A population of Nestin expressing progenitors in the cerebellum exhibits increased tumorigenicity

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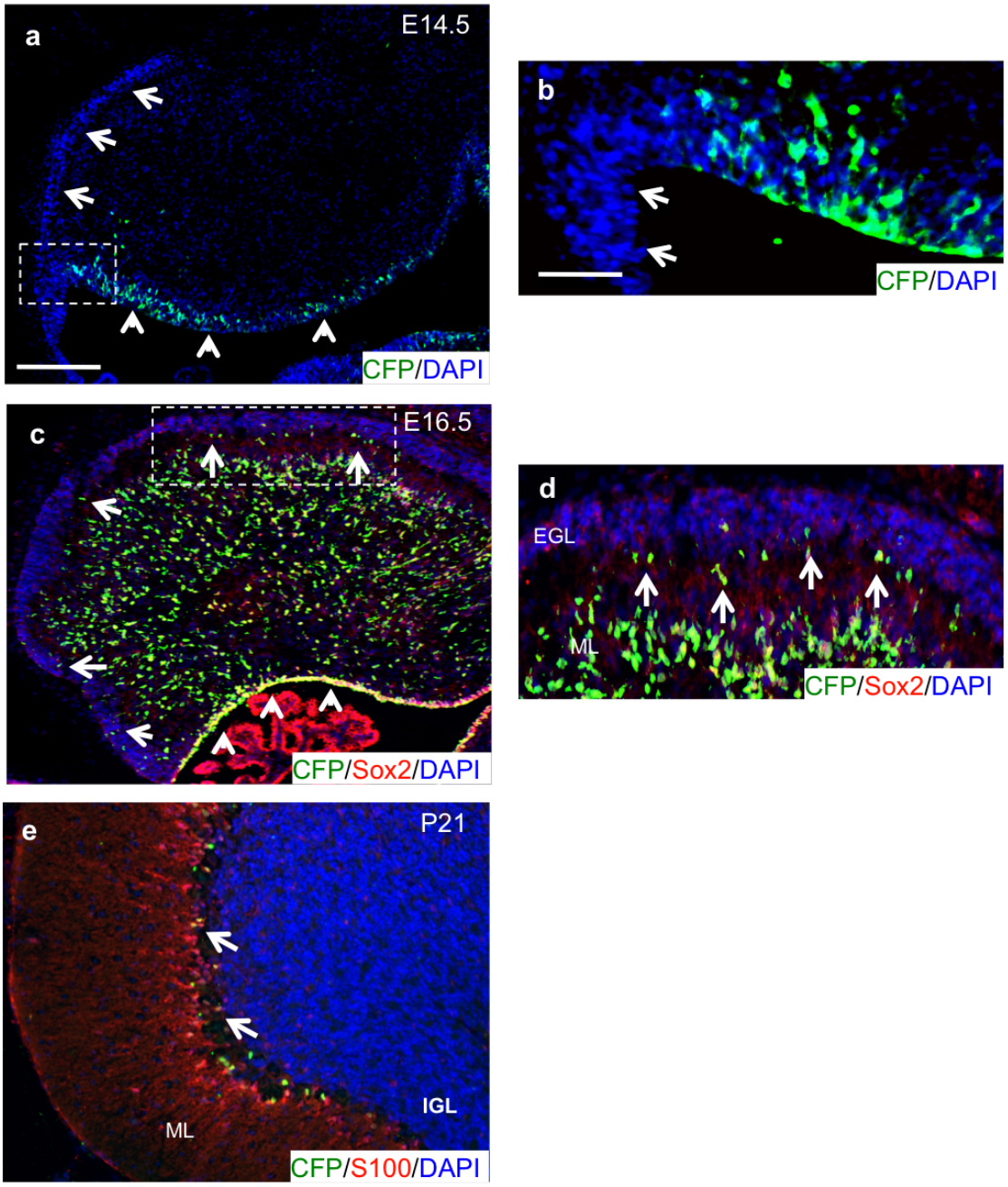
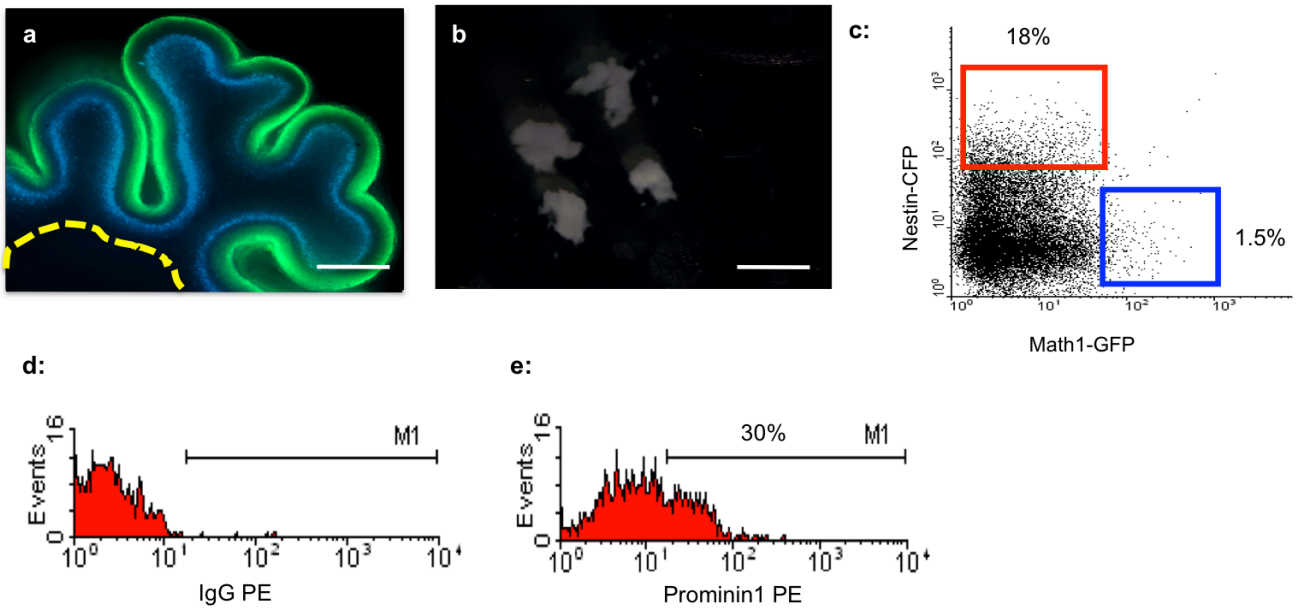
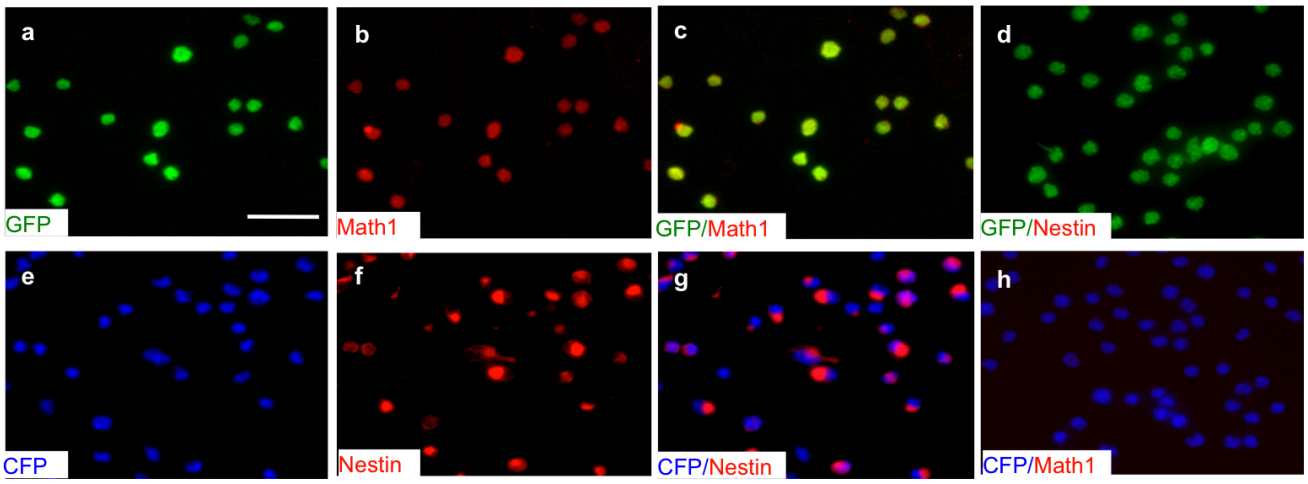


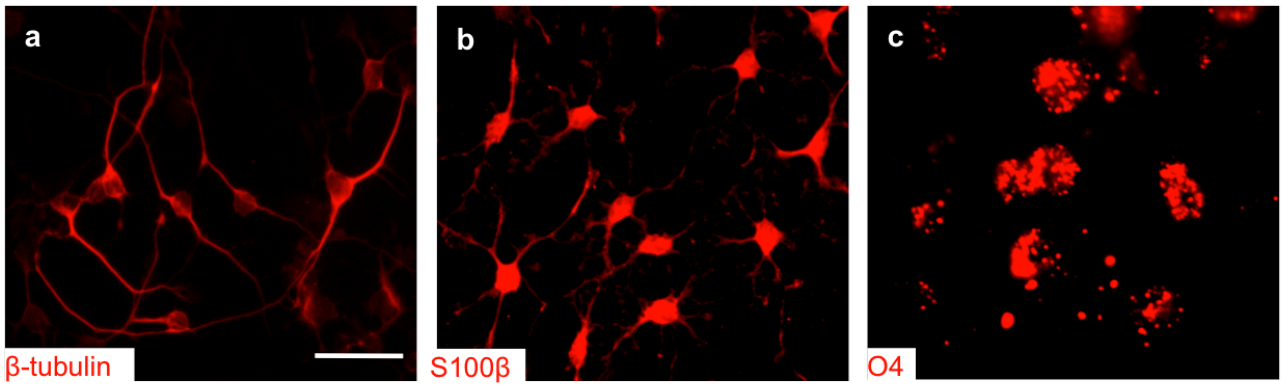
Supplementary Figure 1. Nestin expression in the developing cerebellum
Cerebellar sections from *Nestin-CFP* animals at various stages were immunostained with indicated antibodies. (a) At E14.5, no CFP+ cells were present in the EGL (arrows) and most CFP+ cells were localized in the ventricular zone (arrowheads). (b) Higher magnification of boxed region in figure a reveals the absence of CFP+ cells in the rhombic lip (arrows). (c) At E16.5, NEPs (CFP+, arrows) were found in the deep part of the EGL and NSCs (Sox2+, arrow heads) in the ventricular zone. (d) Higher magnification of boxed region in figure b shows the localization of NEPs in the EGL (arrows). (e) At P21, only astroglial cells (S100β+, arrows) still express CFP. Scale bar: a, c and e (200 μm); b and d (80 μm).



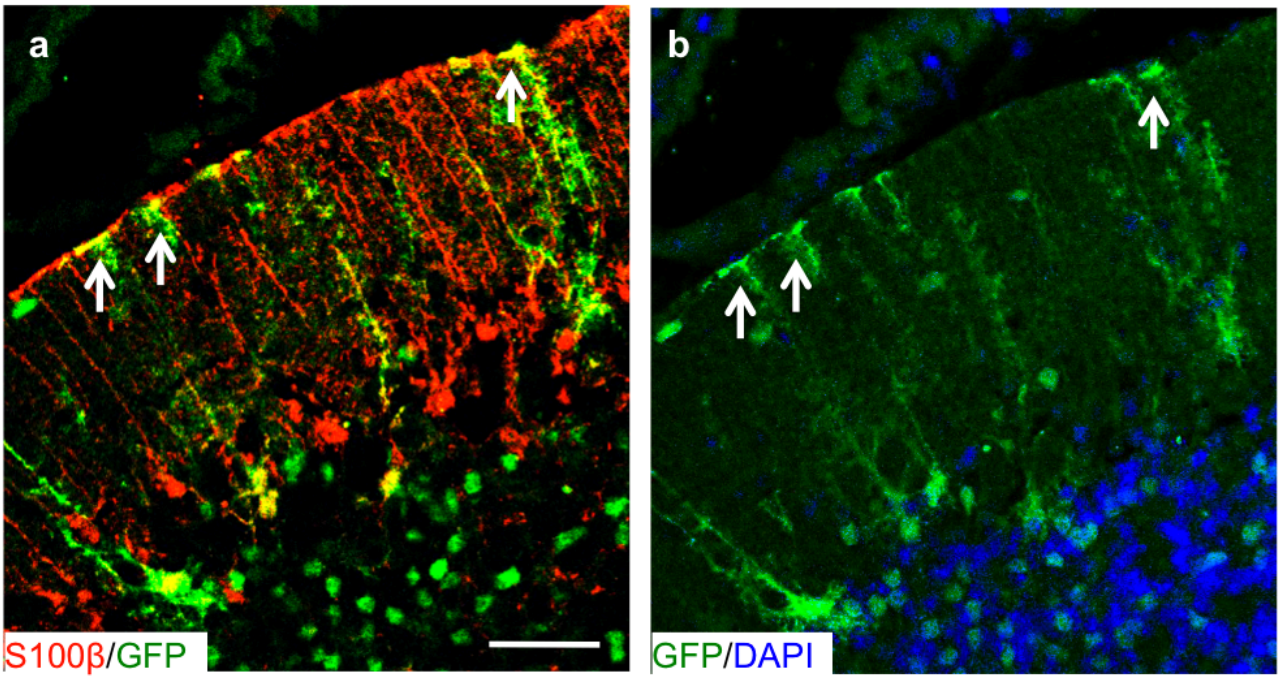
Supplementary Figure 2. Nestin expressing cells in the cerebellar white matter
(a) Cerebellar slices were prepared from *Math1-GFP/Nestin-CFP* animals at P4. The white matter was dissected under a fluorescent microscope (as shown by the dotted yellow line). (b) The dissected white matter was collected for cell dissociation. (c) Cells isolated from the white matter of P4 *Math1-GFP/Nestin-CFP* animals, were analyzed by flow cytometry for expression of GFP and CFP. Approximately 18% of cells in the white matter were positive for Nestin-CFP. (d and e) Nestin-CFP positive cells in the white matter were stained with isotype control (mouse IgG) or anti-Prominin1 prior to FACs analysis. ~30% of CFP positive cells were Prominin1+, suggesting that Nestin-expressing cells in the cerebellar white matter at P4 include NSCs. Scale bar: a (400μm); b (2mm).



Supplementary Figure 3. Expression of Nestin and Math1 in purified CFP+ and GFP+ cells
(a-d) Immediately after being isolated from the EGL of *Math1-GFP/Nestin-CFP* animals at P4, GFP+ cells (a) were stained for Math1 (red, b). Merged image of a and b indicates that all GFP+ cells express Math1 (c). GFP+ cells were also immunostained for Nestin (red, d). No Nestin expression was found among GFP+ cells. (e-g) CFP+ cells (e) from the EGL of *Math1-GFP/Nestin-CFP* cerebellum at P4 were immunostained for Nestin (red, f). The merged image of e and f shows Nestin expression in all purified CFP+ cells (g). CFP+ cells were immunostained for Math1 (red, h). No Math1+ cells were detected among CFP+ cell population. Scale bar: 200μm.

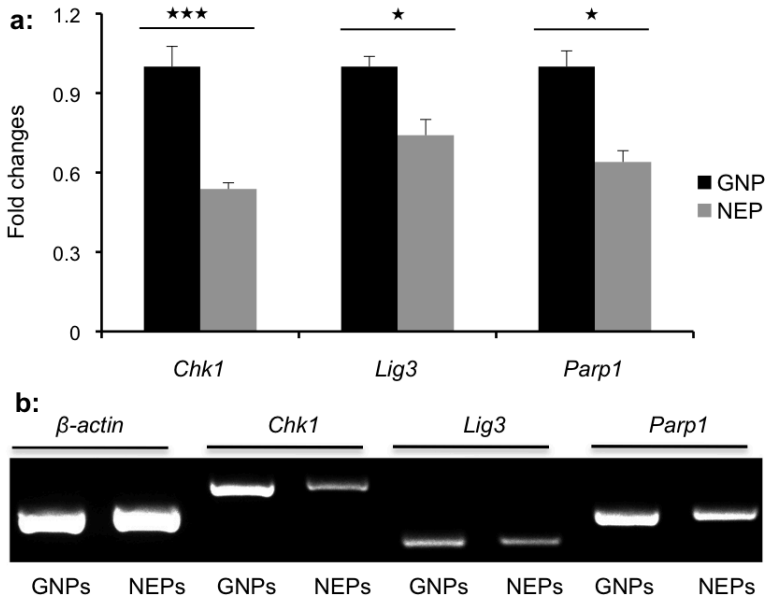


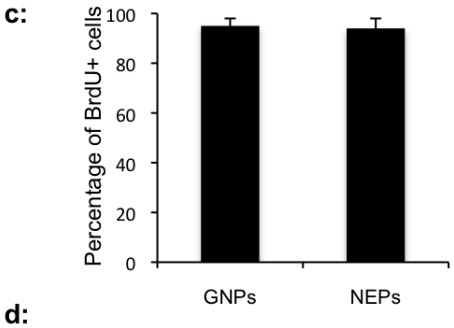
Supplementary Figure 4. Differentiation of purified NSCs
NSCs (Prominin1+, Lin- cells) isolated from P4 *Nestin-CFP/Math1-GFP* cerebellum, were cultured *in vitro* for 3 days and immunostained for neurons (ß-tubulin+, a), Bergmann glia (S100β+, b) and oligodendrocytes (O4+, c). Scale bar: 60μm.



Supplementary Figure 5. Fibers of Bergmann glia remaining on cerebellar surface of *Nestin-CreER^{T2}/R26R-GFP* cerebellum

Cerebellar sections prepared from *Nestin-CreER^{T2}/R26R-GFP* mice at P21 after tamoxifen treatment at P4 were stained for GFP (green, a and b), S100β (red, a), and counterstained with DAPI (blue, b). GFP+ fibers on the cerebellar surface were positive for S100ß, and negative for DAPI (arrows). Scale bar: 67μm.



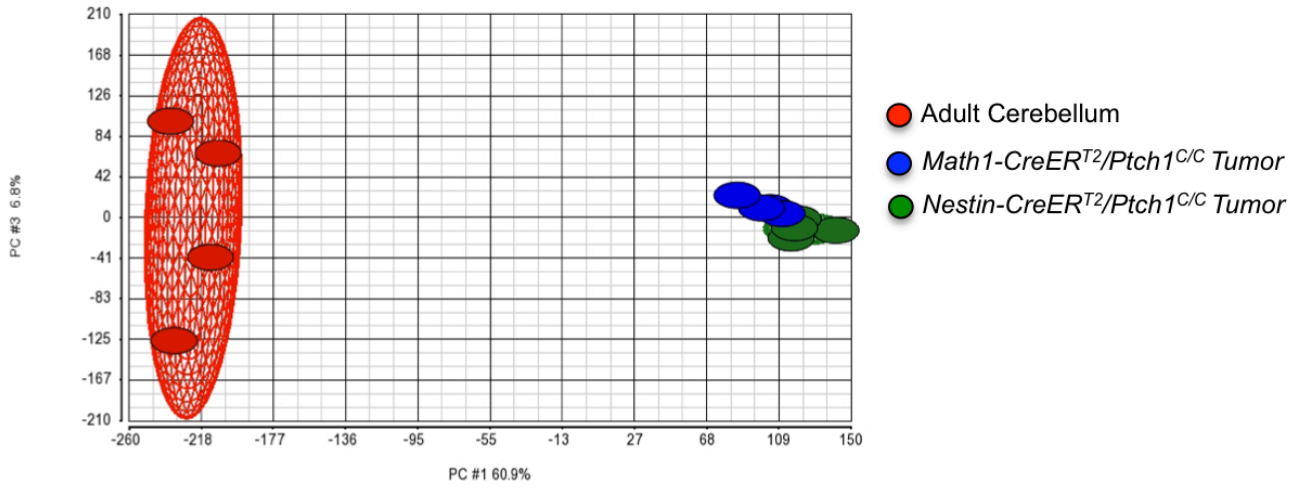


d:

		Breaks	Centromere seperation	Pulverization	Total metaphase analyzed
Number of Metaphases	NEPs	8	10	6	54
	GNPs	3	7	0	50

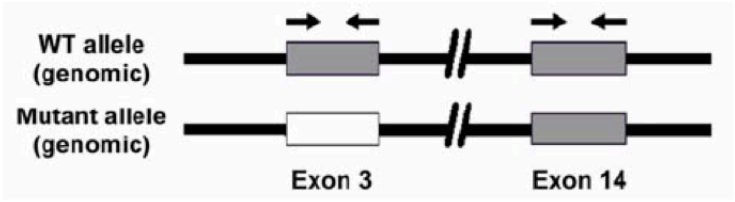
Supplementary Figure 6. DNA instability in proliferating NEPs

NEPs and GNPs purified from *Math1-GFP/Nestin-CFP* cerebella at P4 were treated with recombinant Shh *in vitro* for 48hrs. (a) Proliferating NEPs and GNPs were then harvested to examine the expression of *Chk1*, *Lig3* and *Parp1* by quantitative PCR. Expression of all genes in NEPs is normalized to their relative expression in GNPs. (b) PCR products were examined by gel electrophoresis. (c) Percentage of BrdU-incorporated GNPs and NEPs among Cre-infected cells. (d) The table summarizes the number of metaphases with chromosomal alterastions in metaphase spread from *Ptch1* deficient GNPs and NEPs. More chromosomal abnormalities were detected among NEPs compared with GNPs (Chi-square test, χ^2=7.05, *P*=0.00793, n=50 for GNPs and n=54 for NEPs.). Graphic data in a and c represent means of triplicate experiments ±SEM and significance determined with two-tailed Student's *t* test $^{***}P$<0.001, $^{**}P$<0.01. (a) *Chk1* of NEPs *vs* GNPs, *P*=0.00085; *Lig3* of NEPs *vs* GNPs, *P*=0.0171; *Parp1* of NEPs *vs* GNPs, *P*=0.0106; (b) *P*=0.837.

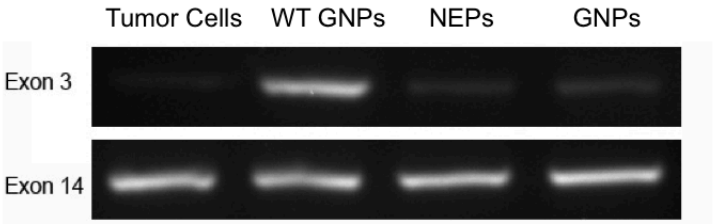


Supplementary Figure 7. The similar genetic profile of NEP- and GNP-derived tumor cells
MB cells were isolated from *Math1-CreER^{T2}/Ptch1$^{C/C}$* mice and *Nestin-CreER^{T2}/Ptch1$^{C/C}$* mice, and total RNAs were extracted from tumor cells for microarray analysis. Genetic profiles of tumor cells were compared with the normal cerebella (downloaded from the NCBI Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo) with the accession number GSE11859) by PCA.

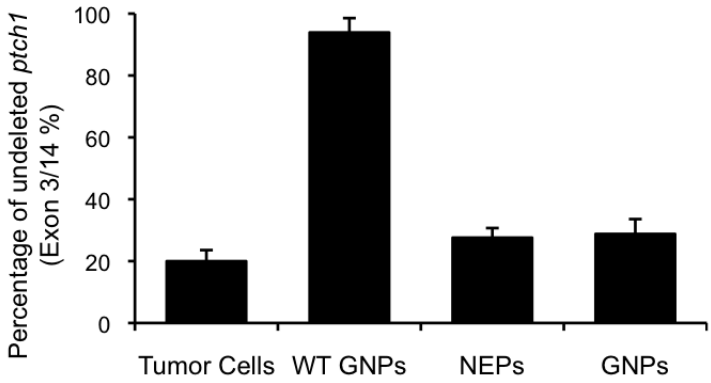
a:



b:



c:



d:

		Number of transplanted cells					
		2K	*5K*	*10K*	*20K*	*50K*	*100K*
Tumor Incidence	NEPs	0/3	4/8	4/6	6/6	4/4	3/3
	GNPs		0/7	0/8	3/5	6/6	5/5

Supplementary Figure 8. The tumorigenicity of transplanted NEPs and GNPs
(a) Two different primer sets were designed to analyze *Ptch1* genomic DNA. Primer set "a" amplifies part of exon 3, which is deleted in the mutant allele; primer set "b" is specific for exon 14, which is present in both wild type and mutant alleles. (b) After tamoxifen treatment at P4, GNPs and NEPs were purified from the EGL of *Math1-GFP/Math1-CreER^{T2}/Ptch1$^{C/C}$* cerebellum and *Nestin-CFP/Nestin-CreER^{T2}/Ptch1$^{C/C}$* cerebellum at P8, respectively. GNPs from wild type cerebellum at P8 and tumor cells from *Math1-Cre/Ptch1$^{C/C}$* at 8 weeks of age were isolated as controls. Genomic DNA extracted from those cells was used for quantitative PCR using primer sets a and b. (c) The amount of undeleted *Ptch1* in each sample was calculated by dividing the level of exon 3 product by the total amount of *Ptch1* DNA (represented by exon 14 product). (d) The table shows the number of animals that developed tumors from different amount of transplanted cells. Data in c represent means of triplicate experiments ± SEM. and significance determined with two-tailed Student's *t* test. *Ptch1* deletion among NEPs *vs* GNPs, *P*=0.486.

Antibodies			
Name	**Species**	**Working Concentration**	**Source**
Anti-Calbindin-D-28K	Mouse-IgG1	1/250	Sigma (C9848)
Anti-Ds-red	Goat-IgG	1/200	Santa Cruz (sc-33354)
Anti-GFP	Chicken-IgY	1/500	Invitrogen (A10262)
Anti-Ki67	Rabbit-IgG	1/500	Abcam (ab15580)
Anti-Math1	Mouse-IgG1	1/100	DSHB
Anti-Musashi-1	Rabbit-IgG	1/500	Millipore (AB5977)
Anti-Nestin	Mouse-IgG1	1/200	Abcam (ab6142)
Anti-NeuN	Mouse-IgG1	1/100	Millipore (MAB377)
Anti-O4	Mouse-IgM	1/50	Millipore (MAB345)
Anti-S100	Mouse-IgG1	1/200	Sigma (S2532)
Anti-Sox2	Rabbit-IgG	1/500	Millipore (AB5603)
Anti-Zic1	Rabbit-IgG	1/500	Gift from Dr. Segal
Anti-β3-tubulin	Mouse-IgG1	1/500	Santa Cruz (sc-58888)
Anti-Mouse CD133 (Prominin-1)	Rat IgG	1μg/100μl	ebioscience 121331-82
Rat IgG1 κ Isotype Control	Rat IgG	1μg/100μl	ebioscience 124301-82

Supplementary Table. List of antibodies used in this study