Supporting Information

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SI Text

Cell Proliferation Assays. For analysis of hippocampus, P8 mice were injected with CldU (equimolar to 75 mg/kg BrdU) or IdU (equimolar to 75 mg/kg BrdU) i.p. at P8 and P12 and killed at P13. Cryosections were counterstained with the proliferation marker, Ki67 (Vector Laboratories), and nuclear marker, DAPI (Sigma). Quantification was performed by calculating the percentage of colocalization of markers in 100 randomly chosen cells in a 1 in 12 series of sections.

***In Situ* Hybridization (ISH).** ISH for *Stumpy* was performed as previously described (1).

Quantitative Real-Time PCR (qRT-PCR). Total RNA was isolated from P8 hippocampus using the TRIzol reagent according to manufacturer's instructions (Invitrogen Life Technologies). Purified RNA was digested with DNase I to remove trace contaminating genomic DNA using the DNAfree kit according to the manufacturer's protocol (Ambion). An aliquot corresponding to 1.4 μg (normalized for hippocampus) of purified total RNA was then used for first-strand cDNA synthesis using SuperScript II reverse transcriptase and oligo(dT) in a final volume of 20 μl according to the manufacturer's instruction (Invitrogen Life Technologies). Complementary DNA (1 μl) was used for subsequent 10 μl qRT-PCR conducted in an Applied Biosystems 7500 Fast Real-Time PCR System. We quantified gene expression using for the following primer-probe sets (Applied Biosystems Assay ID in parentheses): *Shh* (Mm00436527 m1), *Smo* (Mm01162710 m1), *Ptch1* (Mm00436026 m1), *Gli1* (Mm00494645 m1), *Gli2* (Mm01293117 m1), and *Gli3* (Mm00492333 m1). The ratio of the amount of amplified target cDNA to the amount of HPRT cDNA represents the relative levels in each sample.

Electron Microscopy (EM). Postnatal day 0 (P0) control or Δ*Stumpy* mice were transcardially perfused with 0.1M phosphate buffer (PB) followed by 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1M PB. Brains were dissected and fixed overnight at 4°C. Coronal sections (100 μm) were treated with 1% osmium tetroxide, dehydrated in ethanol and propylene oxide, and flat embedded in Durcupan (ACM; Fluka) between glass slides and coverslips coated with Liquid Release Agent (EMS). The comparable regions of the fourth ventricle were dissected, re-embedded and further proceed for electron microscopy investigation as described below.

For Immuno-electron microscopy labeling, P7 C57BL/6 wild-type mice were perfused transcardially with 0.9% saline followed by a fixative containing 4% paraformaldehyde, 0.2% picric acid, and 0.2% glutaraldehyde and immersed overnight in the same fixative. Vibratome sections were taken for pre-embedding immunohistochemistry and incubated in rabbit anti-adenylyl cyclase III (ACIII) (1:1000; Santa Cruz Biotechnology). Sections were incubated in biotinylated anti-rabbit IgG secondary antibody (1:300; Vector Laboratories) and exposed to peroxidase-conjugated avidin-biotin complexes (ABC Elite kit; Vector Laboratories). Sections were reacted with Ni-intensified DAB as a chromogen. Sections were postfixed with 1% OsO_4, dehydrated, and flat-embedded in Durcupan as described above. Selected immunolabeled regions were re-embedded, cut into 70-nm-thick sections using a Reichert ultramicrotome and stained with uranyl acetate and lead citrate. All sections were evaluated using a JEOL 1010 electron microscope equipped with a Multiscan 792 digital camera (Gatan). Three-dimensional (3D) reconstruction of the cilia from the serial images was performed using the computer program Reconstruct (2), publicly available at http://synapses.bu.edu. The reconstructed cilia lengths were measured and the mean ± SE for cilia harbored by each cell type were calculated.

Cell Culture. C6-R radial glial-like cells (gift from N. Sestan, Yale University, New Haven, CT) were grown in 90% DMEM, 10% FBS and 1X Penicillin/Streptomycin in a 37°C incubator with 5%CO_2/95%O_2. Cells were fixed in either 4% paraformaldehyde in 0.1MPB (PFA) for 15 min or briefly (2–3 min) with -20°C methanol. Postnatal neural progenitor cells were cultured according to previously published protocols by Ray and Gage, including a Percoll gradient separation step (3).

Western Blot. P5 hippocampi of control or Δ*Stumpy* brains, or E11.5 whole embryos were lysed in 1X RIPA buffer (Cell Signaling) supplemented with 1X protease inhibitor mixture (Sigma), 1X phosphatase inhibitor cocktails-1 and -2 (Sigma), and 1X PMSF. One hundred μg of total protein was subjected to SDS/PAGE using a 3–8% Tris-Acetate gel (NuPAGE) for 1 h at 150V in 1X Tris-Acetate SDS running buffer (NuPAGE). Gels were incubated in an equilibration buffer [2X transfer buffer (NuPAGE), 10% methanol, antioxidant (1:1000; NuPAGE)] for 20 min at RT followed by protein transfer onto PVDF membranes using an iBlot™ Dry Blotting System (Invitrogen Life Technologies). Membranes were blocked for 1 h in 5% NFDM in 1X Tris-buffered saline containing 0.1%Tween-20 (TBST) and incubated in rabbit anti-Gli3 (1:200; gift from B. Wang) in 2.5% BSA in 1X TBST for 72 h at 4°C. Membranes were washed, incubated in HRP-conjugated goat anti-rabbit IgG secondary antibody (1:10,000; BioRad) diluted in 2.5% non-fat dry milk (NFDM) in 1X TBST for 1 h at RT. Signals were detected using enhanced chemiluminescence (SuperSignal® West Femto Kit; Thermo Fisher Scientific). For loading control, blots were stripped and re-probed using a mouse anti-β-actin antibody (1:5000; Sigma). Blots (repeated in triplicate) were scanned at a resolution of 2400 dpi and the band intensities of Gli3 and β-actin were quantified using Adobe Photoshop CS2. The ratio of Gli3 to β-actin band intensity was calculated for control, Δ*Stumpy* and E11.5 whole embryos.

1. Town T, et al. (2008) The *stumpy* gene is required for mammalian ciliogenesis. *Proc Natl Acad Sci USA* 105(8):2853–2858.
2. Fiala JC, Harris KM (2001) Extending unbiased stereology of brain ultrastructure to three-dimensional volumes. *J Am Med Inform Assoc* 8(1):1–16.
3. Ray J, Gage FH (2006) Differential properties of adult rat and mouse brain-derived neural stem/progenitor cells. Mol Cell Neurosci 31(3):560–573.

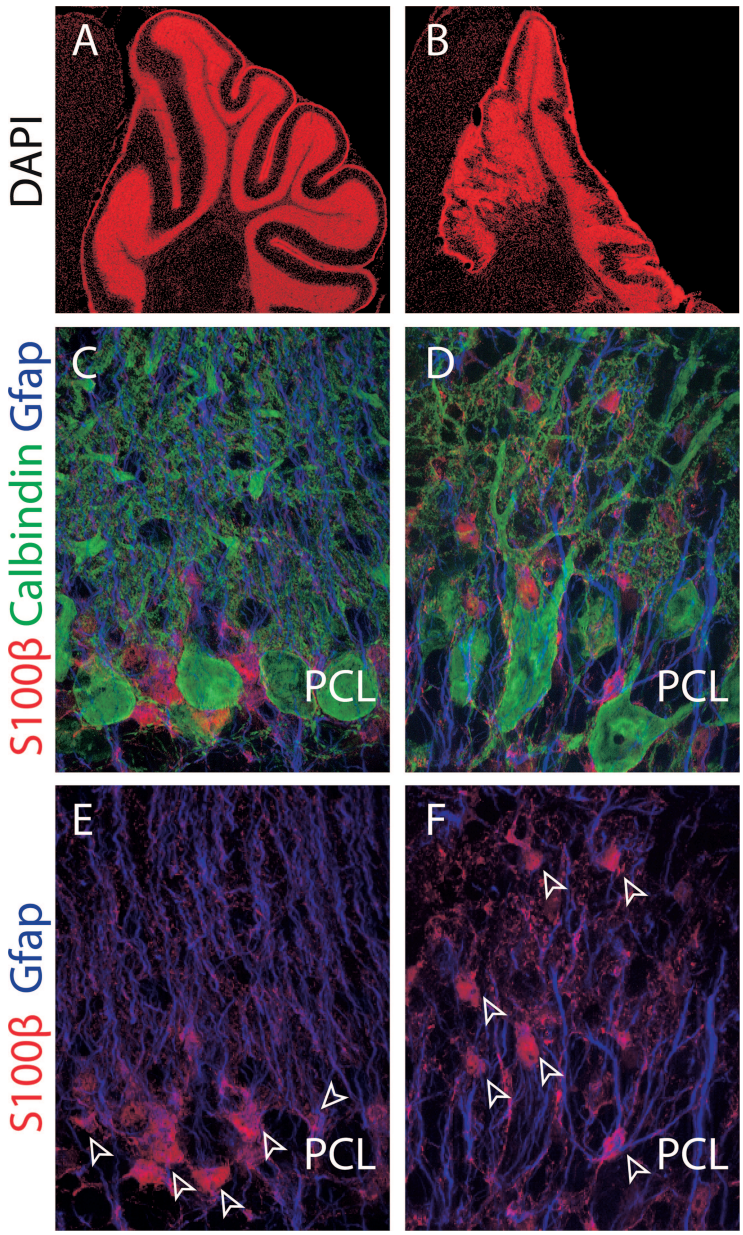

Fig. S1. Cerebellar defects in Δ*Stumpy* brains. (*A* and *B*) DAPI nuclear labeling of P13 control (*A*) and Δ*Stumpy* (*B*) cerebellum. Low magnification showed that in Δ*Stumpy* mice, the cerebellum was smaller and poorly formed including a disrupted inner granule layer (IGL) and abnormal foliation. (*C–F*) Immunostaining for Calbindin (green), GFAP (blue), and S100β (red). The Purkinje cell layer (PCL) showed displaced Calbindin+ cells in Δ*Stumpy* brains (*D*) compared to control mice (*C*). Fewer GFAP+ Bergmann glia were observed in Δ*Stumpy* cerebellum (*D*, *F*) and remaining cells were abnormally located (arrowheads show S100β+ cell bodies) and dysmorphic compared to control (*C*, *E*).

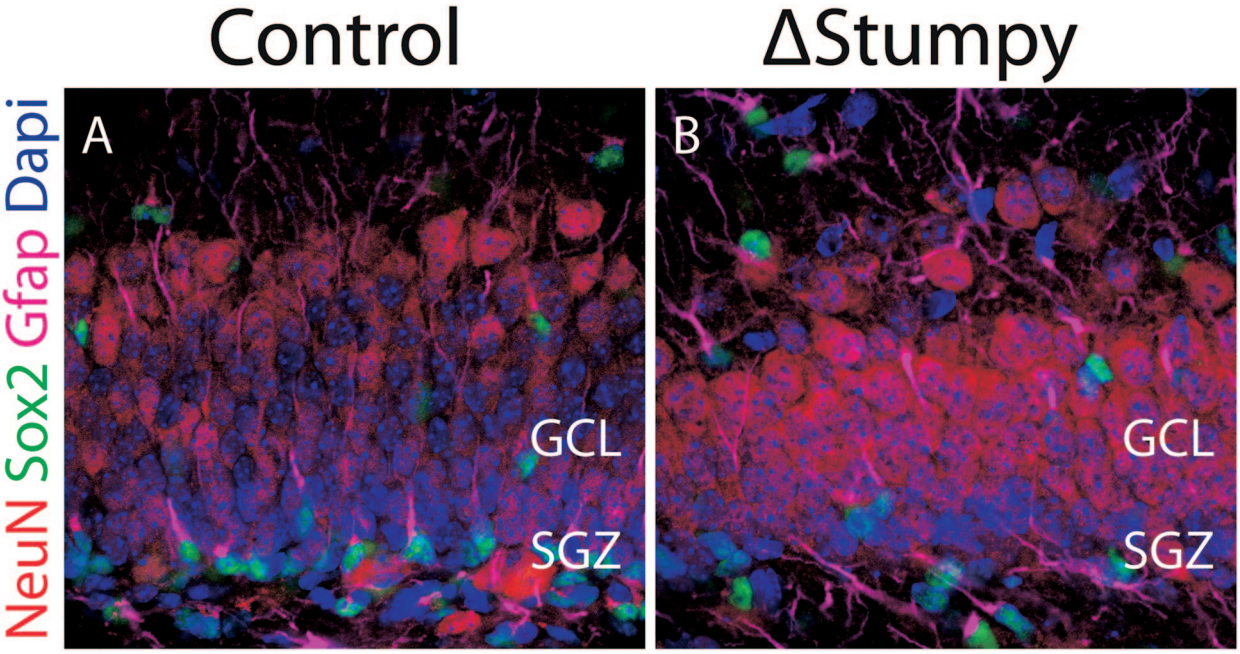

Fig. S2. Merged view from Fig. 1. Immunostaining for NeuN (red), Sox2 (green), and GFAP (magenta). Nuclei are shown in blue.

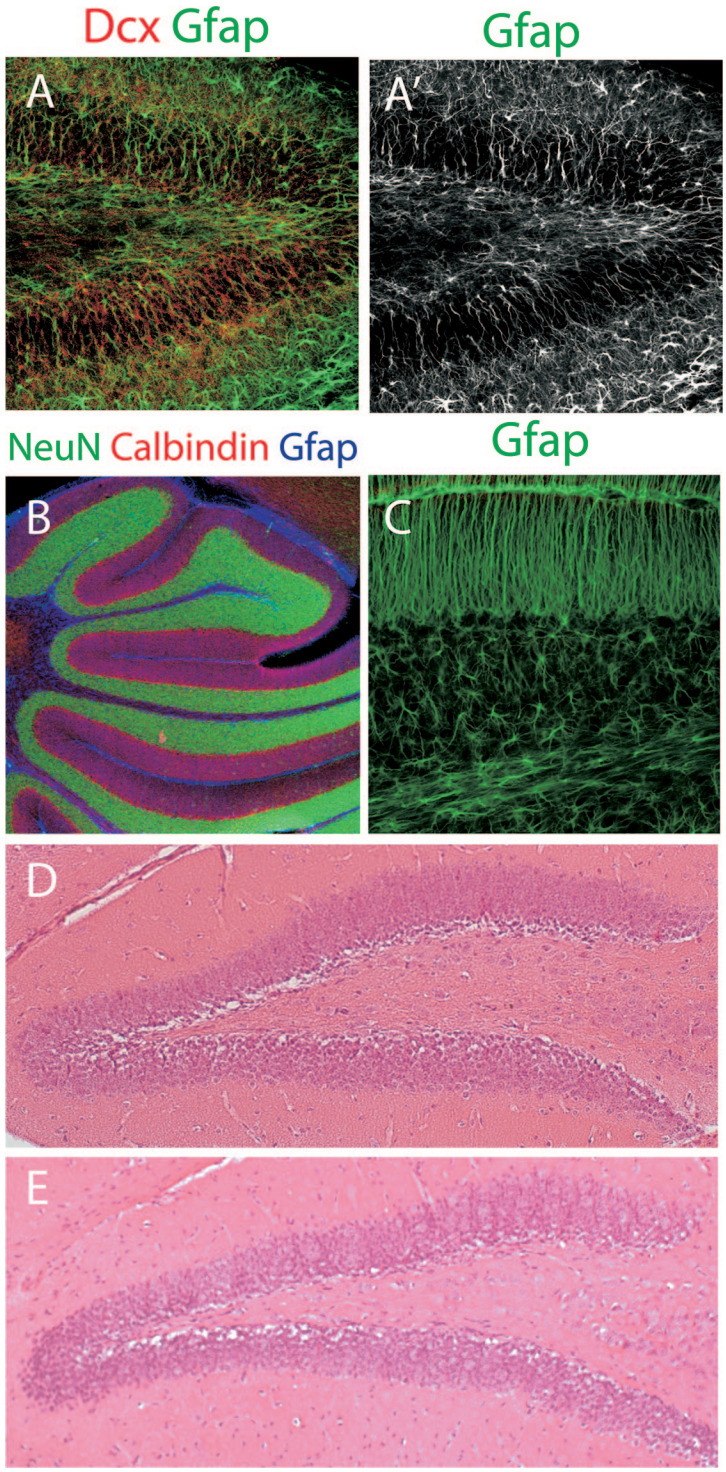

Fig. S3. Normal cerebellar and hippocampal morphology in ''control'' spontaneous hydrocephalic mice. (*A* and *A′*) Immunostaining for DCX (red) and GFAP (green) in the dentate gyrus (DG) from a hydrocephalic *NestinCre*−/*Stumpy fl/fl* brain. (*B* and *C*) Cerebellum immunostained for NeuN (green), Calbindin (red), and GFAP (blue) from a hydrocephalic *NestinCre*−/*Stumpy* +/+ brain. Normal expression patterns were observed with all three antibodies. (*D* and *E*) Hematoxylin and eosin-stained sections show a preserved DG architecture in a wild-type control (*D*) compared to a spontaneous ApoE deficient mouse (*E*) hydrocephalic brain.

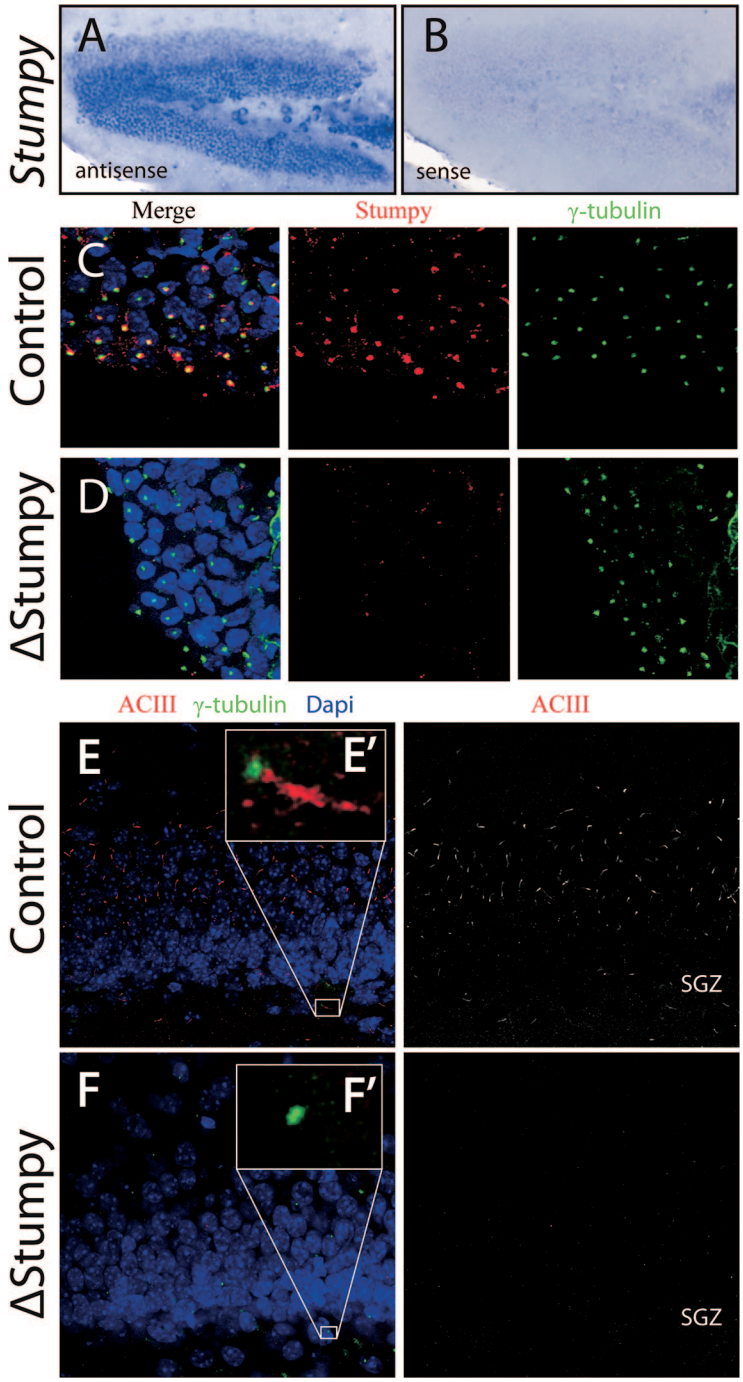

Fig. S4. *Stumpy* expression and lack of cilia in Δ*Stumpy* mutant hippocampus. (*A* and *B*) *In situ* hybridization for *Stumpy* in postnatal mouse brain showed high expression throughout the hippocampus including the dentate gyrus which was absent in the sense control (*B*). (*C* and *D*) Immunostaining of choroid plexus (nuclei labeled with DAPI) showed colocalization of *Stumpy* (red) with the ciliary basal body component, γ-tubulin (green). Note that γ-tubulin expression appears unaffected in the mutant (D). (*E* and *F*) Immunostaining of P13 control (*E* and *E′*) and *Stumpy*-deficient (*F* and *F′*) hippocampus for adenyl cyclase III (ACIII; red) and γ-tubulin (green). ACIII labels ciliary axonemes that were completely absent in *Stumpy* mutants. Insets show higher magnification views.

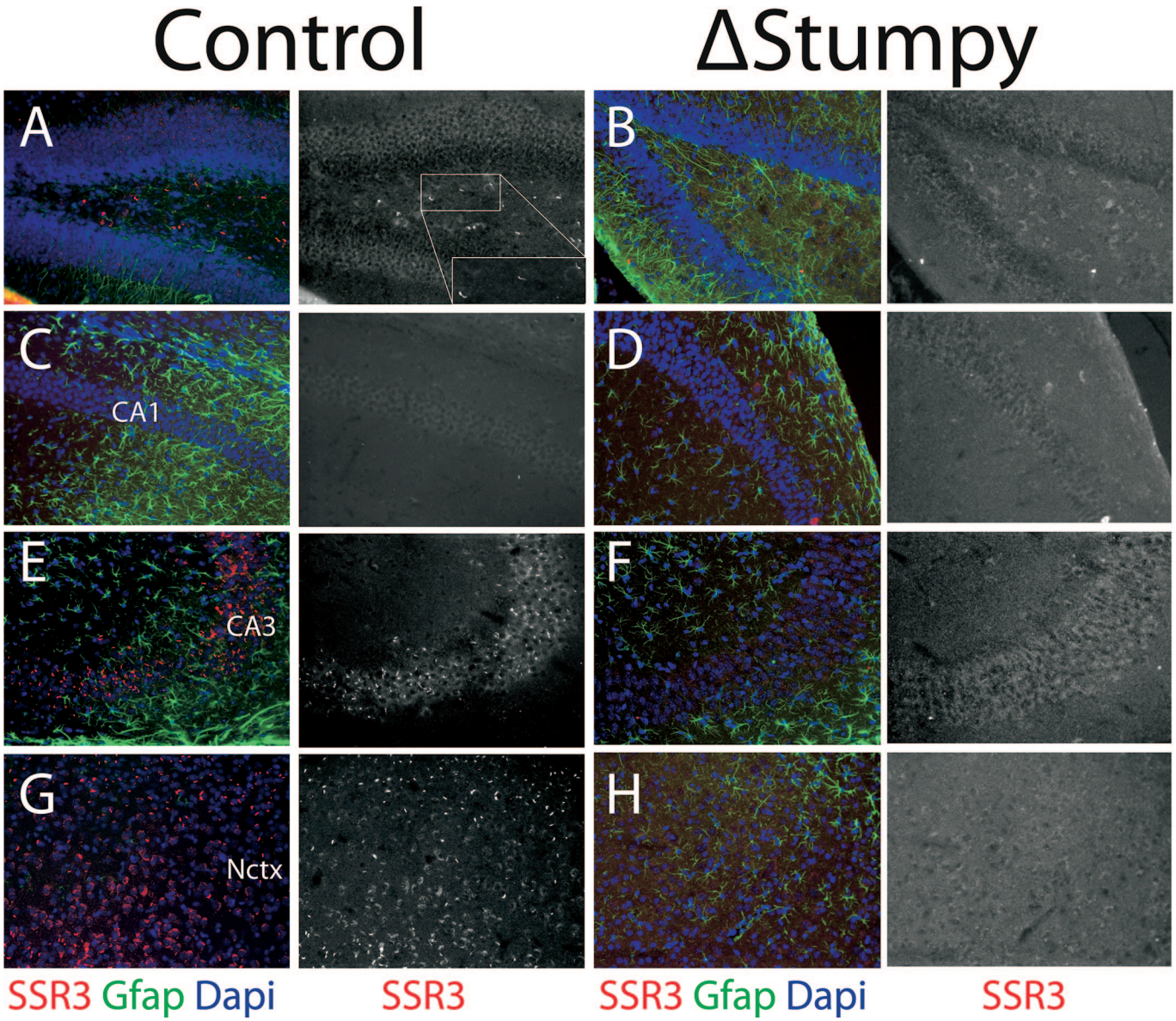

Fig. S5. Somatostatin Receptor S3 (SSR3) localizes to specific neural populations in mouse brain. Immunolabeling for SSR3 (red) and GFAP (green) in control (*A*, *C*, *E*, and *G*) and Δ*Stumpy* mice (*B*, *D*, *F*, and *H*). In control mice, SSR3+ cilia were observed within the hilus (*A*), CA3 (*E*) and neocortex (*G*), but not in the GCL of the DG (*A*) or CA1 (*B*). SSR3+ cilia were not observed in any brain region of Δ*Stumpy* mice (*B*, *D*, *F*, and *H*). Note: Δ*Stumpy* images are intentionally overexposed to demonstrate the lack of detectable cilia.

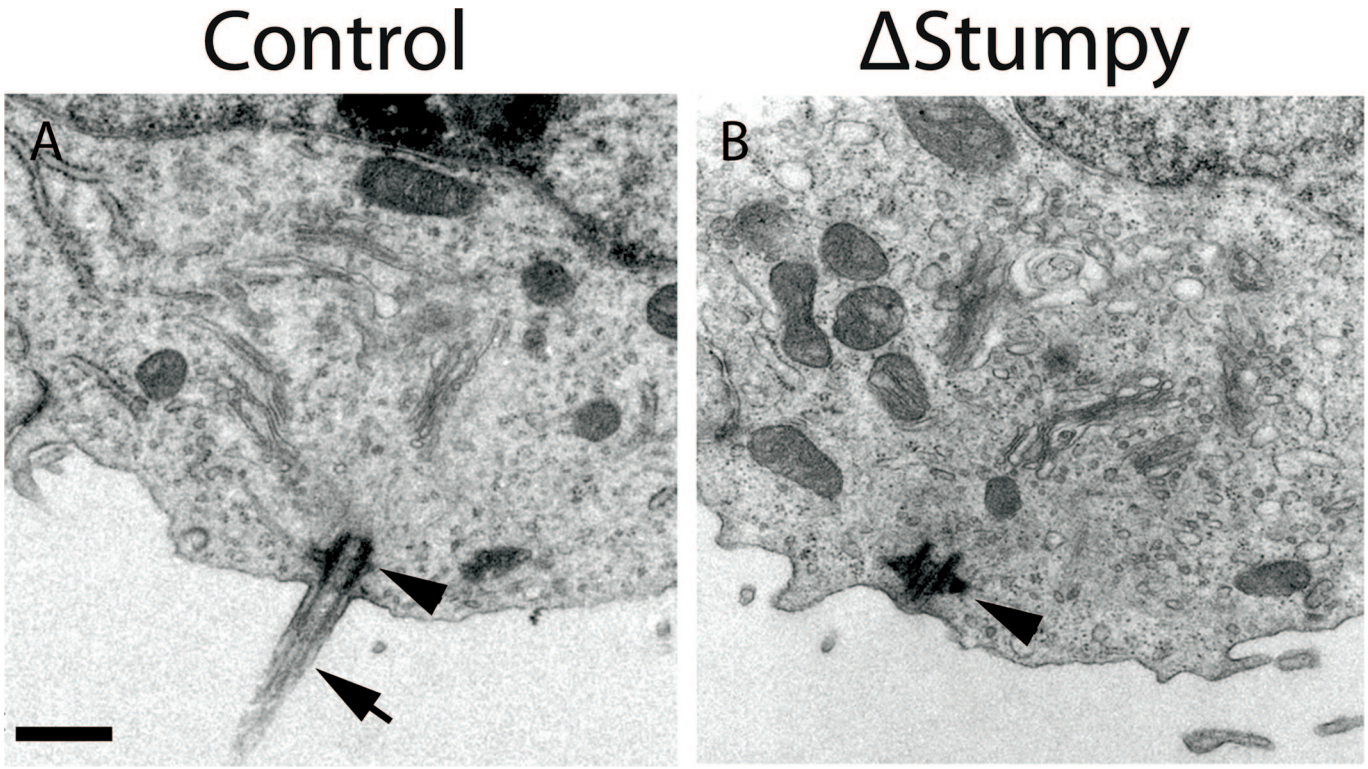

Fig. S6. Lack of ciliary axonemes in Δ*Stumpy* mice. Transmission electron micrograph shows extension of a ciliary axoneme (arrow) in control (*A*) and the lack of an axoneme in P0 Δ*Stumpy* brain (*B*). Basal bodies (arrowheads) appeared to form properly in Δ*Stumpy* mice. Images were taken along the lining of the 4th ventricle at P0. Bar = 0.5 μm

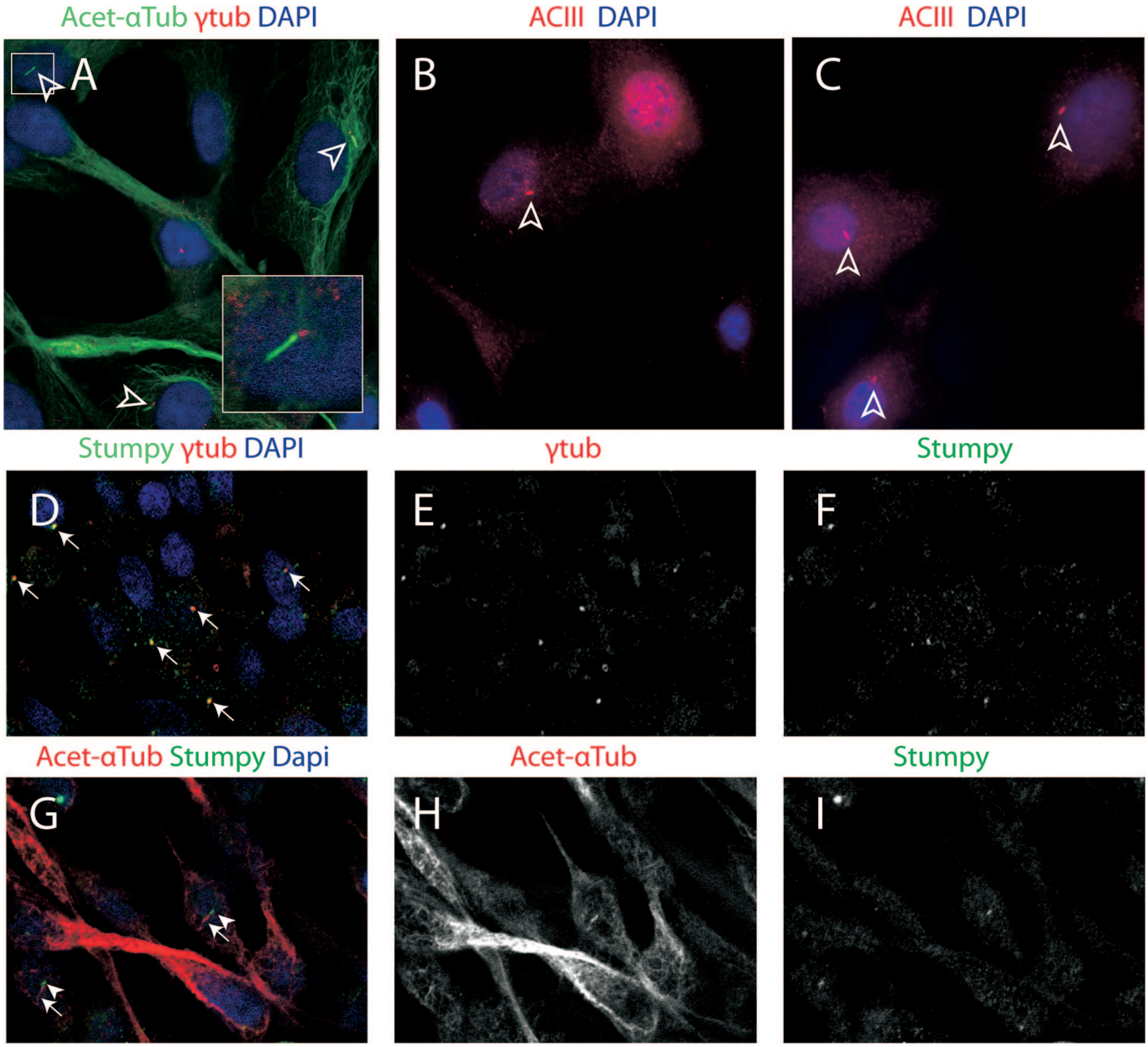

Fig. S7. Presence of cilia on cultured radial glia-like C6-R and adult neural progenitor cells (ANPCs) harvested from postnatal mouse brain. (*A*) Immunostaining of C6-R cells for γ-tubulin (red) and acetylated α-tubulin (green) showed the presence of basal bodies and ciliary axonemes, respectively. Inset shows higher magnification view of a cilium. Nuclei are stained with DAPI (blue). (*B* and *C*) ANPCs treated with EGF, FGF2, and heparin (*B*) or under differentiating conditions (*C*) immunostained for ACIII (red). Cilia (arrowheads) are observed. Nuclei are labeled with DAPI. (*D–F*) C6-R cells immunostained for stumpy (green) and γ-tubulin (red) reveals colocalization (arrows) with centrioles. Nuclei are stained with DAPI (blue). (*G–I*) C6-R cells immunostained for stumpy (green) and acetylated α-tubulin (red) revealed stumpy localization (arrowheads) at the basal body of acetylated α-tubulin-positive ciliary axonemes (arrows). Nuclei are stained with DAPI (blue).

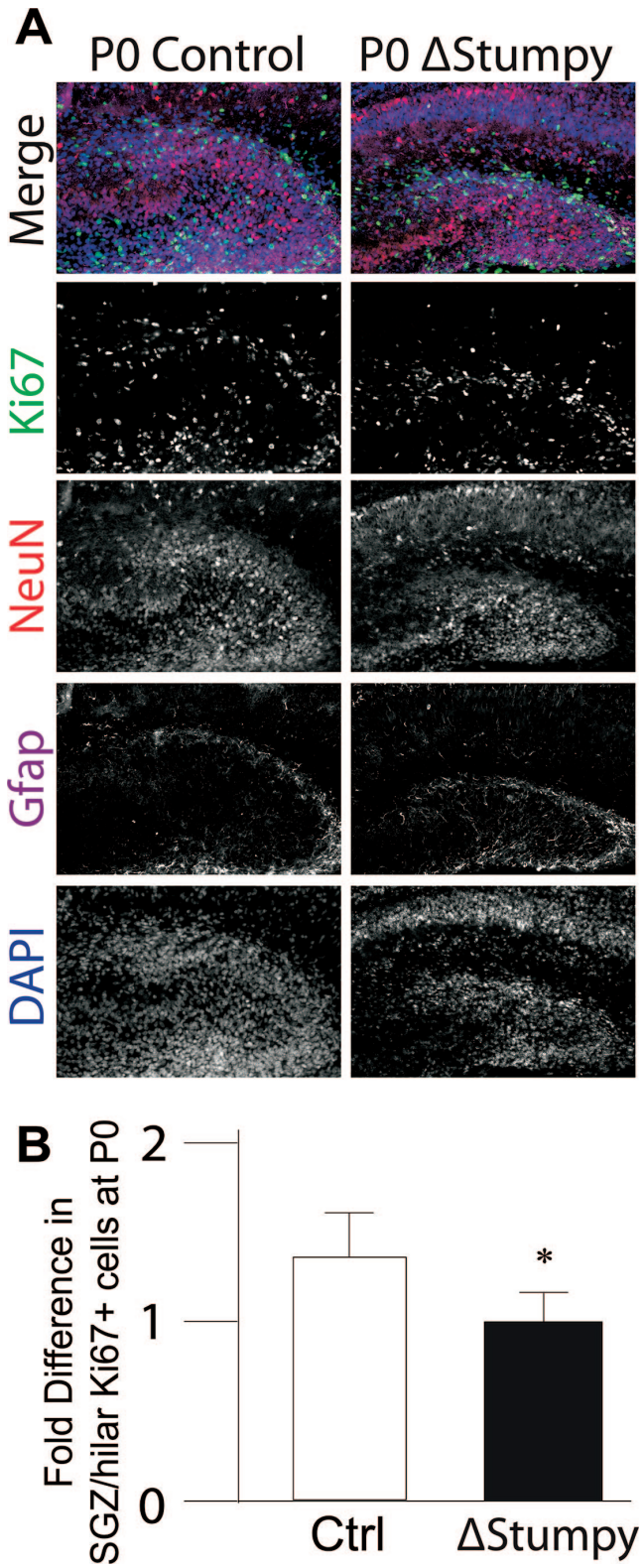

Fig. S8. Proliferation is already altered at P0 in *Stumpy*-deficient hippocampus. (*A*) Immunostaining for NeuN (red), Ki67 (green), and GFAP (purple). Nuclei stained with DAPI are shown in blue. (*B*) Quantification of Ki67+ SGZ/hilar cells shows a significant difference at P0.

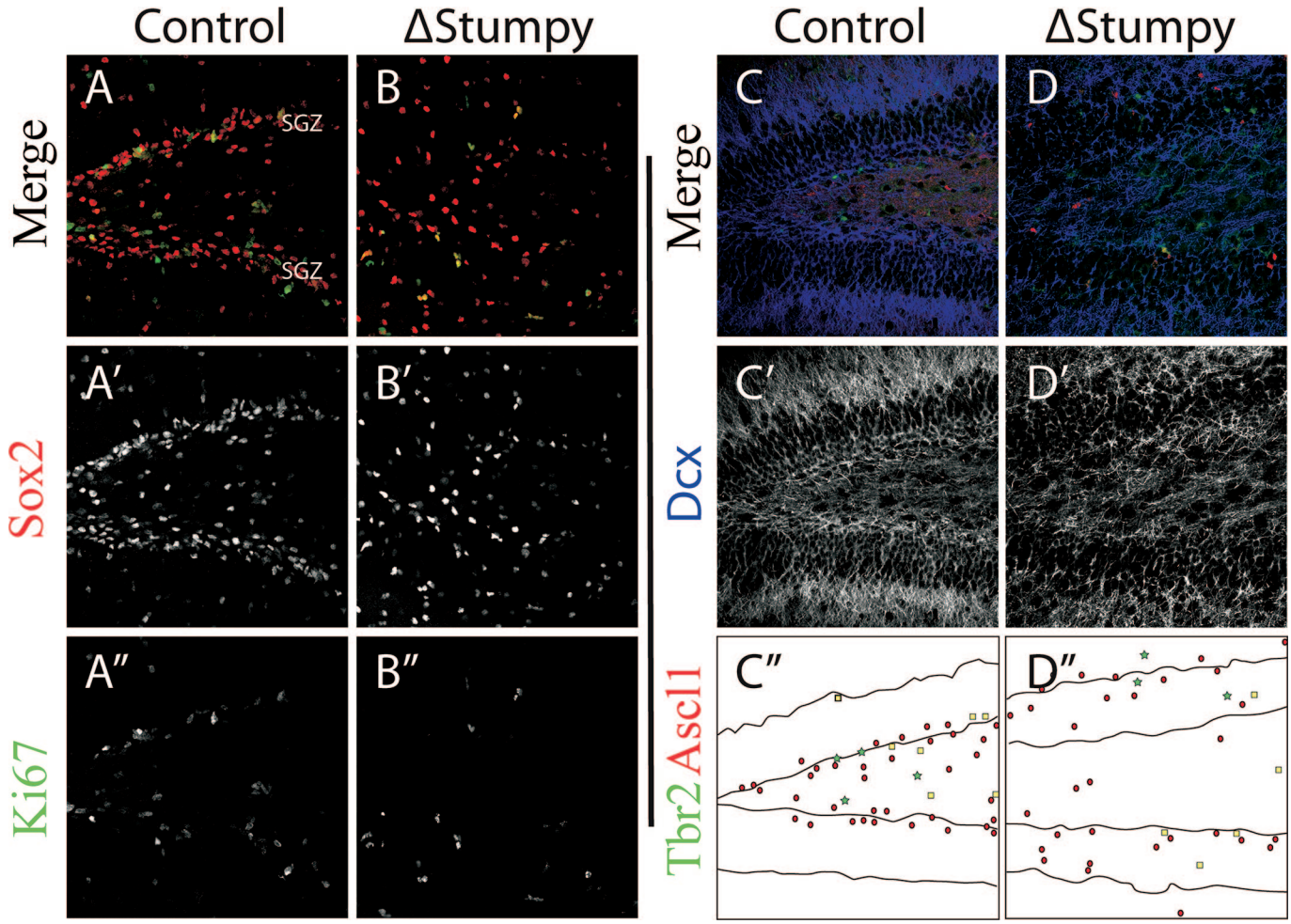

Fig. S9. Altered neurogenesis in *Stumpy*-deficient hippocampus. (*A* and *B*) Immunostaining at P13 for the stem cell marker, Sox2 (red) and proliferation marker, Ki67 (green). Dispersed localization and reduced number of Sox2+ (*B′*) and Ki67+ (*B″*) cells are shown in *Stumpy* mutant (NesCre; fl/fl) compared to control dentate gyrus Sox2+ (*A′*) and Ki67+ (*A″*) cells. (*C* and *D*) Immunostaining for intermediate progenitor markers Tbr2 (green), Ascl1 (red) and DCX (blue). Δ*Stumpy* brains (*D-D″*) showed altered distributions of differentiating neurons compared to control (*C-C″*). The boxes (*C″*,*D″*) show locations of Tbr2 (green stars), Ascl1+ (red circles), and Tbr2+/Ascl1+ (yellow boxes) cells relative to the DG blades (outlined). Note the dispersion of progenitors in Δ*Stumpy* mutants (*D″*).

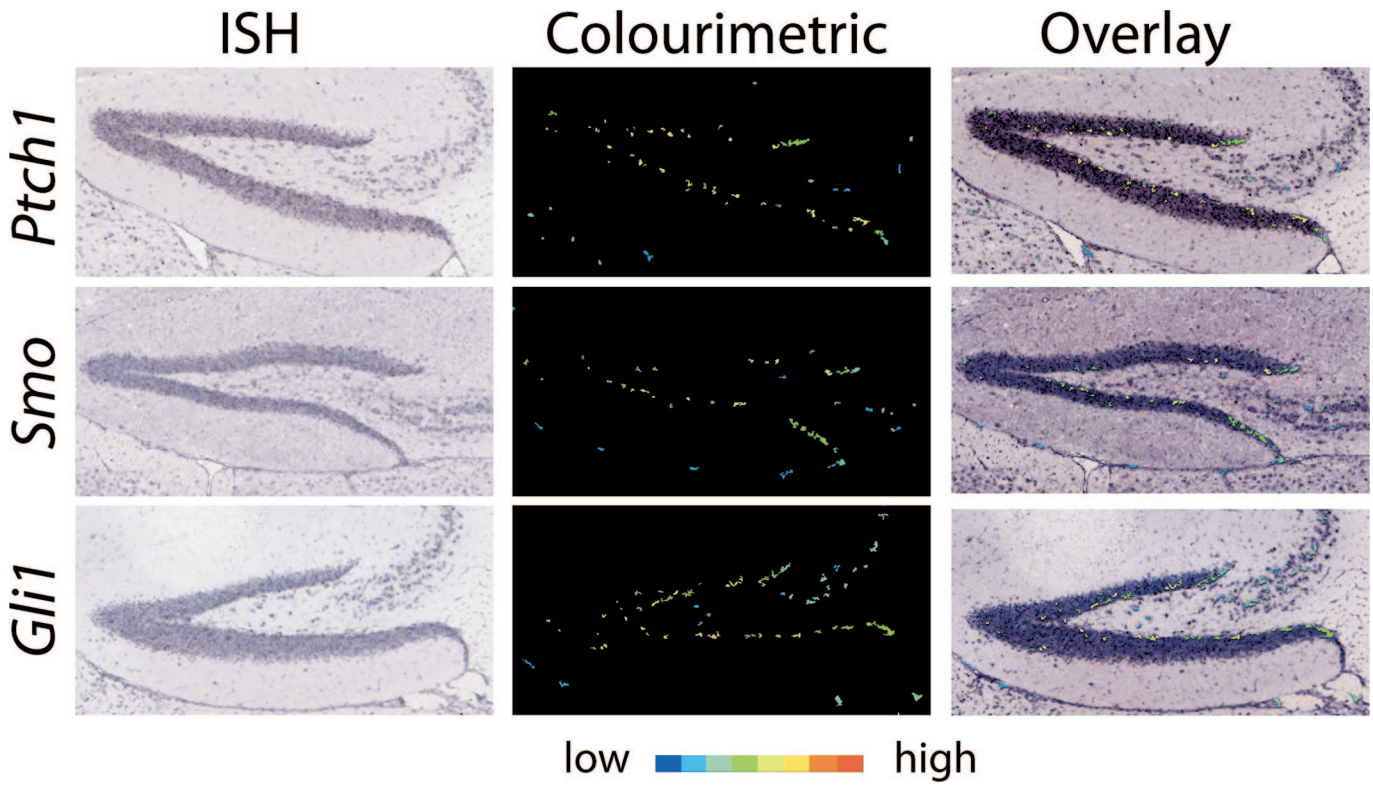

Fig. S10. Expression of *Shh* genes in the SGZ of adult mouse hippocampus. *In situ* hybridization (ISH) of *Ptch1* (*Top*), *Smo* (*Middle*), and *Gli1* (*Bottom*). Colourimetric analysis of expression density is shown in *Middle*. Highest expression is indicated by red while lowest is indicated by blue. Overlay of ISH and colourimetric analysis (*Right*) showed that *Ptch1*, *Smo* and *Gli1* are all highly expressed in the SGZ of the DG. All data were obtained from the Allen Brain Atlas (http://www.brain-map.org). Scale Bar ~100 μm.