The role of RhoA in corticogenesis

Christian Rainer Jacques Böhringer

München 2012

Dissertation zur Erlangung des Doktogrades
der Fakultät für Chemie und Pharmazie
der Ludwig-Maximilians-Universität München

The role of RhoA in corticogenesis

Christian Rainer Jacques Böhringer
aus Donauwörth

2012

Erklärung

Diese Dissertation wurde im Sinne von § 7 der Promotionsordnung vom 28. November 2011 von Frau Prof. Magdalena Götz betreut und von Herrn Prof. Carsten Culmsee vor der Fakultät für Chemie und Pharmazie vertreten.

Eidesstattliche Versicherung

Diese Dissertation wurde eigenständig und ohne unerlaubte Hilfe erarbeitet.

München,

Dissertation eingereicht am	16.2.2012
1. Gutachter	Prof. Dr. Carsten Culmsee
2. Gutachterin	Prof. Dr. Magdalena Götz
Mündliche Prüfung am	8.5.2012

Abstract

Insights into the developmental processes during which the brain forms from the neuroepithelium may provide a deeper understanding how the brain works. The Rho family of small GTPases is known for its many cell biological functions such as regulation of the cytoskeleton, gene expression, cell migration, adhesion, cell polarity and the cell cycle. All of these functions are of importance during the formation of the cerebral neocortex, which consists of the generation of its different cell types, their migration to their destination and their maturation to a functional network. These roles have been mostly established in vitro using dominant negative or constitutively active constructs. Since these approaches are often not entirely specific for single pathways, this work used the Cre/loxP system to genetically delete an individual member of the Rho family, RhoA, to examine its role following a loss-of-function approach. Specifically, we examined a mouse line where part of the RhoA gene has been deleted by means of the Emx1::Cre mouse line. This idea is based on previous experiences with the deletion of Cdc42 in the developing neocortex, which leads to a loss of apical progenitors. RhoA often works as a functional antagonist to Cdc42.

Using immunofluorescence, we could detect a loss of RhoA at embryonic day 12 (E12) in Emx1::Cre-positive offspring carrying the floxed RhoA-construct in both alleles (cKO). At E14, we detected an increase in mitotic cells to 160% (±25%, $p<0,05$) that decreased to 140% (±10%, $p<0,05$) at E16. In addition, these mitoses were no longer restricted to their specific zones, but rather scattered throughout the developing cortex. This change did not coincide with a severely changed proportion of Pax6-pos. apical progenitors and Tbr2-pos. basal progenitors.

Investigating the cellular architecture of the developing cortex, we observed a loss of the radial orientation of radial glial cells, likely due to the disruption of the apical band of adherens junctions, which is the first effect observed after loss of the protein, and the consequent formation of rosette like structures in the brain parenchyma.

Despite the severe cortical malformations at embryonic stages, the mice get born and reach the age of weaning at no apparent difference from the Mendelian rate. These post-natal animals display a phenotype known as subcortical band heterotopia or "double-cortex". The phenotype is characterized by changes in the formation of the cortical layers. Between the characteristic six-layered structure of the cortex (homotopic cortex) and the ventricle, we found a second, unlayered neuronal structure embedded in the white matter (heterotopic cortex). By means of immunofluorescence and BrdU birthdating experiments, we observed that this structure consists of neurons of all layers and generated at all stages of neurogenesis, with late-born neurons of upper-layer identity being the majority. In addition, we found astrocytes and interneurons rather evenly distributed throughout both cortical structures.

Finally, by using in-utero electroporation to delete RhoA in individual cells, we found out that the misplacements of neurons in the heterotopic cortex was not due to an inability of RhoA-neg. neurons to migrate. This lead us to the conclusion, that the neuronal misplacement is a secondary effect, which occurs due to the observed disruption of the radial glial structure.

Looking for molecular pathways that may be at the start of these defects, we could observe a decrease of F-actin levels in RhoA-neg. progenitor cells in culture. Since F-actin stabilizes adherens junctions, RhoA's regulation of actin levels might indeed be at the origin "double-cortex" phenotype.

Taken together, our data show an important role of RhoA in developing cortex. In addition they show, that defects in the radial glial scaffold are enough to induce the formation of a "double-cortex".


INTRODUCTION

1. Introduction

No other organ is as intriguing to us as the brain – the source of our intelligence, personality and hence identity. Yet, after decades of research, we are still far from understanding it.

Understanding the developmental processes during which this complex structure is formed from the relatively simple neuroepithelium, might serve as valuable introduction into the functions of the brain – step-by-step as they are formed. This work tries to provide its own little stone to an immense picture, by examining a small aspect in the development of the neocortex.

1.1. Cortical development

1.1.1. Anatomy of the developing cortex

For future orientation, I will briefly introduce the anatomy of the developing neocortex at around embryonic day 12 (E12), because at this stage, the main cell types covered by this work are already present.

The developing cortex is restricted at its apical side by the liquid-filled ventricle and at its basal side by the basement membrane, an extracellular matrix mainly composed of laminins, collagen IV, nidogen, heparan sulphate proteoglycans and enriched in growth factors (Paulsson M 1992, Timpl R 1996, Erickson AC and Couchman JR 2000, Colognato H and ffrench-Constant C 2004).

The main population of cells is called radial glia. Their cell somata are located at the ventricle, but their processes span through the entire cortex, contacting the ventricular surface apically and the basement membrane basally (Ramon y Cajal S 1995). At E12, they have mostly replaced an earlier cell type, the neuroepithelial progenitors (Hartfuss E et al. 2001, Malatesta P et al. 2003). Radial glia merit a more detailed description that will follow in the next section. The zone closest to the ventricle, where all radial glial somata reside, is called the ventricular zone.

Directly basally to the ventricular zone lies the subventricular zone, which is more distinguishable at later stages (Gray GE et al. 1990). This is a secondary proliferative zone hosting the “basal progenitor” cells that will also be described in more detail further on in the text.

Adjacent to the subventricular zone develops the intermediate zone. It is initially formed by the corticofugal axons of the earliest cortical neurons, which are later met by the thalamocortical axons arriving in the cortex (Price DJ et al. 2006). Also the following waves of both incoming and outgoing axons grow along the same tract. The resulting bundles of axons will later get myelinated and form the adult “white matter”, together with astrocytes and oligodendrocytes.

A thin layer of neurons that have already been produced directly from neuroepithelial cells is present at the basal side. This layer is called the preplate (Marin-Padilla M 1971, Frotscher M 1997, Soriano E and Del Rio JA 2005). At the apical surface, new projection neurons are generated and migrate then into the preplate to form the cortical plate.

The first wave of arriving neurons splits the preplate into the subplate and the marginal zone. Part of the marginal zone remains the most basical layer of the cortical plate. The following neurons will all settle directly underneath the marginal zone, i.e. on top of the earlier-generated neurons, thus forming the cortical plate in an inward-out manner (Fig. 1).

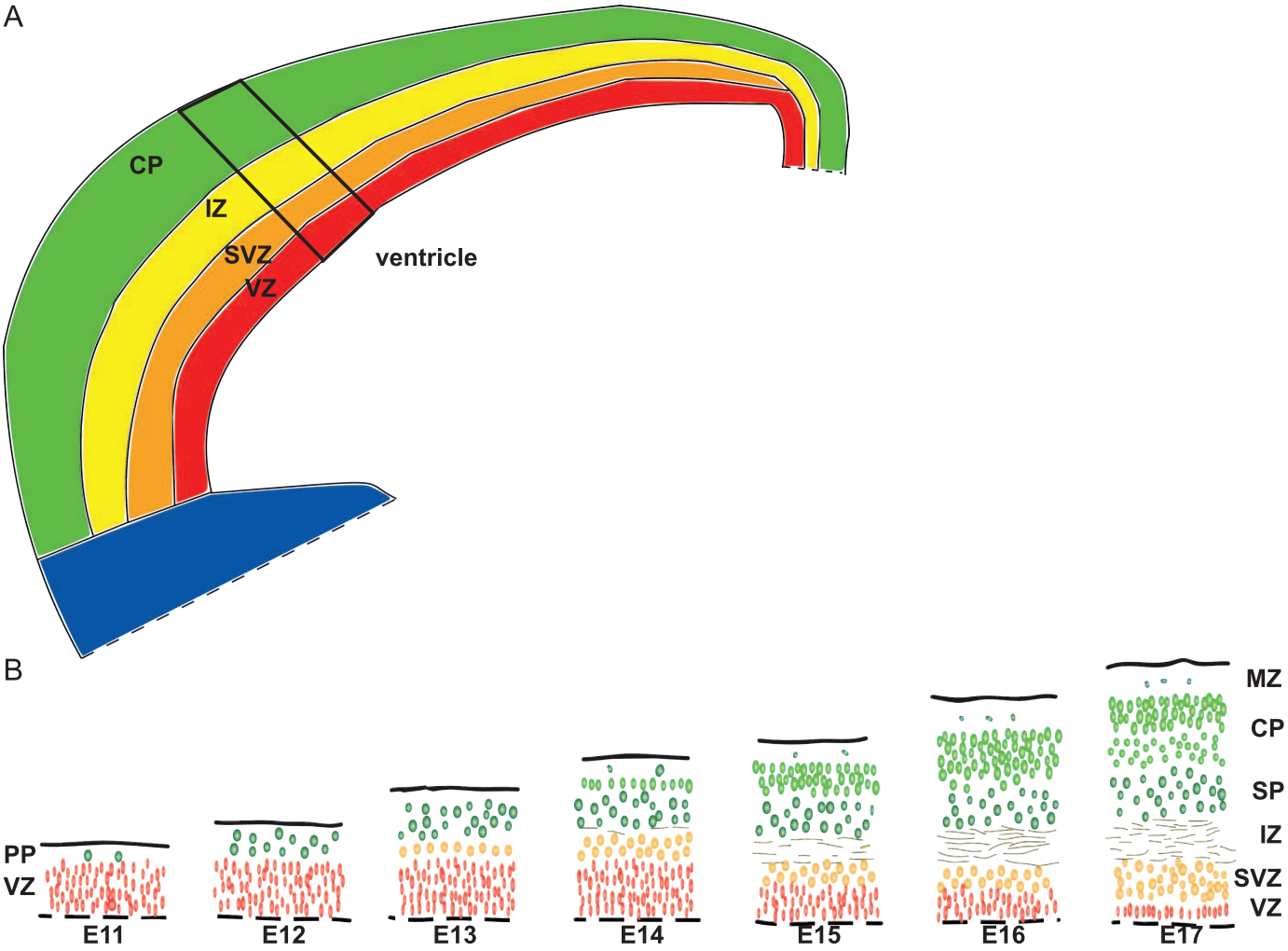


Fig. 1: Schematic drawing of the developing cortex

(A) Overview

The coloured bands indicate the relative positions of the different zones. For simplification, the medial part and most of the ventral telencephalon have been removed (dashed lines). The thicknesses are arbitrary, for details refer to B. The boxed area indicates a radial column as they are shown in detail in B.

(B) The zones of the developing cortex at different stages

The ventricular zone (VZ) is populated by the somata of radial glia (red). The preplate (PP) is formed by the most early-born neurons (dark green). Later, basal progenitors (orange) settle in the subventricular zone (SVZ). The first wave of neurons (light green) generated by radial glia splits the preplate in the outer marginal Zone (MZ) and the inner subplate (SP) and forms the cortical plate (CP) in between. Corticofugal and thalamocortical axons (yellow, elongated) form the intermediate zone (IZ).

The solid black line indicates the basement membrane. The dashed line does not indicate permeability, but illustrates that the ventricle is not separated from the cortex by a membrane.

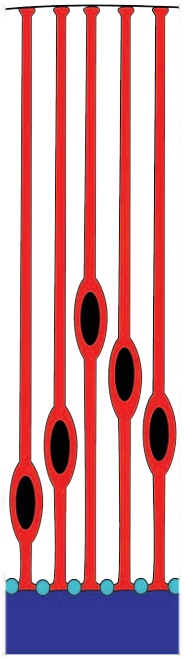
Adapted from (Dehay C and Kennedy H 2007).

1.1.2. Radial glia

As mentioned above, radial glia have a bipolar morphology with two processes that span the entire cortex. The apical process has an endfoot that forms cell-cell junctions with its neighbours; the basal process is anchored to the basement membrane (Shoukimas GM and Hinds JW 1978, Mollgard K et al. 1987, Astrom KE and Webster HD 1991, Aaku-Saraste E et al. 1996) (Fig. 2).

Fig. 2 Schematic drawing of radial glial cells

The radial glia (red) touch the basement membrane (black line) at their basal end. Their apical endfeet form cell-cell junctions (cyan) with the neighbouring cells. The endfeet are in direct contact to the ventricle (purple), which is filled with cerebrospinal fluid (CSF).



Despite their early discovery, the true nature of radial glia has long remained undiscovered. In particular due to their distinct morphology they were presumed to provide some sort of scaffold. As indeed, neurons could be found in close contact with the radial processes, their role in guiding newborn neurons on their migration to the cortical plate seemed revealed (Rakic P 1972). These observations are still valid and further studies, especially those using time-lapse microscopy, have provided more evidence to the role of radial glia in neuronal migration (Hatten ME 1999, Nadarajah B et al. 2003). As a matter of fact, this function also plays an important role in the present study.

Nowadays, however, the main interest in radial glia is due to their role as the progenitors of nearly all neural cells in the mammalian cortex, a site that was long overlooked. Based on the mitotic figures that they display in electron microscopy and their uptake of triturated Thymidine (H3Thy) or the Thymidine analogue 5-Bromo-2-deoxyuridine (BrdU), they had already been recognized as dividing. However, as they transform into astrocytes after the end of neurogenesis, they were thought to be purely astrogenic and to coexist with neuroepithelial progenitors or a still unidentified pool of neuronal precursors (Malatesta P et al. 2000, Noctor SC et al. 2001, Campbell K and Gotz M 2002, Gotz M et al. 2002, Kriegstein AR and Gotz M 2003, Pinto L and Gotz M 2007). Only much later it was discovered, that a culture only consisting of radial glia, purified by fluorescence-activated cell sorting (FACS), would give rise to both astrocytes and neurons in vitro. This result was soon confirmed by using different approaches, such as live imaging, not only in vitro but also in vivo (Malatesta P et al. 2000, Miyata T et al. 2001 , Noctor SC et al. 2001).

Neuroepithelial progenitors, in contrast, had already been well recognized as progenitor cells, so are radial glia just a subtype of neuroepithelial progenitors? Indeed, both cell types share a lot of characteristics. Most strikingly, they both share the same polarized morphology, even though neuroepithelial progenitors naturally have to span a much shorter distance. On the molecular level, they share the expression of the intermediate filament Nestin (Misson JP et al. 1988, Edwards MA et al. 1990, Chanas-Sacre G et al. 2000, Hartfuss E et al. 2001), RC1 and RC2 (Misson JP et al. 1988, Hartfuss E et al. 2001, Mori T et al. 2005). There are, however, many proteins expressed in radial glia, which they rather share with reactive astrocytes (Ridet JL et al. 1997, Doetsch F et al. 1999, Fawcett JW and Asher RA 1999, Seri B et al. 2001, Doetsch F et al. 2002, Seri B et al. 2004), such as GLAST (Shibata T et al. 1997, Malatesta P et al. 2000, Hartfuss E et al. 2001), GS (Akimoto J et al. 1993), S100β, TnC (Gotz M et al. 1998), Vimentin (Schnitzer J et al. 1981) or BLBP (Hartfuss E et al. 2001). This is another reason, why these cells were considered purely astroglial and it added to the surprise when it was proven that precisely these glial-marker-expressing cells gave rise to neurons.

More importantly, also functional differences exist between radial glia and neuroepithelial progenitors. The latter divide mostly symmetrically, producing two neuroepithelial progenitors and thus enlarging the progenitor pool. In contrast, radial glia need a mechanism that allows them to generate differentiated cells without depleting their own population. In principle, this can be achieved with the right balance between self-renewing and differentiating symmetric cell divisions. However, it has been observed that, especially from mid-neurogenesis onwards, the main mode of cell division in radial glia is rather asymmetric (Noctor SC et al. 2004, Pinto L and Gotz M 2007), so that during each division the mother cell can both self-renew and generate a differentiated cell. A common conclusion is, that the lateral expansion of the brain surface is completed before the onset of neurogenesis, and later stages rather increase the thickness of the cortex (Huttner WB and Kosodo Y 2005, Pontious A et al. 2008).

Synchronous with their cell cycle, neuroepithelial progenitors and radial glia display a migratory phenomenon called interkinetic nuclear migration. Their nuclei migrate up and down the ventricular zone in a way such that they undergo S-phase at their basal turning point and divide at the ventricular surface. This phenomenon has been identified relatively early (Sauer FC 1935) and it is the reason for the pseudostratified appearance of the neuroepithelium. Even though this pattern of migration seems noteworthy, it is not a prerequisite for cell division, as progression through the cell cycle can still occur when interkinetic nuclear migration is inhibited (Messier PE 1978, Murciano A et al. 2002, Baye LM and Link BA 2008, Schenk J et al. 2009, Taverna E and Huttner WB 2010). On the other hand, interkinetic nuclear migration is itself dependant on the cell cycle, and changes in the length of the latter do change the speed of interkinetic nuclear migration so that the nuclei will still be at their basal turning point in S-phase and at the ventricular surface in M-phase (Lange C et al. 2009, Pilaz LJ et al. 2009, Taverna E and Huttner WB 2010).

Another peculiarity is the highly polarized morphology, which is again shared by neuroepithelial progenitors and radial glia. This apico-basal polarity continues at the molecular level, where several proteins have been shown to localize distinctly to the apical or the baso-lateral part of the cell. Some amongst them, such as Cdc42 or Par3 (Cappello S et al. 2006, Costa MR et al. 2008) have already been shown to influence the behaviour of the progenitor cells and even change their cell fate. Other approaches have focussed on changing the cells' behaviour by interfering with radial glial morphology (Haubst N et al. 2006), but despite the resulting disorganization of the overall cortical structure, no changes in cell fate could be observed, and on the single-cell level, an apical "pole" was still detectable (Schmid M-T 2008).

1.1.3. Basal progenitors

As early as 1973, another zone of proliferation has been described in the developing cortex, in between ventricular and intermediate zone (Smart IH 1973). It took until the development of sophisticated time-lapse video microscopy, to spark deeper interest in this cell population that then turned out to consist of a very different, multipolar cell type without contact to the ventricle. Unlike neuroepithelial progenitors or radial glia, these cells do not express Pax6 nor genes of the Hes-family, but can rather be characterized by their expression of the non-coding RNA Svet1 (Tarabykin V et al. 2001) and the transcription factors Tbr1/2 (Englund C et al. 2005), Cux1/2

(Nieto M et al. 2004, Zimmer C et al. 2004), Ngn2 (Miyata T et al. 2004) and Satb2 (Britanova O et al. 2005). Albeit detectable as early as E11 (Smart IH 1973, Nieto M et al. 2004, Zimmer C et al. 2004, Englund C et al. 2005, Wu SX et al. 2005) their population increases over time and forms a distinct anatomic region, the subventricular zone, around mid-neurogenesis (E14) (Viti J et al. 2003, Gotz M and Barde YA 2005).
Imaging techniques allowed to observe the division of basal progenitors directly and to follow their fate. Contrary to former believes, that the subventricular zone would contain mainly glial progenitors (Takahashi T et al. 1995), these studies could show that basal progenitors divide symmetrically to produce pairs of neurons (Haubensak W et al. 2004, Miyata T et al. 2004, Noctor SC et al. 2004, Wu SX et al. 2005), or undergo up to three rounds of – also symmetric – proliferative divisions (Noctor SC et al. 2004, Pontious A et al. 2008).
As for their function, they had been speculated to be an independent progenitor population, giving rise to upper layer neurons (Tarabykin V et al. 2001, Zimmer C et al. 2004). This theory emerged mainly because they share expression of Cux1/2, Satb2 and Svet1 with upper layer neurons (Tarabykin V et al. 2001, Nieto M et al. 2004, Zimmer C et al. 2004, Britanova O et al. 2005) but exist already before those neurons are generated (Zimmer C et al. 2004).
In the meantime, it has become clear that basal progenitors cannot self-renew for more than three rounds and that they are themselves generated by radial glia and neuroepithelial progenitors (Miyata T et al. 2004, Noctor SC et al. 2004). As this happens at the ventricular surface, they subsequently have to migrate. The current model is, that basal progenitors are intermediate progenitors that increase the neuronal output of a single radial glia. This way, the output at a given point in time could be regulated by the radial glia producing a basal progenitor instead of directly producing a neuron, and the rounds of proliferative cell divisions that the basal progenitor then undergoes before producing neurons (Pontious A et al. 2008).

1.1.4. The layered structure of the cortex

At the end of development, the mammalian cortex has reached its characteristic six-layered structure. Neurons are considered to belong to a certain layer, if their somata are located within that layer.
The first neurons, which are already generated by neuroepithelial progenitors around E11, form the preplate. The neurons that are generated after the formation of the preplate migrate into the preplate, splitting it into the outer marginal zone and the inner subplate (Caviness VS, Jr. 1982, Wood JG et al. 1992, Del Rio JA et al. 2000, Hevner RF et al. 2003, Casanova MF and Trippe J, 2nd 2006). The marginal zone forms layer I, consisting mostly of Cajal-Retzius cells, which are marked by their expression of Reelin and/or Calretinin (D'Arcangelo G et al. 1997, Frotscher M 1997, Soriano E and Del Rio JA 2005, Casanova MF and Trippe J, 2nd 2006). Those neurons that split the preplate form layer VI and the following neurons settle on top of the previous layers thus generating the next layers in an inward-out manner (Bayer SA et al. 1991).
These layers can be distinguished by their expression of certain transcription factors. The upper layers II and III express Cux1/2, Brn1/2 and the non-coding RNA Svet1. Neurons from layer IV and V express ER81 and layer VI-neurons are positive for Tbr1 and Foxp2 (Molyneaux BJ et al. 2007).

More importantly, each layer has a certain function in the wiring of the neuronal network. Stereotypically, thalamocortical efferents arrive in layer IV, which relays the signals to the upper layers II and III, a connection that forms the so-called "ascending pathway". The layers II/III are the most heavily interconnected layers, including many connections across the corpus callosum, which connects the two brain hemispheres (Elberger AJ 1993, Douglas RJ and Martin KA 2004). Their output is connected to the deep layers V and VI via the "descending pathway". Layer VI provides a feedback signalling to the thalamus, whereas layer V is the source of the subcortical connections to midbrain, brainstem, cerebellum (via the brainstem), striatum and the spinal cord (Gilbert CD and Wiesel TN 1983, Douglas RJ and Martin KA 2004, Shipp S 2007). Obviously, this pattern is a strong generalization. Historically, most information has been gained from the visual cortex of the cat.

1.1.5. Neuronal migration

In order to reach their layer after birth in the ventricular or subventricular zone, neurons have to be able to migrate. Since they migrate radially away from their place of birth, this is referred to as "radial migration", in contrast to "tangential migration", which refers to the horizontal movement of neurons that also occurs (Walsh C and Cepko CL 1992, O'Rourke NA et al. 1995, O'Rourke NA et al. 1997). There are, however, different mechanisms by which radial migration can be achieved.

One of these mechanisms is dependent on radial glia and called "locomotion". The mechanism of neurons migrating along radial glial fibres, which was concluded from electron microscopy pictures showing neurons in close apposition to these processes (Rakic P 1972), may have led to an underestimation of the role of radial glia in neurogenesis, but nevertheless turned out to be correct. Observations, according to which all migrating (pyramidal) neurons were aligned in parallel to radial glial processes (Misson JP et al. 1991) and not to e.g. neuronal processes (Rakic P 1971, 1972, 1990) provided another indication that radial migration was indeed dependant on radial glia. Time-lapse experiments finally allowed direct observation of migrating neurons (Hatten ME 1999). Locomotion could mainly be detected at later stages of development. The migrating cells display a motile leading process with constant length. The movement is not constant but saltatory, reaching an average speed of around 35µm/h (Nadarajah B et al. 2001).

Based on Golgi stainings, another mode of migration had been proposed, where the migrating neurons seemed to have direct contact to the basement membrane (Berry M and Rogers AW 1965). Later, this kind of cells was also discovered by means of immunohistochemistry (Brittis PA et al. 1995). As for locomotion, time-lapse experiments turned out to reveal most information about this mode of migration that is known today as "somal translocation". When a radial glia divides to produce a neuron, this neuron can inherit the radial process and pull itself up towards the basement membrane. Upon entering the subventricular zone, it will lose its connection to the ventricle (Miyata T et al. 2001). Neurons performing somal translocation show a more constant movement than it is seen in locomotion and move at an average speed of around 60µm/h. The radially oriented process, which has a length of 60-95µm when the soma is still in the outer ventricular zone, becomes thicker and shorter during migration and its basal process remains attached to the pial surface. Sometimes a small trailing process is seen in addition. It has to be

noted, that electron microscopy would be needed to confirm a real attachment of the basal process to the basement membrane. Also, there is a lack of agreement concerning the inheritance of the radial process, as also the outgrowth of the process before the onset of migration has been observed (Nadarajah B et al. 2001).
The current model is, that somal translocation is the prevalent mode of migration at the early stages, especially during formation of the preplate, when the distances to be bridged are still rather short. Consequently, locomotion is dominating at later stages and also overall the most frequent mode of radial migration (Nadarajah B et al. 2001).
Mixed modes have been observed as well. In addition to the long-range somal translocation described above, a locomoting neuron can undergo short-range somal translocation, too, as soon as its process has reached the marginal zone (Nadarajah B et al. 2001, Chai X et al. 2009). Also the opposite can happen, as recently basal progenitors have been observed, that use somal translocation to reach the subventricular zone and that become multipolar once they have arrived there (Tabata H et al. 2009).
The third mode of migration, "multipolar migration", has been discovered most recently. From around E14 on, multipolar cells have been described, that also migrate radially. Their population increases and constitutes 20% of all migrating neurons at E15. They form and retract multiple processes that might serve to sense environmental cues. The soma moves rapidly (1-3µm/min) towards the branching point of such a process, pauses there until one process is retracted and then follows the remaining process (Nadarajah B et al. 2001). Initially, these cells were thought to be a small population of dorsally generated interneurons (Anderson SA et al. 1997, Nadarajah B et al. 2001, Anderson SA et al. 2002, Letinic K et al. 2002, Nadarajah B et al. 2003), but in the meantime it has been shown that radially migrating neurons can sojourn in the subventricular zone in a multipolar state, during which they can also spread tangentially (Tabata H and Nakajima K 2003, Noctor SC et al. 2004).

1.2. The Rho GTPases

As of today, the molecular mechanisms involved in the formation of the neocortex are still far from understood. Our lab has performed genetic screens to find genes with expression patterns that suggest an involvement in corticogenesis (Pinto L et al. 2008). In this screen, several members of the Rho GTPases appeared.
The family of Rho GTPases is a subfamily of Ras GTPases that is present in all eukaryotic cells (Jaffe AB and Hall A 2005). There are 22 mammalian genes for Rho GTPases that can be clustered in different subfamilies (Aspenstrom P et al. 2004, Jaffe AB and Hall A 2005). They function as molecular switches that are inactive in their GDP-bound form. Upon exchange of GDP against GTP they get activated and can interact with a big variety of effector molecules. So far, over 50 different effector proteins have been discovered, including scaffold proteins, kinases and other enzymes. With the exception of some atypical Rho GTPases (Pacary E et al. 2011), the active form inactivates itself by its intrinsic GTPase activity (Jaffe AB and Hall A 2005).
Different classes of activating and inactivating proteins can transduce signals to the GTPases and add specificity to the system. Guanosine exchange factors (GEFs) constitute the activators. They catalyze the exchange of GDP against GTP (Schmidt A and Hall A 2002, Jaffe AB and Hall

A 2005). There are two different classes of inactivating proteins. GTPase activating proteins (GAPs) increase the GTPase activity (Bernards A 2003), whereas guanosine dissociation inhibitors (GDIs) block the dissociation of GDP from the GTPase and also sequester the GTPase out of the membrane, which is mostly their place of action (Olofsson B 1999).

1.2.1. Regulation of the cytoskeleton

Rho GTPases are probably best examined for their regulation of the cytoskeleton, especially for the formation of actin-rich protrusions. Activation of Cdc42 typically leads to the formation of filopodia and the activation of Rac to lamellipodia (Etienne-Manneville S and Hall A 2002). To form these protrusions, but also for various other cellular functions, they can induce actin polymerization. The most important actin-binding molecules in this context are Arp2/3 (Millard TH et al. 2004), which is downstream of Cdc42 (Ho HY et al. 2004) and Rac (Eden S et al. 2002, Innocenti M et al. 2004), and the Formins, mDias in mice, which are downstream of Rho (Zigmond SH 2004). To provide dynamics, there must also be means to depolymerize and restructure actin filaments. Both Cdc42/Rac and Rho can do this via the Cofilin pathway (Ohashi K et al. 2000, Dawe HR et al. 2003, Pollard TD and Borisy GG 2003, Ghosh M et al. 2004, DesMarais V et al. 2005, Jaffe AB and Hall A 2005).

Rho GTPases can also regulate the microtubule part of the cytoskeleton. Cdc42/Rac activity typically leads to assembly and stabilization of microtubules (Daub H et al. 2001, Cassimeris L 2002, Etienne-Manneville S and Hall A 2003). Depending on the context, Rho activation can lead to either stabilization (Palazzo AF et al. 2001, Wen Y et al. 2004) or destabilization (Arimura N et al. 2000, Fukata Y et al. 2002) (Fig. 3).

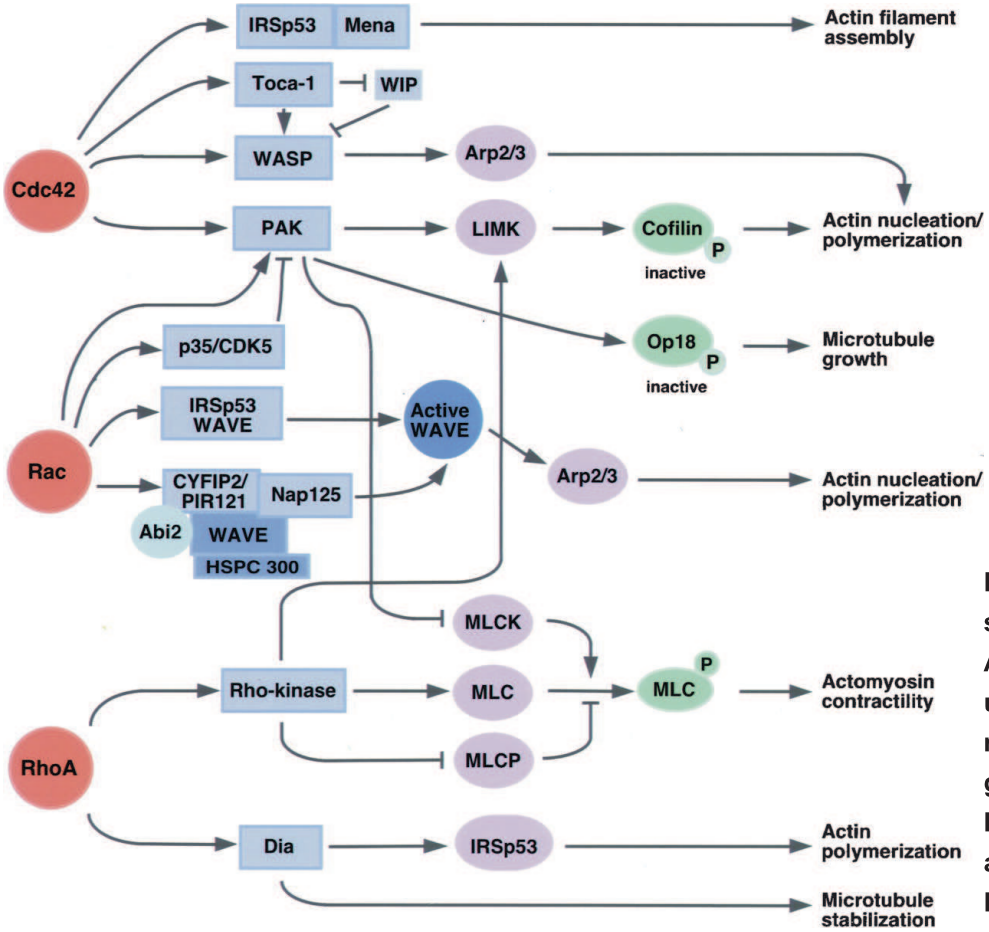


Fig. 3 Regulation of the cytoskeleton
Activation of Rho GTPases usually leads to actin polymerization and microtubule growth.
In addition, RhoA can mediate acto-myosin contractility.
From (Govek EE et al. 2005)

1.2.2. Regulation of gene expression

Besides their effects on the cytoskeleton Rho GTPases are also involved in gene expression. This can, however, also be tightly linked to the cytoskeleton, namely the serum response factor (SRF) pathway. SRF is a transcription factor that activates the corresponding serum response element (SRE) to initiate the transcription of immediate early genes (IEGs) (Jaffe AB and Hall A 2005). To do so, it needs MAL as a coactivator. To function as such, MAL needs to be present in the nucleus, which, in turn, can only happen when MAL is not bound to the monomeric form of actin, G-actin. If Rho activity lowers the amount of G-actin present in the cell by actin polymerization, which is the net outcome of Rho activation in most cells, more MAL will be free to bind SRF (Miralles F et al. 2003).

Besides this mode of regulation, there are other, actin-independent, pathways that can involve Rho GTPases, such as the JNK- and MAPK-pathways, which are known to affect transcription in other molecular pathways (Burbelo PD et al. 1995, Coso OA et al. 1995, Minden A et al. 1995, Teramoto H et al. 1996, Puls A et al. 1999, Gallagher ED et al. 2004).

1.2.3. Function in cell migration

Cell migration typically starts with the extension of a process into new areas. Both Cdc42 and Rac1 are concentrated on the leading edge of migrating cells to promote the actin polymerization needed for this kind of membrane extensions (Arthur WT and Burridge K 2001, Raftopoulou M and Hall A 2004). It has been shown that both GTPases are required for cell migration in scratch wound assays of fibroblast, astrocyte and epithelial cell cultures, where especially Cdc42 regulates the direction (Nobes CD and Hall A 1999, Etienne-Manneville S and Hall A 2001, Tzima E et al. 2003, Watanabe T et al. 2004, Cau J and Hall A 2005, Gomes ER et al. 2005, Robel S et al. 2011), but also in neuronal migration (Kawauchi T et al. 2003, Konno D et al. 2005, Yoshizawa M et al. 2005, Chen L et al. 2007, Tahirovic S et al. 2010). Interestingly, also constitutively active constructs of Cdc42 or Rac1 inhibited migration in these experiments, which shows how crucial the level of activation is.

After extension of the leading process, the process attaches to the environment and the acto-myosin cytoskeleton generates contractile forces to move the nucleus. Especially the Rho-subfamily plays a role in the formation of adhesion and the generation of acto-myosin contractions at the rear of the cell (Luo L 2000, Ridley AJ 2001, Ridley AJ et al. 2003). For this reason, its inhibition blocks migration in certain systems (Paterson HF et al. 1990, Stasia MJ et al. 1991, Hinsch KD et al. 1993, Miura Y et al. 1993, Takaishi K et al. 1993, Jay PY et al. 1995). Nucleokinesis seems to be particularly dependant on acto-myosin function (Hatten ME 2002, Schaar BT and McConnell SK 2005) and consequently, macrophages can still extend processes if RhoA is blocked, but the nucleus is unable to follow (Allen WE et al. 1997, Allen WE et al. 1998). More interestingly (in the context of this work), the same holds true for precerebellar neurons (Causeret F et al. 2004).

But the situation is not as straightforward as it might seem at first and the cell adhesion mediated by RhoA also inhibits cell movement (Couchman JR and Rees DA 1979, Paszek MJ et al. 2005, Marin O et al. 2006). The net outcome of RhoA activity is thus dependent on the cell type. Especially flat cells with stress fibres are strongly attached to the extracellular matrix with focal

adhesions, and are therefore kept in their place by RhoA activity. Examples are MDCK cells migrating along a growth factor gradient (Ridley AJ et al. 1995) or primary fibroblasts closing a scratch wound (Nobes CD and Hall A 1999). In contrast, RhoA can be necessary for the amoeboid movement of round cells without stress fibres (Sahai E and Marshall CJ 2003). But even though neurons do not have stress fibres (Guasch RM et al. 1998), and despite the positive role of RhoA in nucleokinesis of precerebellar neurons (Causeret F et al. 2004), recent data indicate that RhoA usually is a negative regulator for radial migration of cortical neurons (Kholmanskikh SS et al. 2003, Besson A et al. 2004, Hand R et al. 2005, Ge W et al. 2006, Nguyen L et al. 2006).

1.2.4. Function in cell adhesion

Also cell adhesion often starts with cells contacting each other with filopodia or lamellipodia before the actual junctions are formed (Jacinto A et al. 2000, Vasioukhin V et al. 2000, Ehrlich JS et al. 2002). Both Rho and Rac have been found necessary for the formation of adherens junctions since they stabilize actin filaments required to form the actin-mediated adhesion belt (Braga VM et al. 1997, Hordijk PL et al. 1997, Takaishi K et al. 1997, Malliri A et al. 2004). In addition, interactions between Rho and α-catenin, an important part of adherens junctions, are known from work performed in drosophila (Magie CR et al. 2002, Vaezi A et al. 2002).

1.2.5. Function in cell polarity

Cdc42 activates the Par-complex, consisting of Par3, Par6 and the atypical protein kinase C (aPKC), that has been shown to be necessary for adherens junction formation in various cell types (Izumi Y et al. 1998, Itoh M et al. 2001, Suzuki A et al. 2001, Yamanaka T et al. 2001, Hirose T et al. 2002, Gibson MC and Perrimon N 2003).

Besides the maintenance of tissue integrity, these junctions also delineate the apical from the baso-lateral membrane domain (Gotz M and Huttner WB 2005). Cdc42 and the Par-complex seem to play a general role in the establishment of cell polarity. In neuronal cells, these proteins are also enriched in the developing axon. Disruption of this signalling pathway leads to polarity defects like neurons without axons or with multiple axons (Shi SH et al. 2003, Schwamborn JC and Puschel AW 2004, Jaffe AB and Hall A 2005).

These observations fit to other observations where Cdc42 was necessary for polarized behaviour of cells, including budding sites in mating yeast, cells migrating along a chemotactic gradient or monolayer cultures closing a scratch wound (Allen WE et al. 1998, Nobes CD and Hall A 1999, Li Z et al. 2003, Cau J and Hall A 2005, Robel S et al. 2011).

Another effect on polarity is known from drosophila, where Cdc42 and the Par-complex are necessary for asymmetric cell division (Etienne-Manneville S and Hall A 2001, Gotta M et al. 2001, Ahringer J 2003, Peterson FC et al. 2004). In mammals, there is an ongoing discussion about the role of cell polarity in cell fate decisions (Gotz M and Huttner WB 2005, Zhong W and Chia W 2008, Kosodo Y and Huttner WB 2009). It has been found that in mice the Par-complex promotes radial glial cell fate (Costa MR et al. 2008) and that upon loss of Cdc42, which activates the Par-complex, radial glia change into basal progenitors (Cappello S et al. 2006) and thereby lose their ability to divide asymmetrically.

1.2.6. Regulation of the cell cycle

Another role of Rho GTPases is cell cycle progression. Cdc42 and Rac as well as Rho seem to be important for G1 progression (Yamamoto M et al. 1993, Olson MF et al. 1995) by regulating Cdk2 and the expression of cyclins (Weber JD et al. 1997, Westwick JK et al. 1997, Olson MF et al. 1998, Hu W et al. 1999, Joyce D et al. 1999, Mettouchi A et al. 2001, Sahai E et al. 2001, Welsh CF et al. 2001, Lai JM et al. 2002, Vidal A et al. 2002, Chou MM et al. 2003, Roovers K and Assoian RK 2003, Roovers K et al. 2003).

Again, it is also due to their function in the cytoskeleton, that they play another major role in the cell cycle as acto-myosin is needed for centrosome positioning (Rosenblatt J et al. 2004). Furthermore, Rho and some of its effectors localize at the cleavage furrow (Glotzer M 2001), probably to activate myosin which leads to the ring contraction necessary for cytokinesis (Matsumura F et al. 1998, Komatsu S et al. 2000, Yamashiro S et al. 2003).

1.3. Aim of this study

Rho GTPases are involved in all stages of corticogenesis. Their function in the cytoskeleton, cell adhesion and cell polarity means a potential role for the formation and maintenance of the characteristic shapes of both radial glia and neurons, their influence in gene expression can be of consequence for cell fate decisions and regulation of the cell cycle can play a role for the proliferation of neural progenitors and the cell cycle exit of mature neurons. Considering the significance of Cdc42 in the developing cortex (Cappello S et al. 2006), the examination of RhoA, which usually is a functional antagonist of Cdc42 (Postma FR et al. 1996, Kozma R et al. 1997, Hall A 1998), is a logical next step. The opposite effect of loss of Cdc42 function would be a loss of basal progenitors, but due to the variety of effects in different cell types, the outcome is hard to predict.

Even though the functions of RhoA seem to be well characterized at first, it is still not possible to answer its role in cortical development. This has several reasons:

Many of the existing studies used antagonists against Rho effectors, that are relatively specific for the effector itself, but unspecific for the activating GTPase. As we have seen, Rho GTPases are a family of very homologue proteins and there is substantial crosstalk between the different signalling pathways. Especially dominant-negative or constitutively active constructs are prone to influence several pathways. The dominant negative constructs are changed in a way that either always leaves them in their inactive GDP-bound form, or they can no longer bind their effector molecule. However, different Rho GTPases often interact with the same molecules, so that for example a dominant negative RhoA might inhibit an effector that would usually be activated by RhoC. Investigating the role of a single family member without influencing other signalling pathways is therefore challenging. Since even a single subfamily of small GTPases can be involved in so many different cellular functions, it is especially interesting to differentiate between the individual effects of the different family members.

In addition, studies in other regions of the CNS have shown, that the role of RhoA is highly region specific (Herzog D et al. 2011, Katayama K et al. 2011). Therefore it is difficult to transfer insights from other brain regions to the development of the neocortex.

Here, we want to address the role of RhoA in corticogenesis by specifically deleting RhoA.

cells
neurons
cKO
RhoA
adherens
control
cortex
radial
stained
progenitors
migration
junctions
ventricular
observed
white
matter
structure
expression

2. Results

2.1. The Emx1::Cre || RhoA fl mouse model is an appropriate tool to investigate RhoA in the developing cerebral cortex

Much of the work done on Rho GTPase signalling stems from studies conducted in invertebrates, cell culture or even cell-free assays. But the interacting molecules expressed in a certain cell, their localisation and hence the entire pathways have shown to differ significantly from one cell type to another (Jaffe AB and Hall A 2005, Wu X et al. 2006). In addition, the developing brain is a complex three-dimensional structure, and due to its known involvement in the cytoskeleton, this structure may indeed be directly linked with the function of RhoA.

Even regarding only the function of RhoA itself, it is rather likely to be still involved in several cellular functions in parallel. They may, however, differ in their importance or their function may be redundant. It is therefore difficult to predict to which phenotype a loss-of-function approach would lead.

These considerations in mind, we decided for a genetic approach to delete only the gene of interest in the cells and at the stage of interest. For this, we crossed a mouse-line in which the third exon of the RhoA gene, containing the start-codon, is flanked by loxP-sites (RhoA fl) (Jackson B et al. 2011), with the Emx1::Cre-line (Iwasato T et al. 2004) that expresses Cre-recombinase in neuroepithelial progenitors and radial glia from E9,5 on. After recombination, the loss of the start-codon will stop the expression of any RhoA without any truncated proteins being produced.

Though RhoA-expression, on the mRNA level, has been reported to be restricted to the ventricular zone (Ge W et al. 2006, Pinto L et al. 2008), the protein could be detected in the control sections throughout the developing cortex (Fig. 4A, C).

In the conditional knock-out (cKO) brain, RhoA protein has disappeared by E12 (Fig. 4B). Consistent with a recombination that is restricted to neuroepithelial progenitors and radial glia, immunoreactivity is still detectable in meninges, blood vessels and the ventral telencephalon, where the Emx1 promoter is not active (arrows in Fig. 4B, D).

These results confirmed the mouse model as suitable to investigate the role of RhoA in the developing cortex.

2.2. Proliferation in the RhoA-deficient cortex

We started examining cell divisions. Not only is the rate of cell divisions an important feature of progenitor cells, but their localisation also gives us first hints at tissue architecture and the progenitor cell types. This is because usually radial glia only divide at the ventricular surface whereas basal progenitors divide in the subventricular zone.

To detect dividing cells, we performed a staining for PH3, which is detectable in late G2/M-phase of the cell cycle on sections of the cerebral cortex of embroys age E12, E14 and E16. In the control, the signal is indeed restricted to the ventricular surface (Fig. 5A-A'', C-C'', E-E'') and, especially at mid- and late neurogenesis, also present in the subventricular zone (Fig. 5C-C'', E-E''). This is clearly different in the cKO. At E12, clusters of PH3 positive nuclei can be seen inside the cerebral cortex parenchyma and only what seems a minority of divisions are located at the ventricular surface. At this stage, this is restricted to the caudal and intermediate levels. The

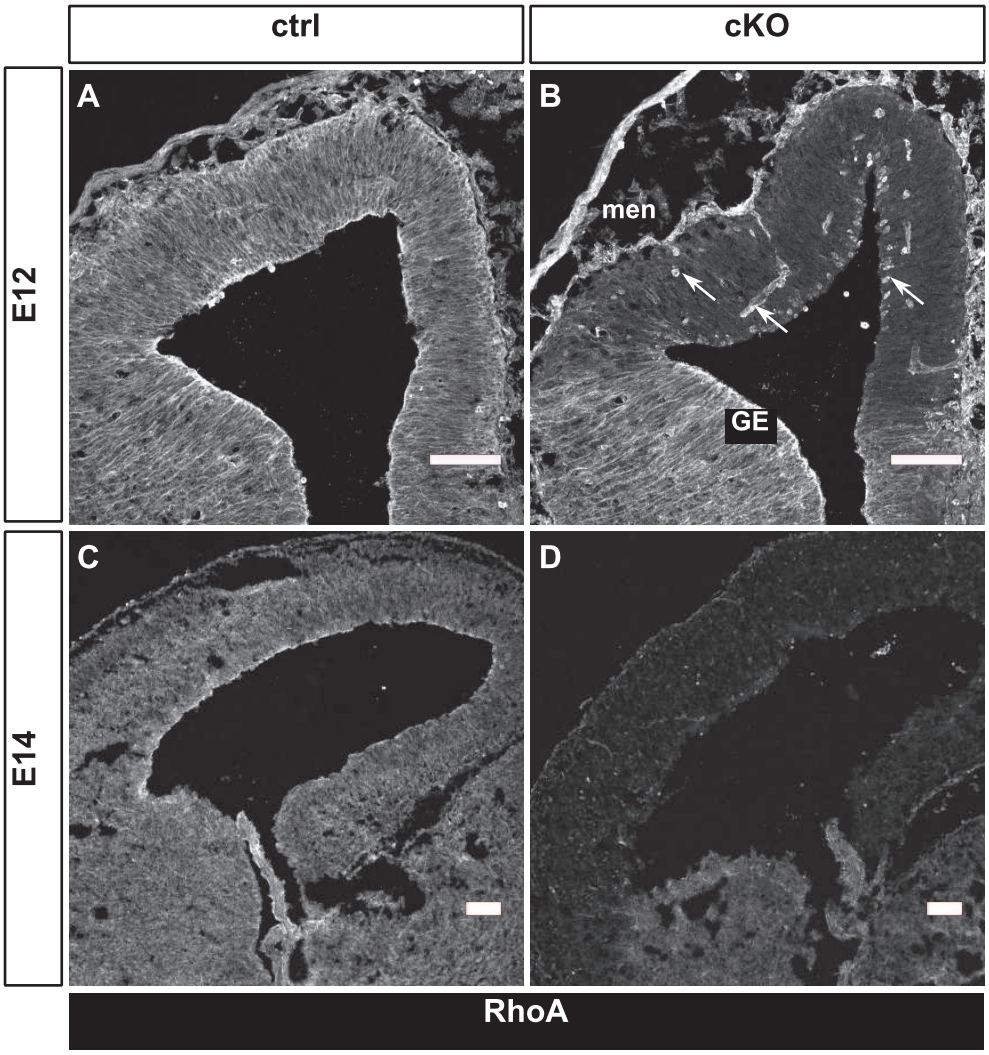


Fig. 4 Tissue specific loss of RhoA protein

(A-D) Coronal sections of embryonic control and cKO brains immunostained for RhoA

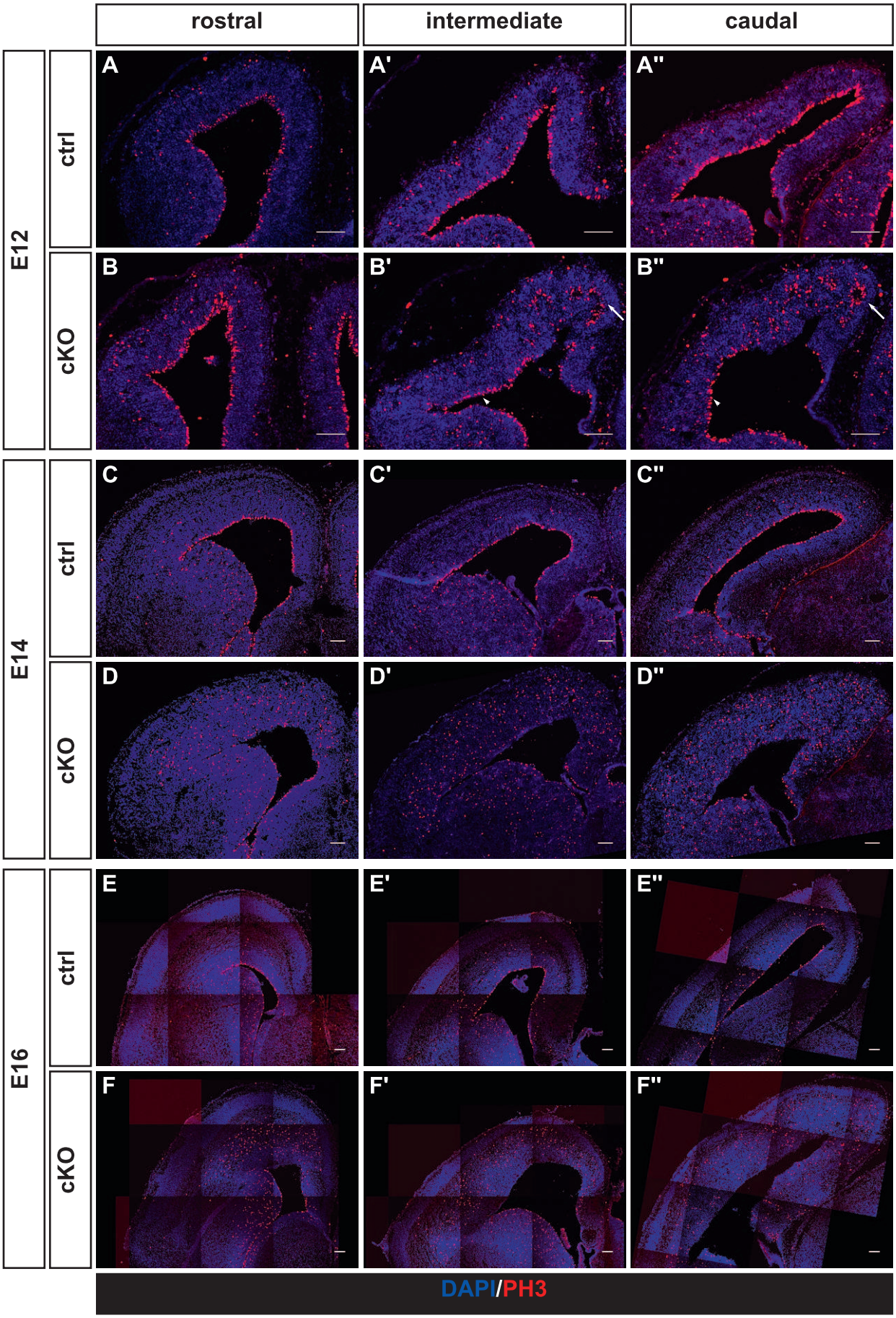
Starting E12 (A-B) the cKO has lost RhoA protein in a region specific manner (B). Meninges (men), ganglionic eminence (GE), blood vessels (arrows, examples) remain immunopositive for RhoA as they do not express Cre-recombinase under the Emx1 promoter.

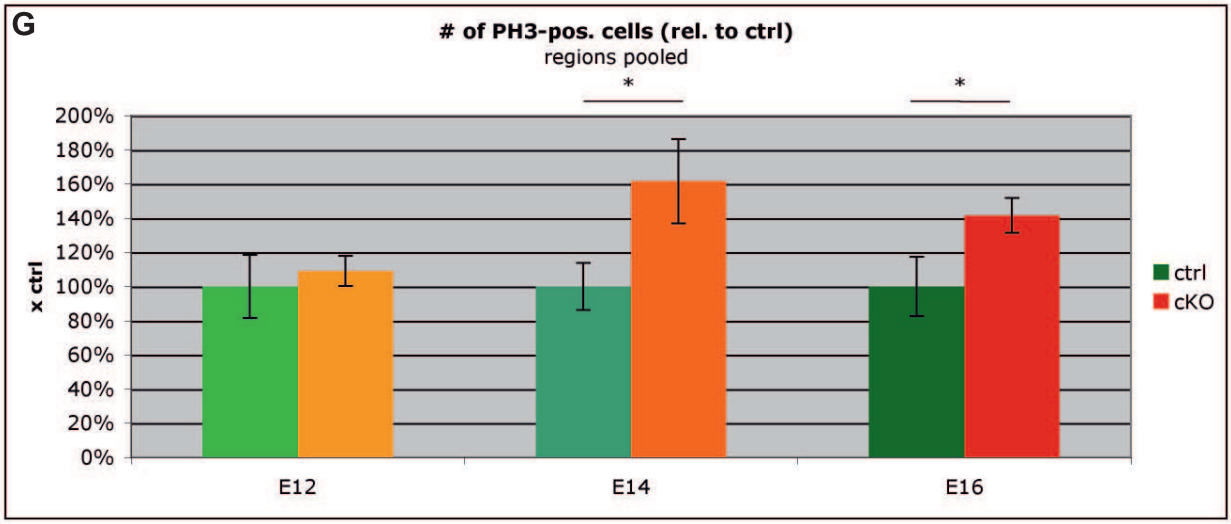
scale bars: 100µm

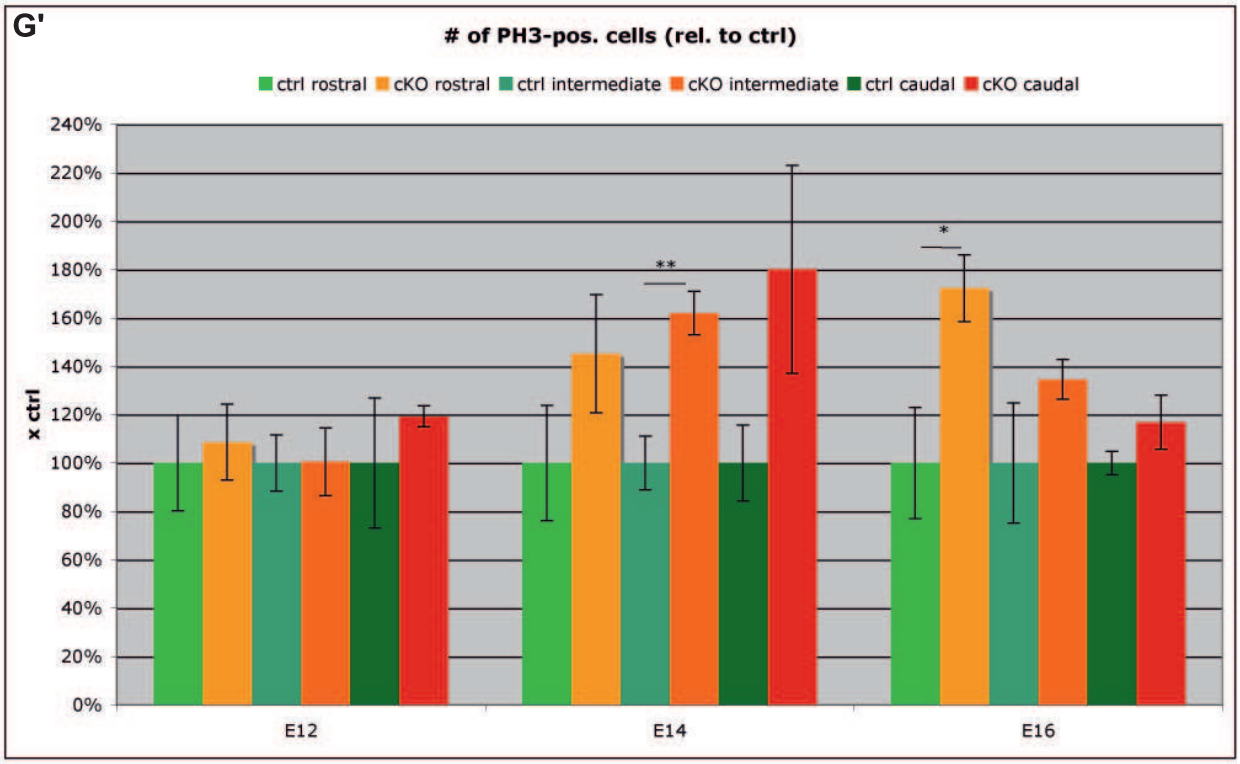
most lateral parts are not affected either (Fig. 5B-B''). At later stages, mitoses are more and more scattered throughout the parenchyma (Fig. 5D-D'') until the ventricular surface is virtually free of cell divisions (Fig. 5F-F'').

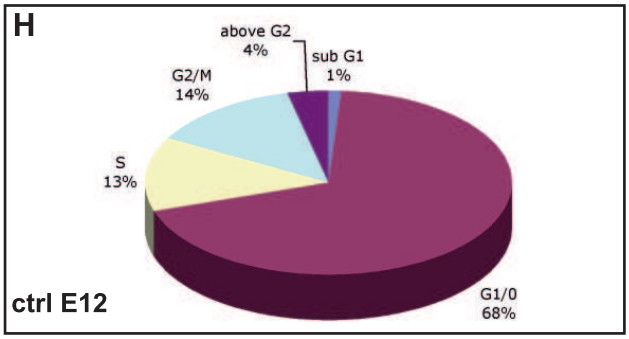
As the number of immunopositive cells seemed increased, we quantified the number of PH3 positive cells per area. They were clearly increased at E14 (162±25%, $p<0,05$) and E16 (142±10%, $p<0,05$) (Fig. 5G). This hyperproliferation follows the same caudal to rostral gradient as the mislocalization. Even though statistic significance could only be shown for intermediate levels at E14 (162±9%, $p<0,01$) and rostral levels at E16 (172±14%, $p<0,05$), a clear trend is visible (Fig. 5G').

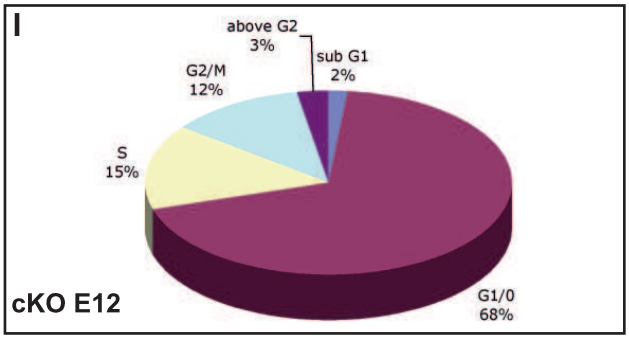
Another method to observe differences in proliferation uses flow cytometry. During S-phase, cells synthesize the DNA for another daughter cell. Therefore, during G2/M-phase, shortly before cell division, a cell has twice the DNA-content of a cell in G1 or G0. The DNA content of an individual cell gives us information about its phase in the cell cycle and the distribution of cell cycle phases over a cell population allows us to determine the rate of proliferation. We fixed and permeabilized cortical cells from E12 and E14 mouse embryos, labelled the DNA with propidium iodide and analysed them. There were no differences detectable at E12 (Fig. 5H-I). Surprisingly, at E14 there was hardly any difference in cells in G2/M-phase, which is a discrepancy to the results of the PH3 staining. However, the population of cells in S-phase increased from 6,3% in the control to 10,7% in the cKO (Fig. 5J-K). This means an increase to 170% (i.e. cKO/ctrl=1,7 n.s.), which fits to the PH3 data.

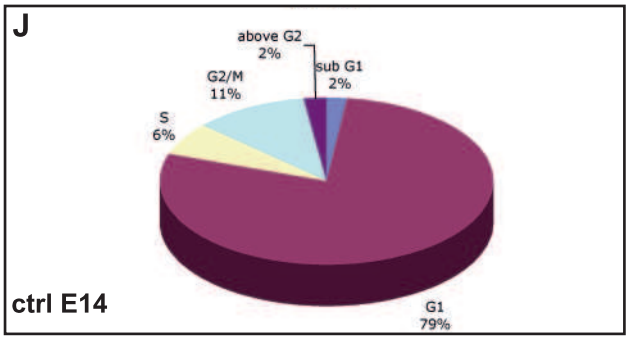

rostral
intermediate
caudal
E12
ctrl
cKO
E14
ctrl
cKO
E16
ctrl
cKO
A
A'
A''
B
B'
B''
C
C'
C''
D
D'
D''
E
E'
E''
F
F'
F''
DAPI/PH3


G
# of PH3-pos. cells (rel. to ctrl)
regions pooled
*
*
200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
x ctrl
E12
E14
E16
ctrl
cKO


G'
# of PH3-pos. cells (rel. to ctrl)
ctrl rostral
cKO rostral
ctrl intermediate
cKO intermediate
ctrl caudal
cKO caudal
240%
220%
200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
x ctrl
**
*
E12
E14
E16


H
above G2
4%
sub G1
1%
G2/M
14%
S
13%
G1/0
68%
ctrl E12


I
above G2
3%
sub G1
2%
G2/M
12%
S
15%
G1/0
68%
cKO E12


J
above G2
2%
sub G1
2%
G2/M
11%
S
6%
G1
79%
ctrl E14

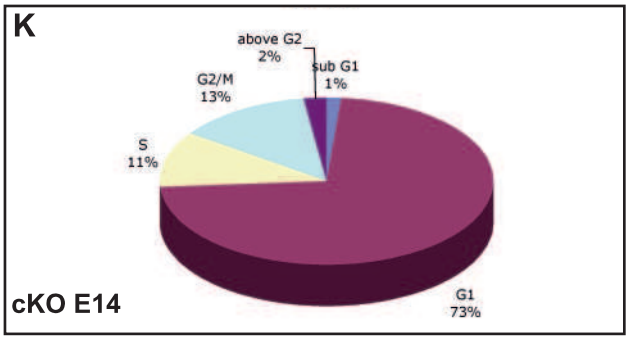

K
above G2
2%
sub G1
1%
G2/M
13%
S
11%
G1
73%
cKO E14

Fig. 5 Loss of RhoA leads to hyperproliferation

(A-F'') Coronal brain sections immunostained for the mitotic marker PH3

DAPI as nuclear counterstain

Note the clusters of mitotic cells (arrows).

At early stages, lateral parts of the cerebral cortex are not affected yet (arrowheads).

scale bars 100µm

(G, G') Quantification of PH3-positive cells/area

Different regions (rostral, intermediate, caudal) were either pooled (G) or compared independently (G').

heteroskedastic t-test; means ± standard deviations; *: p<0,05, **: p<0,01; 3 litters per age and genotype

(H-K) Distribution of cell cycle phases in the cell population

The data was gained by measuring the DNA content of propidium iodide-labelled cells by flow cytometry.

1 litter each

2.3. The increase in basal divisions is not a result of identity

To understand the unusual spread of cell divisions away from the ventricular surface, we examined the proliferating cell types in more detail. Usually, the cells that divide at basal positions are basal progenitors. The change in position could thus be the consequence of a change in cell fate (Cappello S et al. 2006). Therefore, E14 sections were stained for Tbr2 to identify basal progenitors and Pax6 to identify radial glia. None of the populations seems to have drastically changed in amount, but their organisation is completely abolished (Fig. 6). In the control, there is a distinct band of Pax6 positive cells in the ventricular zone and the beginning of the subventricular zone. Apart from presumably newly specified basal progenitors that are migrating towards the subventricular zone, all Tbr2 positive cells form a band in the subventricular zone (Fig. 6A). In the cKO, however, both cell types are completely intermingled (Fig. 6B). Only in some places a pattern is visible, that consists of a ring of radial glia nuclei surrounded by basal progenitors (Fig. 6B' arrowheads).

2.4. Radial glia lose their radial orientation

As Pax6 only reveals the radial glia nuclei, we stained for the intermediate filament Nestin and its post-translationally modified form RC2 to get a better understanding of the morphology and orientation of these cells. In addition, β-III-tubulin was used to investigate localisation and morphology of neurons in the mutant cortex.

As before, no obvious difference could be seen at rostral levels of the E12 cortex (Fig. 7A-B). At caudal levels, however, the most apparent difference are clusters of neurons, which are located at the ventricular surface (Fig. 7B'-B''). In the control, only few neurons are seen outside the cortical plate (Fig. 7B). Presumably, they are either migrating towards the cortical plate or they belong to the β-III-tubulin positive basal progenitors. Upon very close observation, the radial pattern of radial glia seems lost at some places, giving a clumped impression (Fig. 7B'').

Qualitatively, the observations are the same at E14, although in a more progressed state. Throughout the mutant cortex neurons can be seen, many of them outside of the cortical plate (Fig. 7F-H'), which seems thinner in the cKO compared to the control (Fig. 7C-E'). The "radial glia clumps" are now clearly visible as round or elongated "rosettes" (Fig. 7H-H''' arrowheads).

As a consequence of this clustering, part of the radial glia population still reaches the basement membrane, whereas other are clearly misoriented (Fig. 7H''-H''').

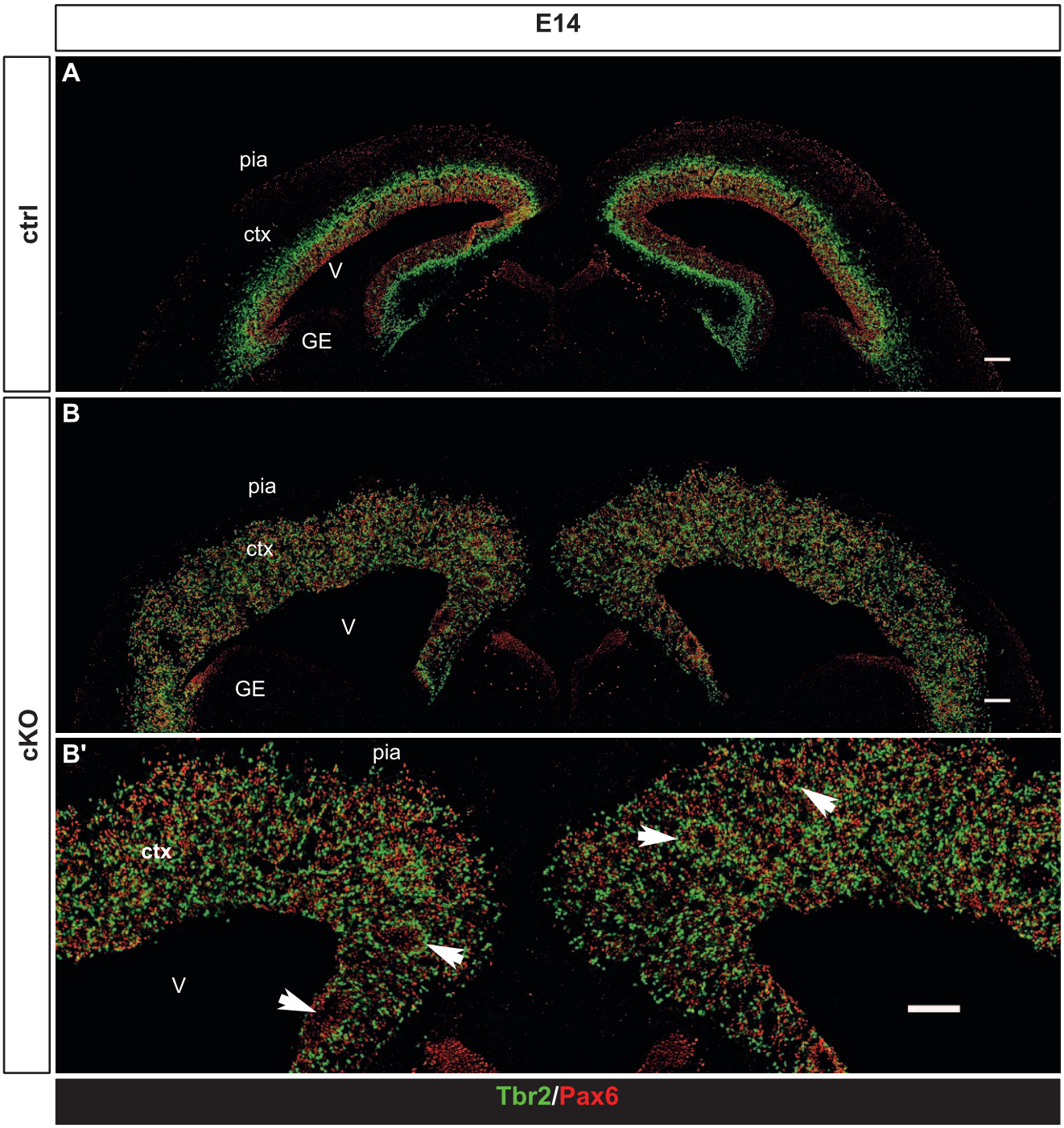


Fig. 6 Progenitor cell identity

(A-B') Cortical E14 brain sections immunostained for different progenitor populations

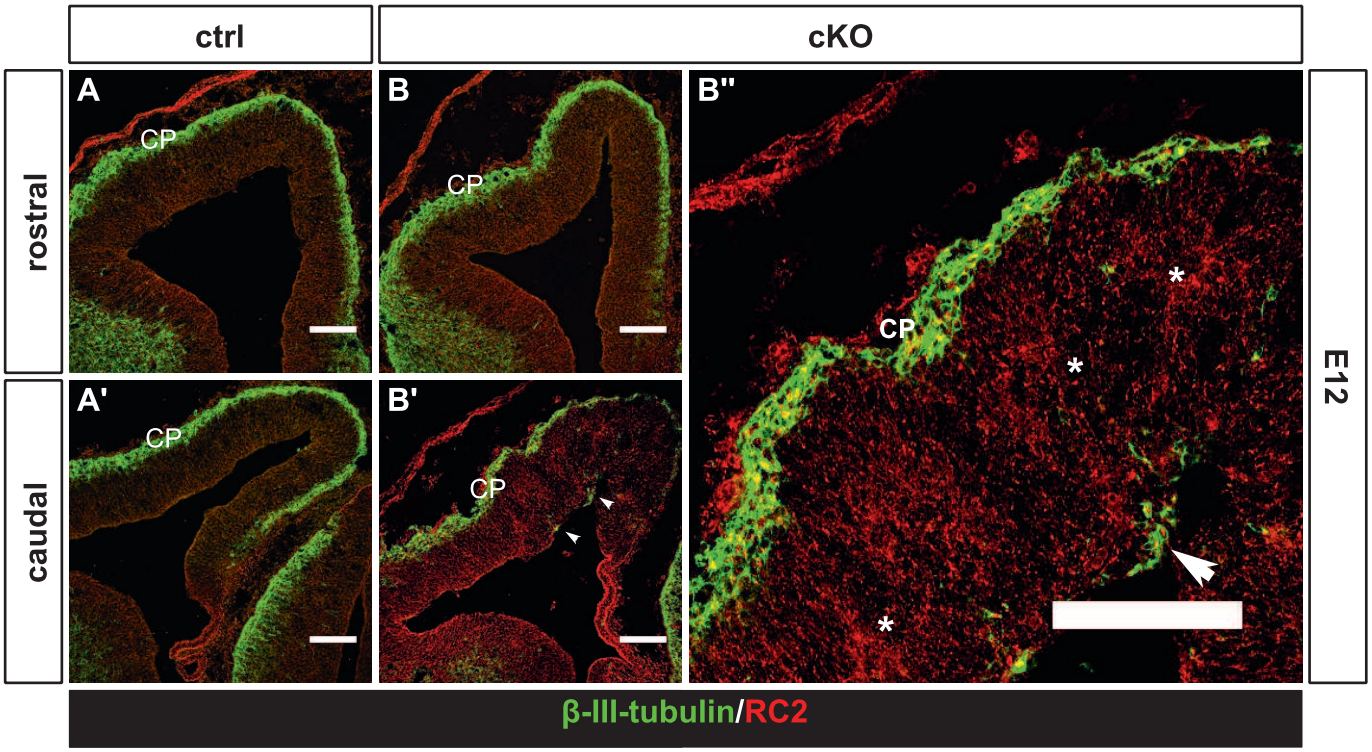
Radial glia were identified by immunostaining for Pax6 and basal progenitors by immunostaining for Tbr2.

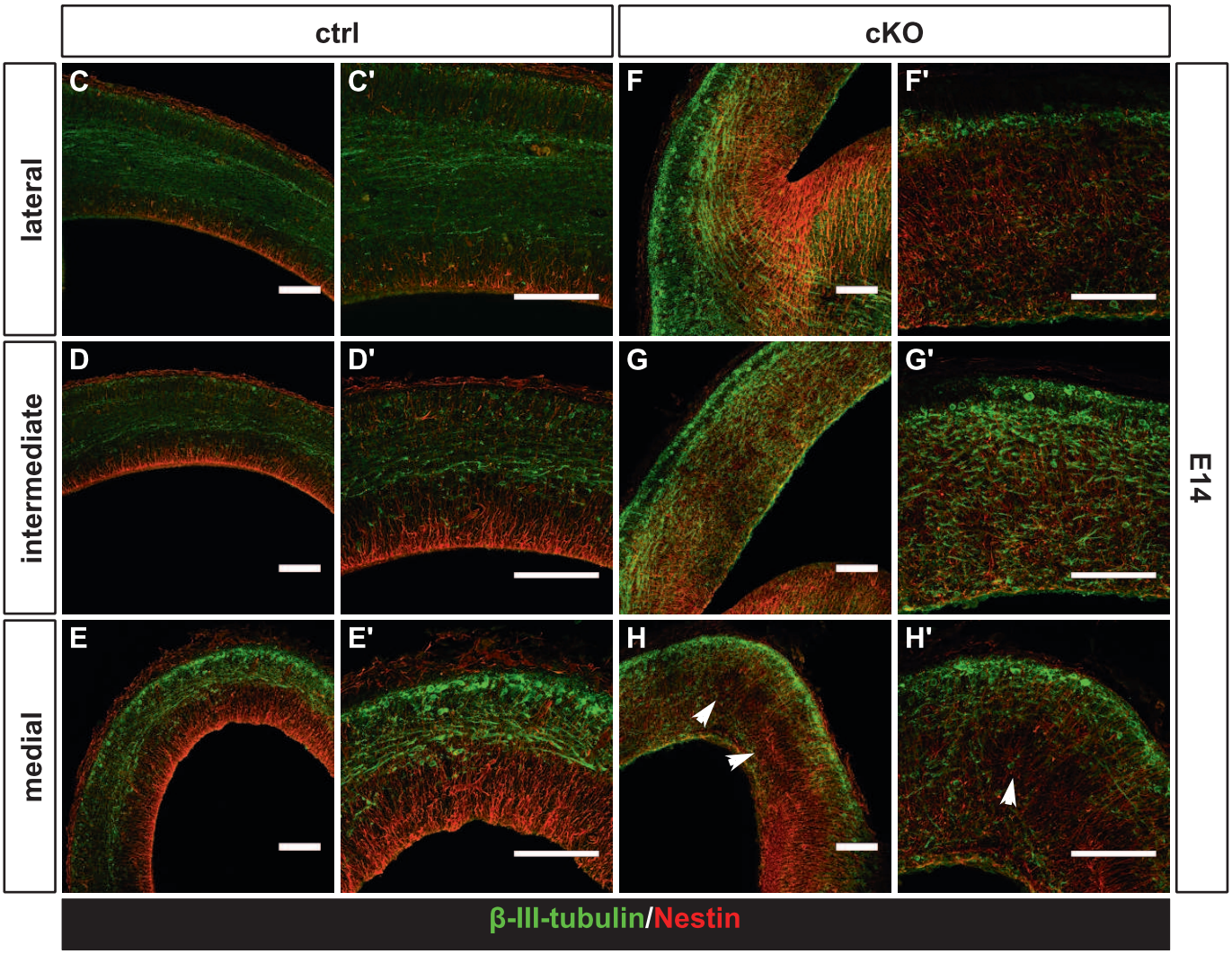
B' is a magnification of B.

Note the rosettes with Pax6-positive cells in the centre surrounded by Tbr2-positive cells (arrowheads).

ctx: cortex, V: ventricle, GE: ganglionic eminence

scale bars: 100µm


ctrl
cKO
rostral
caudal
A
CP
B
CP
B''
CP
*
*
*
A'
CP
B'
CP
E12
β-III-tubulin/RC2


ctrl
cKO
lateral
intermediate
medial
C
C'
F
F'
D
D'
G
G'
E
E'
H
H'
E14
β-III-tubulin/Nestin

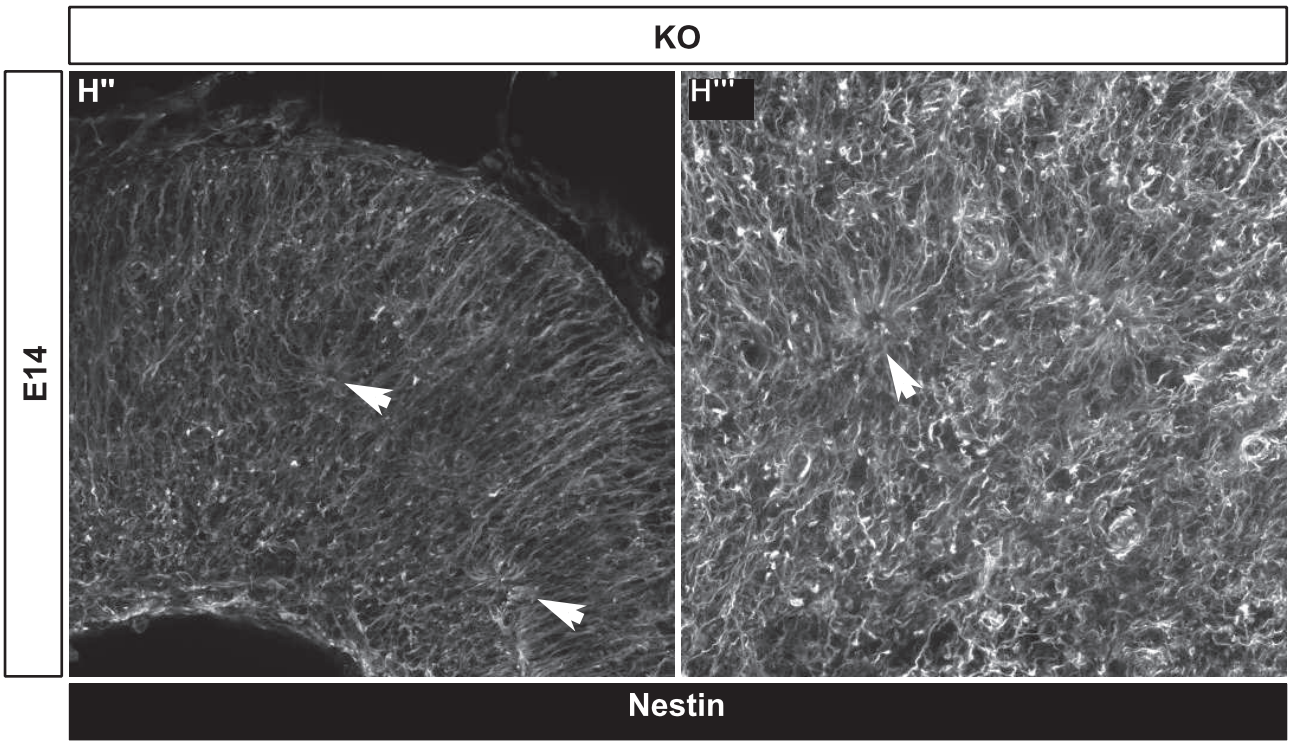


Fig. 7 Structure of radial glia and neurons

(A-B'') Cortical E12 brain sections immunostained for radial glia and neurons

radial glia were detected by RC2 immunostaining and neurons were labelled with β-III-tubulin immunostaining.

Note the misplaced neurons at the ventricular surface (arrowheads) and the clumped radial glia (asterisks).

B'' is a magnification of B'.

CP: cortical plate

scale bars 100µm

(C-H''') Cortical E14 brain sections immunostained for radial glia and neurons

Radial glia were detected by Nestin immunostaining and neurons were labelled with β-III-tubulin immunostaining.

Note the rosette-like clusters of radial glia (arrowheads).

(H''-H''') Higher magnifications of the Nestin immunostaining in H' without β-III-tubulin

The centres of the rosettes (arrowheads) can be clearly seen now. Towards the apical side (down), the radial glia seem misoriented whereas towards the basal side (up), radial glial fibres still reach the basement membrane.

scale bars: 100µm

This mutant shows many misplaced neurons and there are strong indications for a disruption of the radial glia structure. In the context of neuronal migration, the clear bipolar structure of radial glia is an important feature that allows locomoting neurons to use them as a scaffold to migrate from their place of birth at the ventricular surface to the cortical plate. To see if the mutant radial glia are still in contact with both the apical and the basal side of the cortex, a suspension of the red fluorescent lipophilic tracer DiI was injected into the ventricle of a PFA-fixed E14 brain, and crystals of the green fluorescent lipophilic tracer DiO were placed on the pial surface of the cerebral cortex, after removal of the meninges. Due to its lipophilicity these substances can only

diffuse within the membrane of a labelled cell. In the control, the red signal can also be detected at the basal surface (Fig. 8A, A''), which means that cells that could only come in contact with the dye at the ventricle reach all the way up to the basal surface. Conversely, there are also cells that contacted the green dye and reach down to the apical surface (Fig. 8A, A', C). Please note that the green labelling spreads much more tangentially, because also neurons from the cortical plate get labelled with the DiO crystals.

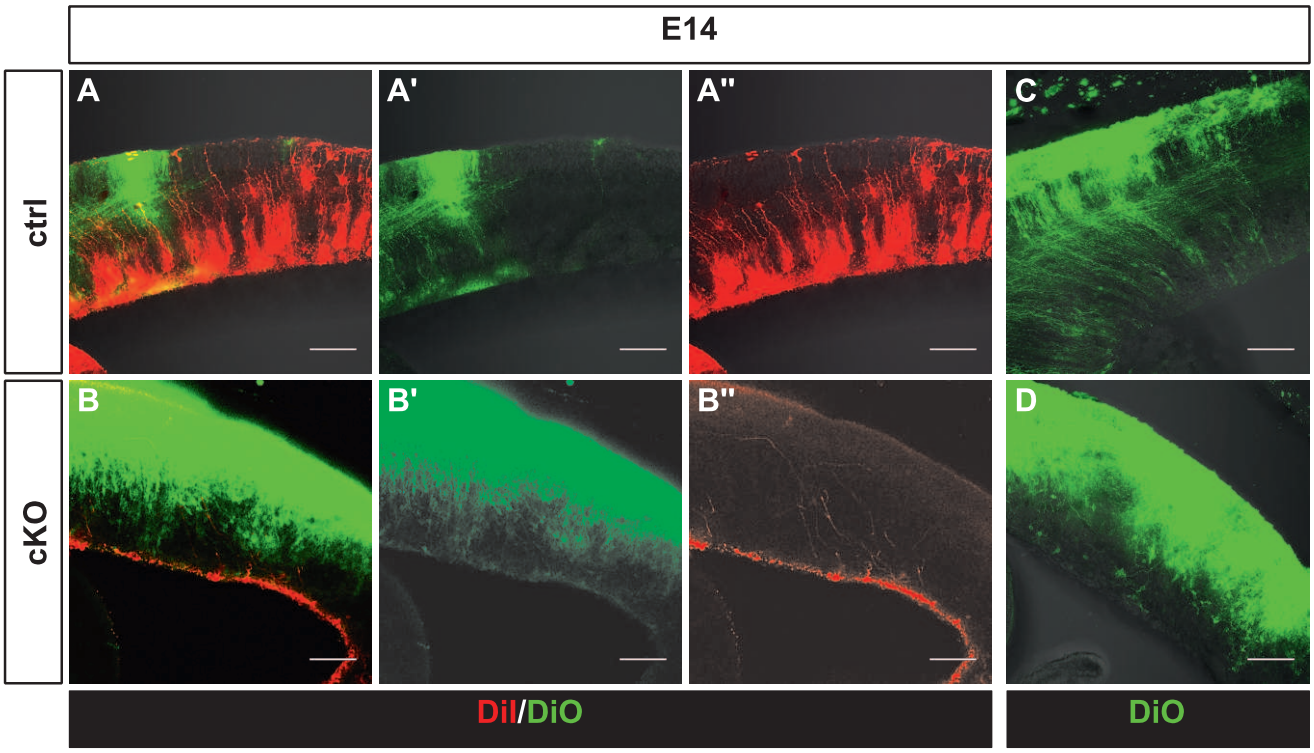


Fig. 8 Tracing of radial glial processes

(A-D) Radial glia stained with lipophilic tracers from the basal and apical surface

DiI suspension was injected into the ventricle of an E14 brain to trace the cells with contact to the ventricle. DiO crystals were placed on the basal surface to trace with contact to the basal surface.

A'-B'' are the single channels of A-B.

C-D are selected examples showing an especially clear view of the radial glial processes in the control (C) and their disturbance (D) in the cKO.

scale bars: 100µm

In the cKO experiment, much less of the dye reaches the opposite side of the cortex, and the labelled cells also don't seem parallely oriented in a radial manner (Fig. 8B-B'', D).

2.5. Loss of RhoA leads to disruption of adherens junctions and formation of rosettes

As the radial orientation is a hallmark of radial glia, we went on to investigate how this change in morphology came about. Usually, the radial glial endfeet are anchored to one another at the ventricular surface by adherens junctions. Defective adherens junctions have been shown to lead to tissue disorganisation (Ganzler-Odenthal SI and Redies C 1998, Machon O et al. 2003, Lien WH et al. 2006).

We stained for cadherins, an extracellular adherens junction component, and for β-catenin, an

internal component that anchors cadherins to the cytoskeleton. In the control, as in rostral E12 cKO cerebral cortex sections, the stainings show an overlapping intense continuous band along the ventricular surface (Fig. 9A, A', B). Again, in caudal cKO sections this is no longer the case. Though not entirely absent, the band at the ventricular surface is disrupted and in some cases continuing into the parenchyma (Fig. 9B'-B'''). In addition, there are again circular structures present inside the parenchyma (Fig. 9B').

At E14, the disruption at the ventricular surface in the cKO looks even more severe, with only some double-stained patches remaining (Fig. 9D). This is, with the exception of the hippocampus anlage, where the β-catenin immunostaining is still present. Also at this stage, the rosettes are visible as clusters of adherens junctions (Fig. 9D'', D'''). Their centre is devoid of nuclei (Fig. 9D'), indicating that all cell somata are on the outside of the rosettes.

When seen from inside the ventricle, the pattern formed by adherens junctions is a honeycomb-like structure outlining the apical membrane framed by adherens junctions of the radial glia. In higher magnification this is sometimes visible due to a slightly oblique cutting angle. To determine with more confidence, that the immunopositive lines are indeed adherens junctions, we scanned the section at a high Z-resolution (oversampled at 0,3µm) and rotated the resulting 3D reconstruction to get an "en face" view of the ventricular surface (Fig. 9E). This was done with β-catenin-stained sections as well as with sections where F-actin, which is present at high concentration in adherens junctions, was revealed by means of labelled Phalloidin.

In the area where the virtual section plane is parallel to the ventricular surface, a clear honeycomb pattern is seen in the control tissue, indicating that the staining indeed reveals adherens junctions (Fig. 9F, H). In the cKO, the staining pattern is very diffuse and only at small remaining spots reminiscent of the control pattern (Fig. 9G, I).

The most reliable method for the detection of adherens junctions, is electron microscopy (EM). We analyzed cerebral cortices of E13 embryos to be sure that the adherens junctions were just disrupted.

Not only did the control show the presence of adherens junctions connecting the radial glial endfeet at the ventricular surface, but in addition numerous mitotic figures showed the cell divisions in this area (Fig. 9J-J').

In some areas, the situation in the cKO (Fig. 9K-K') was still identical to the control. Considering the age of the embryos, the relatively rostral section plane and the presence of parts with remaining adherens junctions even at E14, this is not unexpected. However, there are also areas of the ventricular surface where no more adherens junctions could be identified (Fig. 9L). This confirms the immunostaining and the loss of apical adherens junctions. Consequently, the cells in this region gave a rather disorganized impression, with curled processes and no radial orientation. Also the nuclei did not show the condensed chromatin of dividing cells. Just like in the immunostainings, there were, however, regions inside the parenchyma where several cells were connected with adherens junctions, forming a rosette structure. These cells showed mitotic nuclei in proximity to the junctions and are connected at their endfeet (Fig. 9M-M').

Taken together, these observations show that following the loss of RhoA protein at E12, the band of adherens junctions at the ventricular surface gets disrupted. Some radial glia remain, however, still connected and clump inside the parenchyma to form rosette-like clusters.

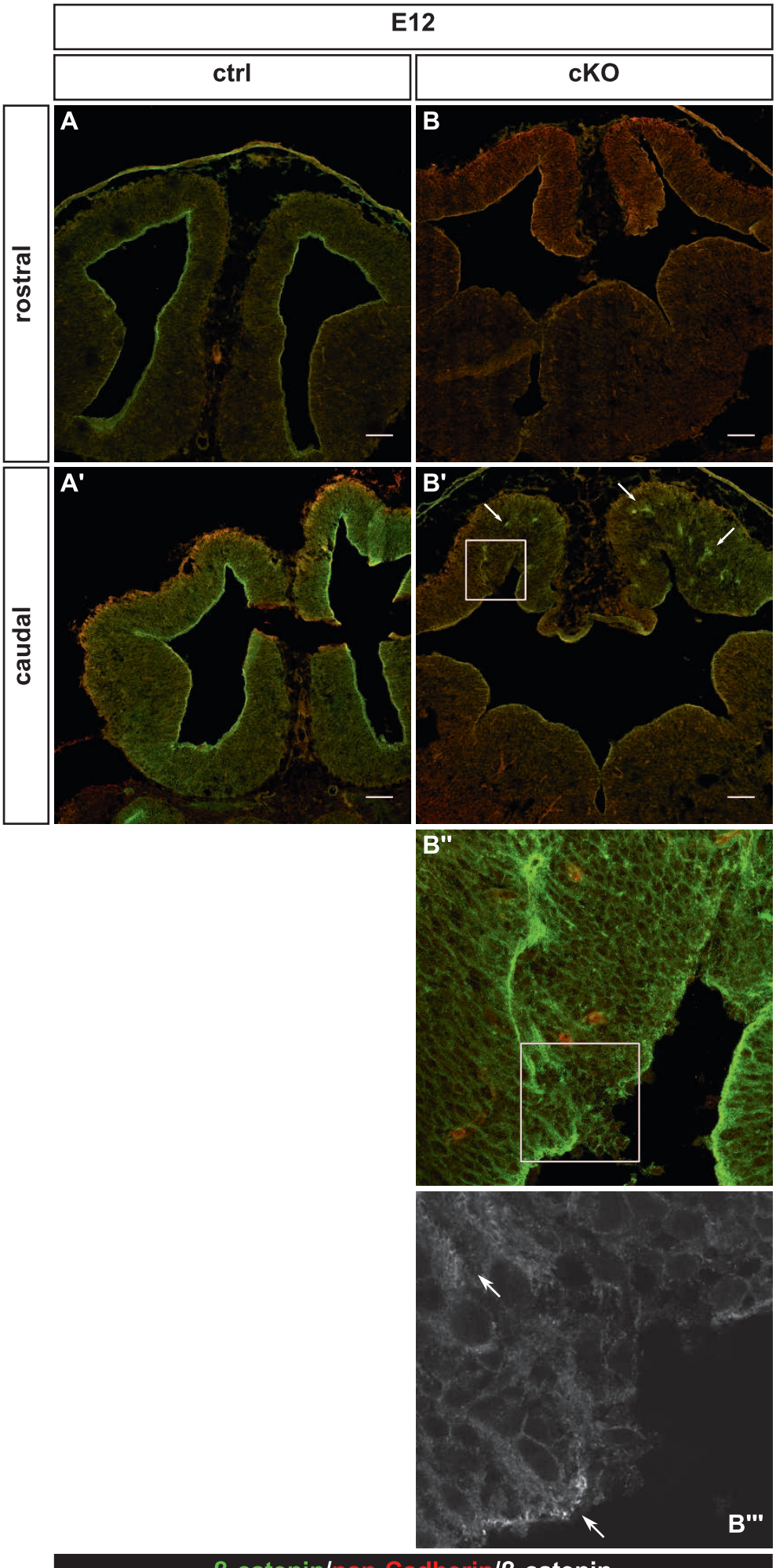


Fig. 9 Disrupted adherens junctions in the cKO

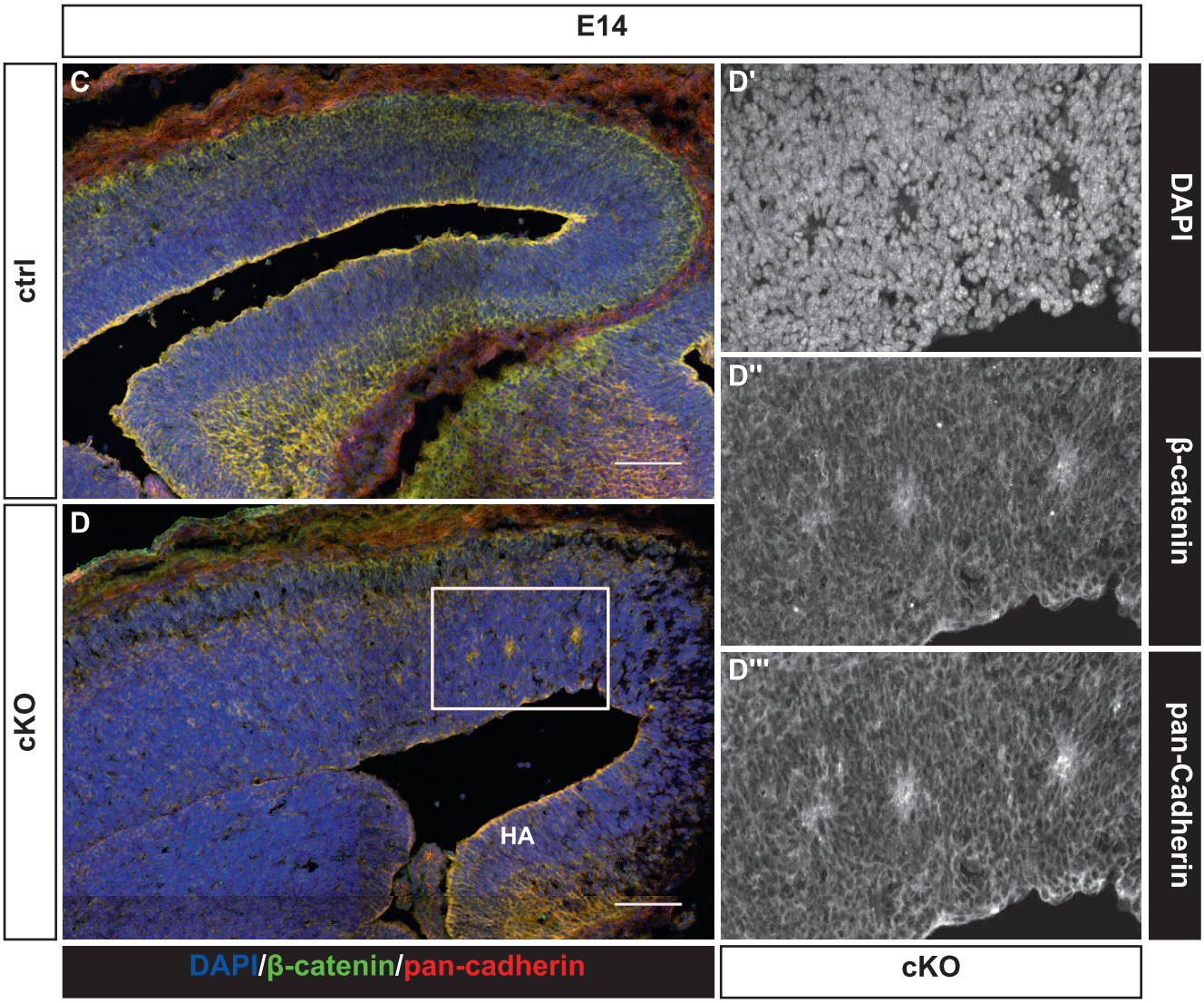
(A-B’’’) E12 brain sections immunostained for adherens junction components

The intracellular adherens junction component β-catenin and the extracellular cadherins were labelled by immunostaining. At this stage, no disruption in the chain of adherens junctions at the ventricular surface is visible at rostral levels (A, B). Caudal section levels (A’-B’) show first disruptions in the chain of adherens junctions (box) and rosettes where adherens junctions are concentrated inside the parenchyma (arrows).

B’’ is the marked region of B’ in higher magnification. The loss of adhesion at the ventricular surface can be seen more clearly (box). A part of the chain of adherens junctions has moved inside the parenchyma.

B’’’ is a higher magnification of the marked region of B’’, only showing the β-catenin immunostaining. The remaining band of adherens junctions, as well as the band inside the parenchyma, show the honeycomb pattern typical of adherens junctions (arrows).

scale bars: 100µm



(C-D’’’) E14 brain sections immunostained for adherens junction components

The sections were stained for β-catenin and pan-cadherins like A-B. DAPI was used as a nuclear counterstain.

The double-positive band of adherens junctions along the ventricular surface is severely disrupted, with the exception of the hippocampus anlage (HA).

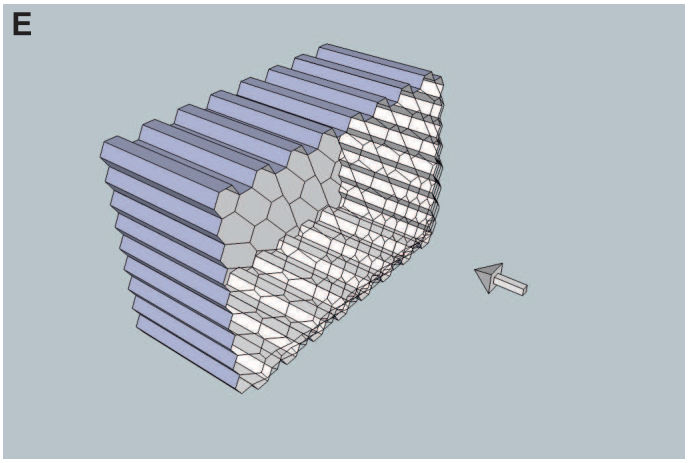
The single channels of a magnification of the highlighted region in D (D’-D’’’) clearly shows that the rosettes consist of an accumulation of adherens junctions in their centre, which is devoid of nuclei.

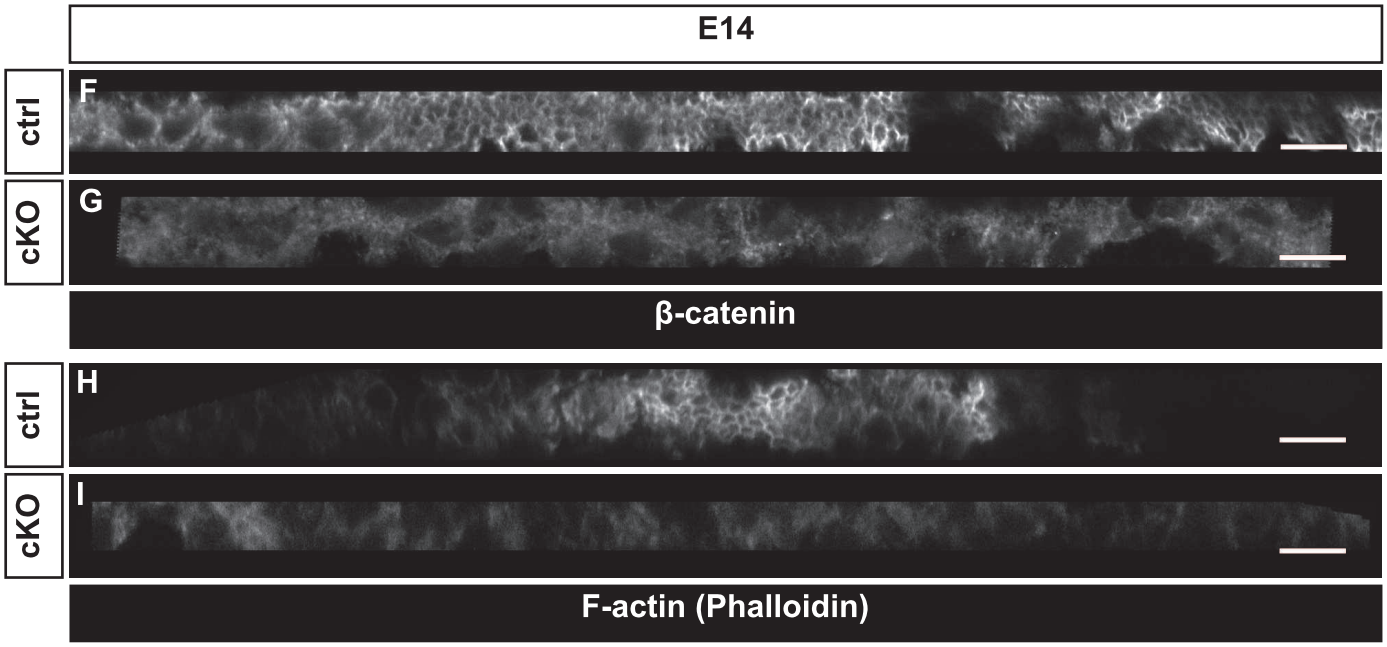
scale bars: 100µm

(E-I) En face view of the apical adherens junctions

E14 brain sections were stained for β-catenin (F-G) or F-actin (H-I), which is also highly concentrated at adherens junctions. A 3D reconstruction was produced of confocal stacks of 20µm cryosections. The stack was rotated so that the ventricular surface is seen en-face from the ventricle (E). Both stainings show the characteristic honeycomb pattern formed by the apical rings of adherens junctions in the control (F, H). The cKO (G, I) shows only diffuse staining at the ventricular surface.

scale bars: 10µm





(J-M') electron microscopy pictures of E13 cortical brain sections

In the control case (J-J'), adherens junctions (arrows) connect the radial glia at the ventricular surface. Almost all nuclei at this apical position have the condensed chromatin structure of dividing cells.

J' is a higher magnification of J.

At some positions, the adherens junctions (arrows) are still intact in the cKO (K-K'). The nuclei also have condensed chromatin here.

K' is a higher magnification of K.

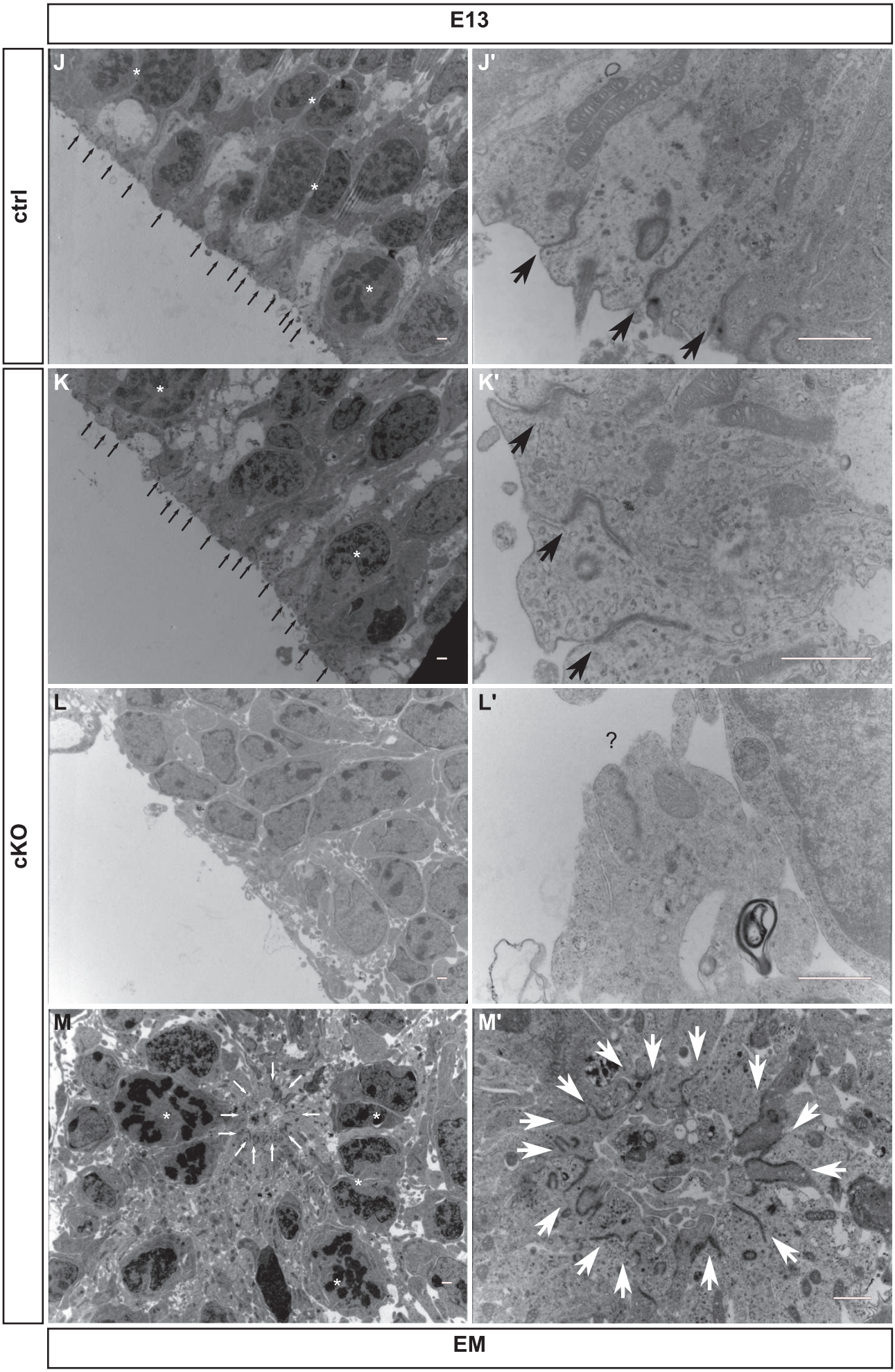
In many places in the cKO, the band of adherens junctions is no longer present at the ventricular surface (L).

L' is a higher magnification of L showing what might be the remnants of an adherens junction (question mark) in the tangle of misoriented processes.

The rosettes (M-M') consist of radial glia with their endfeet connected by adherens junctions (arrows). Near this ectopic ventricular surface mitotic figures can be seen.

M' is a higher magnification of M.

scale bars: 1µm


E13
ctrl
cKO
J
J'
K
K'
L
L'
?
M
M'
EM

2.6. cKO animals are born at expected Mendelian rate

Considering the strong phenotype at embryonic stages, it seemed surprising that mutant pups were born alive and reached adulthood. At the age of weaning, at around 4 weeks of age, no significant differences from the Mendelian ratio could be observed. This way it was possible to gain data about the further progression of the phenotype.

litter	ctrl (no Cre)	cHeKO	cKO
#1	6	1	1
#2	2	0	5
#3	2	2	2
Sum	10	3	8
total animals	21		
expected freq	0,5	0,25	0,25
expected #	10,5	5,25	5,25
Chi-square	2,6578125	p=0,2647	

2.7. Loss of RhoA leads to formation of a “double-cortex”

To get a first impression of the adult brain structure, a staining for the neuronal nucleic marker NeuN was performed. To fully appreciate the extent of the observation, it is useful to briefly recapitulate the wildtype anatomy. Directly overlying the ventricle and the hippocampus we can distinguish the white matter by its absence of neuronal nuclei. On top of the white matter follow the six layers of the neocortex, of which the less dense layer IV and the virtually cell free layer I are appreciable in the staining (Fig. 10A-A'). The cKO shows a stark contrast to this general architecture (Fig. 10B-B’). Here we can distinguish two structures consisting of neurons that are separated by an immunonegative band, reminiscent of the white matter. Only the upper basal structure shows the less dense band of layer IV. The lower structure only shows some areas that are devoid of neurons. This type of brain malformation, where two cortical structures are separated by a band of white matter is often called a “double-cortex” (Bielas S et al. 2004). Furthermore, the recognizable layer I is penetrated by neurons in rostral positions, a phenotype called “cobblestone lissencephaly” (Bielas S et al. 2004).

In order to identify the changes in anatomy more closely and to ensure that the non-neuronal band in the cKO is indeed the white matter, we combined stainings for GFAP and S100β to identify all astrocytes. Like the white matter in the control (Fig. 10C-C’), the band in the cKO was populated by fibrous GFAP-positive cells (Fig. 10D-D’). As in the control, more GFAP signal was visible at the pia. In the cKO, a thin GFAP positive band was seen directly adjacent to the ventricle and the hippocampus (Fig. 10D-D’’). Both “cortices” in the cKO were virtually free of GFAP positive cells, as was the control cortex.

S100β is also expressed in grey matter astrocytes and in ependymal cells (Fig. 10C-C’). This also seems to be the case in the cKO, where these cells populate both cortices at similar density, which also roughly corresponds to the density in the control. Also in the cKO, a very thin band of GFAP-positive cells can be seen adjacent to the ventricle (Fig. 10D’’). This is noteworthy, because two different types of "double-cortices" can be distinguished: periventricular heterotopia

(PH), where the lower cortex is directly adjacent to the ventricle, and subcortical band heterotopia (SBH), where the lower cortex is embedded in the white matter, which seems to be the case here.

Identification of the third neural lineage, oligodendrocytes, would help us to determine if we identified both the white matter and the periventricular heterotopia correctly. We stained for the myelin produced by these cells, namely the myelin antigen MAG. Not only in the control, but also in the cKO was the white matter intensely stained (Fig. 10E-F). It is now safe to assume that also in the cKO we are indeed looking at the white matter. Also a thin band overlying ventricle and hippocampus is stained and meets the white matter at caudal levels (Fig. 10F'), confirming the subcortical band heterotopia case of a "double-cortex".

So far we have learned, that our mouse model has two cortices separated by the white matter, but we still know nothing about the neurons inside these cortices. An important feature of the cortex is its layered structure. Already at early developmental stages, we observed a severe disorganisation in the cKO cortex. Since the structure of the adult cortex is also severely altered, it is important to find out if the neurons of the correct layer identity were generated.

Neurons of different layers can be distinguished by their gene expression. We performed stainings for different proteins to identify the specific neuronal subtype.

Cux1 labels the uppermost and therefore latest-born neurons of layer II-IV (Fig. 10G). They appear to make up a large part of the lower neuronal structure in the cKO cerebral cortex (Fig. 10H-H'). The upper cortex looks surprisingly normal. It is, however, somewhat thinner than in the control and protrudes into layer I at the places where the ectopias have been observed before. At the centre of those ectopias, the band appears particularly thin (Fig. 10H, asterisk).

Tbr1 is expressed by the early born neurons, which make out layer VI. Fewer of them are present in the lower cortex, but they still form a neat band on top of the white matter in the upper cortex (Fig. 10J-J'). Remarkably, they even seem to concentrate towards the bottom of the lower structure. It is interesting to note that at the ectopias they sometimes protrude even past the upper layer neurons into layer I (compare asterisk in Fig. 10H and J).

The last marker shown in Fig. 10 is Ctip2, which labels the lower layers V-VI. It is also only sparsely expressed in the lower cortex of the cKO and forms a band at its expected position in the upper cortex (Fig. 10L-L').

Since the upper cortex shows the same layering as the wildtype and since it is correctly positioned above the white matter, we will further refer to it as “homotopic cortex”. In contrast, the lower structure will be called “heterotopic cortex”, because it is embedded in the white matter.

Usually, the layers are generated in a sequential pattern from the inside towards the outside. After we had seen the distribution of layer markers in the cKO brain, we decided to analyze if they were also generated in the normal sequence. We therefore injected BrdU at different times of gestation (E12, E14, E16) and observed the pattern of BrdU-positive cells at the same postnatal stage (p7).

The inside-out pattern is very clear in the control (Fig. 10M, O, Q). As suggested by the previous set of experiments, it is also preserved in the homotopic cortex (Fig. 10N, P, R). The heterotopic

cortex, in contrast, contains neurons born at all stages of gestation in an intermingled pattern with no obvious organization. The majority of the neurons in the heterotopic cortex are born at E16, the cells born at E12 contribute least to the heterotopic cortex.

One should not forget that not all neurons in the cortex are also generated there. The place of birth of nearly all GABAergic interneurons is the ganglionic eminence. These neurons are not affected by the Emx1::Cre line, as Emx1 and hence also Cre are only expressed in the cortex (Iwasato T et al. 2004). Nevertheless, it is interesting to see where these cells migrate in the mutant environment and whether they would enter the heterotopic cortex. One possibility to identify GABAergic neurons is their expression of GAD67. We performed in-situ hybridisation against GAD67 mRNA to visualize them. Both in the control and the cKO, the difference between cortex and the more intensely labelled basal ganglia (BG) is clearly visible (Fig. 10S-T'). The white matter, which hardly contains neuronal somata, is virtually free of staining in both cases. The control cortex and both of the cKO cortices do not show a noteworthy difference in cell density and distribution.

Figure 10: Cellular identity of the cKO cortex

(A-B') Adult brain sections immunostained for neurons

NeuN shows a heterotopic (het) neuronal mass that is separated from the normotopic cortex by a band of white matter (WM). There are also neurons that protrude into layer I (asterisk).

A' and B' are magnifications of the indicated regions in A and B.

The cell sparse layers I and IV are indicated.

scale bars: 100µm

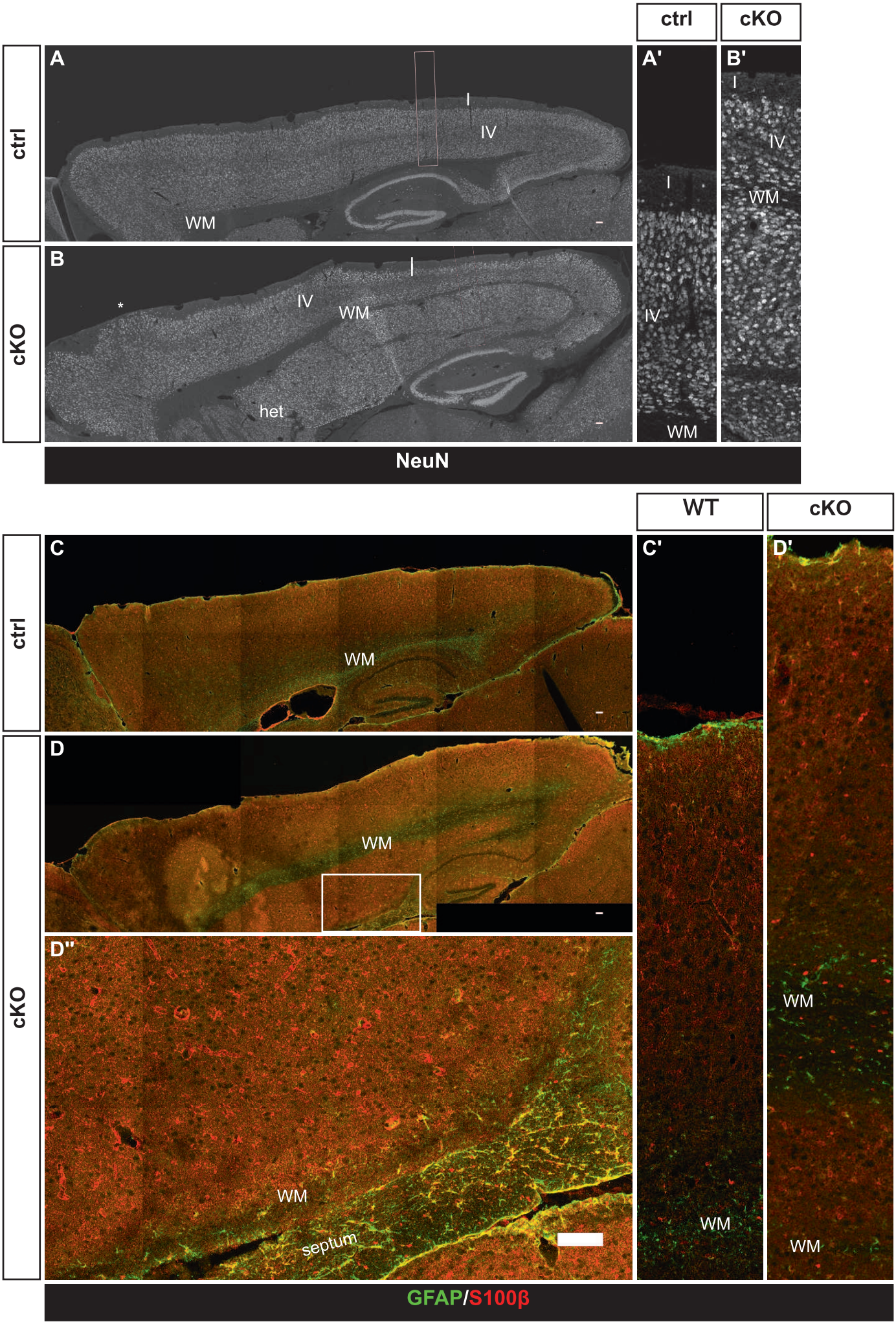
(C-D'') Adult brain sections immunostained for astrocytes

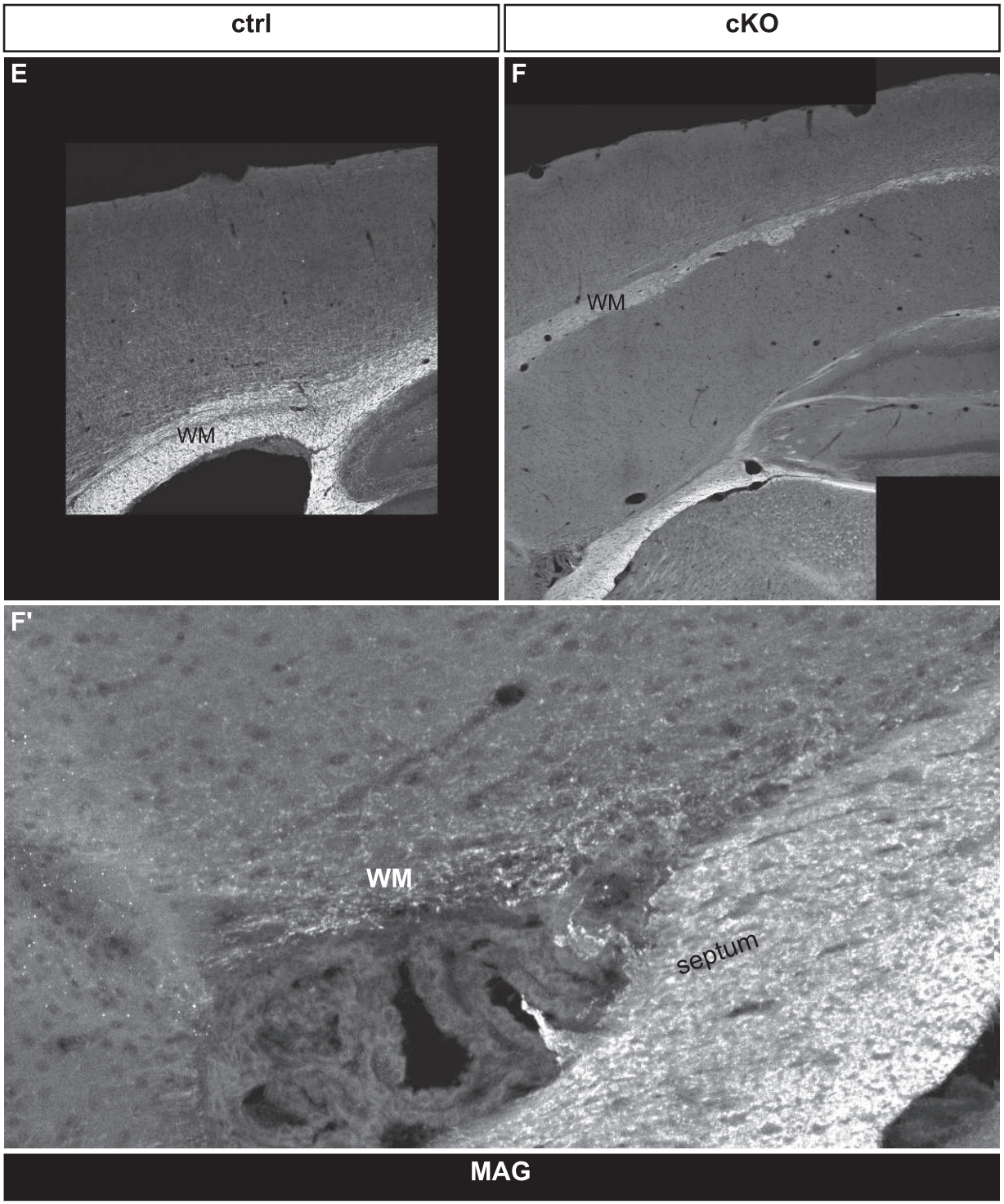
Different types of astrocytes were identified by immunostainings for GFAP and S100β. Like in the control, S100β only labels grey matter astrocytes. Their density is comparable in control and cKO. (C',D' show a magnified area corresponding to the marked regions in A,B) GFAP mainly labels the fibrous astrocytes of the white matter (WM). Between ventricle and the heterotopic cortex appears a thin band of GFAP-positive cells. (D'', magnification of boxed region in D).

scale bars: 100µm

(E-F') Adult sagittal brain sections immunostained for oligodendrocytes

The oligodendrocyte marker MAG clearly identifies the white matter. In higher magnification (F') one can appreciate the thin band of white matter (WM) remaining between ventricle and the heterotopic cortex.


ctrl
cKO
A
A'
B'
B
I
IV
WM
het
NeuN
WT
C
C'
D'
D
D''
septum
GFAP/S100β



NB: In F and F' the septum also produces a strong immunopositive band, which is inside the ventricle.

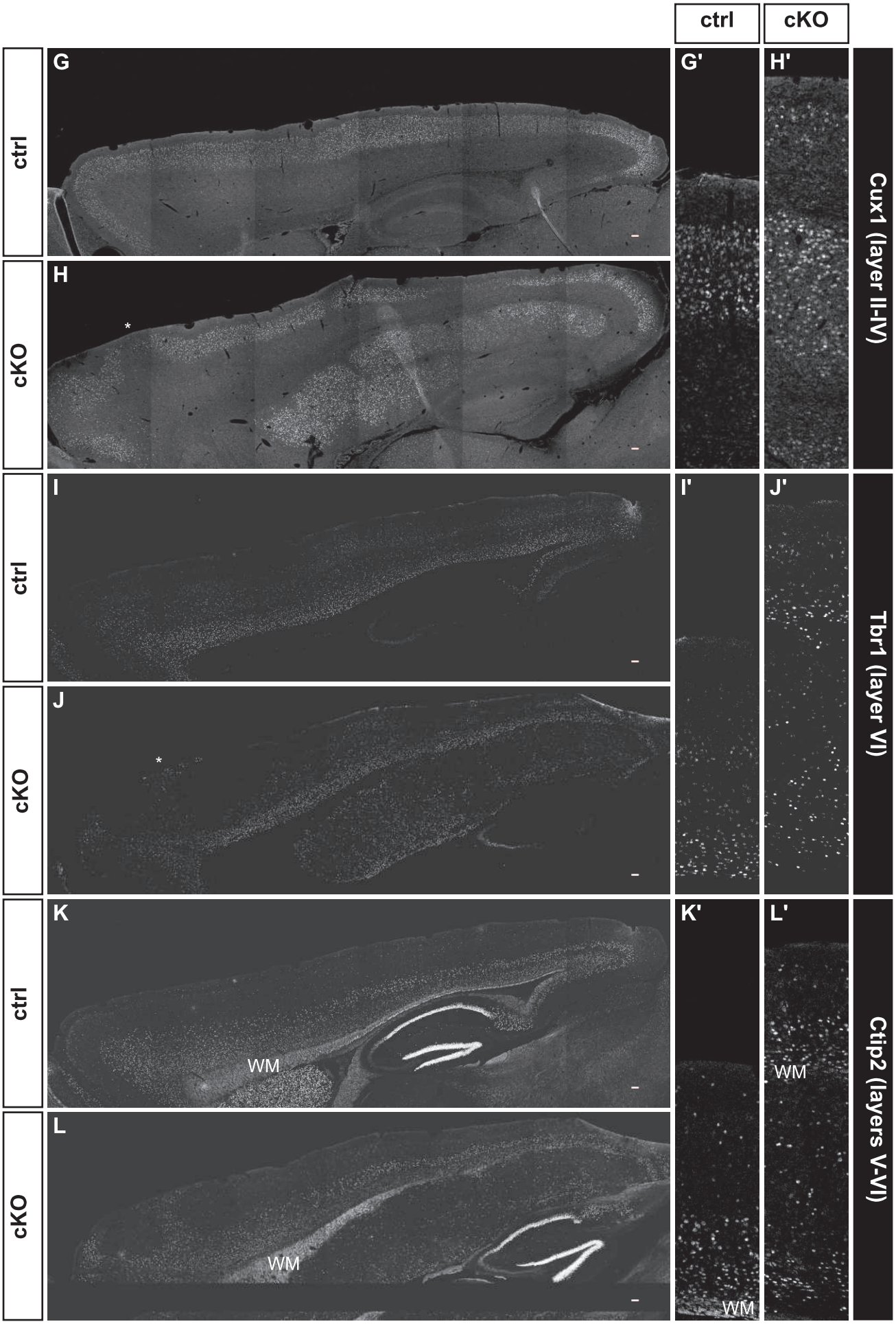
<u>(G-L) Adult sagittal brain sections immunostained for different layer markers</u>

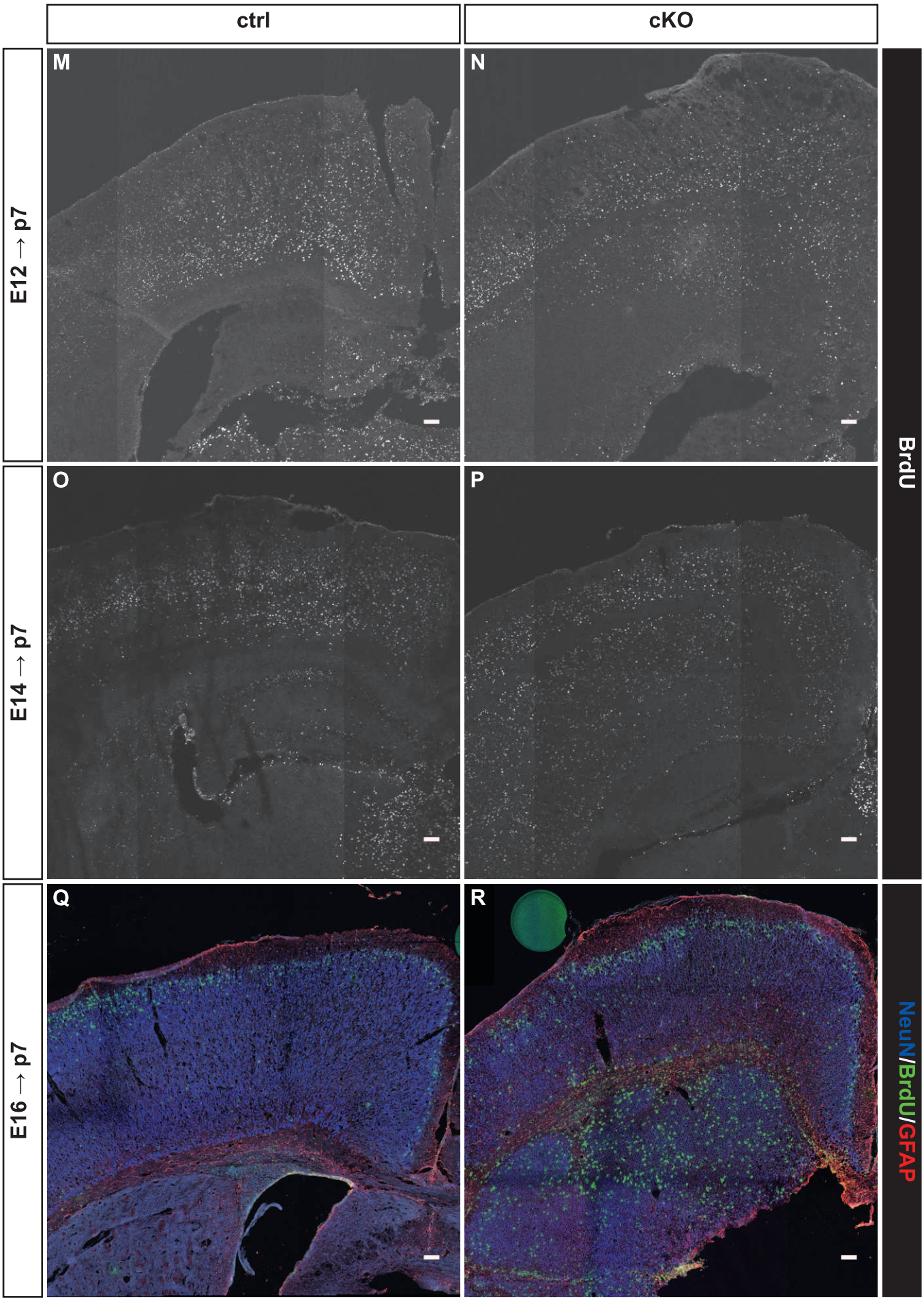
Cux1 (G-H') labels neurons of layer II-IV, Tbr1 (I-J') labels neurons of layer VI and Ctip2 labels neurons of layer V-VI (K-L'). All layer markers are present in the normotopic and the heterotopic cortex, but upper layer neurons clearly form the majority. Apart from the ectopia in layer I, the normotopic cortex is correctly layered like the wildtype.

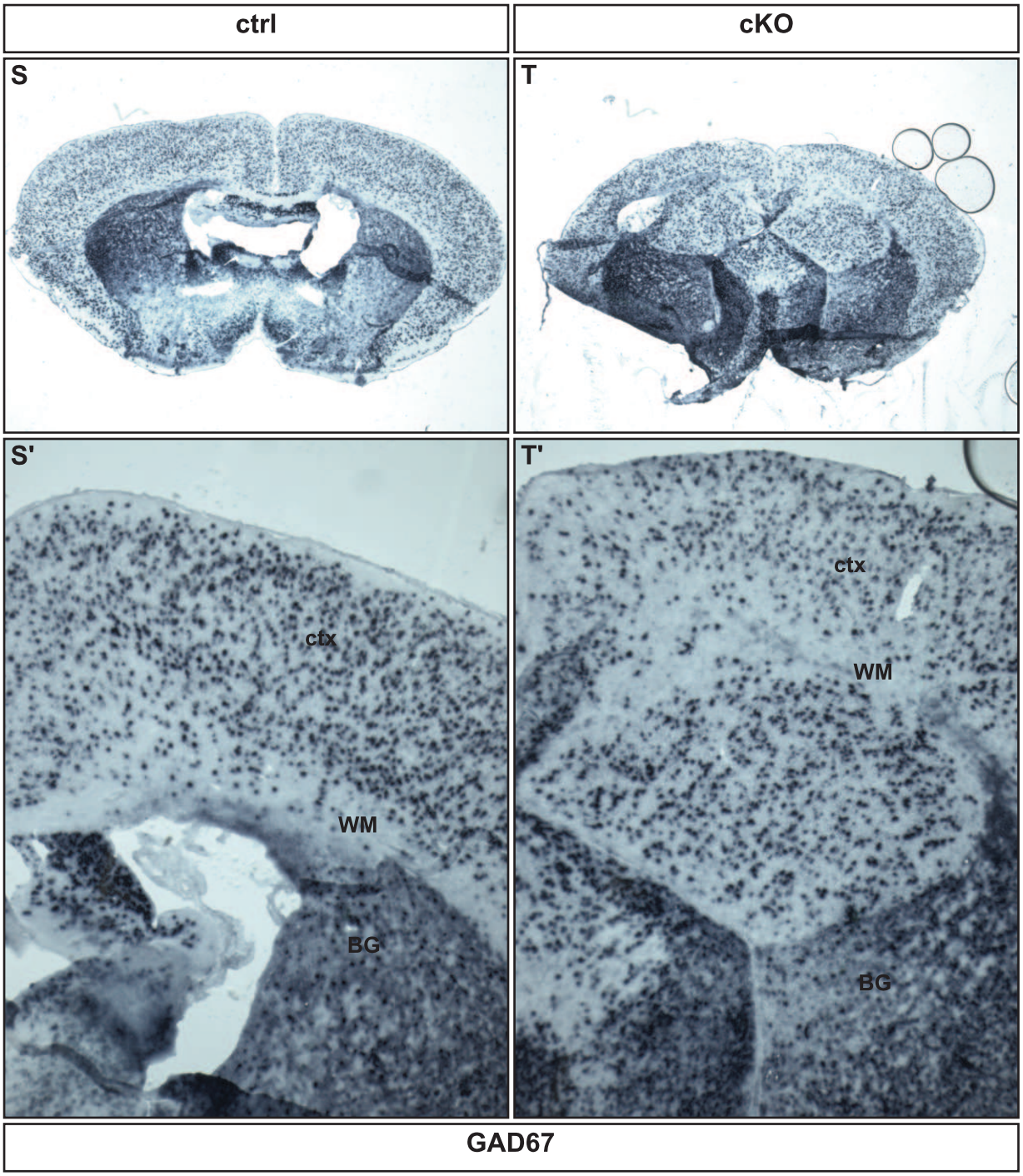
G'-L' are magnifications of G-L that represent areas comparable to the ones highlighted in A-B.

NB: Due to the pre-treatment, the Ctip2 immunostaining produces a strong background in the white matter (WM) (K-L). It can be distinguished from the correct nuclear signal by its fibrous appearance (K'-L').

scale bars: 100µm


ctrl
cKO
G
H
G'
H'
Cux1 (layer II-IV)
I
J
I'
J'
Tbr1 (layer VI)
K
L
K'
L'
Ctip2 (layers V-VI)
WM


ctrl
cKO
E12 → p7
E14 → p7
E16 → p7
M
N
O
P
Q
R
BrdU
NeuN/BrdU/GFAP



(M-R) Postnatal coronal brain sections with neurons labelled according to their date of birth

Pregnant mice were injected with BrdU at different times of gestation. Accordingly, neurons generated at E12, E14 and E16 will be BrdU positive in the corresponding p7 brain sections.

The inside-out formation of the control cortex (M, O, Q) is preserved in the normotopic cortex of the cKO (N, P, Q). The heterotopic cortex contains neurons generated at all examined stages with a clear majority of late-born neurons (Q).

scale bars: 100µm

(S-T') Postnatal coronal brain sections stained for GABAergic neurons

GAD67 mRNA that marks GABAergic neurons was revealed in p7 brain sections by means of in situ hybridisation. These interneurons represent only a minor cell population in the cortex in contrast to the basal ganglion (BG). Their density in both cortices of the cKO is comparable to the control cortex.

2.8. The formation of the "double-cortex" is not cell-autonomous

Obviously, some of the neurons have problems reaching their destination in the cortical plate and thus form the heterotopic cortex. RhoA has been implicated in migration, so the loss of it might well lead to migration defects. On the other hand, we have observed disorganisation of the radial glial scaffold that might also lead to this phenotype. Since the Emx1::Cre line already recombines at the radial glia stage, it is difficult to tell these effects apart. A loss of RhoA in the newborn neurons only, would leave the radial glial scaffold intact. This approach could thus show, if RhoA is indeed needed for neuronal migration. The Ngn2-Cre line (Berger J et al. 2004) does not recombine in radial glia, but recombines in neurons very early after they become postmitotic.

At p3, the animals were perfused and the anatomy of the early postnatal cortex was examined. We used DAPI to reveal overall anatomy, and NeuN to see the distribution of neurons. The pattern of NeuN as an indication for the, albeit not completed, neuronal layering looked very similar in the cKO compared to the control (Fig. 11A-B'). The only peculiarity that we were able to observe in the cKO brain was the white matter, which was not compact but had a loose spider-web like appearance that made cutting difficult (Fig. 11B-B', D-D').

Unluckily, it is difficult to draw conclusions from the absence of an effect, because the reasons might as well be of technical nature. In this case, we cannot be sure, that we have indeed lost RhoA in the young neurons. We could not get the RhoA staining working in postnatal sections either, so we decided to examine embryonic stages for loss of RhoA. However, the examination of E14 sections showed no reduction of (Fig. 11E-F'").

An alternative mouse line, the Nex::Cre line (Goebbels S et al. 2006) showed the same problem that no reduction in RhoA could be observed at E14 (Fig. 11G-H'").

Figure 11: Deletion of RhoA with neuronal Cre-lines

(A-B') Postnatal coronal brain sections of Ngn2-Cre RhoA cKOs immunostained for neurons

p3 coronal brain cryosections of control and Ngn2-Cre +/- | RhoA fl/fl (cKO) mice. The neuronal NeuN immunostaining does not reveal any histological difference that might be indicative of a double-cortex. DAPI was used as a nuclear counterstain.

A' and B' are more caudal sections of the same brain as A and B.

scale bars: 100µm

(C-D') Postnatal coronal brain vibratome sections of Ngn2-Cre RhoA cKOs

p3 coronal brain sections of control and Ngn2-Cre +/- | RhoA fl/fl (cKO) were prepared at the vibratome. The cKO (D, D') seems to have a white matter phenotype. Parts of the brain are not compact but consist of spaced fibres. These structures are virtually impossible to keep intact in cryosections (B, B')

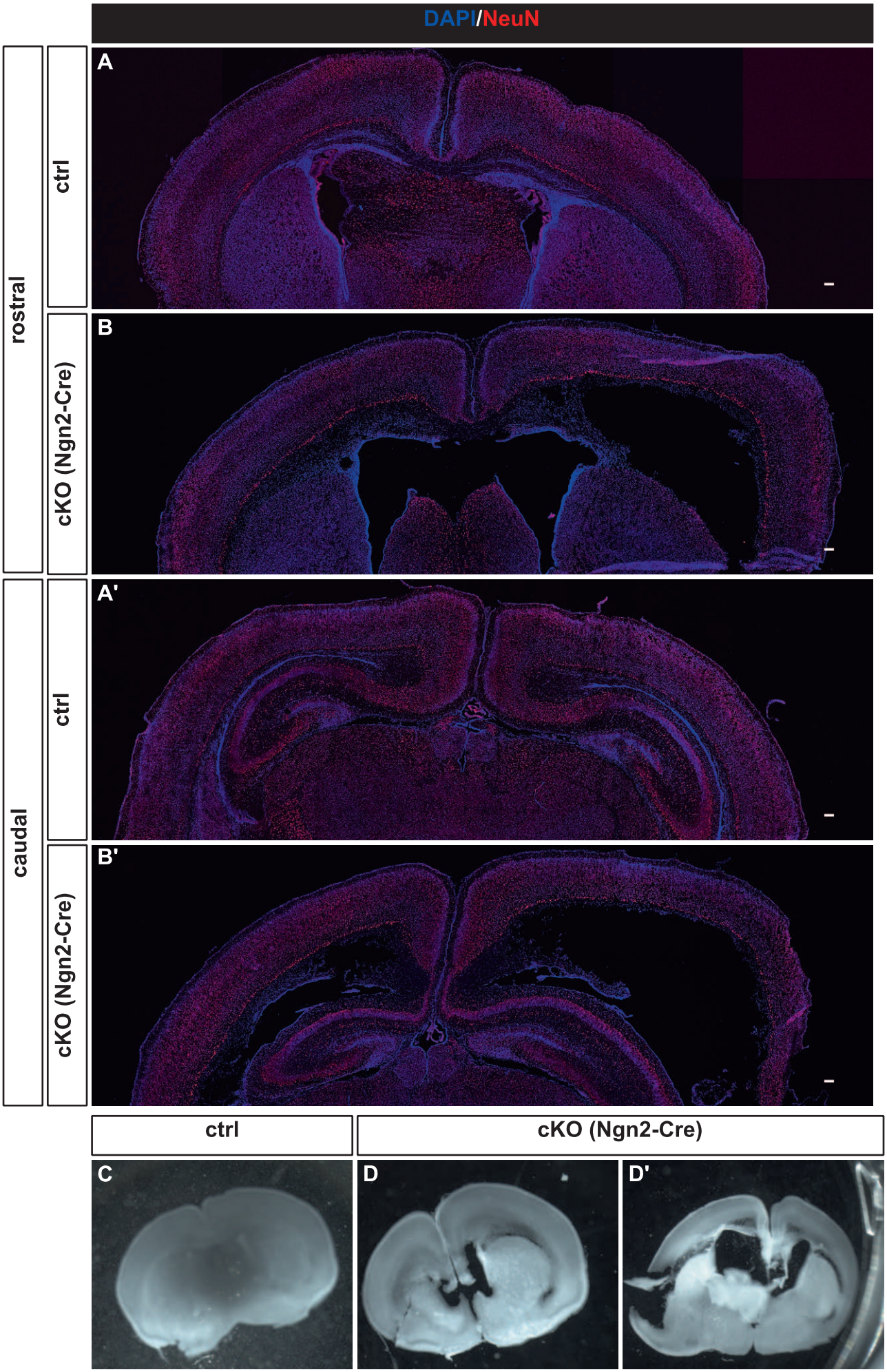
scale bars: 100µm

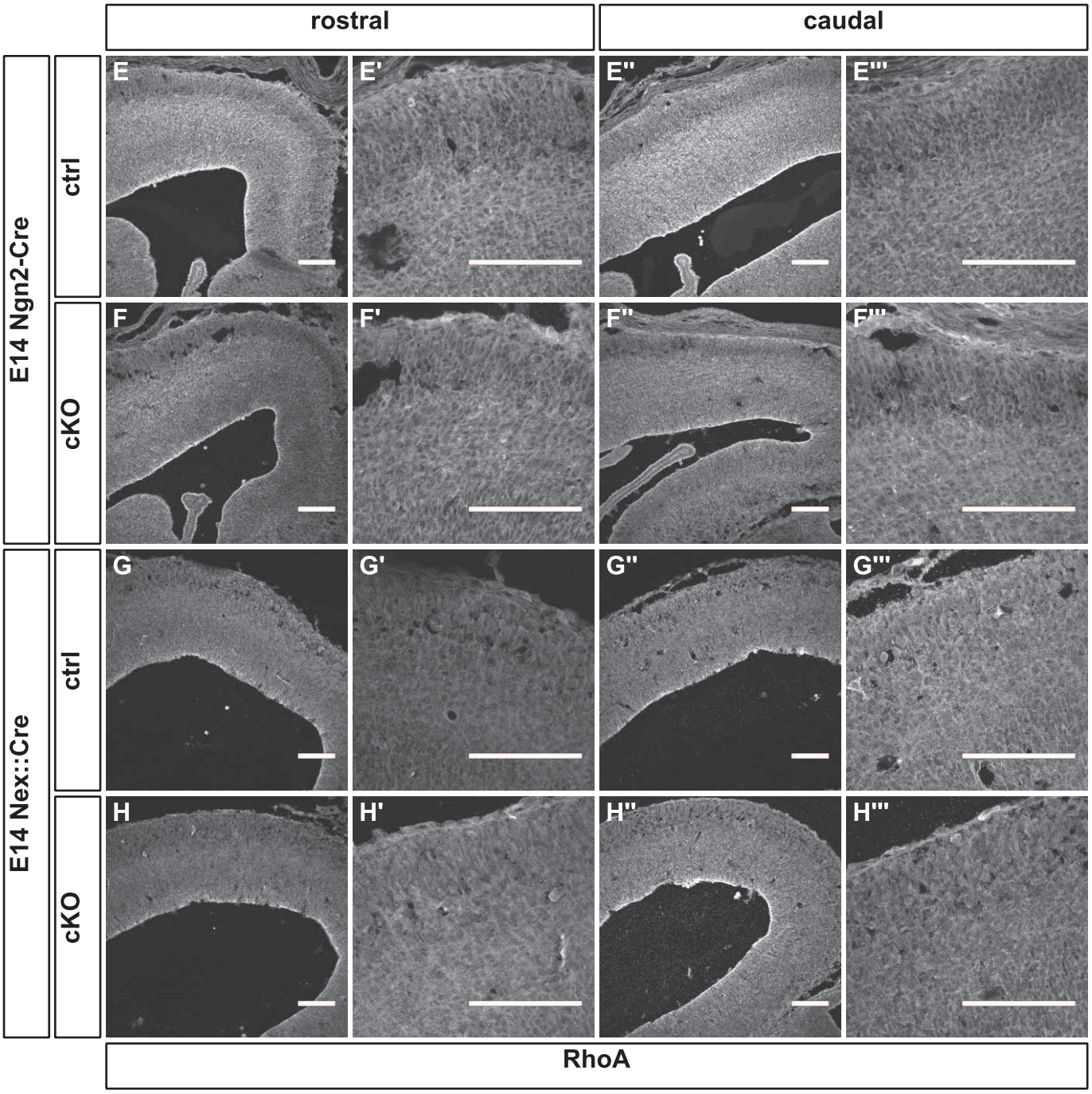
(E-H'") Embryonic sections of neuronal Cre-lines examined for loss of RhoA

The Ngn2-Cre mouse line (E-F'") and the Nex::Cre mouse line (G-H'") were used to generate control and Cre +/- | RhoA fl/fl (cKO) mice. Coronal E14 brain sections of rostral and caudal levels were immunostained for RhoA. No reduction in RhoA protein levels could be observed in the cKOs.

X' and X'" are magnifications of the cortical plate of X and X".

scale bars: 100µm


DAPI/NeuN
rostral
caudal
ctrl
cKO (Ngn2-Cre)
A
B
A'
B'
ctrl
cKO (Ngn2-Cre)
C
D
D'



So we devised another set of experiments to distinguish a migration defect of the neurons themselves from a secondary migration defect that is due to morphological changes in the radial glia. Such a secondary migration defect should not appear if only few radial glia are affected so that enough of the scaffold remains to make migration possible. In utero electroporation gives us the possibility to affect only some of the cells. In addition, the method is known to quickly achieve high levels of Cre recombinase, which enhances the chance of quick and successful recombination. So we electroporated RhoA fl/fl embryos at E14 in utero with pCIG2 expressing Cre-IRES-GFP to delete RhoA and used the same plasmid in wildtype embryos as a control. When the brains were examined three days later, at E17, for localisation of GFP positive cells, we found that the Cre-electroporated RhoA-fl/fl cells were not delayed in their migration, but that even more of them reached cortical plate, than of the electroporated cells in the control (Fig. 12A-B''''). This would rather indicate accelerated migration! In addition, the neurons gave the impression of perfectly healthy, morphologically normal neurons. As in the control, they were

mostly bipolar (Fig. 12B''-B''''). We repeated the experiment with a longer survival of 5 days, i.e. the embryos were electroporated at E14 and examined at E19, just before birth. This time, we used pCIG2 expressing IRES-GFP without Cre as control plasmid and injected it into RhoA fl/fl mice, to avoid delaying the migration in the control by potential side effects of Cre. Nevertheless, this experiment also showed no reduction in Cre-electroporated RhoA-fl/fl cells reaching the cortical plate (Fig. 12C-D'''). These results strongly speak for the scaffold hypothesis, where the neurons in the cKO are not themselves unable to migrate but are rather lacking an intact radial glial scaffold to enable their migration.

One surprising observation in the 5-day-experiment were groups of neurons which would not stop at the basal boundary of the cortex but migrate further, creating mushroom shaped ectopias (Fig. 12E-E'') that very much reminded us of the ectopias in layer I that we saw in the adult cKOs. Apparently this aspect of the adult phenotype does indeed come from the loss of RhoA in the neurons themselves.

Figure 12: Cell-autonomous effects of RhoA-deletion by in-utero electroporation

A-B'''' In utero electroporated embryos after 3 days

E14 embryos were electroporated with a Cre-IRES-GFP construct and examined at E17. Wildtype (WT) embryos were used as control (A-A'''') and RhoA fl/fl embryos to examine the loss of RhoA (B-B''''). A and B and their magnifications A' and B' show comparable radial distributions of the electroporated cells in control and experiment. The higher magnifications of different cortical regions of the experiment (B''-B'''') show morphologically normal neurons with mostly bipolar morphology. The cortical plate (B'') does not show less, but rather more incoming neurons than the wildtype cortical plate (A'').

scale bars: 100µm

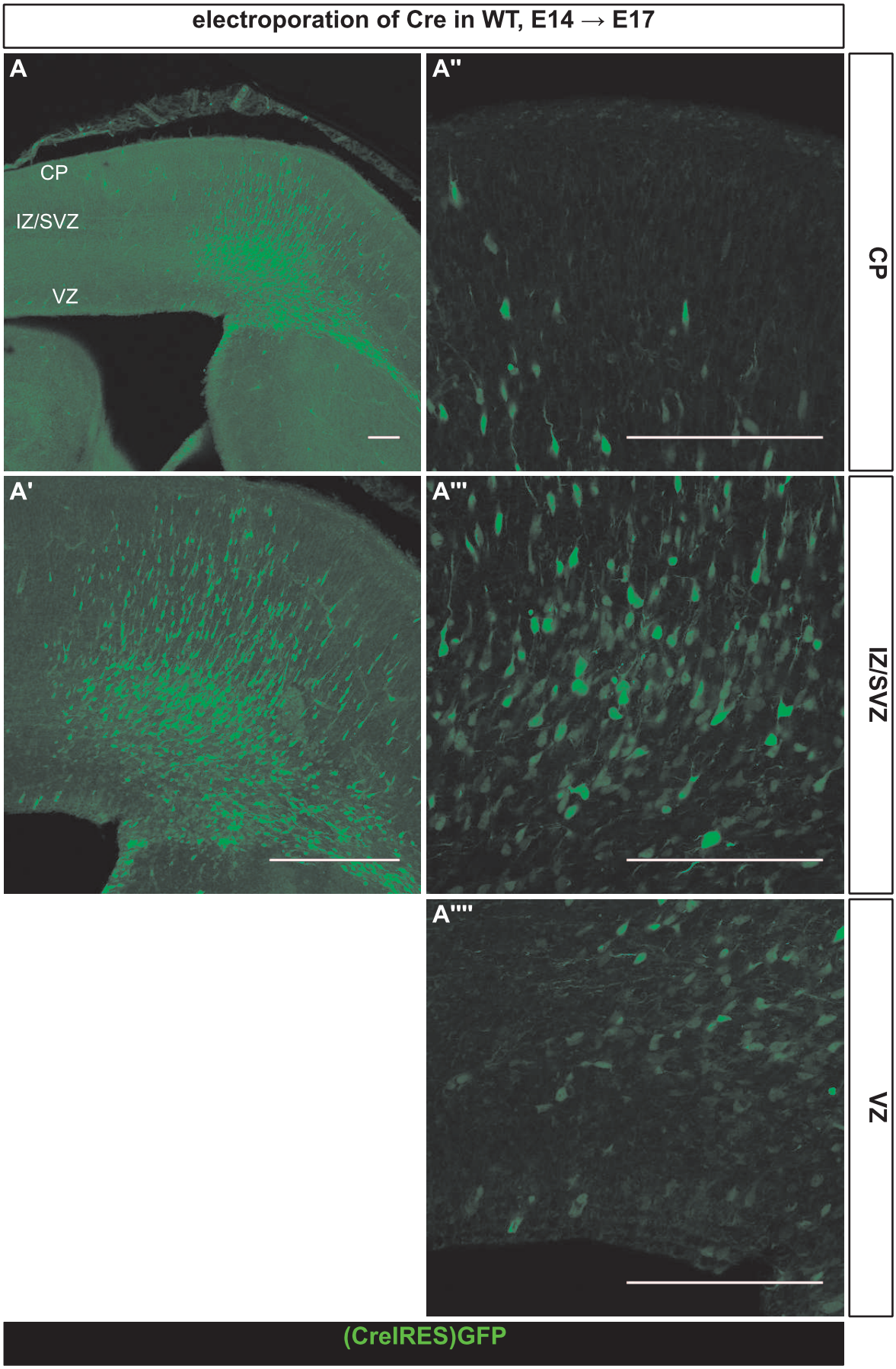
C-E'' In utero electroporated embryos after 5 days

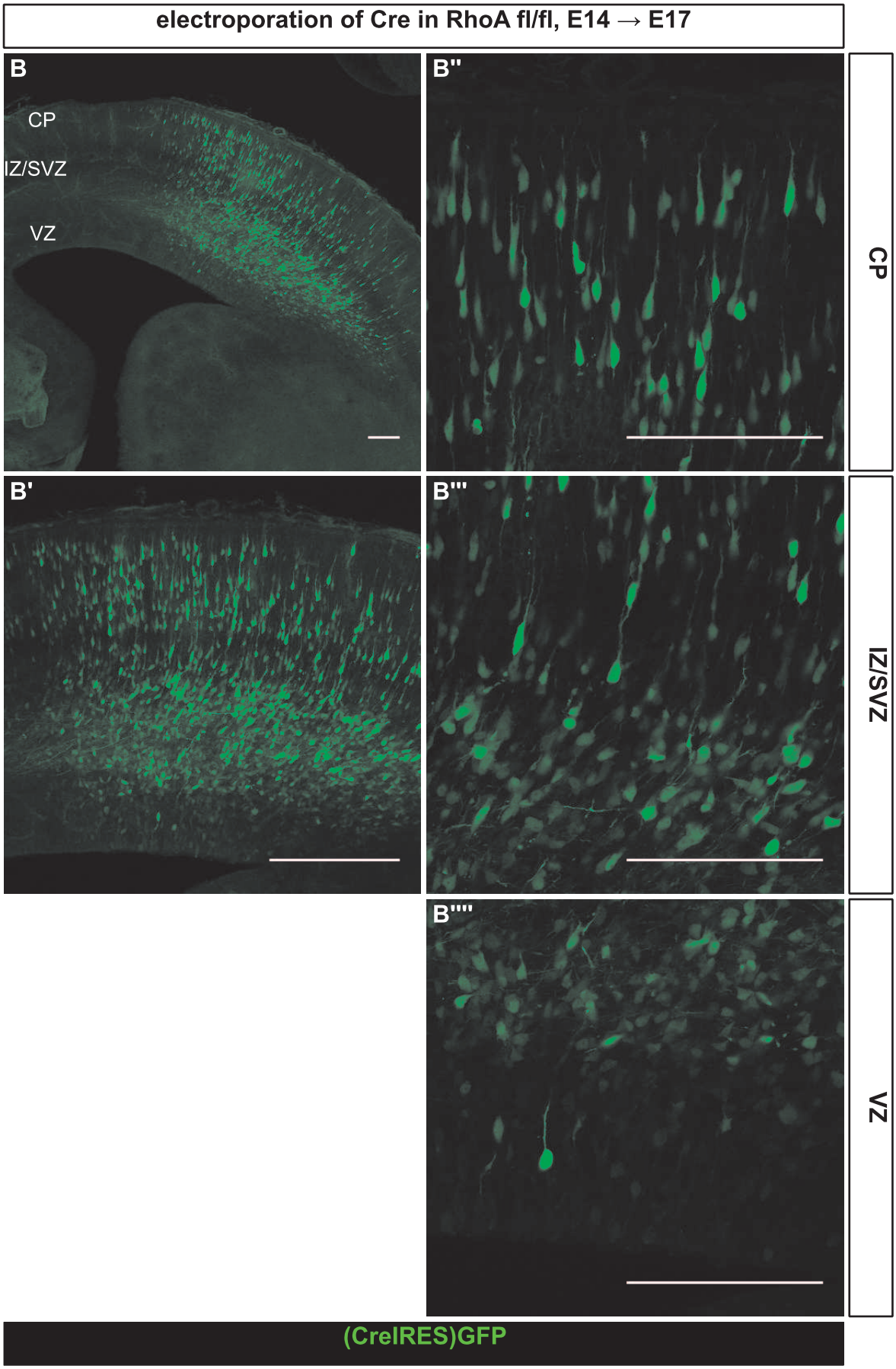
E14 RhoA fl/fl embryos were electroporated with a Cre-IRES-GFP construct (D-E'') or with an IRES-GFP construct as control (C-C'''') and examined just before birth at E19. C and D and their magnifications C' and D' show again comparable radial distributions of the electroporated cells. Higher magnifications of the cortical plate (C''-C''' and D''-D''') show morphologically normal neurons that are not reduced in number in the experiment (D''-D''').

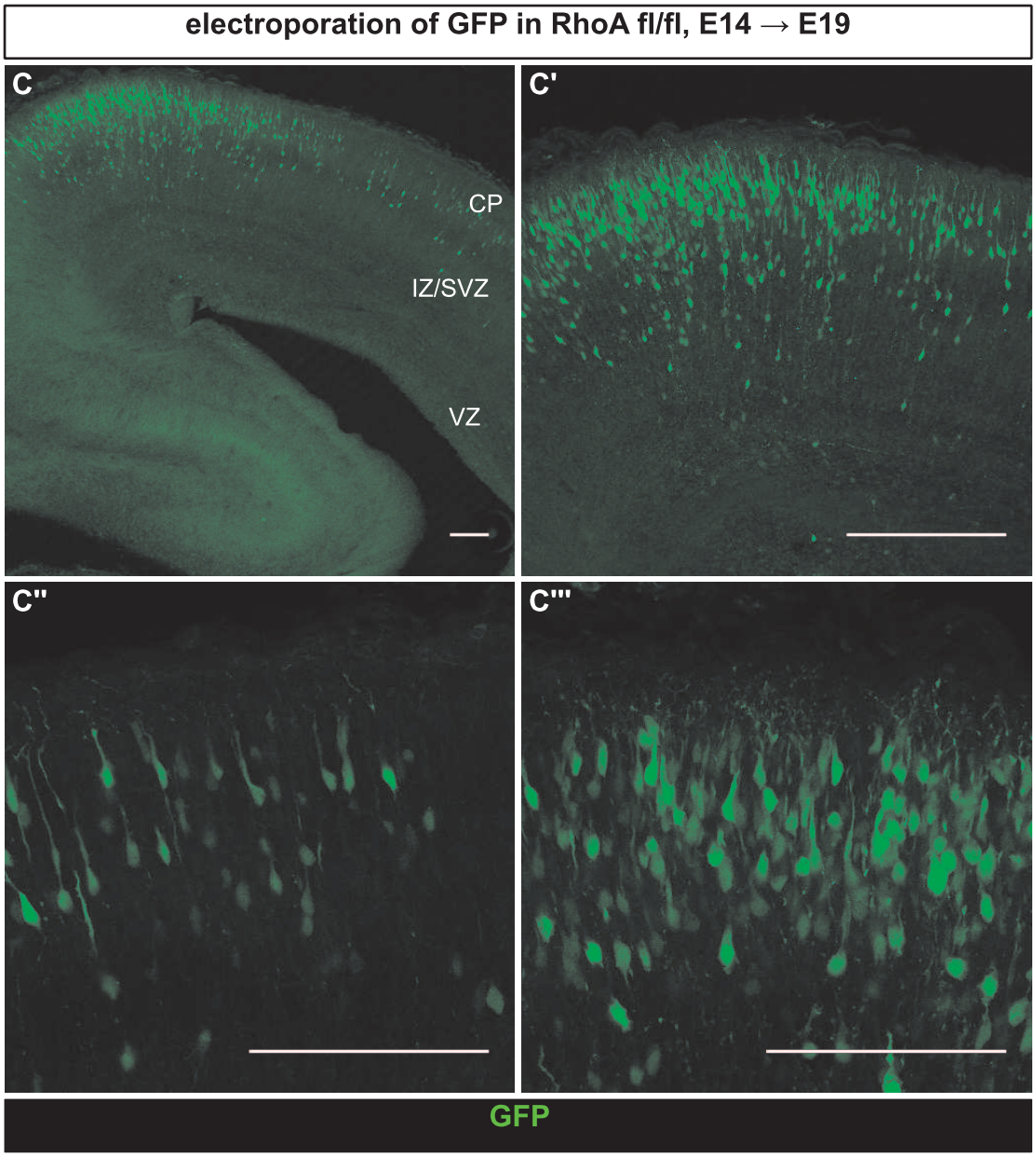
E and its magnifications E'-E'' show RhoA-negative neurons that do not respect the boundaries of the cortex and produce a mushroom shaped ectopia. The neurons themselves are otherwise morphologically normal.

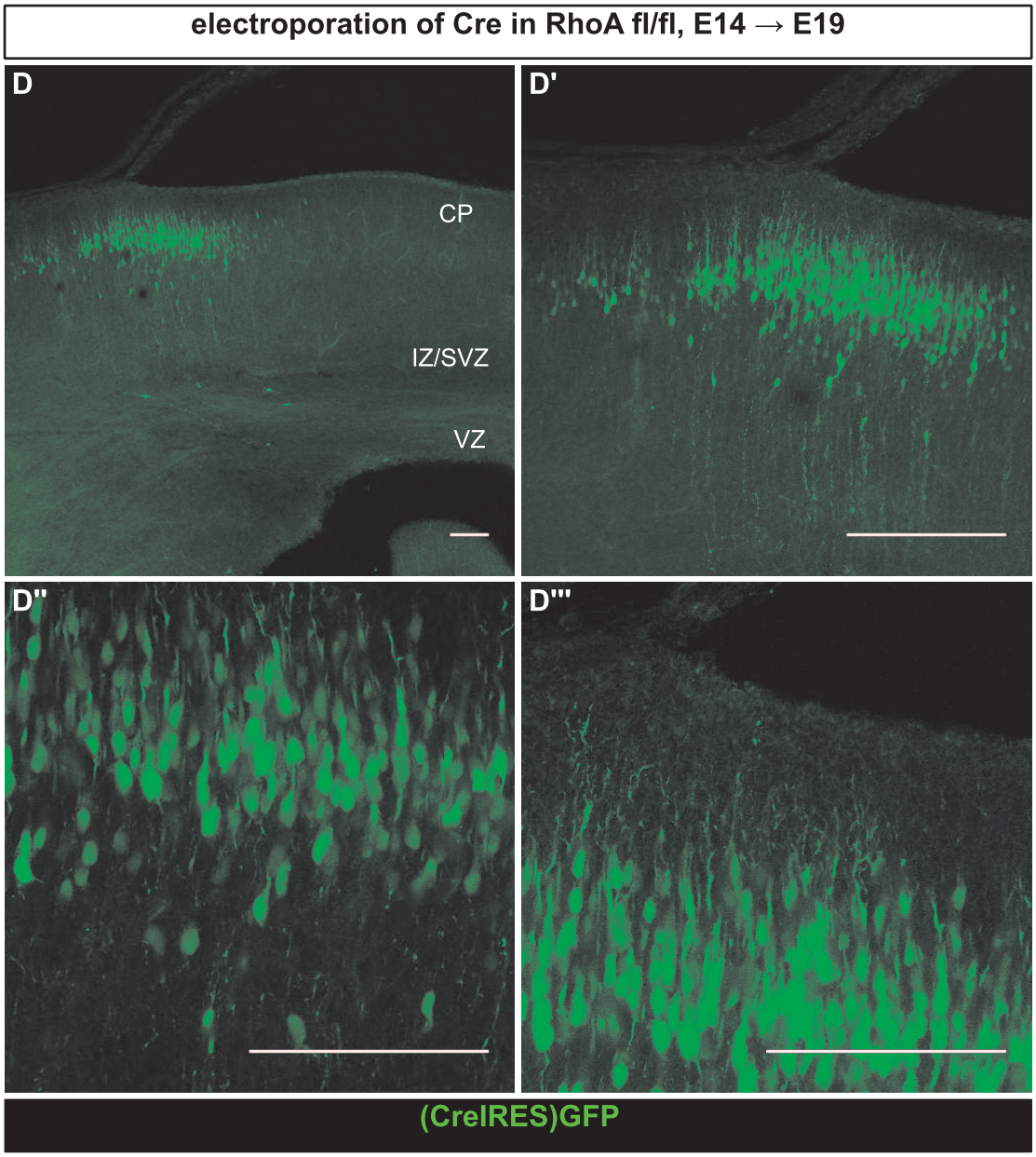
CP: cortical plate, IZ: intermediate zone, SVZ subventricular zone, VZ: ventriculare zone

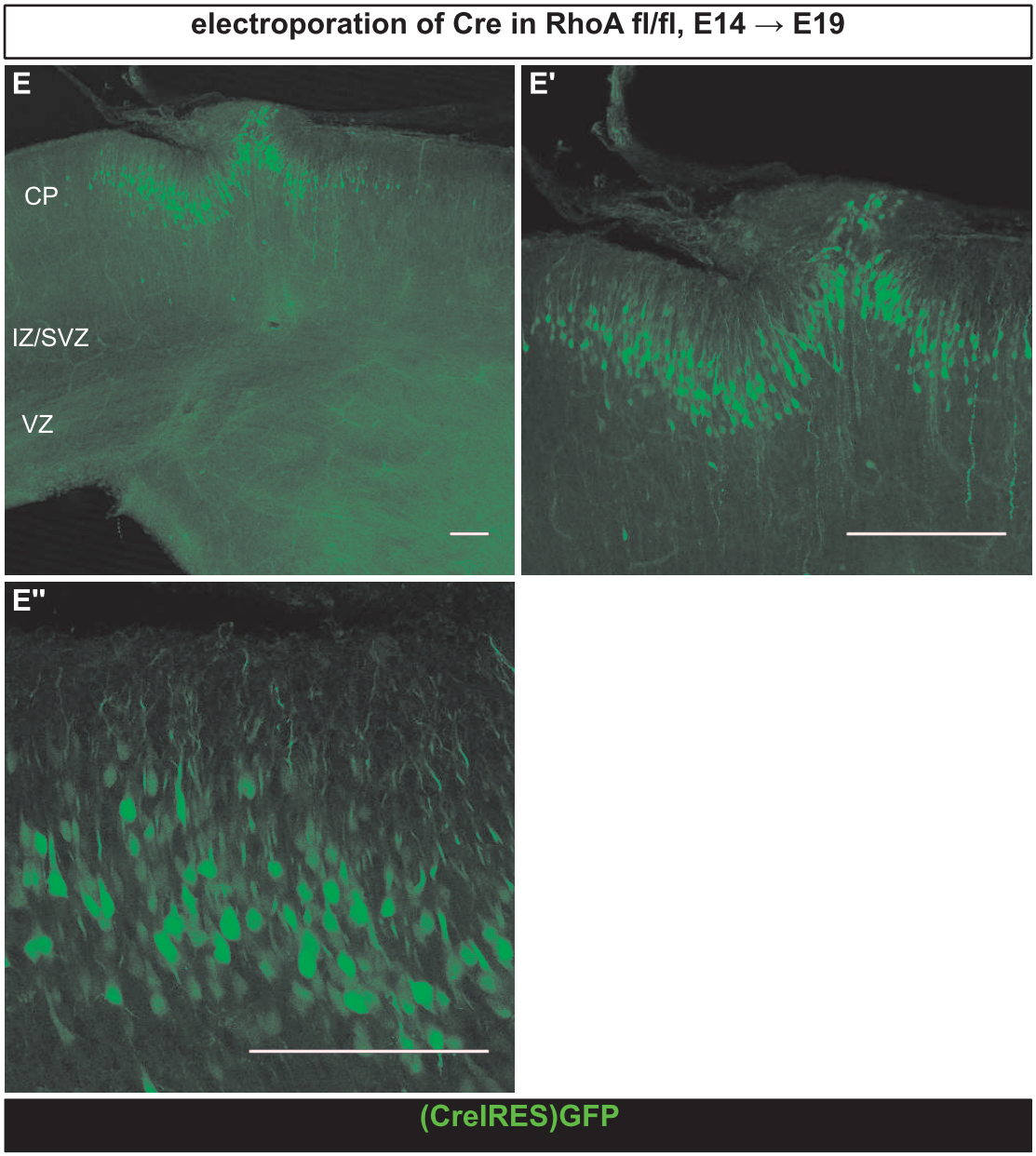
scale bars: 100µm


electroporation of Cre in WT, E14 → E17
A
CP
IZ/SVZ
VZ
A''
A'
A'''
A''''
CP
IZ/SVZ
VZ
(CreIRES)GFP


electroporation of Cre in RhoA fl/fl, E14 → E17
B
CP
IZ/SVZ
VZ
B''
B'
B'''
B''''
CP
IZ/SVZ
VZ
(CreIRES)GFP


electroporation of GFP in RhoA fl/fl, E14 → E19
C
CP
IZ/SVZ
VZ
C'
C''
C'''
GFP


electroporation of Cre in RhoA fl/fl, E14 → E19
D
CP
IZ/SVZ
VZ
D'
D''
D'''
(CreIRES)GFP



2.9. The localisation of Reelin-positive cells is unchanged

We set out to further investigate the cellular mechanisms of the subcortical band heterotopia phenotype. Reelin does not only play an almost historical role in he regulation of migration, which still is far from being elucidated, but there are also crosslinks with RhoA signalling (Bernard O 2006, Chai X et al. 2009). For this reason, we examined its localisation in embryonic cortices to check for disruption of the Reelin positive Cajal-Retzius cell layer or ectopic expression of Reelin at apical locations. Briefly, neither could be observed at the stages examined (Fig. 13).

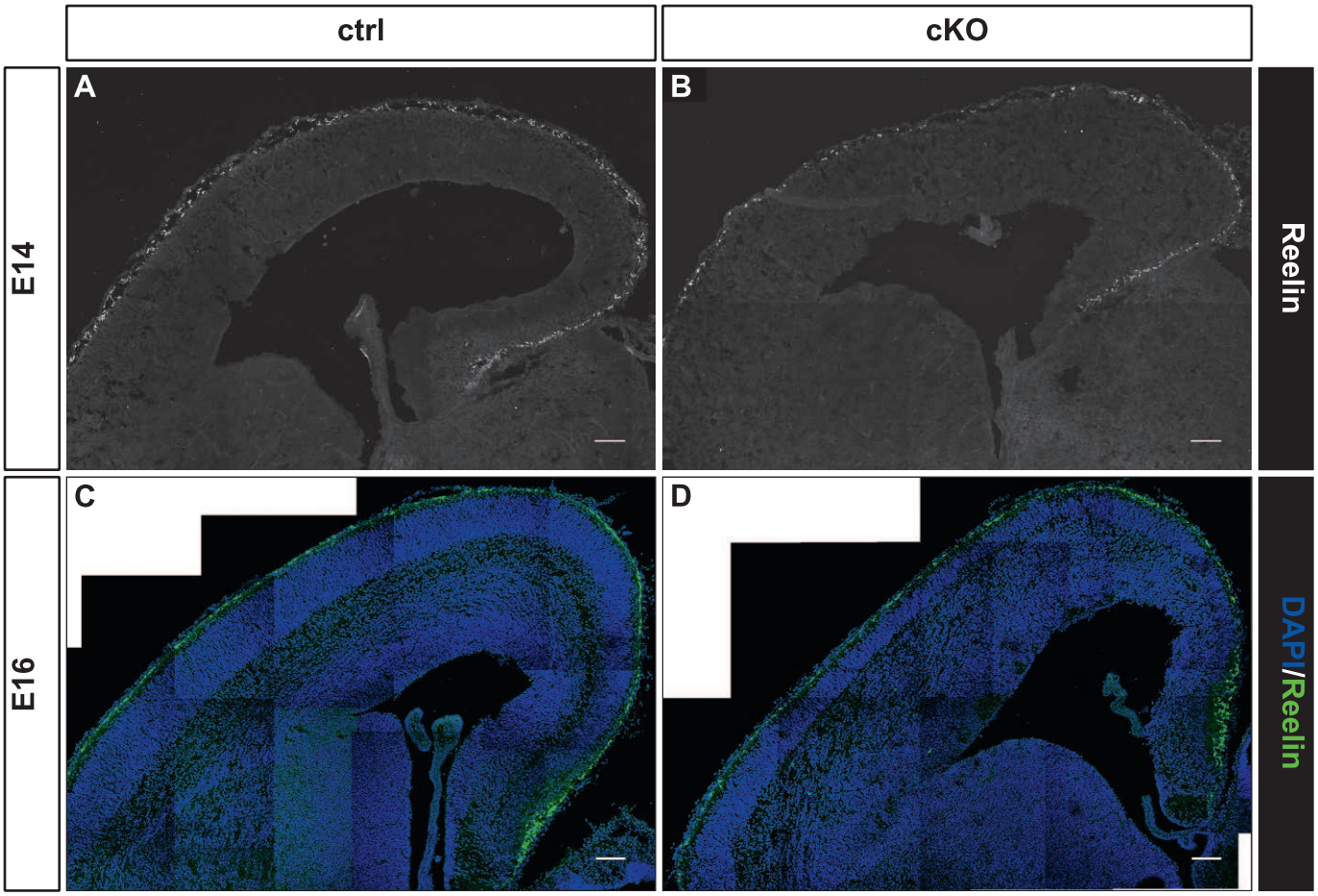


Figure 13: Reelin expression

A-D Embryonic coronal brain sections immunostained for Reelin

E12 (A-B) and E14 (C-D) sections of control and cKO (Emx1::Cre) mice were immunostained for Reelin. At both stages, the cKO (B, D) does not show an altered expression of Reelin, especially no ectopic apical expression.

2.10. F-actin levels are reduced in RhoA-deficient progenitors

As RhoA has been shown to affect the actin cytoskeleton (Ridley AJ and Hall A 1992), we examined actin fibres. They are dynamically regulated and are undergoing constant polymerization and depolymerization. In addition, the actin monomers play an important role in gene expression, including genes that further regulate the cytoskeleton (see introduction). Thus, the pools of monomeric G-actin and F-actin fibres have to remain in constant balance. To examine these pools at the cellular level, we prepared primary cultures of dissociated E14 cortices of control and cKO mice. The cells were kept in culture for differentiation for either one day (1 div) or six days (6 div). The cells were then stained for G- and F-actin. Whereas changes in G-actin levels could not be observed, F-actin was clearly decreased after 1 div (Fig. 14A-D''). Interestingly, the difference in F-actin levels was no longer visible after 6 div (Fig. 14E-H'').

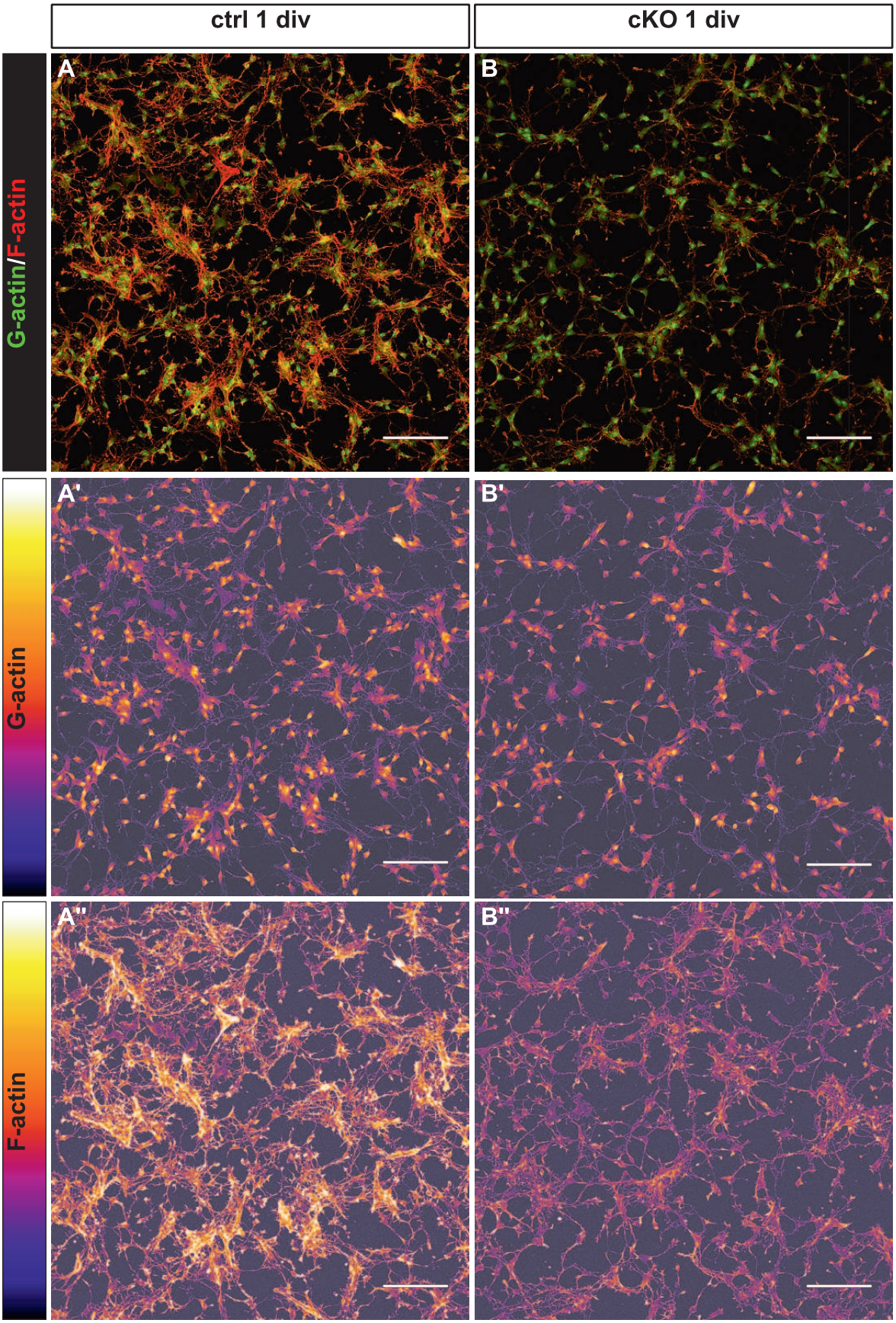
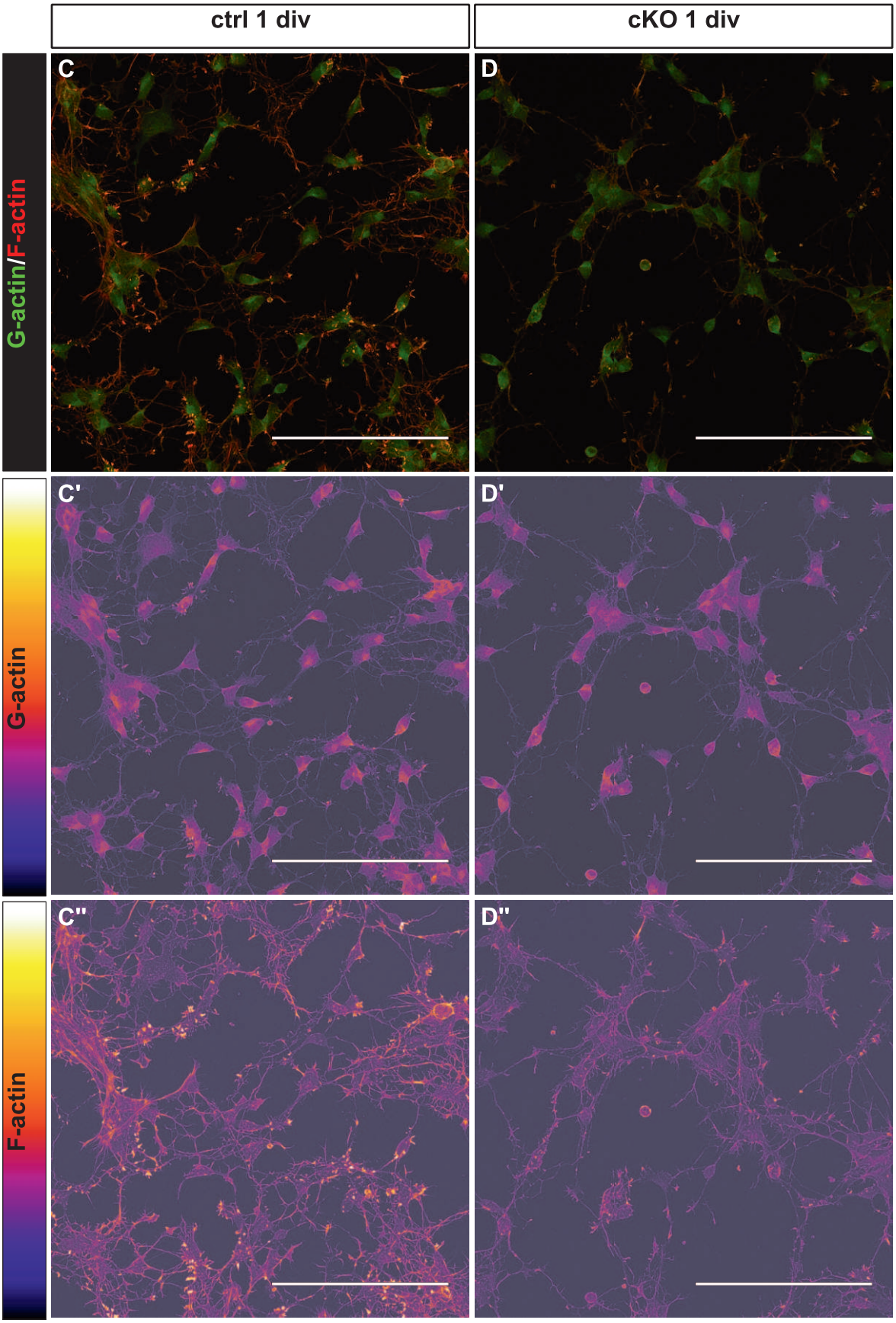


Figure 14: Changes in the G- to F-actin balance

(A-D") Monomeric actin and actin fibres in primary cortical cell cultures after 1div

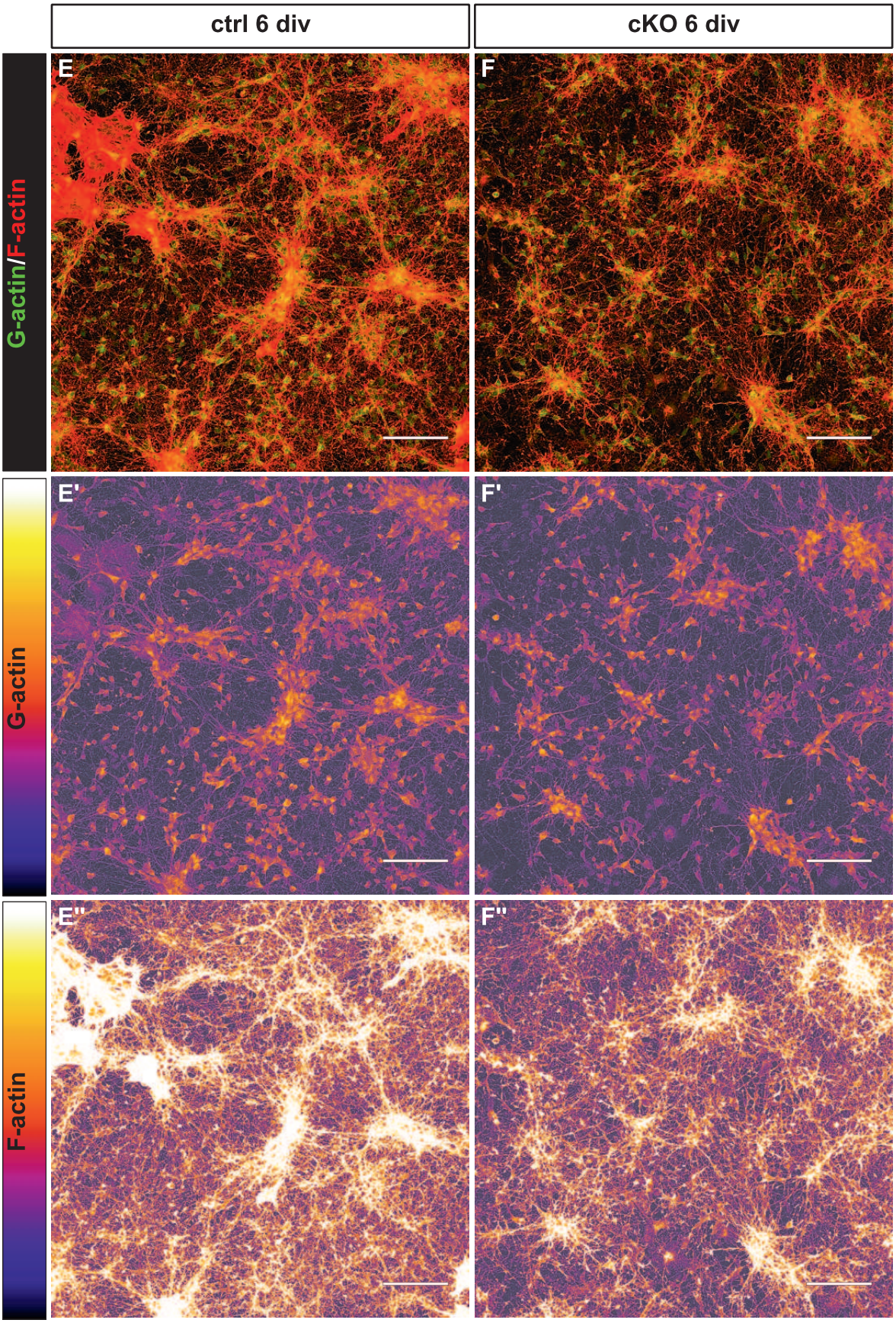
Primary cortical cell cultures were prepared of the cortices of E14 control (A-A", C-C") and cKO mice (B-B", D-D") and kept in vitro for 1 day. They were stained for monomeric actin (G-actin) with Alexa 488-labeled DNAse I and stained for actin fibres (F-actin) with Texas Red-labelled phalloidin. The single channels in X'



and X'' are coloured with the colour scheme displayed on the labels that shows the lowest intensity black-blue and the highest intensity yellow-white. The cKO clearly shows a decreased intensity in F-actin staining (B, B'', D, D'').

C-D'' are magnifications of A-B''.

scale bars: 100µm

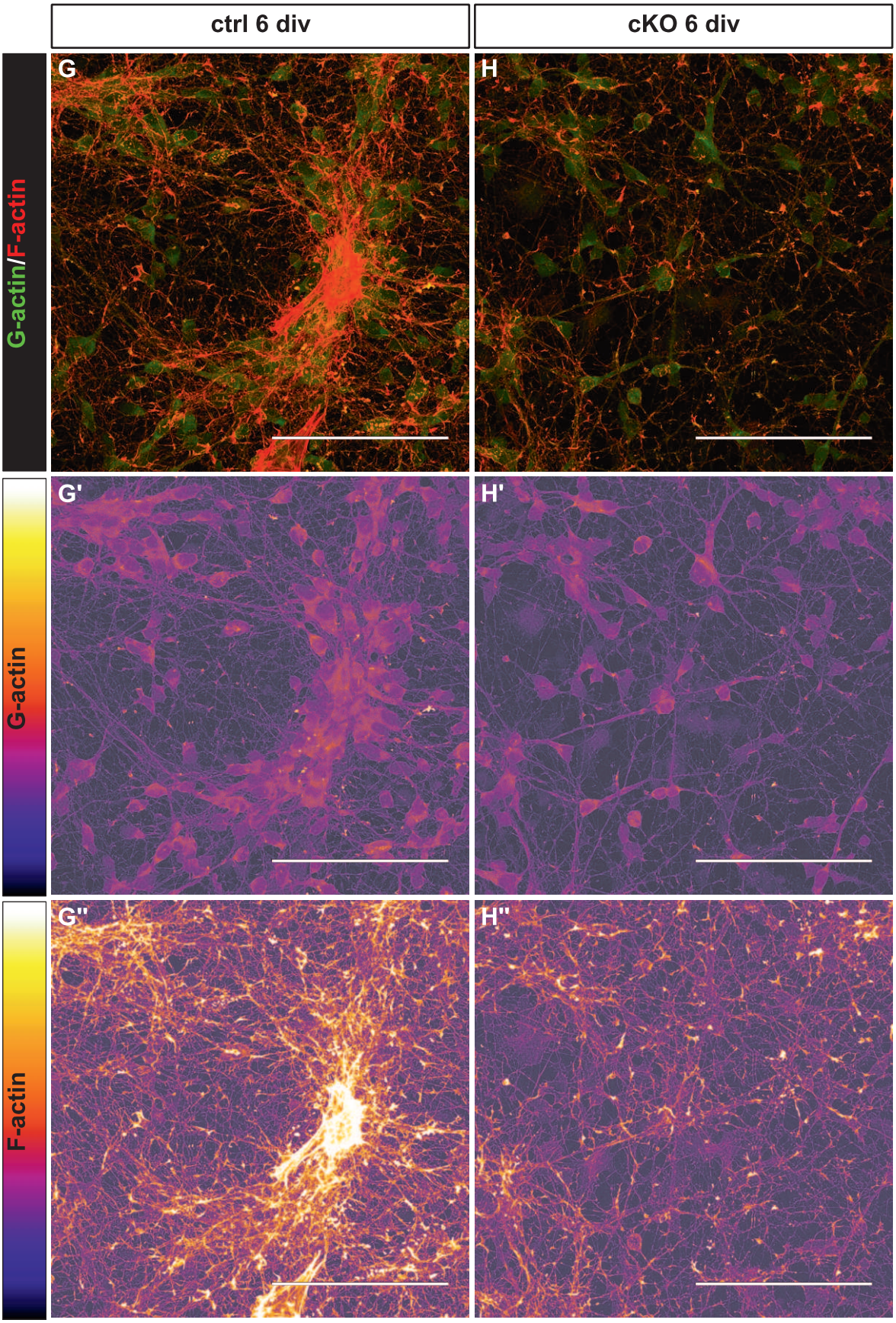


(E-H'') Monomeric actin and actin fibres in primary cortical cell cultures after 6div

Primary cultures identical to the ones used in A-D'' were kept in vitro for differentiation for 6 days and the stained for G- and F-actin as before. The decrease in F-actin is no longer visible (F, F'', H, H'').

G-H'' are magnifications of E-F''.

scale bars: 100µm


ctrl 6 div
cKO 6 div
G-actin/F-actin
G
H
G-actin
G'
H'
F-actin
G''
H''


DISCUSSION
RhoA
model
cortex
cho
junctions
Cappello
subcortical
migration
defects
neurons
cortical
heterotopia
expression
radial
formation
actin
levels
protein
cells
layer
addition
mutant
observed
results
SRF
effect
reduced

3. Discussion

3.1. Summary

Here, we used a cKO approach to assess the role of RhoA in the developing central nervous system in a time and tissue specific manner. Recombination in the brain was restricted to the neural tissue of the dorsal telencephalon, resulting in loss of RhoA protein at E12, an early stage of neurogenesis.

We discovered transient hyperproliferation that reaches its peak at midneurogenesis (E14), and leads to 1,3x increase in adult cortex size.

The hyperproliferation is accompanied by a scattering of progenitor cells throughout the cerebral cortex. This is probably due to a disruption of adherens junctions, which is the first effect we could observe after loss of the protein. This disruption also leads to severe disturbances of the radial glial scaffold, which loses its radial orientation.

In adult animals, we could observe the formation of a characteristic subcortical band heterotopia with a correctly layered, albeit thinner, normotopic cortex and a heterotopic cortex that is embedded inside the white matter. Strikingly, both the normotopic and the heterotopic cortex contain neurons of all layers, even though the late-born upper layer neurons form a clear majority in the heterotopic cortex. In addition, we observed type II cobblestone ectopias protruding into layer I of the cKO.

We used in utero electroporations, which allowed us to delete RhoA in individual cells while leaving the scaffold mainly intact. In the course of those experiments, we found strong evidence that the formation of the heterotopia is not due to a cell-intrinsic incapacity of neurons to migrate. Our results rather favour the hypothesis, that the heterotopia is formed as a consequence of a partially disrupted radial glial scaffold, which can no longer serve all locomoting neurons as a substrate to migrate into the cortical plate.

On a cellular level, we saw a severe lack of F-actin in primary cells made from cKO cerebral cortex, which fits well to RhoA activity leading to actin polymerization.

3.2. Suitability of the RhoA fl mouse line

As only the RhoA gene had been flanked by loxP sites in the first place, no other genes could be affected. This is a strong advantage, especially compared to dominant negative or constitutively active constructs, which by their very nature are prone to interact with similar signalling pathways. Unluckily, those have been widely used to study the role of Rho GTPases so far. Even RNA interference techniques can lead to off-target effects and, in high levels, their exogenous presence alone can have unwanted effects on the cells. So far, there have been few reports on side-effects of Cre (Forni PE et al. 2006), but comparing the heterozygous cKO, retaining only one allele of RhoA, and Cre negative control animals, we never noticed any differences, even on the electron microscopic level.

The recombination is as previously shown (Iwasato T et al. 2000, Cappello S et al. 2006, Schmid M-T 2008), and by E12 RhoA protein has disappeared in all neural tissues of the dorsal telencephalon. Corresponding to the Emx1::Cre expression, we see remaining protein in the ganglionic eminence, blood vessels, the meninges and the choroid plexus. This also confirms the specificity of the antibody used for this study. The loss of RhoA, however, takes place later

than expected. Emx1::Cre expression usually starts around E9,5 (Iwasato T et al. 2004), and another Rho GTPase, Cdc42, disappeared already shortly afterwards (Cappello S et al. 2006). This could be explained by a higher protein stability of RhoA. We were also unable to delete RhoA by using different mouse lines with neuron-specific expression of Cre. This also indicates a high stability of the protein. In proliferating cells, such as radial glia, the protein already present before the deletion of its gene is not only degraded but also diluted with each division of the cell. This is possible in the Emx1::Cre mouse line, which recombines in mitotically active radial glia, but not in mouse lines that express Cre recombinase in postmitotic cells.
We discovered a rather ubiquitous expression pattern of RhoA, which differed from prior studies that reported it to be expressed strongest in the ventricular zone of E12 and E14 embryos (Ge W et al. 2006, Pinto L et al. 2008). These studies had however been carried out by means of in situ hybridisation, which sometimes gives a poor prognosis of the resulting protein levels. Even a low mRNA level, that might be under the detection threshold of an in situ hybridisation, can lead to protein expression. Taking again into account the presumably high protein stability, it can indeed be enough to maintain the protein in neurons.

3.3. Proliferation

At mid-neurogenesis, two days after loss of RhoA protein, we saw a clear hyperproliferation effect. It lasted until E16, but whereas at E14 the proliferation was most prominent at caudal levels, at E16 it was most prominent at rostral levels and had returned almost to normal caudally. Thus, the effect seems to be transient (at least at caudal levels) and follows the caudal to rostral Emx1 expression gradient.
Interestingly, the opposite effect has been found in the spinal cord, where increased apoptosis and cell cycle exit lead to reduced cell numbers (Herzog D et al. 2011), highlighting the region-specific role of RhoA in the development of the nervous system.
Since RhoA has been implicated in cell cycle regulation via various pathways (Bustelo XR et al. 2007), there are a number of ways how the overproliferation phenotype observed in the cKO cortex could be explained. A well known way in which RhoA regulates gene transcription, is the SRF pathway. SRF is a sensor for the balance between monomeric G-actin and polymerized F-actin. Its Co-activator MAL binds to G-actin and can not translocate into the nucleus in this form. Only the unbound form can get into the nucleus and bind to SRF, thus initiating the transcription of SRF target genes (Vartiainen MK et al. 2007, Connelly JT et al. 2010). RhoA activation generally leads to actin polymerization, which means a reduction of G-actin and increased SRF activity. As expected, we observed reduced F-actin formation in cKO cells, which should lead to a reduction of SRF signalling. Consistent with the model, S. Cappello in our lab found a 30% increase in G-actin levels in the cKO cortex. Furthermore, she also detected increased MAL levels in cytoplasmic fractions of cKO cortex tissue (Cappello S et al. 2011). Taken together, the reduced formation of F-actin does lead to an increased G-actin pool, which keeps more MAL outside the nucleus than is the case in the wildtype. SRF activity has already been shown to promote differentiation in epidermal stem cells whereas its inhibition keeps them in a proliferative state (Connelly JT et al. 2010). Also in certain cancer cells, inhibition of SRF is amongst the

mechanisms that lead to aberrant proliferation (Yoshio T et al.). As we found this pathway in RhoA mutant cells, it is a likely cause of the hyperproliferation.
In addition, in the developing midbrain, where proliferation is also increased upon loss of RhoA, an increased expression of target genes of the hedgehog pathway has been found (Katayama K et al. 2011).

3.4. Adherens junctions

Even though actin has an important function in regulating gene expression, its role as a part of the cytoskeleton is more obvious. As such, it also takes part in the stabilization of cell adhesions and is actually necessary for the active formation of adherens junctions in different epithelia (Vasioukhin V and Fuchs E 2001). We could directly visualize the network formed by actin fibres at the ventricular surface, and we could show the loss thereof in the cKO. The reduction of F-actin is a possible cause for the destabilization and later disruption of this network. As we did not directly delete adherens junction proteins, such as catenins and cadherins, the passive formation of adherens junctions, or the maintenance of existing ones, can be affected to a lesser extent than in adherens junction mutants. This way, the adherens junctions at the ventricular surface, that are under tension due to the pressure from the cerebrospinal fluid, can disrupt, but once the resulting stripes of connected tissue have clustered to rosettes inside the parenchyma, they are less stressed and can remain there for longer periods of time. A similar phenomenon has already been observed when cell-cell junctions in the chick spinal chord have been disrupted by means of a cadherin blocking antibody (Ganzler-Odenthal SI and Redies C 1998). The resulting scattering of progenitor cells has also been observed in other mutants with adherens junction defects (Machon O et al. 2003, Cappello S et al. 2006, Lien WH et al. 2006).
These results correspond to findings for the deletion of RhoA in the developing midbrain and spinal cord (Herzog D et al. 2011, Katayama K et al. 2011). Both mutants also show loss of apical adherens junctions and scattering of progenitor cells with the formation of rosettes. In addition, in the spinal cord it was shown, that the formin mDia1, which regulates actin polymerisation downstream of RhoA, is no longer localised at the apical adherens junctions, after loss of RhoA. Further experiments by the same group also showed, that expression of a dominant negative form of mDia1 could reproduce the loss of apical adherens junctions (Herzog D et al. 2011).

3.5. The "double-cortex"

Considering the initial scattering of progenitor cells, the resulting adult cortex is surprisingly well organized. Especially the normotopic cortex shows an intact organization in the typical six layers. Also non-neuronal cells are correctly localized. This is different to other Rho GTPase mutants, such as Cdc42, that show strong astrogliosis (Cappello S et al. 2006, Robel S et al. 2009).
In addition, the GABAergic neurons generated in the GE still arrive in both cortices, so some guidance cues must remain intact.
Even more intriguing than the molecular organization of the heterotopia is its function. For one thing, all layer markers are expressed in the heterotopic cortex, so it is interesting to know, if this part also receives sensory input, and if it is able to respond adequately. In addition, we have

seen that especially the normotopic cortex maintains a high degree of organization, comparable to wildtype cortices, but that does not necessarily mean that it is also functional. We chose the primary visual cortex as a model system and collaborated with M. Caleo to investigate this point (Cappello S et al. 2011). His experiments showed, that visual stimuli could still induce the expression of the immediate early genes (IEG) c-Fos and Egr-1 in a region specific manner in the somatosensory areals of both the normotopic and the heterotopic cortex, albeit at a much lower level than in control animals. This means, that also functional arealization still exists in the cKO.

It should be kept in mind, that the SRF pathway which seems inhibited in the cKO is upstream of IEG expression and both c-Fos and Egr-1 are known SRF targets (Herschman HR 1991). To investigate the response to visual stimuli further, visual evoked potentials (VEPs) were recorded in the cKO cortex. When the visual stimuli consisted of square wave gratings, there was hardly any response at all. However, there were responses to flashes of light, but those were greatly reduced in amplitude and appeared with increased latency, compared to control animals. So although there is a slight response to sensory stimuli in the correct areas of the cKO and even in the corresponding heterotopic cortex, the functional responsiveness of V1 is greatly reduced in mutant mice.

Defects in the innervation of the cortex might be a possible explanation for the reduced responsiveness. However, vGlut2, which is selectively expressed in geniculocortical synapses (Coleman JE et al. 2010) could be labelled in a stripe of layer IV. In addition, it was possible to retrogradely label geniculocortical cells in the thalamus by intracortical injections of FluoroGold, so thalamocortical connectivity was still intact. To exclude any anatomical defects in retino-geniculo-cortical projections at all, intraocular injections of the cholera toxin β-subunit were used to anterogradely label the projections from the retina into the geniculate. As all of these experiments confirmed the intact innervation, the reduced responses have to be due to the altered intracortical connectivity.

In fact, abnormal Rho signalling has been found to be a frequent cause for mental retardation, because the coordination of the actin cytoskeleton is essential for the formation dendrites, spines and synapses (Ramakers GJ 2002).

3.6. Hypothesis

Our results so far indicate that the formation of the subcortical band heterotopia is a consequence of the disorganized scaffold, rather than due to the inability of the neurons to migrate. A potential problem of the electroporation experiments is the high stability that RhoA seems to have. If RhoA would be essential for the initiation of migration, we might have missed this effect if RhoA protein disappeared too late. On the other hand, we do not only electroporate the Cre-construct into neurons, but also into progenitor cells. These cells can still divide before they produce RhoA-deficient neurons, so that in those neurons the protein should be sufficiently diluted. If these neurons were to have a migration defect, they would form a second population in the apical regions. However, we could not see a second population remaining behind, rather all electroporated cells migrated as one group, like in the control experiments.

To exclude any doubt left, S. Cappello performed a beautiful set of transplantation experiments (Cappello S et al. 2011). First, RhoA-deficient neurons from E14 cKO cortices were dissociated, labelled and transplanted in-utero into the ventricle of E14 wildtype embryos. These neurons had already lost RhoA protein by E12 but reached the cortical plate just as well as neurons from wildtype donor animals. This excludes any inability to migrate. Finally, to prove that the scaffold alone can in fact cause subcortical band heterotopia, cortical neurons from E14 wildtype donors were transplanted into E14 cKO hosts. At E17, the majority of the transplanted cells either reached the cortical plate, or virtually all cells remained in apical locations. This finding is consistent with the theory that depending on the environment a neuron is located, it can either reach the cortical plate by translocating along intact radial glial fibres, or remains at its initial location if the radial processes are misoriented. If the host embryos were examined two days after birth, a distinction between heterotopic and normotopic cortex could already be made and 85% of the transplanted cells remained in the lower cortex.

As an additional obstacle, these neurons first had to integrate into the cortex. In addition, they start their migration even more apically than the endogenous neurons of the host embryos, which are born inside rosettes, further basally. Taking this into consideration, we have two populations that form either the normotopic or the heterotopic cortex, even though all of them are wildtype neurons. This observation clearly proves that it is possible to generate subcortical band heterotopia with a normally layered normotopic cortex by only interfering with the radial glial scaffold.

Since RhoA controls the actin polymerization, which is necessary for migration (Rivas RJ and Hatten ME 1995), one might expect that the deletion of RhoA would interfere with cell migration. Especially the myosin II-regulated acto-myosin contractility, which is downstream of RhoA, has been shown to be required for nucleokinesis (Hatten ME 2002, Schaar BT and McConnell SK 2005), and in fact macrophages (Allen WE et al. 1997, Allen WE et al. 1998), but also precerebellar neurons (Causeret F et al. 2004), can still extend processes if RhoA is inhibited, but the nucleus is unable to follow. RhoA also stabilizes cell junctions, which counteract migration if they are too strong (Couchman JR and Rees DA 1979, Nobes CD and Hall A 1995, Lauffenburger DA and Horwitz AF 1996, Kaibuchi K et al. 1999, Vial E et al. 2003), and during the last years, several groups observed that RhoA actually inhibits migration in cortical neurons (Kholmanskikh SS et al. 2003, Besson A et al. 2004, Hand R et al. 2005, Ge W et al. 2006, Nguyen L et al. 2006, Pacary E et al. 2011).

S. Cappello saw that the decrease in F-actin was less severe in neurons (Cappello S et al. 2011) which could explain the lack of difference in F-actin after keeping cortical cells in culture for 6 days, because less progenitors and mostly differentiated cells will remain in the dish. In addition to the changes in the actin pool, S. Cappello found similar results for tubulin. The stable acetylated form is reduced, especially in radial glia, and correspondingly the dynamic tyrosinated form is increased (Cappello S et al. 2011). This means, that the entire cytoskeleton of radial glia becomes less stable and more dynamic, so that it does not longer maintain the radial scaffold needed for migration.

3.7. Clinical relevance of a "double-cortex"

The “double-cortex” is a phenotype of clinical interest, because it occurs in human patients as a brain malformation associated with mental retardation and epilepsy (Guerrini R and Parrini E 2009).

One form is periventricular heterotopia where, unlike in our mouse model, the heterotopic cortex is placed immediately adjacent to the ventricle, without a separating band of white matter. This malformation has been associated with defects in the initiation of migration. Filamin-A is a protein expressed from the X-chromosome. Due to random X-inactivation, female patients develop a mosaic phenotype with normal neurons forming the normotopic and mutant neurons forming the heterotopic cortex. This kind of mosaic expression is the typical and more trivial cause for “double-cortices”. Filamin-A binds to actin and forms actin-cross-links and stress fibres (Ross ME and Walsh CA 2001, Bielas S et al. 2004, Robertson SP 2004, Guerrini R and Parrini E 2009). Another gene that leads to the formation of periventricular heterotopia is Arfgef2. Its product, the Big2 protein is involved in vesicle trafficking. Inhibition of Big2 leads to intracellular mislocalizations of β-catenin and E-cadherin, suggesting that Big2 may less play a role in migration, but migrational defects may rather be due to a scaffold effect as we observed it in our mouse model (Bielas S et al. 2004, Sheen VL et al. 2004, Guerrini R and Parrini E 2009).

The probably most famous gene responsible for “double-cortices” is doublecortin (DCX), named after this phenotype (Gleeson JG et al. 1998). It leads to an X-linked brain malformation that belongs to the subcortical band heterotopia type of “double-cortices”, which is associated with defects in ongoing migration. DCX is a microtubule associated protein whose function in regulating and stabilizing the cytoskeleton is still not entirely understood. Surprisingly, DCX hemizygous mice do not display subcortical band heterotopia, and even the full knockout has only mild defects in hippocampal lamination. Even more intriguing is the acute knock-down of DCX in-utero by siRNA, which does result in subcortical band heterotopia. So apparently, cells can compensate for an early loss of DCX but otherwise it is still needed for migration. A reason might be the upregulation of the doublecortin-like kinase 1 (Dclk1), since the DCX/Dclk1 double-mutant does also display migration defects. The formation of subcortical band heterotopia in this mouse model is not surprising as again a genetic mosaic is created (Gleeson JG et al. 1998, Ross ME and Walsh CA 2001, Bai J et al. 2003, Bielas S et al. 2004, Reiner O and Coquelle FM 2005, Deuel TA et al. 2006, Koizumi H et al. 2006, Guerrini R and Parrini E 2009).

Conversely, in human patients the full loss of DCX, such as in male patients, leads to a more severe form of cortical malformation, which is called lissencephaly, the complete absence of gyri and sulci with only four rudimentary layers formed. But most lissencephalic patients carry a mutation in another microtubule associated protein, Lis1. Again, the heterozygous mutation of Lis1 in mice has only minor defects in layer targeting (Reiner O et al. 1993, Gleeson JG et al. 1998, Ross ME and Walsh CA 2001, Bielas S et al. 2004, Reiner O and Coquelle FM 2005, Guerrini R and Parrini E 2009).

Unlike this classical type 1 lissencephaly, type 2 lissencephaly is a disorder that is accompanied by mushroom shaped ectopias in and beyond layer I, which give the brain surface a cobblestone-like appearance. They are also of clinical interest as they are linked to the congenital muscular dystrophies (CMD) (Bielas S et al. 2004). It is common in mutations of the

glycosyltransferases that act on α-dystroglycan (Satz JS et al. 2010). In addition, it is known from the conditional focal adhesion kinase (FAK) mutant mouse that also displays disruptions in the basement membrane and subsequent migration beyond the boundaries of the cortex (Beggs HE et al. 2003), or defects in other basement membrane proteins like integrins (Georges-Labouesse E et al. 1998, Belvindrah R et al. 2007), Perlecan (Costell M et al. 1999) or laminin-γ1 (Haubst N et al. 2006). This phenotype is also part of the RhoA mutant phenotype described here, and indeed, focal adhesion kinase is an effector of RhoA. But interestingly, this particular aspect most likely has a cell-autonomous cause, as it also appeared in the in utero electroporations (Cappello S et al. 2011). Therefore, it is more likely that the RhoA-deficient neurons do not respond to stop signals any more. One candidate for mediating this effect might be Reelin, that acts as a stop signal via a pathway involving n-Cofilin phosphorylation (Chai X et al. 2009), which probably involves RhoA and its effector LIMK. The fact that the migration defects in Lis1 +/- neurons (Kholmanskikh SS et al. 2003) and in Ngn2 -/- cells (Hand R et al. 2005) can actually be overcome by inhibition of RhoA, also speaks for a role of RhoA in relaying stop signals.
Interestingly, all of these malformations are associated with epilepsies, whereas we so far have no indication of spontaneous seizures or an increased lethality in adult mutant animals. While this should be investigated in more detail, the decreased responsiveness of the mutant cortex might also keep the excitation of the neurons low enough not to reach the seizure threshold. In addition, inhibitory GABAergic neurons can still invade the cortex.

3.8. Importance of this work

Finally, the real surprise of this study is the formation of subcortical band heterotopia without a direct migration defect of the newborn neurons themselves, but solely due to the radial glial scaffold. The fact is, that usually the radial scaffold was not examined at all, which is understandable, because the human patients were only examined after birth, when the (mal)formation of the cortex was completed. As it seems the most straightforward explanation for layering defects, neuronal migration was simply assumed to be the cause (Ross ME and Walsh CA 2001, Bielas S et al. 2004, Guerrini R and Parrini E 2009). This may well be true in some cases, but the present results indicate that the model needs to be challenged and the aetiology of these defects are still far from being understood. Even the seemingly simple cases of mosaic expression of mutant genes might not be so simple to explain after all. For example, in the DCX shRNA model there are also wildtype neurons found that contribute to the heterotopic cortex (Bai J et al. 2003). The necessity of an intact scaffold for neuronal migration is one possibility to explain non cell-autonomous effects and should be examined in more detail, in the future.
The problems to examine these developmental malformations highlight the need for more animal models. There are other animal models for subcortical band heterotopia present, but the aetiology of these “double-cortices” is also not yet fully understood. These examples include the TISH rat (Lee KS et al. 1997, Schottler F et al. 2001, Trotter SA et al. 2006), the HeCo mouse (Croquelois A et al. 2009) and the RA-GEF1 mutant mouse (Bilasy SE et al. 2009). In the first two examples, even the mutated genes are still unknown. They bear, however, some resemblance to the RhoA model, because in both cases progenitor cells are scattered. Still, recent examinations of the TISH rat showed that apical adherens junctions are still intact and

that very early in development a secondary germinal zone close to the preplate is formed. The radial glial scaffold is slightly disorganized in the early heterotopic cortex, but remains intact in the basal part. The basal proliferative zone also still contributes to both cortices (Fitzgerald MP et al. 2011). These results insofar confirm our results, as also in our model neurons generated at different positions can reach the normotopic cortex as long as they are born in an intact environment.

So far, the RhoA mouse looks like a valuable tool to gain a further understanding of the genesis of subcortical band heterotopia. The gene is known and many pathways have been revealed in vitro, and in addition we have a firm idea of how exactly the phenotype comes about.


MATERIALS


primer
diluted
ml
Invitrogen
PBS
Tris
incubated
product
removed
buffer


METHODS
Cre
containing
sections
cell
washing
rabbit
min
mg
brains
staining
mouse
HCl
RhoA
temperature
Sigma
antibody

4. Materials and Methods

4.1. Chemicals

Substance	Supplier	Product no.
Agarose	Biozym	870055
Agarose, low melting	Biozym	840101
Atipamezole	Pfizer	PZN 7575554
B27	Invitrogen	17504044
Blocking reagent	Roche	11096176001
Boric acid	Roth	6943
Buprenorphine	Essex	PZN 0345928
DIG RNA labelling mix	Roche	11277073910
DMEM (high glucose with GlutaMAX)	Invitrogen	61965
EDTA	Merck	1084180250
Ethidium bromide	Roth	7870
EtOH	Merck	107017
Fast Green	Sigma	F7258
FCS	Pan	2602-P290705
Fentanyl	Janssen-Cilag	PZN 4795545
Flumazenil	HEXAL	PZN 4470990
Isopropanole	Merck	1096342500
HBSS	Invitrogen	24020
HCl	Merck	109057
HEPES	Invitrogen	15630
Isopentane	Roth	3927.1
KCl	Merck	104936
Ketamine	Pfizer	PZN 7727024
KH_2PO_4	Merck	104873
Medetomidine	Pfizer	PZN 7575896
Midazolam	HEXAL	PZN 0886423
Na-citrate	Merck	6448
NaCl	Fisher	BP358-212
Na_2HPO_4	Merck	T876
NBT/BCIP	Roche	11681451001
NGS	Vector Labs	S-1000
PDL	Sigma	P-0899
PeSt	Invitrogen	15140
PFA	Merck	104005
RNAse inhibitor	Roche	3335399001
SDS	Roth	2326
T3 RNA polymerase	Stratagene	600111
T7 RNA polymerase	Stratagene	600123
TissueTek	Hartenstein Laborversand	TTEK
TRIS-HCl	Roth	6683
Triton-X100	Roth	3051
Trypsin/EDTA	Invitrogen	25300
Tween-20	Sigma	P-1379

4.2. Solutions

Denhardt's solution	polyvinylchloride	0,02%
	pyrrolidone	0,02%
	BSA	0,04%
ISH blocking solution	blocking reagent	2%
	NGS	20%
	in MABT	
ISH hybridisation buffer	Formamide	50%
	Tween	0,10%
	Dextransulfate	10%
	tRNA	1mg/ml
	Deinhardt's solution	1x
	in SSC	
ISH staining solution	NBT	350µg/ml
	BCIP	175µg/ml
	NaCl	100mM
	$MgCl_2$	50mM
	TRIS	100mM
	Tween	0,10%
	pH 9,5	
ISH washing solution	Formamide	50%
	Tween	0,10%
	in SSC	
Lysis buffer	Tris-HCl	1M
	EDTA	5mM
	SDS	0,20%
	NaCl	200mM
	proteinase K	1mg/ml
	pH 8,5	
MABT	maleic acid	100mM
	NaCl	150mM
	Tween	0,02%
PBS	NaCl	137mM
	KCl	2,7mM
	Na_2HPO_4	8,3mM
	KH_2PO_4	1,4mM
	pH 7,4	
SSC	NaCl	0,15mM
	Na_3-citrate	0,015mM
	pH 7,0	

TBE buffer	TRIS	89mM
	boric acid	89mM
	Na_2-EDTA	2mM
TE buffer	Tris-HCl	10mM
	EDTA	1mM
	pH 8,0	
TNB blocking buffer	Tris-HCl	0,1M
	NaCl	0,15M
	TSA Blocking Reagent	0,50%
	pH 7,5	
TNT wash buffer	Tris-HCl	0,1M
	NaCl	0,15M
	Tween	0,05%
	pH 7,5	

4.3. Commercial kits

Kit	Supplier	Product no.
RNeasy Mini kit	Qiagen	74104
Taq PCR Core kit	Qiagen	201223
TSA kit	Perkin Elmer	NEL741

4.4. Immunohistochemistry

4.4.1. Primary antibodies

Antigen	Host species/subtype	Supplier (product no.)
BrdU	rat	Abcam (ab6326)
pan-cadherin	rabbit	Sigma-Aldrich (C3678)
β-catenin	mouse IgG1	BD Transduction Lab (610153)
Ctip2	rat	Abcam (ab18465)
Cux1	rabbit	Santa Cruz (SC-13024)
DIG	sheep Fab (AP-labelled)	Roche (11093274910)
GFAP	mouse IgG1	Sigma (G3898)
GFAP	rabbit	DAKO (Z0334)
MAG	mouse IgG1	Chemicon/Millipore (MAB1567)
Nestin	mouse IgG1	Developmental Studies Hybridoma
NeuN	mouse IgG1	Chemicon/Millipore (MAB377)
Pax6	rabbit	Chemicon/Millipore (AB2237)
PH3	rabbit	Upstate Biotech/Millipore (05-789)
RC2	mouse IgM	kind gift from P. Leprince (Misson JP et al. 1988, Chanas-Sacre G et al. 2000)
Reelin	mouse IgG1	Developmental Studies Hybridoma Bank (E4, supernatant)

Antigen	Host species/subtype	Supplier (product no.)
RhoA	mouse IgG1	Santa Cruz (sc-418)
S100β	mouse IgG1	Sigma (S2532)
Tbr1	rabbit	Abcam (ab31940)
Tbr2	rabbit	Abcam (ab23345)
β-III-tubulin	mouse IgG2b	Sigma (T5076)

4.4.2. Secondary antibodies

Target	Label	Supplier (product no.)
mouse	Alexa405	Invitrogen (A-31553)
mouse IgG1	Alexa488	Invitrogen (A-21121)
mouse IgG1	Alexa546	Invitrogen (A-21123)
mouse IgG_{2b}	Alexa488	Invitrogen (A-21141)
mouse IgM	Alexa546	Invitrogen (A-21045)
rabbit	Alexa488	Invitrogen (A-11008)
rabbit	Alexa546	Invitrogen (A-11010)
rabbit	biotin	Vector Labs (BA-1000)
rat	Alexa488	Invitrogen (A-11006)
rat	Cy3	Jackson ImmunoResearch (112-165-003)

4.4.3. Chemicals used for histology

Substance	recognizes	Supplier (product no.)
CM-DiI	cell membrane	Invitrogen (C7001)
DAPI	dsDNA	Sigma-Aldrich (D9564)
DiO	cell membrane	Sigma-Aldrich (D4292)
DNAse I-Alexa 488	G-actin	Invitrogen (D12371)
Texas Red-X Phalloidin	F-actin	Invitrogen (T7471)

4.5. Mouse lines

The $Emx1^{tm1(Cre)Ito}$ is a knock-in line that expresses Cre recombinase under the control of the endogenous Emx1 promoter. Expression starts around E10 and is cortex-specific (Iwasato T et al. 2004).

$RhoA^{tm1Brak}$ is a conditional KO line, where exon 3, containing the start codon, is flanked by loxP sites (Jackson B et al. 2011).

$Neurod6^{tm1(cre)Kan}$ mice express Cre-recombinase in the NeuroD6 locus, also called the Nex locus (Goebbels S et al. 2006).

The Tg(Neurog2-cre/GFP) mouse line is a transgenic line in which the E1-Ngn2 enhancer and a human beta-globin minimal promoter drive the expression of Cre and, behind the IRES sequence, GFP (Berger J et al. 2004).

The mice have a heterogeneous genetic background. As wildtype mates, C57/Bl6 mice were used.

official nomenclature	referred to as	kind gift from
Emx1$^{tm1(cre)Ito}$	Emx1::Cre	Iwasato/Itohara
RhoA^{tm1Brak}	RhoA fl	C. Brakebusch
Neurod6$^{tm1(cre)Kan}$	Nex::Cre	S. Göbbels
Tg(Neurog2-cre/GFP)^{1Stoy}	Ngn2-Cre	A. Stoykova

4.6. Plasmids

pCIG2 and PCIG2-Cre express IRES-GFP or Cre-IRES-GFP under control of a CMV-enhancer and a chicken β-actin promoter (Hand R et al. 2005). They are a kind gift from C. Schuurmans.

4.7. Lab animals

4.7.1. Animal husbandry

All mice were kept in the facility “Kleintierhaus” of the Helmholtz Center Munich according to FELASA regulations.

For maintenance of the lines, the following breeding schemes were used:

Parent		Parent	Desired offspring (%)
RhoA fl/fl	x	RhoA fl/fl	RhoA fl/fl (100%)
Emx1::Cre +/+ || RhoA fl/wt	x	Emx1::Cre +/+	Emx1::Cre +/+ || RhoA fl/wt (50%)
Nex::Cre +/wt || RhoA fl/wt	x	RhoA fl/fl	Nex::Cre +/wt || RhoA fl/wt (25%)
Ngn2-Cre +/wt || RhoA fl/wt	x	RhoA fl/fl	Ngn2-Cre +/wt || RhoA fl/wt (25%)

For experiments, the following breeding schemes were used:

Parent		Parent	ctrl	cKO
Emx1::Cre +/+ || RhoA fl/wt	x	RhoA fl/fl	50%	50%
Emx1::Cre +/wt || RhoA	x	RhoA fl/fl	75%	25%
Nex::Cre +/wt || RhoA fl/wt	x	RhoA fl/fl	75%	25%
Ngn2-Cre +/wt || RhoA fl/wt	x	RhoA fl/fl	75%	25%

Since heterozygous cKOs were phenotypically identical to wildtype, both Cre-negative animals and heterozygous cKOs were used as control (ctrl).

4.7.2. Plug check

For the production of embryos, mice were mated in the evening and separated the next morning around 7a.m. To determine if the female might have been impregnated, the presence of a vaginal plug was determined. This day was defined E0.

4.7.3. Genotyping

A small piece of tail was incubated in 500µl lysis buffer over night at 55°C. Hair and remaining tissue were removed by short centrifugation in a tabletop centrifuge. DNA was precipitated with an equal volume of isopropanole, purified by centrifugation and dissolved over night in TE buffer at 55°C.

The actual genotyping was performed by PCR using the following protocols.

Emx1::Cre

10x PCR buffer	2,0µl
Q-solution	4,0µl
primer fw (10µM)	1,0µl
primer rev (10µM)	1,0µl
primer Cre (10µM)	1,0µl
dNTPs (10mM)	0,5µl
Taq polymerase	0,3µl
DNA	2,0µl
H2O	ad 20,0µl

program (touchdown-PCR):

4min @ 95°C
10 cycles:
    30sec @ 95°C
    30sec @ 95-94°C (decrease 0,1°C/cycle)
    1min @ 72°C
30 cycles:
    30sec @ 95°C
    30sec @ 64,5°C
    30sec @ 72°C
7min @ 72°C
hold @ 20°C

primers:

fw 5’ GTGAGTGCATGTGCCAGGCTT G 3’
rev 5’ TGGGGTGAGGATAGTTGAGCGC 3’
Cre 5’ GCGGCATAACCAGTGAAACAGC 3’

expected products:

WT ca. 200bp
TG ca. 500bp

RhoA floxed allele

10x PCR buffer	2,0µl
primer JVH11 (10µM)	2,0µl
primer JVH15 (10µM)	2,0µl
primer Cre (0,2µM)	1,0µl
dNTPs (10mM)	0,4µl
Taq polymerase	0,2µl
DNA	2,0µl
H2O	ad 20,0µl

program

2min @ 94°C

35 cycles:

30sec @ 94°C

30sec @ 55°C

30sec @ 72°C

10min @ 72°C

hold @ 20°C

primers:

JVH11 5' AGCCAGCCTCTTGACCGATTTA 3'

JVH15 5' TGTGGGATACCGTTTGAGCAT 3'

expected products:

WT 297bp

fl 393bp

Cre (used for Ngn2::Cre and Nex::Cre):

10x PCR buffer	2,0µl
Q-solution	4,0µl
primer Cre 3' (10µM)	0,8µl
primer Cre 5' (10µM)	0,8µl
dNTPs (10mM)	0,4µl
Taq polymerase	0,2µl
DNA	2,0µl
H2O	ad 20,0µl

program (step-down PCR)

2min @ 95°C

10 cycles:

30sec @ 95°C

30sec @ 63°C

30sec @ 72°C

35 cycles:

30sec @ 95°C

30sec @ 53°C

30sec @ 72°C

5min @ 72°C

hold @ 20°C

primers:

CRE 3' 5' TTCGGATCATCAGCTACCC 3'

CRE 5' 5' AACATGCTTCATCGTCGG 3'

expected products:

WT no product

Cre 419bp

The products were analysed by gel electrophoresis. The gels were prepared of 1,5% agarose in TBE containing 10µg/mL ethidium bromide.

4.8. Cell culture

4.8.1. Coating

Coverslips were cleaned by rinsing in acetone, 30 min boiling in EtOH/0,7% HCl, washing in 100% EtOH. twice. After drying at RT, they were autoclaved for 2hrs at 180°C. They were placed in 24 well plates and wetted with sterile PBS. PBS was removed and the coverslips incubated in 1% PDL dissolved in PBS for at least 2hrs at 37°C. After washing with three changes of MilliQ water, they were dried at room temperature under a laminar air flow and stored at 4°C.

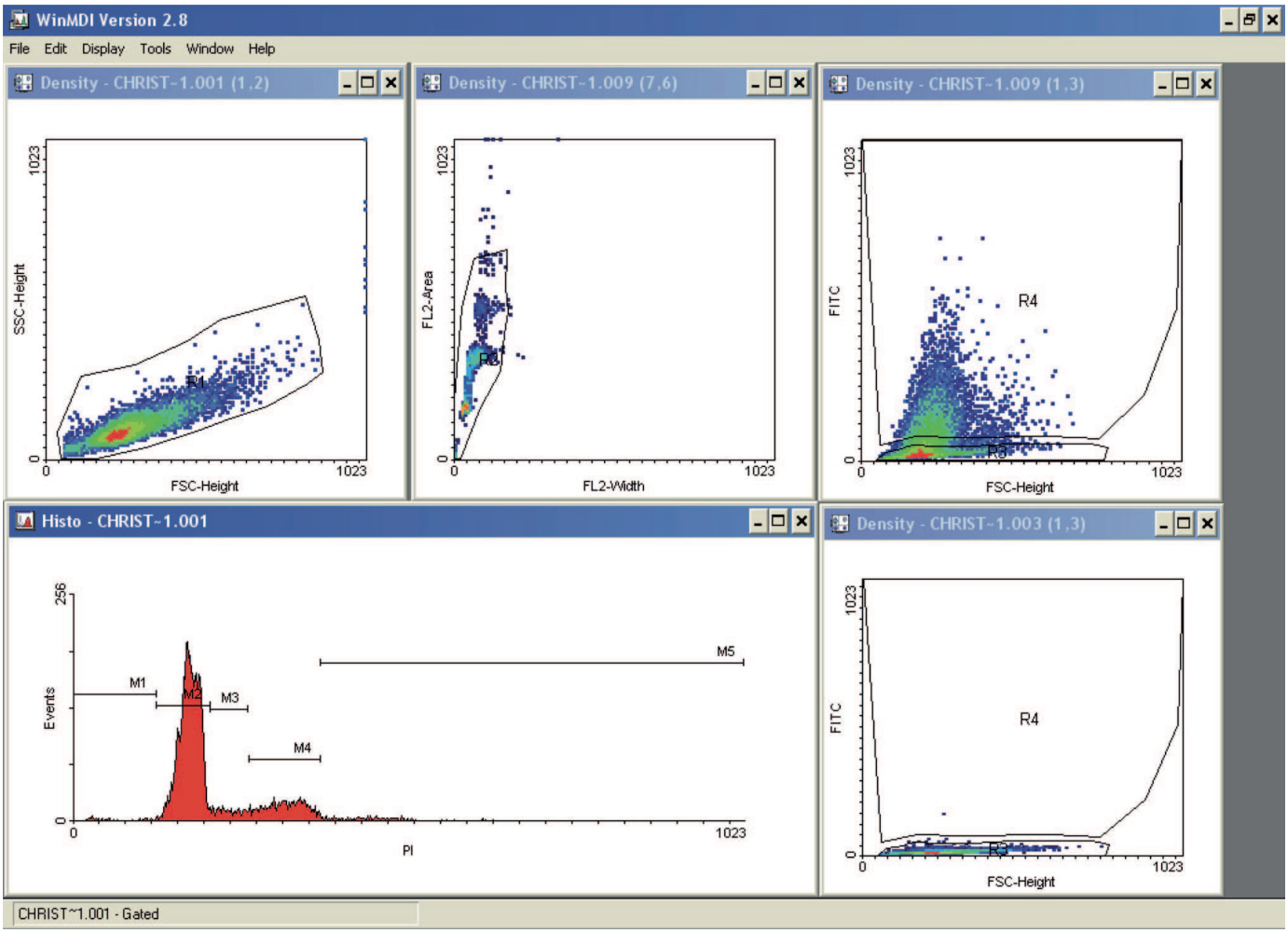
4.8.2. Primary culture

Pregnant mothers were killed by cervical dislocation. The uteri were removed and transported in ice cold HBSS containing 0,01M HEPES. The embryonic brain was taken out. The meninges were removed from the telencephalon as much as possible. Then, the olfactory bulb was removed, the cortex was separated from the rest of the telencephalon and the hippocampus anlage was removed. The whole dissection was carried out in ice cold HBSS/HEPES. The cortices were left in 2ml Eppendorf tubes until they settled. The solution was then replaced with 0,5ml Trypsin/EDTA and the tissue was incubated at 37°C for 15min. Enzymatic dissociation was stopped by adding 1ml DMEM containing 10% FCS and 1% PeSt. The tissue was further

mechanically dissociated by trituration with a flame-polished Pasteur pipette. The tissue was centrifuged for 5min at 172*g and resuspended in DMEM containing 10% FCS and 1% PeSt. 500.000 cells/well were plated in 0,5ml on PDL-coated coverslips.
The next day 0,5ml DMEM/B27/1% PeSt was added. Every second day 0,5ml of the medium was replaced with 0,5ml fresh DMEM containing 1x B27 and 1% PeSt.
At the end of the experiment, cells were fixed with 4% PFA/PBS for 15min at room temperature.

4.8.3. Flow cytometry

Cortices were dissected and dissociated as for primary cultures but suspended in ice cold 70% EtOH instead of cell culture medium. They were kept at -20°C for one week. Afterwards, cells were washed with PBS and suspended in PBS containing 10% FCS. DNA was stained with 1mg/ml propidium iodide for 5min. Cells from E14 cortices were stained against β-III-tubulin in PBS containing 10% FCS for 15min at room temperature to analyze only progenitor cells. The cells were analyzed with a BD FACSCalibur flow cytometer. Cell cycle analysis was carried out with Flow Explorer, WinMDI and the cylchred software (Fig. 15).



4.9. Immunohistochemistry

4.9.1. Preparation of embryonic and early postnatal brains

Brains or heads were removed as described for primary cell cultures. They were fixed in 4% PFA/PBS at 4°C according to the following table:

Age	whole head or brain	duration	slice thickness
E12	head	2hrs	12µm
E14	brain	2hrs	12µm
E16	brain	3hrs	16µm
p3	brain	6hrs	20µm

After fixation they were incubated in 30%(m/m) sucrose/PBS. The solution was changed immediately to avoid dilution by remaining PBS. For cryoprotection, they were incubated at 4°C until they sank. Afterwards, they were carefully dried with a soft tissue and briefly immersed in TissueTek to remove adhering sucrose, which would prevent firm contact with the TissueTek. Then the brains were oriented in a plastic mould filled with TissueTek and rapidly frozen by immersing the lower half of the mould in isopentane cooled to -79°C. The specimen was sealed airtight to prevent drying and stored at -20°C until cutting.

For cutting, the resulting block was oriented and cut coronally on a cryostat. Sections were stored on SuperFrost+ microscopic slides at -20°C.

4.9.2. Preparation of older postnatal/adult brains

Animals were deeply anesthetized with Ketamine (100mg/kg) and Xylazine (20mg/kg) and fixed on a styrofoam board under a fume hood. A needle was inserted into the left ventricle of the heart and the right atrium was cut open. The blood was removed by transcardial perfusion by means of a peristaltic pump, then the mouse was fixated by transcardial perfusion with about 150ml 4% PFA/PBS. The flow rate was kept under 100µl/s to ensure sufficient penetration of PFA into the tissue. The brain was post fixed over night with 4% PFA/PBS at 4°C.

Cryoprotection was carried out essentially as for embryonic brains. For adult brains, the hemispheres were separated along the midline. Brains were frozen analogue to embryonic brains. Adult brains were cut sagittally in 20µm thick sections and postnatal brains coronally in 20µm thick sections on a cryostat. Sections were stored on SuperFrost+ microscopic slides at -20°C.

4.9.3. Preparation of vibratome sections

3% agarose in PBS was melted in a microwave oven and left in a water bath at 55°C to cool down while remaining liquid. For temperature sensitive tissue, especially specimen containing fluorescent proteins, low-melting agarose was used and cooled down to 37°C. The brain was embedded in the agarose in a plastic mould and cooled down at room temperature until the agarose had solidified. The block was then cut in the desired orientation and sectioned on a vibratome. The sections were kept in PBS containing 0,1% Na-azide at 4°C.

4.9.4. General staining procedure

Cryosections were rehydrated with PBS and incubated with the primary antibody diluted in PBS containing 0,5% Triton-X and 10% NGS over night at 4°C. After washing with PBS, the sections were incubated with the secondary antibody diluted in PBS containing 0,5% Triton-X and 10% NGS for 1,5hrs at room temperature. After final washing with PBS, the sections were mounted with AquaPolymount.

Vibratome sections were stained essentially the same way, but the incubations with AB took place for three days at 4°C.

Fixed cells were treated the same way, only rehydration was not necessary. The coverslips were instead quickly rinsed in PBS.

4.9.5. Special treatments

4.9.5.1. Boiling

For unmasking of antigens, sections were boiled in 0,01M sodium citrate (pH6) using a microwave oven. Sections of embryonic brains were boiled for 8min, sections of adult brains were boiled for 30min.

4.9.5.2. HCl treatment

For denaturation of DNA, sections were incubated for 30min with 2M HCl. The pH was readjusted by a 15min incubation in 0,1M Sodium-tetraborate (pH8,5) and washing with PBS.

4.9.5.3. Tyramide signal amplification (TSA)

Tyramide signal amplification can greatly enhance the signal by using an enzymatic reaction to couple the fluorophore to cell organelles. With its help one can also use two primary antibodies generated in the same host on the same section. To do so, one antibody is diluted below the detection limit of a normal secondary antibody (typically 50-100x the standard dilution) and its signal then enhanced by tyramide signal amplification. Afterwards, the next primary antibody can be used.

The sections were washed in TNT wash buffer. Then endogenous peroxidase activity was quenched by a 30min incubation with 0,3% H_2O_2 in TNT. After three washing steps, the sections were incubated with the primary antibody diluted in TNB Blocking Buffer. After washing, a biotinylated secondary antibody was used. Sections were washed again and incubated with a horseradish-peroxidase coupled Streptavidin (1:200). To reveal the signal, Fluorescein-tyramide, diluted 1:100 in the provided amplification buffer, was added for 7min.

4.9.6. Stainings in this work

Antibody	Dilution	Special treatment
RhoA	1:500	none
PH3	1:200	none
Pax6	1:500 (x1:50)	boiling, TSA
Tbr2	1:500	boiling, HCl
β-III-tubulin	1:100	none
Nestin	1:3	none
RC2	1:500	none
β-catenin	1:50	none
pan-cadherin	1:200	none
NeuN	1:100	none
GFAP	1:100	none
S100β	1:500	none
MAG	1:100	none
Cux1	1:100	none
Tbr1	1:100	none
Ctip2	1:200	boiling
BrdU	1:250	HCl
Reelin	1:10	none

Alexa-coupled antibodies were diluted 1:1000, Cy3-coupled antibodies were diluted 1:100 and biotinylated antibodies were diluted 1:200.

Nuclei were visualized by staining with DAPI (0,02mg/ml in PBS) for 10min.

F-actin can be detected by fluorescently labelled phalloidin, whereas G-actin can be detected by labelled DNAse-I. The reagents are diluted in PBS according to the manufacturer's manual and added to the specimen for 15min.

4.10. In situ hybridisation

4.10.1. In-vitro transcription

The cDNA containing plasmid was linearized with an appropriate restriction enzyme. After digestion DNA was purified by use of a column (Qiagen).

Transcription was performed by incubating 1,5ml of the following mixture for 2hrs at 37°C:

1µg DNA
2µl DIG labelled dNTPs
4µl 5x Stratagene buffer
1µl RNAse inhibitor
1µl RNA polymerase

The RNA product was cleaned using the RNeasy mini kit.

4.10.2. Hybridisation

Tissue sections were incubated in hybridisation buffer containing 150ng RNA probe at 65°C over night. Subsequently, sections were washed 3 times in washing solution at 65°C and twice in MABT at room-temperature. Sections were blocked in blocking solution for 1hr and RNA probes were detected by Anti-Digoxigenin-AP Fab fragments (Roche) in blocking solution over night at room temperature. Sections were washed 4 times with MABT and developed in staining solution for 1-3 days at 4°C.

4.11. Nucleophilic tracers

Brains of E14 embryos had their meninges removed and were fixed with PFA. CM-DiI was diluted 1:10 in PBS, thus forming a very fine precipitate, and injected into the ventricles. Some small crystals of DiO were pressed against surface of the brain. The brains were incubated over night on 0,5% PFA/PBS, embedded in agarose and cut into 200µm sections on a vibratome.

4.12. Surgery

4.12.1. Anaesthesia

Mice were anesthetized with Fentanyl (0,05mg/kg), Midazolam (5mg/kg) and Medetomidine (0,5mg/kg). After the operation, the anaesthesia was antagonized with Buprenorphine (0,1mg/kg), Atipamezole (2,5mg/kg) and Flumazenil (0,5mg/kg). The partial antagonist/agonist nature of Buprenorphine ensures sufficient postoperative analgesia.

4.12.2. In utero electroporation

Plasmids were mixed with Fast Green (2.5mg/µl) and injected into the cerebral ventricles at a concentration of 1µg/µl using a glass micropipette. After injection, electroporation was performed using five 50V pulses spaced at 200 ms, which were applied with 5mm tweezer-style electrodes (Protech) using a BTX square-wave electroporator (Harvard Apparatus).

4.13. Image analysis

4.13.1. Image acquisition

Fluorescent images were taken on an Olympus FluoView1000 confocal microscope and saved in the proprietary OIF-format.

Care was taken to use the same settings for control and experimental brain sections.

When a picture was bigger than one field of view, it was stitched together with one of the following software: FluoView Multiple region acquisition, Photoshop or ImageJ with one of the following plugins: MosaicJ, Stitching or TurboReg.

4.13.2. Image processing

When necessary, ImageJ was used to adjust brightness and contrasts and to set thresholds. Colour information was never changed in a non-linear manner. All processing steps were carried out on pictures of control and experiment in the same manner.

4.13.3. Quantitative analysis

Images, including the scaling information, were imported as Hyperstacks into ImageJ, where distances or areas could be measured directly. Cells were counted manually, using the Cell Counter plugin.


REFERENCES

5. References

Aaku-Saraste, E., Hellwig, A. and Huttner, W. B. (1996). "Loss of occludin and functional tight junctions, but not ZO-1, during neural tube closure--remodeling of the neuroepithelium prior to neurogenesis." Dev Biol 180(2): 664-79.

Ahringer, J. (2003). "Control of cell polarity and mitotic spindle positioning in animal cells." Curr Opin Cell Biol 15(1): 73-81.

Akimoto, J., Itoh, H., Miwa, T. and Ikeda, K. (1993). "Immunohistochemical study of glutamine synthetase expression in early glial development." Brain Res Dev Brain Res 72(1): 9-14.

Allen, W. E., Jones, G. E., Pollard, J. W. and Ridley, A. J. (1997). "Rho, Rac and Cdc42 regulate actin organization and cell adhesion in macrophages." J Cell Sci 110 (Pt 6): 707-20.

Allen, W. E., Zicha, D., Ridley, A. J. and Jones, G. E. (1998). "A role for Cdc42 in macrophage chemotaxis." J Cell Biol 141(5): 1147-57.

Anderson, S. A., Eisenstat, D. D., Shi, L. and Rubenstein, J. L. (1997). "Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes." Science 278(5337): 474-6.

Anderson, S. A., Kaznowski, C. E., Horn, C., Rubenstein, J. L. and McConnell, S. K. (2002). "Distinct origins of neocortical projection neurons and interneurons in vivo." Cereb Cortex 12(7): 702-9.

Arimura, N., Inagaki, N., Chihara, K., Menager, C., Nakamura, N., Amano, M., Iwamatsu, A., Goshima, Y. and Kaibuchi, K. (2000). "Phosphorylation of collapsin response mediator protein-2 by Rho-kinase. Evidence for two separate signaling pathways for growth cone collapse." J Biol Chem 275(31): 23973-80.

Arthur, W. T. and Burridge, K. (2001). "RhoA inactivation by p190RhoGAP regulates cell spreading and migration by promoting membrane protrusion and polarity." Mol Biol Cell 12(9): 2711-20.

Aspenstrom, P., Fransson, A. and Saras, J. (2004). "Rho GTPases have diverse effects on the organization of the actin filament system." Biochem J 377(Pt 2): 327-37.

Astrom, K. E. and Webster, H. D. (1991). "The early development of the neopallial wall and area choroidea in fetal rats. A light and electron microscopic study." Adv Anat Embryol Cell Biol 123: 1-76.

Bai, J., Ramos, R. L., Ackman, J. B., Thomas, A. M., Lee, R. V. and LoTurco, J. J. (2003). "RNAi reveals doublecortin is required for radial migration in rat neocortex." Nat Neurosci 6(12): 1277-83.

Baye, L. M. and Link, B. A. (2008). "Nuclear migration during retinal development." Brain Res 1192: 29-36.

Bayer, S. A., Altman, J., Russo, R. J., Dai, X. F. and Simmons, J. A. (1991). "Cell migration in the rat embryonic neocortex." J Comp Neurol307(3): 499-516.

Beggs, H. E., Schahin-Reed, D., Zang, K., Goebbels, S., Nave, K. A., Gorski, J., Jones, K. R., Sretavan, D. and Reichardt, L. F. (2003). "FAK deficiency in cells contributing to the basal lamina results in cortical abnormalities resembling congenital muscular dystrophies." Neuron 40(3): 501-14.

Belvindrah, R., Graus-Porta, D., Goebbels, S., Nave, K. A. and Muller, U. (2007). "Beta1 integrins in radial glia but not in migrating neurons are essential for the formation of cell layers in the cerebral cortex." J Neurosci27(50): 13854-65.
Berger, J., Eckert, S., Scardigli, R., Guillemot, F., Gruss, P. and Stoykova, A. (2004). "E1-Ngn2/Cre is a new line for regional activation of Cre recombinase in the developing CNS." Genesis 40(4): 195-9.
Bernard, O. (2006). "Lim kinases, regulators of actin dynamics." Int J Biochem Cell Biol.
Bernards, A. (2003). "GAPs galore! A survey of putative Ras superfamily GTPase activating proteins in man and Drosophila." Biochim Biophys Acta 1603(2): 47-82.
Berry, M. and Rogers, A. W. (1965). "The migration of neuroblasts in the developing cerebral cortex." J Anat 99(Pt 4): 691-709.
Besson, A., Gurian-West, M., Schmidt, A., Hall, A. and Roberts, J. M. (2004). "p27Kip1 modulates cell migration through the regulation of RhoA activation." Genes Dev 18(8): 862-76.
Bielas, S., Higginbotham, H., Koizumi, H., Tanaka, T. and Gleeson, J. G. (2004). "Cortical neuronal migration mutants suggest separate but intersecting pathways." Annu Rev Cell Dev Biol 20: 593-618.
Bilasy, S. E., Satoh, T., Ueda, S., Wei, P., Kanemura, H., Aiba, A., Terashima, T. and Kataoka, T. (2009). "Dorsal telencephalon-specific RA-GEF-1 knockout mice develop heterotopic cortical mass and commissural fiber defect." Eur J Neurosci.
Braga, V. M., Machesky, L. M., Hall, A. and Hotchin, N. A. (1997). "The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts." J Cell Biol 137(6): 1421-31.
Britanova, O., Akopov, S., Lukyanov, S., Gruss, P. and Tarabykin, V. (2005). "Novel transcription factor Satb2 interacts with matrix attachment region DNA elements in a tissue-specific manner and demonstrates cell-type-dependent expression in the developing mouse CNS." Eur J Neurosci 21(3): 658-68.
Brittis, P. A., Meiri, K., Dent, E. and Silver, J. (1995). "The earliest patterns of neuronal differentiation and migration in the mammalian central nervous system." Exp Neurol 134(1): 1-12.
Burbelo, P. D., Drechsel, D. and Hall, A. (1995). "A conserved binding motif defines numerous candidate target proteins for both Cdc42 and Rac GTPases." J Biol Chem 270(49): 29071-4.
Bustelo, X. R., Sauzeau, V. and Berenjeno, I. M. (2007). "GTP-binding proteins of the Rho/Rac family: regulation, effectors and functions in vivo." Bioessays29(4): 356-370.
Campbell, K. and Gotz, M. (2002). "Radial glia: multi-purpose cells for vertebrate brain development." Trends Neurosci 25(5): 235-8.
Cappello, S., Attardo, A., Wu, X., Iwasato, T., Itohara, S., Wilsch-Brauninger, M., Eilken, H. M., Rieger, M. A., Schroeder, T. T., Huttner, W. B., Brakebusch, C. and Gotz, M. (2006). "The Rho-GTPase cdc42 regulates neural progenitor fate at the apical surface." Nat Neurosci 9(9): 1099-107.
Cappello, S., Böhringer, C. R. J., Bergami, M., Konzelmann, K.-K., Ghanem, A., Tomassy, G. S., Arlotta, P., Mainardi, M., Allegra, M., Caleo, M., van Hengel, J., Brakebusch, C. and Götz, M. (2012). "A radial glia specific role of RhoA in double-cortex formation." Neuron accepted.

Casanova, M. F. and Trippe, J., 2nd (2006). "Regulatory mechanisms of cortical laminar development." Brain Res Rev 51(1): 72-84.
Cassimeris, L. (2002). "The oncoprotein 18/stathmin family of microtubule destabilizers." Curr Opin Cell Biol 14(1): 18-24.
Cau, J. and Hall, A. (2005). "Cdc42 controls the polarity of the actin and microtubule cytoskeletons through two distinct signal transduction pathways." J Cell Sci 118(Pt 12): 2579-87.
Causeret, F., Hidalgo-Sanchez, M., Fort, P., Backer, S., Popoff, M. R., Gauthier-Rouviere, C. and Bloch-Gallego, E. (2004). "Distinct roles of Rac1/Cdc42 and Rho/Rock for axon outgrowth and nucleokinesis of precerebellar neurons toward netrin 1." Development 131(12): 2841-52.
Caviness, V. S., Jr. (1982). "Neocortical histogenesis in normal and reeler mice: a developmental study based upon [3H]thymidine autoradiography." Brain Res 256(3): 293-302.
Chai, X., Forster, E., Zhao, S., Bock, H. H. and Frotscher, M. (2009). "Reelin stabilizes the actin cytoskeleton of neuronal processes by inducing n-cofilin phosphorylation at serine3." J Neurosci 29(1): 288-99.
Chanas-Sacre, G., Thiry, M., Pirard, S., Rogister, B., Moonen, G., Mbebi, C., Verdiere-Sahuque, M. and Leprince, P. (2000). "A 295-kDA intermediate filament-associated protein in radial glia and developing muscle cells in vivo and in vitro." Dev Dyn 219(4): 514-25.
Chen, L., Liao, G., Waclaw, R. R., Burns, K. A., Linquist, D., Campbell, K., Zheng, Y. and Kuan, C. Y. (2007). "Rac1 controls the formation of midline commissures and the competency of tangential migration in ventral telencephalic neurons." J Neurosci 27(14): 3884-93.
Chou, M. M., Masuda-Robens, J. M. and Gupta, M. L. (2003). "Cdc42 promotes G1 progression through p70 S6 kinase-mediated induction of cyclin E expression." J Biol Chem 278(37): 35241-7.
Coleman, J. E., Nahmani, M., Gavornik, J. P., Haslinger, R., Heynen, A. J., Erisir, A. and Bear, M. F. (2010). "Rapid structural remodeling of thalamocortical synapses parallels experience-dependent functional plasticity in mouse primary visual cortex." J Neurosci 30(29): 9670-82.
Colognato, H. and ffrench-Constant, C. (2004). "Mechanisms of glial development." Curr Opin Neurobiol 14(1): 37-44.
Connelly, J. T., Gautrot, J. E., Trappmann, B., Tan, D. W., Donati, G., Huck, W. T. and Watt, F. M. (2010). "Actin and serum response factor transduce physical cues from the microenvironment to regulate epidermal stem cell fate decisions." Nat Cell Biol 12(7): 711-8.
Coso, O. A., Chiariello, M., Yu, J. C., Teramoto, H., Crespo, P., Xu, N., Miki, T. and Gutkind, J. S. (1995). "The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway." Cell 81(7): 1137-46.
Costa, M. R., Wen, G., Lepier, A., Schroeder, T. and Gotz, M. (2008). "Par-complex proteins promote proliferative progenitor divisions in the developing mouse cerebral cortex." Development 135(1): 11-22.
Costell, M., Gustafsson, E., Aszodi, A., Morgelin, M., Bloch, W., Hunziker, E., Addicks, K., Timpl, R. and Fassler, R. (1999). "Perlecan maintains the integrity of cartilage and some basement membranes." J Cell Biol 147(5): 1109-22.

Couchman, J. R. and Rees, D. A. (1979). "The behaviour of fibroblasts migrating from chick heart explants: changes in adhesion, locomotion and growth, and in the distribution of actomyosin and fibronectin." J Cell Sci 39: 149-65.
Croquelois, A., Giuliani, F., Savary, C., Kielar, M., Amiot, C., Schenk, F. and Welker, E. (2009). "Characterization of the HeCo mutant mouse: a new model of subcortical band heterotopia associated with seizures and behavioral deficits." Cereb Cortex 19(3): 563-75.
D'Arcangelo, G., Nakajima, K., Miyata, T., Ogawa, M., Mikoshiba, K. and Curran, T. (1997). "Reelin is a secreted glycoprotein recognized by the CR-50 monoclonal antibody." J Neurosci 17(1): 23-31.
Daub, H., Gevaert, K., Vandekerckhove, J., Sobel, A. and Hall, A. (2001). "Rac/Cdc42 and p65PAK regulate the microtubule-destabilizing protein stathmin through phosphorylation at serine 16." J Biol Chem 276(3): 1677-80.
Dawe, H. R., Minamide, L. S., Bamburg, J. R. and Cramer, L. P. (2003). "ADF/cofilin controls cell polarity during fibroblast migration." Curr Biol 13(3): 252-7.
Dehay, C. and Kennedy, H. (2007). "Cell-cycle control and cortical development." Nat Rev Neurosci 8(6): 438-50.
Del Rio, J. A., Martinez, A., Auladell, C. and Soriano, E. (2000). "Developmental history of the subplate and developing white matter in the murine neocortex. Neuronal organization and relationship with the main afferent systems at embryonic and perinatal stages." Cereb Cortex 10(8): 784-801.
DesMarais, V., Ghosh, M., Eddy, R. and Condeelis, J. (2005). "Cofilin takes the lead." J Cell Sci 118(Pt 1): 19-26.
Deuel, T. A., Liu, J. S., Corbo, J. C., Yoo, S. Y., Rorke-Adams, L. B. and Walsh, C. A. (2006). "Genetic interactions between doublecortin and doublecortin-like kinase in neuronal migration and axon outgrowth." Neuron49(1): 41-53.
Doetsch, F., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (1999). "Regeneration of a germinal layer in the adult mammalian brain." Proc Natl Acad Sci U S A 96(20): 11619-24.
Doetsch, F., Petreanu, L., Caille, I., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (2002). "EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells." Neuron 36(6): 1021-34.
Douglas, R. J. and Martin, K. A. (2004). "Neuronal circuits of the neocortex." Annu Rev Neurosci 27: 419-51.
Eden, S., Rohatgi, R., Podtelejnikov, A. V., Mann, M. and Kirschner, M. W. (2002). "Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck." Nature 418(6899): 790-3.
Edwards, M. A., Yamamoto, M. and Caviness, V. S., Jr. (1990). "Organization of radial glia and related cells in the developing murine CNS. An analysis based upon a new monoclonal antibody marker." Neuroscience 36(1): 121-44.
Ehrlich, J. S., Hansen, M. D. and Nelson, W. J. (2002). "Spatio-temporal regulation of Rac1 localization and lamellipodia dynamics during epithelial cell-cell adhesion." Dev Cell 3(2): 259-70.
Elberger, A. J. (1993). "Distribution of transitory corpus callosum axons projecting to developing cat visual cortex revealed by DiI." J Comp Neurol 333(3): 326-42.

Englund, C., Fink, A., Lau, C., Pham, D., Daza, R. A., Bulfone, A., Kowalczyk, T. and Hevner, R. F. (2005). "Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex." J Neurosci 25(1): 247-51.
Erickson, A. C. and Couchman, J. R. (2000). "Still more complexity in mammalian basement membranes." J Histochem Cytochem 48(10): 1291-306.
Etienne-Manneville, S. and Hall, A. (2001). "Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta." Cell 106(4): 489-98.
Etienne-Manneville, S. and Hall, A. (2002). "Rho GTPases in cell biology." Nature 420(6916): 629-35.
Etienne-Manneville, S. and Hall, A. (2003). "Cell polarity: Par6, aPKC and cytoskeletal crosstalk." Curr Opin Cell Biol 15(1): 67-72.
Fawcett, J. W. and Asher, R. A. (1999). "The glial scar and central nervous system repair." Brain Res Bull 49(6): 377-91.
Fitzgerald, M. P., Covio, M. and Lee, K. S. (2011). "Disturbances in the positioning, proliferation and apoptosis of neural progenitors contribute to subcortical band heterotopia formation." Neuroscience 176: 455-71.
Forni, P. E., Scuoppo, C., Imayoshi, I., Taulli, R., Dastru, W., Sala, V., Betz, U. A., Muzzi, P., Martinuzzi, D., Vercelli, A. E., Kageyama, R. and Ponzetto, C. (2006). "High levels of Cre expression in neuronal progenitors cause defects in brain development leading to microencephaly and hydrocephaly." J Neurosci 26(37): 9593-602.
Frotscher, M. (1997). "Dual role of Cajal-Retzius cells and reelin in cortical development." Cell Tissue Res 290(2): 315-22.
Fukata, Y., Itoh, T. J., Kimura, T., Menager, C., Nishimura, T., Shiromizu, T., Watanabe, H., Inagaki, N., Iwamatsu, A., Hotani, H. and Kaibuchi, K. (2002). "CRMP-2 binds to tubulin heterodimers to promote microtubule assembly." Nat Cell Biol 4(8): 583-91.
Gallagher, E. D., Gutowski, S., Sternweis, P. C. and Cobb, M. H. (2004). "RhoA binds to the amino terminus of MEKK1 and regulates its kinase activity." J Biol Chem 279(3): 1872-7.
Ganzler-Odenthal, S. I. and Redies, C. (1998). "Blocking N-cadherin function disrupts the epithelial structure of differentiating neural tissue in the embryonic chicken brain." J Neurosci 18(14): 5415-25.
Ge, W., He, F., Kim, K. J., Blanchi, B., Coskun, V., Nguyen, L., Wu, X., Zhao, J., Heng, J. I., Martinowich, K., Tao, J., Wu, H., Castro, D., Sobeih, M. M., Corfas, G., Gleeson, J. G., Greenberg, M. E., Guillemot, F. and Sun, Y. E. (2006). "Coupling of cell migration with neurogenesis by proneural bHLH factors." Proc Natl Acad Sci U S A 103(5): 1319-24.
Georges-Labouesse, E., Mark, M., Messaddeq, N. and Gansmuller, A. (1998). "Essential role of alpha 6 integrins in cortical and retinal lamination." Curr Biol 8(17): 983-6.
Ghosh, M., Song, X., Mouneimne, G., Sidani, M., Lawrence, D. S. and Condeelis, J. S. (2004). "Cofilin promotes actin polymerization and defines the direction of cell motility." Science 304(5671): 743-6.
Gibson, M. C. and Perrimon, N. (2003). "Apicobasal polarization: epithelial form and function." Curr Opin Cell Biol 15(6): 747-52.

Gilbert, C. D. and Wiesel, T. N. (1983). "Clustered intrinsic connections in cat visual cortex." J Neurosci 3(5): 1116-33.
Gleeson, J. G., Allen, K. M., Fox, J. W., Lamperti, E. D., Berkovic, S., Scheffer, I., Cooper, E. C., Dobyns, W. B., Minnerath, S. R., Ross, M. E. and Walsh, C. A. (1998). "Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein." Cell 92(1): 63-72.
Glotzer, M. (2001). "Animal cell cytokinesis." Annu Rev Cell Dev Biol 17: 351-86.
Goebbels, S., Bormuth, I., Bode, U., Hermanson, O., Schwab, M. H. and Nave, K. A. (2006). "Genetic targeting of principal neurons in neocortex and hippocampus of NEX-Cre mice." Genesis 44(12): 611-21.
Gomes, E. R., Jani, S. and Gundersen, G. G. (2005). "Nuclear movement regulated by Cdc42, MRCK, myosin, and actin flow establishes MTOC polarization in migrating cells." Cell 121(3): 451-63.
Gotta, M., Abraham, M. C. and Ahringer, J. (2001). "CDC-42 controls early cell polarity and spindle orientation in C. elegans." Curr Biol 11(7): 482-8.
Gotz, M. and Barde, Y. A. (2005). "Radial glial cells defined and major intermediates between embryonic stem cells and CNS neurons." Neuron46(3): 369-72.
Gotz, M., Hartfuss, E. and Malatesta, P. (2002). "Radial glial cells as neuronal precursors: a new perspective on the correlation of morphology and lineage restriction in the developing cerebral cortex of mice." Brain Res Bull57(6): 777-88.
Gotz, M. and Huttner, W. B. (2005). "The cell biology of neurogenesis." Nat Rev Mol Cell Biol 6(10): 777-88.
Gotz, M., Stoykova, A. and Gruss, P. (1998). "Pax6 controls radial glia differentiation in the cerebral cortex." Neuron 21(5): 1031-44.
Gray, G. E., Leber, S. M. and Sanes, J. R. (1990). "Migratory patterns of clonally related cells in the developing central nervous system." Experientia46(9): 929-40.
Guasch, R. M., Scambler, P., Jones, G. E. and Ridley, A. J. (1998). "RhoE regulates actin cytoskeleton organization and cell migration." Mol Cell Biol 18(8): 4761-71.
Guerrini, R. and Parrini, E. (2009). "Neuronal Migration Disorders." Neurobiol Dis.
Hall, A. (1998). "Rho GTPases and the actin cytoskeleton." Science 279(5350): 509-14.
Hand, R., Bortone, D., Mattar, P., Nguyen, L., Heng, J. I., Guerrier, S., Boutt, E., Peters, E., Barnes, A. P., Parras, C., Schuurmans, C., Guillemot, F. and Polleux, F. (2005). "Phosphorylation of Neurogenin2 specifies the migration properties and the dendritic morphology of pyramidal neurons in the neocortex." Neuron 48(1): 45-62.
Hartfuss, E., Galli, R., Heins, N. and Gotz, M. (2001). "Characterization of CNS precursor subtypes and radial glia." Dev Biol 229(1): 15-30.
Hatten, M. E. (1999). "Central nervous system neuronal migration." Annu Rev Neurosci 22: 511-39.
Hatten, M. E. (2002). "New directions in neuronal migration." Science 297(5587): 1660-3.
Haubensak, W., Attardo, A., Denk, W. and Huttner, W. B. (2004). "Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis." Proc Natl Acad Sci U S A 101(9): 3196-201.

Haubst, N., Georges-Labouesse, E., De Arcangelis, A., Mayer, U. and Gotz, M. (2006). "Basement membrane attachment is dispensable for radial glial cell fate and for proliferation, but affects positioning of neuronal subtypes." Development133(16): 3245-54.
Herschman, H. R. (1991). "Primary response genes induced by growth factors and tumor promoters." Annu Rev Biochem 60: 281-319.
Herzog, D., Loetscher, P., van Hengel, J., Knüsel, S., Brakebusch, C., Taylor, V., Suter, U. and Relvas, J.B. (2011). "The small GTPase RhoA is required to maintain spinal cord neuroepithelium organization and the neural stem cell pool." J Neurosci 31(13):5120-30.
Hevner, R. F., Daza, R. A., Rubenstein, J. L., Stunnenberg, H., Olavarria, J. F. and Englund, C. (2003). "Beyond laminar fate: toward a molecular classification of cortical projection/pyramidal neurons." Dev Neurosci25(2-4): 139-51.
Hinsch, K. D., Habermann, B., Just, I., Hinsch, E., Pfisterer, S., Schill, W. B. and Aktories, K. (1993). "ADP-ribosylation of Rho proteins inhibits sperm motility." FEBS Lett 334(1): 32-6.
Hirose, T., Izumi, Y., Nagashima, Y., Tamai-Nagai, Y., Kurihara, H., Sakai, T., Suzuki, Y., Yamanaka, T., Suzuki, A., Mizuno, K. and Ohno, S. (2002). "Involvement of ASIP/PAR-3 in the promotion of epithelial tight junction formation." J Cell Sci 115(Pt 12): 2485-95.
Ho, H. Y., Rohatgi, R., Lebensohn, A. M., Le, M., Li, J., Gygi, S. P. and Kirschner, M. W. (2004). "Toca-1 mediates Cdc42-dependent actin nucleation by activating the N-WASP-WIP complex." Cell 118(2): 203-16.
Hordijk, P. L., ten Klooster, J. P., van der Kammen, R. A., Michiels, F., Oomen, L. C. and Collard, J. G. (1997). "Inhibition of invasion of epithelial cells by Tiam1-Rac signaling." Science 278(5342): 1464-6.
Hu, W., Bellone, C. J. and Baldassare, J. J. (1999). "RhoA stimulates p27(Kip) degradation through its regulation of cyclin E/CDK2 activity." J Biol Chem 274(6): 3396-401.
Huttner, W. B. and Kosodo, Y. (2005). "Symmetric versus asymmetric cell division during neurogenesis in the developing vertebrate central nervous system." Curr Opin Cell Biol 17(6): 648-57.
Innocenti, M., Zucconi, A., Disanza, A., Frittoli, E., Areces, L. B., Steffen, A., Stradal, T. E., Di Fiore, P. P., Carlier, M. F. and Scita, G. (2004). "Abi1 is essential for the formation and activation of a WAVE2 signalling complex." Nat Cell Biol 6(4): 319-27.
Itoh, M., Sasaki, H., Furuse, M., Ozaki, H., Kita, T. and Tsukita, S. (2001). "Junctional adhesion molecule (JAM) binds to PAR-3: a possible mechanism for the recruitment of PAR-3 to tight junctions." J Cell Biol 154(3): 491-7.
Iwasato, T., Datwani, A., Wolf, A. M., Nishiyama, H., Taguchi, Y., Tonegawa, S., Knopfel, T., Erzurumlu, R. S. and Itohara, S. (2000). "Cortex-restricted disruption of NMDAR1 impairs neuronal patterns in the barrel cortex." Nature406(6797): 726-31.
Iwasato, T., Nomura, R., Ando, R., Ikeda, T., Tanaka, M. and Itohara, S. (2004). "Dorsal telencephalon-specific expression of Cre recombinase in PAC transgenic mice." Genesis 38(3): 130-8.

Izumi, Y., Hirose, T., Tamai, Y., Hirai, S., Nagashima, Y., Fujimoto, T., Tabuse, Y., Kemphues, K. J. and Ohno, S. (1998). "An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of Caenorhabditis elegans polarity protein PAR-3." J Cell Biol 143(1): 95-106.

Jacinto, A., Wood, W., Balayo, T., Turmaine, M., Martinez-Arias, A. and Martin, P. (2000). "Dynamic actin-based epithelial adhesion and cell matching during Drosophila dorsal closure." Curr Biol 10(22): 1420-6.

Jackson, B., Peyrollier, K., Pedersen, E., Basse, A., Karlsson, R., Wang, Z., Lefever, T., Ochsenbein, A., Schmidt, G., Aktories, K., Stanley, A., Quondamatteo, F., Ladwein, M., Rottner, K., van Hengel, J. and Brakebusch, C. (2011). "RhoA is dispensable for skin development, but crucial for contraction and directed migration of keratinocytes." Mol Biol Cell.

Jaffe, A. B. and Hall, A. (2005). "Rho GTPases: biochemistry and biology." Annu Rev Cell Dev Biol 21: 247-69.

Jay, P. Y., Pham, P. A., Wong, S. A. and Elson, E. L. (1995). "A mechanical function of myosin II in cell motility." J Cell Sci 108 (Pt 1): 387-93.

Joyce, D., Bouzahzah, B., Fu, M., Albanese, C., D'Amico, M., Steer, J., Klein, J. U., Lee, R. J., Segall, J. E., Westwick, J. K., Der, C. J. and Pestell, R. G. (1999). "Integration of Rac-dependent regulation of cyclin D1 transcription through a nuclear factor-kappaB-dependent pathway." J Biol Chem 274(36): 25245-9.

Kaibuchi, K., Kuroda, S., Fukata, M. and Nakagawa, M. (1999). "Regulation of cadherin-mediated cell-cell adhesion by the Rho family GTPases." Curr Opin Cell Biol 11(5): 591-6.

Katayama, K., Melendez, J., Baumann, J.M., Leslie, J.R., Chauhan, B.K., Nemkul, N., Lang, R.A., Kuan, C.Y., Zheng, Y. and Yoshida, Y. (2011). "Loss of RhoA in neural progenitor cells causes the disruption of adherens junctions and hyperproliferation." Proc Natl Acad Sci U S A 108(18):7607-12.

Kawauchi, T., Chihama, K., Nabeshima, Y. and Hoshino, M. (2003). "The in vivo roles of STEF/Tiam1, Rac1 and JNK in cortical neuronal migration." EMBO J22(16): 4190-201.

Kholmanskikh, S. S., Dobrin, J. S., Wynshaw-Boris, A., Letourneau, P. C. and Ross, M. E. (2003). "Disregulated RhoGTPases and actin cytoskeleton contribute to the migration defect in Lis1-deficient neurons." J Neurosci 23(25): 8673-81.

Koizumi, H., Tanaka, T. and Gleeson, J. G. (2006). "Doublecortin-like kinase functions with doublecortin to mediate fiber tract decussation and neuronal migration." Neuron 49(1): 55-66.

Komatsu, S., Yano, T., Shibata, M., Tuft, R. A. and Ikebe, M. (2000). "Effects of the regulatory light chain phosphorylation of myosin II on mitosis and cytokinesis of mammalian cells." J Biol Chem 275(44): 34512-20.

Konno, D., Yoshimura, S., Hori, K., Maruoka, H. and Sobue, K. (2005). "Involvement of the phosphatidylinositol 3-kinase/rac1 and cdc42 pathways in radial migration of cortical neurons." J Biol Chem 280(6): 5082-8.

Kosodo, Y. and Huttner, W. B. (2009). "Basal process and cell divisions of neural progenitors in the developing brain." Dev Growth Differ 51(3): 251-61.
Kozma, R., Sarner, S., Ahmed, S. and Lim, L. (1997). "Rho family GTPases and neuronal growth cone remodelling: relationship between increased complexity induced by Cdc42Hs, Rac1, and acetylcholine and collapse induced by RhoA and lysophosphatidic acid." Mol Cell Biol 17(3): 1201-11.
Kriegstein, A. R. and Gotz, M. (2003). "Radial glia diversity: a matter of cell fate." Glia 43(1): 37-43.
Lai, J. M., Wu, S., Huang, D. Y. and Chang, Z. F. (2002). "Cytosolic retention of phosphorylated extracellular signal-regulated kinase and a Rho-associated kinase-mediated signal impair expression of p21(Cip1/Waf1) in phorbol 12-myristate-13- acetate-induced apoptotic cells." Mol Cell Biol 22(21): 7581-92.
Lange, C., Huttner, W. B. and Calegari, F. (2009). "Cdk4/cyclinD1 overexpression in neural stem cells shortens G1, delays neurogenesis, and promotes the generation and expansion of basal progenitors." Cell Stem Cell 5(3): 320-31.
Lauffenburger, D. A. and Horwitz, A. F. (1996). "Cell migration: a physically integrated molecular process." Cell 84(3): 359-69.
Lee, K. S., Schottler, F., Collins, J. L., Lanzino, G., Couture, D., Rao, A., Hiramatsu, K., Goto, Y., Hong, S. C., Caner, H., Yamamoto, H., Chen, Z. F., Bertram, E., Berr, S., Omary, R., Scrable, H., Jackson, T., Goble, J. and Eisenman, L. (1997). "A genetic animal model of human neocortical heterotopia associated with seizures." J Neurosci 17(16): 6236-42.
Letinic, K., Zoncu, R. and Rakic, P. (2002). "Origin of GABAergic neurons in the human neocortex." Nature 417(6889): 645-9.
Li, Z., Hannigan, M., Mo, Z., Liu, B., Lu, W., Wu, Y., Smrcka, A. V., Wu, G., Li, L., Liu, M., Huang, C. K. and Wu, D. (2003). "Directional sensing requires G beta gamma-mediated PAK1 and PIX alpha-dependent activation of Cdc42." Cell114(2): 215-27.
Lien, W. H., Klezovitch, O., Fernandez, T. E., Delrow, J. and Vasioukhin, V. (2006). "alphaE-catenin controls cerebral cortical size by regulating the hedgehog signaling pathway." Science 311(5767): 1609-12.
Luo, L. (2000). "Rho GTPases in neuronal morphogenesis." Nat Rev Neurosci 1(3): 173-80.
Machon, O., van den Bout, C. J., Backman, M., Kemler, R. and Krauss, S. (2003). "Role of beta-catenin in the developing cortical and hippocampal neuroepithelium." Neuroscience 122(1): 129-43.
Magie, C. R., Pinto-Santini, D. and Parkhurst, S. M. (2002). "Rho1 interacts with p120ctn and alpha-catenin, and regulates cadherin-based adherens junction components in Drosophila." Development 129(16): 3771-82.
Malatesta, P., Hack, M. A., Hartfuss, E., Kettenmann, H., Klinkert, W., Kirchhoff, F. and Gotz, M. (2003). "Neuronal or glial progeny: regional differences in radial glia fate." Neuron 37(5): 751-64.
Malatesta, P., Hartfuss, E. and Gotz, M. (2000). "Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage." Development127(24): 5253-63.

Malliri, A., van Es, S., Huveneers, S. and Collard, J. G. (2004). "The Rac exchange factor Tiam1 is required for the establishment and maintenance of cadherin-based adhesions." J Biol Chem 279(29): 30092-8.
Marin, O., Valdeolmillos, M. and Moya, F. (2006). "Neurons in motion: same principles for different shapes?" Trends Neurosci 29(12): 655-61.
Marin-Padilla, M. (1971). "Early prenatal ontogenesis of the cerebral cortex (neocortex) of the cat (Felis domestica). A Golgi study. I. The primordial neocortical organization." Z Anat Entwicklungsgesch 134(2): 117-45.
Matsumura, F., Ono, S., Yamakita, Y., Totsukawa, G. and Yamashiro, S. (1998). "Specific localization of serine 19 phosphorylated myosin II during cell locomotion and mitosis of cultured cells." J Cell Biol 140(1): 119-29.
Messier, P. E. (1978). "Microtubules, interkinetic nuclear migration and neurulation." Experientia 34(3): 289-96.
Mettouchi, A., Klein, S., Guo, W., Lopez-Lago, M., Lemichez, E., Westwick, J. K. and Giancotti, F. G. (2001). "Integrin-specific activation of Rac controls progression through the G(1) phase of the cell cycle." Mol Cell 8(1): 115-27.
Millard, T. H., Sharp, S. J. and Machesky, L. M. (2004). "Signalling to actin assembly via the WASP (Wiskott-Aldrich syndrome protein)-family proteins and the Arp2/3 complex." Biochem J 380(Pt 1): 1-17.
Minden, A., Lin, A., Claret, F. X., Abo, A. and Karin, M. (1995). "Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs." Cell 81(7): 1147-57.
Miralles, F., Posern, G., Zaromytidou, A. I. and Treisman, R. (2003). "Actin dynamics control SRF activity by regulation of its coactivator MAL." Cell113(3): 329-42.
Misson, J. P., Austin, C. P., Takahashi, T., Cepko, C. L. and Caviness, V. S., Jr. (1991). "The alignment of migrating neural cells in relation to the murine neopallial radial glial fiber system." Cereb Cortex 1(3): 221-9.
Misson, J. P., Edwards, M. A., Yamamoto, M. and Caviness, V. S., Jr. (1988). "Identification of radial glial cells within the developing murine central nervous system: studies based upon a new immunohistochemical marker." Brain Res Dev Brain Res 44(1): 95-108.
Miura, Y., Kikuchi, A., Musha, T., Kuroda, S., Yaku, H., Sasaki, T. and Takai, Y. (1993). "Regulation of morphology by rho p21 and its inhibitory GDP/GTP exchange protein (rho GDI) in Swiss 3T3 cells." J Biol Chem 268(1): 510-5.
Miyata, T., Kawaguchi, A., Okano, H. and Ogawa, M. (2001). "Asymmetric inheritance of radial glial fibers by cortical neurons." Neuron 31(5): 727-41.
Miyata, T., Kawaguchi, A., Saito, K., Kawano, M., Muto, T. and Ogawa, M. (2004). "Asymmetric production of surface-dividing and non-surface-dividing cortical progenitor cells." Development 131(13): 3133-45.
Mollgard, K., Balslev, Y., Lauritzen, B. and Saunders, N. R. (1987). "Cell junctions and membrane specializations in the ventricular zone (germinal matrix) of the developing sheep brain: a CSF-brain barrier." J Neurocytol 16(4): 433-44.

Molyneaux, B. J., Arlotta, P., Menezes, J. R. and Macklis, J. D. (2007). "Neuronal subtype specification in the cerebral cortex." Nat Rev Neurosci 8(6): 427-37.
Mori, T., Buffo, A. and Gotz, M. (2005). "The novel roles of glial cells revisited: the contribution of radial glia and astrocytes to neurogenesis." Curr Top Dev Biol 69: 67-99.
Murciano, A., Zamora, J., Lopez-Sanchez, J. and Frade, J. M. (2002). "Interkinetic nuclear movement may provide spatial clues to the regulation of neurogenesis." Mol Cell Neurosci 21(2): 285-300.
Nadarajah, B., Alifragis, P., Wong, R. O. and Parnavelas, J. G. (2003). "Neuronal migration in the developing cerebral cortex: observations based on real-time imaging." Cereb Cortex 13(6): 607-11.
Nadarajah, B., Brunstrom, J. E., Grutzendler, J., Wong, R. O. and Pearlman, A. L. (2001). "Two modes of radial migration in early development of the cerebral cortex." Nat Neurosci 4(2): 143-50.
Nguyen, L., Besson, A., Heng, J. I., Schuurmans, C., Teboul, L., Parras, C., Philpott, A., Roberts, J. M. and Guillemot, F. (2006). "p27kip1 independently promotes neuronal differentiation and migration in the cerebral cortex." Genes Dev 20(11): 1511-24.
Nieto, M., Monuki, E. S., Tang, H., Imitola, J., Haubst, N., Khoury, S. J., Cunningham, J., Gotz, M. and Walsh, C. A. (2004). "Expression of Cux-1 and Cux-2 in the subventricular zone and upper layers II-IV of the cerebral cortex." J Comp Neurol 479(2): 168-80.
Nobes, C. D. and Hall, A. (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.
Nobes, C. D. and Hall, A. (1999). "Rho GTPases control polarity, protrusion, and adhesion during cell movement." J Cell Biol 144(6): 1235-44.
Noctor, S. C., Flint, A. C., Weissman, T. A., Dammerman, R. S. and Kriegstein, A. R. (2001). "Neurons derived from radial glial cells establish radial units in neocortex." Nature 409(6821): 714-20.
Noctor, S. C., Martinez-Cerdeno, V., Ivic, L. and Kriegstein, A. R. (2004). "Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases." Nat Neurosci 7(2): 136-44.
O'Rourke, N. A., Chenn, A. and McConnell, S. K. (1997). "Postmitotic neurons migrate tangentially in the cortical ventricular zone." Development 124(5): 997-1005.
O'Rourke, N. A., Sullivan, D. P., Kaznowski, C. E., Jacobs, A. A. and McConnell, S. K. (1995). "Tangential migration of neurons in the developing cerebral cortex." Development 121(7): 2165-76.
Ohashi, K., Nagata, K., Maekawa, M., Ishizaki, T., Narumiya, S. and Mizuno, K. (2000). "Rho-associated kinase ROCK activates LIM-kinase 1 by phosphorylation at threonine 508 within the activation loop." J Biol Chem 275(5): 3577-82.
Olofsson, B. (1999). "Rho guanine dissociation inhibitors: pivotal molecules in cellular signalling." Cell Signal 11(8): 545-54.
Olson, M. F., Ashworth, A. and Hall, A. (1995). "An essential role for Rho, Rac, and Cdc42 GTPases in cell cycle progression through G1." Science 269(5228): 1270-2.

Olson, M. F., Paterson, H. F. and Marshall, C. J. (1998). "Signals from Ras and Rho GTPases interact to regulate expression of p21Waf1/Cip1." Nature394(6690): 295-9.
Pacary, E., Heng, J., Azzarelli, R., Riou, P., Castro, D., Lebel-Potter, M., Parras, C., Bell, D. M., Ridley, A. J., Parsons, M. and Guillemot, F. (2011). "Proneural transcription factors regulate different steps of cortical neuron migration through Rnd-mediated inhibition of RhoA signaling." Neuron 69(6): 1069-84.
Palazzo, A. F., Cook, T. A., Alberts, A. S. and Gundersen, G. G. (2001). "mDia mediates Rho-regulated formation and orientation of stable microtubules." Nat Cell Biol 3(8): 723-9.
Paszek, M. J., Zahir, N., Johnson, K. R., Lakins, J. N., Rozenberg, G. I., Gefen, A., Reinhart-King, C. A., Margulies, S. S., Dembo, M., Boettiger, D., Hammer, D. A. and Weaver, V. M. (2005). "Tensional homeostasis and the malignant phenotype." Cancer Cell 8(3): 241-54.
Paterson, H. F., Self, A. J., Garrett, M. D., Just, I., Aktories, K. and Hall, A. (1990). "Microinjection of recombinant p21rho induces rapid changes in cell morphology." J Cell Biol 111(3): 1001-7.
Paulsson, M. (1992). "Basement membrane proteins: structure, assembly, and cellular interactions." Crit Rev Biochem Mol Biol 27(1-2): 93-127.
Peterson, F. C., Penkert, R. R., Volkman, B. F. and Prehoda, K. E. (2004). "Cdc42 regulates the Par-6 PDZ domain through an allosteric CRIB-PDZ transition." Mol Cell 13(5): 665-76.
Pilaz, L. J., Patti, D., Marcy, G., Ollier, E., Pfister, S., Douglas, R. J., Betizeau, M., Gautier, E., Cortay, V., Doerflinger, N., Kennedy, H. and Dehay, C. (2009). "Forced G1-phase reduction alters mode of division, neuron number, and laminar phenotype in the cerebral cortex." Proc Natl Acad Sci U S A106(51): 21924-9.
Pinto, L. and Gotz, M. (2007). "Radial glial cell heterogeneity-The source of diverse progeny in the CNS." Prog Neurobiol 83(1): 2-23.
Pinto, L., Mader, M. T., Irmler, M., Gentilini, M., Santoni, F., Drechsel, D., Blum, R., Stahl, R., Bulfone, A., Malatesta, P., Beckers, J. and Gotz, M. (2008). "Prospective isolation of functionally distinct radial glial subtypes--lineage and transcriptome analysis." Mol Cell Neurosci 38(1): 15-42.
Pollard, T. D. and Borisy, G. G. (2003). "Cellular motility driven by assembly and disassembly of actin filaments." Cell 112(4): 453-65.
Pontious, A., Kowalczyk, T., Englund, C. and Hevner, R. F. (2008). "Role of intermediate progenitor cells in cerebral cortex development." Dev Neurosci 30(1-3): 24-32.
Postma, F. R., Jalink, K., Hengeveld, T. and Moolenaar, W. H. (1996). "Sphingosine-1-phosphate rapidly induces Rho-dependent neurite retraction: action through a specific cell surface receptor." EMBO J 15(10): 2388-92.
Price, D. J., Kennedy, H., Dehay, C., Zhou, L., Mercier, M., Jossin, Y., Goffinet, A. M., Tissir, F., Blakey, D. and Molnar, Z. (2006). "The development of cortical connections." Eur J Neurosci 23(4): 910-20.
Puls, A., Eliopoulos, A. G., Nobes, C. D., Bridges, T., Young, L. S. and Hall, A. (1999). "Activation of the small GTPase Cdc42 by the inflammatory cytokines TNF(alpha) and IL-1, and by the Epstein-Barr virus transforming protein LMP1." J Cell Sci 112 (Pt 17): 2983-92.
Raftopoulou, M. and Hall, A. (2004). "Cell migration: Rho GTPases lead the way." Dev Biol 265(1): 23-32.

Rakic, P. (1971). "Neuron-glia relationship during granule cell migration in developing cerebellar cortex. A Golgi and electronmicroscopic study in Macacus Rhesus." J Comp Neurol 141(3): 283-312.
Rakic, P. (1972). "Mode of cell migration to the superficial layers of fetal monkey neocortex." J Comp Neurol 145(1): 61-83.
Rakic, P. (1990). "Principles of neural cell migration." Experientia 46(9): 882-91.
Ramakers, G. J. (2002). "Rho proteins, mental retardation and the cellular basis of cognition." Trends Neurosci 25(4): 191-9.
Ramon y Cajal, S. (1995). Histology of the nervous system of man and vertebrates, Oxford University Press, New York.
Reiner, O., Carrozzo, R., Shen, Y., Wehnert, M., Faustinella, F., Dobyns, W. B., Caskey, C. T. and Ledbetter, D. H. (1993). "Isolation of a Miller-Dieker lissencephaly gene containing G protein beta-subunit-like repeats." Nature364(6439): 717-21.
Reiner, O. and Coquelle, F. M. (2005). "Missense mutations resulting in type 1 lissencephaly." Cell Mol Life Sci 62(4): 425-34.
Ridet, J. L., Malhotra, S. K., Privat, A. and Gage, F. H. (1997). "Reactive astrocytes: cellular and molecular cues to biological function." Trends Neurosci 20(12): 570-7.
Ridley, A. J. (2001). "Rho GTPases and cell migration." J Cell Sci 114(Pt 15): 2713-22.
Ridley, A. J., Comoglio, P. M. and Hall, A. (1995). "Regulation of scatter factor/hepatocyte growth factor responses by Ras, Rac, and Rho in MDCK cells." Mol Cell Biol 15(2): 1110-22.
Ridley, A. J. and Hall, A. (1992). "The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors." Cell 70(3): 389-99.
Ridley, A. J., Schwartz, M. A., Burridge, K., Firtel, R. A., Ginsberg, M. H., Borisy, G., Parsons, J. T. and Horwitz, A. R. (2003). "Cell migration: integrating signals from front to back." Science 302(5651): 1704-9.
Rivas, R. J. and Hatten, M. E. (1995). "Motility and cytoskeletal organization of migrating cerebellar granule neurons." J Neurosci 15(2): 981-9.
Robel, S., Bardehle, S., Lepier, A., Brakebusch, C. and Gotz, M. (2011). "Genetic deletion of cdc42 reveals a crucial role for astrocyte recruitment to the injury site in vitro and in vivo." J Neurosci 31(35): 12471-82.
Robel, S., Mori, T., Zoubaa, S., Schlegel, J., Sirko, S., Faissner, A., Goebbels, S., Dimou, L. and Gotz, M. (2009). "Conditional deletion of beta1-integrin in astroglia causes partial reactive gliosis." Glia.
Robertson, S. P. (2004). "Molecular pathology of filamin A: diverse phenotypes, many functions." Clin Dysmorphol 13(3): 123-31.
Roovers, K. and Assoian, R. K. (2003). "Effects of rho kinase and actin stress fibers on sustained extracellular signal-regulated kinase activity and activation of G(1) phase cyclin-dependent kinases." Mol Cell Biol 23(12): 4283-94.
Roovers, K., Klein, E. A., Castagnino, P. and Assoian, R. K. (2003). "Nuclear translocation of LIM kinase mediates Rho-Rho kinase regulation of cyclin D1 expression." Dev Cell 5(2): 273-84.

Rosenblatt, J., Cramer, L. P., Baum, B. and McGee, K. M. (2004). "Myosin II-dependent cortical movement is required for centrosome separation and positioning during mitotic spindle assembly." Cell 117(3): 361-72.
Ross, M. E. and Walsh, C. A. (2001). "Human brain malformations and their lessons for neuronal migration." Annu Rev Neurosci 24: 1041-70.
Sahai, E. and Marshall, C. J. (2003). "Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis." Nat Cell Biol 5(8): 711-9.
Sahai, E., Olson, M. F. and Marshall, C. J. (2001). "Cross-talk between Ras and Rho signalling pathways in transformation favours proliferation and increased motility." Embo J 20(4): 755-66.
Satz, J. S., Ostendorf, A. P., Hou, S., Turner, A., Kusano, H., Lee, J. C., Turk, R., Nguyen, H., Ross-Barta, S. E., Westra, S., Hoshi, T., Moore, S. A. and Campbell, K. P. (2010). "Distinct functions of glial and neuronal dystroglycan in the developing and adult mouse brain." J Neurosci 30(43): 14560-72.
Sauer, F. C. (1935). "Mitosis in the neural tube." The Journal of Comparative Neurology 62(2): 377-405.
Schaar, B. T. and McConnell, S. K. (2005). "Cytoskeletal coordination during neuronal migration." Proc Natl Acad Sci U S A 102(38): 13652-7.
Schenk, J., Wilsch-Brauninger, M., Calegari, F. and Huttner, W. B. (2009). "Myosin II is required for interkinetic nuclear migration of neural progenitors." Proc Natl Acad Sci U S A 106(38): 16487-92.
Schmid, M.-T. (2008). Comparative analysis of the function of alpha- and beta-catenin in cerebral cortical development. Munich, Technische Universität München. Dr. rer. nat.
Schmidt, A. and Hall, A. (2002). "Guanine nucleotide exchange factors for Rho GTPases: turning on the switch." Genes Dev 16(13): 1587-609.
Schnitzer, J., Franke, W. W. and Schachner, M. (1981). "Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system." J Cell Biol 90(2): 435-47.
Schottler, F., Fabiato, H., Leland, J. M., Chang, L. Y., Lotfi, P., Getachew, F. and Lee, K. S. (2001). "Normotopic and heterotopic cortical representations of mystacial vibrissae in rats with subcortical band heterotopia." Neuroscience108(2): 217-35.
Schwamborn, J. C. and Puschel, A. W. (2004). "The sequential activity of the GTPases Rap1B and Cdc42 determines neuronal polarity." Nat Neurosci 7(9): 923-9.
Seri, B., Garcia-Verdugo, J. M., Collado-Morente, L., McEwen, B. S. and Alvarez-Buylla, A. (2004). "Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus." J Comp Neurol 478(4): 359-78.
Seri, B., Garcia-Verdugo, J. M., McEwen, B. S. and Alvarez-Buylla, A. (2001). "Astrocytes give rise to new neurons in the adult mammalian hippocampus." J Neurosci 21(18): 7153-60.
Sheen, V. L., Ganesh, V. S., Topcu, M., Sebire, G., Bodell, A., Hill, R. S., Grant, P. E., Shugart, Y. Y., Imitola, J., Khoury, S. J., Guerrini, R. and Walsh, C. A. (2004). "Mutations in ARFGEF2 implicate vesicle trafficking in neural progenitor proliferation and migration in the human cerebral cortex." Nat Genet 36(1): 69-76.

Shi, S. H., Jan, L. Y. and Jan, Y. N. (2003). "Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity." Cell112(1): 63-75.
Shibata, T., Yamada, K., Watanabe, M., Ikenaka, K., Wada, K., Tanaka, K. and Inoue, Y. (1997). "Glutamate transporter GLAST is expressed in the radial glia-astrocyte lineage of developing mouse spinal cord." J Neurosci 17(23): 9212-9.
Shipp, S. (2007). "Structure and function of the cerebral cortex." Curr Biol 17(12): R443-9.
Shoukimas, G. M. and Hinds, J. W. (1978). "The development of the cerebral cortex in the embryonic mouse: an electron microscopic serial section analysis." J Comp Neurol 179(4): 795-830.
Smart, I. H. (1973). "Proliferative characteristics of the ependymal layer during the early development of the mouse neocortex: a pilot study based on recording the number, location and plane of cleavage of mitotic figures." J Anat116(Pt 1): 67-91.
Soriano, E. and Del Rio, J. A. (2005). "The cells of cajal-retzius: still a mystery one century after." Neuron 46(3): 389-94.
Stasia, M. J., Jouan, A., Bourmeyster, N., Boquet, P. and Vignais, P. V. (1991). "ADP-ribosylation of a small size GTP-binding protein in bovine neutrophils by the C3 exoenzyme of Clostridium botulinum and effect on the cell motility." Biochem Biophys Res Commun 180(2): 615-22.
Suzuki, A., Yamanaka, T., Hirose, T., Manabe, N., Mizuno, K., Shimizu, M., Akimoto, K., Izumi, Y., Ohnishi, T. and Ohno, S. (2001). "Atypical protein kinase C is involved in the evolutionarily conserved par protein complex and plays a critical role in establishing epithelia-specific junctional structures." J Cell Biol 152(6): 1183-96.
Tabata, H., Kanatani, S. and Nakajima, K. (2009). "Differences of Migratory Behavior between Direct Progeny of Apical Progenitors and Basal Progenitors in the Developing Cerebral Cortex." Cereb Cortex.
Tabata, H. and Nakajima, K. (2003). "Multipolar migration: the third mode of radial neuronal migration in the developing cerebral cortex." J Neurosci 23(31): 9996-10001.
Tahirovic, S., Hellal, F., Neukirchen, D., Hindges, R., Garvalov, B. K., Flynn, K. C., Stradal, T. E., Chrostek-Grashoff, A., Brakebusch, C. and Bradke, F. (2010). "Rac1 regulates neuronal polarization through the WAVE complex." J Neurosci 30(20): 6930-43.
Takahashi, T., Nowakowski, R. S. and Caviness, V. S., Jr. (1995). "Early ontogeny of the secondary proliferative population of the embryonic murine cerebral wall." J Neurosci 15(9): 6058-68.
Takaishi, K., Kikuchi, A., Kuroda, S., Kotani, K., Sasaki, T. and Takai, Y. (1993). "Involvement of rho p21 and its inhibitory GDP/GTP exchange protein (rho GDI) in cell motility." Mol Cell Biol 13(1): 72-9.
Takaishi, K., Sasaki, T., Kotani, H., Nishioka, H. and Takai, Y. (1997). "Regulation of cell-cell adhesion by rac and rho small G proteins in MDCK cells." J Cell Biol 139(4): 1047-59.
Tarabykin, V., Stoykova, A., Usman, N. and Gruss, P. (2001). "Cortical upper layer neurons derive from the subventricular zone as indicated by Svet1 gene expression." Development 128(11): 1983-93.
Taverna, E. and Huttner, W. B. (2010). "Neural progenitor nuclei IN motion." Neuron67(6): 906-14.

Teramoto, H., Coso, O. A., Miyata, H., Igishi, T., Miki, T. and Gutkind, J. S. (1996). "Signaling from the small GTP-binding proteins Rac1 and Cdc42 to the c-Jun N-terminal kinase/stress-activated protein kinase pathway. A role for mixed lineage kinase 3/protein-tyrosine kinase 1, a novel member of the mixed lineage kinase family." J Biol Chem 271(44): 27225-8.
Timpl, R. (1996). "Macromolecular organization of basement membranes." Curr Opin Cell Biol 8(5): 618-24.
Trotter, S. A., Kapur, J., Anzivino, M. J. and Lee, K. S. (2006). "GABAergic synaptic inhibition is reduced before seizure onset in a genetic model of cortical malformation." J Neurosci 26(42): 10756-67.
Tzima, E., Kiosses, W. B., del Pozo, M. A. and Schwartz, M. A. (2003). "Localized cdc42 activation, detected using a novel assay, mediates microtubule organizing center positioning in endothelial cells in response to fluid shear stress." J Biol Chem 278(33): 31020-3.
Vaezi, A., Bauer, C., Vasioukhin, V. and Fuchs, E. (2002). "Actin cable dynamics and Rho/Rock orchestrate a polarized cytoskeletal architecture in the early steps of assembling a stratified epithelium." Dev Cell 3(3): 367-81.
Vartiainen, M. K., Guettler, S., Larijani, B. and Treisman, R. (2007). "Nuclear actin regulates dynamic subcellular localization and activity of the SRF cofactor MAL." Science 316(5832): 1749-52.
Vasioukhin, V., Bauer, C., Yin, M. and Fuchs, E. (2000). "Directed actin polymerization is the driving force for epithelial cell-cell adhesion." Cell100(2): 209-19.
Vasioukhin, V. and Fuchs, E. (2001). "Actin dynamics and cell-cell adhesion in epithelia." Curr Opin Cell Biol 13(1): 76-84.
Vial, E., Sahai, E. and Marshall, C. J. (2003). "ERK-MAPK signaling coordinately regulates activity of Rac1 and RhoA for tumor cell motility." Cancer Cell 4(1): 67-79.
Vidal, A., Millard, S. S., Miller, J. P. and Koff, A. (2002). "Rho activity can alter the translation of p27 mRNA and is important for RasV12-induced transformation in a manner dependent on p27 status." J Biol Chem 277(19): 16433-40.
Viti, J., Gulacsi, A. and Lillien, L. (2003). "Wnt regulation of progenitor maturation in the cortex depends on Shh or fibroblast growth factor 2." J Neurosci 23(13): 5919-27.
Walsh, C. and Cepko, C. L. (1992). "Widespread dispersion of neuronal clones across functional regions of the cerebral cortex." Science 255(5043): 434-40.
Watanabe, T., Wang, S., Noritake, J., Sato, K., Fukata, M., Takefuji, M., Nakagawa, M., Izumi, N., Akiyama, T. and Kaibuchi, K. (2004). "Interaction with IQGAP1 links APC to Rac1, Cdc42, and actin filaments during cell polarization and migration." Dev Cell 7(6): 871-83.
Weber, J. D., Hu, W., Jefcoat, S. C., Jr., Raben, D. M. and Baldassare, J. J. (1997). "Ras-stimulated extracellular signal-related kinase 1 and RhoA activities coordinate platelet-derived growth factor-induced G1 progression through the independent regulation of cyclin D1 and p27." J Biol Chem 272(52): 32966-71.
Welsh, C. F., Roovers, K., Villanueva, J., Liu, Y., Schwartz, M. A. and Assoian, R. K. (2001). "Timing of cyclin D1 expression within G1 phase is controlled by Rho." Nat Cell Biol 3(11): 950-7.

Wen, Y., Eng, C. H., Schmoranzer, J., Cabrera-Poch, N., Morris, E. J., Chen, M., Wallar, B. J., Alberts, A. S. and Gundersen, G. G. (2004). "EB1 and APC bind to mDia to stabilize microtubules downstream of Rho and promote cell migration." Nat Cell Biol 6(9): 820-30.

Westwick, J. K., Lambert, Q. T., Clark, G. J., Symons, M., Van Aelst, L., Pestell, R. G. and Der, C. J. (1997). "Rac regulation of transformation, gene expression, and actin organization by multiple, PAK-independent pathways." Mol Cell Biol 17(3): 1324-35.

Wood, J. G., Martin, S. and Price, D. J. (1992). "Evidence that the earliest generated cells of the murine cerebral cortex form a transient population in the subplate and marginal zone." Brain Res Dev Brain Res 66(1): 137-40.

Wu, S. X., Goebbels, S., Nakamura, K., Nakamura, K., Kometani, K., Minato, N., Kaneko, T., Nave, K. A. and Tamamaki, N. (2005). "Pyramidal neurons of upper cortical layers generated by NEX-positive progenitor cells in the subventricular zone." Proc Natl Acad Sci U S A 102(47): 17172-7.

Wu, X., Quondamatteo, F., Lefever, T., Czuchra, A., Meyer, H., Chrostek, A., Paus, R., Langbein, L. and Brakebusch, C. (2006). "Cdc42 controls progenitor cell differentiation and beta-catenin turnover in skin." Genes Dev 20(5): 571-85.

Yamamoto, M., Marui, N., Sakai, T., Morii, N., Kozaki, S., Ikai, K., Imamura, S. and Narumiya, S. (1993). "ADP-ribosylation of the rhoA gene product by botulinum C3 exoenzyme causes Swiss 3T3 cells to accumulate in the G1 phase of the cell cycle." Oncogene 8(6): 1449-55.

Yamanaka, T., Horikoshi, Y., Suzuki, A., Sugiyama, Y., Kitamura, K., Maniwa, R., Nagai, Y., Yamashita, A., Hirose, T., Ishikawa, H. and Ohno, S. (2001). "PAR-6 regulates aPKC activity in a novel way and mediates cell-cell contact-induced formation of the epithelial junctional complex." Genes Cells 6(8): 721-31.

Yamashiro, S., Totsukawa, G., Yamakita, Y., Sasaki, Y., Madaule, P., Ishizaki, T., Narumiya, S. and Matsumura, F. (2003). "Citron kinase, a Rho-dependent kinase, induces di-phosphorylation of regulatory light chain of myosin II." Mol Biol Cell 14(5): 1745-56.

Yoshio, T., Morita, T., Tsujii, M., Hayashi, N. and Sobue, K. "MRTF-A/B suppress the oncogenic properties of v-ras- and v-src-mediated transformants." Carcinogenesis.

Yoshizawa, M., Kawauchi, T., Sone, M., Nishimura, Y. V., Terao, M., Chihama, K., Nabeshima, Y. and Hoshino, M. (2005). "Involvement of a Rac activator,P-Rex1, in neurotrophin-derived signaling and neuronal migration." J Neurosci 25(17): 4406-19.

Zhong, W. and Chia, W. (2008). "Neurogenesis and asymmetric cell division." Curr Opin Neurobiol 18(1): 4-11.

Zigmond, S. H. (2004). "Formin-induced nucleation of actin filaments." Curr Opin Cell Biol 16(1): 99-105.

Zimmer, C., Tiveron, M. C., Bodmer, R. and Cremer, H. (2004). "Dynamics of Cux2 expression suggests that an early pool of SVZ precursors is fated to become upper cortical layer neurons." Cereb Cortex 14(12): 1408-20.

I want to thank

My wife Zoofa for giving me strength: Her love, support, patience and understanding over many years was vital for completing this work.
Magdalena Götz for supervision and Carsten Culmsee for co-supervision.
My dear parents for their kind support.
My helpful colleagues at the ISF, in particular Franziska Weinandy.
My family and friends (including my Persian family) for believing in me.

Thank you!