Supplementary Data

Supplementary Material and Methods

The different markers used in this study are listed in Supplementary Table S1.

Immunofluorescence

Cells in culture or optimal cutting temperature-embedded sections were fixed for 10 min with 2% paraformaldehyde in phosphate-buffered saline (PBS) and then permeabilized 10 min with 0.2% Triton X-100 in PBS. Prior to adding antibodies, the cells were incubated 1 h in buffer containing 2.5% bovine serum albumin (Serva Electrophoresis GmbH). The following antibodies were used in this study: Isl1 (mouse monoclonal antibody; Developmental Studies Hybridoma Bank; 1:25), human Nkx2.5 (goat polyclonal; R&D Systems; 1:100), VEGF-R2/KDR (mouse monoclonal; Abcam; 1:100), sarcomeric α-actinin (mouse monoclonal; Sigma; 1:600), smooth muscle myosin heavy chain (rabbit polyclonal; Biomedical Technologies, Inc.; 1:100), alpha smooth muscle actin (rabbit polyclonal; Abcam; 1:100), human CD31 (mouse monoclonal; BD Pharmingen; 1:200), connexin-43 (mouse monoclonal; Millipore; 1:500), and GFP (rabbit polyclonal; Molecular Probes; 1:200). Alexa Fluor 488, Alexa Fluor 594, or Alexa Fluor 647-conjugated secondary antibodies specific to the appropriate species were used (Molecular Probes; 1:200). Nuclei were stained with DAPI (Molecular Probes). An Axiovision fluorescence microscope (Carl Zeiss), a Nikon LSR Eclipse TE2000S inverted fluorescence microscope, and a Zeiss LSM 710 Quasar Confocal Microscope were used in this study. For confocal microscopy of heart sections, nonspecific autofluorescence was eliminated using a window of detection corresponding to the GFP-specific fluorescence emission spectra excluding the autofluorescence emission spectra (513–523 nm as previously described[30]) and an equivalent thickness of 0.5 μm.

RNA extraction, reverse transcriptase–polymerase chain reaction, and real-time polymerase chain reaction analysis

Cells were washed in PBS and lysed, and total RNA was purified from each sample using Nucleospin RNA II Kit (Macherey Nagel) following the manufacturer's instructions. Total RNA was subjected to reverse transcription using oligodT (microsynth) and Moloney murine leukemia virus reverse transcriptase (Invitrogen). The resulting cDNA was used as a template for reverse transcriptase–polymerase chain reaction (PCR) (Supplementary Table S2) or real-time PCR using specific probes provided by the manufacturer (Applied Biosystems) and the ABI Prism 7500

Supplementary Table S1. Markers Used in This Study

Abbreviation	*Name (h: human; m: mouse)*	*Used as a marker for*
α-Actinin	Alpha-actinin (h; m)	Cardiac
α-MHC	Alpha myosin heavy chain (h; m)	Cardiac
β-MHC	Beta myosin heavy chain (h; m)	Cardiac
Cav1.2	L-type calcium channel (h; m)	Cardiac
CD31	PECAM-1 (platelet/endothelial cell adhesion molecule) (h; m)	Endothelial
CD34	Hematopoietic progenitor cell antigen CD34 (h; m)	Precursors; endothelial
CD45	PTPRC (protein tyrosine phosphatase, receptor type, C) (h; m)	Hematopoietic
CD73	NT5E (ecto-5′-nucleotidase) (h; m)	Mesenchymal
CD90	Thy-1 cell surface antigen (h; m)	Mesenchymal
CD105	Endoglin (h; m)	Mesenchymal
CD117	c-kit (h; m); stem cell factor receptor (h; m)	Precursor
Cx43	Connexin 43 (h; m)	Cardiac
Gata4	GATA binding protein 4 (h; m)	Cardiac
Isl1	islet 1 (h; m)	Cardiac
KDR	VEGF Receptor 2 (h; m); kinase insert domain receptor (h); Flk1 Fetal liver kinase 1 (m)	Precursors;endothelial
Mef2C	Myocyte-specific enhancer factor 2C (h; m)	Cardiac
Nkx2.5	NK2 transcription factor related, locus 5 (h; m)	Cardiac
RYR2	Ryanodine receptor (h; m)	Cardiac
Sca-1	Ly-6A/E (m); stem cell antigen 1 (m)	Precursors
Serca2b	Sarcoplasmic reticulum calcium ATPase (h; m)	Cardiac
SMA	Alpha smooth muscle actin (h; m)	Smooth muscle; mesenchymal
smMHC	Smooth muscle myosin heavy chain (h; m)	Smooth muscle
Troponin I	Troponin I (h; m)	Cardiac

Supplementary Table S2. Primers Used for Reverse Transcriptase–Polymerase Chain Reaction

Gene	*Primers*
Cx43	5′GGGGCAGGCGGGAAGCACCATCTC3′ 5′TCTCTTATCCCCTCCCTCTCCACC CATCTACCC3′
α-MHC	5′TCTCTTCTCCTCCTACGCAAC3′ 5′GCTTCTCTGTCCCCTTCCTG3′
β-MHC	5′CACCAACAACCCCTACGATT3′ 5′ACTCATTGCCCACTTTCACC3′
Troponin I	5′GAGAGATACGACATAGAGGCAAAA3′ 5′CTCCTTCTTCACCTGCTTGA3′
Gata4	5′GTGTCAACTGTGGGGCTATG3′ 5′ATTCAGGTTCTTGGGCTTCC3′
Nkx2.5	5′CGACGCCGAAGTTCACGAAGT3′ 5′CGCCGCTCCAGTTCATAG3′
Mef2c	5′CAGGACAAGGAATGGGAGGA3′ 5′TGAGTAGAAGGCAGGGAGAGA3′
Isl1	5′TGATGAAGCAACTCCAGCAG3′ 5′TAAGCCACCGTCGTGTCTC3′
GAPDH	5′ATCCCATCACCATCTTCCAG3′ 5′TGAGTCCTTCCACGATACCA3′

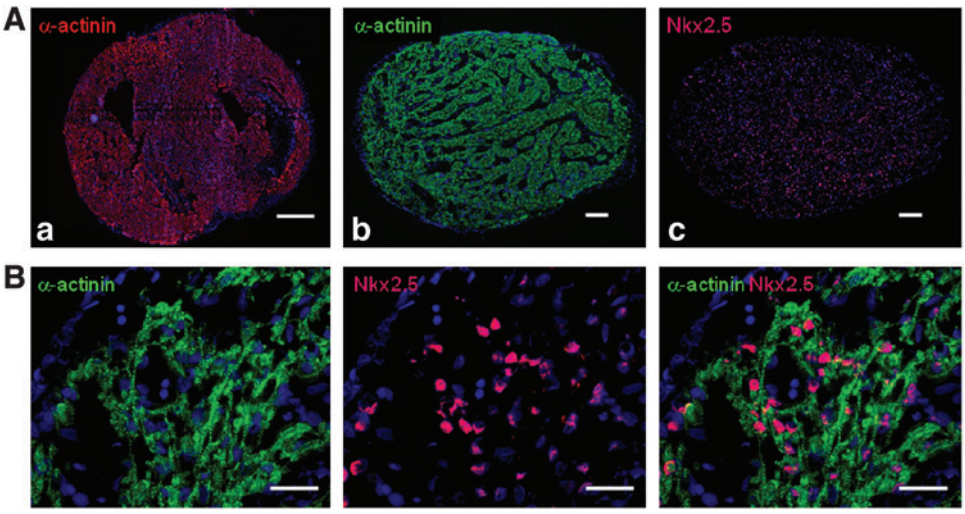
Fast Real-Time PCR System. Results were normalized to the β2-microglobulin reference gene.

Flow cytometry

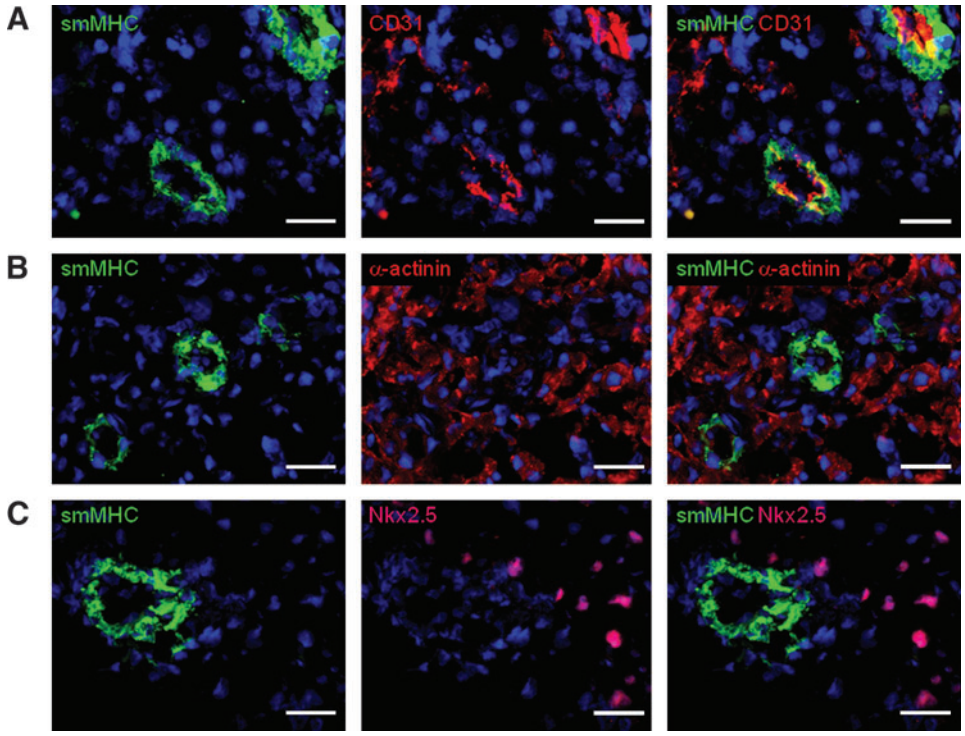
Cells were resuspended in PBS containing 3% PBS and 2 mM ethylenediaminetetraacetic acid (EDTA) and incubated for 30 min on ice with various antibodies. The following antibodies were used: FITC-conjugated anti-human CD31 (mouse monoclonal; BD Pharmingen), R-phycoerythrin (PE)-conjugated anti human CD45 (mouse monoclonal; BD Pharmingen), PE-Cy5-conjugated anti-human CD34 (mouse monoclonal; BD Pharmingen), PE-conjugated anti-human CD117 (mouse monoclonal; BD Pharmingen), APC-conjugated anti-human CD73 (mouse monoclonal; eBioscience), PE-conjugated anti-human CD73 (mouse monoclonal; BioLegend), APC-conjugated anti-human CD90 (mouse monoclonal; BioLegend), FITC-conjugated anti-human CD90 (mouse monoclonal; BioLegend), and APC-conjugated anti-human CD105 (mouse monoclonal; eBioscience). The following antibodies were used as isotype controls: PE-conjugated mouse IgG1κ monoclonal immunoglobulin isotype control (BD Pharmingen), PE-Cy5-conjugated mouse IgG1κ monoclonal immunoglobulin isotype control (BD Pharmingen), FITC-conjugated mouse IgG1κ monoclonal immunoglobulin isotype control (BD Pharmingen), and APC-conjugated mouse IgG1κ monoclonal immunoglobulin isotype control (eBioscience).

Vector production and genetic labeling of cells

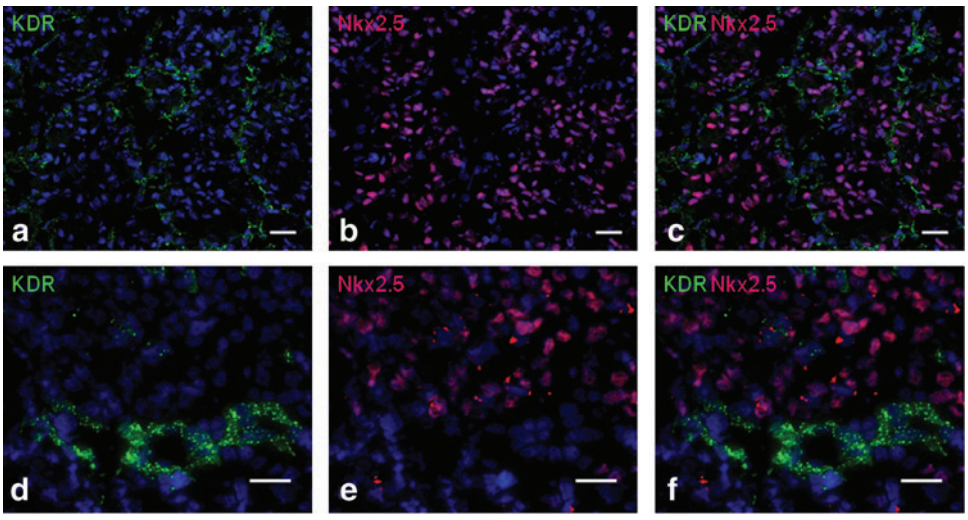
pSD-44 plasmid containing the human phosphoglycerate kinase (PGK) promoter and the puromycin acetyltransferase gene cloned into pRRLhPGK.GFP.SIN18 was kindly provided by Dr. Stephan Duss (Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland). Cells infected with pSD-44 started to express GFP at 3–5 days postinfection. Expression was stable for several passages. This infection was used to label the cells in order to detect them once they were injected into mouse myocardium.



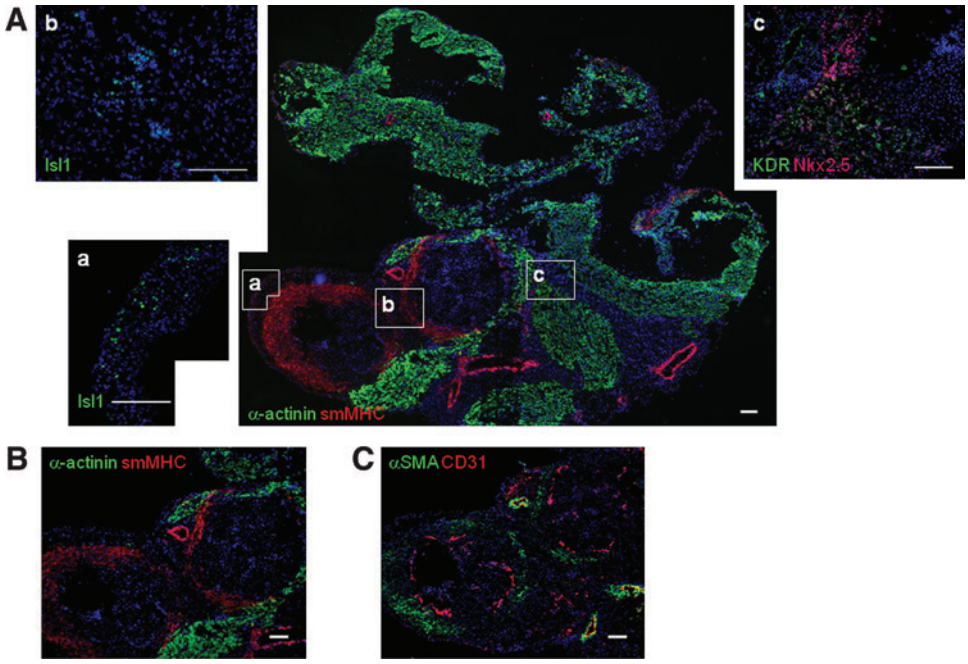
SUPPLEMENTARY FIG. S1. Immunostaining of cardiac markers in the ventricle part of sections of human fetal hearts at 12 weeks of gestation. Staining for α-actinin **(A**, red in "a" and green in "b"**)** and for Nkx2.5 **(A**, red in "c"**)**. **(A**, b and **A**, c**)** Two successive heart sections of the apex region. **(B)** Costaining for α-actinin (green) and Nkx2.5 (red). Nuclei were stained with DAPI (blue). Scale bars in **A**: 100 μm; **B**: 25 μm.



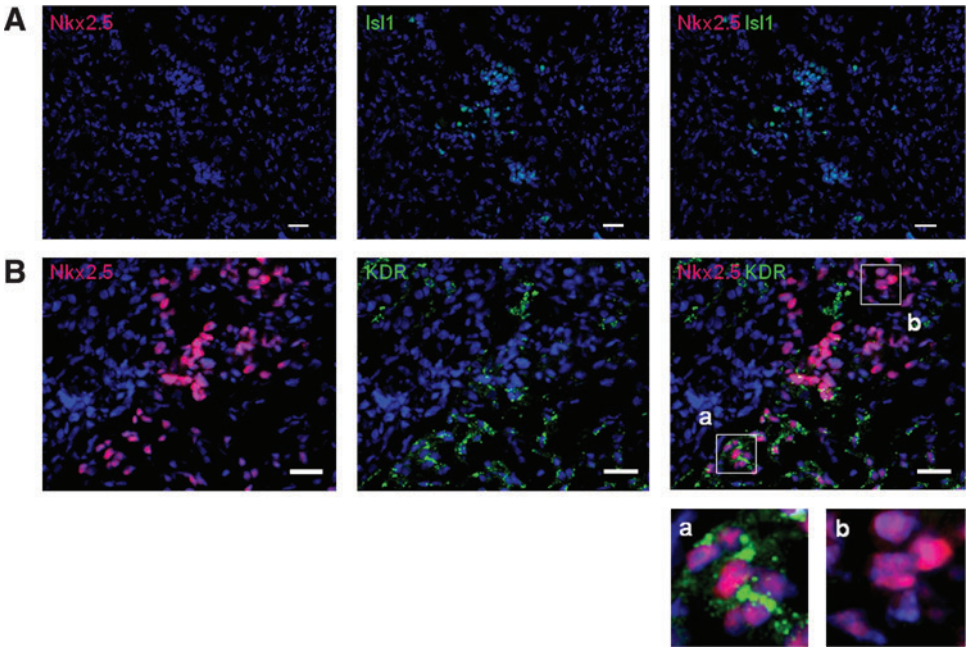
SUPPLEMENTARY FIG. S2. Immunostaining of cardiovascular markers in the ventricle part of sections of human fetal hearts at 12 weeks of gestation. **(A)** Costaining for smMHC (green) and CD31 (red). **(B)** Costaining for smMHC (green) and α-actinin (red). **(C)** Costaining for smMHC (green) and Nkx2.5 (red). Nuclei were stained with DAPI (blue). Scale bars: 25 μm.



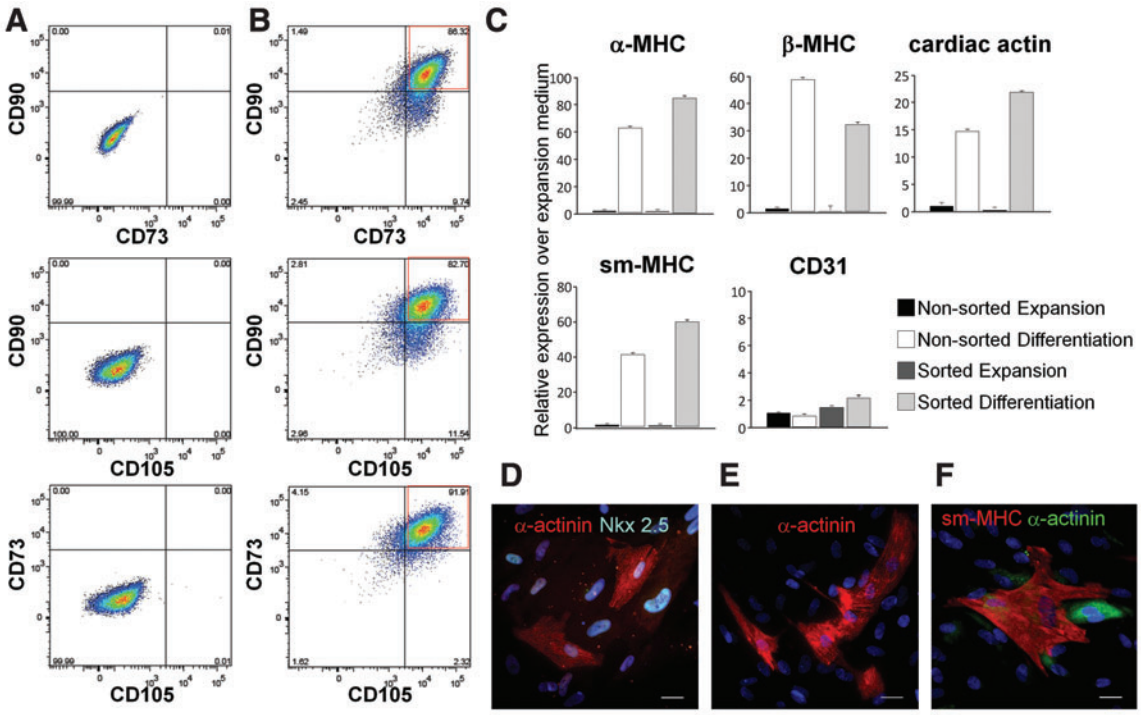
SUPPLEMENTARY FIG. S3. Immunostaining of small growing vessels and capillaries in the ventricle part of sections of human fetal hearts at 12 weeks of gestation. Staining with anti-Nkx2.5 **(a**, **d**; red**)** and anti-KDR **(b**, **e**; green**)**. **(c, f)** Merged images of **a** and **b** and of **d** and **e**, respectively. Nuclei were stained with DAPI (blue). Scale bars: 25 μm.



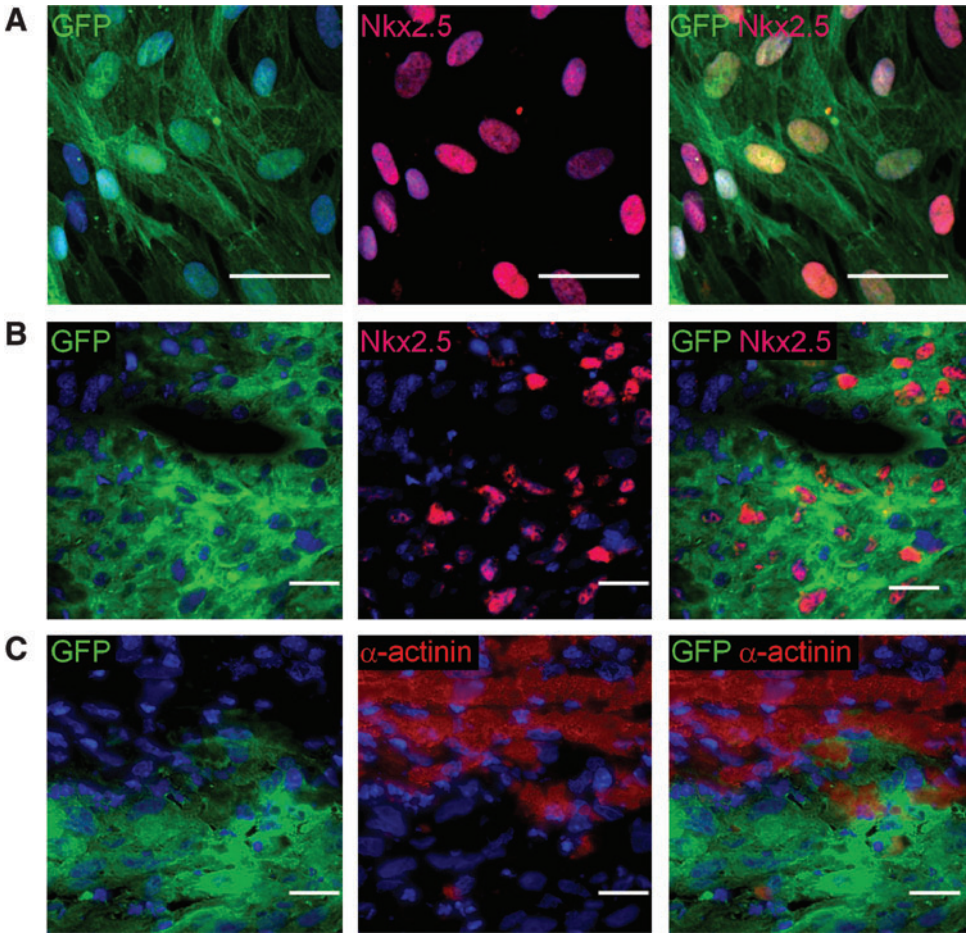
SUPPLEMENTARY FIG. S4. Immunostaining of whole heart sections of human fetal heart at 12 weeks of gestation. Staining for different cardiac, smooth muscle, and endothelial markers was performed on successive heart sections. **(A)** Central picture shows costaining for smMHC (red) and α-actinin (green) and identifies the outflow tract on the left (aorta and pulmonary artery stained for smMHC in red) and the auricles on the top and the right (stained positive for α-actinin in green). The smaller blood vessels stained in red represent the coronary arteries. (a, b) Staining for Isl1 (green); (c) costaining for Nkx2.5 (red) and KDR (green). **(B)** Costaining for α-actinin (green) and smMHC (red). **(C)** Costaining for αSMA (green) and CD31 (red). Nuclei were stained with DAPI (blue). Scale bars: 100 μm.



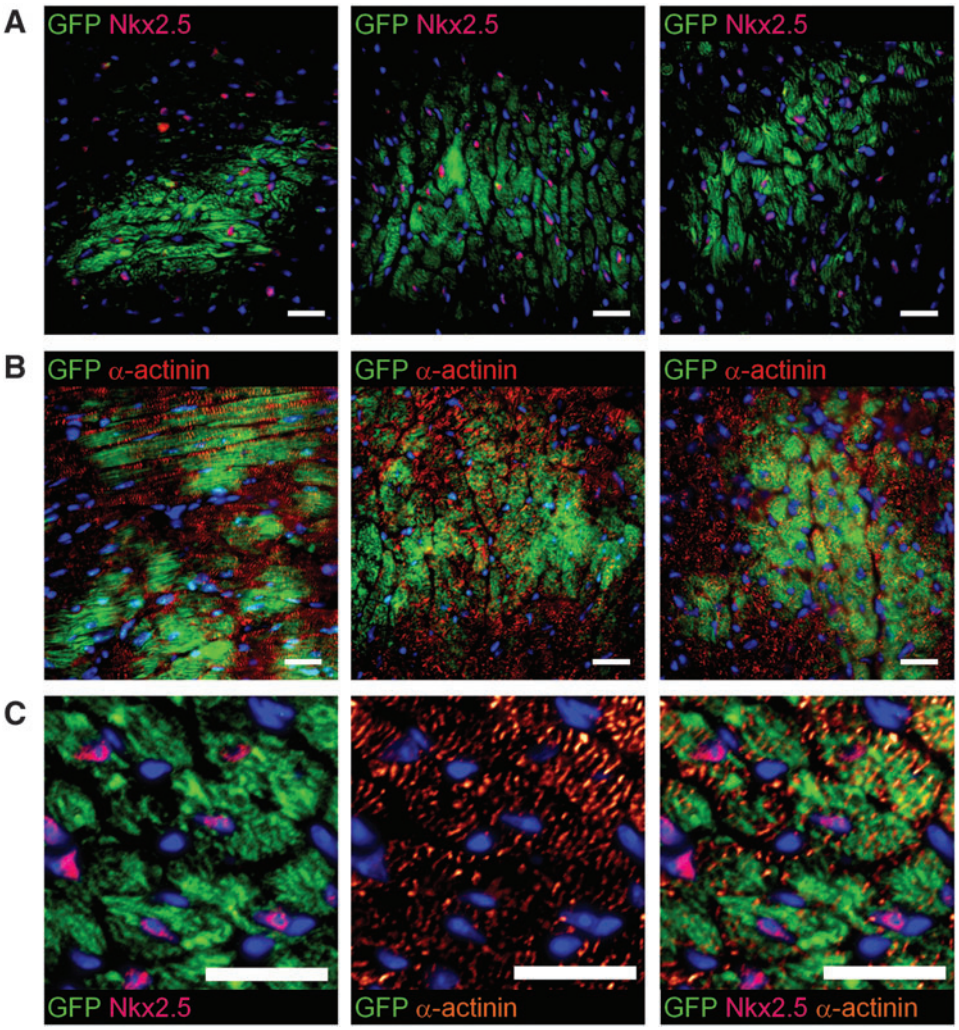
SUPPLEMENTARY FIG. S5. Staining of sections of the human fetal heart at 12 weeks of gestation, representing high magnification of Figure 4**A**, b and c. **(A)** High magnification of Supplementary Figure S4**A**, b: costaining for Nkx2.5 (red) and Isl1 (green). **(B)** High magnification of Supplementary Figure S4**A**, c: costaining for Nkx2.5 (red) and KDR (green). Nuclei were stained with DAPI (blue). a and b represent higher magnification of the area indicated by the squares in (**B**). Scale bars: 25 μm.



SUPPLEMENTARY FIG. S6. Cardiogenic potential of cells expressing CD73, CD90, and CD105. **(A, B)** Fluorescence-activated cell sorting of CD73-positve, CD90-positve, CD105-positve cells. Scatter plots with isotype controls **(A)** and specific antibodies **(B)** are shown. Red squares indicate gating for cell sorting based on CD73, CD90, and CD105 expression. **(C)** Quantitative real-time RT-PCR analysis of cardiac markers (α-MHC, β-MHC, cardiac actin, and Nkx2.5), smooth muscle cell marker (sm-MHC), and endothelial marker (CD31) expression in unsorted CPCs and in CPCs sorted with CD73, CD90, and CD105. Expression in different conditions was compared with expansion medium. Black bars: unsorted cells in expansion medium; white bars: unsorted cells in differentiation medium; dark gray bars: CD73-positve, CD90-positve, CD105-positve cells in expansion medium; light gray bars: CD73-positve, CD90-positve, CD105-positve sorted cells in differentiation medium. **(D, E)** Differentiation of CD73-positve, CD90-positve, CD105-positve sorted cells into cardiomyocytes *in vitro*. Costaining for α-actinin (red), Nkx2.5 (green), and nuclei (DAPI in blue). Scale bars: 25 μm. **(F)** Differentiation of CD73-positve, CD90-positve, CD105-positve sorted cells into smooth muscle cells *in vitro*. Costaining for smMHC (red), α-actinin (red), and nuclei (DAPI in blue). Scale bars: 25 μm.



SUPPLEMENTARY FIG. S7. Cell engraftment in SCID mice heart. **(A)** CPCs infected with a lentivirus expressing GFP and cultured in expansion condition were stained for GFP (green), Nkx2.5 (red), and DAPI (blue). **(B, C)** Staining of heart sections of SCID mice at 1 week after myocardial injection of CPCs labeled with GFP. Heart sections were stained for GFP (green), DAPI (blue), and either Nkx2.5 **(**red; in **B)** or α-actinin **(**red; in **C)**. Scale bars: 25 μm.



SUPPLEMENTARY FIG. S8. Cell engraftment in SCID mice heart. Staining of heart sections of SCID mice at 4 weeks after myocardial injection of CPCs labeled with GFP. **(A)** Heart sections were stained for GFP (green), DAPI (blue), and Nkx2.5. **(B)** Heart sections were stained for GFP (green), DAPI (blue), and α-actinin (red). **(C)** Heart sections were stained for GFP (green), DAPI (blue), Nkx2.5 (red), and α-actinin (brown). Scale bars: 25 μm.