A Transcriptional Mechanism Integrating Inputs from Extracellular Signals to Activate Hippocampal Stem Cells

Jimena Andersen,1 Noelia Urbán,1,* Angeliki Achimastou,1 Ayako Ito,1 Milesa Simic,2 Kristy Ulloom,2 Ben Martynoga,1 Mélanie Lebel,1 Christian Göritz,3 Jonas Fрисén,2 Masato Nakafuku,2 and François Guillemot1,*

1Division of Molecular Neurobiology, MRC National Institute for Medical Research, Mill Hill, London NW7 1AA, UK
2Division of Developmental Biology, Cincinnati Children’s Hospital Research Foundation, Cincinnati, OH 45229-3039, USA
3Department for Cell and Molecular Biology, Karolinska Institute, 17177 Stockholm, Sweden
*Correspondence: nurban@nimr.mrc.ac.uk (N.U.), fguille@nimr.mrc.ac.uk (F.G.)
http://dx.doi.org/10.1016/j.neuron.2014.08.004

SUMMARY

The activity of adult stem cells is regulated by signals emanating from the surrounding tissue. Many niche signals have been identified, but it is unclear how they influence the choice of stem cells to remain quiescent or divide. Here we show that when stem cells of the adult hippocampus receive activating signals, they first induce the expression of the transcription factor Ascl1 and only subsequently exit quiescence. Moreover, lowering Ascl1 expression reduces the proliferation rate of hippocampal stem cells, and inactivating Ascl1 blocks quiescence exit completely, rendering them unresponsive to activating stimuli. Ascl1 promotes the proliferation of hippocampal stem cells by directly regulating the expression of cell-cycle regulatory genes. Ascl1 is similarly required for stem cell activation in the adult subventricular zone. Our results support a model whereby Ascl1 integrates inputs from both stimulatory and inhibitory signals and converts them into a transcriptional program activating adult neural stem cells.

INTRODUCTION

Adult stem cells maintain tissue function and integrity throughout the lifetime of an organism. They produce mature progeny to replace short-lived cells and repair tissue damage while maintaining their numbers through self-renewing divisions (Simons and Clevers, 2011). Many tissue stem cells are relatively quiescent, which delays their attrition and minimizes the accumulation of deleterious mutations (Orford and Scadden, 2008). The transit of stem cells between quiescent and activated states is not well understood in most systems. Elucidating the mechanisms that control the activation of tissue stem cells is an important goal in stem cell biology.

A variety of extracellular signals present in stem cell niches have been shown to influence the activity of tissue stem cells (Fuchs et al., 2004; Goldstein and Horsley, 2012; Kuang et al., 2008). For example, BMP signaling induces quiescence, while Wnts promote proliferation of skin and blood stem cells (Blank et al., 2008; Fuchs et al., 2004). However, the cell-intrinsic mechanisms that mediate the activity of extrinsic signals and promote stem cell quiescence or proliferation are poorly characterized. Niche signals might act by inducing the expression or activity of transcription factors that in turn regulate the large number of genes differentially expressed between quiescent and active stem cells (Lien et al., 2011; Martynoga et al., 2013; Venezia et al., 2004). Transcription factors have indeed been shown to regulate stem cell activity in various tissues by controlling their proliferation, survival, or differentiation (Akala and Clarke, 2006; Goldstein and Horsley, 2012). However, it is not known in most instances how these factors are regulated (Niu et al., 2011; Osorio et al., 2008).

In the adult mammalian nervous system, neural stem cells (NSCs) are found mostly in two regions of the anterior brain, the dentate gyrus (DG) of the hippocampus and the ventricular-subventricular zone (V-SVZ) lining the lateral ventricles, where stem cells produce new neurons that integrate into neuronal circuits of the hippocampus and olfactory bulb, respectively (Fuentealba et al., 2012; Ming and Song, 2011). Most adult NSCs are quiescent and reside in G0, with only a small fraction progressing through the cell cycle at any time. NSC divisions result in the generation of transit-amplifying cells or intermediate progenitor cells (IPCs) that undergo a limited number of rapid divisions before they exit the cell cycle and differentiate into neurons (Ming and Song, 2011; Ponti et al., 2013). Clonal analysis in the adult mouse hippocampus in vivo has provided evidence that hippocampal NSCs, also called radial glia-like cells (RGLs), are multipotent and can generate both neurons and astrocytes, and that they use two modes of divisions to self-renew. Some RGLs divide asymmetrically to generate a new RGL and an IPC or an astrocyte, while others divide symmetrically into two new RGLs (Bonaquidi et al., 2011).

A particularly important feature of hippocampal neurogenesis is its regulation by a variety of physiological stimuli (Ming and Song, 2011). Neurogenesis in the hippocampus declines sharply with age, due in part to a reduction of the fraction of RGLs that divide, and it is suppressed by stress and depression (Lee et al., 2011; Ming and Song, 2011). Conversely, an enriched environment, task learning, or seizures stimulate hippocampal neurogenesis, in part by stimulating RGL divisions (Kronenberg...
et al., 2003; Ming and Song, 2011). Some of the extracellular signal regulating RGL activity have been identified (Ming and Song, 2011). In particular, the BMP and Notch signaling pathways maintain RGLs in a quiescent state (Ables et al., 2010; Ehm et al., 2010; Mira et al., 2010), while the Wnt and IGF-1 pathways, among others, promote RGL divisions and stimulate neurogenesis (Bracko et al., 2012; Jang et al., 2013; Lie et al., 2005; Qu et al., 2010). Little is known, however, of how the activity of physiological stimuli or extrinsic signals is transduced inside RGLs to control their divisions. The orphan nuclear receptor Tlx is required to maintain RGLs in proliferation (Niu et al., 2011; Qu et al., 2010; Zhang et al., 2008), but how Tlx expression and activity are regulated has not been addressed.

The proneural transcription factor achaete-scute homolog 1 (Ascl1/Mash1) is an important regulator of neurogenesis in the embryonic nervous system. It is expressed by dividing progenitors and promotes their proliferation, specification, and differentiation into neurons (Bertrand et al., 2002; Castro et al., 2011). Moreover, ectopic expression of Ascl1 can reprogram various cell types into neurons (Beminger et al., 2007; Yang et al., 2011). Ascl1 is also expressed in the DG and V-SVZ of the adult rodent brain, but its function there has not been examined. Ascl1 adult expression is mostly confined to IPCs (Lugert et al., 2012; Parras et al., 2004; Pastrana et al., 2009), but recent genetic lineage-tracing experiments have established that it is also present in self-renewing stem cells in both the V-SVZ and hippocampus (Kim et al., 2011). Consistent with this finding, Ascl1 was found expressed by a small subset of cycling stem cells in both neurogenic zones (Breunig et al., 2007; Kim et al., 2011). Here we show that Ascl1 expression is rapidly induced by neurogenic signals in hippocampal RGLs, and that Ascl1 has a crucial role in RGL activation in both DG and V-SVZ. Ascl1 is specifically expressed in activated adult stem cells and is specifically required for the exit of stem cells from quiescence.

RESULTS

Ascl1 Is Expressed by Activated Stem Cells in the Adult Hippocampus

To study the function of Ascl1 in hippocampal neurogenesis, we first characterized its expression in the adult hippocampus. Labeling of 2-month-old mouse brains with a monoclonal antibody against Ascl1 showed that in the hippocampus, Ascl1-expressing cells are restricted to the subgranular zone (SGZ) of the DG (Figure 1A). Double labeling for Ascl1 and the cell proliferation marker Ki67 (red) shows that Ascl1 is expressed in proliferating cells in the dentate gyrus (DG) of a P60 mouse. Counterstained by DAPI (white).

Figure 1. Expression of Ascl1 by Proliferating Stem and Progenitor Cells in the Adult Hippocampus

(A) Colocalization of Ascl1 protein (green) and proliferation marker Ki67 (red) shows that Ascl1 is expressed in proliferating cells in the dentate gyrus (DG) of a P60 mouse. Counterstained by DAPI (white).

(B) Ascl1 (green), MCM2 (a marker of cell cycle and G0 to early G1 transition, red), and neural stem cell marker GFAP (white) are colocalized in an activated radial glia-like stem cell (RGL). The z stack of the merged confocal picture is shown along the x axis (top) and the y axis (right).

(C) The percentage of activated (MCM2+) and Ascl1-expressing (Ascl1+) RGLs in the DG of P60 mice show that Ascl1 is expressed in a third of activated RGLs. n = 3.

(D) Labeling for Ascl1 (green), the intermediate progenitor cell (IPC) marker Tbr2 (red), and GFAP (white) in a P60 DG show that Ascl1 is expressed in both Tbr2+ (yellow arrowhead) and Tbr2−/GFAP+ IPCs. Scale bars, 20 μm in (A) and (D) and 10 μm in (B). Values represent mean values, and error bars represent SDs.
Ascl1 Activates Adult Hippocampal Stem Cells

Ascl1 Expression in Hippocampal Stem Cells by Neurogenic Stimuli

Ascl1 is expressed by more mature cells in the lineage, including Tbr2 (Kempermann et al., 2004; Ming and Song, 2011). Ascl1 into two subsets that differ in the expression of the IPC marker IPCs. Ascl1 represents 13.5% ± 4.1% of all IPCs and are subdivided into two subsets that differ in the expression of the IPC marker Tbr2 (Kempermann et al., 2004; Ming and Song, 2011). Ascl1 is not expressed by more mature cells in the lineage, including doublecortin (DCX)+ neuroblasts and NeuN+ granule neurons (Figure S1; data not shown). Together, these data agree with previous reports showing that Ascl1 expression is restricted to the earliest stages of the neurogenic lineage of the adult DG, including proliferating RGLs (Breunig et al., 2007; Kim et al., 2011) and early IPCs (Lugert et al., 2010, 2012), and that it is downregulated before IPCs begin to express neuronal markers and exit the cell cycle.

Ascl1 Expression in Hippocampal Stem Cells Is Induced by Neurogenic Stimuli

The fact that Ascl1 expression is restricted to activated RGLs suggested that this factor might be induced by signals that promote RGL activity and neurogenesis in the hippocampus. To address this possibility, we examined Ascl1 expression in mice treated with the ionotropic glutamate receptor agonist kainic acid (KA), a neurogenic molecule that induces progenitor divisions in the DG (Lugert et al., 2010). A single injection of KA in 8- to 9-week-old wild-type (WT) mice produced, as expected, a robust increase in the number of MCM2+ RGLs in the DG, which became detectable 2 days after injection (Figure S2). Remarkably, KA induced Ascl1 expression in RGLs with more rapid kinetics, as the number of Ascl1+ RGLs was already increased 24 hr after injection (Figure S2). Thus, a significant fraction of quiescent (MCM2+) RGLs expressed Ascl1 at 24 hr after injection (7.8% ± 1.3% Ascl1+ MCM2+ RGLs in WT versus RBPJk cKO mice) (D) show an increase in the number of quiescent Ascl1+ RGLs in RBPJk cKO mice (black bar), suggesting that blocking Notch signaling induces Ascl1 expression before RGLs exit quiescence. p values, Ascl1+ RGLs in WT versus RBPJk cKO < 0.0001; MCM2+ RGLs in WT versus RBPJk cKO < 0.0001; Ascl1+ MCM2+ RGLs in WT versus RBPJk cKO = 0.0911. n = 5 for WT, n = 7 for RBPJk cKO. Scale bars, 20 μm in main panels and 10 μm in enlarged panels. Values represent mean values, and error bars represent SDs.

To determine whether antineurogenic stimuli also regulate Ascl1 expression in the DG, we examined Notch signaling. Deletion of the Notch pathway component RBPJk has been shown to transiently induce the proliferation of hippocampal RGLs, followed later by a depletion of RGLs (Ehm et al., 2010). We deleted RBPJk from RGLs by injecting tamoxifen in 3-month-old mice carrying an inducible RBPJk-mutant allele (Glast::CreERT2; RBPJkloxP/loxP; Rosa26R-stop-YFP, called thereafter RBPJk cKO mice). Examination of the hippocampus 7 days later revealed a dramatic increase in the numbers of MCM2+ activated RGLs and of Ascl1-expressing RGLs in RBPJk cKO mice.
compared with control mice, demonstrating that loss of Notch signaling stimulates both the proliferation of RGLs and the expression of Ascl1 (Figures 2C and 2D). A fraction of RGLs in RBPJk cKO DGs expressed Ascl1, and not MCM2 (3.60% ± 1.50% in RBPJk cKO versus 0.25% ± 0.25% in control mice; Figure 2D), suggesting that like KA administration, inactivation of the Notch pathway in RGLs sequentially promotes Ascl1 expression and quiescence exit. We also examined the effect of voluntary exercise on RGL proliferation and expression of Ascl1 and found that it did not significantly increase proliferation of hippocampal RGLs, but only that of IPCs, as previously reported (Klempin et al., 2013). Together, our results demonstrate that neurogenic stimuli rapidly induce Ascl1 expression in quiescent RGLs, which in turn suggests that this factor might be implicated in RGL activation.

**Ascl1 Is Absolutely Required for the Exit of RGLs from Quiescence**
To directly address the role of Ascl1 in the activation of DG RGLs, we generated triple-transgenic mice that were homozygous for a conditional mutant allele of Ascl1 (Pacary et al., 2011) and also carried the Glast-CreERT2 allele to delete Ascl1 in RGLs in a tamoxifen-dependent manner (Mori et al., 2006) and the Rosa26-floxed stop-YFP reporter transgene to identify cells having undergone Cre-mediated recombination by their expression of YFP (Srinivas et al., 2001). Administration of tamoxifen for 5 days to postnatal day 60 (P60) triple-transgenic mice and control mice (carrying Glast-CreERT2 and Rosa26-floxed stop-YFP, but WT for Ascl1) resulted in widespread induction of YFP in radial GFAP+, Nestin+ RGLs (Figures 3A, 3B, and data not shown). However, examination by immunolabeling revealed that a fraction of SGZ cells that expressed YFP and had therefore recombined the Rosa26-floxed stop-YFP locus also expressed Ascl1 and had therefore not recombined the Ascl1flox locus, indicating that recombination at the two loci was partially uncoupled (Vooijs et al., 2001). We also examined triple-transgenic mice carrying a different conditional mutant allele of Ascl1, in which a PGK promoter-neo cassette remained inserted on the 3' side of the Ascl1 locus (Ascl1neoflox mice; Figures 3C and S3A). Interestingly, even without tamoxifen-induced recombination, Ascl1 RNA and protein expressions were significantly reduced in the DG of these mice compared with WT mice (Figures 3D and S3B), suggesting that Ascl1neoflox is a hypomorphic allele (Nagy et al., 1998). Analysis of Ascl1neoflox mice at P10 did not reveal any overt morphological defect of the DG, and the rate of RGL proliferation was similar to that found in WT mice, indicating that the hypomorphic allele of Ascl1 does not result in a developmental defect in the DG (Figure S3). Tamoxifen administration to these mice at P06–P64 resulted in undetectable Ascl1 expression in the DG at P90 (Ascl1neocKO mice; Figures 3C, 3D, S3A, and S3B). We therefore used Ascl1neocKO mice in the rest of this study to examine the effect of loss of Ascl1 on hippocampal neurogenesis, and we used Ascl1neoflox mice to examine the effect of a reduced expression of Ascl1.

To determine whether Ascl1 deletion has an impact on DG RGLs, we administered tamoxifen at P06–P64 and examined Ascl1neocKO mice 1 month later, at P90. Double labeling for YFP and for MCM2, Ki67, or bromodeoxyuridine (BrdU) after a 2 hr pulse revealed a near-complete absence of proliferating YFP+ cells in the SGZ of Ascl1neocKO mice (Ascl1neocKO mice versus WT mice, 19.65 ± 19.65 versus 4,635 ± 921 YFP+ MCM2+ cells; 0 versus 2,875 ± 815 YFP+ Ki67+ cells; 9.8 ± 4.9 versus 471.6 ± 115.5 YFP+ BrdU+ cells), while proliferating cells were present in Ascl1neoflox mice, albeit in reduced numbers (2,233 ± 632 MCM2+ cells; 1,225 ± 298 Ki67+ cells; 142.8 ± 10.2 BrdU+ cells; Figures 3E–3H). Moreover, triple labeling for YFP, GFAP, and MCM2 to mark activated stem cells or Ki67 to mark proliferating stem cells demonstrated a complete absence of recombined RGLs that were activated or proliferating in Ascl1neocKO mice, while activated and cycling RGLs were present, but less numerous, in Ascl1neoflox mice than in WT mice (Ascl1neocKO versus Ascl1neoflox versus WT mice, 0 versus 126.3 ± 26.3 versus 281.2 ± 70.1 MCM2+ cells; 0 versus 66.0 ± 29.5 versus 161.2 ± 48.5 Ki67+ cells; Figures 3I–3K; see also Figures S3D–S3G for measures of proliferation in the DG of Ascl1neoflox and Ascl1cKO mice). Rarely dividing RGLs, characterized by their capacity to retain BrdU, were labeled in tamoxifen-injected P90 mice by 10 days of BrdU administration followed by 20 days of chase (Figure 3L). BrdU label-retaining cells were present in WT mice and to a lesser extent in Ascl1neoflox mice, but were again completely absent in Ascl1neocKO mice (0 cells in Ascl1neocKO mice; 9.7 ± 4.9 cells in Ascl1neoflox mice; 43.8 ± 6.2 cells in WT mice; Figure 3L). We could thus demonstrate by several independent methods the complete inability of RGLs to exit quiescence and divide in the absence of Ascl1, and therefore establish that Ascl1 is essential for activation of RGLs in the adult hippocampus. Interestingly, Ascl1 deletion had no significant effect on the rate of RGL proliferation in the postnatal DG. When Ascl1 was deleted by tamoxifen administration in Ascl1neocKO mice at P7, RGLs continued to proliferate at P10 at a rate that was not significantly different from that seen in WT mice (Figure S3H). Therefore, the absolute requirement of Ascl1 for RGL activity is specific to the adult DG.

Dividing RGLs in the adult hippocampus generate IPCs that proliferate before producing postmitotic granule neurons (Bonaguidi et al., 2011; Kempermann et al., 2004). Since RGLs require Ascl1 to divide, the production of IPCs and their neuronal progeny might also depend on Ascl1 function. The absence of Ki67+, MCM2+, and BrdU+ cells in the SGZ of Ascl1neocKO mice (Figures 3F–3H) already suggested that IPCs are indeed missing in these mice. We further examined neurogenesis by double labeling the hippocampus of Ascl1neocKO mice for YFP and for Tbr2 to mark IPCs, for DCX to mark neuroblasts, and for NeuN to mark granule neurons (Kempermann et al., 2004; Ming and Song, 2011). No YFP+ cells expressed these markers in the DG of Ascl1neocKO mice at P90, demonstrating that no new IPCs or granule neurons were produced in these mice (Figures S3I–S3K; data not shown). Ascl1 is therefore absolutely required for the generation of IPCs and for neurogenesis in the hippocampus.

**Ascl1 Is Required for RGL Activation in the V-SVZ**
We also examined neurogenesis in the V-SVZ of Ascl1neocKO mice to determine whether the role of Ascl1 in adult RGLs extends to the other main neurogenic region of the adult rodent brain. Analyzing the expression of GFAP, DCX, EGFR, and GFP in Ascl1neocKO and WT mice showed that deletion of
Ascl1 results in a severe decrease in the fraction of RGLs of the V-SVZ that are activated (GFAP+ EGFR+) and proliferate (BrdU label retaining), and in a severe reduction in the production of DCX+ neuroblasts (Figures 4 and S4). Therefore, Ascl1 is essential for NSC activation and proliferation and for neurogenesis in the two main neurogenic regions of the adult brain.

Figure 3. Block of Activation and Proliferation of Hippocampal Stem Cells by Conditional Inactivation of Ascl1

(A and B) Administration of tamoxifen at P60–P64 in Glast-CreERT2; Rosa26-floxed stop-YFP mice followed by the analysis of YFP expression at P64 (A) and the percentage of RGLs that express YFP (B) show the high efficiency of recombination of the YFP reporter allele. n = 3.

(C) Presentation of the five mouse lines analyzed in this study, including (first column) their names, (second column) the Ascl1 allele they carry (WT, flox, or neoflox), (third column) the Rosa26-floxed stop-YFP reporter transgene they all carry (R26 YFP), (fourth column) whether they carry (+) or do not carry (−) the deleter allele Glast-CreERT2 (CreER), and (fifth column) whether the tamoxifen injection they have all received (TAM, +) results in inactivation of Ascl1 and the YFP reporter (Rec, Yes) or not (Rec, No).

(D) Conditional inactivation of the Ascl1 gene by tamoxifen administration at P60–P64 and analysis at P90 of Ascl1 transcripts by quantitative RT-PCR on laser-capture-microdissected SGZ tissue shows that Ascl1 expression is strongly reduced in Ascl1neoflox mice compared to WT mice and is eliminated in Ascl1neocKO mice. The graph shows expression levels normalized to Gapdh and relative to Ascl1 expression in WT. n = 3 in each genotype.

(E–H) Labeling for the proliferation marker Ki67 and for YFP to mark recombined cells (right panels are enlargements of the areas boxed in left panels) (E) and the total numbers per DG of MCM2 + cells (F), Ki67 + cells (G), and BrdU + cells 2 hr after BrdU administration (H) show that cells do not proliferate in the DG of P90 Ascl1neocKO mice, and that the lower level of Ascl1 expression in Ascl1neoflox mice results in reduced proliferation compared to WT mice. p values in WT versus Ascl1neocKO, MCM2 + cells = 0.058; Ki67 + cells = 0.033; BrdU + cells = 0.0162. n = 5 for WT, n = 4 for Ascl1neoflox (F), 4 (G), and 3 (H).

(I–L) Labeling for Ki67 and GFAP (I) and total numbers per DG of MCM2 + and Ki67 + RGLs (J and K) and BrdU label-retaining RGLs following prolonged administration and chase of BrdU (L) demonstrate the absence of activated, proliferating, and BrdU label-retaining RGLs in Ascl1neocKO mice and their reduced numbers in Ascl1neoflox mice. p values in WT versus Ascl1neocKO, MCM2 + cells = 0.016; Ki67 + cells = 0.038; BrdU + cells = 0.0084. n = 5 for WT, n = 4 for Ascl1neoflox (J), 4 (K), 5 (L, WT), 3 (L, Ascl1neoflox), and 3 (L, Ascl1neocKO).

Scale bars, 40 μm in (A) and (E) and 10 μm in (I). Values represent mean values, and error bars represent SDs.
Ascl1 Acts Cell Autonomously to Promote RGL Proliferation

Ascl1 is expressed in proliferating RGLs, and the loss of Ascl1 results in an arrest of RGL proliferation, suggesting that this gene is required in RGLs to promote their divisions. However, Ascl1 is also expressed in some IPCs, raising the alternative possibility that Ascl1 is primarily required for the generation and/or division of IPCs, and that the arrest of RGL divisions is a secondary consequence of the loss of IPCs. In particular, a loss of IPCs might disrupt Notch signaling in the SGZ, resulting in a transient increase in RGL proliferation, followed later by a reduction of proliferation due to RGL exhaustion (Ables et al., 2010; Ehm et al., 2010; Lavado et al., 2010). To address this possibility, RGL proliferation was monitored in Ascl1<sup>neo</sup>-cKO mice just 4 days after the beginning of tamoxifen administration at P60. RGLs had already stopped dividing in P64 Ascl1<sup>neo</sup>-cKO mice, indicating that the effect of Ascl1 deletion on RGL proliferation is rapid and therefore likely direct (Figures 5A and 5B). Moreover, although RGLs in the DG of P64 Ascl1<sup>neo</sup>-cKO mice are mostly YFP<sup>+</sup> and have therefore recombined (Figures 3B and 5A), WT Tbr2<sup>+</sup> IPCs and DCX<sup>+</sup> neuroblasts that were produced by RGLs before tamoxifen administration at P60–P64 are still present (Figure 5C). This suggests that the RGL proliferation defect is not the consequence of a loss of Ascl1-mutant IPCs and neuroblasts, i.e., Ascl1 is required cell autonomously for RGL divisions.

To more rigorously address the cell autonomy or noncell autonomy of Ascl1 function, we performed a mosaic analysis. We activated CreERT2 in a fraction of RGLs with only one injection of tamoxifen at P60, resulting 1 month later in an intermingling of recombined YFP-positive RGLs and nonrecombined YFP-negative RGLs and their progenies (Figure 5D). In WT mice that had received a single tamoxifen injection, a fraction of recombined YFP<sup>+</sup> RGLs was proliferating (Figures 5E and 5F). In contrast, recombined YFP<sup>+</sup> RGLs did not proliferate in mosaic Ascl1<sup>neo</sup>-cKO and Ascl1cKO mice, irrespective of the recombination efficiency (Figures 5E, 5F, and S5). Therefore, the proliferation defect of Ascl1-mutant RGLs cannot be rescued by the presence of nearby WT cells, demonstrating that Ascl1 is required cell autonomously in RGLs for their divisions.

Ascl1-Deficient RGLs Do Not Respond to Mitogenic Stimuli

Next, we examined the phenotype of the cells remaining in the SGZ after Ascl1 deletion. Antibody labeling of the DG of P90 Ascl1<sup>neo</sup>-cKO mice revealed that these cells retain the typical radial morphology of RGLs and maintain expression of the RGL markers GFAP, Nestin, and Sox2 and do not express the astrocytic marker S100β or the oligodendrocyte progenitor marker Olig2 (Figures 6A, 6B, and S6A–S6C). RT-PCR analysis showed that expression of p16<sup>ink4a</sup>/Cdkn2a was not elevated in the DG of Ascl1<sup>neo</sup>-cKO mice, suggesting that RGLs in these mice do not become senescent (Molofsky et al., 2006). Moreover, RGLs in Ascl1<sup>neo</sup>-cKO maintained normal levels of the cyclin-dependent kinase inhibitor p57<sup>Kip2</sup>, which is required for quiescence of hippocampal RGLs (Furutachi et al., 2013). These data, together with the lack of MCM2 expression in Ascl1-deficient RGLs (Figure 3J), show that Ascl1 is not required for the maintenance of RGLs, but specifically for their activation, and that loss of Ascl1 keeps RGLs in an inactive and undifferentiated state.

Although Ascl1-deficient RGLs are unable to proliferate when in a steady state, they might still be able to respond to a potent neurogenic stimulus such as KA. We therefore injected tamoxifen in Ascl1<sup>neo</sup>-cKO mice at P60 to delete Ascl1, then injected KA at P86 and analyzed the DG at P90. KA failed to activate RGLs in Ascl1<sup>neo</sup>-cKO mice, while it strongly activated them in WT mice (Figures 6E and 6F). Therefore, loss of Ascl1 in RGLs results in an inactive state that cannot be reversed by KA stimulation. We also confirmed the inactivated state of
**Ascl1 Activates Adult Hippocampal Stem Cells**

Ascl1-deficient RGLs by performing an in vitro neurosphere assay in the presence of mitogens (Ehm et al., 2010). The DG was dissected from 7- to 8-week-old mice and dissociated, and single-cell suspensions were cultured on clonal density in the presence of FGF2 and EGF. Cultures of WT DG cells produced large numbers of primary neurospheres that generated secondary neurospheres when passaged. In contrast, Ascl1^{neo} cKO DG cell cultures produced very few neurospheres, and antibody labeling showed that these neurospheres maintained expression of Ascl1 and therefore originated from cells that had escaped recombination (Figures 6G, 6H, and S6E). Therefore, Ascl1-deficient RGLs are quiescent and unable to respond to mitogens and divide in vitro.

We next examined whether the inactive state of Ascl1-deficient RGLs was stable over a longer period. Analysis of Ascl1^{neo} cKO mice 5 months after Ascl1 deletion, at P210, showed that RGLs retained a radial morphology and GFAP expression and remained Ki67 negative (Figure 6I). Interestingly, while the total number of RGLs in the DG of WT mice decreased considerably and remained Ki67 negative (Figure 6J), the maintenance of RGL numbers in older Ascl1^{neo} cKO mice supports the finding that RGLs do not divide in these mice.

**Ascl1 Directly Regulates Cell-Cycle Genes in Hippocampal Stem Cells**

To identify target genes that mediate the proliferative role of Ascl1 in hippocampal stem cells, we examined the genome-wide binding of Ascl1 in adult hippocampus-derived NSCs (Knobloch et al., 2013) by chromatin immunoprecipitation-sequencing (ChiP-seq) with an anti-serum against Ascl1 (Figures 7 and S7). Ascl1 was bound to 7,826 high-confidence sites in the genome of AH-NSCs (Figure 7A), a majority of which mapped to enhancers previously identified in cultured NSCs (Figure 7B; Martynoga et al., 2013). Moreover, a large fraction of enhancers active in proliferating NSCs featured an Ascl1-binding event in the AH-NSC genome (Table S1) and enhanced V-SVZ NSCs include, in addition to known targets such as the Notch ligands Dll1 and Dll3 (Castro et al., 2011), several cell-cycle regulators such as E2f1, Ccnd2, Cdc6, and Skp2. To establish whether these genes are regulated by Ascl1 in DG RGLs, we used fluorescence-activated cell
Ascl1 Activates Adult Hippocampal Stem Cells

Figure 6. Unresponsiveness of Ascl1-Deficient Hippocampal Stem Cells to Extrinsic Stimuli

(A and B) Ascl1-deficient RGLs in P90 Ascl1neo cKO mice retain a neural stem cell phenotype characterized by expression of the neural stem/progenitor cell markers Nestin, Sox2, and GFAP and a radial morphology. We note that Nestin remains expressed in Ascl1-mutant quiescent hippocampal RGLs, albeit at a lower level than in WT RGLs (not shown), whereas it is not expressed in quiescent RGLs in the V-SVZ (Codega et al., 2014). n = 3 in each genotype.

(C) Quantitative RT-PCR analysis of the cyclin-dependent kinase inhibitor p16INK4a/Cdkn2a in the laser-capture-microdissected SGZ shows that expression of this marker of RGL senescence is barely detectable and not increased in the Ascl1neo cKO SGZ compared with WT mice. Expression levels normalized to Gapdh. n = 3 in each genotype.

(D) Expression of the cyclin-dependent kinase inhibitor p57Kip2, a marker of quiescent RGLs, is similar in the DG of WT and Ascl1neo cKO mice.

(E and F) KA administration stimulates cell proliferation in the SGZ of WT mice, but not in the SGZ of Ascl1neo cKO mice. Total numbers of MCM2+ cells in the SGZ in each condition (F). p value, MCM2+ cells in WT versus Ascl1neo cKO = 0.0183. n = 3 in each condition.

(G and H) Clonal cultures of dissociated DG cells produced a large number of primary neurospheres from WT DG and only few neurospheres from Ascl1neo cKO DG. Total numbers of spheres obtained from clonal cultures of one dissociated DG. p value = 0.0060. n = 3 in each genotype. Further analysis showed that the few neurospheres from Ascl1neo cKO DG have escaped recombination and express Ascl1 (see Figure S6E).

(I) At 5 months after Ascl1 inactivation, SGZ cells in Ascl1neo cKO mice retain morphological and molecular features of RGLs and remain nonproliferative. The boxed areas are enlarged on the right and show a proliferating RGL in WT and a nonproliferating RGL in Ascl1neo cKO. Scale bars, 40 μm in (A), (D), (E), (G), and (I) and 10 μm in enlargement of (I). Values represent mean values, and error bars represent SDs.
Ascl1 activates adult hippocampal stem cells

Neuron

Figure 7. Direct Targets of Ascl1 in Adult Hippocampal Stem Cells

(A–C) Heat map representation of Ascl1-binding signals in adult hippocampus-derived neural stem cells (AH-NSCs) alongside p300-binding signals in proliferating NSCs at the same genomic locations to mark enhancers (from Martynoga et al. [2013]) (A), and the distribution of Ascl1-binding sites in different classes of NSC enhancers (from Martynoga et al. [2013]) (B) shows that a large fraction of Ascl1-binding sites are located in enhancers present in proliferating NSCs, while reciprocally a large fraction of enhancers present in proliferating NSCs are bound by Ascl1 (C), quies-specific, enhancers specific for quiescent NSCs; pan-NSC, enhancers present in both quiescent and activated NSCs; active-specific, enhancers specific for activated NSCs (from Martynoga et al. [2013]).

(D) ChiP-seq signals in AH-NSCs for Ascl1 and the enhancer marks p300 and H3K27ac (from Martynoga et al. [2013]) at five genes coding for components of the cell-cycle machinery. Significant binding peaks are indicated by green rectangles. Ascl1 binds enhancers in the four genes in AH-NSCs.

(E) Quantitative RT-PCR analysis of fluorescent-activated cell-sorted YFP+ cells from the DG of Ascl1 neo cKO and WT mice 4 days after tamoxifen treatment, and we analyzed gene expression by qRT-PCR. We found that the cyclin Ccnd2 and the ribonucleotide reductase subunit Rrm2 were significantly downregulated when Ascl1 was deleted in RGLs, while E2f1 and Cdc6 expression were not detectable in either WT or mutant cells, and expression of Skp2 and non-cell-cycle genes such as Fbl were not significantly reduced (Figure 7E). Together, these data demonstrate that Ascl1 controls the proliferation of hippocampal RGLs by directly activating the expression of Ccnd2, Rrm2, and possibly additional cell-cycle genes.

DISCUSSION

Stem cells in adult tissues respond to environmental signals by adjusting the production of mature cells to the needs of the tissue. Deciphering the pathways that link physiological stimuli to NSC activity requires characterization of the intrinsic machinery that controls stem cell activation. We show in this study that signals regulating hippocampal stem cell activity control the expression of Ascl1, and that this factor has an essential role in stem cell activation.

Neurogenic and Antineurogenic Signals Converge on Ascl1 Expression

Ascl1 expression in the adult brain has often been described as being restricted to IPCs in the SVZ and DG (Lugert et al., 2012; Parras et al., 2004). We found, however, that Ascl1 is already expressed in proliferating RGLs in the hippocampus, in agreement with earlier studies (Breunig et al., 2007; Kim et al., 2011). Ascl1 expression is then presumably maintained by the IPCs that are produced when RGLs divide. Most Ascl1+ RGLs are activated, but only about a third of activated RGLs express Ascl1 detectably, which might be due to an oscillation of Ascl1 expression in activated RGLs as in embryonic neural progenitor cells (Imayoshi et al., 2013).

The results of our experiments with KA-injected mice suggest that neurogenic stimuli such as neuronal activity promote stem cell activation in the hippocampus by inducing Ascl1 expression in quiescent RGLs. The essential role of Ascl1 in the activation of adult NSCs suggests that other neurogenic signals controlling this step, including Wnt signals in the hippocampus (Jang et al., 2013; Qu et al., 2010, 2013; Seib et al., 2013) and VEGF in the V-SVZ (Calvo et al., 2011), might also act by inducing Ascl1 expression. Our results with RBPJk conditional mutant mice also suggest that antineurogenic stimuli, including the Notch pathway, suppress stem cell activity by repressing Ascl1 expression in RGLs. The mechanism by which the Notch-RBPJk pathway represses Ascl1 expression can be inferred from studies in the embryonic brain and in cancer cells, which have shown that the Notch-induced Hes factors directly repress the Ascl1 gene (Kageyama et al., 2005). Moreover, antineurogenic signals may also target Ascl1 protein activity. The maintenance of hippocampal stem cell quiescence by BMPs (Mira et al., 2010) may involve an inactivation of Ascl1 protein by BMP effectors Id proteins, which are known to block Ascl1 activity by preventing its dimerization with E proteins (Nakashima et al., 2001). FoxO3, which acts downstream of the insulin/IGF-1 receptor pathway, repress the expression of Ascl1, which supports Ascl1 expression in RGLs.
Ascl1 signaling pathway to maintain hippocampal stem cell quiescence, shares many targets with Ascl1 and may also function by antagonizing Ascl1 function (Webb et al., 2013). Inactivation of Ascl1 protein in RGLs that have begun to transcribe the Ascl1 gene may be important to slow down the transition to an active state or to accelerate the return of active RGLs to quiescence.

Mice carrying the hypomorphic allele Ascl1<sup>neo</sup> express Ascl1 in the DG at a reduced level and retain only a small fraction of the proliferating RGLs found in WT mice (Figure 3). The finding that a change in Ascl1 expression level translates into a change in the fraction of RGLs that proliferate suggests that extrinsic signals may fine-tune the rate of hippocampal neurogenesis by modulating the expression level of Ascl1, as shown for PDK1/Akt signaling, which regulates Ascl1 protein stability in the embryonic brain (Oishi et al., 2009).

**Ascl1 Has a Crucial Role in Hippocampal Stem Cell Activation**

A cell that exits quiescence and transits from the G<sub>0</sub> to the early G<sub>1</sub> phase of the cell cycle assembles a prereplication complex that contains minichromosome maintenance proteins, including MCM2. MCM2 expression therefore marks not only cycling cells, but also activated cells that have exited the quiescent state but not yet re-entered the cell cycle (Niu et al., 2011; Stoeber et al., 2001). The glutamate receptor agonist KA induces the expression of Ascl1 before that of MCM2, indicating that Ascl1 induction is one of the first steps in the pathway through which neuronal activity promotes the activation of quiescent RGLs. Since MCM2 is not expressed in Ascl1-deficient RGLs even after KA stimulation, Ascl1 is also absolutely required for the quiescence exit of hippocampal RGLs.

Because Ascl1-deficient RGLs do not exit the quiescent state, it is not possible to ascertain whether Ascl1 also regulates the cell-cycle progression of RGLs. However, Ascl1 promotes the proliferation of progenitor cells in the embryonic brain (which do not enter quiescence), and it directly regulates the expression of cell-cycle regulatory genes in AH-NSCs, including Ccnd2 and Rrm2, suggesting that it promotes the divisions of hippocampal RGLs in addition to their activation. Ascl1 has previously been shown to promote cell proliferation in cancer cells and in the injured zebrafish retina by regulating Wnt-signaling genes (Osada et al., 2008; Ramachandran et al., 2011; Rheinbay et al., 2013). In glioblastoma cancer stem cells (GBM CSCs), Ascl1 has been shown to bind a site near the Wnt antagonist gene Dkk1, whose regulation mediates Ascl1 activity in these cells (Rheinbay et al., 2013). In AH-NSCs, however, Ascl1 does not bind this site and many other sites bound in GBM CSCs, and reciprocally, many Ascl1-bound loci were found in AH-NSCs, and not in GBM CSCs, including sites near the cell-cycle regulators E2f1, Ccna1, Ccnd2, and Skp2 (data not shown), suggesting that Ascl1 controls the proliferation of hippocampal RGLs by regulating different genes from those regulated in cancer cells and the injured retina.

Transcriptomic and genetic studies suggest that NSCs undergo profound changes in their oxygen and lipid metabolisms and cell adhesion properties when they exit quiescence (Knobloch et al., 2013; Martynoga et al., 2013; Renault et al., 2009). Further characterization of Ascl1 target genes in RGLs should elucidate whether Ascl1 directly controls these physiological changes in addition to promoting cell-cycle re-entry and cell-cycle progression. Ascl1 inactivation also blocks RGL activity in the V-SVZ. Whether Ascl1 acts through the same downstream mechanisms in the two adult neurogenic regions remains to be addressed.

In contrast with the complete lack of RGL activity in the adult DG in the absence of Ascl1, RGL proliferation was not significantly affected by Ascl1 deletion in the early postnatal DG, supporting our earlier finding that Ascl1 is not required for DG morphogenesis during embryonic development (Galichet et al., 2008). Interestingly, cell proliferation in the DG becomes also increasingly dependent on the Ascl1 target Ccnd2 between early postnatal and adult stages (Ansorg et al., 2012). Therefore, a switch in the genetic control of DG RGL proliferation occurs during the first few weeks of life, with the activation of a mitogenic pathway involving Ascl1 and Ccnd2.

Ascl1-deficient RGLs remain in Ascl1<sup>neo</sup>−SKO mice for at least 5 months without dividing, differentiating, or dying. The permanent cell-cycle arrest can be readily explained by the cell-autonomous role of Ascl1 in RGL activation. The lack of astrocyte differentiation may also be partially explained by the absence of proliferation, as astrogensis is normally coupled to RGL divisions (Encinas et al., 2011). However, astrocytes can also be generated by direct differentiation of RGLs without cell division (Bonaguidi et al., 2011). In the embryonic brain, activation of Notch signaling by ligands presented by IPCs and young neurons switches neurogenic progenitors to an astrocytic fate (Namihira et al., 2009). In the hippocampal SGZ, Ascl1 deficiency greatly reduces the expression of the Notch effectors Hes1 and Hes5 (Figure S6D), likely due to the elimination of Notch-ligand-presenting IPCs and young neurons. The resulting decrease in Notch activity may thus block the astrocytic differentiation of Ascl1-deficient RGLs.

Numerous transcription factors have been shown to stimulate the self-renewal of stem cells in tissues such as the blood and the skin (Akala and Clarke, 2006; Goldstein and Horsley, 2012). These factors often act by regulating multiple aspects of the biology of the stem cells; for example, by suppressing their differentiation, senescence, or apoptosis (Lieu and Reddy, 2009; Souroullas et al., 2009). Only a few factors, such as Gata3 in hematopoietic stem cells (Ku et al., 2012) and Runx1 in hair follicle stem cells (Osorio et al., 2008), have been proposed to primarily regulate adult stem cell divisions. In the brain, the orphan nuclear receptor Tlx promotes hippocampal NSC proliferation through induction of Wnt7a and repression of p21/WAF1 (Niu et al., 2011; Qu et al., 2010). However, how Tlx is regulated is currently not known. The function of Ascl1 of controlling adult stem cell activity in response to environmental signals is therefore so far unique in the adult brain. It is akin to that of MyoD in muscle satellite stem cells, which is expressed shortly after quiescent satellite cells have been activated, and which in turn induces the expression of the component of the prereplication complex Cdc6 (Zhang et al., 2010). Identifying the molecular pathways that control Ascl1 expression at transcriptional and posttranscriptional levels will be important in order to learn how to manipulate hippocampal neurogenesis for therapeutic purposes.
EXPERIMENTAL PROCEDURES

Animals
Mice were housed, bred, and treated according to the guidelines approved by the Home Office under the Animal (Scientific Procedures) Act 1986. All experimental procedures involving mice have been approved by the Animal Welfare and Ethical Review Panel of the National Institute for Medical Research. RBPJκneo/neo mice were generated as previously described (Han et al., 2003) and bred to Glast-CreERT2 BAC transgenic mice (Slezak et al., 2007). Ascl1floxed/neo mice, in which exon 1 of the Ascl1 gene is flanked by loxP sites (Pacary et al., 2011), were bred with Glast-CreERT2 knockin mice (Mori et al., 2006) and with Rosa26-floxed stop-YFP reporter mice (Srinivas et al., 2001) to generate Glast-CreERT2; Ascl1floxed; R26 YFP mice, which are heterozygous for Glast-CreERT2 and homozygous for Ascl1floxed and R26 YFP. Both Glast-CreERT2 and Glast-CreERT2 lines target both radial and horizontal astrocytes in the DG. In order to remove the PGK promoter-neo cassette from the Ascl1 locus, Ascl1floxed mice were crossed with acti-Fip mice (The Jackson Laboratory).

Tamoxifen, BrdU, and KA Administration
For activation of the CreERT2 recombinase, P60 animals were administered intraperitoneally (i.p.) 4-hydroxytamoxifen (TAM) for 5 consecutive days. For mosaic experiments, P60 animals received a single TAM injection at the same concentration. All animals including WT and Ascl1floxed mice received TAM injections. To examine proliferating progenitors, mice received a single i.p. injection of BrdU 2 hr prior to tissue collection. To examine slowly dividing RGLs, mice received 5 daily BrdU injections followed by 5 consecutive days of BrdU-containing drinking water. Mice were sacrificed 20 days later. Male mice received KA as a single i.p. injection and were monitored for 90 min after KA injection. Animals that did not display rearing and falling were sacrificed 1, 2, or 4 days later and processed as described below.

Tissue Preparation and Immunofluorescence
Animals were transcardially perfused with saline followed by 4% paraformaldehyde (PFA). Brains were postfixed with 4% PFA for 2 hr at 4°C and sectioned coronally at 40 μm with a vibratome. The immunofluorescence procedure and the primary and secondary antibodies are described in Supplemental Information.

Microscopic Analysis and Quantification
Labeled cells were counted in every ninth 40 μm section through the entire rostrocaudal length of the DG (–0.82 mm to –4.24 mm from bregma). Counted cells were divided by the number of z planes counted to obtain the number of cells per 1 μm, and then multiplied by the total length of the DG. To count RGLs (GFAP+ radial cells), cells were deemed radial if the cell body clearly associated with a DAPI-positive nucleus was located in the SGZ and had a single radial process extending through at least two-thirds of the granule layer. In all figures, the cell numbers counted in WT and Ascl1CreKO mice are numbers of YFP+ marker double-labeled cells, while the numbers counted in Ascl1Crelox mice are for K67+ cells only, since YFP is not expressed in these mice.

Laser-Capture Microdissection, FACS Sorting, RNA Isolation, and Quantitative Real-Time PCR
Coronal sections 14 μm long were cut from fresh-frozen brains in OCT with a cryostat and placed on slides. The SGZ of WT, Ascl1Crelox and Ascl1CreKO mice was excised by a PLAM laser-capture microdissection system (Zeiss) and collected in an adhesive cap. RNA from microdissected tissue was extracted and purified using Arcturus Pico Pure RNA Isolation Kit (Applied Biosystems) and reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Gene expression was detected using TaqMan Gene Expression Assays (Applied Biosystems). The protocol used for fluorescent-activated cell sorting is described in Supplemental Information.

Statistical Analyses
Statistical analyses were conducted using a two-sample t test with equal variance in Prism software. Values represent mean values ± SD.

Neurosphere Assay
Clonal primary and secondary neurosphere cultures were performed from dissociated DG dissected from 7- to 8-week-old mice as described (Walker et al., 2013). The number of neurospheres per well was counted 10 days after plating.

ChIP-seq Data Generation and Processing
For chromatin immunoprecipitation, adult hippocampus-derived NSCs were fixed and processed as described (Castro et al., 2011) and immunoprecipitated using a rabbit anti-Ascl1 antibody (Abcam, ab74065, 4.5 μg per ChIP sample). DNA libraries were prepared and sequences analyzed as described (Martynoga et al., 2013). A total of 13.5 million nonredundant reads were used to call peaks, and only peaks with an FDR-corrected q value ≤ 1 x 10−5 were used for the analysis. p300 and H3K27ac data and active enhancer definitions in NSCs were from Martynoga et al. (2013).

ACCESSION NUMBERS
The European Nucleotide Archive accession number for the Ascl1 ChIP-Seq data reported in this paper is PRJEB5023.

SUPPLEMENTAL INFORMATION
Supplemental information includes seven figures, two tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2014.08.004.

ACKNOWLEDGMENTS
We gratefully acknowledge Lan Chen for helping with AH-NSC cultures, Marie Caulfield and Rekha Subramaniam for helping manage the mouse colony, Graham Preece and Wayne Turnbull for helping with flow cytometry, Magdalena Götz for providing Glst-CreERT2 mice, Sebastian Jessberger for providing AH-NSCs, James Brock and Hayley Wood for helping with figure designs, Stephen Martin for advice on statistics, Yukiko Gotoh for advice on p57 immunolabeling, and Bertie Göttsens, Alex Gould, Vivian Li, Robin Lovell-Badge, and the members of the F.G. lab for discussions and comments on the manuscript. J.A. was supported by a Medical Research Council (MRC) Studentship and N.U. by an MRC Career Development Fellowship. This work was supported by a project grant from the Wellcome Trust (082347/Z/07/Z) and a Grant-in-Aid from the MRC (U117570528) to F.G. and a research grant from NIH (1R01NS096980) to M.N.

Accepted: August 1, 2014
Published: September 3, 2014

REFERENCES
Ables, J.L., Decarolis, N.A., Johnson, M.A., Rivera, P.D., Gao, Z., Cooper, D.C., Radtke, F., Hsieh, J., and Eisch, A.J. (2010). Notch1 is required for maintenance of the reservoir of adult hippocampal stem cells. J. Neurosci. 30, 10484–10492.
Akala, O.O., and Clarke, M.F. (2006). Hematopoietic stem cell self-renewal. Curr. Opin. Genet. Dev. 16, 496–501.
Ansorg, A., Witte, O.W., and Urbach, A. (2012). Age-dependent kinetics of dentate gyrus neurogenesis in the absence of cyclin D2. BMC Neurosci. 13, 46.
Benninger, B., Costa, M.R., Koch, U., Schroeder, T., Sutor, B., Grothe, B., and Götz, M. (2007). Functional properties of neurons derived from in vitro reprogrammed postnatal astroglia. J. Neurosci. 27, 8654–8664.
Bertrand, N., Castro, D.S., and Guillemot, F. (2002). Proneural genes and the specification of neural cell types. Nat. Rev. Neurosci. 3, 517–530.
Blank, U., Karlsson, G., and Karlsson, S. (2008). Signaling pathways governing stem-cell fate. Blood 111, 492–503.
Ascl1 Activates Adult Hippocampal Stem Cells

Kim, E.J., Ablen, J.L., Dickel, K.L., Eisch, A.J., and Johnson, J.E. (2011). Ascl1 (Mash1) defines cells with long-term neurogenic potential in subgranular and subventricular zones in adult mouse brain. PLoS ONE 6, e18472.

Klepин, F., Beis, D., Mosienko, V., Kempermann, G., Bader, M., and Alenina, N. (2013). Serotonin is required for exercise-induced adult hippocampal neurogenesis. J. Neurosci. 33, 8270–8275.

Knobloch, M., Braun, S.M., Zurkirchen, L., von Schoutilz, C., Zamboni, N., Araujo-Brazo, M.J., Kovacs, W.J., Karayal, O., Suter, U., Machado, R.A., et al. (2013). Metabolic control of adult neural stem cell activity by Fasn-dependent lipogenesis. Nature 493, 226–230.

Kronenberg, G., Reuter, K., Steiner, B., Brandt, M.D., Jessberger, S., Yamaguchi, M., and Kempermann, G. (2003). Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. J. Comp. Neurol. 467, 455–463.

Ku, C.J., Hosoaya, T., Maillard, I., and Engel, J.D. (2012). GATA-3 regulates hematopoietic stem cell maintenance and cell-cycle entry. Blood 119, 2242–2251.

Kuang, S., Gillespie, M.A., and Rudnicki, M.A. (2008). Niche regulation of muscle satellite cell self-renewal and differentiation. Cell Stem Cell 2, 22–31.

Lavado, A., Lagutin, O.V., Chow, L.M., Baker, S.J., and Oliver, G. (2010). Prox1 is required for granule cell maturation and intermediate progenitor maintenance during brain neurogenesis. PLoS Biol. 8, e1000480.

Lee, S.W., Clemenson, G.D., and Gage, F.H. (2011). New neurons in an aged brain. Behav. Brain Res. 227, 497–507.

Lie, D.C., Colaromino, S.A., Song, H.J., Désiré, L., Mira, H., Consiglio, A., Lein, E.S., Jessberger, S., Lansford, H., Dairie, A.R., and Gage, F.H. (2005). Wnt signalling regulates adult hippocampal neurogenesis. Nature 437, 1370–1375.

Lien, W.H., Guo, X., Polak, L., Lawton, L.N., Young, R.A., Zheng, D., and Fuchs, E. (2011). Genome-wide maps of histone modifications unwind in vivo chromatin states of the hair follicle lineage. Cell Stem Cell 9, 219–232.

Lieu, Y.K., and Reddy, E.P. (2009). Conditional c-myb knockout in adult hematopoietic stem cells leads to loss of self-renewal due to impaired proliferation and accelerated differentiation. Proc. Natl. Acad. Sci. USA 106, 21689–21694.

Lugert, S., Basak, O., Knuckles, P., Haussler, U., Fabel, K., Götz, M., Haas, C.A., Kempermann, G., Taylor, V., and Giachino, C. (2010). Quiescent and active hippocampal neural stem cells with distinct morphologies respond selectively to physiological and pathological stimuli and aging. Cell Stem Cell 6, 445–456.

Lugert, S., Vogt, M., Tchorz, J.S., Müller, M., Giachino, C., and Taylor, V. (2012). Homeostatic neurogenesis in the adult hippocampus does not involve amplification of Ascl1(high) intermediate progenitors. Nat. Commun. 3, 670.

Machida, Y.J., Hamlin, J.L., and Dutta, A. (2005). Right place, right time, and only once: replication initiation in metazoans. Cell 123, 13–24.

Martynoga, B., Mateo, J.L., Zhou, B., Andersen, J., Achimastou, A., Urbán, N., van den Berg, D., Georgopoulou, D., Hadjur, S., Wittbrodt, J., et al. (2013). Epigenomic enhancer annotation reveals a key role for NFIX in neural stem cell quiescence. Genes Dev. 27, 1769–1786.

Ming, G.L., and Song, H. (2011). Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 70, 687–702.

Mira, H., Andreu, Z., Suh, H., Lie, D.C., Jessberger, S., Consiglio, A., San Emeterio, J., Hortiguera, R., Marqués-Torrejón, M.A., Nakashima, K., et al. (2010). Signaling through BMPRIA regulates quiescence and long-term activity of neural stem cells in the adult hippocampus. Cell Stem Cell 7, 78–89.

Molosky, A.V., Slutskey, S.G., Joseph, N.M., He, S., Pardal, R., Krishnamurthy, J., Sharpless, N.E., and Morrison, S.J. (2006). Increasing p18INK4a expression decreases forebrain progenitors and neurogenesis during ageing. Nature 443, 448–452.

Mori, T., Tanaka, K., Buffo, A., Wurst, W., Kühn, R., and Götz, M. (2006). Inducible gene deletion in astroglia and radial glia—a valuable tool for functional and lineage analysis. Glia 54, 21–34.

Nagy, A., Moens, C., Ivarny, E., Pawling, J., Gertsenstein, M., Hadjantonakis, A.K., Pinty, M., and Rossant, J. (1998). Dissecting the role of N-myc in
development using a single targeting vector to generate a series of alleles. Curr. Biol. 8, 661–664.
Nakashima, K., Takizawa, T., Ochiai, W., Yanagisawa, M., Hisatsune, T., Nakafuku, M., Miyazono, K., Kishimoto, T., Kageyama, R., and Taga, T. (2001). BMP2-mediated alteration in the developmental pathway of fetal mouse brain cells from neurogenesis to astrocytogenesis. Proc. Natl. Acad. Sci. USA 98, 5868–5873.
Namihira, M., Kohyama, J., Semi, K., Sanosaka, T., Deneen, B., Taga, T., and Nakashima, K. (2009). Committed neuronal precursors confer astrocytic potential on residual neural precursor cells. Dev. Cell 16, 245–255.
Niu, W., Zou, Y., Shen, C., and Zhang, C.L. (2011). Activation of postnatal neural stem cells requires nuclear receptor TLX. J. Neurosci. 31, 13816–13828.
Oishi, K., Watatani, K., Itoh, Y., Okano, H., Guillemot, F., Nakajima, K., and Gotoh, Y. (2009). Selective induction of neocortical GABAergic neurons by the PDX1-Akt pathway through activation of Mash1. Proc. Natl. Acad. Sci. USA 106, 13064–13069.
Orford, K.W., and Scadden, D.T. (2008). Deconstructing stem cell self-renewal: genetic insights into cell-cycle regulation. Nat. Rev. Genet. 9, 115–128.
Osada, H., Tomida, S., Yatabe, Y., Tatematsu, Y., Takeuchi, T., Murakami, H., Kondo, Y., Sekido, Y., and Takahashi, T. (2008). Roles of achaete-scute homologue 1 in DKK1 and E-cadherin repression and neuroendocrine differentiation in lung cancer. Cancer Res. 68, 1647–1655.
Osorio, K.M., Lee, S.E., McDermitt, D.J., Waghmare, S.K., Zhang, Y.V., Woo, H.N., and Tumbar, T. (2008). Runx1 modulates developmental, but not injury-driven, hair follicle stem cell activation. Development 135, 1059–1068.
Pacary, E., Heng, J., Azzarelle, R., Riou, P., Castro, D., Lebel-Potier, M., Parras, C., Bell, D.M., Ridley, A.J., Parsons, M., and Guillemot, F. (2011). Proneural transcription factors regulate different steps of cortical neuron migration through Rnd-mediated inhibition of RhoA signaling. Neuron 69, 1069–1084.
Parras, C.M., Galli, R., Britz, O., Soares, S., Galichet, C., Battiste, J., Johnson, J.E., Nakafuku, M., Vesovic, A., and Guillemot, F. (2004). Mash1 specifies neurons and oligodendrocytes in the postnatal brain. EMBO J. 23, 4495–4505.
Pastrana, E., Cheng, L.C., and Doetsch, F. (2009). Simultaneous prospective purification of adult subventricular zone neural stem cells and their progeny. Proc. Natl. Acad. Sci. USA 106, 6387–6392.
Ponti, G., Obernier, K., Quinto, C., Jose, L., Bonfanti, L., and Alvarez-Buylla, A. (2013). Cell cycle and lineage progression of neural progenitors in the ventricular-subventricular zones of adult mice. Proc. Natl. Acad. Sci. USA 110, E1045–E1054.
Qu, Q., Sun, G., Li, W., Yang, S., Ye, P., Zhao, C., Yu, R.T., Gage, F.H., Evans, R.M., and Shi, Y. (2010). Orphan nuclear receptor TLX activates Wnt/beta-catenin signalling to stimulate neural stem cell proliferation and self-renewal. Nat. Cell Biol. 12, 31–40.
Qu, Q., Sun, G., Murali, K., Ye, P., Li, W., Asuelime, G., Cheung, Y.T., and Shi, Y. (2013). Wnt7a regulates multiple steps of neurogenesis. Mol. Cell. Biol. 33, 2551–2559.
Ramachandran, R., Zhao, X.F., and Goldman, D. (2011). Ascl1a/Dkk/beta-catenin signalling pathway is necessary andglycogen synthase kinase-3beta inhibition is sufficient for zebrafish retina regeneration. Proc. Natl. Acad. Sci. USA 108, 15858–15863.
Renault, V.M., Rafalski, V.A., Morgan, A.A., Sain, D.A., Brett, J.O., Webb, A.E., Villeda, S.A., Thekkat, P.U., Guillerey, C., Denko, N.C., et al. (2009). FoxO3 regulates neural stem cell homeostasis. Cell Stem Cell 5, 527–539.
Rheinbay, E., Suva, M.L., Gillespie, S.M., Nakafuku, H., Patel, A.P., Shahid, M., Okus, O., Rabkin, S.D., Martuza, R.L., Rivera, M.N., et al. (2013). An aberrant transcription factor network essential for Wnt signaling and stem cell maintenance in glioblastoma. Cell Rep. 3, 1567–1579.
Seib, D.R., Corsini, N.S., Elwanger, K., Plaa, C., Mateos, A., Pitzer, C., Niehrs, C., Celikel, T., and Martin-Villalba, A. (2013). Loss of Dickkopf-1 restores neurogenesis in old age and counteracts cognitive decline. Cell Stem Cell 12, 204–214.
Simons, B.D., and Clevers, H. (2011). Strategies for homeostatic stem cell self-renewal in adult tissues. Cell 145, 851–862.
Slezak, M., Göritz, C., Niemiec, A., Frisén, J., Chambon, P., Metzger, D., and Pfrieger, F.W. (2007). Transgenic mice for conditional gene manipulation in astroglial cells. Glia 55, 1565–1576.
Sourouillas, G.P., Salmon, J.M., Sabilztky, F., Curtis, D.J., and Goodell, M.A. (2009). Adult hematopoietic stem and progenitor cells require either Ly1 or SCl for survival. Cell Stem Cell 4, 180–186.
Srinivas, S., Watanabe, T., Lin, C.S., William, C.M., Tanabe, Y., Jessell, T.M., and Costantini, F. (2001). Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev. Biol. 1, 4.
Stoeber, K., Tisty, T.D., Happerfield, L., Thomas, G.A., Romanov, S., Bobrow, L., Williams, E.D., and Williams, G.H. (2001). DNA replication licensing and neural cell proliferation. J. Cell Sci. 114, 2027–2041.
Suh, H., Consiglio, A., Ray, J., Sawai, T., D’Amour, K.A., and Gage, F.H. (2007). In vivo fate analysis reveals the multipotent and self-renewal capacities of Sox2+ neural stem cells in the adult hippocampus. Cell Stem Cell 1, 515–528.
Venezia, T.A., Merchant, A.A., Ramos, C.A., Whitehouse, N.L., Young, A.S., Shaw, C.A., and Goodell, M.A. (2004). Molecular signatures of proliferation and quiescence in hematopoietic stem cells. PLoS Biol. 2, e301.
Vooijs, M., Jonkers, J., and Berns, A. (2001). A highly efficient ligand-regulated Cre recombinase mouse line shows that LoxP recombination is position dependent. EMBO Rep. 2, 292–297.
Walker, T.L., Wierick, A., Sykes, A.M., Waldau, B., Corbeil, D., Carmeliet, P., and Kempermann, G. (2013). Prominin-1 allows prospective isolation of neural progenitors from the adult murine hippocampus. J. Neurosci. 33, 3010–3024.
Weiss, A.E., Pollina, E.A., Vierbuchen, T., Urbán, N., Ucar, D., Leeman, D.M., Martyrnoya, B., Sewak, M., Rando, T.A., Guillemot, F., et al. (2013). FOXO3 shares common targets with ASCL1 genome-wide and inhibits ASCL1-dependent neurogenesis. Cell Rep. 2, 477–491.
Yang, N., Ng, Y.H., Zhang, Z., Sano, J., and Wernig, M. (2011). Induced neuronal cells: how to make and define a neuron. Cell Stem Cell 9, 517–527.
Zhang, C.L., Zou, Y., He, W., Gage, F.H., and Evans, R.M. (2008). A role for adult TLX-positive neural stem cells in learning and behaviour. Nature 451, 1004–1007.
Zhang, K., Sha, J., and Harter, M.L. (2010). Activation of Cdc6 by MyoD is associated with the expansion of quiescent myogenic satellite cells. J. Cell Biol. 188, 39–48.