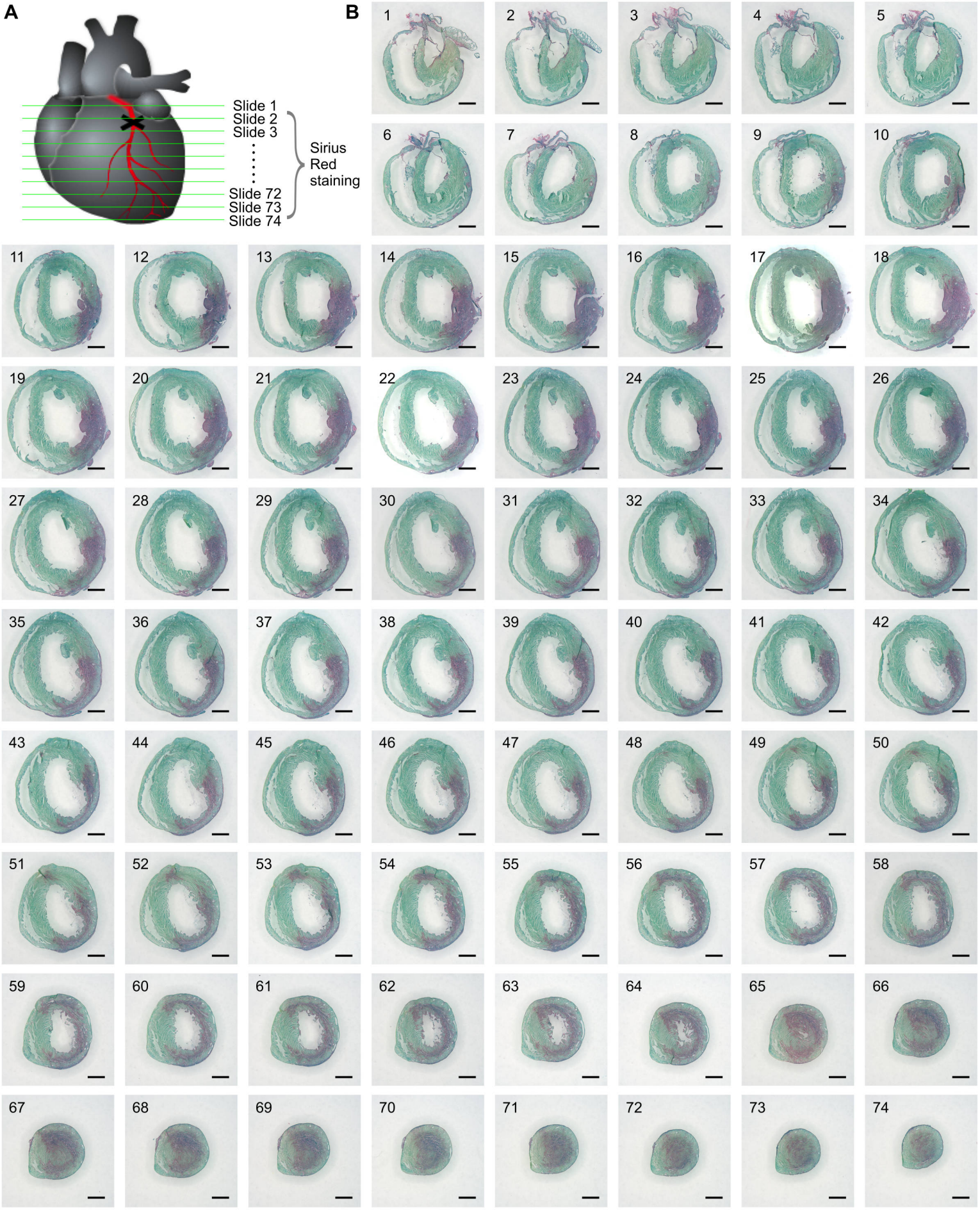
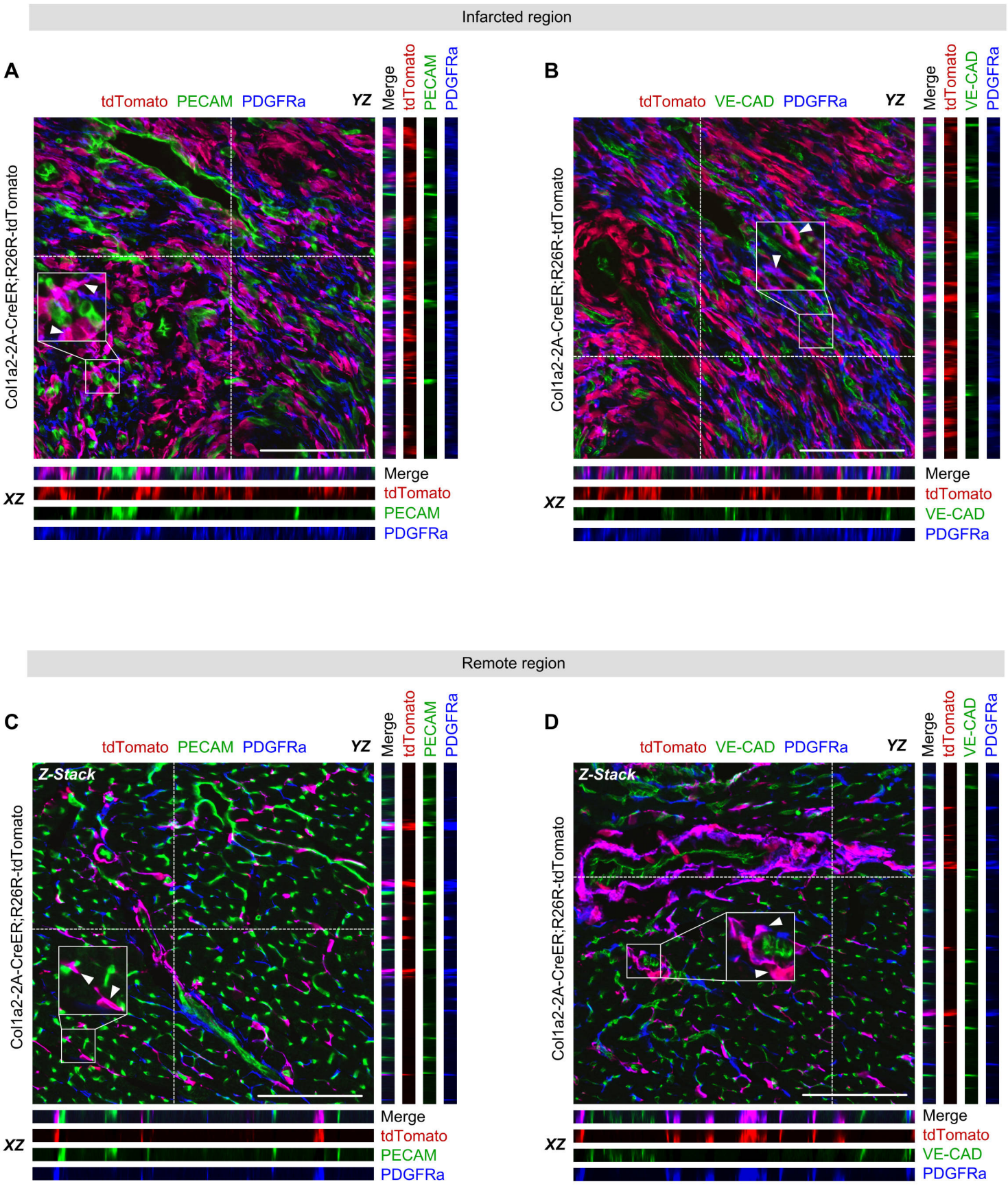
Preexisting coronary endothelial cells mediate cardiac neovascularization after injury

Lingjuan He, Xiuzhen Huang, Onur Kanisicak, Yi Li, Yue Wang, Yan Li, Wenjuan Pu, Qiaozhen Liu, Hui Zhang, Xueying Tian, Huan Zhao, Xiuxiu Liu, Shaohua Zhang, Yu Nie, Shengshou Hu, Xiang Miao, Qing-Dong Wang, Fengchao Wang, Ting Chen, Qingbo Xu, Kathy O Lui, Jeffery D. Molkentin, Bin Zhou

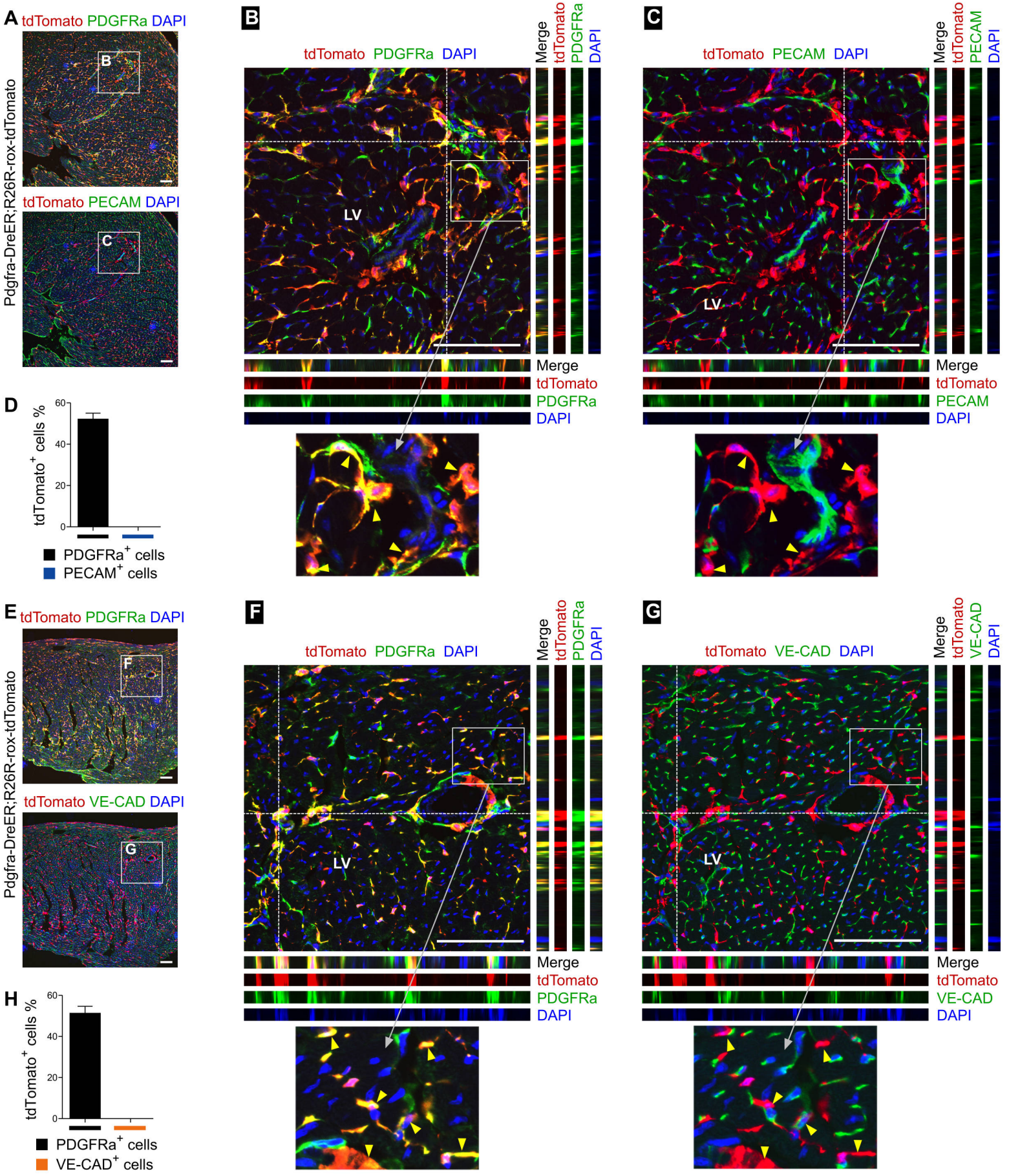
Supplemental Figures 1-9



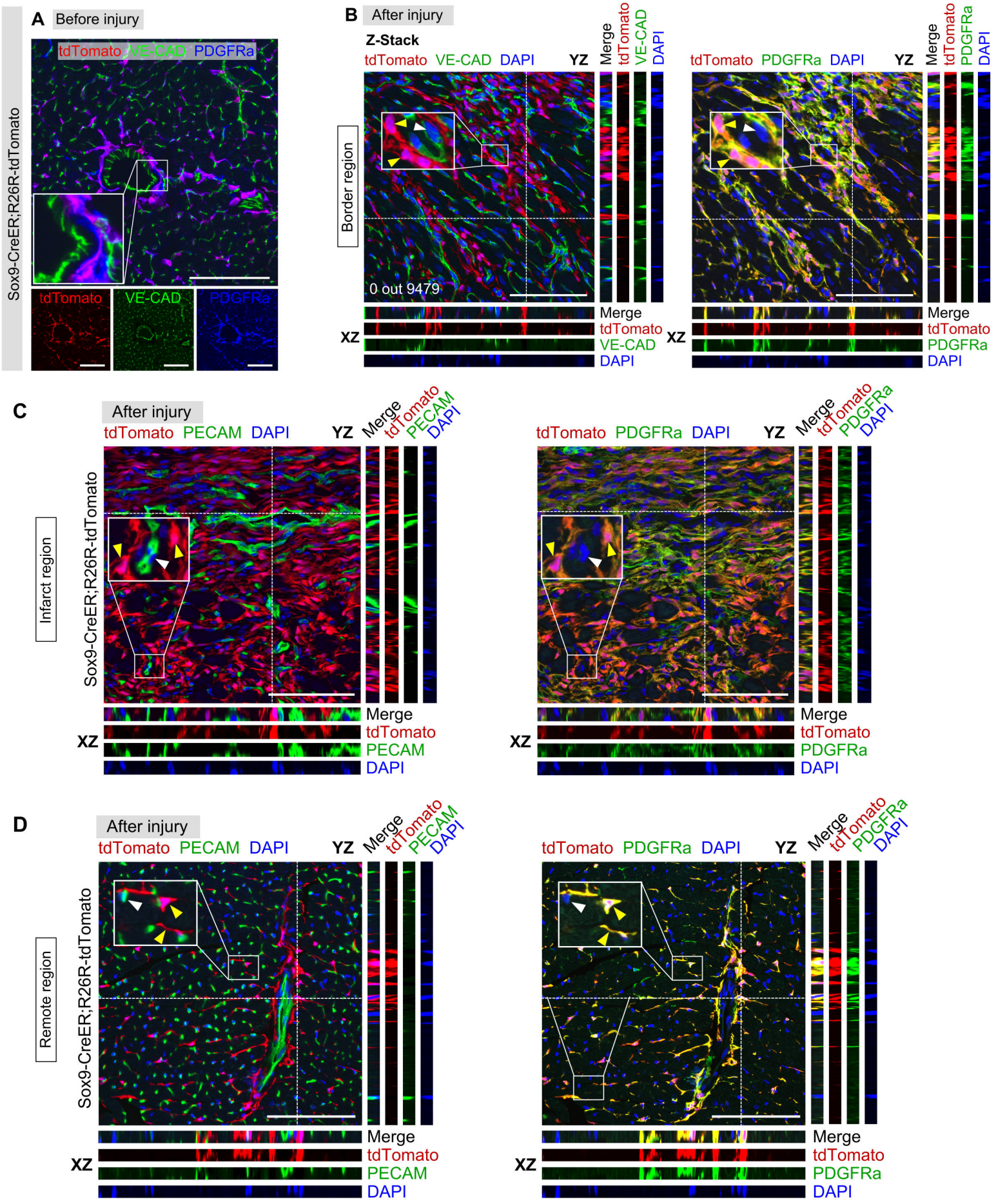
Supplemental Figure 1. Establishment of cardiac injury detected by Sirius Red staining. (**A**) Serial transverse sections of heart at 1 week after myocardial ischemia-reperfusion (IR) model. (**B**) Sirius Red staining of IR heart showed significant injured regions (red color). Representative figure of 3 individual hearts. Scale bars, 1 mm.



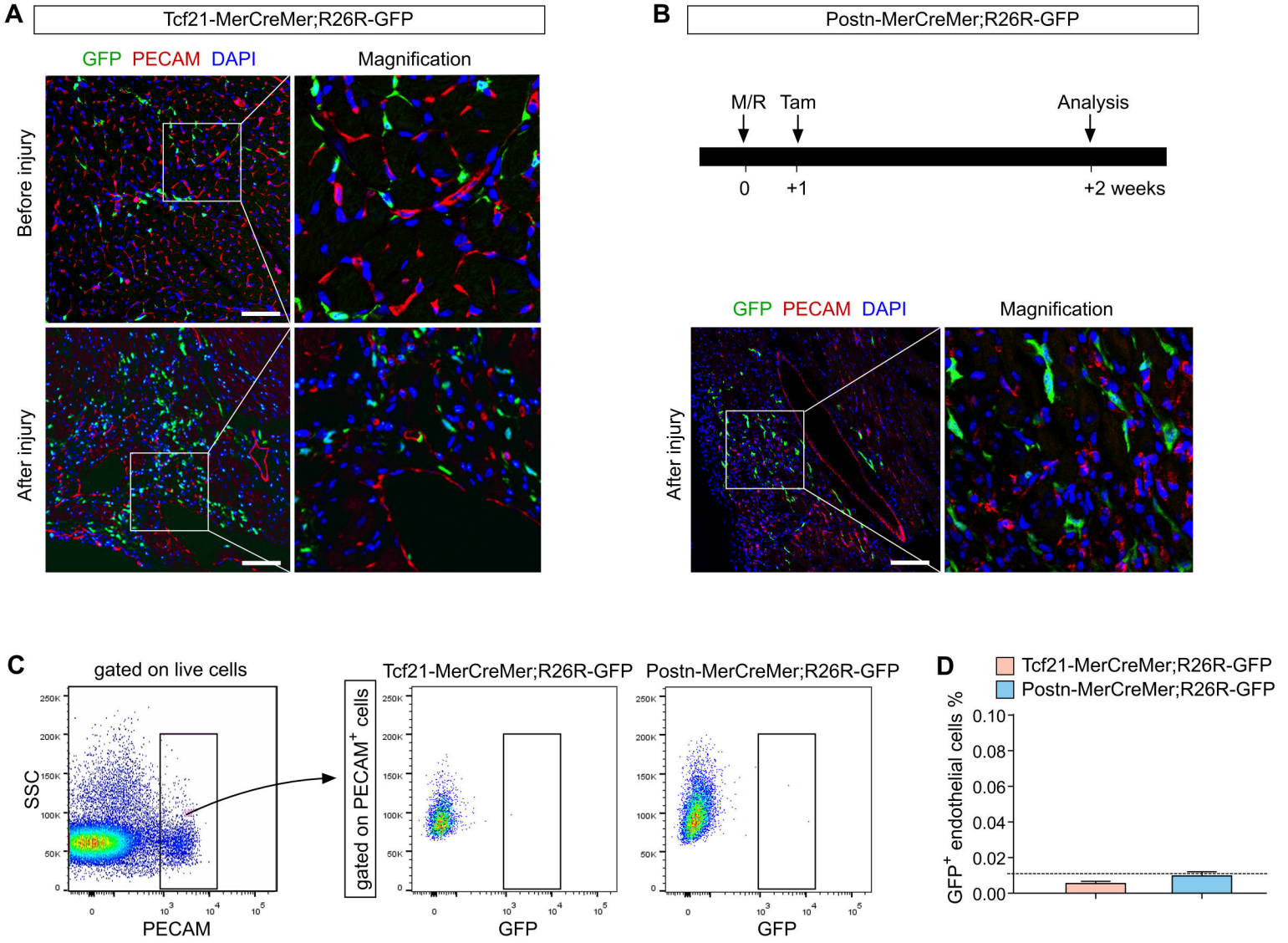
Supplemental Figure 2. Col1a2+ cells adopt fibroblast cell fate and do not differentiate into endothelial cells after injury. (**A,C**) Immunostaining for tdTomato, PECAM and PDGFRa on heart sections. XZ and YZ indicate signals from dotted lines on Z-stack images. Arrowheads indicate tdTomato^{+}PDGFRa^{+}PECAM^{-} cells. (**B,D**) Immunostaining for tdTomato, VE-CAD and PDGFRa on heart sections. Arrowheads indicate tdTomato^{+}PDGFRa^{+}VE-CAD^{-} cells. Scale bars, 100 μm; Each image is a representative of 4 individual samples.



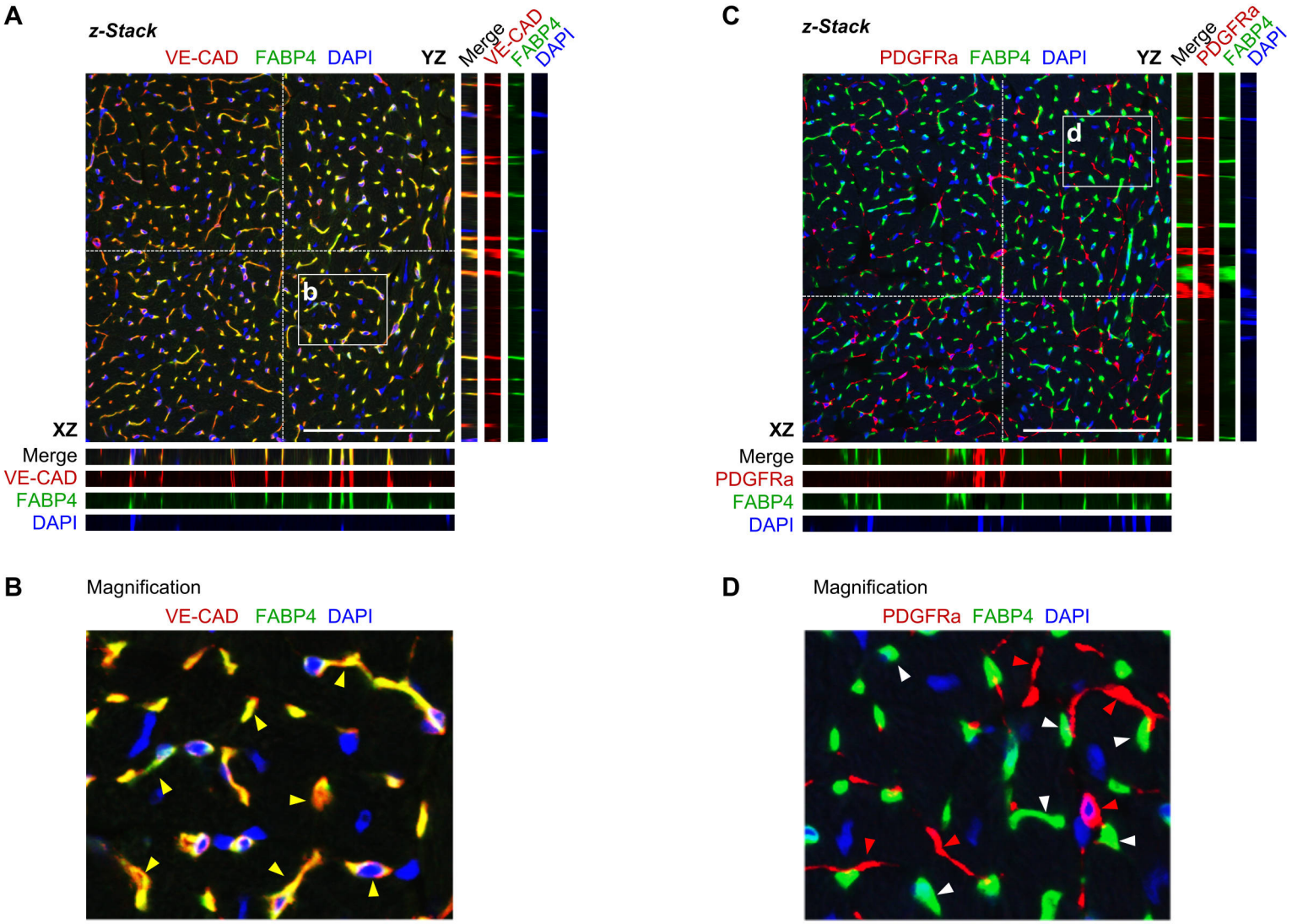
Supplemental Figure 3. PDGFRa-DreER labels fibroblasts but not endothelial cells before injury. (**A-C**) Immunostaining for tdTomato, PDGFRa and PECAM on heart sections. Boxed regions are magnified in B and C. *XZ* and *YZ* indicate signals from dotted lines on *Z*-stack images (B,C). Yellow arrowheads indicate tdTomato$^+$PDGFRa$^+$PECAM$^-$ cells. (**D**) Quantification data shows the percentage of tdTomato$^+$ cells in PDGFRa$^+$ or PECAM$^+$ cell population. (**E-G**) Immunostaining for tdTomato, PDGFRa and VE-CAD on heart sections. Boxed regions are magnified in F and G. *XZ* and *YZ* indicate signals from dotted lines on *Z*-stack images (**F,G**). Yellow arrowheads indicate tdTomato$^+$PDGFRa$^+$VE-CAD$^-$ cells. (**H**) Quantification data shows the percentage of tdTomato$^+$ cells in PDGFRa$^+$ or VE-CAD$^+$ cell population. Scale bars, 100 μm; Each image is a representative of 4 individual samples.



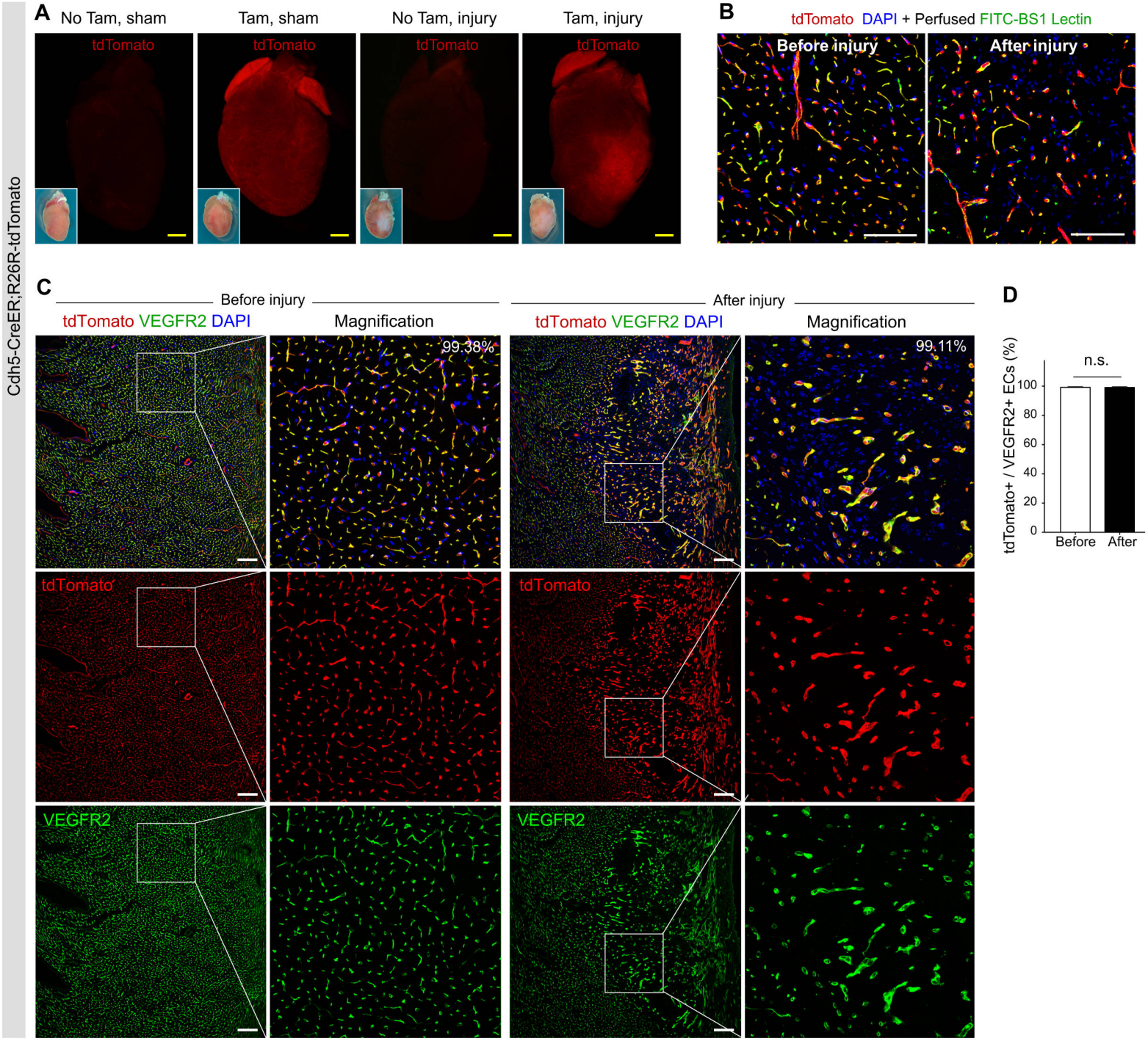
Supplemental Figure 4. Pre-existing Sox9⁺ fibroblasts adopt fibroblast fate but do not form blood vessels in injured heart. (**A**) Immunostaining for tdTomato, VE-CAD and PDGFRa on heart sections before injury. (**B**) Immunostaining for tdTomato, VE-CAD and PDGFRa on heart sections after injury. *XZ* and *YZ* indicate signals from dotted lines on *Z*-stack images. Yellow arrowheads indicate PDGFRa⁺tdTomato⁺ fibroblasts; white arrowheads indicate VE-CAD⁺tdTomato⁻ endothelial cells. (**C,D**) Immunostaining for tdTomato, PECAM and PDGFRa on infarcted (C) and remote (D) regions of heart sections. Yellow arrowheads indicate PDGFRa⁺tdTomato⁺ fibroblasts; white arrowheads indicate PECAM⁺tdTomato⁻ endothelial cells. *XZ* and *YZ* indicate signals from dotted lines on *Z*-stack images. Scale bars, 100 μm; Each image is a representative of 4 individual samples.



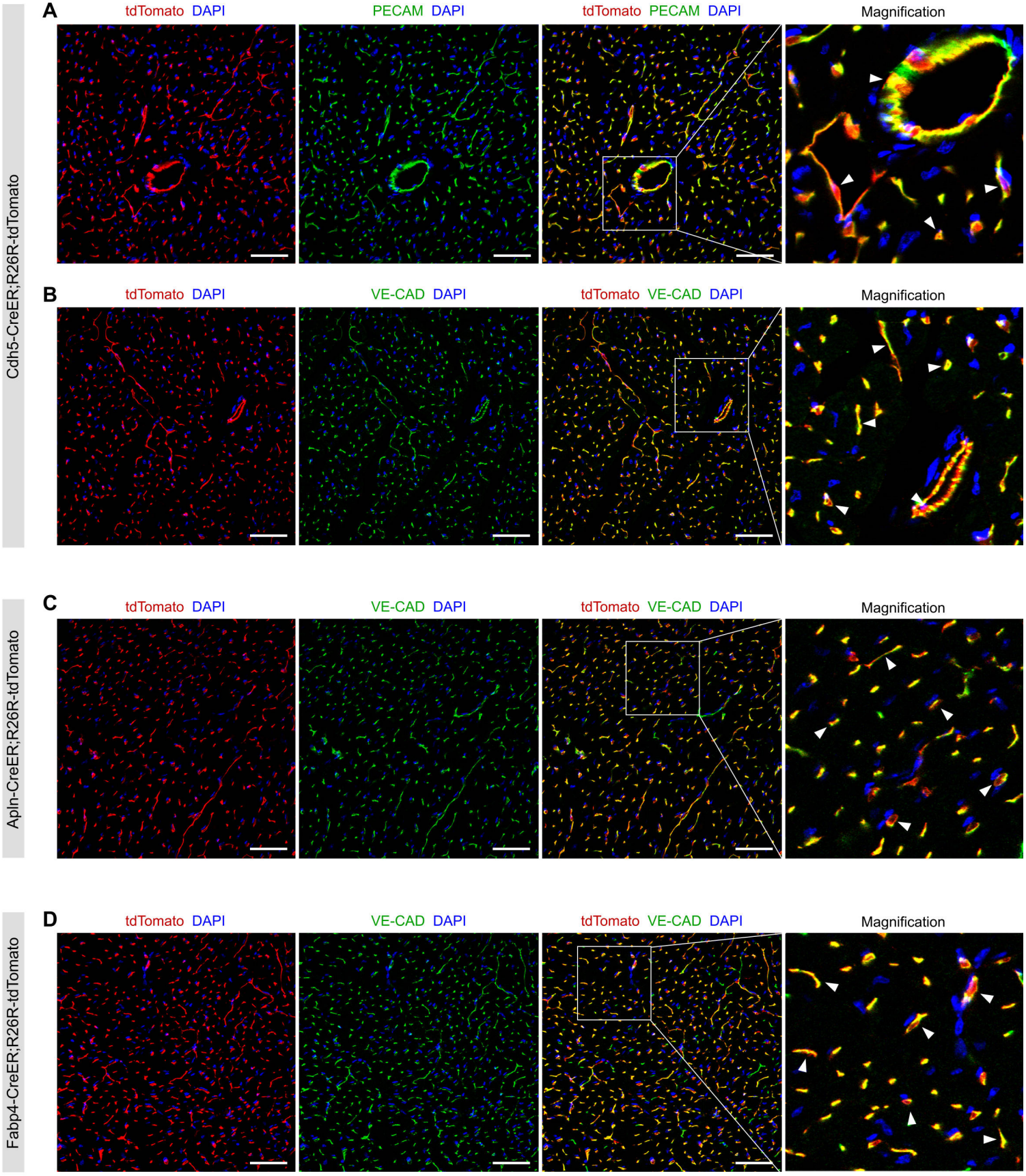
Supplemental Figure 5. Tcf21⁺ and Postn⁺ fibroblasts do not give rise to endothelial cells after cardiac injury. (**A,B**) Immunostaining for GFP and PECAM on heart sections of Tcf21-MerCreMer;R26R-GFP (A) or Postn-MerCreMer;R26R-GFP (B) mice. Samples were collected at 2 weeks after cardiac injury. (**C**) Flow cytometric analysis of GFP⁺ cell percentage in PECAM⁺ cell population of Tcf21-MerCreMer;R26R-GFP or Postn-MerCreMer;R26R-GFP heart after injury. (**D**) Quantification of the percentage of GFP⁺ endothelial cells. Data are mean ± SEM. Dotted line indicates 0.01%. Scale bars, 100 μm.



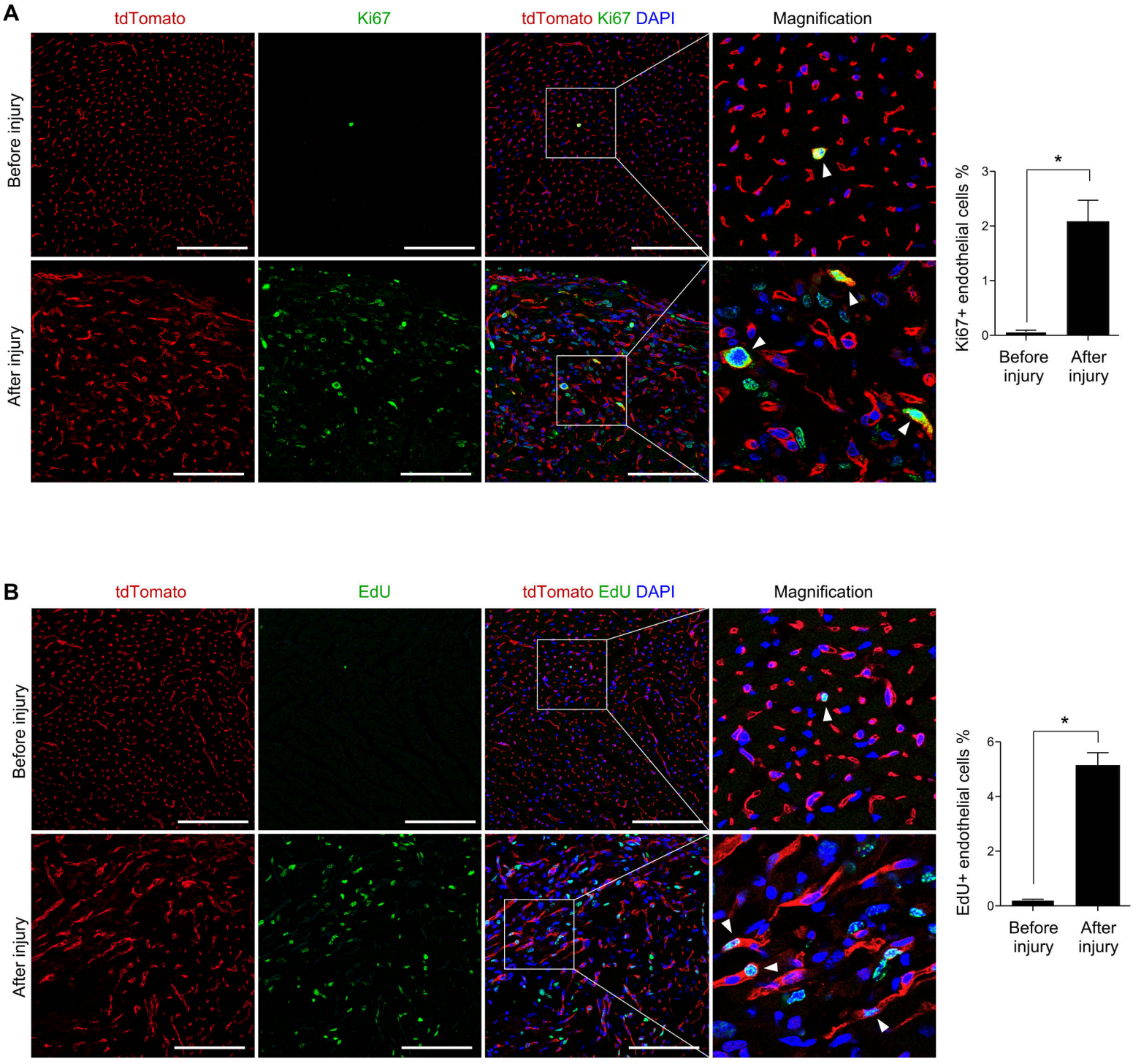
Supplemental Figure 6. FABP4 is expressed in coronary endothelial cells but not in fibroblasts. (**A,C**) Immunostaining for FABP4 with endothelial cell specific marker VE-CAD (A) or fibroblast specific marker PDGFRa (C) on heart sections. XZ and YZ indicate signals from dotted lines on Z-stack confocal images. **(B,D)** Magnified images of boxed regions in A,C. Yellow arrowheads indicate FABP4$^+$VE-CAD$^+$ endothelial cells (B); FABP4$^+$ cells (white arrowheads, D) are close to but not co-localized with PDGFRa$^+$ fibroblasts (red arrowheads, D). Scale bars, 100 μm. Each image is representative of four independent samples.



Supplemental Figure 7. Coronary vessels in the injured heart are derived from preexisting coronary vessels. (**A**) Whole mount fluorescence view of hearts from Cdh5-CreER;R26R-tdTomato mice in sham or after injury. Mice were treated with or without tamoxifen (Tam or No Tam) before operation. (**B**) Immunostaining for tdTomato on heart sections of mice perfused with FITC-labeled BS1 lectin (green) before sacrifice. (**C**) Immunostaining for tdTomato and VEGFR2 on heart sections before and after injury. (**D**) Quantification on the percentage of tdTomato$^+$ endothelial cells in VEGFR2$^+$ endothelial cells before and after injury. n.s., non-significant by 2-tailed Student's *t* test; n = 4. Scale bars, 1 mm in A; 100 μm in B,C. Each figure is representative of four individual samples.



Supplemental Figure 8. Endothelial cells maintain endothelial cell fate in remote regions of injured hearts. (**A**) Immunostaining for PECAM and tdTomato on Cdh5-CreER;R26R-tdTomato heart sections at 2 weeks after injury. (**B-D**) Immunostaining for VE-CAD and tdTomato on Cdh5-CreER;R26R-tdTomato (B), Apln-CreER;R26R-tdTomato (C) and Fabp4-CreER;R26R-tdTomato (D) heart sections. Arrowheads indicate tdTomato$^+$ endothelial cells. Scale bars, 100 μm. Each image is representative of 4 individual heart samples.



Supplemental Figure 9. Pre-labeled endothelial cells proliferate significantly after IR. (**A,B**) Immunostaining for tdTomato and Ki67 or EdU on heart sections of Cdh5-CreER;R26R-tdTomato mice at 3 days after injury. Arrowheads indicate Ki67$^+$tdTomato$^+$ or EdU$^+$tdTomato$^+$ endothelial cells. Quantification of the percentage of Ki67$^+$ or EdU$^+$ endothelial cells is shown on the right panel. Data are mean ± SEM; n = 4; *P < 0.05, by 2-tailed Student's t test. Scale bars, 100 μm.