Circulation Research | 谱系示踪技术揭示胚胎期冠状动脉的起源

国际知名学术期刊《Circulation Research》在线发表了中国科学院上海生命科学研究院营养科学研究所周斌研究组的最新研究成果“Endocardium minimally contributes to coronary endothelium in the embryonic ventricular free walls”。该研究利用遗传谱系示踪技术揭示了心血管研究领域内长期存在的争论性问题，为研究冠状血管的发生发育与再生治疗提供了理论基础。

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心血管领域关于胚胎期冠状血管的起源一直存在争论，静脉窦和心室心内膜是最主要也是最具争议的两个起源。周斌组通过利用传统的心内膜标记基因Nfatc1构建了Nfatc1-Cre等工具小鼠，并对心室心内膜进行了谱系示踪实验。研究发现，虽然Nfatc1-Cre可以标记上大量冠状血管，但Nfatc1基因并不仅仅表达在心室心内膜，还表达在胚胎早期的静脉窦内皮细胞中。由此，研究人员对冠状血管的心室心内膜起源提出了质疑。

为能够对心室心内膜实现特异性标记，研究人员首先利用单细胞实时定量PCR、原位杂交实验等技术，发现并鉴定了特异性表达在心室心内膜的基因Npr3。

通过构建Nfatc1-Cre、 Nfatc1-Dre、 Nfatc1-GFP、 Npr3-CreER和Npr3-GFP多种基因敲入工具小鼠，对心室心内膜开展谱系示踪实验发现，心室心内膜很少贡献到胚胎期冠状血管。通过多种工具小鼠实验，进一步证实静脉窦内皮细胞很可能是胚胎期冠状血管的主要来源。

心内膜细胞和冠状血管内皮细胞虽都是内皮细胞，但两者的基因表达存在很大差异。在该课题中，研究人员还利用多种工具小鼠，分离出了胚胎期心内膜细胞和冠状血管内皮细胞，通过RNA-sequencing实验发现并鉴定出了一系列特异性表达在心内膜细胞或冠状血管内皮细胞上的基因，这对心血管领域内的后续研究具有重要意义。

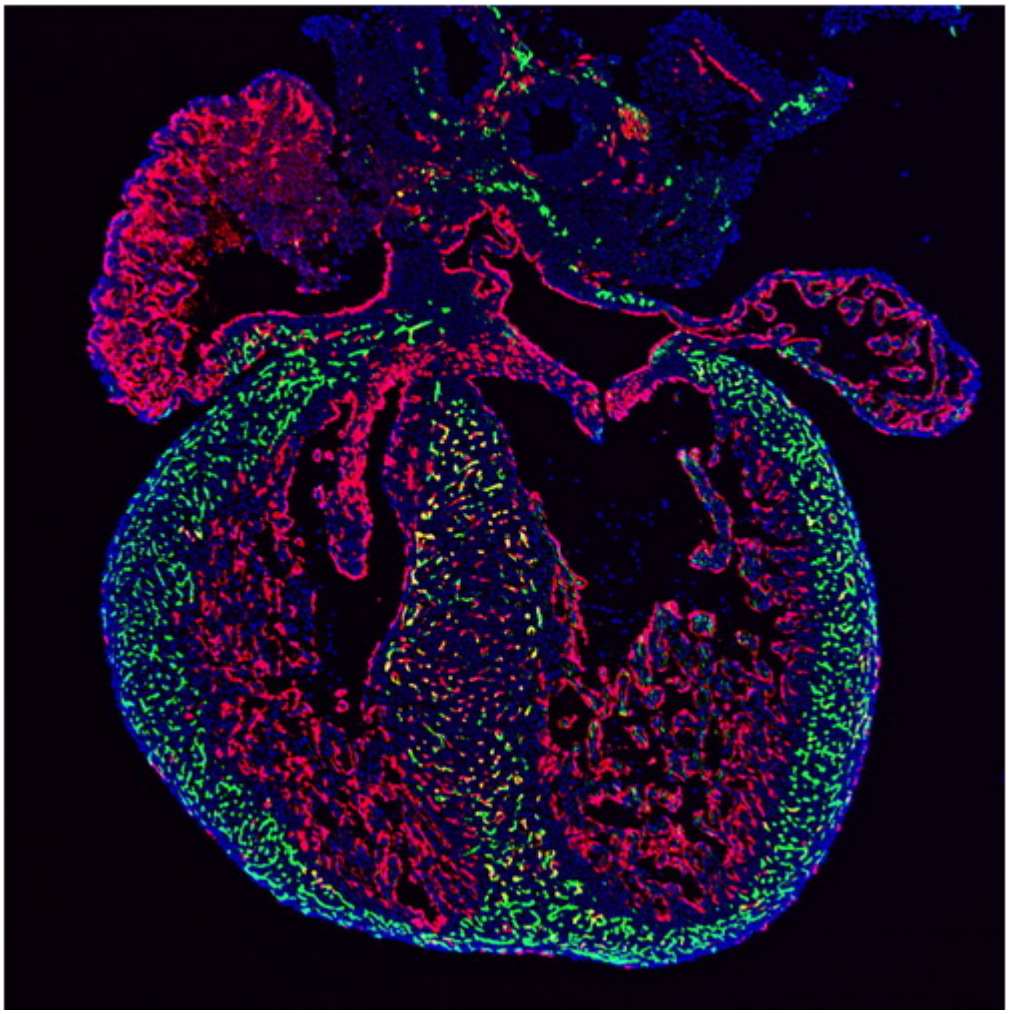

Fig1. 心内膜来源的细胞（红色）很少形成胚胎期心脏壁的冠状血管（绿色）。蓝色为细胞核。

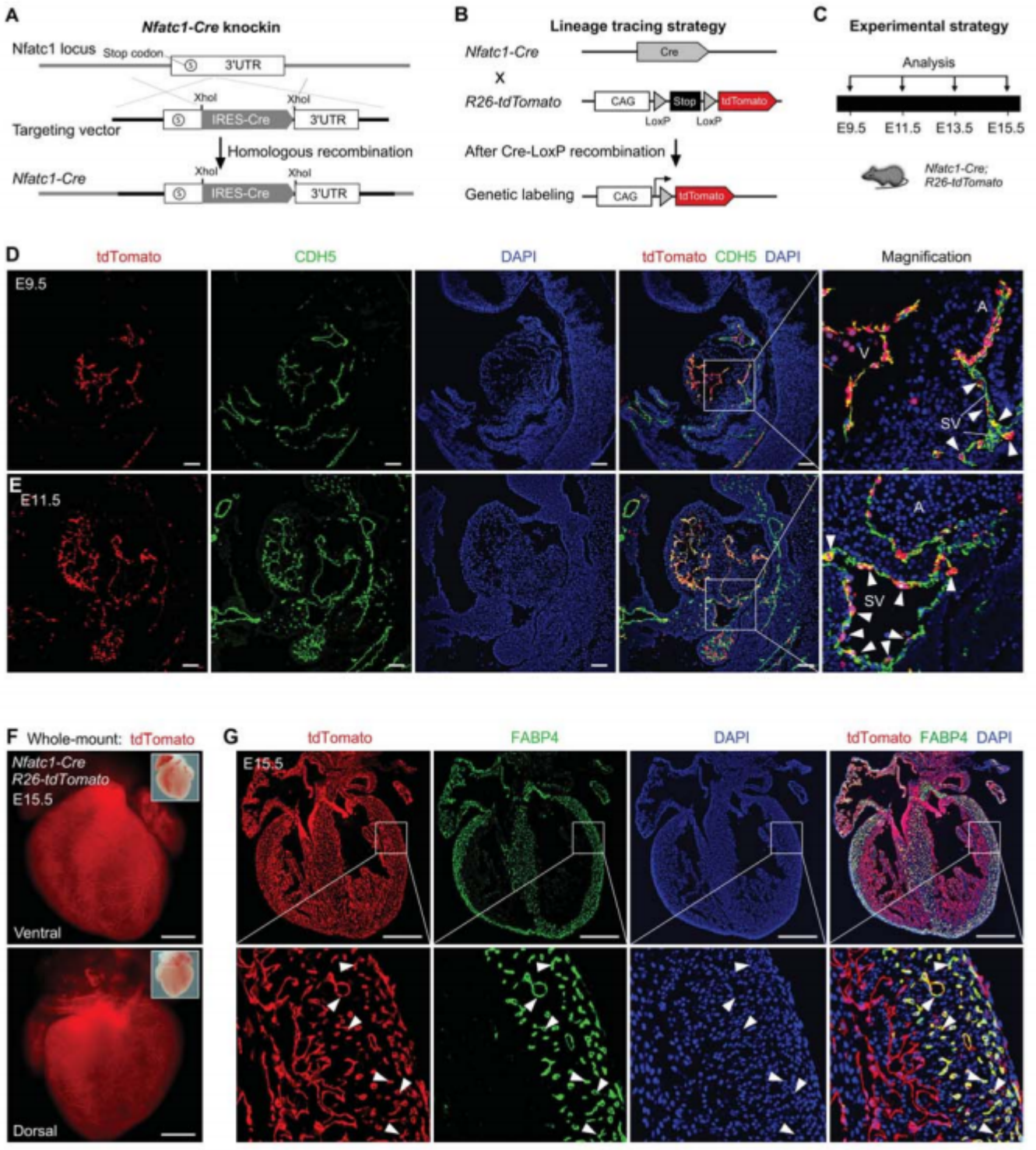

Fig2. **Nfatc1+ cells contribute to both SV and ventricular endocardium.** A-C, Schematic showing the knockin strategy of Nfatc1-Cre allele, lineage tracing principle and experimental procedures. D,E, Immunostaining for tdTomato and CDH5 on sections of E9.5 and E11.5 Nfatc1-Cre;R26-tdTomato embryos showed Nfatc1+ cells contribute to endothelial cells in SV (tdTomato+ CDH5+ , arrowheads), and in ventricle (V) and atrium (A). F, Whole-mount fluorescence view of E15.5 embryos showed labeling of coronary vessels. G, Immunostaining for tdTomato and coronary vascular endothelial cell marker FABP4 on heart sections of Nfatc1-Cre;R26-tdTomato embryo showed Nfatc1+ cells contribute to the majority of coronary vascular endothelial cells (arrowheads) in the compact myocardium. A, atrium, V, ventricle; SV, sinus venosus. Scale bars,

100 μm in D,E; 500 μm in F,G.

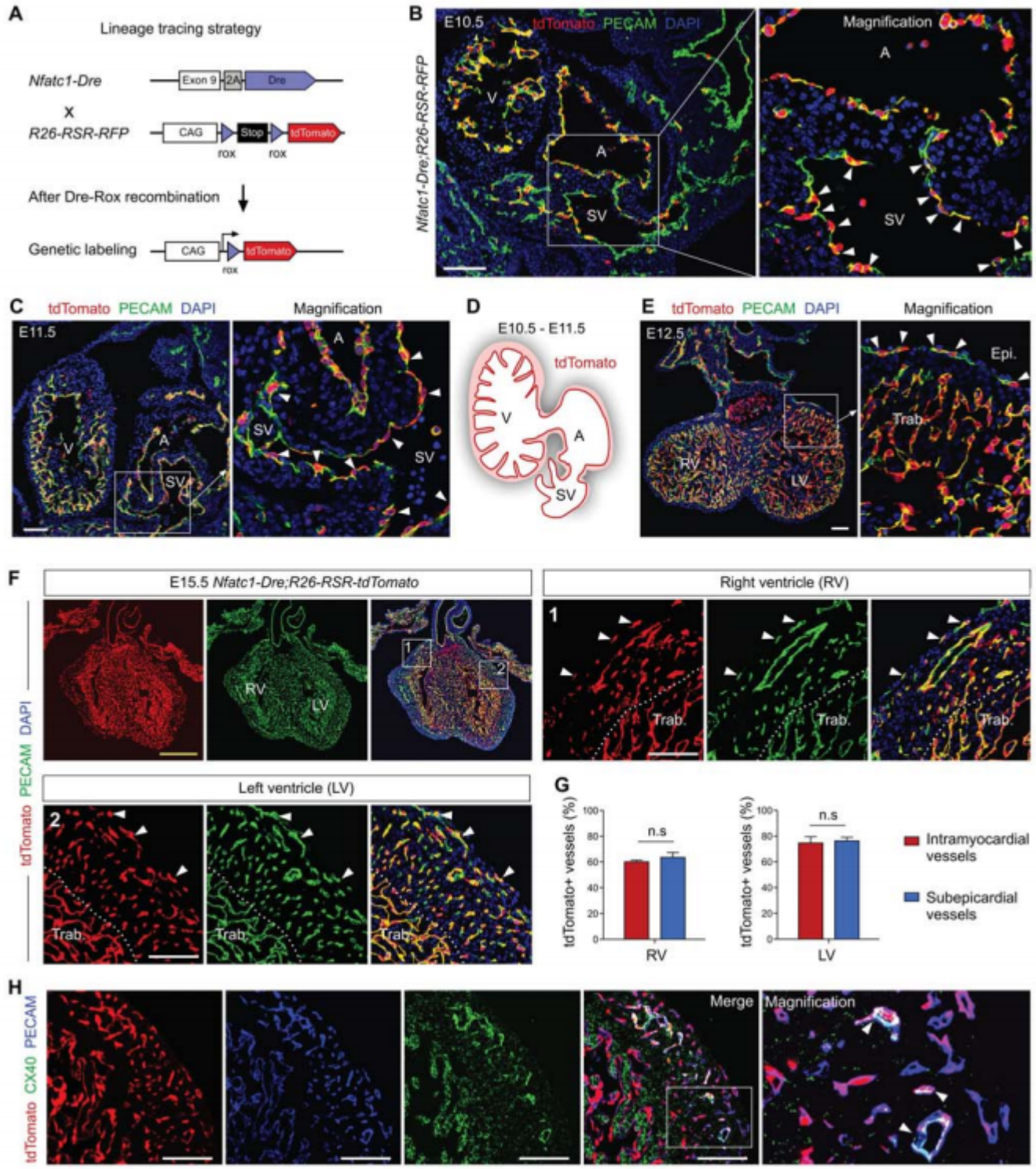

Fig3. **Nfatc1+ cells contribute significantly to both SV and subepicardial vessels.** A, Schematic showing the strategy for lineage tracing of Nfatc1+ cells by Dre-Rox system. B,C, Immunostaining for tdTomato and PECAM on Nfatc1-Dre;R26-RSR-tdTomato embryonic sections. Boxed region is magnification of SV. Nfatc1+ cells contribute to a substantial number of SV endothelial cells (arrowheads). D, Cartoon figure showing atrium, ventricle and sinus venosus are labeled by Nfatc1-Dre. E, Immunostaining for tdTomato and PECAM on E12.5 heart section shows that

Nfatc1-Dre labels subepicardial vessels (arrowheads). F. Immunostaining for tdTomato and PECAM on E15.5 heart sections shows Nfatc1+ cells contribute to subepicardial vessels (arrowheads) and intramyocardial vessels. Dotted line indicates the border between compact myocardium and trabecular myocardium (Trab.). G, Quantification of the percentage of tdTomato+ coronary vessels shows there is no significance (n.s.) in percentage between intramyocardial vessels and subepicardial vessels. The percentage of tdTomato+ cells in coronary vessels was averaged from four E15.5 hearts. H, Immunostaining for tdTomato, PECAM with arterial marker CX40 shows Nfatc1-derived cells contribute to arterial endothelial cells (arrowheads). A, atrium; V, ventricle; SV, sinus venosus; RV, right ventricle; LV, left ventricle. Scale bars, yellow 500 μm; white 100 μm.

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