

RESEARCH REPORT

STEM CELLS AND REGENERATION

Ntf3 acts downstream of Sip1 in cortical postmitotic neurons to control progenitor cell fate through feedback signaling

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ABSTRACT

Cortical progenitors undergo progressive fate restriction, thereby sequentially producing the different layers of the neocortex. However, how these progenitors precisely change their fate remains highly debatable. We have previously shown the existence of cortical feedback mechanisms wherein postmitotic neurons signal back to the progenitors and promote a switch from neurogenesis to gliogenesis. We showed that Sip1 (Zeb2), a transcriptional repressor, controls this feedback signaling. A similar mechanism was also suggested to control neuronal cell type specification; however, the underlying mechanism was not identified. Here, we provide direct evidence that in the developing mouse neocortex, Ntf3, a Sip1 target neurotrophin, acts as a feedback signal between postmitotic neurons and progenitors, promoting both apical progenitor (AP) to basal progenitor (BP) and deep layer (DL) to upper layer (UL) cell fate switches. We show that specific overexpression of *Ntf3* in neocortical neurons promotes an overproduction of BP at the expense of AP. This shift is followed by a decrease in DL and an increase in UL neuronal production. Loss of *Ntf3*, by contrast, causes an increase in layer VI neurons but does not rescue the *Sip1* mutant phenotype, implying that other parallel pathways also control the timing of progenitor cell fate switch.

KEY WORDS: Cell fate, Cortical development, Feedback signaling, *Ntf3*, *Sip1*, *Zeb2*

INTRODUCTION

Cortical progenitors sequentially give rise to the different layers of the cortex (Angevine and Sidman, 1961; Rakic, 1974). Neurons occupying the different layers show a high degree of heterogeneity with respect to their transcriptome, axonal targeting, dendritic complexity and physiology (Fishell and Hanashima, 2008). Understanding how such a heterogeneous population of neurons is born from a smaller, relatively homogeneous population of progenitors has always invited great attention.

Previous work has shown that cortical progenitors undergo progressive fate restriction (Desai and McConnell, 2000). This was proposed to be an intrinsic phenomenon (Temple, 2001; Shen et al., 2006). However, it is also well established that environmental cues influence cell fate decisions (Dehay et al., 2001; Zarbalis et al., 2007; Lehtinen et al., 2011; Siegenthaler and Pleasure, 2011).

Previous work from our laboratory and others showed that cortical progenitors receive cues from postmitotic neurons through a

feedback signal instructing a shift from neurogenesis to gliogenesis (Barnabe-Heider et al., 2005; Seuntjens et al., 2009). We showed that Sip1, a transcription repressor, is a master regulator of this feedback mechanism. Within postmitotic neurons it controls *Fgf9* expression, which in turn regulates this shift. Furthermore, in *Sip1* mutants the neocortex contains more UL and fewer DL neurons (Seuntjens et al., 2009). However, it is not clear whether feedback signaling also controls a shift from producing DL to UL neurons. It is also unknown whether such a shift is due to Sip1 intrinsic effects in postmitotic cells or Sip1 controlled cell extrinsic signals.

In this study, using *in utero* electroporation (IUE), we show that inactivating *Sip1* in a mosaic manner in neocortical neurons could reproduce the *Sip1* mutant phenotype. We show that Ntf3, a Sip1 target neurotrophin, is expressed in the developing cortex. We then overexpressed *Ntf3* specifically in postmitotic neurons to determine whether it could influence progenitor fate. We show that Ntf3 can act as a feedback signal between postmitotic neurons and progenitors to control both AP to BP and DL to UL neuronal shift. This Ntf3-mediated feedback signaling eventually leads to a radial expansion of the cortex. Finally, we show that deleting *Ntf3* leads to an increase in layer VI neurons. Thus, our results demonstrate that postmitotic neurons can indeed influence progenitor fate and that feedback signaling is an important means of regulating cell fate decisions.

RESULTS AND DISCUSSION

Mosaic deletion of *Sip1* in young cortical neurons phenocopies the *Sip1* mutant

We have previously shown that deletion of *Sip1* in postmitotic neurons has severe effects on cortical development (Miquelajuregui et al., 2007; Seuntjens et al., 2009). We had demonstrated that *Sip1* deletion in young neocortical neurons changes the proportion of DL versus UL neurons. In order to test whether this effect is cell intrinsic or cell extrinsic, we decided to employ IUE. We introduced a plasmid encoding GFP-IRES-Cre in E12.5 *Sip1*^{fl/fl} and *Sip1*^{fl/wt} embryos. To verify if this relatively low efficiency deletion of *Sip1* could replicate the phenotype of *Sip1*^{fl/fl}:*Nex*^{Cre} mutants, we stained for the layer V marker Ctip2 (Bcl11b). As in *Sip1*^{fl/fl}:*Nex*^{Cre}, the number of Ctip2+ cells decreased in *Sip1*^{fl/fl} brains when compared with controls (Fig. 1A). Interestingly, within the electroporated region where the loss of Ctip2+ cells was observed, GFP+ cells did not show a preference for being either Ctip2 positive or negative [Fig. 1B; proportion of GFP+/Ctip2+ cells in *Sip1*^{fl/wt}=0.21±0.06 (*n*=4) compared with 0.17±0.02 (*n*=3) in *Sip1*^{fl/fl}, *P*>0.05]. This shows that *Sip1* affects cell fate through cell-extrinsic mechanisms and also that IUE can be used to study these effects.

Ntf3 is upregulated in the *Sip1* mutant cortex

We have previously shown that upon loss of *Sip1*, there is an ectopic upregulation of *Ntf3* in the cortical plate (CP; Fig. 1C) and through ChIP assay that *Sip1* directly binds to the *Ntf3* promoter region

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(Seuntjens et al., 2009). In order to investigate the functionality of this binding, we performed a luciferase assay. The *Ntf3* enhancer region (Fig. 1D) was inserted upstream of the gausia-luciferase gene and transfected with or without *Sip1* in COS7 cells. We observed a 1.9-fold decrease in transcriptional activity of the *Ntf3* enhancer when *Sip1* was co-transfected (Fig. 1D; $n=3$, $P=0.0009$). To check for *Ntf3* expression dynamics in the developing cortex we used qRT-PCR, as the levels of *Ntf3* expression were not quantifiable by *in situ* hybridization. We observed that the expression of *Ntf3* at E13.5 was ~160-fold higher than at E12.5 (Fig. 1E; $P=0.033$). Furthermore, the expression doubled from E13.5 to E15.5 (Fig. 1E; $P=0.027$). This shows that the expression of *Ntf3* correspondingly increases as the cortical plate grows. Additionally, the sudden increase in expression from E12.5 to E13.5 coincides with the appearance of the first neurons in the CP, thus

indicating that the first postmitotic cells entering the cortical plate at E13.5 express *Ntf3*.

Ntf3 promotes an increase in the BP population

To study whether the upregulation of *Ntf3* in the *Sip1* mutant has any effect on the proportion of AP and BP, we overexpressed *Ntf3* in the wild-type (WT) cortex. BPs are situated in the subventricular zone (SVZ) and are responsible for the vast expansion of UL neuronal production, though they have been shown to produce all layers of the cortex (Tarabykin et al., 2001; Haubensak et al., 2004; Englund et al., 2005; Gotz and Huttner, 2005; Arnold et al., 2008; Kowalczyk et al., 2009; Pinto et al., 2009; Vasistha et al., 2014).

In order to restrict the expression of *Ntf3* to postmitotic neurons, we designed a plasmid system in which the *Ntf3*-IRES-EGFP cassette was preceded by a mCherry-polyA sequence flanked by

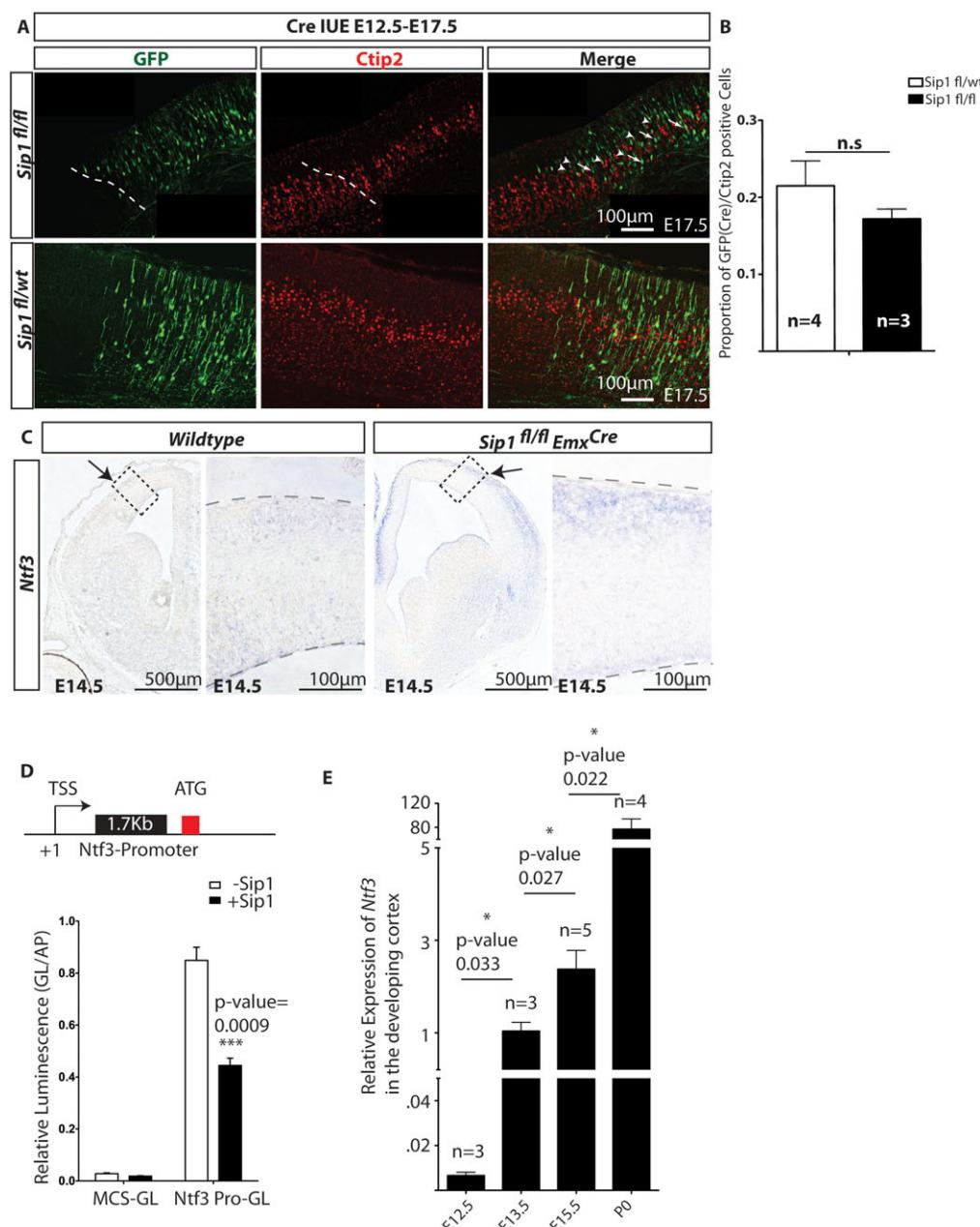


Fig. 1. *Ntf3* is upregulated in the *Sip1* mutant cortex. (A) *In utero* electroporation of Cre in *Sip1*^{fl/fl} and *Sip1*^{fl/wt} embryos at E12.5. Ctip2 decreases in the electroporated region of *Sip1*^{fl/fl} embryos at E17.5, whereas no difference was observed in *Sip1*^{fl/wt} embryos. Arrowheads indicate GFP+/Ctip2+; arrows indicate GFP+/Ctip2– cells. Images are composites of tiled images. (B) Proportion of GFP(Cre)+ cells also positive for Ctip2 in *Sip1*^{fl/wt} = 0.21 ± 0.06 ($n=4$) compared with 0.17 ± 0.02 ($n=3$) in *Sip1*^{fl/fl} ($P>0.05$). (C) *Ntf3* is upregulated in the *Sip1* mutant cortex at E14.5, whereas no expression could be detected in wild type. Higher magnification images correspond to the boxed regions. (D) The *Ntf3* enhancer region used. Luciferase assay shows that, upon co-transfection of *Sip1*, the transcription of the *Ntf3* enhancer reduced by 1.9 fold ($n=3$, relative luminescence without *Sip1* = 0.85 ± 0.05 versus 0.44 ± 0.03 with *Sip1*, $P=0.0009$). (E) qRT-PCR results showing the endogenous expression of *Ntf3* (relative to *Gapdh*) during different stages of development. Expression at E13.5 ($n=3$) is ~160-fold higher compared with E12.5 ($n=3$, $P=0.033$). The expression at E15.5 ($n=5$) is ~2.2-fold higher compared with E13.5 ($P=0.027$). The expression at P0 ($n=4$) is ~74-fold higher compared with E13.5 ($P=0.022$).

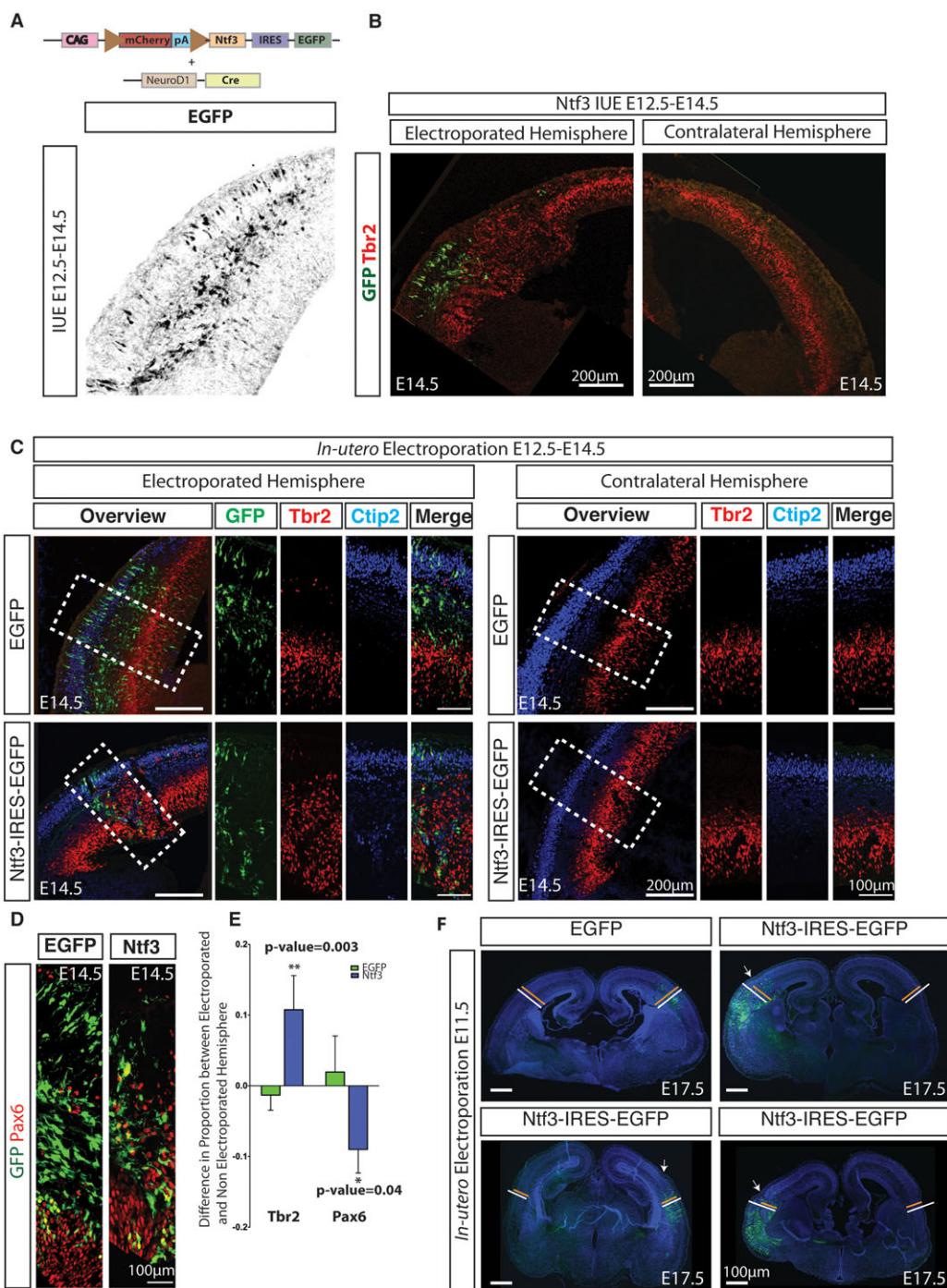


Fig. 2. Ntf3 promotes an increase in BP and decrease in AP populations. (A) Plasmid system for restricting expression to postmitotic neurons. A representative image showing exclusive postmitotic expression of EGFP. (B) Overview image showing that upon *Ntf3* overexpression, the Tbr2+ BP population is expanded. Images are composites of tiled images. (C,E) Electroporation of GFP does not lead to any change in the size or distribution of Tbr2+ (-0.013 ± 0.021 , $n=5$) and Ctip2+ cells. Overexpression of *Ntf3* leads to an expansion of Tbr2+ cells (0.10 ± 0.048 higher proportions of Tbr2+ cells, $n=5$, $P=0.003$). Ctip2+ cells are displaced, mostly as a secondary effect of this increase in Tbr2+ cells. The contralateral hemisphere serves as an internal control. (D,E) Pax6+ APs are also displaced and reduced in *Ntf3* overexpression brains (the difference in the proportion of Pax6+ cells between the electroporated and non-electroporated hemispheres upon *Ntf3* overexpression is 0.9 ± 0.03 compared with 0.01 ± 0.05 in controls; $n=3$, $P=0.04$). All electroporation was carried out at E12.5 and analyzed at E14.5. (F) Effect of radial expansion of the cortex on *Ntf3* overexpression. White and orange bars indicate the thickness of the electroporated and non-electroporated hemisphere, respectively. Bars were placed parallel to the apical process of the cells. Arrows indicate the physical fold in the cortical plate. Images are composites of tiled images.

loxP sites. Cre recombinase was driven by the postmitotic-specific NeuroD1 promoter, which switches expression from mCherry to Ntf3 and EGFP specifically in postmitotic neurons (Fig. 2A; supplementary material Fig. S1A,B). The location of the

EGFP+ cells and the expression of *Ntf3* verified by *in situ* hybridization confirmed exclusive postmitotic expression (Fig. 2A; supplementary material Fig. S2). In order to avoid variations that arise from comparing different mediolateral and

rostrocaudal levels, we present all analysis as the difference between the electroporated and non-electroporated hemispheres. We electroporated wild-type E12.5 embryos and analyzed the brains at E14.5. We observed that upon *Ntf3* overexpression there was a vast expansion in the proportion of Tbr2+ BP and hence the size of the SVZ (Fig. 2B,C,E; the proportion of Tbr2+ cells was 0.10 ± 0.048 more in the electroporated hemisphere compared with the contralateral hemisphere upon *Ntf3* overexpression compared with -0.013 ± 0.02 in *EGFP* electroporations; $n=5$, $P=0.003$). Interestingly, there was also a decrease in the proportion of Pax6+ AP upon *Ntf3* overexpression (Fig. 2D,E; 0.09 ± 0.03 decrease in the proportion of Pax6+ cells in the electroporated hemisphere upon *Ntf3* overexpression compared with 0.01 ± 0.05 in control plasmid electroporations; $n=3$, $P=0.04$). Interestingly, the expansion of Tbr2+ progenitors was not always restricted to the electroporated region (Fig. 2B,C). Furthermore, the number of GFP+ *Ntf3*-expressing cells required for causing the AP to BP switch was not high. However, as each neuron carries multiple copies of the plasmid, a crucial threshold for *Ntf3* concentration might be attained with fewer cells.

Following the expansion in BPs, we also observed a radial expansion of the electroporated cortex compared with the contralateral hemisphere (Fig. 2F). A similar radial expansion of the

cortex has been previously reported (Nonaka-Kinoshita et al., 2013; Stahl et al., 2013). Because in all these experiments *Ntf3* expression was restricted to postmitotic neurons, the changes in progenitor populations are most likely to be the result of feedback signaling.

***Ntf3* promotes UL neurogenesis at the expense of DL neurons**

Next, we asked whether the *Ntf3* initiated AP to BP shift could also lead to an alteration in proportions of different cell types. To test this, we overexpressed *Ntf3* at E11.5 and analyzed the brains at E17.5. We observed a 0.077 ± 0.027 decrease in the proportion of Tbr1+ layer VI neurons in the *Ntf3* electroporated hemisphere compared with the contralateral hemisphere, whereas *EGFP* expression had no effect (0.002 ± 0.008 ; Fig. 3A,D; $n=3$, $P=0.028$). *Ntf3* electroporated hemisphere showed a 0.034 ± 0.01 decrease in Ctip2+ (layer V) proportions compared with the contralateral hemisphere, whereas *EGFP* electroporated brains did not show any difference (-0.0005 ± 0.01 ; Fig. 3B,D; $n=5$, $P=0.003$). We also observed a 0.05 ± 0.03 increase in Brn2+ UL neuron proportions between electroporated and non-electroporated hemispheres in *Ntf3* electroporated brains compared with *EGFP* electroporations (-0.0068 ± 0.006 ; Fig. 3C,D; $n=4$, $P=0.036$). This shows that *Ntf3* promotes a shift from DL to UL neurogenesis,

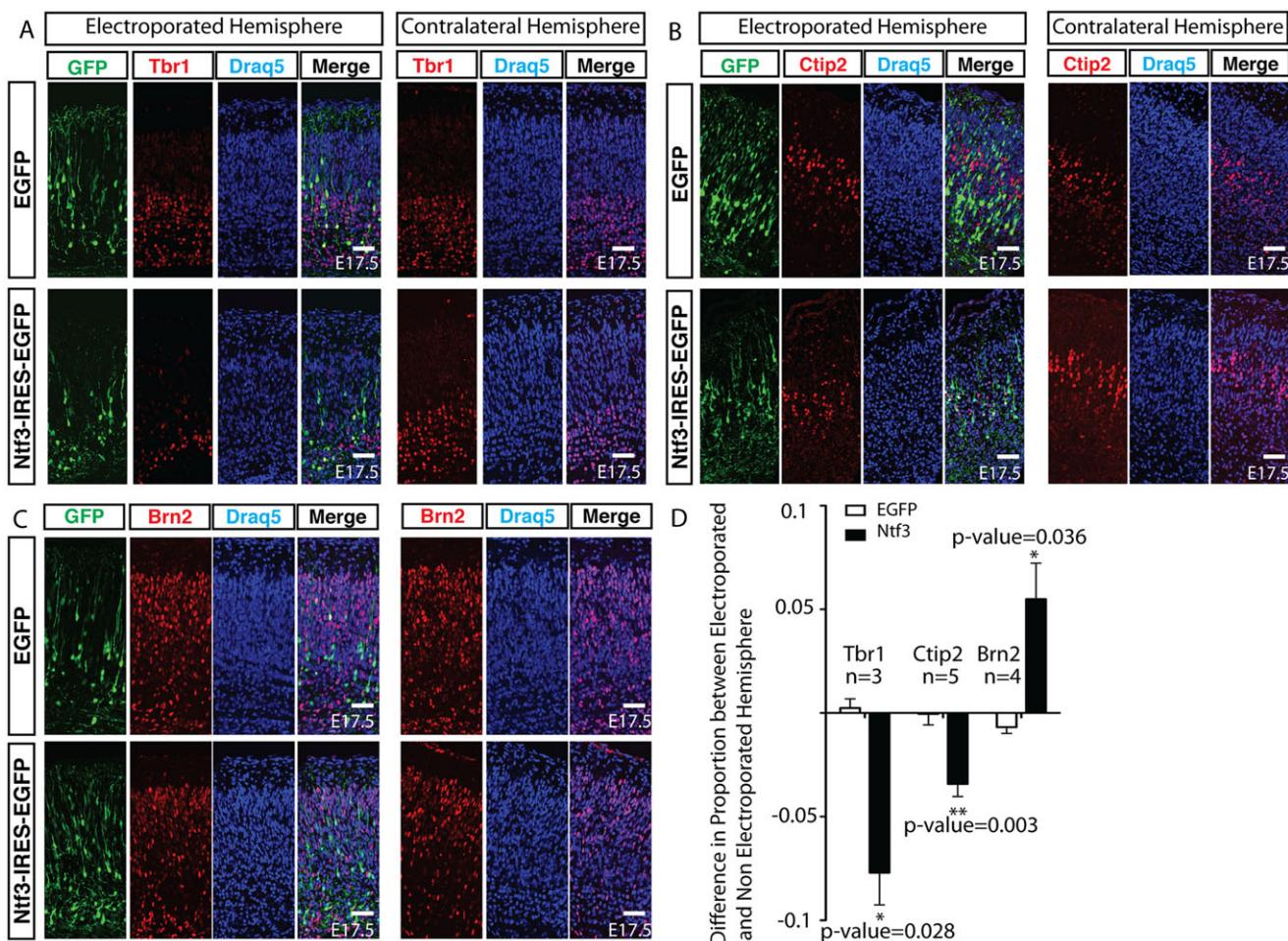


Fig. 3. *Ntf3* promotes UL neurogenesis at the expense of DL neurons. (A,D) Decrease in proportions of Tbr1+ layer VI neurons between electroporated and non-electroporated hemispheres upon *Ntf3* overexpression (-0.07 ± 0.027 versus 0.002 ± 0.008 in *EGFP*-expressing brains); $n=3$, $P=0.028$. (B,D) *Ntf3* overexpression leads to a 0.034 ± 0.013 decrease in Ctip2+ cells compared with the non-electroporated hemisphere, whereas *EGFP* expression does not alter the proportion (-0.0005 ± 0.0102); $n=5$, $P=0.003$. (C,D) *Ntf3* electroporated brains show a 0.054 ± 0.034 increase in proportion of Brn2+ UL neurons, whereas *EGFP* does not affect the proportion (-0.0068 ± 0.006 , $n=4$, $P=0.036$). Scale bars: 50 μ m.

highlighting the presence of cortical feedback signals in layer specification.

Recent work points to the existence of lineage-specific progenitors (Franco et al., 2012). One hypothesis for the switch from DL to UL neuron production mediated by Ntf3 would be the preferential effect of Ntf3 on UL specific progenitors (Franco and Muller, 2013), promoting their premature differentiation.

Deletion of *Ntf3* increases the proportion of layer VI neurons but the *Ntf3-Sip1* compound mutant does not rescue the *Sip1* mutant phenotype

We have previously reported that in the absence of *Sip1*, cortical progenitors precociously produce UL neurons at the expense of DL neurons (Seuntjens et al., 2009). To investigate whether this phenotype can be reverted on *Ntf3* deletion, we generated *Sip1-Ntf3*

double knockout (DKO). We did not find any significant difference in the proportions of Satb2+ (layer II–V), Ctip2+ (layer V), Brn2+ (layer II–IV) and Sox5+ (layer VI) cells between *Sip1* single and *Sip1-Ntf3* DKO (Fig. 4A–D, all $P \geq 0.05$). Furthermore, we did not find any difference between wild type and *Ntf3*^{-/-} with respect to the proportion of Satb2, Brn2 and Ctip2+ populations (Fig. 4A,B,D). Interestingly, we noticed an increase in the proportion of Sox5+ layer VI population in *Ntf3*^{-/-} (0.365 ± 0.029 in *Ntf3*^{-/-} compared with 0.293 ± 0.023 in wild type; $n=3$, $P=0.029$).

The increase in the proportion of Sox5+ layer VI neurons in *Ntf3*^{-/-} indicates that Ntf3 is involved in controlling important aspects of the cell fate switch. It is also noteworthy that the maximum effect of both *Ntf3* overexpression and deletion was on layer VI cells. This also correlates with the onset of *Ntf3* expression.

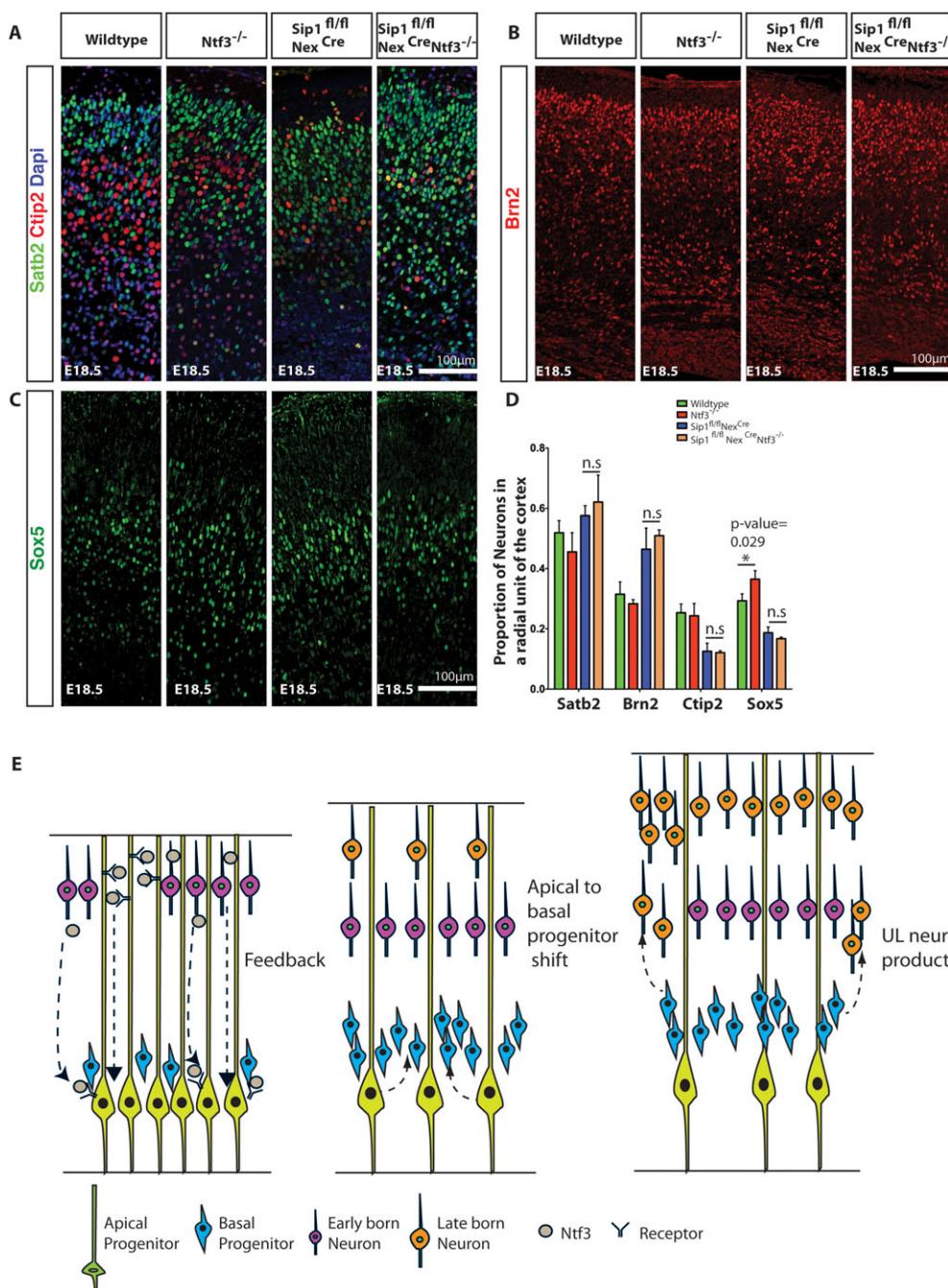


Fig. 4. Deletion of *Ntf3* from the *Sip1* mutant cortex does not rescue the *Sip1* mutant phenotype. (A) Satb2 and Ctip2 immunohistochemistry marking UL and layer V cells in wild type, *Ntf3*^{-/-}, *Sip1*^{fl/fl},*Nex*^{Cre} and *Sip1-Ntf3* DKO. The decrease in Ctip2 cells in *Sip1* mutants is not restored in *Sip1-Ntf3* DKOs. Similarly, the increase in Satb2+ cells is also not restored. (B) Brn2, another UL marker, and (C) Sox5, a layer VI marker, also confirm that the *Sip1-Ntf3* DKO does not rescue the *Sip1* mutant phenotype. (D) Proportion of Sox5+ cells is higher in *Ntf3*^{-/-} compared with wild type [$n=2$ for wild type, *Sip1* and *Ntf3*^{-/-}; $n=2$ for *Sip1-Ntf3* DKO (all $P > 0.05$)]. The proportion of Sox5+ cells in *Ntf3*^{-/-} = 0.36 ± 0.029 compared with 0.29 ± 0.02 in wild type, * $P=0.029$. (E) Model depicting two possible modes of *Ntf3*-mediated signaling. Receptors located either along the radial glial process or the cell body can sense *Ntf3* released by postmitotic neurons. This leads to an increased production of BPs from APs. Finally, the feedback signal initiated by *Ntf3* leads to a shift from DL to UL neuronal production.

However, as the deletion of *Ntf3* from the *Sip1* mutant cortex does not rescue the phenotype, it is probable that multiple pathways exist for controlling cortical feedback signaling. Another possibility is that the receptors for Ntf3 (Trks) are also activated by alternate pathways in the developing cortex (Lee et al., 2002). For example, recent work has shown that EGFR can transactivate both TrkB and TrkC (Ernfors et al., 1994; Puehringer et al., 2013). Interestingly, Bdnf and Ntf3 have opposing functions in laminar fate specification (Fukumitsu et al., 2006). Whereas Ntf3 promotes UL neurogenesis, Bdnf has been shown to promote DL neurogenesis.

The presence of proteins such as Ntf3 in the CP that act as feedback signals influencing progenitors located within the VZ and SVZ raises intriguing issues about how these signals are transmitted. Signals originating from the CP could either bind to cognate receptors along the radial glial process or diffuse to the VZ and bind to receptors on the cell body. Although our results do not answer this question, we believe that using such a system in combination with optical methods such as FRET could address this issue (Fig. 4E).

MATERIALS AND METHODS

Mouse lines

All mouse experiments were carried out in compliance with German law and according to the guidelines approved by the Bezirksregierung Braunschweig or Landesamt für Gesundheit und Soziales Berlin. Both male and female mouse embryos were used. *Sip1* conditional mutants carried a loxP flanked exon 7 crossed to either *Nex* or *Emx1-Cre* (Gorski et al., 2002; Higashi et al., 2002; Goebels et al., 2006). The *Ntf3* mutant was kindly provided by Dr Michael Sendtner (University of Würzburg, Germany).

Tissue processing and antibodies

Tissue was processed as described previously (Seuntjens et al., 2009). The following primary antibodies were used: GFP (Abcam, chicken, 1:1000, ab13970/Rockland, goat, 1:500, 600-101-215), Ctip2 (Abcam, rat, 1:250, ab18465), Tbr2 (Abcam, rabbit, 1:150, ab23345), Pax6 (Millipore, rabbit, 1:300, AB2237), Satb2 (self-generated, rabbit, 1:1000), Brn2 (Santa-Cruz, goat, 1:200, sc-6029), Sox5 (Santa-Cruz, goat, sc-17329) and Tbr1 (Abcam, rabbit, 1:200, ab31940). Dylight/Alexa-coupled secondary antibodies (Jackson ImmunoResearch) were used at 1:500. Hoechst (Sigma, 1:500) or Draq5 (eBioscience, 1:1000) were used for counterstaining.

In utero electroporation (IUE)

IUE was performed as described previously (Saito, 2006).

Luciferase assay

The following primers were used for amplifying the *Ntf3* promoter region: 5'-AGGAATTCTGGCTCCTTCGCCCGCAGCTT-3' and 5'-GTG-GATCCACGTCGACATGAAGAGAAAGGTGGA-3'.

The amplicon was cloned into pMCS-Gaussia luciferase (Thermo Scientific). CMV driven alkaline phosphatase served as the transfection control. COS7 cells were transfected using Lipofectamine 2000 (Invitrogen). Supernatant was collected 48 h post-transfection and luminescence was measured using the Secreta pair dual luminescence kit (Genecopoeia) and read using a luminometer (Promega). Data were collected from three independent experiments and presented as the relative luminescence.

Plasmids

Sip1 and *Ntf3* were cloned from E17.5 forebrain cDNA. NeuroD1 promoter plasmid was a kind gift from Dr Gordon Fishell (New York University, USA). Cre recombinase was subcloned downstream of the NeuroD1 promoter. The Cre activatable plasmid was cloned by inserting loxP sites on either side of a mCherry-pA sequence and thereafter cloning this fragment upstream of the MCS of pCAGIG (Addgene).

Imaging and data analysis

Images were procured on a Zeiss or Leica confocal system and processed using Fiji or Adobe Photoshop. Statistical analysis (Student's *t*-test) was carried out using Excel and Graphpad Prism. Data are presented as mean±s.d.

Quantitative RT-PCR

Total RNA was extracted from E12.5, E13.5, E15.5 and P0 cortices using the RNeasy plus mini kit (Qiagen). FS cDNA was prepared using Maxima reverse transcriptase (Thermo Scientific) using a mixture of oligodT and random primers. qRT-PCR was performed using Fast SYBR Green master mix (Applied Biosystems) on an Applied Biosystems 7500 RT-PCR system. Primers were designed across an exon-exon boundary. A negative cDNA preparation (containing no reverse transcriptase) was used to verify the primer pairs. The following primers were used: *Ntf3*_fwd, 5'-AAGGGATCG-TTGGAGGTGAC-3'; *Ntf3*_Rws, 5'-GCCGCAGCCACTCATCATT-3'; *GAPDH*_fwd, 5'-TACGGCAAATCCGTTACA-3'; *GAPDH*_Rws, 5'-GAGAGTGTTCCTCGTCCC-3'

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Competing interests

The authors declare no competing financial interests.

Author contributions

S.P. and V.T. conceived the project. S.P., S.S. and A.N. performed experiments. S.P., S.S. and V.T. analyzed data. S.P. wrote the manuscript. S.S. and V.T. edited the manuscript.

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Supplementary material

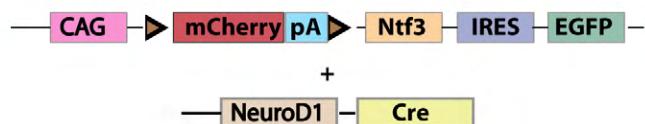
Supplementary material available online at <http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.114173/-DC1>

References

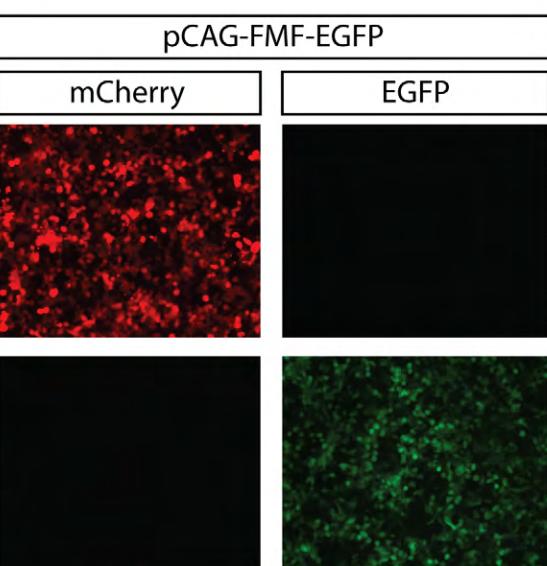
- Angevine, J. B., Jr and Sidman, R. L. (1961). Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* **192**, 766-768.
- Arnold, S. J., Huang, G.-J., Cheung, A. F. P., Era, T., Nishikawa, S.-I., Bikoff, E. K., Molnar, Z., Robertson, E. J. and Groszer, M. (2008). The T-box transcription factor Eomes/Tbr2 regulates neurogenesis in the cortical subventricular zone. *Genes Dev.* **22**, 2479-2484.
- Barnabé-Heider, F., Wasylka, J. A., Fernandes, K. J. L., Porsche, C., Sendtner, M., Kaplan, D. R. and Miller, F. D. (2005). Evidence that embryonic neurons regulate the onset of cortical gliogenesis via cardiotrophin-1. *Neuron* **48**, 253-265.
- Dehay, C., Savatier, P., Cortay, V. and Kennedy, H. (2001). Cell-cycle kinetics of neocortical precursors are influenced by embryonic thalamic axons. *J. Neurosci.* **21**, 201-214.
- Desai, A. R. and McConnell, S. K. (2000). Progressive restriction in fate potential by neural progenitors during cerebral cortical development. *Development* **127**, 2863-2872.
- Englund, C., Fink, A., Lau, C., Pham, D., Daza, R. A. M., Bulfone, A., Kowalczyk, T. and Hevner, R. F. (2005). Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. *J. Neurosci.* **25**, 247-251.
- Ernfors, P., Lee, K.-F. and Jaenisch, R. (1994). Mice lacking brain-derived neurotrophic factor develop with sensory deficits. *Nature* **368**, 147-150.
- Fishell, G. and Hanashima, C. (2008). Pyramidal neurons grow up and change their mind. *Neuron* **57**, 333-338.
- Franco, S. J. and Müller, U. (2013). Shaping our minds: stem and progenitor cell diversity in the mammalian neocortex. *Neuron* **77**, 19-34.
- Franco, S. J., Gil-Sanz, C., Martinez-Garay, I., Espinosa, A., Harkins-Perry, S. R., Ramos, C. and Müller, U. (2012). Fate-restricted neural progenitors in the mammalian cerebral cortex. *Science* **337**, 746-749.
- Fukumitsu, H., Ohtsuka, M., Murai, R., Nakamura, H., Itoh, K. and Furukawa, S. (2006). Brain-derived neurotrophic factor participates in determination of neuronal laminar fate in the developing mouse cerebral cortex. *J. Neurosci.* **26**, 13218-13230.
- Goebels, S., Bormuth, I., Bode, U., Hermanson, O., Schwab, M. H. and Nave, K.-A. (2006). Genetic targeting of principal neurons in neocortex and hippocampus of NEX-Cre mice. *Genesis* **44**, 611-621.

- Gorski, J. A., Talley, T., Qiu, M., Puelles, L., Rubenstein, J. L. and Jones, K. R.** (2002). Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. *J. Neurosci.* **22**, 6309-6314.
- Götz, M. and Huttner, W. B.** (2005). The cell biology of neurogenesis. *Nat. Rev. Mol. Cell Biol.* **6**, 777-788.
- Haubensak, W., Attardo, A., Denk, W. and Huttner, W. B.** (2004). Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. *Proc. Natl. Acad. Sci. USA* **101**, 3196-3201.
- Higashi, Y., Maruhashi, M., Nelles, L., Van de Putte, T., Verschueren, K., Miyoshi, T., Yoshimoto, A., Kondoh, H. and Huylebroeck, D.** (2002). Generation of the floxed allele of the SIP1 (Smad-interacting protein 1) gene for Cre-mediated conditional knockout in the mouse. *Genesis* **32**, 82-84.
- Kowalczyk, T., Pontious, A., Englund, C., Daza, R. A. M., Bedogni, F., Hodge, R., Attardo, A., Bell, C., Huttner, W. B. and Hevner, R. F.** (2009). Intermediate neuronal progenitors (basal progenitors) produce pyramidal-projection neurons for all layers of cerebral cortex. *Cereb. Cortex* **19**, 2439-2450.
- Lee, F. S., Rajagopal, R., Kim, A. H., Chang, P. C. and Chao, M. V.** (2002). Activation of Trk neurotrophin receptor signaling by pituitary adenylate cyclase-activating polypeptides. *J. Biol. Chem.* **277**, 9096-9102.
- Lehtinen, M. K., Zappaterra, M. W., Chen, X., Yang, Y. J., Hill, A. D., Lun, M., Maynard, T., Gonzalez, D., Kim, S., Ye, P. et al.** (2011). The cerebrospinal fluid provides a proliferative niche for neural progenitor cells. *Neuron* **69**, 893-905.
- Miquelajauregui, A., Van de Putte, T., Polyakov, A., Nityanandam, A., Boppana, S., Seuntjens, E., Karabinos, A., Higashi, Y., Huylebroeck, D. and Tarabykin, V.** (2007). Smad-interacting protein-1 (Zfhx1b) acts upstream of Wnt signaling in the mouse hippocampus and controls its formation. *Proc. Natl. Acad. Sci. USA* **104**, 12919-12924.
- Nonaka-Kinoshita, M., Reillo, I., Arregiani, B., Martínez-Martínez, M. Á., Nelson, M., Borrell, V. and Calegari, F.** (2013). Regulation of cerebral cortex size and folding by expansion of basal progenitors. *EMBO J.* **32**, 1817-1828.
- Pinto, L., Drechsel, D., Schmid, M.-T., Ninkovic, J., Irmiger, M., Brill, M. S., Restani, L., Gianfranceschi, L., Cerri, C., Weber, S. N. et al.** (2009). AP2gamma regulates basal progenitor fate in a region- and layer-specific manner in the developing cortex. *Nat. Neurosci.* **12**, 1229-1237.
- Puehringer, D., Orel, N., Lüningschrör, P., Subramanian, N., Herrmann, T., Chao, M. V. and Sendtner, M.** (2013). EGF transactivation of Trk receptors regulates the migration of newborn cortical neurons. *Nat. Neurosci.* **16**, 407-415.
- Rakic, P.** (1974). Neurons in rhesus monkey visual cortex: systematic relation between time of origin and eventual disposition. *Science* **183**, 425-427.
- Saito, T.** (2006). In vivo electroporation in the embryonic mouse central nervous system. *Nat. Protoc.* **1**, 1552-1558.
- Seuntjens, E., Nityanandam, A., Miquelajauregui, A., Debruyn, J., Stryjewska, A., Goebels, S., Nave, K.-A., Huylebroeck, D. and Tarabykin, V.** (2009). Sip1 regulates sequential fate decisions by feedback signaling from postmitotic neurons to progenitors. *Nat. Neurosci.* **12**, 1373-1380.
- Shen, Q., Wang, Y., Dimos, J. T., Fasano, C. A., Phoenix, T. N., Lemischka, I. R., Ivanova, N. B., Stifani, S., Morrissey, E. E. and Temple, S.** (2006). The timing of cortical neurogenesis is encoded within lineages of individual progenitor cells. *Nat. Neurosci.* **9**, 743-751.
- Siegenthaler, J. A. and Pleasure, S. J.** (2011). We have got you 'covered': how the meninges control brain development. *Curr. Opin. Genet. Dev.* **21**, 249-255.
- Stahl, R., Walcher, T., De Juan Romero, C., Pilz, G. A., Cappello, S., Irmiger, M., Sanz-Aquela, J. M., Beckers, J., Blum, R., Borrell, V. et al.** (2013). Trnp1 regulates expansion and folding of the mammalian cerebral cortex by control of radial glial fate. *Cell* **153**, 535-549.
- Tarabykin, V., Stoykova, A., Usman, N. and Gruss, P.** (2001). Cortical upper layer neurons derive from the subventricular zone as indicated by Svt1 gene expression. *Development* **128**, 1983-1993.
- Temple, S.** (2001). The development of neural stem cells. *Nature* **414**, 112-117.
- Vasistha, N. A., García-Moreno, F., Arora, S., Cheung, A. F., Arnold, S. J., Robertson, E. J. and Molnár, Z.** (2014). Cortical and clonal contribution of Tbr2 expressing progenitors in the developing mouse brain. *Cereb. Cortex*, doi:10.1093/cercor/bhu125.
- Zarbalis, K., Siegenthaler, J. A., Choe, Y., May, S. R., Peterson, A. S. and Pleasure, S. J.** (2007). Cortical dysplasia and skull defects in mice with a Foxc1 allele reveal the role of meningeal differentiation in regulating cortical development. *Proc. Natl. Acad. Sci. USA* **104**, 14002-14007.

A



B

*In utero* electroporation E12.5-E14.5

C

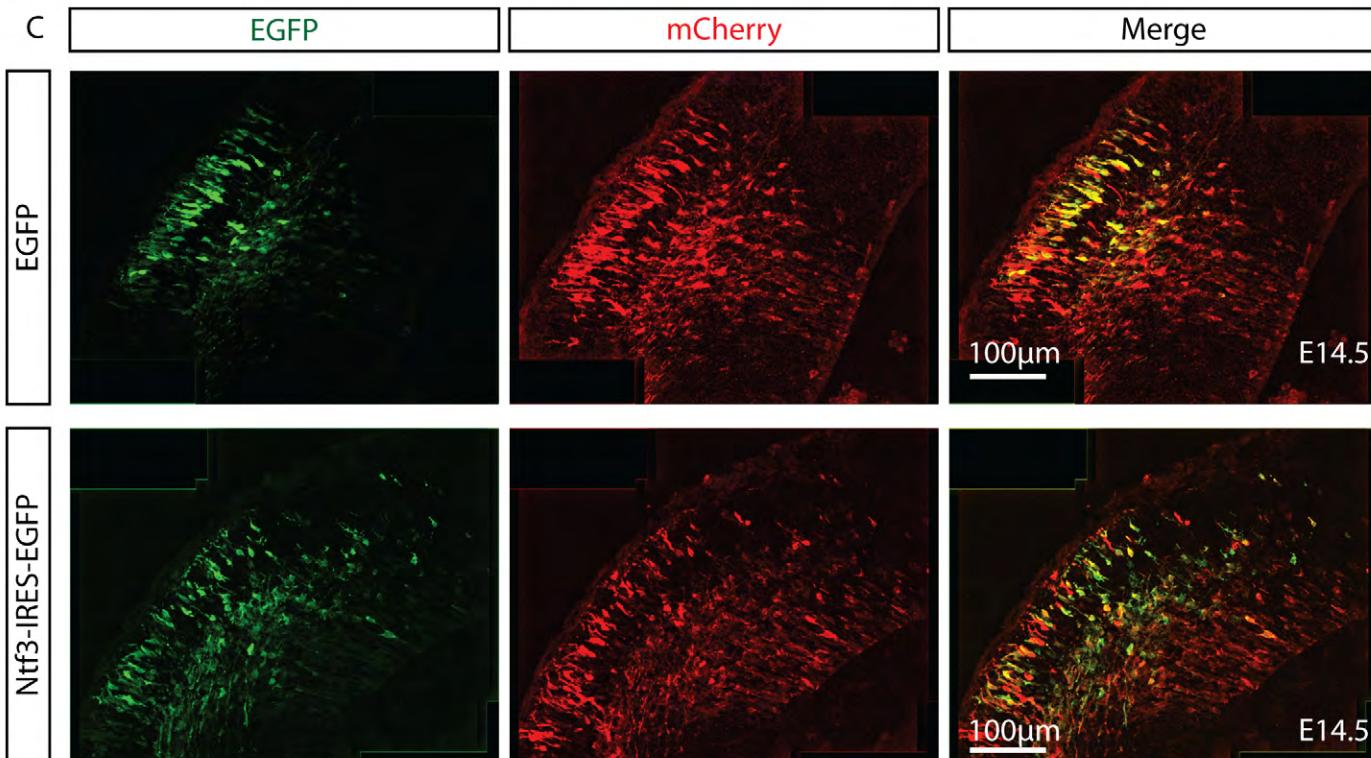


Fig. S1. Validation of plasmid system used for postmitotic expression. (A) Design of the plasmid system used. (B) Validation in HEK293T cells using CAG-Cre shows that all cells express mCherry before and EGFP after recombination. (C) Only mCherry is expressed in the germinal zone, while expression switches to EGFP after NeuroD1 promoter-driven Cre-mediated recombination.

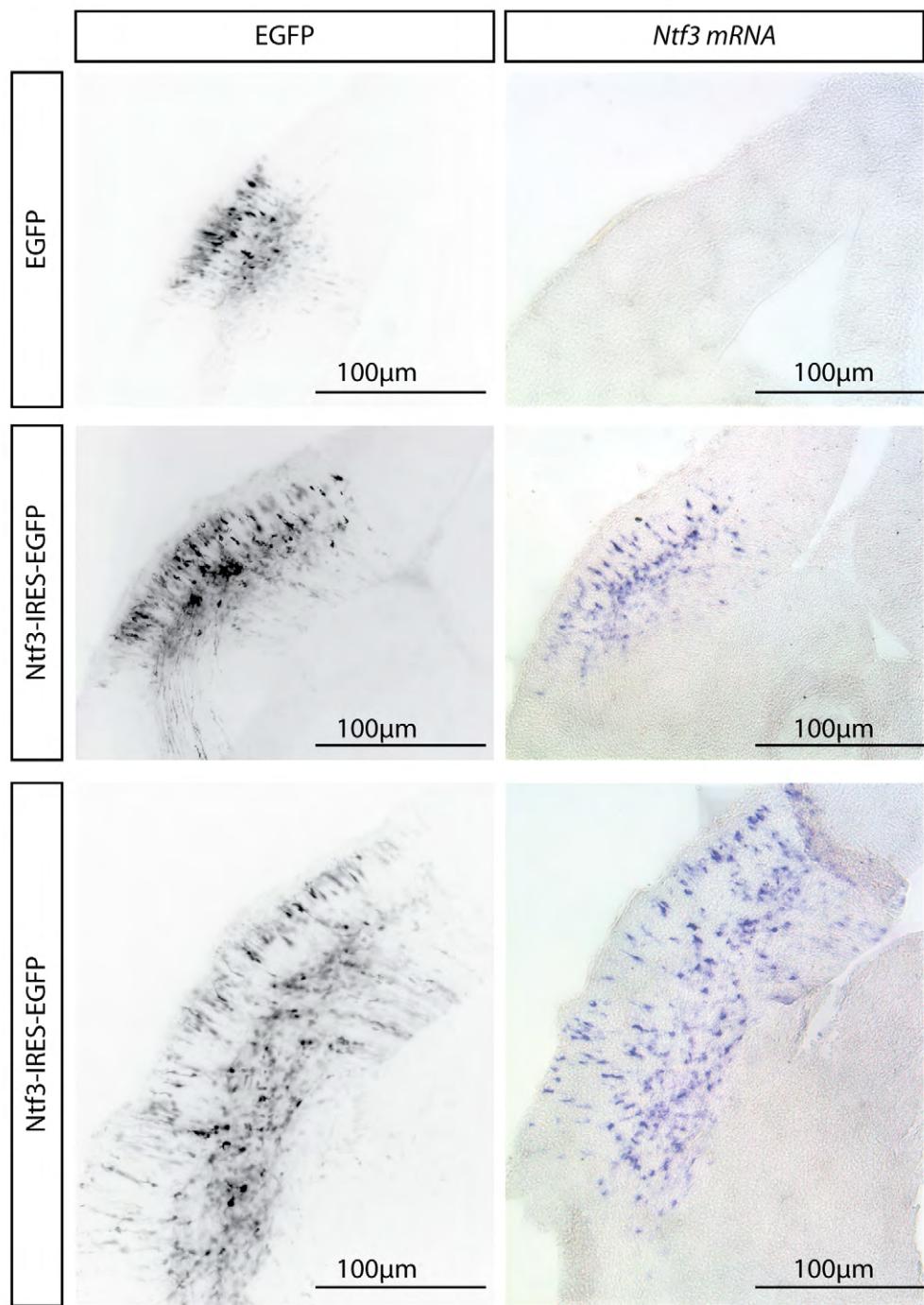


Fig. S2. Validation of *Ntf3* expression. Postmitotic expression of *Ntf3* was validated using *in situ* hybridization. No *Ntf3* expression can be seen in the control brains.