The Role of Bone Morphogenetic Protein Mediated Signalling in the Neurogenic Niche of the Adult Mouse Brain

PhD Thesis

Submitted to the Faculty of Biology

Ludwig-Maximillian-University Munich

Prepared in the group Prof. Dr. Magdalena Götz

at the Helmholtz Zentrum München/Institute for Stem Cell Research

Dilek Colak

1. Gutachter: Prof. Benedikt Grothe

2. Gutachter: Prof. Rudiger Klein

Tag der mündlichen Prüfung: 28/07/2008

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2 ABSTRACT

In the mammalian brain, neurogenesis continues only in few regions of the forebrain. The molecular signals governing neurogenesis in these unique neurogenic niches, however, are still ill-defined. Here we identify a key neurogenic signalling pathway in one of the adult neurogenic niches. I examined the role of Bone Morphogenic Protein (BMP) mediated signalling that is discovered to be selectively active in adult neural stem cells and transit amplifying progenitors. This is in pronounced contrast to proliferating cells outside the neurogenic niche that do not have active BMP signalling as monitored by phosphorylated form of p-Smad1,5 and 8. Here I show that BMP signalling is crucial to initiate the neurogenic lineage in the adult mouse subependymal zone. Interference with the bone morphogenic protein (BMP)-mediated signalling by either genetic deletion of the central signalling mediator of this pathway (the transcription factor Smad4) or the extracellular infusion of Noggin that inhibits BMP-binding to the receptor both reduce adult neurogenesis dramatically. Smad4 deletion in stem, but not progenitor cells, as well as Noggin infusion lead to an increased number of Olig2-expressing progeny that migrate to the corpus callosum and differentiate into oligodendrocytes. Transplantation experiments further verified the cell autonomous nature of this phenotype. I showed that niche aspects are not affected as wild type cells differentiate normally in knockout background. These results therefore demonstrate one of the first key neurogenic signals allowing adult neurogenesis in the mammalian brain. Moreover, they identify the generation of oligodendrocytes as the fate that needs to be suppressed for neurogenesis to occur.

3 INTRODUCTION

3.1 Neurogenesis persists in the adult mammalian brain

It has been believed for long time that neurogenesis only occurs during development and there are no new neurons generated in the adult mammalian brain. During development of mammalian forebrain neurons are derived from proliferating cells of ventricular zone (VZ), a region lining the ventricles, and the subventricular zone (SVZ), a second proliferative zone above the ventricular zone. While VZ shrinks into a cell layer called ependyma, a part of it persists into adulthood as a mitotically active region called Subependyma (Smart, 1961; Lewis, 1968). When these proliferating cells were labelled by [3H]-tyhmidine it has been suggested that neurogenesis continues in this particular region of adult mammalian brain in rodents (Altman, 1963; Altman, 1969). The same labelling technique also suggested neurogenesis in the dentate gyrus (DG) of the hippocampus in rodents (Altman and Das, 1965; Bayer, 1982; Bayer et al., 1982). A study on rhesus monkeys suggested that unlike to rodents there are no neurons being generated in adulthood in primates (Rakic, 1985). However in the 1990s, new protocols for labelling dividing cells in the central nervous system (CNS), such as retrovirus and bromodeoxyuridine (BrdU) labellings, not only confirmed previous studies on rodents (Kuhn et al., 1996; Luskin, 1993; Seki and Arai, 1993) but also provided the first evidence that neurogenesis occurs in the adult primates, human and non-human (Eriksson et al., 1998; Kornack and Rakic, 1999; Curtis et al., 2007).

Furthermore, adult neurogenesis also occurs in non-mammalian vertebrates and insects (Goldman and Nottebohm, 1983; Cayre et al., 1998). In adult canaries new neurons are incorporated throughout most of the telencephalon, but not outside of it (Goldman and Nottebohm, 1983; Paton et al., 1985; Alvarez-Buylla, 1988). This spatial restriction emerges during development (Goldman and Nottebohm, 1983; Alvarez-Buylla et al., 1994; Barami et al., 1995). Neurons are born in the walls of the lateral ventricle (Goldman and Nottebohm, 1983; Barami et al., 1995) from which they migrate long distances to reach most areas of the telencephalon (Alvarez-Buylla, 1988). Old neurons are continuously replaced with new ones (Kim and Nottebohm, 1993) in a process thought to be related to plasticity and learning (Nottebohm, 1985; Alvarez-Buylla et al., 1990).

3.2 Neural stem cells and their progeny in adult SEZ

The SEZ is located throughout most of the lateral wall of the lateral ventricle (**Figure 1**). The presence of large numbers of dividing cells in the SEZ has been known for almost a century (Allen, 1912) and is known to occur in many vertebrates (Lewis et al., 1968; Blakemore et al., 1972; McDermott et al., 1990; Eriksson et al., 1998; Huang et al., 1998; Gould et al., 1999; Kornack and Rakic, 2001; Sanai et al., 2007). However, the fate of the large number of newly generated SEZ cells had been controversial. While pioneering work by Joseph Altman had suggested that the SEZ could generate neurons (Altman, 1969; Das and Altman, 1970), most other studies suggested that dividing SEZ cells serve as a reservoir of precursors for glial cells (Privat, 1972; Privat et al., 1972). Other investigators had suggested that the dividing cells in the SEZ die soon after mitosis (Smart, 1961; Morshead et al., 1992). Direct demonstration that large numbers of neuronal precursors exist in the adult SEZ was obtained by culturing SEZ cells that had been labeled in vivo with [3H]-Thymidine (Lois et al., 1993). In vivo adult SEZ cells generate new neurons that migrate to the olfactory bulb where they constantly replace interneurons (Luskin, 1993; Lois et al., 1994) (**Figure 1**). Recently, in vivo studies showed that not only neurons but also oligodendrocytes are generated in the adult SEZ (Menn et al., 2006; Hack et al., 2006). These immature oligodendorcytes then migrate to the corpus callosum (where oligodendrocytes myelinate axons of cortical neurons) and mature.

These findings suggested that neural stem cells are retained in the SEZ of adult mammals. In vitro studies also supported this notion. Cells that possess the properties of stem cells (self-renewal and multipotency) in culture can be isolated from the SEZ using epidermal growth factor (EGF) and fibroblast growth factor (FGF) (Morshead et al., 1994; Gritti et al., 1999).

Ultrastructural reconstruction of the adult SEZ by electron microscopy (EM) reveals that four major cell types constitute this region (Doetsch et al., 1997). SEZ neuroblasts (neuroblasts are neuronal progenitors that express already neuronal characteristics but still divide. In SEZ they are called type A cells), migrate in homotypic chains (Lois et al., 1996; Wichterle et al., 1997) in a network of interconnecting pathways distributed throughout the wall of the lateral ventricle (Doetsch et al., 1996). These chains are ensheathed by slowly proliferating type B cells or non proliferating astrocytes (Lois et al., 1996). B cells have properties of astrocytes,

including a light cytoplasm, thick bundles of glial fibrillary acidic protein (GFAP)-positive intermediate filaments, gap junctions, glycogen granules, and dense bodies (Doetsch et al., 1997). Scattered along the chains of type A cells are clusters of rapidly dividing type C cells (Doetsch et al., 1997). The SEZ is largely separated from the ventricle by a layer of multiciliated ependymal cells which is the fourth cell type of this region. Many of the chains of type A cells unite in the anterior and dorsal SEZ forming a restricted path called the rostral migratory stream (RMS). The RMS is the pathway along which neuroblasts migrate from the SEZ to the olfactory bulb, where the neuroblasts differentiate into granule and periglomerular neurons (Lois et al., 1994).

A critical step towards understanding adult neurogenesis is the identification of the primary precursors that generate the new neurons in vivo and stem cells in vitro. The identification of the stem cells is also critically important to engineer these cells and thereby to elucidate mechanisms governing neurogenesis.

Previous studies in the developing mammalian brain had suggested that radial glia serve as scaffolding for building the brain (Rakic, 1988) and they were committed progenitors of astrocytes which give them their name glia. Beginning of 2000 it has been understood that radial glia were progenitors for neurons in the developing mammalian brain (Malatesta et al., 2000; Noctor et al., 2001; Miyata et al., 2001; Malatesta et al., 2003). Radial glia arise during early brain development and share an important structural property with the earlier neuroepithelial cells: contact with both the ventricular and pial surfaces. Radial glia have their soma in the VZ, and possess a long process that extends towards the pial surface (Gadisseux et al., 1989; Bentivoglio and Mazzarello , 1999; Cameron and Rakic, 1991).

Radial glia in cold-blooded vertebrates persist into adult life (Horstmann et al., 1954; Stensaas et al., 1968; Stevenson and Yoon, 1982; Connors et al., 1987; Adolf et al., 2006). In amphibians and fish, there is no ependyma in adulthood and radial glia cells line the ventricle throughout adulthood (Adolf et al., 2006; Echeverri and Tanaka et al., 2002; Grandel et al., 2006; Stevenson and Yoon, 1981). Also in song birds radial glia persist in the adulthood (Alvarez-Buylla et al., 1987) and have been shown extending a single short cilium into ventricle (Alvarez-Buylla et al., 1998) like neuroepithelial cells (Cohen et al., 1987). And

finally in all those animals (fish, amphibian, birds, also reptiles) radial glia found to be in adult neurogenic areas and have been identified as progenitors for adult neurogenesis (Alvarez-Buylla et al., 1990; Echeverri and Tanaka et al., 2002; Garcia-Verdugo et al., 2002; Adolf et al., 2006; Grandel et al., 2006).

In contrast to lower vertebrates, radial glia do not exist in adult mammals. Radial glia disappear soon after birth. Within the first two postnatal weeks, radial cells of the lateral wall transform into astrocytes. It has been shown that radial glia in the striatum give rise to SEZ astrocytes (type B cells, see above) (Merkle et al., 2004). The transformation of radial glia in the lateral ventricular wall is similar to that previously described for cortical radial glia (Schmechel and Rakic, 1979; Voigt, 1989). This morphological change is accompanied by changes in the expression of molecular markers. This morphological and molecular transformation is not, however, associated with terminal differentiation for a subpopulation of adult astrocytes in SEZ (see below). Some of the properties of embryonic germinal cells are retained. Several lines of evidence indicate that SEZ astrocytes function as the primary neuronal precursors in vivo. Following infusion of the antimitotic drug cytosine-D-arabinofuranoside (Ara-C) into the adult mouse brain for 6 days, neuroblasts (A cells) and rapidly dividing transit-amplifying progenitors (type C cells) are eliminated. This treatment spares some SEZ astrocytes and the non dividing ependymal cells (Doetsch et al., 1999a). Twelve hours after Ara-C removal, SEZ astrocytes begin dividing. Dividing SEZ astrocytes give rise to type C cells, which in turn generate neuroblasts (Doetsch et al., 1999b) (**Figure 2**). Within 10 days, the entire SEZ regenerates. GFAP-expressing astrocytes can be specifically labeled in transgenic mice carrying the receptor for an avian retrovirus (Holland et al., 1998). SEZ astrocytes labeled with this method generate neurons that migrate to the olfactory bulb, indicating that under non-regenerating conditions SEZ astrocytes are also the primary precursors of the new neurons (Doetsch et al., 1999b). As indicated above, a subpopulation of SEZ cells in the adult rodent brain can respond to EGF to generate neural stem cells in vitro. Cells derived from vitally labeled SEZ astrocytes respond to EGF signaling both in vivo (Doetsch et al., 2002) and in vitro. These cells can undergo self-renewal and can generate both neurons and glia (Doetsch et al., 1999b). These findings prooved that SEZ astrocytes (B cells) correspond to the stem cells in the adult SEZ. Interestingly, some of these astrocytes have access to the ventricle (Doetsch et al., 1999b;

Conover et al., 2000) like neurogenic radial glia in lower vertebrates and also have primary cilia like neuroepithelial cells. Although astrocytes from other brain regions seem to have plasticity in early postnatal days (Laywell et al., 2000; Berninger et al., 2007) they no longer can do so at later stages.

Just before astrocytes were proposed to be neural stem cells, multiciliated ependymal cells had been suggested to function as neural stem cells for the SEZ in vivo and in vitro (Johansson et al., 1999). Several studies did not support this interpretation (Chiasson et al., 1999; Doetsch et al., 1999a; Doetsch et al., 1999b; Capela and Temple,). Ependymal cells do not incorporate mitotic markers at any of the survivals studied after Ara-C treatment (Doetsch et al., 1999a).

Beside the main roles of astrocytes such as interacting closely with blood vessels and neurons and bridging compartments between blood and brain tissue, supporting neurons physically and metabolically, some of them also acts as stem cells in adult mammalian nervous system.

3.3 Neural stem cells (NSCs) as promising cellular source for the treatment of diseases in nervous systems

While the functional roles of adult neurogenesis is not well proved, studies provided convincing evidence that newly generated neurons are able to integrate into the existing neuronal circuits in the adult CNS (van Praag et al., 2002; Carleton et al., 2003; Ninkovic et al., 2007).

However, this capacity of generating new neurons in the adult brain is very limited as most neurons in the adult brain are not renewed not even after brain injury. In this regard, discovery of on going neurogenesis in adulthood was very important even though the capacity of neurogenesis is considerably limited. This was an encouraging finding which prompted researchers to examine neurogenesis in degenerative diseases and following cerebral injury.

Until now, the major approaches for inducing regeneration in the damaged CNS can be classified into two subgroups:

(1) replacement of neurons
(2) support of existing neurons, to prevent excessive degeneration and promote rewiring and plasticity.
The first approach includes 2 main strategies:
(1) activation of endogenous neural stem cells and
(2) cell transplantation therapies.

Stem cells have two defining properties : they can self-renew to produce more stem cells and they can differentiate to generate all cell types of a given tissue.

Discovery of such cells, the so-called neural stem cells (NCSs), in adult and the fast progress in isolation of adult stem cells with neural capacities have renewed researchers' hope to repair the diseased CNS with adult stem cell-based neuronal replacement therapy. Neural stem cells can self-renew and give rise to new neurons, astrocytes, and oligodendrocytes. This was first shown from the subependymal zone (SEZ) (one of the adult neurogenic niche mentioned above) (Reynolds and Weiss, 1992; Richards et al., 1992) and then in the dentate gyrus (the second adult neurogenic niche mentioned above) of the hippocampus (Gage et al., 1995; Palmer et al., 1997), and in most structures of the brain examined (Palmer et al., 1995; Palmer et al., 1999; Shihabuddin et al., 2000; Kondo and Raff, 2000).

Because extensive amplification may be required to generate enough cells for transplantation, neural stem cells have to pass various procedures in vitro and are exposed extrinsic factors mainly mitogens. The standard method of isolating neural stem cells in vitro is to dissect out a region of the fetal or adult brain that has been demonstrated to contain dividing cells in vivo, for example, the subependymal (SEZ) or the hippocampus in the adult or a larger variety of structures in the developing brain. Usually, the tissue is disaggregated and then the dissociated cells are exposed to a high concentration of mitogens such as fibroblast growth factor–2 (FGF-2) or epidermal growth factor (EGF) in either a defined or supplemented medium on a matrix as a substrate for binding. After some proliferation, the cells are either induced to differentiate by withdrawing the mitogens or by exposing the cells to another factor that induces some of the cells to develop into different lineages. Cellular fates are analyzed by staining with antibodies directed against antigens specific for astrocytes,

oligodendrocytes, and neurons. After characterizing in vitro , investigators have grafted cells expanded with mitogenic growth factors and/or genetically marked cells back to the brain.

However numerous attempts to transplant multipotent NSCs directly into the non-neurogenic regions of the adult CNS (such as intact cerebellum or intact striatum) failed to generate neurons but rather generated glial cells (Gage et al., 1995; Suhonen et al., 1996; Shihabuddin et al., 2000).

This failure may be caused by several problems. In vitro propagation of stem cells with mitogenic factors may alter their intrinsic properties. Supporting this idea, transplantion of neuronal restricted progenitors did generate neurons (Han et al., 2002) suggesting that neuronal fate specification is a limiting step. However another work contradicting to this finding shows that even neuronal restricted progenitors can not generate neurons outside of neurogenic niches in the adult nervous system (Seidenfaden et al., 2000). Moreover, interpretation of some of the transplantation experiments are further complicated by the potential fusion events that have occurred between the transplanted cells and the host cells (Temple 2001; Raff, 2001). And finally, on top of this, using of embryonic and fetal tissue to develop stem cells raised ethic issues.

The technical and ethical difficulties associated with the cell therapy approaches promoted a search for alternatives. It had been shown that stem cells obtained from the adult hippocampus can be expanded in vitro and implanted back into the hippocampus, where they generate new neurons and glia, similar to the cells they generate normally in the adult dentate gyrus (Gage et al., 1995; Suhonen et al., 1996; Shihabuddin et al., 2000). Furthermore, these same cells can generate olfactory bulb neurons when implanted in the rostral migratory stream (RMS), expressing neurotransmitter phenotypes, such as tyrosine hydroxylase, which the cells do not make in the hippocampus but which are normally generated in the olfactory bulb (Gage et al., 1995; Suhonen et al., 1996; Shihabuddin et al., 2000).
Considering the incapability of the same stem cells isolated from adult neurogenic areas for generating neurons in non-neruogenic environment (see above), this finding indicates that adult CNS environment limits the neurogenic potentials of neural stem cells. Indeed, this is

consistent with the presence of endogenous progenitor cells in intact and injured CNS which can not generate neurons in their local environment ((Buffo et al., 2005; Horner et al., 2002). Damage to the central nervous system results in glial reaction and eventually formation of a glial scar (Pekny and Nilsson, 2005). Glial cells including astrocytes, NG2-expressing glial progenitors as well as microglia are involved in this process (Alonso, 2005; Magnus et al., 2007; Sofroniew, 2005). Although these cells become highly proliferative after acute stab wound lesion in mouse cerebral cortex they are restricted to a glial fate and fail to generate neurons (Alonso, 2005; Buffo et al., 2005). However, after transplantation into neurogenic niches, these cells can generate neurons indicating that environmental cues play critical role for instructing and/or allowing the progenitors to acquire a neurogenic fate.

Besides emphasizing how crucial it is to identify essential factors for survival, maturation, and full integration of the grafted cells, these findings raise an important question : is it possible that with accurate techniques or appropriate behavioral and environmental stimulation, neurogenesis can be revealed or induced in areas previously considered non-neurogenic, or will they give insight into mechanisms limiting neurogenesis in different regions? The key question therefore is whether it will be possible to convert the fate of progenitors in the adult brain parenchyma by defining the regulators of endogenous neurogenesis. This would mean a self-repair therapy instead of using external sources for cell replacement after injury.

Following this line several studies started to investigate restrictive signalling molecules in the damaged brain as well as the signaling cascades mediating fate choices in the adult neurogenic niches. To shed more light into this we focused on SEZ and tried to understand mechanisms of cell fate specification as until now very little is known about instructive signals governing neurogenesis in the adult SEZ particularly.

3.4 Regulators of adult neurogenesis in SEZ

Studies in a variety of models have revealed genetic, environmental and pharmacological factors that regulate adult neurogenesis in regard to different aspects such as proliferation,

self-renewal and migration. It is clear that stem cells are in a microenvironment, the so-called niche, which allows them to maintain their neurogenic behaviour. Adult SEZ retains many developmental characteristics maintaining the expression of specific extracellular matrix components and growth factors (Gates et al., 1995; Ninkovic and Gotz, 2007).

Factors that determine where the cells reside also likely determine their survival. In 1999 Goldman laboratory suggested that migrating neuroblasts localize to angiogenic vessels within brain parenchyma (Leventhal et al., 1999). Explants of adult rat SEZ raised on endothelial cells generated more neurons which survived longer than the explants raised on astrocytes, fibroblasts or laminin. They showed that endothelial neurotrophism was based on endothelial secretion of BDNF. Previously BDNF had been shown to increase newly generated neuron number in the adult SEZ by intraventricular administration. Shortly after the work of Leventhal, the Gage laboratory described a 'vascular niche' in the adult dendate gyrus (Palmer et al., 2000). According to their observation, dividing cells are found in dense clusters associated with the vasculature and roughly 37% of all dividing cells are immunoreactive for endothelial markers. Most of the newborn endothelial cells disappear over several weeks, suggesting that neurogenesis is intimately associated with a process of active vascular recruitment and following remodelling. Newly formed endothelial cells, resulting from angiogenic and vasculogenic processes, produce an array of neurotrophic factors, among which are BDNF, vascular endothelial growth factor (VEGF), and bFGF. These factors, in addition to being angiogenic, have strong chemotactic effects on stem-like cells and promote the survival and viability of these cells. Additionally, Shen and colleagues in 2004 demonstrated the effects of endothelial cells on both embryonic and adult neural stem cells. In the presence of endothelial cells, a neural stem cell undergoes symmetric, proliferative divisions to produce undifferentiated stem cell sheets that maintain their multipotency and, upon endothelial cell removal, generate neurons as well as astrocytes and oligodendrocytes (Shen et al., 2004). In 2006 Ramirez-Castillejo and colleagues revealed that a growth factor called Pigment epithelium derived growth factor (PEDF) is released by ependymal and vascular cells in the adult SEZ. They demonstrated that this factor stimulates self-renewal of type B cells, namely stem cells in vitro and in vivo as well as induces a molecular state of undifferentiation (Ramirez-Castillejo et al., 2006). This undifferentiated state is result of increased expression of Notch effectors (Hes1, Hes5) and transcription factor

Sox2 (Ramirez-Castillejo et al., 2006) all of which inhibit the actions of proneural bHLH factors (Ohtsuka et al., 2001; Bertrand et al., 2002; Hitoshi et al., 2002; Bylund et al., 2003; Ferri et al., 2004; Parras et al., 2004). Another factor suggested as controlling self-renewal of adult neural stem cells is Sonic Hedgehog (Shh). Palma and colleagues observed mitogenic effect of Shh in SEZ by blocking it with a specific drug (Palma et al., 2005). They also observed that inhibition of Shh decreases the number of neurosphere forming cells and vice versa indicating the effect of Shh on self-renewal. Consistent with this data recent work analyzing conditional Smoothened mutant found similar results. Smoothened (Smo) is a transmembrane protein that mediates Shh signalling. Deletion of Smo showed that Hedgehog is required for the maintenance of type B and C cell populations (Balordi and Fishell, 2007). A carbohydrate-binding protein Galectin is also suggested to act as self-renewing factor for adult neural stem cells (Sakaguchi et al., 2005). Galectin 1 increases the number of neurospheres in vitro and the number of slow dividing cells in vivo.

Besides self-renewal, proliferation of adult neural stem cells has been also extensively studied. As mentioned above, stem cells from the adult SEZ proliferate and make aggregates in the presence of epidermal growth factor (EGF) (Reynolds and Weiss, 1992) or fibroblast growth factor 2 (FGF2) (Richards et al., 1992; Gritti et al., 1996; Johe et al., 1996). The possibility that growth factors also may influence neural progenitors in vivo has been supported by findings that intracerebroventricular administration of EGF expanded proliferative progenitors in the SEZ of adult mice (Craig et al., 1996). This was consistent with the detection of EGF receptor in vivo in adult SEZ (Morshead et al., 1994; Seroogy et al., 1995; Weickert et al., 2000). FGF2 also increased proliferation of SEZ progenitors (Kuhn et al., 1997) when infused into lateral ventricle. This prompted the question whether more than one stem-like cell type may be present in the adult SEZ. (Stemple and Mahanthappa, 1997). Vescovi laboratory showed that both EGF and FGF2-responsive stem cells derive from a single precursor cell type that responds to both growth factors. Additionally, EGF and FGF2 are able to substitute for each other in maintaining and expanding the SEZ stem cell population, although EGF seems to be more effective (Gritti et al., 1999). However the analysis did not explore true identity of cells responding to these factors. Contrary to the commonly accepted hypothesis that EGF-responsive cells derive from a quiescent or slow dividing population, it has been shown that the majority of EGF-responsive cells in the adult

SEZ zone correspond to fast dividing transit amplifying progenitors (Doetsch et al., 2002). According to this study, transit amplifying cells retain stem cell characteristics when induced to continually proliferation by addition of exogenous growth factors.

Notch signalling also seems to be important for adult neurogenesis. The presence of Notch1 mRNA had been shown first in postnatal SEZ (Higuchi et al., 1995; Irvin et al., 2001). Notch1 mRNA has been shown to be expressed only by neuroblasts in SEZ, Rostral migratory stream and Olfactory bulb whereas its ligand Jagged1 is expressed by astrocytes in the same regions (Givogri et al., 2006). Immunohistochemistry for these antigens revealed the same pattern in postnatal SEZ (Nyfeler et al., 2005). Compound reductions in Jagged1 and Notch1 results in reduced proliferation in SEZ (Nyfeler et al., 2005). Jagged1 is also found in a subset of GFAP-positive cells within neurospheres and ablation of Jagged1 blocks neural stem cell self-renewal in vitro (Nyfeler et al., 2005). It has been shown that Jagged1 functions through Notch1 to maintain stem cell self-renewal and this pathway does not have a role in differentiation. As Sonic hedgehog was also proposed to be required for neural stem cell self-renewal in the adult SEZ, this raised the question whether these two pathways are in the same chain or act synergistically. Recent work provided evidence for Notch signalling as the inducer of Shh protein in fetal neural stem cells (Androutsellis-Theotokis et al., 2006). This work revealed that Notch signalling regulates stem cell number in vitro and in vivo by mainly keeping stem cells undifferentiated. Also Platelet-Derived Growth Factor Receptor (PDGFR)α was shown to be expressed in a subset of astrocytes and this population was referred to be the true stem cells as they respond to PDGF by increased proliferation and undifferentiated state (Jackson et al., 2006). However, gain and loss of function experiments revealed no function of this receptor in regard to neurogenesis but rather oligodendrogenesis without altering neurogenesis (Jackson et al., 2006).

Thus many factors have been identified that either regulate proliferation or self-renewal of stem cells or their immediate progeny. However, still very little is known about the factors or mechanisms promoting differentiation of neural stem cells into neurons in these unique niches particularly in SEZ. In the subgranular zone (SGZ) of dentate gyrus (DG) Wnt signalling is suggusted to promote neurogenesis (Lie et al., 2005). And recently the p75 neurotrophin receptor has been shown to be expressed by stem cells and ablation of this receptor leads to

reduction in neurogenesis (Young et al., 2007). Brain derived growth factor (BDNF) seems to be responsible for this function. However, in vivo data for these conclusions were missing as most of the data provided was done in vitro. Beside this work, still little is known about extrinsic factors promoting neurogenesis in the adult SEZ.

However more is known about intrinsic fate determinants of adult neurogenesis. First, the proneural transcription factor Mash1 was found to be expressed in transit-amplifying precursors and neuroblasts (Parras et al., 2004). Analysis of the Mash1 mutant phenotype revealed reduction in both neurogenesis and oligodendrogenesis at the expense of astrogliogenesis in the postnatal SEZ. Transcription factor Pax6 was identified as necessary and sufficient for neurogenesis in the adult SEZ by our laboratory (Hack et al., 2005). Conversely, Olig2 promotes oligodendrogenesis and rather blocks neurogenesis in the SEZ (Hack et al., 2005). In contrast to the expression pattern of Mash1 and Pax6, Olig2 is not expressed by neuroblasts but only by transit-amplifying precursors. High levels of Olig2 in these cells are able to convert neurogenesis to oligodendrogliogenesis in the adult SEZ.

As these transcription factors are expressed in neuroblasts and the transit-amplifying precursors, the obvious question is how their up-regulation in the neurogenic lineage is regulated and conversely how Olig2 up-regulation in the oligodendrogliogenic lineage is regulated. Indeed whereas non-neurogenic transcription factors are up-regulated upon stab wound injury, neurogenic factors are not (Buffo et al., 2005). Moreover overexpression of neurogenic transcription factors such as Pax6 and Ngn2 or supression of non-neurogenic transcription factor Olig2 after injury allows a certain degree of neurogenesis. Therefore to understand the signals allowing neurogenesis within this unique neurogenic niche may help to replacement such a positive environment also in the remaining brain parenchyma.

Previous analysis suggested that the bone morphogenic protein (BMP) signalling would inhibit neurogenesis and secretion of Noggin which is a specific inhibitor of BMP from the ependymal cells was crucial to allow neurogenesis in the adult SEZ (Lim et al., 2000). However, overexpression of BMP receptor1a in the postnatal SEZ did not affect neurogenesis (Coskun et al., 2001). Thus, the exact role of this versatile signalling pathway exerting multiple diverse roles in the developing nervous system (see below) has yet to be resolved in

relation to adult neurogenesis both in a neurogenic and non neurogenic context. As BMPs can also act neurogenic during development (see below), I focused here on the analysis of BMP signalling in the adult SEZ.

3.5 TGFβ superfamily and BMP signalling

BMPs are small cytokines of TGFβ superfamily. TGFβ superfamily cytokines are dimeric proteins with conserved structures, and have pleiotropic functions in vitro and in vivo (Kawabata et al 1998). This superfamily includes nearly 30 proteins in mammals. Beside bone morphogenetic proteins (BMPs) also TGFβs, activins and inhibins, nodal, myostatin, growth/differentiation factors (GDFs), and anti-Müllerian hormone (AMH, also called Müllerian inhibiting substance or MIS) (**see Figure 3 for phylogenetic tree of TGFβ superfamily**) belong to this superfamily. TGFβs/BMP-like proteins are found in various species, including Xenopus, Caenorhabditis elegans and Drosophila melanogaster.

3.5.1 Receptors and intracellular cascade of TGFβ superfamily

Although the biological effects of the cytokines of this superfamily appear to be complicated, two major pathways involving Smad proteins are activated by members of the TGFβ superfamily (Miyazono et al., 2001). Accordingly, the TGFβ superfamily cytokines can be classified into two subfamilies depending on the Smad signalling pathways they activate (**Figure 4 and Figure 5**). Members of TGFβ superfamily bind to two distinct receptor types, known as type II and type I receptors (Wrana et al., 1994; Heldin et al., 1997). Both type II and type I receptors are required for signal transduction (**Figure 4 and Figure 5**). Both type II and type I receptors contain serine/threonine kinase domains in their intracellular portions. The type II receptor kinases are constitutively active; upon ligand binding, hetero-tetrameric complexes composed of two molecules each of type II and type I receptors are formed (Kirsch et al., 2000; Qin et al., 2002). In the tetrameric receptor complexes, type II receptor kinases transphosphorylate the GS domain of type I receptors, which are located between the transmembrane domain and the kinase domain of type I receptors. Following phosphorylation of the GS domain, type I receptor kinases are activated and phosphorylate intracellular

substrates. Thus, type I receptors act as downstream components of type II receptors in the signalling pathways, and determine the specificity of the intracellular signals induced by the TGFβ superfamily cytokines. Five type II receptors and seven type I receptors are present in mammals (**Figure 5**). Among those Activin receptor-like kinase 2 (Alk2), BMPRI-A (Alk3) and BMPRI-B (Alk6) as type I receptors; Activin receptor II (ActRII) and Activin receptor IIB (ActRIIB) are activated upon BMP ligand binding (**Figure 5**).

3.5.2 Signalling by three types of Smad proteins

Smad proteins are the major signalling molecules acting downstream of the serine/threonine kinase receptors (Heldin et al., 1997; Moustakas et al., 2001). Smads are classified into three subclasses, i.e. receptor-regulated Smads (R-Smads), common-partner Smads (Co-Smads), and inhibitory Smads (I-Smads). R-Smads are further divided into two subclasses; Smad2 and Smad3 (referred to as activin/TGF-b activated R-Smads) and Smad1, Smad5 and Smad8 (referred to as BMP activated R-Smads; BR-Smads) (**Figure 5**). In contrast to several R-Smads Smad4 serves as the only Co-Smad in mammals. Smad6 and Smad7 function as I-Smads. It is proposed that Smad6 is more specific for the inhibition of BMP signalling whereas Smad7 serves as the inhibitor of TGFβ (Hanyu et al., 2001).

Smads are composed of the N-terminal Mad homology (MH1) domain, followed by linker regions and the C terminal MH2 domains. MH2 domains are conserved in all three subclasses of Smads, whereas MH1 domains are conserved in R-Smads and Co-Smads, but not in I-Smads. The amino acid sequences of linker regions diverge between Smads. In addition, R-Smads have Ser-Ser-X-Ser motifs in their most C-terminal parts, which are phosphorylated by type I receptors. R-Smads are directly phosphorylated and activated by type I receptor kinases. Without receptor activation, the MH1 and MH2 domains are physically associated with each other, and R-Smads are anchored as dimers to the plasma membrane through SARA and other molecules (Qin et al. 2002). Following the receptor activation and phosphorylation of R-Smads, the interaction between the MH1 and MH2 domains is disrupted, and R-Smads form hetero-oligomers with the Co-Smad through their MH2 domains. Although the exact structures of the Smad oligomers have not been fully determined, and hetero-trimer and hetero-dimer models have been suggested (Kawabata et al.,

1998; Qin et al., 2001; Wu et al. 2001), Qin et al., (2001) proposed that the R-Smad-Co-Smad complexes are hetero-trimers, containing two R-Smad molecules and one Co-Smad molecule. The R-Smad-Co-Smad complexes translocate into the nucleus and regulate the transcription of target genes. In the nucleus, R-Smads and Co-Smad interact with various DNA-binding proteins, which bind to promoter regions of target genes together with the Smads. In addition, R-Smads and Co-Smad bind to transcriptional co-activators and co-repressors, which induce the acetylation and de-acetylation of histones, respectively, and play important roles in transcriptional regulation (Miyazono et al. 2000).

I-Smads associate with activated type I receptors and prevent the activation of R-Smads by the receptors. In addition, Smad6 interacts with activated R-Smads and interferes with the formation of a complex with Smad4.

3.5.3 Versatile roles of BMP signalling in nervous system

The activity of BMPs was first identified in the 1960s (Urist et al., 1965) but proteins responsible for bone induction were unknown until the purification and sequence of bovine BMP-3 and cloning of human BMP-2 and 4 at the end of 1980s (Wozney et al., 1988; Luyten et al., 1989). At the end, 20 family members have been identified and characterized. Although BMPs are secreted from cells, their biological actions are likely to be quite local. For example, BMPs bind to extracellular matrix proteins, which may both limit their diffusion through tissues and function to present the ligands to specific cells in a more biologically active form. The localization of BMP signaling is further refined by the actions of endogenous extracellular inhibitors such as Noggin (Zimmerman et al., 1996) or Chordin (Piccolo et al., 1996) that block BMP functions and limit their availability.

BMP genes were found to be expressed in many embryonic organs and tissues, including those in which reciprocal interactions between epithelial and mesenchymal cells are important for morphogenesis and differentiation (e.g. in forming kidney, lung, tooth, skin and hair). These expression patterns have implicated multiple roles for BMPs at different stages of development. Following studies showed that BMPs are not only involved in bone formation but, like other members of the TGF-β superfamily, are multifunctional proteins with many

effects not related to the formation of bone (Cunningham et al., 1992; Yamashita et al., 1995). Knockout phenotypes for some of the BMP ligands and components of the Smad pathway are summarized with references in **Table 1** and **2**, respectively. BMPs were found to have important roles in directing cell fate choices of mesenchymal cells in vitro. They stimulate osteoblast differentiation and inhibit the differentiation of mesenchymal cells into the myoblast lineage (Vukicevic et al., 1989; Katagiri et al., 1994; Yamaguchi et al., 1991). However the function of BMP signaling is not restricted to development. BMPs have been implicated in maintenance of various organs as well as in other aspects in adulthood.

3.5.3.1 BMPs in early neural development

BMPs are also crucial regulators of nervous system development. BMPs act at different stages of neural development and in different regions of the central nervous system to regulate cell fate, proliferation and differentiation. The development of the CNS begins with the specialization of the ectoderm into either non-neural or neural ectoderm, a transformation that is actively directed by the presence or absence of BMP signalling, respectively (for Xenopus: Smith and Harland, 1992; Smith et al., 1993). While noggin and chordin are normally expressed on the dorsal side of the embryo, BMPs are found on the ventral side. In Xenopus, BMPs allow the formation of ectoderm whereas noggin and chordin induce a neural fate, a process that is inhibited by BMPs (Lamb, 1993; Wilson and Hemmati-Brivanlou, 1995; Sasai et al., 1995). Overexpressing mutant forms of BMP4 or BMP7, which block the normal function of BMPs in Xenopus, also leads to neural induction (Hawley, 1995). In contrast, knockdown of BMP antagonist function results in loss of neural tissue, particularly head structures (Smith et al., 1993; Oelgeschlager et al., 2003; Kuroda et al., 2004; Khokha et al., 2005).

Genetic studies in zebrafish and mice also highlight the importance of BMP signalling in ventral tissue formation (blood and ventral body wall/gut) and the suppression of BMP signalling by endogenous antagonists in the formation of neural tissue. In zebrafish, mutations in the BMP2 and BMP7 genes or the Smad homologues all result in ventral defects, whereas mutation of chordin results in neural defects (Kishimoto et al., 1997; Hild et al., 1999; Dick, 2000). Genetic studies in mice have also demonstrated the importance of BMP antagonism in

head formation. Although single knockouts of noggin and chordin do not produce a phenotype that is revealing in terms of the neural/ectodermal fate decision, mutations in both genes lead to the loss of anterior head structures (Bachiller et al., 2000). The results of these studies indicate that the suppression of BMP signalling on the dorsal side of the embryo is important to induce the ectoderm to form neural tissue although, as described in the discussion, other signalling pathways also contribute to this process.

3.5.3.2 BMPs in the developing spinal cord

Once the neural tissue is established, BMP signalling has positive influence on the regulation of dorsal neural cell type formation. BMPs are strongly expressed in the dorsal midline of the spinal cord, both during early development and at later stages that correlate with periods of oligodendrocyte precursor differentiation (Miller et al., 2004). BMP signalling acts in association with other developmental pathways such as WNTs and Sonic Hedgehog to coordinate cell proliferation and patterning, allowing the formation of the appropriate numbers and types of differentiated neurons. BMPs act as a gradient morphogen, inducing more dorsal fates at higher concentrations and intermediate fates at lower concentrations (Hogan, 1996; Nguyen et al., 2000). In contrast, the ventrally secreted morphogen Shh derived from notochord and floor plate (Echelard et al., 1993; Roelink et al., 1995; Ericson et al., 1997) acts to induce ventrally located cells in a concentration-dependent manner. In chick neural explant cultures, the addition of BMP induces dorsal cell types (Liem et al., 1997). In vivo experiments using in ovo electroporation of an activated BMP receptor (BMPR1A or BMPR1B) into chick embryonic spinal cord showed that BMP signalling is sufficient to transform more ventrally located cell types to a more dorsal pattern of bHLH and dorsal interneuron gene expression (Timmer et al., 2002). Transgenic mouse embryos overexpressing activated BMPR1A or BMPR1B also showed expansion of dorsal cell types in the spinal cord, which provided further evidence that BMPs are important for dorsal spinal cord development (Panchision, 2001).

The region of the ventral neural tube that gives rise to motor neurons afterwards generates oligodendrocytes, the myelinating cells of the CNS (Bunge, 1968). Effects of BMPs and Shh extend beyond motor neuron identity and contribute to the location of the founder cells of the

oligodendrocyte lineage. Spinal cord oligodendrocytes arise from cells in the motor neuron domain (Pringle and Richardson, 1993; Ono et al., 1995; Richardson et al., 1997; Miller, 2002), and this localization is dependent on the local expression of Shh (Pringle et al., 1996; Orentas et al., 1999). Shh signaling in the ventral region induces Olig 1 and Olig 2, Shh-dependent transcription factors that regulate in the development of oligodendrocytes (Lu et al., 2000, 2002; Zhou et al., 2001; Zhou and Anderson, 2002). Implantation of noggin secreting cells adjacent to the dorsal chick neural tube promoted the appearance of oligodendrocyte precursors in dorsal neural tube (Mekki-Dauriac et al., 2002), suggesting that endogenous dorsally expressed BMPs inhibit oligodendrogenesis. Conversely, elevated BMP expression inhibited the appearance of ventral oligodendrocyte precursors (Mekki-Dauriac et al., 2002). The inhibition of early oligodendrogenesis by BMPs is not restricted to chick spinal cord. Implantation of BMP-coated beads into developing Xenopus is sufficient to suppress ventral oligodendrogenesis, whereas implantation of either Shh-coated beads or anti-BMP coated beads is sufficient to induce dorsal oligodendrocytes (Miller et al., 2004). A similar phenomenon was observed in slice cultures of rat spinal cord exposed to soluble ligands in vitro. In these studies, function-blocking antibody specific for BMP4 increased oligodendrocytes, consisting the hypothesis that BMP4 is the endogenous dorsally active factor. BMPs inhibit the development of several stages of oligodendrocyte differentiation, becoming a more effective inhibitor at later stages (Miller et al., 2004).

As in the spinal cord, BMP signalling is also necessary for the development of the most dorsal structure of the forebrain whereas the development of the ventral forebrain is dependent on the inhibition of BMP signalling (Furuta et al., 1997; Anderson et al., 2002; Hebert et al., 2002).

BMPs also help to define the region from which a vertebrate-specific population of neural cells, the neural crest cells, will be generated (Nguyen, 1998; Nguyen, 2000). This multiprogenitor population arises at the border between the neuroectoderm and the non-neural ectoderm. It has been shown that combined actions of BMP and WNT are required for neural crest induction (LaBonne et al., 1998). BMPs also promote neural crest cell migration, mediate neural crest apoptosis in the hindbrain (Graham et al., 1994; Marazzi et al., 1997; Sela-Donenfeld and Kalcheim, 1999; Sela-Donenfeld and Kalcheim, 2000; Smith and

Graham, 2001). The neural crest gives rise to many diverse cell populations, including the peripheral nervous system (PNS) (Farlie et al., 2004). Furthermore, BMPs are essential and sufficient to induce the generation of sympathetic neurons from neural crest cells in vitro and in vivo (Varley et al., 1995; Reissmann et al., 1996; Shah et al., 1996; Shah and Anderson, 1997; Schneider et al., 1999). BMPs induce in vitro expression of the proneural gene Mash1 (Shah et al., 1996) and Phox2a (Reissmann et al., 1996), genes that are essential for sympathetic neuron generation. Schneider and colleagues demonstrated that inhibition of BMPs in the chick embryo by Noggin prevents sympathetic neuron generation. In Noggin-treated embryos, the noradrenergic marker genes tyrosine hydroxylase (TH) and dopamine-beta-hydroxylase (DBH), panneuronal neurofilament 160 (NF160) and panneuronal SCG10 genes, and the transcriptional regulators Phox2b and Phox2a are not expressed in sympathetic ganglia while initial ganglion development is not affected.

3.5.3.3 BMPs in the developing brain

BMP ligands and receptors are present in the developing and adult brain (Gross et al., 1996; Soderstrom et al., 1996; Ebendal et al., 1998; Li et al., 1998; Zhang et al., 1998; Lim et al., 2000; Peretto et al., 2002; Peretto et al., 2004), suggesting that BMP signalling is a key factor for cell fate determination in the brain as well. Indeed, BMPs are important regulators of cerebellar granule neuron fate determination (Alder et al., 1999). BMP treatment of ventral metencephalic explants taken from neurulation-stage mouse embryos results in the upregulation of expression of genes specific to granule neurons, such as Math1, zinc finger protein of the cerebellum 1 (Zic1), Zic2 and Wnt3a. In addition, ventral metencephalic cells, when transplanted to the adult cerebellum, failed to contribute to the granule cell population. However, BMP-treated ventral metencephalic cells contribute to granule cells after transplantation. BMP signalling is therefore sufficient to induce a dorsal cell fate in ventral cerebellar precursors. BMP signalling is involved in regulating granule cell differentiation during embryonic development and postnatal development (Angley et al., 2003; Rios et al., 2004).

BMP stimulation also increases neuronal differentiation in cortical cultures (Li et al., 1998). Li and collegues showed that in cultures of dissociated neocortical neuroepithelial cells (Embryonic day 12, E12), BMPs increase the number of MAP-2– and TUJ1- positive cells

(MAP-2 and TUJ1 are neuron specific antigens) within 24 hr of treatment. In explant cultures, BMP-4 treatment leads to an increase in the number of TUJ1- positive cells within the ventricular zone. Inhibition of BMP signalling in neocortical precursors blocks neurite elaboration and migration out of the VZ. Conversely, Noggin inhibits differentiation of neurons in dissociated cultures from embryonic day 12 cortex. Another group showed that BMPs promote cell death and inhibit the proliferation of early (embryonic day 13, E13) ventricular zone progenitor cells whereas at later embryonic (E16) stages of cerebral cortical development, BMPs exhibit a concentration-dependent dissociation of cellular actions, with either enhancement of neuronal and astroglial determination (at 1–10 ng/ml) or potential cell death (at 100ng/ml). Moreover, several groups also observed that BMPs promote apoptosis of neural cells (Graham et al., 1994; Glozak and Rogers,1996; Song et al., 1998), including the early telencephalic neuroectoderm (Furuta et al., 1997).
Notably, different neural precursors react differently to BMP signalling. BMP treatment of EGF-responsive SVZ progenitor cells in vitro instructs cells to commit to the astroglial lineage (Gross et al., 1996) whereas treatment of FGF-responsive VZ progenitor cells with BMPs initiates neuronal differentiation or apoptosis depending on the concentration of the cytokine and other factors such as cell density (Mabie et al., 1999).

Later in development, during periods of intense gliogenesis, BMP stimulation enhances astrogliogenesis and inhibits oligodendrogenesis from late embryonic subventricular zone precursors or neuroepithelial cells (Gross et al., 1996; Grinspan et al., 2000; Mehler et al., 2000; Nakashima et al., 2001; Yanagisawa et al., 2001). Consistent with the hypothesis that the development of cortical astrocytes and oligodendrocytes is regulated by BMPs, altering the spatial and temporal expression of BMPs changes glial development in vivo. In the cortex of animals that overexpressed BMP4 driven by the neuron-specific endolase promoter, increased numbers of GFAP- or S-100β-positive (Glial fibrillary acidic protein(GFAP) and S100β are astrocyte markers) astrocytes and a smaller, but significant, reduction in the number of oligodendrocytes were observed (Gomes et al., 2003).

The effects of BMP on the differentiation of neuroepithelial or stem cells in the brain seem to be more complex than in the spinal cord, with different BMP effects extremely adjusted to particular stages of neural precursors (Grinspan et al., 2000; Mehler et al., 2000). The exact

cellular responses may depend on both the nature of the target cells and the context in which the different signals are presented. Therefore, tissue-,cell-,stage-specific knockout of a specific BMP ligand, a subtype of BMP receptors or a specific signaling molecule is required to determine the specific role of a BMP ligand, receptor or signaling molecule in a particular tissue, in a particular cell type and stage.

3.5.3.4 BMPs in the adult brain

In the adult CNS, BMP receptor expression persists (Zhang et al., 1998) suggesting that neural cells retain the ability to respond to the ligands. BMP6 mRNA has been reported to be present in hippocampal neurons, cerebral cortex and cerebellar cortex neurons (Martinez et al., 2001). However this data should be confirmed as no evidence was provided for co-localization of this ligand with neurons.

BMP7 mRNA is present at low levels in adult spinal cord but is dramatically increased in glial cells and motor neurons after traumatic injury (Setoguchi et al., 2001) as well as after demyelinating lesion (Fuller et al., 2007). Also BMP receptor expression suggested to be up-regulated on neurons of dentate gyrus after mechanical injury (Lewen et al.,1997) but still little is known about the expression pattern of BMP signalling components particularly in intact or injured cerebral cortex .

Expression of BMP ligands or receptors in the adult SEZ have been also reported (Zhang et al., 1998; Lim et al., 2000; Peretto et al., 2002; see also in results) Previous analysis suggested that the bone morphogenic protein (BMP) signalling would inhibit neurogenesis and secretion of Noggin from the ependymal cells was crucial to allow neurogenesis in the adult SEZ (Lim et al., 2000). However, overexpression of BMP receptor1a in the postnatal SEZ did not affect neurogenesis (Coskun et al., 2001). Thus, the exact role of this versatile signalling pathway exerting multiple diverse roles in the developing nervous system has yet to be resolved in relation to adult neurogenesis. Indeed, it has recently been shown that the same signalling molecule, Sonic Hedgehog, may exert different effect on adult neural stem cells, TAPs or neuroblasts respectively (Balardi and Fishell, 2007).

Here I examined the activity and function of BMP-mediated signalling in the adult SEZ. I discovered that Smad mediated BMP signalling is active in stem cells and their immediate

progeny transit amplifying progenitors (TAPs=type C cells) by using two independent means (see in results). To study the role of BMP signalling in stem cells I disrupted the Smad pathway by genetic deletion of Smad4, the common mediator, in stem cells. To achieve this I used GLAST::CreERT2 mouse line containing the inducible form of Cre (CreERT2) in the GLAST, the astrocyte specific glutamate transporter, locus (Mori et al., 2006) and Smad4 floxed line containing loxP sites flanking exon 8 (Yang et al., 1998). GLAST::CreERT2 mouse line allows efficient targeting of radial glial cells and astrocytes at different developmental stages and adulthood and thereby to address the function of specific genes in astroglial cells. Following recombined cells by expression of a reporter gene in the adult SEZ showed that we can target neural stem cells by using this line (Ninkovic et al., 2007) (see also in the results).

Genetic deletion of the key signalling mediator Smad4 in stem cells of adult SEZ by this way resulted in a diversion of the neurogenic lineage towards the generation of oligodendrocytes. Infusion of Noggin as well resulted in alteration in neurogenesis and increase in Olig2-positive cells indicating BMP specificity of the phenotype.

I could further demonstrate that this lineage switch is initiated in the stem cells while manipulations at later stages were ineffective. Thus, these results imply Smad4-mediated BMP-signalling as the earliest pro-neurogenic signal so far identified in the adult neural stem cell niche.

I also analyzed expression of BMP ligands and other signalling components including BMP specific phoshorylated Smads in intact and injured adult cortex. I found that BMP signalling is active in neurons of cerebral cortex and is not increased after Stab Wound injury indicating that this signalling is one of the key difference between SEZ astrocytes the so called neurogenic astrocytes and cortical non neurogenic astrocytes.

4 ABBREVIATIONS

aCSF	Artificial cerebrospinal fluid
ActRII	Activin receptor 2
Alk	Activin receptor like kinase
APC	Adenomatous polyposis coli
Ara-C	ß-D-arabinofuranisode
Bambi	BMP and Activin membrane bound inhibitor
BMP	Bone morphogenetic protein
BMPRII	Bone morphogenetic protein receptor 2
BRE	Bone morphogenetic protein responsive element
CNPase	2',3'-Cyclic Nucleotide 3'-Phosphodiesterase
Ctx	Cortex
Dapi	4'-6-Diamidino-2-phenylindole
DCX	Doublecortin
DG	Dentate gyrus
Dlx2	Distal less homeobox 2
DW	Dorsal Wall
DNAse	Deoxyribonuclease
dNTP	Deoxynucleotides
EGF	Epidermal growth factor

eGFP	Enhanced green fluorescent protein
ERT2	Estrogen receptor
fl	floxed
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GFAP	Glial fibrillary acidic protein
GLAST	Glutamate astrocyte-specific transporter
HPRT	Hypoxanthine guanine phosphhoribosyl transferase
IRES	Internal ribosome entry site
ip	Intraperitoneal
LI	Labelling index
LTR	Long terminal repeat
LV	Lateral ventricle
LW	Lateral wall
MW	Medial wall
n	Sample number
NGS	Normal goat serum
OB	Olfactory bulb
Olig2	Oligodendrocyte transcription factor 2
PBS	Phosphate buffered saline
PGC	Primordial germ cells
PCR	Polymerase chain reaction
PDL	Poly-D-lysine

PDGF	Platelet derived growth factor
PDGFRα	Platelet derived growth factor receptor alpha
PFA	Paraformaldehyde
PSANCAM	Polysialylated neural cell adhesion molecule
pSmad	Phoshorylated Smads
RMS	Rostral migratory stream
RNAse	Ribonuclease
RT	Room temperature
RT-PCR	Realtime-polymerase chain reaction
R-Smads	Receptor regulated Smads
SARA	Smad anchor for receptor activation
SEM	Standart error of mean
SGZ	Subgranular zone
Str	Striatum
TAP	Transit amplifying progenitor
TGFβ	Transforming growth factor beta
TGFβR	Transforming growth factor beta receptor
TSA	Tyramid signal amplification
Wt	Wild type

5 Materials and Methods

5.1 Animals

5.1.1 Strains

In all experiments 9- to 10- week-old animals were used. The Smad4 floxed mouse line (Yang et al., 1998) was received on a 129/FVB and Black Swiss background and back crossed to C57/Bl6/J. GLAST::CreERT2 mice (Mori et al., 2006), BRE-EGFP mice (Monteiro et al., 2004), Rosa26 reporter mice (Soriano, 1999) and the Tgfbr2 floxed mice (Leveen et al., 2002) were maintained on C57Bl6/J background. The Smad4 floxed mouse line contains loxP sites flanking exon 8 (Yang et al., 1998). The GLAST::CreERT2 mouse line contains the inducible form of Cre (CreERT2) in the GLAST, the astrocyte specific glutamate transporter, locus. The fusion of Cre to the ligand binding domain of the modified estrogen receptor (ERT2) is restricted to the cytoplasm and translocates only upon tamoxifen binding into the nucleus where it can then mediate the recombination. Tgfbr2 floxed mice contain loxP sites flanking exon 2 of the Tgfbr2. The Rosa26 reporter mice carry a triple polyadenylation sequence at the 3′ end of the neocasette in order to prevent transcriptional readthrough which is in this case LacZ. The neocasette is flanked by loxP sites and transcription only occurs when Cre recombinase excises. BRE-eGFP mice carry a Smad1/5-response element derived from Id1 promoter coupled to enhanced-Green Fluorescent Protein (eGFP). While mice positive for BRE-EGFP were identified by visualization of GFP at the fluorescent stereo microscope the others have been genotyped for identification.

5.1.2 Genotyping

For maintenance of the colonies and to identify control or mutant, mice were genotyped by PCR on tail DNA. DNA was obtained following the protocol from Laird et al., (Laird et al., 1991) : Tail biopsies of less than 5mm length were transferred in 0.5ml lysis buffer and incubated rotating for several hours or overnight at 55ºC in a modified hybridization oven. Following complete lysis, hairs and tissue residues were removed by centrifugation in an Eppendorf centrifuge at maximal speed (13.1 x 103g~ 16.000 rpm) for 10-20 minutes. The supernatant was poured into 0.5 ml isopropanol and

mixed well. DNA-precipitates were transferred in 300-500µl TE-buffer. To solve the DNA, tubes were again rotated at 55ºC for several hours.

5.1.2.1 GLAST::CreERT2 PCR protocol

PCR was carried out using approximately 40ng of genomic DNA (~1µl) and 1µM each of the primers GLAST::CreERT2 forward, GLAST::CreERT2 reverse, GLAST::CreERT2_Cer1 (see Table 6.1) in a 30µl reaction containing 0.2mM dNTPs, 1.5 U of HotstarTaq-polymerase, 3µl 10xPCR-buffer and 6µl 5xQ-solution. Cycling conditions were: 2 minutes at 94°C for HotStarTaq-activation and 35 cycles at 94°C for 20 seconds, at 55°C for 20 seconds and at 72°C for 30 seconds followed. Finally, amplicons were extended at 72°C for 5 minutes. 15µl of each PCR-product was analysed on a 1% agarose-TBE-gel. The amplicon obtained from normal WT-DNA 700bp long, whereas the amplicon from GLAST::CreERT2-DNA is 400bp long.

5.1.2.2 Smad4/floxed PCR protocol

PCR was carried out using approximately 80ng of genomic DNA (~2µl) and 2µM each of the primers Smad4-9 and Smad4-10 (see Table 6.1) in a 30µl reaction containing 0.2mM dNTPs, 1.5 U of HotstarTaq-polymerase, 2.5µl 10xPCR-buffer and 5µl 5xQ-solution. Cycling conditions were: 2 minutes at 94°C for HotStarTaq-activation and 35 cycles at 94°C for 30 seconds, at 60°C for 30 seconds and at 72°C for 1 minute followed. Finally, amplicons were extended at 72°C for 10 minutes. 15µl of each PCR-product was analysed on a 1% agarose-TBE-gel. The amplicon obtained from normal WT-DNA 450bp long, whereas the amplicon from Smad4/floxed-DNA is 500bp long.

5.1.2.3 Tgfbr2/floxed PCR protocol

PCR was carried out using approximately 80ng of genomic DNA (~2µl) and 2µM each of the primers tgfbr2/floxed_up and tgfbr2/floxed_down (see Table 6.1) in a 40µl reaction containing 0.2mM dNTPs, 1.5 U of HotstarTaq-polymerase, 2.5µl 10xPCR-buffer and 5µl 5xQ-solution. Cycling conditions were: 5 minutes at 95°C for HotStarTaq-activation and 38 cycles at 95°C for 30 seconds, at 62°C for 30 seconds and at 72°C for 40 seconds followed. Finally, amplicons were extended at 72°C for 7 minutes. 15µl of each PCR-product

was analysed on a 1% agarose-TBE-gel. The amplicon obtained from normal WT-DNA 422bp long, whereas the amplicon from Tgfbr2/floxed-DNA is 575bp long.

5.1.2.4 Rosa26 reporter (R26R) PCR protocol

PCR was carried out using approximately 40ng of genomic DNA (~1µl) and 1.2µM each of the primers R1, R2 and R3 (see Table 6.1) in a 30µl reaction containing 0.2mM dNTPs, 1 U of HotstarTaq-polymerase, 3µl 10xPCR-buffer and 6µl 5xQ-solution. Cycling conditions were: 15 minutes at 94°C for HotStarTaq-activation and 35 cycles at 94°C for 30 seconds, at 58°C for 30 seconds and at 72°C for 30 seconds followed. Finally, amplicons were extended at 72°C for 5 minutes. 15µl of each PCR-product was analysed on a 1% agarose-TBE-gel. The amplicon obtained from normal WT-DNA 500bp long, whereas the amplicon from R26R-DNA is 250bp long.

5.1.2.5 hGFAP-eGFP PCR protocol

The PCR protocol for genotyping of the the hGFAP-eGFP mouse line was adapted from a previous publication (Nolte et al., 2001). PCR was carried out using about 40ng of genomic DNA (~1µl) and 0.4µM of the primers GFAP-LZ1 and GFP-2 (see Table 4.1) in a 30µl reaction containing 0.2mM dNTPs, 1.5 U of Taq-DNA-polymerase, 3µl 10xPCR-buffer and 3µl 5xQ-solution. Cycling conditions were: 4 minutes at 94°C, followed by 30 cycles at 94°C for 30 seconds, at 61.5°C for 30 seconds and at 72°C for 1 minute. Finally, amplicons were extended at 72°C for 5 minutes. 15µl of each PCR-product was analysed on a 1 % agarose-TBE-gel. The amplicon obtained from transgenic animals is 498bp long. In all PCRs water was used as a control sample.

Table 5.1 PCR-Primers

Primer name	Primer sequence
GLAST::CreERT2_F8	5'- GAGGCACTTGGCTAGGCTCTGAGGA-3'
GLAST::CreERT2_R3	5'-GAGGAGATCCTGACCGATCAGTTGG -3'
GLAST::CreERT2_Cer1	5'-GGTGTACGGTCAGTAAATTGGACAT -3'

R1	5’-AAAGTCGCTCTGAGTTGTTAT -3’
R2	5’- GCGAAGAGTTTGTCCTCAACC-3’
R3	5’-GGAGCGGGAGAAATGGATATG -3’
Smad4-9	5’- GGGCAGCGTAGCATATAAGA-3’
Smad4-10	5’- GACCCAAACGTCACCTTCAC-3’
Tgfbr2floxed_up	5’-TGGGGATAGAGGTAGAAAGACATA -3’
Tgfbr2floxed_down	5’- TATGGACTGGCTGCTTTTGTATTC-3’
hGFAP-eGFP-primer1	5’-ACTCCTTCATAAAGCCCTCG-3’
hGFAP-eGFP-primer2	5’-AAGTCGATGCCCTTCAGCTC-3’

5.2 Tamoxifen Administration

Tamoxifen (SIGMA, T-5648) was dissolved in pre-warmed corn oil (SIGMA, C-8267) at 20mg/ml concentration on a shaker at 37ºC overnight. 10 mg tamoxifen is required to achieve recombination efficiently. Following a previously established protocol, 1mg tamoxifen was injected intraperitoneally (i.p) twice a day for 5 consecutive days (Mori et al., 2006). The solution was kept at 4ºC maximum 1 month to avoid precipitation.

5.3 Tissue culture

5.3.1 Neurosphere Assay

Subependymal zone (SEZ, the lateral wall of lateral ventricle) of GLAST::CreERT2/Smad4wt/wt or Smad4wt/floxed as controls and GLAST::CreERT2/Smad4floxed/floxed were dissected immediately or 21 days after the end of tamoxifen application and enzymatically dissociated in 0.7 mg/ml hyaluronic acid, 1.33 mg/ml trypsin in HBSS with 2 mM glucose at 37ºC for 30 minutes and gently triturated. The cells were centrifuged at 200g for 5 minutes, resuspended in 0.9 M sucrose in 0.5XHBSS (GIBCO), and centrifuged for 10 minutes at 750 g. The cell pellet was

resuspended in 2 ml of culture medium, placed on top of 10 ml 4% BSA (GIBCO) in EBSS solution, and centrifuged at 200 g for 7 minutes, followed by washing in Dulbecco's modified eagle medium:F-12 nutrient mixture (DMEM/F12, Gibco). The culture medium consisted of 20 ng/ml EGF (Roche), 20 gn/ml FGF (Roche), B27 supplement (Gibco), 2mM glutamine, 100U/ml penicillin and 10µg/ml streptomycin (Gibco) in DMEM-F12 medium. Dissociated cells were cultured at low density (10 cells/µl) to ensure the clonal origin of neurospheres (Morshead et al., 1994) in a 24 well plate (Falcon, well size: ~ 2cm^2). Every other day EGF and FGF were added to the cultures. Primary neurospheres were quantified 7 days after and the spheres were dissociated mechanically into single cells in order to get secondary neurospheres. Single cells were cultured again 10 cells/µl in the same conditioins in 24 well plates. Secondary neurospheres were quantified 7 days after culturing.

Figure 5.1 Scheme demonstrating neurosphere assay from adult SEZ.

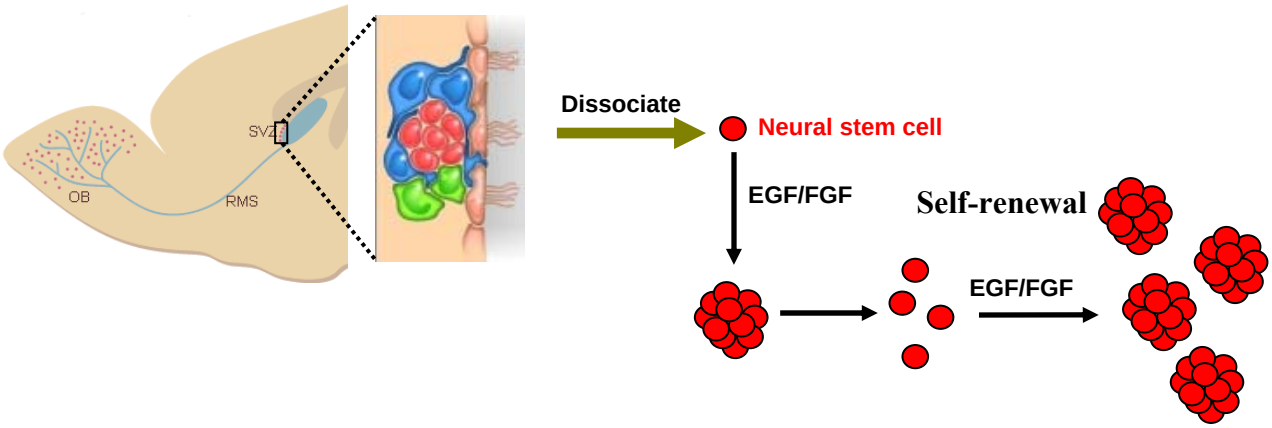
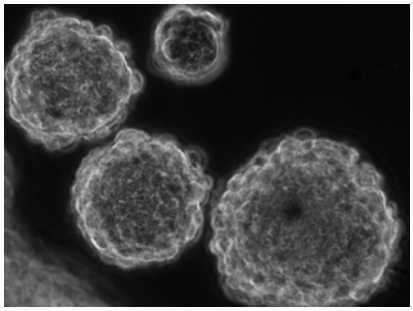


Figure 5.2 Image of primary neurospheres from adult SEZ after 7 days in vitro

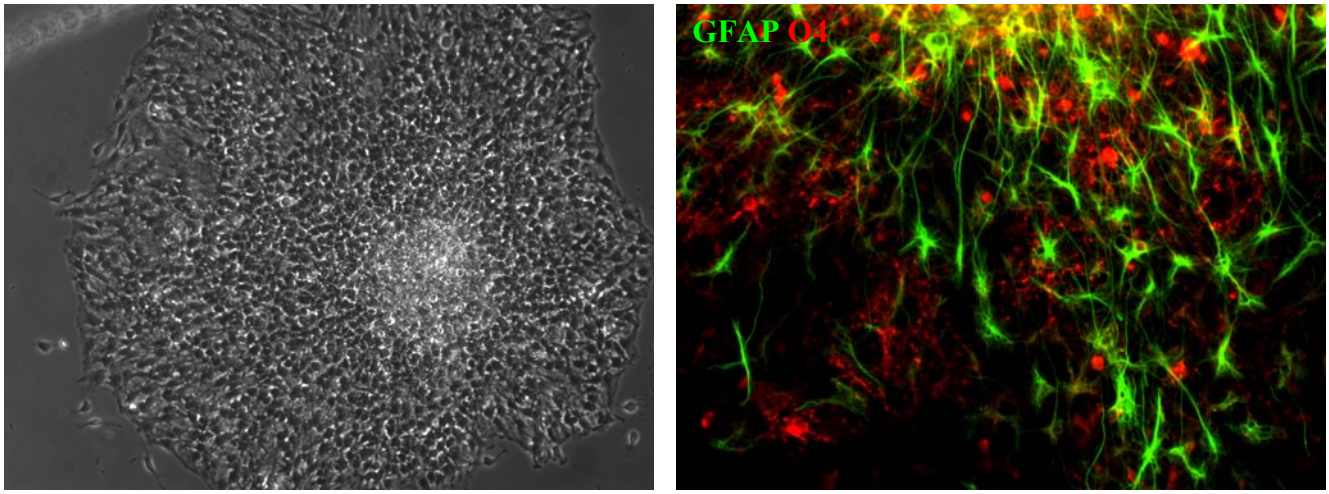


5.3.2 Differentiation of Neurospheres

For differentiation, neurospheres, spun down and either enzymatically or mechanically dissociated. For enzymatic dissociation the spheres were trypsinized at 37ºC for 3 minutes. For mechanic dissociation 200µl pipet tip were used to triturate the cells (30 times up 30 times down). Dissociated cells were plated on PDL-coated glass coverslips at a density of 1-2x10^5 cells/ml and cultured in DMEM-F12 medium with B27 supplement (Gibco), 2mM glutamine, 100U/ml penicillin and 10µg/ml sterptomycin (Gibco). No growth factor was kept in the medium. In some cases the spheres were directly plated on PDL-coated glass coverslips without dissociation. In this case cells migrated from the core of sphere and differentiated (See below).

Figure 5.3 Whole mount neurosphere after differentiation for 5 days in phase contrast (A) and fluorescent (B) images (GFAP stains for astrocytes, O4 stains for oligodendroctyes)

A **B**



5.4 Histological procedures and Immunohistochemistry

5.4.1 Cryosections

Mice were anesthetized with 3% chloralhydratefor perfusing with 4% paraformaldehyde (PFA) in 0.1M phosphate-buffered saline, pH 7.5, (PBS). After overnight post fixation at

4ºC in the same fixative, the brains were incubated for at least 24 hours in 30% sucrose in PBS until they sank to the bottom for cryoprotection. Afterwards the barins were embedded in Tissue Tek and stored at -20ºC. Cryosections (15-20 µm thick) were cut sagital and collected on Superfrost glass slides (Fischer Scientific) and processed for immunohistochemistry. They were stored at -20ºC before staining and 4ºC after staining.

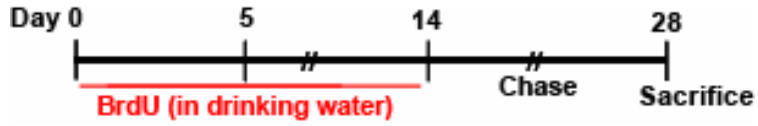
5.4.2 Free Floating Sections

For some in situ studies sections were cut at 30µm thickness at -4ºC and collected in PBS. Those free floating sections were stored at -20ºC in storing solution (30% glycerol, 30% ethylen glycol, 30% distilled water, 10% 10xPO4 buffer) before staining.

5.4.3 BrdU Labelling

For detection of proliferating cells, the DNA base analogue 5-bromodeoxyuridine (BrdU, Sigma) was injected i.p. (100 mg/kg body weight, dissolved in 0.9% NaCl with 0.4 N NaOH) 1h before perfusion to label fast proliferating cells (short pulse). To label slow dividing stem cells in SEZ BrdU was given in the drinking water (1mg/ml) for 2 weeks followed by another 2 weeks with BrdU-free drinking water. This assay was also used to determine postmitotic oligodendrocytes in corpus callosum.

Figure 5.4 Scheme demonstrating label retaining assay with BrdU



The labelling index of TAPs and neuroblasts were calculated as follow. The proportion of cells that have incorporated BrdU (after a single injection) amongst all proliferating cells is the labelling index (LI):

$$LI = \frac{\text{BrdU-positive proliferating cells}}{\text{proliferating cells}}$$

As all TAPs proliferate, I did not use a proliferating marker to detect them. However for neuroblasts I used Ki67 staining to detect the proliferating ones.

5.4.4 Immunostaining

Primary antibodies were directed against: Phospho-Smad1/5/8 (rabbit, Cell Signalling Technology); DCX (guineapig, Chemicon); BrdU (rat, Abcam, Cambridge, MA; 15 minutes in HCl 2N, 15 minutes Borate Buffer, pH 8.5); glial fibrillary acidic protein (GFAP, mouse, Dako, or rabbit, Sigma); Dlx2 (rabbit, Chemicon); Olig2 (rabbit, Chemicon); Mash1 (mouse IgG1, kind gift from F. Guillemot, National Institute for Medical Research, Mill Hill,London, UK); S100β (mouse, Sigma); PDGFRα (rabbit, Spring Biosciences); CNPase (mouse, Sigma); CC1 (mouse, Calbiochem,); MOG (mouse IgG1, kind gift from C. Linington, University of Aberdeen, Aberdeen, UK); TGFβ receptor II (rabbit, Santa Cruz), p-Smad2/3 (rabbit, Cell Signalling Technology) and NeuN (mouse IgG1, Chemicon).

Antibodies were diluted in 0.1M PBS (0.5% Triton X-100, 10% normal goat serum). Slides were kept in antibody solution in most cases overnight at 4°C in a humid chamber but for some antibodies longer incubations were necessary.

For BrdU immunostaining the sections were treated with 2N HCl for 15 minutes at 37ºC for DNA denaturation followed by one washing step of 10 minutes in PBS and were treated with 0.1M Sodium-tetra-borat (PH: 8.5). Overnight incubation with the anti-BrdU antibody at 4°C was followed by 2 hours of incubation at room temperature with the secondary antibody. The primary antibodies were then detected by subclass specific secondary antibodies coupled to FITC, TRITC (1:200, Jackson Laboratory), Cy2- or Cy3-coupled antisera (1:800, Jackson Laboratory) or biotin (1:200, Vector Laboratories, Burlingame, CA).

Nuclei were visualized with DAPI (4', 6' Diamidino-2-phenylindole, Sigma) by incubating sections for 10 minutes with a concentration of 0.1µg/ml DAPI in PBS. Sections were mounted in Aqua Poly/Mount (Polysciences, Northampton, UK), a glycerol-based mounting medium. Stainings were analyzed at Zeiss Axioplan 2 or Olympus FV1000 laser-scanning confocal microscope with optical sections of 1µm intervals.

To rule out any unspecific binding of the secondary antisera, control experiments were performed by either leaving out the primary antibody or by using a primary antibody against an antigen that is not present in the respective tissue or at the respective developmental stage.

5.4.4.1 Tyramid Signal Amplification

Some stainings were enhanced using the tyramide amplification kit (Perkin Elmer) as described in the tyramid signal amplification (TSA) handbook (Perkin Elmer). Briefly, the TSA system uses horse radish peroxidase coupled to a secondary antibody to catalyze the deposition of fluorescein labelled tyramide amplification reagent onto tissue sections. This reaction results in the deposition of numerous fluorescein labels immediately adjacent to the immobilized HRP enzyme. Since this technique results in a significant enhancement of the signal, it was used for weak signals in immunostainings (for p-Smad1/5/8, Dlx2, MOG, endogenous GFP in BRE-eGFP mice and in some cases for Olig2 tyramid system was used).

5.4.4.2 Tunel staining

Tunnel staining was carried out using the in situ cell death kit (Roche). The assay uses an optimized terminal transferase (TdT) to label free 3'OH ends in genomic DNA with fluorescein-dUTP. Staining carried out as following manufacturer's instructions. Briefly, sections were incubated for two minutes in 0.1% Triton X-100, 0.1% sodium citrate on ice. The slides were then incubated with the Tunel reaction mixture containing the fluorescein labelled nucleotides binding to double stranded low molecular weight DNA fragments occurring during apoptosis for 60 minutes at 37º C. The samples were directly analyzed by fluorescence microscopy. Apoptotic TUNEL-positive cells were detected in the green (515-565) wavelength spectrum.

Table 5.2 Primary Antibodies

Name	Host-animal	Marker for	Supplier
Anti-β-galactosidase	Rabbit	Reporter gene	Cappel
Anti-β-tubulin-III	Mouse, monoclonal IgG2b	Postmitotic neurons	Sigma
Anti-BrdU	Rat	S-Phase marker	Abcam
Anti-CC1 (APC)	Mouse Monoclonal IgG2a	Immature oligodendroctytes	Calbiochem
Anti-CNPase	Mouse IgG1	Partially mature oligodendrocyte marker	Sigma
Anti-DCX	Guineapig	Immature neurons (neuroblasts)	Chemicon
Anti-Dlx2	Rabbit	Marker gene for TAPs in Adult SEZ	Chemicon
Anti-GFAP	Rabbit	Precursor cell subtypes	DAKO
Anti-GFAP	Mouse, IgG1	Astrocytes	Sigma
Anti-GFP	Rabbit	Reporter gene	RDI
Anti-GFP	Chicken	Reporter gene	Aves LABS

Anti-Ki67	Rabbit	Precursor cells	Dianova Immundiagnostics
Anti-Ki67 (TEC-3)	Rat	Precursor cells	Dianova Immundiagnostics
Anti-Mash1	Mouse, Monoclonal IgG1	bHLH-transcription factor	F.Guillemot, NIMR, London, UK
Anti-NeuN	Mouse, Monoclonal IgG1	Postmitotic neurons	Chemicon
Anti-O4	Mouse, Monoclonal IgM	Mature oligodendrocyte marker	Jack Price, Kings College, Institute of Psychiatry, London, UK
Anti-PDGFRα	rabbit	Immature oligodendrocytes	Spring Biosciences
Anti-pSmad1/5/8	Rabbit	Phosphorylated Smad 1,5, and 8	Cell Signalling Technology
Anti-pSmad 2/3	Rabbit	phosphorylated Smad 2 and 3	Cell Signalling Technology
Anti-S100β	Mouse,IgG1	Astrocytes	Sigma
Anti-Sox2	Rabbit	Progenitor cells	Chemicon
Anti-Sox9	Rabbit	Astrocytes, astrocyte and oligodendrocyte	Michael Wegner, Friedrich-Alexander University,Institute for Biochemistry,Nürnberg,Germany
Anti-Sox10	Guineapig	Immature and mature oligodendrocytes	Michael Wegner, Friedrich-Alexander University,Institute for Biochemistry,Nürnberg,Germany

Table 5.3 Secondary Antibodies

Name	Supplier
Anti-rabbit Ig FITC / TRIC / biotinylated Anti-rabbit Ig Cy2 / Cy3 Streptavidin AMCA / FITC / Texas Red	Boehringer Ingelheim (Vector Laboratories)
Anti-mouse IgG+M Cy2/Cy3 Anti-mouse IgG1 FITC / TRIC / biotinylated Anti-mouse IgG2b FITC / TRIC / biotinylated Anti-mouse IgM FITC / TRIC / biotinylated	EuroPath Ltd. (Southern Biotechnology Associates)
Anti-guinea pig Ig Cy2 / Cy3 Anti-rat FITC / TRIC / Cy2 / Cy3	Dianova Immundiagnostics (Jackson ImmunoResearch)

Table 6.4 Tyramid Signal Amplification Reagents

Name	Content
TN Buffer (PH 7.5)	0.1 M TRIS-HCl (PH 7.5) and 0.15 M NaCl
TNT Buffer	0.1 M TRIS-HCl (PH 7.5), 0.15 M NaCl, 0.005% Tween 20
Blocking Reagent	Milk powder
TNB Buffer	0.1 M TRIS-HCl (PH 7.5), 0.15 M NaCl and Blocking reagent
SA-HRP	Horseradish Peroxidase-labelled reagent
Amplification Diluent	?
Amplification reagent	Fuorophore

5.5 In Situ Hybridization

5.5.1 Plasmid preparation and *in vitro* transcription

10ng plasmid DNA was added to 25 µl chemically competent Top10 cells and incubated for 30 minutes on ice. Cells obtained the a heat shock at 42°C for 45 seconds. After recovering the bacteria for 10 minutes on ice, 1ml LB-medium was added and cells were incubated on a bacterial shaker for 45 minutes at 37°. Then 50-100µl of bacterial suspension were plated on e.g. ampicillin containing (50µg/ml) LB-agar plates, depending on the resistance encoded by the plasmid, and incubated at 37°C overnight. One colony was picked the next day and grown for about 4 hours in 5ml ampicillin-containing LB-medium. This pre-culture then was added to 50ml LB-ampicillin-medium and was incubated overnight at 37°C on the rotary shaker. Plasmid-DNA was harvested following the Quiagen-Midiprep protocol using a midi Tip100 column. The DNA pellet was dissolved in 200µl ddH2O and 20µg of plasmid-DNA, quantified by spectrophotometry at 260nm, were linearized with the appropriate enzyme (40U) in a total volume of 50µl of the appropriate buffer for 2-3 hours at 37°C. The plasmid-DNA then was purified by phenol extraction. First, water was added to a total volume of 200µl, then 200µl phenol-chloroform-isoamylalcohol (50:49:1) was added and strongly vortexed for about 1 minute. After 5 minutes of centrifugation in an Eppendorf-centrifuge at maximum speed (13.1 x 103rpm ~ 16.000 xg), the water phase was recovered and 1/10x volume 3M sodium acetate and 0.7x volume isopropanol were added and incubated for 10 minutes at RT for precipitation. After a centrifugation at maximum speed in an Eppendorf-centrifuge for 15 minutes the pellet was washed shortly with 70% ethanol and resuspended in 18µl TE (pH8, RNAse-free). For in vitro transcription of the linearized plasmid, 1µl (about 1µg)of the plasmid-DNA was mixed with 2µl NTP-mix containing digoxigenin labelled UTP (DIG-UTP, Roche), 4µl 5xtranscription buffer, 1µl RNAse inhibitor and 1µl (50U/µl) of the respective RNA-polymerase (T3, T7 or SP6, Stratagene). Pure RNAse-free ddH20 was added up to a final volume of 20µl and the plasmid-mix was incubated for 2 hours at 37°C. To stop the reaction 2µl 0.2M EDTA was added followed by addition of 2.5µl 4M LiCl and 75µl pure ethanol to precipitate the RNA either at -20°C overnight or at -80°C for two hours. The RNA-probe was centrifuged for 7 minutes at 4°C

and the pellet was dissolved in 22.5µl ddH20 for 30 minutes at 37°C. RNA-precipitation was repeated by addition of 2.5µl 4M LiCl and 75µl 100% EtOH and incubation for 2 hours at -20°C. The RNA-probe again was centrifuged for 7 minutes at 4°C. The RNA-probe was resuspended in 20µl ddH20 and 200µl hybridisation buffer at a final RNA-concentration of around 100ng/µl.

5.5.1.1 Plasmids

Mouse cDNAs of BMP ligands (kind gift from B.L.M. Hogan, Duke University Medical Center, Durham, USA), Noggin (kind gift from R.M. Harland, University of California, Berkeley, USA), BMPRII (kind gift from M. Ruat, Neurobiology Institute Alfred Fessard, Gif-sur-Yvette,France), Smad4 (kind gift from Y. Chai, University of Southern California, Los Angeles, USA) and Ids (kind gift from K. Miyazono, University of Tokyo, Tokyo, Japan) were used as templates for *In situ* hybridization probes. The probe for Smad4 binds to a region corresponding exon 6-10 of transcript.

Table 5.5 In *situ* Plasmids

Name	**Digestion**	**Trkrp**
BMP2	XbaI	T3
BMP4	EcoR1	Sp6
BMP6	EcoR1	Sp6
BMP7	BamH1	T7
BMPRII	EcoR1	T7
Id1	EcoR1	Sp6
Id2	HindIII	T3
Id3	EcoR1	Sp6
Id4	XbaI	T3
Msx1	PstI	T3

Noggin	NotI	T7
Smad4	EcoR1	T3

5.5.2 Non-radioactive in-situ hybridisation

In situ hybridizations were performed on 20µm thick cyrostat sections with hybridization buffer (1X Salt Solution (10X Salt Solution: 2M NaCl, 90mM Tris HCl, pH7.5, 10mM Tris base, 70mM NaH2PO4, 50mM Na2H PO4, 50mM EDTA, ddH2O), 50% Formamid, 10% Dextran Sulfate (Sigma), 1mg/ml wheat germ tRNA (Sigma, R7876), 1X Denhard's solution (Sigma, D2532), 0.5% CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid) and ddH_2O) at 65ºC overnight. The next day slides were washed first with washing solution containing 1X SSC, 50% Formamid, 0.1% Tween-20 at 65ºC with followed washing with 1X MABT (100mM Maleic Acid, 150mM NaCl-pH 7.5, 0.02 Tween-20). Slides were incubated 1 hour at room temperature in blocking solution (1X MABT, blocking reagent (Boehringer Mannheim), and 20% heat inactivated sheep serum (Sigma, G6767). Anti-digoxigenin Fab fragments coupled to alkaline phosphatase was diluted 1:2500 in blocking solution, and applied on slides after 1 hour blocking for overnight. The third day the slides were again washed with 1X MABT and kept in staining solution containing the substrates for alkaline phosphotase, NBT and BCIP (Sigma) in a concentration 350µg/ml for NBT and BCIP. For double stainings of Smad4mRNA fluorescent *In situ* hybridization (FISH) was performed with HNPP fluorescent detection set (Roche).

5.6 In vivo injections and Cortex Injury

5.6.1 Anesthesia

Animals were anesthetized by intraperitoneal injection of Medetomidine (Domitor, 0.5 mg/kg body weight), Midazolam (Dormicum, 5 mg/kg body weight), and Fentanyl (Fentanyl Hexal, 0.005 mg/kg body weight). The anesthesia was antagonized by intraperitoneal injection of Atipamezol (Antisedan, 5 mg/kg body weight), Flumacenil (Anexate, 0.5 mg/kg body weight) and Naloxon (Narcanti-vet, 1.2 mg/kg body weight) were used. The licence number for operation is 209-211-2531-23/04.

5.6.2 Stereotaxic injections

Injections of virus particules were done stereotactically on 9-10 weeks old control or mutant animals. The adult mice were fixed in a stereotactic apparatus (Kopf). The head was shaved and a 1 cm midline cut was performed with a scalpel. The skull was opened at the anterior-posterior and medio-lateral coordinates (see below) using a drill (Multipro395PR, Dremel). Glass capillaries were filled with virus solution and an oil microinjector (Narashige) was used to inject this solution very slowly. Coordinates (relative to the bregma) : for SEZ, anterior-posterior= 0.7; medio-lateral=1.2; dorso-ventral=1.9 and for the corpus collosum (CC): anterior-posterior= 0.6; medio-lateral=1; dorso-ventral=1.5

5.6.3 Viral Vectors

5.6.3.1 Retroviral vectors and retrovirus production

Murine Leukemia Virus (MLV) derived retrovirus pseudotyped with the vesicular stomatitis virus glycoprotein (VSV-G) expressing either Green Flourescent Protein (GFP) or Cre recombinase were used. In some cases pMXIG (Hack et al., 2004) or CMMP (Hack et al., 2004) were used to trace the cells. For examine the role of olig2 after Smad4 deletion I used the Olig2VP16 virus as described in Hack et al., 2004. The transgene in the plasmid was replaced with enhanced Green Flourescent Protein (eGFP) to visualize the cells. The entire coding sequence of Olig2 with a 675-bp fragment encoding for VP16 was inserted into the EcoRI unique restriction site of the retroviral vector pMXIG (nosaka 1999).

Gpg cells (Ory et al., 1996) were cultured in DMEM (Gibco) containing 10% (v/v) FCS (heat inactivated 30 minutes at 56°C; Gibco), 1% (v/v) Penicillin-streptomycin in DMEM (Gibco), 1 µg/ml tetracycline (Sigma), 2µg/ml puromycin (Sigma) and 0.3 mg/ml G418 (Gibco). Cells were passaged 1/week with PBS and tryipsin containing 1µg/ml tetracycline. These cells allow for the production of high titer amphotropic retrovirus. Many retrovirus packaging cell lines lose packaging efficiency as they are cultured due to the gradual loss of expression of the packaging genes. The packaging plasmids introduced to this cell line were introduced using different selection markers (gentamycin (G418), puromycin). Therefore, expression of packaging proteins can be fairly well maintained by culturing the cells continuously in media containing the corresponding antibiotics. In addition, the vesicular stomatitis virus G (VSV-G) protein is toxic to gpg293 cells so expression of his gene is controlled by tetracycline. Therefore, these cells are maintained in tetracycline, puromycin, and G418 containing

medium. Retroviral vectors pseudotyped with VSV-G differ from standard murine retroviruses by their very broad tropism and the capacity to be concentrated by ultracentrifugation without loss of activity.

Gpg helper-free packaging cells were used for viral production (Peer 1993, yee 1994). For retrovirus production 90-95% confluent gpg cells were transfected using Lipofectamine 2000 (Invitrogen) and opti-MEM I reduced-Serum Medium (Invitrogen) as described in the Lipofectamin 2000 transfection protocol for adherent cells (Invitrogen). Transfection medium was replaced after 8-10 hours, and gpg cells were further cultured in DMEM containing 10% (v/v) FCS (heat inactivated 30 minutes at 56°C; Gibco), 1% (v/v) Penicillin-streptomycin in DMEM (Gibco). After 48 hours the virus containing medium was collected and filtered through a 0.4 μm filter to remove the cell debris but maintain the viral particles(Becton Dickinson) and centrifuged at 50000 x g for 90 minutes at 4°C. The virus pellet was resuspended in TNE (50mM Tris-HCl pH7.8, 130mM NaCl, 1mM EDTA) and aliquoted. Virus aliquots were stored at -80°C. Virus titers (viral particles/ml) were measured by adding viral vectors serially diluted to primary cerebral cortex cells isolated from embryonic day 14 cortices. Cells were cultured for 2 days to allow the expression of the transgene and then the number of infected clones (clusters of GFP-positive cells) was counted. The number of infected clones corresponds to the viral particles used for transduction of primary cells and was then referred to viral particle/μl.

5.6.3.2 Lentiviral vectors and lentivirus production

Cytomegalo-virus (CMV) promoter containing lentiviral vectors expressing either GFP (LV-GFP) or Cre (LV-Cre) are based on a previously described vector system (Pfeifer et al., 2001) and were produced as described (Pfeifer et al., 2002). A third-generation, Tat-free packaging system was used to produce recombinant lentivirus. The GFP-LV plasmid together with the two packaging plasmids (encoding human immunodeficiency virus (HIV) gag,pol, and rev) and the plasmid coding for VSV-G envelope were transfected into 293T(HEK) cells using the calcium phosphate method. In brief, 24 15-cm dishes were transfected and the virus was harvested by collecting the cell culture medium 24,48 and 72 hours. After filtering the collected medium through 0.45-μm filters, we concentrated the virus by spinning at 68,400g for 2 hours followed by a second spin (59,000g, 2.5 hours at room temperature). The resulting pellet was resuspended in 200 μl Hanks' buffer. The titer of lentiviral vectors was determined

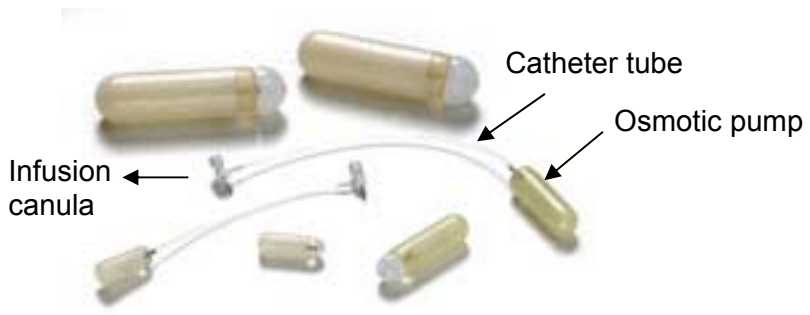
was determined by measuring the amount of HIV p24 Gag antigen by ELISA (Alliance: NEN Life Science Products, Boston, MA).

To make the Cre-lentivirus (LV-Cre), a *Mlu*I fragment containing the nuclear localization signal (nls) of the simian virus 40 large T antigen and the cDNA for Cre recombinase (nlsCre) was excised from pMC-Cre. The nlsCre was first cloned into the EcoRV site of a pBlueskript II KS vector and then inserted *Xba-Sal*I sites of a pRRL lentiviral vector that contains a central polypurine tract (ppt) and the posttranscriptional woodchuck hepatitis virus resulting in LV-Cre.

5.6.4 Noggin Infusion

Noggin (Peprotech) dissolved in artificial Cerebro Spinal Fluid (aCSF, aCSF contains (in mM): 148 NaCl, 3 KCl, 1.4 $CaCl_2$, 0.8 $MgCl_2$, 1.5 Na_2HPO_4 , and 0.2 NaH_2PO_4, pH 7.4 and 100µg/ml rat serum albumin (Sigma, St. Louis, MO)) or aCSF only was infused at a rate of 500ng/day for 7 days into the lateral ventricle of C57Bl6/J mice at the coordinates -0.2 (anterioposterior), 1 (mediolateral), 2 (dorsoventral) by miniature implantable osmotic minipumps (1µl/hour ALZET osmotic minipumps 2001 SIGMA, Brain Infusion Kit II SIGMA). First the brain infusion assembly (catheter tube which has canula at the tip) was filled with the solution to be delivered by a syringe. The flow moderator was attached at the other end of the catheter. After filling the osmotic minipump with the solution of interest the flow moderator (connected to the catheter and infusion canula) was placed in the filled osmotic pump. Then the pumps were kept at 37°C for overnight before implanting into animals. Animals were sacrificed either 3 days or 21 after Noggin infusion. In the case of longer survival, the pumps were removed 3 days after the infusion.

Figure 5.5 Images of osmotic minipumps (for 3, 7 and 14 days delivery)



5.6.5 Stab-Wound Injury

The adult mice (9-10 weeks old male C57BL/6J) were fixed in a stereotactic apparatus (Kopf). The head was shaved and a 1 cm midline cut was performed. The skull was opened at the anterior-posterior and medio-lateral coordinates (see below) using a drill (Multipro395PR, Dremel). Animals then underwent a stab wound in the right cerebral neocortex (Bregma from -0.9 mm to -2.7 mm, latero-lateral 1.5-2.5 mm).

5.6.6 Transplantation

For transplantation experiments Myristoyl (myr)-Venus animals were used. In this mouse line the Venus protein contains Myristoyl protein sequence which is a lipid modified protein present in plasma membrane of all cells (Rhee et al., 2006). SEZ of 6 weeks old myr-Venus animals were dissected and enzymatically dissociated in 0.7 mg/ml hyaluronic acid, 1.33 mg/ml trypsin in HBSS with 2 mM glucose at 37ºC for 15 minutes. After the first 15 minutes of tryipsinization the cells were triturated with a fire polished and fetal calf serum coated Pasteur pipet very strongly to avoid clumps. This step was followed by another 15 minutes digestion at 37ºC. The cells were centrifuged at 200g for 5 minutes, resuspended in 0.9 M sucrose in 0.5XHBSS, and centrifuged for 10 minutes at 750 g. The cell pellet was resuspended in 2 ml of culture medium, placed on top of 10 ml 4% BSA in EBSS solution, and centrifuged at 200 g for 7 minutes, followed by washing in Dulbecco's modified eagle medium:F-12 nutrient mixture (DMEM/F12, Gibco). At the end the cells were dissolved in DMEM/F12 at a concentration of 30.000 cells per µl. 1 µl was injected into the SEZ at the coordinates (relative to the bregma) : anterior-posterior= 0.7; medio-lateral=1.2; dorso-ventral=1.7

5.7 RNA Extraction and Microarray

5.7.1 RNA extraction

For microarray analysis total RNA was prepared from Subependymal zone of control (GLAST::CreERT2/Smad4wt/wt) and Smad4-/- (GLAST::CreERT2/Smad4floxed/floxed) animals, 10 days after tamoxifen administration. RNA was prepared from SEZ tissue with

QIAGEN RNAeasy Kit. SEZ was dissected and collected in lysis buffer (RLT) containing □-mercaptoethanol in an eppendorf tube. After 15 minutes centrifugation at maximum speed (13,200 rpm) the supernatant was collected in a QIAshredder (purple) spin columns placed in 2-mL collection tubes. Following a 2 minutes centrifugation at maximum speed the supernatant of the flow-through fraction was transferred to a new RNase-free 1.5-ml microcentrifuge tube without disturbing the cell-debris pellet. 0.5 volume (half of initial lysis volume) of room temperature 96-100% ethanol was added to the clear lysate and mixed immediately. The the mixture was applied to an RNeasy (pink) mini column placed in a 2-ml collection tube. This was followed by 15 seconds centrifugation at 11.000 rpm. At this stage RNA and DNA are bound to silica gel membrane in the RNeasy column. DNA was digested in the same column with DNAse I 15 minutes. After proper washing steps the columns were trasferred into RNase-free1.5-mL microcentrifuge tubes. 30 □L of RNase-free water (supplied with the kit) was put directly onto the center of the silica-gel membrane of the RNeasy columns for elution. The samples were then centrifuged for 1 minutes 2 times at maximum speed. The eluted RNA was measured at Nanodrop Spectrophotometer.

5.7.2 Microarray

The quality of purified RNA was examined using the Agilent Bioanalyser and revealed high quality of all RNA preparations. 80 ng of total RNA was used for each microarray from 3 biological replicates of control and 2 biological replicates of Smad4-/-. The RNA amplification was performed with MessageAmp II-Biotin Enhanced kit (Ambion, 1791). This single round aRNA amplification kit was used for all samples to avoid variations resulting from multiple rounds of amplification. Hybridization to Affymetrix MOE430 2.0 GeneChips (46 k probe sets) was performed according to standard protocols provided by Affymetrix (www.affymetrix.com). All housekeeping genes were present and number of present calls was determined as 40% or higher. To process the data we calculated probe set summaries (according to RMA (Bolstad et al., 2003) and normalized the data (lmp, nonlinear transformation employing the loess smoother (Cleveland, 1981)). To test the quality and reproducibility of the samples, hierarchical clustering was used to find (dis)similarities between the samples, showing that the replicates of each group of cells analyzed were clustering together. Hierarchical clustering was performed on normalized data (RMA) using packages available from http://cran.r-project.org. For statistical analysis of the expression data

the Bioconductor software package implemented in Carma web (Ref) was employed using the moderated limma test. The Benjamini-Hochberg algorithm (Benjamini, 1997) was used to identify genes with a false discovery rate < 5 %.

5.8 cDNA Preparation and Real Time (RT) PCR

5.8.1 cDNA Synthesis

To confirm the expression differences as predicted by the microarray analysis we compared mRNA expression levels of some of the genes of interest by RT-PCR. For complementary DNA preparation RNA was reverse transcribed by SuperScript™ III Reverse Transcriptase kit from Invitrogen. As shown below (components and their amounts for 1 reaction) after mixing the RNA template with oligo(dT) and dNTPs the samples were kept at 65°C for 5 minutes for annealing of oligo(dT)s to the messenger RNA. This was followed by addition of reverse transcriptase together with other components which were listed below. Transcription took place 50 minutes at 50°C. The samples were then incubated at 85°C for 5 minutes for the inactivation of reverse transcriptase enzyme. cDNA was store at -20°C

Table 5.5 cDNA Reaction Mix

Components	**ONE Reaction**
dNTPs	1µl
Oligo(dT)	1µl
10xRT buffer	2µl
25mM $MgCl_2$	4µl
0.1 M DTT	2µl
RNAaseOUT	1µl
Superscript IIIRT	1µl
RNA template	---- µL
Total volume	**20 µL**

5.8.2 Real Time (RT) PCR

Real-time PCR was done using the iQSYBRGreen kit from BIO-RAD in an Opticon qPCR machine. For 1 reaction see the components listed in the table 6.6. 2X SYBR Green Supermix contains 100mM KCl,40mM Tris-HCl, pH 8.4, 0.4 mM of each dNTP, iTaq DNA polymerase, 50 units/ml, 6mM $MgCl_2$, SYBR Green I, 20 nM fluorescein, and stabilizers. Primers used for genes of interest were listed in table 6.7.

Table 5.6 Real-time PCR reaction mix

Components	**ONE Reaction**
iQSYBR Green Supermix	12.5µl
Primer 1	0.5µl(100nM)
Primer 2	0.5µl(100nM)
Sterile water	Xµl
DNA template	2-5µl(10ng)
Total volume	**25 □L**

Table 5.7 RT-PCR Primers

Primer name	**Primer sequence**
Pou6f1 forward	**5'- -3'** ctgtcaggaagccaaacaca
Pou6f1 reverse	**5'- -3'** ctcatccagacttggggtgt
Sox17 forward	**5'- -3'** ctcggggatgtaaaggtgaa
Sox17 reverse	**5'- -3'** gcttctctgccaaggtcaac
Paip1 forward	**5'- -3'** tgctttgcaagaacttgtgg
Paip1 reverse	**5'- -3'** catactccgtcctgcacctt
Cdk4 forward	**5'- -3'** caatgttgtacggctgatgg
Cdk4 reverse	**5'- -3'** caggccgcttagaaactgac

Sox11 forward	**5'- -3'** gtggtccaagatcgagcgca
Sox11 reverse	**5'- -3'** gcgcctcaagcacatggctg
Olig2 forward	**5'- -3'** cacaggagggactgtgtcct
Olig2 reverse	**5'- -3'** ggtgctggaggaagatgact
Dpp6 forward	**5'- -3'** caagggaaaggccaattaca
Dpp6 reverse	**5'- -3'** ctcctcctctttgctgtgg

5.9 Quantitative Analysis and Statistics

Quantifications (absolute cell numbers, marker coexpression) were performed by means of NEUROLUCIDA connected to an Axiophot Zeiss microscope (×40 objective). The analysis was performed on sagital sections at medio-lateral levels from 0.6 to 1mm relative to midline (3-7 sections per animal). The SEZ area analyzed comprised 60,000 μm^2 limited to a maximum distance of 50-100 μm away from the ependymal cell layer. This was corresponded well to the entire SEZ at these levels as detectable e.g. by the zone comprising DCX-positive cells. For all data sets, the arithmetic average $\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$ was calculated and the standard deviation $s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$ and the standard error of the mean $SEM = \frac{s}{\sqrt{n}}$ were computed. Error bars depict the SEM. The unpaired Student's t-test was used to examine whether data sets differed significantly. Data were considered as significant with $p<0.05$ and as highly significant with $p<0.01$. Calculations of the arithmetic average, the standard deviation, the standard error of the mean were performed with Microsoft Excel.

6 RESULTS

6.1 BMP signalling in adult subependymal zone (SEZ)

6.1.1 BMP pathway compenents are present in the adult subependymal zone

As described in the introduction BMP signalling has important roles in many aspects of development including the nervous system. To search for the differences between the non-neurogenic brain parenchyma and the neurogenic niches I first did an expression analysis. I performed in *situ* hybridization for some of the BMP ligands and Smad pathway elements in postnatal and adult Subependymal Zone **(Figure 6 and 7)**.

I could observe mRNA transcripts of BMP ligands 2,4,6 (**Figure 6A-C**) in the subependymal zone of postnatal (postnatal day 5) as well as BMP specific type II receptor (**Figure 6D)**, Common-Smad Smad4 (**Figure 6E)** and downstream targets Id1 and Id3 (**Figure 6F,G).** In many cases these transcripts were localized all around the wall of the ventricle, including the dorsal or/and medial wall. Similar to postnatal stages, mRNA transcripts of BMP ligands and Smad pathway elements were also present in the adult subependymal zone (SEZ). This includes BMP ligands 2,4,6 and 7 (**Figure 7A-D**), BMP specific type II receptor (BMPRII) (**Figure 7F**), Smad4 (**Figure 7G**) and Id3 (**Figure 7H**). I also detected the mRNA transcript of BMP specific inhibitor Noggin (**Figure 7E**) in the adult SEZ. In contrast to postnatal stage BMP pathway elements seem to be enriched in the lateral wall of the lateral ventricle in the adult.

My observations of the presence of BMP signalling in postnatal and adult SEZ are consistent with previous works (Fan et al., 2003; Lim et al., 2000; Peretto et al., 2002; Peretto et al., 2004) which examined expression of some of the BMP pathway elements in this neurogenic niche.

6.1.2 Activity of BMP-mediated signalling in adult neural stem cells of the SEZ, but not SGZ

Expression pattern revealed by in *situ* hybridization led us to examine if BMP-mediated signalling activity can be detected in the adult SEZ. As described above and shown in the

Figure 4 receptor regulated Smads 1,5 and 8 are specifically phosphorylated and translocated to the nucleus after heterodimerization with Smad4 upon BMP binding to the receptor dimer. Whereas presence of BMP ligands and other pathway elements alone do not indicate the activity of the Smad pathway, detection of phosphorylated BMP specific Smads would reveal the actual activity of BMP signalling. Thereby I monitored BMP activity by immunostaining for the phosphorylated forms of Smads 1, 5 and 8 (p-Smad 1/5/8). Many p-Smad1/5/8-positive nuclei were detected along the lateral wall of the lateral ventricle (**Figure 8A-G**), indicating active BMP-signalling in this zone. In contrast, I did not observe p-Smad 1/5/8-positive cells in the other adult neurogenic niche apart Subependymal zone namely subgranular zone (SGZ) of dentate gyrus (DG) (**Figure 8I**). Pyramidal neurons in the CA regions of hippocampus were positive for p-Smad1/5/8 ((**Figure 8I**). However p-Smad 1/5/8-immunostaining was detectable in some neuronal populations in the forebrain, but not in parenchymal glia (**Figure 10**). Thus, outside the SEZ p-Smad1,5,8-immunoreactivity was largely restricted to postmitotic neurons. Similar to p-Smad1/5/8, Smad4 is also not expressed by parenchymal glial cells (**Figure 10**). Smad4 is present in postmitotic cells in cortex, olfactory bulb and cerebellum. In **Figure 10** there is an example from olfactory bulb.

In the SEZ, the majority of p-Smad1/5/8-positive cells (60%) were astrocytes (GFAP-positive) (**Figure 8**). To determine whether the p-Smad1/5/8-positive astrocytes (GFAP-positive) contain self-renewing stem cells, I used a label-retaining protocol (see materials and methods). Injection of the DNA-base analogue Bromo-deoxy-Uridine (BrdU) intraperitoneally into animal or addition to the drinking water allow labelling proliferating cells. Because BrdU is incorporated into the DNA during the S phase of cell cycle, only cells in S-phase at the time point of BrdU application incorporate it. Because different cells have different cell cycle length, length of BrdU application can allow to identify different cell types. While transit amplifying precursors (TAPs) proliferate fast and can be labelled with a single pulse BrdU given 1 hour prior to sacrifice, stem cells divide very slowly and require long time of BrdU application to be detected. An assay used for identification of slow dividing cells namely stem cells is called label retaining assay (see materials and methods and **Figure 19**). Addition of BrdU 2 weeks to the drinking water of mice allows labelling of slow dividing cells. However not only slow dividing cells but also fast dividing cells incorporate BrdU within this period. To eliminate fast proliferating cells, 2 weeks BrdU period is

followed by another 2 weeks with normal water in which TAPs dilute the label. Besides labelling the slow dividing stem cells, this protocol also labels any cells that leave the cell cycle shortly after incorporating BrdU, such as newly generated doublecortin (DCX)-positive neuroblasts (30% of BrdU-retaining cells are DCX-positive). When I applied this protocol and did a triple immunostaining for BrdU/DCX and p-Smad1/5/8 antigens I found that BrdU-retaining postmitotic neuroblasts were not p-Smad1/5/8-positive whereas 43% (n=48 cells) of the remaining BrdU-retaining cells contained p-Smad1/5/8 (**Figure 8G,H**), suggesting that a high fraction of slow dividing stem cells is subject to BMP signalling. I did not observe p-Smad1/5/8-immunoreactivity in any DCX-positive cell. The remaining (DCX- and GFAP-negative) population is largely composed of transit-amplifying precursors that express transcription factors of the Dlx family (Doetsch et al., 2002). All DCX- and GFAP-negative p-Smad1/5/8-positive cells showed Dlx2-immunoreactivity (**Figure 8D,H**). In contrast, p-Smad 1/5/8-immunoreactivity did not co-localize with immunostaining for the transcription factor Olig2 (**Figure 8E**) expressed by a subset of TAPs (Hack et al., 2005; Menn et al., 2006). p-Smad1/5/8-positive cells responding to BMP-signalling in the adult SEZ are composed of 60% of astrocytes including slow-dividing stem cells and 40% of Dlx2-positive transit amplifying precursors (**Figure 8H**).

6.1.3 BMP Reporter Mouse Line

As an additional approach to monitor BMP signalling activity, I analyzed SEZ of a BMP reporter mouse line (**Figure 9**). In this line green fluorescent protein (GFP) is expressed under the control of the BMP-specific Responsive Element (BRE) containing Smad 1 and 5 binding sites derived from the Id1 promoter (Monteiro, Chuva da Sousa Lopes, Mummery, unpublished). Consistent with the localization of p-Smad 1/5/8, GFP co-localized with GFAP, but not with DCX in the adult SEZ of the BRE-GFP mice (**Figure 9A,B**). To further ensure the identity of these GFP/GFAP-positive cells as stem cells I stained for PDGFRα as a marker for the stem cell subset amongst SEZ astrocytes (Jackson et al., 2006). I could detect PDGFRα antigen in GFP/GFAP-positive cells in the SEZ of BRE-eGFP mice showing that the stem cells were subject to BMP-signalling (**Figure 9C-C'''**). Thus, the Smad-mediated BMP-signalling is active in stem cells of the adult SEZ, but not in neuroblasts.

6.2 BMP Signalling in non-neurogenic areas

As observed in CA3 neurons of hippocampus (**Figure 8I**) p-Smad1/5/8 antigen was restricted to postmitotic neurons outside of subependymal zone in the adult forebrain (**Figure 10**). Both p-Smad1/5/8 and Smad4 were found to be scattered in the cortex and mostly concentrated close to midline. Similar to p-Smad1/5/8, Smad4 mRNA was not found in parenchyma glial cells as shown in cerebellum and olfactory bulb with GFAP double staining (**Figure 10**). Similar expression pattern was also observed in BRE-eGFP mouse.

As a summary Smad pathway mediated BMP signalling is restricted to the progenitors of SEZ but not progenitors of white matter and parenchyma such as oligodendrocyte progenitors (These progenitors are defined by their chondroitin sulphate proteoglycan NG2 expression) as expressions of both p-Smad1/5/8 and Smad4 are restricted to postmitotic neurons outside of neurogenic niche. Therefore BMP signalling may play an important role in the SEZ namely stem cell niche.

6.3 Expression pattern of BMP signalling components in the adult cortex after stab wound

After an injury to the nervous system neurons are not regenerated. In response to injury, reactive astrocytes appear and accumulate in the wounded area, leading to glial scar formation. Quiescent astrocytes become highly proliferative (Buffo et al., 2005) and behave as stem cells (Buffo,Rite,Tripathi, unpublished data). These cells only give rise to astrocytes but not neurons (Buffo,Rite,Tripathi, unpublished data). Signals governing this difference between neurogenic astrocytes such as in SEZ and non-neurogenic astrocytes acting as stem cells after brain injury are not known.

As above results show that BMP signalling promotes neurogenesis we asked the question whether this signalling is active after brain injury. I used stab wound as a model system in adult forebrain (see also materials and methods). Upon stab wound reactive astrocytes and other cell types such as NG2-positive cells and lineage marker negative cells start to proliferate and reach the peak in their proliferation 7 days post injury (Buffo et al., 2005).

I examined BMP signalling components 3 days after stab wound in cortex of wild type mice. I started by examining BMP ligands and performed in situ hybridization for BMP 2,4 and 6

(**Figure 11**). After injury BMP ligands did not seem to be differentially expressed as both ipsilateral (the side of lesion) and contralateral (the control side) sides show similar expression intensity. BMP2 shows a wide expression pattern in cortex whereas BMP4 is scattered. BMP6 seems to be restricted to SEZ and choroid plexus in the forebrain (**Figure 11C**) as the signal hardly detectable in cortex, striatum and hippocampus. I also checked BMPRII expression after stab wound and did not observe a difference between injured and uninjured side (**Figure 12A-A''**). Like BMP2, BMPRII also has a wide expression pattern in cortex. However this expression neither goes down nor goes up after stab wound. Expression pattern of either ligands or receptor seem to be neuronal. Also very round cytoplasmic in situ signal resembles neuron cytoplasm surrounding big nuclues. This is consistent with co-localization of p-Smad 1/5/8 with neuron marker NeuN (**Figure 10**).

Additionally, I also examined p-Smad 1/5/8 immunoreactivity to monitor active BMP signalling after stab wound. Consistent with the BMP ligands and receptor expression after injury, p-Smad1/5/8 activity did not change upon stab wound as well (**Figure 12B-C'**).

Beside analysing signalling components of BMP, I also examined in situ hybridization for Id3 as a downstream target of BMP and TGFβ signalling (**Figure 13**). 3 days post lesion Id3 mRNA expression was up-regulated by the cells around the lesion area (**Figure 13A-A'**) compared to control side lacking stab wound. When I applied in situ hybridization 7 days post lesion the mRNA expression pattern of Id3 seemed to be back to control levels in lesion side suggesting that some cells respond to injury by expressing Id3 transiently (**Figure 13A''**). As I did not observe up-regulation of BMP ligands , BMP specific receptor and p-Smad1/5/8 as the indicator of active BMP signalling 3 days post lesion, this suggests that Id3 was up-regulated by other upstream ligand belonging to TGFβ signalling namely TGFβ itself.

These data altogether suggest that BMP signalling which is shown above as a positive factor for neurogenesis, is not up-regulated by the cells especially proliferating astrocytes after an injury in the cortex.

6.4 Conditional deletion of Smad4 in the adult SEZ

As the functions of BMP signalling are concentration dependent (Mehler et al., 2000) the role of endogenous levels of BMP can only be determined by a loss of function approach. As several BMP ligands and receptors are present in the adult SEZ, the loss of a single BMP

ligand or receptor may be compensated for by others (see introduction and Table 1 and Table 2). Therefore we decided to delete the Co-Smad4. It has been shown that deletion of common mediator Smad4 leads to lethality immediately after gastrulation due to impaired extraembryonic membrane formation and decreased epiblast proliferation (Sirard et al., 1998; Yang et al., 1998). As Smad4 is the only component of the pathway which can carry phosphorylated Smads into nucleus and mediate regulation of transcription, loss of this molecule in the adult SEZ could elucidate the function of BMP signalling via any of the ligands and receptors. To ensure Smad4 expression in the cells found to be responsive to BMP, we examined Smad4 mRNA by fluorescent in *situ* hybridization. Smad4 transcripts colocalized with GFAP in the adult SEZ (**Figure 14**). This allowed us to take advantage of the GLAST::CreERT2 mouse line (Mori et al., 2006) to achieve Smad4 deletion by Cre/loxP system. Cre is a 38 kDa recombinase protein from bacteriophage P1 which mediates intramolecular (excisive or inversional) and intermolecular (integrative) site specific recombination between loxP sites. In the GLAST::CreERT2 mouse line the inducible form of Cre is expressed in the locus of the astrocyte-specific glutamate transporter (GLAST). The fusion of Cre to the ligand binding domain of the modified estrogen receptor (ERT2) is restricted to the cytoplasm and translocates only upon ligand binding (ligand=tamoxifen) into the nucleus where it can then mediate recombination (**Figure 15**) (Feil et al., 1996; Metzger and Chambon, 2001). Thus inducible Cre (CreERT2) in the locus of GLAST allows mediating recombination at specific time points specifically in astroglial cells (**Figure 15**). For deletion of Smad4 based on Cre/loxP system we took advantage of the Smad4 floxed mouse line in which exon 8 was flanked by loxP sites (Smad4 fl/fl) (Yang et al., 2002). Embryos having deletion of this allele resembles that of Smad4-null embryos and die around embryonic day 7 as null embryos (Yang et al., 2002) indicating that deletion of exon 8 is sufficient to interfere with function of Smad4 gene. As Smad4 is not present in astrocytes outside the SEZ, GLAST::CreERT2 mediated excision of exon 8 of Smad4 (Smad4 fl/fl) is specific to the astrocytes and stem cells in the SEZ. Moreover, GLAST is not expressed in other cell types in the SEZ, such as ependymal cells (Mori et al., 2006) (see also below).

I therefore crossed these mouse lines and gave tamoxifen to adult (~10 weeks) animals. All animals were heterozygous for the GLAST::CreERT2 allele. While control animals carried either no (GLAST::CreERT2/Smad4wt/wt, in graphics shown as Control) or only a single

allele of Smad4 floxed (GLAST::CreERT2/Smad4wt/fl), experimental animals had homozygous floxed allele (GLAST::CreERT2/Smad4fl/fl, in graphics shown as Smad4 -/-). All animals received same amount of tamoxifen and as a result all animals had the same amount of Cre recombinase translocating to their nuclei which permits us to control the potential toxicity of Cre (Naiche and Papaioannou, 2007). Moreover, any influence of estrogen analogue tamoxifen on adult neurogenesis is equal in mice heterozygous or homozygous for the Smad4 allele. In order to examine the efficiency of the recombination *in situ* hybridization for Smad4 was performed on sections of GLAST::CreERT2 mice without (**Figure 16A,C**) or with floxed exon 8 of Smad4 (**Figure 16B,D**) 10 days after the end of tamoxifen administration (see materials and methods for the time of tamoxifen application). Smad4 mRNA was present in SEZ of GLAST::CreERT2/Smad4wt/wt mice (**Figure 16A,C**). In contrast, Smad4 mRNA was virtually undetectable in the SEZ of adult GLAST::CreERT2/Smad4fl/fl mice (**Figure 16B,D**). Loss of Smad4 mRNA in GLAST::CreERT2/Smad4fl/fl mice after tamoxifen application was specific to the SEZ as in situ signal for Smad4 in the CA3 region of the hippocampus was not altered in these animals (**Figure 16D**) and remained comparable to the control (**Figure 16C**). Although the mRNA of Smad4 was decreased in GLAST::CreERT2/Smad4fl/fl mice, the protein may still persist longer. To examine the protein level I stained for p-Smad1/5/8 immunostaining. Because phosphorylated Smads require Smad4 to localize to the nucleus (Wrana, 2000), a decrease in Smad4 protein would result in release of p-Smad1/5/8 from the nucleus. Indeed, very few p-Smad1/5/8-positive nuclei could be detected in the SEZ of GLAST::CreERT2/Smad4fl/fl mice in contrast to the many positive nuclei in the control animals (**Figure 17A,B**). In control animals in the presence of Smad4 protein the phosphorylated forms of Smad1/5/8 is translocated into nucleus after making a complex with functional Smad4 protein. Loss of nuclear p-Smad1/5/8 staining in GLAST::CreERT2/Smad4fl/fl mice 10 days after the end tamoxifen application provides a strong evidence that at this stage Smad4 protein is already strongly reduced. Thus, tamoxifen-induced recombination in GLAST-positive cells very efficiently deleted Smad4 and interfered with p-Smad1/5/8 translocation to the nuclei in the adult SEZ only.

6.5 Analysis of adult SEZ stem cells and their progeny after Smad4 deletion

6.5.1 Smad4 deletion does not affect neural stem cell number and properties

When tamoxifen-inducible form of Cre recombinase (CreERT2) in the locus of GLAST (Mori et al.,2006) is induced and the progeny of cells that underwent recombination was followed by expression of a reporter gene (β-galactosidase, see also below) for up to 9 months, reporter-positive cells constituted the majority of label retaining GFAP-positive cells (see above). This data indicates that label retaining astrocytes in adult SEZ are indeed stem cells. As Smad4 was deleted in astrocytes and as a result in stem cells I first examined this population in the adult SEZ of GLAST::CreERT2/Smad4wt/wt and GLAST::CreERT2/Smad4fl/fl mice. Because Smad4 mRNA and p-Smad1/5/8 protein in the nuclei had been lost 10 days after the end of tamoxifen application I started to analyze the phenotype at this time point. As a first step I quantified the number of astrocytes labelled by GFAP in SEZ per area (**Figure 18**) (see materials and methods for detailed description of SEZ area). I observed no significant difference in the number of GFAP-positive cells after Smad4 deletion (**Figure 18A,C**) compared to control animals (**Figure 18B,C**). Although Smad4 deletion did not seem to have an effect on the number of GFAP-positive cells it may act only in the slow-dividing GFAP-positive cells namely stem cells. In order to study this I quantified the number of label-retaining BrdU-positive (DCX-negative) cells (see materials and methods, **Figure 19A** and above) and found it not to be different between the genotypes (**Figure 19B,C,D**). This result indicates that deletion of Smad4 specifically in SEZ astrocytes did not change their number in vivo. However this data does not exclude the possibility that Smad4 deletion may influence their capacity for self renewal. In order to examine the self-renewing capacity of Smad4-/- cells I used the neurosphere in vitro assay.

Neural Stem cells isolated from the adult SEZ can proliferate and keep their stem cell identity in the presence of growth factors (epidermal growth factor (EGF) and basic fibroblast growth factor (FGF2)) in vitro without differentiating . Proliferating stem cells make sphere like structures which are called neurospheres (see materials and methods for picture). In my

hands, the efficiency of neurosphere formation is 1% (5000 cells form around 50 neurospheres in 7 days) from the adult SEZ. Thus many cells contained in the dissected population do not form neurospheres as they are not stem cells. Within the neurosphere stem cells mostly stay undifferentiated and increase their number by self-renewing. Neurospheres can be then dissociated into single cells and maintained in the same conditions to get secondary neurospheres. Because stem cells increase their number by self-renewing within the neurospheres kept in EGF and FGF2, the same number of dissociated cells makes more neurospheres after each passage.

I dissected the SEZ of GLAST::CreERT2/Smad4wt/wt and GLAST::CreERT2/Smad4fl/fl mice either immediately or 21 days after the end of tamoxifen and cultivated 10 cells/µl with EGF and FGF2. 7 days later the cultured neurospheres were quantified per well. No significant difference in the number of primary neurospheres was detected between the genotypes (**Figure 19E**). This data reveals that there is no difference in the proliferation capacity of stem cells while it does not provide information about the self-renewing capacity yet. In order to understand this I dissociated the primary neurospheres into single cells and maintained them in the presence of growth factors 7 days more. Similar to the primary neurospheres, the number of secondary neurospheres did not show a difference between the two genotypes (**Figure 19E**) indicating that deletion of Smad4 has no effect on self-renewal of adult stem cells in vitro (nor in vivo, **Figure 19D**)

6.5.2 Number of transit amplifying precursors is not altered 10 days after Smad4 deletion

Dlx2-positive/DCX-negative cells in the adult SEZ comprises the largest population of transit amplifying precursors (Doetsch et al., 2002). As Dlx2-positive TAPs contained p-Smad1/5/8 they may be altered by Smad4 deletion. Therefore I analyzed TAPs after Smad4 deletion (as I detected p-Smad1/5/8 in all Dlx2-positive/DCX-negative cells indicating activity of BMP signalling in these cells). As described above, TAPs can be labelled by a short pulse BrdU given 1 hour prior to sacrifice. Counting TAPs in that way would give a general idea about their number without discriminating the subtypes (such as Dlx2 or Olig2-expressing sub type). While around 70% of short-pulse BrdU-positive cells are DCX-negative the rest are DCX-positive and hence are proliferating neuroblasts. To distinguish these populations I did

double immunolabelling with BrdU and the neuroblast specific marker DCX and counted only BrdU-positive/DCX-negative cells as TAPs (TAPs=BrdU+DCX- cells) per area in the SEZ. Similar to the number of stem cells, I observed no difference in the number of TAPs between GLAST::CreERT2/Smad4wt/wt and GLAST::CreERT2/Smad4fl/fl 10 days after the end of tamoxifen application (**Figure 20A**). Also in the labelling index of the Dlx2-positive or Olig2-positive population (see materials and methods; LI: short-pulse BrdU-positive cells/all proliferating cells) I could not observe any difference in their proliferating capacity 10 days after Smad4 deletion (Dlx2: Control=0.33±2.1, Smad4-/-=0.3±3.5 (As Dlx2 is also expressed by neuroblasts, to obtain the labelling index of the only Dlx2-TAPs I did triple staining with BrdU/Dlx2/DCX); Olig2: Control=0.37±4.1, Smad4-/-=0.34±1.7, 3 animals per genotype). Taken together the total number of TAPs and proliferation of different subtypes were not affected 10 days after Smad4 deletion.

6.5.3 Neurogenesis is impaired 10 days after Smad4 deletion

After examining stem cells and their immediate progeny, I continued by analyzing neuroblasts. Application of DCX staining on the sections of GLAST::CreERT2/Smad4wt/wt and GLAST::CreERT2/Smad4fl/fl mice 10 days after the end of tamoxifen revealed a consistent decrease in the immuno-reactivity in the Smad4-/- SEZ compared to controls (**Figure 20B,C**). Quantification of the number of DCX-positive cells in the SEZ showed a reduction to half of the control values (**Figure 20D**). As reduction in the number of cells can be due to cell death, I used the TUNEL assay to determine apoptotic cells via DNA fragmentation (for more detail see materials and methods). The number of Tunel-positive cells was not different in the GLAST::CreERT2/Smad4wt/wt or GLAST::CreERT2/Smad4fl/fl mice 10 days after the end tamoxifen application (1.8±0.8 Control, 1.4±0.4 Smad4-/- , analyzed per sagittal sections at medio-lateral levels from 0.6 to 1mm relative to midline, 2 animals each). To understand whether defects in proliferation may lead to the reduction of neruoblasts I examined proliferation of DCX-positive cells as a following step. For this I used both short BrdU labelling and the antisera Ki67. With short pulse BrdU I can detect only the proliferating cells in S-phase while Ki67 staining covers all proliferating cells as the antigen is present in almost all phases of the cell cycle. However, proliferation of neuroblasts in GLAST::CreERT2/Smad4fl/fl mice was similar to control

(**Figure 21A-D**) shown by both BrdU (**Figure 21A,B**) and Ki67 stainings (**Figure 21C,D**) and quantifications for those labellings (%BrdUDCX/DCX in Control 10±1%, n(cells)=729; Smad4-/- 9±1%, n(cells)=534, 3 animals each); %Ki67DCX/DCX in Control=30%±7.7%, Smad4-/-=28%±11%, 3 animals per genotype). These data suggest that Smad4 deletion may affect the neurogenic pathway rather than survival and proliferation.

6.5.4 Fate mapping of Smad4-/- cells

Using reporter mice the efficiency of Cre recombination mediated by GLAST::CreERT2 has been determined as ~70% (Mori et al., 2006; Ninkovic et al., 2007). Despite the high reduction in nuclear p-Smad1/5/8 staining, some stem cells/astrocytes may have escaped recombination. Thus the analysis described above may be showed by overgrowth of non recombined cells. To avoid this I examined only recombined cells after Smad4 deletion by crossing GLAST::CreERT2/Smad4fl mice with the ROSA26 reporter line (R26R) (Soriano, 1999). In this line the LacZ gene is under a ubiquitous promoter. To report Cre recombination, a stop cassette flanked by loxP sites is placed in front of the LacZ gene. Only in the presence of Cre, the stop cassette is deleted and the LacZ gene is transcrined the enzyme β-galactosidase. Recombined astrocytes, transit-amplifying precursors and neuroblasts could therefore be identified by double stainings of cell type specific antigens with β-galactosidase (for neuroblasts see **Figure 22A,B**). When I quantified the number of DCX-positive and GFAP-positive cells among β-galactosidase-positive cell, similar results, namely a reduction in the number of DCX-positive, but not TAPs and GFAP-positive cells, were observed for reporter-positive cells in the SEZ of GLAST::CreERT2/Smad4fl/ROSA26 mice (**Figure 22C**). Thus, the similar numbers of TAPs and GFAP-positive cells can not be explained by a compensatory increase in the number of WT cells at the expense of mutant cells. Rather, these results suggest that deletion of Smad4 does not affect stem cell numbers and properties, but strongly affects neurogenesis.

6.5.5 Deletion of Smad4 alters TAP identity by aberrant expression of Olig2

As reduction in neuroblasts is not due to cell death or impaired proliferation , Smad4 deletion may have lead to a defect at earlier stages in the lineage. However, the above results

demonstrated normal stem cell numbers and self-renewal as well as normal numbers of TAPs. Although the TAPs did not change in their total number 10 days after Smad4 deletion we hypothesized that the molecular identity of TAPs may be altered in a way to interfere with their progression towards the neuroblast fate. To examine the molecular composition of TAPs, I stained sections of GLAST::CreERT2/Smad4wt/wt and GLAST::CreERT2/Smad4fl/fl mice 10 days after Smad4 deletion for Dlx2 and Olig2. Immunostaining for Olig2 revealed a prominent increase in the number of Olig2-positive cells in the SEZ of GLAST::CreERT2/Smad4fl/fl mice (**Figure 23E,G**) to double the number in GLAST::CreERT2/Smad4wt/wt mice (**Figure 23B,G**). As the total number of TAPs was not increased (**Figure 20A**), the up-regulation of Olig2 may either occur in the TAP population or ectopically in astrocytes or neuroblasts. However, neither in control animals nor after Smad4 deletion I could detect the Olig2 antigen in GFAP- or DCX-expressing cells (**Figure 23H**). When I applied Olig2 and Dlx2 staining together, I found Olig2 frequently co-localized with Dlx2 in the SEZ of GLAST::CreERT2/Smad4fl/fl mice (**Figure 23F,G**). While no Olig2/Dlx2 double positive cells were present in the SEZ of control mice (**Figure 23C,G**) indicating that Olig2 was aberrantly expressed in the Dlx2 cell populatio in the GLAST::CreERT2/Smad4fl/fl animals.

As Olig2 overexpression was previously shown to interfere with neurogenesis in the adult SEZ (Hack et al., 2005), I examined whether upregulation of Olig2 among Dlx2-positive cells may divert these cells from their normal neurogenic lineage. Dlx2-positive cells normally comprise about 70% DCX-positive neuroblasts and 30% DCX-negative TAPs (**Figure 23H**). However, 10 days after Smad4 deletion, the DCX-positive neuroblast population amongst Dlx2-positive cells was reduced leading to a reduction in the total number of Dlx2-positive cells (**Figure 23H**). However, amongst Dlx2-positive cells the DCX-negative fraction increased to 46% (**Figure 23H**). Interestingly, the non-neuroblast fraction was largely composed of Dlx2/Olig2-double-positive cells (**Figure 23H**), consistent with the notion that the aberrant expression of Olig2 may interfere with the progression towards the neuroblast stage (Hack et al., 2005).

In order to show whether up-regulation of Olig2 occurred in recombined cells I applied Olig2 staining on sections of GLAST::CreERT2/Smad4fl/fl/ROSA26 mice 10 days after Smad4

deletion. Indeed, the number of recombined cells (detected by β-galactosidase immunostaining) having Olig2-immunoreactivity in the SEZ of Smad4-/- mice was higher than the control ones (**Figure 24A versus B**). This observation indicates the cell autonomous up-regulation of Olig2 after Smad4 deletion.

6.5.6 Proliferation of TAPs is impaired 21 days after Smad4 deletion

To further follow the phenotype upon Smad4 deletion at later stages, I examined GLAST::CreERT2/Smad4wt/wt and GLAST::CreERT2/Smad4fl/fl mice 21 days after the end of tamoxifen application. As a first step I quantified all cells without using a reporter. The decrease in the number of DCX-positive cells after Smad4 deletion was still present 21 days after (**Figure 25C,D,F**) and comparable to the reduction to 50% after 10 days (**Figure 20D**). Similarly, there was no difference in the number of GFAP-positive cells after Smad4 deletion compared to control (**Figure 25A,B,E**). In contrast to the 10 days time point there was a dreduction in the number of short pulse BrdU-positive cells 21 days after Smad4 deletion compared to control (**Figure 26A**). In order to see whether this reduction is due to proliferation defects in TAPs or in some subtypes I examine the labelling index of Olig2-,Dlx2 or Mash1-positive cells at this stage. Because both Dlx2 and Mash1 are also expressed by neuroblasts beside TAPs, I did triple stainings of BrdU with DCX (Dlx2/DCX staining or Mash1/DCX staining) to distinguish these two populations. Indeed, the quantifications revealed a proliferation defect among all subtypes of TAPs, the Olig2-,Dlx2- or Mash1-positive cells (**Figure 26B**, see also C,D for example of Mash1) 21 days after Smad4 deletion. The results of the reduced number of neuroblasts and impaired TAP were confirmed also in reporter-positive cells. Interestingly, the number of recombined cells in the SEZ of GLAST::CreERT2/Smad4fl/ROSA26 mice had further decreased to 53% of the number of reporter-positive cells in control mice 21 days after Smad4 deletion (data not shown). Consistent with above finding, the large fraction of GFAP- and DCX-immunonegative cells (TAPs) had disappeared by this stage and DCX-positive/reporter-positive cells were still strongly reduced among the reporter-positive cells. In contrast, the number of GFAP-positive/ reporter-positive cells was no different between control and Smad4-deficient mice but their relative proportion was increased due to the reduction in reporter-positive cells to half the number observed in control mice. Thus, these data confirmed that the major phenotype upon

Smad4 deletion is a reduction of neuroblasts and an initial increase in aberrant TAPs. These mis-specified TAPs then largely disappear from the reporter-positive population, consistent with the reduction of TAPs observed 21 days after tamoxifen application in GLAST::CreERT2/Smad4fl/fl mice (**Figure 26A**).

6.5.7 BrdU birthdating analysis shows an increase in the number of immature oligodendrocytes in corpus callosum

To examine slow dividing cells in SEZ I had used a label retaining assay (**Figure 19A and Figure 27A**, see also in materials and methods). Addition of BrdU 2 weeks to the drinking water of mice allows labelling of slow dividing cells. However not only slow dividing cells but also fast dividing cells incorporate BrdU within this period. To eliminate fast proliferating cells 2 weeks BrdU period is followed by another 2 weeks with normal water in which TAPs dilute the label (Figure and also see materials and methods). Besides labelling the slow dividing stem cells, this protocol also labels any cells that leave the cell cycle shortly after incorporating BrdU. Thus this assay can also be used to obtain recently generated postmitotic cells in any regions of the brain. I applied BrdU in drinking water of GLAST::CreERT2xSmad4wt/wt or wt/fl and GLAST::CreERT2xSmad4fl/fl mice for 2 weeks and at the end of 2 weeks mice received normal water for additional 2 weeks before sacrifice (**Figure 27A**). When I applied BrdU staining on barin sections of these mice I observed more BrdU-positive cells in the corpus callosum of GLAST::CreERT2xSmad4fl/fl mice compared to control ones (**Figure 27B,C**). These BrdU-positive cells may be slow dividing progenitors committed to certain lineage or/and recently generated postmitotic cells. There are slow proliferating oligodendrocyte precursors outside the neurogenic niches (Horner et al., 2002; Dawson et al., 2003; Buffo etal., 2005) including the corpus callosum. Recently it has been shown that in the adult brain, oligodendrocytes are continuously generated either by committed progenitors within the corpus callosum or by stem cells from SEZ that then migrate and locate in the corpus callosum (Menn et al., 2006). To determine the identity of BrdU-positive cells I used an antibody against the transcription factor Sox10 to label oligodendrocytes or their progenitors (Kuhlbrodt et al., 1998; Stolt et al., 2002) and quantified the number of Sox10/BrdU double positive cells in the corpus callosum area (see materials and methods) of both GLAST::CreERT2xSmad4wt/wt or wt/fl and

GLAST::CreERT2xSmad4fl/fl mice at the end of 4 weeks (**Figure 28**). The number of double positive cells were significantly increased in the corpus callosum of mutant mice compared to the control. Considering the data of elevated number of Olig2-positive cells in the SEZ in the absence of Smad4, this new observation suggested that SEZ is potentially responsible for the higher number of oligodendrocytes in the Smad4-/- corpus callosum. However, with this method we can not distinguish between the oligodendrocytes that migrated from subependymal zone and the ones generated already in corpus callosum. This data does not directly clarify whether progenitors in the SEZ are responsible for the increase in Sox10/BrdU-positive cells in the corpus callosum after Smad4 deletion or whether progenitors in the corpus callosum may be directly affected. However, Smad4 is not expressed in the corpus callosum as revealed by in situ hybridization (**Figure 29**) and so far restricted to neurons as Smad4 is not localized in progenitors outside the SEZ. Therefore these observations support the hypothesis that Olig2-positive cells increased in the Smad4-/- SEZ may migrate into corpus callosum after Smad4 deletion (Moreover there is aberrant expression of Olig2 in SEZ of GLAST::CreERT2xSmad4fl/fl mice further supporting this hypothesis). Indeed, Olig2-positive cells normally migrate from the SEZ to the corpus callosum (Menn et al., 2006) and Olig2 over-expression results in a strong increase in the number of cells migrating to the corpus callosum (Hack et al., 2005).

6.5.8 Deletion of Smad4 results in migration of cells to the corpus callosum and increased oligodendrogliogenesis

To test the hypothesis that TAPs mis-specified after Smad4 deletion may leave the SEZ and head towards the corpus callosum and generate oligodendrocytes, I injected retroviral vectors (MLV-based, see also materials and methods) containing GFP into the SEZ of GLAST::CreERT2/Smad4fl/fl or control mice 10 days after the end of tamoxifen application. At this time point neurogenesis is already impaired and Olig2 is up-regulated by the Dlx2-positive TAPs as demonstrated with a scheme in **Figure 30**. I examined the location of GFP-positive cells 10 days later (20 days after the end of tamoxifen application). In control mice only 2% of GFP-labelled cells migrated from the SEZ to the CC (**Figure 31C**), consistent with previous observations (Hack et al., 2005; Menn et al., 2006). However, up to 30% of all

cells labelled by injection into the Smad4-/- SEZ were located in the corpus callosum 10 days later (**Figure 31A,B,C)**.

Although I did not observe Smad4 mRNA in corpus callosum, nevertheless I did a control injection in corpus callosum of Smad4-/- in order to see whether deletion of Smad4 affects the progenitors within the corpus callosum or not (**Figure 32**). Menn and colleagues showed by injections that progenitors giving rise to oligodendrocytes within the corpus callosum do not migrate very far (~100μm) whereas the ones generated from SEZ migrate much further. Consistent with this data, also after injection of virus into the corpus callosum of Smad4-/- mice, GFP-positive cells were found only close to the injection track (**Figure 32A**) in contrast to the far distance migration of cells labelled by injection into the SEZ (**Figure 32B**). Thus, the increased number of oligodendrocytes found in corpus callosum by BrdU birthdating and Sox10 co-localization after Smad4 deletion can not originate from progenitors within corpus callosum. Rather, many more cells originating in the SEZ migrate far into the corpus callosum upon Smad4 deletion in adult neural stem cells.

To assess the fate of the GFP-positive cells that had migrated from the SEZ into the corpus callosum I stained for PDGFRα to detect immature oligodendrocyte progenitors and CC1 or CNPase for oligodendrocytes at later stages. Sox10 is present in oligodedndrocyte progenitors, immature and mature oligodendrocytes. To follow the maturation of oligodendrocytes after Smad4 deletion I preferred to label different antigens present in different stage of oligodendrogenesis. Indeed, most of the GFP-positive cells in corpus callosum were PDGFRα-positive (80%, **Figure 33A,C**) and about 60% were CC1-positive (**Figure 33B,C**), consistent with their progression along the oligodendroglial lineage. To assess the further maturation of cells that migrated from the SEZ to the corpus callosum, I analyzed the brains at later stages after viral vector injection (21-30 days, **Figure 33D-E''**). Most cells labelled with GFP virus in the SEZ that had migrated into the corpus callosum exhibited further mature features of oligodendrocytes in regard to their morphology (**Figure 33D**) and the expression of MOG (**Figure 33E-E''**), a protein contained only in mature oligodendrocytes. Thus, a large proportion of TAPs up-regulate Olig2 after Smad4 deletion and then give rise to cells migrating to the corpus callosum and differentiating into oligodendrocytes rather than generating neuroblasts migrating to the OB (**Figure 31C**).

This phenotype may obviously depend on Olig2-upregulation, as Olig2 was previously shown to direct SEZ cells towards the generation of cells migrating towards the corpus callosum (Hack et al., 2005). However, Smad4 deletion may result in deregulation of many other direct or indirect down-stream targets in addition to Olig2. We therefore asked whether the up-regulation of Olig2 is really the key factor for this lineage diversion by attempting a rescue via suppression of Olig2 function. The fusion of Olig2 to the strong activator VP16 has previously been shown to antagonize the endogenous function of Olig2 that acts as a repressor (Buffo et al., 2005; Hack et al., 2005; Mizuguchi et al., 2001). I therefore injected a previously described virus containing Olig2VP16, IRES and GFP (Buffo et al., 2005; Hack et al., 2005) into the SEZ of Smad4-/- mice (tamoxifen was applied 10 days prior to virus injection as above). The proportion of Olig2VP16 transduced cells migrating to the corpus callosum was significantly reduced compared to the cells infected with a control virus in Smad4-/- mice (**Figure 31C**). Even more strikingly, the proportion of Olig2VP16-GFP-positive cells migrating via the RMS to the OB was increased to normal WT levels as observed upon GFP virus injection into control mice (**Figure 31C**). Thus, suppression of Olig2 function achieves a rather complete rescue of the defective neurogenesis upon Smad4 deletion.

6.5.9 Wild type cells transplanted into the Smad4-/- SEZ are not impaired in neurogenesis

As deletion of Smad4 in all astrocytes of adult SEZ may change the niche signals and thereby contribute to the defects in neurogenesis, I examined the function of the neurogenic niche by transplanting a small number of wild type cells into 6 weeks old control and Smad4-/- mice (**Figure 34**). Towards this aim, adult SEZ cells were isolated from 6 weeks old animals expressing the green fluorescent protein Venus targeted to the plasma membrane ubiquitously (Rhee et al., 2006) and 30.000 cells were transplanted into the SEZ of GLAST::CreERT2/Smad4wt/wt or GLAST::CreERT2/Smad4fl/fl mice 10 days after the end of tamoxifen application. Notably, at this time point neurogenesis was decreased and Olig2-expression was increased in the Smad4-/- mice (**Figure 20,23**). Consistent with our aim to expose wild type cells to a mutant environment, in most cases only a single Venus-positive cell was detected in each section 7 days after transplantation. When I examined the identity of

the transplanted Venus positive cells (Venus protein is detected by GFP antibody) in the SEZ, RMS and OB, most of them were DCX-positive neuroblasts, in both a control or Smad4-/- SEZ environment (**Figure 34B-D**). These data therefore demonstrate that the neurogenic niche is not altered in the SEZ of GLAST::CreERT2/Smad4fl/fl mice as wild type cells progress normally along their neuronal fate. Thus, Smad4 acts in a cell autonomous manner to allow neurogenesis by Olig2 suppression.

6.5.10 Smad4 is required at early stages in the stem cell-derived lineage

As the above results support a cell-autonomous function of Smad4, we next asked at which stage in the neural stem cell derived lineage Smad4 is required. Towards this end, I deleted Smad4 at different stages in the lineage. In order to delete Smad4 in all fast-proliferating cells of the SEZ (TAPs and neuroblasts) and spare the stem cells we targeted Cre by the Moloney-based viral (MLV) vectors that require the breakdown of the nuclear envelope during cell division to incorporate their genome (**Figure 35**). Indeed, upon injection of MLV-based retroviral vectors containing GFP into the SEZ, virtually no GFP-positive cells were GFAP-positive 2 days (**Figure 35A,** this data was observed by my colleague Monika Brill in our laboratory) or 7 days later (Hack et al., 2005) indicating that neural stem cells were not targeted. In contrast, upon injection of lentiviral vectors containing GFP (Pfeifer et al., 2001; Pfeifer et al., 2002) the vast majority of GFP-positive cells are GFAP-positive 2 days after viral injection (**Figure 35A**). I could therefore use different viral vectors to target Cre to distinct stem or progenitor populations in Smad4fl/fl mice (**Figure 35B**). Indeed, 7 days after injection of lentivirus containing Cre into the SEZ of Smad4fl/fl mice a strong reduction in the number of DCX-positive cells was detected among the Cre-infected cells to less than a third of the DCX+ cells among cells infected by the GFP-containing control virus (**Figure 35C,D,G**). In contrast, however, MLV-retrovirus mediated deletion of Smad4 by Cre expression in TAPs and neuroblasts of Smad4fl/fl mice did not show any effect on the number of DCX+ neuroblasts 7 days after injection (**Figure 35E,F,G**). Thus, these data suggest a role for Smad4 at an early stage in the adult neural stem cell derived neurogenesis.

6.6 Discrimination of BMP and TGFβ signalling in regard to the contribution to Smad4 phenotype

The above results have shown a role of Smad4 in suppressing the oligodendroglial lineage when TAPs are generated. As introduced above Smad4 is a central mediator in TGFβ superfamily. Thus, the phenotype revealed after Smad4 deletion may be due to interference with exclusively with BMP or/and TGFβ pathway signals in this superfamily. Although this phenotype is well consistent with the identity of BMP-responsive cell types, the stem cells and TAPs, it does not exclude the possible contribution of TGFβ signalling. Indeed, cells containing phosphorylated Smad 2 and 3, the receptor regulated Smads of TGFβ signalling, are rather widespread in the adult SEZ (**Figure 38**).

We therefore aimed to discriminate the role of the BMP or TGFβ pathway respectively.

6.6.1 Extracellular inhibition of BMP signalling via Noggin increases Olig2 expression and decreases neurogenesis

To block the BMP selectively I used minipumps to infuse Noggin, an extracellular inhibitor binding to BMP-ligands, but not to TGFβ (Brunet et al., 1998; Shi and Massague, 2003; Zimmerman et al., 1996), for 7 days into the lateral ventricle of wild type mice. The animals examined either at 3 or 21 days after the end of infusion. When mice were examined 3 days after the end of infusion, p-Smad1/5/8 immunostaining showed a severe decrease in the SEZ after Noggin, but not after vehicle (artificial Cerebro-Spinal-Fluid, aCSF) infusion (**Figure 36A,D**), confirming the successful delivery of Noggin. As a first step I checked neurogenesis. The number of DCX-positive cells in the SEZ decreased after Noggin infusion to less than half the number observed in animals receiving vehicle infusion (**Figure 36B,E,G**). In further agreement with the results obtained after Smad4 deletion, Noggin infusion also increased the number of Olig2-positive cells to double the number obtained after vehicle infusion (**Figure 36C,F,H**).

When animals were examined 21 days after the end of infusion, the loss of p-Smad1/5/8 had not yet (**Figure 37A,C**) although the number of p-Smad1/5/8-positive cells was higher compared to the analysis at 3 days after Noggin infusion. Strikingly, impaired neurogenesis (**Figure 37B,E,G**) and increase in the number of Olig2-positive cells (**Figure 37C,F,H**) also persisted until 21 days after the end of Noggin infusion.

6.6.2 Inhibition of TGFβ signalling does not cause Oli2 up-regulation and thereby alteration in neurogenesis

Noggin infusion showed that BMP signalling is required for cell fate choice in favour to neurogenesis versus oligodendrogenesis indicating its contribution to the phenotype in Smad4-/- mice. Taken together, the results imply that the BMP pathway is responsible for the major phenotype after Smad4 deletion. However, given the presence of p-Smad2/3 (**Figure 38**), TGFβ may have some other roles yet unravelled e.g. regulate the later proliferation effects observed after Smad4 deletion. To clarify the role of TGFβ signalling, we used the GLAST::CreERT2 mouse line to delete the type II TGFβ receptor (Tgfbr2fl/fl) (Leveen et al., 2002) in collaboration with Lukas Sommer and Sven Falk. This floxed line is a suitable tool to study the role of TGFβ signalling as TGFβ specific type II receptor (Tgfbr2) is the only secondary receptor and therefore required to mediate TGFβ signalling (Leveen et al., 2002). GLAST::CreERT2/Tgfbr2wt/wt and GLAST::CreERT2/Tgfbr2fl/fl mice received tamoxifen as it has been done in previous experiments (5 days consecutive). One month after the end of tamoxifen application animals were sacrificed. However, TGFβ type II receptor immunostaining in the SEZ of GLAST::CreERT2/Tgfbr2wt/wt and GLAST::CreERT2/Tgfbr2fl/fl was not different in the two genotypes (**Figure 39**). However, when animals were examined three months after the end of tamoxifen application TGFβ type II receptor immunoreactivity had disappeared in the SEZ of animals with homozygous floxed allele of TGFβ type II receptor (**Figure 40, see D versus A**). Notably, however TGFβ type II receptor-positive cells were still observed outside the SEZ within the striatum confirming the specificity mediated by GLAST::CreERT2 (**Figure 40D**) However, despite the loss of TGFβ type II receptor immunoreactivity, I could not observe a difference in the number of neuroblasts between the SEZ of GLAST::CreERT2/Tgfbr2fl/fl and GLAST::CreERT2/Tgfbr2wt/wt (**Figure 40B,E,G**). Similar to the neuroblasts, the number of Olig2-expressing cells also did not differ in the animals with homozygous floxed allele of TGFβ type II receptor compared to ones carrying none (**Figure 40C,F,H**).

Thus, TGFβ-mediated signalling does not contribute to the striking decrease in neurogenesis and diversion of the adult stem cell progeny towards an oligodendroglial fate observed after Smad4 deletion.Conversly, the blockade of BMP-signalling phenocopies the main defects

observed after deletion of Smad4, suggesting that BMP is the main neurogenic signal in this regard.

6.7 Overexpression of BMP ligands in vivo and in vitro

6.7.1 BMP7 reduces proliferation in the adult SEZ

Until now we studied endogenous role of BMP signaling in the adult SEZ either by interfering with the intracellular cascade via Smad4 deletion or by interfering extra cellularly via noggin infusion. In both experiments we decreased the BMP activity. In order to examine the effects of BMP levels elevating, as an opposite approach, we decided to infuse BMP ligand into the lateral ventricle. In order to avoid endogenous inhibition by Noggin, I chose BMP7 as the ligand least sensitive to Noggin (Zimmermann et al., 1996). BMP7 (dissolved in aCSF) was infused for 7 days into the lateral ventricle of wild type mice and animals were examined 3 days after the end of infusion. As control, some animals received only aCSF without BMP7. All animals had BrdU injection 1 hour prior to sacrifice. When proliferation was examined by Ki67 and BrdU (**Figure 41**), I observed less Ki67- or BrdU-positive cells in the animals received BMP7 compared to control (**Figure 41A,B;** Ki67: Control=142±21/SEZ area, BMP7=73±17/SEZ area, 2 animals each). These results show that increasing BMP levels in the adult SEZ result in less proliferation and thereby less neurogenesis.

6.7.2 BMP decreases the proliferation of neurosphere forming cells in vitro

In order to see BMP function on stem cells in vitro I used neurosphere assay. After dissociating SEZ from adult mice I cultured single cells (10 cells/µl) and cultivated them with growth factors EGF and basic FGF (see also in materials and methods) in 24 well plates as explained previously. In these conditions single cells proliferate and make neurospheres. In some wells I added BMP2 at different concentrations (1ng and 10ng). The number of neurospheres per well (5000 cells) was quantified after 7 days. Only in the condition with higher BMP2 concentration the number of neurospheres was reduced compared to control (**Figure 42A,C**). While 1ng BMP2 did not show a significant difference, 10ng BMP2 reduced the number of neurospeheres almost to half of the number observed in control conditions (**Figure 42C**).

Previously it has been shown that Olig2 is highly expressed in neurospheres and required to keep them proliferative (Hack et al., 2004). As interfering with BMP signaling via Noggin infusion and Smad4 deletion lead to increase in Olig2 expression, we asked the question whether BMP addition has influenced Olig2 expression and thereby decreased proliferation. Indeed, Olig2 immunoreactivity in neurospheres treated with BMP2 was undetectable (**Figure 42E**) compared to very prominent expression in control neurospheres (**Figure 42D**).
The negative effect of BMP2 on proliferation of neurosphere cells in vitro correlates with the data observed after BMP7 infusion into the ventricle. These results also show further support for regulation of Olig2 by BMP, as Olig2 is downregulated in the neurospheres treated with BMP2 and conversely up-regulated upon inactivation of BMP signaling via Smad4 deletion and Noggin infusion in vivo.

6.7.3 BMP regulates proliferation via Smad pathway

Having observed proliferation deficiency of neurosphere forming cells in the presence of BMP, I examined whether neurospheres have endogenous BMP signalling per se and whether ectopic BMP signals also through Smad pathway.
However, p-Smad1/5/8 staining was absent in neurospheres cultivated without BMP2 (**Figure 43A-A''**) whereas neurospheres cultured in presence of BMP2 showed a high p-Smad1/5/8 activity (**Figure 43B-B''**). This indicates the absence of notable levels of endogenous BMP signalling within the neurospheres

Interestingly adult neural stem cells do not have BMP activity in vitro in comparison to their very prominent BMP activity in vivo. This observation was confirmed also by using BRE-eGFP mouse line. SEZ of adult BRE-eGFP mice were dissected and dissociated cells were cultured in neurosphere culture conditions. No GFP was visible in neurospheres not even after amplification by antibody staining (**Figure 43C-C''**) consistent with the lack of p-Smad1/5/8 expression in these cells (**Figure 43A-A''**). However, when neurosphere cells from BRE-eGFP were treated with BMP2, neurospheres expressed high levels of GFP indicating the activation of Smad pathway ectopically.

6.7.4 BMP2 blocks oligodendrocyte differentiation from stem cells in vitro

In order to examine the role of BMP during differentiaion of neurosphere cells, I treated differentiating cells with BMP2. Neurospheres were dissociated and plated on Poly-D-Lysine as single cells without growth factors (see also in materials and methods). This allows neurosphere cells to leave their undifferentiated/proliferative state and differentiate into neurons, astrocytes or oligodendrocytes. In vitro adult neural stem cells mostly give rise to astrocytes (~60%) (**Figure 44A**). The remainder is composed of oligodendrocytes (~15%), neurons (~10%) and cells which are not positive for those lineage markers but having astrocytic shape (**Figure 44A**) . When BMP2 was given at the first day and not removed or refreshed until fixation of cells, the number of oligodendrocytes generated by neurosphere cells dropped in a dose dependent manner (**Figure 44A**). In the presence of 10 and 100ng BMP2 very few oligodendrocytes were generated compared to control conditions (**Figure 44A,B-C'**). Conversely higher levels of BMP lead to an increase in the number of marker negative cells probably at the expense of oligodendrogenesis. BMP2 also showed a tendency to increase the number of neurons and decrease the number of astrocytes (**Figure 44A**).

Taken together, BMP2 addition to differentiating neurosphere cells blocks oligodendrogenic lineage consistent with the in vivo data observed after Smad4 deletion and Noggin infusion . However, the effect on promoting neurogenesis was only in vivo, suggesting that in vitro additional factors may be required to promote neurogenesis.

6.8 Monitoring direct or indirect targets of Smad4 via microarray analysis

Given the potent effect of Smad4 deletion in vivo, we were interested to examine potential mediators of Smad4 that elicit Olig2 upregulation. Towards this end, I isolated RNA form the SEZ of GLAST::CreERT2/Smad4wt/wt and GLAST::CreERT2/Smad4fl/fl mice. To test the quality and reproducibility of the samples, hierarchical clustering was used to find (dis)similarities between the samples, showing that the replicates of each group of cells analyzed were clustering together. Differentially expressed messenger RNAs were monitored by microarray analysis (see materials and methods). Around 500 gene were found to be differentially expressed in their mRNA levels at least in one of four different analysis (CI, Carma Mas5, Carma RMA, BC Mas). The main difference between these analysis is the way

of significance calculation. Among the differentially expressed genes, only 12 genes were significant resulted in all of the 4 analysis. Notably, amongst these we detected a higher number of mRNAs downregulated in the Smad4-/- SEZ consistent with activator role of Smad4.

In order to confirm the expression differences observed by microarray, I performed Real Time RT-PCR (**Figure 45**). Complementary DNA (cDNA) was prepared (see materials and methods) from the same RNA with which microarray analysis had been performed. All mRNAs were found to be regulated in the same direction as indicated by our microarry. Among those genes confirmed by RT-PCR, polyadenylate binding protein-interacting protein 1(translational activator) (Paip1), dipeptidylpeptidase 6 (Dpp6), SRY-box containing gene 17 (Sox 17) were up-regulated and cyclin-dependent kinase 4 (Cdk4), POU domain, class 6, transcription factor 1 (translational activator) (Pou6f1), Sox11 were down-regulated in Smad4-/- versus control.

7 DISCUSSION

The aim of this study was to determine the role of BMP signalling in the adult SEZ and also to monitor this signalling after brain injury. I started with an expression analysis for BMP signalling pathway elements and by determining the cell types subjected to BMP signalling. I observed an active BMP signalling in the adult SEZ.

Here I demonstrate that Smad-mediated BMP signalling is active in adult neural stem cells and their immediate progeny, the TAPs and it is required for adult neurogenesis in the adult SEZ. My results show that both genetic deletion of Smad4, a central mediator of BMP signalling, and infusion of Noggin, an extracellular inhibitor of BMP, resulted in an increase in Olig2-positive oligodendrocyte precursors at the expense of neurogenesis. An abnormally high number of oligodendrocyte precursors emerged in the SEZ after Smad4 deletion and migrated then towards the corpus callosum where they started to express mature oligodendrocyte features. The decrease in neurogenesis could be fully rescued by suppression of Olig2 function indicating that the up-regulation of Olig2 was the main cause of increased oligodendrogenesis. Furthermore, transplantation experiments showed that Smad4 deletion does not affect the SEZ niche per se but rather Smad4-mediated BMP-signalling is required in a cell-autonomous manner at an early stage in the adult neural stem cell lineage when stem cells give rise to TAPs. Consistent with its presence in TAPs, BMP signalling also affected TAP proliferation at later stages.

My results therefore imply BMP as one of the earliest key signals in the adult neural stem cell niche allowing the progression towards the neurogenic lineage by inhibiting the generation of oligodendrocytes.

7.1 Smad pathway dependent BMP activity is present in stem cells of adult SEZ but not in SGZ

Previously, some of BMP ligands and receptors as well as Noggin had been described in the adult SEZ (Zhang et al., 1998; Lim et al., 2000; Peretto et al., 2002). However, the cells responding to BMP signalling and its actual activity were not previously examined enough. It had also been suggested that Noggin negatively regulates BMP transcription as Bmp genes

were ectopically expressed in the ventral spinal cord of Noggin mutants (McMahon, 1996; Hammerschmidt et al., 1996). However this additional role of Noggin beside its direct role in preventing the binding of BMP to its receptor does not apply to adult SEZ as mRNA of several BMP ligands and protein of some others are present in the adult SEZ despite the Noggin presence (Lim et al., 2000; Peretto et al., 2002; and **Figure 7**).
Lim and colleagues found Noggin protein exclusively in ependymal cells lining the ventricle and proposed that secretion of Noggin from ependymal cells inhibit BMP signalling and thereby allow neurogenesis. Consistent with this, in adult Noggin-LacZ reporter mice β-galactosidase is found in ependymal cells beside corpus callosum and rostral migratory stream (Peretto et al., 2002). However, expression of Noggin does not exclude a certain degree of BMP activity as BMP signalling acts in a concentration dependent manner (Furuta et al., 1997; Mehler et al., 2000). Moreover it has been shown that Noggin may be required as a feedback mechanism as its expression is up-regulated together with BMP ligands (Gazzero et al., 1998). Similar to Noggin, the intracellular inhibitor of BMP signalling, Smad6, is also strongly induced by BMPs in certain cell types indicating a feedback inhibition mechanism after ligand-induced activation.

Thus, to determine whether there is an active BMP signalling in the adult SEZ zone or not, I focused on BMP mediated Smad pathway as Smad proteins are major signalling molecules acting downstream of the serine/threonine kinase receptors after ligand binding (Heldin et al., 1997; Moustakas et al., 2001). Among these, Smad 1,5 and 8 are phosphorylated by receptors specifically upon BMP binding which can be used to monitor the activity of BMP signaling. Indeed, I found immunoreactivity for phosphorylated Smad1/5/8 in the adult SEZ. Moreover I could also show BMP activity in the adult SEZ by using BRE-driven reporter. This activity is present close to the ependymal layer despite the expression of Noggin in ependymal cells. I showed that stem cells of adult SEZ and a sub population of their immediate progeny TAPs are subject to BMP signalling. Finding BMP specific phosphorylated Smads in the adult SEZ indicates that there must be certain levels of BMP present extracellularly. Thus, rather than fully blocking the binding of BMP ligands to their receptors, Noggin secretion may adjust the level of BMP-signalling activity. This adjustment may depend on the amount as BMPs act in a dose dependent manner both in vitro and in vivo (Hogan, 1996; Nguyen et al., 2000; Mehler, 2000) as well as the identity of the ligands secreted. It is known that Noggin is

relatively insensitive to BMP7 (Zimmerman et al., 1996) which is indeed present in the adult SEZ (Peretto et al., 2002; and **Figure 7D**). As different concentrations of BMP are known to exert different effects (Furuta et al., 1997; Nguyen et al., 2000), a tight balance between BMP ligands and inhibitors of this pathway is particularly important. For example, higher levels of BMP promote cell death and inhibit proliferation of embryonic cortex precursors (Graham et al., 1996; Mehler et al., 2000; Hebert et al., 2002), while lower concentrations promote neuronal and astroglial differentiation and inhibited oligodendrogliogenesis (Mabie et al., 1999; Chojnacki and Weiss, 2004). Thus, the level of BMP-signalling may also be critical in the adult SEZ.

Finding Smad-dependent BMP activity in the adult SEZ raised the question whether this pathway is active also in the SGZ of dentate gyrus. However, I could not detect p-Smad1/5/8 immunoreactivity in this neurogenic niche (**Figure 8I**). This raises 3 possibilities : 1)The activity can be very low to be detectable 2) BMP ligands may not activate Smad pathway and rather activate an alternative pathway 3) BMP signalling may be completely absent in this niche.

Indeed it has been shown that BMP can also act through mitogen activated protein kinase (Nohe et al., 2002). The activation of MAPK pathway instead of Smad is suggested to be regulated by receptor complexes. Combinatorial interactions in the tetrameric receptor complex allow differential ligand binding or differential signalling in response to the same ligand. BMP receptors ALK3, ALK6 and BMPR-II have been shown to assemble either as preformed hetero-oligomeric or homo-oligomeric complexes (Gilboa et al., 2000). Thus, a ligand such as BMP2 has two options to initiate signal transduction. It can either bind to ALK3 or ALK6 and then recruit BMPR-II into a hetero-oligomeric complex or, alternatively, binds simultaneously to the preformed hetero-oligomeric complexes consisting of at least one type I and one type II receptor. This results in the subsequent activation of either the Erk/JNK/p38 mitogen-activated protein kinase (MAPK) or classical Smad pathways, respectively (Nohe et al., 2002). It is know that the receptors in most cases are not preformed prior to ligand binding and therefore the classical Smad pathway is the major pathway mediating BMP signalling. However it is not known in which cases the receptors are

preformed and how this is regulated. MAPK pathway mediated by BMP has mostly been shown in chondrocytes (Lyon, 2004).

I can not exclude the possibility that the MAPK pathway is maybe preferentially activated in SGZ instead of Smad pathway. Besides the MAPK pathway, another novel alternative pathway has been recently suggested to be activated by BMP. Upon BMP4 treatment, the serine-threonine kinase FKBP12/rapamycin-associated protein (FRAP), mammalian target of rapamycin (mTOR), associates with Stat3 and facilitates STAT activation (Rajan and Panchision, 2003). However there is very little known about these alternative pathways, their regulation in different tissues and their final effects.

There are certain differences between two neurogenic niches either in molecular level or in the integration mode of the newly generated neurons (Nincovic et al., 2007). For example in SGZ there is no Olig2 expression and consistent with this there is no oligodendrogenesis. Thus, absence of BMP signaling in SGZ may also be possible. Taken together, BMP signaling activity should be studied in more detail in SGZ.

7.2 BMP signalling is not active in cells responding to stab wound in cerebral cortex

Cytokines are one group of candidates which could direct reactive gliosis, scar formation, microglia activation as well as inhibition of neurogenesis after injury (Owens et al., 2005; Unsicker et al., 1992). It has been shown that BMP ligands are up-regulated upon injury in spinal cord (Chen et al., 2005; Setoguchi et al., 2004; Fuller et al., 2007) and induce scar formation (Fuller et al., 2007). Fuller and colleagues proposed that BMPs mediate a second astrocytic response to a demyelination injury in spinal cord by promoting the expression of chondroitin sulphate proteoglycans (CSPGs) in the glial scar.

Previously, BMPRIA and BMPRII transcripts were found in the intact cerebral cortex (Zhang et al., 1996). However BMP ligands, receptors and cells subjected to BMP signalling in the intact and lesioned cerebral cortex had not been shown. Here, by immunostaining for p-Smad1/5/8, I show that Smad-dependent BMP activity is present in neurons of intact cortex (**Figure 10**). Also in spinal cord p-Smad1/5/8 and NeuN co-localize indicating BMP activity

in neurons (Fuller et al., 2007). I examined in addititon the BMP ligands 2, 4 , 6 and BMPRII transcripts as well as p-Smad1/5/8 immunoreactivity in the intact cortex or after a stab wound (**Figure 11,12**). In contrast to spinal cord, I did not observe an up regulation of any of the ligands, the receptor and also p-Smad1/5/8 immunoreactivity upon stab wound in the cortex (**Figure 11,12**) suggesting that BMPs are not up-regulated by cells responding to injury. Consistent with this a recent study showed that Noggin is up-regulated after knife cut injury (very similar to stab wound) and inhibit BMP activity (Hampton et al., 2007) in the adult cortex. They found expression of Noggin almost exclusively in astrocytes. Interfering with Noggin after injury increased the number of NG2-positive cells and GFAP-positive cells in vivo. NG2-positive cells are known as oligodendrocyte progenitors but they do not give rise to oligodendrocytes in adult cortex but rather mature as NG2-positive cells with no characteristics of oligodendorcytes.

The presence of Noggin after such an injury may explain why I do not observe an increase in BMP activity monitored by p-Smad1/5/8 staining after injury.

However when I examined the Id3 mRNA, I found a transient up-regulation of this gene upon injury (**Figure 13**). This suggests that TGFβ may be responsible for this up-regulation as this ligand is known to up-regulate some of the Id proteins transiently (Hacker, 2003; Sugai, 2003) (except Id1 which is an exclusive target of BMP signalling (Korchynskyi and ten Dijke, 2002; Lopez-Rovira, 2002; Katagiri, 2002)). Consistent with this, it has been shown that endogenous TGFβ1 is expressed by parenchymal microglial cells and exerts a trophic and anti-inflammatory effect in the adult CNS (Makwana et al., 2007) and this effect is increased upon injury (Makwana et al., 2007). Another study also showed that TGFβ signalling is induced after stab wound injury in the adult cortex and interference with it results in more rapid wound closure and reduced scar formation (Wang et al., 2006). Therefore Id3 induction upon stab wound in cortex is likely mediated by TGFβ signalling.

Taken together, BMP signalling seems to be in favor of neurogenesis in the adult SEZ, as it is active in neurogenic stem cells but absent in progenitors upon injury.

7.3 Smad4-mediated BMP signalling positively regulates neurogenesis in the adult neural stem cell niche

Here we propose the BMP-mediated Smad pathway as one of the key factors promoting neurogenesis in the unique neurogenic environment of the SEZ, but not the SGZ.

Beginning of 2000, Lim and colleagues had analyzed the effects of Noggin on adult SEZ cells cultured on top of the astrocyte monolayer. Noggin inhibited BMPs secreted from astrocytes and as a result increased neurogenesis in vitro. To demonstrate this in vivo, they killed stem cells with AraC treatment (see also introduction) and overexpressed BMP7 in ependymal cells. They indeed found no regeneration of neurons in the presence of BMP7 consistent with the in vitro data. Based on these results, they suggested that Noggin secreted from ependymal cells would inhibit BMP signalling and as a result allow neurogenesis as they observed Noggin protein in ependymal cells. However they also observed a decrease in proliferation that may be responsible for the decrease in regeneration of neuroblasts after AraC and BMP treatment. Indeed, when I infused BMP7 into the ventricle without AraC treatment I also observed a decrease in proliferation in the intact SEZ (**Figure 41**) as well as after BMP treatment in cultured SEZ cells (**Figure 42**). Recent data also demonstrated the anti-proliferative role of BMP overexpression on the proliferation of glioblastoma cells in the adult brain (Piccirillo et al., 2006). Moreover, cell death was not examined in Lim et al., 2000 study after overexpression of BMP7 which can also be responsible for the decrease in regeneration of neuroblasts as there is some evindence for high levels of BMP being apoptotic in embryonic cortical cells and neural crest cells (Graham et al., 1996; Furuta et al., 1997; Mehler et al., 2000; Hebert et al., 2002). Additionally, particularly high levels of BMP may also act via Smad-independent pathways (Nohe et al., 2002; Rajan et al., 2003; Nohe et al., 2004; see also above). These considerations therefore imply that raising the levels of BMP may not necessarily reveal the endogenous function of BMP-signalling at the physiological levels. Indeed, lowering the levels of BMP signalling by Noggin infusion or Smad4 deletion did not affect proliferation, consistent with only high levels of BMP acting on proliferation.

Given the importance of a precise level of BMP-signalling activity one may gain better insights by interfering with the endogenous levels of BMP or its signalling mediators. My results obtained by lowering the levels of BMP-activity by Noggin infusion into the ventricle

or deleting the essential signalling mediator Smad4 revealed the generation of oligodendrocytes at the expense of neurogenesis. These findings suggest that the endogenous role of BMP-signalling is to allow neurogenesis by suppressing oligodendrogliogenesis. The endogenous levels of Noggin expressed in the ependymal cells of the adult SEZ may therefore be crucial to allow the low degree of oligodendrogliogenesis occurring normally (Hack et al., 2005; Menn et al., 2006). Interestingly, oligodendrogliogenesis in the SEZ increases after demyelination (Nait-Oumesmar et al., 1999; Nait-Oumesmar et al., 2007) (see below) indicating that alterations in the stem cell niche may mediate the injury triggered signalling. Taken together, my results imply that the fine-tuning of progenitors specified towards neurogenesis or oligodendrogliogenesis crucially depends on BMP-mediated signalling.

Consistent with our observations in the adult SEZ, BMP-signalling inhibits and Noggin application promotes oligodendrogliogenesis in the developing nervous system (Hardy and Friedrich, 1996; Kondo and Raff, 2000; Mekki-Dauriac et al., 2002; Gomes et al., 2003; Samanta and Kessler, 2004; Miller et al., 2004). This suppression of oligodendrogenesis by BMPs does not depend on the stage unlike to other effects of BMP signalling as BMPs inhibit oligodendrogenesis at all stages of development (Miller, 2004). Interestingly, the inhibition of oligodendrogliogenesis observed during development occurs sometimes in favour of neurogenesis (Mabie et al., 1999; Schneider et al., 1999; Muller and Rohrer, 2002; Chojnacki and Weiss, 2004), and sometimes in favor of astroglial differentiation (Gross et al., 1996; Gomes et al., 2003).

In cultured adult SEZ cells BMP suppressed oligodendrogenic fate but did not promote neurogenic fate but instead lead to the generation of lineage marker negative population (**Figure 44**). The nature of the cells responding to BMP signaling in vitro is not fully understood. One candidate is the multipotent neural stem cells capable of giving rise to neurons, astrocytes, and oligodendrocytes (Gage, 2000). Alternatively, more fate-restricted neuron and glia-restricted precursor cells that have been identified in vitro (Rao and Mayer-Proschel, 1997) may respond in quite different ways to the same cues (Gregori et al., 2002). If the former is the case it means that the development of a distinct cell fate under the influence of BMP signaling likely involves both the active promotion of specific pathways and the suppression of alternative fates in a multipotent stem cell. However in cultured SEZ cells BMP does not change neuron number but mostly acts on glial fate suggesting that there might be restricted precursors in SEZ cultures. Not necessarily dependent on the nature of the cell,

these diverse responses to BMPs may also be due to the other signals that modulate the response to BMPs (Sun et al., 2001; White et al., 2001) and/or the activation of different BMP signal transduction pathways (see below).

7.4 Smad4 acts early in the lineage within the adult SEZ

A particularly interesting aspect of my findings is that Smad4-mediated BMP-signalling promotes neurogenesis at very early stages of the lineage. This is evident from the finding that deletion of Smad4 in TAPs and neuroblasts by retroviral expression of Cre had no immediate effect on neurogenesis, while deletion of Smad4 by a lentiviral vector in stem and progenitor cells resulted in a decrease in neurogenesis. Thus, Smad4-mediated signalling apparently promotes neurogenesis prior to the formation of neuroblasts at the level of the stem cells, when the identity of the TAPs is determined. This effect is different from previous effects on adult neural stem cells that mostly altered their proliferation and thereby affected neurogenesis (Ramirez-Castillejo et al., 2006; Sakaguchi et al., 2006). We therefore conclude that Smad4-mediated transcription is required at the transition from stem cells to TAPs.

However, 21 days after Smad4 deletion I observed a proliferation defect exclusively in the TAP population. Interestingly this effect was not observed by retroviral deletion of Smad4 in TAPs and neuroblasts in which also neurogenesis was not altered and TAP identity was not changed. This suggests that the proliferation defect occurs in the cells which changed their fate towards oligodendrogenesis. This may also be explained by the differentially regulated genes observed in microarray analysis after Smad4 deletion (see below) as well as differential expression of BMP type I receptors (see below).

7.5 Possible mechanisms underlying diverse roles of BMP signalling

While the canonical BMP/Smad signaling cascade has been clarified, the pathway by itself does not explain how BMPs exert such diverse functions. The answer may lie in the crosstalk between BMPs and other signaling pathways, the diverse transcription factors used by BMPs as well as alternative pathways activated by BMPs.

7.5.1 BMP type I receptors display different roles

The diverse functions of BMP signalling already can take place at the levels of the receptors. BMP-RII can combine with three type I receptors, ALK2, BMPRIA (ALK3), and BMPRIB (ALK6). Diverse functions of these receptors have been mostly studied in chondrogenesis. For example ALK2 has effects on chondrogenesis that are very distinct from those elicited by ALK3 and ALK6. Constitutively active (CA)-BMPRIA or BMPRIB increases cartilage nodule formation, while CA-ALK-2 does not. In fact, overexpression of CA-ALK2 actually delays differentiation in primary chondrocytes by inducing expression of an inhibitor of hypertrophic differentiation (Zhang et al., 2003). Moreover, when dominant negative (DN)-BMPRIA or IB is overexpressed in vitro, chondrogenic differentiation is blocked, while DN-ALK2 overexpression has no effect (Fujii et al., 1999). These results suggest that although ALK2 can activate the same subset of BMP-specific Smads as BMPRIA and IB in many cell types in vitro, signaling through BMPR1A and 1B promotes chondrogensic differentiation whereas signaling through ALK2 may inhibit it. Consistent with this possibility, ALK2 is highly expressed in the resting and proliferative zones, areas where chondrocytes need mechanisms to prevent premature differentiation (Zhang et al., 2003). Similar to this, in certain cell lines BMPRIB determines osteoblastic differentiation and the BMPRIA determines adipocyte differentiation (Namiki et al., 1997; Chen et al., 1998). Similar results were also obtained in limb bud (Yoon and Lyons, 2004). However, the mechanistic process for these apparent differences is unknown. It may be qualitative; these receptors may activate different non-Smad mediated signaling pathways. The differences may also be quantitative, with distinct outcomes arising as a result of different threshold requirements for BMP signal transduction in distinct aspects of proliferation and differentiation.

Also within the nervous system opposing effects of different BMP type I receptors were demonstrated (Panchision et al., 2001). It was shown that BMPRIA promotes proliferation of neural stem cells whereas BMPRIB limits precursor cell numbers by causing mitotic arrest. This results in apoptosis in early gestation embryos and terminal differentiation in mid-gestation embryos (Panchision et al., 2001). These authors have also shown that BMPRIA activation induces expression of BMPRIB whereas Shh prevents it. They suggested that

sequential actions of these receptors control the production and fate of dorsal precursor cells from neural stem cells.

Whether there is differential expression pattern of type I receptors (ALK2, BMPRIA, BMPRIB) in adult neurogenic niches is not clear yet. However, preliminary data from our laboratory (from Pratibha Tripathi, unpublished observations) indicates that adult SEZ cells have high levels of BMPRIA but not BMPRIB. As BMPRIA promotes proliferation of neural stem cells the proliferation defect in TAP population upon Smad4 deletion may be explained with the disruption of BMPRIA mediated signals.

Comparing expression pattern of BMP type I receptors between SEZ and SGZ may also help to understand whether different pathways possibly exist in these niches.

7.5.2 Potential interactions between BMP-derived signals and other pathways

Other signaling pathways also help to define the responses to BMPs. Studies showed intensive crosstalk between BMP, FGF and IGF signal pathways. For example, FGF and IGF signals activate intracellular MAPKs (mitogen-activated protein kinases) such as Erk, Jnk, and p38 that have been shown to phosphorylate Smad1 in the linker regions connecting MH1 and MH2 domains. Linker phosphorylation strongly decreases nuclear accumulation of Smads and therefore inhibits activation of the BMP pathway in vitro and in vivo (Kretzschmar et al. 1997; Aubin et al., 2004). Significantly, phosphorylation of same linker region was observed in vivo upon treatment of cells with epidermal growth factor (EGF) or platelet-derived growth factor (PDGF) (Moustakas et al., 2001). Experiments in the chick early epiblast shows that FGF signalling represses BMP4 and BMP7 expression and is required for neural induction (Streit et al., 2000; Wilson et al., 2000). Similarly, in Xenopus the downregulation of FGF signalling by overexpression of dominant-negative forms of FGF receptors has a negative impact on neural plate formation, partly due to de-repression of the expression of multiple BMP genes (Launay et al., 1996; Londin et al., 2005). In Xenopus, both FGF and IGF can mediate neural induction at least in part by inhibiting Smad activity through Smad phosphorylation (Pera et a., 2001; Pera et al. 2003). The functional antagonism between BMP and FGF signaling pathways is further confirmed in limb culture studies. BMP

treatment rescues the phenotype of FGF treated growth plates, and FGF treatment neutralizes the effects of BMPs (Minina et al., 2002).
I had emphasized in the introduction that BMPs promote the generation of neural crest cells in the posterior neural tube (Lee and Jessell 1999). In contrast to the negative effect of FGF2 on BMP signalling in many studies, FGF2 is required for BMPs to generate neural-crest like cells in cortical explants or cultured stem cells (Sailer et al., 2005). Co-treatment with FGF2 and BMP2 rapidly upregulates β-catenin, a mediator of Wnt activity (Moon et al., 2004) and of BMP2 itself, suggesting a positive feedback of BMP-signaling.
Similar to above examples of either positive or negative influence of FGF signalling on BMP signalling, it is still not clear whether FGF signalling has positive or negative influence in chondrogenesis due to controversial data (Lyon, 2004).

The phosphorylation of R-Smads in linker region and therefore inhibition of nuclear accumulation may explain why I do not detect nuclear p-Smad1/5/8 in adult neurospheres (**Figure 43**). As adult SEZ cells are kept with high FGF2 and EGF to prevent differentiation in vitro, this may lead alteration in Smad pathway which is active in vivo situation. However, exogenous BMP addition to the neurosphere cultures lead to active BMP signaling as demonstrated with nuclear p-Smad1/5/8 staining (**Figure 43D-D''**) indicating that FGF2 and EGF were not sufficient to fully compete with high amount of R-Smads. In contrast to high levels of FGF2 and EGF in neurosphere cultures, endogenous expressions of these ligands have not yet been described in the adult SEZ consistent with high activity of Smad-mediated BMP signalling in this niche. However, receptors for both FGF and EGF ligands are expressed in the adult SEZ (Morshead et al., 1994; Seroogy et al., 1995; Weickert et al., 2000). Therefore possible negative or positive influences of FGF and EGF on modulation of BMP signalling should be additionally examined in the adult SEZ.

As BMPs and also TGFβ can activate the MAPK pathway (Nohe et al., 2002; Yamaguchi et al., 1999), the balance between direct activation of Smads and MAPK pathways often defines cellular responses. BMPs effect chondrocytes by activating p38MAPK (Nakamura et al., 1999; Ju et al., 2000; Hatakeyama et al., 2003; Seto et al., 2004). In vitro, BMP treatment leads to sustained phosphorylation of p38. The ability of BMPs to promote chondrogenesis requires p38, as p38 inhibitors strongly suppress induction of type II collagen and

chondrogenic differentiation without effecting cell proliferation (Nakamura et al., 1999). Taken together, these data indicates that Smad and p38 mediated BMP signaling play essential and nonoverlapping roles in chondrogenic differentiation. However, existence or biological consequences of MAPK pathway mediated by BMPs are not known in CNS. Therefore studies are required in particular adult neurogenic niches to elucidate the extend to which these pathways mediate distinct aspects of neurogenesis and the extent to which they interact.

Another signaling pathway shown to modulate BMP signaling is Wnt pathway. Activation of Wnt signalling or inhibition of GSK3 can reverse Smad1 inhibition. GSK3 is a constitutively active, multifunctional kinase that plays roles in Wnt and Hedgehog signaling, insulin signaling through PI3K, and numerous other processes (Forde and Dale, 2007). In the Wnt pathway it participates in the destruction complex that targets β-catenin for ubiquitination and degradation. Wnt signaling inhibits GSK3, allowing β-catenin stabilization and activation of target genes with Tcf/Lef transcription factors. Treatment of cells with Wnt3a can inhibit GSK3 and lead to stabilized p-Smad1, with a decrease in p-Smad1GSK3 complex. In Xenopus embryos neural tissue differentiates when BMP signaling is inhibited, while epidermis forms upon high BMP signaling. Expression of the Wnt pathway inhibitor Dickkopf1 (Dkk1) causes neuralized phenotypes like those caused upon inhibition of BMP (Niehrs et al., 2001). Fuentealba et al. showed that injection of either Wnt morpholinos (MO) or Dkk1 mRNA resulted in expansion of neural tissues, and that this effect required intact GSK3 phosphorylation sites in Smad1. Furthermore, elevated Wnt or Dkk1 MO could induce epidermis, and this effect required active BMP/Smad signaling.
Synergism of BMP and Wnt pathways has also been shown in neural crest stem cells. The laboratory of Lukas Sommer showed that Wnt and BMP act synergistically to suppress differentiation and to maintain neural crest stem cell marker expression and multipotency (Kleber et al., 2005). However, it is also demonstrated that BMP signalling antagonizes the sensory fate inducing activity of Wnt/βcatenin within the same system.

In regard to adult neurogenic niches, Wnt signalling has been shown to promote neurogenesis in the SGZ. It has been shown that β-catenin promotes proliferation of Mash1-positive cells and thereby increases neuron production in the adult SEZ (Adachi et al., 2007). As deletion of

Smad4 leads to a decrease in proliferation of TAPs at later stages, Smad pathway and Wnt pathway may act synergistically to regulate proliferation in the adult SEZ. However, whether Wnt signalling is involved in a synergism with BMP in its neurogenic function in the adult SEZ is not clear.

Similar to the neural crest stem cells, BMP is also crucial to maintain the multipotency of embryonic stem cells but this time in combination with leukemia inhibitory factor (LIF) instead of Wnt (Ying et al., 2003). In contrast, in neural stem cell case it has been shown that simultaneous treatment of neuroepithelial cells with LIF and BMP2 synergistically induced astrocytic differentiation. The synergistic effect of the two cytokines is brought about by a complex formation composed of downstream transcription factors signal transducer and activator of transcription (STAT) 3 and Smad1, together with the transcriptional coactivator p300 (Nakashima et al., 1999; Fukuda and taga, 2005). It has been shown that LIF induces expression of BMP2 via STAT3 activation and leads to the consequent activation of Smad1 to efficiently promote astrogliogenic differentiation of neuroepithelial cells (Fukuda et al., 2007). As BMPs themselves can also activate the STAT pathway and thereby promote astrocyte differentiation (Rajan et al., 2003), BMP-mediated effects on astroglial differentiation are thought to be mediated via the STAT signalling pathway. Interestingly, STAT3 is not active in the adult neural stem cell zone (Bauer and Patterson, 2006). This may explain why BMP does not promote astrogliogenesis in the adult SEZ. Furthermore, there is also evidence demonstrating that BMPs can inhibit the phosphorylation and activation of STAT3 by an unknown mechanism (Kawamura et al., 2000). It may therefore be possible that BMP itself may inhibit STAT signalling in the adult SEZ and thereby promote neurogenesis.

Notably, the STAT-signalling pathway is activated after injury in the brain parenchyma (Okada et al., 2006; Fuller et al., 2007). Therefore the coactivation of STAT and Smad-signalling may explain why BMP promotes astroglial fate in the injured brain parenchyma (Okada et al., 2006; Fuller et al., 2007) and why overexpression of Noggin in the striatal or spinal cord parenchyma – causing injury by injection of the viral vectors – allows transplanted neurosphere cells to differentiate into neurons (Lim et al., 2000; Setoguchi et al., 2004). Instead, in the adult SEZ where STAT-signalling seems not to be active, endogenous BMP-mediated signalling via Smad4 promotes neurogenesis and inhibits oligodendrogliogenesis.

Taken together, as interaction between signaling pathways are crucial for fate specification (see above and also for review see: Nakashima et al., 2002; Sommer, 2006; Lillien et al., 2006) and much still needs to be learnt about the pathways interacting in the neurogenic niches in comparison to those active in the parenchyma after brain injury. This is highlighted by the intriguing role of BMP-mediated signaling promoting neurogenesis in the adult stem cell niche, in contrast to its role in other brain regions. Thus, further understanding of the key signals permitting neurogenesis in the adult neural stem cell niche should guide us how to stimulate neurogenesis from proliferating glia after brain injury.

7.5.3 Smad-interacting proteins contribute to diverse functions of BMP signalling

BMP proteins activate transcription through physical interaction and functional cooperation of DNA-binding Smads with sequence specific transcription factors and the coactivators Creb Binding Protein (CBP) and p300. R-Smads and Smad4 bind to preferred DNA sequences with a 100-fold lower affinity than the interacting high-affinity DNA-binding transcription factors, yet their DNA binding is required for transcriptional activation. Selective DNA binding to a subset of promoters that bind a potential Smad interacting transcription factor defines the promoters that are activated in response to the ligand. The number of DNA-binding transcription factors with which Smads can functionally interact is impressive (Massague, 2000; Itoh et al., 2000; Moustakas et al., 2001) and these are also often regulated by multiple signalling pathways. Thus, these factors probably complete the diversity mediated by BMP signalling. Besides the essential CBP or p300 coactivators, other coactivators and co-repressors that interact with Smads define the level of transcriptional activation (Derynck and Zhang et al., 2003). Table 3 shows a summary of Smad-interacting proteins.

7.6 Identifying downstream targets of Smad4 via microarray analysis

Many genes are activated in response to TGFβ signalling, whereas others are transcriptionally repressed. This is consistent with our microaray data in which we had more genes downregulated rather than up regulated ones after Smad4 deletion.

However, what defines transcriptional activation versus repression by Smads is not clear (Derynck and Zhang et al., 2003). Whether Smads repress or activate transcription also

depends on the cell type and the promoter sequence. For example, Smad3 cooperates with Runx proteins to activate transcription in epithelial cells, and represses transcription from the same promoter in mesenchymal cells (Derynck and Zhang et al., 2003).
Thus, distinct mechanisms, depending on the interacting transcription factor, the promoter and the intracellular context lead to Smad-mediated repression and determine whether Smads activate or repress transcription.

TGFβ regulated-Smads transactivate various target genes, including plasminogen activator inhibitor-1 (PAI-1), type I collagen, junB, Smad7 and Mix.2. In contrast, only a few direct target genes for BMPs have been identified, including Id (inhibitor of differentiation or inhibitor of DNA-binding) 1 through 3, Smad6, Vent-2, and Tlx-2. Id proteins act as negative regulators of cell differentiation and positive regulators of cell proliferation (Norton et al. 1998; Yokota & Mori, 2002). Four Id proteins, Id1 through Id4, have been identified in mammals. They have overlapping profiles of expression, and show similar, but not identical biological activities. BMPs may display some of their biological activities through Id proteins (Nakashima et al. 2001; Goumans et al. 2002). Id proteins may work as downstreams of BMP signalling in its neurogenic function in the adult SEZ as transcripts of Ids are present there (see results). However, there was no significant difference between Smad4 -/- and control in Id1-4 expression levels in microarray analysis. This again raises the question whether BMPs induce Id expression via MAPK pathway and therefore Smad4 deletion does not change the the levels of Id proteins. Instead, Cdk4, Pou6f1, Sox17, Sox11 were amongst the genes highly significantly altered in their expression upon Smad4 deletion. The ability of BMPs to regulate Sox gene expression has been investigated in chondrogenesis (Lyon, 2004). Sox9 is the earliest known marker for cells committed to chondrogenesis. BMPs promote the expression of Sox9 and induce chondrogenesis in vitro (Zehentner et al., 1999; Chimal-Monroy et al., 2003; Fernandez-Lloris et al., 2003) and in vivo (Lyon, 2004). However, whether BMPs directly regulate Sox9 is not clear. In our analysis Sox17 is up-regulated whereas Sox11 is down-regulated in Smad4-/- versus control. Loss and gain of function experiments showed that Sox17 stimulates oligodendrocyte precursor cell cycle exit and promotes myelin expression and differentiation (Sohn et al., 2006). Smad4 deletion leads to an increase in Sox17 levels indicating that oligodendrocyte precursors may exit the cell cycle much faster and maturate earlier in the absence of Smad4. This is consistent with the decrease in

proliferation of TAPs which had changed their identity towards oligodendrocyte precursors after Smad4 deletion. Therefore this regulation likely takes place in progenitors but not in stem cells. It has been shown that Sox11 function downstream from proneural basic helix loop helix (bHLH) protein as critical activator of both generic and subtypespecific neuronal properties in the developing spinal cord. Proneural bHLH transcription factors are essential for the progression of neurogenesis and can induce cell cycle exit and commit progenitors to a neurogenic program (Farah et al. 2000; Bertrand et al. 2002; Kintner 2002; Lo et al. 2002). Elimination of Sox11 activity does not disrupt the ability of proneural bHLH proteins to promote cell cycle exit, but blocks their capacity to establish the expression of neuronal properties indicating that the induction of this gene is a critical step in the acquisition of a neuronal phenotype (Bergsland et al., 2006). Down regulation of Sox11 after Smad4 deletion raises the possibility that it may have similar function in adult SEZ consistent with the decreased neurogenesis upon Smad4 deletion. It is also likely that this gene exerts its function prior to progenitor stage as deletion of Smad4 in progenitors does not alter the neurogenesis but does so in stem cells.

For inhibition of cell growth by TGFβ R-Smads induce the transcription of cyclin-dependent kinase (Cdk) inhibitors p21 and p15 (Miyazawa et al., 2002). In contrast to TGFβ, BMP has been shown to both stimulate and inhibit the growth of cells, depending on cell type and culture conditions. In microarray analysis Cdk4 was down-regulated in Smad4-/- compared to control indicating a positive role of BMP on proliferation in the adult SEZ. Indeed proliferation of TAPs decreased 21 days after Smad4 deletion (**Figure 26A**). As Sox17 is up-regulated upon Smad4 deletion and its function also leads to less proliferation and premature differentiation of oligodendrocyte precursors, the proliferation defect in the oligodendrogenic TAPs is likely a secondary effect following cell fate change 21 days after Smad4 deletion.

Beside these genes which have been functionally implicated in neurogenesis or proliferation, there are some genes which have not yet been studied functionally. For example, the class VI POU domain family member known as Emb in the mouse (rat Brn5 or human mPOU/TCFbeta1) is down regulated upon Smad4 deletion. This gene has been shown to be expressed in embryonic and adult neurons 24-48 hours after cell cycle exit (Cui and Bulleit, 1998). However, its function and its transcriptional regulation in progenitor stage are not

known. Our microarray analysis shows that neuronal progenitors already express POU6f1 gene at least in mRNA level and this is down-regulated upon Smad4 deletion. This can be due to a reduction in the number of neuroblasts in Smad4-/- SEZ at the time when microarray analysis has been performed (microarray analysis was performed 10 days after the end of tamoxifen application). Therefore it is difficult to predict whether the change in POU6f1 expression level is a consequence of decreased neuroblast number or a cause of it.

Taken together, microarray analysis gave us hints to study the mechanisms by which Smad4-mediated BMP signalling regulate the cell fate in favor of neurogenesis with the key candidates Sox11, Sox17 and Pou6f1.

7.7 Neurogenesis results from inhibition of oligodendrogenesis in the adult brain

Interestingly, however, interference with Smad4 did not cause a failure to up-regulate the key neurogenic factors, such as Pax6 (Hack et al., 2005) and Dlx2 (Doetsch et al., 2002; Petryniak et al., 2007) but rather lead to the ectopic up-regulation of Olig2. Thus, other signals must be crucial for up-regulation of these neurogenic transcription factors, while Smad4-mediated transcription is crucial to suppress Olig2 and thereby restrict the oligodendroglial lineage. Strikingly, suppression of Olig2 function by the Olig2VP16 construct was sufficient to rescue the defects in neurogenesis observed after Smad4 deletion, consistent with the remnant neurogenic potential in these cells in the absence of Olig2 up-regulation. These data henceforth imply the regulation of the transcription factor Olig2 as a key determinant for the choice between a neuronal or oligodendroglial lineage in the adult neural stem cell-derived progeny, reminiscent of its role in development (Doetsch et al., 2002; Ligon et al., 2006; Petryniak et al., 2007) Moreover, these data also suggest that TAPs and neuroblasts are not yet irreversibly committed to their fate, as their fate can not only be altered by overexpression of Olig2 (Hack et al., 2005) but also by transplantation of PSA-NCAM+ neuroblasts outside the neurogenic niche (Seidenfaden et al., 2006). These data therefore underline the importance of a signalling mechanism maintaining TAPs and neuroblasts within the neurogenic lineage and suppressing Olig2.

Our data imply Smad4-mediated BMP signalling as a key factor for this role. BMP ligands and BMP-specific p-Smads are expressed in the SEZ, but not in the RMS. Moreover, BMP-specific p-Smads are detectable only in stem cells and TAPs, but not in neuroblasts. Finally, blocking of BMP-signalling via Noggin also leads to the up-regulation of Olig2, while interference with the TGFβ-pathway did not, demonstrating the key role of BMP-mediated signalling in the suppression of Olig2. The observation that this pathway is present and required at early stages in the stem cell derived lineage, is consistent with the need of Olig2 suppression at these early stages in the lineage (Hack et al., 2005). Our data therefore imply BMP-mediated signalling as one of the earliest factors in the adult neural stem cell niche to direct the immediate progeny of stem cells towards a neurogenic fate by blocking progression towards the oligodendroglial lineage. Notably, outside the neurogenic niche virtually all progenitors are oligodendrocyte progenitors in the adult mammalian brain (Horner et al., 2002, Dawson et al., 2003) and suppression of Olig2 function in these cells in the cortical parenchyma also leads to upregulation of Pax6 and neurogenesis (Buffo et al., 2005). One may therefore speculate that a key aspect of the adult neurogenic niche is to suppress oligodendrogliogenesis, the apparent default pathway in the adult mammalian brain.

7.8 Future Aspects

As here I showed that the role of endogenous BMP signalling in the adult SEZ is to suppress oligodendrogenesis, manipulation with this pathway may be useful for the diseases related to oligodendrocyte damage. For example, multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease that affects the CNS oligodendrocytes. MS results in a thinning or complete loss of myelin generated by oligodendorcytes. Therefore it is important to replace destroyed oligodendrocytes by new ones and thereby induce the generation of myelin. However, in mouse models, demyelination is not due to lack of repair. For example, lysolecithin injection or cuprizone poisoning produces a local demyelinating lesion that partially repairs over a period of weeks (Woodruff and Franklin, 1999). After such lesion with lysolecithin in corpus callosum, progenitor cells from the adult SEZ contribute to oligodendrogliogenesis in the corpus callosum (Nait-Oumesmar et al., 1999). First SEZ progenitors respond to demyelination with an increase in proliferation. Afterwards the

progenitor cells of SEZ are recruited by lesion where they generate astrocytes and oligodendrocytes (Nait-Oumesmar et al., 1999). However only a small proportion of recruited SEZ cells differentiates into mature oligodendrocytes within the lesion as most of them differentiate into astrocytes. Therefore one important point is to increase the number of mature oligodendrocytes generated by these progenitors. Interestingly, preliminary findings suggest that this type of recruitment also occurs in human multiple sclerosis (Nait-Oumesmar et al., 2008).

As there is a low degree of endogenous oligodendrocyte production in the adult SEZ (Menn et al., 2006; current work), increase in oligodendrogenesis upon demyelination indicates that there should be a mechanism mediating fine-tuning of progenitors specified towards neurogenesis or oligodendrogliogenesis. Here, I could show that BMP signalling is crucial for this fate choice. Whether the response to demyelination will increase after Smad4 deletion is an intriguing question. Therefore we plan to do demyelination injuries after Smad4 deletion and monitor the remyelination. After Smad4 deletion I could show that SEZ progenitors generated more mature oligodendrocytes in corpus callosum, however, the extend of their final myelination capacity is still not clear. Further myelination capacity should be studied by using electron microscopy. Additionally to the number of oligodendrocytes generated after demyelination in Smad4-/- mice, the capacity of myelination with or without demyelination should be also studied. While remyelination can be quite robust, in multiple sclerosis it often fails. Understanding and stimulating the remyelination process are therefore important goals in MS research.

8 FIGURES

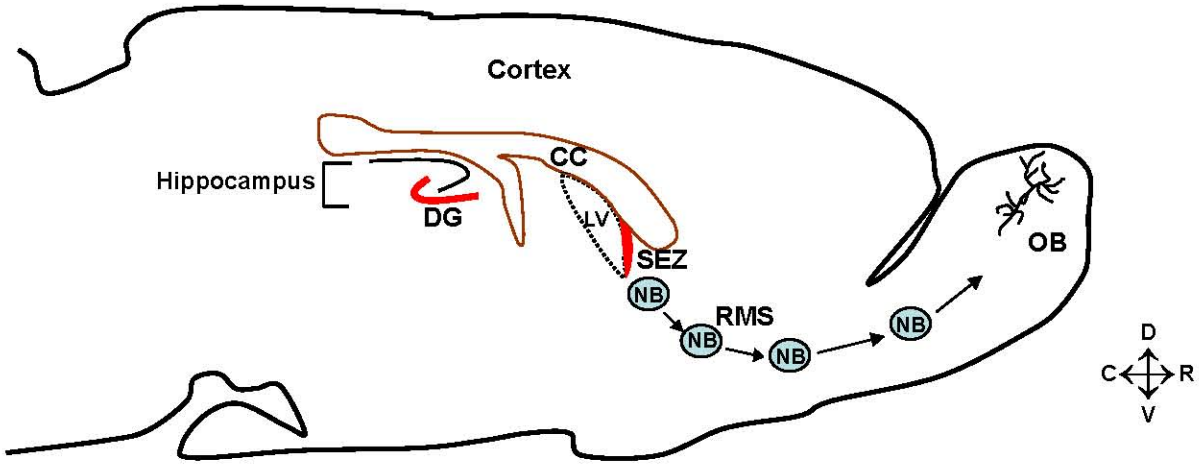


Figure 1. Location of adult neurogenesis in mammalian brain

Scheme of a sagittal section of an adult mouse brain, indicating the two locations of adult neurogenic niches: 1) DG 2) SEZ (in red). Neuroblasts (blue) from SEZ migrate to OB via RMS and generate mature interneurons. SEZ: Subependymal Zone, RMS: Rostral Migratory Stream, LV: Lateral Ventricle, CC: Corpus Callosum, OB: Olfactory Bulb, NB: Neuroblast, DG: Dentate Gyrus. D: Dorsal, V: Ventral, R: Rostral, C: Caudal.

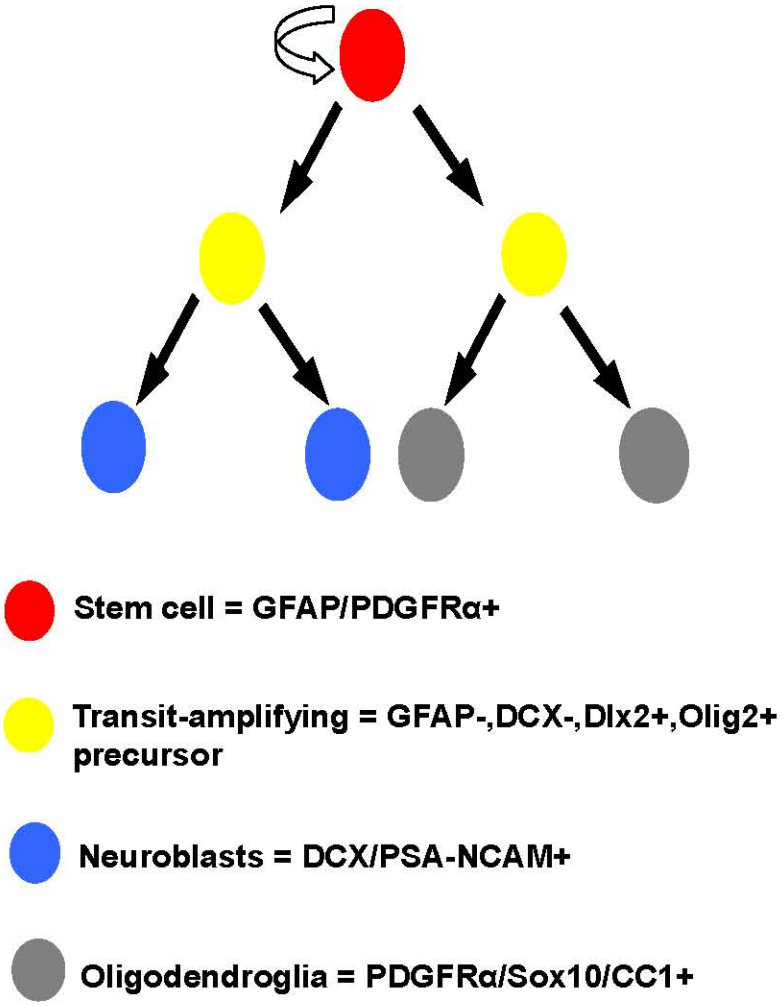
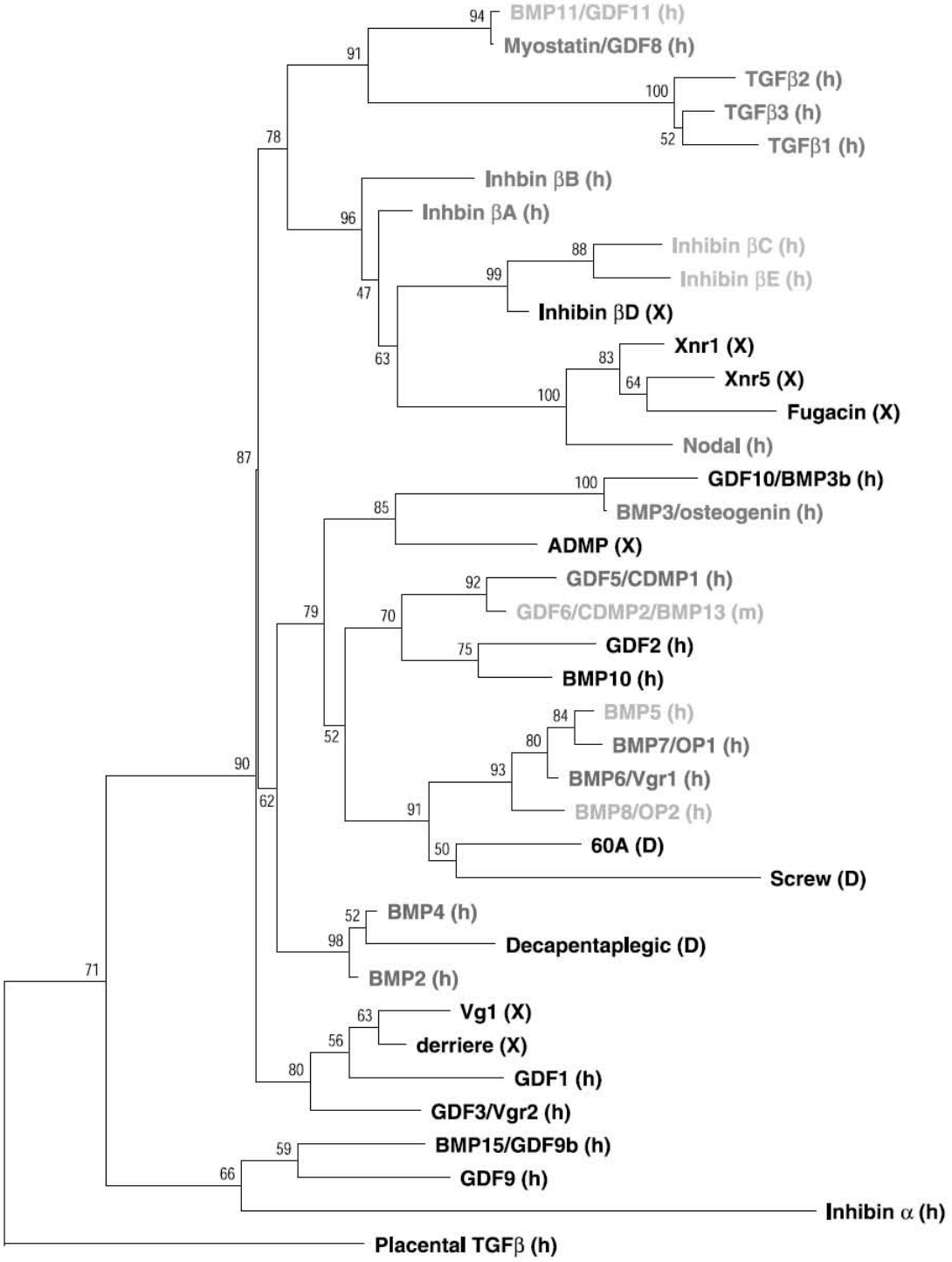


Figure 2. Schematic lineage diagram in adult subependymal zone

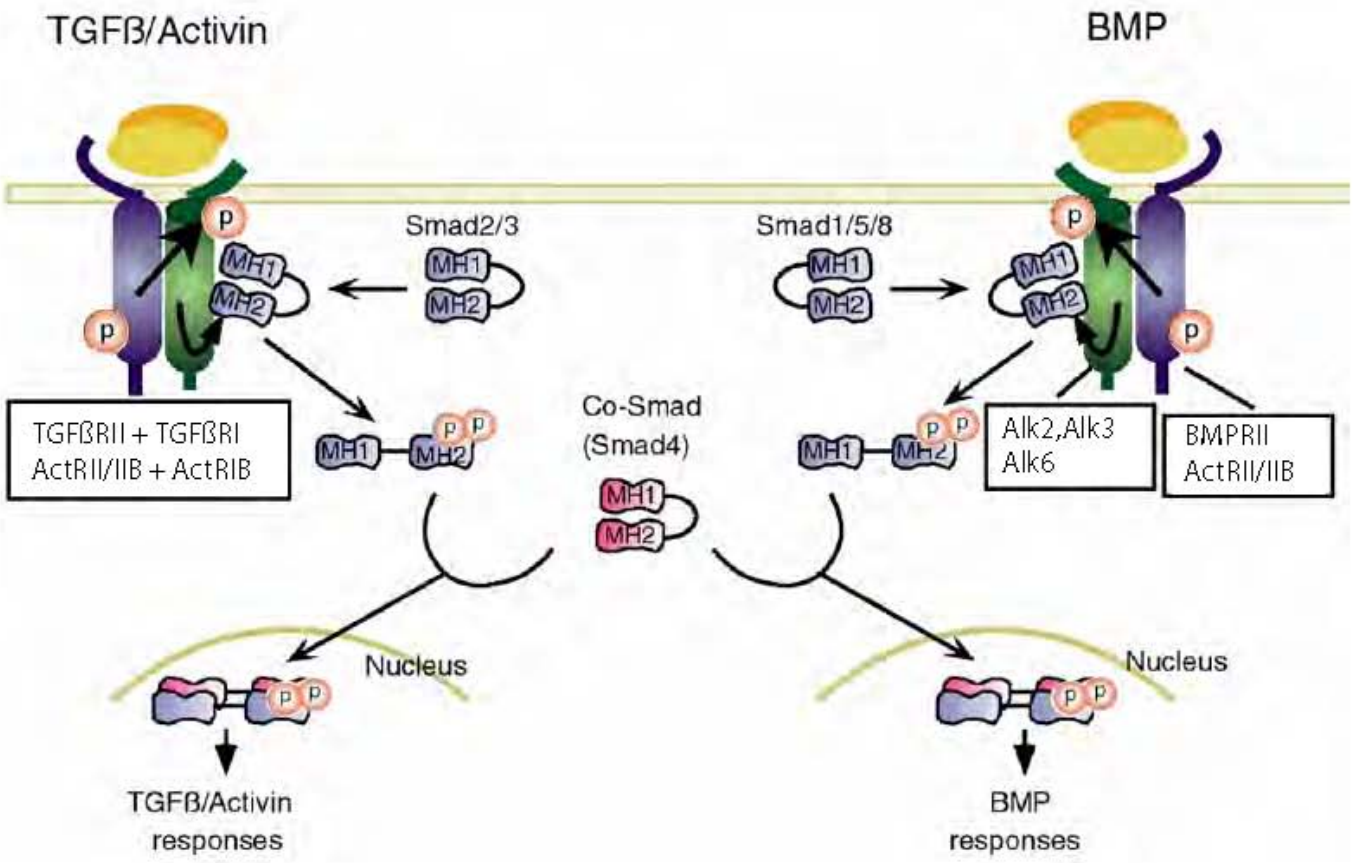
The progenitor population in adult subependymal zone (SEZ) consist of stem cells (red), transit-amplifying precursors (yellow), neural precursors (blue,neuroblasts) and oligodendroglia precursors (grey). Stem cells give rise to fast proliferating precursors which then generate either neuroblasts or oligodendrocyte precursors.



Miyazawa et al., 2002

Figure 3. A phylogenetic tree of the TGFβ superfamily in human (h), mouse (m), Xenopus (X), Drosophila (D)

Ligands that activate BMP receptor Smads are shown in dark blue. Ligands whose receptors and downstream signalling pathways have not yet been fully determined are shown in orange and light blue, respectively. Note that BMP5/6/7/8 and BMP2/4 have high homology.



Modified from Wrana, 2000

Figure 4. Two major Smad pathways of TGFβ superfamily.

TGFβ binds to its receptors (TGFβRII and I) while activins interact with the type II receptors ActRII, ActRIIB, and the type I receptor ActRIB. In contrast, BMPs act through the type II receptors ActRII,ActRIIB, or BMPRII and the the type I receptors Alk2,3 and 6; these receptors propagate the signal to Smads 1,5, and 8. the activated type I receptors phosphorylate a receptor-regulated Smad (R-Smad; blue) at a conserved SSXS motif present at the COOH-terminus of the protein. In the case of TGFβ/activin, Smad2 and Smad3 are phosphorylated, whereas for BMPs, Smads 1,5 and 8 are targeted. This phosphorylation causes dissociation of the R-Smad from the receptor complex and induces formation of a heteromeric Smad complex with the co-Smad Smad4 (red). This complex then translocates into nucleus and regulates transcription.

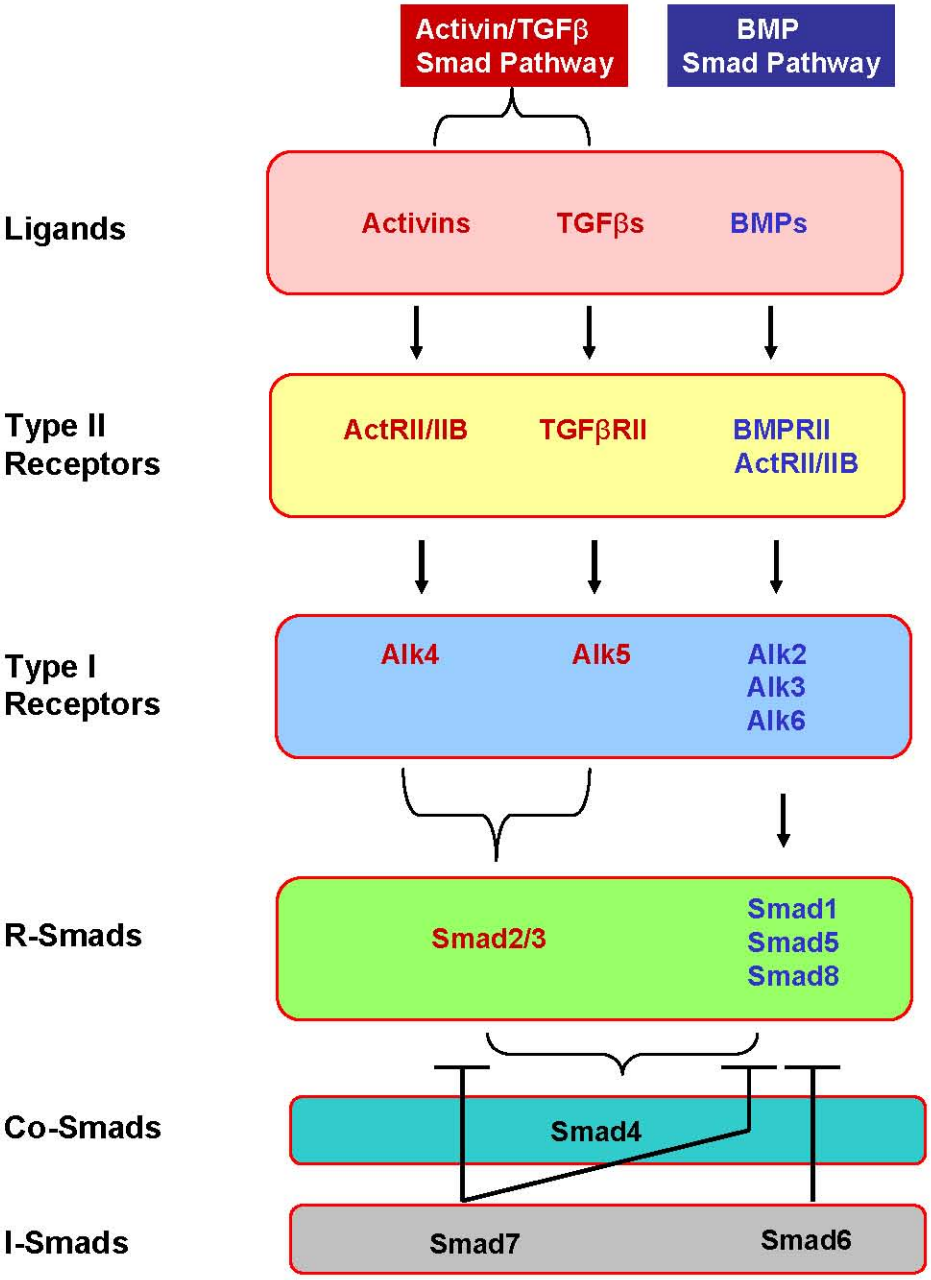


Figure 5. Simplified Scheme of Smad pathway components.

Representative examples of mammalian ligands (pink shading), type II receptors (yellow shading), type I receptors (blue shading), R-Smads (green shading), Co-Smads (turquoise shading) and I-Smads (grey shading) are depicted in pathways linked by arrows or signs of inhibition. Nomenclature of proteins are activin type II and type IIB receptor (ActRII/IIB), TGFβ type IIreceptor (TGFβRII), BMP type II receptor (BMPRII), activin receptor-like kinases 2 to 6 (ALK2-ALK6), R-Smads (Receptor regulated Smads), CoSmads (Common Smad) and I-Smads (Inhibitor Smads).

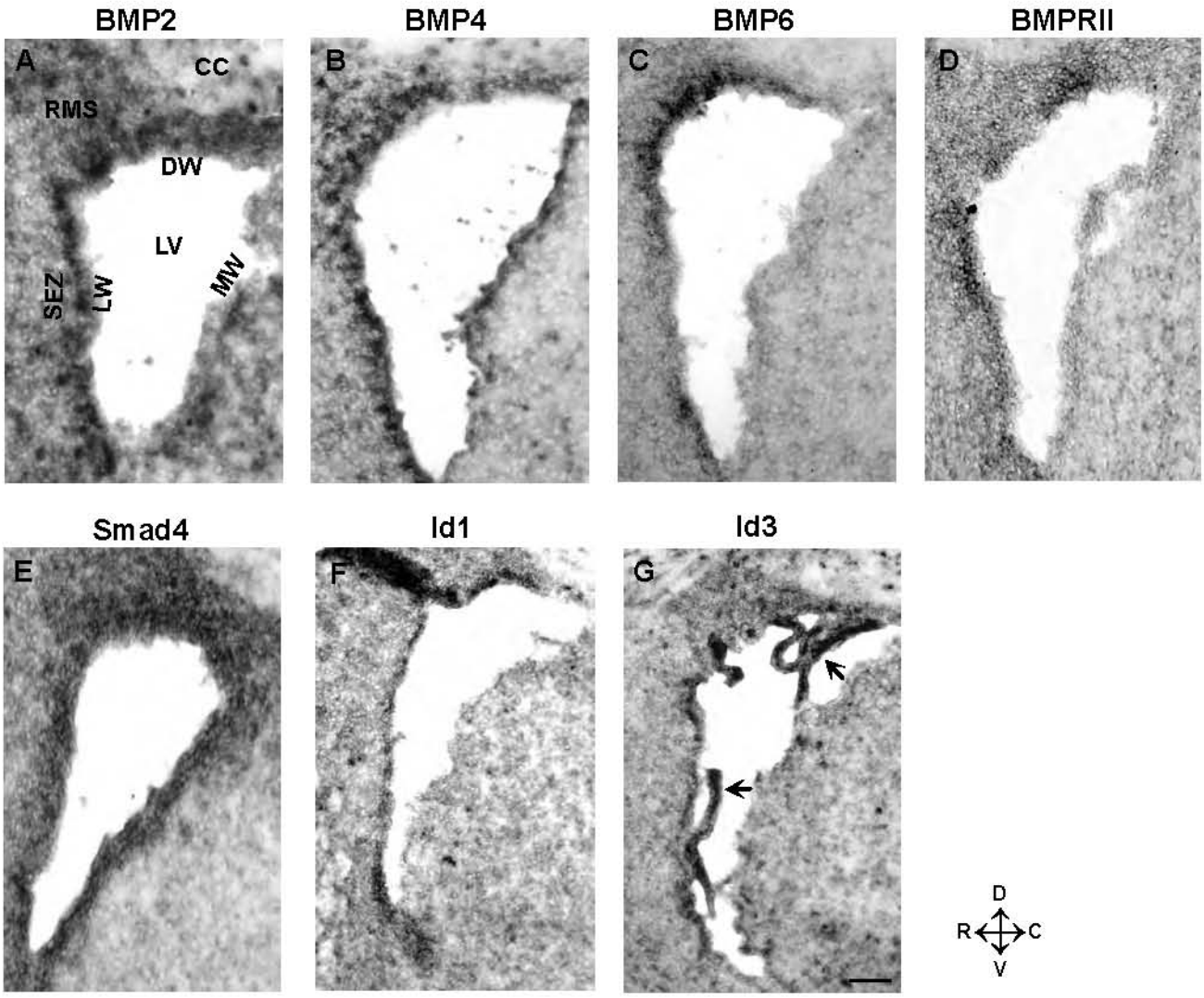


Figure 6. Expression of BMP signalling components in the postnatal subependymal zone (SEZ)

(A-G) In situ hybridization of mRNA transcripts of BMP ligands (A-C), BMP receptor II (D), the central signalling mediator Smad4 (E), and the downstream targets Id1(F) and Id3 (G; Arrows indicate choroid plexus which also expresses BMP downstream targets).
SEZ: Subependymal Zone, RMS: Rostral Migratory Stream, LV: Lateral Ventricle, CC: Corpus Callosum, LW: Lateral Wall, MW: Medial Wall, DW: Dorsal Wall. The orientation is indicated under D depicting Dorsal, V depicting Ventral, R depicting Rostral, C depicting Caudal. Scale bar: 100 µm.

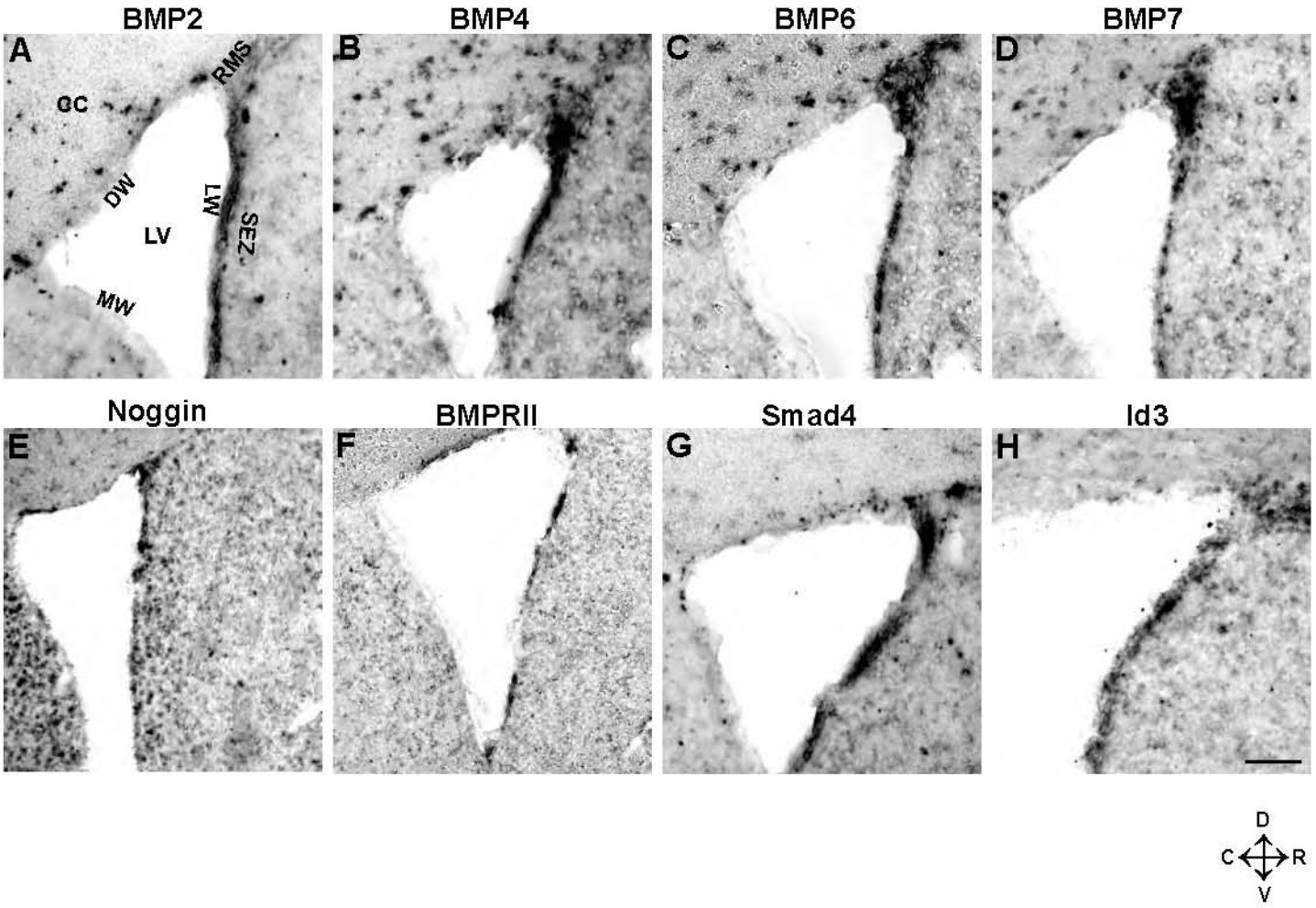


Figure 7. Expression of BMP signalling components in the adult subependymal zone (SEZ)

(A-H) In situ hybridization of mRNA transcripts of BMP ligands (A-D), the extracellular inhibitor noggin (E), BMP receptor II (F), the central signalling mediator Smad4 (G), and the downstream target Id3 (H).

SEZ: Subependymal Zone, RMS: Rostral Migratory Stream, LV: Lateral Ventricle, CC: Corpus Callosum, LW: Lateral Wall, MW: Medial Wall, DW: Dorsal Wall. The orientation is indicated under D depicting Dorsal, V depicting Ventral, R depicting Rostral, C depicting Caudal. Scale bar: 100 µm.

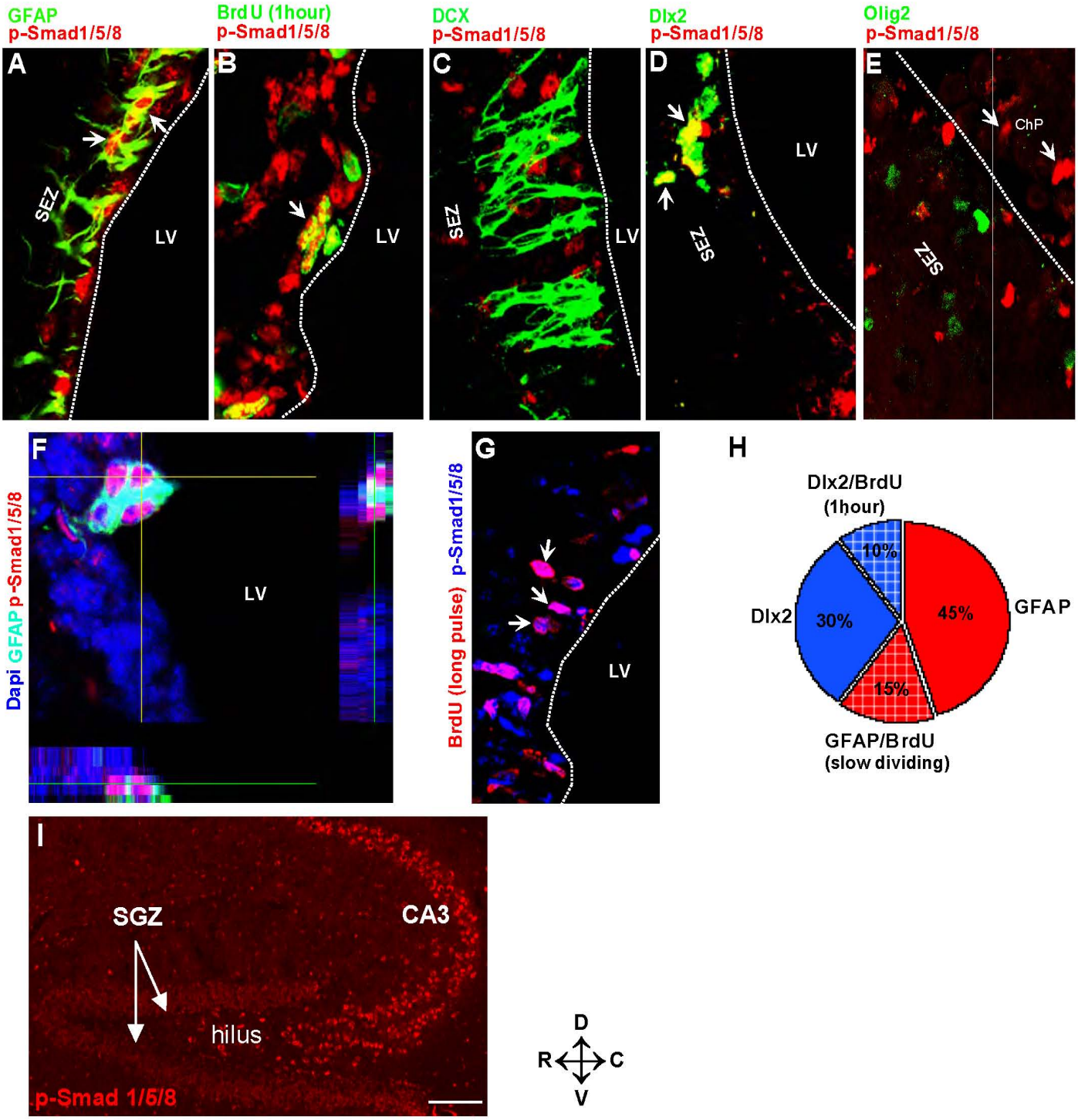


Figure 8. Functional activity of BMP signalling components in the adult SEZ

(A-G) Fluorescent micrographs of double-immunostainings for the BMP ligand specific phosphorylated Smads 1/5/8 (p-Smad1/5/8) with different cell type-specific antigens in the adult SEZ as indicated in the panels. Arrows in panels indicate double labelled cells. Note that p-Smad1/5/8 is contained largely in GFAP-positive (A,F) and some fast proliferating BrdU+ cells (B), but not in doublecortin (DCX)-positive neuroblasts (C). p-Smad1/5/8 also co-localizes with the transcription factor Dlx2 (D), but not Olig2 (E, note that there are p-Smad-1/5/8-positive cells also in the Choroid Plexus). (G) Fluorescent micrograph depicting the co-localization of p-Smad1/5/8 with BrdU in BrdU retaining cells of the SEZ. The quantitative composition of the p-Smad1/5/8 immunopositive cell pool is depicted in the pie diagram in (H). Note that p-Smad1/5/8 is not present in dentate gyrus of hippocampus but expressed by CA3 neurons.

SEZ: Subependymal Zone, LV: Lateral Ventricle, ChP: Choroid Plexus, SGZ: Subgranular Zone
The orientation of the sections is indicated under D depicting Dorsal, V depicting Ventral, R depicting Rostral, C depicting Caudal. Scale bars: A-E and G, 40 µm ; F, 30µm ; I, 200µm as indicated in panel I.

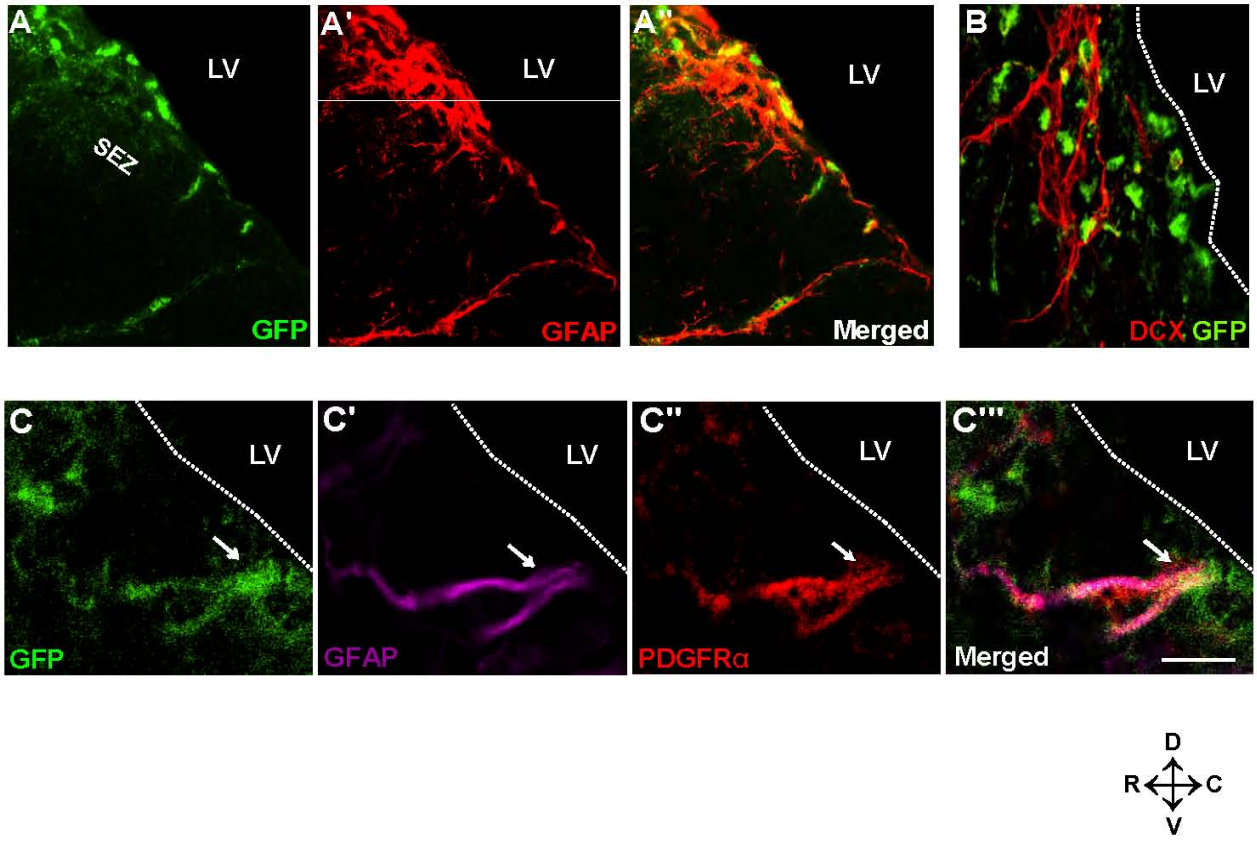


Figure 9. BMP activity indicated by a reporter line in the adult SEZ

(A-C‴) Fluorescent micrographs depicting BMP signalling activity in the SEZ of BRE-eGFP mice by GFP- combined with GFAP- (A-A''), DCX- (B) PDGFRα- and GFAP- (C-C‴ (arrow indicates 1 cell)) immunostaining. Note that GFP co-localizes with GFAP and also PDGFRα but not with DCX in the SEZ consistent with the co-localization of p-Smad1/5/8 and GFAP.

SEZ: Subependymal Zone, LV: Lateral Ventricle, D: Dorsal, V: Ventral, R: Rostral, C: Caudal. Scale bar: A-B, 60µm; C-C‴, 30µm (indicated in panel C‴).

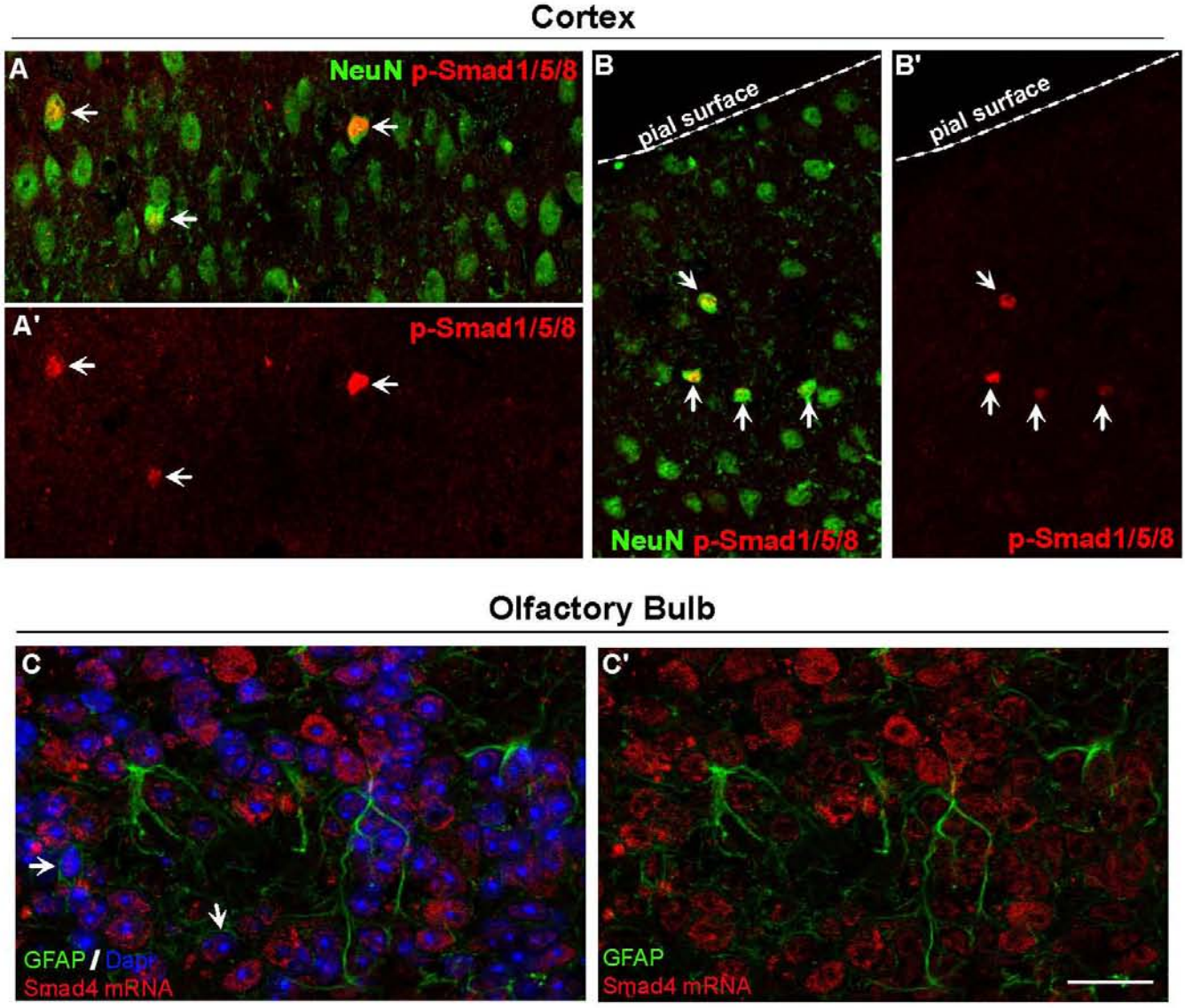


Figure 10. BMP signalling is restricted to postmitotic cells outside neurogenic niche

(A-B') Fluorescent micrographs depicting p-Smad1/5/8 and NeuN-double positive cells in the adult cortex. The arrows indicate double positive cells. All p-Smad1/5/8-expressing cells are post mitotic neurons in cortex. (C-C') Fluorescent images of Smad4 mRNA (obtained by fluorescent in situ hybridization) and GFAP-double labelling in olfactory bulb of adult mouse. Arrows indicate GFAP-positive/Smad4-negative cells. A-B',75µm; C,C',60µm.

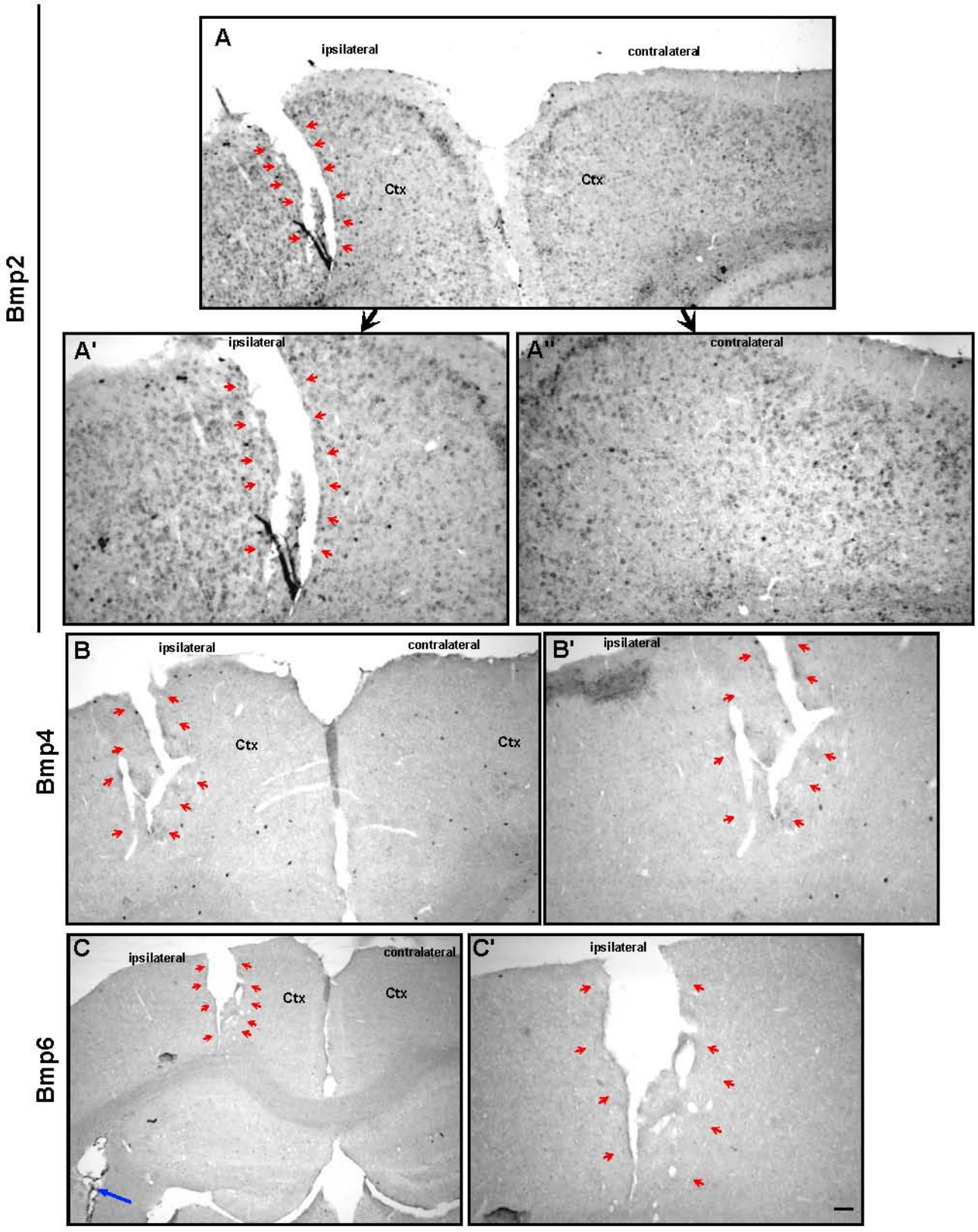


Figure 11. In situ hybridization of BMP ligands after Stab Wound in adult cortex

In situ hybridization for BMP2 (A-A''), BMP4 (B-B') and BMP6 (C-C') 3 days post injury showed no obvious difference in the injured side versus control side. The blue arrow in panel C indicates the choroid plexus and SEZ as region known to express BMP6. A',B',C' represents in situ hybridization for BMP2, BMP4 and BMP6 in the lesioned site respectively. A' and A'' are higher magnification images of panel A representing ipsilateral and contralateral sites respectively. B' and C' are higer magnification images of panel B and C respectively. Red arrows indicate stab wound area. Scale bar : A,125µm; A',A'',60µm; B,125µm; B'100µm; C,150µm; C',100µm.

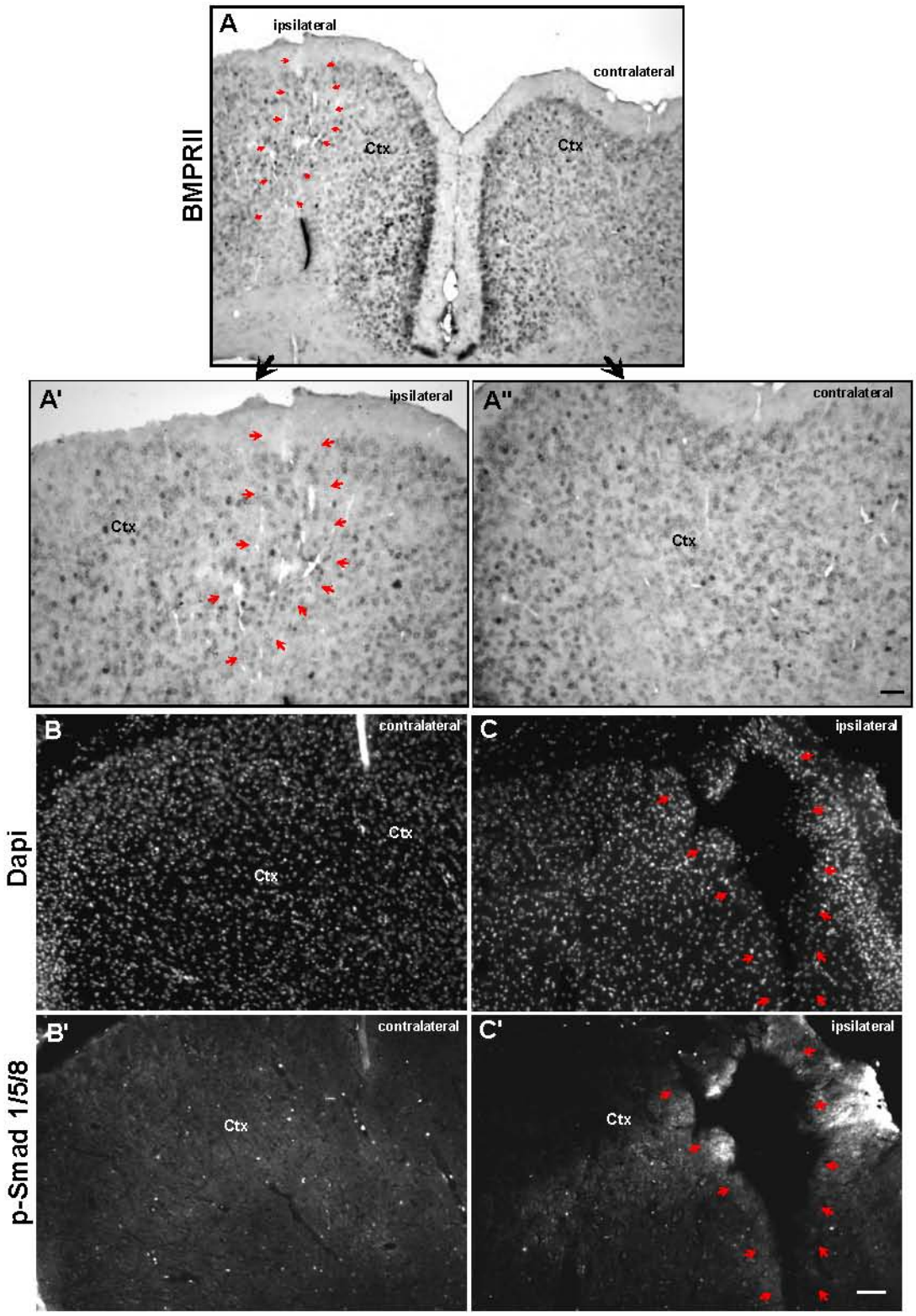


Figure 12. Cells responding to injury do not up-regulate BMP signalling avtivity

In situ hybridization performed for BMPRII (A-A'') 3 days post injury showed no obvious difference in t mRNA level of this receptor in the injured side versus control side (A' versus A''; A' and A'' are higher magnification images of panel A for ipsilateral and contralateral sites respectively). Red arrows indicate lesion area. (B-C') Histograms depicting Dapi staining (B,C) and p-Smad1/5/8 staining (B',C') in lesioned (B,B') and non-lesioned site (C,C'). Red arrows indicate lesion area. Note that p-Smad1/5/8 immunoreactivity does not change upon injury compared to control. Scale bar : A,100µm; A',A'',60µm (indicated in A''); B-C',100µm (indicated in C').

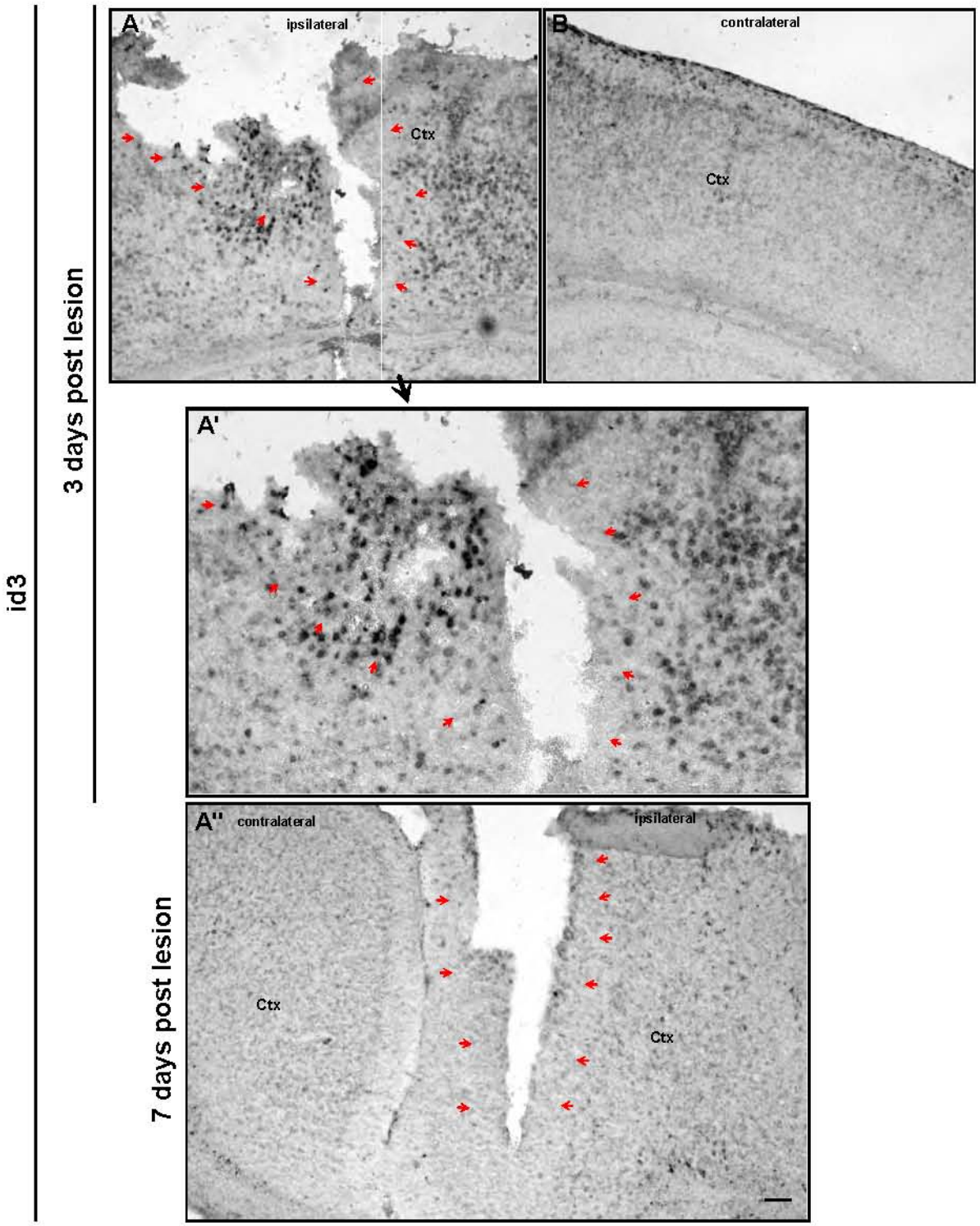


Figure 13. Id3 is transiently up-regulated upon injury in the adult cortex

In situ hybridization performed for Id3 showed more cells positive for this mRNA in lesioned site (A,A'; A' is high magnification of A) compared to control side (B) 3 days post injury. Note the increased number of cells expressing Id3 mRNA around lesion area (lesion is indicated with red arrows). Id3 expression goes back to its normal level 7 days post injury (A''). Red arrows indicate lesion area. Scale bar: A,B,A'',120µm; A',75µm.

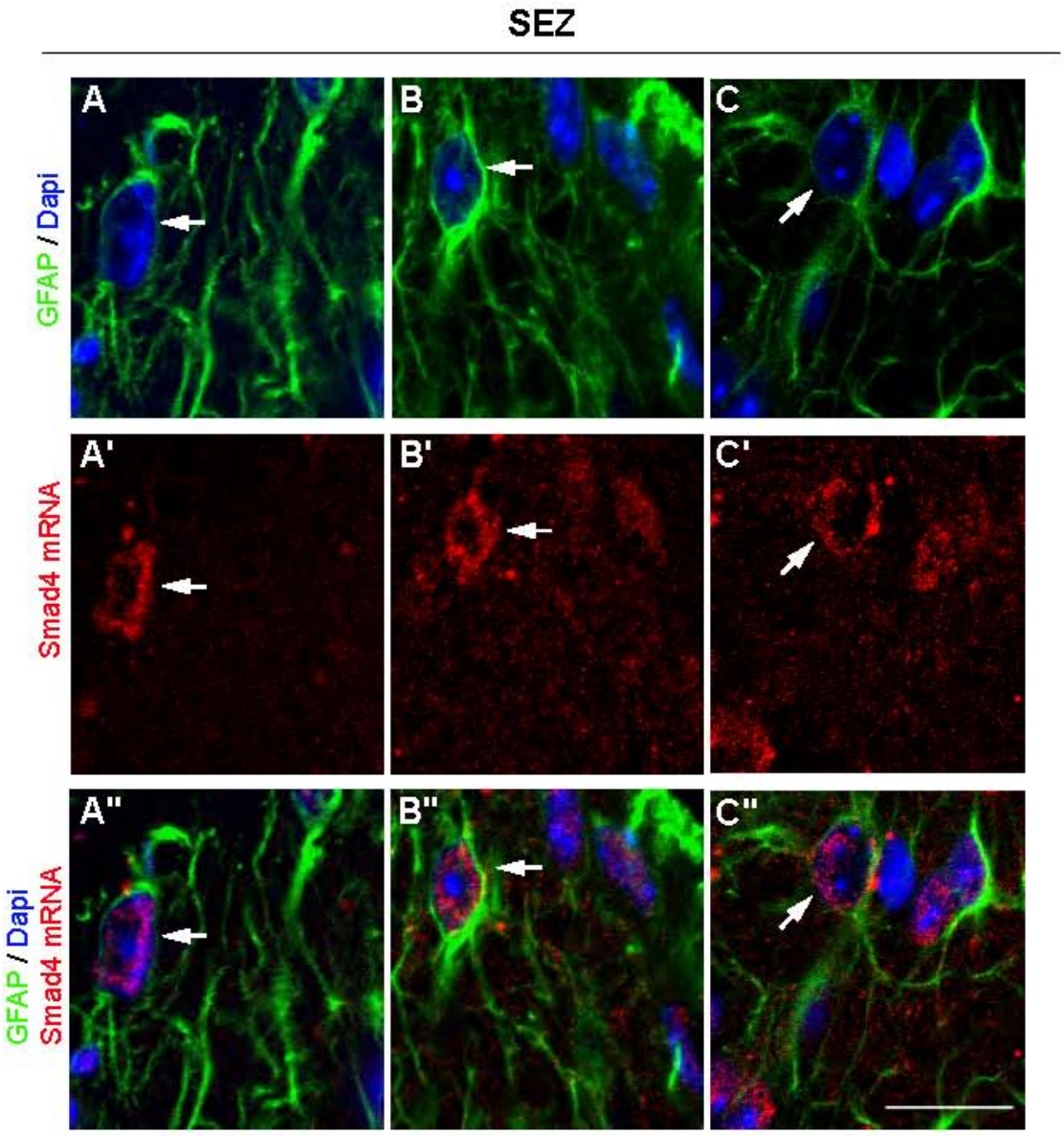


Figure 14. Smad4 is expressed by astrocytes in adult subependymal zone

(A-C") Fluorescent images showing Smad4 mRNA expression in astrocytes of the adult SEZ. mRNA is in red and GFAP protein is in green. Note the localization of Smad4 mRNA in GFAP-positive cells (arrows). Scale bar: 25µm

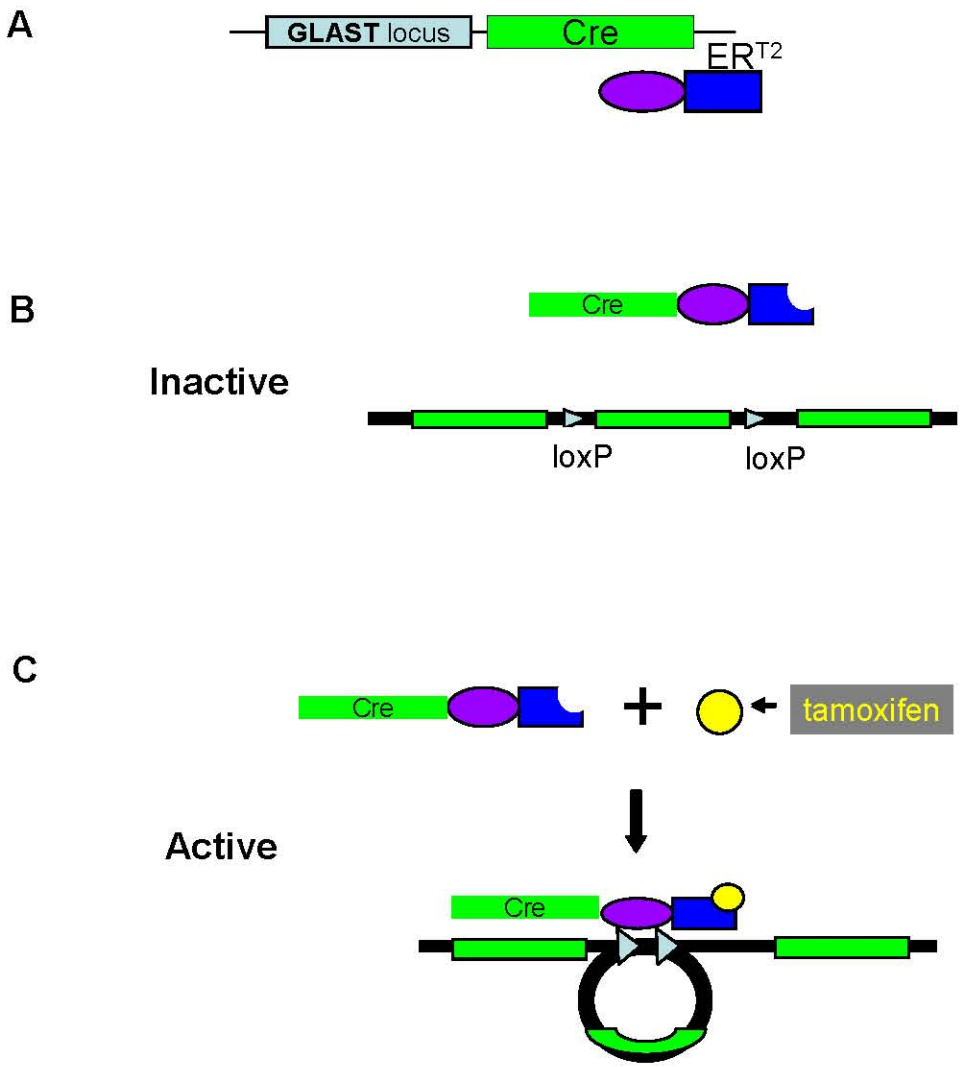


Figure 15. Scheme of tamoxifen-based inducible Cre/loxP system

(A) A drawing illustrating Cre protein fused with receptor binding domain of estrogen (ERT). This domain carries a mutation to allow binding of only estrogen analog tamoxifen but not estrogen. The CreERT2 is knocked in astrocyte specific GLAST locus. In the absence of tamoxifen CreERT2 stays in cytoplasm and can not achieve recombination (B) whereas in the presence of tamoxifen CreERT2 goes to nucleus and recombined the loxp sites (C).

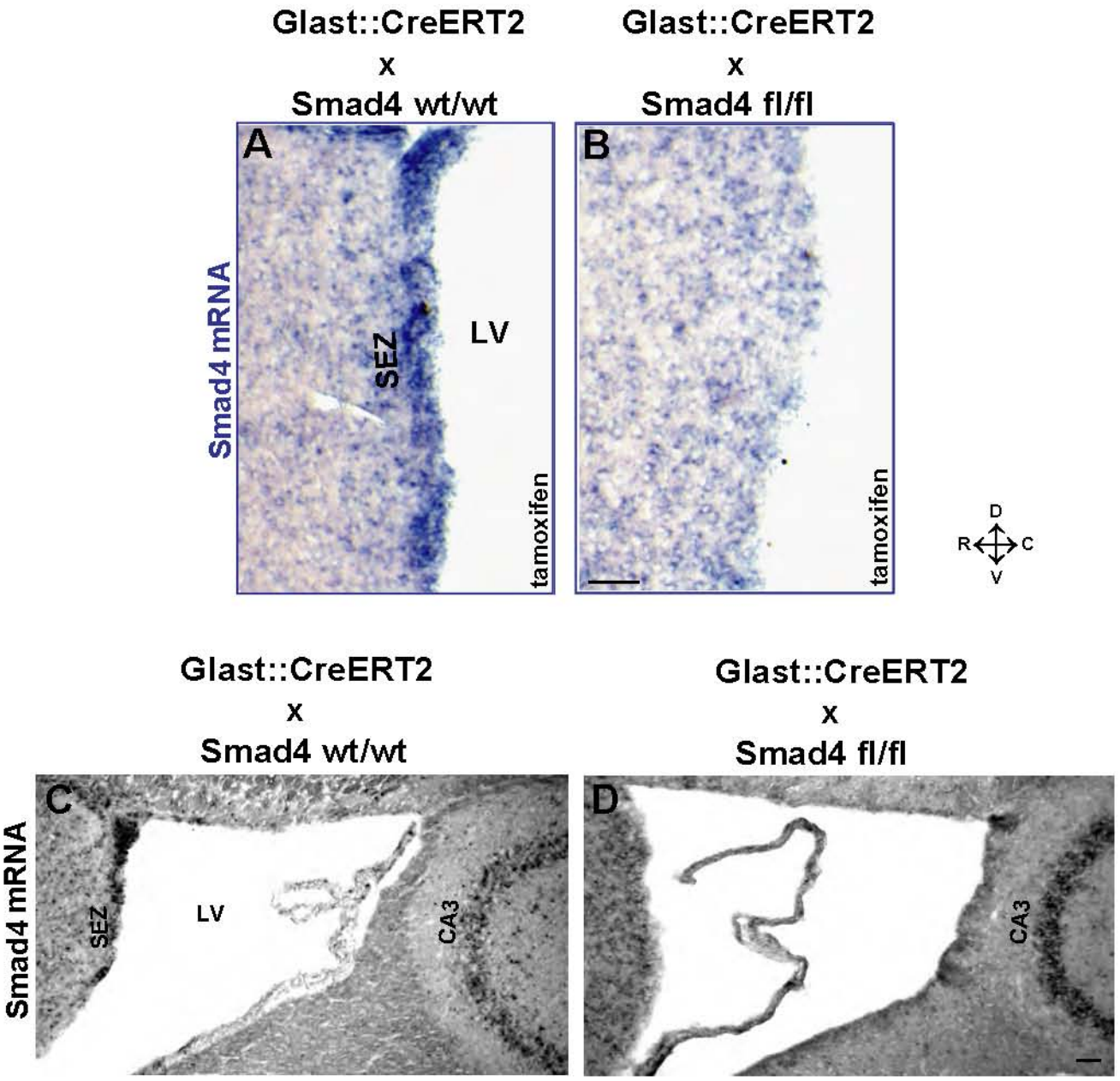


Figure 16. Inducible deletion of Smad4 in adult astrocytes and neural stem cells in the adult SEZ

(A-B) In situ hybridization of Smad4 mRNA in the adult SEZ 10 days after activation of CreERT2 expressed in the GLAST locus (GLAST::CreERT2) by tamoxifen in mice without (A) or with (B) exon 8 of Smad4 flanked by loxP sites. Note the loss of Smad4 mRNA in (B) versus (A). Note that Smad4 mRNA is specifically lost in astrocytes (B,D) whereas its expression persists in CA3 neurons of hippocampus after tamoxifen administration (D). SEZ: Subependymal Zone, LV: Lateral Ventricle, D: Dorsal, V: Ventral, R: Rostral, C: Caudal. Scale bar: A-B, 100µm (indicated in panel B), C-D, 100µm (indicated in panel D).

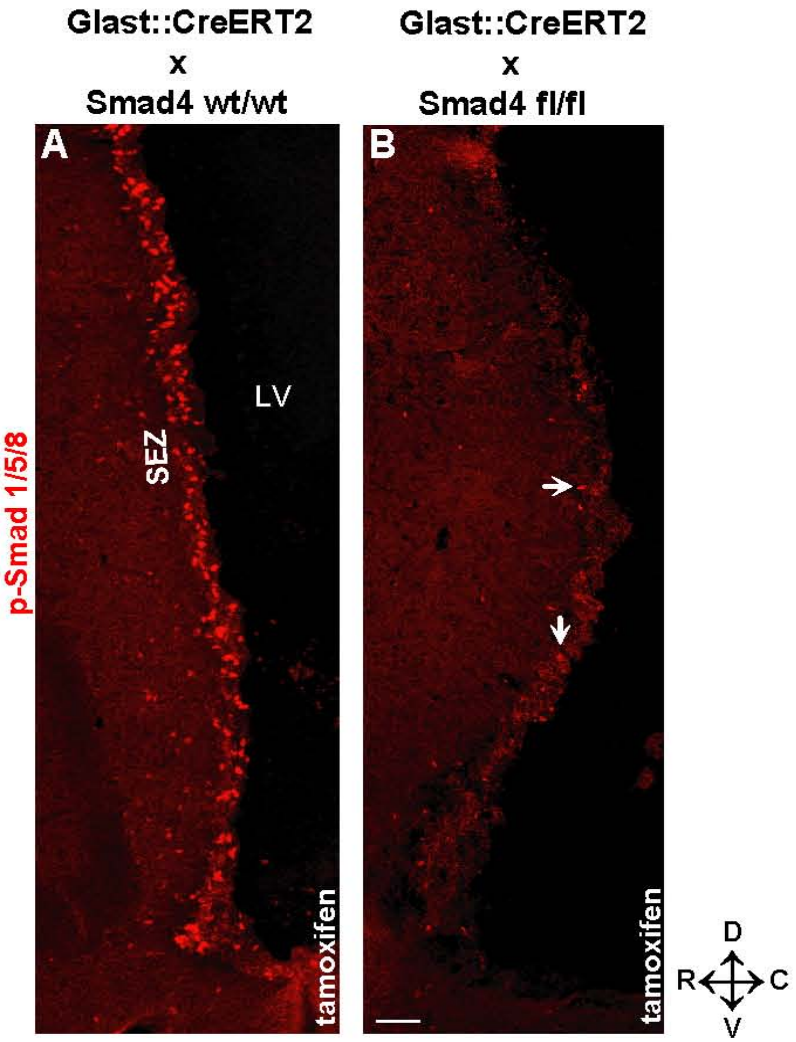


Figure 17. Loss of nuclear p-Smad1/5/8 in the adult SEZ 10 days after induction of Smad4 deletion

(A-B) Fluorescent micrographs of p-Smad1/5/8 immunostaining in GLAST::CreERT2 mice WT for the Smad4 locus (A) or homozygous for the floxed exon 8 of Smad4 (B) 10 days after induction of Cre-mediated recombination by tamoxifen. Note the widespread loss of nuclear localization of p-Smad1/5/8 after deletion of Smad4 (B). Arrows in panel B indicate some of the remaining p-Smad1/5/8+ cells also demonstrating the same quality of immunostaining. SEZ: Subependymal Zone, LV: Lateral Ventricle, D: Dorsal, V: Ventral, R: Rostral, C: Caudal. Scale bar: A-B, 100µm (indicated in panel B).

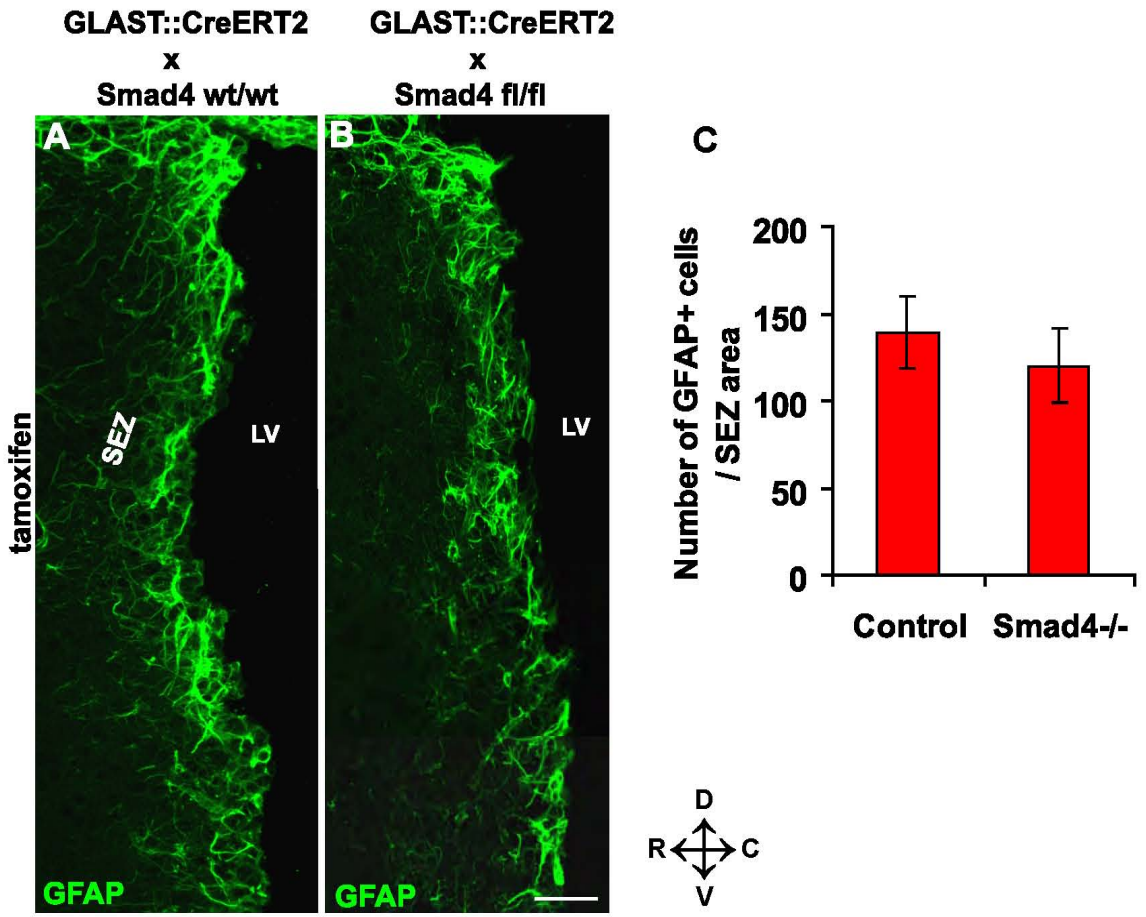


Figure 18. Number of GFAP-positive cells after deletion of Smad4 in adult SEZ

(A,B) Fluorescent micrographs depicting GFAP-immunostaining for astrocytes and stem cells in the adult SEZ 10 days after induction of Cre-mediated recombination.
(C) Histogram depicting the number of GFAP-immunopositive astrocytes and stem cells (n(cells)=2922 (Control); 2531 (Smad4-/-), 4 animals each). SEZ: Subependymal Zone, LV: Lateral Ventricle. D: Dorsal, V: Ventral, R: Rostral, C: Caudal. Scale bar : 75µm

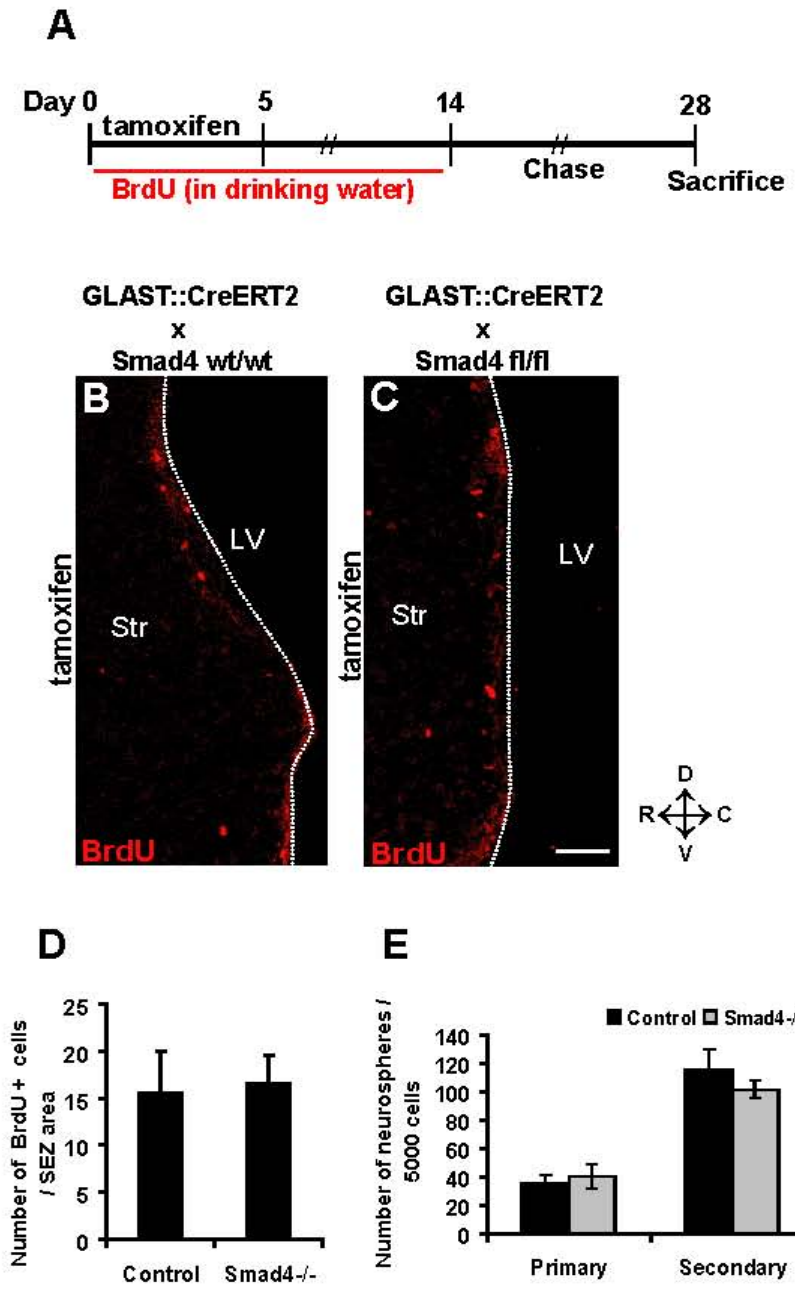


Figure 19. Deletion of Smad4 does not affect neural stem cell self renewal in vivo or in vitro.

(A) Scheme of BrdU-application for labelling of label-retaining neural stem cells in the adult SEZ.
(B,C) Fluorescent micrograph depicting label-retaining BrdU-positive cells in the adult SEZ of GLAST::CreERT2 mice without (A) or with (B) floxed exon 8 of Smad4.
(D) Histogram depicting the quantification of label-retaining neural stem cells (BrdU+/DCX-) in the adult SEZ of GLAST::CreERT2 mice without (n(cells)=116, 2 animals, black bar) or with floxed exon8 of Smad4 (n(cells)=118, 2 animals, grey bar). Note that the number of label-retaining cells is not affected 3 weeks after tamoxifen application.
(E) Histogram depicting the number of primary or secondary neurospheres formed from the adult SEZ of GLAST::CreERT2 without (n(experiment)=3, black bar) or with floxed exon 8 of Smad4 (n(experiment)=3, grey bar). No effect on neurosphere formation or self-renewal was observed 3 weeks after Smad4 deletion. LV:Lateral Ventricle, Str:Striatum, D:Dorsal, V:Ventral, R:Rostral, C:Caudal. Scale bar: B,C, 100µm.

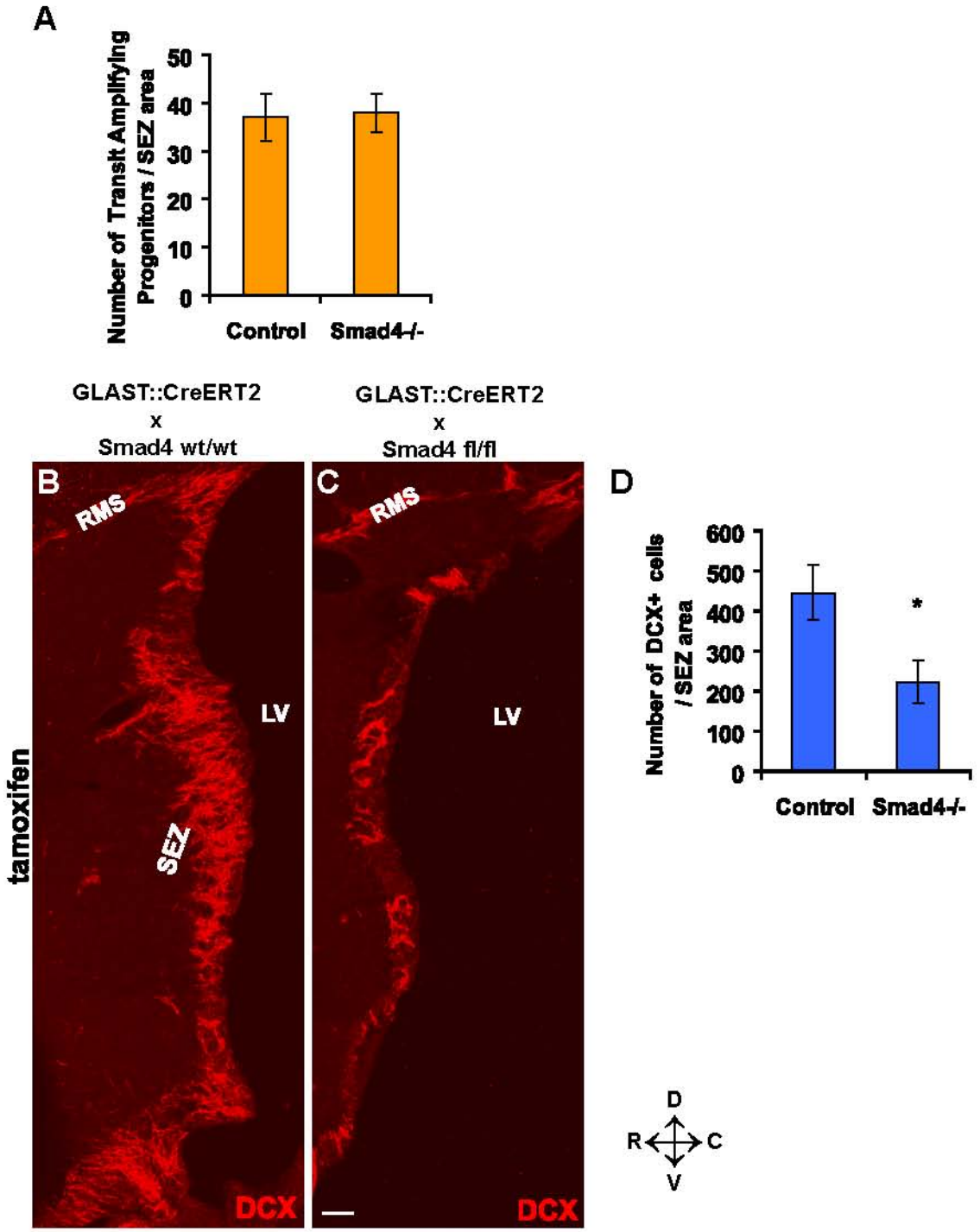


Figure 20. Number of TAPs and neuroblasts after deletion of Smad4 in adult SEZ

(A) Histogram depicting the number of transit-amplifying precursors (TAPs:BrdU+, DCX-negative) labelled with BrdU 1 hour prior to sacrifice (n(cells)= 240 (Control), 426 (Smad4-/-), 3 animals each). (B,C) Fluorescent micrographs depicting DCX-immunostaining for neuroblasts in the adult SEZ 10 days after induction of Cre-mediated recombination. (D) Histogram depicting the number of DCX+ neuroblasts (G; n = 11752 cells (Control), 5400 (Smad4-/-) 4 animals each) 10 days after tamoxifen application. Note the significant reduction of neuroblasts, but not TAPs 10 days after Smad4 deletion. $p < 0.05$ = * SEZ:Subependymal Zone, RMS: Rostral Migratory Stream, LV:Lateral Ventricle. D:Dorsal, V:Ventral, R:Rostral, C:Caudal. Scale bar: 100µm.

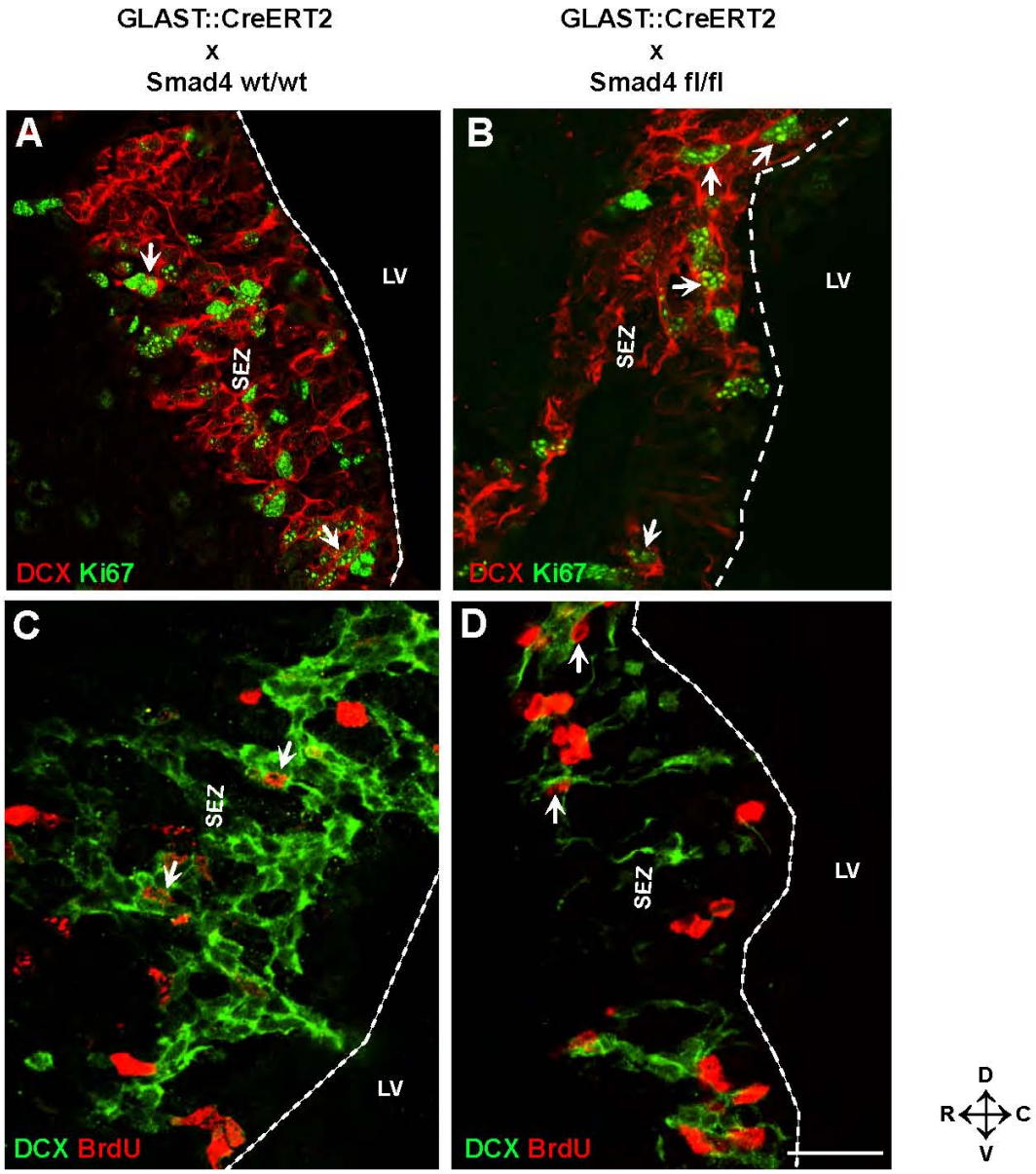


Figure 21. Proliferation of neuroblasts after Smad4 deletion

(A-B) Fluorescent micrographs of double staining for the neuroblast marker DCX and the proliferation marker Ki67 in GLAST::CreERT2 mice wt for the Smad4 locus (A) or homozygous for the floxed exon 8 of Smad4 (B) 10 days after induction of Cre-mediated recombination by tamoxifen. Arrows indicate proliferating cells in both panels. (C-D) Fluorescent micrographs of double staining for DCX and BrdU (short pulse) in GLAST::CreERT2 mice WT for the Smad4 locus (C) or homozygous for the floxed exon 8 of Smad4 (D) 10 days after induction of Cre-mediated recombination by tamoxifen. Note that proliferation of neuroblasts is not impaired after Smad4 deletion (A,C versus B,D respectively). SEZ: Subependymal Zone, LV: Lateral Ventricle, D: Dorsal, V: Ventral, R: Rostral, C: Caudal. Scale bar: A, 75µm, B,C,D, 50 µm (indicated in panel D).

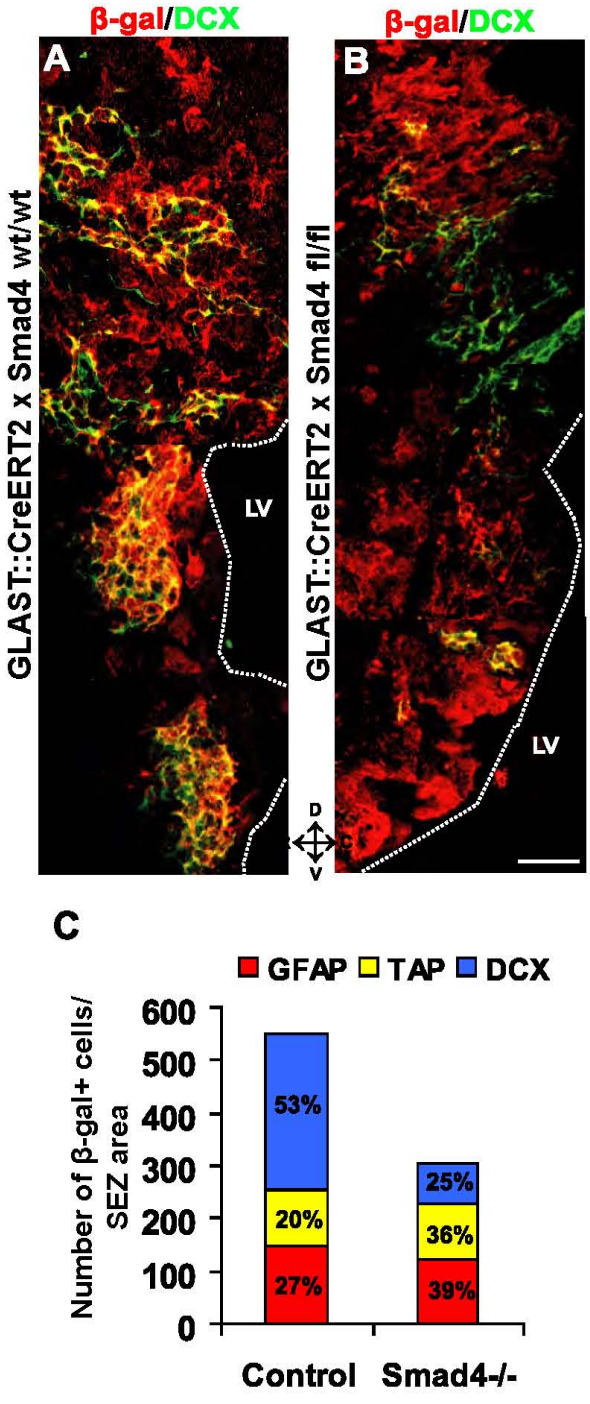


Figure 22. Fate mapping of Smad4-/- stem cell derived progeny by the use of reporter activity

(A-B) Flourescent micrographs of β-galacatosidase- and DCX-immunostaining in the adult SEZ of GLAST::CreERT2/R26R mice without (A) or with floxed exon8 of Smad4 (B) 10 days after tamoxifen application. Note the reduced number of β-gal+ cells that also contain DCX in B versus A shown also by arrows.
(C) Histograms depicting the identity of β-gal+ cells in the adult SEZ of GLAST::CreERT2 / R26R without (Control bar) or with floxed exon8 of Smad4 (Smad4-/- bar) 10 days after tamoxifen application (C ; n(cells)=3306 (Control); 2491 (Smad4-/-), 3 animals each). LV: Lateral Ventricle. The dashed line indicates the ventricular surface of the SEZ. Scale bar: A,B, 75μm.
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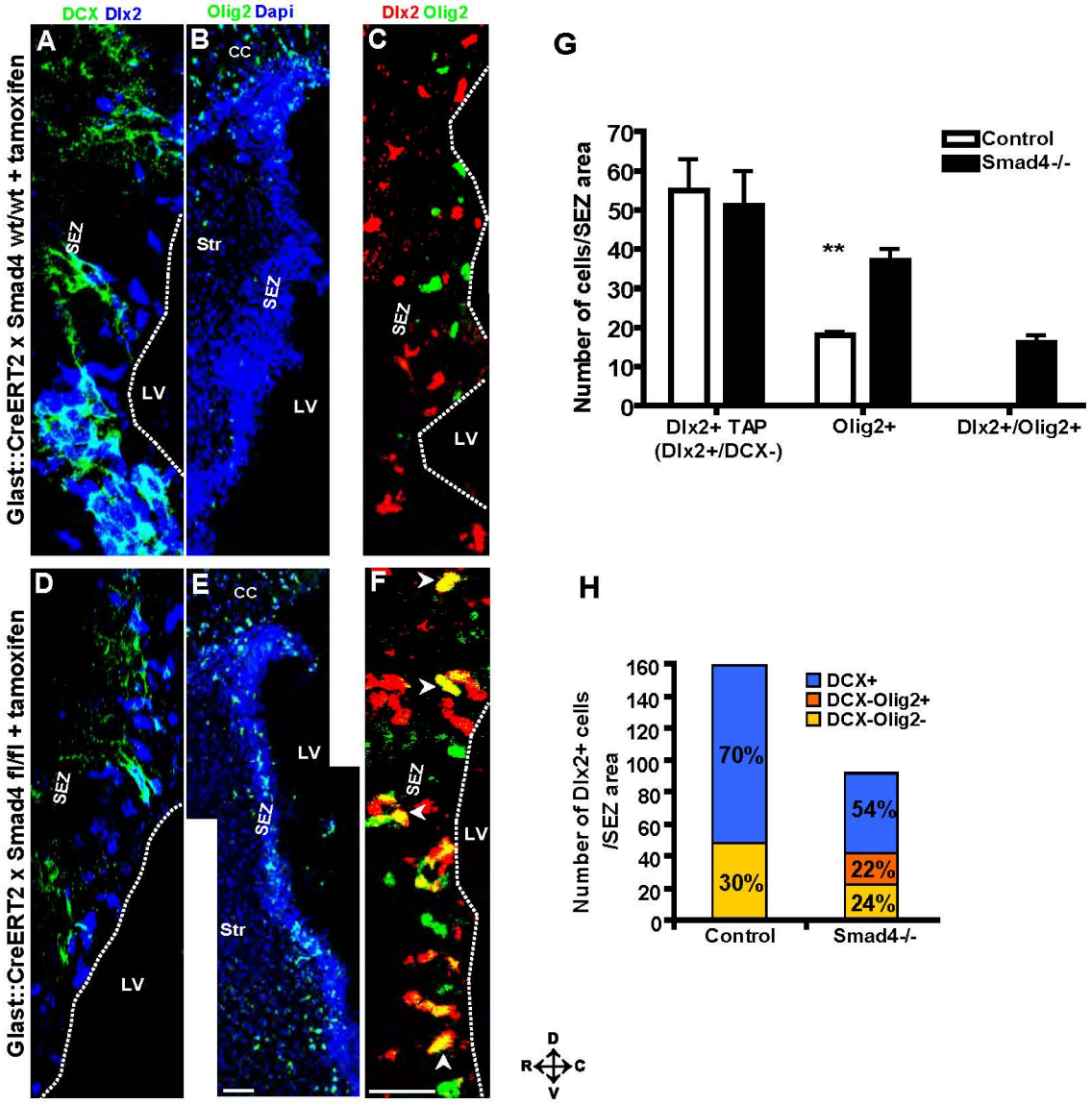

Figure 23. Alteration of Transit-Amplifying Precursor Fate upon Smad4 Deletion

(A-F) Fluorescence micrographs depicting DCX and Dlx2 double staining (A,D), Olig2-immunostaining (B,E), and Dlx2-/Olig2-doublestainings (C,F, double-positive nuclei are indicated by arrowheads) in the adult SEZ of GLAST::CreERT2 mice without (A,B,C) or with (D,E,F) floxed exon 8 of Smad4 10 days after tamoxifen application.

(G,H) Histograms depicting the number of Dlx2+ TAPs (Dlx2+DCX-) (G, n(cells)=652 (Control); 562 (Smad4-/-) 3 animals each), the number of Olig2+ cells (G, n(cells)=255 (Control), 513 (Smad4-/-), 3 animals each), and the number of Olig2- and Dlx2-doublepositive cells (G, n(cells)=107, 3 animals each) or the number of all Dlx2+ cells (H, n(cells)=1430 (Control); 834 (Smad4-/-) 3 animals each) with the percentage of DCX- or Olig2+ co-localization in the adult SEZ of GLAST::CreERT2 without (Control) or with floxed exon8 of Smad4 (Smad4-/-) 10 days after tamoxifen application. Note the increase in Olig2 expression in the Dlx2+DCX- population and corresponding decrease in the fraction of Dlx2+DCX+.

$p < 0.01$ = ** The dashed white line in A,C,D,F depicts the ventricular surface of the SEZ. SEZ: Subependymal Zone, LV: Lateral Ventricle, Str: Striatum, CC: Corpus Callosum, D: Dorsal, V: Ventral, R: Rostral, C: Caudal. Scale Bar: B-E, 100µm (indicated in panel E); A,C,D,F, 60µm (indicated in panel F).

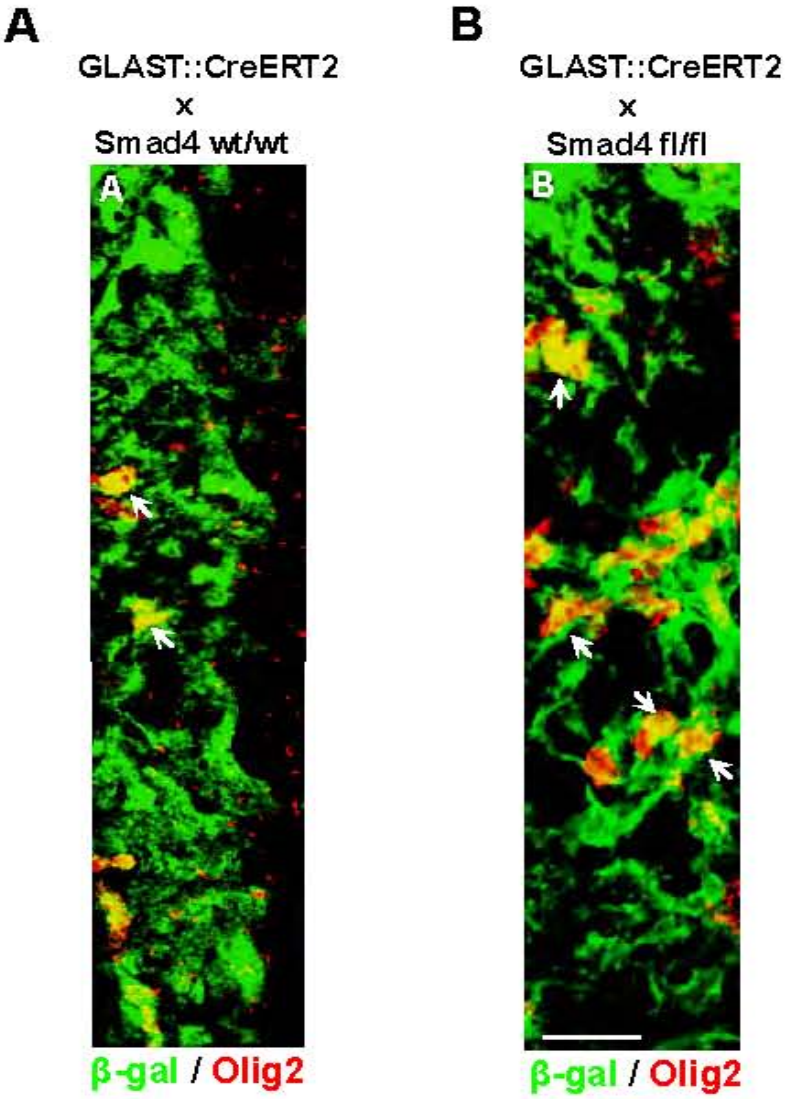

Figure 24. Identity of Smad4-/- stem cell derived progeny failed to generate neuroblast.

(A-B) Fluorescent micrographs of double staining for β-gal and Olig2 in mice without (A) or with (B) exon 8 of Smad4 flanked by loxP sites. Arrows indicate double positive cells in both panels. Note the up-regulation of Olig2 in reporter-positive cells after Smad4 deletion (B versus A) consisting with the increase in the number of Olig2-positive cells and decrease in the number of neuroblasts. Scale bar: 50μm

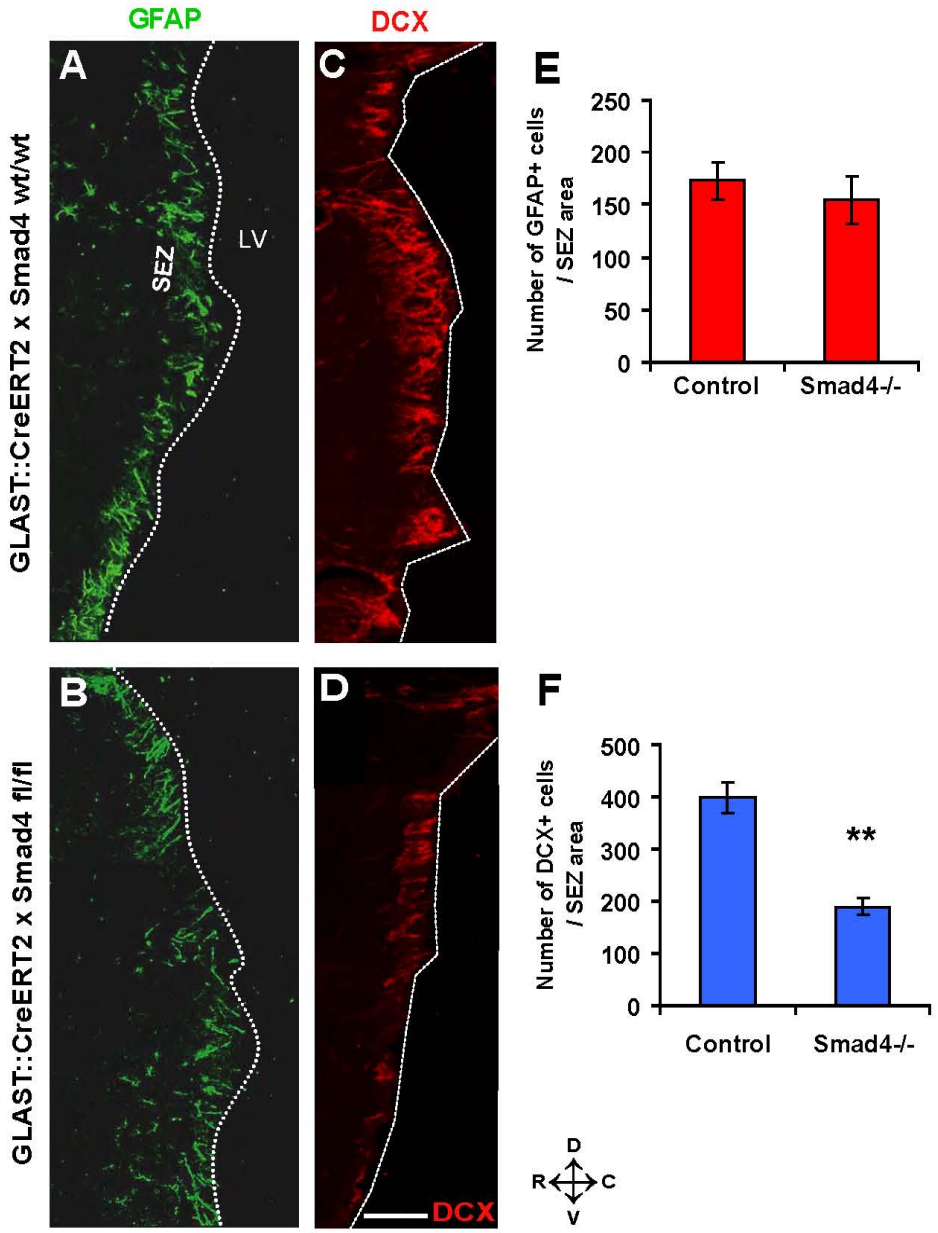

Figure 25. Number of GFAP-positive cells and neuroblasts 21 days after Smad4 deletion in the adult SEZ

(A-D) Fluorescent micrographs depicting GFAP-immunostaining for astrocytes and stem cells (A,B) and DCX-immunostaining for neuroblasts (C,D) in the adult SEZ 21 days after Cre induction by tamoxifen.
(E,F) Histograms depicting the number of GFAP+ astrocytes and stem cells (E, n(cells)=2595 (Control); 2322 (Smad4-/-), 5 animals each) and the number of DCX-immunopositive neuroblasts (F, n = 14306 cells (Control), 5513 (Smad4-/-) of 6 animals each) 21 days after tamoxifen application. Note the significant reduction in the number of neuroblasts, but not GFAP+ cells after Smad4 deletion. SEZ: Subependymal Zone, LV: lateral Ventricle, D:Dorsal, V:Ventral, R:Rostral, C:Caudal. $p < 0.01$ = ** Scale bar: 75µm.

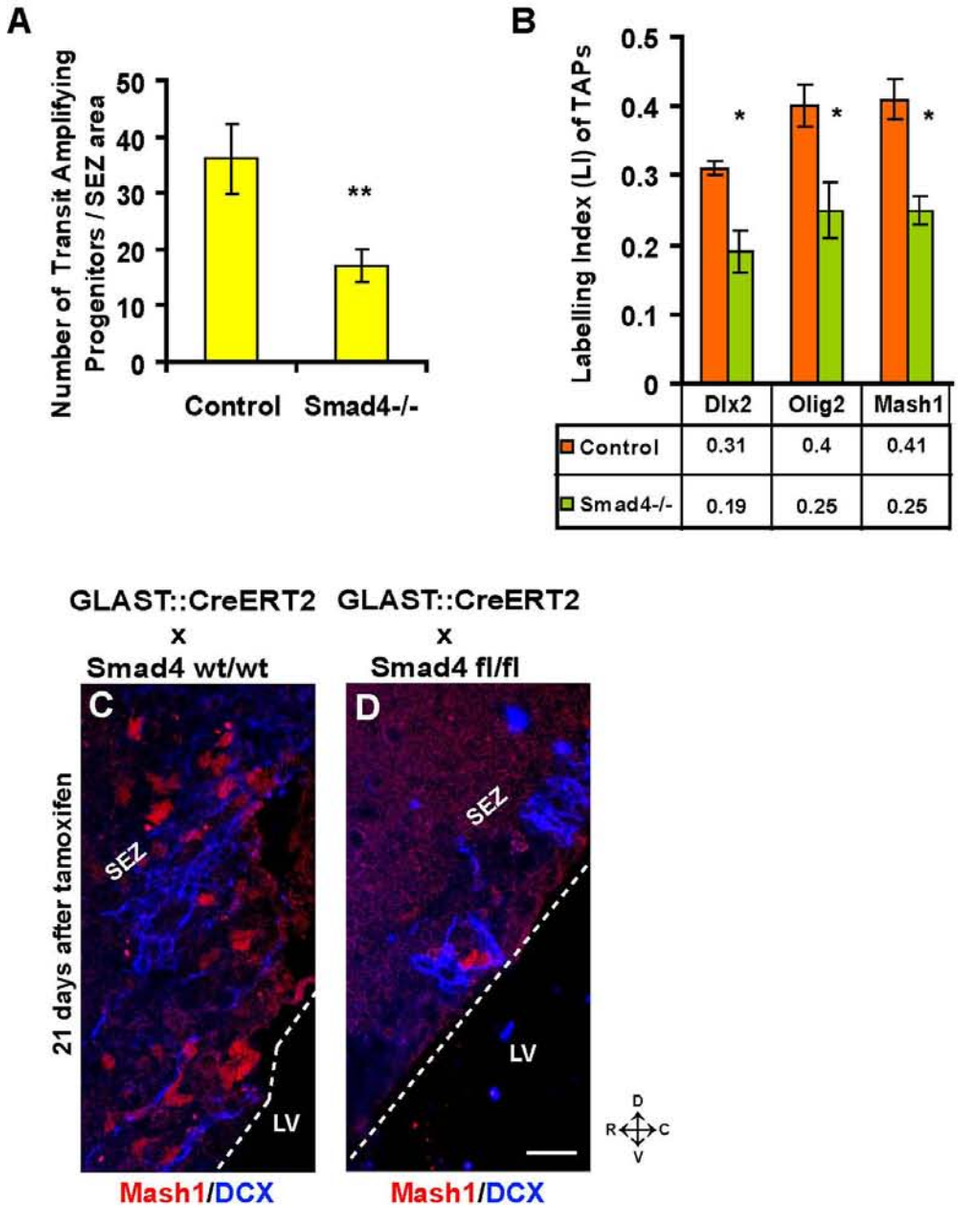

Figure 26. Decrease in the number of transit-amplifying progenitors 21 days after Smad4 deletion

(A,B) Histograms depicting the number of TAPs identified by short pulse BrdU labelling (A, n(cells)=890 (Control), 418 (Smad4-/-), 5 animals each) and labelling index (LI) of different subsets of TAPs identified by transcription factors Dlx2, Olig2 and Mash1 (B) in the SEZ of GLAST::CreERT2 without (Control) or with (Smad4-/-) floxed exon 8 of Smad4 21 days after end of tamoxifen application. Note the reduction in the total number of proliferating TAPs.
(C,D) Fluorescent micrographs of double staining for DCX and Mash1 antigen in control (C) and Smad4-/- (D) 21 days after Smad4 deletion. Note the reduction in the number of Mash1-positive TAPs (Mash1+/DCX-) in D versus (C). SEZ: Subependymal Zone, LV: Lateral Ventricle, D: Dorsal, V: Ventral, R: Rostral, C: Caudal. $p < 0.01$ = ** $p < 0.05$ = * Scale bar: 50µm.

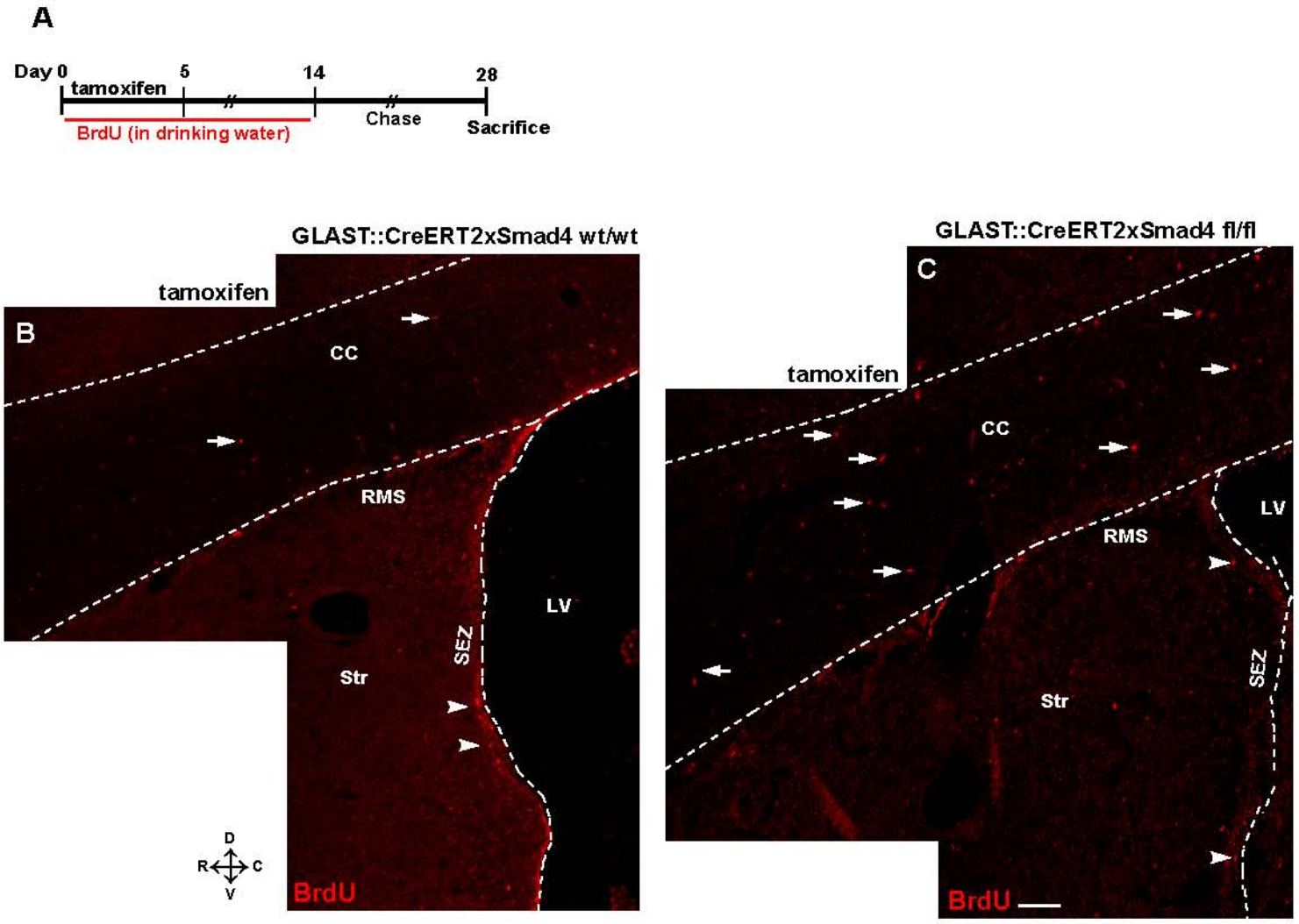

Figure 27. Increased number of immature oligodendroctytes in corpus callosum after Smad4 deletion

(A) Scheme of BrdU-application
(B-C) Fluorescent micrograph depicting BrdU-positive cells in the adult corpus callosum and SEZ of GLAST::CreERT2 mice without (B) or with (C) floxed exon 8 of Smad4. Note that there are many more BrdU+ cells in corpus callosum of GLAST::CreERT2xSmad4floxed/floxed mouse (C) compared to control (B) whereas there is no difference in the number of BrdU-positive cells in the SEZ of both genotypes. BrdU-positive cells are indicated by arrows in corpus callosum and by arrow heads in the SEZ. SEZ: Subependymal Zone, RMS: Rostral Migratory Stream, LV: Lateral Ventricle, CC: Corpus Callosum, Str: Striatum.
The orientation is indicated under D depicting Dorsal, V depicting Ventral, R depicting Rostral, C depicting Caudal. Scale bar: 100µm.

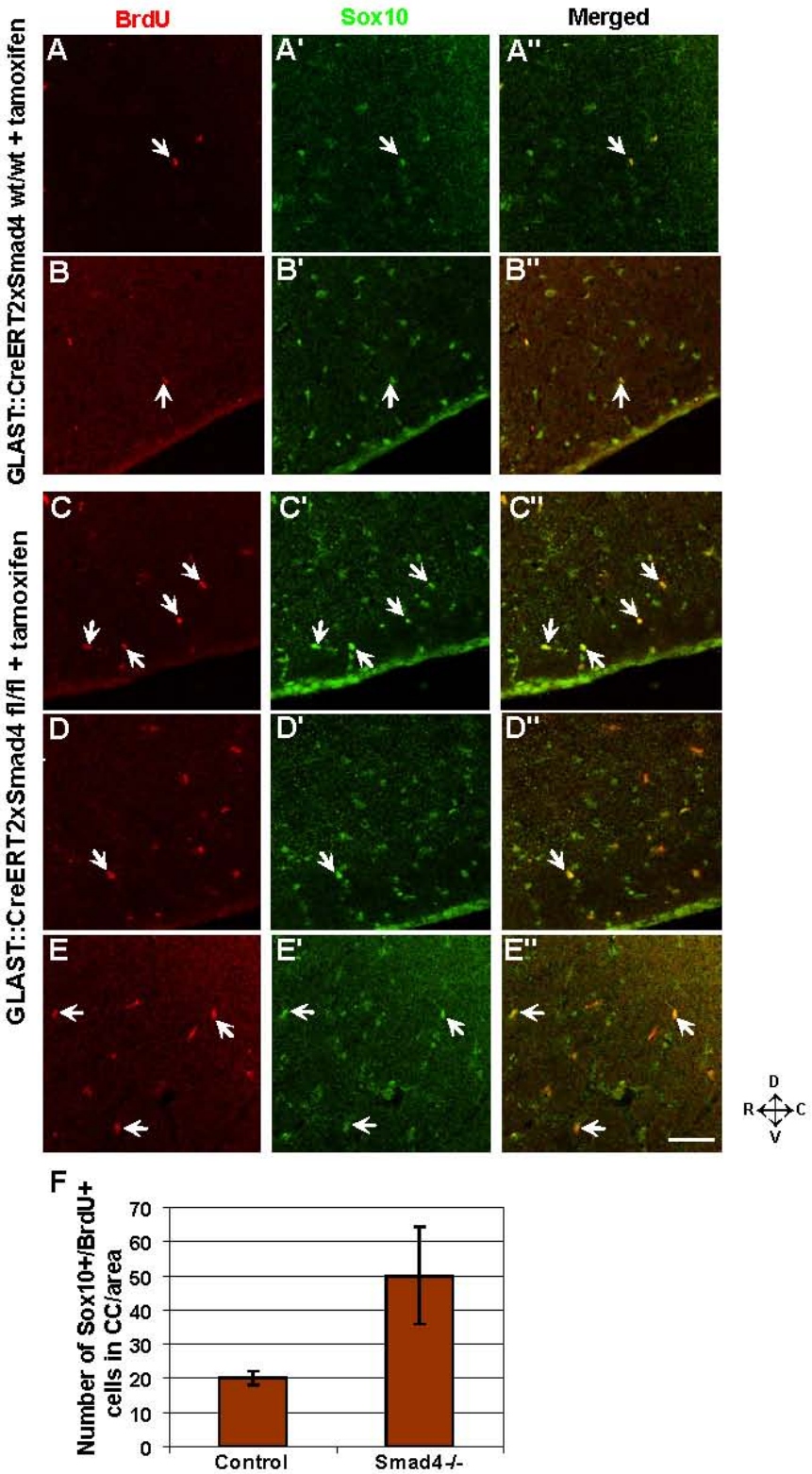

Figure 28. Increase in the number of oligodendrocytes in corpus callosum after Smad4 deletion

(A,E") Fluorescent micrographs of double staining for BrdU (2 weeks BrdU,2 weeks chase) and Sox10 in the corpus callosum of GLAST::CreERT2 mice without (A-B") or with (C-E") floxed exon 8 of Smad4 23 days after the end (28 days after the beginning) of tamoxifen application (see materials and methods and Figure 16 for the application of long pulse BrdU and tamoxifen). Arrows show double positive cells. Note the increase in the number of double positive cells in C-E" versus A-B" showing that there is an increase in generation of oligodendrocytes after deletion of Smad4. (F) Histogram depicting the number of Sox10/BrdU positive cells in the corpus callosum of Control and Smad4-/- mice. Corpus callosum area : 100.000µm². $p < 0.05$ = * Scale bar: 100µm.

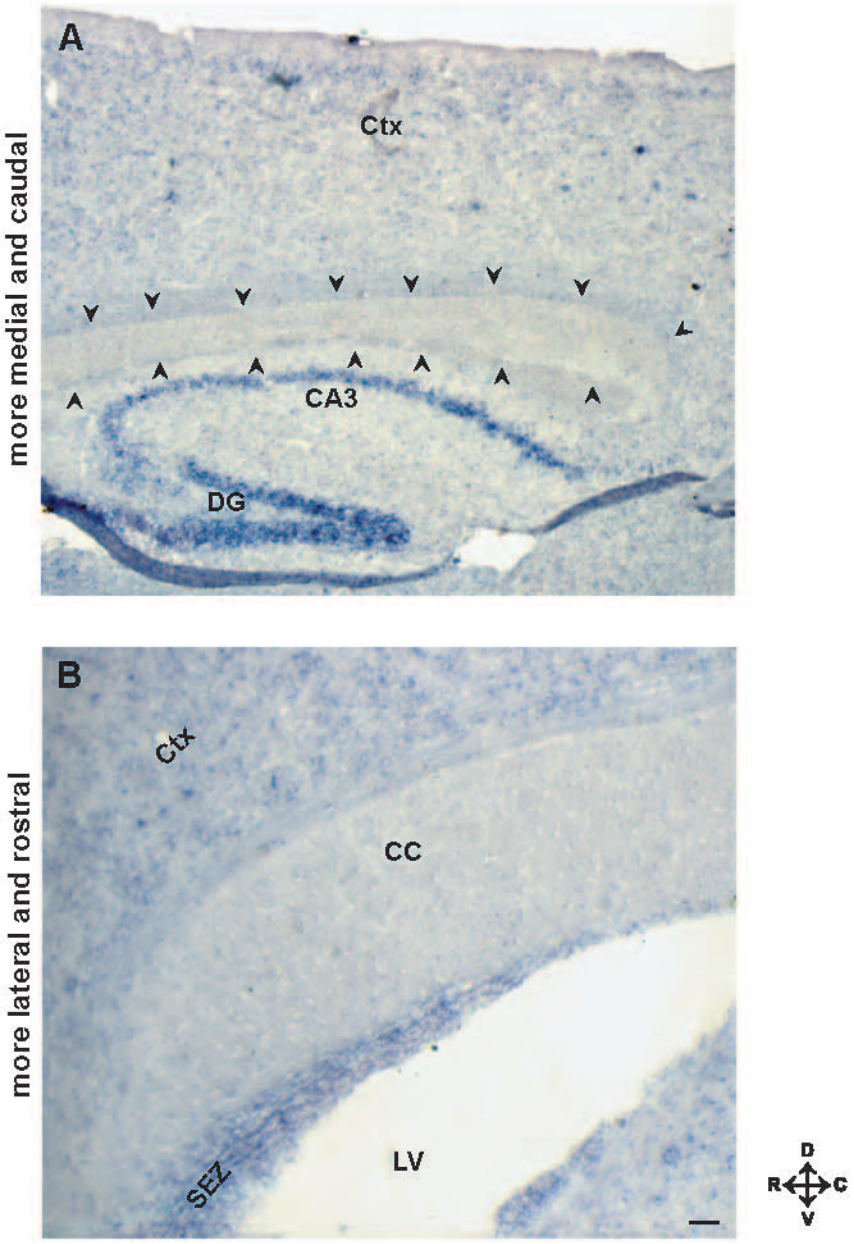

Figure 29. Smad4 expression in corpus callosum

(A,B) In situ hybridization of mRNA transcript of Smad4 in sagital sections of wt adult brain.Corpus callosum is indicated by arrows in A and in a higher magnification in B. Compared to positive signals in SEZ, hippocampus and upper layers of cortex Smad4 does not seem to be expressed in corpus callosum. CC: Corpus Callosum, Ctx: Cortex, SEZ: Subependymal Zone, DG: Dentate Gyrus, LV: Lateral Ventricle. Scale bar: A, 120µm and B, 90µm.

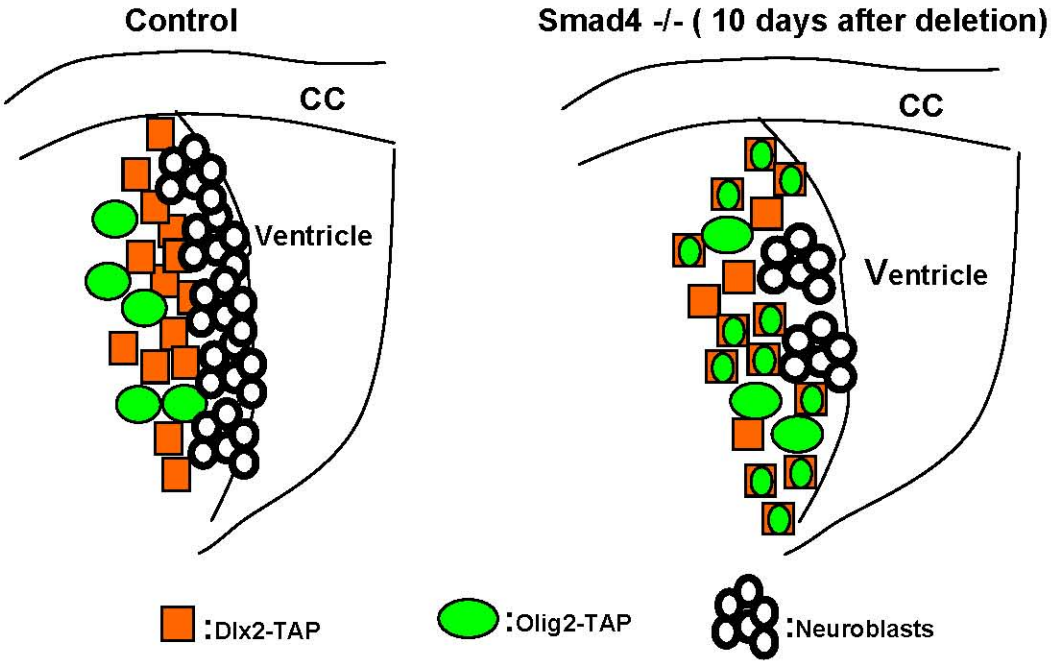

Figure 30 . Scheme summarizing TAP identity 10 days after Smad4 deletion in the adult SEZ

The drawing shows that there is a reduction in the number of neuroblasts and a change in the identity of TAPs. Ater Smad4 deletion a large number of Dlx2-expressing TAP population up-regulates Olig2 antigen. In control animals Dlx2 and Olig2 double positive cells can not be detected.

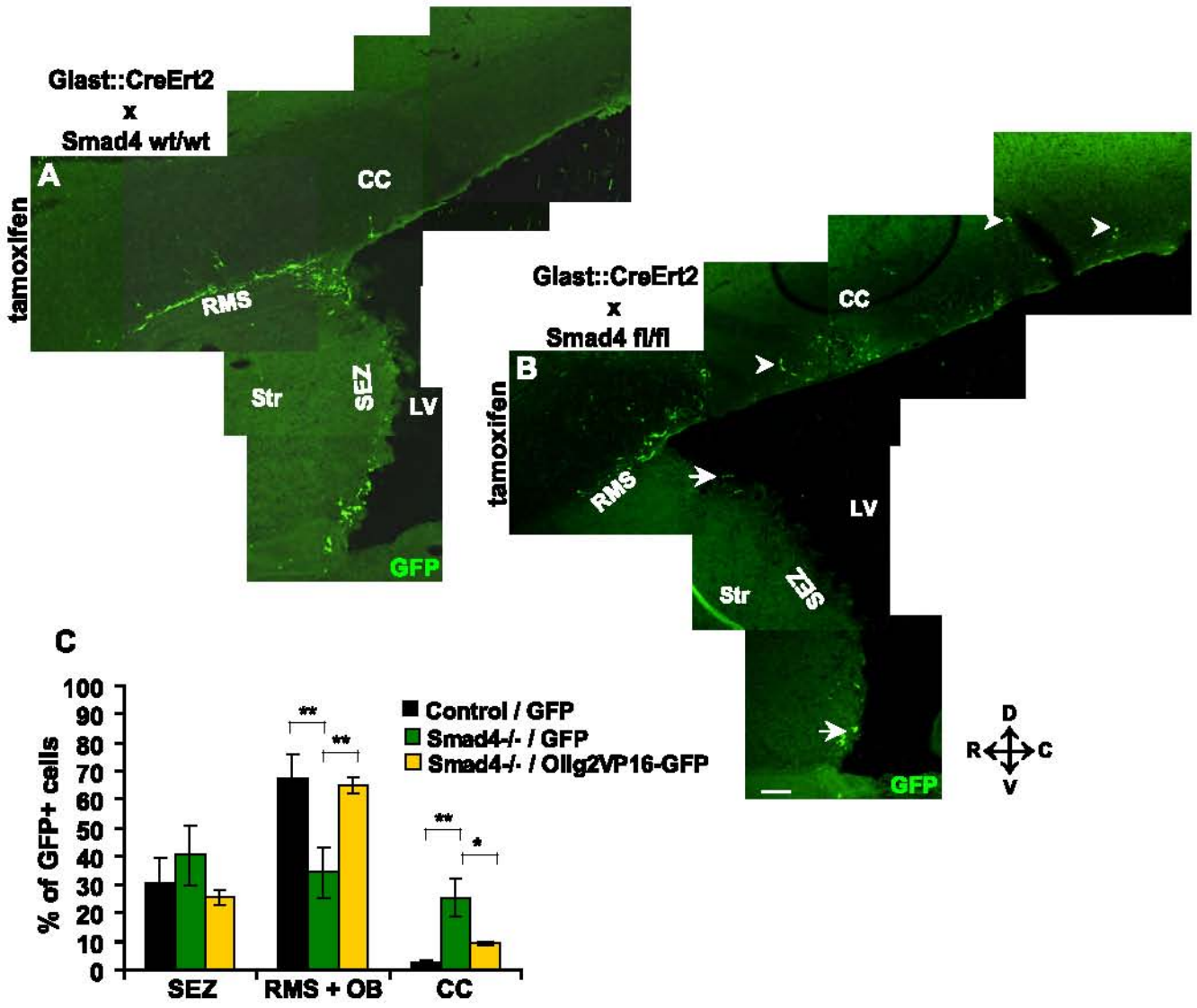

Figure 31. Smad4 deletion results in increased migration of SEZ-derived cells towards the corpus callosum

(A-B) Fluorescent micrographs depicting the location of GFP+ cells 10 days after GFP-virus injection into the SEZ of GLAST::CreERT2 without (A) or with (B) floxed exon 8 of Smad4 10 days after tamoxifen application. Arrowheads indicate GFP+ cells located in the Corpus Callosum (CC). The arrow in B highlights the small number of GFP+ cells remaining in the SEZ after Smad4 deletion.

(C) Histogram depicting the percentage of GFP+ cells located in the subependymal zone (SEZ), rostral migratory stream (RMS), olfactory bulb (OB) and the CC 10 days after injection into the SEZ of GLAST::CreERT2 without (Control, n(animals)=3; n(cells)=1404) or with floxed exon 8 of Smad4 (Smad4-/-, n(animals)=3; n(cells)=440) 10 days after tamoxifen application. Note the increase of GFP-labelled cells in the CC and the corresponding decrease of GFP-labelled cells in the OB upon Smad4 deletion. The third bar represents Olig2VP16-GFP injection into SEZ of GLAST::CreERT2 with floxed exon 8 of Smad4 (n(cells)=528, 2 animals). Note that the interference with Olig2 function fully rescues the generation of neuroblasts migrating via RMS to the OB in Smad4 -/- mice. $p < 0.05$ = *; $p < 0.01$ = ** LV: Lateral Ventricle, Str: Striatum, D: Dorsal, V: Ventral, R: Rostral, C: Caudal. Scale Bar: 100µm

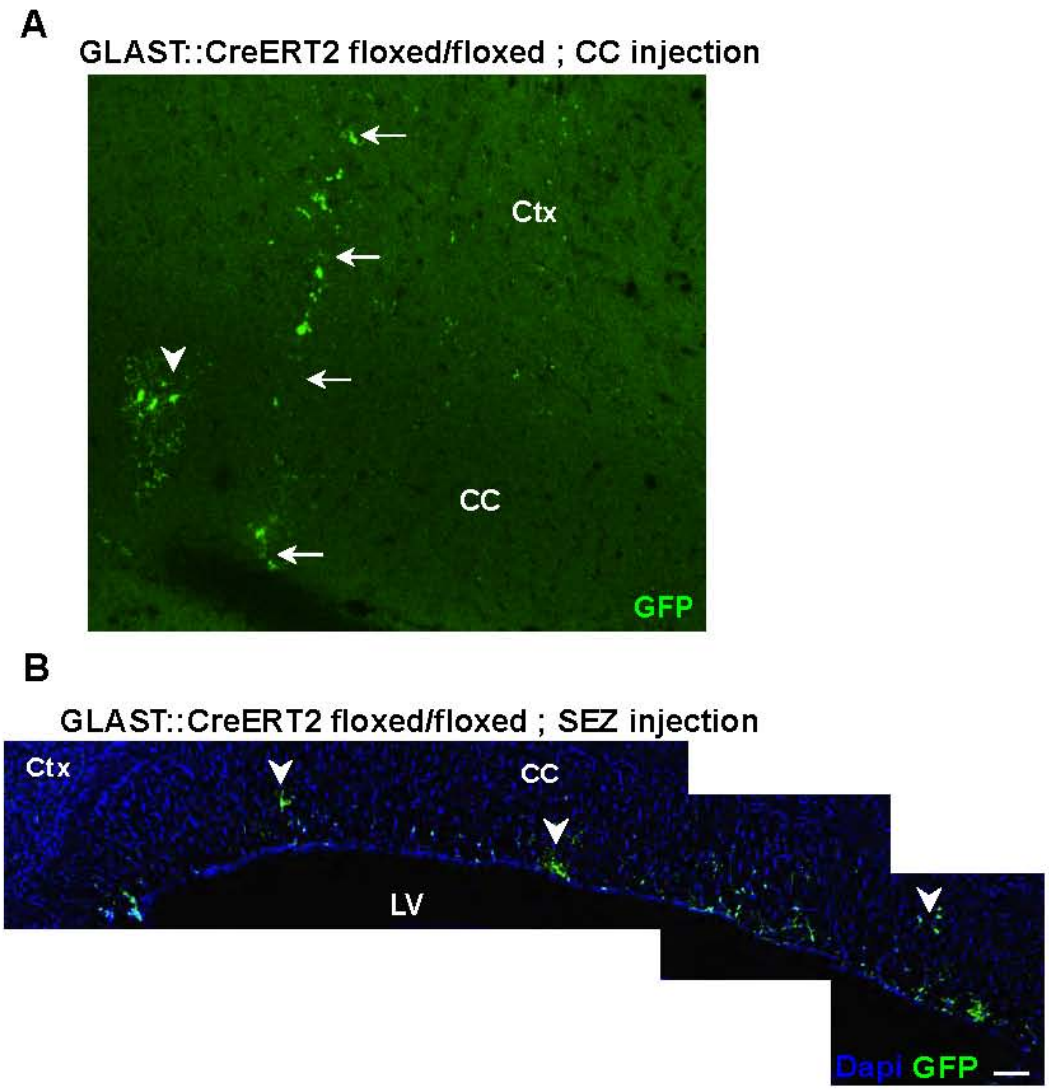

Figure 32. Migration of Oligodendrocyte Precursors from the SEZ or Corpus Callosum after Smad4 Deletion

(A-B) Flourescent micrographs of GFP+ cells 10 days after virus injection into the corpus callosum (CC) (A) or SEZ (B) of GLAST::CreERT2 with homozygous floxed exon 8 of Smad4 10 days after tamoxifen application. Note that oligodendrocyte precursors labelled in the CC (arrowhead indicates oligodendrocyte precursor and arrows indicate injection track in panel A) remain close to the injection site (A), while oligodendrocyte precursors labelled in the SEZ migrate very far from the injection site into caudal regions of the CC (B). Ctx: Cortex, LV: Lateral Ventricle, CC: Corpus Callosum. Scale bar: A,70µm; B, 100µm (indicated in panel B).

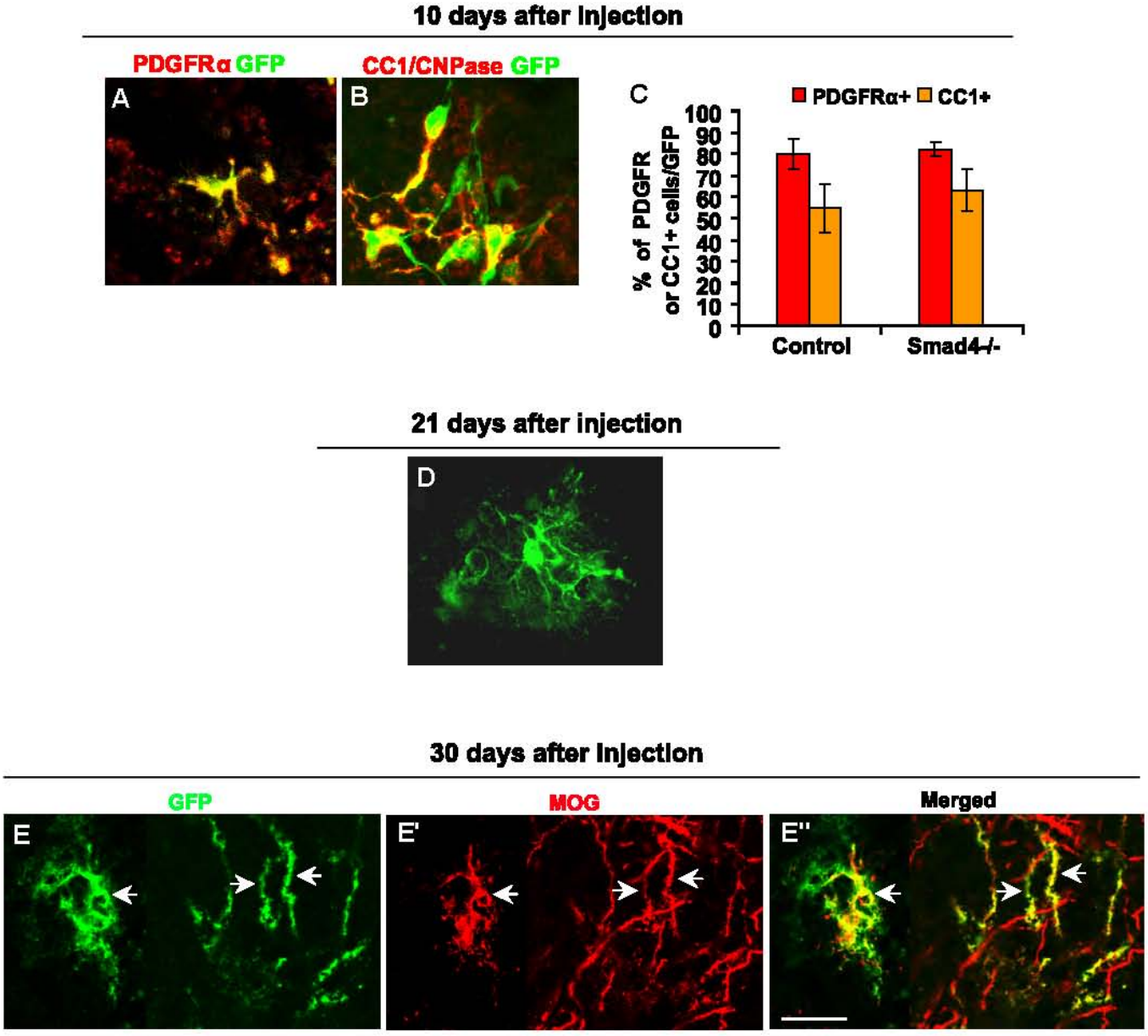

Figure 33. Cells migrating into corpus callosum after Smad4 deletion generate mature oligodendrocytes

(A-B) Fluorescent micrographs of GFP+ cells in CC expressing immature (PDGFR?, D) and partially mature (CC1 and/or CNPase, E) oligodendrocyte markers. (C) Histogram depicting the proportion of PDGFR?- or CC1- and/or CNPase+ cells amongst the GFP+ cells labelled in the SEZ that then migrated into the CC .Note that vitually all the increased number of cells migrated into the CC in the Smad4-/- differentiated along the oligodendroglial lineage. (D) Fluorescent micrograph depicting the morphology of GFP+ cells in CC 21 days after virus injection in the SEZ of Smad4-/- .

(E-E'') Fluorescent micrographs of GFP+ cells expressing MOG, one of the mature oligodendrocyte marker, in CC of Smad4-/- mice 30 days after virus injection into the SEZ. Arrows in panels E-E'' indicate processes positive for both GFP and MOG. Scale Bar: A,D,E-E'', 60µm; B, 30µm (indicated in panel E'').

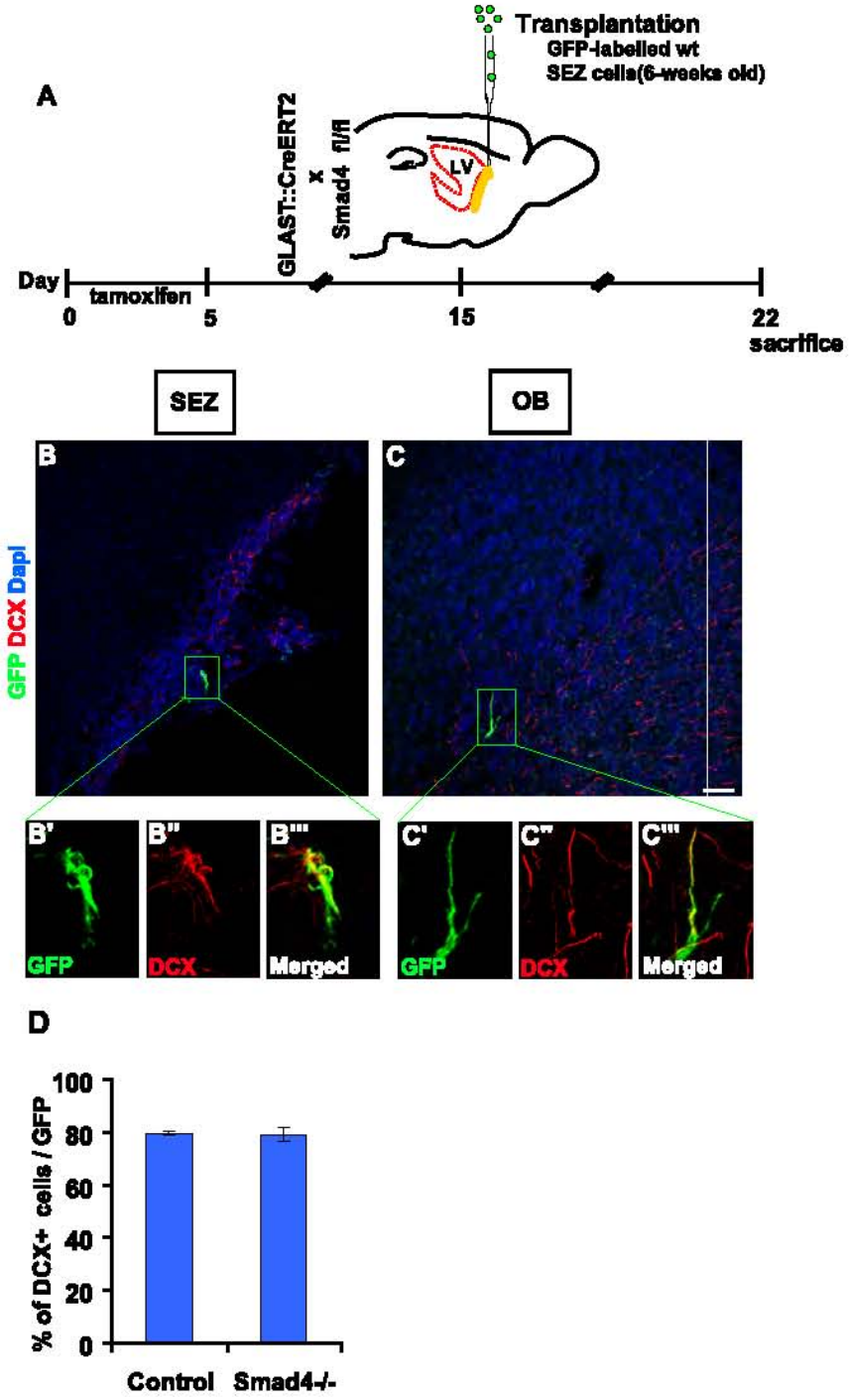

Figure 34. Differentiation of wt cells transplanted into Smad4-/- SEZ

(A) Schematic diagram depicting the experimental design of the Venus labelled cells into the SEZ of Glast::CreERT2/Smad4 fl/fl animals.
(B-C'') Fluorescent micrographs depicting localization and DCX expression of GFP-labelled cells 7 days after transplantation in the SEZ (B- B'''), and OB (C- C''). Note that GFP-labelled cells generated DCX+ neuroblasts and were capable of migrating to the OB after transplantation into the SEZ of Smad4-/-. SEZ: Subependymal Zone, LV: Lateral Ventricle, OB: Olfactory Bulb. Scale bar: B,C, 50μm; B'-C'', 20μm (indicated in panel C).

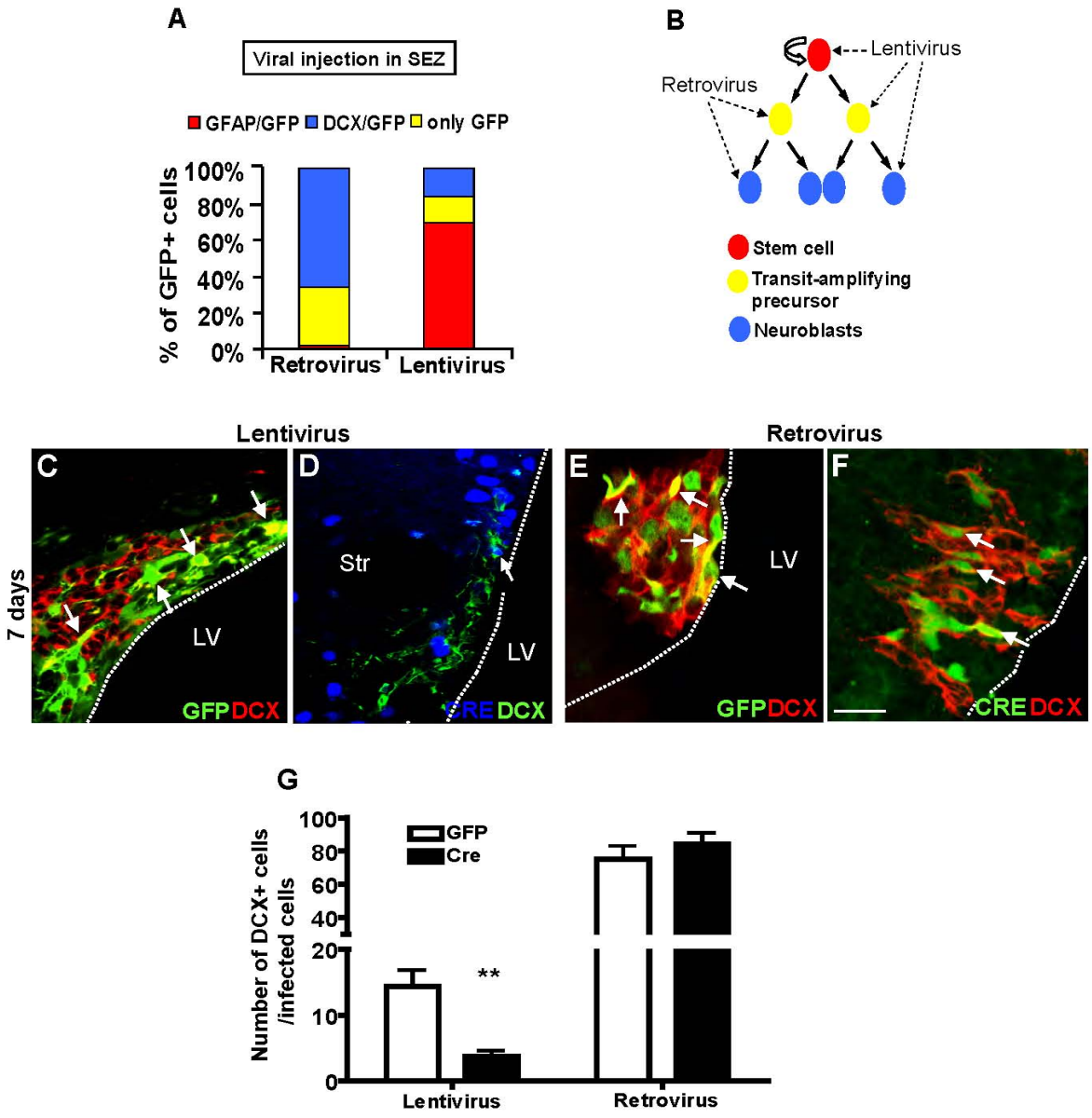

Figure 35. Deletion of Smad4 in stem cells or transit-amplifying cells results in different phenotypes

(A) Histogram depicting the identity of GFP-positive cells 2 days after injection of retroviral or lentiviral vectors into the adult SEZ respectively (This data was obtained by my colleague Monika Brill in our laboratory). Note that retroviral mediated gene transfer occurs only in fast proliferating cells (transit-amplifying precursors and DCX+ neuroblasts), but not in astrocytes and stem cells. The latter are preferentially targeted by lentiviral vectors.
(B) Lineage diagram depicting viral–specific targeting.
(C,D,E,F) Fluorescent micrographs of GFP+ cells infected with lentiviral (C,D) or retroviral (E,F) vectors double–stained for DCX 7 days after injection. Note that fewer Cre+/DCX+ cells were observed when Cre-containing lentivirus (D), but not Cre-containing retrovirus was used (F). Examples of double-positive cells are indicated by arrows.
(G) Histogram depicting the number of DCX+ neuroblasts among lentiviral and retroviral infected GFP or Cre+ cells in the adult SEZ of homozygous floxed Smad4 mice 7 days after injection. Note the strong reduction of neuroblasts generated from lentivirus Cre-infected cells while the neuroblast progeny of retrovirus Cre-infected cells was not affected. (Lentivirus: n(animals)=10 n(cells): GFP=572, Cre=830; Retrovirus: n(animals)=6, n(cells): GFP=372, Cre=559). $p < 0.01$ = ** Scale bar: C,D, 50µm; E,F, 40µm (indicated in panel F).

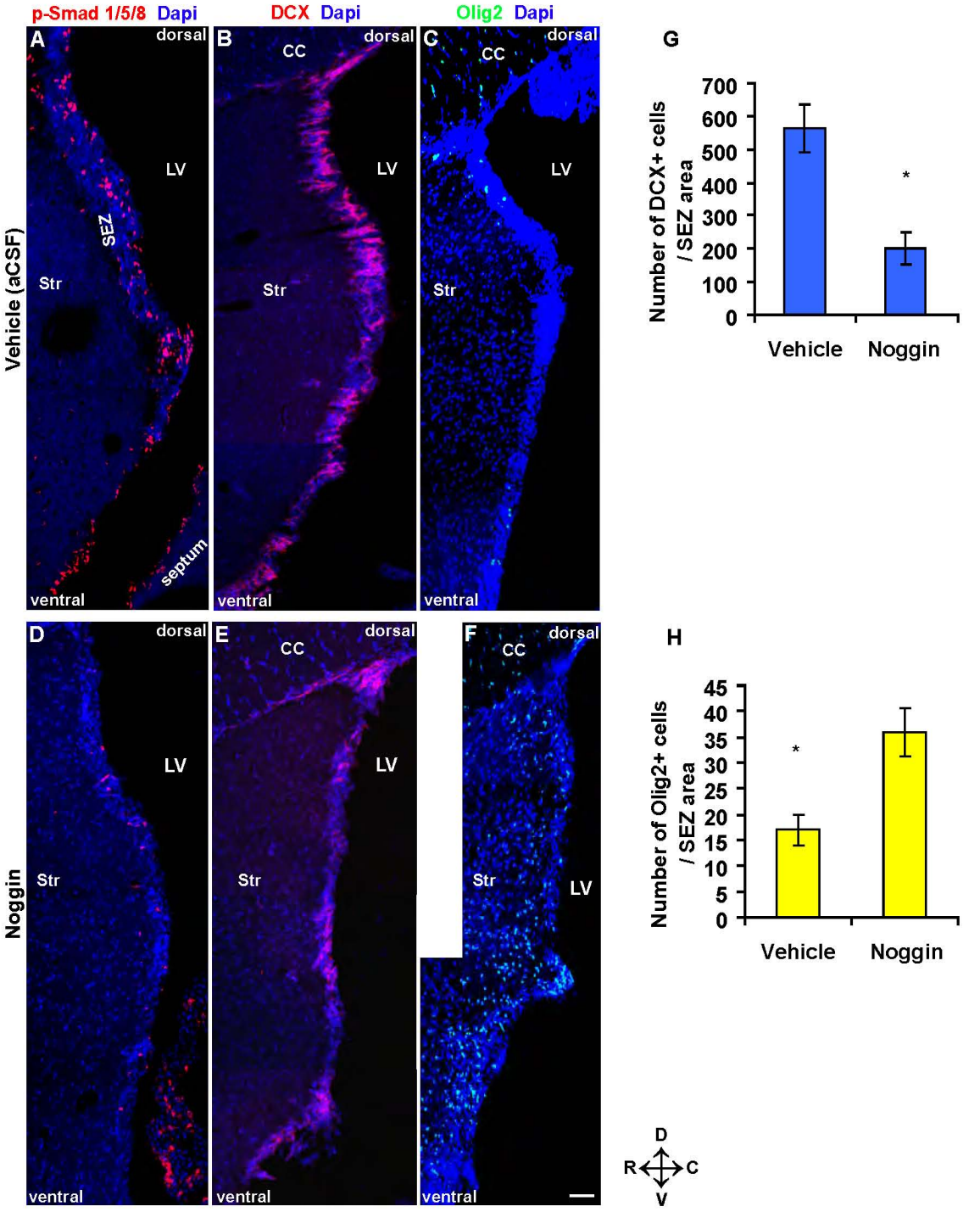

Figure 36. Noggin infusion inhibits adult neurogenesis and up-regulates Olig2

(A-F) Fluorescent micrographs depicting p-Smad1/5/8+ (A,D) , DCX+ (B,E) and Olig2+ (C, F) cells in the SEZ 10 days (7 days infusion + 3 days survival) after artificial Cerebrospinal Fluid (aCSF) (A-C) or infusion of the extracellular inhibitor of BMP ligands, Noggin (D-F) into the lateral ventricle of WT mice. Note the loss of nuclear p-Smad1/5/8 staining after Noggin infusion in D compared to conrol in A. Note the severe reduction in neuroblasts in (B,E) after Noggin infusion also as depicted in the histogram G (n(cells)=13516 (Vehicle) ; 4833 (Noggin), 3 animals each). Conversely, the number of Olig2+ cells is notably increased after Noggin infusion as quantified in the histogram H (n(cells)=168 (Vehicle); 332 (Noggin), 3 animals each). $p < 0.05$ = * SEZ: Subependymal Zone, LV:Lateral Ventricle, Str:Striatum, CC:Corpus Callosum, D:Dorsal, V:Ventral, R:Rostral, C:Caudal. Scale bar: A,C,D,F, 100µm ; B,E, 120µm (indicated in panel F).

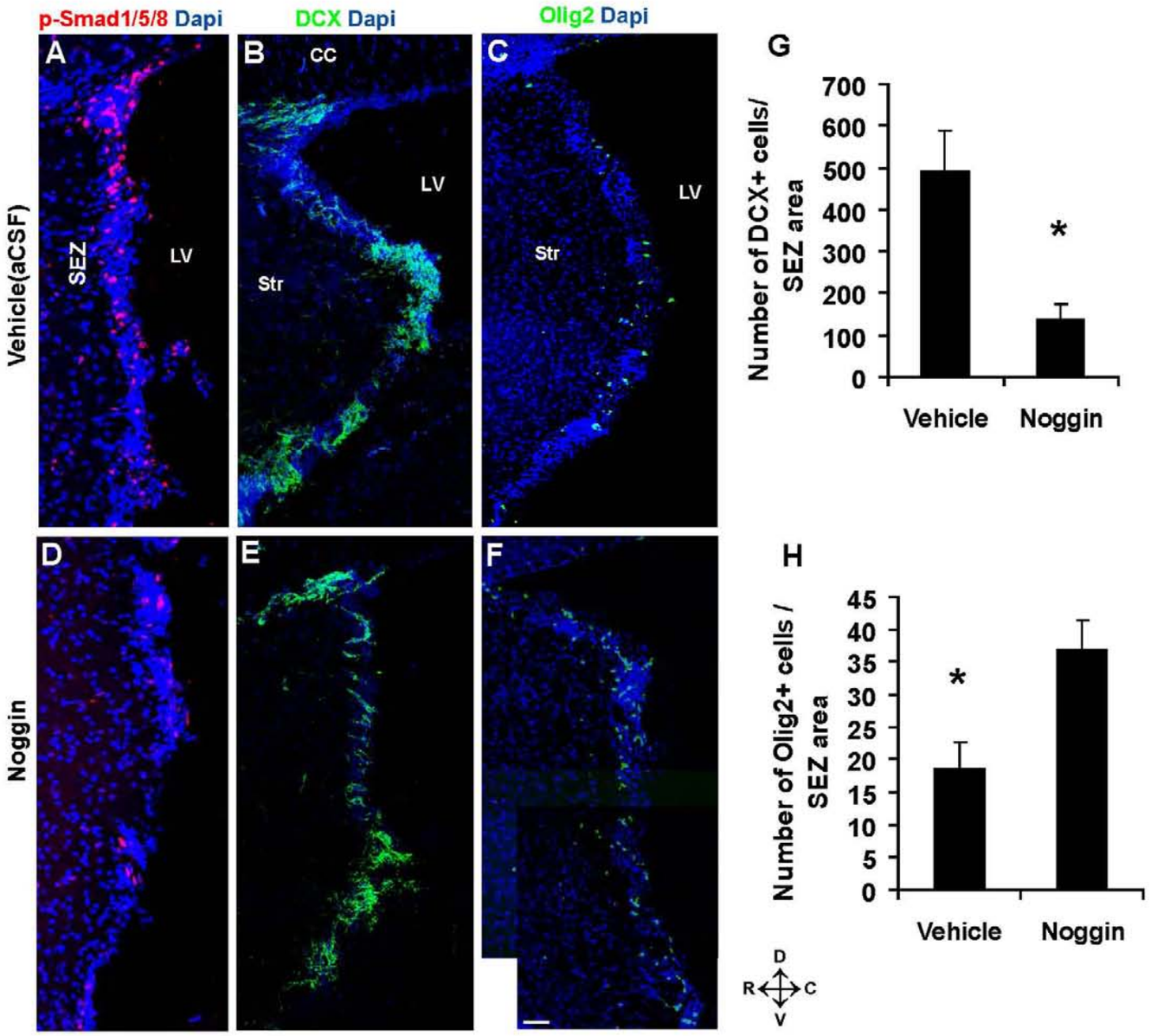

Figure 37. Persistence of impaired neurogenesis 21 days after noggin infusion

(A-F) Fluorescent micrographs depicting p-Smad1/5/8+ (A,D), DCX+ (green in B,E) and Olig2+ (C,F) cells in the SEZ 21 days after artificial Cerebrospinal Fluid (aCSF) (A-C) or Noggin (D-F) into the lateral ventricle of WT mice. Note the slow recovery of nuclear p-Smad1/5/8 staining 21 days after noggin infusion in D compared to 10 days after Noggin infusion in Figure 33 in panel D. Note that impaired neurogenesis still persists 21 days after Noggin infusion in E as depicted in the histogram G (n(animals)=3 ; n(cells)=Vehicle, 5752 ; Noggin, 2065) as well as persistence of increased number of Olig2-positive cells as quantified in the histogram H (n(animals)=3 ; n(cells)=Vehicle, 168 ; Noggin, 332). $p < 0.05$: * LV: Lateral Ventricle ; Str: Striatum ; CC: Corpus Callosum. Scale bar: B,E,100µm ; A,D,C,F,75µm.

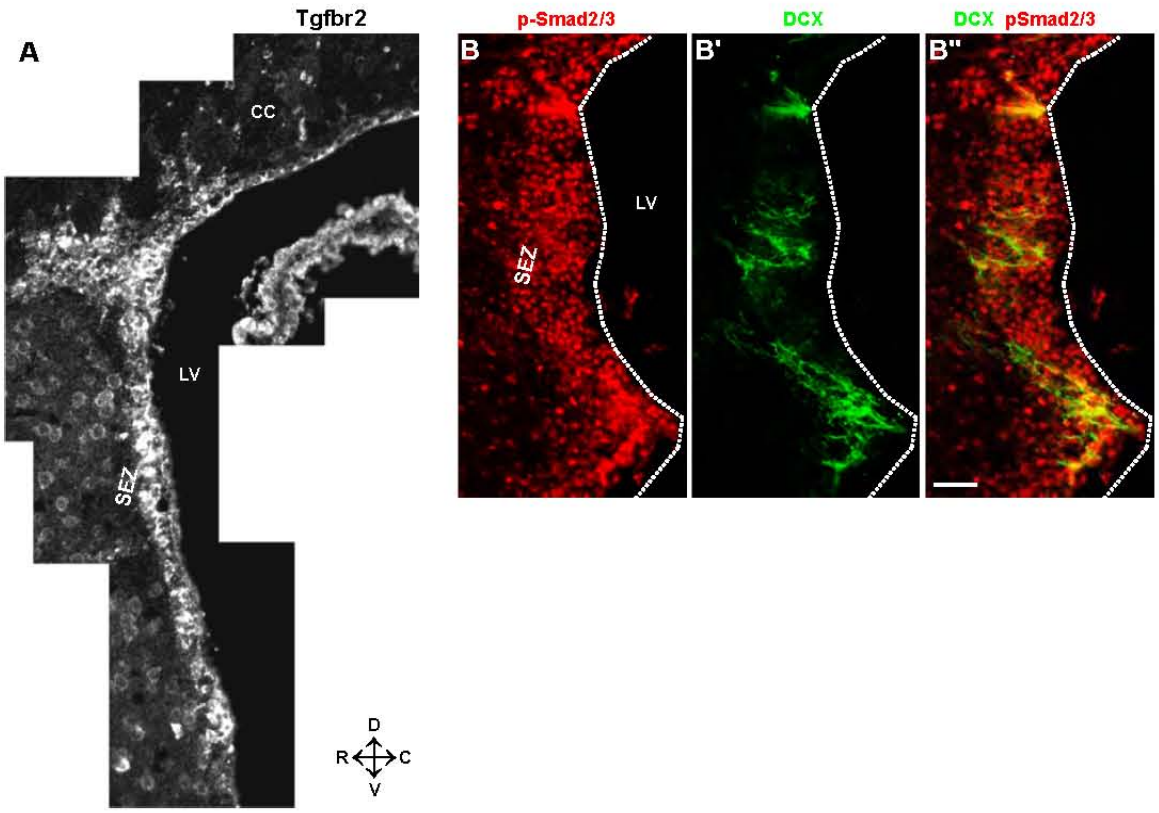

Figure 38. Expression of Tgfbr2 and p-Smad2/3 in the Adult SEZ

(A) Fluorescent micrograph of Transforming growth factor beta type 2 receptor (Tgfbr2) in the adult SEZ. (B-B'') Fluorescent micrographs depicting p-Smad2/3- (B), DCX -(B') and double-(B'') immunostaining in the adult SEZ. Note the enrichment of both Tgfbr2 and p-Smad2/3 protein in the SEZ. The dashed white line in B-B'' depicts the ventricular surface of the SEZ. SEZ:Subependymal Zone, LV:Lateral ventricle, CC: Corpus Callosum. Scale bar: A, 50µm; B- B'', 100µm (indicated in panel B''). These stainings were done by Sven Falk as a collaboration.

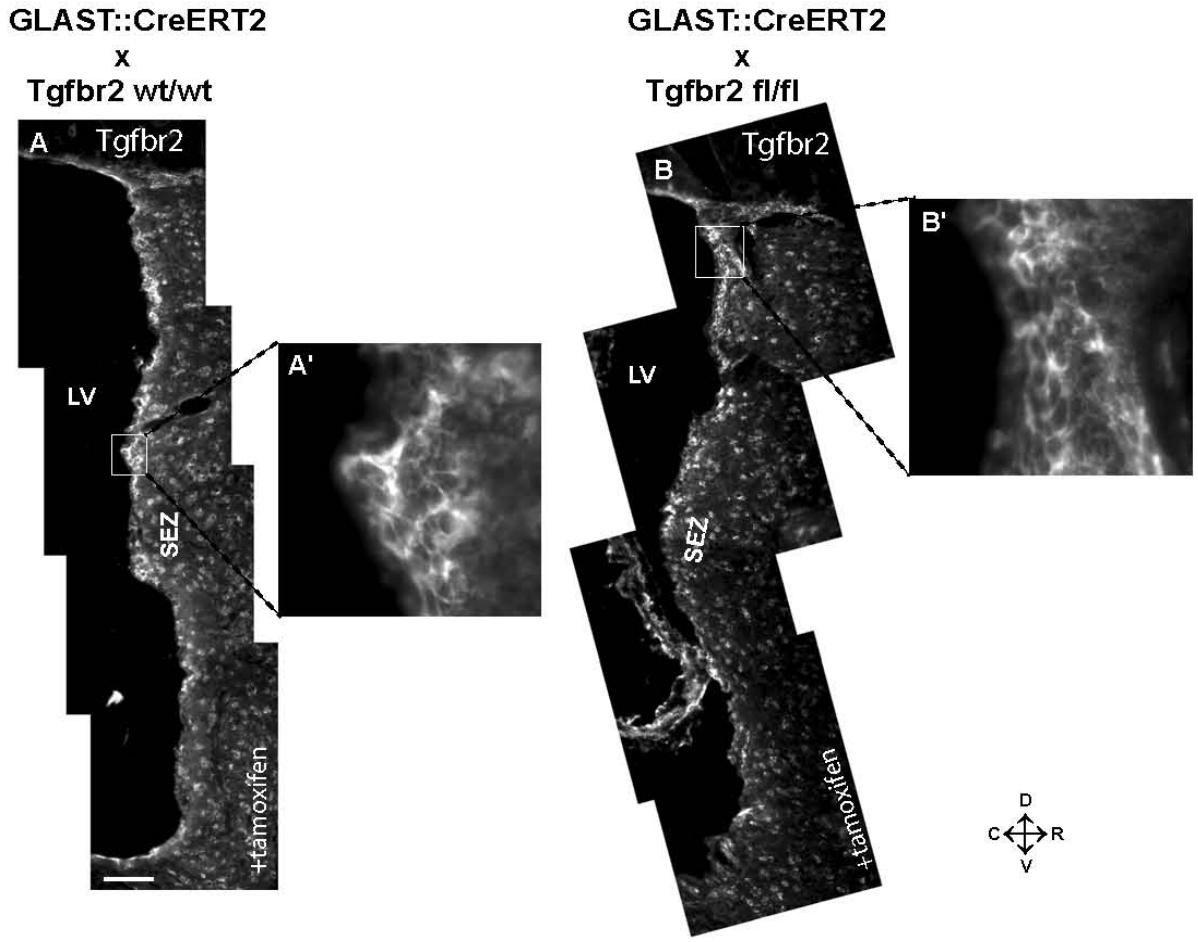

Figure 39. Tgfbr2 expression 1 month after inducible deletion in adult SEZ

(A-B') Tgfβ receptor 2 immunostaining in SEZ of GLAST::CreERT2 without (A-A') or with (B-B') floxed exon 2 of Tgfβ receptor 2. A' and B' are high powers of insets in A and B respectively. Tgfβ receptor 2 protein still persists in SEZ 1 month after Cre induction by tamoxifen application. SEZ: Subependymal Zone, LV: Lateral Ventricle. Scale bar: A,B, 120µm; A',B', 75µm. This experiment was done by Sven Falk as a collaboration.

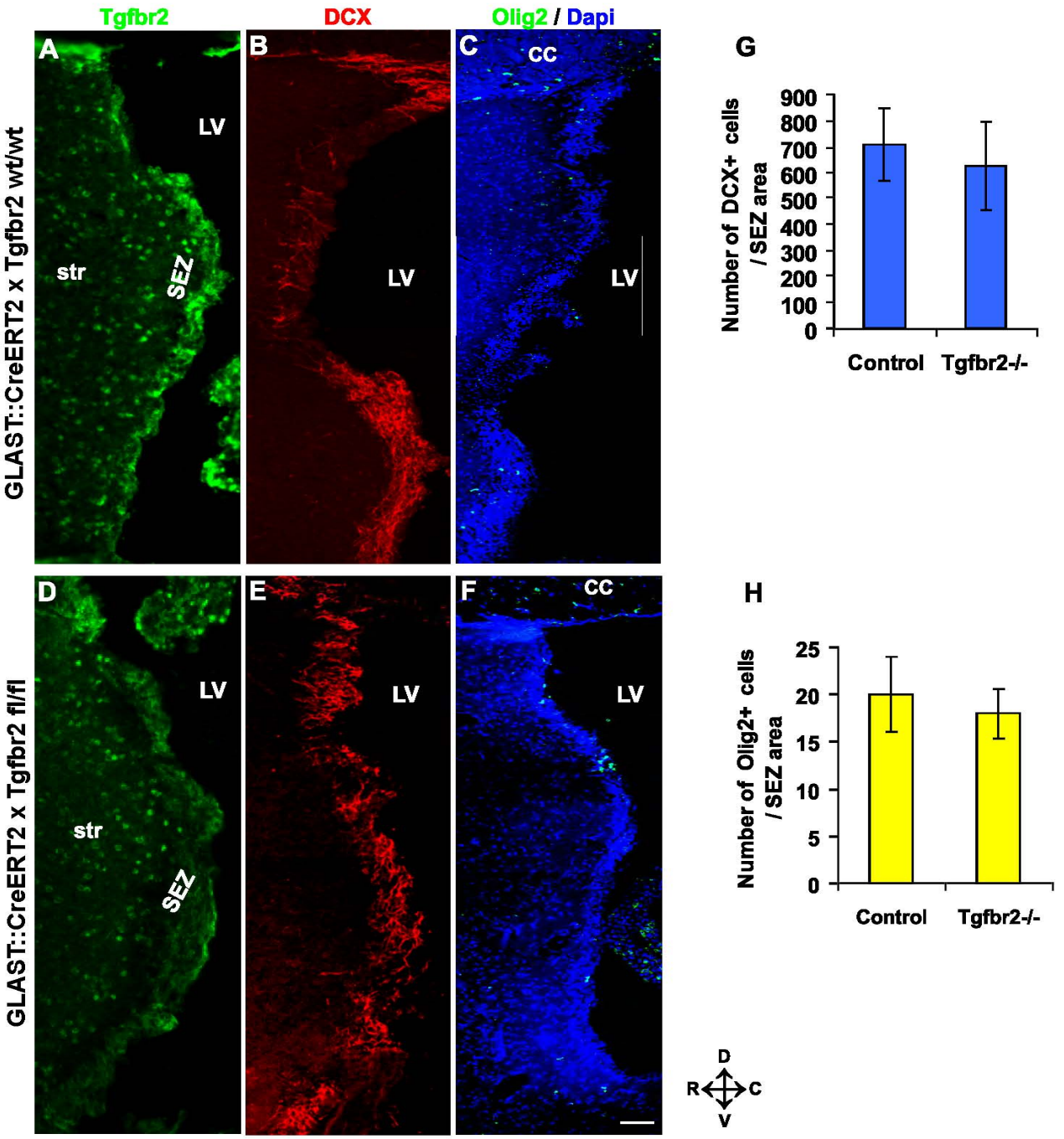

Figure 40. Conditional deletion of Tgfbr2 in adult SEZ

(A-F) Fluorescent micrographs depicting Tgfbr2-(A,D), DCX-(B,E) and Olig2-(C,F) immunostaining in control (A-C) or in mutant animals with the homozygous floxed allele of Tgfbr2 (D-F). Note the loss of Tgfbr2 protein in the SEZ of the latter (D) but not the former (A) mice. Histogram in G depicts the number of neuroblasts and Olig2+ cells (for DCX, n(cells)=13053 (Control), 5016 (Tgfbr2fl/fl), 3 and 1 animal respectively; for Olig2, n(cells)=297 (Control); 92 (Tgfbr2fl/fl), 3 and 1 animal respectively) in the SEZ 3 months after tamoxifen application. LV: Lateral Ventricle, SEZ: Subependymal Zone, CC: Corpus Callosum, Str: Striatum. Scale bar: A,D, 75µm; B,E,C,F, 100µm (indicated in panel F).

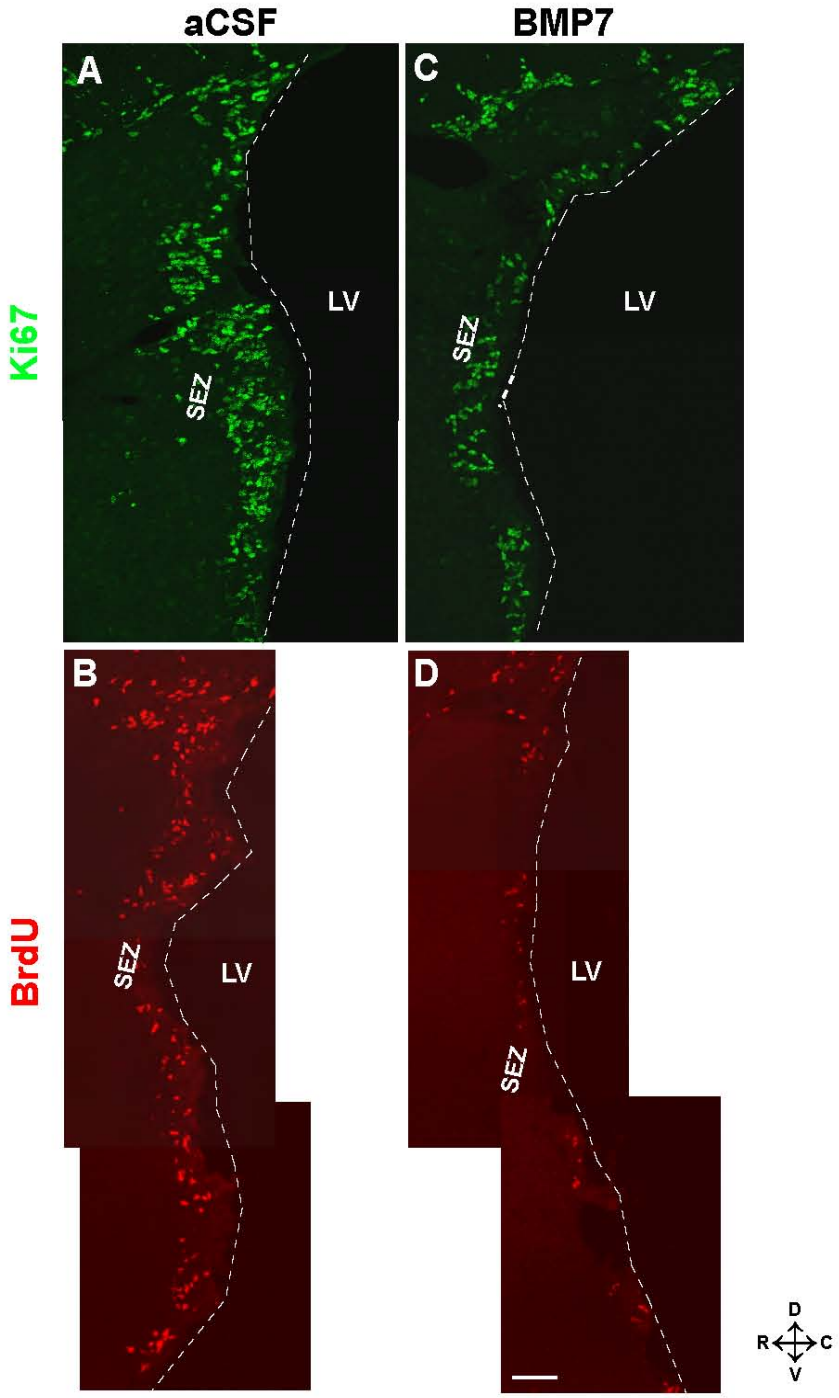

Figure 41. Proliferation in the adult SEZ after BMP7 infusion

(A-D) Fluorescent micrographs of stainings for Ki67 (A,C) and BrdU (injected 1 hour prior to sacrifice) (B,D) in the SEZ after artificial Cerebrospinal Fluid (aCSF) (A-B) or infusion of BMP7 into the lateral ventricle of WT mice. Note the prominent decrease in the number of both BrdU- and Ki67-positive cells (C versus A and D versus B, respectively) after BMP7 infusion. SEZ: Subependymal Zone, LV: Lateral Ventricle. Scale bar: A,C, 100µm; B,D, 125µm.

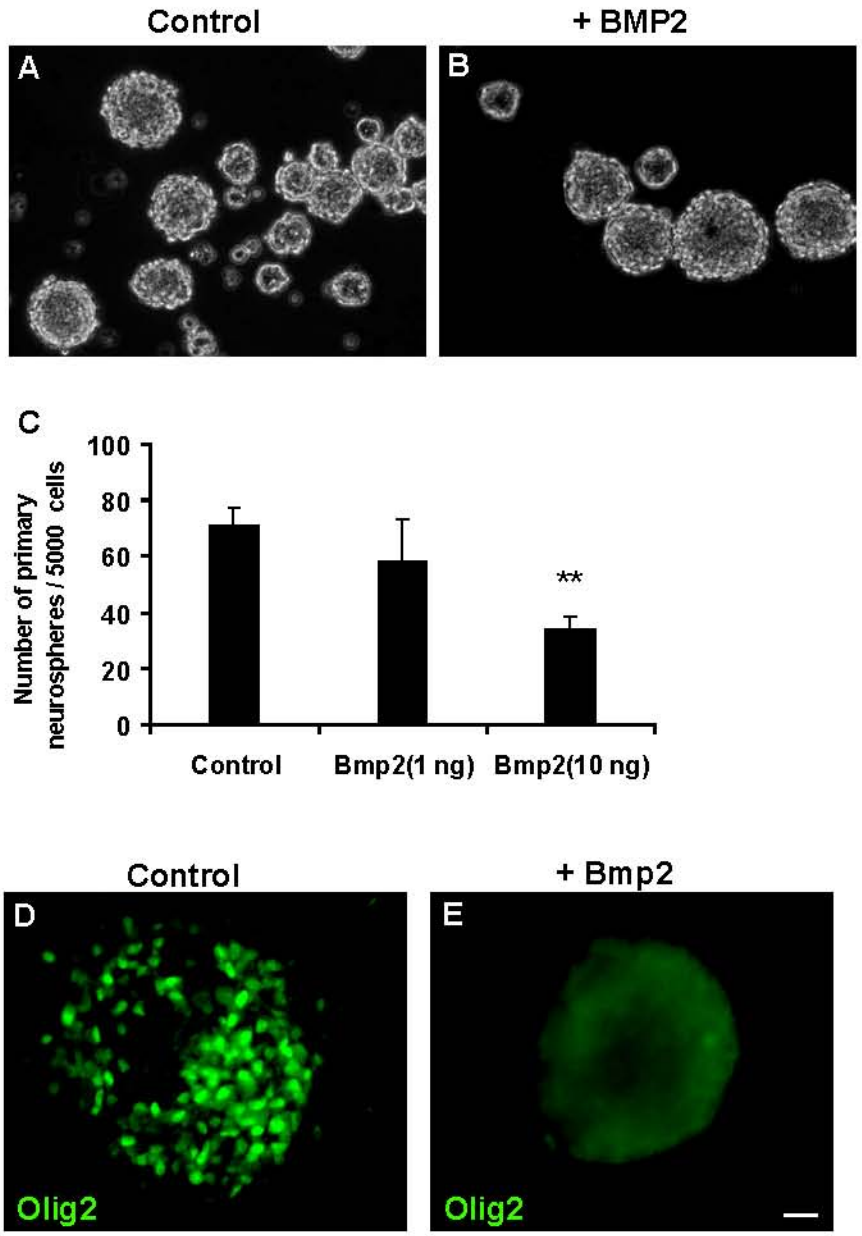

Figure 42. Effects of BMP2 on neurospheres

(A-B) Phase contrast images depicting neurosphere forming capacity of adult SEZ cells either in absence (A) or presence of Bmp2 (B) in vitro. Note the reduction in the number of neurospheres in the presence of Bmp2 as depicted in the histogram C (6 wells per each and 2 sets of experiments). (D,E) Fluorescent micrographs depicting Olig2 immunostaining in neurospheres in the absence (D) or in the presence (E) of Bmp2 ligand. In this case Bmp was added to the culture after neurospheres were already formed (incubation time with Bmp2 was 2 days). $p < 0.01$ = ** Scale bar: A,B, 100µm; D,E, 40µm.

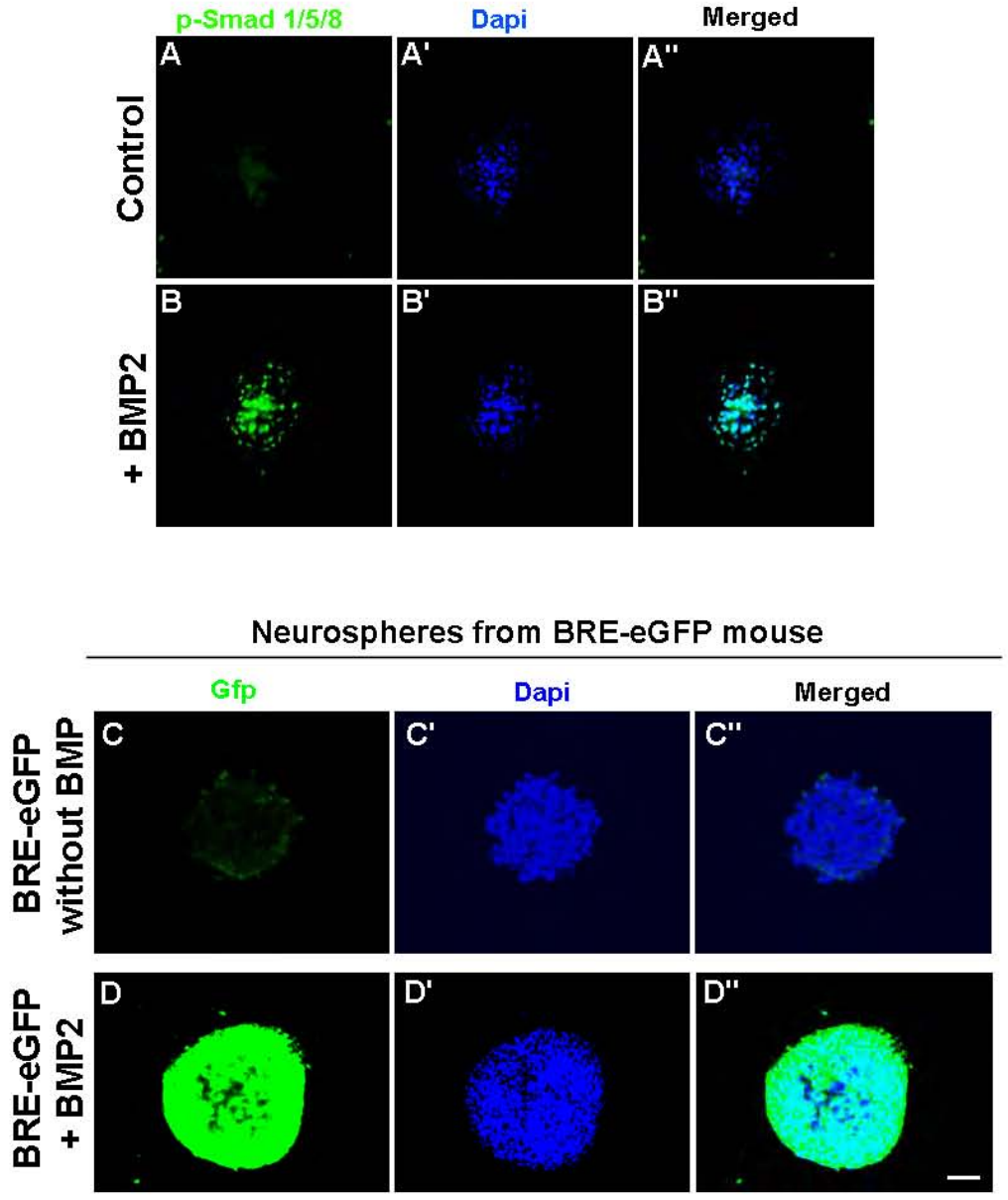

Figure 43. BMP activity in neurospheres

(A-B'') Fluorescent micrographs of staining for p-Smad1/5/8 in a mounted neurosphere in control (A-A'') or in presence of Bmp2 (B-B''). In control situation neurosphere cells do not have active BMP signalling as indicated by absence of p-Smad1/5/8 antigen (A) which can be detected after addition of BMP ligand 2. This is consistent with GFP up-regulation in neurospheres from SEZ of BRE-eGFP mouse only after Bmp2 addition (D versus C). Scale bar: A-B'' and D-D'', 125µm; C-C'', 75µm.

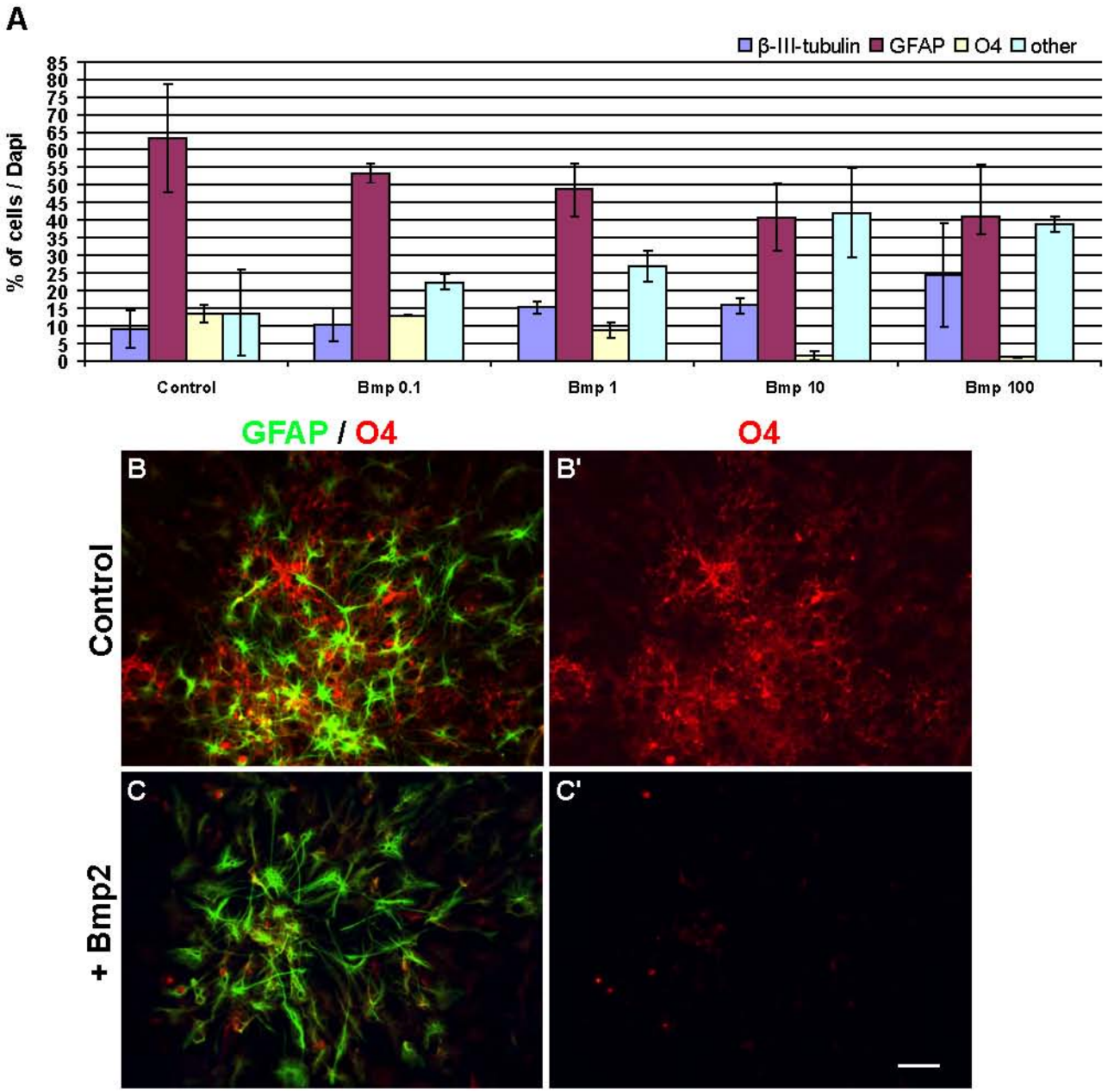

Figure 44. Effects of BMP2 on cell fate decision of differentiating neurospheres

(A) Histogram depicting cell fate decisions of differentiating neurospheres in control and in presence of different concentrations of Bmp2 7 days after plating. for determination of different fates antibodies against βIII-tubulin (for neurons), GFAP (for astrocytes) and O4 (for oligodendrocytes) were used. BMP interferes with Oligodendrogenic fate in a dose dependent manner (yellow bar) while increasing percentage of marker negatif cells (light green). (B-C') Fluorescent micrographs of double staining for GFAP and O4 in differentiated neurospheres without (B-B') or with addition of Bmp2 (C-C'). Note the impaired oligodendrogenesis in C' versus B'. Scale bar: 75µm.

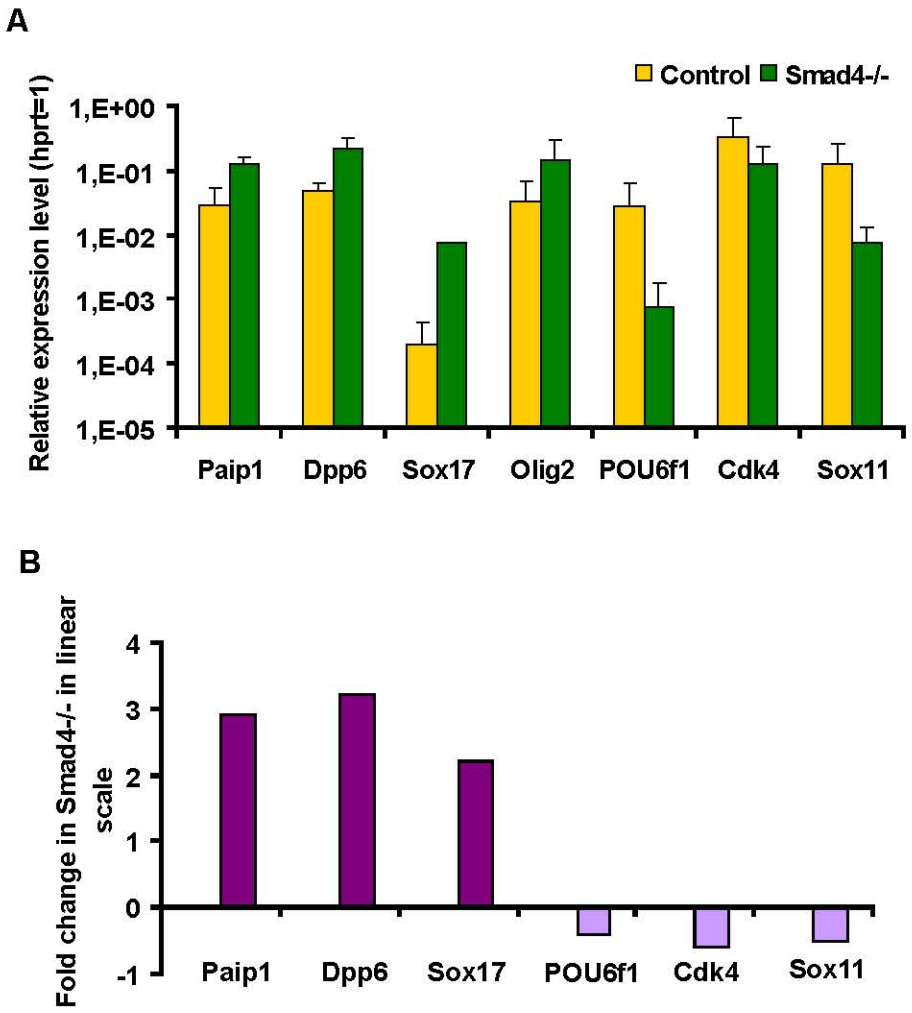

Figure 45. RT-PCR results of differentially expressed genes after Smad4 deletion

Graphics showing the Real Time-PCR results of some of the differentially expressed genes in Smad4-/- SEZ versus control (A) and relative fold changes in Smad4-/- compared to control from microarray analysis (B). Paip1, Dpp6, Sox17, Olig2 mRNA levels are higher in Smad4-/- whereas Pou6f1, Sox11 and Cdk4 mRNA levels are lower compared to control. The decrease in the mRNA levels of Pou6f1 and Sox11 were very prominent as well as the increase in the level of Sox17 in Smad4-/- SEZ.

Table 1 . BMP ligand mutants

Knockout mouse model	Phenotype	References
BMP2	Embryonic lethal; failure to close proamniotic canal; defective cardiac development; role in allantois and PGC development	Ying et al., 2001 Zhang et al., 1996
BMP3	Viable; increased bone density in adult mice	Daluiski et al., 2001
BMP4	Embryonic lethal; most show no mesoderm differentiation; haploinsufficient in C57BL/6 genetic background; no PGCs	Lawson et al., 1999 Winnier et al., 1995 Dunn et al., 1997
BMP5	Viable; skeletal and cartilage abnormalities; role in allantois development and fusion with the chorion along with BMP-7	Solloway et al., 1999 Kingsley et al., 1992 King et al., 1994
BMP6	Viable; slight delay in ossification of sternum; role in cardiac cushion formation and septation along with BMP-7	Solloway et al., 1998 Kim et al., 2001
BMP7	Perinatal lethal; kidney dysgenesis and anophthalmia;skeletal patterning defects; role in cardiac cushion formation and septation along with BMP-6; role in allantois development and fusion with the chorion along with BMP-5	Solloway et al., 1999 Dudley et al., 1995 Kim et al., 2001
BMP8A	Viable; some male infertility due to germ cell degeneration;epididymal epithelial degeneration in a small percentage of males	Zhao et al., 1998 Galloway et al., 2000
BMP8B	Viable; male infertility due to germ cell depletion; defects in PGC and allantois development	Zhao et al., 1996 Ying et al., 2000
BMP-15	Viable; female subfertility; interacting role with GDF-9 in ovarian physiology	Yan et al., 2001

Table 2 . BMP pathway component mutants

Knockout mouse model	Phenotype	References
Activin receptor type IA (ALK2)	Embryonic lethal; gastrulation defective; abnormal visceral endoderm; defective mesoderm formation	Gu et al., 1999 Mishina et al., 1999
BMP type IA receptor (ALK3 or BMPR1A)	Embryonic lethal; no mesoderm formation; role in formation of the apical ectodermal ridge and dorsal-ventral patterning of the limb	Mishina et al., 1995 Ahn et al., 2001
BMP type IB receptor (AL K6)	Viable; defects of chondrogenesis in the limb; female infertility due to defects in ovarian cumulus expansion	Yi et al., 2001 Yi et al., 2000
Activin receptor type IIA (ActR2)	25% die perinatally from craniofacial defects; males have delayed fertility; females are infertile	Matzuk et al., 1995 Matzuk et al., 1996
Activin receptor type IIB (ActR2B)	Perinatal lethal; left-right asymmetry and anteroposterior axis defects	Oh et al., 1997 Song et al., 1999
BMP type II receptor (BMPR2)	Embryonic lethality; arrest at gastrulation	Beppu et al., 2000
Noggin	Perinatal lethal; cartilage hyperplasia; abnormal joint formation	Brunet et al., 1998
SMAD1	Embryonic lethality; death prior to E10.5 due to extraembryonic (allantois formation defect and chorion overproliferation) defects; dramatically reduced or absent PGCs	Tremblay et al., 2001 Lechleider et al., 2001
SMAD4	Embryonic lethal; defective visceral endoderm leading to gastrulation defect	Sirard et al., 1998 Yang et al., 1998
SMAD5	Embryonic lethal; allantois shortening; defective vascular development, ventral closure, cardiac development and craniofacial development; abnormal heart looping and embryonic turning; defective PGC development	Chang et al., 1999 Yang et al., 1999 Chang et al., 2000

Table 3. Smad interacting proteins

Name	Binding to Smads*								Interacting domain†	Signal dependency‡	Description	References
	1	5	8	2	3	4	6	7				
AMSH	□	□	□	□		□	■	■	MH2	YES	cytosolic signalling molecule	Itoh *et al.* 2001
APC (anaphase promoting complex)				■	■	□			MH2	YES	ubiquitin ligase	Stroschein *et al.* 2001
AR (androgen receptor)	◆			□	■	□			ND		transcription activator (nuclear receptor)	Kang *et al.* 2001; Chipuk *et al.* 2002
ARC105	□			■	■	■			MH2	YES	activator-recruited cofactor component	Kato *et al.* 2002
ATF-2					■	■			MH1	YES	transcription activator (b-Zip)	Sano *et al.* 1999
Axin	□	□		■	■	□			MH2	DIS	scaffold protein (Wnt pathway)	Furuhashi *et al.* 2001
BF-1 (brain factor-1)	■			■	■	■			MH2	NO	transcription repressor (winged-helix, c-Qin)	Rodriguez *et al.* 2001
BRCA2				■	■				MH1, 2	YES	tumour suppressor (BRCT domain)	Preobrazhenska *et al.* 2002
Calmodulin	■			■	■	■			MH1		calcium binding protein	Zimmerman *et al.* 1998; Scherer & Graff 2000
CBF-C (CCAAT-binding factor C subunit)				■	■				MH2	NO	transcription activator subunit	Chen *et al.* 2002b
Dab2	□			■	■	□		□	MH2	YES	cytosolic signalling molecule (PTB domain)	Hocevar *et al.* 2001
Dok-1				■	■	■			MH2	YES	ras-GAP binding protein	Yamakawa *et al.* 2002
E1A	■			■	■	□	□		MH2	NO	viral oncoprotein (adenovirus)	Nishihara *et al.* 1999
E2F4/5					■				MH2	NO	transcription repressor	Chen *et al.* 2002a
E7				■	■				MH1	NO	viral oncoprotein (human papilloma virus)	Lee *et al.* 2002b
ER (oestrogen receptor)				■	■	■			MH2	YES	transcription activator (nuclear receptor)	Matsuda *et al.* 2001
Evi-1	□			□	■	□			MH2	YES	co-repressor (Zn finger protein, recruiting HDAC)	Kurokawa *et al.* 1998
FAST1	□			■	■	□			MH2	YES	transcription activator (winged-helix)	Chen *et al.* 1997
FAST2	□			■					ND	YES	transcription activator (winged-helix)	Labbé *et al.* 1998; Liu *et al.* 1999
Filamin	■	■		■		■	■		MH1, L		cytoskeletal actin binding protein	Sasaki *et al.* 2001
GATA-3	◆			□	■	□			MH1		transcription activator (b-Zip)	Blokzijl *et al.* 2002
Gli3 (C-terminally truncated)	■			■	■	■			ND	DIS	transcription repressor (Zn finger, HH target)	Liu *et al.* 1998
GR (glucocorticoid receptor)					■	□			MH2		transcription activator (nuclear receptor)	Song *et al.* 1999
HEF1	◆			■	■	□			MH1, 2		cytoplasmic docking protein (Cas family)	Liu *et al.* 2000
HNF4					■	◆			ND	YES	transcription activator (nuclear receptor, orphan)	Kardassis *et al.* 2000
Hoxa-9				□	□	■			ND	NO	transcription repressor (homeodomain)	Shi *et al.* 2001
Hoxc-8	■					■	■	□	MH2	YES	transcription repressor (homeodomain)	Shi *et al.* 1999; Bai *et al.* 2000
Hrs	■			■	■	□	□		ND	NO	intracellular trafficking (FYVE domain protein)	Miura *et al.* 2000
Importin-b1				□	■	□			MH1	YES	intracellular trafficking	Xiao *et al.* 2000; Kurisaki *et al.* 2001
Jab1					□	■			MH2, L		co-activator (for AP1)	Wan *et al.* 2002
c-Jun				■	■	◆			MH1	YES	transcription activator (b-Zip)	Zhang *et al.* 1998; Liberati *et al.* 1999
JunB	◆	◆		◆	■	■			ND		transcription activator (b-Zip)	Liberati *et al.* 1999
JunD					■	■			ND		transcription activator (b-Zip)	Liberati *et al.* 1999
Lef1	◆			■	■	■			MH1, 2	YES	transcription activator (HMG domain, Wnt target)	Nishita *et al.* 2000; Labbé *et al.* 2000
MEF2				■					ND		transcription activator	Quinn *et al.* 2001
Menin (MEN1 gene product)				□	■	□			MH2	?	tumour suppressor	Kaji *et al.* 2001
Mixer				■		□			MH2	YES	transcription activator (homeodomain)	Germain *et al.* 2000
Milk				■					MH2		transcription activator (homeodomain)	Germain *et al.* 2000
MSG1						■			MH2, L	YES	co-activator	Shioda *et al.* 1998
Msx-1	■			■		■			ND		homeobox protein	Yamamoto *et al.* 2001
c-Myc	□			■	■	□			MH2	YES	transcription activator (bHLH-Zip)	Feng *et al.* 2002
MyoD	■			□	■	□			MH1?		transcription activator (bHLH)	Liu *et al.* 2001
NF-κB					■	□			ND	YES	transcription activator	Lopez-Rovira *et al.* 2000
OAZ	■			□		□			MH2	YES	DNA-binding Zn finger protein	Hata *et al.* 2000
p300/CBP	◆			■	■	◆		□	MH2	YES	co-activator (HAT)	Six references§
P/CAF				◆	■				MH2	NO	co-activator (HAT)	Itoh *et al.* 2000
PPARγ					■	□			ND		transcription activator (nuclear receptor)	Fu *et al.* 2001

continued overleaf

Table 3 continued

Name	Binding to Smads* 1	5	8	2	3	4	6	7	Interacting domain†	Signal dependency‡	Description	References
pX	□			□	□	■			MH1	NO	viral oncoprotein (Hepatitis B viras)	Lee *et al.* 2001
Rb (p107)					■				MH2	NO	tumour suppressor	Chen *et al.* 2002a
ROC1					■				MH2	YES	ubiquitin ligase component (SCF complex)	Fukuchi *et al.* 2001
Runx1	■	■		■	■	■			MH2	YES	transcription activator (Runt domain)	Hanai *et al.* 1999; Pardali *et al.* 2000a
Runx2	■	■		■	■				ND	YES	transcription activator (Runt domain)	Hanai *et al.* 1999
Runx3	■	■		■	■				MH2	YES	transcription activator (Runt domain)	Hanai *et al.* 1999
SARA	□			■	■	□	□	□	MH2	DIS	scaffold protein (FVYE domain protein)	Tsukazaki *et al.* 1998
SIP1 (Smad interacting protein 1)	■	■		■	■	□			MH2	YES	transcription repressor (Zn finger-homeodomain)	Verschueren *et al.* 1999
c-Ski	◆	◆		■	■	■	□	□	MH2	YES	co-repressor (recruiting HDAC)	Akiyoshi *et al.* 1999; Wang *et al.* 2000
SnoN				■	■	■			MH2		co-repressor (recruiting HDAC)	Stroschein *et al.* 1999
SKIP (Ski-interacting protein)				■	■				MH2 +α	?	co-activator	Leong *et al.* 2001
SNIP (Smad nuclear interacting protein)	◆	□		◆	□	■			MH2	YES	p300 inhibitor	Kim *et al.* 2000
SMIF	□	□		□	□	■	□		L	YES	co-activator	Bai *et al.* 2002
Smurf1	■	■	□	□	□	□	■	■	L(PY)	NO	ubiquitin ligase (HECT type)	Zhu *et al.* 1999; Ebisawa *et al.* 2001
Smurf2	■			■	■	□			L(PY)	NO	ubiquitin ligase (HECT type)	Lin *et al.* 2000; Zhang *et al.* 2001
Sp1	□			■	■	■	□	□	MH1(2), 2(4)	YES	transcription activator (Zn finger)	Pardali *et al.* 2000b; Datta *et al.* 2000; Feng *et al.* 2000
STRAP	□			◆	◆	□	◆	■	ND		TβRI, II-associated protein	Datta & Moses 2000
Swift	◆			■					ND	YES	co-activator (BRCT domain)	Shimizu *et al.* 2001
TAK1							■		ND		MAPKKK	Kimura *et al.* 2000
Tax				■	■	■			MH2	YES	viral oncoprotein (HTLV)	Lee *et al.* 2002a
TFE3					■	■			MH1	YES	transcription activator (bHLH-Zip)	Hua *et al.* 1999
TGIF	◆			■	■	□			ND	YES	co-repressor (recruiting HDAC)	Wotton *et al.* 1999
Tob	■	■		□	□	■			MH2	YES	anti-proliferative protein	Yoshida *et al.* 2000
TRAP1	□	□	□	□	□	■	□	□	MH1 +α	YES	TβRI-associated protein	Wurthner *et al.* 2001
tublin				■	■	■			ND	DIS	microtubules	Dong *et al.* 2000
VDR (vitamin D receptor)				□	■				MH1		transcription activator (nuclear receptor)	Yanagisawa *et al.* 1999
Vent-2	■				□	□			MH1		transcription activator/repressor (homeodomain)	Henningfeld *et al.* 2002
WBSCR11				■	■				MH1	YES	transcription activator (bHLH)	Ring *et al.* 2002
YAP65 (Yes-associated protein)							□	■	L(PY) +α		WW domain protein	Ferrigno *et al.* 2002

*■, interaction positive; ◆, weak or non-functional interaction; □, no interaction.
†ND, not deermined; PY, PY motif; +α, other unidentified binding site(s).
‡DIS, interaction dissociated upon receptor activation.
§Feng *et al.* (1998); Janknecht *et al.* (1998); Nishihara *et al.* (1998); Pouponnot *et al.* (1998); Shen *et al.* (1998); Topper *et al.* (1998).

9 REFERENCES

Adolf, B., Chapouton, P., Lam, C. S., Topp, S., Tannhauser, B., Strahle, U., Gotz, M. and Bally-Cuif, L. (2006). Conserved and acquired features of adult neurogenesis in the zebrafish telencephalon. *Dev Biol* **295**, 278-93.

Ahn, K., Mishina, Y., Hanks, M. C., Behringer, R. R. and Crenshaw, E. B., 3rd. (2001). BMPR-IA signaling is required for the formation of the apical ectodermal ridge and dorsal-ventral patterning of the limb. *Development* **128**, 4449-61.

Ahn, S. and Joyner, A. L. (2005). In vivo analysis of quiescent adult neural stem cells responding to Sonic hedgehog. *Nature* **437**, 894-7.

Alder, J., Lee, K. J., Jessell, T. M. and Hatten, M. E. (1999). Generation of cerebellar granule neurons in vivo by transplantation of BMP-treated neural progenitor cells. *Nat Neurosci* **2**, 535-40.

Alonso, G. (2005). NG2 proteoglycan-expressing cells of the adult rat brain: possible involvement in the formation of glial scar astrocytes following stab wound. *Glia* **49**, 318-38.

Altman, J. (1963). Autoradiographic investigation of cell proliferation in the brains of rats and cats. *Anat Rec* **145**, 573-91.

Altman, J. (1969). Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J Comp Neurol* **137**, 433-57.

Altman, J. and Das, G. D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol* **124**, 319-35.

Altman, J. and Das, G. D. (1965). Post-natal origin of microneurones in the rat brain. *Nature* **207**, 953-6.

Alvarez-Buylla, A., Buskirk, D. R. and Nottebohm, F. (1987). Monoclonal antibody reveals radial glia in adult avian brain. *J Comp Neurol* **264**, 159-70.

Alvarez-Buylla, A., Garcia-Verdugo, J. M., Mateo, A. S. and Merchant-Larios, H. (1998). Primary neural precursors and intermitotic nuclear migration in the ventricular zone of adult canaries. *J Neurosci* **18**, 1020-37.

Alvarez-Buylla, A., Garcia-Verdugo, J. M. and Tramontin, A. D. (2001). A unified hypothesis on the lineage of neural stem cells. *Nat Rev Neurosci* **2**, 287-93.

Alvarez-Buylla, A. and Lim, D. A. (2004). For the long run: maintaining germinal niches in the adult brain. *Neuron* **41**, 683-6.

Alvarez-Buylla, A., Ling, C. Y. and Yu, W. S. (1994). Contribution of neurons born during embryonic, juvenile, and adult life to the brain of adult canaries: regional specificity and delayed birth of neurons in the song-control nuclei. *J Comp Neurol* **347**, 233-48.

Alvarez-Buylla, A. and Nottebohm, F. (1988). Migration of young neurons in adult avian brain. *Nature* **335**, 353-4.

Alvarez-Buylla, A., Theelen, M. and Nottebohm, F. (1990). Proliferation "hot spots" in adult avian ventricular zone reveal radial cell division. *Neuron* **5**, 101-9.

Anderson, R. M., Lawrence, A. R., Stottmann, R. W., Bachiller, D. and Klingensmith, J. (2002). Chordin and noggin promote organizing centers of forebrain development in the mouse. *Development* **129**, 4975-87.

Androutsellis-Theotokis, A., Leker, R. R., Soldner, F., Hoeppner, D. J., Ravin, R., Poser, S. W., Rueger, M. A., Bae, S. K., Kittappa, R. and McKay, R. D. (2006). Notch signalling regulates stem cell numbers in vitro and in vivo. *Nature* **442**, 823-6.

Androutsellis-Theotokis, A., Leker, R. R., Soldner, F., Hoeppner, D. J., Ravin, R., Poser, S. W., Rueger, M. A., Bae, S. K., Kittappa, R. and McKay, R. D. (2006). Notch signalling regulates stem cell numbers in vitro and in vivo. *Nature* **442**, 823-6.

Angley, C., Kumar, M., Dinsio, K. J., Hall, A. K. and Siegel, R. E. (2003). Signaling by bone morphogenetic proteins and Smad1 modulates the postnatal differentiation of cerebellar cells. *J Neurosci* **23**, 260-8.

Artavanis-Tsakonas, S., Rand, M. D. and Lake, R. J. (1999). Notch signaling: cell fate control and signal integration in development. *Science* **284**, 770-6.

Attisano, L. and Wrana, J. L. (2002). Signal transduction by the TGF-beta superfamily. *Science* **296**, 1646-7.

Aubin, J., Davy, A. and Soriano, P. (2004). In vivo convergence of BMP and MAPK signaling pathways: impact of differential Smad1 phosphorylation on development and homeostasis. *Genes Dev* **18**, 1482-94.

Bachiller, D., Klingensmith, J., Kemp, C., Belo, J. A., Anderson, R. M., May, S. R., McMahon, J. A., McMahon, A. P., Harland, R. M., Rossant, J. et al. (2000). The organizer factors Chordin and Noggin are required for mouse forebrain development. *Nature* **403**, 658-61.

Baffi, M. O., Slattery, E., Sohn, P., Moses, H. L., Chytil, A. and Serra, R. (2004). Conditional deletion of the TGF-beta type II receptor in Col2a expressing cells results in defects in the axial skeleton without alterations in chondrocyte differentiation or embryonic development of long bones. *Dev Biol* **276**, 124-42.

Balordi, F. and Fishell, G. (2007). Hedgehog signaling in the subventricular zone is required for both the maintenance of stem cells and the migration of newborn neurons. *J Neurosci* **27**, 5936-47.

Barami, K., Iversen, K., Furneaux, H. and Goldman, S. A. (1995). Hu protein as an early marker of neuronal phenotypic differentiation by subependymal zone cells of the adult songbird forebrain. *J Neurobiol* **28**, 82-101.

Bauer, S. and Patterson, P. H. (2006). Leukemia inhibitory factor promotes neural stem cell self-renewal in the adult brain. *J Neurosci* **26**, 12089-99.

Bayer, S. A. (1982). Changes in the total number of dentate granule cells in juvenile and adult rats: a correlated volumetric and 3H-thymidine autoradiographic study. *Exp Brain Res* **46**, 315-23.

Bayer, S. A., Yackel, J. W. and Puri, P. S. (1982). Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life. *Science* **216**, 890-2.

Bentivoglio, M. and Mazzarello, P. (1999). The history of radial glia. *Brain Res Bull* **49**, 305-15.

Beppu, H., Kawabata, M., Hamamoto, T., Chytil, A., Minowa, O., Noda, T. and Miyazono, K. (2000). BMP type II receptor is required for gastrulation and early development of mouse embryos. *Dev Biol* **221**, 249-58.

Bergsland, M., Werme, M., Malewicz, M., Perlmann, T. and Muhr, J. (2006). The establishment of neuronal properties is controlled by Sox4 and Sox11. *Genes Dev* **20**, 3475-86.

Bertrand, N., Castro, D. S. and Guillemot, F. (2002). Proneural genes and the specification of neural cell types. *Nat Rev Neurosci* **3**, 517-30.

Blakemore, W. F. and Jolly, R. D. (1972). The subependymal plate and associated ependyma in the dog. An ultrastructural study. *J Neurocytol* **1**, 69-84.

Brunet, L. J., McMahon, J. A., McMahon, A. P. and Harland, R. M. (1998). Noggin, cartilage morphogenesis, and joint formation in the mammalian skeleton. *Science* **280**, 1455-7.

Buffo, A., Vosko, M. R., Erturk, D., Hamann, G. F., Jucker, M., Rowitch, D. and Gotz, M. (2005). Expression pattern of the transcription factor Olig2 in response to brain injuries: implications for neuronal repair. *Proc Natl Acad Sci U S A* **102**, 18183-8.

Bunge, R. P. (1968). Glial cells and the central myelin sheath. *Physiol Rev* **48**, 197-251.

Bylund, M., Andersson, E., Novitch, B. G. and Muhr, J. (2003). Vertebrate neurogenesis is counteracted by Sox1-3 activity. *Nat Neurosci* **6**, 1162-8.

Cameron, R. S. and Rakic, P. (1991). Glial cell lineage in the cerebral cortex: a review and synthesis. *Glia* **4**, 124-37.

Capela, A. and Temple, S. (2002). LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal. *Neuron* **35**, 865-75.

Carleton, A., Petreanu, L. T., Lansford, R., Alvarez-Buylla, A. and Lledo, P. M. (2003). Becoming a new

neuron in the adult olfactory bulb. *Nat Neurosci* **6**, 507-18.

Cau, E., Gradwohl, G., Fode, C. and Guillemot, F. (1997). Mash1 activates a cascade of bHLH regulators in olfactory neuron progenitors. *Development* **124**, 1611-21.

Cayre, M., Buckingham, S. D., Strambi, A., Strambi, C. and Sattelle, D. B. (1998). Adult insect mushroom body neurons in primary culture: cell morphology and characterization of potassium channels. *Cell Tissue Res* **291**, 537-47.

Chang, H., Huylebroeck, D., Verschueren, K., Guo, Q., Matzuk, M. M. and Zwijsen, A. (1999). Smad5 knockout mice die at mid-gestation due to multiple embryonic and extraembryonic defects. *Development* **126**, 1631-42.

Chang, H., Zwijsen, A., Vogel, H., Huylebroeck, D. and Matzuk, M. M. (2000). Smad5 is essential for left-right asymmetry in mice. *Dev Biol* **219**, 71-8.

Chen, D., Ji, X., Harris, M. A., Feng, J. Q., Karsenty, G., Celeste, A. J., Rosen, V., Mundy, G. R. and Harris, S. E. (1998). Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages. *J Cell Biol* **142**, 295-305.

Chen, J., Leong, S. Y. and Schachner, M. (2005). Differential expression of cell fate determinants in neurons and glial cells of adult mouse spinal cord after compression injury. *Eur J Neurosci* **22**, 1895-906.

Chiasson, B. J., Tropepe, V., Morshead, C. M. and van der Kooy, D. (1999). Adult mammalian forebrain ependymal and subependymal cells demonstrate proliferative potential, but only subependymal cells have neural stem cell characteristics. *J Neurosci* **19**, 4462-71.

Chimal-Monroy, J., Rodriguez-Leon, J., Montero, J. A., Ganan, Y., Macias, D., Merino, R. and Hurle, J. M. (2003). Analysis of the molecular cascade responsible for mesodermal limb chondrogenesis: Sox genes and BMP signaling. *Dev Biol* **257**, 292-301.

Chojnacki, A. and Weiss, S. (2004). Isolation of a novel platelet-derived growth factor-responsive precursor from the embryonic ventral forebrain. *J Neurosci* **24**, 10888-99.

Chytil, A., Magnuson, M. A., Wright, C. V. and Moses, H. L. (2002). Conditional inactivation of the TGF-beta type II receptor using Cre:Lox. *Genesis* **32**, 73-5.

Cohen, E. and Meininger, V. (1987). Ultrastructural analysis of primary cilium in the embryonic nervous tissue of mouse. *Int J Dev Neurosci* **5**, 43-51.

Connors, B. W. and Ransom, B. R. (1987). Electrophysiological properties of ependymal cells (radial glia) in dorsal cortex of the turtle, Pseudemys scripta. *J Physiol* **385**, 287-306.

Conover, J. C., Doetsch, F., Garcia-Verdugo, J. M., Gale, N. W., Yancopoulos, G. D. and Alvarez-Buylla, A. (2000). Disruption of Eph/ephrin signaling affects migration and proliferation in the adult subventricular zone. *Nat Neurosci* **3**, 1091-7.

Coskun, V., Venkatraman, G., Yang, H., Rao, M. S. and Luskin, M. B. (2001). Retroviral manipulation of the expression of bone morphogenetic protein receptor Ia by SVZa progenitor cells leads to changes in their p19(INK4d) expression but not in their
neuronal commitment. *Int J Dev Neurosci* **19**, 219-27.

Craig, C. G., Tropepe, V., Morshead, C. M., Reynolds, B. A., Weiss, S. and van der Kooy, D. (1996). In vivo growth factor expansion of endogenous subependymal neural precursor cell populations in the adult mouse brain. *J Neurosci* **16**, 2649-58.

Cui, H. and Bulleit, R. F. (1998). Expression of the POU transcription factor Brn-5 is an early event in the terminal differentiation of CNS neurons. *J Neurosci Res* **52**, 625-32.

Cunningham, N. S., Paralkar, V. and Reddi, A. H. (1992). Osteogenin and recombinant bone morphogenetic protein 2B are chemotactic for human monocytes and stimulate transforming growth factor beta 1 mRNA expression. *Proc Natl Acad Sci U S A* **89**, 11740-4.

Curtis, M. A., Kam, M., Nannmark, U., Anderson, M. F., Axell, M. Z., Wikkelso, C., Holtas, S., van Roon-Mom, W. M., Bjork-Eriksson, T., Nordborg, C. et al. (2007). Human neuroblasts migrate to the olfactory

bulb via a lateral ventricular extension. *Science* **315**, 1243-9.

Daluiski, A., Engstrand, T., Bahamonde, M. E., Gamer, L. W., Agius, E., Stevenson, S. L., Cox, K., Rosen, V. and Lyons, K. M. (2001). Bone morphogenetic protein-3 is a negative regulator of bone density. *Nat Genet* **27**, 84-8.

Das, G. D. and Altman, J. (1970). Postnatal neurogenesis in the caudate nucleus and nucleus accumbens septi in the rat. *Brain Res* **21**, 122-7.

Dawson, M. R., Polito, A., Levine, J. M. and Reynolds, R. (2003). NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. *Mol Cell Neurosci* **24**, 476-88.

Decker, L., Picard-Riera, N., Lachapelle, F. and Baron-Van Evercooren, A. (2002). Growth factor treatment promotes mobilization of young but not aged adult subventricular zone precursors in response to demyelination. *J Neurosci Res* **69**, 763-71.

Derynck, R. and Zhang, Y. E. (2003). Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* **425**, 577-84.

Dick, A., Hild, M., Bauer, H., Imai, Y., Maifeld, H., Schier, A. F., Talbot, W. S., Bouwmeester, T. and Hammerschmidt, M. (2000). Essential role of Bmp7 (snailhouse) and its prodomain in dorsoventral patterning of the zebrafish embryo. *Development* **127**, 343-54.

Doetsch, F. (2003). The glial identity of neural stem cells. *Nat Neurosci* **6**, 1127-34.

Doetsch, F. and Alvarez-Buylla, A. (1996). Network of tangential pathways for neuronal migration in adult mammalian brain. *Proc Natl Acad Sci U S A* **93**, 14895-900.

Doetsch, F., Caille, I., Lim, D. A., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (1999a). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* **97**, 703-16.

Doetsch, F., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (1997b). Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J Neurosci* **17**, 5046-61.

Doetsch, F., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (1999). Regeneration of a germinal layer in the adult mammalian brain. *Proc Natl Acad Sci U S A* **96**, 11619-24.

Doetsch, F., Petreanu, L., Caille, I., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (2002). EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. *Neuron* **36**, 1021-34.

Dudley, A. T., Lyons, K. M. and Robertson, E. J. (1995). A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes Dev* **9**, 2795-807.

Dunn, N. R., Winnier, G. E., Hargett, L. K., Schrick, J. J., Fogo, A. B. and Hogan, B. L. (1997). Haploinsufficient phenotypes in Bmp4 heterozygous null mice and modification by mutations in Gli3 and Alx4. *Dev Biol* **188**, 235-47.

Ebendal, T., Bengtsson, H. and Soderstrom, S. (1998). Bone morphogenetic proteins and their receptors: potential functions in the brain. *J Neurosci Res* **51**, 139-46.

Echelard, Y., Epstein, D. J., St-Jacques, B., Shen, L., Mohler, J., McMahon, J. A. and McMahon, A. P. (1993). Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* **75**, 1417-30.

Echeverri, K. and Tanaka, E. M. (2002). Ectoderm to mesoderm lineage switching during axolotl tail regeneration. *Science* **298**, 1993-6.

Ericson, J., Briscoe, J., Rashbass, P., van Heyningen, V. and Jessell, T. M. (1997). Graded sonic hedgehog signaling and the specification of cell fate in the ventral neural tube. *Cold Spring Harb Symp Quant Biol* **62**, 451-66.

Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A. and Gage, F. H. (1998). Neurogenesis in the adult human hippocampus. *Nat Med* **4**, 1313-7.

Fan, X., Xu, H., Cai, W., Yang, Z. and Zhang, J. (2003). Spatial and temporal patterns of expression of Noggin and BMP4 in embryonic and postnatal rat hippocampus. *Brain Res Dev Brain Res* **146**, 51-8.

Farah, M. H., Olson, J. M., Sucic, H. B., Hume, R. I., Tapscott, S. J. and Turner, D. L. (2000). Generation of neurons by transient expression of neural bHLH proteins in mammalian cells. *Development* **127**, 693-702.

Farlie, P. G., McKeown, S. J. and Newgreen, D. F. (2004). The neural crest: basic biology and clinical relationships in the craniofacial and enteric nervous systems. *Birth Defects Res C Embryo Today* **72**, 173-89.

Feil, R., Brocard, J., Mascrez, B., LeMeur, M., Metzger, D. and Chambon, P. (1996). Ligand-activated site-specific recombination in mice. *Proc Natl Acad Sci U S A* **93**, 10887-90.

Fernandez-Lloris, R., Vinals, F., Lopez-Rovira, T., Harley, V., Bartrons, R., Rosa, J. L. and Ventura, F. (2003). Induction of the Sry-related factor SOX6 contributes to bone morphogenetic protein-2-induced chondroblastic differentiation of C3H10T1/2 cells. *Mol Endocrinol* **17**, 1332-43.

Ferri, A. L., Cavallaro, M., Braida, D., Di Cristofano, A., Canta, A., Vezzani, A., Ottolenghi, S., Pandolfi, P. P., Sala, M., DeBiasi, S. et al. (2004). Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain. *Development* **131**, 3805-19.

Forde, J. E. and Dale, T. C. (2007). Glycogen synthase kinase 3: a key regulator of cellular fate. *Cell Mol Life Sci* **64**, 1930-44.

Fujii, M., Takeda, K., Imamura, T., Aoki, H., Sampath, T. K., Enomoto, S., Kawabata, M., Kato, M., Ichijo, H. and Miyazono, K. (1999). Roles of bone morphogenetic protein type I receptors and Smad proteins in osteoblast and
chondroblast differentiation. *Mol Biol Cell* **10**, 3801-13.

Fukuda, S., Abematsu, M., Mori, H., Yanagisawa, M., Kagawa, T., Nakashima, K., Yoshimura, A. and Taga, T. (2007). Potentiation of astrogliogenesis by STAT3-mediated activation of bone morphogenetic protein-Smad signaling in neural stem cells. *Mol Cell Biol* **27**, 4931-7.

Fukuda, S. and Taga, T. (2005). Cell fate determination regulated by a transcriptional signal network in the developing mouse brain. *Anat Sci Int* **80**, 12-8.

Fuller, M. L., DeChant, A. K., Rothstein, B., Caprariello, A., Wang, R., Hall, A. K. and Miller, R. H. (2007). Bone morphogenetic proteins promote gliosis in demyelinating spinal cord lesions. *Ann Neurol* **62**, 288-300.

Furuta, Y., Piston, D. W. and Hogan, B. L. (1997). Bone morphogenetic proteins (BMPs) as regulators of dorsal forebrain development. *Development* **124**, 2203-12.

Gadisseux, J. F., Evrard, P., Misson, J. P. and Caviness, V. S. (1989). Dynamic structure of the radial glial fiber system of the developing murine cerebral wall. An immunocytochemical analysis. *Brain Res Dev Brain Res* **50**, 55-67.

Gage, F. H. (2000). Mammalian neural stem cells. *Science* **287**, 1433-8.

Gage, F. H., Coates, P. W., Palmer, T. D., Kuhn, H. G., Fisher, L. J., Suhonen, J. O., Peterson, D. A., Suhr, S. T. and Ray, J. (1995). Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. *Proc Natl Acad Sci U S A* **92**, 11879-83.

Gaiano, N., Nye, J. S. and Fishell, G. (2000). Radial glial identity is promoted by Notch1 signaling in the murine forebrain. *Neuron* **26**, 395-404.

Galloway, S. M., McNatty, K. P., Cambridge, L. M., Laitinen, M. P., Juengel, J. L., Jokiranta, T. S., McLaren, R. J., Luiro, K., Dodds, K. G., Montgomery, G. W. et al. (2000). Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nat Genet* **25**, 279-83.

Garces, A., Haase, G., Airaksinen, M. S., Livet, J., Filippi, P. and deLapeyriere, O. (2000). GFRalpha 1 is required for development of distinct subpopulations of motoneuron. *J Neurosci* **20**, 4992-5000.

Garcia-Verdugo, J. M., Doetsch, F., Wichterle, H., Lim, D. A. and Alvarez-Buylla, A. (1998). Architecture and cell types of the adult subventricular zone: in search of the stem cells. *J Neurobiol* **36**, 234-48.

Garcia-Verdugo, J. M., Ferron, S., Flames, N., Collado, L., Desfilis, E. and Font, E. (2002). The proliferative ventricular zone in adult vertebrates: a comparative study using reptiles, birds, and mammals. *Brain*

Res Bull **57**, 765-75.

Gates, M. A., Thomas, L. B., Howard, E. M., Laywell, E. D., Sajin, B., Faissner, A., Gotz, B., Silver, J. and Steindler, D. A. (1995). Cell and molecular analysis of the developing and adult mouse subventricular zone of the cerebral hemispheres. *J Comp Neurol* **361**, 249-66.

Gilboa, L., Nohe, A., Geissendorfer, T., Sebald, W., Henis, Y. I. and Knaus, P. (2000). Bone morphogenetic protein receptor complexes on the surface of live cells: a new oligomerization mode for serine/threonine kinase receptors. *Mol Biol Cell* **11**, 1023-35.

Givogri, M. I., de Planell, M., Galbiati, F., Superchi, D., Gritti, A., Vescovi, A., de Vellis, J. and Bongarzone, E. R. (2006). Notch signaling in astrocytes and neuroblasts of the adult subventricular zone in health and after cortical injury. *Dev Neurosci* **28**, 81-91.

Glozak, M. A. and Rogers, M. B. (1996). Specific induction of apoptosis in P19 embryonal carcinoma cells by retinoic acid and BMP2 or BMP4. *Dev Biol* **179**, 458-70.

Goldman, S. A. and Nottebohm, F. (1983). Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. *Proc Natl Acad Sci U S A* **80**, 2390-4.

Gomes, W. A., Mehler, M. F. and Kessler, J. A. (2003). Transgenic overexpression of BMP4 increases astroglial and decreases oligodendroglial lineage commitment. *Dev Biol* **255**, 164-77.

Gould, E. (1999). Serotonin and hippocampal neurogenesis. *Neuropsychopharmacology* **21**, 46S-51S.

Goumans, M. J., Valdimarsdottir, G., Itoh, S., Rosendahl, A., Sideras, P. and ten Dijke, P. (2002). Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors. *Embo J* **21**, 1743-53.

Graham, A., Francis-West, P., Brickell, P. and Lumsden, A. (1994). The signalling molecule BMP4 mediates apoptosis in the rhombencephalic neural crest. *Nature* **372**, 684-6.

Graham, A., Koentges, G. and Lumsden, A. (1996). Neural Crest Apoptosis and the Establishment of Craniofacial Pattern: An Honorable Death. *Mol Cell Neurosci* **8**, 76-83.

Grandel, H., Kaslin, J., Ganz, J., Wenzel, I. and Brand, M. (2006). Neural stem cells and neurogenesis in the adult zebrafish brain: origin, proliferation dynamics, migration and cell fate. *Dev Biol* **295**, 263-77.

Gregori, N., Proschel, C., Noble, M. and Mayer-Proschel, M. (2002). The tripotential glial-restricted precursor (GRP) cell and glial development in the spinal cord: generation of bipotential oligodendrocyte-type-2 astrocyte progenitor cells and dorsal-ventral differences in GRP cell function. *J Neurosci* **22**, 248-56.

Grinspan, J. B., Edell, E., Carpio, D. F., Beesley, J. S., Lavy, L., Pleasure, D. and Golden, J. A. (2000). Stage-specific effects of bone morphogenetic proteins on the oligodendrocyte lineage. *J Neurobiol* **43**, 1-17.

Gritti, A., Frolichsthal-Schoeller, P., Galli, R., Parati, E. A., Cova, L., Pagano, S. F., Bjornson, C. R. and Vescovi, A. L. (1999). Epidermal and fibroblast growth factors behave as mitogenic regulators for a single multipotent stem cell-like population from the subventricular region of the adult mouse forebrain. *J Neurosci* **19**, 3287-97.

Gritti, A., Parati, E. A., Cova, L., Frolichsthal, P., Galli, R., Wanke, E., Faravelli, L., Morassutti, D. J., Roisen, F., Nickel, D. D. et al. (1996). Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. *J Neurosci* **16**, 1091-100.

Gross, R. E., Mehler, M. F., Mabie, P. C., Zang, Z., Santschi, L. and Kessler, J. A. (1996). Bone morphogenetic proteins promote astroglial lineage commitment by mammalian subventricular zone progenitor cells. *Neuron* **17**, 595-606.

Gu, Z., Reynolds, E. M., Song, J., Lei, H., Feijen, A., Yu, L., He, W., MacLaughlin, D. T., van den Eijnden-van Raaij, J., Donahoe, P. K. et al. (1999). The type I serine/threonine kinase receptor ActRIA (ALK2) is required for gastrulation of the mouse embryo. *Development* **126**, 2551-61.

Gulacsi, A. and Lillien, L. (2003). Sonic hedgehog and bone morphogenetic protein regulate interneuron development from dorsal telencephalic progenitors in vitro. *J Neurosci* **23**, 9862-72.

Hack, M. A., Saghatelyan, A., de Chevigny, A., Pfeifer, A., Ashery-Padan, R., Lledo, P. M. and Gotz, M.

(2005). Neuronal fate determinants of adult olfactory bulb neurogenesis. *Nat Neurosci* **8**, 865-72.

Hacker, C., Kirsch, R. D., Ju, X. S., Hieronymus, T., Gust, T. C., Kuhl, C., Jorgas, T., Kurz, S. M., Rose-John, S., Yokota, Y. et al. (2003). Transcriptional profiling identifies Id2 function in dendritic cell development. *Nat Immunol* **4**, 380-6.

Hampton, D. W., Asher, R. A., Kondo, T., Steeves, J. D., Ramer, M. S. and Fawcett, J. W. (2007). A potential role for bone morphogenetic protein signalling in glial cell fate determination following adult central nervous system injury in vivo. *Eur J Neurosci* **26**, 3024-35.

Han, S. S., Kang, D. Y., Mujtaba, T., Rao, M. S. and Fischer, I. (2002). Grafted lineage-restricted precursors differentiate exclusively into neurons in the adult spinal cord. *Exp Neurol* **177**, 360-75.

Hanyu, A., Ishidou, Y., Ebisawa, T., Shimanuki, T., Imamura, T. and Miyazono, K. (2001). The N domain of Smad7 is essential for specific inhibition of transforming growth factor-beta signaling. *J Cell Biol* **155**, 1017-27.

Hardy, R. J. and Friedrich, V. L., Jr. (1996). Oligodendrocyte progenitors are generated throughout the embryonic mouse brain, but differentiate in restricted foci. *Development* **122**, 2059-69.

Hawley, S. H., Wunnenberg-Stapleton, K., Hashimoto, C., Laurent, M. N., Watabe, T., Blumberg, B. W. and Cho, K. W. (1995). Disruption of BMP signals in embryonic Xenopus ectoderm leads to direct neural induction. *Genes Dev* **9**, 2923-35.

Hebert, J. M., Mishina, Y. and McConnell, S. K. (2002). BMP signaling is required locally to pattern the dorsal telencephalic midline. *Neuron* **35**, 1029-41.

Heldin, C. H., Miyazono, K. and ten Dijke, P. (1997). TGF-beta signalling from cell membrane to nucleus through SMAD proteins. *Nature* **390**, 465-71.

Higuchi, M., Kiyama, H., Hayakawa, T., Hamada, Y. and Tsujimoto, Y. (1995). Differential expression of Notch1 and Notch2 in developing and adult mouse brain. *Brain Res Mol Brain Res* **29**, 263-72.

Hild, M., Dick, A., Rauch, G. J., Meier, A., Bouwmeester, T., Haffter, P. and Hammerschmidt, M. (1999). The smad5 mutation somitabun blocks Bmp2b signaling during early dorsoventral patterning of the zebrafish embryo. *Development* **126**, 2149-59.

Hitoshi, S., Alexson, T., Tropepe, V., Donoviel, D., Elia, A. J., Nye, J. S., Conlon, R. A., Mak, T. W., Bernstein, A. and van der Kooy, D. (2002). Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. *Genes Dev* **16**, 846-58.

Hitoshi, S., Alexson, T., Tropepe, V., Donoviel, D., Elia, A. J., Nye, J. S., Conlon, R. A., Mak, T. W., Bernstein, A. and van der Kooy, D. (2002). Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. *Genes Dev* **16**, 846-58.

Hogan, B. L. (1996). Bone morphogenetic proteins in development. *Curr Opin Genet Dev* **6**, 432-8.

Horner, P. J., Thallmair, M. and Gage, F. H. (2002). Defining the NG2-expressing cell of the adult CNS. *J Neurocytol* **31**, 469-80.

Horstmann, E. (1954). [The fiber glia of selacean brain.]. *Z Zellforsch Mikrosk Anat* **39**, 588-617.

Huang, L., DeVries, G. J. and Bittman, E. L. (1998). Photoperiod regulates neuronal bromodeoxyuridine labeling in the brain of a seasonally breeding mammal. *J Neurobiol* **36**, 410-20.

Ille, F., Atanasoski, S., Falk, S., Ittner, L. M., Marki, D., Buchmann-Moller, S., Wurdak, H., Suter, U., Taketo, M. M. and Sommer, L. (2007). Wnt/BMP signal integration regulates the balance between proliferation and differentiation of neuroepithelial cells in the dorsal spinal cord. *Dev Biol* **304**, 394-408.

Irvin, D. K., Zurcher, S. D., Nguyen, T., Weinmaster, G. and Kornblum, H. I. (2001). Expression patterns of Notch1, Notch2, and Notch3 suggest multiple functional roles for the Notch-DSL signaling system during brain development. *J Comp Neurol* **436**, 167-81.

Ishibashi, M., Moriyoshi, K., Sasai, Y., Shiota, K., Nakanishi, S. and Kageyama, R. (1994). Persistent expression of helix-loop-helix factor HES-1 prevents mammalian neural differentiation in the central nervous system. *Embo J* **13**, 1799-805.

Itoh, S., Itoh, F., Goumans, M. J. and Ten Dijke, P. (2000). Signaling of transforming growth factor-beta family members through Smad proteins. *Eur J Biochem* **267**, 6954-67.

Jackson, E. L., Garcia-Verdugo, J. M., Gil-Perotin, S., Roy, M., Quinones-Hinojosa, A., VandenBerg, S. and Alvarez-Buylla, A. (2006). PDGFR alpha-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling. *Neuron* **51**, 187-99.

Johansson, C. B., Momma, S., Clarke, D. L., Risling, M., Lendahl, U. and Frisen, J. (1999). Identification of a neural stem cell in the adult mammalian central nervous system. *Cell* **96**, 25-34.

Johe, K. K., Hazel, T. G., Muller, T., Dugich-Djordjevic, M. M. and McKay, R. D. (1996). Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. *Genes Dev* **10**, 3129-40.

Ju, W., Hoffmann, A., Verschueren, K., Tylzanowski, P., Kaps, C., Gross, G. and Huylebroeck, D. (2000). The bone morphogenetic protein 2 signaling mediator Smad1 participates predominantly in osteogenic and not in chondrogenic differentiation in mesenchymal progenitors C3H10T1/2. *J Bone Miner Res* **15**, 1889-99.

Katagiri, T., Imada, M., Yanai, T., Suda, T., Takahashi, N. and Kamijo, R. (2002). Identification of a BMP-responsive element in Id1, the gene for inhibition of myogenesis. *Genes Cells* **7**, 949-60.

Katagiri, T., Yamaguchi, A., Komaki, M., Abe, E., Takahashi, N., Ikeda, T., Rosen, V., Wozney, J. M., Fujisawa-Sehara, A. and Suda, T. (1994). Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage. *J Cell Biol* **127**, 1755-66.

Kawabata, M., Imamura, T. and Miyazono, K. (1998). Signal transduction by bone morphogenetic proteins. *Cytokine Growth Factor Rev* **9**, 49-61.

Kawabata, M., Inoue, H., Hanyu, A., Imamura, T. and Miyazono, K. (1998). Smad proteins exist as monomers in vivo and undergo homo- and hetero-oligomerization upon activation by serine/threonine kinase receptors. *Embo J* **17**, 4056-65.

Kawamura, C., Kizaki, M., Yamato, K., Uchida, H., Fukuchi, Y., Hattori, Y., Koseki, T., Nishihara, T. and Ikeda, Y. (2000). Bone morphogenetic protein-2 induces apoptosis in human myeloma cells with modulation of STAT3. *Blood* **96**, 2005-11.

Khokha, M. K., Yeh, J., Grammer, T. C. and Harland, R. M. (2005). Depletion of three BMP antagonists from Spemann's organizer leads to a catastrophic loss of dorsal structures. *Dev Cell* **8**, 401-11.

Kim, R. Y., Robertson, E. J. and Solloway, M. J. (2001). Bmp6 and Bmp7 are required for cushion formation and septation in the developing mouse heart. *Dev Biol* **235**, 449-66.

King, J. A., Marker, P. C., Seung, K. J. and Kingsley, D. M. (1994). BMP5 and the molecular, skeletal, and soft-tissue alterations in short ear mice. *Dev Biol* **166**, 112-22.

Kingsley, D. M., Bland, A. E., Grubber, J. M., Marker, P. C., Russell, L. B., Copeland, N. G. and Jenkins, N. A. (1992). The mouse short ear skeletal morphogenesis locus is associated with defects in a bone morphogenetic member of the TGF beta superfamily. *Cell* **71**, 399-410.

Kintner, C. (2002). Neurogenesis in embryos and in adult neural stem cells. *J Neurosci* **22**, 639-43.

Kirn, J. R. and Nottebohm, F. (1993). Direct evidence for loss and replacement of projection neurons in adult canary brain. *J Neurosci* **13**, 1654-63.

Kirsch, T., Sebald, W. and Dreyer, M. K. (2000). Crystal structure of the BMP-2-BRIA ectodomain complex. *Nat Struct Biol* **7**, 492-6.

Kishimoto, Y., Lee, K. H., Zon, L., Hammerschmidt, M. and Schulte-Merker, S. (1997). The molecular nature of zebrafish swirl: BMP2 function is essential during early dorsoventral patterning. *Development* **124**, 4457-66.

Kleber, M., Lee, H. Y., Wurdak, H., Buchstaller, J., Riccomagno, M. M., Ittner, L. M., Suter, U., Epstein, D. J. and Sommer, L. (2005). Neural crest stem cell maintenance by combinatorial Wnt and BMP signaling. *J Cell Biol* **169**, 309-20.

Kleber, M., Lee, H. Y., Wurdak, H., Buchstaller, J., Riccomagno, M. M., Ittner, L. M., Suter, U., Epstein,

D. J. and Sommer, L. (2005). Neural crest stem cell maintenance by combinatorial Wnt and BMP signaling. *J Cell Biol* **169**, 309-20.

Kohwi, M., Osumi, N., Rubenstein, J. L. and Alvarez-Buylla, A. (2005). Pax6 is required for making specific subpopulations of granule and periglomerular neurons in the olfactory bulb. *J Neurosci* **25**, 6997-7003.

Kondo, T. and Raff, M. (2000). The Id4 HLH protein and the timing of oligodendrocyte differentiation. *Embo J* **19**, 1998-2007.

Kondo, T. and Raff, M. (2000). Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. *Science* **289**, 1754-7.

Korchynskyi, O. and ten Dijke, P. (2002). Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the Id1 promoter. *J Biol Chem* **277**, 4883-91.

Kornack, D. R. and Rakic, P. (1999). Continuation of neurogenesis in the hippocampus of the adult macaque monkey. *Proc Natl Acad Sci U S A* **96**, 5768-73.

Kornack, D. R. and Rakic, P. (2001). The generation, migration, and differentiation of olfactory neurons in the adult primate brain. *Proc Natl Acad Sci U S A* **98**, 4752-7.

Kretzschmar, M., Doody, J. and Massague, J. (1997). Opposing BMP and EGF signalling pathways converge on the TGF-beta family mediator Smad1. *Nature* **389**, 618-22.

Kuhlbrodt, K., Herbarth, B., Sock, E., Hermans-Borgmeyer, I. and Wegner, M. (1998). Sox10, a novel transcriptional modulator in glial cells. *J Neurosci* **18**, 237-50.

Kuhn, H. G., Dickinson-Anson, H. and Gage, F. H. (1996). Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci* **16**, 2027-33.

Kuhn, H. G., Winkler, J., Kempermann, G., Thal, L. J. and Gage, F. H. (1997). Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. *J Neurosci* **17**, 5820-9.

Kuroda, H., Wessely, O. and De Robertis, E. M. (2004). Neural induction in Xenopus: requirement for ectodermal and endomesodermal signals via Chordin, Noggin, beta-Catenin, and Cerberus. *PLoS Biol* **2**, E92.

LaBonne, C. and Bronner-Fraser, M. (1998). Neural crest induction in Xenopus: evidence for a two-signal model. *Development* **125**, 2403-14.

Lachapelle, F., Avellana-Adalid, V., Nait-Oumesmar, B. and Baron-Van Evercooren, A. (2002). Fibroblast growth factor-2 (FGF-2) and platelet-derived growth factor AB (PDGF AB) promote adult SVZ-derived oligodendrogenesis in vivo. *Mol Cell Neurosci* **20**, 390-403.

Laird, P. W., Zijderveld, A., Linders, K., Rudnicki, M. A., Jaenisch, R. and Berns, A. (1991). Simplified mammalian DNA isolation procedure. *Nucleic Acids Res* **19**, 4293.

Lamb, T. M., Knecht, A. K., Smith, W. C., Stachel, S. E., Economides, A. N., Stahl, N., Yancopolous, G. D. and Harland, R. M. (1993). Neural induction by the secreted polypeptide noggin. *Science* **262**, 713-8.

Launay, C., Fromentoux, V., Shi, D. L. and Boucaut, J. C. (1996). A truncated FGF receptor blocks neural induction by endogenous Xenopus inducers. *Development* **122**, 869-80.

Lawson, K. A., Dunn, N. R., Roelen, B. A., Zeinstra, L. M., Davis, A. M., Wright, C. V., Korving, J. P. and Hogan, B. L. (1999). Bmp4 is required for the generation of primordial germ cells in the mouse embryo. *Genes Dev* **13**, 424-36.

Laywell, E. D., Rakic, P., Kukekov, V. G., Holland, E. C. and Steindler, D. A. (2000). Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain. *Proc Natl Acad Sci U S A* **97**, 13883-8.

Lechleider, R. J., Ryan, J. L., Garrett, L., Eng, C., Deng, C., Wynshaw-Boris, A. and Roberts, A. B. (2001). Targeted mutagenesis of Smad1 reveals an essential role in chorioallantoic fusion. *Dev Biol* **240**, 157-67.

Lee, K. J. and Jessell, T. M. (1999). The specification of dorsal cell fates in the vertebrate central nervous system. *Annu Rev Neurosci* **22**, 261-94.

Leveen, P., Larsson, J., Ehinger, M., Cilio, C. M., Sundler, M., Sjostrand, L. J., Holmdahl, R. and Karlsson, S. (2002). Induced disruption of the transforming growth factor beta type II receptor gene in mice causes a lethal inflammatory disorder that is transplantable. *Blood* **100**, 560-8.

Leventhal, C., Rafii, S., Rafii, D., Shahar, A. and Goldman, S. A. (1999). Endothelial trophic support of neuronal production and recruitment from the adult mammalian subependyma. *Mol Cell Neurosci* **13**, 450-64.

Lewis, P. D. (1968). A quantitative study of cell proliferation in the subependymal layer of the adult rat brain. *Exp Neurol* **20**, 203-7.

Lewis, P. D. (1968). A quantitative study of cell proliferation in the subependymal layer of the adult rat brain. *Exp Neurol* **20**, 203-7.

Li, W., Cogswell, C. A. and LoTurco, J. J. (1998). Neuronal differentiation of precursors in the neocortical ventricular zone is triggered by BMP. *J Neurosci* **18**, 8853-62.

Lie, D. C., Colamarino, S. A., Song, H. J., Desire, L., Mira, H., Consiglio, A., Lein, E. S., Jessberger, S., Lansford, H., Dearie, A. R. et al. (2005). Wnt signalling regulates adult hippocampal neurogenesis. *Nature* **437**, 1370-5.

Liem, K. F., Jr., Tremml, G. and Jessell, T. M. (1997). A role for the roof plate and its resident TGFbeta-related proteins in neuronal patterning in the dorsal spinal cord. *Cell* **91**, 127-38.

Ligon, K. L., Huillard, E., Mehta, S., Kesari, S., Liu, H., Alberta, J. A., Bachoo, R. M., Kane, M., Louis, D. N., Depinho, R. A. et al. (2007). Olig2-regulated lineage-restricted pathway controls replication competence in neural stem cells and malignant glioma. *Neuron* **53**, 503-17.

Ligon, K. L., Kesari, S., Kitada, M., Sun, T., Arnett, H. A., Alberta, J. A., Anderson, D. J., Stiles, C. D. and Rowitch, D. H. (2006). Development of NG2 neural progenitor cells requires Olig gene function. *Proc Natl Acad Sci U S A* **103**, 7853-8.

Lillien, L. and Gulacsi, A. (2006). Environmental signals elicit multiple responses in dorsal telencephalic progenitors by threshold-dependent mechanisms. *Cereb Cortex* **16 Suppl 1**, i74-81.

Lillien, L. and Raphael, H. (2000). BMP and FGF regulate the development of EGF-responsive neural progenitor cells. *Development* **127**, 4993-5005.

Lim, D. A., Tramontin, A. D., Trevejo, J. M., Herrera, D. G., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (2000). Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron* **28**, 713-26.

Lo, L., Dormand, E., Greenwood, A. and Anderson, D. J. (2002). Comparison of the generic neuronal differentiation and neuron subtype specification functions of mammalian achaete-scute and atonal homologs in cultured neural progenitor cells. *Development* **129**, 1553-67.

Lo, L., Sommer, L. and Anderson, D. J. (1997). MASH1 maintains competence for BMP2-induced neuronal differentiation in post-migratory neural crest cells. *Curr Biol* **7**, 440-50.

Lois, C. and Alvarez-Buylla, A. (1993). Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc Natl Acad Sci U S A* **90**, 2074-7.

Lois, C. and Alvarez-Buylla, A. (1994). Long-distance neuronal migration in the adult mammalian brain. *Science* **264**, 1145-8.

Lois, C., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (1996). Chain migration of neuronal precursors. *Science* **271**, 978-81.

Londin, E. R., Niemiec, J. and Sirotkin, H. I. (2005). Chordin, FGF signaling, and mesodermal factors cooperate in zebrafish neural induction. *Dev Biol* **279**, 1-19.

Lopez-Rovira, T., Chalaux, E., Massague, J., Rosa, J. L. and Ventura, F. (2002). Direct binding of Smad1 and Smad4 to two distinct motifs mediates bone morphogenetic protein-specific transcriptional activation of Id1 gene. *J Biol Chem* **277**, 3176-85.

Lu, Q. R., Sun, T., Zhu, Z., Ma, N., Garcia, M., Stiles, C. D. and Rowitch, D. H. (2002). Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. *Cell* **109**, 75-86.

Lu, Q. R., Yuk, D., Alberta, J. A., Zhu, Z., Pawlitzky, I., Chan, J., McMahon, A. P., Stiles, C. D. and Rowitch, D. H. (2000). Sonic hedgehog--regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. *Neuron* **25**, 317-29.

Luskin, M. B. (1993). Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron* **11**, 173-89.

Luskin, M. B. (1993). Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron* **11**, 173-89.

Luyten, F. P., Cunningham, N. S., Ma, S., Muthukumaran, N., Hammonds, R. G., Nevins, W. B., Woods, W. I. and Reddi, A. H. (1989). Purification and partial amino acid sequence of osteogenin, a protein initiating bone differentiation. *J Biol Chem* **264**, 13377-80.

Mabie, P. C., Mehler, M. F. and Kessler, J. A. (1999). Multiple roles of bone morphogenetic protein signaling in the regulation of cortical cell number and phenotype. *J Neurosci* **19**, 7077-88.

Magnus, T., Coksaygan, T., Korn, T., Xue, H., Arumugam, T. V., Mughal, M. R., Eckley, D. M., Tang, S. C., Detolla, L., Rao, M. S. et al. (2007). Evidence that nucleocytoplasmic Olig2 translocation mediates brain-injury-induced differentiation of glial precursors to astrocytes. *J Neurosci Res* **85**, 2126-37.

Makwana, M., Jones, L. L., Cuthill, D., Heuer, H., Bohatschek, M., Hristova, M., Friedrichsen, S., Ormsby, I., Bueringer, D., Koppius, A. et al. (2007). Endogenous transforming growth factor beta 1 suppresses inflammation and promotes survival in adult CNS. *J Neurosci* **27**, 11201-13.

Malatesta, P., Hack, M. A., Hartfuss, E., Kettenmann, H., Klinkert, W., Kirchhoff, F. and Gotz, M. (2003). Neuronal or glial progeny: regional differences in radial glia fate. *Neuron* **37**, 751-64.

Malatesta, P., Hartfuss, E. and Gotz, M. (2000). Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. *Development* **127**, 5253-63.

Marazzi, G., Wang, Y. and Sassoon, D. (1997). Msx2 is a transcriptional regulator in the BMP4-mediated programmed cell death pathway. *Dev Biol* **186**, 127-38.

Massague, J., Blain, S. W. and Lo, R. S. (2000). TGFbeta signaling in growth control, cancer, and heritable disorders. *Cell* **103**, 295-309.

Matzuk, M. M., Kumar, T. R. and Bradley, A. (1995). Different phenotypes for mice deficient in either activins or activin receptor type II. *Nature* **374**, 356-60.

Matzuk, M. M., Kumar, T. R., Shou, W., Coerver, K. A., Lau, A. L., Behringer, R. R. and Finegold, M. J. (1996). Transgenic models to study the roles of inhibins and activins in reproduction, oncogenesis, and development. *Recent Prog Horm Res* **51**, 123-54; discussion 155-7.

McDermott, K. W. and Lantos, P. L. (1990). Cell proliferation in the subependymal layer of the postnatal marmoset, Callithrix jacchus. *Brain Res Dev Brain Res* **57**, 269-77.

Mekki-Dauriac, S., Agius, E., Kan, P. and Cochard, P. (2002). Bone morphogenetic proteins negatively control oligodendrocyte precursor specification in the chick spinal cord. *Development* **129**, 5117-30.

Menn, B., Garcia-Verdugo, J. M., Yaschine, C., Gonzalez-Perez, O., Rowitch, D. and Alvarez-Buylla, A. (2006). Origin of oligodendrocytes in the subventricular zone of the adult brain. *J Neurosci* **26**, 7907-18.

Merkle, F. T., Tramontin, A. D., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (2004). Radial glia give rise to adult neural stem cells in the subventricular zone. *Proc Natl Acad Sci U S A* **101**, 17528-32.

Metzger, D. and Chambon, P. (2001). Site- and time-specific gene targeting in the mouse. *Methods* **24**, 71-80.

Miller, R. H. (2002). Regulation of oligodendrocyte development in the vertebrate CNS. *Prog Neurobiol* **67**, 451-67.

Miller, R. H., Dinsio, K., Wang, R., Geertman, R., Maier, C. E. and Hall, A. K. (2004). Patterning of spinal cord oligodendrocyte development by dorsally derived BMP4. *J Neurosci Res* **76**, 9-19.

Ming, G. L. and Song, H. (2005). Adult neurogenesis in the mammalian central nervous system. *Annu Rev Neurosci* **28**, 223-50.

Minina, E., Kreschel, C., Naski, M. C., Ornitz, D. M. and Vortkamp, A. (2002). Interaction of FGF, Ihh/Pthlh, and BMP signaling integrates chondrocyte proliferation and hypertrophic differentiation. *Dev Cell* **3**, 439-49.

Mishina, Y., Crombie, R., Bradley, A. and Behringer, R. R. (1999). Multiple roles for activin-like kinase-2 signaling during mouse embryogenesis. *Dev Biol* **213**, 314-26.

Mishina, Y., Suzuki, A., Gilbert, D. J., Copeland, N. G., Jenkins, N. A., Ueno, N. and Behringer, R. R. (1995). Genomic organization and chromosomal location of the mouse type I BMP-2/4 receptor. *Biochem Biophys Res Commun* **206**, 310-7.

Miyata, T., Kawaguchi, A., Okano, H. and Ogawa, M. (2001). Asymmetric inheritance of radial glial fibers by cortical neurons. *Neuron* **31**, 727-41.

Miyazawa, K., Shinozaki, M., Hara, T., Furuya, T. and Miyazono, K. (2002). Two major Smad pathways in TGF-beta superfamily signalling. *Genes Cells* **7**, 1191-204.

Miyazono, K. (2000). Positive and negative regulation of TGF-beta signaling. *J Cell Sci* **113 (Pt 7)**, 1101-9.

Miyazono, K. (2001). [Recent advances in the research on TGF-beta/Smad signaling pathways]. *Tanpakushitsu Kakusan Koso* **46**, 105-10.

Miyazono, K. and Miyazawa, K. (2002). Id: a target of BMP signaling. *Sci STKE* **2002**, PE40.

Mummery, C. L. (2004). Spatio-temporal activation of Smad1 and Smad5 in vivo: monitoring transcriptional activity of Smad proteins. *J Cell Sci* **117**, 4653-63.

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

Morshead, C. M., Reynolds, B. A., Craig, C. G., McBurney, M. W., Staines, W. A., Morassutti, D., Weiss, S. and van der Kooy, D. (1994). Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. *Neuron* **13**, 1071-82.

Morshead, C. M. and van der Kooy, D. (1992). Postmitotic death is the fate of constitutively proliferating cells in the subependymal layer of the adult mouse brain. *J Neurosci* **12**, 249-56.

Moustakas, A., Souchelnytskyi, S. and Heldin, C. H. (2001). Smad regulation in TGF-beta signal transduction. *J Cell Sci* **114**, 4359-69.

Muller, F. and Rohrer, H. (2002). Molecular control of ciliary neuron development: BMPs and downstream transcriptional control in the parasympathetic lineage. *Development* **129**, 5707-17.

Nait-Oumesmar, B., Decker, L., Lachapelle, F., Avellana-Adalid, V., Bachelin, C. and Van Evercooren, A. B. (1999). Progenitor cells of the adult mouse subventricular zone proliferate, migrate and differentiate into oligodendrocytes after demyelination. *Eur J Neurosci* **11**, 4357-66.

Nait-Oumesmar, B., Picard-Riera, N., Kerninon, C. and Baron-Van Evercooren, A. (2008). The role of SVZ-derived neural precursors in demyelinating diseases: From animal models to multiple sclerosis. *J Neurol Sci* **265**, 26-31.

Nait-Oumesmar, B., Picard-Riera, N., Kerninon, C., Decker, L., Seilhean, D., Hoglinger, G. U., Hirsch, E. C., Reynolds, R. and Baron-Van Evercooren, A. (2007). Activation of the subventricular zone in multiple sclerosis: evidence for early glial progenitors. *Proc Natl Acad Sci U S A* **104**, 4694-9.

Nakamura, K., Shirai, T., Morishita, S., Uchida, S., Saeki-Miura, K. and Makishima, F. (1999). p38 mitogen-activated protein kinase functionally contributes to chondrogenesis induced by growth/differentiation factor-5 in ATDC5 cells. *Exp Cell Res* **250**, 351-63.

Nakashima, K. and Taga, T. (2002). Mechanisms underlying cytokine-mediated cell-fate regulation in the nervous system. *Mol Neurobiol* **25**, 233-44.

Nakashima, K., Takizawa, T., Ochiai, W., Yanagisawa, M., Hisatsune, T., Nakafuku, M., Miyazono, K., Kishimoto, T., Kageyama, R. and Taga, T. (2001). BMP2-mediated alteration in the developmental pathway of fetal mouse brain cells from neurogenesis to astrocytogenesis. *Proc Natl Acad Sci U S A* **98**, 5868-73.

Nakashima, K., Yanagisawa, M., Arakawa, H., Kimura, N., Hisatsune, T., Kawabata, M., Miyazono, K. and Taga, T. (1999). Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300. *Science* **284**, 479-82.

Namiki, M., Akiyama, S., Katagiri, T., Suzuki, A., Ueno, N., Yamaji, N., Rosen, V., Wozney, J. M. and Suda, T. (1997). A kinase domain-truncated type I receptor blocks bone morphogenetic protein-2-induced signal transduction in C2C12 myoblasts. *J Biol Chem* **272**, 22046-52.

Nguyen, V. H., Schmid, B., Trout, J., Connors, S. A., Ekker, M. and Mullins, M. C. (1998). Ventral and lateral regions of the zebrafish gastrula, including the neural crest progenitors, are established by a bmp2b/swirl pathway of genes. *Dev Biol* **199**, 93-110.

Nguyen, V. H., Trout, J., Connors, S. A., Andermann, P., Weinberg, E. and Mullins, M. C. (2000). Dorsal and intermediate neuronal cell types of the spinal cord are established by a BMP signaling pathway. *Development* **127**, 1209-20.

Niehrs, C., Kazanskaya, O., Wu, W. and Glinka, A. (2001). Dickkopf1 and the Spemann-Mangold head organizer. *Int J Dev Biol* **45**, 237-40.

Ninkovic, J., Mori, T. and Gotz, M. (2007). Distinct modes of neuron addition in adult mouse neurogenesis. *J Neurosci* **27**, 10906-11.

Noctor, S. C., Flint, A. C., Weissman, T. A., Dammerman, R. S. and Kriegstein, A. R. (2001). Neurons derived from radial glial cells establish radial units in neocortex. *Nature* **409**, 714-20.

Nohe, A., Hassel, S., Ehrlich, M., Neubauer, F., Sebald, W., Henis, Y. I. and Knaus, P. (2002). The mode of bone morphogenetic protein (BMP) receptor oligomerization determines different BMP-2 signaling pathways. *J Biol Chem* **277**, 5330-8.

Nohe, A., Keating, E., Knaus, P. and Petersen, N. O. (2004). Signal transduction of bone morphogenetic protein receptors. *Cell Signal* **16**, 291-9.

Norton, J. D., Deed, R. W., Craggs, G. and Sablitzky, F. (1998). Id helix-loop-helix proteins in cell growth and differentiation. *Trends Cell Biol* **8**, 58-65.

Nottebohm, F. (1985). Neuronal replacement in adulthood. *Ann N Y Acad Sci* **457**, 143-61.

Nyfeler, Y., Kirch, R. D., Mantei, N., Leone, D. P., Radtke, F., Suter, U. and Taylor, V. (2005). Jagged1 signals in the postnatal subventricular zone are required for neural stem cell self-renewal. *Embo J* **24**, 3504-15.

Oelgeschlager, M., Kuroda, H., Reversade, B. and De Robertis, E. M. (2003). Chordin is required for the Spemann organizer transplantation phenomenon in Xenopus embryos. *Dev Cell* **4**, 219-30.

Oh, S. P. and Li, E. (1997). The signaling pathway mediated by the type IIB activin receptor controls axial patterning and lateral asymmetry in the mouse. *Genes Dev* **11**, 1812-26.

Ohtsuka, T., Sakamoto, M., Guillemot, F. and Kageyama, R. (2001). Roles of the basic helix-loop-helix genes Hes1 and Hes5 in expansion of neural stem cells of the developing brain. *J Biol Chem* **276**, 30467-74.

Okada, S., Nakamura, M., Katoh, H., Miyao, T., Shimazaki, T., Ishii, K., Yamane, J., Yoshimura, A., Iwamoto, Y., Toyama, Y. et al. (2006). Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. *Nat Med* **12**, 829-34.

Ono, K., Bansal, R., Payne, J., Rutishauser, U. and Miller, R. H. (1995). Early development and dispersal of oligodendrocyte precursors in the embryonic chick spinal cord. *Development* **121**, 1743-54.

Orentas, D. M., Hayes, J. E., Dyer, K. L. and Miller, R. H. (1999). Sonic hedgehog signaling is required during the appearance of spinal cord oligodendrocyte precursors. *Development* **126**, 2419-29.

Owens, T., Babcock, A. A., Millward, J. M. and Toft-Hansen, H. (2005). Cytokine and chemokine inter-regulation in the inflamed or injured CNS. *Brain Res Brain Res Rev* **48**, 178-84.

Palma, V., Lim, D. A., Dahmane, N., Sanchez, P., Brionne, T. C., Herzberg, C. D., Gitton, Y., Carleton, A., Alvarez-Buylla, A. and Ruiz i Altaba, A. (2005). Sonic hedgehog controls stem cell behavior in the postnatal and adult brain. *Development* **132**, 335-44.

Palmer, T. D., Markakis, E. A., Willhoite, A. R., Safar, F. and Gage, F. H. (1999). Fibroblast growth factor-2

activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. *J Neurosci* **19**, 8487-97.

Palmer, T. D., Ray, J. and Gage, F. H. (1995). FGF-2-responsive neuronal progenitors reside in proliferative and quiescent regions of the adult rodent brain. *Mol Cell Neurosci* **6**, 474-86.

Palmer, T. D., Takahashi, J. and Gage, F. H. (1997). The adult rat hippocampus contains primordial neural stem cells. *Mol Cell Neurosci* **8**, 389-404.

Palmer, T. D., Willhoite, A. R. and Gage, F. H. (2000). Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol* **425**, 479-94.

Panchision, D. M., Pickel, J. M., Studer, L., Lee, S. H., Turner, P. A., Hazel, T. G. and McKay, R. D. (2001). Sequential actions of BMP receptors control neural precursor cell production and fate. *Genes Dev* **15**, 2094-110.

Parras, C. M., Galli, R., Britz, O., Soares, S., Galichet, C., Battiste, J., Johnson, J. E., Nakafuku, M., Vescovi, A. and Guillemot, F. (2004). Mash1 specifies neurons and oligodendrocytes in the postnatal brain. *Embo J* **23**, 4495-505.

Paton, J. A., O'Loughlin, B. E. and Nottebohm, F. (1985). Cells born in adult canary forebrain are local interneurons. *J Neurosci* **5**, 3088-93.

Pekny, M. and Nilsson, M. (2005). Astrocyte activation and reactive gliosis. *Glia* **50**, 427-34.

Pera, E. M., Ikeda, A., Eivers, E. and De Robertis, E. M. (2003). Integration of IGF, FGF, and anti-BMP signals via Smad1 phosphorylation in neural induction. *Genes Dev* **17**, 3023-8.

Pera, E. M., Wessely, O., Li, S. Y. and De Robertis, E. M. (2001). Neural and head induction by insulin-like growth factor signals. *Dev Cell* **1**, 655-65.

Peretto, P., Cummings, D., Modena, C., Behrens, M., Venkatraman, G., Fasolo, A. and Margolis, F. L. (2002). BMP mRNA and protein expression in the developing mouse olfactory system. *J Comp Neurol* **451**, 267-78.

Peretto, P., Dati, C., De Marchis, S., Kim, H. H., Ukhanova, M., Fasolo, A. and Margolis, F. L. (2004). Expression of the secreted factors noggin and bone morphogenetic proteins in the subependymal layer and olfactory bulb of the adult mouse brain. *Neuroscience* **128**, 685-96.

Petryniak, M. A., Potter, G. B., Rowitch, D. H. and Rubenstein, J. L. (2007). Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. *Neuron* **55**, 417-33.

Pfeifer, A., Brandon, E. P., Kootstra, N., Gage, F. H. and Verma, I. M. (2001). Delivery of the Cre recombinase by a self-deleting lentiviral vector: efficient gene targeting in vivo. *Proc Natl Acad Sci U S A* **98**, 11450-5.

Pfeifer, A., Ikawa, M., Dayn, Y. and Verma, I. M. (2002). Transgenesis by lentiviral vectors: lack of gene silencing in mammalian embryonic stem cells and preimplantation embryos. *Proc Natl Acad Sci U S A* **99**, 2140-5.

Picard-Riera, N., Decker, L., Delarasse, C., Goude, K., Nait-Oumesmar, B., Liblau, R., Pham-Dinh, D. and Evercooren, A. B. (2002). Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. *Proc Natl Acad Sci U S A* **99**, 13211-6.

Piccirillo, S. G., Reynolds, B. A., Zanetti, N., Lamorte, G., Binda, E., Broggi, G., Brem, H., Olivi, A., Dimeco, F. and Vescovi, A. L. (2006). Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. *Nature* **444**, 761-5.

Piccolo, S., Sasai, Y., Lu, B. and De Robertis, E. M. (1996). Dorsoventral patterning in Xenopus: inhibition of ventral signals by direct binding of chordin to BMP-4. *Cell* **86**, 589-98.

Pringle, N. P. and Richardson, W. D. (1993). A singularity of PDGF alpha-receptor expression in the dorsoventral axis of the neural tube may define the origin of the oligodendrocyte lineage. *Development* **117**, 525-33.

Pringle, N. P., Yu, W. P., Guthrie, S., Roelink, H., Lumsden, A., Peterson, A. C. and Richardson, W. D.

(1996). Determination of neuroepithelial cell fate: induction of the oligodendrocyte lineage by ventral midline cells and sonic hedgehog. *Dev Biol* **177**, 30-42.

Privat, A. (1972). [Glycogen-rich cells in the lateral ventricle ependyma of the rat brain. Optical and electromicroscopical study]. *Z Zellforsch Mikrosk Anat* **123**, 356-68.

Privat, A. and Leblond, C. P. (1972). The subependymal layer and neighboring region in the brain of the young rat. *J Comp Neurol* **146**, 277-302.

Qin, B. Y., Chacko, B. M., Lam, S. S., de Caestecker, M. P., Correia, J. J. and Lin, K. (2001). Structural basis of Smad1 activation by receptor kinase phosphorylation. *Mol Cell* **8**, 1303-12.

Qin, B. Y., Lam, S. S., Correia, J. J. and Lin, K. (2002). Smad3 allostery links TGF-beta receptor kinase activation to transcriptional control. *Genes Dev* **16**, 1950-63.

Raff, M., Apperly, J., Kondo, T., Tokumoto, Y. and Tang, D. (2001). Timing cell-cycle exit and differentiation in oligodendrocyte development. *Novartis Found Symp* **237**, 100-7; discussion 107-12, 158-63.

Rajan, P., Panchision, D. M., Newell, L. F. and McKay, R. D. (2003). BMPs signal alternately through a SMAD or FRAP-STAT pathway to regulate fate choice in CNS stem cells. *J Cell Biol* **161**, 911-21.

Rakic, P. (1985). Limits of neurogenesis in primates. *Science* **227**, 1054-6.

Rakic, P. (1988). Defects of neuronal migration and the pathogenesis of cortical malformations. *Prog Brain Res* **73**, 15-37.

Ramirez-Castillejo, C., Sanchez-Sanchez, F., Andreu-Agullo, C., Ferron, S. R., Aroca-Aguilar, J. D., Sanchez, P., Mira, H., Escribano, J. and Farinas, I. (2006). Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. *Nat Neurosci* **9**, 331-9.

Rao, M. S. and Mayer-Proschel, M. (1997). Glial-restricted precursors are derived from multipotent neuroepithelial stem cells. *Dev Biol* **188**, 48-63.

Reissmann, E., Ernsberger, U., Francis-West, P. H., Rueger, D., Brickell, P. M. and Rohrer, H. (1996). Involvement of bone morphogenetic protein-4 and bone morphogenetic protein-7 in the differentiation of the adrenergic phenotype in developing sympathetic neurons. *Development* **122**, 2079-88.

Reynolds, B. A. and Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255**, 1707-10.

Richards, L. J., Kilpatrick, T. J. and Bartlett, P. F. (1992). De novo generation of neuronal cells from the adult mouse brain. *Proc Natl Acad Sci U S A* **89**, 8591-5.

Richardson, W. D., Pringle, N. P., Yu, W. P. and Hall, A. C. (1997). Origins of spinal cord oligodendrocytes: possible developmental and evolutionary relationships with motor neurons. *Dev Neurosci* **19**, 58-68.

Rios, I., Alvarez-Rodriguez, R., Marti, E. and Pons, S. (2004). Bmp2 antagonizes sonic hedgehog-mediated proliferation of cerebellar granule neurones through Smad5 signalling. *Development* **131**, 3159-68.

Roelink, H., Porter, J. A., Chiang, C., Tanabe, Y., Chang, D. T., Beachy, P. A. and Jessell, T. M. (1995). Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of sonic hedgehog autoproteolysis. *Cell* **81**, 445-55.

Ross, S. E., Greenberg, M. E. and Stiles, C. D. (2003). Basic helix-loop-helix factors in cortical development. *Neuron* **39**, 13-25.

Ruzinova, M. B. and Benezra, R. (2003). Id proteins in development, cell cycle and cancer. *Trends Cell Biol* **13**, 410-8.

Saghatelyan, A., de Chevigny, A., Schachner, M. and Lledo, P. M. (2004). Tenascin-R mediates activity-dependent recruitment of neuroblasts in the adult mouse forebrain. *Nat Neurosci* **7**, 347-56.

Sailer, M. H., Hazel, T. G., Panchision, D. M., Hoeppner, D. J., Schwab, M. E. and McKay, R. D. (2005). BMP2 and FGF2 cooperate to induce neural-crest-like fates from fetal and adult CNS stem cells. *J Cell Sci* **118**, 5849-60.

Sakaguchi, M., Shingo, T., Shimazaki, T., Okano, H. J., Shiwa, M., Ishibashi, S., Oguro, H., Ninomiya, M.,

Kadoya, T., Horie, H. et al. (2006). A carbohydrate-binding protein, Galectin-1, promotes proliferation of adult neural stem cells. *Proc Natl Acad Sci U S A* **103**, 7112-7.

Sakai, T., Li, S., Docheva, D., Grashoff, C., Sakai, K., Kostka, G., Braun, A., Pfeifer, A., Yurchenco, P. D. and Fassler, R. (2003). Integrin-linked kinase (ILK) is required for polarizing the epiblast, cell adhesion, and controlling actin accumulation. *Genes Dev* **17**, 926-40.

Samanta, J. and Kessler, J. A. (2004). Interactions between ID and OLIG proteins mediate the inhibitory effects of BMP4 on oligodendroglial differentiation. *Development* **131**, 4131-42.

Sanai, N., Berger, M. S., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (2007). Comment on "Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension". *Science* **318**, 393; author reply 393.

Sanai, N., Tramontin, A. D., Quinones-Hinojosa, A., Barbaro, N. M., Gupta, N., Kunwar, S., Lawton, M. T., McDermott, M. W., Parsa, A. T., Manuel-Garcia Verdugo, J. et al. (2004). Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. *Nature* **427**, 740-4.

Sasai, Y., Lu, B., Steinbeisser, H. and De Robertis, E. M. (1995). Regulation of neural induction by the Chd and Bmp-4 antagonistic patterning signals in Xenopus. *Nature* **377**, 757.

Schneider, C., Wicht, H., Enderich, J., Wegner, M. and Rohrer, H. (1999). Bone morphogenetic proteins are required in vivo for the generation of sympathetic neurons. *Neuron* **24**, 861-70.

Seidenfaden, R., Desoeuvre, A., Bosio, A., Virard, I. and Cremer, H. (2006). Glial conversion of SVZ-derived committed neuronal precursors after ectopic grafting into the adult brain. *Mol Cell Neurosci* **32**, 187-98.

Seki, T. and Arai, Y. (1993). Highly polysialylated neural cell adhesion molecule (NCAM-H) is expressed by newly generated granule cells in the dentate gyrus of the adult rat. *J Neurosci* **13**, 2351-8.

Sela-Donenfeld, D. and Kalcheim, C. (1999). Regulation of the onset of neural crest migration by coordinated activity of BMP4 and Noggin in the dorsal neural tube. *Development* **126**, 4749-62.

Sela-Donenfeld, D. and Kalcheim, C. (2000). Inhibition of noggin expression in the dorsal neural tube by somitogenesis: a mechanism for coordinating the timing of neural crest emigration. *Development* **127**, 4845-54.

Seroogy, K. B., Gall, C. M., Lee, D. C. and Kornblum, H. I. (1995). Proliferative zones of postnatal rat brain express epidermal growth factor receptor mRNA. *Brain Res* **670**, 157-64.

Seto, H., Kamekura, S., Miura, T., Yamamoto, A., Chikuda, H., Ogata, T., Hiraoka, H., Oda, H., Nakamura, K., Kurosawa, H. et al. (2004). Distinct roles of Smad pathways and p38 pathways in cartilage-specific gene expression in synovial fibroblasts. *J Clin Invest* **113**, 718-26.

Setoguchi, T., Nakashima, K., Takizawa, T., Yanagisawa, M., Ochiai, W., Okabe, M., Yone, K., Komiya, S. and Taga, T. (2004). Treatment of spinal cord injury by transplantation of fetal neural precursor cells engineered to express BMP inhibitor. *Exp Neurol* **189**, 33-44.

Shah, N. M. and Anderson, D. J. (1997). Integration of multiple instructive cues by neural crest stem cells reveals cell-intrinsic biases in relative growth factor responsiveness. *Proc Natl Acad Sci U S A* **94**, 11369-74.

Shah, N. M., Groves, A. K. and Anderson, D. J. (1996). Alternative neural crest cell fates are instructively promoted by TGFbeta superfamily members. *Cell* **85**, 331-43.

Shen, Q., Goderie, S. K., Jin, L., Karanth, N., Sun, Y., Abramova, N., Vincent, P., Pumiglia, K. and Temple, S. (2004). Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* **304**, 1338-40.

Shi, Y., Chichung Lie, D., Taupin, P., Nakashima, K., Ray, J., Yu, R. T., Gage, F. H. and Evans, R. M. (2004). Expression and function of orphan nuclear receptor TLX in adult neural stem cells. *Nature* **427**, 78-83.

Shi, Y. and Massague, J. (2003). Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* **113**, 685-700.

Shihabuddin, L. S., Horner, P. J., Ray, J. and Gage, F. H. (2000). Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus. *J Neurosci* **20**, 8727-35.

Shou, J., Murray, R. C., Rim, P. C. and Calof, A. L. (2000). Opposing effects of bone morphogenetic proteins on neuron production and survival in the olfactory receptor neuron lineage. *Development* **127**, 5403-13.

Sirard, C., de la Pompa, J. L., Elia, A., Itie, A., Mirtsos, C., Cheung, A., Hahn, S., Wakeham, A., Schwartz, L., Kern, S. E. et al. (1998). The tumor suppressor gene Smad4/Dpc4 is required for gastrulation and later for anterior development of the mouse embryo. *Genes Dev* **12**, 107-19.

Smith, A. and Graham, A. (2001). Restricting Bmp-4 mediated apoptosis in hindbrain neural crest. *Dev Dyn* **220**, 276-83.

Smith, W. C. and Harland, R. M. (1992). Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in Xenopus embryos. *Cell* **70**, 829-40.

Smith, W. C., Knecht, A. K., Wu, M. and Harland, R. M. (1993). Secreted noggin protein mimics the Spemann organizer in dorsalizing Xenopus mesoderm. *Nature* **361**, 547-9.

Soderstrom, S., Bengtsson, H. and Ebendal, T. (1996). Expression of serine/threonine kinase receptors including the bone morphogenetic factor type II receptor in the developing and adult rat brain. *Cell Tissue Res* **286**, 269-79.

Sofroniew, M. V. (2005). Reactive astrocytes in neural repair and protection. *Neuroscientist* **11**, 400-7.

Sohn, J., Natale, J., Chew, L. J., Belachew, S., Cheng, Y., Aguirre, A., Lytle, J., Nait-Oumesmar, B., Kerninon, C., Kanai-Azuma, M. et al. (2006). Identification of Sox17 as a transcription factor that regulates oligodendrocyte development. *J Neurosci* **26**, 9722-35.

Solloway, M. J., Dudley, A. T., Bikoff, E. K., Lyons, K. M., Hogan, B. L. and Robertson, E. J. (1998). Mice lacking Bmp6 function. *Dev Genet* **22**, 321-39.

Solloway, M. J. and Robertson, E. J. (1999). Early embryonic lethality in Bmp5;Bmp7 double mutant mice suggests functional redundancy within the 60A subgroup. *Development* **126**, 1753-68.

Sommer, L. (2006). Growth factors regulating neural crest cell fate decisions. *Adv Exp Med Biol* **589**, 197-205.

Song, J., Oh, S. P., Schrewe, H., Nomura, M., Lei, H., Okano, M., Gridley, T. and Li, E. (1999). The type II activin receptors are essential for egg cylinder growth, gastrulation, and rostral head development in mice. *Dev Biol* **213**, 157-69.

Song, Q., Mehler, M. F. and Kessler, J. A. (1998). Bone morphogenetic proteins induce apoptosis and growth factor dependence of cultured sympathoadrenal progenitor cells. *Dev Biol* **196**, 119-27.

Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* **21**, 70-1.

Stemple, D. L. and Mahanthappa, N. K. (1997). Neural stem cells are blasting off. *Neuron* **18**, 1-4.

Stensaas, L. J. and Stensaas, S. S. (1968). Light microscopy of glial cells in turtles and birds. *Z Zellforsch Mikrosk Anat* **91**, 315-40.

Stevenson, J. A. and Yoon, M. G. (1982). Morphology of radial glia, ependymal cells, and periventricular neurons in the optic tectum of goldfish (Carassius auratus). *J Comp Neurol* **205**, 128-38.

Stolt, C. C., Rehberg, S., Ader, M., Lommes, P., Riethmacher, D., Schachner, M., Bartsch, U. and Wegner, M. (2002). Terminal differentiation of myelin-forming oligodendrocytes depends on the transcription factor Sox10. *Genes Dev* **16**, 165-70.

Streit, A., Berliner, A. J., Papanayotou, C., Sirulnik, A. and Stern, C. D. (2000). Initiation of neural induction by FGF signalling before gastrulation. *Nature* **406**, 74-8.

Stump, G., Durrer, A., Klein, A. L., Lutolf, S., Suter, U. and Taylor, V. (2002). Notch1 and its ligands Delta-like and Jagged are expressed and active in distinct cell populations in the postnatal mouse brain. *Mech Dev* **114**, 153-9.

Sugai, M., Gonda, H., Kusunoki, T., Katakai, T., Yokota, Y. and Shimizu, A. (2003). Essential role of Id2 in negative regulation of IgE class switching. *Nat Immunol* **4**, 25-30.

Suhonen, J. O., Peterson, D. A., Ray, J. and Gage, F. H. (1996). Differentiation of adult hippocampus-derived progenitors into olfactory neurons in vivo. *Nature* **383**, 624-7.

Takizawa, T., Ochiai, W., Nakashima, K. and Taga, T. (2003). Enhanced gene activation by Notch and BMP signaling cross-talk. *Nucleic Acids Res* **31**, 5723-31.

Taupin, P. and Gage, F. H. (2002). Adult neurogenesis and neural stem cells of the central nervous system in mammals. *J Neurosci Res* **69**, 745-9.

Temple, S. (2001). Stem cell plasticity--building the brain of our dreams. *Nat Rev Neurosci* **2**, 513-20.

Timmer, J. R., Wang, C. and Niswander, L. (2002). BMP signaling patterns the dorsal and intermediate neural tube via regulation of homeobox and helix-loop-helix transcription factors. *Development* **129**, 2459-72.

Tokuda, H., Hatakeyama, D., Shibata, T., Akamatsu, S., Oiso, Y. and Kozawa, O. (2003). p38 MAP kinase regulates BMP-4-stimulated VEGF synthesis via p70 S6 kinase in osteoblasts. *Am J Physiol Endocrinol Metab* **284**, E1202-9.

Tremblay, K. D., Dunn, N. R. and Robertson, E. J. (2001). Mouse embryos lacking Smad1 signals display defects in extra-embryonic tissues and germ cell formation. *Development* **128**, 3609-21.

Tropepe, V., Sibilia, M., Ciruna, B. G., Rossant, J., Wagner, E. F. and van der Kooy, D. (1999). Distinct neural stem cells proliferate in response to EGF and FGF in the developing mouse telencephalon. *Dev Biol* **208**, 166-88.

Unsicker, K., Grothe, C., Westermann, R. and Wewetzer, K. (1992). Cytokines in neural regeneration. *Curr Opin Neurobiol* **2**, 671-8.

Urist, M. R. (1965). Bone: formation by autoinduction. *Science* **150**, 893-9.

van Praag, H., Schinder, A. F., Christie, B. R., Toni, N., Palmer, T. D. and Gage, F. H. (2002). Functional neurogenesis in the adult hippocampus. *Nature* **415**, 1030-4.

Varga, A. C. and Wrana, J. L. (2005). The disparate role of BMP in stem cell biology. *Oncogene* **24**, 5713-21.

Varley, J. E., Wehby, R. G., Rueger, D. C. and Maxwell, G. D. (1995). Number of adrenergic and islet-1 immunoreactive cells is increased in avian trunk neural crest cultures in the presence of human recombinant osteogenic protein-1. *Dev Dyn* **203**, 434-47.

Viti, J., Gulacsi, A. and Lillien, L. (2003). Wnt regulation of progenitor maturation in the cortex depends on Shh or fibroblast growth factor 2. *J Neurosci* **23**, 5919-27.

Voigt, T. (1989). Development of glial cells in the cerebral wall of ferrets: direct tracing of their transformation from radial glia into astrocytes. *J Comp Neurol* **289**, 74-88.

Vukicevic, S., Luyten, F. P. and Reddi, A. H. (1989). Stimulation of the expression of osteogenic and chondrogenic phenotypes in vitro by osteogenin. *Proc Natl Acad Sci U S A* **86**, 8793-7.

Wang, S., Sdrulla, A., Johnson, J. E., Yokota, Y. and Barres, B. A. (2001). A role for the helix-loop-helix protein Id2 in the control of oligodendrocyte development. *Neuron* **29**, 603-14.

Wang, Y., Moges, H., Bharucha, Y. and Symes, A. (2007). Smad3 null mice display more rapid wound closure and reduced scar formation after a stab wound to the cerebral cortex. *Exp Neurol* **203**, 168-84.

Weickert, C. S., Webster, M. J., Colvin, S. M., Herman, M. M., Hyde, T. M., Weinberger, D. R. and Kleinman, J. E. (2000). Localization of epidermal growth factor receptors and putative neuroblasts in human subependymal zone. *J Comp Neurol* **423**, 359-72.

Wichterle, H., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (1997). Direct evidence for homotypic, glia-independent neuronal migration. *Neuron* **18**, 779-91.

Wilson, P. A. and Hemmati-Brivanlou, A. (1995). Induction of epidermis and inhibition of neural fate by Bmp-4. *Nature* **376**, 331-3.

Wilson, S. I., Graziano, E., Harland, R., Jessell, T. M. and Edlund, T. (2000). An early requirement for FGF signalling in the acquisition of neural cell fate in the chick embryo. *Curr Biol* **10**, 421-9.

Winnier, G., Blessing, M., Labosky, P. A. and Hogan, B. L. (1995). Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev* **9**, 2105-16.

Woodruff, R. H. and Franklin, R. J. (1999). The expression of myelin protein mRNAs during remyelination of lysolecithin-induced demyelination. *Neuropathol Appl Neurobiol* **25**, 226-35.

Wozney, J. M., Rosen, V., Celeste, A. J., Mitsock, L. M., Whitters, M. J., Kriz, R. W., Hewick, R. M. and

Wang, E. A. (1988). Novel regulators of bone formation: molecular clones and activities. *Science* **242**, 1528-34.

Wrana, J. L. (2000). Crossing Smads. *Sci STKE* **2000**, RE1.

Wrana, J. L., Attisano, L., Wieser, R., Ventura, F. and Massague, J. (1994). Mechanism of activation of the TGF-beta receptor. *Nature* **370**, 341-7.

Wu, J. W., Fairman, R., Penry, J. and Shi, Y. (2001). Formation of a stable heterodimer between Smad2 and Smad4. *J Biol Chem* **276**, 20688-94.

Yamaguchi, A., Katagiri, T., Ikeda, T., Wozney, J. M., Rosen, V., Wang, E. A., Kahn, A. J., Suda, T. and Yoshiki, S. (1991). Recombinant human bone morphogenetic protein-2 stimulates osteoblastic maturation and inhibits myogenic differentiation in vitro. *J Cell Biol* **113**, 681-7.

Yamaguchi, K., Nagai, S., Ninomiya-Tsuji, J., Nishita, M., Tamai, K., Irie, K., Ueno, N., Nishida, E., Shibuya, H. and Matsumoto, K. (1999). XIAP, a cellular member of the inhibitor of apoptosis protein family, links the receptors to TAB1-TAK1 in the BMP signaling pathway. *Embo J* **18**, 179-87.

Yamashita, H., ten Dijke, P., Huylebroeck, D., Sampath, T. K., Andries, M., Smith, J. C., Heldin, C. H. and Miyazono, K. (1995). Osteogenic protein-1 binds to activin type II receptors and induces certain activin-like effects. *J Cell Biol* **130**, 217-26.

Yan, C., Wang, P., DeMayo, J., DeMayo, F. J., Elvin, J. A., Carino, C., Prasad, S. V., Skinner, S. S., Dunbar, B. S., Dube, J. L. et al. (2001). Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. *Mol Endocrinol* **15**, 854-66.

Yanagisawa, M., Nakashima, K., Takizawa, T., Ochiai, W., Arakawa, H. and Taga, T. (2001). Signaling crosstalk underlying synergistic induction of astrocyte differentiation by BMPs and IL-6 family of cytokines. *FEBS Lett* **489**, 139-43.

Yanagisawa, M., Nakashima, K., Takizawa, T., Ochiai, W., Arakawa, H. and Taga, T. (2001). Signaling crosstalk underlying synergistic induction of astrocyte differentiation by BMPs and IL-6 family of cytokines. *FEBS Lett* **489**, 139-43.

Yang, X., Castilla, L. H., Xu, X., Li, C., Gotay, J., Weinstein, M., Liu, P. P. and Deng, C. X. (1999). Angiogenesis defects and mesenchymal apoptosis in mice lacking SMAD5. *Development* **126**, 1571-80.

Yang, X., Li, C., Herrera, P. L. and Deng, C. X. (2002). Generation of Smad4/Dpc4 conditional knockout mice. *Genesis* **32**, 80-1.

Yang, X., Li, C., Xu, X. and Deng, C. (1998). The tumor suppressor SMAD4/DPC4 is essential for epiblast proliferation and mesoderm induction in mice. *Proc Natl Acad Sci U S A* **95**, 3667-72.

Yi, S. E., Daluiski, A., Pederson, R., Rosen, V. and Lyons, K. M. (2000). The type I BMP receptor BMPRIB is required for chondrogenesis in the mouse limb. *Development* **127**, 621-30.

Yi, S. E., LaPolt, P. S., Yoon, B. S., Chen, J. Y., Lu, J. K. and Lyons, K. M. (2001). The type I BMP receptor BmprIB is essential for female reproductive function. *Proc Natl Acad Sci U S A* **98**, 7994-9.

Ying, Q. L., Nichols, J., Chambers, I. and Smith, A. (2003). BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell* **115**, 281-92.

Ying, Y., Liu, X. M., Marble, A., Lawson, K. A. and Zhao, G. Q. (2000). Requirement of Bmp8b for the generation of primordial germ cells in the mouse. *Mol Endocrinol* **14**, 1053-63.

Ying, Y. and Zhao, G. Q. (2001). Cooperation of endoderm-derived BMP2 and extraembryonic ectoderm-derived BMP4 in primordial germ cell generation in the mouse. *Dev Biol* **232**, 484-92.

Yokota, Y. and Mori, S. (2002). Role of Id family proteins in growth control. *J Cell Physiol* **190**, 21-8.

Yoon, B. S. and Lyons, K. M. (2004). Multiple functions of BMPs in chondrogenesis. *J Cell Biochem* **93**, 93-103.

Young, K. M., Merson, T. D., Sotthibundhu, A., Coulson, E. J. and Bartlett, P. F. (2007). p75 neurotrophin receptor expression defines a population of BDNF-responsive neurogenic precursor cells. *J Neurosci* **27**, 5146-55.

Zehentner, B. K., Dony, C. and Burtscher, H. (1999). The transcription factor Sox9 is involved in BMP-2 signaling. *J Bone Miner Res* **14**, 1734-41.

Zhang, C. and Evans, T. (1996). BMP-like signals are required after the midblastula transition for blood cell development. *Dev Genet* **18**, 267-78.

Zhang, D., Mehler, M. F., Song, Q. and Kessler, J. A. (1998). Development of bone morphogenetic protein receptors in the nervous system and possible roles in regulating trkC expression. *J Neurosci* **18**, 3314-26.

Zhang, D., Schwarz, E. M., Rosier, R. N., Zuscik, M. J., Puzas, J. E. and O'Keefe, R. J. (2003). ALK2 functions as a BMP type I receptor and induces Indian hedgehog in chondrocytes during skeletal development. *J Bone Miner Res* **18**, 1593-604.

Zhao, G. Q., Deng, K., Labosky, P. A., Liaw, L. and Hogan, B. L. (1996). The gene encoding bone morphogenetic protein 8B is required for the initiation and maintenance of spermatogenesis in the mouse. *Genes Dev* **10**, 1657-69.

Zhao, G. Q., Liaw, L. and Hogan, B. L. (1998). Bone morphogenetic protein 8A plays a role in the maintenance of spermatogenesis and the integrity of the epididymis. *Development* **125**, 1103-12.

Zhou, Q. and Anderson, D. J. (2002). The bHLH transcription factors OLIG2 and OLIG1 couple neuronal and glial subtype specification. *Cell* **109**, 61-73.

Zhou, Q., Choi, G. and Anderson, D. J. (2001). The bHLH transcription factor Olig2 promotes oligodendrocyte differentiation in collaboration with Nkx2.2. *Neuron* **31**, 791-807.

Zimmerman, L. B., De Jesus-Escobar, J. M. and Harland, R. M. (1996). The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. *Cell* **86**, 599-606.

10 ACKNOWLEDGMENTS

I would like to thank to

Magdalena Götz for being a great supervisor. She had always time for me which is very important as time is the most precious thing nowadays ! She encouraged me a lot and taught me many things.

Mücella Öcalan for helping me in many cases

Annalisa Buffo, Silvia Cappello, Pratibha Tripathi, Benedikt Berninger, Marcos Costa for being great colleagues and friends

Ali Ertürk for encouraging me to start my PhD and supporting me despite all problems

and most importantly my parents for everything.

11 Curriculum Vitae

Personal Data:

Name: Dilek COLAK

Birth data / place: 24 April 1980 / Sakarya, Turkey

Contact info:

E-mail: dilek.colak@helmholtz-muenchen.de ; erturkdilek@yahoo.com

Call: +49 0176 22796690 or +49 89 3187 3755

Present Address:

Institute of Stem Cell Research

Helmholtz Zentrum München

German Research Center for Environmental Health

Ingolstädter Landstraße 1 D-85764 Neuherberg

Education:

- 1994-1998: Sakarya Super High School
- 1998-2003: Bachelor of Science, Hacettepe University, Faculty of Biology,
- 01.01.2004-01.06.2004 : started PhD in Max-Planck Institute for Neurobilogy
  01.06.2004-01.03.2008 : finished PhD in Helmholtz Centrum Munich in Department of Stem Cell Research

Achievements and Awards:

- Graduated from the primary and high school amongst the most successful students.
- Awarded as winner once by city ruler, once by high school discipline in poetry competitions.

- Scholarship awarded by ANACEV (Anatolian Modern Education Foundation) (1999-2003).
- Scholarship awarded by CYDD (Foundation of Education Supporters), (2000-2003).
- Graduated from Biology faculty in the second rank among 120 students in 2003.

Publications:

***** **Colak D**, Mori T, Brill MS, Pfeifer A, Falk S, Deng C, Monteiro R, Mummery C, Sommer L, Götz M (2008) Adult neurogenesis requires Smad4-mediated bone morphogenic protein signaling in stem cells. J Neurosci Jan 9, 28(2) :434-46.

Buffo A, Rite I, Tripathi P, Lepier A, **Colak D**, Horn AP, Mori T, Götz M (2008) Origin and progeny of reactive gliosis - a novel source of multipotent cells in the injured brain. Proc Natl Acad Sci U S A (Accepted).

Melanie Jawerka, **Colak D**, Spiller C, Lagger S, Wurst W, Göttlicher M, Götz M (2008) Specific requirement of HDAC2 for survival of neurons generated in the adult brain. (submitted Nature Neuroscience)

Ma L, Cantrup R, Varrault A, **Colak D**, Klenin N, Götz M, McFarlane S, Journot L, Schuurmans C (2007) Zac1 functions through TGFbetaII to negatively regulate cell number in the developing retina. Neural Develop June 8; 2:11.

****** Buffo A, Vosko MR, ***Ertürk D***, Hamann GF, Jucker M, Rowitch D, Götz M (2005) Expression pattern of the transcription factor Olig2 in response to brain injuries: implications for neuronal repair. Proc Natl Acad Sci U S A. 2005 Dec 13;102(50):18183-8.

***** : this publication has a cover image in the issue January 23, Vol. 28, Num. 4
****** : erturk was my previous name.