Investigating the Roles of Poly (ADP-Ribose) Polymerase 1 in Cortical Development

by

Megan M. Nelson

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Neuroscience)
in the University of Michigan
2021

Doctoral Committee:

Professor Gabriel Corfas, Chair
Professor Peter F. Hitchcock
Associate Professor Shigeki Iwase
Professor Donna Martin
Professor Michael Uhler

Megan M. Nelson

mmnels@umich.edu

ORCID iD: 0000-0002-5377-7601

© Megan M. Nelson 2021

Dedication

This dissertation is dedicated to my parents, who have continuously inspired, guided, and supported me through all of my academic endeavors.

Acknowledgments

Numerous people have supported me during my graduate career. First, thank you to my mentor, Dr. Gabriel Corfas. I am so grateful for his continuous encouragement as he allowed me the freedom to explore my interests. I am thankful that he remained patient with the many roadblocks I encountered while going out of his way to provide his expertise or resources for aid. During my time in his lab, I have grown into a more careful, thoughtful scientist, and I am confident his influence has led directly to that growth. I would also like to thank the members of the Corfas lab, past and present, who went above and beyond to teach me, help me, and encourage me. Their support eased my transition into graduate school and fostered an environment where I felt safe to explore my interests and discuss my failures as well as my successes. I particularly want to thank my undergraduate students, Mya, Brenna, and Nada, who were a delight to interact with and teach. I very much appreciated their dedication and willingness to take on any task. They brought joy to the lab, for which I am thankful.

Thank you to my thesis committee – Dr. Donna Martin, Dr. Peter Hitchcock, Dr. Michael Uhler, and Dr. Shigeki Iwase. Their advice, expertise, and guidance shaped the course of this dissertation and made me a better scientist. I appreciated their encouragement during my lows and their enthusiasm during my highs. I would also like to thank my undergraduate research mentor, Dr. Stephanie Gardner. Through working in her lab, I discovered my love for neuroscience, which directly led me on the path to pursuing a neuroscience Ph.D. Her support as I applied to graduate programs and searched for graduate mentors was invaluable. Thank you to the staff of the Neuroscience Graduate Program and the Kresge Hearing Research Institute for taking care of all of my administrative needs, helping me focus on research. I would also like to thank the members of the Kresge family – graduate students, postdocs, and faculty. They were always willing to lend a reagent in a pinch and offer their expertise, which I very much appreciated.

Thank you so much to my family – specifically my mom and dad, brothers, and grandparents. They were nothing but patient and encouraging during the highs and lows of my career, constantly reiterating how proud they were of the person and scientist I had become. My parents inspired me to become a scientist and helped all of my dreams to come to fruition. Last but not least, thank you to my friends – my constant cheerleaders, the people without which I could not have done this. Through my failures and successes, they were there every step of the way. Minh, Kim, Ketki, Colleen, Amanda, Kristin, Mihir – thank you for never wavering in your support, always lending an ear when I needed a friend, and sticking by my side through everything. I could never describe in words how much each of you means to me.

For everyone mentioned above and countless others, I am exceedingly thankful.

Table of Contents

Dedication ii

Acknowledgments iii

List of Tables ix

List of Figures x

Abstract xii

Chapter 1: Introduction 1

- 1.1 Overview 1
- 1.2 Cortical Development 3
  - 1.2.1 Neurogenesis and Gliogenesis 3
  - 1.2.2 Neuronal Migration 11
  - 1.2.3 Cajal-Retzius Cells 16
  - 1.2.4 The Extracellular Matrix 25
  - 1.2.5 Human Cortical Development 28
- 1.3 NRG1/ErbB4 Signaling 29
  - 1.3.1 ErbB/EGFR Family of Receptor Tyrosine Kinases 29
  - 1.3.2 ErbB4 Structure and Signaling 30
  - 1.3.3 Roles in Brain Development 31
  - 1.3.4 Associations with Neurological and Psychiatric Diseases 37
- 1.4 Poly (ADP-Ribose) Polymerase 1 (PARP1) 38
  - 1.4.1 PARP Family of Proteins 39
  - 1.4.2 PARP1 Protein Structure and Catalytic Activity 40
  - 1.4.3 PARP1 Functions 40

1.4.4 Roles in Brain Development 50
1.4.5 Roles in Neurological and Psychiatric Diseases 51
1.5 Summary and Dissertation Outline 52
1.6 Figures 54
Chapter 2: ErbB4 Interacts with PARP1 to Regulate Astrogenesis 57
2.1 Introduction 57
2.2 Materials and Methods 60
2.2.1 PARP1 KO Mice 60
2.2.2 Generation of ErbB4-JMa$^{-/-}$ mice 60
2.2.3 Cell Culture and Treatments 61
2.2.4 shRNA-mediated PARP1 knockdown 62
2.2.5 Luciferase Assay 62
2.2.6 PARylation Assay 63
2.2.7 RNA Isolation 63
2.2.8 RT-PCR and RT-qPCR 63
2.2.9 ErbB4 Immunoprecipitation 64
2.3 Results 65
2.3.1 NRG1-induced PARylation in mouse NPCs is dependent upon PARP1 and ErbB4 65
2.3.2 Generation and Validation of the ErbB4-JMa$^{-/-}$ mouse 66
2.3.3 NRG1 repression of GFAP expression following FGF removal depends upon PARP1 and ErbB4-JMa 66
2.3.4 Loss of PARP1 or ErbB4 increases cortical *GFAP* expression at birth 68
2.4 Discussion 68
2.5 Author Contributions 70
2.6 Acknowledgements 71
2.7 Figures 72
Chapter 3: PARP1 Regulates Cajal-Retzius Cell Development and Neural Precursor Cell Adhesion 79
3.1 Introduction 79
3.2 Materials and Methods 81
3.2.1 PARP1 KO Mice 81

3.2.2 Cell Culture and Treatments ... 81
3.2.3 RNA-Sequencing and Analysis ... 82
3.2.4 Immunofluorescence and Quantification ... 83
3.2.5 Cresyl Violet Staining and Brain Volume Quantification ... 84
3.2.6 EdU Labeling *in vivo* ... 85
3.2.7 RNA Isolation and RT-qPCR ... 85
3.2.8 shRNA-mediated PARP1 Knockdown ... 86
3.2.9 Luciferase Assay ... 87
3.2.10 Western Blot ... 87
3.2.11 Reelin Conditioned Media ... 88
3.2.12 Chromatin Immunoprecipitation ... 89
3.2.13 RNA Immunoprecipitation ... 89
3.2.14 Atomic Force Microscopy (AFM) ... 90
3.2.15 Statistics ... 91
3.3 Results ... 91
3.3.1 PARP1 KO mice have brain development defects ... 91
3.3.2 Loss of PARP1 increases the expression levels of genes associated with cell migration and adhesion in the E15.5 cortex ... 93
3.3.3 Loss of PARP1 results in an increased number of Cajal-Retzius cells in the cortex ... 94
3.3.4 Loss of PARP1 increases levels of genes expressed by CR cells and Reelin protein in NPCs ... 94
3.3.5 PARP1 loss of function increases CR cell abundance in NPC cultures ... 96
3.3.6 PARP1 loss increases NPC adhesiveness to N-cadherin ... 96
3.4 Discussion ... 97
3.5 Conflict of Interest Statement ... 100
3.6 Author Contributions Statement ... 100
3.7 Funding ... 100
3.8 Acknowledgments ... 100
3.9 Figures ... 102
3.10 Tables ... 115
Chapter 4: Discussion and Future Directions ... 118
4.1 Summary of Findings ... 118

4.2 Future Directions 121
4.2.1 Mechanism of PARP1-ErbB4 Regulation of Astrogenesis 121
4.2.2 Consequences of Precocious Astrogenesis 125
4.2.3 Potential Mechanisms of PARP1 Regulation of Cajal-Retzius Cell Development.... 126
4.2.4 Phenotypes Associated with Reelin Function 131
4.2.5 Phenotypes Associated with Extracellular Matrix Function and Abnormal Neuronal Migration 132
4.2.6 Final Thoughts and Conclusions 135
References 136

List of Tables

Table 3.1 Sequences of primers used for quantitative RT-PCR. 116

Table 3.2 Sequences of primers used for chromatin immunoprecipitation. 116

Table 3.3 PARP1 loss upregulates the expression of genes associated with cell adhesion, axon development, dendrite morphogenesis, and cell migration in the E15.5 cortex. 117

List of Figures

Figure 1.1 Overview of neurogenesis and gliogenesis in the rodent brain.. 54

Figure 1.2 *ErbB4* alternative splicing. 55

Figure 1.3 PARP1 protein structure. 55

Figure 1.4 PARP1 enzymatic function 56

Figure 2.1 PARP1 interacts with E4ICD in a kinase-specific manner 73

Figure 2.2 NRG1 treatment induces PARP1 PARylation in an E4ICD-specific manner 73

Figure 2.3 PARP1 DNA binding and enzymatic activity are necessary to repress CNTF-induced *GFAP* promoter activity in rat NPCs 74

Figure 2.4 PARP1 and ErbB4 are necessary for NRG1-induced PARylation in mouse NPCs.... 75

Figure 2.5 ErbB4-JMa$^{-/-}$ mutant mice lack ErbB4-JMa and E4ICD expression. 76

Figure 2.6 PARP1 and ErbB4-JMa are necessary for NRG1-induced repression of *GFAP* expression after FGF removal in mouse NPCs 77

Figure 2.7 PARP1 or ErbB4 loss increases *GFAP* expression in the mouse cortex at birth 78

Figure 3.1 Loss of PARP1 results in reduced brain weight and cortical surface area at birth ... 103

Figure 3.2 PARP1 KO mice have defects in cortical development 105

Figure 3.3 Expression levels of genes associated with neuronal migration and cell adhesion are increased at E15.5 in the cortex of PARP1 KOs 107

Figure 3.4 Loss of PARP1 increases the number of Cajal-Retzius cells in the E15.5 and P5 cortex 108

Figure 3.5 PARP1 loss by KO, shRNA knockdown, or pharmacological inhibition increases mRNA levels of genes expressed by Cajal-Retzius cells 109

Figure 3.6 PARP1 KO NPC cultures overexpress Reelin protein.. 110

Figure 3.7 Reelin induces Dab1 phosphorylation in NPCs 111

Figure 3.8 PARP1 loss increases CR cell abundance in NPC cultures 112

Figure 3.9 PARP1 loss of function does not alter the activity of the *Reln* promoter or the stability of the *Reln* transcript.. 113

Figure 3.10 Reelin increases NPC adhesiveness to N-cadherin 114

Figure 3.11 PARP1 loss increases NPC adhesiveness to N-cadherin 115

Abstract

Poly (ADP-ribose) polymerase 1 (PARP1) is a ubiquitously expressed enzyme that post-translationally modifies proteins via poly (ADP-ribosylation) (PARylation). PARP1 serves various functions, including DNA damage repair, regulation of cell death pathways, chromatin modification, RNA processing, and transcriptional regulation. Accordingly, mutations in *Parp1* or *Adprhl2* (encoding the protein ADP-ribosylhydrolase 3, which removes PAR polymers) cause intellectual disability, ataxia, episodic psychosis, neurodegeneration, and developmental delay. Altered PARP1 expression is also associated with numerous neurodegenerative and neuroimmune disorders, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, rheumatoid arthritis, major depressive disorder, and epilepsy. Despite ubiquitous expression and an apparent connection with brain disorders, PARP1's role in neurodevelopment has not been widely studied.

Our lab has recently uncovered a novel interaction between PARP1 and the receptor tyrosine kinase ErbB4, which binds its ligand NRG1 to mediate numerous functions during neurodevelopment, including radial migration of excitatory neurons, tangential migration of inhibitory neurons, synaptogenesis, and differentiation. Additionally, ErbB4 has multiple splice forms that confer different signaling modalities. Specifically, the ErbB4-juxtamembrane (JM)-a isoform is cleavable via the enzymes tumor necrosis factor-α (TACE) and presenilin/γ-secretase. Upon NRG1 binding and ErbB4-JMa cleavage, the ErbB4 intracellular domain (E4ICD) is released, which regulates transcription through direct promoter binding. Previous findings have

shown that E4ICD complexes with co-factors to repress gliogenesis during early development. Due to PARP1's prominent roles in chromatin modification and transcriptional control, this begs the question as to whether PARP1 is likewise regulating glial gene expression via E4ICD.

The aims of this dissertation are two-fold: 1) investigate the role of PARP1 in regulating astrocytic gene expression via E4ICD and 2) further characterize the effect of PARP1 loss on brain development. To explore the role of PARP1-E4ICD in the regulation of astrogenesis, I utilized mouse primary embryonic neural precursor cell (NPC) cultures and transgenic mice with a germline knockout of PARP1, ErbB4, or ErbB4-JMa. I found that NRG1-mediated repression of *GFAP* expression upon FGF removal from NPC cultures was dependent upon the presence of PARP1, ErbB4, and ErbB4-JMa. Additionally, I showed that PARP1 KO and ErbB4 KO mice overexpress *GFAP* at birth, indicating the importance of both proteins *in vivo*.

To investigate the effect of PARP1 loss on neurodevelopment more broadly, I analyzed the brain and cortical size of PARP1 KO mice at birth, finding a reduction in brain weight relative to body size, which is associated with a thinner cortex and a reduced cortical surface area. Furthermore, I discovered that PARP1 loss alters early-born neuron migration and increases the density of deeper-layer neurons. To investigate changes in gene expression associated with these findings, I performed RNA-sequencing of the embryonic PARP1 KO cortex. I found that PARP1 loss increases the expression of genes involved in neuronal migration and adhesion, including *Reln*, which encodes the glycoprotein Reelin. Accordingly, my findings indicate that PARP1 loss increases the abundance of Reelin-expressing cells in the developing (E15.5) and adolescent (P5) mouse brain. I further demonstrated that PARP1 loss, inhibition, or acute knockdown increases Cajal-Retzius cell abundance *in vitro*, suggesting PARP1 regulates Cajal-Retzius cell development via a cell-autonomous mechanism. Finally, atomic force microscopy showed that NPCs isolated from the PARP1 KO cortex adhere more strongly to the cell adhesion molecule N-cadherin, likely due to excess Reelin. Overall, these findings demonstrate that PARP1 regulates astrogenesis, Cajal-Retzius cell development, and cell adhesion in the developing brain.

Chapter 1: Introduction

1.1 Overview

Cerebral cortex development is a highly ordered and temporally controlled process that involves the coordinated expression of countless proteins, including transcription factors, extracellular signaling molecules, trophic factors, enzymes, and more (Guillemot, 2005; Florio and Huttner, 2014). Misexpression or misregulation of single proteins can severely impair brain development to render the organism inviable, or it can result in more subtle effects that increase an individual's susceptibility for psychiatric disorders later in life, including schizophrenia (Pablo V. Bejman et al., 2011; Huang et al., 2014). Several single nucleotide polymorphisms (SNPs) in the genes encoding Neuregulin 1 (*NRG1*) or its receptor, ErbB4 (*ErbB4*), have been associated with schizophrenia (Munafò et al., 2006; Norton et al., 2006; Nicodemus et al., 2009; Feng et al., 2017). Their connection to psychiatric disorders is thought to be due to their critical roles in regulating inhibitory interneuron migration during development (Flames et al., 2004; Li et al., 2012). Accordingly, mice lacking ErbB4 or NRG1 have fewer Parvalbumin-expressing interneurons in the cortex, disrupting the balance between excitatory pyramidal neurons and inhibitory neurons, resulting in excess electrical activity (Fazzari et al., 2010; Neddens and Buonanno, 2010). Disrupted excitatory-inhibitory balance has likewise been found in patients diagnosed with psychiatric disorders (Selten et al., 2018).

Additionally, NRG1 signaling through ErbB4 regulates the timing of astrogenesis onset in the developing cortex (Sardi et al., 2006). The primary cell types in the brain include neurons and glial cells, such as astrocytes and oligodendrocytes. During development, neural precursor cells differentiate first into neurons, then switch to astrocyte and oligodendrocyte differentiation during later stages (Sauvageot, 2002; Guillemot, 2005; Jiang and Nardelli, 2016). NRG1 signaling through ErbB4 represses the onset of astrogenesis at early developmental stages by inhibiting the transcription of glial-specific genes, such as glial fibrillary acidic protein (GFAP). Data suggests that this occurs by direct binding of the ErbB4 intracellular domain (E4ICD) to the promoter region of these genes (Sardi et al., 2006). Intriguingly, E4ICD is generated by full-length ErbB4 cleavage by presenilin/γ-secretase, a causative factor for Alzheimer's disease when disrupted (De Strooper, 2007), indicating Alzheimer's pathology may be associated with altered ErbB4 expression and signaling.

More recent unpublished data collected in the Corfas lab indicates that E4ICD's interaction with the *GFAP* promoter may be mediated through binding to Poly (ADP-Ribose) Polymerase 1 (PARP1). PARP1 is a ubiquitously expressed enzyme that binds and post-translationally modifies proteins and DNA via the addition of ADP-ribose polymers, regulating diverse cellular processes, from DNA repair to transcriptional regulation to cell survival (Kraus, 2008; Krishnakumar and Kraus, 2010b). As ErbB4 lacks a DNA binding domain, this interaction between E4ICD and PARP1 suggests that PARP1 may serve as a bridge to bind E4ICD to the *GFAP* promoter, thus inhibiting transcription. Similar to NRG1/ErbB4, PARP1 is implicated in the pathogenesis of numerous neurological disorders (Mao and Zhang, 2021). Furthermore, mutations in proteins affecting PARylation cause disorders that include symptoms such as episodic psychosis, ataxia, intellectual disability, and neurodegeneration (Najmabadi et al., 2011; Danhauser et al., 2018; Durmus et al., 2021). Together, these studies indicate that PARP1 may have other roles in brain development apart from regulating astrogenesis. A better understanding of how PARP1 loss-of-function alters cortical development will ultimately allow scientists to understand its etiology further and develop more targeted therapies for associated neurological disorders.

1.2 Cortical Development

1.2.1 Neurogenesis and Gliogenesis

Neurogenesis is the process by which progenitor cells within the embryonic brain generate neurons and expand the neocortex during development. In the rodent brain, the earliest born neurons arise around embryonic day 10.5 (E10.5), forming the preplate (Angevine and Sidman, 1961; Meyer et al., 1998). A few days later, the preplate splits to form the deeper subplate and superficial marginal zone. As neurogenesis begins, progenitor cells divide and produce immature neurons, which form the cortical plate in a layer between the subplate and marginal zone. These neurons eventually mature into excitatory pyramidal neurons that comprise the multilayered neocortex in mature animals (Marín-Padilla, 1992; Olson, 2014). On the other hand, gliogenesis is the process by which non-neuronal subtypes, or glial cells, are produced in the brain. These encompass astrocytes and oligodendrocytes, among others not discussed in this dissertation. This section will review mechanisms of proliferation and differentiation of these cell types within the cerebral cortex (overview depicted in Figure 1.1).

1.2.1.1 Timeline and Mechanisms of Proliferation

Just before the onset of neurogenesis at E10.5, the neuronal pool rapidly increases through the symmetric division of highly polarized neuroepithelial cells within the neural tube, expanding the cortex laterally and radially. At the onset of neurogenesis around E12.5 – E13.5, neuroepithelial cells become radial glial cells. Like neuroepithelial cells, radial glial cells have long processes that connect the basal lamina at the pial surface of the brain to the apical ventricular zone, spanning the width of the early cortex (Rakic, 1995; Huttner and Brand, 1997; Hartfuss et al., 2001). Radial glial cells divide asymmetrically along the ventricular wall to generate two distinct cell types: a new radial glial cell and an intermediate progenitor cell (also known as a basal progenitor) or an immature neuron (Hartfuss et al., 2001; Malatesta et al., 2003; Noctor et al., 2004). Intermediate progenitor cells (IPCs), in turn, migrate dorsally outside of the ventricular zone, where most divide symmetrically to produce two immature neurons (Noctor et al., 2004). However, there is evidence that some IPCs undergo a round of symmetric proliferative division to increase the progenitor pool before producing neurons (Noctor et al., 2004). Most neurons are generated in the neocortex through IPCs (Kowalczyk et al., 2009). While both symmetric IPC division and asymmetric radial glial cell division are occurring throughout neurogenesis (through E17.5), the predominant origin of newborn neurons during later stages of cortical development is via symmetric division of IPCs (Noctor et al., 2004; Zimmer et al., 2004; Wu et al., 2005). This switch allows the cortex to expand both radially and laterally during the later stages of brain development.

The onset of gliogenesis in the rodent brain begins around E16.5-E17.5 as neurogenesis completes (Qian et al., 2000; Sauvageot, 2002; Mallamaci, 2013). The initial glial cells produced are astrocytes, which have many critical functions in the cortex, including regulation of synaptic function and neural homeostasis, formation and function of the blood-brain barrier, assistance in migration of some neural progenitors, and regulation of neuronal dendrite morphology (Abbott, 2002; Haim and Rowitch, 2017; Vasile et al., 2017). Astrogenesis peaks around P2-3 in the rodent then recedes by P7-9 (Qian et al., 2000; Ge et al., 2012). Unlike neurons and other glial cell subtypes, astrocytes are generated exclusively in the pallium, otherwise known as the dorsal portion of the developing telencephalon. Like neurons, they can arise directly from former radial glial cells or indirectly from IPCs within the subventricular zone (Noctor et al., 2004). Mechanisms regulating the neurogenic to gliogenic fate switch of IPCs will be discussed further below.

As astrogenesis declines, oligodendrocytes begin to differentiate from oligodendrocyte precursor cells (OPCs), with peak production near P14 in the cortex (Sauvageot, 2002). Oligodendrocytes form the myelin sheaths surrounding axons of neurons within the central nervous system. These myelin sheaths insulate axons to improve signal transduction while providing metabolic support (Kuhn et al., 2019). Unlike astrocytes, oligodendrocytes develop from precursors within the subpallium, or ventral telencephalon, as well as the pallium. In addition, OPC production occurs in three waves within each brain region: early embryonic stages (E11.5-12.5) within the medial ganglionic eminence (MGE), later embryonic stages (E15.5) within the lateral ganglionic eminence, and early postnatally within the cortex (Kessaris et al., 2006). OPCs then migrate dorsally into the forebrain, where they eventually differentiate and mature into myelinating oligodendrocytes (Jiang and Nardelli, 2016)

1.2.1.2 Molecular Regulation of Stem Cell Proliferation and Differentiation

Cortical development is a highly temporally regulated process influenced by numerous transcription factors, morphogens such as Wnt, Fgf, and Shh, and epigenetic modifications (Jiang and Nardelli, 2016). Slight disruptions in genes that regulate this process can result in microcephaly, macrocephaly, and other neurodevelopmental disorders (Lindy et al., 2018; Santos-Cortez et al., 2018). In this section, many of the more critical molecular regulators of neurogenesis and astrogenesis will be discussed.

1.2.1.2.1 Neurogenesis

The Notch signaling pathway is a well-established mechanism of stem cell renewal within the developing neocortex. Through its effectors Hes1 and Hes5, Notch maintains the undifferentiated state of radial glial cells by repressing other transcription factors that promote neural differentiation, including Neurogenin 1 (Ngn1), Neurogenin 2 (Ngn2), and Ascl1 (also known as Mash1) (Ohtsuka et al., 1999; Gaiano et al., 2000; Nieto et al., 2001; Gaiano and Fishell, 2002; Ochiai et al., 2009). The influence of Notch on stemness is demonstrated by experiments showing that after an asymmetric radial glial cell division, the daughter cell with higher Notch signaling remains a radial glial cell, while the lower Notch-expressing cell differentiates into a neuron (Ochiai et al., 2009; Dong et al., 2012). Interestingly, an autoinhibitory feedback loop continually oscillates Hes1 expression in radial glial cells (Imayoshi et al., 2013). This oscillation is hypothesized to contribute to the preservation of radial glial cell pluripotency, as maintained Hes expression at later developmental stages induces astrogenesis in radial glial cells. (Imayoshi et al., 2013).

Wnt signaling through β-catenin is also an essential regulator of neurogenesis. Similar to Notch, Wnt has contrasting roles during neurodevelopment depending upon the timing of its expression and transcriptional partners. For example, Wnt-mediated upregulation of Empty Spiracles Homeobox 2 (Emx2) expression promotes stem cell proliferation (Muzio et al., 2005), while Wnt-mediated upregulation of Paired Box 6 (Pax6), Ngn1/Ngn2, and N-Myc expression stimulates neuronal differentiation (Gunhaga et al., 2003; Hirabayashi et al., 2004; Kuwahara et al., 2010). Interestingly, the regulation of this switch in Wnt function may be mediated by Fibroblast Growth Factor 2 (FGF2) presence (which promotes proliferation) or absence (which promotes differentiation) (Israsena et al., 2004). In the chick developing brain, N-Myc represses

Notch signaling to promote neuronal differentiation, indicating that Wnt additionally promotes neurogenesis through N-Myc-mediated Notch repression (Zinin et al., 2014).

In addition to Wnt and FGF, another signaling molecule that regulates neurogenesis is Sonic Hedgehog (Shh). Both Shh and FGF act in part through the Notch effector Hes1 to promote symmetric division and radial glial cell proliferation (Dave et al., 2011; Rash et al., 2011). At the onset of neurogenesis, Shh decreases, which increases the activity of the Gli3 repressor and promotes neuronal differentiation (Ruiz i Altaba, 1998; Wang et al., 2011a). Similarly, the absence of FGF signaling during development causes radial glial cell differentiation into IPCs, beginning the process of neurogenesis (Kang et al., 2009). Taken together, these studies display the interactive nature of Notch, Wnt, Shh, and FGF signaling in regulation of stem cell proliferation and neurogenesis.

The transcription factors Pax6, Ngn2, and Ascl1 are some of the primary regulators of neurogenesis and cerebral cortex patterning (Nieto et al., 2001; Osumi et al., 2008, 6). Pax6 promotes neurogenesis by increasing the expression of proneural genes such as Ngn2 and T-box Brain Protein 2 (Tbr2) while promoting stem cell proliferation (Warren et al., 1999; Sansom et al., 2009). These dual roles of Pax6 are hypothesized to be mediated in part by alternative splicing of the *Pax6* transcript, which generates proteins that differ in their DNA binding subdomains, thus altering their gene targets (Walcher et al., 2013). Ascl1 is an additional bHLH transcription factor (along with Hes1) that oscillates its expression in neural progenitor cells while sustaining its expression in differentiated neurons (Imayoshi et al., 2013). Thus, loss of Ascl1 significantly impairs neurogenesis and causes precocious astrogenesis, while its overexpression induces rapid neuronal differentiation (Casarosa et al., 1999; Nieto et al., 2001; Nakada et al., 2004; Berninger et al., 2007). Genome-wide characterization of Ascl1 targets further demonstrates that Ascl1 directly regulates genes involved in neuronal differentiation (Castro and Guillemot, 2011). Ngn2, in the same family of transcription factors as Ascl1, likewise induces neuronal differentiation when overexpressed, while its loss causes defects in radial glial cell organization and differentiation (Nieto et al., 2001; Berninger et al., 2007). Together, these studies demonstrate the importance of Pax6, Ngn2, and Ascl1 during early development to promote neural differentiation.

1.2.1.2.1.1 Dorsal-Ventral Patterning

The dorsoventral axis of the telencephalon is primarily regulated by Shh and Wnt signaling through mutual repression of opposing transcription factors. Ventral progenitor cells in the subpallium generally mature into GABAergic inhibitory interneurons, while dorsal progenitors in the pallium predominantly produce glutamatergic excitatory neurons. Shh, which is secreted by cells within the ventral cortex, represses the pro-dorsal activity of the Gli3 repressor, which results in upregulated expression of ventral cell fate-specific genes, including Nk2 Homeobox 1 (Nkx2.1) and Gamma Glutamylcysteine Synthetase1 and 2 (Gsh1/2) (Rallu et al., 2002). In turn, Nkx2.1 induces LIM Homeobox 6 (Lhx6) expression while Gsh1/2 promotes transcription of Ascl1 and Distal-Less Homeobox 1 and 2 (Dlx1/2), which repress the dorsal cell fate and promote differentiation into inhibitory neurons (Casarosa et al., 1999; Toresson et al., 2000; Du et al., 2008; Long et al., 2009; Wang et al., 2009).

Meanwhile, Wnt secretion in the dorsal telencephalon induces the expression of Pax6 and Ngn1 (Gunhaga et al., 2003; Hirabayashi et al., 2004), which along with Ngn2 and Emx1/2 specify the dorsal neuron fate by repressing the expression of ventral-specific transcription factors, including previously discussed Ascl1, Gsh2, and Dlx2 (Stoykova et al., 2000; Yun et al., 2001, 6; Muzio et al., 2002; Quinn et al., 2007). Therefore, the absence of Pax6 and Emx2 results in the complete lack of a cortex, leaving only ventral progenitor regions (Muzio et al., 2002). Other transcription factors involved in dorsal neuron specification include LIM Homeobox 2 (Lhx2) and Forkhead Box G1 (Foxg1). Similar to Pax6 and Emx2, the absence of either of these proteins results in brains with expanded ventral regions (Bulchand et al., 2001; Monuki et al., 2001; Muzio and Mallamaci, 2005), suggesting a similar mechanism. Through these complex opposing pathways, the dorsal telencephalon forms the mature neocortex composed of pyramidal neurons, while the ventral telencephalon develops into the basal ganglia and parts of the amygdala (Medina and Abellán, 2012).

1.2.1.2.2 Astrogenesis

One of the significant effectors regulating astrogenesis is Janus Kinase (JAK)/STAT signaling (Bonni et al., 1997), which is initiated through binding of pro-astrocytic cytokines, such as Ciliary Neurotrophic Factor (CNTF), Cardiotropin 1 (Ct1), and Leukemia Inhibiting Factor (LIF), to receptors gp130 and one of its partners (Davis et al., 1993). Ligand binding induces receptor dimerization and receptor-associated JAK autophosphorylation and activation (Lutticken et al., 1994). Activated JAK, in turn, phosphorylates its associated receptors, which recruit STAT3 to be phosphorylated by JAK (Stahl et al., 1995). Phosphorylation of STAT3 then induces its association with the co-factor complex CREB binding protein (CBP)/p300, which binds to promoter regions of astrocyte-specific genes, including Glial Fibrillary Acidic Protein (GFAP), to promote gene transcription (Bonni et al., 1997; Nakashima et al., 1999). Accordingly, STAT3 deletion or repression stimulates neuronal differentiation and prevents astrocytic differentiation (Kamakura et al., 2004; Gu et al., 2005; Cao et al., 2006, 2010).

Additionally, astrogenesis is negatively regulated by transcription factors that promote neurogenesis, including Ngn1, Ngn2, Neuronal Differentiation 1 (NeuroD1), and Ascl1, by their repression of glial-specific genes (Tomita et al., 2000; Nakashima et al., 2001, 2; Nieto et al., 2001; Sun et al., 2001). For example, one mechanistic hypothesis postulates that Ngn1 and STAT compete for CBP/p300 binding. Thus, Ngn1 can sequester these co-factors away from gene promoter regions when its expression is elevated (Sun et al., 2001). At the same time, Ngn1 inhibits STAT phosphorylation, preventing it from binding CBP/p300 (Sun et al., 2001). Therefore, as Ngn1 expression declines following neurogenesis, STAT is phosphorylated by JAK, and STAT instead binds CBP/p300 to promote gliogenesis (Sun et al., 2001).

As previously discussed, Notch is crucial to repress neurogenesis and promote stem cell proliferation during early development stages (Gaiano and Fishell, 2002; Dave et al., 2011; Dong et al., 2012). However, in later stages of development, Notch activation induces astrocytic differentiation (Chambers et al., 2001; Grandbarbe et al., 2003). This switch from Notch promoting stem cell renewal to astrogenesis remains unclear. However, evidence shows that sustained rather than oscillating Hes1 expression promotes gliogenesis rather than proliferation (Imayoshi et al., 2013). Mechanistically, Notch promotes astrogenesis partially through activation of the JAK/STAT3 pathway. The Notch effector Hes1 binds nuclear STAT3, facilitating its interaction with JAK (Kamakura et al., 2004). In turn, JAK induces STAT3 phosphorylation and the initiation of *GFAP* transcription (Kamakura et al., 2004). In addition to its influence on STAT3 activation, Notch epigenetically modifies the *GFAP* promoter region. Studies show that overexpressing the Notch intracellular domain (NICD), cleaved from full-length Notch after ligand binding, promotes *GFAP* transcription through its downstream target Nuclear Factor 1A (NF1A). NF1A releases DNA Methyltransferase 1 (DNMT1) from the *GFAP* promoter, resulting in promoter demethylation, opening the chromatin, and allowing transcription to occur (Namihira et al., 2009). In addition to epigenetically modifying the *GFAP* promoter, NICD also forms a complex with the transcription factor RBPJk/CSL and directly binds the *GFAP* promoter to promote transcription (Ge et al., 2002).

Additionally, Neuregulin 1 (NRG1) signaling via its ErbB4 receptor inhibits astrogenesis, previously identified by the Corfas lab (Sardi et al., 2006). NRG1 binding to ErbB4 stimulates ErbB4 cleavage by TACE and presenilin/γ-secretase, releasing the ErbB4 intracellular domain (E4ICD) (Rio et al., 2000; Ni et al., 2001). E4ICD then binds to co-factors Nuclear Receptor Co-Repressor (N-CoR) and TAK Binding Protein 2 (TAB2), which translocate to the nucleus to prevent *GFAP* transcription through its promoter (Sardi et al., 2006). Accordingly, ErbB4 knockout mice display precocious astrogenesis (Sardi et al., 2006). Furthermore, it has been proposed that NICD and E4ICD/N-CoR/TAB2 interact with the *GFAP* promoter at the same binding site (RBPJ-k/CSL). Therefore, high levels of NRG1 and ErbB4 may prevent Notch-induced astrogenesis during early stages of brain development (Ge et al., 2002; Hermanson et al., 2002). When E4ICD levels decrease, the RBPJk/CSL binding site would be freed from N-CoR and bound by NICD, promoting glial gene expression. However, the mechanism behind *GFAP* repression by ErbB4 remains unclear and is the focus of Chapter 2 of this dissertation.

1.2.2 Neuronal Migration

As newborn excitatory neurons differentiate from radial glial cells and intermediate progenitors within the dorsal telencephalon, they migrate in an inside-out fashion within the developing neocortex to reach their final destinations (Rakic, 1995). Thus, early-born neurons are situated in deeper cortical layers (layers V and VI), and later-born neurons migrate past early-born neurons to localize to more superficial cortical layers (II/III and IV). Excitatory neurons migrate radially through two different mechanisms: glial-independent migration (somal translocation) or glial-dependent migration (Nadarajah et al., 2001; Noctor et al., 2004). In addition, GABAergic inhibitory interneurons migrate tangentially from the ventral subpallium to the dorsal pallium (Marín, 2013). The mechanisms of migration for excitatory and inhibitory neurons are discussed further below.

1.2.2.1 Glial-independent migration

During the early stages of brain development, the primary mode of neuron migration is glial-independent (Nadarajah and Parnavelas, 2002). To accomplish this, the migrating neuron's leading process attaches to the basal lamina at the pial surface of the brain and then retracts, which translocates the neuron's soma to its final position within the cortex (Nadarajah et al., 2001). Thus, this type of migration is also called "somal translocation". This migration technique is also utilized at later developmental stages to terminate migration as later-born migrating neurons approach the brain's pial surface (Nadarajah et al., 2001).

1.2.2.2 Glial-dependent migration

The predominant method of newborn neuron migration within the developing cortex is glial-dependent migration, where radial glial cells form a "scaffold" for which neurons can migrate. This scaffold is increasingly crucial as the cortex expands radially during the latter stages of development when neurons migrate longer distances (Nadarajah et al., 2001). Neurons are born in the ventricular and subventricular zones, where they attach to radial glia fibers and migrate toward the brain's pial surface (Noctor et al., 2004). Both internal and external cues regulate glial-dependent migration. Intrinsic modifications involve modulating cytoskeleton elements, including the neuron's growth cone, leading, and trailing processes. In general, leading process extension is followed by nuclear translocation, then trailing process retraction (Tsai and Gleeson, 2005). Leading process extension is facilitated primarily by actin polymerization, while nuclear translocation is managed by interactions between microtubules within the cytoskeleton and motor proteins such as myosin and dynein (Kriegstein and Noctor, 2004; Solecki et al., 2009; Tsai et al., 2010). Cytoskeletal stabilization during nuclear translocation is aided by proteins including Lissencephaly 1 (Lis1) and Doublecortin (DCX) (Bai et al., 2003; Shu et al., 2004, 1). The mechanisms behind trailing process retraction are not widely studied and remain unclear.

Extrinsic factors controlling migration involve complex interactions between cell adhesion molecules, signaling molecules, scaffolding proteins, and neurons (Franco and Müller, 2011; Cooper, 2013). One of the most essential and well-characterized signaling molecules regulating cell migration is Reelin, secreted by Cajal-Retzius cells (Ogawa et al., 1995). Further details regarding Reelin signaling will be described in section 1.2.3.3. In addition to Reelin, the cell adhesion molecule integrin and its ligand laminin promote adhesion between migrating neurons and radial glial cells (Cox and Huttenlocher, 1998). Accordingly, loss of β1 integrin or its ligand laminin α2 in mice disrupts radial glial anchorage to the basement membrane and slows neuronal migration, resulting in abnormal neocortical lamination (Graus-Porta et al., 2001; Loulier et al., 2009). In contrast, the deletion of laminin γ1 completely prevents radial neuron migration, suggesting that different laminin isoforms have varying effects on migration (Chen et al., 2009). Other cell adhesion molecules involved in regulating cell migration include NCAM, L1, and N-cadherin (Schmid and Maness, 2008; Shikanai et al., 2011; Lutz et al., 2017). Further discussion of cell adhesion molecules and their interaction with the brain’s extracellular matrix and influence on cell migration is addressed in later sections.

Additionally, signaling by trophic factors such as soluble NRG1 promotes neuronal migration by directing migration along radial glial fibers while maintaining and elongating radial glia (Anton et al., 1997; Rio et al., 1997). This interaction appears to be mediated in part by brain lipid-binding protein (BLBP), which is vital for radial glial fiber establishment and maintenance (Anton et al., 1997). An additional trophic signal that stabilizes leading processes of migrating neurons is Semaphorin 3A (Sema3A). This molecule is secreted near the cortical plate, forming a gradient (Chen et al., 2008). As ablating Sema3A receptor Neuropilin 1 on migrating neurons or overexpressing Sema3A in the ventricular zone disrupts radial migration, this molecule likely serves as a chemoattractant for migrating neurons (Chen et al., 2008). Together, intrinsic and extrinsic cues guide migrating neurons to their proper positions in the developing cortex.

1.2.2.3 Tangential Interneuron Migration

Unlike excitatory neurons, inhibitory interneurons develop from precursors within the ventral telencephalon, specifically within the medial and caudal ganglionic eminences. These cells then migrate tangentially to their final locations in the dorsal cortex (Anderson et al., 2002; Marín, 2013; Chu and Anderson, 2015). Unlike unipolar migrating excitatory neurons, interneurons adopt a branched morphology to their leading processes and move by creating a new leading process in the direction of chemoattractants. This process appears to be the primary mechanism that drives the directionality of the migrating neuron (Martini et al., 2009). The initial movement of interneurons out of the ventral brain region is likely due to chemorepulsive rather than attractive cues, as the expression of Slit1 (which binds to its receptor Robo) and Ephrin-A5 (which binds to Ephrin receptors) within the ganglionic eminence repel interneurons (Zhu et al., 1999; Marillat et al., 2002; Zimmer et al., 2008; Rudolph et al., 2010).

There are also chemoattractive cues, however, which include the trophic factors Brain-Derived Neurotrophic Factor (BDNF), Glial-Derived Neurotrophic Factor (GDNF), Neurotrophin-4 (NT4), and NRG1 (Polleux et al., 2002; Pozas and Ibáñez, 2005). *In vitro* experiments demonstrate that BDNF, GDNF, and NT4 stimulate interneuron migration (Polleux et al., 2002; Pozas and Ibáñez, 2005). However, the effects of these proteins *in vivo* remain less clear, as mice lacking the receptor for BDNF, TrkB, have unaltered tangential interneuron migration (Carmona et al., 2006; Sánchez-Huertas and Rico, 2011). In contrast, NRG1 signaling through its receptor ErbB4 has demonstrated effects *in vivo*, as ErbB4 KO mice have a decreased number of MGE-derived interneurons in their dorsal cortex and hippocampus (Flames et al., 2004; Neddens and Buonanno, 2010). Interestingly, the membrane-bound and soluble isoforms of NRG1 are hypothesized to serve as short-range and long-range cues for migrating neurons, respectively, creating a gradient for tangentially migrating neurons to follow (Flames et al., 2004).

Once the interneurons migrate from the ventral to dorsal telencephalon, different guidance cues direct their migration through the cortex to their final locations. Migratory streams of interneurons appear to be restricted to the marginal zone, subventricular zone, and subplate of the developing cortex, altogether avoiding the cortical plate where radial neuron migration is ongoing (Lavdas et al., 1999; Wichterle et al., 2001). Studies have shown that this is mediated through the chemoattractant molecules Netrin and Cxcl12 (Stumm et al., 2003). Cxcl12 is expressed in the meninges, in IPCs within the subventricular zone, and at lower levels in the subplate (Stumm et al., 2003; Daniel et al., 2005; Tiveron et al., 2006; Li et al., 2008). It signals through its G-protein coupled receptors, Cxcr4 and Cxcr7, which are expressed in migrating neurons (Stumm et al., 2003; Tiveron et al., 2006; Li et al., 2008). Cxcl12 also regulates the tangential-to-radial switch of interneurons during migration, as loss of Cxcl12 promotes the invasion of interneurons into the cortical plate as soon as they reach the cortex (López-Bendito et al., 2008). This finding indicates that a timely decrease in Cxcl12 expression allows neurons to respond to attractive cues within the cortical plate and reach their final destinations. Aligned with their importance are studies showing that loss of Cxcl12, Cxcr4, or Cxcr7 *in vivo* impairs intracortical interneuron migration (Tiveron et al., 2006; Li et al., 2008; Sánchez-Alcañiz et al., 2011; Wang et al., 2011b). On the other hand, Netrin attracts interneurons migrating specifically within the marginal zone migratory stream (Stanco et al., 2009). This combination of attractive and repulsive cues facilitates the tangential migration of neurons from their birthplaces in the ventral telencephalon to their final locations in the cortex.

1.2.3 Cajal-Retzius Cells

Cajal-Retzius cells are among the earliest-born neurons during cortical development, arising between E10.5 and E12.5 in rodents (Meyer et al., 1998). They localize to the brain's marginal zone and secrete Reelin, a critical signaling molecule for neuron migration and patterning of the cortex (Ogawa et al., 1995; Meyer et al., 1998). Mice with mutations in Reelin (known as *reeler*) have a severe disruption in cortical and cerebellar lamination, indicating the singular importance of Cajal-Retzius cells and Reelin signaling in regulating neuronal migration (Meier and Hoag, 1962; Hamburgh, 1963; Goffinet et al., 1984). In addition to Reelin, subsets of Cajal-Retzius cells express the calcium-binding proteins Calretinin and Calbindin, though the expression of these proteins is not restricted to Cajal-Retzius cells within layer I (Meyer et al., 1998; Bielle et al., 2005). The tumor suppressor protein P73 additionally co-localizes with Reelin, and within layer I it is exclusively expressed in Cajal-Retzius cells (Meyer et al., 2002).

1.2.3.1 Cajal-Retzius Cell Development

Cajal-Retzius cells arise from progenitors within multiple areas of the developing brain, including the septum and ventral pallium, but most progenitors arise from the cortical hem (caudomedial region of the neocortex) (Takiguchi-Hayashi et al., 2004; Bielle et al., 2005; Griveau et al., 2010; Barber et al., 2015). Cajal-Retzius cells tangentially migrate until they reach their final positions within the marginal zone of the neocortex (Soriano and del Río, 2005; Kirischuk et al., 2014). The marginal zone will eventually mature to form Layer I, the uppermost cortical layer. Like the tangential migration of inhibitory interneurons, Cajal-Retzius cell migration is regulated by Cxcl12 through its receptor Cxcr4, which is expressed in Cajal-Retzius cells (Borrell and Marín, 2006). These chemokines are especially critical for the migration of cortical hem-derived Cajal-Retzius cells toward the meninges, where Cxcl12 is secreted (Borrell and Marín, 2006). However, some Cajal-Retzius cells migrate to the marginal zone in the absence of Cxcr4, indicating that this is not the only chemoattractant stimulating progenitor cell migration (Borrell and Marín, 2006). Accordingly, recent data demonstrate that extracellular Pax6 also regulates the tangential migration of progenitors that originate from the cortical hem and septum (Kaddour et al., 2020). Furthermore, other transcription factors that control Cajal-Retzius cell development are hypothesized to influence progenitor migration. These transcription factors are discussed in more detail below.

Cajal-Retzius cell development and differentiation are regulated by signaling molecules, transcription factors, and miRNAs. Transcription factors include negative modulators Pax6, Lhx2, Foxg1, Hes1/3/5, and COUP Transcription Factor 1 (CoupTFI) (Stoykova et al., 2003; Studer et al., 2005; Imayoshi et al., 2008; Shibata et al., 2008), and positive modulators Emx1/2, Tbr1, Lhx5, and Ascl1 (Hevner et al., 2001; Shinozaki et al., 2002; Miquelajáuregui et al., 2010; Dixit et al., 2011). Loss of Pax6 disrupts preplate formation and doubles the number of Reelin-expressing cells in the developing brain's marginal zone. This finding is hypothesized to be partially due to the increased migration of Cajal-Retzius progenitor cells (Stoykova et al., 2003; Kaddour et al., 2020). Lhx2 and Foxg1 indirectly repress Cajal-Retzius cell differentiation by regulating the development of the cortical hem and progenitors that arise there (Roy et al., 2014;

Liu et al., 2018). CoupTFI acts more directly, as CoupTFI overexpression downregulates multiple Cajal-Retzius cell proteins, including Reelin and Calretinin (Studer et al., 2005). Finally, the Notch effectors Hes1/3/5 repress the Cajal-Retzius cell fate by downregulating Ngn2, instead promoting a choroid plexus cell fate (Imayoshi et al., 2008). These findings also indicate that Ngn2 promotes Cajal-Retzius cell differentiation (Imayoshi et al., 2008).

As previously discussed, Ascl1 is highly expressed in ventral progenitor neurons and has a definable role in specifying the ventral cell fate. However, it is also expressed to a lesser extent in dorsal progenitors, promoting Cajal-Retzius cell differentiation. Accordingly, Ascl1 depletion slightly but significantly decreases the number of Cajal-Retzius cells at E15.5 (Dixit et al., 2011). Lhx5 loss likewise decreases the density of Reelin-expressing cells in the cortex (Miquelajáuregui et al., 2010). Emx1/2 double knockout completely prevents Cajal-Retzius cells from developing, which may be due to defective tangential migration (Shinozaki et al., 2002). Rather than a loss of Cajal-Retzius cells, loss of Tbr1 decreases the expression of Reelin within existing Cajal-Retzius cells (Hevner et al., 2001). Interestingly, Tbr1 loss disrupts preplate formation similar to the loss of Pax6, indicating that decreased or increased Reelin expression has comparable effects on preplate development (Hevner et al., 2001; Stoykova et al., 2003). Taken together, these studies show that Cajal-Retzius cell development is a complex process that can be disrupted through the altered expression of many different transcription factors.

Signaling molecules that influence Cajal-Retzius cell development include BDNF and Transforming Growth Factor β (TGFβ) (Ringstedt et al., 1998; Siegenthaler and Miller, 2008). BDNF expression is detected at low levels during embryonic development, starting as early as E13.5, and signals through its receptor TrkB, which is expressed in Cajal-Retzius cells (Friedman et al., 1991; Timmusk et al., 1994; Marty et al., 1996; Brunstrom et al., 1997). BDNF treatment *in vitro* downregulates Reelin expression, while BDNF overexpression *in vivo* reduces Reelin expression within Cajal-Retzius cells and causes Cajal-Retzius cells with abnormal morphology to cluster within the marginal zone (Ringstedt et al., 1998). Similarly, BDNF loss increases Reelin expression within Cajal-Retzius cells (Ringstedt et al., 1998). In addition, TGFβ signals through p21 and Foxo3a to promote the production of Cajal-Retzius cells (Siegenthaler and Miller, 2008). Together, these studies demonstrate that extracellular and intracellular cues influence the generation of Cajal-Retzius cells.

A total disruption in miRNA biogenesis through the loss of Dicer increases Cajal-Retzius cell abundance, indicating that miRNAs are an essential regulator of Cajal-Retzius cell development (McLoughlin et al., 2012). miRNAs are short single-stranded RNAs produced from double-stranded RNA folded into a hairpin loop. This hairpin is cleaved from the remaining RNA molecule by Drosha and Pasha to form pre-miRNA, then further cleaved by the enzyme Dicer to eventually form mature miRNA (O'Brien et al., 2018). miRNAs disrupt mRNAs by binding and cleaving them, destabilizing them, or hindering transcription (Huntzinger and Izaurralde, 2011; O'Brien et al., 2018). Specifically, miRNA-9 indirectly regulates Cajal-Retzius cell development by targeting Foxg1, which, as previously discussed, suppresses Cajal-Retzius cell differentiation by regulating cortical hem development (Shibata et al., 2008). miRNA-200c, however, has been demonstrated to directly target *Reln* mRNA following ischemic stroke (Stary Creed M. et al., 2015), and miRNA-128 targets *Reln* in neuroblastoma cells (Evangelisti et al., 2009); however, it remains unclear if these miRNAs affect Cajal-Retzius cell development in the embryonic brain.

1.2.3.2 Cajal-Retzius Cell Apoptosis

Cajal-Retzius cells are programmed to undergo apoptosis within the first two weeks of postnatal life, as Cajal-Retzius cells are not present in the adult brain (del Río et al., 1995). This process is not well understood, but evidence shows Cajal-Retzius cell death is mediated partially through changes in neuronal activity. In cell culture experiments, inhibition of neuronal activity using tetrodotoxin prevents Cajal-Retzius cell death (Blanquie et al., 2017). Similarly, *in vitro* inhibition of glutamate receptors, which mediate excitatory neuronal signaling, has a similar effect, while activating glutamate or GABA receptors causes Cajal-Retzius cells to disappear *in vivo* (Blanquie et al., 2017). Cell death through GABA receptors occurs through an NKCC1-dependent process, whereby blockade of the chloride transporter NKCC1 rescues Cajal-Retzius cells from apoptosis (Blanquie et al., 2017). Additionally, hyperpolarization prevents the death of septal-derived Cajal-Retzius cells (Riva et al., 2019).

P73, whose TAp73 and ΔNp73 isoforms promote neuronal survival (Miller, 2016), also prevents Cajal-Retzius cell apoptosis. Accordingly, deletion of P73 or ΔNp73 in mice reduces Cajal-Retzius cell abundance significantly (Meyer et al., 2004; Tissir et al., 2009). Furthermore, programmed cell death of septal-derived Cajal-Retzius cells is regulated via the proapoptotic protein Bax (Ledonne et al., 2016). Accordingly, conditional knockout of Bax in ΔNp73-expressing septal-derived Cajal-Retzius cells significantly increases the abundance of Cajal-Retzius cells at P24, long after Cajal-Retzius cell death occurs in wild-type mice (Ledonne et al., 2016). In contrast, conditional knockout of Bax from Wnt3a-expressing cortical hem-derived Cajal-Retzius cells does not affect Cajal-Retzius cell survival, indicating normal apoptosis (Ledonne et al., 2016). These findings suggest that Cajal-Retzius cell origin dictates its mechanism of programmed cell death. Interestingly, no changes in caspase 3 expression have been detected in Cajal-Retzius during apoptosis (Blanquie et al., 2017), nor is hippocampal Cajal-Retzius cell degeneration dependent upon caspase 3 activation (Anstötz et al., 2016), indicating that Cajal-Retzius cell apoptosis occurs mainly through caspase 3-independent mechanisms.

1.2.3.3 Roles of Reelin Signaling in Brain Development

Reelin signals predominantly by bindings to its receptors Very Low Density Lipoprotein Receptor (VLDLR) and ApoE Receptor Type 2 (ApoER2), stimulating phosphorylation of the adapter protein DAB Adapter Protein 1 (Dab1) and resulting in the initiation of corresponding intracellular kinase cascades, including Crk/C3G, PI3K/Akt, and Src family kinases, each of which has different targets (D'Arcangelo et al., 1999; Hiesberger et al., 1999; Howell et al., 1999; Lambert de Rouvroit et al., 1999; Bock et al., 2003; Huang et al., 2004). Accordingly, mice deficient in VLDLR and ApoER2, Dab1, or Src family kinases have phenotypes similar to *reeler* (Sweet et al., 1996; Sheldon et al., 1997; Trommsdorff et al., 1999; Howell et al., 2000; Kuo et al., 2005). Functions of Reelin include regulation of migration, cell adhesion, and dendrite development (Niu et al., 2004; Hirota and Nakajima, 2017). Accordingly, changes in Reelin expression are associated with numerous psychiatric and neurodevelopmental diseases, including schizophrenia, autism, and depression (Ovadia and Shifman, 2011; Ishii et al., 2016). Further details regarding each of Reelin's functions in the developing brain will be discussed below.

1.2.3.3.1 Migration

As previously discussed, newborn neurons migrate independently of radial glia or dependent upon radial glia (Nadarajah et al., 2001). Studies have found that Reelin has roles in regulating both modes of migration during development. One significant indication that Reelin is required for somal translocation is that preplate splitting is impaired in *reeler* mice. As previously discussed, preplate splitting occurs very early in development, when most neurons migrate via somal translocation (Nadarajah et al., 2001). Instead of a clear division between the marginal zone and subplate in *reeler* mice, the subplate and marginal zone cells are mixed and disorganized (Sheppard and Pearlman, 1997). During somal translocation, Reelin stabilizes the actin cytoskeleton through PI3-Kinase (PI3K)-mediated phosphorylation of N-cofilin after LIM Kinase (LIMK) activation (Chai et al., 2009). N-cofilin phosphorylation prevents the protein from depolymerizing F-actin, preventing actin's disassembly and stabilizing the neuron's leading process (Chai et al., 2009). Additionally, Reelin signaling through Dab1 and Rap1 activates integrin $\alpha5\beta1$, promoting neuronal processes' binding to fibronectin localized to the marginal zone (Sekine et al., 2012). Finally, Reelin stabilizes the leading process of migrating neurons through Rap1 regulation of the cell adhesion molecule N-cadherin, similarly promoting the attachment of leading processes of migrating neurons (Franco et al., 2011). Taken together, these studies suggest that Reelin aids in stabilizing and anchoring leading neuronal processes to the marginal zone, which is a crucial step during somal translocation.

There is also evidence that Reelin regulates aspects of glial-dependent migration prior to locomotion onset. Glial-dependent migration is the primary form of neuronal migration from the subventricular and intermediate zones of the developing brain (Nadarajah and Parnavelas, 2002), and evidence from *reeler* mice suggests that migration through these areas is altered (Hamburgh, 1963). Indeed, studies show a low level of Reelin expression within the intermediate zone of the brain (Yoshida et al., 2006). It remains unclear, however, how this Reelin pool specifically contributes to neuron migration. Changes in neuronal migration may be due to regulation of N-cadherin's cell surface expression by Rap1 via Reelin (Franco et al., 2011), as N-cadherin is important for glial-dependent neuronal migration (Shikanai et al., 2011; Gärtner et al., 2012). Furthermore, overexpression of LIMK or N-cofilin within the brain's intermediate zone partially rescues migration defects in *reeler* mice (Chai et al., 2016), suggesting that Reelin-induced N-cofilin phosphorylation partially contributes to radial glial-dependent migration in addition to somal translocation.

1.2.3.3.2 Adhesion

Reelin modulates the function of cell adhesion molecules to promote interactions between neurons and Cajal-Retzius cells. For example, Reelin facilitates interactions between neurons and Cajal-Retzius cells through Nectins and N-cadherin (Gil-Sanz et al., 2013). Other studies have shown that Reelin transiently strengthens adhesive bonds between N-cadherin and neurons such that Reelin overexpression causes neuronal clustering (Matsunaga et al., 2017). Further studies demonstrated that this effect is dependent upon Dab1 and ApoER2, but not VLDLR (Matsunaga et al., 2017; Hirota et al., 2018, 2; Hirota and Nakajima, 2020). This functionality may regulate the aggregation of neurons at the primitive cortical zone, which is localized at the top of the cortical plate and regulates layer formation in the mature cortex (Ajioka and Nakajima, 2005). However, the significance of neuronal clustering within this area for normal brain development remains unclear. Reelin also cleaves the cell adhesion molecule L1, promoting neurite outgrowth and neuronal migration independently of canonical signaling through its receptors or Dab1 (Lutz et al., 2017). Accordingly, L1 loss causes abnormalities in neuronal migration partially consistent with *reeler* mice, including abnormal neuronal orientation and misplaced neurons (Lutz et al., 2017). These experiments demonstrate the importance of Reelin-regulated adhesion as an additional mechanism by which it controls neuronal migration.

1.2.3.3.3 Dendrite morphogenesis

In addition to regulation of neuronal migration and aggregation, Reelin influences dendrite development and morphology. Reelin appears to be particularly important in regulating dendrite morphology within the pyramidal neurons of the hippocampus, both during development and postnatally (Niu et al., 2004, 2008). Similar to its regulation of migration, this process depends upon Dab1 signaling (Niu et al., 2004). Therefore, in the absence of Reelin, hippocampal neurons have impaired dendrite development (Stanfield et al., 1979; Niu et al., 2008), while Reelin haploinsufficiency causes a relatively mild decrease in dendritic spine density within the motor prefrontal cortex (Liu et al., 2001). These experiments suggest that Reelin also regulates synapse formation and neurotransmission in the adult brain by modulating dendrite development and morphology.

1.2.3.4 Reelin-independent functions of Cajal-Retzius cells

Reelin is specifically and highly expressed in Cajal-Retzius cells, so it can be challenging to identify the specific functions of Cajal-Retzius cells independently of Reelin expression. One such study to address this divide utilized a transgenic P73 knockout mouse. As discussed previously, P73 is expressed in Cajal-Retzius cells and promotes survival (Tissir et al., 2009). Loss of P73, therefore, causes a severe decrease in the abundance of Reelin-expressing Cajal-Retzius cells yet maintains a low level of Reelin expression within the marginal zone (Meyer et al., 2004). Despite the near-total loss of Cajal-Retzius cells, P73 knockout mice have relatively minor defects in cortical development (Meyer et al., 2004). Cortical lamination is mostly normal despite a reduced cortical thickness, which is more prevalent in the posterior cortex. The preplate also split into the marginal zone and subplate at early development stages without defects (Meyer et al., 2004). Similarly, ablation of cortical-hem-derived Cajal-Retzius cells does not result in large-scale disruptions in cortical layering despite a near-complete loss of Reelin in the marginal zone (Yoshida et al., 2006). These results suggest that 1) Reelin is responsible for a majority of the cortical development defects associated with Cajal-Retzius cell loss and 2) low levels of Reelin expression or compensation in later-appearing Reelin-expressing interneurons is sufficient for relatively normal development.

In a separate study that wished to differentiate the effects of Reelin on migration independently of abnormal preplate formation, researchers pharmacologically ablated Cajal-Retzius cells at birth (Supèr et al., 2000). They identified that ablation at birth impaired the migration of later-born neurons, reduced the number of radial glial cells, and increased astrocyte abundance (Supèr et al., 2000). These results suggest that Cajal-Retzius cells protect the radial glial cell identity and prevent premature differentiation into astrocytes. They also demonstrate that disruption of preplate formation is not solely responsible for neuronal migration deficits present with loss of Reelin (Supèr et al., 2000). However, this study does not desegregate Reelin signaling from Cajal-Retzius cells, so it is not known how many of these findings are attributed to the loss of Reelin signaling.

1.2.4 The Extracellular Matrix

The brain's extracellular matrix comprises glycoproteins, such as chondroitin and heparin Sulfate proteoglycans, laminins, tenascins, and Reelin (Barros et al., 2011; Franco and Müller, 2011). This network of extracellular cues is critical for neuronal migration, synapse formation, and myelination (Franco and Müller, 2011). Laminins are expressed within the brain's basal lamina at the pial surface and within the ventricular zone. They maintain radial glial cell orientation and function (Ferent et al., 2020). Heparin and chondroitin sulfate proteoglycans include Perlecan, Glypican, Phosphacan, Versican, and Neurocan (Maeda, 2015). Finally, the glycoproteins Tenascin-C, Tenascin-R, and Reelin are critical to extracellular matrix composition and function (Barros et al., 2011; Franco and Müller, 2011).

1.2.4.1 Extracellular Matrix Regulation of Migration

The major proteins that comprise the basal lamina at the brain's pial surface are the laminins, which are crucial to maintaining radial glial cell attachment and survival (Timpl et al., 1979; Radakovits et al., 2009). Studies demonstrate that removing the basal lamina severely disrupts brain lamination, indicating its necessity for proper neuronal migration (Sievers et al., 1994; Radakovits et al., 2009). Similarly, mutations in or loss of laminins or their integrin receptors cause defects in cortical layering (Smyth et al., 1999; Graus-Porta et al., 2001; Halfter et al., 2002; Chen et al., 2009). These defects are associated with the detachment of radial glial endfeet from the basal lamina (Halfter et al., 2002; Chen et al., 2009). The specific deletion of integrin from migrating neurons does not cause severe changes in lamination, suggesting that disruptions in migration after integrin loss are most likely due to radial glial cell endfeet detachment rather than a direct effect on migrating neurons (Graus-Porta et al., 2001). Laminins are also expressed within the brain's ventricular region, promoting neural stem cell expansion and differentiation. Similar to loss of integrin within the basal lamina, disruption in integrin function within the ventricular zone causes detachment of radial glial cell apical processes (Loulier et al., 2009).

Proteoglycans make up a large portion of the brain's extracellular matrix (Maeda, 2015). Similar to laminin and integrin, disruption of specific heparin sulfate proteoglycans or heparin sulfate biosynthesis overall result in basal lamina disruptions with microcephaly, altered neurogenesis, and minor lamination defects (Inatani et al., 2003; Haubst et al., 2006; Girós et al., 2007; Jen et al., 2009). In addition, evidence suggests that chondroitin sulfate proteoglycans

(CSPGs) regulate aspects of neuronal migration, as depletion of sulfotransferases that generate the sulfate groups on CSPGs disrupts the multipolar-to-bipolar transition of migrating neurons (Ishii and Maeda, 2008). These findings indicate that sulfate groups on CSPGs may be binding sites for trophic factors that guide migrating neurons. Likewise, there is evidence that CSPGs located in the striatum regulate tangential interneuron migration through binding to the repulsive cue Semaphorin 3A (Sema3A), suggesting that CSPGs bind and retain Sema3A in the striatum to repel tangentially migrating neurons away from this area of the brain (Zimmer et al., 2010). In addition to Sema3A, CSPGs bind many attractive and repulsive guidance cues, including Cxcl12, neurotrophins NT4, NT3, BDNF, and NGF, Slit2, Netrin, and multiple Ephrins (Mbemba et al., 2000; Shipp and Hsieh-Wilson, 2007; Rogers et al., 2011; Mizumoto et al., 2013; Maeda, 2015), suggesting CSPGs may additionally regulate tangential migration through binding with these molecules. However, these studies were completed *in vitro* using biochemical assays, so it remains to be seen whether these interactions occur in the developing brain.

Glycoprotein tenascin family members Tenascin-C (TNC) and Tenascin-R (TNR) are highly expressed in the developing cortex (Garcion et al., 2004; Ayachi et al., 2011). TNC is predominantly expressed in radial glial cells and is thought to regulate radial glial cell differentiation, as TNC-deficient mice have a reduced number of RC2-expressing radial glial cells (Garcion et al., 2004). Likewise, a separate study found that TNC knockout mice have an increased density of neurons and astrocytes, suggesting enhanced neurogenesis and gliogenesis (Irintchev et al., 2005). This study also found that TNC loss causes a reduced density of Parvalbumin-expressing interneurons, reduced percentage of oligodendrocytes, and alterations in dendritic spine morphology (Irintchev et al., 2005), indicating TNC has a broad range of effects on brain development. Despite all of these changes in brain composition, current evidence does not support a role for TNC in modulating neuronal migration. TNR, however, has been specifically demonstrated to inhibit the migration of neurons derived from neural progenitor cells *in vitro* (Huang et al., 2009); however, its effects on migration *in vivo* remain unclear.

1.2.5 Human Cortical Development

Human and primate cortical development differ from rodent development in several crucial ways. One of the main distinctions between human and rodent brains is the additional gyrification in primates. It has been hypothesized that gyrification evolved to increase the brain's surface area and the number of neurons without drastically increasing skull size (Zilles et al., 2013). Accordingly, human brains have significantly more neurons than rodent brains (Azevedo et al., 2009). There are a couple of different ways that the neuronal pool is expanded in humans. First, neurogenesis onset is delayed in humans relative to rodents (Rakic, 1995; Kornack and Rakic, 1998). As previously discussed, the earliest precursors of radial glial cells, neuroepithelial cells, undergo rounds of cell division to expand the cortex during the early stages of brain development (Rakic, 1995). In the human brain, delayed neurogenesis allows neuroepithelial cells to undergo many more divisions, producing more progenitor and radial glial cells than are present in rodents (Rakic, 1995; Kornack and Rakic, 1998).

Additionally, the neurogenic period is expanded ten-fold in primates, further increasing the progenitor pool that produces neurons (Caviness et al., 1995; Rakic, 1995). Moreover, while rodent IPCs generally undergo one or zero rounds of symmetric cell division before terminal differentiation, human IPCs undergo multiple rounds of division, drastically increasing the progenitor pool and the number of neurons that can be produced (Fietz et al., 2010; Hansen et al., 2010; Betizeau et al., 2013). Finally, the expanded radial size of the human cortex correlates with an additional progenitor cell type localized closer to the apical surface of the brain. These cells are known as outer radial glia; their endfeet attach to the pial surface of the brain, while their soma lies outside of the ventricular zone in a new layer called the outer subventricular zone (Smart et al., 2002; Lukaszewicz et al., 2005; Howard et al., 2006; Fietz et al., 2010; Betizeau et al., 2013). These cells make up the vast majority of progenitor cells in the human cortex, with four times as many cells as present in the ventricular and inner subventricular zones (Smart et al., 2002). Thus, although the rodent is a suitable model system to study aspects of brain development, human brain development differs in its complexity and scale.

1.3 NRG1/ErbB4 Signaling

As previously discussed, NRG1 signaling through ErbB4 is a crucial regulator of brain development. Studies from the Corfas lab and others show that these molecules are critically important to regulate astrogenesis, radial neuron migration, interneuron migration, and myelination in the developing brain (Rio et al., 1997; Flames et al., 2004; Sardi et al., 2006; Neddens and Buonanno, 2010; Makinodan et al., 2012). However, the specifics of ErbB4 intracellular domain (E4ICD)-mediated regulation of astrogenesis remain to be identified.

1.3.1 ErbB/EGFR Family of Receptor Tyrosine Kinases

ErbB4 is a member of the ErbB/EGFR family of receptor tyrosine kinases, which are receptors that contain extracellular, transmembrane, and intracellular domains that have intrinsic tyrosine kinase activity (Plowman et al., 1993a). Additional family members include EGFR/ErbB1, ErbB2, and ErbB3. These receptors bind growth factors such as Neuregulins (NRG), Epidermal Growth Factor (EGF), Epiregulin, Amphiregulin, and others. ErbB4 specifically binds NRG1, NRG2, NRG3, NRG4, and NRG5 as well as β-cellulin (BTC), Epiregulin, and Heparin-binding EGF-like Growth Factor (HB-EGF) (Mei and Nave, 2014).

Ligand binding stimulates receptor phosphorylation and downstream kinase cascades (Plowman et al., 1993a, 1993b).

Typically, receptor tyrosine kinases dimerize to function. Interestingly, the EGFR family of receptors can heterodimerize or homodimerize; however, some homodimers are not functionally active (Guy et al., 1994; Tzahar et al., 1996). For example, EGFR and ErbB2 lack a ligand-binding domain, whereas ErbB3 lacks a tyrosine phosphorylation domain (Guy et al., 1994; Tzahar et al., 1996). Therefore, homodimers of these receptors cannot bind ligands or are catalytically inactive (Guy et al., 1994). ErbB4, on the other hand, has a ligand-binding domain and a phosphorylation site, which means it is the only EGFR family member that can homodimerize, bind ligands, and phosphorylate itself (Plowman et al., 1993b, 1993a).

1.3.2 ErbB4 Structure and Signaling

ErbB4 has multiple alternative splice forms that vary in the juxtamembrane (JM) domain and the intracellular cytoplasmic (CYT) domain, with two primary alternative splice forms for each (Figure 1.2). Thus, alternative splicing of ErbB4 predominantly generates four different proteins (Mei and Nave, 2014). CYT-1 is produced by the inclusion of exon 26, whereas CYT-2 is generated upon its exclusion (Sawyer et al., 1998). Furthermore, the PI3K binding domain localizes within exon 26, so only ErbB4-CYT-1 can activate PI3K (Sawyer et al., 1998). The two primary JM splice forms are JMa and JMb, which either include exon 16 or exon 15, respectively (Elenius et al., 1997). There is also some evidence of ErbB4-JMc and JMd isoforms, which exclude or include both exons, respectively (Zeng et al., 2009). However, these isoforms are not well characterized or studied. Significantly, ErbB4-JMa and ErbB4-JMb differ in their potential signaling mechanisms, which are discussed further below (Elenius et al., 1997).

1.3.2.1 ErbB4 Canonical Signaling

As is the case for other receptor tyrosine kinases, ligand binding to ErbB4 causes receptor dimerization, tyrosine autophosphorylation of the receptor intracellular domain, and activation of intracellular signaling pathways through adapter proteins. These signaling pathways include Raf/MEK/ERK, PI3K/Akt/S6K, and activation of Src family kinases, which stimulate transcription factors that modulate cell function (Yarden and Sliwkowski, 2001; Mei and Nave, 2014). Specific functions associated with these signaling pathways are discussed in later sections.

1.3.2.2 ErbB4 Non-Canonical Intracellular Domain Signaling

In addition to canonical signaling, ErbB4 functions "non-canonically" via ErbB4-JMa. Exon 16, only expressed in ErbB4-JMa, contains a cleavage site for tumor necrosis factor α-converting enzyme (TACE) (Elenius et al., 1997; Rio et al., 2000). As with canonical signaling, NRG1 binding to ErbB4-JMa stimulates receptor dimerization and autophosphorylation. However, instead of adapter protein stimulation of intracellular kinase cascades, ErbB4 is cleaved by TACE within the JM domain (Rio et al., 2000), followed by γ-secretase in the transmembrane domain (Lee et al., 2002). These cleavage events release the soluble ErbB4 intracellular domain (E4ICD), which translocates to the cell's nucleus to directly influence transcription (Lee et al., 2002; Sardi et al., 2006).

1.3.3 Roles in Brain Development

NRG1/ErbB4 signaling regulates various aspects of brain development, including differentiation, migration, synapse formation, and myelination (Mei and Xiong, 2008; Mei and Nave, 2014; Kataria et al., 2019). During development, ErbB4 is expressed in progenitor cells within the subventricular zone and the MGE (Rio et al., 1997; Flames et al., 2004; Fox and

Kornblum, 2005). In the adult brain, ErbB4 is expressed in inhibitory somatostatin and parvalbumin-expressing neurons, where it localizes to the cell's postsynaptic synapse, binding to postsynaptic density protein 95 (PSD-95) (Huang et al., 2000; Vullhorst et al., 2009; Fazzari et al., 2010).

1.3.3.1 Transcriptional Regulation of Gliogenesis

One of the functions associated with E4ICD nuclear signaling is repression of astrogenesis, identified previously by the Corfas lab. Upon NRG1 binding to ErbB4-JMa in neural progenitor cells, ErbB4 is phosphorylated and cleaved, releasing E4ICD as described above (Elenius et al., 1997). Our lab further demonstrated that E4ICD binds with the adapter protein TAB2 and the co-repressor N-CoR and shuttles to the nucleus to repress glial gene transcription through direct promoter binding (Sardi et al., 2006). Furthermore, this process is dependent upon ErbB4-JMa cleavage and tyrosine phosphorylation. Expression of ErbB4-JMb, inhibition of ErbB4-JMa cleavage, or expression of a kinase-dead form of JMa inhibit complex formation and promoter binding (Sardi et al., 2006). This function of ErbB4-JMa is hypothesized to repress astrogenesis while neurogenesis is ongoing during the early stages of brain development. Accordingly, ErbB4 KO mice have increased GFAP and S100b protein expression at E17.5, the age of astrogenesis onset in rodents (Sardi et al., 2006).

1.3.3.2 Neuron Migration

In addition to regulation of differentiation, NRG1/ErbB4 signaling regulates both radial migration of immature excitatory neurons and tangential migration of interneurons from the ganglionic eminences to the dorsal telencephalon (Rio et al., 1997; Flames et al., 2004).

1.3.3.2.1 Radial Migration

NRG1 is secreted by neurons and promotes radial glial cell formation and process extension (Anton et al., 1997; Schmid et al., 2003). NRG1 also stimulates the migration of neurons along radial glia fibers, such that *in vitro* NRG1 treatment of migrating neurons attached to radial glial fibers causes a dose-dependent increase in their migration speed (Anton et al., 1997). However, NRG1 regulation of radial glia development and neuron migration in the cortex is mediated through ErbB2, which is expressed in radial glial cells, rather than ErbB4 (Schmid et al., 2003). ErbB4 instead is important in cerebellar development. Like in the developing cortex, newborn cerebellar granule neurons migrate along Bergmann glial fibers. Inhibition of ErbB4 in cerebellar glial cells impairs migration of NRG1-expressing granule cells *in vitro* (Rio et al., 1997). However, despite NRG1's alleged role in cell migration *in vitro*, *in vivo* evidence has failed to recapitulate this significance. First, conditional deletion of NRG1 from neuronal precursors does not result in apparent changes in cortical lamination (Brinkmann et al., 2008). Furthermore, loss of ErbB2, ErbB4, or both ErbB2 and ErbB4 does not cause significant changes in cortical or cerebellar structures (Barros et al., 2009). These findings underlie potential compensatory mechanisms *in vivo*, indicating that redundancies in signaling mechanisms that regulate migration may make NRG1 dispensable for radial neuron migration.

1.3.3.2.2 Tangential Migration

In contrast to radial migration, the role of NRG1/ErbB4 signaling in regulating tangential interneuron migration is much more substantiated. Studies show that NRG1 is secreted from neurons in the dorsal telencephalon, while ErbB4 is expressed in migrating interneurons (Flames et al., 2004). The mechanism of migration, however, has been debated. In one study, researchers found through *in vitro* and *in vivo* experiments that migrating interneurons are attracted by soluble NRG1 and membrane-bound NRG1. They hypothesize that membrane-bound NRG1 expressed in lateral ganglionic eminence (LGE) neurons serves as a short-term cue to permit migrating neurons to travel from the MGE and through the LGE, while soluble NRG1 that is secreted from neurons in the dorsal telencephalon functions as an attractive long-range signal (Flames et al., 2004). In contrast, a separate study found that NRG1 acts as a repulsive cue to funnel inhibitory neurons through the cortex to their final destinations (Li et al., 2012). *In vitro*, NRG1 repelled neurons derived from the MGE. *In vivo*, in utero electroporation of NRG1-expressing plasmids repelled and even blocked migrating interneurons from reaching the dorsal telencephalon (Li et al., 2012). In any case, loss of ErbB4 or NRG1 reduces the number of inhibitory interneurons present in the adult cortex, indicating a defect in interneuron migration or survival (Flames et al., 2004; Neddens and Buonanno, 2010; Li et al., 2012).

Interestingly, NRG1 signaling through ErbB4 also promotes the tangential migration of the second wave (arising around E16.5) of ErbB4-expressing OPCs that colonize the optic nerve (Ortega et al., 2012). NRG1 treatment stimulates OPCs to extend extra and lengthier processes, which may be altering their migration. These findings correspond with impaired migration of OPCs in ErbB4 KO mice (Ortega et al., 2012).

1.3.3.3 Synapse Formation

ErbB4 localizes to the postsynaptic density of neurons at the subcellular level, indicating that it may regulate synapse formation or transmission (Huang et al., 2000; Krivosheya et al., 2008; Fazzari et al., 2010). Indeed, through its interaction with and potential stabilization of PSD-95, it contributes to the formation and maturation of excitatory synapses on interneurons (Abe et al., 2011; Ting et al., 2011; del Pino et al., 2013). Accordingly, NRG1 increases the intensity and frequency of miniature excitatory postsynaptic potentials in brain slices from juvenile mice and interneurons *in vitro* (Abe et al., 2011; Ting et al., 2011). Furthermore, overexpression of ErbB4 increases the intensity of proteins that localize to excitatory axon terminals, while loss of ErbB4 reduces miniature excitatory postsynaptic potential frequency and the density of glutamatergic axon terminals in inhibitory interneurons (Krivosheya et al., 2008; Ting et al., 2011). Together, these experiments demonstrate the necessity of NRG1 signaling through ErbB4 in excitatory synapse formation on ErbB4-expressing GABAergic interneurons.

Evidence suggests that NRG1/ErbB4-mediated regulation of synaptogenesis is mediated partially through schizophrenia-associated protein Disrupted in schizophrenia 1 (DISC1) binding to ErbB4 in inhibitory neurons. Researchers found that a NRG1-induced increase in glutamatergic synapse formation onto inhibitory neurons is blocked by DISC1 loss (Unda et al., 2016). Furthermore, NRG1 induces DISC1 expression and localization to glutamatergic synapses, where it binds to ErbB4 (Unda et al., 2016). In contrast to excitatory-inhibitory neuron synapses, NRG1/ErbB4 signaling does not appear to have an essential role in synapses between interneurons (Yang et al., 2013).

1.3.3.4 Axon and Dendrite Development

NRG1 treatment has been shown to promote neurite outgrowth, dendritic arborization, axon elongation, and branching of processes in ErbB4-expressing neurons (Krivosheya et al., 2008; Fazzari et al., 2010). Accordingly, ErbB4 knockdown *in vitro* decreases the number of primary neurites of inhibitory neurons. This process appears to be mediated through PI3K phosphorylation, which is a common mechanism of canonical ErbB4 signaling (Krivosheya et al., 2008). In addition to dendritic outgrowth and branching, NRG1 regulates dendritic spine formation. Accordingly, double knockout of ErbB2 and ErbB4 decreases dendritic spine density in neurons derived from the cortex and hippocampus (Barros et al., 2009), and single knockdown of ErbB4 reduces dendritic spine formation (Li et al., 2007). Likewise, the specific loss of ErbB4 from parvalbumin-expressing interneurons decreases neuronal dendritic spine density (del Pino et al., 2013; Yin et al., 2013).

1.3.3.5 Central Nervous System Myelination

One of the final stages of brain development is myelination, whereby oligodendrocytes mature and myelinate axons in the central nervous system. NRG1/ErbB4 signaling promotes the maturation of OPCs, which may be facilitated through E4ICD nuclear signaling (Lai and Feng, 2004; Ortega et al., 2012). In the central nervous system, NRG1 regulates myelination during an early postnatal critical period (Makinodan et al., 2012). In this previous study out of the Corfas lab, mouse social isolation immediately after weaning reduces NRG1 expression and causes hypomyelination. These phenotypes are recapitulated in mice that lack ErbB3 within oligodendrocytes, indicating that NRG1 may be acting through ErbB3 to facilitate this process (Makinodan et al., 2012). In addition, mice with blocked ErbB4 signaling in oligodendrocytes have cortical hypomyelination, altered oligodendrocyte morphology, and decreased conduction velocity, suggesting that ErbB4 function is critical for normal oligodendrocyte development and myelination (Roy et al., 2007). However, other studies of overt loss of NRG1, ErbB3, or ErbB4 in mice show no impairments in myelination *in vivo* (Brinkmann et al., 2008; Barros et al., 2009), while a significant overexpression of NRG1 appears to cause hypermyelination (Brinkmann et al., 2008). These studies show that while NRG1 influences myelination, the role of NRG1 and ErbB4 in central nervous system myelination is context-dependent and may be compensated for after overt loss.

1.3.4 Associations with Neurological and Psychiatric Diseases

Many studies demonstrate that altered NRG1 and ErbB4 expression are associated with schizophrenia (Roy and Corfas, 2008). Multiple single nucleotide polymorphisms (SNPs) have been found in the *NRG1* gene, mostly intronic, within the noncoding regions, and a few exonic (Mei and Nave, 2014). Phenotypically, these SNPs are associated with reduced white matter and gray matter or increased ventricle volume in schizophrenia patients, possibly indicating that NRG1-induced myelination is disrupted (Mata et al., 2010; Barnes et al., 2012; Cannon et al., 2012). SNPs in the *NRG1* gene are associated with either increased or decreased NRG1 expression, suggesting downregulation or upregulation can contribute to schizophrenia (Mei and Nave, 2014). In addition, there are many *ErbB4* SNPs associated with schizophrenia, mostly in intronic regions of the gene. In general, ErbB4 expression is upregulated in schizophrenia patients, predominantly ErbB4-JMa, implicating the potential effect of proteolytic ErbB4 cleavage in contributing to schizophrenia etiology (Silberberg et al., 2006; Law et al., 2007; Joshi et al., 2014; Mei and Nave, 2014; Chung et al., 2016). Furthermore, behavioral studies of NRG1 and ErbB4 knockout transgenic mice indicate that they have deficits associated with schizophrenia, including hyperactivity and impaired social behavior (Golub et al., 2004; O'Tuathaigh et al., 2007). In addition to schizophrenia, SNPs in *NRG1* have been identified in patients with bipolar disorder, major depressive disorder, and Hirschsprung's disease, while SNPs in *ErbB4* have been associated with bipolar disorder (Mei and Nave, 2014).

Altered expression of NRG1 and ErbB4 have also been found in human studies of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and Amyotrophic lateral sclerosis (ALS) (Chaudhury et al., 2003; Woo et al., 2010; Depboylu et al., 2012; Takahashi et al., 2019; Sun et al., 2020). For example, in Alzheimer's disease patients, NRG1 and ErbB4 expression co-localize with neuritic plaques and apoptotic hippocampal neurons, while NRG1 levels are elevated in the patient's cerebrospinal fluid (Chaudhury et al., 2003; Woo et al., 2010; Mouton-Liger et al., 2020). Accordingly, mouse models of Alzheimer's disease show increased ErbB4 expression within the cortex and hippocampus and in neuritic plaques (Chaudhury et al., 2003; Woo et al., 2011, 4). Genetically, one study identified a *NRG1* SNP that is associated with psychosis in patients with late-onset Alzheimer's disease (Go et al., 2005); however, a more recent study found no association between *NRG1* SNPs and Alzheimer's-associated psychosis (Middle et al., 2010), disputing the influence of *NRG1* SNPs in Alzheimer's disease. Elevated ErbB4 expression has also been identified in dopaminergic midbrain neurons in human patients with Parkinson's disease. However, unlike in Alzheimer's disease, ErbB4 upregulation is hypothesized to be a protective mechanism (Depboylu et al., 2012).

1.4 Poly (ADP-Ribose) Polymerase 1 (PARP1)

Recently, our lab identified a direct interaction between the intracellular domain of ErbB4 (E4ICD) and Poly (ADP-ribose) Polymerase 1 (PARP1). PARP1 is a ubiquitously expressed enzyme with various functions within and outside of the nervous system and has the capability to directly bind DNA and regulate transcription (Kraus, 2008; Krishnakumar and Kraus, 2010b). This suggests that PARP1 may facilitate E4ICD's interaction with the *GFAP* promoter to repress astrogenesis. Additionally, a PARP1 mutation has been linked to Autosomal Recessive Intellectual Disability (ARID) in humans, and PARP1 knockout mice display neurological deficits (Najmabadi et al., 2011; Plane et al., 2012; Hong et al., 2019). Together, these studies suggest that PARP1 has roles in brain development.

1.4.1 PARP Family of Proteins

PARP1 is the first identified member of a family of 17 PARP proteins, also known as the ADP-ribosyl transferase (ART) family (Alkhatib et al., 1987; Cherney et al., 1987; Suzuki et al., 1987; Amé et al., 2004). These proteins are characterized by a signature “PARP” motif within their catalytic domain, which contains an acceptor site for adenosine and a donor site for nicotinamide (Amé et al., 2004; Krishnakumar and Kraus, 2010b). PARP1s 1-5 post-translationally modify proteins via poly-ADP-ribosylation (PARylation), whereas PARPs 6-8, 10-12, and 14-16 catalyze only mono-ADP-ribosylation (MARylation) reactions. PARP9 and PARP13 are missing nicotinamide binding residues within their catalytic domain, rendering them likely inactive (Krishnakumar and Kraus, 2010b). The different PARP family members also localize to various subcellular components. While PARP1, PARP2, and PARP3 are primarily nuclear, other PARPs are also found outside the cell’s nucleus, including within the cytoplasm and mitochondria (Krishnakumar and Kraus, 2010b). PARP1 is responsible for 90% of PARylation activity in response to DNA damage in cells, with additional events catalyzed by PARP2 (Shieh et al., 1998; D’Amours et al., 1999). Therefore, double knockout of PARP1 and PARP2 is embryonic lethal, and embryos die at the onset of gastrulation (Ménissier de Murcia et al., 2003). However, loss of PARP1 or PARP2 alone is not lethal in mice (Shieh et al., 1998; D’Amours et al., 1999; Shall and de Murcia, 2000; Ménissier de Murcia et al., 2003; Beck et al., 2014). This finding demonstrates the importance of PARP1 and PARP2 in regulating PARylation events and the significance of PARylation during early development, as the lack of both enzymes renders organisms non-viable.

1.4.2 PARP1 Protein Structure and Catalytic Activity

The PARP1 protein contains a DNA-binding domain which includes a nuclear localization sequence, an automodification domain with a BRCT motif to mediate protein-protein interactions, a WGR motif that may facilitate nucleic acid binding, and a catalytic domain, responsible for facilitating PARylation (D'Amours et al., 1999; Amé et al., 2004) (Figure 1.3). The DNA binding domain and nuclear localization sequences allow PARP1 to localize to the cell's nucleus and directly bind to DNA to facilitate various functions, including chromatin modifications to regulate transcription and DNA repair (Kraus, 2008; Ray Chaudhuri and Nussenzweig, 2017). The BRCT motif allows PARP1 to bind to other proteins to PARylate them, thus regulating their activity, and the auto-modification domain allows PARP1 to PARylate itself (D'Amours et al., 1999).

PARP1 catalyzes PARylation reactions using NAD^+ as a substrate to synthesize ADP-ribose polymers (PAR) on acceptor proteins (D'Amours et al., 1999) (Figure 1.4). PAR groups are large negatively charged polymers linked together via glycosidic ribose-ribose bonds in linear or branched chains (Chambon et al., 1966; Reeder et al., 1967). The process of PARylation is reversible through the enzymatic activity of poly (ADP-ribose) glycohydrolase (PARG) and ADP-ribosylhydrolase 3 (ARH3) which remove PAR groups from proteins (Figure 1.4) (Miwa et al., 1975; Ono et al., 2006). PARP1 auto-PARylation has been shown to inhibit its DNA binding and catalytic activity, which is one mechanism through which PARP1 might self-regulate its activity (Cervantes-Laurean et al., 1996; D'Amours et al., 1999; Kauppinen et al., 2005).

1.4.3 PARP1 Functions

The various domains that are included within the PARP1 protein confer a wide variety of functions. These include the regulation of DNA damage repair and transcription, processing and regulation of mRNAs and miRNAs, control of apoptosis and necrosis, and modulation of immune system functionality (Kraus, 2008; Krishnakumar and Kraus, 2010b; Beck et al., 2014; Ke et al., 2019). Each of these functions will be discussed further in the sections below.

1.4.3.1 DNA Damage Repair

The most widely studied and well-known function of PARP1 is as a facilitator of DNA damage repair, including base excision repair, single and double-strand break repair, and homologous recombination (Durkacz et al., 1980; Bouchard et al., 2003; Krishnakumar and Kraus, 2010b; Beck et al., 2014). PARP1 recognizes DNA single-strand and double-strand breaks, activates itself, and PARylates nucleosome proteins H1 and H2B at breakage sites (Gilbert de Murcia and Josiane Menissier de Murcia, 1994). These histone modifications cause the chromatin to unravel and relax, allowing it to be accessible by DNA repair proteins (Poirier et al., 1982; Kraus, 2008; Ray Chaudhuri and Nussenzweig, 2017). PARylation of chromatin also serves as a signal for DNA repair proteins to localize to the break site and repair the DNA. For example, for PARP1-mediated repair via repair protein XRCC1, PARP1 is necessary for XRCC1 to be recruited to the site of damaged DNA, where XRCC1 serves as a scaffold for other repair proteins such as DNA ligase 3 (Caldecott et al., 1996, 1; Masson et al., 1998; El-Khamisy et al., 2003). During DNA double-strand break repair, ataxia telangiectasia mutated (ATM) recruits repair proteins such as p53 and SMC1. ATM is PARylated by PARP1, which stimulates its activity (Murcia et al., 2001; Aguilar-Quesada et al., 2007). PARP1 also facilitates homologous recombination by recruiting meiotic recombination protein 11 (Mre11) and the breast cancer susceptibility protein BRCA1 to the DNA (Haince et al., 2008; Li and Yu, 2013; Hu et al., 2014). Mre11 is critical during the end resection process in homologous recombination

(Haince et al., 2008), and PARP1 regulation of BRCA1 has been hypothesized to fine-tune the homologous recombination process (Hu et al., 2014).

Given the importance of PARP1 in regulating various aspects of DNA damage repair, cells deficient in PARP1 display greater intensities of damage upon treatment with DNA damaging agents (Wang et al., 1995; de Murcia et al., 1997; Trucco et al., 1998; Masutani et al., 1999; Shall and de Murcia, 2000). Accordingly, mice lacking PARP1 are more susceptible to DNA damage due to genotoxic insults, exhibit spontaneous carcinogenesis, and age at an accelerated rate (Wang et al., 1995; de Murcia et al., 1997; Masutani et al., 1999; Piskunova et al., 2008). The process of DNA repair is critical for cancer cell replication; thus, PARP inhibitors are widely used in the treatment of various cancers (Dziadkowiec et al., 2016; Pommier et al., 2016; Rose et al., 2020; Wengner et al., 2020).

1.4.3.2 Transcriptional Regulation

PARP1 modulates transcription through several different mechanisms. For example, it can directly modify chromatin to facilitate transcription factor binding and gene activation, PARylate transcription factors or chromatin modifiers to activate or deactivate them, and bind to enhancer regions of the genome (Kraus, 2008).

1.4.3.2.1 Chromatin Binding

Given PARP1's ability to directly modify chromatin at the sites of DNA damage to facilitate DNA damage repair, it is unsurprising that chromatin modifications via PARP1 also regulate gene transcription (Ray Chaudhuri and Nussenzweig, 2017). Similar to other post-translational chromatin modifications, such as acetylation or methylation, histone PARylation modifies chromatin structure (Kim et al., 2004; Ray Chaudhuri and Nussenzweig, 2017). For example, PARylation of linker Histone H1 or PARP1 binding to the nucleosome causes Histone

H1’s eviction from the target gene’s promoter, which results in chromatin relaxation, allowing for transcription factors to bind to the gene promoter and promote transcription (Poirier et al., 1982; Huletsky et al., 1989; Kim et al., 2004). This is the mechanism by which PARP1 regulates transcription of *Doublecortin* (*Dcx*) in neuronal cells, specifically by PARP1 recruitment to its promoter via transcription factors PBX and MEIS (Hau et al., 2017). In contrast, PARP1 represses transcription by binding to nucleosomes and causing chromatin to compact. Interestingly, this function of PARP1 only occurs when the protein is unmodified in the absence of NAD^+ (Kim et al., 2004). PARP1 may also facilitate chromatin compaction through a chromatin-binding protein called DEK. PARP1 activation after the addition of NAD^+ causes PARP1 PARylation of DEK and the removal of PARP1 and DEK from chromatin, allowing transcription to occur (Gamble and Fisher, 2007).

1.4.3.2.2 Regulation of Chromatin Modifying Enzymes

In addition to directly modifying chromatin, PARP1 can PARylate chromatin-modifying proteins, such as enzymes that regulate DNA or histone methylation and acetylation (Kraus, 2008). The process of methylation and acetylation can either repress or activate transcription depending upon the specific nature and site of modification by opening or condensing chromatin to allow or prevent transcription factors from accessing promoter regions of genes (Rothbart and Strahl, 2014; Tessarz and Kouzarides, 2014; Barnes et al., 2019).

DNA methyltransferase 1 (DNMT1) is an enzyme that methylates cytosine residues on DNA, which prevents transcription. PARP1 binds to the promoter of the *Dnmt1* gene to protect it from silencing via DNA methylation, thus promoting transcription of the gene when PARP1 is activated (Zampieri et al., 2009). PARP1 PARylation of the DNMT1 protein, in contrast, inhibits DNMT1’s catalytic activity, thus preventing DNA methylation and promoting transcription

(Reale et al., 2005). PARP1 also interacts with Ten-eleven-translocation 1 (Tet1), which catalyzes the formation of hydroxymethyl groups on cytosine residues (Zhang et al., 2010; Ciccarone et al., 2015). These are epigenetic modifications associated with transcription factor binding sites (Zhang et al., 2010). Through Tet1 PARylation, PARP1 can increase or decrease its enzymatic activity and alter downstream functions associated with Tet1 (Ciccarone et al., 2015).

PARylation of a lysine methyltransferase, Ezh2, inhibits its activity and decreases its association with chromatin, preventing its methylation of lysine residues on histone H3 (H3K27me3), a repressive epigenetic modification (Caruso et al., 2018). Therefore, PARP1 activation promotes transcription via decreased H3K27me3. PARP1 also regulates H3K27me3 levels via repression of *Ezh2* gene transcription (Martin et al., 2015). In addition to methylases, PARP1 interacts with histone-associated lysine demethylases Kdm5b and Kdm4d (Krishnakumar and Kraus, 2010a; Le May et al., 2012). Kdm5b is a lysine-specific demethylase that regulates methylation levels of lysine four on histone H3 (H3K4me3), which are found at sites of active transcription (Tan et al., 2003; Sims and Reinberg, 2006; Christensen et al., 2007). PARylation of Kdm5b by PARP1 inhibits its recruitment to gene promoters, preventing it from silencing genes, which leads to increased transcription of Kdm5b-dependent genes (Krishnakumar and Kraus, 2010a). PARP1 also PARylates Kdm4d, which impairs its recruitment to promoters that are responsive to retinoic acid (RA). Loss of Kdm4d retains the methylated state of lysine residues on Histone H3 (H3K9me2), which is an epigenetic modification that represses transcription (Le May et al., 2012; Ninova et al., 2019). Consequently, co-localization of the enzyme that de-PARylates proteins (known as PARG) with Kdm4d at RA-responsive gene promoters restores demethylation and allows transcription to occur (Le May et al., 2012). Taken together, these studies indicate that PARP1 inhibition of different demethylases can either promote or repress transcription.

Another histone modification that controls the accessibility of chromatin is acetylation (Barnes et al., 2019). PARP1 interacts with the protein deacetylase known as Sirt1, which regulates the expression of genes that control cell differentiation, survival, and stress responses (Herskovits and Guarente, 2014; Cai et al., 2016). Experimental evidence demonstrates that Sirt1 and PARP1 have an antagonistic relationship, whereby Sirt1 knockout increases PARP1 activity while its activation decreases PARP1 activity (Kolthur-Seetharam et al., 2006; Rajamohan et al., 2009). This antagonism is hypothesized to be due to their competition for available NAD^+ to catalyze reactions. Additionally, activated Sirt1 also represses transcription of the *Parp1* gene through deacetylation to further suppress the activity of PARP1 (Rajamohan et al., 2009).

1.4.3.2.3 Modification of Transcription Factors

PARP1 modifies transcription factors that regulate gene expression in many cell types, including neurons. One such transcriptional repressor is MeCP2, a methyl binding protein that associates with corepressor complexes and directs their binding to methylated sites on DNA, compacting chromatin (Cheng and Qiu, 2014). Endogenous MeCP2 protein within the mouse brain is PARylated, which prevents it from binding to chromatin and inducing chromatin aggregation (Becker et al., 2016). Thus, in the absence of PARP1, MeCP2 binding to heterochromatin increases, and chromatin is more prone to aggregate (Becker et al., 2016). PARP1 also indirectly influences transcription factor Elk1 activity via Erk2 (Cohen-Armon et al., 2007). Erk2 phosphorylation stimulates its binding to and subsequent activation of PARP1. Activated PARP1 then increases Erk2-mediated phosphorylation of the transcription factor Elk1, promoting the expression of its target gene, *c-fos*, an immediate-early gene essential for neuronal function (Cohen-Armon et al., 2007).

PARP1 is also a binding partner of subunits of the transcription factor NF-ƙB, which regulates genes that contribute to immune functions in cells. Its interaction with subunits p50, p65, and p300 facilitates NF-ƙB-dependent gene expression (Hassa and Hottiger, 1999; Hassa et al., 2003). Thus, following immune system activation in PARP1 KO cells, the expression of genes that NF-ƙB control, including IL-6, are dysregulated (Hassa et al., 2003; Minotti et al., 2015). Examples of other transcription factors that PARP1 modifies include STAT3, SRY-Box Transcription Factor 2 (Sox2), Hes1, CCAAT Enhancer Binding Protein (C/EBP), and Liver X Receptors (LXRs) (Ju et al., 2004; Gao et al., 2009; Shrestha et al., 2016; Luo et al., 2017; Ding et al., 2019). Taken together, these studies demonstrate that PARP1 regulates transcription factors that alter pathways necessary for cell differentiation, survival, and immune function.

1.4.3.2.4 Direct Promoter or Enhancer Binding

Direct PARP1 binding to DNA within putative gene promoter or enhancer regions regulates the expression of the chemokine CXCL1 and the transcription factor BCL6 (Amiri et al., 2006; Ambrose et al., 2007). Inactive PARP1 binding to DNA upstream of the *CXCL1* promoter inhibits binding of its associated transcription factor, NF-ƙB (Amiri et al., 2006). Upon PARP1 activation and auto-PARylation, PARP1 becomes unbound from the promoter, allowing transcription of CXCL1 to occur. Therefore, PARP1 loss increases CXCL1 expression, while PARP1 inhibition decreases CXCL1 expression (Amiri et al., 2006). Additionally, chromatin immunoprecipitation assays demonstrate that PARP1 binds to a DNA sequence within the first intron of the *BCL6* gene that represses its expression. Therefore, PARP1 knockdown and inhibition increase the expression of BCL6 (Ambrose et al., 2007). Interestingly, PARP1 also binds to hairpin structures associated with its own promoter region to decrease its expression. In cells lacking PARP1, the *PARP1* promoter region is overactive, while the reintroduction of PARP1 into these cells downregulates its promoter activity (Soldatenkov et al., 2002).

1.4.3.3 mRNA Processing and Regulation

Recent evidence shows that PARP1 can post-transcriptionally modify mRNA expression by regulating mRNA stability, alternative splicing, and 3’ polyadenylation (Ke et al., 2019). For example, PARP1 loss in mouse fibroblast cells decreases the stability of the *IP-10* transcript without altering its promoter activity (Galbis-Martínez et al., 2010). Similarly, PARP1 loss or inhibition in *Drosophila* cells decreases the stability of *AKAP200* and *CAPER* (Matveeva et al., 2019). This effect may be mediated through changes in alternative splicing, as PARP1 knockdown alters the splicing products of these mRNAs (Matveeva et al., 2019). In addition, a study of PARP1 transcriptome-wide binding sites to RNAs showed that it binds primarily to mRNAs and within introns. This study also found that loss of PARP1 alters alternative splicing of pre-mRNAs for a significant number of genes in human HeLa cells (Melikishvili et al., 2017). This finding agrees with a previous study in *Drosophila* showing that PARP1 localizes to intron/exon boundaries, where it possibly recruits splicing factors to pre-mRNAs (Matveeva et al., 2016).

PARP1 also indirectly modulates RNA stability through the RNA-binding protein Human Antigen R (HuR) (Ke et al., 2017). HuR is a ubiquitously expressed protein that binds and stabilizes various mRNA transcripts. A recent study showed that PARP1 binding and subsequent PARylation of HuR enhance its binding to mRNAs, promoting mRNA stability (Ke et al., 2017). This is purportedly the mechanism by which PARP1 regulates the stability of the pro-inflammatory cytokine *Cxcl2* in primary macrophages isolated from mice (Ke et al., 2017).

Interestingly, PARP1 also affects the process of 3’ polyadenylation through modification of Poly(A) Polymerase (PAP) (Di Giammartino et al., 2013). PAP is an enzyme that catalyzes the synthesis of the poly(A) tail onto the 3’ end of pre-mRNA and is supported by ~80 proteins, including PARP1 (Shi et al., 2009). PARP1 binding and PARylation of PAP inhibit its polyadenylation activity due to decreased PAP binding to RNA (Di Giammartino et al., 2013). In the stressful environment of heat shock, this mechanism is crucial to inhibit polyadenylation and repress mature mRNA production (Di Giammartino et al., 2013).

1.4.3.4 miRNA Regulation

miRNAs are small RNA molecules that bind to and degrade specific mRNAs (Huntzinger and Izaurralde, 2011; O’Brien et al., 2018). PARP1 has been demonstrated to regulate the expression of several miRNAs, including miR-204, miR-365, miR-196, miR-203, and miR-98 (Nozaki et al., 2018; Wang et al., 2019). In exosomes derived from PARP1 KO embryonic stem cells, researchers identified 329 miRNAs that were altered by more than 2-fold, either upregulated or downregulated (Nozaki et al., 2018). In vascular smooth muscle cells, PARP1 suppresses the expression of miR-204 via IL-6/STAT3 phosphorylation, which results in overexpression of its target gene, Runx2. Further experiments demonstrated that PARP1 loss increases Runx2 expression without altering its promoter activity (Wang et al., 2019). Additionally, a transcriptome-wide assay for PARP1-RNA binding identified miRNAs bound directly by PARP1, though it was only 1% of all bound RNAs (Melikishvili et al., 2017). Therefore, miRNA regulation is an additional method by which PARP1 post-transcriptionally modifies mRNAs.

1.4.3.5 Regulation of Cell Death

PARP1 can regulate cell death through two different methods. In one mechanism, PARP1 plays a crucial role in programmed cell death signaling cascades, otherwise known as apoptosis. Cells initiate apoptosis by activating suicide proteases, including caspases, calpain, cathepsins, and granzymes, that cleave PARP1 and other molecules that are critical for cell survival (Fischer et al., 2003). PARP1 cleavage into multiple fragments prevents it from binding and repairing DNA, ultimately resulting in cell death (Kaufmann et al., 1993; Soldani et al., 2001). PARP1 overactivation also causes cell death in a process known as necrosis, which occurs when cells have severely damaged DNA (Eguchi et al., 1997). As previously discussed, PARP1 utilizes NAD^+ to catalyze PARylation reactions. PARP1 overactivation due to severe DNA damage overutilizes NAD^+, depleting it from cells. Therefore, cells are prevented from using NAD^+ to produce ATP during glycolysis, ultimately resulting in cell death (Eguchi et al., 1997). In addition, excessive PAR production activates the mitochondrial protein Apoptosis Inducing Factor (AIF), releasing it from mitochondria, after which it travels to the nucleus to stimulate endonucleases that cause apoptosis (Yu et al., 2002).

1.4.3.6 Immune Functions

During the immune system's response to a threat, inflammation is the initial response that activates the body's innate immune response. As previously discussed, PARP1 activates the transcription factor NF-ƙB (Hassa and Hottiger, 1999; Hassa et al., 2003), which regulates the inflammatory response during immune activation via transcriptional regulation of TNFα, IL-1β, IL-2, IL-5, and IL-8, as well as some adhesion molecules (Bhatt and Ghosh, 2014). Accordingly, PARP1 promotes inflammatory cytokine expression, including IL-6, IL-1β, and TNFα (Huang et al., 2008, 1; Robaszkiewicz et al., 2016). Interestingly, extracellularly released PARs have been shown to stimulate macrophages to induce cytokine and chemokine production, a method by which cells may be able to communicate that they have been damaged (Krukenberg et al., 2015). Accordingly, loss of PARP1 is protective in disorders that are associated with chronic inflammation, such as arthritis, inflammatory bowel disease, asthma, diabetes, ischemia, and some neurodegenerative diseases (Eliasson et al., 1997; Jijon et al., 2000, 1; Szabó, 2005; Matsuura et al., 2011; García and Conde, 2015; Kam et al., 2018; Sethi et al., 2019; Mao and Zhang, 2021). Likewise, overexpressed PARP1 is associated with multiple sclerosis (MS), a disease of excessive inflammation (Kauppinen et al., 2005). Accordingly, PARP1 inhibition in a mouse model of MS ameliorates symptoms (Farez et al., 2009); however, the genetic deletion of PARP1 before the onset of experimental autoimmune encephalomyelitis (EAE) worsens symptoms, disputing the potential therapeutic effect of PARP1 inhibition in MS treatment (Selvaraj et al., 2009).

1.4.4 Roles in Brain Development

Very little is known about the roles of PARP1 in brain development. Multiple studies have found that the brains of PARP1 KO mice tend to weigh less than controls at postnatal ages (Plane et al., 2012; Hong et al., 2019). Embryonically, PARP1 loss causes enlarged ventricles at E14.5 and increased cell death at E16.5 and E18.5 (Hong et al., 2019). PARP1 loss also impairs the proliferation of neural stem cells derived from the embryonic cortex, likely through regulation of PDGFRα and embryonic stem cell phosphatase (ESP) (also known as PTPRV) expression (Hong et al., 2019; Son et al., 2020). Studies have also found increased or decreased adult stem cell proliferation in the subventricular zone or hippocampal dentate gyrus in PARP1 KO mice, respectively (Plane et al., 2012; Hong et al., 2019). Adult stem cells from the subventricular zone or embryonic-derived neural progenitor cells are also more prone to differentiate into glial cells, either oligodendrocytes or astrocytes, in the absence of PARP1 (Plane et al., 2012; Hong et al., 2019). Glial differentiation occurs at the expense of neuronal differentiation, as PARP1 KO cells show decreased differentiation into MAP2-expressing neurons in both studies (Plane et al., 2012; Hong et al., 2019). Taken together, loss of PARP1 causes minor defects in brain development, but the few studies examining PARP1's role fail to go into great depth regarding potential embryonic developmental abnormalities.

1.4.5 Roles in Neurological and Psychiatric Diseases

PARP1 has been implicated in numerous neurological and psychiatric disorders. These include ischemic stroke, Alzheimer's disease (AD), Parkinson's disease (PD), MS, major depressive disorder, glioblastoma, and epilepsy (Mao and Zhang, 2021). In many of these disorders, PARP1 is overexpressed or overactive, contributing to associated cellular stress and neurodegeneration (Chiarugi, 2005; Kauppinen et al., 2005; Farez et al., 2009; Szebeni et al., 2016). For example, in PD, a recent study suggests that aggregation of pathological α-Synuclein in dopaminergic neurons, a hallmark of PD, is driven by PARP1 PARylation (Kam et al., 2018). Furthermore, PAR-driven aggregation increases the toxicity of pathological α-Synuclein and subsequent cell death in cell culture experiments. These effects were abrogated in both PARP1 KO neurons and with inhibition of PARP1 (Kam et al., 2018). Accordingly, PAR levels are elevated in the cerebrospinal fluid and substantia nigra of PD patients (Kam et al., 2018). In postmortem brain tissue from PD patients, researchers found a dramatic reduction in PARP1 expression in the nucleus of affected cells in the substantia nigra, dorsal motor nucleus of vagus, and frontal and cingulate cortices, suggesting an altered subcellular location of PARP1 in diseased cells (Salemi et al., 2021). They also observed a colocalization between PARP1 and α-Synuclein in the cytoplasm of affected neurons (Salemi et al., 2021).

Similarly, PARP1 co-localizes with Tau tangles and Aβ plaques in the brain of AD patients, is further activated by Aβ, and promotes the formation of Tau tangles that contribute to AD pathogenesis (Abeti et al., 2011; Mao and Zhang, 2021). In mice, PARP1 loss or inhibition causes Schizophrenia-associated behaviors, including increased anxiety, decreased social interaction, and impaired pre-pulse inhibition, as well as defects in short-term and long-term memory (Goldberg et al., 2009; Hong et al., 2019). In human studies, mutations in genes that affect PARylation, such as *Parp1* or *Adprhl2*, are associated with increased risk of stroke, cognitive dysfunction, ataxia, episodic psychosis, and neurodegeneration (Najmabadi et al., 2011; Danhauser et al., 2018; Durmus et al., 2021). Taken together, PARP1 loss of function or overexpression can negatively impact brain development and function in multiple ways.

1.5 Summary and Dissertation Outline

While PARP1 has various functions in different tissues, it is clear that it is implicated in brain development and function and that alterations in its protein activity result in brain development defects and related disorders. Therefore, further study of its specific roles in the developing brain will help scientists understand the etiology of associated neurodevelopmental diseases. Consequently, the main aims of my dissertation are to 1) further validate the role of PARP1 in NRG1/ErbB4 regulation of astrogenesis and 2) identify other roles for PARP1 in the developing brain.

Chapter 2 will examine the role of PARP1 in the regulation of astrogenesis through E4ICD nuclear signaling. This chapter expands upon previous work in the Corfas lab utilizing neural precursor cells isolated from rats and establishes methods to investigate this interaction in mouse cells. Specifically, I show that NRG1 induces PARylation in mouse NPCs and that PARP1 is essential for NRG1 to repress *GFAP* expression *in vitro*. Accordingly, loss of PARP1 or ErbB4 increases the expression of *GFAP* in the mouse cortex at birth. Chapter 3 identifies changes in gene expression associated with PARP1 loss in the embryonic brain, including the glycoprotein Reelin. I further demonstrate that PARP1 regulates Cajal-Retzius cell development, cortical morphology, neuronal migration, and adhesion to N-cadherin *in vitro*. Chapter 4 summarizes and discusses the findings of the previous two chapters and delineates future directions. Overall, these data demonstrate that PARP1 regulates the expression of numerous genes in the developing brain and that its loss causes changes in brain morphology that may contribute to the etiology of neurodevelopmental or neurodegenerative disorders.

1.6 Figures

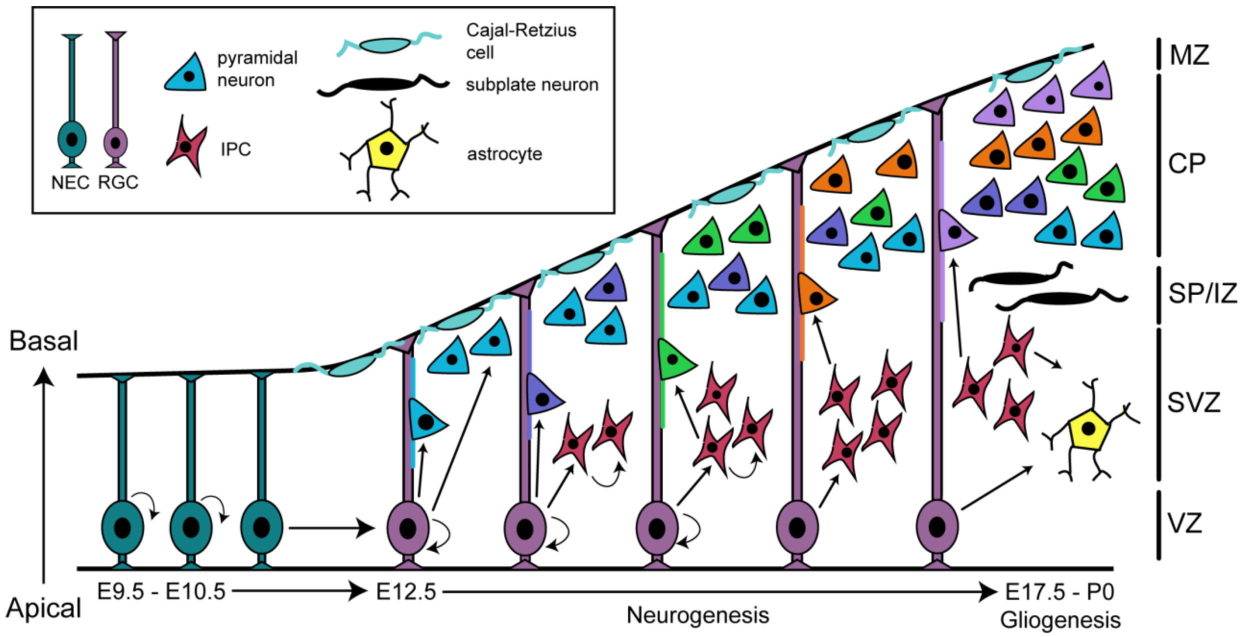


Figure 1.1 Overview of neurogenesis and gliogenesis in the rodent brain. Just before the onset of neurogenesis, neural epithelial cells (NECs) divide symmetrically to expand their pool rapidly. The earliest born neuronal subtypes are Cajal-Retzius cells (born around E10.5), which are located in the developing brain's marginal zone (MZ) and direct neuronal migration. At the onset of neurogenesis (around E12.5), NECs become radial glial cells (RGCs), whose cell bodies are localized to the ventricular zone (VZ) and have endfeet that extend to the basal and apical surfaces of the brain. RGCs divide symmetrically to form two new radial glial cells or asymmetrically to form one radial glial cell and one neuron or one radial glial cell and one intermediate progenitor cell (IPC). RGCs can also divide into two IPCs, which are located in the subventricular zone (SVZ). IPCs, in turn, proliferate to form additional IPCs (in rare cases) or two migrating neurons. In early development, neurons migrate independently of radial glial cells or dependent upon radial glial cell fibers, which serve as scaffolding. Migration at later stages of development when the cortical plate is thicker usually occurs along radial glial cell fibers. Neurons that have completed migration form the cortical plate (CP), splitting the MZ from the subplate (SP). The brain develops inside-out so that earlier-born neurons migrate to deeper cortical layers, while later-born neurons migrate beyond early-born neurons to more superficial brain layers. At the end of neurogenesis, around E17.5, IPCs and RGCs differentiate into astrocytes, starting the process of gliogenesis, which continues until 7 or 8 days following birth.

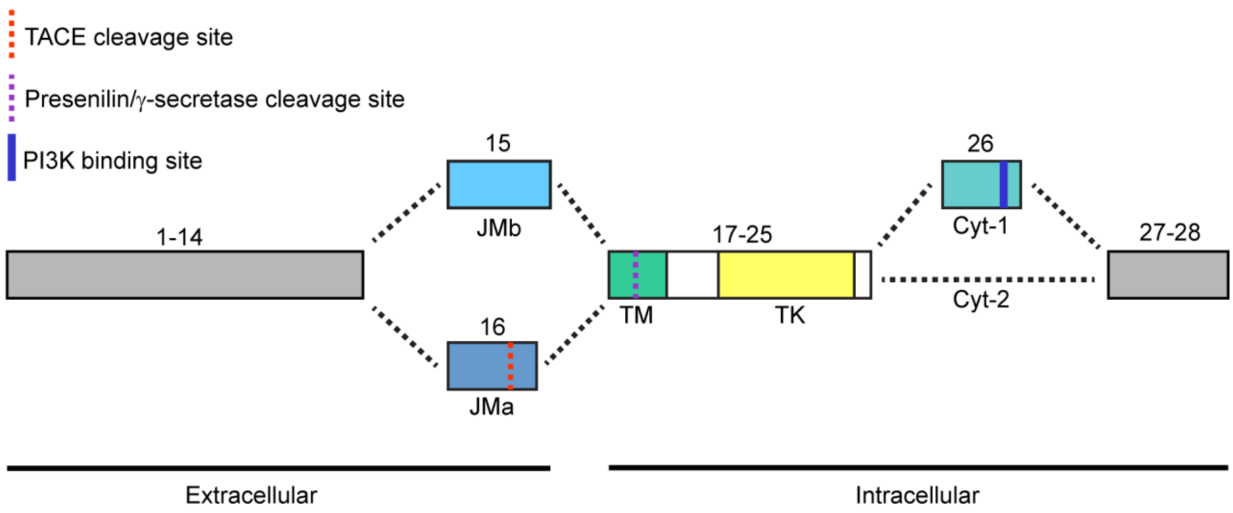


Figure 1.2 *ErbB4* alternative splicing. Alternative splicing of ErbB4 generates different isoforms with the inclusion of exon 15 (ErbB4-JMb) or exon 16 (ErbB4-JMa), exon 26 (Cyt-1) or lack of exon 26 (Cyt-2). The TACE cleavage site is located within exon 16, while the presenilin/γ-secretase cleavage site is within the transmembrane (TM) domain. Only ErbB4-JMa can be cleaved by both TACE and γ-secretase to release the ErbB4 intracellular domain. Exon 26 (therefore ErbB4-Cyt1) includes the PI3K binding site. TK = tyrosine kinase domain.

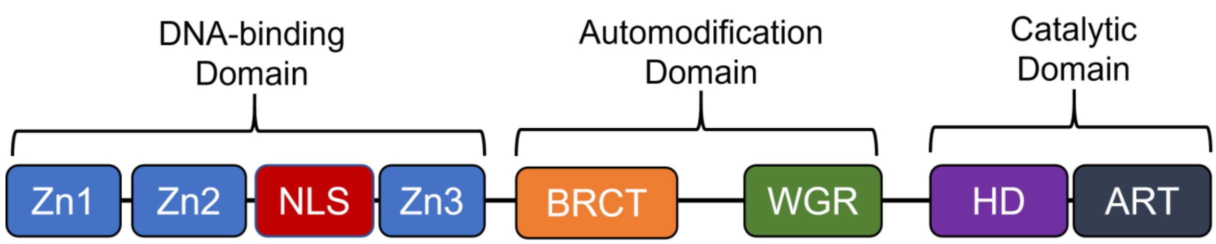


Figure 1.3 PARP1 protein structure. PARP1 contains a DNA-binding domain, automodification domain, and catalytic domain. The DNA-binding domain contains three Zinc-finger binding motifs (Zn1, Zn2, and Zn3) and a nuclear localization sequence (NLS). The automodification domain contains the breast-cancer-susceptibility protein carboxy terminus (BRCT) and WGR domain. The catalytic domain contains the helical subdomain (HD) and ADP-ribosyl transferase subdomain (ART).

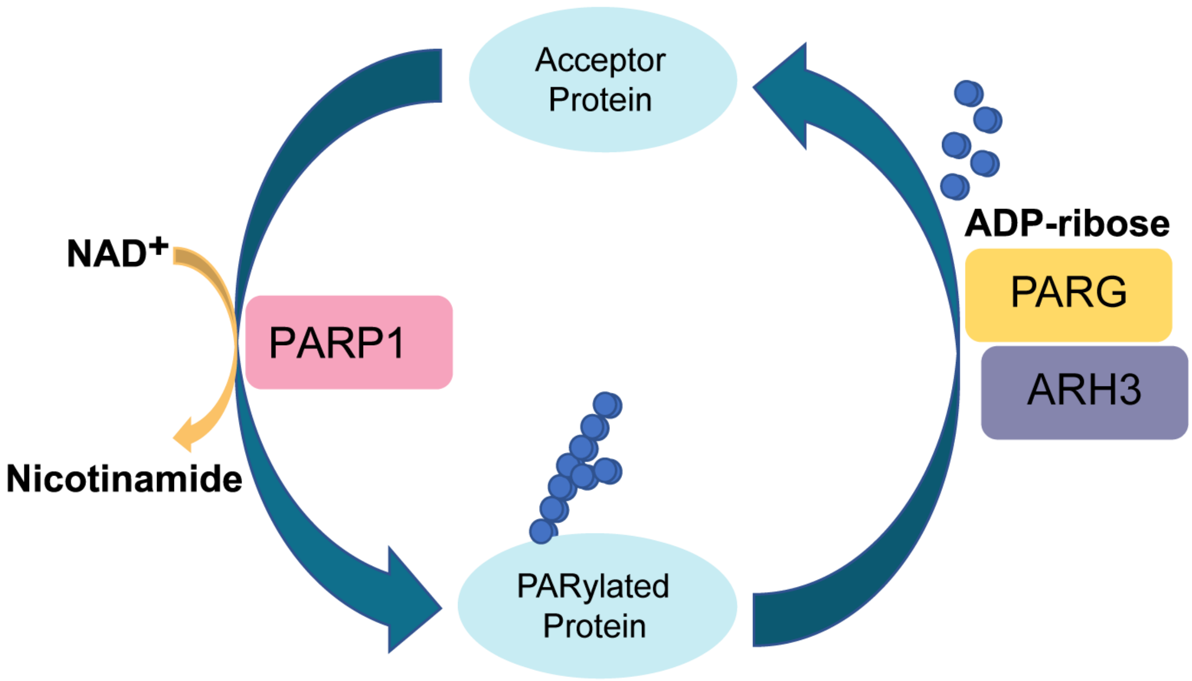


Figure 1.4 PARP1 enzymatic function. PARP1 uses NAD^+ as a substrate to add ADP-ribose polymers to acceptor proteins, converting NAD^+ to Nicotinamide in the process. ADP-ribose polymers are removed from proteins by reverse enzymes Poly (ADP-ribose) Glycohydrolase (PARG) or ADP-Ribose Glycohydrolase 3 (ARH3).

Chapter 2: ErbB4 Interacts with PARP1 to Regulate Astrogenesis[1]

2.1 Introduction

ErbB4 is a receptor tyrosine kinase highly expressed in the developing brain, of which Neuregulin 1 (NRG1) is a ligand (Fox and Kornblum, 2005). NRG1 binding to ErbB4 induces the receptor to dimerize and autophosphorylate within the protein's intracellular domain, activating other kinases to stimulate an intracellular signaling cascade which ultimately regulates transcriptional activity in the cell (Plowman et al., 1993b, 1993a). This type of signaling is known as canonical ErbB4 signaling. However, an additional splice form of ErbB4, which differs in the juxtamembrane (JM) domain of the transcript (known as JMa), can signal directly via the protein's intracellular domain (Elenius et al., 1997; Ni et al., 2001). ErbB4-JMa contains a cleavage site for TACE, while ErbB4-JMb does not (Rio et al., 2000). Thus, NRG1 binding and ErbB4-JMa autophosphorylation induce cleavage by TACE in the JM domain, allowing a secondary cleavage by presenilin-1/γ-secretase within the intramembrane domain (Ni et al., 2001; Lee et al., 2002). These sequential cleavage events release ErbB4's intracellular domain (E4ICD), which complexes with TAB2 and N-CoR, and together they travel directly to the cell's nucleus and bind to the promoter of astrocytic genes to repress their transcription (Sardi et al., 2006). We hypothesize that this complex serves as a "break" on astrogenesis while neurogenesis is ongoing at the early stages of neurodevelopment. Therefore, a reduction in ErbB4 expression at later stages of neurodevelopment would release this "break" and promote the expression of glial-specific genes.

[1] Some data and figures presented in this chapter were collected and prepared by previous Corfas lab members Dr. Falak Sher, Dr. Pablo Sardi, and Dr. Anna Kane and current lab member Robert Doherty.

Unpublished data from the Corfas lab implicates Poly (ADP-Ribose) Polymerase 1 (PARP1) in this complex as well. PARP1 is an enzyme that post-translationally modifies proteins and chromatin via poly(ADP-ribosyl)ation (PARylation) using NAD^+ as a substrate. These modifications can influence transcription via altering protein activity, promoter accessibility, or chromatin compaction (Kraus, 2008). As ErbB4 contains a nuclear localization signal but lacks a DNA-binding domain, E4ICD, TAB2, and N-CoR may bind to PARP1, which then binds to the gene promoter of astrocytic genes. Indeed, our data indicate that a constitutively active form of E4ICD (LexA-E4ICD) interacts with PARP1 as indicated via a yeast 2-hybrid assay and a proteomic screening analysis of E4ICD binding partners (Figure 2.1A,B). This interaction is specific to active E4ICD, as E4ICD with a mutated kinase domain (kinase-dead) does not interact with PARP1 (Figure 2.1C,D). Further co-immunoprecipitation experiments in transfected N2A cells and mouse neural progenitor cells (NPCs) or cortical lysates indicate that this interaction is specific to ErbB4-JMa, requires ErbB4 cleavage by TACE and presenilin-1/γ-secretase, and is dependent upon NRG1 stimulation and ErbB4 phosphorylation (Figure 2.1E-H). Together, these experiments suggest that NRG1-induced activation of ErbB4 and subsequent cleavage by TACE and presenilin-1/γ-secretase preclude its binding to PARP1.

Corfas lab members next sought to determine if ErbB4-JMa activation and cleavage stimulate PARP1 activity, finding that NRG1 induces PARylation in mouse NPCs to a level similar to H_2O_2, a known DNA damaging molecule that stimulates PARP1 (Figure 2.2A). Additionally, inhibition of ErbB4 cleavage via treatment with TACE inhibitor TAPI or presenilin-1/γ-secretase inhibitor DAPT prevents NRG1 from activating PARP1 in ErbB4-JMa transfected N2A cells (Figure 2.2B) or mouse NPCs (Figure 2.2C), suggesting that E4ICD mediates PARP1 activation, likely through its binding to PARP1. After confirming the interaction between E4ICD and PARP1, Corfas lab members questioned whether PARP1 was necessary for E4ICD/TAB2/N-CoR to repress transcription of the gene glial fibrillary acidic protein (*GFAP*). Indeed, while ciliary neurotrophic factor (CNTF)-induced *GFAP* promoter activity was reduced by NRG1 treatment in NPCs isolated from rat cortex, this effect was blocked by co-transfection of PARP1 constructs with mutations in either the DNA-binding domain or catalytic domain. Similarly, PARP1 inhibition via 3-ABA blocked NRG1 function, indicating both PARP1's ability to bind DNA and PARylate proteins are critical for NRG1-mediated *GFAP* repression (Figure 2.3A,B). Finally, chromatin immunoprecipitation experiments show that both PARP1 and ErbB4 bind to the same region of the *GFAP* promoter following treatment with NRG1 (Figure 2.3C). Together with the evidence that NRG1 influences *GFAP* promoter activity, this finding suggests that E4ICD-PARP1 regulates *GFAP* expression by modulating its transcription through the promoter.

Taken together, these findings suggest that NRG1-induced autophosphorylation of ErbB4, cleavage and release of E4ICD, binding to N-CoR and TAB2, translocation to the nucleus, and subsequent binding and activation of PARP1 represses transcription of astrocyte-specific genes, such as *GFAP*. However, many of these experiments need to be replicated and validated in mouse NPCs to take advantage of transgenic mice as negative controls. Furthermore, it is unknown if loss of PARP1 causes precocious astrogenesis as previously observed in ErbB4 KO mice. To validate the role of PARP1 in regulating astrogenesis, I developed a model of NRG1-mediated repression of *GFAP* expression in mouse NPCs. We further demonstrated that this repression is dependent upon the presence of PARP1 and ErbB4-JMa. Finally, I found that *GFAP* expression is increased to a similar extent in PARP1 KO and ErbB4 KO cortex of mice at birth, suggesting E4ICD-PARP1-mediated repression of astrogenesis has an essential role *in vivo*.

2.2 Materials and Methods

2.2.1 PARP1 KO Mice

The PARP1 KO mouse line 129S-*Parp1*^{tm1Zqw}/J[64] was obtained from the Jackson Laboratory and maintained on a 129S1/SvImJ background. All animals were kept under a 12/12 hr light/dark cycle and allowed food ad libitum. Animal procedures were reviewed and approved by the University of Michigan Institutional Animal Care and Use Committee. Embryonic dating was performed with vaginal plugging denoted as embryonic day 0.5 (E0.5).

2.2.2 Generation of ErbB4-JMa$^{-/-}$ mice

Two single guide RNAs (sgRNA) were designed to create cut sites in the ErbB4-JMa sequence within Exon 16. Repair of the chromosome breaks by non-homologous end joining was expected to create mutations in ErbB4 Exon 16 and introduce a premature termination codon which could lead to nonsense-mediated decay (NMD) and therefore result in specific loss of ErbB4-JMa expression. The sgRNAs were co-injected into fertilized C57BL/6 embryos with the CRISPR/Cas9 components and ultramer oligonucleotide bearing the mutations. From more than 300 injections with both sgRNAs, 39 putative founder mice were created. Genotyping of Exon 16 by PCR and sequencing identified 20 founder mice with other uninterpretable mutations.

To generate founders carrying a single mutant allele, 12 potential founders were crossed with wild-type C57BL/6 mice, and ErbB4 Exon 16 was sequenced in the progeny, two of which had a single base pair deletion creating a premature termination codon within ErbB4 Exon 16. Male and female heterozygote offspring from the founders were back-crossed, a step that generated viable homozygote offspring for each line with the expected Mendelian ratio, indicating that the mutations do not compromise viability. The mutant allele was named ErbB4-JMa^{-}, indicative of the mice bearing a premature termination codon, resulting in NMD of ErbB4-JMa transcript.

Mouse generation was completed with the aid of the University of Michigan Transgenic Animal Core. All animals were kept under a 12/12 hr light/dark cycle and allowed food ad libitum. Animal procedures were reviewed and approved by the University of Michigan Institutional Animal Care and Use Committee.

2.2.3 Cell Culture and Treatments

For primary NPC cell cultures, pregnant females were euthanized via cervical dislocation. Telencephalons were dissected from E14.5 embryos in ice-cold phosphate-buffered saline (PBS), meninges were removed, and cortices were dissociated into single cells with StemPro Accutase (ThermoFisher) for 5 min. NPCs were seeded as neurospheres in T75 flasks at 500,000 cells/mL and expanded for 2 days in NPC media (DMEM with GlutaMAX, 1% penicillin/streptomycin, and 2% B27 without RA) supplemented with epidermal growth factor (EGF, 20 ng/mL) and basic fibroblast growth factor (bFGF, 20 ng/mL) in a humidified 5% CO_2/95% air incubator at 37°C. Half of the media was changed every day, with replenishment of EGF and bFGF each day. On day 3, neurospheres were dissociated with Accutase into a single cell suspension and plated in NPC media supplemented with bFGF. Plates were pre-prepared by incubating in Poly-L-Lysine (Sigma) 30 min or overnight followed by Fibronectin (1 µg/mL, Corning) for 2 hrs.

To inhibit PARP1 enzymatic activity, NPCs were treated with Olaparib (a gift of C. Brenner) at the indicated concentrations (10, 30, 50 nM) for 48 hrs starting on day 1 following plating. Half of the media was replenished each day, and cells were re-treated with Olaparib every 24 hrs. To measure the effect of NRG1 on *GFAP* expression, NPCs were cultured as adherent cells as previously described. 24 hrs following culturing, bFGF was removed from media by replacing with NPC media containing only B27 without RA. Simultaneously, NPCs were treated with NRG1 (2 nM). 24 hrs later, NPCs were lysed, and RNA was extracted.

2.2.4 shRNA-mediated PARP1 knockdown

Lentiviral packaging plasmids and scramble shRNA or *Parp1* shRNA constructs were transfected into HEK 293T cells using Lipofectamine 3000 (Invitrogen). Lentiviral supernatants were collected and concentrated using Lenti-X Concentrator (Clontech) and titered with puromycin selection. *Parp1* shRNA plasmid was obtained from Sigma (clone ID TRCN0000305949) with the following target sequence: 5'- GGTTCATCTTTGCTTTAATTT-3'. Lentiviral packaging plasmids and scramble shRNA plasmid were gifts of S. Iwase. To knockdown PARP1, NPCs were transduced with *Parp1* shRNA expressing lentivirus or scramble shRNA lentivirus (at multiplicity of infection 3) for 48 hrs prior to FGF removal and NRG1 (2 nM) treatment, following the protocol described above.

2.2.5 Luciferase Assay

NPCs were cultured as adherent cells as previously described. 24 hrs following plating, NPCs were co-transfected with a construct expressing the rat *GFAP* promoter upstream of firefly luciferase and a construct expressing *CMV*-renilla (50:1 ratio). 24 hrs later, media was replaced with NPC media containing only B27 without RA with simultaneous treatment of NRG1 (2 nM). 24 hrs later, media was replenished, and NPCs were re-treated with NRG1. 48 hrs following initial treatment, luciferase activity was measured using the Dual-Glo Luciferase Kit (Promega), following the manufacturer's protocol. Luciferase intensity was measured using a BioTek plate reader. Final values were obtained by normalizing firefly luciferase to Renilla luciferase. Technical replicates were measured in triplicate.

2.2.6 PARylation Assay

NPCs were plated onto round glass coverslips as adherent NPCs. The next day following plating, NPCs were treated with NRG1 (2 nM) or H_2O_2 (100 µM) for 5 min. NPCs were then fixed in 4% paraformaldehyde (PFA) in PBS for 10 min, then washed 3x in PBS. For fluorescent immunostaining, coverslips were blocked in 10% normal donkey serum in 0.2% triton in PBS then incubated in mouse anti-PAR primary antibody (Trevigen #4335-MC-100, 1:500 dilution) overnight diluted in blocking buffer. The following day, coverslips were washed 3x in 0.2% triton in PBS then incubated in Alexa-Fluor donkey anti-mouse 568 secondary antibody (Invitrogen, 1:500 dilution). Z-stack images were taken with Leica SP8 confocal microscope at 63x magnification.

2.2.7 RNA Isolation

RNA was isolated from P0 cortex after removal of meninges and ganglionic eminences or cultured NPCs using Qiagen RNeasy Kit following the manufacturer’s instructions. RNA extraction from tissue was completed with on-column DNase digestion (Qiagen).

2.2.8 RT-PCR and RT-qPCR

For RT-qPCR, equal amounts of RNA (1 µg) were reverse transcribed into cDNA using an iScript cDNA Synthesis Kit (Bio-Rad) in 20 µL reaction volume following the manufacturer’s instructions. cDNA was diluted 5-fold for the PCR reaction. Quantitative PCR was performed using an iTaq SYBR Green Supermix (Bio-Rad) with a Bio-Rad CFX96 Thermocycler. Each sample was run in duplicate. Each well of the 96-well plate contained 5 µL iTaq SYBR Green

Supermix, 2.5 µL diluted cDNA, and 3 pmol of each forward and reverse primer. The cycling conditions were as follows: 95°C for 30 s followed by 39 cycles of 95°C for 5 s and 60°C for 30 s. Normalized Gene Expression (NGE) was calculated using the efficiency of each primer with the following formula: [$\text{efficiency\_target}^{-CT_{target}}/\text{efficiency\_reference}^{-CT_{ref}}$]. Fold changes in mRNA level were then calculated relative to controls for each experiment.

For non-quantitative RT-PCR, equal amounts of RNA were reverse transcribed into cDNA using an iScript cDNA Synthesis kit (Bio-Rad), as previously described. Equal amounts of cDNA were amplified with primers spanning the ErbB4 juxtamembrane domain with the following PCR cycling conditions: 94° for 3 min, then 30 cycles of 63°C for 30 s, 72°C for 30 s, and 94°C for 30 s, followed by 72°C for 20 min. PCR products were run on 2% agarose gels stained with Ethidium Bromide. The expected size for ErbB4 JMa is 273 bp, and the expected size for ErbB4 JMb is 243 bp.

Primer sequences are as follows (5'-3'): *GFAP* F: GCAGGAGTACCAGGATCTACT; *GFAP* R: TGGAGGTTGGAGAAAGTCTGT; *GAPDH* F: TCACTGCCACCCAGAAGA; *GAPDH* R: GCCAAGCCCTGAGCATAA; *PARP1* F: GGCAGCCTGATGTTGAGGT; *PARP1* R: GCGTACTCCGCTAAAAAGTCAC; *DCX* F: GCCAGGGAGAACAAGGACTTT; *DCX* R: CACCCCACTGCGGATGA; *NeuN* F: CCAGGCACTGAGGCCAGCACACAGC *NeuN* R: CTCCGTGGGGTCGGAAGGGTGG *RPL19* F: ACCTGGATGAGAAGGATGAG; *RPL19* R: ACCTTCAGGTACAGGCTGTG; *ErbB4* JM F: GAAATGTCCAGATGGCCTACAGGG ; *ErbB4* JM R: CTTTTTGATGCTCTTTCTTCTGAC

2.2.9 ErbB4 Immunoprecipitation

For NPCs, two 10 cm dishes of adherent NPCs were treated with TPA (100 ng/mL for 45 mins) or PBS control. Cells were washed 2x with PBS and lysed in RIPA buffer (Sigma) with protease and phosphatase inhibitors for 15 mins, then centrifuged for 10 mins at 14,000 rpm, and supernatant was collected. Cerebellum was lysed directly in RIPA buffer (Sigma) with protease and phosphatase inhibitors by homogenization on ice for 15 mins, then centrifuged for 10 mins at 14,000 rpm, and supernatant was collected. Samples were normalized to 1 µg/µl by BCA assay, and 500 µl lysate was incubated overnight at 4°C with 30 µl washed anti-ErbB4-conjugated beads (ErbB4 antibody (C-7) agarose conjugated, sc-8050 AC, Santa Cruz). The following day, beads were washed 4x in RIPA buffer, and the sample was eluted in 1.5x Laemmli sample buffer for 5 mins at 95°C. Western blot from input, IP, and supernatant was carried out with ErbB4 antibody (HER4/ErbB4 (111B2) Rabbit mAb #4795, Cell Signaling Technology) according to standard procedures.

2.3 Results

2.3.1 NRG1-induced PARylation in mouse NPCs is dependent upon PARP1 and ErbB4

PARP1 functions enzymatically by post-translationally modifying proteins via the addition of (ADP-Ribose) polymers (PAR). Typically, PARP1 is activated by DNA damage; however, there are incidences in which PARP1 is stimulated by other proteins (Cohen-Armon et al., 2007; Visochek et al., 2016). Our lab previously observed in WT mouse NPCs that NRG1 activates PARP1, resulting in increased PARylation. To test if this effect is specific to ErbB4 and PARP1, I treated NPCs isolated from WT, PARP1 KO, and ErbB4 KO mice with NRG1 or H_2O_2 as a positive control. As observed previously, NRG1 increased cellular PAR levels in WT NPCs, but not in ErbB4 KO or PARP1 KO NPCs (Figure 2.4), indicating that both ErbB4 and PARP1 are required for NRG1-induced PARylation.

2.3.2 Generation and Validation of the ErbB4-JMa$^{-/-}$ mouse

To further investigate the role of the cleavable form of ErbB4, ErbB4-JMa, *in vivo*, our lab developed a transgenic mouse using CRISPR/Cas9 to edit ErbB4 exon 16. This directed targeting introduced a mutation that resulted in predicted nonsense-mediated decay of the ErbB4-JMa transcript while retaining ErbB4-JMb. To test the validity of this prediction, we isolated NPCs from WT or ErbB4-JMa$^{-/-}$ embryos and assessed the expression of the ErbB4-JMa and JMb transcripts using RT-PCR with primers flanking the juxtamembrane (JM) region. In WT NPCs, we detected both JMa and JMb, however in ErbB4-JMa$^{-/-}$ NPCs, we identified only ErbB4-JMb (Figure 2.5A), consistent with our expectation of ErbB4-JMa nonsense-mediated decay. Furthermore, stimulation of TACE with tissue-type plasminogen activator (TPA) treatment causes ErbB4 intracellular domain cleavage, indicated by the accumulation of the 80 kD E4ICD. As expected, no E4ICD is detectable in ErbB4 KO or ErbB4-JMa$^{-/-}$ NPCs upon TPA treatment, indicating no cleavable form of ErbB4 remains in JMa$^{-/-}$ NPCs (Figure 2.5B). Notably, full-length ErbB4 is expressed in ErbB4-JMa$^{-/-}$ tissues (Figure 2.5B). Similarly, we confirmed the lack of E4ICD *in vivo* with cerebellar lysates from adult ErbB4-JMa$^{-/-}$ mice (Figure 2.5C,D). Taken together, these experiments verify that ErbB4-JMa and E4ICD are lost in the ErbB4-JMa$^{-/-}$ mouse brain, indicating that these mice are suitable to assess the effect of E4ICD loss without the loss of ErbB4-JMb canonical signaling.

2.3.3 NRG1 repression of GFAP expression following FGF removal depends upon PARP1 and ErbB4-JMa

Given previous observations of NRG1-mediated repression of *GFAP* expression after induction with CNTF in rat NPCs, we sought to establish a similar model in mouse NPCs. I found that removal of bFGF from NPC media drastically increases *GFAP* expression 24 hrs later. However, concurrent removal of FGF with NRG1 treatment suppresses the increase in *GFAP* expression by about 50% in WT NPCs (Figure 2.6A). To assess the necessity of PARP1 and ErbB4 for NRG1-mediated repression, I measured *GFAP* transcript levels after FGF removal and NRG1 treatment in ErbB4 KO and PARP1 KO NPCs. Similar to our findings in rat NPCs, I found that loss of either PARP1 or ErbB4 abolished NRG1-mediated *GFAP* repression (Figure 2.6B). Importantly, this repression was specific to glial genes, as I observed no change in *DCX* or *NeuN*, which are expressed in immature or mature neurons, respectively. I observed similar results with PARP1 knockdown in WT NPCs via a PARP1 shRNA-expressing lentivirus (Figure 2.6C), further validating the necessity of PARP1.

To test if NRG1-mediated repression of *GFAP* is specific to ErbB4-JMa, as our lab had found previously in rat NPCs, we removed FGF and treated NPCs isolated from ErbB4-JMa$^{-/-}$ mice with NRG1. As previously discussed, NPCs isolated from this mouse express ErbB4-JMb normally. As expected, the lack of the cleavable form of ErbB4 abolished the effect of NRG1, strongly suggesting that ErbB4 cleavage and its interaction with PARP1 are necessary to repress *GFAP* expression (Figure 2.6B). We verified that NRG1 suppresses *GFAP* expression after FGF removal through the *GFAP* promoter by transfecting WT NPCs with a *GFAP*-luciferase construct which expresses luciferase downstream of the *GFAP* promoter. Similar to *GFAP* transcript levels, removal of FGF increases *GFAP*-luciferase activity while concurrent NRG1 treatment represses the induction (Figure 2.6D). Furthermore, PARP1 inhibition alone increases *GFAP* expression, suggesting that lack of PARP1's enzymatic function interrupts *GFAP* repression by NRG1 (Figure 2.6E). Together, these results indicate that PARP1 PARylation is critical for NRG1/ErbB4-JMa repression of *GFAP* expression and that this effect is mediated through the *GFAP* promoter.

2.3.4 Loss of PARP1 or ErbB4 increases cortical *GFAP* expression at birth

In mice, astrogenesis begins around E17.5 and continues through several days after birth (Sauvageot, 2002). Previous data suggest that ErbB4 KO animals have precocious astrogenesis, or increased expression of glial genes in the developing cortex (Sardi et al., 2006). Given the interaction between E4ICD and PARP1, this finding suggests that loss of PARP1 also causes precocious astrogenesis. To test this hypothesis, we measured *GFAP* expression in the P0 cortex of ErbB4 KO and PARP1 KO mice. Indeed, we observed an upregulation in *GFAP* expression in both transgenic mice (Figure 2.7), suggesting that the inability of NRG1 to repress *GFAP* expression in cells lacking ErbB4 or PARP1 results in overexpression of the transcript *in vivo*.

2.4 Discussion

Here, we identified a novel role for PARP1 in mediating the repression of astrogenesis through ErbB4 nuclear signaling. We show that this repression depends on NRG1-induced phosphorylation of ErbB4-JMa and PARP1 activation, which represses *GFAP* expression through *GFAP* promoter activity. We also identified a mechanism for PARP1 activation that is not triggered by DNA damage but by NRG1 binding and subsequent phosphorylation of the ErbB4 receptor and cleavage of E4ICD. PARP1 activation independently of DNA damage has been shown to occur in other instances as well. For example, neuronal stimulation with a high-frequency electrical signal causes Erk2 phosphorylation, which induces PARP1 binding to Erk2 and subsequent PARP1 activation. PARP1 then PARylates itself and chromatin at promoters of Erk2-associated immediate-early neuronal genes. PARylation of Histone H1 at these promoter sites causes the linker histone to dissociate from the complex, allowing transcription to occur (Cohen-Armon et al., 2007; Visochek et al., 2016). We propose a similar mechanism, whereby stimulation of ErbB4 with NRG1 and subsequent ErbB4 phosphorylation and E4ICD cleavage induces PARP1 binding to E4ICD and promotes PARylation. However, it remains unclear whether PARP1 PARylates itself, other proteins in the complex, chromatin at the *GFAP* promoter, or multiple of these. Further studies are needed to elucidate the specifics of PARP1 activation and its effects on the *GFAP* promoter.

In accordance with our findings, previous studies investigating the role of PARP1 in differentiation have identified that progenitor cells lacking PARP1 are more prone to differentiate into glial cells at the expense of neuronal cells, including GFAP-expressing astrocytes and Olig2-expressing oligodendrocytes (Plane et al., 2012; Hong et al., 2019). Hong *et al*. hypothesized that increased glial gene expression in the absence of PARP1 is caused by decreased neurogenesis due to reduced expression of Ascl1 and Ngn2, two crucial transcriptional regulators of neurogenesis (Hong et al., 2019). Similarly, Plane and colleagues hypothesized that PARP1 promotes neurogenesis by regulating Sox2 expression; thus, PARP1 loss disrupts neurogenesis and causes progenitor differentiation into glial subtypes (Plane et al., 2012). Here, we propose an additional mechanism for glial cell fate repression by PARP1. Specifically, via direct repression of *GFAP* expression through promoter modulation. As ErbB4-JMa expression declines at later stages of development (Fox and Kornblum, 2005), repression of *GFAP* by E4ICD and PARP1 would be released, allowing astrogenesis to occur.

Intriguingly, a previous study found that PARP1 PARylates STAT3, a well-known transcription factor promoting astrogenesis (Bonni et al., 1997; Kamakura et al., 2004; Ding et al., 2019). They identified that PARP1 PARylation of STAT3 inhibits STAT3 phosphorylation and its ability to promote transcription, ultimately repressing the expression of *PD-L1* in cancer cells (Ding et al., 2019). If this pathway is also active in NPCs, it may be an additional mechanism by which activated PARP1 represses *GFAP* expression during neurogenesis. By suppressing STAT3 activity, STAT3 would be unable to bind to the *GFAP* promoter and promote transcription. However, further studies are needed to examine the interaction between PARP1 and STAT3 in neuronal cells.

The *GFAP* promoter region has several sites where transcription factors bind to repress or promote its expression. Our data indicate that E4ICD and PARP1 bind to the region of the *GFAP* promoter where the Notch intracellular domain (NICD) has been suggested to bind. Similar to ErbB4, ligand binding to the Notch receptor induces its cleavage and the release of its intracellular domain. NICD then forms a complex with transcriptional activator CSL (also known as RBPJk), which directly binds to the *GFAP* promoter to activate transcription (Ge et al., 2002). N-CoR also complexes with CSL/RBPJk; however, binding of this complex to the *GFAP* promoter represses transcription (Hermanson et al., 2002). Given that E4ICD binds N-CoR, these findings suggest that N-CoR/CSL may facilitate E4ICD binding to the CSL/RBPJk binding site of the *GFAP* promoter. Therefore, our data indicate that E4ICD-PARP1 binding during neurogenesis may prevent NICD/CSL from binding to the promoter and activating *GFAP* transcription. However, future studies will be needed to ascertain whether E4ICD participates in reciprocal binding with NICD to regulate astrogenesis.

2.5 Author Contributions

G.C. and M.M.N. designed the study. P.S., A.K., and F.S. performed experiments presented in the introduction section. M.M.N. performed most experiments described in the results section and analyzed the data; R.D. generated, validated, and performed all experiments with the ErbB4-JMa$^{-/-}$ mouse line.

2.6 Acknowledgements

We thank Eric Cho and Michael Hayes for their assistance with the mouse colony and genotyping. We also thank Dr. Chad Brenner and Dr. Shigeki Iwase for reagents and plasmids and Dr. Thom Saunders with the University of Michigan Transgenic Animal Core for technical support.

2.7 Figures

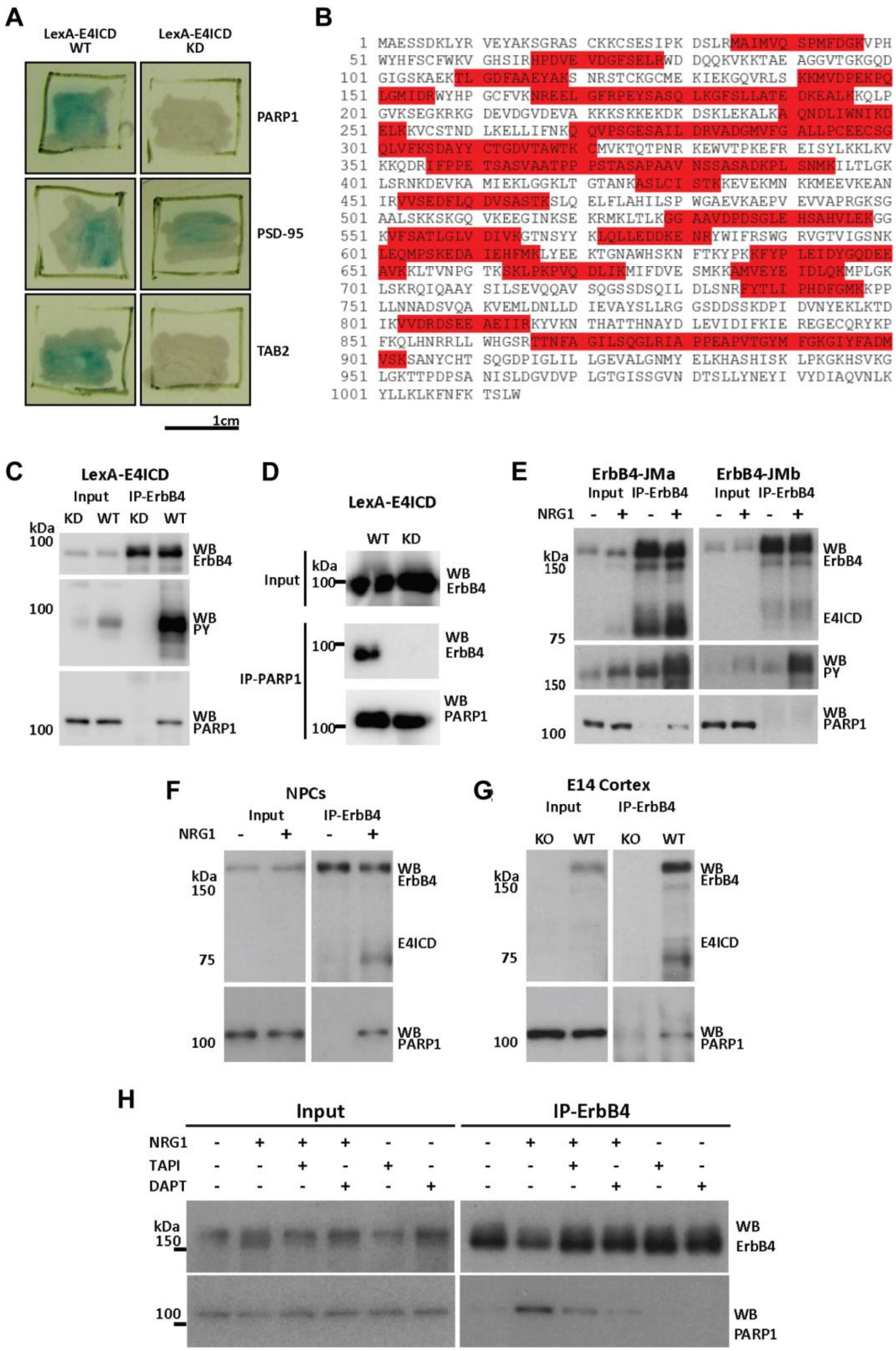

A
LexA-E4ICD WT
LexA-E4ICD KD
PARP1
PSD-95
TAB2
1cm
B
C
LexA-E4ICD
Input
IP-ErbB4
KD WT KD WT
kDa
WB ErbB4
WB PY
WB PARP1
D
LexA-E4ICD
WT KD
Input
IP-PARP1
E
ErbB4-JMa
ErbB4-JMb
NRG1
E4ICD
F
NPCs
G
E14 Cortex
KO WT KO WT
H
TAPI
DAPT

Figure 2.1 PARP1 interacts with E4ICD in a kinase-specific manner (A) Yeast 2 hybrid screen indicates that PARP1 interacts with a wild-type (WT), but not kinase dead (KD) LexA-E4ICD construct. Blue coloration indicates interaction. Interaction between TAB2 and LexA-E4ICD included as a positive control. **(B)** Mass spectometry analysis of proteins that bind LexA-E4ICD shows large coverage of PARP1 amino acids (highlighted in red). **(C)** N2A cells transfected with WT LexA-E4ICD, but not KD LexA-E4ICD, show an interaction between E4ICD and PARP1. PY = phosphotyrosine. **(D)** PARP1 co-IP shows PARP1 binding to WT E4ICD in LexA-E4ICD-transfected N2A cells. **(E)** Only N2A cells transfected with cleavable ErbB4-JMa and treated with NRG1 demonstrate an interaction between ErbB4 and PARP1 via ErbB4 co-IP. **(F)** ErbB4 co-IP in mouse NPCs shows binding to PARP1 following NRG1 treatment. **(G)** ErbB4 co-IP in E14.5 cortical lysates demonstrates an interaction between ErbB4 and PARP1 in wild-type (WT) cortex, but not ErbB4 knockout (KO) cortex. **(H)** Inhibition of TACE with TAPI (100 µM) or presenilin/γ-secretase with DAPT (1 µM) 30 min prior to NRG1 treatment (2 nM, 40 min.) prevents interaction between ErbB4 and PARP1 in N2A cells transfected with ErbB4-JMa. These experiments were performed by Pablo Sardi, Falak Sher, and Anna Kane.

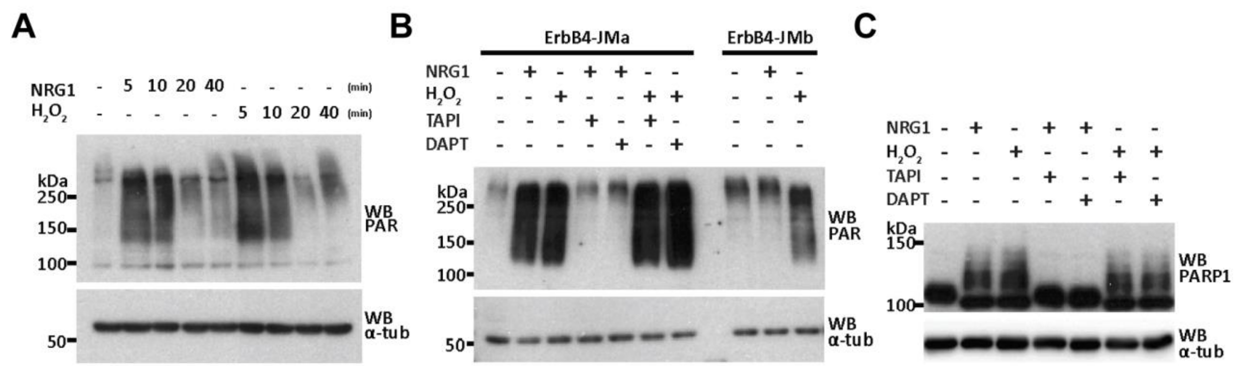


Figure 2.2 NRG1 treatment induces PARP1 PARylation in an E4ICD-specific manner (A) NRG1 (2 nM) treatment induces PARylation in a similar time frame as H_2O_2 (80 µM) in mouse NPCs. **(B)** Inhibition of TACE cleavage with TAPI (100 µM) or presenilin/γ-secretase cleavage with DAPT (1 µM) prevents NRG1-induced PARylation in ErbB4-JMa transfected N2A cells. TAPI nor DAPT prevent PARylation in H_2O_2 treated cells. NRG1 does not induce PARylation in ErbB4-JMb transfected N2A cells. **(C)** Western blotting for PARP1 shows increased PARylated PARP1 following NRG1 treatment in the absence of inhibitors of ErbB4 cleavage in mouse NPCs. These experiments were completed by Pablo Sardi and Falak Sher.

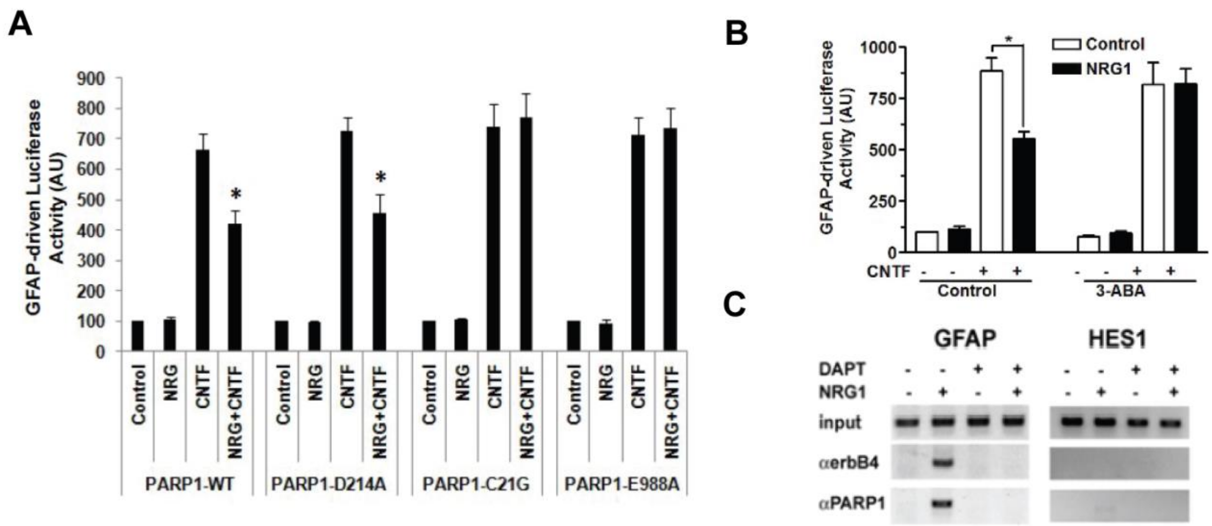


Figure 2.3 PARP1 DNA binding and enzymatic activity are necessary to repress CNTF-induced *GFAP* promoter activity in rat NPCs (A) NRG1 (1 nM) pre-treatment represses CNTF (0.3 ng/mL)-induced *GFAP* promoter activity in rat NPCs transfected with WT PARP1-expressing plasmids, but fails to repress *GFAP* promoter activity in rat NPCs transfected with PARP1 constructs with mutations in the DNA binding domain (C21G) or catalytic domain (E988A). PARP1 cleavage by caspase 3 is not necessary for NRG1-induced repression of GFAP as indicated by transfection with a PARP1 constructed with a mutated caspace 3 cleavage site (D214A). **(B)** Inhibition of PARP1 with 3-ABA prevents NRG1 from repressing CNTF-induced *GFAP* promoter activity in rat NPCs. **(C)** PARP1 and ErbB4 localize to the *GFAP* promoter, but not the *Hes1* promoter, following NRG1 stimulation in rat NPCs as indicated by chromatin immunoprecipitation. Pre-treatment with DAPT to inhibit presenilin/γ-secretase prevents promoter localization. These experiments were completed by Pablo Sardi.

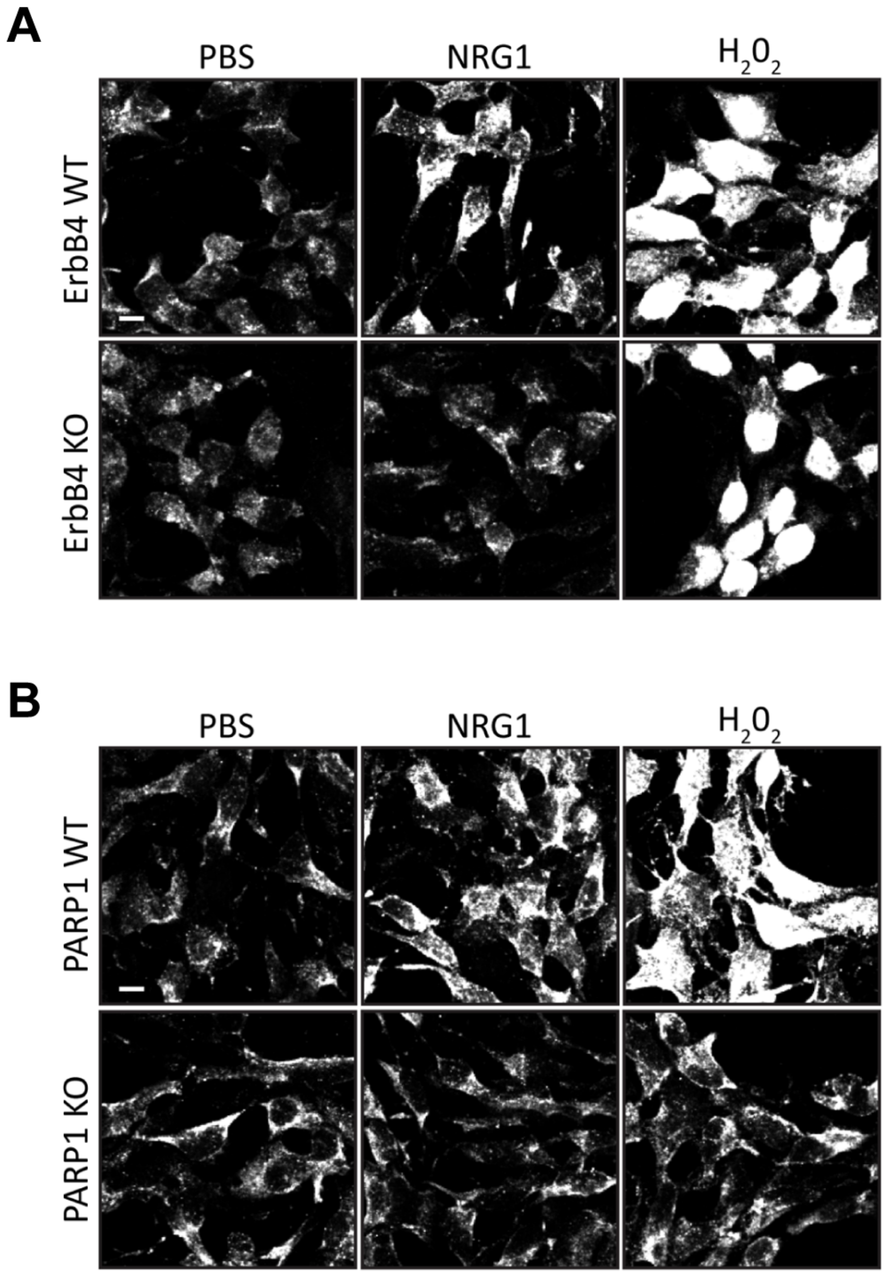


Figure 2.4 PARP1 and ErbB4 are necessary for NRG1-induced PARylation in mouse NPCs (A) 5 min NRG1 (2 nM) treatment increases PAR in WT but not ErbB4 KO mouse NPCs. 5 min H_2O_2 treatment (100 µM) included as positive control. **(B)** PARP1 KO abolishes NRG1 and H_2O_2-induced PARylation in NPCs. Scale bar = 10 µM. n = 2 ErbB4 WT, n = 4 ErbB4 KO, n = 2 PARP1 WT, n = 2 PARP1 KO (independent embryos).

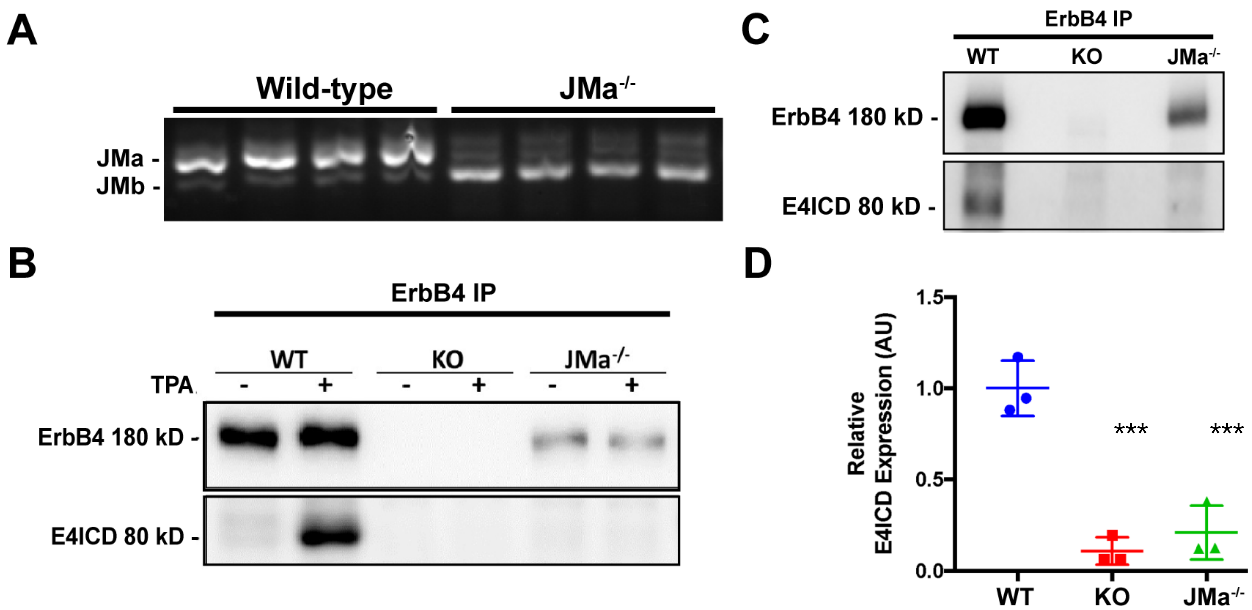


Figure 2.5 ErbB4-JMa$^{-/-}$ mutant mice lack ErbB4-JMa and E4ICD expression (A) NPCs isolated from JMa$^{-/-}$ mouse cortex express *ErbB4-JMb*, but not *ErbB4-JMa* via RT-PCR with primers that span the ErbB4 juxtamembrane domain. **(B)** Enrichment of ErbB4 with ErbB4 immunoprecipitation (IP) in mouse NPCs shows increased E4ICD following TACE stimulation with TPA treatment (100 ng/mL) for 45 min in WT, but not ErbB4 knockout (KO) or JMa$^{-/-}$ NPCs. **(C)** ErbB4 IP from adult cerebellar lysates shows presence of E4ICD in WT, but not KO or JMa$^{-/-}$ mice. **(D)** Quantification of E4ICD expression in cerebellar lysates in multiple replicates (n = 3 of each genotype). ***$p < 0.001$ by one-way ANOVA with multiple comparisons. These experiments were completed by Robert Doherty.

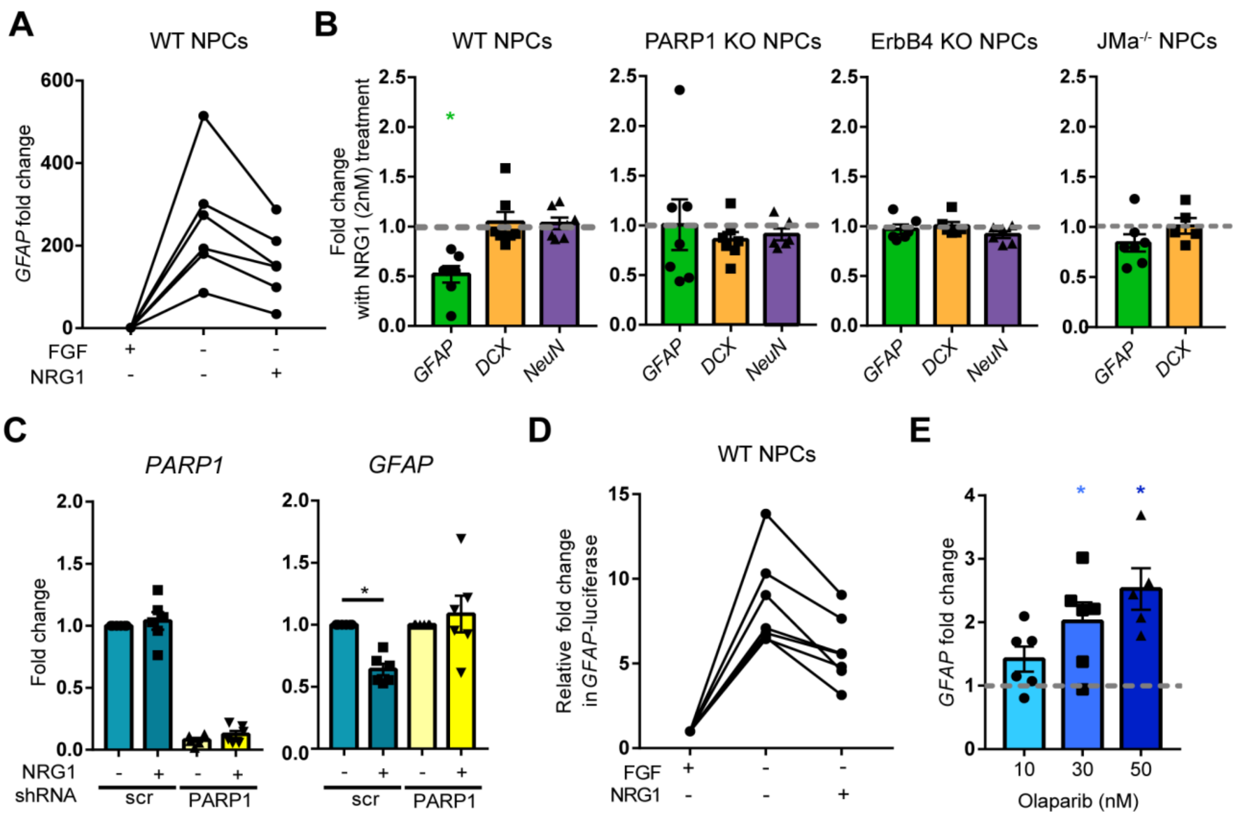


Figure 2.6 PARP1 and ErbB4-JMa are necessary for NRG1-induced repression of *GFAP* expression after FGF removal in mouse NPCs (A) Removal of FGF (20 ng/mL) from NPC media increases *GFAP* expression 100-500 fold after 24 hrs, while concurrent NRG1 (2 nM) treatment represses this increase in WT mouse NPCs. **(B)** PARP1 KO, ErbB4 KO, or ErbB4-JMa loss abolish the ability of NRG1 to repress *GFAP* expression following FGF removal. Repression is specific to glial gene *GFAP*, as neuronal genes *DCX* and *NeuN* are unaffected by NRG1 treatment. Gene expression is normalized to reference gene *GAPDH*. *p < 0.05 by Wilcoxon test. (n = 7 WT, n = 7 PARP1 KO, n = 6 ErbB4 KO, and n = 7 JMa$^{-/-}$ independent cultures) **(C)** Knockdown of *PARP1* in WT NPCs prior to FGF removal abolishes repression of *GFAP* expression by NRG1. *p < 0.05 by Wilcoxon test. Gene expression is normalized to reference gene *GAPDH* **(D)** NRG1 represses *GFAP*-promoter activity induced by FGF removal in WT NPCs. *p < 0.05 by Student's paired t-test (n = 6 cultures). Relative *GFAP*-luciferase values were normalized with *CMV*-Renilla. **(E)** PARP1 inhibition with Olaparib at the indicated concentrations increases *GFAP* expression in WT NPCs. Gene expression is normalized to reference gene *RPL19* (n = 6 independent cultures). *p < 0.05 by repeated measures one-way ANOVA.

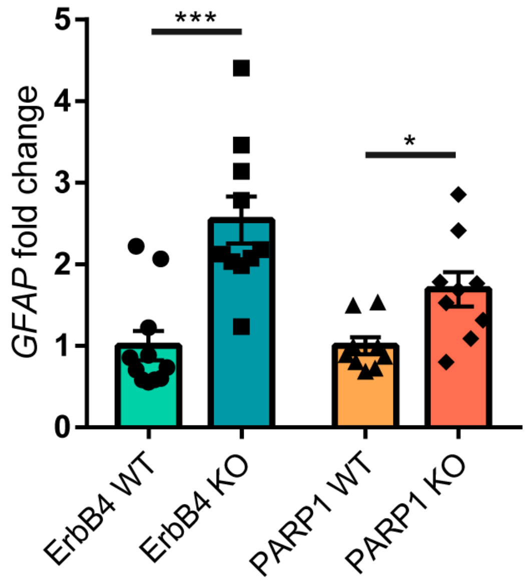


Figure 2.7 PARP1 or ErbB4 loss increase *GFAP* expression in the mouse cortex at birth. P0 cortex isolated from ErbB4 KO or PARP1 KO mice overexpress *GFAP* relative to their respective WT controls. Gene expression is normalized to *RPL19*. *p < 0.05, ***p < 0.001 by Mann-Whitney test.

Chapter 3: PARP1 Regulates Cajal-Retzius Cell Development and Neural Precursor Cell Adhesion[2]

3.1 Introduction

Poly (ADP-ribose) Polymerase 1 (PARP1) is a ubiquitously expressed enzyme that plays roles in a variety of key biological processes, including DNA repair, inflammation, transcription, and programmed cell death (Kraus, 2008; Krishnakumar and Kraus, 2010b; Jubin et al., 2017). PARP1 exerts its functions by protein PARylation, a post-translational modification consisting of the covalent attachment of ADP-ribose polymers (PAR) to itself and other proteins using NAD^+ as a substrate (Krishnakumar and Kraus, 2010b). Extensive evidence implicates PARP1 in a number of nervous system diseases, including neurodegenerative disorders (Mao and Zhang, 2021), ischemic stroke (Endres et al., 1997; Chiarugi, 2005), glioma (Galia et al., 2012; Murnyák et al., 2017), epilepsy (Kim et al., 2014), traumatic brain injury (Stoica et al., 2014), and psychiatric disorders (Szebeni et al., 2016). Furthermore, pharmacological inhibition of PARP1 *in vivo* causes defects in long-term memory (Goldberg et al., 2009), while complete loss of PARP1 causes impaired short-term memory formation (Hong et al., 2019). Additionally, adult PARP1 KO mice have a reduced brain weight, altered neuronal proliferation within the brain's dentate gyrus (Plane et al., 2012) and subventricular zone (Hong et al., 2019), and display

---

[2] This chapter reflects the following manuscript:

Nelson, MN, Hoff, JD, Zeese, ML, Corfas, G. Poly (ADP-Ribose) Polymerase 1 Regulates Cajal-Retzius Cell Development and Neural Precursor Cell Adhesion. In revision with *Frontiers in Cell and Developmental Biology*.

schizophrenia-like behaviors, including defects in pre-pulse inhibition, decreased social interaction, and increased anxiety-like behaviors (Hong et al., 2019). Accordingly, human studies have linked mutations in genes affecting PARylation to episodic psychosis, intellectual disability, peripheral neuropathy, ataxia, and increased risk of stroke (Najmabadi et al., 2011; Danhauser et al., 2018; Meng et al., 2018; Durmus et al., 2021).

Despite evidence that PARP1 dysregulation contributes to aberrant brain function and related disorders in humans and mice, very few studies have examined the roles of PARP1 in brain development. It has been reported that PARP1 loss in mice causes enlarged ventricles at E14.5, increases cortical cell death at E16.5 and E18.5, and impairs the proliferation of neural stem cells derived from embryonic telencephalon (Hong et al., 2019). This study also found that PARP1 influences neural stem cell differentiation by repressing a glial cell fate *in vitro* (Hong et al., 2019). While it is apparent that loss of PARP1 has detrimental effects on brain development and function, little is known about its role in the regulation of neuronal migration and cortical patterning. Furthermore, no studies have yet assessed the effect of PARP1 loss on gene expression in the embryonic brain.

Here, we show that PARP1 loss causes alterations in early-born neuron migration, decreases cortical thickness, and increases neuronal density in deeper cortical layers at birth. These changes are associated with increased Cajal-Retzius (CR) cell abundance and *Reln* mRNA levels in the PARP1 KO embryonic brain and neural progenitor cells (NPCs) derived from the embryonic mutant telencephalon. Accordingly, PARP1 KO NPCs show excess adhesion to N-cadherin, likely through Reelin signaling. Additionally, RNA-sequencing of the PARP1 KO E15.5 cortex demonstrates that PARP1 loss increases expression levels of many genes associated with neuronal migration and adhesion during embryonic brain development. Taken together, our findings uncover a new role for PARP1 in regulating CR cell development, neuronal migration, and cell adhesion.

3.2 Materials and Methods

3.2.1 PARP1 KO Mice

The PARP1 KO mouse line 129S-*Parp1*^{tm1Zqw}/J[64] was obtained from the Jackson Laboratory and maintained on a 129S1/SvImJ background. All animals were kept under a 12/12 hr light/dark cycle and allowed food ad libitum. Animal procedures were reviewed and approved by the University of Michigan Institutional Animal Care and Use Committee. Embryonic dating was performed with vaginal plugging denoted as embryonic day 0.5 (E0.5). Following vaginal plugging, females were separated and sacrificed at the indicated time points.

3.2.2 Cell Culture and Treatments

For primary NPC cell cultures, pregnant females were euthanized via cervical dislocation. Telencephalons were dissected from E14.5 embryos in ice-cold phosphate-buffered saline (PBS), meninges were removed, and cortices were dissociated into single cells with StemPro Accutase (ThermoFisher) for 5 min. NPCs were seeded as neurospheres in T75 flasks at 500,000 cells/mL and expanded for 2 days in NPC media (DMEM with GlutaMAX, 1% penicillin/streptomycin, and 2% B27 without RA) supplemented with epidermal growth factor (EGF, 20 ng/mL) and basic fibroblast growth factor (bFGF, 20 ng/mL) in a humidified 5% CO_2/95% air incubator at 37°C. Half of the media was changed every day, with replenishment of EGF and bFGF each day. On day 3, neurospheres were dissociated with Accutase into a single cell suspension and plated in NPC media supplemented with bFGF. Plates were pre-prepared by incubating in Poly-L-Lysine (Sigma) 30 min or overnight followed by Fibronectin (1 µg/mL, Corning) for 2 hrs. Experiments on adherent NPCs were performed on day 2 after plating unless otherwise indicated.

To inhibit PARP1 enzymatic activity, NPCs were treated with Olaparib (a gift of C. Brenner) at the indicated concentrations (30, 50, or 100 nM) for 48 hrs starting on day 1 following plating. Half of the media was replenished each day, and cells were re-treated with Olaparib every 24 hrs. For Reelin-positive cell quantification, NPCs were plated on coverslips and treated with 50 nM Olaparib for 72 hrs. To test Olaparib efficacy, NPCs were pre-treated with 30, 50, or 100 nM Olaparib for 1 hr prior to 10 min treatment with 50 µM H_2O_2. To assess RNA stability, NPCs were treated with Actinomycin D (10 µg/mL) for 2 hrs or 4 hrs in the presence or absence of Olaparib (100 nM). HEK293T cells were maintained in DMEM with GlutaMAX, 1% penicillin/streptomycin, and 10% fetal bovine serum in a humidified 5% CO_2/95% air incubator at 37°C.

3.2.3 RNA-Sequencing and Analysis

Pregnant females were euthanized via cervical dislocation. E15.5 embryos were placed in ice-cold PBS, brains were dissected, and the cortical hemispheres were isolated. The meninges and ganglionic eminences were removed from the cortex, and the tissue was stored in RNAlater (Invitrogen) until RNA extraction. RNA was extracted from wild-type (WT) and PARP1 KO dorsal cortex using the Qiagen RNeasy Mini Kit with on-column DNase digestion (Qiagen), then analyzed with a BioAnalyzer to measure RNA quality. RNA with RNA integrity numbers (RINs) greater than 8 were sequenced (n = 4 of each genotype). Non-strand specific polyA-selected cDNA libraries were prepared. Single-end sequencing was then completed with read lengths of 50 nucleotides using an Illumina HiSeq-4000 Sequencing System. cDNA library preparation and sequencing were carried out by the University of Michigan DNA Sequencing Core. Sequences were mapped to the mouse genome (mm9) using HISAT, transcript counts obtained with HTseq-count, and differential gene expression analysis completed using DESeq2. All analysis was carried out with Galaxy (www.usegalaxy.org). The volcano plot was generated with RStudio. P-values adjusted for multiple comparisons (q-value) < 0.05 indicated genes with statistically significant differences. Gene Ontology Analysis of dysregulated genes was performed using the Panther Classification system (www.pantherdb.org). Protein interaction analysis was completed using Cytoscape software.

3.2.4 Immunofluorescence and Quantification

Whole brains were dissected from E15.5 embryos or P0 pups in ice-cold PBS, fixed in 4% paraformaldehyde (PFA) in 1x PBS for 24 hrs, cryoprotected in 30% sucrose, embedded in OCT Compound (Fisher), and snap frozen in isopentane on dry ice. Frozen brains were cryosectioned at a thickness of 14 µm onto Superfrost plus slides (Fisher). Sections were blocked in 5% Normal Goat Serum (Jackson ImmunoResearch) with 2% Triton in PBS for 1 hr, then incubated overnight at 4°C in primary antibody diluted in blocking buffer. The next day, sections were washed 3x in 1x PBS, incubated in corresponding Alexa-Fluor secondary antibodies (Invitrogen) diluted in blocking buffer (1:500) 1-2 hrs, and coverslipped with Fluoro-Gel II with DAPI (Electron Microscopy Services). Confocal z-stack images through the full depth of each section with a z-step of 2 µm were taken at a magnification of 20x or 40x with a Leica SP8 confocal microscope. For each biological replicate, cells from 3 sections and 3 images per section were quantified by an individual blind to the genotype and averaged. Cell number and area quantification were completed using Fiji software.

For Reelin NPC immunostaining, NPCs were plated on round glass coverslips. On day 2 after plating, coverslips were fixed in 4% PFA for 10 min, washed 3x in 1x PBS, then blocked in 10% Normal Goat Serum (Jackson ImmunoResearch) with 0.2% Triton in PBS for 1 hr. Coverslips were then incubated in Reelin primary antibody overnight diluted in blocking buffer. Following 3 washes in 0.2% triton in PBS, coverslips were incubated in the corresponding Alexa-Fluor secondary antibody diluted in blocking buffer (1:500, Invitrogen) for 1-2 hrs, then mounted on slides with Fluoro-Gel II with DAPI (Electron Microscopy Services). For Reelin-expressing cell quantification in WT and PARP1 KO NPCs, images of whole coverslips were taken with a Leica SP8 confocal at 20x magnification with image stitching. For quantification of Reelin-expressing cells after PARP1 inhibition with Olaparib (50 µM) or shRNA-mediated knockdown, images of whole coverslips were taken with a Nikon TE300 inverted fluorescent microscope equipped with Stereoinvestigator (MBF Bioscience), using the slide scan module. For each biological replicate, all Reelin-expressing cells from 3 coverslips were quantified and averaged. Quantification and mean fluorescence intensity analysis were completed using Fiji. The following primary antibodies and concentrations were used in this study: mouse anti-Reelin (1:500, clone G10, Millipore #MAB5364), rabbit anti-TBR1 (1:250, Abcam #ab31940), and rat anti-CTIP2 (1:500, Abcam #ab18465).

3.2.5 Cresyl Violet Staining and Brain Volume Quantification

WT and PARP1 KO littermates were sacrificed at birth, and their brains were dissected then fixed in 4% paraformaldehyde for 24 hrs. Brains were cryoprotected in 30% sucrose, embedded in OCT, then cryosectioned with a section thickness of 70 µm. Serial sections were stained with 0.5% cresyl violet using standard procedures. Slides were digitally scanned with the assistance of the University of Michigan In-Vivo Animal Core (IVAC), and surface areas of each brain section were quantified with Fiji. To calculate the brain volume, the surface areas of each section were summed together and multiplied by the section thickness. Cortical surface area and thickness were quantified with Fiji.

3.2.6 EdU Labeling *in vivo*

Pregnant dams from PARP1 heterozygous crosses were injected intraperitoneally with EdU (50 mg/kg) at E13.5 or E15.5. Littermate P0 pups were transcardially perfused at birth with PBS followed by 4% PFA, then whole brains were dissected and postfixed in 4% PFA for 24 hrs. Brains were then either cryoprotected in 30% sucrose, embedded in OCT, and cryosectioned at a thickness of 20 µm or embedded in 4% agarose and sectioned on a vibratome at a thickness of 50 µm. EdU was visualized using the Click-iT EdU Cell Proliferation Kit (Invitrogen), following the manufacturer's protocol. Nuclei were counterstained with Hoechst 33342 (1:2000, Invitrogen). Slides were coverslipped using Aqua Poly/Mount (Polysciences). Confocal z-stack images through the full depth each section were taken at 20x magnification with a Leica SP8 confocal microscope. Each image was separated vertically into 8 bins of equal size and the number of EdU positive cells within each bin was counted by an individual blind to the genotype. For each biological replicate, 3 sections and 3 images per section were quantified and averaged. Quantification was completed using Fiji.

3.2.7 RNA Isolation and RT-qPCR

RNA was isolated from the cortex after removal of meninges and ganglionic eminences or from cultured NPCs using a Qiagen RNeasy Kit or ThermoFisher PureLink RNA Mini Kit, following the manufacturer's instructions. RNA extraction from tissue was completed with on-column DNase digestion (Qiagen). Equal amounts of RNA (400 ng -1 µg) were reverse transcribed into cDNA using an iScript cDNA Synthesis Kit (Bio-Rad) following manufacturer's instructions. cDNA was diluted 5-fold for the PCR reaction. Quantitative PCR was performed using iTaq SYBR Green Supermix (Bio-Rad) with a Bio-Rad CFX96 Thermocycler. Each sample was run in duplicate. Each well of the 96-well plate contained 5 µL iTaq SYBR Green Supermix, 2.5 µL diluted cDNA, and 3 pmol of each forward and reverse primer. The cycling conditions were as follows: 95°C for 30 s followed by 39 cycles of 95°C for 5 s and 60°C for 30 s. Normalized Gene Expression (NGE) was calculated using the efficiency of each primer with the following formula: [$\text{efficiency\_target}^{-CT_{target}}/\text{efficiency\_reference}^{-CT_{ref}}$]. Fold changes in mRNA level were then calculated relative to controls for each experiment. All primer sequences are in Table 1.

3.2.8 shRNA-mediated PARP1 Knockdown

Lentiviral packaging plasmids and scramble shRNA or *Parp1* shRNA constructs were transfected into HEK 293T cells using Lipofectamine 3000 (Invitrogen). Lentiviral supernatants were collected and concentrated using Lenti-X Concentrator (Clontech) and titered with puromycin selection. To test the efficacy of PARP1 protein knockdown, NPCs were transduced with scramble or *Parp1* shRNA-expressing lentivirus at multiplicity of infection (MOI) 2 for 48 hrs. To assess the effects of PARP1 knockdown on gene expression, NPCs were transduced with scramble or *Parp1* shRNA-expressing lentivirus at MOI 3 for 48 hrs, then media was replaced with fresh NPC media and cells were lysed 24 hrs later. bFGF (20 ng/mL) was supplemented every 24 hrs. *Parp1* shRNA plasmid was obtained from Sigma (clone ID NM_007415.2-3021s21c1) with the following sequence: 5'-CCGGGAGTACATTGTCTACGACATTCTCGAGAATGTCGTAGACAATGTACTCTTTTTG-3'. Lentiviral packaging plasmids and scramble shRNA plasmid were gifts of S. Iwase.

3.2.9 Luciferase Assay

NPCs were co-transfected with a *Reln*-promoter luciferase construct that contained 2600 bp upstream of the *Reln* transcription start site (Chen et al., 2002) (a gift of D. Grayson) and *CMV*-Renilla at a 50:1 ratio with Lipofectamine 3000. NPCs were treated with either Olaparib (100 nM) for 24 hrs or 48 hrs or Valproic Acid (VPA, 1 mM) for 12 hrs or 24 hrs. Luciferase activity was measured using a Dual-Luciferase Assay Kit (Promega), following the manufacturer's instructions. Luciferase intensity was measured with a BioTek plate reader. Final values were obtained by normalizing firefly luciferase to Renilla luciferase. Technical replicates were obtained in triplicate.

3.2.10 Western Blot

For Reelin western blots, NPCs were lysed in RIPA buffer (Sigma) containing protease and phosphatase inhibitors. Equal amounts of protein were diluted in 4x Laemmli buffer with 10% β-mercaptoethanol (BME) and run on 4-15% SDS polyacrylamide gels. For western blotting of conditioned media, protein concentrations were quantified, and equal amounts of protein were diluted in 4x Laemmli buffer with 10% BME and run on 6% SDS polyacrylamide gels. Reelin in conditioned media was normalized to GAPDH in corresponding cell lysates. Gels were transferred onto PVDF membranes overnight at 4°C (wet transfer at 40 mA). Blots were subsequently blocked in Intercept Blocking Buffer (Licor) (for Reelin) or 5% Bovine Serum Albumin (BSA) (for GAPDH), incubated in primary antibody for 3 hrs, washed in 0.2% tween in PBS, then incubated in HRP-conjugated secondary antibody (Cell Signaling Technology). For pDab1 and PARP1 western blot, samples were lysed in RIPA buffer (Sigma) containing protease and phosphatase inhibitors. For PAR western blot, PARG inhibitor (1 μM ADP-HPD) was also added to RIPA lysis buffer. Equal amounts of protein were diluted in 4x Laemmli buffer with

10% BME and run on 7.5% or 8% SDS polyacrylamide gels, then transferred for 1.5 hrs onto PVDF membrane with a semi-dry transfer unit. Blots were then blocked in 5% BSA and immunoblotted for primary antibody overnight. The next day, blots were washed in 0.2% tween in PBS, then incubated in HRP-conjugated secondary antibody (Cell Signaling Technology). All blots were exposed with Pierce ECL Chemiluminescent Substrate and imaged on a BioRad Chemidoc.

The following primary antibodies and concentrations were used: mouse anti-Reelin (1:2000, Millipore #MAB5364), mouse anti-PAR (1:1000, Trevigen #4335), mouse anti-GAPDH (1:2000, ThermoFisher #MA5-15738), rabbit anti-PARP1 (1:2000, Cell Signaling Technology #9532) and rabbit anti-pDab1 (1:1000, Cell Signaling Technology #3325). For all antibodies used, linearity was assessed, and the amount of total protein loaded onto gels was within the linear range of the antibody. Band densities were quantified using Image Lab software (Bio-Rad) and normalized to internal control GAPDH.

3.2.11 Reelin Conditioned Media

HEK 293T cells were transfected with pcDNA3 or Reelin ORF (10 kb)-expressing plasmids (D'Arcangelo et al., 1997) (Addgene plasmid #122443) with Lipofectamine 3000 (Invitrogen). 24 hrs after transfection, media was replaced with serum-free media. 24 hrs later, conditioned media was collected and stored at -80°C. For pDab1 induction, NPCs were treated with 200 or 400 µL conditioned media for 10 min. Conditioned media from WT and KO NPCs was collected from adherent cultures 48 hrs after plating. For induction of pDab1, protein secretion was blocked by pre-treating NPCs with Brefeldin A (BFA, 0.75 µg/mL, Sigma) for 3 hrs. NPCs were then treated with 2 mL conditioned media from wild-type NPCs (supplemented with 0.75 µg/mL BFA) for 20 min.

3.2.12 Chromatin Immunoprecipitation

PARP1 chromatin immunoprecipitation was carried out using a SimpleChIP Plus Enzymatic Chromatin IP Kit with magnetic beads (Cell Signaling Technology, #9005), following the manufacturer's instructions. 2 μg of each antibody was incubated with magnetic beads overnight, and 0.5 μL micrococcal nuclease was used per sample to digest chromatin. The following antibodies were used: rabbit anti-PARP1 (Cell Signaling Technology, #9532S), rabbit anti-Histone H3 (CST #4620), and normal rabbit IgG (CST #2729). Primer sequences are indicated in Table 3.2.

3.2.13 RNA Immunoprecipitation

RNA immunoprecipitation was completed as described previously (Dahm et al., 2012) with the lysate pre-clearing step. Briefly, NPCs were lysed in passive lysis buffer containing protease, phosphatase, and RNAse inhibitors. Pre-swelled Protein A Sepharose beads were incubated with 30 μg rabbit anti-PARP1 antibody (CST #9532S) overnight at 4°C. Following 5 washes in NT2 buffer, beads were resuspended in RNAse inhibitors and 1 mg NPC lysate, incubating at room temperature for 2.5 hours. Following 5 washes in NT2 buffer, beads were resuspended in RNase-free DNase and incubated at 37°C for 10 min. Beads were then resuspended in proteinase K at 55°C for 30 min to release ribonucleoprotein complexes. Beads were pelleted, and the supernatant was collected. RNA was then isolated from the supernatant via phenol-chloroform extraction, eluting in 11 μL H_2O per sample. 10 μL RNA was reverse transcribed using the iScript cDNA synthesis kit in a 20 μL reaction volume. Quantitative PCR for the *Reln* transcript was completed in 50 μL reaction volume with 20 μL cDNA, 25 μL iTaq SYBR green, 1.5 μL of each forward and reverse primer (10 μM stock), and 2 μL H_2O with standard iTaq qPCR cycling parameters for 40 cycles. Primer sequences are indicated in Table 3.1. Fold enrichment was quantified relative to rabbit IgG control antibody.

3.2.14 Atomic Force Microscopy (AFM)

AFM force-distance (F-D) measurements were performed using a TT-AFM (AFM Workshop, South Carolina, USA) using contact mode AFM probes of 0.2 N/m nominal stiffness (PPP-CONTAuD from Nanosensors). Probes were functionalized by incubating the tip overnight at 4°C in 100 µg/mL N-cadherin. Immediately prior to measurements, probes were rinsed 4x in PBS. Probe stiffness was determined by the Sader method in air (Sader, 1998). The optical lever sensitivity was determined in fluid for each sample using the thermal noise method (Heim et al., 2004; Hutter, 2005).

Cells were cultured on round glass coverslips. Approximately 30 min prior to the beginning of measurements, a cell-coated coverslip was epoxied to an AFM stub and transferred to a fluid AFM imaging chamber with NPC media containing 10 mM HEPES and bFGF (20 ng/mL) and maintained at 37°C throughout measurements, which were taken within approximately 1 hr for each sample. For conditioned media treatment, cells were treated with control or Reelin conditioned media for 30 min prior to the beginning of measurements. For each F-D measurement, the probe was engaged with the surface with approximately 9 nN force for 5 s before retracting at a rate of 2000 nm/s. F-D curves were evaluated using a custom Matlab script to objectively parse the maximum adhesion force developed, the adhesive force for stepwise detachments, and integrated work performed for each trace. These experiments were completed in collaboration with the Single Molecule Analysis in Real-Time (SMART) Center at the University of Michigan. Statistical outliers were excluded from the datasets (<Q1-1.5*interquartile range (IQR) or >Q3+1.5*IQR). Statistical significance in average values was assessed via Mann-Whitney analysis and differences in histogram distributions were assessed with a Kolmogorov-Smirnov test.

3.2.15 Statistics

All statistical analyses were performed using GraphPad Prism version 7.0, except for two-way ANOVA analysis of litter size, genotype, and P0 brain weight, which was performed using SAS in collaboration with the University of Michigan Center for Cancer Biostatistics. Bars for each of the graphs represent mean ± standard error of the mean. Legends for each figure contain statistical tests performed, number of biological replicates, and specific significance values.

3.3 Results

3.3.1 PARP1 KO mice have brain development defects

Previous studies reported that brain size is reduced in PARP1 KO mice from P11 to adulthood (Plane et al., 2012; Hong et al., 2019). We now find that both body and brain weights are smaller in PARP1 KO mice at birth (Figure 3.1A,B), but that brain weight is reduced to a larger relative extent than the mass of the entire body (Figure 3.1C). While there is an inverse correlation between litter size and brain weight in wild-type mice, two-way ANOVA analysis showed no significant interaction between genotype and litter size (Figure 3.1D), indicating that decreased brain weight in PARP1 KO animals is driven exclusively by genotype. To assess whether decreased brain weight is due to a reduction in brain size in a specific area, we collected serial coronal sections from WT and PARP1 KO P0 brains, measuring the surface area of each section on the rostral-caudal axis. We found that the surface area of PARP1 KO brain sections tends to be smaller than their WT littermates at each level along the rostral-caudal axis, indicating that brain size is reduced overall rather than in a specific area (Figure 3.1E). This corresponds with a reduction in brain volume in PARP1 KO animals (Figure 3.1F). Finally, we found that decreased brain size in PARP1 KO mice is associated with a reduced cortical surface area and thickness (Figure 3.1G-I).

These findings suggest that PARP1 loss alters brain development. One of the key events in brain formation is neuronal migration, the process by which newborn neurons reach their final destinations in the cortex (Valiente and Marín, 2010). During mouse brain development, neurogenesis begins near E11.5 and concludes around E17.5 (Guillemot, 2005). To determine if the migration of early-born neurons is affected by the loss of PARP1, we injected EdU at E13.5 and analyzed the distribution of the labeled cells at birth. PARP1 loss leads to a decreased number of early-born neurons in the deepest portion of the cortex (Figure 3.2A,B) without altering the total number of EdU-positive cells (Figure 3.2C). In contrast, we found no differences in the position of neurons born at E15.5 (data not shown). Similar to previous, we observed a reduction in cortical thickness in PARP1 KO animals (Figure 3.2D). To further characterize the impact of PARP1 KO on cortical development, we immunostained brains at birth for markers of layer V and VI neurons (CTIP2 and TBR1, respectively) (Figure 3.2E). We found that PARP1 loss increases neuronal density but decreases the surface area each layer occupies, resulting in a normal number of more densely packed cells (Figure 3.2F-H). Together, these results suggest that reduced brain weight in PARP1 KO mice reflects altered neuronal migration, decreased cortical thickness, and increased cell density.

3.3.2 Loss of PARP1 increases the expression levels of genes associated with cell migration and adhesion in the E15.5 cortex

To gain insights into the mechanisms through which loss of PARP1 affects brain development, we performed RNA-sequencing of the E15.5 wild-type and KO cortex and identified 48 genes whose levels are significantly altered by PARP1 loss-of-function. Remarkably, in contrast to reports that PARP1 promotes transcription in neuronal cells (Tapia-Páez et al., 2008; Hau et al., 2017; Azad et al., 2018), most of the changes in expression in the brains of PARP1 KOs reflect increases in mRNA levels (Figure 3.3A). RT-qPCR validated the altered expression of *Reln*, *Nav1*, *Tnc*, and *Txnip* in the embryonic cortex (Figure 3.3B). Furthermore, a subset of these genes (*Tnc* and *Reln*) continue to be increased to a similar extent in the P0 cortex of PARP1 KO mice (data not shown). The genes altered by PARP1 loss encode proteins involved in various processes, with a particular enrichment for cell adhesion, axon development, dendrite development/morphogenesis, and cell migration (Table 3.3). Similarly, molecular interaction network analysis of the differentially expressed genes suggested direct interactions among proteins which comprise parts of the brain extracellular matrix (ECM) (Figure 3.3C), an important component in the regulation of neuronal migration and lamination (Franco and Müller, 2011). These include Laminins (*Lamb1*, *Lama2*, and *Lamc1*), Reelin (*Reln*), Tenascin C (*Tnc*), Versican (*Vcan*), and Phosphacan (*Ptprz1*). These proteins in turn interact with Collagen Type XII Alpha 1 Chain (*Col12a1*) and several cell adhesion molecules (*Astn1*, *Nrcam*, and *Dscaml1*). Taken together, these findings suggest that the brain defects in neonatal PARP1 KO mice could result from alterations in ECM function.

3.3.3 Loss of PARP1 results in an increased number of Cajal-Retzius cells in the cortex

Reln encodes the glycoprotein Reelin, which is specifically expressed by CR cells within the developing brain's marginal zone (Ogawa et al., 1995). Reelin is critical for cortical layering and neuronal migration, and mice lacking Reelin (known as *Reeler*) have severely malformed brains and die prematurely (Meier and Hoag, 1962; Hamburgh, 1963; Goffinet et al., 1984). Quantification of Reelin-expressing cells in the E15.5 brain revealed an increased number of CR cells along the marginal zone and a reduction in marginal zone area per field in PARP1 KO brains, resulting in increased density of CR cells within the marginal zone (Figure 3.4A-D), suggesting that PARP1 regulates the abundance of CR cells in the embryonic brain. To test if the number of CR cells is increased through the entire process of embryonic neurodevelopment, we immunostained P5 brains for Reelin, finding a similar increase in the abundance and density of CR cells (Figure 3.4E-H).

3.3.4 Loss of PARP1 increases levels of genes expressed by CR cells and Reelin protein in NPCs

To interrogate the mechanism by which PARP1 regulates CR cell numbers, we analyzed telencephalon-derived NPCs in culture. RT-qPCR demonstrated that neurospheres and adherent NPCs from PARP1 KO mice have increased mRNA levels of several genes expressed by CR cells, including *Reln*, the *Trp73* isoforms *TAp73* and *ΔNp73,* as well as *Car10* and *Calb2* (Yamazaki et al., 2004) (Figure 3.5A, B). Moreover, acute PARP1 knockdown in wild-type adherent NPCs using *Parp1* shRNA expressing lentivirus (Figure 3.5C) led to a significant increase in *Reln*, *Calb2*, and *Car10*, with a trend of increased *Trp73* isoforms (Figure 3.5D). Furthermore, treatment with the PARP1 inhibitor Olaparib, which blocks PARylation (Figure 3.5E), had similar effects on the expression of *Reln*, *Car10*, and *Calb2* in wild-type NPCs

(Figure 3.5F). These results indicate that PARP1 PARylation influences the mRNA levels of genes expressed by CR cells.

Reelin is proteolytically cleaved at three sites, resulting in multiple protein fragments (Lambert de Rouvroit et al., 1999; Krstic et al., 2012) (Figure 3.6A). CR cells regulate neuronal migration in part through secretion of Reelin, which binds to its receptors Apolipoprotein E Receptor 2 (ApoER2) and Very Low Density Lipoprotein Receptor (VLDLR) on nearby migrating neurons (Hirota and Nakajima, 2017). Full-length Reelin is hypothesized to be the most catalytically active, whereas N-terminal (N-t) cleavage (Kohno et al., 2009; Ogino et al., 2017) and loss of the C-terminal region of Reelin (Nakano et al., 2007; Kohno et al., 2015) reduce its catalytic activity. To identify the Reelin fragments expressed in NPC cultures, we immunoblotted NPC lysates and conditioned media for Reelin using an N-terminal antibody. Analysis of cell lysates demonstrated a significant upregulation in the 430 kDa full-length Reelin and the 160 kDa NR2 fragment in PARP1 KO NPCs (Figure 3.6B,C). Similarly, western blotting of conditioned media collected from NPCs showed increased immunoreactivity for the full length Reelin and the NR6 and NR2 fragments, indicating increased Reelin secretion in PARP1 KO NPC cultures (Figure 3.6D,E). Importantly, similar to the effects of medium conditioned by HEK293T cells transfected with a Reelin expression plasmid (Figure 3.7A,B), NPC conditioned medium induced Dab1 tyrosine phosphorylation (Figure 3.7C), a key component of the intracellular signaling cascades initiated by Reelin (Hiesberger et al., 1999; Lambert de Rouvroit et al., 1999), indicating that NPCs secrete active Reelin. Furthermore, the state of Dab1 phosphorylation in NPCs was reduced when cells were incubated with Brefeldin A (BFA) to block protein secretion (Figure 3.7C), indicating that the Reelin acts in a paracrine or autocrine fashion in NPC cultures.

3.3.5 PARP1 loss of function increases CR cell abundance in NPC cultures

To explore if the changes in Reelin levels in tissue culture reflect differences in levels of Reelin expression per cell or, alternatively, in the number of Reelin expressing cells, we immunostained adherent NPCs for Reelin. Cultures of PARP1 KO NPCs contained a higher proportion of Reelin-positive cells without changes in the intensity of Reelin staining per cell (Figure 3.8A-C). Similarly, shRNA-mediated PARP1 knockdown and pharmacological PARP1 inhibition increased the proportion of Reelin-positive cells in wild-type NPC cultures (Figure 3.8D,E). These results suggest that PARP1 and its enzymatic function are critical for regulating CR-like cell abundance *in vitro* rather than having a direct transcriptional effect on gene expression in existing CR cells. This finding is consistent with results from *in situ* hybridization showing that the number of *Reln* transcripts per CR cell in the E15.5 brain is not altered after PARP1 loss (data not shown). Mechanistically, this finding is further supported by the observation that the activity of a firefly luciferase *Reln* promoter reporter construct (Chen et al., 2002) transfected into wild-type NPCs is not altered by PARP1 inhibition (Figure 3.9A). In addition, we observed no alteration in *Reln* mRNA stability in PARP1 KO NPCs (Figure 3.9B) and found no evidence of PARP1 binding to the *Reln* gene or its mRNA via chromatin or RNA immunoprecipitation, respectively (Figure 3.9C or data not shown). Together, these results suggest that PARP1 regulates the abundance of Reelin-expressing cells *in vivo* and *in vitro* and that this function of PARP1 is not mediated by direct transcriptional regulation of the *Reln* gene.

3.3.6 PARP1 loss increases NPC adhesiveness to N-cadherin

To test if the increased levels of Reelin present in media conditioned by PARP1 KO NPCs has functional consequences, we focused on cell adhesion since a prior study showed that Reelin increases neuronal adhesion to N-cadherin using atomic force microscopy (AFM)

(Matsunaga et al., 2017). Using a similar approach (Figure 3.10A), we found that treatment with medium conditioned by Reelin-transfected HEK293T cells increases adhesion of N-cadherin in wild-type NPCs (Figure 3.10B-D). Furthermore, we found that adhesion to N-cadherin is increased in PARP1 KO NPCs (Figure 3.11), suggesting that the increased Reelin levels alter the adhesive state of PARP1 KO cells.

3.4 Discussion

Our findings uncover a new role for PARP1, i.e., the regulation of CR cell development. Loss of PARP1 leads to increases in the number of Reelin-expressing cells in the cortex *in vivo* and cultured NPCs. While relative increases in *Reln* transcript levels were demonstrated in bulk RNA from the brain of PARP1 KOs, we did not detect any effects of PARP1 loss-of-function on *Reln* transcript levels per CR cell *in vivo* or identify any evidence of PARP1 binding to the *Reln* gene or its mRNA to directly influence *Reln* transcription in cultured cells. These results suggest that PARP1 influences CR cell generation rather than more directly impacting the expression of CR cell genes. The mechanism by which this occurs is unclear, but the overabundance of CR cells in PARP1 KO brains at E15.5, together with the fact that these cells do not undergo programmed cell death until P8 in wild-type mice (del Río et al., 1995), indicate that PARP1 does not influence the number of CR cells by regulating the time-course of apoptosis. A potential mechanism by which PARP1 could regulate CR cell generation is through miRNAs. PARP1 has been shown to regulate the expression of many miRNAs (Chacon-Cabrera et al., 2015; Nozaki et al., 2018; Wang et al., 2019). Additionally, disruption of miRNA biogenesis through Dicer depletion in Nestin-expressing cells causes a similar increase in CR cell abundance and *Reln* expression (McLoughlin et al., 2012). Similar to findings in PARP1 KOs (Hong et al., 2019), McLoughlin *et al.* reported that Dicer depletion reduces cell proliferation and increases apoptosis in the E15.5 cortex. Further studies will be necessary to test the links between PARP1, miRNAs, and CR cell generation.

In contrast to PARP1 KOs, in which we found the number of Reelin-positive cells to be increased in the marginal zone, its normal site of expression, previous studies explored the effects of ectopic Reelin expression during embryonic development. The Nakajima group tested the effects of ectopic Reelin expression in deeper cortical layers using *in utero* electroporation of a Reelin-expressing plasmid into the lateral ventricle of E14.5 embryos (Kubo et al., 2010; Matsunaga et al., 2017). In line with our findings that Reelin overexpression is associated with increased NPC adhesion, this ectopic Reelin expression induced the formation of aberrant neuronal aggregates that appeared to be mediated by N-cadherin-dependent neuronal adhesion. In a transgenic mouse line with Nestin-driven Reelin expression, ectopic Reelin expression in ventricular and subventricular zone NPCs was shown to increase NPC proliferation, possibly through alterations in Notch signaling, and to result in an increased number of TBR1, TBR2, and CTIP2-positive neurons (Lakoma et al., 2011). These findings contrast with previous observations in PARP1 KOs of decreased NPC proliferation (Hong et al., 2019) and our observation of increased TBR1 and CTIP2-positive neuronal density without changes in cell number. Taken together, these findings highlight the different consequences that Reelin overexpression has on brain development depending upon the timing, location, and nature of its overexpression.

Reelin influences cell migration in part through regulating cell adhesion via integrin (Sekine et al., 2012), L1 (Lutz et al., 2017), Nectins (Gil-Sanz et al., 2013), and N-cadherin (Franco et al., 2011; Matsunaga et al., 2017). In accordance with this, we found that Reelin treatment and PARP1 loss increase NPC adhesiveness to N-cadherin. Loss of PARP1 also increases the expression of other cell adhesion molecules in the embryonic brain, including *Nrcam*, *Dscaml1*, and *Astn1*. In line with reports that cell adhesion molecules are critical for normal neuronal migration (Valiente and Marín, 2010; Franco et al., 2011; Gärtner et al., 2012; Hirota and Nakajima, 2017; Mitsogiannis et al., 2021), the likely increase in cellular adhesion in the PARP1 KO cortex is associated with the increased neuronal density and decreased number of early-born neurons in the deeper layers of the brain. Interestingly, alterations in Reelin expression (Liu et al., 2001; Niu et al., 2008), cellular adhesion complex function (Seong et al., 2015), and neuronal density (Selemon and Goldman-Rakic, 1999) have all been previously associated with changes in dendritic arborization, and our findings indicate that PARP1 loss alters the expression of genes that are important for regulating dendritic development and morphology. Together, these findings indicate a possible connection between cellular adhesion, neuronal density, and dendritic morphology. Further studies will be needed to explore the mechanistic links between these findings and PARP1 function.

PARP1 KO mice display endophenotypes associated with Schizophrenia (Hong et al., 2019), and mutations in genes affecting PARylation have been linked to intellectual disability and episodic psychosis in humans (Najmabadi et al., 2011; Durmus et al., 2021). Likewise, defects in neurodevelopment and altered Reelin expression have been associated with schizophrenia and other neuropsychiatric disorders (Ayhan et al., 2011; Folsom and Fatemi, 2013; Muraki and Tanigaki, 2015). These observations raise the possibility that the brain development defects and schizophrenia-like behaviors in PARP1 KO mice may be a consequence of increased CR cell abundance. Intriguingly, both neuropsychiatric disorders (Selemon et al., 1998; Selemon and Goldman-Rakic, 1999; Chana et al., 2003) and reduced Reelin expression (Liu et al., 2001) have been associated with increased neuronal density in deeper layers of the cortex, similar to our findings in PARP1 KO mice. Together, these findings suggest that the regulation of Reelin expression during embryogenesis is critical for normal development, and slight increases or decreases in Reelin levels or patterns of expression might impact brain development in ways that lead to neuropsychiatric disorders.

3.5 Conflict of Interest Statement

G.C. is a scientific founder of Decibel Therapeutics, has an equity interest in and has received compensation for consulting. The company was not involved in this study. M.M.N. and J.D.H. declare no competing financial interest.

3.6 Author Contributions Statement

G.C. and M.M.N. designed the study. M.M.N. performed most experiments and analyzed the data; J.D.H. performed the AFM experiments. M.Z. provided technical assistance. G.C. and M.M.N. interpreted the data and wrote the manuscript with the help of the other authors.

3.7 Funding

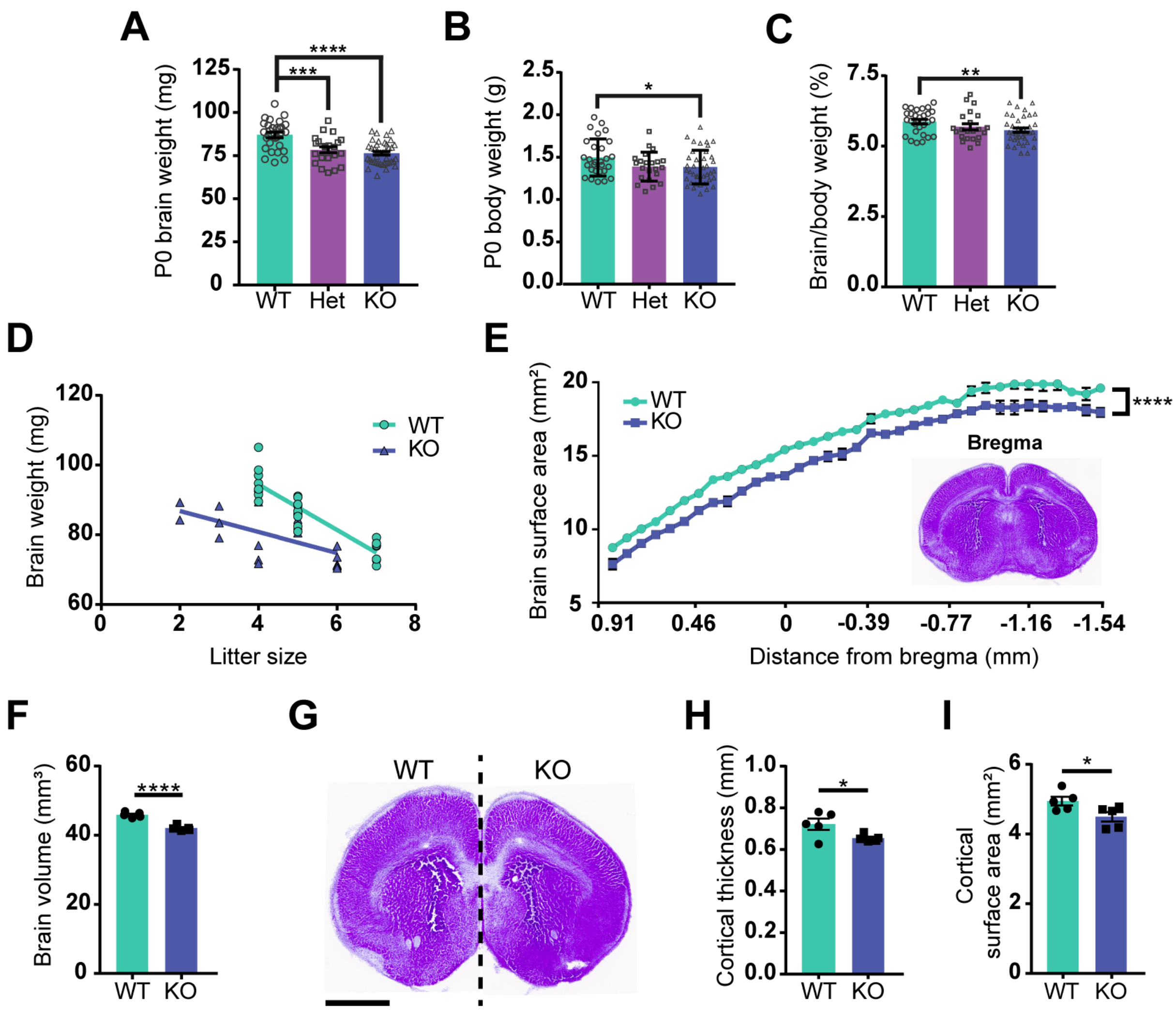
This study was supported in part by the NIH Training Grant 5T32NS076401 and Hearing, Balance, and Chemical Senses (HBCS) Training Grant 5T32DC000011.

3.8 Acknowledgments

We thank Eric Cho and Michael Hayes for assistance with the mouse colony and genotyping, Robert Doherty, Nada Jaradat, and Dr. Anna Kane for technical assistance, and Dr. David Kohrman, Robert Doherty, and Dr. Beatriz de Carvalho Borges del Grande for comments and critical reading of the manuscript. We also thank Dr. Chad Brenner, Dr. Dennis Grayson, and Dr. Shigeki Iwase for reagents and plasmids, Dr. Ulus Atasoy for help with RNA immunoprecipitation experiments, the University of Michigan In-Vivo Animal Core, SMART

Center, and the DNA Sequencing Core for technical support, and Emily Bellile from the Center for Cancer Biostatistics for advice.

3.9 Figures



A
B
C
P0 brain weight (mg)
125
100
75
50
25
0
WT Het KO
***
****
P0 body weight (g)
2.5
2.0
1.5
1.0
0.5
0.0
*
Brain/body weight (%)
7.5
5.0
2.5
0.0
**
D
Brain weight (mg)
120
100
80
60
0 2 4 6 8
Litter size
WT
KO
E
Brain surface area (mm²)
20
15
10
5
0.91 0.46 0 -0.39 -0.77 -1.16 -1.54
Distance from bregma (mm)
****
Bregma
F
Brain volume (mm³)
60
40
20
0
****
WT KO
G
WT
KO
H
Cortical thickness (mm)
1.0
0.8
0.6
0.4
0.2
0.0
*
I
Cortical surface area (mm²)
6
4
2
0
*

Figure 3.1 Loss of PARP1 results in reduced brain weight and cortical surface area at birth. (A) Brain weight of wild-type (WT) (n = 28), PARP1 Heterozygous (Het) (n = 22), and PARP1 KO mice (n = 39) at birth (P0) shows a reduction in Het and KO mice compared with WT controls. ***p = 0.006; ****p < 0.0001 by Student's unpaired t-test. **(B)** P0 body weight is reduced in KO mice compared to WT and Het animals. *p = 0.0315 by Student's unpaired t-test. **(C)** The ratio of brain to body weight for each group shows a significant reduction in the relative size of KO brains compared to WT. **p = 0.0099 by Student's unpaired t-test. **(D)** Litter size is inversely correlated with P0 brain weight in mice. WT: $R^2 = 0.811$, $p < 0.0001$; KO: $R^2 = 0.3725$, $p = 0.0043$. p-values indicate the significance of correlation within each genotype. Slopes are significantly different (p = 0.0029). Two-way ANOVA analysis indicated no significant interaction between litter size and genotype (p = 0.249). **(E)** Surface area measurement from rostral to caudal portions of the PARP1 KO brain indicates a reduced size throughout the rostral-caudal brain axis. The section designated as bregma is indicated in the inset. Distance from bregma for the remaining sections is estimated. ****p <0.0001 via two-way ANOVA. **(F)** Brain volume is reduced in PARP1 KO mice. **(G)** Representative image showing reduced brain surface area in PARP1 KO mouse. This section corresponds with bregma 1.045 mm in the adult brain. Scale bar = 1 mm. **(H)** PARP1 KO reduces cortical surface area and **(I)** cortical thickness. *p <0.05; ****p < 0.0001 by Student's unpaired t-test**.** Quantification shown represents brain slices corresponding with bregma 1.045 mm in the adult mouse brain. **(E-I)** n = 5 WT and 5 KO brains from 4 litters.

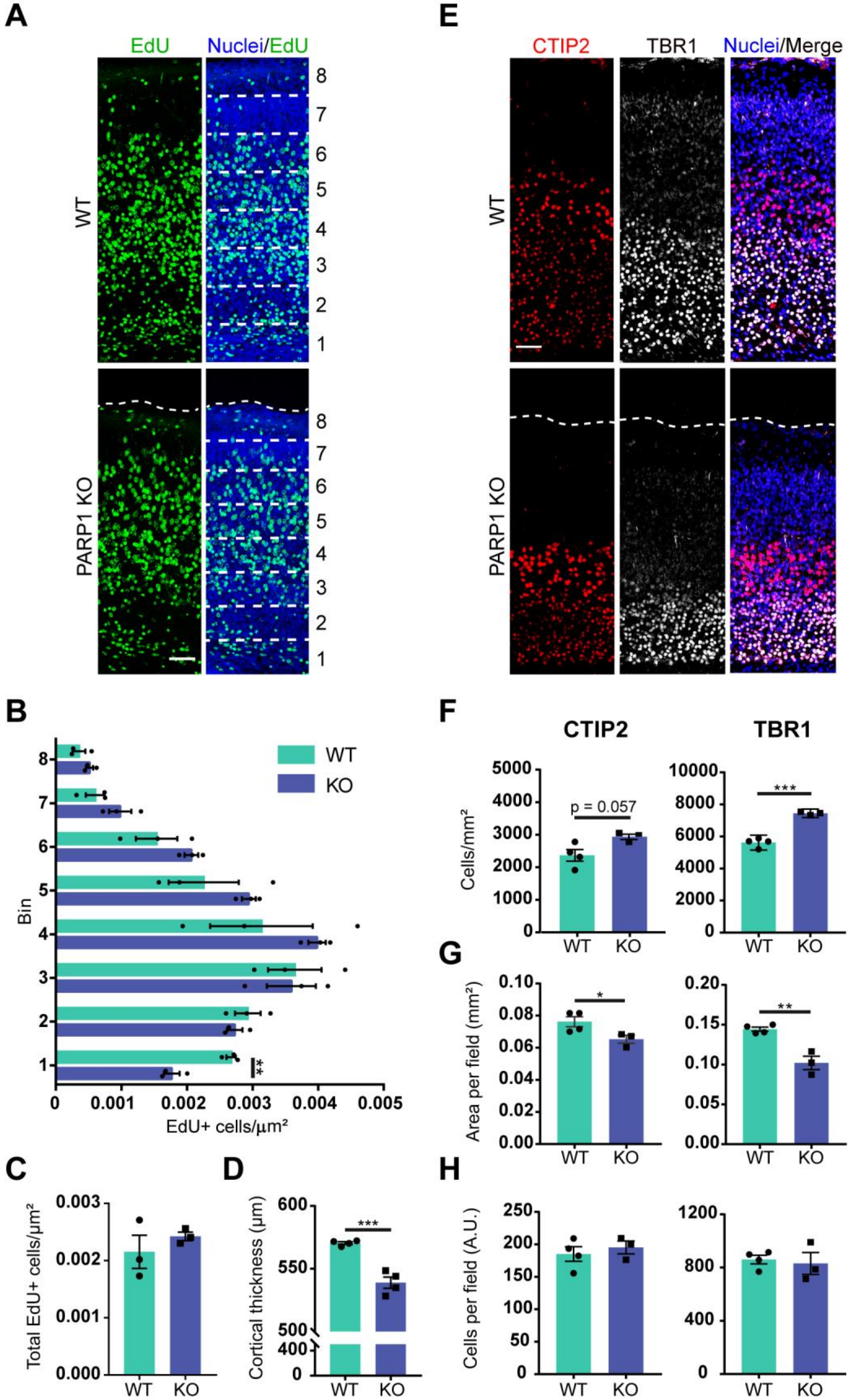

A
EdU
Nuclei/EdU
WT
PARP1 KO
8
7
6
5
4
3
2
1
E
CTIP2
TBR1
Nuclei/Merge
B
WT
KO
Bin
EdU+ cells/μm²
0
0.001
0.002
0.003
0.004
0.005
**
F
CTIP2
TBR1
Cells/mm²
p = 0.057
***
G
Area per field (mm²)
*
**
C
Total EdU+ cells/μm²
D
Cortical thickness (μm)
***
H
Cells per field (A.U.)
WT
KO

Figure 3.2 PARP1 KO mice have defects in cortical development. (A) Representative images of coronal cortical sections at P0 of mice in which proliferating cells (green) were labeled by injecting EdU (IP, 50 mg/kg) to the dam at day 13.5 of pregnancy. Nuclei were labeled with Hoechst 33342 (blue). Scale bar = 50 µm. The cortex was subdivided into 8 bins of equal size for quantification. **(B)** PARP1 loss decreases the number of EdU+ cells in the deepest portion of the cortex (bin #1) (n = 3 animals per genotype from 2 litters, average of 3 sections in the somatosensory cortex region per animal, and 3 images per section). **p = 0.003 by Student's unpaired t-test. **(C)** The number of EdU+ cells per field in KO animals does not change. **(D)** Cortical thickness is decreased in KO P0 brains. ***p = 0.005 by Student's unpaired t-test. **(E)** Representative images from CTIP2+ (red, layer V) and TBR1+ (white, layer VI) cells in the cortex of P0 mice. Nuclei were labeled with DAPI (blue). Scale bar = 50 µm. **(F)** The density of CTIP2+ and TBR1+ cells is increased in KO P0 brains. ***p = 0.0002 by Student's unpaired t-test. **(G)** The area of TBR1 and CTIP2-expressing cells per field is decreased in KO P0 brains. *p = 0.0498 and **p = 0.0026 by Student's unpaired t-test. **(H)** The number of TBR1 and CTIP2 positive cells per field in P0 brains does not differ between genotypes. **(E-H)** n = 4 WT and 3 KO animals from 2 litters, average of 3 sections in the somatosensory cortex region per animal, and 3 images per section.

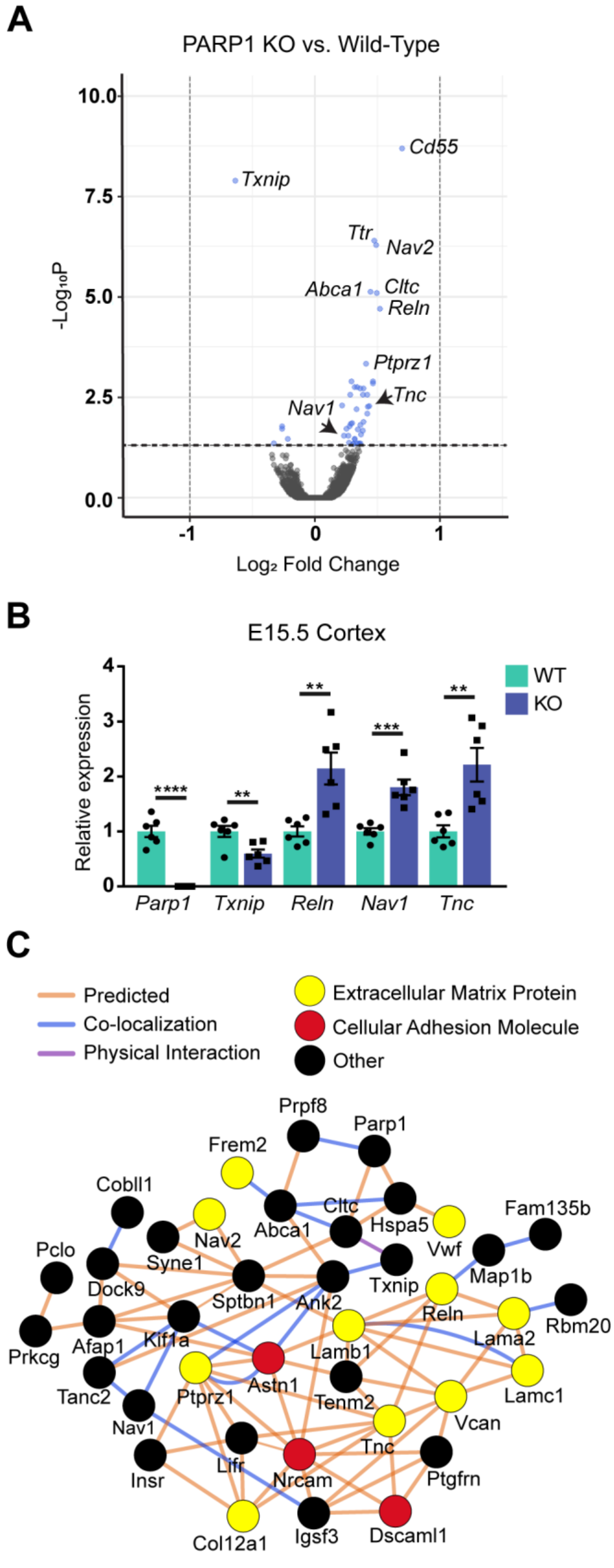

A
PARP1 KO vs. Wild-Type
10.0
7.5
5.0
2.5
0.0
-Log10P
-1
0
1
Log2 Fold Change
Cd55
Txnip
Ttr
Nav2
Abca1
Cltc
Reln
Ptprz1
Tnc
Nav1
B
E15.5 Cortex
Relative expression
WT
KO
****
**
**
***
**
Parp1
Txnip
Reln
Nav1
Tnc
C
Predicted
Co-localization
Physical Interaction
Extracellular Matrix Protein
Cellular Adhesion Molecule
Other
Prpf8
Parp1
Frem2
Cobll1
Cltc
Fam135b
Nav2
Abca1
Hspa5
Vwf
Pclo
Syne1
Map1b
Dock9
Txnip
Sptbn1
Ank2
Reln
Rbm20
Kif1a
Lama2
Prkcg
Afap1
Lamb1
Astn1
Lamc1
Tanc2
Ptprz1
Tenm2
Nav1
Vcan
Lifr
Tnc
Insr
Nrcam
Ptgfrn
Col12a1
Igsf3
Dscaml1

Figure 3.3 Expression levels of genes associated with neuronal migration and cell adhesion are increased at E15.5 in the cortex of PARP1 KOs. (A) Volcano plot of the differentially expressed genes in the PARP1 KO cortex at E15.5 identified by RNA-sequencing (n = 4 biological replicates per genotype). Genes with significantly altered expression are indicated in blue (q-value < 0.05). Upregulated genes comprised 88% (42/48 total) of all genes exhibiting significant differential expression in KO embryonic cortex. **(B)** RT-qPCR validation of a subset of differentially expressed genes (n = 6 of each genotype). Levels of target mRNAs were normalized to *β-actin* levels. **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ by Student's unpaired t-test. **(C)** Interactions among proteins encoded by differentially expressed genes were identified with Cytoscape software based upon bioinformatic prediction (orange lines), co-localization (blue lines), or physical interaction (purple lines). Proteins involved in the extracellular matrix (yellow) and cell adhesion (red) are highlighted. All others are colored in black.

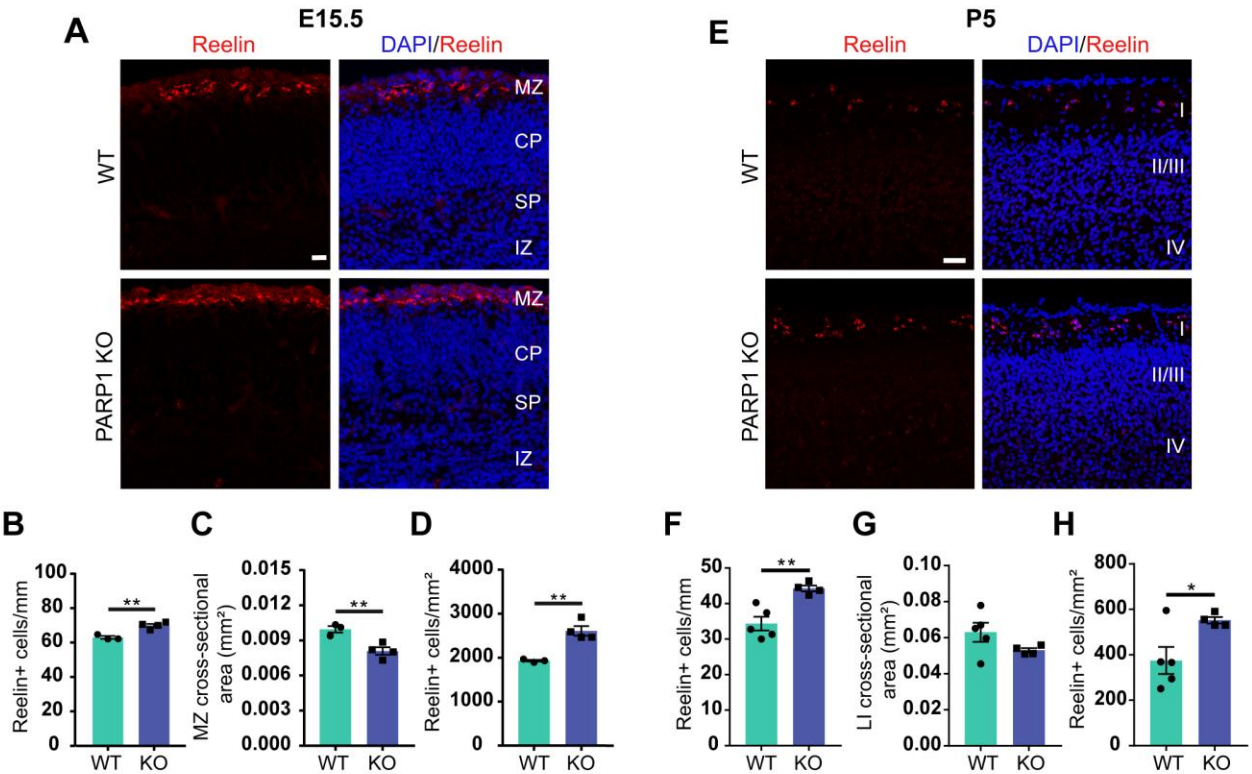


Figure 3.4 Loss of PARP1 increases the number of Cajal-Retzius cells in the E15.5 and P5 cortex. (**A**) Representative images of coronal sections of brain cortex from E15.5 WT and PARP1 KO embryos following Reelin immunostaining (red). Nuclei are labeled with DAPI (blue). Scale bar = 20 µm. Marginal zone (MZ), cortical plate (CP), subplate (SP), and intermediate zone (IZ) are labeled. (**B**) The number of Reelin-expressing cells along the marginal zone is increased, (**C**) marginal zone surface area is decreased, and (**D**) Reelin+ cell density is increased in the KO E15.5 brain (n = 4 for each genotype from 7 litters, 3 sections per animal including rostral, medial, and caudal regions, and 3 images per section). **p < 0.01 by Student's unpaired t-test. **(E)** Representative images of coronal sections of cortex from P5 WT and PARP1 KO embryos showing Reelin immunostaining in red. Nuclei are labeled with DAPI (blue). Scale bar = 50 µm. **(F)** The number of Reelin-expressing cells in layer I is increased, **(G)** surface area of layer I (LI) shows a decreasing trend, and **(H)** density of Reelin-expressing cells in layer I is increased in the P5 KO brain (n = 4 WT and 5 KO from 8 litters, 3 sections per animal from rostral, medial, and caudal brain regions, and 3 images per section). *p < 0.05 and **p <0.01 by Student's unpaired t-test. p = 0.15 for P5 LI surface area.

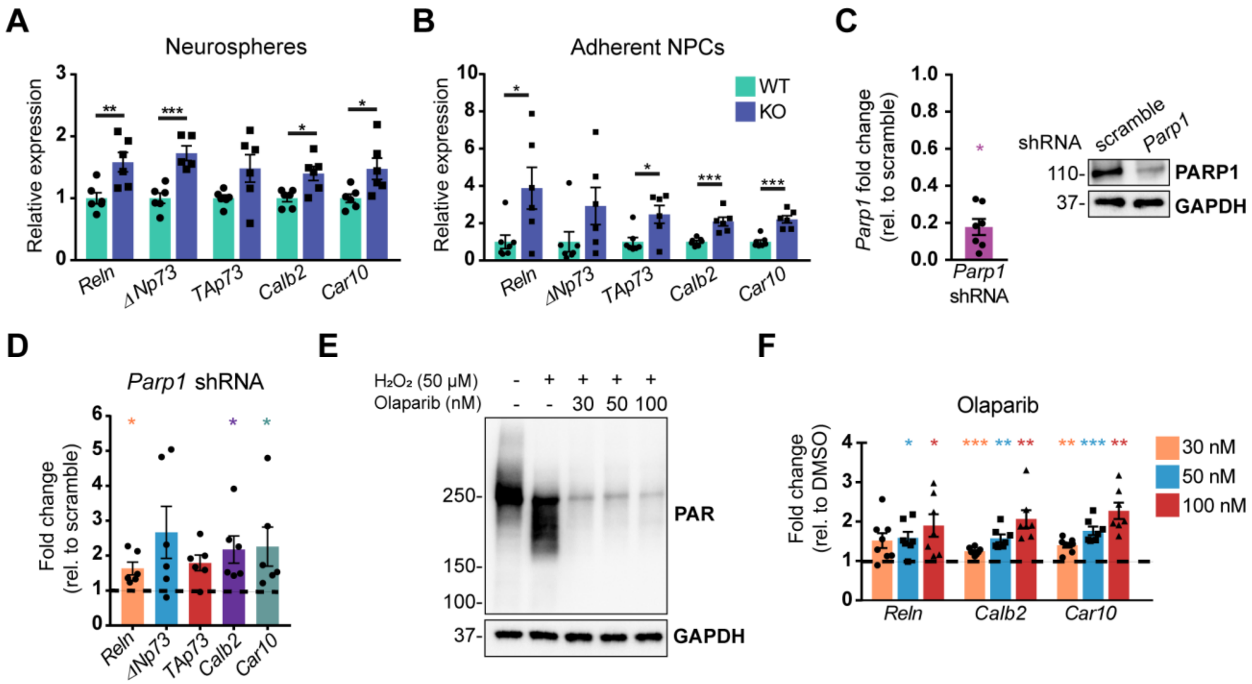


Figure 3.5 PARP1 loss by KO, shRNA knockdown, or pharmacological inhibition increases mRNA levels of genes expressed by Cajal-Retzius cells. (A,B) Quantitative RT-PCR for genes expressed by CR cells in neurospheres **(A)** and adherent NPCs **(B)** from WT and KO cultures (n = 6 of each genotype) demonstrate increased expression of genes expressed by CR cells. Target mRNA levels were normalized to *Rpl19*. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ by Student's unpaired t-test. Relative WT versus KO expression of *TAp73* in (A) is p = 0.06 and of *ΔNp73* in (B) is p = 0.01. **(C)** *Parp1* transcript (left) and protein (right) levels are substantially reduced following transduction of wild-type NPCs with *Parp1* shRNA expressing lentivirus. Fold change in transcript levels was calculated relative to scramble (scr) shRNA control (n = 7). *Parp1* expression was normalized to *Gapdh*. *p = 0.0156 by Wilcoxon test. **(D)** mRNA transcripts expressed by CR cells are increased after wild-type NPC transduction with *Parp1* shRNA-expressing lentivirus (MOI 3) for 72 hrs. Gene expression was normalized to *Gapdh.* Fold change relative to scramble (scr) shRNA-transduced NPCs is plotted (n = 6); *ΔNp73* and *TAp73*: p = 0.06; *p = 0.0313 by Wilcoxon test. **(E)** PAR western blot of wild-type NPCs after Olaparib pre-treatment (at indicated concentrations) for 1 hr followed by 10 min H_2O_2 treatment (50 µM) shows that Olaparib inhibits H_2O_2-induced PARylation. **(F)** Olaparib-mediated inhibition of PARP1 in wild-type NPCs for 48 hrs increases mRNA levels of genes expressed by CR cells. Gene expression was normalized to *Rpl19*. Fold changes were calculated relative to DMSO control treated cells (n = 7-8). *Reln* (30 nM): p = 0.056; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ by repeated measures one-way ANOVA.

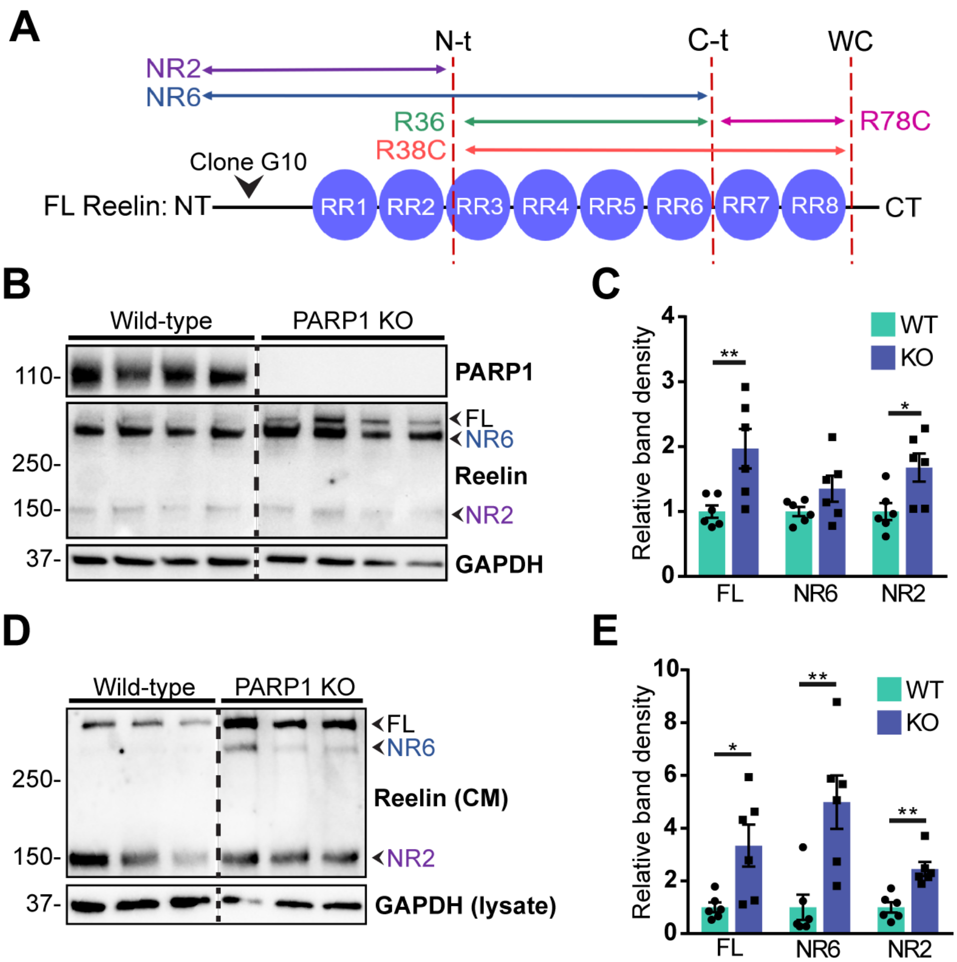


Figure 3.6 PARP1 KO NPC cultures overexpress Reelin protein. (**A**) Schematic drawing of Reelin depicting its proteolytic cleavage sites (vertical dotted lines) and the site of recognition for the N-terminal clone G10 Reelin antibody. This antibody recognizes full-length (FL) Reelin as well as the NR6 and NR2 Reelin fragments. (**B**) Reelin western blot from WT and PARP1 KO NPC lysates shows increased Reelin protein in KO NPC cultures. Arrows indicate the FL, NR2, and NR6 fragments. (**C**) Quantification of relative band density of FL Reelin, the NR2 fragment, and the NR6 fragment normalized to GAPDH (n = 6). NR6: p = 0.24; *p = 0.026; **p = 0.0087 by Mann-Whitney test. (**D**) Western blot for Reelin in conditioned media (CM) and GAPDH in cell lysates of WT and KO NPCs. (**E**) Quantification of band densities of FL Reelin, NR2, and NR6 fragments normalized to GAPDH in cell lysates (n = 6 separate cultures) indicate increased Reelin present in media conditioned by KO NPC cultures. *p < 0.05; **p < 0.01 by Mann-Whitney test.

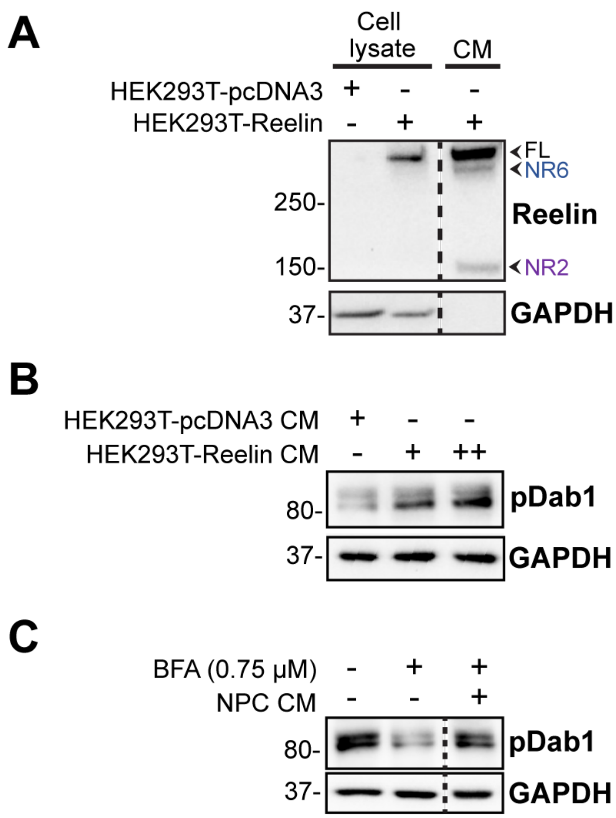


Figure 3.7 Reelin induces Dab1 phosphorylation in NPCs. (A) Western blot of medium conditioned by HEK293T cells transfected with a Reelin-expressing plasmid or empty vector (pcDNA3) and their cell lysates indicates expression and secretion of Reelin. Lysates and conditioned medium (CM) were collected 24 hrs after transfection. Arrows indicate full-length (FL) Reelin and NR2 and NR6 fragments. **(B)** WT NPCs treated with 200 µL (+) or 400 µL (++) conditioned medium (CM) from Reelin-transfected HEK293T cells for 10 min have increased Dab1 phosphorylation (pDab1). **(C)** 20 min treatment with CM from WT NPC cultures increases Dab1 phosphorylation in WT NPCs (n = 4 separate cultures). Pretreatment with Brefeldin A (BFA, 0.75 µg/mL) for 3 hrs prior to CM treatment reduces Dab1 phosphorylation in WT NPCs, indicating that the state of Dab1 phosphorylation depends on the ability of cells to secrete proteins. Addition of BFA to CM after harvesting does not affect pDab1 induction. Relative band density was normalized to GAPDH. Fold change was 2.19 with $p = 0.023$ by one-sample t-test.

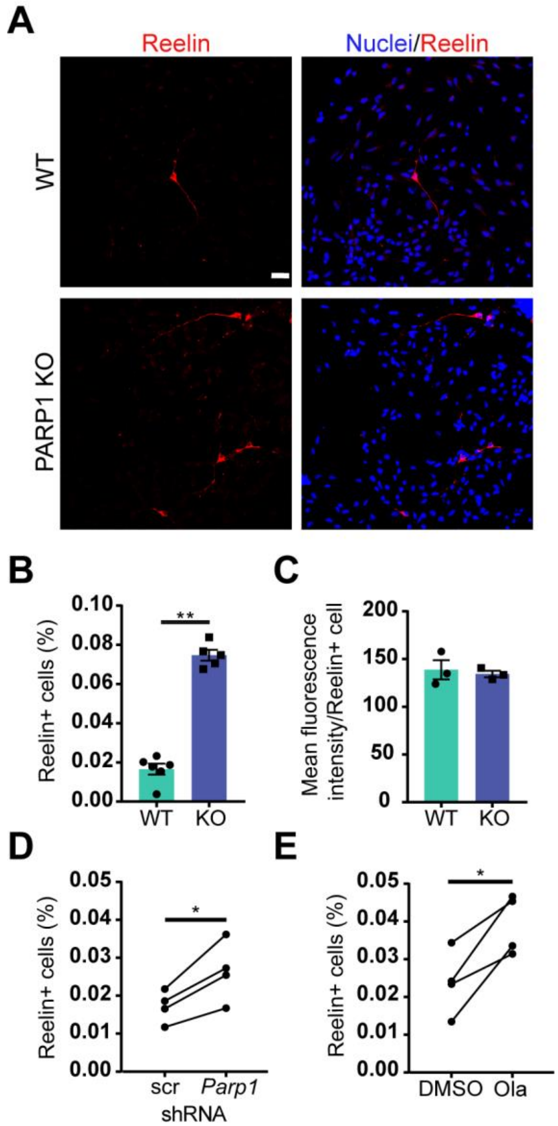


Figure 3.8 PARP1 loss increases CR cell abundance in NPC cultures. (A) Immunostaining for Reelin (red) demonstrates presence of Reelin-expressing cells in WT and PARP1 KO NPC cultures. Nuclei were labeled with DAPI (blue). Scale bar = 20 µm. **(B)** Quantification of the percentage of Reelin-expressing cells within NPC cultures shows increased proportion of Reelin+ cells in KO cultures (n = 6 WT and 5 KO biological replicates and 3 coverslips per replicate). **p = 0.0013 by Mann-Whitney test. **(C)** The mean fluorescence intensity of Reelin expression per cell does not differ between genotypes. Each point on graph represents average Reelin intensity per cell from 3 biological replicates of each genotype. **(D)** The percentage of Reelin-expressing cells in wild-type NPC cultures increases after shRNA-mediated PARP1 knockdown for 72 hrs (n = 4 biological replicates per group and 3 coverslips per replicate). *p = 0.0174 by Student's paired t-test. **(E)** The percentage of Reelin-expressing cells in wild-type NPC cultures increases with PARP1 inhibition via Olaparib (50 nM) for 72 hrs. Olaparib was re-treated every 24 hrs (n = 4 biological replicates per group and 3 coverslips per replicate). *p = 0.022 by Student's paired t-test.

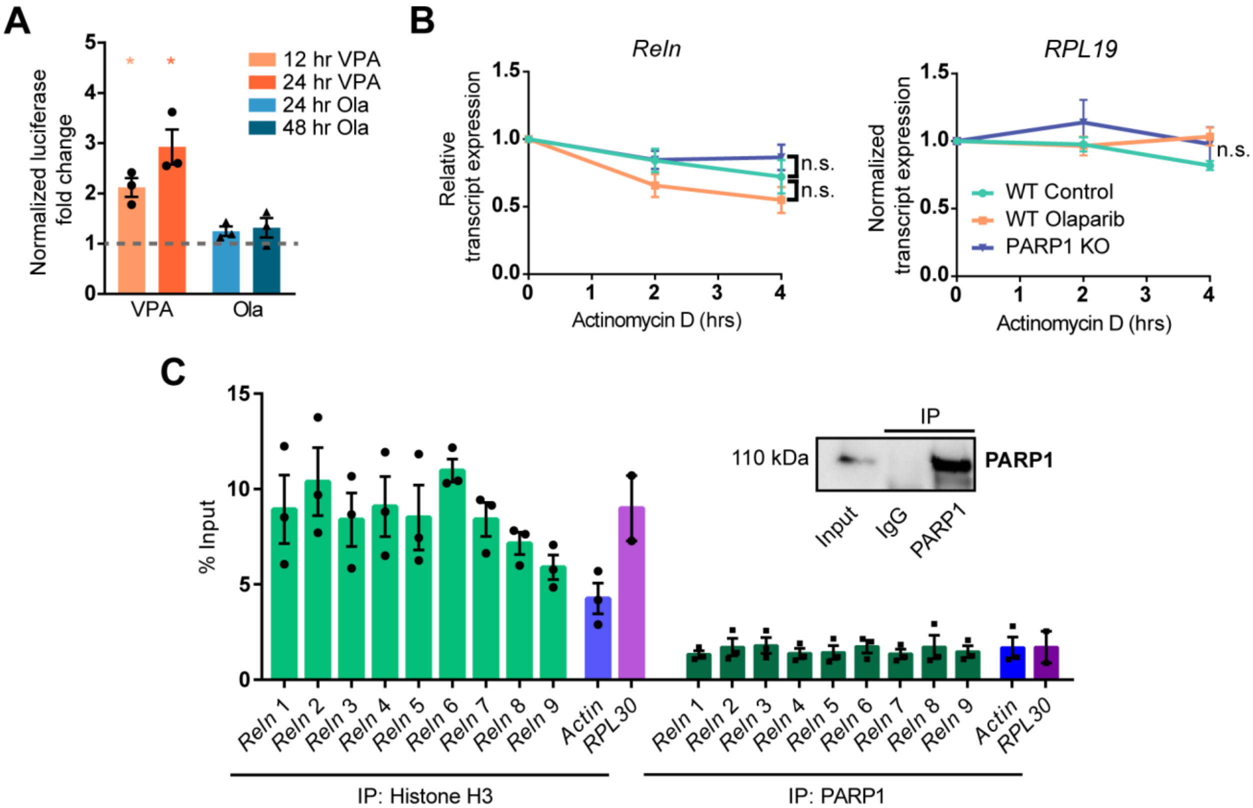


Figure 3.9 PARP1 loss of function does not alter the activity of the *Reln* promoter or the stability of the *Reln* transcript. (A) NPCs transfected with plasmids expressing the putative *Reln* promoter (2600 base pairs prior to the transcription start site) upstream of firefly luciferase did not show increased luciferase activity after treatment with Olaparib (Ola, 100 nM) for 24 hrs or 48 hrs. Valproic Acid (VPA) is a known positive regulator of the *Reln* transcript and was used as a positive control. *$p < 0.05$ by One-Way ANOVA. **(B)** PARP1 KO or PARP1 inhibition with Olaparib (100 nM) does not alter the stability of the *Reln* transcript. RNA polymerase II was inhibited by treatment with Actinomycin D (10 µg/mL) for 2 hrs and 4 hrs and transcript levels were assessed. *Reln* was normalized to *RPL19* expression, which did not decline over 4 hrs. No significant difference was observed between slopes for any of the groups. **(C)** PARP1 Chromatin IP showed no enrichment for portions of the *Reln* promoter from 2600 base pairs upstream of the transcription start site through exon 1 (numbered from 1, most distal to transcription start site, to 9, within exon 1). Histone H3 ChIP was used as a positive control. Inset western blot shows pulldown of PARP1 protein.

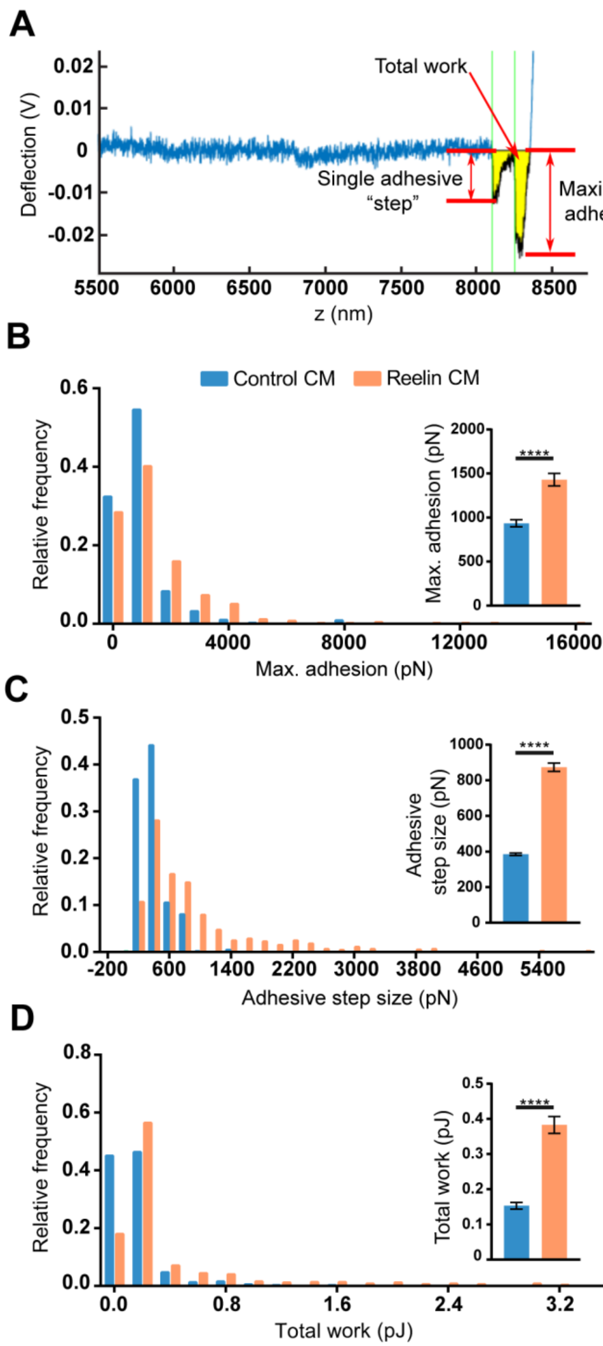


Figure 3.10 Reelin increases NPC adhesiveness to N-cadherin. (A) Example trace generated by AFM from an N-cadherin-coated cantilever in contact with a single cell. From each trace, adhesive step size, maximum adhesion, and total work (yellow shading) were calculated. Thirty-minute treatment of WT NPCs with conditioned media (CM) from Reelin-transfected HEK293T cells increases **(B)** maximum adhesion, **(C)** adhesive step size, and **(D)** total work compared to CM from pcDNA3-transfected HEK293T cells (control CM) (n = 402 - 536 from 2 biological replicates). Distributions are significantly different ($p < 0.0001$ by Kolmogorov-Smirnov test). Inset graphs show average values. ****$p < 0.0001$ by Mann-Whitney test.

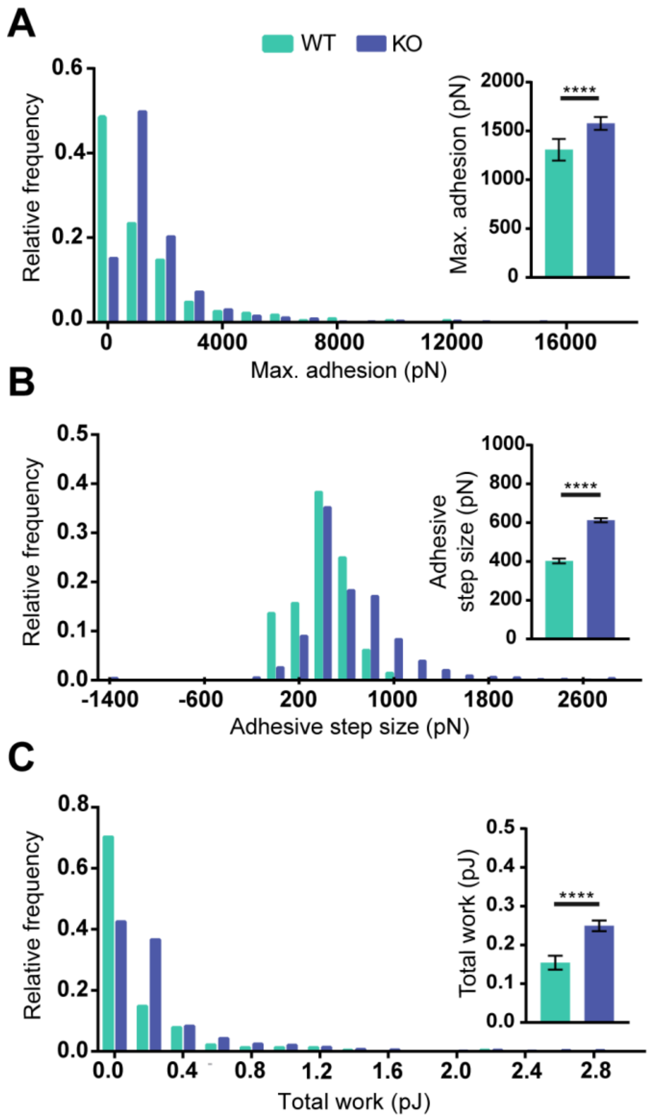


Figure 3.11 PARP1 loss increases NPC adhesiveness to N-cadherin. Maximum adhesion **(A)**, adhesive step size **(B)**, and total work **(C)** are higher in NPCs from KOs than from WTs (n = 229 - 742 from 4 WT and 5 KO biological replicates). Distributions are significantly different ($p < 0.0001$ by Kolmogorov-Smirnov test). Inset graphs show average values. ****$p < 0.0001$ by Mann-Whitney test.

3.10 Tables

Table 3.1 Sequences of primers used for quantitative RT-PCR.

Gene	**Forward Sequence (5’ - 3’)**	**Reverse Sequence (5’ - 3’)**
Reln	GTCGTGTCTTCTGGATCTTCTC	CAGCACTCTCTCCTCCTATCT
Nav1	CCAGCCACCAAGTTAGCAGA	CATGGGTGTCGCTGGAAGAT
Tnc	ACCATGCTGAGATAGATGTTCCAAA	CTTGACAGCAGAAACACCAATCC
Txnip	GTCAGTGTCCCTGGCTCCAAGA	AGCTCATCTCAGAGCTCGTCCG
ΔNp73	CTACCATGCTTTACGTCGG	CTGCCCATCTGGTCCAT
TAp73	GCACCTACTTTGACCTCCCC	GCACTGCTGAGCAAATTGAAC
Car10	GAGAGCAAGAGCCCAGAACTC	CTCACCAGTGGCAGAAATGGC
Calb2	CGGAGCTGGCGCAGAT	CTGCCTGAAGCACAAAAGGAA
Parp1	GGCAGCCTGATGTTGAGGT	GCGTACTCCGCTAAAAAGTCAC
Gapdh	TCACTGCCACCCAGAAGA	GCCAAGCCCTGAGCATAA
Rpl19	ACCTGGATGAGAAGGATGAG	ACCTTCAGGTACAGGCTGTG
β-actin	TCCCATTGAACACGGAGTG	CCTCGGTGAGAAGAATAGATGT

Table 3.2 Sequences of primers used for chromatin immunoprecipitation.

Gene	**Forward Sequence (5’ - 3’)**	**Reverse Sequence (5’ - 3’)**
Reln 1	CACATGTCGGCTACAGCTCA	GAGAAAAGGCCAATGTGAGGTC
Reln 2	TGCAGCTAAACCGAAGCTAATC	ACCGGACCACCTACTTTGG
Reln 3	GTCAGCCTTCGTCTTACTTGG	ATCCTAACACCACCACCGGAA
Reln 4	CCAACAGGCAGTTAGGTCCTT	GAGTTTGGGAGAAGGGCGTC
Reln 5	GCTCTGTTCTCCCGTCTCTG	TGAAACCGGCGTTAATGAGC
Reln 6	CGCGCGCGGGGCACCGTC	AGAGACCGACGGGCTGCC
Reln 7	GGGCGGCGGGCCCCGAGG	AGAGACCGACGGGCTGCC
Reln 8	GGGCTTTAAGAAGGTGCGGAG	CGGTGTGCACGCGACG
Reln 9	GTAACTTCGGGAGCCTCGGT	CTCTCTCATCCACTTTCGGAGG
Actin	CAGCCAACTTTACGCCTAGC	TTTGGACAAAGACCCAGAGG

Table 3.3 PARP1 loss upregulates the expression of genes associated with cell adhesion, axon development, dendrite morphogenesis, and cell migration in the E15.5 cortex.

GO Biological Process	Fold Enrichment	False Discovery Rate
cell adhesion (GO:0007155)	7.95	0.00001
cell morphogenesis involved in differentiation (GO:0000904)	8.51	0.0002
axon development (GO:0061564)	10.38	0.00072
regulation of dendrite morphogenesis (GO:0048814)	20.51	0.00293
neuron projection morphogenesis (GO:0048812)	8.24	0.00302
plasma membrane bounded cell projection morphogenesis (GO:0120039)	8.15	0.00304
cell projection morphogenesis (GO:0048858)	8.05	0.00322
axonogenesis (GO:0007409)	9.91	0.00327
regulation of cell morphogenesis involved in differentiation (GO:0010769)	9.74	0.00355
regulation of dendrite development (GO:0050773)	12.47	0.0206
establishment of organelle localization (GO:0051656)	8.77	0.0235
cerebral cortex radially oriented cell migration (GO:0021799)	39.25	0.0276
axon guidance (GO:0007411)	11.05	0.0336
neuron projection guidance (GO:0097485)	10.95	0.0343
glial cell migration (GO:0008347)	34.57	0.0361
gliogenesis (GO:0042063)	10.25	0.041

Chapter 4: Discussion and Future Directions

4.1 Summary of Findings

The primary aim of this thesis was to identify roles for PARP1 during the process of brain development. PARP1 is a ubiquitously expressed protein with many critical functions in various cell types and tissues and has previously been linked with brain-associated disorders and diseases (Mao and Zhang, 2021). These findings suggest that PARP1 loss of function affects brain development; however, this has not been widely studied. Given PARP1's important role in regulating the DNA repair response following DNA damage, inhibitors for PARP1 are widely utilized to treat certain cancers (Dziadkowiec et al., 2016; Pommier et al., 2016; Rose et al., 2020) and have been proposed to treat pediatric cancers (Barton et al., 2009; Valanejad et al., 2018). Therefore, we must understand the consequences of PARP1 loss of function in the brain, especially during developmental stages. In this thesis, I further validated that PARP1 regulates *GFAP* expression through an interaction with the receptor tyrosine kinase ErbB4 in cultured mouse NPCs and the developing mouse brain (Chapter 2), I discovered that PARP1 loss reduces cortical thickness, disrupts early-born neuron migration, and increases neuronal density at birth (Chapter 3), and I found that PARP1 regulates Cajal-Retzius cell development and neuronal adhesion in the developing brain (Chapter 3). Together, these findings highlight the impacts of PARP1 loss on brain development, potentially contributing to PARP1-linked brain disorders observed in humans and mice.

In Chapter 2, I described previously unpublished data that suggests a novel interaction between PARP1 and the ErbB4 intracellular domain (E4ICD), which is cleaved from the ErbB4-JMa isoform of this protein. Previous data indicate that E4ICD binds to the promoter region of glial genes to repress their expression during neurogenesis, thereby controlling the timing of astrogenesis during brain development (Sardi et al., 2006). These experiments were completed mainly with NPCs isolated from rat cortices. However, we wished to assess the role of PARP1 in this pathway using NPCs isolated from transgenic mice, so I developed an *in vitro* assay to test the effect of NRG1 on *GFAP* expression during differentiation in mouse NPCs. Upon FGF removal from NPC cultures, *GFAP* expression increases over 100-fold. However, concurrent NRG1 treatment with FGF removal reduces this increase by 50% on average in WT NPCs. Repeating this experiment using PARP1 KO NPCs, NRG1 lost the ability to repress *GFAP* expression, indicating that PARP1 is necessary to mediate NRG1-induced repression of *GFAP* expression.

To assess the specific role of ErbB4-JMa in mice, our lab produced mice using CRISPR-Cas9 gene editing that lack ErbB4-JMa but retain ErbB4-JMb. This novel mouse allowed us to further interrogate the specific roles ErbB4-JMa and E4ICD *in vivo* and *in vitro* without utilizing toxic pharmacological inhibitors or transfection-mediated overexpression, as had been previously completed in WT NPCs. Repeating FGF removal with concurrent NRG1 treatment in NPCs isolated from ErbB4-JMa$^{-/-}$ mice, we found that NRG1 did not repress *GFAP* expression, indicating the necessity for the cleavable isoform of ErbB4. Repeating this assay in WT NPCs transfected with constructs expressing the *GFAP* promoter region upstream of firefly luciferase indicated that PARP1 and ErbB4 regulate *GFAP* promoter activity to repress gene expression. Finally, we identified overexpressed *GFAP* in ErbB4 KO and PARP1 KO cortices at birth, suggesting that PARP1-E4ICD regulates astrogenesis in the developing brain *in vivo*. Further studies are needed to identify the specific mechanism of PARP1-E4ICD regulation of the *GFAP* promoter region, which is discussed in section 4.2.

In Chapter 3, I described altered brain morphology in the developing PARP1 KO mouse. Previous studies indicate that postnatal mouse brains that lack PARP1 tend to be smaller than their wild-type littermates (Plane et al., 2012; Hong et al., 2019). We found a similar reduction in brain size in PARP1 KO mice at birth, suggesting a developmental role for PARP1 in the brain. Furthermore, we identified that PARP1 KO brains have altered early-born neuron migration and increased neuronal density with a thinner cortex. To identify changes in gene expression caused by PARP1 loss that contribute to these alterations, we performed RNA-sequencing of the E15.5 cortex. We identified an upregulation in genes associated with neuronal migration and adhesion in the PARP1 KO mouse brain, including the glycoprotein Reelin. Reelin is expressed in Cajal-Retzius cells early in development and is critically important for regulating neuronal adhesion and migration in the developing brain (Ogawa et al., 1995; Meyer et al., 1998). We found that loss of PARP1 increases the abundance of Cajal-Retzius cells *in vivo* at E15.5 and P5 and in NPC cultures *in vitro.* We also demonstrated that regulation of Cajal-Rezius cell abundance is dependent upon PARP1 enzymatic activity. After interrogating the involvement of the Reelin promoter, we did not find any indication that PARP1 loss of function affected promoter activity, indicating an indirect or non-promoter-associated mechanism. Additionally, we found no evidence that PARP1 regulates *Reln* transcript stability. Further experiments are needed to identify the mechanism of regulation, which is discussed in section 4.2

In Chapter 3, I also showed that NPCs isolated from PARP1 KO embryonic cortex secrete excess Reelin. A previous study indicates that Reelin increases neuronal adhesion to cell adhesion molecule N-cadherin (Matsunaga et al., 2017). We repeated this experiment in WT NPCs using a similar experimental setup by incubating them with Reelin-containing conditioned media for 30 min. As found by Matsunaga and colleagues, NPCs treated with Reelin had an increased adhesion to N-cadherin. Since PARP1 KO NPC cultures contain excess Reelin, we hypothesized that PARP1 KO NPCs would similarly adhere more strongly to N-cadherin. As expected, PARP1 loss and exogenous Reelin increase NPC adhesion to N-cadherin to a similar extent. These findings suggest that PARP1 influences NPC adhesion to N-cadherin through Reelin. Further experiments are needed to address the potential effects of this role for PARP1 *in vivo* and are discussed below.

4.2 Future Directions

The findings described above lead to many interesting questions regarding the interaction between PARP1 and ErbB4 and their functions in regulating astrogenesis in the developing brain, the mechanism of PARP1 regulation of Cajal-Retzius cell development, and potential changes in PARP1 KO brain morphology and function associated with increased Cajal-Retzius cell abundance and cell adhesion. Future directions to address these questions are outlined in the sections below.

4.2.1 Mechanism of PARP1-ErbB4 Regulation of Astrogenesis

Our previously published data suggest that NRG1 stimulation of ErbB4-JMa induces phosphorylation, E4ICD cleavage, subsequent E4ICD interaction with N-CoR and TAB2, and translocation to the cell's nucleus (Sardi et al., 2006). Our new data indicate that this protein complex binds to and stimulates PARP1, then acts on the *GFAP* promoter to repress its activity. However, it remains unclear whether the complex binds PARP1 in the cytoplasm or nucleus and whether PARP1 or ErbB4 is necessary for binding to the *GFAP* promoter. Additionally, while

NRG1 stimulation increases PARylation, it is unclear what molecules in the cell become PARylated, whether it is PARP1 itself, other proteins that make up this complex, chromatin, or multiple of these. Furthermore, our findings suggest that this complex binds to the same area of the *GFAP* promoter region as NICD, suggesting E4ICD complex occupation prevents NICD from binding to promote astrogenesis, yet this hypothesis has not been tested.

PARP1 is mainly located in the cell's nucleus, but it is sometimes localized to the cytoplasm (Chen et al., 2018; Xu et al., 2019). To determine if PARP1 is binding to E4ICD in the cytoplasm or nucleus, I would isolate cytoplasmic and nuclear cellular fractions and repeat the ErbB4 and PARP1 co-immunoprecipitation (co-IP) experiments. Since PARP1 is predominantly nuclear, I expect that the E4ICD-Tab2-NCoR complex travels to the nucleus, where it binds to PARP1. If this is the case, I anticipate only seeing an interaction between ErbB4 and PARP1 in nuclear fractions upon stimulation with NRG1. Additionally, our data suggest that NRG1 stimulation of ErbB4-JMa and subsequent cleavage of its intracellular domain activates PARP1, facilitating PARP1 binding to the *GFAP* promoter. To confirm the necessity of ErbB4-JMa, I would perform chromatin immunoprecipitation (ChIP) in ErbB4-JMa$^{-/-}$ NPCs after NRG1 stimulation, predicting that the absence of ErbB4-JMa would prevent PARP1 from binding to the *GFAP* promoter. Furthermore, we hypothesize that PARP1 is necessary for E4ICD to bind the *GFAP* promoter. To test this, I would repeat the *GFAP* ChIP in PARP1 KO NPCs. In the absence of PARP1, I expect to see no E4ICD promoter binding upon NRG1 stimulation.

Given that NRG1 induces E4ICD cleavage and PARylation, we expect that PARP1 is activated following E4ICD binding to PARP1. To confirm this, I would repeat the PARP1-ErbB4 co-IP experiments in the presence of a PARP1 inhibitor. If inactive PARP1 can bind

E4ICD, it strengthens the notion that E4ICD binding activates PARP1. Additionally, our previous experiments suggest that PARP1 activity is necessary to subsequently repress *GFAP* promoter activity. To test if PARP1 enzymatic function is essential for binding to the *GFAP* promoter, I would pharmacologically inhibit PARP1 and repeat the ChIP. I expect that PARP1 inhibition would prevent it from binding the *GFAP* promoter after treatment with NRG1, yet it remains possible that PARP1-E4ICD can bind the promoter but cannot repress its activity in the absence of PARylation.

We found that NRG1 induces PARylation, yet it remains unclear what molecules are becoming PARylated. To test whether PARP1 PARylates the *GFAP* promoter, I would perform a PAR ChIP following NRG1 treatment. Similar to PARP1 ChIP, if PARP1 PARylates the region of the *GFAP* promoter at which this complex binds, I should identify the *GFAP* promoter region in chromatin pulled down with PAR following NRG1 stimulation. Similarly, I would determine if other proteins associated with E4ICD and PARP1 are PARylated by performing a PAR co-IP and immunoblotting for ErbB4 and PARP1. If any one of the proteins in the complex is PARylated, PAR would co-precipitate with both of these proteins. I would also execute the reverse IP by pulling down each of these proteins and immunoblotting for PAR. Studies indicate PARP1 PARylates itself and chromatin to regulate transcription (Visochek et al., 2016), so I expect to identify PARylation of both PARP1 and chromatin at the *GFAP* promoter.

Our findings indicate that the E4ICD-PARP1 complex binds to the same region of the *GFAP* promoter as the Notch intracellular domain (NICD), which stimulates *GFAP* transcription via binding to CSL/RBPJk (Ge et al., 2002). N-CoR, which binds E4ICD, also complexes with CSL/RBPJk to regulate *GFAP* transcription; however, N-CoR binding to the *GFAP* promoter represses its transcription (Hermanson et al., 2002). Therefore, we hypothesize that E4ICD/N-

CoR/TAB2 and PARP1 bind to CSL/RBPJk, which facilitates binding to the *GFAP* promoter during neurogenesis, preventing NICD from binding and promoting gliogenesis. Downregulation of ErbB4 expression later in development may allow NICD binding to CSL/RBPJk and *GFAP* activation. To test this hypothesis, I would perform a Notch ChIP for this promoter region after ErbB4 knockdown via the expression of a dominant-negative ErbB4 or in ErbB4 KO NPCs. If we observe increased NICD binding after ErbB4 knockdown or loss, that would indicate reciprocal binding at this site between NICD and E4ICD. To further confirm this interaction, I would transfect N2A cells with a *GFAP*-luciferase promoter construct and assess the effect of co-transfection of NICD and LexA-E4ICD, NICD alone, or LexA-E4ICD alone. I expect that NICD would stimulate *GFAP* activity, but that co-transfection of E4ICD would repress this stimulation because of competition for the promoter. If these experiments indicated a reciprocal occupation of the promoter, I would perform a co-IP to confirm that the ErbB4-PARP1 complex binds with CSL/RBPJk to facilitate transcriptional repression.

STAT3 is well-known for its role in regulating *GFAP* expression (Bonni et al., 1997). Interestingly, a recent study found that PARP1 binds and PARylates STAT3, inhibiting its activity in a cancer cell line (Ding et al., 2019). This study indicates that PARP1 may additionally inhibit STAT3 function in neural stem cells. To test this, I would perform PARP1 and PAR co-IPs with STAT3 in NPCs. If I observed PARP1 binding and PARylation of STAT3, I would then perform ChIP at the STAT3 binding sites of the *GFAP* promoter in PARP1 KO NPCs. If there is an increase in STAT3 occupation at the promoter following PARP1 loss, that would suggest PARP1 has an inhibitory effect on STAT3 function and promoter-binding ability. I would next test if the PARP1-STAT3 interaction occurs independently of ErbB4 by performing a co-IP between ErbB4 and STAT3. To further confirm the necessity of ErbB4, I would repeat the co-IP between PARP1 and STAT3 in ErbB4 KO NPCs. If PARP1 interacts with STAT3 in the absence of ErbB4, that would suggest PARP1 binds and PARylates STAT3 as an independent mechanism of astrogenesis repression.

4.2.2 Consequences of Precocious Astrogenesis

Our findings show that ErbB4 KO and PARP1 KO cortices express excess *GFAP* at birth relative to their respective WT controls. However, it is unclear whether this corresponds with increased GFAP-expressing astrocytes or increased GFAP expression per astrocyte. To test this, I would collect P0 brains and perform a fluorescent *in situ* hybridization for *GFAP* mRNA, quantifying both the relative number of transcripts per cell and the number of GFAP-expressing cells. I would next determine if this change corresponded with an increase in GFAP protein expression or the number of GFAP-expressing astrocytes via immunohistochemistry.

Additionally, precocious astrogenesis has previously been associated with decreased neurogenesis in other mouse models, as astrocytes are produced at the expense of neurons at the end of neurogenesis (Gauthier et al., 2007; Wu et al., 2017). Our findings show that despite a thinner cortex, the PARP1 KO brain has no difference in the quantity of earlier-born Tbr1 or Ctip2-expressing neurons at birth. However, it remains unclear if there are fewer overall neurons or fewer later-born neurons, such as those that express Cux1 or Cux2. Precocious astrogenesis is more likely to negatively impact neurons born just before the onset of astrogenesis. Therefore, if astrogenesis begins prematurely, IPCs abnormally differentiate into astrocytes instead of Cux1 or Cux2-expressing neurons. To test if specific neuronal populations are affected, I would collect cortical lysates from PARP1 KO and ErbB4 KO mice at various stages of embryonic development – E13.5 through P5, and quantify transcript levels of neuronal genes, such as *DCX*, *NeuN*, *Cux1*, *Cux2*, etc. using RT-qPCR. If I identified any changes in transcript expression, I would look further at their associated proteins in brain slices at different developmental stages.

4.2.3 Potential Mechanisms of PARP1 Regulation of Cajal-Retzius Cell Development

One of the major outstanding questions from my findings is through what mechanism PARP1 regulates Cajal-Retzius cell development. As described, we observed an increased abundance and density of Cajal-Retzius cells at E15.5 and P5 with PARP1 loss. Cajal-Retzius cells undergo programmed cell death starting around P8 (del Río et al., 1995); therefore, our findings suggest that PARP1 is not responsible for mediating Cajal-Retzius cell apoptosis. We also found that PARP1 knockdown or inhibition increases the quantity of Cajal-Retzius-like cells in culture. However, we found no indication that PARP1 regulates the activity of the *Reln* promoter or stability of the transcript. Together, these findings suggest that PARP1 regulates Cajal-Retzius cell development through a cell-autonomous, Reelin-independent mechanism, likely through altered expression or activity of transcription factors or miRNAs associated with Cajal-Retzius cell development, differentiation, and migration.

4.2.3.1 Cajal-Retzius Cell Subpopulations

To further assess Cajal-Retzius cell development, it will be critical to characterize the different populations of Cajal-Retzius cells in the PARP1 KO brain. Cajal-Retzius cell progenitors arise from several portions of the developing brain, including the cortical hem, pallial septum, and pallial-subpallial boundary (PSB), then tangentially migrate to different areas of the cortex on the rostral-caudal and dorsal-ventral axes (Barber and Pierani, 2016). Interestingly, Cajal-Retzius cells from each of these origins express different combinations of proteins at the progenitor stage and following completion of migration, which makes identifying the subpopulations of Cajal-Retzius cells relatively straightforward (Bielle et al., 2005). Dbx1^{+}

progenitors arise from the pallial septum and the PSB and migrate to the rostral-medial cortex and the lateral cortex, respectively. In addition, $Dbx1^+$ Cajal-Retzius cell progenitors that arise from the septum co-express Emx1 (Bielle et al., 2005). While both neuronal subtypes express Reelin, only $Dbx1^+$ cells that arise from the PSB also express Calretinin (Bielle et al., 2005). In contrast, Cajal-Retzius cell progenitors derived from the cortical hem do not express Dbx1 and migrate to the caudal-medial cortex and hippocampus (Barber and Pierani, 2016) and express p73 and Calretinin in addition to Reelin (Barber et al., 2015). In summary, cortical hem-derived Cajal-Retzius cells express Reelin, p73, and Calretinin, septal pallium-derived Cajal-Retzius cells express p73 and Reelin, and PSB-derived Cajal-Retzius cells express Reelin and Calretinin (Takiguchi-Hayashi et al., 2004; Bielle et al., 2005; Barber et al., 2015).

To interrogate whether specific populations of Cajal-Retzius cells are altered in the PARP1 KO mice, I would immunolabel these different cell populations and quantify the number of cells in regions of the developing cortex on the dorsal-ventral and rostral-medial axis. This would entail collecting rostral, medial, and caudal sections from the developing brain (around E12.5) and quantifying the number of Reelin, p73, and Calretinin-expressing and co-expressing cells both medially and laterally within each section. Since we observed increased Reelin-expressing cells in multiple brain regions on the rostral-caudal axis and increased *Calretinin* and *p73* transcript expression *in vitro,* I do not expect a specific Cajal-Retzius cell population is altered. However, if I were to observe changes in one or multiple of the progenitor populations, I could then assess migration patterns by injecting the affected brain area(s) at E9.5-E10.5 with DiI and evaluating the migration of the labeled cells over time. Suppose migration is unaffected, but quantity is increased. In that case, I could instead assess the number of Dbx1 or Emx1-expressing progenitors from their associated brain region(s) to determine if increased Cajal-

Retzius cell abundance arises from an increase in the progenitor pool due to altered differentiation. If there is an increase in only some subtypes of Cajal-Retzius cells, that would also give clues as to potential dysregulated proteins, as some proteins regulating Cajal-Retzius cell development affect only subsets of progenitors (Stoykova et al., 2003; Borrell and Marín, 2006; Kaddour et al., 2020).

4.2.3.2 Proteins Associated with PARP1 and Cajal-Retzius Cell Development

As described in section 1.2.3.1, many proteins influence the development or migration of Cajal-Retzius cells. Interestingly, some of these proteins are also linked to PARP1. This includes the transcription factor Ascl1 (also known as Mash1), which PARP1 represses in rat neural stem cells in a complex containing Hes1. PARP1 knockdown, therefore, de-represses Ascl1, increasing its expression (Ju et al., 2004). A mouse model of Ascl1 loss indicates that Ascl1 promotes Cajal-Retzius cell development, specifically cells expressing Reelin and p73, suggesting that Ascl1 regulates cortical hem and septal-derived progenitors (Dixit et al., 2011). Therefore, overexpression of Ascl1 may increase Cajal-Retzius cell abundance, similar to our observation in PARP1 KO mice. However, it remains to be seen if Ascl1 is overexpressed in PARP1 KO developing cortex or NPCs. If I found Ascl1 to be overexpressed, that would indicate that PARP1 may indirectly affect Cajal-Retzius cell development through Ascl1.

PARP1 also regulates Pax6 expression during neuroectoderm specification early in neurodevelopment. Specifically, FGF activation of ERK1/2 causes PARP1 activation, which then binds to the Pax6 promoter to promote neuroectoderm specification and neural differentiation. Accordingly, inhibition of PARP1 suppresses Pax6 expression (Yoo et al., 2011). Among other functions, Pax6 has previously been shown to negatively regulate Cajal-Retzius cell development in a non-cell-autonomous mechanism, potentially influencing cell migration via extracellular Pax6 (Stoykova et al., 2003; Kaddour et al., 2020). In the Pax6 knockout mouse brain, predominantly Reelin and Calretinin-expressing Cajal-Rezius cells are increased in abundance, indicating Pax6 loss causes an increase in cells that arise from the cortical hem or PSB (Stoykova et al., 2003); however, extracellular Pax6 was shown to alter the migration of cortical hem and septal-derived progenitors, though PSB-derived progenitor migration was not tested (Kaddour et al., 2020). These studies indicate that Pax6 may be a master regulator of Cajal-Retzius cell development and migration. Therefore, PARP1 loss or inhibition may decrease Pax6 expression at very early stages of development, leading to excess Cajal-Retzius cells through changes in progenitor cell differentiation or migration. To test this, I would measure Pax6 mRNA and protein expression in PARP1 KO brains at multiple time points during early development, as changes in its expression profile may contribute to our observations.

The chemokine Cxcl12, signaling through its G-protein coupled receptor Cxcr4, regulates the migration of cortical hem-derived Cajal-Retzius progenitor cells (Borrell and Marín, 2006). Cxcl12 is secreted from cells located in the meninges and is chemoattractive for Cajal-Retzius progenitor cells that express Cxcr4. Therefore, loss of Cxcr4 disrupts Cajal-Retzius cell tangential migration and results in ectopic clusters of cells in deeper portions of the cortex (Borrell and Marín, 2006). PARP1 negatively regulates *Cxcl12* transcription by binding to the gene promoter, first identified in pancreatic beta cells (Marković et al., 2013). Further experiments showed that loss of PARP1 from mouse embryonic fibroblasts decreases the methylation state of the Cxcl12 promoter, likely due to increased expression of Tet1, a DNA de-methylase (Tolić et al., 2019). DNA methylation represses transcription; therefore, PARP1-mediated repression of Tet1 expression increases the methylation state of the *Cxcl12* promoter, repressing transcription of the gene. These studies present the intriguing possibility that loss of

PARP1 increases Cxcl12 expression and secretion from the marginal zone, increasing the migration of Cajal-Retzius cell progenitors toward the meninges. To test this, I would measure the mRNA and protein of Cxcl12 between E10.5-E12.5 in the developing brain and cultured NPCs. Cortical hem-derived progenitors express p73 and Calretinin in addition to Reelin (Barber et al., 2015), so if this population of cells is increased in the PARP1 KO brain and Cxcl12 is overexpressed, PARP1 may be regulating Cajal-Retzius cell abundance through Cxcl12.

Cajal-Retzius cell development is also regulated through miRNAs, small RNAs that bind to target mRNAs, cleave them, destabilize them, or prevent transcription (Huntzinger and Izaurralde, 2011; O'Brien et al., 2018). A total disruption of miRNA biogenesis through knockout of Dicer, which cleaves immature miRNAs into mature miRNAs, increases Cajal-Retzius cell abundance, indicating a critical role for miRNAs in their development (McLoughlin et al., 2012). Specifically, miRNA-9 affects Foxg1 expression, a transcription factor that regulates cortical hem development, which is a vital source of Cajal-Retzius cell progenitors (Shibata et al., 2008; Liu et al., 2018). Additionally, miRNA-128 and miRNA-200c specifically target *Reln*, which may affect Cajal-Retzius cell development and function, although this has yet to be tested (Evangelisti et al., 2009; Stary Creed M. et al., 2015). PARP1 regulates the expression of miRNAs, presenting an additional possibility by which it modulates Cajal-Retzius cell abundance (Nozaki et al., 2018). I would test this hypothesis by measuring the expression of miRNAs known to influence Cajal-Retzius cell development or *Reln* expression, such as miRNA-9, miRNA-128, or miRNA-200c, in the PARP1 KO cortex. I would also perform RNA-sequencing to identify altered miRNA expression in the developing PARP1 KO cortex, comparing the profile of altered miRNAs in the PARP1 KO brain to altered miRNAs after Dicer loss. Any overlapping miRNAs could indicate candidate miRNAs that influence Cajal-Retzius cell development due to PARP1 loss.

4.2.4 Phenotypes Associated with Reelin Function

In addition to regulation of neuronal migration and adhesion, Reelin is well-known for its role in regulating preplate splitting and cortical plate formation very early during neurodevelopment, around E12.5. Without Reelin to anchor migrating neurons to the marginal zone during somal translocation, the preplate fails to form correctly (Magdaleno et al., 2002). We observed changes in early-born neuron migration, so there may be alterations in cortical plate formation in the PARP1 KO embryonic brain. To test this, I would immunostain E14.5-E15.5 PARP1 KO brains for MAP2 and CSPGs, which label the subplate and marginal zone. In a wild-type brain, there is a lack of labeling in the cortical plate between the subplate and marginal zone, indicating proper splitting of the preplate. In *reeler* mice, MAP2 and CSPG staining is diffuse and spread throughout the cortex, indicating the presence of an abnormal "superplate" (Sheppard and Pearlman, 1997; Magdaleno et al., 2002). If early-born neuron migration in PARP1 KO mice is altered due to abnormal preplate splitting, I would expect to see changes in the distribution or abundance of subplate and marginal zone cells.

Reelin also regulates dendrite development and dendritic spine density (Stanfield et al., 1979; Liu et al., 2001; Niu et al., 2004, 2008). This function is intriguing because we found that PARP1 KO results in increased neuronal density, similar to that observed in *reeler* heterozygous mice (Liu et al., 2001). Increased neuronal density has also been associated with decreased dendritic arborization in schizophrenic patients (Selemon and Goldman-Rakic, 1999). Additionally, schizophrenic-like behaviors have been observed in PARP1 KO mice (Hong et al., 2019). These findings suggest that dendritic morphology or dendritic spine density is abnormal in the PARP1 KO cortex. To test this, I would assess dendritic morphology and quantify dendritic spines of neurons in the developing and adult cortex and hippocampus of PARP1 KO mice utilizing Golgi staining and 3D reconstruction of individual neurons. This experiment could be completed in parallel *in vitro* in neuron cultures derived from WT and PARP1 KO cortex and/or hippocampus. Interestingly, Reelin overexpression in the adult mouse brain causes an opposite phenotype as Reelin haploinsufficiency, causing enlarged dendritic spines and increased numbers of synapses per dendritic spine (Pujadas et al., 2010); however, it remains unclear how Reelin overexpression may affect dendritic spine density in the developing brain.

4.2.5 Phenotypes Associated with Extracellular Matrix Function and Abnormal Neuronal Migration

Loss of PARP1 causes changes in gene expression of corresponding proteins that make up the extracellular matrix, including scaffolding proteins, CSPGs, signaling molecules, and cell adhesion molecules. These changes in gene expression are associated with the altered migration of early-born neurons in the PARP1 KO cortex. The extracellular matrix regulates many aspects of cortical morphology and neuronal migration, including radial glial cell organization and neuronal process orientation (Barros et al., 2011). Alterations in radial glia cause many phenotypes, including misorientation of migrating neuron apical and basal processes, disruptions in endfeet formation, microcephaly, defective proliferation, and premature differentiation (Ferent et al., 2020). To further assess the organization of radial glial cells in the embryonic PARP1 KO cortex, I would immunostain E13.5 cortex for RC2 and GFAP, which are commonly expressed in radial glial cells at this stage of development, and co-label with BLBP, which is expressed in the endfeet of radial glial cells (Haubst et al., 2006). The resulting stain would allow me to determine if radial glial cell orientation and endfeet attachment are affected. With altered Reelin and laminin expression, attachment of radial glial endfeet to the basement membrane at the pial surface of the PARP1 KO brain may be disrupted, especially early in development when neuronal migration is altered.

Adhesion molecules are also critical components of the extracellular matrix. We found that PARP1 loss increases the adhesion of NPCs to N-cadherin *in vitro*, and the expression of several adhesion molecules is upregulated in the developing PARP1 KO cortex. Together, these findings suggest altered neuronal adhesion *in vivo*. N-cadherin overexpression has been previously shown to reduce the distance between a migrating cell and radial glial fiber and reduce neuronal migration into the cortical plate (Shikanai et al., 2011). To test whether neurons are more adhesive to radial glial fibers in the PARP1 KO brain, I would electroporate E13.5 embryos with a GFP-expressing construct into the lateral ventricle, collect embryos the following day, and co-label for a radial glial cell marker, such as Nestin or RC2. Then, I would quantify the distance between the RC2-labeled radial glial fiber and the GFP-transfected migrating neuron, with shorter distances indicating increased adhesion. If I found changes in adhesion *in vivo*, I would test if N-cadherin is mediating this interaction by electroporating embryos with a dominant-negative N-cadherin-GFP construct and repeat the experiment in WT and KO embryos. If N-cadherin knockdown restored the distance between the neuron and radial glial cell fiber back to baseline, that would suggest N-cadherin is mediating increased adhesion *in vivo*. If I saw no difference, I would repeat this experiment with knockdown of adhesion molecules Dscaml1 or Nrcam, which are overexpressed in the PARP1 KO cortex.

If I observe changes in adhesion *in vivo*, that may suggest downstream changes in the orientation of neuronal processes. N-cadherin is essential for orienting the leading process of migrating neurons radially; thus, neurons that express a mutated dominant-negative N-cadherin protein have processes that tend to be more horizontally oriented than vertical (Gärtner et al., 2012). To test the orientation of migrating neurons in PARP1 KO mice, I would perform a similar experiment as previously described, electroporating a construct expressing GFP into the lateral ventricle of WT and KO embryos at E13.5. GFP would be expressed in ventricular neurons and their processes, allowing me to assess their respective positioning with regards to the ventricular wall. If migration is disrupted due to misorientation of neuronal processes, I should observe an increased proportion of neuronal processes that lack a vertical orientation in the PARP1 KO cortex.

Given that we observed changes in neuronal migration *in vivo*, I would assess the migration rate of PARP1 KO NPCs or premature neurons *in vitro*. Our data suggest that migrating neurons in the PARP1 KO brain tend to mislocalize to more superficial brain areas *in vivo*, which may indicate an increased migration rate. I would test migration *in vitro* using a scratch assay, whereby a scratch in the cell monolayer removes neurons from a specific area. I would then image the scratch area over many hours and quantify the time it takes for neurons to repopulate the area. If PARP1 KO causes increased migration rate cell-autonomously, I would expect to see PARP11 KO neurons move into the scratch area at an earlier time point than WT neurons. This finding would suggest that migration rate is regulated by molecules secreted from or present within the neurons, which then act upon other nearby neurons. However, if migration rate is controlled by factors present *in vivo*, or non-cell autonomously, I may not observe changes in cell migration *in vitro*. Therefore, I could utilize *ex vivo* time-lapse imaging of migrating neurons. With this method, GFP-expressing vectors are electroporated into the lateral ventricle of embryonic brains, where it is taken up by dividing neurons. Migrating neurons expressing GFP can then be live imaged in brain slice cultures via time-lapse imaging to assess the dynamics of migrating cells more carefully.

4.2.6 Final Thoughts and Conclusions

Taken together, the data presented in this dissertation show that PARP1 has a more significant and complex role in neurodevelopment than previously known. Specifically, I have demonstrated that PARP1 regulates cortical thickness, Cajal-Retzius cell development, neuronal adhesion, and gliogenesis. Although the effects of PARP1 loss are relatively minor in scale, the accumulation of multiple of these defects may be contributing to PARP1-associated neurological disorders. Additionally, inhibition of PARP1 is currently utilized to treat cancers and has been proposed to treat conditions associated with PARP1 overexpression (Dziadkowiec et al., 2016; Pommier et al., 2016; Rose et al., 2020; Mao and Zhang, 2021). Our findings, however, show that PARP1 loss of function during neurodevelopment negatively impacts brain formation, perhaps indicating that PARP1 inhibition should be used sparingly to treat pediatric cancer patients. Future experiments outlined in this dissertation to elucidate the specific mechanisms behind PARP1 regulation of Cajal-Retzius cell development and astrogenesis will increase our understanding of PARP1's roles in brain development and further expand our knowledge of PARP1's diversity of functions in the brain.

References

Abbott, N. J. (2002). Astrocyte–endothelial interactions and blood–brain barrier permeability. *J Anat* 200, 629–638. doi:10.1046/j.1469-7580.2002.00064.x.

Abe, Y., Namba, H., Kato, T., Iwakura, Y., and Nawa, H. (2011). Neuregulin-1 Signals from the Periphery Regulate AMPA Receptor Sensitivity and Expression in GABAergic Interneurons in Developing Neocortex. *J Neurosci* 31, 5699–5709. doi:10.1523/JNEUROSCI.3477-10.2011.

Abeti, R., Abramov, A. Y., and Duchen, M. R. (2011). β-amyloid activates PARP causing astrocytic metabolic failure and neuronal death. *Brain* 134, 1658–1672. doi:10.1093/brain/awr104.

Aguilar-Quesada, R., Muñoz-Gámez, J. A., Martín-Oliva, D., Peralta, A., Valenzuela, M. T., Matínez-Romero, R., et al. (2007). Interaction between ATM and PARP-1 in response to DNA damage and sensitization of ATM deficient cells through PARP inhibition. *BMC Molecular Biology* 8, 29. doi:10.1186/1471-2199-8-29.

Ajioka, I., and Nakajima, K. (2005). Birth-date-dependent segregation of the mouse cerebral cortical neurons in reaggregation cultures. *European Journal of Neuroscience* 22, 331–342. doi:10.1111/j.1460-9568.2005.04214.x.

Alkhatib, H. M., Chen, D. F., Cherney, B., Bhatia, K., Notario, V., Giri, C., et al. (1987). Cloning and expression of cDNA for human poly(ADP-ribose) polymerase. *Proc Natl Acad Sci U S A* 84, 1224–1228.

Ambrose, H. E., Papadopoulou, V., Beswick, R. W., and Wagner, S. D. (2007). Poly-(ADP-ribose) polymerase-1 (Parp-1) binds in a sequence-specific manner at the Bcl-6 locus and contributes to the regulation of Bcl-6 transcription. *Oncogene* 26, 6244–6252. doi:10.1038/sj.onc.1210434.

Amé, J.-C., Spenlehauer, C., and de Murcia, G. (2004). The PARP superfamily: Review articles. *Bioessays* 26, 882–893. doi:10.1002/bies.20085.

Amiri, K., Ha, H., Smulson, M., and Richmond, A. (2006). Differential regulation of CXC ligand 1 transcription in melanoma cell lines by poly(ADP-ribose) polymerase-1. *Oncogene* 25, 7714–7722. doi:10.1038/sj.onc.1209751.

Anderson, S. A., Kaznowski, C. E., Horn, C., Rubenstein, J. L. R., and McConnell, S. K. (2002). Distinct Origins of Neocortical Projection Neurons and Interneurons In Vivo. *Cerebral Cortex* 12, 702–709. doi:10.1093/cercor/12.7.702.

Angevine, J. B., and Sidman, R. L. (1961). Autoradiographic Study of Cell Migration during Histogenesis of Cerebral Cortex in the Mouse. *Nature* 192, 766–768. doi:10.1038/192766b0.

Anstötz, M., Huang, H., Marchionni, I., Haumann, I., Maccaferri, G., and Lübke, J. H. R. (2016). Developmental Profile, Morphology, and Synaptic Connectivity of Cajal–Retzius Cells in the Postnatal Mouse Hippocampus. *Cereb Cortex* 26, 855–872. doi:10.1093/cercor/bhv271.

Anton, E. S., Marchionni, M. A., Lee, K. F., and Rakic, P. (1997). Role of GGF/neuregulin signaling in interactions between migrating neurons and radial glia in the developing cerebral cortex. *Development* 124, 3501–3510.

Ayachi, I. E., Fernandez, C., Baeza, N., Paula, A. M. D., Pesheva, P., and Figarella-Branger, D. (2011). Spatiotemporal distribution of tenascin-R in the developing human cerebral cortex parallels neuronal migration. *Journal of Comparative Neurology* 519, 2379–2389. doi:10.1002/cne.22632.

Ayhan, Y., Abazyan, B., Nomura, J., Kim, R., Ladenheim, B., Krasnova, I. N., et al. (2011). Differential effects of prenatal and postnatal expressions of mutant human DISC1 on neurobehavioral phenotypes in transgenic mice: evidence for neurodevelopmental origin of major psychiatric disorders. *Molecular Psychiatry* 16, 293–306. doi:10.1038/mp.2009.144.

Azad, G. K., Ito, K., Sailaja, B. S., Biran, A., Nissim-Rafinia, M., Yamada, Y., et al. (2018). PARP1-dependent eviction of the linker histone H1 mediates immediate early gene expression during neuronal activation. *Journal of Cell Biology* 217, 473–481. doi:10.1083/jcb.201703141.

Azevedo, F. A. C., Carvalho, L. R. B., Grinberg, L. T., Farfel, J. M., Ferretti, R. E. L., Leite, R. E. P., et al. (2009). Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *Journal of Comparative Neurology* 513, 532–541. doi:10.1002/cne.21974.

Bai, J., Ramos, R. L., Ackman, J. B., Thomas, A. M., Lee, R. V., and LoTurco, J. J. (2003). RNAi reveals doublecortin is required for radial migration in rat neocortex. *Nat Neurosci* 6, 1277–1283. doi:10.1038/nn1153.

Barber, M., Arai, Y., Morishita, Y., Vigier, L., Causeret, F., Borello, U., et al. (2015). Migration Speed of Cajal-Retzius Cells Modulated by Vesicular Trafficking Controls the Size of Higher-Order Cortical Areas. *Current Biology* 25, 2466–2478. doi:10.1016/j.cub.2015.08.028.

Barber, M., and Pierani, A. (2016). Tangential migration of glutamatergic neurons and cortical patterning during development: Lessons from Cajal-Retzius cells. *Developmental Neurobiology* 76, 847–881. doi:10.1002/dneu.22363.

Barnes, A., Isohanni, M., Barnett, J. H., Pietiläinen, O., Veijola, J., Miettunen, J., et al. (2012). Neuregulin-1 genotype is associated with structural differences in the normal human brain. *NeuroImage* 59, 2057–2061. doi:10.1016/j.neuroimage.2011.10.007.

Barnes, C. E., English, D. M., and Cowley, S. M. (2019). Acetylation & Co: an expanding repertoire of histone acylations regulates chromatin and transcription. *Essays Biochem* 63, 97–107. doi:10.1042/EBC20180061.

Barros, C. S., Calabrese, B., Chamero, P., Roberts, A. J., Korzus, E., Lloyd, K., et al. (2009). Impaired maturation of dendritic spines without disorganization of cortical cell layers in mice lacking NRG1/ErbB signaling in the central nervous system. *Proc Natl Acad Sci U S A* 106, 4507–4512. doi:10.1073/pnas.0900355106.

Barros, C. S., Franco, S. J., and Muller, U. (2011). Extracellular Matrix: Functions in the Nervous System. *Cold Spring Harbor Perspectives in Biology* 3, a005108–a005108. doi:10.1101/cshperspect.a005108.

Barton, V. N., Donson, A. M., Kleinschmidt-DeMasters, B. K., Gore, L., Liu, A. K., and Foreman, N. K. (2009). PARP1 expression in pediatric central nervous system tumors. *Pediatric Blood & Cancer* 53, 1227–1230. doi:10.1002/pbc.22141.

Beck, C., Robert, I., Reina-San-Martin, B., Schreiber, V., and Dantzer, F. (2014). Poly(ADP-ribose) polymerases in double-strand break repair: Focus on PARP1, PARP2 and PARP3. *Experimental Cell Research* 329, 18–25. doi:10.1016/j.yexcr.2014.07.003.

Becker, A., Zhang, P., Allmann, L., Meilinger, D., Bertulat, B., Eck, D., et al. (2016). Poly(ADP-ribosyl)ation of Methyl CpG Binding Domain Protein 2 Regulates Chromatin Structure. *J. Biol. Chem.* 291, 4873–4881. doi:10.1074/jbc.M115.698357.

Berninger, B., Guillemot, F., and Götz, M. (2007). Directing neurotransmitter identity of neurones derived from expanded adult neural stem cells. *European Journal of Neuroscience* 25, 2581–2590. doi:10.1111/j.1460-9568.2007.05509.x.

Betizeau, M., Cortay, V., Patti, D., Pfister, S., Gautier, E., Bellemin-Ménard, A., et al. (2013). Precursor Diversity and Complexity of Lineage Relationships in the Outer Subventricular Zone of the Primate. *Neuron* 80, 442–457. doi:10.1016/j.neuron.2013.09.032.

Bhatt, D., and Ghosh, S. (2014). Regulation of the NF-κB-Mediated Transcription of Inflammatory Genes. *Front. Immunol.* 5. doi:10.3389/fimmu.2014.00071.

Bielle, F., Griveau, A., Narboux-Nême, N., Vigneau, S., Sigrist, M., Arber, S., et al. (2005). Multiple origins of Cajal-Retzius cells at the borders of the developing pallium. *Nature Neuroscience* 8, 1002–1012. doi:10.1038/nn1511.

Blanquie, O., Liebmann, L., Hübner, C. A., Luhmann, H. J., and Sinning, A. (2017). NKCC1-Mediated GABAergic Signaling Promotes Postnatal Cell Death in Neocortical Cajal–Retzius Cells. *Cereb Cortex* 27, 1644–1659. doi:10.1093/cercor/bhw004.

Bock, H. H., Jossin, Y., Liu, P., Förster, E., May, P., Goffinet, A. M., et al. (2003). Phosphatidylinositol 3-Kinase Interacts with the Adaptor Protein Dab1 in Response to Reelin Signaling and Is Required for Normal Cortical Lamination*. *Journal of Biological Chemistry* 278, 38772–38779. doi:10.1074/jbc.M306416200.

Bonni, A., Sun, Y., Nadal-Vicens, M., Bhatt, A., Frank, D. A., Rozovsky, I., et al. (1997). Regulation of Gliogenesis in the Central Nervous System by the JAK-STAT Signaling Pathway. *Science* 278, 477–483. doi:10.1126/science.278.5337.477.

Borrell, V., and Marín, O. (2006). Meninges control tangential migration of hem-derived Cajal-Retzius cells via CXCL12/CXCR4 signaling. *Nat Neurosci* 9, 1284–1293. doi:10.1038/nn1764.

Bouchard, V. J., Rouleau, M., and Poirier, G. G. (2003). PARP-1, a determinant of cell survival in response to DNA damage. *Experimental Hematology* 31, 446–454. doi:10.1016/S0301-472X(03)00083-3.

Brinkmann, B. G., Agarwal, A., Sereda, M. W., Garratt, A. N., Müller, T., Wende, H., et al. (2008). Neuregulin-1/ErbB signaling serves distinct functions in myelination of the peripheral and central nervous system. *Neuron* 59, 581–595. doi:10.1016/j.neuron.2008.06.028.

Brunstrom, J. E., Gray-Swain, M. R., Osborne, P. A., and Pearlman, A. L. (1997). Neuronal Heterotopias in the Developing Cerebral Cortex Produced by Neurotrophin-4. *Neuron* 18, 505–517. doi:10.1016/S0896-6273(00)81250-7.

Bulchand, S., Grove, E. A., Porter, F. D., and Tole, S. (2001). LIM-homeodomain gene Lhx2 regulates the formation of the cortical hem. *Mechanisms of Development* 100, 165–175. doi:10.1016/S0925-4773(00)00515-3.

Cai, Y., Xu, L., Xu, H., and Fan, X. (2016). SIRT1 and Neural Cell Fate Determination. *Mol Neurobiol* 53, 2815–2825. doi:10.1007/s12035-015-9158-6.

Caldecott, K. W., Aoufouchi, S., Johnson, P., and Shall, S. (1996). XRCC1 polypeptide interacts with DNA polymerase beta and possibly poly (ADP-ribose) polymerase, and DNA ligase III is a novel molecular "nick-sensor" in vitro. *Nucleic Acids Res* 24, 4387–4394.

Cannon, D. M., Walshe, M., Dempster, E., Collier, D. A., Marshall, N., Bramon, E., et al. (2012). The association of white matter volume in psychotic disorders with genotypic variation in NRG1, MOG and CNP: a voxel-based analysis in affected individuals and their unaffected relatives. *Transl Psychiatry* 2, e167. doi:10.1038/tp.2012.82.

Cao, F., Hata, R., Zhu, P., Ma, Y.-J., Tanaka, J., Hanakawa, Y., et al. (2006). Overexpression of SOCS3 inhibits astrogliogenesis and promotes maintenance of neural stem cells. *J Neurochem* 98, 459–470. doi:10.1111/j.1471-4159.2006.03890.x.

Cao, F., Hata, R., Zhu, P., Nakashiro, K., and Sakanaka, M. (2010). Conditional deletion of Stat3 promotes neurogenesis and inhibits astrogliogenesis in neural stem cells. *Biochem Biophys Res Commun* 394, 843–847. doi:10.1016/j.bbrc.2010.03.092.

Carmona, M. A., Pozas, E., Martínez, A., Espinosa-Parrilla, J. F., Soriano, E., and Aguado, F. (2006). Age-dependent Spontaneous Hyperexcitability and Impairment of GABAergic Function in the Hippocampus of Mice Lacking trkB. *Cerebral Cortex* 16, 47–63. doi:10.1093/cercor/bhi083.

Caruso, L. B., Martin, K. A., Lauretti, E., Hulse, M., Siciliano, M., Lupey-Green, L. N., et al. (2018). Poly(ADP-ribose) Polymerase 1, PARP1, modifies EZH2 and inhibits EZH2 histone methyltransferase activity after DNA damage. *Oncotarget* 9. doi:10.18632/oncotarget.24291.

Casarosa, S., Fode, C., and Guillemot, F. (1999). Mash1 regulates neurogenesis in the ventral telencephalon. *Development* 126, 525–534. doi:10.1242/dev.126.3.525.

Castro, D. S., and Guillemot, F. (2011). Old and new functions of proneural factors revealed by the genome-wide characterization of their transcriptional targets. *Cell Cycle* 10, 4026–4031. doi:10.4161/cc.10.23.18578.

Caviness, V. S., Takahashi, T., and Nowakowski, R. S. (1995). Numbers, time and neocortical neuronogenesis: a general developmental and evolutionary model. *Trends in Neurosciences* 18, 379–383. doi:10.1016/0166-2236(95)93933-O.

Cervantes-Laurean, D., Jacobson, E. L., and Jacobson, M. K. (1996). Glycation and Glycoxidation of Histones by ADP-ribose. *Journal of Biological Chemistry* 271, 10461–10469. doi:10.1074/jbc.271.18.10461.

Chacon-Cabrera, A., Fermoselle, C., Salmela, I., Yelamos, J., and Barreiro, E. (2015). MicroRNA expression and protein acetylation pattern in respiratory and limb muscles of Parp-1−/− and Parp-2−/− mice with lung cancer cachexia. *Biochimica et Biophysica Acta (BBA) - General Subjects* 1850, 2530–2543. doi:10.1016/j.bbagen.2015.09.020.

Chai, X., Förster, E., Zhao, S., Bock, H. H., and Frotscher, M. (2009). Reelin Stabilizes the Actin Cytoskeleton of Neuronal Processes by Inducing n-Cofilin Phosphorylation at Serine3. *J Neurosci* 29, 288–299. doi:10.1523/JNEUROSCI.2934-08.2009.

Chai, X., Zhao, S., Fan, L., Zhang, W., Lu, X., Shao, H., et al. (2016). Reelin and cofilin cooperate during the migration of cortical neurons: a quantitative morphological analysis. *Development* 143, 1029–1040. doi:10.1242/dev.134163.

Chambers, C. B., Peng, Y., Nguyen, H., Gaiano, N., Fishell, G., and Nye, J. S. (2001). Spatiotemporal selectivity of response to Notch1 signals in mammalian forebrain precursors. *Development* 128, 689–702. doi:10.1242/dev.128.5.689.

Chambon, P., Weill, J. D., Doly, J., Strosser, M. T., and Mandel, P. (1966). On the formation of a novel adenylic compound by enzymatic extracts of liver nuclei. *Biochemical and*

Biophysical Research Communications 25, 638–643. doi:10.1016/0006-291X(66)90502-X.

Chana, G., Landau, S., Beasley, C., Everall, I. P., and Cotter, D. (2003). Two-dimensional assessment of cytoarchitecture in the anterior cingulate cortex in major depressive disorder, bipolar disorder, and schizophrenia: evidence for decreased neuronal somal size and increased neuronal density. *Biological Psychiatry* 53, 1086–1098. doi:10.1016/S0006-3223(03)00114-8.

Chaudhury, A. R., Gerecke, K. M., Wyss, J. M., Morgan, D. G., Gordon, M. N., and Carroll, S. L. (2003). Neuregulin-1 and ErbB4 Immunoreactivity Is Associated with Neuritic Plaques in Alzheimer Disease Brain and in a Transgenic Model of Alzheimer Disease. *Journal of Neuropathology & Experimental Neurology* 62, 42–54. doi:10.1093/jnen/62.1.42.

Chen, G., Sima, J., Jin, M., Wang, K., Xue, X., Zheng, W., et al. (2008). Semaphorin-3A guides radial migration of cortical neurons during development. *Nat Neurosci* 11, 36–44. doi:10.1038/nn2018.

Chen, Y., Chen, S., Liang, H., Yang, H., Liu, L., Zhou, K., et al. (2018). Bcl-2 protects TK6 cells against hydroquinone-induced apoptosis through PARP-1 cytoplasm translocation and stabilizing mitochondrial membrane potential. *Environmental and Molecular Mutagenesis* 59, 49–59. doi:10.1002/em.22126.

Chen, Y., Sharma, R. P., Costa, R. H., Costa, E., and Grayson, D. R. (2002). On the epigenetic regulation of the human reelin promoter. *Nucleic Acids Res* 30, 2930–2939.

Chen, Z.-L., Haegeli, V., Yu, H., and Strickland, S. (2009). Cortical deficiency of laminin γ1 impairs the AKT/GSK-3β signaling pathway and leads to defects in neurite outgrowth and neuronal migration. *Dev Biol* 327, 158–168. doi:10.1016/j.ydbio.2008.12.006.

Cheng, T.-L., and Qiu, Z. (2014). MeCP2: multifaceted roles in gene regulation and neural development. *Neurosci Bull* 30, 601–609. doi:10.1007/s12264-014-1452-6.

Cherney, B. W., McBride, O. W., Chen, D. F., Alkhatib, H., Bhatia, K., Hensley, P., et al. (1987). cDNA sequence, protein structure, and chromosomal location of the human gene for poly(ADP-ribose) polymerase. *Proc Natl Acad Sci U S A* 84, 8370–8374.

Chiarugi, A. (2005). Poly(ADP-ribosyl)ation and stroke. *Pharmacological Research* 52, 15–24. doi:10.1016/j.phrs.2005.02.018.

Christensen, J., Agger, K., Cloos, P. A. C., Pasini, D., Rose, S., Sennels, L., et al. (2007). RBP2 Belongs to a Family of Demethylases, Specific for Tri-and Dimethylated Lysine 4 on Histone 3. *Cell* 128, 1063–1076. doi:10.1016/j.cell.2007.02.003.

Chu, J., and Anderson, S. A. (2015). Development of Cortical Interneurons. *Neuropsychopharmacol* 40, 16–23. doi:10.1038/npp.2014.171.

Chung, D. W., Volk, D. W., Arion, D., Zhang, Y., Sampson, A. R., and Lewis, D. A. (2016). Dysregulated ErbB4 Splicing in Schizophrenia: Selective Effects on Parvalbumin Expression. *Am J Psychiatry* 173, 60–68. doi:10.1176/appi.ajp.2015.15020150.

Ciccarone, F., Valentini, E., Zampieri, M., and Caiafa, P. (2015). 5mC-hydroxylase activity is influenced by the PARylation of TET1 enzyme. *Oncotarget* 6. doi:10.18632/oncotarget.4476.

Cohen-Armon, M., Visochek, L., Rozensal, D., Kalal, A., Geistrikh, I., Klein, R., et al. (2007). DNA-Independent PARP-1 Activation by Phosphorylated ERK2 Increases Elk1 Activity: A Link to Histone Acetylation. *Molecular Cell* 25, 297–308. doi:10.1016/j.molcel.2006.12.012.

Cooper, J. A. (2013). Mechanisms of cell migration in the nervous system. *Journal of Cell Biology* 202, 725–734. doi:10.1083/jcb.201305021.

Cox, E. A., and Huttenlocher, A. (1998). Regulation of integrin-mediated adhesion during cell migration. *Microscopy Research and Technique* 43, 412–419. doi:https://doi.org/10.1002/(SICI)1097-0029(19981201)43:5<412::AID-JEMT7>3.0.CO;2-F.

Dahm, G. M., Gubin, M. M., Magee, J. D., Techasintana, P., Calaluce, R., and Atasoy, U. (2012). Method for the Isolation and Identification of mRNAs, microRNAs and Protein Components of Ribonucleoprotein Complexes from Cell Extracts using RIP-Chip. *J Vis Exp*. doi:10.3791/3851.

D’Amours, D., Desnoyers, S., D’Silva, I., and Poirier, G. G. (1999). Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. *Biochem J* 342, 249–268.

Danhauser, K., Alhaddad, B., Makowski, C., Piekutowska-Abramczuk, D., Syrbe, S., Gomez-Ospina, N., et al. (2018). Bi-allelic ADPRHL2 Mutations Cause Neurodegeneration with Developmental Delay, Ataxia, and Axonal Neuropathy. *The American Journal of Human Genetics* 103, 817–825. doi:10.1016/j.ajhg.2018.10.005.

Daniel, D., Rossel, M., Seki, T., and König, N. (2005). Stromal cell-derived factor-1 (SDF-1) expression in embryonic mouse cerebral cortex starts in the intermediate zone close to the pallial–subpallial boundary and extends progressively towards the cortical hem. *Gene Expression Patterns* 5, 317–322. doi:10.1016/j.modgep.2004.10.007.

D’Arcangelo, G., Homayouni, R., Keshvara, L., Rice, D. S., Sheldon, M., and Curran, T. (1999). Reelin Is a Ligand for Lipoprotein Receptors. *Neuron* 24, 471–479. doi:10.1016/S0896-6273(00)80860-0.

D’Arcangelo, G., Nakajima, K., Miyata, T., Ogawa, M., Mikoshiba, K., and Curran, T. (1997). Reelin Is a Secreted Glycoprotein Recognized by the CR-50 Monoclonal Antibody. *J. Neurosci.* 17, 23–31. doi:10.1523/JNEUROSCI.17-01-00023.1997.

Dave, R. K., Ellis, T., Toumpas, M. C., Robson, J. P., Julian, E., Adolphe, C., et al. (2011). Sonic Hedgehog and Notch Signaling Can Cooperate to Regulate Neurogenic Divisions of Neocortical Progenitors. *PLoS One* 6. doi:10.1371/journal.pone.0014680.

Davis, S., Aldrich, T. H., Stahl, N., Pan, L., Taga, T., Kishimoto, T., et al. (1993). LIFR beta and gp130 as heterodimerizing signal transducers of the tripartite CNTF receptor. *Science* 260, 1805–1808. doi:10.1126/science.8390097.

de Murcia, J. M., Niedergang, C., Trucco, C., Ricoul, M., Dutrillaux, B., Mark, M., et al. (1997). Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. *PNAS* 94, 7303–7307.

De Strooper, B. (2007). Loss-of-function presenilin mutations in Alzheimer disease. Talking Point on the role of presenilin mutations in Alzheimer disease. *EMBO Rep* 8, 141–146. doi:10.1038/sj.embor.7400897.

del Río, J. A., Martinez, A., Fonseca, M., Auladell, C., and Soriano, E. (1995). Glutamate-like Immunoreactivity and Fate of Cajal-Retzius Cells in the Murine Cortex as Identified with Calretinin Antibody. *Cerebral Cortex* 5, 13–21. doi:10.1093/cercor/5.1.13.

del Pino, I., García-Frigola, C., Dehorter, N., Brotons-Mas, J. R., Alvarez-Salvado, E., Martínez de Lagrán, M., et al. (2013). Erbb4 Deletion from Fast-Spiking Interneurons Causes Schizophrenia-like Phenotypes. *Neuron* 79, 1152–1168. doi:10.1016/j.neuron.2013.07.010.

Depboylu, C., Höllerhage, M., Schnurrbusch, S., Brundin, P., Oertel, W. H., Schrattenholz, A., et al. (2012). Neuregulin-1 receptor tyrosine kinase ErbB4 is upregulated in midbrain dopaminergic neurons in Parkinson disease. *Neuroscience Letters* 531, 209–214. doi:10.1016/j.neulet.2012.10.050.

Di Giammartino, D. C., Shi, Y., and Manley, J. L. (2013). PARP1 REPRESSES PAP AND INHIBITS POLYADENYLATION DURING HEAT SHOCK. *Mol Cell* 49, 7–17. doi:10.1016/j.molcel.2012.11.005.

Ding, L., Chen, X., Xu, X., Qian, Y., Liang, G., Yao, F., et al. (2019). PARP1 Suppresses the Transcription of PD-L1 by Poly(ADP-Ribosyl)ating STAT3. *Cancer Immunol Res* 7, 136–149. doi:10.1158/2326-6066.CIR-18-0071.

Dixit, R., Zimmer, C., Waclaw, R. R., Mattar, P., Shaker, T., Kovach, C., et al. (2011). Ascl1 Participates in Cajal–Retzius Cell Development in the Neocortex. *Cerebral Cortex* 21, 2599–2611. doi:10.1093/cercor/bhr046.

Dong, Z., Yang, N., Yeo, S.-Y., Chitnis, A., and Guo, S. (2012). Intra-lineage Directional Notch Signaling Regulates Self-renewal and Differentiation of Asymmetrically Dividing Radial Glia. *Neuron* 74, 65–78. doi:10.1016/j.neuron.2012.01.031.

Du, T., Xu, Q., Ocbina, P. J., and Anderson, S. A. (2008). NKX2.1 specifies cortical interneuron fate by activating Lhx6. *Development* 135, 1559–1567. doi:10.1242/dev.015123.

Durkacz, B. W., Omidiji, O., Gray, D. A., and Shall, S. (1980). (ADP-ribose) n participates in DNA excision repair. *Nature* 283, 593–596. doi:10.1038/283593a0.

Durmus, H., Mertoğlu, E., Sticht, H., Ceylaner, S., Kulaksızoğlu, I. B., Hashemolhosseini, S., et al. (2021). Episodic psychosis, ataxia, motor neuropathy with pyramidal signs (PAMP syndrome) caused by a novel mutation in ADPRHL2 (AHR3). *Neurol Sci*. doi:10.1007/s10072-021-05100-w.

Dziadkowiec, K. N., Gąsiorowska, E., Nowak-Markwitz, E., and Jankowska, A. (2016). PARP inhibitors: review of mechanisms of action and BRCA1/2 mutation targeting. *pm* 4, 215–219. doi:10.5114/pm.2016.65667.

Eguchi, Y., Shimizu, S., and Tsujimoto, Y. (1997). Intracellular ATP Levels Determine Cell Death Fate by Apoptosis or Necrosis. *Cancer Res* 57, 1835–1840.

Elenius, K., Corfas, G., Paul, S., Choi, C. J., Rio, C., Plowman, G. D., et al. (1997). A Novel Juxtamembrane Domain Isoform of HER4/ErbB4: ISOFORM-SPECIFIC TISSUE DISTRIBUTION AND DIFFERENTIAL PROCESSING IN RESPONSE TO PHORBOL ESTER*. *Journal of Biological Chemistry* 272, 26761–26768. doi:10.1074/jbc.272.42.26761.

Eliasson, M. J. L., Sampei, K., Mandir, A. S., Hurn, P. D., Traystman, R. J., Bao, J., et al. (1997). Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. *Nat Med* 3, 1089–1095. doi:10.1038/nm1097-1089.

El-Khamisy, S. F., Masutani, M., Suzuki, H., and Caldecott, K. W. (2003). A requirement for PARP-1 for the assembly or stability of XRCC1 nuclear foci at sites of oxidative DNA damage. *Nucleic Acids Res* 31, 5526–5533. doi:10.1093/nar/gkg761.

Endres, M., Wang, Z.-Q., Namura, S., Waeber, C., and Moskowitz, M. A. (1997). Ischemic Brain Injury is Mediated by the Activation of Poly(ADP-Ribose)Polymerase. *J Cereb Blood Flow Metab* 17, 1143–1151. doi:10.1097/00004647-199711000-00002.

Evangelisti, C., Florian, M. C., Massimi, I., Dominici, C., Giannini, G., Galardi, S., et al. (2009). MiR-128 up-regulation inhibits Reelin and DCX expression and reduces neuroblastoma cell motility and invasiveness. *FASEB j.* 23, 4276–4287. doi:10.1096/fj.09-134965.

Farez, M. F., Quintana, F. J., Gandhi, R., Izquierdo, G., Lucas, M., and Weiner, H. L. (2009). Toll-like receptor 2 and poly(ADP-ribose) polymerase 1 promote central nervous system neuroinflammation in progressive EAE. *Nat Immunol* 10, 958–964. doi:10.1038/ni.1775.

Fazzari, P., Paternain, A. V., Valiente, M., Pla, R., Luján, R., Lloyd, K., et al. (2010). Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling. *Nature* 464, 1376–1380. doi:10.1038/nature08928.

Feng, Y., Cheng, D., Zhang, C., Li, Y., Zhang, Z., Wang, J., et al. (2017). Association between ErbB4 single nucleotide polymorphisms and susceptibility to schizophrenia. *Medicine (Baltimore)* 96. doi:10.1097/MD.0000000000005920.

Ferent, J., Zaidi, D., and Francis, F. (2020). Extracellular Control of Radial Glia Proliferation and Scaffolding During Cortical Development and Pathology. *Front. Cell Dev. Biol.* 8. doi:10.3389/fcell.2020.578341.

Fietz, S. A., Kelava, I., Vogt, J., Wilsch-Bräuninger, M., Stenzel, D., Fish, J. L., et al. (2010). OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. *Nat Neurosci* 13, 690–699. doi:10.1038/nn.2553.

Fischer, U., Jänicke, R. U., and Schulze-Osthoff, K. (2003). Many cuts to ruin: a comprehensive update of caspase substrates. *Cell Death Differ* 10, 76–100. doi:10.1038/sj.cdd.4401160.

Flames, N., Long, J. E., Garratt, A. N., Fischer, T. M., Gassmann, M., Birchmeier, C., et al. (2004). Short- and Long-Range Attraction of Cortical GABAergic Interneurons by Neuregulin-1. *Neuron* 44, 251–261. doi:10.1016/j.neuron.2004.09.028.

Florio, M., and Huttner, W. B. (2014). Neural progenitors, neurogenesis and the evolution of the neocortex. *Development* 141, 2182–2194. doi:10.1242/dev.090571.

Folsom, T. D., and Fatemi, S. H. (2013). The involvement of Reelin in neurodevelopmental disorders. *Neuropharmacology* 68, 122–135. doi:10.1016/j.neuropharm.2012.08.015.

Fox, I. J., and Kornblum, H. I. (2005). Developmental profile of ErbB receptors in murine central nervous system: Implications for functional interactions. *J. Neurosci. Res.* 79, 584–597. doi:10.1002/jnr.20381.

Franco, S. J., Martinez-Garay, I., Gil-Sanz, C., Harkins-Perry, S. R., and Müller, U. (2011). Reelin Regulates Cadherin Function via Dab1/Rap1 to Control Neuronal Migration and Lamination in the Neocortex. *Neuron* 69, 482–497. doi:10.1016/j.neuron.2011.01.003.

Franco, S. J., and Müller, U. (2011). ECM Functions During Neuronal Migration and Lamination in the Mammalian Central Nervous System. *Dev Neurobiol* 71, 889–900. doi:10.1002/dneu.20946.

Friedman, W. J., Olson, L., and Persson, H. (1991). Cells that Express Brain-Derived Neurotrophic Factor mRNA in the Developing Postnatal Rat Brain. *European Journal of Neuroscience* 3, 688–697. doi:10.1111/j.1460-9568.1991.tb00854.x.

Gaiano, N., and Fishell, G. (2002). The Role of Notch in Promoting Glial and Neural Stem Cell Fates. *Annu. Rev. Neurosci.* 25, 471–490. doi:10.1146/annurev.neuro.25.030702.130823.

Gaiano, N., Nye, J. S., and Fishell, G. (2000). Radial Glial Identity Is Promoted by Notch1 Signaling in the Murine Forebrain. *Neuron* 26, 395–404. doi:10.1016/S0896-6273(00)81172-1.

Galbis-Martínez, M., Saenz, L., Ramírez, P., Parrilla, P., and Yélamos, J. (2010). Poly(ADP-ribose) polymerase-1 modulates interferon-γ-inducible protein (IP)-10 expression in murine embryonic fibroblasts by stabilizing IP-10 mRNA. *Molecular Immunology* 47, 1492–1499. doi:10.1016/j.molimm.2010.01.022.

Galia, A., Calogero, A. E., Condorelli, R. A., Fraggetta, F., La Corte, C., Ridolfo, F., et al. (2012). PARP-1 protein expression in glioblastoma multiforme. *Eur J Histochem* 56. doi:10.4081/ejh.2012.e9.

Gamble, M. J., and Fisher, R. P. (2007). SET and PARP1 remove DEK from chromatin to permit access by the transcription machinery. *Nature Structural & Molecular Biology* 14, 548–555. doi:10.1038/nsmb1248.

Gao, F., Kwon, S. W., Zhao, Y., and Jin, Y. (2009). PARP1 Poly(ADP-ribosyl)ates Sox2 to Control Sox2 Protein Levels and *FGF4* Expression during Embryonic Stem Cell Differentiation. *J. Biol. Chem.* 284, 22263–22273. doi:10.1074/jbc.M109.033118.

García, S., and Conde, C. (2015). The Role of Poly(ADP-ribose) Polymerase-1 in Rheumatoid Arthritis. *Mediators Inflamm* 2015. doi:10.1155/2015/837250.

Garcion, E., Halilagic, A., Faissner, A., and ffrench-Constant, C. (2004). Generation of an environmental niche for neural stem cell development by the extracellular matrix molecule tenascin C. *Development* 131, 3423–3432. doi:10.1242/dev.01202.

Gärtner, A., Fornasiero, E. F., Munck, S., Vennekens, K., Seuntjens, E., Huttner, W. B., et al. (2012). N-cadherin specifies first asymmetry in developing neurons. *EMBO J* 31, 1893–1903. doi:10.1038/emboj.2012.41.

Gauthier, A. S., Furstoss, O., Araki, T., Chan, R., Neel, B. G., Kaplan, D. R., et al. (2007). Control of CNS Cell Fate Decisions by SHP-2 and its Dysregulation in Noonan Syndrome. *Neuron* 54, 245–262. doi:10.1016/j.neuron.2007.03.027.

Ge, W., Martinowich, K., Wu, X., He, F., Miyamoto, A., Fan, G., et al. (2002). Notch signaling promotes astrogliogenesis via direct CSL-mediated glial gene activation. *Journal of Neuroscience Research* 69, 848–860. doi:10.1002/jnr.10364.

Ge, W.-P., Miyawaki, A., Gage, F. H., Jan, Y. N., and Jan, L. Y. (2012). Local generation of glia is a major astrocyte source in postnatal cortex. *Nature* 484, 376–380. doi:10.1038/nature10959.

Gilbert de Murcia and Josiane Menissier de Murcia (1994). Poly(ADP-ribose) polymerase: a molecular nick-sensor. *Trends in Biochemical Sciences* 19, 172–176. doi:10.1016/0968-0004(94)90280-1.

Gil-Sanz, C., Franco, S. J., Martinez-Garay, I., Espinosa, A., Harkins-Perry, S., and Müller, U. (2013). Cajal-Retzius Cells Instruct Neuronal Migration by Coincidence Signaling between Secreted and Contact-Dependent Guidance Cues. *Neuron* 79, 461–477. doi:10.1016/j.neuron.2013.06.040.

Girós, A., Morante, J., Gil-Sanz, C., Fairén, A., and Costell, M. (2007). Perlecan controls neurogenesis in the developing telencephalon. *BMC Dev Biol* 7, 29. doi:10.1186/1471-213X-7-29.

Go, R. C. P., Perry, R. T., Wiener, H., Bassett, S. S., Blacker, D., Devlin, B., et al. (2005). Neuregulin-1 polymorphism in late onset Alzheimer's disease families with psychoses. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 139B, 28–32. doi:https://doi.org/10.1002/ajmg.b.30219.

Goffinet, A. M., So, K.-F., Yamamoto, M., Edwards, M., and Caviness, V. S. (1984). Architectonic and hodological organization of the cerebellum in reeler mutant mice. *Developmental Brain Research* 16, 263–276. doi:10.1016/0165-3806(84)90031-2.

Goldberg, S., Visochek, L., Giladi, E., Gozes, I., and Cohen-Armon, M. (2009). PolyADP-ribosylation is required for long-term memory formation in mammals. *Journal of Neurochemistry* 111, 72–79. doi:10.1111/j.1471-4159.2009.06296.x.

Golub, M. S., Germann, S. L., and Lloyd, K. C. K. (2004). Behavioral characteristics of a nervous system-specific erbB4 knock-out mouse. *Behavioural Brain Research* 153, 159–170. doi:10.1016/j.bbr.2003.11.010.

Grandbarbe, L., Bouissac, J., Rand, M., Hrabé de Angelis, M., Artavanis-Tsakonas, S., and Mohier, E. (2003). Delta-Notch signaling controls the generation of neurons/glia from neural stem cells in a stepwise process. *Development* 130, 1391–1402. doi:10.1242/dev.00374.

Graus-Porta, D., Blaess, S., Senften, M., Littlewood-Evans, A., Damsky, C., Huang, Z., et al. (2001). β1-Class Integrins Regulate the Development of Laminae and Folia in the Cerebral and Cerebellar Cortex. *Neuron* 31, 367–379. doi:10.1016/S0896-6273(01)00374-9.

Griveau, A., Borello, U., Causeret, F., Tissir, F., Boggetto, N., Karaz, S., et al. (2010). A Novel Role for Dbx1-Derived Cajal-Retzius Cells in Early Regionalization of the Cerebral Cortical Neuroepithelium. *PLOS Biology* 8, e1000440. doi:10.1371/journal.pbio.1000440.

Gu, F., Hata, R., Ma, Y.-J., Tanaka, J., Mitsuda, N., Kumon, Y., et al. (2005). Suppression of Stat3 promotes neurogenesis in cultured neural stem cells. *Journal of Neuroscience Research* 81, 163–171. doi:10.1002/jnr.20561.

Guillemot, F. (2005). Cellular and molecular control of neurogenesis in the mammalian telencephalon. *Current Opinion in Cell Biology* 17, 639–647. doi:10.1016/j.ceb.2005.09.006.

Gunhaga, L., Marklund, M., Sjödal, M., Hsieh, J.-C., Jessell, T. M., and Edlund, T. (2003). Specification of dorsal telencephalic character by sequential Wnt and FGF signaling. *Nat Neurosci* 6, 701–707. doi:10.1038/nn1068.

Guy, P. M., Platko, J. V., Cantley, L. C., Cerione, R. A., and Carraway, K. L. (1994). Insect cell-expressed p180erbB3 possesses an impaired tyrosine kinase activity. *PNAS* 91, 8132–8136.

Haim, L. B., and Rowitch, D. H. (2017). Functional diversity of astrocytes in neural circuit regulation. *Nat Rev Neurosci* 18, 31–41. doi:10.1038/nrn.2016.159.

Haince, J.-F., McDonald, D., Rodrigue, A., Déry, U., Masson, J.-Y., Hendzel, M. J., et al. (2008). PARP1-dependent Kinetics of Recruitment of MRE11 and NBS1 Proteins to Multiple DNA Damage Sites*. *Journal of Biological Chemistry* 283, 1197–1208. doi:10.1074/jbc.M706734200.

Halfter, W., Dong, S., Yip, Y.-P., Willem, M., and Mayer, U. (2002). A Critical Function of the Pial Basement Membrane in Cortical Histogenesis. *J Neurosci* 22, 6029–6040. doi:10.1523/JNEUROSCI.22-14-06029.2002.

Hamburgh, M. (1963). Analysis of the postnatal developmental effects of “reeler,” a neurological mutation in mice. A study in developmental genetics. *Developmental Biology* 8, 165–185. doi:10.1016/0012-1606(63)90040-X.

Hansen, D. V., Lui, J. H., Parker, P. R. L., and Kriegstein, A. R. (2010). Neurogenic radial glia in the outer subventricular zone of human neocortex. *Nature* 464, 554–561. doi:10.1038/nature08845.

Hartfuss, E., Galli, R., Heins, N., and Götz, M. (2001). Characterization of CNS Precursor Subtypes and Radial Glia. *Developmental Biology* 229, 15–30. doi:10.1006/dbio.2000.9962.

Hassa, P. O., Buerki, C., Lombardi, C., Imhof, R., and Hottiger, M. O. (2003). Transcriptional Coactivation of Nuclear Factor-κB-dependent Gene Expression by p300 Is Regulated by Poly(ADP)-ribose Polymerase-1*. *Journal of Biological Chemistry* 278, 45145–45153. doi:10.1074/jbc.M307957200.

Hassa, P. O., and Hottiger, M. O. (1999). A Role of Poly (ADP-Ribose) Polymerase in NF- B Transcriptional Activation. 380, 953–959. doi:10.1515/BC.1999.118.

Hau, A.-C., Grebbin, B. M., Agoston, Z., Anders-Maurer, M., Müller, T., Groß, A., et al. (2017). MEIS homeodomain proteins facilitate PARP1/ARTD1-mediated eviction of histone H1. *Journal of Cell Biology* 216, 2715–2729. doi:10.1083/jcb.201701154.

Haubst, N., Georges-Labouesse, E., De Arcangelis, A., Mayer, U., and Götz, M. (2006). Basement membrane attachment is dispensable for radial glial cell fate and for proliferation, but affects positioning of neuronal subtypes. *Development* 133, 3245–3254. doi:10.1242/dev.02486.

Heim, L.-O., Kappl, M., and Butt, H.-J. (2004). Tilt of Atomic Force Microscope Cantilevers: Effect on Spring Constant and Adhesion Measurements. *Langmuir* 20, 2760–2764. doi:10.1021/la036128m.

Hermanson, O., Jepsen, K., and Rosenfeld, M. G. (2002). N-CoR controls differentiation of neural stem cells into astrocytes. *Nature* 419, 934–939. doi:10.1038/nature01156.

Herskovits, A. Z., and Guarente, L. (2014). SIRT1 in neurodevelopment and brain senescence. *Neuron* 81, 471–483. doi:10.1016/j.neuron.2014.01.028.

Hevner, R. F., Shi, L., Justice, N., Hsueh, Y.-P., Sheng, M., Smiga, S., et al. (2001). Tbr1 Regulates Differentiation of the Preplate and Layer 6. *Neuron* 29, 353–366. doi:10.1016/S0896-6273(01)00211-2.

Hiesberger, T., Trommsdorff, M., Howell, B. W., Goffinet, A., Mumby, M. C., Cooper, J. A., et al. (1999). Direct Binding of Reelin to VLDL Receptor and ApoE Receptor 2 Induces Tyrosine Phosphorylation of Disabled-1 and Modulates Tau Phosphorylation. *Neuron* 24, 481–489. doi:10.1016/S0896-6273(00)80861-2.

Hirabayashi, Y., Itoh, Y., Tabata, H., Nakajima, K., Akiyama, T., Masuyama, N., et al. (2004). The Wnt/β-catenin pathway directs neuronal differentiation of cortical neural precursor cells. *Development* 131, 2791–2801. doi:10.1242/dev.01165.

Hirota, Y., Kubo, K., Fujino, T., Yamamoto, T. T., and Nakajima, K. (2018). ApoER2 Controls Not Only Neuronal Migration in the Intermediate Zone But Also Termination of Migration in the Developing Cerebral Cortex. *Cerebral Cortex* 28, 223–235. doi:10.1093/cercor/bhw369.

Hirota, Y., and Nakajima, K. (2017). Control of Neuronal Migration and Aggregation by Reelin Signaling in the Developing Cerebral Cortex. *Front. Cell Dev. Biol.* 5, 40. doi:10.3389/fcell.2017.00040.

Hirota, Y., and Nakajima, K. (2020). VLDLR is not essential for reelin-induced neuronal aggregation but suppresses neuronal invasion into the marginal zone. *Development* 147. doi:10.1242/dev.189936.

Hong, S., Yi, J. H., Lee, S., Park, C.-H., Ryu, J. H., Shin, K. S., et al. (2019). Defective neurogenesis and schizophrenia-like behavior in PARP-1-deficient mice. *Cell Death Dis* 10, 943. doi:10.1038/s41419-019-2174-0.

Howard, B., Chen, Y., and Zecevic, N. (2006). Cortical progenitor cells in the developing human telencephalon. *Glia* 53, 57–66. doi:10.1002/glia.20259.

Howell, B. W., Herrick, T. M., and Cooper, J. A. (1999). Reelin-induced tryosine phosphorylation of Disabled 1 during neuronal positioning. *Genes Dev* 13, 643–648.

Howell, B. W., Herrick, T. M., Hildebrand, J. D., Zhang, Y., and Cooper, J. A. (2000). Dab1 tyrosine phosphorylation sites relay positional signals during mouse brain development. *Current Biology* 10, 877–885. doi:10.1016/S0960-9822(00)00608-4.

Hu, Y., Petit, S. A., Ficarro, S. B., Toomire, K. J., Xie, A., Lim, E., et al. (2014). PARP1-Driven Poly-ADP-Ribosylation Regulates BRCA1 Function in Homologous Recombination–Mediated DNA Repair. *Cancer Discov* 4, 1430–1447.

Huang, D., Yang, C., Yao, L., Wang, Y., Liao, Y., and Huang, K. (2008). Activation and Overexpression of PARP-1 in Circulating Mononuclear Cells Promote TNF-α and IL-6 Expression in Patients with Unstable Angina. *Archives of Medical Research* 39, 775–784. doi:10.1016/j.arcmed.2008.09.003.

Huang, W., Zhang, L., Niu, R., and Liao, H. (2009). Tenascin-R distinct domains modulate migration of neural stem/progenitor cells in vitro. *In Vitro Cell.Dev.Biol.-Animal* 45, 10–14. doi:10.1007/s11626-008-9145-6.

Huang, Y., Magdaleno, S., Hopkins, R., Slaughter, C., Curran, T., and Keshvara, L. (2004). Tyrosine phosphorylated Disabled 1 recruits Crk family adapter proteins. *Biochemical and Biophysical Research Communications* 318, 204–212. doi:10.1016/j.bbrc.2004.04.023.

Huang, Y., Yu, S., Wu, Z., and Tang, B. (2014). Genetics of hereditary neurological disorders in children. *Transl Pediatr* 3, 108–119. doi:10.3978/j.issn.2224-4336.2014.03.04.

Huang, Y. Z., Won, S., Ali, D. W., Wang, Q., Tanowitz, M., Du, Q. S., et al. (2000). Regulation of Neuregulin Signaling by PSD-95 Interacting with ErbB4 at CNS Synapses. *Neuron* 26, 443–455. doi:10.1016/S0896-6273(00)81176-9.

Huletsky, A., de Murcia, G., Muller, S., Hengartner, M., Ménard, L., Lamarre, D., et al. (1989). The Effect of poly(ADP-ribosyl)ation on Native and H1-depleted Chromatin. *Journal of Biological Chemistry* 264, 8878–8886. doi:10.1016/S0021-9258(18)81875-0.

Huntzinger, E., and Izaurralde, E. (2011). Gene silencing by microRNAs: contributions of translational repression and mRNA decay. *Nat Rev Genet* 12, 99–110. doi:10.1038/nrg2936.

Hutter, J. L. (2005). Comment on Tilt of Atomic Force Microscope Cantilevers: Effect on Spring Constant and Adhesion Measurements. *Langmuir* 21, 2630–2632. doi:10.1021/la047670t.

Huttner, W. B., and Brand, M. (1997). Asymmetric division and polarity of neuroepithelial cells. *Current Opinion in Neurobiology* 7, 29–39. doi:10.1016/S0959-4388(97)80117-1.

Imayoshi, I., Isomura, A., Harima, Y., Kawaguchi, K., Kori, H., Miyachi, H., et al. (2013). Oscillatory Control of Factors Determining Multipotency and Fate in Mouse Neural Progenitors. *Science* 342, 1203–1208. doi:10.1126/science.1242366.

Imayoshi, I., Shimogori, T., Ohtsuka, T., and Kageyama, R. (2008). Hes genes and neurogenin regulate non-neural versus neural fate specification in the dorsal telencephalic midline. *Development* 135, 2531–2541. doi:10.1242/dev.021535.

Inatani, M., Irie, F., Plump, A. S., Tessier-Lavigne, M., and Yamaguchi, Y. (2003). Mammalian Brain Morphogenesis and Midline Axon Guidance Require Heparan Sulfate. *Science* 302, 1044–1046. doi:10.1126/science.1090497.

Irintchev, A., Rollenhagen, A., Troncoso, E., Kiss, J. Z., and Schachner, M. (2005). Structural and Functional Aberrations in the Cerebral Cortex of Tenascin-C Deficient Mice. *Cerebral Cortex* 15, 950–962. doi:10.1093/cercor/bhh195.

Ishii, K., Kubo, K., and Nakajima, K. (2016). Reelin and Neuropsychiatric Disorders. *Front. Cell. Neurosci.* 10. doi:10.3389/fncel.2016.00229.

Ishii, M., and Maeda, N. (2008). Oversulfated Chondroitin Sulfate Plays Critical Roles in the Neuronal Migration in the Cerebral Cortex*. *Journal of Biological Chemistry* 283, 32610–32620. doi:10.1074/jbc.M806331200.

Israsena, N., Hu, M., Fu, W., Kan, L., and Kessler, J. A. (2004). The presence of FGF2 signaling determines whether β-catenin exerts effects on proliferation or neuronal differentiation of neural stem cells. *Developmental Biology* 268, 220–231. doi:10.1016/j.ydbio.2003.12.024.

Jen, Y.-H. L., Musacchio, M., and Lander, A. D. (2009). Glypican-1 controls brain size through regulation of fibroblast growth factor signaling in early neurogenesis. *Neural Dev* 4, 33. doi:10.1186/1749-8104-4-33.

Jiang, X., and Nardelli, J. (2016). Cellular and molecular introduction to brain development. *Neurobiology of Disease* 92, 3–17. doi:10.1016/j.nbd.2015.07.007.

Jijon, H. B., Churchill, T., Malfair, D., Wessler, A., Jewell, L. D., Parsons, H. G., et al. (2000). Inhibition of poly(ADP-ribose) polymerase attenuates inflammation in a model of chronic colitis. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 279, G641–G651. doi:10.1152/ajpgi.2000.279.3.G641.

Joshi, D., Fullerton, J. M., and Weickert, C. S. (2014). Elevated ErbB4 mRNA is related to interneuron deficit in prefrontal cortex in schizophrenia. *Journal of Psychiatric Research* 53, 125–132. doi:10.1016/j.jpsychires.2014.02.014.

Ju, B.-G., Solum, D., Song, E. J., Lee, K.-J., Rose, D. W., Glass, C. K., et al. (2004). Activating the PARP-1 Sensor Component of the Groucho/ TLE1 Corepressor Complex Mediates a CaMKinase II□-Dependent Neurogenic Gene Activation Pathway. 15.

Jubin, T., Kadam, A., Gani, A. R., Singh, M., Dwivedi, M., and Begum, R. (2017). Poly ADP-ribose polymerase-1: Beyond transcription and towards differentiation. *Seminars in Cell & Developmental Biology* 63, 167–179. doi:10.1016/j.semcdb.2016.07.027.

Kaddour, H., Coppola, E., Di Nardo, A. A., Le Poupon, C., Mailly, P., Wizenmann, A., et al. (2020). Extracellular Pax6 Regulates Tangential Cajal–Retzius Cell Migration in the Developing Mouse Neocortex. *Cerebral Cortex* 30, 465–475. doi:10.1093/cercor/bhz098.

Kam, T.-I., Mao, X., Park, H., Chou, S.-C., Karuppagounder, S. S., Umanah, G. E., et al. (2018). Poly (ADP-ribose) Drives Pathologic α-Synuclein Neurodegeneration in Parkinson's Disease. *Science* 362. doi:10.1126/science.aat8407.

Kamakura, S., Oishi, K., Yoshimatsu, T., Nakafuku, M., Masuyama, N., and Gotoh, Y. (2004). Hes binding to STAT3 mediates crosstalk between Notch and JAK–STAT signalling. *Nat Cell Biol* 6, 547–554. doi:10.1038/ncb1138.

Kang, W., Wong, L. C., Shi, S.-H., and Hébert, J. M. (2009). The Transition from Radial Glial to Intermediate Progenitor Cell Is Inhibited by FGF Signaling during Corticogenesis. *J Neurosci* 29, 14571–14580. doi:10.1523/JNEUROSCI.3844-09.2009.

Kataria, H., Alizadeh, A., and Karimi-Abdolrezaee, S. (2019). Neuregulin-1/ErbB network: An emerging modulator of nervous system injury and repair. *Progress in Neurobiology* 180, 101643. doi:10.1016/j.pneurobio.2019.101643.

Kaufmann, S. H., Desnoyers, S., Ottaviano, Y., Davidson, N. E., and Poirier, G. G. (1993). Specific Proteolytic Cleavage of Poly(ADP-ribose) Polymerase: An Early Marker of Chemotherapy-induced Apoptosis. *Cancer Res* 53, 3976–3985.

Kauppinen, T. M., Suh, S. W., Genain, C. P., and Swanson, R. A. (2005). Poly(ADP-ribose) polymerase-1 activation in a primate model of multiple sclerosis. *Journal of Neuroscience Research* 81, 190–198. doi:https://doi.org/10.1002/jnr.20525.

Ke, Y., Han, Y., Guo, X., Wen, J., Wang, K., Jiang, X., et al. (2017). PARP1 promotes gene expression at the post-transcriptional level by modulating the RNA-binding protein HuR. *Nat Commun* 8, 14632. doi:10.1038/ncomms14632.

Ke, Y., Zhang, J., Lv, X., Zeng, X., and Ba, X. (2019). Novel insights into PARPs in gene expression: regulation of RNA metabolism. *Cell. Mol. Life Sci.* 76, 3283–3299. doi:10.1007/s00018-019-03120-6.

Kessaris, N., Fogarty, M., Iannarelli, P., Grist, M., Wegner, M., and Richardson, W. D. (2006). Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. *Nat Neurosci* 9, 173–179. doi:10.1038/nn1620.

Kim, J.-E., Kim, Y.-J., Kim, J. Y., and Kang, T.-C. (2014). PARP1 activation/expression modulates regional-specific neuronal and glial responses to seizure in a hemodynamic-independent manner. *Cell Death Dis* 5, e1362. doi:10.1038/cddis.2014.331.

Kim, M. Y., Mauro, S., Gévry, N., Lis, J. T., and Kraus, W. L. (2004). NAD+-Dependent Modulation of Chromatin Structure and Transcription by Nucleosome Binding Properties of PARP-1. *Cell* 119, 803–814. doi:10.1016/j.cell.2004.11.002.

Kirischuk, S., Luhmann, H. J., and Kilb, W. (2014). Cajal–Retzius cells: Update on structural and functional properties of these mystic neurons that bridged the 20th century. *Neuroscience* 275, 33–46. doi:10.1016/j.neuroscience.2014.06.009.

Kohno, S., Kohno, T., Nakano, Y., Suzuki, K., Ishii, M., Tagami, H., et al. (2009). Mechanism and significance of specific proteolytic cleavage of Reelin. *Biochemical and Biophysical Research Communications* 380, 93–97. doi:10.1016/j.bbrc.2009.01.039.

Kohno, T., Honda, T., Kubo, K., Nakano, Y., Tsuchiya, A., Murakami, T., et al. (2015). Importance of Reelin C-Terminal Region in the Development and Maintenance of the Postnatal Cerebral Cortex and Its Regulation by Specific Proteolysis. *J. Neurosci.* 35, 4776–4787. doi:10.1523/JNEUROSCI.4119-14.2015.

Kolthur-Seetharam, U., Dantzer, F., McBurney, M. W., Murcia1, G. de, and Sassone-Corsi, P. (2006). Control of AIF-mediated Cell Death by the Functional Interplay of SIRT1 and PARP-1 in Response to DNA Damage. *Cell Cycle* 5, 873–877. doi:10.4161/cc.5.8.2690.

Kornack, D. R., and Rakic, P. (1998). Changes in cell-cycle kinetics during the development and evolution of primate neocortex. *PNAS* 95, 1242–1246.

Kowalczyk, T., Pontious, A., Englund, C., Daza, R. A. M., Bedogni, F., Hodge, R., et al. (2009). Intermediate Neuronal Progenitors (Basal Progenitors) Produce Pyramidal–Projection Neurons for All Layers of Cerebral Cortex. *Cereb Cortex* 19, 2439–2450. doi:10.1093/cercor/bhn260.

Kraus, W. L. (2008). Transcriptional control by PARP-1: chromatin modulation, enhancer-binding, coregulation, and insulation. *Current Opinion in Cell Biology* 20, 294–302. doi:10.1016/j.ceb.2008.03.006.

Kriegstein, A. R., and Noctor, S. C. (2004). Patterns of neuronal migration in the embryonic cortex. *Trends in Neurosciences* 27, 392–399. doi:10.1016/j.tins.2004.05.001.

Krishnakumar, R., and Kraus, W. L. (2010a). PARP-1 Regulates Chromatin Structure and Transcription through a KDM5B-Dependent Pathway. *Molecular Cell* 39, 736–749. doi:10.1016/j.molcel.2010.08.014.

Krishnakumar, R., and Kraus, W. L. (2010b). The PARP Side of the Nucleus: Molecular Actions, Physiological Outcomes, and Clinical Targets. *Mol Cell* 39, 8–24. doi:10.1016/j.molcel.2010.06.017.

Krivosheya, D., Tapia, L., Levinson, J. N., Huang, K., Kang, Y., Hines, R., et al. (2008). ErbB4-Neuregulin Signaling Modulates Synapse Development and Dendritic Arborization through Distinct Mechanisms*. *J Biol Chem* 283, 32944–32956. doi:10.1074/jbc.M800073200.

Krstic, D., Rodriguez, M., and Knuesel, I. (2012). Regulated Proteolytic Processing of Reelin through Interplay of Tissue Plasminogen Activator (tPA), ADAMTS-4, ADAMTS-5, and Their Modulators. *PLoS One* 7. doi:10.1371/journal.pone.0047793.

Krukenberg, K. A., Kim, S., Tan, E. S., Maliga, Z., and Mitchison, T. J. (2015). Extracellular poly(ADP-ribose) is a pro-inflammatory signal for macrophages. *Chem Biol* 22, 446–452. doi:10.1016/j.chembiol.2015.03.007.

Kubo, K., Honda, T., Tomita, K., Sekine, K., Ishii, K., Uto, A., et al. (2010). Ectopic Reelin Induces Neuronal Aggregation with a Normal Birthdate-Dependent “Inside-Out”

Alignment in the Developing Neocortex. *J Neurosci* 30, 10953–10966. doi:10.1523/JNEUROSCI.0486-10.2010.

Kuhn, S., Gritti, L., Crooks, D., and Dombrowski, Y. (2019). Oligodendrocytes in Development, Myelin Generation and Beyond. *Cells* 8. doi:10.3390/cells8111424.

Kuo, G., Arnaud, L., Kronstad-O'Brien, P., and Cooper, J. A. (2005). Absence of Fyn and Src Causes a Reeler-Like Phenotype. *J Neurosci* 25, 8578–8586. doi:10.1523/JNEUROSCI.1656-05.2005.

Kuwahara, A., Hirabayashi, Y., Knoepfler, P. S., Taketo, M. M., Sakai, J., Kodama, T., et al. (2010). Wnt signaling and its downstream target N-myc regulate basal progenitors in the developing neocortex. *Development* 137, 1035–1044. doi:10.1242/dev.046417.

Lai, C., and Feng, L. (2004). Implication of γ-secretase in neuregulin-induced maturation of oligodendrocytes. *Biochemical and Biophysical Research Communications* 314, 535–542. doi:10.1016/j.bbrc.2003.12.131.

Lakoma, J., Garcia-Alonso, L., and Luque, J. M. (2011). Reelin sets the pace of neocortical neurogenesis. *Development* 138, 5223–5234. doi:10.1242/dev.063776.

Lambert de Rouvroit, C., de Bergeyck, V., Cortvrindt, C., Bar, I., Eeckhout, Y., and Goffinet, A. M. (1999). Reelin, the Extracellular Matrix Protein Deficient in Reeler Mutant Mice, Is Processed by a Metalloproteinase. *Experimental Neurology* 156, 214–217. doi:10.1006/exnr.1998.7007.

Lavdas, A. A., Grigoriou, M., Pachnis, V., and Parnavelas, J. G. (1999). The Medial Ganglionic Eminence Gives Rise to a Population of Early Neurons in the Developing Cerebral Cortex. *J Neurosci* 19, 7881–7888. doi:10.1523/JNEUROSCI.19-18-07881.1999.

Law, A. J., Kleinman, J. E., Weinberger, D. R., and Weickert, C. S. (2007). Disease-associated intronic variants in the ErbB4 gene are related to altered ErbB4 splice-variant expression in the brain in schizophrenia. *Human Molecular Genetics* 16, 129–141. doi:10.1093/hmg/ddl449.

Ledonne, F., Orduz, D., Mercier, J., Vigier, L., Grove, E. A., Tissir, F., et al. (2016). Targeted Inactivation of Bax Reveals a Subtype-Specific Mechanism of Cajal-Retzius Neuron Death in the Postnatal Cerebral Cortex. *Cell Reports* 17, 3133–3141. doi:10.1016/j.celrep.2016.11.074.

Lee, H.-J., Jung, K.-M., Huang, Y. Z., Bennett, L. B., Lee, J. S., Mei, L., et al. (2002). Presenilin-dependent γ-Secretase-like Intramembrane Cleavage of ErbB4*. *Journal of Biological Chemistry* 277, 6318–6323. doi:10.1074/jbc.M110371200.

Le May, N., Iltis, I., Amé, J.-C., Zhovmer, A., Biard, D., Egly, J.-M., et al. (2012). Poly (ADP-Ribose) Glycohydrolase Regulates Retinoic Acid Receptor-Mediated Gene Expression. *Molecular Cell* 48, 785–798. doi:10.1016/j.molcel.2012.09.021.

Li, B., Woo, R.-S., Mei, L., and Malinow, R. (2007). ErbB4, a receptor of the schizophrenia-linked protein neuregulin-1, controls glutamatergic synapse maturation and plasticity. *Neuron* 54, 583–597. doi:10.1016/j.neuron.2007.03.028.

Li, G., Adesnik, H., Li, J., Long, J., Nicoll, R. A., Rubenstein, J. L. R., et al. (2008). Regional Distribution of Cortical Interneurons and Development of Inhibitory Tone Are Regulated by Cxcl12/Cxcr4 Signaling. *J Neurosci* 28, 1085–1098. doi:10.1523/JNEUROSCI.4602-07.2008.

Li, H., Chou, S.-J., Hamasaki, T., Perez-Garcia, C. G., and O'Leary, D. D. (2012). Neuregulin repellent signaling via ErbB4 restricts GABAergic interneurons to migratory paths from ganglionic eminence to cortical destinations. *Neural Dev* 7, 10. doi:10.1186/1749-8104-7-10.

Li, M., and Yu, X. (2013). Function of BRCA1 in the DNA Damage Response Is Mediated by ADP-Ribosylation. *Cancer Cell* 23, 693–704. doi:10.1016/j.ccr.2013.03.025.

Lindy, A. S., Stosser, M. B., Butler, E., Downtain-Pickersgill, C., Shanmugham, A., Retterer, K., et al. (2018). Diagnostic outcomes for genetic testing of 70 genes in 8565 patients with epilepsy and neurodevelopmental disorders. *Epilepsia* 59, 1062–1071. doi:10.1111/epi.14074.

Liu, B., Xiao, H., and Zhao, C. (2018). Forced Expression of Foxg1 in the Cortical Hem Leads to the Transformation of Cajal-Retzius Cells into Dentate Granule Neurons. *J Dev Biol* 6. doi:10.3390/jdb6030016.

Liu, W. S., Pesold, C., Rodriguez, M. A., Carboni, G., Auta, J., Lacor, P., et al. (2001). Down-regulation of dendritic spine and glutamic acid decarboxylase 67 expressions in the reelin haploinsufficient heterozygous reeler mouse. *PNAS* 98, 3477–3482. doi:10.1073/pnas.051614698.

Long, J. E., Cobos, I., Potter, G. B., and Rubenstein, J. L. R. (2009). Dlx1&2 and Mash1 Transcription Factors Control MGE and CGE Patterning and Differentiation through Parallel and Overlapping Pathways. *Cereb Cortex* 19, i96–i106. doi:10.1093/cercor/bhp045.

López-Bendito, G., Sánchez-Alcañiz, J. A., Pla, R., Borrell, V., Picó, E., Valdeolmillos, M., et al. (2008). Chemokine Signaling Controls Intracortical Migration and Final Distribution of GABAergic Interneurons. *J. Neurosci.* 28, 1613–1624.

Loulier, K., Lathia, J. D., Marthiens, V., Relucio, J., Mughal, M. R., Tang, S.-C., et al. (2009). β1 Integrin Maintains Integrity of the Embryonic Neocortical Stem Cell Niche. *PLoS Biol* 7. doi:10.1371/journal.pbio.1000176.

Lukaszewicz, A., Savatier, P., Cortay, V., Giroud, P., Huissoud, C., Berland, M., et al. (2005). G1 Phase Regulation, Area-Specific Cell Cycle Control, and Cytoarchitectonics in the Primate Cortex. *Neuron* 47, 353–364. doi:10.1016/j.neuron.2005.06.032.

Luo, X., Ryu, K. W., Kim, D.-S., Nandu, T., Medina, C. J., Gupte, R., et al. (2017). PARP-1 Controls the Adipogenic Transcriptional Program by PARylating C/EBPβ and Modulating Its Transcriptional Activity. *Molecular Cell* 65, 260–271. doi:10.1016/j.molcel.2016.11.015.

Lutticken, C., Wegenka, U. M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., et al. (1994). Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. *Science* 263, 89–92. doi:10.1126/science.8272872.

Lutz, D., Sharaf, A., Drexler, D., Kataria, H., Wolters-Eisfeld, G., Brunne, B., et al. (2017). Proteolytic cleavage of transmembrane cell adhesion molecule L1 by extracellular matrix molecule Reelin is important for mouse brain development. *Scientific Reports* 7, 15268. doi:10.1038/s41598-017-15311-x.

Maeda, N. (2015). Proteoglycans and neuronal migration in the cerebral cortex during development and disease. *Front. Neurosci.* 9. doi:10.3389/fnins.2015.00098.

Magdaleno, S., Keshvara, L., and Curran, T. (2002). Rescue of Ataxia and Preplate Splitting by Ectopic Expression of Reelin in reeler Mice. *Neuron* 33, 573–586. doi:10.1016/S0896-6273(02)00582-2.

Makinodan, M., Rosen, K. M., Ito, S., and Corfas, G. (2012). A Critical Period for Social Experience–Dependent Oligodendrocyte Maturation and Myelination. *Science* 337, 1357–1360. doi:10.1126/science.1220845.

Malatesta, P., Hack, M. A., Hartfuss, E., Kettenmann, H., Klinkert, W., Kirchhoff, F., et al. (2003). Neuronal or Glial Progeny: Regional Differences in Radial Glia Fate. *Neuron* 37, 751–764. doi:10.1016/S0896-6273(03)00116-8.

Mallamaci, A. (2013). Developmental control of cortico-cerebral astrogenesis. *Int. J. Dev. Biol.* 57, 689–706. doi:10.1387/ijdb.130148am.

Mao, K., and Zhang, G. (2021). The role of PARP1 in neurodegenerative diseases and aging. *The FEBS Journal* n/a. doi:https://doi.org/10.1111/febs.15716.

Marillat, V., Cases, O., Nguyenf-Ba-Charvet, K. T., Tessier-Lavigne, M., Sotelo, C., and Chédotal, A. (2002). Spatiotemporal expression patterns of slit and robo genes in the rat brain. *Journal of Comparative Neurology* 442, 130–155. doi:10.1002/cne.10068.

Marín, O. (2013). Cellular and molecular mechanisms controlling the migration of neocortical interneurons. *Eur J Neurosci* 38, 2019–2029. doi:10.1111/ejn.12225.

Marín-Padilla, M. (1992). Ontogenesis of the pyramidal cell of the mammalian neocortex and developmental cytoarchitectonics: A unifying theory. *Journal of Comparative Neurology* 321, 223–240. doi:10.1002/cne.903210205.

Marković, J., Grdović, N., Dinić, S., Karan-Djurašević, T., Uskoković, A., Arambašić, J., et al. (2013). PARP-1 and YY1 Are Important Novel Regulators of CXCL12 Gene

Transcription in Rat Pancreatic Beta Cells. *PLoS One* 8, e59679. doi:10.1371/journal.pone.0059679.

Martin, K. A., Cesaroni, M., Denny, M. F., Lupey, L. N., and Tempera, I. (2015). Global Transcriptome Analysis Reveals That Poly(ADP-Ribose) Polymerase 1 Regulates Gene Expression through EZH2. *Mol Cell Biol* 35, 3934–3944. doi:10.1128/MCB.00635-15.

Martini, F. J., Valiente, M., López Bendito, G., Szabó, G., Moya, F., Valdeolmillos, M., et al. (2009). Biased selection of leading process branches mediates chemotaxis during tangential neuronal migration. *Development* 136, 41–50. doi:10.1242/dev.025502.

Marty, S., Carroll, P., Cellerino, A., Castren, E., Staiger, V., Thoenen, H., et al. (1996). Brain-derived neurotrophic factor promotes the differentiation of various hippocampal nonpyramidal neurons, including Cajal-Retzius cells, in organotypic slice cultures. *J Neurosci* 16, 675–687. doi:10.1523/JNEUROSCI.16-02-00675.1996.

Masson, M., Niedergang, C., Schreiber, V., Muller, S., Menissier-de Murcia, J., and de Murcia, G. (1998). XRCC1 Is Specifically Associated with Poly(ADP-Ribose) Polymerase and Negatively Regulates Its Activity following DNA Damage. *Mol Cell Biol* 18, 3563–3571.

Masutani, M., Nozaki, T., Nishiyama, E., Shimokawa, T., Tachi, Y., Suzuki, H., et al. (1999). Function of poly(ADP-ribose) polymerase in response to DNA damage: gene-disruption study in mice. *Mol Cell Biochem* 193, 149–152.

Mata, I., Perez-Iglesias, R., Roiz-Santiañez, R., Tordesillas-Gutierrez, D., Gonzalez-Mandly, A., Berja, A., et al. (2010). Additive effect of NRG1 and DISC1 genes on lateral ventricle enlargement in first episode schizophrenia. *NeuroImage* 53, 1016–1022. doi:10.1016/j.neuroimage.2009.11.010.

Matsunaga, Y., Noda, M., Murakawa, H., Hayashi, K., Nagasaka, A., Inoue, S., et al. (2017). Reelin transiently promotes N-cadherin–dependent neuronal adhesion during mouse cortical development. *PNAS* 114, 2048–2053. doi:10.1073/pnas.1615215114.

Matsuura, S., Egi, Y., Yuki, S., Horikawa, T., Satoh, H., and Akira, T. (2011). MP-124, a novel poly(ADP-ribose) polymerase-1 (PARP-1) inhibitor, ameliorates ischemic brain damage in a non-human primate model. *Brain Research* 1410, 122–131. doi:10.1016/j.brainres.2011.05.069.

Matveeva, E. A., Mathbout, L. F., and Fondufe-Mittendorf, Y. N. (2019). PARP1 is a versatile factor in the regulation of mRNA stability and decay. *Sci Rep* 9, 3722. doi:10.1038/s41598-019-39969-7.

Matveeva, E., Maiorano, J., Zhang, Q., Eteleeb, A. M., Convertini, P., Chen, J., et al. (2016). Involvement of PARP1 in the regulation of alternative splicing. *Cell Discov* 2, 15046. doi:10.1038/celldisc.2015.46.

Mbemba, E., Gluckman, J. C., and Gattegno, L. (2000). Glycan and glycosaminoglycan binding properties of stromal cell-derived factor (SDF)-1α. *Glycobiology* 10, 21–29. doi:10.1093/glycob/10.1.21.

McLoughlin, H. S., Fineberg, S. K., Ghosh, L. L., Tecedor, L., and Davidson, B. L. (2012). Dicer is required for proliferation, viability, migration and differentiation in corticoneurogenesis. *Neuroscience* 223, 285–295. doi:10.1016/j.neuroscience.2012.08.009.

Medina, L., and Abellán, A. (2012). "Chapter 7 - Subpallial Structures," in *The Mouse Nervous System*, eds. C. Watson, G. Paxinos, and L. Puelles (San Diego: Academic Press), 173–220. doi:10.1016/B978-0-12-369497-3.10007-X.

Mei, L., and Nave, K.-A. (2014). Neuregulin-ERBB Signaling in the Nervous System and Neuropsychiatric Diseases. *Neuron* 83, 27–49. doi:10.1016/j.neuron.2014.06.007.

Mei, L., and Xiong, W.-C. (2008). Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat Rev Neurosci* 9, 437–452. doi:10.1038/nrn2392.

Meier, H., and Hoag, W. G. (1962). The Neuropathology of "Reeler", a Neuro-Muscular Mutation in Mice*†. *Journal of Neuropathology & Experimental Neurology* 21, 649–654. doi:10.1097/00005072-196210000-00011.

Melikishvili, M., Chariker, J. H., Rouchka, E. C., and Fondufe-Mittendorf, Y. N. (2017). Transcriptome-wide identification of the RNA-binding landscape of the chromatin-associated protein PARP1 reveals functions in RNA biogenesis. *Cell Discov* 3, 17043. doi:10.1038/celldisc.2017.43.

Meng, D., He, W., Huang, P., Liu, D., Zhong, L., Yu, R., et al. (2018). Polymorphism of PARP-1 indicates an increased risk and a worse initial severity of ischemic stroke. *Personalized Medicine* 15, 355–360. doi:10.2217/pme-2018-0007.

Ménissier de Murcia, J., Ricoul, M., Tartier, L., Niedergang, C., Huber, A., Dantzer, F., et al. (2003). Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse. *The EMBO Journal* 22, 2255–2263. doi:10.1093/emboj/cdg206.

Meyer, G., Perez-Garcia, C. G., Abraham, H., and Caput, D. (2002). Expression of p73 and Reelin in the Developing Human Cortex. *J Neurosci* 22, 4973–4986. doi:10.1523/JNEUROSCI.22-12-04973.2002.

Meyer, G., Socorro, A. C., Garcia, C. G. P., Millan, L. M., Walker, N., and Caput, D. (2004). Developmental Roles of p73 in Cajal-Retzius Cells and Cortical Patterning. *J Neurosci* 24, 9878–9887. doi:10.1523/JNEUROSCI.3060-04.2004.

Meyer, G., Soria, J. M., Martínez-Galán, J. R., Martín-Clemente, B., and Fairén, A. (1998). Different origins and developmental histories of transient neurons in the marginal zone of the fetal and neonatal rat cortex. *Journal of Comparative Neurology* 397, 493–518. doi:10.1002/(SICI)1096-9861(19980810)397:4<493::AID-CNE4>3.0.CO;2-X.

Middle, F., Pritchard, A. L., Handoko, H., Haque, S., Holder, R., Bentham, P., et al. (2010). No Association Between Neuregulin 1 and Psychotic Symptoms in Alzheimer's Disease Patients. *Journal of Alzheimer's Disease* 20, 561–567. doi:10.3233/JAD-2010-1405.

Miller, W. B. J., Gregory S. Walsh, Freda D. (2016). Neuronal Survival and p73/p63/p53: A Family Affair - W. Bradley Jacobs, Gregory S. Walsh, Freda D. Miller, 2004. *The Neuroscientist*. Available at: http://journals.sagepub.com/doi/10.1177/1073858404263456?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed [Accessed March 4, 2021].

Minotti, R., Andersson, A., and Hottiger, M. O. (2015). ARTD1 suppresses interleukin 6 expression by repressing MLL1-dependent histone H3 trimethylation. *Mol. Cell. Biol.*, MCB.00196-15. doi:10.1128/MCB.00196-15.

Miquelajáuregui, A., Varela-Echavarría, A., Ceci, M. L., García-Moreno, F., Ricaño, I., Hoang, K., et al. (2010). LIM-Homeobox Gene Lhx5 Is Required for Normal Development of Cajal–Retzius Cells. *J. Neurosci.* 30, 10551–10562. doi:10.1523/JNEUROSCI.5563-09.2010.

Mitsogiannis, M. D., Pancho, A., Aerts, T., Sachse, S. M., Vanlaer, R., Noterdaeme, L., et al. (2021). Subtle Roles of Down Syndrome Cell Adhesion Molecules in Embryonic Forebrain Development and Neuronal Migration. *Front Cell Dev Biol* 8. doi:10.3389/fcell.2020.624181.

Miwa, M., Nakatsugawa, K., Hara, K., matsushima, T., and Sugimura, T. (1975). Degradation of poly(adenosine diphosphate ribose) by homogenates of various normal tissues and tumors of rats. *Archives of Biochemistry and Biophysics* 167, 54–60. doi:10.1016/0003-9861(75)90440-3.

Mizumoto, S., Fongmoon, D., and Sugahara, K. (2013). Interaction of chondroitin sulfate and dermatan sulfate from various biological sources with heparin-binding growth factors and cytokines. *Glycoconj J* 30, 619–632. doi:10.1007/s10719-012-9463-5.

Monuki, E. S., Porter, F. D., and Walsh, C. A. (2001). Patterning of the Dorsal Telencephalon and Cerebral Cortex by a Roof Plate-Lhx2 Pathway. *Neuron* 32, 591–604. doi:10.1016/S0896-6273(01)00504-9.

Mouton-Liger, F., Dumurgier, J., Cognat, E., Hourregue, C., Zetterberg, H., Vanderstichele, H., et al. (2020). CSF levels of the BACE1 substrate NRG1 correlate with cognition in Alzheimer's disease. *Alzheimers Res Ther* 12. doi:10.1186/s13195-020-00655-w.

Munafò, M. R., Thiselton, D. L., Clark, T. G., and Flint, J. (2006). Association of the NRG1 gene and schizophrenia: a meta-analysis. *Mol Psychiatry* 11, 539–546. doi:10.1038/sj.mp.4001817.

Muraki, K., and Tanigaki, K. (2015). Neuronal migration abnormalities and its possible implications for schizophrenia. *Front. Neurosci.* 9. doi:10.3389/fnins.2015.00074.

Murcia, J. M., Mark, M., Wendling, O., Wynshaw-Boris, A., and de Murcia, G. (2001). Early Embryonic Lethality in PARP-1 Atm Double-Mutant Mice Suggests a Functional Synergy in Cell Proliferation during Development. *Mol Cell Biol* 21, 1828–1832. doi:10.1128/MCB.21.5.1828-1832.2001.

Murnyák, B., Kouhsari, M. C., Hershkovitch, R., Kálmán, B., Marko-Varga, G., Klekner, Á., et al. (2017). PARP1 expression and its correlation with survival is tumour molecular subtype dependent in glioblastoma. *Oncotarget* 8, 46348–46362. doi:10.18632/oncotarget.18013.

Muzio, L., Di Benedetto, B., Stoykova, A., Boncinelli, E., Gruss, P., and Mallamaci, A. (2002). Conversion of cerebral cortex into basal ganglia in Emx2 −/− Pax6 Sey/Sey double-mutant mice. *Nat Neurosci* 5, 737–745. doi:10.1038/nn892.

Muzio, L., and Mallamaci, A. (2005). Foxg1 Confines Cajal-Retzius Neuronogenesis and Hippocampal Morphogenesis to the Dorsomedial Pallium. *J. Neurosci.* 25, 4435–4441.

Muzio, L., Soria, J. M., Pannese, M., Piccolo, S., and Mallamaci, A. (2005). A Mutually Stimulating Loop Involving Emx2 and Canonical Wnt Signalling Specifically Promotes Expansion of Occipital Cortex and Hippocampus. *Cerebral Cortex* 15, 2021–2028. doi:10.1093/cercor/bhi077.

Nadarajah, B., Brunstrom, J. E., Grutzendler, J., Wong, R. O. L., and Pearlman, A. L. (2001). Two modes of radial migration in early development of the cerebral cortex. *Nature Neuroscience* 4, 143–150. doi:10.1038/83967.

Nadarajah, B., and Parnavelas, J. G. (2002). Modes of neuronal migration in the developing cerebral cortex. *Nat Rev Neurosci* 3, 423–432. doi:10.1038/nrn845.

Najmabadi, H., Hu, H., Garshasbi, M., Zemojtel, T., Abedini, S. S., Chen, W., et al. (2011). Deep sequencing reveals 50 novel genes for recessive cognitive disorders. *Nature* 478, 57–63. doi:10.1038/nature10423.

Nakada, Y., Hunsaker, T. L., Henke, R. M., and Johnson, J. E. (2004). Distinct domains within Mash1 and Math1 are required for function in neuronal differentiation versus neuronal cell-type specification. *Development* 131, 1319–1330. doi:10.1242/dev.01008.

Nakano, Y., Kohno, T., Hibi, T., Kohno, S., Baba, A., Mikoshiba, K., et al. (2007). The Extremely Conserved C-terminal Region of Reelin Is Not Necessary for Secretion but Is Required for Efficient Activation of Downstream Signaling*. *Journal of Biological Chemistry* 282, 20544–20552. doi:10.1074/jbc.M702300200.

Nakashima, K., Takizawa, T., Ochiai, W., Yanagisawa, M., Hisatsune, T., Nakafuku, M., et al. (2001). BMP2-mediated alteration in the developmental pathway of fetal mouse brain cells from neurogenesis to astrocytogenesis. *Proc Natl Acad Sci U S A* 98, 5868–5873. doi:10.1073/pnas.101109698.

Nakashima, K., Yanagisawa, M., Arakawa, H., Kimura, N., Hisatsune, T., Kawabata, M., et al. (1999). Synergistic Signaling in Fetal Brain by STAT3-Smad1 Complex Bridged by p300. *Science* 284, 479–482. doi:10.1126/science.284.5413.479.

Namihira, M., Kohyama, J., Semi, K., Sanosaka, T., Deneen, B., Taga, T., et al. (2009). Committed Neuronal Precursors Confer Astrocytic Potential on Residual Neural Precursor Cells. *Developmental Cell* 16, 245–255. doi:10.1016/j.devcel.2008.12.014.

Neddens, J., and Buonanno, A. (2010). Selective populations of hippocampal interneurons express ErbB4 and their number and distribution is altered in ErbB4 knockout mice. *Hippocampus* 20, 724–744. doi:https://doi.org/10.1002/hipo.20675.

Ni, C.-Y., Murphy, M. P., Golde, T. E., and Carpenter, G. (2001). γ-Secretase Cleavage and Nuclear Localization of ErbB-4 Receptor Tyrosine Kinase. *Science* 294, 2179–2181. doi:10.1126/science.1065412.

Nicodemus, K. K., Law, A. J., Luna, A., Vakkalanka, R., Straub, R. E., Kleinman, J. E., et al. (2009). A 5′ Promoter Region SNP in NRG1 is Associated with Schizophrenia Risk and Type III Isoform Expression. *Mol Psychiatry* 14, 741–743. doi:10.1038/mp.2008.150.

Nieto, M., Schuurmans, C., Britz, O., and Guillemot, F. (2001). Neural bHLH Genes Control the Neuronal versus Glial Fate Decision in Cortical Progenitors. *Neuron* 29, 401–413. doi:10.1016/S0896-6273(01)00214-8.

Ninova, M., Fejes Tóth, K., and Aravin, A. A. (2019). The control of gene expression and cell identity by H3K9 trimethylation. *Development* 146. doi:10.1242/dev.181180.

Niu, S., Renfro, A., Quattrocchi, C. C., Sheldon, M., and D’Arcangelo, G. (2004). Reelin Promotes Hippocampal Dendrite Development through the VLDLR/ApoER2-Dab1 Pathway. *Neuron* 41, 71–84. doi:10.1016/S0896-6273(03)00819-5.

Niu, S., Yabut, O., and D’Arcangelo, G. (2008). The Reelin Signaling Pathway Promotes Dendritic Spine Development in Hippocampal Neurons. *J. Neurosci.* 28, 10339–10348. doi:10.1523/JNEUROSCI.1917-08.2008.

Noctor, S. C., Martínez-Cerdeño, V., Ivic, L., and Kriegstein, A. R. (2004). Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat Neurosci* 7, 136–144. doi:10.1038/nn1172.

Norton, N., Moskvina, V., Morris, D. W., Bray, N. J., Zammit, S., Williams, N. M., et al. (2006). Evidence that interaction between neuregulin 1 and its receptor erbB4 increases susceptibility to schizophrenia. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 141B, 96–101. doi:10.1002/ajmg.b.30236.

Nozaki, T., Sasaki, Y., Fukuda, I., Isumi, M., Nakamoto, K., Onodera, T., et al. (2018). Next-generation sequencing-based miRNA expression analysis in Parp1-deficient embryonic stem cell-derived exosomes. *Biochemical and Biophysical Research Communications* 499, 410–415. doi:10.1016/j.bbrc.2018.03.073.

O'Brien, J., Hayder, H., Zayed, Y., and Peng, C. (2018). Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Front. Endocrinol.* 9. doi:10.3389/fendo.2018.00402.

Ochiai, W., Nakatani, S., Takahara, T., Kainuma, M., Masaoka, M., Minobe, S., et al. (2009). Periventricular notch activation and asymmetric Ngn2 and Tbr2 expression in pair-generated neocortical daughter cells. *Molecular and Cellular Neuroscience* 40, 225–233. doi:10.1016/j.mcn.2008.10.007.

Ogawa, M., Miyata, T., Nakajima, K., Yagyu, K., Seike, M., Ikenaka, K., et al. (1995). The reeler Gene-Associated Antigen on CajaI-Retzius Neurons Is a Crucial Molecule for Laminar Organization of Cortical Neurons. 14.

Ogino, H., Hisanaga, A., Kohno, T., Kondo, Y., Okumura, K., Kamei, T., et al. (2017). Secreted Metalloproteinase ADAMTS-3 Inactivates Reelin. *J Neurosci* 37, 3181–3191. doi:10.1523/JNEUROSCI.3632-16.2017.

Ohtsuka, T., Ishibashi, M., Gradwohl, G., Nakanishi, S., Guillemot, F., and Kageyama, R. (1999). Hes1 and Hes5 as notch effectors in mammalian neuronal differentiation. *EMBO J* 18, 2196–2207. doi:10.1093/emboj/18.8.2196.

Olson, E. C. (2014). Analysis of Preplate Splitting and Early Cortical Development Illuminates the Biology of Neurological Disease. *Front Pediatr* 2. doi:10.3389/fped.2014.00121.

Ono, T., Kasamatsu, A., Oka, S., and Moss, J. (2006). The 39-kDa poly(ADP-ribose) glycohydrolase ARH3 hydrolyzes O-acetyl-ADP-ribose, a product of the Sir2 family of acetyl-histone deacetylases. *Proc Natl Acad Sci U S A* 103, 16687–16691. doi:10.1073/pnas.0607911103.

Ortega, M. C., Bribián, A., Peregrín, S., Gil, M. T., Marín, O., and de Castro, F. (2012). Neuregulin-1/ErbB4 signaling controls the migration of oligodendrocyte precursor cells during development. *Experimental Neurology* 235, 610–620. doi:10.1016/j.expneurol.2012.03.015.

Osumi, N., Shinohara, H., Numayama-Tsuruta, K., and Maekawa, M. (2008). Concise Review: Pax6 Transcription Factor Contributes to both Embryonic and Adult Neurogenesis as a Multifunctional Regulator. *STEM CELLS* 26, 1663–1672. doi:10.1634/stemcells.2007-0884.

O'Tuathaigh, C. M. P., Babovic, D., O'Sullivan, G. J., Clifford, J. J., Tighe, O., Croke, D. T., et al. (2007). Phenotypic characterization of spatial cognition and social behavior in mice with 'knockout' of the schizophrenia risk gene neuregulin 1. *Neuroscience* 147, 18–27. doi:10.1016/j.neuroscience.2007.03.051.

Ovadia, G., and Shifman, S. (2011). The Genetic Variation of RELN Expression in Schizophrenia and Bipolar Disorder. *PLoS One* 6. doi:10.1371/journal.pone.0019955.

Pablo V. Bejman, Alan R. Sanders, and Kenneth S. Kendler (2011). Genetics of Schizophrenia: New Findings and Challenges. *Annual Review of Genomics and Human Genetics* 12, 121–144. doi:10.1146/annurev-genom-082410-101459.

Piskunova, T. S., Yurova, M. N., Ovsyannikov, A. I., Semenchenko, A. V., Zabezhinski, M. A., Popovich, I. G., et al. (2008). Deficiency in Poly(ADP-ribose) Polymerase-1 (PARP-1) Accelerates Aging and Spontaneous Carcinogenesis in Mice. *Current Gerontology and Geriatrics Research* 2008, 1–11. doi:10.1155/2008/754190.

Plane, J. M., Grossenbacher, S. K., and Deng, W. (2012). PARP-1 deletion promotes subventricular zone neural stem cells toward a glial fate. *J. Neurosci. Res.* 90, 1489–1506. doi:10.1002/jnr.23040.

Plowman, G. D., Culouscou, J. M., Whitney, G. S., Green, J. M., Carlton, G. W., Foy, L., et al. (1993a). Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family. *Proc Natl Acad Sci U S A* 90, 1746–1750.

Plowman, G. D., Green, J. M., Culouscou, J. M., Carlton, G. W., Rothwell, V. M., and Buckley, S. (1993b). Heregulin induces tyrosine phosphorylation of HER4/p180erbB4. *Nature* 366, 473–475. doi:10.1038/366473a0.

Poirier, G. G., de Murcia, G., Jongstra-Bilen, J., Niedergang, C., and Mandel, P. (1982). Poly(ADP-ribosyl)ation of polynucleosomes causes relaxation of chromatin structure. *Proc Natl Acad Sci U S A* 79, 3423–3427.

Polleux, F., Whitford, K. L., Dijkhuizen, P. A., Vitalis, T., and Ghosh, A. (2002). Control of cortical interneuron migration by neurotrophins and PI3-kinase signaling. *Development* 129, 3147–3160.

Pommier, Y., OConnor, M. J., and de Bono, J. (2016). Laying a trap to kill cancer cells: PARP inhibitors and their mechanisms of action. *Science Translational Medicine* 8, 362ps17-362ps17. doi:10.1126/scitranslmed.aaf9246.

Pozas, E., and Ibáñez, C. F. (2005). GDNF and GFRα1 Promote Differentiation and Tangential Migration of Cortical GABAergic Neurons. *Neuron* 45, 701–713. doi:10.1016/j.neuron.2005.01.043.

Pujadas, L., Gruart, A., Bosch, C., Delgado, L., Teixeira, C. M., Rossi, D., et al. (2010). Reelin Regulates Postnatal Neurogenesis and Enhances Spine Hypertrophy and Long-Term Potentiation. *Journal of Neuroscience* 30, 4636–4649. doi:10.1523/JNEUROSCI.5284-09.2010.

Qian, X., Shen, Q., Goderie, S. K., He, W., Capela, A., Davis, A. A., et al. (2000). Timing of CNS Cell Generation: A Programmed Sequence of Neuron and Glial Cell Production from Isolated Murine Cortical Stem Cells. *Neuron* 28, 69–80. doi:10.1016/S0896-6273(00)00086-6.

Quinn, J. C., Molinek, M., Martynoga, B. S., Zaki, P. A., Faedo, A., Bulfone, A., et al. (2007). Pax6 controls cerebral cortical cell number by regulating exit from the cell cycle and specifies cortical cell identity by a cell autonomous mechanism. *Dev Biol* 302, 50–65. doi:10.1016/j.ydbio.2006.08.035.

Radakovits, R., Barros, C. S., Belvindrah, R., Patton, B., and Müller, U. (2009). Regulation of Radial Glial Survival by Signals from the Meninges. *J Neurosci* 29, 7694–7705. doi:10.1523/JNEUROSCI.5537-08.2009.

Rajamohan, S. B., Pillai, V. B., Gupta, M., Sundaresan, N. R., Birukov, K. G., Samant, S., et al. (2009). SIRT1 Promotes Cell Survival under Stress by Deacetylation-Dependent Deactivation of Poly(ADP-Ribose) Polymerase 1. *Mol Cell Biol* 29, 4116–4129. doi:10.1128/MCB.00121-09.

Rakic, P. (1995). A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. *Trends Neurosci* 18, 383–388. doi:10.1016/0166-2236(95)93934-p.

Rallu, M., Machold, R., Gaiano, N., Corbin, J. G., McMahon, A. P., and Fishell, G. (2002). Dorsoventral patterning is established in the telencephalon of mutants lacking both Gli3 and Hedgehog signaling. *Development* 129, 4963–4974. doi:10.1242/dev.129.21.4963.

Rash, B. G., Lim, H. D., Breunig, J. J., and Vaccarino, F. M. (2011). FGF Signaling Expands Embryonic Cortical Surface Area by Regulating Notch-Dependent Neurogenesis. *J Neurosci* 31, 15604–15617. doi:10.1523/JNEUROSCI.4439-11.2011.

Ray Chaudhuri, A., and Nussenzweig, A. (2017). The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. *Nature Reviews Molecular Cell Biology* 18, 610–621. doi:10.1038/nrm.2017.53.

Reale, A., Matteis, G. D., Galleazzi, G., Zampieri, M., and Caiafa, P. (2005). Modulation of DNMT1 activity by ADP-ribose polymers. *Oncogene* 24, 13–19. doi:10.1038/sj.onc.1208005.

Reeder, R. H., Ueda, K., Honjo, T., Nishizuka, Y., and Hayaishi, O. (1967). Studies on the Polymer of Adenosine Diphosphate Ribose. *Journal of Biological Chemistry* 242, 3172–3179. doi:10.1016/S0021-9258(18)95948-X.

Ringstedt, T., Linnarsson, S., Wagner, J., Lendahl, U., Kokaia, Z., Arenas, E., et al. (1998). BDNF Regulates Reelin Expression and Cajal-Retzius Cell Development in the Cerebral Cortex. *Neuron* 21, 305–315. doi:10.1016/S0896-6273(00)80540-1.

Rio, C., Buxbaum, J. D., Peschon, J. J., and Corfas, G. (2000). Tumor Necrosis Factor-α-converting Enzyme Is Required for Cleavage of erbB4/HER4*. *Journal of Biological Chemistry* 275, 10379–10387. doi:10.1074/jbc.275.14.10379.

Rio, C., Rieff, H. I., Qi, P., and Corfas, G. (1997). Neuregulin and erbB Receptors Play a Critical Role in Neuronal Migration. *Neuron* 19, 39–50. doi:10.1016/S0896-6273(00)80346-3.

Riva, M., Genescu, I., Habermacher, C., Orduz, D., Ledonne, F., Rijli, F. M., et al. (2019). Activity-dependent death of transient Cajal-Retzius neurons is required for functional cortical wiring. *eLife* 8, e50503. doi:10.7554/eLife.50503.

Robaszkiewicz, A., Qu, C., Wisnik, E., Ploszaj, T., Mirsaidi, A., Kunze, F. A., et al. (2016). ARTD1 regulates osteoclastogenesis and bone homeostasis by dampening NF-κB-dependent transcription of IL-1β. *Sci Rep* 6. doi:10.1038/srep21131.

Rogers, C. J., Clark, P. M., Tully, S. E., Abrol, R., Garcia, K. C., Goddard, W. A., et al. (2011). Elucidating glycosaminoglycan–protein–protein interactions using carbohydrate microarray and computational approaches. *Proc Natl Acad Sci U S A* 108, 9747–9752. doi:10.1073/pnas.1102962108.

Rose, M., Burgess, J. T., O’Byrne, K., Richard, D. J., and Bolderson, E. (2020). PARP Inhibitors: Clinical Relevance, Mechanisms of Action and Tumor Resistance. *Front. Cell Dev. Biol.* 8. doi:10.3389/fcell.2020.564601.

Rothbart, S. B., and Strahl, B. D. (2014). Interpreting thelanguage of histone and DNA modifications. *Biochim Biophys Acta* 1839, 627–643. doi:10.1016/j.bbagrm.2014.03.001.

Roy, A., Gonzalez-Gomez, M., Pierani, A., Meyer, G., and Tole, S. (2014). Lhx2 Regulates the Development of the Forebrain Hem System. *Cereb Cortex* 24, 1361–1372. doi:10.1093/cercor/bhs421.

Roy, C., and Corfas, G. (2008). “Neuregulin-erbB Signaling and the Pathogenesis of Schizophrenia,” in *Cortical Deficits In Schizophrenia*, ed. P. O’Donnell (Boston, MA: Springer US), 73–96. doi:10.1007/978-0-387-74351-6_4.

Roy, K., Murtie, J. C., El-Khodor, B. F., Edgar, N., Sardi, S. P., Hooks, B. M., et al. (2007). Loss of erbB signaling in oligodendrocytes alters myelin and dopaminergic function, a potential mechanism for neuropsychiatric disorders. *Proc Natl Acad Sci U S A* 104, 8131–8136. doi:10.1073/pnas.0702157104.

Rudolph, J., Zimmer, G., Steinecke, A., Barchmann, S., and Bolz, J. (2010). Ephrins guide migrating cortical interneurons in the basal telencephalon. *Cell Adh Migr* 4, 400–408. doi:10.4161/cam.4.3.11640.

Ruiz i Altaba, A. (1998). Combinatorial Gli gene function in floor plate and neuronal inductions by Sonic hedgehog. *Development* 125, 2203–2212. doi:10.1242/dev.125.12.2203.

Sader, J. E. (1998). Frequency response of cantilever beams immersed in viscous fluids with applications to the atomic force microscope. *Journal of Applied Physics* 84, 64–76. doi:10.1063/1.368002.

Salemi, M., Mazzetti, S., De Leonardis, M., Giampietro, F., Medici, V., Poloni, T. E., et al. (2021). Poly (ADP-ribose) polymerase 1 and Parkinson's disease: A study in post-mortem human brain. *Neurochemistry International* 144, 104978. doi:10.1016/j.neuint.2021.104978.

Sánchez-Alcañiz, J. A., Haege, S., Mueller, W., Pla, R., Mackay, F., Schulz, S., et al. (2011). Cxcr7 Controls Neuronal Migration by Regulating Chemokine Responsiveness. *Neuron* 69, 77–90. doi:10.1016/j.neuron.2010.12.006.

Sánchez-Huertas, C., and Rico, B. (2011). CREB-Dependent Regulation of GAD65 Transcription by BDNF/TrkB in Cortical Interneurons. *Cerebral Cortex* 21, 777–788. doi:10.1093/cercor/bhq150.

Sansom, S. N., Griffiths, D. S., Faedo, A., Kleinjan, D.-J., Ruan, Y., Smith, J., et al. (2009). The Level of the Transcription Factor Pax6 Is Essential for Controlling the Balance between Neural Stem Cell Self-Renewal and Neurogenesis. *PLoS Genet* 5. doi:10.1371/journal.pgen.1000511.

Santos-Cortez, R. L. P., Khan, V., Sher Khan, F. S., Mughal, Z.-N., Chakchouk, I., Lee, K., et al. (2018). Novel candidate genes and variants underlying autosomal recessive neurodevelopmental disorders with intellectual disability. *Hum Genet* 137, 735–752. doi:10.1007/s00439-018-1928-6.

Sardi, S. P., Murtie, J., Koirala, S., Patten, B. A., and Corfas, G. (2006). Presenilin-Dependent ErbB4 Nuclear Signaling Regulates the Timing of Astrogenesis in the Developing Brain. *Cell* 127, 185–197. doi:10.1016/j.cell.2006.07.037.

Sauvageot, C. (2002). Molecular mechanisms controlling cortical gliogenesis. *Current Opinion in Neurobiology* 12, 244–249. doi:10.1016/S0959-4388(02)00322-7.

Sawyer, C., Hiles, I., Page, M., Crompton, M., and Dean, C. (1998). Two erbB-4 transcripts are expressed in normal breast and in most breast cancers. *Oncogene* 17, 919–924. doi:10.1038/sj.onc.1202015.

Schmid, R. S., and Maness, P. F. (2008). L1 and NCAM ADHESION MOLECULES AS SIGNALING CO-RECEPTORS IN NEURONAL MIGRATION AND PROCESS OUTGROWTH. *Curr Opin Neurobiol* 18, 245–250. doi:10.1016/j.conb.2008.07.015.

Schmid, R. S., McGrath, B., Berechid, B. E., Boyles, B., Marchionni, M., Šestan, N., et al. (2003). Neuregulin 1–erbB2 signaling is required for the establishment of radial glia and their transformation into astrocytes in cerebral cortex. *Proc Natl Acad Sci U S A* 100, 4251–4256. doi:10.1073/pnas.0630496100.

Sekine, K., Kawauchi, T., Kubo, K., Honda, T., Herz, J., Hattori, M., et al. (2012). Reelin Controls Neuronal Positioning by Promoting Cell-Matrix Adhesion via Inside-Out Activation of Integrin α5β1. *Neuron* 76, 353–369. doi:10.1016/j.neuron.2012.07.020.

Selemon, L. D., and Goldman-Rakic, P. S. (1999). The reduced neuropil hypothesis: a circuit based model of schizophrenia. *Biological Psychiatry* 45, 17–25. doi:10.1016/S0006-3223(98)00281-9.

Selemon, L. D., Rajkowska, G., and Goldman-Rakic, P. S. (1998). Elevated neuronal density in prefrontal area 46 in brains from schizophrenic patients: Application of a three-dimensional, stereologic counting method. *Journal of Comparative Neurology* 392, 402–412. doi:https://doi.org/10.1002/(SICI)1096-9861(19980316)392:3<402::AID-CNE9>3.0.CO;2-5.

Selten, M., van Bokhoven, H., and Nadif Kasri, N. (2018). Inhibitory control of the excitatory/inhibitory balance in psychiatric disorders. *F1000Res* 7. doi:10.12688/f1000research.12155.1.

Selvaraj, V., Soundarapandian, M. M., Chechneva, O., Williams, A. J., Sidorov, M. K., Soulika, A. M., et al. (2009). PARP-1 Deficiency Increases the Severity of Disease in a Mouse Model of Multiple Sclerosis. *J Biol Chem* 284, 26070–26084. doi:10.1074/jbc.M109.013474.

Seong, E., Yuan, L., and Arikkath, J. (2015). Cadherins and catenins in dendrite and synapse morphogenesis. *Cell Adh Migr* 9, 202–213. doi:10.4161/19336918.2014.994919.

Sethi, G. S., Sharma, S., and Naura, A. S. (2019). PARP inhibition by olaparib alleviates chronic asthma-associated remodeling features via modulating inflammasome signaling in mice. *IUBMB Life* 71, 1003–1013. doi:10.1002/iub.2048.

Shall, S., and de Murcia, G. (2000). Poly(ADP-ribose) polymerase-1: what have we learned from the deficient mouse model? *Mutation Research/DNA Repair* 460, 1–15. doi:10.1016/S0921-8777(00)00016-1.

Sheldon, M., Rice, D. S., D'Arcangelo, G., Yoneshima, H., Nakajima, K., Mikoshiba, K., et al. (1997). Scrambler and yotari disrupt the disabled gene and produce a reeler -like phenotype in mice. *Nature* 389, 730–733. doi:10.1038/39601.

Sheppard, A. M., and Pearlman, A. L. (1997). Abnormal reorganization of preplate neurons and their associated extracellular matrix: An early manifestation of altered neocortical development in the reeler mutant mouse. *Journal of Comparative Neurology* 378, 173–179. doi:10.1002/(SICI)1096-9861(19970210)378:2<173::AID-CNE2>3.0.CO;2-0.

Shi, Y., Di Giammartino, D. C., Taylor, D., Sarkeshik, A., Rice, W. J., Yates, J. R., et al. (2009). Molecular architecture of the human pre-mRNA 3′ processing complex. *Mol Cell* 33, 365–376. doi:10.1016/j.molcel.2008.12.028.

Shibata, M., Kurokawa, D., Nakao, H., Ohmura, T., and Aizawa, S. (2008). MicroRNA-9 Modulates Cajal–Retzius Cell Differentiation by Suppressing Foxg1 Expression in Mouse Medial Pallium. *J. Neurosci.* 28, 10415–10421. doi:10.1523/JNEUROSCI.3219-08.2008.

Shieh, W. M., Amé, J.-C., Wilson, M. V., Wang, Z.-Q., Koh, D. W., Jacobson, M. K., et al. (1998). Poly(ADP-ribose) Polymerase Null Mouse Cells Synthesize ADP-ribose Polymers*. *Journal of Biological Chemistry* 273, 30069–30072. doi:10.1074/jbc.273.46.30069.

Shikanai, M., Nakajima, K., and Kawauchi, T. (2011). N-Cadherin regulates radial glial fiber-dependent migration of cortical locomoting neurons. *Communicative & Integrative Biology* 4, 326–330. doi:10.4161/cib.4.3.14886.

Shinozaki, K., Miyagi, T., Yoshida, M., Miyata, T., Ogawa, M., Aizawa, S., et al. (2002). Absence of Cajal-Retzius cells and subplate neurons associated with defects of tangential cell migration from ganglionic eminence in Emx1/2 double mutant cerebral cortex. *Development* 129, 3479–3492. doi:10.1242/dev.129.14.3479.

Shipp, E. L., and Hsieh-Wilson, L. C. (2007). Profiling the Sulfation Specificities of Glycosaminoglycan Interactions with Growth Factors and Chemotactic Proteins Using Microarrays. *Chemistry & Biology* 14, 195–208. doi:10.1016/j.chembiol.2006.12.009.

Shrestha, E., Hussein, M. A., Savas, J. N., Ouimet, M., Barrett, T. J., Leone, S., et al. (2016). Poly(ADP-ribose) Polymerase 1 Represses Liver X Receptor-mediated ABCA1 Expression and Cholesterol Efflux in Macrophages. *J. Biol. Chem.* 291, 11172–11184. doi:10.1074/jbc.M116.726729.

Shu, T., Ayala, R., Nguyen, M.-D., Xie, Z., Gleeson, J. G., and Tsai, L.-H. (2004). Ndel1 Operates in a Common Pathway with LIS1 and Cytoplasmic Dynein to Regulate Cortical Neuronal Positioning. *Neuron* 44, 263–277. doi:10.1016/j.neuron.2004.09.030.

Siegenthaler, J. A., and Miller, M. W. (2008). GENERATION OF CAJAL-RETZIUS NEURONS IN MOUSE FOREBRAIN IS REGULATED BY TRANSFORMING GROWTH FACTOR β-FOX SIGNALING PATHWAYS. *Dev Biol* 313, 35–46. doi:10.1016/j.ydbio.2007.09.036.

Sievers, J., Pehlemann, F. W., Gude, S., and Berry, M. (1994). A time course study of the alterations in the development of the hamster cerebellar cortex after destruction of the overlying meningeal cells with 6-hydroxydopamine on the day of birth. *J Neurocytol* 23, 117–134. doi:10.1007/BF01183866.

Silberberg, G., Darvasi, A., Pinkas-Kramarski, R., and Navon, R. (2006). The involvement of ErbB4 with schizophrenia: Association and expression studies. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 141B, 142–148. doi:10.1002/ajmg.b.30275.

Sims, R. J., and Reinberg, D. (2006). Histone H3 Lys 4 methylation: caught in a bind? *Genes Dev.* 20, 2779–2786. doi:10.1101/gad.1468206.

Smart, I. H. M., Dehay, C., Giroud, P., Berland, M., and Kennedy, H. (2002). Unique Morphological Features of the Proliferative Zones and Postmitotic Compartments of the

Neural Epithelium Giving Rise to Striate and Extrastriate Cortex in the Monkey. *Cerebral Cortex* 12, 37–53. doi:10.1093/cercor/12.1.37.

Smyth, N., Vatansever, H. S., Murray, P., Meyer, M., Frie, C., Paulsson, M., et al. (1999). Absence of Basement Membranes after Targeting the LAMC1 Gene Results in Embryonic Lethality Due to Failure of Endoderm Differentiation. *J Cell Biol* 144, 151–160.

Soldani, C., Lazzè, M. C., Bottone, M. G., Tognon, G., Biggiogera, M., Pellicciari, C. E., et al. (2001). Poly(ADP-ribose) Polymerase Cleavage during Apoptosis: When and Where? *Experimental Cell Research* 269, 193–201. doi:10.1006/excr.2001.5293.

Soldatenkov, V. A., Chasovskikh, S., Potaman, V. N., Trofimova, I., Smulson, M. E., and Dritschilo, A. (2002). Transcriptional Repression by Binding of Poly(ADP-ribose) Polymerase to Promoter Sequences*. *Journal of Biological Chemistry* 277, 665–670. doi:10.1074/jbc.M108551200.

Solecki, D. J., Trivedi, N., Govek, E.-E., Kerekes, R. A., Gleason, S. S., and Hatten, M. E. (2009). Myosin II Motors and F-Actin Dynamics Drive the Coordinated Movement of the Centrosome and Soma During CNS Glial-Guided Neuronal Migration. *Neuron* 63, 63–80. doi:10.1016/j.neuron.2009.05.028.

Son, D. I., Hong, S., Shin, K. S., and Kang, S. J. (2020). PARP-1 regulates mouse embryonic neural stem cell proliferation by regulating PDGFRα expression. *Biochemical and Biophysical Research Communications* 526, 986–992. doi:10.1016/j.bbrc.2020.03.166.

Soriano, E., and del Río, J. A. (2005). The Cells of Cajal-Retzius: Still a Mystery One Century After. *Neuron* 46, 389–394. doi:10.1016/j.neuron.2005.04.019.

Stahl, N., Farruggella, T. J., Boulton, T. G., Zhong, Z., Darnell, J. E., and Yancopoulos, G. D. (1995). Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. *Science* 267, 1349–1353. doi:10.1126/science.7871433.

Stanco, A., Szekeres, C., Patel, N., Rao, S., Campbell, K., Kreidberg, J. A., et al. (2009). Netrin-1–α3β1 integrin interactions regulate the migration of interneurons through the cortical marginal zone. *Proc Natl Acad Sci U S A* 106, 7595–7600. doi:10.1073/pnas.0811343106.

Stanfield, B. B., Caviness, V. S., and Cowan, W. M. (1979). The organization of certain afferents to the hippocampus and dentate gyrus in normal and reeler mice. *Journal of Comparative Neurology* 185, 461–483. doi:https://doi.org/10.1002/cne.901850304.

Stary Creed M., Xu Lijun, Sun Xiaoyun, Ouyang Yi-Bing, White Robin E., Leong Jason, et al. (2015). MicroRNA-200c Contributes to Injury From Transient Focal Cerebral Ischemia by Targeting Reelin. *Stroke* 46, 551–556. doi:10.1161/STROKEAHA.114.007041.

Stoica, B. A., Loane, D. J., Zhao, Z., Kabadi, S. V., Hanscom, M., Byrnes, K. R., et al. (2014). PARP-1 Inhibition Attenuates Neuronal Loss, Microglia Activation and Neurological

Deficits after Traumatic Brain Injury. *J Neurotrauma* 31, 758–772. doi:10.1089/neu.2013.3194.

Stoykova, A., Hatano, O., Gruss, P., and Götz, M. (2003). Increase in Reelin-positive Cells in the Marginal Zone of Pax6 Mutant Mouse Cortex. *Cerebral Cortex* 13, 560–571. doi:10.1093/cercor/13.6.560.

Stoykova, A., Treichel, D., Hallonet, M., and Gruss, P. (2000). Pax6 Modulates the Dorsoventral Patterning of the Mammalian Telencephalon. *J. Neurosci.* 20, 8042–8050.

Studer, M., Filosa, A., and Rubenstein, J. L. R. (2005). The nuclear receptor COUP-TFI represses differentiation of Cajal-Retzius cells. *Brain Research Bulletin* 66, 394–401. doi:10.1016/j.brainresbull.2004.11.027.

Stumm, R. K., Zhou, C., Ara, T., Lazarini, F., Dubois-Dalcq, M., Nagasawa, T., et al. (2003). CXCR4 Regulates Interneuron Migration in the Developing Neocortex. *J. Neurosci.* 23, 5123–5130. doi:10.1523/JNEUROSCI.23-12-05123.2003.

Sun, L., Cheng, B., Zhou, Y., Fan, Y., Li, W., Qiu, Q., et al. (2020). ErbB4 Mutation that Decreased NRG1-ErbB4 Signaling Involved in the Pathogenesis of Amyotrophic Lateral Sclerosis/Frontotemporal Dementia. *Journal of Alzheimer's Disease* 74, 535–544. doi:10.3233/JAD-191230.

Sun, Y., Nadal-Vicens, M., Misono, S., Lin, M. Z., Zubiaga, A., Hua, X., et al. (2001). Neurogenin Promotes Neurogenesis and Inhibits Glial Differentiation by Independent Mechanisms. *Cell* 104, 365–376. doi:10.1016/S0092-8674(01)00224-0.

Supèr, H., Del Río, J. A., Martínez, A., Pérez-Sust, P., and Soriano, E. (2000). Disruption of Neuronal Migration and Radial Glia in the Developing Cerebral Cortex Following Ablation of Cajal–Retzius Cells. *Cerebral Cortex* 10, 602–613. doi:10.1093/cercor/10.6.602.

Suzuki, H., Uchida, K., Shima, H., Sato, T., Okamoto, T., Teruyuki Kimura, et al. (1987). Molecular cloning of cDNA for human poly(ADP-ribose) polymerase and expression of its gene during HL-60 cell differentiation. *Biochemical and Biophysical Research Communications* 146, 403–409. doi:10.1016/0006-291X(87)90543-2.

Sweet, H. O., Bronson, R. T., Johnson, K. R., Cook, S. A., and Davisson, M. T. (1996). Scrambler, a new neurological mutation of the mouse with abnormalities of neuronal migration. *Mammalian Genome* 7, 798. doi:10.1007/s003359900240.

Szabó, C. (2005). Roles of poly(ADP-ribose) polymerase activation in the pathogenesis of diabetes mellitus and its complications. *Pharmacological Research* 52, 60–71. doi:10.1016/j.phrs.2005.02.015.

Szebeni, A., Szebeni, K., DiPeri, T. P., Johnson, L. A., Stockmeier, C. A., Crawford, J. D., et al. (2016). Elevated DNA Oxidation and DNA Repair Enzyme Expression in Brain White

Matter in Major Depressive Disorder. *Int J Neuropsychopharmacol* 20, 363–373. doi:10.1093/ijnp/pyw114.

Takahashi, Y., Uchino, A., Shioya, A., Sano, T., Matsumoto, C., Numata-Uematsu, Y., et al. (2019). Altered immunoreactivity of ErbB4, a causative gene product for ALS19, in the spinal cord of patients with sporadic ALS. *Neuropathology* 39, 268–278. doi:10.1111/neup.12558.

Takiguchi-Hayashi, K., Sekiguchi, M., Ashigaki, S., Takamatsu, M., Hasegawa, H., Suzuki-Migishima, R., et al. (2004). Generation of Reelin-Positive Marginal Zone Cells from the Caudomedial Wall of Telencephalic Vesicles. *J. Neurosci.* 24, 2286–2295. doi:10.1523/JNEUROSCI.4671-03.2004.

Tan, K., Shaw, A. L., Madsen, B., Jensen, K., Taylor-Papadimitriou, J., and Freemont, P. S. (2003). Human PLU-1 Has Transcriptional Repression Properties and Interacts with the Developmental Transcription Factors BF-1 and PAX9*. *Journal of Biological Chemistry* 278, 20507–20513. doi:10.1074/jbc.M301994200.

Tapia-Páez, I., Tammimies, K., Massinen, S., Roy, A. L., and Kere, J. (2008). The complex of TFII-I, PARP1, and SFPQ proteins regulates the *DYX1C1* gene implicated in neuronal migration and dyslexia. *FASEB j.* 22, 3001–3009. doi:10.1096/fj.07-104455.

Tessarz, P., and Kouzarides, T. (2014). Histone core modifications regulating nucleosome structure and dynamics. *Nat Rev Mol Cell Biol* 15, 703–708. doi:10.1038/nrm3890.

Timmusk, T., Belluardo, N., Persson, H., and Metsis, M. (1994). Developmental regulation of brain-derived neurotrophic factor messenger RNAs transcribed from different promoters in the rat brain. *Neuroscience* 60, 287–291. doi:10.1016/0306-4522(94)90242-9.

Timpl, R., Rohde, H., Rennard, S. I., Foidart, J. M., and Martin, G. R. (1979). Laminin–a glycoprotein from basement membranes. *Journal of Biological Chemistry* 254, 9933–9937. doi:10.1016/S0021-9258(19)83607-4.

Ting, A. K., Chen, Y., Wen, L., Yin, D.-M., Shen, C., Tao, Y., et al. (2011). Neuregulin 1 Promotes Excitatory Synapse Development and Function in GABAergic Interneurons. *J Neurosci* 31, 15–25. doi:10.1523/JNEUROSCI.2538-10.2011.

Tissir, F., Ravni, A., Achouri, Y., Riethmacher, D., Meyer, G., and Goffinet, A. M. (2009). DeltaNp73 regulates neuronal survival in vivo. *PNAS* 106, 16871–16876. doi:10.1073/pnas.0903191106.

Tiveron, M.-C., Rossel, M., Moepps, B., Zhang, Y. L., Seidenfaden, R., Favor, J., et al. (2006). Molecular Interaction between Projection Neuron Precursors and Invading Interneurons via Stromal-Derived Factor 1 (CXCL12)/CXCR4 Signaling in the Cortical Subventricular Zone/Intermediate Zone. *J Neurosci* 26, 13273–13278. doi:10.1523/JNEUROSCI.4162-06.2006.

Tolić, A., Grdović, N., Dinić, S., Rajić, J., Đorđević, M., Sinadinović, M., et al. (2019). Absence of PARP-1 affects Cxcl12 expression by increasing DNA demethylation. *J Cell Mol Med* 23, 2610–2618. doi:10.1111/jcmm.14154.

Tomita, K., Moriyoshi, K., Nakanishi, S., Guillemot, F., and Kageyama, R. (2000). Mammalian achaete–scute and atonal homologs regulate neuronal versus glial fate determination in the central nervous system. *EMBO J* 19, 5460–5472. doi:10.1093/emboj/19.20.5460.

Toresson, H., Potter, S. S., and Campbell, K. (2000). Genetic control of dorsal-ventral identity in the telencephalon: opposing roles for Pax6 and Gsh2. *Development* 127, 4361–4371. doi:10.1242/dev.127.20.4361.

Trommsdorff, M., Gotthardt, M., Hiesberger, T., Shelton, J., Stockinger, W., Nimpf, J., et al. (1999). Reeler/Disabled-like Disruption of Neuronal Migration in Knockout Mice Lacking the VLDL Receptor and ApoE Receptor 2. *Cell* 97, 689–701. doi:10.1016/S0092-8674(00)80782-5.

Trucco, C., Oliver, F. J., de Murcia, G., and Ménissier-de Murcia, J. (1998). DNA repair defect in poly(ADP-ribose) polymerase-deficient cell lines. *Nucleic Acids Res* 26, 2644–2649.

Tsai, J.-W., Lian, W.-N., Kemal, S., Kriegstein, A. R., and Vallee, R. B. (2010). Kinesin 3 and cytoplasmic dynein mediate interkinetic nuclear migration in neural stem cells. *Nat Neurosci* 13, 1463–1471. doi:10.1038/nn.2665.

Tsai, L.-H., and Gleeson, J. G. (2005). Nucleokinesis in Neuronal Migration. *Neuron* 46, 383–388. doi:10.1016/j.neuron.2005.04.013.

Tzahar, E., Waterman, H., Chen, X., Levkowitz, G., Karunagaran, D., Lavi, S., et al. (1996). A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. *Mol Cell Biol* 16, 5276–5287.

Unda, B. K., Kwan, V., and Singh, K. K. (2016). Neuregulin-1 Regulates Cortical Inhibitory Neuron Dendrite and Synapse Growth through DISC1. *Neural Plast* 2016. doi:10.1155/2016/7694385.

Valanejad, L., Cast, A., Wright, M., Bissig, K.-D., Karns, R., Weirauch, M. T., et al. (2018). PARP1 activation increases expression of modified tumor suppressors and pathways underlying development of aggressive hepatoblastoma. *Commun Biol* 1. doi:10.1038/s42003-018-0077-8.

Valiente, M., and Marín, O. (2010). Neuronal migration mechanisms in development and disease. *Current Opinion in Neurobiology* 20, 68–78. doi:10.1016/j.conb.2009.12.003.

Vasile, F., Dossi, E., and Rouach, N. (2017). Human astrocytes: structure and functions in the healthy brain. *Brain Struct Funct* 222, 2017–2029. doi:10.1007/s00429-017-1383-5.

Visochek, L., Grigoryan, G., Kalal, A., Milshtein-Parush, H., Gazit, N., Slutsky, I., et al. (2016). A PARP1-ERK2 synergism is required for the induction of LTP. *Sci Rep* 6, 24950. doi:10.1038/srep24950.

Vullhorst, D., Neddens, J., Karavanova, I., Tricoire, L., Petralia, R. S., McBain, C. J., et al. (2009). Selective Expression of ErbB4 in Interneurons, But Not Pyramidal Cells, of the Rodent Hippocampus. *J Neurosci* 29, 12255–12264. doi:10.1523/JNEUROSCI.2454-09.2009.

Walcher, T., Xie, Q., Sun, J., Irmler, M., Beckers, J., Öztürk, T., et al. (2013). Functional dissection of the paired domain of Pax6 reveals molecular mechanisms of coordinating neurogenesis and proliferation. *Development* 140, 1123–1136. doi:10.1242/dev.082875.

Wang, B., Waclaw, R. R., Allen, Z. J., Guillemot, F., and Campbell, K. (2009). Ascl1 is a required downstream effector of Gsx gene function in the embryonic mouse telencephalon. *Neural Development* 4, 5. doi:10.1186/1749-8104-4-5.

Wang, C., Xu, W., An, J., Liang, M., Li, Y., Zhang, F., et al. (2019). Poly(ADP-ribose) polymerase 1 accelerates vascular calcification by upregulating Runx2. *Nat Commun* 10. doi:10.1038/s41467-019-09174-1.

Wang, H., Ge, G., Uchida, Y., Luu, B., and Ahn, S. (2011a). Gli3 Is Required for Maintenance and Fate Specification of Cortical Progenitors. *J Neurosci* 31, 6440–6448. doi:10.1523/JNEUROSCI.4892-10.2011.

Wang, Y., Li, G., Stanco, A., Long, J. E., Crawford, D., Potter, G. B., et al. (2011b). CXCR4 and CXCR7 Have Distinct Functions in Regulating Interneuron Migration. *Neuron* 69, 61–76. doi:10.1016/j.neuron.2010.12.005.

Wang, Z. Q., Auer, B., Stingl, L., Berghammer, H., Haidacher, D., Schweiger, M., et al. (1995). Mice lacking ADPRT and poly(ADP-ribosyl)ation develop normally but are susceptible to skin disease. *Genes Dev.* 9, 509–520. doi:10.1101/gad.9.5.509.

Warren, N., Caric, D., Pratt, T., Clausen, J. A., Asavaritikrai, P., Mason, J. O., et al. (1999). The Transcription Factor, Pax6, is Required for Cell Proliferation and Differentiation in the Developing Cerebral Cortex. *Cerebral Cortex* 9, 627–635. doi:10.1093/cercor/9.6.627.

Wengner, A. M., Scholz, A., and Haendler, B. (2020). Targeting DNA Damage Response in Prostate and Breast Cancer. *Int J Mol Sci* 21. doi:10.3390/ijms21218273.

Wichterle, H., Turnbull, D. H., Nery, S., Fishell, G., and Alvarez-Buylla, A. (2001). In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. *Development* 128, 3759–3771. doi:10.1242/dev.128.19.3759.

Woo, R.-S., Lee, J.-H., Yu, H.-N., Song, D.-Y., and Baik, T.-K. (2010). Expression of ErbB4 in the apoptotic neurons of Alzheimer's disease brain. *Anat Cell Biol* 43, 332–339. doi:10.5115/acb.2010.43.4.332.

Woo, R.-S., Lee, J.-H., Yu, H.-N., Song, D.-Y., and Baik, T.-K. (2011). Expression of ErbB4 in the neurons of Alzheimer's disease brain and APP/PS1 mice, a model of Alzheimer's disease. *Anat Cell Biol* 44, 116–127. doi:10.5115/acb.2011.44.2.116.

Wu, S.-X., Goebbels, S., Nakamura, K., Nakamura, K., Kometani, K., Minato, N., et al. (2005). Pyramidal neurons of upper cortical layers generated by NEX-positive progenitor cells in the subventricular zone. *PNAS* 102, 17172–17177.

Wu, Z.-Q., Li, D., Huang, Y., Chen, X.-P., Huang, W., Liu, C.-F., et al. (2017). Caspr Controls the Temporal Specification of Neural Progenitor Cells through Notch Signaling in the Developing Mouse Cerebral Cortex. *Cerebral Cortex* 27, 1369–1385. doi:10.1093/cercor/bhv318.

Xu, F., Sun, Y., Yang, S.-Z., Zhou, T., Jhala, N., McDonald, J., et al. (2019). Cytoplasmic PARP-1 promotes pancreatic cancer tumorigenesis and resistance. *International Journal of Cancer* 145, 474–483. doi:10.1002/ijc.32108.

Yamazaki, H., Sekiguchi, M., Takamatsu, M., Tanabe, Y., and Nakanishi, S. (2004). Distinct ontogenic and regional expressions of newly identified Cajal-Retzius cell-specific genes during neocorticogenesis. *Proc Natl Acad Sci U S A* 101, 14509–14514. doi:10.1073/pnas.0406295101.

Yang, J.-M., Zhang, J., Chen, X.-J., Geng, H.-Y., Ye, M., Spitzer, N. C., et al. (2013). Development of GABA circuitry of fast-spiking basket interneurons in the medial prefrontal cortex of erbb4-mutant mice. *J Neurosci* 33, 19724–19733. doi:10.1523/JNEUROSCI.1584-13.2013.

Yarden, Y., and Sliwkowski, M. X. (2001). Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* 2, 127–137. doi:10.1038/35052073.

Yin, D.-M., Sun, X.-D., Bean, J. C., Lin, T. W., Sathyamurthy, A., Xiong, W.-C., et al. (2013). Regulation of Spine Formation by ErbB4 in PV-Positive Interneurons. *J. Neurosci.* 33, 19295–19303.

Yoo, Y. D., Huang, C. T., Zhang, X., Lavaute, T. M., and Zhang, S.-C. (2011). Fibroblast Growth Factor Regulates Human Neuroectoderm Specification Through ERK1/2-PARP-1 Pathway. *STEM CELLS* 29, 1975–1982. doi:10.1002/stem.758.

Yoshida, M., Assimacopoulos, S., Jones, K. R., and Grove, E. A. (2006). Massive loss of Cajal-Retzius cells does not disrupt neocortical layer order. *Development* 133, 537–545. doi:10.1242/dev.02209.

Yu, S.-W., Wang, H., Poitras, M. F., Coombs, C., Bowers, W. J., Federoff, H. J., et al. (2002). Mediation of Poly(ADP-Ribose) Polymerase-1-Dependent Cell Death by Apoptosis-Inducing Factor. *Science* 297, 259–263.

Yun, K., Potter, S., and Rubenstein, J. L. (2001). Gsh2 and Pax6 play complementary roles in dorsoventral patterning of the mammalian telencephalon. *Development* 128, 193–205. doi:10.1242/dev.128.2.193.

Zampieri, M., Passananti, C., Calabrese, R., Perilli, M., Corbi, N., De Cave, F., et al. (2009). Parp1 Localizes within the Dnmt1 Promoter and Protects Its Unmethylated State by Its Enzymatic Activity. *PLoS ONE* 4, e4717. doi:10.1371/journal.pone.0004717.

Zeng, N., Liu, L., McCabe, M. G., Jones, D. T. W., Ichimura, K., and Collins, V. P. (2009). Real-time quantitative PCR analysis with FRET probes reveals differential expression of the four ERBB4 juxtamembrane-region variants between medulloblastoma and pilocytic astrocytoma. *Neuropathol Appl Neurobiol* 35, 353–366. doi:10.1111/j.1365-2990.2008.01001.x.

Zhang, H., Zhang, X., Clark, E., Mulcahey, M., Huang, S., and Shi, Y. G. (2010). TET1 is a DNA-binding protein that modulates DNA methylation and gene transcription via hydroxylation of 5-methylcytosine. *Cell Res* 20, 1390–1393. doi:10.1038/cr.2010.156.

Zhu, Y., Li, H., Zhou, L., Wu, J. Y., and Rao, Y. (1999). Cellular and Molecular Guidance of GABAergic Neuronal Migration from an Extracortical Origin to the Neocortex. *Neuron* 23, 473–485. doi:10.1016/S0896-6273(00)80801-6.

Zilles, K., Palomero-Gallagher, N., and Amunts, K. (2013). Development of cortical folding during evolution and ontogeny. *Trends in Neurosciences* 36, 275–284. doi:10.1016/j.tins.2013.01.006.

Zimmer, C., Tiveron, M.-C., Bodmer, R., and Cremer, H. (2004). Dynamics of Cux2 Expression Suggests that an Early Pool of SVZ Precursors is Fated to Become Upper Cortical Layer Neurons. *Cerebral Cortex* 14, 1408–1420. doi:10.1093/cercor/bhh102.

Zimmer, G., Garcez, P., Rudolph, J., Niehage, R., Weth, F., Lent, R., et al. (2008). Ephrin-A5 acts as a repulsive cue for migrating cortical interneurons. *European Journal of Neuroscience* 28, 62–73. doi:10.1111/j.1460-9568.2008.06320.x.

Zimmer, G., Schanuel, S. M., Bürger, S., Weth, F., Steinecke, A., Bolz, J., et al. (2010). Chondroitin Sulfate Acts in Concert with Semaphorin 3A to Guide Tangential Migration of Cortical Interneurons in the Ventral Telencephalon. *Cerebral Cortex* 20, 2411–2422. doi:10.1093/cercor/bhp309.

Zinin, N., Adameyko, I., Wilhelm, M., Fritz, N., Uhlén, P., Ernfors, P., et al. (2014). MYC proteins promote neuronal differentiation by controlling the mode of progenitor cell division. *EMBO Rep* 15, 383–391. doi:10.1002/embr.201337424.