Figure S1  Differentiation of MB cells *in vitro*
MB cells isolated from *Math1-Cre/Pch1*/*/* mice (at 8 weeks of age) were plated on PDL-coated coverslips and harvested immediately after plating (0hr, A) or 72hrs of culturing. βIII-tubulin expression was examined via indirect immunofluorescence. Note that the increase in βIII-tubulin positive cells indicates *in vitro* differentiation of adherent MB cells.
Figure S2  The presence of tumor stromal cells in the adherent culture
Tumor cells dissociated from Math1-Cre/Ptch1fl/fl mice (at 8 weeks of age) were plated on PDL-coated coverslips, and indirect immunofluorescence was conducted immediately after plating (A-C) or after 48hrs (D-F). The presence of tumor-associated stromal cells including astrocytes (S100β+, A and D), oligodendrocyte precursor cells (NG2+, B and E) or microglia (CD68+, C and F) was examined. The percentage of stromal cells in A-F was quantified and shown in the graph in G. Note that the percentage of astrocytes, oligodendrocyte precursor cells and microglia was significantly reduced in adherent cultures at 48hrs, compared with that immediately following harvesting from in vivo tumor tissues.
Figure S3  Effective deletion of Ptch1 in MB cells
Genomic DNA was extracted from cultured MB cells or GNPs isolated from wild type cerebella at postnatal day 6, respectively. The presence of the exon 3 and exon 14 of Ptch1 gene was examined by q-PCR. β-actin gene was used as a control. PCR products were examined by DNA electrophoresis (A). The ratio of Ptch1 exon 3 relative to exon 14 of Ptch1 gene in the abundance, was calculated from the CT values of the q-PCR (B).
Tumor cells dissociated from Math1-Cre/Ptch1\textsuperscript{fl/fl} mice (at 8 weeks of age) were plated on PDL-coated coverslips and cultured with NB-B27 in the presence/absence of 1\%FBS. Cells were collected immediately after plating (0hr) or after adherent culturing for 48hrs and 72hrs. Graphs represent measured mRNA levels of Hh pathway target genes including Gli1 (A), Ptc1h (B), Sfrp1 (C). Note that the expression of Gli1, Ptc1h and Sfrp1 mRNAs progressively declined in MB cells, regardless of the presence or absence of 1\%FBS.
Figure S5  Astrocytes are present in MB tumoroids
MB tumoroids were examined for the presence of astrocytes, based on the expression of S100β (A) and BLBP (B) via indirect immunofluorescence. A merged image (C) shows that majority of S100β+ cells simultaneously expressed BLBP, indicative of the astrocyte identity of S100β+ cells in MB tumoroids.
Figure S6  Hh signaling in tumoroids from putative monocellular MB culture
Fibronectin (A and B) and Nestin (C and D) expression/content was examined in monocellular MB tumoroids via indirect immunofluorescence and spheres were counterstained with DAPI to note all nuclei. Harvested tumoroids were analyzed for the expression of Gli1 and Ptc2 mRNAs by q-PCR (E and F). Adherent MB cells, cultured for 72hrs, were used as a control (E and F). The presence of astrocytes in the few detected/remaining tumoroids was revealed by indirect immunofluorescence using S100β as before (G and H). DAPI was used to counterstain cell nuclei in B, D and H.
Figure S7 MB cell proliferation is repressed by fibroblast-derived ECM

MB cells were cultured in pancreatic fibroblast-derived ECM or on PDL-coated coverslips for 48hrs, and pulse-labeled with BrdU for 2hrs prior to indirect immunofluorescence (A-B). Percentage of BrdU positive cells is shown in C. Note that less than 10% of tumor cells were found positive for BrdU on the fibroblast-derived ECM, whereas more than 30% MB cells incorporated BrdU when adherently cultured on PDL coated coverslips. DAPI was used to counterstain for total cell nuclei counts.
Figure S8 Shh ligand in astrocyte-derived ECM stimulates MB cell proliferation

MB cells were adherently cultured on PDL-coated coverslips (A-D) or into astrocyte-derived ECM (E-H) for 48hrs, in the presence of vehicle (NB-B27), 1% NS1 conditioned culture medium, 1% isotype hybridoma supernatant (IgG1), or functional Shh binding antibody present in 1% 5E1 hybridoma supernatant. Tumor cells were pulse-labeled with EdU for 2hrs and subjected to indirect immunofluorescence. The percentage of EdU+ cells was quantified in I, using DAPI to detect total nuclei counts as before. Note that 5E1 significantly inhibited the proliferation of MB cells cultured into ECM, but not on PDL-coated coverslips.
Figure S9 Compromised ECM formation from Shh-deficient astrocytes

Astrocytes isolated from the cerebella of Cas9 mice at postnatal day 2, were infected with a lentivirus carrying scrambled RNA or guide RNA specific for Shh. Infected astrocytes were cultured on gelatin-coated plates (A-B) to achieve 7 day astrocyte-derived ECM production, and the extent of ECM produced was gauged via fibronectin indirect immunofluorescence (C-D) and collagen fiber polymers imaged using backward SHG (E-F; see Materials and Methods). Representative images shown, in E and F, are presented as pseudo colored SHG signal “intensity maps” in which warm colors indicate high SHG signal (e.g., significant collagen fibers). Inserts show original SHG reconstituted monochromatic images for reference. Graph in G denotes SHG signals acquired from images like in E and F. Note that the number of Shh-knockout astrocytes was dramatically reduced, with much less fibronectin as well as significantly low levels of collagen in the culture of Shh-deficient astrocytes, compared with control astrocytes that formed substantial amounts of ECM, as expected.
Figure S10 Fibronectin and collagen support MB cell proliferation in the ECM
MB cells were treated with functional blocking antibodies known to limit the activation of integrins α5β1 (A1-A3), β1 (B1-B3) or αv (C1-C3) as well as with corresponding isotype controls, and plated onto astrocyte-derived ECM for 48hrs. MB cells were pulsed with EdU for 2hrs and subjected to indirect immunofluorescence accompanied by DAPI counterstained. Percentages of EdU+ cells are shown in A3, B3 and C3. Note that blocking all types of integrins significantly limited MB cell attachment as well as proliferation suggesting ECM proteins other than fibronectin play important roles in the observed astrocyte-derived ECM sustained MB growth.