Supplemental Methods

Primary and secondary antibodies

Primary antibodies used in this study include (I) rabbit polyclonal antibodies: anti-alpha smooth muscle actin (αSMA; ab5694 (1:400); abcam, Cambridge, MA, www.abcam.com); anti-GFP (sc-8334 (1:100); Santa Cruz Biotechnology, Inc., Santa Cruz, CA, www.scbt.com/); anti-Troponin C (sc-48347 (1:50); Santa Cruz); anti-Von Willebrand Factor (vWF; ab6994 (1:400); abcam); anti-NOS3 (eNOS; sc-654 (1:200); Santa Cruz); (II) mouse monoclonal antibody anti-sarcomeric myosin (MF-20 (1:50); Developmental Studies Hybridoma Bank (DSHB), Iowa City, IA, http://dshb.biology.uiowa.edu/); (III) rat monoclonal antibodies: anti-Pecam1 (CD31, 550274 (1:50); BD Pharmingen, San Diego, CA, www.bdbiosciences.com/pharmingen/); and anti-Cadherin 5/VE-Cadherin (CD144, 550548 (1:50); BD Pharmingen); as well as (IV) rabbit monoclonal antibodies: anti-calponin (ab46794 (1:200); abcam) and anti-caldesmon (ab32330 (1:250); abcam).

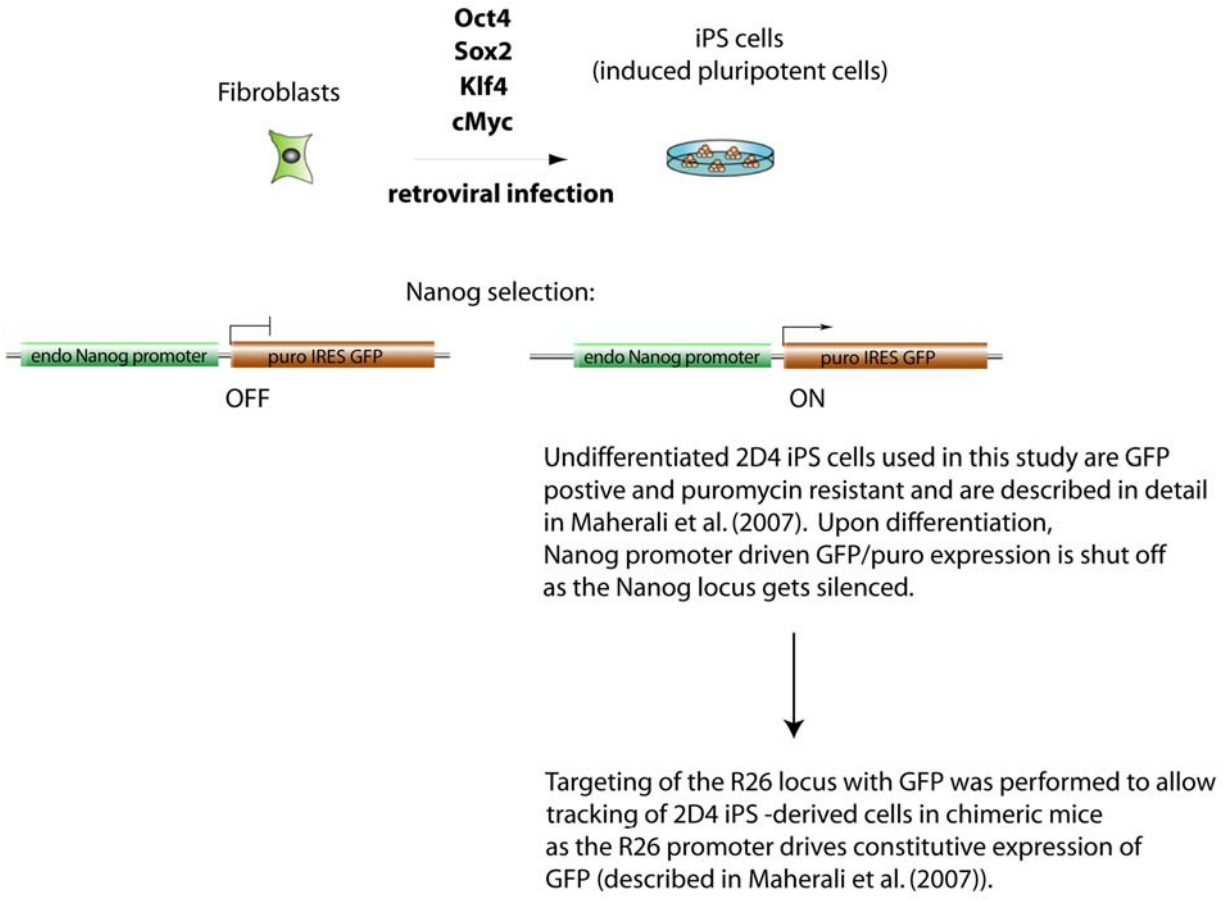
Secondary antibodies included Alexa Fluor 488-, Alexa Fluor 594-, Alexa Fluor 647-conjugated goat-anti mouse IgG (H+L); goat-anti rabbit IgG (H+L); goat-anti rat IgG (H+L) and goat-anti mouse IgM (H+L) (1:250; all from Molecular Probes, Eugene, OR, probes.invitrogen.com).

SMC contractility, acetylated low-density lipoprotein (acLDL)-uptake and Matrigel assay

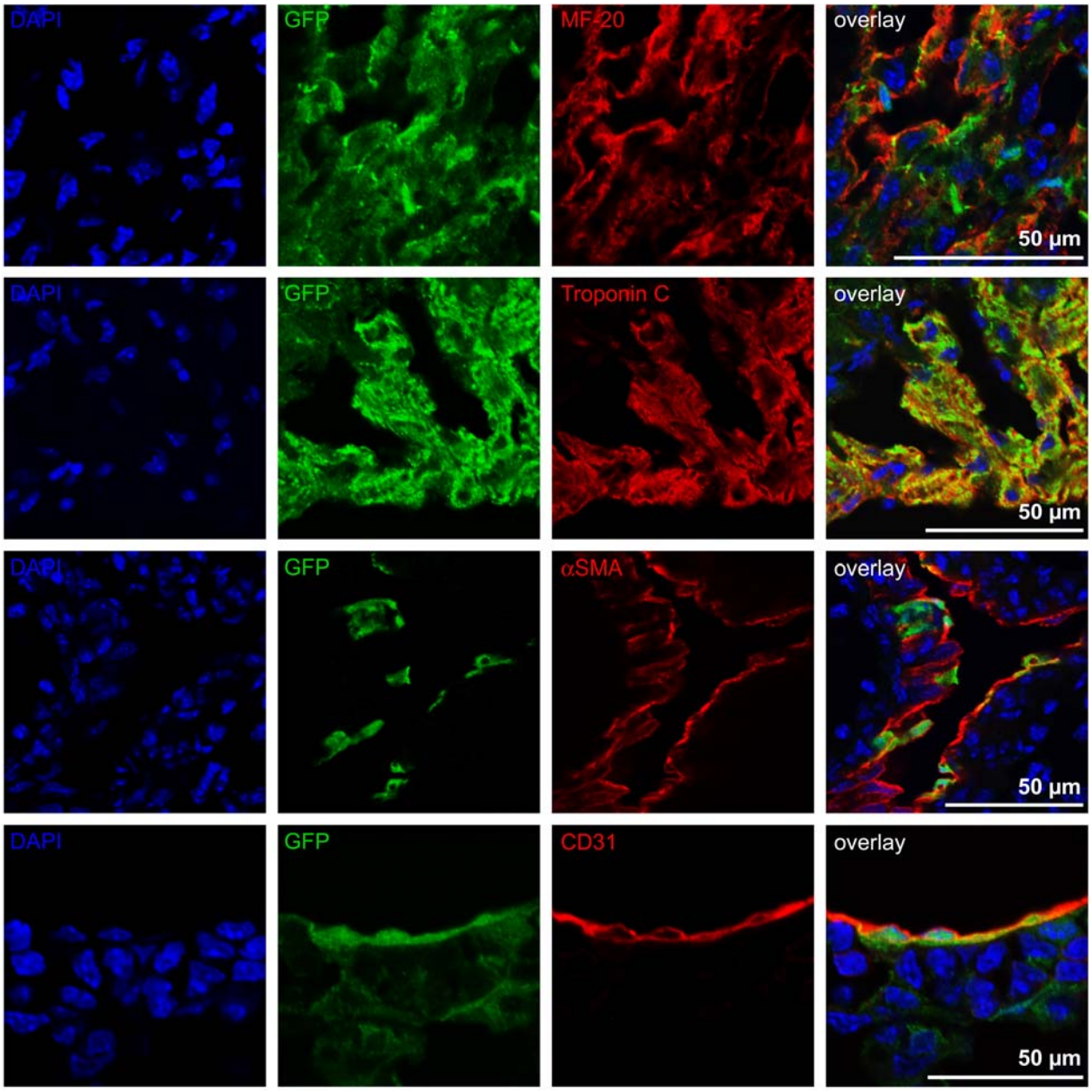
To assess cell functionality, SMC contractility, uptake of acLDL in EC, and Matrigel EC *in vitro* tube formation were determined. Briefly, cells were subjected to the effect of 10^{-5} M carbachol (C-4382, Sigma-Aldrich Corp., St. Louis, MO, www.sigma.com) in SMGM-2 medium (Lonza, Walkersville, MD, www.lonza.com) for 30 minutes. Contraction was calculated by the difference of cell area at time zero and after 30 minutes using bright-field images. To determine the uptake of acLDL, cells were incubated with 10 µg/ml Alexa Fluor 594-labeled acLDL

(Molecular Probes) for 4 hours at 37°C, washed in PBS, fixed with 4% paraformaldehyde (Sigma), counterstained with 4'-6-diamidino-2-phenylindole (DAPI; Sigma) and visualized. For Matrigel assays, 2.5 x 10^4 cells/ 350 µl/ well were plated onto Matrigel-coated 24-well plates (BD Biocoat, BD Bioscience Discovery Labware, Bedford, MA, www.bdbiosciences.com/discoverylabware/) and cultured for 24 hours at 37°C, 5% CO_2. Tube formation was analyzed by phase-contrast microscopy. All imaging was performed using a Zeiss Axiovert 200 microscope (Carl Zeiss MicroImaging Inc., Thornwood, NY, www.zeiss.com). To serve as controls, primary murine vascular smooth muscle cells (mSMC), isolated from thoracic aortas of C57BL/6 mice, and human umbilical vein endothelial cells (HUVEC), obtained from Lonza, were cultured in either SMGM-2 or EGM-2 media (Lonza).

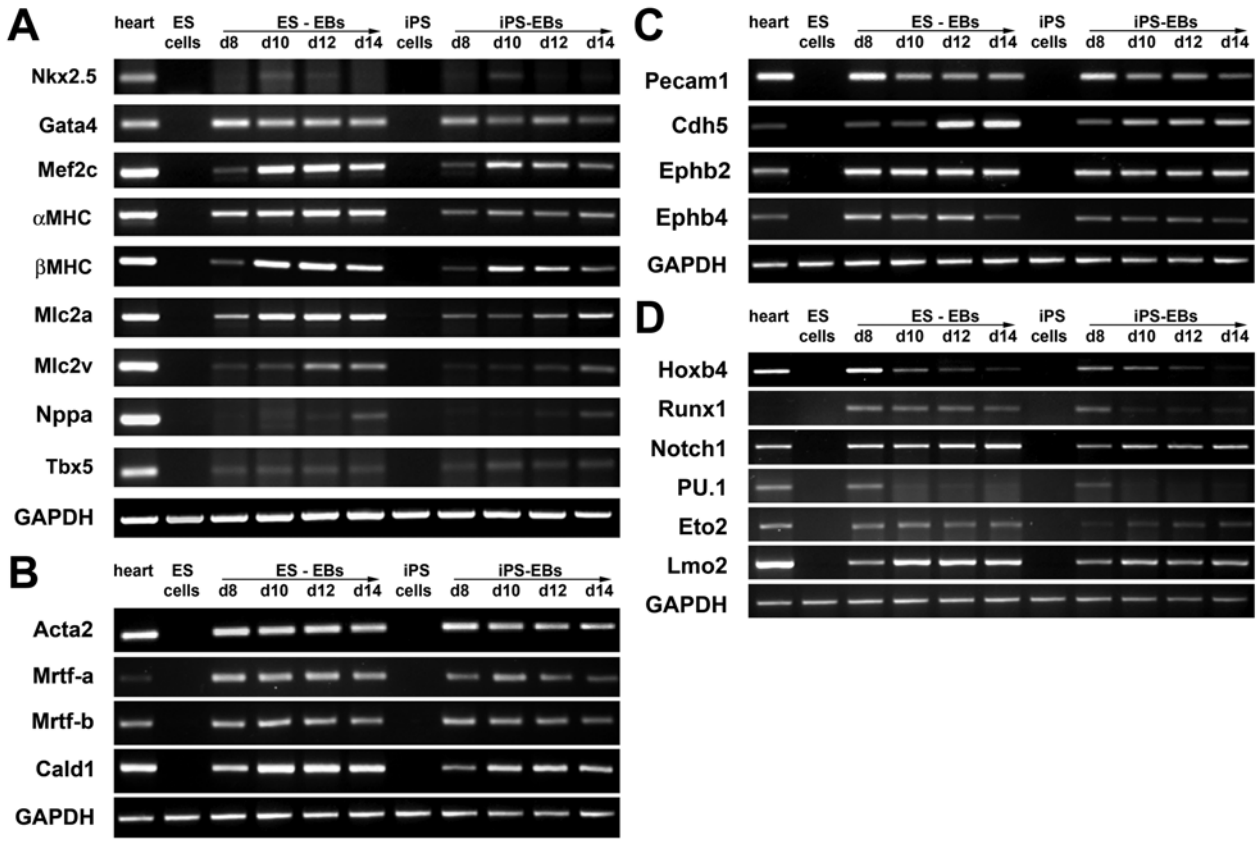
Supplemental Figures



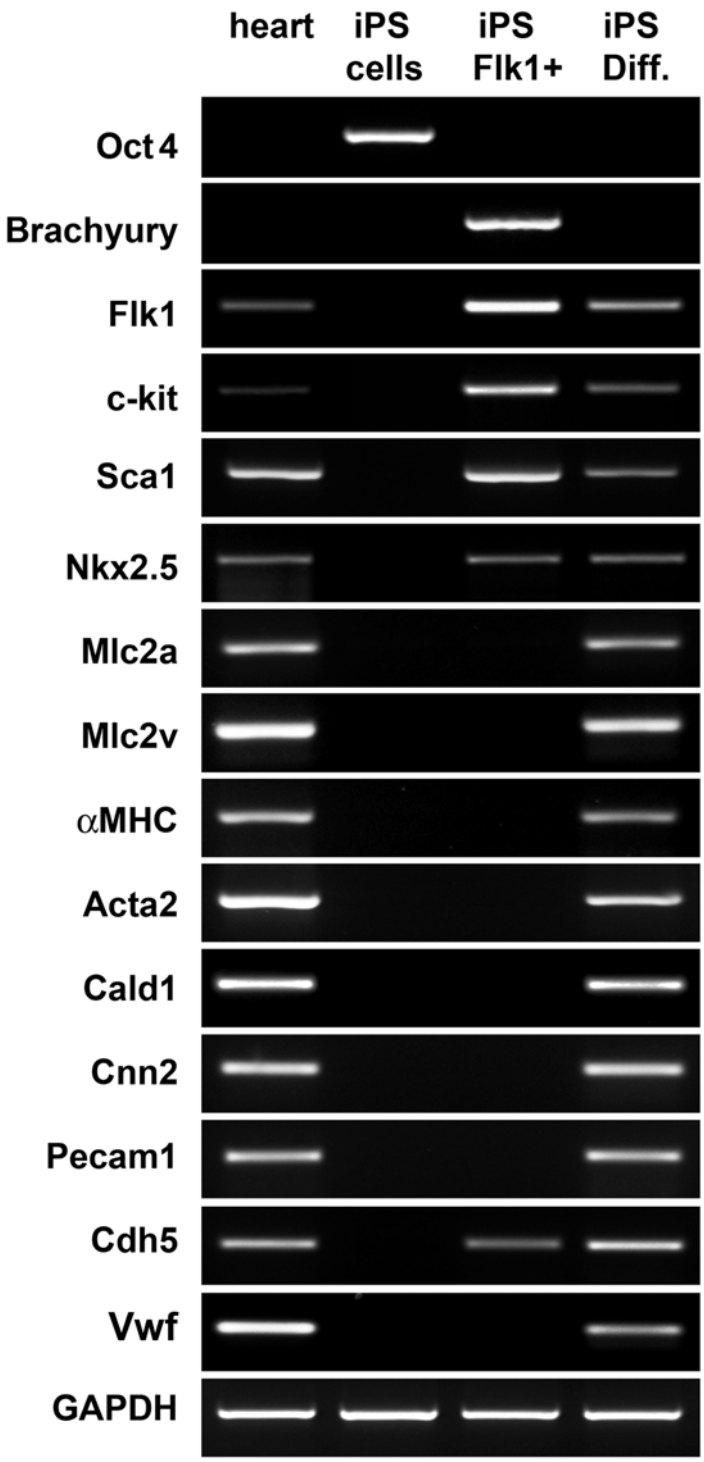
Suppl. Fig. 1 Generation of 2D4 iPS cells.



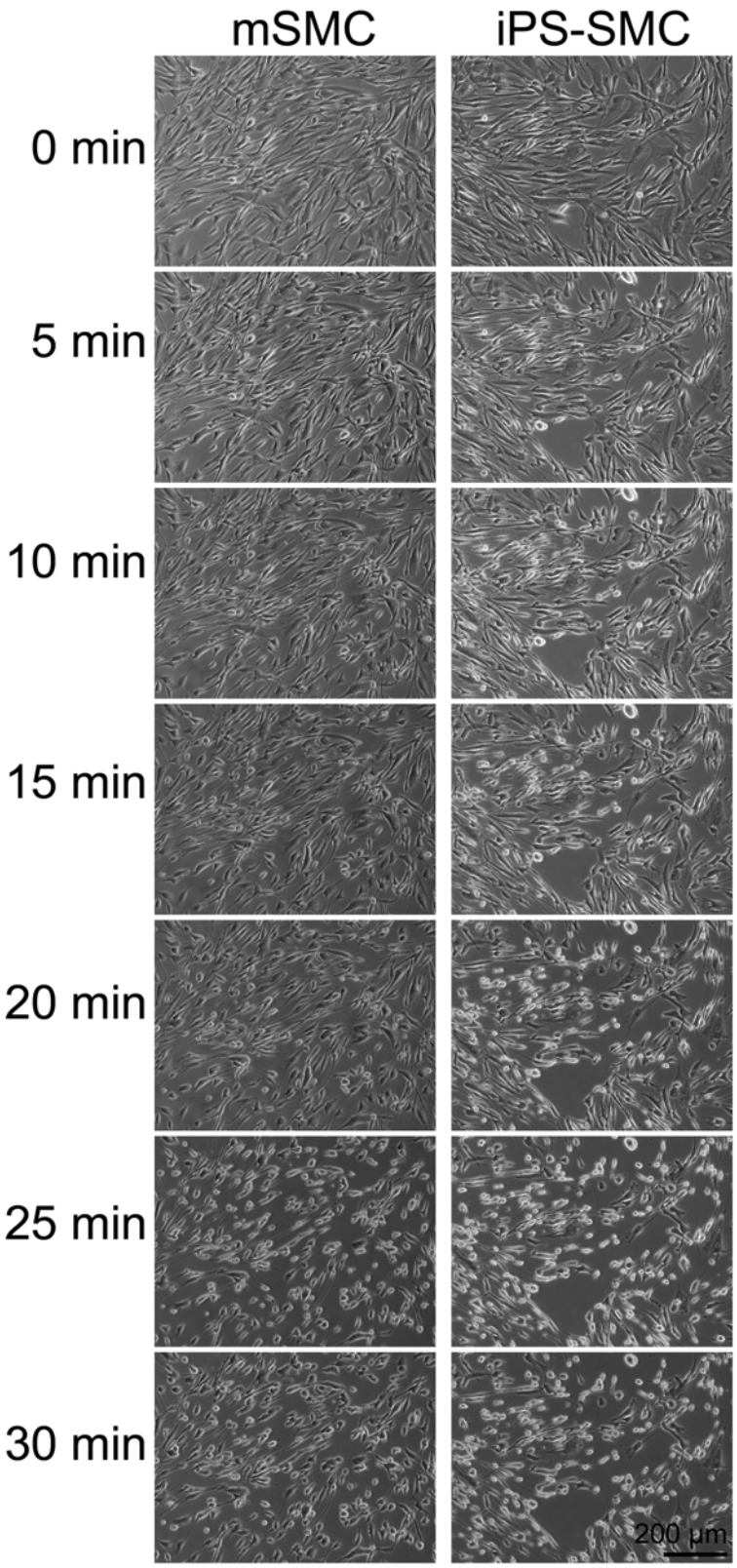
Suppl. Fig. 2 ***In vivo*** **developmental potential of 2D4 iPS cells.** Immunofluorescence staining shows GFP-expressing 2D4 iPS cells (green) that contribute *in vivo* to cells of the cardiovascular lineage including cardiomyocytes (sarcomeric myosin (MF-20) and Troponin C staining (red)), smooth muscle cells (αSMA staining (red)), and endothelial cells (CD31 staining (red)). DAPI-staining was performed to show cell nuclei (blue). Scale bars equal 50 µm.



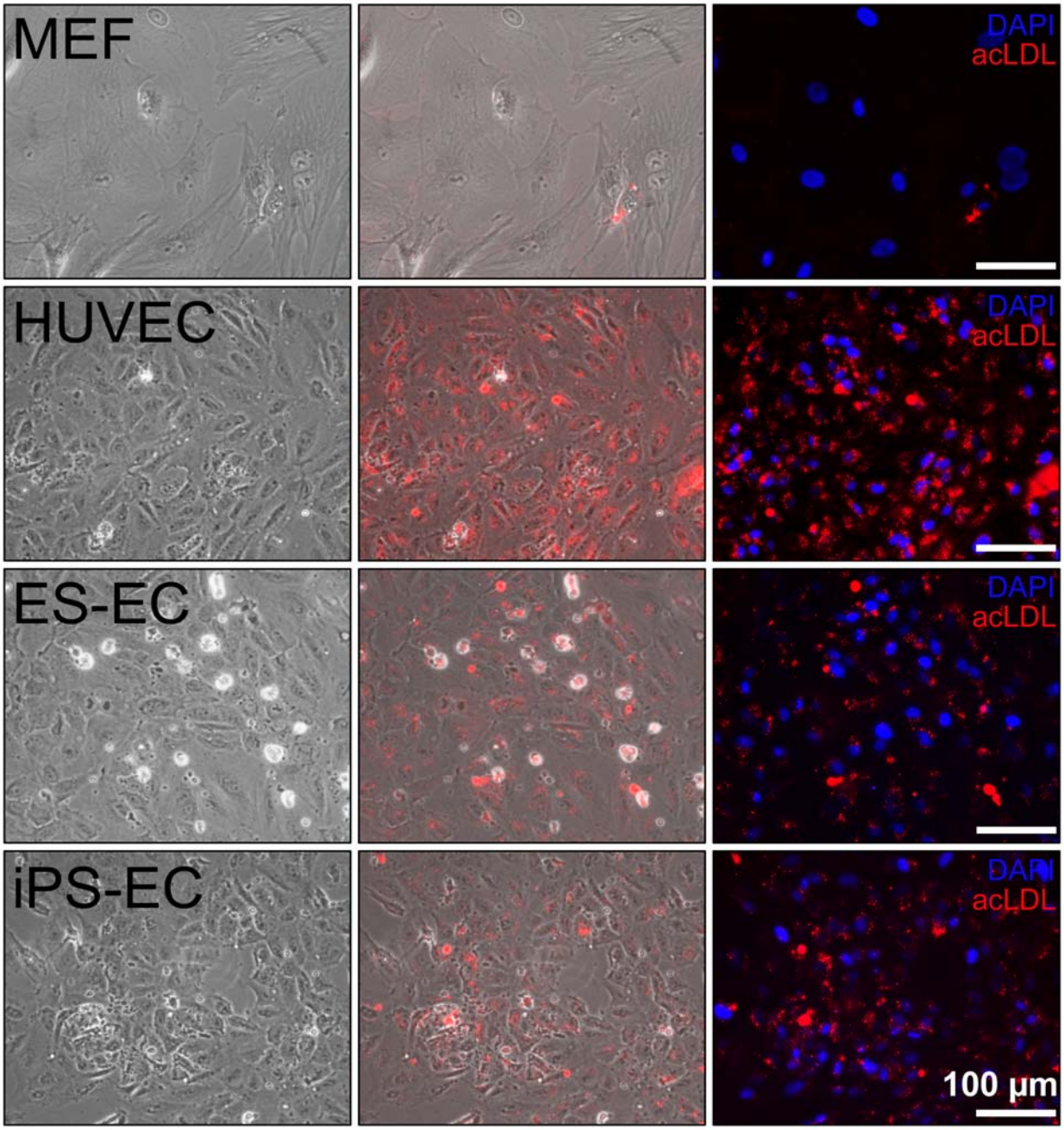
Suppl. Fig. 3 **Cardiovascular and hematopoietic gene expression in ES and iPS cell-derived EBs**. Semi-quantitative RT-PCR analysis shows the upregulation of (**A**) cardiac, (**B**) smooth muscle, (**C**) endothelial and (**D**) hematopoietic cell markers.



Suppl. Fig. 4 RT-PCR analysis shows mRNA expression profiles of heart tissue, undifferentiated iPS cells, MACS-isolated iPS cell-derived Flk1-positive progenitor cells, and differentiated Flk1-positive cells after 12 days of culture in alpha-MEM, PDFG-BB and VEGF medium (pooled samples).



Suppl. Fig. 5 After exposure to 10^{-5} M carbachol for 30 minutes, mSMC and iPS-SMC contracted between 66.9 ± 3.4% and 71.1 ± 2.4%. Scale bar equals 200 µm.



Suppl. Fig. 6 In contrast to murine embryonic fibroblasts (MEF), human umbilical vein endothelial cells (HUVEC), ES cell-derived EC (ES-EC) and iPS cell-derived EC (iPS-EC) display a typical cobblestone morphology and have the ability to uptake acetylated low-density lipoprotein (acLDL; red). DAPI-staining was performed to show cell nuclei (blue). Scale bars equal 100 µm.

Supplemental Tables

Supplemental Table 1. Primer sets used for RT-PCR.

Gene	Primer Sequences	AT in °C	Cycles	Reference
Oct3/4	F5'-agcacgagtggaaagcact-3' R5'-ctcattgttgtcggcttcct-3'	58	36	*
Nanog	F5'-agggtctcgtactgagatgctctg-3' R5'-caaccactggtttttctgccaccg-3'	55	35	*
Brachyury	F5'-aatgggggtggcttgttcct-3' R5'-aggctttgggccgtgtcata-3'	65	38	1
Scl/Tal1	F5'-attgcacacacgggattctg-3' R5'-catacagtacgacactgacg-3'	58	38	*
Flk1	F5'-tctgtggttctgcgtggaga-3' R5'-gtatcatttccaaccaccc-3'	57	38	*
Flt1	F5'-tgtggagaaacttggtgacct-3' R5'-tggagaacagcaggactcctt-3'	65	36	*
Tie2	F5'-aagacatacgtgaacaccacact-3' R5'-actctagagtcagaacacactgcagat-3'	53	34	*
Gata1	F5'-cattggccccttgtgaggccag-3' R5'-cgctccagccagattcgaccc-3'	65	36	*
c-kit	F5'-ccatgtggctaaagatgaac-3' R5'-ctgctggtgctcgggtttg-3'	56	34	*
Sca1	F5'-tctgaggatggacacttctc-3' R5'-ctcaggctgaacagaagcac-3'	56	34	*
Isl1	F5'-agatatgggagacatgggcgat-3' R5'-acacagcggaaacactcgatg-3'	65	36	*
Nkx2.5	F5'-caagtgctctcctgctttcc-3' R5'-ggctttgtccagctccact-3'	60	38	*
Gata4	F5'-tctcactatgggcacagcag-3' R5'-gcgatgtctgagtgacagga-3'	60	32	*
Mef2c	F5'-aggtgttgctcaagtacaccgagt-3' R5'-atctcaaagctgggaggtggaaca-3'	65	32	*
αMHC	F5'-ctgctggagaggttattcctcg-3' R5'-ggaagagtgagcggcgcatcaagg-3'	68	34	*
βMHC	F5'-tgcaaaggctccaggtctgagggc-3' R5'-gccaacaccaacctgtccaagttc-3'	68	36	*

Mlc2a	F5'-cagacctgaaggagacct-3' R5'-gtcagcgtaaacagttgc-3'	54	36	*
Mlc2v	F5'-tgtgggtcacctgaggctgtggttcag-3' R5'-gaaggctgactatgtgtccgggagatgc-3'	68	36	*
Nppa	F5'-tgatagatgaaggcaggaagccgc-3' R5'-aggattggagcccagagtggactagg-3'	68	36	*
Tbx5	F5'-ggagcctgattccaaagaca-3' R5'-ttcagccacagttcacgttc-3'	60	38	*
Mrtf-a	F5'-ttgtcccagcctggttctcca-3' R5'-atctgctgaaatctctccactctg-3'	60	38	*
Mrtf-b	F5'-ccccagcagtttgttgttcagcactctt-3' R5'-gatgctggctgtcactggtttcatcttg-3'	60	38	*
Ephb2	F5'-tccaggagggactctgtgtggaag-3' R5'-cggggtattctccttcttaattgt-3'	65	36	*
Ephb4	F5'-cccaaataggagacgagtcc-3' R5'-ctcaaaaggaggtggtccag-3'	62	36	*
Hoxb4	F5'-ggagtttcactacaaccgctacctg-3' R5'-ctacccccttctctgtgtttattc-3'	65	36	*
Runx1	F5'-ccagcaagctgaggagcggcg-3' R5'-ccgacaaacctgaggtcgttg-3'	65	36	*
Notch1	F5'-gcagccacagaacttaccactccag-3' R5'-taaatgcctctggaatgtgggtgat-3'	65	36	*
PU.1	F5'-atggaagggttttccctcaccgcc-3' R5'-gtccacgctctgcagctctgtgaa-3'	65	36	*
Eto2	F5'-acggcctcgctctccac-3' R5'-ggtgcaggaccgcttactg-3'	60	35	*
Lmo2	F5'-atgtcctcggccatcgaaagg-3' R5'-agatgatcccattgatcttgg-3'	60	35	*
Nes	F5'-ccctcaccactctatttta-3' R5'-actatctaaacctttaggagaa-3'	58	36	*
Afp	F5'-tcgtattccaacaggagg-3' R5'-aggcttttgcttcaccag-3'	54	36	*
GFP	F5'-tgttctgctggtagtggtcg-3' R5'-tatatcatggccgacaagca-3'	55	36	*
GAPDH	F5'-gcaaattcaacggcac-3' R5'-gatgacccgtttggct-3'	60	32	1

*DNA sequence for this gene was obtained from the GenBank database (http://www.ncbi.nlm.nih.gov). The PCR primers were designed using OligoPerfect™ Designer (Invitrogen).

References

1. Schenke-Layland K, Angelis E, Rhodes KE, et la. Collagen IV induces trophoectoderm differentiation of mouse embryonic stem cells. Stem Cells 2007;25:1529-1538.

Supplemental Table 2. Morphometric analysis of ES and iPS cell-derived EBs. The cross-sectional area was measured for at least 50 ES and iPS cell-derived EBs. For statistical analysis all data are presented as mean EB size in µm ± SEM. Statistical significance was assessed by Student's *t* test. *P*-values less than 0.05 were defined as statistically significant.

	d2	**d4**	**d6**	**d8**	**d10**	**d12**	**d14**
ES-EBs	(254 ± 8.6)	(301 ± 12)	(377 ± 15)	(415 ± 14)	(434 ± 13)	(433 ± 13)	(452 ± 13)
iPS-EBs	(184 ± 7.6)	(252 ± 9.5)	(403 ± 12)	(403 ± 12)	(410 ± 10)	(460 ± 12)	(476 ± 10)
***p*-value**	0.0001	0.0007	0.0853	0.2667	0.0718	0.0632	0.0768