Profilin1 is required for glial cell adhesion and radial migration of cerebellar granule neurons

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Cerebellar granule neurons (CGNs) exploit Bergmann glia (BG) fibres for radial migration, and cell–cell contacts have a pivotal role in this process. Nevertheless, little is known about the mechanisms that control CGN–BG interaction. Here we demonstrate that the actin-binding protein profilin1 is essential for CGN–glial cell adhesion and radial migration. Profilin1 ablation from mouse brains leads to a cerebellar hypoplasia, aberrant organization of cerebellar cortex layers and ectopic CGNs. Conversely, neuronal progenitor proliferation, tangential migration of neurons and BG morphology appear to be independent of profilin1. Our mouse data and the mapping of developmental neuropathies to the chromosomal region of *PFN1* suggest a similar function for profilin1 in humans.

Keywords: profilin1; actin dynamics; radial migration; cerebellum; lissencephaly

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INTRODUCTION

Neuronal migration defects are an important aspect in human diseases such as lissencephaly, mental retardation and epilepsy (Ayala *et al*, 2007). Hence, a comprehensive understanding of the mechanisms controlling neuronal migration is essential for diagnosing and treating human neuropathies. Two types of neuronal migration dominate during the development of layered brain structures: glial cell (GC)-independent tangential migration and GC-guided radial migration (Chedotal, 2010). In higher vertebrates, radial migration is performed by principal output neurons in the cerebral cortex, as well as Purkinje cells (PCs) and

cerebellar granule neurons (CGNs). After exiting from the cell cycle, CGNs migrate tangentially within the external granule cell layer (EGL) and then radially along Bergmann glia (BG) fibres, whereby they cross the molecular layer (ML) and reach the internal granule cell layer (IGL; Chedotal, 2010). Thus, CGNs perform tangential and radial migration, and, as both occur solely after birth, they provide a very suitable model to experimentally approach neuronal migration.

Pharmacological and genetic studies have emphasized a role of actin in neuronal migration (Rivas & Hatten, 1995; Bellenchi *et al*, 2007). Similarly, mutations in actin regulators can cause neuronal migration defects in humans (Ayala *et al*, 2007). However, the mechanisms that control actin dynamics in migrating neurons remain largely elusive. Genetic studies have established a key role for profilins in actin dynamics (Witke, 2004). Although profilins were implicated in cell motility in endothelial cells or mouse chondrocytes (Ding *et al*, 2006; Bottcher *et al*, 2009), their specific function in neuronal migration has not been investigated to date. Of the four mammalian profilins, only profilin1 and profilin2 are expressed in the brain (Witke, 2004). Brain development is normal in profilin2 mutants, and hence a role in neuronal migration was hypothesized for profilin1 (Pilo Boyl *et al*, 2007). We here analysed brain-specific profilin1 mouse mutants and found that profilin1 is crucial for radial migration of CGNs. Moreover, we provide evidence that radial migration of CGNs depends on profilin1 through a mechanism that involves GC adhesion. Profilin1 mutants develop cerebellar hypoplasia, aberrant organization of cerebellar cortex layers and ectopic CGNs. Our data demonstrate that profilin1 is indispensable for normal mouse brain development and imply that profilin1 dysfunction might contribute to human developmental neuropathies.

RESULTS AND DISCUSSION

Cerebellar hypoplasia in brain-specific profilin1 mutants

Systemic profilin1 deletion in mice results in early embryonic lethality (Witke *et al*, 2001). To test whether profilin1 is relevant for neuronal migration, we deleted profilin1 during brain development by crossing a conditional profilin1 mouse model with a nestin-cre transgenic line (Tronche *et al*, 1999; Bottcher

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Fig 1 | Cerebellar hypoplasia in profilin1 mutants. (A) Immunoblot analysis proved efficient profilin1 deletion in mutant brains. Profilin2 levels were unchanged. Actin served as a loading control. (B) The cerebellum (arrow) was smaller in P60 mutants. Scale bar, 3 mm. (C,D) Nissl-stained parasagittal sections of a control and a mutant at P8 (C) and P60 (D). Asterisks depict folia 4/5 that were used for morphometric analysis (Table 1) and are shown at a higher magnification in Fig 2. Scale bar, 1 mm. (E) In adult huGE mice expressing GFP actin under the control of the profilin1 promoter, GFP signal was intense in the CGN-containing IGL and the ML, but not in the white matter. Scale bar, 50 μ m. (F,G) Moreover, GFP signal was present in both calbindin-positive PC (F) and GFAP-positive BG (G). Scale bar, 20 μ m in F and G. BG, Bergmann glia; CGN, cerebellar granule neuron; E, embryonic day; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; IGL, internal granule cell layer; ML, molecular layer; P, postnatal day; PC, Purkinje cells.

et al, 2009). Immunoblot analysis demonstrated efficient deletion of profilin1 in mutant brains without compensatory profilin2 overexpression (Fig 1A). Hence, our mutants proved to be suitable for investigating the role of profilin1 in brain development.

Adult mutants showed a reduced brain size; the cerebellum especially appeared considerably smaller (Fig 1B). Indeed,

analysis of parasagittal sections showed a roughly 50% reduction of cerebellum size, whereas the area of the cerebral cortex was reduced only by about 25% (supplementary Fig S1A,B online). As the latter change roughly reflects the body-weight reduction seen in adult mutants (supplementary Fig S1C online), we concluded that profilin1 might be specifically relevant in the cerebellum and

Table 1 | Morphometric analysis of folia 4/5 at P8 and P60

	P8			P60		
	Pfn1 ^{flx/flx}		Pfn1 ^{flx/flxNes-cre}	Pfn1 ^{flx/flx}		Pfn1 ^{flx/flxNes-cre}
IGL (μm)	102.9 ± 3.3	*	83.2 ± 1.4	187.5 ± 4.9	**	151.1 ± 3.5
ML (μm)	68.6 ± 6.6	*	53.2 ± 1.1	185.8 ± 7.1	*	159.6 ± 4.0
EGL (μm)	50.9 ± 3.0	NS	45.9 ± 1.6			
Cells in EGL (100 μm ⁻²)	401.5 ± 9.5	NS	410.7 ± 8.2			

Shown are the mean values (± s.e.m.) of folia 4/5 length and width, the widths of the ML, IGL and EGL in folium 4 as well as the EGL cell density. **P* < 0.05, ***P* < 0.01. EGL, external granule cell layer; IGL, internal granule cell layer; ML, molecular layer; NS, not significant.

investigated its development in further detail. Nissl staining revealed the presence of all cerebellar folia and cell layers (IGL, PC layer (PCL), ML, EGL) in mutants (Fig 1C,D), and a reduction of the cerebellum size by 35–50% throughout postnatal development (supplementary Fig S1D online). Notably, IGL and ML widths were significantly reduced in mutants at postnatal day (P) 8 and P60, whereas EGL width and cell density were unchanged (Table 1).

To examine which cells of the cerebellar cortex express profilin1, we used huGE mice that express green fluorescent protein (GFP)-tagged actin under the control of the profilin1 promoter (Gurniak & Witke, 2007). In adult huGE mice, GFP signal intensity was abundant in the CGN-containing IGL and the ML, but not in the cerebellar white matter (Fig 1E). Further analysis revealed the presence of profilin1-driven GFP in calbindin-positive PC, as well as in glial fibrillary acidic protein (GFAP)-positive BG (Fig 1F,G).

Normal differentiation of ectopic CGN in mutant ML

At high magnification, Nissl-stained sections revealed various defects in the cerebellar cortex cytoarchitecture in profilin1 mutants (Fig 2A–C). First, we found an aberrant organization of the cerebellar layers, a difference that was most evident when viewing the PCL. Whereas no change was found in P8 mutants, as confirmed by calbindin staining (supplementary Fig S2A online), the PCL organization was clearly irregular at P60 (Fig 2C; supplementary Fig S2B online). Second, gaps within the PCL, which were not present in controls or P8 mutants, were detectable in adult mutants. Most strikingly, we noted an increase in the ML cell density in mutants at P12 and P60 (Fig 2D).

As CGNs are generated in the EGL and migrate across the ML during postnatal development (Carletti & Rossi, 2008), the surplus cells in the mutant ML might be CGNs. To test this hypothesis, we used an antibody against neuron-specific nuclear protein (NeuN), as NeuN is solely expressed by CGNs in the cerebellar cortex (Weyer & Schilling, 2003). We found a roughly fivefold increase in the number of NeuN-positive cells in the mutant ML, whereas the density of NeuN-negative cells, probably representing interneurons (stellate and basket cells), was unchanged (Fig 2E,F). Thus, our data revealed normal interneuron numbers in the mutant ML and the presence of ectopic CGNs.

To address the important question of whether these ectopic CGNs differentiate and mature normally, we next analysed the immunoreactivity of CaMKIV (Ca²⁺/calmodulin-dependent

protein kinase IV), a marker for mature, postmigratory CGNs, and of VGluT2 (vesicular glutamate transporter 2), which is expressed in CGNs innervating mossy fibres (Kerjan *et al*, 2005). In contrast to the control ML, we found CaMKIV-positive CGNs in the ML of mutants (supplementary Fig S2C online). In addition, we frequently found cells surrounded by rosette-shaped VGluT2 signals typical for mossy fibre terminals in the mutant ML, but not in the control ML (supplementary Fig S2D online). Together, these data suggest that the ectopic CGNs in profilin1 mutants differentiate normally, and that they receive synaptic input.

Impaired radial CGN migration in profilin1 mutants

The increased ML CGN count is in agreement with an elevated CGN production and defective radial migration. To pinpoint these two possibilities, we performed 5-bromo-2-deoxyuridine (BrdU) pulse-labelling experiments *in vivo*. By counting the numbers of BrdU-positive (BrdU⁺) cells in the EGL 3 h after injection, we quantified CGN production in the mutant EGL at P8 and P12 and found unchanged numbers (supplementary Fig S3A,B online). Similarly, numbers of cells positive for the mitotic marker phospho-histoneH3 (pH3) were similar in control and mutant EGL (supplementary Fig S3C,D online). Moreover, normal pH3-positive cell counts in the cerebellar white matter at P8 revealed that profilin1 is also not relevant for interneuron production (supplementary Fig S3E online). From these data we conclude that profilin1 is dispensable for the proliferation of interneuron or CGN progenitors. Conversely, profilin1 was implicated in cell-cycle regulation in early embryos and chondrocytes (Witke *et al*, 2001; Bottcher *et al*, 2009). Thus, our data suggest cell-type-specific functions of profilin1, which is relevant for cell division in certain cell types, but not in neuronal progenitors.

To test whether profilin1 is relevant for radial migration of CGNs, we injected BrdU at P8—a time point at which CGN proliferation is high (supplementary Fig S3B online)—and quantified migration of BrdU⁺ cells 48, 72 and 96 h after BrdU injection (Fig 3A–C). We found the mean migration distances to be reduced in mutants at all three time points (Fig 3D). Moreover, the relative distribution of BrdU⁺ cells in the mutant cerebellar cortex was significantly altered (supplementary Fig S4A–C online). Together, our BrdU tracing experiments allowed us to conclude that profilin1 is important for radial migration of CGNs. In agreement with our conclusion, we found the relative number of rounded BrdU⁺ cells increased in the mutant ML (supplementary Fig S4D online). A rounded shape is typical for non-migrating

Fig 2 | Ectopic CGNs in profilin1 mutants. (A) Nissl staining revealed normal cerebellar cortex cytoarchitecture in P8 mutants. Scale bar, 50 μm in A–C. (B) In P12 mutants, layering of the cerebellar cortex was normal, whereas the ML cell density appeared higher. (C) Increased ML cell density, an irregularly shaped PCL (dashed lines) and gaps within the PCL (arrowhead) were noted in P60 mutants. (D) ML cell densities were significantly increased in mutants at P12 (controls: $4,702 \pm 222$ cells per mm^2 ; mutants: $7,411 \pm 206$ cells per mm^2 ; $n = 12$ images from 6 mice; $P < 0.001$) and P60 (controls: $1,975 \pm 150$ cells per mm^2 ; mutants: $3,397 \pm 119$ cells per mm^2 ; $n = 16/8$; $P < 0.001$), but not at P8 (controls: $5,182 \pm 221$ cells per mm^2 ; mutants: $6,069 \pm 387$ cells per mm^2 ; $n = 6/3$; $P = 0.07$). (E) NeuN immunoreactivity (green) and PI counterstaining (magenta) in the ML at P60. Scale bar, 40 μm . Yellow arrowheads indicate PI⁺/NeuN⁻ cells, whereas blue arrowheads indicate PI⁺/NeuN⁺ cells. (F) Although the number of PI⁺/NeuN⁻ cells was unchanged (controls: $1,971 \pm 88$ cells per mm^2 ; mutants: $1,789 \pm 115$ cells per mm^2 ; $n = 6/3$; $P = 0.242$), the number of PI⁺/NeuN⁺ cells was increased in the mutant ML (controls: 292 ± 42 cells per mm^2 ; mutants: $1,459 \pm 76$ cells per mm^2 ; $P < 0.001$). CGN, cerebellar granule neuron; EGL, external granule cell layer; IGL, internal granule cell layer; ML, molecular layer; NeuN, neuron-specific nuclear protein; NS, not significant; P, postnatal day; PCL, Purkinje cell layer; PI, propidium iodide.

CGNs, whereas for migrating CGNs a characteristic elongated shape was described (Adams *et al*, 2002). Notably, absolute numbers of BrdU⁺ cells were similar in controls and mutants 96 h after BrdU injection, indicating normal CGN survival rates in the absence of profilin1 (supplementary Fig S4E online).

Profilin1 in CGN and GC is required for radial migration

CGNs exploit BG processes for their radial migration across the ML. Nestin-mediated cre expression removes profilin1 from all brain cells, neurons and GCs (Tronche *et al*, 1999). Thus, impaired migration of mutant CGNs could be cell autonomous and/or caused by an abnormal BG organization or morphology. To test whether profilin1 is relevant for BG organization, we visualized BG by using a GFAP antibody. Doing so, we found no changes in

the organization or density of mutant BG, either at the early postnatal stages at which CGN migration was disturbed or in the adult cerebellum (supplementary Fig S5A–C online). Analysis of Alexa Fluor 488-labelled mutant BG revealed that their morphology was grossly normal. In mutant BG, the number of radial processes and maximal width were unchanged (supplementary Fig S5D–F online). In correlation with the reduced ML width (Table 1), the length of BG processes was reduced by 15% in mutants. Together, our data suggest that the defect in radial migration of CGNs is not caused by an abnormal BG organization or morphology.

To conclusively test whether profilin1 activity in CGNs and/or in GCs is required for radial migration, we next performed time-lapse experiments with isolated CGNs that migrate along

Fig 3 | Impaired radial migration of cerebellar granule neurons (CGNs) in profilin1 mutants. (A–C) Representative images of BrdU⁺ cells (green) in the cerebellar cortex (A) 48 h, (B) 72 h and (C) 96 h after BrdU injection at postnatal day (P) 8. Sections were counterstained with PI (magenta). Examples for rounded (white arrowheads) and elongated (yellow arrowheads) CGNs located in the ML are shown in C. Scale bar, 50 μm for A–C. (D) Mean migration distance of BrdU⁺ cells was reduced in mutants at all time points (48 h: controls: 79.4 ± 3.4 μm; mutants: 55.0 ± 4.4 μm; $n = 6$ images from 3 mice; $P < 0.01$; 72 h: controls: 109.7 ± 3.7 μm; mutants: 97.6 ± 8.6 μm; $n = 6/3$; $P < 0.05$; 96 h: controls: 169.5 ± 8.7 μm; mutants: 111.5 ± 7.5 μm; $n = 6/3$; $P < 0.001$). BrdU, 5-bromo-2-deoxyuridine; CGN, cerebellar granule neuron; EGL, external granule cell layer; IGL, internal granule cell layer; ML, molecular layer; PI, propidium iodide.

Fig 4 | Profilin1 activity in CGNs and GC is required for CGN-GC adhesion and radial migration. (A) Representative time-lapse images showing reduced migration of profilin1-deficient CGNs (CGN^{-/-}) along processes of control GC (GC^{+/+}) and of control CGN (CGN^{+/+}) along processes of profilin1-deficient GC (GC^{-/-}). Scale bar, 10 μm . (B) When profilin1 was absent in CGNs (CGN^{-/-}/GC^{+/+}) or in GC (CGN^{+/+}/GC^{-/-}), mean CGN migration velocity was significantly reduced (CGN^{+/+}/GC^{+/+}: $56.3 \pm 4.5 \mu\text{m h}^{-1}$, $n = 17$; CGN^{-/-}/GC^{+/+}: $38.4 \pm 2.7 \mu\text{m h}^{-1}$, $n = 21$, $P < 0.01$; CGN^{+/+}/GC^{-/-}: $39.7 \pm 3.1 \mu\text{m h}^{-1}$, $n = 16$, $P < 0.01$). (C) Compared with adhesion of control CGNs and control GC (CGN^{+/+}/GC^{+/+}: 22.6 ± 1.7 cells per mm^2 ; $n = 20$), CGN-GC adhesion was significantly impaired when profilin1 was absent from either CGNs (CGN^{-/-}/GC^{+/+}: 11.6 ± 1.0 cells per mm^2 ; $n = 11$; $P < 0.001$) or GC (CGN^{+/+}/GC^{-/-}: 7.4 ± 1.5 cells per mm^2 ; $n = 8$; $P < 0.001$). (D) Mena and vinculin immunoreactivity in isolated CGN^{+/+} and CGN^{-/-} attached to processes of GC^{+/+} (GCP). Scale bar, 5 μm . Dashed line in the upper left micrograph exemplarily illustrates CGN-GC junctions as chosen for further analyses. (E) Mean Mena and vinculin fluorescence intensities in CGN-GC junctions were reduced when profilin1 was absent from CGNs (Mena: CGN^{+/+}/GC^{+/+}: 48.0 ± 5.1 AU; CGN^{-/-}/GC^{+/+}: 26.3 ± 4.5 AU; $n = 8$; $P < 0.01$; vinculin: CGN^{+/+}/GC^{+/+}: 37.1 ± 3.8 AU; CGN^{-/-}/GC^{+/+}: 19.5 ± 3.7 AU; $n = 9$; $P < 0.01$). AU, arbitrary units; CGN, cerebellar granule neuron; GC, glial cell.

GC processes (Fig 4A). We chose a complementary experimental setup to distinguish between profilin1 activity in CGNs and GCs. Profilin1-deficient CGNs (CGN^{-/-}) were plated on top of control GCs (GC^{+/+}), and, vice versa, control CGNs (CGN^{+/+}) on mutant GCs (GC^{-/-}). As described (Edmondson & Hatten, 1987), CGN cell bodies were elongated while migrating along GC fibres

(supplementary Fig S6A online). Compared with CGN^{+/+} plated on top of GC^{+/+}, migration velocity was significantly reduced when profilin1 was absent from either CGNs or GCs (Fig 4B). Our data confirm the radial migration defect that we found *in vivo*, and show that profilin1 activity in both CGNs and GCs is required for radial migration of CGNs.

Normal tangential migration and axonal outgrowth

Apart from GC-guided radial migration, CGNs also perform tangential migration that is GC independent (Komuro & Yacubova, 2003). In a subsequent series of experiments, we tested whether profilin1 is relevant for tangential migration as well. To do so, we adopted a well-established *in vitro* approach for tangential migration that relies on CGN's exit out of cultured EGL explants (Chedotal, 2010). EGL explants were prepared at P4, and the migration distance of Hoechst-stained CGNs that had exited from the explants 4 days later was measured (supplementary Fig S6B online). Quantitative analysis showed no difference between control and mutant EGL explants (supplementary Fig S6C online). Further, we stained EGL explants with an antibody against β -tubulin to test whether the loss of profilin1 in CGNs has an effect on axonal outgrowth (supplementary Fig S6D online). Similar axon numbers and lengths in control and mutant EGL explants argue against a gross defect of axonal outgrowth in mutant CGNs (supplementary Fig S6E,F online).

Together, our data suggest that profilin1 is dispensable for axonal outgrowth and tangential migration of CGNs. In agreement with normal tangential migration, EGL formation that depends on the tangential migration of CGN progenitors and ML interneuron numbers that depends on their tangential migration were not altered in mutants. Hence, our mutants represent a valuable tool to dissect the molecular mechanisms that control radial versus tangential migration.

Profilin1 is required for CGN–GC adhesion

CGN–GC adhesion is a critical parameter for radial migration (Adams *et al*, 2002). As profilin1-deficient fibroblasts display adhesion defects (Bottcher *et al*, 2009), it is conceivable that profilin1 also controls CGN–GC interaction. To test this hypothesis, we performed a GC-binding assay with isolated CGNs that were allowed to settle on top of a confluent GC layer (Adams *et al*, 2002). Again, we plated CGN^{−/−} on GC^{+/+} and CGN^{+/+} on GC^{−/−} to assess whether profilin1 in CGNs and/or in GC is relevant for CGN–GC adhesion. We found a strong impairment in CGN–GC adhesion in both assays (Fig 4C), and conclude that profilin1 is required for CGN–GC adhesion because of its activity in CGNs and GC.

Through which pathway does the actin-binding protein profilin1 control CGN–GC adhesion? Dynamic actin turnover is essential to coordinate the formation of cell–cell contacts with cell movements (Bershadsky, 2004), and a role of profilin1 in this specific aspect is conceivable. In fact, profilin1 was previously implicated in cell–cell contact formation in cancer cells (Zou *et al*, 2007). We wanted to know whether profilin1 also fulfils a similar function in CGNs, and what molecules might be involved in profilin1-dependent CGN–GC adhesion. We again plated CGN^{−/−} on GC^{+/+} and investigated the distribution of the known profilin1 ligands Mena and vinculin at CGN–GC junctions. We chose Mena and vinculin as both are known to be concentrated at cell–cell contacts of various cell types (Ziegler *et al*, 2006; Trichet *et al*, 2008) including neuronal cells (Arregui *et al*, 1994; van Horck *et al*, 2002), and as both molecules were suggested to be important for neuron–glia interaction (Jockusch *et al*, 1995). In particular, Mena has been implicated in radial migration (Goh *et al*, 2002), and analysis of mouse mutants proved the importance of profilin1–Mena interaction *in vivo* (Lanier *et al*,

1999). Mena and vinculin both seemed to be concentrated in puncta at the junctions of CGN^{+/+} and GC^{+/+} as shown by representative images (Fig 4D). Conversely, both Mena and vinculin levels were significantly reduced at CGN^{−/−}–GC^{+/+} junctions (Fig 4E). Thus, our data suggest (i) that profilin1 interacts with Mena and vinculin at CGN–GC contacts, and (ii) that these interactions are critical for the GC-binding capacity of CGNs.

Concluding remarks

Our study demonstrates that the actin-binding protein profilin1 is critical for radial migration of CGNs, but not for other fundamental aspects of cerebellar development, such as tangential migration, neuron proliferation or BG morphology. Our data provide evidence that radial migration of CGNs depends on profilin1 through a mechanism that involves adhesion to GC. We show that genetic ablation of profilin1 from mice impairs radial migration and brain development, whereas no human profilin1 mutations have been described to date. However, the human profilin1 gene *PFN1* is located on a chromosomal region that has been linked to the Miller–Dieker Lissencephaly Syndrome (Kwiatkowski *et al*, 1990), as well as to a hereditary cerebellar hypoplasia (Turkmen *et al*, 2006). The genetic bases for both disorders have not been fully resolved yet. It is tempting to speculate that *PFN1* mutations contribute to these human neuropathies.

METHODS

Mice. Brain-specific profilin1 mutants were generated by crossing conditional profilin1 mice with transgenic mice that express cre under the control of the rat nestin promoter (Tronche *et al*, 1999; Bottcher *et al*, 2009). Generation of GFP–actin-expressing mice (huGE) has been described previously (Gurniak & Witke, 2007).

A list of antibodies used in this study and a description of standard techniques (immunoblots, Nissl staining, immunohistochemistry) is given in the supplementary information online.

BrdU experiments. Mice received a single BrdU injection (0.1 mg BrdU per gram body weight) and were killed 3 h later for quantifying cell proliferation. Others were killed 48, 72 and 96 h after the injection for migration studies. For details, see supplementary information online.

BG morphology. Individual BGs were labelled by adding Alexa Fluor 488 hydrazide (Invitrogen) to the patch pipette. For details, see supplementary information online.

***In vitro* CGN assays.** For isolation of astrocytes, cerebella of P2–4 mice were excised, chopped and digested with dispase and DNase. After washing, cerebellar pieces were triturated using gauge needles and transferred to coated coverslips. CGNs were removed by discarding the supernatant 30 min after plating. For details, see supplementary information online. For isolation of CGNs, a two-step Percoll gradient (30% and 60%) was loaded with a single-cell suspension that was generated as described above. CGNs were harvested from the 30%/60% interphase of the gradient after centrifugation. For details, see supplementary information online. ***In vitro* CGN migration:** Astrocytes, purified as described above, were grown for 1 day. Thereafter, freshly purified granule cells were seeded on top and culturing was continued for another day. Elongated CGNs that were flattened against GC processes were imaged. Migration of CGNs on stationary GC was quantified as described previously (Edmondson & Hatten, 1987). For details and for the analysis of Mena and

vinculin immunoreactivity, see supplementary information online. *EGL explants*: Cerebellar grey matter from P4 mice was excised, chopped and transferred to poly-L-lysine-coated coverslips. Explants were incubated for 4 days before processing for immunocytochemistry. For details, see supplementary information online. *CGN-binding assay*: CGNs were isolated from P6–7 mice; CGN-binding assay was performed essentially as described previously (Adams *et al*, 2002). In brief, 30,000 Hoechst-stained CGNs per well were added to an astrocyte carpet. CGNs were allowed to settle for 120 min before the dish was shaken. The supernatant was drawn off and the remaining cells were processed for immunocytochemistry. For details, see supplementary information online.

Data analysis and statistics. Data are presented as mean values $\pm$ standard error of the mean. For some experiments, the median and the 1%, 25%, 75% and 99% values are given in the box plots. The unpaired two-tailed Student's *t*-test was used when comparing two sets of data with normal distributions. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Supplementary information is available at EMBO reports online (<http://www.emboreports.org>).

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Author contributions: J.A.K., M.B.R., A.N. and C.B.G. conducted the experiments and analysed the data. M.B.R., W.W., E.F. and J.A.K. designed the project. M.B.R., W.W. and E.F. wrote the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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