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Supplemental Data

Tbr2 Directs Conversion of Radial Glia into Basal Precursors and Guides Neuronal Amplification by Indirect Neurogenesis in the Developing Neocortex

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Figure S1. Tbr2 deficient cortices do not show increase in cell death
(A and B) E13.5 and (C and D) E14.5 forebrain coronal sections subjected to TUNEL assay to highlight apoptotic events (red signal) in control and Tbr2 mutant cortices. (E) TUNEL cell quantification at both stages of development does not reveal a statistically significant change between the two different genotypes.

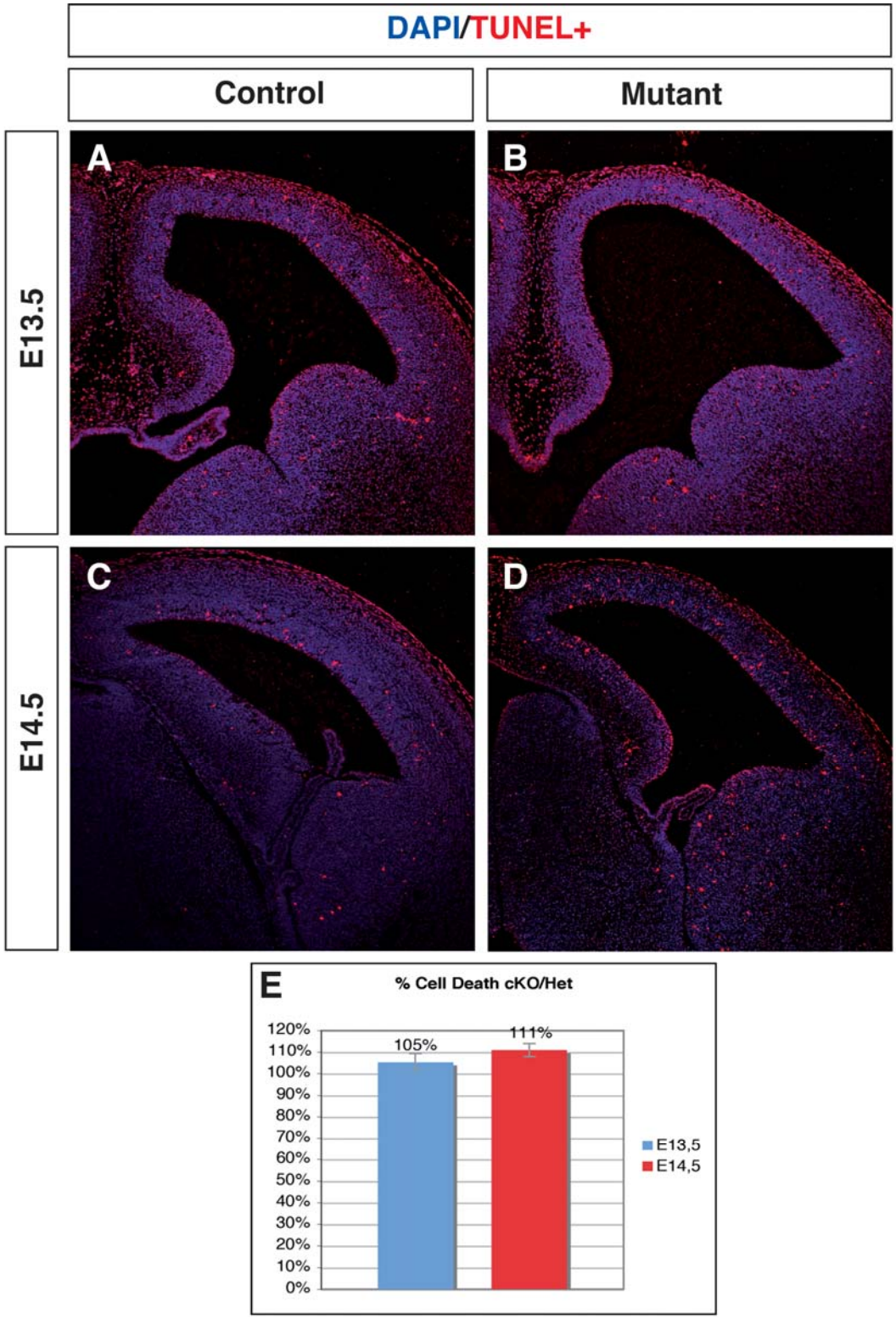
DAPI/TUNEL+
Control
Mutant
E13.5
E14.5
A
B
C
D
E
% Cell Death cKO/Het
120%
110%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
105%
111%
E13,5
E14,5

Figure S2. Gene expression analysis of deeper cortical layer markers in E18.5 mutant and wt cortices
Reduction of the mutant deeper cortical layers is assessed by gene expression analysis of Math2 (pan-neuronal marker), FoxP2 (layers VI and V), Tle4 (layers VI and V) and ER81 (layer V). Consecutive sections along the rostro-caudal axis and higher magnifications of medial, parietal and lateral cortex are shown for each gene. Note that the expression domains in the medial cortex are less affected respect to more lateral areas (FoxP2, P and V; Tle4, AB and AF, ER81; AL and AR). Conversely, similar reduction in gene expression was found at the different levels along the rostro-caudal cortical axis of the Tbr2 mutant respect to wt cortices. cx, cerebral cortex; hy, hypothalamus; str, striatum; t, thalamus; te, tegmentum.

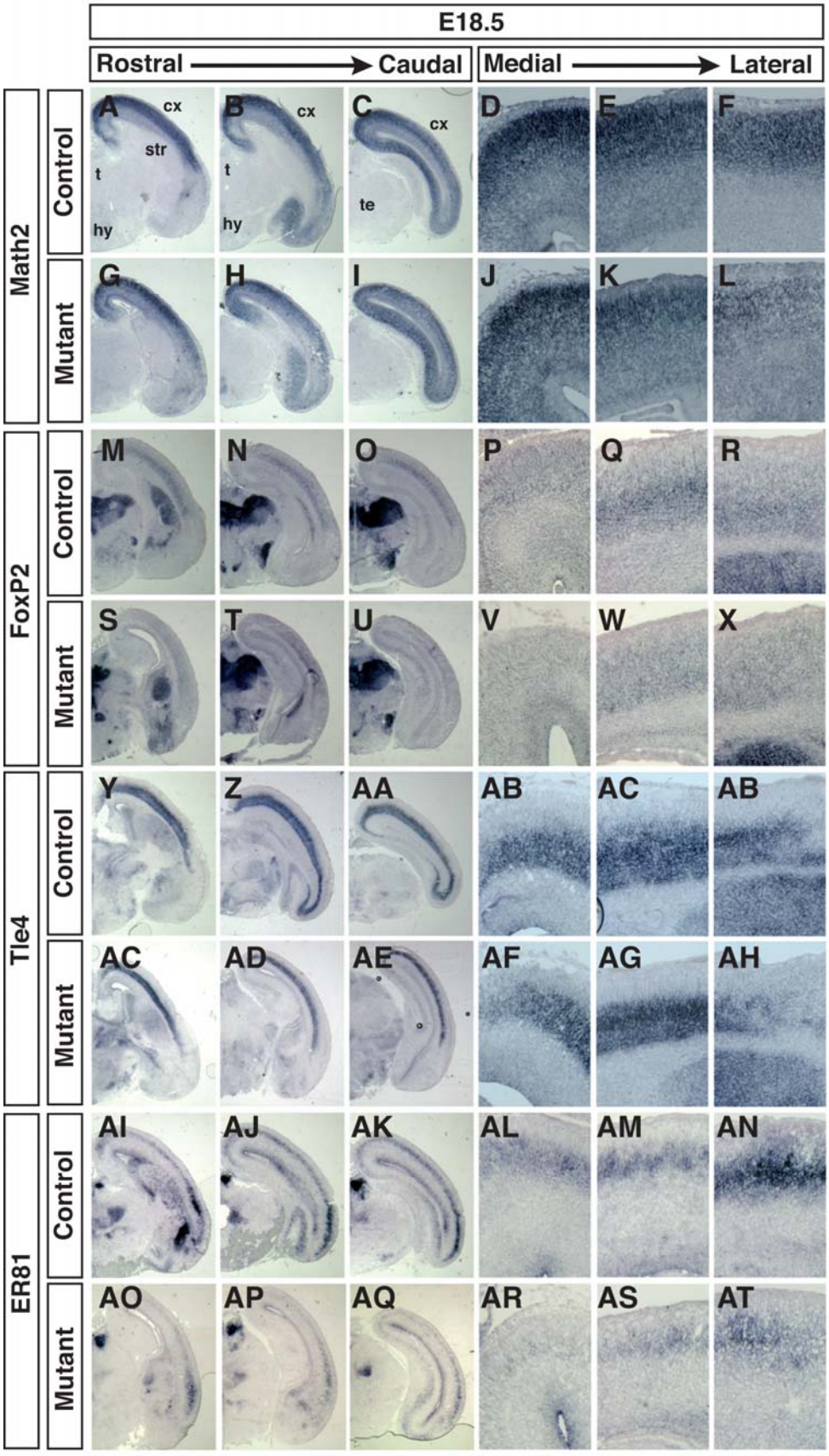
E18.5
Rostral
Caudal
Medial
Lateral
Math2
FoxP2
Tle4
ER81
Control
Mutant
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
Y
Z
AA
AB
AC
AB
AC
AD
AE
AF
AG
AH
AI
AJ
AK
AL
AM
AN
AO
AP
AQ
AR
AS
AT
cx
str
t
hy
te

Figure S3. Gene expression analysis of upper cortical layer markers in E18.5 mutant and wt cortices
Reduction of the mutant upper cortical layers is assessed by gene expression analysis of Lhx2 (layers II-III), Lmo3 (layers II-III, IV) and Rorβ (layer IV). Consecutive sections along the rostro-caudal axis and higher magnifications of medial, parietal and lateral cortex are shown for each gene. Note that the expression domains in the medial cortex are less affected respect to more lateral areas (Lhx2, D and J; Lmo3, P and V, Rorβ; AB and AF). Conversely, similar reduction in gene expression was ascertained at the different levels along the rostro-caudal cortical axis of the Tbr2 mutant respect to wt cortices. cx, cerebral cortex; hi, hippocampus; hy, hypothalamus; str, striatum; t, thalamus; te, tegmentum.

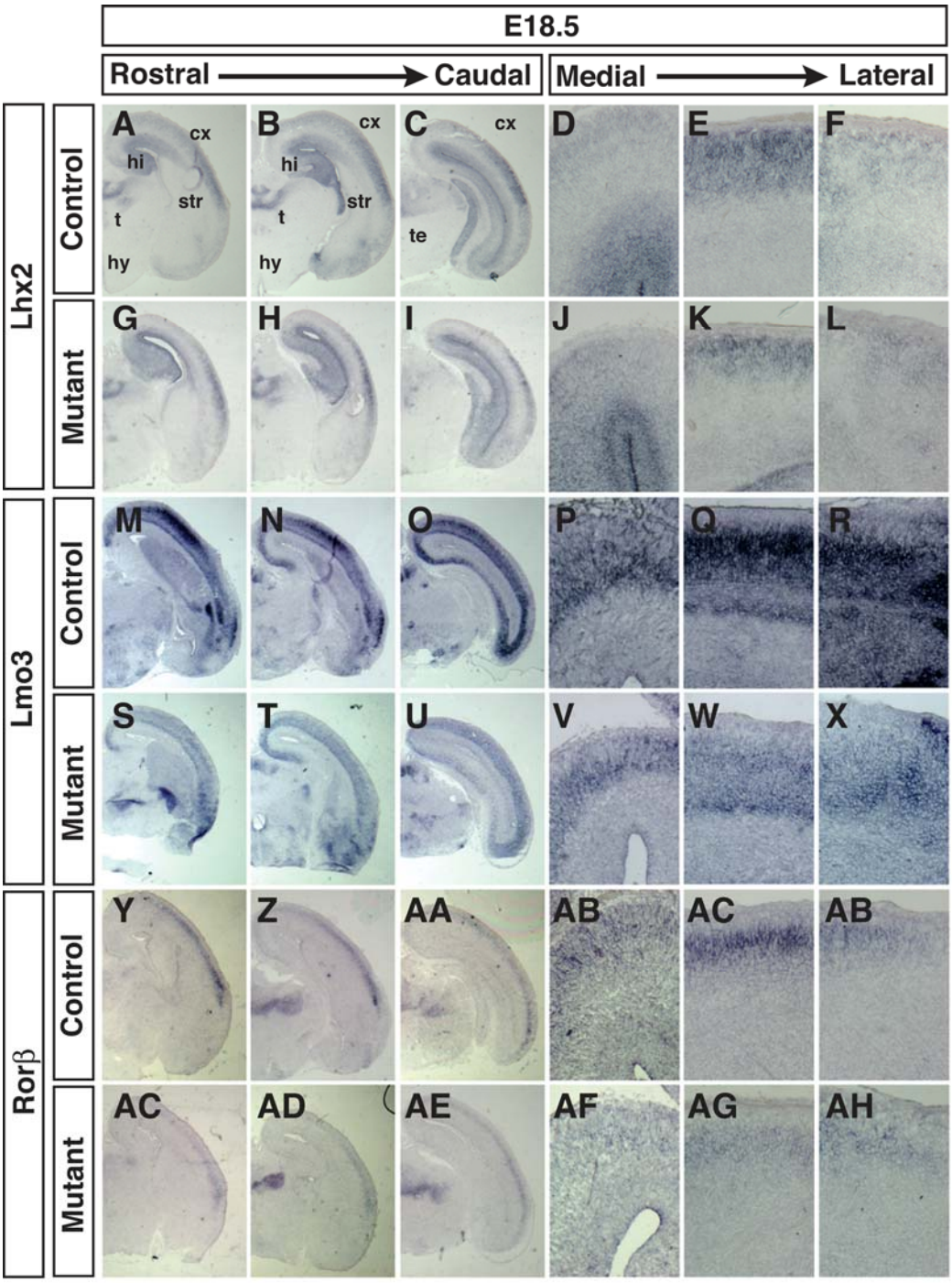

Figure S4. Long-term cell fate analysis of Tbr2-derived neuronal progeny in Tbr2::GFP transgenic mice

(A–C) Immunohistochemistry on coronal sections of E11.5 Tbr2::EGFP cerebral cortex. GFP^+ cells are detectable in the cortical marginal domain (A) and show close correlation with Tbr2 expressing IPCs (B, arrowheads). Few GFP^+ are β-III-tubulin (TuJ1) positive young neurons (C, arrow). (D–H) Immunohistochemistry on coronal section of E13.5 transgenic cortex showing GFP expression along the entire SVZ and CP neocortical domains (D). GFP expression domain includes most of the Tbr2 expressing cells (E, arrowheads). GFP^+ cells don't show co-localization with the radial glia markers Pax6 (G) but some of them, in the marginal cortical domain, are positive for markers of neural differentiation like Tbr1 (F) and β-III-tubulin (TuJ1) (H). (I-K) Immunohistochemistry on coronal sections of P0 cerebral cortex reveals that $EGFP^+$ cells are found spread in all the cortical layers (I) and colocalize with both Satb2 (J) and Tbr1 (K) positive nuclei. cx, cerebral cortex; dg, dentate gyrus; hi, hippocampus; lge, lateral ganglionic eminence; mge, medial ganglionic eminence.

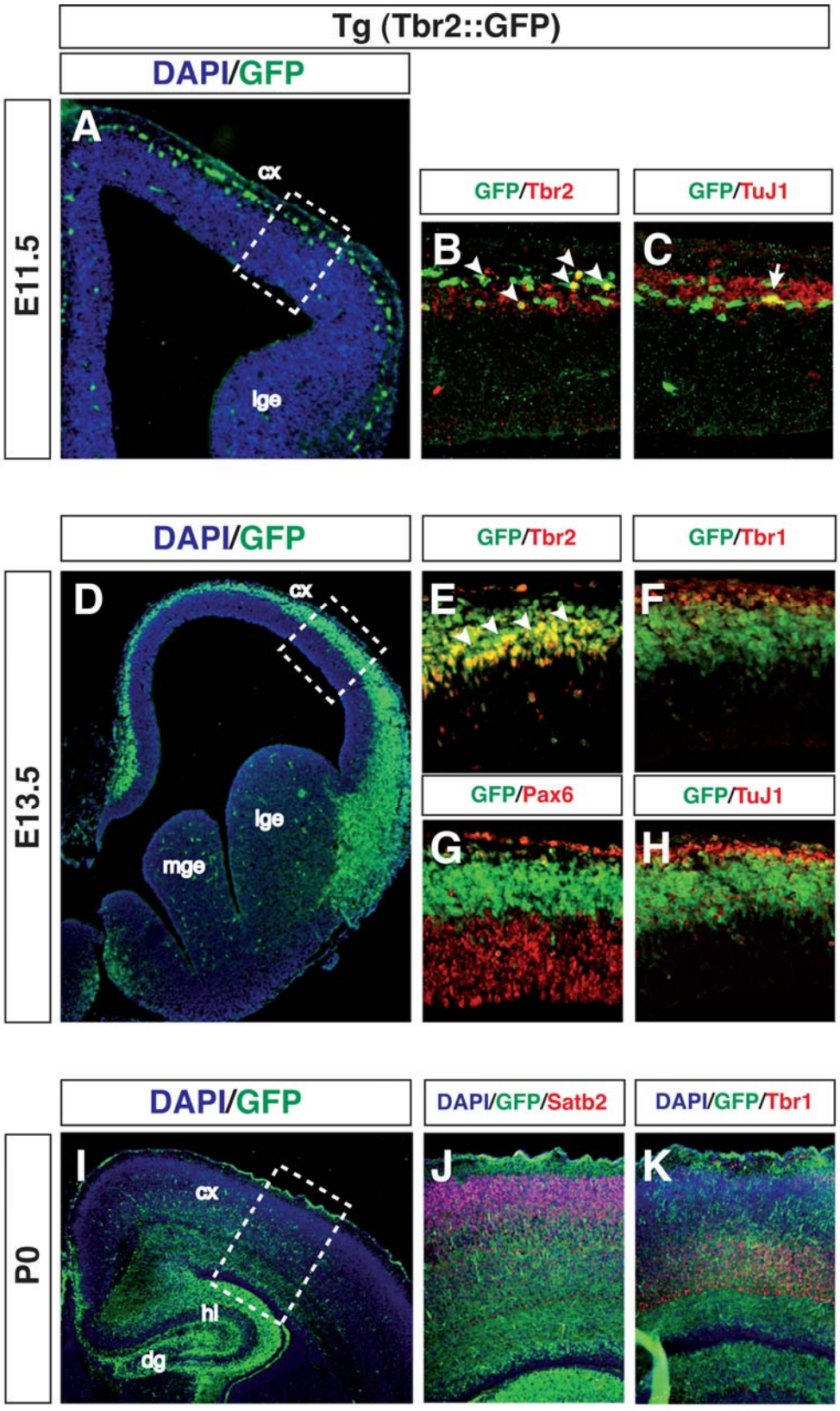
Tg (Tbr2::GFP)
DAPI/GFP
E11.5
A
cx
lge
GFP/Tbr2
B
GFP/TuJ1
C
DAPI/GFP
E13.5
D
cx
lge
mge
GFP/Tbr2
E
GFP/Tbr1
F
GFP/Pax6
G
GFP/TuJ1
H
DAPI/GFP
P0
I
cx
hl
dg
DAPI/GFP/Satb2
J
DAPI/GFP/Tbr1
K

Figure S5. Tbr2 misexpression induces cell accumulation in the SVZ and multipolar-like morphology
(A–F) High magnification view of E15.5 cerebral cortex electroporated at E13.5 with GFP alone or coupled with Tbr2. (D) Tbr2 targeted cortical tissue shows a clear expansion of the cellular compacted VZ/SVZ region visible by DAPI counterstaining at the extent of the overlying IZ (arrows). Similar alterations are not detectable when only EGFP is electroporated (A). (B, C, E and F) While many EGFP targeted cells in periventricular area display a classical bipolar shape (C), Tbr2 misexpressing cells are accumulated in the SVZ regions and present a multipolar-like morphology (F).

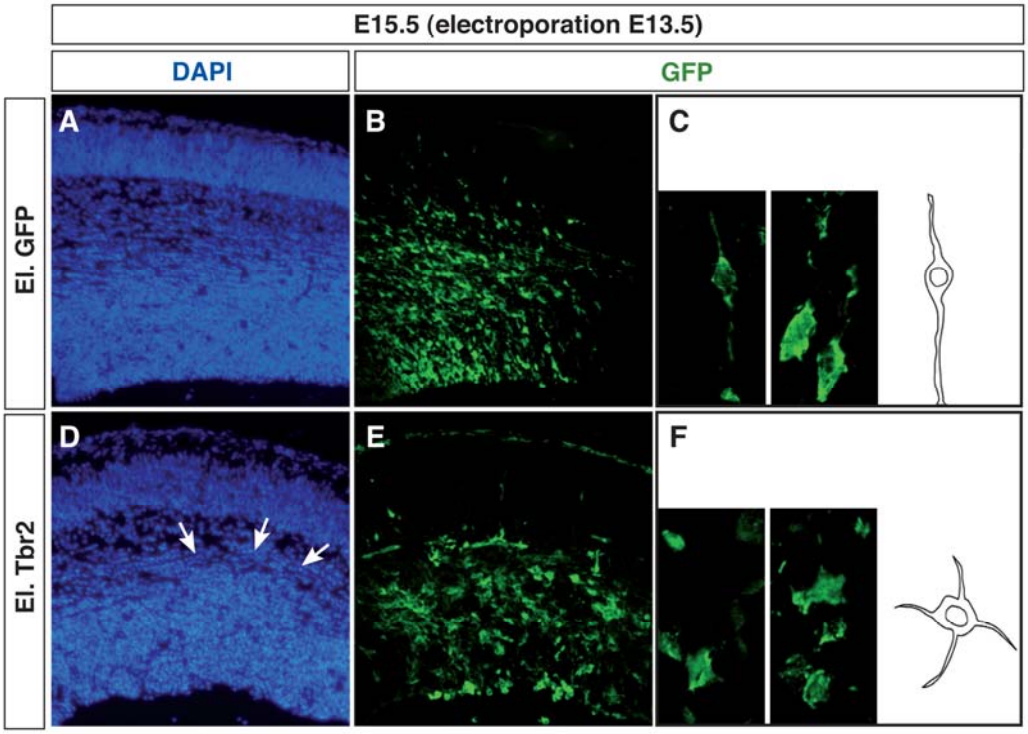

Figure S6. Tbr2 misexpression does not cause any increase in cell death in the targeted cortical tissue
(A-F) Coronal sections of the cortical domain targeted by GFP (A-C) or Tbr2iresGFP (D-F) electroporation at E13.5 and subjected to TUNEL assay at E15.5. TUNEL^{+} apoptotic events are highlighted with a red signal in control (B) and Tbr2 mutant (E) cortices. (F) TUNEL cell quantification does not reveal a statistically significant change comparing GFP or Tbr2 missexpressing cortical tissues.

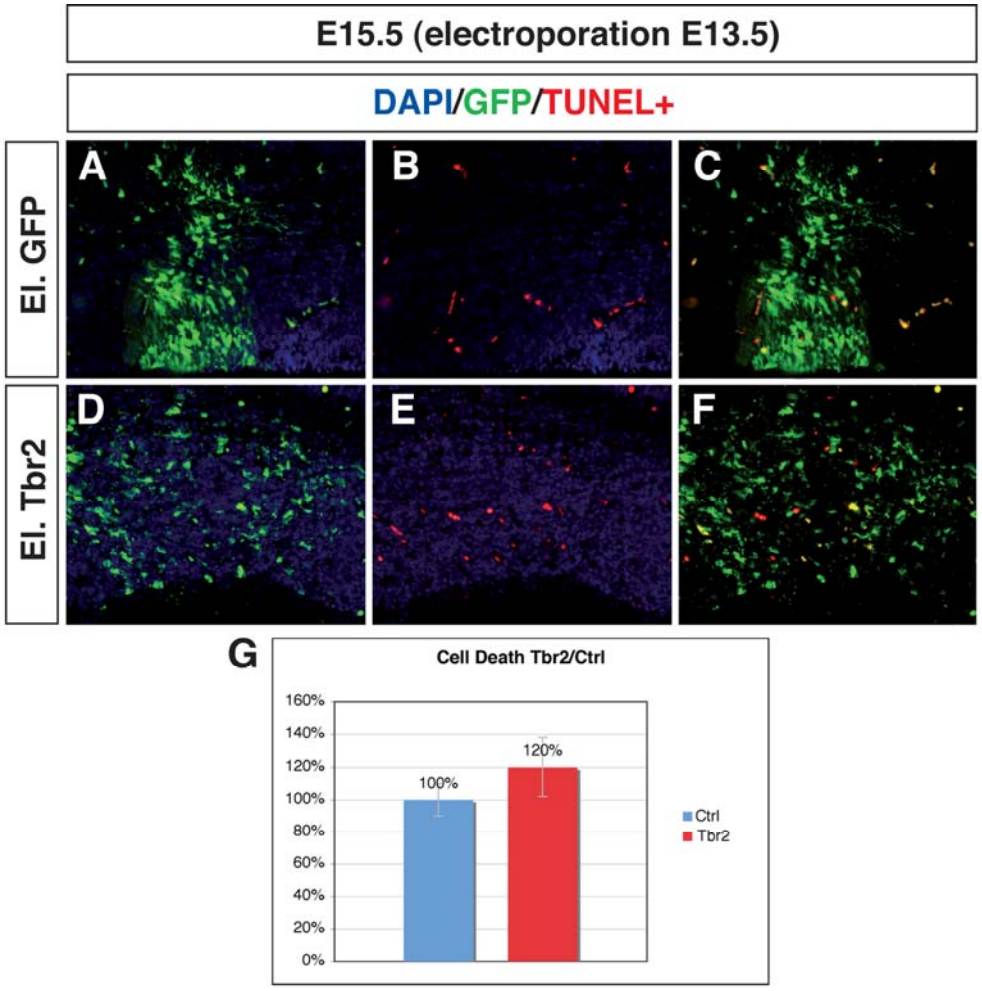

Figure S7. Tbr2 misexpression in the ventricular layer causes impaired of neuroepithelial integrity, loss of cell junctions and RGC detachment

(A–R) Coronal sections of E15.5 cerebral cortices electroporated with GFP (control) or Tbr2iresGFP expressing plasmids at E13.5. (A–F) Immunostaining for Nestin reveals a reduction of radial glial cells coupled with a severe derangement of their columnar structural organization in overexpressing Tbr2 tissue. To note, differently from EGFP, Tbr2 overexpressing cells are not $Nestin^{+}$ and display a multi-polar like morphology. (G–L) Pals1 immunofluorescent staining, specifically labeling radial glia apical cell junctions, is largely disappeared in Tbr2 targeted cortical areas (J–L) while it remains unaltered upon EGFP gene reporter misexpression (G–I). (M–R) β-catenin immunoreactivity is severely compromised upon Tbr2 misexpression in radial glia cells (P-R), while EGFP misexpression does not exert any notable alteration (M – O).

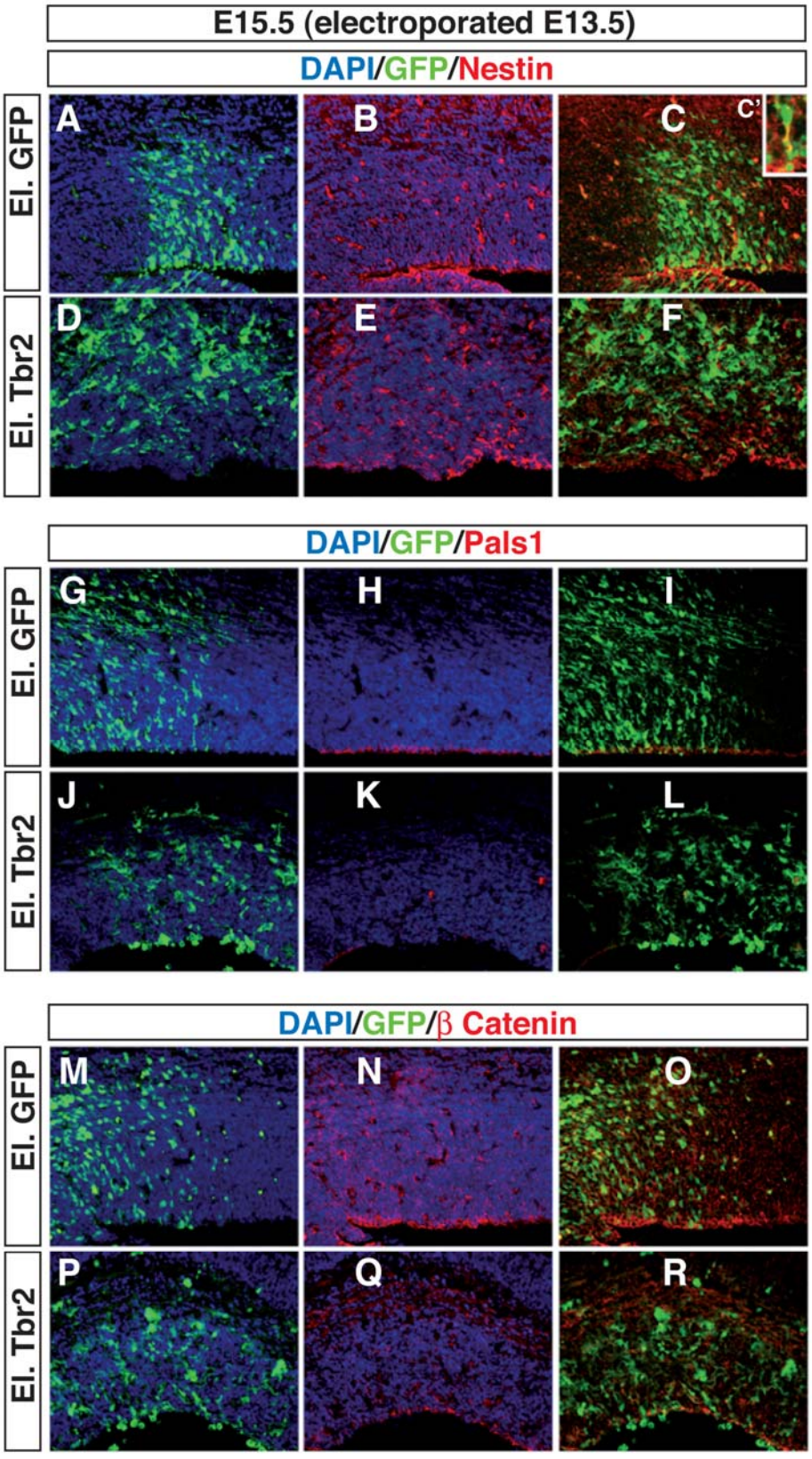
E15.5 (electroporated E13.5)
DAPI/GFP/Nestin
El. GFP
El. Tbr2
A
B
C
C'
D
E
F
DAPI/GFP/Pals1
G
H
I
J
K
L
DAPI/GFP/β Catenin
M
N
O
P
Q
R

Table S1.

Gene	qPCR Primers
NeuroD1	F: CTCAGCATCAATGGCAACTTCTC
	R: GACTCGCTCATGATGCGAATGCC
Cux2	F: GTCCCCAGAGCTGGACACATAC
	R: CTTCAAGCTCAGTTTGTGCCAG
Lmo4	F: GGAGATCGGTTTCACTACATCAATG
	R: GATGAGCAATTCTGAGATGAAGGCAC
Svet1	F: GTCGTAGCAACAGGATAGATGAG
	R: GGCAAACCATTGGGAACTCGTG
Tis21	F: CATTACAAACACCACTGGTTTCCAG
	R: GCTGGCTGAGTCCAATCTGGCTG