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# The role of Chd7 & Chd8 chromatin remodelers in oligodendrogenesis and (re)myelination

Corentine Marie

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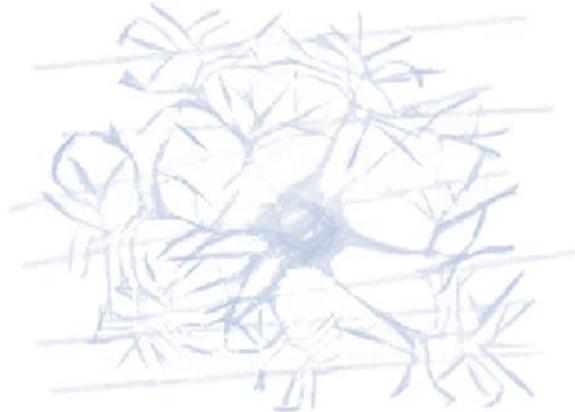
École doctorale CERVEAU, COGNITION et COMPORTEMENT

Laboratoire Développement oligodendrocytaire et interactions neurovasculaires

Institut du Cerveau et de la Moelle Epinière, Hôpital de la Pitié Salpêtrière

UPMC UMR\_S 975 - Inserm U 1127 - CNRS UMR 7225 - ICM

# **THE ROLE OF CHD7 & CHD8 CHROMATIN REMODELERS** **IN OLIGODENDROGENESIS AND (RE)MYELINATION**



**Par Corentine Marie**

Thèse soutenue le 29 septembre 2017 pour obtenir le titre de Docteur en neurosciences de  
l'Université Pierre et Marie Curie

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*A mes regrettés grands-parents,  
Claude, Denise et Paul*



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## Acronyms list

Ascl1	Achaete-scute complex-like 1).
bHLH	Basic Helix-Loop-Helix
CC	Corpus callosum
Chd	Chromodomain helicase DNA binding protein
ChIP	Chromatin immuno-precipitation
ChIP-seq	ChIP followed by New Generation Sequencing
Cnp	2',3'-Cyclic nucleotide phosphodiesterase
CNS	Central nervous system
CR	Chromatin remodeler
E	Embryonic day
GOF	Gain-of-function
Gpr17	G-protein-coupled receptor 17
iOL	Immature oligodendrocytes
LOF	Loss-of-function
LPC	Lysolecithin or LysoPhosphatidylCholine
Mag	Myelin associated glycoprotein
Mbp	Myelin basic protein
MN	Motor neurons
Mog	Myelin oligodendrocyte glycoprotein
mOL	Mature oligodendrocytes
MS	Multiple Sclerosis
Myrf	Myelin regulatory factor
NSCs	Neural Stem Cells
OL	Oligodendrocytes
Omg	Oligodendrocyte Myelin Glycoprotein
OPC	Oligodendrocytes Precursor Cells
P	Postnatal age
PDGFR $\alpha$	Platelet derived growth factor receptor $\alpha$
Plp	Proteolipid protein
PNS	Peripheral Nervous System
RT	Room Temperature
SVZ	Subventricular zone
TFs	Transcription Factors

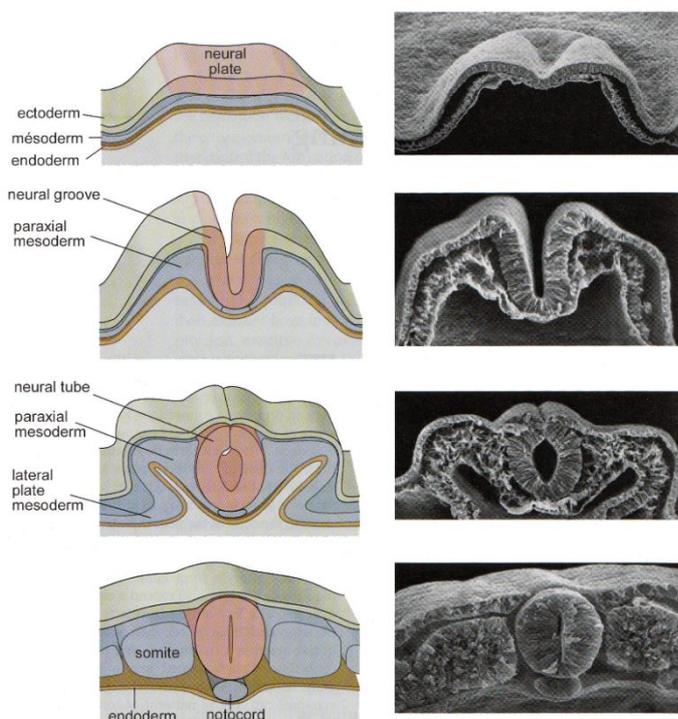


# **Introduction**



## Part I: Neural subtype determination

Since Schwann (1810-1882) enunciated "All living things are composed of cells and cell products", biologists have worked to catalog cell-types and their functions. With the invention of microscopy in 1595 and all the new technics now available, it became easier to observe tissues and their components. The discovery of different cell-types within an organism leads to question their generation. How does one cell with one genome give rise to a plethora of different cell-types expressing different markers and factors? That question has also been asked for the nervous system. What are the different cell-types composing the nervous system? How are they generated?

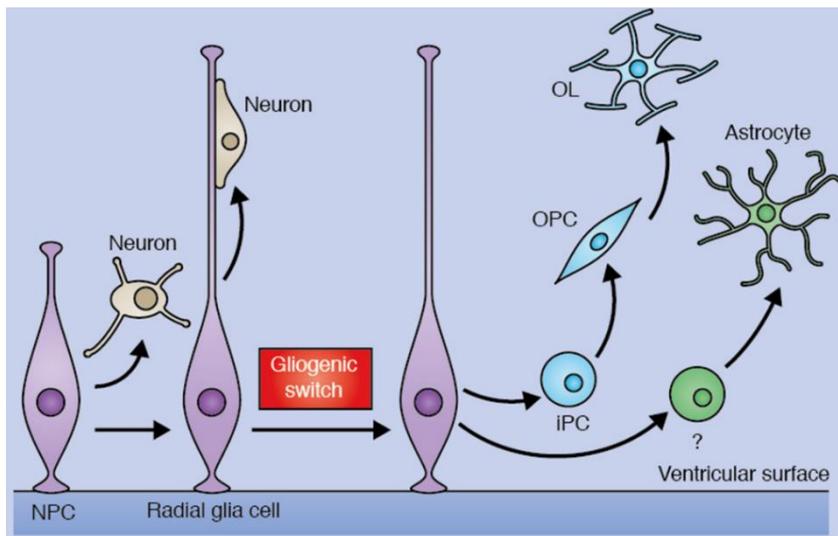


**Figure 1. Neural tube development.** During neurulation, neural folds close at the dorsal midline. The initial medio-lateral orientation of the neural plate becomes the dorso-ventral organization of the neural tube.

plate along antero- posterior axis and its bending allow the formation of neural folds which elevate (neural groove) and close at the midline to form the neural tube. NSCs form a single layer of cells that line the center of neural tube.

NSCs are highly proliferative and pluripotent. In the central nervous system (CNS), they transform into radial glia cells and first generate neurons (Fig. 2). Then, they go under a "gliogenic switch" and start to generate glial cells: astrocytes and oligodendrocytes. This step of choosing its "cell fate" is called specification and is highly controlled.

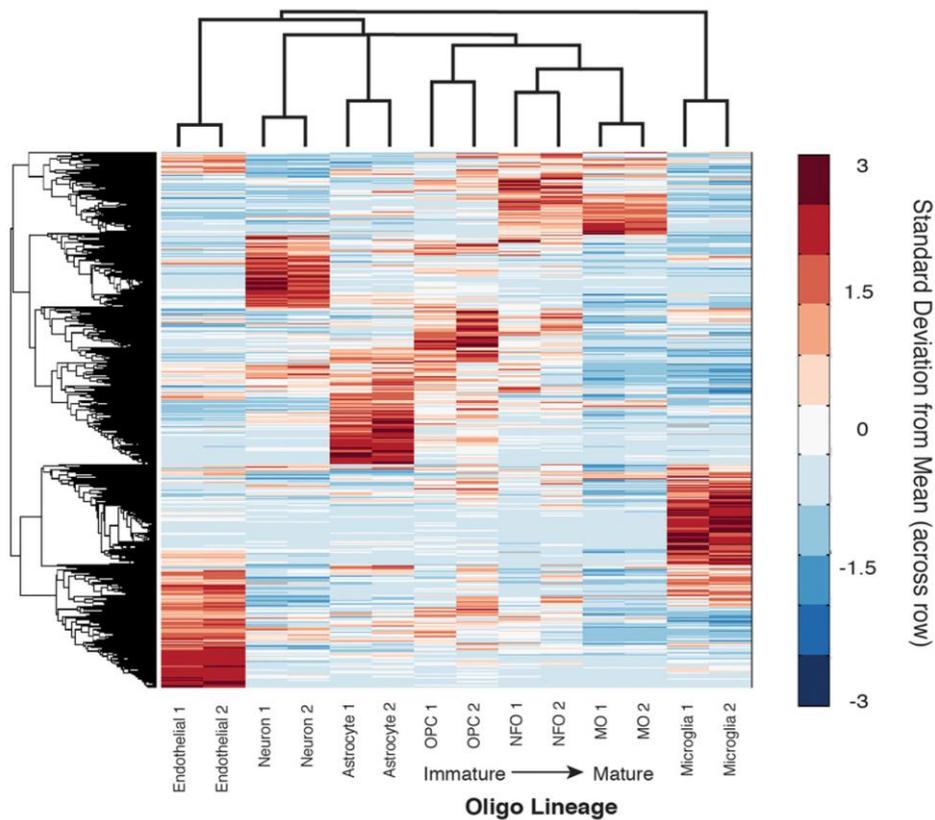
Embryonic development has been extensively studied and even if a lot of mechanisms are yet to be discovered, the different development stages have been described. We know that at the stage of gastrulation, three layers can be identified (Fig. 1). One of them, the ectoderm, is composed of cells that specified into neuroectodermal cells which represent the origin of the entire nervous system. Then, pseudostratification of the neuroectoderm give rise to the neural plate which contain neural stem cells (NSCs). The lengthening of neural



**Figure 2. Specification of neurons, OLs and astrocytes from neural precursor cells (NPC).** During early development, NPCs from neural tube and forebrain give rise to radial glial cells generating first neurons and then oligodendrocytes and astrocytes following a gliogenic switch. Figure from Zuchero and Barres, 2015.

Each of these cell-types has its own function in the CNS. Astrocytes promote neuronal survival, metabolic support, neurovascular coupling. They also play an important role in synapse formation and transmission (review: Zuchero and Barres, 2015). Oligodendrocytes are responsible of myelin sheaths formation which permit saltatory conduction along the axon. They also play a role in plasticity and axonal support. Microglia are another important glial cells of the CNS. They have a different origin than astrocytes and oligodendrocytes as they are generated at embryonic day (E)7.5 from the yolk sac. Their functions are primarily immune surveillance, apoptotic clearance, neurogenesis and synaptic pruning (review: Casano and Peri, 2015). However, we know that all the cells within one organism share the same genome. To create different cell-types, it is essential for cells to regulate their gene expression. Each cell-type can be distinguished from another one by their markers and functions, which translate the expression of specific set of genes. Indeed, when we compare gene expression between the different cell-types within the CNS, we can find differential gene expression (Fig. 3; Zhang et al., 2014).

## A Clustergram of RNA-Seq



**Figure 3. Genes showing cell-type specific expression in the CNS.** Clustering heatmap representing the differential gene expression between CNS cell-types: neurons, astrocytes, oligodendrocytes precursor cells (OPC), newly formed oligodendrocytes (NFO), mature oligodendrocytes (MO), microglia and endothelial cells. Figure from Zhang et al., 2014.

The regulation of these specific genes is key during differentiation. Genes coding proteins promoting differentiation have to be activated, whereas genes coding proteins inhibiting differentiation have to be repressed. How these gene regulations are controlled? And more generally, what control cell fate? These questions are essential and are still very much active aspects of research in biology. As CNS and, more generally, multicellular organism are very complex, we focus our study on one cell-type, oligodendrocytes, and try to understand what determine at the molecular level their generation.

## **Part II: Oligodendrocyte Biology and Oligodendrogenesis Regulation**

### **II.A – Oligodendrocytes are myelin-forming cells of the Central Nervous System**

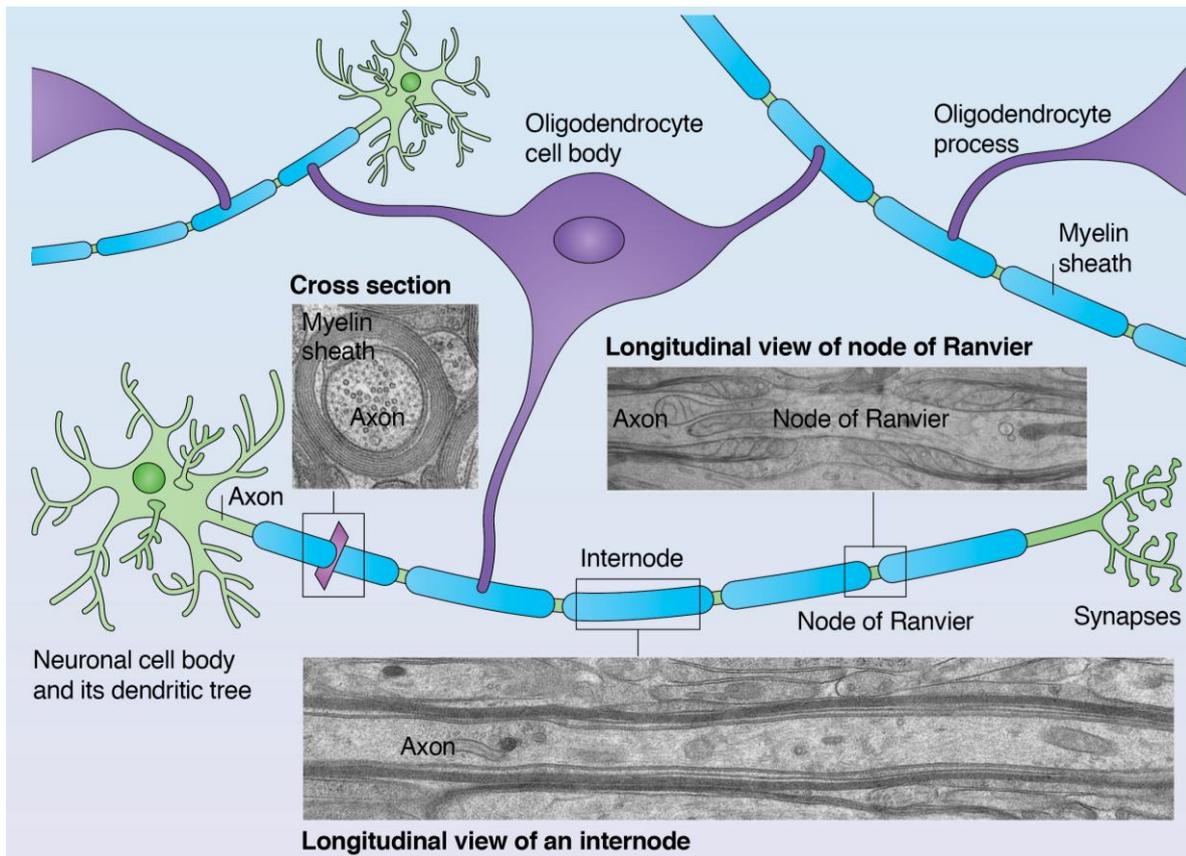
Oligodendrocytes (OLs) are cells that form myelin sheaths around the axons. The term “myelin” was first introduced by Virchow in 1854 and comes from *myelos* which means marrow in Greek (review: Boullerne, 2016).

#### **II.A.1 – Oligodendrocytes are cells of the Central Nervous System**

OLs are present in the CNS, mostly in the white matter where axon tracts are more present. They are distinguished from Schwann cells, the myelin-forming cell of the peripheral nervous system (PNS), which originate from neural crest cells, express P0 (myelin protein zero) and can ensheath only 1 axon. In comparison, one OL can ensheath up to 40 axons (review: Zuchero and Barres, 2015).

#### **II.A.2 – Oligodendrocytes are forming myelin sheath**

In 1956, it was for the first time demonstrated that myelin was not formed by neurons but by myelin-forming glial cells (Uzman, 1956). OLs form myelin sheaths by extending numerous processes that will wrap in a tightly compacted spiral of plasma membrane around axons, structuring it (Fig. 4): portions of myelin sheath are called internodes which are regularly spaced along the axon and come from different OLs. Each myelin sheath is separated by a myelin-free portion called node of Ranvier. Myelin is essentially made of plasma membrane but has a unique molecular composition and architecture. The amount of lipids is higher than in other cell membrane (73 to 81% of dry weight; Folch and Lees, 1951). Some proteins are also highly specific of myelin as Proteolipids represent 20-25% of wet tissue (Folch and Lees, 1951). 342 myelin-associated proteins has been identified by proteomic analysis (Jahn et al., 2009) such as Plp (proteolipid protein) and Mbp (myelin basic protein) which represent, respectively, 17% and 8% of myelin proteins (Jahn et al., 2009), but also: Cnp (2',3'-cyclic nucleotide phosphodiesterase), Mog (Myelin Oligodendrocyte Glycoprotein), Mag (Myelin Associated Glycoprotein), Omg (Oligodendrocyte Myelin Glycoprotein).



**Figure 4. Oligodendrocytes and myelin sheaths in the CNS.** Oligodendrocytes (purple) forms myelin sheaths (blue) by wrapping their processes around axons, generating one internode each time. Compact myelin can be visualized by electron microscopy. Two internodes are separated by a Node of Ranvier. Figure from Snaidero and Simons, 2014.

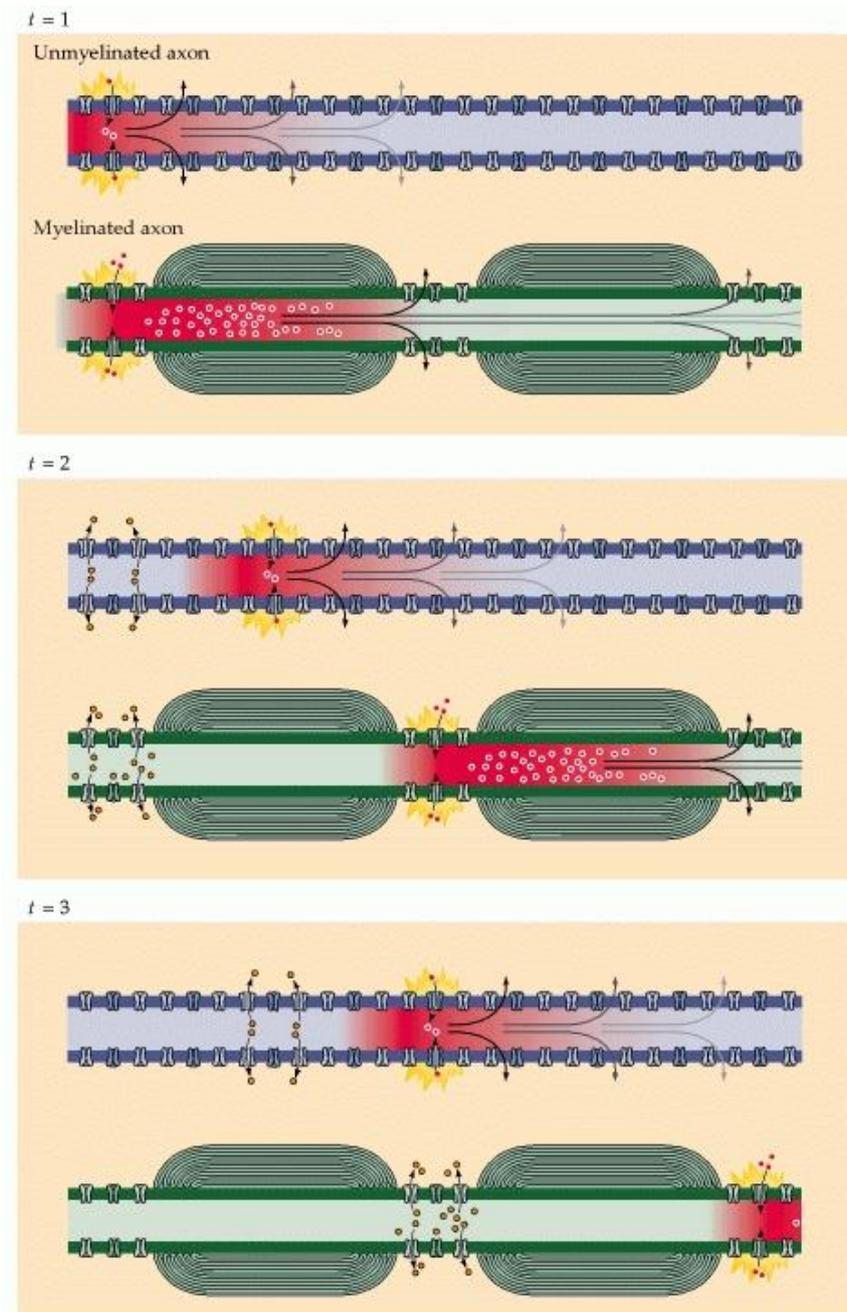
### II.A.3 – Myelin has different essential functions

OLs and myelin sheaths have different functions. First, myelin allows rapid transmission of the signal through the axon. Then, myelin plasticity allow adjustment of these conduction velocity. Finally, myelin has an important role of support for the axon.

#### II.A.2.a - Rapid transmission

The first studied function of myelin is its capacity to permit rapid and efficient signal transmission through the axon. Different strategies has been used by different species to increase conduction speed (Review: Hartline and Colman, 2007). The first one was to increase the internal diameter leading to giant axons, like in giant squids in which axon diameter can measure up to 500 $\mu\text{m}$  permitting a conduction speed of 25m/s. The other strategy is to use myelin sheaths to increase velocity of signal transmission, allowing species to have axon with diameter of only few  $\mu\text{m}$ . This strategy, used in vertebrates, had allow them to increase their body size (Zalc et al., 2008). Indeed, myelinated axons have a conduction velocity increased up to 100 fold compared to unmyelinated axons (Franz and Iggo, 1968) as myelin works as an

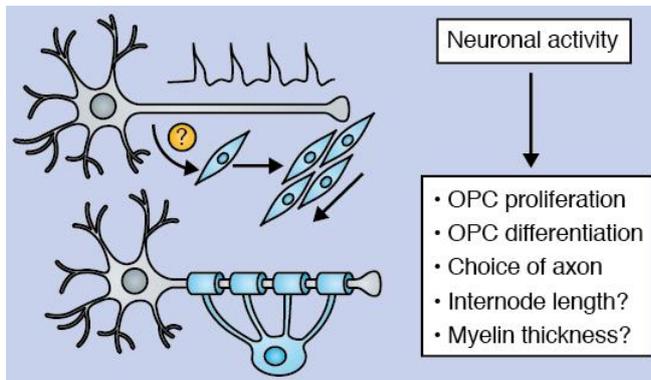
insulator, being a non-conductive sheath, and the low exposure of axon membrane through the nodes of Ranvier decrease the area of membrane into which the current must flow. The current travel through the axon by “jumping” from one node of Ranvier to another one, where ion channels are clusters, causing saltatory conduction (Fig. 5). Moreover, the thickness of myelin influences the conduction velocity, thus the importance of the g-ratio which is the ratio of the inner axonal diameter on the total outer diameter. The optimal g-ratio is 0.6 and any deviation (lower or higher) leads to a conduction velocity drop.



**Figure 5. Saltatory conduction through the axon.** Comparison of the conduction velocity between an unmyelinated (blue) and a myelinated (green) axons. Myelin isolated the axon and reduce the axon membrane surface having to propagate the action potential.

### II.A.2.b – Plasticity

The capacity of myelin to affect conduction velocity through the axon is combined by the fact that myelin can modulate this activity, depending on the signals and environment. This process is called myelin plasticity (Fig. 6). Indeed, it was observed that, in mice, two months isolation after weaning provoke alteration of myelination (Makinodan et al., 2012), making



**Figure 6. Myelin plasticity.** Neuron activity can promote OPC proliferation and differentiation, playing an important role in myelin plasticity. Figure from (Zuchero and Barres, 2015).

myelin thinner and modify g-ratio. Myelin genes are also less expressed like *Mbp* (Makinodan et al., 2012). More importantly, in adult mice, short-term isolation induce reduction of myelin thickness (Liu et al., 2012), showing that environment can have consequences on myelin, even in adults. Furthermore, learning new skills rely, on part, on

myelin plasticity. It was shown in human that piano training can induce white matter plasticity during maturation and a correlation exists between practice and fiber tract organization (Bengtsson et al., 2005). Also, in adult, six weeks of juggle training show structural changes in white matter compared to control group (Scholz et al., 2009). It was therefore hypothesized that neuronal activity during maturation promote myelin plasticity, and thus learning (Bengtsson et al., 2005). Indeed, some evidences agree with this hypothesis as blocking of action potential in neurons inhibits myelination, whereas increase of electrical activity promote myelination (Demerens et al., 1996). Moreover, in mice, stimulation of neurons by optogenetic promotes oligodendrogenesis and myelination as adult mice showed an increase of the number of  $CC1^+$  cells (marker of OLs) as well as *Mbp* expression, four weeks after end of stimulation (Gibson et al., 2014). An *in vitro* study showed that glutamate released along axons of active neurons promote myelin formation by increasing local MBP synthesis (Wake et al., 2011).

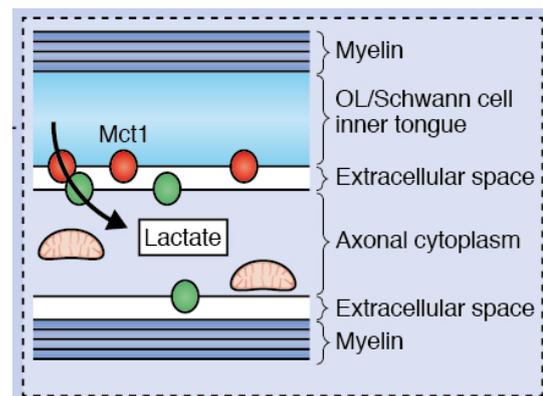
As axonal activity promote myelination, its plasticity also have consequences on neurons as increased myelination is associated with improved motor functions (Gibson et al., 2014). Furthermore, blocking the generation of new OLs by deletion of *Myrf*, a transcription factor required for myelination, keep mice from mastering new skills like complex wheel (McKenzie et al., 2014), suggesting that generation of new OLs is important for skill learning

and to form a feedback with axon. This concept is called adaptative myelination and cover the fact that neuronal activity can modify myelin, through myelin plasticity and, in turn, myelin changes can modulate neuronal activity and behavior.

### ***II.A.2.c - Axonal support***

The drawback of being surround by myelin is that axons will have difficulties to communicate with the extracellular space. It can have significant consequences regarding the access to nutrients. As neurons express  $\text{Na}^+/\text{K}^+ - \text{ATPase}$  all along the axon, not only in nodes of Ranvier, to conduct signals (McGrail et al., 1991; Young et al., 2008), they need an important energy input. Also, axons present mitochondria all along the axon (Morris and Hollenbeck, 1993) which implies that mitochondria must have access to the necessary metabolites directly in the axon to produce ATP at the site, where it is needed. Importantly, myelin play a direct role as metabolic support of the axon. OLs have a highly active glucose metabolism (Amaral et al., 2016) and perturbation of mitochondrial respiration in OLs did not affect them (Funfschilling et al., 2012). In this experiment, neurons were not affected either, showing that OLs does not transfer ATP molecules to neurons. However, an increase of lactate concentration was observed only when animals where anesthetized (Funfschilling et al., 2012). Furthermore, MCT1 which is a lactate transporter (Fig. 7) is highly expressed in OLs and cause axon damage and neuronal loss when downregulated *in vivo* and *in vitro* (Lee et al., 2012b). Astrocytes, as glycogen

storage, are known to play a role in metabolic support of neurons (review: (Belanger et al., 2011), but whether their role is direct or not is not clear, as they have very limited possible contact points in nodes of Ranvier. It is however possible that astrocytes provide lactate to neurons via OLs as it is known that astrocytes and OLs can bind through Gap junctions made with Cx47/Cx43 and Cx32/Cx30 channels that can transport glucose and their metabolites (Rouach et al., 2008). Lack of OL connexins Cx47 or Cx32 in mice leads to OL loss and axonal death (Menichella et al., 2003).



**Figure 7. Myelin play an important role in axonal support.** Lactate can be directly transported from myelin to axon via Mct1. Figure from Zuchero and Barres, 2015.

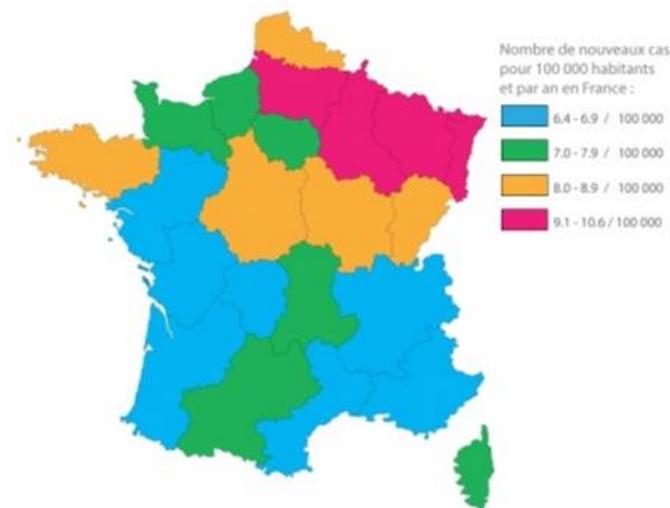
storage, are known to play a role in metabolic support of neurons (review: (Belanger et al., 2011), but whether their role is direct or not is not clear, as they have very limited possible contact points in nodes of Ranvier. It is however possible that astrocytes provide lactate to neurons via OLs as it is known that astrocytes and OLs can bind through Gap junctions made with Cx47/Cx43 and Cx32/Cx30 channels that can transport glucose and their metabolites (Rouach et al., 2008). Lack of OL connexins Cx47 or Cx32 in mice leads to OL loss and axonal death (Menichella et al., 2003).

Axonal support by OLs is not only metabolic, OLs also play a role in maintaining the axon integrity. OL can secrete endosome-derived vesicles that are endocytosed by neurons, promoting neuron survival under oxidative stress and starvation (Frühbeis et al., 2013).

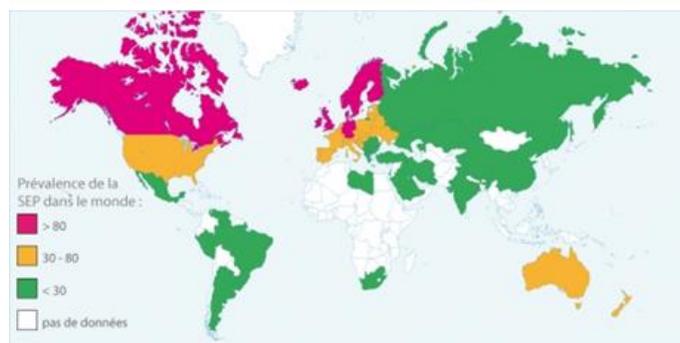
All these functions are very important for the axon to function correctly and survive and are highlighted when myelin is lost in demyelinating diseases such as Multiple Sclerosis.

## **II.B – Multiple Sclerosis is characterized by a loss of myelin sheaths**

Multiple Sclerosis (MS) is an acquired disabling neurological disease and is characterized by a loss of myelin. 2.3 million People worldwide have been diagnosed with MS and 100.000 in France with 4.000 to 6.000 new cases each year (from ARSEP, [www.arsep.org](http://www.arsep.org)). The symptoms are multiple and different depending on the CNS region touched: sensitive deficit, motor deficit, visual deficit, language deficit, fatigue, anxiety... It is heterogeneous as 3/4 of patients are women and dependent of the geographical region as people living in North regions are more prone to develop MS (Fig. 8). MS is an inflammatory disease of CNS and the causes are



multifactorial (Oksenberg and Baranzini, 2010). There is a genetic susceptibility (International Multiple Sclerosis Genetics et al., 2013), but epigenetics and environmental factors are to take in account (reviews: Handel et al., 2010; Oksenberg and Baranzini, 2010).



**Figure 8. Heterogeneous repartition of Multiple Sclerosis in France and worldwide.** Top; incidence of MS in France and worldwide. Bottom; prevalence of MS worldwide. Figure from ARSEP ([www.arsep.org](http://www.arsep.org))

### **II.B.1 – Demyelination/Remyelination mechanism**

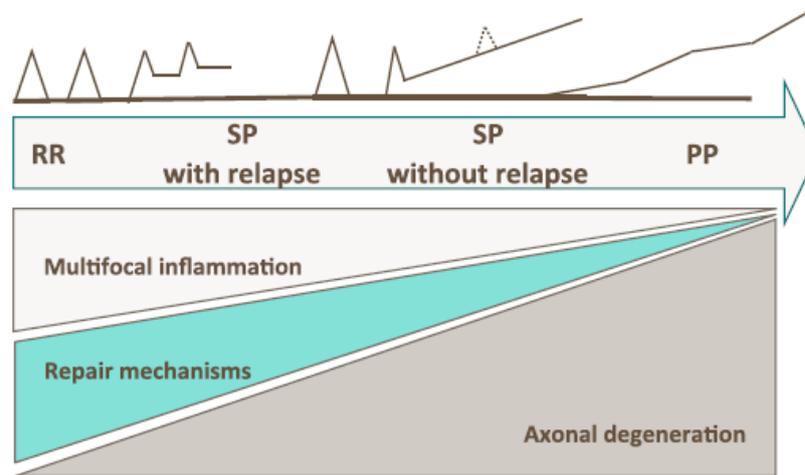
Demyelination which characterize MS is a pathological degradation of myelin. The first element of this disease is inflammation due to immune dysregulation (review: Grigoriadis et al., 2015). Dendritic cells, antigen-presenting cells present in the blood, become activated, cross the blood-brain barrier and differentiate (Ifergan et al., 2008). They activate T-cells which secrete cytokines and activate macrophages (Murphy et al., 2010). It has been shown that MS lesions contains both resident-microglia and blood-derived monocytes that will initiate the immune response against myelin antigen (Trebst et al., 2001). They secrete pro-inflammatory

factors and reactive oxygen species that will provoke demyelination and axonal loss (review: Raivich and Banati, 2004). However, microglia are also important for protection as they clear out the lesion of death cells and debris and can produce growth factors to protect the axons (Yamasaki et al., 2014). Recently, it was shown that B-cells also play a role in autoimmune response and participate in the development of the CNS lesions (review: Wekerle, 2017).

Myelin sheaths loss means loss of their functions. The axons are not insulated anymore, resulting in loss of saltatory conduction and even conduction block. Normally, a regeneration process called remyelination takes place in order to reform new OLs and new myelin sheaths. Despite that myelin sheaths generated after remyelination are thinner and shorter than the developmental ones (Blakemore, 1974; Ludwin and Maitland, 1984), remyelination allows recovery of saltatory conduction and other myelin functions (Smith et al., 1979). The efficiency of this process is particularly important to prevent axonal loss (review: Trapp and Nave, 2008)

### **II.B.2 – Evolution of the disease**

This mechanism of demyelination/remyelination is characteristic of the relapsing-remitting (RRMS; Fig. 9) phase of the disease where attacks or relapses are correlated with demyelination and recovery or remission are correlated with remyelination. For the patients, relapses are associated with apparition of disabilities depending of the region of the lesion and remissions are associated with disappearance (complete or partial) of the symptoms (from NMSS, [www.nationalmssociety.org](http://www.nationalmssociety.org)). With age, this remyelination is less and less efficient (Shields et al., 1999) leading to secondary progressive MS (SPMS) in which there is no remission. It is different from Primary progressive MS (PPMS) in which the absence of remission is visible from the onset of the disease.



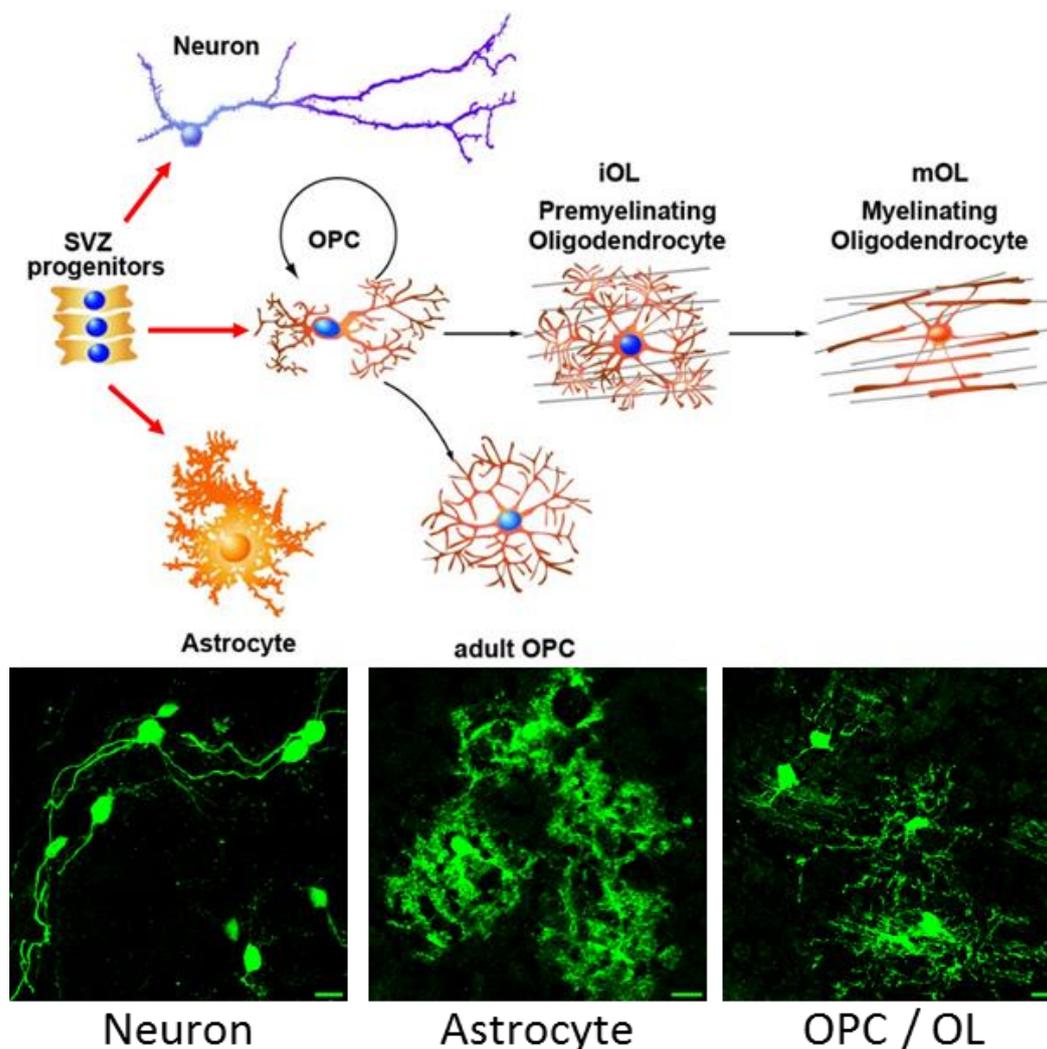
**Figure 9. Evolution of MS.** Type of MS (RR, relapsing-remitting; SP, secondary progressive; PP, primary progressive) correlated to inflammation, repair and axonal degeneration. Figure from (Grigoriadis et al., 2015).

Immunosuppressant, immune-modulator and anti-inflammatory drugs are the current therapies used against MS (Compston and Coles, 2002). However, they are not sufficient to avoid neuronal loss, due to inefficient remyelination. Promoting efficient remyelination is therefore a challenge to produce functional recovery in MS patients. Using a culture assay for myelination potential, Clemastine has been identified as a good candidate for pro-remyelination drug as it promote OL differentiation and myelination (Mei et al., 2014) and its therapeutic potential is being tested in clinical trials.

To understand how remyelination occurs, why it fails and how to promote it, we have to better understand how oligodendrocytes are (re)generated physiologically and in disease.

## II.C – Oligodendrogenesis and its transcriptional regulation

Oligodendrogenesis is a complex process in which we can distinguish three different steps: 1) Specification which correspond to the oligodendroglial lineage commitment of NSCs. 2) OPC differentiation which will give rise to immature OLs (iOLs). 3) Maturation which is the step of myelin formation and axonal wrapping by mature OLs (mOLs). These three steps will be detailed below as they are highly regulated at different levels by transcription factors, signaling pathways, non-coding RNAs, post-translational modification... Our attention will be ported to the profound global changes in gene expression occurring during this process and how transcription factors and chromatin modifiers affect and regulate each of these steps in a time and space controlled manner.



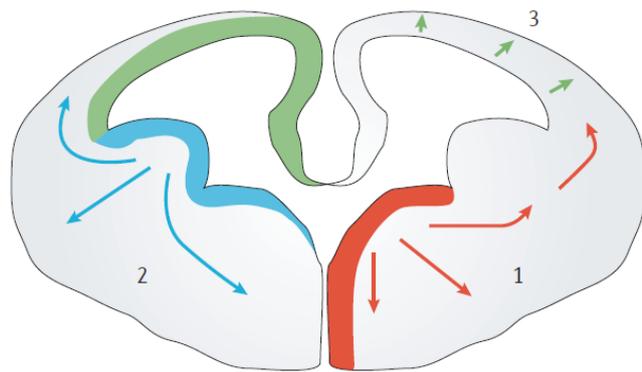
**Figure 10. Specification of SVZ progenitors.** Top, diagram showing how a pool of progenitors can give rise to neurons, OPCs or astrocytes. Bottom, morphology of neurons, astrocytes and OPC/OL expressing GFP after postnatal electroporation. Scale bar represent 5µm.

## II.C.1 – Specification

The step where a pool of NSCs select their fate to become either neurons or glial cells, OLs or astrocytes, is called specification (Fig. 10). However, OLs are not directly generated like neurons or astrocytes. An in-between step is necessary: the generation of oligodendrocyte precursor cells (OPCs).

### II.C.1.a – Sources of OPCs

OPCs start to appear in development at embryonic stage. We can distinguish three waves of OPC generation in the forebrain during development (Fig. 11). In the first wave at embryonic day (E)12.5, OPCs arise from the medial ganglionic eminence (MGE) in the ventral telencephalon (Tekki-Kessarlis et al., 2001). In the second wave at E15.5, OPCs are produced in the lateral and caudal ganglionic eminence (LGE/CGE). Finally, after birth, OPCs are generated from the subventricular zone (SVZ) to migrate in the dorsal cortex (Kessarlis et al., 2006).



**Figure 11. OPC generation in the developmental forebrain in 3 waves:** the first wave (1) at E12.5 in the medial ganglionic eminence (MGE); the second wave (2) at E15.5 in the lateral and caudal ganglionic eminence (LGE/CGE); the third wave (3) after birth in the subventricular zone (SVZ). From Richardson et al., 2006.

### II.C.1.b – What is an OPC?

OPCs are oligodendrocyte precursor cells expressing particular markers such as PDGFR $\alpha$  (Platelet derived growth factor receptor  $\alpha$ ), NG2 (chondroitin sulphate proteoglycan; *CSPG4* gene; Ozerdem et al., 2001) and O4. They also express key TFs involved in their generation such as Sox10, Sox9 & Sox8, Olig2 & Olig1 and Ascl1. These ramified cells represent a significant population of the brain as they don't differentiate directly into OLs (Dawson et al., 2003), being present all over the adult brain (Ffrench-Constant and Raff, 1986) where they represent 5% of the cells. Their uniform distribution is highly controlled and they can then detect perturbation within the whole CNS (Birey and Aguirre, 2015; Hughes et al., 2013).

One of the particularity of OPCs is their capacity to proliferate as they are the most actively cycling cell population after NSCs. All OPCs can still proliferate in adult but the timing depend on the brain region and on the age of mice: the cell-cycle time is 3 days in white matter at postnatal age (P)21, but is 36 days in grey matter at P60 (Young et al., 2013). PDGF (platelet-

derived growth factor) drives OPC proliferation through its receptor, PDGFR $\alpha$ , expressed by OPCs (Pringle et al., 1992). Overexpression of PDGF leads to hyperproliferation (Calver et al., 1998) and *PDGF-A-null* mice present less OPCs than control leading to hypomyelination with associated phenotypes like tremors (Fruttiger et al., 1999). Another growth factor, FGF (fibroblast growth factor) maintains PDGFR $\alpha$  expression in OPCs and can block differentiation (McKinnon et al., 1990). Gpr56 is a member of the adhesion G protein-coupled receptor family and is expressed in early stages of OL development (Ackerman et al., 2015). Ablation of Gpr56 in mouse or zebrafish decrease OPC proliferation leading to a decrease in OL number and hypomyelination (Ackerman et al., 2015; Giera et al., 2015). Transcription factors are also involved in the regulation of OPC proliferation. In spinal cord injured model, Olig2 overexpression was shown to foster OPC proliferation to the extent of leading to tumor formation (Kim et al., 2011). Indeed, Olig2 is expressed in many brain tumors (Bouvier et al., 2003; Lu et al., 2001; Marie et al., 2001; Ohnishi et al., 2003) and Olig2 is required for proliferation of neural progenitors and glioma formation in mouse models (Ligon et al., 2007; Lu et al., 2016).

Another important characteristic of OPCs is their time-control differentiation that mainly happen during postnatal life and permit the generation of mature OLs and myelin sheaths.

### **II.C.1.c – OPC functions**

As OPCs represent a cell population in the adult brain, it is unlikely that their function stop at OL generation; the question of their own functions arise.

#### ***Differentiation in physiological cell renewal and plasticity***

OPCs can still generate OLs in adult to permit physiological cell renewal and plasticity. Using *Cre* inducible transgenic mouse, it was shown that adult OPCs could generated new OLs (Rivers et al., 2008). New OLs are generated in adult CNS either in region where some axons are still myelin-free (corpus callosum; CC) enhancing plasticity or in region where axon are almost all myelinated (optic nerve) enhancing renewal or subtle remodeling (Young et al., 2013). Stimulation of neurons with optogenetic promotes OPC proliferation and differentiation in adult mouse (Gibson et al., 2014). Myelin regulatory factor (MyRF) is a factor necessary for myelination but is also required for new myelination in adult. Deletion of *Myrf*

in OPCs prevents new myelination and prevents from learning new skills (McKenzie et al., 2014), showing the importance of the OPC presence in adult brain for myelin plasticity and its participation in skill learning.

### ***Neuronal activity***

A cross-talk exists between neurons and OPCs. As seen above, neuronal activity plays a role in myelin plasticity as it promotes proliferation and differentiation of OPCs (Gibson et al., 2014). But OPCs can also influence neuronal activity. NG2 immunoreactivity was found within the synaptic cleft (Ong and Levine, 1999) and electron microscopy showed the existence of synaptic junction between axon and OPC processes (Bergles et al., 2000), highlighting the capacity of OPCs to make contact at synapses. NG2<sup>+</sup> cells can also contact neurons at nodes of Ranvier where they could detect changes in axonal function prior to demyelination or maintain nodes function (Butt et al., 1999). As it was shown that OLs could transfer their exosome to neurons through glutamate binding on NMDA and AMPA receptors (Frühbeis et al., 2013), we can wonder if OPCs could communicate with neurons with the same mechanism, especially when OPCs express NMDAR and AMPAR (Karadottir et al., 2005). Interestingly, more recent work showed a direct effect of NG2<sup>+</sup> cells on neuronal activity. OPCs were ablated in the prefrontal cortex of mice, leading to a deficit in glutamatergic signaling in pyramidal neurons and behavioral defects characterized as depressive-like (Birey et al., 2015). Furthermore, it was shown that chronic social stress in mice leads to a reduction of NG2 cells and MDD (Major Depressive Disorder) patients appear with a reduction of NG2 glia density (Birey et al., 2015).

### **II.C.1.d – OPC specification is highly regulated at the transcriptional level**

Specification is highly controlled in a time-specific manner, involving different key transcription factors.

### ***Olig1 and Olig2***

Transcriptional regulation is essential during cell specification. In 2000, Olig1 and Olig2, both expressed in OPC nuclei, were identified as the first basic Helix-Loop-Helix (bHLH) TFs to be linked to oligodendrogenesis (Lu et al., 2000). *Olig2KO* mice die at birth and present a complete absence of OPCs in the spinal cord and most of the brain by E18.5, showing

importance of Olig2 in specification (Lu et al., 2002). However, in the brain, Olig1 compensated for Olig2 deletion in *Olig2KO* and some OPCs were formed in the hindbrain region (Lu et al., 2002). On the other hand, the number of OPCs generated was decreased in *Olig1KO* brain but not in spinal cord (Dai et al., 2015), showing the role of Olig1 in OPC specification. Interestingly, *Olig1/2* double mutant doesn't generate any PDGFR $\alpha$ <sup>+</sup> or Sox10<sup>+</sup> cells in the brain (Zhou and Anderson, 2002), showing the requirement of both factors for OPC generation.

Moreover, *Olig2KO* mice present a defect in motor neurons (MNs) generation both in spinal cord and hindbrain (Lu et al., 2002), suggesting a dual role of Olig2 in promoting both MN and OPC fate. Importantly, it is the phosphorylation state of Olig2 that determine the fate as Olig2 serine 147 (Ser147) is phosphorylated during MN generation and dephosphorylated during OL generation (Li et al., 2011). To explain how this post-translational modification can regulate NSCs fate, it was shown that phosphorylated-Olig2 tends to form homodimers whereas dephosphorylated-Olig2 forms heterodimers with Neurogenin 2 (Ngn2) or other TFs (Li et al., 2011). Sequestration of Ngn2, an important pro-neuronal bHLH factor (review: Bertrand et al., 2002), by dephosphorylated-Olig2 prevent activation of downstream Ngn2 target genes involved in neurogenesis and OPCs can be generated. Interestingly, it seems unlikely that one progenitor can switch between phosphorylated and dephosphorylated Olig2 to produce MNs and OPCs, successively. Indeed, a recent study done in zebrafish spinal cord, showed that MNs and OLs are generated from distinct progenitors expressing Olig2 in a sequential manner (Ravanelli and Appel, 2015).

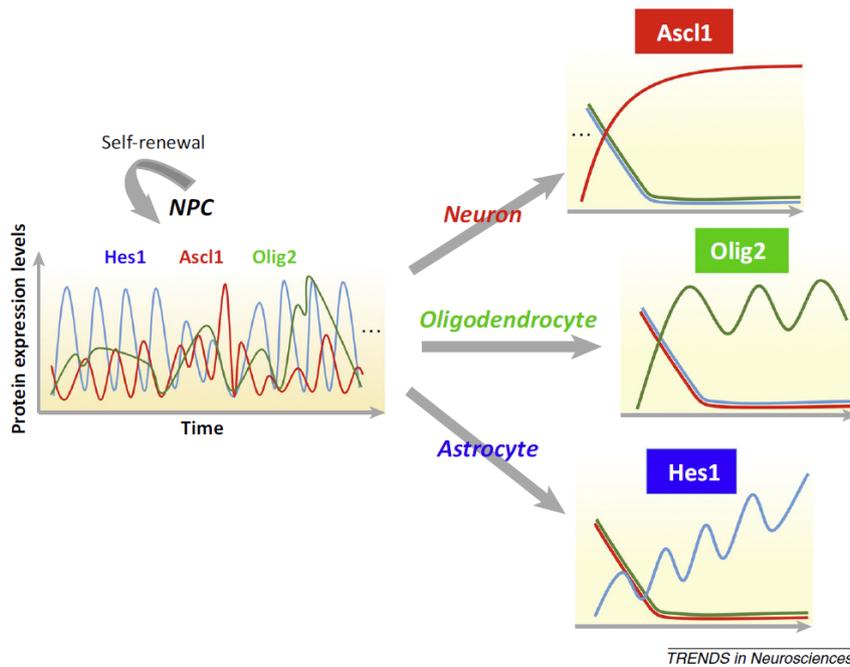
### ***Ascl1***

Another bHLH TF involved in OPC specification is *Ascl1* (Achaete-Scute Complex-Like 1), identified as a proneural TF because of its homology with achaete-scute complex (*asc*) which regulate early step of neural development in *drosophila*. As proneural factors, the members of the Achaete-Scute family are expressed in ectoderm even before neural differentiation and they are required and sufficient to generate neural progenitor cells (review: Bertrand et al., 2002). *Ascl1KO* mice die in the 24 hours after birth and they present severe defects in the generation of olfactory neurons and autonomic neurons (Guillemot et al., 1993). In the CNS, *Ascl1KO* mice have a severe loss of neuronal precursors in the MGE which lacks its rostral part at embryonic stage (Casarosa et al., 1999). A more recent paper showed that *Ascl1* targets genes of proliferation and differentiation of progenitors during neurogenesis and acts as

pioneer factor (Raposo et al., 2015). Indeed, *Ascl1* can bind to either open or close DNA and promote local chromatin opening in order to activate genes involved neurogenesis (Raposo et al., 2015). *Ascl1* also plays a role in NSC quiescence exit as shown in the mouse hippocampus, where *Ascl1* is expressed by cycling NSCs and directly activates cell-cycle genes like *Ccnd2* or *Rrm2* and *Ascl1cKO* show an absence of proliferative NSCs (Andersen et al., 2014).

*Ascl1* also play a role in OPC generation. Expression of *Ascl1* in OPCs was first shown in culture by western blot after purification of OPCs (Kondo and Raff, 2000a). Later, It was shown *in vivo* by immunostaining in combination with Olig2, NG2 and PDGFR $\alpha$  (Parras et al., 2004) (Parras et al., 2007). Newborn *Ascl1KO* mice present a defect in the generation of both neuronal precursors and OPCs in the olfactory bulb indicating a major role of *Ascl1* in the neonatal SVZ for both neuronal and oligodendroglial generation (Parras et al., 2004). *Ascl1KO* E12.5 embryos showed severe decrease in OPC numbers at the moment of their first wave of generation from the VZ of the ventral telencephalon, strongly suggesting a role for *Ascl1* in OPC specification (Parras et al., 2007). More recently, using a conditional *Ascl1<sup>Flox</sup>* allele and the postnatal electroporation technic (Boutin et al., 2008), *Ascl1* has been specifically deleted in neonatal SVZ NSCs and the generation of different neural subtypes assessed. A strong decrease in postnatally-generated OPCs and oligodendrocytes have been shown at 4 weeks post-electroporation, demonstrating the requirement of *Ascl1* in oligodendrocyte specification from postnatal SVZ NSCs (Nakatani et al., 2013).

Some studies have also shown the synergy between *Ascl1* and Olig2. *Ascl1* interacts genetically with *Olig2* as loss of one allele of *Olig2* aggravate the reduction in OPCs generated in the ventral telencephalon of *Ascl1KO* heterozygous or homozygous embryos (Parras et al., 2007). A study showed that the expression of *Ascl1* and *Olig2* (as well as *Hes1* which has a role in keeping undifferentiated NSCs and in astrocyte generation) oscillate inside NSCs with different periods (*Ascl1* and *Hes1*: 2 to 3 hours; *Olig2*: 5 to 8 hours) and that these expression levels evolves depending on the cell fate choice (Imayoshi et al., 2013; Fig. 12). For example, it was shown by optogenetics that sustained expression of *Ascl1* promote neuronal differentiation whereas oscillatory expression promote NSCs proliferation (Imayoshi et al., 2013). That would explain why overexpression of *Ascl1* in NSCs cannot induce the whole program of OPCs determination (Parras et al., 2007).



**Figure 12. TF oscillation is determining during specification.** Diagram showing how TF oscillation can be determining during specification. From Imayoshi and Kageyama, 2014.

### **Sox9 and Sox10**

Members of the SoxE protein group are also essential for OPC specification. This group contains three proteins: Sox8, Sox9, Sox10 and are characterized by the presence of a DNA-binding high mobility group (HMG) domain.

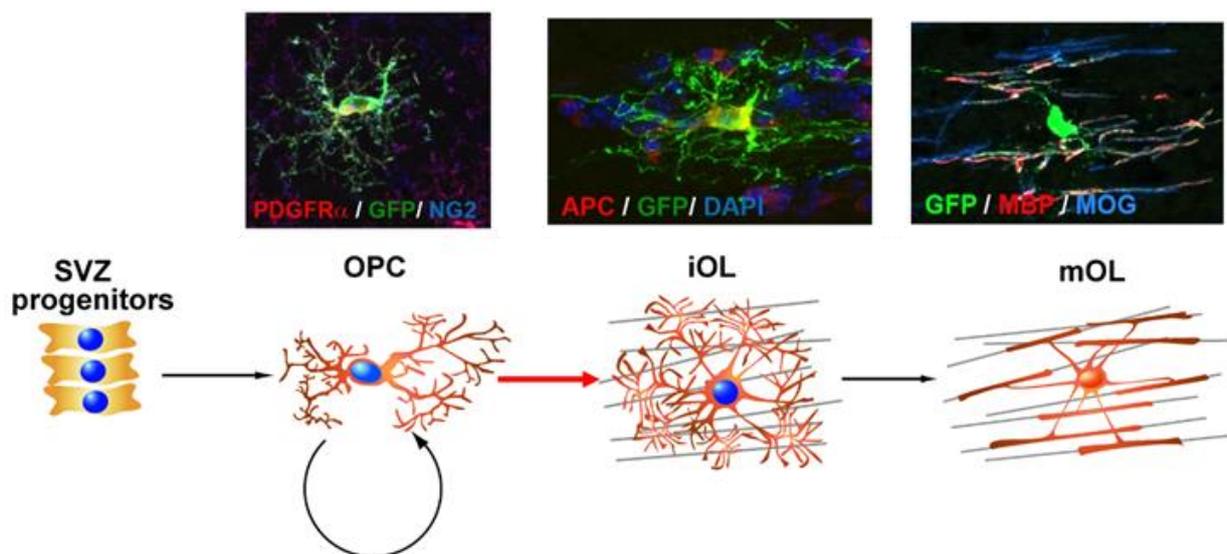
*Sox9* is expressed in NSCs (Scott et al., 2010) and, after specification, in OPCs and astrocyte precursors (Stolt et al., 2003). *Sox9* conditional ablation showed severe decrease of Olig2<sup>+</sup>-Sox10<sup>+</sup> cells in E12.5 spinal cord. Further analysis showed a delay in oligodendroglial development in mutant (Stolt et al., 2003). Furthermore, it was shown that Sox9 induce NFIA (nuclear factor-1A), a TFs promoting onset of gliogenesis (Deneen et al., 2006), and that Sox9 and NFIA form a complex to initiate gliogenesis (Kang et al., 2012).

In the CNS, *Sox10* expression is restricted to oligodendroglial cells. It starts to express in OPCs (Stolt et al., 2002) before *PDGFRα* and after *Sox9* (Stolt et al., 2004). Olig2 has the capacity to bind to an enhancer of *Sox10* (called *U2*) and activate expression of *Sox10* in OPCs (Kuspert et al., 2011); (Yu et al., 2013b). *Sox10KO* mice show no reduction of OPCs in the CNS at embryonic stages (Stolt et al., 2002) and die at neonatal stages (Britsch et al., 2001). However, a study showed the redundancy of Sox10 and Sox9 to generate OPCs. Double mutant showed an alteration of OPC distribution and a reduction in number at embryonic

stage (Finzsch et al., 2008). Also, Sox9 and Sox10 regulate expression of PDGFR $\alpha$  in OPCs (Finzsch et al., 2008), consistently with previous observations where *in situ* hybridization showed a decrease of PDGFR $\alpha$  intensity in Sox10KO mice (Stolt et al., 2002). Sox8, another SoxE factor, is also involved in OPC specification as it was shown that , in spinal cord, double mutant for Sox8 and Sox9 provoke a loss of oligodendrocytes (Stolt et al., 2005).

## II.C.2 - Differentiation

To form myelin sheaths, OPCs will have to exit cell cycle and differentiate. We can distinguish two steps in this process. First, actual differentiation where OPCs stop proliferating, modify their programs and go under morphological changes in order to become an immature oligodendrocyte (iOL) also called premyelinating OL. Second, iOLs will mature and ensheath axons to form myelin sheath to become mature oligodendrocytes (mOLs) or myelinating OLs, in the step of maturation (or myelination; Fig. 13).



**Figure 13. OPC differentiation.** Two different step are necessary to generate OLs, first OPCs (PDGFR $\alpha^+$ -NG2 $^+$ ) differentiate (red arrow) to give rise to iOLs and then they mature to become mOLs (Mbp $^+$ -Mog $^+$ ) and form myelin sheaths.

### II.C.2.a – Immature oligodendrocyte markers

During differentiation, PDGFR $\alpha$  and Olig1 expression decrease and new markers start to be strongly expressed, such as Nkx2.2 and Gpr17 (Bhat et al., 1996; Fumagalli et al., 2011; Nakatani et al., 2013). Recently, new markers of iOLs has been identified using single-cell transcriptomic strategies: Itpr2 (Inositol 1,4,5-Trisphosphate Receptor Type 2), an intracellular

Ca<sup>2+</sup> channel (Marques et al., 2016) and Enpp6 (Ectonucleotide Pyrophosphatase/ Phosphodiesterase Family Member 6; (Xiao et al., 2016).

### II.C.2.b – OPC differentiation regulation

As specification, OPC differentiation is highly regulated by TFs.

#### ***Olig2***

*Olig2* important function in specification made it difficult to determine its role in differentiation without using a time-controlled *Olig2* deletion. Cortical *Olig2cKO* (*Foxg1-Cre*, telencephalon progenitors) mice present a severe deficit of myelination at postnatal stages (P12, P18 and P28; Yue et al., 2006), while *in vivo Olig2* overexpression leads to increased number of OLs generated as well as precocious myelination (Wegener et al., 2015). However, these studies didn't answer if *Olig2* was important for differentiation, maturation or both. In another study, authors deleted *Olig2* in a more controlled manner at early stages (*Cnp-Cre*) or at later stage (*Plp-Cre*) of differentiation (Mei et al., 2013). They found that *Olig2* deletion in early stage (*Cnp-Cre*), decreases the number of OLs and leads to myelin defects, whereas *Olig2* deletion in later stage (*Plp-Cre*) accelerates myelination due to subsequent *Olig1* upregulation (Mei et al., 2013). Taken together, these results show that *Olig2* play a role in activating differentiation but seems to slow down myelination. Interestingly, conditional deletion of *Olig2* in OPCs (*NG2-Cre/Olig2<sup>Flox</sup>* mice) not only impaired oligodendrocyte differentiation but it induced OPCs to differentiate into astrocytes, indicating that *Olig2* is essential not only for OL differentiation but also to maintain oligodendroglial fate (Zhu et al., 2012). Indeed, in tumor cells, *Olig2* deletion causes transition from OPC-proneural glioma to astroglioma-like phenotypes (Lu et al., 2016).

To go further in the molecular mechanisms, *Olig2* induces the expression of *Brg1*, coding a chromatin remodeling factor that is recruited by *Olig2* to genes involved in OPC differentiation in order to active the differentiation program (Yu et al., 2013b). Indeed, *Brg1cKO* (using *Olig1<sup>Cre</sup>*) mice present decrease OL numbers and it was shown that *Brg1* regulate directly the expression of myelinating and lipid-synthesis genes as *Brg1* binds to myelinating genes (Yu et al., 2013b). However, *Brg1* is not absolutely required for OL differentiation as *Brg1cKO* (*Cnp1<sup>Cre</sup>*) mice show a mild reduction of oligodendrocytes after birth (Bischof et al., 2015). It contrasts with observations in the PNS where deletion of *Brg1*

impaired Schwann cells differentiation (Weider et al., 2012). Furthermore, it was shown that Brg1 and Sox10 interact genetically and functionally in Schwann cells (Weider et al., 2012) and that this relationship was not found in the CNS (Bischof et al., 2015) showing different mechanisms of TF/chromatin remodeler interactions between OL and Schwann cells.

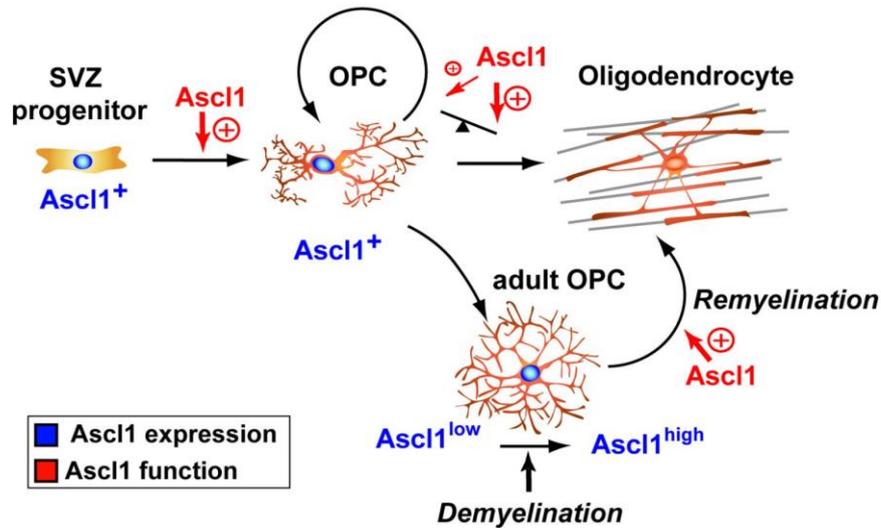
### **Sox10**

As a target of Olig2 (Yu et al., 2013b), *Sox10* also play an important role in OPC differentiation. *Sox10* is still expressed in differentiating and mature OLs while *Sox9* is downregulated at these stages (Stolt et al., 2002). Interestingly, a recent study shows that *Sox10* drive *Sox9* downregulation during differentiation by directly activating microRNA *miR335* and *miR338* which recognize *Sox9*, leading to a reduction of *Sox9* protein (Reiprich et al., 2017). Therefore, *Sox9* cannot compensate *Sox10* LOF anymore during OL differentiation. Indeed, *Sox10* is required for OL differentiation and maturation as mice lacking *Sox10* have no myelin-protein expressing cells (i.e. Plp<sup>+</sup>, Mag<sup>+</sup> or Mbp<sup>+</sup> cells; Stolt et al., 2002). On a more mechanistic note, *Sox10* can interact with the mediator complex (Vogl et al., 2013) showing the function of *Sox10* as a TFs to recruit the pre-initiation complex (PIC) to initiate transcription in myelin genes. Interestingly, *Sox8* play a similar role than *Sox10* during differentiation, although its function is less required as *Sox8*-deficient mice showed a less severe decrease of generated OLs (Stolt et al., 2004).

Differentiation inhibitors such as *Hes5* can prevent *Sox10* activity at myelin genes (such as *Mbp*; (Liu et al., 2006) but *Hes5* downregulation induced by microRNAs during OPC differentiation (Zhao et al., 2010) permits a strong *Sox10* activity promoting OL differentiation and myelination. Similarly, *Sox10* also competes with *SoxD* TFs, *Sox5* and *Sox6*, which are repressors of specification and OPC differentiation, but are also downregulated during differentiation (Stolt et al., 2006).

### **Ascl1**

*Ascl1* expression decreases with OPC differentiation and *Ascl1* is required in the balance between OPC proliferation and differentiation. Indeed, OPCs in *Ascl1cKO* proliferate more, privileging symmetrical OPC/OPC division over asymmetrical OPC/iOL ones, leading to a decrease of differentiating OLs both during myelination of the postnatal brain and during remyelination of the adult (Nakatani et al., 2013; Fig. 14).



**Figure 14. Model for Ascl1 function in oligodendrogenesis.** Ascl1 is expressed in cortical SVZ progenitors and the OPCs they generate, being downregulated upon oligodendrocyte differentiation. At postnatal stages, Ascl1 function is required in dorsal SVZ cells for normal OPC specification, and it is required in OPCs to balance OPC differentiation and proliferation. Ascl1 is maintained at low levels in adult OPCs, and its levels are upregulated upon demyelination being Ascl1 function required for normal OPC differentiation during remyelination. Arrow with plus symbol indicates functional requirement. Triangle with inclined line indicates Ascl1 function in the balance between OPC proliferation and differentiation, weighting more in the differentiation process. From Nakatani et al., 2013.

## ***Nkx2.2***

*Nkx2.2*, a member of the homeobox transcription factor, is another target of *Olig2* (Liu et al., 2007) and is transiently expressed during OPC differentiation (Fu et al., 2002; Nakatani et al., 2013; Qi et al., 2001). Interestingly, *Nkx2.2* negatively regulates the expression of *Pdgfra* in OPCs by directly binding to its promoter (Zhu et al., 2014), showing the function of *Nkx2.2* in the transition of OPCs to iOLs. Indeed, it was shown that *Nkx2.2KO* mice present severe delay in myelination as *Mbp* expression was not detected before E18.5 (compared to E15.5 in control) in the spinal cord (Qi et al., 2001).

## ***Gpr17***

*Gpr17* (G-protein-coupled receptor 17) has been identified as regulator of OPC differentiation by transcriptome comparison of *Olig1KO* and wild-type optic nerves (Chen et al., 2009). The presence of *Gpr17* seems to be required for proper OPC differentiation as knock-down with *siRNA* decreases the number of OLs (Fumagalli et al., 2011). Importantly, *Gpr17* presence increases both expression and nuclear translocation of *Id2* and *Id4* (Chen et al., 2009), where they interact with *Olig1/2* blocking their activity therefore inhibiting OL differentiation

(Samanta and Kessler, 2004). A recent paper has suggested that Gpr17 play an important role in restricting OPCs from neurogenic potential (Boccazzi et al., 2016) that could be deregulated in ischemic/traumatic lesions (Honsa et al., 2012). Authors used an antagonist of Gpr17, cangrelor, and reproduce the increased neurogenesis that they observed when OPCs are cultured in neurogenic conditions (Boccazzi et al., 2016). Moreover, Gpr17 is expressed in “hybrid” form of OPCs which start to express neuronal markers ( $\beta$ III-tub<sup>+</sup> cells; (Boccazzi et al., 2016).

### **HDACs**

Like mentioned above for Brg1, other chromatin modifiers has been identified as regulators of OPC differentiation such as histone deacetylases (HDACs). HDAC1/2, that are members of the class I Histone Deacetylase family, are required for OPC specification and differentiation (Ye et al., 2009). Administration of trichostatin A (TSA), an inhibitor of class I and II deacetylases, on cultured OPCs affects their differentiation and decreases *PLP* expression (Marin-Husstege et al., 2002). *In vivo* injection of another HDAC1/2 inhibitor, valproic acid (VPA), in postnatal rats showed a severe hypomyelination and a decrease in OL (CC1<sup>+</sup> cells) numbers (Shen et al., 2005). Mechanistically, it seems that HDAC1/2 block activity or expression of OPC differentiation inhibitors. It was shown that HDAC1 can be recruited by the transcription factor Yin Yang 1 (YY1) on the promoter of *Hes5*, *Id2*, *Id4* and *Tcf7l2/Tcf4*, inhibitors of OPC differentiation (He et al., 2007; Kondo and Raff, 2000b) in order to repress their expression during OPC differentiation (He et al., 2007). HDAC1/2 can also interact in a competitive way with TCF7L2 protein to block its interaction with  $\beta$ -catenin which inhibit OPC differentiation (Ye et al., 2009).

Finally, a recent study using genome-wide occupancy analysis and conditional knockout mice revealed that HDAC3 interacts with p300 to activate oligodendroglial lineage-specific genes, and that modulating the acetylation state of Stat3, HDAC3 competes with Stat3 for p300 binding to antagonize astroglialogenesis (Zhang et al., 2016).

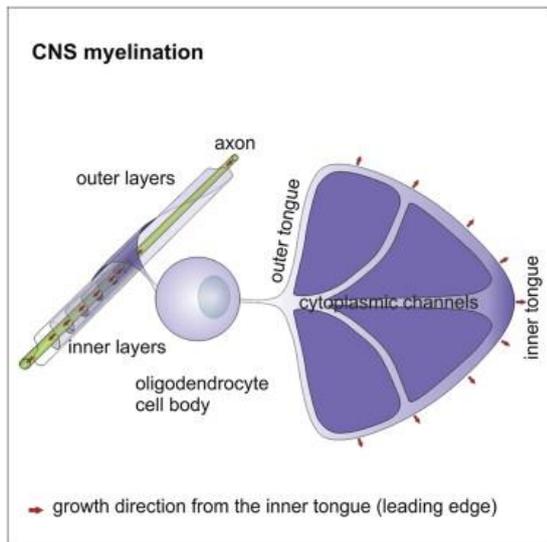
### **Non-coding RNAs**

MicroRNAs (*miRNAs*) play different role at the different steps of OL generation. Dicer1, an enzyme that catalyze the cleavage of miRNAs to permit them to be incorporate into the RISC complex (RNA-induced silencing complex), is necessary for myelination, highlighting the fact

that miRNAs presence and processing are required for correct OL programming (Dugas et al., 2010). Other non-coding RNAs are beginning to be studied. In a recent work, authors compared transcriptomes of oligodendroglial cells at different stages and identified long non-coding RNAs (*lncRNAs*) that could play a role in OPC differentiation. Among these, they showed that *lncOL1* regulate OPC differentiation by interacting with Suz12, a subunit of polycomb repressive complex 2 (PRC2) and repressing OPC genes (He et al., 2017).

### II.C.3 – Maturation/Myelination

Maturation is the last step of oligodendrogenesis when myelination occurs taking place mostly during postnatal life. OLs have to go through a lot of morphological changes to create and wrap myelin sheaths around axons (Fig. 15). To that purpose, OLs have to express myelin proteins such as MBP (Myelin Basic Protein), PLP (Proteolipid Protein), MOG (Myelin



**Figure 15. Model of Myelination.** From *Snaidero et al.*, 2014.

Oligodendrocyte Glycoprotein), MAG (Myelin Associated Glycoprotein) and OMG (Oligodendrocyte Myelin Glycoprotein). OLs also need other components like cytoskeletal proteins or lipid metabolism enzymes and synthesis of all these components needs a consistent energy support in the form of lactate (ATP) and carbon-skeleton (for lipids; Rinholm et al., 2011). Each step has to be finely controlled in order to myelinate the correct axon with efficient myelin sheaths (correct internodal length, correct compaction, correct g-ratio...). To achieve that, OLs have to do three things: contact the

correct axon, wrap it and compact the myelin around the axon.

#### II.C.3.a - Contact with axon

First, OLs extend a multitude of microfilament-rich processes called filipodia in order to find axons. This is done in response to several signals from the extra-cellular matrix (ECM) or from axons. Some signals expressed by OLs are inhibitors like PSA-NCAM, Lingo-1 or Jagged-1; and other signals are pro-myelinating such as binding of axonal ligands to oligodendroglial receptors (L1 ligand to contactin, netrin-1 to DCC; review: Mitew et al., 2014). It was shown

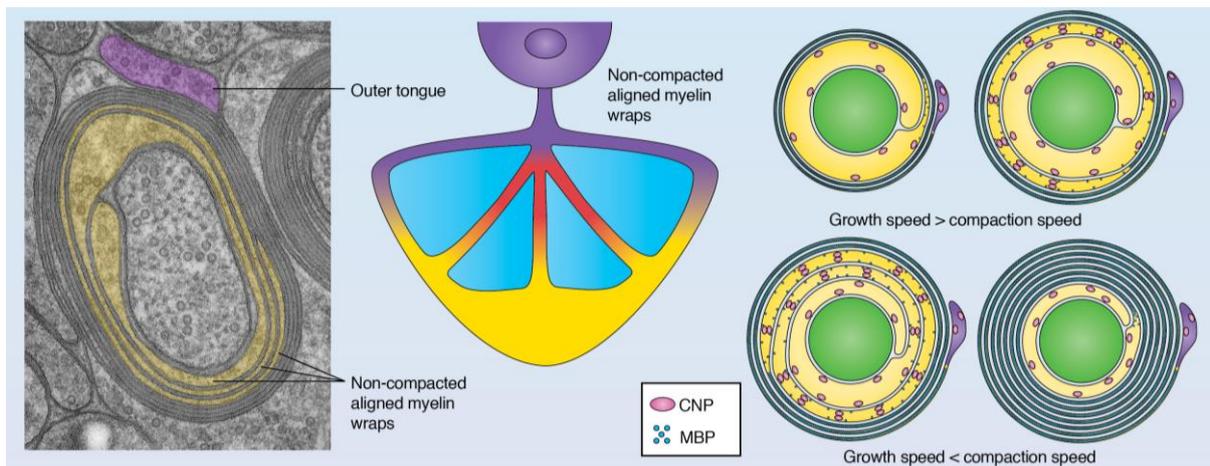
that both axonal activity and diameter are main factors controlling its myelination (Friede, 1972). Surprisingly, in culture, OLs can myelinate fixed axons (Rosenberg et al., 2008) or even synthetic nanofibers only if they are bigger than 0.3 microns (Lee et al., 2012a), supporting the role of axonal size in triggering myelination. However, *in vivo*, not all neurons are myelinated and how an iOLs choose which axon to myelinate is still unclear (Osanai et al., 2017). This step seems particularly important in humans because compared to rodent in which iOLs will rapidly mature to become mOLs (Barres et al., 1992a; Trapp et al., 1997), human iOLs can persist several months before maturing (Back et al., 2001) while making contact with axons (Back et al., 2002). This timing ensures that the correct axon is myelinated and contributes to brain plasticity. Interestingly, Sirt2 (sirtuin 2), a class III HDAC protein, plays a role at this control of axonal myelination. Indeed Sirt2 is expressed in OLs and myelin sheaths colocalizing with CNP (Li et al., 2007b; Vanrobaeys et al., 2005) and while *Sirt2* overexpression inhibits OL arborization, its knockdown using *siRNA* have the opposing effect (Li et al., 2007b). It was shown that  $\alpha$ -tubulin which composed microtubules is the main substrate of Sirt2 and deacetylation of  $\alpha$ -tubulin by Sirt2 impacts on OL cytoskeleton and processes arborization (Li et al., 2007b). Furthermore, Sirt2 is transported into the myelin compartment in presence of PLP (Proteolipid Protein) another myelin protein (Werner et al., 2007). *PlpKO* mice have no myelin defect but they present paralysis at adult stage (Edgar et al., 2004), showing the importance of Plp and Sirt2 in myelin functions.

### **II.C.3.b - Wrapping**

The process of myelin wrapping around the axon is complex. Since the first demonstration that it is axon-associated glial cells which form myelin sheaths around the axons (Uzman, 1956), different models have been proposed (review: Snaidero and Simons, 2014). However, we had to wait for the emergence, optimization and combination of certain technics such as live imaging or electron microscopy to visualize and understand myelin generation and wrapping processes. The most recent and complete model proposes that “myelin grows by two coordinated motions: the wrapping of the leading edge at the inner tongue in a triangle-like shape around the axon underneath the previously deposited membrane, together with the lateral extension of myelin membrane layers toward the nodal regions” (Snaidero et al., 2014; Fig. 15).

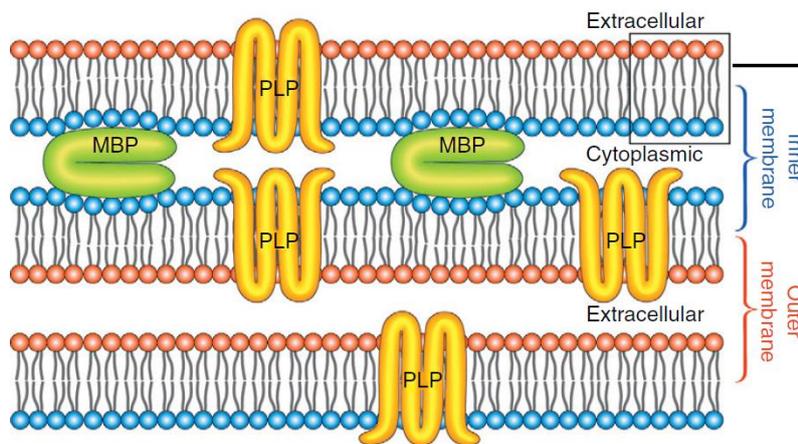
### II.C.3.c - Compaction

During this step, myelin sheaths compact their layers on each other, eliminating their cytoplasm. Myelin sheaths reach their optimal g-ratio and, thus, fully gain their functions. To achieve compaction, two kinds of interaction have to occur: between extracellular leaflets and between intracellular leaflets. MBP helps to compact the intracellular sheets together. For the extracellular ones, it is the loss of electrostatic repulsion that normally prevent the non-specific interaction between cells that permit compaction (Bakhti et al., 2013). Importantly, wrapping and compaction have to be coordinate. To that purpose, both processes occur at different myelin regions. Wrapping occurs in innermost region, whereas compaction occurs in outermost region and progresses inward (review: Snaidero and Simons, 2014; Fig. 16). However, as compact myelin prevent molecules from travelling through it, we can wonder how the material that will serve to grow the inner tongue is delivered. It was shown that myelin sheaths contain non-compacted cytoplasmic “pockets” (Velumian et al., 2011) or cytoplasmic channels which permit to deliver the membrane material to the inner tongue (Snaidero et al., 2014).



**Figure 16. Model of myelin wrapping and compaction.** As wrapping proceeds from inner tongue, compaction proceeds from outer to inner layers. From Snaidero and Simons, 2014.

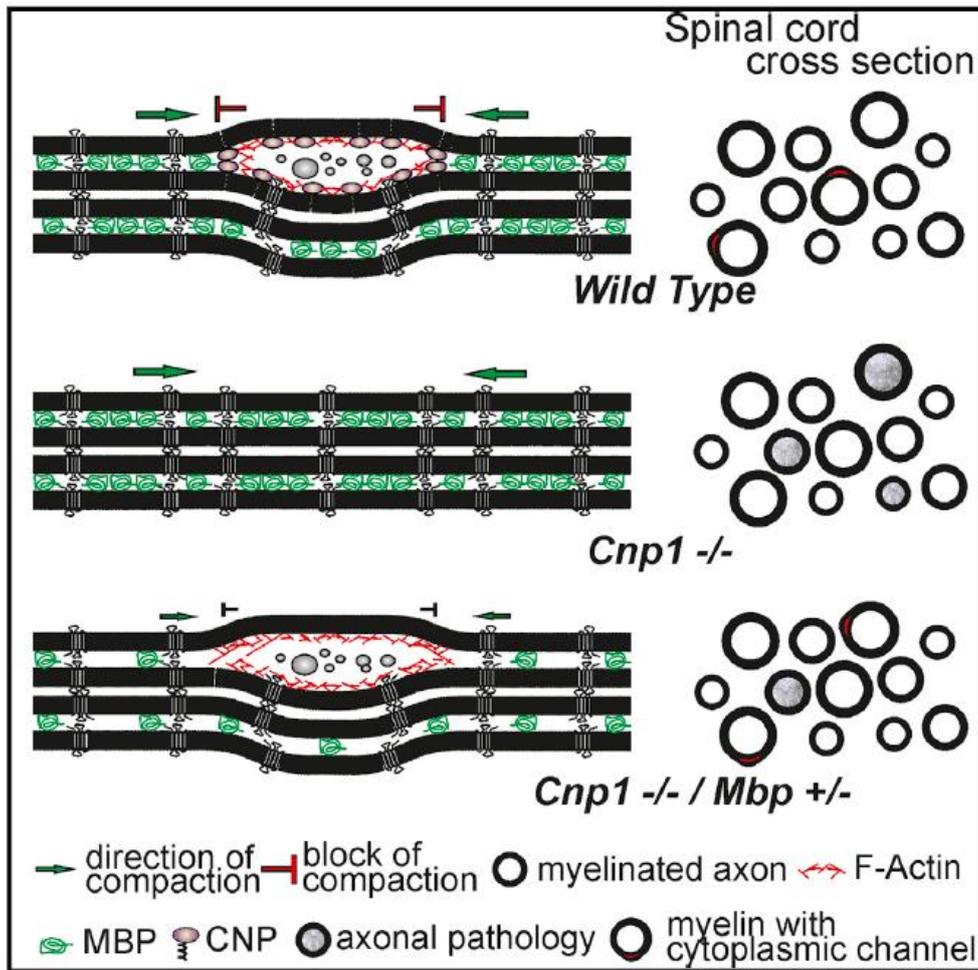
Historically, myelination defect was studied using *Shiverer* mice, recessive autosomal mutants, first discovered by Biddle et al. in 1973. In these mice, myelin compaction is impaired (Inoue et al., 1981) causing tremor, convulsions and premature death. This defect was attributed to absence of Myelin Basic Protein (MBP) in the mice brain (Roach et al., 1983). *Mbp* gain-of-function in the *Shiverer* mice rescue their phenotype and myelin compaction (Readhead et al., 1987). This analysis demonstrated the essential function of Mbp in myelin compaction. Mechanistically, MBP bind to the cytoplasmic face of the cell membrane and polymerize to form a network that will bring together two adjacent cell membranes (Aggarwal et al., 2013; Fig. 17). MBP acts as a barrier against diffusion of large protein in the cytoplasm



**Figure 17.** Myelin proteins. Scheme representing myelin membranes with positions of Mbp (green) and Plp (yellow). From Aggarwal et al., 2011a.

between membrane sheets. Cytoplasmic GFP, which cannot diffuse between membrane sheets in normal myelin, can on the contrary diffuse in the myelin of *Shiverer* mice (Aggarwal et al., 2011b).

CNP (2',3'-cyclic nucleotide phosphodiesterase) is a myelin protein used as a marker of pre-myelinating and myelinating OLs (Braun et al., 1988). *CnpKO* mice present motor deficit and premature death due to axonal loss, however, at first glance, these mice seem to have normal OL numbers and normal myelin (Lappe-Siefke et al., 2003). In fact, it was shown that *CnpKO* present a severe decrease of non-compacted myelin in the innermost tongue (Snaidero et al., 2014), leading to a more compact myelin. Moreover, overexpression of *Cnp* lead to defect in myelin compaction (Gravel et al., 1996; Yin et al., 1997). A very recent work shows that *Cnp* prevent compaction in cytoplasmic channels, permitting transport of molecules from OL cell body to axon, and thus allowing support and survival of the axon (Snaidero et al., 2017). An equilibrium of MBP and CNP expression has to be reached for the myelin to be totally functional: enough compaction providing insulation and cytoplasmic channels permitting wrapping and axon survival (Fig. 18).



**Figure 18. *Cnp* function in cytoplasmic channels formation and maintenance.** *Cnp* is required to form cytoplasmic channels in myelin sheath, with an Antagonistic functions with MBP. From Snaidero et al., 2017.

### II.C.3.c – Transcriptional regulation of myelination

#### ***Olig2* and *Sox10***

As shown above, *Sox10* and *Olig2* are involved in OPC differentiation and myelination. *Olig2* conditional KO in iOLs slows down myelination (Mei et al., 2013), whereas *Sox10* is required for myelination (Stolt et al., 2002).

#### ***Olig1***

*Olig1*KO mice have a more subtle phenotype than *Olig2*KO. During development, they show no obvious effect on specification in the spinal cord but there is a delay of expression of *MBP* and *PLP*, showing an effect on maturation (Lu et al., 2002). At postnatal stages, *Olig1*-null mice

present severe myelination deficit leading to axonal degeneration provoking tremor and premature death (Xin et al., 2005). However, a more recent work indicate that Olig1 is important for terminal differentiation but not for initiation of myelination, as myelin wrapping and elongation were normal in *Olig1-null* mice (Dai et al., 2015). After a transient silencing during differentiation (Dai et al., 2015; Nakatani et al., 2013), Olig1 is expressed again during OL maturation. Interestingly, in OPCs, Olig1 is localized mainly in nucleus whereas in OLs Olig1 is exclusively in the cytoplasm (Arnett et al., 2004). It has been shown that Olig1 partner with Sox10 to promote *Mbp* expression (Li et al., 2007a; Xin et al., 2005) and that forced nuclear localization of Olig1 increases *Mbp* expression (Niu et al., 2012). Together these results clearly demonstrates that nuclear Olig1 controls myelin protein gene expression. Cytoplasmic translocation of Olig1 is achieved after phosphorylation of Serine 138 and forced nuclear localization limit membrane extension (Niu et al., 2012), suggesting a contribution of cytoplasmic Olig1 function in myelination.

### ***Myrf***

Myrf is a TF identified rather recently using transcriptome comparison of astrocytes, neurons and OLs. *Gm98* (gene model 98) was identified as specific of postmitotic OLs (Cahoy et al., 2008). Later, *Gm98* has been renamed Myelin Regulatory Factor (Myrf) as it controls myelin genes expression (Emery et al., 2009). Indeed, in *Myrf iKO* mice OLs are generated but myelin is lacking (Emery et al., 2009) and Myrf is also required for maintenance of myelin and its ablation in adult OLs leads to loss of myelin with loss of myelin genes expression (*Plp*, *Mbp*, *Mag*, *Mog*) and axonal damages (Koenning et al., 2012). Even though the link between Myrf function and myelin genes expression was well established, whether this link was direct or not was not clear as Myrf expression didn't seems to be nuclear. However, more recently, it was shown that Myrf is cleaved in two fragments with C-terminal fragment assigned to cell membrane and N-terminal fragment is translocated to the nucleus where it binds to enhancer regions of myelin genes (Bujalka et al., 2013). Binding sites found for Myrf overlaps with those of Sox10 (Bujalka et al., 2013). Furthermore, it was shown that Myrf is a target gene of Sox10 (Hornig et al., 2013) as well as Olig2 and Brg1 (Yu et al., 2013b), and when Myrf is expressed, it will work in synergy with Sox10 to activate myelin-specific genes (Hornig et al., 2013).

### ***Nkx2.2***

*Nkx2.2* is transiently expressed in iOLs (Gokhan et al., 2005) and has to be downregulated to permit the myelination process to occur. *Nkx2.2* is required for OPC differentiation (Qi et al., 2001) but acts as an inhibitor of myelination by repressing *Mbp* expression (Gokhan et al., 2005; Wei et al., 2005). Downregulation of *Nkx2.2* is followed by expression of *Nkx6.2* (Cai et al., 2010; Southwood et al., 2004) which is involved in proper formation of paranodes (Southwood et al., 2004).

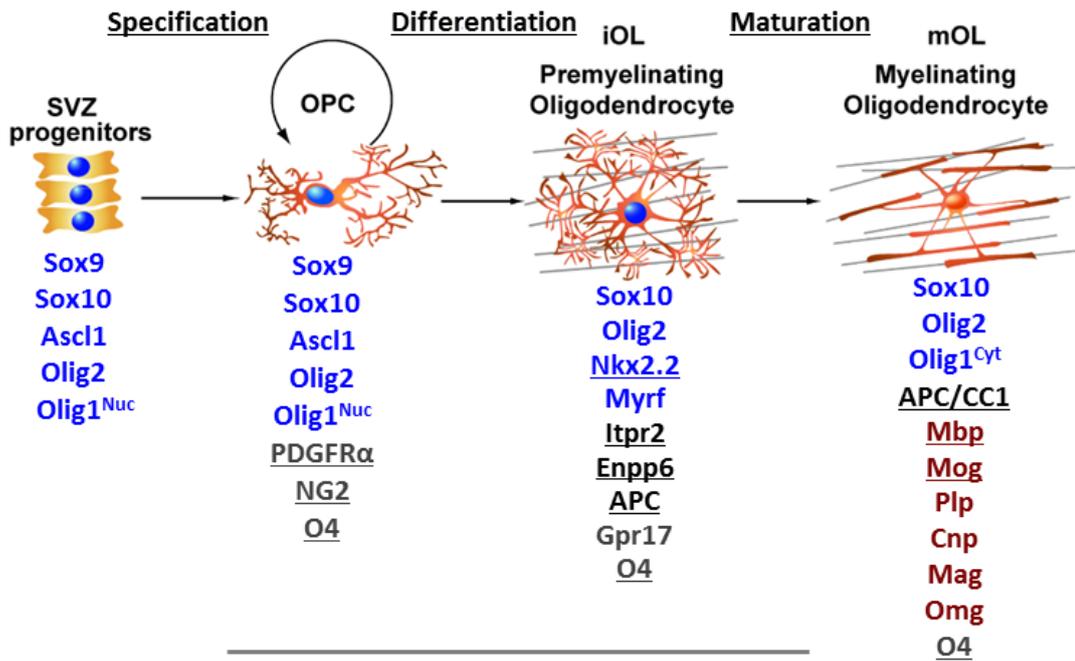
### ***Gpr17***

With a similar expression pattern as *Nkx2.2*, *Gpr17* is also important for OPC differentiation but inhibits OL maturation. Overexpression of *Gpr17* inhibits myelination with reduction of *Mbp* and *Plp* expression. Moreover, *Gpr17KO* accelerate onset of myelination (Chen et al., 2009), showing that *Gpr17* has to be turned down to permit normal maturation. Another G-protein coupled receptor, *Gpr37*, is also an inhibitor of myelination. Deletion of *Gpr37* leads to precocious OL differentiation and hypermyelination (Yang et al., 2016a). Therefore, G-protein coupled receptors serve as checkpoint mechanisms to make sure that the correct axon is myelinated.

### ***Zfp488 and Zfp191***

*Zfp488* is a zinc-finger transcription regulator that starts to express in OLs and increases with myelination (Wang et al., 2006). This factor can interact with *Olig2* and promote expression of myelin genes (Wang et al., 2006). Another zinc-finger protein is *Zfp191* is also required for myelination (Howng et al., 2010).

## Expression



## Regulation

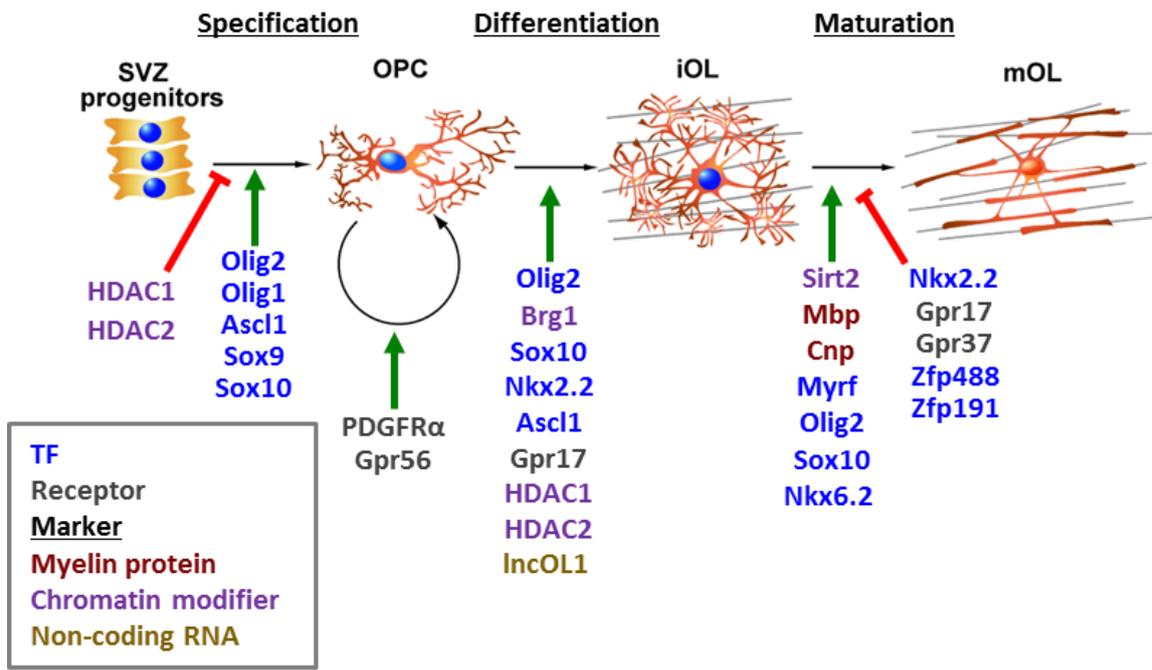


Figure 19. Summary of expression and regulation in OLs by TF and other factors.

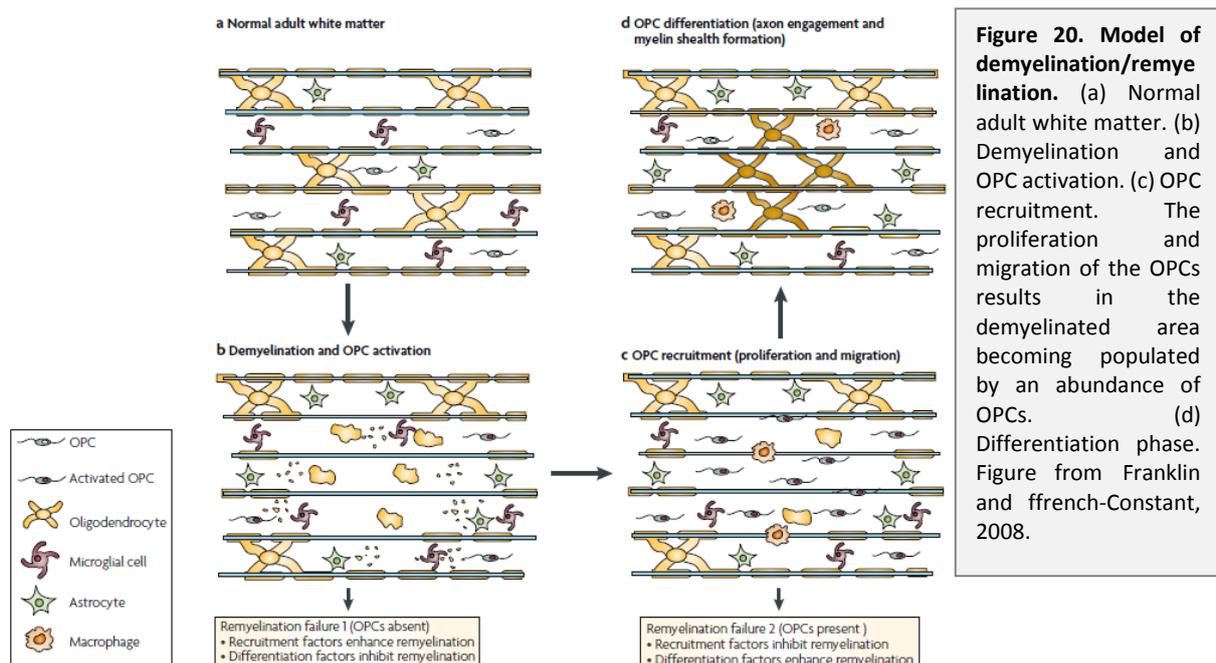
## II.C.4 – Remyelination

Remyelination, as myelination, aims to wrap axons in myelin sheaths in order to give axons their full functions (saltatory conduction, trophic and metabolic support). Mechanism of remyelination share a lot of aspects with myelination, making comprehension of developmental oligodendrogenesis and myelination an important tool to understand remyelination and promote it.

### II.C.4.a – Remyelination needs OPC differentiation

Remyelination start with OPC recruitment in which adult OPCs proliferate and migrate to the lesion region (Fig. 20). They upregulate factors like Olig2 or Nkx2.2 (Fancy et al., 2004) and start to differentiate. New OLs can then form new myelin that will wrap around naked axons and recovers their functions.

As mentioned above, remyelination is less effective with age (Shields et al., 1999). Why remyelination fails is still a debated question, however different hypotheses has been formulated (review: Franklin and ffrench-Constant, 2008). It seems that failure of remyelination is not due to absence of OPCs (Penderis et al., 2003) as they can repopulate regions lacking OPCs (Chari and Blakemore, 2002). More likely, remyelination fails due to OPCs differentiation defects (Kuhlmann et al., 2008). Therefore, studying oligodendrogenesis and how this process is regulated is essential to promote efficient remyelination in pathological conditions such as MS.



### II.C.4.b – Remyelination models

Using different animal models, we can study transcriptional regulation of OL differentiation in the context of remyelination. As MS is a complex disease, a lot of different animals models have been generated and optimized to cover the whole spectrum of aspects of the disease (review: Procaccini et al., 2015; Table 1). Depending on what we want to observe or test, animal models are chosen accordingly.

Characteristics of the different mouse models of multiple sclerosis.

Model of MS	Mechanism	Application	Involved cells	Translational value	Main references
<b>Relapsing–remitting EAE in SJL/J mice</b>	Immunization of SJL/J mice with PLP <sub>139–151</sub>	Study of neuroinflammation and immune system activation	CD8, CD4, Th17, monocytes, macrophages, B cells, Treg cells	Relapsing–remitting MS, study of the relapse rate, testing therapeutical agents	Zamvil et al., 1985; McRae et al., 1995; Whitham et al., 1991; Miyagawa et al., 2010; Adlard et al., 1999
<b>Chronic EAE in C57BL/6j mice</b>	Immunization of C57BL/6j mice with MOG <sub>35–55</sub>	Study of neuroinflammation and immune system activation	CD8, CD4, Th17, monocytes, macrophages, B cells, Treg cells	Primary progressive MS, secondary progressive MS, testing therapeutical agents	Mendel et al., 1995; Berard et al., 2010; Hjelmstrom et al., 1998; Bullard et al., 2007; Koh et al., 1992; Baron et al., 1993
<b>EAE in transgenic mice</b>	T cell clone (2D2) expressing V $\alpha$ and V $\beta$ chains reacting specifically to MOG <sub>35–55</sub> , or B cell heavy chain knock-in mouse strain (IgH MOG)	Study of neuroinflammation and immune system activation	CD8, CD4, Th17, monocytes, macrophages, B cells, Treg cells	<i>In vitro</i> study of immune cell activation and function	Bettelli et al., 2003; Litzemberger et al., 1998; Jäger et al., 2009; Encinas et al., 1999; Anderson et al., 2012
<b>Theiler's murine encephalomyelitis virus (TMEV)</b>	Infection with picornavirus, such as Theiler's murine encephalomyelitis virus (TMEV)	Study of axonal damage and inflammatory-induced demyelination	Macrophage/microglia, oligodendrocyte, astrocytes and CD4, CD8	Primary progressive MS, study of brain, brainstem and spinal cord lesions, study of new therapeutic approaches targeting adhesion molecules, axonal degeneration	Tsunoda and Fujinami, 2010; Libbey and Fujinami, 2003; Owens, 2006; Tsunoda et al., 1996; Tsunoda et al., 2003
<b>Cuprizone-induced MS</b>	Feeding C57BL/6 mice with 0.2% cuprizone for 6 weeks	Study of the de- and re-myelination processes	Oligodendrocytes, astrocytes, microglia	Therapeutical trials designed to repress demyelination or accelerate remyelination	Matsushima and Morell, 2001; Blakemore and Franklin, 2008; Lucchinetti et al., 2000
<b>Lysolecithin-induced MS</b>	Lysolecithin injection in SJL/J mice	Study of the de- and re-myelination processes	Oligodendrocytes, astrocytes, microglia	Therapeutical trials designed to repress demyelination or accelerate remyelination	Jeffery and Blakemore, 1995; Shields et al., 1999; Bieber et al., 2003

**Table 1. Characteristics of different MS mouse models.** Figure from Procaccini et al., 2015.

To study remyelination, two mice models are classically used: cuprizone-induced demyelination or lysolecithin (LPC)-induced demyelination. Cuprizone is a neurotoxicant that can be incorporated in mice food. It is a copper chelator that will provoke copper deficiency and will damage OLs in the CNS leading to demyelination after some weeks with this diet. Removal of cuprizone after a short administration (4-6 weeks) allows spontaneous remyelination (review: Matsushima and Morell, 2001). Lysolecithin (LPC) is used to induce focal demyelinating lesions. Demyelination occurs on the site of injection due to the toxic effect of detergent on oligodendrocytes which are very sensitive cells (Procaccini et al., 2015). These models can be coupled with transgenic mice to study the effect of removal of factors on remyelination.

#### ***II.C.4.c – Remyelination regulation***

It was shown that adult overexpression of *Olig2* accelerates remyelination (Wegener et al., 2015) as it accelerates myelination when overexpressed during postnatal stages (Maire et al., 2010). *Ascl1* is required for OPC differentiation after LPC-induced focal lesion as it is required during OPC differentiation in postnatal development (Nakatani et al., 2013). *Olig1* seems to have a more important role in remyelination than myelination as remyelination fails in *Olig1* *KO* mice after LPC-lesion or Cuprizone diet (Arnett et al., 2004). These observations validate the importance of these factors and highlight the importance of understanding developmental oligodendrogenesis to find new ways to promote remyelination.

In both myelination and remyelination, major and profound gene regulations are occurring during OL differentiation. Transcription factors and chromatin modifiers are more and more well-known to be involved in these processes. However, we also have to understand how these factors work at the mechanistic level. How do they promote myelin genes activation? How do they work together? Which ones work as partners to regulate transcription of certain genes? To answer these questions, we have to look at how gene expression is regulated in a general way. We are particularly interested in the transcription initiation when TFs are involved.

## **Part III: Mechanism of Transcription Initiation**

### **III.A – From chromatin to gene expression**

Understanding cell-type specific gene expression has demanded big method breakthroughs like DNA sequencing and associated techniques like RNA-seq or ChIP-seq. It also had demanded a lot of human resources as one lab couldn't handle alone the amount of work that was necessary to finish such ambitious projects. To overcome that difficulty, different labs collaborated in consortiums to join forces. In that context, The ENCODE project started in 2003, with the aim of mapping functional elements of the human genome (Consortium et al., 2007; Consortium, 2012). Some years after, the Mouse ENCODE Consortium was created to map transcriptional regions, DNase I hypersensitivity sites, transcription factor binding sites, chromatin modifications and replication domains throughout the mouse genome in diverse cells and tissue types (Yue et al., 2014). These data provide precious information as a base to understand mechanism of transcription.

#### **III.A.1 – From DNA to chromatin**

Each cell of the human body contains 2 meters of DNA that have to be highly compacted to be contained in a 2-10 $\mu$ m diameter nucleus. To that purpose, there are different levels of compaction for the chromatin which is the association of DNA and proteins like histones or transcription factors. Histones permit to attain the first level of compaction as they form together a protein structure composed of eight histones (2 heterodimers of H2A and H2B and 2 heterodimers of H3 and H4) wrapped by 147 base pairs (bp) of DNA. This wrapping of DNA around one complex of histones is called nucleosome (review: (Ordu et al., 2016)). Positioning of nucleosome is important and non-random as it affects gene expression (Simpson, 1990) and they reformed themselves on promoters during minutes after DNA replication (Fennessy and Owen-Hughes, 2016).

Nucleosomes can also condense themselves and two chromatin domains can be distinguished. On one hand, euchromatin is transcriptionally permissive as it is loosely compacted. On the other hand, heterochromatin is transcriptionally repressive as it is more condensed (review: Rothbart and Strahl, 2014). Importantly, these degrees of compaction depends on different factors. For example, the presence of histone H1 permit higher-order chromatin organization as H1 is a linker that bind to nucleosome to form chromatosome

(Harshman et al., 2013). Another way to change chromatin compaction is to replace histones within nucleosome by histone variants which have roles in different process like DNA repair, meiotic recombination but also in transcription initiation and chromatin condensation (review: Talbert and Henikoff, 2010). For instance, H2A.Z is a histone variant found mostly in promoter regions (Guillemette et al., 2005), and more specifically in nucleosomes flanking nucleosome-free regions (NFRs; Raisner et al., 2005). H2A.Z play a role in transcription as it was shown to recruit RNA pol II (Adam et al., 2001). Finally, histones can also be affected by post-translational modifications that forms a “code” (review: Rothbart and Strahl, 2014). N-terminal sequences of histones called tails are structurally out of the nucleosome and can be modified on their lysine by methylation or acetylation.

All of these changes of the chromatin structure have consequences on the cell biology. For example, a reduction of heterochromatin in oligodendrocytes was observed in isolated mice which present less thick myelin (Liu et al., 2012).

### **III.A.2 – Gene expression**

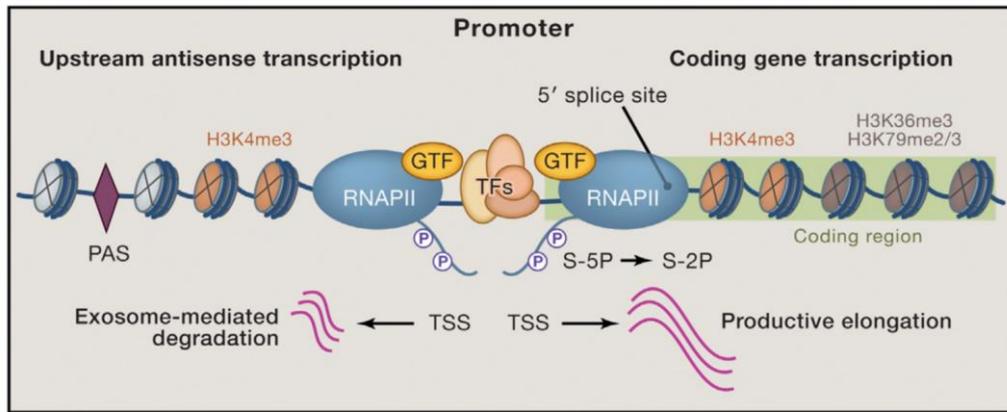
A gene is a DNA sequence defined by “a union of genomic sequences encoding a coherent set of potentially overlapping functional products” (Gerstein et al., 2007). Protein-coding genes start at transcription start sites (TSS) and stop at transcription stop sites (TTS). They are made of succession of exons which can be translate and introns which are excised during splicing. Two kinds of protein-coding genes exist: housekeeping genes and cell-type specific genes. The latest have a spatiotemporal expression that is highly regulated at the transcriptional level. Indeed, as we just mentioned, chromatin is a compact structure made of proteins wrapping DNA and limiting access to DNA. We can thus wonder which kind of regulatory elements, factors and chromatin changes are necessary for the transcription machinery to access the TSS to activate gene expression.

## **III.B – Regulatory Elements of transcription initiation**

### **III.B.1 – Promoter**

In 1961, Jacob and Monod first introduced the concept of “promoter” as a binding site for RNA polymerase. As promoters are more and more studied and understood, a new definition can be made: “DNA region that allow accurate transcription initiation of a gene” (Kim and Shiekhattar, 2015b).

The core promoter is composed of different DNA sequences (~100bp) positioned around the TSS and that can extend up to 35bp from TSS (Smale and Kadonaga, 2003). It represents the minimal regulatory sequence needed for the binding of transcriptional machinery and transcription initiation. Accordingly, it has a basal activity on its own but need other regulatory elements like enhancers to properly initiate transcription. It's particularly important to communicate to the transcription machinery the correct position and direction to begin transcription. Interestingly, core promoter composition is different depending on the gene type. Housekeeping genes core promoter contains DNA replication-related elements (DREs) and Ohler motifs 1, 6, 7 and 8; whereas, spatiotemporal-controlled genes have core promoter containing mostly TATA box, Initiator or downstream promoter element (DPE) (review: Zabidi and Stark, 2016). Crucially, it was shown that the position of TATA box relatively to other regulatory elements is determinant to the directionality of the transcription (Xu et al., 1991). Indeed, transcription at promoters is bidirectional, meaning that transcription starts from core promoters and goes in opposing direction. However, as opposed to divergent transcription, two type of RNAs are synthesized: mRNAs are generated downstream of core promoters and PROMPTs (promoter upstream transcript) which are short, unstable and rapidly degraded RNAs are generated upstream (review: Bagchi and Iyer, 2016).



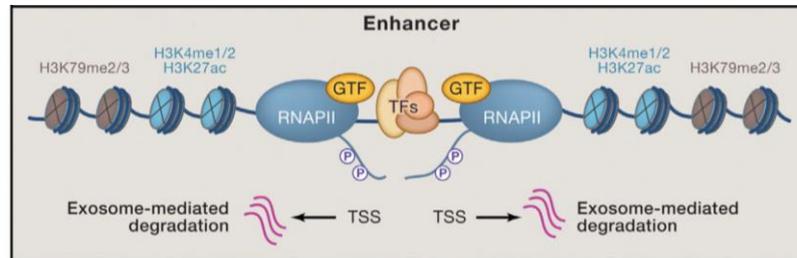
**Figure 21. Scheme of a promoter region.** From Kim and Shiekhattar, 2015b.

Promoter is also used as a term referring to the core promoter region together with near regulatory elements at a distance of 1-2Kb upstream of the TSS (Fig. 21). Activity level of these promoter regions can be measured by detection of specific histone marks like H3K4me3 (Ghavi-Helm et al., 2014; Guenther et al., 2007; Fig. 23) or be studied by placing it in front of a sequence coding a reporter gene, such as *GFP* or *LacZ*. That way, different mouse line have been generated to study OL-specific promoters like *Pip-GFP* (Le Bras et al., 2005). Its activity can be studied in different condition like development or remyelination (Feret et al., 2013). Furthermore, it can become a powerful tool to study a cell population or doing conditional deletion by inserting a Cre-recombinase gene instead of a reporter gene, like *PDGFR $\alpha$ -CreER<sup>T</sup>* (Kang et al., 2010).

### III.B.2 – Enhancer

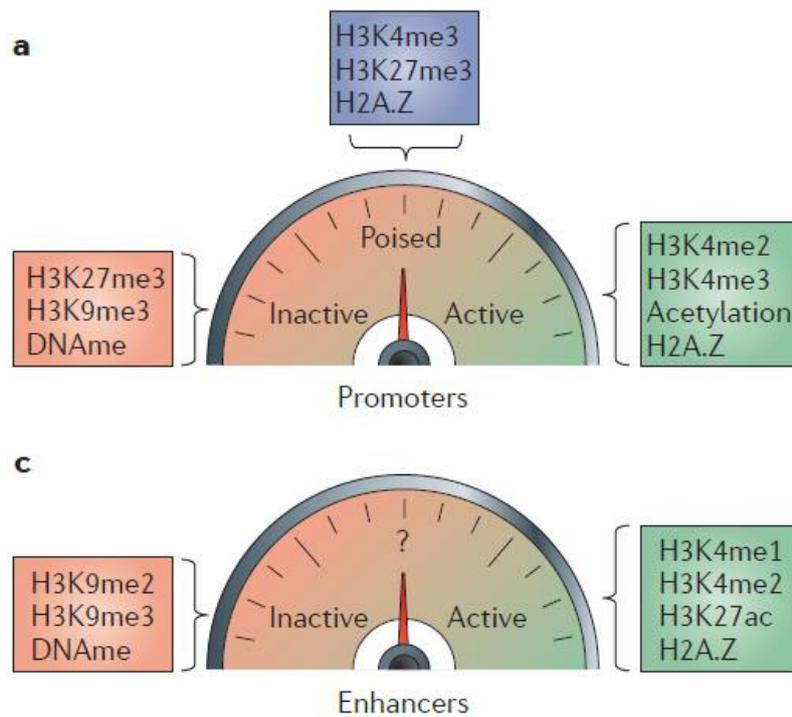
Enhancers are distal regulatory elements, discovered in 1980s in SV40 (de Villiers et al., 1982), which enhance transcription at their target promoters. It has been estimated that up to 1 million enhancer elements with gene regulatory potential are present in mammalian genomes (Consortium, 2012). They provide a way to bring specificity of gene expression and permit cell-type and time-controlled gene expression. Over the years, different characteristics of enhancers has been enunciated to define it (review Kim and Shiekhattar, 2015b, Fig. 22): 1) Enhancers can targets promoters over short or long distance (from few kb to 1Mb) and they can be intergenic or intronic. 2) Importantly, enhancers are “independent of their position or orientation” from their target promoters. 3) Enhancers contains in their sequences transcription factor (TF) binding sites which are responsible of the spatiotemporal pattern of gene expression. 4) Bidirectional transcription also occurs in enhancers. It will produce short,

unstable, unspliced RNAs called enhancer RNAs (eRNAs). The generation of these eRNAs can be a good predictor of the activity of an enhancer. 5) Enhancers are DNA regions where chromatin is less compact. It is usually detected as DNaseI hypersensitive.



**Figure 22. Scheme of an enhancer region.** From Kim and Shiekhattar, 2015b.

As identification of promoters can be done by searching specific sequences near the core promoter, it is however more difficult for enhancers. Different ways has been proposed but none of them are perfect and often need to be combined (review: Kim and Shiekhattar, 2015b). For example, we can look for RNA Pol II binding sites outside promoters as the RNA Pol II can bind to enhancers to produce eRNAs. Another way is to look at low compaction regions with technics like DNase-seq, FAIRE-seq or ATAC-seq; or to look at TF binding sites or coactivators binding sites (p300/CBP). Like in promoters, histone marks in enhancers can be identify to determine their activity level. Active enhancers tend to present high levels of H3K4me1/2 and low levels of H3K4me3 with an enrichment of H3K27ac marks (Ghavi-Helm et al., 2014); Fig.23).



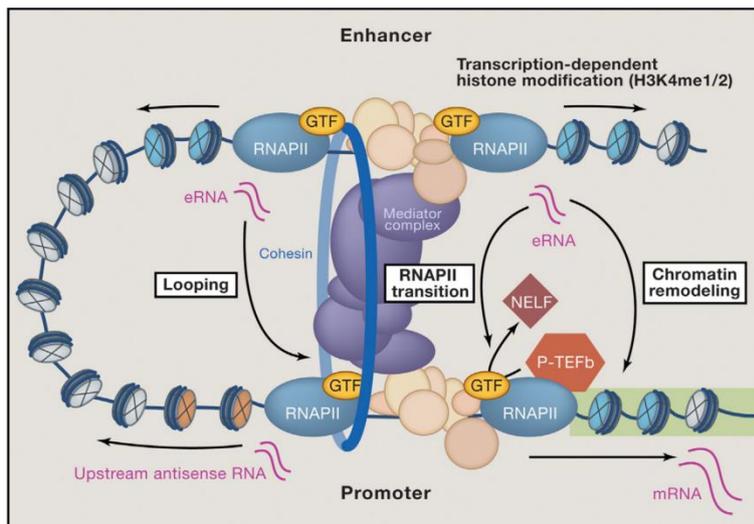
**Figure 23. Characteristic histones modifications found in promoter and enhancer regions.** From Zhou et al., 2011

Interestingly, one gene can need different enhancers to be activated depending of the cell type or the cell differentiation stage inside a given cell lineage. A study showed that enhancers that permit *Mbp* expression in oligodendrocytes do not activate its expression in Schwann cells where *Mbp* needs other regulatory elements (Gow et al., 1992). In the case of *Sox10* enhancers, one of them called *U6* permit expression of *Sox10* in OPCs where it is bound directly by Olig2 (Kuspert et al., 2011). On the contrary, in mOLs, *Sox10* expression is not regulated by *U6* enhancer which is inhibited by Nkx6.2 binding (Kuspert et al., 2011).

Finally, we can see a lot of common features between promoters and enhancers: they can both produce RNAs in a bidirectional way, they are both in less compact regions (DNAase I hypersensitive) and TFs binding sites are found in both. The main difference between promoter and enhancer is the capacity of promoter to drive the transcription of a spliced, poly-A transcript which is the mRNA. That make the promoter the most important site as excision of promoter stop gene expression even if enhancer is still present (Kim et al., 2010). However, enhancers are the elements that give cell-type specificity. But, how is it achieved? How an enhancer, which can be far away from its target promoter, can activate transcription?

### III.C – Physical interaction between promoters and enhancers

To activate promoters, enhancers have to be in close proximity. When enhancers are far away from their target promoters, the only way to bring enhancers and promoters close to each other is to gather the whole DNA regions in a 3D conformation (Carter et al., 2002), forming what is called a loop (Javierre et al., 2016; Fig. 24). It permits the direct binding of enhancers to cognate promoters via different proteins like TFs, cofactors, mediator complex and



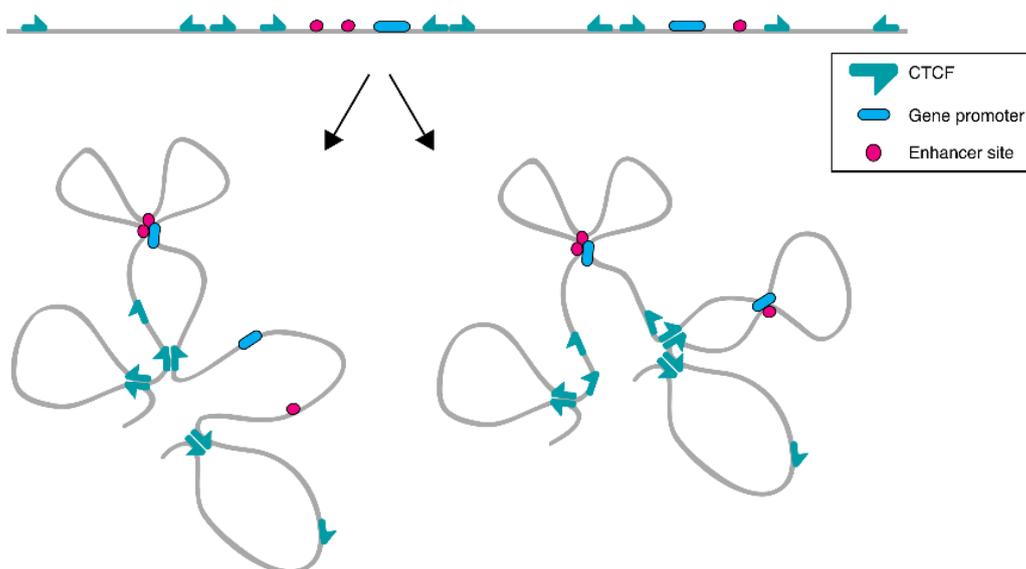
**Figure 24. Model of the promoter-enhancer loop formation and composition.** From Kim and Shiekhhattar, 2015a.

remodeling factors (Deng et al., 2014). Loops can be pre-formed (Jin et al., 2013b), creating a microenvironment where factors concentration is higher, permitting a fast expression initiation when needed. However, loops can also be formed *de novo* (de Laat and Duboule, 2013) corresponding to cell-type specific genes.

Anyway, this interaction between promoters and enhancers is highly dynamic and can be flexible as it changes with gene regulation.

As mentioned above, DNA is a big molecule highly compacted in a small nucleus which is a 3D structure. How an enhancer can find its cognate promoter in a 3D compacted environment? In fact, chromatin is organized within the nucleus in different structural domains called TADs (topological associated domain; (Dixon et al., 2012); Fig. 25), creating microenvironments where interactions – DNA-DNA, DNA-Protein or Protein-Protein – are more frequent (Nora et al., 2012). It was shown that genes inside one TAD shared the same pattern of expression and present the same histone modifications (Dixon et al., 2012; Nora et al., 2012; Sexton et al., 2012). Moreover, TADs are bordered by TAD boundaries which have a role of barrier (Matharu and Ahituv, 2015) as they prevent spreading of heterochromatin (Dixon et al., 2012) and the level of chromatin interaction is very low inside TAD boundaries. In these chromatin region, high levels of H3K27me3 can be found (Pope et al., 2014) showing their low gene expression activity.

To form these TADs, two factors are needed: CTCF (CCCTC-Binding Factor) and cohesin (Tang et al., 2015). CTCF binds to DNA sequences called insulators as an enhancer present on one side of this sequence cannot interact with a promoter present on the other side. CTCF is critical for the formation of loops and chromatin organization as 92% of chromatin loops contain CTCF (Jin et al., 2013a). Importantly, the orientation of CTCF on the DNA is crucial for the formation of loops as only two convergently oriented CTCFs will dimerize (Tang et al., 2015; Fig. 25). Moreover, another factors playing a role in loop formation is Cohesin. It is a protein complex that form a large ring-like structure which slide along the chromatin until finding CTCF that stabilize cohesin position (Parelho et al., 2008; Rubio et al., 2008; Wendt et al., 2008).



**Figure 25. Model of TAD formation.** Loops preferentially form between convergent CTCF sites. From Bouwman and de Laat, 2015.

### **III.D – Regulation of interaction**

Different regulatory factors will interact with promoters and/or enhancers to create the loop. They are of different kinds and have all different roles. We can distinguish three categories: transcription factors, cofactors and chromatin modification complexes.

#### **III.D.1 – Transcription factors**

Transcription factors (TFs) are proteins that contain a DNA binding domain which can be of different kind and determine the protein family of the TF. In the oligodendrocyte lineage, Olig1, Olig2 and Ascl1 are part of the basic helix-loop-helix (bHLH) family (Bertrand et al., 2002), whereas Sox10 is part of the HMG (high mobility group)-box family (Malarkey and Churchill, 2012). These domains usually bind to specific DNA sequences present in enhancers and composed of 4-10 nucleotides called TF binding motifs (Inukai et al., 2017). The role of TFs is to recruit the basal transcription machinery that will link enhancers to promoters and permit the initiation of transcription (Panne, 2008). On that note, it was shown that Sox10 can directly interact with the mediator complex in order to recruit RNA pol II (Vogl et al., 2013). The main model of TF binding is called “enhanceosome” (Panne, 2008): TFs bind together in an ordered and cooperatively way to form a protein complex. It means that all the proteins of this complex are needed to have the most efficient and precise expression of the associated genes (Panne, 2008).

The major problem for the TFs is to overcome the compact structure of the chromatin. As mentioned above, chromatin is made of nucleosomes spaced along DNA (Schalch et al., 2005). This limit the amount of free DNA available and the presence of histone can hide the binding site of a TF as steric hindrance. It means that chromatin is structurally and intrinsically repressive (Zaret and Mango, 2016), preventing unwelcomed gene expression and precocious cell fate transition. To overcome this barrier, some TFs have a role of initiators and are called pioneer transcription factors (Cirillo et al., 2002). Their particularity is to be able to bind to DNA in the context of nucleosome (Cirillo et al., 2002; Cirillo et al., 1998) and often have a reduced binding motif exposed at the surface of the nucleosome (Soufi et al., 2015). That first binding will permit the recruitment of other factors that will allow changes in the local chromatin structure (Zaret and Mango, 2016). The binding of the pioneer TFs will facilitate the binding of a second factors in a cooperative and hierarchical way. Concerning OL

differentiation, it was shown that Olig2 and Ascl1 present pioneer TF characteristics (Raposo et al., 2015; Yu et al., 2013b).

### **III.D.2 – Cofactors**

TFs are helped by cofactors which can be activating or repressive. They have not sequence-specific binding, it's the partner TF that determine the recruitment site (Malik and Roeder, 2005; Soufi et al., 2012). CBP (CREB-binding Protein) and p300 are two coactivators binding to enhancers (Holmqvist and Mannervik, 2013; Tie et al., 2009).

### **III.D.3 – Chromatin modifying factors**

As mentioned, pioneer TFs are able to bind to compact chromatin (Cirillo et al., 2002). However, to permit binding of other TFs, chromatin have to be in a less compact conformation in order to allow access to binding sites. This is where remodeling factors are particularly important. Their aim is to move nucleosomes in order to create nucleosome depleted regions (NDRs). Interestingly, a study has shown the chromatin reorganization undergoing during differentiation of OPCs to mOLs (Nielsen et al., 2002), showing the importance of these mechanisms. There are different type of remodeling factors that will affect chromatin at different levels: DNA methylations, histone modifications and ATP-dependent chromatin alterations.

#### ***III.D.3.a – DNA methylation***

DNA methylation occurs mostly in CpG sites in 5'promoters to repress gene expression. We can distinguish three classes of enzymes that are associated with DNA methylation (review: Moore et al., 2013): 1) the writers that catalyzes the addition of a methyl group onto a cytosine residue. They are the DNA Methyltransferases: Dnmt1, Dnmt3a and Dnmt3b; 2) the erasers like Ten-eleven translocation (TET) enzymes which add a hydroxyl group to the methylated cytosine. 3) the readers are proteins that have the capacity to bind to DNA methylation like MBD (Methyl-CpG-Binding domains) proteins which prevent the binding of other factors, thus inhibiting transcription.

DNA methylation has a cell-specific distribution and we can note that it is essential for oligodendrogenesis as deletion of Dnmt1 in OPCs results in hypomyelination (Moyon et al., 2016). Moreover, an alteration of DNA methylation was found in MS patients (Huynh et al.,

2014) and a very recent study showed the importance of DNA methylation for remyelination (Moyon et al., 2017), showing the growing interest of these mechanisms in pathological processes.

### **III.D.3.b – Histone modifications**

As shown above, histone modifications can characterize the level of activity of promoters and enhancers (Fig. 23) as they modify the chromatin conformation (Marmorstein and Zhou, 2014). For instance, acetylation of lysine residues (K) reduce the affinity of histone for negative charges present on DNA, reducing chromatin compaction and increasing accessibility. It is controlled by Histone acetyltransferase (HATs) and Histone deacetylase (HDACs; Marmorstein and Zhou, 2014; Table 2). Interestingly, HDACs have roles in OPC generation and differentiation like HDAC1, HDAC2 and HDAC3 (Marin-Husstege et al., 2002; Ye et al., 2009; Zhang et al., 2016). Also, Sirt2 play a role in myelination (Li et al., 2007b; Vanrobaeys et al., 2005).

Classe I	Classe II	Classe III	Classe IV
HDAC 1	HDAC 4	Sirt 1	HDAC 11
HDAC 2	HDAC 5	Sirt 2	
HDAC 3	HDAC 6	Sirt 3	
HDAC 8	HDAC 7	Sirt 4	
	HDAC 9	Sirt 5	
	HDAC 10	Sirt 6	
		Sirt 7	

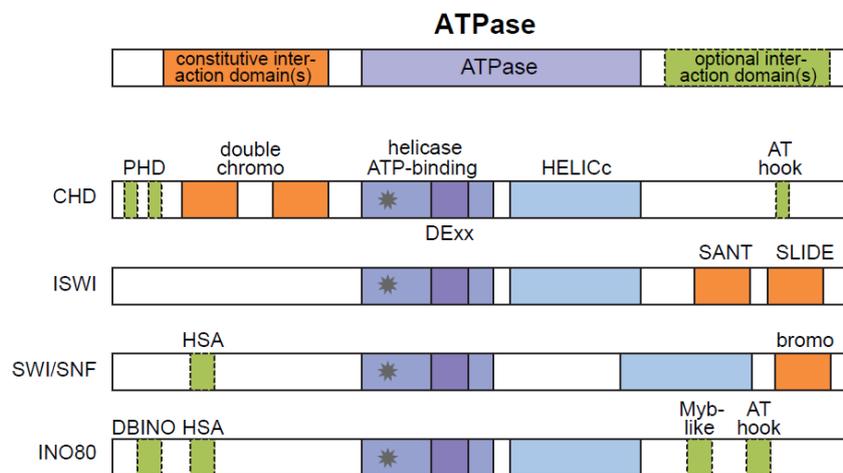
**Table 2. The four classes of HDACs**

Methylation of lysine residues of histone H3 is another post-translational modification that can control transcription. We correlate H3K4me3 with gene activation and H3K9me3 and H3K27me3 with repression (Shilatifard, 2006). These modifications are catalyzed by histone methyltransferases and removed by demethylases (Hu et al., 2015).

### **III.D.3.c – ATP-dependent alteration of chromatin**

ATP-dependent chromatin remodelers use the energy from ATP hydrolysis to modify chromatin structure, permitting the local dynamic of nucleosomes as they can translocate, evict, add or change histones on the DNA. They also work in a non-ATP manner as they interact

with TFs to regulate transcription or Histone-modifying complexes. These ATP-dependent chromatin remodeler are part of the SF2 superfamily and present a common helicase ATPase domains. They are then subdivided in 4 major subfamilies depending on their other domains (Fig. 26).



**Figure 26. The subfamily of ATP-dependent chromatin remodeler.** Scheme of the proteic domains of each member of the ATP-dependent chromatin remodelers. From Erdel et al., 2011

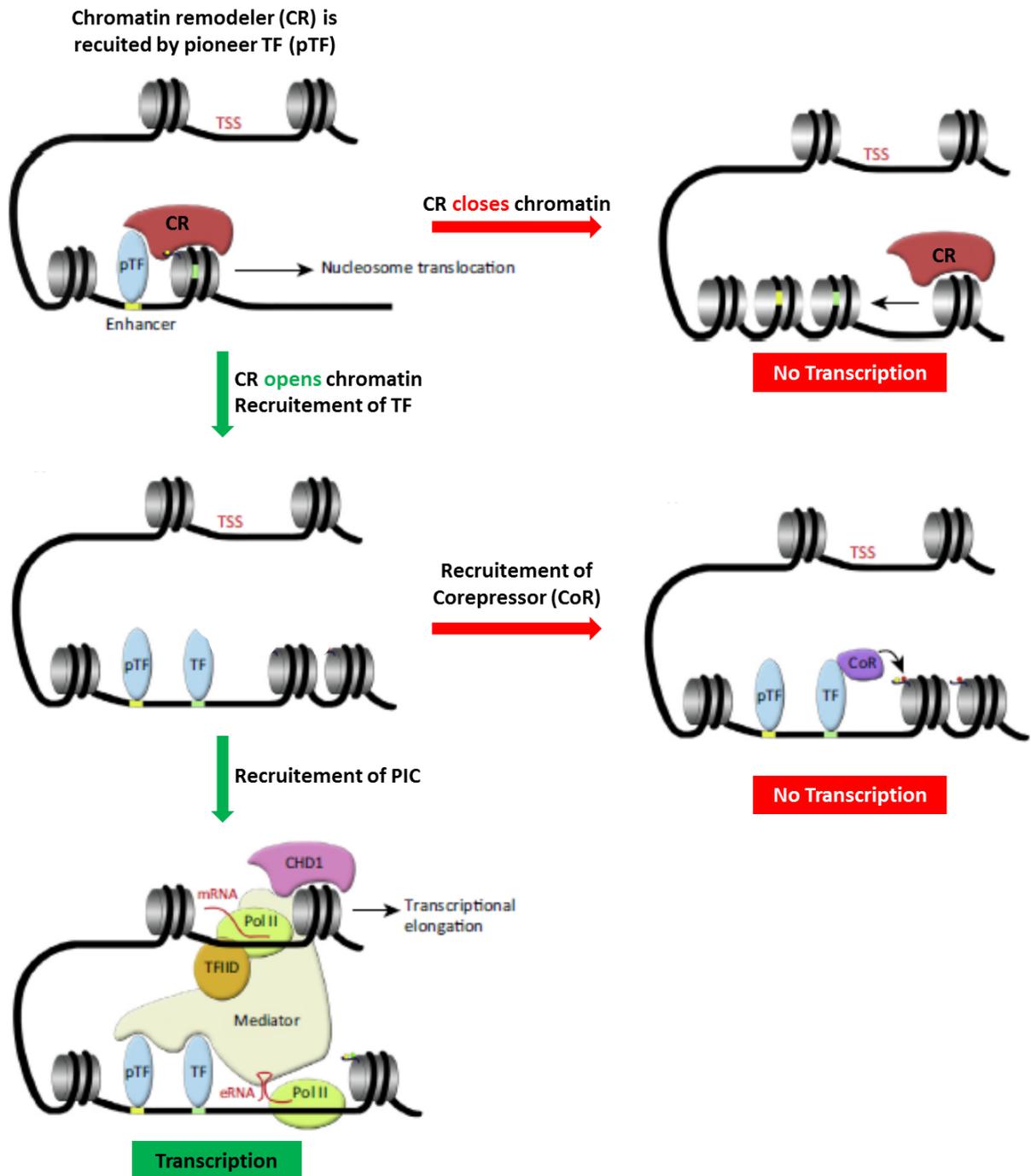
The subfamily **SWI/SNF** (switch/sucrose non-fermentable) is composed of Brahma (Brm, gene *Smarca2*) and Brahma-related gene 1 (Brg1, gene *Smarca4*), two homologs which contain a bromodomain. This domain is important to bind to acetylated histones (Ferri et al., 2016). On note, Brg1 is a part of a complex called BAF (BRM/BRG1-associated factors; Narayanan and Tuoc, 2014) and was shown to play a role in oligodendrogenesis (Yu et al., 2013b). The subfamily **ISWI** (imitation switch) is characterized by the presence of a HAND-SANT-SLIDE domains which bind to nucleosome and nucleosomal DNA. It is composed of SNF2H (*Smarca5*) which is essential for cell survival (Alvarez-Saavedra et al., 2014) and SNF2L (*Smarca1*) which participate in brain development (Yip et al., 2012). The third subfamily is **CHD** (chromodomain helicase DNA binding protein) and is characterized by the presence of two chromodomains which have been shown to bind to methylated histones. There are nine different Chd identified in three different subgroups (Table 3). The last subfamily is **INO80/SWR** (inositol auxotroph mutant 80 / SWI/SNF related) and its members can exchange histones like H2A.Z (Yen et al., 2013), playing a role in gene repression.

Subgroup I	Subgroup II	Subgroup III
Chd1	Chd3	Chd6
Chd2	Chd4	Chd7
	Chd5	Chd8
		Chd9

**Table 3. The three subgroups of Chd proteins.**

Some of them have been shown to be involved in formation of proteic complexes in which we find one chromatin remodeler and different associated subunits that provide specificity. The combination of proteins inside the complex can be different depending on the cell-type or the stage of differentiation. For instance, BAF complexes with contain Brg1 goes through a switch in the composition of its subunits during differentiation of neural progenitor to mature neurons, permitting the expression of different genes (Son and Crabtree, 2014). In neural progenitors, BAF is composed of BAF45a and BAF53a and is involved in self renewal and proliferation; whereas, in mature neurons, nBAF is composed of BAF45b/c and BAF53b and is involved in neurite outgrowth and synapse formation (Son and Crabtree, 2014).

When all these different elements (promoter and enhancers; TFs, cofactors and chromatin modifying factors; mediator complex) are present for transcription initiation (Fig. 27), RNA pol II still have to go through elongation. This is a critical step for transcription as +1 nucleosome represent, for RNA pol II, a “roadblock” where it is going to pause. Different factors are needed to overcome this roadblock like general transcription factors or activators (Kwak and Lis, 2013). Interestingly, chromatin remodeling factors also play a role at this step as it was shown that Chd1 can be recruited by the elongation complex like PAF1 (Lee et al., 2017; Simic et al., 2003).



**Figure 27. Diagram representing the mechanism of transcription initiation.** Pioneer transcription factor (pTF) can bind to closed chromatin and recruits chromatin remodeler (CR). CR either close chromatin repressing transcription or open chromatin to expose binding sites of other TF which is recruited, followed by either a corepressor inhibiting transcription or coactivators permitting the formation of the pre-initiation complex (PIC) and transcription activation. Modified From Basson and van Ravenswaaij-Arts, 2015.

### **III.E – Methods to study chromatin**

Since the emergence of sequencing technics and with the developing of next-generation sequencing, different methods have been developed to study DNA and chromatin. The levels of gene expression in a given cell-type or tissue can be measured by their transcripts. There are different technics to quantify the amount of produced RNAs like microarrays or RNA-seq (Mortazavi et al., 2008). Microarrays (Schena et al., 1995) which consist of matching probes, are more limiting as it cannot detect new sequences. RNA-seq permit a deep analysis of transcripts and permitted to discover non-coding RNAs (Arrigoni et al., 2016). However, these technics give no indications about the proteic level as a gene can be expressed but post-transcriptional regulation can prevent the production of the protein. Proteomic analysis will have to be done to have this information (Humphery-Smith and Blackstock, 1997).

To determine if these targets are direct or indirect, binding sites of the given factor on the chromatin have to be found. To that purpose, a method called chromatin immunoprecipitation (ChIP; Crane-Robinson et al., 1999) permit to select fragments of chromatin bound by a given factor using specific antibodies. We can then identify the fragments after PCR or by deep-sequencing.

Rather recent methods have been developed to determine the opening state of the chromatin. One of them is called FAIRE-seq for Formaldehyde-Assisted Isolation of Regulatory Elements (Nammo et al., 2011). It consist of fixation of proteins on the DNA with formaldehyde followed by sonication. The important step is the separation of free DNA fragments (in aqueous phase) and DNA fragments linked to proteins (in organic phase). Free DNA fragments are then purified and sequenced. Another method to observe the opening state of the chromatin is ATAC-seq (Assay for transposase-accessible chromatin using sequencing; Buenrostro et al., 2013). This technic use a Tn5 transposase to integrate adaptors in accessible region of chromatin causing cuts in the DNA and creating small fragments which are selected and sequenced.

## **Part IV: Chd7 is a chromatin remodeling factors involved in CNS biology**

As mentioned in Part III, transcriptional regulation by TFs starts to be well described during oligodendrogenesis. The use of mutant mice has permit to characterize the functions of Olig2, Sox10, Nkx2.2 or Myrf (Mei et al., 2013; Stolt et al., 2002; Qi et al., 2001; Emery et al., 2009). However, little is known about the mechanisms of their action. The increased understanding of the transcription initiation made us realize that other factors should be included in these study. These other factors has to be determined and characterized to untangle the whole process occurring during oligodendrogenesis. A growing body of recent studies are showing the interest in these factors. Indeed, it was shown that Olig2 works together with Brg1, a chromatin remodeling factor, to promote OL differentiation (Yu et al., 2013b). However, other chromatin remodelers must be involved. Interestingly, *Chd7* (chromodomain helicase DNA binding protein 7) has been identified as an Olig2 target gene and is expressed in oligodendrocyte lineage cells (He et al., 2016). Chd7 is part of the CHD (chromodomain Helicase DNA binding) protein family that are ATP-dependent chromatin remodeling factors and have roles in modifying chromatin compaction in order to activate or inhibit initiation of transcription. In the following part, we focus on what is known about Chd7 function and mechanisms in the CNS.

### **IV.A – CHD7 is involved in CHARGE syndrome**

Mutations of *CHD7* have been associated with CHARGE syndrome in human (Vissers et al., 2004), and are nowadays known to be responsible for most if not all CHARGE syndrome cases (Basson and van Ravenswaaij-Arts, 2015).

#### **IV.A.1 – What is CHARGE syndrome?**

The name CHARGE is an acronym for its symptoms: Coloboma, Hear defects, choanal Atresia, Retardation, Genitourinary malformation and Ear abnormalities (Pagon et al., 1981); review: Hsu et al., 2014) and affects 1/10.000 births (Issekutz et al., 2005). Coloboma is a malformation of the eye characterized by the presence of a hole in one the eye structure (iris, retina...). Heart defects are found in the form of cardiac malformations that can be of all the types (except heterotaxy and cardiomyopathy). Choanal atresia is an obstruction of the back of the nasal structure, leading to airway obstruction. Retardation can involve growth retardation, impaired

cognitive ability and/or affected communication and language ability. Delay or arrest in pubertal development can lead to genital hypoplasia. Ear abnormalities is commonly characterized by the absence of the lateral semicircular canals leading to poor balance and a delay in walking (Hsu et al., 2014). This combination of symptoms and disorders can be life-threatening during neonatal and postnatal periods. Diagnosis is done on the basis of clinical criteria defined by Blake (Blake et al., 1998) and modified later by Verloes (Verloes, 2005; Table 4). Interestingly, using MRI, it was suggested that some CHARGE patients could present thinner corpus callosum (Gregory et al., 2013) that could be due to a possible OLS number decrease.

<b>Table 1</b> CHARGE syndrome diagnostic criteria, revised and updated	
Blake criteria (1998) <sup>6</sup>	Verloes criteria (2005) <sup>7</sup>
Definite (4 major or 3 major and 3 minor)	Typical: 3 major or 2 major and 2 minor
Probable/possible CHARGE (1 or 2 major & several minor features)	Partial: 2 major and 1 minor Atypical: 2 major but no minor, or 1 major and 2 minor
<b>Major criteria</b>	<b>Major criteria</b>
Coloboma	Coloboma (ocular)
Choanal atresia/stenosis	Choanal atresia/stenosis
Characteristic ear anomalies	Hypoplasia/aplasia of semicircular canals
Cranial nerve dysfunction (especially CN VII and VIII)	
<b>Minor criteria</b>	<b>Minor criteria</b>
Genital hypoplasia	Rhombencephalic dysfunction (brainstem and cranial nerve anomalies)
Developmental delay	Hypothalamo-hypophyseal dysfunction
Cardiovascular malformation	Malformation of the internal/external ear
Growth deficiency	Malformation of mediastinal organs (heart, oesophagus)
Orofacial cleft	Intellectual disability
Tracheo-oesophageal fistula	
Distinctive facial features	
CN, cranial nerve.	

**Table 4. CHARGE syndrome criteria.** From Hsu et al., 2014

#### **IV.A.2 – *CHD7* mutations cause CHARGE syndrome**

In 2004, *CHD7* mutations were, for the first time, associated with CHARGE syndrome (Vissers et al., 2004). Diagnosis for this disease is firstly based on clinical features but *CHD7* analysis can be done for mildly affected patients (Bergman et al., 2011). *CHD7* mutations are found in 90% of patients (Janssen et al., 2012), but it is a possibility that for the other 10%, *CHD7* alterations are not detectable by common genotyping strategies (Basson and van Ravenswaaij-Arts, 2015). CHARGE syndrome is an autosomal dominant syndrome with variable expressivity and mutations appear mostly *de novo* but some familial cases have been observed (Jongmans et al., 2008). Moreover, *CHD7* mutations can be of different types: “nonsense and frameshift mutations occur in over 75% of the patients. Missense and splice site mutations comprise an additional 20%, while complete and partial deletions/duplications and chromosomal rearrangements are rare” (Janssen et al., 2012). A database listing *CHD7* mutations has been built and can be found at [www.CHD7.org](http://www.CHD7.org) (Janssen et al., 2012). However, no correlation between *CHD7* mutation type and CHARGE syndrome expressivity and penetrance has been found yet. Importantly, *CHD7* expression pattern in body correlate with abnormalities observed in CHARGE as it is expressed in neural crest cells, brain, developing inner ear, nasal and oral epithelium, heart, kidneys, stomach, gut, and lungs (review: Janssen et al., 2012).

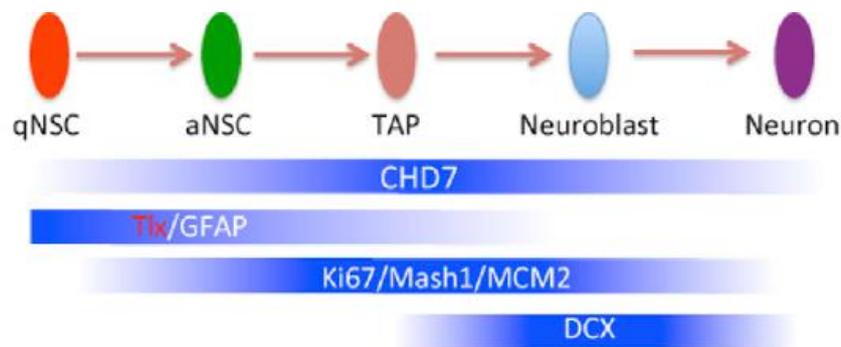
#### **IV.A.3 – Other Syndromes involving *CHD7* mutations**

Some *CHD7* mutations has been associated with autism (O’Roak et al., 2012). It is consistent with the fact that autism behavior have been found in CHARGE syndrome patients (Betancur, 2011). *CHD7* mutations have also been found in Kallmann syndrome (Marcos et al., 2014). It is an inherited developmental disease characterized by hypogonadism due to gonadotropin-releasing hormone (GnRH) deficiency. It affect 1/8.000 boys and 1/40.000 girls, but this number can be underestimated (Dode and Hardelin, 2009). Interestingly, mutations in other genes like *FGF8* or *SOX10* (Pingault et al., 2013) has been associated with this disease (in less than 30% of patients). A recent work also identified *CHD7* as a putative schizophrenia risk gene (Whitton et al., 2016).

## IV.B – Chd7 involvement in CNS biology

### IV.B.1 – Expression of Chd7 in the brain

*Chd7* is expressed in neural crest cells (Bajpai et al., 2010), as well as NSCs during embryonic development (Layman et al., 2009) and in adult brain, co-expressed with *Ascl1* (Feng et al., 2013). Expression of *Chd7* persists in the neuronal fate, in progenitor and neuroblasts (Feng et al., 2013; Micucci et al., 2014), as well as in cerebellar granule cells (Feng et al., 2017), but is downregulated in most mature neurons (Feng et al., 2013; Layman et al., 2009; Fig. 28). However, *Chd7* expression in the oligodendroglial lineage had not been assessed yet.



**Figure 28. Chd7 expression during neurogenesis.** NSCs start to express CHD7 upon exiting the quiescent state, and Chd7 expression persists in neural progenitors and neuroblasts. From (Feng et al., 2013)

### IV.B.2 – Chd7 functions in the brain

As CHARGE syndrome provoke CNS defects showing up as impaired cognitive ability and/or affected communication and language ability, Chd7 function has been studied in the CNS.

#### ***Chd7 is involved in early development***

Homozygous *Chd7* mutant mice cannot survive past E10.5 (Hurd et al., 2007). In mouse ES cells, Chd7 co-localize with Oct4, Sox2 and Nanog pluripotent factors and act as a fine-tuner of expression levels of ES-specific genes (Schnetz et al., 2010). Microarray comparing wild-type and *Chd7KO* mouse E9.5 showed a decrease of expression of genes involved in neural crest cells and axon guidance (Schulz et al., 2014). Moreover, *Chd7* depletion decreased neural crest cells migration in *Xenopus* tadpole, leading to CHARGE features like Coloboma, malformations of the craniofacial cartilage and heart defects (Bajpai et al., 2010). Sox9 and Twist, two factors involved in migration (Sauka-Spengler and Bronner-Fraser, 2008), are

targets of Chd7 in neural crest cells (Bajpai et al., 2010), showing the involvement of Chd7 in cell migration. *Chd7KO* in *Xenopus* also leads to a decrease of *Sema3a* expression which is involved in Kallmann syndrome (Schulz et al., 2014). Taken together, these data show that Chd7 is required during early development for expression of ES-specific genes and neural crest cell migration.

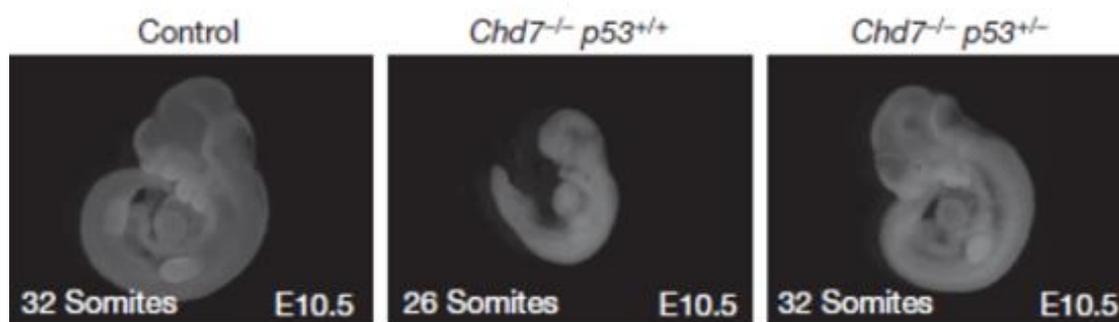
### ***Chd7 regulates Neurogenesis***

Chd7 plays an important role in neurogenesis in different brain regions. Loss of Chd7 in NSCs in SVZ and in SGZ (subgranular zone of the dentate gyrus in the hippocampus) leads to a reduction of neurogenesis in adult mice (Feng et al., 2013). Physical exercise can rescue this defect in SGZ by increasing proliferation and survival (Feng et al., 2013). Loss of Chd7 leads to proliferative and cell-renewal defects in SVZ, in addition to neurogenic deficits (Micucci et al., 2014). Consequently, in *Chd7* deficient mice, olfactory bulbs are smaller with less olfactory sensory neurons and disorganized epithelium (Layman et al., 2009). Furthermore, Chd7 is important for cerebellum embryonic specification in mouse and in human, as cerebellar defects are observed in CHARGE patients (Yu et al., 2013a). In the cerebellum, *Chd7* deletion in granule cells leads to cerebellar hypoplasia with abnormal distribution of Purkinje cells (Feng et al., 2017). Granule neuron progenitors lacking *Chd7* also fail to differentiate with direct downregulation of cerebellar development genes such as *Reln* (Feng et al., 2017). In mice, it translates to developmental delay and motor deficits (Whittaker et al., 2017). However, no effect on proliferation was observed in this context (Feng et al., 2017). On a more mechanistic note, Sox2, a TFs required for NSCs maintenance and neurogenesis (Pevny and Nicolis, 2010) – but also oligodendrogenesis (Hoffmann et al., 2014) – was shown to physically interact with Chd7 to regulate genes of the Notch and Shh pathways (Engelen et al., 2011). Taken together, these data suggest a strong role of Chd7 during neurogenesis explaining the developmental deficit that is observed in CHARGE syndrome patients.

### ***Chd7 inhibits cell death***

Some evidence show apoptosis occurrence in *Chd7* mutant cells. In adult mice, it was shown that *Chd7iKO* NSCs had more contact with microglia than control ones (Feng et al., 2013), suggesting that *Chd7iKO* cells are dying. More recently, it was shown that *Chd7* deletion in granule neuron progenitors increased cell death with expression of Caspase3 (Feng et al.,

2017). Moreover, a direct link between *Chd7* and p53 (*Trp53* gene), which activate apoptosis pathway, has been made previously. Hyperactivation of p53 in mice results in the same phenotype than *Chd7KO* with CHARGE syndrome features like coloboma, ear malformations, heart defects and craniofacial defects (Van Nostrand et al., 2014). Interestingly, deletion of one allele of *Trp53* partially rescue the *Chd7KO* phenotype in mice (Van Nostrand et al., 2014), showing that p53 participate in the phenotype (Fig. 29). Also, p53 is upregulated in *Chd7KO* mouse neural crest cells, as well as in fibroblast from patients with CHARGE syndrome (Van Nostrand et al., 2014). Moreover, a direct genetic interaction exists and has been shown as *Chd7* binds to p53 promoter in mouse neural crest cells (Van Nostrand et al., 2014).



**Figure 29. *Chd7* and apoptosis in neural crest cells.** Results showing the link between *Chd7* and p53 as hyperactivated p53 rescues partially *Chd7KO* phenotype in mice. From (Van Nostrand et al., 2014)

All of these results show that *Chd7* functions in a context-specific manner as its role and targets are different depending on the tissue, cell-type and partners. Indeed, comparison of *Chd7* binding sites in two human cell lines (colorectal carcinoma cells, DLD1 and a neural-like cell line derived from a metastatic neuroblastoma, SH-SY5Y) indicates that *Chd7* binds in a cell-specific manner (Schnetz et al., 2009).

#### IV.B.3 – Mechanisms of *Chd7* function

*Chd7* can act as an activator in ES cells (Schnetz et al., 2010), in neural crest cells (Schulz et al., 2014) and in granule neuron progenitors (Feng et al., 2017). But also as a repressor like in mouse ES cells where *Chd7* deletion leads to increased gene expression (Schnetz et al., 2010), or to repressed *Trp53* expression in neural crest cells (Van Nostrand et al., 2014). Data show that *Chd7* seems to preferentially bind to enhancers. Indeed, in mouse ES cells, *Chd7* binds to more than 10,000 sites (Schnetz et al., 2010) and only 14% of them are in TSSs (Schnetz et al., 2009). The other sites present enhancer features (H3K4me co-localization, hypersensitive to

DNaseI, co-localization with p300) (Schnetz et al., 2010). Moreover, in human colorectal carcinoma cell line (DLD1), Chd7 binds to DNase I hypersensitive sites that are conserved and located distal to TSS (Schnetz et al., 2009). It was also shown that Chd7 binds directly to H3K4me activation marks with its chromodomain, in DLD1 and SH-SY5Y cells (Schnetz et al., 2009). It was recently shown *in vitro*, that Chd7 bind to 155 bp 'core' nucleosomes and that Chd7 slides nucleosomes along the DNA (Manning and Yusufzai, 2017).

In 2012, Chd7 was identified as an ATP-dependent nucleosome remodeling factor (Bouazoune and Kingston, 2012) and ATP hydrolysis of Chd7 was confirmed in a recent work (Manning and Yusufzai, 2017). After looking at chromatin state in mutants, it seems that Chd7 works as a chromatin opener. MNase used on *Chd7iKO* NSCs showed that deletion of Chd7 leads to more compacted chromatin in Sox4 and Sox11 promoters, two targets of Chd7 in NSCs (Feng et al., 2013). Furthermore, using ChIP-seq and ATAC-seq in *Chd7iKO* granule neuron progenitors, it was shown that Chd7 participate at keeping an open chromatin state in genes involved in neuronal differentiation like *Reln* (Feng et al., 2017; Whittaker et al., 2017). All these data show that Chd7 binds mostly in regions presenting enhancer features and is an activator of gene expression by opening chromatin.

### ***Chd7 can associates with Brg1***

Proteomic analysis of Chd7 in human neural crest-like cells showed that Chd7 associates with several subunits of PBAF (polybromo- and BRG1-associated factor-containing complex), chromatin remodeling complexes which contain Brg1 (Bajpai et al., 2010). Moreover, Brg1 depletion in *Xenopus* tadpole leads to a phenotype close to the Chd7 depletion one, including Coloboma and craniofacial malformations (Bajpai et al., 2010). Comparison of Chd7 (Schnetz et al., 2009) and Brg1 (Ho et al., 2009) binding sites in mouse embryonic stem cells (ESCs) showed that most of these regions are common (81%) and that most of them are bound within 1kb from a TSS (89%; Bajpai et al., 2010). A recent analysis of more than 200 datasets from ChIP experiments in ESCs discovered that Chd7 and Brg1 co-localize extensively in distal but not proximal regions (Yang et al., 2017). These co-localization sites are also bound by master TFs of NSCs (Nanog, Sox2, and Oct4) and present active chromatin architecture (H3K27ac, p300, and H3K4me1 sites; Yang et al., 2017). As it was shown that Brg1 play a role during oligodendrogenesis by interacting with Olig2, we can wonder if Chd7 can have a similar role than Brg1 in OL differentiation.

#### **IV.C – Other members of Chd protein family**

Chd7, due to its implication in CHARGE syndrome in human, has been well studied in the CNS. However, its involvement in oligodendrogenesis had not been explored. As explained above, Chd7 is part of the Chd protein family which include 9 members divided in 3 different subfamilies. To better understand how Chd proteins work and their different functions, we can have overview of each members.

##### **IV.B.1 – Kismet is the *Drosophila* homologue of Chd7**

Kismet (Kis) is the *Drosophila* homologue of Chd7 and other Chd subgroup III members. Kis interacts genetically with Polycomb and is important for determination of body segment identity (Daubresse et al., 1999). It was shown that Kis and Brahma (BRM) contains conserved domains, putting Kis in the family of chromatin remodeling factors (Daubresse et al., 1999). Indeed, Kis contains ATPase but also BRK and Chromodomains (Srinivasan et al., 2005). Interestingly, Kis loss-of-function leads to defect in locomotion, memory and axon development in *Drosophila* (Melicharek et al., 2010). It was shown that Kis is expressed in neurons and is important for motor neuron synaptic morphology and transmission (Ghosh et al., 2014). Mechanistically, little is known about the action of Kis on the chromatin. Looking at its distribution in larval salivary gland, it was shown that Kis is present in active chromatin (Srinivasan et al., 2005). Kis play a role during early step of transcriptional elongation, as deletion of Kis reduce the level of elongating RNA Pol II and Chd1 present in chromosomes (Srinivasan et al., 2005; Srinivasan et al., 2008). Kis also play in the recruitment of histone methyltransferases to chromatin reducing H3 lysine 27 methylation and transcription repression (Dorigi and Tamkun, 2013; Srinivasan et al., 2008).

##### **IV.B.2 – Chd Subgroup III**

As Chd7 is a member of the Subgroup III, we can expect to find more similarity with these factors.

###### **IV.B.2.a – Chd8**

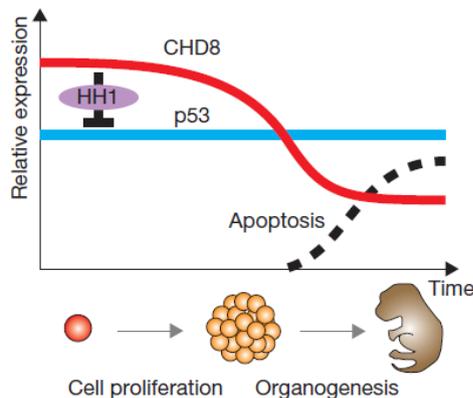
Chd8, with Chd7, is the most studied Chd protein. Interestingly, an interaction between Chd7 and Chd8 has been shown and, like Chd7, Chd8 is involved in regulation of apoptosis and mutation of *Chd8* is implicated in a disease affecting CNS in humans.

### ***Chd8 interacts with Chd7***

Using a yeast two-hybrid library screen, Chd8 was found as a binding partner of Chd7 (Batsukh et al., 2010). Induced missense mutation in Chd7 that are known in CHARGE syndrome, showed a disruption of the Chd7-Chd8 interaction only in yeast two-hybrid experiment. This was not observed in co-immunoprecipitation experiment showing that this interaction could be both direct and indirect (Batsukh et al., 2010). The authors hypothesized that disruption of Chd7-Chd8 interaction could be a mechanism involved in CHARGE syndrome (Batsukh et al., 2010).

### ***Chd8 inhibits cell death during development***

Mouse lacking Chd8 die from massive apoptosis at E7.5 (Nishiyama et al., 2004). Moreover,



**Figure 30. Chd8 inhibits apoptosis during development by recruitment of histone H1.** From (Nishiyama et al., 2009)

overexpression of Chd8 induces apoptosis *in vitro* with upregulation of p53 target genes (Nishiyama et al., 2009). It was shown that Chd8 forms a complex with p53 and histone H1 in the promoter of p53 target genes to inhibit their transcription (Nishiyama et al., 2009; Fig. 30). Double mutant *Chd8*<sup>-/-</sup> *p53*<sup>-/-</sup> die at E10.5 – against E7.5 in *Chd8*<sup>-/-</sup> – from severe hemorrhages (Nishiyama et al., 2009), highlighting the functions of Chd8 in cardiovascular system (Shanks et al., 2012).

### ***Chd8 is a risk gene in Autism Spectrum Disorder (ASD)***

The early death of *Chd8*-null mice and the lack of conditional mouse line made it difficult to study Chd8 function *in vivo*. However, evidences show clearly that Chd8 has an important role in the CNS. *CHD8* mutations has been identified as important risk factors of autism spectrum disorder (ASD). *CHD8* regulated genes that have been defined as ASD risk genes (Cotney et al., 2015), highlighting the gene network regulated by *CHD8* and associated with ASD.

Autism was described by Kanner in 1943 (Kanner, 1968) and Asperger in 1944. ASD affect 1/100 births worldwide and boys are four time more affected than girls. Symptoms vary

between patients but three core behavioral symptoms have been defined: impairment in social interactions, communication, and the propensity for repetitive behaviors (Miles, 2011).

Patient characteristics	Number (%)
ASD	13/15 (87%)
Tall stature	12/14 (86%)
Macrocephaly*	12/15 (80%)
GI problems*	12/15 (80%)
Sleep problems	10/15 (67%)
Intellectual disability	9/15 (60%)

\*Indicates significantly different from the typical ASD population.

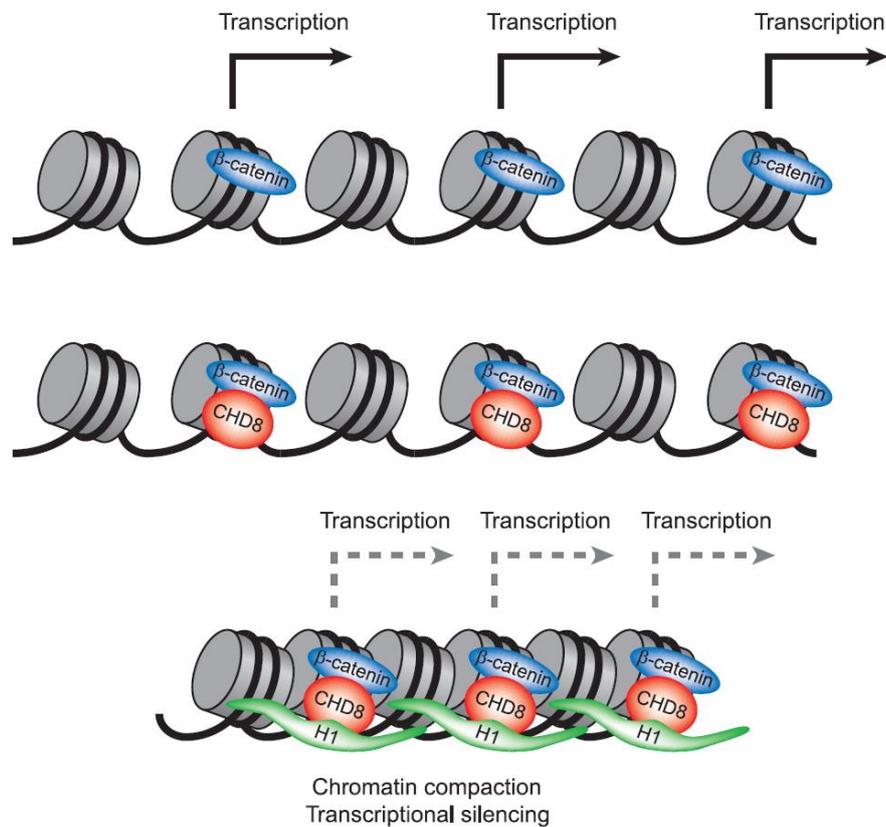
**Table 5. Phenotypic summary of patients with CHD8 mutations.** From (Barnard et al., 2015).

*CHD8* disruption has been defined as a subtype of ASD as patients develop characteristic symptoms like macrocephaly, distinct faces, and gastrointestinal complaints (Barnard et al., 2015; Bernier et al., 2014a; Table 5). These features have been recapitulated in zebrafish after *Chd8* LOF due to forebrain/midbrain expansion and decrease of postmitotic enteric neurons (Bernier et al., 2014a). Macrocephaly has also been observed in mouse model (Platt et al., 2017). Interestingly, ASD has also been linked to CHD7 mutations (O'Roak et al., 2012) and autistic features are found in CHARGE syndrome patients (Betancur, 2011).

### ***Wnt pathway***

*Chd8* has first been identified as a nuclear protein that inhibits Wnt signaling pathway in *Xenopus* (Kobayashi et al., 2002; Sakamoto et al., 2000). *Chd8* was then called Duplin (axis duplication inhibitor) because it prevents  $\beta$ -catenin induced axis duplication in *Xenopus* embryos as *Chd8* inhibits the binding of Tcf in  $\beta$ -catenin gene (Sakamoto et al., 2000). Furthermore, it was shown that *Chd8* can interact directly with  $\beta$ -catenin and can bind to promoter of  $\beta$ -catenin target genes to inhibit them (Thompson et al., 2008) by recruitment of histone H1 to the promoters (Nishiyama et al., 2012) (Fig. 31). However, it seems that *Chd8* regulate Wnt signaling pathway in a cell-type specific manner as in mouse neural progenitors, *Chd8* knock-down leads to downregulation of its components (Durak et al., 2016).

Interestingly, other genes involved in Wnt pathway has been shown to be mutated in ASD patients, showing the implication of this pathway in autism (Kalkman, 2012).



**Figure 31. Chd8 driven gene repression. Scheme representing repression of β-catenin target genes by Chd8 recruiting histone H1. From (Nishiyama et al., 2012).**

### ***Chd8 is involved in progenitor proliferation and neurogenesis***

*Chd8* is highly expressed in the cortex in mouse at E12 and in Human during fetal stage before decreasing with corticogenesis (Durak et al., 2016). Knock down of *Chd8* at embryonic stage in mice, shows a decrease of proliferation of neural progenitors associated with cell cycle exit (Durak et al., 2016), leading to a premature depletion of neural progenitor pool. Chd8 promote expression of genes involved in cell cycle progression by directly binding to promoters of cell cycle genes (Durak et al., 2016). These results are consistent with another study showing that depletion of Chd8 in C33A cell line reduce proliferation as Chd8 activate expression of *Ccne2* and *Tyms*, two genes involved in G1/S transition (Rodriguez-Paredes et al., 2009). A very recent work confirm the involvement of Chd8 in cell cycle regulation as well as histone and chromatin modification in adult mice (Platt et al., 2017). Furthermore, overexpression of *Chd8*

in neural progenitors reduces cell cycle exit and neuronal differentiation (Durak et al., 2016). Transcriptional analysis in human neural progenitors showed that Chd8 regulate genes involved in brain development, including synapse formation, neuron differentiation, cell adhesion and axon guidance (Sugathan et al., 2014). Also, analysis of transcriptome of patients with loss of functional Chd8, show a decrease of expression of genes involved in neuronal development (Wilkinson et al., 2015). Interestingly, Chd8 LOF mutations leads to synaptic dysfunctions (Platt et al., 2017). Chd8 has been recently reported to be expressed in the CNS in mature neurons, interneurons, and in macroglial cells (oligodendrocytes and astrocytes; Platt et al., 2017). But a detailed characterization of Chd8 expression and functions in glial cells has not been investigated yet.

### ***Mechanisms of Chd8 function***

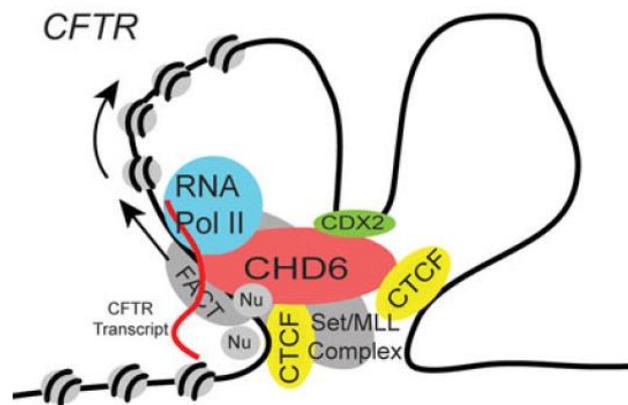
Mechanistically, Chd8 has been shown to act mostly as a repressor (Nishiyama et al., 2009; Sakamoto et al., 2000; Yates et al., 2010) but also as an activator (Durak et al., 2016; Rodriguez-Paredes et al., 2009) of gene expression, depending of the cell type. As a Chd protein, Chd8 possesses an ATPase activity stimulated by nucleosomal DNA (Thompson et al., 2008) and, like Chd7, slides nucleosomes along the DNA (Manning and Yusufzai, 2017). Interestingly, it was shown that Chd8 interacts directly with CTCF *in vitro*. They bind together at the same sites and have an enhancer-blocking activity (Ishihara et al., 2006). It was also shown in multiple studies that Chd8 binds mostly to promoters (Nishiyama et al., 2009; Sugathan et al., 2014; Thompson et al., 2008; Yates et al., 2010) at the binding site of RNA Pol II (Rodriguez-Paredes et al., 2009). In ES cells, Chd8 can bind to H3K4me3-enriched sites (de Dieuleveult et al., 2016) as it had been shown in other cell lines like HeLa (Rodriguez-Paredes et al., 2009; Yuan et al., 2007).

### ***IV.B.2.b – Chd6***

Chd6 was discovered in 2002 and defined the new CHD subfamily III (Schuster and Stoger, 2002). Chd6 was first named Chd5 before being renamed when Chd5, a part of subfamily II, was discovered in 2003 (Thompson et al., 2003). *Chd6* is expressed in human and mouse in various tissues with highest level of expression in the brain (Lein et al., 2007; Su et al., 2004). Accordingly, some rare cases of Chd6 mutations in the human population have been reported associated with moderate to severe mental retardation (Kalscheuer et al., 2008; Yamada et al., 2010). Deletion of Chd6 exon 12 which encode a part of its ATPase domain, reveals a

decrease in coordination in mice but no difference has been found yet in the brain of mutant mice compared to controls (Lathrop et al., 2010).

*In vitro* study on cell lines showed that Chd6 colocalizes with RNA Pol II at the sites of mRNA synthesis and is a part of a bigger complex of high molecular weight (Lutz et al., 2006). Chd6 interact with CTCF in cooperation with Chd8 in CF-PAC cell line to participate in the organization and formation of chromatin loops (Sancho et al., 2015; Fig. 32). In ES cells, Chd6 binding to nucleosome is not linked to H3K4me3 presence and Chd6 follows the same pattern of binding than Chd8 (de Dieuleveult et al., 2016). However, as Chd7 and Chd8 both slide nucleosome along the DNA, Chd6 disrupts nucleosomes (Manning and Yusufzai, 2017).



**Figure 32. Model of Chd6 involvement in loop formation.**  
From (Sancho et al., 2015)

#### **IV.B.2.c – Chd9**

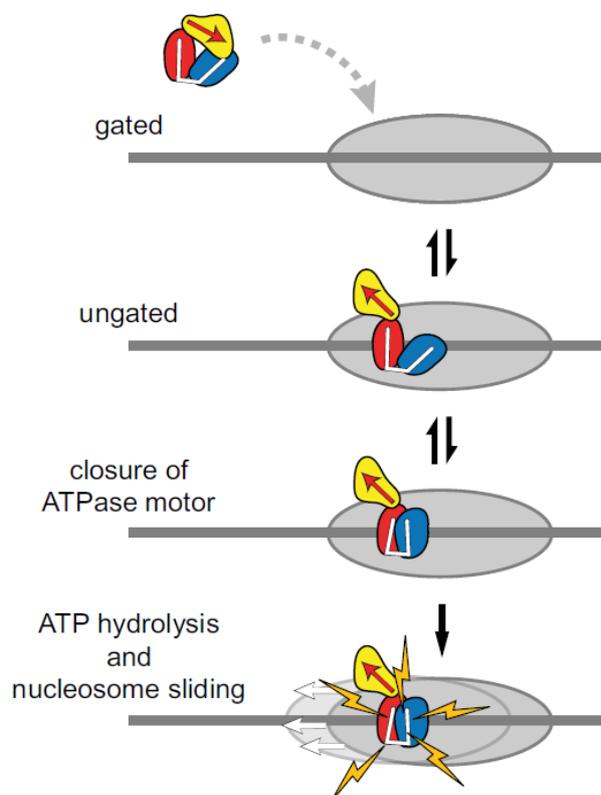
Chd9 is the last member of Chd family. *Chd9* mutations has been found in neuroblastoma (Lasorsa et al., 2016). However, the link between neuroblastoma and Chd9 mutations seems to be indirect as it is associated with metastatic spread in bones which is common in neuroblastoma and leads to low survival rate. Indeed, it was shown that Chd9 regulate osteogenesis (Shur et al., 2006).

#### **IV.B.3 – Chd Subgroup I**

##### **IV.B.3.a – Chd1**

*Chd1* is already expressed at 1-cell stage (Suzuki et al., 2015). Knock down of *Chd1* in mouse embryos, severely decreased expression of key regulators of cell fate like Pou5f1, Nanog and Cdx2 (Suzuki et al., 2015). However, *Chd1*<sup>-/-</sup> mice die at E5.5 due to defect in proliferation and cell death (Guzman-Ayala et al., 2015) and, after removal of p53, *Chd1*<sup>-/-</sup> mice die at E7.5 (Guzman-Ayala et al., 2015).

Interestingly, Chd1 is required to open chromatin in embryonic stem cells and to maintain pluripotency (Gaspar-Maia et al., 2009; Piatti et al., 2015). Chd1 mechanism has been studied in yeast, showing that Chd1 is required to maintain the nucleosome structure (Gkikopoulos et al., 2011; Park et al., 2014) and spacing (Lusser et al., 2005; Pointner et al., 2012; Stockdale et al., 2006). Chd1 participate in transcription elongation as it is recruited by elongation complex like PAF1 (Lee et al., 2017; Simic et al., 2003). Also, in mouse ES cells, Chd1 is enriched at nucleosome +1 where it must play a role in helping the RNA Pol II to cross the nucleosome barrier (de Dieuleveult et al., 2016). Importantly, Chd1 is the Chd protein for which we have the most information about the structure as its ATPase domains in association with its chromodomain has been resolved by crystallography in 2010 (Hauk et al., 2010). A



**Figure 33. Model of Chd1 ATPase action.** From (Hauk et al., 2010).

very recent work studied the Chd1 conformation when bound to nucleosome. They found a significant conformational difference that allow Chd1 activity only if bound to nucleosome (Sundaramoorthy et al., 2017; Fig. 33). Furthermore, another recent study investigated the domain-domain communication of Chd1 on the nucleosome (Nodelman et al., 2017). They showed that chromodomain and ATPase bind to specific location on the nucleosome suggesting a role of Chd1 in nucleosome assembly and spacing (Nodelman et al., 2017).

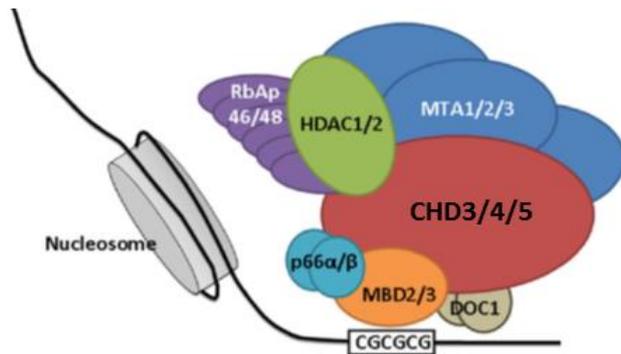
#### **IV.B.3.b – Chd2**

*Chd2* mutations are found in human in epileptic encephalopathies and associated with photosensitive epileptic encephalopathy (Helbig and Tayoun, 2016). LOF of *Chd2* in mice leads to growth delay and perinatal lethality (Marfella et al., 2006). Heterozygous mice show

decrease viability and abnormalities in most primary organs like in kidneys, heart or liver (Marfella et al., 2006).

#### IV.B.4 – Chd Subgroup II – NuRD Complex

The Chd subgroup II is composed of Chd3, Chd4 and Chd5. All three Chds can be part of the NuRD complex, named for Nucleosome Remodeling and histone Deacetylation. It is a complex made of multiple subunits in which we can find Chd3/4/5, histone deacetylases HDAC1/2,

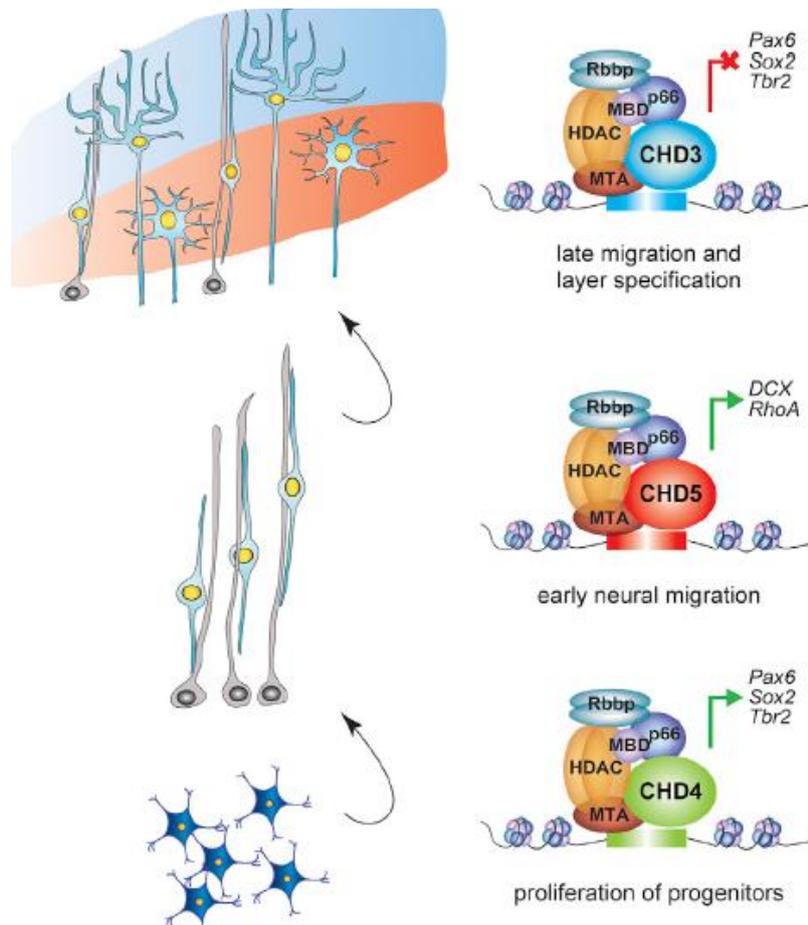


**Figure 34. Scheme of the NuRD complex.** Modified from (Torchy et al., 2015).

histone chaperones RbAp46/48, CpG-binding proteins MBD2/3, the GATAD2a (p66a) and/or GATAD2b (p66b) and specific DNA-binding proteins MTA1/2/3 (Torchy et al., 2015; Fig. 34). The coupling of Chd and Hdac proteins highlights the need of open chromatin for Hdac activity (Verreault et al., 1998).

In ESCs, Chd4 is required for maintenance of self-renewal (Zhao et al., 2017) and it was shown that, in CNS, NuRD complex is important for synapse formation and that *Chd4cKO* mice present a decrease of granule neuron parallel fiber/Purkinje cell synapses in the cerebellum (Yamada et al., 2014). Moreover, Knock-Out of Chd4 impairs dendrite pruning, resulting in granule neurons hypersensitivity (Yang et al., 2016b). Interestingly, in mouse PNS, NuRD complex is required for myelination as *Chd4cKO* in Schwann cells develop hypomyelination in the peripheral nerve (Hung et al., 2012). However, no functions for Chd4 and NuRD complex have been shown for oligodendrocytes yet.

In a recent paper, authors decide to dissect the role of NuRD complex in mouse cortical development and showed a switch of Chd proteins during this process (Nitarska et al., 2016). Indeed, Chd composition of NuRD change during neurogenesis and their occupancy in the complex are exclusive (Nitarska et al., 2016; Fig. 35). Importantly, their functions are non-redundant as deletion of each factors alters cortical development in different ways (Nitarska et al., 2016). *Chd4cKO* in neural progenitors leads to premature exit of cell-cycle (Nitarska et al., 2016). Loss of *Chd5* affect cell migration (Nitarska et al., 2016). And Knock-Down of *Chd3* by shRNA affects neural radial migration (Nitarska et al., 2016).

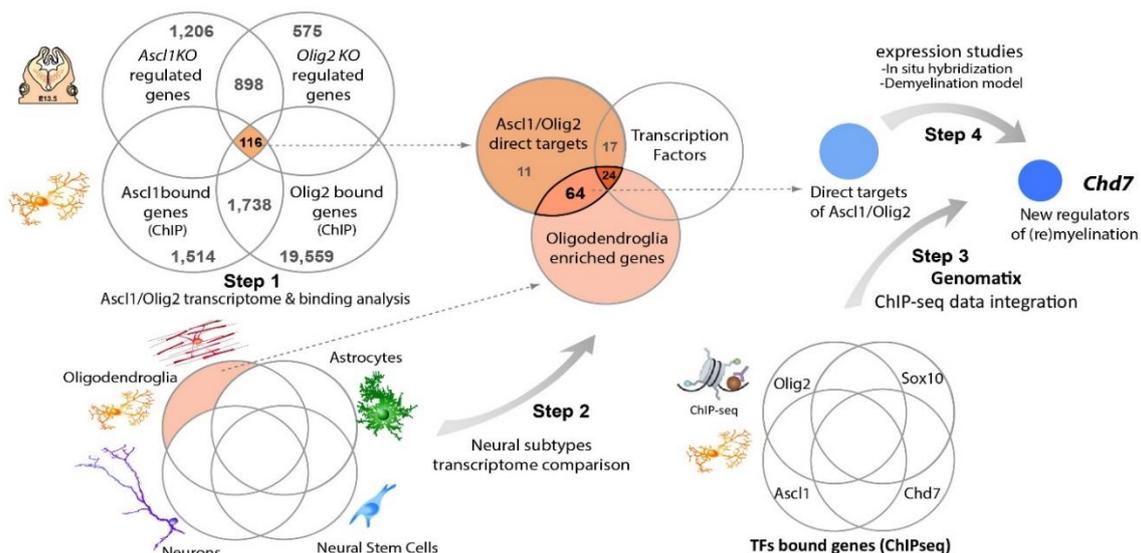


**Figure 35. Model of Chd switch in NuRD complex during cortical development.**  
From (Nitarska et al., 2016).

Overall, these data show that Chd proteins have distinct functions that depend on context and cell-type. In the future, more should be known about these factors as a lot of them are involved in human pathologies.

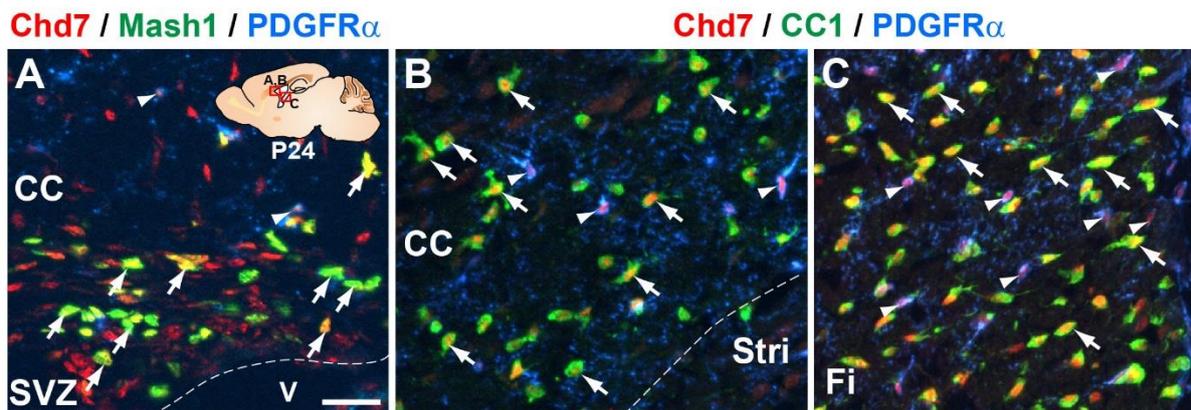
# Objectives

Our lab has recently focused on understanding the transcription program controlling neural stem cells differentiation into oligodendrocytes. The rationale of this work relies on previous studies demonstrating that the bHLH transcription factors Olig2 and Ascl1 work in synergy to specify OPCs, the oligodendrocyte progenitor cells (Lu et al., 2002; Parras et al., 2004; Parras et al., 2007; Sugimori et al., 2007; Sugimori et al., 2008; Wang et al., 2006; Xin et al., 2005; Zhou and Anderson, 2002). In order to understand at a genomic and transcriptomic level how Ascl1 and Olig2 work together to specify OPCs, we followed a strategy using genome-wide transcriptome analysis and chromatin immunoprecipitation. We characterized Ascl1 and Olig2 regulated genes comparing genome-wide the transcriptomes (microarrays) from *Ascl1KO*, *Olig2KO* and wild-type (WT) embryonic ventral telencephalon (at embryonic day 13, E13.5). To identify Ascl1&Olig2 common direct target genes, we used chromatin-immunoprecipitation and sequencing (ChIP-Seq) to assess Ascl1&Olig2 genome-wide occupancy in OPCs. In order to perform an integrative analysis of the neural stem cell (NSCs) and oligodendroglial lineage differentiation processes, we coupled our Ascl1/Olig2 analysis with epigenetic information for active transcriptional enhancers in NSCs and OPCs that we combined with a cell-type specific transcriptomic analysis of the major cell types in the brain (Zhang et al., 2014; Fig. 1; *Clavairolly and Parras, unpublished*).



**Figure 1. Project experimental design for identification of Ascl1/Olig2 common direct targets.** Scheme representation of the different sets of data generated or processed to identify both the direct targets common to Ascl1 and Olig2, and those that are enriched in the oligodendroglial cells. Each Venn circle represents a gene dataset. Time flows from left to right. *In silico* studies are depicted with grey arrows and biological experiments with red arrows. Final focus in few selected genes is shown with relative size corresponding to the functional data cumulated for each selected gene (e.g. *Chd7*).

We identified new specific candidate genes involved in OPCs differentiation. For this thesis project, we focused on *Chd7*, a gene whose expression is driven by *Ascl1* & *Olig2* and is strongly expressed in *Ascl1*<sup>+</sup> and *Olig2*<sup>+</sup> cells (Fig. 2). Moreover, as a member of the CHD protein family, *Chd7* is an ATP-dependent chromatin remodeling factors which, together with TFs, play important roles for gene expression initiation by changing chromatin conformation. Studying expression and function of *Chd7* and paralogs (i.e. *Chd8*) in OLs could therefore have a high impact to better understand the mechanism of gene activation during OL differentiation.



**Figure 2. Chd7 protein in Mash1<sup>+</sup> cells and oligodendroglia (Olig2<sup>+</sup> cells).** Sagittal sections of P24 brains immunostained for Chd7 antibody. Arrows and arrowheads indicate some examples of labelled cells. (A) Coexpression of Chd7 and Mash1 in many cells in the SVZ (transit amplifying precursors, arrows) and in PDGFRα<sup>+</sup> OPCs (arrowheads) in the CC. Schematic inset indicates territories shown in each panel. (B,C) Chd7-expressing cells in the CC (B) or in the fimbria (C) are either OPCs (PDGFRα<sup>+</sup> cells, arrowheads) or maturing oligodendrocytes (APC<sup>+</sup> cells, arrows). Note that the highest levels of Chd7 protein expression are found in APC<sup>+</sup> cells. V, ventricle; Fi, fimbria; CC, corpus callosum, Stri, striatum. Scale bar, 20 μm.

# **Methods**



## ***Animals***

Mice homozygous for floxed alleles of *Chd7* (*Chd7<sup>Flox/Flox</sup>*) were crossed with PDGFR $\alpha$ -CreERT mice to generate the OPC-specific *Chd7iKO* (*iKO*) mice. *Chd7* deletion was controlled by immunostaining and RTqPCR. Animals of either sex were used in the study and Cre negative littermates were used as controls. The mouse strains used in this study were generated and housed (six or less animals per cage) in a vivarium with a 12-h light/dark cycle. Wild type Swiss mice have been ordered from Janvier. All animal studies were conducted following protocols approved by local ethical committees and French regulatory authorities (#03860.07).

## ***Tamoxifen administration and Tissue processing***

For tamoxifen treatment, tamoxifen (T5648) was dissolved in corn oil (Sigma, C-8267) and injected subcutaneously at 20mg/ml concentration at postnatal stages (40 $\mu$ l at P1 and 30 $\mu$ l at P4). For pifithrin- $\alpha$  (PFT) treatment, PFT was dissolved in DMSO (less than 10% final) and NaCl and injected subcutaneously at 0.8 mg/ml concentration at postnatal stages (5 $\mu$ l at P3, P4 and P5). P7 Mice were anesthetized with euthasol and perfused with 2% paraformaldehyde (PFA). Brains were dissected, dehydrated in 20% sucrose at 4°C, embedded in OCT and cryosectioned at 14  $\mu$ m.

## ***Demyelinating lesions***

Before surgery, adult (2-3months) mice were weighted and anesthetized by intraperitoneal injection of mixture of ketamine (0.1 mg/g) and xylazine (0.01 mg/g). Focal demyelinating lesions were induced by stereotaxic injection of 1 $\mu$ l of lysolecithin solution (LPC, Sigma, 1% in 0.9%NaCl) into the corpus callosum (CC; at coordinates: 1 mm lateral, 1.3 mm rostral to bregma, 1.7 mm deep to brain surface) using a glass-capillary connected to a 10 $\mu$ l Hamilton syringe. Animals were left to recover in a warm chamber before being returned into their housing cages. Brains were collected 2 or 4 days after lesions (2-4 dpi).

## ***MS and non-neurological control tissues***

Autopsy brain tissue samples from patients with confirmed secondary progressive MS were obtained from the United Kingdom MS tissue bank (Richards Reynolds, Imperial College, London). MS tissue block containing active lesions and periplaque white matter were selected for analysis.

### ***Immunohistochemistry***

Cryosections (14- $\mu$ m thick) were permeabilized and blocked in blocking buffer (0.05% Triton X-100 and 10% normal goat serum in PBS) for 1 h and overlaid with primary antibodies overnight at 4 °C. Antibodies used in the study were: rat anti-PDGFR $\alpha$  (BD Bioscience, 558774, 1:250), mouse anti-APC (CC1, Oncogene Research, OP80, 1:100), rabbit anti-Chd7 (Cell signaling, 6505, 1:1000), sheep anti-Chd7 (R&D, AF350, 1:100) rabbit anti-APC (Santa Cruz, sc-896, 1:100), mouse anti-Nkx2.2 (gift from Brahim Nait-Oumesmar's lab, 1:4), mouse anti-Olig1 (NeuroMab, 75-180, 1:500), rabbit anti-Itpr2 (Millipore, AB3000, 1:40), rabbit anti-Chd8 (Bethyl, A301-224A, 1:1000), mouse-anti MCM2 (BD biosciences, 610701, 1:500), rabbit anti-p53 (Leica, P53-CM5P-L, 1:500), mouse anti-CNP (Millipore, MAB326R, 1:250). After washing with 0.05% Triton X-100 in PBS, sections were incubated with secondary antibodies conjugated to Alexa488, Alexa594 or Alexa647 (Thermo, 1:1,000) and DAPI for 1h at room temperature, washed in PBS and mounted with Fluoromount-G (SouthernBiotech).

For cells, coverslips were fixed in 4% paraformaldehyde for 10 minutes at room temperature and washed in PBS. They were blocked for 1h at room temperature in blocking buffer (0.05% Triton X-100 and 10% normal goat serum in PBS) and overlaid with primary antibodies for 30min at room temperature. After washing with 0.05% Triton X-100 in PBS, cells were incubated with secondary antibodies conjugated to Alexa488, Alexa594 or Alexa647 (Thermo, 1:1,000) for 30min at room temperature, stained in DAPI for 5 min, washed in PBS and mounted with Fluoromount-G (SouthernBiotech).

Photos of were taken with Zeiss microscope using Apotome system optical sectioning and deconvolution. Z-stack was used. Photo are treated and cells were counted using Zen and ImageJ software packages.

### ***OPCs MAC sorting***

Cortex and corpus callosum were dissected from P7 mouse brains from control, *Chd7iKO* or wild type mice. Dissociation of tissues was done using neural tissue dissociation kit (T) (Miltenyi biotec) and dissociator (gentleMACS Octo Dissociator, Miltenyi Biotec). Magnetic sorting was done using anti-O4-coupled-beads and the MultiMACS Cell24 Separator Plus (Miltenyi biotec). To control obtained sorted cells, cells were put on coverslips coated with poly-ornithine for a couple of hours and fixed in 4% paraformaldehyde for 10 minutes, before doing immunostaining. For RNA-seq, at least 1.10<sup>5</sup> cells of each sample (controls and mutants)

were directly processed after MACs. For ChIP-seq, O4<sup>+</sup>-cells from wild type mice were sorted and directly fixed in 1% formaldehyde.

### ***RNA extraction and qRT-PCR.***

Analyses were conducted with RNA extracts from MACsorted O4<sup>+</sup> cells from P7 mutant mice and their littermate controls. Total RNA was extracted using Nucleospin RNA kit (Macherey-Nagel). cDNAs were generated with SuperScript cDNA Synthesis Kit (Invitrogen). RTqPCR was performed using LightCycler® 96 real-time PCR system (Roche) and primers for mouse gene sequences were: Chd7-f, CAGCAGCATCTGCATCATCT, Chd7-r, GACCCAGGTGTCCAGAAGAG; Ascl1-f, ACTTGA ACTCTATGGCGGGTT, Ascl1-r, CCAGTTGGTAAAGTCCAGCAG; Olig2-f, GAAGCAGATGACTGAGCCCGAG, Olig2-r, CCCGTAGATCTCGCTCACCAG; Pdgfra-f, ACAGAGAC TGAGCGCTGACA, Pdgfra-r, CTCGATGGTCTCGTCCTCTC; Sox10-f, CAGGTGTGGCTCTGCCACG, Sox10-r, GTGTAGAGGGGCCGCTGGGA; Nkx2-2-f, TGGCCATGTACACGTTCTGA, Nkx2-2-r, CCGATGCTCAGGAGACGAAA; Gpr17-f, ACACAGTTGTCTGCCTGCAA, Gpr17-r, GCCGTAGTGGGT AGTTCTTG; Myrf-f, CCTGTGTCCGTGGTACTGTG, Myrf-r, TCACACAGGCGGTAGAAAGTG; Cnp-f, TCCACGAGTGCAAGACGCTATTCA, Cnp-r, TGTAAGCATCAGCGGACACCATCT; Mbp-f, CCAAGTTCACCCCTACTCCA, Mbp-r, TAAGTCCCCGTTTCCTGTTG; Trp53-f, GGGGAGGAGCCAGG CCATCA, Trp53-r, CCGCGCCATGGCCATCTACA;  $\beta$ -actin-f, TCCTAGCACCATGAAGATCAAGATC,  $\beta$ -actin-r, CTGCTTGCTGATCCACATCTG.  $\beta$ -actin was used to normalize.

### ***RNA-seq and data analysis.***

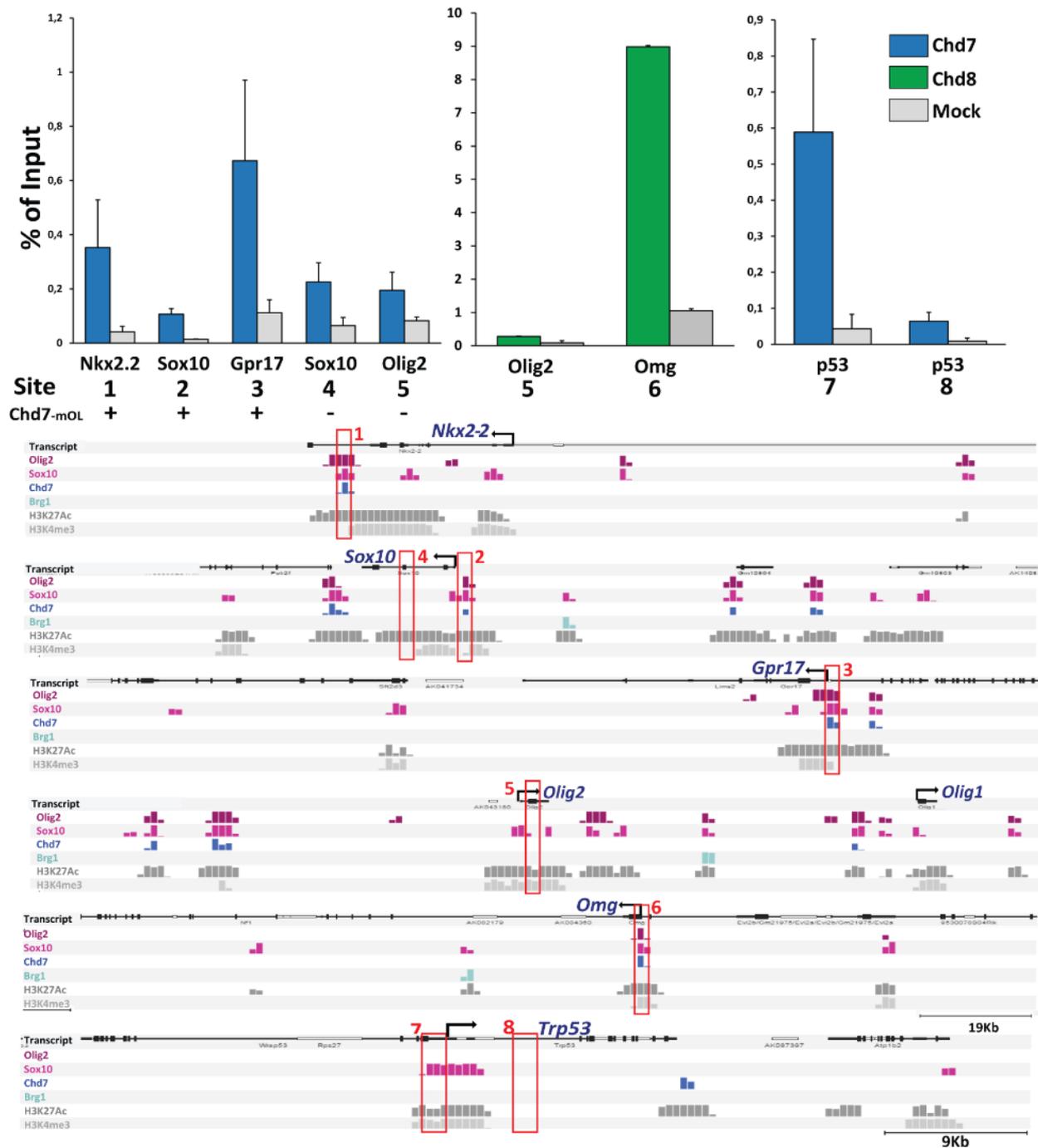
RNA-seq libraries from control (n=7) and Chd7iKO (n=5) O4<sup>+</sup> cells were prepared and sequenced. All RNA-Seq data were aligned to mm10 using TopHat59 with default settings. We used featureCounts for the gene-scaled counting and edgeR to analyze differentially expressed transcripts. In all differential expression tests, genes were considered regulated when p-value < 0.05 and Fold change > 1.2. Heatmap of gene expression was generated using R language (<http://www.r-project.org>). GO analysis of genes repressed and increased in Chd7iKO mutants was performed using Pathway.

### ***ChIP-PCR***

ChIP assays were performed using iDeal ChIP-seq kit for Transcription Factors (Diagenode). Briefly, fresh sorted O4<sup>+</sup> cells were fixed in 1% formaldehyde for 10 min at room temperature.

Lysate were sonicated with a Bioruptor Pico sonicator (Diagenode, total time 8 min) and 4µg of antibodies were added to chromatin and incubated at 4°C overnight. Rabbit anti-Chd7 (Cell signaling, 6505) and anti-Chd8 (Bethyl, A301-224A) antibodies were used for immunoprecipitation experiments. Chromatin-protein complexes were immunoprecipitated with protein A/G magnetic beads and washed sequentially.

To determine the success of the experiment, we took advantage of the ChIP data already published for Chd7 in OLs (He et al., 2016) and for Chd8 in E17.5 cortical tissue (Cotney et al., 2015). Chd7 binding sites in OLs (Nkx2.2, Sox10, Gpr17), Chd8 binding sites (Omg) and negative controls (Olig2 and exonic region of Sox10) were chosen as they were also bound by Sox10 & Olig2 and had active epigenetic marks (H3K27Ac, H3K4me3) and tested by qPCR after Chd7 and Chd8 ChIP in MACS sorted OPCs. Primers for Chd7 binding mouse sequences were: Nkx2.2(1)-f, GATAAGCGCGCTGAATGGTG; Nkx2.2(1)-r, TGGAGATGTTAGAGGCGTGC; Sox10(2)-f, GCAGAGCCCAGTGAATTAGGA; Sox10(2)-r, GGAGATTGTCCAAGGCCAGC; Gpr17(3)-f, CAGAGGCCAAGTGTGTGACT; Gpr17(3)-r, GTGTCTGTCAACTCTCGCCA. Primers for control mouse sequences were: Sox10(4)-f, CATGGGGGAGCCTTCTTCTG; Sox10(4)-r, TACAAGTACCAACCTCGGCG; Olig2-f, GAACCCCGAAAGGTGTGGAT; Olig2-r, GGAGGAGAACCTGGCTCTGG. Primers for Chd8 binding mouse sequences were: Omg-f, TTGTTGTGGGAGTCGGAAGG; Omg-r, GACAGCTCTGCAGTCCTCCT. We also took advantage for primers already published {Van Nostrand, 2014 #4233} to test Chd7 binding to p53 promoter: p53(7)-f, TTCACAAAGCGTTCCTGCTG; p53(7)-r, CGCCATAACAAGTAAGGGCAAG; with negative control: p53(8)-f, CCCACCAGTGTGTTGTGATTTC; p53(8)-r, CCCACCAGTGTGTTGTGATTTC. Interestingly, both Nkx2-2 and Gpr17 bound regions in OLs were also enriched in our OPC Chd7 ChIP while Sox10 region was not, like in negative controls (Fig. 1). Similarly, Chd8 in sorted OPCs bound to Omg region. Binding of Chd7 in p53 promoter was also confirmed in OPCs. These validation allowed us to be confident about the success of the ChIP experiment and we proceeded to sequences obtained DNA fragments.



**Figure 1: Validation of Chd7 and Chd8 binding sites by ChIP-PCR.** ChIP-qPCR of Chd7 and Chd8 compared to Mock for different binding sites. Sites 4, 5 and 7 are negative controls. The data are presented as mean  $\pm$  s.e.m. (Chd7, n=2; Chd8, n=1)

### ***ChIP-Seq and data analysis.***

The ChIP-seq libraries were sequenced and mapped using bowtie2 with option -X 2000 to mm10. Data were filtered with SAM tools with options -q 10 -F 4 and duplicated were removed via “picard MarkDuplicates” with options ASSUME\_SORTED=true, REMOVE\_DUPLICATES=true, CREATE\_INDEX=false and VALIDATION\_STRINGENCY=LENIENT. Peak calling was performed using MACS (Model-based Analysis of ChIP-Seq) (<http://liulab.dfci.harvard.edu/MACS>) with options: keep-dup all, nolambda, broad, nomodel, extsize 75 -q 0.01 and peaks were filtered to obtains length<100bp and P-Value>5%. Two and one separate experiments were done for Chd7 and Chd8, respectively. Input and Mock ChIP were used as controls in each individual experiments.

Representation of the data and correlations were done using Genomatix. Promoters correspond to regions 1000bp upstream of transcription start site (TSS) and 10bp downstream of TSS (Genomatix). Enhancers correspond to the binding of Sox10 and Olig2 (He et al., 2017; Yu et al., 2013). Gene expression profiles have been analyzed from OL stage specific transcriptome and single-cell analysis (Marques et al., 2016; Zhang et al., 2014).

### ***ATAC-seq and data analysis.***

O4+ cells from Control and Chd7iKO P7 mice were purified as described above and 105 cells were lysate in lysis buffer (0.1% Igepal (CA-630, Sigma), 10mM Tris-HCl pH7.4, 10mM NaCl, 3mM MgCl<sub>2</sub>) before ATAC-seq reaction was done as described before (Buenroostro et al., 2015). Libraries were done using Nextera DNA sample kit (Illumina) and sequenced.

Reads from 5 controls and 5 Chd7iKO were uniquely aligned to the mouse reference genome (mm10) using bowtie2 with option -X 2000 to mm10. Data were filtered with SAM tools with options -q 10 -F 4, reads from mitochondria were suppressed and duplicated were removed via “picard MarkDuplicates” with options ASSUME\_SORTED=true, REMOVE\_DUPLICATES=true, CREATE\_INDEX=false and VALIDATION\_STRINGENCY=LENIENT. Peak calling was performed using MACS (Model-based Analysis of ChIP-Seq) (<http://liulab.dfci.harvard.edu/MACS>) with options: keep-dup all, nolambda, broad, nomodel, extsize 75 -q 0.01 and peaks were filtered to obtains length<100bp and P-Value>5%. Peaks from Chd7 ChIP were correlated to ATAC signal via bedops. Genomatix genome browser was used to visualize ATAC and ChIP peaks.

### ***Statistical Analysis***

All statistical analyses were done using Prism. Data are shown as mean  $\pm$  s.e.m. Data distribution was assumed to be normal, but this was not formally tested. Statistical significance was determined using two-tailed Student's t tests. One-way ANOVA test was performed by multiple comparisons or pairwise comparisons following Turkey's ranking tests when comparing multiple groups. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those generally employed in the field. Quantifications were performed from at least three independent experiments. No randomization was used to collect all the data, but they were quantified blindly.

### ***Postnatal electroporation***

Postnatal brain electroporation (Boutin et al., 2008) was adapted to target the dorsal SVZ. Briefly, postnatal day 2 (P2) pups were cryoanesthetized for 2 min on ice and 1.5  $\mu$ l of plasmid were injected into the left lateral ventricle using a glass capillary. Plasmids were injected at a concentration of 2-2.5 $\mu$ g/ $\mu$ l. Electrodes (Nepagene CUY650P10) were positioned in the dorso-ventral axis with the positive pole dorsal. Five electric pulses of 100V, 50ms pulse ON, 850ms pulse OFF were applied using a Nepagene CUY21-SC electroporator. Pups were immediately warmed up in a heating chamber and brought to their cages at the end of the experiment.

### ***Neurospheres cultures***

Neurospheres were isolated from *Chd7<sup>LoxP/LoxP</sup>* ; *Rosa<sup>YFP/YFP</sup>* mice at 2 days postnatal (P2). Briefly, pups were euthanized by putting them in ice for 2 minutes then rapidly decapitated and the brains were collected in PBS 1X (Invitrogen) containing 1% of penicillin/streptomycin and washed the tissue three time in PBS before dissecting the SVZ. Then the SVZ was dissected and transferred to neural stem cell fresh medium as following DMEM/F12 (Gibco) up to 50ml, 250 $\mu$ l HEPES buffer 1M (Gibco), 666 $\mu$ l glucose 45% (Sigma), 0.5ml penicillin/streptomycin (Sigma), 0.5ml N2 supplement (Gibco), 1ml B27 (Gibco), EGF to a final concentration 20ng/ml (Peprotech), FGF to a final concentration 10ng/ml (Peprotech) and Insulin to a final concentration 20 $\mu$ g/ml (Sigma), and mechanically dissociated using a pipette. Approximately  $1 \times 10^6$  cells/mL were cultured in 5 ml NSC fresh medium in a 25 ml flask, and maintained at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>. Under these proliferating conditions, the cells grow as free-floating neurospheres. After mechanical dissociation of neurospheres,

proliferating medium was changed after 2 DIV. For differentiation analysis, cells were plated on coated coverslips pre-coated with poly-d-lysine (PDL, Sigma) or matrigel (Corning BV) at  $6 \times 10^4$  cells/well for 24 wells plate.

### ***Transduction and Differentiation***

Neurospheres were dissociated and plated in 24-wells plates on poly-ornithine. Transduction with Cre-Adenovirus was done putting virus in Neurosphere medium (MOI 1 and 15). After 1 day in Neurosphere medium (EGF/FGF), PDGFa was added to the medium for 2 more days. Differentiation was induced by withdrawing the growth factors from the medium and adding 2% calf fetal serum (CFS). Cells are fixed with 4% PFA at different time-point.

# **Results**



**Article I – Chd7 cooperates with Sox10 and regulates the onset of CNS myelination and remyelination**





















































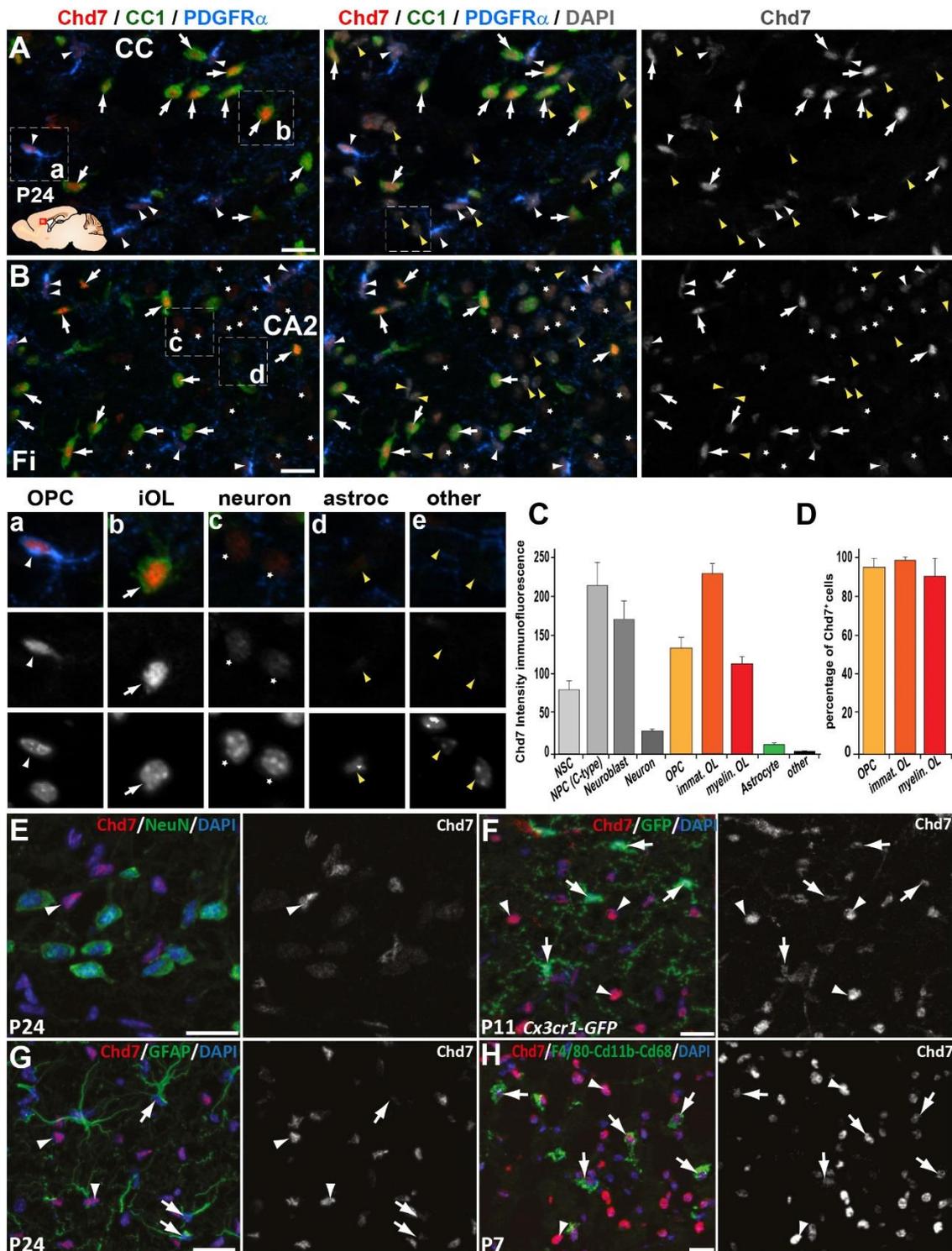






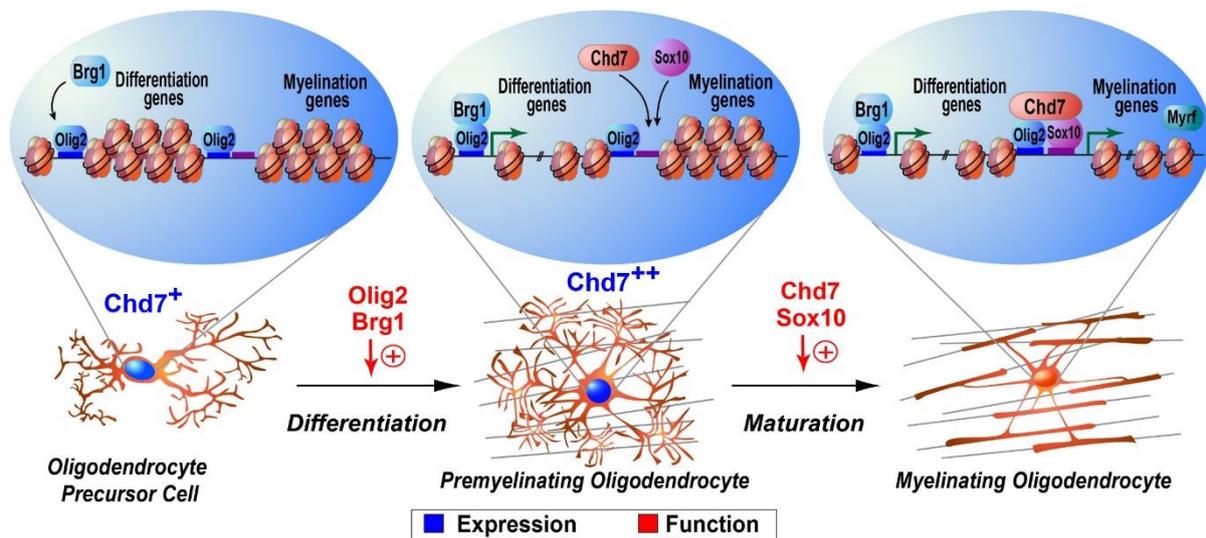
## Comments

This first paper was done in collaboration with Richard Lu's lab. In this context, we shared some of our data to complete their work in order to finish this paper. We first helped in characterizing *Chd7* expression in the postnatal brain. We showed that, in oligodendroglia, *Chd7* is expressed in OPCs ( $PDGFR\alpha^+$  cells, Fig. 1, white arrow heads), with the highest levels of expression found in iOLs ( $CC1^{\text{high}}$  cells, Fig. 1, arrows) and almost not detected in mature OLs. In the neuronal lineage, *Chd7* has a peak of expression in neuroblasts but it is hardly detected in neurons (Fig. 1C and 2A; (Feng et al., 2013)). In parenchymal astrocytes, *Chd7* was almost not detected (P24,  $CC1^{\text{low}}$  cells, Fig. 1C or  $GFAP^+$  cells, Fig. 2B), while in microglia, *Chd7* was clearly detected at P7 ( $F4/80\text{-}Cd68\text{-}Cd11b^+$  cells, Fig. 2D) and P11 ( $Cx3cr1\text{-}GFP^+$  cells; Fig. 2C) but not detected at later stages (P24). These results are in agreement with the expression of *Chd7* mRNA reported in brain cell subtypes from RNA-seq at P7 (astrocytes, neurons, microglia, endothelial cells) and oligodendroglia (at P17) by Barres' lab (Zhang et al., 2014). We then determined *Chd7* function in myelination using a time-controlled deletion of *Chd7* in OPCs using  $PDGFR\alpha\text{CreER}^T$  mice, assessing effect of postnatal *Chd7* loss-of-function on the OL differentiation and myelination. We demonstrated that conditional deletion of *Chd7* in neonatal OPCs impacts the onset of myelination. Finally, we also investigated *Chd7* requirement during remyelination after LPC focal lesion in the corpus callosum by specifically deleting *Chd7* in oligodendroglia using  $Olig1^{\text{Cre}}$  mice demonstrating that *Chd7**CKO* mice had a reduction in newly-formed OLs in and around the lesion. Therefore, we contribute to this paper showing that: 1) *Chd7* expression is highly enriched in OL lineage cells in the CNS, with a peak of expression in differentiating OLs; 2) Inactivation of *Chd7* causes defects in OL differentiation and myelination; 3) *Chd7* is required for OL remyelination after demyelinating injury; and our collaborators further investigated genome-occupancy analyses coupled with transcriptome profiling revealing that *Chd7* cooperates with Sox10 to target the enhancers of key myelination genes. Collectively, these data provide evidence that the chromatin remodeler *Chd7* coordinates with Sox10 to regulate OL differentiation and consequent myelination or remyelination by directly activating myelination programs.



**Figure 1. Chd7 expression in the brain subtypes.** (A-B) Immunostaining of Chd7, CC1 and PDGFR $\alpha$  in the corpus callosum (CC, A) and hippocampus (B) of P24 mice. Arrows represent immature oligodendrocytes (iOLs). White arrow heads represent OPCs. Stars represent neurons. Yellow arrow heads represent astrocytes and other cell-types. (C) Quantification of Chd7 intensity of immunofluorescence in different CNS cell-types at P24. (D) Quantification of Chd7<sup>+</sup> cells as a percentage of total OPCs, iOLs and mOLs in P24 mice. (E) Immunostaining of Chd7, NeuN and DAPI in the cortex of P24 mice. (F) Immunostaining of Chd7, GFAP and DAPI in the cortex of P24 mice. Arrows represent Chd7<sup>LOW</sup> astrocytes. (G) Immunostaining of Chd7, GFP and DAPI in the cortex of P11 Cx3cr1-GFP mice. Arrows represent Chd7<sup>LOW</sup> microglia (H) Immunostaining of Chd7, F4/80, Cd11b, Cd68 and DAPI in the cortex of P7 mice. Arrows represent Chd7<sup>+</sup> microglia. (E-F) Arrow heads represent Chd7<sup>HIGH</sup> cells. Scale bar, 20 $\mu$ m.

Integrating these results with data from (Yu et al., 2013b), we constructed a model where, Olig2 acts a pioneer factor in OPCs binding to OPC differentiation genes allowing chromatin opening and activate transcription of these genes (step 1). Similarly, in iOLs, Chd7 is recruited by Olig2 and in turn Chd7 recruits Sox10 to activate myelination genes (step 2, He et al., 2017). Some aspects of this model are supported from data included in these two papers, such as the cooperation between Sox10 and Chd7 in iOLs to activate genes involved in myelination. However, some key aspects has not been addressed yet. Our work has shown that Chd7 is a gene target of Ascl1 and Olig2 in OPCs and therefore we wonder about Chd7 function in OPCs. Could Chd7 play roles in the control of OPC proliferation, survival and the start of differentiation? Furthermore, although in *Chd7* loss-of-function there was a reduction in OLs and myelination, it was only a partial loss of OLs indicating that Chd7 is not completely required for this process, suggesting a possible compensation by another chromatin remodeler. To answer these questions and go deeper in the understanding of this process, we assessed Chd7 function in OPC differentiation, proliferation and survival and looked for other chromatin remodelers expressed in the oligodendroglial lineage and that could play redundant functions with Chd7.



**Figure 2. Model of OL differentiation regulation.** Olig2, a pioneer TF bind to close chromatin and recruit Brg1, a chromatin remodeler, to open chromatin allowing driving of expression of differentiation genes (Yu et al., 2013b). Chd7 is recruited by Olig2 in myelinating genes to open chromatin permitting the binding of Sox10 and together activate expression of myelination genes (He et al., 2016).



**Article II - Chd7 and Chd8 chromatin remodelers  
cooperate to induce oligodendrocyte precursor  
differentiation and survival**



# Chd7 and Chd8 chromatin remodelers cooperate to induce oligodendrocyte precursor differentiation and survival

**Abbreviated title:** Chd7 is required for OPC differentiation and survival

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**Number of pages:** 46

**Number of figures:** 14

**Words count:** Abstract: 1,435; Introduction: 5,318; Discussion: 12,084

## HIGHLIGHTS

- Chd7 function is required to induce OPC differentiation but not OL stage progression
- Chd7 protects OPC from apoptosis by p53 direct repression
- Chd8 & Chd7 bind together to OPC differentiation, proliferation and survival genes
- Chd7/8 and Olig2/Sox10 synergize to activate oligodendroglia stage-specific genes

## ABSTRACT

In Multiple Sclerosis, remyelination fails in part due to oligodendrocytes precursor cells (OPCs) differentiation defects, and mechanisms underlying this process remain poorly understood. Chd7 and Chd8 are two chromatin remodelers of the ATP-dependent chromodomain helicase DNA-binding (CHD) protein family that regulate the formation of the promoter-enhancer loop prior to transcription, and mutations of *Chd7* and *Chd8* leads to developmental defect associated with CHARGE syndrome and Autism spectrum disorder (ASD), respectively. Here using genome wide chromatin and transcriptomic analyses from *in vivo* purified OPCs, we

show that Chd7 promotes OPC differentiation without affecting its progression once initiated, by directly activating genes involved in oligodendrocyte differentiation and maturation. Furthermore, Chd7 protects non-proliferative OPCs from apoptosis, independently of its OPC differentiation function, by directly inhibiting *p53* expression. Fetching for factors with similar functions, we found that Chd8 is expressed, similarly to Chd7, in oligodendroglia with a peak of expression in maturing oligodendrocytes during myelination and remyelination, both in mouse and human. Moreover, genome-occupancy analyses from *in vivo* mouse sorted-OPCs indicate that Chd7 and Chd8 bind, together with Sox10 and Olig2, to enhancer and promoters of genes involved in oligodendrocyte differentiation in a stage-specific manner.

## INTRODUCTION

Multiple Sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) characterized by oligodendrocyte (OL) loss, myelin sheath destruction and axonal degeneration leading to motor and neurological disabilities (Compston and Coles, 2002). MS affects around 2.3 million persons worldwide, 400,000 in Europe and more than 100,000 in France with 3 out of 4 patients being females ([www.arsep.org](http://www.arsep.org)). Currently, MS therapies are mainly based on immuno-suppressant, immuno-modulatory and anti-inflammatory drugs (Compston and Coles, 2002; Kieseier et al., 2007) but no treatment are yet available to directly promote remyelination and therefore improve patient disabilities. Recovery is achieved through remyelination by new OLs generated from oligodendrocyte precursor cells (OPCs) which are present all over the brain (Franklin and French-Constant, 2008). Unfortunately, remyelination becomes less and less efficient with disease progression supposedly due to OL differentiation defects (Kuhlmann et al., 2008). It is thus crucial to better understand the mechanisms involved in OPCs differentiation to promote efficient remyelination.

Cell differentiation is a process requiring profound changes of gene expression programs and is highly regulated, particularly in the case of OL differentiation (for review see, Küspert and Wegner, 2016; Wheeler and Fuss, 2016). Key regulators of OL differentiation are transcription factors (TFs) such as Sox10, Olig2, Nkx2.2 or Ascl1, which can directly control transcription initiation and by this mean considered to regulate OL differentiation (Mei et al., 2013; Nakatani et al., 2013; Stolt et al., 2002). However, the mechanisms of their action are still poorly understood. On that note, a growing body of evidence suggests that TFs work together with chromatin remodeling factors during transcription initiation, setting up the

correct chromatin state that allows recruitment of the pre-initiation complex and induces robust transcription (Zaret and Mango, 2016). Accordingly, Olig2 and Brg1, a SWI/SNF chromatin remodeler expressed in oligodendroglia, have been found to cooperate in promoting OPC differentiation gene expression (Yu et al., 2013). Recently, we identified Chd7 as a target of Olig2 and Brg1 (He et al., 2016). As a member of the subgroup III of the chromodomain helicase DNA-binding (CHD) family of SNF2H-like ATP-dependent nucleosome remodeling factors, CHD7 has been shown to modulates chromatin configurations to regulate temporal and spatial gene expression during development (Schnetz et al., 2010; Schulz et al., 2014) and neurogenesis (Feng et al., 2017; Feng et al., 2013; Layman et al., 2009; Micucci et al., 2014; Whittaker et al., 2017). Importantly, Chd7 mutations are the cause of CHARGE syndrome, an autosomal dominant syndrome with variable expressivity, impairing normal development of different tissues with frequent brain developmental defects leading to cognitive disabilities (Jongmans et al., 2008). We previously reported that Chd7 expression is highly enriched in OL lineage cells in the CNS, with a peak of expression in differentiating OLs. By Chd7 loss-of-function (LOF) experiments we showed that Chd7 is partially required for proper myelination and remyelination. Moreover, we presented evidence that mechanistically, Chd7 cooperate with Sox10 in OLs to activate expression of myelin-associated genes (He et al., 2016). In this study we investigated the role of Chd7 in OPCs, that are highly proliferative cells also present in the adult brain (Ffrench-Constant and Raff, 1986) having their own functions (Fernandez-Castaneda and Gaultier, 2016), and explore the possible redundant function of its paralog gene *Chd8*. Indeed, Chd8 is another member of subgroup III of the CHD protein family, which has been shown to interact with Chd7 (Batsukh et al., 2010; Feng et al., 2017). Importantly, Chd8 mutations are typical of a subgroup of patients with Autism spectrum disorder (ASD; Barnard et al., 2015; Bernier et al., 2014; O'Roak et al., 2012; RK et al., 2017) and autism features can be found in some CHARGE syndrome patients (Betancur, 2011). Moreover, Chd8 plays roles in CNS progenitor proliferation and neurogenesis (Durak et al., 2016; Platt et al., 2017; Sugathan et al., 2014; Wilkinson et al., 2015) but nothing is known of its function in glial cells. Here, using a time controlled deletion in OPCs, we show that inactivation of *Chd7* leads to a decrease in OPC differentiation but not OL stage progression. Furthermore, Chd7 genome wide binding profiles from *in vivo* OPCs coupled to transcriptomic analysis indicates that Chd7 targets and activates genes involved in OL differentiation. Moreover, we found that Chd7 promotes survival of post-mitotic OPC by directly inhibiting

*Trp53*. Finally, we report that *Chd8* has the same expression pattern than *Chd7* in oligodendroglia and that *Chd8* binding together with *Chd7* to genes involved in OPC differentiation, proliferation and survival, suggesting some overlapping functions of these two chromatin remodelers. Collectively, these data provide evidence that *Chd7* chromatin remodeler binds regulatory regions together with *Chd8*, *Sox10* and *Olig2*, in an OL stage-specific manner to promote expression of genes involved in OPC differentiation and OL maturation.

## RESULTS

*Chd7* is expressed in OPCs (Fig. 1A, Fig. S2B) and its protein levels accumulate in immature oligodendrocytes (iOLs, Fig. S2B,C; He et al., 2016) questioning about *Chd7* role(s) in oligodendrocyte lineage cells. We recently showed that *Chd7* is required for the normal onset of myelination (He et al., 2016) but *Chd7* involvement in different aspects of OPC biology has not been explored. OPCs have a tight balance between proliferation & differentiation by mechanisms not completely understood (Fernandez-Castaneda and Gaultier, 2016). We therefore, investigated the role of *Chd7* in OPCs by specifically generating a time-controlled *Chd7* deletion in neonatal OPCs (hereafter mentioned as *Chd7iKO*, for *Chd7* induced knockout) and assessed *Chd7* requirement in OPC proliferation, survival and differentiation.

### ***Chd7* regulated genes are involved in OPCs proliferation, differentiation and survival**

To assess *Chd7* regulated genes in OPCs, we induced a *Chd7* conditional deletion (*Chd7iKO*) in neonatal OPCs, by combining *PDGFR $\alpha$ -CreER<sup>T</sup>* and *Chd7<sup>Flox</sup>* alleles and administrating tamoxifen at postnatal day 1 (P1) and P4 (Fig. 1A). Indeed, this protocol allowed us to delete *Chd7* in 85-95% of OPCs as shown by *Chd7* immunofluorescence and RT-qPCR at P7 (Fig. 1A-B; Fig. 2A). We then purified O4<sup>+</sup> cells from P7 cortices using MACS (Magnetic Assisted Cell Sorting; Fig. 1C) which enabled us to obtain almost pure (98%) oligodendroglia (~80% of *PDGFR $\alpha$* <sup>+</sup> OPCs and ~20% of *Nkx2.2*<sup>+</sup>/*CNP*<sup>+</sup> iOLs; Fig. S1A-C). Interestingly, the proportion of OPCs and iOLs was not altered by the loss of *Chd7* (Fig. S1C, E) allowing us to get comparable oligodendroglial populations from mutant and control brains. We therefore investigated the impact of *Chd7* loss-of-function on gene transcription by genome wide transcriptome analysis (RNA-seq, Fig. 1C). Surprisingly, despite *Chd7* being described as an activator of transcription (Feng et al., 2017; Schnetz et al., 2010; Schulz et al., 2014), the majority of genes regulated

upon *Chd7* deletion in OPCs (3,689 genes, fold-change > 1.2 & p-value < 0.05) were upregulated (63%, 2,315 genes) and only 37% were downregulated (1,374 genes, Fig. 1D). Gene ontology (GO) analysis indicated that many upregulated genes were associated with apoptosis and cell cycle/proliferation (Fig. 1F; Fig. S1F). By contrast, genes associated with oligodendrocyte differentiation were exclusively found among downregulated genes (Fig. 1F). Indeed, the 100 most differentially expressed genes included several well-known regulators of oligodendrocyte differentiation being all downregulated (Fig.1E). Therefore, we investigate in more detail the role of *Chd7* in these three biological processes.

### ***Chd7* is required to induce OPC differentiation but not for OL stage progression**

Taking advantage that our purified O4<sup>+</sup> cells from P7 cortices included 80% of OPCs and 20% of iOLs (Fig. S1A,C) and that oligodendroglial transcriptomes indicate that OPCs & iOLs already express transcripts from proteins found at later stages of the lineage (Marques et al., 2016; Zhang et al., 2014), we assessed how *Chd7* deletion impacts the expression of genes involved in different stages of differentiation. Interestingly, loss of *Chd7* in OPCs (Fig. 2A) led to downregulation of transcript of key regulators of OPC differentiation (*Sox10*, *Gpr17*, *Nkx2.2*, *Tcf7l2*, Fig. 2B,D) but also of key regulators of OL maturation (*Sirt2*, *Myrf*, *Zfp488*) and myelin proteins (*Cnp*, *Omg*, Fig. 2C,D) while other genes involved in these processes were not affected (such as *Ascl1*, *Olig2*, *Brg1*, *Pdgfra*, *Nkx6.2*, *Mbp*, *Plp1*, *Mag*, *Mog*, Fig. 2B-D). This suggest that *Chd7* activates part of the genetic program of oligodendrocyte differentiation/maturation. Accordingly, P7 *Chd7*<sup>iKO</sup> corpus callosum presented a strong decreased (~40%) of Nkx2-2<sup>+</sup> iOLs compared to control brains (Fig. 2E-G) supporting the fact that *Chd7* deletion in OPCs impairs normal differentiation. Notably, taking advantage of other iOL markers successively expressed in the lineage (Itpr2<sup>+</sup> and APC<sup>+</sup>; Fig. S2), we found a similar decrease in each subset of iOLs expressing these markers (Fig. 2F,G), suggesting that loss of *Chd7* does not alter OL stage progression once OPC differentiation is started. Supporting this, CC1/Olig1 immunofluorescence that allows to separate different OL stages of differentiation (Nakatani et al., 2013) confirmed that all oligodendroglial stages were similarly reduced in *Chd7*<sup>iKO</sup> compared to controls (Fig. S3). All together this data indicates that *Chd7* deletion affects OPC capacity to start differentiation but not the normal progression once differentiation is initiated.

## **Chd7 promotes OPC survival through p53 downregulation independently of its role in OPC differentiation**

Surprisingly, analysis of the density and OPC numbers in P7 *Chd7iKO* cortices revealed a reduction of ~30% of OPCs in the CC and ~60% in the cortex (Fig. 3A,B) suggesting that *Chd7* deletion affects either OPC proliferation or survival. Accordingly, we observed a reduced number of O4<sup>+</sup> MACSorted cells from *Chd7iKO* cortices compared to controls (Fig. S1D). Notably, the iOL/OPC ratio was not modified in *Chd7iKO* brains due to a similar reduction in OPCs and iOL populations (Fig. S1E). We therefore analyzed the impact of *Chd7* deletion in the expression of genes linked to proliferation/cell cycle, and found that key regulators of cell cycle check points (*Ccnd1*, *Cdk6*, *Ccne1*, *Ccna1*, *Cdk2*, *Ccnb1*, *Cdk1*) were upregulated as well as proliferation markers (*Mki67*, *Mcm2*, Fig. 3C). Surprisingly, when measuring proliferation of OPCs in P7 brains, we did not find any difference between *Chd7iKO* and controls in the number of Mcm2<sup>+</sup> or Ki67<sup>+</sup> proliferating OPCs both in CC and Ctx (Mcm2, Fig. 3D,F and Fig. S4A,B; Ki67, Fig. S4E-G). The upregulation of cell cycle genes could be thus explained by an increase in the proportion of cycling OPCs due to a reduction of non-proliferating OPCs (Fig. S4B,M). Indeed, we found a reduction of Mcm2<sup>-</sup> OPCs (PDGFRα<sup>+</sup> cells) in *Chd7iKO* brains compared to controls (Fig. 3D,F and Fig. S4A) suggesting a possible function of Chd7 in survival of post-mitotic OPCs and consistent with the enrichment of GO apoptosis category in our *Chd7iKO* RNA-seq (Fig. 1F). Notably, we found that p53 (*Trp53*), a main regulator of apoptosis which has been linked to Chd7-mediated defects in CHARGE syndrome (Van Nostrand et al., 2014), was among the upregulated genes, as well as some of its targets (i.e. *p21* and *Noxa*, Fig. 3C). *Trp53* mRNA upregulation in *Chd7iKO* OPCs was confirmed by RT-qPCR (Fig. 3H). At the protein level, 15% of CC OPCs and 40% cortical OPCs were p53<sup>+</sup> in *Chd7iKO* brains while p53 immunofluorescence was not detectable in P7 control OPCs (Fig. 3E,G and Fig. S4C,D). The apoptotic marker Casp3 also confirmed the specific cell death of *Chd7iKO* OPCs (Fig. S4H-J). Interestingly, 90% of *Chd7iKO* OPCs expressing p53 were post-mitotic (Mcm2<sup>-</sup> /PDGFRα<sup>+</sup> cells; Fig. S4K-M), confirming that loss of Chd7 results in a specific apoptosis of non-cycling OPCs likely mediated by p53 upregulation. Altogether, these results strongly indicates that *Chd7* deletion do not affect proliferating OPCs but leads to apoptosis of non-proliferating OPCs (Fig. 3L).

These findings led us to assess whether OPC apoptosis would be sufficient to account for the reduction of differentiating OLs in *Chd7iKO* brains or if the two functions could be separated. To this end, we made use of an inhibitor of apoptosis (Pifithrin-α, PFT, Fig. 3J) to

rescue the p53-mediated loss of *Chd7iKO* OPCs (PDGFR $\alpha$ <sup>+</sup> cells; Fig. 3I,K) and assess the impact in oligodendrocyte differentiation. Remarkably, the number of iOLs (Nkx2.2<sup>+</sup> cells) in *Chd7iKO* corpus callosum treated with PFT was the same than non-treated *Chd7iKO* brains (Fig. 3I,K) indicating that rescue of OPC survival is not sufficient to rescue normal differentiation of *Chd7iKO* OPCs. Together these results indicate that Chd7 is not only required in OPC differentiation but also contributes to the survival of post-mitotic OPCs by inhibiting p53 (Fig. 3L).

### **Chd7 bind to enhancers/promoter of genes involved in OPC differentiation**

To better understand the mechanisms implicated in Chd7 control of OPC differentiation and survival, we performed genome wide Chd7 binding analysis by ChIP-seq (chromatin immunoprecipitation [ChIP] combined with high-throughput sequencing) from *in vivo* mouse purified OPCs, using the same O4<sup>+</sup> cell purification protocol described for RNA-seq (Fig. 4A). We identified 11,655 Chd7 binding sites in OPCs (Fig. 4B). To investigate whether Chd7 binding in OPCs was stage-specific, we compared our Chd7 ChIP-seq data with Chd7 binding in OLs that we previously generated (He et al., 2016). Interestingly, Chd7 binds different regions in OPCs and OLs with only 10% of common sites at both stages (Fig. 4B) corresponding to 20% commonly bound genes (Fig. 4C), which nevertheless include key regulators of OL differentiation (*Ascl1*, *Olig1*, *Sox10*, *Nkx2.2*). On the contrary, myelin-genes were only bound by Chd7 in OLs (*Mobp* and *Omg*) while Chd7 bound genes involved in cell cycle and apoptosis only in OPCs (Fig. 4C). Therefore, Chd7 mostly binds to regulatory regions of genes expressed in oligodendroglial cells in a stage-specific manner.

Given that Chd7 has been reported to preferentially bind enhancer elements in other cell types such as ESCs (Schnetz et al., 2009a; Schnetz et al., 2010), we assessed Chd7 binding enrichment in active enhancers in OPCs and OLs. To do so, we took advantage of Sox10 & Olig2 binding in OPCs and OLs (He et al., 2016; Yu et al., 2013) and found that regions outside promoters commonly bound by Sox10/Olig2 presented H3K27ac marks (Fig. S5A-B), indicating that they correspond to active enhancers (Creyghton et al., 2010). Accordingly, we found that Chd7 binds these enhancers (Sox10/Olig2 bound regions) both in OPCs and OLs with Chd7 binding a larger number of enhancers in OLs (Fig. 4D). Unexpectedly, Chd7 binds also to many promoter regions (as defined in Genomatix portal, see M&M) in OPCs but not in OLs (Fig. 4E). Indeed, many regions bound by Chd7 in OPCs had H3K4me3/H3K27ac histone marks, commonly used to define active promoters (Guenther et al., 2007); Fig. 4F), confirming that

Chd7 binds promoter regions in OPCs. Together this data suggest that Chd7 binding to promoters is temporally controlled during oligodendroglial stages.

We then, studied if Chd7 was directly bound to genes down- or up-regulated in *Chd7* loss-of-function. Indeed, 47% of downregulated genes were bound by Chd7 including genes involved in oligodendrocyte differentiation (e.g. *Sox10*, *Nkx2.2*, *Gpr17*, *Mbp*, Fig. 4G) indicating that OL differentiation genes are direct target genes activated in the presence of Chd7. In contrast, only 30% of upregulated genes were bound by Chd7, including cell cycle genes controlling the restriction check point (early G1 to late G1 transition; *Ccnd1*, *Cdk4*, *Cdk6*) and *Trp53*, a regulator of apoptosis (Fig. 4G). Therefore, Chd7 binding and transcriptome analysis together indicate that Chd7 directly regulate genes crucial for OPC differentiation, proliferation and survival.

To get some inside into the mechanistic action of Chd7, that is an ATP-dependent nucleosome remodeler (Bouazoune and Kingston, 2012), we decided to assess the chromatin status of some genes involved in oligodendrogenesis and bound by Chd7. To map open chromatin sites genome-wide, we performed open chromatin profiling by ATAC-seq (Tn5 transposase-accessible chromatin with high-throughput sequencing [ATAC] combined with sequencing, Buenrostro et al., 2013) in purified O4<sup>+</sup>-cells obtained from either *Chd7*<sup>TKO</sup> or control cortices (Fig. 4H). We obtained a good genome wide correlation between transcript expression and ATAC signal in the TSS indicating efficient open chromatin mapping (Fig. 4I). Interestingly, we found several regulatory regions bound by Chd7 having decreased ATAC signal in *Chd7* mutant OPCs including genes encoding for key oligodendrogenic TFs (e.g. *Olig2*, *Olig1*), G protein-coupled receptors involved in OL differentiation (i.e. *Gpr17*) and myelin proteins (e.g. *Mbp*; Fig. 4J). We also found that *Trp53*, which was bound by Chd7 and upregulated upon its deletion, showed an increased ATAC signal. Therefore, our findings point to a role of Chd7 in regulating chromatin opening in genes related with OPC differentiation, survival and myelination.

### **Chd8 is coexpressed with Chd7 in oligodendroglia during myelination and remyelination**

*Chd7* genome wide binding analysis combined with transcriptional changes in *Chd7* mutant OPCs strongly suggest that Chd7 can bind to many genes involved in different OPC functions but only a fraction of these genes are deregulated upon *Chd7* deletion. We therefore asked if another chromatin remodeler could cooperate with Chd7 and compensate for its loss-of-

function in regulating Chd7 bound genes. Chd7 is a member of the CHD family subgroup III, together with Chd6, Chd8 & Chd9, all being paralogues of a unique *Drosophila* orthologue, called Kismet (Daubresse et al., 1999). Of note, Chd8 protein have been shown to interact with Chd7 in a yeast two-hybrid screen (Batsukh et al., 2010) and recently in HEK293T cells (Feng et al., 2017). Interestingly, Chd8 is one of the nine high-confidence Autistic Spectrum Disorders (ASD) risk genes (Neale et al., 2012; O'Roak et al., 2012; Talkowski et al., 2012) and autistic symptoms are also found in some CHARGE syndrome patients (Betancur, 2011). We therefore, investigated Chd8 expression in the postnatal brain. In accordance with our hypothesis, we found that Chd8 protein was detected in the postnatal brain (P21) at low levels in OPCs (PDGFR $\alpha$ <sup>+</sup> cells) and at strong levels in differentiating OLs (CC1<sup>+</sup> cells and MOG<sup>+</sup> cells; Fig. 5A-C) similarly to Chd7 expression pattern, making Chd8 a good candidate to compensate for Chd7 loss-of-function. We also found a strong Chd8 expression in neurons (Fig. 5A) while Chd7 was restricted to progenitors in the neuronal lineage (*data not shown*), in accordance with previously studies (Feng et al., 2013; Micucci et al., 2014; Platt et al., 2017). We then investigated the expression pattern of Chd8 in pathological conditions both in a mouse model of de/remyelination and in MS brain samples. Interestingly, in mice, Chd8 was strongly expressed in OPCs around the demyelinated lesion as early as 2 days post-demyelination while it was hardly detectable in adult OPCs (Fig. 5F). Furthermore, at 4 days post-demyelination when new OLs are generated to remyelinate the lesion, Chd8 was strongly expressed in immature OLs (CC1<sup>high</sup>/Olig1<sup>high</sup> cells) in and around the lesion (Fig. 5G), suggesting a crucial role for Chd8 the generation of new oligodendrocytes during remyelination. Additionally, in humans, immunohistological analysis of MS brain tissue showed that CHD8 is expressed in many small nuclei in shadow plaques likely corresponding to oligodendroglial cells (Fig. 5H-J). Indeed, immunolabeling with oligodendrocyte lineage markers showed that CHD8<sup>+</sup> cells corresponded to maturing oligodendrocytes (Nogo-A<sup>+</sup> cells) that could be found at the border of actively remyelinating lesions (MBP<sup>+</sup> cells, Fig.5K-N), suggesting a relevant role of CHD8 during remyelination in the context of MS pathology. Altogether, the expression pattern of Chd8 in oligodendroglia during myelination and remyelination suggest that it could regulate either similar or different programs of oligodendrogenesis and remyelination to those controlled by Chd7.

### **Chd8 binds together with Chd7 to OPC differentiation, proliferation and survival genes**

To investigate whether Chd7 and Chd8 regulate common or different OPC gene programs, we generated a genome wide map of Chd8 binding from *in vivo* purified OPCs (Fig. 6A) and compared it to Chd7 binding sites. Chd8 was bound to 18,415 sites in the genome (Fig. 6B) and many (57.6%) of them were found in promoter regions (Fig. S5D) in accordance with studies in other cell types (Nishiyama et al., 2009; Sugathan et al., 2014; Thompson et al., 2008; Yates et al., 2010) but we also found Chd8 in a large number of OPC active enhancers (Fig. S5D). Interestingly, Chd8 shared many sites in common with Chd7 (6,418 regions) representing 55% of Chd7 bound regions (Fig. 6B), indeed suggesting that Chd7 and Chd8 may play redundant functions. By contrast, Brg1 chromatin remodeler, that cooperates with Olig2 to control OL differentiation (Yu et al., 2013), had almost no common binding sites with Chd7 (750/11655; 1%; Fig. S5C) suggesting that Chd7 and Brg1 regulate different aspects of OPC differentiation. The large overlap between Chd7/Chd8 binding sites suggests that both remodeling factors regulate similar genetic functions. Supporting this scenario, we found that most (84%) of genes bound by Chd7 were also bound by Chd8 (Fig. 6C). Furthermore, all well-known regulators (e.g. *Ascl1*, *Nkx2.2*, *Olig1*, *Olig2*, *Sox10*) and markers of oligodendroglial stages (e.g. *Pdgfra*, *Cspg4*, *Gpr17*, *APC*, *Itpr2*, *Mbp*) were commonly bound by Chd7 & Chd8 (Fig. 6C), strongly supporting that they commonly regulate key programs controlling OPC biology and differentiation. Of note, other genes involved in oligodendrocyte lineage (e.g. *Sox9*, *Zfp488*, *Gpr37*) and myelin protein genes (e.g. *Cnp*, *Mog*, *Omg*) were bound by Chd8 alone (Fig. 6C) suggesting that Chd8 can play additional roles to those shared with Chd7.

With respect to cell cycle regulation, Chd8 & Chd7 commonly bind to the R check-point regulators (*Ccnd1*, *Cdk4*, *Cdk6*) and Chd8 alone to genes involved in other phases of cell cycle (*Ccnb1*, *Cdk1*, *Cdk2*, *Mcm2*, Fig. 6C), suggesting that even if Chd8 control R-check point together with Chd7, Chd8 is likely to have a stronger role in cell cycle regulation. In addition, *Trp53* that is upregulated in *Chd7* loss-of-function (Fig. 3C,H and Van Nostrand et al., 2014), was also commonly bound by Chd7 & Chd8 in the promoter of purified OPCs but not in OLs (Fig. 6C, 4C & S8) suggesting an OPC-specific regulation of apoptosis by these remodelers. All these results suggest that part of the OPC gene networks controlling OPC differentiation, proliferation and survival are commonly regulated by Chd7 and Chd8, strongly supporting a possible compensatory mechanism of *Chd8* in *Chd7* loss-of-function.

### **Chromatin remodelers and key TFs synergize to activate time-controlled gene expression**

To better understand the mechanisms of Chd7-Chd8 gene regulation, we hypothesized that Chd7 & Chd8 could contribute to time-controlled gene expression at different stages of OL differentiation. To assess this possibility, we subdivided genes in three groups according to their expression timing in oligodendroglia based in single cell transcriptomes (Fig. 6D; Marques et al., 2016). We thus defined: i) *OPC genes* as those expressed in OPCs and downregulated upon differentiation (e.g. *Ascl1*, *Cspg4*, *Pdgfra*); ii) *iOL genes* as those upregulated and maintain upon early OPC differentiation (COPs of Marques et al., 2016; e.g. *Nkx2.2*, *Gpr17*, *Itpr2*); and iii) *mOL genes* as those upregulated only in maturing OLs (NFOLs & MOLs; e.g. *Mbp*, *Mog*, *Omg*, Fig. 6D). To study the temporally-controlled binding of key regulators to the three gene groups in OPCs and OLs, we made use of ChIP-seq datasets we had generated for key regulators (Sox10, Olig2, Ascl1), chromatin remodelers (Brg1, Chd7 & Chd8) and active histone marks (H3K4me3/H3K27ac; this study & He et al., 2016; Yu et al., 2013; Fig. 6E, S6-S8). Interestingly, by this integrative approach, we found that in OPCs but not in OLs, most OPC genes were bound by all regulators (Olig2, Sox10, Chd7 & Chd8) both at enhancers and their cognate promoters, suggesting that together these factors drive active gene transcription (Fig. 6F, S6). Accordingly, most mOL genes which are not yet expressed in OPCs, were not bound by Chd7, Chd8, Sox10 or Ascl1 (only by Olig2), while in OLs most of mOL genes were bound at enhancer regions by both Sox10 and Olig2 with an intermediate presence of Chd7, supporting the hypothesis that only the presence of all the factors can drive robust gene expression (Fig. 6F and S6). To test if this hypothesis holds true, we analyze binding to iOL genes that are just starting to be expressed in OPCs but are strongly expressed in the next stage (OLs; Marques et al., 2016). According to our hypothesis, we expected an intermediate binding of Chd7/Chd8 & key TFs, compared to full binding to active OPC genes and almost no binding to inactive mOL genes. Indeed, we found that iOL genes were bound only at enhancers in OPCs by Chd8 (not many bound by Chd7) and Olig2 & Ascl1 pioneer TFs (i.e. that bind close/poised chromatin; (Raposo et al., 2015; Yu et al., 2013)) while in OLs all regulators (Olig2, Sox10, Chd7 & Chd8) were bound in most enhancers and to a less extend in their cognate promoters (Fig. 6F and S6). Given all these results, we propose that a coordinated binding of Chd7/Chd8 remodellers and key oligodendrogenic TFs (Olig2, Sox10 and Ascl1) is required in enhancers to drive robust gene expression at each stage of the OL lineage.

## DISCUSSION

CHD chromatin remodelers, such as Chd7/Chd8, make use of their helicase-like ATPase motor to regulate nucleosome positioning (Bouazoune and Kingston, 2012; Manning and Yusufzai, 2017) and by this way control DNA accessibility in eukaryotic cells either activating or repressing transcription in a context and cell-type dependent manner (Garcia-Gonzalez et al., 2016). Mutations in chromatin remodeling genes have been associated with neurodevelopmental diseases (Yoo and Crabtree, 2009). In this context, *CHD7* haploinsufficiency causes CHARGE syndrome (Vissers et al., 2004), an autosomal dominant syndrome with variable expressivity, both in humans and mouse models including neurodevelopmental defects leading to progressive neurological pathology (Martin, 2010; Janssen et al., 2012). Accordingly, *CHD8*, paralogue of *CHD7*, is one of the genes most strongly associated with ASD and *CHD8* disruptions represent a distinct ASD subtype characterized by macrocephaly, a facial phenotype marked by prominent forehead, wide-set eyes, and pointed chin as well as constipation (Bernier et al., 2014). Moreover, mice heterozygous for *Chd8* mutations manifest ASD-like behaviors including increased anxiety, repetitive behavior and altered social behavior (Katayama et al., 2016; Platt et al., 2017). Interestingly, autism features can be found in CHARGE syndrome (Betancur, 2011) and *Chd8* was found to bind *Chd7* both in a yeast two-hybrid screen (Batsukh et al., 2010) and more recently in HEK293T cells (Feng et al., 2017). It is therefore, a relevant question for human health to unravel the mechanisms controlled by *Chd7/8* remodelers and understand how their haploinsufficiency leads to developmental brain pathology involved in these diseases. We previously reported that *Chd7* is required for proper onset of OL myelination, as well as remyelination (He et al., 2016). Here, integrating data from genome wide transcriptomics (RNA-seq), chromatin binding (ChIP-seq) and chromatin opening (ATAC-seq) profiles, we have studied the mechanisms by which *Chd7* together with *Chd8* controls oligodendrogenesis and myelination. Our results indicate that: i) *Chd7* is required to induce oligodendrocyte differentiation but is not necessary for oligodendrocyte stage progression; ii) *Chd7* protects OPCs from cell death through p53 direct transcriptional repression; iii) *Chd7* and *Chd8* together bind to OPC differentiation, proliferation and survival genes. Finally, by integrating this data with other ChIP-seq datasets we propose a model where both *Chd7/8* chromatin remodelers and key oligodendrogenic TFs (i.e. *Olig2* & *Sox10*) cooperate at enhancer and promoter regions to regulate gene expression in a stage-specific manner.

### **Chd7 OPC-specific mutation reduces differentiation but it is not necessary for oligodendrocyte stage progression**

To address Chd7 function and mechanisms in OPCs, here we performed a transcriptomic analysis of purified OPCs obtained from MACS-sorted O4<sup>+</sup> cells of postnatal (P7) mouse cortex after *Chd7* conditional deletion. Noteworthy, we found no difference between control and mutant in proportion of oligodendroglial cells (80% OPCs and 20% iOLs), allowing us to compare transcriptomes of similar cell populations. Interestingly, upon *Chd7* deletion OPC differentiation genes were downregulated, in accordance with the decrease in OL numbers generated from *Chd7-iOK* OPCs observed at later points. Furthermore, chromatin binding (ChIP-seq) analysis showed Chd7 binding in most OPC differentiation genes downregulated upon *Chd7* deletion, indicating that Chd7 is a direct regulator of OPC differentiation process. Remarkably, using combination of markers expressed in successive stages of differentiating OLs (Marques et al., 2016; Nakatani et al., 2013), similar proportions of each stage were found both in *Chd7iKO* brains than controls, indicating that those *Chd7* mutant OPCs that started differentiation progress through those stages with the same timing than wild-type oligodendroglia.

Given that Chd7 is also expressed in neuronal precursors and is required for adult neurogenesis (Feng et al., 2017), we wondered if similar or different genetic programs were regulated by Chd7 during neuronal and oligodendroglial differentiation. Comparing our Chd7 ChIP-seq in OPCs with a recently published Chd7 ChIP-seq from granule neuron progenitors (GNPs; Feng et al., 2017), we observed that only ~4% (500 out of 11,000 genes in OPCs) were commonly bound in OPCs and GNPs (*data not shown*) indicating that Chd7 regulated diverse genetic programs involved in cell differentiation in different cell-types. These results suggest that Chd7 does not bind to specific DNA sequences but is recruited to regulatory elements by other cell-type specific transcription factors in agreement with *in vitro* data from two human cell lines (DLD1 colorectal carcinoma cells, and SH-SY5Y metastatic neuroblastoma Schnetz et al., 2009b).

### **Chd8 binds together with Chd7 to OPC genes**

Our Chd7 loss-of-function experiments demonstrate that Chd7 is only partially required for OPC differentiation and the Chd7 genome wide chromatin profiling in OPCs indicates that not all genes bound by Chd7 are deregulated upon *Chd7* deletion, suggesting the presence of a

compensatory mechanism/factor. We selected Chd8 as a candidate because of interaction with Chd7 and their involvement in neurodevelopmental diseases mentioned above. Here, we have shown that Chd8 protein similarly to Chd7 is expressed in OPCs and OLs with a peak of expression in iOLs. Moreover, our Chd7 & Chd8 genome wide chromatin binding profiling from in vivo OPCs shows that Chd7 & Chd8 share many common binding sites at active (H3K27ac marks) regulatory elements (enhancers and promoters) of genes, including OL differentiation genes (e.g. *Sox10*, *Nkx2.2*), suggesting a possible Chd8-mediated compensation in *Chd7* mutant OPCs. The precise Chd8 function in OPC differentiation and myelination awaits for the generation of specific *Chd8* LOF in the oligodendroglial lineage, given the lethality of *Chd8* null alleles and that the generation of *Chd8<sup>Flox</sup>* alleles has not been obtained. Furthermore, it would be also interesting to investigate the synergy between *Chd7* and *Chd8* by generating compound mutations affecting *Chd7* and *Chd8* alleles in oligodendroglia.

Beside sharing many binding sites suggesting common functions between Chd7 and Chd8, however, Chd8 present a large number of binding sites that are not shared with Chd7, suggesting that Chd8 has also some specific functions in OPCs. Firstly, it seems that, in OPCs, Chd8-only (without Chd7) binds in genes not yet expressed (iOL genes Marques et al., 2016) which will be later bound by Chd7 in OLs, where these genes are expressed. Thus, Chd8 could have a precocious function and prepare the promoters of Chd7-activated genes. Otherwise, the presence of Chd8 alone could prevent the “precocious” expression of these not-yet-expressed genes. These possibilities should be addressed through a functional study of Chd8.

### **Chd7 promotes OPC survival through p53 downregulation independently of its role in OPC differentiation**

Time-controlled *Chd7* deletion in OPCs has also allowed us to look at other OPC functions like proliferation and survival. Surprisingly, we found that after *Chd7* LOF, apoptosis was mostly restricted to non-cycling OPCs. Importantly, Chd7 binds to *Trp53* promoter in OPCs, and p53 is upregulated in *Chd7* mutant OPCs and in turn promotes the apoptotic pathway. Similarly, apoptosis in neural crest cells has been linked to *Chd7* mutations and CHARGE syndrome (Van Nostrand et al., 2014). Consistently with these results, *Chd7* deletion in granule neuron progenitors has been recently shown to increase cell death by expression of Caspase3 (Feng et al., 2017). These studies altogether suggest a general role of Chd7 to inhibit apoptosis pathway in different tissues and cell types. Interestingly, our rescue experiments with cell

death inhibitors did not rescue the reduce number of differentiating OLS indicating that Chd7 function in OPC survival and differentiation can be separated.

Our Chd8 ChIP-seq analysis also indicates that Chd8 can bind, together with Chd7, to *Trp53* promoter, suggesting a role for Chd8 in OPC survival. This is particularly interesting as a direct regulation of *Trp53* by Chd8 has never been identify before, while it has been shown that Chd8 can complex with p53 and histone H1 at the promoter of p53 target genes to inhibit their transcription (Nishiyama et al., 2009). It would therefore of interest to see if *Chd8* LOF induces OPC cell death.

Cell death in non-proliferative OPCs lead to unbalance the proportion of cycling and non-cycling OPCs. This is directly translated as an overrepresentation of cell-cycle genes in mutant transcriptome (RNA-seq) compared to control. Nevertheless, we did not find any difference in the number of proliferating (MCM2<sup>+</sup> and Ki67<sup>+</sup> cells) OPCs between mutant and control conditions showing that cell-cycle entry is not affected by *Chd7* LOF. Accordingly, no difference were observed in the proportion of Ki67<sup>+</sup> OPCs in *Olig1<sup>Cre</sup>; Chd7<sup>Flox/Flox</sup>* mice (He et al., 2016). However, we could find that Chd7 binds, together with Chd8, to some cell-cycle regulator genes (*Cdk4*, *Cdk6*). Therefore, we cannot exclude a subtle roles of Chd7 in OPC proliferation as the timing of cell-cycle in Chd7 mutant OPCs has not been assessed in depth.

### **Chromatin remodelers and TFs synergize at regulatory regions to activate time-controlled gene expression**

Our study aimed to achieve a better understanding of the mechanisms involved in the transcription regulation of oligodendrocyte differentiation. To this goal, we have used an integrative analysis of different genome wide chromatin profiling (ChIP-seq datasets) in oligodendroglial cells in order to integrate Chd7 & Chd8 binding with that of key TFs involved in OPC differentiation: Sox10, Olig2 and Ascl1 (He et al., 2016; He et al., 2017; Yu et al., 2013). By this method, we have observed a timely controlled binding to stage-specific genes in OPCs and OLS (Marques et al., 2016) and built a transcription regulatory model where: i) Olig2 and Ascl1 pioneer TFs (Raposo et al., 2015; Yu et al., 2013) would bind to enhancers and ii) their binding will allow the recruitment of Chd7 & Chd8, with Chd8 likely binding before Chd7; iii) Chd7/Chd8 remodelers will open the chromatin to allow the binding of Sox10 which in turn using its DNA bending activity, helps to recruit other transcription cofactors and the mediator complex to form the promoter-enhancer loop leading to iv) robust gene transcription. That

timing of events could explain the crucial importance of Sox10 and Olig2 in activating expression of these genes (Stolt et al., 2002; Yu et al., 2013), but also the apparent compensation of Chd7 by Chd8. Notably, we could observed that most genes not-anymore-expressed in OLs (“OPC genes”) are still bound by Olig2 and Sox10. Interestingly, most of the expressed genes are those commonly bound by Sox10, Olig2, Chd7 and Chd8 (at least in OPCs). In agreement to what we reported previously (He et al., 2016), Chd7 and Sox10 seems to bind together to regulatory elements already bound by Olig2 and suggest that together this binding activate gene expression. This could imply that the lack of Chd7 and Chd8 binding together with the binding of some repressors (e.g. Sox5, Sox6, Stolt et al., 2006) is required for OPC gene downregulation to allow OL differentiation to proceed to the next step.

Altogether, our study allows to propose that Chd7 is a chromatin remodeling factor required for cell-subtype differentiation and that together with Chd8 and other transcription factors, they form a complex that binds to enhancers and promoters of differentiation genes in cell-subtype specific manner. A better understanding of this mechanism is precious to be able to overcome OPC differentiation failure in MS and find new remyelination therapies. It will also permit to investigate new pathways involved in CHARGE syndrome and ASD, upon Chd7 and Chd8 mutations.

## **MATERIAL & METHODS**

### ***Animals***

Mice homozygous for floxed alleles of *Chd7* (*Chd7<sup>Flox/Flox</sup>*) were crossed with PDGFR $\alpha$ -CreERT mice to generate the OPC-specific *Chd7iKO* (*iKO*) mice. *Chd7* deletion was controlled by immunostaining and RTqPCR. Animals of either sex were used in the study and Cre negative littermates were used as controls. The mouse strains used in this study were generated and housed (six or less animals per cage) in a vivarium with a 12-h light/dark cycle. Wild type Swiss mice have been ordered from Janvier. All animal studies were conducted following protocols approved by local ethical committees and French regulatory authorities (#03860.07).

### ***Tamoxifen administration and Tissue processing***

For tamoxifen treatment, tamoxifen (T5648) was dissolved in corn oil (Sigma, C-8267) and injected subcutaneously at 20mg/ml concentration at postnatal stages (40 $\mu$ l at P1 and 30 $\mu$ l at P4). For pifithrin- $\alpha$  (PFT) treatment, PFT was dissolved in DMSO (less than 10% final) and NaCl and injected subcutaneously at 0.8 mg/ml concentration at postnatal stages (5 $\mu$ l at P3, P4 and P5). P7 Mice were anesthetized with euthasol and perfused with 2% paraformaldehyde (PFA). Brains were dissected, dehydrated in 20% sucrose at 4°C, embedded in OCT and cryosectioned at 14  $\mu$ m.

### ***Demyelinating lesions***

Before surgery, adult (2-3months) mice were weighted and anesthetized by intraperitoneal injection of mixture of ketamine (0.1 mg/g) and xylazine (0.01 mg/g). Focal demyelinating lesions were induced by stereotaxic injection of 1 $\mu$ l of lysolecithin solution (LPC, Sigma, 1% in 0.9%NaCl) into the corpus callosum (CC; at coordinates: 1 mm lateral, 1.3 mm rostral to bregma, 1.7 mm deep to brain surface) using a glass-capillary connected to a 10 $\mu$ l Hamilton syringe. Animals were left to recover in a warm chamber before being returned into their housing cages. Brains were collected 2 or 4 days after lesions (2-4 dpi).

### ***MS and non-neurological control tissues***

Autopsy brain tissue samples from patients with confirmed secondary progressive MS were obtained from the United Kingdom MS tissue bank (Richards Reynolds, Imperial College, London). MS tissue block containing active lesions and periplaque white matter were selected for analysis.

### ***Immunohistochemistry***

Cryosections (14- $\mu$ m thick) were permeabilized and blocked in blocking buffer (0.05% Triton X-100 and 10% normal goat serum in PBS) for 1 h and overlaid with primary antibodies overnight at 4 °C. Antibodies used in the study were: rat anti-PDGFR $\alpha$  (BD Bioscience, 558774, 1:250), mouse anti-APC (CC1, Oncogene Research, OP80, 1:100), rabbit anti-Chd7 (Cell signaling, 6505, 1:1000), sheep anti-Chd7 (R&D, AF350, 1:100) rabbit anti-APC (Santa Cruz, sc-896, 1:100), mouse anti-Nkx2.2 (gift from Brahim Nait-Oumesmar's lab, 1:4), mouse anti-Olig1 (NeuroMab, 75-180, 1:500), rabbit anti-Itpr2 (Millipore, AB3000, 1:40), rabbit anti-Chd8

(Bethyl, A301-224A, 1:1000), mouse-anti MCM2 (BD biosciences, 610701, 1:500), rabbit anti-p53 (Leica, P53-CM5P-L, 1:500), mouse anti-CNP (Millipore, MAB326R, 1:250). After washing with 0.05% Triton X-100 in PBS, sections were incubated with secondary antibodies conjugated to Alexa488, Alexa594 or Alexa647 (Thermo, 1:1,000) and DAPI for 1h at room temperature, washed in PBS and mounted with Fluoromount-G (SouthernBiotech).

For cells, coverslips were fixed in 4% paraformaldehyde for 10 minutes at room temperature and washed in PBS. They were blocked for 1h at room temperature in blocking buffer (0.05% Triton X-100 and 10% normal goat serum in PBS) and overlaid with primary antibodies for 30min at room temperature. After washing with 0.05% Triton X-100 in PBS, cells were incubated with secondary antibodies conjugated to Alexa488, Alexa594 or Alexa647 (Thermo, 1:1,000) for 30min at room temperature, stained in DAPI for 5 min, washed in PBS and mounted with Fluoromount-G (SouthernBiotech).

Photos of were taken with Zeiss microscope using Apotome system optical sectioning and deconvolution. Z-stack was used. Photo are treated and cells were counted using Zen and ImageJ software packages.

### ***OPCs MAC sorting***

Cortex and corpus callosum were dissected from P7 mouse brains from control, *Chd7iKO* or wild type mice. Dissociation of tissues was done using neural tissue dissociation kit (T) (Miltenyi biotec) and dissociator (gentleMACS Octo Dissociator, Miltenyi Biotec). Magnetic sorting was done using anti-O4-coupled-beads and the MultiMACS Cell24 Separator Plus (Miltenyi biotec). To control obtained sorted cells, cells were put on coverslips coated with poly-ornithine for a couple of hours and fixed in 4% paraformaldehyde for 10 minutes, before doing immunostaining. For RNA-seq, at least 1.105 cells of each sample (controls and mutants) were directly processed after MACs. For ChIP-seq, O4<sup>+</sup>-cells from wild type mice were sorted and directly fixed in 1% formaldehyde.

### ***RNA extraction and qRT-PCR.***

Analyses were conducted with RNA extracts from MACsorted O4<sup>+</sup> cells from P7 mutant mice and their littermate controls. Total RNA was extracted using Nucleospin RNA kit (Macherey-Nagel). cDNAs were generated with SuperScript cDNA Synthesis Kit (Invitrogen). RTqPCR was

performed using LightCycler® 96 real-time PCR system (Roche) and primers for mouse gene sequences were: Chd7-f, CAGCAGCATCTGCATCATCT, Chd7-r, GACCCAGGTGTCCAGAAGAG; Ascl1-f, ACTTGA ACTCTATGGCGGGTT, Ascl1-r, CCAGTTGGTAAAGTCCAGCAG; Olig2-f, GAAGCAGATGACTGAGCCCGAG, Olig2-r, CCCGTAGATCTCGCTCACCAG; Pdgfra-f, ACAGAGACTGAGCGCTGACA, Pdgfra-r, CTCGATGGTCTCGTCCTCTC; Sox10-f, CAGGTGTGGCTCTGCCACG, Sox10-r, GTGTAGAGGGGCCGCTGGGA; Nkx2-2-f, TGGCCATGTACACGTTCTGA, Nkx2-2-r, CCGATGCTCAGGAGACGAAA; Gpr17-f, ACACAGTTGTCTGCCTGCAA, Gpr17-r, GCCGTAGTGGGTAGTTC TTG; Myrf-f, CCTGTGTCCGTGGTACTGTG, Myrf-r, TCACACAGGCGGTAGAAGTG; Cnp-f, TCCACGAGTGCAAGACGCTATTCA, Cnp-r, TGTAAGCATCAGCGGACACCATCT; Mbp-f, CCAAGTTC ACCCCTACTCCA, Mbp-r, TAAGTCCCCGTTTCTGTG; Trp53-f, GGGGAGGAGCCAGGCCATCA, Trp53-r, CCGCGCCATGGCCATCTACA;  $\beta$ -actin-f, TCCTAGCACCATGAAGATCAAGATC,  $\beta$ -actin-r, CTGCTTGCTGATCCACATCTG.  $\beta$ -actin was used to normalize.

#### ***RNA-seq and data analysis.***

RNA-seq libraries from control (n=7) and Chd7iKO (n=5) O4+ cells were prepared and sequenced. All RNA-Seq data were aligned to mm10 using TopHat59 with default settings. We used featureCounts for the gene-scaled counting and edgeR to analyze differentially expressed transcripts. In all differential expression tests, genes were considered regulated when p-value < 0.05 and Fold change > 1.2. Heatmap of gene expression was generated using R language (<http://www.r-project.org>). GO analysis of genes repressed and increased in Chd7iKO mutants was performed using Pathway

#### ***ChIP-Seq and data analysis.***

ChIP-seq assays were performed using iDeal ChIP-seq kit for Transcription Factors (Diagenode). Briefly, fresh sorted O4+ cells were fixed in 1% formaldehyde for 10 min at room temperature. Lysate were sonicated with a Bioruptor Pico sonicator (Diagenode, total time 8 min) and 4 $\mu$ g of antibodies were added to chromatin and incubated at 4°C overnight. Rabbit anti-Chd7 (Cell signaling, 6505) and anti-Chd8 (Bethyl, A301-224A) antibodies were used for immunoprecipitation experiments. Chromatin-protein complexes were immunoprecipitated with protein A/G magnetic beads and washed sequentially.

The ChIP-seq libraries were sequenced and mapped using bowtie2 with option -X 2000 to mm10. Data were filtered with SAM tools with options -q 10 -F 4 and duplicated were

removed via “picard MarkDuplicates” with options ASSUME\_SORTED=true, REMOVE\_DUPLICATES=true, CREATE\_INDEX=false and VALIDATION\_STRINGENCY=LENIENT. Peak calling was performed using MACS (Model-based Analysis of CHIP-Seq) (<http://liulab.dfci.harvard.edu/MACS>) with options: keep-dup all, nolambda, broad, nomodel, extsize 75 -q 0.01 and peaks were filtered to obtains length<100bp and P-Value>5%. Two and one separate experiments were done for Chd7 and Chd8, respectively. Input and Mock CHIP were used as controls in each individual experiments.

Representation of the data and correlations were done using Genomatix. Promoters correspond to regions 1000bp upstream of transcription start site (TSS) and 10bp downstream of TSS (Genomatix). Enhancers correspond to the binding of Sox10 and Olig2 (He et al., 2017; Yu et al., 2013). Gene expression profiles have been analyzed from OL stage specific transcriptome and single-cell analysis (Marques et al., 2016; Zhang et al., 2014).

#### ***ATAC-seq and data analysis.***

O4+ cells from Control and Chd7iKO P7 mice were purified as described above and 105 cells were lysate in lysis buffer (0.1% Igepal (CA-630, Sigma), 10mM Tris-HCl pH7.4, 10mM NaCl, 3mM MgCl<sub>2</sub>) before ATAC-seq reaction was done as described before (Buenrostro et al., 2015). Libraries were done using Nextera DNA sample kit (Illumina) and sequenced.

Reads from 5 controls and 5 Chd7iKO were uniquely aligned to the mouse reference genome (mm10) using bowtie2 with option -X 2000 to mm10. Data were filtered with SAM tools with options -q 10 -F 4, reads from mitochondria were suppressed and duplicated were removed via “picard MarkDuplicates” with options ASSUME\_SORTED=true, REMOVE\_DUPLICATES=true, CREATE\_INDEX=false and VALIDATION\_STRINGENCY=LENIENT. Peak calling was performed using MACS (Model-based Analysis of CHIP-Seq) (<http://liulab.dfci.harvard.edu/MACS>) with options: keep-dup all, nolambda, broad, nomodel, extsize 75 -q 0.01 and peaks were filtered to obtains length<100bp and P-Value>5%. Peaks from Chd7 CHIP were correlated to ATAC signal via bedops. Genomatix genome browser was used to visualize ATAC and CHIP peaks.

#### ***Statistical Analysis***

All statistical analyses were done using Prism. Data are shown as mean  $\pm$  s.e.m. Data distribution was assumed to be normal, but this was not formally tested. Statistical

significance was determined using two-tailed Student's t tests. One-way ANOVA test was performed by multiple comparisons or pairwise comparisons following Turkey's ranking tests when comparing multiple groups. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those generally employed in the field. Quantifications were performed from at least three independent experiments. No randomization was used to collect all the data, but they were quantified blindly.

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## FIGURE LEGENDS

### Figure 1. *Chd7* regulated genes are involved in OPCs proliferation, differentiation and survival

A – Left, Diagram representing tamoxifen (Tam) administration to *control* (*Ctrl*) and *Chd7iKO* (*iKO*) mice at P1 and P4 followed by analysis at P7. Right, Immunostaining showing PDGFR $\alpha$  and *Chd7* expression in the corpus callosum of *Ctrl* and *iKO* P7 mouse brains. Stars show OPCs still expressing *Chd7* in *iKO*. Scale bar 10  $\mu$ m.

B – Quantification of *Chd7*<sup>+</sup> cells as a percentage of total PDGFR $\alpha$ <sup>+</sup> cells in the corpus callosum and cortex of *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (n= 5 *Ctrl* and 5 *iKO*; CC, P < 0.001, t = 28.73; Ctx, P < 0.001, t = 64.92; two-tailed unpaired Student's t test).

C – Diagram representing MACsorting of O4<sup>+</sup> cells in *Ctrl* and *iKO* P7 brains after tamoxifen (Tam) administration at P1 and P4, followed by RNA-seq.

D – Pie chart showing relative percentage and number of genes that were significantly upregulated or downregulated in P7 *iKO* O4<sup>+</sup> cells compared to *Ctrl* (Fold Change>1.2; p-value<0.05).

E – Heatmap representing the expression of 100 most different genes in *Ctrl* and *iKO* O4<sup>+</sup> cells (n=7 *Ctrl* and 5 *iKO*).

F – Diagram representing the gene ontology (GO) analysis of the significantly upregulated and downregulated genes between *iKO* and *Ctrl*. The numbers indicate the number of genes of each category. \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001.

### Figure 2. *Chd7* promotes the expression of genes involved in OPC differentiation and maturation

A – Top, scheme of exons 2 and 3 of *Chd7* gene. Orange triangle represent *LoxP* sites, blue arrows represent primers used for RT-qPCR. Bottom, RT-qPCR analysis of *Chd7* deletion in P7 O4<sup>+</sup> cells of *Chd7iKO* compared to Control. Normalization with  $\beta$ -actin. The data are presented as mean  $\pm$  s.e.m. (n = 7 Controls and 7 *Chd7iKO*; P < 0.001, t = 50.97; two-tailed unpaired Student's t test).

B – Barplot of the Log Fold Change (LogFC) of genes involved in OPC differentiation of *iKO* compared to *Ctrl*. Dashed gray line represent FC = 1.2 (n=7 *Ctrl* and 5 *iKO*; NG2, P = 0.07; Pdgfra, P = 0.16; Ascl1, P = 0.31; Sox10, P < 0.001; OLig2, P = 0.11; Brg1, P = 0.09; Gpr17, P < 0.001; Nkx2-2, P < 0.001; Enpp6, P < 0.001; Itpr2, P < 0.001; Tcf7l2, P < 0.001).

C – Barplot the LogFC of genes involved in OL maturation and myelination of *iKO* compared to *Ctrl*. Dashed gray line represent FC = 1.2 (n=7 *Ctrl* and 5 *iKO*; *Olig1*, P = 0.08; *Nkx6-2*, P =0.16; *Sirt2*, P < 0.001; *Myrf*, P < 0.001; *Zf488*, P < 0.001; *Cnp*, P < 0.001; *Mbp*, P = 0.7; *Omg*, P < 0.001; *Plp1*, P =0.05; *Mag*, P =0.14; *Mog*, P =0.31)

D – RT-qPCR analysis of OL differentiation- and maturation-related genes in P7 O4<sup>+</sup> cells of *Chd7iKO* compared to Control. Normalization with  $\beta$ -actin. The data are presented as mean  $\pm$  s.e.m. (n = 4 Controls and 4 *Chd7iKO*; *Ascl1*, P = 0.126, t = 1.83; *Olig2*, P = 0.266, t = 1.25; *PDGFR $\alpha$* , P = 0.251, t = 1.30; *Sox10*, P = 0.022, t = 3.61; *Nkx2.2*, P = 0.012, t = 3.825; *Gpr17*, P = 0.013, t = 3.29; *Myrf*, P < 0.001, t = 6.47; *Cnp*, P = 0.035, t = 3.13; *Mbp*, P = 0.059, t = 2.42; two-tailed unpaired Student's t test).

E –Diagram representing tamoxifen (Tam) administration to control and *Chd7iKO* mice at P1 and P4 followed by analysis at P7.

F – Immunostaining of PDGFR $\alpha$ , Nkx2.2, Itpr2 and APC in the corpus callosum from control (*Ctrl*) and *Chd7iKO* (*iKO*) mice at P7. Scale bar 10  $\mu$ m.

F – Quantification of Nkx2.2<sup>+</sup>, Itpr2<sup>+</sup> and APC<sup>+</sup> cells density (/mm<sup>2</sup>) in the corpus callosum of P7 *Ctrl* and *iKO* mice. The data are presented as mean  $\pm$  s.e.m. (*Nkx2.2*, n= 4 *Ctrl* and 5 *iKO*, P < 0.001, t = 7.37; *Itpr2*, n= 3 *Ctrl* and 4 *iKO*, P < 0.001, t = 9.26; *APC*, n= 3 *Ctrl* and 3 *iKO*, P = 0.0024, t = 9.58; two-tailed unpaired Student's t test). \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001.

### Figure 3. Chd7 promotes OPC survival through p53 downregulation

A – Immunostaining of PDGFR $\alpha$  in the corpus callosum (CC) and cortex (Ctx) from Control (*Ctrl*) and *Chd7iKO* (*iKO*) P7 mice. Scale bar 10  $\mu$ m.

B - Quantification of PDGFR $\alpha$ <sup>+</sup> cells density (/mm<sup>2</sup>) in the corpus callosum and cortex of *Ctrl* and *iKO* mice at P7. The data are presented as mean  $\pm$  s.e.m. (CC, n= 5 *Ctrl* and 5 *iKO*, P =0.0182, t = 2.96; Ctx, n= 5 *Ctrl* and 5 *iKO*, P < 0.001, t = 6.44; two-tailed unpaired Student's t test).

C – Barplot the Log Fold Change (LogFC) of genes involved in cell cycle and apoptosis of *iKO* compared to *Ctrl*. Dashed gray line represent FC = 1.2 (n=7 *Ctrl* and 5 *iKO*; *Ccnd1*, P < 0.001; *Cdk4*, P = 0.74; *Cdk6*, P = 0.004; *Ccne1*, P < 0.001; *Ccna1*, P = 0.012; *Cdk2*, P = 0.002; *Ccnb1*, P < 0.001; *Cdk1*, P < 0.001; *Mki67*, P < 0.001; *Mcm2*, P < 0.001; *Trp53*, P < 0.001; *Noxa*, P < 0.001; *p21*, P < 0.001)

D – Immunostaining of MCM2 and PDGFR $\alpha$  in the corpus callosum from *Ctrl* and *iKO* P7 mice. Scale bar 10  $\mu$ m.

E – Immunostaining of p53 and PDGFR $\alpha$  in the corpus callosum from *Ctrl* and *iKO* P7 mice. Scale bar 10  $\mu$ m.

F – Quantification of MCM2<sup>+</sup> and MCM2<sup>-</sup> OPCs (PDGFR $\alpha$ <sup>+</sup>) density (/mm<sup>2</sup>) in the CC of *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (MCM2<sup>+</sup>, n= 3 *Ctrl* and 3 *iKO*, P = 0.128, t = 1.91; MCM2<sup>-</sup>, n= 3 *Ctrl* and 3 *iKO*, P = 0.019, t = 3.79; two-tailed unpaired Student's t test).

G – Quantification of p53<sup>+</sup> OPCs (PDGFR $\alpha$ <sup>+</sup>) density (/mm<sup>2</sup>) in the CC of *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (n= 3 *Ctrl* and 4 *iKO*, P = 0.0027, t = 5.52; two-tailed unpaired Student's t test).

H – RT-qPCR analysis of *Trp53* genes (coding p53) in P7 O4<sup>+</sup> cells of *Chd7iKO* compared to Control. Normalization with  $\beta$ -*actin*. The data are presented as mean  $\pm$  s.e.m. (n = 4 controls and 4 mutant tissues; P = 0.006, t = 4.14; two-tailed unpaired Student's t test).

I – Immunostaining of PDGFR $\alpha$  and Nkx2.2 in the corpus callosum from *Ctrl*, *iKO* and *iKO*+PFT P7 mice. Scale bar 10  $\mu$ m.

J – Diagram representing tamoxifen (Tam) administration at P1 and P4 and pifithrin- $\alpha$  (PFT) injection at P3, P4 and P5, followed by tissue collection at P7.

K – Quantification of PDGFR $\alpha$ <sup>+</sup> and Nkx2.2<sup>+</sup> cell density (/mm<sup>2</sup>) in the corpus callosum of *Ctrl*, *iKO* and *iKO*+PFT P7 mice. The data are presented as mean  $\pm$  s.e.m. (PDGFR $\alpha$ <sup>+</sup>, n= 4 *Ctrl*, 4 *iKO* and 3 *iKO*+PFT, ANOVA F (3, 12) = 5.509; multiple comparisons with t test, q*Ctrl* vs *iKO* = 4.39, q*Ctrl* vs *iKO*+PFT = 0.42, q*iKO* vs *iKO*+PFT = 3.39; Nkx2.2<sup>+</sup>, n= 3 *Ctrl*, 4 *iKO* and 3 *iKO*+PFT, ANOVA F (3, 10) = 28.68; multiple comparisons with t test, q*Ctrl* vs *iKO* = 8.23, q*Ctrl* vs *iKO*+PFT = 10.28, q*iKO* vs *iKO*+PFT = 3.27). \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001

J – Scheme representing proliferative OPC population (light blue), non-cycling OPC population (middle blue) and OL population (dark blue) in Control, *Chd7iKO* and *Chd7iKO*+PFT.

#### **Figure 4. Chd7 bind to genes involved in OPCs proliferation, differentiation and survival.**

A – Diagram representing MACsorting of O4<sup>+</sup> cells of P7 wild-type mice followed by Chd7 ChIP-seq.

B – Venn diagrams depicting overlap of Chd7 binding sites in OPCs and OLs.

C – Venn diagrams depicting overlap of Chd7 bound genes in OPCs and OLs with examples of genes involved in OL differentiation (blue), cell death (green) and cell cycle (orange).

D – Graph showing the number of correlation of Chd7 peaks in OPCs (blue) and in OLs (black) compared to the central position of enhancer regions.

E – Graph showing the number of correlation of Chd7 peaks in OPCs (blue) and in OLs (black) compared to the central position of promoter regions.

F – Graph showing the number of correlation of Chd7 peaks in OPCs (blue) and in OLs (black) compared to the central position of active histone marks H3K4me3/H3K27ac regions.

G – Pie chart showing the proportion of Chd7 bound genes among *Chd7iKO* downregulated and upregulated genes with examples of Chd7 bound genes involved in OL differentiation (blue), cell death (green) and cell cycle (orange).

H – Diagram representing MACsorting of O4<sup>+</sup> cells of P7 *Chd7iKO* and control mice followed by Chd7 ATAC-seq.

I – Graph showing the ATAC signal normalized coverage in TSS of genes differentially expressed in OPCs from the 25% of less expressed genes (blue) to the 25% most expressed genes (red).

J-K – Schematic representation from Genomatix genome browser of gene locus integrating ChIP data for main oligodendroglial TFs (Olig2 and Sox10) and chromatin remodeling factors (Chd7, Chd8) in OPCs and ATAC data from *Ctrl* and *Chd7iKO* P7 OPCs. Locus of genes involved in oligodendrogenesis (J) and *Trp53* (K).

**Figure 5. Chd8/CHD8 is highly enriched in immature OLs during myelination and remyelination**

A – Chd8 immunofluorescence in P14 brain sections showing expression in all maturing OLs (CC1<sup>high</sup> expressing cells, arrows) and in cortical neurons. A1 is a higher magnification of the inset in A, showing that, beside the high Chd8 expression in maturing OLs (white arrows) and neurons (grey arrows), low levels of Chd8 expression is detected in some OPC (PDGFR<sup>+</sup> cells, white arrowheads) and hardly detectable in astrocytes (CC1<sup>low</sup> expressing cells, grey arrowheads).

B – Immunofluorescence at P21 showing young mature MOG<sup>+</sup> OLs still express Chd8 (white arrows). B1 is a higher magnification of the inset shown in B; grey arrows correspond to Chd8<sup>+</sup> neurons.

C – Summary schematic showing Chd8 and Chd7 expression at different stages of the oligodendroglial lineage identified by PDGFR $\alpha$ , CC1 and MOG markers. Ctx, cortex; CC, corpus callosum; Scale bar 20 $\mu$ m.

D-E – P140 brain sagittal sections at 2 days after LPC lesion in the corpus callosum (2 dpi) showing increased levels of Chd8 expression close to the lesion.

D – Graphic representing Chd8<sup>+</sup> cells showing increased number of Chd8<sup>+</sup> cells in/around lesion.

E – Chd8 immunofluorescence levels presented in green-blue gradient color code showing high levels of Chd8 in cells in/around the lesion.

F – Immunofluorescence showing the lesion area at 2 dpi (dotted line) by the absence of CC1<sup>+</sup> OLs where PDGFR $\alpha$ <sup>+</sup>-OPCs express strong levels of Chd8. Comparison of Chd8 expression found in OPCs present at a distance from the lesion (E2) and OPCs inside lesions (E1). Arrow heads represent PDGFR $\alpha$ <sup>+</sup>-OPCs. Asterisk indicate Chd8 cells not expressing oligodendroglial markers, most likely corresponding to microglial/macrophage cells.

G - Immunofluorescence showing the lesion area and remyelinating area at 4 dpi (dotted line) by the presence of iOLs (CC1<sup>high</sup>/Olig1<sup>-</sup>) where OPCs (Olig1<sup>+</sup> cells) and iOLs express strong levels of Chd8. G1 is a higher magnification of the inset in G, in remyelinating area. Arrows represent iOLs, arrow heads represent OPCs. Asterisk indicate Chd8 cells not expressing oligodendroglial markers, most likely corresponding to microglial/macrophage cells.

H – Oil-Red staining showing white matter demyelinated areas as darker zones (arrows).

I – Luxol staining in adjacent section to tissue in A showing demyelinated areas (light blue staining). Insets indicated areas used in immunofluorescence staining following panels.

J – Demyelinated white matter track showing many small nuclei expressing CHD8 with typical OLs alignments.

K – High magnification picture showing that CHD8+ cells are labelled by an anti-NogoA antibody recognizing differentiating/immature OLs.

L – MBP staining showing the border area of re/demyelination.

M,N – High magnification showing CHD8+ nuclei (arrows) in remyelinating areas depicted in D. Scale bar 20µm.

**Figure 6. Chd8 binds together with Chd7 to OPC differentiation, proliferation and survival genes**

A – Diagram representing MACsorting of O4+ cells of P7 wild-type mice followed by Chd8 ChIP-seq.

B – Venn diagrams depicting overlap of Chd7 and Chd8 binding sites in OPCs.

C – Venn diagrams depicting overlap of Chd7 and Chd8 bound genes in OPCs with examples of genes involved in OL differentiation (blue), cell death (green) and cell cycle (red).

D – Diagram representing example of genes with time-controlled expression (Data from (Marques et al., 2016)) divided in three groups: OPC genes (*Ascl1* and *Pdgfra*), iOL genes (*Nkx2-2* and *Gpr17*) and mOL genes (*Mbp* and *Omg*)

E – Schematic representation from Genomatix genome browser of examples of locus (OPC, iOL and mOL gene) integrating ChIP data for main oligodendroglial TFs (*Ascl1*, *Olig2* and *Sox10*), chromatin remodeling factors (*Chd7*, *Chd8* and *Brg1*) and active epigenetic marks (*H3K27ac* and *H3K4me3*) in OPCs and OLs.

F – Model of regulation of time-controlled gene expression by binding of *Sox10*, *Olig2*, *Ascl1*, *Chd7*, *Chd8*, and *Brg1* in OPCs and mOLs.

**Supplemental figure 1** (related to figure 1)

A – Immunostaining showing PDGFR $\alpha$ , CNP and Nkx2.2 expression in MACsorted O4+ cells of P7 mice. Scale bar 10µm.

B – Quantification of PDGFR $\alpha$ -CNP+ cells as a percentage of total cells (DAPI) in O4+ cells of *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (n=6 *Ctrl* and 7 *iKO*; P = 0.376, t = 0.92; two-tailed unpaired Student's t test).

C – Quantification of PDGFR $\alpha$ +, Nkx2.2+ and CNP+ cells as a percentage of total cells (DAPI) in O4+ cells of *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (PDGFR $\alpha$ +, n=5 *Ctrl* and 3 *iKO*; P = 0.17, t = 1.56; Nkx2.2+, n=5 *Ctrl* and 4 *iKO*; P = 0.46, t = 0.78; CNP+, n=5 *Ctrl* and 5 *iKO*; P = 0.58, t = 0.57; two-tailed unpaired Student's t test).

D – Quantification of the number of sorted cells per sample after MACs in *Ctrl* and *iKO*. The data are presented as mean  $\pm$  s.e.m. (n=7 *Ctrl* and 7 *iKO*; P = 0.0016, t = 4.05; two-tailed unpaired Student's t test).

E – Ratio between Nkx2.2+ and PDGFR $\alpha$ + cells in the corpus callosum of *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (n= 3 *Ctrl* and 3 *iKO*, P =0.31, t = 1.16; two-tailed unpaired Student's t test).

F – Barplot showing the gene ontology (GO) analysis of the significantly upregulated and downregulated genes between control and *Chd7iKO* with Log of p-value. \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001.

### Supplemental figure 2 (related to figure 2)

A – Immunostaining showing APC and *Itpr2* expression during OL differentiation compared to PDGFR $\alpha$ , Olig1, Nkx2.2 and CC1 staining in the corpus callosum of wild-type P21 mice. Scale bars represent 10  $\mu$ m.

B- Immunostaining showing *Chd7* expression with co-labels PDGFR $\alpha$ , Olig1, Nkx2.2, CC1, APC and *Itpr2*. Scale bar 10  $\mu$ m.

C – Scheme representing expression levels of different markers depending on OL stage.

### Supplemental figure 3 (related to figure 2)

A – Top, diagram of the Tamoxifen (Tam) administration at P3 followed by tissue collecting at P14. Bottom, immunostaining of Olig2, APC/CC1 and Olig1 in the corpus callosum from control (*Ctrl*, A) and *Chd7iKO* (*ckO<sup>OPC</sup>*, A') mice at P14. A2 and A2' are a higher magnification of the inset in A and A', respectively. Scale bar 20  $\mu$ m.

B – Immunostaining of Olig2, APC/CC1 and Olig1 used to distinguish OL stages. OPC are Olig1<sup>nuclear</sup>, iOL1 are CC1<sup>high</sup>-Olig1<sup>-</sup>, iOL2 are CC1<sup>high</sup>-Olig1<sup>cyto</sup>, mOLs are CC1<sup>+</sup>-Olig1<sup>cyto</sup> and astrocytes are CC1<sup>Low</sup>.

C - Quantification of Olig1<sup>nuclear</sup> (OPCs), CC1<sup>high</sup> (OL) and CC1<sup>Low</sup> (astrocytes) cell density (/mm<sup>2</sup>) in *Ctrl* and *Chd7* cKO P14 mice. The data are presented as mean  $\pm$  s.e.m.

D - Quantification of iOL1 (CC1<sup>high</sup>-Olig1<sup>-</sup>), iOL2 (CC1<sup>high</sup>-Olig1<sup>cyto</sup>) and mOLs (CC1<sup>+</sup>-Olig1<sup>cyto</sup>) as a percentage of total OL cells in *Ctrl* and *Chd7* cKO P14 mice. The data are presented as mean  $\pm$  s.e.m.

### Supplemental figure 4 (refers to figures 3)

A – Quantification of MCM2+ and MCM2- OPCs (PDGFR $\alpha$ +) density (/mm<sup>2</sup>) in the Ctx of *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (MCM2+, n= 3 *Ctrl* and 3 *iKO*, P = 0.21, t = 1.51; MCM2-, n= 3 *Ctrl* and 3 *iKO*, P = 0.017, t = 3.96; two-tailed unpaired Student's t test).

B – Quantification of MCM2+ cells as a percentage of total PDGFR $\alpha$ + cells in the Ctx of *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (n= 3 *Ctrl* and 3 *iKO*, P = 0.003, t = 6.37; two-tailed unpaired Student's t test).

C - Quantification of p53 OPCs (PDGFR $\alpha$ +) density (/mm<sup>2</sup>) in the Ctx of *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (n= 3 *Ctrl* and 4 *iKO*, P < 0.001, t = 7.93; two-tailed unpaired Student's t test).

D – Quantification of p53+ cells as a percentage of total PDGFR $\alpha$ + cells in the Ctx of *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (n= 3 *Ctrl* and 4 *iKO*, P < 0.001, t = 10.84; two-tailed unpaired Student's t test).

E – Immunostaining of Ki67 and PDGFR $\alpha$  in the corpus callosum of P7 mice. Scale bar 10  $\mu$ m.

F – Quantification of Ki67+ and Ki67- OPCs (PDGFR $\alpha$ +) density (/mm<sup>2</sup>) in the CC and Ctx of *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (CC, Ki67+, n= 3 *Ctrl* and 3 *iKO*, P = 0.445, t = 0.86; Ki67-, n= 3 *Ctrl* and 4 *iKO*, P = 0.271, t = 1.24; Ctx, Ki67+, n= 3 *Ctrl* and 3 *iKO*, P = 0.113, t = 2.02; Ki67-, n= 3 *Ctrl* and 3 *iKO*, P < 0.001, t = 12.60; two-tailed unpaired Student's t test).

G – Quantification of Ki67+ cells as a percentage of total PDGFR $\alpha$ + cells in the CC and Ctx of *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (CC, n= 3 *Ctrl* and 4 *iKO*, P = 0.50, t = 0.72; Ctx, n= 3 *Ctrl* and 3 *iKO*, P < 0.001, t = 9.48; two-tailed unpaired Student's t test).

H – Immunostaining of Casp3 and PDGFR $\alpha$  in the cortex of *iKO* P7 mice. Scale bar 10  $\mu$ m.

I – Quantification of Casp3+ OPCs (PDGFR $\alpha$ +) density (/mm<sup>2</sup>) in the *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (n= 3 *Ctrl* and 4 *iKO*, P = 0.0147, t = 3.65; two-tailed unpaired Student's t test).

J – Quantification of Casp3+ cells as a percentage of total PDGFR $\alpha$ + cells in the *Ctrl* and *iKO* P7 mice. The data are presented as mean  $\pm$  s.e.m. (n= 3 *Ctrl* and 4 *iKO*, P = 0.011, t = 3.91; two-tailed unpaired Student's t test).

K – Immunostaining of MCM2 co-labelled with p53 in the CC of *iKO* P7 mice. Scale bar 10  $\mu$ m.

L – Pie chart showing the percentage of MCM2+ cells among the p53+ cells in *iKO* P7 mouse.

M – Barplot showing the density of the different population of PDGFR $\alpha$ + cells in the CC and Ctx of *Ctrl* and *iKO* P7 mice. \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001

#### **Supplemental figure 5** (related to figure 4 & 6)

A – Venn diagrams depicting overlap of binding sites between Sox10 and Olig2 in OLs.

B – Graph showing the number of correlation of Sox10 (blue), Olig2 (red) and Sox10-Olig2 (purple) peaks in OLs compared to the central position of promoter (left) and H3K27ac mark regions (right).

C – Venn diagrams depicting overlap of binding sites between Chd7, Chd8 and Sox10 (left), Chd7, Sox10 and Olig2 (middle) and Chd7, Brg1 and Ascl1 (right) in OPCs.

D – Venn diagrams depicting overlap of binding sites between Chd7, Sox10 and Olig2 in OLs.

E – Graph showing the number of correlation of Chd7 (blue), Chd8 (red) and Chd7-Chd8 (green) peaks in OPCs compared to the central position of enhancer (left), promoter (middle) and H3K4me3/H3K27ac mark regions (right).

**Supplemental figure 6** (related to figure 6)

Quantification of the percentage of binding of key transcription factors (Sox10, Olig2, Ascl1) and chromatin remodelers (Chd7, Chd8 and Brg1) in each gene group (OPC, iOL and mOL) in OPCs and OLs.

**Supplemental figure 7** (related to figure 6)

Schematic representation from Genomatix genome browser of example of locus integrating ChIP data for main oligodendroglial TFs (Ascl1, Olig2 and Sox10), chromatin remodeling factors (Chd7, Chd8 and Brg1) and active epigenetic marks (H3K27ac and H3K4me3) in OPCs and OLs.

**Supplemental figure 8** (related to figure 6)

Schematic representation from Genomatix genome browser of example of locus integrating ChIP data for main oligodendroglial TFs (Ascl1, Olig2 and Sox10), chromatin remodeling factors (Chd7, Chd8 and Brg1) and active epigenetic marks (H3K27ac and H3K4me3) in OPCs and OLs.



# FIGURES

## Figure 1

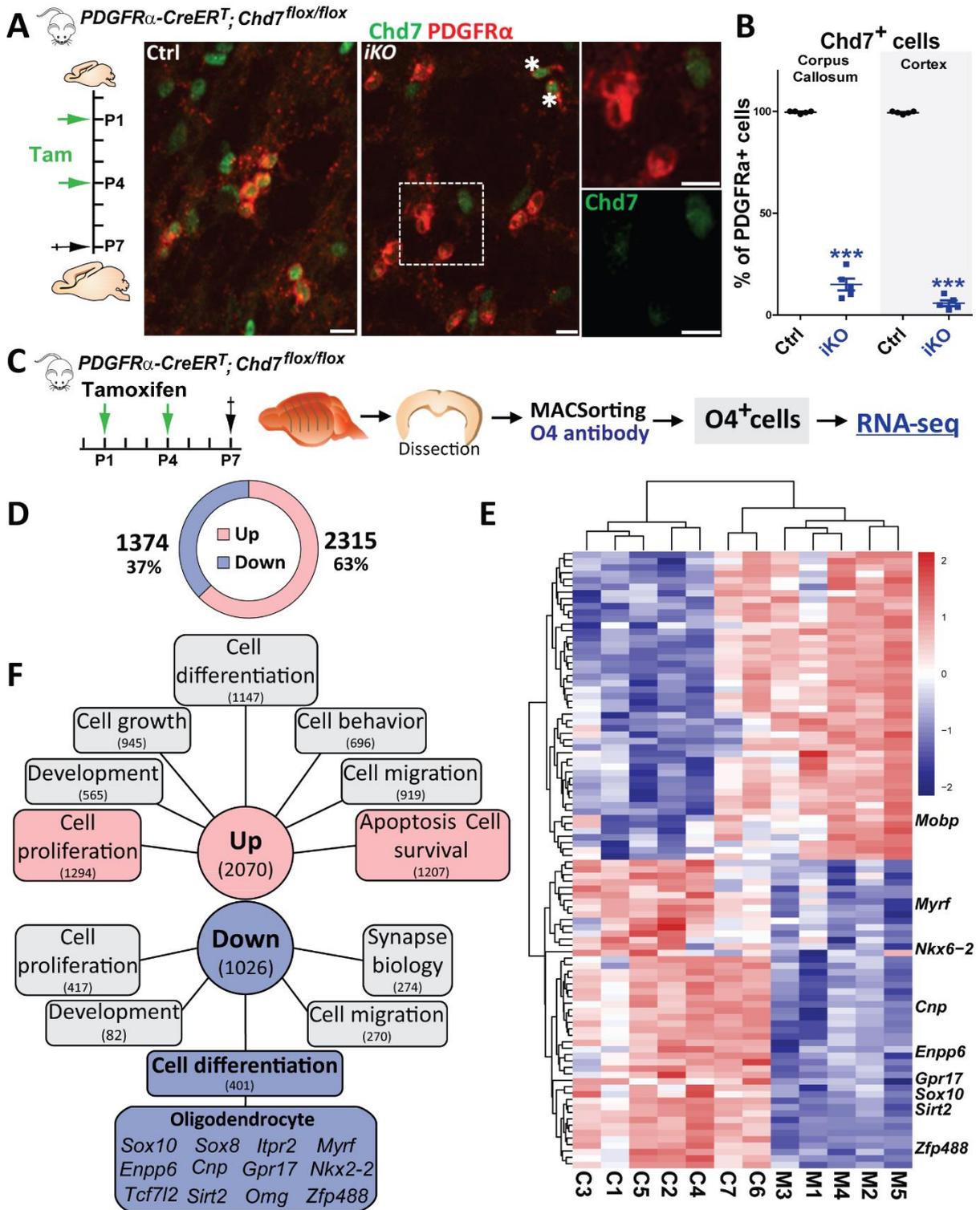


Figure 2

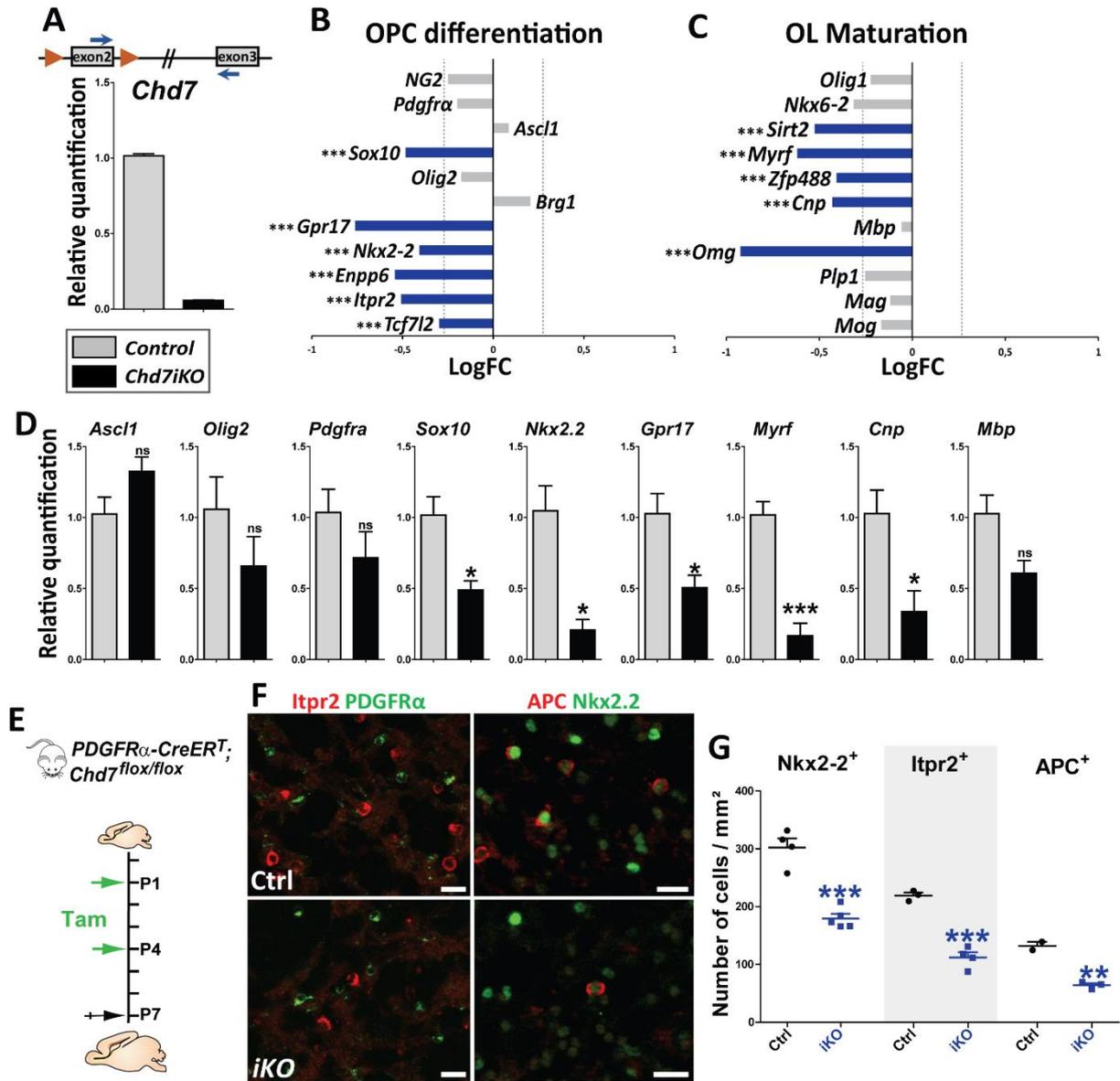
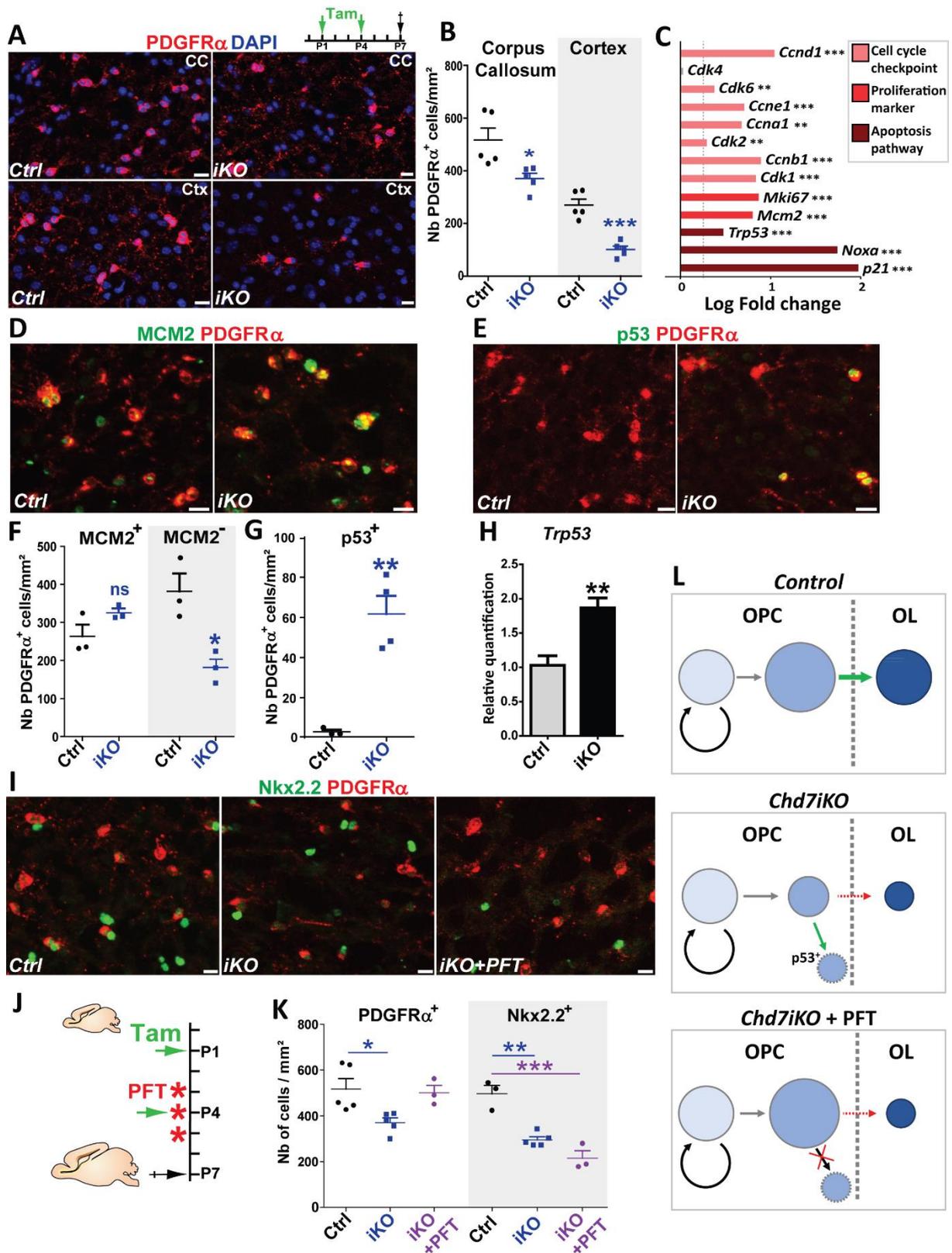


Figure 3



**Figure4**

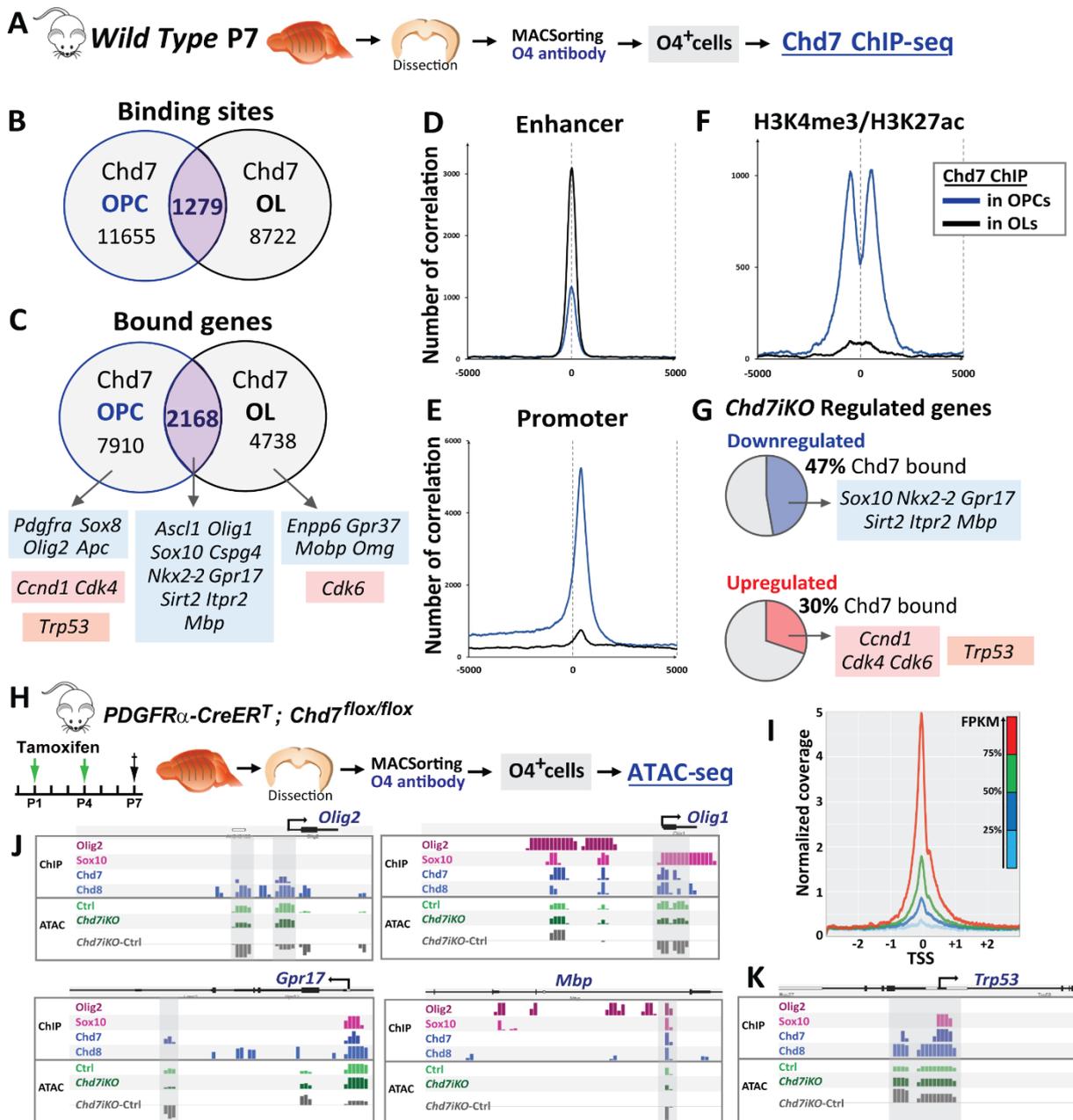


Figure 5

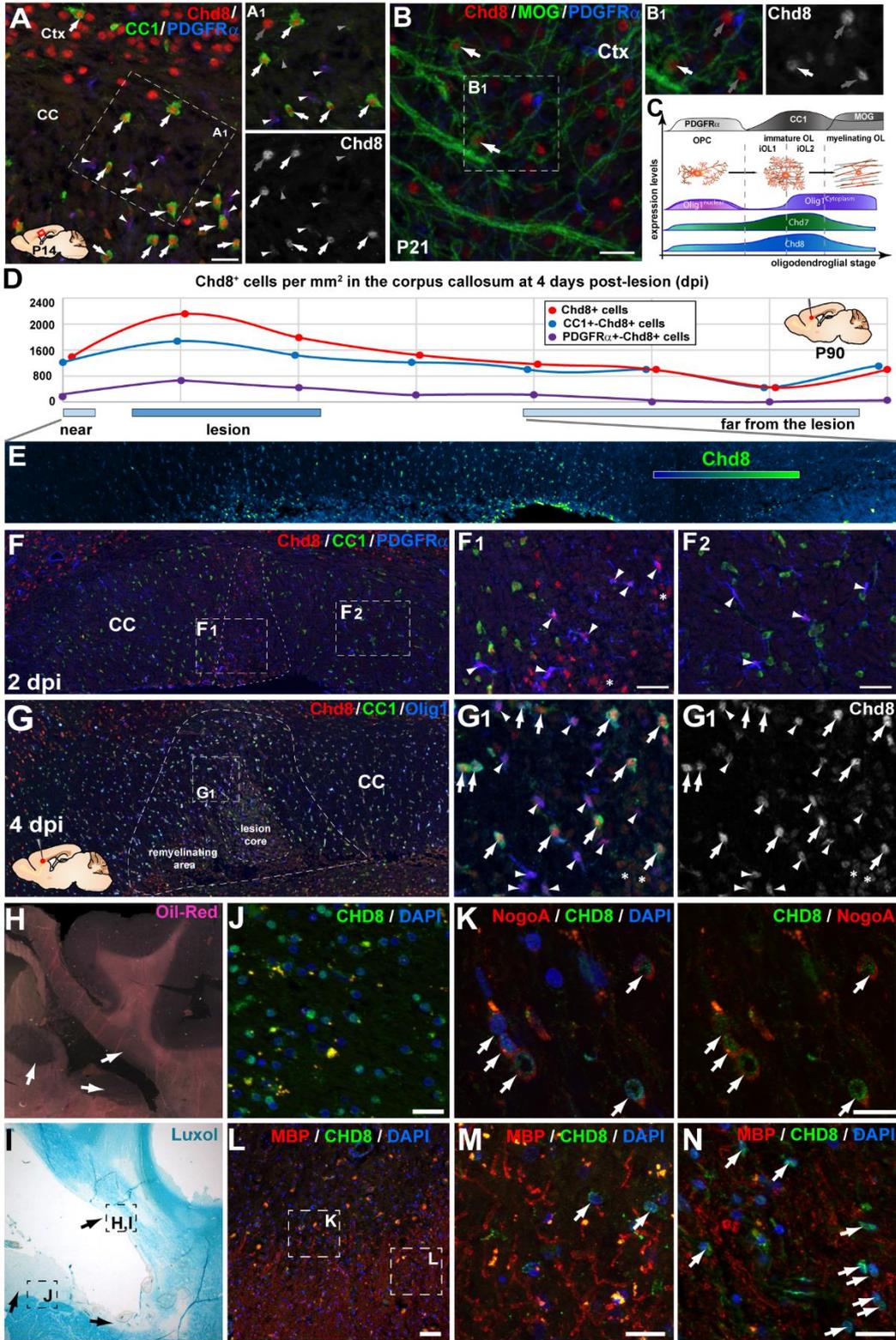
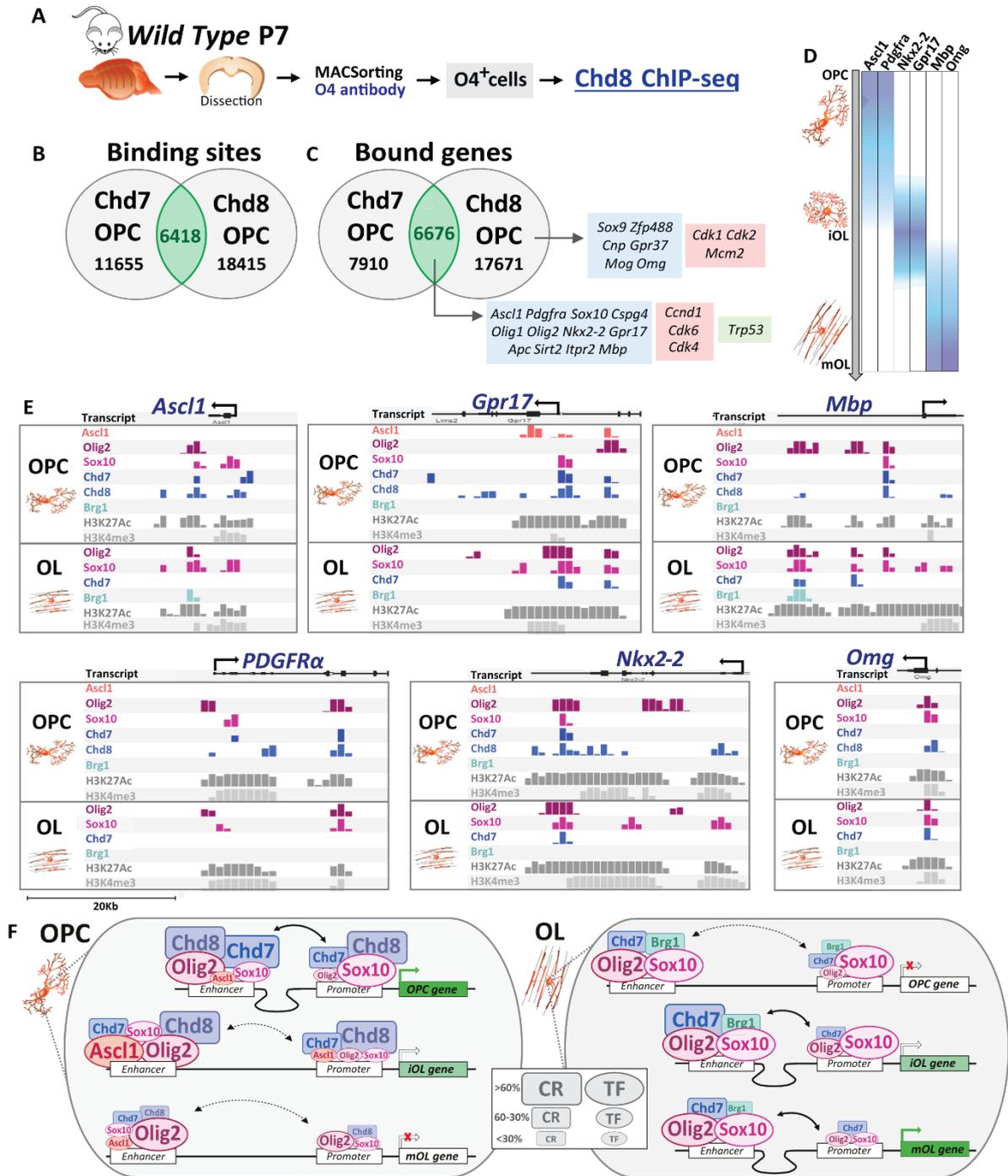
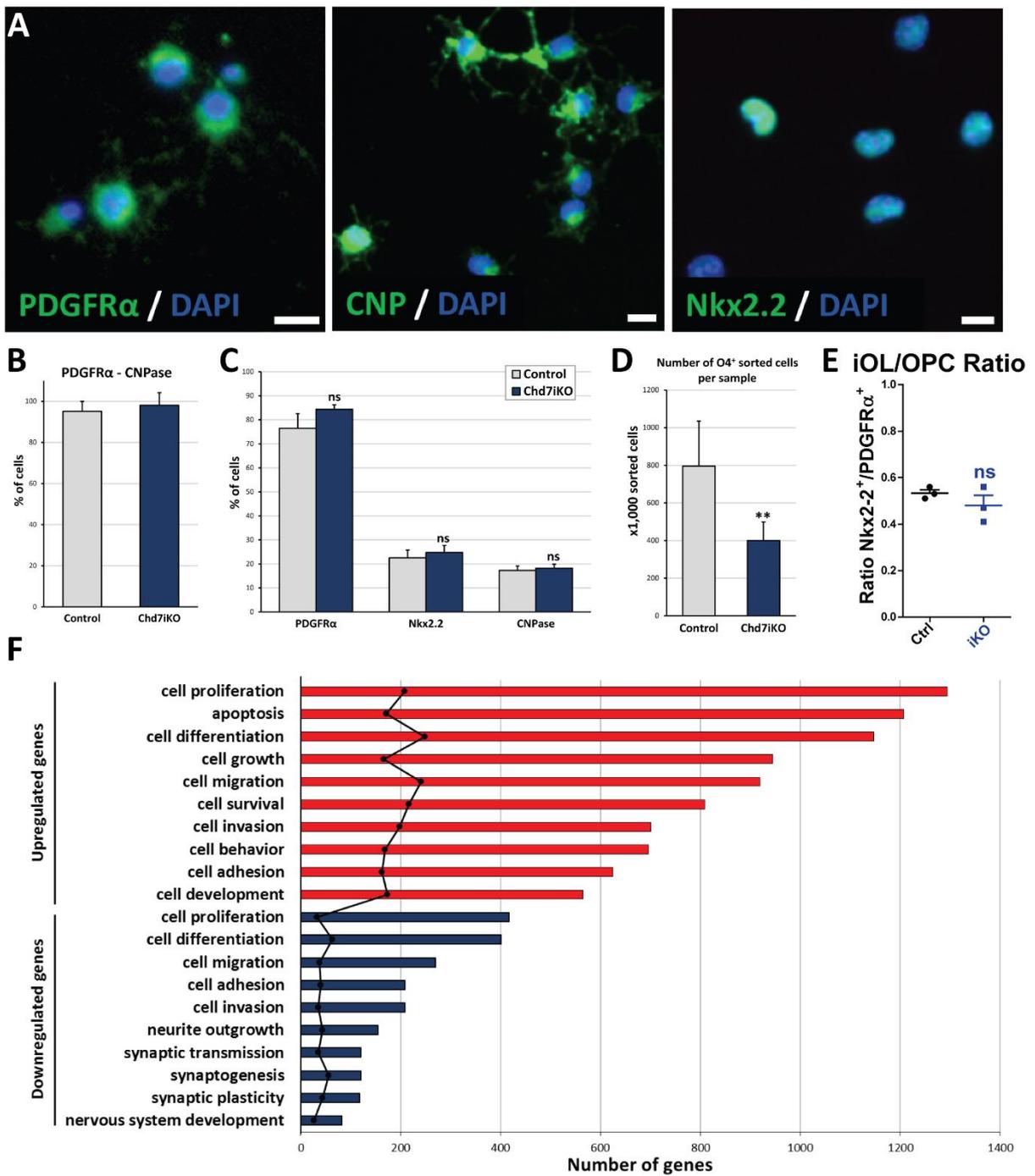


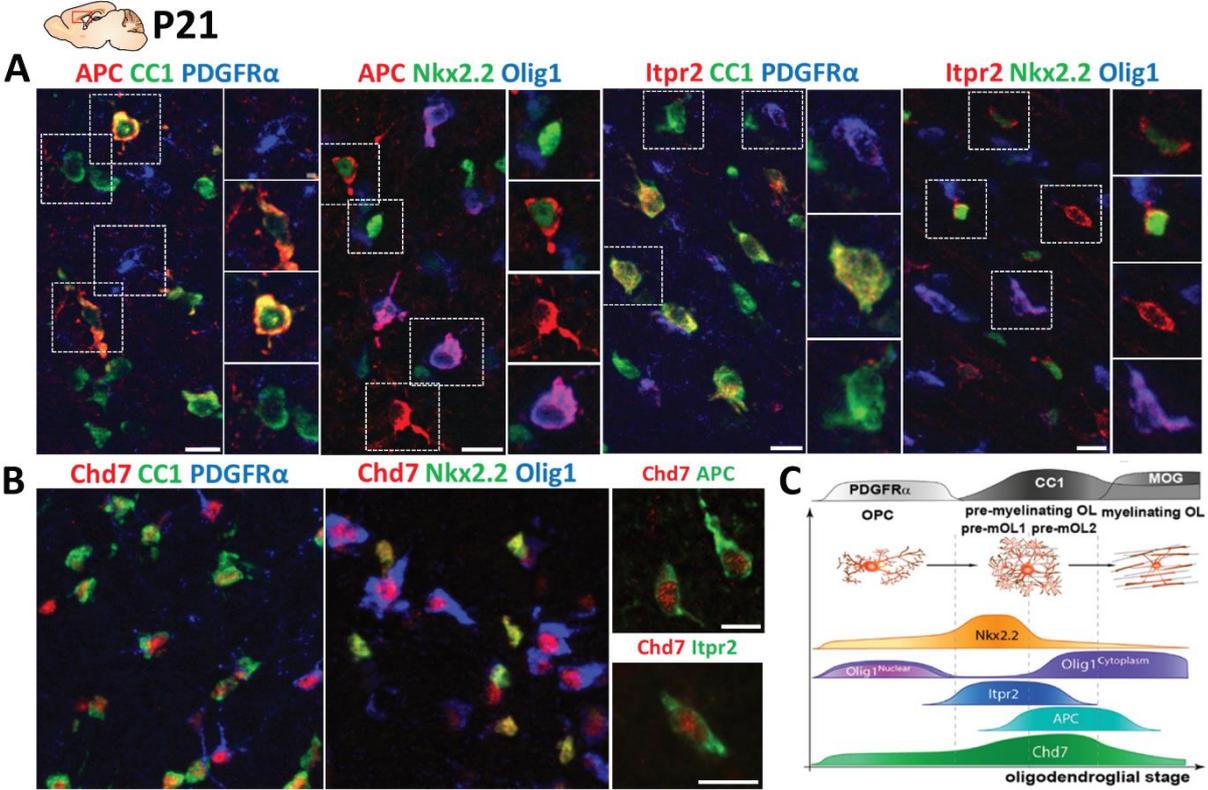
Figure6



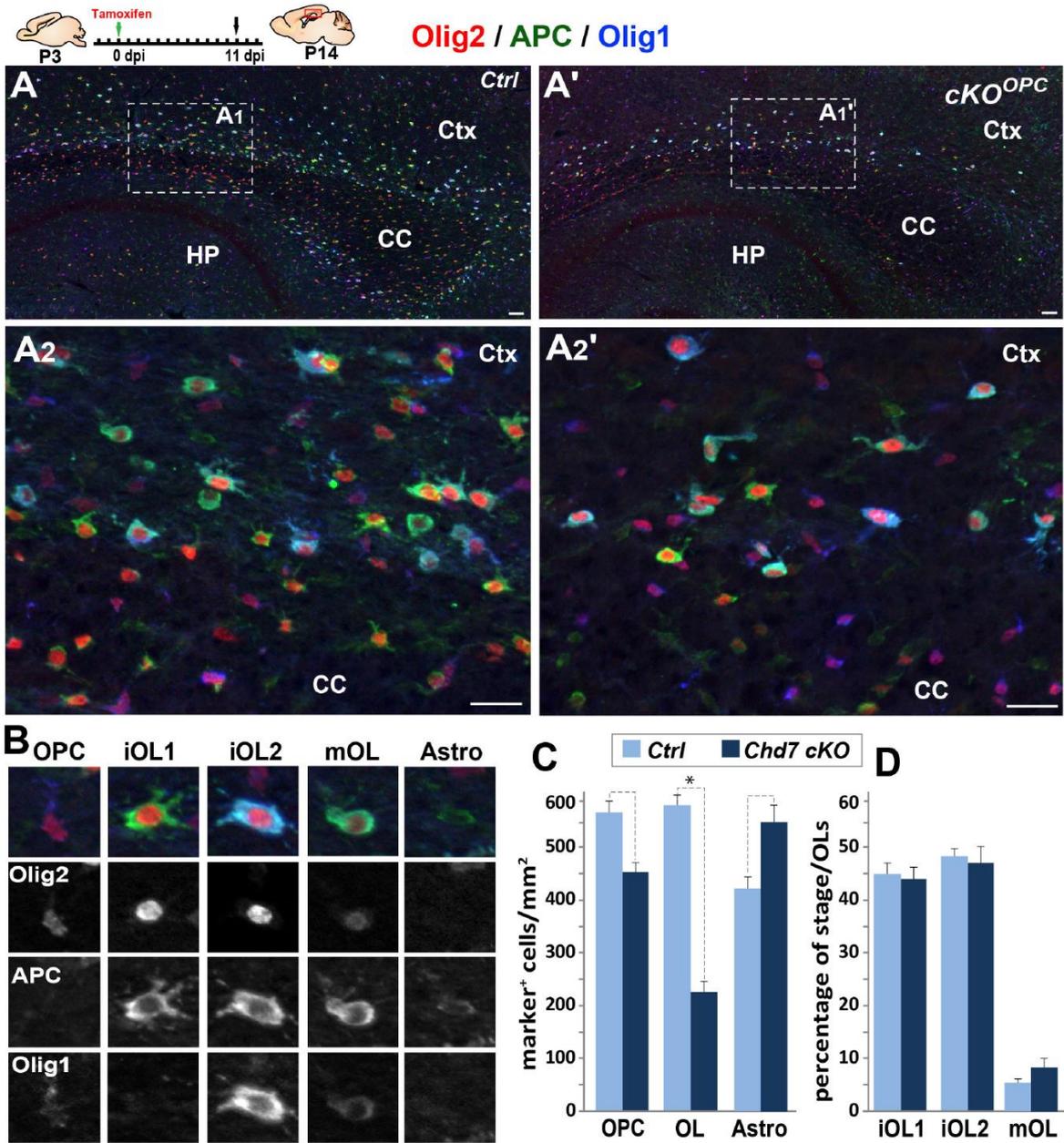
FigureS1



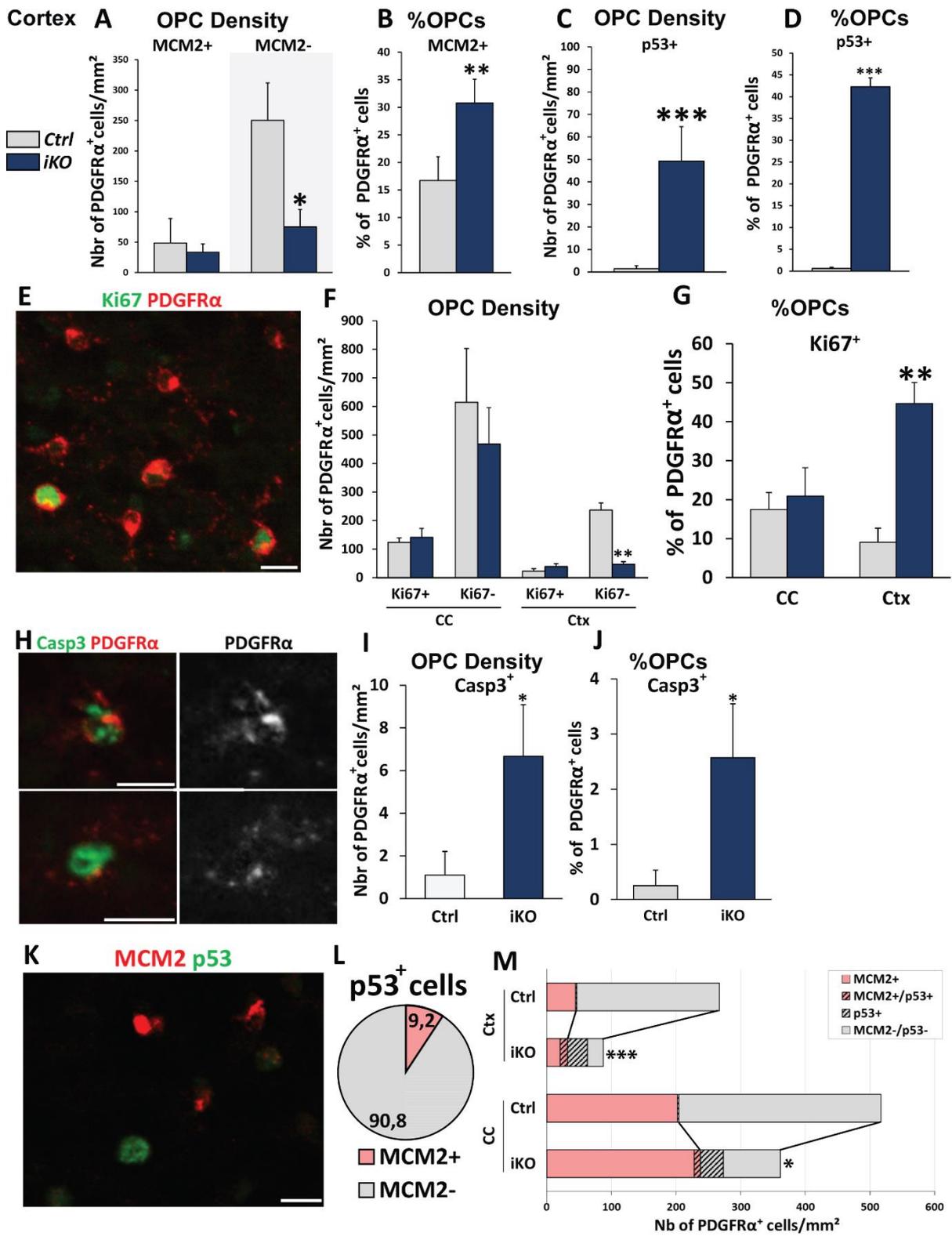
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FigureS3

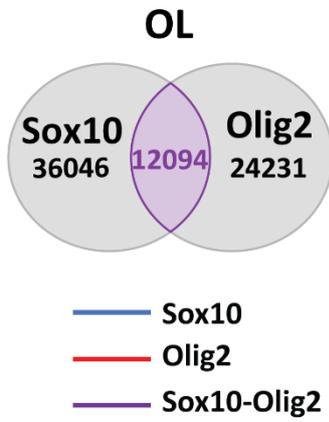


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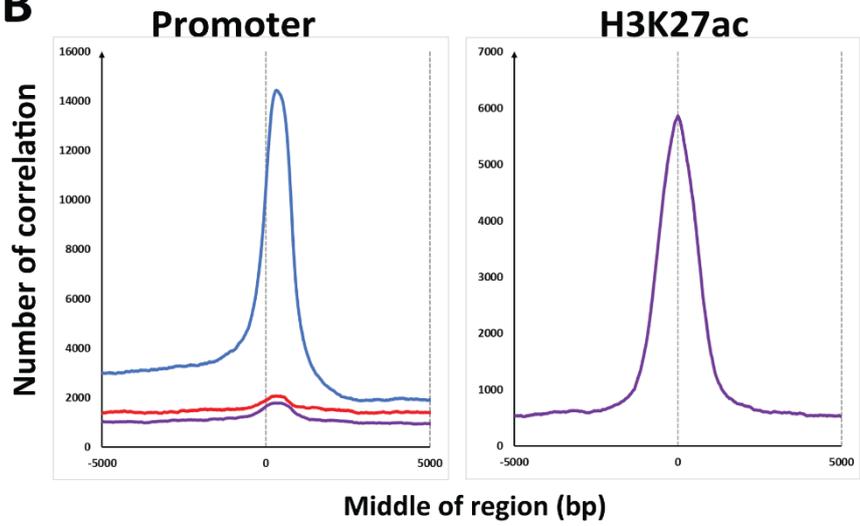


FigureS5

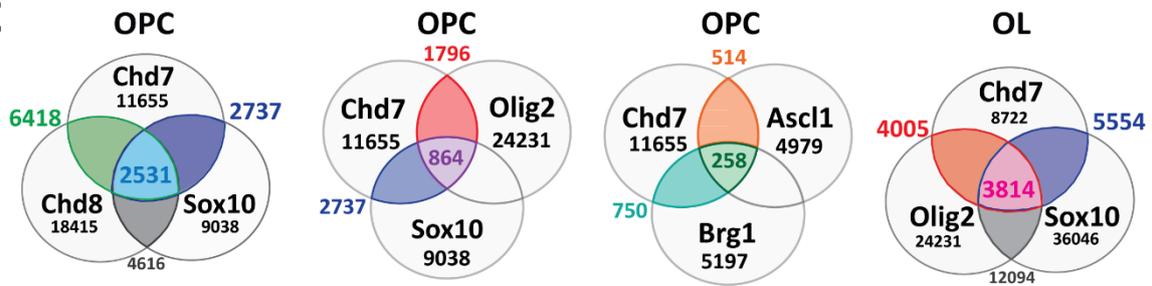
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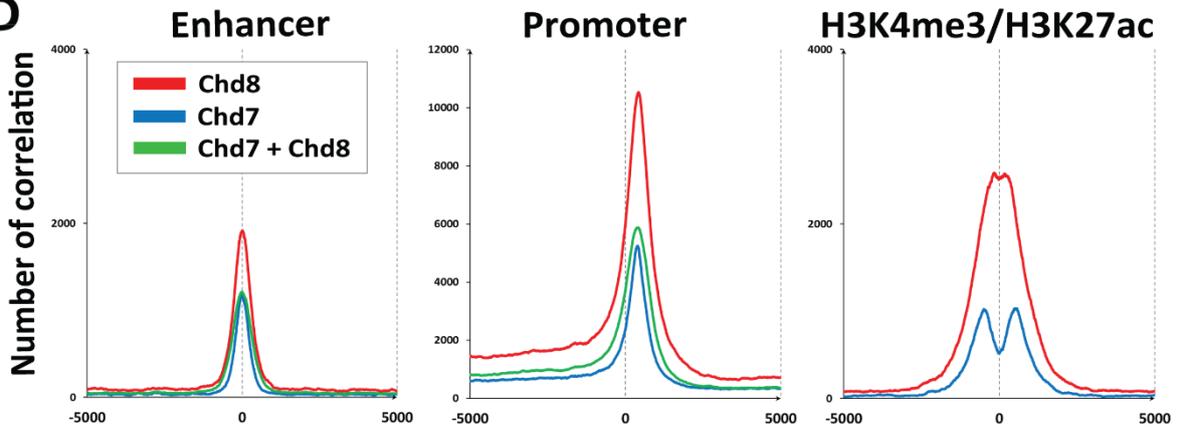
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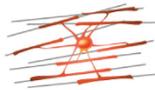
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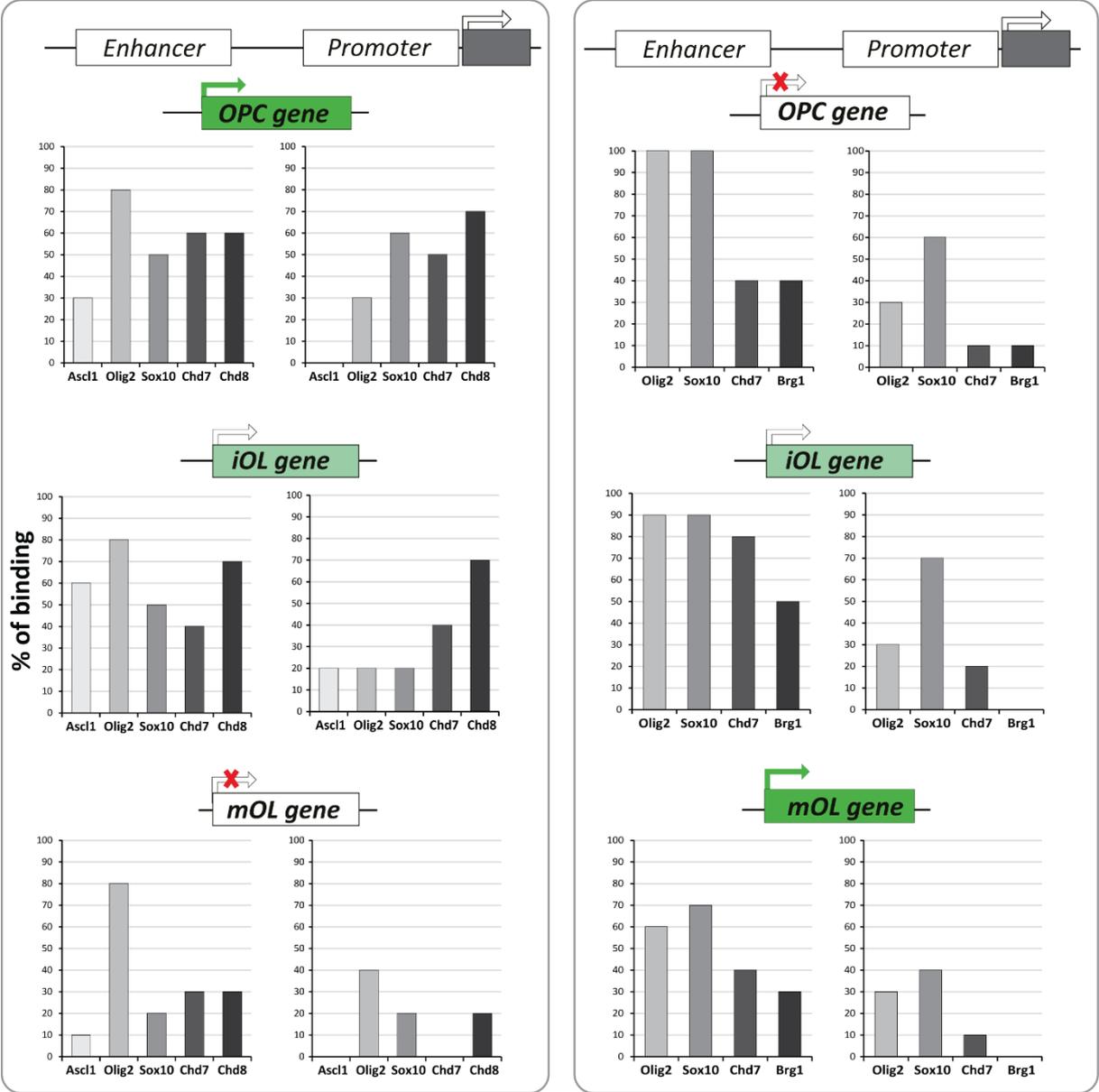
FigureS6



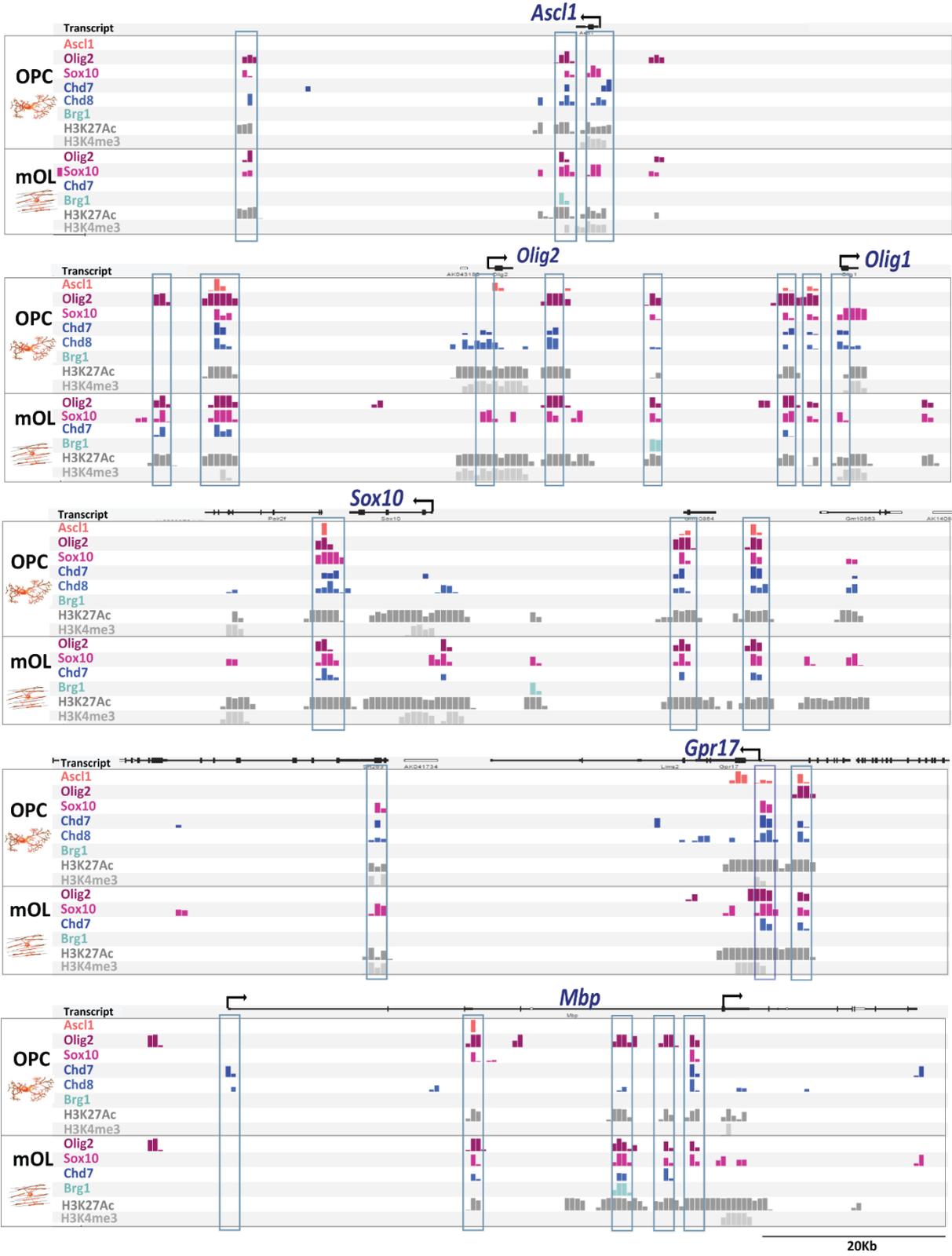
OPC



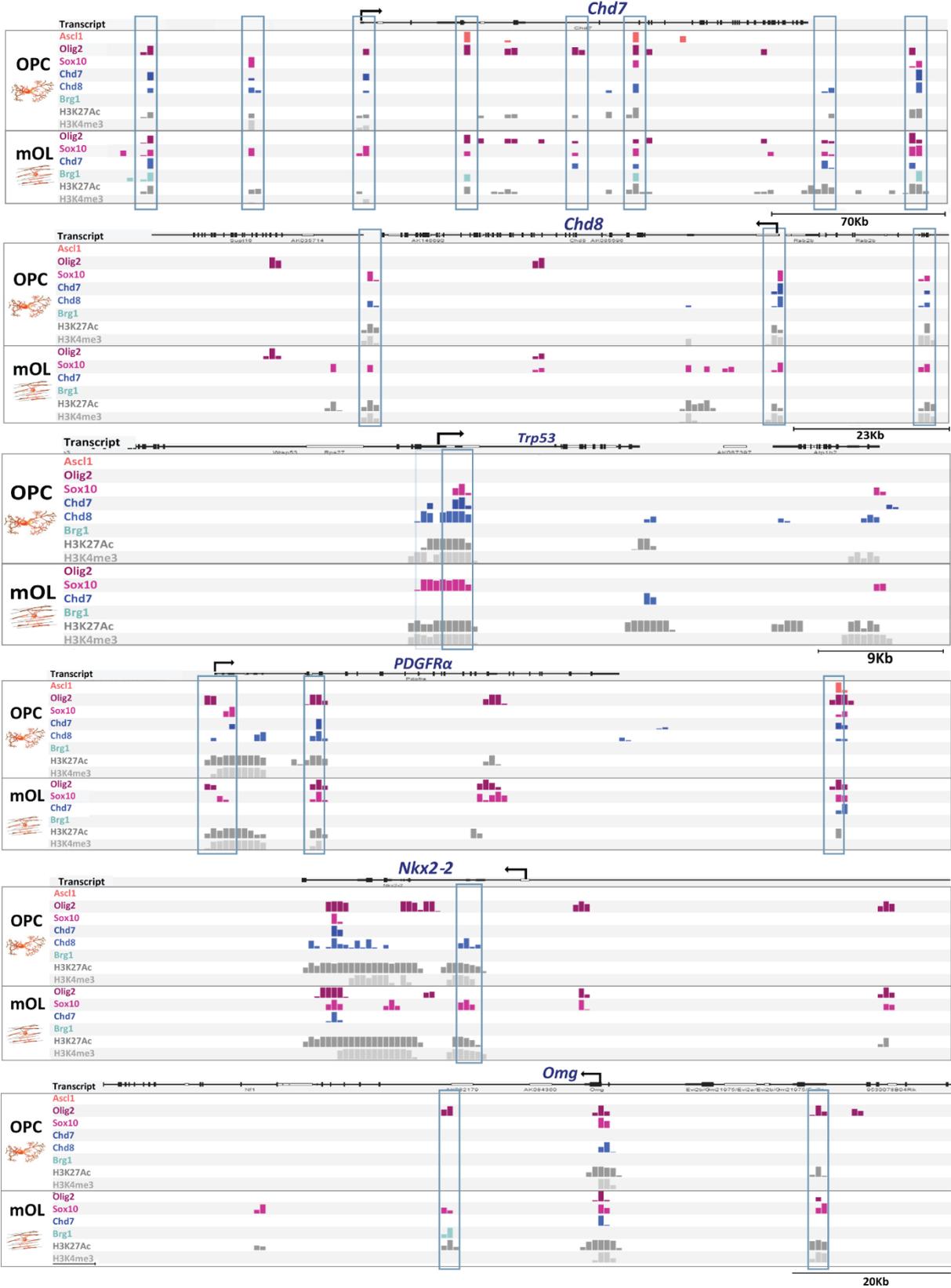
OL



FigureS7



FigureS8



## **Additional Results**

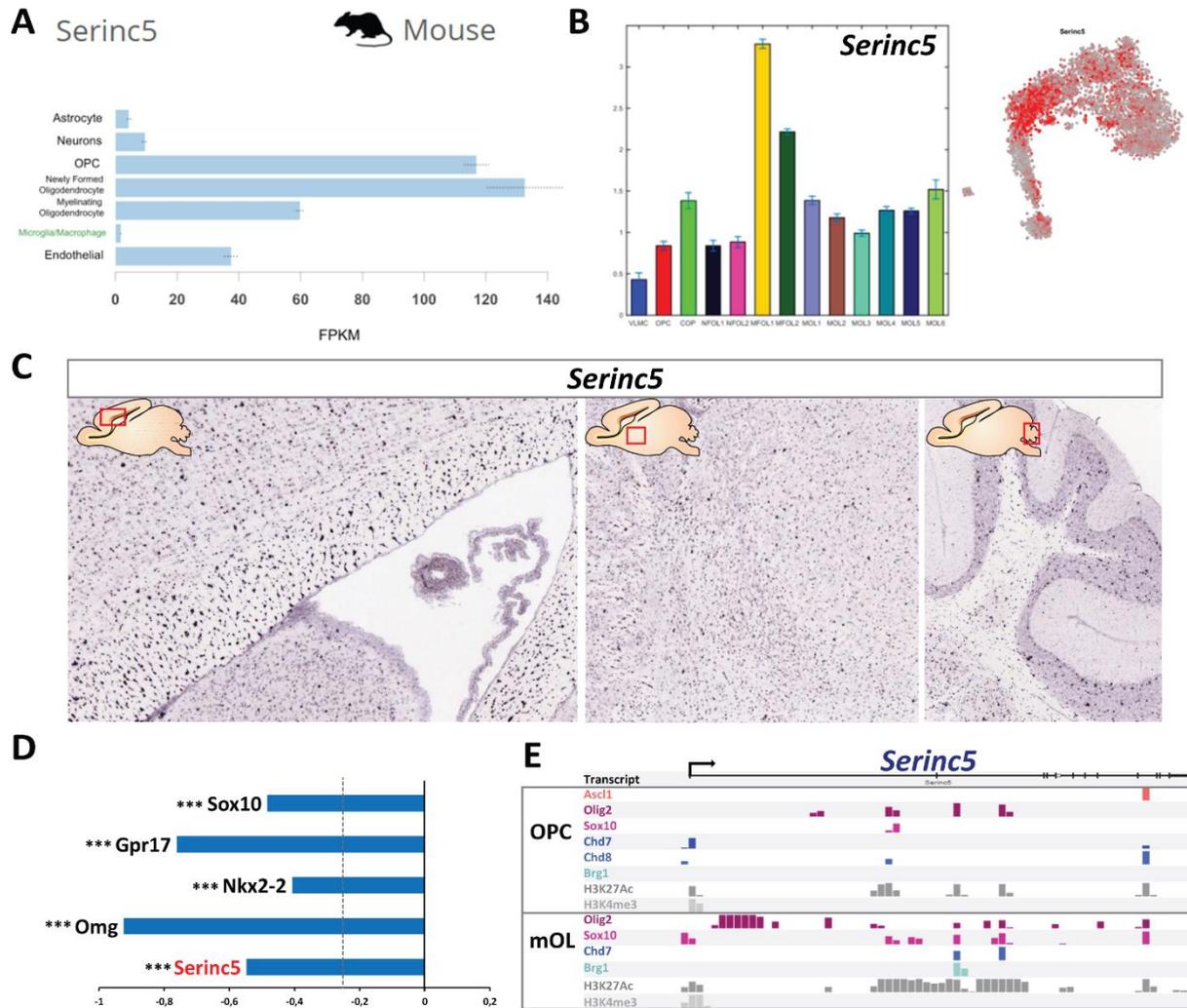


## Serinc5, an ASD risk gene, is a target of Chd7 and Chd8

In this last study, we focused on the understanding of Chd7 and Chd8 function in OPC differentiation, proliferation and survival based on their regulation of known factors involved in these processes. However, this study could also be an opportunity to find new factors involved in OL differentiation and myelination and which could be involved in pathologies associated with Chd7 and/or Chd8 mutations. As myelin defect has been found in Autism (Deoni et al., 2015), we considered genes that have been identified as ASD risk genes (Liu et al., 2014) and looked at their levels of expression in OPCs and OLs compared to other neural cell-types (Zhang et al., 2014). One of them, Serinc5 (also called TPO1) was found to be expressed in oligodendroglial cells with a peak in differentiating OLs (iOL / MFOL1; Fig. 1A-C) (Marques et al., 2016; Zhang et al., 2014).

Serinc5 is a transmembrane protein of the SERINC protein family that is conserved from yeast to mammals (Murrell et al., 2016). Serinc5 has been identified as a host cell restriction factor that could impair the infectivity of HIV-1 virions (Usami et al., 2015) and have been mostly studied for this capacity (Sood et al., 2017; Trautz et al., 2017). Concerning the CNS, expression of Serinc5 has been characterized in the OL lineage *in vitro* and *in vivo*, showing high expression in myelin and OL cell body but not in neurons or astrocytes (Fukazawa et al., 2006; Krueger et al., 1997). In shiverer mice (Mbp deficient), Serinc5 is downregulated and its myelin localization is altered, suggesting a function in myelin sheath formation (Fukazawa et al., 2006). Furthermore, *in vitro* analysis in Cos7 cells showed that Serinc5 promoted Fyn auto-phosphorylation that could explain its function in myelin formation (Fukazawa et al., 2006). However, additional experiments using Serinc5 loss-of-function to prove and detail these functions are still lacking.

Interestingly, we found Serinc5 downregulated in *Chd7-iKO* OPCs (Fig. 1D) and bound by Chd7 and Chd8 in its promoter region in OPCs and in enhancers (Sox10-Olig2 bound regions) in OLs (Fig. 1E), suggesting a direct regulation of Serinc5 by Chd7 and Chd8, as well as Sox10 and Olig2. All together, these results suggest a role of Serinc5 in myelination and, if it was demonstrated, its mutation in ASD together with Chd8 could explain the myelin defect observed in ASD patients.



**Figure 1: Expression and regulation of Serinc5.** (A) Expression at RNA level of Serinc5 in different mouse CNS cell-type from (Zhang et al., 2014). (B) Expression at RNA level of Serinc5 in mouse oligodendroglial lineage cells from (Marques et al., 2016). (C) In situ of Serinc5 in adult mouse brain from Allen brain atlas. (D) Barplot showing downregulated (Log Fold Change) genes in *Chd7iKO* P7 OPCs compared to control ones. Dotted grey line represent FC = 1.2. (Ctrl, n=7; *Chd7iKO*, n=5; *Serinc5*, p-value < 0.001). (E) Schematic representation from Genomatix genome browser of *Serinc5* locus integrating ChIP data for main oligodendroglial TFs (*Ascl1*, *Olig2* and *Sox10*), chromatin remodeling factors (*Chd7*, *Chd8* and *Brg1*) and active epigenetic marks (*H3K27ac* and *H3K4me3*) in OPCs and OLs.

## Function of Chd7 in OPC generation

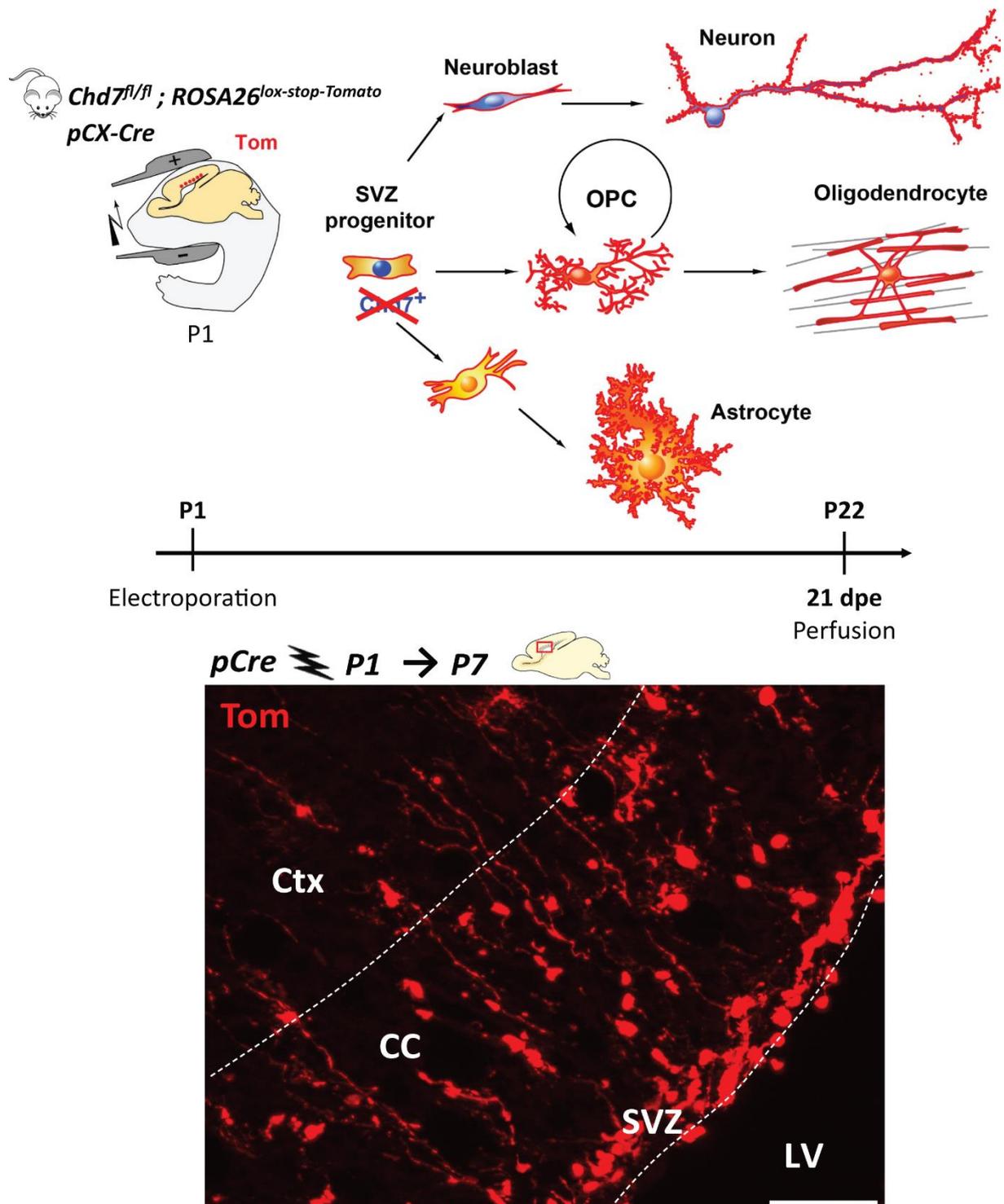
As it was shown in the above mentioned results, conditional deletion of *Chd7* in mouse OPCs demonstrates that Chd7 is required for OPC differentiation, myelination and remyelination. However, with these experiments, we couldn't address Chd7 function in the prior step which is the generation of OPCs (i.e. specification). Chd7 is expressed in NSCs (Feng et al., 2013) and Chd7 binding (ChIP-seq) in OPCs showed that Chd7 can bind, together with Sox10 and Olig2, to genes involved in OPC specification (*Ascl1* and *Olig2*). Even if no downregulation for these genes was observed in purified OPCs (O4<sup>+</sup> cells) from *Chd7iKO* at P7, we cannot exclude a regulation at an earlier stage. To explore the question of a potential function of Chd7 in OPC specification, we used *in vivo* and *in vitro* loss-of-function strategies to delete *Chd7* in NSCs and observed the effect on OPC specification.

We first deleted Chd7 *in vivo* in NSCs of the SVZ of neonatal mice. To that purpose, we electroporated a plasmid expressing the Cre recombinase in the dorsal SVZ of P1 *Chd7<sup>flox/flox</sup>; Rosa<sup>flox-stop-Tomato</sup>* (*Chd7cKO*) mice and *Rosa<sup>flox-stop-Tomato</sup>* (Control) mice. We then waited for the cells to differentiate for 21 days post-electroporation (21dpe) before collecting the brains and analyzing the electroporated cells (Tom<sup>+</sup> cells; Fig 1). Importantly, while nearly all of Tom<sup>+</sup>-Olig2<sup>+</sup> cells expressed Chd7 in control at 21dpe, some (22%) still expressed Chd7 in *Chd7cKO* mice (Fig. 2A,B) indicating a non-complete loss of Chd7 in electroporated cells. We then used markers to distinguish OPCs (Olig1<sup>+</sup>-CC1<sup>-</sup>), OLs (CC1<sup>+</sup>) and astrocytes (Olig1<sup>-</sup>-CC1<sup>-</sup>; Fig. 2D) to determine the fate of Tom<sup>+</sup> electroporated cells in the corpus callosum and overlying cortex. Consistent with previous results (OPC *Cdh7 iKO*), we observed a reduction in the proportion of generated OLs in *Chd7cKO*, increasing the proportion of observed astrocytes (Fig. 2C,E). However, no change in the proportion of OPCs was observed (Fig. 2E). These data supports the Chd7 function in OPC differentiation but at this time point analysis (21dpe) we found no evidence of a role of Chd7 in specification.

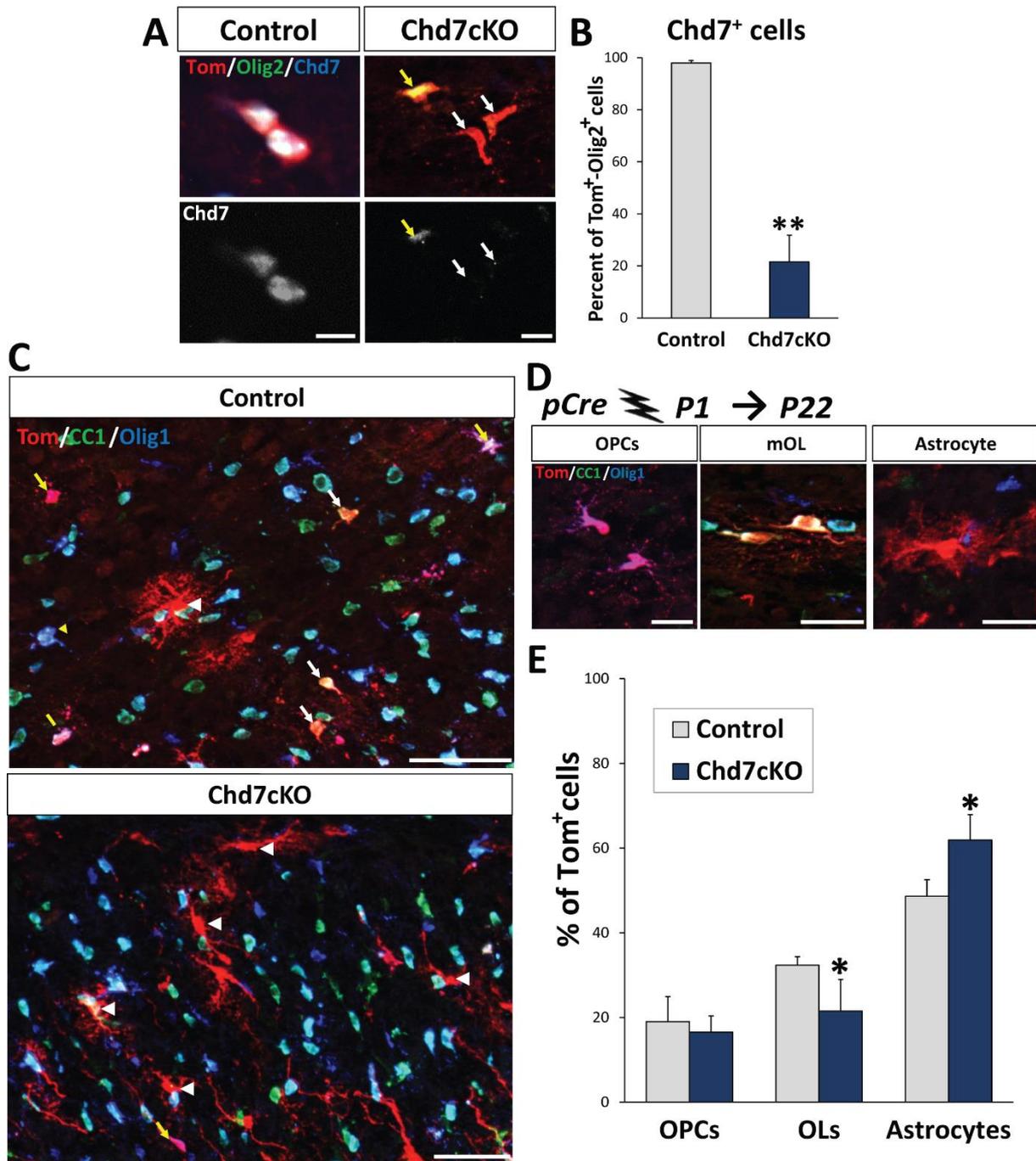
As the *in vivo* study didn't allow a complete loss of Chd7, we switched to an *in vitro* model in which we can massively delete Chd7 using viruses. We generated neurospheres from P1-P2 *Chd7<sup>flox/flox</sup>; Rosa<sup>flox-stop-YFP</sup>* (*Chd7cKO*) mice and *Rosa<sup>flox-stop-YFP</sup>* (control) mice. We then transduced them with an Adenovirus expressing the Cre recombinase (Ad-Cre) and we put

them in proliferation medium for 2 days before switching to differentiation conditions (Fig. 3). As a control of the efficiency of Chd7 deletion, we looked at Chd7 expression 2 days after transduction (Fig. 4A) and found a complete loss of Chd7 in the *Chd7cKO* cells (Fig. 4B), showing the efficiency of Chd7 deletion with this strategy. To determine the fate of these cells, they were fixed at different time-points after differentiation (2, 4 or 6 days in differentiation medium – DID) and cells of the OL lineage were immunostained using a combination of PDGFR $\alpha$  and CNP antibodies (Fig. 4C). At 2 DID, no changes were observed in the proportion of generated oligodendroglial cells between control and *Chd7cKO*. At 4 and 6 DID, that proportion tended toward a reduction (no statistical evaluation; Fig. 4D) compared to control cells, suggesting either a defect in specification that start to be visible at 4DID or a defect in proliferation. To investigate the potential role of Chd7 on proliferation and cell death, staining were done using MCM2 (cell cycle, Figure 4E) and Casp3 (apoptosis, Figure 4G) antibodies. No changes were observed in the number of proliferating YFP<sup>+</sup> cells (Figure 4F), and Casp3 staining was barely observed in YFP<sup>+</sup> cells (Figure 4G).

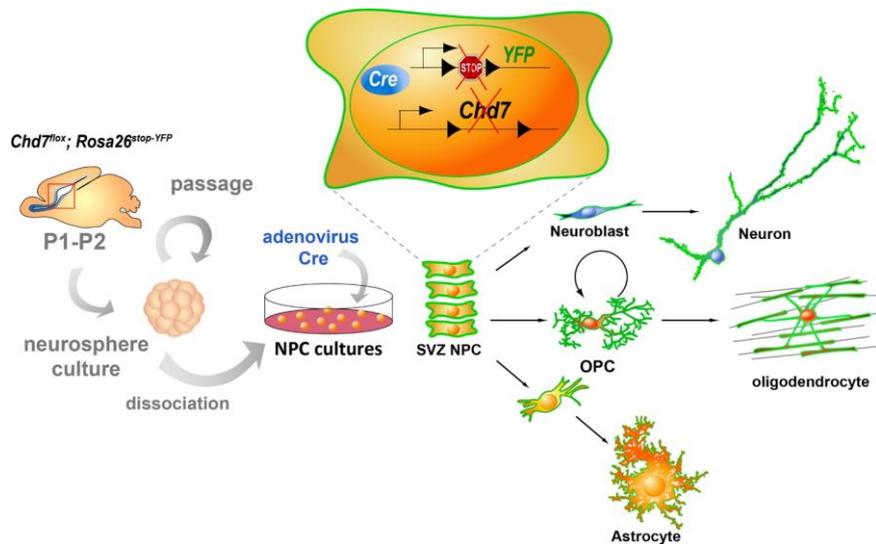
Altogether, from these preliminary results, we cannot exclude a possible role of Chd7 in OPC specification and complementary experiments will be needed to conclude on the requirement of Chd7 during OPC generation. On that note, it would be interesting to do the same experiment of *Chd7* LOF by postnatal electroporation but looking at earlier time-points (dpe7 and dpe14) to observe the OPC population before differentiation and myelination.



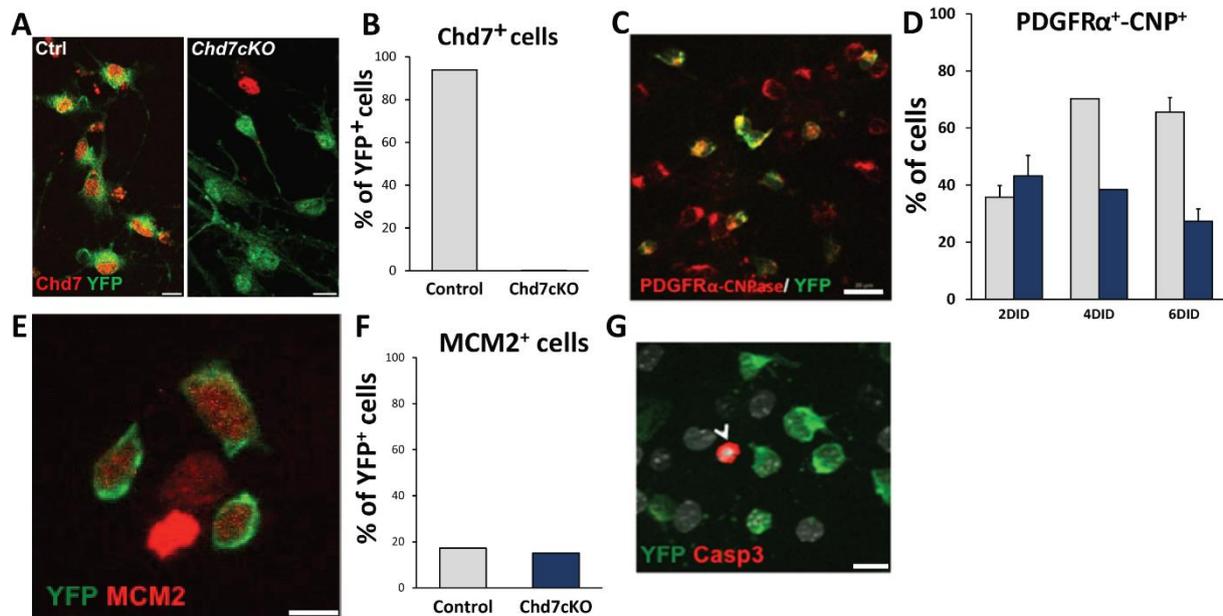
**Figure 1: Postnatal electroporation.** Top, diagram representing the postnatal electroporation experiment. Control and *Chd7*cKO pups (P1) were electroporated with pCX-Cre plasmid, targeting the dorsal SVZ. Cre catalyzes the recombination at LoxP sites, resulting in deletion of exon2 of *Chd7* gene and deletion of stop sequence in *Rosa* locus, activating Tomato expression. Electroporated (Tomato<sup>+</sup>) SVZ progenitors then differentiate to neurons that migrate to the olfactory bulbs and glial cells that migrate to the CC and Ctx. Bottom, sagittal brain section at 6dpe (days post-electroporation) showing targeted cells (Tomato<sup>+</sup>) in the SVZ and radial glial cells. Scale bars represent 50  $\mu$ m.



**Figure 2: Chd7 deletion in NSCs doesn't affect OPC proportion.** (A) Immunostaining showing Chd7 expression in Tom<sup>+</sup>-Olig2<sup>+</sup> cells. White arrows show Chd7 efficiently deleted cells, yellow arrow shows cell still expressing Chd7. Scale bars represent 10  $\mu$ m. (B) Quantification of Chd7<sup>+</sup> cells as a percentage of Tom<sup>+</sup>-Olig2<sup>+</sup> cells of Control and Chd7cKO mice at P22 (21dpe). The data are presented as mean  $\pm$  s.e.m. (n= 2 Controls and 4 Chd7cKO;  $P = 0.0014$ ,  $t = 7.85$ ; two-tailed unpaired Student's  $t$  test). (C) Immunostaining of Olig1 and CC1 in the CC and Ctx from Control and Chd7cKO mice at P22 (21dpe). White arrows show OLs (CC1<sup>+</sup>), yellow arrows show OPCs (Olig1<sup>+</sup>; CC1<sup>-</sup>) and white arrow heads show Astrocytes (Olig1<sup>-</sup>; CC1<sup>-</sup>). Scale bars represent 50  $\mu$ m. (D) Details showing OPCs (Olig1<sup>+</sup>; CC1<sup>-</sup>), OLs (CC1<sup>+</sup>) and Astrocytes (Olig1<sup>-</sup>; CC1<sup>-</sup>). Scale bars represent 20  $\mu$ m. (E) Quantification of OPCs (Olig1<sup>+</sup>; CC1<sup>-</sup>), OLs (CC1<sup>+</sup>) and Astrocytes (Olig1<sup>-</sup>; CC1<sup>-</sup>) as a percentage of total Tom<sup>+</sup> cells in Control and Chd7cKO mice at P22 (21dpe). The data are presented as mean  $\pm$  s.e.m. (n= 2 control and 4 Chd7cKO; OPCs,  $P = 0.52$ ,  $t = 0.68$ ; OLs,  $P = 0.04$ ,  $t = 2.82$ ; Astrocytes,  $P = 0.037$ ,  $t = 2.82$ ; two-tailed unpaired Student's  $t$  test).



**Figure 3: In vitro deletion of Chd7 in neurospheres.** Diagram representing in vitro experiment. Neurospheres were generated from Control (*Rosa<sup>YFP</sup>*) and Chd7cKO (*Chd7<sup>fllox/fllox</sup>; Rosa<sup>YFP</sup>*) P1-P2 pups, amplified and plated in adherent conditions. At that moment, transduction with Ad-Cre was performed, resulting in Chd7 deletion and expression of YFP. Cells were then put in differentiation medium.



**Figure 4: Chd7 deletion in neurospheres doesn't affect OPC generation.** (A) Immunostaining showing Chd7 expression in YFP<sup>+</sup> cells in control and Chd7cKO cells 2 days post-transduction. Scale bars represent 5 μm. (B) Quantification of Chd7<sup>+</sup> cells as a percentage of YFP<sup>+</sup> cells of Control and Chd7cKO mice 2 days post-transduction (n= 1). (C) Immunostaining of PDGFRα-CNPase and YFP in Chd7cKO cells at 2 DID (days in differentiation conditions). Scale bars represent 20 μm. (D) Quantification of OPCs/OLs as a percentage of total YFP<sup>+</sup> cells in Control (grey) and Chd7cKO (blue) cells at 2, 4 and 6DID. The data are presented as mean ± s.e.m. (2 and 6DID, n= 2; 4DID, n=1). (E) Immunostaining of MCM2 and YFP in Chd7cKO cells at 4 DID. Scale bars represent 5 μm. (F) Quantification of MCM2<sup>+</sup> cells as a percentage of YFP<sup>+</sup> cells of Control and Chd7cKO mice at 4 DID (n= 1). (G) Immunostaining of Casp3 and YFP in Chd7cKO cells at 4 DID. Arrow head shows Casp3<sup>+</sup> cells. Scale bars represent 5 μm.



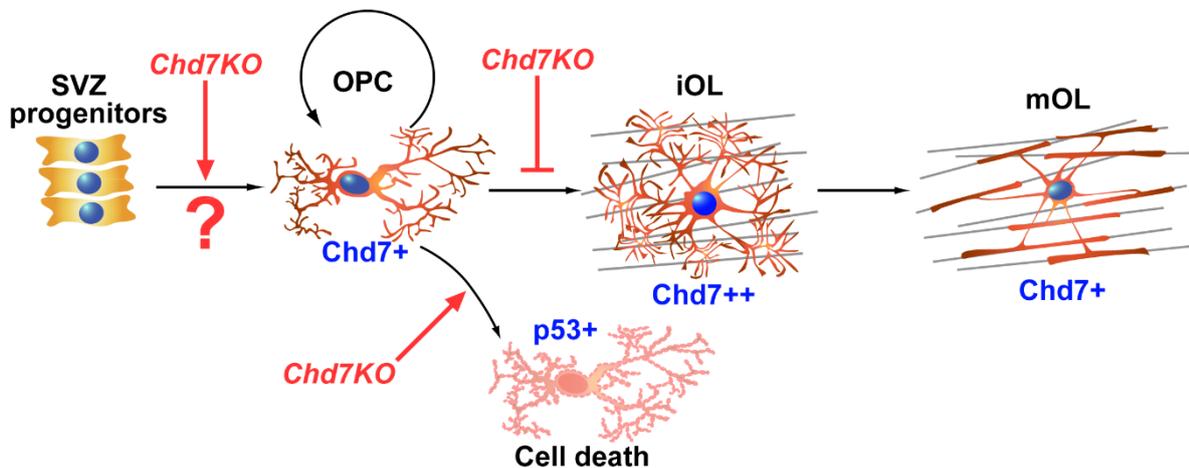
# Discussion



Oligodendrocytes (OLs) are the myelin-forming cells in the central nervous system (CNS), making them responsible for saltatory conduction in axons, myelin plasticity and axon support. These functions are highlighted when myelin is lost in demyelinating disease such as Multiple Sclerosis (MS). Partial recovery can be achieved by remyelination due to oligodendrocyte precursor cells (OPCs), present all over the brain and which can migrate, proliferate and differentiate to give rise to new OLs and new myelin sheaths. Unfortunately, remyelination becomes less and less efficient with the progression of disease. It is thus crucial to better understand mechanisms that are involved in OPCs differentiation to promote more efficient remyelination. Cell differentiation is a process that demands profound changes in the gene expression program and is promoted by many factors. Knowing which factors promote these changes and the mechanisms of their action is key to deeply understand this process and to find druggable targets to promote differentiation and remyelination. Transcription factors (TFs) involved in OL differentiation such as Sox10 (Stolt et al., 2002), Olig2 (Mei et al., 2013) or Ascl1 (Nakatani et al., 2013) have been well studied and show key roles in this process. However, even if we have many data about their functions, their mechanisms of action are still poorly understood. Transcription initiation is a mechanism largely studied for which general mechanisms are known. For instance, it is known that particular TFs called pioneer factors can recruit other factors such as cofactors or chromatin modifying factors to remodel the chromatin in the correct state enabling transcription initiation by recruitment of other TFs and stabilization of the pre-initiation complex. Therefore, to understand how these TFs work, we have to look for cofactors and chromatin remodelers and how they all interact together. Even if some chromatin modifying factors have been studied in the context of OL differentiation like HDACs or Brg1 (Shen et al., 2005; Ye et al., 2009)(Yu et al., 2013b), nothing was known about the function of factors from the CHD family in this process. Interestingly, Chd7 was found as a target of Olig2 (He et al., 2016) and Ascl1 (*Clavairoly and Parras, unpublished*).

In this work, we are showing that: 1) Chd7 expression is highly enriched in OL lineage cells in the CNS, with a peak of expression in differentiating OLs; 2) Chd7 is required for proper myelination and remyelination; 3) Inactivation of Chd7 leads to a decrease in OPC differentiation but not OL stage progression; 4) Genome wide binding analysis and transcriptomics indicate that Chd7 targets and activates genes involved in OL differentiation; 5) Chd7 is involved in OPC survival by directly inhibiting *Trp53*; 6) Chd8, another CHD subgroup

III factor, is highly co-expressed with Chd7 in oligodendroglia and enriched in differentiating OLs and 7) Chd7 and Chd8 binds to common genes involved in OPC differentiation, proliferation and survival suggesting some overlapping functions of these two chromatin remodelers.



**Figure 1. Summary of Chd7 functions in OPC differentiation, survival and specification.**  
Writing in blue represent expression and writing in red represent function.

### Chd7 is required to promote expression of genes involved in both OPC differentiation and OL maturation

*Chd7* conditional knockout leads to a decrease in newly-formed OLs during both myelination and remyelination. Our collaboration with Richard Lu and colleagues has permitted to show similar results and observations in two different labs with two different models, making us confident with our conclusions on the requirement of Chd7 in myelination, as well as in remyelination. In that first study, as nothing was known about the function of Chd7 in OLs, we investigated the question of the requirement of Chd7 in OL differentiation and myelination. Indeed, both *Chd7* conditional deletion in oligodendroglia (*Olig1<sup>Cre</sup>* driver) and timely induced deletion in OPCs (*PDGFR $\alpha$ ::CreER<sup>T</sup>* driver) led to a decrease in OLs, associated with less myelin in mutant brain compared to control. Furthermore, Chd7 ChIP-seq analysis revealed that Chd7 binds together with Sox10, to myelin-associated genes like *Mbp*, suggesting the role of Chd7 in myelination. However, as Chd7 is already expressed in OPCs, we wondered what could be its function in this oligodendroglial stage. Therefore, in a second study, we focused on OPC stage to characterize Chd7 chromatin function prior to myelination. As the use of CC1 marker

alone does not allow to distinguish iOLs from mOLs, we used a combination of more restrictive markers of iOLs (Nkx2.2, Itpr2 and APC) and showed that *Chd7* deletion impaired early phases of differentiation but once differentiation was initiated *Chd7* deletion did not affect its progression towards mature OL. To specifically address whether *Chd7-cKO* observed defect in myelin is due to the loss of OLs or from a direct role of Chd7 in the myelination process, one would need to use Cre drivers expressed after OL differentiation and delete *Chd7* at the iOL or mOL stages, such as *Cnp<sup>Cre</sup>* or *Plp::Cre* mice, respectively (Lappe-Siefke et al., 2003) (Doerflinger et al., 2003). Therefore, one would expect to see defects in normal myelination as Chd7 binds to myelin-associated genes.

To address in depth Chd7 function and mechanisms in OPCs, we decided to do transcriptomic analysis after *Chd7* conditional deletion in purified OPCs. To be more precise and more specific in our analysis, we decided to sort O4<sup>+</sup> cells from P7 mouse cortex using MACs instead of using whole brain tissue, which would reduce sensitivity. O4<sup>+</sup> sorted cells included 80% of OPCs (PGDFR $\alpha$ <sup>+</sup> cells) and 20% of iOLs (CNP<sup>+</sup> cells). Noteworthy, we found no difference between control and mutant in OPC/iOL ratio, allowing us to compare transcriptomes of similar cell populations. Interestingly, we found that both OPC differentiation and OL maturation genes were downregulated following Chd7 loss, which could explain the decrease in OLs we observed. Indeed, chromatin binding (ChIP-seq) analysis showed that Chd7 binds most of these downregulated genes indicating that Chd7 is a direct regulator of OPC differentiation process. This downregulation was however partial, as we could still detect these transcripts in mutant cells, suggesting a possible compensation by another (co)factor.

It was interesting to note that, as Chd7 is expressed in neuroblasts and involved in neurogenesis (Feng et al., 2013), totally different genes are regulated by Chd7 in both processes. Indeed, a recent ChIP dataset is available for Chd7 in granule neuron progenitors (GNPs) (Feng et al., 2017) and less than 500 binding sites were found in common between OPCs and GNPs (*data not shown*), suggesting that Chd7 does not bind to specific DNA sequences but is recruited to regulatory elements by other cell-type specific factors. Altogether, our study allows to propose that Chd7 is a chromatin remodeling factor required for cell-subtype differentiation that needs other factors (likely TFs) to be recruited to the correct regulatory elements depending on the cell-subtype, suggesting that Chd7 must work as part of a transcription complex.

### Chd7 protects OPCs from apoptosis

Time-controlled *Chd7* deletion in OPCs has also allowed us to look at other OPC functions like proliferation and survival. Surprisingly, we found that after *Chd7* LOF cell death was mostly restricted to non-cycling OPCs. Importantly, Chd7 binds in OPCs to *Trp53* promoter, a gene coding for p53 which promote the pathway and that in neural crest cells has been linked to *Chd7* and CHARGE syndrome (Van Nostrand et al., 2014). Consistently with these results, *Chd7* deletion in granule neuron progenitors has been recently shown to increase cell death by expression of Caspase3 (Feng et al., 2017). These studies altogether suggest a general role of Chd7 to inhibit apoptosis pathway in different tissues and cell types. Of note, no decrease of OPCs was observed in the spinal cord nor in the cortex of *Olig1<sup>Cre</sup>; Chd7<sup>Flox</sup>* mice at P0, P7 and P21 (He et al., 2016). The question of cell death was therefore not assessed in this context. However, as this deletion occur during development, we could imagine that some compensatory mechanisms to maintain the pool of OPCs had the time to set up. Nevertheless, it should be noted that in our model (*PDGFR $\alpha$ CreER<sup>T</sup>*), tamoxifen must be injected to induce *Chd7* deletion which could lead to a different environment for the cells that may be more propitious for apoptosis. It suggest that it may not be the deletion of *Chd7* that activate cell death but that, in a suboptimal context where cell death might occur, the presence of Chd7 can have protective action against cell death. It can be particularly important during OL differentiation when cell death can occurs more easily (Barres et al., 1992a, b) as premyelinating OLs can either myelinate axons or degenerate (Trapp et al., 1997). This could explain why we see cell death mostly in non-cycling OPCs ready to differentiate. That is supported by the very few (9.6%, *data not shown*) p53<sup>+</sup> iOLs (Nkx2.2<sup>+</sup>) observed in *Chd7iKO* brains and the fact that Chd7 does not bind anymore to *Trp53* promoter in OLs, suggesting that once OPCs are differentiated to iOLs, they are less susceptible to die from apoptosis and therefore doesn't need Chd7 protection anymore.

Cell death in non-proliferative OPCs lead to unbalance the proportion of cycling and non-cycling OPCs. This is directly translated as an overrepresentation of cell-cycle genes in mutant transcriptome (RNA-seq) compared to control. We didn't find any difference in the number of MCM2<sup>+</sup> and Ki67<sup>+</sup> OPCs between mutant and control showing that cell-cycle entry is not affected by *Chd7* LOF. Also in *Olig1<sup>Cre</sup>* mice, no difference were observed in the proportion of Ki67<sup>+</sup> OPCs (He et al., 2016). However, we could find that Chd7 binds, together

with Chd8, to some cell-cycle regulator genes (Cdk4, Cdk6). Therefore, we cannot exclude a role of Chd7 in OPC proliferation as the timing of cell-cycle in Chd7 mutant OPCs has not been assessed yet.

Gene ontology analysis of Chd7 regulated genes in *Chd7-iKO* OPCs, indicated that genes related with “migration” category were present in both upregulated and downregulated genes. The function of Chd7 in OPC migration has not been assessed in our study as no hint of a defect in OPC migration has been observed in our mutation paradigm. The decrease of OPCs observed in *Chd7-iKO* mice is more important in the cortex compared to the corpus callosum, however, it seems that this decrease is due to more cell death in the cortex than a migration defect. Interestingly, it was shown that *Chd7* depletion decreased neural crest cell migration in *Xenopus* tadpole, leading to CHARGE features like coloboma, malformations of the craniofacial cartilage and heart defects (Bajpai et al., 2010). Also, Sox9 and Twist, two factors involved in migration (Sauka-Spengler and Bronner-Fraser, 2008), are targeted by Chd7 in neural crest cells (Bajpai et al., 2010). From these data, we cannot exclude a role of Chd7 in OPC migration and complementary experiments will be required to fully address this question.

### **Chd7 function in OPC specification**

Our genome wide Chd7 chromatin binding analysis (ChIP-seq dataset) in OPCs showed that Chd7, together with Sox10 and Olig2, binds to genes involved in OPC specification such as *Ascl1* and *Olig2*. We thus wondered if Chd7 could have a function in this process. It has been shown that Chd7 is expressed in NSCs (Layman et al., 2009) and that Chd7 play a role in neurogenesis to specify neurons (Feng et al., 2013). To study Chd7 function in OPC generation, we deleted *Chd7* in NSCs by two approaches and we obtained some preliminary data. *In vivo*, *Chd7* LOF by neonatal electroporation targeting NSCs of the subventricular zone (SVZ) didn't show any reduction in OPC numbers three weeks after Chd7 deletion. However, we observed a reduction of OLs likely consequence of a defect in OPC differentiation, consistent with our results of OPC-specific *Chd7* deletion. However, as 22% of Olig2<sup>+</sup> targeted cells (Tomato<sup>+</sup>) were still Chd7<sup>+</sup>, we can wonder if the *Chd7* deletion was efficient enough to observe small changes. This problem was countered in the *in vitro* experiments in which Chd7 deletion was almost complete in NSC cultures (neonatal SVZ-derived neurospheres) prior to induce their differentiation. A decrease of the oligodendroglial cell proportion was observed at 4 and 6DID

(days in differentiation medium) but not at 2DID suggesting a possible specification and/or proliferation defect. None of these results have permitted to put in evidence a clear effect of Chd7 in OPC specification.

We can however note that although we don't see any decrease in OPC proportion, we don't see any increase either. What do happen to OPCs that don't differentiate? We looked for a possible proliferation defect or cell death but we found no evidence of changes compared to control cultures. These were however very preliminary data and complementary experiments should be done. Another explanation is the possibility that the defect in OPC differentiation hide a reduction in OPC generation. We cannot exclude this, especially in the *in vivo* experiment, where less OPCs could be generated from the mutant NSCs but the defect of OPC differentiation could lead to an accumulation of OPCs "rescuing" their proportion. Therefore, more studies need to be done to conclude on the possible function of Chd7 in OPC generation. On that note, it would be interesting to perform similar experiments of *Chd7* LOF by postnatal electroporation but analyzing them at earlier time-points (7dpe and 14dpe) to quantify the OPC population before their onset of differentiation. We could further analyze possible OPC proliferation or survival defects in this context and conclude on the Chd7 role in OPC specification.

### **Chd7 and Chd8 bound common genes in OPCs**

Our Chd7 LOF experiments demonstrate that Chd7 is partially required but not totally necessary for OPC differentiation. Moreover, Chd7 genome wide chromatin profiling (ChIP-seq) from *in vivo* postnatal brain OPCs indicates that Chd7 binds to genes that we are not deregulated in Chd7 mutant OPCs. These results suggested a compensatory mechanism and therefore, we looked into another factor that could have similar expression pattern and functions. Interestingly, Chd8, that like Chd7 is a chromatin remodeler of the CHD subgroup III, can physically interact with Chd7 (Batsukh et al., 2010; Feng et al., 2017). Furthermore, Chd8 mutations are typical of a subgroup of patients with Autism spectrum disorder (ASD; (Bernier et al., 2014b; Barnard et al., 2015; O'Roak et al., 2012; RK et al., 2017) and autism features can be found in CHARGE syndrome (Betancur, 2011) in which Chd7 mutations are found (Vissers et al., 2004). For these reasons, we looked at Chd8 expression in the oligodendroglial lineage and found that Chd8 expressed in OPCs and OLs with a peak of expression in iOLs, similarly to Chd7. Genome wide chromatin binding analysis (ChIP-seq) from

*in vivo* brain OPCs showed that Chd7 and Chd8 shared many binding sites and are bound to common genes including those involved in OL differentiation such as *Sox10* or *Nkx2.2*, suggesting a role of Chd8 in this process. To determine Chd8 function in OPC differentiation and myelination, analysis of Chd8 LOF in the oligodendroglial lineage will have to be conducted. It would also be interesting to look at the synergy between Chd7 and Chd8 by generating mutations affecting *Chd7* and *Chd8* alleles.

Our Chd8 ChIP-seq analysis also indicates that Chd8 can bind, together with Chd7, to *Trp53* promoter, suggesting a role for Chd8 in OPC survival. This is particularly interesting as a direct regulation of *Trp53* by Chd8 has never been identified before, while it has been shown that Chd8 can complex with p53 and histone H1 at the promoter of p53 target genes to inhibit their transcription (Nishiyama et al., 2009). It would therefore be very interesting to see if *Chd8* LOF induce OPC cell death.

We expect to find common functions between Chd7 and Chd8, however, Chd8 present many binding sites that are not shared with Chd7, suggesting that Chd8 can also have some specific functions in OPCs. Firstly, it seems that, in OPCs, Chd8-alone (without Chd7) binds in genes not yet expressed (iOL genes; Marques et al., 2016) which will be later bound by Chd7 in OLs, when these genes are expressed. Could Chd8 have a more precocious function than Chd7 and prepare the promoter of Chd7 activated genes? Otherwise, could the presence of Chd8 alone in these not-yet-expressed genes prevent their “precocious” expression? These are questions that should be addressed through a functional study of Chd8. Secondly, we showed that Chd8 binds to genes involved in cell cycle regulation. Correspondingly, it was recently showed that *Chd8* LOF leads to decrease proliferation of neural progenitors associated with cell cycle exit and that Chd8 binds directly to promoters of cell cycle genes (Durak et al., 2016). From these results, we could therefore expect a proliferation defect in *Chd8* mutant OPCs. Thirdly, different studies has shown the inhibitory effect of Chd8 in the Wnt pathway (Durak et al., 2016; Kobayashi et al., 2002; Nishiyama et al., 2012; Sakamoto et al., 2000; Thompson et al., 2008). In agreement to them, our ChIP-seq data indicates that Chd8 binds to  $\beta$ -catenin promoter (*data not shown*), suggesting a possible regulation of the Wnt pathway by Chd8 in OPCs, which could consequently affect OPC differentiation and myelination.

## **Chd7 and Chd8 bind together with Olig2 and Sox10 to activate oligodendroglia stage-specific genes**

A combination of RNA-seq, ChIP-seq and ATAC-seq analysis have been used to investigate the mechanisms involving Chd7 functions in OPCs. Our experiments have highlight the function of Chd7 in OPC differentiation, proliferation and survival. In our first study, a very convincing work have been done by our collaborators to demonstrate the synergy between Chd7 and Sox10. They also showed that in OLs, Chd7 and Sox10 bind to myelinating genes which are expressed at this stage. To go further, we wondered if Chd7 could bind to the same genomic sites at the earlier OPC stage, prior to differentiation. We were surprised to find few common binding sites between the two oligodendroglial stages. Interestingly, we found that in OPCs Chd7 binds to genes expressed at the OPC stage, suggesting a stage-specific binding of Chd7 to promote gene expression.

To have a more global view of the mechanism of stage-specific gene expression, we also integrate Chd7/Chd8 binding with ChIP-seq datasets of key TFs involved in OPC differentiation: Sox10, Olig2 and Ascl1 (He et al., 2016; He et al., 2017; Yu et al., 2013b). We could observe timely controlled binding to stage-specific genes from OPC to OL (Marques et al., 2016) and build a transcription regulatory model (Fig. 2). Interestingly, most of the expressed genes are those commonly bound by Sox10, Olig2, Chd7 and Chd8 (at least in OPCs). Chd7 and Sox10 seems to bind together, in agreement to what we reported previously (He et al., 2016) to regulatory elements already bound by Olig2 and suggest that together this binding activate gene expression.

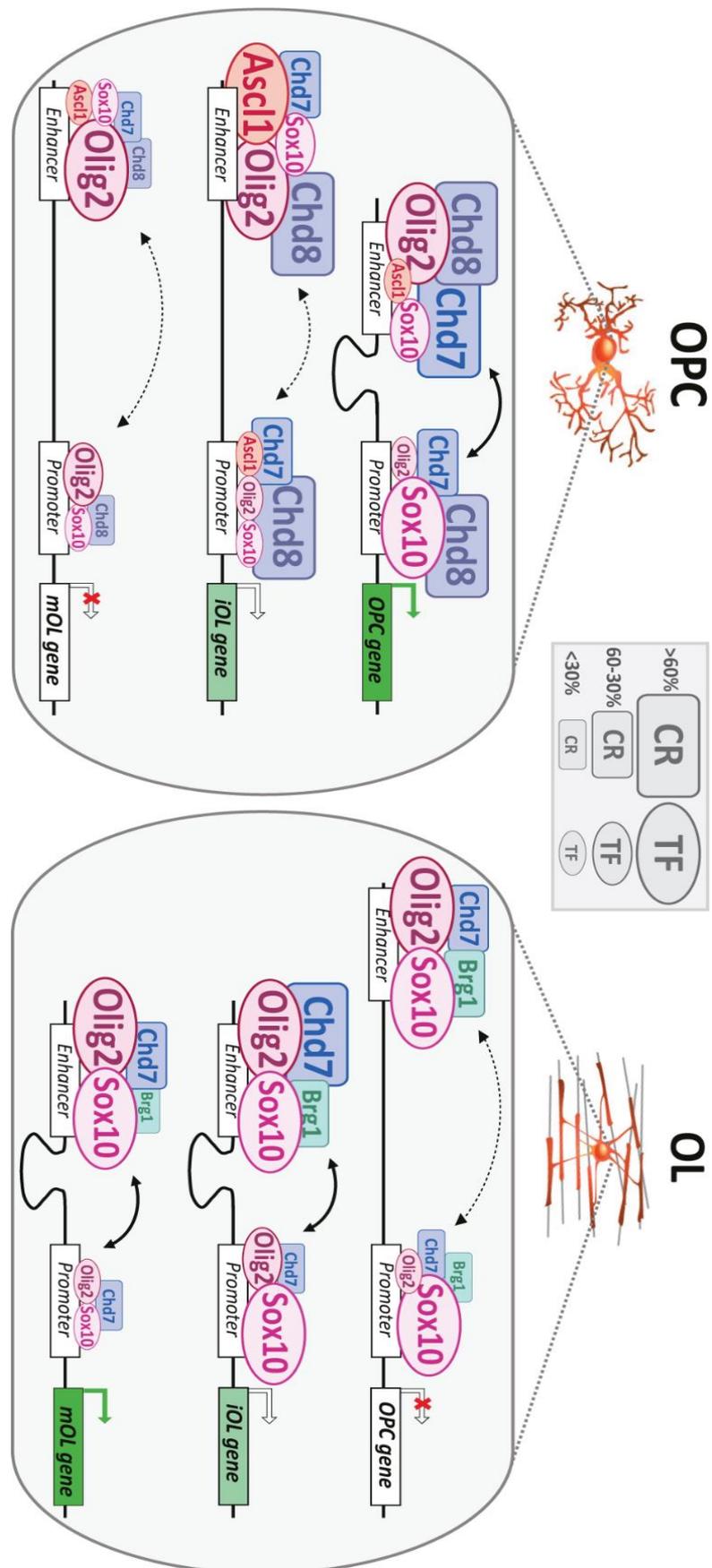


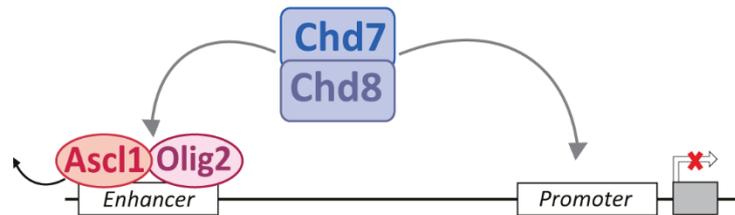
Figure 2. Model of regulation of time-controlled gene expression by binding of Sox10, Olig2, Ascl1, Chd7, Chd8, and Brg1 in OPCs and mOLs.

From these results, we can hypothesize successive binding stages to activate gene expression (Fig. 3): i) Olig2 and Ascl1 pioneer TFs (Raposo et al., 2015) would bind to enhancers; ii) Their binding allows the recruitment of Chd7 and Chd8, with Chd8 likely prior to Chd7; iii) Chd7/Chd8 remodelers will open the chromatin to allow the binding of Sox10 which in turn using its DNA bending activity, help to recruit other transcription cofactors and the mediator complex to form the promoter-enhancer loop leading to iv) robust gene transcription. That timing could explain the crucial importance of Sox10 and Olig2 in activating expression of these genes (Stolt et al., 2002; Yu et al., 2013b), but also explain the apparent compensation of Chd7 by Chd8. Interestingly, we could note that in OLs, most genes not expressed anymore (“OPC genes”) are still bound by Olig2 and Sox10. Does the lack of Chd7 and Chd8 are enough to prevent gene expression? More likely, some repressors must also be involved such as chromatin modifiers (HDACs; He et al., 2007) or other TFs (e.g. Nkx2.2, Nkx6.2). This study is a first step in trying to understand how these factors work together and what could be the complex needed for sustained transcription during OPC differentiation. However, the mechanisms regulating this time-controlled binding of factors still need to be unravel. Why is Chd7 recruited in OPCs to only to OPC-specific genes when Olig2 is also present in OL-specific genes? Which others factors are involved? These are questions that we cannot answer yet but are fundamental to fully understand these mechanisms leading to OL differentiation.

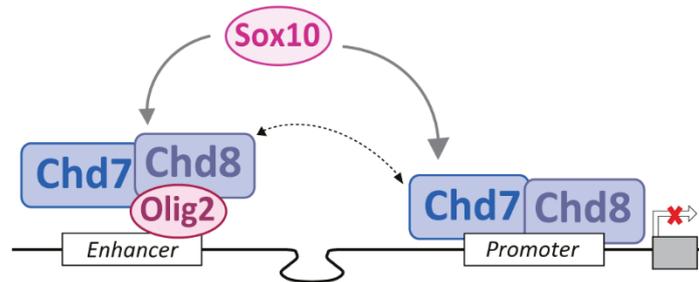
### 1. Pioneer TFs (Ascl1 & Olig2) bind to enhancer



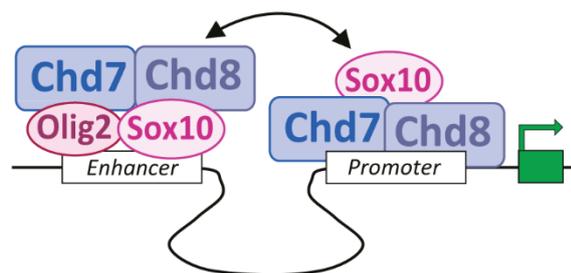
### 2. Recruitment of chromatin remodelers (Chd7 & Chd8)



### 3. Recruitment of Sox10 Promoter-Enhancer Loop formation



### 4. Gene expression



### 5. Gene downregulation

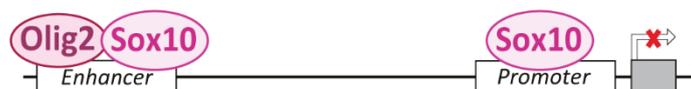
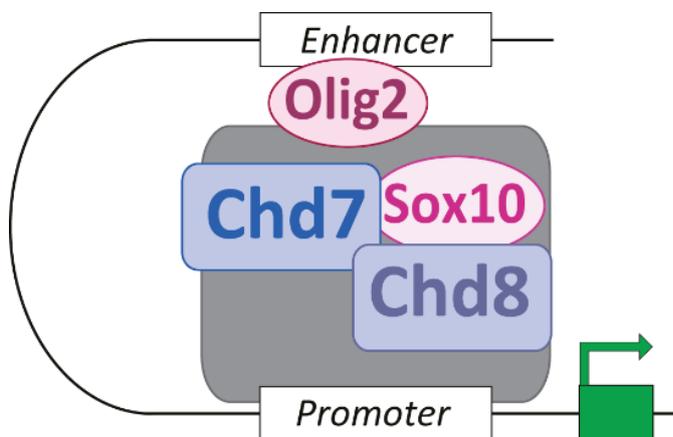


Figure 3. Model of time-controlled steps of factors binding that lead to robust gene expression.

We were particularly surprised to find Chd7 binding to many promoters in OPCs. In previous studies, Chd7 have been mostly found in enhancers (Schnetz et al., 2009; Schnetz et al., 2010). A possible explanation would be that Chd7 binds first in enhancers but when the gene is expressed, Chd7 binding to both enhancer and cognate promoter can be detected due to the loop formation (Fig. 4). To confirm that, one could use a technic named 3C-ChIP which have been used for Chd6 (Sancho et al., 2015), and permits to sequence together associated regions of the chromatin which would permit to picture the 3D structure of the chromatin in OPCs.



**Figure 4. Model of promoter-enhancer loop in OL lineage.** Scheme showing hypothetical complex forming promoter-enhancer loop in OPCs and OLs. Box in grey represent additional factors and complexes like Mediator complex.

### **Chd7 acts as an activator in OPC differentiation genes and as a repressor in *Trp53***

Chd7 is usually called an activator (Schnetz et al., 2010; Schulz et al., 2014; Feng et al., 2017). In OPCs, we have shown that in *Chd7* deletion affect expression of some genes. We were surprised to find a majority of them upregulated, that could suggest that Chd7 acts as a repressor in OPCs. However, it is of note that many (i.e. 575) of the upregulated genes found after *Chd7* LOF were involved in cell cycle and proliferation. We showed that this supposed “upregulation” is the consequence of an increased in the proportion of proliferating OPCs to due to the cell death of non-cycling OPCs. Indeed, we found that only 30% of upregulated genes found from RNA-seq were bound by Chd7. It shows that these numbers of up- and downregulated genes have to be interpreted carefully and only validation can allow us to conclude on the function of Chd7. Regarding OPC differentiation, all the regulated-genes that we found were actually downregulated, accordingly to the phenotype (reduced OPC differentiation) and indicating that Chd7 acts as an activator of these genes. Moreover, in

Chd7 mutant OPCs, we found a decreased ATAC signal in some of these genes, suggesting a chromatin opening defect due to the absence of Chd7. Chd7 is therefore an activator of gene expression by opening the chromatin of genes involved in OPC differentiation, as it was described before in other cell-types and genes (Schnetz et al., 2010; Schulz et al., 2014; Feng et al., 2017). However, can Chd7 be a repressor for other functions? It has been shown before that Chd7 could work as a repressor (Schnetz et al., 2010; Van Nostrand et al., 2014) and we have the example of *Trp53* which promoter is bound by Chd7 and which is upregulated after Chd7 LOF. It seems that Chd7 loss increase ATAC signal in *Trp53* promoter, suggesting a direct effect of Chd7 in closing chromatin to prevent gene expression. Altogether, these results show that Chd7 binding is cell-subtype dependent and its activity is gene-dependent. Further studies are required to understand how Chd7 activity is regulated and how is it recruited at the correct timing to enhancer and promoter elements.



# Perspectives



In this study, we investigated the function of Chd7 in OPC differentiation, proliferation and survival and how different key factors share with Chd7 and Chd8 binding sites on genes involved in these functions. From these conclusions, different questions and hypothesis rose. Does Chd7 and Chd8 cooperate to regulate the same functions in OPCs? Do they work in synergy? If they share common binding site together with TFs, do they all form a big complex? Do they physically interact with each other? These are questions we aim to answer.

### Chd8 function in oligodendrogenesis

To asses this question, we will do *Chd8* LOF in OPCs. However, as no *Chd8<sup>Flox</sup>* animals has been generated yet (we are currently trying to obtain a *Chd8<sup>Flox</sup>* allele by microinjection of zygotes using a homologous recombination template targeting *Chd8* exon 9 using CRISPR/Cas9 technology), we project to use shRNAs against Chd8. Two already validated shRNAs for Chd8 (Matthieu Gerard, CEA, Paris, *unpublished results*) will be inserted into lentivirus co-expressing Cre recombinase. MACS purified OPCs obtained from P7 cortices of *Rosa26<sup>stop-YFP</sup>* mice will be transfected with *shChd8-Cre* or control (*shScramble-Cre*) virus. After proliferation, OPCs will be put in differentiation medium for 3 days and OL differentiation will be assessed using OL markers (CC1, Nkx2.2 and *Itpr2*) and myelin proteins immunostaining (CNP, MBP and MOG) in YFP<sup>+</sup> cells. We will also assess OPC proliferation using MCM2 and Ki67 markers and cell death using p53 and Caspase3 immunostaining. In order to evaluate the functional specificity or redundancy between Chd8 & Chd7, we will used these *shChd8-Cre* viruses on OPCs purified from *Chd7<sup>Flox/Flox</sup>; Rosa26<sup>stop-YFP</sup>* mice. Their differentiation potential will be compared with that of *Chd8* knock-down alone. We expect to show that Chd8 function is required of normal OL differentiation and that its function is associated with Chd7. We also expect to find cell death in OPCs as Chd8 binds to Trp53 promoter similarly to Chd7. Finally, we expect to find a greater impact on OPC proliferation as Chd8 binds to more genes involved in cell-cycle than Chd7 and the role of Chd8 in cell-cycle have been shown before in neural progenitors (Durak et al., 2016).

To complete our binding model, this study should be also completed with Chd8 genome wide binding profile in OLs. To this aim, we will purify by MACS O4<sup>+</sup> cells from P14 and deplete them from OPCs with PDGFR $\alpha$  antibody (second MACSort). If the quality (cells being only OLs) or quantity is not sufficient to do ChIP-seq, alternatively, we will purify O4<sup>+</sup> cells from P7 and put them in OL differentiation medium. After 3 day in differentiation, we

will perform ChIP-seq with Chd8 antibody. We expect to find that genes bound by Chd8 in OLs are involved in myelination, such as *Mbp* or *Mog*.

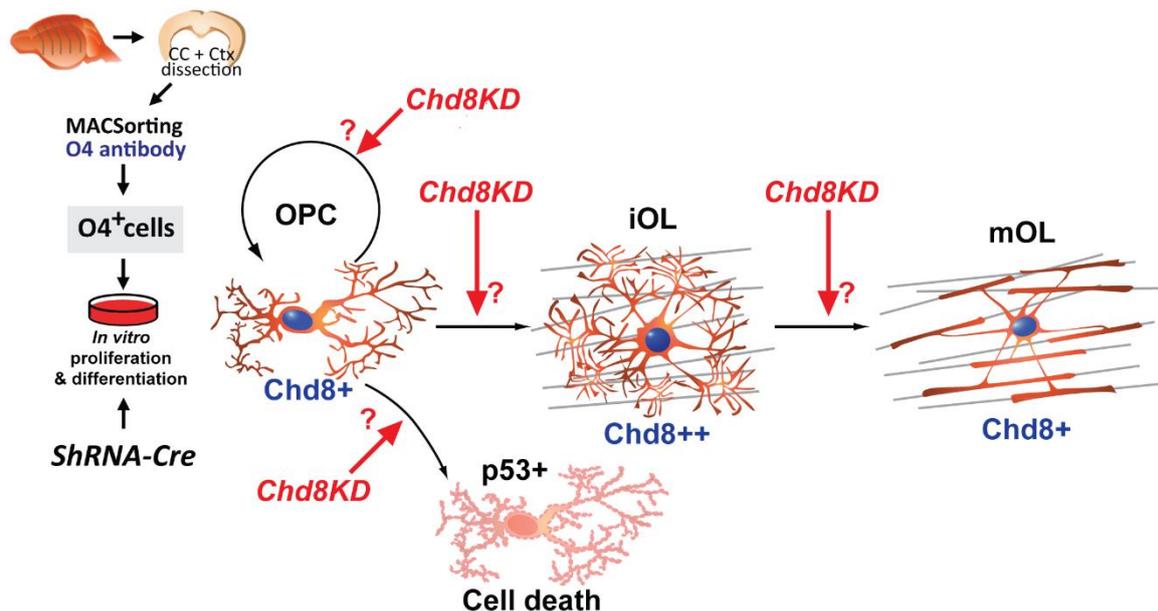


Figure 5. Diagram representing the in vitro experiment to assess Chd8 function in OPC differentiation, proliferation and survival.

### Validation of the *Olig2/Sox10/Chd7/Chd8* complex

ChIP data can describe where a factor can bind on the chromatin. However, when multiple factors bind to the same sites, these experiment are limited in the understanding of how these factors interact with each other. It has been shown that Chd7 and Chd8 can interact (Batsukh et al., 2010; Feng et al., 2017). To identify partner of Chd7, we started RIME experiments (Rapid Immunoprecipitation Mass spectrometry of Endogenous proteins, Active Motif) which consist of chromatin immunoprecipitation (ChIP) followed by Mass spectrometry. Our antibody against Chd7 is in the process of validation and we should know soon if the technic can be apply for our problematic. We expect to find binding partners such as Chd8, Sox10 and Olig2.

Alternatively, we could perform sequential ChIP which consist of doing ChIP with antibodies against one factor (like Chd7) and immunoprecipitate again the obtained chromatin with antibodies against another factor (like Chd8). We should that way obtained DNA sequenced bound by both factors. We could also use Duolink<sup>®</sup> technologies to visualize by imaging the interaction between two factors.

### Identification of *Serinc5* as a target of Chd7 and Chd8

We have identified *Serinc5* as a target of Chd7 and Chd8 as it is downregulated after Chd7 LOF in OPCs and it is bound directly by Chd7 and Chd8. Interestingly, *Serinc5* is part of the ASD risk genes (ref) and is expressed in myelin (ref). To better understand *Serinc5* function, it would be interesting to look at OPC differentiation and myelination potential after *Serinc5* deletion. We could also wonder if *Serinc5* KO mice, which have not been studied yet, would develop specific phenotype associated with hypomyelination like shaking or behavioral modification associated to autism.

Altogether, this study aimed to a better understand the mechanisms of gene expression regulation during OL differentiation. A clinical application of this knowledge is to work out the mechanisms promoting OL differentiation and remyelination in the context of diseases such as Multiple Sclerosis. Myelination is also affected in other syndromes like in Autism (ASD) (Fields, 2008) for which Chd8 is one major risk gene (Barnard et al., 2015; O/'Roak et al., 2012; RK et al., 2017). Interestingly, Chd7 mutations are the major cause of CHARGE syndrome which can present some autistic features (Betancur, 2011) and myelin defects (He et al., 2016). How these two factors interact together is therefore a crucial question to better understand these pathologies. Moreover, we showed a direct effect of Chd7 and Chd8 on *Trp53* and it was very recently shown that Chd7 have an oncogenic role as Chd7 overexpression in breast cancer correlates with aggressive subtypes (Chu et al., 2017), suggesting a link between Chd7 and p53 in cancer. The involvement of Chd7 and Chd8 in such severe pathologies shows the importance to study their functions and mechanisms of action.



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# Abstract

Oligodendrocytes (OLs) are myelin-forming cells of the central nervous system wrapping axons and allowing the saltatory conduction of action potentials. In Multiple sclerosis (MS), myelin sheath is destroyed and effective remyelination by oligodendrocyte precursor cells (OPCs) diminishes with disease progression. Therefore, a better understanding of the mechanisms controlling OPC generation and differentiation is essential to develop efficient remyelinating therapies. Oligodendrogenesis, involving the steps of OPC generation, OPC differentiation and maturation of OLs, is a process controlled by specific transcription factors including *Ascl1*, *Olig2* and *Sox10* but the mechanisms involved are poorly understood. As it is known that chromatin remodelers are regulatory factors necessary in the formation of the promoter-enhancer loop prior to transcription, we focused our study on *Chd7* (Chromodomain-Helicase-DNA-Binding 7), a member of the CHD protein family. In a first study, we showed that *Chd7* is highly enriched in the oligodendroglial lineage cells with a peak of expression during OL differentiation and that *Chd7* OPC-conditional deletion impairs OL differentiation during (re)myelination. In a second study, we used unbiased genome wide technics in purified OPCs to study *Chd7* regulation of genes involved in OPC differentiation, proliferation and survival. To this aim, we have generated OPC-specific inducible *Chd7* knock-out (*Chd7-iKO*) and analyse the transcriptome (RNA-seq) of purified OPCs from P7 mouse cortices compared to control littermates. We found that *Chd7* promote the expression genes involved in OPC differentiation and myelination and inhibits apoptosis, without affecting OPC proliferation. Furthermore, we investigated *Chd8*, a paralog of *Chd7*, showing that it is expressed in the oligodendroglial lineage with a peak of expression in differentiating oligodendrocytes, similar to *Chd7*. Genome wide binding (ChIP-seq) profiling for *Chd7* and *Chd8* indicate that these two chromatin remodelers bind to common genes related to OPC differentiation, survival and proliferation. Integrating these datasets with other key transcriptional regulators of oligodendrogenesis (*Olig2*, *Ascl1* & *Sox10*), we have built a model accounting for the time-controlled regulate expression of genes involved in each step of OL differentiation.

## Résumé en Français

Les oligodendrocytes (OLs) sont les cellules myélinisantes du système nerveux central, s'enroulant autour des axones et permettant la conduction saltatoire du potentiel d'action. Dans la Sclérose en Plaques, des gaines de myélines sont détruites et l'efficacité de la remyélinisation par les précurseurs d'oligodendrocytes (OPCs) diminue avec la progression de la maladie. Une meilleure compréhension du mécanisme qui contrôle la génération des OPCs et leur différenciation est donc essentielle pour développer des thérapies efficaces de remyélinisation. L'oligodendrogenèse, qui comprend les étapes de génération des OPCs, de différenciation et de maturation des OLs, est un processus contrôlé par des facteurs de transcription spécifiques incluant *Ascl1*, *Olig2* and *Sox10* mais le mécanisme impliqué est encore peu connu. Sachant que les facteurs du remodelage de la chromatine sont des régulateurs nécessaires à la formation de la boucle promoteur-enhancer permettant l'initiation de la transcription, nous nous sommes focalisés sur *Chd7* (Chromodomain-Helicase-DNA-Binding 7), un membre de la famille de protéine CHD. Dans une première étude, nous avons montré que *Chd7* est hautement enrichi dans le lignage oligodendrogial avec un pic d'expression pendant la différenciation des OLs. Nous avons également montré que la délétion conditionnelle de *Chd7* diminuait la différenciation des OLs pendant la (re)myélinisation. Dans une seconde étude, nous avons utilisé des techniques de génomique sur les OPCs purifiés pour étudier la régulation par *Chd7* de gènes impliqués dans la différenciation, la survie et la prolifération des OPCs. Dans ce but, nous avons généré des délétions inducible de *Chd7* spécifiquement dans les OPCs (*Chd7-*i*KO*) et nous avons analysé le transcriptome (RNA-seq) d'OPCs purifiés à partir de cerveaux de souris P7 comparé à des contrôles. Nous avons trouvé que *Chd7* activait l'expression des gènes impliqués dans la différenciation des OPCs et la myélinisation et inhibait l'apoptose, sans montrer de défaut de prolifération. Pour aller plus loin, nous avons étudié *Chd8*, un paralogue de *Chd7*, et nous avons montré qu'il est exprimé dans le lignage oligodendrocytaire avec un pic d'expression dans les OLs en différenciation, similairement à *Chd7*. Les données de fixation (ChIP-seq) de *Chd7* et *Chd8* indiquent que ces deux facteurs du remodelage de la chromatine se fixent sur des gènes communs reliés au processus de différenciation, de survie et de prolifération des OPCs. Intégrant ces données avec celles de facteurs transcriptionnels clés dans l'oligodendrogenèse (*Olig2*, *Ascl1* et *Sox10*), nous avons construit un modèle de la régulation de l'expression de gènes contrôlés dans le temps et impliqués dans chacune des étapes de la différenciation des oligodendrocytes.