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Supplemental Data

Neural Stem Cells Confer Unique Pinwheel Architecture to the Ventricular Surface in Neurogenic Regions of the Adult Brain

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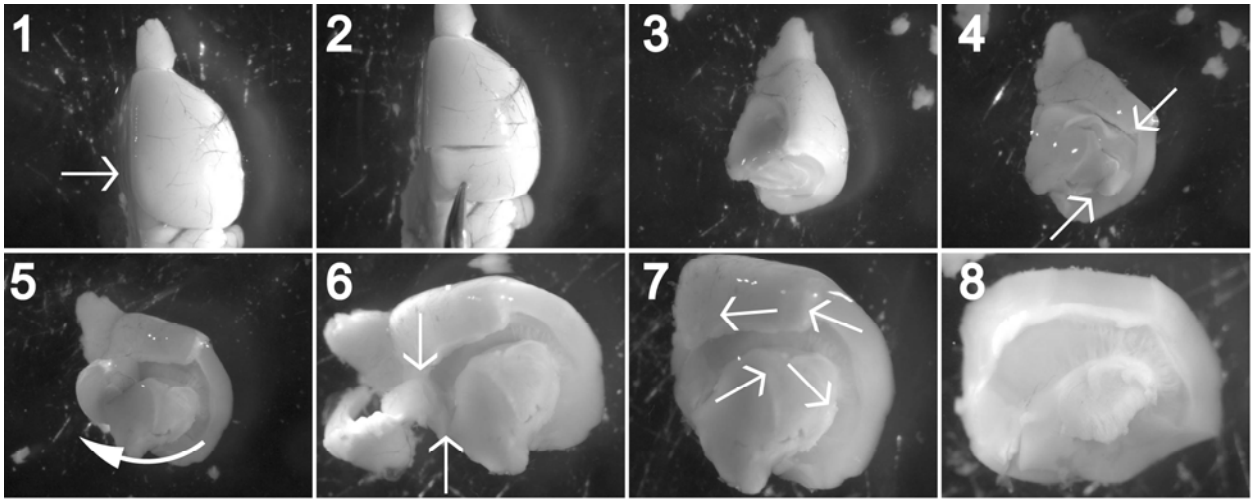

Figure S1. Stereomicroscopic images showing wholemount dissection of the lateral wall of the LV. Open arrows indicate positions where the brain was cut and the closed arrow indicates the direction of the pulling motion applied to separate the LV walls.

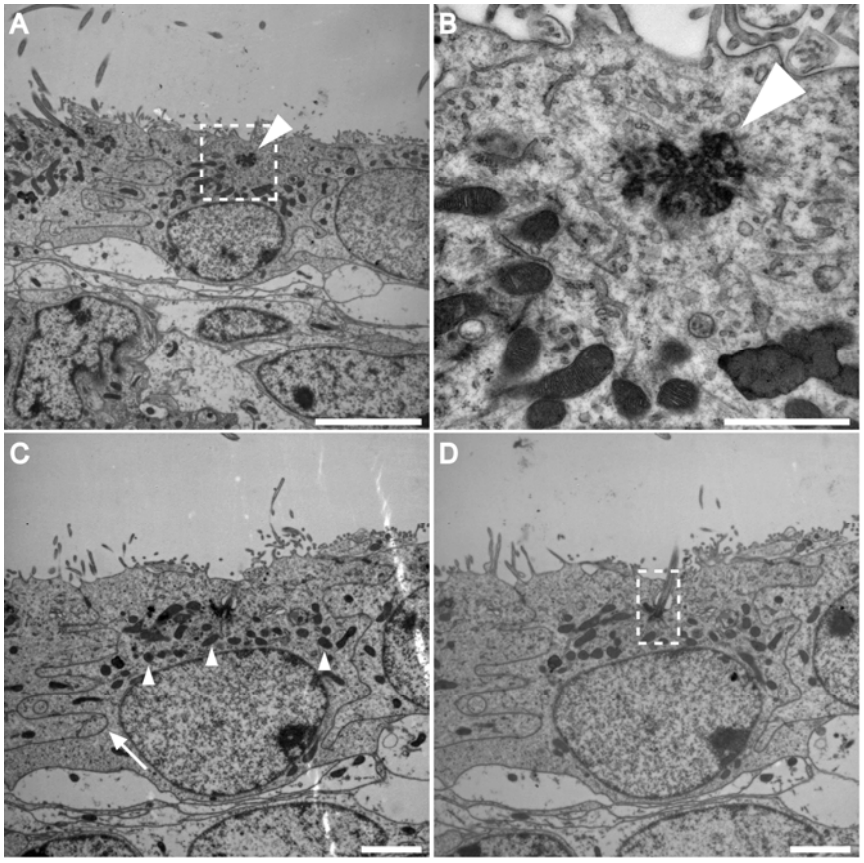

Figure S2. Ultrastructure of a serially reconstructed E2 cell in transverse sections.

(**A, B**) Boxed region in A is enlarged in B. The distinguishing feature of E2 cells was their heavily electron dense basal bodies (arrowheads in A and B). Scale bar = 5 um (A), 1 um (B).

(**C**) E2 nuclei were spherical with no invaginations and dispersed chromatin. Note many mitochondria (arrowheads) in a supranuclear position and deep interdigitations of the lateral membranes (arrow). Scale bar = 2 um.

(**D**) Low magnification image of the ultrathin section shown in Figure 1E (boxed region). Scale bar = 2 um.

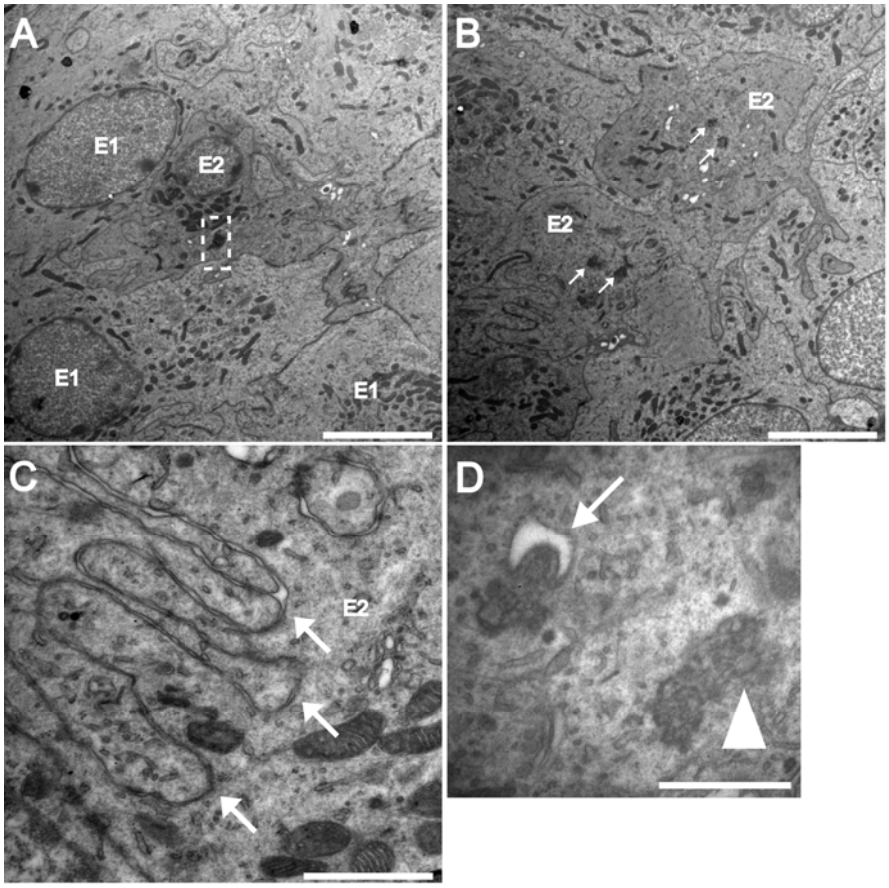

Figure S3. Ultrastructure of E2 cells in en-face sections.

(**A**) E2 cells had light cytoplasm that was slightly darker than E1 cells. Boxed region in (A) is enlarged in Figure 1F. Scale bar = 5 um.

(**B**) Two E2 cells observed en-face, each with two electron dense basal bodies (arrows). Scale bar = 5 um.

(**C**) High magnification image of the deeply interdigitating lateral membranes (arrows) of E2 cells. Scale bar = 1 um.

(**D**) An E2 cilium (arrow) emanating from one side of the invaginated apical membrane. Note complex lateral extensions of the basal bodies (arrowhead indicates the basal body barrel in cross-section). Scale bar = 5 um.

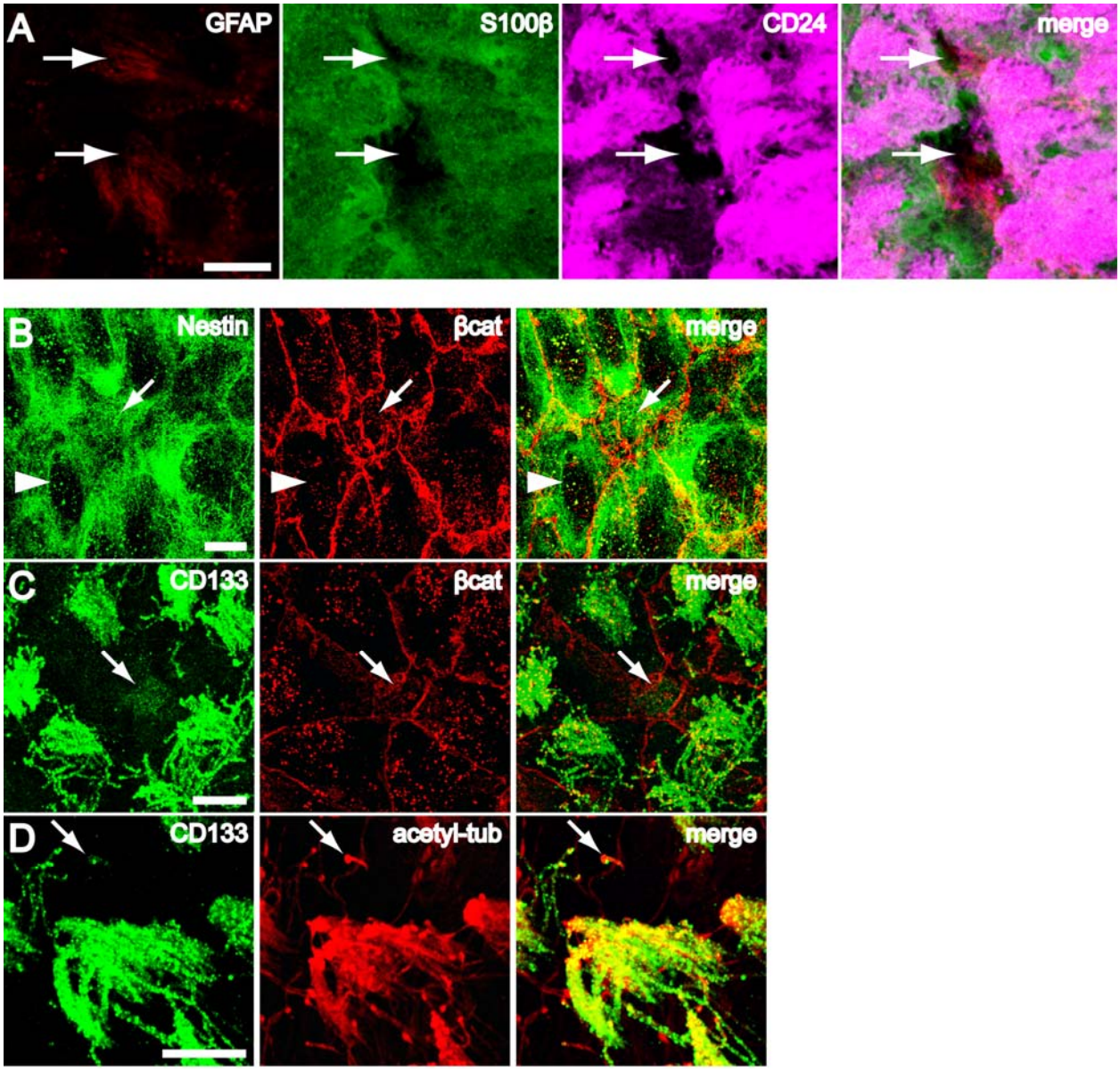

Figure S4. Molecular markers expressed by B1 cells.

(**A**) Triple labeling confirms that B1 cells (arrows) are GFAP+/S100β-/CD24-.

(**B**) Both B1 cells (arrow) and E1 cells (arrowhead) express Nestin.

(**C**) Confocal image of the surface of the lateral wall of the LV stained for CD133 (green) and β-catenin (red). Diffuse CD133 expression is observed at the B1 apical surface (arrow) in the center of the pinwheel. E1 cells have intense expression in cilia.

(**D**) CD133 localizes to the primary cilium (arrow) in some B1 cells. Scale bar = 10 um (A-D).

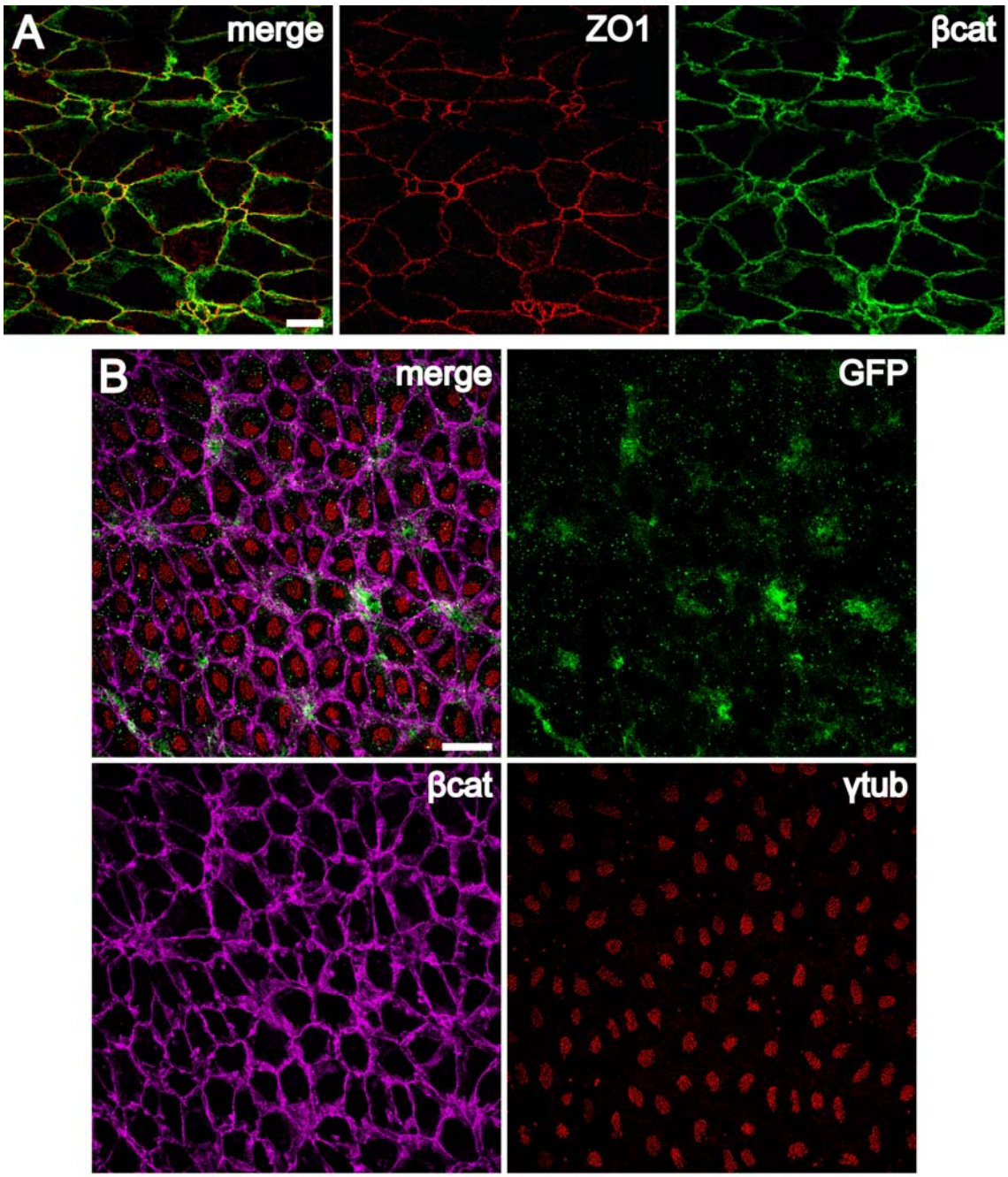

Figure S5. Pinwheel organization of the ventricular surface revealed by ZO-1 staining and Aldh1L-GFP transgenic mice.

(**A**) Confocal image of the surface of the lateral wall of the LV stained for ZO-1 (red) and β-catenin (green). ZO-1 sharply delineates the cell boundaries and reveals the pinwheels. Scale bar = 10 um.

(**B**) GFP+ B1 apical surfaces are located at the core of pinwheel structures throughout the lateral wall in Aldh1L-GFP transgenic mice. Scale bar = 20 um.

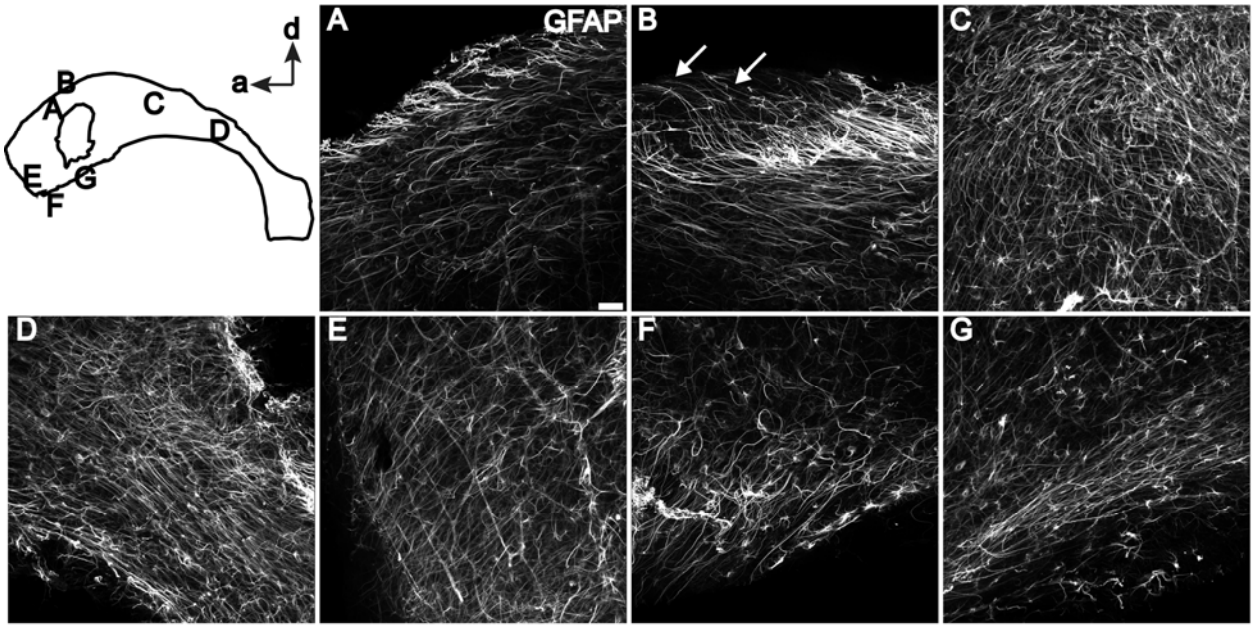

Figure S6. Long GFAP+ fibers are oriented in different directions depending on the region of the lateral wall of the LV.

Z-projections of confocal stacks (50 um thick) from the lateral wall stained for GFAP. Diagram of the lateral wall indicates where the images are from and compass shows anterior (a) and dorsal (d) directions. Arrowheads in (B) indicate fibers oriented obliquely to the surface that ran deep into the underlying striatum beneath the corpus callosum (dissected away in image). Scale bar = 50 um.

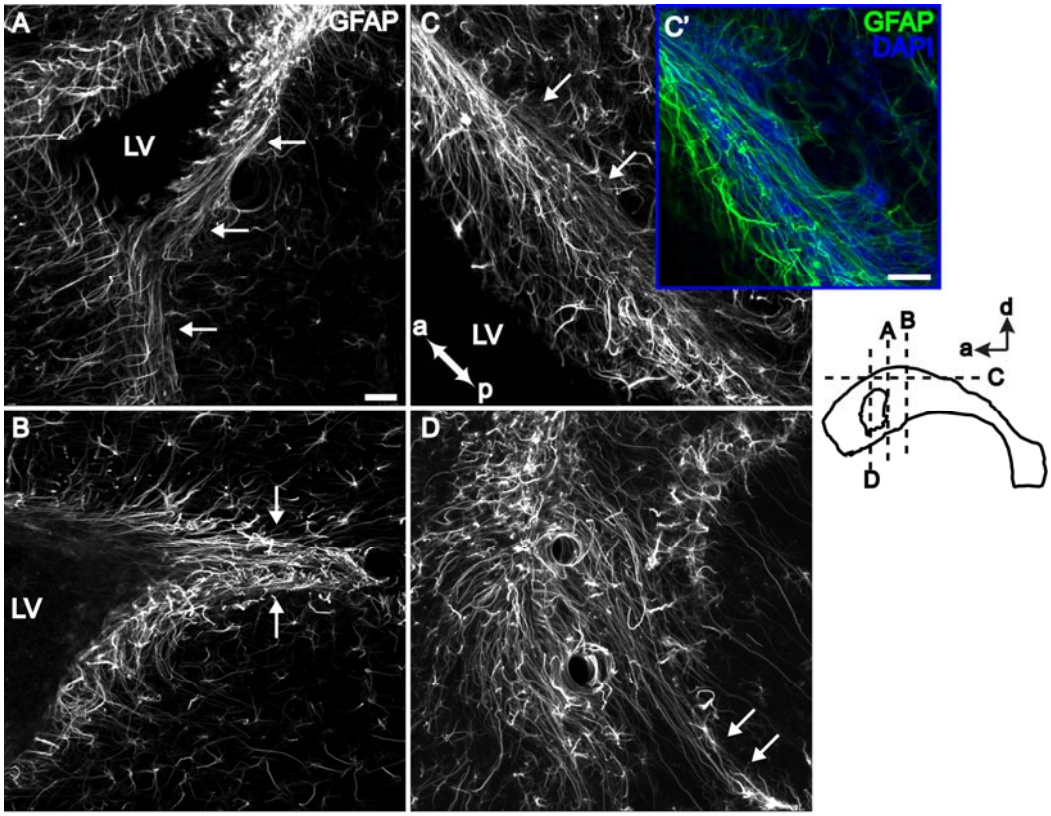

Figure S7. Long GFAP+ fibers in 100 um SVZ sections.

(**A-D**) Long GFAP+ fibers observed in the dorsal SVZ in coronal (A, B) and horizontal (C) sections and the ventral SVZ (D) in coronal sections. The diagram of the lateral wall indicates the section level. The compass shows anterior (a) and dorsal (d) directions. LV is lateral ventricle. Scale bar = 50 um.

(**C**) The dorsal corridor of the SVZ contains many chains of neuroblasts (elongated DAPI nuclei in C'). GFAP+ fibers surround these nuclei and form gliotubes.

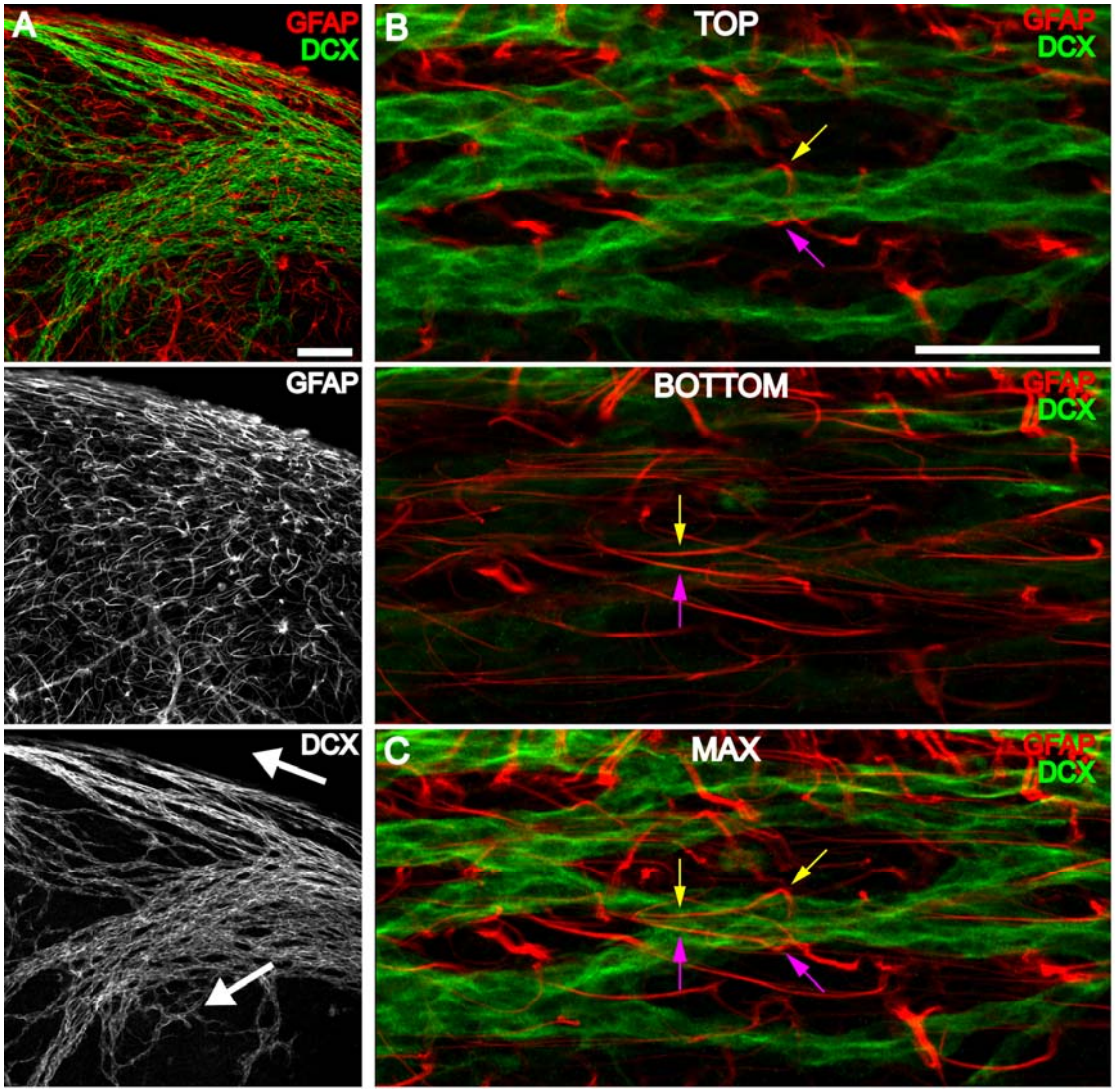

Figure S8. Chains of migrating neuroblasts in the SVZ are oriented parallel to long GFAP+ fibers.

(**A**) Chains of DCX+ neuroblasts (green) are oriented in two general directions (arrows), parallel to the direction of long GFAP+ fibers (red). Scale bar = 100 um.

(**B**) Top and bottom images of a high power confocal stack from the dorsal corridor of the SVZ. DCX+ chains ran largely along GFAP+ fibers, which wrapped around the chains closer to the ventricle surface (TOP, yellow and magenta arrows indicate 2 B1 cells) and were oriented tangentially to the chains deeper in the SVZ (BOTTOM). Scale bar = 50 um.

(**C**) Maximum projection of the confocal stack shown in (B).

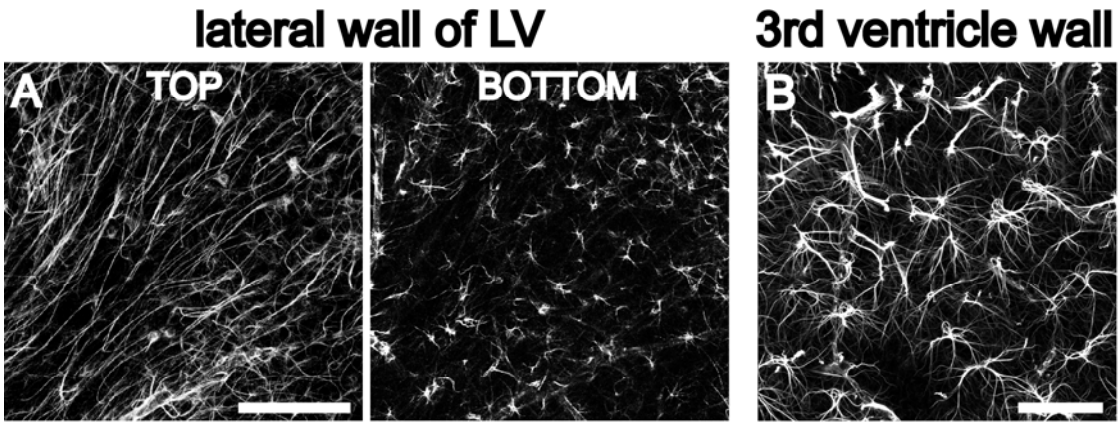

Figure S9. GFAP staining in neurogenic v. non-neurogenic adult ventricular walls.

(**A**) Two images from a single confocal stack of the lateral wall of the LV stained for GFAP. Closer to the ventricular surface (TOP), B1 cells are observed with long GFAP+ fibers. Beneath this layer of fibers (BOTTOM) are GFAP+ multipolar astrocytes corresponding to B2 cells.

(**B**) GFAP staining in the 3rd ventricle wall reveals GFAP+ multipolar astrocytes beneath the ependyma. Scale bar = 50 um (A, B).

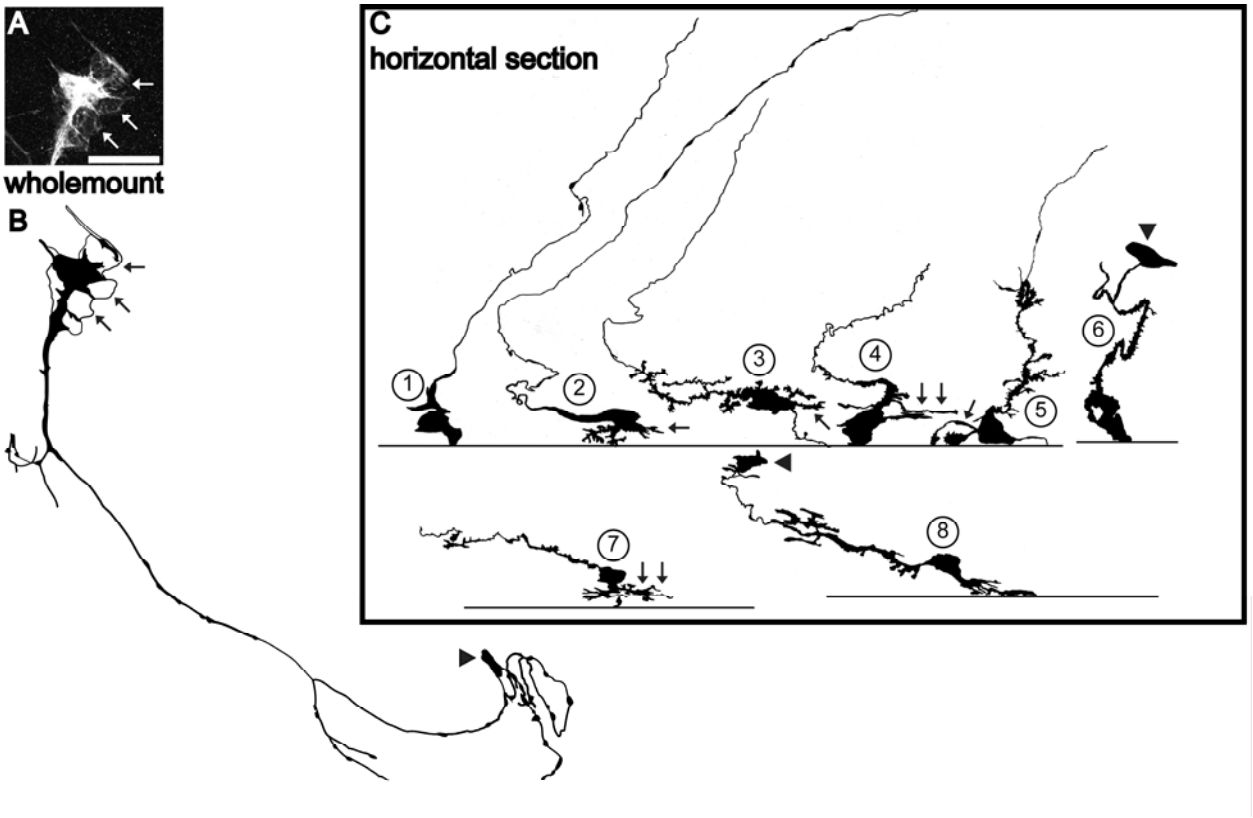

Figure S10. Morphology of ventricle-contacting B1 cells in wholemounts and sections.

These GFP-filled B1 cells were observed in the lateral wall of the adult LV. They were derived from radial glia targeted in neonatal Z/EG mice.

(**A**) Z-projection of a confocal stack reveals web-like extensions of membrane from the B1 cell body (arrows), which cover the basal side of adjacent ependymal cells.

(**B**) Tracing of the complete morphology of the cell shown in (A) including specialized contact (arrowhead) with a blood vessel.

(**C**) Tracings of B1 cells observed in horizontal sections. Arrows indicate lateral extensions and arrowheads indicate contacts with blood vessels.

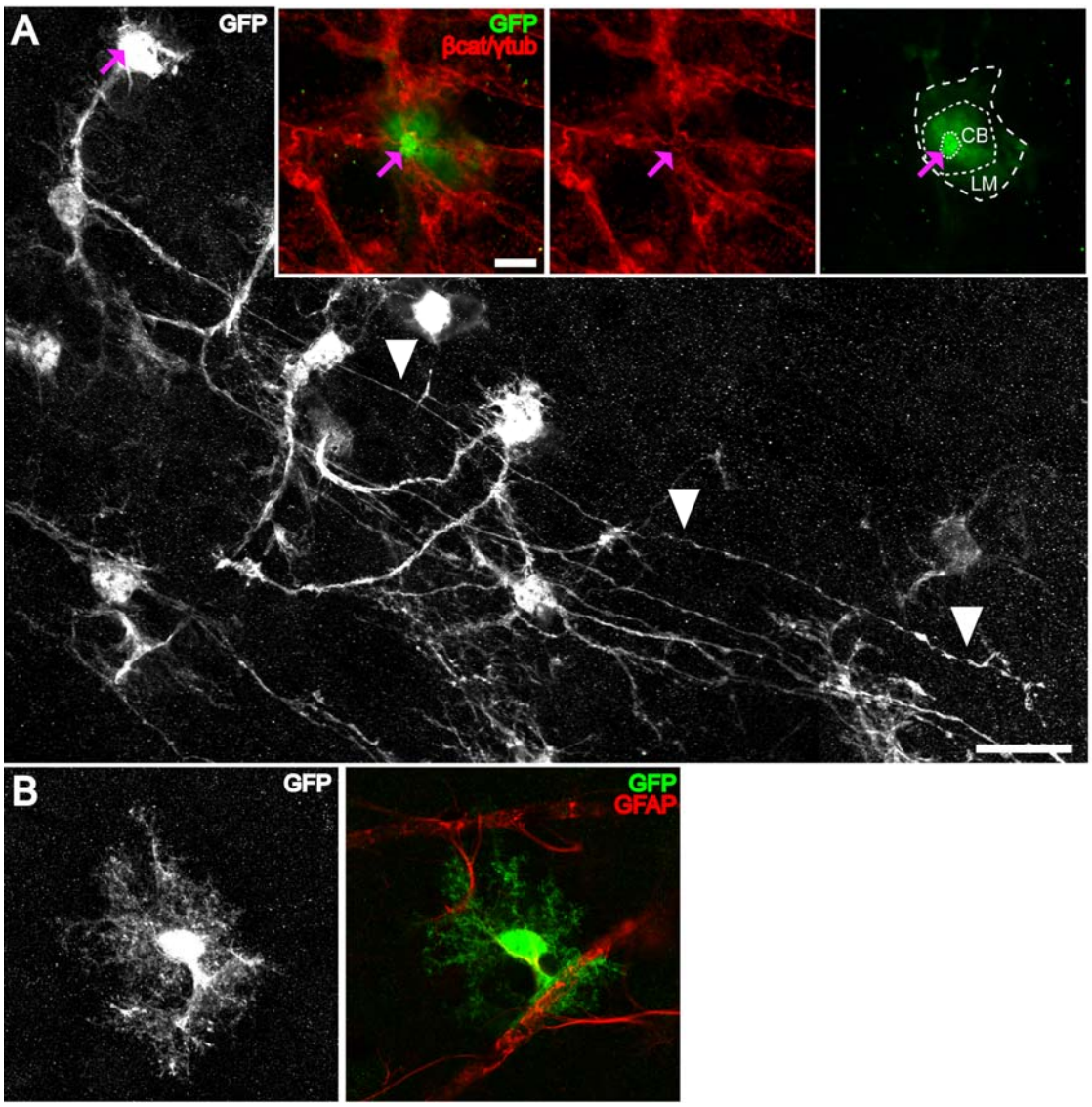

Figure S11. Morphology of ventricle-contacting B1 vs. SVZ B2 cells.

(**A, B**) GFP-labeled cells observed in the lateral wall of the adult LV. These cells were derived from radial glia targeted in neonatal Z/EG mice. Scale bar = 10 um (A, B), 5 um (A inset).

(**A**) Arrow indicates apical ending of a B1 cell with a long basal process (arrowheads). Inset is a confocal optical section showing the B1 apical ending surrounded by ependymal cells. The apical surface (arrow), cell body (CB), and thin lateral membrane (LM) are outlined.

(**B**) An SVZ B2 cell at the SVZ-striatal border, 30-40 um beneath the ventricular surface. B2 cells do not contact the ventricle but have specialized contact with blood vessels.

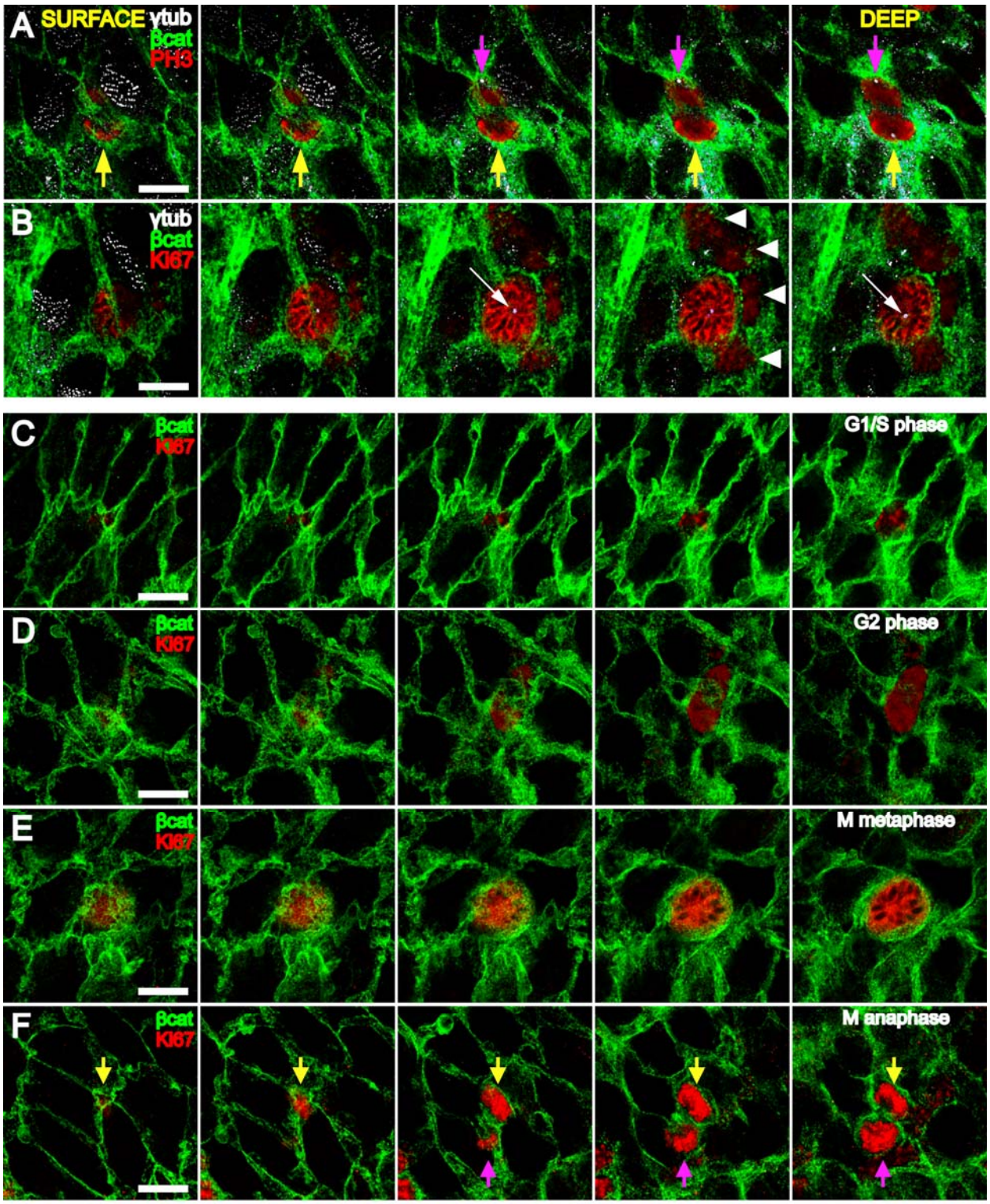

Figure S12. B1 cells with apical ventricular contact in different stages of the cell cycle.

(**A-F**) are confocal stacks used to reconstruct the apical surface and cell body (delineated by β-catenin, green) of B1 cells expressing PH3 (A) or Ki67 (B-F). The first optical section in each series was taken at the ventricular surface and the step size between sections is 0.5 um. Scale bar = 10 um.

(**A**) PH3+ ventricle-contacting B1 cell in anaphase. Yellow and magenta arrows indicate two sister chromatids.

(**B**) Arrows indicate the two centrosomes of a Ki67+ metaphase ventricle-contacting B1 cell. This cell is flanked by other Ki67+ cells (arrowheads) beneath the ependyma.

(**C-F**) Ki67+ B1 apical surfaces were found with staining patterns corresponding to late G1/S (C), G2 (D), metaphase (E), and anaphase (F).

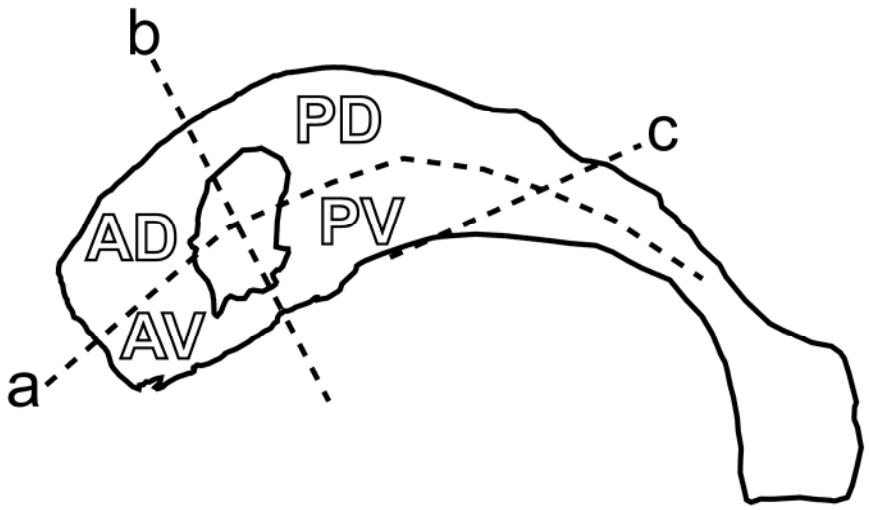

Figure S13. Regions quantified for B1, E1, and E2 cell density. Line (a) bisects the wholemount into a dorsal and ventral half. Line (b) bisects the adhesion point orthogonal to line (a). Line (c) is tangential to the ventral boundary of the anterior half of the wholemount. These lines divide the lateral wall into four regions: anterior-dorsal (AD), anterior-ventral (AV), posterior-dorsal (PD), and posterior-ventral (PV).