UC Riverside
UC Riverside Electronic Theses and Dissertations

Title

Regulation of Cellular Adhesion and Cytoskeletal Remodeling in Neurodevelopment: Functional Roles for Beta-Chimaerins and the Crk-Associated Substrate (Cas) Family of Proteins

Permalink
https://escholarship.org/uc/item/3xp8d9gq

Author
Estep, Jason Allen

Publication Date
2022

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

UNIVERSITY OF CALIFORNIA
RIVERSIDE

Regulation of Cellular Adhesion and Cytoskeletal Remodeling in Neurodevelopment: Functional Roles for Beta-Chimaerins and the Crk-Associated Substrate (Cas) Family of Proteins

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Cell, Molecular, and Developmental Biology

by

Jason A Estep

December 2022

Dissertation Committee:
Dr. Martin M Riccomagno, Chairperson
Dr. Anupama Dahanukar
Dr. Martin Garcia-Castro

Copyright by
Jason A Estep
2022

The Dissertation of Jason A Estep is approved:

____

____

____

Committee Chairperson

University of California, Riverside

The text of this dissertation, in part, is a reprint of the material as it appears in "The RacGAP beta-chimaerin is essential for cerebellar granule cell migration" published on January 12, 2018 in Scientific Reports. The co-author Martin M Riccomagno listed in this publication directed and supervised the research which forms the basis for this dissertation.

The author would like to thank Alyssa Treptow, Niloofar Rajabli, Wenny Wong, Yiu-Cheung E Wong, Brian M Loui, and Lu O Sun for their contributions to this work, as well as the National Institute of Neurological Disorders and Stroke and the University of California Office of the President's Cancer Research Coordinating Committee for their generous financial support. The author would like to personally thank Teresa Ubina and Jane Kim for their ever-present advice, friendship, and support. Finally, the author would like to thank Martin M Riccomagno for his unwavering commitment to his students' growth, in the laboratory and beyond. I would not be the scientist I am today without his mentorship, and I am deeply grateful for his many lessons in scientific rigor, academic scholarship, and the pursuit of knowledge with compassion and integrity.

I dedicate this dissertation to my mother, Carolyn M Estep, for her unfailing love and support; my pursuit of higher education would never have been possible without her many sacrifices, endless wisdom, gentle encouragement, and her many care packages filled with home-baked treats.

ABSTRACT OF THE DISSERTATION

Regulation of Cellular Adhesion and Cytoskeletal Remodeling in Neurodevelopment: Functional Roles for beta-Chimaerins and the Crk-Associated Substrate (Cas) Family of Proteins

by

Jason A Estep

Doctor of Philosophy, Graduate Program in Cell, Molecular, and Developmental Biology
University of California, Riverside, December 2022
Dr. Martin M Riccomagno, Chairperson

Proper nervous system function requires neurons to make synaptic connections with the appropriate partners, ensuring the formation of precise circuits. Even transient developmental delays in cell migration, lamination, neurite outgrowth, axon guidance, synapse assembly or refinement might have lasting consequences, as manifested in a wide range of neurodevelopmental disorders. Underscoring each of these cellular processes is the requirement of newborn neurons and glia to dynamically alter their morphology in response to extracellular cues. Failure to appropriately remodel the cytoskeleton during any of the above processes may result in changes to nervous system architecture, circuit organization and physiology, and consequently behavioral or cognitive function. Therefore, to understand how neurons organize themselves into functional circuits, it is imperative to understand how neural cells remodel their cytoskeleton in response to instructive and permissive cues from the environment. Many

neuronal guidance cues that regulate circuit assembly have been functionally characterized, yet little is known regarding the intracellular effectors that these ligand-receptor pairs utilize to modulate cellular adhesion and cytoskeletal remodeling during neuronal guidance events. Here, I present the combined studies of three intracellular effectors at the crossroads of adhesion and guidance signaling. In chapter 1, I provide genetic evidence the Crk-Associated Substrate (Cas) family of adaptor proteins are required for the proper fasciculation of several forebrain tracts. In chapter 2, I present the design of a novel luminescent-based, genetically-encoded tool for live cell analysis and quantification of adhesion signaling events upstream of Focal Adhesion Kinase. In chapter 3, I provide genetic evidence that the RacGAP β-chimaerin is required for cerebellar lamination. Collectively, these studies provide novel insights into the cytoplasmic effectors essential for the regulation of cell adhesion and cytoskeletal remodeling during circuit building events.

TABLE OF CONTENTS

Introduction……………………………………………………………………………..1

Chapter 1: Cas proteins are required for cortical white matter tract fasciculation

Abstract……………..……………………………………………………………24

Introduction………………………………………………………………………25

Results……………………………………………………………………………29

Discussion……………………………………………………………………….40

Materials & Methods……………………………………………………………48

Figures……………………………………………………………………………52

Chapter 2: A bimolecular complementation tool for monitoring adhesion signaling

Abstract………………………………………………………………………….61

Introduction………………………………………………………………………62

Results……………………………………………………………………………66

Discussion……………………………………………………………………..…71

Materials & Methods……………………………………………………………81

Figures & Tables…………………………………………………………………85

Chapter 3: β-chimaerin proteins are required for cerebellar granule cell radial migration

Abstract………………………………………………………………………….92

Introduction………………………………………………………………………93

Results……………………………………………………………………………95

Discussion………………………………………………………………………104

Materials & Methods…………………………………………………………...108

Figures……………………………………………………………………..111

Summary & Conclusion…………………………………………………………...121

References…………………………………………………………………………125

LIST OF FIGURES

Figure 1.1: Cas family proteins are expressed in the cortical plate during lamination and axonal tract formation……………………………………………………………52

Figure 1.2: *p130Cas* is expressed in the cortical white matter…………………………..53

Figure 1.3: *Cas TcKO* mutants display errors in cortical and thalamocortical connections…………………………………………………………………………54

Figure 1.4: Developmental progression of white matter defects in *Cas TcKO* mutants……………………………………………………………………………...55

Figure 1.5: Neuronal-autonomous and non-neuronal autonomous roles for *Cas* genes in white matter tract fasciculation………………………………………………..56

Figure 1.6: Genetic labeling confirms cortical origins of AC defasciculating axons in *Cas TcKO* mutants…………………………………………………………………57

Figure 1.7: *Igtb1* mutants phenocopy non-neuronal autonomous white matter tract defects observed in *Cas TcKO* mutants………………………………………………..58

Figure 1.8: Non-neuronal autonomous phenotypes in *Igtb1* and *Cas TcKO* mutants correlate with displaced subplate cells…………………………………………...59

Figure 2.1: Design of pyFAK BiLuc Constructs………………………………………...85

Figure 2.2: pyFAK BiLuc constructs are recruited to actively signaling Focal Adhesions…………………………………………………………………………...86

Figure 2.3: pyFAK BiLuc constructs respond to pharmacological blocking of adhesion signaling…………………………………………………………………………….87

Figure 2.4: pyFAK BiLuc signal is dependent on specific FAK tyrosine residues……………………………………………………………………..88
Figure 2.5: pyFAK BiLuc recruitment to Focal Adhesion complexes is not dependent on tyrosine phosphorylation………………………………………………………89
Figure 3.1: β-chimaerin expression in the postnatal cerebellum……………………….111
Figure 3.2: β-chimaerin deficiency causes neuronal ectopic clusters to form along the cerebellar folia in an asymmetrical pattern……………………………………..112
Figure 3.3: Ectopic clusters contain mature granule cells, but not other types of cerebellar neurons………………………………………………………………………114
Figure 3.4: Arrested migration of GCs in β-chimaerin deficient animals……………...115
Figure 3.5: Neuronal Ectopias are contacted by pontine mossy fibers…………………116
Figure 3.6: Early postnatal cerebellar structure in unaltered in β-chimaerin deficient animals………………………………………………………………………117
Figure 3.7: Tangential migration is unaffected in β-chimaerin deficient mice…………118
Figure 3.8: Cell proliferation in β-chimaerin deficient animals………………………..119
Figure 3.9: Cerebellar structure is unaffected in β-chimaerin hyperactive mutants……120

Introduction

Proper histogenesis of the vertebrate Central Nervous System (CNS) relies on the sequential regionalization of the embryonic neural tube into distinct transcriptionally defined domains. Cells within each domain, in turn, are guided by both cell-autonomous programs and extrinsic signals to direct proliferation, migration, differentiation, axonal and dendritic outgrowth, synaptic establishment and refinement events – thus building the elaborate architecture and connectivity of the adult CNS. Failure of any of these neurodevelopmental processes may result in dramatic changes in CNS organization or function, and thus each have become areas of interest within modern cellular neuroscience. Despite decades of research, fundamental questions regarding the cellular mechanisms underlying CNS morphogenesis and histogenesis persists. In the context of neuronal guidance, several ligand-receptor systems that act as attractive or repulsive cues have been functionally characterized (H. T. Park, Wu, and Rao 2002; Kolodkin and Tessier-Lavigne 2011), yet relatively little is understood regarding how these signaling events are relayed intracellularly to converge on regulators of the cytoskeletal system. Further, as development advances new cohorts of neurons must correctly navigate an increasingly elaborate milieu of transcriptionally defined domains, instructive substrates, neighboring cell types, and diffusible extracellular cues. How individual cells integrate this complex array of extracellular signals to achieve directed cytoskeletal responses remains an important and outstanding question in developmental neuroscience.

Cellular Adhesion Molecules underlie most neurodevelopmental processes

Early *in vitro* studies established that neurons display strong adhesive preferences for particular extracellular matrix (ECM) substrates (HARRISON 1959; Weiss 1934; WEISS 1945; M. Singer, Nordlander, and Egar 1979), and require contact with such permissive substrates for adhesion, neurite outgrowth, axonal extension, and migration (Bonner and O'Connor 2001; Walter et al. 1987; Bozyczko and Horwitz 1986; Gundersen 1987; Hatten, Furie, and Rifkin 1982; Husmann, Faissner, and Schachner 1992). Such cell-cell and cell-ECM associations are mediated by broad classes of Cell Adhesion Molecules (CAMs), including the four classical families: Cadherins, Selectins, Integrins, and the Immunoglobulin superfamily (IgSF) of receptors (R O Hynes and Lander 1992; Letourneau, Condic, and Snow 1994; Kenneth M. Yamada and Geiger 1997); as well as the more recently described Neuroligins, Neurexins, Teneurins, and Leucine-Rich Repeat (LRR) Proteoglycans (Moreland and Poulain 2022). As a group, CAMs have been demonstrated to participate in nearly every aspect of neurodevelopment, including neurogenesis (Homan et al. 2018; R. Huang et al. 2020), neuronal migration (Schmid and Maness 2008; Solecki 2012; Y.-A. Chen, Lu, and Tsai 2018), neurite formation (Pollerberg et al. 2013; Missaire and Hindges 2015; Fischer, Künemund, and Schachner 1986), synaptogenesis (Gerrow and El-Husseini 2006; Yogev and Shen 2014; Duncan, Murphy, and Maness 2021), and myelination (Rasband and Peles 2021).

Some of the best studied CAMs have been the integrin family of transmembrane receptors, due to their demonstrated roles in mediating cell-ECM associations, acting to

physically link the extracellular environment to the cytoskeletal network, and thereby relay anchorage-dependent growth and survival signals to the cell (B. Geiger 1989; E. Zamir and Geiger 2001; Brakebusch and Fässler 2003; Reichardt and Tomaselli 1991). However, the roles of integrins and their ECM ligands during neurodevelopmental events was not always clear. Early investigators quickly recognized that ECM components such as laminin, fibronectin, collagen, thrombospondin, and tenascin participate in many aspects of neurodevelopment; histological studies showed these molecules were present in many basement membranes, line the borders of tissues and axon tracts, and are expressed by diverse glial populations, suggesting they have important roles in organizing tissues (Nakanishi 1983; Nagata and Nakatsuji 1990; Hausmann and Sievers 1985; Halfter et al. 2002; M. Singer, Nordlander, and Egar 1979). Similarly, early genetic and *in vitro* studies using purified substrates showed these molecules are required for neurite outgrowth (Davis et al. 1985; Edgar, Timpl, and Thoenen 1988; Gundersen 1987), neuronal migration (Dufour et al. 1988; Hatten, Furie, and Rifkin 1982; O'Shea, Rheinheimer, and Dixit 1990; Bartsch et al. 1992; Husmann, Faissner, and Schachner 1992; J R Sanes 1989; Liesi 1985), and axonal pathfinding (Carbonetto, Gruver, and Turner 1983; Stephen S. Easter et al. 1994; Lefcort and Bentley 1987; J. Cohen et al. 1986; J R Sanes 1989). Initially these findings led to a view that neurons could navigate to their targets simply by displaying selective adhesion to particular substrates, until it was demonstrated that adhesive strength alone was not sufficient to predict substrate choice or axonal growth rates *in vitro* (Lemmon et al. 1992); while these ECM substrates were shown to be 'permissive' for neurite and axonal growth, they were not 'instructive'

in the same way as the classical guidance molecules like Netrins, Slits, Semaphorins, and Ephrins (Tessier-Lavigne and Goodman 1996). However, the role of the ECM could not be described as purely mechanical, as it was observed that ECM components and their integrin receptors could induce signaling cascades independent of classical guidance molecules, eliciting the same second messenger systems as growth factor receptors (Damsky and Werb 1992; Richard O Hynes 1992; R O Hynes and Lander 1992; Schweighoffer and Shaw 1992). Further, early evidence showed that ECM molecules could modulate a neuron's response to a given guidance cue (Nguyen-Ba-Charvet et al. 2001; Höpker et al. 1999) or even response to ECM components of other families (Snow, Brown, and Letourneau 1996; Carbonetto, Gruver, and Turner 1983). Collectively, such observations engendered a nuanced view of cell-ECM adhesion and integrin receptors during neurodevelopmental events; while they are not sufficient to instruct guidance events, integrins nevertheless underlie almost all investigated examples of neuronal guidance due to their fundamental roles in sensing the environment and physically linking the extracellular environment to the cytoskeletal network (Letourneau, Condic, and Snow 1994). Therefore, from the earliest days of the field of neuronal guidance, researchers have sought to understand how guidance molecule signaling modulates such integrin-mediated cellular adhesion pathways in ways that direct neuronal migration and axonal pathfinding (Myers, Santiago-Medina, and Gomez 2011a).

Regulation of Cellular Adhesion during Neuronal Migration and Axon Pathfinding

Two key steps during the proper histogenesis of the CNS are: 1) the establishment of laminated structures, and 2) the precise and regulated assembly of circuits upon these structures. Lamination of the CNS has been proposed as a fundamental organizational mechanism, as it provides a means of grouping cells with similar properties, functions, or connectivity (Caviness, Bhide, and Nowakowski 2008; Joshua R Sanes and Zipursky 2010; Rakic and Sidman 1970). Indeed, the laminar organization of tissues through the assembly upon, or anchorage to, basement membranes appears to be a deeply conserved organizational strategy across species and tissue types (Kruegel and Miosge 2010; Sekiguchi and Yamada 2018; Halfter et al. 2002; Hausmann and Sievers 1985; Yurchenco 2011). Disruptions to lamination during human CNS development are associated with behavioral and cognitive dysfunction, as evidenced by multiple neurodevelopmental disorders like lissencephaly or focal dysplasia (Barkovich 2022; Blümcke et al. 2011; Palmini and Holthausen 2013; Bahi-Buisson and Guerrini 2013; Guerrini, Dobyns, and Barkovich 2008). Laminar organization of the CNS is principally achieved through the sequential production of transcriptionally defined cohorts of neurons (Chédotal and Rijli 2009; Nóbrega-Pereira and Marín 2009), followed by their directed migration to form discrete tissues or structures. Guided by a broad suite of extracellular cues (H. T. Park, Wu, and Rao 2002), postmitotic neurons must navigate away from their point of origin, often a highly proliferative germinal zone, to their final target structure, thus ensuring proper stratification of discrete layers and spatial-temporal relationships between cells (Valiente and Marín 2010; Kriegstein and Noctor 2004).

Much of our understanding of the cellular mechanisms orchestrating cell migration comes from live imaging and biochemical studies of isolated fibroblasts or mesenchymal cells (Ridley et al. 2003; SenGupta, Parent, and Bear 2021). These studies have elucidated a discrete series of steps that occur to ensure processive movement, termed the 'Migration Cycle' (Lauffenburger and Horwitz 1996). In this framework, cells first become polarized in response to some attractant or repulsive signal, appropriately partitioning the subcellular machinery and orienting the cytoskeletal network, followed by the extension of a leading process in the direction of migration, adhesion of that leading process to the substrate, the coordinated production of traction and contractile forces to propel the cell forward, and finally detachment of the cell from the substrate (Ridley et al. 2003; Lauffenburger and Horwitz 1996; T. Kawauchi and Hoshino 2007; Trivedi et al. 2014). In this way, progressive cellular locomotion is achieved through the cyclic regulation of leading process extension, attachment, force generation, and detachment.

In contrast to migrating fibroblasts, neurons display diverse strategies and morphologies to achieve directed migration (Marin et al. 2010; Valiente and Marín 2010; Cooper 2013; Evsyukova, Plestant, and Anton 2013; Nguyen and Hippenmeyer, n.d.; Trivedi and Solecki 2011; Kriegstein and Noctor 2004). Some neurons will adopt multipolar morphologies with highly branched leading processes, such as that observed in tangentially migrating interneurons as they travel from the ganglionic eminences to populate the neocortex (O Marín and Rubenstein 2001), tangentially migrating precerebellar pontine neurons (Watanabe and Murakami 2009), or the multipolar stage of

pyramidal neurons of the neocortex (LoTurco and Bai 2006). *In vitro* observations of such multipolar neurons have shown that they can dynamically extend and retract these leading processes, and that exposure to guidance molecules can influence the rate and directionality of branching and new process formation (Ward, Jiang, and Rao 2005; Martini et al. 2009). Migration is achieved through the subsequent shuttling of the nucleus and small organelles into a newly formed extension in a two-step process termed 'nucleokinesis' (Bellion et al. 2005; Kappeler et al. 2006; Nasrallah et al. 2006), wherein centrosome repositioning precedes movement of the nucleus into a newly formed process. Alternatively, neurons migrating through the assistance of a glial scaffold will often display a bipolar morphology, extending a single elongated leading process with which they closely associate with the scaffold, as is seen in the radial migration of cerebellar granule cells (Komuro and Rakic 1998; Edmondson et al. 1988) and during the radial migration of more superficial neurons of the neocortex (Rakic 1972). Migration of these cells more closely mirrors the 'migration cycle' model of fibroblast cells, with neurons advancing through the cyclic regulation of leading process extension, centrosome repositioning, nucleokinesis, and trailing process retraction (Oscar Marín, Valdeolmillos, and Moya 2006). An individual neuron may display both multipolar, glial-independent and bipolar, gliophilic modes of locomotion over the course of its navigation from its germinal zone to its final destination, as is observed with cerebellar granule cells (Komuro and Rakic 1998; Leto et al. 2016), precerebellar pontine neurons (Yee et al. 1999; D. Kawauchi et al. 2006), and cortical pyramidal neurons (LoTurco and Bai 2006). However, the cellular mechanisms that orchestrate such changes in locomotive strategies

remain poorly understood and highlight the complex interplay between cellular adhesion, guidance signaling, cell polarity, and cytoskeletal dynamics.

A second developmental event required for proper CNS histogenesis is the assembly of precise circuits. Proper connectivity between neurons and structures is achieved through the regulated guidance of axons from the cell body towards the innervation target, followed by the establishment and refinement of synaptic contacts (Kolodkin and Tessier-Lavigne 2011). Axonal navigation is mediated by a specialized structure known as the growth cone (Lowery and Van Vactor 2009; Huber et al. 2003), which must properly regulate timely adhesion and detachment from the underlaying substrate in response to long- and short-range guidance cues, thereby achieving axonal steering and proper fasciculation and defasciculation events (Breau and Trembleau 2022; Myers, Santiago-Medina, and Gomez 2011b; Nichol et al. 2016; Zang, Chaudhari, and Bashaw 2021; Craig 2018). The growth cone is a highly organized and dynamic structure, consisting of three layers of distinct cytoskeletal organization. Closest to the axon body is the microtubule-dense Central (C) domain, which is enriched with organelles and vesicles and is the main site of axon elongation (Lowery and Van Vactor 2009; Nozumi and Igarashi 2018; Sánchez-Huertas and Herrera 2021). Most distal to the cell body is the Peripheral (P) domain, which contains a dense, branched network of filamentous actin (F-actin) and myosin motors that creates a lamellipodial-like structure at the tip of the axon (Lowery and Van Vactor 2009; Omotade, Pollitt, and Zheng 2017). The P domain also contains arrays of long, unbranched F-actin bundles that produce finger-like filopodial projections that contain many guidance receptors (Lowery and Van

Vactor 2009; Snow, Brown, and Letourneau 1996); this unique combination of lamellipodia and filopodial projections gives the growth cone its characteristic ‘hand’ shape. Sandwiched between the C and P domains is the Transitory (T) zone, which contains short F-actin polymers that run perpendicular to the dense, branched F-actin network in the P zone and which encircle the C domain; this ring-like structure controls the advancement of the microtubule network in the C domain during axon elongation (Medeiros, Burnette, and Forscher 2006; Burnette et al. 2008). New F-actin is polymerized against the leading edge of the lamellipodium in the P domain, producing force that pushes already assembled F-actin backward towards the C domain, a process that is facilitated by the pulling of myosin motor proteins, and older F-actin is depolymerized in the C domain (Bray and White 1988; C.-H. Lin and Forscher 1995). This coordinated polymerization, pulling, and depolymerization causes a constant treadmilling effect that pushes F-actin towards the C domain in a process termed ‘F-actin retrograde flow’ (Bray and White 1988; C.-H. Lin and Forscher 1995; Kerstein, Nichol, and Gomez 2015; Craig et al. 2012). In many ways, the axonal growth cone may be considered analogous to the lamellipodial leading edge of a migrating fibroblast, as this cytoskeletal organization closely mirrors the lamellipodial of non-neuronal migrating cells (Craig 2018; Zang, Chaudhari, and Bashaw 2021; Sánchez-Huertas and Herrera 2021; C. H. Lin, Thompson, and Forscher 1994; Huber et al. 2003; Lewis, Courchet, and Polleux 2013; Kerstein, Nichol, and Gomez 2015; Schneider, Metz, and Rust 2022), suggesting conserved cellular mechanisms and effectors coordinate cellular movement in each process.

Much of our current understanding of the interplay between neuronal guidance signals and adhesion signaling comes from *in vitro* studies of growth cone cytoskeletal dynamics and *in vivo* genetic studies that ablate specific guidance receptors or the adhesive machinery of the cell (Moreland and Poulain 2022; Huber et al. 2003). Sites where neurons must distinguish and selectively respond to multiple instructive cues are known as ‘Choice Points’ (Stoeckli and Landmesser 1998; Tessier-Lavigne and Goodman 1996; Raper and Mason 2010), and are often sites of axonal turning, sorting, or regulated fasciculation. In the mammalian CNS, some classical examples of choice points include axonal sorting at the optic chiasm (Erskine et al. 2000; Plump et al. 2002) and subsequent target selection to produce visual maps in the optic tectum and superior colliculus (Feldheim and O’Leary 2010), commissural axon crossing in the spinal cord (Comer et al. 2019), midline-crossing of forebrain tracts like the corpus callosum or anterior commissure (Julien et al. 2005; Piper et al. 2009; Hatanaka et al. 2009; Shu and Richards 2001; Shu, Sundaresan, et al. 2003; Bagri et al. 2002), and subplate regulation of thalamocortical axon entry into the cortex (McConnell, Ghosh, and Shatz 1989; Ghosh et al. 1990; Ghosh and Shatz 1992; Kanold and Luhmann 2010). During development, choice points present a broad array of permissive, attractive and repulsive signals, in the form of diffusible cues or contact-mediated interactions with other cell types or ECM substrates (Kolodkin and Tessier-Lavigne 2011; Raper and Mason 2010; H. T. Park, Wu, and Rao 2002). On the cellular level, these cues are integrated within the growth cone to direct steering or fasciculation by causing asymmetrical differences in its adhesion to the substrate, a model referred to as the ‘Clutch Hypothesis’ (Mitchison and Kirschner 1988;

Jay 2000; Short, Suarez-Zayas, and Gomez 2016). In this framework, guidance cues direct axonal steering by selectively strengthening or weakening growth cone adhesion to its substrate, causing the growth cone to turn towards or away from the guidance cue. In support of this, coupling of F-actin to the substrate through Integrin receptors has been shown to allow growth cone advancement through the production of traction forces (C.-H. Lin and Forscher 1995; Craig et al. 2015; Nichol et al. 2016; Elosegui-Artola et al. 2016; 2014) and asymmetric remodeling of F-actin and microtubules is associated with growth cone turning (Cammarata, Bearce, and Lowery 2016; Omotade, Pollitt, and Zheng 2017; Craig 2018; Geraldo and Gordon-Weeks 2009). However, the exact cellular pathways that permit the coordination between guidance receptor activation and strengthening or weakening of integrin-based attachments remains poorly understood.

While commonly discussed separately, both neuronal migration and axonal pathfinding are often paired developmental processes: great overlap exists between the guidance factors, cellular machinery, signaling, and mechanical forces orchestrating each (Oscar Marín, Valdeolmillos, and Moya 2006; T. Kawauchi and Hoshino 2007), and an individual neuron may be engaged in both processes simultaneously. Indeed, the tight spatiotemporal regulation of cellular adhesion is crucial to coordinate complex cellular activities like locomotion or growth cone turning; in each case neurons must tightly adhere to the substrate to anchor themselves and generate traction forces, detach themselves from the substrate once advanced, and cyclically regulate these two processes to ensure processive movement until the destination is reached (Webb, Parsons, and Horwitz 2002; Moreland and Poulain 2022; Craig 2018). Due to their roles mediating

cell-ECM interactions, the Integrin family of transmembrane receptors have been extensively studied in the context of cellular locomotion (Huttenlocher and Horwitz 2011; Webb, Parsons, and Horwitz 2002; Askari et al. 2010; Ballestrem et al. 2001), and have been shown to play integral roles in adhesion signaling (Richard O Hynes 2002; Reichardt and Tomaselli 1991; Schwartz 2001; Askari et al. 2010; Damsky and Werb 1992; Benjamin Geiger and Yamada 2011; J. L. Guan, Trevithick, and Hynes 1991; Yurchenco 2011), cytoskeletal reorganization (Miyamoto et al. 1995; K M Yamada and Miyamoto 1995; Schoenwaelder and Burridge 1999; Palazzo et al. 2004; Brakebusch and Fässler 2003; DeMali, Wennerberg, and Burridge 2003), and force generation (Keith Burridge and Chrzanowska-Wodnicka 1996; Beningo et al. 2001; Sheetz, Felsenfeld, and Galbraith 1998; J. Lee et al. 1994; Oliver, Dembo, and Jacobson 1999). Similarly, the Rho family of GTPases have been extensively studied for their roles in regulating cell polarization and remodeling the cytoskeletal network in both migrating cells and axonal growth cones (Nobes and Hall 1995; Watabe-Uchida, Govek, and Van Aelst 2006; E.-E. Govek, Hatten, and Van Aelst 2011; Stankiewicz and Linseman 2014; Guilluy, Garcia-Mata, and Burridge 2011). However, the cellular pathways that link integrin receptors and Rho family GTPases, and how these pathways are influenced by guidance receptor signaling, remain largely uncharacterized.

Integrin Adhesion Complexes mediate cell-ECM adhesion signaling events

Integrin Adhesion Complexes (IACs, or simply ‘adhesion complexes’) are cellular structures that permit cells to tightly adhere to the extracellular matrix (ECM) by

mechanically linking the cellular exterior to the intracellular actin cytoskeletal system (K. Burridge et al. 1988; B. Geiger 1989; Jockusch et al. 1995; Miyamoto et al. 1995; Winograd-Katz et al. 2014), thus anchoring the cell to its substrate. Highly dynamic structures, IACs encompass at least four types of adhesive complexes: focal complexes (FXs, also referred to as nascent adhesions), focal adhesions (FAs), fibrillar adhesions (FBs), and cellular podosomes (Benjamin Geiger and Yamada 2011; Nobes and Hall 1995; Vicente-Manzanares and Horwitz 2011). The role of IACs and their components in the sensing and mediating mechanical forces has been under increasing investigation in recent years (J. Thomas Parsons, Horwitz, and Schwartz 2010; Sun, Guo, and Fässler 2016), and has prompted reexamination of the role of force production in tissue morphogenesis (Yusko and Asbury 2014; Eyckmans et al. 2011), cell and neuronal migration (Sun, Guo, and Fässler 2016; Minegishi and Inagaki 2020), and axon guidance (Kerstein, Nichol, and Gomez 2015; Suter and Miller 2011; Raffa 2022). While it has long been appreciated that the formation of actinomyosin ‘stress fibers’ is associated with stabilization and maturation of IAC complexes (Chrzanowska-Wodnicka and Burridge 1996; Choi et al. 2008; Shemesh et al. 2005; Flinn and Ridley 1996), recent studies have demonstrated that intracellular forces indeed increase integrin receptor affinity for ECM substrates (Katsumi et al. 2005; Thodeti et al. 2009; Sheetz, Felsenfeld, and Galbraith 1998; Galbraith, Yamada, and Sheetz 2002), that many IAC components are recruited and signal in a force-dependent manner (del Rio et al. 2009; Satz et al. 2010; Galbraith, Yamada, and Sheetz 2002), and experience stretch-induced molecular rearrangements that expose binding motifs and enable force-induced interactions (Goult, Yan, and

Schwartz 2018; Kadaré et al. 2015; Braniš et al. 2017; Zhao et al. 2016; Janoštiak et al. 2014). Collectively, these studies have added a novel layer of complexity to our understanding of adhesion-based neuronal guidance, and highlight the work that must be done to delineate the chemical and mechanical cues that drive cytoskeletal responses during guidance events.

IACs are named for the integrins, which are a large family of transmembrane receptors that include 18 alpha (α) and 8 beta (β) subunits, which are known to form 24 combinations of αβ heterodimers (Takada, Ye, and Simon 2007; Schwartz 2001). The integrin-β1 subunit has been particularly well studied, as it is found in 12 of the known heterodimer combinations and appears to mediate most intracellular signaling functions during CNS development (Richard O Hynes 2002; Takada, Ye, and Simon 2007; Milner and Campbell 2002; Lilja and Ivaska 2018; Jaudon, Thalhammer, and Cingolani 2021; Myers, Santiago-Medina, and Gomez 2011a; Riccomagno et al. 2014; Schmid and Anton 2003)**.** Integrin-β1 deficiency results in embryonic lethality (Fässler and Meyer 1995). CNS-specific ablation of Integrin-β1 results in disrupted cortical lamination, caused by destabilization of glial endfoot processes (Graus-Porta et al. 2001; Belvindrah et al. 2007), speaking to its role in maintaining cell-ECM interactions. Other roles for Integrin-β1 in CNS development include regulating cerebellar granule cell proliferation (Blaess et al. 2004), cortical neural progenitor proliferation (Leone et al. 2005), cortical neuron radial migration (Marchetti et al. 2010), neurite outgrowth of DRG axons (Tomaselli et al. 1993) and retinal ganglion cells in xenopus retina (Sakaguchi and Radke 1996),

remodeling of hippocampal dendritic spines (Ning et al. 2013) and hippocampal synapse function (Zhen Huang et al. 2006).

Proteomic approaches on fibroblast cells have identified over 200 proteins that make up IACs, termed the 'integrin adhesome' (Byron et al. 2011; Winograd-Katz et al. 2014; Zaidel-Bar and Geiger 2010; Zaidel-Bar et al. 2007), although a conserved set of 147 proteins organized into five signaling modules has been proposed (Chastney et al. 2020). The regulation of IAC assembly, turnover, signaling, and disassembly is crucial to coordinate complex cellular behaviors such as cell migration, axonal guidance, epithelial-to-mesenchymal transitions, and podosome formation, and tissue morphogenesis (J. T. Parsons et al. 2000; J. Thomas Parsons, Horwitz, and Schwartz 2010; Webb, Parsons, and Horwitz 2002; Long and Huttner 2019; Robles and Gomez 2006). These cellular adhesive complexes rapidly remodel in response to internal or external stimuli, and have been shown to signal bi-directionally through 'outside-in' and 'inside-out' mechanisms (Hoffmann et al. 2014; Lele et al. 2008; Wolfenson et al. 2009).

One of the earliest observable events in IAC assembly is the recruitment and tran-autophosphorylation of Focal Adhesion Kinase (FAK), a nonreceptor tyrosine kinase that mediates many of the scaffolding and signaling functions of IACs and also regulates their turnover in migrating cells (L. Kornberg et al. 1992; Miyamoto et al. 1995; Webb, Brown, and Horwitz 2003; Zaidel-Bar et al. 2003). Like the integrins, FAK and its homologue Pyk2 have been shown to have diverse roles in neuronal guidance, with demonstrated roles in neurite outgrowth (Ivankovic-Dikic et al. 2000), axonal adhesion (Woo, Rowan, and Gomez 2009), growth cone remodeling and turning (Myers and

Gomez 2011; Chacón, Fernández, and Rico 2010; Moore Dr. et al. 2012), and axon pathfinding (Robles and Gomez 2006; Kerstein, Patel, and Gomez 2017). In recent years, FAK has also gained attention for its potential roles in tumorigenesis or metastasis due to its unique role in mediating anchorage-dependent survival signaling (Chauhan and Khan 2021; Chuang et al. 2022; Dawson et al. 2021; B. Y. Lee et al. 2015; Murphy et al. 2020; Sulzmaier, Jean, and Schlaepfer 2014). These findings make phospho-activation of FAK a desirable target to monitor during IAC assembly and signaling either during development or disease, yet traditional approaches to monitor FAK activity rely on western blot or fluorescently-tagged genetic constructs, neither of which allow for the sensitive, quantitative observation of FAK phosphorylation in living cells.

In Chapter 2, I describe the generation of a novel luminescent-based tool to monitor IAC activation in living cells. We call this tool pYFAK BiLuc due to its bi-molecular complementation design to produce firefly luciferase (BiLuc) and its sensitivity in recognizing tyrosine-phosphorylated Focal Adhesion Kinase (pYFAK). I propose that this genetically-encoded set of probes fulfills a current need for a tool that provides simple, specific, and sensitive reporting of FAK activation during IAC assembly and signaling, with the potential to be expanded for high-throughput drug screens, genetic screens, or other *in vivo* or *in vitro* applications.

Cas proteins as integrators of adhesive and neuronal guidance signals

An intriguing and understudied group of IAC constituents are the Cas family of cytoplasmic adaptors. The Cas family of proteins comprises of four paralogues: *P130Cas*

(*Bcar1*), *Cas-L* (*HEF1*, *NEDD9*), *Sin* (*EFS*), and *Cass4* (Defilippi, Di Stefano, and Cabodi 2006; Deneka, Korobeynikov, and Golemis 2015). The more recently discovered fourth family member, *Cass4*, initially believed to be a pseudogene, appears to only be expressed in adult tissues (Deneka, Korobeynikov, and Golemis 2015). As scaffolding proteins, all Cas members share a common secondary structure consisting of multiple interaction domains: an N-terminal Src Homology 3 (SH3) domain, a tyrosine-repeat rich substrate domain, a serine rich domain, and a C-terminal Focal Adhesion Targeting (FAT) domain (Deneka, Korobeynikov, and Golemis 2015). Except for Cass4, all Cas homologues have been shown to interact with Src-family kinases via their C-terminal domain (Deneka, Korobeynikov, and Golemis 2015). During integrin-mediated adhesion signaling, Cas is recruited to IACs through SH2-mediated binding to Focal Adhesion Kinase (FAK) and FAT-mediated targeting to Paxillin (Calalb, Polte, and Hanks 1995b; Harte et al. 1996; Nakamoto et al. 1997; Hayashi, Vuori, and Liddington 2002; Arold, Hoellerer, and Noble 2002). Once recruited, these highly structurally similar scaffolding proteins may be phosphorylated on tyrosine residues within the substrate-binding domain, which contain repeats of a YxxP sequence, thus permitting docking of SH2 and PTB-containing partners like Crk1, Crk2, and CrkL (Defilippi, Di Stefano, and Cabodi 2006; Ruest et al. 2001; Fonseca et al. 2004; Bouton, Riggins, and Bruce-Staskal 2001). A signaling complex of Cas-Crk-DOCK180 is then able to activate Rho family GTPases, leading to local actin polymerization and lamellipodial extension, promoting cell motility and spreading (Defilippi, Di Stefano, and Cabodi 2006; Honda et al. 1999; Gustavsson, Yuan, and Fällman 2004; Tomar and Schlaepfer 2009). In this way, Cas proteins act as

upstream positive regulators of Rac-mediated F-actin assembly, cell spreading, and cell migration (Cary et al. 1998; Honda et al. 1999).

In the context of neuronal guidance signaling events, phosphotyrosine activation of Cas proteins has been observed downstream of Integrin (Bargon, Gunning, and O'Neill 2005; Z. Huang et al. 2007), CXCL12/CXCR4 (J. F. Wang, Park, and Groopman 2000), Neuropilin (Evans et al. 2011), and Netrin (Liu et al. 2007) signaling. Conversely, Eph/Ephrin (Bourgin et al. 2007) signaling events have been demonstrated to decrease Cas phosphorylation. These findings indicate Cas may have additional neurodevelopmental roles, yet its functions during CNS development remain largely unexplored. The Drosophila homologue DCas is required for proper motor axon projection and fasciculation (Z. Huang et al. 2007). Consistent with this, Cas proteins function in the mammalian PNS to regulate DRG axon fasciculation and pathfinding (Vahedi-Hunter et al. 2018). In the mammalian CNS, Cas proteins are required for Retinal Ganglion Cell migration and monolayer resolution within the mouse retina (Riccomagno et al. 2014), and have been recently shown to participate in cortical lamination (Wong et al. 2022), demonstrating the dual-roles for Cas proteins in neuronal migration and axonal guidance events. Within this framework, the study of Cas protein function could provide a unique opportunity to understand how the interplay between adhesive and instructive cues regulates neuronal guidance events.

In Chapter 1, I provide evidence that Cas proteins are required for the proper fasciculation and guidance for two forebrain white matter tracts: the Anterior Commissure (AC) and Thalamocortical (TCA) projections. Using conditional mouse

genetics, I demonstrate that Cas proteins are required cell-autonomously for the proper fasciculation of the AC in a manner that is not phenocopied by Integrin-β1 mutants, suggesting Cas proteins are signaling independently of their canonical receptor. Additionally, I show that Cas proteins are required in a cortical-autonomous manner to regulate the guidance of TCA afferents, in a manner that is phenocopied by Integrin-β1 mutants. I go on to demonstrate that in both Cas and Integrin-β1 mutants, lamination of the subplate is disrupted, providing a developmental mechanism to explain the mis-projection of TCA afferents in these animals.

Rho-family GTPases as effectors of guidance signaling

The ultimate consequence of any neuronal guidance signaling event is to affect a change in the cytoskeletal system (Mitchison and Kirschner 1988). As mentioned above, the Rho family of GTPases are attractive candidates as common targets of guidance and adhesive signaling events, due to their well-demonstrated roles in actin assembly and microfilament organization (Jaffe and Hall 2005). The mammalian Rho family consists of approximately 23 members, while over 70 negative regulators and 80 activators of Rho GTPases have been identified (Bai et al. 2015; Cherfils and Zeghouf 2013). Errors in Rho GTPase activity or regulation underlay a breadth of neurodevelopmental and psychiatric disorders (DeGeer and Lamarche-Vane 2013; G. H. Huang et al. 2017; Zamboni et al. 2018; Stankiewicz and Linseman 2014), as they have been found to participate in almost every aspect of CNS circuit formation (G. H. Huang et al. 2017; Stankiewicz and Linseman 2014). In the context of neuronal migration, Rho family GTPases have been

implicated in neuronal polarization, neurite outgrowth, leading process dynamics, adhesion to substrates, the regulation of contractile bundles, and somal translocation (G. H. Huang et al. 2017; Stankiewicz and Linseman 2014; E.-E. Govek, Hatten, and Van Aelst 2011; Bai et al. 2015). Coordinated activities of Rac1 and RhoA have been shown to promote neuritogenesis through stabilizing focal contacts (Woo and Gomez 2006). Studies have shown a requirement for RhoA (Mulherkar et al. 2014b), Cdc42 (E.-E. Govek et al. 2018), and Rac1/Rac3 (Nakamura et al. 2017) GTPases during cerebellar granule cell migration.

Chimaerin proteins as negative regulators of RhoGTPases

As small G-proteins, Rho GTPases exist in both active (GTP-bound) and inactive (GDP-bound) states (Cherfils and Zeghouf 2013). Therefore, to ensure proper migratory behaviors, cells must precisely control the subcellular activity of GTPases in response to guidance cues. This is generally achieved through the action of local positive regulators (Guanine Nucleotide Exchange Factors, of GEFs) and negative regulators (GTPase Activating Proteins, GAPs), which induce conversion to either the GTP- or GDP-bound form, respectively (Cherfils and Zeghouf 2013; Kutys and Yamada 2015). Two main families of GEFs exists: the DH-PH (Dbl homology-pleckstrin homology) domain family (Rossman, Der, and Sondek 2005), and the DHR (Dock homology region) domain family (Meller, Merlot, and Guda 2005; Bai et al. 2015). In contrast, the diversity of GAPs has prevented their classification into related families, and they are instead grouped by their respective targets, which may or may not reflect actual evolutionary relatedness

(Bernards 2003). Notably, a third class of Rho GTPase regulators, called Guanine Disassociation Inhibitors, or GDIs, have been described to regulate the membrane recruitment of some classes of GTPases (Cherfils and Zeghouf 2013). The target promiscuity of these regulators, coupled with their overlapping expression profiles and placements in diverse signaling cascades, have made elucidation of their individual *in vivo* roles a difficult task.

The chimaerin family of RacGAPs consists of two genes: α-chimaerin (*CHN1*) and β-chimaerin (*CHN2*), each of which are known to produce two isoforms (Yang and Kazanietz 2007). Among the estimated 70 mammalian RhoGAPs, the chimaerins are unique for their specificity as negative regulators of Rac1 GTPase, and for their C1 binding domain, which allows interactions with the second messenger diacylglycerol (DAG) and consequent recruitment to the plasma membrane inner leaflet (G. H. Huang et al. 2017; Yang and Kazanietz 2007). Of the two homologues, α-chimaerin has been more thoroughly investigated in neurodevelopmental processes, and has been demonstrated to play roles in hippocampal dendritic spine remodeling (Van de Ven 2005; Buttery et al. 2006), Ephrin-mediated growth cone collapse, (Brown 2004; Beg et al. 2007; Iwasato et al. 2007; Wegmeyer et al. 2007) and optic tract axon guidance (Ferrario et al. 2012). The homologue β-chimaerin is less well studied but was recently shown to act downstream of Sema3F/neuropilin-2 signaling to regulate Rac1 activity during hippocampal Dentate Gyrus axon pruning (Riccomagno et al. 2012). Of note, β-chimaerin has been shown to be strongly expressed in cerebellar granule cells (GCs) in the adult (Leung et al. 1994), but its function during cerebellar morphogenesis is unknown.

A new functional role for β-chimaerin during cerebellar lamination is the main topic of study of Chapter 3. Here I demonstrate that a small population of cerebellar granule cell progenitors (GCPs) express β-chimaerin prior to completing their radial migration from the External Germinal Layer (EGL) inward to the mature Granule Cell Layer (GCL). Mouse mutants for β-chimaerin have disrupted lamination of the cerebellar cortex, displaying aggregated ectopic clusters of neurons well into adulthood. I show that these clusters are composed principally of post-mitotic granule cells and not other cerebellar neurons, and that they are able to recruit mossy fiber afferents from the pontine nucleus. These results expand on our current knowledge of the regulation of Rac1 during cerebellar development.

Chapter 1

Cas proteins are required for cortical white matter tract fasciculation

Abstract

Proper neural circuit organization requires individual neurons to project to their targets with high specificity. While several guidance molecules have been shown to mediate axonal fasciculation and pathfinding, little is understood regarding how neurons intracellularly integrate these cues. Here we provide genetic evidence that the Crk-Associated Substrate (Cas) family of intracellular adaptor proteins are required for proper fasciculation and guidance of two cortical white matter tracts, the Anterior Commissure (AC) and thalamocortical axons (TCAs). Using a Triple Conditional Knock Out (*Cas TcKO*) model generated in our lab, we show evidence that Cas proteins are required non-neuronal-autonomously for TCA projection, in a manner that phenocopies a non-neuronal requirement for the integrin-β1 receptor. Additionally, analysis of *Cas TcKO* mutants reveals a neuronal-autonomous role in AC fasciculation that is not phenocopied in integrin-β1 deficient mutants, suggesting that Cas proteins are signaling downstream of a different receptor during this axon pathfinding event. These findings build on a growing literature implicating Cas proteins as key mediators of axon fasciculation and guidance, acting downstream of a variety of receptors.

Introduction

During Central Nervous System (CNS) development, axons often traverse complex routes to meet their synaptic targets, interacting with a diverse array of attractive, repulsive, and permissive substrates (Tessier-Lavigne and Goodman 1996; Kolodkin and Tessier-Lavigne 2011). One developmental mechanism proposed to ensure proper guidance of large tracts is the concept of "pioneer" vs "follower" axons (Stephen S. Easter et al. 1994; S S Easter, Ross, and Frankfurter 1993; Caudy and Bentley 1986; Lefcort and Bentley 1987; L. Wang and Marquardt 2013). In this framework, initial pioneering axons are able to carve out exquisitely precise and stereotyped trajectories thanks to the presence of intermediate targets or guidepost cells, their passage laying tracks by which subsequent follower axons may use to reach a common target (Chédotal and Richards 2010). Both the guidepost-to-guidepost advancement of pioneering axons and the regulated fasciculation and defasciculation of follower axons require neurons to properly sense and respond to adhesive signals (Moreland and Poulain 2022; Breau and Trembleau 2022), in the forms of cell-cell and cell-extracellular matrix (ECM) interactions. In the vertebrate nervous system, two well established examples of guidepost-assisted axonal guidance are thalamocortical axon (TCA) projections (Garel and López-Bendito 2014; López-Bendito et al. 2006) and the commissural tracts of the forebrain, such as the Anterior Commissure (AC) (Squarzoni, Thion, and Garel 2015). However, the exact cellular and molecular mechanisms that permit the timely regulation of adhesive signals during TCA guidance or AC formation remains to be elucidated.

During normal development, TCA projections are guided by a diverse array of secreted gradients, multiple guidepost populations, and intermediate targets as they carve out their complex trajectories to cross the pallio-subpallial boundary, fasciculate to form the internal capsule, innervate the cortical plate, and defasciculate to reach their individual targets (Molnár et al. 2012). TCAs reach the cortex before their target layer (IV) is fully formed, and their processes arrest for several days before entering the cortical plate, temporarily targeting a cortical region known as the subplate (Ghosh et al. 1990; Ayoub and Kostovic 2009; Allendoerfer and Shatz 1994). Entry of thalamic axons into the cortical layers and coordinated target selection is facilitated by subplate neurons, a developmentally transient population of the earliest-born telencephalic neurons that may be considered another type of intermediate target for TCA pathfinding (McConnell, Ghosh, and Shatz 1989; Ghosh et al. 1990; Ghosh and Shatz 1992; Allendoerfer and Shatz 1994; Kanold and Luhmann 2010). The formation of commissural tracts in the forebrain is also known to involve the assistance of intermediate targets, often in the form of transient midline glial populations (Silver, Edwards, and Levitt 1993; Marcus et al. 1995; Shu and Richards 2001; Shu, Li, et al. 2003), such as the glial “tunnel” that facilitates the formation of the AC (Pires-Neto, Braga-De-Souza, and Lent 1998; Lent et al. 2005). The AC is composed of two tracts that share a midline crossing point: axons of the anterior AC (aAC) originate in the Anterior Olfactory Nucleus and form reciprocal connections to each olfactory bulb, while axons of the posterior AC (pAC) originate from the ventrolateral cortex and join with the stria terminalis to form reciprocal connections between the two hemispheres (Jouandet and Hartenstein 1983; Fenlon et al. 2021).

Recent developmental studies have identified a population of pioneering axons that precede the formation of a primary fascicle and midline crossing during pAC formation and suggest a role for ECM molecules in the sorting of aAC and pAC axons (Martin-Lopez, Meller, and Greer 2018).

The Cas family of signal adapter proteins comprises of four paralogues: *p130Cas* (*Bcar1*), *Cas-L* (*HEF1*, *NEDD9*), *Sin* (*EFS*), and *Cass4* (Defilippi, Di Stefano, and Cabodi 2006; Deneka, Korobeynikov, and Golemis 2015). Upon integrin receptor stimulation, these highly structurally similar scaffolding proteins may be phosphorylated on tyrosine residues within the substrate-binding domain, which contain repeats of a YxxP sequence, thus permitting docking of SH2 and PTB-containing partners like Crk (Defilippi, Di Stefano, and Cabodi 2006; Ruest et al. 2001; Fonseca et al. 2004; Bouton, Riggins, and Bruce-Staskal 2001; Donato et al. 2010). This induces the assembly of a Cas-Crk-DOCK180 scaffold complex that subsequently recruits and activates Rac GTPases, leading to local actin polymerization (Defilippi, Di Stefano, and Cabodi 2006; Honda et al. 1999; Gustavsson, Yuan, and Fällman 2004). In this way, Cas proteins act as an essential link between external adhesion signals and the actin cytoskeleton, permitting cells to reorganize their actin network in response to ECM engagement. Developmentally, Cas proteins have been demonstrated to regulate motor axon fasciculation and projection in *Drosophila* (Z. Huang et al. 2007), with similar roles in the fasciculation and pathfinding of DRG axons in the mammalian PNS (Vahedi-Hunter et al. 2018). In the mammalian CNS, Cas proteins coordinate commissural projections in the spinal cord (Liu et al. 2007), are required for Retinal Ganglion Cell positioning in the

mouse retina (Riccomagno et al. 2014) and have been recently shown to participate in cortical lamination (Wong et al. 2022), demonstrating the multifaceted roles for Cas proteins in neuronal migration and axonal guidance events. Biochemical studies have shown that Cas proteins may be phosphorylated downstream of Integrin (Bargon, Gunning, and O'Neill 2005; Z. Huang et al. 2007), Dystroglycan (Wong et al. 2022), CXCL12/CXCR4 (J. F. Wang, Park, and Groopman 2000), Neuropilin (Evans et al. 2011), and Netrin (Liu et al. 2007) signaling. Conversely, activation of the Eph/Ephrin pathway has been demonstrated to decrease Cas protein phosphorylation (Bourgin et al. 2007), suggesting these scaffolding proteins may serve as versatile mediators of both adhesion and neuronal guidance signaling events.

In this study, we sought to investigate the potential roles for Cas proteins during the formation of forebrain white matter tracts. Using conditional reverse genetics, we show that Cas proteins are broadly required for proper guidance of TCA projections and for fasciculation of pAC axons. We go on to show that the TCA projection phenotype is forebrain-autonomous but non-neuronal-autonomous, with mis-projecting TCA processes closely associating with misplaced subplate neurons. Further, we demonstrate a neuronal-autonomous role for Cas genes during AC fasciculation, and show that defasciculating axons originate, in part, from dorsolateral cortex. Interestingly, these defects in AC fasciculation are not phenocopied by integrin-β1 mutants, suggesting this receptor is dispensable for AC fasciculation and pathfinding, and that Cas proteins act at least in part independently of integrin-β1 in this process.

Results

To investigate the potential roles for Cas family proteins during cortical tract formation, we began by assessing the expression of *Cas* family paralogues during cortical development. Recently, we established that transcripts for the paralogues *p130Cas*, *CasL/Nedd9*, and *Sin/Efs* are all expressed in the developing cortex, with overlapping expression in the subventricular and ventricular zones (Wong et al. 2022). Additionally, the localization of *p130Cas* transcript is also dynamically enriched in the cortical plate (embryonic day 12.5, E12.5) and intermediate zone (embryonic day 14.5, E14.5), consistent with expression in postmitotic migrating neurons and projecting axons (Wong et al. 2022). We next performed immunohistochemistry for the *Cas* proteins p130Cas and Nedd9 on C57BL/6J embryos at embryonic days 12.5, 14.5, and 16.5 (E12.5, E14.5, E16.5), as these represent the window when many cortical tracts are established (Wahlsten 1981; Pires-Neto, Braga-De-Souza, and Lent 1998; Martin-Lopez, Meller, and Greer 2018) (**Figure 1.1**). Initially, p130Cas protein is restricted to the marginal and ventricular zones (**Figure 1.1 D and D'**), subsequently expanding to include ventricular/subventricular and intermediate zones by E14.5 (**Figure 1.1 E and E'**). By E16.5, p130Cas protein remains diffusely expressed throughout the cortical plate, with the most intense signal observable in the marginal zone and white matter (**Figure 1.1 F and F'**). In a similar manner, Nedd9 (*CasL* paralogue) protein is restricted to the marginal zone at E12.5 (**Figure 1.1 G and G'**), expanding to include the cortical plate at E14.5 (**Figure 1.1 H and H'**), before again becoming restricted to the marginal zone at E16.5 (**Figure 1.1 I and I'**). Unfortunately, commercial antibodies for Sin and Cass4

proteins are not available, but the mRNA distribution for *Sin* indicates that this paralogue is indeed developmentally expressed in the cortex (Wong et al. 2022). Overall, these initial expression analysis support the idea that *Cas* genes may participate in cortical circuit assembly.

To better visualize axons originating from neurons expressing *p130Cas*, and to test whether these axons contribute to major cortical tracts, we took advantage of the *p130Cas-EGFP-BAC* transgenic line produced by the GENSAT BAC transgenic project (Gong et al. 2003). These mice carry a bacterial artificial chromosome (BAC) containing an enhanced green fluorescent protein (EGFP) expression cassette driven by the *p130Cas* promoter and 10kb of upstream regulatory sequences, thereby causing all cells with *p130Cas* promoter activity to accumulate cytosolic EGFP in a pattern that recapitulates endogenous expression. We collected *p130Cas-EGFP-BAC* pups at E14.5, E16.5 and neonates (P0) and performed immunohistochemistry for Neural Cell Adhesion Molecule L1 (NCAM-L1) (Fischer, Künemund, and Schachner 1986; Moos et al. 1988; Munakata et al. 2003) to visualize cortical white matter tracts (**Figure 1.2**). Indeed, we find at E14.5 *p130Cas*-driven EGFP signal is co-expressed with NCAM-L1 throughout the cortical plate, in the lateral cortex and in a group of axons fasciculating to form the presumptive Anterior Commissure (AC) (**Figure 1.2 A-A''**, white arrowheads denote forming AC). By E16.5, robust *p130Cas*-driven EGFP signal is observed in all layers of the cortex, marginal zone, and white matter, with clear expression in the External Capsule (EC, **Figure 1.2 B**, white star), striatal axons, and continued expression in the now midline-crossing AC (**Figure 1.2 B**, white arrowheads). In neonates (P0), we observe strong

p130Cas-driven EGFP in all major cortical tracts, including the corpus callosum, striatum, EC and AC (**Figure 1.2 C-C''**, white star denotes EC and white arrowheads denote AC). High-magnification images are shown for the pallio-subpallial boundary (**Figure 1.2 D-D''**, white dotted line indicates boundary) and cingulate cortex (**Figure 1.2 E-E''**) in *p130Cas-EGFP-BAC* neonates. We can see many diffuse NCAM-L1^{+} processes innervate the subpallium that are not expressing *p130Cas*-driven EGFP, although many EGFP^{+} cell bodies occupy this region (**Figure 1.2 D-D'**). Similarly, the cortex shows robust expression of *p130Cas*-driven EGFP in all cortical layers and in the white matter (**Figure E-E'**).

Having confirmed the developmental expression of *Cas* genes during the formation of cortical white matter, we next sought to test the requirement for Cas proteins during cortical tract formation. To this end, we generated *Cas* triple conditional knockouts (*Cas TcKO*) (Riccomagno et al. 2014; Vahedi-Hunter et al. 2018; Wong et al. 2022) to assess the broad requirements for Cas proteins during cortical tract development. In brief, these animals bear homozygous null alleles for the Cas homologues CasL (*CasL*$^{-/-}$) and Sin (*Sin*$^{-/-}$), while also harboring homozygous floxed alleles for p130Cas (*p130Cas*$^{fl/fl}$), permitting Cre-mediated excision upon crossing to different Cre-driver lines. As such, the *Cas TcKO* model is a powerful resource to study the broad developmental requirements of *Cas* homologues in a cell- and tissue-specific manner.

To determine the requirement *Cas* genes during brain development, we chose to induce a broad deletion of *Cas* gene function by crossing *Cas TcKO* animals with *Nestin-Cre* mice, which drives early (E9.5) recombination in neural stem cells and intermediate

progenitors (Tronche et al. 1999). As such, *Nestin-Cre* is often employed by investigators to produce CNS-wide deletions in both neural and glial populations. We generated *Nestin-Cre;CasL*$^{-/-}$*;Sin*$^{-/-}$*;p130Cas*$^{fl/\Delta}$ (or more simply *Nes-Cre;TcKO*) mutants and performed immunohistochemistry for the axon marker NCAM-L1 to examine the organization of major cortical tracts (**Figure 1.3**). We observe notable disruptions to two major white matter tracts in *Nes;TcKO* mutants: the Anterior Commissure (AC) and cortical white matter. Axons of the External Capsule (EC) that, upon exit from the pallium, turn medially to join the posterior Anterior Commissure (pAC), fail to fasciculate properly in *Nes-Cre;TcKO* mutants and project ventrally into the subpallium (**Figure 1.3 A' vs D'**). These mis-projecting axons connect to the dorsolateral cortex, as they are labelled by tracing experiments using DiA crystals placed into the Somatosensory 2 (S2) region of the cortex (**Figure 1.3 B vs E**, white arrowheads). This is notable, as in mice the pAC has only been described to connect projections from lateroventral areas, mainly the piriform and entorhinal cortex (Fenlon et al. 2021). *Nes;TcKO* mutants also display severe defects in white matter within the cortical plate, with large fasciculated bundles of axons mis-projecting to the pial surface. (**Figure 1.3 A'' vs D''**). We hereafter refer to these ectopic projections as Cortical Bundles (CBs). Given the cortex receives complex input from multiple cortical and subcortical areas, including contralateral connections via commissural tracts like the corpus callosum or AC (Chédotal and Richards 2010) and subcortical thalamic input from Thalamocortical (TCA) projections (López-Bendito and Molnár 2003; Hanashima, Molnár, and Fishell 2006), we asked if the CB phenotype presented by *Nes-Cre;TcKO* mutants consists of

axons originating from cortical or subcortical areas. We performed lipophilic tracing experiments using DiI by placing crystals into the laterodorasal nucleus of the thalamus and found robust labeling of CBs in the ipsilateral hemisphere in *Nes-Cre;TcKO* mutant neonates (**Figure 1.3 C, F**, white stars). To assess if cortical axons contribute to either CB formation, we performed stereotactic injection of an AAV-tdTomato fluorescent reporter into the endopiriform cortex of P21 *Nes-Cre;TcKO* and cre-negative control littermates (Fenlon et al. 2021), and allowed viral expression for two weeks before collecting tissue for immunohistochemistry. We found robust labeling of callosal, EC and AC tracts with this method (**Figure 1.3 G and H**). Surprisingly, we did not observe CB labeling from cortical axons, including those crossing through the corpus callosum (**Figure 1.3 G' and H'**), suggesting that these CBs form primarily from TCA afferents. Similarly, we did not observe AC defasciculating axons contralateral to the injection site (**Figure 1.3 G'' and H''**), demonstrating the AC defasciculating axons in *Nes-Cre;TcKO* mutants originate only from ipsilateral cortex. These data point to a strong requirement for *Cas* family genes during cortical white matter tract formation.

To better assess the developmental progression of *Nes-Cre;TcKO* white matter tract defects, we collected mutant embryos at E14.6, E16.5, and E18.5 and performed immunohistochemistry for NCAM-L1 (**Figure 1.4**). Interestingly, we observed different developmental trajectories for each phenotype. Initial formation of the EC-AC tract was unperturbed in *Nes-Cre;TcKO* animals, with no observable defasciculating axons at E14.5 and E16.5 (**Figure 1.4 A' vs B', C' vs D'**). It was not until late embryonic development (E18.5) that axons aberrantly defasciculating from the EC were observed

(**Figure 1.4 E' vs F'**, white arrowhead). In contrast, the CB phenotype is apparent in *Nes-Cre;TcKO* mutants as early as E14.5 (**Figure 1.4 A'' vs B''**, white stars), with the phenotype growing in severity as development continues (**Figure 1.4 C''-F''**, white stars denote CBs). These results suggest subtly different developmental trajectories for each of the presented white matter tract phenotypes.

Due to the apparent differences in developmental timing for each of the white matter phenotypes observed in *Nes-Cre;TcKO* mutants, we next asked if *Cas* family genes are functioning cortical- or neuronal-autonomously during establishment of these white matter tracts. As such, we chose to generate two new classes of *Cas TcKO* mutants by crossing into *Emx1-Cre* (Gorski et al. 2002) and *Nex-Cre* (Goebbels et al. 2006) driver lines. In contrast to the broad expression of *Nestin-cre,*, *Emx1-Cre* and *Nex-Cre* recombination events are largely restricted to the cortex. While *Emx1-Cre* induces recombination in cortical intermediate progenitors, thus affecting both cortical neurons and most glial populations, *Nex-Cre* is active only in postmitotic neurons within the cortex. As such, comparisons between the three classes of *Cas TcKOs* (*Nestin-Cre* vs *Emx1-Cre* vs *Nex-Cre*) allow us to elucidate the tissue- and cell-specific requirements of *Cas* family genes during cortical white matter tract establishment.

Following this genetic reasoning, we generated *Emx1-Cre;CasL*$^{-/-}$*;Sin*$^{-/-}$*;p130Cas*$^{fl/\Delta}$ (*Emx1-Cre;TcKO*) mutants and collected neonates for immunohistochemistry using the axon marker NCAM-L1 (**Figure 1.5**). *Emx1-Cre;TcKO* animals phenocopy the AC defasciculation errors observed in *Nes-Cre;TcKO* neonates (**Figure 1.5 A',vs D'**, white arrowhead). These defasciculating AC axons were labeled by

lipophilic tracing from DiA crystal placement into the S2 cortex (**Figure 1.5 B vs E**, white arrowheads), reproducing the unusually dorsolateral origin of these projections in both classes of mutants. *Emx1-Cre;TcKO* animals also present the CB phenotype (**Figure 1.5 A'' vs D''**, white stars). We performed axon tracing experiments using lipophilic dyes in *Emx1;TcKO* neonates and confirmed that these CBs are primarily formed by thalamic axons in these mutants. (**Figure 1.5 C vs F**, white stars). Given *Emx1-Cre* is not expressed in the thalamic territory (Gorski et al. 2002), and we have established that the CBs in *Nes-Cre;TcKO* mutants are primarily TCA afferents originating from the thalamic nuclei (**Figure 1.3 C vs F**), these results suggest a non-cell autonomous role for *Cas* genes in TCA innervation, possibly through the positioning of, or in response to, some local guidance cue or intermediate target population. Collectively, analysis of *Emx1-Cre;TcKO* mutants demonstrates that both white matter tract phenotypes in *Cas TcKO* animals are cortical-autonomous, i.e. requiring *Cas* gene function in the cortical plate itself, and reproduce a dorsolateral origin of the defasciculating AC axons.

We next generated *Nex-Cre;CasL*$^{-/-}$*;Sin*$^{-/-}$*;p130Cas*$^{fl/\Delta}$ (*Nex-Cre;TcKO*) and collected neonates for immunohistochemistry using the axon marker NCAM-L1 (**Figure 1.5**). In contrast to *Emx1-Cre;TcKO* mutants, *Nex-Cre;TcKO* mutant neonates only present the AC defasciculation phenotype (**Figure 1.5 G' vs J'**, white arrowheads), demonstrating a cortical- and neuronal-autonomous role for *Cas* genes during AC fasciculation. These stray AC axons were again confirmed dorsolateral in origin by lipophilic tracing from DiA crystal placement into the S2 cortex (**Figure 1.5 H vs K**, white arrowheads). Interestingly, *Nex-Cre;TcKO* mutant neonates do not present the CB

phenotype. Collectively, analysis of the three classes of *Cas TcKO* mutants implicate a multifaceted requirement for *Cas* genes during cortical white matter tract formation. The proper fasciculation of AC axons appears to require *Cas* in a cortical- and neuronal-autonomous manner, likely reflecting a requirement for *Cas* genes in the projecting axons themselves. In contrast, the proper projection of TCA afferents into the cortex appears to have a cortical-autonomous, but non-neuronal-autonomous requirement for *Cas* genes. As such, we infer that *Cas* genes are required by some cortical-resident cell population to properly coordinate TCA guidance. We chose to examine more closely both the neuronal-autonomous role of *Cas* genes in AC fasciculation and the non-cell autonomous role of *Cas* genes in TCA projection into the cortex in subsequent experiments.

To further assess the neuronal-autonomous role of *Cas* genes during AC fasciculation, we decided to genetically label these axons and confirm their cortical origins. This also allowed us to more closely examine their relationship to neighboring tracts. To this end, we made use of the *Ai14/R26*$^{LSL\text{-}tdTomato}$ cre reporter line (or simply *Ai14* in text), which produces tdTomato fluorescent protein upon cre-mediated excision of upstream translational STOP sequences, permitting visualization of cells or tissues that have or previously had cre recombinase expression (Madisen et al. 2010). We crossed the *Ai14* reporter line into *Emx1-Cre;TcKO* line to generate *Emx1-Cre;Ai14*$^{+/-}$*;TcKO* mutants and collected neonates for immunohistochemistry with the marker NCAM-L1 (**Figure 1.6**). As expected, we found robust labeling of the EC-AC tract in *Emx1-Cre*$^{+}$ controls and observed tight fasciculation of the AC tract in these animals (**Figure 1.6 A-B'' ** and **C-D''**). Examination of *Emx1-Cre;Ai14*$^{+/-}$*;TcKO* mutants revealed that all of the

defasciculating AC processes were tdTomato^{+}, genetically confirming the cortical origin of these mis-projections (**Figure 1.6 E-F''**, white arrowheads). Interestingly, these tdTomato^{+}NCAM-L1^{+} processes appear to interpolate with tdTomato^{-}NCAM-L1^{+} processes that diffusely populate the subpallium, suggesting that these *Cas* deficient (tdTomato^{+}) axons are improperly associating with other axonal processes.

We next crossed the *Ai14* reporter line into *Nex-Cre;TcKO* line to generate *Nex-Cre;Ai14$^{+/-}$;TcKO* mutants and collected neonates for immunohistochemistry with the marker NCAM-L1 (**Figure 1.6**). As expected, we found robust labeling of the EC-AC tract in *Nex-Cre^{+}* controls (**Figure 1.6 G-H''** and **I-J''**). Interestingly however, we observed a dose-dependent effect of the loss of *Cas* gene function in the severity of the AC defasciculation phenotype in *Nex-Cre* crosses. While *Nex-Cre;Ai14$^{+/-}$;CasL$^{+/-}$;Sin$^{+/-}$;p130Cas$^{+/+}$* animals display normal AC fasiculation (**Figure 1.6 H-H''**), *Nex-Cre;Ai14$^{+/-}$;CasL$^{-/-}$;Sin$^{-/-}$;p130Cas$^{fl/+}$* animals begin to display a less severe AC defasciculating phenotype (**Figure 1.6 I-I''**), with the full severity of the phenotype presented by *Nex-Cre;Ai14$^{+/-}$;TcKO* (full genotype written as *Nex-Cre;Ai14$^{+/-}$;CasL$^{-/-}$;Sin$^{-/-}$;p130Cas$^{fl/fl}$*) mutants (**Figure 1.6 J-J''**). Like seen with *Emx1-Cre;Ai14$^{+/-}$;TcKO* mutants, all defasciculating AC processes are tdTomato^{+} and appear to interpolate with with tdTomato^{-}NCAM-L1^{+} processes that diffusely populate the subpallium (**Figure 1.6 I-I''** and **J-J''**). Collectively, these data genetically confirm the cortical origins of EC defasciculating axons while also suggesting that defasciculating AC projections are incorrectly associating with other NCAM-L1^{+} processes.

We next sought to further investigate the non-neuronal-autonomous role for *Cas* genes during TCA projection into the cortex. During canonical adhesion signaling events, Cas proteins are recruited to early focal complexes downstream of integrin-β1 receptor stimulation and activation of the non-receptor tyrosine kinases Focal Adhesion Kinase (FAK) and Src (Defilippi, Di Stefano, and Cabodi 2006). In the brain, the integrin-β1 receptor (encoded by the *Itgb1* gene) appears to be the most prevalent β-integrin subunit mediating adhesion signaling events (Yan and Cui 2022; Myers, Santiago-Medina, and Gomez 2011a; Jaudon, Thalhammer, and Cingolani 2021; Lilja and Ivaska 2018; Milner and Campbell 2002), with established roles in axonal guidance and growth cone dynamics (Myers, Santiago-Medina, and Gomez 2011a; Kerstein, Nichol, and Gomez 2015; Bixby and Harris 1991; Renaudin et al. 1999). Given the cortical-autonomous but non-cell-autonomous role for *Cas* genes during TCA projection (**Figure 1.5**), we asked if this non-neuronal-autonomous requirement was recapitulated by *Itgb1* mutants, or if these mutants display similar deficits in other white matter tracts. Therefore, we generated *Emx1-Cre;Itgb1*$^{fl/fl}$ and *Nex-Cre;Itgb1*$^{fl/fl}$ mice and performed immunohistochemistry for NCAM-L1 (**Figure 1.7**). We observe that *Emx1-Cre;Itgb1*$^{fl/fl}$ mutants strongly phenocopy the CB phenotype presented by *Cas TcKO* mutants (**Figure 1.7 A'' vs B''**, white stars), whereas *Nex-Cre;Itgb1*$^{fl/fl}$ do not (**Figure 1.7 C'' vs D''**). These results suggest a similar non-neuronal-autonomous role for integrin-β1 during TCA projection. Having generated these two classes of *Itgb1* mutants, we also took the opportunity to examine the EC-AC tract in these neonates. Interestingly, we found that neither class of *Itgb1* mutants phenocopy the AC defasciculation defects observed in *Cas*

TcKO mutants (**Figure 1.7 A' vs B'** and **C' vs D'**). These observations confirm a non-neuronal-autonomous role for *Itgb1* during TCA guidance and fasciculation and demonstrate the integrin-β1 receptor is dispensable for AC fasciculation.

Finally, we sought to identify a developmental mechanism by which TCA afferents mis-project to form CBs in *Cas TcKO* mutants. Informed by recent research in our lab establishing a role for Cas proteins during cortical lamination (Wong et al. 2022), we asked if the cortical-autonomous, non-cell-autonomous CB phenotype presented by *Emx1-Cre;TcKO* and *Emx1-Cre;Igtb1*$^{fl/fl}$ mutants could be explained as a secondary phenotype resulting from the cortical lamination defects we observe in these mutants. As previously mentioned, the subplate is a transient structure that acts as an intermediate target for TCAs that coordinates the entry of these thalamic axons into the cortical plate (Ghosh and Shatz 1992; López-Bendito and Molnár 2003). Given the cortical requirement of *Cas* genes for TCA guidance, and the previously described errors in cortical lamination presented by *Cas TcKO* mutants (Wong et al. 2022), we hypothesized that one possible explanation for the CB phenotype presented here could be the displacement of subplate neurons in *Cas TcKO* and *Igtb1* mutants. Therefore, we generated *Emx1-Cre;CasTcKO* and *Emx1-Cre;Itgb*$^{fl/fl}$ mutants and collected E16.5 cortex for immunohistochemistry with the markers NCAM-L1 and Tbr1, a transcription factor known to regulate the specification of the subplate and layer VI cortical layers (Hevner et al. 2001) (**Figure 1.8**). Indeed, we find that in both *Emx1-Cre;CasTcKO* and *Emx1-Cre;Itgb*$^{fl/fl}$ mutants lamination of Tbr1^{+} cells is highly disrupted, displaying focal dysplasia containing columns of Tbr1^{+} cells that reach the marginal zone (**Figure 1.8 B'**

vs D' and **F' vs H'**). We observe that NCAM-L1$^+$ CBs strongly correlate with these displaced Tbr1$^+$ cells, at times with clear "tubes" of Tbr1$^+$ cells containing CBs projecting to the marginal zone (**Figure 1.8 D-D''** and **H-H''**, white stars). These results demonstrate that the subplate and deep cortical layer organization is disrupted in both *Cas TcKO* and *Itgb1* mutants, with breakages in the subplate correlating strongly with the CB phenotype in these animals.

Discussion

In this study we used conditional mouse genetics to assess the cortical-autonomous and neuronal-autonomous roles of *Cas* genes during forebrain white matter tract formation. Using a triple conditional knockout (*Cas TcKO*) model developed in the Kolodkin and our labs (Riccomagno et al. 2014; Vahedi-Hunter et al. 2018; Wong et al. 2022), we provide strong genetic evidence Cas proteins are required neuronal-autonomously for proper fasciculation of the posterior branch of the Anterior Commissure (pAC) (**Figure 1.3 D' and E**) and cortical-autonomously for thalamocoritical axon (TCA) guidance (**Figure 1.3 D'' and F**). Each of these phenotypes is discussed below, beginning with the AC fasciculation.

The AC is highly evolutionarily conserved forebrain tract that, in mice, makes reciprocal connections between the Anterior Olfactory Nuclei of each olfactory bulb (via the anterior AC tract, aAC), as well as mediating reciprocal connections between the piriform and entorhinal cortices of the two hemispheres (via the posterior AC tract, pAC) (Fenlon et al. 2021). Recent developmental studies have identified a small group of

pioneering axons with unusual elongation kinetics that advance towards the embryonic midline at E13 that precede the majority of pAC axons arriving later at E15 (Martin-Lopez, Meller, and Greer 2018). These later arriving processes join with the stalled pioneers to form a primary fascicle that crosses the embryonic midline in unison at E16, with contralateral innervation occurring at E17 and specific target selection achieved at E18 (Martin-Lopez, Meller, and Greer 2018). In the present study, we find that *Cas TcKO* mutants display defasciculation and ventral targeting of axons of the pAC tract (**Figure 1.3 D' and E**). However, these defects are only observable at later embryonic (E18.5) and postnatal (P0) stages (**Figure 1.3** and **Figure 1.4 F'**). Considering the developmental timeline of pAC development, this suggests that it is a population of late "follower" axons that arrive at the AC, possibly reflecting slower axonal extension or more distant cell body positioning. Indeed, we find that the defasciculating AC processes originate from dorsolateral cortex, as they are labeled by lipophilic tracing from the somatosensory S2 cortex in all three classes of *Cas TcKO* mutants analyzed (**Figure 1.3 E, Figure 1.5 E and K**). These results are unusual, in that the pAC has only been described to carry axons from ventrolateral cortical areas in mice (Fenlon et al. 2021), suggesting these axons may be mis-routed from their normal tracts, possibly the corpus callosum. We go on to genetically confirm the cortical origin of these defasciculating AC processes, as they are labeled by the *Ai14* fluorescent cre-dependent reporter when crossed to either *Emx1-Cre* or *Nex-Cre* (**Figure 1.6 F-F'' and L-L''**). Further, we observe that the defasciculating tdTomato$^+$ AC processes appear to interpolate with other NCAM-L1$^+$ processes in the subpallium, suggesting these axons are failing to properly

regulate homotypic vs heterotypic associations (Spead and Poulain 2020). Of note, we observe a dose-dependent effect of the loss of *Cas* genes in the *Nex-Cre* crosses, with animals containing a single wildtype copy of *p130Cas* presenting an intermediate phenotype (**Figure 1.6 J-J''**). We believe that these observations may be explained in part by the highly sensitized and somewhat outbred background of $Ai14^{+/-};CasL^{-/-};Sin^{-/-}$ animals; we postulate that the differences seen here in sensitivity to *Cas* gene dosage may reflect either different efficiencies of the *Emx1-Cre* and *Nex-Cre* lines, or simply random intractable differences in sensitizing genetic background when generating these crosses from outbred lines, or both. Nevertheless, a single allelic copy of wildtype or floxed *p130Cas* appears generally sufficient for normal cortical tract development in a cre-negative *Cas TcKO* background, as evidenced by the littermate controls presented elsewhere in this manuscript. Taken together, these data support a cell autonomous requirement for Cas proteins for guidance of a subset of cortical axons, that in the absence of these genes mis-project into the ventral subpallium.

During canonical adhesion signaling, Cas proteins are recruited to cellular adhesion complexes following integrin receptor-ECM engagement (Defilippi, Di Stefano, and Cabodi 2006; Vuori et al. 1996; Donato et al. 2010). The integrins are a large family of transmembrane receptors that include 18 alpha (α) and 8 beta (β) subunits, which are known to form 24 combinations of αβ heterodimers (Takada, Ye, and Simon 2007; Schwartz 2001). The integrin-β1 subunit has been particularly well studied, as it is found in 12 of the known heterodimer combinations and appears to mediate most intracellular signaling functions during CNS development (Richard O Hynes 2002; Takada, Ye, and

Simon 2007; Milner and Campbell 2002; Lilja and Ivaska 2018; Jaudon, Thalhammer, and Cingolani 2021; Myers, Santiago-Medina, and Gomez 2011a; Riccomagno et al. 2014; Schmid and Anton 2003). Therefore, we asked if Cas proteins are signaling downstream of the integrin-β1 receptor during the formation of cortical white matter tracts. We analyzed the structure of the AC in *Igtb1* mutant neonates, which are deficient for the integrin-β1 subunit. We found that conditional deletion of *Igtb1* using *Emx1-Cre* or *Nex-Cre* failed to phenocopy the AC defasciculation defects observed in *Cas TcKO* mutants, suggesting that integrin-β1 is dispensable for the fasciculation of this tract. This raises two intriguing possibilities: either there is compensation from another β subunit in *Igtb1* deficient animals, or Cas proteins are signaling downstream of a non-canonical receptor system. Indeed, Integrin-β5, Integrin-β6, and Integrin-β8 have all been shown to be expressed in the embryonic cortex (Schmid and Anton 2003). These three subunits are known to form heterodimers with Integrin-αV, permitting recognition of the ECM molecules fibronectin and vitronectin (Lilja and Ivaska 2018). Development of the pAC is coordinated by a population of midline glial cells that dynamically express the ECM molecules CSPG, tenascin, fibronectin, and laminin (Pires-Neto, Braga-De-Souza, and Lent 1998), making it plausible that Cas proteins are mediating an αV-fibronectin signaling cascade, a novel function for these versatile scaffolding proteins. The second possible explanation for the failure of *Igtb1* mutants to phenocopy the AC defasciculation defects presented by *Cas TcKO* animals is if Cas proteins are signaling downstream of a different receptor system. In support of this, it has been shown that phospho-activation of Cas can be modulated by CXCL12/CXCR4 (J. F. Wang, Park, and Groopman 2000),

Neuropilin (Evans et al. 2011), Netrin (Liu et al. 2007), and Eph/Ephrin (Bourgin et al. 2007) signaling. Mutations in several genes have been shown to affect the development of the aAC (Fazeli et al. 1997), pAC (Henkemeyer et al. 1996; Robichaux et al. 2016; Kwon, Tsai, and Crandall 1999), or the entire AC tract (Islam et al. 2009; Ito et al. 2010; Serafini et al. 1996; Klingler et al. 2015; Julien et al. 2005; Dottori et al. 1998; H. Chen et al. 2000; Giger et al. 2000; Sahay et al. 2003; Suto et al. 2005; Abudureyimu et al. 2018; Deuel et al. 2006; Shen et al. 2002; Tissir et al. 2005; L. Chen et al. 2007; Tole et al. 2006; Hua et al. 2014), although most observed mutations result in thinning or absence of the AC tracts, not defasciculation of AC axons. Notably, mutants of the Eph ligand *Nuk* (Henkemeyer et al. 1996) as well as *EphB1/EphB2* double-mutants (Robichaux et al. 2016) display similar defasciculation and ventral targeting of pAC axons as those we observe in *Cas TcKO* mutants. Similarly, the defasciculating AC axons observed in *EphB1/EphB2* double-mutants have an unusually dorsolateral origin, which the authors suggest demonstrates axonal guidance defects in these animals (Robichaux et al. 2016). Some biochemical studies have implicated cross-talk between Integrin-β1 and EphB/EphrinB receptor pathways in various cell types (Stein, Huynh-Do, et al. 1998; Stein, Lane, et al. 1998; Huynh-Do et al. 1999; Becker et al. 2000). In the context of CNS biology, the adhesion molecules laminin and NCAM-L1 were shown to differentially modulate EphB-stimulated growth cone collapse in cultured retinal ganglion cells (Suh et al. 2004). Similarly, Ephrin-B reverse signaling was shown to induce stereotyped mossy fiber axon pruning via the recruitment of the SH2/SH3-domain containing scaffolding protein Grb4, leading to the formation of a Grb4-Pak-Dock180 complex to induce Rac

activity (Xu and Henkemeyer 2009). This latter signaling cascade is quite similar to the canonical Cas-Crk-Dock180 complex that induces Rac activation downstream of integrin receptor stimulation (Defilippi, Di Stefano, and Cabodi 2006), suggesting multiple potential points of cross-talk between EphB/Ephrin-B and Integrin signaling could exists in neurons. Differentiating if Cas proteins are working downstream of either Integrin or Eph/Ephrin receptor systems during AC projection and fasciculation will be an interesting goal for future studies.

The second white matter tract phenotype presented by *Cas TcKO* mutants is the appearance of large fasciculated bundles of axons mis-projecting to the pial surface of the cortex (**Figure 1.3 A'' vs D''**), which we termed Cortical Bundles (CBs). Lipophilic and adenoviral tracing experiments reveal the origin of these mis-projections to be thalamic (**Figure 1.3 F and H'**). We go on to demonstrate that this phenotype is cortical- but non-neuronal-autonomous, as conditional deletion of *Cas* genes using *Nex-Cre* fail to recapitulate the CB phenotype (**Figure 1.5 G'', I, J'', L**). From this, we infer that *Cas* gene function is not required in TCA projections themselves, but rather some cortical-resident population that is responsible for coordinating TCA entry. Analysis of *Igtb1* mutants similarly revealed a cortical-autonomous but non-neuronal-autonomous role for integrin-β1, as conditional deletion of *Igtb1* using *Nex-Cre* did not result in the formation of CBs (**Figure 1.7 D''**).

During normal development, TCA innervation of the cortex is regulated by a transient structure known as the subplate, which acts as an intermediate target for TCAs until their target layer IV is fully formed (Molnár et al. 2012). Arriving TCA projections

will stall in the subplate for several days and grow extensive side processes within the subplate during this waiting period (Ghosh et al. 1990; Ayoub and Kostovic 2009; Allendoerfer and Shatz 1994). Upon entering the cortical plate, TCAs will form transient microcircuits with subplate neurons and maturing cortical layer IV neurons, a process which appears to facilitate the proper targeting of layer IV and thus the organization of mature circuits (Kanold and Luhmann 2010; Tolner et al. 2012; Viswanathan et al. 2012). Similarly, subplate neurons display surprising heterogeneity in morphology over the course of development (Hoerder-Suabedissen and Molnár 2012), suggesting they are dynamically involved in coordinating TCA regionalization and partner choice, yet the exact cellular mechanisms that regulate the formation and remodeling of these connections is not yet well elucidated. Nevertheless, mutations that disrupt the laminar organization of the subplate, such as *Reeler* mutants (Molnár et al. 1998) and *Cdk5* mutants (Gilmore et al. 1998) result in aberrant TCA projection patterns within the cortical plate, speaking to the developmental importance of the subplate in TCA guidance. Previous work from our lab and others has demonstrated that *Cas TcKO* and *Igtb1* mutants have severely disrupted laminar organization of the cortex, caused by breakages in the basal lamina that destabilize radial glial endfeet attachment, thus resulting in improper migration of cortical neurons that results in severe cortical dysplasia (Wong et al. 2022; Graus-Porta et al. 2001; Belvindrah et al. 2007). Therefore, we asked if the subplate organization were disrupted in *Cas TcKO* and *Igtb1* mutants, as this could suggest a developmental mechanism to explain the cortical-autonomous defects we observe in TCA guidance. Indeed, using the subplate/Layer VI marker Tbr1, we observe

severe disruption to the deep cortical layers at E16.5 in both *Cas TcKO* and *Igtb1* mutants (**Figure 1.8 D' and H'**). Co-labeling with NCAM-L1 reveals that the CB phenotype correlates strongly with displaced Tbr1^{+} cells. These results suggest a model wherein *Cas* genes are required by cortical radial glia to regulate their endfeet attachment and subsequent migration of the cortical layers, including the subplate. Mispositioned subplate neurons, in turn, recruit TCAs to innervate improper cortical layers, resulting in the CB phenotype.

Collectively, these studies demonstrate multifaceted roles for the *Cas* family of signal adaptor proteins to function cell-autonomously and non-cell autonomously to direct axonal guidance and fasciculation of cortical white matter tracts. We build on previous evidence that *Cas* genes function radial glial-autonomously downstream of the integrin-β1 receptor to ensure proper lamination of the cortex (Wong et al. 2022), showing that such errors in lamination result in disruptions to the subplate, causing aberrant TCA guidance and the formation of fasciculated bundles that mis-project to the pial surface. Further, we provide genetic evidence that *Cas* genes function independently of the integrin-β1 receptor during fasciculation and guidance of the Anterior Commissure, demonstrating that a subset of late-arriving axons fail to properly fasciculate with the posterior branch of the Anterior Commissure. These mis-projecting axons are, in part, originating in the Somatosensory 2 (S2) cortex and display improper ventral targeting following crossing of the pallio-subpallio boundary. This study builds on a rapidly growing literature implicating Cas proteins as key mediators of axon

fasciculation and guidance downstream of multiple receptor systems, suggesting these versatile scaffolding proteins uniquely mediate both adhesion and guidance events.

Materials & Methods

Animals and Genotyping: For embryonic collections, the morning of vaginal plug observation was designated as embryonic day 0.5 (E0.5) and the day of birth was postnatal day 0 (P0). The generation of the *Cas TcKO* model has been previously described (Riccomagno et al. 2014; Seo et al. 2005; Donlin et al. 2005). The following lines were purchased from The Jackson Laboratory: *Nestin-Cre* (Stock: 003771), *Emx1-Cre* (Stock: 005628), *Ai14/R26*$^{LSL\text{-}tdTomato}$ (Stock: 007914), and *Itgb1*$^{fl/fl}$ (Stock: 004605). Genotyping for *Nestin-Cre* was carried out using the following primers: F: CCT TCC TGA AGC AGT AGA GCA R: GCC TTA TTG TGG AAG GAC TG. Genotyping for *Emx1-Cre* was carried out using the following primers: F:CCA TAT CAA CCG GTG GCG CAT C and R: TCG ATA AGC TTG GAT CCG GAG AG. Genotyping for *Ai14/R26*$^{LSL\text{-}tdTomato}$ was carried out using the following primers: WTF:AAG GGA GCT GCA GTG GAG TA, WTR:CCG AAA ATC TGT GGG AAG TC, MutF: CTG TTC CTG TAC GGC ATG G, and MutR: GGC ATT AAA GCA GCG TAT CC. Genotyping for *Itgb1*$^{fl/fl}$ mice was carried out using the primers: F: CGG CTC AAA GCA GAG TGT CAG TC and R: CCA CAA CTT TCC CAG TTA GCT CTC. *Nex-Cre* mice were kindly provided by Drs. Nave and Goebbels (Goebbels et al. 2006; S.-X. Wu et al. 2005). Genotyping for *Nex-Cre* was carried out using the following primers: Primer 4: GAG TCC TGG AAT CAG TCT TTT TC, Primer 5: AGA ATG TGG AGT AGG GTG AC,

Primer 6: CCG CAT AAC CAG TGA AAC AG. Tail biopsies were digested with Quanta tail extraction reagent kit (cat: 95091-025) and PCR reactions prepared using GoTaq Master Mix (Promega cat: PRM7123). All animal procedures were performed according to the University of California, Riverside's Institutional Animal Care and Use Committee (IACUC) guidelines. All procedures were approved by UC Riverside IACUC.

Immunohistochemistry: Embryos E14.5 and older, neonates, and adults were transcardially perfused with ice cold 1xPBS, dissected in cold 1xPBS, and brain tissue was post-fixed in 4% PFA in 1xPBS for 2hrs – overnight at 4°C. E12.5 embryos were directly dissected in ice cold 1xPBS with no perfusion. Tissue samples were processed immediately as floating sections or cryopreserved in a solution of 30% sucrose in 1xPBS before embedding in Tissue Plus O.C.T. Compound (Fisher HealthCare, cat:4585) for long-term storage. Tissue processed as floating sections were embedded in 3% agarose and sectioned coronally as 100-150μm sections on a vibratome (Lecia, cat: VT1000S). Floating sections were incubated with a permeabilization solution containing 3% bovine serum albumin (BSA), 0.3% TritonX-100 in 1xPBS for 4hrs-overnight at 4°C. Primary and secondary antibody mixes were diluted in a solution of 5% goat serum made with permeabilization solution. All antibody incubations were overnight at 4°C with gentle agitation. Cryoprotected samples were sectioned coronally at 25-50μm on a cryostat (Leica cat: CM3050). Cryosections were dried for 20min at room temperature before blocking in freshly prepared blocking buffer (10% goat serum and 0.1% TritonX-100 in 1xPBS) for 1hr prior to primary antibody addition. Antibodies were then prepared in

blocking buffer with reduced serum (5%) and incubated in humidified chamber overnight at 4°C. The antibodies and concentrations used in this study: chicken anti-GFP (1:500, Aves cat:GFP-1020), rabbit anti-p130Cas c-20 (1:250, SCBT sc-17), rabbit anti-Nedd9 (1:250, Sigma cat: HPA038768), rat anti-NCAM-L1 (1:500, Millipore cat: MAB5272), rabbit anti-dsRed (1:500, Takara Bioscience cat: 632496), rabbit anti-Tbr1 (1:500, Abcam cat: ab31940). Secondary antibodies were all purchased from ThermoFisher and diluted to 1:1000 from the stock concentration: goat anti-chicken 488 (cat: A-11039), goat anti-rabbit 546 (cat: A-11035), goat anti-rat 488 (cat: A-21208), goat anti-rat 546 (cat: A-11081). Nuclear conterstain was achieved by co-incubation with 1ug/ml DAPI (ThermoFisher cat: 50850585) in all secondary antibody incubations. Tissue was mounted with Fluorogel with DABCO (Electron Microscopy Sciences, cat:17985-02). Confocal scanning images were acquired on a Leica DMi8 using a 10x objective (NA=0.40) or 20x objective (NA=0.75) and z-stacks acquired with 20-40 steps of sizes of 2-4μm.

Axonal Tracing:

Lipophilic tracing of white matter tracts was performed by manually placing small crystals of either DiA (Invitrogen cat: D3883) or DiI (Invitrogen cat: D282) into neonate tissue dissected as described above for immunohistochemistry and postfixed in 4% PFA in 1xPBS overnight. Lipophilic crystals were allowed to diffuse for 8 weeks at 37°C in a solution of 4% PFA in 1xPBS, with the solution changed the daily the first four days and thereafter weekly. Tissue was embedded in 3% agarose in 1xPBS and sectioned as

150μm sections on a vibratome (Lecia, cat: VT1000S). Sections were imaged freshly sliced on a Zeiss Axio Imager 2 microscope. Injections of AAV8-CAG-tdTomato were obtained from the University of North Carolina viral core. The concentrated viral solution (0.2 μl), was delivered into the endopiriform cortex by stereotactic injection (0.25 μl per min), using the following coordinates from Bregma: anterior-posterior, +0.014 mm; lateral, -0.34 mm; and depth, -0.175 mm. Virus was allowed to express for two weeks before tissue collection for immunohistochemistry.

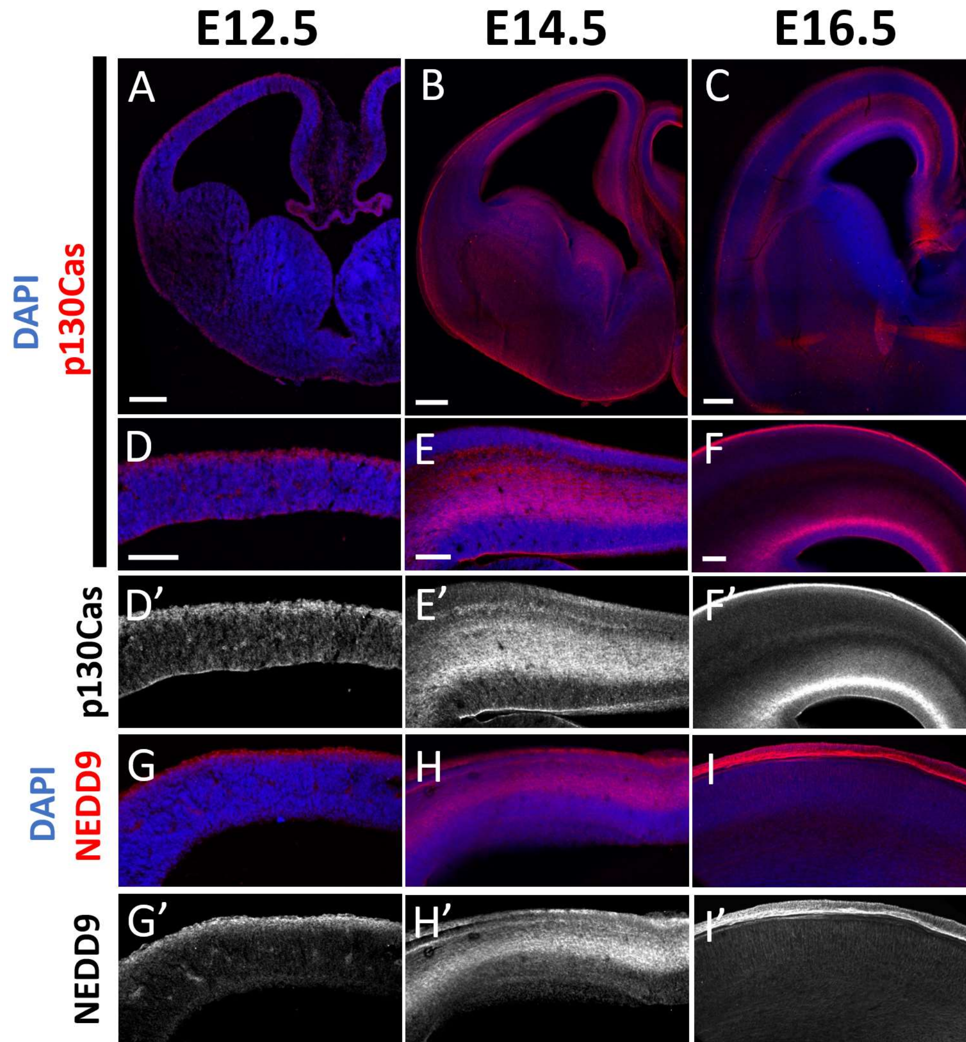


Figure 1.1: Cas family proteins are expressed in the cortical plate during lamination and axonal tract formation. (**A-F'**) Immunohistochemistry depicting the developmental expression pattern of p130Cas protein during cortical development. At E12.5, p130Cas expression is limited to the marginal and ventricular zones (**A, D, D'**). By E14.5, expression is expanded throughout the ventricular, subventricular, and intermediate zones and marginal zone (**B, E, E'**). By E16.5, persistent expression remains in the ventricular, subventricular, marginal zones and white matter (**C, F, F'**). Similar developmental expression is seen for the p130Cas homologue NEDD9 (*CasL* paralogue) (**G-I'**), with particularly strong expression in the white matter and pial surface (**H-I'**). Scale bar = 250µm for all. n=3 animals examined for each developmental timepoint.

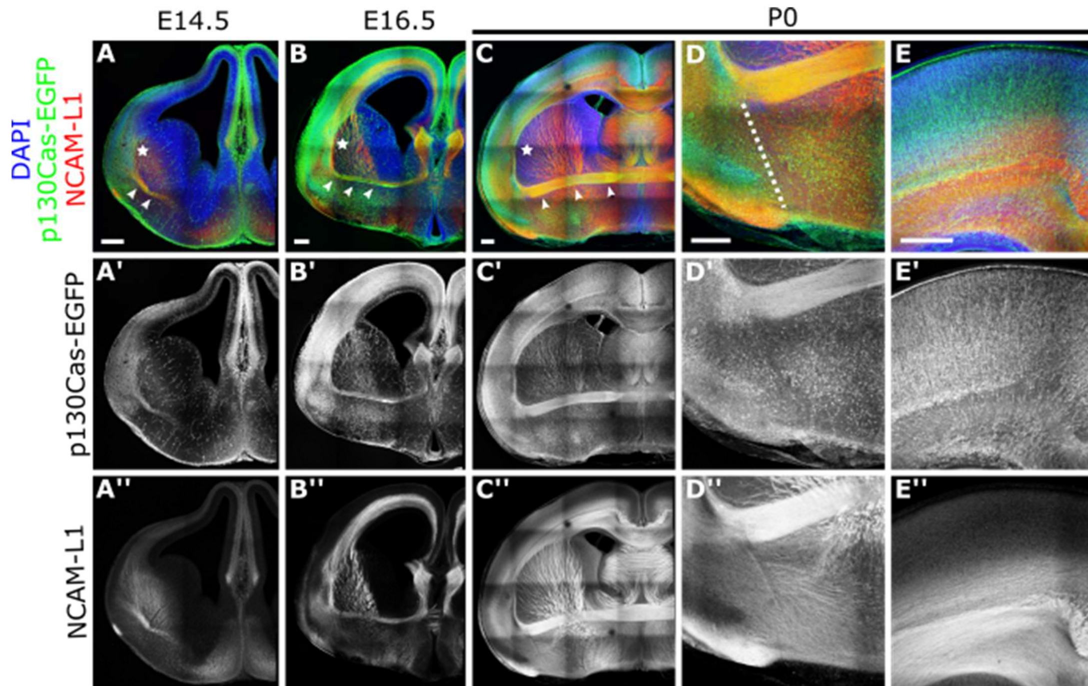


Figure 1.2: *p130Cas* is expressed in the cortical white matter. (**A-E**) Analysis of EGFP (green) expression in *p130Cas-EGFP-BAC* transgenic embryos (columns **A-A''** and **B-B''**) and neonates (column **C-C''**), co-immunolabeled with the axonal marker NCAM-L1 (red) reveals strong *p130Cas* promoter activity during the formation of cortical white matter tracts. Robust EGFP expression is observed in the External Capsule (EC, star in row **A-C**) and Anterior Commissure (AC, arrowheads in row **A-C**). High resolution images of neonate EGFP (row **D'-E'**) and NCAM-L1 (row **D''-E''**) expression is shown for the pallio-subpallial boundary (dotted line, column **D-D''**) and the cortical plate (column **E-E''**). Scale bar = 250μm for all. n=3 animals examined for each developmental timepoint.

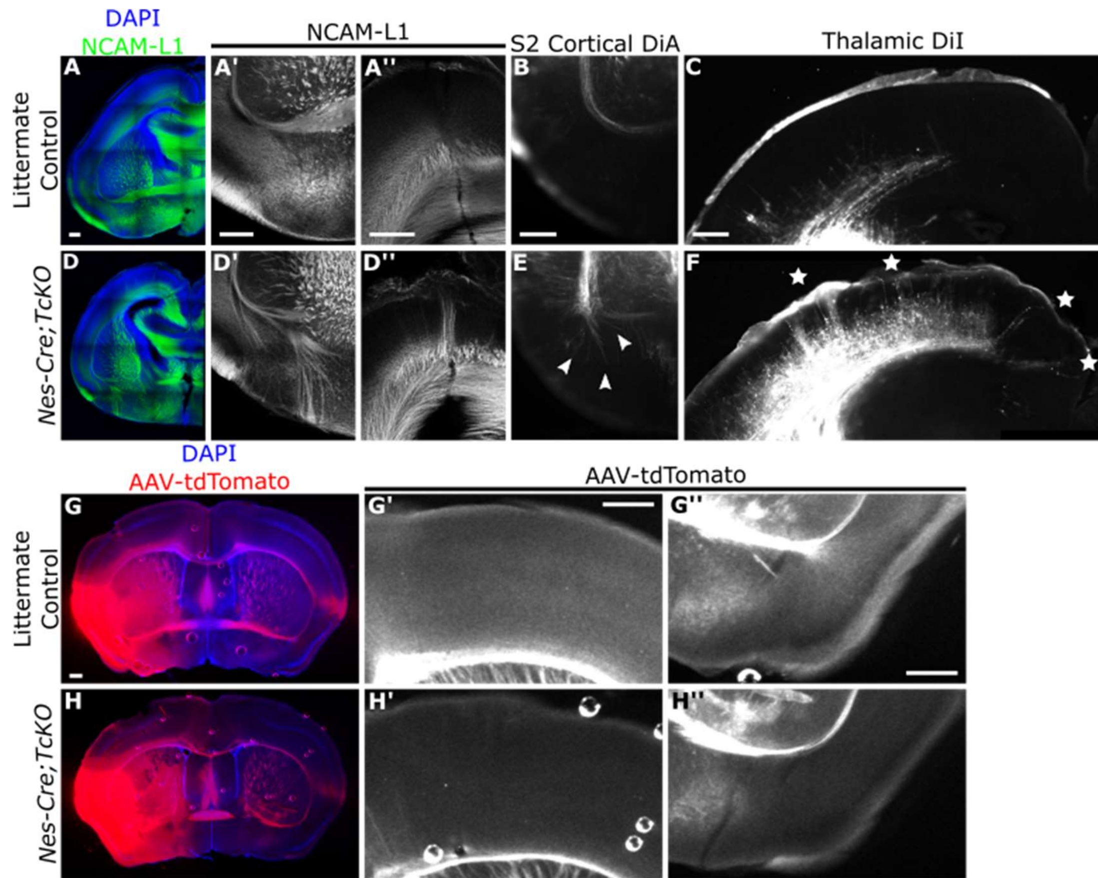


Figure 1.3: ***Cas TcKO*** **mutants display errors in cortical and thalamocortical connections.** (**A-D''**) Immunohistological analysis of major cortical tracts using the axonal marker NCAM-L1 in *Nes-Cre;TcKO* mutant neonates (P0) reveals two major fasciculation and projection phenotypes: 1) efferents originating in the cortex traveling along the pAC tracts fail to fasciculate properly and erroneously project into the subpallium (**A' vs D'**) and 2) thalamocortical connections meant to innervate layer IV overproject to the subpial boundary, forming large fasciculated Cortical Bundles (**A'' vs D''**). We find each of these phenotypes to be fully penetrant in *Nes-Cre;TcKO* mutants (n=8 animals of each genotype assessed). (**B-C, E-F, G-H''**) The origin of mis-projecting axons is confirmed via anterograde lipophilic and adenoviral tracing experiments. DiA crystals placed into the S2 cortex label ventrally defasciculating AC axons (**B vs E**, arrowheads) and DiI crystals placed in the lateraldorsal thalamus label Cortical Bundles (**C vs F**, white stars) (n=7 pairs of mutants and littermate controls examined for each lipophilic tracing experiment). Stereotactic injection of an AAV-tdTomato reporter into adult (P21) Endopiriform cortex induces robust labeling of commissural tracts (**G-H''**). We observe no labeling of CBs (**G' and H'**) or defasciculating AC axons (**G''-H''**) in the contralateral hemisphere, n=4 pairs of mutants and littermate controls examined. Scale bar = 250µm for all.

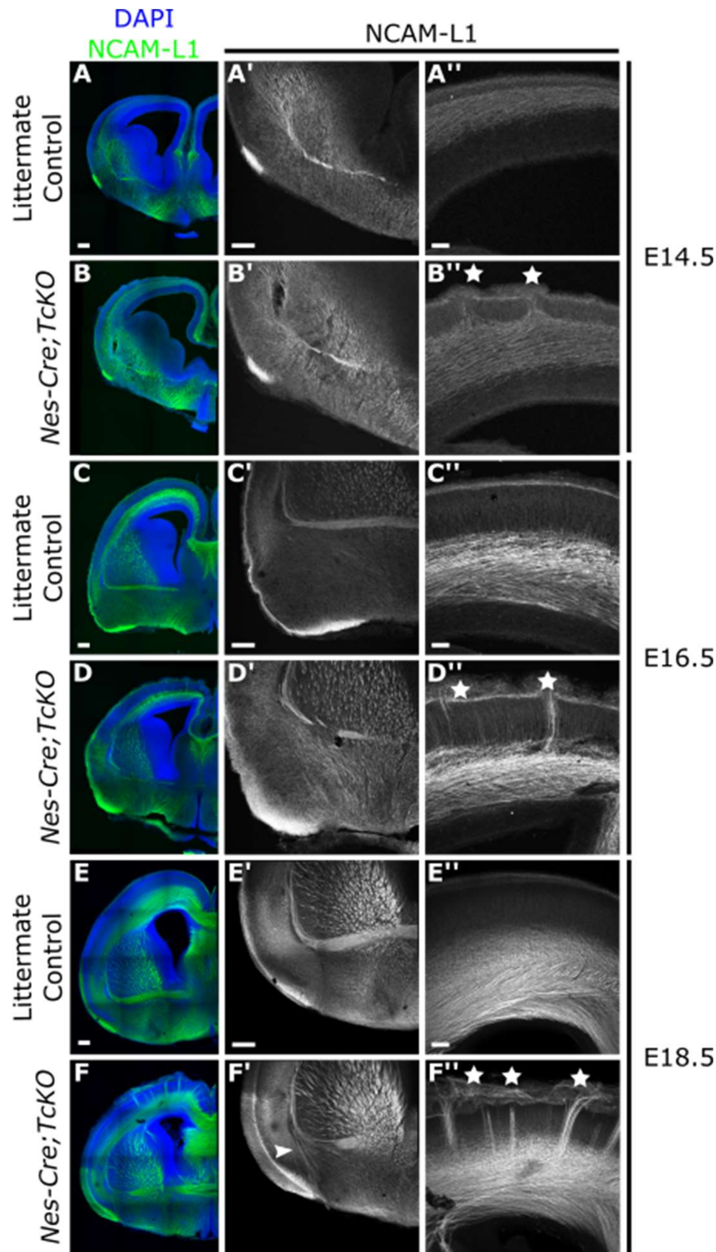


Figure 1.4: Developmental progression of white matter defects in *Nes-Cre;TcKO* mutants. Immunohistochemistry for the axonal marker NCAM-L1 in *Nes-Cre;TcKO* embryos (E14.4, **A-B''**, n=6 pairs of mutants and littermate controls examined; E16.5, **C-D''**, n=10 pairs; and E18.5, **E-F''**, n=3 pairs) reveals different developmental timelines for each fasciculation phenotype. The EC and AC tracts begin developing at E14.5 (**A',B'**) but *Nes-Cre;TcKO* mutants display no AC fasciculation or projection errors until E18.5 (**E' vs F'**, white arrowhead). Cortical bundles are apparent at E14.5 in *Nes-Cre;Tcko* mutants (**A''vs B''**, white stars) and worsen in severity as development continues (**D'' and F''**, white stars). Scale bar = 250µm for all.

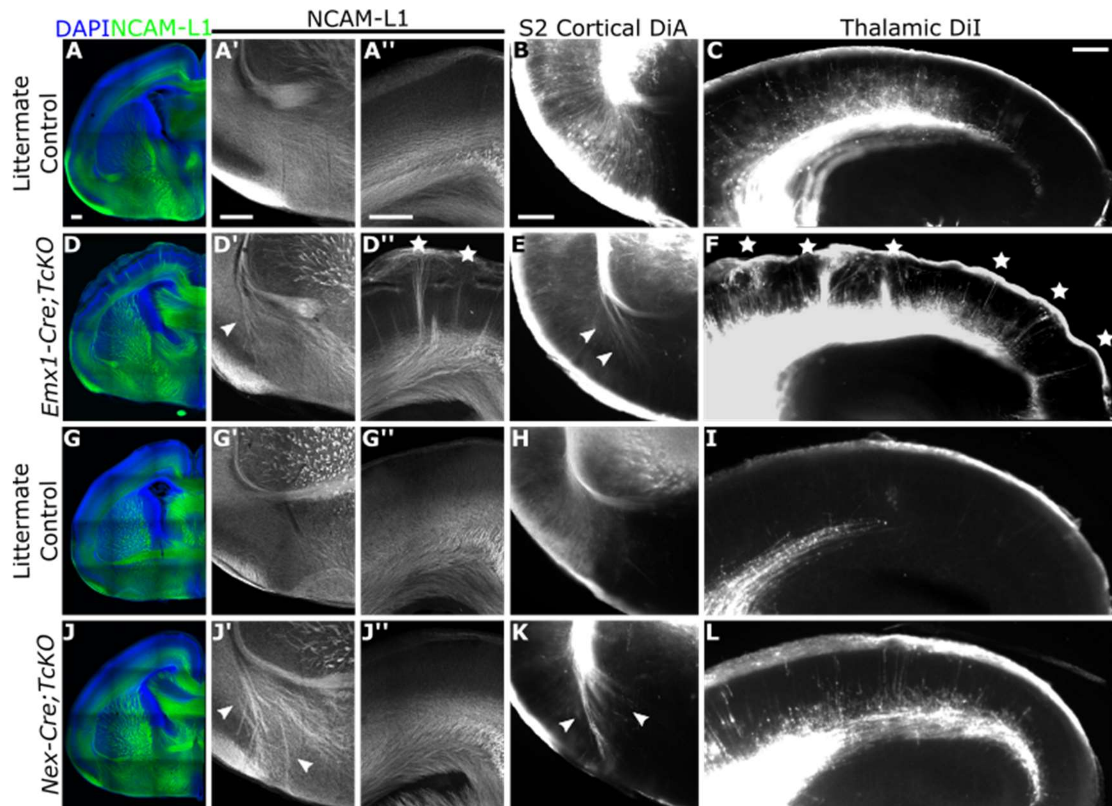


Figure 1.5: Neuronal-autonomous and non-neuronal autonomous roles for *Cas* genes in white matter tract fasciculation. Immunohistochemistry for the axonal marker NCAM-L1 in *Emx1-Cre;TcKO* and *Nex-Cre;TcKO* neonates reveals both classes of conditional knockouts present the AC defasciculation phenotype (**A' vs D'** and **G' vs J'**, white arrowheads), indicating this phenotype is neuronal-autonomous. Again, we find this phenotype fully penetrant (n=3 *Emx1-Cre;TcKO* mutants examined, n=4 *Nex-Cre;TcKO* mutants examined). Like *Nes-Cre;TcKO* mutants (**Fig 1.3**), these defasciculating axons are cortical in origin as revealed by anterograde DiA tracing from crystal placement in the S2 cortex (**B vs E** and **H vs K**, white arrowheads, n=5 *Emx1-Cre;TcKO* mutants examined, n=5 *Nex-Cre;TcKO* mutants examined). In contrast, only *Emx1-Cre;TcKO* mutants display the Cortical Bundle phenotype (**A'' vs D''**, white stars). These Cortical Bundles are again confirmed to be TCA projections by anterograde labeling from DiI crystals placed in the lateraldorsal thalamus (**F**, white stars). The absence of the Cortical Bundle phenotype in *Nex-Cre;TcKO* mutants (**G'' vs J''** and **I vs L**) indicates the role of Cas genes during TCA fasciculation and projection is non-neuronal autonomous. Scale bar = 250μm for all.

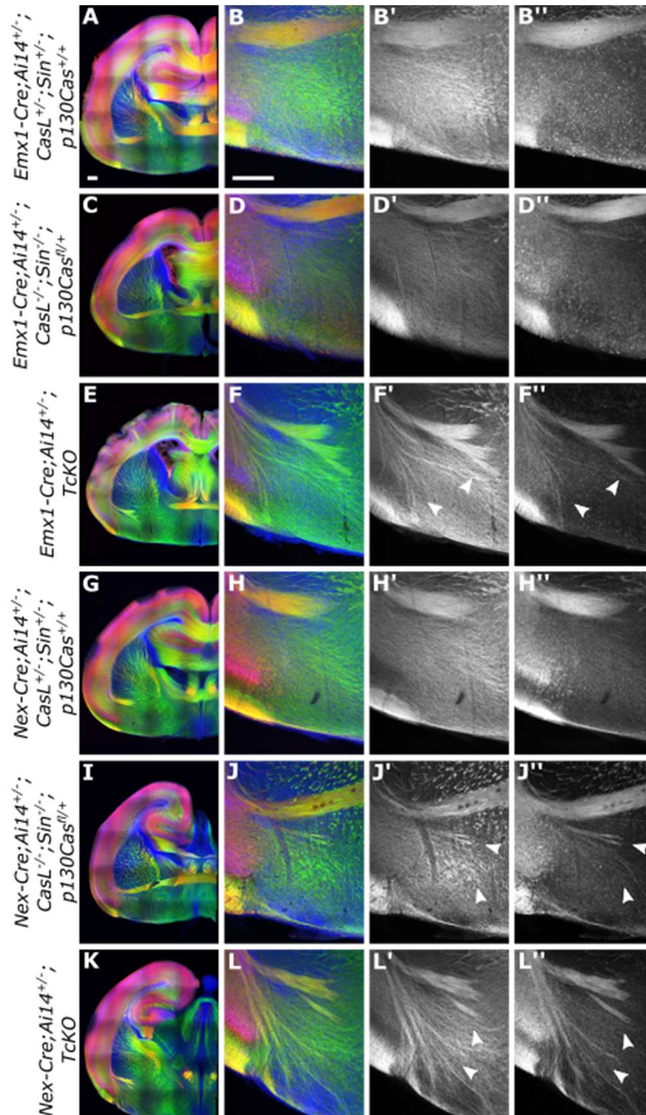


Figure 1.6: Genetic labeling confirms cortical origins of AC defasciculating axons in *Cas TcKO* mutants. Immunohistochemistry for NCAM-L1 and the tdTomato fluorescent cre-dependent reporter reveals extensive co-labeling in defasciculating AC axons in both *Emx1-Cre;Ai14$^{+/-}$;TcKO* (**E-F''**) and *Nex-Cre;Ai14$^{+/-}$;TcKO* (**K-L''**) mutant neonates. Interestingly, in both classes of conditional mutants, tdTomato^{+} defasciculating axons appear to interpolate with diffuse NCAM-L1^{+} processes that occupy the subpallium (**F' vs F''**, **J' vs J''**, and **L' vs L''**, white arrowheads). Further, a dose-dependent effect of *Cas* gene function is observed in *Nex-Cre* crosses, but not *Emx1-Cre* crosses. An intermediate AC defasciculation phenotype may be observed in *Nex-Cre;Ai14$^{+/-}$;CasL$^{-/-}$;Sin$^{-/-}$;p130Cas$^{fl/+}$* neonates (**J' and J''**, white arrowheads). For reader comparison, NCAM-L1 and tdTomato reporter signal are shown for *Emx1-Cre;Ai14$^{+/-}$;CasL$^{+/-}$;Sin$^{+/-}$;p130Cas$^{+/+}$* (**A-B''**) and *Nex-Cre;Ai14$^{+/-}$;CasL$^{+/-}$;Sin$^{+/-}$;p130Cas$^{+/+}$* (**G-H''**), where no tdTomato^{+} processes may be seen invading the subpallium. Scale bar = 250µm for all. n=2 animals of each above genotype examined.

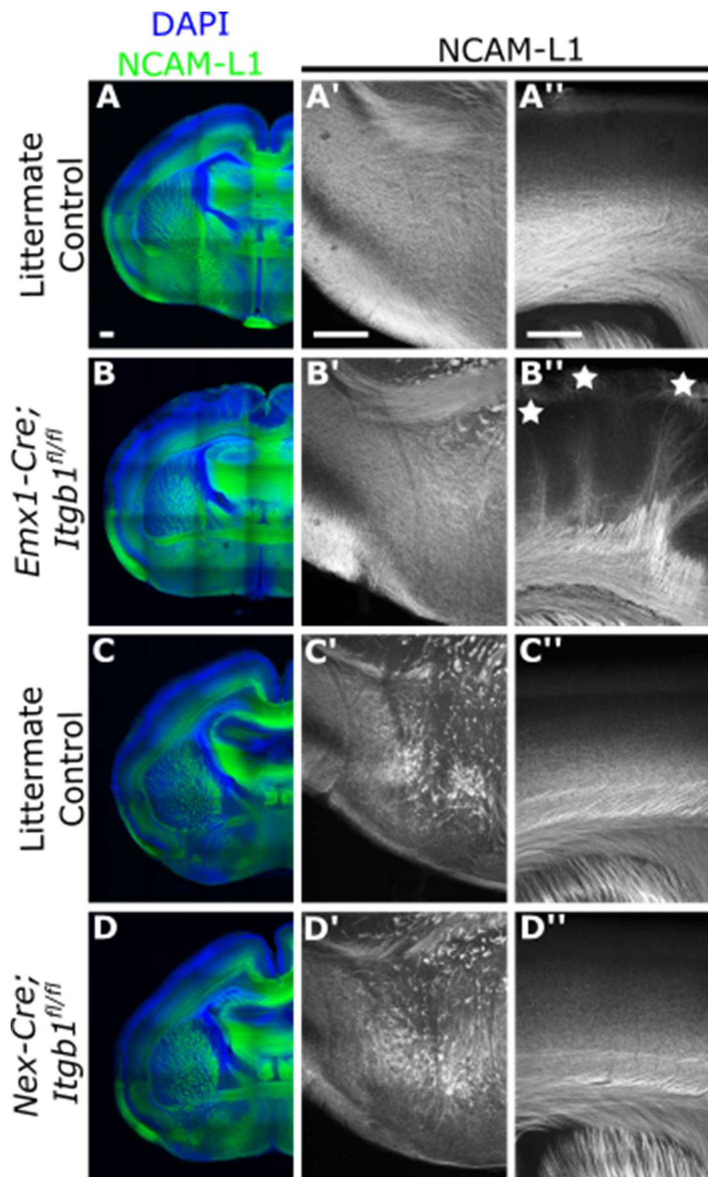


Figure 1.7: *Itgb1* mutants phenocopy non-neuronal autonomous white matter tract defects observed in *Cas TcKO* mutants. Immunohistological analysis of NCAM-L1 in *Emx1-Cre;Itgb1*$^{fl/fl}$ and *Nex-Cre;Itgb1*$^{fl/fl}$ neonates reveals that neither class of conditional mutant phenocopies the AC defasciculation defects observed in *Cas TcKO* mutants (**A' vs B'** and **C' vs D'**). Only *Emx1-Cre;Itgb1*$^{fl/fl}$ mutants phenocopy the Cortical Bundle phenotype (**A'' vs B''**, white stars). Scale bar = 250µm for all. n=4 *Emx1-Cre;Itgb1*$^{fl/fl}$ and n=4 *Nex-Cre;Itgb1*$^{fl/fl}$ mutants examined.

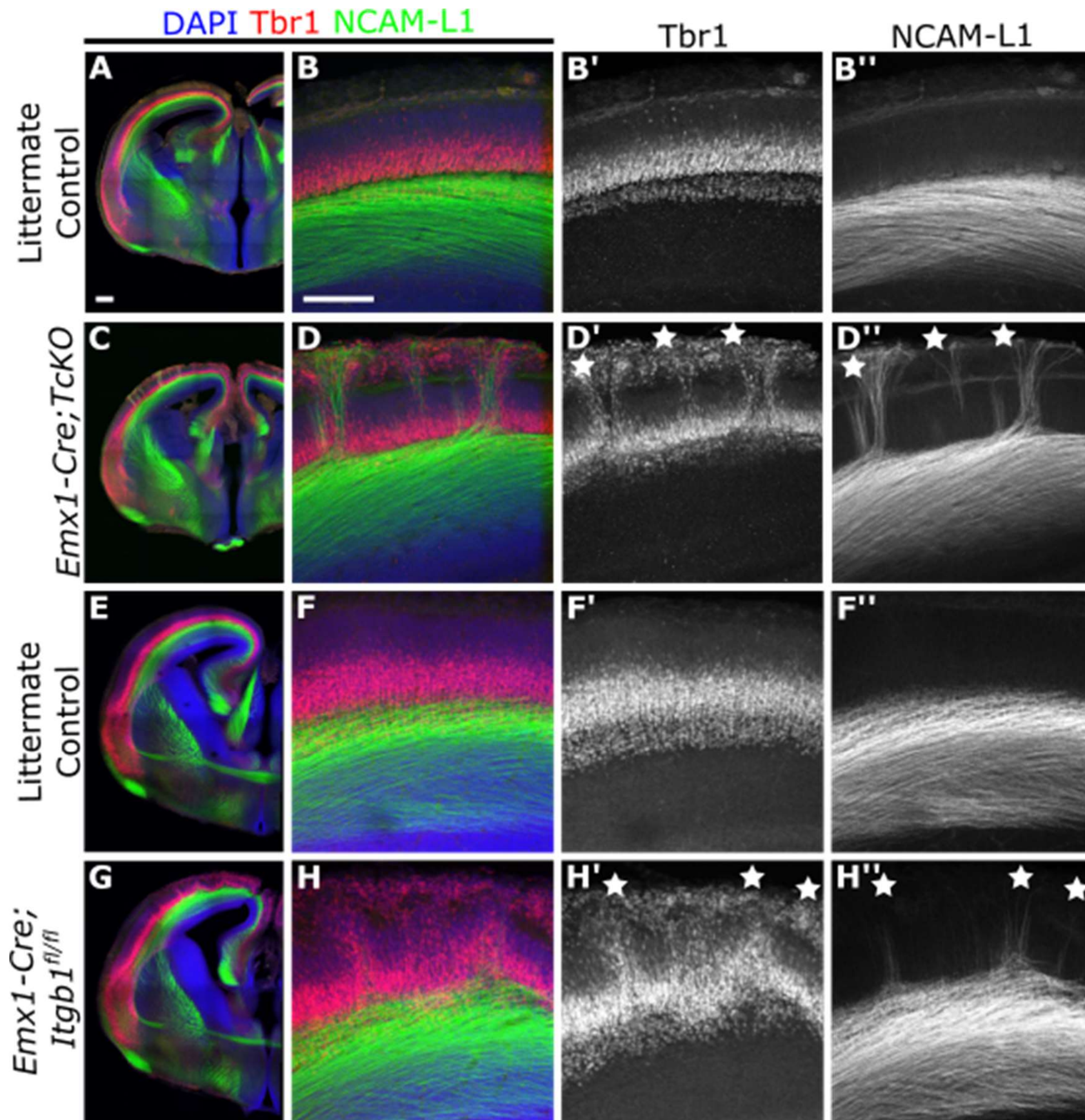


Figure 1.8: Non-neuronal autonomous phenotypes in *Itgb1* and *Cas TcKO* mutants correlate with displaced subplate cells. Immunohistological analysis of Tbr1 and NCAM-L1 in embryonic (E16.5) *Emx1-Cre;TcKO* and *Emx1-Cre;Itgb1$^{fl/fl}$* mutants reveals severe cortical dysplasia in regions where Cortical Bundles form. In both classes of conditional mutants, Tbr1^{+} subplate cells are seen to be displaced to more superficial layers (**B' vs D'** and **F' vs H'**, white stars). Cortical Bundles correlate very closely with these superficially displaced subplate cells (**B'' vs D''** and **F'' vs H''**, white stars). Scale bar = 250μm for all. n=3 *Emx1-Cre;TcKO* and *Emx1-Cre;Itgb1$^{fl/fl}$* mutants examined.

Chapter 2

A bimolecular complementation tool for monitoring adhesion signaling

Abstract

Integrin Adhesion Complexes (IACs) serve as links between the cytoskeleton and extracellular environment, acting as mechanosensing and signaling hubs. As such, IACs participate in many aspects of cellular motility, tissue morphogenesis, anchorage-dependent growth and cell survival. Focal Adhesion Kinase (FAK) has emerged as a critical organizer of IAC signaling events due to its early recruitment and diverse substrates, and thus has become a key genetic and therapeutic target. Here we present the design and characterization of simple, reversible, and scalable Bimolecular Complementation sensors to monitor FAK phosphorylation in living cells. These probes provide novel means to quantify IAC signaling, expanding on the currently available toolkit for interrogating FAK phosphorylation during diverse cellular processes.

Introduction

Cell-Matrix adhesions are critical regulators of cellular motility and structure, tissue morphogenesis, anchorage-dependent growth, and cell survival (Critchley 2000; B. Geiger 1989; Wehrle-Haller 2012). Integrin Adhesion Complexes (IACs, or simply 'adhesion complexes') are the best studied examples of cellular-extracellular adhesions, serving to physically link the extracellular matrix (ECM) to the actin cytoskeletal system (K. Burridge et al. 1988; B. Geiger 1989; Jockusch et al. 1995; Miyamoto et al. 1995; Winograd-Katz et al. 2014). Highly dynamic structures, IACs encompass at least four types of adhesive complexes, each with distinct molecular compositions and signaling capacity. These include focal complexes (FXs, also referred to as nascent adhesions), focal adhesions (FAs), fibrillar adhesions (FBs), and cellular podosomes (Benjamin Geiger and Yamada 2011; Nobes and Hall 1995; Vicente-Manzanares and Horwitz 2011). To date, proteomic approaches have identified over 200 IAC components, termed the 'integrin adhesome' (Byron et al. 2011; Winograd-Katz et al. 2014; Zaidel-Bar and Geiger 2010; Zaidel-Bar et al. 2007), although a consensus set of 60-147 proteins organized into four or five signaling modules has been recently proposed by different groups (Horton, Astudillo, et al. 2016; Horton et al. 2015; Chastney et al. 2020). Additionally, adhesive complexes continually exchange components with the cytosol, allowing them to rapidly remodel in response to internal or external stimuli (Hoffmann et al. 2014; Lele et al. 2008; Wolfenson et al. 2009). Given this molecular diversity and dynamism, IACs are able to act as signaling hubs, permitting mechanosensation of traction forces (Benjamin Geiger and Bershadsky 2002; Miller, Hu, and Barker 2020;

Riveline et al. 2001) and relaying anchorage-dependent growth and survival signals to the cell (Keith Burridge and Chrzanowska-Wodnicka 1996; Benjamin Geiger et al. 2001; Mitra, Hanson, and Schlaepfer 2005; Playford and Schaller 2004); while their regulated establishment and turnover are essential for processive cell migration, cellular podosome formation, and epithelial-to-mesenchymal transitions (J. T. Parsons et al. 2000; J. Thomas Parsons, Horwitz, and Schwartz 2010; Webb, Parsons, and Horwitz 2002).

Due to their functions linking the extracellular environment to the cytoskeleton, IACs and the proteins that form them have been shown to be involved in many aspects of development and disease. Many of these structural and signaling functions of IACs are mediated through Focal Adhesion Kinase (FAK), a nonreceptor tyrosine kinase that is recruited to the inner plasma membrane upon integrin receptor clustering and ECM engagement (J. L. Guan, Trevithick, and Hynes 1991; Hanks et al. 1992; L. Kornberg et al. 1992; L. J. Kornberg et al. 1991; Lipfert et al. 1992; J. Thomas Parsons 2003; Schaller et al. 1992a) Increased FAK expression and kinase activity have been associated with cancer survival, the formation of invadopodia, and tumor progression (Agochiya et al. 1999; Alexander et al. 2008; Chan, Cortesio, and Huttenlocher 2009; Feng et al. 2019; Hauck et al. 2002; Kim et al. 2019; B. Y. Lee et al. 2015; Provenzano and Keely 2009; Sulzmaier, Jean, and Schlaepfer 2014). Conversely, FAK deficiency in mice results in embryonic lethality (Ilić et al. 1995), with cellular defects in FA turnover, cell migration, and growth factor response (B. H. Chen et al. 2002; Ren et al. 2000; Sieg, Hauck, and Schlaepfer 1999; Webb et al. 2004). Phospho-regulation of FAK has emerged as a central theme in coordinating FAK activity, and extensive studies have elucidated the

biochemical regulation of FAK during IAC assembly and turnover. A key phosphorylated FAK residue is Tyr-397, which is trans-autophosphorylated upon integrin receptor stimulation and FAK oligimerization (Acebrón et al. 2020; Brami-Cherrier et al. 2014; Brod and Fässler 2020; J. L. Guan and Shalloway 1992; Hanks et al. 1992; L. Kornberg et al. 1992; Lipfert et al. 1992; Schaller et al. 1994), allowing subsequent associations with Src-Homology 2 (SH2) domain containing partners (Calalb, Polte, and Hanks 1995a; Cobb et al. 1994; Eide, Turck, and Escobedo 1995; Schaller et al. 1994; Xing et al. 1994). FAK recruitment and autophosphorylation of Tyr-397 are some of the earliest observable steps in FA assembly (L. Kornberg et al. 1992; Miyamoto et al. 1995; Webb, Brown, and Horwitz 2003; Zaidel-Bar et al. 2003) and overexpression of mutant Y397F-FAK results in longer FA occupancy times and inhibited FA turnover in migrating cells (Hamadi et al. 2005; Webb et al. 2004). Following Tyr-397 phosphorylation, full activation of FAK is achieved by Src-mediated phosphorylation of Tyr-576 and Tyr-577 residues within the kinase domain activation loop (Calalb, Polte, and Hanks 1995a; Owen et al. 1999; Ruest et al. 2000). These modifications destabilize inhibitory interactions between the N-terminal FERM domain and the C-terminal catalytic domain, resulting in a more open conformation and full FAK kinase activity (Xinming et al. 2008; Dunty et al. 2004; Dunty and Schaller 2002; Lietha et al. 2007). Previous studies have demonstrated that mutating these three tyrosine residues to phenylalanine (Y397-576-577F) reduces FAK in vitro kinase activity to 20% of wildtype levels (Calalb, Polte, and Hanks 1995a). Collectively, these studies demonstrate that phospho-regulation of these

tyrosine residues is essential in regulating FAK kinase activity, making these post-translational modifications attractive targets for monitoring IAC signaling events.

While many methodologies have been developed to monitor FAK conformational changes and activity (Xinming et al. 2008; Damayanti et al. 2017; De Virgilio, Kiosses, and Shattil 2004; Giannone et al. 2002; Hamadi et al. 2005; Horton, Humphries, et al. 2016; Kirchner et al. 2003; Nollau and Mayer 2001; Papusheva et al. 2009; Ritt, Guan, and Sivaramakrishnan 2013; Seong et al. 2011; Yiqian Wu et al. 2016), we sought to develop simple and reliable sensors that would detect FAK tyrosine phosphorylation. Bi-molecular Complementation Assays (also called Split-Reporter Assays) have emerged as powerfully adaptable genetically-encodable tools that allow for live-cell monitoring of diverse molecular, cellular, and physiological processes (Kerppola 2008; I Remy, Galarneau, and Michnick 2002; Rossi, Charlton, and Blau 1997). Conceptually, these systems comprise of a reporter – often a fluorescent molecule (Hu and Kerppola 2003), luminescent (Luker et al. 2004) or colorimetric enzyme (Rossi, Charlton, and Blau 1997) – that is rendered undetectable by genetic dissection into complementary fragments, which are separately tethered to putative interacting proteins. Associations between interaction targets results in spontaneous reassembly of the reporter molecule and generation of a detectable signal. Split luciferase systems (also termed Luciferase Fragment Complementation Assays, or LFCAs) in particular, enjoy widespread use in both clinical and preclinical settings due to their unrivaled sensitivity, rapid assembly and disassembly kinetics, amenability to *in vivo* and *in vitro* systems, and low costs of implementation (Azad, Tashakor, and Hosseinkhani 2014; Lake and Aboagye 2014).

Here, we present the design and characterization of novel luciferase-based Bi-molecular Complementation probes to quantify FAK tyrosine phosphorylation during IAC signaling events in living cells. We find these tools faithfully report IAC signaling in a manner that is sensitive and dynamic along physiologically relevant timescales. These tools have the potential to be scalable for screening purposes, and as such will be a valuable addition to the expanding toolkit to monitor FAK activity and IAC dynamics.

Results

To monitor FAK tyrosine phosphorylation (pYFAK) events, we designed a bimolecular luciferase (BiLuc) system that pairs a phospho-tyrosine sensor with rapid luciferase assembly and disassembly kinetics to produce a sensitive, reversible reporter of FAK activation (**Figure 2.1 A**). In this system, hereafter referred to as pYFAK BiLuc, complementary C-terminal (cLuc, aa401-550) and N-terminal (nLuc, aa1-415) fragments of firefly luciferase enzyme (Luker et al. 2004) are tethered by flexible RSIAT (Arg-Ser-Ala-Ile-Thr) linker sequences to c-myc tagged FAK and to a phospho-tyrosine sensor, respectively. This phospho-tyrosine sensor consists of a double repeat of Src Homology 2 motifs (double SH2, or dSH2) derived from pp60(c-Src), which has been previously shown to display high affinity for tyrosine-phosphorylated targets in IACs, including FAK, Paxillin, and p130Cas (Ballestrem et al. 2006; Iyer et al. 2005; Kirchner et al. 2003). Tyrosine phosphorylation of FAK is detected by direct binding of the dSH2 sensor, thereby allowing the N- and C-terminal fragments of luciferase to reassemble into a catalytically active enzyme.

To determine the proper configurations of split-luciferase constructs, we designed two alternate pYFAK split-luciferase construct pairs by tethering either N- or C-terminal fragments of firefly luciferase enzyme to the C-terminal of a myc-taged FAK coding sequence (CNFAK and CCFAK, respectively) and complementary luciferase fragments to the N-terminal of the dSH2 phospho-tyrosine sensor (NCLucdSH2 and NNLucdSH2, respectively) (**Figure 2.1 B**). We determined only one pair of constructs (CCFAK+NNdSH2) was able to reconstitute firefly luciferase enzymatic activity (**Figure 2.1 C**), and subsequent experiments were carried out using this set. Importantly, the split luciferase fragments alone display no detectable enzymatic activity without the interaction of the FAK-dSH2 fusion products, as neither individually transfected constructs (CNFAK, CCFAK, NNdSH2, NCdSH2) nor co-transfected but untethered luciferase fragments (NNVector-NCVector) were able to produce luminescent activity in transfected HEK293 cells (**Figure 2.1 C**).

We next sought to determine if the pYFAK BiLuc constructs were incorporated into IACs *in vivo*. We transfected the pYFAK BiLuc constructs into COS-7 cells grown on laminin-coated coverslips and performed immunocytochemistry for the N-terminal myc tag in the CCFAK probe (**Figure 2.2**, green). In all cases, we observe the myc tag robustly labels small, peripheral Focal Contacts (FXs), Focal Adhesions (FAs) and larger, elongated fibrillar bodies (FBs) (**Figure 2.2 A', B', C'**) (Benjamin Geiger & Yamada, 2011; Nobes & Hall, 1995). The identity of these structures is confirmed by co-labeling with p397-FAK (**Figure 2.2 A-A''**) and pY118-Paxillin (**Figure 2.2 B-B''**). Interestingly, pY165-p130Cas co-labeled some FXs and FAs, but not all, and is absent

from larger FBs (**Figure 2.2 C-C''**), consistent with previous descriptions of this scaffolding protein being present in nascent FXs and FAs, but not more mature FBs (Vicente-Manzanares & Horwitz, 2011). To quantify the degree of pYFAK BiLuc incorporation into IACs, we performed whole-cell colocalization analysis between the myc-tagged CCFAK probe and the forementioned phosphospecies. Colocalization analysis revealed strong correlation between myc and pY397-FAK (**Figure 2.2 A-A''**, **Table 1**) (Pearson's R=0.77±0.02 and Spearman's rank correlation ρ=0.83±0.01). Similarly, strong correlation is observed between myc and pY118-Paxillin (**Figure 2.2 B-B''**, **Table 1**) (Pearson's R=0.73±0.10 and Spearman's rank correlation ρ=0.77±0.09). A weaker correlation was observed between myc and pY165-P130Cas (Figure **2.2 C-C''**, **Table 1**) (Pearson's R=0.40±0.02 and Spearman's rank correlation ρ=0.57±0.02), possibly reflecting the more restricted localization of this phosphoprotein. Collectively, these results confirm efficient recruitment of pYFAK BiLuc probes to various actively signaling IAC structures.

Next, we sought to evaluate if the pYFAK BiLuc system was functionally responsive to endogenous integrin signaling events. Extensive studies have established the Src family of kinases as the primary kinases responsible for phosphorylating FAK Y576-577 residues during FA signaling events *in vivo*, leading to full activation of FAK kinase activity (Calalb, Polte, and Hanks 1995a; Hanks et al. 1992; Ruest et al. 2000). Therefore, it was expected that luciferase activity from the pYFAK BiLuc system would be proportionally responsive to the degree of Src kinase activity in the cell. Indeed, we found that pharmacological treatment with the Src-family kinase inhibitor PP2 resulted in

progressively diminished luciferase reporter activity in transfected COS-7 cells, with a maximal decrease of 55.6% observed after 15min incubation (**Figure 2.3**; ANOVA $p<0.01$; Tukey Post-hoc test $p<0.05$ 5-minutes vs control; $p<0.01$ 15-minutes vs control; n=4 biological replicates). Similar levels of inhibition were observed after only 1min incubation with the Src-family inhibitor SU6656 (**Figure 2.3**; $p<0.05$ 1min vs control; $p<0.01$ 5mins vs control; $p<0.05$ 15mins vs control). Importantly, Src family kinases may be activated by several endogenous pathways and therefore result in FAK phosphorylation independent of integrin receptor activity. Therefore, to address if the pYFAK BiFC system was also responsive to integrin receptor activity, cells were treated with an integrin-β1 function blocking antibody (Ha2/5) (Mendrick and Kelly 1993) and luciferase signal was measured. Treated cells displayed a robust 61.8% reduction in normalized luciferase signal 15mins following Ha2/5 addition to the culture medium (**Figure 2.3**; $p<0.01$ vs control), confirming that the pYFAK BiLuc system is sensitive to both Src family activity and endogenous integrin receptor activation. These results also demonstrate the reversibility of the luciferase signal, along the order of minutes, in response to pharmacological treatment, which mirrors IAC assembly and turnover rates observed in live imaging studies (Kirchner et al. 2003; Zaidel-Bar et al. 2003; Eli Zamir et al. 2000). These results suggest the pYFAK BiLuc system could indeed be applicable for quantifying changes in steady-state levels of adhesion signaling in response to other pharmacological perturbations, or in the design of genetic screens.

Finally, we sought to test the specificity of the pYFAK BiLuc system towards FAK tyrosine phosphorylation events. Given that the dSH2 phospho-tyrosine sensor used

in this design has previously been shown to display affinity to several IAC phospho-tyrosine species, including the other core scaffolding proteins Paxillin and p130Cas (Ballestrem et al. 2006; Iyer et al. 2005; Kirchner et al. 2003; Zaidel-Bar et al. 2003), we wanted to differentiate if our tool was specifically reporting FAK tyrosine phosphorylation, or phosphotyrosine accumulation within IACs more broadly. To this end, we designed an alternate CCFAK construct that contains tyrosine-to-phenylalanine point mutations at the three key tyrosine residues regulating FAK kinase activity: Y397, Y576 and Y577 (CCFAK-3YF) (**Figure 2.4 A**) (Calalb, Polte, and Hanks 1995a; Owen et al. 1999). We observed an almost complete attenuation of luciferase activity in CCFAK-3YF + NNdSH2 transfected HEK293 cells, at levels comparable to controls (**Figure 2.4 B**). We confirmed robust expression of the CCFAK-3YF probe by Western Blot for the N-terminal myc tag (**Figure 2.4 C**), indicating that the marked reduction in luciferase signal observed was indeed the result of abolishing pYFAK-dSH2 interactions and not variable construct expression. We next sought to determine whether CCFAK-3YF was recruited to IACs, as abolishment of the scaffolding functions of CCFAK-3YF could potentially explain the reduction in luciferase signal. We again performed immunocytochemistry for either myc (green) or for the core IAC phosphoproteins Y397-FAK, Y118-Paxillin, and Y165-p130Cas (red) (**Figure 2.5**). Similar to the unmodified CCFAK probe, we observe the myc tag of the CCFAK-3YF probe robustly labels FXs, FAs, and larger, elongated FBs (**Figure 2.5 B'**, **D'**, **F'**). Whole cell colocalization analysis showed strong correlation between myc and pY397-FAK (**Figure 2.5 B-B''**, **Table 1**) (Pearson's R=0.79±0.03 and Spearman's rank correlation ρ=0.85±0.03) as well

as between myc and pY118-Paxillin (**Figure 2.5 D-D''**, **Table 1**) (R=0.65±0.18 and Spearman's rank correlation ρ=0.72±0.12). Again, a weaker correlation was observed between myc and pY165-P130Cas (**Figure 2.5 F-F''**, **Table 1**) (Pearson's R=0.43±0.07 and Spearman's rank correlation ρ=0.57±0.06). These correlations were not found to be significantly different from those measured for the CCFAK construct (**Figure 2.2**, **Table 1**) suggesting that the three-point mutation introduced did not affect recruitment of the CCFAK-3YF construct to integrin complexes (Mann-Whitney comparing Spearman's rank correlations; pY397-FAK CCFAK median=0.8353114, 3YF median=0.85287551, U=1158, p=0.1127; pY118-Paxillin CCFAK median=0.77363353, 3YF median=0.73749172, U=676.5, p=0.1768; pY165-P130Cas CCFAK median=0.57599749, 3YF median=0.56036582, U=702, p=0.6949). Collectively, these data confirm that the pYFAK BiLuc system is specific and sensitive to the relative levels of FAK tyrosine phosphorylation and pYFAK BiLuc luciferase signal does not reflect the tyrosine phosphorylation status of other core IAC scaffolding proteins.

Discussion

In this study we made use of the bimolecular complementation approach to design a sensitive, specific reporter of FAK tyrosine phosphorylation, a posttranslational modification that has emerged as a critical regulator of IAC signaling (Keith Burridge, Turner, and Romer 1992; Eide, Turck, and Escobedo 1995; L. Kornberg et al. 1992). Based on the system's reliance on bimolecular complementation of firefly luciferase (BiLuc) and demonstrated specificity toward tyrosine phosphorylated FAK (pYFAK)

(**Figure 2.4 B**), we term this tool the pYFAK BiLuc system. To our knowledge, this is the first luciferase-based reporter system developed to monitor FAK activation during IAC signaling events.

pYFAK BiLuc Design, Specificity, and Sensitivity

In approaching the design of a sensor that specifically recognizes FAK tyrosine phosphorylation, we chose to take advantage of the Src Homology 2 (SH2) domain of pp60(c-Src), as this domain mediates the interaction between FAK and Src during adhesion signaling events (Schaller et al. 1994; Xing et al. 1994). Indeed, several other groups have used probes derived from the Src SH2 domain to investigate IAC dynamics and monitor phosphotyrosine accumulation in a variety of contexts (Kubow, Conrad, and Horwitz 2013; Ballestrem et al. 2006; Guo and Wang 2007; Hoffmann et al. 2014; Humphries et al. 2007; Kirchner et al. 2003; Felkl et al. 2012; Logue, Cartagena-Rivera, and Chadwick 2018; McKenzie et al. 2019; Myers and Gomez 2011; Nollau and Mayer 2001; Santiago-Medina, Myers, and Gomez 2012; J. Wu et al. 2015). Many of these probes, including the pYFAK BiLuc system presented here, make use of a double tandem repeat of the Src SH2 domain (dSH2), an approach originally developed in Dr. Benny Geiger's laboratory (Weitzmann Institute; Israel) (Ballestrem et al. 2006; Kirchner et al. 2003). Importantly, the initial author's report this dSH2 sensor shows broad binding to pY residues in adhesion complexes, displaying a linear correlation with an anti-pY antibody and demonstrating the capability of co-precipitating both p130Cas and paxillin (Kirchner et al. 2003). This observation of broad affinity of the dSH2 sensor to several

phosphotyrosine species in adhesion complexes has been reproduced by subsequent users, and exploited by some in the design of FRET-based assays to examine interactions between multiple adhesion complex components or monitor phosphotyrosine content during adhesion signaling events (Ballestrem et al. 2006; Xinming et al. 2008). However, this broad affinity for phosphotyrosine species presents a challenge in designing a tool that is specific to FAK tyrosine phosphorylation, but insensitive to the phosphorylation status of other adhesion components. Therefore, we chose to incorporate the dSH2 sensor into a bi-molecular complementation design.

Bi-molecular complementation systems (also called split reporter systems) are powerful programmable tools due to their modular nature and ability to be genetically encoded. These systems often display high specificity, as the split reporter approach requires the stoichiometric balance, proximity, and proper orientation of both putative interaction targets to reconstitute reporter signal (Kerppola 2008; 2009; I Remy, Galarneau, and Michnick 2002). As applied to the detection of tyrosine phosphorylated FAK, we reasoned that tethering the two halves of a reporter molecule to the dSH2 sensor and to FAK itself would permit reporter reconstitution only in adhesive complexes where FAK and Src interact (Cobb et al. 1994; Hanks et al. 1992; Schaller et al. 1992b; Xing et al. 1994), in a phosphorylation-dependent manner (Eide, Turck, and Escobedo 1995; J. L. Guan and Shalloway 1992; Schaller et al. 1994), generating the specificity we desired. Indeed, we show that only one pair of split-luciferase constructs designed and tested was able to reconstitute luciferase activity (**Figure 2.1 C**), suggesting that the luciferase fragments cannot drive self-reassembly and that proper relative positioning mediated by

pYFAK-dSH2 interactions is essential for reporter activity. Reconstitution of luciferase activity is dependent on the presence of both the dSH2 and FAK products, as untethered luciferase fragments are unable to spontaneously reassemble into functional enzyme when co-transfected (NNVector-NCVector, **Figure 2.1 C**); nor can a soluble nLuc fragment spontaneously associate with the FAK-cLuc fusion product when co-expressed (NNVector-CCFAK, **Figure 2.1 C**). We go on to demonstrate the specificity of the pYFAK BiLuc system to FAK tyrosine phosphorylation, as mutation of three key regulatory tyrosine residues to phenylalanine (Y397-576-577F) (Calalb, Polte, and Hanks 1995a) nearly abolishes luciferase activity (**Figure 2.4**). This strong attenuation of luciferase signal cannot be explained by loss of recruitment to IACs, as colocalization analysis revealed no significant difference in the correlations calculated between each pYFAK BiLuc construct (CCFAK vs CCFAK-3YF) and the three IAC components pY397-FAK, pY118-Paxillin, and pY165-p130Cas (**Figure 2.5**, **Table 1**). These results are in accordance with previous studies that have shown that mutating tyrosine residues on FAK, or pharmacological blocking of the FAK-Src complex, does not alter IAC composition, but does affect signaling properties and cellular phenotypes (Hamadi et al. 2005; Horton, Humphries, et al. 2016; Webb et al. 2004; Westhoff et al. 2004). Collectively, these results indicate that the pyFAK BiLuc system presented is highly specific to FAK phosphorylation, and not responsive to other resident IAC phosphoproteins.

When designing a bi-molecular complementation system, the investigator must carefully consider the benefits and limitations of the reporter molecule chosen. Here, we

chose to make use of a split-luciferase reporter because of the unrivaled sensitivity, signal-to-noise ratio, and rapid assembly and disassembly kinetics these split-reporter enzymes provide (Azad, Tashakor, and Hosseinkhani 2014; Lake and Aboagye 2014; Paulmurugant and Gambhir 2003). In contrast to split-fluorescent systems, where reassembly of the reporter is irreversible due to the high intrinsic stability of the reconstituted fluorophore (Kerppola 2008; 2009), split-luciferase systems demonstrate ready reversibility (Luker et al. 2004; Ingrid Remy and Michnick 2006; Stefan et al. 2007; Villalobos et al. 2010). Importantly, this ensures that the observable signal reflects steady-state associations between interaction targets and avoids potential experimental artifacts stemming from the production of interaction target aggregates, which may result in the cellular accumulation signaling complexes, resulting in above physiological-levels of activity. We find that pYFAK BiLuc signal is rapidly attenuated, on the order of minutes, by pharmacological block of Src-family kinases or with incubation of an integrin-β1 function blocking antibody (**Figure 2.3**). These results demonstrate that the pYFAK BiLuc system is responsive along FA disassembly and turnover timescales (Kirchner et al. 2003; Zaidel-Bar et al. 2003; Eli Zamir et al. 2000), while confirming that these tools are responsive to the endogenous integrin receptor signaling axis. Additionally, we note that the degree of luciferase attenuation by pharmacological block of Src family kinases (maximal 55.6% reduction after 15mins of PP2 treatment, **Figure 2.3**) or antibody-mediated block of integrin receptor (61.8% reduction with 15mins Ha2/5 treatment, **Figure 2.3**) is not as dramatic as the near complete abolishment of luciferase activity observed by mutating the three regulatory tyrosine residues to phenylalanine

(Y397-576-577F) (CCFAK+NNdSH2 vs. CCFAK-3YF+NNdSH2, **Figure 2.4**). During IAC assembly, engagement of ECM ligands induces integrin receptor clustering and recruitment of FAK, which in turn trans-autophosphorylates residue Y397, providing a docking site for Src kinase (J. L. Guan, Trevithick, and Hynes 1991; L. Kornberg et al. 1992; L. J. Kornberg et al. 1991; Schaller et al. 1994; Xing et al. 1994). Therefore, it is possible that the residual luciferase activity observed after pharmacological treatment reflects dSH2 sensor binding to pY397-FAK, as this tyrosine phosphorylation event would still be expected to occur in the presence of PP2 or SU6665. Similarly, FAK may still be recruited to adhesive complexes in a cell that expresses a heterogenous population of integrin receptors, as the Ha2/5 antibody only targets one class (integrin-β1 subunit) of receptor complexes (Mendrick and Kelly 1993). Notably, expression data accessed from the Human Protein Atlas (proteinatlas.org) indicates expression of 11 integrin receptor subunits by HEK293 cells: integrin-α2 (*ITGA2*), integrin-α3 (*ITGA3*), integrin-α4 (*ITGA4*), integrin-α5 (*ITGA5*), integrin-α6 (*ITGA6*), integrin-α7 (*ITGA7*), integrin-α8 (*ITGA8*), integrin-αV (*ITGAV*), integrin-β1 (*ITGB1*), integrin-β3 (*ITGB3*), and integrin-β5 (*ITGB5*) (Pontén, Jirström, and Uhlen 2008). As such, 10 known heterodimer receptor combinations may potentially be formed in HEK293 cells, 8 of which include the integrin-β1 receptor subunit (αVβ3 and αVβ5 being the two exceptions). We speculate that the residual luciferase signal observed following Ha2/5 treatment may represent the fraction of integrin receptor complexes unaffected by this function-blocking monoclonal antibody, or simply in inability of the antibody to target all available integrin-β1 receptor complexes available. Collectively, we propose these observations demonstrate the

sensitivity of the pYFAK BiLuc system to detect varying degrees of FAK tyrosine phosphorylation *in vivo*, and suggest it may therefore be applied to quantitatively measure, by proxy, the strength of IAC signaling (Calalb, Polte, and Hanks 1995a; Hanks et al. 1992).

Comparison to Benchmark and potential applications

Traditional methodologies for monitoring phosphorylation in IACs include western blotting, co-immunoprecipitation experiments, and immunofluorescence using antibodies to detect particular phosphotyrosine residues (Iyer et al. 2005; Ruest et al. 2000). For live-cell analysis, dSH2-YFP and dSH2-CFP constructs were developed by Dr. Benny Geiger's laboratory (Kirchner et al. 2003), and have since been employed for standard fluorescence imaging, as well as FRET-based and FRAP-based assays to monitor phosphotyrosine accumulation during IAC assembly and component exchange with the cytosol (Ballestrem et al. 2006; Xinming et al. 2008; Horton, Humphries, et al. 2016). As technology advanced, several approaches designed to specifically target FAK functions or interactions were developed. The first were fluorescent-fusion proteins that allowed live-cell monitoring of FAK recruitment to IACs, and have been used to investigate the role of tyrosine phosphorylation in adhesion turnover (Giannone et al. 2002; Hamadi et al. 2005; Webb et al. 2004). Later, a bimolecular fluorescence complementation (BiFC) approach was developed to monitor interactions between FAK and Src-family kinases Src and Syk during IAC assembly (De Virgilio, Kiosses, and Shattil 2004). Led by several lines of research indicating an important regulatory

interaction between the N-terminal FERM domain and C-terminal catalytic domain (L. Cohen and Guan 2005; Dunty and Schaller 2002; Lietha et al. 2007), several groups developed FRET-based probes to investigate FAK intramolecular rearrangements and partner coupling during adhesion assembly (Xinming et al. 2008; Papusheva et al. 2009; Ritt, Guan, and Sivaramakrishnan 2013). More recently, fluorescent-based biosensors were developed to monitor FAK kinase activity *in vivo*, again using FRET-based biosensors that would change conformation in response to FAK-mediated phosphorylation (Seong et al. 2011; Yiqian Wu et al. 2016), or by monitoring the differential fluorescence decay rates of a FAK phosphorylation-dependent biosensor (Damayanti et al. 2017). Collectively, these various approaches have contributed greatly to our understanding of FAK regulation and function during IAC assembly and signaling, yet none have permitted a simple, quantitative, scalable approach to monitor FAK phosphorylation and IAC signaling.

For these reasons, we sought to fill an apparent gap in the literature by designing a Luciferase Fragment Complementation Assay (LFCA) to detect FAK phosphorylation. Whereas the above approaches have relied on genetically encoded fluorescent reporters, we felt that the inherit limitations of these systems prevent their widespread adoption in basic research and clinical settings. While these systems bring many benefits for multiplexing with other cellular markers and visualizing sub-cellular interactions, split-fluorescent and FRET-based systems are relatively insensitive to discrete signaling events, often requiring overexpression of interaction targets to detect appreciable signal (Kerppola 2009). Similarly, FRET-based and split-fluorescent reporters display large

background signal, resulting in a comparatively poor signal-to-noise ratio (Lake and Aboagye 2014). Further, FRET and split-fluorescent systems are poorly translated to *in vivo* models, and require expensive, specialized microscopy equipment and computational analysis to process. In contrast, luciferase reporters display outstanding signal-to-noise ratios, as neither luciferase fragment produce bioluminescence, and the reconstituted enzyme requires investigator-supplied substrate for signal development (Lake and Aboagye 2014; Paulmurugant and Gambhir 2003). Similarly, split-luciferase reporters display high dynamic range, over several orders of magnitude, and permit repeat measurements from a biological sample without worry of signal degradation, lending themselves to quantitative applications where both sensitivity and scale are desired (Lake and Aboagye 2014). Finally, split-luciferase reporters are relatively easy to measure, requiring a more accessible luminometer or plate reader, and have been successfully translated into in vivo models (Lake and Aboagye 2014; Paulmurugan, Umezawa, and Gambhir 2002).

We feel that the beforementioned advantages of the pYFAK BiLuc system may be useful in basic research, pre-clinical, or clinical settings where the monitoring of FAK activation is desirable. In recent years, FAK has gained considerable attention for its potential roles in tumorigenesis or metastasis (Chauhan and Khan 2021; Chuang et al. 2022; Dawson et al. 2021; B. Y. Lee et al. 2015; Murphy et al. 2020; Sulzmaier, Jean, and Schlaepfer 2014), given its established roles in mediating adhesion signaling and its integration with growth factor response pathways (Keith Burridge and Chrzanowska-Wodnicka 1996; Chrzanowska-Wodnicka and Burridge 1996; B Geiger and Zamir 2001;

Mitra, Hanson, and Schlaepfer 2005; Westhoff et al. 2004). FAK has been found to be overexpressed in numerous human cancers (Agochiya et al. 1999; Kanteti et al. 2018; Kim et al. 2019; Rigiracciolo et al. 2021; Zakaria et al. 2020; Zhang et al. 2021) and its activity has been correlated with the development of the formation of invasive podosomes and increased cellular motility and proliferation (Alexander et al. 2008; Chan, Cortesio, and Huttenlocher 2009; Feng et al. 2019; Hauck, Hsia, and Schlaepfer 2002; B. Y. Lee et al. 2015; Provenzano and Keely 2009; Sulzmaier, Jean, and Schlaepfer 2014; Y. Wang and McNiven 2012). As such, methods to inhibit FAK recruitment and kinase activity have been suggested as a therapeutic approaches in treating cancer progression and metastasis (Antoniades et al. 2021; Dawson et al. 2021; Mousson et al. 2018; Murphy et al. 2020; Pang et al. 2021; Yueling Wu et al. 2021). While many of the associations between FAK and cancer progression have been established by comparing gene or protein expression from tumor biopsies, a robust method to monitor FAK activity *in vivo* remains lacking.

For these reasons, we believe the pYFAK BiLuc system represents a first-in-its-class approach to monitoring FAK tyrosine phosphorylation and IAC signaling. Collectively, our results indicate that the pyFAK BiLuc system is a dynamic, specific, and sensitive reporter of FAK activation, with the potential to be expanded for high-throughput drug screens, genetic screens, or other *in vivo* or *in vitro* applications to monitor cellular behavior. Our hope is that these tools will add versatility to the existing toolkit to monitor IAC dynamics in both basic research and clinical settings.

Materials & Methods

Plasmids: Generation of the split Luciferase backbones: The split Luciferase backbones were generated by cloning the nLuc and cLuc split fragments into pCDNA3.1+. NNVector (containing nLuc) was generated by amplifying the nLuc fragment from pGL2-Basic (Promega) using the following primers: F:CCCGGATCCACCATGGAAGACGCCAAAAACATAAAG; R:CCCCTCGAGTGAATTCGCGGCCGCCGTGGCGATGGAGCGTCCATCCTTGTCAATCAAGGCG. The amplified fragment was then cloned into pCDNA3.1+ using the BamHI-XhoI sites. NCVector (containing cLuc) was generated by PCR amplifying cLuc using the following primers: F: CCCTCTAGACCGGCCTGCAAGATCCCGAACGACCTGAAACAGAAGGTCATGAACCACTCCGGTTATGTAAACAATCCGGAAG; R: GAAGGGCCCCTACACGGCGATCTTTCCGCCCTTC. The amplified fragment was then subcloned into pCDNA3.1+ using the XbaI-ApaI sites.

Generation of CCFAK: CCFAK was generated by subcloning N-terminal myc-tagged FAK into NCVector. We first amplified N-terminal Myc-tagged FAK from pRcCMV-FAK-ntMyc (kindly provided by S.K. Hanks) using the following oligos: F:CCCTAAGCGGCCGCGAATTCAATGGAGCAGAAGCTGATCTCCG; R:CGCTCTAGAGTGTGGCCGTGTCTGCCCTAG. The amplified fragment was then subcloned into NCVector using the NotI-XbaI sites.

Generation of CCFAK-3YF: CCFAK-3YF was ordered from Vector builder (XXX).

Genreation of dSH2 phosphotyrosine sensor: The SH2 domain from pp60(c-Src) was amplified by PCR from embryonic cDNA two independent times using the following primers: for SH2a F:CCCTAAGCGGCCGCGAATTCAACCATGGGGAGCAACTATGTGGCGCCCTCC ; R:GCGGGATCCTACGGTAGTGAGGCGGTGACAC ; for SH2b F:GCGGGATCCAGCAACTATGTGGCGCCCTCC; R:CGCTCTAGATACGGTAGTGAGGCGGTGACAC. The resulting amplicons were combined and cloned by 3-way ligation into a NdCGFP backbone construct using the EcorI and XbaI sites. The NdCGFP backbone was previously generated by PCR amplification C-terminal EGFP and PEST domain of pd2EGFP-Basic (Clontech) using the following primers: F:CCCGGATCCGCCATGGGGAAGAACGGCATCAAGGTGAAC; R:CCCCTCGAGTGAATTCGCGGCCGCCGTGGCGATGGAGCGCACATTGATCCTAGCAGAAGC and subcloning into pCDNA3.1+ using the BamHI/XhoI sites. Generation of NNdSH2: NNdSH2 was generated by amplifying the dSH2 sequence from NdCGFP using the following primers: F:CCCTAAGCGGCCGCGGGAGCAACTATGTGGCGCCCTCC; R:CGCGGGCCCTACGGTAGTGAGGCGGTGACAC, and subcloning into the NotI-ApaI sites of the NNVector.

Luciferase assays: HEK293 cells were seeded at 9.6x103 cells/ml in tissue culture treated 96-well flat-bottom plates and co-transfected with pYFAK BiLuc constructs and

pRL-TK at 24hrs using Lipofectamine 2000 (ThermoFisher) following manufacturer's instructions. A total of 130ng DNA was transfected per well (60ng each split construct and 10ng pRL-TK control). Cultures were allowed to express for a subsequent 36hrs before lysis and measurement. Luciferase was measured in a GloMax 20/20 Luminometer (Promega) using the Dual Luciferase Kit (Promega) following manufacturer's instructions. Pharmacological treatment was performed immediately before passive lysis with PLB buffer for luminescence detection. Drugs were diluted to 10μM concentrations in prewarmed culture media prior to addition to the cells. Treatment with vehicle for 15min was used as control. Firefly luciferase activity was normalized to Renilla Luciferase activity. Four independent experiments were quantified for all treatments.

Immunocytochemistry and Colocalization Analysis: COS-7 cells were seeded at 5.7e4 cells/well in a 24-well plate on glass coverslips that had been coated overnight with a solution of 10ug/ml laminin. Each well was co-transfected with 1ug DNA per well (500ng of each half construct: 500ng CCLucFAK + 500ng NNLucdSH2 or 500ng CCLucFAK-3YF + 500ng NNLucdSH2) using Lipofectamine 2000 (ThermoFisher) reagent following the manufacturer's protocol. Cells were allowed to express for 24 hours after transfection before fixation with 4% paraformaldehyde in 1xPBS for 5mins at room temperature (RT). Coverslips were then blocked/permeabilized for 20min at RT in a solution of 10% goat serum in 1xPBST (1xPBS+0.1%TritonX-100). Following blocking, coverslips were incubated in primary antibody overnight at 4C with gentle

agitation. Coverslips were then washed for 4 ten-minute washes in 1xPBS prior to secondary antibody incubation for 3 hours at RT. Coverslips were washed again for 4 ten-minute washes in 1xPBS before mounting with Flourogel+DABCO mounting medium. All primary antibodies were diluted at 1:250 and all secondary antibodies were diluted at 1:1000 in 1xPBS+5% goat serum. Antibodies used: mouse anti-myc (Cell Signaling Technologies, cat # 2276S), rabbit anti-p130CasY165 (Cell Signaling Technologies, cat # 4015S), rabbit anti-PaxillinY118 (Cell Signaling Technologies, cat # 69363S), rabbit anti-FAKY397 (ThermoFisher, cat # 44624G), alexafluor 488 goat anti-mouse (ThermoFisher, cat # A-11001), alexaflour 647 goat anti-rabbit (ThermoFisher, cat # A-21244). Three independent transfections were quantified for colocalization analysis, and 10-15 cells were analyzed per condition. Confocal scanning images were acquired on a Leica DMi8 using an oil-immersion 63x objective (NA=1.3) with a 1.85x digital zoom and z-stack acquired with 40-50 steps of sizes of 0.15µm. Correlations of individual cells were calculated from maximally projected images. Individual cells were selected by manually drawing a ROI around the cell perimeter in FIJI, and auto-thresholding and Pearson's R and Spearman's rank ρ correlations were calculated using the Coloc2 plugin

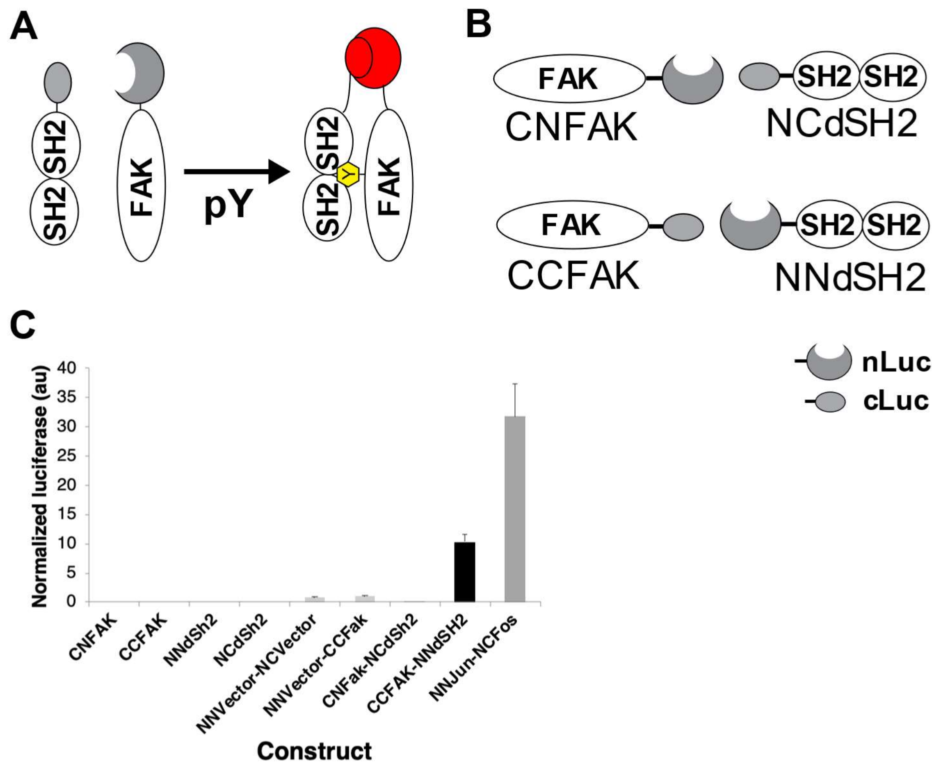


Figure 2.1: Design of pyFAK BiLuc constructs. (**A**) Schematic representation of pYFAK BiLuc general approach. Intramolecular interactions between phosphotyrosine-FAK (pYFAK) and a phosphotyrosine sensor (double SH2, dSH2) permit reconstitution of enzymatically active firefly luciferase. (**B**) Design of pYFAK split luciferase construct pairs. The first letter of the construct name describes the attachment point of split luciferase fragments on FAK and dSH2 interaction targets (N-terminal vs C-terminal), while the second letter indicates which fragment of split luciferase was attached at this position: N-terminal Luciferase (nLuc, aa1-416) and C-terminal Luciferase (cLuc, aa401-550). (**C**) Tests of different pyFAK split luciferase construct pairs in HEK293 cells identify an effective pyFAK probe pair (CCFAK+NNdSH2) which displays high specificity and low background activity. Individual constructs (CNFAK, CCFAK, NNdSH2, NCdSH2) do not produce detectable luciferase activity, nor do untethered luciferase constructs when co-transfected (NNVector+NCVector). Similarly, luciferase activity is dependent on the presence of both FAK and dSH2 interaction partners (NNVector+CCFAK). The activity of a previously described split-luciferase reporter system (NNJun+NCFos) is shown for comparison. Renilla luciferase was co-transfected as a control in all cases. Luciferase activity is normalized to baseline firefly/renilla luciferase ratio of the NNVector+CCFAK pair (ANOVA $p<0.01$, followed by Tukey Post-hoc **$p<0.01$; ***$p<0.001$ compared to baseline; n=4 biological replicates).

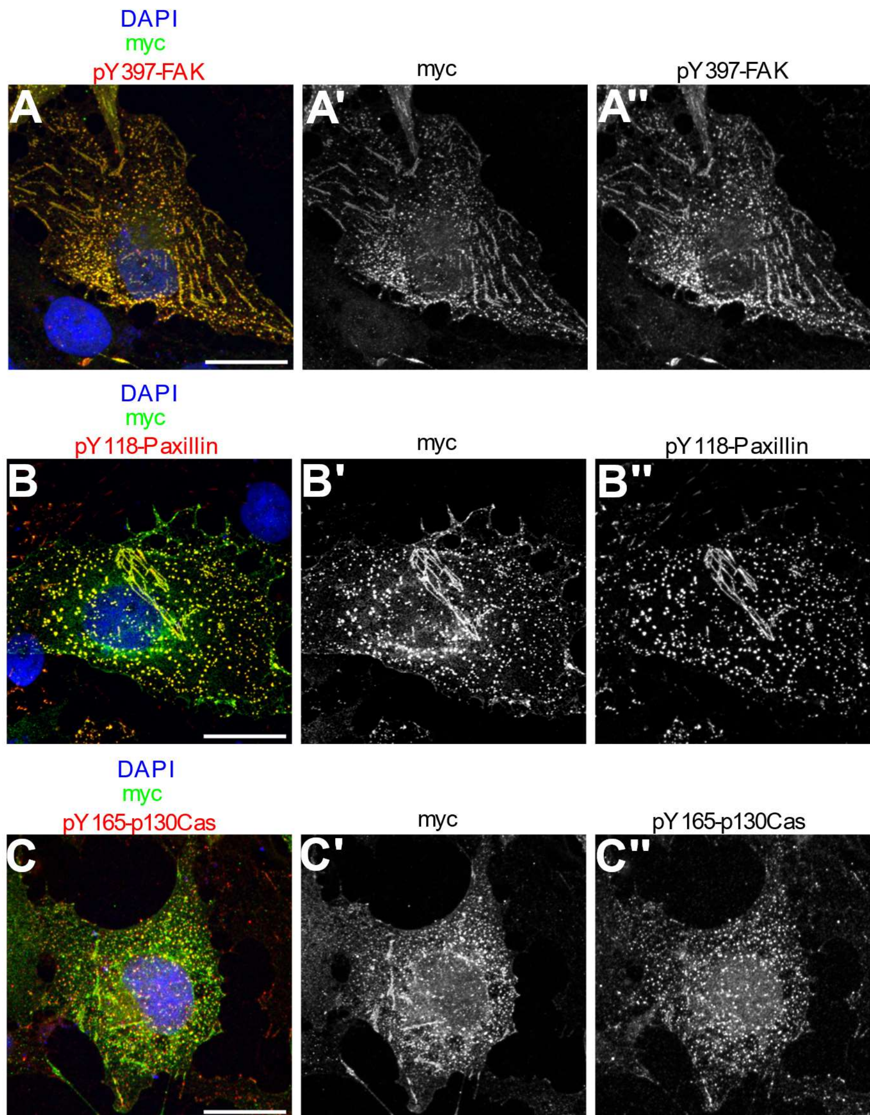


Figure 2.2: pyFAK BiLuc constructs are recruited to actively signaling FAs. Colocalization analysis confirms myc-tagged FAK constructs (green) incorporate into actively signaling FA complexes in transfected COS-7 cells, as revealed by phospho-specific antibodies (red). Strong correlation is observed between myc (**A, A'**) and pY397-FAK (**A, A''**) (Pearson's R=0.77±0.02 and Spearman's rank correlation ρ=0.83±0.01) indicating the CCFAK construct is incorporating into adhesion complexes. Similar correlation is observed between myc (**B, B'**) and pY118-Paxillin (**B, B''**) (R=0.73±0.10 and Spearman's rank correlation ρ=0.77±0.09), another early marker of active FAs. Correlation is weaker between myc and pY165-P130Cas (Pearson's R=0.40±0.02 and Spearman's rank correlation ρ=0.57±0.02). Scale bar = 20μm for all. n=3 biological replicates for each stain.

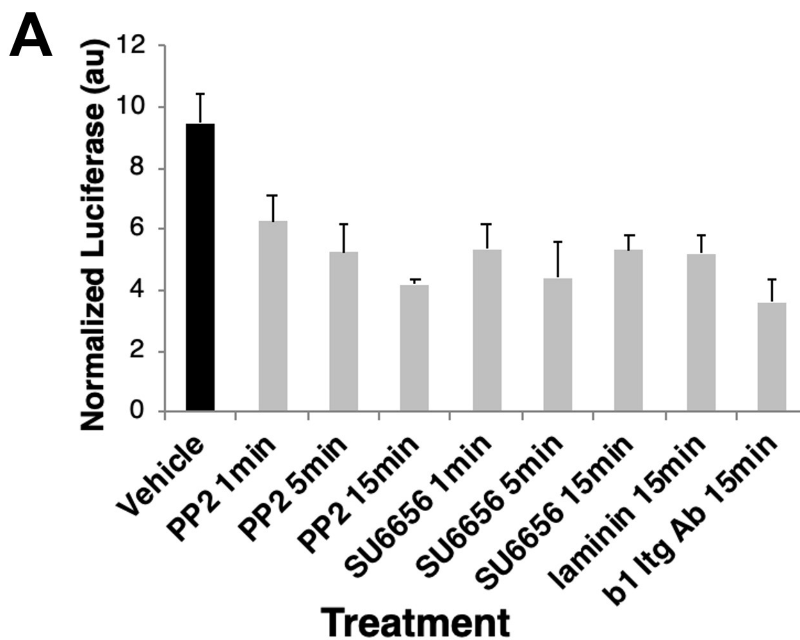


Figure 2.3: pyFAK BiLuc constructs respond to pharmacological blocking of adhesion signaling. Quantification of pYFAK BiLuc (CCFAK+NNdSH2) enzyme activity following treatments with adhesion signaling blocking agents. Signal was normalized to baseline firefly/renilla luciferase ratio of untreated NNVector + CCFAK pair. Application of 10µM PP2 results in a progressive reduction in luciferase activity following application, with a 33% reduction after 1min (NS), 43% reduction after 5mins, and maximal repression of 55% after 15mins. Application of 10µM SU6656 results in a significant 43% decrease in luciferase activity after 1min, 54% after 5mins, and return to 43% reduction after 15mins. A 15min incubation with Ha2/5 β1-integrin function blocking antibody (Itgb1 Ab) results in a 61% decrease in luciferase activity compared to vehicle treatment (ANOVA $p<0.01$, followed by Tukey Post-hoc $*p<0.05$; $**p<0.01$). Error bars = SEM. n=4 biological replicates.

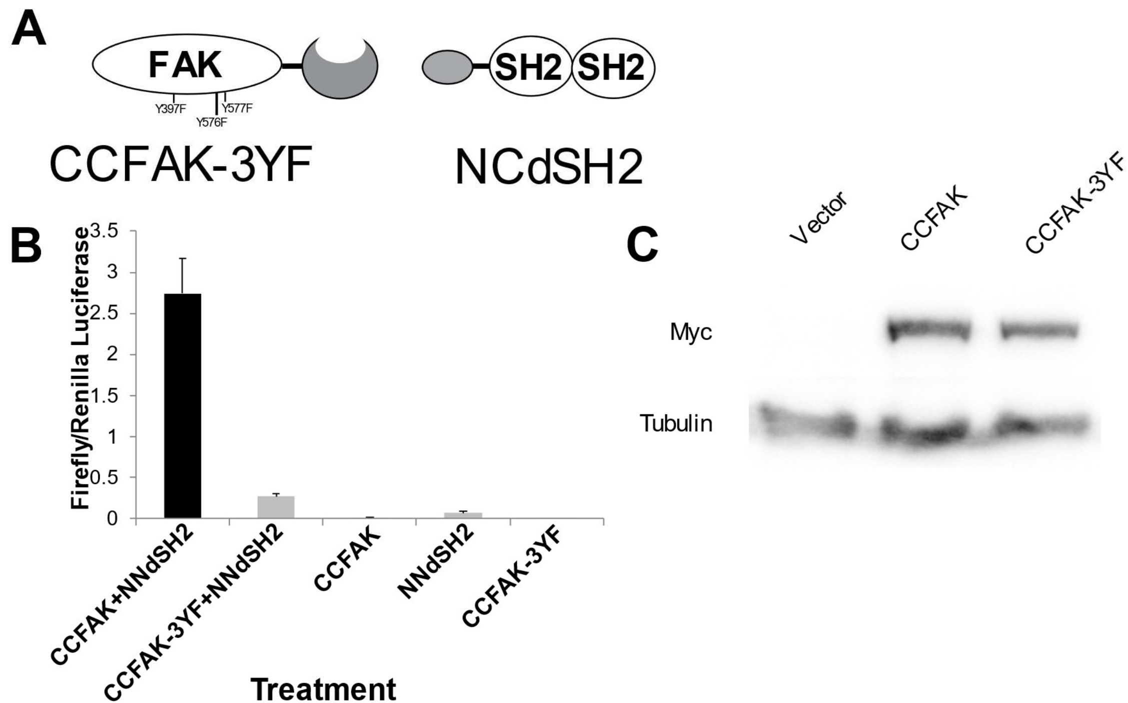
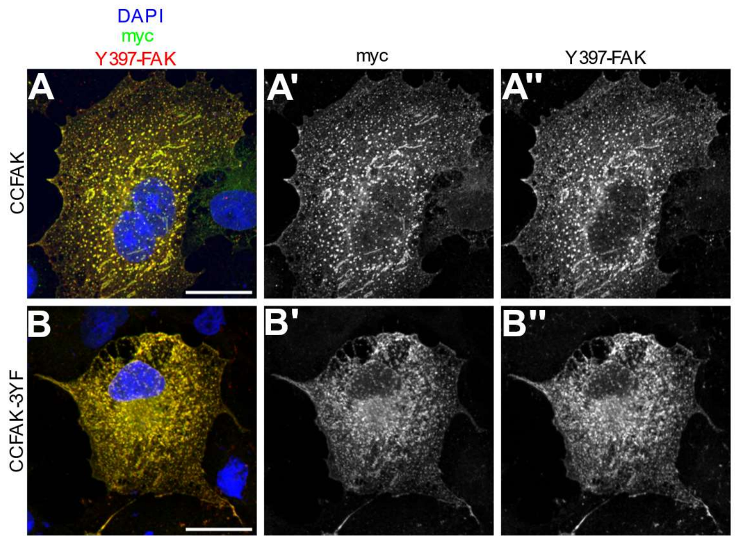
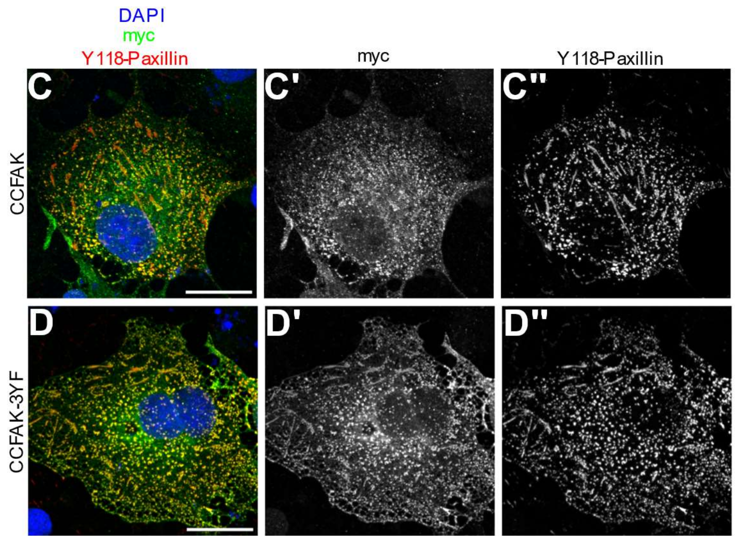


Figure 2.4: pyFAK BiLuc signal is dependent on specific FAK tyrosine residues. (**A**) Schematic representation of the three-point mutation introduced to produce the CCFAK-YF construct. Tyrosine to phenylalanine missense mutations were introduced to tyrosine residues Y397, Y576, and Y577. (**B**) Quantification of pyFAK BiLuc enzyme activity for tested constructs. Luciferase activity is normalized to baseline firefly/renilla luciferase ratio of the NNdSH2 (ANOVA $p<0.01$, followed by Tukey Post-hoc **$p<0.01$; ***$p<0.001$ compared to baseline; n=4 biological replicates). (**C**) Western Blot and quantification from transfected HEK293 cells showing robust myc signal from both CCFAK and CCFAK-3YF transfected cells. 20ng of total protein was ran for each lane.


DAPI
myc
Y397-FAK
myc
Y397-FAK
A
A'
A''
B
B'
B''
CCFAK
CCFAK-3YF


DAPI
myc
Y118-Paxillin
myc
Y118-Paxillin
C
C'
C''
D
D'
D''
CCFAK
CCFAK-3YF

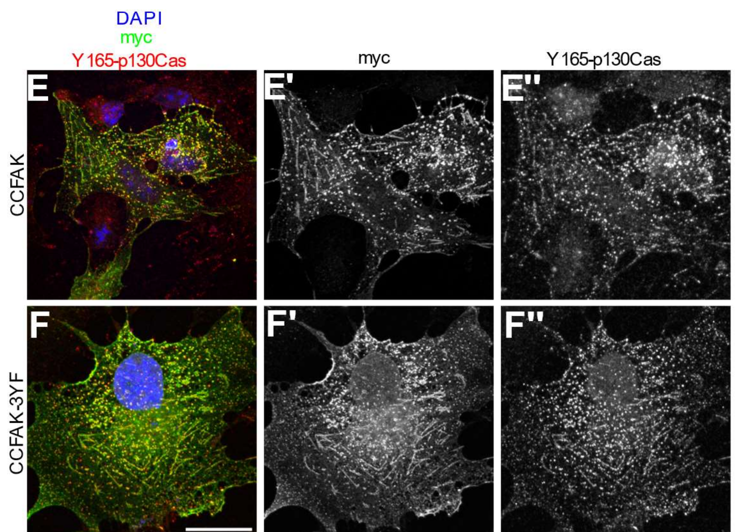

DAPI
myc
Y165-p130Cas
myc
Y165-p130Cas
E
E'
E''
F
F'
F''
CCFAK
CCFAK-3YF

Figure 2.5: pyFAK BiLuc recruitment to FA complexes is not dependent on tyrosine phosphorylation. Colocalization analysis confirms myc-tagged CCFAK-3YF constructs (green) incorporate into actively signaling FA complexes in transfected COS-7 cells, as revealed by phospho-specific antibodies (red). Strong correlation is observed between myc (**A, A'**) and pY397-FAK (**A, A''**) (Pearson's R=0.79±0.03 and Spearman's rank correlation ρ=0.85±0.03) indicating the CCFAK-3YF construct half is incorporating into FA structures. Similar correlation is observed between myc (**B, B'**) and pY118-Paxillin (**B, B''**) (R=0.65±0.18 and Spearman's rank correlation ρ=0.72±0.12), another early marker of active FAs. Correlation is weaker between myc and pY165-P130Cas (Pearson's R=0.43±0.07 and Spearman's rank correlation ρ=0.57±0.06). These correlations were found to be not significantly different from those collected for the CCFAK construct (**Figure 2.2**) ((Mann-Whitney comparing Spearman's rank correlations; pY397-FAK CCFAK median=0.8353114, 3YF median=0.85287551, U=1158, p=0.1127; pY118-Paxillin CCFAK median=0.77363353, 3YF median=0.73749172, U=676.5, p=0.1768; pY165-P130Cas CCFAK median=0.57599749, 3YF median=0.56036582, U=702, p=0.6949).). Scale bar = 20μm for all.

Chapter 3

β-chimaerin proteins are required for cerebellar granule cell radial migration

Abstract

During mammalian cerebellar development, postnatal granule cell progenitors (GCPs) proliferate in the outer part of the External Granule Layer (EGL). Upon cell cycle exit, GCPs migrate tangentially in the inner EGL before switching to migrate radially inward, past the Purkinje cell layer, to achieve their final position in the mature Granule Cell Layer (GCL). Here, we show that the RacGAP β-chimaerin is expressed by a small population of late-born, pre-migratory GCPs. β-chimaerin deficiency causes a subset of GCPs to become arrested in the EGL, where they differentiate and form ectopic neuronal clusters. These clusters of granule cells recruit aberrantly projecting mossy fibers. Collectively, these data suggest a role for β-chimaerin as an intracellular mediator of Cerebellar GCP radial migration.

Introduction

Proper morphogenesis of the vertebrate Central Nervous System (CNS) relies on the tight spatiotemporal control of cell proliferation, differentiation, migration and guidance events. In the mammalian cerebellum, Granule Cells (GCs) undergo a prolonged and highly stereotyped migration that begins embryonically and completes late postnatally (Chédotal 2010). In the mouse, beginning at embryonic day 12 (E12), granule cell precursors (GCPs) are born from the rhombic lip and migrate tangentially to cover the cerebellar anlage (Wingate 2005), forming a secondary germinal zone, the External Granule Layer (EGL). Postnatally, GPCs in the EGL exit the cell cycle and travel inwards, splitting the EGL into an upper, mitotically active (outer EGL, oEGL) and a lower, migratory layer (inner EGL, iEGL) (**Fig 3.1 A**). These postmitotic GCPs grow two horizontal processes and migrate tangentially before growing a third perpendicular leading process. Using this third leading process GCPs migrate radially inward along Bergmann Glial fibers, past the Purkinje Cell (PC) Layer, to occupy their final location in the mature Granule Cell Layer (GCL) (Yacubova and Komuro 2003; Kawaji et al. 2004). Cerebellar GCP migration has been shown to be influenced by a wide set of guidance cues, including the chemokine SDF-1 (Zhu et al. 2002), Slit2/Robo (C. bing Guan et al. 2007), Plexin/Semaphorin (Friedel et al. 2007; Kerjan et al. 2005; Renaud et al. 2008), brain-derived neurotrophic factor (BDNF) (Borghesani et al. 2002), and Vascular Endothelial Growth Factor (Ruiz de Almodovar et al. 2010). However, the cytosolic machinery responsible for directing the cellular response downstream of these ligand-receptor pairs remains largely unexplored.

The Rho family of small G-Proteins, or GTPases, play essential roles in vertebrate CNS development, influencing a wide range of developmental processes, including cell migration, cell polarity, axon pathfinding, and dendritic remodeling through their ability to modulate cytoskeletal structure (E.-E. Govek, Hatten, and Van Aelst 2011; E. E. Govek, Newey, and Van Aelst 2005). GTPases exist in two states: an active GTP-bound state and an inactive GDP-bound state (Cherfils and Zeghouf 2013). Precise subcellular regulation of GTPase activity is essential in maintaining proper cellular function, and neurons achieve this using positive regulators, Rho Guanine Nucleotide Exchange Factors (or RhoGEFs) and negative regulators, Rho GTPase Activating Proteins (or RhoGAPs) (Cherfils and Zeghouf 2013; G. H. Huang et al. 2017). Disruption of RhoGTPase activity or their regulators' function has been associated with a broad array of developmental and behavioral disorders (G. H. Huang et al. 2017; Bai et al. 2015). The chimaerin family of RhoGAPs consists of two genes: α-chimaerin (*CHN1*) and β-chimaerin (*CHN2*). They possess specific GAP activity toward Rac family GTPases, which are key modulators of actin filaments (Yang and Kazanietz 2007). In neural development, α-chimaerin has been shown to play roles in Ephrin-mediated circuit formation (Beg et al. 2007; Iwasato et al. 2007; Wegmeyer et al. 2007; Kao et al. 2015), cortical migration (Ip et al. 2012), optic tract axon guidance (Ferrario et al. 2012; Miyake et al. 2009), and hippocampal dendritic arbor pruning (Buttery et al. 2006). The *in vivo* role of β-chimaerin in neural development was unexplored until recently, where it was shown to affect hippocampal dentate gyrus axon pruning by regulating Rac1 activity downstream of Sema3F/Neuropilin-2 signaling (Riccomagno et al. 2012). Of note, β-

chimaerin has been shown to be strongly expressed in GCs in the adult rat (Leung et al. 1994), but its function during cerebellar morphogenesis is unknown. Here, we show a functional requirement for β-chimaerin during mouse cerebellar development. We find that β-chimaerin is necessary for a small subset of granule cells to complete radial migration from the EGL to GCL.

Results

β-chimaerin is specifically expressed in the Granule Cell Layer of the mouse cerebellum

β-chimaerin has been previously shown to be expressed in the adult cerebellum (Leung et al. 1994). To explore the developmental expression profile of β-chimaerin in the cerebellum, we performed *in situ* hybridization in C57/BL6J mice to visualize β-chimaerin (*Chn2*) messenger RNA (mRNA) at various postnatal stages (**Fig3.1 B-H**). We found *Chn2* mRNA was strongly expressed in the GCL at all the postnatal stages examined. Interestingly, we observed *Chn2* expression in small clusters of cells in the Molecular Layer (ML) of postnatal day 18 (P18) animals, but not earlier (**Fig 3.1 F**). This stage represents one of the last postnatal stages before the EGL dissolves entirely as GCPs complete their radial migration to the GCL. This ML expression did not persist into adulthood, disappearing by P35 (**Fig 3.1 G**).

β-chimaerin deficient mice display ectopic neuronal clusters on the cerebellar surface

As *Chn2* transcript was found to be robustly expressed in the cerebellum at all postnatal stages examined, we asked whether β-chimaerin played a functional role during cerebellar development. We took advantage of a previously generated knock-in/knock-out mouse that expresses beta-galactosidase (βgal) from the endogenous *Chn2* promoter, simultaneously disrupting the reading frame of the endogenous *Chn2* coding sequence and thus rendering the gene product inactive (Riccomagno et al. 2012). We generated adult (P35) mice homozygous for this knock-in/knock-out *Chn2* null allele (simplified in text as *Chn2*$^{-/-}$) and compared the cerebellar structure to heterozygous or wildtype (WT) (*Chn2*$^{+/-}$ or *Chn2*$^{+/+}$, respectively) littermate controls (**Fig 3.2 A-C**). We observed no gross alterations to cerebellar lobule formation or overall lamination in *Chn2*$^{-/-}$ mutants. However, we did observe large ectopic clusters of cells aggregating in the ML of mutant animals (**Fig 3.2 B**, white arrows). These clusters strongly co-labeled with the pan-neuronal marker NeuN and an antibody raised against βgal, indicating that these clusters consist of ectopic cells that normally display *Chn2* promoter activity (**Fig 3.2 D-E**). Sparse NeuN labeling was also seen in the ML of both WT and *Chn2*$^{-/-}$ genotypes (**Fig 3.2 D-E**), and likely represents the stellate and basket cells known to occupy this region. We next asked if β-chimaerin function is required in a dose-dependent manner for normal cerebellar development. We quantified the number of neuronal ectopias in *Chn2*$^{+/+}$, *Chn2*$^{+/-}$ and *Chn2*$^{-/-}$ adult animals and found a significant increase in the number of

ectopias in *Chn2*$^{-/-}$ animals compared to either other genotype ($p<0.01$ for both comparisons) (**Fig 3.2 C**).

The adult cerebellum can be organizationally divided into four domains: Anterior, Central, Posterior, and Nodular. Each region, in turn, is physically divided into lobules, numbered I-X in mice (Sudarov and Joyner 2007). Closely examining the P18 *in situ* hybridization data, we noticed that the majority of ML *Chn2* transcript expression occurred in more posterior sections, particularly Lobules VII-IX and the fissures separating them (data not shown). Therefore, we asked if the NeuN-positive clusters we observe in *Chn2*$^{-/-}$ animals follow a similar pattern of distribution. Indeed, we found that NeuN-positive ectopias were more prevalent in the fissure separating lobules VII and VIII and on the posterior side of lobule IX (**Fig 3.2 F** for schematic and percent distribution). These two locations collectively account for approximately 45% of all ectopic clusters scored (n=1068 ectopias across nine *Chn2*$^{-/-}$ animals). Collectively, these data suggest that *Chn2* is expressed by a small subset of more caudally positioned GCPs prior to their arrival in the GCL, and that loss of β-chimaerin function causes these cells to fail to complete radial migration, resulting in thier arrest in the ML.

The ectopic clusters contain mature granule cells, but not other types of cerebellar neurons

While the prior data demonstrate that the neuronal ectopias observed in *Chn2*$^{-/-}$ mutants contain *Chn2* promotor activity, suggesting their identity as cerebellar GCPs, we sought to thoroughly examine the cellular composition of these ectopias. To test for the

presence of mature GCs, we made use of the marker Gamma-Amino Butyric Acid Receptor subunit α6 (GABARα6), confirming the presence of mature, fully differentiated GCs (71 ± 5% of NeuN+ cells, mean ± SD). To explore the possibility of other cell types contributing to the composition of these ectopic clusters, we immunolabeled with antibodies raised against the Purkinje Cell marker Calbindin, but did not identify any Calbindin^{+} cells within the ectopias (**Fig 3.3 C-D**). Interestingly, Purkinje Cell dendrites failed to invade the space occupied by the neuronal clusters, often wrapping around or outlining them, suggesting these ectopias were able to repel outgrowth from these dendritic processes (**Fig 3.3 D**). We also immunolabeled for the general GABAergic interneuron marker Parvalbumin (**Fig 3.3 E-F**) and found no co-labeling in the neuronal ectopias. Finally, we immunolabeled with the GABAergic marker Glutamic Acid Decarboxylase 67 (GAD67) (**Fig 3.3 G-H**). No GAD67^{+} cell bodies were detected in the ectopic clusters. However, evenly spaced GAD67^{+} processes were detected invading the ectopias, suggesting potential GABAergic input from stellate or basket cells (**Fig 3.3 G-H**). To address the cellular composition of the neuronal ectopias another way, we performed fate-mapping by pulse labeling proliferating cells with Bromo-deoxy-Uridine (BrdU), a thymidine analog that incorporates specifically into cells in the S-phase of mitosis. WT and *Chn2*$^{-/-}$ animals were injected with BrdU at P10, a developmental stage when the GCP population is rapidly expanding (Espinosa and Luo 2008), and cerebella were collected at adult (P35) stages. Whereas BrdU labeling in WT animals is largely restricted to the mature GCL with few cells scattered in the ML, *Chn2*$^{-/-}$ animals display considerable accumulation of BrdU^{+} cells in the ectopias (**Fig 3.4 A-B**. arrows). This

accumulation of BrdU^{+} cells in the ectopias of *Chn2*$^{-/-}$ mutants suggests both that the major contributing cell type are GCPs. Collectively, these data suggest that the neuronal ectopias found in *Chn2*$^{-/-}$ animals are composed primarily of GCs, but not other cerebellar neuronal types.

During radial migration, GCPs in the iEGL migrate along Bergmann glial fibers to navigate towards the GCL (Yacubova and Komuro 2003). Failure of GCPs to properly associate with glial tracts, or errors in glial scaffold architecture itself, could inhibit GC radial migration and explain the ectopic GC accumulation observed in *Chn2*$^{-/-}$ mutants. Therefore, we examined the structure of the glial scaffold surrounding neuronal ectopias using an antibody against Glial Fibrillary Acidic Protein (GFAP) (**Fig 3.4 C-D**). We observed no gross alterations to Bergmann Glial structure, arguing against the possibility of an architectural cause underlying the phenotype. However, upon co-labeling with βgal, which strongly marks most cells in neuronal clusters (**Fig 3.2 E**), we observe many individual cells clinging to single GFAP^{+} tracts even in the adult (**Fig 3.4 E**, white arrowheads). We observe a significant increase in βgal^{+} cells arrested in the ML of *Chn2*$^{+/-}$ and *Chn2*$^{-/-}$ animals (**Fig 3.4 E**; Tukey HSD $p<0.01$ for WT vs *Chn2*$^{+/-}$, WT vs *Chn2*$^{-/-}$, and *Chn2*$^{+/-}$ vs *Chn2*$^{-/-}$). This observation reinforces the idea that GCPs lacking β-chimaerin function stall during radial migration.

Granule cell ectopias recruit presynaptic partners

In the mature cerebellar circuit, GCs in the GCL receive glutamatergic input from mossy fibers originating from the spinal cord, pontine nucleus, and other CNS regions.

GCs, in turn, provide glutamatergic output via parallel fibers onto local Purkinje Cell dendrites (Chédotal 2010). Since the neuronal ectopias in *Chn2*$^{-/-}$ animals contain differentiated, GABARα6^{+} GCs (**Fig 3.3 B**), we asked if they could form local circuits. We assayed for the expression of the synaptic marker Vesicular Glutamate Transporter 2 (Vglut2), which labels a subset of cerebellar glutamatergic synapses formed by climbing and mossy fibers, and found robust co-labeling with βgal^{+} cells within neuronal ectopias (**Fig 3.5 A-B**). Furthermore, Vglut2 staining in the ectopias displayed a pattern highly reminiscent of the rosette structures formed by mossy fiber terminals with full penetrance and expressivity (100% of ectopias examined displayed this pattern).

To test whether the Vglut2^{+} staining in the ectopic neuronal clusters indeed represented mossy fiber synaptic terminals, we performed stereotactic injections of an Adenosine Associated Virus expressing a Synapsin-driven Enhanced Green Fluorescent Protein cassette (AAV-Syn-EGFP) into the pontine nucleus. In contrast to WT controls, where all observed EGFP^{+} axon terminals were restricted to the GCL, we observed EGFP^{+} axons extending beyond the GCL to contact neuronal ectopias in *Chn2*$^{-/-}$ mutants (**Figure 3.5 C-D**; dotted line demarks outer boundary of the GCL). Under high magnification we found that these terminals co-label with Vglut2, suggesting that these indeed represent true pontine mossy fiber terminals and are forming mature synapses (**Fig 3.5 E-F**, white arrowheads).

External Germinal Layer structure and proliferation is normal in early postnatal Chn2$^{-/-}$ mice

During cerebellar development, granule cells undergo a stepwise maturation process. At embryonic stages, mitotically active GCPs expand across the cerebellar anlage from their point of origin at the rhombic lip to generate the EGL proper. Postnatally, these precursors become postmitotic and extend two horizontal processes, moving inward to generate the inner EGL (iEGL) as a distinct population from the more superficial precursors that remain mitotically active in the outer EGL (oEGL). In the iEGL these postmitotic GCPs will migrate tangentially, eventually arresting and growing a third perpendicular process they then use to radially migrate inward, past the PC layer, to form the mature inner GCL. Given the complex migratory path GCPs take in their development, we asked if earlier, subtler defects in EGL structure may precede the development of neuronal ectopias.

We examined P10 cerebella from WT and *Chn2*$^{-/-}$ animals to assess the overall distribution of GCPs. We first immunolabeled using an antibody against the transcription factor Pax6, which is active in GCPs in the EGL and maturing GCs in the GCL. We noticed no major difference in Pax6 distribution between WT control and *Chn2*$^{-/-}$ mutants in the lobules that most frequently develop ectopias at later developmental stages (**Fig 3.6 A-B**). We also examined the expression profile of the cell adhesion molecule NCAM-L1 (L1), which labels the horizontal processes of tangentially migrating GCPs in the iEGL (Kerjan et al. 2005). We found no major difference in its distribution between WT controls and *Chn2*$^{-/-}$ mutants (**Fig 3.6 C-D**). These results suggest that there is no altered

distribution of GCPs preceding the development of neuronal ectopias. As stated earlier, one possible explanation of ectopia formation is alterations to Bergmann glial tract adhesiveness or architecture. We analyzed the structure of the Bergmann glial scaffold using an antibody against GFAP and found no obvious structural differences in the lobules that more frequently develop ectopias (**Fig 3.6 E-F**). Collectively, these results suggest that there are no major early postnatal lamination or architectural defects that could predispose certain GCs to arrest.

The data presented in **Fig 3.4** suggest that GCs in *Chn2*$^{-/-}$ animals arrest during radial migration. However, defects in tangential migration and/or proliferation could indirectly contribute to neuronal ectopia formation by affecting GCP positioning or mitotic exit. Initial embryonic GCP tangential migration to cover the cerebellar anlage appears normal in *Chn2*$^{-/-}$ animals, as the sagittal length of the EGL in WT and *Chn2*$^{-/-}$ animals is not significantly different in neonates (P0) (**Fig 3.7 A-C**; two-tail t-test, p=0.082, n=5 per genotype) (Nakamura et al. 2017). GCPs undergo a second phase of tangential migration postnatally after precursors become postmitotic and move inward to generate the iEGL. If GCPs are arresting during tangential migration or fail to complete the tangential-to-radial migration switch, it could be predicted that the iEGL would become thicker in the folia that are prone to develop ectopias in *Chn2*$^{-/-}$ animals compared to WT controls. Therefore, to assess the thickness of the iEGL and total EGL, we performed immunostaining for Sema6a on P10 cerebella (**Fig 3.7 D-E**) and calculated the iEGL/totalEGL ratio in caudal folia. The iEGL/totalEGL ratio was comparable between WT and *Chn2*$^{-/-}$ animals (**Fig 3.7 F**; two-tail t-test, p=0.528, n=10 each

genotype), suggesting that postnatal tangential migration is not notably disrupted in *Chn2*$^{-/-}$ mice.

During mammalian cerebellar development, GCPs normally continue to proliferate postnatally in the oEGL (Fujita 1967). To assess the proliferative capacity of GCPs in *Chn2*$^{-/-}$ animals, we analyzed the distribution of proliferating GCPs in early postnatal animals (P4 and P10) two hours after BrdU injection (**Fig 3.8 A-F**). As expected, most proliferating cells were found in the oEGL in both WT control and *Chn2*$^{-/-}$ animals. The density of proliferating GCPs in WT controls and *Chn2*$^{-/-}$ animals was comparable (**Fig 3.8 C-F**; two tail t-test, n=5; p=0.32 for P4 and p=0.952 for P10) suggesting that early postnatal GCPs proliferate at normal rates. To test whether the cells that form neuronal ectopias in *Chn2*$^{-/-}$ animals continue to proliferate into adult stages, we performed BrdU injections in WT control and *Chn2*$^{-/-}$ at P35 (**Fig 3.8 G-H**). No proliferating cells were detected in neuronal ectopias at this stage, suggesting that neuronal ectopias consist entirely of post-mitotic cells. Overall, these data suggest that the formation of ectopias and the arrest of GCs in the molecular layer of *Chn2*$^{-/-}$ animals are specifically due to defects in radial migration and not errors in proliferation or tangential migration.

Cerebellar Structure in mice expressing hyperactive β-chimaerin

Genetic ablation of *Rac1* and *Rac3* results in severe disruption of cerebellar granule cell migration (Nakamura et al. 2017; Tahirovic et al. 2010). Could increasing β-chimaerin RacGAP activity cause a similar phenotype? To test this we made use of a

knock-in mouse that harbors a hyperactive *Chn2* allele in which a single amino acid substitution (I130A) yields a protein with a more "open" confirmation and thus renders it more sensitive to induction (Riccomagno et al. 2012; Canagarajah et al. 2004). We collected adult (P35) mice that were homozygous for the hyperactive allele ($Chn2^{I130A/I130A}$) and stained for the mature granule cell marker GABARα6 (**Fig 3.9 A-B**) and the glutamatergic synapse marker Vglut2 (**Fig 3.9 C-D**) to label mature GCs and their synapses, respectively. In contrast to $Chn2^{-/-}$ mutants, $Chn2^{I130A/I130A}$ animals did not develop ectopic clusters of cells. Further, GC lamination and cerebellar foliation appeared normal. Similarly, we found no difference in Bergmann glial scaffold architecture using the marker GFAP (**Fig 3.9 E-F**) nor in the distribution of GABAergic cell populations using the markers Parvalbumin (**Fig 3.9 G-H**) and GAD67 (**Fig 3.9 I-J**). Collectively, these data suggest that hyperactivity of β-chimaerin does not negatively affect cerebellar morphogenesis.

Discussion

Here we show that the RacGAP β-chimaerin is essential for cerebellar GC radial migration. Many ligand-receptor pairs have been shown to regulate GC proliferation and migration, but less is known about the cytoplasmic effectors that link these extracellular signals with the cytoskeleton (Zhu et al. 2002; C. bing Guan et al. 2007; Friedel et al. 2007; Kerjan et al. 2005; Renaud et al. 2008). Guided by the previously reported robust expression of *Chn2* in the adult GCL (Leung et al. 1994), we examined whether this cytoplasmic protein could be playing a functional role during cerebellar development. We

found that the genetic ablation of *Chn2* results in the formation of ectopic clusters of neurons in the outer ML. These ectopias are primarily formed by mature GCs. Since we initially established that *Chn2* transcript was mainly expressed in the mature interior GCL of early postnatal and adult cerebella (**Fig 3.1**), how could the mispositioned ectopic GCs appear on the outside edge of the cerebellum? Interestingly, a fine-windowed developmental study revealed a small subset of late pre-migratory GCPs display *Chn2* expression in the oEGL in WT animals (**Fig 3.1 F**). Based on the distribution of these *Chn2*$^{+}$ cells and the co-localization of βgal with NeuN and GABARα6 in the ectopias of *Chn2*$^{-/-}$ animals (**Fig 3.2** and Fig **3.3 A-B**), it is likely that it is this sub-population of *Chn2*$^{+}$ late pre-migratory neurons are the ones that fail to radially migrate inward upon ablation of Chn2, thus forming neuronal ectopias. The regulatory mechanisms that restrict *Chn2* expression to this small subset of pre-migratory GCPs is currently unknown, making this an intriguing question for future studies. The small subset of cells (25-30%) that are part of the ectopias but fail to express the GABARα6 or markers of other neuronal types might represent an intermediate step in GC maturation, as they do express *Chn2* promoter-driven βgal.

RhoGTPases have been shown to regulate migration in a variety of neuronal systems (E.-E. Govek, Hatten, and Van Aelst 2011; E. E. Govek, Newey, and Van Aelst 2005; G. H. Huang et al. 2017). In particular, the small G-proteins Rac1 and Rac3 are required for proper GCP migration (Nakamura et al. 2017; Tahirovic et al. 2010). RhoA is also necessary for cerebellar development (Mulherkar et al. 2014a). To our knowledge, β-chimaerin is one of the first RacGAPs to be shown to participate in GCP migration (E.-

E. Govek, Hatten, and Van Aelst 2011; G. H. Huang et al. 2017). Notably, only a small set of GCPs in the more caudal cerebellum is affected by loss of *Chn2*. Given the essential role of Rac and Rho during cerebellar morphogenesis and GCP migration specifically, it is likely that other RhoGAPs and GEFs are also involved in regulating GCP migratory events. *Chn1* expression in the developing and adult cerebellum appears to be restricted to the Purkinje Cell Layer (Buttery et al. 2006; Hall et al. 2001; 1993), making it unlikely that this homologue is substituting function for loss of β-chimaerin in *Chn2*$^{-/-}$ animals. However, with over 80 GEFs and 70 GAP reported in mammals (Azzarelli, Kerloch, and Pacary 2015), there are many potential candidates to regulate Rho GTPases during GCP migration. For example, the RacGAPs Abr and Bcr have been shown to participate in GC migration, although they likely act by regulating glial-scaffold development (Kaartinen et al. 2001). While genetic ablation of Rac1 and Rac3 reduce the overall level of active Rac, *Chn2* deficiency is removing a negative regulator of Rac-GTP function (Riccomagno et al. 2012; Griner et al. 2010), and is probably moving the scale in the other direction. Thus, balanced Rac activity may be essential for proper GCP migration. In this regard, expression of a hyperactive version of β-chimerin from the endogenous *Chn2* locus (the I130A point mutation) was not enough to disrupt GCP migration (**Fig 3.9**). This could be in part due to the regionally and temporally restricted expression of *Chn2* in pre-migratory GCPs or the presence of efficient positive regulators of Rac activity.

This novel role for *Chn2* during cerebellar development is the newest addition to a growing list of functional requirements for these RacGAPs during neuronal

development: chimaerins have been shown to regulate axon guidance, pruning in the hippocampus, and cortical lamination (Beg et al. 2007; Iwasato et al. 2007; Wegmeyer et al. 2007; Kao et al. 2015; Ip et al. 2012; Ferrario et al. 2012; Miyake et al. 2009; Buttery et al. 2006). While in the cortex *Chn1* is required for radial migration of most excitatory neurons (Ip et al. 2012), we show that in the cerebellum *Chn2* is required for migration and positioning of a small subpopulation of GCPs, displaying remarkable specificity. The functional requirement of chimaerins during a variety of developmental processes in a wide array of CNS circuits highlights the importance of this small family of RacGAPs during neural circuit formation. Previous studies have demonstrated that chimaerin function can be modulated by Class 3 Semaphorins during axon guidance and pruning (Ferrario et al. 2012). In particular, β-chimaerin's RacGAP activity has been shown to be regulated by Sema3F/Neuropilin2 function during hippocampal pruning (Riccomagno et al. 2012). Even though cerebellar circuitry proceeds normally in *Sema3F*$^{-/-}$ animals (Matsuda et al. 2010), other Semaphorins and their Plexin receptors have well-established roles during GCP proliferation and migration (Friedel et al. 2007; Kerjan et al. 2005; Renaud et al. 2008; Deng et al. 2007; Maier et al. 2011). It is plausible that some of these other members of the Semaphorin family could mediate β-chimaerin function in GCPs, since it is still mostly unclear how Plexin receptors regulate the actin cytoskeleton (Kerjan et al. 2005; Renaud et al. 2008).

As mentioned above, only a subset of granule cells are susceptible to an arrest in migration in *Chn2*$^{-/-}$ cerebella, while the GC population at large is phenotypically normal. Are these ectopic cells able to recruit the right presynaptic partners in a sea of normally

positioned GCs? The surprising answer to this question appears to be yes. Anterograde labeling of the pons using viral approaches revealed that the ectopic clusters found in *Chn2*$^{-/-}$ cerebella were innervated by pontine axon fibers, one of the normal presynaptic partners for cerebellar GCs (**Fig 3.5**). These ectopic presynaptic terminals are Vglut2^{+} and display the rosette morphology characteristic of normal pontine mossy fibers. Whether these synaptic terminals are mature and active remains to be explored. While the data presented here provides developmental insight into cerebellar circuit assembly at the anatomical level, it is unlikely that the small number of ectopias present in *Chn2*$^{-/-}$ animals will result in obvert physiological or behavioral changes. Far more severe histological defects are observed in other classes of cerebellar mutants without any measurable behavioral motor changes, speaking to the resilience of this model system to regulate and fine-tune motor behaviors (Friedel et al. 2007; Kerjan et al. 2005). Exploration of potentially subtle changes in behavior and physiology in *Chn2*$^{-/-}$ animals will be the subject of future studies.

Materials & Methods

Animals and Genotyping: The day of birth in this study is designated as postnatal (P) day 0. The generation of *Chn2*$^{-/-}$ and *Chn2*$^{I130A/+}$ mice has been described elsewhere (Riccomagno et al. 2012). Genotyping of *Chn2*$^{-/-}$ mice was performed by PCR using the following primers: *Chn2*KO1: 5′-CAGCCTGGTCTACAGAGTGAG-3′; *Chn2*KO2: 5′-GCATTCCACCACTGAGCTAGG-3′; *Chn2*KO3: 5′-GTAGGCTAAGCATTGGCTGGC-3′. Genotyping of the *Chn2*$^{I130A/+}$ knock-in mice was

performed by PCR using the following primers: *Chn2*KIF: 5′-CCAAGCCCAGCTTTAGAGTGGGC-3′; *Chn2*KIR: 5′-GAAGGCCCTCCTTTGCTCTGAG-3′. All animal procedures presented here were performed according to the University of California, Riverside's Institutional Animal Care and Use Committee (IACUC) guidelines. All procedures were approved by UC Riverside IACUC.

Immunohistochemistry: Mice were perfused and fixed with 4% paraformaldehyde for 2 hours at 4 °C, rinsed and sectioned on a vibratome (150 μm). Immunohistochemistry of floating parasagittal cerebellar sections was carried out essentially as described (Polleux and Ghosh 2002). The primary antibodies used were: rabbit anti-calbindin (Swant at 1:2500), anti-parvalbumin (Swant at 1:2000), rabbit anti-calretinin (Swant at 1:2000), chicken anti-βGal (AVES labs at 1:2000), chicken anti-GFP (AVES labs at 1:1000), rabbit anti-GFAP (abcam at 1:1000), guinea pig anti-vGlut2 (Millipore at 1:1000), rabbit anti-GABARα6 (Millipore at 1:1000, discontinued), Mouse anti-GAD67 (Millipore at 1:500), rat anti-L1 (Millipore at 1:500) and mouse anti-pax6 (Developmental Studies Hybridoma Bank at 1:200). Sections were then washed in 1× PBS and incubated with secondary antibodies and DAPI (Molecular Probe at 1:600 and 1:2000, respectively). Sections were washed in PBS and mounted using vectorshield hard-set fluorescence mounting medium (Vector laboratories). Confocal fluorescence images were taken using a Leica SPE II microscope. Area and length were measured using ImageJ. For cell counts, the ImageJ cell counter plugin was used.

***In situ* Hybridization:** *In situ* hybridization was performed on floating cerebellar vibratome sections (150 µm thickness) using digoxigenin-labeled cRNA probes, essentially as described for whole-mount RNA in situ hybridization (H. L. Park et al. 2000). Generation of the Chn2 cRNA probes has been described (Riccomagno et al. 2012).

Injections of AAV-Synapsin-EGFP AAV8 was obtained from the University of North Carolina viral core. The concentrated viral solution (0.2 µl), was delivered into the pons by stereotactic injection (0.25 µl per min), using the following coordinates: anterior-posterior, –5.1 mm; lateral, ± 0.6 mm; and vertical, –4.1 mm. For all injections, Bregma was the reference point.

BrdU labeling: BrdU labeling agent was purchased from Life Technologies (#000103) and was delivered via intraperitoneal injection at 1 ml BrdU solution/100 g animal weight, following manufacturer instructions. Brains were perfused and collected 2hrs post injection for proliferation assessment, or as adults for pulse-chase experiments. Perfused brains were fixed for 2 hours and sectioned on a vibratome (150 µm thickness). Sections underwent antigen retrieval: incubated in 1 M HCl in 1xPBS for 30 mins at room temperature, washed 3 × 10 min in 1xPBS, incubated in 10 mM sodium citrate for 30 min at 80 C, and washed 3 × 10 min in 1xPBS. Following antigen retrieval, immunohistochemistry was performed as described above using a mouse monoclonal antibody anti-BrdU (Invitrogen, clone BU-1, MA3-071 at 1:250).

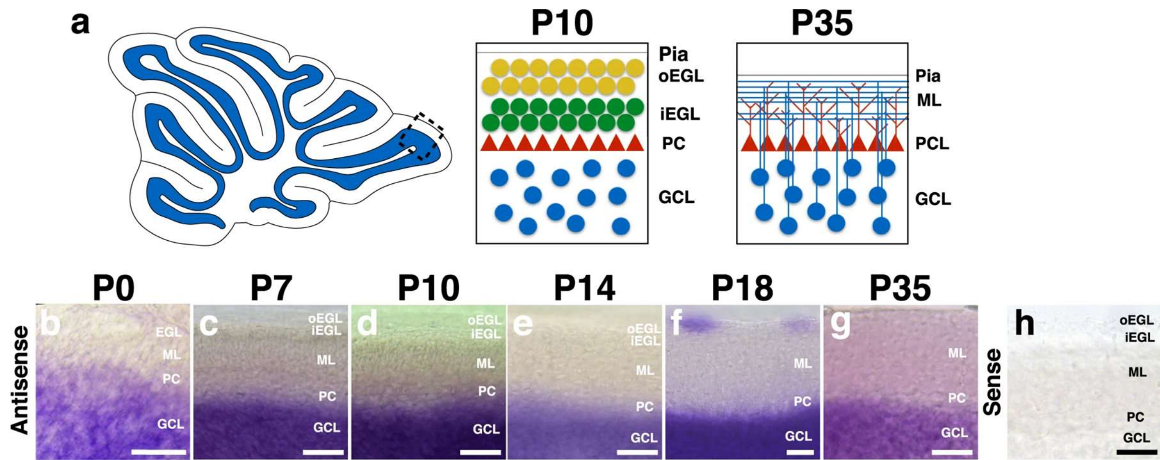
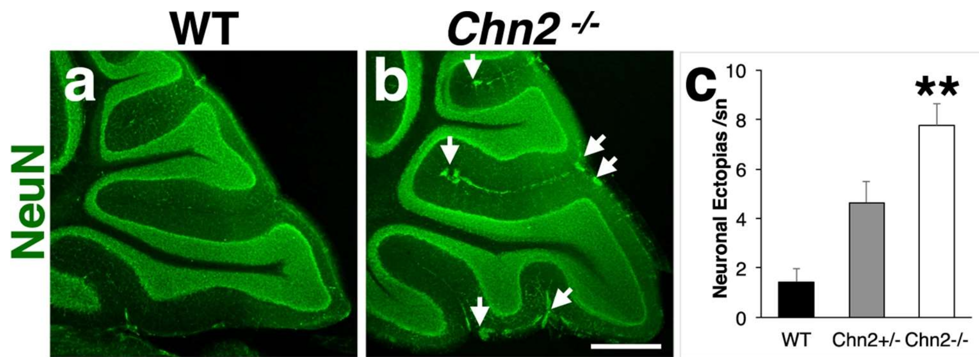
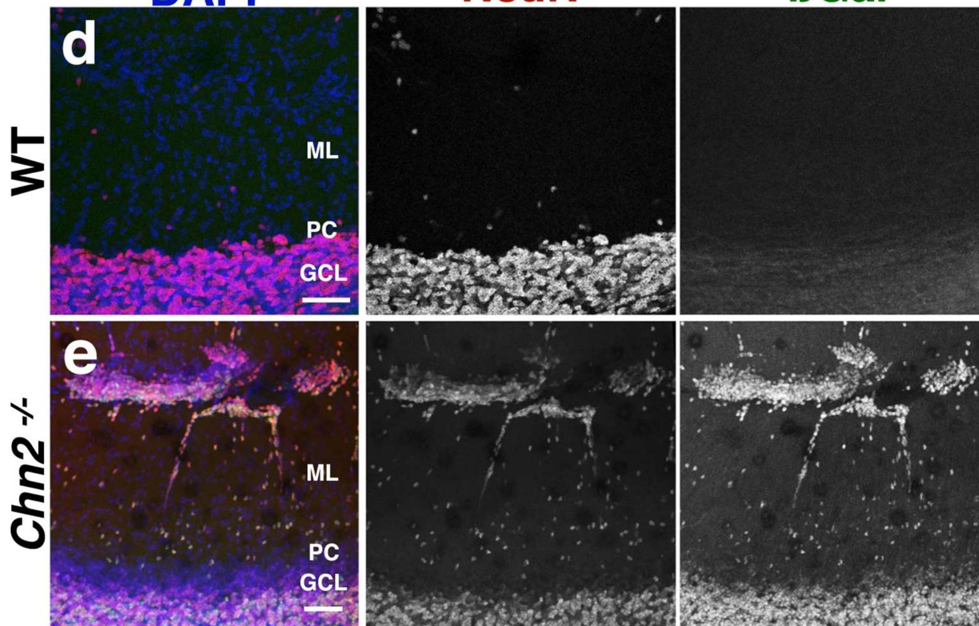


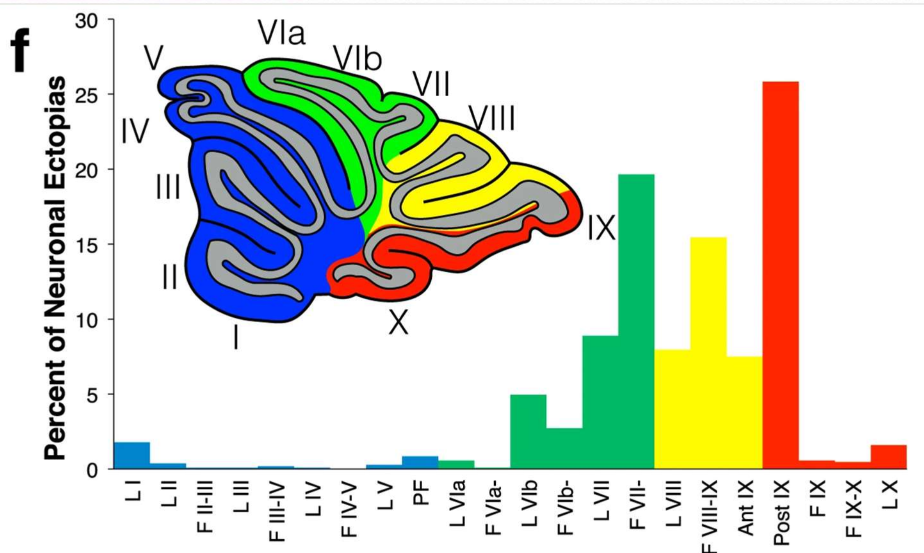
Figure 3.1: β-chimaerin expression in the postnatal cerebellum. (**a**) Developmental maturation of cerebellar granule cells. At early postnatal stages, mitotically active granule cell precursors (GCPs, yellow) populate the outer External Granule Layer (EGL). Postmitotic GCPs (green) move to the inner EGL, where they grow two horizontal processes and migrate tangentially to expand across the surface of the cerebellum. These cells eventually grow a third perpendicular process and begin migrating radially inward along Bergmann glial fibers, past the Purkinje Cell layer (PCL, red triangles), to form the mature inner Granule Cell Layer (GCL). Mature granule cells (blue) extend their axons back to the Molecular Layer (ML) to produce parallel fibers that provide Glutamatergic inputs on Purkinje Cell dendrites. (**b–h**) *In situ* hybridization in *C57/BL6J* mice using a probe against β-chimaerin (*Chn2*) transcript. *Chn2* shows robust expression in the GCL at all postnatal stages. Notably, we detected *Chn2* expression in the EGL at P18 (**f**), but this expression did not persist in adult (**g**) animals. Hybridization with a sense probe does not result in any detectable signal at any of these stages (P14 is shown in **h**). Scale bar, 50 μm for all.



WT
Chn2 -/-
NeuN
a
b
c
Neuronal Ectopias /sn
0
2
4
6
8
10
**
WT
Chn2+/-
Chn2-/-
NeuN ßGal



DAPI
NeuN
ßGal
d
e
WT
Chn2 -/-
ML
PC
GCL



f
Percent of Neuronal Ectopias
0
5
10
15
20
25
30
I
II
III
IV
V
VIa
VIb
VII
VIII
IX
X
L I
L II
F II-III
L III
F III-IV
L IV
F IV-V
L V
PF
L VIa
F VIa-
L VIb
F VIb-
L VII
F VII-
L VIII
F VIII-IX
Ant IX
Post IX
F IX
F IX-X
L X

Figure 3.2: β-chimaerin deficiency causes neuronal ectopic clusters to form along the cerebellar folia in an asymmetrical pattern. (**a,b**) Immunoflourescence of the pan-neuronal marker NeuN in adult (P35) WT and *Chn2*$^{-/-}$ animals. Ectopic clusters of neurons are observed in the ML in *Chn2*$^{-/-}$ animals (white arrows), but these mutants display no other changes in overall cerebellar structure. Scale bar, 500 μm. (**c**) Quantification of the average number of neuronal ectopias per 150 μm section across genotypes. There is a highly significant difference among the three genotypes ($n = 9$; One-way ANOVA, $p = 4.4996e\text{-}05$). While there appears to be a step-wise increase in the average number of ectopias found in *Chn2*$^{+/+}$, *Chn2*$^{+/-}$, and *Chn2*$^{-/-}$ mice, only *Chn2*$^{-/-}$ show a significant increase in the frequency of ectopias as compared to *Chn2*$^{+/+}$ and *Chn2*$^{+/-}$ animals (**$p < 0.01$ for both comparisons, Tukey HSD test). Error bars represent SEM. (**d,e**) Immunoflourescence with antibodies recognizing both NeuN and betagalactosidase (βgal) reveal that these neuronal ectopias strongly co-label with both markers. (**e**) Schematic and graph representing the percent distribution of ectopic clusters across the cerebellum in *Chn2*$^{-/-}$ animals. The cerebellum may be divided into four principle regions: Anterior (blue), Central (Green), Posterior (Yellow), and Nodal (Red); each region may be further divided into several individual folds, or Lobules (I–X). We found that ectopias most commonly occur in posterior and nodal lobules and fissures, with enrichment in the fissure separating lobules VII–VIII and on the posterior side of lobule IX (collectively accounting for 45% of all ectopias scored; $n = 1068$ ectopias across nine animals).

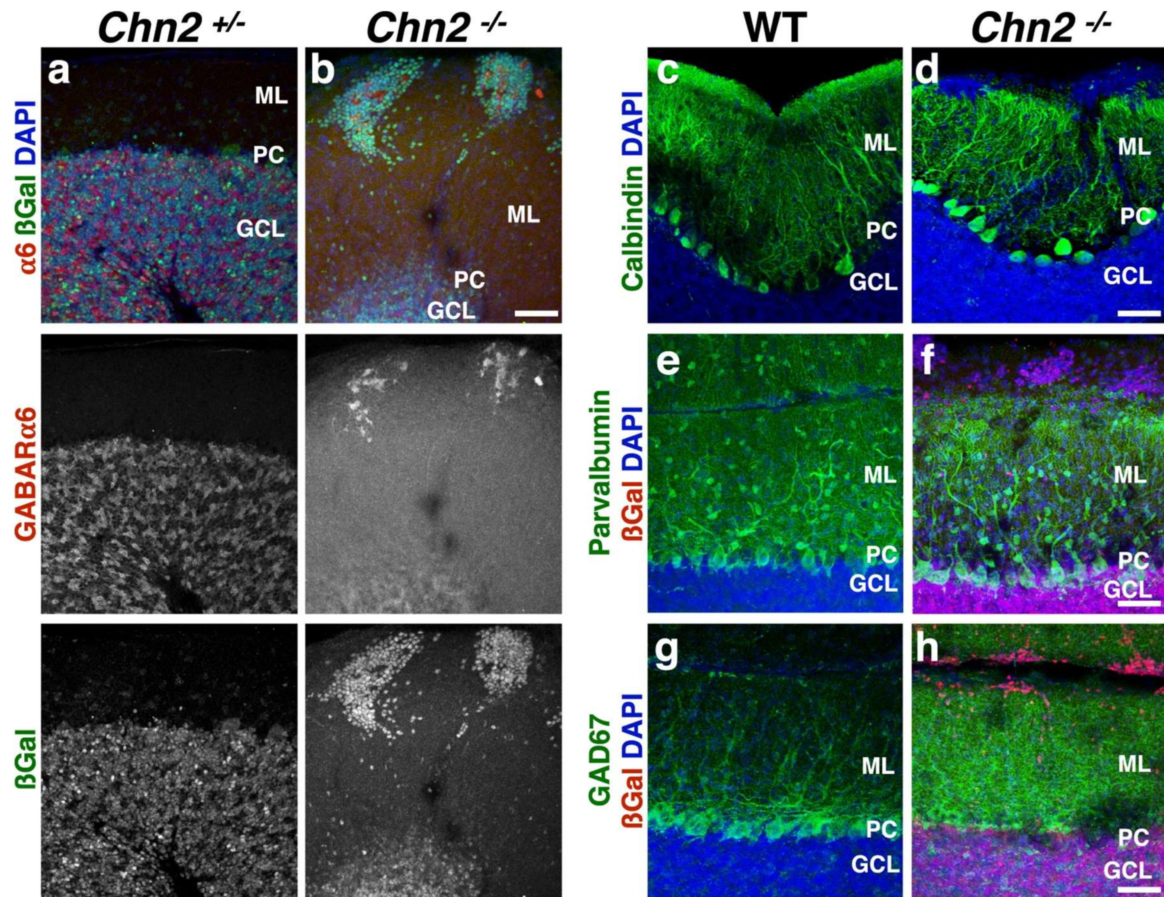


Figure 3.3: Ectopic clusters contain mature granule cells, but not other types of cerebellar neurons. (**a,b**) Immunofluoresence of the mature granule cell-specific marker GABARa6, with βgal and DAPI as counterstains. βgal positive ectopias contain large numbers of differentiated granule cells. (**c–h**) Immunofluorescence for the PC marker Calbindin (**c,d**) and the general interneuron markers Parvalbumin (**e,f**) and GAD67 (**g,h**), with βgal and DAPI as counterstains. Neuronal ectopias do not co-label with any of these three markers and therefore do not contain PCs, stellate, or basket cells that normally occupy the ML. Interestingly, Purkinje cell dendrites appear to avoid invading the clusters. Scale bar, 50 μm for all.

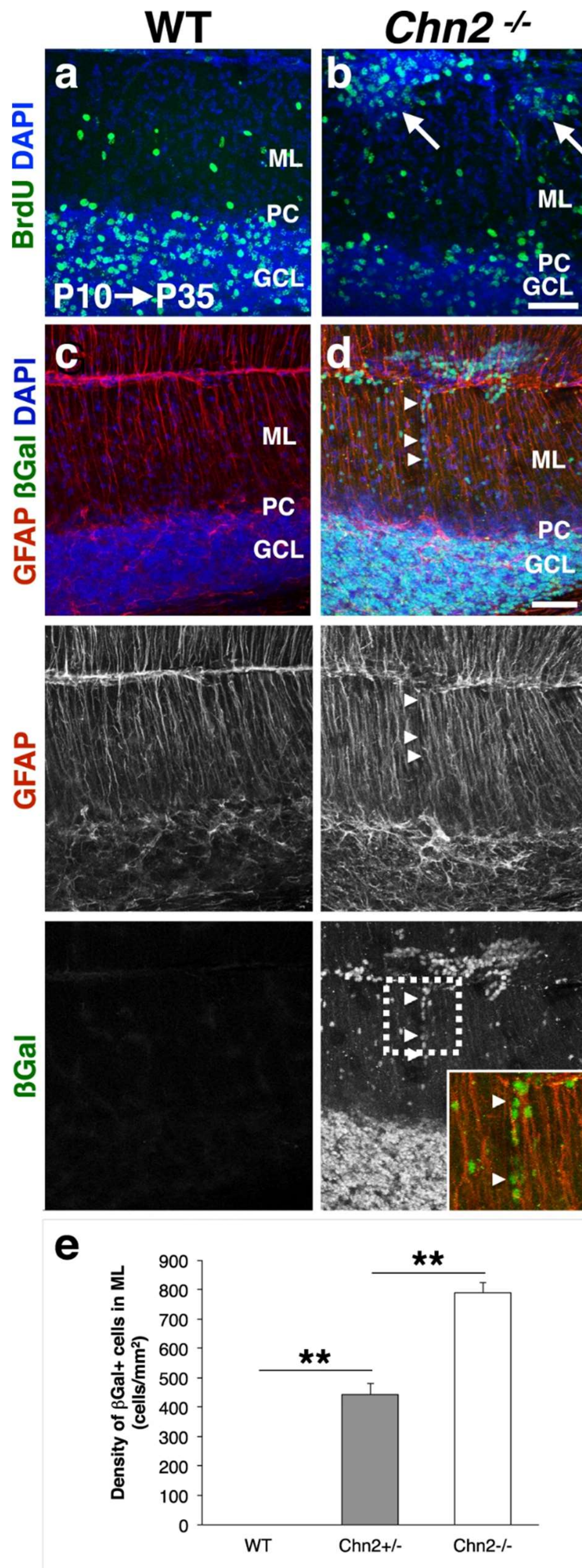


Figure 3.4: Arrested migration of GCs in β-chimaerin deficient animals. (**a,b**) Cerebellar BrdU pulse labeling at P10, collected at P35. $BrdU^{+}$ cells accumulate in the ectopic clusters present in *Chn2*$^{-/-}$ mutants (white arrows). (**c,d**) Immunofluorescence for βgal and the glial cell marker GFAP, with DAPI as counterstain. The Bergmann Glial scaffold, which radially migrating GCPs adhere to during their migration from the iEGl to GCL, does not appear disrupted in *Chn2*$^{-/-}$ mutants. Of note, βgal immunoreactive cells may be seen collected on individual glial tracts (white arrowheads), suggesting some β-chimaerin deficient GCPs may initiate but fail to complete radial migration. Insert shows a higher magnification view of the dotted area (green: βgal; red: GFAP). Scale bars, 50 μm. (**e**) Quantification of βgal^{+} cells arrested in the molecular layer of WT, *Chn2*$^{+/-}$ and *Chn2*$^{-/-}$ adult mice. One-way ANOVA, $p = 1.1102e\text{-}16$, $n = 10$. $^{**}p < 0.01$, Tukey HSD test. Error bars represent SEM.

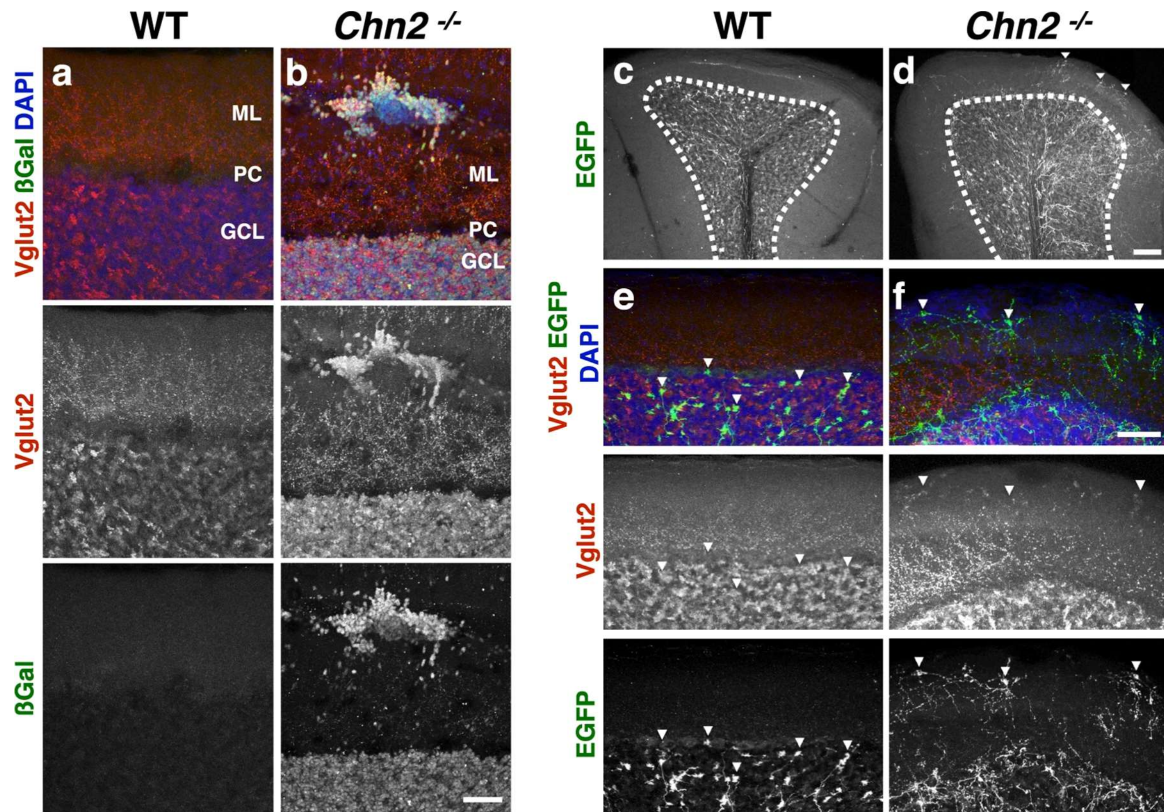


Figure 3.5: Neuronal Ectopias are contacted by pontine mossy fibers. (**a,b**) Immunofluorescence of the presynaptic marker glutamate vesicular transporter (Vglut2), and βgal, with DAPI as counterstains. 100% of the ectopias robustly label with Vglut2, suggesting they may form synapses with mossy fibers. Scale bar, 50 μm. (**c,d**) Injection of AAV-Syn-EGFP into the pons reveals that neuronal ectopias are innervated by aberrant mossy fibers. AAV-syn-EGFP injections into the pontine nucleus of adult (P35) animals label mossy fibers innervating the cerebellar cortex. We found that mossy fibers improperly projected into the ML in *Chn2*$^{-/-}$ animals (white arrowheads). Scale bar, 100 μm. (**e,f**) Higher-resolution image showing that these mossy fibers make direct contact with neuronal ectopias and are surrounded by Vglut2^{+} processes. Scale bar, 50 μm.

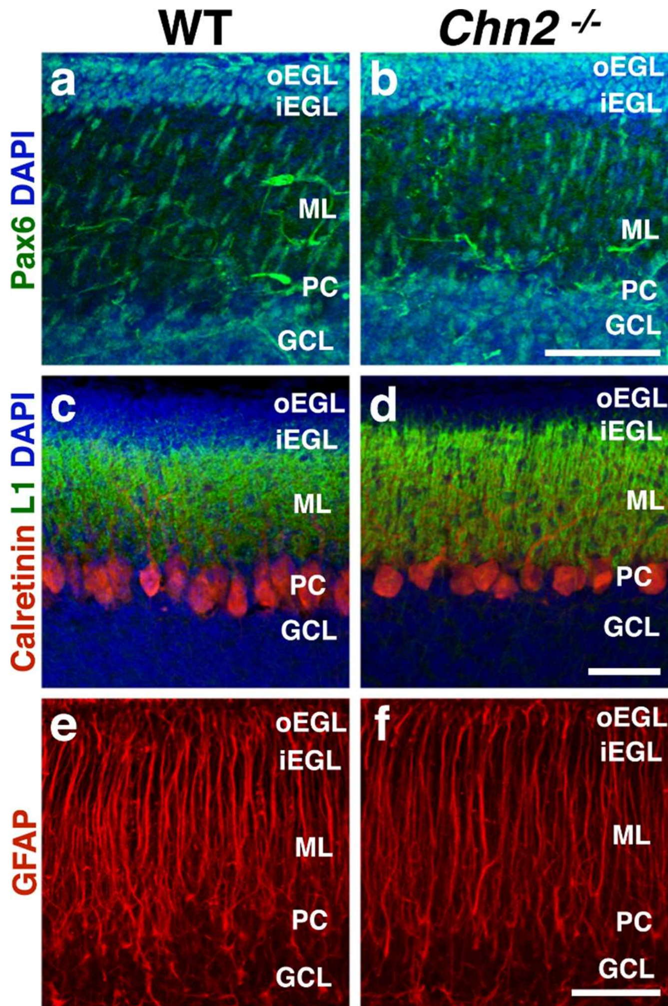


Figure 3.6: Early postnatal cerebellar structure is unaltered in β-chimaerin deficient animals. (**a**–**d**) Immunofluorescence on early postnatal (P10) *Chn2*$^{-/-}$ mutants with an antibody targeting the transcription factor Pax6, which identifies both GCPs in the EGL as well as mature GCs in the GCL (**a**,**b**) or the cell adhesion molecule NCAM-L1 (L1), which labels migrating GCPs in the iEGL (**c**,**d**). At these early postnatal stages, neither Pax6 nor L1 reveal any differences in GCP distribution. (**e**,**f**) Immunofluorescence with the glial marker GFAP. The Bergmann glial scaffold appears unaffected. Scale bar, 50 µm for **a–d**; 25 µm for **e–f**.

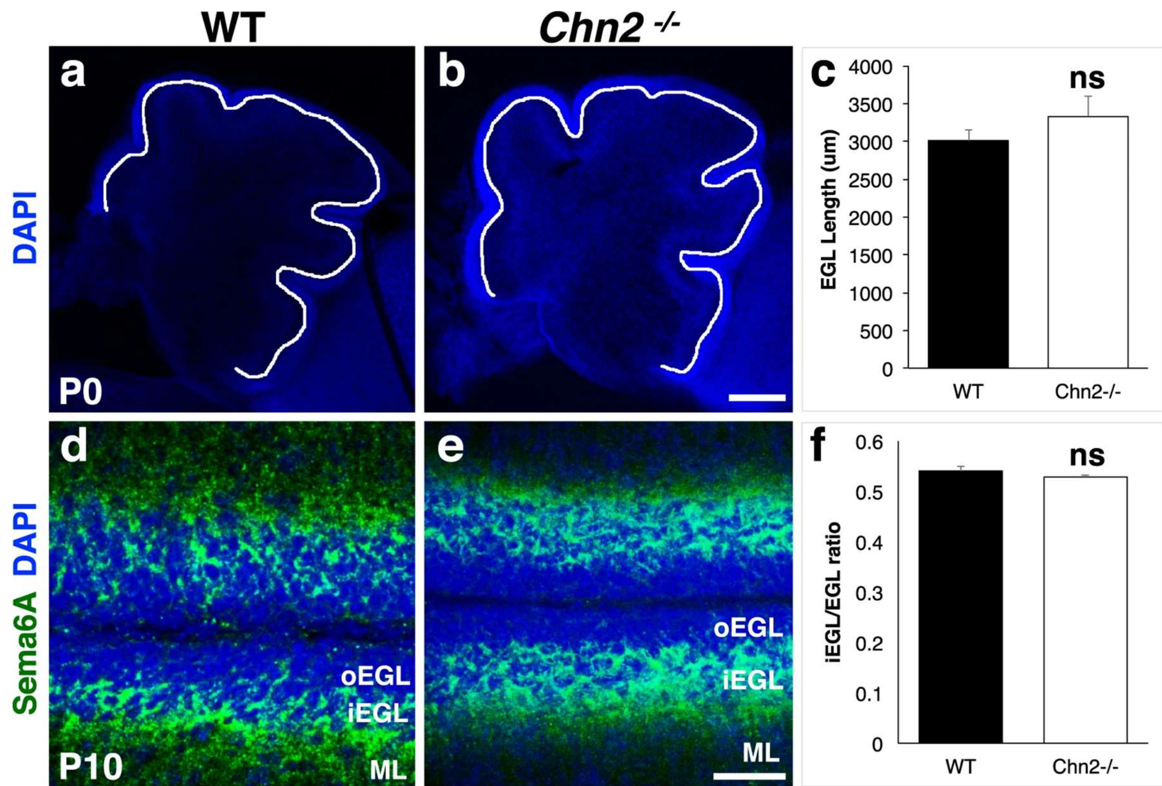


Figure 3.7: Tangential migration is unaffected in β-chimaerin deficient mice. (**a–c**) Measurement of EGL length in P0 WT (**a**) and *Chn2*$^{-/-}$ (**b**) animals. Scale bar, 200 μm. (**c**) Quantification of EGL length. No difference was observed between groups (two-tail t-test, n = 5; p = 0.082). (**d,e**) Immunostaining for the iEGL marker Sema6A in WT (**d**) and *Chn2*$^{-/-}$ (**e**) P10 cerebella. Scale bar, 25 μm (**f**) Quantification of iEGL (Sema6A^{+}) thickness, relative to overall EGL thickness. Two-tail t-test, n = 10; p = 0.528. Error bars represent SEM.

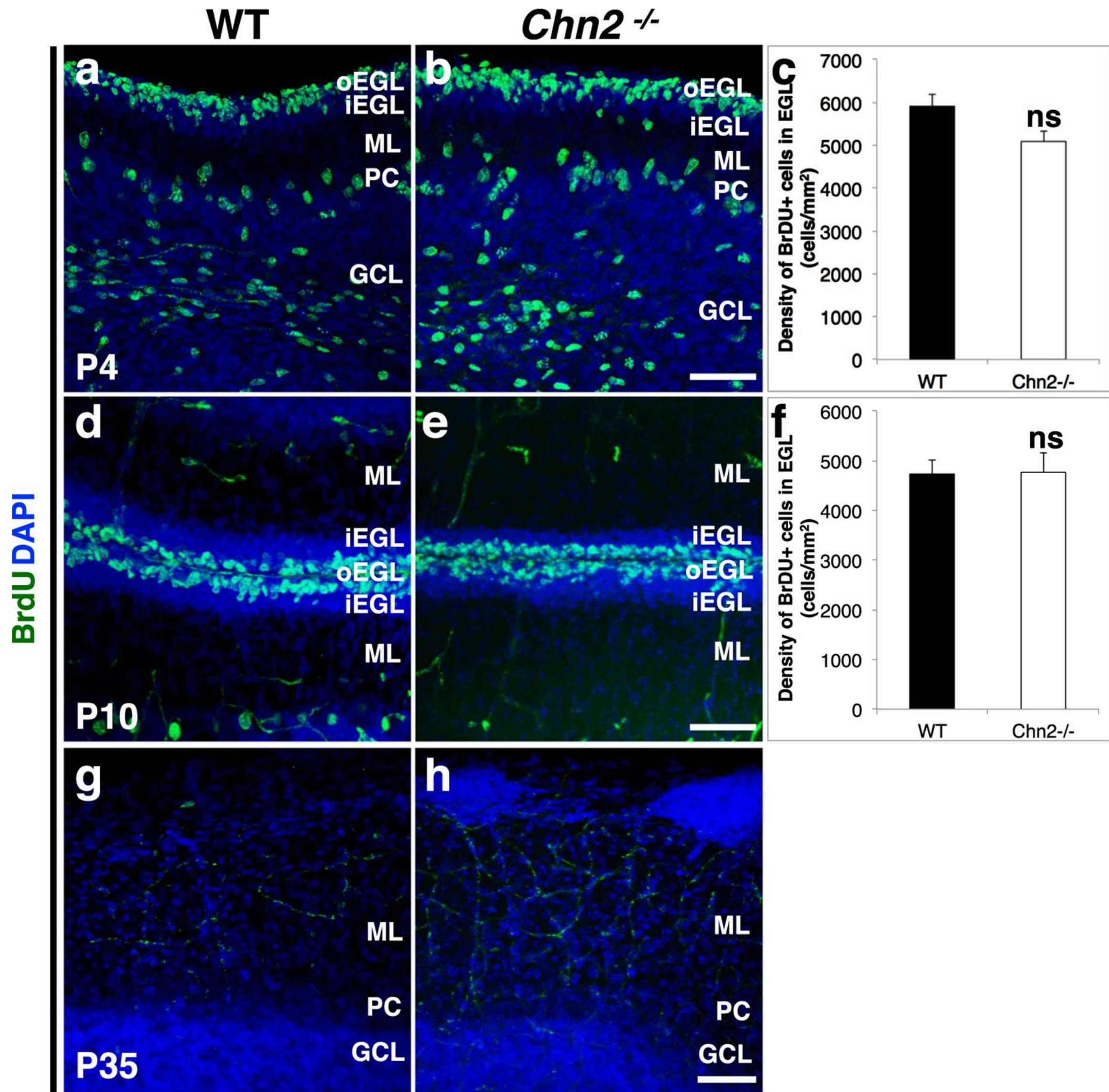


Figure 3.8: Cell proliferation in β-chimaerin deficient animals. (**a–h**) BrdU was injected into either early postnatal stages (P4 and P10, **a,b,d,e**) or adult (P35, **g,h**) and allowed to incorporate for 2 hours prior to animal collection. (**c,f**) Quantification of BrdU pulse-chase experiments. We found there is no significant difference in the density of proliferating, BrdU$^+$ GCPs in the oEGL between *Chn2*$^{-/-}$ and WT animals (n = 5 animals per genotype, per stage, 4–5 sections 4 μm-thick per animal, two-tail t-test; P4: p = 0.32; P10: p = 0.952). In adult (P35) animals, neuronal ectopias do not contain proliferating cells, suggesting that they are composed entirely of postmitotic GCs (**h**). Scale bar, 50 μm for all. Error bars represent SEM.

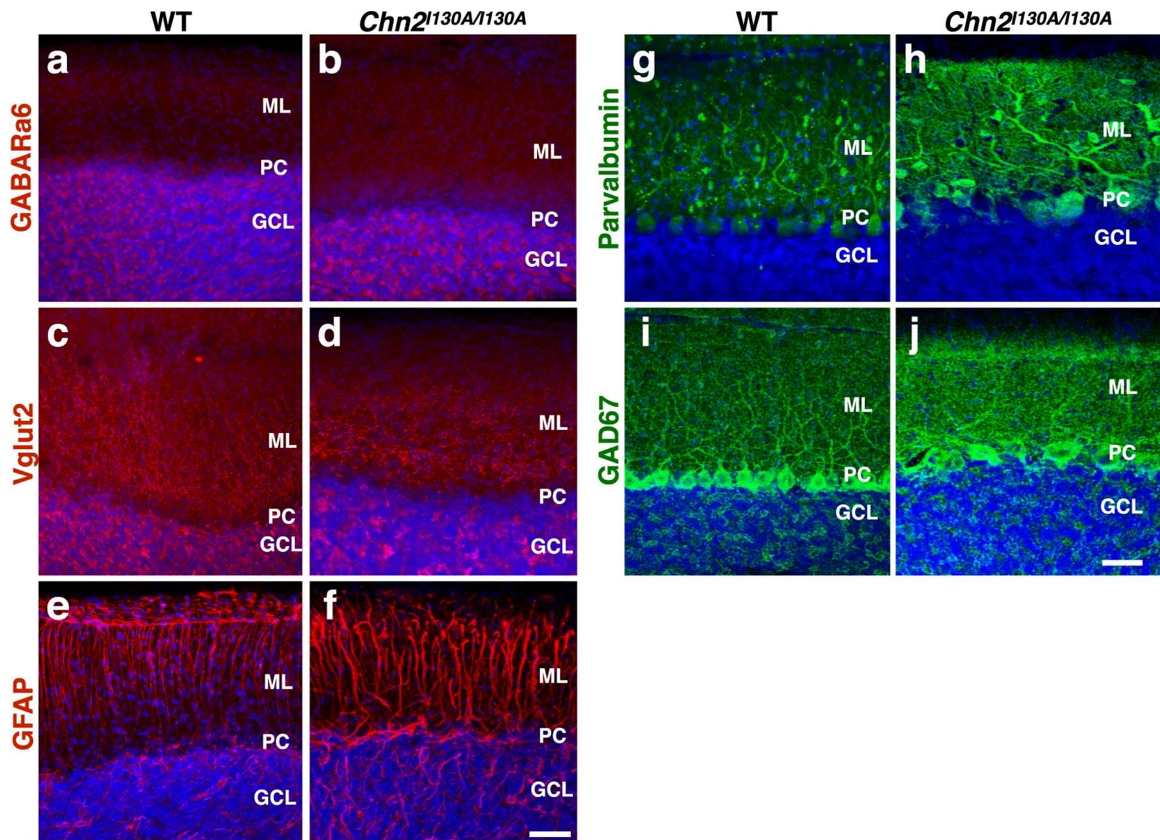


Figure 3.9: Cerebellar structure is unaffected in β-chimaerin hyperactive mutants. (**a**–**j**) Histological analysis of adult (P35) cerebellar structure in WT mice and mice homozygous for a hyperactive allele of the β-chimaerin gene (*Chn2*$^{I130A/I130A}$). We observe no notable differences in the mature granule cell marker GABARα6 (**a,b**), the glutamatergic synaptic marker Vglut2 (**c,d**), the glial marker GFAP (**e,f**), or the interneuron markers Parvalbumin (**g,h**) and GAD67 (**i,j**). Scale bar, 50 μm for all.

SUMMARY & CONCLUSION

Proper development of the Central Nervous System (CNS) requires that neurons make appropriate connections with their synaptic partners. To ensure this, neurons in the developing brain must migrate from their sites of germination to their final structures, guided by a broad suite of guidance cues(H. T. Park, Wu, and Rao 2002). Proper connectivity between neurons and structures is achieved through the regulated guidance of axons, followed by the establishment and refinement of synaptic contacts (Kolodkin and Tessier-Lavigne 2011). The tight spatiotemporal regulation of cellular adhesion is crucial to coordinate each of these activities; in each case neurons must tightly adhere to the substrate to anchor themselves and generate traction forces, detach themselves from the substrate once advanced, and cyclically regulate these two processes to ensure processive movement until the destination is reached (Webb, Parsons, and Horwitz 2002; Moreland and Poulain 2022; Craig 2018). In order to gain a deeper understanding of the cytoplasmic effectors that link extracellular signals to changes in adhesion and cytoskeletal organization during neuronal migration and axon pathfinding, I investigated the roles of cytoplasmic adaptors and regulators, and designed a tool to monitor adhesion signaling events, outlined in the three chapters presented.

In Chapter 1, I established the role of Crk-Associated Substrate (Cas) family of cytosolic adapter proteins during cortical axon guidance and fasciculation. During development, the timely regulation of growth cone adhesion to its substrate is vital to ensuring proper neuronal responses to guidance cues. While many ECM ligands and their cognate receptors have been identified, less is known about how these signals are relayed

to the effectors of the cytoskeletal network of the cell. Using conditional mouse genetics, I provide evidence that Cas proteins are required cell-autonomously for proper fasciculation of the Anterior Commissure, an evolutionarily conserved forebrain tract that, in mice, mediates reciprocal connections between the piriform and entorhinal cortices of the two hemispheres and the olfactory bulbs (Fenlon et al. 2021). Further, I provide evidence that Cas proteins are required cortical-autonomously to regulate the entry of thalamocortical projections originating in the thalamus (Molnár et al. 2012), by ensuring proper lamination of the cortex and integrity of the subplate, an intermediate target used by thalamic axons that regulates their entry into the cortex (Allendoerfer and Shatz 1994). These data provide novel functions for the Cas family of signal adapter proteins during CNS development, and build on a growing body of literature implicating them in axonal fasciculation and guidance downstream of multiple receptors (Z. Huang et al. 2007; Vahedi-Hunter et al. 2018; Wong et al. 2022)

In Chapter 2, I present the development of a novel tool to probe Integrin Adhesion Compex (IAC) dynamics, which we name pYFAK BiLuc. Using a bi-molecular complementation approach, we design a set of genetically-encoded probes to reconstitute firefly luciferase downstream of IAC signaling. We provide evidence that these probes are properly recruited to IAC complexes, are responsive to canonical integrin ligands, and are specific to phoshotyrosine activation of Focal Adhesion Kinase (FAK), one of the earliest observable events in IAC assembly (L. Kornberg et al. 1992; Miyamoto et al. 1995; Webb, Brown, and Horwitz 2003; Zaidel-Bar et al. 2003). This genetically-encoded set of probes fulfills a current need for a tool that provides simple, quantifiable,

and sensitive reporting of IAC signaling, with the potential to be expanded for high-throughput drug screens, genetic screens, or other *in vivo* or *in vitro* applications in both basic research and clinical settings.

Finally, in Chapter 3 I established the requirement for RacGAP β-chimaerin during cerebellar granule cell progenitor (GCP) radial migration. During development, neurons must often navigate complex routes from their points of origin to their final sites of connection, guided by many classes of guidance molecules and adopting diverse locomotive strategies (H. T. Park, Wu, and Rao 2002; Valiente and Marín 2010). This is true of cerebellar granule cells, which sequentially undergo a glial-independent, tangential migration and gliophilic, radial migration during their navigation from the External Germinal Layer (EGL) inward towards the mature Granule Cell Layer (EGL) (Leto et al. 2016). The cellular pathways that orchestrate such complex locomotive decisions are poorly understood, but the Rho family of GTPases have been shown to influence cell polarity and cytoskeletal dynamics in response to neuronal guidance cues (Stankiewicz and Linseman 2014; Bai et al. 2015), making them attractive targets in the interplay between adhesion and guidance signaling. In this chapter, I show *in vivo* evidence that the negative regulator of Rac1 GTPase, β-chimaerin, is required for proper lamination of the cerebellar cortex. Mutants for β-chimaerin accumulate ectopic clusters of neurons on the surface of the cerebellar folia. I demonstrate these clusters are composed principally of post-mitotic cerebellar granule cells and not other types of cerebellar neurons. I go on to show that these ectopias express mature synaptic markers and recruit mossy fiber afferents from the pontine nucleus. Interestingly, examination of

mutants containing a hyperactive allele for β-chimaerin have no gross deficits in cerebellar lamination, suggesting that the correct balance of Rac GTPase activity is required for proper GCP migratory events.

Collectively, these studies shed light on how neurons interpret extracellular signals to modulate cellular adhesion and cytoskeletal remodeling pathways during neuronal migration and axonal guidance events. Given the potential for these proteins as integrators of diverse guidance cues, our work opens new avenues for further study.

REFERENCES

Abudureyimu, Shaniya, Naoya Asai, Atsushi Enomoto, Liang Weng, Hiroki Kobayashi, Xiaoze Wang, Chen Chen, Shinji Mii, and Masahide Takahashi. 2018. "Essential Role of Linx/Islr2 in the Development of the Forebrain Anterior Commissure." *Scientific Reports* 8 (1): 7292. https://doi.org/10.1038/s41598-018-24064-0.

Acebrón, Iván, Ricardo D Righetto, Christina Schoenherr, Svenja Buhr, Pilar Redondo, Jayne Culley, Carlos F Rodríguez, et al. 2020. "Structural Basis of Focal Adhesion Kinase Activation on Lipid Membranes." *The EMBO Journal* 39 (19): 1–21. https://doi.org/10.15252/embj.2020104743.

Agochiya, Mahima, Valerie G. Brunton, Dewi W. Owens, E. Kenneth Parkinson, Christos Paraskeva, W. Nicol Keith, and Margaret C. Frame. 1999. "Increased Dosage and Amplification of the Focal Adhesion Kinase Gene in Human Cancer Cells." *Oncogene* 18 (41): 5646–53. https://doi.org/10.1038/sj.onc.1202957.

Alexander, Nelson R., Kevin M. Branch, Aron Parekh, Emily S. Clark, Izuchukwu C. Iwueke, Scott A. Guelcher, and Alissa M. Weaver. 2008. "Extracellular Matrix Rigidity Promotes Invadopodia Activity." *Current Biology* 18 (17): 1295–99. https://doi.org/10.1016/j.cub.2008.07.090.

Allendoerfer, K L, and C J Shatz. 1994. "The Subplate, a Transient Neocortical Structure: Its Role in the Development of Connections between Thalamus and Cortex." *Annual Review of Neuroscience* 17: 185–218. https://doi.org/10.1146/annurev.ne.17.030194.001153.

Antoniades, Ioanna, Maria Kyriakou, Anna Charalambous, Katerina Kalalidou, Andri Christodoulou, Maria Christoforou, and Paris A Skourides. 2021. "FAK Displacement from Focal Adhesions: A Promising Strategy to Target Processes Implicated in Cancer Progression and Metastasis." *Cell Communication and Signaling* 19 (1): 3. https://doi.org/10.1186/s12964-020-00671-1.

Arold, Stefan T., Maria K. Hoellerer, and Martin E.M. Noble. 2002. "The Structural Basis of Localization and Signaling by the Focal Adhesion Targeting Domain." *Structure* 10 (3): 319–27. https://doi.org/10.1016/S0969-2126(02)00717-7.

Askari, Janet A., Christopher J. Tynan, Stephen E.D. Webb, Marisa L. Martin-Fernandez, Christoph Ballestrem, and Martin J. Humphries. 2010. "Focal Adhesions Are Sites of Integrin Extension." *Journal of Cell Biology* 188 (6): 891–903. https://doi.org/10.1083/jcb.200907174.

Ayoub, Albert E, and Ivica Kostovic. 2009. "New Horizons for the Subplate Zone and Its Pioneering Neurons." *Cerebral Cortex (New York, N.Y. : 1991)*. United States.

https://doi.org/10.1093/cercor/bhp025.

Azad, Taha, Amin Tashakor, and Saman Hosseinkhani. 2014. “Split-Luciferase Complementary Assay: Applications, Recent Developments, and Future Perspectives.” *Analytical and Bioanalytical Chemistry* 406 (23): 5541–60. https://doi.org/10.1007/s00216-014-7980-8.

Azzarelli, Roberta, Thomas Kerloch, and Emilie Pacary. 2015. “Regulation of Cerebral Cortex Development by Rho GTPases: Insights from in Vivo Studies.” *Frontiers in Cellular Neuroscience* 8 (January): 1–18. https://doi.org/10.3389/fncel.2014.00445.

Bagri, Anil, Oscar Marín, Andrew S Plump, Judy Mak, Samuel J Pleasure, John L R Rubenstein, and Marc Tessier-Lavigne. 2002. “Slit Proteins Prevent Midline Crossing and Determine the Dorsoventral Position of Major Axonal Pathways in the Mammalian Forebrain.” *Neuron* 33 (2): 233–48. https://doi.org/10.1016/s0896-6273(02)00561-5.

Bahi-Buisson, Nadia, and Renzo Guerrini. 2013. “Diffuse Malformations of Cortical Development.” *Handbook of Clinical Neurology* 111: 653–65. https://doi.org/10.1016/B978-0-444-52891-9.00068-3.

Bai, Yanyang, Xiaoliang Xiang, Chunmei Liang, and Lei Shi. 2015. “Regulating Rac in the Nervous System: Molecular Function and Disease Implication of Rac GEFs and GAPs.” *BioMed Research International* 2015. https://doi.org/10.1155/2015/632450.

Ballestrem, Christoph, Noam Erez, Joachim Kirchner, Zvi Kam, Alexander Bershadsky, and Benjamin Geiger. 2006. “Molecular Mapping of Tyrosine-Phosphorylated Proteins in Focal Adhesion Using Flourescence Resonance Energy Transfer.” *Journal of Cell Science* 119 (5): 866–75. https://doi.org/10.1242/jcs.02794.

Ballestrem, Christoph, Boris Hinz, Beat A. Imhof, and Bernhard Wehrle-Haller. 2001. “Marching at the Front and Dragging behind: Differential AVβ3-Integrin Turnover Regulates Focal Adhesion Behavior.” *Journal of Cell Biology* 155 (7): 1319–32. https://doi.org/10.1083/jcb.200107107.

Bargon, Sharmilla D., Peter W. Gunning, and Geraldine M. O’Neill. 2005. “The Cas Family Docking Protein, HEF1, Promotes the Formation of Neurite-like Membrane Extensions.” *Biochimica et Biophysica Acta - Molecular Cell Research* 1746 (2): 143–54. https://doi.org/10.1016/j.bbamcr.2005.10.008.

Barkovich, Matthew J. 2022. “Pediatric Brain Maturation and Migration Disorders.” *Diagnostics (Basel, Switzerland)* 12 (5). https://doi.org/10.3390/diagnostics12051123.

Bartsch, S, U Bartsch, U Dörries, A Faissner, A Weller, P Ekblom, and M Schachner. 1992. “Expression of Tenascin in the Developing and Adult Cerebellar Cortex.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 12 (3): 736–49. https://doi.org/10.1523/JNEUROSCI.12-03-00736.1992.

Becker, E, U Huynh-Do, S Holland, T Pawson, T O Daniel, and E Y Skolnik. 2000. “Nck-Interacting Ste20 Kinase Couples Eph Receptors to c-Jun N-Terminal Kinase and Integrin Activation.” *Molecular and Cellular Biology* 20 (5): 1537–45. https://doi.org/10.1128/MCB.20.5.1537-1545.2000.

Beg, Asim A., Julia E. Sommer, John H. Martin, and Peter Scheiffele. 2007. “A2-Chimaerin Is an Essential EphA4 Effector in the Assembly of Neuronal Locomotor Circuits.” *Neuron* 55 (5): 768–78. https://doi.org/10.1016/j.neuron.2007.07.036.

Bellion, Arnaud, Jean-Pierre Baudoin, Chantal Alvarez, Michel Bornens, and Christine Métin. 2005. “Nucleokinesis in Tangentially Migrating Neurons Comprises Two Alternating Phases: Forward Migration of the Golgi/Centrosome Associated with Centrosome Splitting and Myosin Contraction at the Rear.” *The Journal of Neuroscience* 25 (24): 5691 LP – 5699. https://doi.org/10.1523/JNEUROSCI.1030-05.2005.

Belvindrah, Richard, Diana Graus-Porta, Sandra Goebbels, Klaus Armin Nave, and Ulrich Müller. 2007. “B1 Integrins in Radial Glia But Not in Migrating Neurons Are Essential for the Formation of Cell Layers in the Cerebral Cortex.” *Journal of Neuroscience* 27 (50): 13854–65. https://doi.org/10.1523/JNEUROSCI.4494-07.2007.

Beningo, K A, M Dembo, I Kaverina, J V Small, and Y L Wang. 2001. “Nascent Focal Adhesions Are Responsible for the Generation of Strong Propulsive Forces in Migrating Fibroblasts.” *The Journal of Cell Biology* 153 (4): 881–88. https://doi.org/10.1083/jcb.153.4.881.

Bernards, André. 2003. “GAPs Galore! A Survey of Putative Ras Superfamily GTPase Activating Proteins in Man and Drosophila.” *Biochimica et Biophysica Acta* 1603 (2): 47–82. https://doi.org/10.1016/s0304-419x(02)00082-3.

Bixby, J L, and W A Harris. 1991. “Molecular Mechanisms of Axon Growth and Guidance.” *Annual Review of Cell Biology* 7: 117–59. https://doi.org/10.1146/annurev.cb.07.110191.001001.

Blaess, Sandra, Diana Graus-Porta, Richard Belvindrah, Randor Radakovits, Sebastian Pons, Amanda Littlewood-Evans, Mathias Senften, et al. 2004. “B1-Integrins Are Critical for Cerebellar Granule Cell Precursor Proliferation.” *The Journal of Neuroscience* 24 (13): 3402 LP – 3412. https://doi.org/10.1523/JNEUROSCI.5241-

03.2004.

Blümcke, Ingmar, Maria Thom, Eleonora Aronica, Dawna D Armstrong, Harry V Vinters, Andre Palmini, Thomas S Jacques, et al. 2011. "The Clinicopathologic Spectrum of Focal Cortical Dysplasias: A Consensus Classification Proposed by an Ad Hoc Task Force of the ILAE Diagnostic Methods Commission." *Epilepsia* 52 (1): 158–74. https://doi.org/10.1111/j.1528-1167.2010.02777.x.

Bonner, J, and T P O'Connor. 2001. "The Permissive Cue Laminin Is Essential for Growth Cone Turning in Vivo." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 21 (24): 9782–91. https://doi.org/10.1523/JNEUROSCI.21-24-09782.2001.

Borghesani, Paul R, Jean Michel Peyrin, Robyn Klein, Joshua Rubin, Alexandre R Carter, Phillip M Schwartz, Andrew Luster, Gabriel Corfas, and Rosalind a Segal. 2002. "BDNF Stimulates Migration of Cerebellar Granule Cells." *Development (Cambridge, England)* 129 (6): 1435–42.

Bourgin, Caroline, Keith K. Murai, Melanie Richter, and Elena B. Pasquale. 2007. "The EphA4 Receptor Regulates Dendritic Spine Remodeling by Affecting Β1-Integrin Signaling Pathways." *Journal of Cell Biology* 178 (7): 1295–1307. https://doi.org/10.1083/jcb.200610139.

Bouton, Amy H., Rebecca B. Riggins, and Pamela J. Bruce-Staskal. 2001. "Functions of the Adapter Protein Cas: Signal Convergence and the Determination of Cellular Responses." *Oncogene* 20 (44): 6448–58. https://doi.org/10.1038/sj.onc.1204785.

Bozyczko, D, and A F Horwitz. 1986. "The Participation of a Putative Cell Surface Receptor for Laminin and Fibronectin in Peripheral Neurite Extension." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 6 (5): 1241–51. https://doi.org/10.1523/JNEUROSCI.06-05-01241.1986.

Brakebusch, Cord, and Reinhard Fässler. 2003. "The Integrin-Actin Connection, an Eternal Love Affair." *The EMBO Journal* 22 (10): 2324–33. https://doi.org/10.1093/EMBOJ/CDG245.

Brami-Cherrier, Karen, Nicolas Gervasi, Diana Arsenieva, Katarzyna Walkiewicz, Marie Claude Boutterin, Alvaro Ortega, Paul G. Leonard, et al. 2014. "FAK Dimerization Controls Its Kinase-Dependent Functions at Focal Adhesions." *EMBO Journal* 33 (4): 356–70. https://doi.org/10.1002/embj.201386399.

Braniš, Jaroslav, Csilla Pataki, Marina Spörrer, Richard C Gerum, Astrid Mainka, Vladimir Cermak, Wolfgang H Goldmann, Ben Fabry, Jan Brabek, and Daniel Rosel. 2017. "The Role of Focal Adhesion Anchoring Domains of CAS in

Mechanotransduction.” *Scientific Reports* 7 (April): 46233. https://doi.org/10.1038/srep46233.

Bray, D, and J G White. 1988. “Cortical Flow in Animal Cells.” *Science* 239 (4842): 883–88. https://doi.org/10.1126/science.3277283.

Breau, Marie Anne, and Alain Trembleau. 2022. “Chemical and Mechanical Control of Axon Fasciculation and Defasciculation.” *Seminars in Cell & Developmental Biology*. https://doi.org/https://doi.org/10.1016/j.semcdb.2022.06.014.

Brod, Florian, and Reinhard Fässler. 2020. “ A FAK Conundrum Is Solved: Activation and Organization of Focal Adhesion Kinase at the Plasma Membrane .” *The EMBO Journal* 39 (19): 1–3. https://doi.org/10.15252/embj.2020106234.

Brown, M. 2004. “ 2-Chimaerin, Cyclin-Dependent Kinase 5/P35, and Its Target Collapsin Response Mediator Protein-2 Are Essential Components in Semaphorin 3A-Induced Growth-Cone Collapse.” *Journal of Neuroscience* 24 (41): 8994–9004. https://doi.org/10.1523/JNEUROSCI.3184-04.2004.

Burnette, Dylan T, Lin Ji, Andrew W Schaefer, Nelson A Medeiros, Gaudenz Danuser, and Paul Forscher. 2008. “Myosin II Activity Facilitates Microtubule Bundling in the Neuronal Growth Cone Neck.” *Developmental Cell* 15 (1): 163–69. https://doi.org/https://doi.org/10.1016/j.devcel.2008.05.016.

Burridge, K., K. Fath, T. Kelly, G. Nuckolls, and C. Turner. 1988. “Focal Adhesions: Transmembrane Junctions between the Extracellular Matrix and the Cytoskeleton.” *Annual Review of Cell Biology* 4: 487–525. https://doi.org/10.1146/annurev.cb.04.110188.002415.

Burridge, Keith, and Magdalena Chrzanowska-Wodnicka. 1996. “Focal Adhesions, Contractility, and Signaling.” *Annual Review of Cell and Developmental Biology* 12 (1): 463–519. https://doi.org/10.1146/annurev.cellbio.12.1.463.

Burridge, Keith, Christopher E. Turner, and Lewis H. Romer. 1992. “Tyrosine Phosphorylation of Paxillin and Pp125FAK Accompanies Cell Adhesion to Extracellular Matrix: A Role in Cytoskeletal Assembly.” *Journal of Cell Biology* 119 (4): 893–903. https://doi.org/10.1083/jcb.119.4.893.

Buttery, P., A. A. Beg, B. Chih, A. Broder, C. A. Mason, and P. Scheiffele. 2006. “The Diacylglycerol-Binding Protein 1-Chimaerin Regulates Dendritic Morphology.” *Proceedings of the National Academy of Sciences* 103 (6): 1924–29. https://doi.org/10.1073/pnas.0510655103.

Byron, Adam, Jonathan D. Humphries, Mark D. Bass, David Knight, and Martin J.

Humphries. 2011. “Proteomic Analysis of Integrin Adhesion Complexes.” *Science Signaling* 4 (167): 1–5. https://doi.org/10.1126/scisignal.2001827.

Calalb, M B, T R Polte, and S K Hanks. 1995a. “Tyrosine Phosphorylation of Focal Adhesion Kinase at Sites in the Catalytic Domain Regulates Kinase Activity: A Role for Src Family Kinases.” *Molecular and Cellular Biology* 15 (2): 954–63. https://doi.org/10.1128/mcb.15.2.954.

Cammarata, Garrett M, Elizabeth A Bearce, and Laura Anne Lowery. 2016. “Cytoskeletal Social Networking in the Growth Cone: How +TIPs Mediate Microtubule-Actin Cross-Linking to Drive Axon Outgrowth and Guidance.” *Cytoskeleton* 73 (9): 461–76. https://doi.org/https://doi.org/10.1002/cm.21272.

Canagarajah, Bertram, Federico Coluccio Leskow, Jonathan Yew Seng Ho, Harald Mischak, Layla F. Saidi, Marcelo G. Kazanietz, and James H. Hurley. 2004. “Structural Mechanism for Lipid Activation of the Rac-Specific GAP, B2-Chimaerin.” *Cell* 119 (3): 407–18. https://doi.org/10.1016/j.cell.2004.10.012.

Carbonetto, S, M M Gruver, and D C Turner. 1983. “Nerve Fiber Growth in Culture on Fibronectin, Collagen, and Glycosaminoglycan Substrates.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 3 (11): 2324–35. https://doi.org/10.1523/JNEUROSCI.03-11-02324.1983.

Cary, Leslie A., Dong Cho Han, Thomas R. Polte, Steven K. Hanks, and Jun Lin Guan. 1998. “Identification of P130(Cas) as a Mediator of Focal Adhesion Kinase-Promoted Cell Migration.” *Journal of Cell Biology* 140 (1): 211–21. https://doi.org/10.1083/jcb.140.1.211.

Caudy, M, and D Bentley. 1986. “Pioneer Growth Cone Steering along a Series of Neuronal and Non-Neuronal Cues of Different Affinities.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 6 (6): 1781–95. https://doi.org/10.1523/JNEUROSCI.06-06-01781.1986.

Caviness, V S, P G Bhide, and R S Nowakowski. 2008. “Histogenetic Processes Leading to the Laminated Neocortex: Migration Is Only a Part of the Story.” *Developmental Neuroscience* 30 (1–3): 82–95. https://doi.org/10.1159/000109854.

Chacón, Mariola R., Gloria Fernández, and Beatriz Rico. 2010. “Focal Adhesion Kinase Functions Downstream of Sema3A Signaling during Axonal Remodeling.” *Molecular and Cellular Neuroscience* 44 (1): 30–42. https://doi.org/10.1016/j.mcn.2010.02.001.

Chan, Keefe T., Christa L. Cortesio, and Anna Huttenlocher. 2009. “Fak Alters Invadopodia and Focal Adhesion Composition and Dynamics to Regulate Breast

Cancer Invasion." *Journal of Cell Biology* 185 (2): 357–70. https://doi.org/10.1083/jcb.200809110.

Chastney, Megan R, Craig Lawless, Jonathan D Humphries, Stacey Warwood, Matthew C Jones, David Knight, Claus Jorgensen, and Martin J Humphries. 2020. "Topological Features of Integrin Adhesion Complexes Revealed by Multiplexed Proximity Biotinylation." *The Journal of Cell Biology* 219 (8). https://doi.org/10.1083/jcb.202003038.

Chauhan, Akshita, and Tabassum Khan. 2021. "Focal Adhesion Kinase—An Emerging Viable Target in Cancer and Development of Focal Adhesion Kinase Inhibitors." *Chemical Biology and Drug Design* 97 (3): 774–94. https://doi.org/10.1111/cbdd.13808.

Chédotal, Alain. 2010. "Should I Stay or Should I Go? Becoming a Granule Cell." *Trends in Neurosciences* 33 (4): 163–72. https://doi.org/10.1016/j.tins.2010.01.004.

Chédotal, Alain, and Linda J. Richards. 2010. "Wiring the Brain: The Biology of Neuronal Guidance." *Cold Spring Harbor Perspectives in Biology* 2 (6): 1–18. https://doi.org/10.1101/cshperspect.a001917.

Chédotal, Alain, and Filippo M Rijli. 2009. "Transcriptional Regulation of Tangential Neuronal Migration in the Developing Forebrain." *Current Opinion in Neurobiology* 19 (2): 139–45. https://doi.org/10.1016/j.conb.2009.04.005.

Chen, Bor Huah, Jason T.C. Tzen, Anne R. Bresnick, and Hong Chen. 2002. "Roles of Rho-Associated Kinase and Myosin Light Chain Kinase in Morphological and Migratory Defects of Focal Adhesion Kinase-Null Cells." *Journal of Biological Chemistry* 277 (37): 33857–63. https://doi.org/10.1074/jbc.M204429200.

Chen, Hang, Anil Bagri, Joel A Zupicich, Yimin Zou, Esther Stoeckli, Samuel J Pleasure, Daniel H Lowenstein, William C Skarnes, Alain Chédotal, and Marc Tessier-Lavigne. 2000. "Neuropilin-2 Regulates the Development of Select Cranial and Sensory Nerves and Hippocampal Mossy Fiber Projections." *Neuron* 25 (1): 43–56. https://doi.org/https://doi.org/10.1016/S0896-6273(00)80870-3.

Chen, Lei, Guanghong Liao, Ronald R Waclaw, Kevin A Burns, Diana Linquist, Kenneth Campbell, Yi Zheng, and Chia-Yi Kuan. 2007. "Rac1 Controls the Formation of Midline Commissures and the Competency of Tangential Migration in Ventral Telencephalic Neurons." *The Journal of Neuroscience* 27 (14): 3884 LP – 3893. https://doi.org/10.1523/JNEUROSCI.3509-06.2007.

Chen, Yi-An, I-Ling Lu, and Jin-Wu Tsai. 2018. "Contactin-1/F3 Regulates Neuronal Migration and Morphogenesis Through Modulating RhoA Activity ." *Frontiers in*

Molecular Neuroscience .
https://www.frontiersin.org/articles/10.3389/fnmol.2018.00422.

Cherfils, J., and M. Zeghouf. 2013. "Regulation of Small GTPases by GEFs, GAPs, and GDIs." *Physiological Reviews* 93 (1): 269–309. https://doi.org/10.1152/physrev.00003.2012.

Choi, Colin K, Miguel Vicente-Manzanares, Jessica Zareno, Leanna A Whitmore, Alex Mogilner, and Alan Rick Horwitz. 2008. "Actin and Alpha-Actinin Orchestrate the Assembly and Maturation of Nascent Adhesions in a Myosin II Motor-Independent Manner." *Nature Cell Biology* 10 (9): 1039–50. https://doi.org/10.1038/ncb1763.

Chrzanowska-Wodnicka, Magdalena, and Keith Burridge. 1996. "Rho-Stimulated Contractility Drives the Formation of Stress Fibers and Focal Adhesions." *Journal of Cell Biology* 133 (6): 1403–15. https://doi.org/10.1083/jcb.133.6.1403.

Chuang, Hsiang-Hao, Yen-yi Zhen, Yu-chen Tsai, Cheng-hao Chuang, Michael Hsiao, Ming-shyan Huang, and Chih-jen Yang. 2022. "FAK in Cancer: From Mechanisms to Therapeutic Strategies." *International Journal of Molecular Sciences* 23 (3): 1726. https://doi.org/10.3390/ijms23031726.

Cobb, B S, M D Schaller, T H Leu, and J T Parsons. 1994. "Stable Association of Pp60src and Pp59fyn with the Focal Adhesion-Associated Protein Tyrosine Kinase, Pp125FAK." *Molecular and Cellular Biology* 14 (1): 147–55. https://doi.org/10.1128/mcb.14.1.147-155.1994.

Cohen, J, J F Burne, J Winter, and P Bartlett. 1986. "Retinal Ganglion Cells Lose Response to Laminin with Maturation." *Nature* 322 (6078): 465–67. https://doi.org/10.1038/322465a0.

Cohen, Lee, and Jun-Lin Guan. 2005. "Mechanisms of Focal Adhesion Kinase Regulation." *Current Cancer Drug Targets* 5 (8): 629–43. https://doi.org/10.2174/156800905774932798.

Comer, J. D., S. Alvarez, S. J. Butler, and J. A. Kaltschmidt. 2019. "Commissural Axon Guidance in the Developing Spinal Cord: From Cajal to the Present Day." *Neural Development* 14 (1): 1–16. https://doi.org/10.1186/s13064-019-0133-1.

Cooper, Jonathan A. 2013. "Mechanisms of Cell Migration in the Nervous System." *Journal of Cell Biology* 202 (5): 725–34. https://doi.org/10.1083/jcb.201305021.

Craig, Erin M. 2018. "Model for Coordination of Microtubule and Actin Dynamics in Growth Cone Turning." *Frontiers in Cellular Neuroscience* 12 (October): 1–7. https://doi.org/10.3389/fncel.2018.00394.

Craig, Erin M., David Van Goor, Paul Forscher, and Alex Mogilner. 2012. “Membrane Tension, Myosin Force, and Actin Turnover Maintain Actin Treadmill in the Nerve Growth Cone.” *Biophysical Journal* 102 (7): 1503–13. https://doi.org/10.1016/j.bpj.2012.03.003.

Craig, Erin M, Jonathan Stricker, Margaret Gardel, and Alex Mogilner. 2015. “Model for Adhesion Clutch Explains Biphasic Relationship between Actin Flow and Traction at the Cell Leading Edge.” *Physical Biology* 12 (3): 35002. https://doi.org/10.1088/1478-3975/12/3/035002.

Critchley, David R. 2000. “Focal Adhesions - The Cytoskeletal Connection.” *Current Opinion in Cell Biology* 12 (1): 133–39. https://doi.org/10.1016/S0955-0674(99)00067-8.

Damayanti, Nur P, Kevin Buno, Nagarajan Narayanan, Sherry L Voytik Harbin, Meng Deng, and Joseph M K Irudayaraj. 2017. “Monitoring Focal Adhesion Kinase Phosphorylation Dynamics in Live Cells.” *The Analyst* 142 (15): 2713–16. https://doi.org/10.1039/c7an00471k.

Damsky, C H, and Z Werb. 1992. “Signal Transduction by Integrin Receptors for Extracellular Matrix: Cooperative Processing of Extracellular Information.” *Current Opinion in Cell Biology* 4 (5): 772–81. https://doi.org/10.1016/0955-0674(92)90100-q.

Davis, George E, Silvlo Varon, Eva Engvall, and Marston Manthorpe. 1985. “Substratum-Binding Neurite-Promoting Factors: Relationships to Laminin.” *Trends in Neurosciences* 8: 528–32. https://doi.org/https://doi.org/10.1016/0166-2236(85)90189-4.

Dawson, John C., Alan Serrels, Dwayne G. Stupack, David D. Schlaepfer, and Margaret C. Frame. 2021. “Targeting FAK in Anticancer Combination Therapies.” *Nature Reviews Cancer* 21 (5): 313–24. https://doi.org/10.1038/s41568-021-00340-6.

Defilippi, Paola, Paola Di Stefano, and Sara Cabodi. 2006. “P130Cas: A Versatile Scaffold in Signaling Networks.” *Trends in Cell Biology* 16 (5): 257–63. https://doi.org/10.1016/j.tcb.2006.03.003.

DeGeer, Jonathan, and Nathalie Lamarche-Vane. 2013. “Rho GTPases in Neurodegeneration Diseases.” *Experimental Cell Research* 319 (15): 2384–94. https://doi.org/10.1016/j.yexcr.2013.06.016.

DeMali, Kris A., Krister Wennerberg, and Keith Burridge. 2003. “Integrin Signaling to the Actin Cytoskeleton.” *Current Opinion in Cell Biology* 15 (5): 572–82. https://doi.org/10.1016/S0955-0674(03)00109-1.

Deneka, Alexander, Vladislav Korobeynikov, and Erica A. Golemis. 2015. "Embryonal Fyn-Associated Substrate (EFS) and CASS4: The Lesser-Known CAS Protein Family Members." *Gene* 570 (1): 25–35. https://doi.org/10.1016/j.gene.2015.06.062.

Deng, S., A. Hirschberg, T. Worzfeld, J. Y. Penachioni, A. Korostylev, J. M. Swiercz, P. Vodrazka, et al. 2007. "Plexin-B2, But Not Plexin-B1, Critically Modulates Neuronal Migration and Patterning of the Developing Nervous System In Vivo." *Journal of Neuroscience* 27 (23): 6333–47. https://doi.org/10.1523/JNEUROSCI.5381-06.2007.

Deuel, Thomas A S, Judy S Liu, Joseph C Corbo, Seung-Yun Yoo, Lucy B Rorke-Adams, and Christopher A Walsh. 2006. "Genetic Interactions between Doublecortin and Doublecortin-like Kinase in Neuronal Migration and Axon Outgrowth." *Neuron* 49 (1): 41–53. https://doi.org/https://doi.org/10.1016/j.neuron.2005.10.038.

Donato, Dominique M, Larisa M Ryzhova, Leslie M Meenderink, Irina Kaverina, and Steven K Hanks. 2010. "Dynamics and Mechanism of P130Cas Localization to Focal Adhesions." *The Journal of Biological Chemistry* 285 (27): 20769–79. https://doi.org/10.1074/jbc.M109.091207.

Donlin, L. T., N. M. Danzl, C. Wanjalla, and K. Alexandropoulos. 2005. "Deficiency in Expression of the Signaling Protein Sin/Efs Leads to T-Lymphocyte Activation and Mucosal Inflammation." *Molecular and Cellular Biology* 25 (24): 11035–46. https://doi.org/10.1128/MCB.25.24.11035-11046.2005.

Dottori, Mirella, Lynne Hartley, Mary Galea, George Paxinos, Mark Polizzotto, Trevor Kilpatrick, Perry F Bartlett, Mark Murphy, Frank Köntgen, and Andrew W Boyd. 1998. "EphA4 (Sek1) Receptor Tyrosine Kinase Is Required for the Development of the Corticospinal Tract." *Proceedings of the National Academy of Sciences* 95 (22): 13248–53. https://doi.org/10.1073/pnas.95.22.13248.

Dufour, S, J L Duband, M J Humphries, M Obara, K M Yamada, and J P Thiery. 1988. "Attachment, Spreading and Locomotion of Avian Neural Crest Cells Are Mediated by Multiple Adhesion Sites on Fibronectin Molecules." *The EMBO Journal* 7 (9): 2661–71. https://doi.org/10.1002/j.1460-2075.1988.tb03119.x.

Duncan, Bryce W, Kelsey E Murphy, and Patricia F Maness. 2021. "Molecular Mechanisms of L1 and NCAM Adhesion Molecules in Synaptic Pruning, Plasticity, and Stabilization ." *Frontiers in Cell and Developmental Biology* . https://www.frontiersin.org/articles/10.3389/fcell.2021.625340.

Dunty, Jill M., Veronica Gabarra-Niecko, Michelle L. King, Derek F. J. Ceccarelli, Michael J. Eck, and Michael D. Schaller. 2004. "FERM Domain Interaction

Promotes FAK Signaling." *Molecular and Cellular Biology* 24 (12): 5353–68. https://doi.org/10.1128/mcb.24.12.5353-5368.2004.

Dunty, Jill M., and Michael D. Schaller. 2002. "The N Termini of Focal Adhesion Kinase Family Members Regulate Substrate Phosphorylation, Localization, and Cell Morphology." *Journal of Biological Chemistry* 277 (47): 45644–54. https://doi.org/10.1074/jbc.M201779200.

Easter, S S, L S Ross, and A Frankfurter. 1993. "Initial Tract Formation in the Mouse Brain." *The Journal of Neuroscience* 13 (1): 285 LP – 299. https://doi.org/10.1523/JNEUROSCI.13-01-00285.1993.

Easter, Stephen S., John Burrill, Riva C Marcus, Linda S Ross, Jeremy S.H. Taylor, and Stephen W Wilson. 1994. "Initial Tract Formation in the Vertebrate Brain." In *Progress in Brain Research*, 102:79–93. Netherlands. https://doi.org/10.1016/S0079-6123(08)60533-6.

Edgar, D, R Timpl, and H Thoenen. 1988. "Structural Requirements for the Stimulation of Neurite Outgrowth by Two Variants of Laminin and Their Inhibition by Antibodies." *The Journal of Cell Biology* 106 (4): 1299–1306. https://doi.org/10.1083/jcb.106.4.1299.

Edmondson, James C, Ronald K H Liem, Joan E Kuster, and Mary E Hatten. 1988. "Astrotactin : A Novel Neuronal Cell Surface Antigen That Mediates." *The Journal of Cell Biology* 106 (February): 505–17.

Eide, B L, C W Turck, and J A Escobedo. 1995. "Identification of Tyr-397 as the Primary Site of Tyrosine Phosphorylation and Pp60src Association in the Focal Adhesion Kinase, Pp125FAK." *Molecular and Cellular Biology* 15 (5): 2819–27. https://doi.org/10.1128/MCB.15.5.2819.

Elosegui-Artola, Alberto, Elsa Bazellières, Michael D. Allen, Ion Andreu, Roger Oria, Raimon Sunyer, Jennifer J. Gomm, et al. 2014. "Rigidity Sensing and Adaptation through Regulation of Integrin Types." *Nature Materials* 13 (6): 631–37. https://doi.org/10.1038/nmat3960.

Elosegui-Artola, Alberto, Roger Oria, Yunfeng Chen, Anita Kosmalska, Carlos Pérez-González, Natalia Castro, Cheng Zhu, Xavier Trepat, and Pere Roca-Cusachs. 2016. "Mechanical Regulation of a Molecular Clutch Defines Force Transmission and Transduction in Response to Matrix Rigidity." *Nature Cell Biology* 18 (5): 540–48. https://doi.org/10.1038/ncb3336.

Erskine, L, S E Williams, K Brose, T Kidd, R A Rachel, C S Goodman, M Tessier-Lavigne, and C A Mason. 2000. "Retinal Ganglion Cell Axon Guidance in the

Mouse Optic Chiasm: Expression and Function of Robos and Slits." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 20 (13): 4975–82. https://doi.org/10.1523/JNEUROSCI.20-13-04975.2000.

Espinosa, J Sebastian, and Liqun Luo. 2008. "Timing Neurogenesis and Differentiation: Insights from Quantitative Clonal Analyses of Cerebellar Granule Cells." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 28 (10): 2301–12. https://doi.org/10.1523/JNEUROSCI.5157-07.2008.

Evans, I. M., M. Yamaji, G. Britton, C. Pellet-Many, C. Lockie, I. C. Zachary, and P. Frankel. 2011. "Neuropilin-1 Signaling through P130Cas Tyrosine Phosphorylation Is Essential for Growth Factor-Dependent Migration of Glioma and Endothelial Cells." *Molecular and Cellular Biology* 31 (6): 1174–85. https://doi.org/10.1128/MCB.00903-10.

Evsyukova, Irina, Charlotte Plestant, and E S Anton. 2013. "Integrative Mechanisms of Oriented Neuronal Migration in the Developing Brain." *Annual Review of Cell and Developmental Biology* 29 (0 1): 299–353. https://doi.org/10.1146/annurev-cellbio-101512-122400.

Eyckmans, Jeroen, Thomas Boudou, Xiang Yu, and Christopher S Chen. 2011. "A Hitchhiker's Guide to Mechanobiology." *Developmental Cell* 21 (1): 35–47. https://doi.org/10.1016/j.devcel.2011.06.015.

Fässler, R, and M Meyer. 1995. "Consequences of Lack of Beta 1 Integrin Gene Expression in Mice." *Genes & Development* 9 (15): 1896–1908. https://doi.org/10.1101/gad.9.15.1896.

Fazeli, Amin, Stephanie L. Dickinson, Michelle L. Hermiston, Robert V. Tighe, Robert G. Steen, Clayton G. Small, Esther T. Stoeckli, et al. 1997. "Phenotype of Mice Lacking Functional Deleted in Colorectal Cancer (Dcc) Gene." *Nature* 386 (6627): 796–804. https://doi.org/10.1038/386796a0.

Feldheim, David A, and Dennis D M O'Leary. 2010. "Visual Map Development: Bidirectional Signaling, Bifunctional Guidance Molecules, and Competition." *Cold Spring Harbor Perspectives in Biology* 2 (11): a001768. https://doi.org/10.1101/cshperspect.a001768.

Felkl, Marco, Kazmar Tomas, Matej Smid, Julian Mattes, Reinhard Windoffer, and Rudolf E Leube. 2012. "Monitoring the Cytoskeletal EGF Response in Live Gastric Carcinoma Cells." *PLOS ONE* 7 (9): e45280. https://doi.org/10.1371/journal.pone.0045280.

Feng, Xiaodong, Nadia Arang, Damiano Cosimo Rigiracciolo, Joo Sang Lee, Huwate

Yeerna, Zhiyong Wang, Simone Lubrano, et al. 2019. “A Platform of Synthetic Lethal Gene Interaction Networks Reveals That the GNAQ Uveal Melanoma Oncogene Controls the Hippo Pathway through FAK.” *Cancer Cell* 35 (3): 457-472.e5. https://doi.org/10.1016/j.ccell.2019.01.009.

Fenlon, Laura R, Rodrigo Suarez, Zorana Lynton, and Linda J Richards. 2021. “The Evolution, Formation and Connectivity of the Anterior Commissure.” *Seminars in Cell & Developmental Biology* 118: 50–59. https://doi.org/https://doi.org/10.1016/j.semcdb.2021.04.009.

Ferrario, J. E., P. Baskaran, C. Clark, A. Hendry, O. Lerner, M. Hintze, J. Allen, J. K. Chilton, and S. Guthrie. 2012. “Axon Guidance in the Developing Ocular Motor System and Duane Retraction Syndrome Depends on Semaphorin Signaling via Alpha2-Chimaerin.” *Proceedings of the National Academy of Sciences* 109 (36): 14669–74. https://doi.org/10.1073/pnas.1116481109.

Fischer, G, V Künemund, and M Schachner. 1986. “Neurite Outgrowth Patterns in Cerebellar Microexplant Cultures Are Affected by Antibodies to the Cell Surface Glycoprotein L1.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 6 (2): 605–12. https://doi.org/10.1523/JNEUROSCI.06-02-00605.1986.

Flinn, Helen M., and Anne J. Ridley. 1996. “Rho Stimulates Tyrosine Phosphorylation of Focal Adhesion Kinase, P130 and Paxillin.” *Journal of Cell Science* 109 (5): 1133–41.

Fonseca, Priscila M., Nah Young Shin, Jan Brábek, Larisa Ryzhova, Jiong Wu, and Steven K. Hanks. 2004. “Regulation and Localization of CAS Substrate Domain Tyrosine Phosphorylation.” *Cellular Signalling* 16 (5): 621–29. https://doi.org/10.1016/j.cellsig.2003.10.004.

Friedel, R. H., G. Kerjan, H. Rayburn, U. Schuller, C. Sotelo, M. Tessier-Lavigne, and A. Chedotal. 2007. “Plexin-B2 Controls the Development of Cerebellar Granule Cells.” *Journal of Neuroscience* 27 (14): 3921–32. https://doi.org/10.1523/JNEUROSCI.4710-06.2007.

Fujita, S. 1967. “Quantitative Analysis of Cell Proliferation and Differentiation in the Cortex of the Postnatal Mouse Cerebellum.” *The Journal of Cell Biology* 32 (2): 277–87. https://doi.org/10.1083/jcb.32.2.277.

Galbraith, Catherine G, Kenneth M Yamada, and Michael P Sheetz. 2002. “The Relationship between Force and Focal Complex Development.” *The Journal of Cell Biology* 159 (4): 695–705. https://doi.org/10.1083/jcb.200204153.

Garel, Sonia, and Guillermina López-Bendito. 2014. "Inputs from the Thalamocortical System on Axon Pathfinding Mechanisms." *Current Opinion in Neurobiology* 27: 143–50. https://doi.org/10.1016/j.conb.2014.03.013.

Geiger, B. 1989. "Cytoskeleton-Associated Cell Contacts." *Current Opinion in Cell Biology* 1 (1): 103–9. https://doi.org/10.1016/S0955-0674(89)80045-6.

Geiger, B, and E Zamir. 2001. "Molecular Complexity and Dynamics of Cell-Matrix Adhesions." *Journal of Cell Science* 114 (20): 3577–79.

Geiger, Benjamin, and Alexander Bershadsky. 2002. "Exploring the Neighborhood: Adhesion-Coupled Cell Mechanosensors." *Cell* 110 (2): 139–42. https://doi.org/10.1016/S0092-8674(02)00831-0.

Geiger, Benjamin, Alexander Bershadsky, Roumen Pankov, and Kenneth M. Yamada. 2001. "Transmembrane Extracellular Matrix-Cytoskeleton Crosstalk." *Nature Reviews Molecular Cell Biology* 2 (11): 793–805. https://doi.org/10.1038/35099066.

Geiger, Benjamin, and Kenneth M. Yamada. 2011. "Molecular Architecture and Function of Matrix Adhesions." *Cold Spring Harbor Perspectives in Biology* 3 (5): 1–21. https://doi.org/10.1101/cshperspect.a005033.

Geraldo, Sara, and Phillip R. Gordon-Weeks. 2009. "Cytoskeletal Dynamics in Growth-Cone Steering." *Journal of Cell Science* 122 (20): 3595–3604. https://doi.org/10.1242/jcs.042309.

Gerrow, Kimberly, and Alaa El-Husseini. 2006. "Cell Adhesion Molecules at the Synapse." *FBL* 11 (3): 2400–2419.

Ghosh, Anirvan, Antonella Antonini, Susan K McConnell, and Carla J Shatz. 1990. "Requirement for Subplate Neurons in the Formation of Thalamocortical Connections." *Nature* 347 (6289): 179–81. https://doi.org/10.1038/347179a0.

Ghosh, Anirvan, and Carla J Shatz. 1992. "Involvement of Subplate Neurons in the Formation of Ocular Dominance Columns." *Science* 255 (5050): 1441–43. https://doi.org/10.1126/science.1542795.

Giannone, Gregory, Philippe Rondé, Mireille Gaire, Jacques Haiech, and Kenneth Takeda. 2002. "Calcium Oscillations Trigger Focal Adhesion Disassembly in Human U87 Astrocytoma Cells." *Journal of Biological Chemistry* 277 (29): 26364–71. https://doi.org/10.1074/jbc.M203952200.

Giger, Roman J, Jean-François Cloutier, Amar Sahay, Rabinder K Prinjha, Dorothy V Levengood, Stephen E Moore, Susan Pickering, et al. 2000. "Neuropilin-2 Is

Required In Vivo for Selective Axon Guidance Responses to Secreted Semaphorins.” *Neuron* 25 (1): 29–41. https://doi.org/https://doi.org/10.1016/S0896-6273(00)80869-7.

Gilmore, E C, T Ohshima, A M Goffinet, A B Kulkarni, and K Herrup. 1998. “Cyclin-Dependent Kinase 5-Deficient Mice Demonstrate Novel Developmental Arrest in Cerebral Cortex.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 18 (16): 6370–77. https://doi.org/10.1523/JNEUROSCI.18-16-06370.1998.

Goebbels, Sandra, Ingo Bormuth, Ulli Bode, Ola Hermanson, Markus H Schwab, and Klaus-Armin Nave. 2006. “Genetic Targeting of Principal Neurons in Neocortex and Hippocampus of NEX-Cre Mice.” *Genesis (New York, N.Y. : 2000)* 44 (12): 611–21. https://doi.org/10.1002/dvg.20256.

Gong, Shiaoching, Chen Zheng, Martin L Doughty, Kasia Losos, Nicholas Didkovsky, Uta B Schambra, Norma J Nowak, et al. 2003. “A Gene Expression Atlas of the Central Nervous System Based on Bacterial Artificial Chromosomes.” *Nature* 425 (6961): 917–25. https://doi.org/10.1038/nature02033.

Gorski, Jessica A, Tiffany Talley, Mengsheng Qiu, Luis Puelles, John L R Rubenstein, and Kevin R Jones. 2002. “Cortical Excitatory Neurons and Glia, but Not GABAergic Neurons, Are Produced in the Emx1-Expressing Lineage.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 22 (15): 6309–14. https://doi.org/10.1523/JNEUROSCI.22-15-06309.2002.

Goult, Benjamin T., Jie Yan, and Martin A. Schwartz. 2018. “Talin as a Mechanosensitive Signaling Hub.” *Journal of Cell Biology* 217 (11): 3776–84. https://doi.org/10.1083/jcb.201808061.

Govek, Eve-Ellen, Mary E. Hatten, and Linda Van Aelst. 2011. “The Role of Rho GTPase Proteins in CNS Neuronal Migration.” *Developmental Neurobiology* 71 (6): 528–53. https://doi.org/10.1002/dneu.20850.

Govek, Eve-Ellen, Zhuhao Wu, Devrim Acehan, Henrik Molina, Keith Rivera, Xiaodong Zhu, Yin Fang, Marc Tessier-Lavigne, and Mary Elizabeth Hatten. 2018. “Cdc42 Regulates Neuronal Polarity during Cerebellar Axon Formation and Glial-Guided Migration.” *IScience* 1: 35–48. https://doi.org/10.1016/j.isci.2018.01.004.

Govek, Eve Ellen, Sarah E. Newey, and Linda Van Aelst. 2005. “The Role of the Rho GTPases in Neuronal Development.” *Genes and Development*. https://doi.org/10.1101/gad.1256405.

Graus-Porta, D, S Blaess, M Senften, A Littlewood-Evans, C Damsky, Z Huang, P

Orban, R Klein, J C Schittny, and U Müller. 2001. “Beta1-Class Integrins Regulate the Development of Laminae and Folia in the Cerebral and Cerebellar Cortex.” *Neuron* 31 (3): 367–79. https://doi.org/10.1016/s0896-6273(01)00374-9.

Griner, Erin M., M. Cecilia Caino, Maria Soledad Sosa, Francheska Colón-González, Michael J. Chalmers, Harald Mischak, and Marcelo G. Kazanietz. 2010. “A Novel Cross-Talk in Diacylglycerol Signaling: The Rac-GAP B2-Chimaerin Is Negatively Regulated by Protein Kinase Cδ-Mediated Phosphorylation.” *Journal of Biological Chemistry* 285 (22): 16931–41. https://doi.org/10.1074/jbc.M109.099036.

Guan, Chen bing, Hua tai Xu, Ming Jin, Xiao bing Yuan, and Mu ming Poo. 2007. “Long-Range Ca2+ Signaling from Growth Cone to Soma Mediates Reversal of Neuronal Migration Induced by Slit-2.” *Cell* 129 (2): 385–95. https://doi.org/10.1016/j.cell.2007.01.051.

Guan, Jun Lin, and David Shalloway. 1992. “Regulation of Focal Adhesion-Associated Protein Tyrosine Kinase by Both Cellular Adhesion and Oncogenic Transformation.” *Nature* 358 (6388): 690–92. https://doi.org/10.1038/358690a0.

Guan, Jun Lin, Jane E. Trevithick, and Richard O. Hynes. 1991. “Fibronectin/Integrin Interaction Induces Tyrosine Phosphorylation of a 120-KDa Protein.” *Molecular Biology of the Cell* 2 (11): 951–64. https://doi.org/10.1091/mbc.2.11.951.

Guerrini, Renzo, William B Dobyns, and A James Barkovich. 2008. “Abnormal Development of the Human Cerebral Cortex: Genetics, Functional Consequences and Treatment Options.” *Trends in Neurosciences* 31 (3): 154–62. https://doi.org/10.1016/j.tins.2007.12.004.

Guilluy, Christophe, Rafael Garcia-Mata, and Keith Burridge. 2011. “Rho Protein Crosstalk: Another Social Network?” *Trends in Cell Biology* 21 (12): 718–26. https://doi.org/10.1016/j.tcb.2011.08.002.

Gundersen, R W. 1987. “Response of Sensory Neurites and Growth Cones to Patterned Substrata of Laminin and Fibronectin in Vitro.” *Developmental Biology* 121 (2): 423–31. https://doi.org/10.1016/0012-1606(87)90179-5.

Guo, Wei-hui, and Yu-li Wang. 2007. “Retrograde Fluxes of Focal Adhesion Proteins in Response to Cell Migration and Mechanical Signals.” *Molecular Biology of the Cell* 18 (11): 4519–27. https://doi.org/10.1091/mbc.e07-06-0582.

Gustavsson, Anna, Ming Yuan, and Maria Fällman. 2004. “Temporal Dissection of B1-Integrin Signaling Indicates a Role for P130CAS-Crk in Filopodia Formation.” *Journal of Biological Chemistry* 279 (22): 22893–901. https://doi.org/10.1074/jbc.M309693200.

Halfter, Willi, Sucai Dong, Yi-Ping Yip, Michael Willem, and Ulrike Mayer. 2002. "A Critical Function of the Pial Basement Membrane in Cortical Histogenesis." *The Journal of Neuroscience* 22 (14): 6029 LP – 6040. https://doi.org/10.1523/JNEUROSCI.22-14-06029.2002.

Hall, Christine, GregoryJ Michael, Nansi Cann, Giovanna Ferrari, Mabel Teo, Tom Jacobs, Clinton Monfries, and Louis Lim. 2001. "α-Chimaerin, a Cdc42/Rac1 Regulator, Is Selectively Expressed in the Rat Embryonic Nervous System and Is Involved in Neuritogenesis in N1E-115 Neuroblastoma Cells." *The Journal of Neuronscience* 21 (14): 5191–5202. https://doi.org/21/14/5191 [pii].

Hall, Christine, W U N Chey Sin, Mabel Teo, Gregory J Michael, Paul Smith, Jing Ming Dong, Hong H W A Lim, et al. 1993. "O2-Chimerin , an SH2-Containing GTPase-Activating Protein for the Ras-Related Protein P2lrac Derived by Alternate Splicing of the Human n-Chimerin Gene , Is Selectively Expressed in Brain Regions and Testes." *Molecular and Cellular Biology* 13 (8): 4986–98.

Hamadi, Abdelkader, Maya Bouali, Monique Dontenwill, Herrade Stoeckel, Kenneth Takeda, and Philippe Rondé. 2005. "Regulation of Focal Adhesion Dynamics and Disassembly by Phosphorylation of FAK at Tyrosine 397." *Journal of Cell Science* 118 (19): 4415–25. https://doi.org/10.1242/jcs.02565.

Hanashima, Carina, Zoltán Molnár, and Gord Fishell. 2006. "Building Bridges to the Cortex." *Cell* 125 (1): 24–27. https://doi.org/10.1016/j.cell.2006.03.021.

Hanks, S. K., M. B. Calalb, M. C. Harper, and S. K. Patel. 1992. "Focal Adhesion Protein-Tyrosine Kinase Phosphorylated in Response to Cell Attachment to Fibronectin." *Proceedings of the National Academy of Sciences of the United States of America* 89 (18): 8487–91. https://doi.org/10.1073/pnas.89.18.8487.

HARRISON, R G. 1959. "The Outgrowth of the Nerve Fiber as a Mode of Protoplasmic Movement." *The Journal of Experimental Zoology* 142: 5–73. https://doi.org/10.1002/jez.1401420103.

Harte, Mary T., Jeffrey D. Hildebrand, Mary Rose Burnham, Amy H. Bouton, and J. Thomas Parsons. 1996. "P130Cas, a Substrate Associated with v-Src and v-Crk, Localizes to Focal Adhesions and Binds to Focal Adhesion Kinase." *Journal of Biological Chemistry* 271 (23): 13649–55. https://doi.org/10.1074/jbc.271.23.13649.

Hatanaka, Yumiko, Tomoko Matsumoto, Yuchio Yanagawa, Hajime Fujisawa, Fujio Murakami, and Masayuki Masu. 2009. "Distinct Roles of Neuropilin 1 Signaling for Radial and Tangential Extension of Callosal Axons." *The Journal of Comparative Neurology* 514 (3): 215–25. https://doi.org/10.1002/cne.22021.

Hatten, M E, M B Furie, and D B Rifkin. 1982. "Binding of Developing Mouse Cerebellar Cells to Fibronectin: A Possible Mechanism for the Formation of the External Granular Layer." *The Journal of Neuroscience* 2 (9): 1195 LP – 1206. https://doi.org/10.1523/JNEUROSCI.02-09-01195.1982.

Hauck, Christof R., Datsun A. Hsia, Dusko Ilic, and David D. Schlaepfer. 2002. "V-Src SH3-Enhanced Interaction with Focal Adhesion Kinase at B1 Integrin-Containing Invadopodia Promotes Cell Invasion." *Journal of Biological Chemistry* 277 (15): 12487–90. https://doi.org/10.1074/jbc.C100760200.

Hauck, Christof R, Datsun A Hsia, and David D Schlaepfer. 2002. "The Focal Adhesion Kinase—A Regulator of Cell Migration and Invasion Structural Characteristics of FAK-like Protein Tyrosine Kinases Focal Adhesion Kinase (FAK) 1 Together with Pyk2 (1) Form a Subfamily of FAK-like Protein-Tyrosine Kinases (PTKs). FAK." *IUBMB Life* 53: 115–19. https://doi.org/10.1080/10399710290039007.

Hausmann, B, and J Sievers. 1985. "Cerebellar External Granule Cells Are Attached to the Basal Lamina from the Onset of Migration up to the End of Their Proliferative Activity." *The Journal of Comparative Neurology* 241 (1): 50–62. https://doi.org/10.1002/cne.902410105.

Hayashi, Ikuko, Kristiina Vuori, and Robert C. Liddington. 2002. "The Focal Adhesion Targeting (FAT) Region of Focal Adhesion Kinase Is a Four-Helix Bundle That Binds Paxillin." *Nature Structural Biology* 9 (2): 101–6. https://doi.org/10.1038/nsb755.

Henkemeyer, Mark, Donata Orioli, Jeffrey T Henderson, Tracy M Saxton, John Roder, Tony Pawson, and Rüdiger Klein. 1996. "Nuk Controls Pathfinding of Commissural Axons in the Mammalian Central Nervous System." *Cell* 86 (1): 35–46. https://doi.org/https://doi.org/10.1016/S0092-8674(00)80075-6.

Hevner, R F, L Shi, N Justice, Y Hsueh, M Sheng, S Smiga, A Bulfone, A M Goffinet, A T Campagnoni, and J L Rubenstein. 2001. "Tbr1 Regulates Differentiation of the Preplate and Layer 6." *Neuron* 29 (2): 353–66. https://doi.org/10.1016/s0896-6273(01)00211-2.

Hoerder-Suabedissen, Anna, and Zoltán Molnár. 2012. "Morphology of Mouse Subplate Cells with Identified Projection Targets Changes with Age." *The Journal of Comparative Neurology* 520 (1): 174–85. https://doi.org/10.1002/cne.22725.

Hoffmann, Jan-Erik, Yessica Fermin, Ruth L O Stricker, Katja Ickstadt, and Eli Zamir. 2014. "Symmetric Exchange of Multi-Protein Building Blocks between Stationary Focal Adhesions and the Cytosol." Edited by Robert H Singer. *ELife* 3: e02257. https://doi.org/10.7554/eLife.02257.

Homan, Claire C, Stephen Pederson, Thu-Hien To, Chuan Tan, Sandra Piltz, Mark A Corbett, Ernst Wolvetang, Paul Q Thomas, Lachlan A Jolly, and Jozef Gecz. 2018. “PCDH19 Regulation of Neural Progenitor Cell Differentiation Suggests Asynchrony of Neurogenesis as a Mechanism Contributing to PCDH19 Girls Clustering Epilepsy.” *Neurobiology of Disease* 116 (August): 106–19. https://doi.org/10.1016/j.nbd.2018.05.004.

Honda, Hiroaki, Tetsuya Nakamoto, Ryuichi Sakai, and Hisamaru Hirai. 1999. “P130(Cas), an Assembling Molecule of Actin Filaments, Promotes Cell Movement, Cell Migration, and Cell Spreading in Fibroblasts.” *Biochemical and Biophysical Research Communications* 262 (1): 25–30. https://doi.org/10.1006/bbrc.1999.1162.

Höpker, V H, D Shewan, M Tessier-Lavigne, M Poo, and C Holt. 1999. “Growth-Cone Attraction to Netrin-1 Is Converted to Repulsion by Laminin-1.” *Nature* 401 (6748): 69–73. https://doi.org/10.1038/43441.

Horton, Edward R., Pablo Astudillo, Martin J. Humphries, and Jonathan D. Humphries. 2016. “Mechanosensitivity of Integrin Adhesion Complexes: Role of the Consensus Adhesome.” *Experimental Cell Research* 343 (1): 7–13. https://doi.org/10.1016/j.yexcr.2015.10.025.

Horton, Edward R., Adam Byron, Janet A. Askari, Daniel H.J. Ng, Angélique Millon-Frémillon, Joseph Robertson, Ewa J. Koper, et al. 2015. “Definition of a Consensus Integrin Adhesome and Its Dynamics during Adhesion Complex Assembly and Disassembly.” *Nature Cell Biology* 17 (12): 1577–87. https://doi.org/10.1038/ncb3257.

Horton, Edward R., Jonathan D. Humphries, Ben Stutchbury, Guillaume Jacquemet, Christoph Ballestrem, Simon T. Barry, and Martin J. Humphries. 2016. “Modulation of FAK and Src Adhesion Signaling Occurs Independently of Adhesion Complex Composition.” *Journal of Cell Biology* 212 (3): 349–64. https://doi.org/10.1083/jcb.201508080.

Hu, Chang Deng, and Tom K. Kerppola. 2003. “Simultaneous Visualization of Multiple Protein Interactions in Living Cells Using Multicolor Fluorescence Complementation Analysis.” *Nature Biotechnology* 21 (5): 539–45. https://doi.org/10.1038/nbt816.

Hua, Zhong L., Sangmin Jeon, Michael J. Caterina, and Jeremy Nathans. 2014. “Frizzled3 Is Required for the Development of Multiple Axon Tracts in the Mouse Central Nervous System.” *Proceedings of the National Academy of Sciences of the United States of America* 111 (29). https://doi.org/10.1073/pnas.1406399111.

Huang, Guo Hui, Zhao Liang Sun, Hong Jiang Li, and Dong Fu Feng. 2017. “Rho

GTPase-Activating Proteins: Regulators of Rho GTPase Activity in Neuronal Development and CNS Diseases." *Molecular and Cellular Neuroscience* 80: 18–31. https://doi.org/10.1016/j.mcn.2017.01.007.

Huang, Rui, De-Juan Yuan, Shao Li, Xue-Song Liang, Yue Gao, Xiao-Yan Lan, Hua-Min Qin, et al. 2020. "NCAM Regulates Temporal Specification of Neural Progenitor Cells via Profilin2 during Corticogenesis." *The Journal of Cell Biology* 219 (1). https://doi.org/10.1083/jcb.201902164.

Huang, Z., U. Yazdani, K. L. Thompson-Peer, A. L. Kolodkin, and J. R. Terman. 2007. "Crk-Associated Substrate (Cas) Signaling Protein Functions with Integrins to Specify Axon Guidance during Development." *Development* 134 (12): 2337–47. https://doi.org/10.1242/dev.004242.

Huang, Zhen, Kazuhiro Shimazu, Newton H Woo, Keling Zang, Ulrich Müller, Bai Lu, and Louis F Reichardt. 2006. "Distinct Roles of the Beta 1-Class Integrins at the Developing and the Mature Hippocampal Excitatory Synapse." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 26 (43): 11208–19. https://doi.org/10.1523/JNEUROSCI.3526-06.2006.

Huber, Andrea B, Alex L Kolodkin, David D Ginty, and Jean-François Cloutier. 2003. "Signaling at the Growth Cone: Ligand-Receptor Complexes and the Control of Axon Growth and Guidance." *Annual Review of Neuroscience* 26: 509–63. https://doi.org/10.1146/annurev.neuro.26.010302.081139.

Humphries, Jonathan D, Pengbo Wang, Charles Streuli, Benny Geiger, Martin J Humphries, and Christoph Ballestrem. 2007. "Vinculin Controls Focal Adhesion Formation by Direct Interactions with Talin and Actin." *Journal of Cell Biology* 179 (5): 1043–57. https://doi.org/10.1083/jcb.200703036.

Husmann, K., A. Faissner, and M. Schachner. 1992. "Tenascin Promotes Cerebellar Granule Cell Migration and Neurite Outgrowth by Different Domains in the Fibronectin Type III Repeats." *The Journal of Cell Biology* 116 (6): 1475–86. https://doi.org/10.1083/jcb.116.6.1475.

Huttenlocher, Anna, and Alan Rick Horwitz. 2011. "Integrins in Cell Migration." *Cold Spring Harbor Perspectives in Biology* 3 (9): a005074. https://doi.org/10.1101/cshperspect.a005074.

Huynh-Do, U, E Stein, A A Lane, H Liu, D P Cerretti, and T O Daniel. 1999. "Surface Densities of Ephrin-B1 Determine EphB1-Coupled Activation of Cell Attachment through Alphavbeta3 and Alpha5beta1 Integrins." *The EMBO Journal* 18 (8): 2165–73. https://doi.org/10.1093/emboj/18.8.2165.

Hynes, R O, and A D Lander. 1992. "Contact and Adhesive Specificities in the Associations, Migrations, and Targeting of Cells and Axons." *Cell* 68 (2): 303–22. https://doi.org/10.1016/0092-8674(92)90472-o.

Hynes, Richard O. 1992. "Integrins: Versatility, Modulation, and Signaling in Cell Adhesion." *Cell* 69 (1): 11–25. https://doi.org/https://doi.org/10.1016/0092-8674(92)90115-S.

Ilić, Duško, Yasuhlde Furuta, Satoshi Kanazawa, Naoki Takeda, Kenji Sobuet, Norio Nakatsuji, Shintaro Nomura, et al. 1995. "Reduced Cell Motility and Enhanced Focal Adhesion Contact Formation in Cells from FAK-Deficient Mice." *Nature* 377 (6549): 539–44. https://doi.org/10.1038/377539A0.

Ip, Jacque P K, Lei Shi, Yu Chen, Yasuhiro Itoh, Wing Yu Fu, Andrea Betz, Wing Ho Yung, Yukiko Gotoh, Amy K Y Fu, and Nancy Y. Ip. 2012. "A2-Chimaerin Controls Neuronal Migration and Functioning of the Cerebral Cortex through CRMP-2." *Nature Neuroscience* 15 (1): 39–47. https://doi.org/10.1038/nn.2972.

Islam, Shahidul M, Yohei Shinmyo, Tatsuya Okafuji, Yuhong Su, Iftekhar Bin Naser, Giasuddin Ahmed, Sanbing Zhang, et al. 2009. "Draxin, a Repulsive Guidance Protein for Spinal Cord and Forebrain Commissures." *Science* 323 (5912): 388–93. https://doi.org/10.1126/science.1165187.

Ito, Ayako, Yohei Shinmyo, Takaya Abe, Naoko Oshima, Hideaki Tanaka, and Kunimasa Ohta. 2010. "Tsukushi Is Required for Anterior Commissure Formation in Mouse Brain." *Biochemical and Biophysical Research Communications* 402 (4): 813–18. https://doi.org/https://doi.org/10.1016/j.bbrc.2010.10.127.

Ivankovic-Dikic, Inga, Eva Grönroos, Andree Blaukat, Bernd Uwe Barth, and Ivan Dikic. 2000. "Pyk2 and FAK Regulate Neurite Outgrowth Induced by Growth Factors and Integrins." *Nature Cell Biology* 2 (9): 574–81. https://doi.org/10.1038/35023515.

Iwasato, Takuji, Hironori Katoh, Hiroshi Nishimaru, Yukio Ishikawa, Haruhisa Inoue, Yoshikazu M. Saito, Reiko Ando, et al. 2007. "Rac-GAP α-Chimerin Regulates Motor-Circuit Formation as a Key Mediator of EphrinB3/EphA4 Forward Signaling." *Cell* 130 (4): 742–53. https://doi.org/10.1016/j.cell.2007.07.022.

Iyer, Vidhya V., Christoph Ballestrem, Joachim Kirchner, Benjamin Geiger, and Michael D. Schaller. 2005. "Measurement of Protein Tyrosine Phosphorylation in Cell Adhesion." *Methods in Molecular Biology (Clifton, N.J.)* 294: 289–302. https://doi.org/10.1385/1-59259-860-9:289.

Jaffe, Aron B., and Alan Hall. 2005. "RHO GTPASES: Biochemistry and Biology." *Annual Review of Cell and Developmental Biology* 21 (1): 247–69.

https://doi.org/10.1146/annurev.cellbio.21.020604.150721.

Janoštiak, Radoslav, Jan Brábek, Vera Auernheimer, Zuzana Tatárová, Lena A Lautscham, Tuli Dey, Jakub Gemperle, et al. 2014. “CAS Directly Interacts with Vinculin to Control Mechanosensing and Focal Adhesion Dynamics.” *Cellular and Molecular Life Sciences : CMLS* 71 (4): 727–44. https://doi.org/10.1007/s00018-013-1450-x.

Jaudon, Fanny, Agnes Thalhammer, and Lorenzo A Cingolani. 2021. “Integrin Adhesion in Brain Assembly: From Molecular Structure to Neuropsychiatric Disorders.” *The European Journal of Neuroscience* 53 (12): 3831–50. https://doi.org/10.1111/ejn.14859.

Jay, D G. 2000. “The Clutch Hypothesis Revisited: Ascribing the Roles of Actin-Associated Proteins in Filopodial Protrusion in the Nerve Growth Cone.” *Journal of Neurobiology* 44 (2): 114–25. https://doi.org/10.1002/1097-4695(200008)44:2<114::aid-neu3>3.0.co;2-8.

Jockusch, Brigitte M., Peter Bubeck, Klaudia Giehl, Martina Kroemker, Jutta Moschner, Martin Rothkegel, Manfred Rudiger, Kathrin Schluter, Gesa Stanke, and Jörg Winkler. 1995. “The Molecular Architecture of Focal Adhesions.” *Annual Review of Cell and Developmental Biology* 11 (1): 379–416. https://doi.org/10.1146/annurev.cb.11.110195.002115.

Jouandet, M L, and V Hartenstein. 1983. “Basal Telencephalic Origins of the Anterior Commissure of the Rat.” *Experimental Brain Research* 50 (2): 183–92. https://doi.org/10.1007/BF00239182.

Julien, Falk, Ahmad Bechara, Roberto Fiore, Homaira Nawabi, Heather Zhou, Carolina Hoyo-Becerra, Muriel Bozon, et al. 2005. “Dual Functional Activity of Semaphorin 3B Is Required for Positioning the Anterior Commissure.” *Neuron* 48 (1): 63–75. https://doi.org/https://doi.org/10.1016/j.neuron.2005.08.033.

Kaartinen, V, I Gonzalez-Gomez, J W Voncken, L Haataja, E Faure, A Nagy, J Groffen, and N Heisterkamp. 2001. “Abnormal Function of Astroglia Lacking Abr and Bcr RacGAPs.” *Development.* 128 (21): 4217–27.

Kadaré, Gress, Nicolas Gervasi, Karen Brami-Cherrier, Heike Blockus, Said El Messari, Stefan T. Arold, and Jean Antoine Girault. 2015. “Conformational Dynamics of the Focal Adhesion Targeting Domain Control Specific Functions of Focal Adhesion Kinase in Cells.” *Journal of Biological Chemistry* 290 (1): 478–91. https://doi.org/10.1074/jbc.M114.593632.

Kanold, Patrick O, and Heiko J Luhmann. 2010. “The Subplate and Early Cortical

Circuits.” *Annual Review of Neuroscience* 33: 23–48. https://doi.org/10.1146/annurev-neuro-060909-153244.

Kanteti, Rajani, Tamara Mirzapoiazova, Jacob J Riehm, Immanuel Dhanasingh, Bolot Mambetsariev, and Jiale Wang. 2018. “Focal Adhesion Kinase a Potential Therapeutic Target for Pancreatic Cancer and Malignant Pleural Mesothelioma.” *Cancer Biology & Therapy* 19 (4): 316–27. https://doi.org/10.1080/15384047.2017.1416937.

Kao, T.-J., G. C. B. Nicholl, J. A. Johansen, A. Kania, and A. A. Beg. 2015. “A2-Chimaerin Is Required for Eph Receptor-Class-Specific Spinal Motor Axon Guidance and Coordinate Activation of Antagonistic Muscles.” *Journal of Neuroscience* 35 (6): 2344–57. https://doi.org/10.1523/JNEUROSCI.4151-14.2015.

Kappeler, Caroline, Yoann Saillour, Jean-Pierre Baudoin, Françoise Phan Dinh Tuy, Chantal Alvarez, Christophe Houbron, Patricia Gaspar, et al. 2006. “Branching and Nucleokinesis Defects in Migrating Interneurons Derived from Doublecortin Knockout Mice.” *Human Molecular Genetics* 15 (9): 1387–1400. https://doi.org/10.1093/hmg/ddl062.

Katsumi, Akira, Tomoki Naoe, Tadashi Matsushita, Kozo Kaibuchi, and Martin Alexander Schwartz. 2005. “Integrin Activation and Matrix Binding Mediate Cellular Responses to Mechanical Stretch.” *The Journal of Biological Chemistry* 280 (17): 16546–49. https://doi.org/10.1074/jbc.C400455200.

Kawaji, Kousuke, Hiroki Umeshima, Mototsugu Eiraku, Tomoo Hirano, and Mineko Kengaku. 2004. “Dual Phases of Migration of Cerebellar Granule Cells Guided by Axonal and Dendritic Leading Processes.” *Molecular and Cellular Neuroscience* 25 (2): 228–40. https://doi.org/https://doi.org/10.1016/j.mcn.2003.10.006.

Kawauchi, Daisuke, Hiroki Taniguchi, Haruyasu Watanabe, Tetsuichiro Saito, and Fujio Murakami. 2006. “Direct Visualization of Nucleogenesis by Precerebellar Neurons: Involvement of Ventricle-Directed, Radial Fibre-Associated Migration.” *Development* 133 (6): 1113–23. https://doi.org/10.1242/dev.02283.

Kawauchi, Takeshi, and Mikio Hoshino. 2007. “Molecular Pathways Regulating Cytoskeletal Organization and Morphological Changes in Migrating Neurons.” *Developmental Neuroscience* 30 (1–3): 36–46. https://doi.org/10.1159/000109850.

Kerjan, Géraldine, Jackie Dolan, Cécile Haumaitre, Sylvie Schneider-Maunoury, Hajime Fujisawa, Kevin J. Mitchell, and Alain Chédotal. 2005. “The Transmembrane Semaphorin Sema6A Controls Cerebellar Granule Cell Migration.” *Nature Neuroscience* 8 (11): 1516–24. https://doi.org/10.1038/nn1555.

Kerppola, Tom K. 2008. “Bimolecular Fluorescence Complementation (BiFC) Analysis as a Probe of Protein Interactions in Living Cells.” *Annual Review of Biophysics* 37 (1): 465–87. https://doi.org/10.1146/annurev.biophys.37.032807.125842.

Kerppola, Tom K. 2009. “Visualization of Molecular Interactions Using Bimolecular Fluorescence Complementation Analysis: Characteristics of Protein Fragment Complementation.” *Chemical Society Reviews* 38 (10): 2876–86. https://doi.org/10.1039/b909638h.

Kerstein, Patrick C., Robert H. Nichol, and Timothy M. Gomez. 2015. “Mechanochemical Regulation of Growth Cone Motility.” *Frontiers in Cellular Neuroscience* 9 (JULY): 1–16. https://doi.org/10.3389/fncel.2015.00244.

Kerstein, Patrick C., Kevin M. Patel, and Timothy M. Gomez. 2017. “Calpain-Mediated Proteolysis of Talin and FAK Regulates Adhesion Dynamics Necessary for Axon Guidance.” *Journal of Neuroscience* 37 (6): 1568–80. https://doi.org/10.1523/JNEUROSCI.2769-16.2016.

Kim, Young Ho, Hyun Kyoung Kim, Hee Yeon Kim, Hyeran Gawk, Seung Hyun Bae, Hye Won Sim, Eun Kyung Kang, Ju Young Seoh, Hyonchol Jang, and Kyeong Man Hong. 2019. “FAK-Copy-Gain Is a Predictive Marker for Sensitivity to Fak Inhibition in Breast Cancer.” *Cancers* 11 (9): 1–14. https://doi.org/10.3390/cancers11091288.

Kirchner, Jochen, Zvi Kam, Gila Tzur, Alexander D. Bershadsky, and Benjamin Geiger. 2003. “Live-Cell Monitoring of Tyrosine Phosphorylation in Focal Adhesions Following Microtubule Disruption.” *Journal of Cell Science* 116 (6): 975–86. https://doi.org/10.1242/jcs.00284.

Klingler, Esther, Pierre-Marie Martin, Marta Garcia, Caroline Moreau-Fauvarque, Julien Falk, Fabrice Chareyre, Marco Giovannini, Alain Chédotal, Jean-Antoine Girault, and Laurence Goutebroze. 2015. “The Cytoskeleton-Associated Protein SCHIP1 Is Involved in Axon Guidance, and Is Required for Piriform Cortex and Anterior Commissure Development.” *Development* 142 (11): 2026–36. https://doi.org/10.1242/dev.119248.

Kolodkin, Alex L, and Marc Tessier-Lavigne. 2011. “Mechanisms and Molecules of Neuronal Wiring: A Primer.” *Cold Spring Harbor Perspectives in Biology* 3 (6). https://doi.org/10.1101/cshperspect.a001727.

Komuro, H, and P Rakic. 1998. “Distinct Modes of Neuronal Migration in Different Domains of Developing Cerebellar Cortex.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 18 (4): 1478–90. https://doi.org/10.1523/JNEUROSCI.18-04-01478.1998.

Kornberg, L., H. S. Earp, J. T. Parsons, M. Schaller, and R. L. Juliano. 1992. "Cell Adhesion or Integrin Clustering Increases Phosphorylation of a Focal Adhesion-Associated Tyrosine Kinase." *Journal of Biological Chemistry* 267 (33): 23439–42. https://doi.org/10.1016/s0021-9258(18)35853-8.

Kornberg, L J, H S Earp, C E Turner, C Prockop, and R L Juliano. 1991. "Signal Transduction by Integrins: Increased Protein Tyrosine Phosphorylation Caused by Clustering of Beta 1 Integrins." *Proceedings of the National Academy of Sciences* 88 (19): 8392–96. https://doi.org/10.1073/pnas.88.19.8392.

Kriegstein, Arnold R, and Stephen C Noctor. 2004. "Patterns of Neuronal Migration in the Embryonic Cortex." *Trends in Neurosciences* 27 (7): 392–99. https://doi.org/10.1016/j.tins.2004.05.001.

Kruegel, Jenny, and Nicolai Miosge. 2010. "Basement Membrane Components Are Key Players in Specialized Extracellular Matrices." *Cellular and Molecular Life Sciences : CMLS* 67 (17): 2879–95. https://doi.org/10.1007/s00018-010-0367-x.

Kubow, Kristopher E, Sarah K Conrad, and A Rick Horwitz. 2013. "Matrix Microarchitecture and Myosin II Determine Adhesion in 3D Matrices." *Current Biology : CB* 23 (17): 1607–19. https://doi.org/10.1016/j.cub.2013.06.053.

Kutys, Matthew L, and Kenneth M Yamada. 2015. "Rho GEFs and GAPs: Emerging Integrators of Extracellular Matrix Signaling." *Small GTPases* 6 (1): 16–19. https://doi.org/10.4161/21541248.2014.989792.

Kwon, Y T, L H Tsai, and J E Crandall. 1999. "Callosal Axon Guidance Defects in P35(-/-) Mice." *The Journal of Comparative Neurology* 415 (2): 218–29. https://doi.org/10.1002/(sici)1096-9861(19991213)415:2<218::aid-cne6>3.0.co;2-f.

Lake, Madryn C., and Eric O. Aboagye. 2014. "Luciferase Fragment Complementation Imaging in Preclinical Cancer Studies." *Oncoscience* 1 (5): 310–25. https://doi.org/10.18632/oncoscience.45.

Lauffenburger, D A, and A F Horwitz. 1996. "Cell Migration: A Physically Integrated Molecular Process." *Cell* 84 (3): 359–69. https://doi.org/10.1016/s0092-8674(00)81280-5.

Lee, Brian Y., Paul Timpson, Lisa G. Horvath, and Roger J. Daly. 2015. "FAK Signaling in Human Cancer as a Target for Therapeutics." *Pharmacology and Therapeutics* 146: 132–49. https://doi.org/10.1016/j.pharmthera.2014.10.001.

Lee, J, M Leonard, T Oliver, A Ishihara, and K Jacobson. 1994. "Traction Forces Generated by Locomoting Keratocytes." *The Journal of Cell Biology* 127 (6 Pt 2):

1957–64. https://doi.org/10.1083/jcb.127.6.1957.

Lefcort, Frances, and David Bentley. 1987. "Pathfinding by Pioneer Neurons in Isolated, Opened and Mesoderm-Free Limb Buds of Embryonic Grasshoppers." *Developmental Biology* 119 (2): 466–80. https://doi.org/https://doi.org/10.1016/0012-1606(87)90050-9.

Lele, Tanmay P, Charles K Thodeti, Jay Pendse, and Donald E Ingber. 2008. "Investigating Complexity of Protein–Protein Interactions in Focal Adhesions." *Biochemical and Biophysical Research Communications* 369 (3): 929–34. https://doi.org/https://doi.org/10.1016/j.bbrc.2008.02.137.

Lemmon, V, S M Burden, H R Payne, G J Elmslie, and M L Hlavin. 1992. "Neurite Growth on Different Substrates: Permissive versus Instructive Influences and the Role of Adhesive Strength." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 12 (3): 818–26. https://doi.org/10.1523/JNEUROSCI.12-03-00818.1992.

Lent, Roberto, Daniela Uziel, Marie Baudrimont, and Cathérine Fallet. 2005. "Cellular and Molecular Tunnels Surrounding the Forebrain Commissures of Human Fetuses." *The Journal of Comparative Neurology* 483 (4): 375–82. https://doi.org/10.1002/cne.20427.

Leone, Dino P, João B Relvas, Lia S Campos, Silvio Hemmi, Cord Brakebusch, Reinhard Fässler, Charles Ffrench-Constant, and Ueli Suter. 2005. "Regulation of Neural Progenitor Proliferation and Survival by Beta1 Integrins." *Journal of Cell Science* 118 (Pt 12): 2589–99. https://doi.org/10.1242/jcs.02396.

Leto, Ketty, Marife Arancillo, Esther B E Becker, Annalisa Buffo, Chin Chiang, Baojin Ding, William B Dobyns, et al. 2016. "Consensus Paper: Cerebellar Development." *Cerebellum (London, England)* 15 (6): 789–828. https://doi.org/10.1007/s12311-015-0724-2.

Letourneau, Paul C, Maureen L Condic, and Diane M Snow. 1994. "Interactions of Developing Neurons with the Extracellular Matrix." In *Journal of Neuroscience*.

Leung, Thomas, Bee-eng How, Edward Manser, and Louis Lim. 1994. "Cerebellar B2-Chimaerin, a GTPase-Activating Protein for P21 Ras-Related Rac Is Specifically Expressed in Granule Cells and Has a Unique N-Terminal SH2 Domain." *THE JOURNAL OF BIOLOGICAL CHEMISTRY*, 12888–92.

Lewis, Tommy L Jr, Julien Courchet, and Franck Polleux. 2013. "Cell Biology in Neuroscience: Cellular and Molecular Mechanisms Underlying Axon Formation, Growth, and Branching." *The Journal of Cell Biology* 202 (6): 837–48.

https://doi.org/10.1083/jcb.201305098.

Liesi, P. 1985. "Do Neurons in the Vertebrate CNS Migrate on Laminin?" *The EMBO Journal* 4 (5): 1163–70. https://doi.org/10.1002/j.1460-2075.1985.tb03755.x.

Lietha, Daniel, Xinming Cai, Derek F.J. Ceccarelli, Yiqun Li, Michael D. Schaller, and Michael J. Eck. 2007. "Structural Basis for the Autoinhibition of Focal Adhesion Kinase." *Cell* 129 (6): 1177–87. https://doi.org/10.1016/j.cell.2007.05.041.

Lilja, Johanna, and Johanna Ivaska. 2018. "Integrin Activity in Neuronal Connectivity." *Journal of Cell Science* 131 (12). https://doi.org/10.1242/jcs.212803.

Lin, C H, C A Thompson, and P Forscher. 1994. "Cytoskeletal Reorganization Underlying Growth Cone Motility." *Current Opinion in Neurobiology* 4 (5): 640–47. https://doi.org/10.1016/0959-4388(94)90004-3.

Lin, Chi-Hung, and Paul Forscher. 1995. "Growth Cone Advance Is Inversely Proportional to Retrograde F-Actin Flow." *Neuron* 14 (4): 763–71. https://doi.org/https://doi.org/10.1016/0896-6273(95)90220-1.

Lipfert, L., B. Haimovich, M. D. Schaller, B. S. Cobb, J. T. Parsons, and J. S. Brugge. 1992. "Integrin-Dependent Phosphorylation and Activation of the Protein Tyrosine Kinase Pp125(FAK) in Platelets." *Journal of Cell Biology* 119 (4): 905–12. https://doi.org/10.1083/jcb.119.4.905.

Liu, G., W. Li, X. Gao, X. Li, C. Jurgensen, H.-T. Park, N.-Y. Shin, et al. 2007. "P130CAS Is Required for Netrin Signaling and Commissural Axon Guidance." *Journal of Neuroscience* 27 (4): 957–68. https://doi.org/10.1523/JNEUROSCI.4616-06.2007.

Logue, Jeremy S, Alexander X Cartagena-Rivera, and Richard S Chadwick. 2018. "C-Src Activity Is Differentially Required by Cancer Cell Motility Modes." *Oncogene* 37 (16): 2104–21. https://doi.org/10.1038/s41388-017-0071-5.

Long, Katherine R., and Wieland B. Huttner. 2019. "How the Extracellular Matrix Shapes Neural Development." *Open Biology* 9 (1). https://doi.org/10.1098/rsob.180216.

López-Bendito, Guillermina, Aline Cautinat, Juan Antonio Sánchez, Franck Bielle, Nuria Flames, Alistair N. Garratt, David A. Talmage, et al. 2006. "Tangential Neuronal Migration Controls Axon Guidance: A Role for Neuregulin-1 in Thalamocortical Axon Navigation." *Cell* 125 (1): 127–42. https://doi.org/10.1016/j.cell.2006.01.042.

López-Bendito, Guillermina, and Zoltán Molnár. 2003. "Thalamocortical Development:

How Are We Going to Get There?” *Nature Reviews Neuroscience* 4 (4): 276–89. https://doi.org/10.1038/nrn1075.

LoTurco, Joseph J., and Jilin Bai. 2006. “The Multipolar Stage and Disruptions in Neuronal Migration.” *Trends in Neurosciences* 29 (7): 407–13. https://doi.org/10.1016/j.tins.2006.05.006.

Lowery, Laura Anne, and David Van Vactor. 2009. “The Trip of the Tip: Understanding the Growth Cone Machinery.” *Nature Reviews. Molecular Cell Biology* 10 (5): 332–43. https://doi.org/10.1038/nrm2679.

Luker, Kathryn E., Matthew C.P. Smith, Gary D. Luker, Seth T. Gammon, Helen Piwnica-Worms, and David Piwnica-Worms. 2004. “Kinetics of Regulated Protein-Protein Interactions Revealed with Firefly Luciferase Complementation Imaging in Cells and Living Animals.” *Proceedings of the National Academy of Sciences of the United States of America* 101 (33): 12288–93. https://doi.org/10.1073/pnas.0404041101.

Madisen, Linda, Theresa A Zwingman, Susan M Sunkin, Seung Wook Oh, Hatim A Zariwala, Hong Gu, Lydia L Ng, et al. 2010. “A Robust and High-Throughput Cre Reporting and Characterization System for the Whole Mouse Brain.” *Nature Neuroscience* 13 (1): 133–40. https://doi.org/10.1038/nn.2467.

Maier, Viola, Christine Jolicoeur, Helen Rayburn, Noriko Takegahara, Atsushi Kumanogoh, Hitoshi Kikutani, Marc Tessier-Lavigne, Wolfgang Wurst, and Roland H. Friedel. 2011. “Semaphorin 4C and 4G Are Ligands of Plexin-B2 Required in Cerebellar Development.” *Molecular and Cellular Neuroscience* 46 (2): 419–31. https://doi.org/10.1016/j.mcn.2010.11.005.

Marchetti, Giovanni, Sarah Escuin, Arjan van der Flier, Adèle De Arcangelis, Richard O Hynes, and Elisabeth Georges-Labouesse. 2010. “Integrin Alpha5beta1 Is Necessary for Regulation of Radial Migration of Cortical Neurons during Mouse Brain Development.” *The European Journal of Neuroscience* 31 (3): 399–409. https://doi.org/10.1111/j.1460-9568.2009.07072.x.

Marcus, R C, R Blazeski, P Godement, and C A Mason. 1995. “Retinal Axon Divergence in the Optic Chiasm: Uncrossed Axons Diverge from Crossed Axons within a Midline Glial Specialization.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 15 (5 Pt 2): 3716–29. https://doi.org/10.1523/JNEUROSCI.15-05-03716.1995.

Marin, O., M. Valiente, X. Ge, and L.-H. Tsai. 2010. “Guiding Neuronal Cell Migrations.” *Cold Spring Harbor Perspectives in Biology* 2 (2): a001834–a001834. https://doi.org/10.1101/cshperspect.a001834.

Marín, O, and J L Rubenstein. 2001. "A Long, Remarkable Journey: Tangential Migration in the Telencephalon." *Nature Reviews. Neuroscience* 2 (11): 780–90. https://doi.org/10.1038/35097509.

Marín, Oscar, Miguel Valdeolmillos, and Fernando Moya. 2006. "Neurons in Motion: Same Principles for Different Shapes?" *Trends in Neurosciences* 29 (12): 655–61. https://doi.org/10.1016/j.tins.2006.10.001.

Martin-Lopez, Eduardo, Sarah J Meller, and Charles A Greer. 2018. "Development of Piriform Cortex Interhemispheric Connections via the Anterior Commissure: Progressive and Regressive Strategies." *Brain Structure & Function* 223 (9): 4067–85. https://doi.org/10.1007/s00429-018-1741-y.

Martini, Francisco J, Manuel Valiente, Guillermina López Bendito, Gábor Szabó, Fernando Moya, Miguel Valdeolmillos, and Oscar Marín. 2009. "Biased Selection of Leading Process Branches Mediates Chemotaxis during Tangential Neuronal Migration." *Development (Cambridge, England)* 136 (1): 41–50. https://doi.org/10.1242/dev.025502.

Matsuda, Ikuo, Masahiro Fukaya, Harumi Nakao, Kazuki Nakao, Hideyuki Matsumoto, Kensaku Mori, Masahiko Watanabe, and Atsu Aiba. 2010. "Development of the Somatosensory Cortex, the Cerebellum, and the Main Olfactory System in Semaphorin 3F Knockout Mice." *Neuroscience Research* 66 (3): 321–29. https://doi.org/10.1016/j.neures.2009.12.001.

McConnell, Susan K, Anirvan Ghosh, and Carla J Shatz. 1989. "Subplate Neurons Pioneer the First Axon Pathway from the Cerebral Cortex." *Science* 245 (4921): 978–82. https://doi.org/10.1126/science.2475909.

McKenzie, Andrew J, Kathryn V Svec, Tamara F Williams, and Alan K Howe. 2019. "Protein Kinase A Activity Is Regulated by Actomyosin Contractility during Cell Migration and Is Required for Durotaxis." *Molecular Biology of the Cell* 31 (1): 45–58. https://doi.org/10.1091/mbc.E19-03-0131.

Medeiros, Nelson A., Dylan T. Burnette, and Paul Forscher. 2006. "Myosin II Functions in Actin-Bundle Turnover in Neuronal Growth Cones." *Nature Cell Biology* 8 (3): 215–26. https://doi.org/10.1038/ncb1367.

Meller, Nahum, Sylvain Merlot, and Chittibabu Guda. 2005. "CZH Proteins: A New Family of Rho-GEFs." *Journal of Cell Science* 118 (Pt 21): 4937–46. https://doi.org/10.1242/jcs.02671.

Mendrick, D L, and D M Kelly. 1993. "Temporal Expression of VLA-2 and Modulation of Its Ligand Specificity by Rat Glomerular Epithelial Cells in Vitro." *Laboratory*

Investigation; a Journal of Technical Methods and Pathology 69 (6): 690–702.

Miller, Andrew E., Ping Hu, and Thomas H. Barker. 2020. “Feeling Things Out: Bidirectional Signaling of the Cell–ECM Interface, Implications in the Mechanobiology of Cell Spreading, Migration, Proliferation, and Differentiation.” *Advanced Healthcare Materials* 9 (8). https://doi.org/10.1002/adhm.201901445.

Milner, Richard, and Iain L Campbell. 2002. “The Integrin Family of Cell Adhesion Molecules Has Multiple Functions within the CNS.” *Journal of Neuroscience Research* 69 (3): 286–91. https://doi.org/10.1002/jnr.10321.

Minegishi, Takunori, and Naoyuki Inagaki. 2020. “Forces to Drive Neuronal Migration Steps.” *Frontiers in Cell and Developmental Biology* 8: 863. https://doi.org/10.3389/fcell.2020.00863.

Missaire, Mégane, and Robert Hindges. 2015. “The Role of Cell Adhesion Molecules in Visual Circuit Formation: From Neurite Outgrowth to Maps and Synaptic Specificity.” *Developmental Neurobiology* 75 (6): 569–83. https://doi.org/https://doi.org/10.1002/dneu.22267.

Mitchison, T, and M Kirschner. 1988. “Cytoskeletal Dynamics and Nerve Growth.” *Neuron* 1 (9): 761–72. https://doi.org/10.1016/0896-6273(88)90124-9.

Mitra, Satyajit K., Daniel A. Hanson, and David D. Schlaepfer. 2005. “Focal Adhesion Kinase: In Command and Control of Cell Motility.” *Nature Reviews Molecular Cell Biology* 6 (1): 56–68. https://doi.org/10.1038/nrm1549.

Miyake, Noriko, John Chilton, Maria Psatha, Long Cheng, Wai-man Chan, Krystal Law, Moira Crosier, et al. 2009. “Human CHN1 Mutations Hyperactivate A2-Chimaerin and Cause Duane’s Retraction Syndrome” 321 (5890): 839–43. https://doi.org/10.1126/science.1156121.Human.

Miyamoto, Shingo, Hidemi Teramoto, Omar A. Coso, J. Silvio Gutkind, Peter D. Burbelo, Steven K. Akiyama, and Kenneth M. Yamada. 1995. “Integrin Function: Molecular Hierarchies of Cytoskeletal and Signaling Molecules.” *Journal of Cell Biology* 131 (3): 791–805. https://doi.org/10.1083/jcb.131.3.791.

Molnár, Zoltán, Richard Adams, AndréM. Goffinet, and Colin Blakemore. 1998. “The Role of the First Postmitotic Cortical Cells in the Development of Thalamocortical Innervation in the <Em>Reeler</Em>Mouse.” *The Journal of Neuroscience* 18 (15): 5746 LP – 5765. https://doi.org/10.1523/JNEUROSCI.18-15-05746.1998.

Molnár, Zoltán, Sonia Garel, Guillermina López-Bendito, Patricia Maness, and David J.

Price. 2012. “Mechanisms Controlling the Guidance of Thalamocortical Axons through the Embryonic Forebrain.” *European Journal of Neuroscience* 35 (10): 1573–85. https://doi.org/10.1111/j.1460-9568.2012.08119.x.

Moore Dr., Simon W., Xian Zhang, Christopher D. Lynch, and Michael P. Sheetz. 2012. “Netrin-1 Attracts Axons through FAK-Dependent Mechanotransduction.” *Journal of Neuroscience* 32 (34): 11574–85. https://doi.org/10.1523/JNEUROSCI.0999-12.2012.

Moos, Marion, Roland Tacke, Herta Scherer, David Teplow, Klaus Früh, and Melitta Schachner. 1988. “Neural Adhesion Molecule L1 as a Member of the Immunoglobulin Superfamily with Binding Domains Similar to Fibronectin.” *Nature* 334 (6184): 701–3. https://doi.org/10.1038/334701a0.

Moreland, Trevor, and Fabienne E Poulain. 2022. “To Stick or Not to Stick: The Multiple Roles of Cell Adhesion Molecules in Neural Circuit Assembly.” *Frontiers in Neuroscience* 16: 889155. https://doi.org/10.3389/fnins.2022.889155.

Mousson, Antoine, Emilie Sick, Philippe Carl, Denis Dujardin, Jan De Mey, and Philippe Rondé. 2018. “Targeting Focal Adhesion Kinase Using Inhibitors of Protein-Protein Interactions.” *Cancers* 10 (9): 1–29. https://doi.org/10.3390/cancers10090278.

Mulherkar, Shalaka, Mohammad Danish Uddin, Anthony D. Couvillon, Roy V. Sillitoe, and Kimberley F. Tolias. 2014a. “The Small GTPases RhoA and Rac1 Regulate Cerebellar Development by Controlling Cell Morphogenesis, Migration and Foliation.” *Developmental Biology* 394 (1): 39–53. https://doi.org/10.1016/j.ydbio.2014.08.004.

Munakata, Hana, Yukiko Nakamura, Kazumasa Matsumoto-Miyai, Kouichi Itoh, Hironobu Yamasaki, and Sadao Shiosaka. 2003. “Distribution and Densitometry Mapping of L1-CAM Immunoreactivity in the Adult Mouse Brain – Light Microscopic Observation.” *BMC Neuroscience* 4 (1): 7. https://doi.org/10.1186/1471-2202-4-7.

Murphy, James M., Yelitza A.R. Rodriguez, Kyuho Jeong, Eun Young Erin Ahn, and Ssang Taek Steve Lim. 2020. “Targeting Focal Adhesion Kinase in Cancer Cells and the Tumor Microenvironment.” *Experimental and Molecular Medicine* 52 (6): 877–86. https://doi.org/10.1038/s12276-020-0447-4.

Myers, Jonathan P., Miguel Santiago-Medina, and Timothy M. Gomez. 2011a. “Regulation of Axonal Outgrowth and Pathfinding by Integrin-Ecm Interactions.” *Developmental Neurobiology* 71 (11): 901–23. https://doi.org/10.1002/dneu.20931.

Myers, Jonathan P, and Timothy M Gomez. 2011. “Focal Adhesion Kinase Promotes

Integrin Adhesion Dynamics Necessary for Chemotropic Turning of Nerve Growth Cones." *The Journal of Neuroscience* 31 (38): 13585 LP – 13595. https://doi.org/10.1523/JNEUROSCI.2381-11.2011.

Myers, Jonathan P, Miguel Santiago-Medina, and Timothy M Gomez. 2011b. "Regulation of Axonal Outgrowth and Pathfinding by Integrin-ECM Interactions." *Developmental Neurobiology* 71 (11): 901–23. https://doi.org/10.1002/dneu.20931.

Nagata, I, and N Nakatsuji. 1990. "Granule Cell Behavior on Laminin in Cerebellar Microexplant Cultures." *Brain Research. Developmental Brain Research* 52 (1–2): 63–73. https://doi.org/10.1016/0165-3806(90)90222-k.

Nakamoto, T, R Sakai, H Honda, S Ogawa, H Ueno, T Suzuki, S Aizawa, Y Yazaki, and H Hirai. 1997. "Requirements for Localization of P130cas to Focal Adhesions." *Molecular and Cellular Biology* 17 (7): 3884–97. https://doi.org/10.1128/mcb.17.7.3884.

Nakamura, Takashi, Takehiko Ueyama, Yuzuru Ninoyu, Hirofumi Sakaguchi, Narantsog Choijookhuu, Yoshitaka Hishikawa, Hiroshi Kiyonari, et al. 2017. "Novel Role of Rac-Mid1 Signaling in Medial Cerebellar Development." *Development* 144 (10): 1863–75. https://doi.org/10.1242/dev.147900.

Nakanishi, S. 1983. "Extracellular Matrix during Laminar Pattern Formation of Neocortex in Normal and Reeler Mutant Mice." *Developmental Biology* 95 (2): 305–16. https://doi.org/10.1016/0012-1606(83)90031-3.

Nasrallah, Ilya M, Matthew F McManus, Maclean M Pancoast, Anthony Wynshaw-Boris, and Jeffrey A Golden. 2006. "Analysis of Non-Radial Interneuron Migration Dynamics and Its Disruption in Lis1+/- Mice." *The Journal of Comparative Neurology* 496 (6): 847–58. https://doi.org/10.1002/cne.20966.

Nguyen-Ba-Charvet, Kim T, Katja Brose, Valérie Marillat, Constantino Sotelo, Marc Tessier-Lavigne, and Alain Chédotal. 2001. "Sensory Axon Response to Substrate-Bound Slit2 Is Modulated by Laminin and Cyclic GMP." *Molecular and Cellular Neuroscience* 17 (6): 1048–58. https://doi.org/https://doi.org/10.1006/mcne.2001.0994.

Nguyen, Laurent, and Simon Hippenmeyer. n.d. *Cellular and Molecular Control of Neuronal Migration*. https://doi.org/10.1007/978-94-007-7687-6_4.

Nichol, Robert H., Kate M. Hagen, Derek C. Lumbard, Erik W. Dent, and Timothy M. Gómez. 2016. "Guidance of Axons by Local Coupling of Retrograde Flow to Point Contact Adhesions." *Journal of Neuroscience* 36 (7): 2267–82. https://doi.org/10.1523/JNEUROSCI.2645-15.2016.

Ning, Lin, Li Tian, Sergei Smirnov, Helena Vihinen, Olaya Llano, Kyle Vick, Ronald L Davis, Claudio Rivera, and Carl G Gahmberg. 2013. "Interactions between ICAM-5 and Β1 Integrins Regulate Neuronal Synapse Formation." *Journal of Cell Science* 126 (Pt 1): 77–89. https://doi.org/10.1242/jcs.106674.

Nobes, Catherine D., and Alan Hall. 1995. "Rho, Rac, and Cdc42 GTPases Regulate the Assembly of Multimolecular Focal Complexes Associated with Actin Stress Fibers, Lamellipodia, and Filopodia." *Cell* 81 (1): 53–62. https://doi.org/10.1016/0092-8674(95)90370-4.

Nóbrega-Pereira, Sandrina, and Oscar Marín. 2009. "Transcriptional Control of Neuronal Migration in the Developing Mouse Brain." *Cerebral Cortex (New York, N.Y. : 1991)* 19 Suppl 1 (July): i107-13. https://doi.org/10.1093/cercor/bhp044.

Nollau, Peter, and Bruce J. Mayer. 2001. "Profiling the Global Tyrosine Phosphorylation State by Src Homology 2 Domain Binding." *Proceedings of the National Academy of Sciences of the United States of America* 98 (24): 13531–36. https://doi.org/10.1073/pnas.241215998.

Nozumi, Motohiro, and Michihiro Igarashi. 2018. "Vesicular Movements in the Growth Cone." *Neurochemistry International* 119: 71–76. https://doi.org/https://doi.org/10.1016/j.neuint.2017.09.011.

O'Shea, K. S., J. S.T. Rheinheimer, and V. M. Dixit. 1990. "Deposition and Role of Thrombospondin in the Histogenesis of the Cerebellar Cortex." *Journal of Cell Biology* 110 (4): 1275–83. https://doi.org/10.1083/jcb.110.4.1275.

Oliver, T, M Dembo, and K Jacobson. 1999. "Separation of Propulsive and Adhesive Traction Stresses in Locomoting Keratocytes." *The Journal of Cell Biology* 145 (3): 589–604. https://doi.org/10.1083/jcb.145.3.589.

Omotade, Omotola F, Stephanie L Pollitt, and James Q Zheng. 2017. "Actin-Based Growth Cone Motility and Guidance." *Molecular and Cellular Neuroscience* 84: 4–10. https://doi.org/https://doi.org/10.1016/j.mcn.2017.03.001.

Owen, J D, P J Ruest, D W Fry, and S K Hanks. 1999. "Induced Focal Adhesion Kinase (FAK) Expression in FAK-Null Cells Enhances Cell Spreading and Migration Requiring Both Auto- and Activation Loop Phosphorylation Sites and Inhibits Adhesion-Dependent Tyrosine Phosphorylation of Pyk2." *Molecular and Cellular Biology* 19 (7): 4806–18. https://doi.org/10.1128/MCB.19.7.4806.

Palazzo, Alexander F., Christina H. Eng, David D. Schlaepfer, Eugene E. Marcantonio, and Gregg G. Gundersen. 2004. "Localized Stabilization of Microtubules by Integrin- and FAK-Facilitated Rho Signaling." *Science (New York, N.Y.)* 303 (5659):

836–39. https://doi.org/10.1126/SCIENCE.1091325.

Palmini, André, and Hans Holthausen. 2013. "Focal Malformations of Cortical Development: A Most Relevant Etiology of Epilepsy in Children." *Handbook of Clinical Neurology* 111: 549–65. https://doi.org/10.1016/B978-0-444-52891-9.00058-0.

Pang, Xiao-Jing, Xiu-Juan Liu, Yuan Liu, Wen-Bo Liu, Yin-Ru Li, Guang-Xi Yu, Xin-Yi Tian, et al. 2021. "Drug Discovery Targeting Focal Adhesion Kinase (FAK) as a Promising Cancer Therapy." *Molecules* . https://doi.org/10.3390/molecules26144250.

Papusheva, Ekaterina, Fernanda Mello de Queiroz, Jeremie Dalous, Yunyun Han, Alessandro Esposito, Elizabeth A. Jares-Erijman, Thomas M. Jovin, and Gertrude Bunt. 2009. "Dynamic Conformational Changes in the FERM Domain of FAK Are Involved in Focal-Adhesion Behavior during Cell Spreading and Motility." *Journal of Cell Science* 122 (5): 656–66. https://doi.org/10.1242/jcs.028738.

Park, H L, C Brian Bai, K a Platt, M P Matise, a Beeghly, Chi-Chung Hui, M Nakashima, and Alexandra L Joyner. 2000. "Mouse Gli1 Mutants Are Viable but Have Defects in SHH Signaling in Combination with a Gli2 Mutation." *Development (Cambridge, England)* 127 (8): 1593–1605. https://doi.org/10.1101/gad.10.16.2003.

Park, Hwan Tae, Jane Wu, and Yi Rao. 2002. "Molecular Control of Neuronal Migration." *BioEssays* 24 (9): 821–27. https://doi.org/10.1002/bies.10141.

Parsons, J. T., K. H. Martin, J. K. Slack, J. M. Taylor, and S. A. Weed. 2000. "Focal Adhesion Kinase: A Regulator of Focal Adhesion Dynamics and Cell Movement." *Oncogene* 19 (49): 5606–13. https://doi.org/10.1038/sj.onc.1203877.

Parsons, J. Thomas. 2003. "Focal Adhesion Kinase: The First Ten Years." *Journal of Cell Science* 116 (8): 1409–16. https://doi.org/10.1242/jcs.00373.

Parsons, J. Thomas, Alan Rick Horwitz, and Martin A. Schwartz. 2010. "Cell Adhesion: Integrating Cytoskeletal Dynamics and Cellular Tension." *Nature Reviews Molecular Cell Biology* 11 (9): 633–43. https://doi.org/10.1038/nrm2957.

Paulmurugan, R., Y. Umezawa, and S. S. Gambhir. 2002. "Noninvasive Imaging of Protein-Protein Interactions in Living Subjects by Using Reporter Protein Complementation and Reconstitution Strategies." *Proceedings of the National Academy of Sciences of the United States of America* 99 (24): 15608–13. https://doi.org/10.1073/pnas.242594299.

Paulmurugant, R., and Sanjiv S. Gambhir. 2003. "Monitoring Protein-Protein Interactions

Using Split Synthetic Renilla Luciferase Protein-Fragment-Assisted Complementation." *Analytical Chemistry* 75 (7): 1584–89. https://doi.org/10.1021/ac020731c.

Piper, Michael, Céline Plachez, Oressia Zalucki, Thomas Fothergill, Guy Goudreau, Reha Erzurumlu, Chenghua Gu, and Linda J Richards. 2009. "Neuropilin 1-Sema Signaling Regulates Crossing of Cingulate Pioneering Axons during Development of the Corpus Callosum." *Cerebral Cortex (New York, N.Y. : 1991)* 19 Suppl 1 (Suppl 1): i11-21. https://doi.org/10.1093/cercor/bhp027.

Pires-Neto, M A, S Braga-De-Souza, and R Lent. 1998. "Molecular Tunnels and Boundaries for Growing Axons in the Anterior Commissure of Hamster Embryos." *The Journal of Comparative Neurology* 399 (2): 176–88. https://doi.org/10.1002/(sici)1096-9861(19980921)399:2<176::aid-cne3>3.0.co;2-y.

Playford, Martin P., and Michael D. Schaller. 2004. "The Interplay between Src and Integrins in Normal and Tumor Biology." *Oncogene* 23 (48 REV. ISS. 7): 7928–46. https://doi.org/10.1038/sj.onc.1208080.

Plump, Andrew S, Lynda Erskine, Christelle Sabatier, Katja Brose, Charles J Epstein, Corey S Goodman, Carol A Mason, and Marc Tessier-Lavigne. 2002. "Slit1 and Slit2 Cooperate to Prevent Premature Midline Crossing of Retinal Axons in the Mouse Visual System." *Neuron* 33 (2): 219–32. https://doi.org/10.1016/s0896-6273(01)00586-4.

Pollerberg, G E, K Thelen, M O Theiss, and B C Hochlehnert. 2013. "The Role of Cell Adhesion Molecules for Navigating Axons: Density Matters." *Mechanisms of Development* 130 (6): 359–72. https://doi.org/https://doi.org/10.1016/j.mod.2012.11.002.

Polleux, F., and A. Ghosh. 2002. "The Slice Overlay Assay: A Versatile Tool to Study the Influence of Extracellular Signals on Neuronal Development." *Science Signaling* 2002 (136): pl9–pl9. https://doi.org/10.1126/stke.2002.136.pl9.

Pontén, F, K Jirström, and M Uhlen. 2008. "The Human Protein Atlas—a Tool for Pathology." *The Journal of Pathology* 216 (4): 387–93. https://doi.org/https://doi.org/10.1002/path.2440.

Provenzano, Paolo P., and Patricia J. Keely. 2009. "The Role of Focal Adhesion Kinase in Tumor Initiation and Progression." *Cell Adhesion and Migration* 3 (4): 1–5. https://doi.org/10.4161/cam.3.4.9458.

Raffa, Vittoria. 2022. "Force: A Messenger of Axon Outgrowth." *Seminars in Cell & Developmental Biology*.

https://doi.org/https://doi.org/10.1016/j.semcdb.2022.07.004.

Rakic, P. 1972. “Mode of Cell Migration to the Superficial Layers of Fetal Monkey Neocortex.” *The Journal of Comparative Neurology* 145 (1): 61–83. https://doi.org/10.1002/cne.901450105.

Rakic, P, and R L Sidman. 1970. “Histogenesis of Cortical Layers in Human Cerebellum, Particularly the Lamina Dissecans.” *The Journal of Comparative Neurology* 139 (4): 473–500. https://doi.org/10.1002/cne.901390407.

Raper, Jonathan, and Carol Mason. 2010. “Cellular Strategies of Axonal Pathfinding.” *Cold Spring Harbor Perspectives in Biology* 2 (9): a001933. https://doi.org/10.1101/cshperspect.a001933.

Rasband, Matthew N, and Elior Peles. 2021. “Mechanisms of Node of Ranvier Assembly.” *Nature Reviews Neuroscience* 22 (1): 7–20. https://doi.org/10.1038/s41583-020-00406-8.

Reichardt, L F, and K J Tomaselli. 1991. “Extracellular Matrix Molecules and Their Receptors: Functions in Neural Development.” *Annual Review of Neuroscience* 14 (1): 531–70. https://doi.org/10.1146/annurev.ne.14.030191.002531.

Remy, I, A Galarneau, and S W Michnick. 2002. “Detection and Visualization of Protein Interactions with Protein Fragment Complementation Assays.” *Methods in Molecular Biology (Clifton, N.J.)* 185: 447–59. https://doi.org/10.1385/1-59259-241-4:447.

Remy, Ingrid, and Stephen W Michnick. 2006. “A Highly Sensitive Protein-Protein Interaction Assay Based on Gaussia Luciferase.” *Nature Methods* 3 (12): 977–79. https://doi.org/10.1038/nmeth979.

Ren, X. D., W. B. Kiosses, D. J. Sieg, C. A. Otey, D. D. Schlaepfer, and M. A. Schwartz. 2000. “Focal Adhesion Kinase Suppresses Rho Activity to Promote Focal Adhesion.” *Journal of Cell Science* 113 (20): 3673–78.

Renaud, Julie, Géraldine Kerjan, Itsuko Sumita, Yvrick Zagar, Virginie Georget, Doyeun Kim, Coralie Fouquet, et al. 2008. “Plexin-A2 and Its Ligand, Sema6A, Control Nucleus-Centrosome Coupling in Migrating Granule Cells.” *Nature Neuroscience* 11 (4): 440–49. https://doi.org/10.1038/nn2064.

Renaudin, A., M. Lehmann, J. A. Girault, and L. McKerracher. 1999. “Organization of Point Contacts in Neuronal Growth Cones.” *Journal of Neuroscience Research* 55 (4): 458–71. https://doi.org/10.1002/(SICI)1097-4547(19990215)55:4<458::AID-JNR6>3.0.CO;2-D.

Riccomagno, Martin M., Lu O. Sun, Colleen M. Brady, Konstantina Alexandropoulos, Sachiko Seo, Mineo Kurokawa, and Alex L. Kolodkin. 2014. "Cas Adaptor Proteins Organize the Retinal Ganglion Cell Layer Downstream of Integrin Signaling." *Neuron* 81 (4): 779–86. https://doi.org/10.1016/j.neuron.2014.01.036.

Riccomagno, Martin M, Andrés Hurtado, HongBin Wang, Joshua G J Macopson, Erin M Griner, Andrea Betz, Nils Brose, Marcelo G Kazanietz, and Alex L Kolodkin. 2012. "The RacGAP Beta-Chimaerin Selectively Mediates Stereotyped Hippocampal Axonal Pruning." *Cell* 149 (7): 1594–1606. https://doi.org/10.1016/j.cell.2012.05.018.The.

Ridley, Anne J., Martin A. Schwartz, Keith Burridge, Richard A. Firtel, Mark H. Ginsberg, Gary Borisy, J. Thomas Parsons, and Alan Rick Horwitz. 2003. "Cell Migration: Integrating Signals from Front to Back." *Science* 302 (5651): 1704–9. https://doi.org/10.1126/SCIENCE.1092053/ASSET/81EC31A6-8853-425C-92A3-DBB2ED5C159B/ASSETS/GRAPHIC/ZSE4730320900002.JPEG.

Rigiracciolo, Damiano Cosimo, Francesca Cirillo, Marianna Talia, Lucia Muglia, Jorge Silvio Gutkind, Marcello Maggiolini, and Rosamaria Lappano. 2021. "Focal Adhesion Kinase Fine Tunes Multifaced Signals toward Breast Cancer Progression." *Cancers* 13 (4): 1–29. https://doi.org/10.3390/cancers13040645.

Rio, Armando del, Raul Perez-Jimenez, Ruchuan Liu, Pere Roca-Cusachs, Julio M Fernandez, and Michael P Sheetz. 2009. "Stretching Single Talin Rod Molecules Activates Vinculin Binding." *Science (New York, N.Y.)* 323 (5914): 638–41. https://doi.org/10.1126/science.1162912.

Ritt, Michael, Jun Lin Guan, and Sivaraj Sivaramakrishnan. 2013. "Visualizing and Manipulating Focal Adhesion Kinase Regulation in Live Cells." *Journal of Biological Chemistry* 288 (13): 8875–86. https://doi.org/10.1074/jbc.M112.421164.

Riveline, Daniel, Eli Zamir, Nathalie Q. Balaban, Ulrich S. Schwarz, Toshimasa Ishizaki, Shuh Narumiya, Zvi Kam, Benjamin Geiger, and Alexander D. Bershadsky. 2001. "Focal Contacts as Mechanosensors: Externally Applied Local Mechanical Force Induces Growth of Focal Contacts by an MDia1-Dependent and ROCK-Independent Mechanism." *Journal of Cell Biology* 153 (6): 1175–85. https://doi.org/10.1083/jcb.153.6.1175.

Robichaux, Michael A, George Chenaux, Hsin-Yi Henry Ho, Michael J Soskis, Michael E Greenberg, Mark Henkemeyer, and Christopher W Cowan. 2016. "EphB1 and EphB2 Intracellular Domains Regulate the Formation of the Corpus Callosum and Anterior Commissure." *Developmental Neurobiology* 76 (4): 405–20. https://doi.org/10.1002/dneu.22323.

Robles, Estuardo, and Timothy M. Gomez. 2006. "Focal Adhesion Kinase Signaling at Sites of Integrin-Mediated Adhesion Controls Axon Pathfinding." *Nature Neuroscience* 9 (10): 1274–83. https://doi.org/10.1038/nn1762.

Rossi, Fabio, Carol A. Charlton, and Helen M. Blau. 1997. "Monitoring Protein-Protein Interactions in Intact Eukaryotic Cells by β-Galactosidase Complementation." *Proceedings of the National Academy of Sciences of the United States of America* 94 (16): 8405–10. https://doi.org/10.1073/pnas.94.16.8405.

Rossman, Kent L, Channing J Der, and John Sondek. 2005. "GEF Means Go: Turning on RHO GTPases with Guanine Nucleotide-Exchange Factors." *Nature Reviews. Molecular Cell Biology* 6 (2): 167–80. https://doi.org/10.1038/nrm1587.

Ruest, P J, S Roy, E Shi, R L Mernaugh, and S K Hanks. 2000. "Phosphospecific Antibodies Reveal Focal Adhesion Kinase Activation Loop Phosphorylation in Nascent and Mature Focal Adhesions and Requirement for the Autophosphorylation Site." *Cell Growth & Differentiation : The Molecular Biology Journal of the American Association for Cancer Research* 11 (1): 41–48.

Ruest, P J, N Y Shin, T R Polte, X Zhang, and S K Hanks. 2001. "Mechanisms of CAS Substrate Domain Tyrosine Phosphorylation by FAK and Src." *Molecular and Cellular Biology* 21 (22): 7641–52. https://doi.org/10.1128/MCB.21.22.7641-7652.2001.

Ruiz de Almodovar, C., C. Coulon, P. A. Salin, E. Knevels, N. Chounlamountri, K. Poesen, K. Hermans, et al. 2010. "Matrix-Binding Vascular Endothelial Growth Factor (VEGF) Isoforms Guide Granule Cell Migration in the Cerebellum via VEGF Receptor Flk1." *Journal of Neuroscience* 30 (45): 15052–66. https://doi.org/10.1523/JNEUROSCI.0477-10.2010.

Sahay, Amar, Mark E Molliver, David D Ginty, and Alex L Kolodkin. 2003. "Semaphorin 3F Is Critical for Development of Limbic System Circuitry and Is Required in Neurons for Selective CNS Axon Guidance Events." *The Journal of Neuroscience* 23 (17): 6671 LP – 6680. https://doi.org/10.1523/JNEUROSCI.23-17-06671.2003.

Sakaguchi, D S, and K Radke. 1996. "Beta 1 Integrins Regulate Axon Outgrowth and Glial Cell Spreading on a Glial-Derived Extracellular Matrix during Development and Regeneration." *Brain Research. Developmental Brain Research* 97 (2): 235–50. https://doi.org/10.1016/s0165-3806(96)00142-3.

Sánchez-Huertas, Carlos, and Eloísa Herrera. 2021. "With the Permission of Microtubules: An Updated Overview on Microtubule Function During Axon Pathfinding." *Frontiers in Molecular Neuroscience* 14: 759404.

https://doi.org/10.3389/fnmol.2021.759404.

Sanes, J R. 1989. "Extracellular Matrix Molecules That Influence Neural Development." *Annual Review of Neuroscience* 12: 491–516. https://doi.org/10.1146/annurev.ne.12.030189.002423.

Sanes, Joshua R, and S Lawrence Zipursky. 2010. "Design Principles of Insect and Vertebrate Visual Systems." *Neuron* 66 (1): 15–36. https://doi.org/10.1016/j.neuron.2010.01.018.

Santiago-Medina, Miguel, Jonathan P Myers, and Timothy M Gomez. 2012. "Imaging Adhesion and Signaling Dynamics in Xenopus Laevis Growth Cones." *Developmental Neurobiology* 72 (4): 585–99. https://doi.org/https://doi.org/10.1002/dneu.20886.

Satz, Jakob S., Adam P. Ostendorf, Shangwei Hou, Amy Turner, Hajime Kusano, Jane C. Lee, Rolf Turk, et al. 2010. "Distinct Functions of Glial and Neuronal Dystroglycan in the Developing and Adult Mouse Brain." *Journal of Neuroscience* 30 (43): 14560–72. https://doi.org/10.1523/JNEUROSCI.3247-10.2010.

Schaller, M. D., C. A. Borgman, B. S. Cobb, R. R. Vines, A. B. Reynolds, and J. T. Parsons. 1992a. "Pp125(FAK), a Structurally Distinctive Protein-Tyrosine Kinase Associated with Focal Adhesions." *Proceedings of the National Academy of Sciences of the United States of America* 89 (11): 5192–96. https://doi.org/10.1073/pnas.89.11.5192.

Schaller, M D, J D Hildebrand, J D Shannon, J W Fox, R R Vines, and J T Parsons. 1994. "Autophosphorylation of the Focal Adhesion Kinase, Pp125FAK, Directs SH2-Dependent Binding of Pp60src." *Molecular and Cellular Biology* 14 (3): 1680–88. https://doi.org/10.1128/mcb.14.3.1680.

Schmid, Ralf S, and E S Anton. 2003. "Role of Integrins in the Development of the Cerebral Cortex." *Cerebral Cortex (New York, N.Y. : 1991)* 13 (3): 219–24. https://doi.org/10.1093/cercor/13.3.219.

Schmid, Ralf S, and Patricia F Maness. 2008. "L1 and NCAM Adhesion Molecules as Signaling Coreceptors in Neuronal Migration and Process Outgrowth." *Current Opinion in Neurobiology* 18 (3): 245–50. https://doi.org/https://doi.org/10.1016/j.conb.2008.07.015.

Schneider, Felix, Isabell Metz, and Marco B Rust. 2022. "Regulation of Actin Filament Assembly and Disassembly in Growth Cone Motility and Axon Guidance." *Brain Research Bulletin* 192 (November): 21–35. https://doi.org/10.1016/j.brainresbull.2022.10.019.

Schoenwaelder, S M, and K Burridge. 1999. "Bidirectional Signaling between the Cytoskeleton and Integrins." *Current Opinion in Cell Biology* 11 (2): 274–86. https://doi.org/10.1016/s0955-0674(99)80037-4.

Schwartz, Martin A. 2001. "Integrin Signaling Revisited." *Trends in Cell Biology* 11 (12): 466–70. https://doi.org/10.1016/S0962-8924(01)02152-3.

Schweighoffer, Tamas, and Stephen Shaw. 1992. "Adhesion Cascades: Diversity through Combinatorial Strategies." *Current Opinion in Cell Biology* 4 (5): 824–29. https://doi.org/https://doi.org/10.1016/0955-0674(92)90106-M.

Sekiguchi, Rei, and Kenneth M Yamada. 2018. "Basement Membranes in Development and Disease." *Current Topics in Developmental Biology* 130: 143–91. https://doi.org/10.1016/bs.ctdb.2018.02.005.

SenGupta, Shuvasree, Carole A Parent, and James E Bear. 2021. "The Principles of Directed Cell Migration." *Nature Reviews. Molecular Cell Biology* 22 (8): 529–47. https://doi.org/10.1038/s41580-021-00366-6.

Seo, Sachiko, Takashi Asai, Toshiki Saito, Takahiro Suzuki, Yasuyuki Morishita, Tetsuya Nakamoto, Motoshi Ichikawa, et al. 2005. "Crk-Associated Substrate Lymphocyte Type Is Required for Lymphocyte Trafficking and Marginal Zone B Cell Maintenance." *The Journal of Immunology* 175 (6): 3492 LP – 3501. https://doi.org/10.4049/jimmunol.175.6.3492.

Seong, Jihye, Mingxing Ouyang, Taejin Kim, Jie Sun, Po Chao Wen, Shaoying Lu, Yue Zhuo, et al. 2011. "Detection of Focal Adhesion Kinase Activation at Membrane Microdomains by Fluorescence Resonance Energy Transfer." *Nature Communications* 2 (1). https://doi.org/10.1038/ncomms1414.

Serafini, Tito, Sophia A Colamarino, E.David Leonardo, Hao Wang, Rosa Beddington, William C Skarnes, and Marc Tessier-Lavigne. 1996. "Netrin-1 Is Required for Commissural Axon Guidance in the Developing Vertebrate Nervous System." *Cell* 87 (6): 1001–14. https://doi.org/https://doi.org/10.1016/S0092-8674(00)81795-X.

Sheetz, Michael P, Dan P Felsenfeld, and Catherine G Galbraith. 1998. "Cell Migration: Regulation of Force on Extracellular-Matrix-Integrin Complexes." *Trends in Cell Biology* 8 (2): 51–54. https://doi.org/https://doi.org/10.1016/S0962-8924(98)80005-6.

Shemesh, Tom, Benjamin Geiger, Alexander D. Bershadsky, and Michael M. Kozlov. 2005. "Focal Adhesions as Mechanosensors: A Physical Mechanism." *Proceedings of the National Academy of Sciences of the United States of America* 102 (35): 12383–88. https://doi.org/10.1073/pnas.0500254102.

Shen, Yiping, Shyamala Mani, Stacy L Donovan, James E Schwob, and Karina F Meiri. 2002. “Growth-Associated Protein-43 Is Required for Commissural Axon Guidance in the Developing Vertebrate Nervous System.” *The Journal of Neuroscience* 22 (1): 239 LP – 247. https://doi.org/10.1523/JNEUROSCI.22-01-00239.2002.

Short, Caitlin A, Edwin A Suarez-Zayas, and Timothy M Gomez. 2016. “Cell Adhesion and Invasion Mechanisms That Guide Developing Axons.” *Current Opinion in Neurobiology* 39 (August): 77–85. https://doi.org/10.1016/j.conb.2016.04.012.

Shu, Tianzhi, Ying Li, Asaf Keller, and Linda J Richards. 2003. “The Glial Sling Is a Migratory Population of Developing Neurons.” *Development (Cambridge, England)* 130 (13): 2929–37. https://doi.org/10.1242/dev.00514.

Shu, Tianzhi, and Linda J Richards. 2001. “Cortical Axon Guidance by the Glial Wedge during the Development of the Corpus Callosum.” *The Journal of Neuroscience* 21 (8): 2749 LP – 2758. https://doi.org/10.1523/JNEUROSCI.21-08-02749.2001.

Shu, Tianzhi, Vasi Sundaresan, Margaret M McCarthy, and Linda J Richards. 2003. “Slit2 Guides Both Precrossing and Postcrossing Callosal Axons at the Midline in Vivo.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 23 (22): 8176–84. https://doi.org/10.1523/JNEUROSCI.23-22-08176.2003.

Sieg, David J., Christof R. Hauck, and David D. Schlaepfer. 1999. “Required Role of Focal Adhesion Kinase (FAK) for Integrin-Stimulated Cell Migration.” *Journal of Cell Science* 112 (16): 2677–91.

Silver, J, M A Edwards, and P Levitt. 1993. “Immunocytochemical Demonstration of Early Appearing Astroglial Structures That Form Boundaries and Pathways along Axon Tracts in the Fetal Brain.” *The Journal of Comparative Neurology* 328 (3): 415–36. https://doi.org/10.1002/cne.903280308.

Singer, M, R H Nordlander, and M Egar. 1979. “Axonal Guidance during Embryogenesis and Regeneration in the Spinal Cord of the Newt: The Blueprint Hypothesis of Neuronal Pathway Patterning.” *The Journal of Comparative Neurology* 185 (1): 1–21. https://doi.org/10.1002/cne.901850102.

Snow, D M, E M Brown, and P C Letourneau. 1996. “Growth Cone Behavior in the Presence of Soluble Chondroitin Sulfate Proteoglycan (CSPG), Compared to Behavior on CSPG Bound to Laminin or Fibronectin.” *International Journal of Developmental Neuroscience : The Official Journal of the International Society for Developmental Neuroscience* 14 (3): 331–49. https://doi.org/10.1016/0736-5748(96)00017-2.

Solecki, David J. 2012. "Sticky Situations: Recent Advances in Control of Cell Adhesion during Neuronal Migration." *Current Opinion in Neurobiology* 22 (5): 791–98. https://doi.org/10.1016/j.conb.2012.04.010.

Spead, Olivia, and Fabienne E Poulain. 2020. "Trans-Axonal Signaling in Neural Circuit Wiring." *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms21145170.

Squarzoni, Paola, Morgane Thion, and Sonia Garel. 2015. "Neuronal and Microglial Regulators of Cortical Wiring: Usual and Novel Guideposts ." *Frontiers in Neuroscience* . https://www.frontiersin.org/articles/10.3389/fnins.2015.00248.

Stankiewicz, Trisha R., and Daniel A. Linseman. 2014. "Rho Family GTPases: Key Players in Neuronal Development, Neuronal Survival, and Neurodegeneration." *Frontiers in Cellular Neuroscience* 8 (October): 1–14. https://doi.org/10.3389/fncel.2014.00314.

Stefan, E, S Aquin, N Berger, C R Landry, B Nyfeler, M Bouvier, and S W Michnick. 2007. "Quantification of Dynamic Protein Complexes Using Renilla Luciferase Fragment Complementation Applied to Protein Kinase A Activities in Vivo." *Proceedings of the National Academy of Sciences of the United States of America* 104 (43): 16916–21. https://doi.org/10.1073/pnas.0704257104.

Stein, E, U Huynh-Do, A A Lane, D P Cerretti, and T O Daniel. 1998. "Nck Recruitment to Eph Receptor, EphB1/ELK, Couples Ligand Activation to c-Jun Kinase." *The Journal of Biological Chemistry* 273 (3): 1303–8. https://doi.org/10.1074/jbc.273.3.1303.

Stein, E, A A Lane, D P Cerretti, H O Schoecklmann, A D Schroff, R L Van Etten, and T O Daniel. 1998. "Eph Receptors Discriminate Specific Ligand Oligomers to Determine Alternative Signaling Complexes, Attachment, and Assembly Responses." *Genes & Development* 12 (5): 667–78. https://doi.org/10.1101/gad.12.5.667.

Stoeckli, E T, and L T Landmesser. 1998. "Axon Guidance at Choice Points." *Current Opinion in Neurobiology* 8 (1): 73–79. https://doi.org/10.1016/s0959-4388(98)80010-x.

Sudarov, Anamaria, and Alexandra L. Joyner. 2007. "Cerebellum Morphogenesis: The Foliation Pattern Is Orchestrated by Multi-Cellular Anchoring Centers." *Neural Development* 2 (1). https://doi.org/10.1186/1749-8104-2-26.

Suh, Leejee H, Stephen F Oster, Sophia S Soehrman, Gabriele Grenningloh, and David W Sretavan. 2004. "L1/Laminin Modulation of Growth Cone Response to EphB

Triggers Growth Pauses and Regulates the Microtubule Destabilizing Protein SCG10." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 24 (8): 1976–86. https://doi.org/10.1523/JNEUROSCI.1670-03.2004.

Sulzmaier, Florian J., Christine Jean, and David D. Schlaepfer. 2014. "FAK in Cancer: Mechanistic Findings and Clinical Applications." *Nature Reviews Cancer* 14 (9): 598–610. https://doi.org/10.1038/nrc3792.

Sun, Zhiqi, Shengzhen S Guo, and Reinhard Fässler. 2016. "Integrin-Mediated Mechanotransduction." *The Journal of Cell Biology* 215 (4): 445–56. https://doi.org/10.1083/jcb.201609037.

Suter, Daniel M, and Kyle E Miller. 2011. "The Emerging Role of Forces in Axonal Elongation." *Progress in Neurobiology* 94 (2): 91–101. https://doi.org/10.1016/j.pneurobio.2011.04.002.

Suto, Fumikazu, Keisuke Ito, Masato Uemura, Masayuki Shimizu, Yutaka Shinkawa, Makoto Sanbo, Tomoyasu Shinoda, et al. 2005. "Plexin-A4 Mediates Axon-Repulsive Activities of Both Secreted and Transmembrane Semaphorins and Plays Roles in Nerve Fiber Guidance." *The Journal of Neuroscience* 25 (14): 3628 LP – 3637. https://doi.org/10.1523/JNEUROSCI.4480-04.2005.

Tahirovic, S., F. Hellal, D. Neukirchen, R. Hindges, B. K. Garvalov, K. C. Flynn, T. E. Stradal, A. Chrostek-Grashoff, C. Brakebusch, and F. Bradke. 2010. "Rac1 Regulates Neuronal Polarization through the WAVE Complex." *Journal of Neuroscience* 30 (20): 6930–43. https://doi.org/10.1523/JNEUROSCI.5395-09.2010.

Takada, Yoshikazu, Xiaojing Ye, and Scott Simon. 2007. "The Integrins." *Genome Biology* 8 (5): 215. https://doi.org/10.1186/gb-2007-8-5-215.

Tessier-Lavigne, M, and C S Goodman. 1996. "The Molecular Biology of Axon Guidance." *Science (New York, N.Y.)* 274 (5290): 1123–33. https://doi.org/10.1126/science.274.5290.1123.

Thodeti, Charles K, Benjamin Matthews, Arvind Ravi, Akiko Mammoto, Kaustabh Ghosh, Abigail L Bracha, and Donald E Ingber. 2009. "TRPV4 Channels Mediate Cyclic Strain-Induced Endothelial Cell Reorientation through Integrin-to-Integrin Signaling." *Circulation Research* 104 (9): 1123–30. https://doi.org/10.1161/CIRCRESAHA.108.192930.

Tissir, Fadel, Isabelle Bar, Yves Jossin, Olivier De Backer, and Andre M Goffinet. 2005. "Protocadherin Celsr3 Is Crucial in Axonal Tract Development." *Nature Neuroscience* 8 (4): 451–57. https://doi.org/10.1038/nn1428.

Tole, Shubha, Grigoriy Gutin, Lahar Bhatnagar, Ryan Remedios, and Jean M Hébert. 2006. “Development of Midline Cell Types and Commissural Axon Tracts Requires Fgfr1 in the Cerebrum.” *Developmental Biology* 289 (1): 141–51. https://doi.org/https://doi.org/10.1016/j.ydbio.2005.10.020.

Tolner, Else A, Aminah Sheikh, Alexey Y Yukin, Kai Kaila, and Patrick O Kanold. 2012. “Subplate Neurons Promote Spindle Bursts and Thalamocortical Patterning in the Neonatal Rat Somatosensory Cortex.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 32 (2): 692–702. https://doi.org/10.1523/JNEUROSCI.1538-11.2012.

Tomar, Alok, and David D Schlaepfer. 2009. “Focal Adhesion Kinase: Switching between GAPs and GEFs in the Regulation of Cell Motility.” *Current Opinion in Cell Biology* 21 (5): 676–83. https://doi.org/10.1016/j.ceb.2009.05.006.

Tomaselli, K J, P Doherty, C J Emmett, C H Damsky, F S Walsh, and L F Reichardt. 1993. “Expression of Beta 1 Integrins in Sensory Neurons of the Dorsal Root Ganglion and Their Functions in Neurite Outgrowth on Two Laminin Isoforms.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 13 (11): 4880–88. https://doi.org/10.1523/JNEUROSCI.13-11-04880.1993.

Trivedi, Niraj, Joseph S Ramahi, Mahmut Karakaya, Danielle Howell, Ryan A Kerekes, and David J Solecki. 2014. “Leading-Process Actomyosin Coordinates Organelle Positioning and Adhesion Receptor Dynamics in Radially Migrating Cerebellar Granule Neurons.” *Neural Development* 9 (1): 26. https://doi.org/10.1186/1749-8104-9-26.

Trivedi, Niraj, and David J. Solecki. 2011. “Neuronal Migration Illuminated: A Look under the Hood of the Living Neuron.” *Cell Adhesion and Migration* 5 (1): 42–47. https://doi.org/10.4161/cam.5.1.13609.

Tronche, François, Christoph Kellendonk, Oliver Kretz, Peter Gass, Katrin Anlag, Paul C Orban, Rudolf Bock, Rüdiger Klein, and Günther Schütz. 1999. “Disruption of the Glucocorticoid Receptor Gene in the Nervous System Results in Reduced Anxiety.” *Nature Genetics* 23 (1): 99–103. https://doi.org/10.1038/12703.

Vahedi-Hunter, Tyler A, Jason A Estep, Kylee A Rosette, Michael L Rutlin, Kevin M Wright, and Martin M Riccomagno. 2018. “Cas Adaptor Proteins Coordinate Sensory Axon Fasciculation.” *Scientific Reports* 8 (1): 5996. https://doi.org/10.1038/s41598-018-24261-x.

Valiente, Manuel, and Oscar Marín. 2010. “Neuronal Migration Mechanisms in Development and Disease.” *Current Opinion in Neurobiology* 20 (1): 68–78. https://doi.org/10.1016/j.conb.2009.12.003.

Ven, T. J. Van de. 2005. "The Nonkinase Phorbol Ester Receptor 1-Chimerin Binds the NMDA Receptor NR2A Subunit and Regulates Dendritic Spine Density." *Journal of Neuroscience* 25 (41): 9488–96. https://doi.org/10.1523/JNEUROSCI.2450-05.2005.

Vicente-Manzanares, Miguel, and Alan Rick Horwitz. 2011. "Adhesion Dynamics at a Glance." *Journal of Cell Science* 124 (23): 3923–27. https://doi.org/10.1242/jcs.095653.

Villalobos, Victor, Snehal Naik, Monique Bruinsma, Robin S Dothager, Mei-Hsiu Pan, Mustapha Samrakandi, Britney Moss, Adnan Elhammali, and David Piwnica-Worms. 2010. "Dual-Color Click Beetle Luciferase Heteroprotein Fragment Complementation Assays." *Chemistry & Biology* 17 (9): 1018–29. https://doi.org/10.1016/j.chembiol.2010.06.018.

Virgilio, Maddalena De, William B. Kiosses, and Sanford J. Shattil. 2004. "Proximal, Selective, and Dynamic Interactions between Integrin AIIbβ3 and Protein Tyrosine Kinases in Living Cells." *Journal of Cell Biology* 165 (3): 305–11. https://doi.org/10.1083/jcb.200402064.

Viswanathan, Sarada, Sharba Bandyopadhyay, Joseph P Y Kao, and Patrick O Kanold. 2012. "Changing Microcircuits in the Subplate of the Developing Cortex." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 32 (5): 1589–1601. https://doi.org/10.1523/JNEUROSCI.4748-11.2012.

Vuori, Kristiina, Hisamaru Hirai, Shinichi Aizawa, and Erkki Ruoslahti. 1996. "Introduction of P130cas Signaling Complex Formation upon Integrin-Mediated Cell Adhesion: A Role for Src Family Kinases." *Mol Cell Biol* 16 (6): 2606–13. https://doi.org/10.1128/MCB.16.6.2606.

Wahlsten, Douglas. 1981. "Prenatal Schedule of Appearance of Mouse Brain Commissures." *Developmental Brain Research* 1 (4): 461–73. https://doi.org/https://doi.org/10.1016/0165-3806(81)90001-8.

Walter, J, B Kern-Veits, J Huf, B Stolze, and F Bonhoeffer. 1987. "Recognition of Position-Specific Properties of Tectal Cell Membranes by Retinal Axons in Vitro." *Development (Cambridge, England)* 101 (4): 685–96. https://doi.org/10.1242/dev.101.4.685.

Wang, J F, I W Park, and J E Groopman. 2000. "Stromal Cell-Derived Factor-1alpha Stimulates Tyrosine Phosphorylation of Multiple Focal Adhesion Proteins and Induces Migration of Hematopoietic Progenitor Cells: Roles of Phosphoinositide-3 Kinase and Protein Kinase C." *Blood* 95 (8): 2505–13. https://doi.org/10.1126/science.8342023.

Wang, Liang, and Till Marquardt. 2013. "What Axons Tell Each Other: Axon–Axon Signaling in Nerve and Circuit Assembly." *Current Opinion in Neurobiology* 23 (6): 974–82. https://doi.org/https://doi.org/10.1016/j.conb.2013.08.004.

Wang, Yu, and Mark A. McNiven. 2012. "Invasive Matrix Degradation at Focal Adhesions Occurs via Protease Recruitment by a FAK-P130Cas Complex." *Journal of Cell Biology* 196 (3): 375–85. https://doi.org/10.1083/jcb.201105153.

Ward, Michael E, Hui Jiang, and Yi Rao. 2005. "Regulated Formation and Selection of Neuronal Processes Underlie Directional Guidance of Neuronal Migration." *Molecular and Cellular Neurosciences* 30 (3): 378–87. https://doi.org/10.1016/j.mcn.2005.08.002.

Watabe-Uchida, Mitsuko, Eve Ellen Govek, and Linda Van Aelst. 2006. "Regulators of Rho GTPases in Neuronal Development." *Journal of Neuroscience* 26 (42): 10633–35. https://doi.org/10.1523/JNEUROSCI.4084-06.2006.

Watanabe, Haruyasu, and Fujio Murakami. 2009. "Real Time Analysis of Pontine Neurons during Initial Stages of Nucleogenesis." *Neuroscience Research* 64 (1): 20–29. https://doi.org/10.1016/j.neures.2009.01.007.

Webb, Donna J., Claire M. Brown, and Alan F. Horwitz. 2003. "Illuminating Adhesion Complexes in Migrating Cells: Moving toward a Bright Future." *Current Opinion in Cell Biology* 15 (5): 614–20. https://doi.org/10.1016/S0955-0674(03)00105-4.

Webb, Donna J., Karen Donais, Leanna A. Whitmore, Sheila M. Thomas, Christopher E. Turner, J. Thomas Parsons, and Alan F. Horwitz. 2004. "FAK-Src Signalling through Paxillin, ERK and MLCK Regulates Adhesion Disassembly." *Nature Cell Biology* 6 (2): 154–61. https://doi.org/10.1038/ncb1094.

Webb, Donna J., J. Thomas Parsons, and Alan F Horwitz. 2002. "Adhesion Assembly, Disassembly and Turnover in Migrating Cells – over and over and over Again." *Nature Cell Biology* 4 (4): E97–100. https://doi.org/10.1038/ncb0402-e97.

Wegmeyer, Heike, Joaquim Egea, Nadine Rabe, Henrik Gezelius, Alessandro Filosa, Anders Enjin, Frederique Varoqueaux, et al. 2007. "EphA4-Dependent Axon Guidance Is Mediated by the RacGAP A2-Chimaerin." *Neuron* 55 (5): 756–67. https://doi.org/10.1016/j.neuron.2007.07.038.

Wehrle-Haller, Bernhard. 2012. "Assembly and Disassembly of Cell Matrix Adhesions." *Current Opinion in Cell Biology* 24 (5): 569–81. https://doi.org/10.1016/j.ceb.2012.06.010.

WEISS, P. 1945. "Experiments on Cell and Axon Orientation in Vitro; the Role of

Colloidal Exudates in Tissue Organization." *The Journal of Experimental Zoology* 100 (December): 353–86. https://doi.org/10.1002/jez.1401000305.

Weiss, Paul. 1934. "In Vitro Experiments on the Factors Determining the Course of the Outgrowing Nerve Fiber." *Journal of Experimental Zoology* 68 (3): 393–448. https://doi.org/https://doi.org/10.1002/jez.1400680304.

Westhoff, M. A., B. Serrels, V. J. Fincham, M. C. Frame, and N. O. Carragher. 2004. "Src-Mediated Phosphorylation of Focal Adhesion Kinase Couples Actin and Adhesion Dynamics to Survival Signaling." *Molecular and Cellular Biology* 24 (18): 8113–33. https://doi.org/10.1128/mcb.24.18.8113-8133.2004.

Wingate, Richard. 2005. "Math-Map(Ic)S." *Neuron* 48 (1): 1–4. https://doi.org/10.1016/j.neuron.2005.09.012.

Winograd-Katz, Sabina E., Reinhard Fässler, Benjamin Geiger, and Kyle R. Legate. 2014. "The Integrin Adhesome: From Genes and Proteins to Human Disease." *Nature Reviews Molecular Cell Biology* 15 (4): 273–88. https://doi.org/10.1038/nrm3769.

Wolfenson, Haguy, Ariel Lubelski, Tamar Regev, Joseph Klafter, Yoav I Henis, and Benjamin Geiger. 2009. "A Role for the Juxtamembrane Cytoplasm in the Molecular Dynamics of Focal Adhesions." *PLOS ONE* 4 (1): e4304. https://doi.org/10.1371/journal.pone.0004304.

Wong, Wenny, Jason A Estep, Teresa Ubina, Jennifer N Jahncke, Kevin M Wright, and Martin M Riccomagno. 2022. "An Adhesive Signaling Axis Regulates the Establishment of the Cortical Glial Scaffold." *BioRxiv*, January, 2022.08.02.502565. https://doi.org/10.1101/2022.08.02.502565.

Woo, Stephanie, and Timothy M Gomez. 2006. "Rac1 and RhoA Promote Neurite Outgrowth through Formation and Stabilization of Growth Cone Point Contacts." *The Journal of Neuroscience* 26 (5): 1418 LP – 1428. https://doi.org/10.1523/JNEUROSCI.4209-05.2006.

Woo, Stephanie, Daniel J. Rowan, and Timothy M. Gomez. 2009. "Retinotopic Mapping Requires Focal Adhesion Kinase-Mediated Regulation of Growth Cone Adhesion." *Journal of Neuroscience* 29 (44): 13981–91. https://doi.org/10.1523/JNEUROSCI.4028-09.2009.

Wu, Jui-chung, Yu-chen Chen, Chih-ting Kuo, Helen Wenshin Yu, Yin-quan Chen, Arthur Chiou, and Jean-cheng Kuo. 2015. "Focal Adhesion Kinase-Dependent Focal Adhesion Recruitment of SH2 Domains Directs SRC into Focal Adhesions to Regulate Cell Adhesion and Migration." *Nature Publishing Group*, no. December:

1–13. https://doi.org/10.1038/srep18476.

Wu, Sheng-Xi, Sandra Goebbels, Kouichi Nakamura, Kazuhiro Nakamura, Kouhei Kometani, Nagahiro Minato, Takeshi Kaneko, Klaus-Armin Nave, and Nobuaki Tamamaki. 2005. “Pyramidal Neurons of Upper Cortical Layers Generated by NEX-Positive Progenitor Cells in the Subventricular Zone.” *Proceedings of the National Academy of Sciences* 102 (47): 17172–77. https://doi.org/10.1073/pnas.0508560102.

Wu, Yiqian, Kaiwen Zhang, Jihye Seong, Jason Fan, Shu Chien, Yingxiao Wang, and Shaoying Lu. 2016. “In-Situ Coupling between Kinase Activities and Protein Dynamics within Single Focal Adhesions.” *Scientific Reports* 6 (February): 1–13. https://doi.org/10.1038/srep29377.

Wu, Yueling, Ning Li, Chengfeng Ye, Xingmei Jiang, Hui Luo, Baoyuan Zhang, Ying Zhang, and Qingyu Zhang. 2021. “Focal Adhesion Kinase Inhibitors, a Heavy Punch to Cancer.” *Discover Oncology* 12 (1). https://doi.org/10.1007/s12672-021-00449-y.

Xing, Zheng, Hong Chen Chen, Julie K. Nowlen, Stephen J. Taylor, David Shalloway, and Jun Lin Guan. 1994. “Direct Interaction of V-Src with the Focal Adhesion Kinase Mediated by the Src SH2 Domain.” *Molecular Biology of the Cell* 5 (4): 413–21. https://doi.org/10.1091/mbc.5.4.413.

Xinming, Cai, Lietha Daniel, Ceccarelli Derek F., Karginov Andrei V., Rajfur Zenon, Jacobson Ken, Hahn Klaus M., Eck Michael J., and Schaller Michael D. 2008. “Spatial and Temporal Regulation of Focal Adhesion Kinase Activity in Living Cells.” *Molecular and Cellular Biology* 28 (1): 201–14. https://doi.org/10.1128/MCB.01324-07.

Xu, Nan-Jie, and Mark Henkemeyer. 2009. “Ephrin-B3 Reverse Signaling through Grb4 and Cytoskeletal Regulators Mediates Axon Pruning.” *Nature Neuroscience* 12 (3): 268–76. https://doi.org/10.1038/nn.2254.

Yacubova, Elina, and Hitoshi Komuro. 2003. “Cellular and Molecular Mechanisms of Cerebellar Granule Cell Migration.” *Cell Biochemistry and Biophysics* 37 (3): 213–34. http://www.ncbi.nlm.nih.gov/pubmed/12625628.

Yamada, K M, and S Miyamoto. 1995. “Integrin Transmembrane Signaling and Cytoskeletal Control.” *Current Opinion in Cell Biology* 7 (5): 681–89. https://doi.org/10.1016/0955-0674(95)80110-3.

Yamada, Kenneth M., and Benjamin Geiger. 1997. “Molecular Interactions in Cell Adhesion Complexes.” *Current Opinion in Cell Biology* 9 (1): 76–85. https://doi.org/10.1016/S0955-0674(97)80155-X.

Yan, Lei, and Zhiming Cui. 2022. “Integrin B1 and the Repair after Nervous System Injury.” *European Neurology*, October, 1–11. https://doi.org/10.1159/000526690.

Yang, Chengfeng, and Marcelo G. Kazanietz. 2007. “Chimaerins: GAPs That Bridge Diacylglycerol Signalling and the Small G-Protein Rac.” *Biochemical Journal* 403 (1): 1–12. https://doi.org/10.1042/BJ20061750.

Yee, Kathleen T., Horst H. Simon, Marc Tessier-Lavigne, and Dennis D.M. O’Leary. 1999. “Extension of Long Leading Processes and Neuronal Migration in the Mammalian Brain Directed by the Chemoattractant Netrin-1.” *Neuron* 24 (3): 607–22. https://doi.org/10.1016/S0896-6273(00)81116-2.

Yogev, Shaul, and Kang Shen. 2014. “Cellular and Molecular Mechanisms of Synaptic Specificity.” *Annual Review of Cell and Developmental Biology* 30 (1): 417–37. https://doi.org/10.1146/annurev-cellbio-100913-012953.

Yurchenco, Peter D. 2011. “Basement Membranes: Cell Scaffoldings and Signaling Platforms.” *Cold Spring Harbor Perspectives in Biology* 3 (2): 1–27. https://doi.org/10.1101/cshperspect.a004911.

Yusko, Erik C, and Charles L Asbury. 2014. “Force Is a Signal That Cells Cannot Ignore.” *Molecular Biology of the Cell* 25 (23): 3717–25. https://doi.org/10.1091/mbc.E13-12-0707.

Zaidel-Bar, Ronen, Christoph Ballestrem, Zvi Kam, and Benjamin Geiger. 2003. “Early Molecular Events in the Assembly of Matrix Adhesions at the Leading Edge of Migrating Cells.” *Journal of Cell Science* 116 (22): 4605–13. https://doi.org/10.1242/jcs.00792.

Zaidel-Bar, Ronen, and Benjamin Geiger. 2010. “The Switchable Integrin Adhesome.” *Journal of Cell Science* 123 (9): 1385–88. https://doi.org/10.1242/jcs.066183.

Zaidel-Bar, Ronen, Shalev Itzkovitz, Avi Ma’ayan, Ravi Iyengar, and Benjamin Geiger. 2007. “Functional Atlas of the Integrin Adhesome.” *Nature Cell Biology* 9 (8): 858–67. https://doi.org/10.1038/ncb0807-858.

Zakaria, Muhammad Asyaari, Nor Fadilah Rajab, Eng Wee Chua, Gayathri Thevi Selvarajah, and Siti Fathiah Masre. 2020. “The Roles of Tissue Rigidity and Its Underlying Mechanisms in Promoting Tumor Growth.” *Cancer Investigation* 38 (8–9): 445–62. https://doi.org/10.1080/07357907.2020.1802474.

Zamboni, Valentina, Rebecca Jones, Alessandro Umbach, Alessandra Ammoni, Maria Passafaro, Emilio Hirsch, and Giorgio R. Merlo. 2018. *Rho GTPases in Intellectual Disability: From Genetics to Therapeutic Opportunities*. *International Journal of*

Molecular Sciences. Vol. 19. https://doi.org/10.3390/ijms19061821.

Zamir, E., and B. Geiger. 2001. "Components of Cell-Matrix Adhesions." *Journal of Cell Science* 114 (20): 3577–79. https://doi.org/10.1242/jcs.114.20.3577.

Zamir, Eli, Menachem Katz, Yehudit Posen, Noam Erez, Kenneth M. Yamada, Ben Zion Katz, Shin Lin, et al. 2000. "Dynamics and Segregation of Cell-Matrix Adhesions in Cultured Fibroblasts." *Nature Cell Biology* 2 (4): 191–96. https://doi.org/10.1038/35008607.

Zang, Yixin, Karina Chaudhari, and Greg J Bashaw. 2021. "New Insights into the Molecular Mechanisms of Axon Guidance Receptor Regulation and Signaling." *Current Topics in Developmental Biology* 142: 147–96. https://doi.org/10.1016/bs.ctdb.2020.11.008.

Zhang, Yumeng, Shu Liu, Shu Zhou, Dandan Yu, Junjie Gu, Qin Qin, Yu Cheng, and Xinchen Sun. 2021. "Focal Adhesion Kinase: Insight into Its Roles and Therapeutic Potential in Oesophageal Cancer." *Cancer Letters* 496 (October 2020): 93–103. https://doi.org/10.1016/j.canlet.2020.10.005.

Zhao, Zhihai, Song Hui Tan, Hiroaki Machiyama, Keiko Kawauchi, Keigo Araki, Hiroaki Hirata, and Yasuhiro Sawada. 2016. "Association between Tensin 1 and P130Cas at Focal Adhesions Links Actin Inward Flux to Cell Migration." *Biology Open* 5 (4): 499–506. https://doi.org/10.1242/bio.016428.

Zhu, Yan, Tao Yu, Xiao-Chun Zhang, Takashi Nagasawa, Jane Y. Wu, and Yi Rao. 2002. "Role of the Chemokine SDF-1 as the Meningeal Attractant for Embryonic Cerebellar Neurons." *Nature Neuroscience* 5 (8): 719–20. https://doi.org/10.1038/nn881.