Data supplement

Visualization of endothelial cell cycle dynamics in mouse using the Flt-1/eGFP-anillin system

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Figure S1: EGFP-anillin construct and expression in embryonic stem (ES) cells. (A) Cloning strategy of flt-1/eGFP-anillin BAC construct. The eGFP-anillin insert together with the neomycin/kanamycin resistance genes were cloned into the first exon of the flt-1 gene in the flt-1 BAC. (B) EGFP-anillin localization during cell cycle. (C-H) EGFP-anillin expression in embryoid bodies (EBs) derived from flt-1/eGFP-anillin ES cells. (C-E) Plated EB on day 5+4 of development derived from flt-1/eGFP-anillin ES cells, clone 10. (F-H) Plated EB on day 5+4 of development derived from flt-1/eGFP-anillin ES cells, clone 30. Arrows indicate native eGFP-anillin expression (green) of hoechst$^+$ nuclei in PECAM$^+$ vascular network. Green = eGFP-anillin; red = PECAM; blue = Hoe. Scale bar = 100µm.

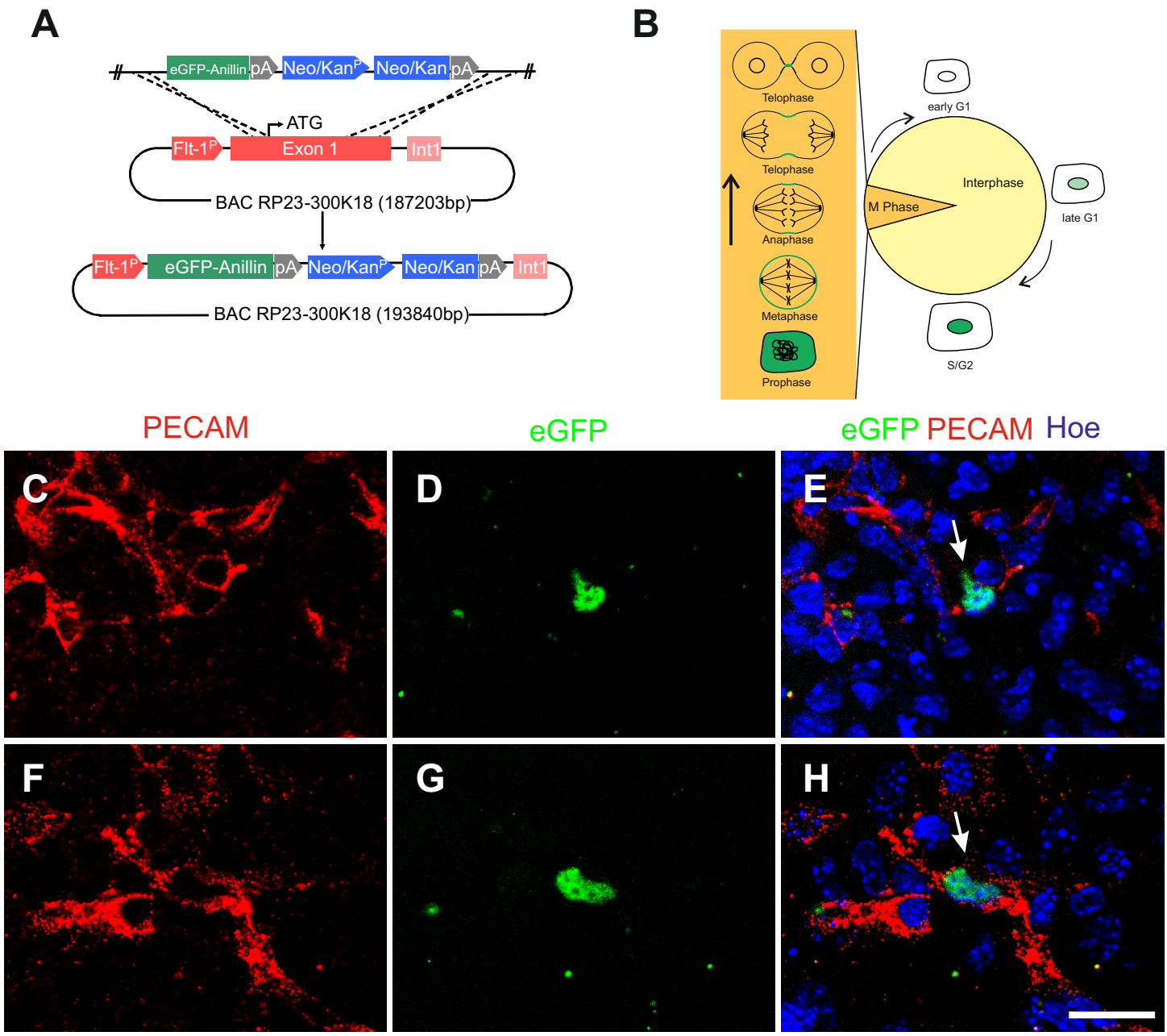
Figure S2: EGFP-anillin expression in adult uterus of flt-1/eGFP-anillin mice. (A) Overview of a cryosection of an adult uterus derived from the flt-1/eGFP-anillin mouse stained with anti-PECAM antibody (red) and hoechst (blue). Arrows indicate native eGFP-anillin$^+$ (green) cells in PECAM$^+$ vessels. (B) Close up of vessel indicated by the square in (A). (C) Dividing PECAM$^+$ cell with eGFP-anillin$^+$ midbody, inset shows higher magnification of cells labelled with arrow. (D-F) Contractile ring in dividing endothelial cell, arrows indicate colocalization of native eGFP-anillin$^+$ (green) and PECAM$^+$ (red). (G-I) Cryosection of uterus stained with anti-Ki67 antibody (H) and hoechst (I). Arrow indicates colocalization of native eGFP-anillin+ (green) and Ki67+ (white) expression in a nucleus. Green = eGFP-anillin; red = PECAM; white = Ki67; purple = autofluorescence; blue = Hoe. Scale bars = 200µm (A), 20 µm (B,C), 25µm (D-F), 15µm (G-I), 10 µm (inset C).

Figure S3: Quantification of eGFP-anillin expression in E12.5 embryos. The lung region is shown. (A,B) Cryosection stained with anti-PECAM antibody (red, A,B) and hoechst (blue, B), arrows indicate the colocalization of native eGFP expression (green, A) and hoechst staining in PECAM$^+$ networks. (C) Quantification of PECAM$^+$ eGFP$^+$ cells. (D) Quantification of eGFP$^+$ and eGFP$^-$ endothelial cells. (E,F) Cryosection displaying native eGFP expression (E) stained with anti-Ki67 antibody (white), arrows indicate the colocalization of native eGFP expression (green, E) and Ki67 staining (F). (G) Quantification of Ki67$^+$ and Ki67$^-$ eGFP$^+$

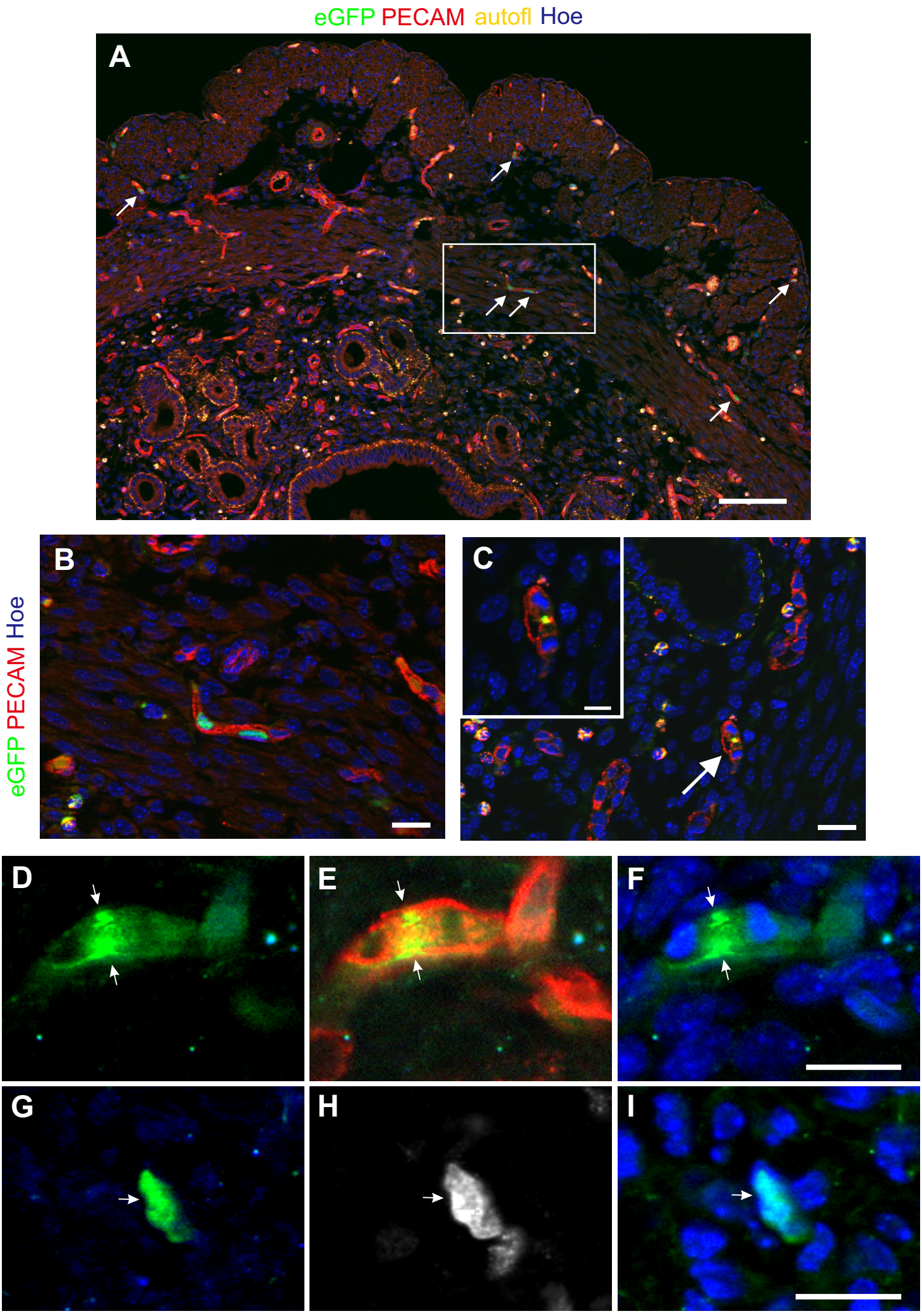
cells. (H,I) Cryosection stained with anti-PECAM antibody (red, H,I), anti-Ki67 antibody (white, H) and hoechst (blue, I), arrows indicate the coloclization Ki67 staining and hoechst staining in PECAM^{+} networks. (J) Quantification of Ki67^{+} and Ki67^{-} endothelial cells. (K) Quantification of eGFP^{+} and eGFP^{-} PECAM^{+}Ki67^{+} cells. N indicates the number of embryos, n indicates the number of cells analyzed. Green = eGFP-anillin; red = PECAM; white = Ki67; purple = autofluorescence; blue = Hoe. Scale bar= 50µm.

Figure S4: Quantification of eGFP-anillin expression in E14.5 embryos. The midbrain region is shown. (A,B) Cryosection stained with anti-PECAM antibody (red, A,B) and hoechst (blue, B), arrows indicate the colocalization of native eGFP expression (green, A) and hoechst staining in PECAM^{+} networks. (C) Quantification of PECAM^{+}eGFP^{+} cells. (D) Quantification of eGFP^{+} and eGFP^{-} endothelial cells. (E,F) Cryosection displaying native eGFP expression (E) stained with anti-Ki67 antibody (white), arrows indicate the colocalization of native eGFP expression (green, E) and Ki67 staining (F). (G) Quantification of Ki67^{+} and Ki67^{-} eGFP^{+} cells. (H,I) Cryosection stained with anti-PECAM antibody (red, H,I), anti-Ki67 antibody (white, H) and hoechst (blue, I), arrows indicate the coloclization Ki67 staining and hoechst staining in PECAM^{+} networks. (J) Quantification of Ki67^{+} and Ki67^{-} endothelial cells. (K) Quantification of eGFP^{+} and eGFP^{-} PECAM^{+}Ki67^{+} cells. N indicates the number of embryos, n indicates the number of cells analyzed. Green = eGFP-anillin; red = PECAM; white = Ki67; purple = autofluorescence; blue = Hoe. Scale bar = 50µm.

Supplementary Movie 1: Time-lapse microscopy of Flt-1/eGFP-anillin E9.5 embryo. Time course of Flt-1/eGFP-anillin signals in the head region of a E9.5 embryo. Endothelial cells in G1, S/G2 phase demonstrate nuclear signals, in M phase eGFP-anillin translocates to the cytoplasm, cell cortex, contractile ring and midbody. Time is indicated in hh:mm:ss, bar = 40 µm.



Suppl. Figure 1



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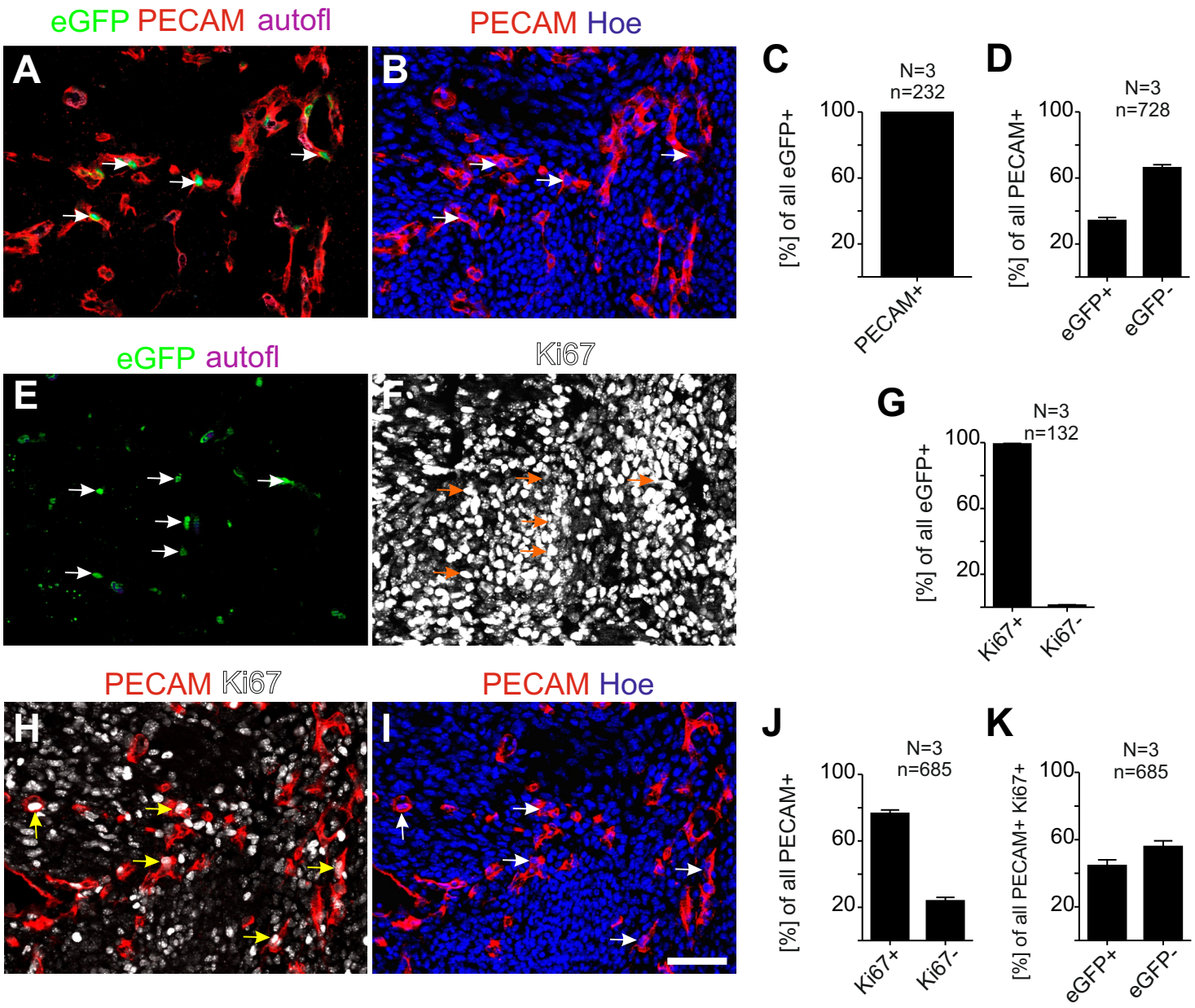

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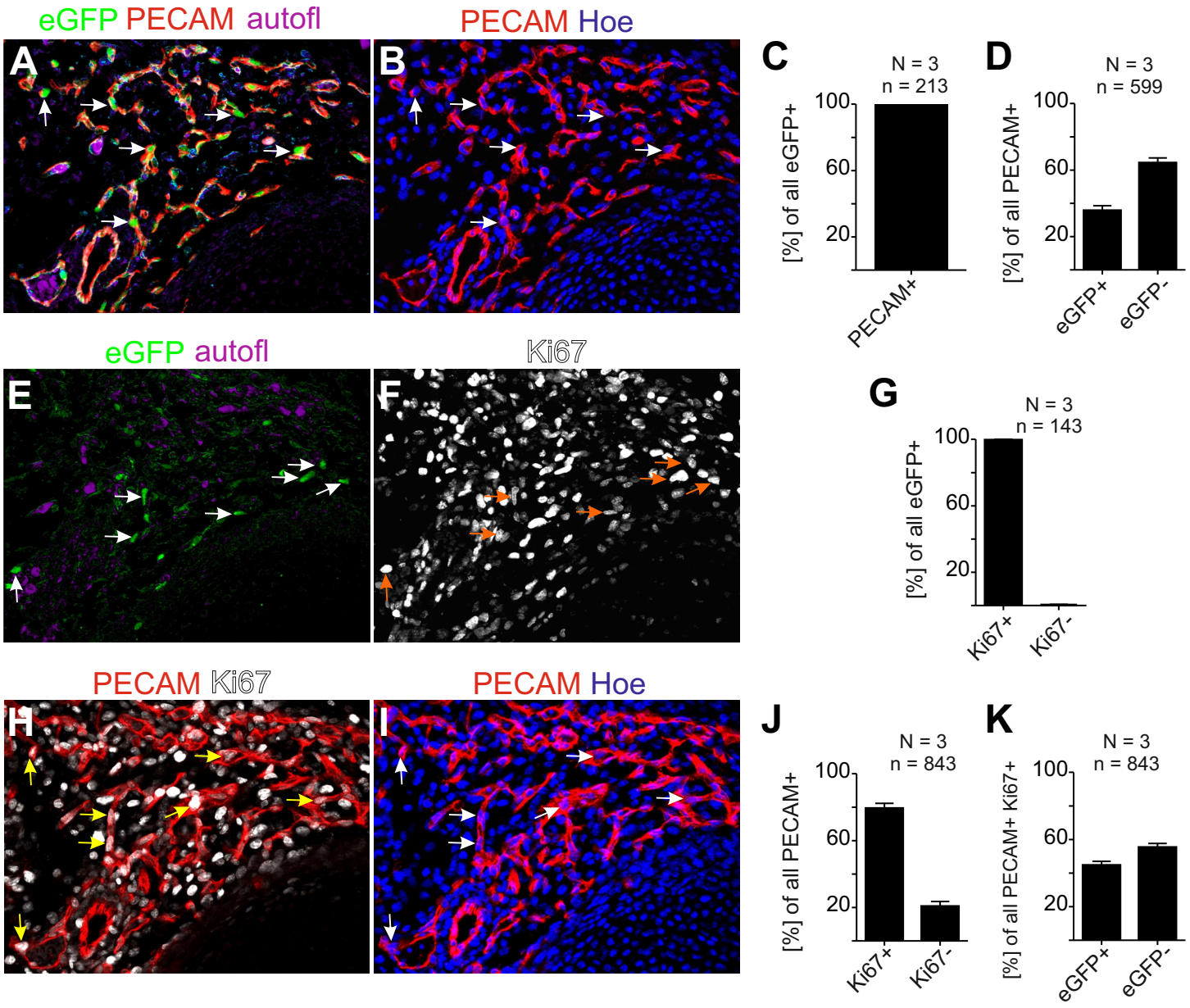

Suppl. Figure 4