Regulation of Olfactory Ensheathing Cell Development by the Transcription Factor Runx1

Manjari Murthy

Department of Neurology and Neurosurgery

Faculty of Medicine, McGill University

Montreal, Quebec, Canada

Submitted January 2013

This thesis is submitted in partial fulfillment of the requirements for the degree of

Master of Science (Neuroscience)

Preface

I declare that I prepared this Master's Thesis, entitled "Regulation of Olfactory Ensheathing Cell Development by the Transcription Factor Runx1" on my own and without any aid or outside sources other than those cited. I performed all of the experiments, with the following exceptions:

The Montreal Neurological Institute Animal Care Facility technicians provided excellent technical support in the breeding and maintenance of the mouse colonies used in these studies. Dr. Federica Verginelli and Rola Dali provided invaluable assistance in the performance of the statistical analyses (Figures 10-13), and Laurent Méthot and Anthony Cotter trained me in the use of Adobe Photoshop CS5 software (all figures). Rita Lo provided indispensible technical assistance and troubleshooting genius.

Dr. Stefano Stifani edited this Thesis, and Laurent Méthot provided assistance in the translation of the abstract into French.

Acknowledgements

First, I thank my supervisor, Dr. Stefano Stifani, for his mentorship and his patience throughout these past two years. I thank my committee, Dr. Alyson Fournier and Dr. Tim Kennedy, for your encouragement and your guidance, as well as my mentor Dr. Ken Hastings. Thanks also to Dr. Joe Makkerh, for your words of wisdom over many a coffee break.

Thanks to all the members of the Stifani lab, past and present – Adèle, Rola, Laurent, Federica, Rita, Yeman and Anthony -, as well as my friends at the MNI, for your insights about science and about life, and for your friendship during those many hours in the lab.

I extend my heartfelt gratitude to my parents and my brother, for your endless amounts of love and support throughout these years, even from afar. Having a cheering squad on my side made all the difference in the world - I could not have accomplished this without you. Thanks to my friends, both in Montreal and scattered all over North America, for making me laugh and for reminding me that every road has its bumps. And thank you to Matt, for your love and your unwavering confidence in me.

Abstract

Olfactory ensheathing cells (OECs) are a unique class of glia in the olfactory system. They exhibit characteristics of both Schwann cells and astrocytes and have the ability to cross the boundary between central (CNS) and peripheral (PNS) nervous systems. The indispensible contributions of OECs to the lifelong regeneration of the olfactory system has identified these cells as promising candidates for cell transplantation therapies to repair CNS injuries. However, initial studies have yielded highly variable results on the suitability of OECs for CNS regeneration strategies. The inherent heterogeneity of OECs is believed to underlie this problem, as only a subpopulation of OECs is thought to have axonal ensheathing properties. Learning how to harness the potential of the right subpopulation has been hindered by the lack of information regarding their specific functions and molecular properties. In this regard, we have previously shown that a particular transcription factor, termed Runx1, is expressed in cells thought to correspond to a subpopulation of OECs in the olfactory bulb (OB) and have set forth to explore its expression pattern and role in this region. We demonstrate Runx1 is preferentially expressed in OECs of the inner olfactory nerve layer (ONL) and molecularly characterization of this subset of cells. Additionally, we show that Runx1 knockdown *in vivo* perturbs the proliferation of presumptive OEC precursors and leads to an increase in Runx1-expressing OEC precursors, with a parallel decrease in the number of more developmentally mature OECs. Finally, we provide initial evidence suggesting that

Runx1 might be involved in the topological organization of Runx1-expressing OECs within the inner ONL of the OB. These results raise the possibility that Runx1 might be involved in OEC fate specification and provide an avenue for further exploration into the molecular mechanisms underlying OEC subpopulation identity, specification and function.

Résumé

Les cellules olfactives engainantes (COE) sont une classe unique de cellules gliales dans le système olfactif. Elles possèdent certaines caractéristiques antigéniques et fonctionnelles des deux grandes classes de cellules gliales, soit les cellules de Schwann et les astrocytes. De plus, elles peuvent traverser la frontière entre le système nerveux central (SNC) et périphérique (SNP). Les contributions indispensables des COE à la régénération continuelle du système olfactif tout au long de la vie adulte font de ces cellules des candidats extrêmement prometteurs pour les thérapies de transplantation cellulaire pour la régénération du SNC. Toutefois, les études préliminaires ont généré des résultats variables, ce qui remet en question l'utilité thérapeutique des COE. Une telle variation dans les résultats pourrait être causée par l'hétérogénéité des COE. Cette explication est fondée sur l'hypothèse que seules certaines sous-populations de COE auraient le potentiel de régénérer et d'engainer les axones. Cependant, notre compréhension des fonctions et des propriétés moléculaires spécifiques aux sous-populations de COE est limitée, ce qui restreint l'exploitation de leur potentiel. Nous avons déjà établi que le facteur de transcription Runx1 est exprimé spécifiquement dans une population de cellules que nous croyons être les COE dans le bulbe olfactif. Ici, nous montrons que Runx1 est exprimé dans une sous-population de COE dans la région interne de la couche du nerf olfactif (CNO) et nous présentons une caractérisation moléculaire de ces cellules. Nous montrons aussi qu'une diminution du niveau de la protéine Runx1 chez la souris augmente de façon significative la prolifération des COE présomptives

et des précurseurs de COE. De plus, nous montrons qu'il y a une diminution concomitante dans le nombre de COE qui ont atteint la maturité développementale. Finalement, nous présentons des évidences initiales qui suggèrent que Runx1 est impliqué dans l'organisation topologique des COE qui expriment Runx1 dans la région interne du CNO. Ces résultats soulèvent la possibilité que Runx1 est impliqué dans la spécification du destin cellulaire des COE. Nous fournissons donc une avenue pour l'exploration future des mécanismes moléculaires qui contrôlent l'acquisition de l'identité, la spécification et la fonction des sous-populations spécifiques de COE.

Table of Contents

Index of abbreviations

AGM: Aorta-gonad-mesoephros

ALS: Amyotrophic lateral sclerosis

AML: Acute myeloid leukemia

AOB: Accessory olfactory bulb

BBB: Basso – Beattie – Bresnahan motor scale

BDNF: Brain-derived neurotrophic factor

CBFβ: Core binding factor

CNS: Central nervous system

DRG: Dorsal root ganglion

Gap-43: Growth-associated protein 43

GFAP: Glial fibrillary acidic protein

Groucho/TLE: Grouche/transducin-like enhancer of split

HAT: Histone acetyl transferase

HDAC: Histone deacetylase

MM: Migratory mass

MOB: Main olfactory bulb

MS: Multiple sclerosis

NPY: Neuropeptide-Y

OB: Olfactory bulb

OE: Olfactory epithelium

OEC: Olfactory ensheathing cell

ONL: Olfactory nerve layer

ORN: Olfactory receptor neuron

PEBP2β: Polyoma enhancer binding protein 2 β

PNS: Peripheral nervous system

Runx1-3: Runt-related transcription factor 1-3

1. Hypothesis

Hypothesis

Olfactory ensheathing cells (OECs) are a heterogeneous population of glial cells that have promising therapeutic potential for neural regeneration. However, the mechanisms underlying their development are not well understood, and little is known about the molecular characteristics that distinguish individual OEC subpopulations from one another. In light of its preferential expression in the inner portion of the olfactory nerve layer (ONL), we have hypothesized that the transcription factor Runx1 is expressed by a distinct subpopulation of OECs in the inner ONL. In addition, given the function of Runx1 in the regulation of cell proliferation and differentiation in other biological contexts, we have hypothesized that Runx1 plays a similar role in the developmental and specification of inner ONL OECs.

2. Introduction and Aims

Introduction and Aims

1. Central nervous system injury: a heavy burden

Central nervous system (CNS) injury is one of the leading causes of death and long-term disability in North America (Dana Alliance for Brain Initiatives – http://www.dana.org/news/publications/detail.aspx?id=4220) with significant socioeconomic consequences. It can occur as a result of both traumatic and non-traumatic events; examples of the former include injury from an external blow to the spinal cord or the head, while the latter include degenerative diseases such as amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS). In the United States alone, over 11,000 people suffer a spinal cord injury (SCI) every year (US Centre for Disease Control – http://www.cdc.gov/healthcommunication/ToolsTemplates/EntertainmentEd/Tips/SpinalCordInjury.html) with the median age of injury being 33 years. Over 3,000 Canadians currently live with ALS (ALS Society of Alberta - http://www.alsab.ca/facts.aspx), and 3 Canadians are diagnosed with MS every day (MS Society of Canada - http://mssociety.ca/en/information/default.htm).

1.1 CNS response to injury

In response to injury, the CNS mounts a swift and robust response, inducing the activation, migration and proliferation of many diverse classes of cells, both local and external to the injury site (Fitch 2008). The glial cells of the CNS – oligodendrocytes, astrocytes and microglia – all play crucial roles in the normal

development and maintenance of the CNS. Oligodendrocytes are vital for the myelination of axons, insulating and increasing conductivity of axon fibers; they have also been shown to secrete growth factors and neurotrophic factors such as brain-derived neurotrophic factor (BDNF) which support locally developing neurons (Dai 2003). Astrocytes, the most abundant type of glia in the CNS, maintain the blood-brain barrier by apposing end-feed to cerebral blood vessels, thus contributing to the transport of materials to neurons (Vise 1975). They are involved in glutamate uptake and release (Drejer 1982), the modulation of cytokines and extracellular pH and K^+ levels (Benveniste 1998), synapse formation and maintenance (Ullian 2004), and T-cell activation as part of the immune response Dong (Dong 2001). Microglia are the resident innate immune cells of the CNS and play a crucial part in the first response to injury through phagocytosis and the recruitment of cells associated with the adaptive immune system through antigen presentation (Aloisi 2001; Kim 2005; Lehnardt 2010). They have also been shown to play a neuroprotective role, for instance minimizing NMDA-induced excitotoxicity in their resting state (Howe 2012).

These and other cells, including meningeal cells and phagocytes, react forcefully to injury, proliferating and migrating to the site of injury (Arvin 1996; Wang 2000; Fitch 2008). The breakdown products of oligodendrocyte-produced myelin include Nogo (Caroni 1988), myelin-associated glycoprotein (Mukhopadhyay 1994), Tenascin (Pesheva 1989) and Semaphorins (Moreau-Fauvarque 2003), and largely inhibit axonal growth. The activation of microglia by molecules such as Toll-like receptors results in the release of a barrage of pro-

inflammatory factors including cytokines, chemokines and other related enzymes. This creates a neurotoxic environment and contributes to secondary degeneration of the lesion site (Pais 2008; Lehnardt 2010). Microglia, once activated, release inflammatory molecules such as reactive oxygen species, excitatory amino acids, cytokines and protease inhibitors, leading to secondary degeneration of the lesion and further destroying surrounding tissue, neurons and remaining axons. In addition to this, the presence of inflammatory factors creates a non-permissive environment for neurogenesis and axonal re-growth (Bovolenta 1993; Ferguson 2011). Astrocytes present a particular problem for regeneration; upon injury they hypertrophy, a process known as "reactive astrocytosis", and in doing so dramatically increase the number of processes emanating from their cell bodies. These processes form a dense network, held together by tight junctions and surrounded by an extracellular matrix; this is collectively known as the glial scar (Fitch 1997; Fawcett 1999) and prevents penetration of neuroprotective substances (Figure 1).

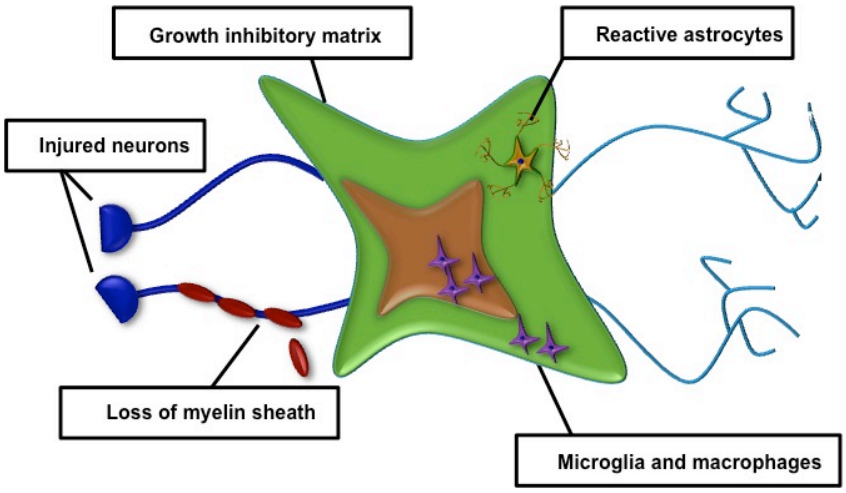

Figure 1: The CNS response to injury. Upon injury, severed neurons degenerate while resident and invading cells secrete molecules that create an inhibitory environment for regeneration. Additionally, reactive astrocytes hypertrophy to form a glial scar. Based on Rolls 2008.

The repair of CNS injury is thus complex and multi-faceted – all of these challenges must be surmounted for any therapy to be successful. Throughout the past two decades cell-based therapies for the repair of the injured CNS have emerged as a promising alternative to non-biological grafts and other similar treatments, circumventing the problems associated with immune rejection. The development of this novel therapy is ongoing, and a notable approach that shows particular clinical promise is the use of a unique cell population which exists within the central and peripheral nervous systems, a cell population with the remarkable ability to support regeneration in the normal adult nervous system: olfactory ensheathing cells (OECs).

2. An introduction to olfactory ensheathing cells

OECs are a class of glia found exclusively in the olfactory system. They are unique in that they exhibit characteristics of both PNS (Schwann cells) and CNS (oligodendrocytes and astrocytes) glia; thus, their membership to either category is heavily debated. Interestingly, the transcription factors that control the development of Schwann cells, astrocytes and oligodendrocytes do not appear to control the development of OECs. Sox10, for example, is known to be critical for the development of peripheral glia in the neural crest lineage, including Schwann cells, as well as the progression of immature Schwann cells towards their mature form (Britsch 2001; Finzsch 2010). In contrast, Sox10 does not as of yet have a demonstrable role in OECs development. Similarly, Olig2 is required for the specification of oligodendrodocytes from neuronal precursors in the embryonic

neural tube, but is not expressed by developing OECs. Another example is provided by Pax6, a transcription factor that is expressed in astrocyte progenitor populations, but has no known expression in OECs at any stage of development (Hochstim 2008).

Similar to Schwann cells, OECs ensheath olfactory receptor neuron (ORN) axons not by myelinating them but by extending tongues of cytoplasm to form a compact bundle (de Lorenzo 1957; Doucette 1984). However, *in vitro* they have been shown to have the capacity to myelinate dorsal root ganglion (DRG) axons (Babiarz 2011). In agreement with the above observations, proteomic (Boyd 2006) and microarray (Franssen 2008) analyses have revealed key differences in the OEC and Schwann cells proteomes that might explain the observed differences. One key characteristic of OECs is their ability to intermingle freely with astrocytes, a characteristic that is not shared by Schwann cells. In co-culture assays, OECs have been shown to migrate towards an area inhabited by astrocytes, while Schwann cells to not, and astrocytes have been shown to hypertrophy in the presence of Schwann cells but not OECs (Laktos 2000). In addition, when cultured on an astrocyte monolyer, OECs have been shown to migrate more quickly than Schwann cells (Wilby 1999). Evidence has been gathered by various groups suggesting that multiple sub-populations of OECs exist, distinguishable by their position, molecular marker expression and function; this will be discussed more fully later in the text.

3. The utilization of olfactory ensheathing cells for the treatment of CNS injury

A number of cell populations have been used in therapies for the treatment of CNS injury in animal models of trauma or neurodegenerative disease (Tetzlaff

2011). The major classes of cells tested - Schwann cells, neural stem/progenitor cells and OECs – were initially identified by virtue of their important roles in the upkeep of nervous system integrity or, in the case of neural stem/progenitors, to generate new neurons.

3.*1. Schwann cells and neural stem/progenitor cell-based therapies have had limited success in clinical trials*

Schwann cells have long been thought to have promising reparative potential due to their natural ability to remyelinate axons *in vivo* and to create a substrate upon which axons can extend (Lavdas 2008). However, studies have shown that their reparative abilities are limited; in one trial, Xu and colleagues showed that Schwann cells can promote the outgrowth of sensory axons from DRG as well as propriospinal axons adjacent to the site of injury (Xu 1995). They can only stimulate limited regeneration of brain stem axons (Menei 1998) and they do not have the ability to the promote re-entry of axons from the Schwann cell graft into the spinal cord (Ramon-Cueto 1998; Vroemen 2007), greatly reducing their therapeutic usefulness. Importantly, they cannot co-exist with astrocytes; they cause the latter to hypertrophy and aggravate the injury site through the additional release of inflammatory and reactionary factors from the glial scar (Fitch 2008).

Neural stem/progenitor cells have also been tested as candidates for therapy. Their multipotency, a key attractive feature, is illustrated by the fact that stem/progenitor cells harvested from the subventricular zone of the brain contain the precursors for neurons, astrocytes and oligodendrocytes (Mothe 2008; Karimi-

Abdolrezaee 2010; Mothe 2011). A number of studies have reported that the majority of cells that differentiated from these isolated neural stem/progenitor cells were of the glial lineage, with few to no neurons at the injection site (Cao 2001). Furthermore, although some studies reported an increase in myelin compaction and functional improvements (Karimi-Abdolrezaee 2006; Parr 2008) on the Basso, Beattie and Bresnahan (BBB) locomotor scale, evidence of decreased sensory thresholds suggests that the use of these cells might result in allodynia and neuropathic pain, which is a serious cause for concern (Hofstetter 2005).

3.2. Olfactory ensheathing cell-based therapies are promising but clinical trials have yielded mixed outcomes

OEC-based therapies have perhaps shown the most clinical promise through both their ability to support various aspects of regeneration and, importantly, the fact that they do not elicit reactive astrocytosis or similar negative outcomes (Lu 2002; Boyd 2005; King-Robson 2011). A number of studies were conducted to examine the effects of OECs on the repair of various types of injuries, including weight drop injury (Deng 2008), full transection (Ramon-Cueto 2000; Guest 2008; Kubasak 2008), hemisection (Deumens 2006), electrolytic lesion (Li 1998) and compression (Boyd 2004) at various levels of the spinal cord. The results from many of these studies have been encouraging. Ramon-Cueto and colleagues found that, upon spinal cord transection, motor axons were regenerated over a long distance, and animals regained locomotor and sensorimotor functions, as assessed by skin prick and climbing tests, three months post-injection (Ramon-Cueto 2000).

Upon performing a spinal cord transection on adult rats, Kubasak and colleagues noted tissue sparing and the regeneration of noradrenergic and seratonergic fibers at the injury site after OEC transplantation, as well as an increase in stepping ability as assessed by a treadmill test (Kubasak 2008). Similarly, Imaizumi and colleagues noted robust axonal growth and myelination in addition to a restoration of impulse conduction upon injection of OECs into dorsal column transection injury site (Imamura 2011). This was similar to results seen by Richter and colleagues, who noted significant outgrowth of axonal populations upon transplantation (Richter 2005).

However, a significant number of studies did not see these beneficial effects, noting little to no axonal regeneration, remyelination or functional recovery. For example, Toft and colleagues examined rats with complete transection and noted that, although there was axonal growth at the lesion site and electrophysiological function was maintained in the dorsal column, there was no regrowth of ascending sensory fibers from the lesion site (Toft 2007). Post OEC injection, Steward and colleagues found no regeneration of descending axons from the site of transection, and no evidence of functional recovery (Steward 2006). Deumens and colleagues did not note any regeneration of corticospinal tract axons across the lesion site after OEC treatment, though axon growth was robust, and no functional recovery (Deumens 2006). Similarly, Pearse and colleagues demonstrated poor OEC cell survival post-injection, no axonal in-growth into the OEC transplants and limited functional recovery (Pearse 2007).

A number of research groups have attempted to duplicate the methodology employed by their peers in an effort to reproduce the positive experimental outcomes demonstrated previously. However, these efforts resulted in neutral or negative outcomes. An example of this is the study conducted by Steward and colleagues, who replicated a study previously published by Lu and colleagues in 2002 (Lu 2002). In their original study, these authors had shown that, upon implantation of pieces of lamina propria into the site of a spinal cord transection injury, serotonergic fibers sprouted and extended up to 4 mm from the site of implantation. In contrast, Steward and colleagues showed little to no axonal regeneration or extension from the injury site (Steward 2006).

3.3. Why are the results from OEC-based transplantation therapies so variable?

One major variable that may explain the contradictory results obtained from OEC-based transplantation studies is the source of the OECs themselves. OECs used in many of these studies were isolated from one of their two areas of residence, the olfactory nerve layer (ONL) of the olfactory bulb (OB) (Deumens 2006; Pearse 2007; Toft 2007) or the lamina propria of the olfactory epithelium (OE) ((Richter 2005; Steward 2006). Alternatively, OEC cell lines were cultured and immortalized (Moreno-Flores 2006). Several groups have shown there to be intrinsic variability in therapeutic outcomes among different populations of OECs (Richter 2005; Guerout 2010); moreover, the culturing OECs has been shown to alter their antigenic profiles and morphologies in vitro (Doucette 2001; Jani 2004; Higginson 2011).

Another variable is the type of experimental animal model used. The species from which OECs were isolated varied from study to study; in the majority of cases, rat-derived OECs were used for transplantation, though some groups used OECs from pigs (Imamura 2011) or primates (Guest 2008). In addition, the time period between experimentally induced injury of the CNS and the implantation of OECs ranged from a few minutes (Guest 2008) to up to 2 weeks (Deng 2008), dramatically altering the biological conditions into which OECs were transplanted. Furthermore, the stage of development of the donor or recipient was not consistent between trials. In some trials, OECs were isolated from prenatal or neonatal rodents (Imaizumi 2000; Lopez-Mascaraque 2002; Boyd 2004; Richter 2005), while in others, the OECs were derived from adult rodents (Ramon-Cueto 2000; Pearse 2007; Kubasak 2008) for transplantation in rat models of CNS trauma.

The inconsistencies that exist in both the methodology employed during the execution of the trials and the results obtained from separate experiments point to a gap in our fundamental understanding of the biology of OECs. This lack of understanding has prevented researchers from being able to successfully and reproducibly use this promising class of cells in therapies for the repair of CNS injury. We do not sufficiently understand the nature of the differences between cells that are leading to such dramatic differences in therapeutic outcomes; thus, we cannot with any degree of certainty determine which population would be maximally beneficial for repair. Some of the key questions that will help us understand this variability include the following: 1) How are OECs obtained from different parts of the olfactory system different, and what unique properties do they

possess? 2) How do these different subtypes of OECs develop and establish their unique properties? 3) How can we distinguish OECs that are optimally beneficial for use in CNS repair from those that may have unnecessary, unwanted or harmful effects (Richter 2005)?

The key to answering these questions lies in acquiring a detailed understanding of OEC biology and, more specifically, unraveling the elements that might make a subset of OECs especially useful for therapeutic use.

4. The biology of the olfactory system

To begin to understand the complex biology of OECs, we must take a step back and examine the olfactory system as a whole. The olfactory system is one of the only biological systems that retain the capacity for self-renewal throughout the lifetime of an organism (Freeman 1997). The structure, function and development of the olfactory system have been studied for almost 50 years; thus, many, but not all, of its components are fairly well understood.

The olfactory system has both a PNS and a CNS component. The PNS component, the OE, was thought to have an exclusively peripheral origin, arising from the olfactory placodes, non-neural ectodermal thickenings that are the sites from which the first pioneering ORNs emerge in the developing embryo (Mendoza 1982; Couly 1985). However, recent work has revealed that there may be a CNS contribution to OE development, in the form of cells derived from the neural crest, a transient neuroepithelial structure that contributes to structural elements of the nose (Katoh 2011). Cells from the neural crest are believed to intermingle with

placode-derived cells (Shimizu 2002; Barraud 2010; Forni 2011; Forni 2012), adding a new dimension to the development of OECS that will be discussed later.

Once the OE has been structurally established in the developing organism, it becomes the site of neurogenesis in the olfactory system, playing host to horizontal and globose basal cells, ORNs, and sustentacular, or support, cells (Graziadei 1979; Carr 1991; Chen 2004; Beites 2005). ORNs that originate in the OE extend axons that bundle together into *fila olfactoria*, or olfactory fascicles, and course towards the CNS component of the olfactory system, the OB. Here, they will ultimately penetrate the two most external layers of the bulb and synapse with second order neurons (Greer 1991) to relay odorant information to the piriform, or olfactory, cortex (Lledo 2005).

The OB is the CNS component of the olfactory system, located in the most anterior part of the forebrain within the rhinencephalon. The OB proper is made up of 2 distinct parts: the accessory OB (AOB) and the main OB (MOB). The AOB is innervated by ORNS from the vomeronasal organ, which forms part of the accessory olfactory system in the PNS, and processes information related to pheromones via connectivity to the amygdala. The MOB is innervated by axons from the OE and relays general odor information to the piriform cortex and hippocampus. Henceforth, I will focus on the MOB, designated for sake of clarity as OB.

Development of the OB begins around embryonic day (E) 11, and continues until about E16 in the mouse (Hinds 1972; Doucette 1989). This process is mediated by intrinsic transcription factor-mediated cues (Jiminez 2000; Harrison 2008) and extrinsic cues from neurotrophic factors (Mackay-Sim 2000), soluble proteins

(Lambert 1988), and extending “pioneer” axons, which induce changes in cell cycle kinetics selectively within the rostral telencephalon that cause it to evaginate (Gong 1995).

The mature OB has a definitive layered organization and a number of well-defined cell populations with distinct structural characteristics and functions within the olfactory sensory pathway. These cells are generated from progenitors, which reside in the subventricular zone of the walls of the lateral ventricles in the forebrain. From here, they migrate to the OB via the rostral migratory stream, where they will reach their final positions in a given layer of the bulb and terminally differentiate (Altman 1969; Lledo 2008). The most internal OB layer is the granule cell layer, followed by the internal plexiform layer, the mitral cell layer, external plexiform layer, glomerular layer and finally, the ONL (Figure 2).

The granule cell layer and glomerular layer are populated by granule cells and periglomerular cells, respectively; these cells types comprise the two broad classes of interneurons within the OB. Eight subtypes of interneurons exist, each with characteristic morphologies and functions (Schneider 1978). The interneurons of the OB are GABA-ergic and form synapses with primary neurons, laterally inhibiting excitatory inputs and allowing for odor discrimination (Parrish-Aungst 2007; Lledo 2008). The principle neurons of the OB are mitral and tufted cells, glumatergic neurons that reside in the mitral cell layer and external plexiform layer, respectively (Halasz 1990), and extend their processes into the glomerular layer. Each mitral/tufted cell has only 1 dendrite, which will synapse with ORN axons in a single glomerulus; these cells project their axons to the olfactory cortex, mediating

responses to odor stimuli based on both their projections and differences in their locations within the OB (Nagayama 2004). Finally, the ONL plays host to a population of interfasicular astrocytes as well as OECs, which accompany incoming ORN axons from the OE into the OB and guide them towards their target neurons in individual glomeruli (Ramon-Cueto 1998) (Figure 2). A more detailed review of OEC biology will be given later in the text.

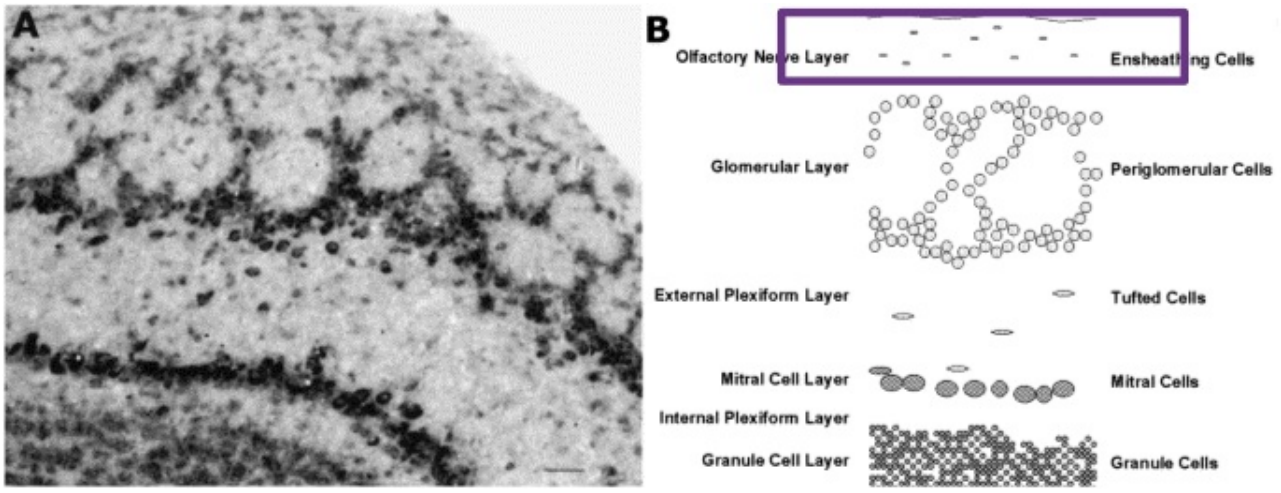

Figure 2. An electromicograph (A) and graphical depiction (B) of the layers of the olfactory bulb. The six layers of the bulb contain cell populations that in concert relay odorant information from the olfactory bulb to the pirifom (olfactory) cortex. The box indicates the ONL, in which OECs reside (Modified from Mackay-Sim 2000)

Different cell populations within the OE and the OB regenerate throughout the lifetime of the organism due to the continual loss of the neurons and supporting cells that occurs with the assault from sustained use. Turnover of ORNs is estimated to occur roughly every 40 days (Coppola 2012); newly born neurons arise from the previously-mentioned population of globose basal cells, a stem cell population maintained deep within the adult OE (Suzuki 1993; Caggiano 1994; Huard 1998; Beites 2005). Globose basal cells and the closely related horizontal basal cells also give rise to sustentacular cells within the OE, which are vital for structural support,

detoxification of the OE environment and phagocytosis of dead ORNs (Suzuki 1996; Beites 2005). The OE is also believed to contain the stem cells that give rise to OECs in the adult organism; evidence for this comes from *in vitro* work showing that OECs can arise from cultured globose basal cells, the stem cells in the OE (Calof 1999; Iwai 2008), as well as from *in vivo* studies showing the migration and replenishment of OECs from precursors in the OE after bulbectomy (Chehrehasa 2012). In the OB, regeneration of the neuronal population also occurs, but new cells arise from a population of progenitors that travels from the subventricular zone to the OB via the rostral migratory stream. This constant replenishment of interneurons is believed to be essential for the fine-tuning and optimization of olfaction in response to environmental cues (Alvarez-Buylla 2001; Mouret 2009).

4.1. Olfactory ensheathing cells: the glia of the olfactory system

The known biology of OECs belies the remarkable diversity in both their presence and function in the olfactory system. As mentioned earlier, OECs can be found in the lamina propria of the OE as well as in the ONL of the OB. First identified by the histologists Golgi (Golgi 1875) and Blanes (Blanes 1898) by virtue of their location apposed to ORNs, OECs are unique in their ability to both intermingle with astrocytes as well as to ensheath axons in a compact bundle, reminiscent of Schwann cells (de Lorenzo 1957; Doucette 1984).

The embryonic origin of OECs is a matter of intense debate (Chuah 1991; Ramon-Cueto 1998; Barraud 2010; Forni 2011). For almost three decades, the widely accepted dogma stated that these cells arise from the migratory mass (MM),

a highly heterogeneous group of proliferating cells composed of ORN and OEC progenitors that originates in the peripheral environment of the olfactory placodes (Graziadei 1979; Mendoza 1982; Farbman 1985; Marin-Padilla 1989; Chuah 1991; Miller 2010). These cells are believed to leave the placodes at around E11 and travel towards the OB, first making contact around E12.5. Upon reaching the rostral telencephalon, neurons from within the mass extend axons towards the presumptive OB while early-born OECs begin to populate the outer layer of the developing bulb till the end of OB development, around E18.

If OECs are indeed of placodal origin, they would be unique among all glia, as the olfactory placodes are composed of non-neural ectoderm; all other glia originate from the neural crest (Schwann cells) or from progenitors in the telencephalon, subventricular zone and other deep brain structures (oligodendrocytes and astrocytes) (Rowitch 2004; Jessen 2005; Menn 2006). However, recent evidence has suggested that at least certain OECs may arise from the neural crest. Grafting experiments in the chick have shown that OECs derive from this transient neuroepithelial structure (Barraud 2010), and lineage tracing and immunohistochemical evidence show that some OECs display neural crest markers within the MM (Forni 2011). This is in agreement with early work that suggested that the neural crest might contribute to the placode itself by virtue of their close proximity during development (Couly 1985; Smith 1994). These findings add another dimension of complexity to OEC biology and demonstrate that further study into the origin of these remarkable cells needs to be conducted.

4.2. Olfactory ensheathing cells play a wide variety of roles in the developing and adult olfactory system.

The diverse roles of OECs within the olfactory system, both in the developing and adult organism, are the key characteristics that make these cells so attractive as therapeutic candidates. They have a well-defined role in promoting the regeneration and survival of neurites from ORNs (Goodman 1993). Studies such as those performed by Jiao and colleagues have demonstrated that, when co-cultured with adult brainstem slices, OECs are capable of not only promoting neurite outgrowth, but also supporting axon extension by allowing axons to growth along their processes (Jiao 2011). OECs also create a substrate upon which extending axons can grow: Chehrehasa and colleagues showed that, upon unilateral bulbectomy, OECs invade the resultant cavity prior to axon extension, creating a permissive environment for growth in addition to a substrate of processes over which axons can extend (Chehrehasa 2010). In this vein, Windus and colleagues demonstrated that OECs from the olfactory epithelium promote axon adhesion and outgrowth via the secretion of glial cell-derived neurotrophic factor (GDNF), which induces the formation of lamellipodial waves. These waves stimulate outgrowth through increased growth cone activity of the tips of axons directly in contact with OECs, greatly enhancing axon motility (Windus 2010).

OECs also secrete a number of neurotrophic factors, which further contributes to their regenerative capabilities (Sonigra 1999; Runyan 2009). These factors include BDNF (Pastrana 2007), which promotes the regeneration of the axons of adult CNS neurons and adult retinal ganglion cells in vitro. In addition,

OECs express axonal growth-enhancing molecules such as neuropeptide Y (Ubink 2000), neurotrophin receptors such as the p75 neutrophin receptor (Vickland 1991), and a variety of axon guidance molecules, including EphB2 (St. John 2001) and Semaphorin 3-A (Schwarting 2000; Schwarting 2004). The highly specific spatiotemporal expression pattern of these guidance molecules reveals the important role they play in another key OEC function, which is influencing precise innervation of afferents in the OB. For example, Schwarting and colleagues demonstrated that the ventro-medial population of OECs expressing Sema3A was vital to the targeting of ORN axons expressing the corresponding receptor neuropilin-1 (Npn1) to the proper glomeruli. Accordingly, loss of Sema3A resulted in mis-targeting of these axons (Schwarting 2004).

OECs are similarly instrumental for the proper migration of neuronal precursors from the subventricular zone towards the OB via the rostral migratory stream by secretion of diffusible factors (Zhu 2010) such as Netrin1 (Hakanen 2011). Interestingly, Windus and colleagues noted that OECs from different anatomical regions have distinct cell-cell interactions when placed in culture – OECs from the olfactory epithelium appeared to grow in closer contact to one another, while those from the OB had a more dispersed spatial distribution. In addition, they showed that ONL-derived OECs displayed adhesive, attractive and repulsive interactions with each other during axonal growth *in vitro*, while olfactory epithelium-derived OECs showed only adhesive behaviour (Windus 2010). These distinct behaviors are representative of the functional differences that exist in this

highly heterogeneous population of cells, and perhaps to some degree to explain the variability in results obtained from transplantation of these OECs (Richter 2005).

4.3. Molecular characterization of olfactory ensheathing cells

The differences among the results obtained from different OEC populations used during clinical trials, the wide range of functions performed by OECs in the olfactory system, and their unique glial characteristics are strongly suggestive of the existence of multiple subpopulations of OECs. Indeed, evidence has been gathered by various groups alluding to the existence of multiple OEC subpopulations, distinguishable by their position, molecular marker expression and function. A study by Au and colleagues, for example, studied the ONL of the OB and molecularly characterized the OEC populations that exist within its boundaries. They performed immunohistochemical analyses, staining for markers of various glia, both peripheral and central (the calcium binding protein S100β, and neuropeptide Y [NPY], both expressed in astrocytes, and the neurotrophin receptor p75, expressed in Schwann cells, among others). Interestingly, they found a distinct expression within the ONL; S100β, for example, was expressed by all OECs, but p75 appeared to be expressed only by those in the outer portion of the ONL (Au 2002).

By contrast, NPY was only expressed by OECs in the inner portion of the ONL**,** a finding previously reported by Ubink and colleagues (Gimpl 1993). Spatiotemporal analyses of the emergence of the OEC markers GFAP and S100β demonstrated the distinct developmental progression of OECs throughout embryonic and neonatal nervous system development (Astic 1998). In addition to

OECs, the inner ONL also plays host to astrocytes. These glia can be distinguished from OECs in large part by their electron micrograph profiles; astrocytes have electron lucent cytoplasm that contain dense bundles of filaments, while OECs are less electron dense and have fewer filaments (Doucette 1984). A number of proteins, including NCAM, GFAP, O4, Gal-C and Aquaporin 1, have been shown to be expressed by OECs throughout the olfactory system (Franceschini 1996; Ramon-Cueto 1998; Barnett 2002; Wang 2008; Smithson 2009; Shields 2010). These findings are a further demonstration of the complexity of OECs, which express markers of both CNS and PNS glia in varying, unique combinations (Figure 3).

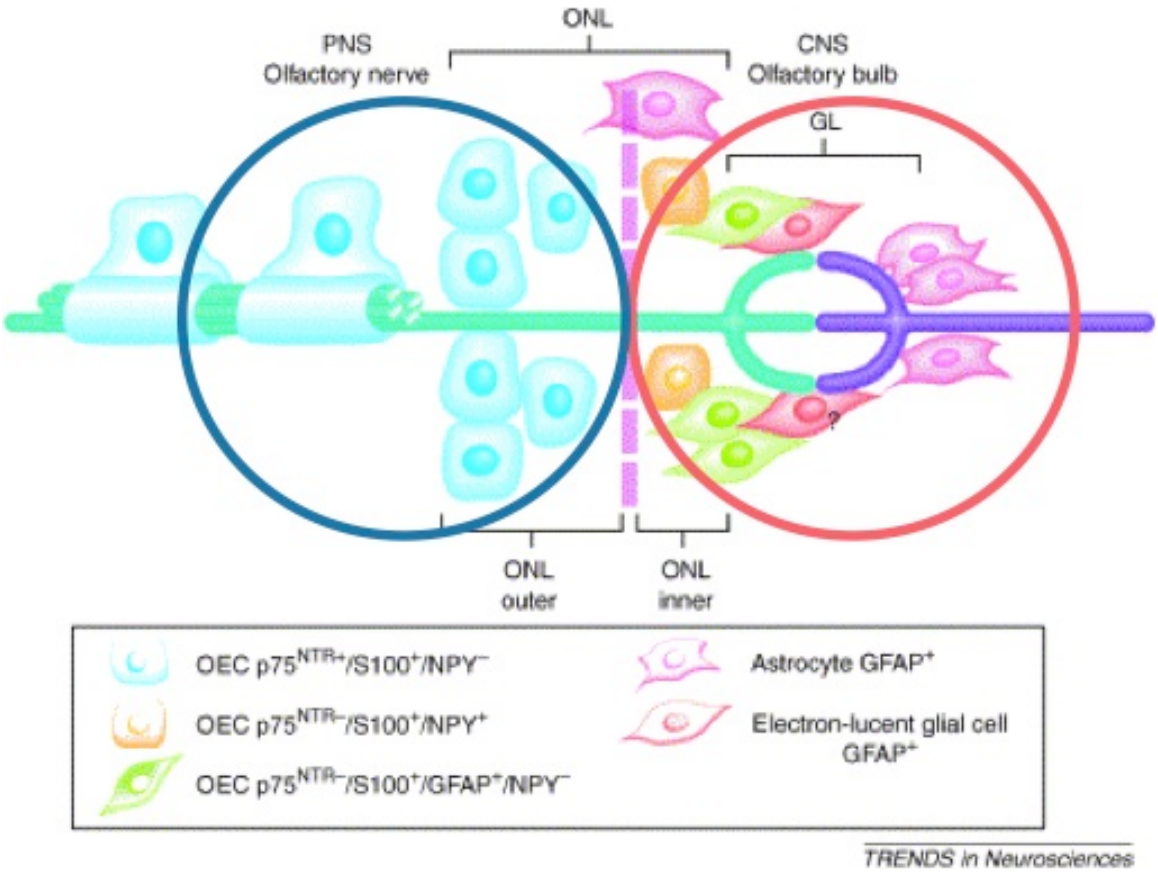

Figure 3: A graphical depiction of the diversity of the biomarkers expressed by different populations of OECs in the OB. OECs express markers of both Schwann cells (eg. p75) and astryocytes (eg. GFAP) in various combinations, strongly suggesting the presence of multiple sub-populations of cells. The circles denote the inner ONL and outer ONL. (Adapted from Franceschini 2004).

It is interesting to note that the heterogeneity of the OEC population is not maintained in culture. An example of this is a study performed by Audisio and colleagues; upon dissociating and immortalizing OECs from the OBs of neonatal rats, they found that the cells in culture were homogeneous in their antigenic profiles (Audisio 2009), even though OECs from the bulb have been previously shown to be antigenically heterogeneous. In this vein, OECs' molecular characteristics are demonstrably plastic as they can be induced to express antigenic markers in the presence of various molecules, such as cAMP (Doucette 1994; Vincent 2005) and soluble factors from various types of conditioned media (Chuah 1993; Pollock 1999; Barnett 2002; Au 2003).

However, despite the growing number of proteins shown to be expressed by OECs (Pellitteri 2010), there is a paucity of information on the mechanisms underlying the formation of individual OEC subpopulations. Thus, we cannot make the finer distinctions between particular populations, nor can we learn how to study these different populations and possibly refine their use in clinical trials. Our understanding of how the generation of OEC subtypes is developmentally regulated is also woefully vague. This makes the elucidation of the mechanisms underlying development of specific OEC subtypes difficult, and the deciphering of these subtypes' roles in the context of CNS regeneration near impossible.

With this in mind, we have focused our research on the search for such novel markers and developmental regulators, in an attempt to better understand the

biology underlying OEC development and subtype specification. The Gene Expression Nervous System Atlas (GENSAT) project (http://www.gensat.org/index.html), a public NIH-funded multi-institute initiative, has previously characterized eGFP transgenic mice exhibiting a striking eGFP expression pattern localized specifically on the outer portion of the OB, in what appears to be the ONL. The gene targeted in these mice was of particular interest to us due to its previously demonstrated role in OE development (Theriault 2005). This gene encodes a transcription factor known as Runx1.

5. The Runx family of transcription factors

Runx1 is a member of the Runt-related family of transcription factors, evolutionarily conserved DNA-binding proteins that are context-dependent transcriptional activators or repressors (Coffman 2003; Evans 2003; Durst 2004; Ito 2004; Inoue 2008; Zagami 2009). All Runx proteins contain a conserved 128-amino acid DNA binding domain, known as the Runt-homology domain, and have distinct expression patterns in organisms ranging from sponges to higher mammals, including humans (Simeone 1995; Coffman 2003; Levanon 2004; Zagami 2009).

5.1. The Drosophila *runt genes*

The *Drosophila* ortholog of mammalian *Runx*, the *runt* gene, was first identified in 1988 as an important regulator of body patterning, acting as a "pair rule gene" required for body segmentation during embryogenesis. The absence of *runt* leads to a runt-like phenotype, lending to the gene its unique moniker (Gergen

1988). Another *Drosphilia runt*-related gene has since been shown to be involved in other types of patterning; specifically, *Lozenge* is important for the specification and pre-patterning of photoreceptor cells in the developing larval eye (Daga 1996; Canon 2000). *runt* also regulates the acquisition of the female sex during *Drosophila* embryogenesis (Duffy 1991).

Various aspects of neurogenesis require the activity of *runt in Drosophila*; this gene regulates the specification of a subset of sensory neurons, and its expression in neuroblasts is vital to the development of this specific neuronal subtype (Duffy 1991). Additionally, *runt* is required for the expression of the *even-skipped* gene in presumptive EL neurons in abdominal hemisegments and thoracic segments of the developing embryo, giving rise to EL neurons that are required for the proper formation of the nerve cord (Dormand 1998). The many roles played by *runt* in *Drosophila* - from segmentation to cell fate specification to cellular patterning – demonstrate the wide range of developmental processes in which it is implicated.

5.2. Mammalian Runx genes

In mammals, there are three Runx family members, each with mostly distinct but sometimes overlapping expression patterns (Levanon 2004). Indeed, in certain developmental processes, such as craniofacial development, concerted activity of all three Runx proteins is required (Yamashiro 2002). Not surprisingly, due to their important biological functions, dysfunction of Runx proteins has been shown to lead

to a wide range of developmental disorders as well as cancers (Evans 2003; Durst 2004; Ito 2004; Inoue 2008).

Of the three *Runx* genes, *Runx3* is the shortest and contains the fewest number of exons (Levanon 2004). Runx3 plays an important role in development of the gastric epithelium, suppressing the proliferation of epithelial cells. Loss-of-function mutations in *Runx3* have been shown to result in certain gastric tumors (Ito 2003; Bae 2004; Friedrich 2006), though the recent findings showing, through lineage tracing, that Runx3 is absent from gastrointestinal tract epithelium, has called into question its role in cancer pathogenesis (Levanon 2011). Runx3 is also vital for the development and survival of TrkC+ nociceptors in the DRG; in the absence of Runx3, TrkC+ cells do not survive long enough to extend axons towards their target cells (Levanon 2002). In addition to this, Runx3 has been shown to mediate epigenetic silencing in cytotoxic T cells during their development (Taniuchi 2002). Together these findings demonstrate a role for Runx3 in functions ranging from proliferation, differentiation, cell fate specification and gene regulation, proving it to be an important regulator of development.

Runx2, sometimes referred to as the "master regulator of bone development", is vital for skeletogenesis, promoting the transition of preosteoblasts from proliferating progenitors to mature osteoblasts. Absence of Runx2 leads to a complete loss of osteoblasts (Komori 1997) and a hereditary congenital disorder known as cleidocranial dysplasia in humans (Otto 2002), which is characterized by underdeveloped bones and partial or absent clavicles (Mendoza-Londono 2006). In addition, Runx2 is expressed in chondrocytes and is required for the production of

chondrocyte precursors; a reduction in Runx2 expression results in the underdevelopment or absence of a bone matrix (Ducy 1999; Long 2011). Runx2 is important for FGF signaling from the epithelium to the early tooth bud, mediating developmental maturation of the tooth; the loss of Runx2 prevents molar odontogenesis (Ryoo 2006). Due to its role in the activation of matrix metalloproteinases, angiogenic factors and bone matrix proteins, Runx2 has been identified as an early regulator of metastasis in various bone, breast and prostate cancers (Pratap 2006). Thus, in a similar vein to the important role of Runx3 in development, Runx2 is notable for its many roles in the coordination of vital aspects of bone development and skeletal maturation.

5.3 Runx1

Runx1 is best known as a gene with a large frequency of chromosomal translocations causing acute myeloid leukemia (AML), lending to Runx1 is alternative moniker of AML1 in humans (Michaud 2003; Goyoma 2011; Liddiard 2012). For consistency, the Runx1 nomenclature will be used throughout the text hereafter.

Runx1 has two promoters, P1 and P2, which are common among all vertebrates; alternative splicing from these promoters yields three isoforms (Runx1a-c), which all contain the Runt domain but differ in protein size, abundance and transcriptional competence (Bae 1994; Miyoshi 1995; Fujita 2001; Kanaykina 2010; Challen 2011). In mice, Runx1a has a strong affinity for DNA but lacks both a transcriptional activator and repressor domain. Thus, in binding to target genes, it

has no transcriptional activity but instead, by not allowing the binding of transcriptionally competent isoforms, acts as a dominant negative form (Miyoshi 1991; Kanaykina 2010). Runx1b is the most abundant isoform and has a unique N-terminal 5 amino acid sequence that is not present in Runx1c. Both are transcriptionally competent, but Runx1c is less frequently expressed and appears to be present only during certain period of early embryonic development (Bae 1994; Fujita 2001; Zambidis 2005; Challen 2011).

These three isoforms of Runx1 are believed to play different roles in cellular development at various stages of maturation. For example, in the hematopoetic system, Runx1a, the transcriptionally incompetent version of Runx1, is highly expressed in the immature hematopietic cell compartments in cord blood, and is thought to play a role in the maintenance of stem/progenitor cells. Indeed, when over-expressed in this population, Runx1a has been shown to increase self renewal and induce an expansion of the hematopoetic stem cell population by the selective up-regulation of genes such has *Hox9a I, Meis1* and *Stat1* (Tsuzuki 2012). This is of particular clinical relevance, as the relative abundance of Runx1a in comparison to Runx1b or Runx1c is higher in patients with leukemia than in healthy individuals. Interestingly, Tanaka and colleagues showed that Runx1a prevents the transactivation of genes by Runx1b by binding to Runx consensus sites on DNA, effectively blocking Runx1b, and that Runx1a has a higher affinity for these sites (Tanaka 1995). This demonstrates an elegant system whereby, in acting antagonistically in a temporally controlled manner, Runx1 isoforms control the expression of developmentally regulated genes.

Beyond its well-defined role as an oncogene, the functions of Runx1 can be broadly divided into the regulation of two major developmental processes: proliferation and differentiation/cell fate specification (Coffman 2003; Zagami 2009). Hereafter, the use of Runx1 in the text will refer to the Runx1b isoform, unless otherwise stated.

5.3.1. Runx1 is a context-dependent activator or inhibitor of proliferation

The dichotomous role of Runx1 in proliferation, as both an activator and suppressor, has been well documented in a number of biological systems (Coffman 2003; Yamagata 2005; Inoue 2008; Zagami 2009). In the hematopoetic system, Runx1 promotes the proliferation of myeloid progenitor cells: cyclin-dependent phosphorylation of Runx1 on three serine residues stimulates G1-S cell cycle progression and proliferation in these cells (Zhang 2008). Similarly, activation of Runx1 by the Notch signaling pathway in the aorta-gonad-mesenephros (AGM) region of the developing embryo is required for expansion of the resident hematopoietic progenitor population (Burns 2005). In hair follicle stem cells, Runx1 enhances proliferation by promoting their progression from the G1 to S phase of the cell cycle through direct binding and suppression of the cell cycle inhibitor $p21^{Cip1}$. Accordingly, conditional deletion of Runx1 leads to an increase in $p21^{Cip1}$ expression and maintains progenitors in the telogen, or quiescent, phase for a longer period of time, resulting in patchy, late-growing fur (Hoi 2010).

The role of Runx1 in proliferation is similarly prominent in the OE. Theriault and colleagues demonstrated that Runx1 is expressed in a population of mitotic

ORN precursor cells in the OE, though is not required for their initial generation. They showed a decrease in the proliferation of ORN precursors in Runx1-deficient embryos *in vivo*, and a concomitant increase in differentiating ORNs, indicating that Runx1 is important for maintaining ORN precursors in their actively cycling state (Theriault 2005). As in hair follicle stem cells, Runx1 may promote proliferation in ORN precursors through the suppression of $p21^{Cip1}$ expression (Theriault 2005).

In certain contexts, however, Runx1 plays the role of inhibitor of proliferation. An example of this is the situation seen in microglia in the forebrain of the developing mouse; Zusso and colleagues demonstrated that Runx1 is expressed in mitotic microglia, and expression of Runx1 correlates with a gradual exit from the cell cycle. These studies also demonstrated both *in vitro* and *in vivo* that down-regulation of Runx1 causes a significant increase in microglia proliferation and that over-expression delays cell cycle exit and transition to the ramified state. Conversely, exogenous expression of Runx1 *in vitro* causes a decrease in microglia proliferation. This effect is correlated with the ability of Runx1 to activate $p21^{Cip1}$ transcription (Zusso 2012). Similarly, in the epidermis, Runx1 induces expression of $p21^{Cip1}$ in keratinocyte progenitors, leading to their exit from the cell cycle and progression towards differentiation pathways (Masse 2012).

In DRG neuronal progenitors, Kobayashi and colleagues demonstrated that there was an increase in the number of mitotic cells in the G2/S phases of the cell cycle in Runx1-deficient mice, and a concomitant decrease in the number of neurons expressing differentiation markers such as NeuN and Isl1 (Kobayashi 2012). Further to this, they showed an increase in the expression of Hes1, a negative

regulator of neuronal differentiation; this suggests that Runx1 normally represses expression of this negative regulator of differentiation, thereby promoting the transition of neurons from a proliferative to a differentiating state (Yoshikawa 2007; Kobayashi 2012). These and other studies demonstrate the dual nature of the regulation of proliferation by Runx1, as well as the wide variety of direct and indirect methods employed by this transcription factor to exert its promotional or inhibitory effect.

5.3.2. Runx1 is involved in the regulation of cell differentiation and fate specification

The second major biological process in which Runx1 plays important roles is cell differentiation and the specification of cell fate (Coffman 2003; Yamagata 2005; Inoue 2008; Zagami 2009). In the epidermis, in addition to suppressing proliferation, Runx1 concomitantly induces the expression of the *KRT1* gene, which promotes the differentiation of keratinocyte progenitors (Masse 2012). Runx1 is well established as a critical regulator of differentiation in cell populations within the hematopoietic system. It plays a crucial role in definitive and primitive erythropoiesis through activation of *GATA-1* expression, an essential regulator of these two processes. It also activates a number of other genes, including *ELKF* and *Ter119*, which are involved in adult erythropoiesis and maintenance of adult erythrocyte morphology (Yokomizo 2008). Additionally, it is indispensible for definitive fetal liver-derived hematopoiesis, promoting the expression of a number of genes required for this developmental process; Runx1-deficient mice die at E12.5 due to massive hemorrhaging resulting from blocked fetal hematopoiesis (Okuda 1996). Similarly,

Sakai and colleagues showed that Runx1 directly promotes the expression of the receptor tyrosine kinase Flk, which is involved in vascular endothelial tissue development, thus promoting the developmental maturation of hematopoietic tissues (Sakai 2009). Finally, in the hindbrain, Runx1 is required posmitotically for the differentiation of cholinergic branchiovisceral motor neurons as well as sensory neurons in the trigeminal and vestibulocochlear ganglia (Theriault 2004).

Runx1 also has a demonstrable role in the selection of cell fate in neural and non-neural systems. In the thymus, Runx1 is responsible for the bifurcation of the CD4 and CD8 thymocytes from a common double positive precursor, through the selective repression of the *CD4* gene (Taniuchi 2002; Taniuchi 2004). In the spinal cord, Runx1 is expressed in specific subtypes of spinal cord motor neurons, where it is required to consolidate the motor neuron fate, in part by suppressing the expression of interneuron-specific genes, such as *Pax2* and *Chx10* (Stifani 2008).

In the DRG, Runx1 is vital for the suppression of the peptidergic fate in selected nociceptors. It serves to specify the non-peptidergic phenotype by suppressing the expression of the neurotrophin receptor TrkA and concurrently maintaining expression of Ret in the DRG neurons in which it is expressed. Further, Runx1 has been shown to positively regulate the expression of nociceptive ion channels, such as the TRPV1 channel, and G-protein coupled receptors, such as Mrgpr, through modulation of gene expression. It also influences the laminar targeting of a specific class of $IB4^{+}$ nociceptive afferents (Chen 2006; Kramer 2006; Marmigere 2006; Kobayashi 2012), demonstrating a role in the acquisition of a terminally mature phenotype. Taken together, these findings show that Runx1 is

important for the promotion of developmental progression as well as cellular fate decision in many biological systems, both neural and non-neural.

6. *Runx1 expression in the olfactory bulb*

Previous studies in the Stifani laboratory have provided evidence that Runx1 is expressed in the ONL of developing mice. These studies have also suggested that Runx1 is expressed in certain OECs located preferentially in the inner portion of the ONL (Bocking and Stifani, 2010). Based on these observations, the main aims of the studies that will be described in this Thesis were two-fold:

1) To precisely characterize the identity of the subpopulation of OECs expressing *Runx1*;
2) To investigate the possible involvement of *Runx1* in the proliferation and/or developmental maturation of the OECs in which this gene is expressed.

3. Materials and Methods

Materials and methods

1. *Animals*

All animal procedures were conducted in accordance with the guidelines of the Canadian Council for Animal Care and were approved by the Animal Care Committee of the Montreal Neurological Institute. *Runx1*$^{LacZ/+}$ mice were generated as described by North *et al*. (1999). In these mice, a bacterial *LacZ* gene encoding β-galactosidase (β-gal) is knocked into exons 7 and 8 of *Runx1*. The splice acceptor site from exon 7 is preserved, allowing the translation of a fusion protein of the N-terminal 242 amino acids of Runx1, containing a nuclear localization sequence, and β-gal. As a result, the β-gal-containing fusion protein expressed in *Runx1*$^{LacZ/+}$ mice is nuclearly localized (Figure 1). Previous studies have shown that β-gal expression in *Runx1*$^{LacZ/+}$ mice faithfully recapitulates the expression of the Runx1 protein (Stifani 2008; Zagami 2010; Zusso 2012).). The fusion protein expressed in *Runx1*$^{LacZ/+}$ mice has no functional Runx1 activity and, as a result, *Runx1*$^{LacZ/LacZ}$ mice are Runx1-null (North 1999).

The genotype of *Runx1*$^{LacZ/+}$ mice was determined by polymerase chain reaction (PCR) amplification of a sequence of the *LacZ* gene as described (North 1999). Genotyping was confirmed by subjecting tail clippings from embryos or post-natal mice to histological detection of β-gal activity. Tissues were rinsed three times in a solution containing 80 mM Na_2HPO_4, 20 mM NaH_2PO_4 (pH 7.4), 2 mM $MgCl_2$, 0.2% IGEPAL, 0.1% sodium deoxycholate, followed by incubation for 1-2 hours at 37 ^oC in a solution containing 5 mM potassium ferricyanide, 5 mM potassium

ferrocyanide, and 1 mg/ml 5-bromo-4-chloro-3-indolyl-β galactopyranoside (X-gal) (Invitrogen). The day of appearance of a vaginal plug was considered to be embryonic day (E) 0.5, and the day on which pups were born was considered to be postnatal day (P)0.

2. Histology

Upon collection, embryonic and postnatal tissue were washed 3 times in ice-cold phosphate-buffered solution (PBS) and fixed in 2% paraformaldehyde, 70 mM-L-Lysine and 10 mM sodium periodate (PLP solution) at 4°C with slow shaking. Different fixation times were used, according to age at which tissue was collected (Table 1). After fixation, tissue was rinsed 3 times in ice-cold PBS for 10 minutes, and then immersed in 30% sucrose at 4°C for 48 hours or as long as needed for tissue to sink to the bottom of the well. Tissue was removed from sucrose, rinsed and embedded on dry ice in Tissue-Tek® O.C.T™ compound (Sakura Finetek), and stored at -80°C. Frozen tissue was cryosectioned using a Leica cryostat to yield 12 μm coronal sections; representative (index) slides were incubated in X-gal solution for 24 hours to detect β-gal activity, as described above. Sections were counterstained with eosine and mounted in Fluoromount-G mounting medium (Southern Biotech).

3. Immunofluorescence

Immunofluorescence staining involving mouse primary antibodies was performed using a "Mouse on Mouse" Kit (Vector Laboratories). All other single- and double-label immunofluorescence experiments were performed by first blocking non-specific staining for 1 hour with blocking solution containing either 5% normal donkey serum (v/v) and 0.2% Triton X-100 (v/v) in PBS (for antibodies detecting intracellular proteins) or without Triton X-100 (for antibodies detecting surface proteins). Sections were then incubated sequentially with primary (16-18 hours at 4°C) (Table 2) and secondary (45-60 min at room temperature) (Table 3) antibodies in blocking solution. Secondary antibody solutions were removed and sections rinsed three times for 10 minutes in locking solution, followed by incubation with Hoechst 33258 (1:5000, Sigma) for 5 minutes, after which Hoechst solution was removed and sections rinsed three times for 2 minutes in PBS. Sections were mounted in the dark using Fluoromount-G mounting solution and allowed to dry at room temperature prior to analysis or being stored short-term (1-2 days) at 4°C or long-term at -20°C in the dark. All images were acquired using a Digital Video Camera mounted on an Axioskop 2 fluorescence microscope (Zeiss) and Northern Eclipse software (Empix Imaging Inc.). Image manipulation (brightness, image size) was performed using Adobe Photoshop CS5.

4. Cell counting studies

Coronal sections through the anatomical area containing OBs and OE of *Runx1*$^{LacZ/+}$ and *Runx1*$^{+/+}$ mouse embryos at E14.5 and E15.5 were subjected to

immunofluorescence analysis with anti-Runx1 and anti-Nestin, anti-S100β or anti-Ki67 antibodies. For these analyses, the region containing presumptive migratory OECs was operationally defined based on the position of the OE and nasal septum, in relation to the position of the OBs (Figure 2) This area was further separated into Regions A and B.

Definition of Region A: The developing olfactory system was visualized using Hoechst staining, with hematoxylin/eosine (H&E)-stained sections as a histological reference. The ventral-most point of the ONL of the left and right OB was identified, and a line was drawn between these two points; this was the dorsal limit of Region A. Similarly, the dorsal-most points of the OE on the left and right side were identified, and a line was drawn between these two points; this was the ventral limit of Region A. The lateral limits were designated by lines drawn between the ventral-most points of the ONL and the dorsal-most points of the OE on the left and right sides. Only cells found within the area described above were included in analyses (Figure 3a).

Definition of Region B: The dorsal-most points of the ONL of the left and right OB were identified, and a line was drawn between them: this was the dorsal limit of Region B. Similarly, the ventral-most points of the ONL of the left and right OB were identified, and a line was drawn between them: this was the ventral limit of Region B. The lateral limits were defined as the outer medial edges of the ONLs as they curved from the lowest ventral point to the highest dorsal point of the left and right OB. Only the cells between the two bulbs, but not incorporated into the ONLs, and within the dorsal and ventral limits, were included in analyses (Figure 3b).

Within regions A and B, the numbers of cells co-expressing Runx1 and Nestin, Runx1 and Ki67, and Runx1 and S100β were counted on every fifth coronal section (every 60 μm), derived from at least three different pups for each genotype (at least 14 sections were analyzed per genotype). Pups were obtained from eight different litters. Cell counts were performed digitally using Adobe Photoshop CS5 software. Data is presented as a percentage of Runx1+ cells co-expressing one of either Ki67, Nestin or S100β; statistical significance was determined using Student's 2-tailed T-test, and error bars represent standard error of the means.

5. *Analysis of OEC topology*

The topological distribution of Runx1-expressing OECs in the ONL of *Runx1*$^{+/+}$ and *Runx1*$^{+/-}$ (e.g., *Runx1*$^{LacZ/+}$) mouse embryos was examined at E18.5. Coronal sections were subjected to single-label immunohistochemistry staining for Runx1; the mediolateral width of the Runx1 immunoreactive domain (the distance between the inner medial margin and the outer lateral margin of Runx1 expression) was measured at the widest point of the OB. Measurements were taken at every tenth coronal section (every 120 μm), derived from at least four pups for each genotype (at least 14 sections were counted per genotype). Pups were obtained from four different litters. Data is presented as the average width of the Runx1 expression domain in μm; statistical significance was determined using Student's 2-tailed T-test, and error bars represent standard error of means.

4. Results

Results

1. Characterization of Runx1-expressing cells in the main olfactory bulb nerve layer

Previous studies have provided evidence suggesting that Runx1 is expressed in OECs in the ONL of the main OB (Bocking and Stifani, 2010). To examine this possibility further, we performed double-label immunohistochemistry on coronal sections through the OB of *Runx1*$^{LacZ/+}$ mice, in which β-gal expression faithfully recapitulates the expression of the Runx1 protein (Stifani 2008; Zagami 2010; Zusso 2012). β-gal expression was initially compared to that of calretinin, a calcium-binding protein expressed by ORN axons in the ONL (Malz 2002), to determine whether β-gal immunoreactivity was associated with neurons and/or axons. Calretinin had a punctate expression pattern in the inner portion of the ONL, suggesting that it was localized on incoming ORN axons. The expression of β-gal did not overlap with that of calretinin in a detectable manner. This result is in agreement with previous studies showing that β-gal expression in the ONL did not overlap with that of another ORN axonal marker, olfactory marker protein (Bocking and Stifani, 2010). Together, these observations suggests that in the ONL Runx1 is expressed in cells that are adjacent to incoming ORN axons (Figure 6).

We next examined Runx1 expression in ONL cell types. OECs, along with GFAP-positive interfascicular astrocytes (from which they are morphologically distinct) (Bailey 1993), are the main cell types present in the ONL (Doucette 1993). We performed double-label immunohistochemistry on *Runx1*$^{LacZ/+}$ mice at two

separate developmental stages: E18.5, when the ONL is still developing, and P3, when the ONL has almost achieved its mature histology (Doucette 1989).

As previously shown (Bocking and Stifani, 2010), we observed that virtually all β-gal-positive cells in the ONL of *Runx1*$^{LacZ/+}$ mice co-expressed S100β, a calcium-binding protein expressed by all OECs (Cummings 1995; Franceschini 1996), as well as by OB astrocytes (Donato 1986). The β-gal/S100β-double positive cells were detected preferentially in the inner ONL (Figure 7).

We next compared expression of β-gal to that of NPY, a neurotransmitter previously shown to be expressed by OECs in the inner, but not outer, ONL, as well as by astrocytes (Ubink 2000; Au 2002). We observed that nearly all β-gal+ cells co-expressed NPY and were located in the inner ONL (Figure 8); these results were seen at both E18.5 and P3.

To further characterize Runx1+ cells in the ONL, we compared the expression of β-gal to that of Gap-43, a protein expressed in most OECs (Pellitteri 2010), as well as in neurons and neuronal growth cones in the OB (Verhaagen 1989). Many, but not all, β-gal immunoreactive cells also expressed Gap-43 (Figure 9). Interestingly, the expression of Gap-43 appeared to be dynamic between E18.5 and P3. At E18.5, Gap-43 immunoreactivity was largely in the inner ONL, but at P3 it was preferentially located in the outer ONL. This suggests that Gap-43 expression is developmentally regulated, and may mark a subset of early inner ONL OECs in which Runx1 is also expressed.

We next examined the expression of GFAP, an intermediate filament protein expressed mainly by OECs in the outer ONL (Doucette 1994; Au 2002), as well as by

astrocytes (Jessen 1980). This analysis revealed that the majority of β-gal+ cells in the ONL of *Runx1*$^{LacZ/+}$ mice did not express GFAP, suggesting that most Runx1+ cells are neither OECs of the outer ONL subtype nor astrocytes (Figure 10). To examine the former possibility further, we compared the expression of β-gal to that of p75, a low-affinity neurotrophin receptor expressed by OECs in the outer ONL (Franceschini 1996; Au 2002). The vast majority of β-gal+ cells did not express p75 (Figure 11), providing further evidence that Runx1+ cells are not OECs of the outer ONL subtype of *Runx1*$^{LacZ/+}$ mice. Taken together, these results demonstrate that Runx1+ cells in the ONL are OECs of the inner ONL subtype (Table 4).

2. Involvement of Runx1 in the proliferation of olfactory ensheathing cells

Having demonstrated that Runx1 is expressed by OECs of the inner ONL subtype, we next sought to determine what role Runx1 plays in these glial cells. Runx1 is a context-dependent transcriptional activator or repressor and controls a number of crucial developmental processes (Coffman 2003; Zagami 2009), including proliferation (Blyth 2005; Theriault 2005; Matheny 2007; Hoi 2010) and cell fate specification (Okuda 1996; Hayashi 2000; Chen 2006; Stifani 2008; Yokomizo 2008). Thus, we tested whether Runx1 might play a role in the proliferation and/or differentiation of developing inner ONL OECs.

OEC precursors are believed to originate from olfactory placodes (Mendoza 1982; Marin-Padilla 1989), the structural precursors of the OE from which later-born OECs arise (Chuah 1991; Carter 2004). These precursors travel towards the OB starting at approximately E11.5 in the mouse (Mendoza 1982; Miller 2010;

Blanchart 2011), as part of a MM of cells (Ramon-Cueto et al., 1998; Miller 2010). During their initial migration toward the OB, most cells within the MM are mitotic (Miller et al., 2010); moreover, OEC precursors have been shown to express proteins also present in neural progenitor cells, such as Nestin (Messam 2000; Wang 2008) and Sox10 (Barraud 2010; Forni 2011). Previous studies have suggested that Runx1 is expressed in OEC precursors of the MM starting at ~E13.5 (Bocking and Stifani, 2010). We therefore focused our attention on Runx1-expressing presumptive OEC precursors, defined as mitotic, Nestin+ cells within the MM at E14.5 (Valverde 1992; Miller 2010).

The area defining the migratory pathway of the MM was operationally divided into two regions, termed A and B. Region A represents the portion of the migratory pathway between the OE and the OB and Region B represents the portion between the outer limits of the ONL, between the left and right OB. We first sought to determine whether Runx1+ cells along the migratory path were OEC precursors; to do so, we performed double-label immunohistochemistry for Runx1 and S100β, which is expressed by astrocytes and all early differentiating OECs (Astic 1998), but not by axons or ORNs. We observed that there was a gradual increase in the number of Runx1+/S100β+ cells along the MM migration pathway between E14.5 and E15.5 (Figure 12). Runx1+/S100β+ cells were unlikely to be OB astrocytes, as this class of glia derives from the subventricular zone of the developing brain and is not part of the MM (Rowitch 2004). These observations provide evidence that Runx1+ cells within the MM are presumptive OECs that are proceeding through development towards a mature differentiated fate in the OB.

To investigate the possible involvement of Runx1 in the proliferation of presumptive OECs within the MM, we took advantage of the previous demonstration that *Runx1* heterozygosity is haploinsufficient in both the myeloid lineage and in microglia (Cai 2000). To test whether reduced Runx1 dosage would perturb the proliferation of Runx1+ presumptive OECs, we performed double-label immunohistochemistry to determine the percentage of Runx1+ cells that also expressed Ki67, a nuclear protein expressed during the G1-S phases of the cell cycle and thus used as a proliferation marker (Scholzen 2000). We observed a significant increase in the percentage of Runx1+ cells that co-expressed Ki67 in *Runx1*$^{+/-}$ embryos, compared to their wild-type littermates, in Region A (Figure 13). This result suggests that Runx1 might negatively regulate the proliferation of Runx1+ OECs by promoting cell cycle exit or decreasing the rate of proliferation (or both), as it does in other cell types.

3. Involvement of Runx1 in the developmental maturation of olfactory ensheathing cells

We next sought to determine whether the observed increase in proliferating Runx1+ OEC progenitors was accompanied by an alteration in their progression towards a more developmentally mature state. We compared the expression of Nestin, an intermediate filament protein expressed early in development by OEC precursors (Ramon Cueto 1998; Wang 2008), and S100β in OECs from the OBs of Runx1$^{+/+}$ and Runx1$^{+/-}$ littermates. We observed that, at E14.5, there was a significantly higher percentage of Runx1+ cells that co-expressed Nestin in Region B

of the migratory pathway in *Runx1* heterozygotes as compared to their wild-type littermates (Figure 14). When taken together with the increase in mitotic OECs, this observation suggests that *Runx1* may be involved in promoting the developmental progression of Runx1+ OECs from an immature, Nestin-expressing and mitotic, precursor stage to a more differentiated stage. Alternatively, an increase in the proliferation of Runx1+ cells as a result of reduced gene dosage might lead to a general increase in mitotic Runx1+/Nestin+ OECs.

To examine further whether *Runx1* heterozygosity might perturb the development of Runx1+ presumptive OECs, we next examined the effect of Runx1 reduction in OECs on the expression of S100β, which marks both early differentiating OECs and more mature OECs (Astic 1998). We observed that a reduced dosage of Runx1 resulted in a significant decrease in the percentage of Runx1+ cells co-expressing S100β in Region B of the migratory pathway at E15.5 (Figure 15). Taken together with the increase in proliferating and Nestin+ OEC precursors, this finding suggests that Runx1 may be involved in promoting the progression of OEC precursors through development and that a reduced dosage of Runx1 leads to the a developmental arrest/delay of Runx1+ presumptive OECs. More specifically, it is possible that Runx1 may both play a repressive role in the proliferation of presumptive Runx1+ OEC precursors and promote the adoption of a more mature OEC phenotype (Figure 14).

4. Involvement of Runx1 in the topological organization of Runx1+ olfactory ensheathing cells in the olfactory bulb nerve layer

Runx1 is implicated in cell migration; an example of this in the keratinocytes, where it is responsible for the emergence and migration of keratinocyte progenitors to their proper skin compartments (Osorio 2011). Therefore, we sought to examine the effect of *Runx1* heterozygosity on the organization of Runx1+ OECs in the ONL. As Runx1+ cells are preferentially located in the inner ONL of the OB, we examined whether Runx1 plays a role in this distinct topological segregation by examining the ONL of $Runx1^{+/+}$ and $Runx1^{+/-}$ mice at E18.5, when the OB is nearly mature. To this end, we compared the width of the Runx1 immunoreactive region (from the inner margin to the outer margin of Runx1 expression) in the ONL of the OB of mice from both genetic backgrounds. We did not observe any evident anatomical alterations in the olfactory system of the $Runx1^{+/-}$ mice as compared to their wild type littermates. However, we did observe that *Runx1* heterozygosity was correlated with a trend towards an increase in the diameter of the Runx1-expression domain (Figure 16). As a result, the outer limit of Runx1 expression appeared to move closer to the area normally designated as the "outer ONL". Although this result was not statistically significant among the animals that were studied, it suggests the possible involvement of Runx1 in the settling of Runx1+ OECs to their appropriate locations within the inner ONL, Thus, decreased Runx1 dosage might cause Runx1+ OECs to fall short of their final destinations in the inner ONL and, consequently, to spread in an outward fashion within the confines of the ONL.

5. Discussion

Discussion

OECs are promising candidates for cell-based neuronal regeneration therapies to treat CNS injury, due to the diverse range of functions they serve in the olfactory system (Goodman 1993; Schwarting 2004; Richter 2005; Runyan 2009; Cherehasa 2010; Jiao 2011; Windus 2010, 2011). However, the ambiguous results of previous clinical trials (reviewed in Tetzlaff 2010) point to a gap in our fundamental understanding of the biology of these cells and how to best attempt to utilize their significant potential. Previous work has shown that multiple sub-populations of OECs exist in both the OE and OB (Francescini 1996; Au 2002; Barnett 2004; Pellitteri 2010). It would thus follow that distinct subtypes of OECs might play distinct roles in the context of olfactory system development and regeneration. However, the nature of these unique roles has yet to be elucidated, in large part due to a paucity of molecular markers associated exclusively with one OEC subtype or another (Barnett 2004). Furthermore, the factors that control OEC specification and function have not been well studied, nor has the manner in which subtype diversification is achieved.

Recent work has revealed that the transcription factor Runx1 is expressed preferentially by OECs in the inner portion of the ONL of the OB (Bocking and Stifani, 2010). We therefore focused our studies on Runx1 to first perform a molecular characterization of the Runx1-expressing sub-population of OECs in the developing OB. We then examined the effect of Runx1 reduction on OEC proliferation and developmental maturation *in vivo*. Our studies have provided evidence that a

reduction in Runx1 dosage results in a significant increase in the percentage of proliferating presumptive OEC precursors in which Runx1 is expressed. This alteration is correlated with a significant increase in the percentage of immature Runx1+ OECs and a parallel decrease in the percentage of differentiating Runx1+ OECs. In addition, our initial analysis of the effect of Runx1 reduction on the topology of Runx1+ cells in the olfactory bulb suggests an increase in the diameter of the Runx1 immunoreactive region, possibly consistent with changes in settling position within the ONL. These findings identify Runx1 as a molecular marker of inner ONL OECs of the OB and suggest a role for this protein in mechanisms important for OEC development.

1. *Expression of Runx1 in olfactory ensheathing cells of the inner olfactory nerve layer*

To characterize the population of Runx1+ OECs in the ONL, we performed an immunohistochemical study in which Runx1 expression was compared to that of a number of proteins known to be expressed by OECs, including S100β, Gap-43, NPY, p75, and GFAP (Au 2002; Barnett 2004; Pellitteri 2010). This analysis has shown that Runx1+ OECs also express S100β, NPY and Gap-43, but do not express p75 or GFAP (Figures 7-11). Because it was reported by a number of groups that the vast majority of NPY staining in the ONL is present in inner ONL OECs (Gimpl 1993; Ubink 2000), while p75 is preferentially expressed by OECs in the outer portion of the ONL (Au 2002), our results provide evidence that most, if not all, Runx1+ OECs

are part of the inner ONL (Table 4). This finding identifies Runx1 as a novel molecular marker by which this sub-population can be distinguished from other OECs.

We have previously shown that Runx1 is expressed early in OEC development, first appearing in cells along the OEC migratory pathway around E13.5 (Bocking and Stifani, 2010). As expression of Runx1 occurs early in OEC development, prior to the expression of proteins found in more mature OECs, such as S100β, NPY and Gap-43 (Astic 1998; Ubink 2000), it is possible that Runx1 may serve as an early indicator of inner ONL OEC identity, before Runx1-expressing OECs have reached the OB. Using Runx1 as a specific marker, it might thus be possible to purify inner ONL OECs, for example by utilizing commercially available Runx1-GFP mice in combination with FACS sorting approaches. This strategy might allow more detailed studies into the function of this subset of OECs, potentially shedding light on the functional characteristics that distinguish unique OEC subpopulations. Having a more homogenous and better-characterized population of OECs will also offer the important opportunity to more definitively evaluate their usefulness as a therapeutic treatment option.

In addition to being a new OEC marker, Runx1 might also play a part in OEC development, including the acquisition of the inner ONL OEC identity. The association of Runx1 with the acquisition of subtype identity is a common theme in several biological systems (Hayashi 2000; Chen 2006; Marmigiere 2006; Stifani 2008; Hoi 2010; Osorio 2011). For example, in DRG neurons, Runx1 is essential for the separation of peptidergic and non-peptidergic sensory neuron phenotypes from

a common precursor state. Runx1 is initially expressed by all embryonic nociceptors, but through development its expression persists only in a subset of nociceptors that undergo a transition from TrkA+ to Ret+ status. In addition to this, Runx1 activates the expression of a plethora of ion channels and sensory receptors and suppresses that of CGRP, which all together define the non-peptidergic nociceptor phenotype (Chen 2006; Kramer 2006; Marmigere 2006).

A similar situation exists in the thymic cortex, where Runx1 is required for the bifurcation of CD4 and CD8 thymocytes early in their development from a common double-positive precursor through the silencing of CD4 expression and concomitant promotion of that of CD8 (Taniuchi 2002; Bosselut 2004; de Bruijn 2004; Taniuchi 2004). Given the important role played by Runx1 in the control of binary cell fate decisions, it is reasonable to speculate that Runx1 may play a similar role in the specification of inner OECs from an initial common state by controlling the expression of genes encoding proteins specific to the inner ONL OEC fate. In addition, or alternatively, Runx1 may repress the expression of proteins specific to outer ONL OECs. If this were the case, one would predict that the absence of Runx1 would perturb OEC subtype specification. It is possible that heterozygous Runx1 mice might not display significant alterations in OEC subtype development because in many cases Runx1 heterozygosity does not lead to a significant difference in phenotype. Therefore, future studies to further explore this hypothesis would include analyzing the effect of conditional Runx1 inactivation in OECs, combined with a detailed marker and topological analysis, to ascertain the full effect of Runx1 on OEC subtype acquisition.

The potential role of Runx1 as an important modulator of OEC subtype diversity is significant, as individual subtypes are believed to have distinct functions in the olfactory system. The specific role played by inner ONL OECs has not been fully elucidated, but there is functional and histological evidence that suggests that this subpopulation is involved in sorting ORN axons to their appropriate glomeruli (Mombaerts 2006). The selection of a glomerular target by ORN afferents is pre-determined (Mori 1999; Auffarth 2011; Imamura 2011) and axon sorting occurs as a result of the spatiotemporal expression of axon guidance molecules, chemorepellants and their receptors (Mombaerts 2006). A prime example of this is the expression of Semaphorin 3A by inner ONL OECs (Schwarting 2004). Expression of this chemorepellant is vital for the repulsion of neuropilin-1- and P2 odorant receptor -expressing axons from the caudomedial OB towards their corresponding P2 glomeruli in the rostrolateral OB (Crandall 2000; Taniguchi 2003; Schwarting 2004).

Our characterization of Runx1-expressing OECs revealed that this subset expresses NPY, a neurotransmitter implicated in the targeting of GABAergic neurons to cells expressing the neurokinin-1 receptor in specific laminae of the spinal cord (Polgar 1999). Similarly, *in vitro* studies have shown that NPY elicits a significant turning response in the growth cones of DRG neurons and is involved in the attraction of neuroblasts from the rostral migratory stream (Hokfelt 2008). The expression of NPY in other neural cells and the role of these cells within their respective systems is reminiscent of the role played by inner ONL OECs; in light of this, it is reasonable to speculate that Runx1 might be involved in the

spatiotemporal expression of molecules such as NPY and, potentially, Semaphorins. This would in turn control selection, sorting and innervation of incoming afferents to glomeruli in distinct anatomical regions of the OB. Future studies might include the use of mice in which Runx1 will be conditionally inactivated in OECs to examine ORN axon sorting and glomerular targeting. The identification of Runx1 target genes in OECS will also provide important information; additionally, *in vitro* co-culture studies could be used to elucidate other potential roles of inner ONL OECs.

2. *Role of Runx1 on the developmental maturation of inner olfactory nerve layer olfactory ensheathing cells*

Given the important roles played by Runx1 and other Runx family members in cell proliferation and development, we investigated the possible involvement of Runx1 in these events in OECs. The proliferation and developmental maturation of Runx1-expressing OECs was examined in *Runx1*$^{LacZ/+}$ mice and their wild-type littermates in order to determine whether halving the amount of Runx1 protein in Runx1-expressing OECs might result in informative phenotypes. The rationale for this approach stems from the observation that Runx1 heterozygosity perturbs proliferation and fate specification in various cell types in which Runx1 is expressed (Hayashi 2000; Zusso 2012).

2.1. Runx1 heterozygosity is correlated with perturbation of olfactory ensheathing cell proliferation

To study the role of Runx1 on OEC development and maturation, we examined Runx1+ cells as they traveled as part of the MM originating in the olfactory placodes between E14.5 and E15.5. We showed that Runx1+ cells were indeed presumptive OECs by double-labeling analysis of the expression of Runx1 and S100β, the earliest known marker of differentiating OECs (Astic 1998). We found that Runx1+ cells co-expressed S100β in increasing numbers between E14.5 and E15.5 along this developmental pathway (Figure 12), suggesting that these cells were of the glial lineage and were differentiating into OECs.

Our studies have also shown that Runx1 heterozygosity is correlated with an increase in the percentage of proliferating Runx1+ cells as compared to wild-type littermates, in the region of the migration pathway containing cells that are early in their development (Figure 13). We did not see a significant difference in the proliferation of Runx1-negative cells, suggesting a cell-autonomous effect. Our *in vivo* findings are consistent with previous *in vitro* studies in which Runx1 was over-expressed in primary cultures of OECs; these studies showed that this manipulation resulted in decreased proliferation (Bocking and Stifani, 2010). Taken together, these data suggest a role for Runx1 in the negative regulation of OEC proliferation. Runx1 might exert its effect by promoting OEC cell cycle exit, as it does in a number of other cell types. A role for Runx1 in the regulation of OEC proliferation is consistent with the manner in which Runx1 participates in the modulation of proliferation in other cell types. For example, in the hematopoetic system (Strom

2000; Burel 2001; Bernardin-Fried 2004) and the nervous system (Theriault 2005; Zusso 2012), Runx1 regulates proliferation by the selective activation or repression of key cell cycle regulatory genes. Thus, it is possible that Runx1 may perform similar functions in mitotic OEC precursors. Several groups have shown that Runx1 binds to and transactivates *cyclin D3* (Burel 2001; Bernardin-Fried 2004), a gene that promotes the progression of cells from the G_1 phase to the S phase of cell cycle. Abnormal forms of Runx1, such as the Runx1-ETO fusion oncoprotein, induce cell cycle arrest by repressing *cyclin D3* (Burel 2001), while over-expression of Runx1 *in vitro* was shown to shorten the G_1 phase of the cell cycle, dramatically increasing the rate of proliferation (Strom 2000). Therefore in OECs, Runx1 might be acting on genes that regulate entry into the cell cycle, such as *cylin D3*, repressing their expression and thus controlling the rate of proliferation of presumptive OECs.

Alternatively, Runx1 may be regulating genes encoding proteins that prevent cell cycle progression, such as the *cyclin-dependent kinase inhibitor 1* (*$p21^{Cip1}$*), which encodes a protein implicated in cell cycle exit (Harper 1993). *$p21^{Cip1}$* is a known transcriptional target of Runx1; in some instances in which it promotes proliferation, Runx1 represses *$p21^{Cip1}$* (Lutterbach 2000); this is the case in neurons of the olfactory system (Theriault 2005). However, in other cells, such as forebrain microglia (Zusso 2012) and in keratinocytes of the epidermis (Masse 2012), Runx1 activates *$p21^{Cip1}$* transcription. In light of this, it is also possible that Runx1 may regulate cell cycle inhibitors similar to *$p21^{Cip1}$* to repress OEC proliferation. Avenues for further study might include conducting a search for Runx1 target genes associated with cell cycle regulation in OECs. It would be interesting to explore

whether known transcriptional co-factors of Runx1 are involved in modulating Runx1 anti-proliferative function in OECs.

2.2. A role for Runx1 in the differentiation of olfactory ensheathing cells?

The observed increase in proliferating Runx1+ cells in response to reduced protein dosage might alternatively be due to a role for Runx1 in the developmental transition. These cells may be transitioning from proliferating progenitors to postmitotic precursors, which might potentially promote the expression of factors influencing their differentiation. These two possible mechanisms of Runx1 activity – repression of proliferation and promotion of differentiation - might also not be mutually exclusive; in keratinocytes, for example, in addition to suppressing proliferation by activating the expression of $p21^{Cip1}$, Runx1 promotes expression of *KRT1,* which encodes a keratin protein that is an early differentiation marker (Masse 2012).

To follow the developmental progression of Runx1+ OECs, we examined the expression of Nestin, an intermediate filament protein expressed by neural progenitors (Messam 2000), and S100β, an early marker of differentiating OECs (Astci 1998; Ramon-Cuet 1998; Barnett 2004). We observed a significant increase in the percentage of Runx1+ cells that co-expressed Nestin in Region B of the migration pathway at E14.5 (Figure 14), suggesting that an increased percentage of Runx1+ cells had remained as OEC precursors. The expansion of the immature Runx1+ OEC precursor population is consistent with both hypotheses regarding mechanism of Runx1 activity mentioned above – namely, that Runx1 may be

involved in modulating OEC progenitor proliferation, or that Runx1 is involved in promoting developmental progression and differentiation.

To further examine whether this expansion was a result of a blocked or delayed transition towards a differentiating, maturing OEC state (S100β+), we examined the expression of S100β and found a significant decrease in the percentage of Runx1+ cells co-expressing S100β in response to a lower dosage of Runx1. This indicates that in a *Runx1* heterozygous background, significantly fewer Runx1+ cells were attaining developmental maturity, remaining instead in an immature precursor or progenitor state.

Our finding that Runx1 heterozygosity is correlated with an increase in the percentage of Runx1+ cells co-expressing precursor markers and a parallel decrease in the percentage of Runx1+ OECs expressing the early differentiation marker S100β suggests that at least a part of Runx1 function is to promote developmental progression in OECs. In some systems, the induction of differentiation by Runx1 can occur by the direct up-regulation of differentiation genes specific to that lineage. For example, in the megakaryocyte lineage, Runx1 is responsible for binding to and activating the expression of the transcription factor EVI1, which is important for the progression of megakaryocyte differentiation (Shimizu 2002; Maicas 2012). Runx1 may be transactivating the expression of OEC-specific differentiation genes. It is also possible that Runx1 may be acting to repress the expression of genes that are negative regulators of differentiation. Avenues for future study may include the identification of candidate OEC-specific genes regulated by Runx1 and of potential modulators of differentiation that respond to changes in Runx1 protein levels.

Recent work on megakaryocyte differentiation has revealed that Runx1 plays an elegant regulatory role in the development of these cells. In this system, Runx1 and an HDAC-containing co-repressor complex are recruited to the promoters of certain key megakaryocyte differentiation genes and keep these genes in a primed, "intermediate" state. Upon the induction of differentiation, co-repressor binding is lost, and sustained Runx1 expression is required for the recruitment of a co-activator complex which together initiate expression of the differentiation genes in question (Herglotz 2012). This interesting mechanism of action of Runx1 raises the question of whether its role in OECs could be similarly multi-layered. It is possible that initially, Runx1 might suppress certain differentiation genes and that later on in development may be required to activate gene expression. In this scenario, a reduced dosage of Runx1 may not be enough to alleviate the initial repression, or this alleviation might occur at a slower pace, leading to a developmental delay. Future studies may involve examining Runx1 co-factor recruitment during different stages of development *in vitro* and the binding of different co-factors during development.

Finally, it is interesting to note that in many systems, the level of Runx1 protein is quite variable throughout different stages of development. A prominent example of this is in myeloid lineage cells, where Runx1 protein levels are modulated within the cell cycle, increasing during the G_1 phase to promote cell cycle entry and then decreasing when cells are in G_1 arrest (Bernardin-Fried 2004). Similar modulations may be occurring in the levels of Runx1 protein in OECs during their development, and different levels of Runx1 expression might be required to

exert specific effects on various aspects of development, from the proliferation to the differentiation stage. Thus, it might be interesting to investigate the possibility of variations in the level of Runx1 protein in presumptive OECs during different stages of development, perhaps by FACS sorting, fractionation and Western blot analysis of protein expression.

3. A role for Runx1 in the establishment of olfactory ensheathing cell topology within the olfactory nerve layer?

Lastly, we examined the topological organization of Runx1-expressing OECs in the ONL of the mature OB. We found that there was a trend towards an expansion in the diameter of the Runx1 immunoreactive region in the ONL in *Runx1* heterozygotes compared to their wild-type littermates (Figure 16). As the overall width of the ONL did not change upon Runx1 dosage reduction, the trend towards an increase in the diameter of the Runx1 immunoreactive region of the ONL was concomitant with a trend towards a decrease in the diameter of the Runx1-negative region. This finding is in agreement with previous data showing a significant expansion of the β-gal immunoreactive region in the ONL of Runx1 knockout mice (Bocking and Stifani, 2010).

The observed increase in the diameter of the Runx1 immunoreactive region of the ONL might be in part attributable to a change in the identity of Runx1+ OECs. It is possible that as a result of reduced Runx1 dosage, OECs that would normally acquire an inner ONL OEC fate might instead develop into outer ONL OECs. Alternatively, or in addition to this, cells that would ordinarily develop into inner

ONL OECs might, upon Runx1 dosage reduction, begin to express markers of both inner and outer ONL OECs (for example, co-express NPY and p75), indicative of a shift towards an intermediate identity. To test this hypothesis, further immunohistochemical analyses will have to be conducted to ascertain the identities of the OECs residing the inner and outer ONLs of *Runx1*$^{+/+}$ and *Runx1*$^{+/-}$. Our studies did not reveal any OECs with a mixed inner and outer ONL phenotype; however, based on the initial findings presented in this thesis, it is reasonable to believe that in a Runx1-null background, the diameter of the Runx1 immunoreactive region of ONL might be significantly greater that that of the wild-type mouse.

The increase in the diameter of the Runx1 immunoreactive region of the ONL also raises the possibility that Runx1 is involved in the mechanisms underlying the topological organization of OECs in the ONL. In the epidermis, Runx1 modulates the emergence of keratinocytes from individual skin compartments. In these cells, Runx1 mediates its effect on cell migration via regulation of both the Lef1 and Wnt proteins, which trigger downstream signaling pathways that, through complex cross-talk, promote the migration of cells from the mesenchyme to the epithelium (Osorio 2011). Runx1 might similarly be involved in modulating signaling pathways in OECs to help direct their migration to the proper inner layer of the ONL. One of the pathways mediating Runx1+ OEC migration to the inner ONL might be that downstream of Wnt1, which has been previously shown to be activated in OECs that, by virtue of their position in the ONL and their molecular signatures, appear to be inner ONL OECs (Wang 2008). Activation of the Wnt1 pathway may lead to the downstream induction of expression of genes involved in migration, as is the case in

the rostral-caudal migration of facial branchiomotor neurons of the hindbrain (Vivancos 2009). Future studies may involve examining the activity of signaling pathway, such as that mediated by Wnt1, that may control the expression of genes associated with cell migration and the expression of positional cues.

The possibility also exists that the migration of Runx1+ OECs to the inner ONL of the mature OB might be a product of their stage of maturation. In the hematopoetic lineage, the emigration of thymocytes from their origin in the thymus to their final destinations in the peripheral tissues is dependent on their state of maturation and Runx1 knockdown *in vivo* leads to a significant decrease in cell emigration, as well as on cell development (Hayashi 2000). Taken together, the effect of Runx1 on OEC topology might be direct, via modulation of cues affecting migration and positioning, or indirect, via the proper developmental progression of OECs. A proposed model of Runx1 function is presented in Figure 17.

Future directions

Our findings raise interesting questions about the role of Runx1 in OECs of the inner ONL as well as its role in the proliferation and differentiation of OECs in the developing olfactory system. Our work was focused around a heterozygous model of perturbation of Runx1 expression; it would be a natural extension of this work to build upon the findings presented in this Thesis and examine the phenotype of Runx1-null mice. As Runx1 loss is embryonic lethal (Okuda 1996), Runx1 would have to be conditionally inactivated in OECs by crossing a $Runx1^{Flox/Flox}$ mouse with

a mouse that had Cre-recombinase under the control of the promoter of a gene that is preferentially expressed in OECs. Such a gene has yet to be definitively identified, but candidates such as *Wnt1* and *Sox10* could be considered although they are expressed in numerous other cell types and thus are not ideal (Wang 2008; Barraud 2010).

More broadly, the model of Runx1 inactivation would add a valuable and interesting dimension to our current work, especially as it is well known that the phenotype of heterozygous animals is sometimes not as pronounced as that of their null counterparts. Using this model, we could further address questions pertaining to OEC fate acquisition and OEC topology by performing similar studies on OB tissue from Runx1-null mice as those presented in this Thesis. Examination of a Runx1 conditional knockout model might help us elucidate why Runx1 is expressed in such a specific subset of OECs and whether OEC subtype specification is perturbed on a Runx1-null background. These and other studies, detailed throughout the Discussion, might also aid us in pinpointing the role, or roles, and importantly, the mechanism of action of Runx1 in the various stages of OEC developmental maturation, including the proliferation, differentiation and specification of OECs.

Finally, an intriguing extension of this Thesis might be to further examine the role of Runx1+ OECs in the context of olfactory system regeneration. This work might include culturing OB and ONL explants from *Runx1*$^{+/+}$ *mice and then establish co-cultures* with ORNs to examine whether inner ONL OECs may influence the targeting behavior or innervation of incoming ORN axons. Refining our

understanding of the role of Runx1+ OECs might help determine for which aspect of CNS regenerative therapy these cells could be most effective.

Conclusions

This Thesis has provided evidence that Runx1 is expressed in a subset of OECs in the inner ONL of the OB. We have also shown that Runx1 is involved in the developmental maturation of Runx1+ OECs outside of the OB. Together, these data implicate Runx1 in the development and possibly function of inner ONL OECs, and shed light on the regulatory mechanisms underlying the diversification of this unique glial cell type.

6. Tables

Tables

Table 1

Age of tissue collection	Fixation time
E14.5	25 min
E15.5	35 min
E18.5	45 min
P3	60 min

Table 1: Fixation times used for tissues from animals at various ages. All tissue fixation was performed at 4°C in 2% PLP solution with shaking.

Table 2

Antibody	Animal of origin	Dilution	Manufacturer/product information	Localization
β-gal	Goat	1:500	Cappel	Nuclear
β-gal	Rabbit	1:1000	Cappel	Nuclear
Calretinin	Rabbit	1:1000	Millipore	Cytoplasmic
Gap-43	Rabbit	1:1000	Millipore	Cytoplasmic
GFAP	Rabbit	1:200	DakoCytomation	Cytoplasmic
Ki67	Rabbit	1:1000	Abcam	Nuclear
Ki67	Mouse	1:1000	BD Pharmigen	Nuclear
Rat-401 (Nestin)	Mouse	1:50	Developmental Hybridoma Bank	Cytoplasmic
NPY	Rabbit	1:750	Abcam	Secreted
p75	Rabbit	1:1000	Millipore	Cell surface
Runx1	Rabbit	1:300	Epitomics	Nuclear
S100β	Mouse	1:500	Sigma-Aldrich	Cytoplasmic

Table 2: Optimal dilutions of antibodies used for immunostaining. Antibodies against proteins with nuclear or cytoplasmic localization were diluted in blocking buffer containing Triton X-100; antibodies against proteins with cell surface expression were diluted in Triton X-100-free blocking buffer.

Table 3

Antibody	Animal of origin	Dilution	Manufacturer
Anti-goat	Donkey	1:1000	Alexa-488 (Invitrogen)
Anti-mouse	Donkey	1:1000	Alexa-488 (Invitrogen)
Anti-mouse	Donkey	1:1000	Alexa-555 (Invitrogen)
Anti-rabbit	Donkey	1:1000	Alexa-488 (Invitrogen)
Anti-rabbit	Donkey	1:1000	Alexa-555 (invitrogen)

Table 3: Optimal dilutions of secondary antibodies used for immunostaining. Secondary antibodies used for the detection of nuclear or cytoplasmic proteins were diluted in blocking buffer containing Triton X-100; secondary antibodies detecting cell surface proteins were diluted in Triton X-100-free blocking buffer**.**

Table 4

	Inner ONL	Outer ONL
Runx1	+	-
S100β	+	+
NPY	+	-
Gap-43	+/-	-
GFAP	+/-	+
p75	-	+

Table 4: Summary of the expression pattern of molecular markers of OECs in the ONL of mice at E18.5. Runx1 was expressed preferentially by OECs in the inner ONL by cells that also expressed S100β, NPY and Gap-43. Runx1+ OECs did not express GFAP or p75.

7. Figures

Figure 4

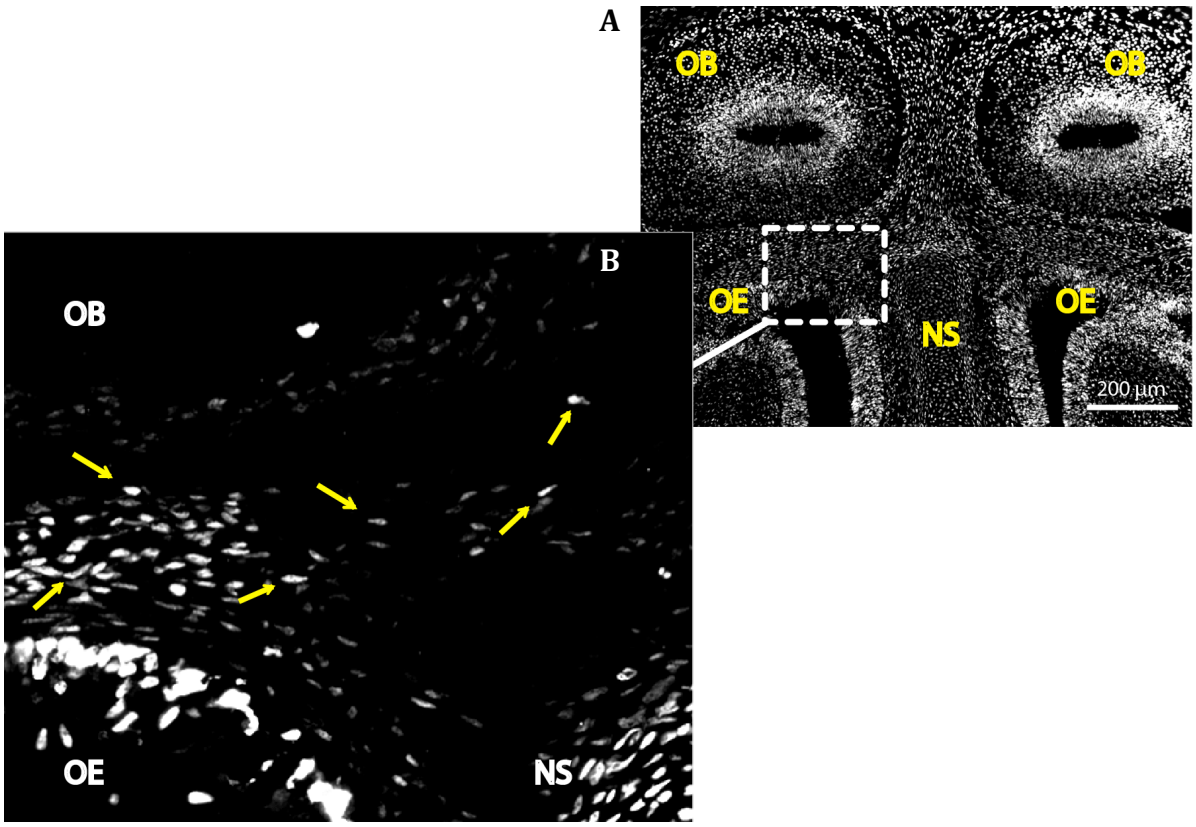

Figure 4: The migration path of OECs. Coronal sections of E14.5 embryos were subjected to immunohistochemistry to examine the expression of Runx1 within the pathway taken by the MM. Panel A: Hoechst-stained section detailing the anatomy of the olfactory of the olfactory pathway. Panel B: A higher magnification view of OECs travelling along the olfactory pathway towards their final destinations in the ONL. A grey scale image was assigned to the red channel to denote Runx1 expression. Robust Runx1 expression can be seen on the basal side of the OE, as described by Theriault 2005, as well as by cells in the nasal septum. Yellow arrows indicate cells along the migratory pathway that express Runx1. OB = olfactory bulb; OE = Olfactory epithelium; NS = nasal septum. Scale bar = 200 µm

Figure 5

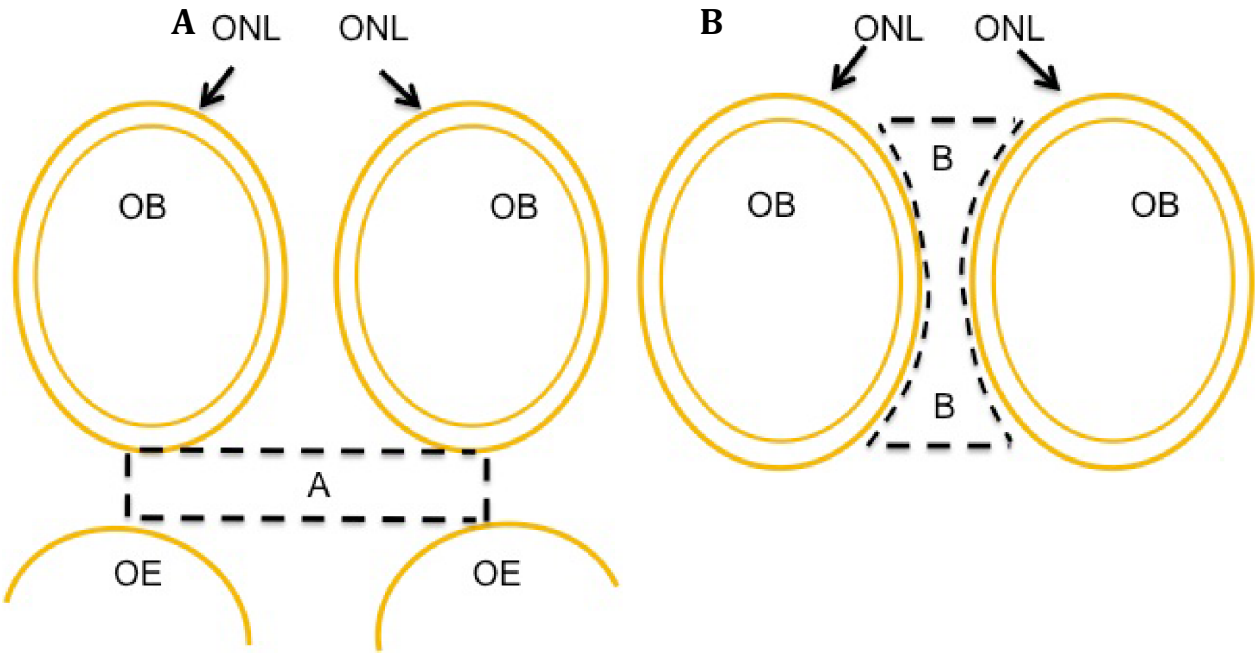

Figure 5: A graphical depiction of Regions A and B of the olfactory ensheathing cell migration pathway. A: Region A of the anatomical region occupied by developing, migrating OECs is defined as the aear between the OE and the OB. B: Region A is defined as the anatomical region occupied by developing, migrating OECs between the outer limits, medial to the left and right OB.

Figure 6

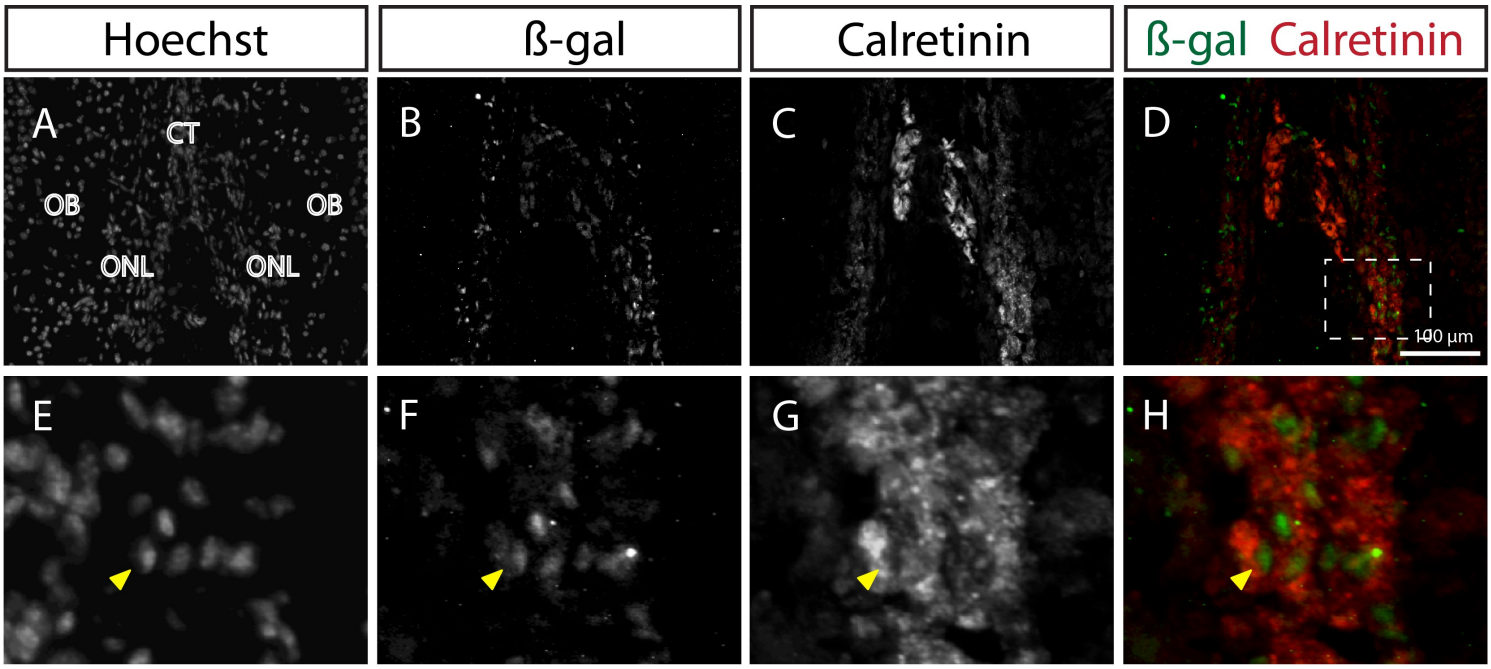

Figure 6: Analysis of β-gal and Calretinin expression in the main OB of *Runx1*$^{LacZ/+}$ mice at E18.5. Coronal sections prepared from the main OB of *Runx1*$^{LacZ/+}$ mice were subjected to double-label immunohistochemistry to examine the expression of β-gal and Calretinin. Panels E-H are higher magnification views of panels A-D. Grey scale images were assigned to green (β-gal) or red (Calretinin) channels, which were merged to generate panels D and H. Hoechst staining is used in panels A and E to visualize cell nuclei. β-gal is preferentially expressed in the nuclei of cells in the inner portion of the ONL (B), while Calretinin has a punctate expression pattern at the ends of axons in the inner ONL (C). The merged image demonstrates that both β-gal and Calretinin are expressed in the inner ONL, but do not overlap. Scale bar = 100 µm.

Figure 7

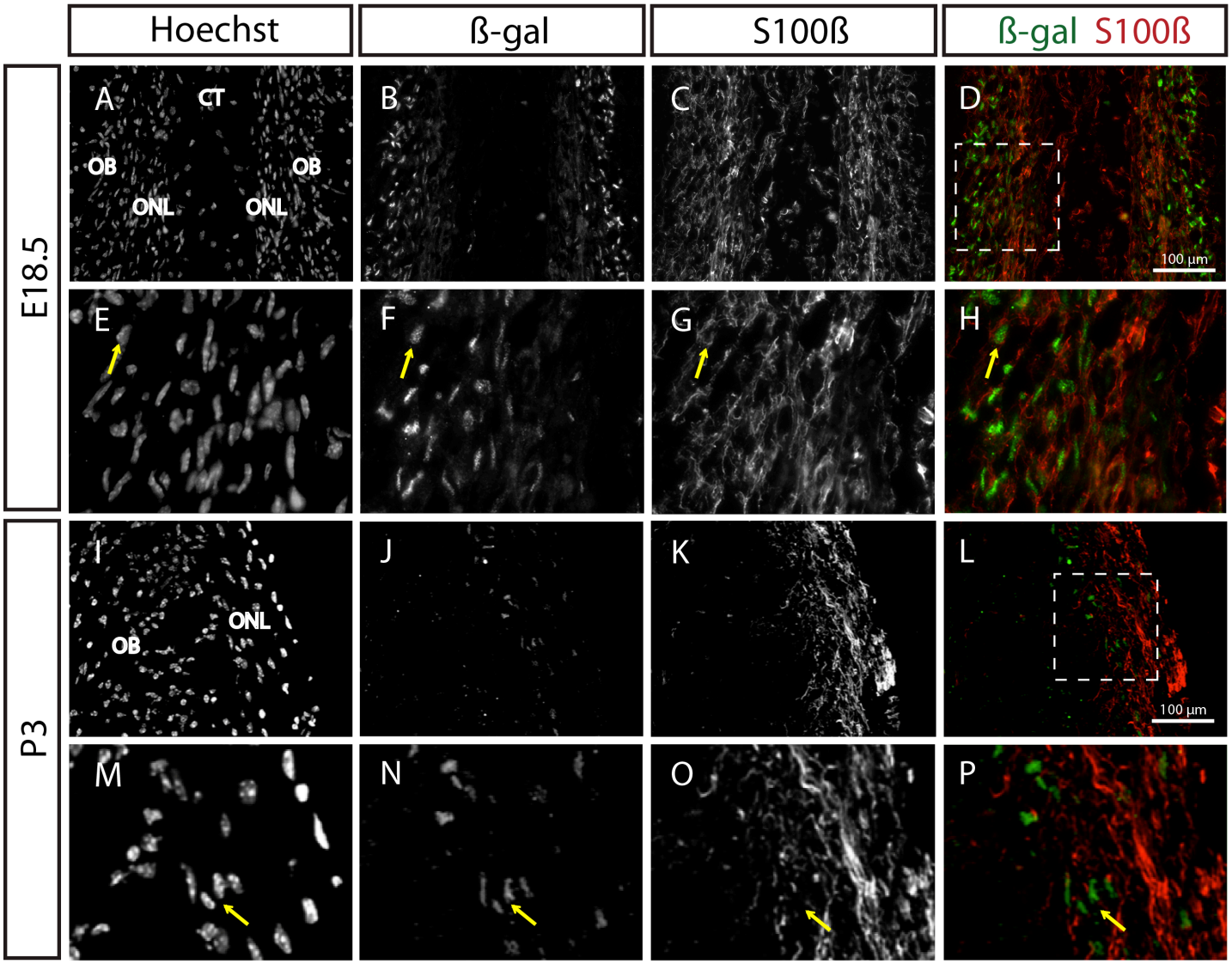

Figure 7: Analysis of β-gal and S100β expression in the main OB of *Runx1*$^{LacZ/+}$ mice. Coronal sections from the main OB of *Runx1*$^{LacZ/+}$ mice at E18.5 (A-H) and P3 (I-P) were subjected to double-label immunohistochemistry to examine the expression of β-gal and S100β. Panels E-H and M-P are higher magnification views of panels A-D and I-L, respectively. Grey scale images were assigned to green (β-gal) and red (S100β) channels, which were digitally merged to generate panels D, H, L and P. Hoechst staining is shown in grey scale in panels A, E, I and M to depict cell nuclei. β-gal expression is preferentially expressed in the nuclei of cells located in the inner ONL (B, F, J, N) (yellow arrows), while S100β is expressed in the cytoplasm of cells throughout the ONL (C, G, K, O). The merged images and arrows demonstrate that almost all β-gal+ cells co-express S100β (D, H, L, P) at both gestational ages. Dorsal is up, medial is right. Scale bar= 100 µm.

Figure 8

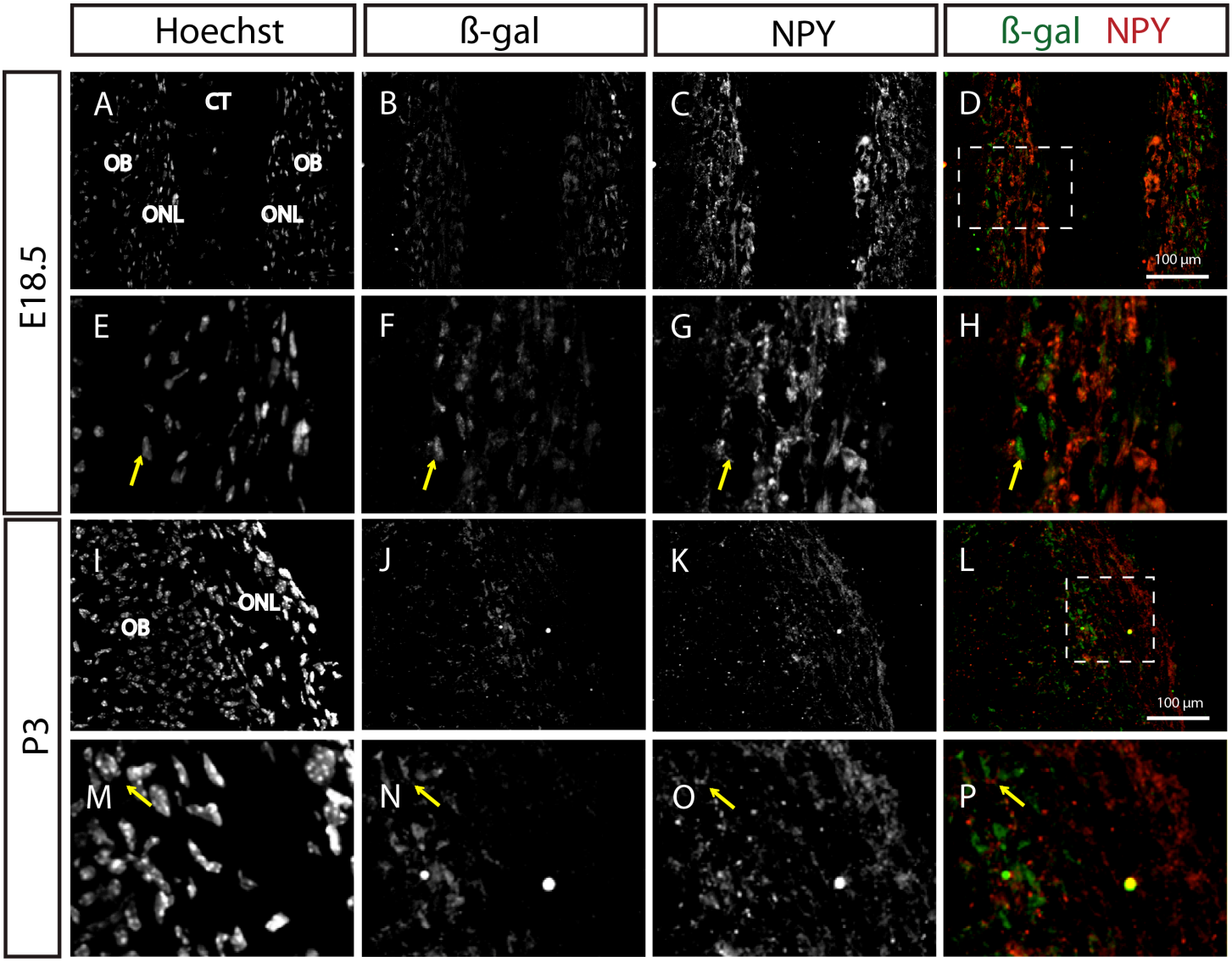

Figure 8: Analysis of β-gal and NPY expression in the main OB of *Runx1*$^{LacZ/+}$ mice. Coronal sections from the main OB of *Runx1*$^{LacZ/+}$ mice at E18.5 (A-H) and P3 (I-P) were subjected to double-label immunihistochemistry to examine the expression of β-gal and NPY. Panels E-H and M-P are higher magnification views of panels A-D and I-L, respectively. Grey scale images were assigned to the green (β-gal) and red (GFAP) channels, which were digitally merged to generate panels D, H, L and P. Hoechst staining is shown in grey scale in panels A, E, I and M to depict cell nuclei. β-gal was preferentially expressed by the nuclei of cells in the inner ONL (B, F, J, N) (yellow arrows) ; NPY was expressed in the inner ONL (C, G, K, O). The merged images and yellow arrows illustrate that almost all β-gal+ cells co-express NPY at both gestational ages. Dorsal is up, medial is right. Scale bar = 100 µm.

Figure 9

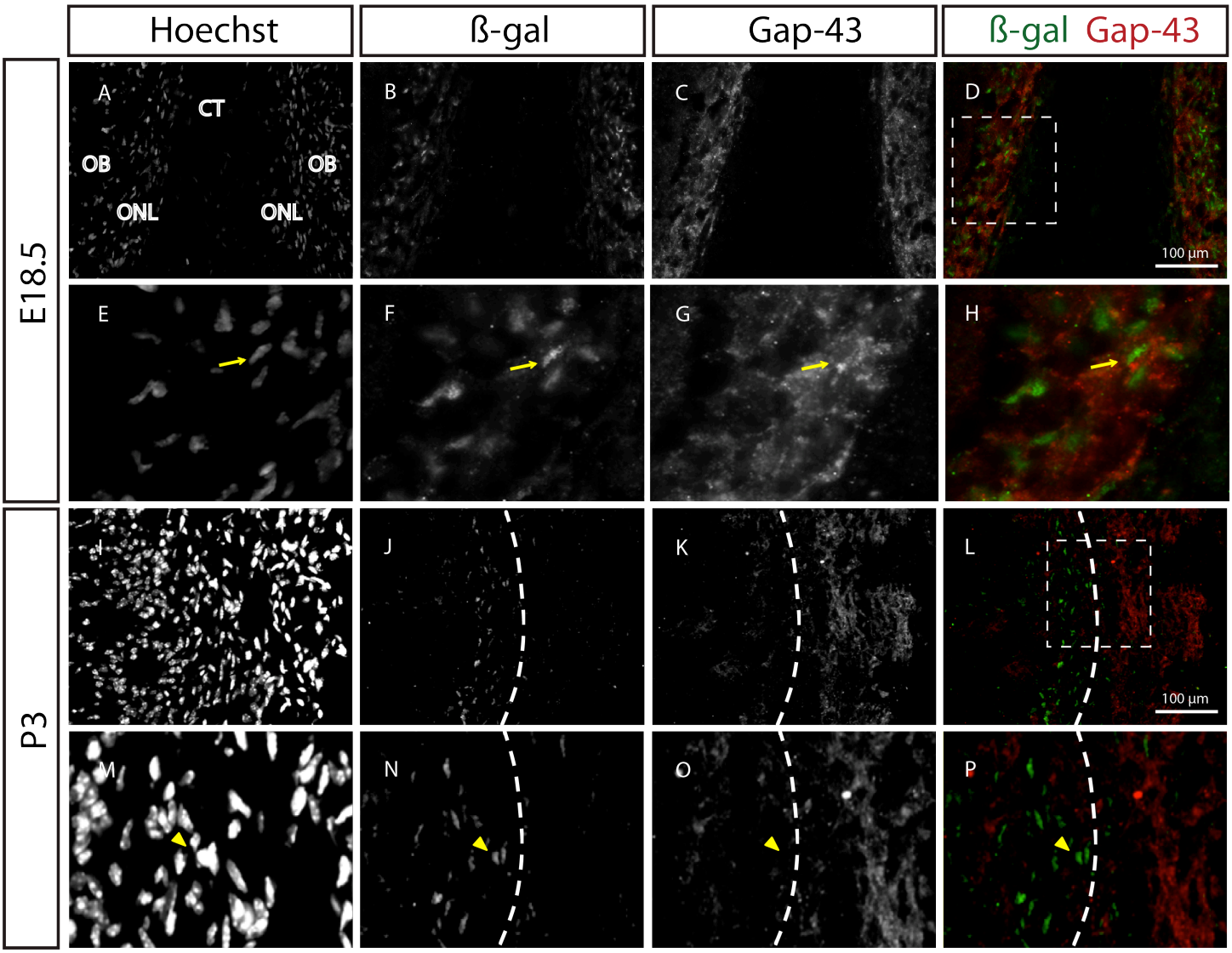

Figure 9: Analysis of β-gal and Gap-43 expression in the main OB of *Runx1*$^{LacZ/+}$ mice. Coronal sections from the main OB of *Runx1*$^{LacZ/+}$ mice at E18.5 (A-H) and P3 (I-P) were subjected to double-label immunohistochemistry to examine the expression of β-gal and Gap 43. Panels E-H and M-P are higher magnification views of panels A-D and I-L, respectively. Grey scale images were assigned to the green (β-gal) and red (Gap-43) channels, which were digitally merged to generate panels D, H, L and P. Hoechst staining is shown in grey scale in panels A, E, I and M to depict cell nuclei. β-gal was preferentially expressed in the nuclei of cells in the inner ONL (B, F, J, N) (yellow arrows and arrowheads) .Gap-43 staining was present in the cytoplasm of cells in the inner ONL at E18.5 (C, G), but at P3 expression was instead seen in the outer ONL (K, O). The merged images D and H along with yellow arrows illustrate that at E18.5, almost all β-gal+ cells co-express Gap-43 at E18.5, but merged images L and P and yellow arrowheads illustrate that β-gal+ cells do not express Gap-43 at P3. Dorsal is up, medial is right. Scale bar = 100 µm.

Figure 10

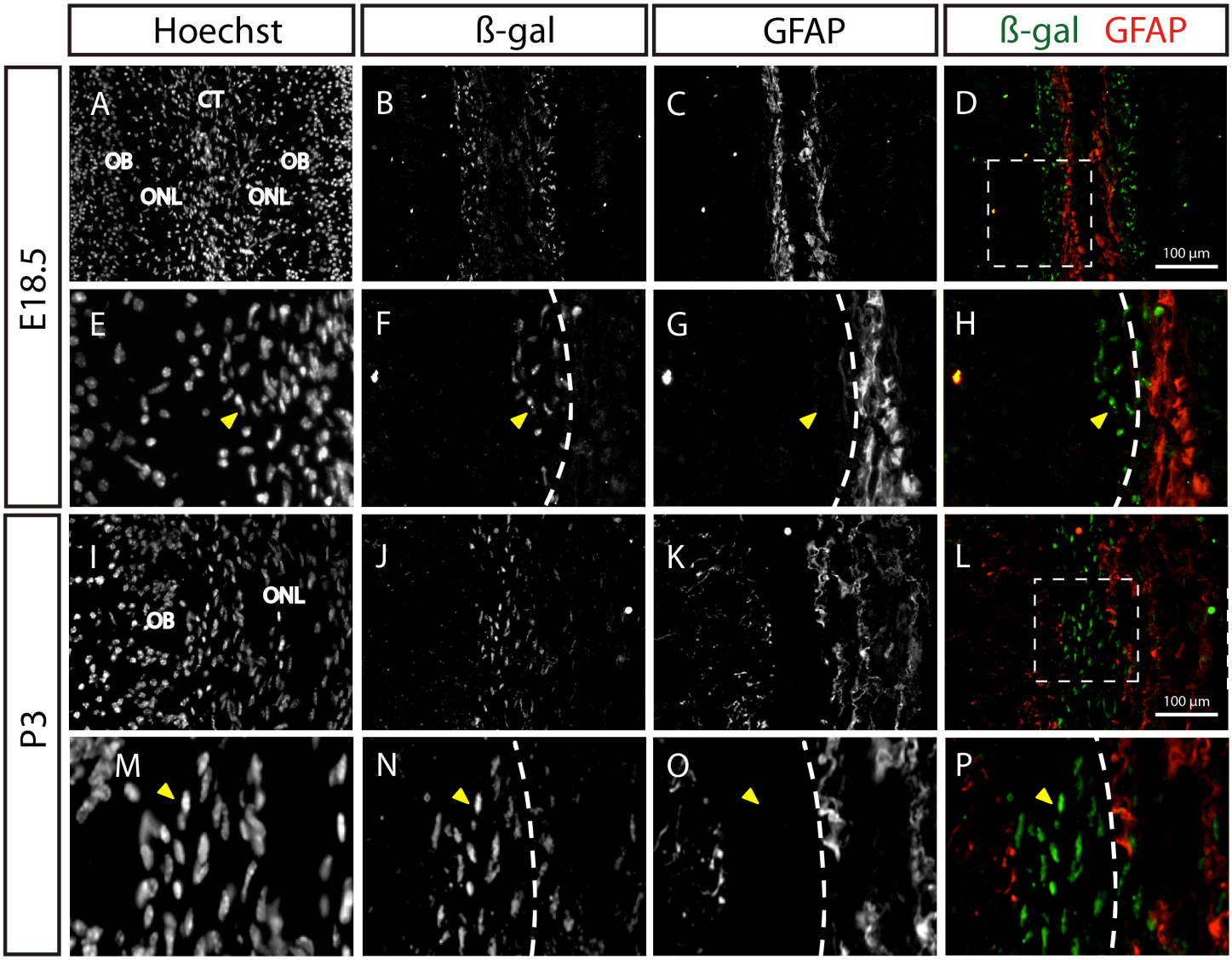

Figure 10: Analysis of β-gal and GFAP expression in the main OB of *Runx1*$^{LacZ/+}$ mice. Coronal sections from the main OB of *Runx1*$^{LacZ/+}$ mice at E18.5 (A-H) and P3 (I-P) were subjected to double-label immunohistochemistry to examine the expression of beta-gal and GFAP. Panels E-H and M-P are higher magnification views of panels A-D and I-L, respectively. Grey scale images were assigned to the green (β-gal) and red (GFAP) channels, which were digitally merged to generate panels D, H, L and P. Hoechst staining is shown in grey scale in panels A, E, I and M to depict cell nuclei. β-gal was preferentially expressed in the nuclei of cells in the inner ONL (B, F, J, N) (yellow arrowheads), while GFAP was preferentially expressed in the cytoplasm of cells in the outer ONL (C, G, K, O). The hashed line indicates the separation between the β-gal and GFAP expression domains. The merged images and yellow arrowheads illustrate that almost no β-gal+ cells co-express GFAP at either gestational age. Dorsal is up, medial is right. Scale bar = 100 µm.

Figure 11

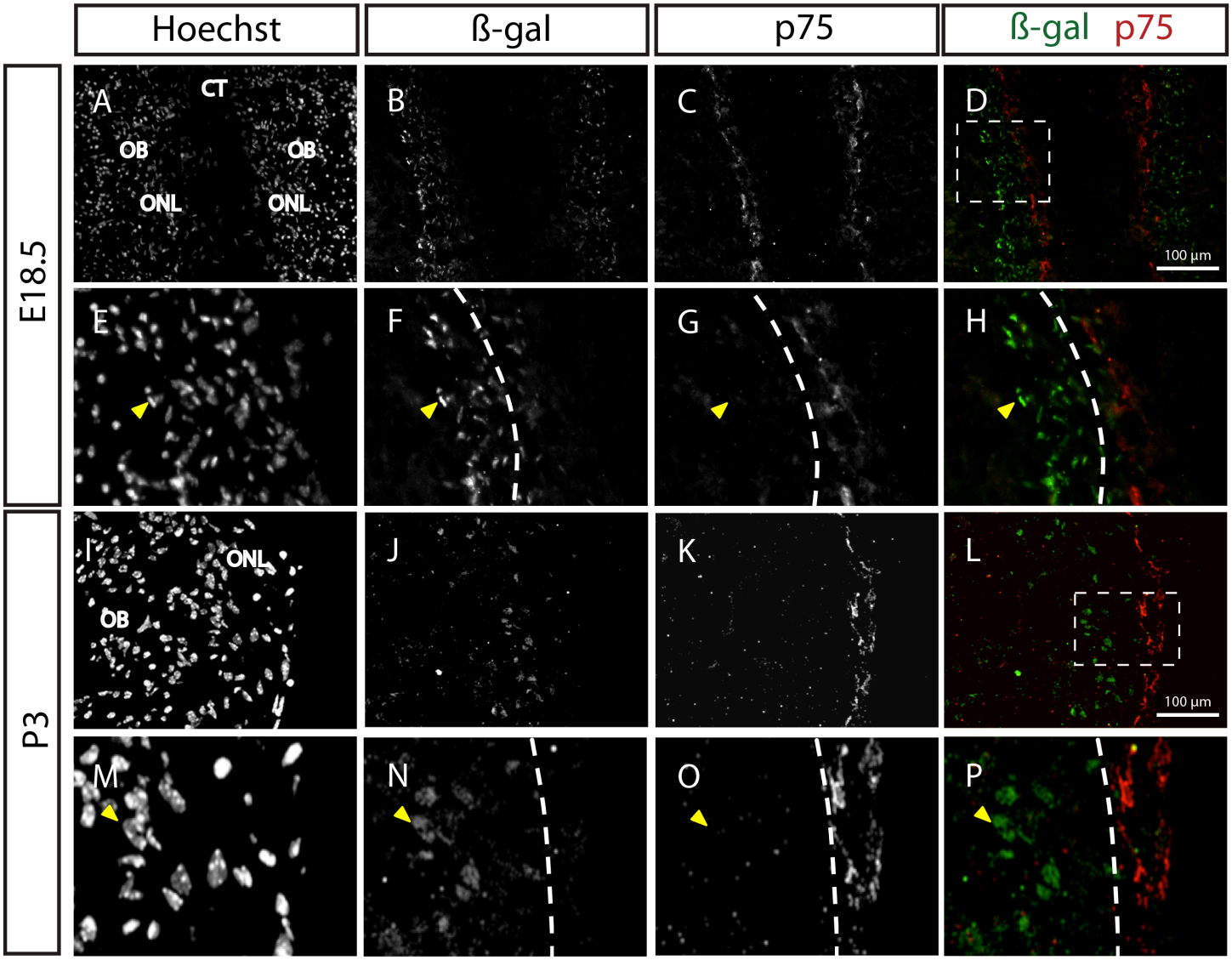

Figure 11: Analysis of β-gal and p75 expression in the main OB of *Runx1*$^{LacZ/+}$ mice. Coronal sections from the main OB of *Runx1*$^{LacZ/+}$ mice at E18.5 (A-H) and P3 (I-P) were subjected to double-label immunihistochemistry to examine the expression of β-gal and p75. Panels E-H and M-P are higher magnification views of A-D and I-L, respectively. Grey scale images were assigned to the green (β-gal) and red (p75) channels, which were digitally merged to generate panels D, H, L and P. Hoechst staining is shown in grey scale in panels A, E, I and M to depict cell nuclei. β-gal was preferentially expressed in the nuclei of cells in the inner portion of the ONL (B, F, J, N) (yellow arrowheads), while p75 was preferentially expressed on the surfaces of cells in the outer ONL (C, G, K, O). The merged images and yellow arrowheads indicate that almost no β-gal+ cells co-expressed p75 at either gestational age (D, H, L, P). Dorsal is up, medial is right. Scale bar = 100 µm.

Figure 12

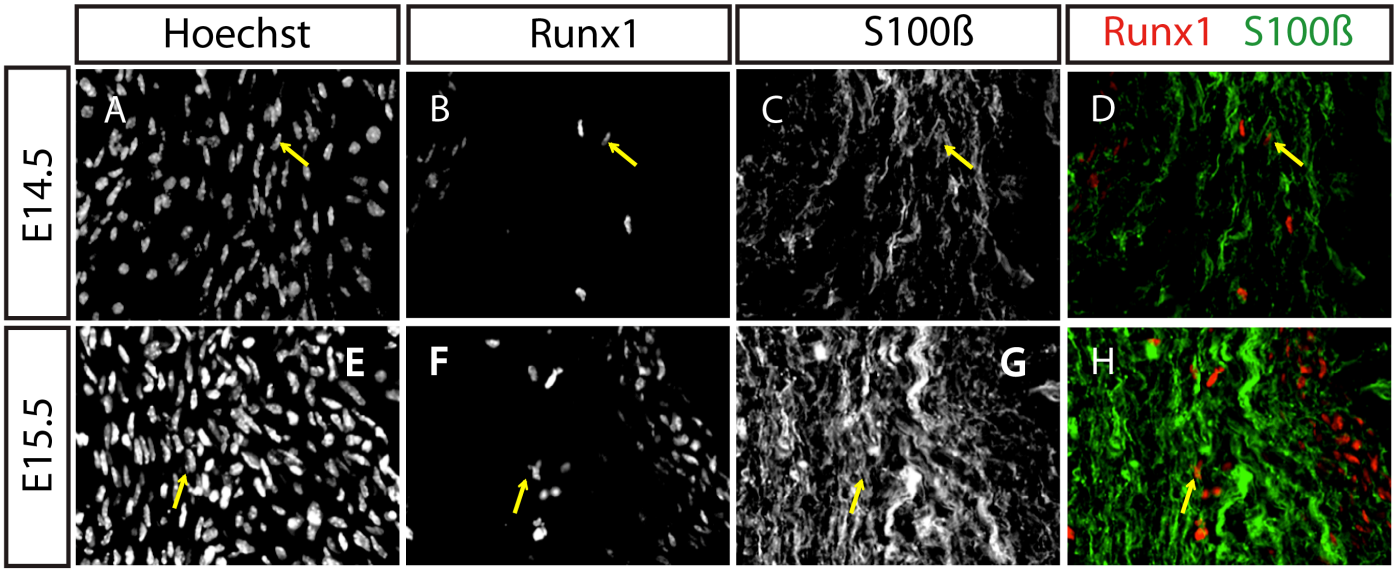

Figure 12: Runx1+ cells along the migratory pathway are presumptive OECs. Coronal sections from the main OB of wild-type mice at E14.5 (A-D) and E15.5 (E-H) were subjected to double-label immunohistochemistry to examine the expression of Runx1 and S100β, an early marker of OECs, in the OB and in region B of the migratory pathway. Grey scale was assigned to red (Runx1) and green (S100β) channels which were digitally merged to generate panels D and H. Hoechst staining is shown in grey scale in panels A and E to depict cell nuclei. Yellow arrows indicate Runx1+ cells along the migratory pathway. From panels A-H, it is apparent that Runx1+ cells, indicated arrows, both increase in number and also increasingly express S100β en route to their final destinations in the ONL.

Figure 13

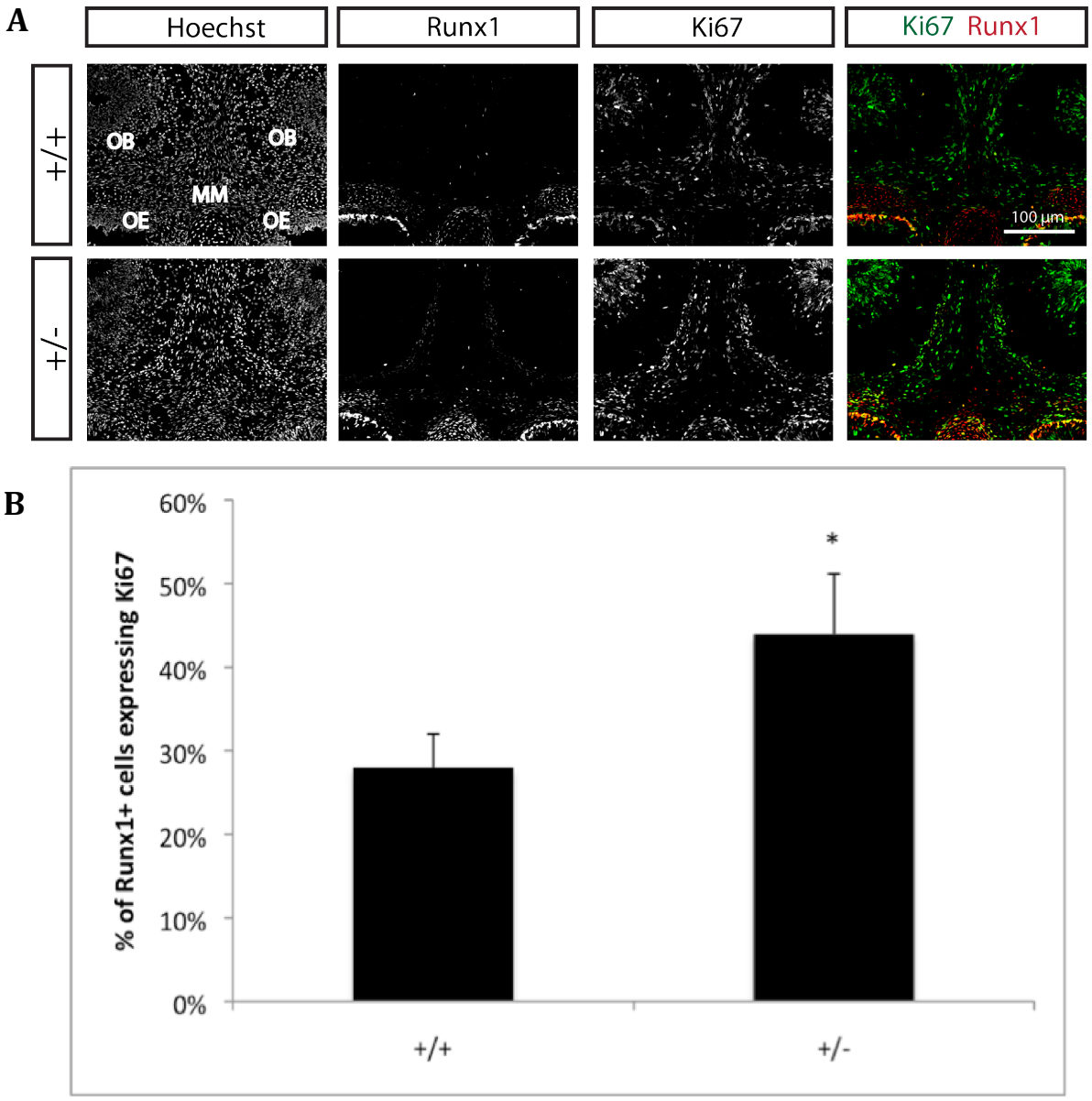

Figure 13: Runx1 heterozygosity leads to a significant increase in the percentage of proliferating Runx1+ cells at E14.5 in Region A of the MM pathway. A) Coronal sections through the OB of *Runx1*$^{+/+}$ and *Runx1*$^{+/-}$ embryos at E14.5 were subjected to immunohistochemical staining for Runx1 and Ki67 and double-labeled cells in Region A were analyzed. B) Quantification of the percentage of Runx1+/Ki67+ cells in response to a decrease in Runx1 protein dosage (*, p=0.05). The increase observed in heterozygous embryos suggests that Runx1 plays a role in the proliferation of OEC precursors. MM= migratory mass. Dorsal is up, medial is right. Scale bar = 100 µm.

Figure 14

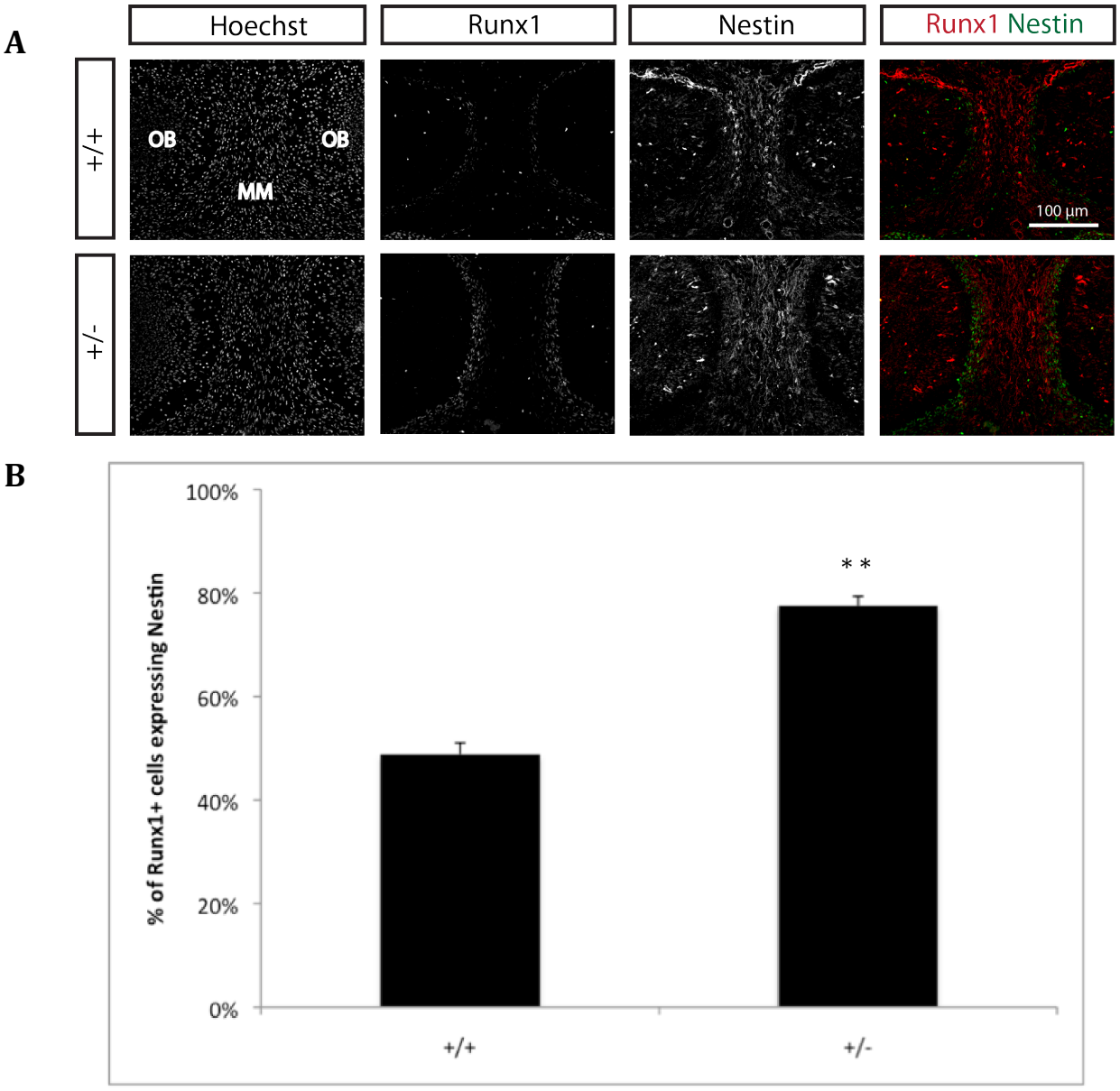

Figure 14: Runx1 heterozygosity leads to a significant increase in the percentage of immature Runx1+ OEC precursors at E14.5 in Region B of the MM pathway. A) Coronal sections through the OB of *Runx1*$^{+/+}$ and *Runx1*$^{+/-}$ embryos at E14.5 were subjected to immunohistochemical staining for Runx1 and Nestin, and double-labeled cells in Region B were analyzed. B) Quantification of the percentage of Runx1+/Nestin+ cells in response to a reduction in Runx1 protein dosage (**, p=0.008). The increase observed in heterozygous embryos suggests that Runx1 plays a role in promoting the maturation of OEC precursors. Dorsal is up, medial is right. Scale bar = 100 µm.

Figure 15

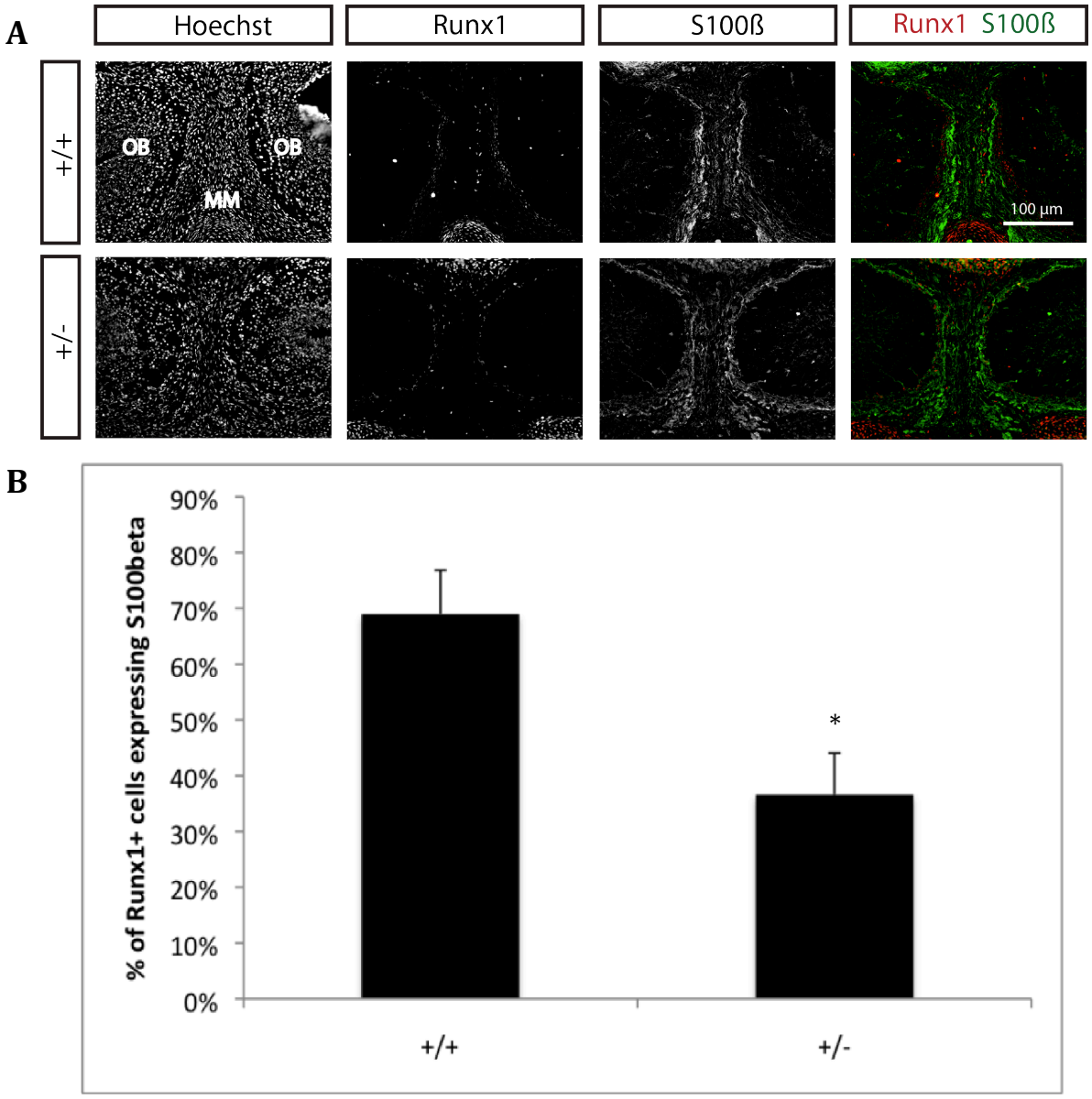

Figure 15: Runx1 heterozygosity perturbs the transition from immature OEC precursors to differentiating OECs in Region B of the MM pathway. A) Coronal sections through the OB of *Runx1*$^{+/+}$ and *Runx1*$^{+/-}$ embryos at E15.5 were subjected to immunohistochemical staining for Runx1 and S100β and double-labeled cells from Region B were analyzed. B) Quantification of the percentage of Runx1+/S100β+ cells in response to a reduction in Runx1 protein dosage (*, p=0.04). The decrease observed in heterozygous embryos suggests that Runx1 is involved in the transition of OECs from the immature precursor state to the differentiating state. Dorsal is up, medial is right. . Scale bar = 100 µm.

Figure 16

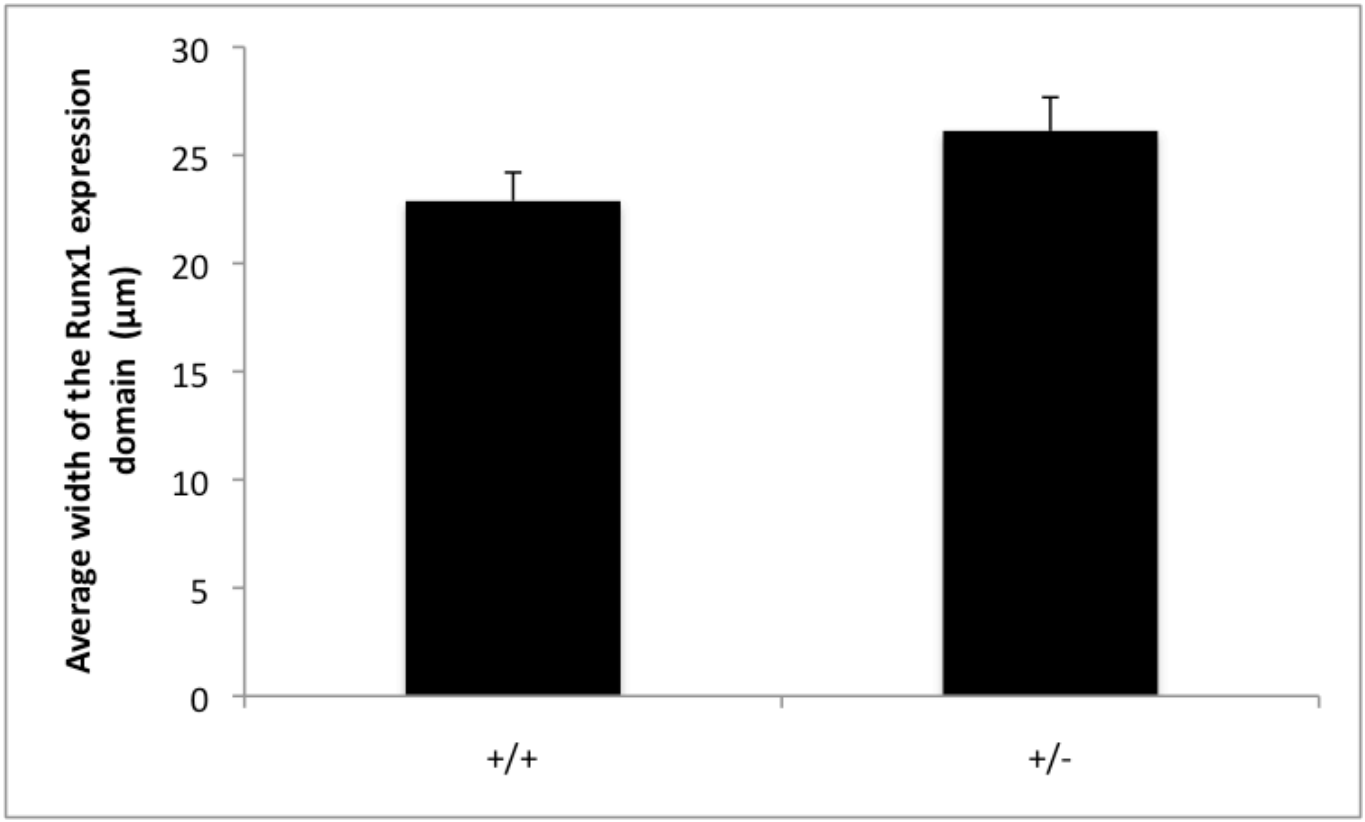

Figure 16: Runx1 heterozygosity leads to a disorganization of the Runx1-expression domain in the inner ONL of the OB. Coronal sections from the OB of *Runx1*$^{LacZ/+}$ (i.e. *Runx1*$^{+/-}$) and *Runx1*$^{+/+}$ mice were subjected to immunohistochemical staining to examine the organizational topology of the Runx1-immunoreactive region of the ONL. A reduction in Runx1 protein dosage led to a trend, through insignificant, towards an increase in the width of the Runx1-expressing domain – Runx1+ cells spread medially from the inner ONL towards the region denoted as the "outer ONL". This suggests that Runx1 may be playing a role in the proper migration of Runx1+ OECs to their appropriate positions within the inner ONL (T-test; n=5).

Figure 17

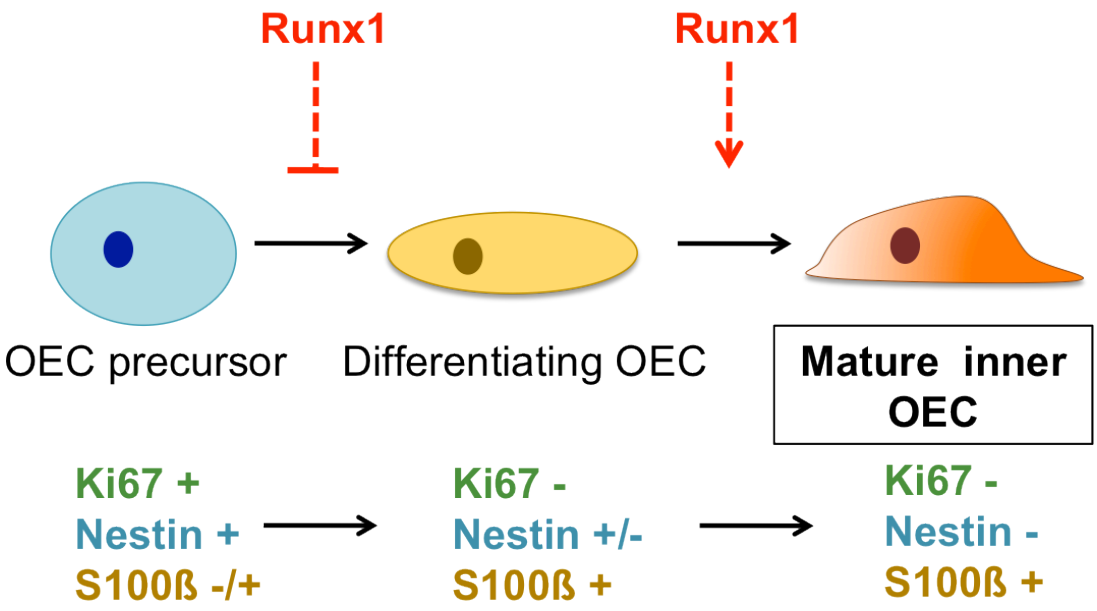

Figure 17: Proposed model of Runx1 activity in OEC development. From our data, we propose that Runx1 acts on two stages of the OEC differentiation pathway. First, it inhibits the proliferation of Runx1+ OEC precursors; subsequently, it promotes the transition of immature OECs from the precursor to the differentiating state. Runx1 may also be playing a role in the specification of the Runx1+ inner ONL OECs from the larger OEC population by virtue of its sustained and highly specific expression in this subset of cells

7. References

References

Aloisi, F. (2001). "Immune function of microglia." Glia 36(2): 165-179.

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." Journal of Comparative Neurology 137(4): 433-457.

Alvarez-Buylla, A., Garcia-Verdugo, J.M., Tamontin, A.D. (2001). "A unified hypothesis on the linease of neural stem cells." Nature Reviews Neuroscience 2(4): 287-293.

Arvin, B., Neville, L.F., Barone, F.C., Feuerstein, G.Z. (1996). "The role of inflammation and cytokines in brain injury." Neuroscience and Biobehavioural Reviews 20(3): 445-452.

Astic, L., Pellier-Monnin, V., Godinot, F. (1998). "Spatio-temporal patterns of ensheathing cell differentiation in the rat olfactory system during development." Neuroscience 83(1): 295-307.

Au, E., Roskams, A.J. (2003). "Olfactory ensheathing cells of the lamina propria in vivo and in vitro." Glia 41: 224-236.

Au, W. A., Treloar, H.B., Greer, C.A. (2002). "Sublaminar organization of the mouse olfactory bulb nerve layer." Journal of Comparative Neurology 446: 68-80.

Audisio, C., Raimondo, S., Nicolino, S., Gambarotta, G., Di Scipio, F., Macri, L., Montarolo, F., Giacobini-Rocecchi, M.G., Porporato, P., Filighueddu, L., Graziani, A., Geuna, S., Perroteau, I. (2009). "Morphological and biomolecular characterization of the neonatal olfactory bulb ensheathing line." Journal of Neuroscience Methods 185(89-98).

Auffarth, B., Kaplan, B., Lansner, A. (2011). "Map formation in the olfactory bulb by axon guidance of olfactory neurons." Frontiers in Systems Neuroscience 5(84).

Babiarz, J., Kane-Goldsmith, N., Basak, S., Lui, K., Young, W., Grumet, M. (2011). "Juvenile and adult olfactory ensheathing cells bundle and myelinate dorsal root glanglion axons in culture " Experimenal Neurology 229(1): 72-79.

Bae, S.-C., Choi, J-K. (2004). "Tumor suppressor activity of RUNX3." Oncogene 23: 4336-4340.

Bae, S.-C., Ogawa, E., Maruyama, M., Oka, H., Satake, M., Shigesada, K., Jenkins, N.A., Gilbert, D.J., Copeland, N.G., Ito, Y. (1994). "PEBP2 alpha B/mouse AML1 consists of

multiple isoforms that possess differential transactivation potentials." Molecular and Cellular Biology 14(5): 3242-3252.

Bailey, M. S., Shipley, M.T. (1993). "Astrocyte subtypes in the rat olfactory bulb: morphological heterogenity and differential laminar distribution." Journal of Comparative Neurology 328(4): 501-526.

Barnett, S. C., Roskams, A.J. (2002). "Olfactory ensheathing cells. Isolation and culture from the rat olfactory bulb." Methods in Molecular Biology 198: 41-48.

Barraud, P., Seferiadis, A.A., Tyson, L.D., Zwart, M.F., Szabo-Rogers, H.L., Ruhrberg, C., Liu, K.J., Baker, C.V.H. (2010). "Neural crest origin of olfactory ensheathing glia." Proceedings of the National Academy of Science of the United States of America 107(49): 21040-21045.

Beites, C. L., Kawauchi, S., Crocker, C.E., Calof, A.L. (2005). "Identification and molecular regulation of neural stem cells in the olfactory epithelium." Experimental Cell Research 306(2): 309-316.

Benveniste, E. N. (1998). "Cytokine actions in the central nervous system." Cytokine Growth Factor Review 8(3-4): 259-275.

Bernardin-Fried, F., Kummalue, T., Leijen, S., Collector, M.I., Ravid, K., Friendman, A.D. (2004). "AML1/RUNX1 increases during G1 to S cycle cell progression independant of cytokine-dependant phosphorylation and induces cyclin D3 gene expression." Journal of Biological Chemistry 279(15): 12678-15687.

Blanchart, A. M.-L., E., De Carlos, J.A., Lopez-Mascaraque, L. (2011). "Peripheral contributions to olfactory bulb cell populations (migratiosn towards the olfactory bulb)." Glia 59(2): 278-292.

Blanes, T. (1898). "Sobre algunos puntos dudosos de la estructura del bulbo olfatorio." Rev Trim Micrografia 3: 99-127.

Blyth, K., Cameron, E.R., Neil, J.C. (2005). "The RUNX genes: Gain or loss of function in cancer." Nature Reviews Cancer 5: 376-387.

Bosselut, R. (2004). "CD4/CD8-lineage differentiation in the thymus: from nuclear effectors to membrane signals." Nature Reviews Immunology 4(7): 529-540.

Bovolenta, P., Wandosell, F., Nieto-Sampedrio, M. (1993). "Neurite outgrowth inhibitors associated with glial cells and glial cell lines." Neuroreports 13;5(3): 345-348.

Boyd, J. G., Doucette, R., Kawaja, M.D. (2005). "Defining the role of olfactory ensheathing cells in facilitating axon remyelination following damage to the spinal cord." The FASEB Journal 19(7): 694-703.

Boyd, J. G., Jahed, A., McDonald, T.G., Krol, K.M., Van Eyk, J.E., Doucette, D., Kawaja, M.D. (2006). "Proteomic evaluation reveals that olfactory ensheathing cells but not Schwann cells express calponin." Glia 53(4): 434-440.

Boyd, J. G., Lee, J., Skihar, V., Doucette, R., Kawaja, M.D. (2004). "LacZ-expressing olfactory ensheathing cells do not associate with myelinated axons after implantation into the compressed spinal cord." Proceedings of the National Academy of Science of the United States of America 101(7): 2162-2166.

Britsch, s., Georich, D.E., Riethmacher, D., Peirano, R.I., Rossner, M., Nave, K.A., Birchmeler, C., Wegner, M. (2001). "The transcription factor Sox10 is a key regulator of peripheral glial development." Genes and Development 15(1): 66-78.

Burel, S. A., Harakawa, N., Zhou, L., Pabst, T., Tenen, D.G., Zhang, D.E. (2001). "Dichotomy of AML1-ETO functions: growth arrest versus block of differentiation." Molecular and Cellular Biology 21(16): 5577-5590.

Burns, C. E., Travel, D., Mayhall, E., Shepard, J.L., Zon, L.I. (2005). "Hematopoetic stem cell fate is establised by the Notch-Runx pathway." Genes and Development 19(19): 2331-2342.

Caggiano, M., Kauer, J.S., Hunter, D.D. (1994). "Globose basal cells are neuronal progenitors in the olfactory epithelium: a lineage analysis using a replication-incompetent retrovirus." Neuron 13: 339-352.

Cai, Z., de Bruijn, M., Ma, X., Dortland, B., Lutejin, T., Downing, R.J., Dzierzak, E. (2000). "Haploinsufficiency of AML1 affects the temporal and spatial generation of hematopoetic stem cells in the mouse embryo." Immunity 13(4): 423-431.

Calof, A. L., Mumm, J.S., Rim, P.C., Shou, J. (1999). "The neuronal stem cell of the olfactory epithelum." Journal of Neurobiology 36(2): 190-205.

Canon, J., Banerjee, U. (2000). "Runt and Lozenge function in Drosophila development." Seminars in Cell & Developmental Biology 11(5): 327-336.

Cao, Q. L., Zhang, Y.P., Howard, R.M., Walters, W.M., Tsoulfas, P., Whittemore, S.R. (2001). "Pluripotent stem cells engrafter into the normal or lesioned adult rat spinal cord are restricted to a glial lineage." Experimenal Neurology 167(1): 48-58.

Caroni, P., Schwab, M.E. (1988). "Two membrane protein fractions from rat central myelin with inhibitory properties for neurite growth and fibroblast spreading." Journal of Cell Biology 106(4): 1281-1288.

Carr, V. M., Farbman, A.I., Colletti, L.M., Morgan, J.I. (1991). "Identification of a new non-neuronal cell type in rat olfactory epithelium." Neuroscience 45(2): 433-449.

Carter, L. A., MacDonald, J.L., Roskams, A.J. (2004). "Olfactory horizontal basal cells demonstrate a conserved multipotent progenitor phenotype." Journal of Neuroscience 24(25): 5670-5683.

Challen, G. A., Goodell, M.A. (2011). "Runx1 isoforms show differential expression patterns during hematopoetic development but have similar functional effects in adult hematopoetic stem cells." Experimental Hematology 38(5): 403-416.

Chehrehasa, F., Ekberg, J.A.K., Lineburg, K., Amaya, D., Mackay-Sim, A., St. John, J.A. (2012). "Two phases of replacement replenish the olfactory ensheathing cell population after injury in postnatal mice." Glia 60(2): 322-332.

Chehrehasa, F., Windus, L.C.E., Ekberg, J.A.K., Scott, S.E., Amaya, D., Mackay-Sim, A., St John, J.A. (2010). "Olfactory glia enhance neonatal axon regeneration." Molecular and Cellular Neuroscience 45(3): 277-288.

Chen, C. L., Broom, D.C., Liu, Y., de Nooij, J.C., Cen, C., Samad, O.A., Jessell, T.M., Woolf, C.J., Ma, Q. (2006). "Runx1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain." Neuron 49(3): 365-377.

Chen, X., Fang, H., Schwob, J.E. (2004). "Multipotency of purified, transplanted globose basal cells in olfactory epithelium." The Journal of Comparative Neurology 469(4): 457-474.

Chuah, M. I., Au, C. (1991). "Olfactory Schwann cells are derived from precursor cells in the olfactory epithelium." Journal of Neuroscience Research 29: 172-180.

Chuah, M. I., Au, C. (1993). "Cultures of ensheathing cells from neonatal rat olfactory bulbs." Brain Research 601(1-2): 213-220.

Coffman, J. A. (2003). "Runx1 transcription factors and the developmental balance between cell proliferation and differentiation." Cell Biology International 27(4): 315-324.

Coppola, D. M. (2012). "Studies of olfactory system neural plasticity: the contribution of the unilateral naris occlusion technique." Neural Plasticity.

Couly, G. F., Le Douarin, N.M. (1985). "Mapping of the early primordium in quail-chick chimeras I: Developmental relationships between placodes, facial ectoderm and prosencephalon." Developmental Biology 110: 422-439.

Crandall, J. E., Dibble, C., Butler, D., Pays, L., Ahmad, N., Kostek, C., Puschel, A.W., Schwartin, G.A. (2000). "Patterning of olfactory sensory connections is mediated by extracellular matrix proteins in the nerve layer of the olfactory bulb." Journal of Neurobiology 45(4): 195-206.

Cummings, D. A., Brunjes, P.C. (1995). "Migrating leuteinizing hormon-releasing hormone (LHRH) neurons and processes are associated with a substrate that expresses S100." Developmental Brain Research 88(2): 148-157.

Daga, A., Karlovich, C.A., Dumstrei, K., Bannerjee, U. (1996). "Patterning of cells in the Drosophila eye by Lozenge, which shares homologous domains with AML1." Genes and Development 10(10): 1194-1205.

Dai, X., Lercher, L.D., Clinton, P.M., Du, Y., Livingston, D.L., Vieira, C., Yang, L., Shen, M.M., Dreyfus, C.F. (2003). "The trophic role of oligodendrocytes in the basal forebrain." Journal of Neuroscience 23(13): 5846-5853.

de Bruijn, M. F., Speck, N.A. (2004). "Core-binding factors in hematopoesis and immune function." Oncogene 23(24): 4238-4248.

de Lorenzo, A. J. (1957). "Electron microscopic observations of the olfactory mucosa and olfactory nerve." Journal of Cell Biology 3(6): 839-850.

Deng, Y. B., Liu, Y., Zhu, W.B., Bi, X.B., Wang, Y.Z., Ye, M.H., Zhou, G.Q. (2008). "The co-transplantation of human bone marrow stromal cells and embryo olfactory ensheathing cells as a new approach to treat spinal cord injury in a rat model." Cryotherapy 10: 551-564.

Deumens, R., Koopmans, G.C., Honig, W.M., Maquet, V., Jerome, R., Steinbusch, H.W., Joosten, E.A. (2006). "Chronically injured corticospinal axons do not cross large spinal lesion gaps after a multifactorial transplantation strategy using olfactory ensheathing cell/olfactory nerve fibroblast-biomatrix bridges." Journal of Neuroscience Research 83(5): 811-820.

Donato, R. (1986). "S-100 proteins." Cell Calcium 7(3): 123-145.

Dong, Y., Benveniste, E.N. (2001). "Immune function of astrocytes." Glia 36(2): 180-190.

Dormand, E. L., Brand, A.H. (1998). "Runt determines cell fates in the Drosophila embryonic CNS." Development 125(9): 1659-1667.

Doucette, J. R. (1984). "The glial cells in the nerve fibre layer of the rat olfactory bulb." Anatomical Record 210(2): 385-391.

Doucette, J. R. (1993). "Glial cells in the nerve fiber layer of the main olfactory bulb of embryonic and adult mammals." Microscopy Research and Technique 24(2): 113-130.

Doucette, J. R., Kott, J., Westrum, L. (1994). "The development of glial fibrillary acidic protein-positive cells and the appearance of laminin-like immunoreactivity in fetal olfactory bulb transplants." Brain Research 649(1-2): 334-338.

Doucette, R. (1989). "Development of the nerve fiber layer in olfactory bulb of mouse embryos." Journal of Comparative Neurology 285: 514-527.

Doucette, R. E. F. a. R. (2001). "Chapter 13: Olfactory ensheathing cell cultures " Protocols for Neural Cell Culture 3rd edition.

Drejer, J., Larsson, O.M., Schousboe, A. (1982). "Characterization of L-glutamate uptake into and release from astrocytes nad neurons cultured from different brain regions." Experimental Brain Research 47: 259-269.

Ducy, P., Starbuck, M., Priemel, M., Shen, J., Pinero, G., Geoffroy, V., Amling, M., Karsenty, G. (1999). "A Cbfa1-dependant genetic pathway controls bone formation beyond embryonic development." Genes and Development 13(8): 1025-1036.

Duffy, J. B., Gergen, J.P. (1991). "The Drosophila segmentation gene runt acts as a position-specific numerator element recessary of the uniform expression of the sex-determining gene Sex-lethal." Genes and Development 5(12A): 2176-2187.

Durst, K. L., Hiebert, S.W. (2004). "Role of RUNX family members in transcriptional repression and gene silencing." Oncogene 23(24): 4220-4224.

Evans, C. J., Banerjee, U. (2003). "Transcriptional regulation of hematopoesis in *Drosophila*." Blood Cells, Molecules and Disease 30(2): 223-228.

Farbman, A., Squinto, L.M. (1985). "Early development of olfactory receptor cell axons." Developmental Brain Research 19: 205-213.

Fawcett, J. W., Asher, R.A. (1999). "The glial scar and central nervous system repair." Brain Research Bulletin 49(6): 377-391.

Ferguson, T. A., Son, Y.J. (2011). "Extrinsic and intrinsic determinants of nerve regeneration." Journal of Tissue Engineering 2(1).

Finzsch, M., Schreiner, S., Kichko, T., Reeh, P., Tamm, E.R., Bosl, M.R., Meijer, D., Wegner, M. (2010). "Sox10 is required for Schwann cell identity and progression beyond the immature Schwann cell stage." Journal of Cell Biology 189(4): 701-712.

Fitch, M. T., Silver, J. (1997). "Activated macrophages and the blood-brain barrier: inflammation after CNS injury leads to increases in putative inhibitory molecules." Experimenal Neurology 148(2): 587-603.

Fitch, M. T., Silver, J. (2008). "CNS injury, glial scars and inflammation." Experimenal Neurology 209(2): 294-301.

Forni, P. E., Taylor-Burds, C., Melvin, V.S., Williams, T., Wray, S. (2011). "Neural crest and ectodermal cells intermix in the nasal placode to give rise to GnRH-1 neurons, sensory neurons and olfactory ensheathing cells." Journal of Neuroscience 31(18): 6915-6927.

Forni, P. E., Wray, S. (2012). "Neural crest and olfactory system: New prospective." Molecular Neurobiology.

Franceschini, I. A., Barnett, S.C. (1996). "Low-affinity NGF-receptor and E-N-CAM expression define two types of olfactory nerve ensheathing cells that share a common lineage." Develomental Biology 173: 327-343.

Franssen, E. H. P., De Bree, F.M., Essing, A.H.W., Ramon-Cueto, A., Verhaagen, J. (2008). "Comparative gene expression profiling of olfactory ensheathing glia and Schwann cells indicates distinct tissue repair characteristics of olfactory ensheathing glia." Glia 56(12): 1285-1298.

Freeman, J. W. (1997). "Chapter 2: Olfactory system: Odorant detection and classification." Building Blocks for Intelligent Systems: Brain Components as Elements of Intelligent Function 3(2): Ed. Amit, D., Parisi, G.

Friedrich, M. J., Rad, R., Langer, R., Voland, P., Hoefler, H., Schmid, R.M., Pinz, C., Gerhard, M. (2006). "Lack of RUNX3 regulation in human gastric cancer." Journal of Pathology 210(2): 141-146.

Fujita, Y., Nishimura, M., Taniwaki, M/, Abe, T., Okuda, T. (2001). "Identification of an alternative spliced form of the mouse AML1/RUNX1 gene transcript AML1c and its expression in early hematopoetic development." Biochemical and Biophysical Research Communications 281(5): 1248-1255.

Gergen, J. P., Bulter, B.A. (1988). "Isolation of the Drosophila segmentation gene runt and analysis of its expression during embryogenesis." Genes and Development 2(9): 1179-1193.

Gimpl, G., Kirchhoff, F., Lang, R.E, Kettenmann, H. (1993). "Identification of neuropeptide Y receptors in cultured astocytes from neonatal rat brain." Journal of Neuroscience Research 34(2): 198-205.

Golgi, C. (1875). "Sulla fina anatomia del bulbi olfactorii." Ti Rivista Sperimentale di Freniatria 1: 403-425.

Gong, Q., Shipley, M.T. (1995). "Evidence that pioneer olfactory axons regulate telencephalon cell cycle kintetics to induce the formation of the olfactory bulb." Neuron 14(91-101).

Goodman, M. N., Jacobberger, J.W. (1993). "Establishment and neurite outgrowth properties of neonatal and adult rat olfactory bulb glial cell lines." Brain Research 619(1-2): 199-213.

Goyoma, S., Mullow, J.C. (2011). "Molecular pathogenesis of core binding factor leukemia: current knowledge and future prospects." International Journal of Hematology 94(2): 126-133.

Graziadei, G. A., Graziadei, P.P.C. (1979). "Neurogenesis and neuron regeneration in the olfactory system of mammals. II. Degeneration and reconstitution of the olfactory sensory neurons after axotomy." Journal of Neurocytology 8(2): 197-213.

Greer, C. A. (1991). "Structural organization of the olfactory system." Smell and Taste in Health and Disease New York, New York: 65-81.

Guerout, N., Derambure, C., Drouot, L., Bon-Mardion, N., Duclos, C., Boyer, O., Marie, J.P. (2010). "Comparative gene expression profiling of olfactory ensheathing cells from olfactory bulb and olfactory mucosa." Glia 58(13): 1570-1580.

Guest, J. D., Herrera, L., Margitich, I., Oliveria, M., Marcillo, A., Casas, C.E. (2008). "Xenografts of expanded primate olfactory ensheathing glia support transient behevioral recovery that is independant of seratonergic or corticospinal axonal regneration in nude rats forllowing spinal cord transection." Experimenal Neurology 212(2): 261-274
.

Hakanen, J., Duprat, S., Salimen, M. (2011). "Netrin1 is required for neural and glial precursor migration into the olfactory bulb." Develomental Biology 355(1): 101-114.

Halasz, N. (1990). "The vertebrate olfactory system." Akadimia Kiado Budapest.

Harper, J. W., Adami, G.R., Wei, N., Keyomarsi, K., Elledge, S.J. (1993). "the p21 CDK-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases." Cell 75(4): 805-816.

Harrison, S. J., Nishinakamura, R., Monaghan, A.P. (2008). "Sall1 regulates mitral cell development and olfactory nerve extension in the developing olfactory bulb." Cerebral Cortex 18: 1604-1617.

Herglotz, J., Kuvardina, O.N., Kolodziej, S., Kumar, A., Hussong, H., Grez, M., Lausen, J. (2012). "Histone arginine methylation keeps RUNX1 target genes in an intermediate state." Oncogene.

Higginson, J. R., Barnett, S.C. (2011). "The culture of olfactory ensheathing cells (OECs) - a distinct glial cell type " Experimental Neurology 229(1): 2-9.

Hinds, J. W. (1972). "Early neuron differentiation in the mouse olfactory bulb. I. Light microscopy." Journal of Comparative Neurology 146(2): 233-252.

Hochstim, C., Deenan, B., Lukaszemicz, A., Zhou, Q., Anderson, D.J. (2008). "Identification of positionally distinct astrocyte subtypes whose identities are specified by a homeodomain code." Cell 133(2): 510-522.

Hofstetter, C. P., Holstrom, N.A., Lija, J.A., Scheinhardt, P., Hao, J., Spenger, C., Wiesenfeld-Hallin, Z., Kurpad, S.N., Frisen, J., Olson, L. (2005). "Alloydinia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome." Nature Neuroscience 8(3): 346-353.

Hoi, C. S. L., Lee, S.E., Lu, S-Y., McDermitt, D.J., Osorio, K.M., Piskun, C.M., Peters, R.M., Paus, R., Tumbar, T. (2010). "Runx1 directly promotes proliferation of hair follicle stem cells and epithelial tumor formation in mouse skin." Molecular and Cellular Biology 30(10): 2518-2536.

Hokfelt, T., Stanic, D., Sanford, S.D., Gatlin, J.C., Nilsson, I., Praratcha, G., Ledda, F., Fetissov, S., Lindfors, C., Herzog, H., Johansen, J.E., Ubink, R., Pfenninger, K.H. (2008). "NPY and its involvement in axon guidance, neurgenesis and feeding." Nutrition 24(9): 860-868.

Howe, M. L., Barres, B.A. (2012). "A novel role for microglia in minimizing excitotoxicity." BMC Biology 10(2): 1-3.

Huard, J. M., Youngentob, S.L., Goldstein, B.J., Luskin, M.B., Schwob, J.E. (1998). "Adult olfactory epithelium contains multipotent progenitors that give rise to neurons and non-neural cells." Journal of Comparative Neurology 400(2): 469-486.

Imaizumi, T., Lankford, K.L., Burton, W.V., Fodor, W.L., Kocsis, J.D. (2000). "Xenotransplantation of transgenic pig olfactory ensheathing cells promotes axonal regeneration in rat spinal cord." Nature Biotechnology 18: 949-953.

Imamura, F., Ayoub, A.e., Rakic, P., Greer, C.A. (2011). "Timing for neurogenesis is a determinant of olfactory circuitry." Nature Neuroscience 14(3): 331-337.

Inoue, K., Shiga, T., Ito, Y. (2008). "Runx transcription factors in neuronal development." Neural Development 3(20).

Ito, Y. (2004). "Oncogenic potential of the RUNX gene family: 'overview'." Oncogene 23(24): 4198-4208.

Ito, Y., Miyazono, K. (2003). "RUNX transcriptin factors as key targets of TGF-beta superfamily signaling." Current Opinion in Genetics & Development 13(1): 43-47.

Iwai, N., Zhou, Z., Roop, D.R., Behringer, R.R. (2008). "Horizontal basal cells are multipotent progenitors in normal and injured adult olfactory epithelium." Stem Cells 26(5): 1298-1306.

Jani, H. R., Raisman, G. (2004). "Ensheathing cell cultures from the olfactory bulb and mucosa." Glia 47(2): 130-137.

Jessen, K. R., Mirsky, R. (2005). "The origin and development of glial cells in the peripheral nerves." Nature Reviews Neuroscience 6: 671-682.

Jiao, Y., Novoshilova, E., Karlen, A., Muhr, J., Olivius, P. (2011). "Olfactory ensheathing cells promote neurite outgrowth from co-cultured brain stem slice." Experimenal Neurology 229(1): 65-71.

Jiminez, D., Garcia, C., de Castro, F., Chedotal, A., Sotelo, C., de Carlos, J.A., Valverde, F., Lopez-Mascaraque, L. (2000). "Evidence for intrinsic development of olfactory structures in Pax-6 mutant mice." Journal of Comparative Neurology 428(3): 511-526.

Kanaykina, N., Abelson, K., King, D., Laikhovitskaia, A., Schreiner, S., Wegner, M., Kozlova, E.N. (2010). "*In vitro* and *in vivo* effects on neural crest stem cell differentiation by conditional activation of Runx1 short isoform and its effect on neuropathic pain behavior." Upsala Journal of Medical Sciences 115(1): 56-64.

Karimi-Abdolrezaee, S., Eftekharpour, E., Wang, J., Corshead, C.M., Fehlings, M.G. (2006). "Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury." Journal of Neuroscience 26(13): 3377-3389
.

Karimi-Abdolrezaee, S., Eftekharpour, E., Wang, J., Schut, D., Fehlings, M.G. (2010). "Synergistic effects of transplanted adult neural stem/progenitor cells, chondroitinase, and growth factors promote functional repair and plasticity of the chronically injured spinal cord." Journal of Neuroscience 30(5): 1657-1676.

Katoh, H., Shibata, S., Fukada, K., Sato, M., Satoh, E., Nagoshi, N., Minematsu, T., Matsuzaki, Y., Akazawa, C., Toyama, Y., Nakamura, M., Okano, H. (2011). "The dual origin of the peripheral olfactory system: placode and neural crest." Molecular Brain 4: 34.

Kim, S. U., de Vellis, J. (2005). "Microglia in health and disease." Journal of Neuroscience Research 81: 302-313.

King-Robson, J. (2011). "Encouraging regeneration in the central nervous system: is there a role for olfactory ensheathing cells?" Neuroscience Research 69(4): 263-275.

Kobayashi, A. S., K., Ozaki, S., Yoshikawa, M., Shiga, T. (2012). "Runx1 promotes neuronal differentiation in dorsal root ganglion." Molecular and Cellular Neuroscience 49(1): 23-31.

Komori, T., Yagi, H., Nomura, S., Yamaguchi, A., Sasaki, K., Deguchi, K., Shimizu, Y., Bronson, R.T., Gao, Y.H., Inada, M., Sato, M., Okamoto, R., Kitamura, Y., Yoshiki, S., Kishimoto, T. (1997). "Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts." Cell 89(5): 755-764.

Kramer, I., Sigrist, M., de Nooji, J.C., Taniuchi, I., Jessell, T.M., Arber, S. (2006). "A role for Runx transcription factor signaling in dorsal root ganglion sensory neuron diversification." Neuron 49(3): 379-393.

Kubasak, M. D., Jindrich, D.L., Zhong, H., Takeoka, A., McFarland, K.C., Munoz-Quiles, C., Roy, R.R., Edgerton, V.R., Ramon-Cueto, A., Phelps, P.E. (2008). "OEC implantation and step training enhance hindlimb-stepping ability in adult spinal transected rats." Brain 131: 264-276.

Laktos, A., Franklin, R.J., Barnett, S.C. (2000). "Olfactory ensheathing cells and Schwann cells differ in their in vitro interactions with astrocytes." Glia 32(2): 214-225.

Lambert, M. P., Megerian, T., Garden, G., Klein, W.L. (1988). "Soluble proteins from rat olfactory bulb promote the survival and differentiation of cultured basal forebrain neurons." Brain Research 469(1-2): 263-276.

Lavdas, A. A., Papastefanaki, F., Thomaidou, D., Matsas, R. (2008). "Schwann cell transplantation for CNS repair." Current Medicinal Chemistry 15(2): 151-160.

Lehnardt, S. (2010). "Innate immunity and neuroinflammation in the CNS: the role of microglia in Toll-like receptor-mediated neruonal injury." Glia 58(3): 253-263.

Levanon, D., Bernstein, Y., Negreanu, V., Bone, K.R., Pozner, A., Eilam, R., Brenner, O., Groner, Y. (2011). "Absence of Runx3 expression in normal gastrointestinal epithelium calls into question its tumour supressor function." EMBO Molecular Medicine 3(10): 593-604.

Levanon, D., Bettoun, D., Haris-Cerruti, C., Woolf, E., Negreanu, V., Ellam, R., Bernstein, Y., Goldenberg, D., Xiao, C., Fliegauf, M., Kremer, E., Otto, F., Brenner, O., Lev-Tov, A., Groner, Y. (2002). "The Runx3 transcription factor regulates

development and survival of TrkC dorsal root ganglia neurons." The EMBO Journal 21(13): 3454-3463.

Levanon, D., Groner, Y. (2004). "Structure and regulated expression of mammalian *RUNX* genes." Oncogene 23: 4211-4219.

Li, Y., Field, P.M., Raisman, G. (1998). "Regeneration of adult rat corticospinal axons induced by transplanted olfactory ensheathing cells." Neuroscience 18(24): 10514-10524.

Liddiard, K., Burnett, A.K., Darley, R.L., Tonks, A. (2012). "RUNX1-ETO deregulates the proliferation and growth factor responsiveness of human hematopoetic progenitor cells downstream of the myeloid transcription factor, MYCT1." Leukemia 26(1): 177-179.

Lledo, P.-M., Gheusi, G., Vincent, J.D. (2005). "Information processing in the mammalian olfactory system." Physiological Reviews 85(1): 281-317.

Lledo, P.-M., Merkle, F.T., Alvarez-Buylla, A. (2008). "Origin and function of olfactory bulb interneuron diversity." Trends in Neuroscience 31(8): 392-400.

Long, F. (2011). "Building strong bones: molecular regulation of the osteoblast lineage." Nature Reviews Molecular and Cell Biology 13(1): 27-38.

Lopez-Mascaraque, L., de Castro, F. (2002). "The olfactory bulb as an independant developmental domain." Cell Death and Differentiation 9(12): 1279-1286.

Lu, J., Ashwell, K. (2002). "Olfactory ensheathing cells: their potential use for repairing the injured spinal cord." Spine 27(8): 887-892.

Lutterbach, B., Westendorf, J.J., Linggi, B., Isaac, S., Seto, E., Hiebert, S.W. (2000). "A mechanism of repression by acute myeloid leukemia-1, the target of multiple chromosomal translocations in acute myeloid leukemia." Journal of Biological Chemistry 275(1): 651-656.

Mackay-Sim, A., Chuah, M.I. (2000). "Neurotrophic factors in the primary olfactory pathway." Progress in Neurobiology 62(5): 527-559.

Maicas, M., Vazquez, I., Vincente, C., Garcia-Sanchez, M.A., Marcotegui, N., Urquiza, L., Calasanz, M.J., Odero, M.D. (2012). "Functional characterization of the promoter region of the human EVI1 gene in acute myeloid leukemia: RUNX1 and ELK1 directly regulate its transcription." Oncogene.

Malz, C. R., Knabe, W., Kuhn, H-J. (2002). "Calretinin immunoreactivity in the prenatally developing olfactory systems of the tree shrew Taupaia belangeri." Anatomy and Embryology 205(7): 83-97.

Marin-Padilla, M., Amieva. B., M.R. (1989). "Early neurogenesis of the mouse olfactory nerve: Golgi and electron microscopy studies." The Journal of Comparative Neurology 288: 339-352.

Marmigere, F., Monterlius, A., Wegner, M., Groner, Y., Reichardt, L.F., Ernfors, P. (2006). "The Runx1/AML1 transcription factor selectively regulates development and survival of TrkA nociceptive sensory neurons." Nature Neuroscience 9(2): 180-187.

Masse, I., Barbollat-Boutrand, L., Molina, M., Berthier-Vergnes, O., Joly-Tonetti, N., Martin, M.T., de Promentel, C.C., Kanitakis, J., Lamartine, J. (2012). "Functional interplay between p63 and p53 controls RUNX1 function in the transition from proliferation to differentiation in human keratinocytes." Cell Death and Disease 3(318).

Matheny, C. J., Speck, M.E., Cushing, P.R., Zhou, Y., Corpora, T., Regan, M., Newman, M., Roudaia, L., Speck, C.L., Gu, T-L., Griffey, S.M., Bushweller, J.H., Speck, N.A. (2007). "Disease mutations in *RUNX1* and *RUNX2* create non-functional, dominant-negatie or hypomorphic alleles." THe EMBO Journal 26(4): 1163-1175.

Mendoza, A. S., Breipohl, W., Miragall, F. (1982). "Cell migration from the chick olfactory placode: a light and electron microscope study." Journal of Embyrology and Experimental Morphology 69: 47-59.

Mendoza-Londono, R., Lee, B. (2006). "Cleidocranial Dysplasia." Gene Reviews Ed: Pagon, R.A., Bird, T.D., Dolan, C.R., Stephens, K., Adam, M.P.(Seattle, WA).

Menei, P., Montero-Menei, C., Whittemore, S.R., Bunge, R.P., Bunge, M.B. (1998). "Schwann cells genetically modified to secrete human BDNF promote enhanced axonal regrowth across transected adult rat spinal cord." European Journal of Neuroscience 10(2): 607-621.

Menn, B., Garcia-Verdugo, J.M., Yaschine, C., Gonzalez-Perez, O., Rowitch, D., Alvarez-Buylla, A. (2006). "Origin of oligodendrocytes in the subventricular zone of the adult brain." Journal of Neuroscience 26(30): 7907-7918.

Messam, C. A., Hou, J., Major, E.O. (2000). "Coexpression of nestin in neural and glial cells in the developing human CNS defined by human-specific anti-nestin antibody." Experimenal Neurology 161(2): 585-596.

Michaud, J., Scott, H.S., Escher, R. (2003). "AML1 interconnected pathways of leukemogenesis." Cancer Investigations 21(1): 105-136.

Miller, A. M., Treloar, H.B., Greer, C.A. (2010). "Composition of the migratory mass during development of the olfactory nerve." The Journal of Comparative Neurology 518(24): 4825-4841.

Miyoshi, H., Ohira, M., Shimizu, K., Mitani, K., Hirai, H., Imai, T., Yokoyama, K., Soeda, E., Ohki, M. (1995). "Alternative splicing and genomic struture of the *AML1* gene involved in actue myeloid leukemia." Nucleic Acids Research 23(14): 2762-2769.

Miyoshi, H., Shimizu, K., Kozu, T., Maseki, N., Kaneko, Y., Ohki, M. (1991). "t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered wtihin a limited region of a single gene, AML1." Proceedings of the National Academy of Science of the United States of America 88(23): 10431-10434.

Mombaerts, P. (2006). "Axonal writing in the mouse olfactory system." Annual Reviews of Cell and Developmental Biology 22(713-737).

Moreau-Fauvarque, C., Kumanogoh, A., Camand, E., Jaillard, C., Barbin, G., Boquet, I., Love, C., Jones, E.Y., Kikutani, H., Lubetzki, C., Dusart, I., Chedotal, A. (2003). "The transmembrane semaphorin Sema4D/CD100, an inhibitor of axonal growth, is expressed on oligodendrocytes and upregulated after CNS lesion." Journal of Neuroscience 23(27): 9228-9239.

Moreno-Flores, M. T., Bradbury, E.J., Martin-Bermejo, M.J., Agudo, M., Lim, F., Pastrana, E., Avila, J., Diaz-Nido, J., McMahon, S.B., Wandosell, F. (2006). "A clonal cell line from immortalized olfactory ensheathing clia promotes functional recovery in the injured spinal cord." Molecular Therapy 13(3): 598-608.

Mori, K., Nagao, H., Yoshihara, Y. (1999). "The olfactory bulb: coding and processing of odor molecule information." Science 286(5440): 711-715.

Mothe, A. J., Kulbatshi, I., Parr, A., Mohareb, M., Tator, C.H. (2008). "Adult spinal cord stem/progenitor cells transplanted as neurospheres preferentially differentiate into oligodendrocytes in the adult rat spinal cord." Cell Transplantation 17(7): 735-751.

Mothe, A. J., Zahir, T., Santaguida, C., Cook, D., Tator, C.H. (2011). "Neural stem/progenitor cells from the adult human spinal cord are multipotent and self-renewing and differentiate after transplantation." PLoS One 6(11).

Mouret, A., Lepousez, G., Gras, J., Gabellec, M.-M., Lledo, P.-M. (2009). "Turnover of neurborn olfactory bulb neurons optimizes olfaction." Journal of Neuroscience 29(39): 12302-12314.

Mukhopadhyay, G., Doherty, P., Walsh, F.S., Crocker, P.R., Filbin, M.T. (1994). "A novel role for myelin-associate glycoprotein as an inhibitor of axonal regeneration." Neuron 12(3): 757-767.

Nagayama, S., Takahashi, Y.L., Yoshihara, Y., Mori, K. (2004). "Mitral and tufted cells differ in the decoding manner of odor maps in the rat olfactory bulb." Journal of Neurophysiology 91(6): 2532-2540.

North, T., Gu, T-L., Stacy, T., Wang, Q., Howard, L., Binder, M., Marin-Padilla, M., Speck, N.A. (1999). "*Cbfa2* is required for the formation of intra-aortic heatopoetic clusters." Development 126: 2563-2575.

Okuda, T., van Deursen, J., Hiebert, S.Q., Grosveld, G., Downing, J.R. (1996). "AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoesis." Cell 84(2): 321-330.

Osorio, K. M., Lilja, K., Tumbar, T. (2011). "Runx1 modulates adult hair follicle stem cell emergence and maintenance from distinct embryonic skin compartments " Journal of Cell Biology 193(1): 235-250.

Otto, F., Kanegane, H., Mundlos, S. (2002). "Mutations in the RUNX2 gene in patients with cleidocranial dysplasia." Human Mutations 19(3): 209-216.

Pais, T. F., Figueiredo, C., Peixoto, R., Braz, M.H., Chatterjee, S. (2008). "Necrotic neurons enhance microglial neurotoxicity through induction of glutaminase by a MyD88-dependant pathway." Journal of Neuroinflammation 5(43): 1-12.

Parr, A. M., Kulbatski, I., Sahir, T., Wang, X., Yue, C., Keating, A., Tator, C.H. (2008). "Transplanted adult spinal cord-derived neural stem/progenitor cells promote early functional recovery after rat spinal cord injury." Neuroscience 155(3): 760-770.

Parrish-Aungst, S., Shipley, M.T., Erdelyi, F., Szabo, G., Puche, A.C. (2007). "Quantitative analysis of neuronal diversity in the mouse olfactory bulb." Journal of Computational Neurology 501(6): 825-836.

Pastrana, E., Moreno-Flores, M.T., Avila, J., Wandosell, F., Minichiello, L., Diaz-Nido, J. (2007). "BDNF production by olfactory ensheathing cells contributes to axonal regneration of culture adult CNS neurons." Neurochemistry International 50(3): 491-498.

Pearse, D. D., Sanchez, A.R., Pereira, F.C., Andrade, C.M., Puzis, R., Pressman, Y., Golden, K., Kitay, B.M., Blits, B., Wood, P.M., Bunge, M.B. (2007). "Transplantation of Schwann cells and/or olfactory ensheathing glia into the contused spinal cord: Survival, migration, axon association, and functional recovery." Glia 55: 976-1000.

Pellitteri, R., Spatuzza, M., Stanzani, S., Zaccheo, D. (2010). "Biomarkers expression in rat olfactory ensheathing cells." Frontiers in Bioscience 2: 289-298.

Pesheva, P., Spiess, E., Schachner, M. (1989). "J10160 and J1-180 are oligodendrocyte-secrete nonpermissive subtrates for cell adhesion." Journal of Cell Biology 109(Part 4): 1765-1778.

Polgar, E., Shehab, S.A.S., Watt, C., Todd, A.J. (1999). "GABAergic neurons that contain neuropeptide Y selectively target cells with the neurokinin 1 receptor in laminae III and IV of the rat spinal cord." Journal of Neuroscience 19(7): 2637-2646.

Pollock, G. S., Franceschini, I.A., Graham, G., Marchionni, M.A., Barnett, S.C. (1999). "Neuregulin is a mitogen and survival factor for olfactory bulb ensheathing cells and an isoform produced by astrocytes." European Journal of Neuroscience 11(3): 769-780.

Pratap, J., Lian, J.B., Javed, A., Barnes, G.L., van Wiknen, A.J., Stein, J.L., Stein, G.S. (2006). "Regulatory roles of Runx1 in metastatic tumor and cancer cell interactions with bone." Cancer Metastasis Reviews 25(4): 589-600.

Ramon-Cueto, A., Avila, J. (1998). "Olfactory ensheathing glia: properties and function." Brain Research Bulletin 46(3): 175-187.

Ramon-Cueto, A., Cordero, M.I., Santos-Benito, F.F., Avila, J. (2000). "Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia." Neuron 25: 425-435.

Richter, M. W., Fletcher, P.A., Liu, J., Tetzlaff, W., Roskams, A.J. (2005). "Lamina propria and olfactory bulb ensheathing cells exhibit differential integration and migration and promote differential sprouting in the lesioned spinal cord." Journal of Neuroscience 25(46): 10700-10711.

Rowitch, D. H. (2004). "Glial specification in the vertebrate neural tube." Nature Reviews Neuroscience 5: 409-419.

Runyan, S. A., Phelps, P.E. (2009). "Mouse olfactory ensheathing glia enhance axon outgrowth on a myelin substrate in vitro." Experimenal Neurology 216(1): 95-104.

Ryoo, H. M., Wang, X.P. (2006). "Control of tooth morphogenesis by Runx2." Critical Reviews in Eukaryotic Gene Expression 16(2): 143-154.

Sakai, E., Kitajima, K., Sato, A., Nakano, T. (2009). "Increase of hematopoetic progenitor and supression of endothelial gene expression by Runx1 expression during in vitro ES differentiation." Experimental Hematology 37(3): 334-345.

Schneider, S. P., Cacrides, F. (1978). "Laminar distributions of interneurons in the main olfactory bulb of the adult hamster." Brain Research Bulletin 3(1): 73-82.

Scholzen, T., Gerdes, J. (2000). "TheKi-67 protein: from the known and the unknown." Journal of Cell Physiology 182(3): 311-322.

Schwarting, G. A., Kostek, C., Ahmad, N., Dibble, C., Pays, L., Puschel, A.W. (2000). "Semephorin 3A is required for guidance of olfactory axons in mice." Journal of Neuroscience 20(20): 7691-7697.

Schwarting, G. A., Raitcheva, D., Crandall, J.E., Burkhardt, C., Puschel, A.W. (2004). "Semaphorin 3A-mediated axon guidance regulates convergence and targeting of P2 odorant receptor axons." European Journal of Neuroscience 19: 1800-1810.

Shields, S. D., Moore, K.D., Phelps, P.E., Basbaum, A.I. (2010). "Olfactory ensheathing glia express aquaporin 1." Journal of Comparative Neurology 518(21): 4329-4341.

Shimizu, S., Nagasawa, T., Katoh, O. Komatsu, N., Yokota, J., Morishita, K. (2002). "EVI1 is expressed in megakaryocyte cell lineage and forced expression of EVI1 in UT-7/GM cells induces megakaryocyte differentiation." Biochemical and Biophysical Research Communications 292(3): 609-616.

Simeone, A., Daga, A., Calabi, F. (1995). "Expression of runt in the mouse embryo." Developmental Dynamics 203(1): 61-70.

Smith, S. C., Graveson, A.C., Hall, B.K. (1994). "Evidence for a developmental and evolutionary link between placodal ectoderm and neural crest." Journal of Experimental Zoology 270: 292-301.

Smithson, L. J., Kawaja, M.D. (2009). "A comparative examination of biomarkers for olfactory ensheathing cells in cats and guinea pigs." Brain Research 1284: 41-53.

Sonigra, R. J., Brighton, P.A., Jacoby, J., Hall, S., Wigley, C.B. (1999). "Adult rat olfactory nerve ensheathing cells are effective promoters of adult central nervous system neurite outgrowth in coculture." Glia 25(3): 256-269.

St. John, J. A., Key, B. (2001). "EphB2 and two of its ligands have dynamic protein expression patterns in the developing olfactory system." Brain research - Developmental Brain Research 126(1): 43-56.

Steward, O., Sharp, K., Selvan, G., Hadden, A., Hofstadter, M., Au, E., Roskams, J. (2006). "A re-assessment of the consequences of delayed transplantation of olfactory lamina propria follwing complete spinal cord trasection in rats." Experimenal Neurology 198(2): 483-499.

Stifani, N., Freitas, A.R., Liakhovitskaia, A., Medvinsky, A., Kania, A., Stifani, S. (2008). "Suppression of interneuron programs and maintenance of selected spinal motor neuron fates by the transcription factor AML1/Runx1." Proceedings of the National Academy of Science of the United States of America 105(17): 6451-6456.

Strom, D. K., Westendorf, J.J., Linggi, B., Lutterbach, B., Downing, J.R., Lenny, N., Hiebert, S.W. (2000). "Expresion of the AML-1 oncogene shortens the G(1) phase of the cell cycle." Journal of Biological Chemistry 275(5): 3438-3445.

Suzuki, Y., Takeda, M. (1993). "Basal cells in the mouse olfactory epithelium during development: immunohistochemical and electron-microscopic studes." Developmental Brain Research 73: 107-113.

Suzuki, Y., Takeda, M., Farbman, A.I. (1996). "Supporting cells as phagocytes in the olfactory epithelium after bulbectomy." Journal of Comparative Neurology 376(4): 509-517.

Tanaka, T., Tanaka, K., Ogawa, S., Kurokawa, M., Mitani, K., Nishida, J., Shibata, Y., Yazaki, Y., Hirai, H. (1995). "An acute myeloid leukemia gene, AML1, regulates hematopoetic myeloid cell differentiation and transcriptional activation antagonistically by two alternative spliced forms." The EMBO Journal 14(2): 341-350.

Taniguchi, M., Nagao, H., Takahashi, Y.K., Yamaguchi, M., Mitsui, S., Yagi, T., Mori, K., Shimizu, T. (2003). "Distorted odor maps in the olfactory bulb of semaphorin 3A-deficient mice." Journal of Neuroscience 23(4): 1390-1397.

Taniuchi, I., Littman, D.R. (2004). "Epigenetic gene silencing by Runx proteins." Oncogene 23(24): 4341-4345.

Taniuchi, I., Osato, M., Egawa, T., Sunshine, M.J., Bae, S.C., Komori, T., Ito, Y., Littman, D.R. (2002). "Differential requirements for Runx proteins in CD4 repression and epigenetic silencing during T lymphocyte development." Cell 111(5): 621-633.

Tetzlaff, W., Okon, E.B., Karimi-Abdolrezaee, S., Hill, C.E., Sparling, J.S., Plemel, J.R., Plunet, W.T., Tsai, E.C., Baptiste, D., Smithson, L.J., Kawaka, M.D., Fehlings, M.G., Kwon, B.K. (2011). "A systematic review of cellular transplantation therapies for spinal cord injury." Journal of Neurotrauma 28: 1611-1682.

Theriault, F. M., Nuthall, H.N., Dong, Z., Lo, R., Barnabe-Heider, F., Miller, F.D., Stifani, S. (2005). "Role for Runx1 in the proliferation and neuronal differentiation of selected progenitor cells in the mammalian nervous system." Journal of Neuroscience 25(8): 2050-2061.

Theriault, F. M., Roy, P., Stifani, S. (2004). "AML1/Runx1 is important for the development of hindbrain cholinergic branchiovisceral motorneurons and selected cranial sensory neurons." Proceedings of the National Academy of Science of the United States of America 101(28): 10343-10348.

Toft, A., Scott, D.T., Barnett, S.C., Riddell, J.S. (2007). "Electrophysiological evidence that olfactory cell transplants improve function after spinal cord injury." Brain 130(4): 970-984.

Tsuzuki, S., Seto, M. (2012). "Expansion of functionally defined mouse hematopoetic stem and progenitor cells by a short isoform of RUNX1/AML1." Blood 119(3): 727-735.

Ubink, R., Hokfelt, T. (2000). "Expression of neuropeptide Y in olfactory ensheathing cells during prenatal development." The Journal of Comparative Neurology 423: 13-25.

Ullian, E. M., Christopherson, K.S., Barres, B.A. (2004). "Role for glia in synaptogenesis." Glia 47(3): 209-216.

Verhaagen, J., Oestreicher, A.B., Gipsen, W.H., Margolis, F.L. (1989). "The expression of the growth associated protein B50/GAP43 in the olfactory system of neonatal and adult rats." Journal of Neuroscience 9(2): 683-691.

Vickland, H., Westrum, L.E., Kott, J.N., Patterson, S.L., Bothwell, M.A. (1991). "Nerve growth factor receptor expression in the young and adult rat olfactory system." Brain Research 565(2): 269-279.

Vincent, A. J. m. W., A.K., Chuah, M.I. (2005). "Morphological and functional plasticity of olfactory ensheathing cells." Journal of Neurocytology 34: 65-80.

Vise, W. M., Yashon, D., Hunt, W.E. (1975). "Astrocytic processes: A route between vessels and neurons following blood-brain barrier injury." Journal of Neuropathology and Experimental Neurology 34(4): 324-334.

Vivancos, V., Chen, P., Spassky, N., Qian, D., Dabdoub, A., Kelley, M., Studer, M., Guthrie, S. (2009). "Wnt activity guides facial branchiomotor neuron migration, and involves the PCP pathway and JNK and ROCK kinases." Neural Development 4(7).

Vroemen, M., Caloni, M., Bogdahn, U., Weidner, N. (2007). "Failure of Schwann cells as supporting cells for adult neural progenitor cell grafts in the acutely injured spinal cord." Cell and Tissue Research 327(1): 1-13.

Wang, Y.-Z., Molotkov, A., Song, L., Lo, Y., Pleasure, D.E., Zhou, C-J. (2008). "Activation of the Wnt/Beta-catenin signaling reporter in developing mouse olfactory nerve layer marks a specialized subgroup of olfactory ensheathing cells." Developmental Dynamics 237: 3157-3168.

Wang, Z., Feurerstein, G.Z. (2000). "Role of immune and inflammatory mediators in CNS injury." Drug News Perspectives 13(3): 133-140.

Wilby, M. J., Muir, E.M., Fok-Seang, J., Gour, B.J., Blaschuk, O.W., Fawcett, J.W. (1999). "N-Cadherin inhibits Schwann cells migration on astrocytes." Molecular and Cellular Neuroscience 14(1): 66-84.

Windus, L. C. E., Lineburg, K.E., Scott, S.E., Claxton, C., Mackay-Sim, A., Key, B., St John, J.A. (2010). "Lamellipodia mediate the heterogeneity of central olfactory ensheathing cell interactions." Cellular and Molecular Life Sciences 67: 1735-1750.

Xu, X. M., Guenard, V., Kleitman, N., Bunge, M.B. (1995). "Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord." Journal of Comparative Neurology 51(1): 145-160.

Yamagata, T., Maki, K., Mitani, K. (2005). "Runx1/AML1 in normal and abnormal hematopoesis." International Journal of Hematology 82(1): 1-8.

Yamashiro, T., Aberg, T., Levanon, D., Groner, Y., Thesleff, I. (2002). "Expression of Runx1, -2 and -3 during tooth, palate and craniofacial bone development." Gene Expression Patterns 21(1-2): 109-112.

Yokomizo, T., Hasegawa, K., Ishitobi, H., Osato, M., Ema, M., Ito, Y., Yamamoto, M., Takahashi, S. (2008). "Runx1 is involved in primitive hematopoesis in the mouse." Bloog 111: 4075-4080.

Yoshikawa, M., Senzaki, K., Tomomasa, Y., Takahashi, S., Ozaki, S., Shiga, T. (2007). "Runx1 selectively regulates cell fate specification and axonal projections of dorsal root ganglion neurons." Developmental Biology 202(2): 663-674.

Zagami, C. J., Zusso, M., Stifani, S. (2009). "Runx transcription factors: lineage-specific regulators of neuronal precursor cell proliferation and post-mitotic neuron subtype development." Journal of Cellular Biochemistry 107(6): 1063-1072.

Zambidis, E. T., Peault, B., Park, T.S., Bunz, F., Civin, C.I. (2005). "Hematopoetic differentiation of human embryonic stem cells progresses through sequential hematoendothelial, primitive and definitive stages resembling human yolk sac development." Blood 106(3): 860-870.

Zhang, L., Fried, F.B., Guo, H., Friedman, A.D. (2008). "Cyclin-dependent kinase phosphorylation of RUNX1/AML1 on 3 sites increases transactivation potency and stimulates cell proliferation." blood 111(3): 1193-1200.

Zhu, Y., Cao, L., Su, Z., Yuan, Y., Gao, L., Qiu, Y., He, C. (2010). "Olfactory ensheathing cells: attractant of neural progenitor migration to olfactory bulb." Glia 58(6): 716-729.

Zusso, M., Methot, L., Lo, R., Grehalgh, A.D., David, S., Stifani, S. (2012). "Runx1 regulates postnal forebrain amoeboid microglial proliferation and ramification." Journal of Neuroscience.; in press