

Antibodies	Host	Source	Cat. Number	IF/IHC Dilution
Ac-Tubulin	Mouse	Sigma-Aldrich	T7541	1:250
γ -Tubulin	Rabbit	Sigma-Aldrich	T5192	1:250
Numb	Rabbit	Novus	NB500-178	1:500
Phospho-Histone H3 (Ser10)		Cell Signaling	9706	1:100
Laminin	Rabbit	Sigma-Aldrich	L9393	1:30
aPKC ζ	Rabbit	Abcam	ab108970	1:500
pan PKC (γ -pT514)	Rabbit	Cell Signaling	9379	
aPKC ζ -pT410	Rabbit	Invitrogen	MA5-15060	
aPKC ζ -pT560	Rabbit	Abcam	ab59412	
pNumb	Rabbit	Cell Signaling	9878	
Total Numb	Rabbit	Cell Signaling	2756	
β -Actin	Mouse	Sigma-Aldrich	A2228	
HA	Mouse	Sigma-Aldrich	H3663	
Six1	Rabbit	Cell Signaling	12891	
GST	Rabbit	Abcam	ab19256	
PP2A-B55 α	Mouse	Santa Cruz	sc-81606	

Table S1. List of antibodies used in the study.

WB Dilution
1:1000
1:1000
1:1000
1:500
1:1000
1:1000
1:200
1:1000
1:1000
1:5000
1:1000

Peptide	Sequence	C-Terminal
aPKC ζ -T410	GDTTS(T ₄₁₀)FCGTPN	Amide
aPKC ζ -T560	EPVQL(T ₅₆₀)PDDEDV	Amide
Numb-S7	NKLRQ(S ₇)FRRKKD	Amide
Numb-S276	LARQG(S ₂₇₆)FRGFPA	Amide
Numb-S295	FKRQL(S ₂₉₅)LRINEL	Amide
H2AX-Y	GPKAPSGGKKATQASQR(Y)	Amide

Table S2. List of peptide sequences used in the study

Fig. S1. Spindle orientation of GCPs is developmentally regulated. (A) Immunostainings for phospho-Histone H3 (pH3) of cerebellar sections from P3 and P6 wild type mice and representative images for vertically (left; angle 0°) and horizontally (right; angle 90°) dividing GCPs in the EGL. (B) Violin plots illustrating the distribution of angles of division of GCPs during the course of postnatal development (Kruskal-Wallis test with Dunn's post hoc test for multiple comparisons). (C) Angles of division of GCPs in relation to the distance from the pial surface as measured in P3 animals.

Fig. S2. GCP proliferation is increased in *Ptch1*^{+/-} mice used in these studies. Quantification of the fraction of phospho-histone H3 (pH3)-positive cells in the EGL of *Ptch1*^{+/-} mice (n=19) as compared to control mice (n=12). Fisher's exact test. Data are mean±s.e.m.

Fig. S3. Laminin staining identifies the pial surface on top of the EGL. Co-immunostainings for Laminin, phospho-Histone H3, and DAPI on sagittal sections from P3 and P6 wildtype mice. Scale bar, 250 μm.

Fig. S4. Live imaging of dividing GCPs in *Atoh1-creER::GFP*^{F/+} mice and after GFP transduction. (A, B) Representative images of individual, GFP-labeled GCPs followed over a period of 9 hours. Single arrows indicate cells before mitosis and double arrows indicate position of daughter cells. Dotted lines indicate boundaries of the different layers of the cerebellar cortex. In vertical divisions (A), both daughter cells remain in the EGL. In contrast, in horizontal divisions (B), one daughter cell remains in the EGL while the other one migrates into the IGL. (C) Table summarizing the migrational behavior of daughter cells after GCP mitosis at P3 and P6. (D, E) Representative images of newborn neurons at time zero (t = 0) labelled with the PNIT-GFP retrovirus. Neurons were distinguished from GCPs by their migration to the molecular layer and complex branched morphology (red arrows). At P3, 25 out of 101 cells labeled at time zero were neurons (25%), compared to P6, where 54 out of 110 cells labeled were neurons (49%).

Fig. S5. Active Shh signaling is recapitulated in MEF cells by SAG induction. Mouse embryonic fibroblast (MEF) cells were induced with SAG (300nM) for 24h (3 technical replicates) and *Gli1* mRNA levels were quantified using quantitative real time PCR. Statistical analysis was performed using an unpaired *t* test. Data are mean±s.e.m.

Fig. S6 Full western blots from the manuscript. Uncropped western blots from Fig. 5A (A), Fig. 5C (B), Fig. 6A (C), and Fig. 6F (D). Boxes indicate the areas shown in the corresponding main figures.

Fig. S7 aPKC is expressed in GCPs. Co-immunostainings for phospho-histone H3 (pH3), atypical protein kinase C (aPKC), and DAPI on sagittal sections from a P3 wildtype mouse. Scale bar, 25 μm.

Movie 1. Immunohistochemical approach to determine angle of division of mitotic GCPs in the EGL. The movie was generated from a z-stack of images from P3 sagittal cerebellum taken from the prepyramidal fissure (lobes 7-8). A 250 um slice was immunostained with anti-acetylated alpha tubulin (green) and anti-beta tubulin (red) to image the centriole and spindle microtubules respectively, and counterstained with DAPI.

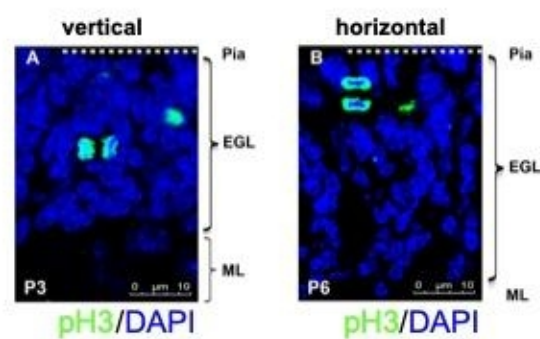
Movie 2. Movie of vertical division. Retroviral GFP labeled cell shown in Figure 2A illustrating vertical division of GCP. Both daughters remain in the EGL and maintain morphology consistent with progenitors. Cells were captured for a total of 420min, and imaged at 10min intervals.

Movie 3. Movie of horizontal division. Retroviral GFP labeled cells shown in Figure 2B illustrating horizontal division of GCP. After division one daughter cell remains in the EGL, while the other daughter extends a long process and migrates toward the IGL, exhibiting morphology consistent with newborn granule neurons. Cells were captured for a total of 400min, and imaged at 10min intervals.

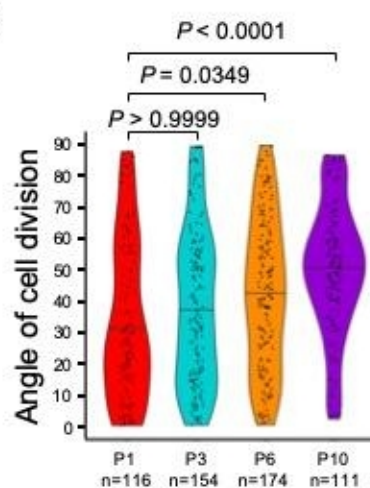
Movie 4. Movie of horizontal division. Retroviral GFP labeled cells illustrating horizontal cell division of GCP. Presumptive neuronal daughter cell migrates toward the IGL while the other daughter remains in IGL. Cells were captured for a total of 370min, and imaged at 10min intervals.

Supplementary Information

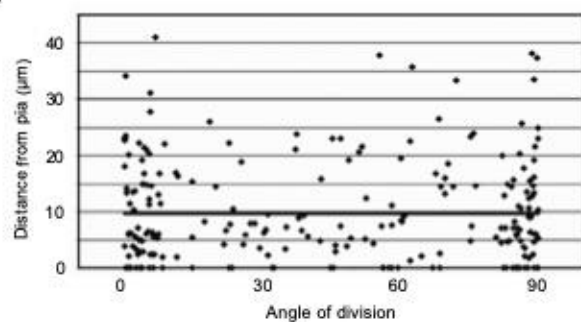
A

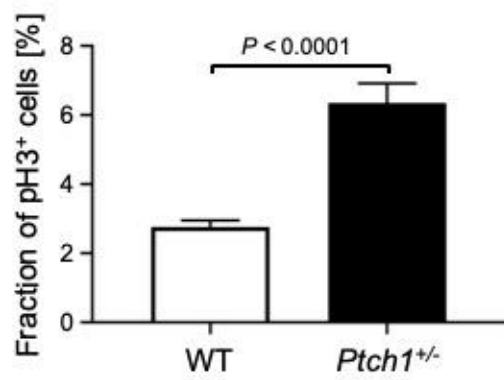


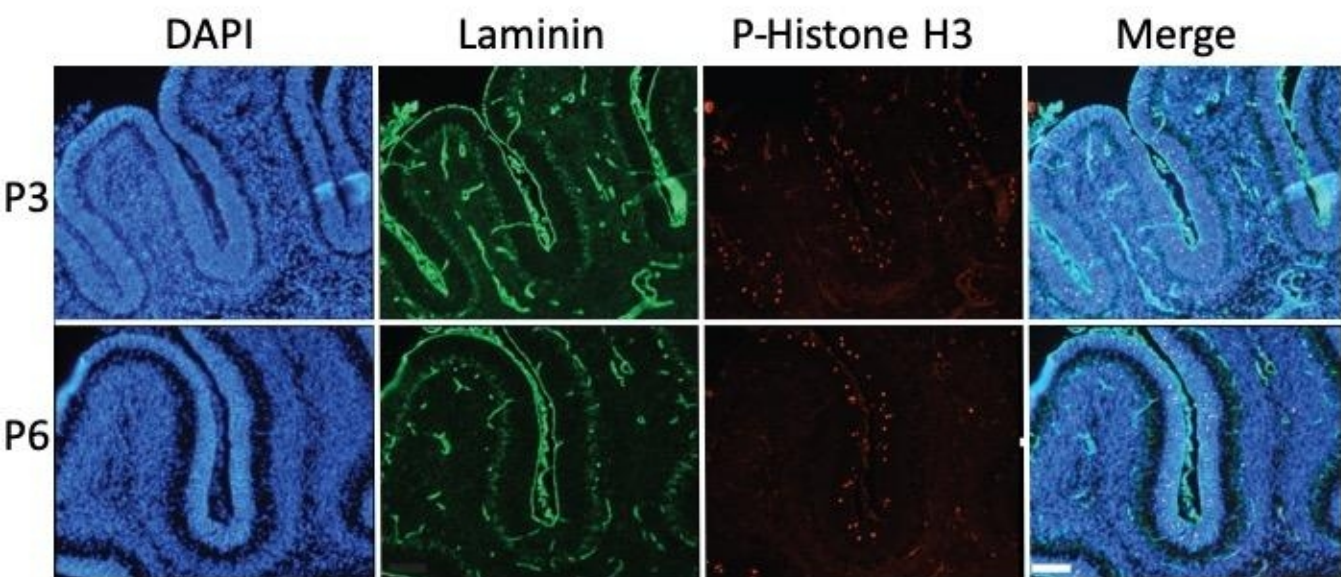
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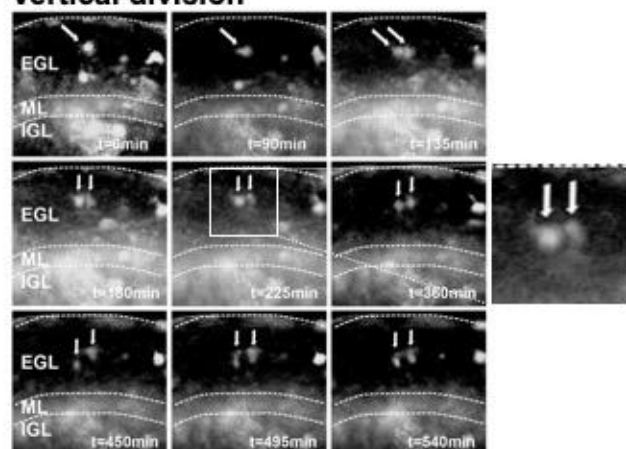
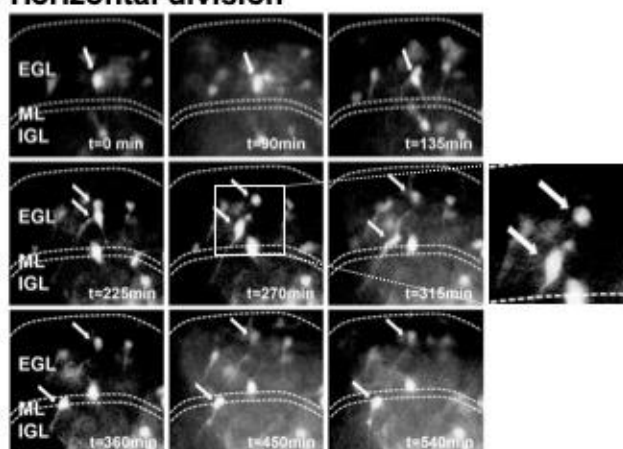


C

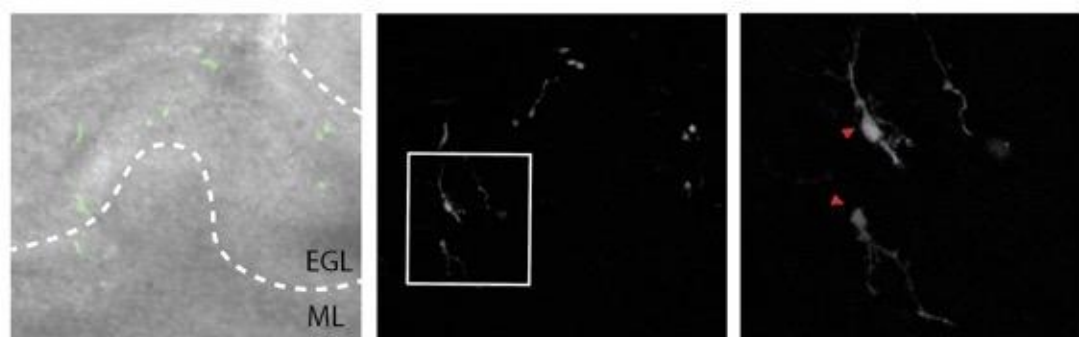






A**Vertical division****B****Horizontal division****C**

	Total number of vertical divisions detected		Total number of horizontal divisions detected	
	Cells stay in EGL	One cell leaves EGL	Cells stay in EGL	One cell leaves EGL
P3	34	0	6	6
P6	10	0	13	5

D**P3 Newborn neurons****E****P6 Newborn neurons**