

JCI | 谱系示踪技术揭示心脏修复再生中新血管形成的机制

2017年6月26日，中国科学院生物化学与细胞生物学研究所周斌研究组在国际学术期刊《Journal of Clinical Investigation》上发表了题为“Preexisting endothelial cells mediate cardiac neovascularization after injury”的论文，揭示了成体心脏修复再生中新血管的来源。

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为了回答成体心脏损伤后新生血管的起源问题，研究人员设计了遗传谱系示踪实验，构建了Pdgfra-DreER、Col1a2-2A-CreER和Sox9-CreER等6组成纤维细胞转基因或基因敲入小鼠模型，通过将上述小鼠分别与报告基因小鼠Rosa26-LSL-tdTomato交配来追踪成纤维细胞和间充质细胞在成体心脏损伤过程中的细胞命运，结果并没有发现tdTomato阳性的血管内皮细胞，说明成体心脏中成纤维细胞并不具有转分化形成血管内皮细胞的能力。

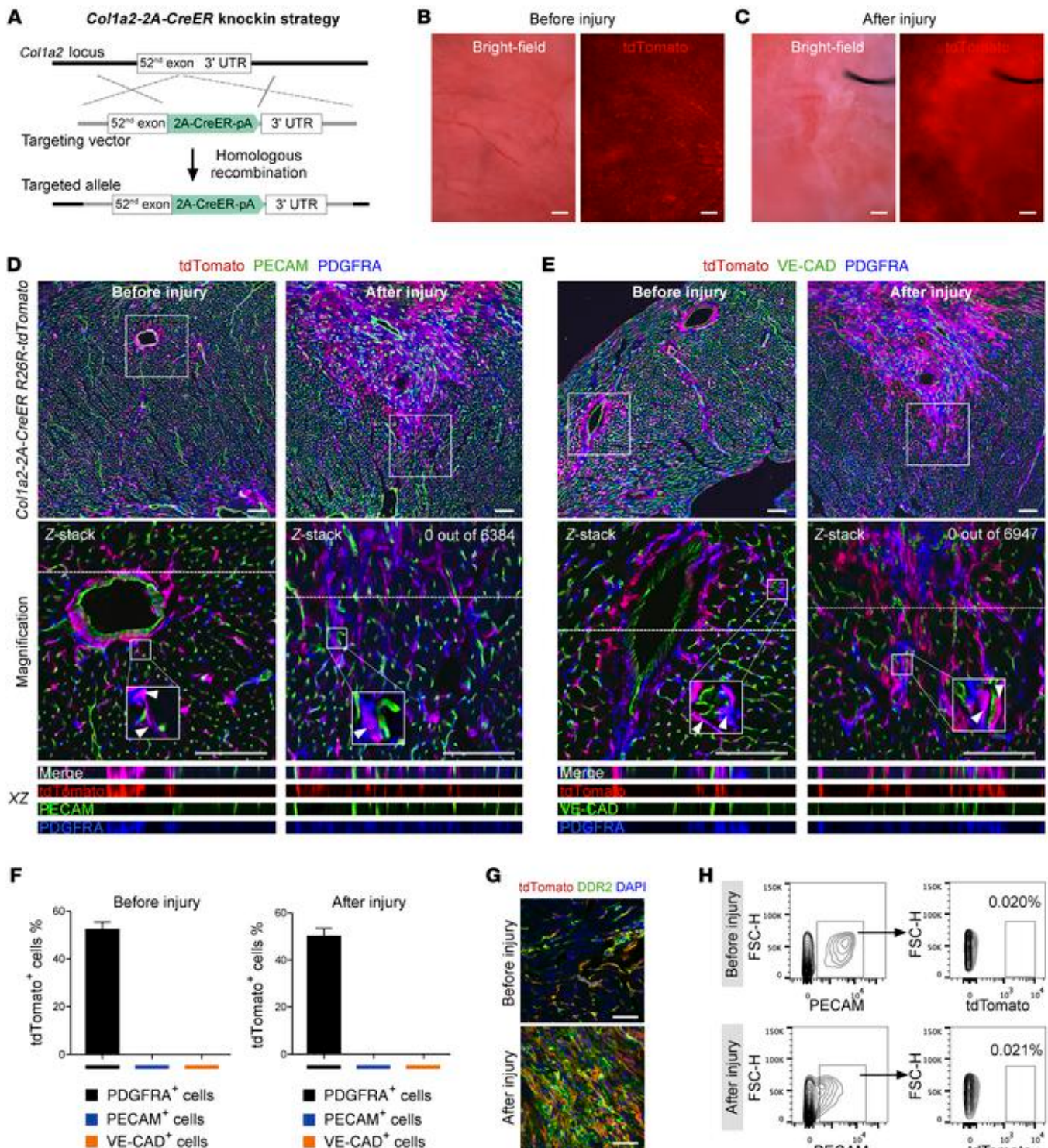


Fig1. COL1A2⁺ fibroblasts do not contribute to coronary endothelial cells after cardiac injury. (A) Schematic figure showing strategy for generation of Col1a2-2A-CreER allele. (B and C) Whole-mount fluorescence images showing tdTomato in hearts before or after injury. (D and E) Immunostaining for tdTomato, PDGFRA, and PECAM (D) or VE-CAD (E) on sections of injured heart. Boxed regions are magnified in bottom panels. Arrowheads point to tdTomato⁺PDGFRA⁺PECAM⁻ (D) or tdTomato⁺PDGFRA⁺VE-CAD⁻ cells (E). XZ indicates signals from dotted line in Z-stack images. (F) Quantification of the percentage of tdTomato⁺ cells in different lineages (PDGFRA⁺, PECAM⁺, or VE-CAD⁺ cell populations). Data are represented as mean $\pm$ SEM. n = 4. (G) Immunostaining for tdTomato and DDR2 on heart sections before or after

injury. (H) Flow cytometric analysis of the percentage of tdTomato+ endothelial cells. FSC-H, forward scatter-height. Scale bars: 200 μ m (B, C); 100 μ m (D, E, G).

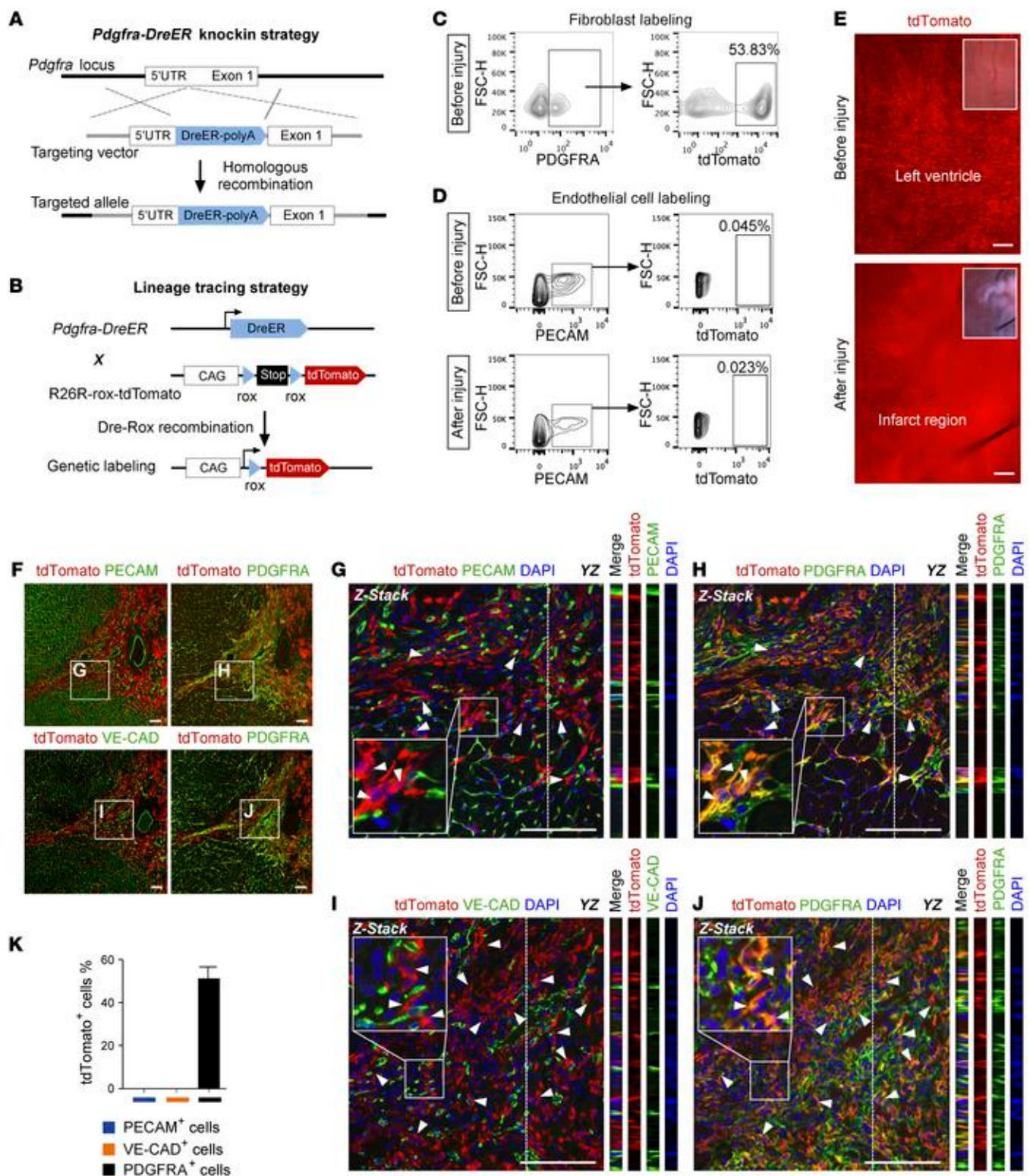


Fig2. PDGFRA+ fibroblasts do not contribute to coronary endothelial cells after cardiac injury. (A) Schematic figure showing strategy for generation of Pdgrfra-DreER allele. (B) Schematic figure showing genetic-lineage-tracing strategy for PDGFRA+ cells by Dre-rox recombination. (C and D) Flow cytometric analysis of the percentage of tdTomato+ fibroblasts (C)

or tdTomato+ endothelial cells (D). (E) Whole-mount fluorescence image showing tdTomato in hearts before and after injury. (F-J) Immunostaining for tdTomato, PDGFRA, PECAM, or VE-CAD on sections of injured heart. Boxed regions in F are magnified in G-J. Arrowheads point to tdTomato+PDGFRA+PECAM- (G, H) or tdTomato+PDGFRA+VE-CAD- cells (I and J). YZ indicates signals from dotted line in Z-stack images in G-J. (K) Quantification of the percentage of tdTomato+ cells in different lineages (PECAM+, VE-CAD+, or PDGFRA+ cell populations). Data are represented as mean $\pm$ SEM. n = 4. Scale bars: 200 μ m (E); 100 μ m in (F-J).

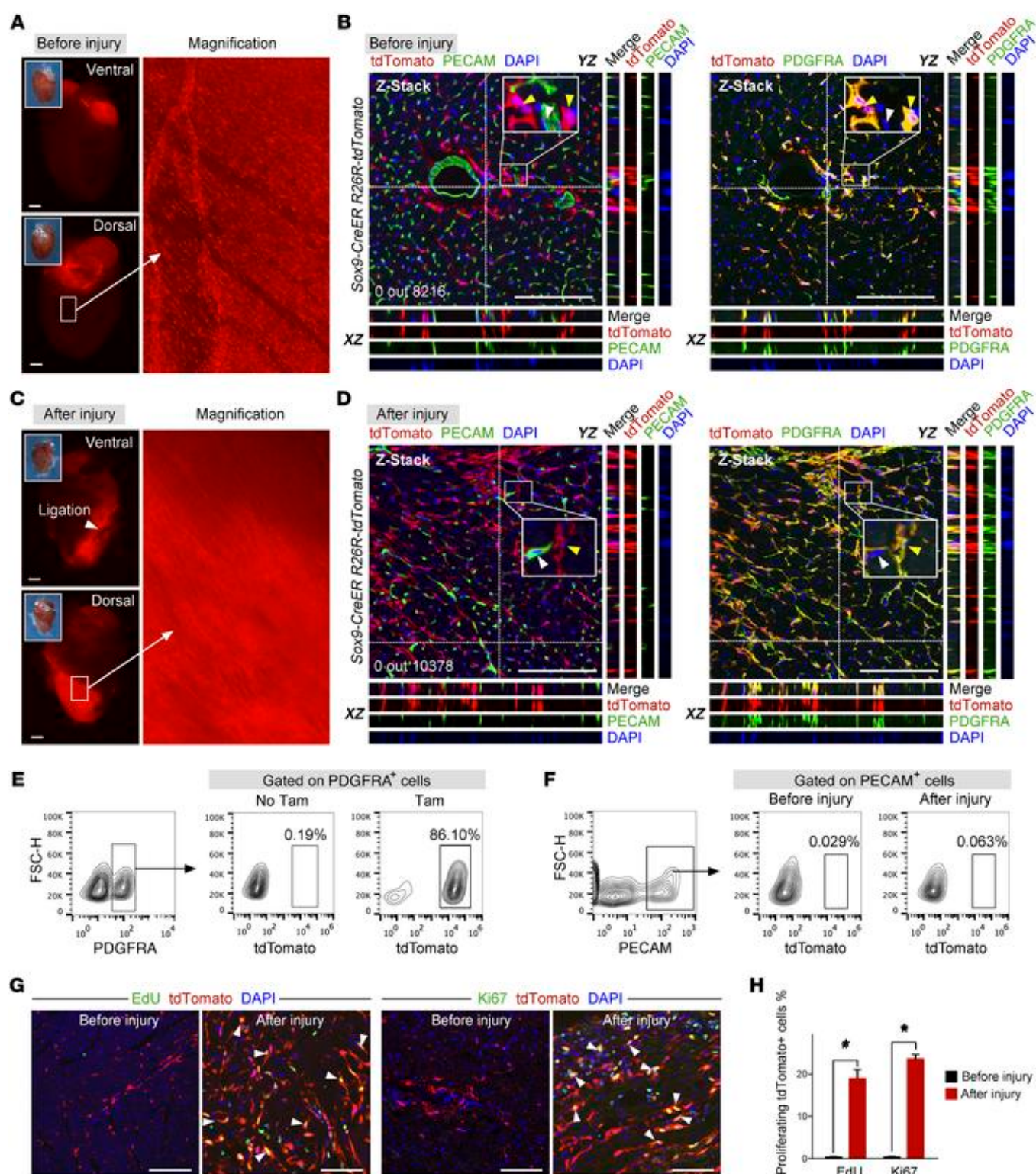


Fig3. Cardiac fibroblasts expand after injury without giving rise to endothelial cells. (A and C) Whole-mount fluorescence view of Sox9-CreER R26R-tdTomato hearts before (A) and after injury (C). Inserts indicate bright-field view of hearts. (B and D) Immunostaining for tdTomato, PDGFRA, and PECAM on heart sections before (B) and after injury (D). XZ and YZ indicate signals from dotted lines on Z-stack images. Yellow arrowheads indicate PDGFRA⁺tdTomato⁺ fibroblasts; white arrowheads indicate PECAM⁺tdTomato⁻ endothelial cells. (E) Flow cytometric analysis of percentage of tdTomato⁺ cells in PDGFRA⁺ cell population. (F) Flow cytometric analysis of tdTomato⁺ cells in PECAM⁺ endothelial cells from heart before or after injury. (G) Immunostaining

for tdTomato and EdU or Ki67 on heart sections before or 3 days after injury. Arrowheads indicate proliferating tdTomato+ cells. (H) Quantification of percentage of proliferating tdTomato+ cells. Data are represented as mean $\pm$ SEM. $n = 4$. * $P < 0.05$, 2-tailed Student's t test. Scale bars: 1 mm (A, C); 100 μ m (B, D, G).

为了进一步研究是否有除了血管内皮细胞以外的所有其它类型细胞能够在成体心脏损伤后形成血管内皮细胞，研究人员又建立了3种血管内皮细胞的遗传谱系示踪实验，通过将血管内皮细胞特异性表达CreER的小鼠（*Cdh5*-CreER、*Apln*-CreER、*Fabp4*-CreER）与报告基因小鼠*Rosa26*-LSL-tdTomato交配，在损伤前标记上大部分血管内皮细胞，然后观察损伤后血管内皮细胞的标记百分比是否降低，如果标记百分比没有发生变化，说明新生的血管内皮细胞来源于原来已存在的血管内皮细胞的自我增殖过程，如果标记的百分比降低了，则说明存在其它类型细胞转分化成为血管内皮细胞。

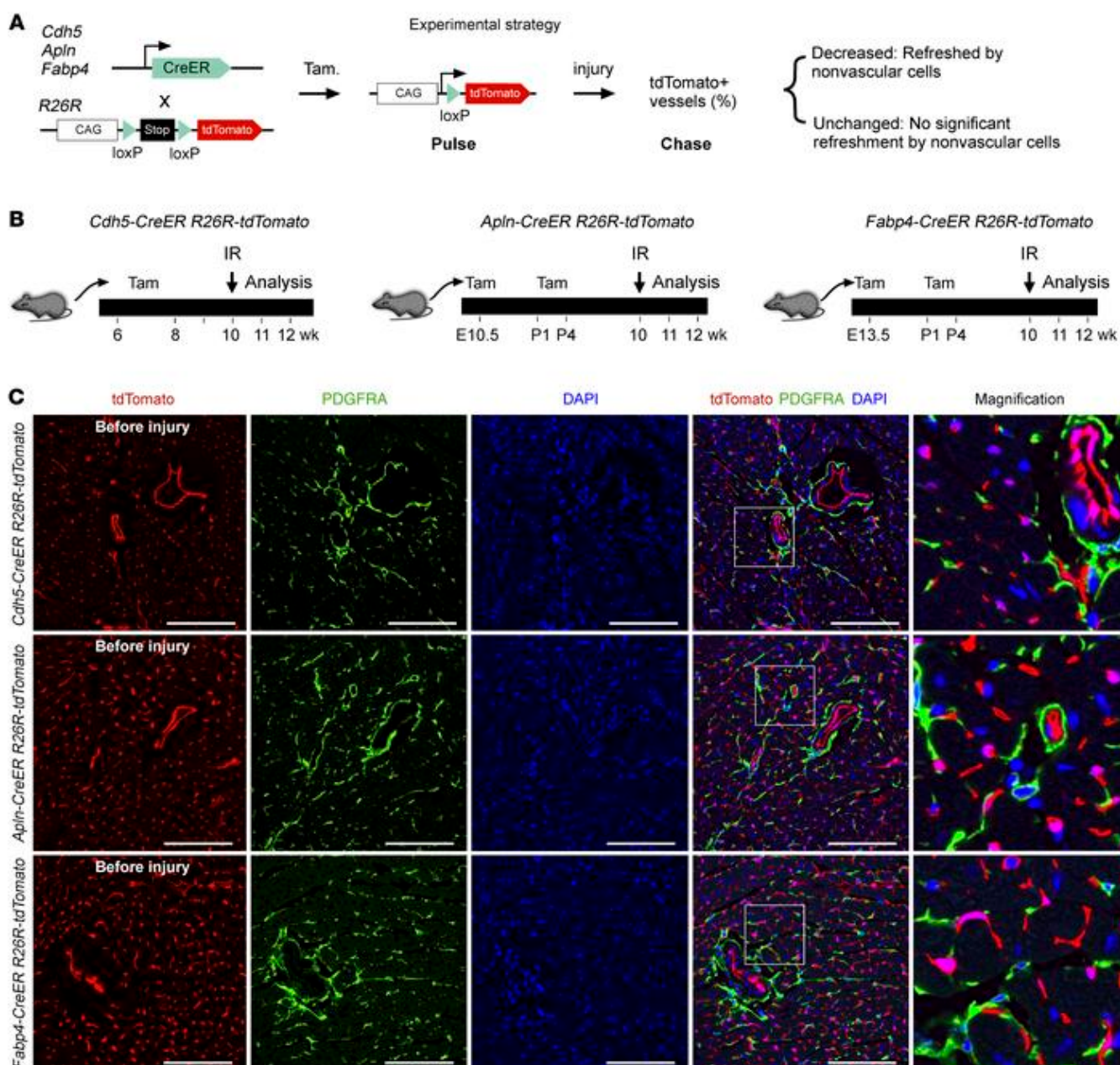


Fig4. Establishment of pulse-chase strategy for labeling of coronary endothelial cells. (A) Schematic figure showing the pulse-chase experimental strategy for measuring refreshment of nonvascular cells. (B) Experimental design for tamoxifen induction, myocardial IR, and tissue analysis. wk, postnatal weeks. (C) Immunostaining for tdTomato and PDGFRA on tissue sections shows that these Cre lines do not label fibroblasts before injury. Each image is representative of 4 individual samples. Scale bars: 100 μ m.

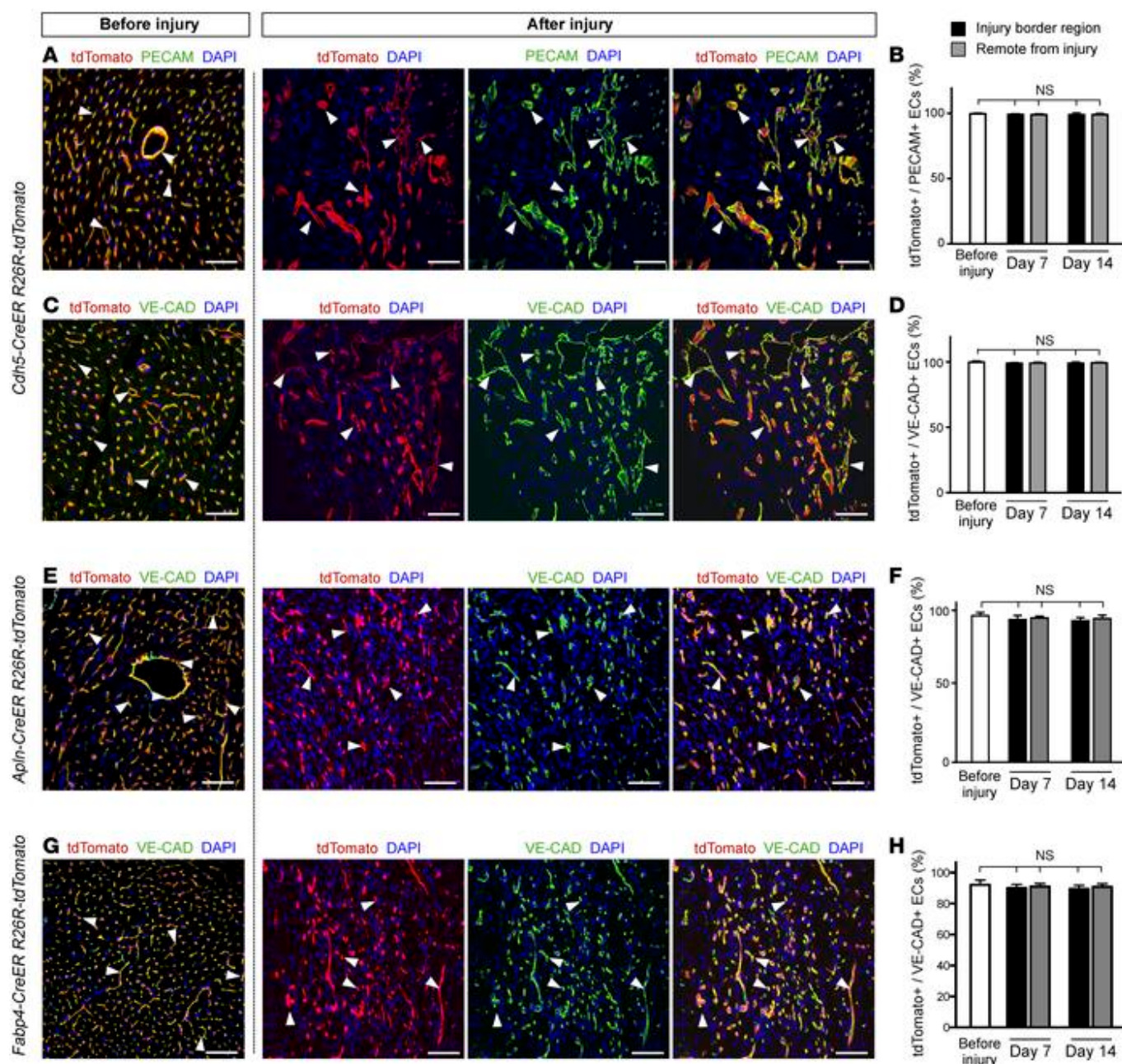


Fig5. Coronary vessels in the injured heart are derived from preexisting coronary vessels. (A, C, E, G) Immunostaining for tdTomato and PECAM or VE-CAD on heart sections before and after injury. Arrowheads indicate labeled endothelial cells (tdTomato+PECAM+ or tdTomato+VE-CAD+). (B, D, F, H) Quantification of the percentage of tdTomato+ endothelial cells (ECs) in PECAM+ or VE-CAD+ endothelial cells in hearts before injury and 7 or 14 days after injury. Data are represented

as mean $\pm$ SEM. n = 4. ANOVA test was used. Scale bars: 50 μ m.

结果显示，损伤后，血管内皮细胞的标记百分比没有发生变化，即没有发生其他类型的细胞向血管内皮细胞的转分化过程。上述实验结果说明，在成体心脏损伤过程中，新生血管主要来自于血管内皮细胞的自我复制增殖过程。

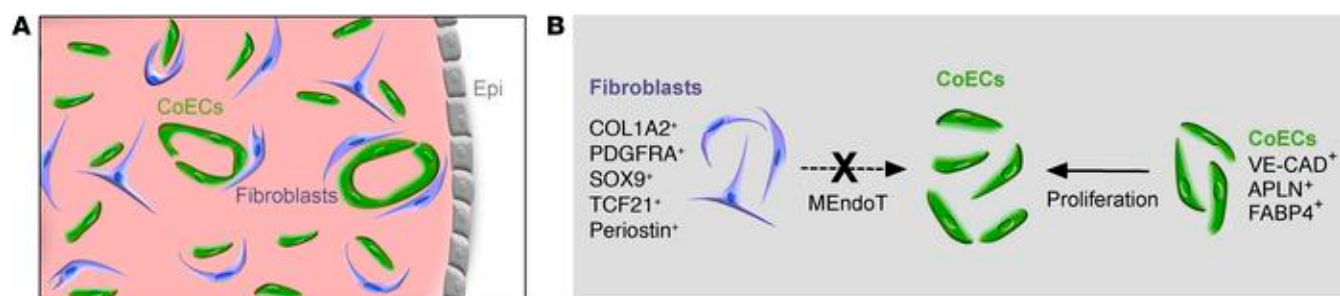


Fig6. **Preexisting coronary endothelial cells but not fibroblasts contribute to blood vessels after injury.** (A) Image showing coronary endothelial cells (CoECs) and fibroblasts in the adult heart. Epi, epicardium. (B) Image showing contribution of different lineages to coronary endothelial cells after injury.

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