Supplemental Materials

Molecular Biology of the Cell

Sato et al.

SUPPLEMENTAL FIGURES

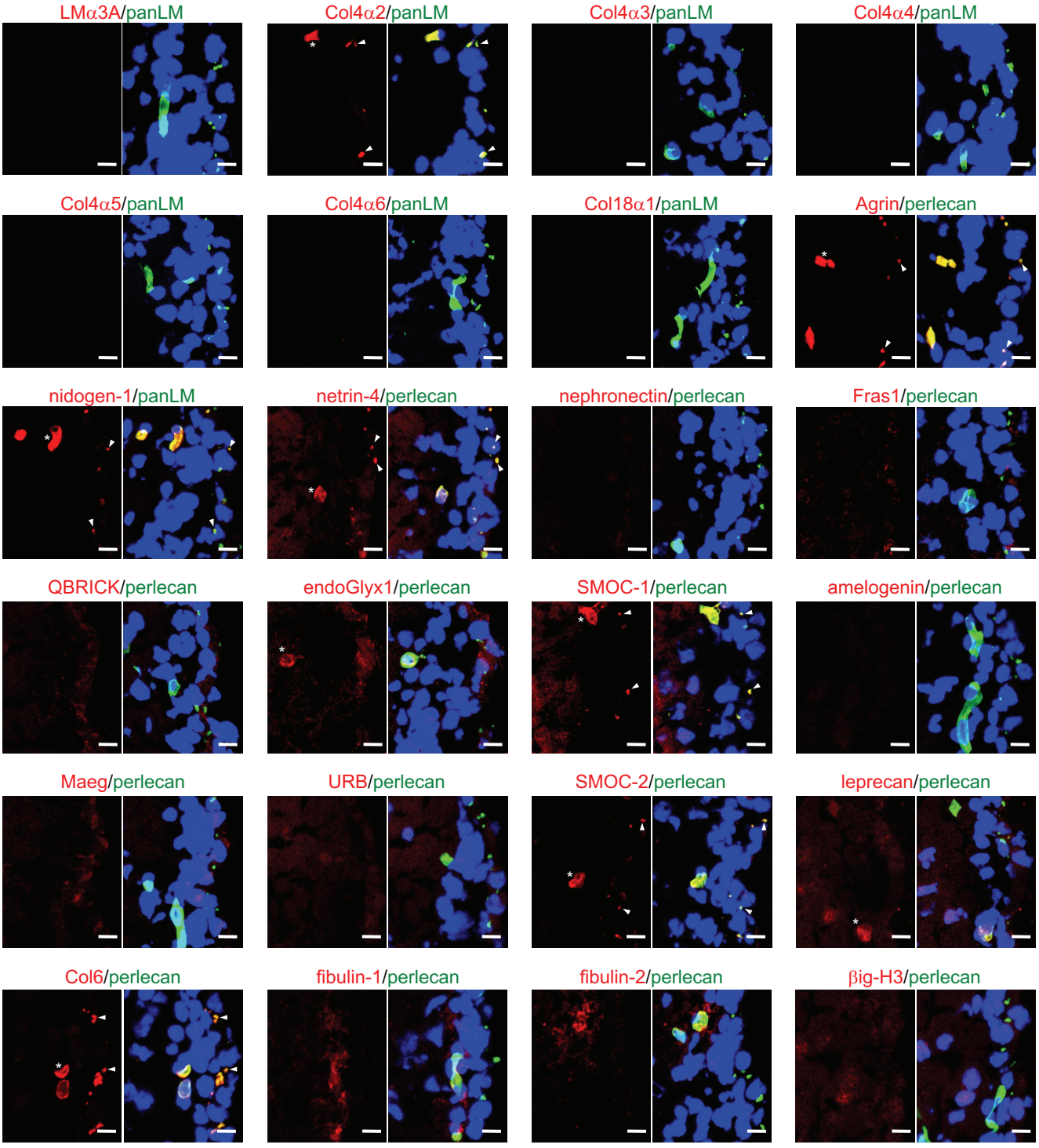

Figure S1. Molecular profiling of BM proteins in adult mouse V-SVZs. (Related to Figure 1) Immunohistochemical localization of BM proteins in adult mouse V-SVZs. Coronal sections of 7-week-old mouse brains were labeled with antibodies against individual BM proteins (*red*). Anti-panLM or anti-perlecan antibodies (*green*) were used as markers for BMs. Nuclei were stained with Hoechst 33342 (*blue*). *Asterisks* and *arrowheads* indicate vascular BMs and fractones, respectively. Scale bar, 10 μm.

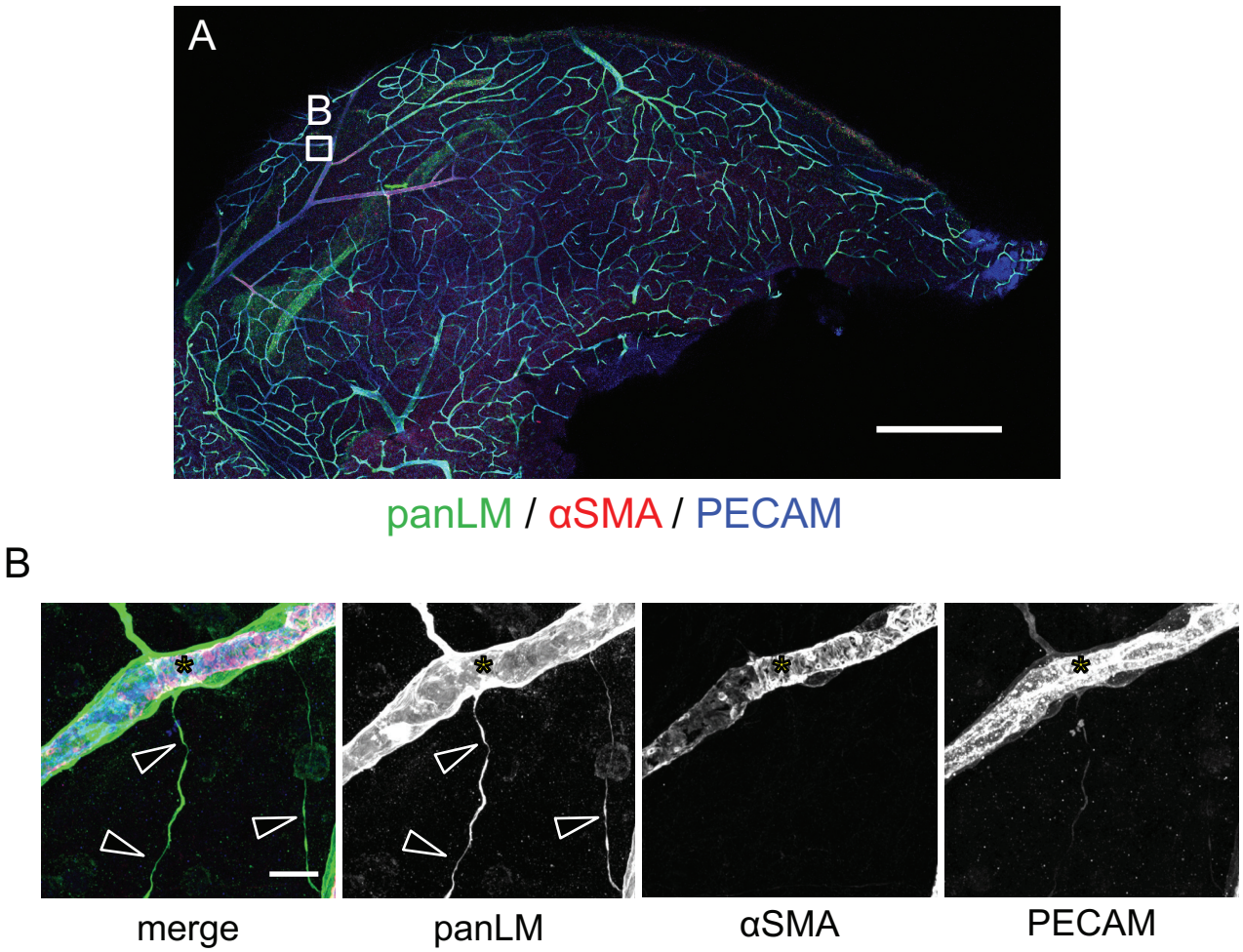

Figure S2. Fibrous BMs extend from blood vessels that are surrounded by smooth muscle cells. (Related to Figure 2)
(A) Optical slice image of a whole-mount V-SVZ labeled with antibodies against panLM (*green*), α-smooth muscle actin (αSMA, smooth muscle cell marker, *red*), and PECAM (endothelial cell marker, *blue*). Scale bar, 500 μm. (B) Higher magnification images of the *boxed* area in (A). Data are shown as maximal projection images. *Arrowheads* represent fibrous BMs extending from blood vessels (*asterisks*). Scale bar, 10 μm.

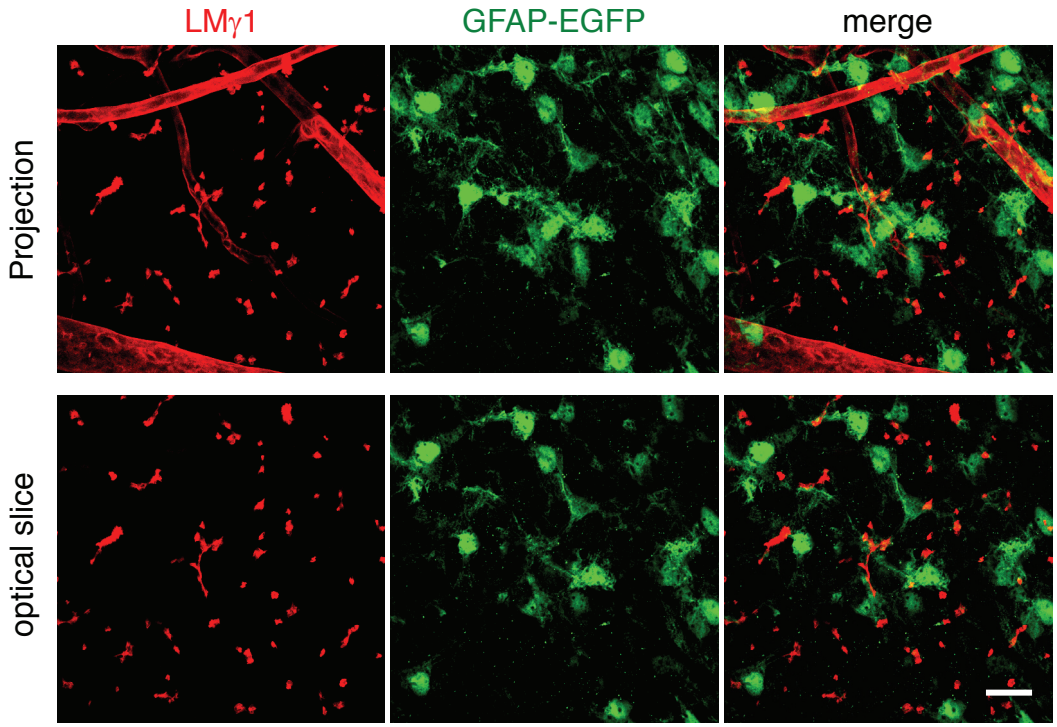

Figure S3. Close association of speckled BMs with GFAP-EGFP cells in V-SVZs. (Related to Figure 3)
Representative images of V-SVZs obtained from GFAP-EGFP reporter mice. The V-SVZs were labeled with antibodies against LMγ1 (*red*) and EGFP (*green*). *Upper* and *lower* figures represent projection images and optical slices focused on the ependymal cell layer, respectively. Scale bar, 10 μm.

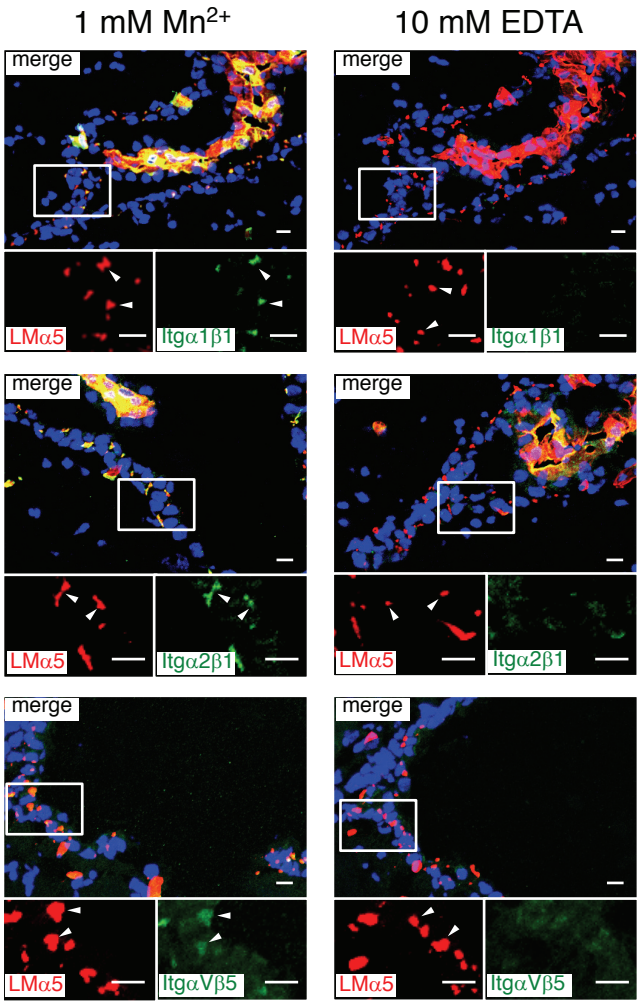

Figure S4. α1β1, α2β1, and αVβ5 integrin-binding activities of speckled BMs. (Related to Figure 5)

Cryosections of adult mouse brains were incubated with recombinant integrins (*green*) in the presence of 1 mM $MnCl_2$ (*left panels*) or 10 mM EDTA (*right panels*). An anti-LMα5 antibody (*red*) was used as a marker for speckled and vascular BMs. Nuclei were stained with Hoechst 33342 (*blue*). Each panel represents a merged image (*upper*) and higher magnification images (*lower*) of each channel in the *boxed* area. *Arrowheads*, speckled BMs. Scale bar, 10 μm.

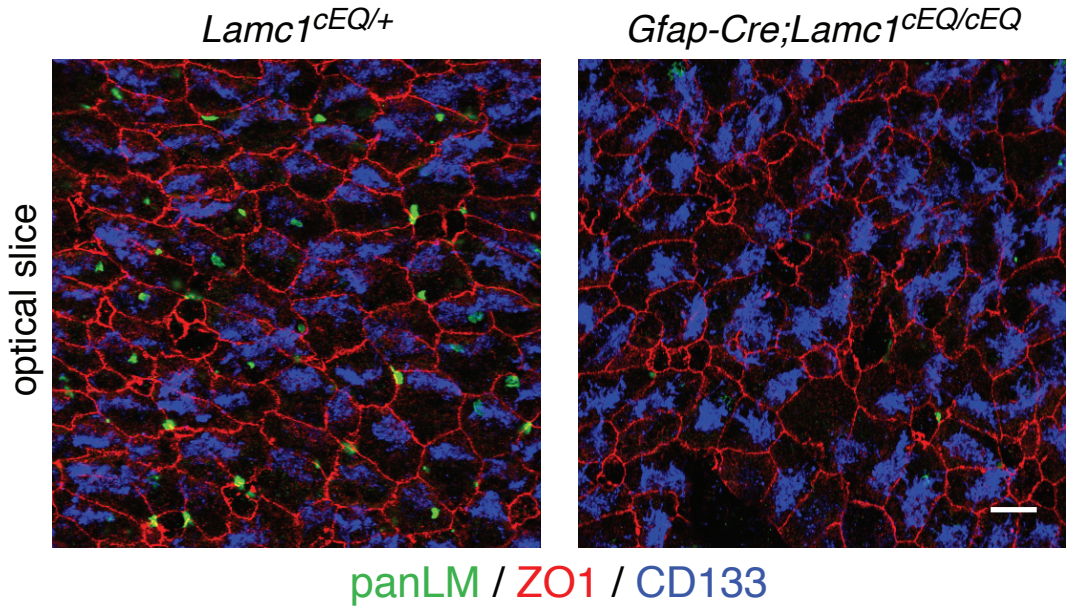

Figure S5. Inactivation of speckled BMs does not affect tight junction formation and ciliogenesis in ependymal cells. (Related to Figure 7)
Whole-mount V-SVZs from *Gfap-Cre;Lamc1*$^{cEQ/cEQ}$ and control mice were labeled with antibodies against panLM (*green*), ZO1 (tight junction marker, *red*), and CD133/prominin-1 (ependymal cilia marker, *blue*). Images are optical slices of the ependymal cell layer. Scale bar, 10 μm.