**Multipotency of Adult Hippocampal NSCs In Vivo Is Restricted by Drosha/NFIB**

**Highlights**
- Drosha regulates adult hippocampal stem cell maintenance
- Drosha inhibits oligodendrocytic differentiation of adult stem cells
- Drosha targets NFIB mRNA hairpin to inhibit expression and enable neurogenesis
- NFIB expression induces oligodendrocytic fate in adult hippocampal stem cells

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**In Brief**
Rolando et al. investigated the function of the RNaseIII Drosha in the regulation of adult hippocampal stem cell maintenance and differentiation. They found that Drosha directly inhibits the expression of the transcription factor NFIB through a miRNA-independent mechanism, thereby permitting neurogenesis and preventing oligodendrocyte fate commitment.

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**Multipotency of Adult Hippocampal NSCs In Vivo Is Restricted by Drosha/NFIB**

Chiara Rolando, Andrea Erni, Alice Grison, Robert Beattie, Anna Engler, Paul J. Gokhale, Marta Milo, Thomas Wegleiter, Sebastian Jessberger, and Verdon Taylor

**INTRODUCTION**

Adult neural stem cells (NSCs) are defined by their inherent capacity to self-renew and give rise to neurons, astrocytes, and oligodendrocytes. In vivo, however, hippocampal NSCs do not generate oligodendrocytes for reasons that have remained enigmatic. Here, we report that deletion of Drosha in adult dentate gyrus NSCs activates oligodendrogenesis and reduces neurogenesis at the expense of gliogenesis. We further find that Drosha directly targets NFIB to repress its expression independently of Dicer and microRNAs. Knockdown of NFIB in Drosha-deficient hippocampal NSCs restores neurogenesis, suggesting that the Drosha/NFIB mechanism robustly prevents oligodendrocyte fate acquisition in vivo. Taken together, our findings establish that adult hippocampal NSCs inherently possess multilineage potential but that Drosha functions as a molecular barrier preventing oligodendrogenesis.

**RESULTS**

Drosha Deletion from Adult DG NSCs Impairs Neurogenesis

NSCs in the DG of the adult mouse are Notch dependent and express the Notch target Hes5 (Lugert et al., 2010, 2012). Drosha is expressed by most cells in the DG, including GFAP+ and Hes5+ radial NSCs (Figures S1A and S1B). To address the functions of Drosha in neurogenic DG NSCs, we treated Hes5::CreER<sup>12</sup> mice carrying floxed Drosha (Drosha cKO) and wild-type (wt) Drosha (ctrl) alleles with tamoxifen (TAM) and followed cell fate by lineage tracing (Rosa26-CAG::EGFP) (Figures 1A and S1A) (Lugert et al., 2012). Twenty-one days after TAM administration, Hes5+ NSCs produce only granule neurons, which contribute to cognition, and loss or dormancy of stem cells during aging can result in psychological disorders and disease (Kronenberg et al., 2003; Petrus et al., 2009; Santarelli et al., 2003; Steiner et al., 2008). Whereas SVZ NSCs make a significant number of oligodendrocytes (Hack et al., 2004; Menn et al., 2006), new oligodendrocytes are normally not produced in the adult DG (Bonaguidi et al., 2011; Encinas et al., 2011; Lugert et al., 2010). In vitro, DG NSCs also rarely produce oligodendrocytes, although oligodendrocytic differentiation can be induced by their co-culture with neurons and in vivo by inactivation of the Neurofibromin 1 gene or reprogramming with the transcription factor Ascl1 (Braun et al., 2015; Jessberger et al., 2008; Song et al., 2002; Suh et al., 2007; Sun et al., 2015). This suggests an intrinsic and niche-independent fate restriction of DG NSCs that prevents oligodendrocyte formation. How DG NSC potency and particularly oligodendrocytic fate are restricted remains unclear.

Drosha is part of the microRNA (miRNA) microprocessor (Ha and Kim, 2014). However, Drosha can also cleave and directly destabilize mRNAs encoding proteins that regulate cell fate decisions (Chong et al., 2010; Han et al., 2009; Knuckles et al., 2012; Macias et al., 2012). During embryonic development, Drosha maintains embryonic NSCs in an undifferentiated, multipotent state by targeting and cleaving the mRNA of the proneural factor Ngn2 (Knuckles et al., 2012). This non-canonical function of Drosha does not require Dicer or miRNAs, and is a rapid mechanism for fate regulation.

Here, we examined how Drosha is involved in the regulation of DG NSC fate. We found that Drosha controls DG NSC maintenance and cell fate acquisition through a non-canonical regulation of the transcription factor nuclear factor IB (NFIB). We show that NFIB is required for the oligodendrocytic commitment by DG NSCs and propose that Drosha promotes neurogenesis and inhibits oligodendrocyte fate acquisition in the hippocampus by repressing NFIB.

SUMMARY

Adult neural stem cells (NSCs) are defined by their inherent capacity to self-renew and give rise to neurons, astrocytes, and oligodendrocytes. In vivo, however, hippocampal NSCs do not generate oligodendrocytes for reasons that have remained enigmatic. Here, we report that deletion of Drosha in adult dentate gyrus NSCs activates oligodendrogenesis and reduces neurogenesis at the expense of gliogenesis. We further find that Drosha directly targets NFIB to repress its expression independently of Dicer and microRNAs. Knockdown of NFIB in Drosha-deficient hippocampal NSCs restores neurogenesis, suggesting that the Drosha/NFIB mechanism robustly prevents oligodendrocyte fate acquisition in vivo. Taken together, our findings establish that adult hippocampal NSCs inherently possess multilineage potential but that Drosha functions as a molecular barrier preventing oligodendrogenesis.
NSCs and their progeny were Drosha deficient and generated fewer cells compared with controls (Figures S1B–S1D). Furthermore, the number of radial GFAP+, Sox2+, and mitotic (PCNA+) NSC/progenitors and neuroblasts (DCX+) was reduced in Drosha cKO animals (Figures 1B–1F and S1E). Decreased neurogenesis persisted in Drosha cKO animals at 100 days, and the reduction in newborn neurons (GFP+NeuN+) was accompanied by an increase in S100b+ parenchymal astrocytes compared with controls (Figures 1G–1I and S1F–S1J). In addition, GFAP+ putative radial NSCs were lost in Drosha cKO animals (Figures 1G, 1J, and 1K). Together these data suggest that Drosha is required for NSC maintenance and promotes neurogenesis in the DG at the expense of gliogenesis.

Quiescent DG NSCs activate, proliferate, and produce neuroblasts in response to seizures (Hüttmann et al., 2003; Sierra et al., 2015; Steiner et al., 2008). We addressed whether NSC-like progenitors remain in the Drosha cKO and can still respond to activating stimuli. We administered epileptogenic kainic acid (KA) to induce seizures in Hes5::CreERT2 Drosha cKO and control animals (Figures 1G–1I). Whereas KA induced proliferation and an increase in neuroblasts in control animals (Figures S1L and S1M), neither proliferation (PCNA+) nor neuroblast (DCX+) production was increased following KA treatment of Drosha cKO mice (Figures S1L and S1N). This suggests that Drosha cKO diminishes the DG NSC pool and compromises progenitor reactivation.

**Drosha cKO Induces Oligodendrocyte Commitment of NSCs**

To examine whether Drosha controls neurogenesis by acting on quiescent NSCs, we ablated Drosha specifically in radial GFAP+ NSCs by stereotactic infection with adenoviruses expressing Cre-recombinase under the control of the gfaP promoter (adeno-gfap::Cre) (Figure S2A) (Merkle et al., 2007). Six days post-infection (dpi), most GFAP-labeled, adenov-gfap::Cre-infected cells in the subgranular zone in control mice were GFAP+ radial process. Together these data suggest that Drosha is required for NSC maintenance and promotes neurogenesis in the DG at the expense of gliogenesis.

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newly formed neuroblasts were reduced in Drosha cKO animals (Figures 2A–2E). Therefore, Drosha cKO DG NSCs lose stem cell potential, demonstrating that Drosha is essential for NSC maintenance and neurogenesis.

DG NSCs normally generate glutamatergic granule neurons and astrocytes but not oligodendrocytes (Bonaguidi et al., 2010). Following adeno-gfap::Cre-mediated Drosha cKO, a significant number of the newborn cells expressed Olig2 and Sox10, markers of oligodendrocyte progenitor cells (OPCs) (Figures 2D–2G). Similarly, we observed newly generated Sox10+, Olig2+, and NG2+ OPCs in Hes5::CreERT2 Drosha cKO animals (Figures S2E–S2G). Thus, Drosha cKO induces a fate switch in DG NSCs to oligodendrocytes.

We performed clonal analysis of Hes5::CreERT2 Drosha cKO NSC fate. Two days after low-dose TAM induction, labeled NSCs were sparse in the DG (mean distance between clones = 184.3 ± 17.2 μm; Figures S2H and S2I). Twenty-one days post-TAM, 6 of the 41 clones examined in Drosha cKO animals contained OPCs but none in the controls (Figures 2H, 2I, S2J, and S2K). Interestingly, 1 clone contained neuroblasts, astrocytes, and oligodendrocytes, indicating tri-lineage potential of Drosha cKO NSC in vivo (Figure 2H).

We addressed whether Drosha controls oligodendrocyte production from mitotic GFAP− stem/progenitor cells. We infected dividing cells in the DG with a Cre-expressing retrovirus. We did not see oligodendrocyte formation in the Drosha cKO after retro-Cre virus infection, and active progenitors continued to generate neuroblasts (Figures S2L and S2M). These data suggest that Drosha deletion induces a fate shift in the quiescent NSC pool to oligodendrocyte production but not in active NSC/progenitors.

Dicer regulates miRNA maturation downstream of Drosha. To investigate whether Drosha regulates oligodendrocyte commitment of NSCs via miRNAs, we deleted Dicer (Dicer cKO) from radial DG NSCs with the adeno-gfap::Cre virus (Figure S2A). Dicer cKO did not affect the number of Sox2+ progenitors (data not shown) and caused a minor decrease in neuroblasts, consistent with the role of Dicer in neuronal survival and maturation (Figures 2G, S2N, and S2O) (Davis et al., 2008). Unlike Drosha cKO, Dicer cKO did not induce oligodendrocytic differentiation of DG NSCs (ctrl versus Dicer cKO, p = 0.56; Figures 2F and 2G). Therefore, Drosha but not Dicer inhibits oligodendrocyte differentiation of adult DG NSCs in vivo, indicating that the mechanism of induced fate switching caused by the loss of Drosha does not primarily involve miRNAs.

**Drosha cKO DG NSCs Produce Oligodendrocytes In Vitro**

To investigate the mechanisms of Drosha-regulated NSC fate acquisition, we generated a self-renewing DG NSC culture system that recapitulates in vivo features of neurogenesis including expression of the progenitor markers Sox2 and BLBP (Figure S2P). Upon growth factor removal (−FGF2/−EGF), DG NSCs differentiated into neurons and astrocytes but not oligodendrocytes, indicating conserved intrinsic cell fate restriction (Figure S2Q; data not shown) (Bonaguidi et al., 2011; Lugert et al., 2010). We cultured DG NSCs from adult Droshafl/fl, Dicer+/−, and Drosha+/−Dicer+/− (control) animals that carried the Rosa26-CAG::EGFP Cre reporter. Following adeno-Cre viral transduction, we investigated the effects of Drosha and Dicer cKO (Figures S2R and S2S). Two days post-infection, BLBP+ progenitors were reduced in the Drosha cKO compared with control and Dicer cKO cultures, similar to the reduction in progenitors after Drosha ablation in vivo (Figures 2J–2M). Both differentiated Drosha cKO and Dicer cKO NSCs generated fewer neurons in vitro (Figures 2M and S2T–S2V). However, we observed an increase in apoptotic cells (cleaved Caspase3+) in the Dicer cKO cultures compared with Drosha cKO and control, confirming that Dicer is crucial for neuronal survival and providing an explanation for the reduction in neurons in its absence (Figure S2W). Drosha cKO induced an increase in NG2+ OPCs in the cultures and this at the expense of neuron and astrocyte production (Figures 2K, 2M, and S2X). Dicer cKO induced a slight but not significant increase in NG2+ OPCs in the cultures (ctrl versus Dicer cKO, p = 0.27; Figures 2L and 2M). Hence, DG NSCs retain a cell-intrinsic bias against oligodendrocyte differentiation in vitro, and Drosha controls this fate decision. We sorted Drosha cKO, Dicer cKO, and control DG NSCs 48 hr after adeno-Cre virus infection in vitro and determined the expression profiles of 381 miRNAs by microarray. Two hundred sixty miRNAs were detected in control DG NSCs (mean Ct values < 32), and their levels were not significantly changed 48 hr after Drosha cKO (R² = 0.81; Figure S2Y), even though the phenotypes were well established by this time. Dicer cKO resulted in moderate changes in miRNA levels after 48 hr (R² = 0.66; Figure S2Z), although Dicer cKO NSCs did not display an obvious phenotype at this time. Hence, Drosha cKO did not cause major global changes in miRNA levels, and any changes were less than in Dicer cKO DG NSCs. These data support that the mechanism of Drosha suppression of oligodendrocyte production by DG NSCs is independent of Dicer and miRNAs.

**Drosha Binds and Cleaves the NFIB mRNA Regulating Expression**

Drosha can bind and cleave hairpin loops in miRNAs (Chong et al., 2010; Han et al., 2009; Knuckles et al., 2012; Macias et al., 2012). In silico analysis (Evofold) (Pedersen et al., 2006) revealed two evolutionarily conserved hairpins in the mRNA of NFIB, a short 20-base hairpin in the 5′ UTR (5′ UTR HP) and a longer hairpin of 83 bases in the 3′ UTR bases in the 3′ UTR HP (Figure 3A). NFIB plays roles in the development of glial cells and myelin tracts (Barry et al., 2008; Deneen et al., 2006; Harris et al., 2015; Kang et al., 2012; Steele-Perkins et al., 2005). To examine whether Drosha binds directly to NFIB mRNA in DG NSCs, we performed cross-linked immunoprecipitation (CLIP) for endogenous Drosha protein and examined the bound RNAs (Figures 3A and 3B). NFIB mRNA cross-linked immunoprecipitated with Drosha from DG NSCs, as did the known target DGC8 mRNA (Figures 3B and 3B) (Han et al., 2009; Knuckles et al., 2012).

In order to address whether either of the two NFIB mRNA hairpins convey Drosha association, we placed the 5′ UTR HP and 3′ UTR HP into the SV40 3′ UTR downstream of the Renilla Luciferase (rLuc) coding region of the psiCheck reporter vector (Figure 3C). We expressed 5′ UTR HP and 3′ UTR HP containing rLuc miRNAs in N2a cells and performed CLIP to address binding by Drosha. Both the 5′ UTR HP and 3′ UTR HP of NFIB bound to Drosha more efficiently than the SV40 3′ UTR sequence alone.
suggesting that Drosha suppresses NFIB mRNA expression in

Drosha cKO-Induced Oligodendrocytic Differentiation Depends on NFIB

To evaluate whether Drosha affects translation of NFIB 3' UTR HP mRNAs, we performed Luciferase assays in cultured adult DG NSCs (Figure S3E). Drosha cKO increased Luciferase activity of an NFIB 3' UTR HP containing synthetic mRNA (Figure S3F). Surprisingly, Dicer cKO also increased translation of the NFIB 3' UTR HP containing Luciferase mRNA by an unknown mechanism, indicating that under these experimental conditions, Dicer can also regulate NFIB 3' UTR HP containing mRNAs.

To determine whether Drosha affects the stability of NFIB 3' UTR HP mRNAs, we performed qRT-PCR over the 3' UTR HP to determine the relative levels of non-cleaved NFIB transcripts, confirming the Drosha-dependent destabilization of NFIB RNAs in vivo (Figure 3G). To evaluate whether Drosha affects translation of NFIB 3' UTR HP mRNAs, we performed Luciferase assays in cultured adult DG NSCs (Figure S3E). Drosha cKO increased Luciferase activity of an NFIB 3' UTR HP containing synthetic mRNA (Figure S3F). Surprisingly, Dicer cKO also increased translation of the NFIB 3' UTR HP containing Luciferase mRNA by an unknown mechanism, indicating that under these experimental conditions, Dicer can also regulate NFIB 3' UTR HP containing mRNAs.

Drosha interaction with hairpins in mRNAs can result in destabilization of the transcripts (Han et al., 2009; Knuckles et al.; 2012). We isolated Hes5::CreER<sup>T2</sup> Drosha cKO and Hes5::CreER<sup>T2</sup> Drosha<sup>cre<sup>h<sub>cre<sup>h<sub>cre</sub></sub></sub></sup> DG NSCs by fluorescence-activated cell sorting (FACS) based on GFP expression from the Cre-activated Rosa26-CAG::EGFP locus following acute induction with TAM (Figure S3G). The Drosha cKO-induced oligodendrocytic differentiation of Drosha cKO cells (Figures 4C and 4F). Like their control counterparts, NFIB knockdown Drosha cKO NSCs adopted a neuronal fate or remained as progenitors (Figures 4G and 4H). Thus, Drosha negatively regulates DG NSC differentiation toward an oligodendrocytic fate by suppressing NFIB mRNA levels (Figure S4). Upon Drosha cKO, inhibition of NFIB is released, and an oligodendrocytic differentiation program is activated (Figure S4J).

DISCUSSION

Adult NSC identity is orchestrated by complex regulatory gene networks and neurogenic niche microenvironments. Post-transcriptional modifications add an additional level of

**Figure 2. Drosha Deletion from DG NSCs Induces Oligodendrocyte Fate Commitment**

(A and B) GFP::Sox2<sup>+</sup> progenitors and GFP::PCNA<sup>+</sup> mitotic cells in control (A) and Drosha cKO (B) animals at day 21 post-adeno-gfap::Cre virus infection. (C and D) GFP::DCX<sup>+</sup> neuroblasts and GFP::Olig2<sup>+</sup> oligodendrocytes in control (C) and Drosha cKO (D) animals at day 21. (E) Quantification of GFP::Sox2<sup>+</sup>, GFP::PCNA<sup>+</sup> progenitors and GFP::Olig2<sup>+</sup> oligodendrocytes in control and Drosha cKO day 21 after adeno-gfap::Cre virus infection (control, n = 3; Drosha cKO, n = 3). Two-sided Student's t test: "p = 0.05, "p = 0.01. (F) GFP::Sox10<sup>+</sup> oligodendrocytes in Drosha cKO and Dicer cKO animals. (G) Quantification of GFP::DCX<sup>+</sup> neuroblasts and GFP::Sox10<sup>+</sup> oligodendrocytes upon Drosha cKO and Dicer cKO (control, n = 3; Drosha cKO, n = 3; Dicer cKO, n = 3). ANOVA with Bonferroni post hoc test: "p = 0.05, "p = 0.01. (H) Tripotent clone derived from a single Drosha cKO NSC. A, astrocyte; N, neuron; O, oligodendrocyte; R, radial NSC. (I) Quantification of clone composition in control and Drosha cKO (control clones, n = 28; Drosha cKO clones, n = 41). Two-sided Student's t test: "p = 0.05, "p = 0.01. (J–L) GFP::BLBP<sup>+</sup> and GFP::NG2<sup>+</sup> expression in cultured control (J), Drosha cKO (K), and Dicer cKO (L) NSCs 2 dpi with adeno-Cre virus. (M) Quantification of neural lineage marker expression by adeno-Cre-infected (GFP<sup>+</sup>) control, Drosha cKO, and Dicer cKO NSCs 2 dpi (n = 4). Kruskal-Wallis with Dunn post hoc test: "p = 0.05, "p = 0.01.

Data are mean ± SEM. The scale bars represent 20 μm. See also Figure S2 and Tables S2 and S3.
regulation to NSC maintenance and differentiation. Growing evidence suggest that miRNA-independent functions of the microprocessor are conserved mechanisms that regulate several cellular processes in the nervous system and other tissues (Chong et al., 2010; Han et al., 2009; Karginov et al., 2010; Knuckles et al., 2012; Macias et al., 2012).

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**Figure 3. Drosha Binds and Cleaves NFIB mRNA in DG NSCs**

(A) Evolutionary conserved hairpins 5’ UTR HP (blue) and 3’ UTR HP (red) in the NFIB mRNA sequence.

(B) Drosha CLIP-qRT-PCR of NFIB mRNA from DG NSCs. DGCR8 and Six3 mRNAs were used as positive and negative control CLIP targets, respectively (n = 3 replicates). Mann-Whitney test: *p < 0.05.

(C) Scheme of the psiCheck Renilla Luciferase constructs (rLuc) containing the NFIB 5’ UTR HP or 3’ UTR HP sequence in the SV40 UTR.

(D) qRT-PCR analysis of rLuc mRNA pulled down with Drosha from psiCheck-NFIB 5’ UTR HP and psiCheck-NFIB 3’ UTR HP transfected N2a cells relative to the pull-down from psiCheck-rLuc transfected cells (n = 3 replicates). Two-sided Student’s t test: *p < 0.05, **p < 0.01.

(E) Scheme of the in vitro processing procedure.

(F) Capillary electrophoresis electropherograms of NFIB 3’ UTR HP RNA (probe) incubated with the beads alone (ctrl), incubated with mock IP sample, or flag-tagged Drosha IP (Drosha FLAG IP). Arrow points to degraded 3’ UTR HP probe. Loading marker (LM) and probe (P) are indicated.

(G) qRT-PCR analysis of the NFIB 3’ UTR HP in control and Drosha cKO NSCs 2 days after adeno-Cre infection. Data are mean ± SEM.
Figure 4. NFIB Knockdown Rescues Drosha cKO-Induced Oligodendrocyte Differentiation
(A) Quantification of lineage marker expression by NFIB overexpressing DG NSCs after 5-days of differentiation (n = 3 replicates). Mann-Whitney test: *p < 0.05, ***p < 0.001.
(B) Experimental paradigm of the nucleofection experiments.
(C) Quantification of adeno-Cre virus infected (GFP+) mCherry+ NG2+ OPCs in Drosha cKO and control NSCs nucleofected with control rLuc esiRNA or NFIB esiRNA.
(D–F) mCherry+, GFP+, and NG2+ cells in adeno-Cre virus infected control NSC cultures nucleofected with the control esiRNA, Drosha cKO NSCs nucleofected with the control esiRNA (E), and Drosha cKO NSCs nucleofected with the NFIB esiRNA (F).
(G) Quantification of adeno-Cre virus infected (GFP+) mCherry+ βtub+ neurons from Drosha cKO and control NSCs nucleofected with rLuc esiRNA or NFIB esiRNA.
(H) Quantification of adeno-Cre virus infected (GFP+) mCherry+ BLBP+ progenitors from Drosha cKO and control NSCs nucleofected with control rLuc esiRNA or NFIB esiRNA.
Data are mean ± SEM. Biological replicates, n = 3. Kruskal-Wallis with Dunn post hoc test: *p < 0.05, **p < 0.01. The scale bars represent 20 μm.
Here we show that Drosha plays a central role in regulating progenitors of the adult DG by sustaining NSC potential. Upon Drosha ablation, DG NSCs are depleted, and gliogenesis increases at the expense of neurogenesis. By comparing Drosha cKO and Dicer cKO mice, we identified the transcription factor NFIB as a target of Drosha and showed that the blockade of NFIB expression is necessary for inhibiting oligodendrocyte formation and enabling neurogenesis in the adult DG. Therefore, Drosha regulates DG neurogenesis and gliogenesis at least partially through a miRNA and Dicer-independent, cell-intrinsic fate program.

CLIP experiments revealed that the microprocessor targets different RNA classes, including pri-miRNAs, small nucleolar RNA, long non-coding RNA, and mRNAs (Macias et al., 2012). The microprocessor interactome has been defined in human embryonic stem cells and indicates the importance of cell type and biological context (Seong et al., 2014). However, it is clear that several miRNAs are processed by the microprocessor, resulting in their destabilization (Chong et al., 2010; Johanson et al., 2015; Knuckles et al., 2012). The non-canonical functions of the microprocessor represent a rapid and efficient way to influence gene expression. Our understanding of the mechanisms underlying these alternative functions of Drosha and the microprocessor need further investigation. The Drosha-DGCR8 complex is required for miRNA biogenesis, but it is possible that other protein-protein interactions underlie the alternate functions of Drosha (Macias et al., 2015).

DG NSCs are fate committed to glutamatergic granule neuron and astrocytic fates in vivo (Bonaguidi et al., 2011; Lugert et al., 2010). How this intrinsic fate restriction is controlled remains unclear. In vitro studies showed that DG NSCs are able to generate oligodendrocytes only under specific conditions, including co-culture with neurons (Song et al., 2002; Suh et al., 2007). Furthermore, reprogramming of adult DG NSCs by Ascl1 overexpression leads to a shift in fate from neuronal to oligodendrocyte differentiation (Braun et al., 2015; Jessberger et al., 2008). A potential link between Drosha and Ascl1 remains to be shown, but Ascl1 mRNA was not cross-linked immunoprecipitated with Drosha from DG NSCs (data not shown).

Clonal lineage tracing of DG NSCs in vivo showed symmetric and asymmetric neuron and astrocytic fates (Bonaguidi et al., 2011). Drosha cKO NSCs exited the stem cell pool and the cell cycle and generated few progeny. However, at the population and single-cell levels, DG NSCs retain the potential to generate all three cell lineages of the brain, but Drosha mediates the intrinsic restriction of oligodendrocyte differentiation potential.

NF1 transcription factors can activate and repress gene transcription depending on the gene and cellular context (Chang et al., 2013; Gronostajski, 2000; Messina et al., 2010). NFIB influences stem cell maintenance and differentiation in several tissues, including in the SVZ, as part of a cross-regulatory network together with Pax6/Brng1 (Chang et al., 2013; Ninkovic et al., 2013). In addition, NFIB can repress Notch signaling in embryonic hippocampal NSCs by repressing Hes1 promoter activity (Piper et al., 2010). Therefore, we speculate that induction of NFIB expression might lead to inhibition of stem cell genes and block of Notch signaling resulting in exhaustion of the DG NSC pool and differentiation. Moreover, we also show for the first time that NFIB has a central function in regulating oligodendrocyte fate commitment in the adult DG. It remains to be shown which genes are regulated downstream of NFIB. Although we cannot exclude that NFIB acts as a transcriptional repressor of genes required for neuronal differentiation and therefore indirectly promotes gliogenesis, NG2 is upregulated in response to Drosha cKO in an NFIB-dependent manner. Interestingly, Cspg4 (the gene encoding NG2) has NF1 binding motifs that are bound by NFIB, suggesting a direct regulation in DG NSCs (Chang et al., 2013). We believe this is the first demonstration of a non-canonical Drosha-mediated regulation of adult stem cell fate through a niche-independent intrinsic pathway. In the future, it will be important to understand the targets of this post-transcriptional pathway and whether stem cells are able to modulate Drosha activity to control cell fate in order to satisfy demand.

**EXPERIMENTAL PROCEDURES**

**Animal Husbandry**

The mice used have been described previously (Supplemental Experimental Procedures). Mice were maintained on a 12 hr day-night cycle with free access to food and water under specific pathogen-free conditions and according to Swiss federal regulations. All procedures were approved by the Basel Cantonal Veterinary Office (license numbers 2537 and 2538).

**Hippocampal NSC Cultures, Adenoviral Infection, and Nucleofection**

DG NSCs were isolated from 8-week-old mice as described previously (Lugert et al., 2010). DG NSCs were infected with an adeno-Cre adenovirus at a multiplicity of infection of 100 and fixed after 24 or 48 hr. DG NSC cultures were nucleofected using a mouse neural stem cell kit (Lonza) (Supplemental Experimental Procedures).

**FACS**

After TAM induction, NSCs were isolated from Hes5CreER<sup>fl/fl</sup>Rosa26-CAG::EGFP<sup>0</sup> and Hes5::CreER<sup>fl/fl</sup>Drosha<sup>0</sup>Rosa26-CAG::EGFP<sup>0</sup> using a FACsAriaII (BD Biosciences) (Supplemental Experimental Procedures).

**RNA Isolation, qRT-PCR, and Analysis of miRNA Expression**

Total RNA was isolated from cultured or sorted DG NSCs using Trizol reagent (Life Technologies). Analysis of gene expression was performed as described in Supplemental Experimental Procedures. miRNAs were isolated using mirVANA kit (ThermoFisher) following the miRNA enrichment procedure and quantified by TaqMan arrays (Life Technologies) (Supplemental Experimental Procedures).

**In Vitro Processing of NFIB HP RNAs**

In vitro processing was performed on 5' and 3' UTR NFIB HP RNAs as described previously with minor adaptations (Supplemental Experimental Procedures) (Lee and Kim, 2007).

**5' RACE**

5' RACE experiments were performed on 3 μg of total RNA of control and Drosha cKO NSCs following the manufacturer’s instructions (Invitrogen) (Supplemental Experimental Procedures).

**Luciferase Assay**

DG NSCs were transduced with an adeno-Cre adenovirus at a multiplicity of infection of 100 with or without subsequent nucleofection 2 days later with the psiCheck2 containing the 3' UTR HP or 5' UTR HP or control psiCheck2 vectors (Supplemental Experimental Procedures).

**Quantification and Statistical Analysis**

Randomly selected, stained cells were analyzed with fixed photomultiplier settings on a Zeiss LSM510 confocal and Apotome2 microscope. For clonal
analysis, the entire hippocampus was sectioned and reconstructed as described previously (Bonaguidi et al., 2011) (Supplemental Experimental Procedures). Percentages were converted by arcsine transformation. Statistical comparisons were conducted by two-tailed unpaired Student’s t test, Mann-Whitney test, one-way ANOVA, or Kruskal-Wallis with Dunn post hoc test as indicated. Statistical significance was assessed using GraphPad Prism software (GraphPad Software). Significance was established at p < 0.05.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.stem.2016.07.003.

AUTHOR CONTRIBUTIONS

C.R., A. Emi, A.G., R.B., A. Engler, P.J.G., and M.M. designed and performed experiments and evaluated and interpreted the data. T.W. and S.J. contributed reagents. V.T. conceived and designed the project and evaluated the data. C.R., A. Emi, and V.T. wrote the paper and prepared the figures. All authors edited and proofread the manuscript.

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