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# Hopx distinguishes hippocampal from lateral ventricle neural stem cells

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ABSTRACT

In the adult dentate gyrus (DG) and in the proliferative zone lining the lateral ventricle (LV-PZ), radial glia-like (RGL) cells are neural stem cells (NSCs) that generate granule neurons. A number of molecular markers including glial fibrillary acidic protein (GFAP), Sox2 and nestin, can identify quiescent NSCs in these two niches. However, to date, there is no marker that distinguishes NSC origin of DG versus LV-PZ. Hopx, an atypical homeodomain only protein, is expressed by adult stem cell populations including those in the intestine and hair follicle. Here, we show that Hopx is specifically expressed in RGL cells in the adult DG, and these cells give rise to granule neurons. Assessed by non-stereological quantitation, Hopx-null NSCs exhibit enhanced neurogenesis evident by an increased number of BrdU-positive cells and doublecortin (DCX)-positive neuroblasts. In contrast, Sox2-positive, quiescent NSCs are reduced in the DG of Hopx-null animals and Notch signaling is reduced, as evidenced by reduced expression of Notch targets Hes1 and Hey2, and a reduction of the number of cells expressing the cleaved, activated form of the Notch1 receptor, the Notch intracellular domain (NICD) in Hopx-null DG. Surprisingly, Hopx is not expressed in RGL cells of the adult LV-PZ, and Hopx-expressing cells do not give rise to interneurons of the olfactory bulb (OB). These findings establish that Hopx expression distinguishes NSCs of the DG from those of the LV-PZ, and suggest that Hopx potentially regulates hippocampal neurogenesis by modulating Notch signaling.



## 1. Introduction

Adult neural stem cells (NSCs) reside within the hippocampal subgranular zone (SGZ) of the dentate gyrus (DG) and in the proliferative zone (PZ) of the lateral ventricles (LV; herein abbreviated as LV-PZ), where they generate new neurons throughout adult life (Braun & Jessberger, 2014). Radial glia-like (RGL) cells, the putative adult NSCs, express a number of markers including glial fibrillary acidic protein (GFAP), brain lipid-binding protein (BLBP), Sox2 and nestin (Ming & Song, 2011). However, these markers do not distinguish between hippocampal-derived versus LV-PZ-derived NSCs.

Both intrinsic and extrinsic factors facilitate and regulate adult neurogenesis (Faigle & Song, 2013). In particular, Notch signaling is crucial for NSC maintenance as demonstrated by numerous pharmacological or genetic experiments (Giachino & Taylor, 2014), although the regulation of Notch signaling during adult neurogenesis is complex.

Hopx is an atypical homeodomain protein in that it is unable to bind DNA, instead functioning in association with other DNA binding factors to repress transcription by recruiting class I histone deacetylases (Hdacs) (Chen et al., 2002; Trivedi et al., 2010; Kook et al., 2006). Hopx has been recently identified as a marker for adult stem cells in several tissues, including the intestine and hair follicle (Takeda et al., 2011; Takeda et al., 2013). We previously reported that Hopx is expressed in radial astrocytes of the DG, suggesting that these Hopx-positive cells might function as NSCs (De Toni et al., 2008).

In this study, we more precisely characterize the expression of Hopx within the adult NSC niches. Lineage tracing experiments with a $Hopx^{ERCre/+}$ mouse and a reporter allele show that Hopx cells in the DG are NSCs that self-renew and can also give rise to granule neurons. Based upon non-stereological analyses, loss of Hopx increases DG neurogenesis, and is accompanied by both a reduction in Notch

*Abbreviations:* Hopx, Homeodomain only protein X; NSC, Neural stem cells; RGL cell, Radial glia-like cell; CC, Corpus callosum; CN, Caudate nucleus; DG, Dentate gyrus; LV, Lateral ventricle; OB, Olfactory bulb; LV-PZ, LV proliferative zone; SGZ, Subgranular zone; GFAP, Glial fibrillary acidic protein; BLBP, Brain lipid-binding protein; BrdU, 5-Bromo-2′-deoxyuridine; TAM, Tamoxifen; IHC, Immunohistochemistry; Tbr2, T-box brain protein 2; ASCL1 or Mash1, Achaete-scute homolog 1; DCX, Doublecortin; GFP, Green fluorescent protein; RFP, Red fluorescent protein; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase.

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signaling in the DG and in the quiescent NSC population. Remarkably, Hopx is not expressed by the LV NSC population, and Hopx-expressing cells do not generate olfactory bulb (OB) interneurons. Since hippocampal neurogenesis is associated with the regulation of memory, mood (Cameron & Glover, 2015), the hippocampal NSC-selective expression of Hopx represents a novel inroad into signaling mechanisms that distinguish translationally relevant subregions of adult neurogenesis.

## 2. Materials and methods

### 2.1. Animals

Hopx$^{ERCre/+}$ (Takeda et al., 2011) and Hopx$^{3FlagGFP/+}$ (Takeda et al., 2013) mice were previously described. R26tdTomato mice (abbreviated R26$^{Tom/+}$ in this manuscript, B6.Cg-Gt (ROSA)26Sortm14(CAG-tdTomato)Hze/J) and C57BL/6-Tg(Nes-cre/ERT2)KEisc/J (abbreviated Nestin-ERCre in this manuscript,) were purchased from Jackson Labs (stock numbers are 007914 and 016261 respectively). All mice were maintained on a mixed genetic background. All animal protocols were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

### 2.2. Tamoxifen and 5-bromo-2′-deoxyuridine (BrdU) administration

Ten mg/ml tamoxifen (Sigma, St. Louis, MO) was dissolved in corn oil and given intraperitoneally (i.p.) to adult mice (100 mg/kg body weight) daily for 5 consecutive days. BrdU (Roche, Indianapolis, IN) solution was prepared at 10 mg/ml in sterile PBS and was injected i.p. into mice (100 mg/kg body weight). For short-term BrdU labeling, 2-month-old mice were injected with BrdU every 3 h for 15 h and euthanized 1 h after the last injection. For BrdU-label retaining experiments, mice were injected once per day for 4 consecutive days (P64-67), then euthanized 30 days after the last injection (Hitoshi et al., 2002). For BrdU incorporation in P78 Hopx null and control mice, BrdU was injected i.p. once a day for 4 consecutive days, then the mice were euthanized on the fifth day.

### 2.3. Histology and immunohistochemistry (IHC)

All brain specimens were fixed in 2% paraformaldehyde overnight, dehydrated through an ethanol series, paraffin embedded, and sectioned (6 μm). Primary antibodies are listed in supplemental Table 1. Primary antibodies were incubated at 4 °C overnight and secondary antibodies (Alexa 488 or Alexa 555, Life technologies, Grand Island, NY) were incubated at room temperature for 1 h. Stained slides were imaged on a Zeiss LSM 710 confocal microscope. Epi-fluorescence was imaged on an Olympus MVX10 stereomicroscope. For the quantitative IHC analyses, cells were counted from three coronal sections (representing 3 distinct dorsal hippocampal anatomical levels: interaural 2.1 mm, interaural 1.5 mm and interaural 0.6 mm) (Franklin & Paxinos, 2008) and were averaged from each animal. Three to six animals per genotype were used in the analyses. The three anatomical levels had highly similar morphologies across brains both within and between genotypes (Jadavji et al., 2012). This work represents non-stereological determinations of brain volume and cell number.

### 2.4. Quantitative real-time PCR (qRT-PCR)

Adult DGs were dissected in cold PBS as previously described (Hagihara et al., 2009) and snap frozen in liquid nitrogen. TRIzol reagent (Life technologies, Grand Island, NY) was used to extract total RNA from DGs and complementary DNA (cDNA) was generated with the Superscript III kit (Life Technologies, Grand Island, NY). SYBR Green quantitative RT-PCR was performed using StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, CA). Primers used for quantitative RT-PCR are listed in Supplemental Table 2.

## 3. Statistical analysis

Data are presented as mean ± SEM. Differences between groups were detected for statistical significance using the unpaired Student's t-test. $P < 0.05$ was considered significant.

## 4. Results

### 4.1. Hopx is expressed in the subgranular zone of the dentate gyrus and co-localizes with quiescent neural stem cell markers

In the adult brain, Hopx is expressed in the cerebellum (Fig. 1A) in both Purkinje cells and Bergmann glial cells (Fig. 1B). In the hippocampus, Hopx is found in mature astrocytes, but not in mature oligodendrocytes or neurons (Fig. 1C–E). We note that Hopx + astrocytes are primarily located in the CA regions, but rare Hopx + astrocytes are present throughout the cerebrum (Supplemental Fig. 1). Interestingly, Hopx is strongly expressed in the SGZ of the DG (Fig. 1F), whereas it is not detectable in the LV-PZ where NSC markers such as Sox2 are expressed (Fig. 1G–H). In the SGZ, Hopx co-localizes with quiescent NSC markers including GFAP, Nestin and Sox2 (Fig. 2A–C). In contrast, Hopx is not co-expressed with either transit-amplifying cell progenitor markers such as T-box brain protein 2 (Tbr2), and achaete-scute homolog 1 (ASCL1 or Mash1), or with the neuroblast marker Doublecortin (DCX) (Fig. 2E–G). These findings suggest that Hopx is expressed in NSCs of the DG but not in NSCs of the LV-PZ. We note that while Hopx-expressing cells also express Sox2 in the SGZ (yellow arrows in Fig. 2C), there are Sox2 + cells that do not express Hopx (red arrows in Fig. 2C). This finding raises the possibility that the Hopx + population represents a subpopulation of SGZ NSCs. Hence, our findings support the growing body of evidence suggesting NSC heterogeneity (Fiorelli et al., 2015).

Most NSCs in the hippocampus remain quiescent during normal physiological conditions, although there is a small active population (Lugert et al., 2010). In the adult hippocampus, most Hopx + cells are not proliferating, as assessed by Ki67 staining and short-term BrdU labeling (BrdU every 3 h × 5, fix 1 h later) (Fig. 2G–H, quantified in J). However, Hopx + cells do meet the criteria for long-term label retaining cells following exposure to BrdU over several consecutive days and assessed a month later (BrdU given daily × 4 days and assayed 30 days later) (Fig. 2I, quantified in J). Taken together, these data indicate that Hopx is expressed primarily in the slowly cycling or quiescent stem cell population of the SGZ that is characterized by Sox2, GFAP and Nestin expression (Braun et al., 2003).

### 4.2. Lineage tracing of Hopx + cells in the adult DG

To determine whether Hopx defines a population of cells with long term potential to generate neurons in the adult, we used Hopx$^{ERCre/+}$; R26$^{Tom/+}$ mice for lineage tracing experiments. First, we confirmed that no cells are labeled in the brain in the absence of tamoxifen (TAM) induction when animals are injected with vehicle alone (Supplemental Fig. 2). Hopx$^{ERCre/+}$; R26$^{Tom/+}$ mice were pulsed with TAM or vehicle alone at 2 months (100 mg/kg BW i.p.) once per day for 5 consecutive days. One hour after the last TAM treatment, the animals were sacrificed and tissue was collected. After this short-term pulse, progeny of Hopx-expressing cells expresses GFAP and Sox2 (Fig. 3A–B, quantified in L), consistent with the expression analysis of Hopx + cells above (Fig. 2A––C). In parallel experiments, we allowed for longer periods of time after TAM exposure to identify the progeny of Hopx-expressing cells. Two weeks post-TAM injection, some tdTomato-expressing cells express DCX (Fig. 3C and L) suggesting that some Hopx + NSCs give rise to committed neuronal progenitors. By six weeks after the initial TAM induction, the Hopx + lineage differentiates into granule neurons (NeuN+, Fig. 3G and L), although the descendants of Hopx-expressing NSCs (GFAP+, Sox2+) persist (Fig. 3D–F and L). Two months after TAM induction, many mature neurons

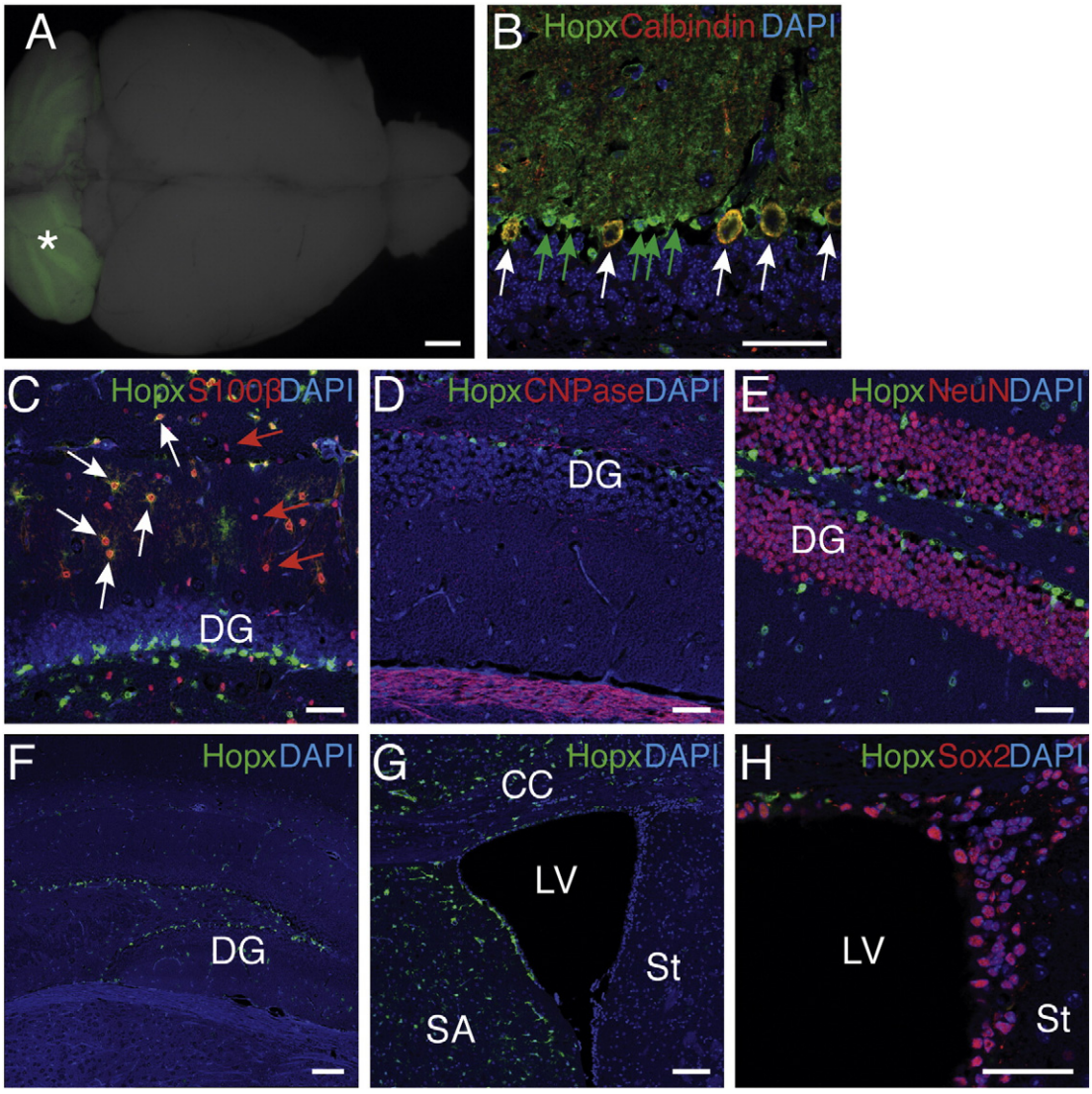


**Fig. 1.** Hopx expression in the adult brain. (A) Epifluorescence image of an adult Hopx$^{3FlagGFP/+}$ brain. Asterisk demarks GFP (Hopx) expression in the cerebellum. (B) Coronal section of adult Hopx$^{3FlagGFP/+}$ cerebellum stained for GFP (Hopx, green) and Calbindin (red), which is expressed by Purkinje cells. White arrows indicate GFP/Calbindin co-positive cells; green arrows indicate Bergmann glial cells. (C-H) Immunostaining of coronal sections through the dorsal hippocampus (C–F) and the lateral ventricle (G–H). (C) GFP (Hopx, green) and the astrocyte marker S100β (red). White arrows indicate GFP/S100β co-positive cells and red arrows indicate cells expressing S100β alone. (D) GFP (Hopx, green) and CNPase (red), a marker of oligodendrocytes. (E) GFP (Hopx, green) and the neuronal marker NeuN (red). (F) Flag (Hopx, green) in the SGZ of the DG. (G) GFP (Hopx, green) in the LV. Hopx is expressed in the septal area (SA). (H) GFP (Hopx, green) and Sox2 (red). Hopx does not co-label with Sox2 in the proliferative zone of the lateral ventricles (LV-PZ). CC = corpus callosum, St. = striatum. Scale bars: A, 1 mm, B–H, 50 μm.

have differentiated from Hopx + NSCs (Fig. 3K, L, Supplemental Fig. 2A), while Sox2+, GFAP+ NSCs derived from Hopx + NSCs are still identifiable (Fig. 3H, I and L).

### 4.3. Lineage tracing of Hopx + cells in the adult olfactory bulb (OB)

Hopx expression is not detectable in the NSCs in the LV-PZ (Fig. 1G–H). In order to determine if Hopx + cells give rise to new neurons in the OB, known to arise from Nestin + NSC in the LV-PZ (Ming & Song, 2011), we performed lineage tracing in the OB comparing the fate of Hopx + and nestin + derivatives. We examined progeny of Hopx + cells two months after the initial 5-day TAM pulse, and found only astrocytes labeled in the OB (Fig. 4A–E). This finding is consistent with the fact that Hopx is expressed by astrocytes but not by neurons in this region (Supplemental Fig. 3) and with the absence of Hopx expression in the Sox2+ cells of the LV-PZ (Fig. 1G–H). In parallel studies, Nestin-ERCre; R26$^{Tom/+}$ mice were induced with TAM using a protocol identical to that used for Hopx$^{ERCre/+}$; R26$^{Tom/+}$ mice. In this case, we observed robust neurogenesis in the OB derived from Nestin + precursors, as previously reported (Lagace et al., 2007; Giachino & Taylor, 2009). The specificity of Hopx + cells behaving as NSCs in the adult hippocampus but not in the LV is further underscored by the observation of astrocyte labeling in the OB as opposed to neuronal labeling in the DG in the same Hopx$^{ERCre/+}$; R26$^{Tom/+}$ animal (Supplemental Fig. 4A). In contrast, neurons in both regions are labeled in a Nestin-ERCre; R26$^{Tom/+}$ brain (Supplemental Fig. 4B). Thus, these data unambiguously demonstrate that Hopx labels NSCs in the adult DG, but not in the LV-PZ.

### 4.4. Hopx is required for normal neurogenesis and NSC maintenance in the hippocampus

In the murine DG neurogenesis continues into adulthood (Spalding et al., 2013; Christie & Cameron, 2006). Our data suggest that Hopx labels quiescent NSCs in the adult hippocampus and that these Hopx + cells give rise to new neurons over time in the adult. Hence, we sought to understand whether Hopx plays a functional role during hippocampal neurogenesis. As previously reported, approximately 50% of Hopx null embryos die in utero with poorly developed hearts (Chen

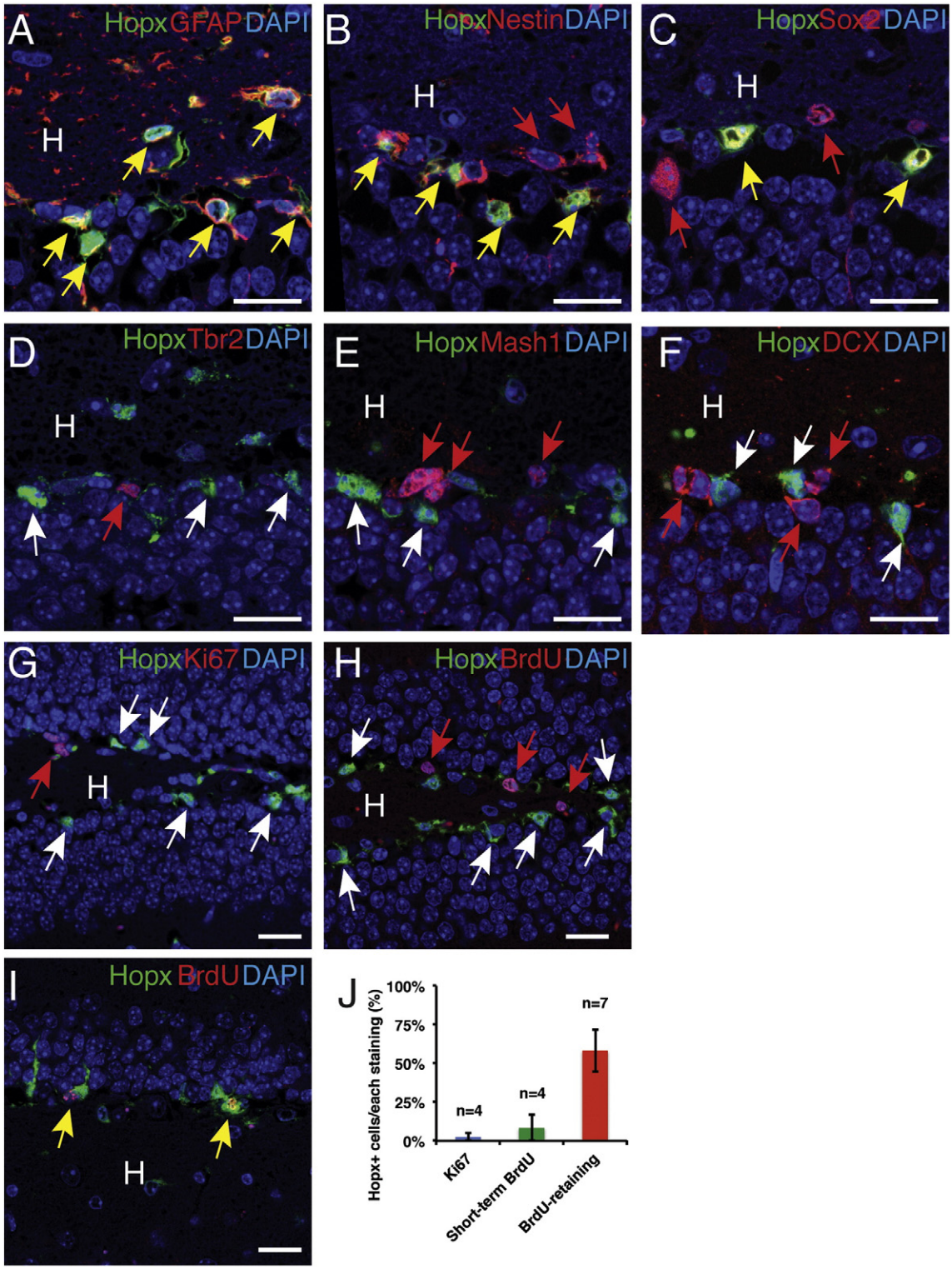


**Fig. 2.** Hopx is expressed by label-retaining NSCs in the adult DG. (A-I) Immunostaining of coronal sections through the SGZ of adult Hopx$^{3FlagGFP/+}$ brains. White arrows indicate cells expressing only Hopx. Red arrows indicate cells positive for the marker indicated in red only. Yellow arrows indicated cells co-expressing both markers. (A) GFP (Hopx, green) and neural stem cell marker GFAP (red). (B) GFP (Hopx, green) and neural stem cell marker Nestin (red). (C) Flag (Hopx, green) and neural stem cell marker Sox2 (red). (D) GFP (Hopx, green) and Tbr2 (red), a marker of transit-amplifying cells. No co-positive cells were identified. (E) GFP (Hopx, green) and achaete-scute Homolog 1 (Mash1, red), a marker of transit-amplifying cells. No co-positive cells were identified. (F) Flag (Hopx, green) and the neuroblast marker DCX (red). No co-positive cells were identified. (G) Hopx (GFP, green) is not co-expressed in actively proliferating Ki67 + cells (red). (H) Hopx (GFP, green) is not expressed by cells labeled with by short-term BrdU incorporation (red). (I) Hopx is expressed in long-term (30 day) BrdU-label retaining cells after loading with BrdU over 4 days. (J) Quantification of the percentage of double positive Hopx/Ki67 cells, short-term BrdU labeled cells, and long-term BrdU-label retaining cells as a percentage of the total number of cells labeled by Ki67 or BrdU under each condition in the SGZ. The average total number of cells labeled by Ki67, short-term BrdU and long-term BrdU per section, per mouse in the SGZ are 5.3, 3.5 and 2.3 respectively. n, number of mice. H = hilus. Scale bars: 25 μm.

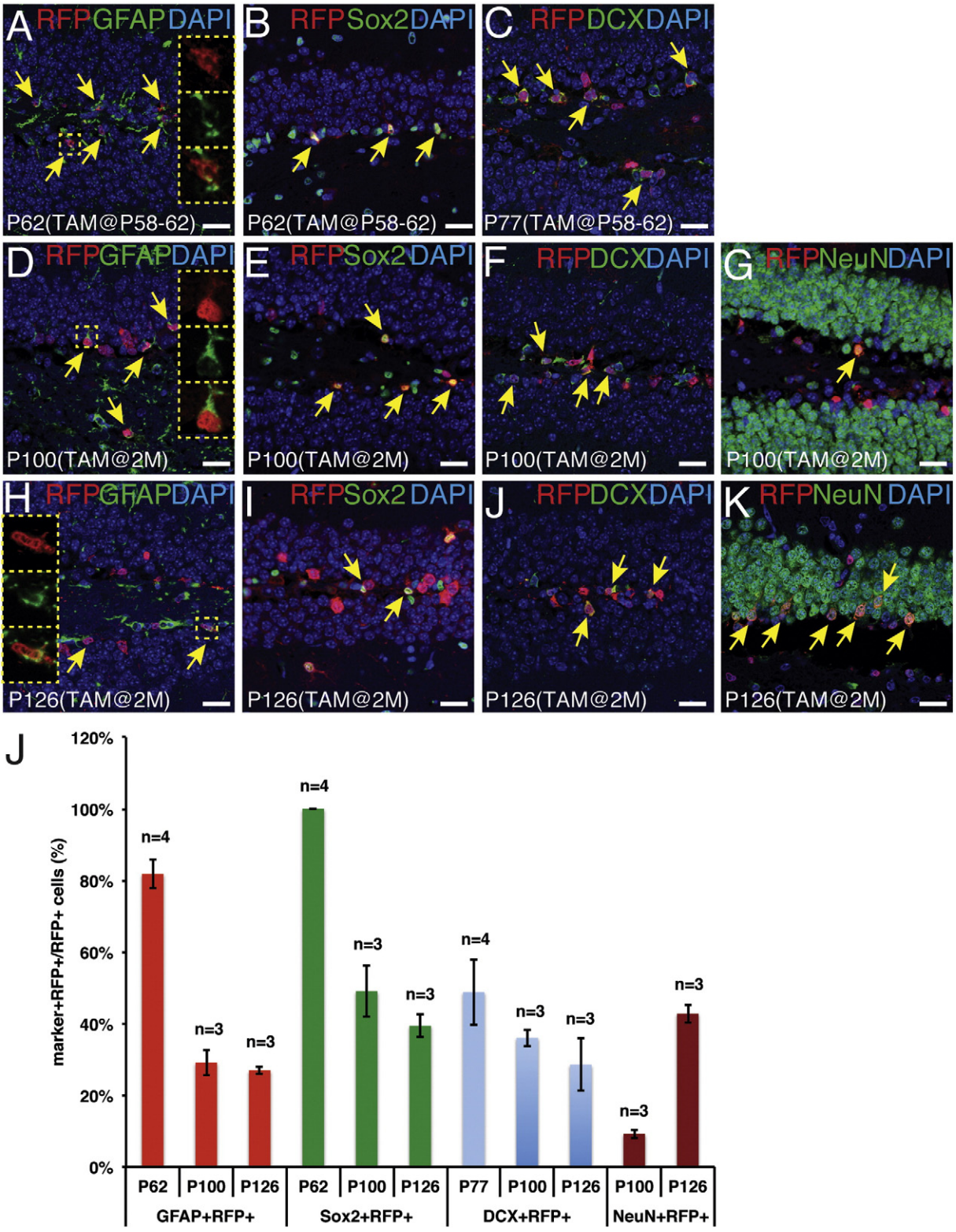


**Fig. 3.** Lineage tracing of Hopx + cells in the SGZ. (A–K) Hopx$^{ERCre/+}$; R26$^{Tom/+}$ mice were given daily TAM i.p. for 5 days (P58-62). (A, B) At P62, Cre-reporter activity (RFP) is present in SGZ cells that also express the neural stem cell markers GFAP (A, green, yellow arrows) and Sox2 (B, green, yellow arrows). (C) At P77, some descendants of Hopx-expressing cells (red, RFP+) give rise to DCX (green)-expressing neuroblasts (yellow arrows). (D–F) At P100, TAM exposure 6 weeks previously labels some GFAP+ and Sox2+ neural stem cells and neuroblasts (yellow arrows). (G) The same labeling protocol demonstrates that some NeuN+ granule neurons (yellow arrows) also derive from Hopx-expressing NSCs. (H-K) Two months (P126) after labeling, the descendants of Hopx-expressing cells (red, RFP+) express NSC markers GFAP and Sox2 (yellow arrows in H and I) the neuroblast marker DCX (yellow arrows in J), and the granule cell marker NeuN (yellow arrows in K). Scale bars in A-K: 25 μm. (L) Lineage analysis in A-K is quantified. n, number of mice analyzed. The average number of RFP+ cells/section/mouse at P62, P77, P100 and P126 are 8.8, 19.3, 18.3, and 23.0 respectively.

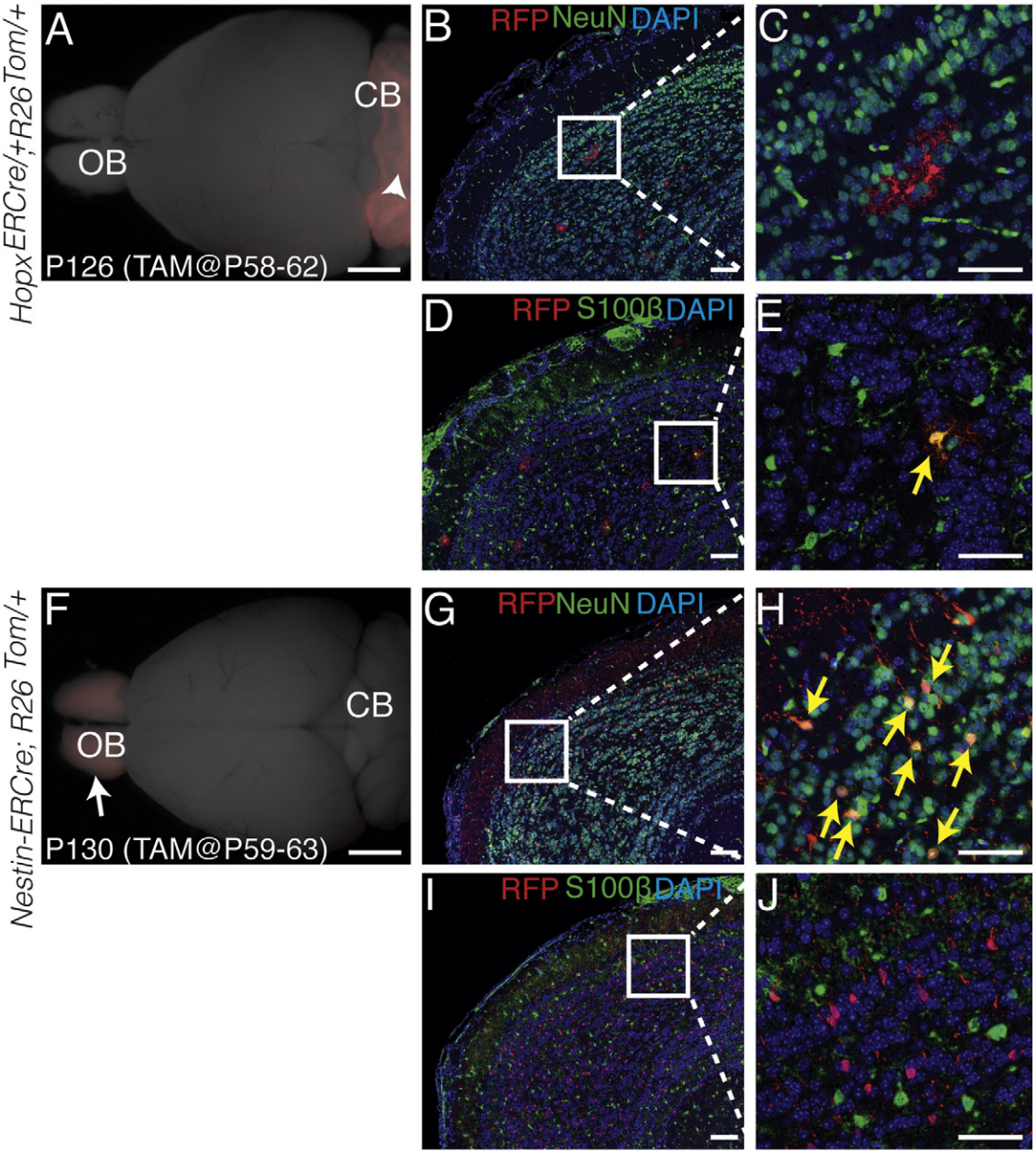


**Fig. 4.** Hopx does not label LV-PZ NSC derivatives in the olfactory bulb (OB). (A–E) $Hopx^{ERCre/+}$; $R26^{Tom/+}$ mice were given daily i.p. injections of TAM for 5 days at P58-62 and analyzed at P126. The experiment was performed in n = 3 mice with similar results. (A) Epi-fluorescence of a P126 $Hopx^{ERCre/+}$; $R26^{Tom/+}$ brain. Arrowhead demarks RFP signal in the cerebellum (CB). (B-E) Sagittal sections of P126 $Hopx^{ERCre/+}$; $R26^{Tom/+}$ forebrain. The descendants of Hopx-expressing cells (red, RFP +) do not give rise to NeuN+ mature neurons (green, B,C) but do label S100β + astrocytes (green, D, E) as indicated by the yellow arrow in E. (F-J) Nestin-ERCre/+; $R26^{Tom/+}$ mice were given daily i.p. injections of TAM for 5 days at P59-63 and analyzed at P130. The experiment was performed in n = 4 mice with similar results. (F) Epi-fluorescence of a P130 Nestin-ERCre; $R26^{Tom/+}$ brain. Arrow points to the RFP signal in the OB. (G-J) Sagittal sections of P126 Nestin-ERCre; $R26^{Tom/+}$ forebrain. Nestin descendants (red) are NeuN+ neurons (green, G, H) as indicated by yellow arrows in H, but not S100β + astrocytes (green, I, J). Scale bars, A, F, 2 mm; B, D, G, I, 100 μm; C, E, H, J, 50 μm.

et al., 2002). Hopx null survivors appear phenotypically normal, are fertile and have a life span comparable to wild type littermates. Hopx heterozygous mice are developmentally normal and fertile, and behaviorally and socially appear indistinguishable from wild type littermates. Neurogenesis of Hopx heterozygous mice appears similar to that of wild type, and we designate both Hopx heterozygous and wild type mice as controls in this study. First, we examined whether adult Hopx null brains are morphologically and histologically different than Hopx heterozygous brains. P78 Hopx null brains appear similar to Hopx heterozygous brains as assessed by overall size and morphology, and histologically (Supplemental Fig. 5). To assess DG proliferation in adult Hopx null and heterozygous mice we administered BrdU for 4 consecutive days and then analyzed BrdU incorporation on the fifth day. Remarkably, as assessed by quantitation on anatomically equivalent histological sections, the number of BrdU + cells in the dorsal DG is increased in Hopx nulls as compared to Hopx heterozygotes (Fig. 5Aa–b and Ag). We also observed significantly more DCX + cells in Hopx null dorsal DGs (Fig. 5Ac–d and Ag). In contrast, the number of Sox2 + NSCs is reduced in Hopx null dorsal DGs (Fig. 5Ae–g). We did not observe any differences in apoptosis (Supplemental Fig. 6).

Many signaling pathways are requisite in the regulation of neurogenesis and NSC maintenance in the DG including Wnt, Bmp, and Notch (Faigle & Song, 2013). Analysis of gene expression in adult Hopx null and heterozygous DGs by qRT-PCR reveals no significant changes in Wnt targets (c-Myc, CyclinD1 and CyclinD2), Bmp4 or known Bmp4 transcriptional targets, Id1 and Id4 (Martynoga et al.,

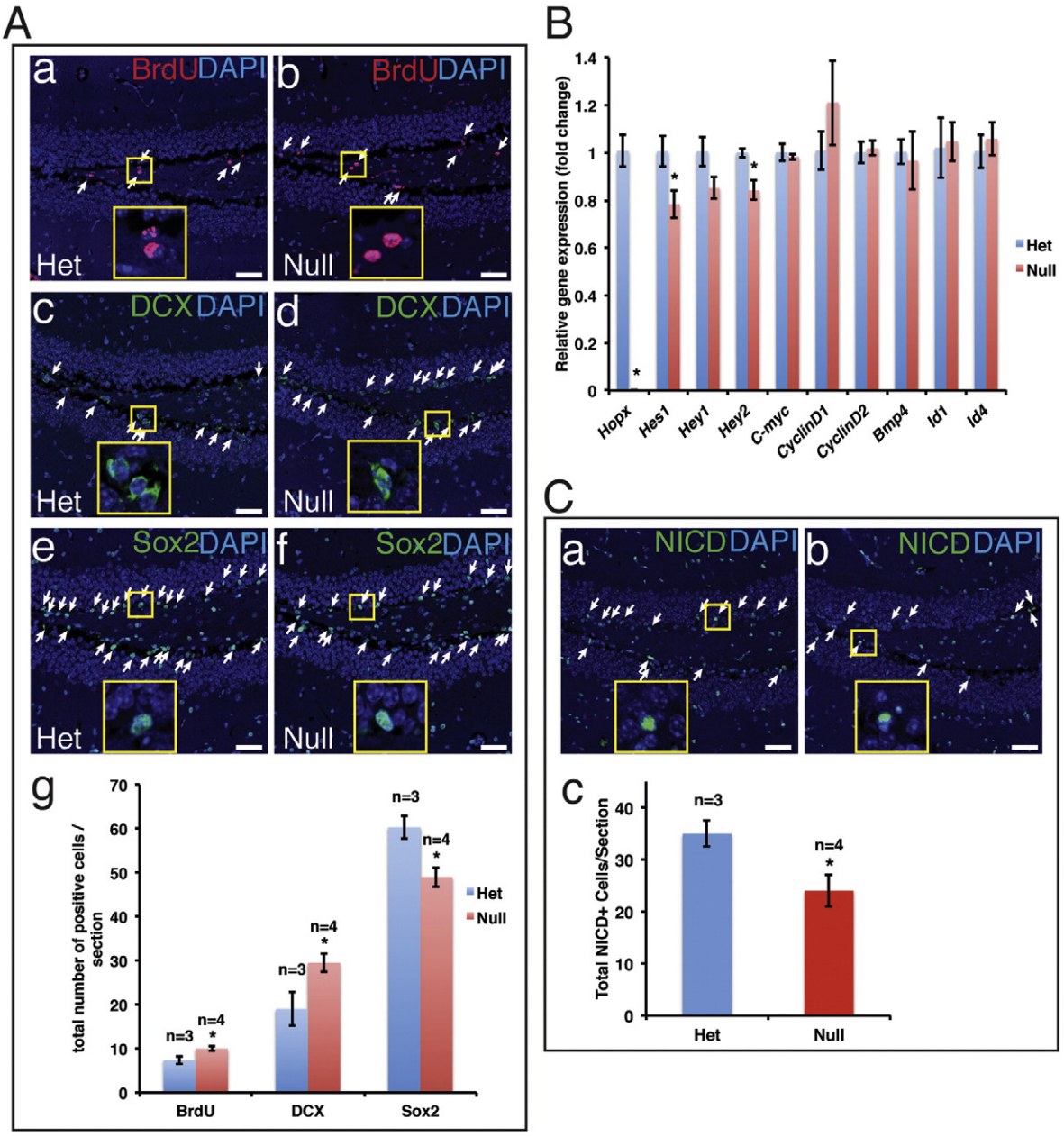


**Fig. 5.** Enhanced neurogenesis and reduced numbers of quiescent NSCs in Hopx null DGs. (A) (a–f) Coronal sections of P78 Hopx null and heterozygous DGs. (a–b) BrdU+ cells in the SGZ marked with arrows. (c–f) Immunofluorescence for DCX (c, d) and Sox2 (e, f). Arrows indicate labeled cells. Insets show higher magnification. (g) Quantification of staining in a–f. Asterisks denote significant differences, $P < 0.05$. Scale bars in a–f, 50 μm. (B) QRT-PCR of P75 Hopx null ($n = 4$) and heterozygous ($n = 4$) microdissected DGs. Gene expression was normalized to Gapdh expression. Expression in heterozygotes is assigned a value of 1. Asterisks denote significant differences in nulls, $P < 0.05$. (C) (a, b) Coronal sections of P78 Hopx null and heterozygous DGs stained for NICD (green, arrows). (c) Quantification of NICD+ cells. Asterisk indicates a significant difference in nulls compared to heterozygotes, $P < 0.05$. Scale bars in a, b, 50 μm.

2013) (Fig. 5B). Interestingly, the Notch signaling targets, Hes1 and Hey2, are significantly decreased in Hopx null compared to heterozygous DGs. Furthermore, the number of cells expressing the cleaved, activated form of the Notch1 receptor, the Notch intracellular domain (NICD), is significantly reduced in Hopx null dorsal DGs (Fig. 5C). Thus, we conclude that Hopx could play a role in maintaining the normal complement of NSCs in the DG, perhaps, at least in part, through regulation of Notch.

## 5. Discussion

We have previously postulated that Hopx might label NSCs in the dentate gyrus based upon its co-expression with GFAP in the SGZ (De Toni et al., 2008). Here we demonstrate that Hopx in the SGZ of the DG co-localizes with NSC markers including GFAP, Sox2 and Nestin, but not with transit-amplifying cell markers Mash1 or Tbr2. Our lineage tracing experiments demonstrate that Hopx + cells in the adult DG give rise to DCX-expressing neuroblasts and then granule neurons over time, confirming that these Hopx + cells are indeed NSCs. Collectively, these data establish that Hopx is a novel marker of quiescent NSCs in the adult DG.

Hopx also plays a functional role in NSCs in the adult hippocampus. Our previous work has shown that neuronal production is increased in Hopx null hippocampi (De Toni et al., 2008). This is consistent with our current findings demonstrating that neurogenesis is enhanced in Hopx null DGs, whereas quiescent NSCs are reduced in number. This finding suggests that Hopx modulates neurogenesis and NSC self-renewal. Of note, our current data were quantified using anatomically matched histological sections. Future stereological analyses of Hopx heterozygous and Hopx null DGs will allow for more definitive measures of cell

numbers and structural volumes between genotypes (West, 1999; Lagace et al., 2007; Gao et al., 2007).

Clearly, however, there are additional and alternative pathways regulating these processes since the phenotypes we observe are relatively mild, and some degree of NSC self-renewal persists in null animals. Indeed, many signaling pathways have been implicated in adult neurogenesis, including Notch, Wnt, Bmp, and Shh (Faigle & Song, 2013). Notch signaling plays a particularly important role in the maintenance of quiescent NSCs. Genetic deletion of Notch signaling components including Jagged1, Notch1, and Rbp-J, or exposure to γ-secretase inhibitors, disturb the balance between NSC maintenance and sustained neurogenesis (Giachino & Taylor, 2014; Hitoshi et al., 2002; Lavado & Oliver, 2014; Breunig et al., 2007). Notch signaling is down-regulated in the Hopx null DGs and decreased Notch activation likely contributes to the neurogenic phenotypes that we have described. Alternatively, the reduction of Hey2 may be attributed to Notch-independent pathways such as FGF, in which Hopx might serve as an intermediate (Doetzlhofer et al., 2009; Al Alam et al., 2015). Future studies will focus on how Hopx functions to regulate Notch and NICD activation, and whether this pathway is relevant to Hopx function in other adult stem cell populations in multiple organs and tissues. Future studies will also focus on differential analyses of dorsal and ventral hippocampi at both the histological and gene expression levels that could reveal additional differences between Hopx mutants and controls.

Interestingly, Hopx is not expressed in NSC of the LV-PZ. Consistent with this finding, Hopx expressing cells do not give rise to interneurons of the olfactory bulb. To our knowledge, this is the first demonstration of a factor that distinguishes between the quiescent NSC populations that generate hippocampal and olfactory bulb neurons in adults. Since Hopx also has functional influences on hippocampal neurogenesis, Hopx may represent a novel tool for discovering mechanistic insights into the neurogenic events most relevant to neuropsychiatric conditions, including cognitive and mood disturbances.

## 6. Conclusion

Our study demonstrates that Hopx is expressed in the quiescent NSC population in the adult hippocampus, but not in the proliferative zone of lateral ventricle. Loss of Hopx function possibly reduces quiescence and enhances neurogenesis by modulating Notch signaling. As such, Hopx represents a novel inroad to the selective regulation of hippocampal neurogenesis.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2015.09.015.

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