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Supplementary Information for

N-CADHERIN PROVIDES A CIS AND TRANS LIGAND FOR ASTROTACTIN THAT FUNCTIONS IN GLIAL-GUIDED NEURONAL MIGRATION

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Supplementary Methods

Animals

B6.129S6(SJL) *Cdh2*$^{fl/fl}$ mice (backcrossed to C57Bl/6) carrying loxP sites flanking exon 1 of the *Cdh2* gene (Jackson Laboratory, Stock # 007611) were crossed with *Tg(NeuroD1-Cre) RZ24*, *Tg(hGFAP-Cre) PK90* (both provided by Dr. Nathaniel Heintz/Gensat) or *Tg(mGFAP-Cre)* (provided by Dr. Alexandra Joyner) lines. The NeuroD1 promoter commences at E16 in postmitotic granule cells (1), the mGFAP promoter after P0 in Bergmann glial cells (2), and the hGFAP promoter at E13.5 in neural progenitors (3). *Cdh2*$^{fl/+}$*;NeuroD1-Cre*, *Cdh2*$^{fl/+}$*;mGFAP-Cre* and *Cdh2*$^{fl/+}$*;hGFAP-Cre* progeny were crossed back to *Cdh2*$^{fl/fl}$ mice. The *Cdh2*$^{fl/fl}$*;Cre*$^{+/–}$ experimental mice were only compared with *Cre*-negative *Cdh2*$^{fl/fl}$ control littermates. Genotyping of the *Cdh2*$^{fl/fl}$ allele was carried out by PCR using 5’-CCA AAG CTG AGT GTG ACT TG and 5’-TAC AAG TTT GGG TGA CAA GC primers, and of the *Cre* allele using 5’-GGA CAT GTT CAG GGA TCG CCA GGC G and 5’-GCC AGA TTA CGT ATA TCC TGG CAG CG primers. The number of animals for each phenotypic analysis is stated in the Results.

All animal work was performed as required by the United States Animal Welfare Act and the National Institutes of Health's policy to ensure proper care and use of laboratory animals for research, and under established guidelines and supervision by the Institutional Animal Care and Use Committee (IACUC) of The Rockefeller University. Mice were housed in accredited facilities of the Association for Assessment of Laboratory Animal Care (AALAC) in accordance with the National Institutes of Health guidelines.

DNA constructs

Venus cDNA was PCR generated from *pMSCXβ-Venus* (4) template with the following primers: Venus forward (EcoRI) primer 5’-GAG AAG GAA TTC ACC *ATG* GTG AGC AAG GGC GAG GAG and Venus reverse (NotI) primer 5’-GAG AAG GCG GCC GC*T TA*C TTG TAC AGC TCG TCC ATG CCG (restriction sites underlined and start/stop codons in italics). The PCR product was inserted into *pCIG2* plasmid (kindly provided by Dr. Franck Polleux) digested with EcoRI and NotI. The *pCIG2-Astn1-Venus* plasmid was generated by fusing the *Venus* sequence in frame with the 3’ end of the *Astn1* coding sequence by joining PCR. The resulting *Astn1-Venus* fusion insert (EcoRI/NotI) was subcloned, along with the *Astn1* cDNA fragment (XmaI/EcoRI), into the *pCIG2* vector. Finally, the 5’ region (231 bp) of the *Astn1* cDNA was inserted between the XhoI and XmaI sites. For *pCIG2-Astn1-ΔCTD-Venus*, the *pCIG2-Astn1-Venus* plasmid was digested with PstI and the larger fragment was purified. Thereafter, a gBlock DNA fragment (Integrated DNA Technologies, Coralville, IA), flanked by PstI sites and containing a fusion between *Astn1* coding sequence base pair number 2160 and the *Venus* start site, was inserted, which generated a 1749 bp deletion of the *Astn1* 3’ region resulting in a 583 amino acid deletion in the ASTN1 C-terminus. The *pRK5-Cdh2-Myc* and *pRK5-Cdh2-Δ390-Myc* plasmids were kindly provided by Dr. Richard Huganir. The *pAc5-STABLE2-Neo* plasmid containing the *Drosophila* Actin5C promoter (5) was kindly provided by Dr. Leslie Vosshall. To generate the *pAc5-Astn1-mCherry* plasmid, *Astn1* cDNA was amplified by PCR with the following primers: Astn1 forward (XbaI)

primer 5'-CTT TCT AGA *ATG* GCT TTA GCC GGG CTC TG and Astn1 reverse (XhoI) primer 5'-CTT CTC GAG *CTA* GAT GTC TTT GCT GTC CC (restriction sites underlined and start/stop codon in italics). The PCR product was inserted between the XbaI and XhoI sites in the *pAc5-STABLE2-Neo* plasmid. The *pAc5-Cdh2-GFP* and *pAc5-Cdh2-Δ390-GFP* plasmids were generated by amplifying the *Cdh2* and *Cdh2-Δ390* cDNA with the primers: Cdh2 forward (EcoRI) 5'-GCG AAT TC*A TG*T GCC GGA TAG CGG GA and Cdh2 reverse (NotI) 5'-TTG CGG CCG CTG TCG TCA CCA CCG CC (restriction sites underlined and start codon in italics). The *Cdh2* and *Cdh2-Δ390* PCR products were inserted into the *pAc5-STABLE2-Neo* plasmid digested with EcoRI and NotI. The *pAc5-STABLE2-Neo* plasmid contains T2A sequences to allow bicistronic expression of GFP and mCherry instead of protein fusions.

Granule cell/Bergmann glia co-cultures

Cerebella were dissected out from P7 mice and the meninges were carefully removed. The cerebella were then incubated in Trypsin-DNase for 5 minutes at 37 °C followed by gentle trituration in DNase to dissociate the tissue into single cells. The cell suspension was filtered through a 40 µm nylon mesh filter (BD Biosciences) and then applied to a two-step gradient of 35 %/60 % Percoll (Sigma-Aldrich) in Tyrode's solution containing 2 mM EDTA. The Percoll gradient was centrifuged at 3000 rpm for 10 minutes at 4 °C. The large cell fraction at the interface of the Tyrode's solution and 35 % Percoll (Bergmann glia) and the small cell fraction at the interface between the layer of 35 % Percoll and 60 % Percoll (granule cells) were transferred to Tyrode's solution and centrifuged at 2000 rpm for 5 minutes at 4 °C. The supernatant was removed and the cell pellets were resuspended in granule cell medium [Basal Medium Eagle (BME; Gibco, cat # 21010) supplemented with 30 % D-glucose (Sigma-Aldrich), 10 % horse serum (Gibco), 2 mM L-glutamine (Gibco) and 100 U/ml penicillin/streptomycin (Gibco)]. The cell suspensions were transferred to untreated petri dishes and incubated at 35 °C/5 % CO_2 for 20 minutes to remove fibroblasts.

For the glial cell fraction, the unbound cell suspension was removed and cultured on 0.1 mg/ml poly D-lysine (Millipore) pre-coated 12 mm coverslips for 1 h at 35 °C/5 % CO_2. The plate was then tapped to dislodge granule cells that had accompanied the glial cell fraction, and the unbound cell suspension was removed. The remaining glial cells, which attached well to the coated coverslips, were cultured in granule cell medium at 35 °C/5 % CO_2. For the granule cell fraction, the cell suspension was transferred from the petri dish to a 60 mm tissue culture dish and incubated for 30 minutes at 35 °C/5 % CO_2. The dish was then tapped to dislodge the granule cells and the cell suspension was transferred to a new tissue culture dish and the process repeated for maximal purification of granule cells. The purified granule cells were then electroporated with an Amaxa Mouse Neuron Nucleofection kit (Lonza, Cologne, Germany), using $3x10^6$ cells, 6 µg *pCIG2-Venus* DNA or 15 µg *pCIG2-Astn1-Venus* DNA, and the O-005 setting in the Amaxa Nucleofector II. Immediately after electroporation, granule cells were recovered in granule cell medium at 35 °C/5 % CO_2 for 15 minutes, centrifuged at 2000 rpm for 5 minutes at 4 °C, resuspended in granule cell medium and subsequently added to the Bergmann glia cell cultures at a ratio of 5:1 to the number of glial cells. The total numbers were ~750,000 granule cells and ~150,000 glial cells per 12 mm coverslip. The

co-cultures were incubated at 35 °C/5 % CO_2 for 48 – 72 h before analyzing neuron-glial attachment and granule cell migration along glial fibers.

Organotypic slice cultures

P8 cerebella from *Cdh2*$^{fl/fl}$ and *Cdh2* cKO littermates were dissected out in Hank's Balanced Salt Solution (HBSS) containing 2.5 mM HEPES (pH 7.4), 46 mM D-glucose, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 4 mM $NaHCO_3$, and Phenol Red on ice. The dissection medium was then removed and *pCIG2-Venus* DNA was diluted to 0.5 µg/µl and *pCIG2-Astn1-FL-Venus* or *pCIG2-Astn1-ΔCTD-Venus* DNA diluted to 1 µg/µl in HBSS. The cerebella were soaked in the DNA for 15 minutes on ice, and were then transferred one at a time into the well of an electroporation chamber (Protech International Inc. CUY520P5 platinum electrode L8xW5xH3 mm, 5 mm gap) that was placed on ice. The cerebella were electroporated dorsal to ventral for 50 ms at 80 V, for a total of 5 pulses with an interval of 500 ms between pulses, using an *electro-square*-porator, *ECM* 830 (BTX Genetronics). The cerebella were then removed from the chamber and placed on ice to recover for 10 minutes.

Subsequent to electroporation, the cerebella were embedded in 3 % agarose in HBSS, and 250 µm horizontal slices were made using a Leica VT1000S vibratome set at a speed of 3 and frequency of 6. Slices were then placed on MilliCell CM 0.4 µm culture plate inserts (Millipore) in a 6 well plate with 1.5 ml of culture medium [BME (Gibco), 25 mM D-glucose (Sigma-Aldrich), 2 mM L-glutamine (Gibco), 1X insulin/transferrin/selenium (Sigma-Aldrich), 100 U/ml penicillin/streptomycin (Gibco)] below the insert. The organotypic slices were incubated at 35 °C/5 % CO_2 for 60 hours before fixation with 4 % paraformaldehyde (PFA)/4 % sucrose in phosphate-buffered saline (PBS) for 2 hours at room temperature.

For immunostaining, the agarose was carefully removed from the slices and the slices were permeabilized and blocked overnight at 4 °C in 10 % normal donkey serum (NDS)/0.3 % Triton X-100 in PBS. The slices were then incubated overnight at 4 °C with a rabbit anti-GFP antibody (1:2000; Invitrogen) diluted in 10 % NDS/0.3 % Triton X-100 in PBS, followed by washes in 10 % NDS/0.3 % Triton X-100 in PBS and overnight incubation at 4 °C with an Alexa Fluor 488 conjugated secondary antibody (Life Technologies) diluted in 10 % NDS/0.3 % Triton X-100 in PBS. The following day, the slices were washed in PBS and mounted with ProLong Gold anti-fade reagent (Molecular Probes). The slices were imaged with a Carl Zeiss Axiovert 200M/ Perkin Elmer Ultraview spinning disk confocal microscope equipped with a 25X objective.

Nissl staining

Brains were dissected out and fixed in 4 % PFA in PBS at 4 °C overnight and thereafter cryoprotected in 20 % sucrose in PBS at 4 °C overnight. The brains were then embedded in Tissue-Tek O.C.T. compound (Sakura Finetek, Torrance, CA) and rapidly frozen. 25 µm sagittal sections were prepared using a Leica CM 3050S cryostat. Sections were postfixed in 4 % PFA in PBS for 15 minutes, rinsed in PBS and incubated in 0.1 % cresyl violet for 5 minutes. The sections were then rinsed in water and dehydrated in a dilution series of ethanol (50, 70, 95, 100 %) and Xylene before mounting the slides in Eukitt

mounting medium (Sigma-Aldrich). The sections were imaged on a Carl Zeiss Axioplan 2 microscope with a 5X objective.

Immunohisto/cytochemistry

Brains were prepared as described above. Sagittal cryosections (25 µm) or granule cell/Bergmann glia co-cultures were fixed in 4 % PFA in PBS for 15 min, rinsed in PBS and blocked/permeabilized in 5 % NDS/0.3 % Triton X-100 in PBS for 45 minutes. Primary antibodies were incubated overnight at 4 ^{o}C, followed by PBS washes and incubation with Alexa Fluor 488 or 555 conjugated secondary antibodies (1:400; Life Technologies) for 2 h at room temperature. After subsequent PBS washes, sections were mounted in Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and cell cultures were mounted in ProLong Gold anti-fade reagent (Molecular Probes). The primary antibodies used were mouse anti-N-cadherin (1:500; BD Transduction Laboratories), rabbit anti-Astn1 (1:200) (6), rabbit anti-BLBP (1:300; Millipore), mouse anti-GFAP (1:500; Sigma-Aldrich), mouse anti-NeuN (1:200; Chemicon), sheep anti-BrdU (1:100; Abcam), rabbit anti-active Caspase-3 (1:400; Cell Signaling Technology), mouse anti-phospho-histone H3 (1:100; Cell Signaling Technology), mouse anti-Calbindin D28-k (1:500; Swant) and rabbit anti-GFP (1:1000; Invitrogen). Primary antibodies were titrated to determine the optimal dilutions, and negative control labelings were included with the respective primary antibody omitted. The slides were imaged on a Carl Zeiss Axiovert 200M/ Perkin Elmer Ultraview spinning disk confocal microscope or a Leica DMI 6000 (TCS SP8) confocal microscope with 25X and 40X objectives.

BrdU labeling

50 µg of BrdU in PBS (BD Biosciences) per gram of body weight was injected subcutaneously in the neck of P5 *Cdh2$^{fl/fl}$* and *Cdh2* cKO littermates. The brains were dissected out 48 h later and fixed in 4 % PFA in PBS at 4 ^{o}C overnight, followed by cryoprotection in 20 % sucrose in PBS at 4 ^{o}C overnight. The brains were then embedded in Tissue-Tek O.C.T. compound (Sakura Finetek, Torrance, CA) and rapidly frozen. 25 µm sagittal sections were prepared using a Leica CM 3050S cryostat. The sections were postfixed in 4 % PFA in PBS for 15 min, washed in PBS, and then incubated in 2N HCl for 30 minutes at 37˚C followed by 0.1 M Sodium Borate, pH 8.5, for 10 minutes at room temperature. The sections were then processed for immunohistochemistry as described above.

Cell culture

Human embryonic kidney (HEK) 293T cells (clone 17; ATCC CRL-11268) were cultured at 37 ^{o}C/5 % CO_2 in Dulbecco's modified Eagle's medium (Gibco, cat # 11995) supplemented with 10 % fetal bovine serum (FBS; Gibco), 2 mM L-glutamine (Gibco) and 100 U/ml penicillin/streptomycin (Gibco). Cells at 50-60 % confluency were transfected with 4 µg DNA constructs using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Drosophila Schneider 2 (S2) cells (Life Technologies) were cultured at 28 ^{o}C in Schneider's *Drosophila* Medium (Gibco, cat # 21720) supplemented with 10% FBS (Gibco) and 100 U/ml penicillin/streptomycin (Gibco). Cells at a 1x10^6/ml density were

transfected with 19 µg DNA constructs using 2 M $CaCl_2$ and 2X HEPES-buffered saline (50 mM HEPES, 1.5 mM Na_2HPO_4, 280 mM NaCl, pH 7.1). Protein expression was verified by Western blotting (see below).

Flow cytometry

Transfected HEK 293T cells were harvested in 1 mM EDTA in PBS. The surface fraction of Venus-linked ASTN1 variants was labeled with rabbit anti-GFP (1:5,000; Invitrogen) for 20 minutes at 4 ^{o}C followed by Alexa Fluor 647 donkey anti-rabbit (1:5,000; Life Technologies) for 25 minutes at 4 ^{o}C. After each antibody incubation, the cells were washed in cold 10 % NDS in PBS. Cells were stained with Propidium Iodide (100 ng/ml; Sigma-Aldrich) for dead cell exclusion. Flow cytometry analysis on BD Accuri C6 (BD Biosciences) was carried out using 488 nm and 640 nm lasers and the CFlow Sampler software (BD Biosciences). A total of 20,000 single viable cells, identified by size and lack of Propidium Iodide staining, were analyzed per condition. Gates were set using non-transfected control cells and cells expressing cytosolic Venus, which were processed for live GFP labeling as described above. Data were analyzed by FlowJo v.9.3.3 (TreeStar Inc., Ashland, OR). Identical gates were applied to each condition.

Immunoprecipitation

Transfected HEK 293T cells or whole cerebella were extracted in ice-cold lysis buffer [50 mM Tris, pH 7.4, 150 mM sodium chloride, 0.5 % sodium deoxycholate, 1 % NP-40, 1 mM EDTA and 1X protease inhibitor cocktail (Sigma-Aldrich)]. The extracts were triturated several times, incubated 20 minutes on ice and centrifuged at 14,000 rpm for 20 minutes at 4˚C. The HEK 293T protein lysates (300-400 µg) or cerebellar protein lysates (700-750 µg) were then precleared with 25 µl Protein G/A Agarose beads (Calbiochem). After removing the beads, the lysates were incubated with 3 µg of a rabbit GFP antibody (Invitrogen), rabbit Astn1 antibody (6), or normal rabbit IgG (Santa Cruz Biotechnology) for 2 h at 4 ^{o}C. Immunoprecipitates were collected on 50 µl Protein G/A Agarose beads by overnight rotation at 4 ^{o}C, washed with lysis buffer and resuspended in 50 µl 2X Laemmli buffer. Western blotting was performed as described below.

Western blotting

Whole cerebella or purified cerebellar granule cells and Bergmann glial cells were extracted in ice-cold lysis buffer [50 mM Tris, pH 7.4, 150 mM sodium chloride, 0.5 % sodium deoxycholate, 1 % NP-40, 1 mM EDTA and 1X protease inhibitor cocktail (Sigma-Aldrich)]. The extracts were triturated several times, incubated 20 minutes on ice and centrifuged at 14,000 rpm for 20 minutes at 4˚C. Protein concentrations were determined using the Pierce BCA Protein Assay Kit (Thermo Scientific). Protein samples in 2X Laemmli buffer were subjected to SDS-PAGE followed by transfer to Immobilon-P PVDF transfer membranes (Millipore). The membranes were blocked with 5% non-fat milk and labeled with primary and secondary antibodies in tris-buffered saline/0.1 % Tween, pH 7.6 (TBST), with each incubation for 1 h followed by washes in TBST. Primary antibodies were rabbit anti-GFP (1:10,000; Invitrogen) mouse anti-c-Myc (1:50; Calbiochem), mouse anti-N-cadherin (1:3000; BD Transduction Laboratories), rabbit anti-Astn1 (1:500), and mouse anti-GAPDH (1:10,000; Millipore). Secondary antibodies were horseradish peroxidase-conjugated anti-mouse (1:10,000; Jackson

ImmunoResearch) or anti-rabbit (1:5,000; Jackson ImmunoResearch). Blots were developed with an ECL Western Blotting Detection kit (GE Healthcare) and exposed to Biomax XAR films (Carestream Health).

S2 cell adhesion assay

After transfecting the S2 cells for 24 h, 1.5×10^6 cells from each transfection were mixed together in four conditions (Fig. 2) at a density of 3×10^6 cells/well (1×10^6 cells/ml) in ultra-low attachment 6-well plates (Corning Inc.). The cells were shaken gently at 28 ^{o}C for up to 2 h to allow aggregation. Fab fragments of the Astn1 antibody were prepared and purified using Pierce Fab Micro Preparation Kit (Thermo Scientific) according to the manufacturer's instructions. The Fab fragments were concentrated to 22 µg/ml using Amicon Ultra 2 ml Ultracel-10K centrifugal filters (Millipore) and were added to the S2 cells at a final concentration of 0.4 µg/ml (6). Cells were imaged on a Carl Zeiss Axiovert 135 fluorescent microscope with a 20X objective immediately after the conditions were set up (T = 0) and after 30 minutes, 1 h and 2 h. Longer incubations resulted in more dense cell cultures and aggregates forming in non-transfected control cells. Co-expression of the fluorophores, rather than tagged expression, ensured that they did not interfere with a potential *trans* binding. Cells expressing GFP (CDH2) or mCherry (control or ASTN1) were quantified in each aggregate and the proportion of mCherry-positive cells per aggregate was calculated for each condition and time point. The experiment was repeated six times.

Statistical analyses

The shape of the granule cells in the ML of control and cKO mice (Fig. 3) was quantified by counting elongated and rounded NeuN-labeled cells and calculating the percentage of the respective cell shape in the ML (n = 7 per genotype). The migration distance in the organotypic cultures (Figs. 4 and 5) was calculated by measuring the distance from the parallel fibers at the edge of the slice to the granule cell soma center using the "ruler" tool in Adobe Photoshop CS6. The number of migrating cells and non-migrating cells (round or multipolar) were quantified based on cell morphology, using the "count" tool in Adobe Photoshop CS6. In total, 700-1200 cells were quantified per control condition and 800-1300 cells per mutant condition from 5-7 slices per cerebellum. The experiments were repeated at least three times. The neuron-glia distance (Fig. 6) was quantified in Adobe Photoshop CS6 by measuring the distance from the granule cell soma center to the nearest glial fiber. 30-45 cells were quantified for each condition and the experiment was repeated three times. Cerebellar size (Suppl Fig. S2) was measured using the freehand selection tool in ImageJ (National Institutes of Health) to encircle the whole cerebellum in midsagittal Nissl-stained sections of control and *Cdh2* cKO mice (n = 7 per genotype). For BrdU experiments (Fig. S4), the number of BrdU-positive cells in the EGL, ML, and IGL of the cerebellar cortex of control and *Cdh2* cKO mice (n = 4 per genotype) were counted using the "count" tool in Adobe Photoshop CS6 and the percentage in each layer determined. A total of 1600-2000 cells were counted per control mouse and 1500-1800 cells were counted per mutant mouse. Proliferating and apoptotic cells were quantified in phospho-histone H3 and Caspase-3 labeled sections of control and *Cdh2* cKO mice (n = 7 per genotype) by counting the cells in Adobe Photoshop CS6 and dividing the number of cells with the mm length (proliferation) or with the mm^2 area (apoptosis) of the

cerebellar lobe. Microsoft Excel 2011 and IBM SPSS Statistics v21.0 were used for the data quantifications and statistical analyses. Differences between conditions were determined using unpaired *t*-tests for equal or unequal variances, except for migration distance in the slice cultures where Kruskal-Wallis and Mann-Whitney U non-parametric tests were used. Significance was set at $P < 0.05$ (two-sided). In the bar diagrams, data are presented as means with error bars representing the standard deviations (Figs. 2, 3, 5H and S4) or standard error of the mean (Fig. 6). The migration distance data from the slice cultures are presented in box plots (Figs. 4 and 5G).

Supplementary Figures

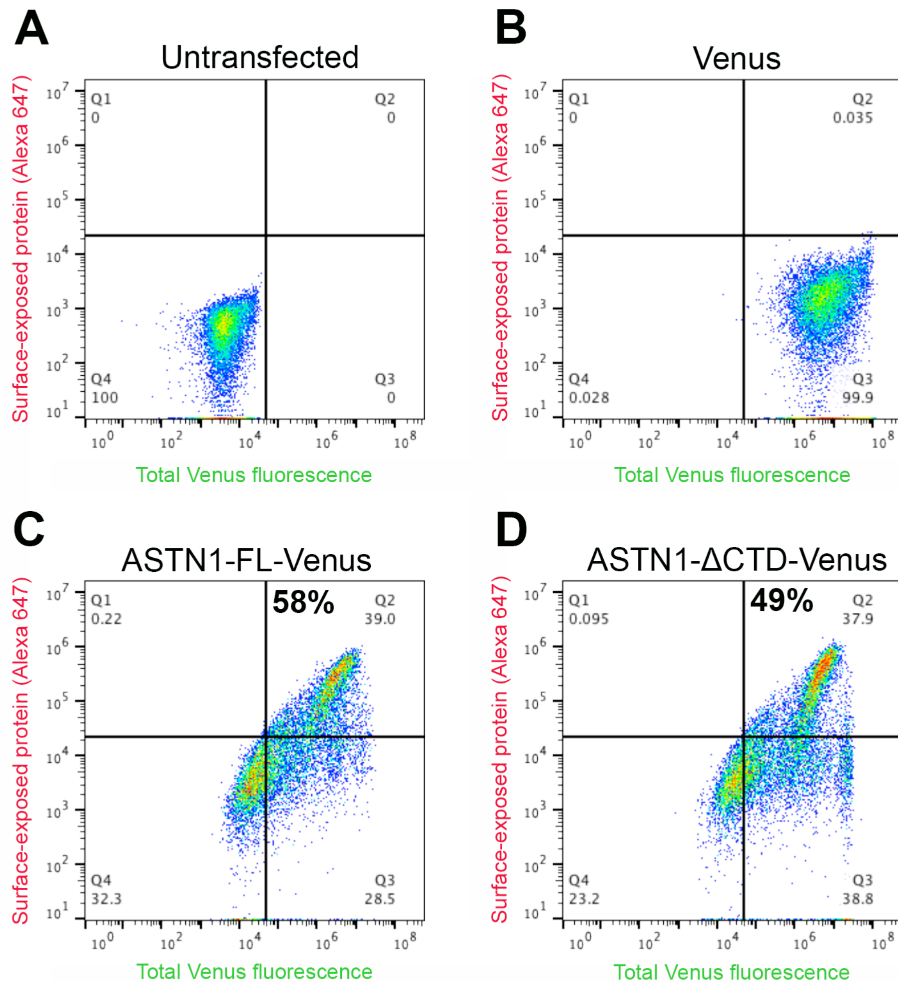

Fig. S1 | ASTN1-FL and ASTN1-ΔCTD are both expressed on the cell surface.

Flow cytometry of live HEK 293T cells labeled with GFP and Alexa Fluor 647 antibodies. Control, untransfected cells **(A)** or cells transfected with *Venus* **(B)**, *Astn1-FL-Venus* **(C)**, or *Astn1-ΔCTD-Venus,* lacking the MACPF, FNIII and ANX-like domains in the C-terminus **(D)**. The x-axis shows total GFP fluorescence and the y-axis shows surface labeling (Alexa Fluor 647). Thus, cells expressing cytosolic Venus-tagged proteins are indicated in the lower right quadrant (Q3), while double positive cells (GFP+/Alexa Fluor 647+) in the upper right quadrant (Q2) express Venus-tagged proteins exposed on the cell surface. The percentage of cells expressing ASTN1 on the cell surface is depicted. Both ASTN1-FL and ASTN1-ΔCTD were significantly expressed on the cell surface (58 % and 49 % of live transfected cells, respectively).

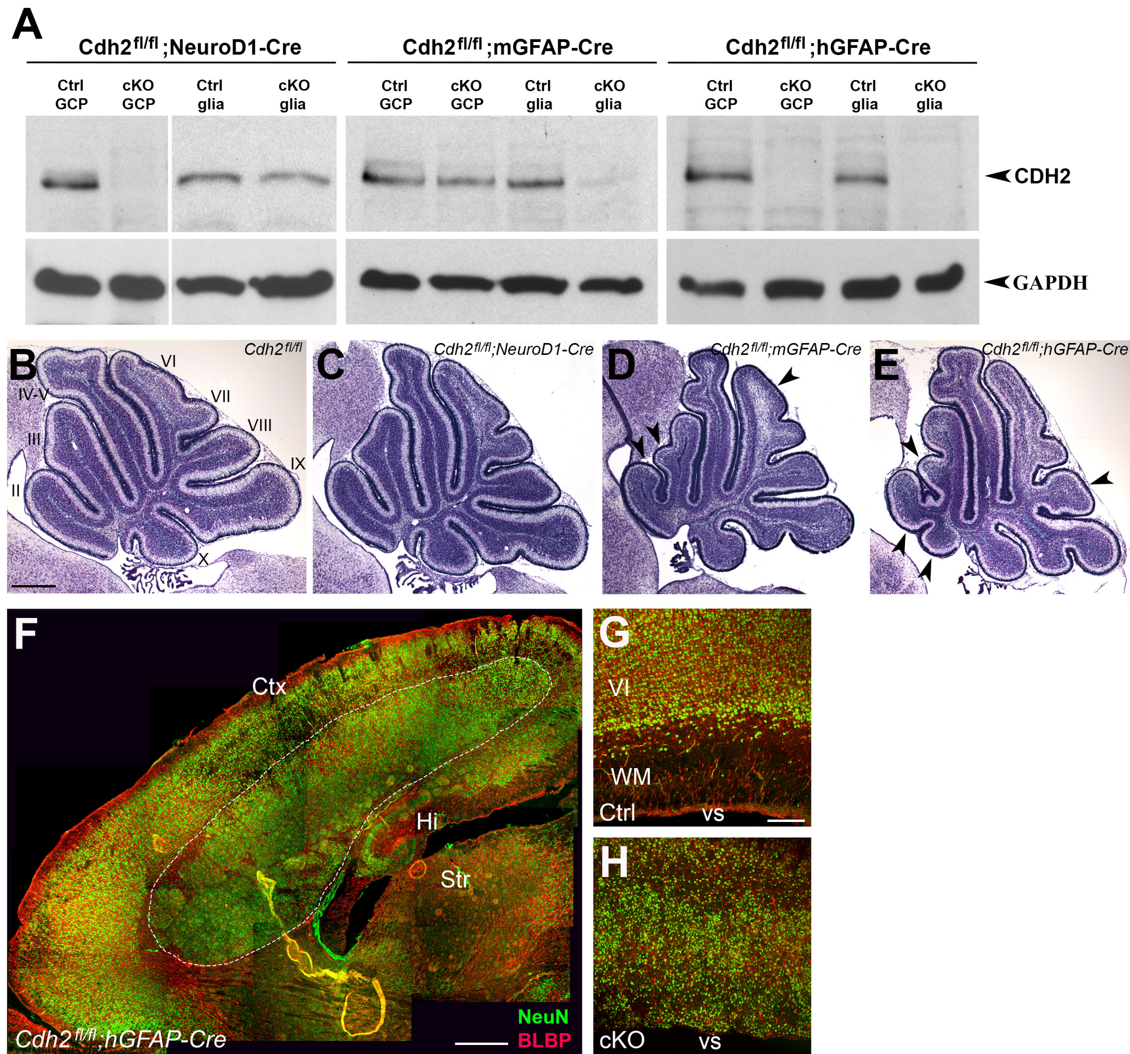

Fig. S2 | Cerebellar foliation defects and double cortex in mutants lacking *Cdh2*.

(A) Western blots of protein lysates of GCPs and BG purified at P7 showing the absence of CDH2 expression in the three *Cdh2$^{fl/fl}$* cKO lines. Loss of CDH2 was observed in GCPs from the *Cdh2$^{fl/fl}$;NeuroD1-Cre* mice, in BG from the *Cdh2$^{fl/fl}$;mGFAP-Cre* mice, and in both GCPs and glia from the *Cdh2$^{fl/fl}$;hGFAP-Cre* mice. Protein expression was compared to GAPDH. **(B – E)** Nissl staining of P7 cerebella of a *Cdh2$^{fl/fl}$* control mouse **(B)**, *Cdh2$^{fl/fl}$;NeuroD1-Cre* mouse **(C)**, *Cdh2$^{fl/fl}$;mGFAP-Cre* mouse **(D)** and *Cdh2$^{fl/fl}$;hGFAP-Cre* mouse **(E)**. The cerebellar lobes are labeled with roman numerals in (B). Note the foliation defects in mice lacking *Cdh2* in BG or in both GCPs and BG (arrowheads in D, E). In particular, the ventral (I-III) lobes contained additional fissures resulting in extra lobules, while medio-dorsal (VI-VIII) lobes had fewer fissures and irregularly shaped folia. **(F – H)** Double cortex and perturbed lamination were observed in the cerebral cortex of *Cdh2$^{fl/fl}$;hGFAP-Cre* mice only. Ctx: cerebral cortex; Hi: hippocampus; Str: striatum; VI: layer 6; WM: white matter; vs: ventricular surface. Scale bar represents 500 μm in (B – F) and 200 μm in (G, H).

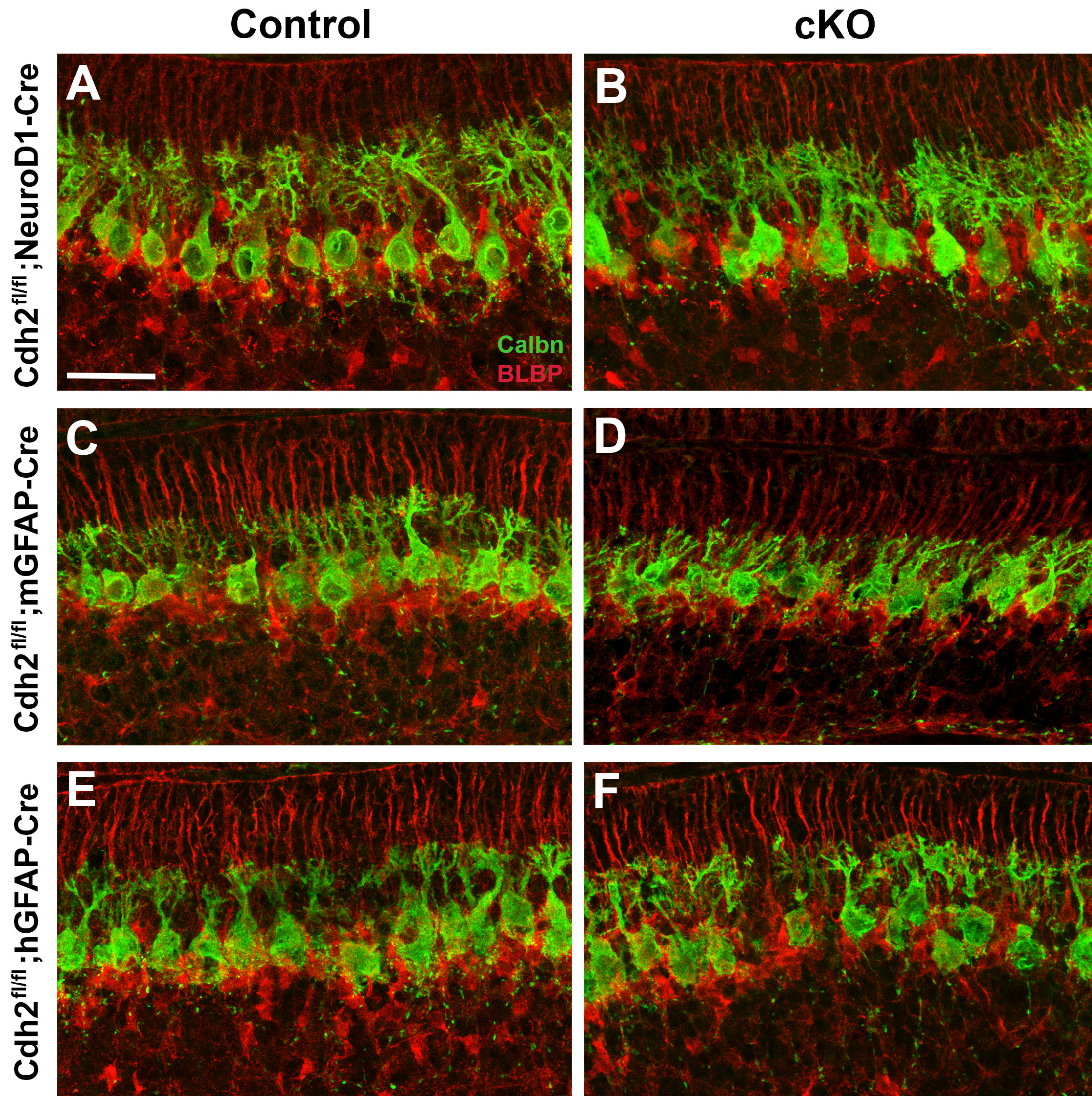

Fig. S3 | Purkinje cell layer formation is normal in *Cdh2* cKO mice.

Calbindin and BLBP immunolabeling of cerebellar sections of P7 *Cdh2$^{fl/fl}$* control mice **(A, C, E)**, and *Cdh2$^{fl/fl}$;NeuroD1-Cre* **(B)**, *Cdh2$^{fl/fl}$;mGFAP-Cre* **(D)**, and *Cdh2$^{fl/fl}$;hGFAP-Cre* **(F)** cKO mice. Purkinje cells aligned normally in the Purkinje cell layer in all three lines. Compared to control littermates, the dendritic morphology was similar in cKO Purkinje cells, although structural detail was not studied. Scale bar represents 50 µm.

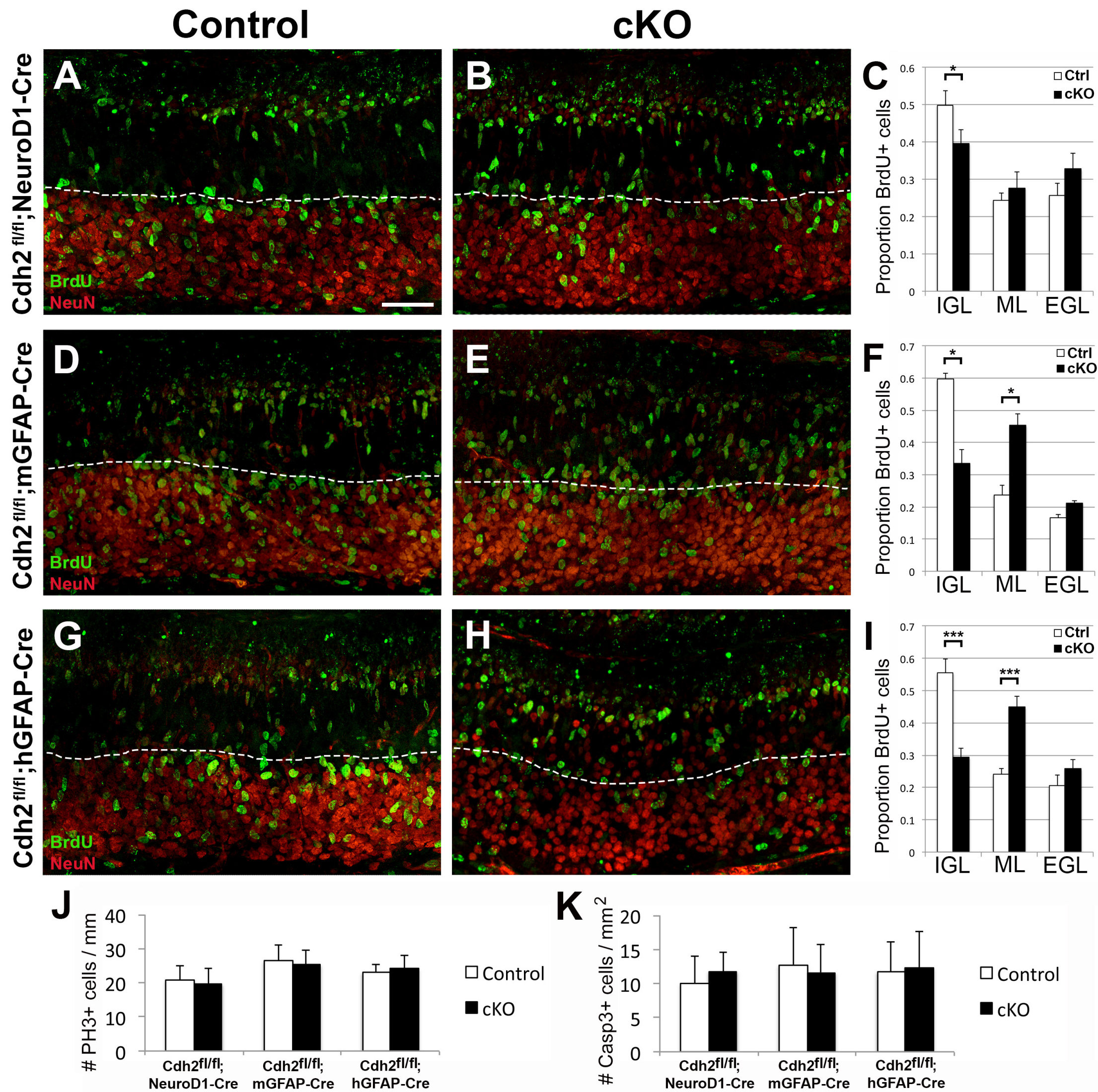

Fig. S4 | Birth-dating of GCs in *Cdh2* cKO mice.

BrdU (green) and NeuN (red) labeling of P7 cerebella after BrdU injection at P5 (48 h). **(A – C)** Loss of *Cdh2* in GCPs in *Cdh2$^{fl/fl}$;NeuroD1-Cre* mice resulted in 10 % decrease in the proportion of GCs reaching the IGL (dotted lines). **(D – F)** Severe migration defects were observed in *Cdh2$^{fl/fl}$;mGFAP-Cre* mice lacking *Cdh2* in BG, with a 27 % decrease in the proportion of GCs in the IGL and a 22 % increase in GCs in the ML. **(G – I)** Similar GCP migration defects were observed in *Cdh2$^{fl/fl}$;hGFAP-Cre* mice, with a 26 % decrease in GCs in the IGL and a 21 % increase in GCs stalled in the ML. While the NeuN labeling showed a slightly more irregular IGL in the *Cdh2$^{fl/fl}$;hGFAP-Cre* line, BrdU labeling revealed similar migration defects between the *Cdh2$^{fl/fl}$;mGFAP-Cre* and *Cdh2$^{fl/fl}$;hGFAP-Cre* lines, suggesting that the IGL patterning difference is dependent on the onset of promoter activity (E13.5 for *hGFAP* and after P0 for *mGFAP*). Proliferation (phospho-histone H3; **J**) and apoptosis (Caspase-3; **K**) did not differ in the cerebellum of either cKO line. * $P < 0.05$; *** $P < 0.001$. Scale bar represents 50 µm.

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