

Figure S1, related to Figure 2 and 3. BAI3 is localized in spines and partially colocalized with the postsynaptic density in cultured hippocampal neurons. DIV20 hippocampal neurons co-transfected at DIV18 with BAI3 and mCherry constructs were immunostained for BAI3 and the postsynaptic density marker PSD95. Higher magnifications of regions (a-c) are shown in the insets on the bottom right. Scale bars, 5 μ m and 0.5 μ m for insets.

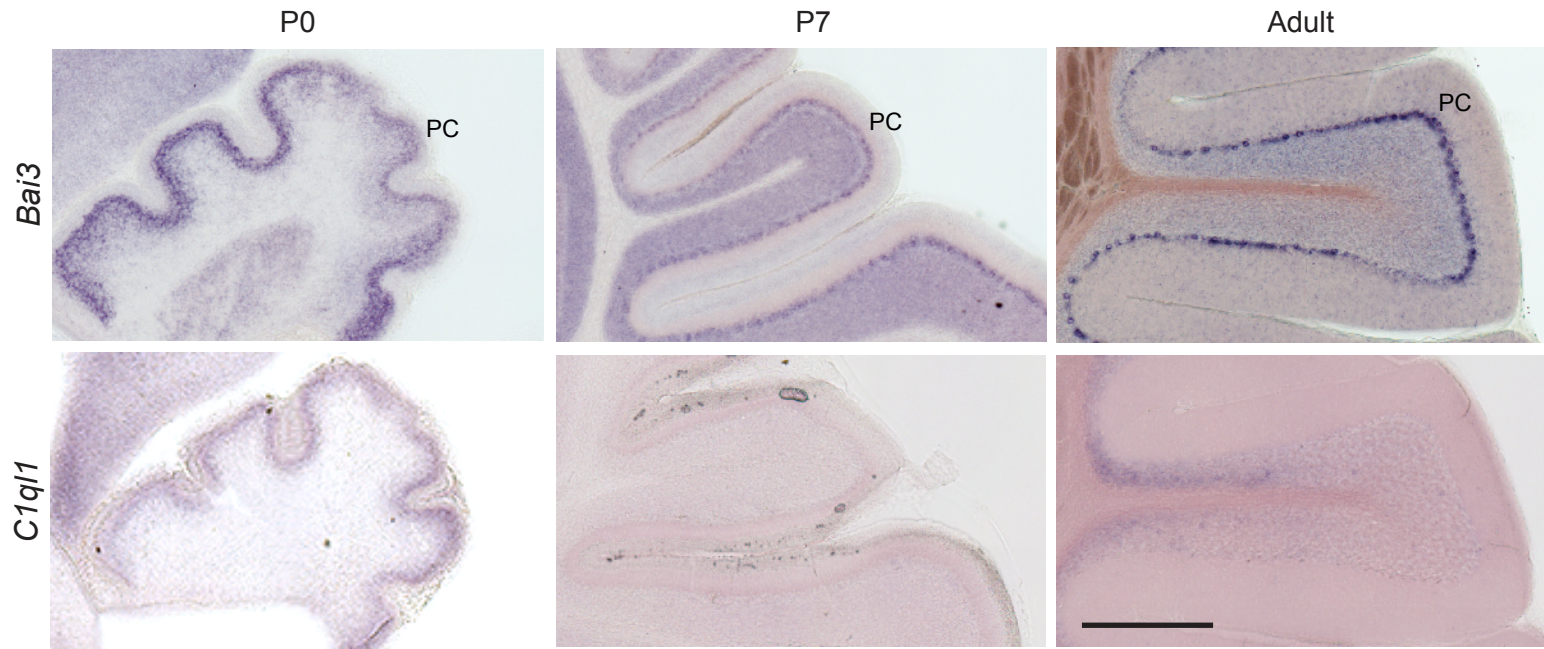
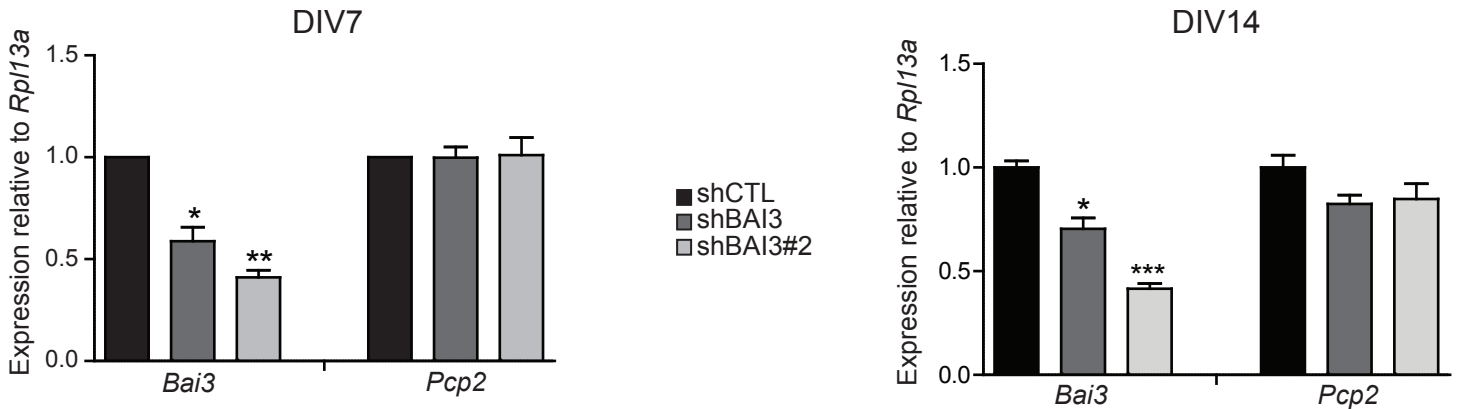


Figure S2, related to Figure 1. *In situ* hybridization analysis of *Bai3* and *C1ql1* mRNA expression in the cerebellar cortex during postnatal development.

In situ hybridization experiments performed using a probe specific for *Bai3* or *C1ql1* on sagittal sections of mouse brain taken at postnatal day 0, 7 and adult. PC, Purkinje cell. Scale bar, 500 μ m.

A



B

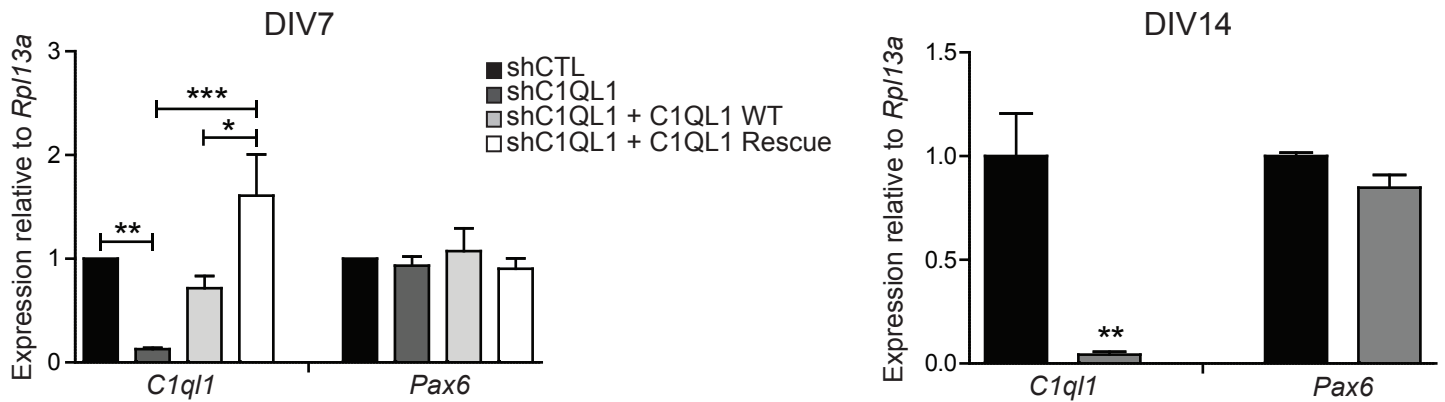


Figure S3, related to Figures 2, 3 and 6. Characterization of knockdown and rescue efficiency for our RNAi approach in cerebellar mixed cultures.

(A) Expression of *Bai3* and *Pcp2* mRNAs was assessed using quantitative RT-PCR on extracts from cerebellar mixed cultures. Transduction was performed at days in vitro 4 (DIV4) with lentiviruses driving the expression of two different shRNAs directed against *Bai3*, or the control shCTL and analysis at DIV7 (left) or DIV14 (right). Expression levels are normalized to the *Rpl13a* gene. Note that twice as many lentiviral particles are used for shBAI3#2. **(B)** Expression of *C1ql1* and *Pax6* mRNAs was assessed using quantitative RT-PCR on cerebellar mixed cultures at DIV7 or DIV14, respectively after 3 days or 10 days of transduction by lentiviruses driving the expression of a shRNA directed against *C1ql1* alone or in combination with lentiviruses expressing *C1ql1* WT or *C1ql1* Rescue, or the shCTL as control. Expression levels are normalized to the *Rpl13a* gene. N=3-7 independent experiments (Data are presented as mean \pm SEM; One-way ANOVA followed by Newman-Keuls posthoc test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

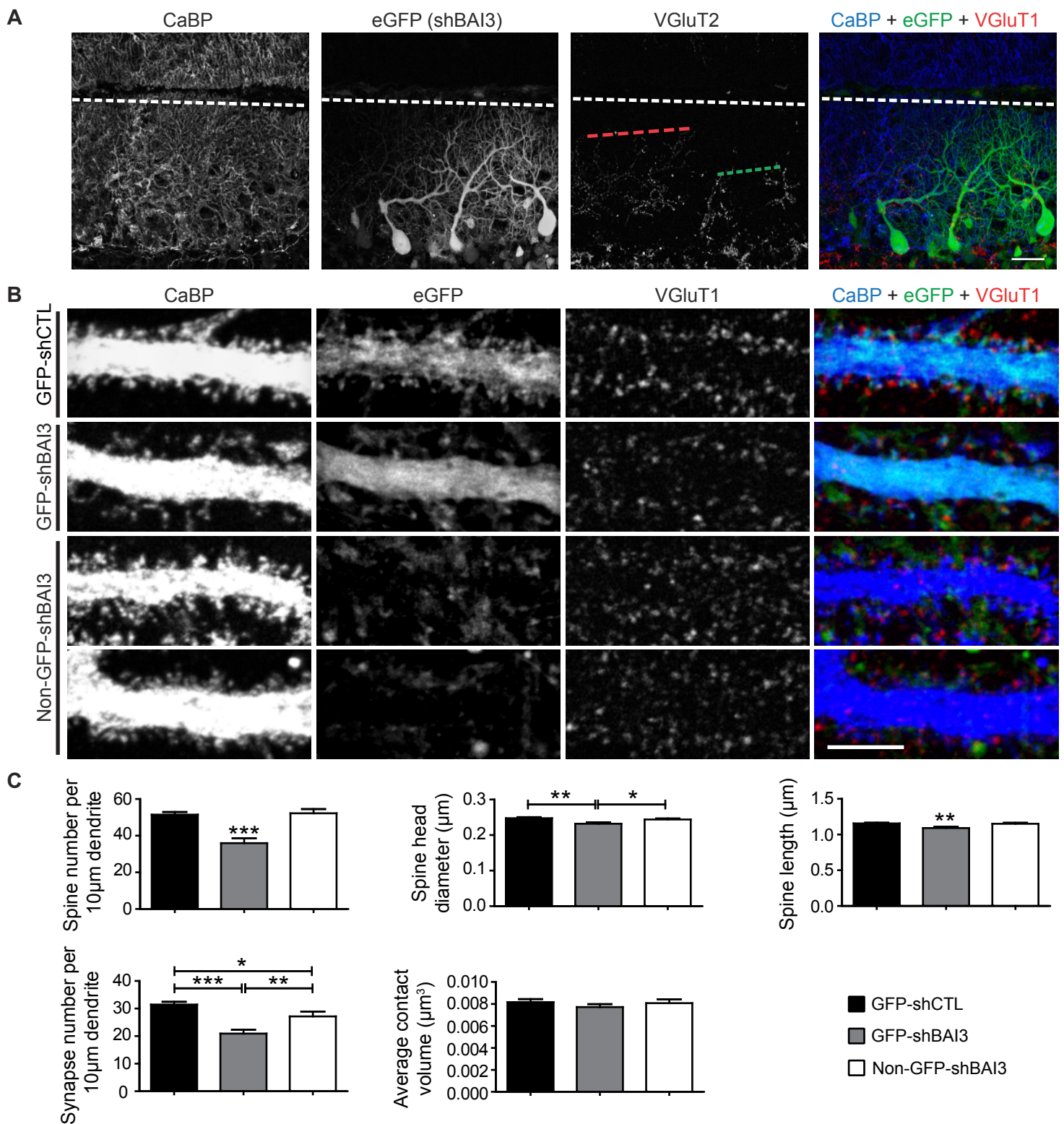


Figure S4, related to Figure 2 and 3. Cell autonomous role of BAI3 in Purkinje cells. (A) P21 immunostaining for vesicular glutamate transporter 2 antibody (VGlut2), a marker specific for climbing fiber synapses, on non-transduced PCs (GFP negative) or transduced PCs (GFP positive). Stereotaxic injections of recombinant lentiviral particles driving expression of a small hairpin RNA against *Bai3* (shBAI3) or control shRNA (shCTL) were performed at P7. Normal VGlut2 extension was observed in GFP-negative cells in contrast to the decreased extension visible on GFP-positive PCs. Granule cells are GFP-positives in the region of both GFP positive and negative cells. Pial surface: white dashed lines. Scale bar, 30µm. **(B)** Cerebellar mixed cultures were transduced at DIV4 with recombinant lentiviral particles driving expression of GFP together with a shRNA targeting *Bai3* (shBAI3) or a control shRNA (shCTL). Dendritic spines and parallel fiber synapses in transduced Purkinje cells (GFP) or non-transduced PCs (Non-GFP) were imaged at DIV14 after immunostaining for calbindin (CaBP) and vesicular glutamate transporter 1 antibody (VGlut1). Scale bar: 5µm. **(C)** Quantitative assessment of the number and morphology of spines was performed in cultured Purkinje cells using the NeuronStudio software. Quantitative assessment of the number and size of vGlut1 synaptic contacts was performed using ImageJ. $N \geq 18$ cells per condition, 3 independent experiments (Data are presented as means \pm SEM; One-way ANOVA followed by Newman-Keuls posthoc test, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

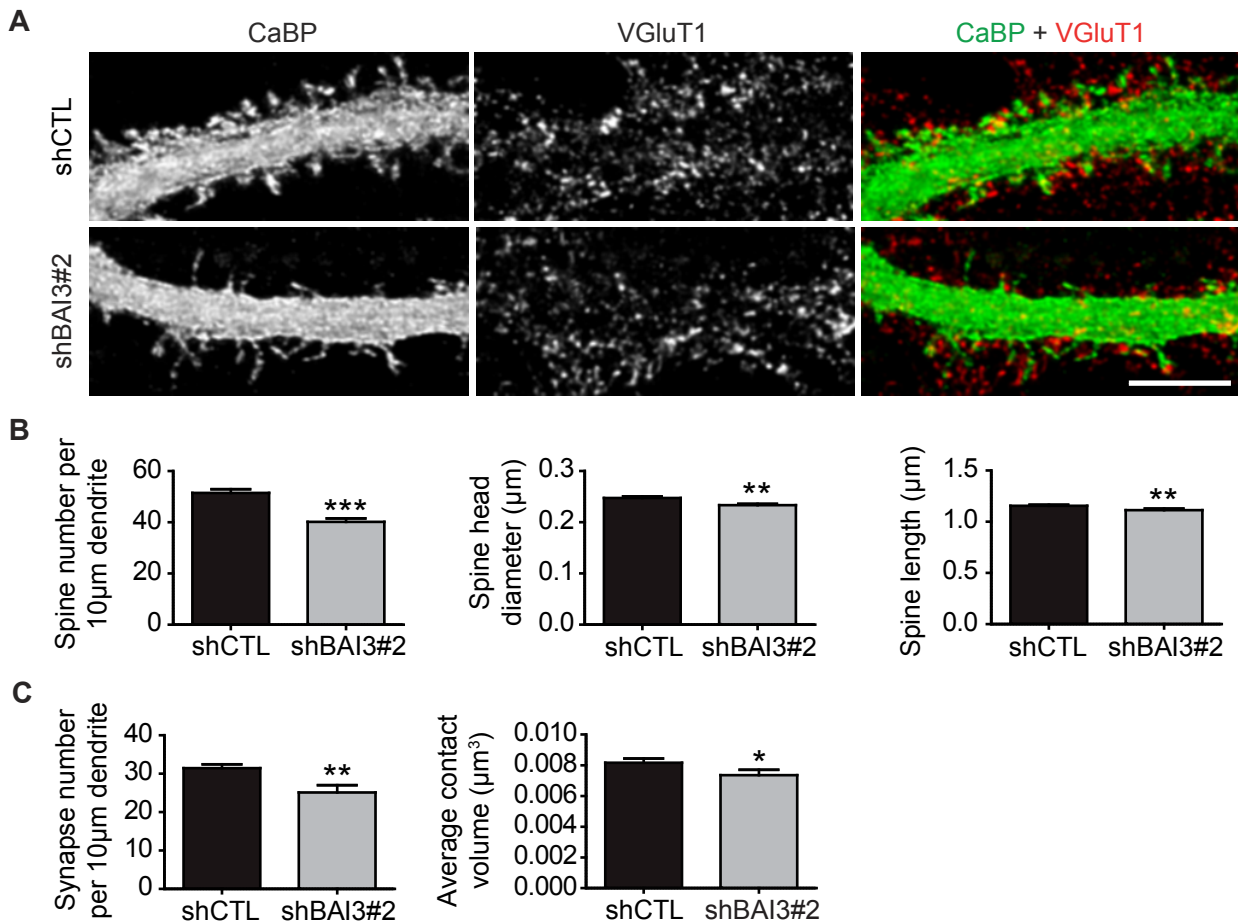
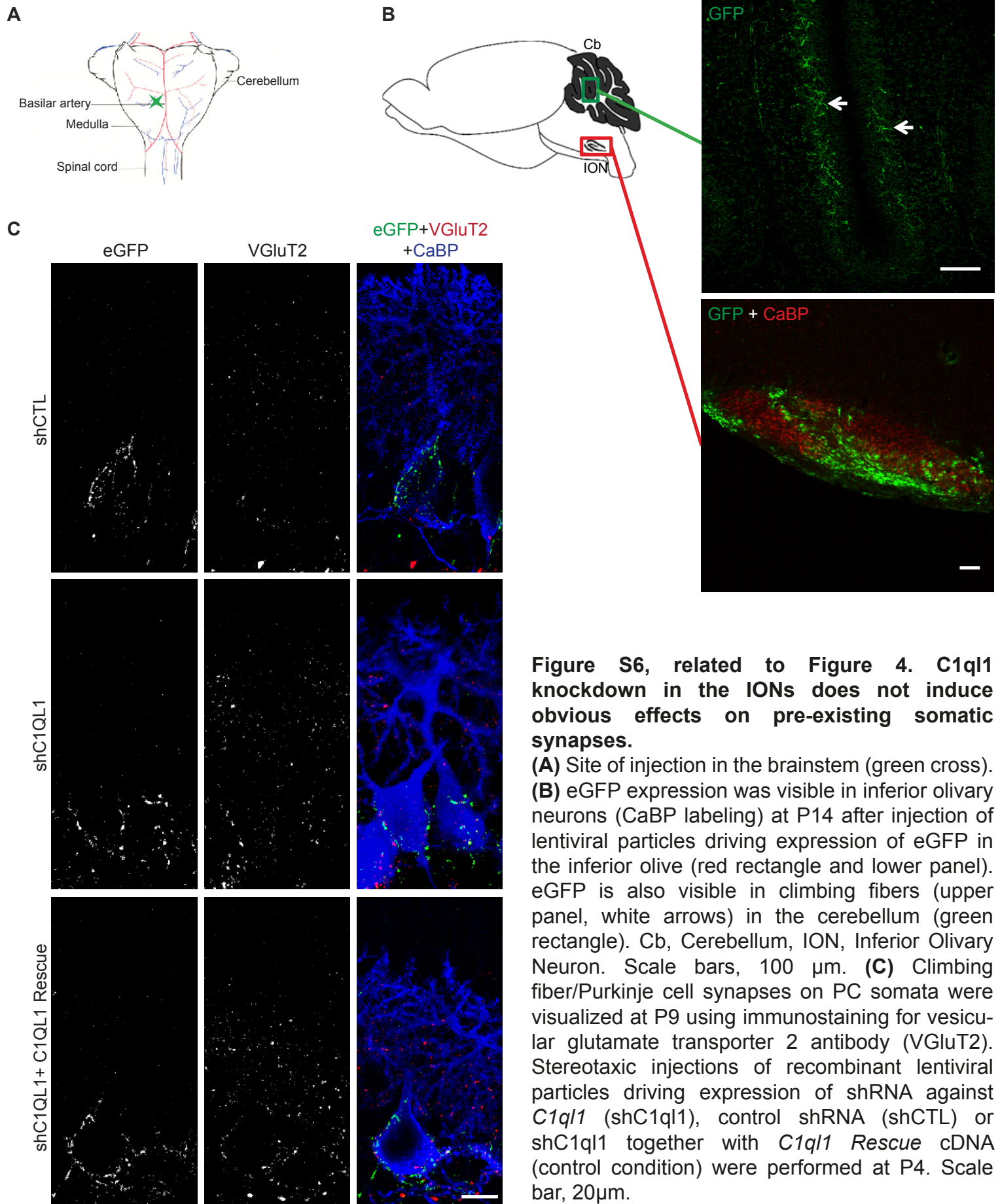


Figure S5, related to Figure 3. Data obtained with a second shRNA targeting BAI3 show similar effects on Parallel fiber/Purkinje cell spinogenesis and synaptogenesis in vitro.

(A) Cerebellar mixed cultures were transduced at DIV4 with lentiviral particles driving expression of GFP together with a small hairpin RNA targeting *Bai3* (shBAI3#2) or a control shRNA (shCTL). Dendritic spines and parallel fiber synapses in transduced Purkinje cells (GFP positive) were imaged at DIV14 after immunostaining for calbindin (CaBP) and vesicular glutamate transporter 1 antibody (VGlut1), respectively. Scale bar: 5μm **(B)** Quantitative assessment of the number and morphology of Purkinje cell spines was performed using the NeuronStudio software. N≥40 cells per condition, 4 independent experiments (Data are presented as means ± SEM; unpaired Student t test and Mann-Whitney U test for spine length, **p < 0.01; ***p < 0.001). **(C)** Quantitative assessment of the number and size of vGlut1 synaptic contacts in DIV14 transduced Purkinje cells was performed using ImageJ. N≥40 cells per condition, 4 independent experiments (Data are presented as means ± SEM; unpaired Student t test, *p < 0.05; **p < 0.01).



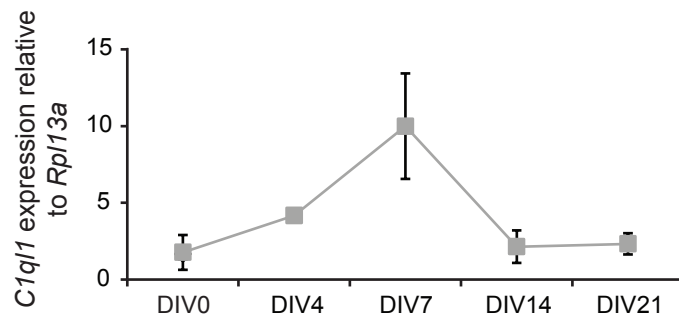


Figure S7, related to Figure 1. *C1q1* expression in cerebellar cultures.

Expression of *C1q1* was assessed at different stages of cerebellar culture development using quantitative RT-PCR on cell extracts (days in vitro, DIV0 to 21). Expression levels are normalized to the *Rpl13a* gene. N=2 samples per stage.

-Supplemental Experimental Procedures

cDNA and RNAi constructs

The *BAI3-WT* construct was cloned into the pEGFP-C2 vector from mouse cDNA clone #BC099951. The shRNA #2 sequence for BAI3 was: 5'tgcagaattaccctttga3', and was subcloned under the H1 promoter in a lentiviral vector that also drives eGFP expression (Avci et al., 2012).

RTqPCR

RNA samples were obtained from mixed cerebellar cultures using the RNeasy Mini kit (QIAGEN, Hilden, Germany), cDNA were amplified using the SuperScript® VILO™ cDNA Synthesis kit (Life technologies, Paisley, UK) according to manufacturer's instructions. Quantitative PCR was performed using the TaqMan Universal Master Mix II with UNG (Applied Biosystems, Courtaboeuf, France) and the following TaqMan probes: *Bai3* (#4331182_Mm00657451_m1), *C1ql1* (#4331182_Mm00657289_m1), *Rpl13a* (#4331182_Mm01612986_gH), *Pcp2* (#4331182_Mm00435514_m1), *Pax6* (#4331182_Mm00443081_m1).

In situ Hybridization

In situ hybridization was performed using a previously described protocol with minor modifications (Bally-Cuif et al., 1992). Briefly, paraformaldehyde-fixed freely floating vibratome sections were obtained (100 µm thickness) from mouse brains at postnatal day 0 (P0), P7 and adult (more than 6 weeks). The probe sequences corresponded to the following nucleotide residues for the indicated mouse cDNA: 3955-4708 bp for *Bai3* (NM_175642.4), 641-1200 bp for *C1ql1* (NM_011795.2) and 501-1017 bp for *C1ql3* (NM_153155.2). The riboprobes were used at a final concentration of 2 µg/µL. The proteinase K (10µg/mL)

treatment was given for 30 seconds for P0 and P7 brain sections, and 10 minutes for adult brain sections. The anti-digoxigenin-AP antibody was used at a dilution of 1/2000.

Primary neuronal cultures

Cerebellar mixed cultures were prepared from P0 Swiss mouse cerebella and were dissected and dissociated according to previously published protocol (Tabata et al., 2000). Neurons were seeded at a density of 5×10^6 cells/ml. Hippocampal cultures were prepared from E18 Swiss mouse embryos as previously published with minor modifications (Fath et al., 2008). Hippocampal neurons were transfected at DIV18 using Lipofectamine-2000 (Life technologies, Carlsbad, USA) according to the manufacturer's protocol.

Immunocytochemistry, immunohistochemistry and antibodies

Immunostaining was performed on cells fixed with 2% paraformaldehyde in PBS or on 30 micrometer thick sagittal cerebellar sections obtained using a freezing microtome from brains of mice perfused with 4% paraformaldehyde in PBS. The following antibodies were used: anti-CaBP mouse antibody (Swant, Marly, Switzerland, #300), anti-VGluT1 guinea pig antibody (Millipore, Molsheim, France, #AB5905), anti-VGluT2 guinea pig antibody (Millipore, Molsheim, France, #AB2251), anti-BAI3 rabbit antibody (Sigma, St Louis, USA, #HPA015963) and anti-PSD95 rabbit antibody (Cell signaling, Leiden, The Netherlands, #3450).

Image acquisition and analysis

Image stacks were acquired using a Confocal Microscope (SP5, Leica), using either 40x (1.25 NA, oil immersion, pixel size: 211 nm and 144 nm for vGluT2 extension in shBAI3 and C1QL1 misexpression experiments, respectively) or 63x (1.4 NA, oil immersion, pixel size: 57 nm for *in vitro* imaging, pixel size: 38 nm for *in vivo* imaging of vGluT2 staining) or 20X (0.7 NA, pixel size 60 nm for hippocampal neurons) objectives. The pinhole aperture was set

to 1 Airy Unit and a z-step of 200 nm was used. Laser intensity and photomultiplier tube (PMT) gain was set so as to occupy the full dynamic range of the detector. Images were acquired in 16-bit range. For spine and synapse analysis, deconvolution was performed with Huygens 4.1 software (Scientific Volume Imaging) using Maximum Likelihood Estimation algorithm. 40 iterations were applied in classical mode, background intensity was averaged from the voxels with lowest intensity, and signal to noise ratio values were set to a value of 25.

Electrophysiology

Responses to parallel (PF) and climbing fibers (CF) stimulation were recorded in Purkinje cells in acute cerebellar slices from Swiss mice (P18 to P23) after lentivirus injection at P7 in the cerebellum and from Swiss mice (P14 to P19) after lentivirus injection at P4 in the inferior olive. Briefly: mice were anesthetized by exposure to isoflurane 4% and sacrificed by decapitation. Cerebellum was dissected in ice cold oxygenated Bicarbonate Buffered Solution (BBS) containing (in mM): NaCl 120, KCl 3, NaHCO₃ 26, NaH₂PO₄ 1.25, CaCl₂ 2 mM, MgCl₂ 1 and glucose 35. 280 μ m sagittal slices were cut with a vibratome in the NMDG-based cutting buffer (in mM): NMDG 93, KCl 2.5, NaH₂PO₄ 1.2, NaHCO₃ 30, HEPES 20, glucose 25, sodium ascorbate 5, thiourea 2, sodium pyruvate 3, MgSO₄ 10 and CaCl₂ 0.5 (pH 7.3). Immediately after cutting, slices were allowed to briefly recovery at 34°C in the oxygenated sucrose-based buffer (in mM): sucrose 230, KCl 2.5, NaHCO₃ 26, NaH₂PO₄ 1.25, glucose 25, CaCl₂ 0.8 and MgCl₂ 8. D-APV and minocycline at a final concentration of 50 μ M and 500 nM respectively were added to the sucrose-based and cutting buffers. Slices were allowed to fully recover in 95% O₂/5% CO₂ bubbled BBS at 34°C for at least 30 minutes before starting experiments.

Patch clamp borosilicate pipettes with 3-5 M Ω resistance were filled with the internal solution containing (in mM): CsMeSO₃ 135, NaCl 6, MgCl₂ 1, HEPES 10, MgATP 4, Na₂GTP 0.4,

EGTA 1.5 and QX314Cl 5 (pH 7.3). Stimulation electrodes with 5 M Ω resistance were pulled from borosilicate pipettes and filled with HEPES Buffered Solution (HBS) containing (in mM): NaCl 120, KCl 3, HEPES 10, NaH₂PO₄ 1.25, CaCl₂ 2, MgCl₂ 1 and glucose 10 (pH7.3). The IsoStim A320 (WPI Inc, USA) stimulator was used to elicit CF and PF mediated responses in PC. Patch clamp experiments were conducted in voltage clamp mode using a MultiClamp 700B amplifier (Molecular Devices Inc, USA). Currents were low-pass filtered at 2 kHz and digitized at 20 kHz. Recordings were performed at room temperature on slices continuously perfused with 95% O₂/5% CO₂ bubbled BBS and in presence of picrotoxin 100 μ M. CF and PF currents were monitored at a holding potential of respectively -10 mV and -60 mV.

During CF recordings the stimulation electrode was placed in the granule cells layer below the clamped cell; CF-mediated responses were identified by the typical all or nothing response and strong depression displayed by the second response elicited during paired pulse stimulations (20 Hz).

PF stimulation was achieved by placing the stimulation electrode in the molecular layer at the minimum distance required to avoid direct stimulation of the dendritic tree of the recorded PC. The input/output curve was obtained by incrementally increasing the stimulation strength. Peak EPSC values for PF were obtained following averaging of five consecutive traces and were normalized to the EPSC recorded at the lowest stimulus intensity in order to determine the fold increase in PF responses. Data analyses were performed with the scientific data analysis software Igor Pro (WaveMetrics, USA).

-Supplemental References

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