Supplementary Figures and Legends

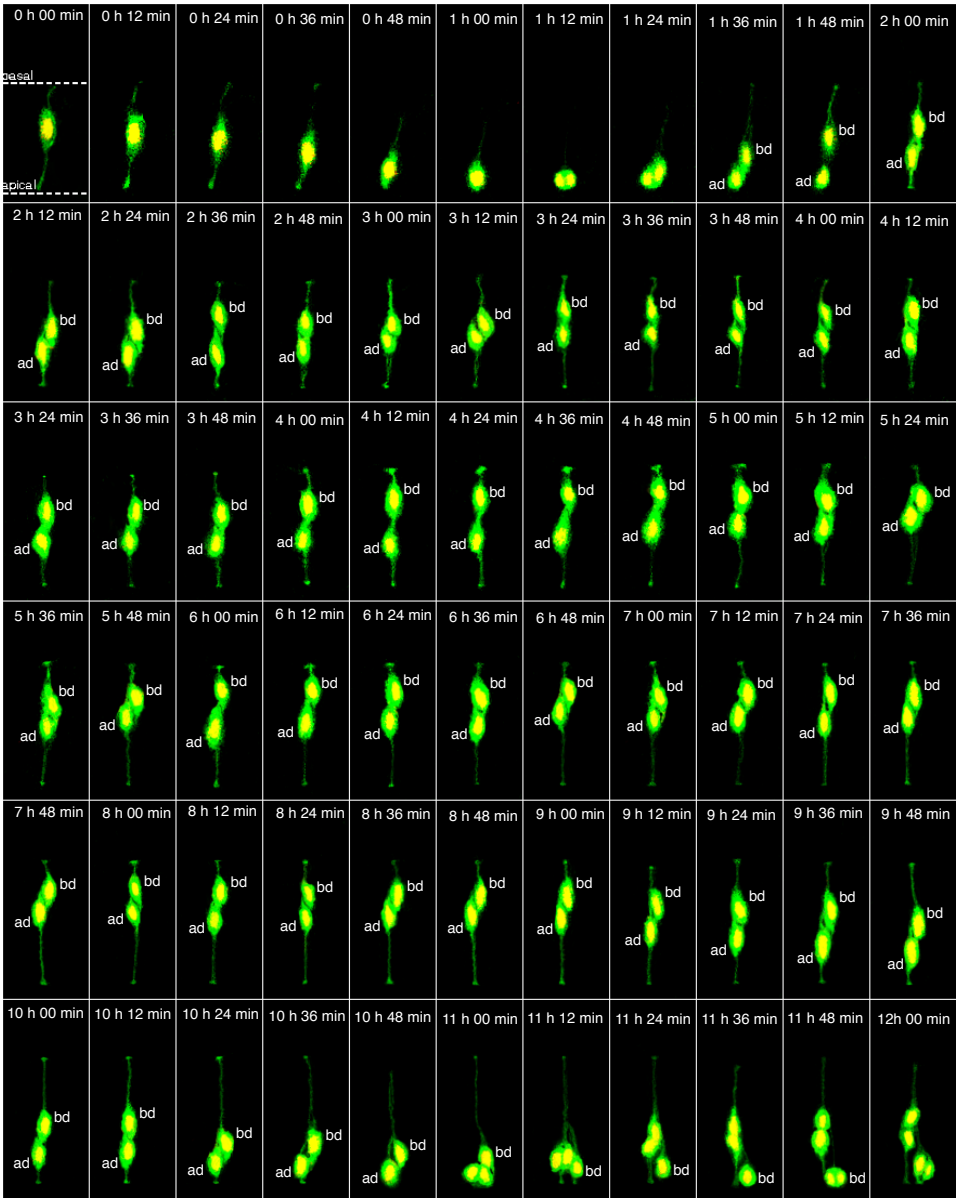

Figure S1. Representative montage of sequential frames from *in vivo* time-lapse imaging of a single fluorescently-labeled mother cell in the developing zebrafish forebrain, related to Figure 1. From these images, the following can be observed: 1) 0 – 48 min, the mother cell undergoes apically directed INM to reach the apical surface. 2) 1h - 1h 24min: The mother cell divides, and its division axis is largely perpendicular to the apical basal axis. 3) 1h 36 min: The two daughter cells assume a differential positioning along the apical basal neural axis. 4) 1h 48min - 9h: The two daughter cells undergo basally directed INM while maintaining their relative apical basal positions. 5) 9h 12min – 10h 48min: The two daughter cells undergo apically directed INM while maintaining their relative apical basal positions. 6) 11h – 11h 36min: The apical daughter divides with its division axis largely perpendicular to the apical basal axis and generates two granddaughter cells that migrate basally. 7) 11h 48 min – 12h: the basal daughter divides with its division axis largely perpendicular to the apical basal axis and generates two granddaughter cells. ad: apical daughter cell which maintains a more apical position, bd: basal daughter cell which migrates to and maintains a more basal position.

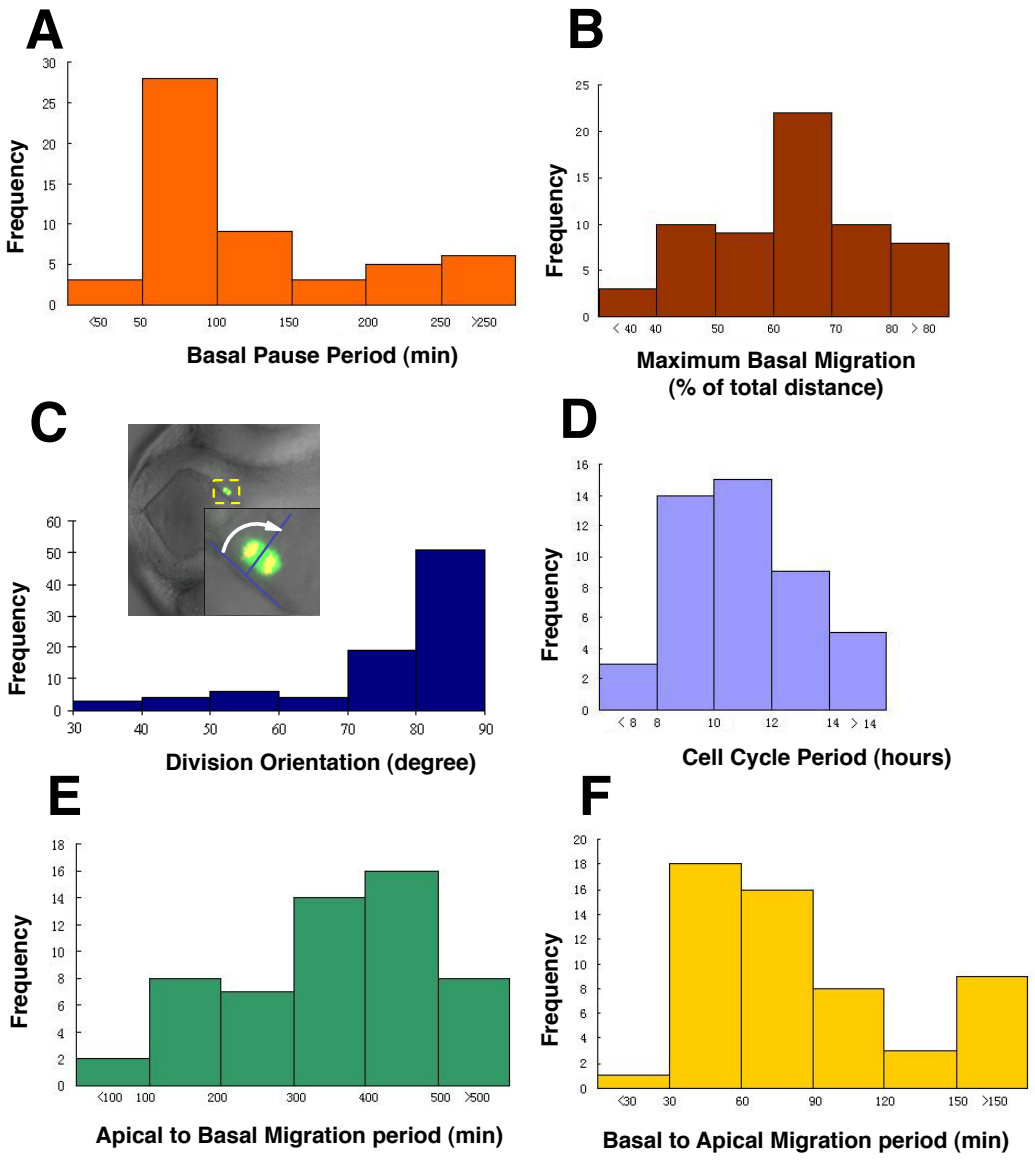

Figure S2. Heterogeneity of radial glia progenitor cell behavior observed through *in vivo* time-lapse imaging in the developing zebrafish forebrain from 28 hpf to 60 hpf, related to Figure 2. (A-F) Graphs show the frequency distributions of basal pause period (A), maximum basal migration (B), division orientation (C), cell cycle period (D), apical to basal migration period (E), and basal to apical migration period (F). Please refer to the Experimental Procedures for the measurement of these parameters.

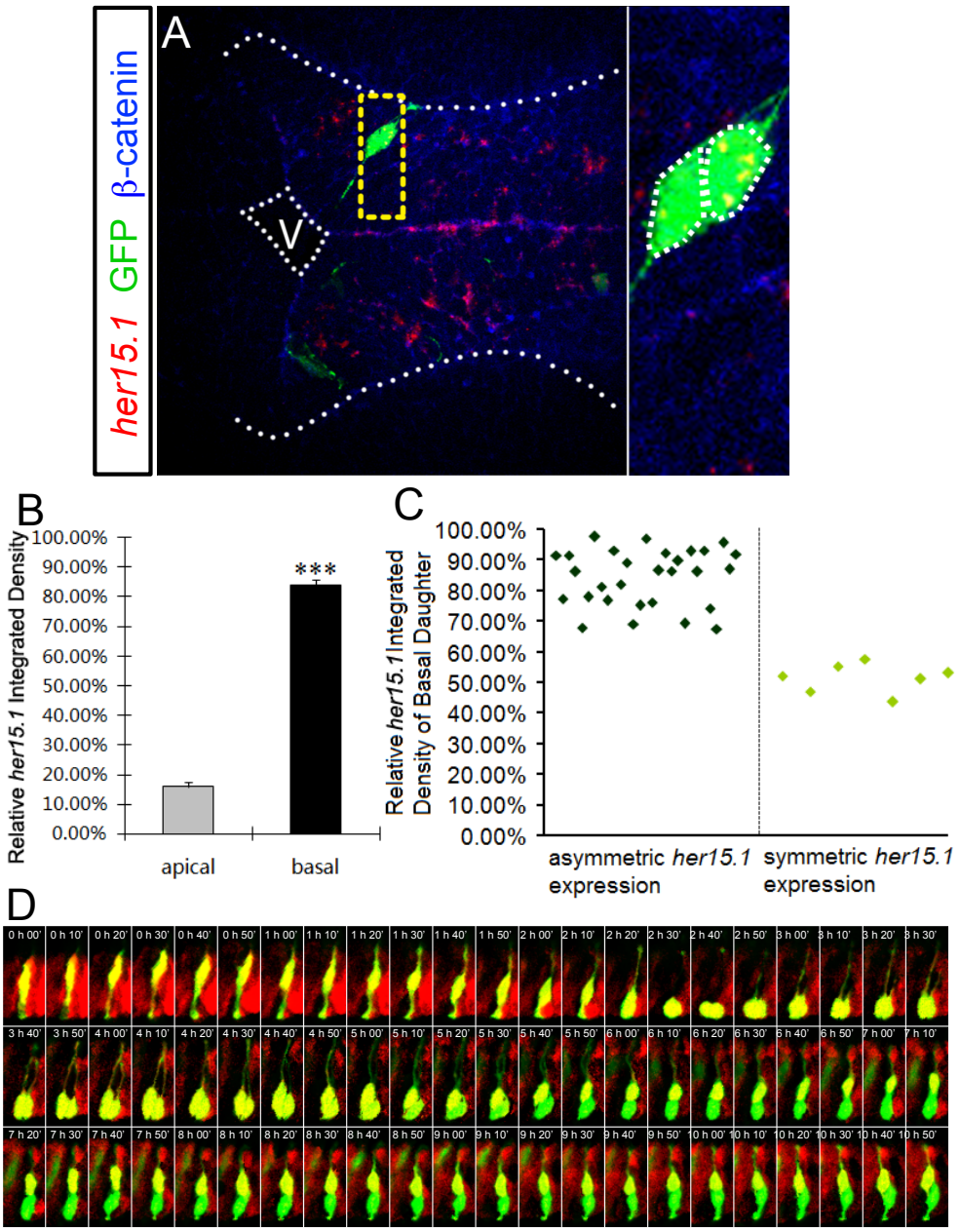

Figure S3. FISH coupled with clonal analyses and live imaging with *her4:dRFP* transgenic embryo reveal that the basal daughter cell exhibits higher Notch activity, related to Figure 4. (A) FISH of *her15.1* (red) coupled with immunohistochemistry of GFP (green) and β-catenin (blue) in forebrain paired daughter cells. The profiles of each daughter cell and the apical and basal surfaces are highlighted by dashed white lines. Enlargement of the yellow-boxed area is shown on the right of each panel. V: ventricle. (B) Quantification the FISH signal of *her15.1* in paired daughter cells. The relative integrated density is the ratio of the integrated density in each daughter cell to the sum of the integrated densities of both daughter cells*** $p < 0.001$ *vs* apical, t-test. (C) Scatter plot shows the relative *her15.1* integrated density of the basal daughter cell of each analyzed pair of daughter cells. 29 pairs of daughter cells show asymmetric *her15.1* expression, while 7 pairs of daughter cells show symmetric *her15.1* expression. (D) Representative montage of sequential frames from *in vivo* time-lapse imaging of a single fluorescently-labeled mother cell in the

developing zebrafish hindbrain of *her4:dRFP* transgenic embryo. The dRFP signal reveals dynamic Notch activity in the mother cell and the paired daughter cells. The following can be observed: 1) 0 – 2h 20min, the mother cell undergoes apically directed INM to reach the apical surface. The mother cell has high Notch activity, as shown by the strong dRFP signal. 2) 2h 30min - 2h 50min: The mother cell divides to generate two paired daughter cells. Notch activity is not biased in the paired daughter cells at this stage, since dRFP signal evenly distributes in the paired daughter cells. 3) 3h 00min – 4h 50min, the paired daughter cells embark on basally directed INM while maintaining their relative apical basal positions. They show similar Notch activity. 4) 5h 00min – 10h 50min: The biased Notch activity in the paired daughter cells is observed as a down-regulation of dRFP signal in the apical daughter. There also appears to be a time-dependent decrease of the overall dRFP signal in the imaged tissues, which may reflect a global down-regulation of Notch activity as development progresses, although signal bleaching due to imaging cannot be ruled out.

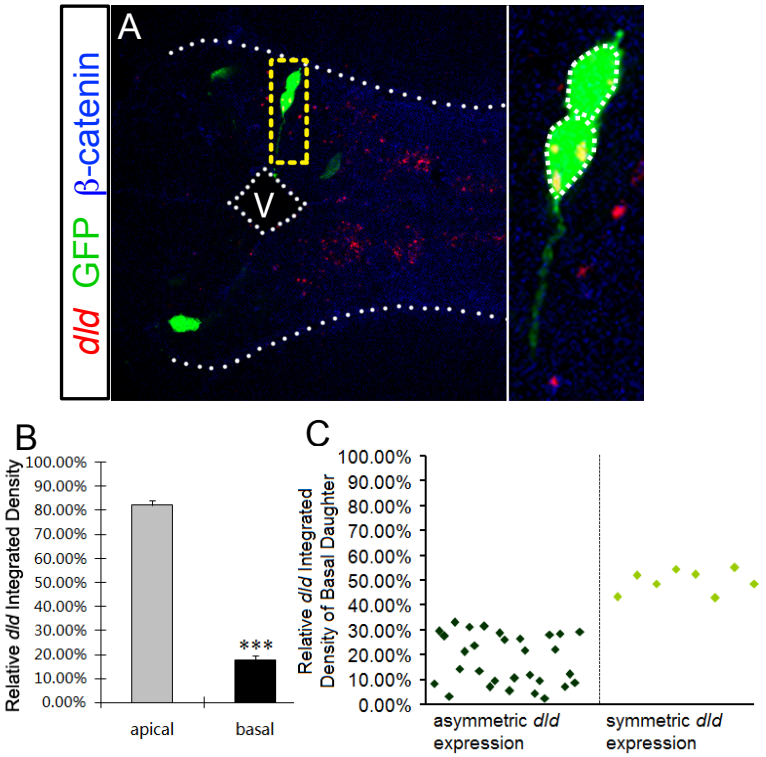

Figure S4. FISH coupled with clonal analyses reveals that the apical daughter cell expresses a higher level of *dld*, related to Figure 5. (A) FISH of *dld* (red) coupled with immunohistochemistry of GFP (green) and β-catenin (blue) in forebrain paired daughter cells. The profiles of each daughter cell and the apical and basal surfaces are highlighted by dashed white lines. Enlargement of the yellow-boxed area is shown on the right of each panel. V: ventricle. (B) Quantification the FISH signal of *dld* in paired daughter cells. *** $p < 0.001$ *vs* apical, t-test. (C) Scatter plot shows the relative *dld* integrated density of the basal daughter cell of each analyzed pair of daughter cells. 30 pairs of daughter cells show asymmetric *dld* expression, while 8 pairs of daughter cells show symmetric *dld* expression.

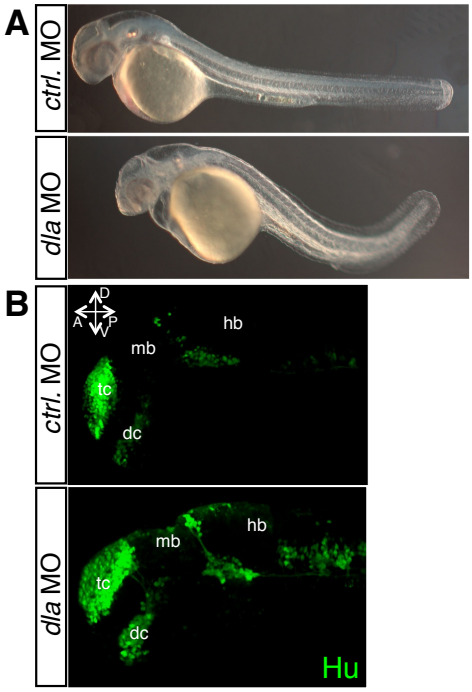

Figure S5. Validation of the *dla* morpholino antisense oligonucleotides, related to Figure 6. (A) Effect of the *dla* morpholino on general morphology of 36 hpf embryos. Control morpholino (upper) and *dla* morpholino (lower) are injected at the 1-cell stage. (B) Effect of the *dla* morpholino on neurogenesis in 24 hpf embryos. Immunohistochemistry of Hu indicates that Hu positive cells are significantly increased in *dla* morpholino injected embryos (lower) compared with control morpholino-injected embryos (upper). tc: telencephalon, dc: diencephalon, mb: midbrain, hb: hindbrain.

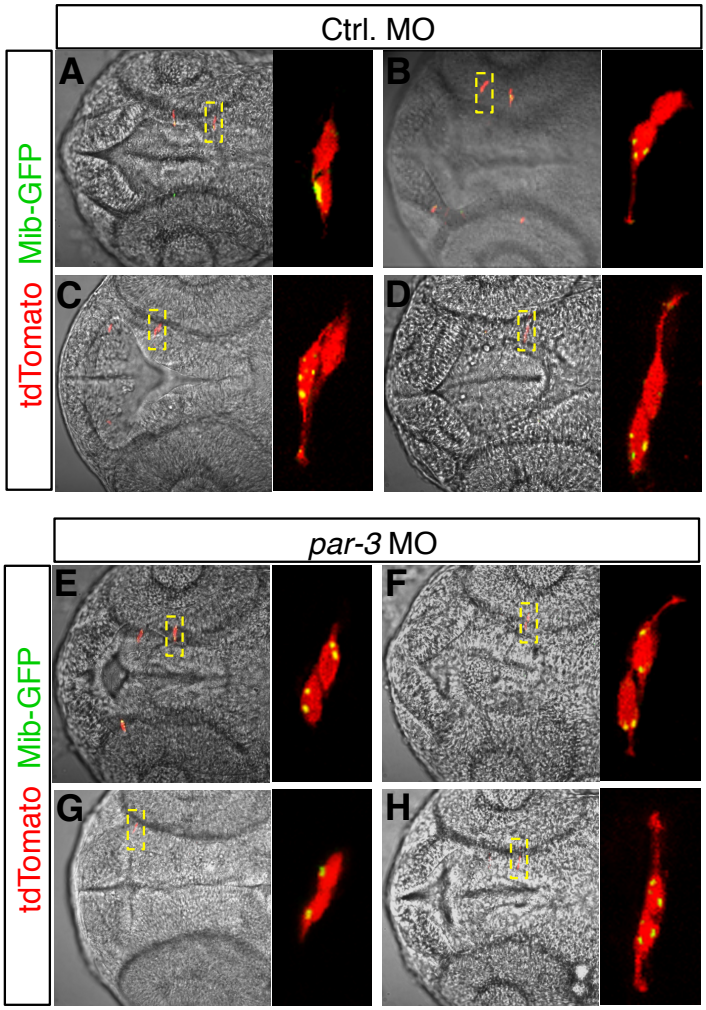

Figure S6. Unequal segregation of Mib depends on Par-3, related to Figure 7. Four representative images showing the localization of Mib-GFP in paired daughter cells in Control (A-D) and *par-3* (E-H) morphants.

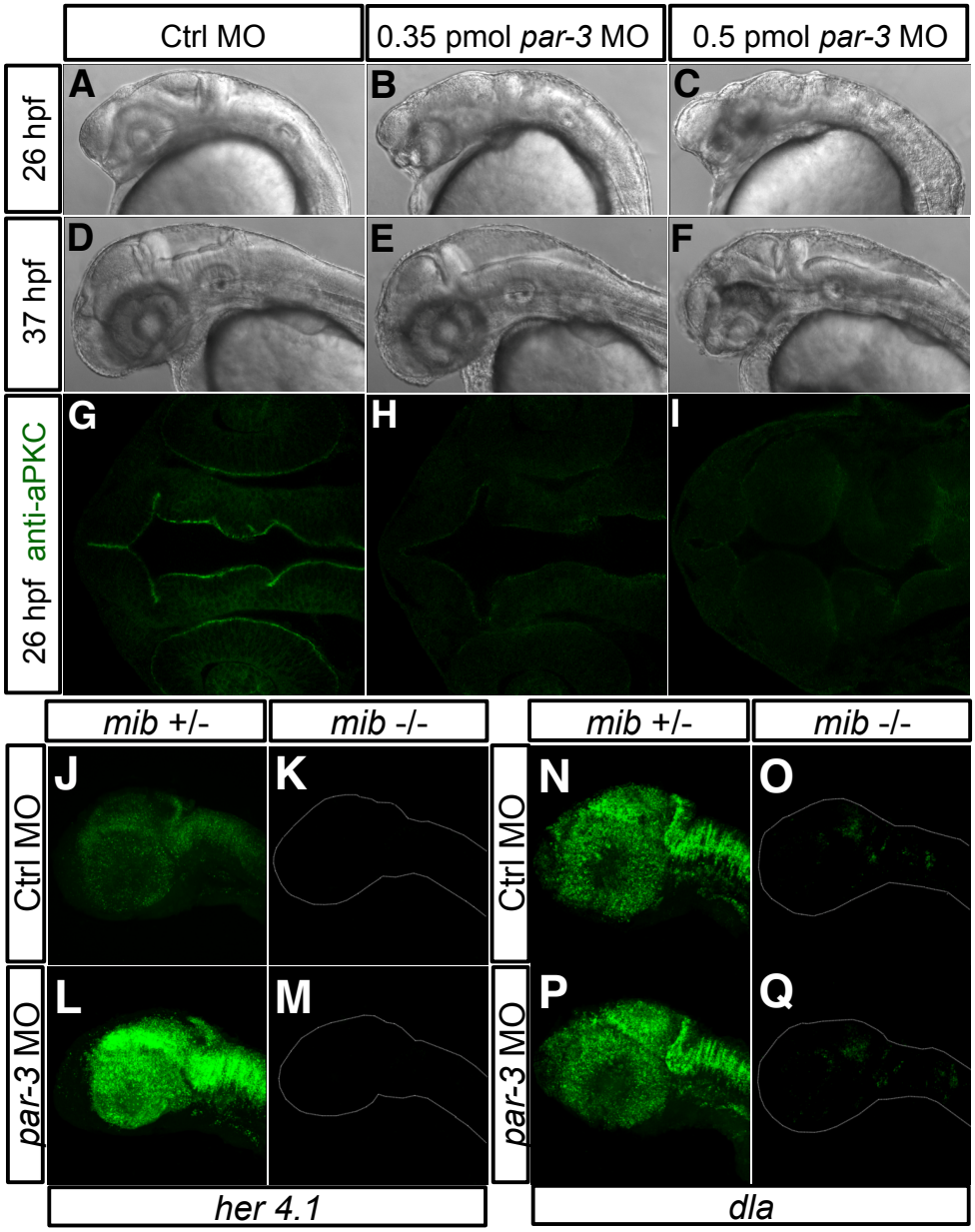

Figure S7. Validation of *par-3* morpholino antisense oligonucleotides and expression of *her4.1* and *dla* in *mib*/*par-3* double deficient embryos, related to Figure 8. Injection of 0.35 pmoles *par-3* morpholino results in mild defect in brain morphology at 26 hpf (B), while 0.5 pmoles par-3 morpholino results in severe defect in brain morphology at 26 hpf (C), compared with injection of control morpholino (A). At 37 hpf, the morphologic defects of both 0.35 pmoles (E) and 0.5 pmoles *par-3* morpholino (F) recover to certain extent, compared with injection of control morpholino (D). (A) and (D), (B) and (E), (C) and (F) represent the same embryo at different developmental stages respectively. Immunostaining of aPKC shows that injection of both 0.35 pmoles (H) and 0.5 pmoles *par-3* morpholino (I) can lead to loss of apico-basal cell polarity, as compared with injection of control morpholino (G). (J and K) FISH of *her4.1* in control morpholino-injected embryo of *mib* +/- (J) or *mib* -/- (K). (L and M) FISH of *her4.1* in *par-3* morpholino-injected embryo of *mib* +/- (L) or *mib* -/- (M). (N and O) FISH of *dla* in control morpholino-injected embryo of *mib* +/- (N) or *mib* -/- (O). (P and Q) FISH of *dla* in *par-3* morpholino-injected embryo of *mib* +/- (P) or *mib* -/- (Q).

Supplemental Experimental Procedures

qRT-PCR Primer Sequences

Gene	Forward primer	Reverse primer
her4.1	AGCAGCfAGCCCGACTCCAGA	GCTGACGGCCTCCTGCACAC
her6	AGAATCAACGAAAGCTTGGGTCAGC	AGGGCAGCGGTCATTTGTGCG
dla	CCGTCATGGTGATGTCGGAGGA	CGAGCAGCAGCATTTTCTCAGGG
dld	TTCCGGGGTGTGATGAAGACCA	TGCCATGCAAGCAGCCTGGG
notch1a	GCCACCGCGGGCTGTCTTTAT	CCGTCCCATTCACACTCTGCGT
notch1b	CAGTGCCACAACACGGGATGC	AGCCCTGGTCACAGTAGCCGT

Measurement and Analysis of *in vivo* Progenitor Behavior

In vivo time-lapse imaging data were analyzed using NIS-Elements imaging software (Nikon). In total, data were collected from 80 mother cells and 427 progenitor cells in 58 independent time-lapse experiments. The following parameters were measured. 1) *Cell cycle period*: the time required for an individual progenitor cell to cycle from M phase to M phase. 2) *Division orientation*: the angle of an M phase cell between the cleavage furrow and the ventricular surface. 3) Relative *maximum basal migration*: the total distance from the middle of the cell nucleus to the apical surface at the apex of basal migration, normalized to the total apical-basal thickness of the neuroepithelial germinal zone. 4) *Basal pause period*: the time the nuclei remained at the apex of their basal migration, as defined by movement that did not exceed 5 μm. 5) *Apical to basal migration period*: the time required for an individual progenitor cell to migrate from the ventricular surface to the apex of its basal migration. 6) *Basal to apical migration period*. The time required for an individual progenitor cell to migrate from the apex of its basal migration to the ventricular surface.

Supplementary Movies

Movie S1 Time-lapse of a single fluorescently labeled mother cell followed through two rounds of division, related to Figure 1. The mother cell gives rise to two paired daughter cells. One daughter cell undergoes a differentiation division, whereas the other daughter cell undergoes a self-renewal division. The interval between each frame is 12 minutes.

Movie S2 Time-lapse of a single fluorescently labeled mother cell in *her4.1:dRFP* transgenic embryo, related to Figure 4. The interval between each frame is 10 minutes.

Move S3

Time-lapse of a single fluorescently labeled mother cell expressing both tdTomato and Mib-GFP, related to Figure 7. The embryo was injected with control morpholino at 1-cell stage. Only the apical daughter inherits Mib-GFP, while the basal daughter does not. The interval between each frame is 6 minutes. The dashed line indicates the ventricular surface (apical side).

Movie S4

Time-lapse of a single fluorescently labeled mother cell expressing both tdTomato and Mib-GFP, related to Figure 7. The embryo was injected with *par-3* morpholino at 1-cell stage. Both the apical daughter and the basal daughter inherit Mib-GFP. The interval between each frame is 6 minutes. The dashed line indicates the ventricular surface (apical side).