

Expanded View Figures

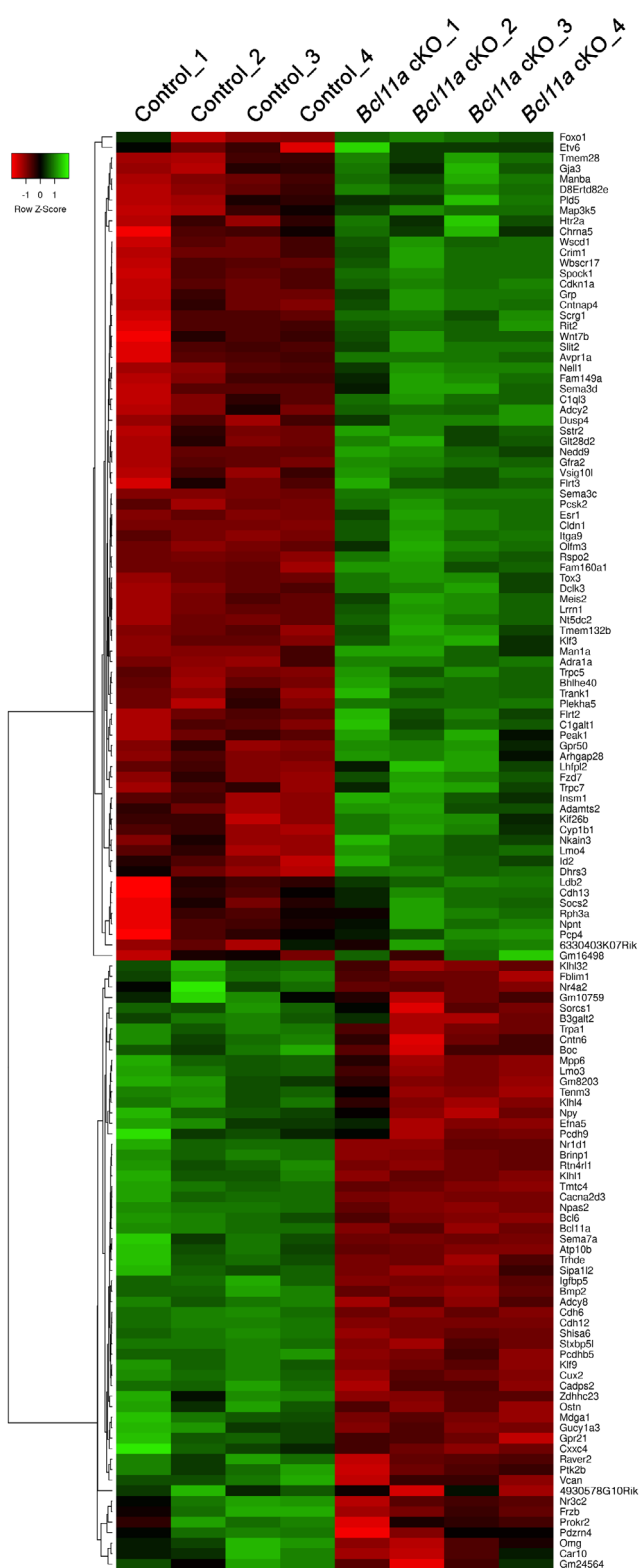


Figure EV1. Heat map of differentially expressed genes in upper layers of P2 *Bcl11a^{Fl/F};Emx1^{iresCre}* compared with control neocortex.

Heat map showing 79 upregulated genes (green) and 58 downregulated genes (red) in upper neocortical layers of conditional *Emx1^{iresCre};Bcl11a^{Fl/F}* mutants (*Bcl11a* cKO) compared with controls at postnatal day 2.

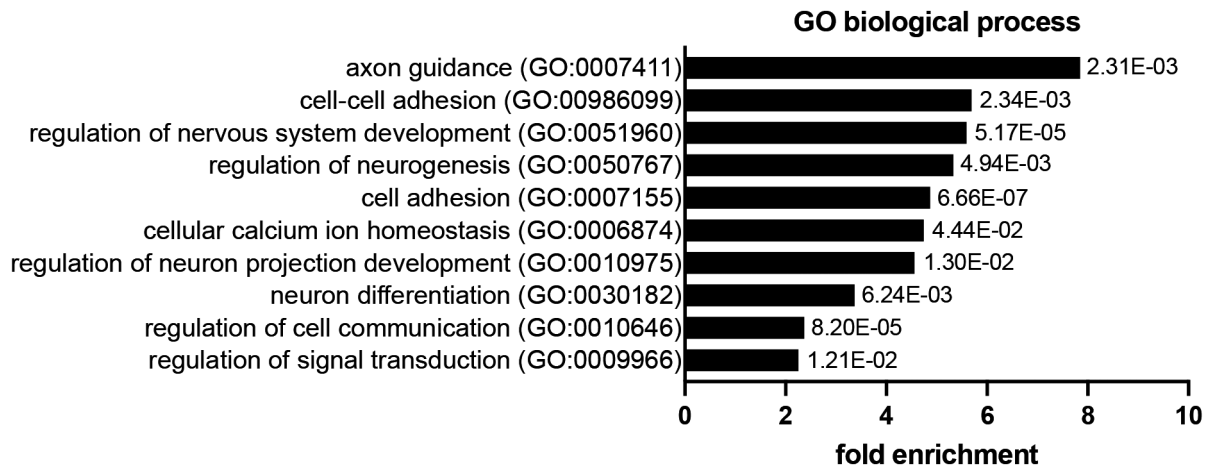


Figure EV2. Gene ontology enrichment analysis of differentially expressed genes in upper layers of P2 *Bcl11a*^{FF};*Emx1*^{IRESCre} compared with control neocortex.

Graph shows selected GO terms for biological process, GO term accession numbers, fold enrichment, and *P*-value. Data were obtained by GO overrepresentation test using PANTHER.

Figure EV3. Selected candidate downstream target genes of *Bcl11a* in superficial cortical layers at early postnatal development.

- A Volcano plot showing differentially expressed (DE) genes in laser-microdissected cortical layers 2–4 of *Bcl11a*^{FF};*Emx1*^{IRESCre} neocortex compared with controls. DE genes not significantly changed (fold change < 1.5; *P* > 0.05) are shaded black. *Cdh6*, *Cdh12*, *EfnA5*, *Pcdh9*, *Cdh13*, *Flrt2*, *Flrt3*, and *Slit2* are highlighted in green.
- B Relative mRNA expression levels of selected DE genes determined by quantitative real-time PCR in laser-microdissected cortical tissue of P2 *Bcl11a*^{FF};*Emx1*^{IRESCre} and control brains (*n* = 4). Graph represents mean ± s.e.m.; Student's *t*-test; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.
- C RNA *in situ* hybridization of selected DE genes with decreased expression in P2 *Bcl11a*^{FF};*Emx1*^{IRESCre} compared with control neocortex.
- D RNA *in situ* hybridization of selected DE genes with increased expression in P2 *Bcl11a*^{FF};*Emx1*^{IRESCre} compared with control neocortex.

Data information: Scale bar, 50 μm.

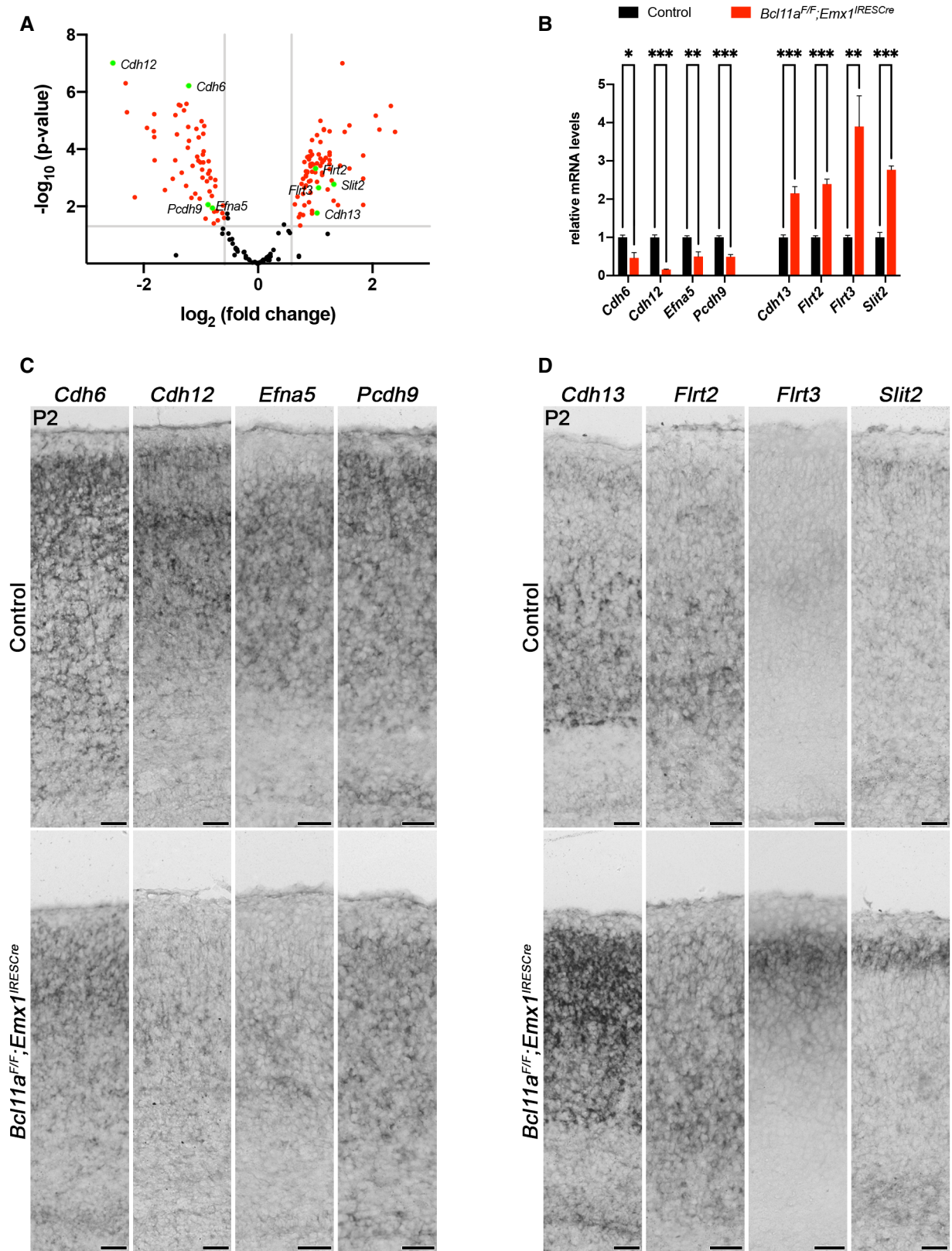


Figure EV3.

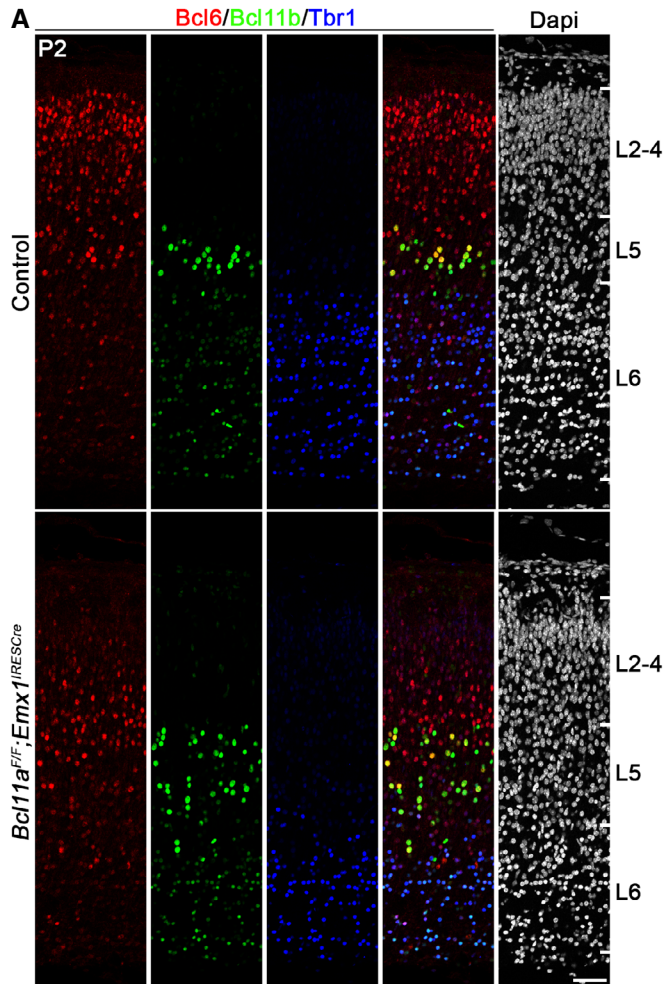


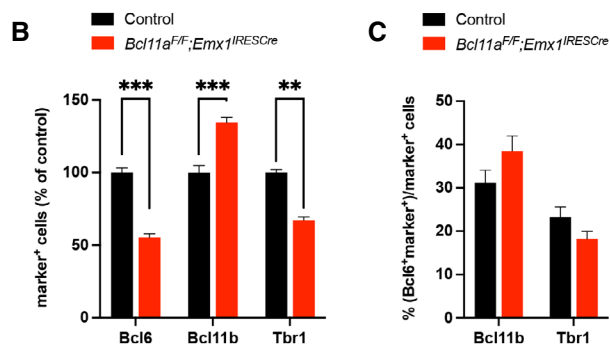
Figure EV4. Relative Bcl6 expression is unchanged in deep cortical layers of *Bcl11a^{Fl/Fl};Emx1^{iresCre}* compared with control neocortex.

A Immunohistochemistry of Bcl6 (red), Bcl11b (green), and Tbr1 (blue) in P2 *Bcl11a^{Fl/Fl};Emx1^{iresCre}* and control neocortex. Bcl6 and Tbr1 expressions are downregulated, while Bcl11b expression is upregulated in *Bcl11a^{Fl/Fl};Emx1^{iresCre}* compared with control neocortex. Nuclei are stained with Dapi (white).

B Relative quantification of Bcl6⁺, Bcl11b⁺, and Tbr1⁺ cells in *Bcl11a^{Fl/Fl};Emx1^{iresCre}* and control neocortex ($n = 4$).

C Numbers of Bcl11b⁺ or Tbr1⁺ cells that coexpress Bcl6 are normal in *Bcl11a^{Fl/Fl};Emx1^{iresCre}* compared with control neocortex ($n = 4$).

Data information: All graphs represent mean \pm s.e.m.; Student's *t*-test; ** $P < 0.01$, *** $P < 0.001$. Scale bar, 50 μ m.



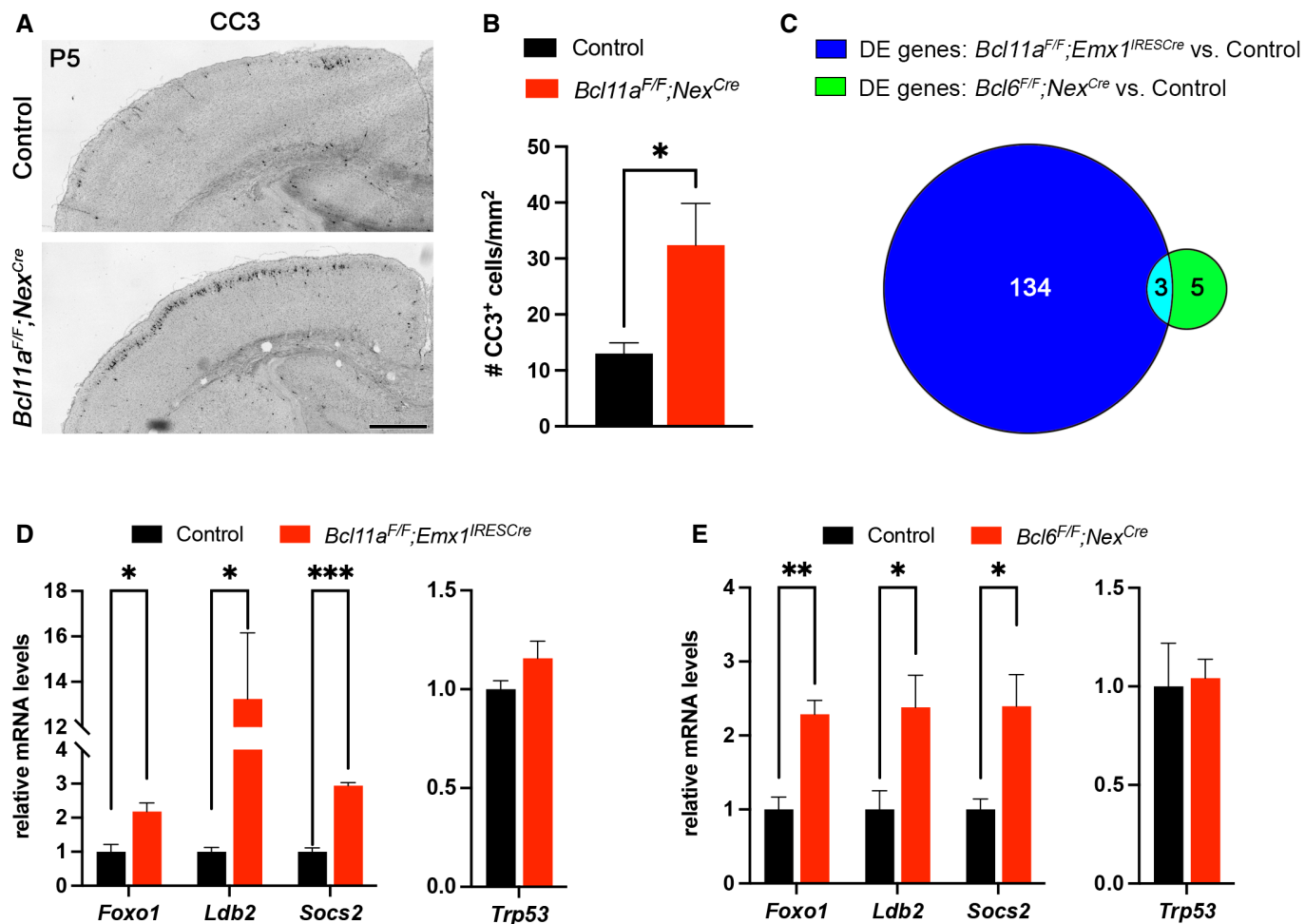


Figure EV5. Increased cell death in *Bcl11a^{F/F};Nex^{Cre}* and overlapping differentially expressed genes in upper cortical layers of *Bcl11a^{F/F};Emx1^{iresCre}* and *Bcl6^{F/F};Nex^{Cre}* compared with controls.

- A Immunohistochemistry of cleaved caspase 3 (CC3) shows that the number of CC3⁺ cells is increased in P5 *Bcl11a^{F/F};Nex^{Cre}* compared with control neocortex.
 B Quantification of the experiment shown in (A).
 C Venn diagram showing the number of overlapping differentially expressed (DE) genes in P2 *Bcl11a^{F/F};Emx1^{iresCre}* and P5 *Bcl6^{F/F};Nex^{Cre}* upper cortical layers.
 D, E Relative mRNA expression levels of the overlapping DE genes *Foxo1*, *Ldb2*, and *Socs2* as well as *Trp53* determined by quantitative real-time PCR in laser-microdissected cortical tissue of P2 *Bcl11a^{F/F};Emx1^{iresCre}* compared with control brains (D) or P5 *Bcl6^{F/F};Nex^{Cre}* compared with control brains (E).

Data information: All graphs represent mean ± s.e.m.; n = 4; Student's t-test; *P < 0.05; **P < 0.01; ***P < 0.001. Scale bar, 500 μm.