Figure EV1. Heat map of differentially expressed genes in upper layers of P2 Bcl11aαF/Emx1IRESCre compared with control neocortex.

Heat map showing 79 upregulated genes (green) and 58 downregulated genes (red) in upper neocortical layers of conditional Emx1IRESCre;Bcl11aαF mutants (Bcl11α cKO) compared with controls at postnatal day 2.
Figure EV2. Gene ontology enrichment analysis of differentially expressed genes in upper layers of P2 Bcl11a<sup>F10</sup>;Emx1<sup>IRESCre</sup> 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<sup>F10</sup>;Emx1<sup>IRESCre</sup> neocortex compared with controls. DE genes not significantly changed (fold change < 1.5, P > 0.05) are shaded black. Cadh6, Cadh12, Efn5, Pcdh9, Cadh13, Flr2, Flr3, 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<sup>F10</sup>;Emx1<sup>IRESCre</sup> 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<sup>F10</sup>;Emx1<sup>IRESCre</sup> compared with control neocortex.

D RNA in situ hybridization of selected DE genes with increased expression in P2 Bcl11a<sup>F10</sup>;Emx1<sup>IRESCre</sup> compared with control neocortex.

Data information: Scale bar, 50 μm.
Figure EV3.
Relative Bcl6 expression is unchanged in deep cortical layers of Bcl11a<sup>FL/F</sup>;Emx1<sup>IRESCre</sup> compared with control neocortex.

A Immunohistochemistry of Bcl6 (red), Bcl11b (green), and Tbr1 (blue) in P2 Bcl11a<sup>FL/F</sup>;Emx1<sup>IRESCre</sup> and control neocortex. Bcl6 and Tbr1 expressions are downregulated, while Bcl11b expression is upregulated in Bcl11a<sup>FL/F</sup>; Emx1<sup>IRESCre</sup> compared with control neocortex. Nuclei are stained with Dapi (white).

B Relative quantification of Bcl6<sup>+</sup>, Bcl11b<sup>+</sup>, and Tbr1<sup>+</sup> cells in Bcl11a<sup>FL/F</sup>; Emx1<sup>IRESCre</sup> and control neocortex (n = 4).

C Numbers of Bcl11b<sup>+</sup> or Tbr1<sup>+</sup> cells that coexpress Bcl6 are normal in Bcl11a<sup>FL/F</sup>; Emx1<sup>IRESCre</sup> compared with control neocortex (n = 4).

Data information: All graphs represent mean ± s.e.m.; Student’s t-test; **P < 0.01; ***P < 0.001. Scale bar, 50 μm.
Figure EV5. Increased cell death in Bcl11αF/F;NexCre and overlapping differentially expressed genes in upper cortical layers of Bcl11αF/F;Emx1IRESCre and Bcl6F/F;NexCre compared with controls.

A Immunohistochemistry of cleaved caspase 3 (CC3) shows that the number of CC3+ cells is increased in P5 Bcl11αF/F;NexCre 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 Bcl11αF/F;Emx1IRESCre and P5 Bcl6F/F;NexCre upper cortical layers.
D, E Relative mRNA expression levels of the overlapping DE genes Foxo1, Ldb2, Socs2 as well as Trp53 determined by quantitative real-time PCR in laser-microdissected cortical tissue of P2 Bcl11αF/F;Emx1IRESCre compared with control brains (D) or P5 Bcl6F/F;NexCre 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.