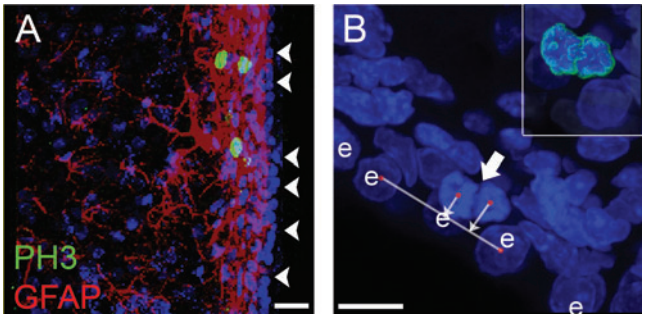
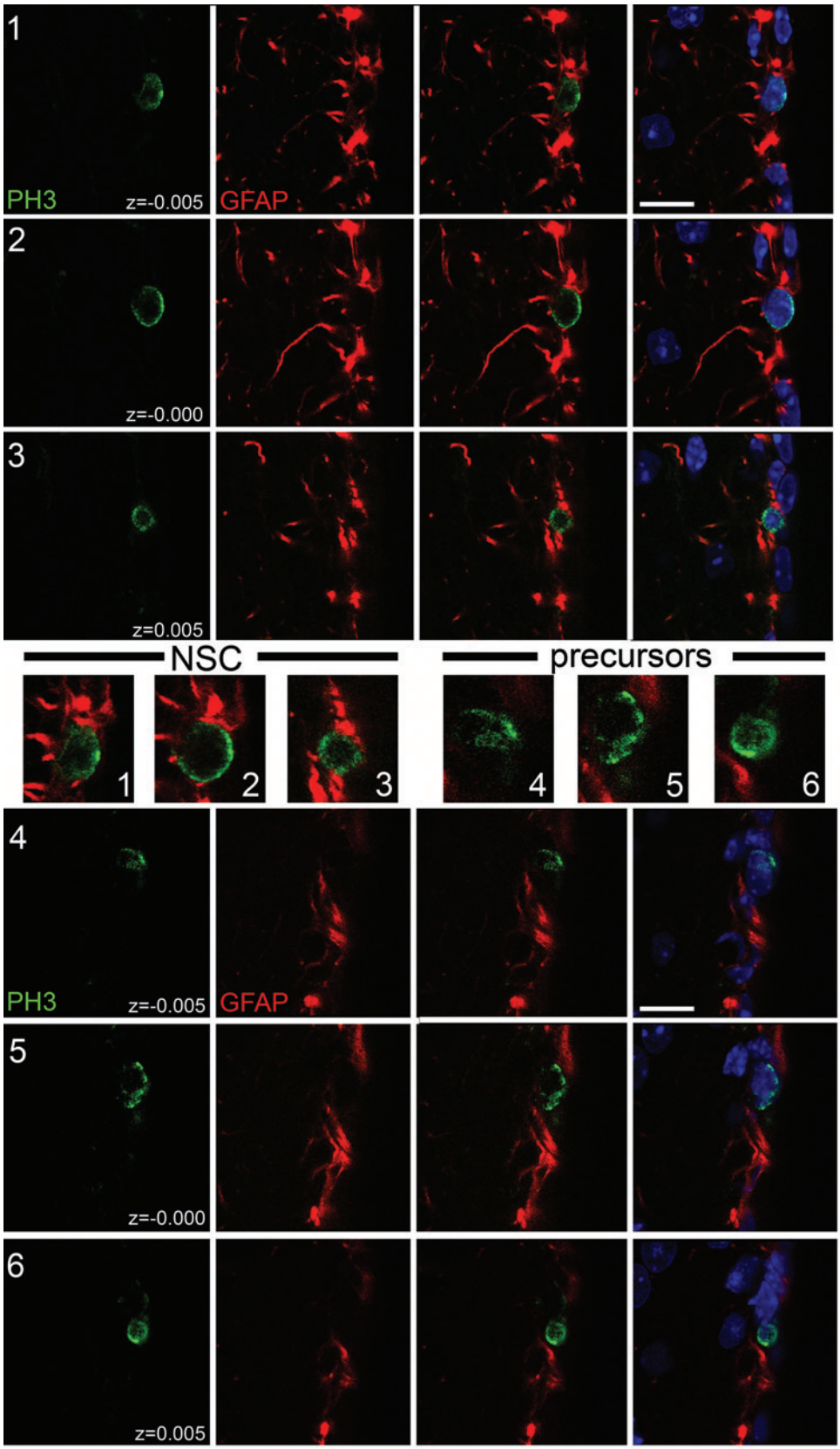
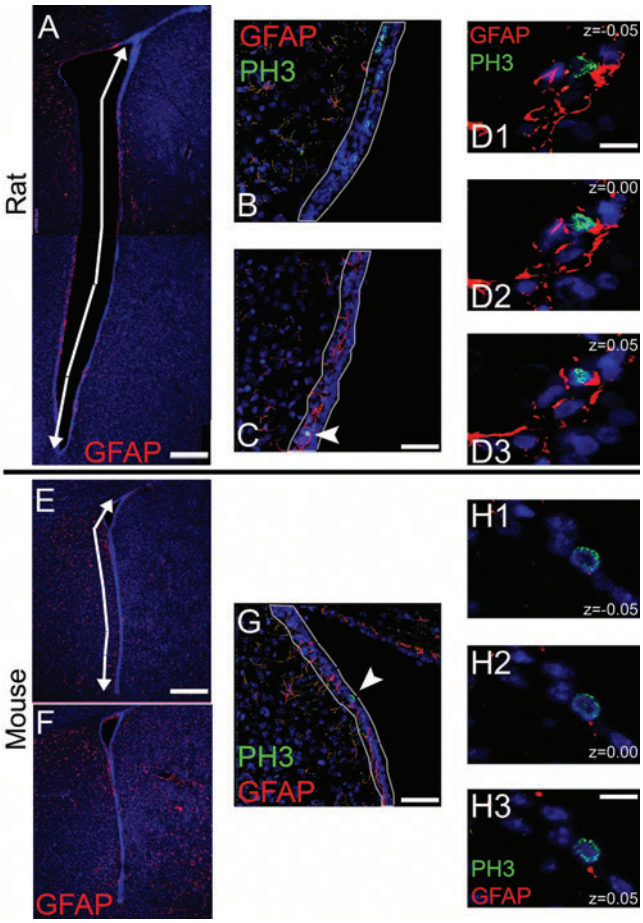
Supplementary Data



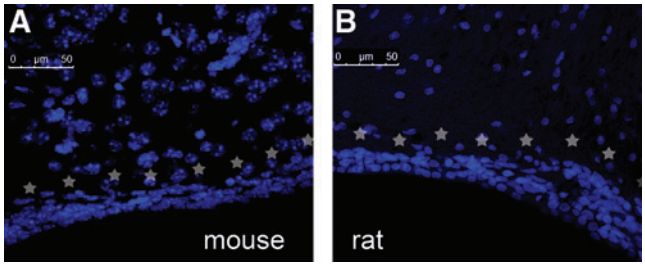
SUPPLEMENTARY FIG. S1. Identification of ependymal cells and measurement of the distance of mitotic nuclei from the ventricular wall. The images show parts of the SEZ from coronal sections of rat brains immunostained for GFAP (in *red*, in **A**), to mark astrocytes and PH3 (in *green*, in **A** and **B**), to mark cells in M phase. Note in "**A**" that the ependymal cell layer (indicated by *white arrowheads*) is immuno-negative for GFAP. In "**B**," 2 newly divided cells (indicated by the *white arrow* and expressing PH3, as shown in the *inset*) are located near ependymal cells (annotated with an "e"). To calculate their distance from the ventricular wall, we measured (using ImageJ software) the shortest distance from the center of their nucleus (*red spot*) up to the line connecting the centers of the 2 neighboring ependymal cells. Scale bars: 20 μm in **A**; 10 μm in **B.** GFAP, glial fibrillary acidic protein; PH3, phosphohistone 3.



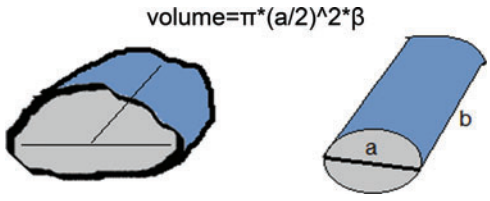
SUPPLEMENTARY FIG. S2. Identification of mitotic NSCs and precursors. The images show parts of the SEZ from coronal sections of mice brains immunostained for GFAP (in *red*), to mark astrocytes and PH3 (in *green*), to mark cells in M phase. *Panels* **1** to **3** show 3 consecutive optical sections (obtained with a confocal microscope) focused on a mitotic astrocyte, considered a dividing NSC. *Panels* **4** to **6** show 3 consecutive optical sections focused on a group of dividing cells that are GFAP negative, thus considered to be precursors. In the center are shown higher magnifications of the mitotic cells of *panels* **1** to **6**, with GFAP and PH3 immunostainings having been merged. Scale bars = 20 μm. NSC, neural stem cell.



SUPPLEMENTARY FIG. S3. Methodology of the analysis. **(A, E, F)** Stack of confocal-generated images showing the whole rat **(A)** and mouse **(E, F)** SEZ, at the same magnification, and after immunostaining for GFAP (in *red*). Note that in **F**, a post-AraC treatment SEZ is shown, characterized by increased expression of GFAP. The *white line* indicates the dorso-ventral length of the SEZ, as calculated using ImageJ software. **(B, C, G)** Single-optical planes, generated with confocal microscopy, showing higher magnifications of the rat **(B, C)** and mouse **(G)** SEZ, after immunostaining for GFAP (in *red*) and PH3 (in *green*). The pale *white line* outlines the area of the niche, as calculated using the ImageJ software. *White arrowheads* indicate examples of mitotic cells that are shown in even higher magnification at the *right panels*. (*Panels* **D** and **H**) High magnifications (×63 objective lens and ×3 digital zoom) of the cells shown by *arrowheads* in **(C)** and **(G)**. Each *panel* includes 3 optical planes generated by confocal microscopy, to assess coexpression of GFAP (*red*) and PH3 (*green*). The rat cell in *panel* **D** was GFAP+/PH3+, and the mouse cell in *panel* **H** was GFAP−/PH3+. Scale bars: 250 μm in **A, E, F**; 50 μm in **B, C, G**; 10 μm in **D, H.**



SUPPLEMENTARY FIG. S4. Delineation of the SEZ according to cell architecture. Images show coronal sections taken from normal adult mouse **(A)** and rat **(B)** brains, focusing at the SEZ. *Gray stars* indicate the hypocellular, mitoses-free zone considered to be at the border of the SEZ. SEZ, subependymal zone.



SUPPLEMENTARY FIG. S5. Graphic illustration of the generic shape of the OBs based on which their volume was calculated. The assumption was that OBs resemble cylinders and volume was calculated using the shown formula. OB, olfactory bulb.