SUPPLEMENTAL DATA

Figure S1. SLN-Cre lineage tracing

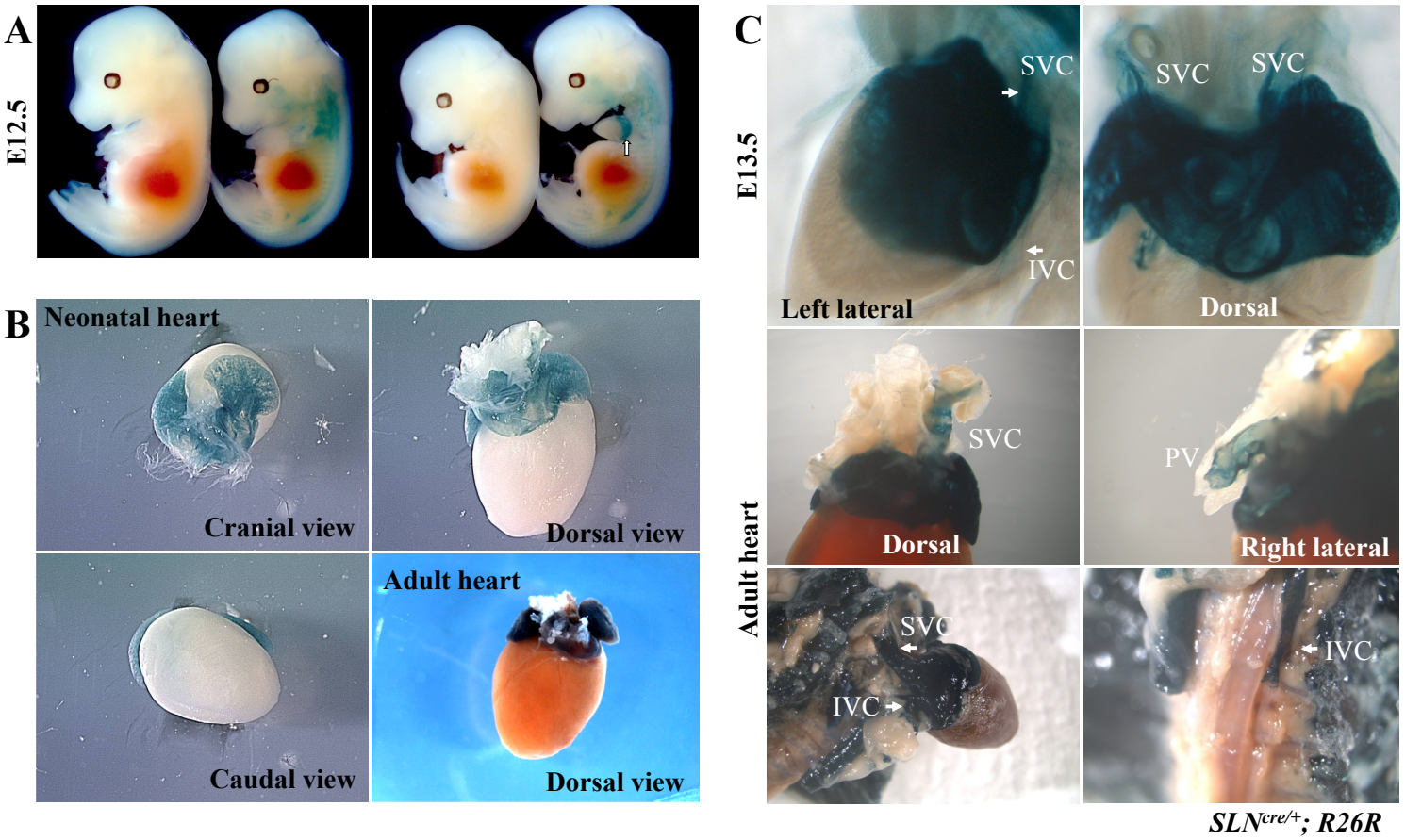


Figure S2. SA nodal cells are labeled by SLN-Cre

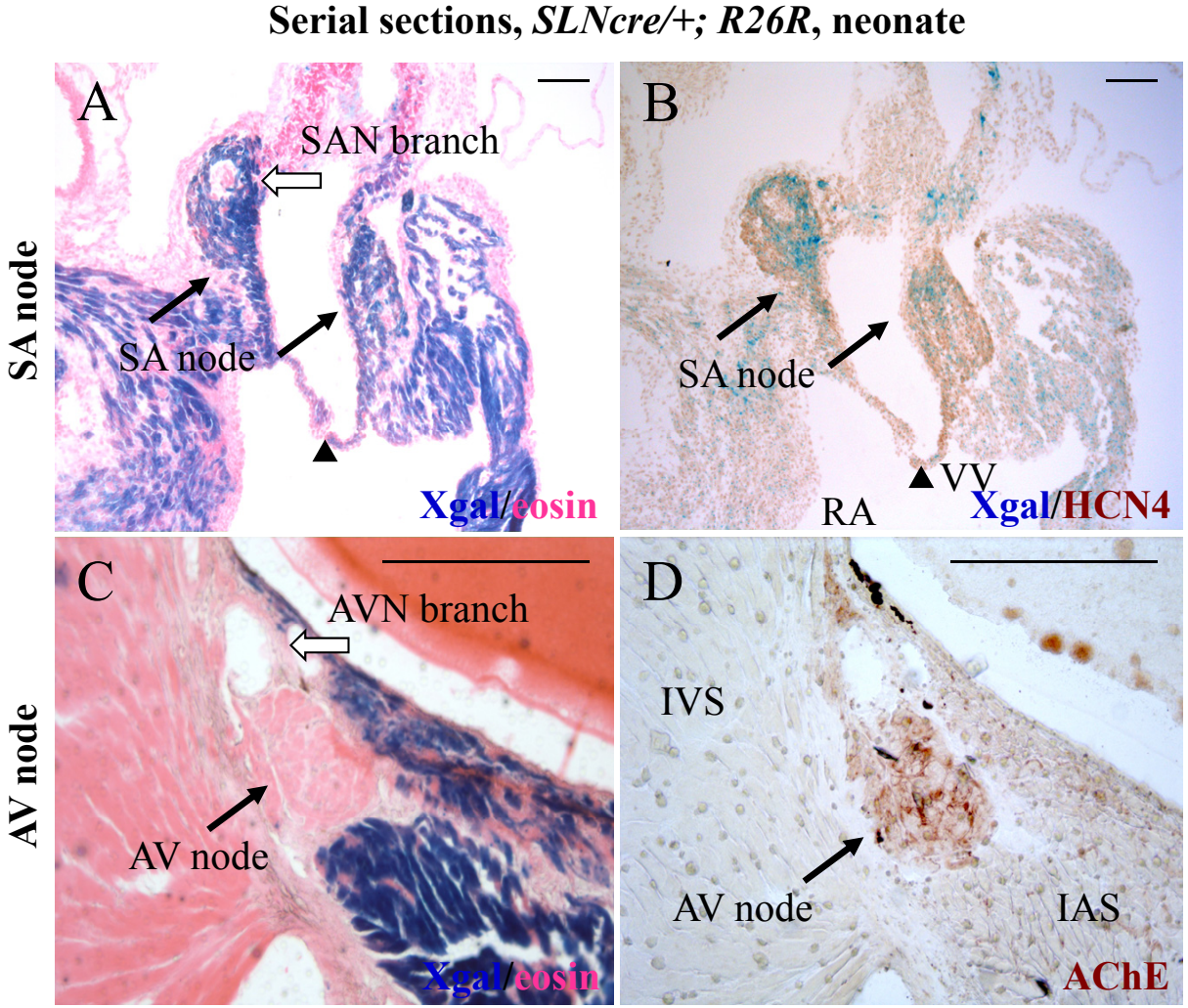
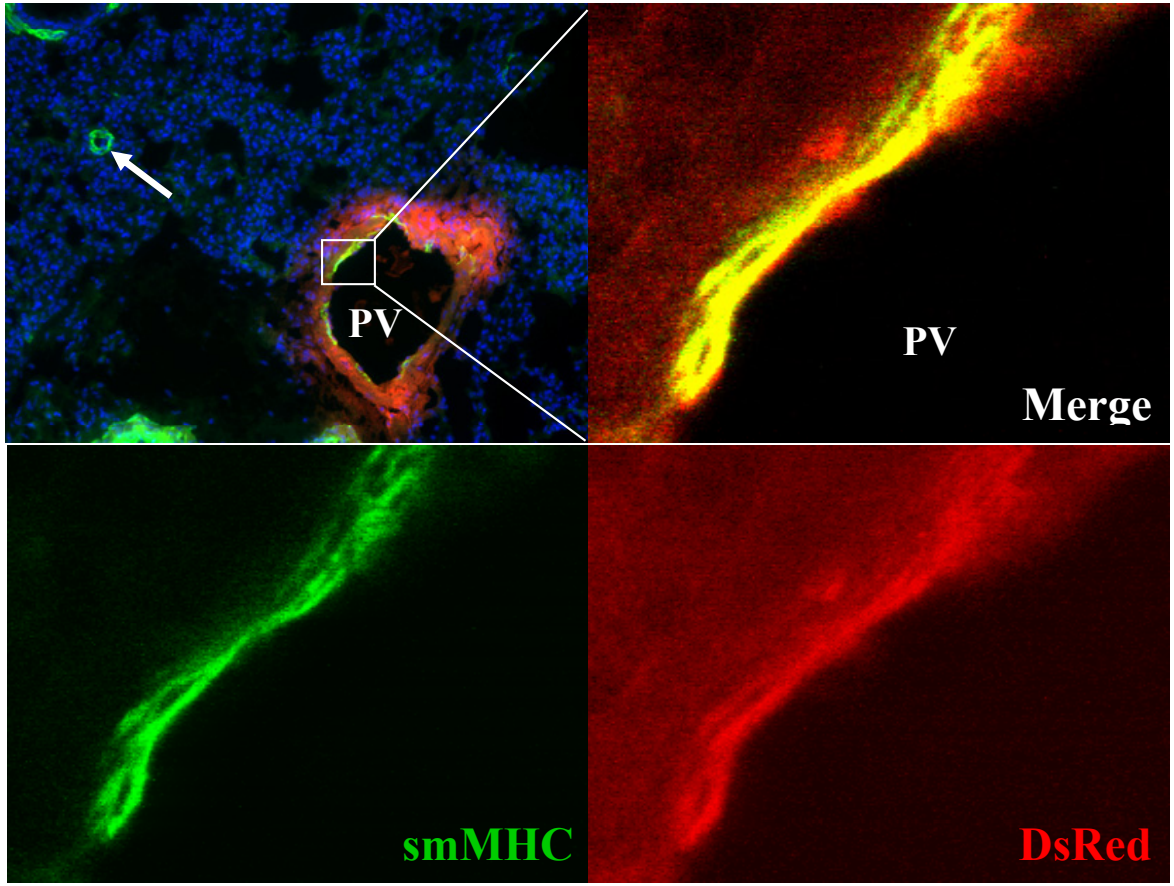


Figure S3. SLN-positive cells give rise to smooth muscle cells in pulmonary veins.



Supplemental Table 1. Summary of atrial-specific SLN-cre lineage tracing analysis

Working Myocyte	
Atrial myocytes	95-100%
Ventricular myocytes	-
Conduction System	
Sinoatrial node	95-100%
Atrioventricular node	-
Purkinje fiber	-
Endocardium	-
Epicardium	-
Cardiac ganglia	-
Blood vessels	
Endothelium	-
Smooth muscle	
Aorta	-
Pulmonary trunk	-
Large Coronary	-
Superior vena cava	5~10%
Inferior vena cava	5~10%
Pulmonary vein	5~10%
Valves	
Mitral valve	-
Tricuspid valve	-
Aortic valve	-
Pulmonary valve	-
Venous Valves between RA and vena cava	80-90%

FO, foramen ovale; IAS, intraatrial septum; IVC, inferior vena cava; IVS, intraventricular septum; LA, left atrium; LV, left ventricle; PV, pulmonary vein; RA, right atria; RV, right ventricle; SVC, superior vena cava; VV, venous valves.

Supplemental Table 2. Quantification of atrial progenitor colonies

	E9.5	E12.5	E15.5
Isl1 expression (positive/total)	**90.3% (65/72)**	**57.9% (44/76)**	**39.7% (23/58)**
SMC differentiation (positive/total)	**51.3% (20/39)**	**19.6% (14/51)**	**17.9% (10/39)**

Atrial progenitor colonies derived from E9.5, 12.5 and 15.5 *SLNcre/+*x *R26R* embryos were scored for the number of Isl1 positive blue colonies per total blue colonies and smMHC-positive bgal-labeled colonies per total blue colonies. Note that Isl1 is expressed in the atrial cell colonies derived from E15.5 atria where Isl1 is already downregulated *in vivo*.

Figure legend for supplementary information

Fig. S1. SLN-Cre lineage tracing.

Whole embryo and tissues from *SLN*$^{cre/+}$*; R26R* mice are stained by Xgal.

A. E12.5 embryo. The forelimbs are removed in the right panel to show the atrial specific staining (white arrow).

B. Neonatal and adult hearts showing atrial specific staining.

C. Inflow tract of embryonic and adult hearts. Note that superior and inferior vena cavae and pulmonary vein are stained in blue. The Xgal staining tapers off.

IVC, inferior vena cava; PV, pulmonary vein; SVC, superior vena cava

Fig. S2. SA nodal cells are labeled by SLN-Cre

Hearts from *SLN*$^{cre/+}$*; R26R* neonates are stained for Xgal and markers for conduction system.

A, B. Serial sections at the junction between right atrium and vena cava. Blue staining is found in working atrium, venous valves, and SA node (black arrows) surrounding SA nodal branch (white arrow). SA node is stained with HCN4 on the adjacent section (**B**).

C, D. Serial sections at the level of AV node. Atrial septum is stained by Xgal (**C**). AV node (black arrows) and AV nodal branch (white arrow) are not stained. AV node is stained by AChE on the adjacent section (**D**).

AV, atrioventricular; AVN atrioventricular node; IAS, intraatrial septum; IVS, intraventricular septum; SA, sinoatrial; SAN, sino-atrial node; VV, venous valve.

Fig. S3. SLN-positive cells give rise to smooth muscle cells in pulmonary veins

Hearts from *SLN*$^{cre/+}$*; CAG-DsRed reporter* neonates are stained with anti-smMHC and anti-DsRed antibodies. smMHC-positive cells (green) are co-labeled with DsRed (red). White arrow indicates a small pulmonary vessel that is not labeled with DsRed.