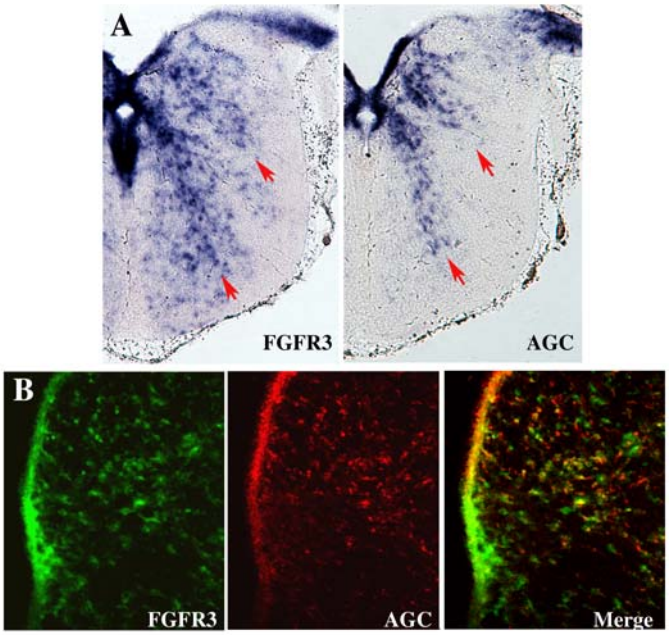
A
FGFR3
AGC
B
FGFR3
AGC
Merge

Figure S1. **A**, *FGFR3* and aggrecan (*AGC*) gene expression by *in situ* hybridization (blue) in E9 hindbrain cross-sections, showing labeled cells (arrows) that appear to be migrating from the VZ. **B**, Confocal images of a two-color fluorescent *in situ* hybridization on E9 hindbrain cross-sections for aggrecan/*AGC* (red) and FGFR3 (green).

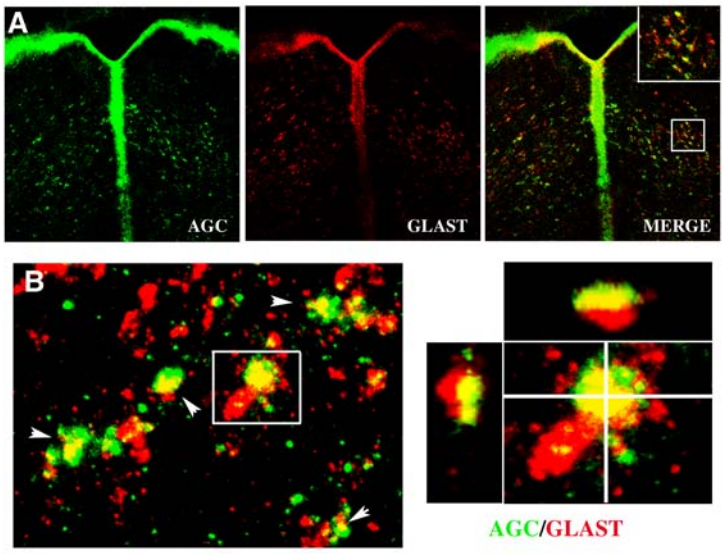
A
AGC
GLAST
MERGE
B
AGC/GLAST

Figure S2. A, Confocal image of two-color fluorescent *in situ* hybridization on E9 midbrain cross-sections for aggrecan/*AGC* (green) and the glial marker *GLAST* (red). Higher magnification of the box area in the confocal merge is shown in the upper right corner. **B,** Confocal overlay XY projections of an independent field identifying cells expressing aggrecan and GLAST (arrowheads). The area in the square box is magnified in the right panel, and Z projections through the X and Y axes (white lines) are shown on the top and left sides of the panels, respectively.

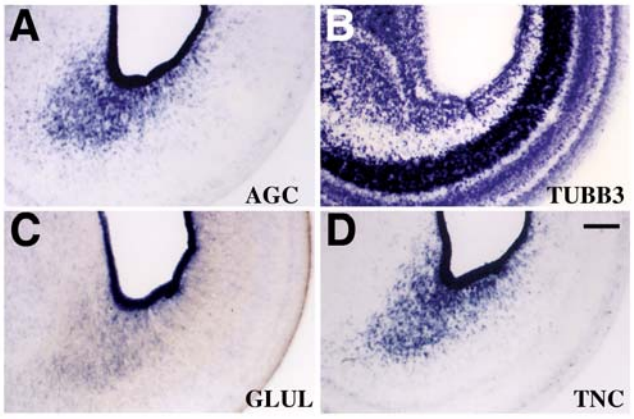
A
AGC
B
TUBB3
C
GLUL
D
TNC

Figure S3. Aggrecan message is strongly expressed in the VZ of the E12 chick midbrain. Illustrated are high power views of the ventrolateral corner of the midbrain, with the optic tectum to the right. Serial sections were processed for mRNA expression of *AGC* (**A**), the neuronal marker *TUBB3* (**B**), and the glial markers *GLUL* (**C**) and *TNC* (**D**). Note the streams of AGC-, GLUL- and TNC-positive cells stretching away from the fundus of the midbrain ventricle. Scale bar: 250 μm.

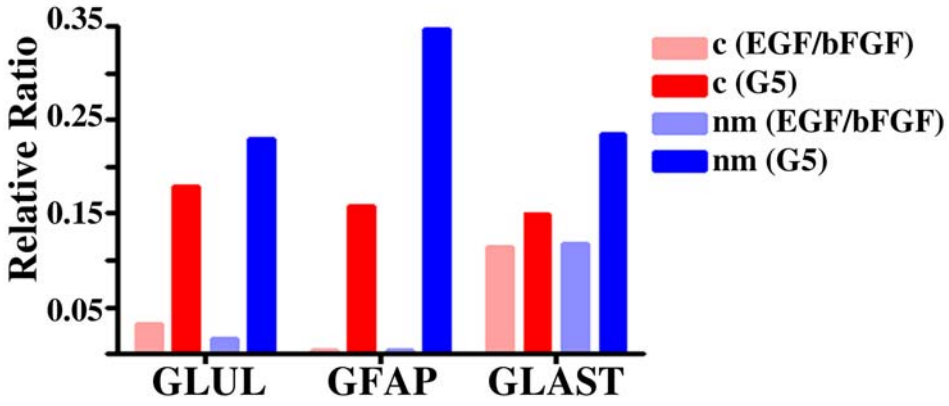
Relative Ratio
0.35
0.25
0.15
0.05
GLUL
GFAP
GLAST
c (EGF/bFGF)
c (G5)
nm (EGF/bFGF)
nm (G5)

Figure S4. Elevation of astroglial gene expression levels in E11 midbrain VZ cultures from chick embryos lacking functional aggrecan. Northern blot analysis of *GLUL, GFAP* and *GLAST* mRNA from flock mate (c, pink) and nanomelic (nm, light blue) E11 midbrain VZ cultures harvested after nine days *in vitro* in DMEM/10% fetal calf serum with 40ng/ml EGF and 40ng/ml bFGF added, or from flock mate (c, red) and nanomelic (nm, blue) parallel cultures incubated for seven days in EGF/bFGF medium then shifted to F12/DMEM with G5 and 1.5 μM *trans*-retinoic acid for two days. Relative ratios were calculated with respect to *GAPDH* mRNA levels to correct for loading differences.

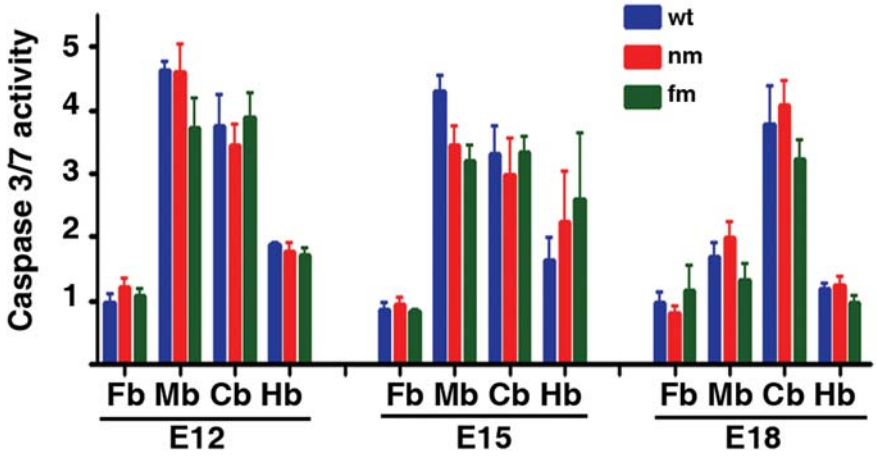
wt
nm
fm
Caspase 3/7 activity
5
4
3
2
1
Fb Mb Cb Hb
E12
Fb Mb Cb Hb
E15
Fb Mb Cb Hb
E18

Figure S5. Nanomelic brains show no significant increase in apoptotic cell death relative to controls as assessed by caspase 3/7 activity levels. E12, E15 and E18 nanomelic, control flock mate and control wild type brains were dissected and homogenized on lysis buffer (AnaSpec, Ca). Caspase 3/7 catalytic activity was determined by measuring the proteolytic cleavage of the fluorogenic substrate Ac-DEVD-AMC (AnaSpec, Ca). Production of AMC was monitored continuously in a Victor3 fluorescent plate reader (PerkinElmer). Total protein content was normalized using the BCA protein assay (Pierce). Initial velocities are represented and expressed as relative fluorescent units per minute per μg of protein. Data was analyzed for statistical significance using the Student's *t*-test. Cb, cerebellum; Fb, forebrain; fm, flock mate control; Hb, hindbrain; Mb, midbrain; nm, nanomelic; wt, wild type control.