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## Selective neuronal expression of the SoxE factor, Sox8, in direct pathway striatal projection neurons of the developing mouse brain

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### Abstract

The striatum is the major component of the basal ganglia and is well known to play a key role in the control of motor function via balanced output from the indirect (iSPNs) and direct pathway striatal projection neurons (dSPNs). Little is known, however, about the molecular genetic mechanisms that control the formation of the iSPNs versus dSPNs. We show here that the SoxE family member, Sox8, is co-expressed with dSPN marker, Isl1, in the developing striatum. Moreover, dSPNs, as marked by *Isl1-cre* fate map, express Sox8 in the embryonic striatum and *Sox8-EGFPBAC* transgenic mice specifically reveal the direct pathway axons during development. These EGFP<sup>+</sup> axons are first observed to reach their midbrain target, the substantia nigra pars reticulata (SNr), at E14 in the mouse with a robust connection observed already at birth. The selective expression of EGFP in dSPNs of *Sox8-EGFPBAC* mice is maintained at postnatal time points. Sox8 is known to be expressed in oligodendrocyte precursor cells (OPCs) together with other SoxE factors and we show here that the EGFP signal co-localizes with the OPC markers throughout the brain. Finally, we show that *Sox8-EGFPBAC* mice can be used to interrogate the altered dSPN development in *Isl1* conditional mutants including aberrant axonal projections detected already at embryonic time points. Thus Sox8 represents an early and specific marker of embryonic dSPNs and the *Sox8-EGFPBAC* transgenic mice are an excellent tool to study the development of basal ganglia circuitry.

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#### ROLE OF AUTHORS

All authors contributed significantly to the research that led to preparation of this article. All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: PMS, RRW, KC. Acquisition of data: PMS, DN, RRW. Analysis and interpretation of data: PMS, RRW, KC. Drafting of the manuscript: PMS, RRW, KC. Critical revision of the manuscript for important intellectual content: PMS, RRW, KC. Obtained funding: KC. Administrative, technical, and material support: PMS, DN, RRW, KC. Study supervision: RRW, KC.

#### CONFLICT OF INTEREST

None of the authors have a known or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, the work presented in this article.

## Keywords

Basal ganglia; Development; Neural circuit; Striatonigral pathway

Basal ganglia circuits play a central role in the processing of motor, emotional and intellectual brain functions (Macpherson et al., 2014; Obeso et al., 2014). The principal structure of the basal ganglia, the striatum (a.k.a. caudate-putamen), contributes to this processing through the two major GABAergic output pathways, which comprise the indirect pathway striatal projection neurons (iSPNs) and direct SPNs (dSPNs) (Gerfen and Surmeier, 2011). Balanced activity in these two pathways is thought to be essential for normal motor control (Albin et al., 1989; Albin, 1995; Albin and Tagle, 1995; Gerfen and Surmeier, 2011). Moreover, alterations in the development and/or function of basal ganglia circuits are believed to underlie aspects of childhood neuropsychiatric disorders such as obsessive compulsive disorder (OCD) and attention deficit hyperactivity disorder (ADHD) (Leisman and Melillo, 2013). Despite this, little is known about the development of these SPN pathways at embryonic or postnatal stages.

SPNs are known to arise from the lateral ganglionic eminence (LGE) located in the ventral telencephalon, largely during the second half of embryogenesis (Deacon et al., 1994; Olsson et al., 1995, 1998; Wichterle et al., 2001). A number of developmental control genes including *Gsx1/2*, *Asc11* and *Dlx* genes are expressed by LGE progenitors and have been shown to be required for the normal development of LGE-derived neuronal subtypes, including the SPNs (Anderson et al., 1997; Casarosa et al., 1999; Corbin et al., 2000; Toresson et al., 2000; Toresson and Campbell, 2001; Yun et al., 2001; Yun et al., 2002, 2003; Long et al., 2009; Waclaw et al., 2009; Wang et al., 2009, 2011, 2013; Pei et al., 2011). A number of transcription factors mark differentiating SPNs in both the indirect and direct pathways including *Nolz1/Zfp503*, *Ctip2* and *Foxp1* (Chang et al., 2004; Tamura et al., 2004; Arlotta et al., 2008; Ko et al., 2013). Moreover, *Ctip2* and *Foxp1* have been shown to be required for the normal differentiation of SPNs (Arlotta et al., 2008; Bacon et al., 2015). The zinc finger transcription factor *Sp9* is expressed by most subventricular zone cells of the LGE, however, its expression resolves to the iSPNs at postnatal stages and is required for the proliferation and postnatal survival of this SPN subtype (Zhang et al., 2016). Conversely, the early B cell factor *Ebf1*, is known to be required for the normal development and connectivity of dSPNs, particularly in the matrix compartment (Garel et al., 1999; Lobo et al., 2006; Lobo et al., 2008). More recently, the LIM homeobox gene *Isl*, has been shown to be selectively expressed in dSPN progenitors and is required for the survival of newborn dSPNs, already at embryonic stages (Ehrman et al., 2013; Lu et al., 2014). Not all dSPNs require *Isl1* for their survival/differentiation as a portion remain in the *Isl1* mutants marked by *Ebf1* expression (Ehrman et al., 2013).

In this study, we have examined the embryonic and postnatal striatal neuron expression of the SoxE factor, *Sox8*, which has previously been associated with oligodendrocyte precursor cells (OPCs) (Sock et al., 2001; Stolt et al., 2004, 2005). In addition to OPCs, we show here that *Sox8* specifically marks dSPNs, particularly in the dorsal striatum. Additionally, we have characterized *Sor8*-EGFP mice and show that the EGFP faithfully represents *Sox8* in

dSPNs while filling the entire cytoplasm (including processes), making the mice a valuable tool to study development of the direct pathway during development.

## MATERIALS AND METHODS

### Animals

*Drd1-EGFP* (RRID: MMRRC\_000297-MU), *Drd2-EGFP* (RRID: MMRRC\_000230-UNC) and *Sox8-EGFP* (RRID: MMRRC\_031855-UCD) BAC transgenic mice were obtained from the Mutant Mouse Regional Resource Center (MMRRC), a NCRR-NIH funded strain repository. These mouse lines were donated to the MMRRC by the NINDS funded GENSAT BAC transgenic project (The Gene Expression Nervous System Atlas Project, NINDS Contracts N01NS02331 & HHSN271200723701C to The Rockefeller University (New York, NY). *Drd1-tdTomato* and *Rosa-tdTomato (Ai14)* mice were obtained from Jackson laboratories (RRID:IMSR\_JAX:016204 and RRID:IMSR\_JAX:007914). All EGFP expressing reporter mice were genotyped as described (Ehrman et al., 2013) while tdTomato expressing mice were genotyped as described in (Qin et al., 2016). Two *cre* mouse lines were used: *Isl1<sup>cre/+</sup>* mice (Srinivas et al., 2001), provided by T. Jessell (Columbia University, New York, NY) and *Dlx1-cre* BAC transgenic mice (RRID: MMRRC\_036076-UCD) obtained from the MMRRC donated by the GENSAT BAC transgenic project, as above. Both were genotyped using cre primers as described (Qin et al., 2016). The *Isl1<sup>flx/flx</sup>* mice (Mu et al., 2008) were obtained from X. Mu (University of Buffalo, Buffalo, NY) and W. H. Klein (MD Anderson Cancer Center, Houston, TX) and genotyped as described (Mu et al., 2008). *Gsx2<sup>RA/+</sup>* mice were crossed and maintained as described (Waclaw et al., 2009). All animal care was approved by the Institutional Animal Care and Use Committee (IACUC) at the Cincinnati Children's Hospital Medical Center (CCHMC).

### Tissue preparation

Embryos were staged by designating the morning of vaginal plug detection as E0.5. The date of birth was considered as P0. Embryos and postnatal brains were fixed overnight in 4% paraformaldehyde at 4° C and thoroughly rinsed in PBS. Before sectioning, embryos were cryoprotected in 30% sucrose before cryostat sectioning at 12µM. Postnatal brains were cryoprotected in 15% sucrose and sectioned at 35µM on a freezing sliding microtome.

### Immunohistochemistry

Standard immunostaining protocol was performed as previously described (Waclaw et al., 2009). Briefly, cryosections were rinsed in KPBS 0.1% Triton followed by 1h blocking with specific serums (NDS, NGS from Jackson Immunoresearch laboratories) and incubated with primary antibody over night at room temperature (see Table 1 for details). Sections were rinsed in KPBS with 0.1% triton and incubated for 2-3h at room temperature with fluorescent secondary antibody (1:300 dilution, Jackson Immunoresearch laboratories) (Table 1) then rinsed and cover slipped using Fluoromount-G (Southern Biotech). For floating sections 0.3% Triton was used instead of 0.1%.

## Antibody characterization

The **Isl1 antibody** was obtained from R and D systems (cat# AF1837, RRID:AB\_2126324). It has been previously used to mark a variety of neuronal populations in the mouse brain. The expression pattern observed in this work is consistent with the previously published reports (Toresson et al., 2000; Toresson and Campbell, 2001; Waclaw et al, 2009)

The **Sox8 antibody** was provided by Dr. Michael Wegner (University of Erlangen-Nuremberg; Bavaria; Germany RRID: AB\_2571872). The antibody was generated in guinea pig against a purified bacterially expressed protein consisting of amino acids 2-60 of mouse Sox8 fused to glutathione S-transferase (Stolt et al., 2005).

The tdTomato immunostaining was performed using a rabbit polyclonal **DsRed antibody** from Clontech Laboratories, Inc. (cat# 632496, RRID: AB\_10015246). The antibody is raised against DsRed-Express, a variant of *Discosoma* sp. red fluorescent protein. This antibody recognizes DsRed-Express, DsRed-Express2, DsRed-Monomer, mCherry, DsRed2, E2-Crimson, tdTomato, mStrawberry, and mBanana, and both N- and C-terminal fusion proteins containing these fluorescent proteins in mammalian cell lysates (manufacturer's datasheet). Specificity was confirmed by immunostaining of tdTomato<sup>-</sup> brains.

The **EGFP antibody** was purchased from Aves Labs (cat# GFP-1020, RRID:AB\_10000240). IgY fractions obtained from immunized chickens were affinity-purified using an agarose matrix to which the recombinant protein was attached. The antiserum was analyzed by western blot analysis and immunohistochemistry using transgenic mice expressing the GFP gene product (manufacturer's datasheet). No staining was observed in sections of GFP<sup>-</sup> brains from litter mates.

Anti-**FoxP1** was purchased from Abcam and was used to identify the striatal projection neurons (cat# ab57216, RRID:AB\_941648). Our immunostaining for FoxP1 shows a distribution pattern in the mouse brain consistent with published reports (Wang et al., 2011)

The rabbit **Ebf1 antibody** was purchased from Millipore (cat# AB10523, RRID: AB\_2636856). According to the manufacturer's datasheet it recognizes Ebf1 but not Ebf2 or Ebf3, from human and mouse origin, in dot blot and in Western blot analysis of COS7 transfected cells. Our expression pattern correlates with previous reports (Hua et al., 2014).

The **Nkx2.1 antibody** was obtained from Seven Hills Bioreagents (cat# WRAB-1231, RRID: AB\_451727). This polyclonal antibody is raised in rabbit and was used to define striatal interneurons as previously described in Marin et al., (2000). Our staining is consistent with previous published reports (Marin et al., 2000; Xu et al., 2008).

The **Sox9 antibody** is a rabbit polyclonal from Santa Cruz Biotechnology which was raised against amino acids 407-496 mapping near the C-terminus of Sox-9 of human origin. A single specific 65 kDa band was observed by Western blot on mouse Sox9 transfected cells (cat# sc-20095, RRID:AB\_661282).

The goat polyclonal **Sox10 antibody** was obtained from Santa Cruz Biotechnology (cat# sc-17342, RRID: AB\_2195374). This antibody recognizes a single band of about 60 kDa on mouse brain lysates in Western blot analysis (manufacturer's datasheet).

The **Olig2 antibody** was purchased from Millipore (cat# AB9610, RRID:AB\_570666). This polyclonal antibody was raised in rabbit and recognizes a 32-kDa band by western blotting on mouse brain lysate (manufacturer's information) and has been previously described to reveal distribution of oligodendrocytes and their precursor cells (OPCs) in the mouse brain (see e.g. (Ehrman et al., 2014).

**PDGFR $\alpha$  antibody** was obtained from Santa Cruz Biotechnology (cat# sc-338, RRID: AB\_631064). This is an affinity purified rabbit polyclonal antibody raised against a peptide mapping to the C-terminal region of human PDGFR $\alpha$ . In Western blot, it recognizes a single 170 kDa band on NIH/3T3 whole cell lysates (manufacturer's datasheet). This antibody has previously been used to identify OPCs in the mouse telencephalon (see e.g. (Ehrman et al., 2014)

The **TH antibody** was purchased from Aves Labs (cat# TYH, RRID:AB\_10013440). Chickens were immunized with two synthetic peptide/keyhole limpet hemocyanin (KLH) conjugates. These synthetic peptides corresponded to different regions of the Tyrosine Hydroxylase gene product (EC 1.14.16.2). These peptide sequences are shared between the mouse (P24529) and human (P07101) gene products (Erickson et al., 2012)

**Substance P antibody** was obtained from Millipore (cat# MAB356, RRID:AB\_94639) has been previously used to identify the direct pathway medium-sized spiny neuron collaterals (Gonzales et al., 2013). The specificity of the substance P antibody has been demonstrated by showing the equal displacement of labeled Substance P by five-, six-, and eight-amino acid COOH-terminal fragments of Substance P, as well as full-length Substance P, and by the specific binding of these antibodies with cell bodies and terminals located in well-defined Substance P-containing nuclei in the central nervous system. Our staining results were consistent with that of Gonzales et al. (2013).

### In situ hybridization

The *in situ* hybridization procedure was performed as previously described (Toresson et al., 1999). Digoxigenin-labeled antisense probes against *Sox8* (Clone ID: 6306276: Dharmacon) were used on 12  $\mu$ m sections of E11.5, E13.5 and E18.5 embryos.

### Imaging and cell counting

Images were captured using a Nikon AIRSi or C2 Inverted Confocal Microscope or an Olympus BX-51 microscope equipped with epifluorescence and processed when needed for brightness and contrast using Adobe Photoshop CS6 (Adobe Systems Inc., San Jose, CA, USA). Cell counts were done using NIS Elements AR 4.5 software (Nikon Instruments Inc.) on three consecutive 400 $\times$  confocal pictures from 2-3 animals each. Between 1001 and 1325 DAPI<sup>+</sup> cells were analyzed for each staining condition. Images were taken from the central portion of the dorsal striatum at E18.5.

## RESULTS

### Sox8 is present in dSPN progenitors

The HMG box transcription factor, Sox8 is known to be expressed in OPCs and to play a compensatory role in oligodendrogenesis in the absence of either Sox9 or Sox10 (Sock et al., 2001; Stolt et al., 2004; Stolt et al., 2005), however, no detailed report on the expression of this factor in neuronal populations exists. A previous study by Sock et al. (2001), in which *lacZ* was used to replace the *Sox8* locus, showed extensive beta-galactosidase staining in the developing striatum. Hence, we set out to carefully analyze Sox8 expression within the ventral telencephalon and in particular in developing striatal projection neurons (SPNs) of the direct (dSPN) and indirect (iSPN) pathways.

Isl1 (a LIM homeobox factor) has recently been shown to be expressed in progenitors of the direct pathway and is required for the survival/differentiation of a subpopulation of dSPNs (Ehrman et al., 2013; Lu et al., 2014). Double immunostaining for Isl1 and Sox8 shows significant co-expression of these factors within the subventricular zone (SVZ) of the LGE as well as in the forming striatum, particularly in the dorsal and medial region at E15.5 (Fig. 1C). While co-expression in the medial striatum was widespread, Sox8 exhibited unique expression within the ventricular zone (VZ) and was absent from the superficial SVZ appearing again deep in the SVZ and maintaining a high to low gradient of expression in SPNs across the medial-lateral aspect of the striatum (Fig. 1B,C). Isl1 is expressed in the superficial SVZ and continues with a less pronounced medial-lateral gradient in the striatum (Fig. 1A,C). The early B cell factor, Ebf1 is also known to be essential for correct formation of the dSPNs (Lobo et al., 2006; Lobo et al., 2008). Ebf1 is expressed in deep portions of the SVZ as well as in a rather uniform manner throughout the mediolateral extent of the developing striatum at E18.5 (Fig. 1D). At E18.5, Sox8 shows a very similar pattern of expression with a pronounced high-medial to low-lateral gradient in the striatum (Fig. 1 E, F). Quantification of double labeling with the dSPN markers in the central portion of the dorsal striatum at E18.5 showed that 59% of Isl1<sup>+</sup> cells co-expressed Sox8 (163 cells Isl1<sup>+</sup>/Sox8<sup>+</sup> out of 278 total Isl1<sup>+</sup> cells), while 56% of Ebf1<sup>+</sup> cells co-labeled with Sox8 (170 cells Ebf1<sup>+</sup>/Sox8<sup>+</sup> out of 305 total Ebf1<sup>+</sup> cells).

Using the *Isl1*<sup>cre</sup> mice (Srinivas et al., 2001), we fate mapped the dSPNs (Ehrman et al., 2013; Lu et al., 2014) and co-labeled with Sox8 at E15.5 and E18.5 (Fig. 1G-K). The vast majority of tdTomato expressing *Isl1* fate mapped cells (i.e. dSPNs) co-express Sox8 at both stages (Fig. 1H,I,K). Indeed, at E15.5, the vast majority of tdTomato-positive dSPNs in the dorsal striatum were observed to express Sox8 (Fig 1I). Thus Sox8 exhibits a complex expression pattern within the LGE/striatum that partially overlaps with that of Isl1 and Ebf1, particularly in the developing dSPNs (Fig. 1L). Hence, these results suggest that Sox8 represents a new marker of dSPNs in addition to the previously established Isl1 (Ehrman et al., 2013; Lu et al., 2014) and Ebf1 (Lobo et al., 2006; Lobo et al., 2008).

We also performed a comparative analysis of *Sox8* gene and protein expression over 3 stages; E11.5 prior to SPN differentiation, E13.5 an early stage of striatal neurogenesis and E18.5 near the completion of striatogenesis (Fig. 2). Interestingly, at E11.5, *Sox8* gene and protein expression is largely confined to the VZ of the ventral telencephalon (Fig. 2A,C). By



E13.5, *Sox8* gene and protein expression in the VZ is expanded dorsally to the pallium with a gradient of expression from ventral to dorsal (Fig. 2E,G). At this stage, expression in the LGE SVZ and developing striatum is evident (Fig. 2E,G). This pattern remains until E18.5, however, the medial-lateral gradient in the maturing striatum, as described above, becomes evident (Fig. 2I,K). Additionally, scattered *Sox8* gene and protein expressing cells were observed in adjacent cortical regions similar in their distribution to migrating OPCs (Fig. 2I,K), which have been shown to express *Sox8* at multiple levels of the neuraxis (Sock et al., 2001; Stolt et al., 2004, 2005). The homeobox gene *Gsx2* is known to be required for correct dorsal-ventral patterning of the LGE and subsequent formation of the striatum (Corbin et al., 2000; Toresson et al., 2000; Toresson and Campbell, 2001; Yun et al., 2001, 2003; Waclaw et al., 2009). We examined *Sox8* gene and protein expression in the *Gsx2<sup>RA/RA</sup>* null mutant (Waclaw et al., 2009) and found an initial ventral shift of its dorsal limit (Fig. 2B,D), similar to that observed for other ventral telencephalic genes (e.g. *Ascl1*, *Dlx* factors) in these mutants (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001). No noticeable change in the VZ expression was observed at later stages in the *Gsx2* mutants, however, expression in the forming striatum was notably reduced (Fig. 2F,H, J, L), as seen for other striatal markers such as, *Isl1*, in these mutants (Toresson et al., 2000; Toresson and Campbell, 2001; Waclaw et al., 2009; Wang et al., 2011). Thus, *Sox8* initially behaves similar to ventral telencephalic genes within the VZ at early stages corresponding with the observed patterning defect in striatal development but at later stages the VZ expression appears to be independent of *Gsx2* gene function.

### Characterization of *Sox8-EGFP* BAC mice

A number of BAC transgenic lines have previously been used to fluorescently mark either the iSPNs or dSPNs together with their axonal projections (Lobo et al., 2006; Wang et al., 2006; Lobo et al., 2008; Shuen et al., 2008; Ehrman et al., 2013) Many of these BAC transgenes, however, contain genes expressed highly in mature SPNs and thus may not provide robust labeling of the developing SPNs and their axonal trajectories. Given our findings that *Sox8* appears to mark the dSPNs at embryonic stages, we examined EGFP expression in *Sox8-EGFP* BAC transgenic mice generated by the GENSAT project (Gong et al., 2003) to determine if these mice provide a reliable tool to visualize the development of the direct pathway already at embryonic time points. Although we were able to detect EGFP expression as early as E12.5 (see Fig. 4) we initially focused on E16.5 and E18.5 when the striatal complex is well delineated. First, we compared the expression of EGFP and *Sox8* at E16.5 and found that, with the exception of the VZ expression of *Sox8* (Fig. 3A), EGFP immunostaining co-labels extensively with *Sox8* in the SVZ and developing striatum (particularly the dorsomedial striatal region) (Fig. 3A-C). In addition to the mediolateral gradient of *Sox8* (see Fig. 1), both *Sox8* and EGFP also show a high-dorsal to low-ventral gradient within the striatal complex (Fig. 3A-C).

The SPNs comprise around 90-95% of the cells in the mature striatum (Kemp and Powell, 1971) with the remaining 5-10% representing different subtypes of interneurons (Kawaguchi, 1993). The forkhead transcription factor *Foxp1* is known to mark the vast majority of SPNs belonging to both the direct and indirect pathway (Arlotta et al., 2008). In the *Sox8-EGFP* striatum at E18.5 staining for *Foxp1* showed that the vast majority of the

EGFP<sup>+</sup> cells were FoxP1<sup>+</sup> (Fig. 3D-F). We next co-stained the *Sox8-EGFP* striatum with dSPN markers and found that 76% of Isl1<sup>+</sup> cells co-express EGFP (Fig. 3G-I, 212 Isl1<sup>+</sup>/EGFP<sup>+</sup> cells out of a total of 278 Isl1<sup>+</sup> cells) and 78% of the Ebf1<sup>+</sup> cells co-labeled EGFP (Fig. 3J-L, 239 Ebf1<sup>+</sup>/EGFP<sup>+</sup> cells out of a total of 305 Ebf1<sup>+</sup> cells). Striatal interneurons are known to migrate tangentially from the medial ganglionic eminence (MGE) and settle in the forming striatum (Marin et al., 2000). These neurons express the transcription factor Nkx2.1 (Marin et al., 2000). Double immunostaining for Nkx2.1 and EGFP showed that there is no detectable colocalization between the two markers (Fig. 3M-O), indicating that Sox8-expressing striatal cells are not interneurons. Taken together, Sox8 appears to mark dSPNs and their progenitors from early stages and the *Sox8-EGFP* BAC mice represent a useful tool for studying this neuronal population at embryonic time points.

The defining feature of dSPNs is their axonal projections to the substantia nigra including the pars reticulata (SNr) (Gerfen, 2004). Thus we decided to carefully analyze the origin and destination of the EGFP<sup>+</sup> axonal projections in *Sox8-EGFP* embryos in sagittal sections at different embryonic stages. We first observed EGFP<sup>+</sup> axons emanating from the developing dorsal striatum at E12.5, however, they only extend to the diencephalon at this stage (Fig. 4A). By E14.5, some of the EGFP<sup>+</sup> axons can be observed reaching all the way to the SNr (Fig. 4B). At E16.5, the EGFP<sup>+</sup> axons projecting to the substantia nigra are numerous and begin arborizing within the SNr (Fig 4C,D). The neuropeptide substance P is an established marker of the direct pathway SPNs (Gerfen, 2004). We were able to identify EGFP/  
Substance P double positive processes in the SNr as early as E16.5 (Fig 4D), further supporting the notion that Sox8-EGFP neurons are dSPNs. In agreement with the fact that most SPNs are generated by birth, the direct pathway appears to be largely complete by E18.5 (Fig. 4F). The reciprocal nature of the striatonigral and nigrostriatal pathways has been well documented (reviewed in Gerfen, 2004) thus we examined the formation of these intimately associated pathways in the *Sox8-EGFP* mice. Indeed, a clear SNc/SNr structure is evident at E16.5 and E18.5 (Fig. 4E,I). Moreover, the close association of descending dSPN axons and ascending dopamine fibers (marked by tyrosine hydroxylase) can be seen before birth within the internal capsule (Fig. 4H). This is also true at striatal levels where dopamine terminals show close association with the EGFP<sup>+</sup> (i.e. dSPN) cell bodies (Fig. 4G). Thus the *Sox8-EGFP* mice represent a very useful tool to study the formation of basal ganglia circuitry especially as it relates to the direct pathway.

A widely exploited approach to identify dSPNs and iSPNs in the postnatal/adult brain has been to utilize the *Drd1-EGFP* and *Drd2-EGFP BAC* transgenic mice (Lobo et al., 2006, 2008; Wang et al., 2006; Shuen et al., 2008; Ehrman et al., 2013). At perinatal time points, however, the *Drd1-EGFP* transgene is only expressed in subsets of SPNs, apparently in the patch (striosomal) compartment (Fig. 5A). Despite the low number of SPNs expressing *Drd1-EGFP* at birth, most of these neurons also co-expressed Sox8 protein (Fig. 5B). In contrast, the *Drd2-EGFP* BAC transgene marks large numbers of SPNs throughout the newborn striatal complex (Fig. 5C) and none of these EGFP<sup>+</sup> neurons co-expressed Sox8 (Fig. 5D). To determine how specific the *Sox8-EGFP* expression was to dSPNs in the postnatal brain, we crossed the mice with *Drd1-tdTomato* mice (Shuen et al., 2008) and analyzed them at P7, when the *Drd1* BAC transgene is expressed throughout the striatal complex (Fig. 5F). Again, at this time point, the EGFP showed a higher expression level



within the dorsal striatum and only weak expression in the ventral striatum, including the nucleus accumbens (Fig. 5E). In line with the embryonic studies described above, we observed a high degree of overlap between the *Sox8* driven EGFP and *Drd1* driven tdTomato within the dorsal striatum as well as along the direct pathway trajectories (Fig. 5G-K), supporting the notion that Sox8 is an early marker of dSPNs and that *Sox8-EGFP*BAC mice represent an excellent tool to follow the formation of the direct pathway from embryonic to postnatal stages.

As mentioned above, Sox8 (and *Sox8-EGFP*) marks a population of cells that are scattered throughout the brain parenchyma including the telencephalon, diencephalon and midbrain resembling migrating OPCs (Figs. 2I,K and 4F). To determine if these Sox8 (and *Sox8-EGFP*) cells were, in fact, OPCs, we co-stained with glial markers. These scattered EGFP<sup>+</sup> cells exhibited a morphology resembling OPCs and expressed Sox8 in cortex, hypothalamus and dorsal thalamus (Fig. 6A,B,C). Moreover, these cells were double positive for Sox9 (Fig. 6D,E), Sox10 (Fig. 6F,G), Olig2 (Fig. 6H,I) and PDGFR $\alpha$  (Fig. 6J,K) in the cortical areas examined. In fact, cortical EGFP<sup>+</sup> cells were approximately 90% positive for Olig2 and PDGFR $\alpha$ . Moreover, similar cells were observed within the parenchyma of the hypothalamus (Fig. 6B), diencephalon (Fig. 6C) and the white matter tracts as well as midbrain/hindbrain regions (data not shown). Thus, *Sox8-EGFP*BAC mice faithfully label OPCs throughout the brain in addition to the specific labeling of dSPNs within the striatum.

### **Sox8-EGFP BAC transgene on the *Isl1* conditional mutant background**

Our previous work suggested that *Isl1* is required cell autonomously for survival of a significant subpopulation of dSPNs as well as non-cell autonomously for the normal formation of the striatonigral axonal trajectory via the internal capsule (Ehrman et al., 2013). However, we were only able to examine these axonal trajectories at adult time points as we were unable to specifically and robustly label the forming direct pathway in embryonic and early postnatal *Isl1* conditional mutants (cKOs).

In this study, we have utilized the *Sox8-EGFP* allele to examine the formation of the direct pathway in *Dlx1-cre; Isl1<sup>fl/fl</sup>* animals at birth (i.e. P0). In contrast to the control animals (Fig. 7A), a clear reduction in EGFP<sup>+</sup> striatal cells and dSPN axons was apparent in the *Isl1* cKO brain (Fig. 7B). In addition to the reduced number of EGFP<sup>+</sup> dSPNs in the *Isl1* cKO striatum, we observed considerable alterations in the EGFP<sup>+</sup> axonal trajectories along the direct pathway of the conditional mutants (Fig. 7B). We previously showed that deletion of *Isl1* using *Dlx5/6-CIE* mice resulted in a major loss of reticular thalamus neurons (Ehrman et al., 2013). Using the *Dlx1-cre* BAC mice, *Isl1* is also severely reduced in the reticular thalamus of the cKO (Fig. 7D). Notably, at the level of the reticular thalamus in these mutants, many EGFP<sup>+</sup> axons were observed to invade the dorsal thalamus in the cKOs, but not the controls (compare Fig. 7D with C). Furthermore, Sox8 protein expression was reduced in the *Isl1* cKO striatum (Fig. 7F), as compared to the control (Fig. 7E). This reduction in Sox8 (and EGFP) expression likely reflects the significant cell death phenotype observed in dSPNs of the *Isl1* cKOs (Ehrman et al., 2013; Lu et al., 2014). Therefore the *Sox8-EGFP*BAC mice represent an excellent tool to study perturbations to the direct pathway in defined genetic lesions.

## DISCUSSION

The SoxE factors, including Sox8, Sox9 and Sox10 are well known regulators of glial development and in particular OPCs and oligodendrogenesis (Sock et al., 2001; Stolt et al., 2004, 2005). In this study we have characterized novel neuronal expression of the SoxE factor, Sox8, specifically within dSPNs of the embryonic and postnatal mouse striatum. Additionally, we have characterized EGFP expression in *Sox8-EGFP*BAC transgenic mice and demonstrated that the EGFP accurately reflects Sox8 expression in dSPNs and their axonal projections as well as in OPCs throughout the perinatal brain. Thus Sox8 represents a novel marker of dSPNs from embryonic to postnatal time points and the *Sox8-EGFP*BAC mice provide a useful tool to study assembly of basal ganglia circuitry.

Although Sox8 is expressed in OPCs, no significant glial phenotypes have been reported in the *Sox8* mutants (Sock et al., 2001). Sox9 has been shown to be required for OPC specification while Sox10 plays important roles in oligodendrocyte differentiation (Stolt et al., 2002, 2003). While *Sox8* mutants have only been shown to exhibit a transient delay in terminal differentiation and myelination within the mutant spinal cord (Stolt et al., 2004), removal of *Sox8* from either the *Sox9* or *Sox10* mutant backgrounds exacerbates both of the phenotypes (Stolt et al., 2004; Stolt et al., 2005). Despite this, it appears that Sox8 is not completely redundant with these SoxE factors, as the replacement of the *Sox10* locus with *Sox8*, does not fully rescue the *Sox10* mutant phenotype (Stolt et al., 2005; Kellerer et al., 2006). Interestingly, this appears to be the first description of a SoxE factor in a defined neuronal subtype lineage. Using *Isl1-cre* mice (Srinivas et al., 2001) to mark the direct pathway (Ehrman et al., 2013; Lu et al., 2014) we demonstrate here that Sox8 is expressed by newborn SPNs and specifically those that belong to the direct pathway (i.e. dSPNs).

Despite the reduction in Sox8 expressing cells within the *Isl1* cKO striatum, it appears that at least a portion of the remaining dSPNs in this mutant do express Sox8 and that the loss of Sox8-expressing cells is likely due to the considerable neuronal death occurring within the mutant dSPNs (Ehrman et al., 2013; Lu et al., 2014). This suggests that Sox8 is not directly regulated by *Isl1* and may thus control a parallel pathway in dSPN differentiation. This is in line with the maintained expression of *Ebf1* in the remaining dSPNs of the *Isl1* cKO mutants (Ehrman et al., 2013; Lu et al., 2014). Therefore, it will be interesting to determine the relationship between Sox8 and *Ebf1*, which represents the other major regulator of dSPN development (Lobo et al., 2006; Lobo et al., 2008). *Ebf1* has been shown to be expressed in, and required for development of the matrix compartment (Garel et al., 1999). Moreover, it regulates dSPN development rather specifically within this compartment of the striatum (Lobo et al., 2008). However, it does not appear that Sox8 is differentially expressed in the matrix versus the patch (striosome) compartment (data not shown) and thus may be regulated by other factors within the developing striatal patches.

We were surprised to observe the ventral restriction of *Sox8* gene and protein expression in the VZ of the E11.5 telencephalon given its broad dorsoventral extent within the VZ at later stages. At this early time point, *Sox8* is subject to dorsoventral patterning constraints typical of most other ventral telencephalon restricted genes. The homeobox gene *Gsx2* represents an important regulator of ventral patterning in the telencephalon and is required for normal

ventral telencephalic gene expression (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001; Waclaw et al., 2009; Wang et al., 2013). In *Gsx2* mutants, we found that the dorsal limit of Sox8 expression is shifted ventral as is the case with other ventral telencephalic regulators such as *Ascl1* and *Dlx* factors. By E13.5, however, the VZ expression of Sox8 appears to be independent of *Gsx2* function showing a similar dorsal extent to that in control embryos. This suggests a complex regulation of *Sox8* gene expression within the telencephalic VZ and possibly different roles at early versus late stages, perhaps in neurogenesis versus gliogenesis.

*Sox8-EGFPBAC* mice have been generated by the GENSAT project (Gong et al., 2003). Characterizing these mice showed that they mark dSPNs from early stages of striatogenesis as well as considerable numbers of OPCs throughout the brain. Interestingly, the VZ expression of Sox8 was not observed in the *Sox8-EGFPBAC* mice at any stage analyzed. Thus the enhancer elements driving this expression are not represented in the BAC used to create these mice. The majority of EGFP<sup>+</sup> neurons in the striatum expressed the dSPN markers, *Isl1* (Ehrman et al., 2013; Lu et al., 2014) and *Ebf1* (Lobo et al., 2006; Lobo et al., 2008) but not the striatal interneuron marker *Nkx2.1* (Marin et al., 2000). At postnatal stages, the *Sox8-EGFP* transgene showed considerable overlap with the *Drd1-tdTomato* transgene (Shuen et al., 2008), at least in dorsal striatal regions where the EGFP signal was most abundant. Similarly, when the EGFP<sup>+</sup> cells with OPC morphologies were investigated, the overwhelming majority co-expressed all of the typical OPC markers. Thus *Sox8-EGFP* BAC mice represent a very useful tool to mark the dSPNs from embryonic to postnatal stages as well as large populations of OPCs. Presumably the developmental history of these EGFP<sup>+</sup> neural subtypes is quite distinct given the restricted generation and migration of dSPNs and the widespread distribution of labeled OPCs. These reporter mice, however, may be useful tools to dissect the lineage relationship between dSPNs and LGE-derived OPCs.

In addition to the cell body labeling, the EGFP expressed in the *Sox8-EGFP* mice also fills the neuronal processes of labeled cells and thus marks the axons and terminals. This characteristic also helped to define the EGFP<sup>+</sup> cells as dSPNs due to the axonal projections through the internal capsule towards, and into, the SNr. As mentioned above, there is considerable overlap in the EGFP and tdTomato signals in axons of the direct pathway in *Sox8-EGFP; Drd1-tdTomato* double transgenic mice at postnatal stages. Unlike the *Drd2-EGFPBAC* transgene which appears to be expressed in large numbers of iSPNs already at embryonic time points (Morello et al., 2015), the *Drd1-EGFPBAC* transgene is only found in a small subpopulation of dSPNs. Therefore, the *Sox8-EGFPBAC* transgene allows us to mark the direct pathway already at embryonic stages. Indeed, we observe EGFP expression in neurons within the E12.5 LGE mantle (i.e. forming striatum) of the *Sox8-EGFP* embryos. At this time point, the EGFP<sup>+</sup> axons only reach as far as the diencephalon, however, 2 days later at E14.5 the first EGFP<sup>+</sup> axons can be seen to invade the ventral midbrain in the vicinity of the SNr. A previous study, using retrograde tracing, indicated that the striatonigral axons first reach the midbrain around E17 in the rat (Fishell and van der Kooy, 1987). This would correlate to about E14.5 in the mouse according to the *Translating Time* website (<http://www.translatingtime.net>), as the mouse is approximately 2 days shorter gestation (Workman et al., 2013). Thus our findings in the *Sox8-EGFPBAC* mice support the timing of dSPN innervation of the SNr shown by Fishell and van der Kooy (1987) and indicate that

these mice represent an excellent tool to study the formation of this crucial basal ganglia circuit component.

Formation of the direct pathway depends on two major transcription factors *Isl1* and *Ebf1* (Garel et al., 1999; Lobo et al., 2006, 2008; Ehrman et al., 2013; Lu et al., 2014). *Isl1* plays a cell autonomous role in regulating survival of newborn dSPNs and a non-cell autonomous role in regulating the normal formation of the internal capsule in which the direct pathway axons travel (Ehrman et al., 2013; Lu et al., 2014). Inactivation of *Isl1* in the embryonic ventral thalamus results in the loss of the reticular thalamus neurons (Ehrman et al., 2013), which correlates well with the observed defects in the internal capsule and direct pathway trajectory at postnatal times. We bred the *Sox8-EGFP* allele onto the *Dlx1-cre; Isl1* cKO background to examine the direct pathway at perinatal time points and found that numerous EGFP<sup>+</sup> axons aberrantly invade the dorsal thalamus at the level of the reticular thalamus in the *Isl1* cKOs at birth. Repulsive Plexin D1-Sema 3e signaling has been proposed to regulate normal internal capsule and direct pathway formation (Chauvet et al., 2007; Ehrman et al., 2013). In fact, Sema 3e is expressed by the ventral thalamus (precursor to the reticular thalamus) and Plexin D1 uniquely defines the dSPNs (Chauvet et al., 2007; Ehrman et al., 2013; Lu et al., 2014). Chauvet et al. (2007) showed that *Sema 3e* mutants exhibit aberrant innervation of Plexin D1 axons originating from the internal capsule within the dorsal thalamus. Moreover, *Sema 3e* mutants show disruptions in direct pathway axonal trajectories at postnatal stages (Ehrman et al., 2013). The direct pathway defects observed at the level of the subthalamic nucleus and SNr in postnatal *Dlx1-cre* driven *Isl1* cKO appear similar to that observed in the *Sema 3e* mutants (Ehrman et al., 2013). However, the aberrant axonal innervation of the dorsal thalamus was not observed in *Isl1* cKOs at the postnatal time points examined. Using the *Sox8-EGFPBAC* transgenic mice, we did observe abnormal dSPN innervation of the dorsal thalamus in *Dlx1-cre; Isl1* cKOs at birth, suggesting that these aberrantly projecting direct pathway axons are pruned back from the dorsal thalamus at postnatal times. These findings demonstrate the utility of the *Sox8-EGFPBAC* mice to study the development of basal ganglia circuitry in both wild type and genetic mutant backgrounds.

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## Abbreviations used in the text and figures

<b>Ctx</b>	Cortex
<b>dSPN</b>	Direct pathway striatal projection neuron
<b>EP</b>	Entopeduncular nucleus
<b>GP</b>	Globus pallidus
<b>Hyp</b>	Hypothalamus

<b>iSPN</b>	Indirect pathway striatal projection neuron
<b>LGE</b>	Lateral ganglionic eminence
<b>MGE</b>	Medial ganglionic eminence
<b>OPC</b>	Oligodendrocyte precursor cell
<b>SNe</b>	Substantia nigra compacta
<b>SNr</b>	Substantia nigra reticulata
<b>STN</b>	Subthalamic nucleus
<b>SVZ</b>	Subventricular zone
<b>rTh</b>	Reticular thalamus
<b>Th</b>	Thalamus
<b>VTA</b>	Ventral tegmental area
<b>VZ</b>	Ventricular zone

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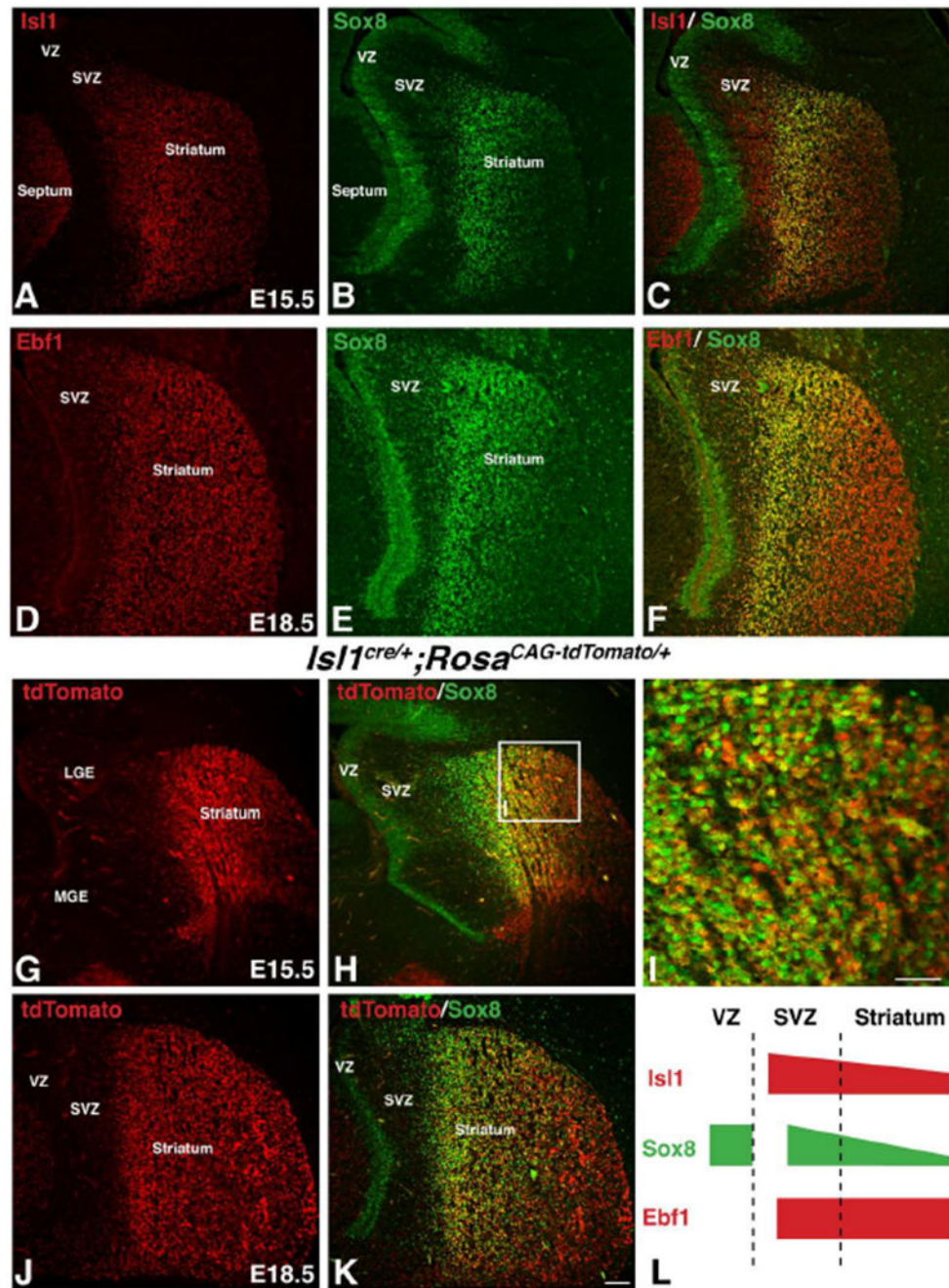
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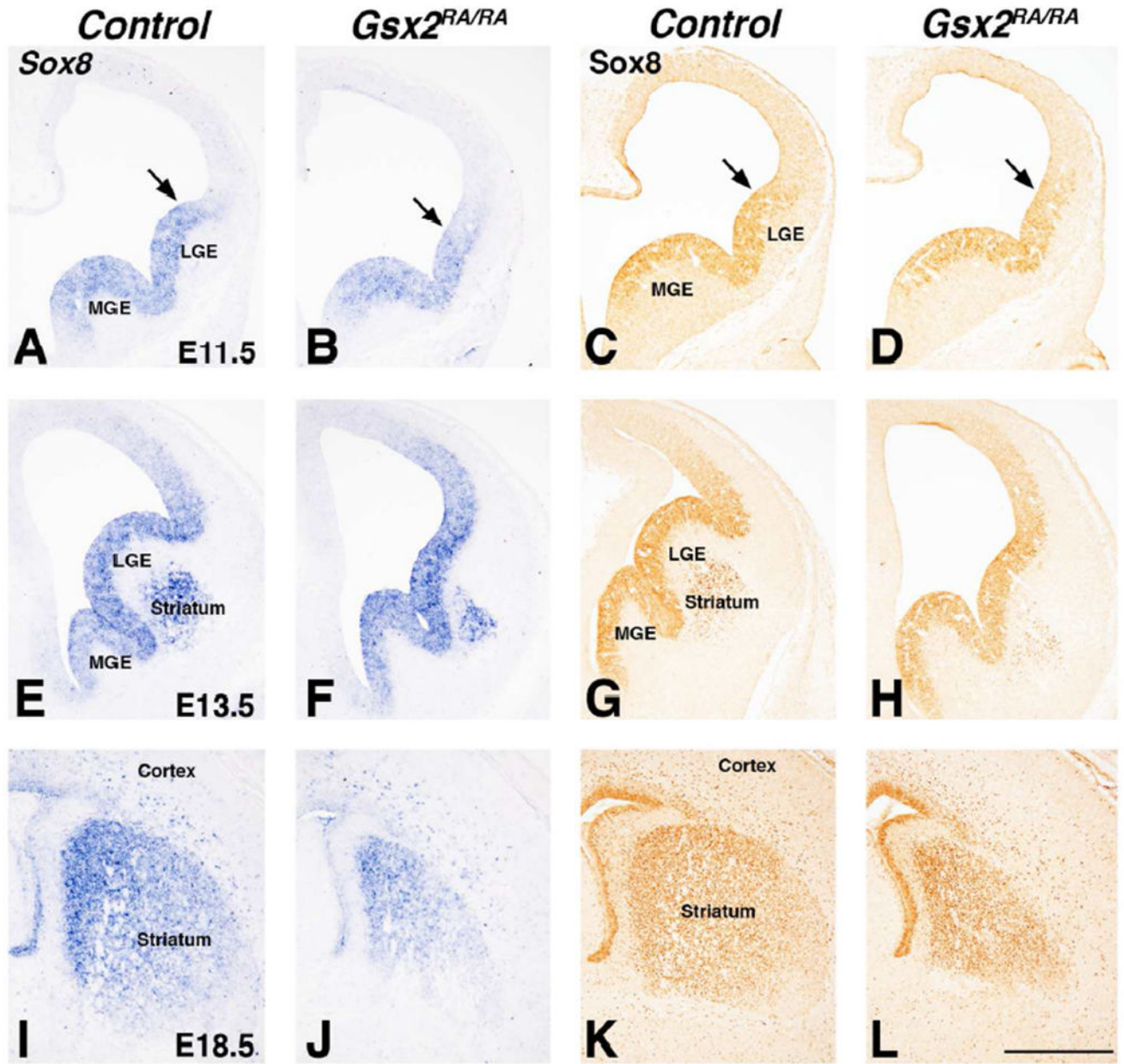


**Figure 1.**

(A-C) Confocal fluorescent images of coronal hemisections of E15.5 mouse brains immunostained for *Isl1* (A) and *Sox8* (B) illustrate the basal ganglia distribution of both proteins. *Sox8* and *Isl1* are co-expressed in the deep portions of the SVZ and in a decreasing gradient from medial to lateral within the striatum (C). (D-F) *Sox8* (D) and *Ebf1* (E) are co-expressed in the deep portions of the SVZ as well as in the medial region of the striatum at E18.5. Note, *Ebf1* shows a rather uniform expression throughout the striatum (F). (G-K) *Isl1* fate mapped striatal cells (*tdTomato*<sup>+</sup>) showing co-localization of *Sox8* at E15.5 (G-I)

and E18.5 (**J,K**). (**I**) High power image of boxed area in **H** showing extensive co-localization Sox8 and tdTomato in newborn SPNs. (**L**) Schematic illustration of the expression of Sox8 in relation to the dSPN markers Isl1 and Ebf1 in the developing LGE showing the different gradients of expression in the SVZ and striatum as well as VZ. Abbreviations: VZ: ventricular zone; SVZ: subventricular zone; MGE: medial ganglionic eminence; LGE: lateral ganglionic eminence. Scale bar: A-H, J, K = 100  $\mu\text{m}$ ; I = 30  $\mu\text{m}$ .





**Figure 2.**

Comparison of *Sox8* gene and protein expression in control and *Gsx2* mutants during striatal development. Mouse brain coronal hemisections *in situ* stained for *Sox8* (A-B, E-F, I-J) and immunostained for Sox8 (C-D, G-H, K-L). *Sox8* gene and protein expression is restricted to the ventricular zone (VZ) of the MGE and LGE at E11.5 (A,C). The *Sox8* expression domain is shifted ventrally (compare arrows in B,D with A,C) in the VZ of the LGE in *Gsx2* mutants (B,D) as compared to controls (*Gsx2*<sup>RA/+</sup>) (A,C). By E13.5, *Sox8* gene and protein expression expands dorsally in the VZ and is also detected in the developing striatum (E, G). At this stage, *Gsx2* mutants (F,H) show no difference in the VZ expression of *Sox8*, but display a clear reduction in the forming striatum (compare F,H to E,G). At E18.5, *Sox8*

remains robustly expressed in the striatum (**I,K**) and is reduced in *Gsx2* mutants (**J,L**).  
Abbreviations: MGE: medial ganglionic eminence; LGE: lateral ganglionic eminence. Scale bar = 500µm.

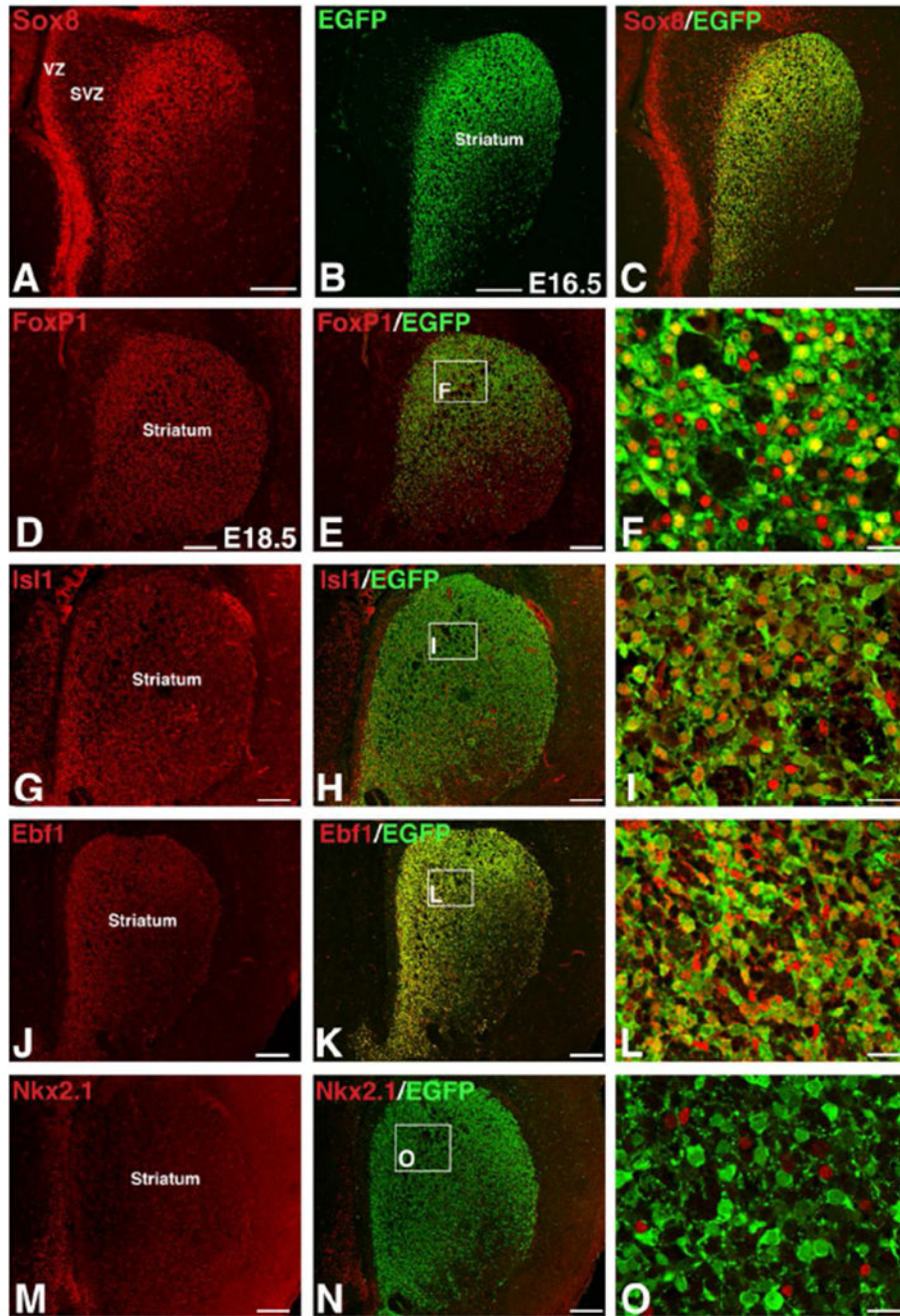
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**Figure 3.**

(A-C) Coronal hemisections from E16.5 *Sox8-EGFP*BAC transgenic mouse brain stained for Sox8 (A) and EGFP (B), respectively. (C) Merged image showing EGFP recapitulates the expression of Sox8 within the subventricular zone (SVZ) and striatum but not in the ventricular zone (VZ). (D-O) Confocal pictures of E18.5 *Sox8-EGFP* coronal hemisections double stained for EGFP (green) and: FoxP1 (D-F), Isl1 (G-I), Ebf1 (J-L) and Nkx2.1 (M-O) in red. (F,I,L,O) High power images of the respective boxed areas in the center column panels from E,H,K and N. As expected, FoxP1/EGFP staining reveals that EGFP<sup>+</sup> cells

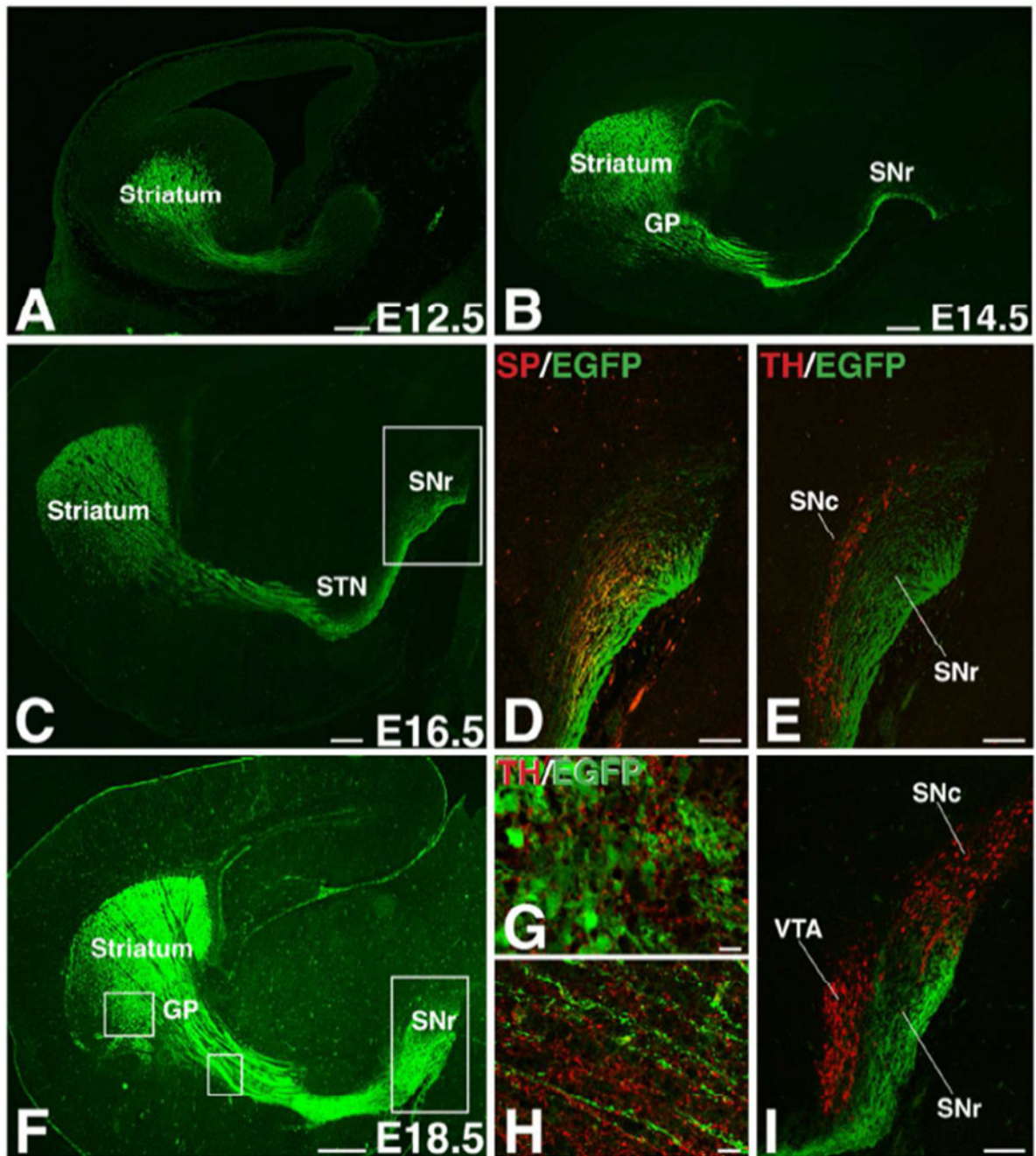
coexpress FoxP1 (**E-F**). Moreover, extensive co-labelling with the dSPN markers markers Isl1 (**H-I**) and Ebf1 (**K-L**) is also observed. No colocalization was found with the interneuron marker Nkx2.1 (**N-O**). Abbreviations: VZ: ventricular zone; SVZ: subventricular zone; Scale bar: A-E, G, H, J, K, M, N = 200 $\mu$ m. Scale bar in F, I, L, O = 20 $\mu$ m.

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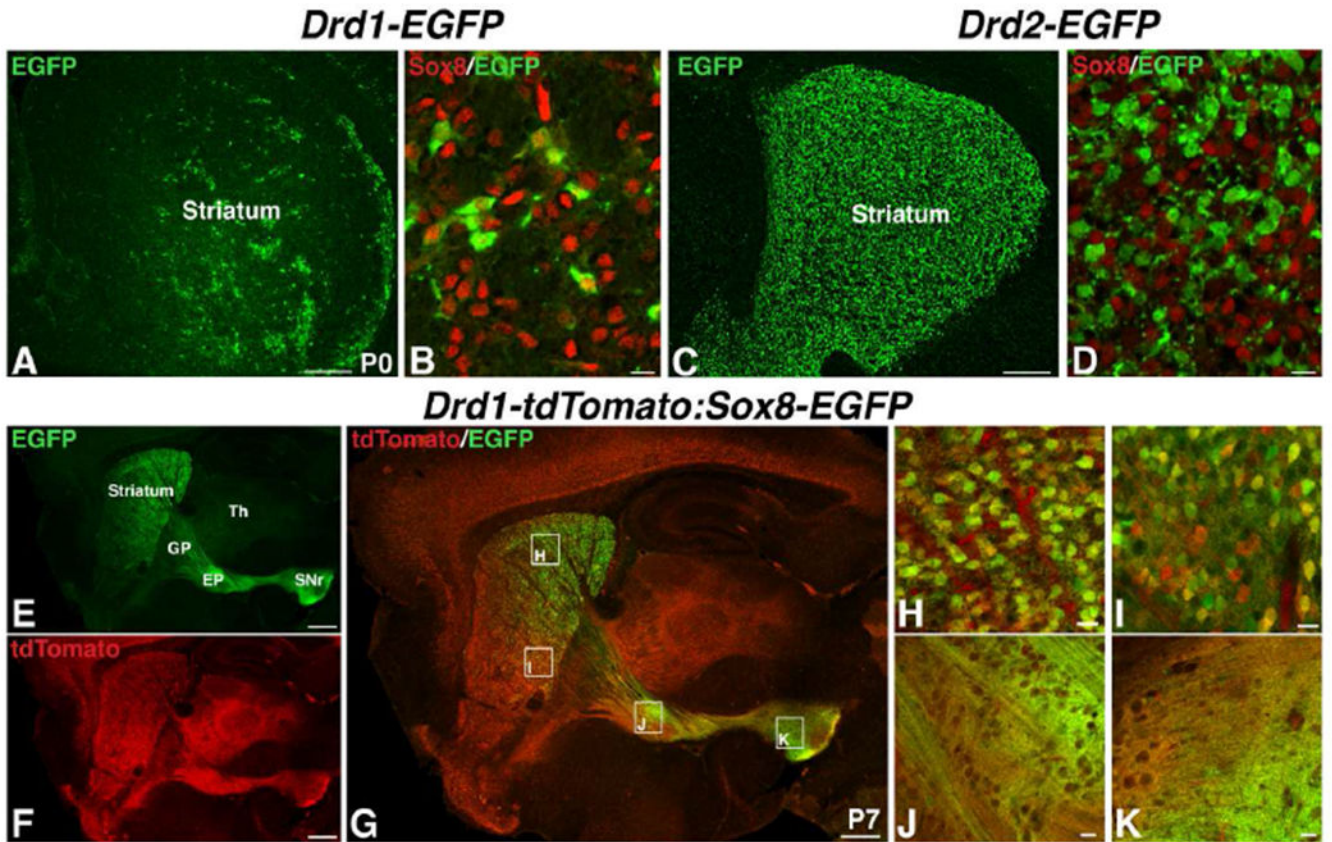


**Figure 4.**

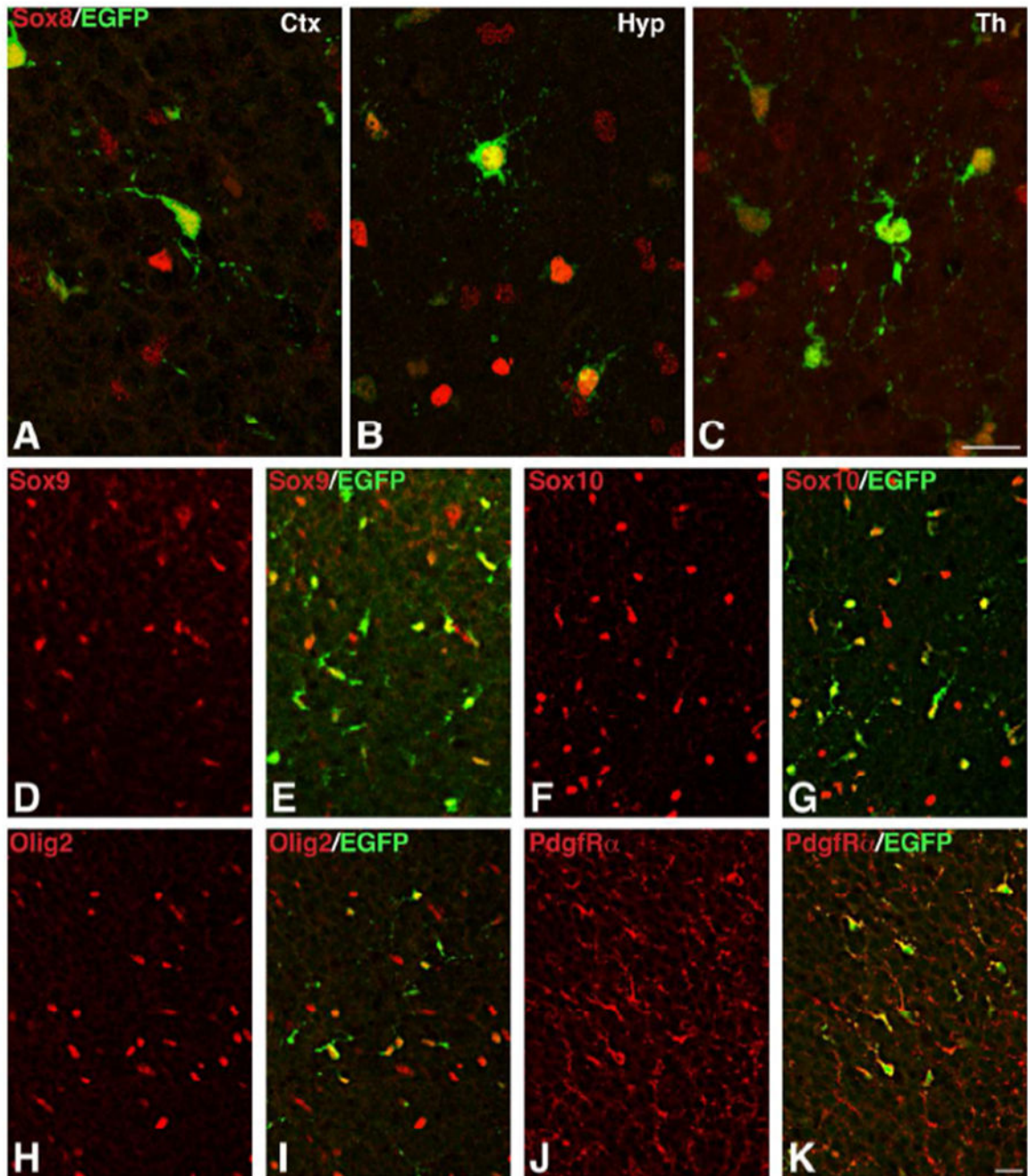
(**A, B, C, F**) Low power images of sagittal sections of *Sox8-EGFP* brains from E12.5 to E18.5. (**A**) At E12.5, EGFP fluorescence is found in dSPNs as well as in their axons which, at this stage, only reach the telencephalon/diencephalon boundary. (**B**) By E14.5, EGFP<sup>+</sup> fibers are observed to reach the midbrain with the first terminals invading the SNr. (**C**) At E16.5, the internal capsule is well formed and many axons show a stereotypic trajectory on their way to the SNr. (**D-E**) High power confocal images of sagittal sections at the SNr level represented by the boxed area in **C**. (**D**) Double immunostaining with substance P, shows

colocalization with the dSPN marker in EGFP<sup>+</sup> terminals of the developing SNr. **(E)** TH neurons of the VTA and SNc can be observed dorsal to the EGFP terminals arborizing within the SNr at E16.5. **(F)** Low power sagittal view of the E18.5 *Sox8-EGFP* brain, showing a fully formed direct pathway. **(G,H,I)** High power confocal images from representative areas boxed in **F**. **(G)** EGFP<sup>+</sup> striatal neurons (i.e. dSPNs) are surrounded by TH<sup>+</sup> terminals. **(H)** Close association between descending EGFP<sup>+</sup> (i.e. direct pathway) and ascending TH<sup>+</sup> (i.e. dopaminergic) fibers is observed along the direct pathway trajectory. **(I)** Typical organization of the substantia nigra with a TH<sup>+</sup> SNc/VTA dorsal and lateral to the SNr containing EGFP<sup>+</sup> terminals. Abbreviations: GP: globus pallidus; SNr: substantia nigra reticulata; SNc: substantia nigra pars compacta; STN: subthalamic nucleus; SP; substance P; TH; tyrosine hydroxylase; VTA: ventral tegmental area. Scale bar: in A-C = 200µm, G-H = 10µm. D-E, I = 100µm F = 500µm



**Figure 5.**

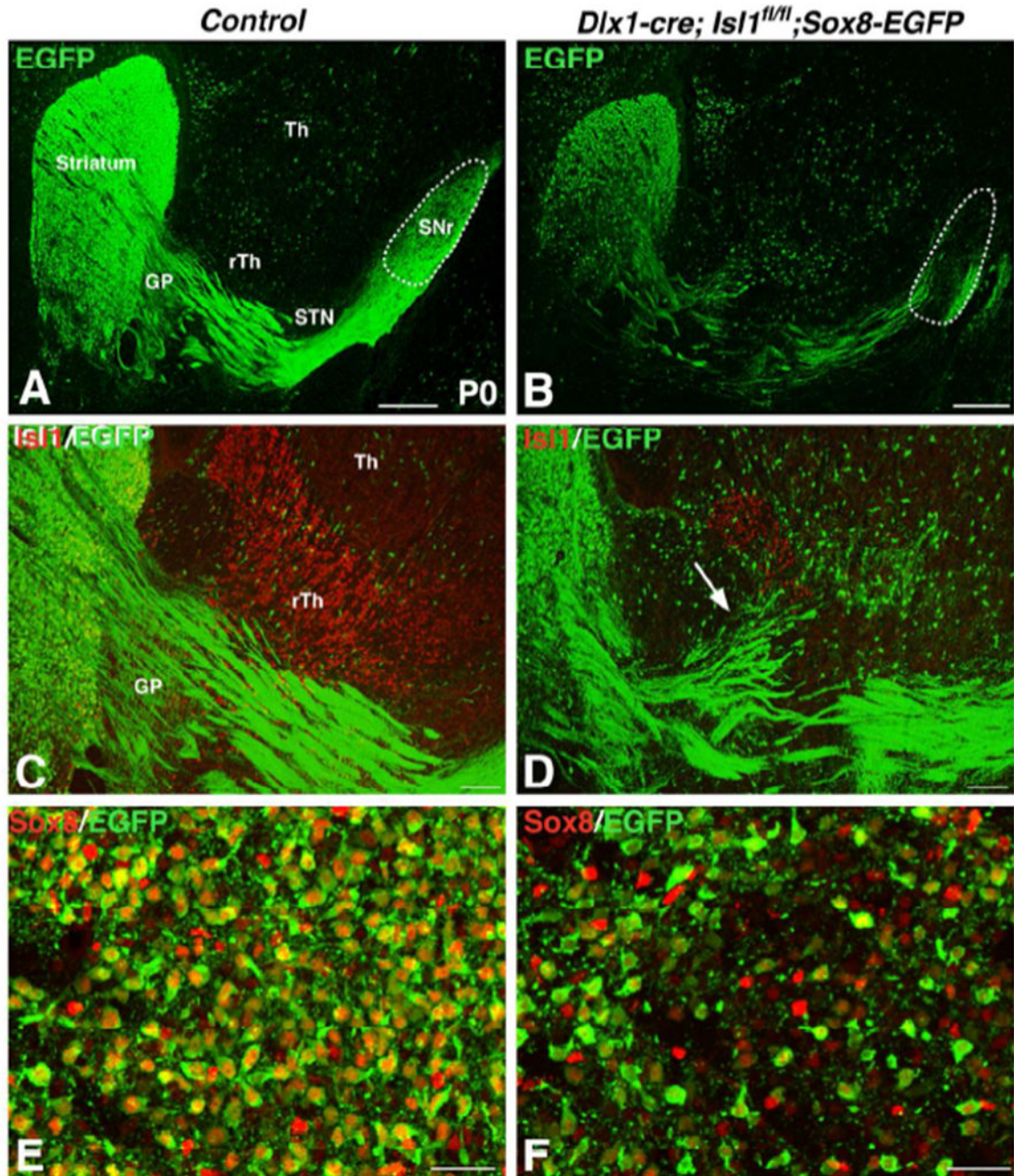
(A) Coronal hemisection of *Drd1-EGFP* brain showing scattered patch-like EGFP expression in the P0 striatum. (B) High power confocal image of Sox8/EGFP double staining demonstrating that at P0, Sox8<sup>+</sup> cells are more numerous than EGFP<sup>+</sup> cells, however, most colocalize with the EGFP<sup>+</sup> SPNs. (C) Coronal section of a *Drd2-EGFP* brain showing homogeneous distribution of EGFP<sup>+</sup> cells (i.e. iSPNs) throughout the striatal complex. (D) High power image showing a lack of co-labeling between EGFP and Sox8 indicating that iSPNs do not express Sox8. (E-G) Sagittal section from a P7 *Drd1-tdTomato; Sox8-EGFP* double transgenic brain show a high degree of overlap reporter expression pattern in the direct pathway. *Sox8-EGFP* appears to be more highly expressed in the dorsal versus ventral striatal complex. (H,I,J,K) High power confocal images from representative boxed areas along the direct pathway in G. A more extensive colocalization between tdTomato and EGFP in the dorsal striatum dSPNs (H) was observed as compared to those in the ventral region (I). (J,K) EGFP<sup>+</sup> fibers and terminals co-label with tdTomato in the EP (J) and SNr (K) both of which represent targets of the direct pathway. Abbreviations: EP; entopeduncular nucleus; GP: globus pallidus; SNr: substantia nigra pars reticulata; Th: thalamus. Scale bar: in A, C = 200µm; B, D = 10µm; E-G = 500µm; H-K = 20µm



**Figure 6.**

(A, B, C) High power confocal images of *Sox8-EGFP* brains showing EGFP<sup>+</sup> cells in the cerebral cortex (A), hypothalamus (B) and thalamus (C) with oligodendrocyte progenitor cells (OPC) morphology and double stained with Sox8. (D-K) Double staining Sox8-EGFP sections with Sox9 (D,E), Sox10 (F,G), Olig2 (H,I) or PDGFR $\alpha$  (J,K) shows that the vast majority of EGFP<sup>+</sup> cells in the cerebral cortex express typical OPC markers. Abbreviations: Ctx: cerebral cortex; Hyp: hypothalamus; Th: thalamus. Scale bar: A-K = 20  $\mu$ m





**Figure 7.** Sagittal views of control (*Is11<sup>fl/fl</sup>; Sox8-EGFP*) (A,C,E,G) and *Is11* cKO (B,D,F,H) brains showing the direct pathway as marked by EGFP. The loss of *Is11* results in a severe reduction of EGFP<sup>+</sup> cells within the striatum as well as EGFP<sup>+</sup> fibers in the direct pathway (B), as compared to control (A). (C,D) Higher power confocal images of A and B double stained for *Is11* and EGFP show the extent of *Is11* deletion in the conditional mutant (D). Note that the reduced *Is11* expression (red) in the reticular thalamic nucleus (rTh) corresponds with the misdirection of EGFP axons towards the thalamus (arrow in D) as compared to that seen in

the control (**C**). Double staining for EGFP and Sox8 in the *Isl1* cKO striatum (**F**) shows a severe reduction in positive cells (in line with the reported cell death phenotype), as compared to the control (**E**). Abbreviations: rTh: reticular thalamus; SNr: substantia nigra pars reticulata; STN: subthalamic nucleus; Th: thalamus. Scale bar: A, B = 300 $\mu$ m; C, D = 100 $\mu$ m; E, F = 20 $\mu$ m.

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**Table 1.**

## Antibodies used for immunohistochemistry

Antigen	Description of immunogen	Host species, Source, catalog No., RRID	Dilution
Isl1	E. coli-derived recombinant human Islet-1Met4-Ala349	Goat polyclonal, R and D Systems Cat# AF1837, RRID:AB_2126324	1:500
Sox8	Purified bacterially expressed protein consisting of amino acids 2–60 of mouse Sox8 fused to glutathione-S-transferase	Guinea pig polyclonal, Dr. Michael Wegner , RRID: AB_2571872	1:500
GFP	Recombinant GFP protein	Chicken polyclonal, Aves Labs Cat# GFP-1020, RRID:AB_10000240	1:1000
Ebf1	KLH-conjugated linear peptide corresponding to mouse EBF-1	Rabbit polyclonal, Millipore Cat# AB10523, RRID: 2636856	1:1000
FoxP1	Synthetic peptide derived from human FoxP1 within residues 650 to the C-terminus	Rabbit polyclonal, Abcam Cat# ab57216, RRID:AB_941648	1:4000
Nkx2.1	Recombinant rat TTF-1 6his fusion	Rabbit polyclonal, Seven Hills Bioreagents Cat# WRAB-1231, RRID:AB_451727	1:500
TH	Synthetic peptides from human and mouse TH gene product (P07101 and P24529)	Chicken polyclonal, Aves Labs Cat# TYH, RRID:AB_10013440	1:1000
SubsP	Substance P conjugated to BSA	Rat monoclonal, Millipore Cat# MAB356, RRID:AB_94639	1:500
Sox9	Amino acids 407-496 of Sox-9 of human origin	Rabbit polyclonal, Santa Cruz Biotechnology Cat# sc-20095, RRID:AB_661282	1:2000
Sox10	Human Sox10 N-terminus	Goat polyclonal, Santa Cruz Biotechnology Cat# sc-17342, RRID:AB_2195374	1:500
Olig2	Recombinant mouse Olig2	Rabbit polyclonal, Millipore Cat# AB9610, RRID:AB_570666	1:2000
PDGFR $\alpha$	Human PDGFR $\alpha$ C-terminus	Rabbit polyclonal, Santa Cruz Biotechnology Cat# sc-338, RRID:AB_631064	1:200
Tomato	DsRed-Express, Discosoma sp. Variant of red fluorescent protein	Rabbit polyclonal, Clontech Laboratories, Inc. Cat# 632496, RRID:AB_10015246	1:1000