed at P13 by immunolabeling of the indicated molecular markers. **f, g,** Cortical layers of control and *Brn1/2-cKO* mice analyzed by TLE4 (layer VI, red), CTIP2 (layer VI, V and interneurons, grey), RORβ (layer IV, red) and CUX1 (layer IV and II/III, grey) immunolabeling (TLE4: n=4 mice/group, CTIP2: n=5 mice/group, RORβ: n=3 CT, n=4 cKO mice, CUX1: n=4 CT, n=6 cKO mice; values are mean ± SEM; two-sided unpaired t-test: TLE4-$p=0.0060$, CTIP2-$p<0.0001$, RORβ-$p=0.0007$, CUX1-$p<0.0001$). Neocortical cell layers I-VI are indicated. Lines represent the limits of the cortical plate (CP); dashed lines represent the limits of the cortical heterotopia (Ht). **h,** Schematic of cortical projection neurons in *Brn1/2-cKO* mice (red) and controls (black). CPN, Callosal Projection Neurons; SCPN, Subcortical Projection Neurons; CThPN, Cortico-Thalamic Projection Neurons. Scale bars: 500 μm (b, d, d1-d5), 100 μm (f). Source data are provided as a Source Data file.

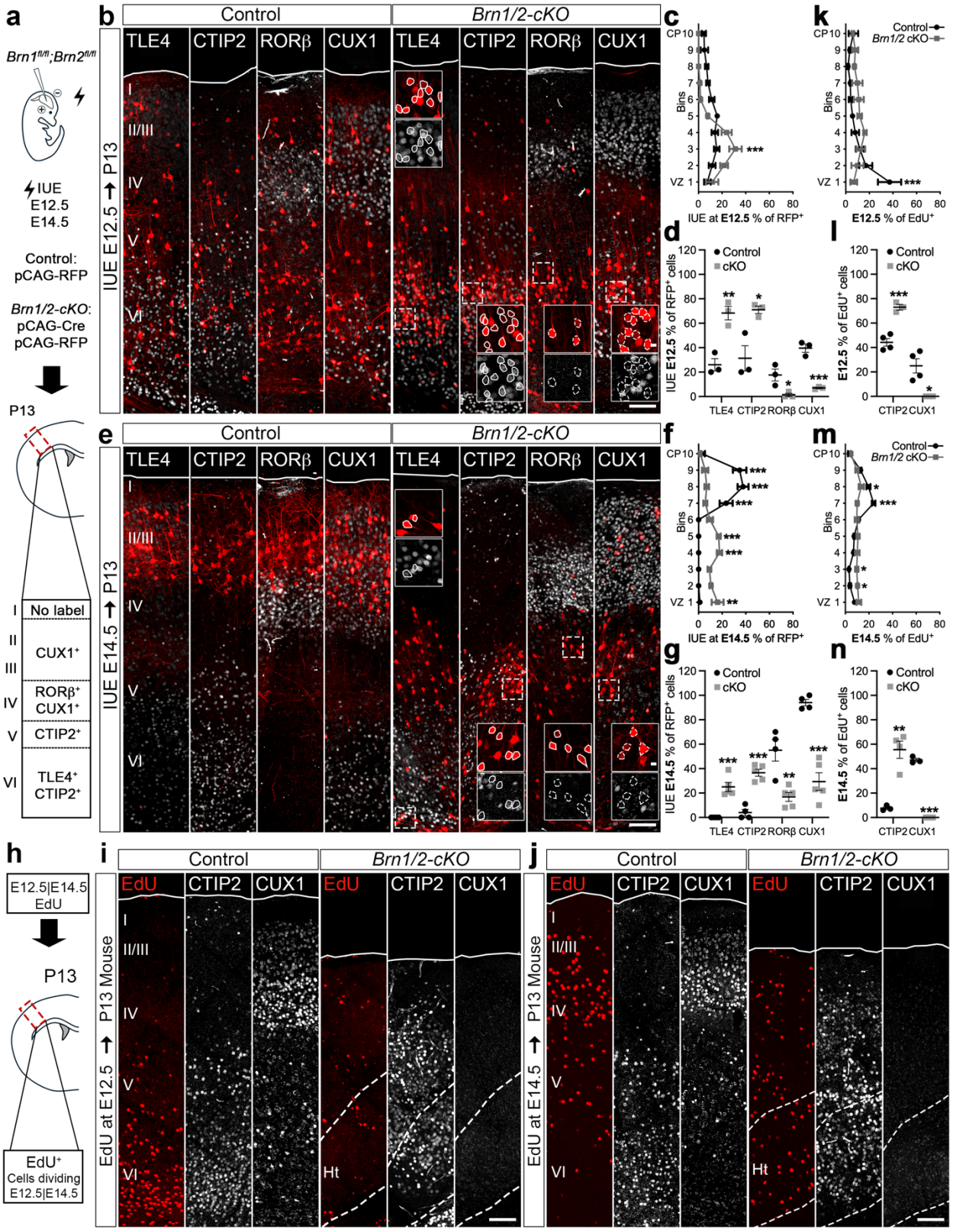
Supplementary Figure 3



Supplementary Fig. 3 Glial cells are increased in *Brn1/2-cKO*. a, a', SOX9 (red), OLIG2 (grey) and GFAP (red) immunolabeling in cortical sections of control (CT) and *Brn1/2-cKO* (cKO) mice at P13. Neocortical cell layers I-VI are indicated. Lines represent the limits of the cortical plate (n=5 CT, n= 4 cKO mice; two-sided unpaired t-test: SOX9-p=0.0001, OLIG2-p=0.0002, GFAP-p<0.0001). **b,** P13 brains were analyzed by EdU and SOX9 or OLIG2 immunolabeling after intraperitoneal injection of EdU at E12.5 (c) and E14.5 (d). **c-f,** EdU (red) and SOX9 or OLIG2 (grey) immunolabeling in P13 control and *Brn1/2-cKO* cortical sections after EdU injection at

E12.5 (c, e) and E14.5 (d, f) (E12.5: n=4 mice/group; E14.5: n=3 mice/group; two-sided unpaired t-test: E12.5-SOX9 p=0.0004, E12.5-OLIG2 p=0.0020, E14.5-SOX9 p=0.0003, E14.5-OLIG2 p=0.0086). Green dotted lines circulating the cells outline cells co-expressing EdU and the indicated glia marker. **g,** In utero electroporation (IUE) in mice from *Brn1*$^{fl/+}$;*Brn2*$^{fl/+}$ crossings at E14.5 with the indicated plasmid. **h,** Cell identities of control and *Brn1/2-cKO* condition immunolabeled for GFP (green; to identify electroporated cells) and OLIG2 (grey) at P13. Lines represent the limits of the CP. Boxed area: higher magnification in inserts. Lines outline cells expressing OLIG2. **i,** GFP^{+} cells expressing OLIG2 at P13 in the control and *Brn1/2-cKO* condition (n=4 CT, n=3 cKO mice; two-sided unpaired t-test: p=0.0106). Values are mean ± SEM; *p<0.05, **p<0.01, ***p<0.001. Top lines represent the limits of the CP. Neocortical cell layers I-VI are indicated. Scale bars: 100 µm (lower magnification), 10 µm (higher magnification). Source data are provided as a Source Data file.

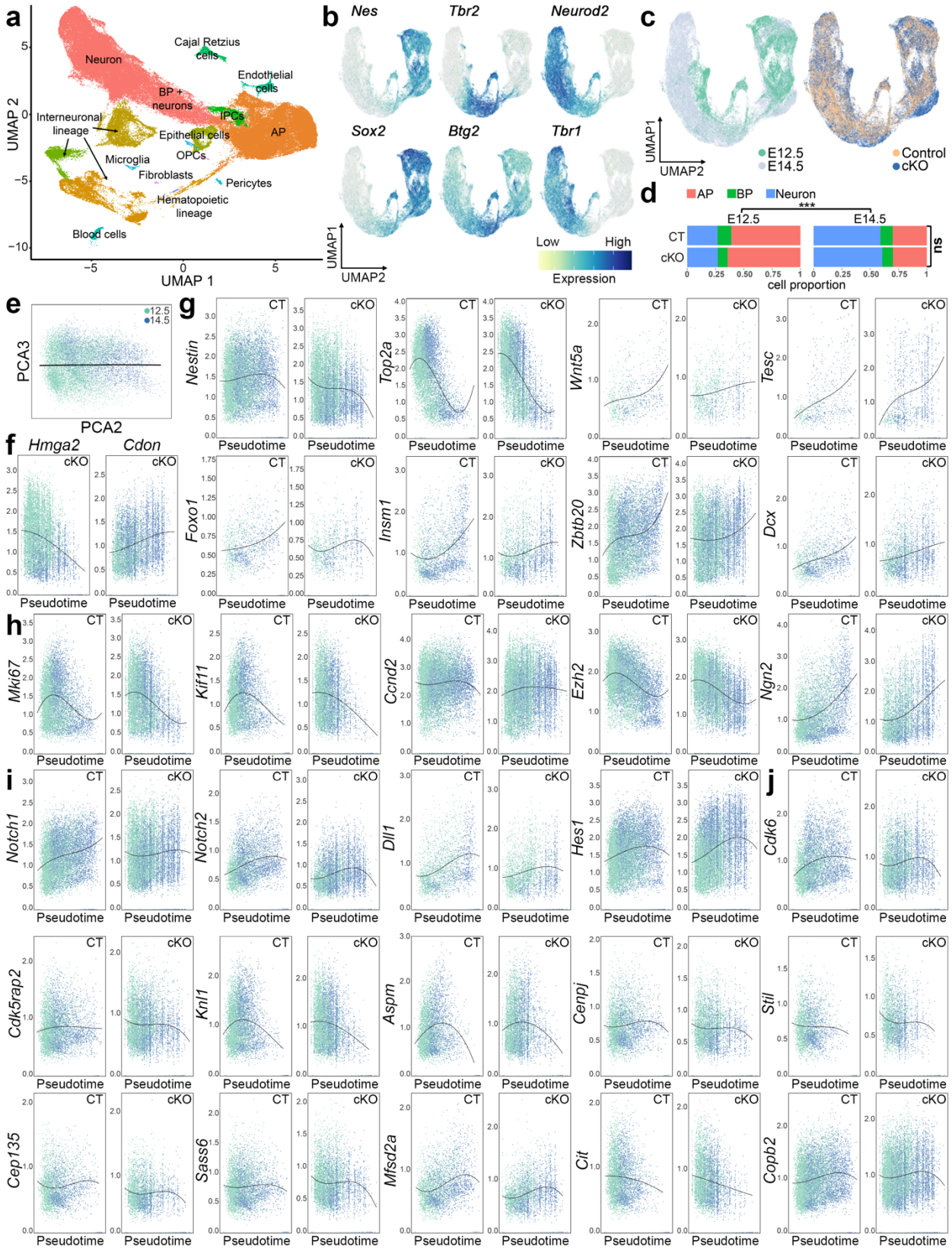
Supplementary Figure 4



Supplementary Fig. 4 BRN1/2 regulate the competence of progenitor cells to generate ULNs. **a,** IUE in *Brn1*$^{fl/fl}$;*Brn2*$^{fl/fl}$ mice at E12.5 and E14.5 with the indicated plasmids. **b, e,** Neuronal identities of control (CT) and *Brn1/2-cKO* (cKO) condition analyzed by co-immunolabeling of RFP (red) with TLE4, CTIP2, RORβ and CUX1 (grey) at P13 after in utero electroporation (IUE) at E12.5 (b) or E14.5 (e). Boxed area: higher magnification in inserts. Lines outline cells expressing DLN markers; dashed lines outline cells not expressing ULN markers. **c, f,** Distribution of RFP$^+$ neurons in the somatosensory cortex at P13 after IUE at E12.5 (c) and E14.5 (f) (E12.5: n=3 mice/group; E14.5: n=4 CT, n=5 cKO mice; two-way ANOVA-Šídák's multiple comparisons test: E12.5 – 3-p=0.0008, $F_{9,60}$=5.980; E14.5 – 1-p=0.0018, 4-p=0.0001, 5-p=0.0003, 7-p=0.0002, 8-p<0.0001, 9-p<0.0001, $F_{9,70}$=24.90). **d, g,** RFP$^+$ neurons expressing the indicated marker genes at P13 after IUE at E12.5 (d) and E14.5 (g) (E12.5: n=3 mice/group, E14.5: n=4 CT, n=5 cKO mice; two-sided unpaired t-test: E12.5 – TLE4-p=0.0048, CTIP2-p=0.0213, RORβ-p=0.0326, CUX1-p=0.0007; E14.5 – TLE4-p=0.0006, CTIP2-p<0.0001, RORβ-p=0.0033, CUX1-p=0.0001). **h,** P13 brains were analyzed by EdU and CTIP2 or CUX1 immunolabeling after intraperitoneal injection of EdU at E12.5 (i) and E14.5 (j). **k, m,** Distribution of EdU$^+$ cells in the somatosensory cortex at P13 after EdU injection at E12.5 (k) and E14.5 (m) (E12.5: n=4 CT, n=3 cKO mice; E14.5: n=3 CT, n=4 cKO mice; two-way ANOVA-Šídák's multiple comparisons test: E12.5 – 1-p<0.0001, $F_{9,50}$=4.323; E14.5 – 2-p=0.0127, 3-p=0.0101, 7-p<0.0001, 8-p=0.0217, $F_{9,60}$=11.15). **l, n,** EdU$^+$ cells expressing the indicated marker genes at P13 after EdU injection at E12.5 (l) and E14.5 (n) (E12.5: n=4 CT, n=3 cKO mice; E14.5: n=3 CT, n=4 cKO mice; two-sided unpaired t-test: E12.5 – CTIP2-p=0.0010, CUX1-p=0.0157; E14.5 – CTIP2-p=0.0023, CUX1-p<0.0001). Top lines represent the limits of the CP. Neocortical cell layers I-VI are indicated. Ht, Cortical neuronal heterotopia. Values are mean ± SEM; *p<0.05, **p<0.01, ***p<0.001. Scale

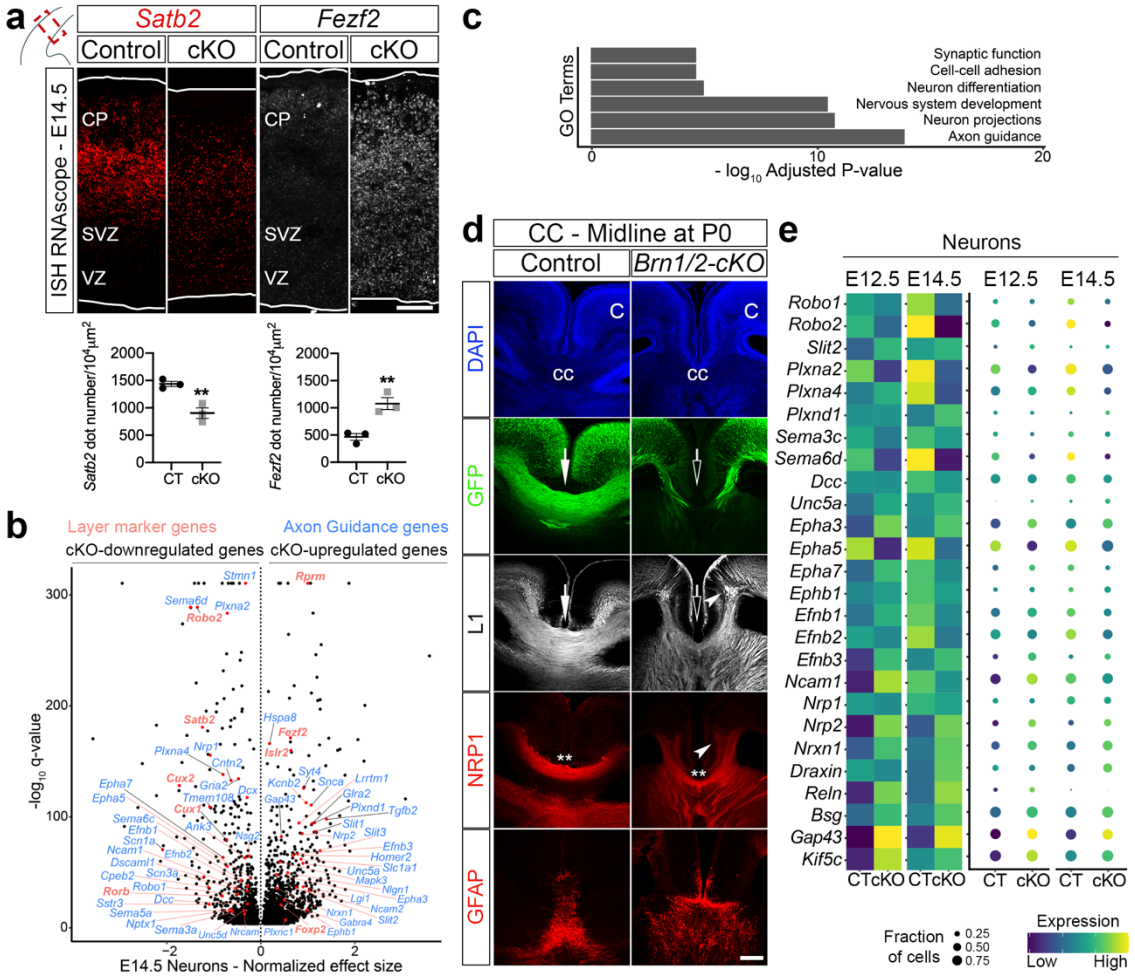
bars: 100 μm (lower magnification), 10 μm (higher magnification). Source data are provided as a Source Data file.

Supplementary Figure 5



Supplementary Fig. 5 Altered transcriptional programs in cortical progenitors of *Brn1/2-cKO* mice a, UMAP of scRNAseq data of all cortical cells sequenced at E12.5 and E14.5. BP, basal progenitors; IPCs, intermediate progenitor cells; AP, apical progenitors; OPCs, oligodendrocyte precursor cells. **b,** UMAP of gene signatures for AP (e.g., *Nes*, *Sox2*), BP (e.g., *Tbr2*, *Btg2*) and neurons (e.g., *Neurod2*, *Tbr1*). (**c,**) UMAP of scRNAseq data from control (CT) and *Brn1/2-cKO* (cKO) cortices at E12.5 and E14.5, by age and genotype. **d,** Proportion of cell types by genotype and age (two sided unpaired t-test: E12.5 CT vs cKO – AP p=0.9946, BP p=0.3965, Neuron p=0.7221; E14.5 CT vs cKO – AP p=0.9977, BP p=0.4026, Neuron p=0.8598; E12.5vsE14.5 – AP p=3.317e-05, BP p=0.8653, Neuron p=1.171e-05; ns, not significant, ***p<0.001). **e,** PCA of control AP age identity organization along the pseudotime axis. **f,** Expression of *Hmga2* and *Cdon* in *Brn1/2-cKO* APs along the pseudotime axis. **g,** Examples of different gene expression dynamics in control and *Brn1/2-cKO* APs along the pseudotime axis. **h, i, j,** Expression of cell cycle-associated genes (h), NOTCH signaling-associated genes (i) and microcephaly-associated genes (j) in control and *Brn1/2-cKO* APs along the pseudotime axis. Source data are provided as a Source Data file.

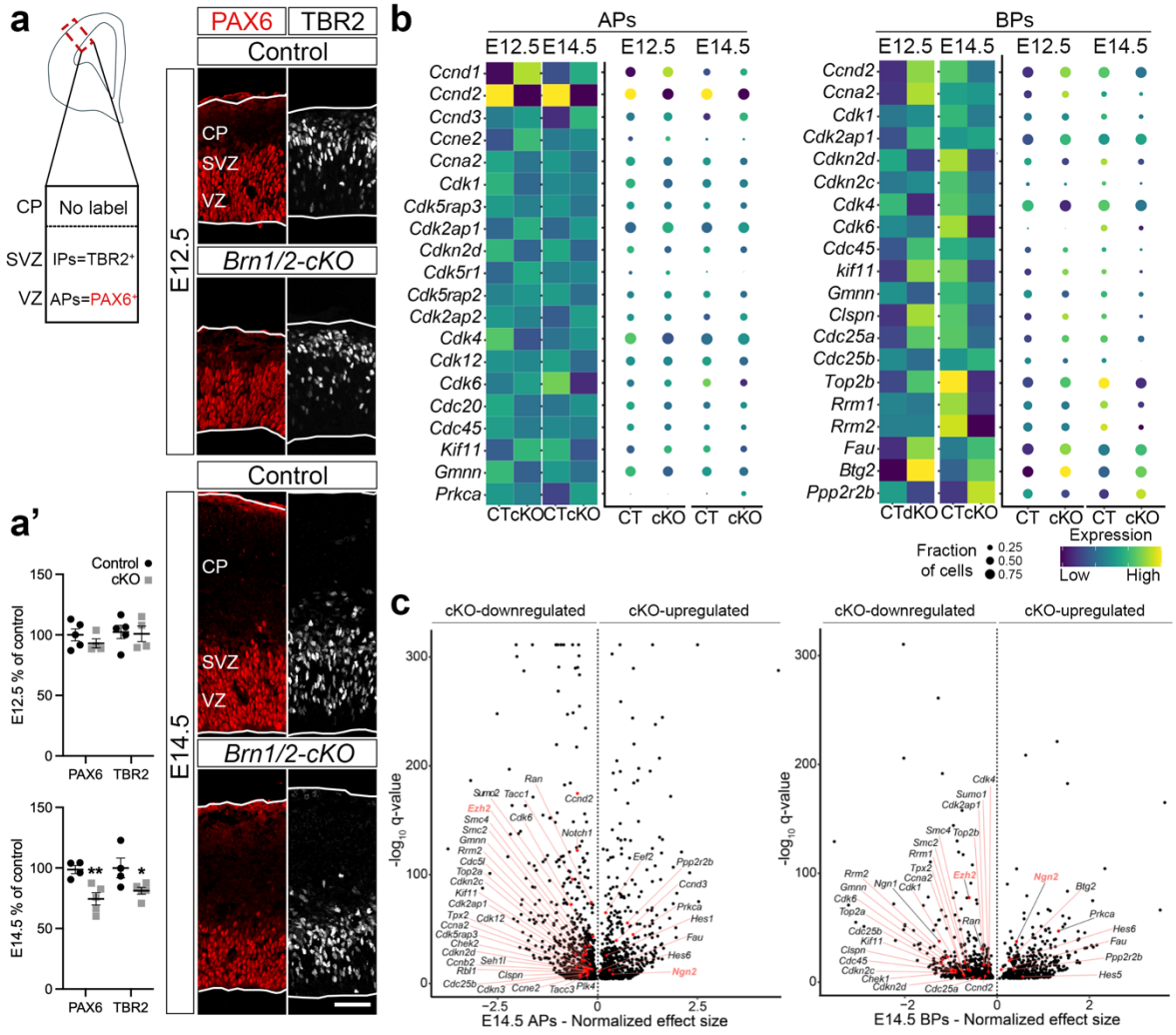
Supplementary Figure 6



Supplementary Fig. 6 Altered transcriptional programs in *Brn1/2-cKO* cortical neurons. a, RNAscope for *Fezf2* and *Satb2* in control (CT) and *Brn1/2-cKO* (cKO) cortical sections at E14.5 (n=3 mice/group; values are mean ± SEM; two-sided unpaired t-test: *Satb2*-p=0.0077, *Fezf2*-p=0.0084). Low and top lines represent the limits of the ventricular zone (VZ) and cortical plate (CP), respectively. SVZ, Subventricular Zone. Scale bar: 50 µm. **b,** Volcano plot: DEG between *Brn1/2-cKO* and control neurons at E14.5 highlighted for layer marker and axon guidance genes (Monocle3 VGAM test; SD=0.15; q<0.001; Supplementary Data 2). **c,** Some of the most relevant gene ontology (GO) terms differentially affected in *Brn1/2-cKO* neurons compared to controls at E14.5. **d,** DAPI (blue), GFP (BRN2^{+} callosal axons, green), L1 (callosal axons; grey), NRP1

(pioneer axons; red) and GFAP (glia guidepost cells; red) immunolabeling in control and *Brn1/2-cKO* brains at P0. Open arrows indicate absent corpus callosum (cc) and ventral misrouting of GFP^+ and $L1^+$ projections in *Brn1/2-cKO* mice. Arrowheads indicate misrouting and abnormal defasciculation of $L1^+$ and $NRP1^+$ neurons in *Brn1/2-cKO* mice. Double asterisks indicate that some $NRP1^+$ pioneer axons still cross the midline in *Brn1/2-cKO* mice. C, cortex. Scale bar: 200 μm. **e,** Expression of axon guidance-associated genes in *Brn1/2-cKO* neurons compared to control at E12.5 and E14.5. Source data are provided as a Source Data file.

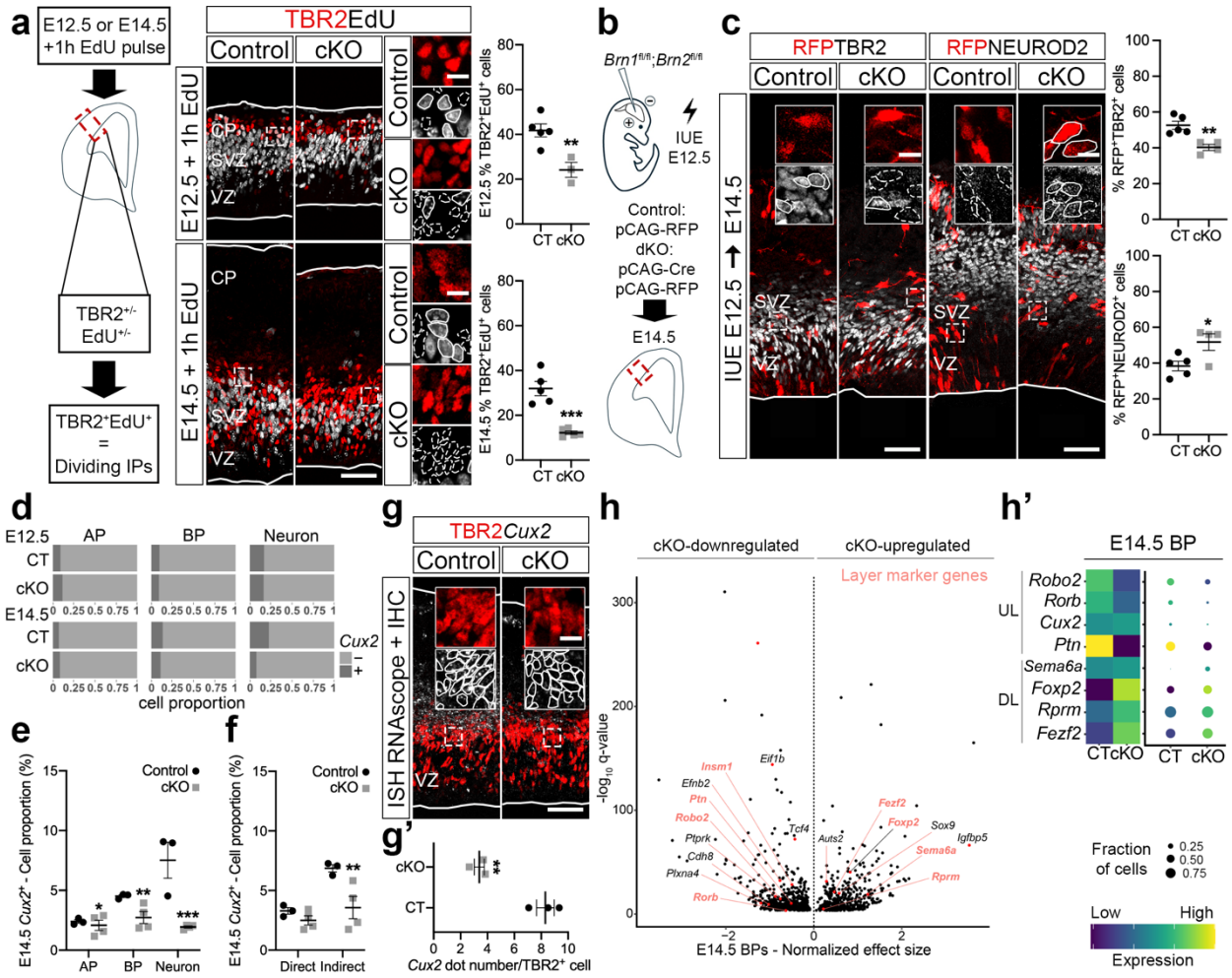
Supplementary Figure 7



Supplementary Fig. 7 Reduction in proliferation rate and precocious cell cycle exit of cortical progenitors in *Brn1/2-cKO* mice. a, a' PAX6 (apical progenitors (APs); red) and TBR2 (intermediate progenitors (IPs); grey) immunolabeling in control (CT) and *Brn1/2-cKO* (cKO) cortical sections at E12.5 and E14.5 (E12.5: n=5 CT, n=4 cKO mice; E14.5: n=4 CT, n=5 cKO mice; values are mean ± SEM; two-sided unpaired t-test: E12 – PAX6-p=0.3095, TBR2-p=0.8582; E14.5 – PAX6-p=0.0069, TBR2-p=0.0493). Low and top lines represent the limits of the ventricular zone (VZ) and cortical plate (CP), respectively. SVZ, Subventricular Zone. Scale bar: 50 µm. **b,** Expression of cell cycle-associated genes in *Brn1/2-cKO* APs and basal progenitors

(BPs) compared to control at E12.5 and E14.5. **d,** Volcano plots: DEG between *Brn1/2-cKO* and control APs and BPs at E14.5 highlighting cell cycle-associated genes (Monocle3 VGAM test; SD=0.15; q<0.001; Supplementary Data 3). Source data are provided as a Source Data file.

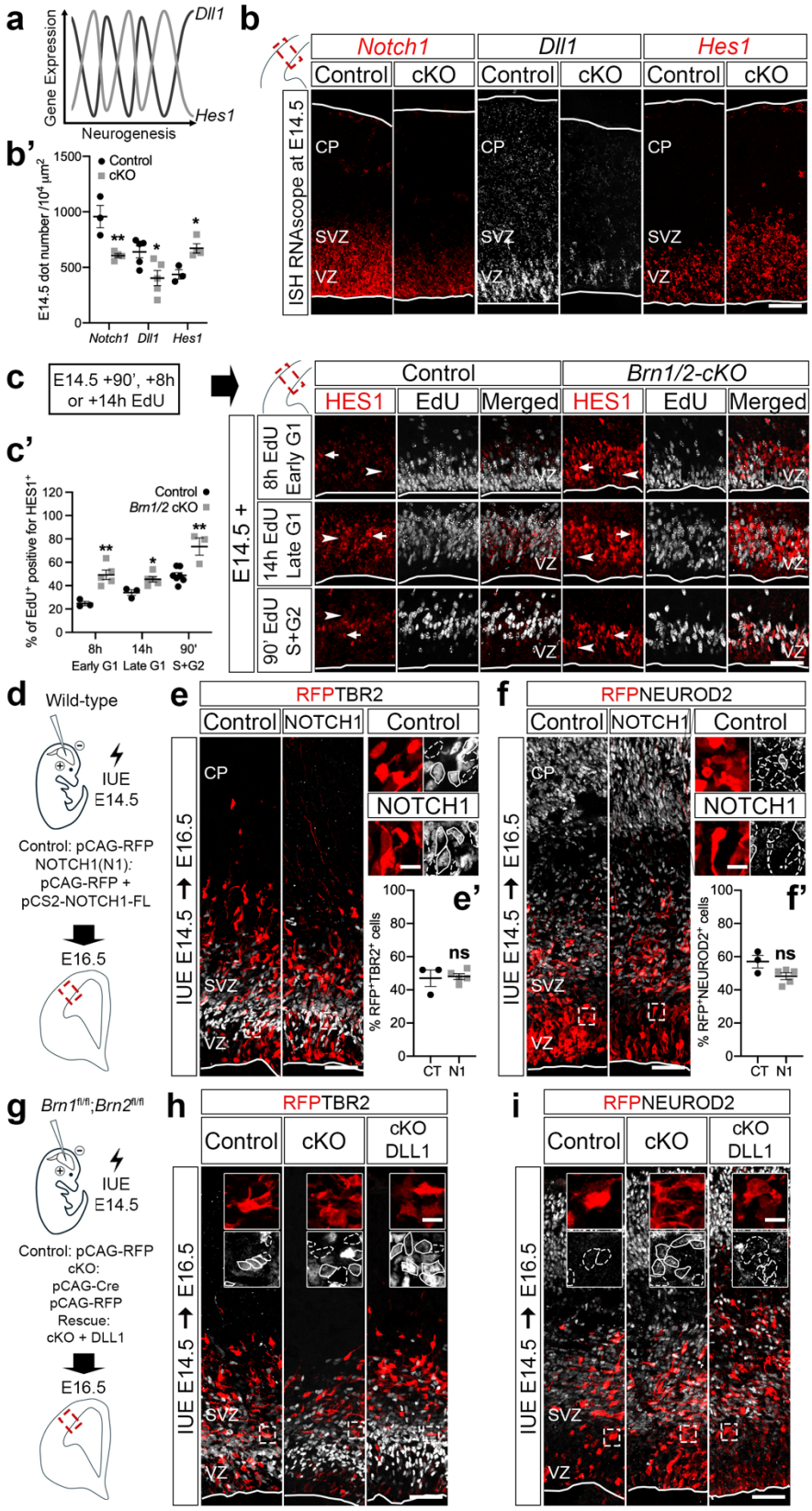
Supplementary Figure 8



Supplementary Fig. 8 BRN1/2 regulate the balance between direct and indirect neurogenesis affecting the generation of a specific type of BPs. a, TBR2 (red) and EdU (grey) immunolabeling in control (CT) and *Brn1/2-cKO* (cKO) cortical sections at E12.5 and E14.5 (E12.5: n=5 CT, n=3 cKO mice; E14.5: n=5 mice/group; two-sided unpaired t-test: E12.5-p=0.0084, E14.5-p=0.0003). Boxed area at higher magnification on the right. Lines and dashed lines circulating cells: expression or absence of EdU, respectively. **b,** In utero electroporation (IUE) in *Brn1*$^{fl/fl}$;*Brn2*$^{fl/fl}$ mice at E12.5 with the indicated plasmids. **c,** Cell identities of the control and *Brn1/2-cKO* condition immunolabeled for RFP (red; to identify electroporated cells), TBR2 and NEUROD2 (grey) at E14.5 (n=5 CT, n=4 cKO mice; two-sided unpaired t-test: TBR2-p=0.0041, NEUROD2-

p=0.0328). **d,** Proportion of apical progenitors (AP), basal progenitors (BP) and neurons expressing *Cux2* in control and *Brn1/2-cKO* cortices at E12.5 and E14.5. **e,** Proportion of AP, BP and neurons expressing *Cux2* in control and *Brn1/2-cKO* cortices at E14.5 (n=3 CT, n=4 cKO mice; Pearson's Chi-squared test: AP-p=0.004047, BP-p=0.001862, Neuron-p=2.2e-16). **f,** Proportion of cells undergoing direct and indirect neurogenesis expressing *Cux2* in control and *Brn1/2-cKO* cortices at E14.5 ((n=3 CT, n=4 cKO mice; Pearson's Chi-squared test: direct-p=0.9413, indirect-p=0.001154). **g, g',** *Cux2* (grey*)* expression by RNAscope and TBR2 (red) expression by immunolabeling in control and *Brn1/2-cKO* cortical sections at E14.5 (n=3 mice/group; two-sided unpaired t-test: p=0.0029). **h,** Volcano plot: DEG between *Brn1/2-cKO* and control BPs at E14.5 highlighted for layer marker genes (Monocle3 VGAM test; SD=0.15; q<0.001; Supplementary Data 3). **h',** Expression of the indicated cortical upper layer (UL) and deep layer (DL) marker genes in control and *Brn1/2-cKO* BPs compared to controls at E14.5. Low and top lines represent the limits of the ventricular zone (VZ) and cortical plate (CP), respectively. Boxed area: higher magnification in inserts. Lines and dashed lines outlining cells expressing or lacking expression of the indicated marker, respectively. SVZ, Subventricular Zone. Values are mean ± SEM; *p<0.05, **p<0.01, ***p<0.001. Scale bars: 50 µm (lower magnification), 10 µm (higher magnification). Source data are provided as a Source Data file.

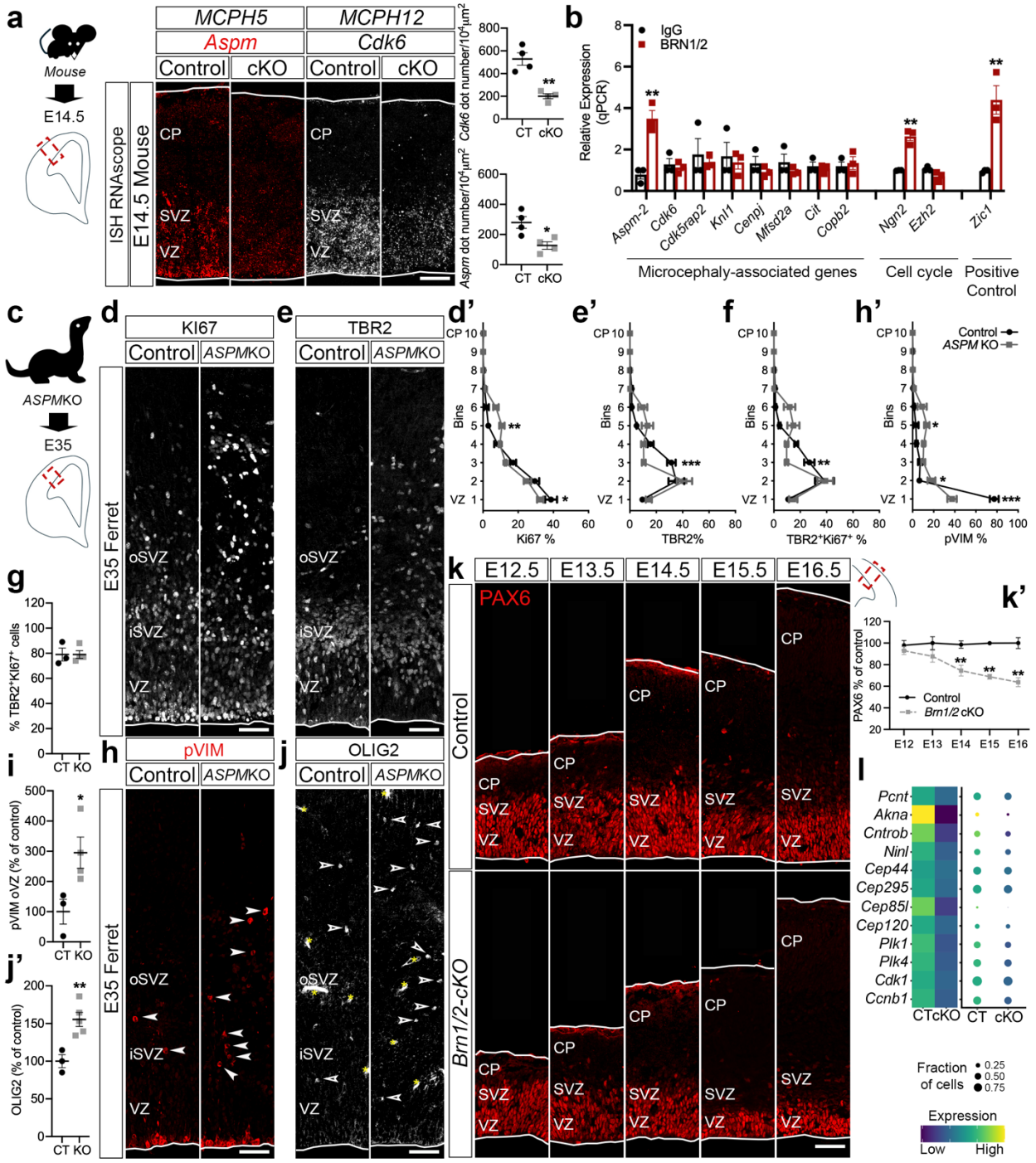
Supplementary Figure 9



Supplementary Fig. 9 BRN1/2 are essential to maintain NOTCH signaling balanced during neurodevelopment. a, Expression of HES1 and DLL1 in neural progenitors is oscillatory, which is critical for the maintenance of neural progenitors[46,47]. **b, b',** RNAscope for *Notch1* (red), *Dll1* (grey) and *Hes1* (red) in control (CT) and *Brn1/2-cKO* (cKO) cortical sections at E14.5 (*Notch1* and *Hes1*: n=3 CT, n=4 cKO mice; *Dll1*: n=5 mice/group; two-sided unpaired t-test: *Notch1*-p=0.01, *Dll1*-p=0.0266, *Hes1*-p=0.0122). **c,** E14.5 brains were collected 90', 8h or 14h after intraperitoneal injection of EdU. HES1 (red) and EdU (grey) immunolabeling in control and *Brn1/2-cKO* cortical sections at early G1 (8h EdU), late G1 (14h EdU) and S+G2 (90' EdU). Cells expressing HES1 at high and low levels are indicated by arrows and arrowheads, respectively. **c',** $HES1^{+}/EdU^{+}$ cells in control and *Brn1/2-cKO* cortices at early G1 (8h EdU), late G1 (14h EdU) and S+G2 (90' EdU) (8h and 14h: n=3 CT, n=5 cKO mice; 90': n=7 CT, n=3 cKO mice; two-sided unpaired t-test: 8h-p=0.0047, 14h-p=0.0202, 90'-p=0.0029). **d,** In utero electroporation (IUE) in wild-type mice at E14.5 with the indicated plasmids. **e, f,** Cell identities of the control and NOTCH1 (N1) condition immunolabeled for RFP (red) with TBR2 (e, grey) and NEUROD2 (f, grey) at E16.5. Boxed area at higher magnification on the right. **e', f',** RFP^{+} cells expressing TBR2 (e') and NEUROD2 (f') (n=3 CT, n=5 N1 mice; two-sided unpaired t-test: TBR2-p=0.8230, NEUROD2-p=0.0612). **g,** IUE in $Brn1^{fl/fl}$;$Brn2^{fl/fl}$ mice at E14.5 with the indicated plasmids. **h, i,** Cell identities of the control, *Brn1/2-cKO* and *Brn1/2-cKO*+DLL1 condition immunolabeled for RFP (red) with TBR2 (h, grey) and NEUROD2 (i, grey) at E16.5. Boxed area: higher magnification in inserts. Lines and dashed lines outlining cells expressing or lacking expression of the indicated marker, respectively. Low and top lines represent the limits of the ventricular zone (VZ) and cortical plate (CP), respectively. SVZ, Subventricular Zone. Values are mean ± SEM;

ns, not significant; *p<0.05, **p<0.01. Scale bars: 50 μm (lower magnification), 10 μm (higher magnification). Source data are provided as a Source Data file.

Supplementary Figure 10



Supplementary Fig. 10 BRN1/2 are required for the expression of microcephaly-associated genes and maintenance of the neuronal progenitor pool. a, *Aspm* (red) and *Cdk6* (grey) expression in control (CT) and *Brn1/2-cKO* (cKO) cortices at E14.5 (n=4 mice/group; two-sided

unpaired t-test: *Cdk6*-p=0.0014, *Aspm*-p=0.0156). **b,** ChIP-qPCR analysis of BRN1/2 binding to the indicated promoters/enhancers at E14.5 (n=3/condition; two-sided unpaired t-test: *Aspm-2*-p=0.0038, *Cdk6*-p=0.6722, *Cdk5rap2*-p=0.6558, *Knl1*-p=0.7183, *Cenpj*-p=0.3186, *Mfsd2a*-p=0.3344, *Cit*-p=0.6262, *Copb2*-p=0.7034, *Ngn2*-p=0.0014, *Ezh2*-p=0.0754, *Zic1*-p=0.0077). **c,** Histological analysis at E35 in cortices of control and *ASPM*KO ferrets. **d, e,** Ki67 (d) and TBR2 (e) immunolabeling in control and *ASPM*KO. **d', e', f,** Distribution of $Ki67^+$ (d'), $TBR2^+$ (e') and $TBR2^+Ki67^+$ (f) cells in control and *ASPM*KO (Ki67: n=3 CT, n=5 KO ferrets; TBR2 and $TBR2^+Ki67^+$: n=3 CT, n=4 KO ferrets; two-way ANOVA-Šídák's multiple comparisons test: Ki67 – 1-p=0.0251, 5-p=0.0023, $F_{9,60}$=4.772; TBR2 – 3-p<0.0001, $F_{9,50}$=5.233; $TBR2^+Ki67^+$ – 3-p=0.0014, $F_{9,50}$=3.735). **g,** $TBR2^+Ki67^+$ cells in control and *ASPM*KO (n=3 CT, n=4 KO ferrets; two-sided unpaired t-test: p>0.9999). **h,** pVIM immunolabeling in control and *ASPM*KO ferrets. Arrowheads: $pVIM^+$ cells outside the VZ (oVZ). **h',** Distribution of $pVIM^+$ cells in control and *ASPM*KO (n=3 CT, n=4 KO ferrets; two-way ANOVA-Šídák's multiple comparisons test: 1-p<0.0001, 2-p=0.0137, 5-p=0.0354, $F_{9,50}$=19.02). **i,** $pVIM^+$ cells in control and *ASPM*KO oVZ (n=3 CT, n=4 KO ferrets; two-sided unpaired t-test: p=0.0391). **j, j',** OLIG2 immunolabeling in control and *ASPM*KO (n=3 CT, n=5 KO ferrets; two-sided unpaired t-test: p=0.0072). Empty arrowheads indicate $OLIG2^+$ cells. **k, k',** PAX6 immunolabeling in control and *Brn1/2-cKO* cortices from E12.5 to E16.5 (E12.5: n=6 CT and n=4 cKO mice; E13.5: n=5 CT and n=6 cKO mice; E14.5: n=4 CT and n=5 cKO mice; E15.5: n=5 CT and n=3 cKO mice; E16.5: n=5 CT and n=3 cKO mice; two-sided unpaired t-test: E12-p=0.4550, E13-p=0.1640, E14-p=0.0069, E15-p<0.0001, E16-p=0.0028). **l,** Expression of centrosome-associated genes in *Brn1/2-cKO* compared to control progenitors at E14.5. Low and top lines represent the limits of the ventricular zone (VZ) and cortical plate (CP), respectively. SVZ, Subventricular Zone; iSVZ, Inner Subventricular Zone;

oSVZ, Outer Subventricular Zone. Values are mean ± SEM; *p<0.05, **p<0.01, ***p<0.001. Scale bar: 50 μm. Source data are provided as a Source Data file.

Supplementary Table 1

ChIP-qPCR primers		
Gene Fragment	**Forward Primer**	**Reverse Primer**
Notch1	CTGCCTGTCTGTCTCGAGG	GTGCCTCCAGTCCTCAGATG
Dll1	CGCCTGCCACCCTTAAATC	AGGGTTGCACATTTTACAGCT
Hes1	ACGCTGTGACCCTTTAATACA	CCTGTGGGTAGAGACTGCT
Aspm_1	AACTCAGTTGACGGGCTTTG	CGCCTGCCAAACGTCTATTT
Aspm_2	CGGAAAAGAAAGAGCCACGG	TGCCCTTCTTTCTTTGTGGT
Stil	CATTGTGACGTGCCTCAGAT	GTACGTCCACGCCTCACTTT
Cep135	AACAACCATTAAGCCGCATC	TGCCCTACAGGGGACTAT
Sass6	CTGATGTATTGAGTCGCTGGA	AGTGAGGGCACCACTTCAAC
Cdk6	TTAAAGCCACCTCAGGCACT	AGGCCCTCTCCCATATTCAT
Cdk5rap2	TAACCCCTGGTGAACCTGAA	CTCCAGATAGCCCAAACAGC
Knl1	TGTGAATGGCTGGAAACCTT	ACCATGTTGGACAGTGCAGA
Cenpj	GGAGACACAGGAGGCTGGTA	CCTATGTCCACAAGAAGCCCTA
Mfsd2a	ATCAGGTGTAGTGGCACCAA	CTTTGTATCCCTGGCTGACC
Cit	AAGGTAAGCCAGCATACAGCA	AAAAGTTGTCCTCCGACTGC
Copb2	AAAAATGGATCTTTGAAAACTATCTGA	GGAAGTTGCCCTTAGCTCCT
Ngn2	CCTTCACACAGTTACCTAATGCA	GGTGCATTCCAAAGCTGTCA
Ezh2	TGGTGTGATTTGGAAAATCTGAC	TGACATGTGTAGCTAGAAGTGTT
Zic1	ATTTTGGGAGTCAGGCCAGA	GGCTCCACGTGCTAATGAAT