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Transcription factors COUP-TFI and COUP-TFII are required for the production of granule cells in the mouse olfactory bulb

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ABSTRACT

Neural stem cells (NSCs) persist in the adult mammalian subventricular zone (SVZ) of the lateral ventricle. Primary NSCs generate rapidly dividing intermediate progenitor cells, which in turn generate neuroblasts that migrate along the rostral migratory stream (RMS) to the olfactory bulb (OB). Here, we have examined the role of the COUP-TFI and COUP-TFII orphan nuclear receptor transcription factors in mouse OB interneuron development. We observed that COUP-TFI is expressed in a gradient of low rostral to high caudal within the postnatal SVZ neural stem/progenitor cells. COUP-TFI is also expressed in a large number of migrating neuroblasts in the SVZ and RMS, and in mature interneurons in the OB. By contrast, very few COUP-TFII-expressing (+) cells exist in the SVZ-RMS-OB pathway. Conditional inactivation of COUP-TFI resulted in downregulation of tyrosine hydroxylase expression in the OB periglomerular cells and upregulation of COUP-TFII expression in the SVZ, RMS and OB deep granule cell layer. In COUP-TFI/COUP-TFII double conditional mutant SVZ, cell proliferation was increased through the upregulation of the proneural gene *Ascl1*. Furthermore, COUP-TFI/II-deficient neuroblasts had impaired migration, resulting in ectopic accumulation of calretinin (CR)+ and NeuN+ cells, and an increase in apoptotic cell death in the SVZ. Finally, we found that most Pax6+ and a subset of CR+ granular cells were lost in the OB. Taken together, these results suggest that COUP-TFI/II coordinately regulate the proliferation, migration and survival of a subpopulation of Pax6+ and CR+ granule cells in the OB.

KEY WORDS: SVZ, Neural stem cells, Neurogenesis, Interneurons, Olfactory bulb, COUP-TFI (Nr2f1), COUP-TFII (Nr2f2)

INTRODUCTION

The subventricular zone (SVZ) of the lateral ventricle is the largest germinal zone in most adult mammalian brains. GFAP+ NSCs directly give rise to rapidly dividing intermediate progenitor cells, which in turn give rise to migratory immature interneurons called neuroblasts. Neuroblasts in the SVZ tangentially migrate into the olfactory bulb (OB) via the rostral migratory stream (RMS) and then radially migrate in the granule cell layer (GCL), external plexiform layer (EPL) and periglomerular layers, and differentiate into OB mature interneurons (Doetsch et al., 1999; Mirzadeh et al., 2008). Neural stem cells (NSCs) in the SVZ also generate

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oligodendrocytes and astrocytes (Merkle et al., 2004; Menn et al., 2006; Xing et al., 2014). Notably, NSCs in the embryonic ventricular zone and adult SVZ of the lateral ventricle are remarkably heterogeneous in terms of their location, transcription factor expression and potential to generate different subtypes of OB interneurons (Merkle et al., 2007, 2014; Young et al., 2007; Batista-Brito et al., 2008; Yang, 2008; Kriegstein and Alvarez-Buylla, 2009; Li et al., 2011).

Here, we examine the expression and functions of the COUP-TFI (NR2F1 – Mouse Genome Informatics) and COUP-TFII (NR2F2 – Mouse Genome Informatics) orphan nuclear receptor transcription factors during the embryonic and postnatal development of OB interneurons. The COUP-TFs belong to the steroid/thyroid hormone receptor superfamily. They can act as either transcriptional activators or repressors in a cell type-dependent manner (Lin et al., 2011). In general, COUP-TFs are expressed in a low-rostral to high-caudal gradient in the embryonic telencephalon (Kanatani et al., 2008; Lodato et al., 2011). The functions of COUP-TFs in neural development, cell-fate specification, organogenesis, angiogenesis and metabolism have been extensively investigated (Lin et al., 2011; Alfano et al., 2014). However, to our knowledge, how they may cooperate with each other has not been studied. In the present study, using OB interneurons as a model system, we have demonstrated that COUP-TFI and COUP-TFII coordinately regulate the proliferation, migration and survival of a subpopulation of granular cells (GCs) in the OB.
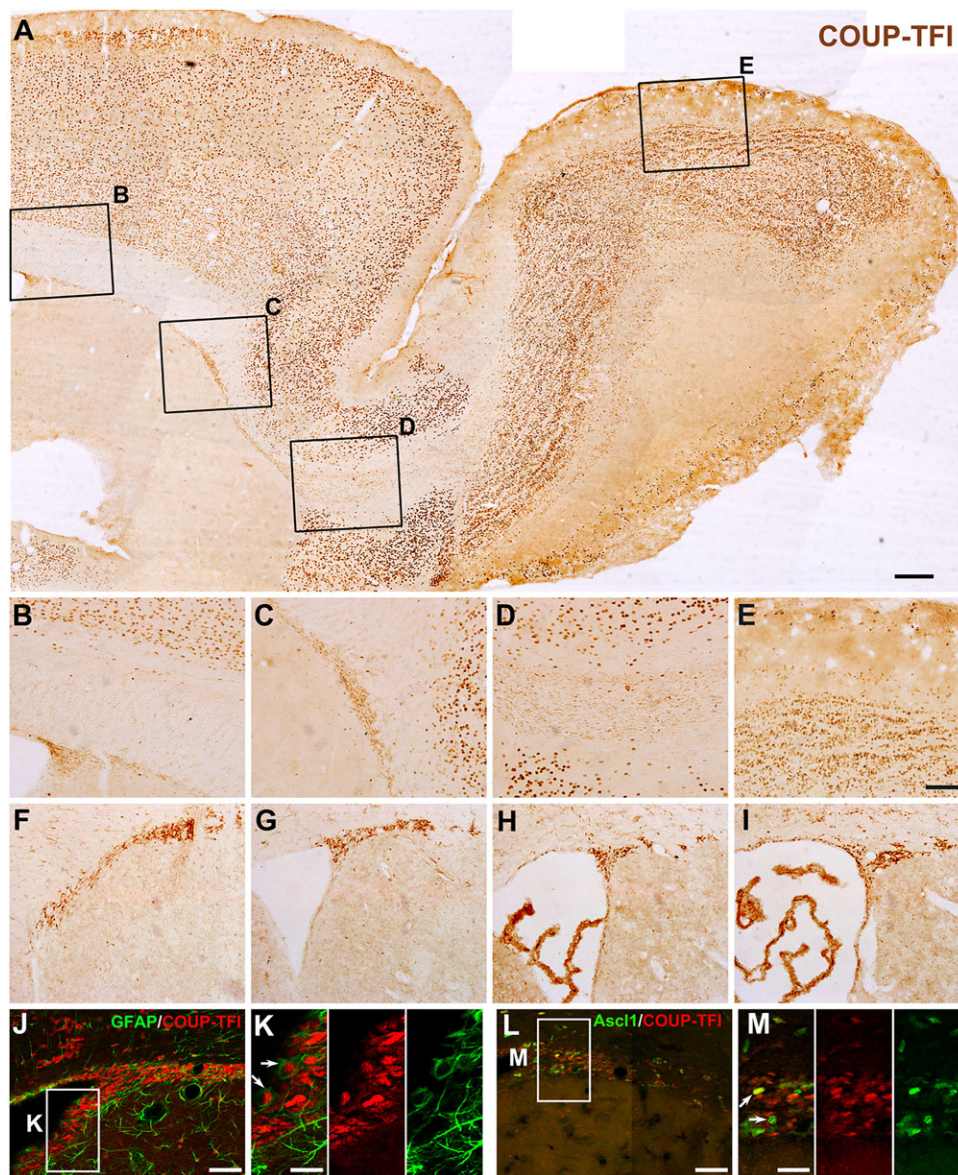
RESULTS

COUP-TFI is expressed in a gradient of low-rostral to high-caudal within the postnatal SVZ

During early brain development, COUP-TFI is expressed in the cerebral cortex primordium (pallium) and several restricted progenitor domains in the subpallium, including the lateral, medial and caudal ganglionic eminence (LGE, MGE and CGE, respectively) (Faedo et al., 2008; Lodato et al., 2011). To examine the function of COUP-TFI in the OB interneuron development, we first analyzed the expression pattern of COUP-TFI in the SVZ-RMS-OB pathway of adult mice (postnatal day 42, P42) using immunoperoxidase and double immunofluorescence staining (Fig. 1). In sagittal brain sections, we observed that COUP-TFI was widely expressed in the SVZ (Fig. 1A,B), RMS (Fig. 1C,D) and OB (Fig. 1E). In coronal sections, we also observed a mass of COUP-TFI+ cells in the dorsal-lateral SVZ for each of the rostral and caudal coronal slices (Fig. 1F-I). Few, if any, COUP-TFI+ cells were observed in the ventral or medial SVZ (Fig. 1F-I). A recent study did not observe COUP-TFI expression in the migrating neuroblasts in the adult SVZ and RMS (Bovetti et al., 2013). However, our immunostaining (following antigen retrieval) revealed a mass of COUP-TFI+ cells in the adult SVZ and RMS (Fig. 1A-I). We confirmed this observation with

DEVELOPMENT

Fig. 1. COUP-TFI is expressed in the SVZ-RMS-OB pathway of the adult mouse brain. (A) Photomicrograph of COUP-TFI+ cells in one immunoperoxidase-stained sagittal section from a P42 CD1 mouse brain. (B-E) Higher-magnification images of boxed areas in A show COUP-TFI+ cells in the SVZ (B), RMS (C,D) and OB (E). (F-I) COUP-TFI+ cells in the SVZ of the adult mouse coronal brain sections at different rostrocaudal levels. (J,K) GFAP+/COUP-TFI+ neural stem cells (arrows in K) in the SVZ. (L,M) Ascl1+/COUP-TFI+ cells (arrows in M) in the SVZ. Scale bars: 200 µm in A; 100 µm in E for B-I; 100 µm in J,L; 20 µm in K,M.

COUP-TFI/doublecortin (DCX) double immunostaining, which showed that a subpopulation of COUP-TFI+ cells expressed DCX (supplementary material Fig. S1A-F), a neuroblast-expressing microtubule-associated protein required for neuronal migration (Koizumi et al., 2006; Baek et al., 2014). In the OB, large numbers of both strongly and weakly stained COUP-TFI+ cells were found in the glomerular layer, EPL and GCL (Fig. 1E). Although ~12.6% CR+ cells in the GCL expressed COUP-TFI (supplementary material Fig. S1G), they did not express COUP-TFI in the periglomerular layer, consistent with previous observations (Bovetti et al., 2013). We also found that some COUP-TFI+ cells in the SVZ expressed GFAP (NSC marker) (Fig. 1J,K). A subset of NSCs and rapidly dividing intermediate progenitor cells have been shown to express the proneural basic helix-loop-helix transcription factor achaete-scute homolog 1 (Ascl1, also known as Mash1) (Parras et al., 2004; Kohwi et al., 2005; Kim et al., 2011; Andersen et al., 2014; Mich et al., 2014). By immunostaining for COUP-TFI and Ascl1, we found that many COUP-TFI+ cells in the adult SVZ expressed Ascl1 (Fig. 1L,M). Taken together, our results suggest that a subpopulation of NSCs, neural progenitor cells, migrating

neuroblasts and mature OB interneurons express COUP-TFI in the adult SVZ-RMS-OB pathway.

Different lineages of NSCs in the postnatal SVZ arise from distinct dorsoventral and anteroposterior subdomains of embryonic ventricular zone of the lateral ventricle (Kriegstein and Alvarez-Buylla, 2009). During early brain development, COUP-TFI is also expressed in a gradient of low-rostroventral to high-caudodorsal in general (Zhou et al., 2001; Alfano et al., 2011; Lodato et al., 2011; Borello et al., 2014). In support of this notion, COUP-TFI and Pax6 double-immunostaining on P0 and P10 mouse brain sections revealed that COUP-TFI is weakly expressed in the rostral SVZ but strongly expressed in the caudal SVZ neural stem/progenitor cells (supplementary material Fig. S2). At P21, the percentage of DCX+ and Ascl1+ cells that express COUP-TFI is also shown in a low-rostral to high-caudal gradient in the SVZ (Fig. 2). Indeed, at the caudalmost SVZ, virtually all DCX+ and Ascl1+ cells express COUP-TFI (Fig. 2F-W). Interestingly, we also found that COUP-TFI is expressed in ependymal cells in the caudal lateral wall of the lateral ventricle (supplementary material Fig. S3A-C). This result suggests that the expression pattern of COUP-TFI in
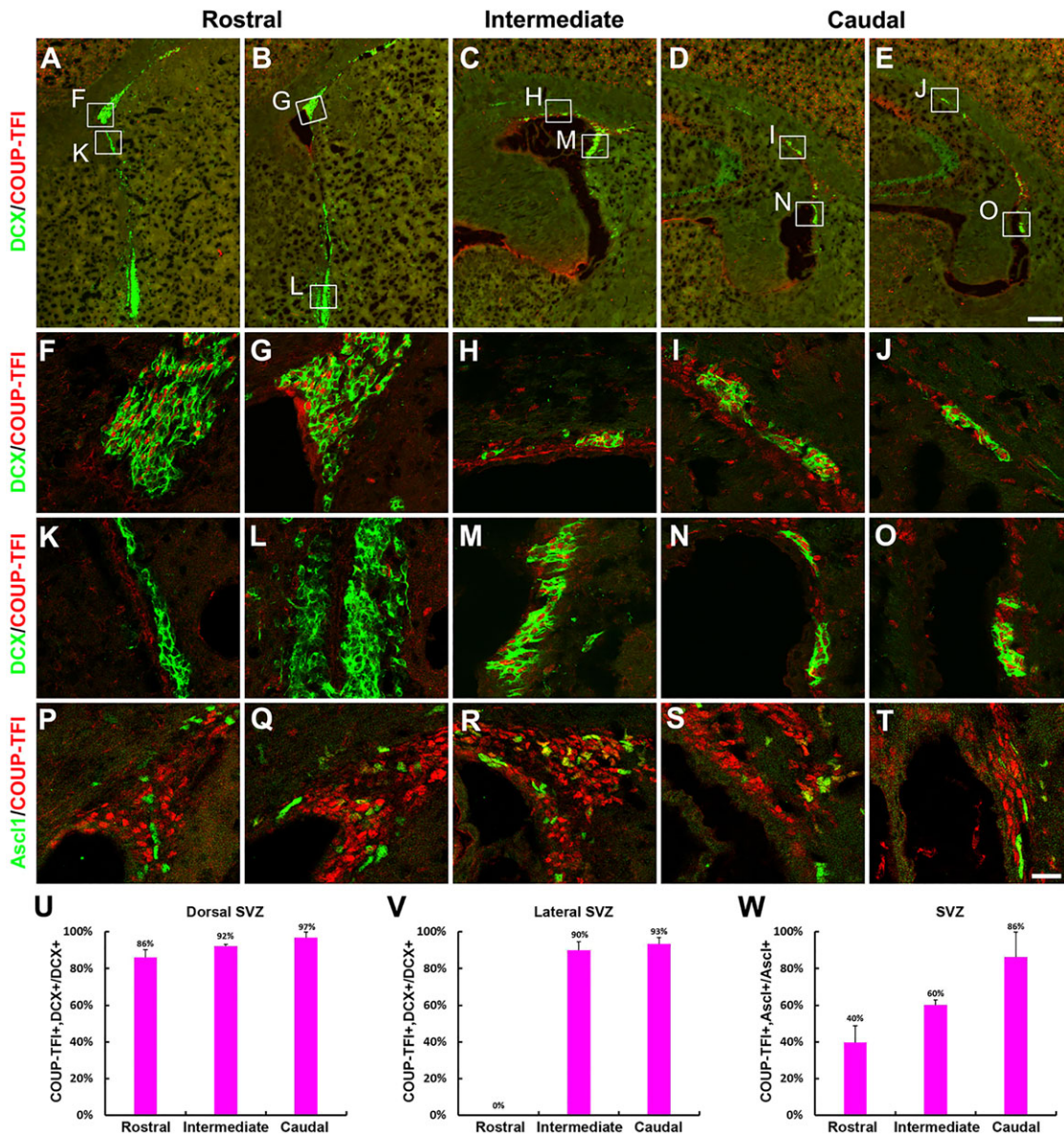
Fig. 2. COUP-TFI is expressed in a gradient of low rostral to high caudal in SVZ neural stem/progenitor cells. (A-E) COUP-TFI/DCX double-immunostained coronal sections at different rostrocaudal levels of the SVZ of control mice at P21. (F-O) Higher-magnification images of boxed areas in A-E show COUP-TFI+/DCX+ cells in the SVZ. (P-T) COUP-TFI/Ascl1 double-immunostained P21 mouse brain sections. (U-W) The percentage of DCX+ cells that expressed COUP-TFI in the dorsal (U) and lateral SVZ (V), and the percentage of Ascl1 that expressed COUP-TFI (W) in the SVZ at different rostrocaudal levels. Data are mean±s.e.m. Scale bars: 200 μm in E for A-E; 20 μm in T for F-T.

the postnatal SVZ shares common features with the embryonic germinal zone.

Few COUP-TFII+ cells exist in the adult SVZ-RMS-OB pathway

We then analyzed the expression pattern of COUP-TFII in the adult SVZ-RMS-OB pathway as it is widely expressed in the CGE during development (Kanatani et al., 2008; Cai et al., 2013). In contrast to COUP-TFI expression, we found very few COUP-TFII+ cells in the adult SVZ and RMS (supplementary material Fig. S3D-F). Accordingly, only scattered COUP-TFII+ cells were identified in the glomerular layer, EPL and GCL of the OB (supplementary material Fig. S3G). COUP-TFII expression in the choroid plexus was observed as previously described (supplementary material Fig. S3H-J) (Cai et al., 2013). Although OB interneurons seldom

expressed COUP-TFII, cells in the olfactory nerve layer that are strongly COUP-TFII+ were observed; these cells are olfactory ensheathing cells as all of them expressed S100β (supplementary material Fig. S3G and Fig. S4A-C).

Our recent study has detected rare COUP-TFII+ cells in the RMS of the rhesus monkey and human fetal brain (Ma et al., 2013). Consistent with this observation, we found a very small number of COUP-TFII+ cells in the GCL, EPL and glomerular layer of the adult rhesus monkey and human OB (supplementary material Fig. S4E,G). By contrast, COUP-TFI was widely expressed in these regions (supplementary material Fig. S4D,F). Once again, olfactory ensheathing cells in the olfactory nerve layer strongly expressed COUP-TFII (supplementary material Fig. S4E,G). This result suggests conserved expression patterns of COUP-TFI and COUP-TFII in the mammalian OB across species.

COUP-TFII expression is upregulated in the SVZ and OB of hGFAP-Cre; COUP-TFI$^{flox/flox}$ conditional mutant mice

To investigate the function of COUP-TFI in the OB interneuron development, we conditionally deleted COUP-TFI in SVZ NSCs using a transgenic hGFAP-Cre mouse (Zhuo et al., 2001), which exhibits excision of floxed alleles in a subset of neural stem/progenitor cells around E13.5 (Malatesta et al., 2003; Lim et al., 2009). hGFAP-Cre; COUP-TFI$^{flox/flox}$ mice were indistinguishable from their COUP-TFI$^{flox/+}$ littermates (referred to as controls). In P21 hGFAP-Cre; COUP-TFI$^{flox/flox}$ mouse SVZ and RMS, few if any COUP-TFI+ cells were observed (supplementary material Fig. S5A,B). The absence of COUP-TFI staining in the hGFAP-Cre; COUP-TFI$^{flox/flox}$ mouse SVZ and RMS further supported the validity of our staining method. In the neocortex, except in layer VI, most cells also did not express COUP-TFI (supplementary material Fig. S5B). In the OB, only scattered COUP-TFI+ cells were observed in the GCL (70-fold decrease compared with controls), EPL and glomerular layer (supplementary material Fig. S5C); these COUP-TFI+ cells were likely produced before E13.5. These results suggest that COUP-TFI is successfully deleted in the SVZ NSCs and their progeny in hGFAP-Cre; COUP-TFI$^{flox/flox}$ mice. In control mice, ~90% Pax6+ cells in the glomerular layer expressed COUP-TFI (Bovetti et al., 2013) and 74.8% Pax6+ cells in the GCL expressed COUP-TFI. As previously described, the number of tyrosine hydroxylase (TH)+ dopaminergic cells was significantly reduced in the glomerular layer due to the downregulation of TH expression in the hGFAP-Cre; COUP-TFI$^{flox/flox}$ mouse OB (supplementary material Fig. S6A-E) (Bovetti et al., 2013), but the number of Pax6+ cells in the GCL and glomerular layer was comparable to those in controls (supplementary material Fig. S7A,B). The numbers of calbindin+ and CR+ cells in the glomerular layer and GCL were also not different from those in controls (data not shown) (Bovetti et al., 2013).

Interestingly, we found that COUP-TFII expression was significantly upregulated in the SVZ and RMS of hGFAP-Cre; COUP-TFI$^{flox/flox}$ mice (supplementary material Fig. S5D-G). DCX/COUP-TFII double-immunostaining on P21 brain sections of hGFAP-Cre; COUP-TFI$^{flox/flox}$ mice revealed that virtually all COUP-TFII+ cells in the SVZ expressed DCX, but not Ascl1 (Fig. 3A-S), suggesting that COUP-TFII expression is upregulated only in neuroblasts but not neural stem/progenitor cells. Moreover, the percentage of DCX+ cells that expressed COUP-TFII exhibited in a low-rostral to high-caudal gradient (Fig. 3S). Although very few COUP-TFII+ cells were observed in the rostral and caudal SVZ of control mice (supplementary material Fig. S3D and Fig. S5D), the vast majority of DCX+ cells in the caudal SVZ expressed COUP-TFII in COUP-TFI conditional mutants (Fig. 3S). In the OB, although there was no difference in COUP-TFII expression in the olfactory nerve layer, glomerular layer and EPL compared with controls, COUP-TFII expression was significantly upregulated in
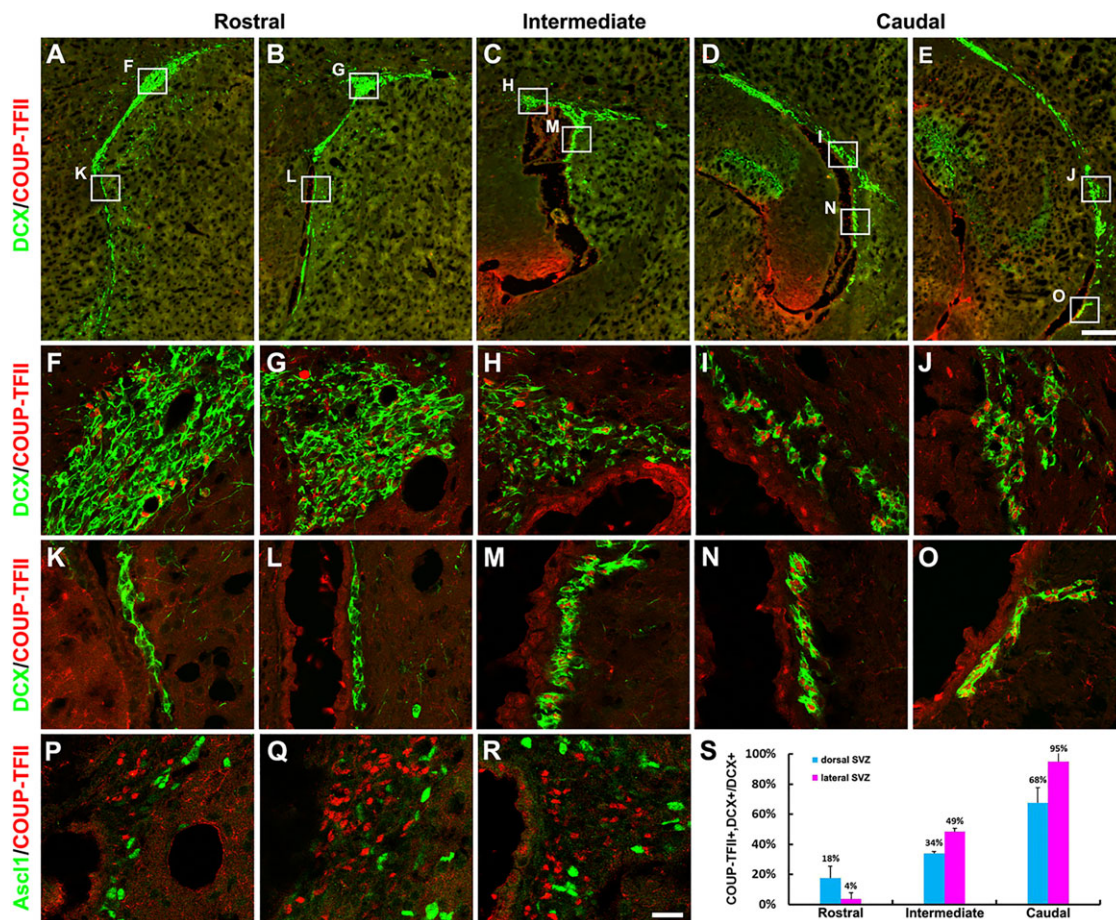
Fig. 3. COUP-TFII expression is upregulated in the SVZ of COUP-TFI conditional mutant mice. (A-E) COUP-TFII/DCX double-immunostained coronal sections of hGFAP-Cre; COUP-TFI$^{flox/flox}$ mouse brain at P21. (F-O) Higher-magnification images of boxed areas in A-E show COUP-TFII+/DCX+ cells in the SVZ. (P-R) COUP-TFII+ cells did not express Ascl1. (S) The percentage of DCX+ cells that expressed COUP-TFII in the rostral, intermediate and caudal SVZ of hGFAP-Cre; COUP-TFI$^{flox/flox}$ mice. Data are mean±s.e.m. Scale bars: 200 μm in E for A-E; 20 μm in R for F-R.

the deep GCL of the OB of hGFAP-Cre; COUP-TFI$^{flox/flox}$ mice (4.5-fold increase, supplementary material Fig. S7D-F). Moreover, the staining of these COUP-TFII+ cells was much stronger than those in controls. Approximately 78.1% of COUP-TFII+ cells were Pax6+, and these COUP-TFII+/Pax6+ cells were mature deep GCs as they expressed NeuN (supplementary material Fig. S7D-I). Taken together, these results suggest that COUP-TFI and COUP-TFII may have redundant functions in making OB GCs (e.g. Pax6+ cells). To test this hypothesis, we deleted both COUP-TFI and COUP-TFII alleles in SVZ NSCs again using hGFAP-Cre.

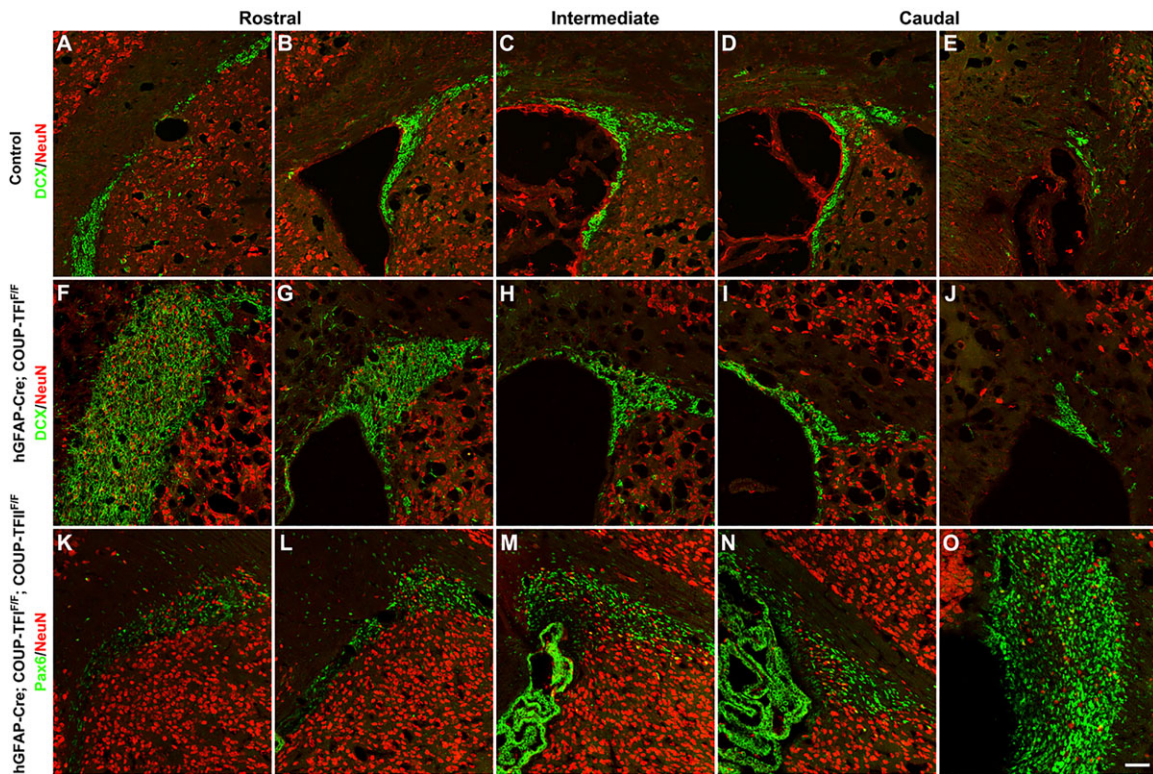### DCX+, NeuN+ and CR+ cells accumulate in the SVZ of COUP-TF conditional mutants

Before P7, hGFAP-Cre; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ double conditional mutant mice (hereafter referred to as COUP-TF double conditional mutants) were indistinguishable from their littermates. However, by P12, COUP-TF double conditional mutants developed progressive growth retardation and the mice usually died around P24. We therefore analyzed COUP-TF double conditional mutants and controls at P21. In the COUP-TF double conditional mutant SVZ, the most prominent phenotype was accumulation of DCX+ and DCX+/Pax6+ cells in the caudal SVZ (Fig. 4A-J). About 82.1% of these Pax6+ cells did not express Ki67 in the caudal SVZ (Fig. 4K-O), further suggesting that they are postmitotic neuroblasts. Although few, if any, NeuN+ or CR+ cells were observed in the normal control mouse SVZ (Fig. 5A-E; supplementary material Fig. S8A-E) (Doetsch et al., 1997, 2002), a subset of NeuN+ (Fig. 5K-O) and CR+ (supplementary material Fig. S8K-O) cells accumulated in the COUP-TF double conditional mutant SVZ. About 16.2% of these NeuN+ and 9.0% of these CR+ cells expressed Pax6 (supplementary material Fig. S8F-O,T).

By contrast, 91% of CR+ cells expressed Sp8 (supplementary material Fig. S8P-T). This suggests that at least two subpopulations of neurons accumulate in the SVZ of COUP-TF double conditional mutant mice; they are NeuN+/Pax6+ and CR+/Sp8+ cells, respectively, as Sp8+ cells in the SVZ seldom express Pax6 (Waclaw et al., 2006). Interestingly, compared with DCX+ cells in the caudal SVZ of control mice, we also found an increase in the accumulation of DCX+ cells in the caudal SVZ of hGFAP-Cre; COUP-TFI$^{flox/flox}$ single conditional mutants (Fig. 2C-E versus Fig. 3C-E). Accordingly, ectopic NeuN+ (Fig. 5F-J) and CR+ (supplementary material Fig. S8F-J) cell accumulation was also observed. Whereas NeuN+ and CR+ cells accumulated mainly in the caudal SVZ of COUP-TF double conditional mutants (Fig. 5K-O; supplementary material Fig. S8K-O), in hGFAP-Cre; COUP-TFI$^{flox/flox}$ single conditional mutants, these cells accumulated mainly in the rostral SVZ (Fig. 5F-J; supplementary material Fig. S8F-J).

We have also deleted COUP-TF alleles in SVZ cells using Emx1-Cre mice (Gorski et al., 2002; Bovetti et al., 2013). Emx1 and Emx1-cre are not only expressed in almost all progenitors in neocortical and septal VZ/SVZ (Gorski et al., 2002), but are also expressed in a large number of progenitors in the LGE and CGE (Cocas et al., 2009; Waclaw et al., 2009). Whereas Emx1-cre; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ double conditional mutant mice were lethal, Emx1-cre; COUP-TFI$^{flox/flox}$ single conditional mutants were indistinguishable from COUP-TFI$^{flox/+}$ controls. Consistent with our above results from hGFAP-Cre; COUP-TFI$^{flox/flox}$ mice, DCX+, NeuN+ and CR+ cells accumulation in the rostral SVZ were clearly observed in Emx1-cre; COUP-TFI$^{flox/flox}$ conditional mutant mice (supplementary material Fig. S9).
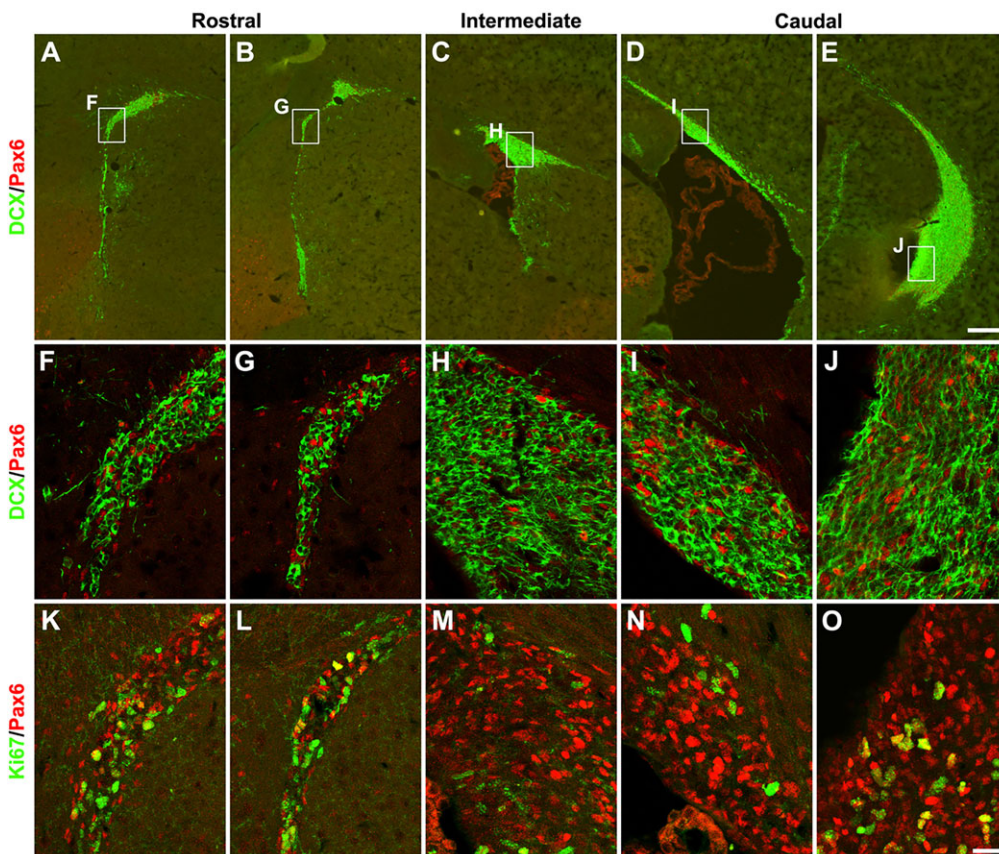
Fig. 4. Neuroblasts accumulate in the SVZ of the COUP-TF double conditional mutants. (A-E) DCX/Pax6 double-immunostained coronal sections of hGFAP-Cre; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ mouse brain at P21. (F-J) Higher-magnification images of boxed areas in A-E show DCX+, Pax6+ and DCX+/Pax6+ cells in the rostral, intermediate and caudal SVZ. (K-O) Ki67/Pax6 double-immunostained coronal sections of hGFAP-Cre; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ mouse brain at P21. Scale bars: 200 µm in E for A-E; 20 µm in O for F-O.

Fig. 5. NeuN+ cells accumulate in the SVZ of the COUP-TF conditional mutants. DCX/NeuN (A-J) and Pax6/NeuN (K-O) double-immunostained coronal sections of P21 control and COUP-TF conditional mutant mice. NeuN+ cells were rarely identified in the control SVZ (A-E), but a subset of NeuN+ cells was observed in the hGFAP-Cre; COUP-TFI$^{flox/flox}$ and hGFAP-Cre; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ mouse SVZ (F-O). Scale bar: 50 µm.

An increase in cell proliferation in the caudal SVZ of COUP-TF conditional mutants through the upregulation of Ascl1

To investigate the cause of immature and mature neuron accumulation in the SVZ of COUP-TFI single and COUP-TF double conditional mutants, we first examined the proliferation of SVZ cells. BrdU was injected into hGFAP-Cre; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ double conditional mutants, hGFAP-Cre; COUP-TFI$^{flox/flox}$ single conditional mutants and control mice at P10, and cell proliferation in the SVZ was analyzed 2 h after BrdU injection. Compared with the rostral SVZ, only a small number of BrdU+/Ki67+ cells were found in the caudal SVZ of control mice (supplementary material Fig. S10A-F). By contrast, a mass of BrdU+/Ki67+ cells were observed in the caudal SVZ of hGFAP-Cre; COUP-TFI$^{flox/flox}$ mice and hGFAP-Cre; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ mice (supplementary material Fig. S10G-R). Notably, cell proliferation in the caudal SVZ appears to be more prominent in COUP-TF double conditional mutants (supplementary material Fig. S10Q,R) compared with COUP-TFI single conditional mutants (supplementary material Fig. S10K,L). This analysis indicates that the rate of neurogenesis in the caudal SVZ was increased in COUP-TF conditional mutant mice.

The proneural gene Ascl1 has been shown to promote neural stem/progenitor cell proliferation and neurogenesis in the embryonic and adult brain (Bertrand et al., 2002; Castro et al., 2011; Andersen et al., 2014; Imayoshi and Kageyama, 2014). In particular, Ascl1 plays a crucial role in activating neural stem/progenitor cells in the adult SVZ (Andersen et al., 2014). Analyzing the expression of Ascl1 at P10 and P21 showed a significant increase in the number of Ki67+/Ascl1+ proliferating neural stem/progenitor cells in the caudal SVZ of COUP-TF double conditional mutant mice compared with control mice (8.7-fold increase at P10

and 10.8-fold increase at P21, Fig. 6A-H). Once again, we also found a significant increase in the number of Ascl1+/Ki67+ cells in the caudal SVZ of hGFAP-Cre; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ double conditional mutants compared with hGFAP-Cre; COUP-TFI$^{flox/flox}$ single conditional mutants (Fig. 6D,H).

Defective migration of COUP-TFI/COUP-TFII-deficient SVZ cells

We then examined the migration of neuroblasts in COUP-TF double conditional mutant SVZ using Nestin-CreER mice that express a tamoxifen-inducible Cre-recombinase under the Nestin enhancer and promoter (Lagace et al., 2007). In the COUP-TFII floxed allele, a *lacZ* reporting cassette was inserted behind the second LoxP site (Takamoto et al., 2005). The *lacZ* reporter gene is silent without COUP-TFII deletion, and will be activated in cells where the COUP-TFII promoter is active and COUP-TFII allele is deleted by Cre recombinase. We injected tamoxifen once into P10 Nestin-CreER; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ mice. This suggested that COUP-TFI and COUP-TFII were intact embryonically but conditionally inactivated around P10. These Nestin-CreER; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ mice were analyzed at P20 (10 days after tamoxifen treatment). We chose this time window on the basis of previous studies in which it was shown that neuroblasts in the caudal SVZ take ~7-10 days to migrate into the OB (Doetsch and Alvarez-Buylla, 1996; Petreanu and Alvarez-Buylla, 2002). DCX/β-galactosidase (β-gal) double-immunostaining revealed that COUP-TFI/COUP-TFII-deficient neuroblasts (DCX+/β-gal+ cells) mainly accumulated in the caudal SVZ (Fig. 7M-O,R-T), whereas very few β-gal+ cells were observed in the rostral SVZ at P20 (Fig. 7K,L,P,Q). We also found that more DCX+ cells accumulated in the caudal SVZ (Fig. 7M-O) than in control SVZ (Fig. 7C-E).
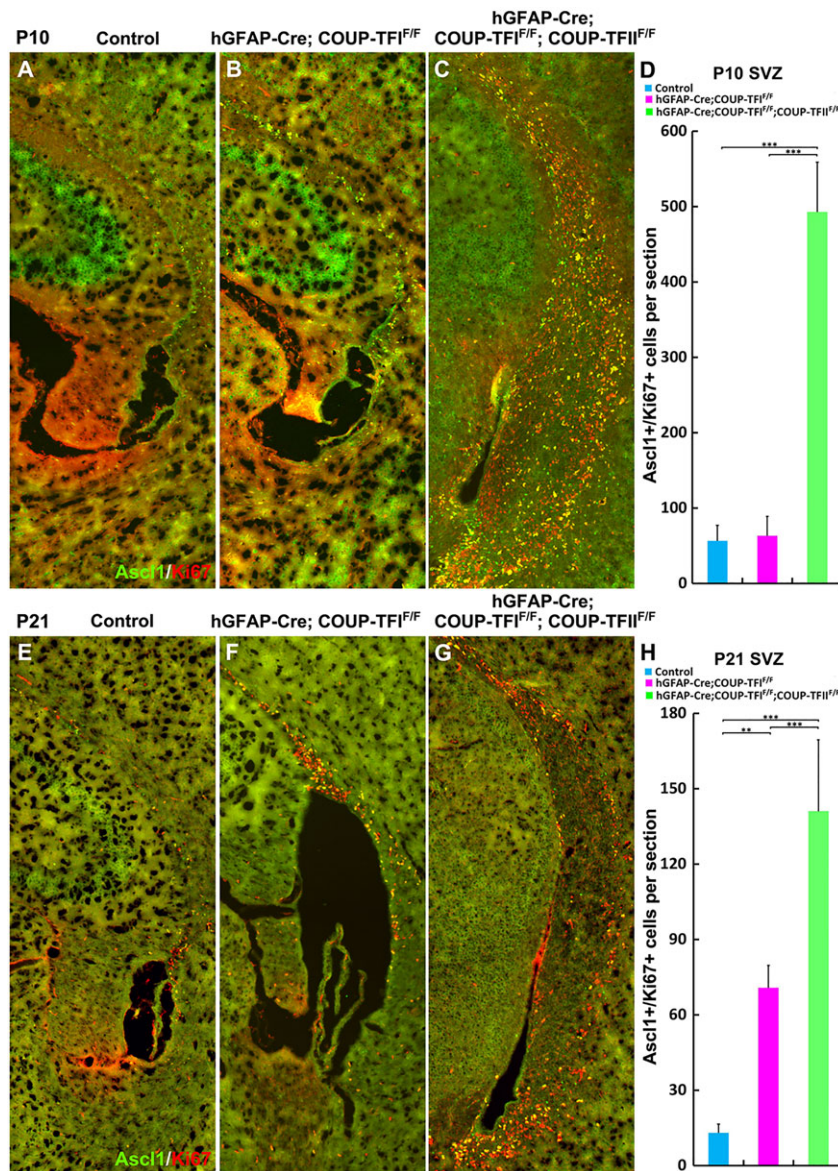
Fig. 6. Increase in cell proliferation in the SVZ of COUP-TF conditional mutants through upregulating Ascl1 expression. (A-H) Ascl1/Ki67 double-immunostaining showing a significant increase in the number of Ascl1+/Ki67+ cells per section in the caudal SVZ of COUP-TF conditional mutants compared with controls at P10 (A-D) and P21 (E-H). There was a significant increase in the number of Ascl1+/Ki67+ cells per section in the caudal SVZ of COUP-TF double conditional mutants compared with COUP-TFI single conditional mutants. Data are mean±s.e.m. **P<0.01; ***P<0.001.

β-Gal+ cells were never found in the SVZ of control mice (Fig. 7A-J). Thus, without COUP-TFI and COUP-TFII, neuroblasts in the caudal SVZ did not migrate to the rostral SVZ, suggesting that COUP-TFs are required for the migration of neuroblasts. Consistently, we also observed significant increase in the number of Ki67+/Ascl1+ cells in the caudal SVZ of Nestin-CreER; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ mice compared with controls (supplementary material Fig. S11A-C).

To further investigate whether this tangential migration defect of neuroblasts in the COUP-TF double conditional mutant SVZ is a cell-autonomous defect, SVZ explants from P1 COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ mice were cultured in Matrigel and infected with Cre-expressing adenovirus for 4 days (Wichterle et al., 1997). At the end of the culture period, cultured explants were fixed and the position of COUP-TFI/COUP-TFII-deficient SVZ cells was assessed by β-gal staining. Consistent with previous reports (Wichterle et al., 1997), neonatal SVZ cells (β-gal-negative cells) migrated along each other in all explants analyzed (five explants from five pups) (supplementary material Fig. S11D-F). Although cell migration was also observed in COUP-TFI/COUP-TFII-deficient SVZ cells (β-gal+ cells), nearly all of these cells were located closer to the explants. The average distance of β-gal-negative cells from the outmost location to the explant edge was about twice that of β-gal+ cells (supplementary material Fig. S11F). These experiments suggest that COUP-TFI and COUP-TFII are essential for the migration of specific subpopulations of OB interneurons, at least partly, in a cell-autonomous manner. Taken together, our results suggest that, without expression of COUP-TFI and COUP-TFII, immature neurons (neuroblasts) may have impaired and/or abnormal migration, resulting in an ectopic accumulation of migrating neuroblasts and mature neurons (NeuN+ and CR+ cells) in an expanded SVZ.

Apoptotic cell death is increased in the SVZ of hGFAP-Cre; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ double conditional mutant mice

As cells that accumulated in the COUP-TF conditional mutant SVZ still expressed DCX and NeuN, it was unlikely that their neuronal fate had changed. We therefore analyzed cell death, as marked by cleaved caspase 3 expression. In all developmental stages analyzed
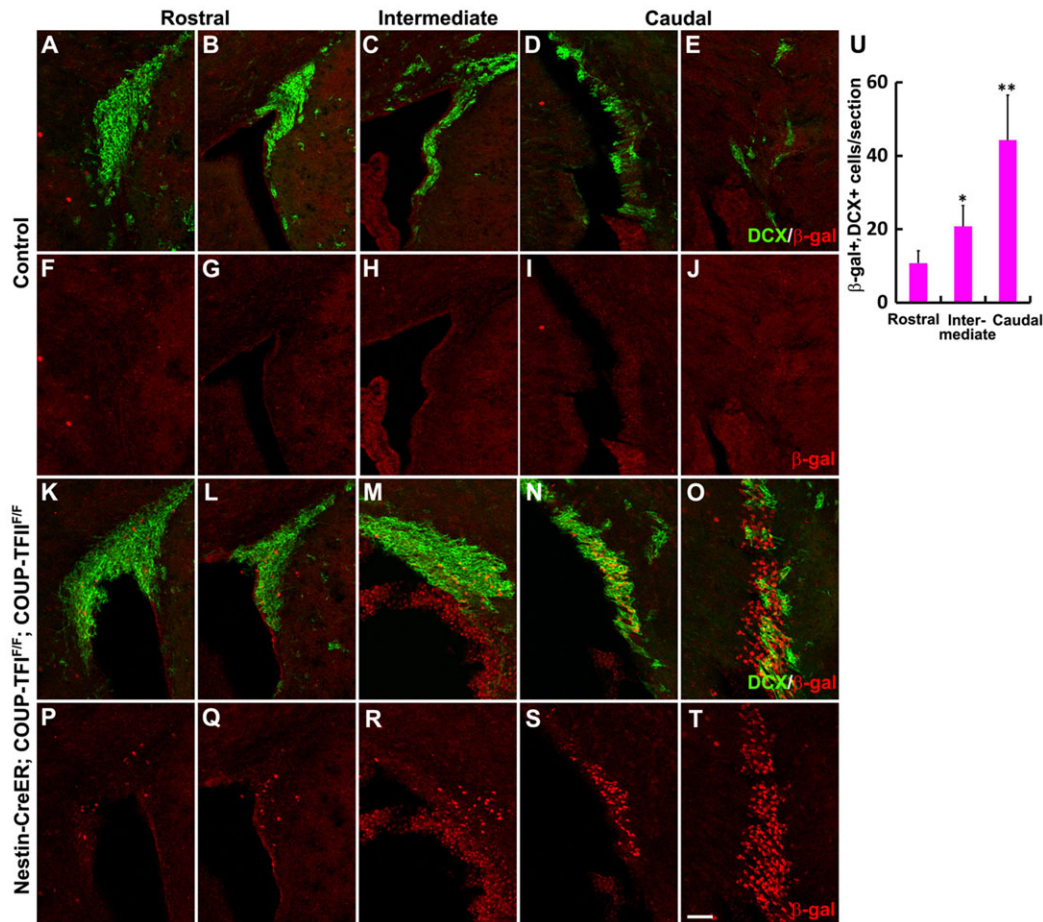
Fig. 7. COUP-TFI/COUP-TFII-deficient SVZ cells in the caudal SVZ are not able to migrate into the rostral SVZ. (A-T) DCX/β-gal double-immunostained coronal sections of P20 control (A-J) and Nestin-CreER; COUP-TFI^flox/flox; COUP-TFII^flox/flox mice (K-T). No β-gal+ cells were observed in the control SVZ (A-J). A large number of β-gal+/DCX+ cells were in the caudal SVZ of Nestin-CreER; COUP-TFI^flox/flox; COUP-TFII^flox/flox mice (N,O), but very few β-gal+/DCX+ cells were in the rostral SVZ (K,L). (U) Quantification of β-gal+/DCX+ cells in the SVZ from rostral to caudal level. *P<0.05; **P<0.01. Data are mean±s.e.m. Scale bar: 50 μm.

(P0, P7, P14 and P21), we found more apoptotic cells (caspase 3+) in the COUP-TF double conditional mutant SVZ than in the control (Fig. 8A-M). These caspase 3+ cells had a neuron-like appearance, as cleaved caspase 3 was expressed both in soma and processes (Southwell et al., 2012). There were also more caspase 3+ cells in the hGFAP-Cre; COUP-TFI^flox/flox and hGFAP-Cre; COUP-TFI^flox/flox; COUP-TFII^flox/+ compound conditional mutant SVZ compared with controls (Fig. 8J-M, data not shown). A few caspase 3+ cells were also observed in the RMS and OB, but no significant difference was observed between the control and mutant mice (data not shown). These results indicate that COUP-TFI and COUP-TFII are required to promote cell survival in the SVZ.

### Pax6+ cells are lost in the deep GCL of COUP-TF double conditional mutant OB

In COUP-TF double conditional mutant OB, very few COUP-TFI+ and COUP-TFII+ cells were found (supplementary material Fig. S7). Once again, there was no significant difference in numbers of calbindin+ or CR+ in the glomerular layer between COUP-TF conditional mutants and controls (supplementary material Fig. S12A-G, data not shown). Consistent with the defect observed in the hGFAP-Cre; COUP-TFI^flox/flox mouse OB, TH+ dopaminergic cells were again significantly reduced in the COUP-TF double conditional mutant OB (data not shown). In contrast to COUP-TFI single

conditional mutants, the most prominent phenotype in COUP-TF double conditional mutant OB was the loss of strongly stained Pax6+ cells in the deep GCL (Fig. 9). Indeed, very few strongly stained Pax6+ cells remained in the GCL (Fig. 9C,F,J) in COUP-TF double conditional mutant OB compared with controls (Fig. 9A,D,J) and hGFAP-Cre; COUP-TFI^flox/flox; COUP-TFII^flox/+ compound conditional mutant mice (Fig. 9B,E,J). Our above results showed that these strongly Pax6+ cells are mature deep GCs (supplementary material Fig. S7F). Therefore, loss of both COUP-TFI and COUP-TFII function resulted in the elimination of strongly Pax6+ granule interneurons in the GCL, especially in the deep GCL (Fig. 9H-J). Compared with controls, Pax6+ periglomerular cells were also reduced in hGFAP-Cre; COUP-TFI^flox/flox; COUP-TFII^flox/flox double conditional mutant mice (Fig. 9G). Furthermore, we also observed that CR+ GCs were slightly but statistically significant reduced in number in COUP-TF double conditional mutants compared with controls (supplementary material Fig. S12H).

DISCUSSION

The main findings of this study are: (1) COUP-TFI is expressed in a gradient of low rostral to high caudal within the SVZ neural stem/ progenitor cells; (2) COUP-TFI, but not COUP-TFII, is expressed in the adult mouse, monkey and human SVZ, RMS and OB; (3) genetic conditional inactivation of COUP-TFI results in

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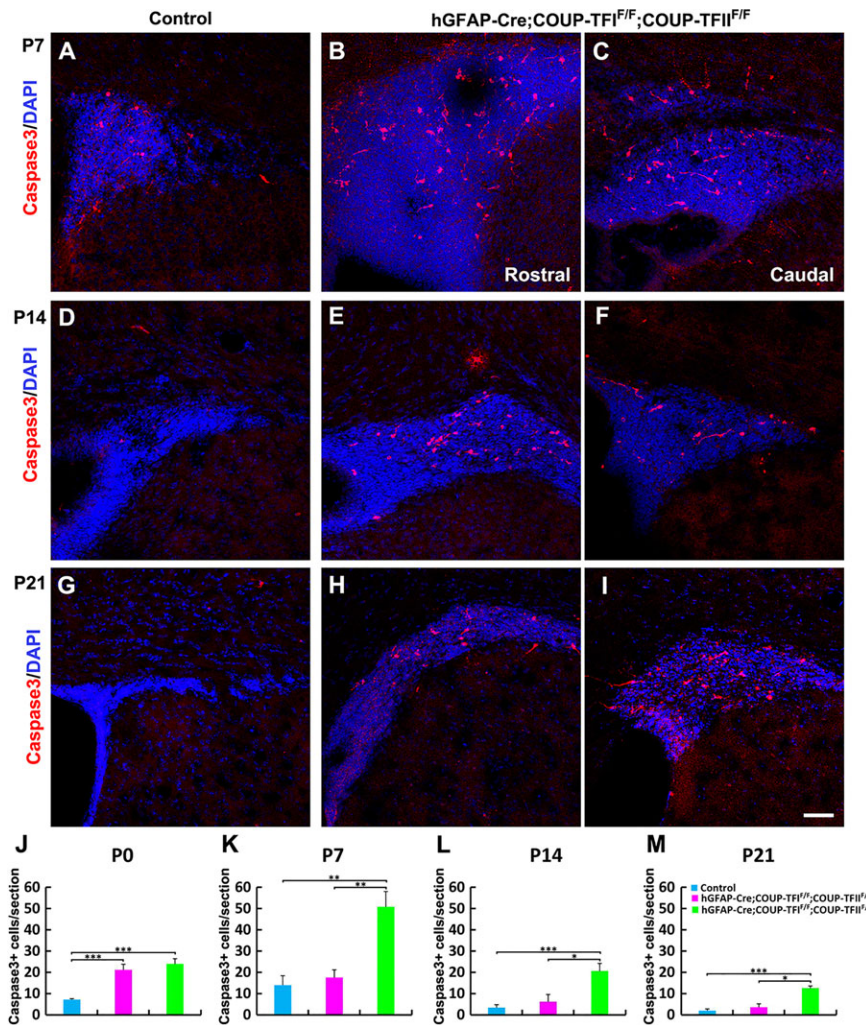
Fig. 8. Apoptotic cells are significantly increased in number in COUP-TF double conditional mutant SVZ. (A-I) Cleaved caspase 3 staining showed an increase in programmed cell death in the SVZ of the hGFAP-Cre; COUP-TFI[flox/flox]; COUP-TFII[flox/flox] mouse (B,C,E,F,H,I) compared with control mice (A,D,G) at P7, P14 and P21. (J-M) Quantification of caspase 3+ cells in the SVZ per section; *$P<0.05$; **$P<0.01$; *** $P<0.001$. Data are mean±s.e.m. Scale bar: 50 µm.

upregulation of COUP-TFII in the SVZ, RMS and deep GCL of the OB; (4) genetic conditional inactivation of only COUP-TFI reduced the expression of TH in periglomerular cells in the OB without reducing Pax6 expression in these cells (Bovetti et al., 2013); (5) genetic conditional inactivation of both COUP-TFI and COUP-TFII in the SVZ results in severe loss of Pax6+ cells and slightly reduced numbers of CR+ interneurons in the GCL; (6) cell proliferation is increased in the COUP-TF conditional mutant SVZ via upregulation of expression of the proneural gene *Ascl1*; (7) COUP-TF-deficient SVZ neuroblasts have impaired migration, resulting in ectopic accumulation of DCX+, CR+ and NeuN+ cells in the SVZ; (8) programmed cell death is significantly increased in the SVZ of COUP-TF conditional mutants. Taken together, these results suggest that transcription factors COUP-TFI and COUP-TFII coordinately regulate the proliferation, migration and survival of SVZ cells that mainly give rise to OB GCs.

Redundant functions of COUP-TFI and COUP-TFII

A recent study demonstrates that COUP-TFI controls the expression of TH in adult dopaminergic interneurons in the OB glomerular layer in an activity-dependent manner (Bovetti et al., 2013), suggesting that COUP-TFI has its intrinsic function in the maintenance of the dopaminergic interneurons in the adult OB. COUP-TFI is also expressed in the GCL of the OB (Fig. 1) (Bovetti et al., 2013), but it has remained largely unknown whether COUP-TFI has physiological functions in the GCL.

COUP-TFI is expressed in the pallial and subpallial VZ and SVZ during development (Faedo et al., 2002; Lodato et al., 2011). NSCs in the adult SVZ are heterogeneous and extensively distributed; they are derived from the radial glia from the cortex, septum, LGE, MGE and CGE (Merkle et al., 2007, 2014; Young et al., 2007; Alvarez-Buylla et al., 2008; Kriegstein and Alvarez-Buylla, 2009). Consistent with this notion, in the present study, we first identified COUP-TFI expression mainly in a subpopulation of neural stem/progenitor cells (GFAP+ and Ascl1+) in the caudal SVZ. To our knowledge, this is the first time heterogeneity among NSCs in the rostral and caudal SVZ of the postnatal brain has been shown with respect to COUP-TFI expression, much like its expression in the embryo (Zhou et al., 2001; Faedo et al., 2008; Alfano et al., 2011; Lodato et al., 2011; Borello et al., 2014). In addition, COUP-TFI+ migratory neuroblasts (DCX+) in the SVZ and RMS, COUP-TFI+ mature interneurons in the glomerular layer and GCL of the OB were also observed (Fig. 1A) (Bovetti et al., 2013). Therefore, COUP-TFI may also play a functional role in regulating GC development in the OB. However, in the hGFAP-Cre; COUP-TFI[flox/flox] mouse OB where COUP-TFI expression was successfully removed, only TH expression was reduced in the OB glomerular layer without reducing the number of Pax6+ periglomerular cells (see Fig. 9) (Bovetti et al., 2013). Other phenotypic abnormalities in OB GCs were not observed, perhaps due to redundancy with COUP-TFII.
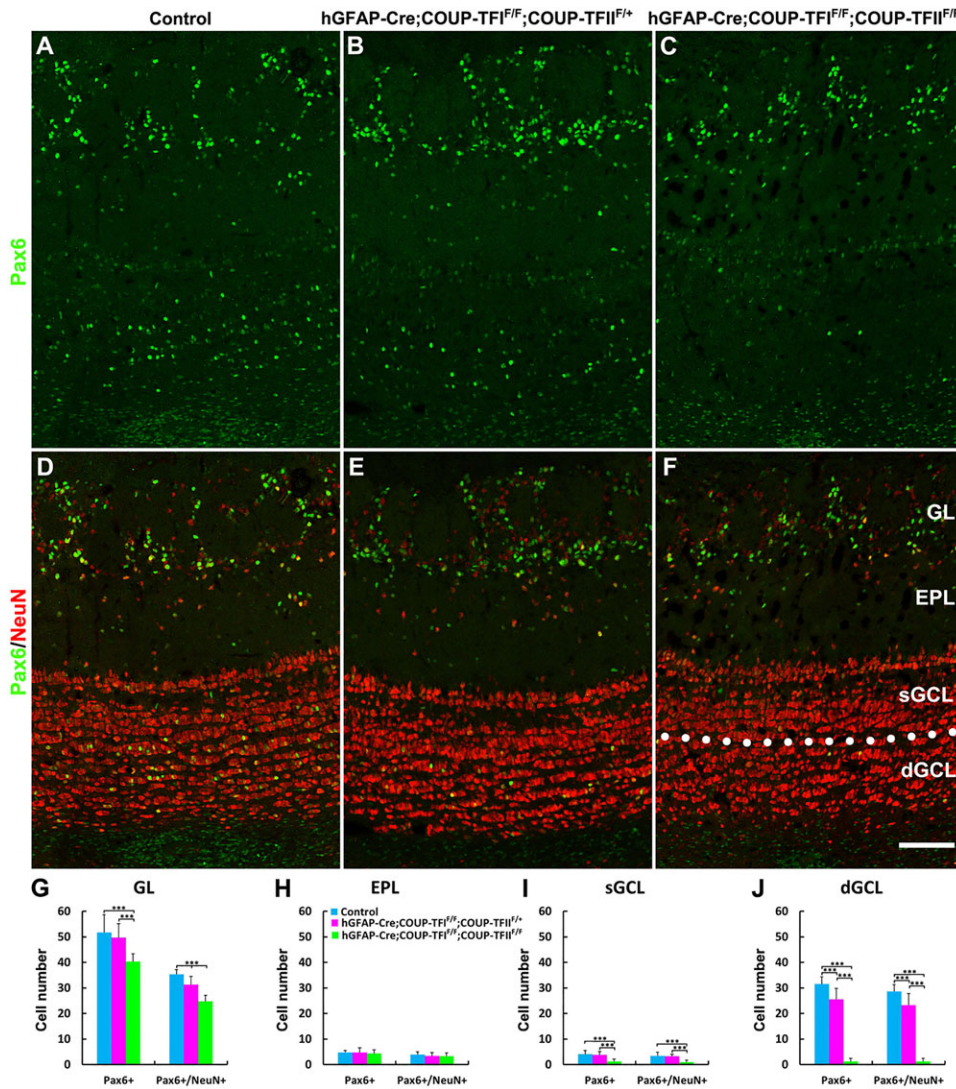
Fig. 9. Pax6+ mature interneurons in the deep GCL of the OB are lost in COUP-TF double conditional mutants. (A-F) In hGFAP-Cre; COUP-TFI[flox/flox]; COUP-TFII[flox/flox] double conditional mutant mice, very few Pax6+/NeuN+ cells in the deep GCL of the OB were detected (C,F). Pax6+/NeuN+ cells in the deep GCL of the OB were also reduced in number in hGFAP-Cre; COUP-TFI[flox/flox]; COUP-TFII[flox/+] mice (B,E) compared with controls (A,D). All OB sections were from P21 mice. (G-J) Quantification data from above experiments. ***$P<0.001$. Data are mean± s.e.m. dGCL, deep GCL; sGCL, superficial GCL; GL, glomerular layer; EPL, external plexiform layer. Scale bar: 100 µm.

Only a few studies are available on COUP-TFII function in the OB, as the expression of COUP-TFII is detected in only a few cells. However, we found that COUP-TFII expression was upregulated in the SVZ, RMS and deep GCL of the OB when COUP-TFI was selectively inactivated in these regions. This indicates that the function of COUP-TFII is likely to compensate in the absence of COUP-TFI expression in the GCL of the OB. Indeed, although conditional inactivation of only COUP-TFI in the NSCs could result in an increase in cell proliferation, DCX+, CR+ and NeuN+ cell accumulation in the SVZ, we observed more prominent features of the increase in cell proliferation, neuroblast impaired migration, ectopic maturation and cell death in the SVZ of COUP-TF double conditional mutants. This is why Pax6+ cells were severely reduced and CR+ cells were mildly reduced in the GCL of COUP-TF double conditional mutant OB, but not in COUP-TFI single conditional mutant OB. Because the expression of COUP-TFII was not upregulated in the superficial GCL and glomerular layer of the OB, the numbers of Pax6+ cells were only slightly reduced in these regions (Fig. 9).

Proneural transcription factor Ascl1 is required for stem/progenitor cell activation in the adult SVZ (Andersen et al., 2014). Ascl1 is able to initiate Notch signaling as it can directly induce expression of Notch ligands, such as Dll1 and Dll3, which bind to the Notch receptor on neighboring cells (Castro et al., 2006; Henke et al., 2009; Castro and Guillemot, 2011). Moreover, Ascl1 can also directly regulate a large number of genes that control cell cycle progression (Castro et al., 2011; Andersen et al., 2014). In the present study, after conditionally inactivating COUP-TFI or COUP-TFI/II, we found significant increase in the number of Ascl1+ neural stem/progenitor cells, leading to further increase in DCX+ cells in the SVZ. Thus, the increase in cell proliferation in the COUP-TF mutant SVZ could be due to upregulating Ascl1 expression. Notably, loss of function of COUP-TFI that caused increased cell proliferation in both pallium (Faedo et al., 2008) and subpallium (Lodato et al., 2011) was also observed during mouse early embryogenesis. COUP-TFI and COUP-TFII expression patterns in the adult rhesus monkey and human OB are very similar to those in mice, further suggesting the conserved molecular mechanisms that may regulate the development of Pax6+ interneurons in the OB across mammalian species.

Cell migration defect and cell death of COUP-TFI/COUP-TFII-deficient SVZ neuroblasts

Previously, gain-of-function studies have provided evidence that COUP-TFs regulate GABAergic interneuron migration in distinct migratory paths (Tripodi et al., 2004). Moreover, the siRNA-

mediated knockdown strategy showed that COUP-TFII is required for CGE cell migration in the caudal direction (Kanatani et al., 2008). In our study, loss-of-function studies in embryonic and postnatal SVZ (using hGFAP-Cre and Nestin-CreER mice) revealed that COUP-TFI and COUP-TFII regulated specific subpopulation of SVZ neuroblast migration. Matrigel SVZ explant assay showed that this process occurred in a cell-autonomous manner. Currently, we do not know the cause of the defective migration of COUP-TFI/COUP-TFII-deficient SVZ cells. The molecular mechanisms underlying this phenotype need to be elucidated in future research.

An expanded SVZ observed in the COUP-TF single and double conditional mutants may directly result from the accumulation of DCX+ immature neuroblasts, and CR+ and NeuN+ mature neurons. However, because most excitatory projection neurons in the neocortex also lost COUP-TFI expression, which can lead to the changes in cortical area patterning and altered axonal projections (Zhou et al., 1999, 2001; Armentano et al., 2006, 2007; Faedo et al., 2008; Borello et al., 2014), these phenotypes may also indirectly result in an expanded SVZ.

Apoptotic cell death was significantly increased in the COUP-TF double conditional mutant SVZ. This may be due to migration defect of neuroblasts. Because ectopic accumulation of CR+ and NeuN+ mature neurons were observed in the SVZ, this suggests that COUP-TFI and COUP-TFII are not required for the maturation of these cells. However, COUP-TFI and COUP-TFII may directly regulate the survival of these cells, as a significant increase in apoptotic cells in the SVZ was observed as early as P0. Both a significant increase in apoptotic cells in the SVZ and a significant decrease in Pax6+ cells in the GCL in hGFAP-Cre; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/+}$ compound conditional mutant mice were also observed in our study, suggesting that COUP-TFs regulate these processes in a dose-dependent manner. Indeed, we even found a slight upregulation of COUP-TFII expression in the SVZ of the hGFAP-Cre; COUP-TFI$^{flox/+}$ mice (data not shown).

In summary, our work showed that transcription factors COUP-TFI and COUP-TFII coordinately regulate the proliferation, migration and survival of adult SVZ cells. We hypothesize that the SVZ defects may specifically or preferentially affect progenitors of GCs in the OB.

MATERIALS AND METHODS

Animals and tissue preparation
Mouse colonies were maintained and experiments were conducted in accordance with the guidelines set by the institutions where the work was carried out. hGFAP-Cre mice (Zhuo et al., 2001), Emx1-Cre (Gorski et al., 2002) and Nestin-CreER mice (Lagace et al., 2007) were obtained from the Jackson Laboratory. COUP-TFI$^{flox/flox}$ and COUP-TFII$^{flox/flox}$ mice were genotyped as previously described (Takamoto et al., 2005; Tang et al., 2010). Mice used in this study were of mixed background. Only littermates were used for the comparisons. COUP-TFI and COUP-TFII heterozygous and/or homozygous floxed mice (without Cre or CreER) were used as controls.

Mice (either sex) were deeply anesthetized before intracardiac perfusion with 4% paraformaldehyde. Twelve μm or free-floating 30 μm coronal sections of the mouse brain were collected. Adult human and rhesus monkey OB were collected as previously described with informed consent and in accordance with institutional guidelines (Wang et al., 2011; Ma et al., 2013).

BrdU injections
To pulse-label newly born cells, BrdU (50 mg/kg; Sigma) was administered once into P10 control and conditional mutant mice by intraperitoneal injection and these mice were sacrificed 2 h after BrdU injection.

Tamoxifen injections
P10 control and conditional mutant mice were given 0.5 mg tamoxifen solution (Sigma) at 20 mg/ml in corn oil by intraperitoneal injection and these mice were sacrificed at P20 (10 days after tamoxifen treatment).

Immunohistochemistry
Immunohistochemistry was performed on cryostat 12 μm or 30 μm sections according to previous studies (Li et al., 2011; Ma et al., 2013). Sections were briefly boiled in 10 mM sodium citrate (pH 6.0) for COUP-TFI, COUP-TFII and Ascl1 antigen retrieval. The following primary antibodies were used: rabbit anti-caspase 3 (1:500; Cell Signaling, #9662); rabbit anti-calbindin (1:5000; Swant, CB38a); mouse anti-COUP-TFI (1:500; Perseus Proteomics, PP-H8132-00); mouse anti-COUP-TFII (1:500; Perseus Proteomics, PP-H7147-00); mouse anti-CR (1:1000; Swant, 6B3); rabbit anti-CR (1:1200; Swant, 7697); goat anti-DCX (1:500; Santa Cruz, sc-8066); rabbit anti-GFAP (1:700; Dako, Z 033401); mouse anti-Ki67 (1:1000; BD Pharmingen, 550609); rabbit anti-Ascl1 (1:500; Cosmo Bio, SK-T01-003); mouse anti-NeuN (1:500; Millipore, MAB377); rabbit anti-NeuN (Fox3, 1:1000; Biosensis, R-3770-100); rabbit anti-Pax6 (1:1200; MBL, PD022); mouse anti-TH (1:500; Millipore, Mab318); rabbit anti-S100β (1:2000; Dako, Z0311); goat-anti Sp8 (1:500; Santa Cruz, sc-104661); rat anti-BrdU (1:50; Accurate Chemical, OBT0030); and chicken anti-β-gal (1:2000; Abcam, ab9361).

The following secondary antibodies (Jackson, 1:300, unless stated otherwise) were used: donkey anti-mouse Cy3 (715-165-151), donkey anti-mouse Cy2 (715-225-151), donkey anti-mouse Biotin (715-065-150), donkey anti-rabbit Cy3 (711-165-152), donkey anti-rabbit Cy2 (711-225-152), donkey anti-goat Alexa Fluor 488 (Molecular Probes, A-11055; 1:600), donkey anti-chicken Cy3 (703-165-155) and donkey anti-rat Alexa Fluor 488 (712-545-150). Secondary antibodies were incubated for 4 h at room temperature. Fluorescently stained sections were stained with DAPI (Sigma, 200 ng/ml) for 2-3 min and coverslipped with Gel/Mount (Biomeda). Diaminobenzidine (DAB) was used to visualize the immunoperoxidase staining.

Matrigel explant assay
SVZ explants from postnatal day 1 (P1) COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ pups were dissected and cultured in Matrigel in DMEM/F12 media (Invitrogen) supplemented with B27, N2, FGF2 (Invitrogen) and adenovirus expressing Cre recombinase (3 μl, 1×10^{10} infectious particles/ml) for 4 days (Wichterle et al., 1997). Cultures were then fixed for 5 min in 4% paraformaldehyde. After washing off the PFA with PBS, X-gal staining was performed as described previously (Wichterle et al., 1999).

Microscopy
All immunofluorescence staining images were acquired using an Olympus FV1000 confocal microscope. Z-stack confocal images were reconstructed using FV10-ASW software. Immunoperoxidase and X-gal staining images were acquired using an Olympus BX 51 microscope. Images were cropped, adjusted and optimized in Photoshop CS3.

Data quantification
The SVZ of P21 mice was divided into the rostral SVZ (relative to bregma: 1.2 mm to 0 mm), intermediate SVZ (0 mm to −1.2 mm) and caudal SVZ (−12 mm to −2.7 mm) based on previous studies (Doetsch and Alvarez-Buylla, 1996). A total of 3-5 samples for each group were used. Three sections from the rostral, intermediate and caudal SVZ were quantified. For the quantification of OB cells, 3-5 sections were counted. In this study, images were first captured using an Olympus FV1000 confocal microscope (20× objective) and immunostained cells were then counted using Photoshop CS3. For quantification of cells in the OB section, we counted 300 μm×650 μm area that includes the glomerular layer, EPL and GCL per section.

To quantify the effect of COUP-TFI/COUP-TFII double mutation on the migration postnatal SVZ cells in Matrigel cultures, we selected 10 *lacZ*+ cells and 10 *lacZ*-negative cells positioned furthest away from the center of each explant and measured their distance from its edge. Five explants from

5 P1 SVZ were analyzed. Results are presented as means±s.e.m. The statistical significance of the difference was evaluated by one-way ANOVA or Student's *t*-test. *P*-values of less than 0.05 were considered significant.

Supplementary material
Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.115279/-/DC1

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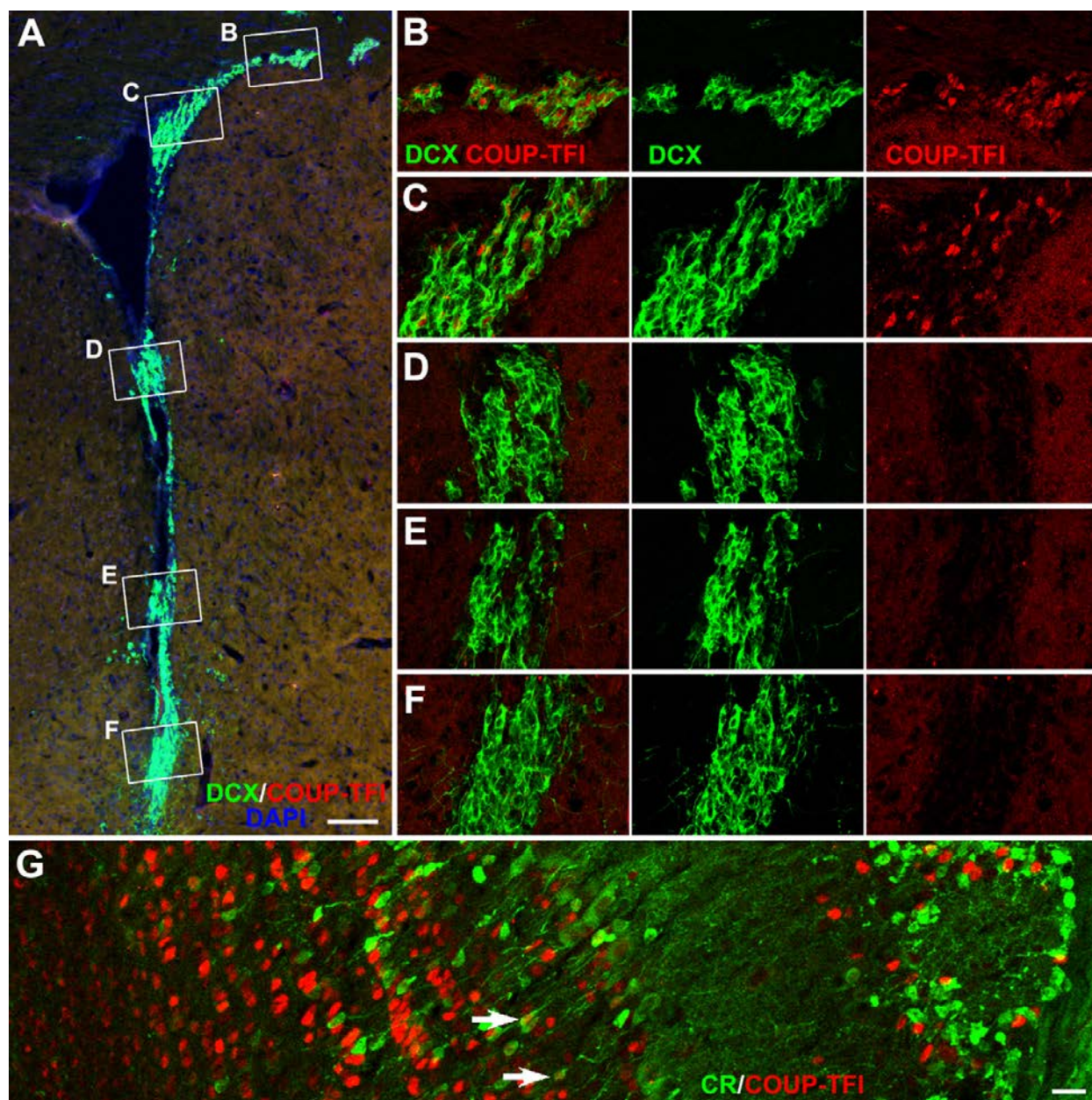
Figure S1. COUP-TFI is expressed in a subpopulation of neuroblasts in the adult mouse SVZ. (A) COUP-TFI/DCX double-immunostained P42 mouse brain section. **(B-F)** Higher-magnification images of the boxed areas in (**A**) showing COUP-TF+/DCX+ cells in the dorsal SVZ (**B, C**); COUP-TFI+ cells were not in the lateral, ventral or medial SVZ at the rostral part of the lateral ventricle. **(G)** COUP-TFI was expressed in about 12% CR+ cells in the GCL, but not in the periglomerular layer. Scale bars: 200 μm in A; 100 μm in G for B-G.
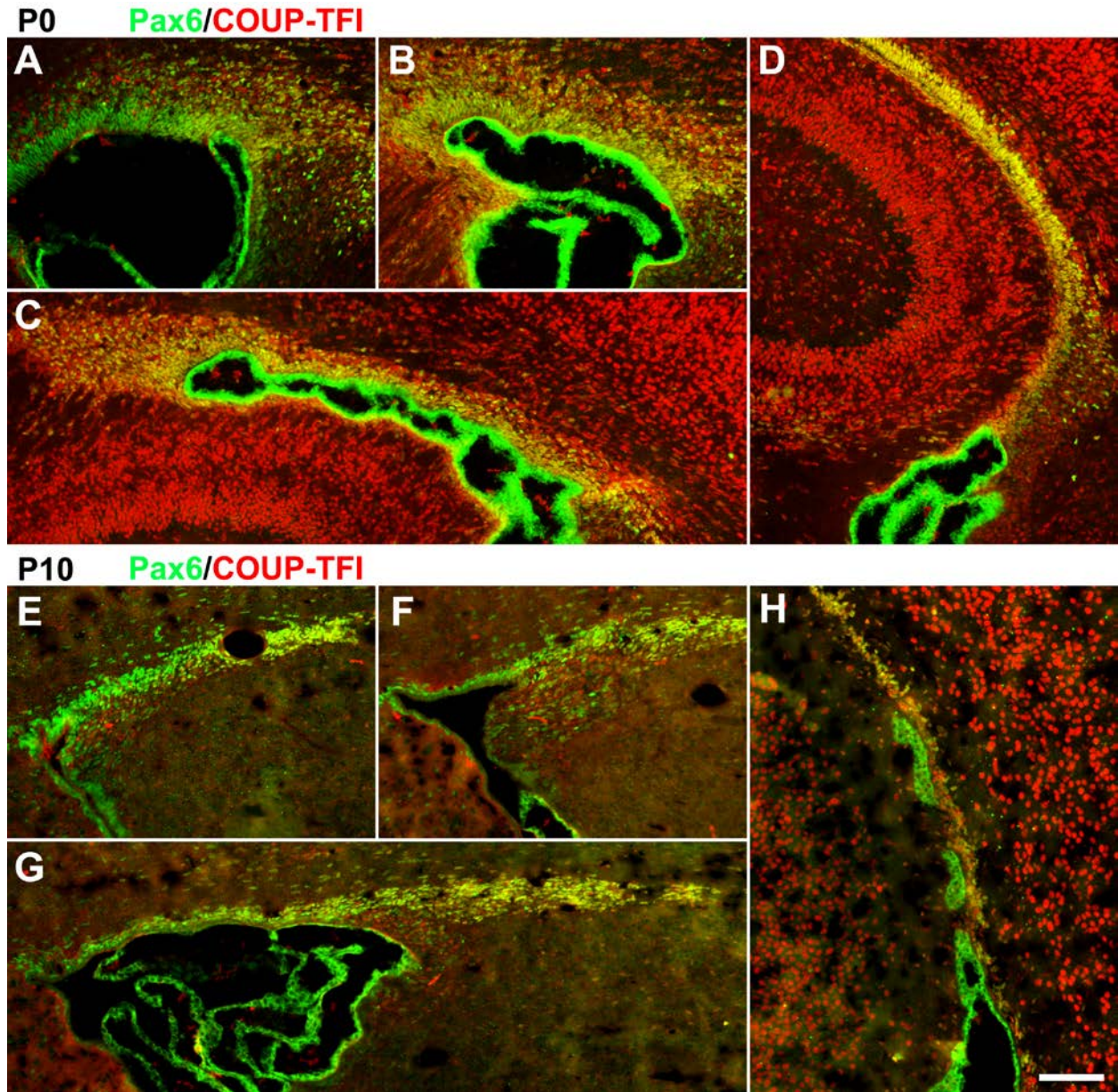
Figure S2. COUP-TFI is expressed in a gradient of low rostral to high caudal within the P0 and P10 SVZ. (A-H) Pax6/COUP-TFI double-immunostained coronal brain sections at different rostrocaudal levels of the SVZ at P0 (**A-D**) and P10 (**E-H**). Scale bar: 100 μm in H for A-H.
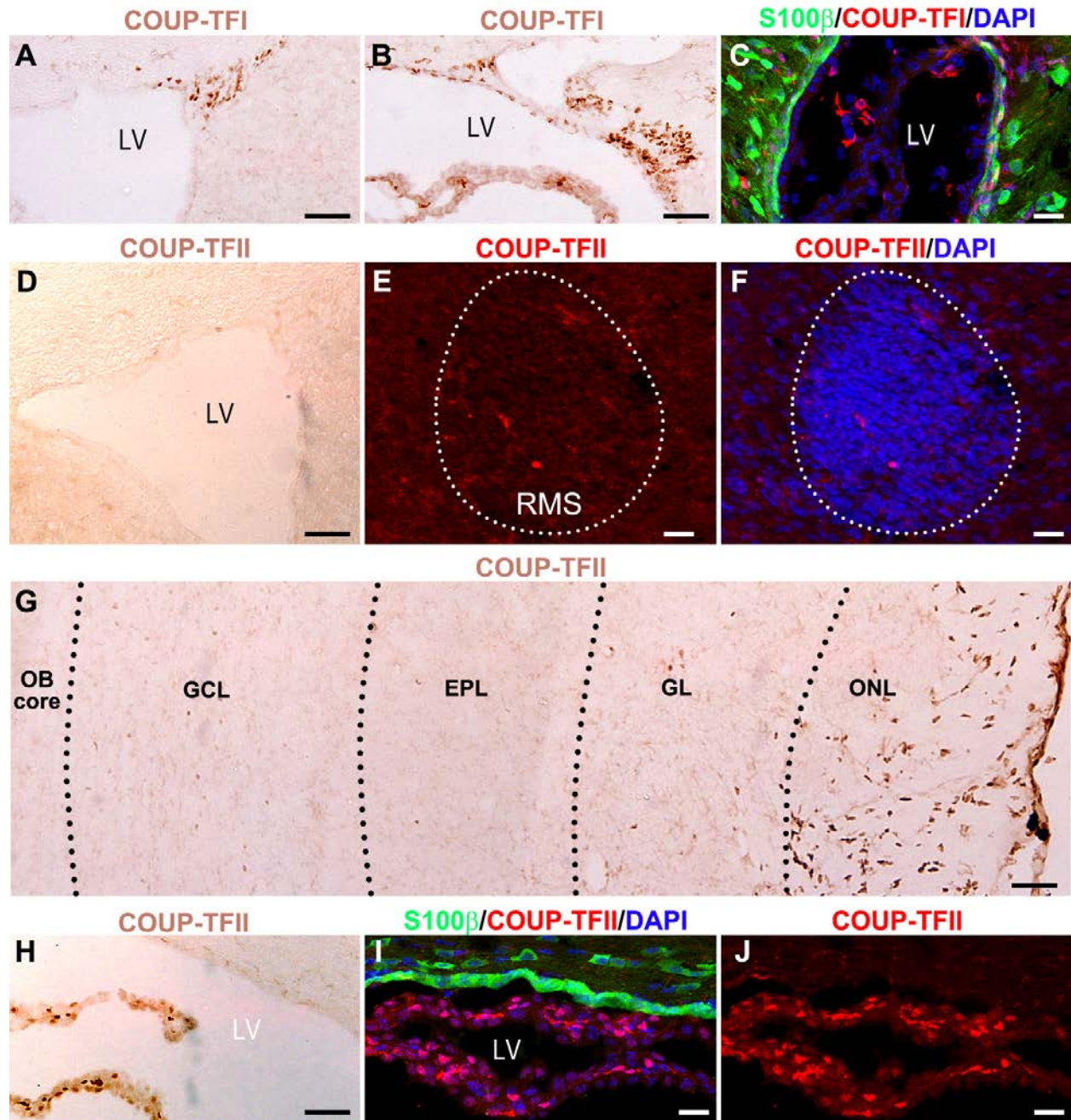
Figure S3. Few COUP-TFII+ cells exist in the adult SVZ-RMS-OB pathway. (A-C) COUP-TFI is expressed in ependymal cells in the caudal lateral wall of the lateral ventricle in adult mice (**B, C**) but not in ependymal cells at the rostral level (**A**). (**D-G**) Very few COUP-TFII+ cells were in the adult SVZ-RMS-OB pathway. Note that COUP-TFII is strongly expressed in the ONL (**G**). (**H-J**) COUP-TFII is expressed in the choroid plexus. LV: lateral ventricle; GCL: granule cell layer; EPL: external plexiform layer; GL: periglomerular layer; ONL: olfactory nerve layer. Scale bars: 50 µm in A, B, D, G, H; 20 µm in C, E, F, I, J.
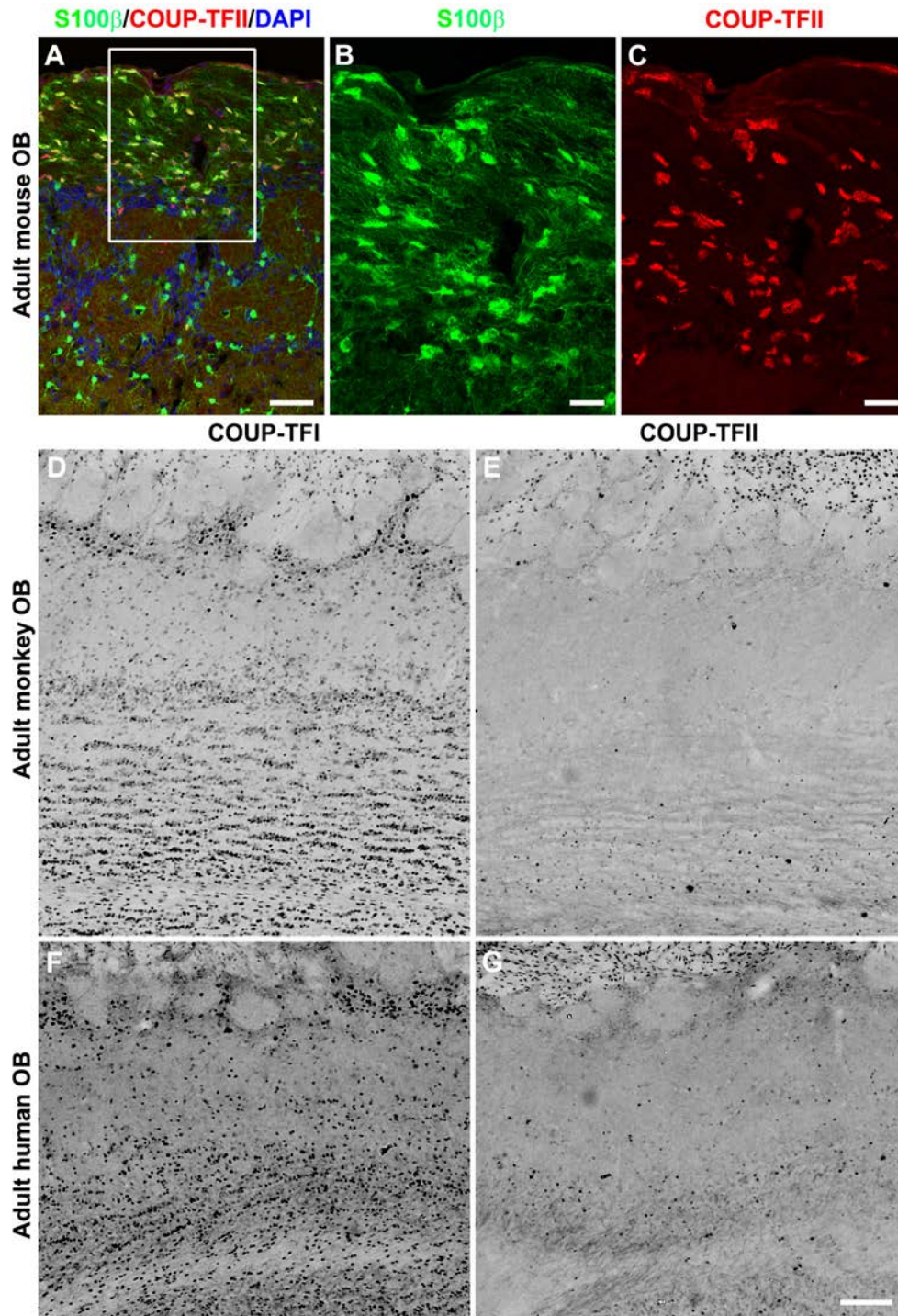
Figure S4. Expression patterns of COUP-TFI and COUP-TFII in the adult human and rhesus monkey OB are similar to those in adult mice. (**A-C**) COUP-TFII is strongly expressed in olfactory ensheathing cells (S100β+) in the olfactory nerve layer of the adult mouse OB. (**D-G**) COUP-TFI+ cells were abundant (**D, F**), whereas only a small number of COUP-TFII+ cells (**E, G**) were in the adult human and monkey OB. Scale bars: 50 μm in A; 20 μm in B, C; 100 μm in G for D-G.
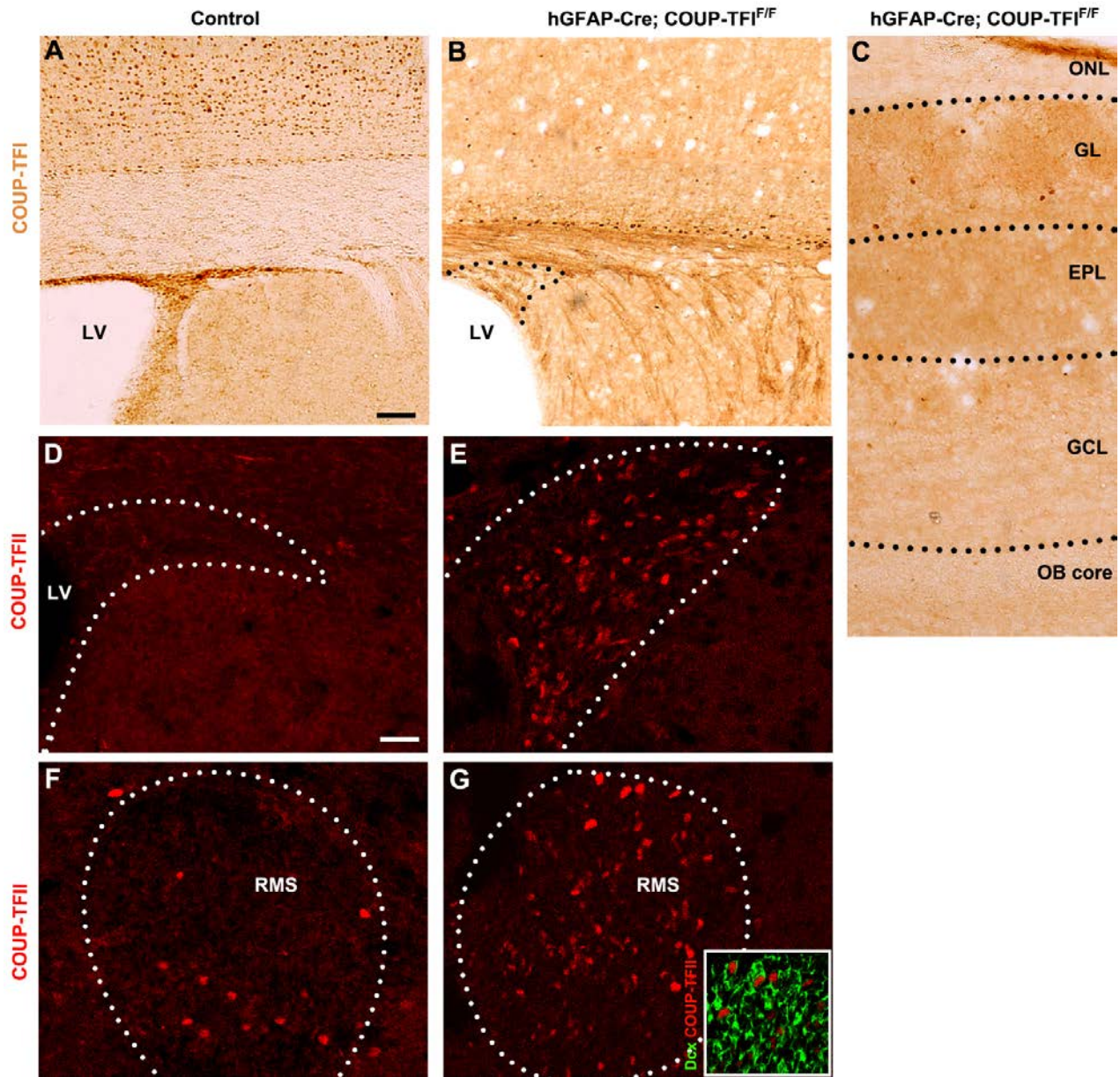
Figure S5. COUP-TFII expression is upregulated in the SVZ and RMS of the COUP-TFI conditional mutant mice. (**A-C**) COUP-TFI expression was eliminated in the SVZ and OB of adult hGFAP-Cre; COUP-TFI^(flox/flox) mice. Note that many projection neurons in neocortical layer VI still expressed COUP-TFI in hGFAP-Cre; COUP-TFI^(flox/flox) mice, as the hGFAP-Cre exhibits excision of floxed alleles in mouse cortical radial glia around E13.5. (**D-G**) In control mice, very few COUP-TFII+ cells were identified in the SVZ and RMS (**D, F**), but COUP-TFII expression in the SVZ and RMS was significantly upregulated in hGFAP-Cre; COUP-TFI^(flox/flox) mice (**E, G**). Insert in (**G**) showed that a subpopulation of DCX+ neuroblasts in the RMS expressed COUP-TFII. All brain sections were from P21 mice. Scale bars: 50 μm in A for A-C; 100 μm in D for D-G.
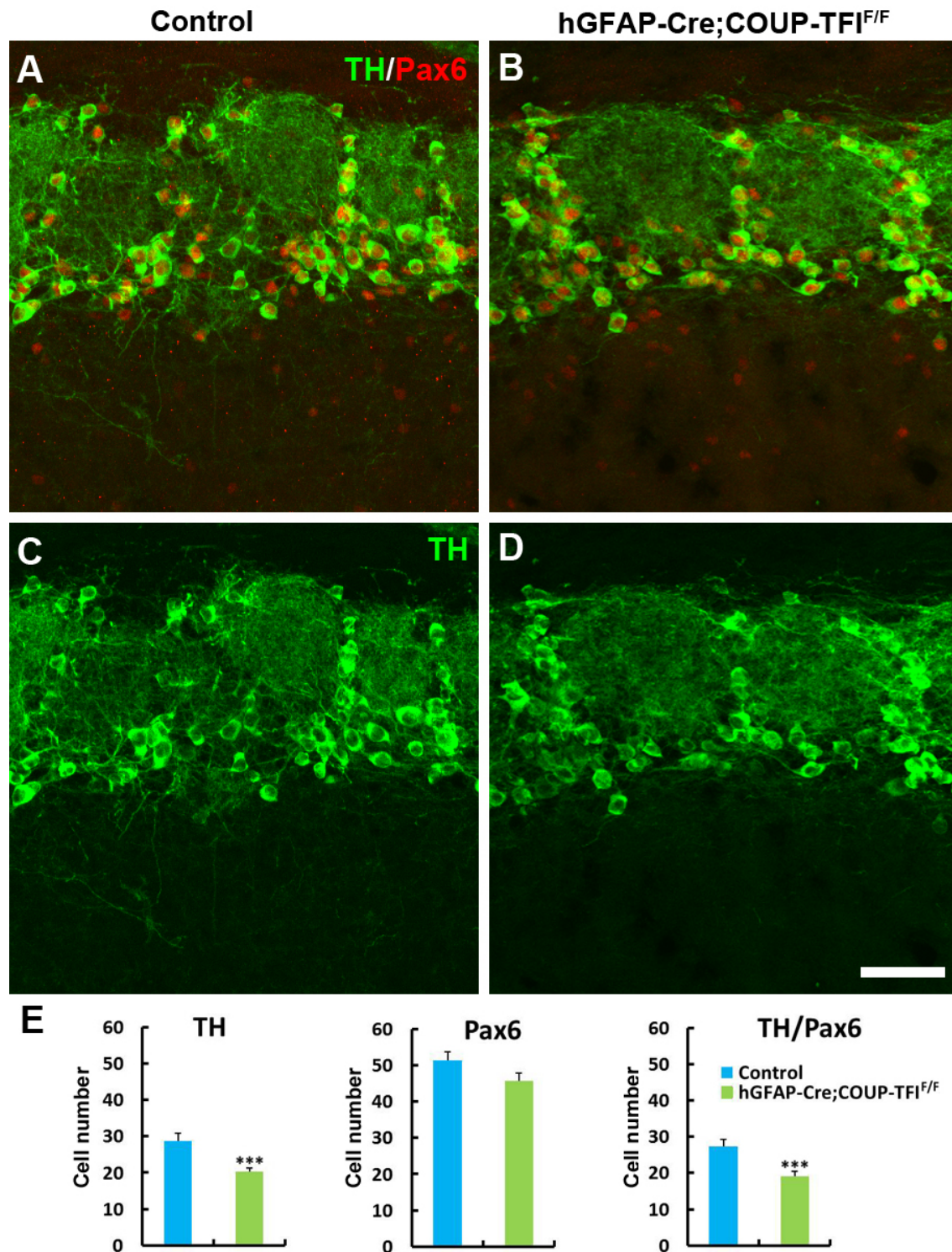
Figure S6. TH expression is reduced in the glomerular layer of the COUP-TFI conditional mutant OB. (**A-D**) OB sections double immunostained for TH and Pax6. (**E**) The number of TH+ and TH+/Pax6+, but not Pax6+ periglomerular cells was significantly reduced in the hGFAP-Cre; COUP-TFI$^{flox/flox}$ mouse OB compared to controls at P21. *** $p<0.001$. Scale bar: 50 μm in D for A-D.
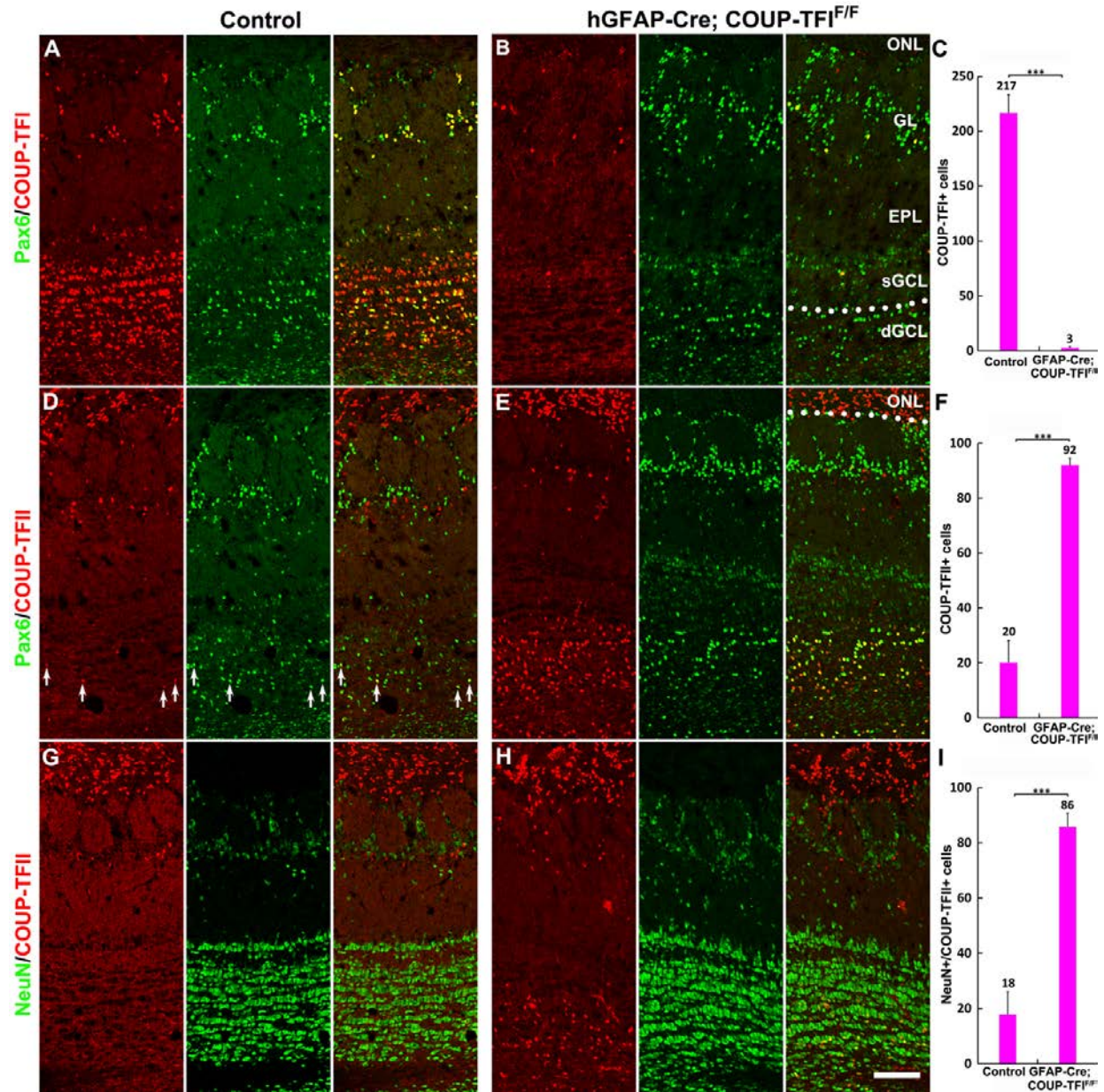
Figure S7. COUP-TFII expression is upregulated in the deep GCL of the COUP-TFI conditional mutant OB. (**A-C**) Only a few COUP-TFI+ cells remained in hGFAP-Cre; COUP-TFI^flox/flox mouse OB compared to controls. Approximately 90% Pax6+ cells in the glomerular layer and about 74.8% Pax6+ cells in the GCL expressed COUP-TFI in the control OB. (**D-I**) A very small number of COUP-TFII+ cells were in the GL and GCL of the control mouse OB, whereas COUP-TFII expression was significantly upregulated in the mature GCs in the deep GCL of hGFAP-Cre; COUP-TFI^flox/flox mice (**F, I**); about 78% of COUP-TFII+ cells in the GCL in the control (**D,** arrows) and COUP-TFI conditional mutant (**E**) mouse OB expressed Pax6. Note that COUP-TFII expression was not upregulated in the superficial GCL, EPL and GL (**E, H**). All OB sections were from P21 mice. dGCL, deep GCL; sGCL, superficial GCL; *** p<0.001. Scale bar: 100 µm in H for A-H.
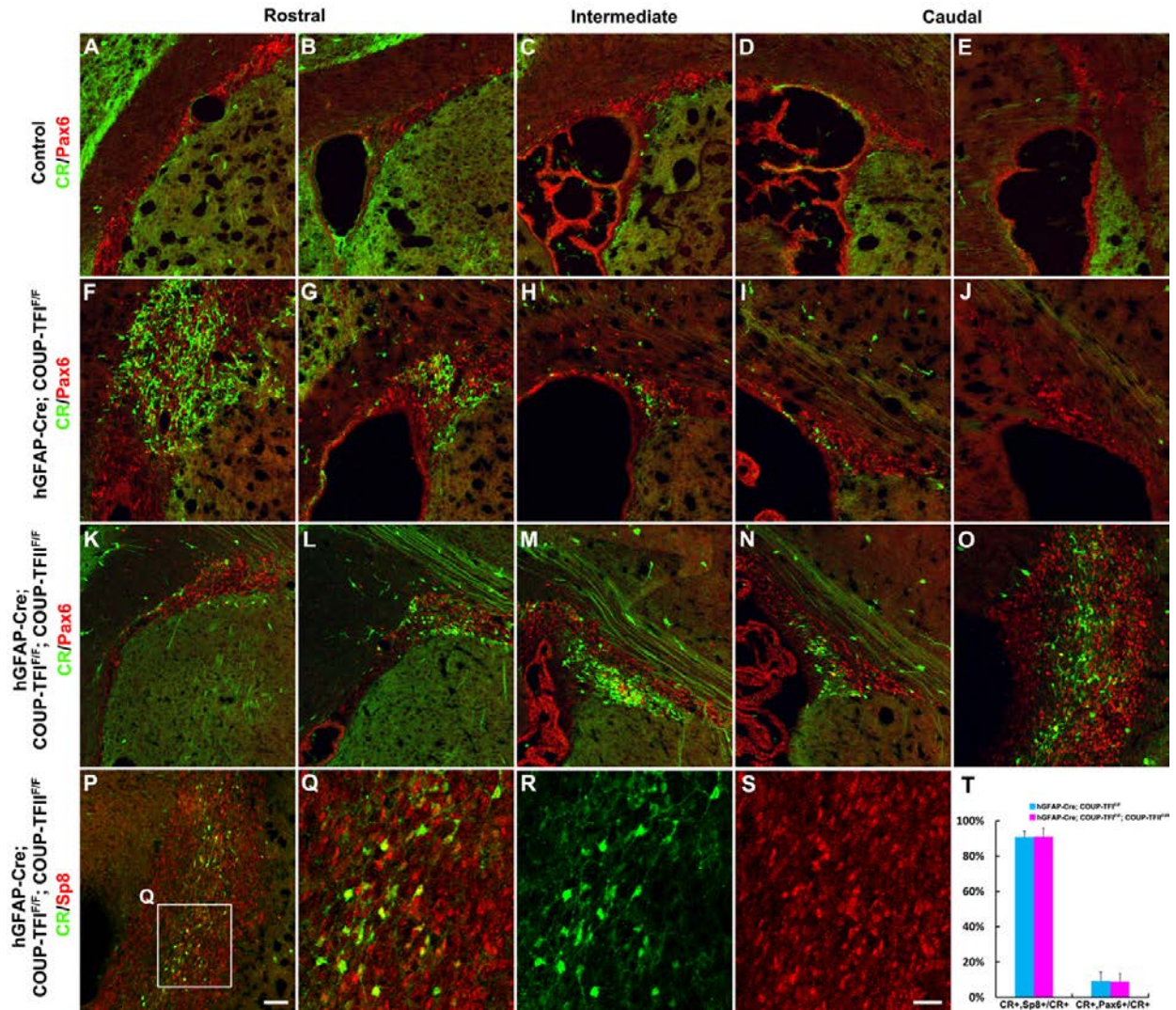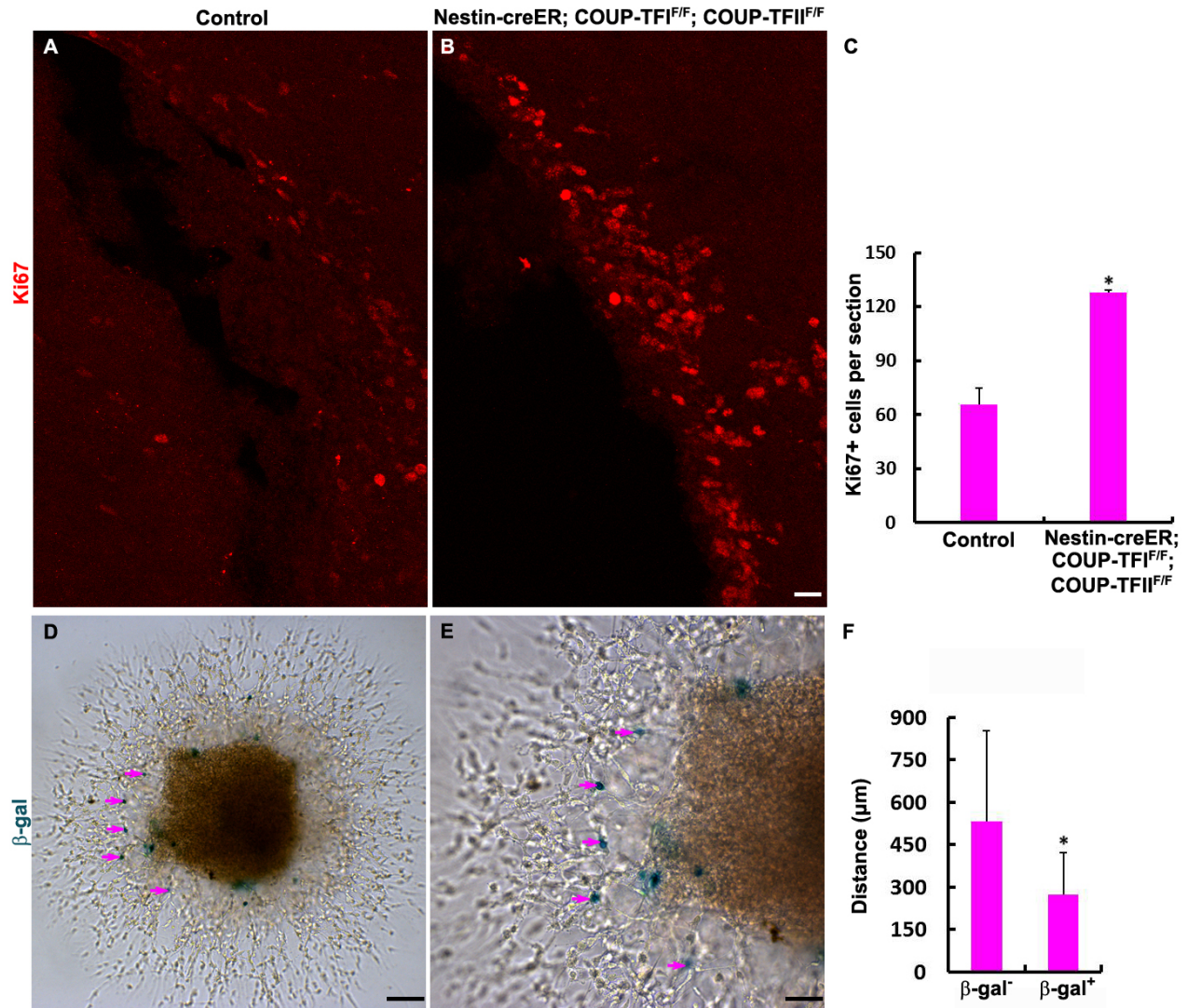
Figure S8. CR+ cells accumulate in the SVZ of COUP-TFs conditional mutant mice. (A-O)
CR/Pax6 double-immunostaining showed that very few CR+ cells were in the control SVZ (**A-E**), whereas a subset of CR+ cells accumulate in the SVZ of hGFAP-Cre; COUP-TFI$^{flox/flox}$ mice (**F-J**) and hGFAP-Cre; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ mice (**K-O**). (**P-S**) CR/Sp8 double immunostained sections of COUP-TFs double conditional mutant SVZ. (**T**) Quantification data showed that about 91% of CR+ cells expressed Sp8 and about 9% of CR+ cells expressed Pax6. All brain sections were from P21 mice. Scale bars: 50 μm in P for A-P; 20 μm in S for Q-S.
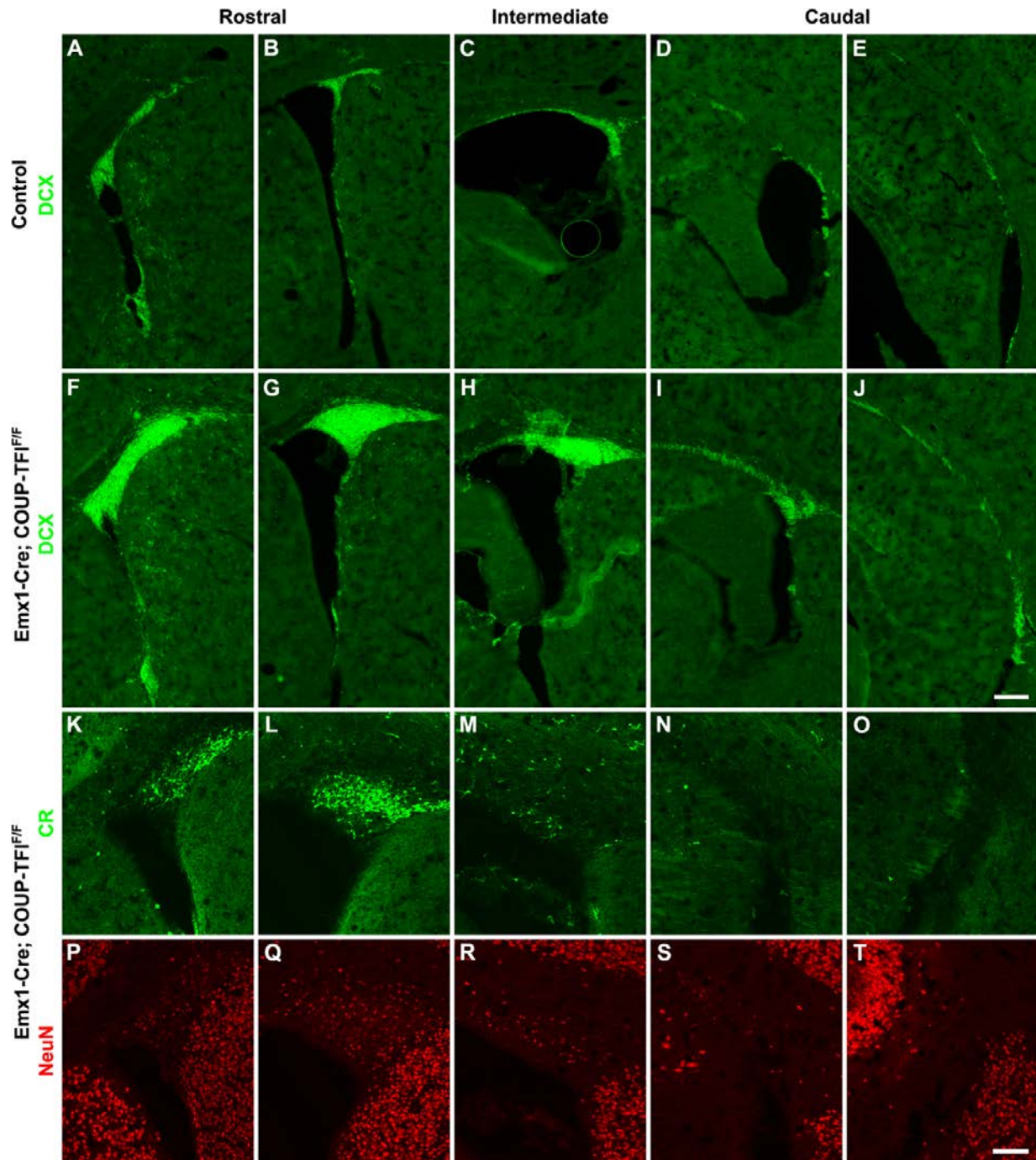
Figure S9. DCX+, CR+ and NeuN+ cells accumulate in the SVZ of Emx1-Cre; COUP-TFI^{flox/flox} conditional mutants. (A-E) DCX+ cells in the P21 control mouse SVZ. **(F-J)** More DCX+ cells were in the SVZ of P21 Emx1-Cre; COUP-TFI^{flox/flox} conditional mutant mice compared to controls. **(K-T)** A subset of CR+ (**K-O**) and NeuN+ (**P-T**) cells accumulate in the SVZ of Emx1-Cre; COUP-TFI^{flox/flox} conditional mutant mice at P21. Scale bars: 200 µm in J for A-J; 100 µm in T for K-T.
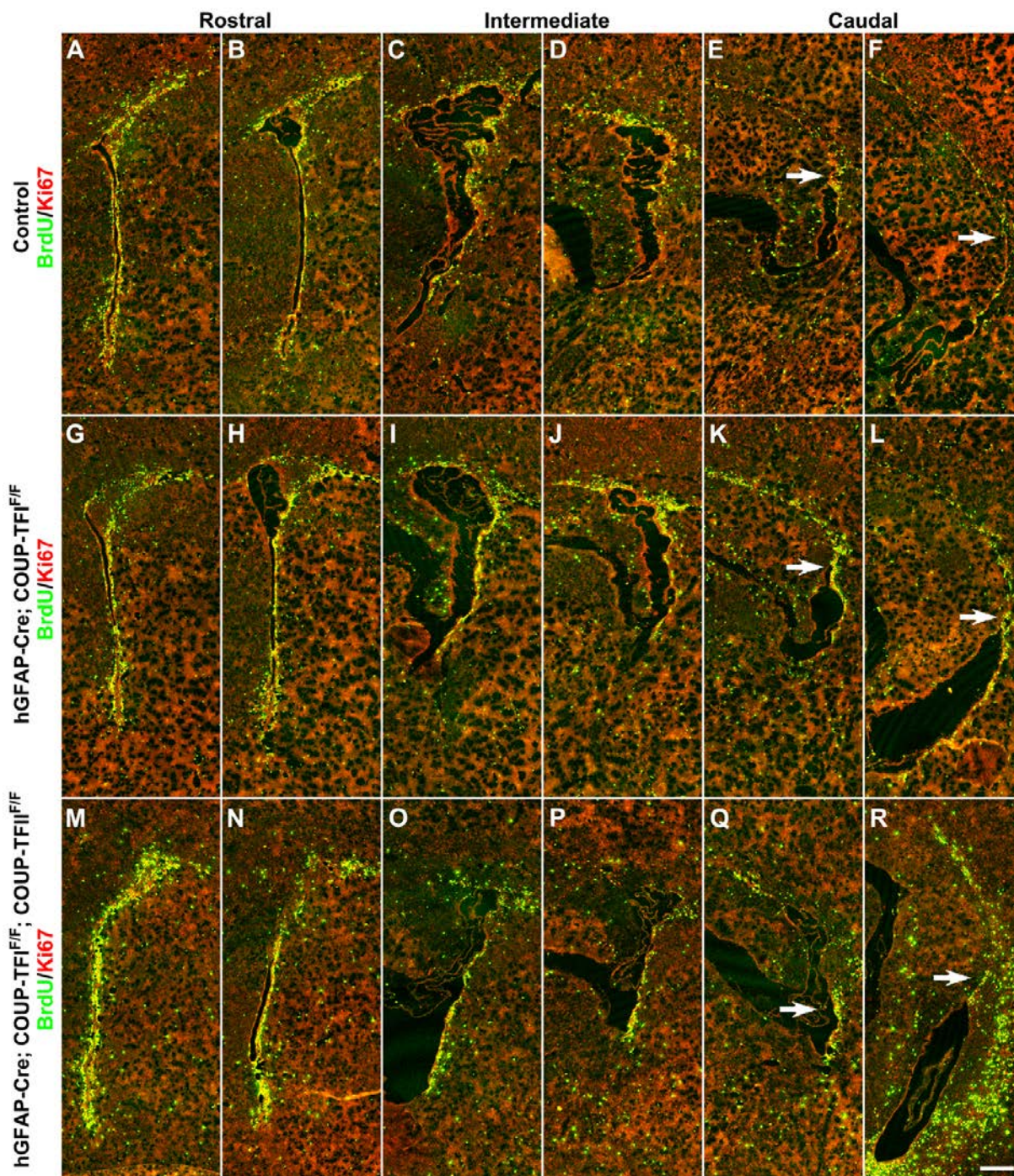
Figure S10. Cell proliferation in the SVZ of COUP-TFs conditional mutant mice. (A-R) BrdU was injected into control and COUP-TFs conditional mutant mice at P10 and mice were sacrificed 2 hours after BrdU injection. Compared to the caudal SVZ of control mice (**E, F,** arrows), more BrdU+/Ki67+ cells were observed in the caudal SVZ of hGFAP-Cre; COUP-TFI[flox/flox] mice (**K, L,** arrows) and hGFAP-Cre; COUP-TFI[flox/flox]; COUP-TFII[flox/flox] mice (**Q, R,** arrows). Scale bar: 200 μm in R for A-R.

Figure S11. Migration deficit of COUP-TFI/COUP-TFII-deficient SVZ cells ex vivo. (A-C)
Control and Nestin-CreER; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ mice were given tamoxifen at P10 by intraperitoneal injection and mice were sacrificed at P20. In the SVZ of Nestin-CreER; COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ mice, significant increase in the number of Ki67+ cells per section were observed compared to controls. **(D)** SVZ explants from P1 COUP-TFI$^{flox/flox}$; COUP-TFII$^{flox/flox}$ pups were cultured with an adenovirus expressing Cre recombinase for 4 days. Most cells (β-gal-negative) showed profuse migration into the Matrigel, whereas reduced migration of COUP-TFI-/COUP-TFII-deficient cells (β-gal+, arrows) was observed. **(E)** Higher-magnification image of β-gal+ cells in **(D)**. **(F)** Quantification of cell migration distance. * p<0.05. Scale bar: 20 μm in B for A, B; 100 μm in D; 50 μm in E.

Figure S12. CR+ GCs are reduced in the hGFAP-Cre; COUP-TFI[flox/flox]; COUP-TFII[flox/flox] mouse OB. (A-F) Most CR+ cells in the OB GCL did not express Pax6. **(G, H).** The number of CR+ GCs in the hGFAP-Cre; COUP-TFI[flox/flox]; COUP-TFII[flox/flox] mouse OB were significantly reduced compared to controls at P21. * $p < 0.05$. Scale bar: 100 μm in F for A-F.