

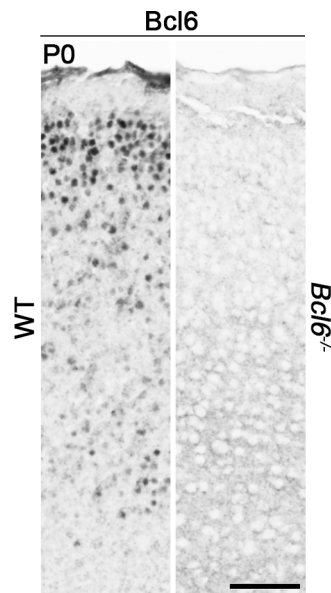
Appendix

Developmental cell death of cortical projection neurons is controlled by a Bcl11a/Bcl6-dependent pathway

Wiegreffe C., Wahl T., Joos N., S., Bonnefont J., Liu P., and Britsch, S.

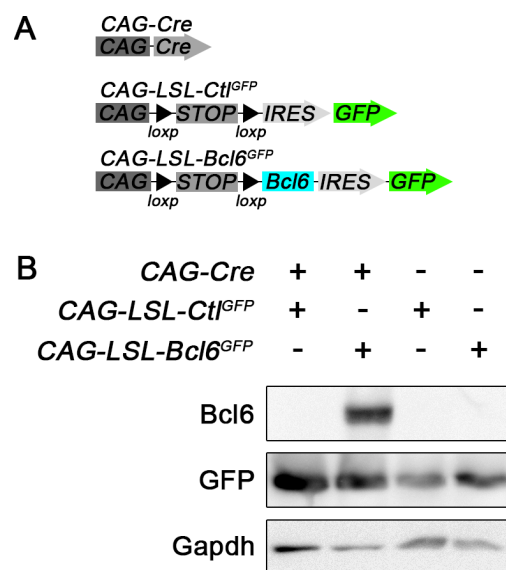
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Appendix Figure S1. Specificity of Bcl6 antibody shown by immunohistochemistry

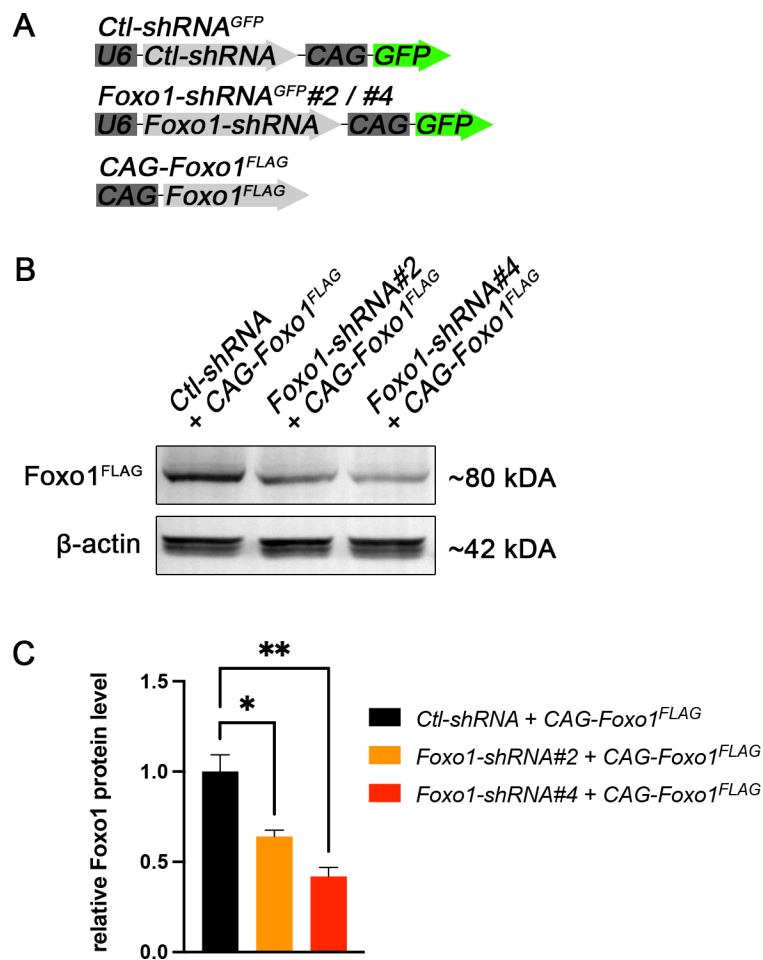
Immunohistochemistry of Bcl6 using guinea pig anti-Bcl6 antibody in P0 *Bcl6*^{-/-} and wildtype neocortex. Bcl6 expression is detectable in upper layers and at lower levels in deep layers of wildtype neocortex. In *Bcl6*^{-/-} mutants Bcl6 expression is not detectable. Scale bar, 50 μ m.



Appendix Figure S2. Western blot analysis of Cre-dependent DNA plasmids for overexpression of Bcl6 cDNA

(A) DNA plasmids used for transfection of HEK293 cells.

(B) Western blot analysis of total protein lysates shows that *CAG-LSL-Bcl6^{GFP}* plasmid expresses *Bcl6* only in the presence *CAG-Cre* plasmid. Note that GFP is expressed independent of *CAG-Cre*. Gapdh served as protein loading control.

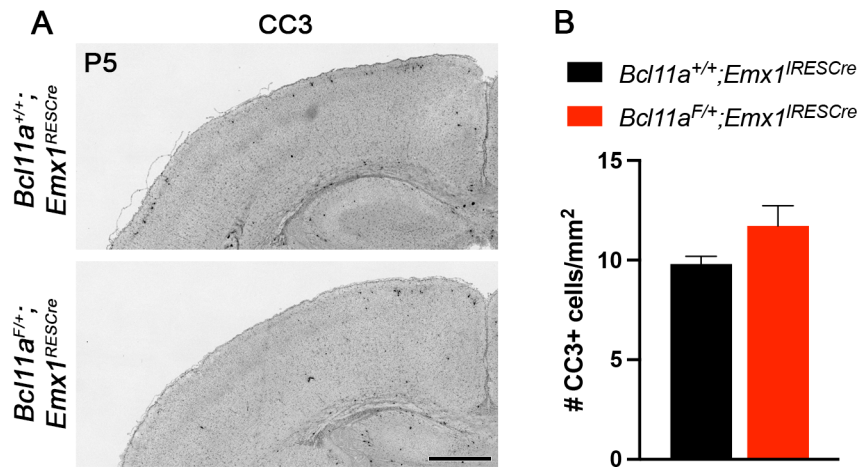


Appendix Figure S3. *In vitro* knockdown of *Foxo1* by shRNA

(A) DNA plasmids used for transfection of HEK293 cells.

(B) Western blot analysis of HEK293 cell co-transfected as indicated and probed using anti-FLAG and anti-β-actin antibodies. Foxo1-shRNA#2 and #4 reduce Foxo1 expression to a different extent (shRNA#2 < shRNA#4) 48 h after transfection.

(C) Quantification of the experiment shown in (B). The experiment was independently repeated 3 times. Results are expressed as mean ± s.e.m.; one-way ANOVA followed by Tukey's post hoc test; * p < 0.05; ** p < 0.01.



Appendix Figure S4. Unchanged cell death in *Bcl11a*^{F/+};*Emx1*^{IRESCre} compared to *Bcl11a*^{+/+};*Emx1*^{IRESCre} neocortex

(A) Immunohistochemistry of cleaved caspase 3 (CC3) shows that the number of CC3⁺ cells is unchanged in P5 *Bcl11a*^{+/+};*Emx1*^{IRESCre} compared to *Bcl11a*^{F/+};*Emx1*^{IRESCre} neocortex.

(B) Quantification of the experiment shown in (A) (n = 4). Results are expressed as mean ± s.e.m.; Student's t test; * p < 0.05. Scale bar, 500 μm.