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Supplemental Information

THY-1 Receptor Expression Differentiates Cardiosphere-Derived Cells with Divergent Cardiogenic Differentiation Potential

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FIGURE S1

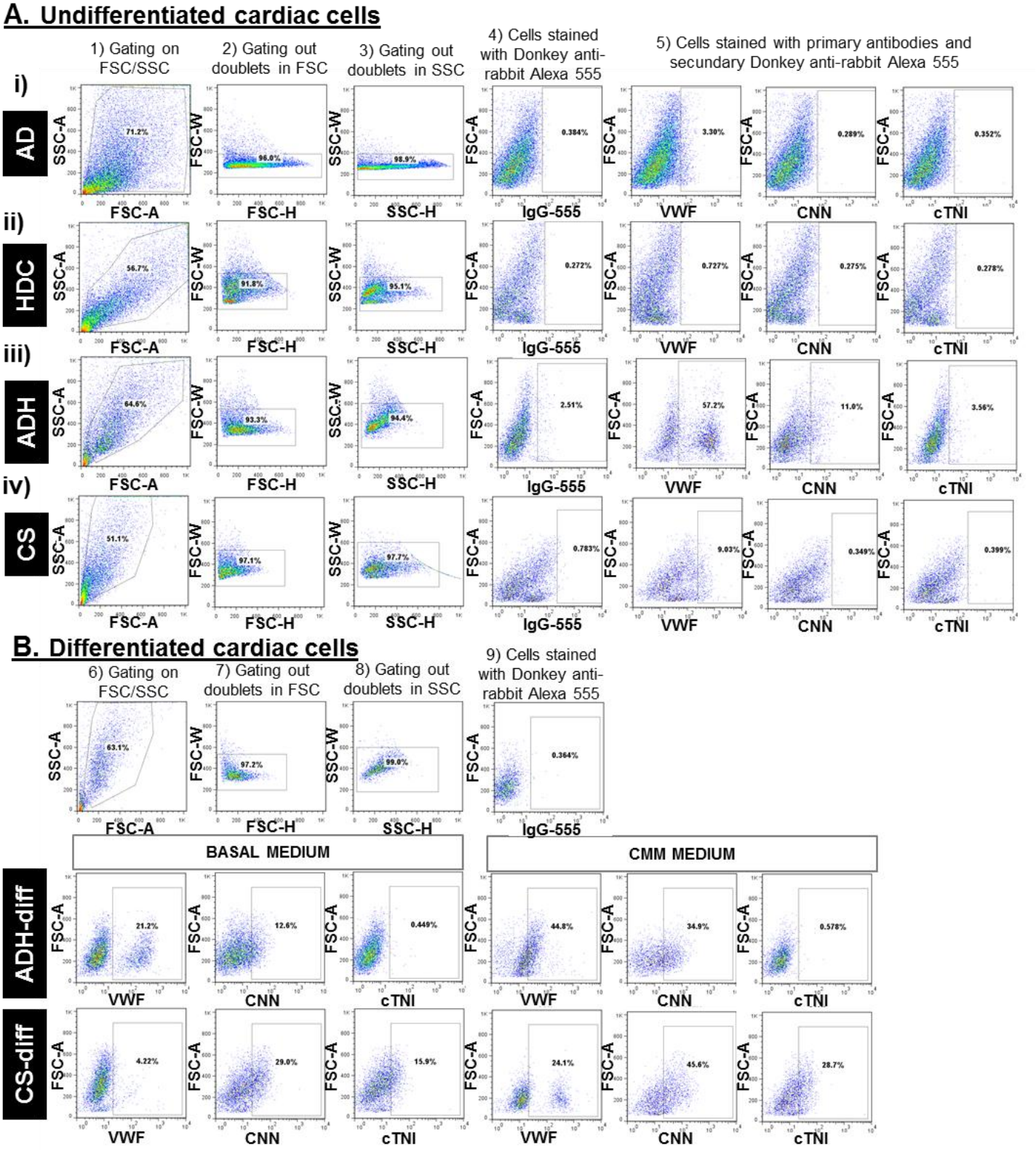
A. Undifferentiated cardiac cells
1) Gating on FSC/SSC
2) Gating out doublets in FSC
3) Gating out doublets in SSC
4) Cells stained with Donkey anti-rabbit Alexa 555
5) Cells stained with primary antibodies and secundary Donkey anti-rabbit Alexa 555
i)
AD
ii)
HDC
iii)
ADH
iv)
CS
SSC-A
FSC-A
FSC-W
FSC-H
SSC-W
SSC-H
IgG-555
VWF
CNN
cTNI
71.2%
96.0%
98.9%
0.384%
3.30%
0.289%
0.352%
56.7%
91.8%
95.1%
0.272%
0.727%
0.275%
0.278%
64.6%
93.3%
94.4%
2.51%
57.2%
11.0%
3.56%
51.1%
97.1%
97.7%
0.783%
9.03%
0.349%
0.399%
B. Differentiated cardiac cells
6) Gating on FSC/SSC
7) Gating out doublets in FSC
8) Gating out doublets in SSC
9) Cells stained with Donkey anti-rabbit Alexa 555
BASAL MEDIUM
CMM MEDIUM
ADH-diff
CS-diff
63.1%
97.2%
99.0%
0.364%
21.2%
12.6%
0.449%
44.8%
34.9%
0.578%
4.22%
29.0%
15.9%
24.1%
45.6%
28.7%

Figure S1. Gating strategy and FACS analysis of basal and differentiated cells. A, Gating strategy for AD, HDC, ADH, and CS cells stained with VWF, CNN and cTNI in undifferentiated conditions. B, Gating strategy for quantification post-differentiation of ADH-diff versus CS-diff cells. All experiments were repeated with cells with three independent heart samples.

FIGURE S2

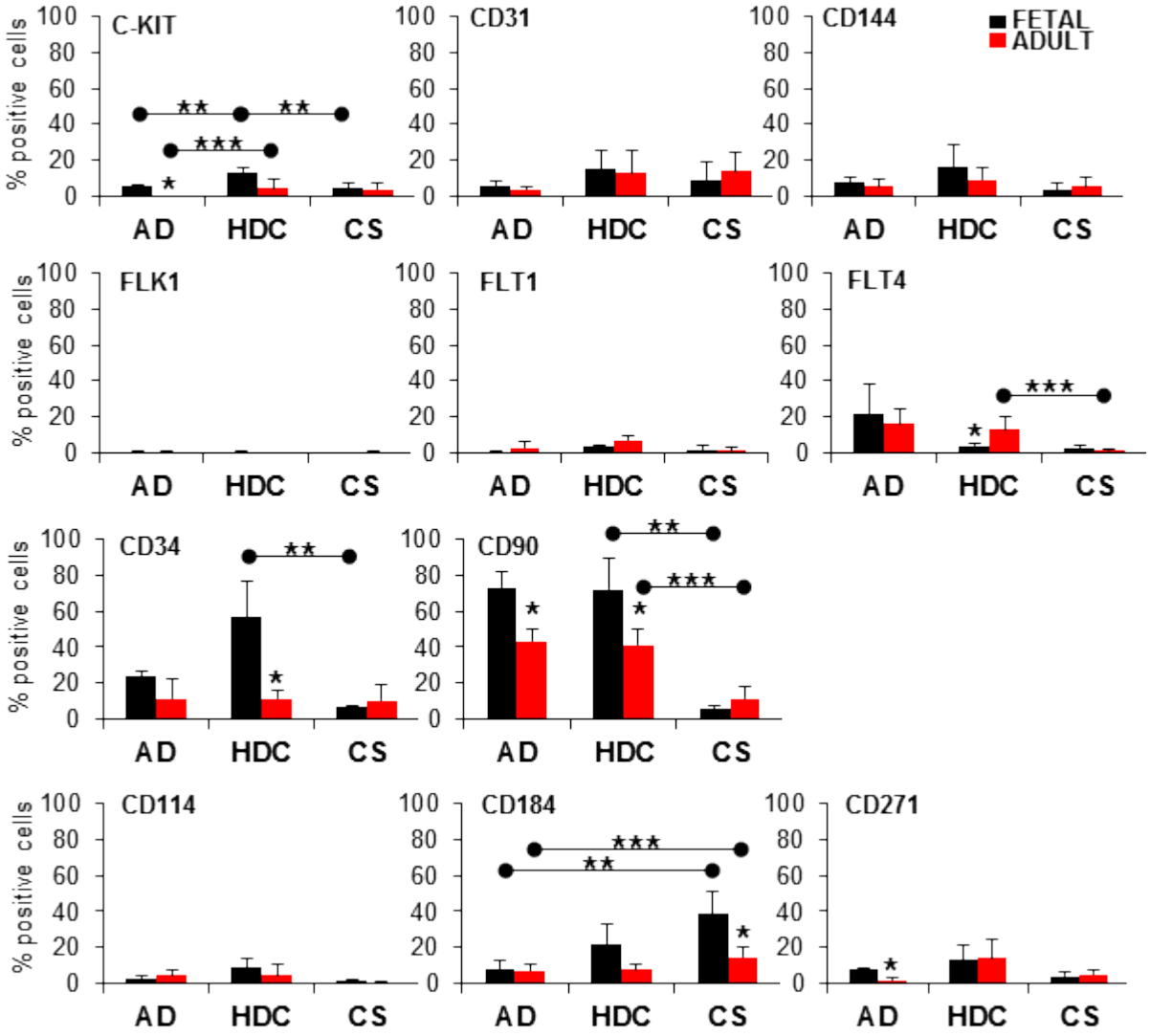

Figure S2. Cell surface marker expression in fetal and adult AD, HDC and CS-derived cells. A) Percentage of cells (% positive cells) for specific surface markers (C-KIT, CD34, CD31, CD144, CD90, CD114, CD184, CD271, FLK1, FLT1, and FLT4) from fetal and adult AD, HDC and CS cells by flow cytometry. *P<0.05 for comparisons between fetal and adult cells within a culture condition, **P<0.05 for

comparisons between fetal cells across the three culture conditions, and ***$P<0.05$ for comparisons between adult cells across the three culture conditions.

FIGURE S3

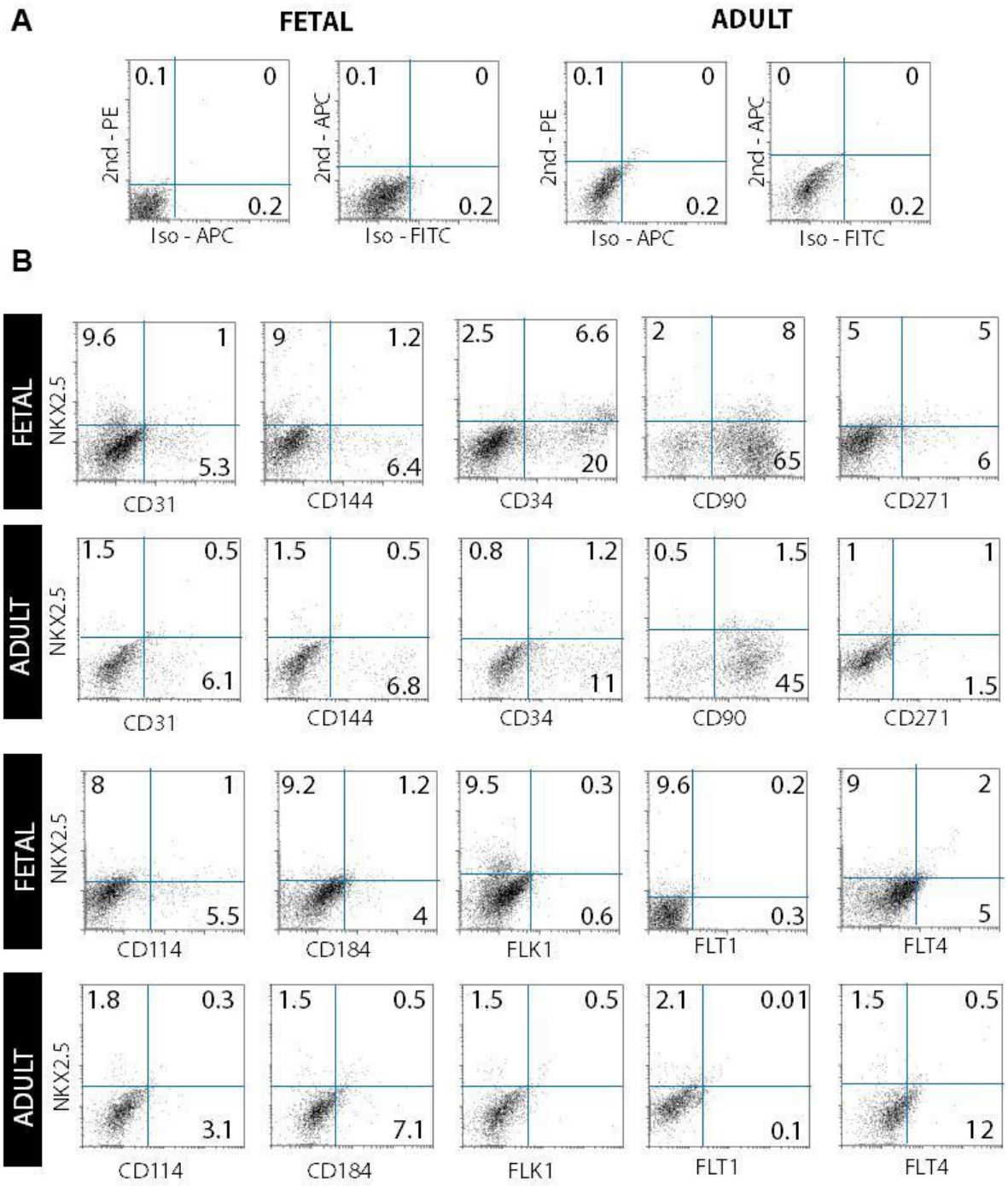

Figure S3. Analysis of NKX2.5 subpopulations from fetal and adult hearts. A, Representative dot plot graphs of negative controls (secondary antibody versus isotypes) for each fluorochrome used in this

analysis. B, Flow cytometry analysis of HDC cells for a panel of surface markers (X-axis) and NKX2.5 (Y-axis) from fetal and adult human heart samples. Total percentage of positive cells for each marker is shown. All experiments were repeated with cells from three independent heart samples.

FIGURE S4

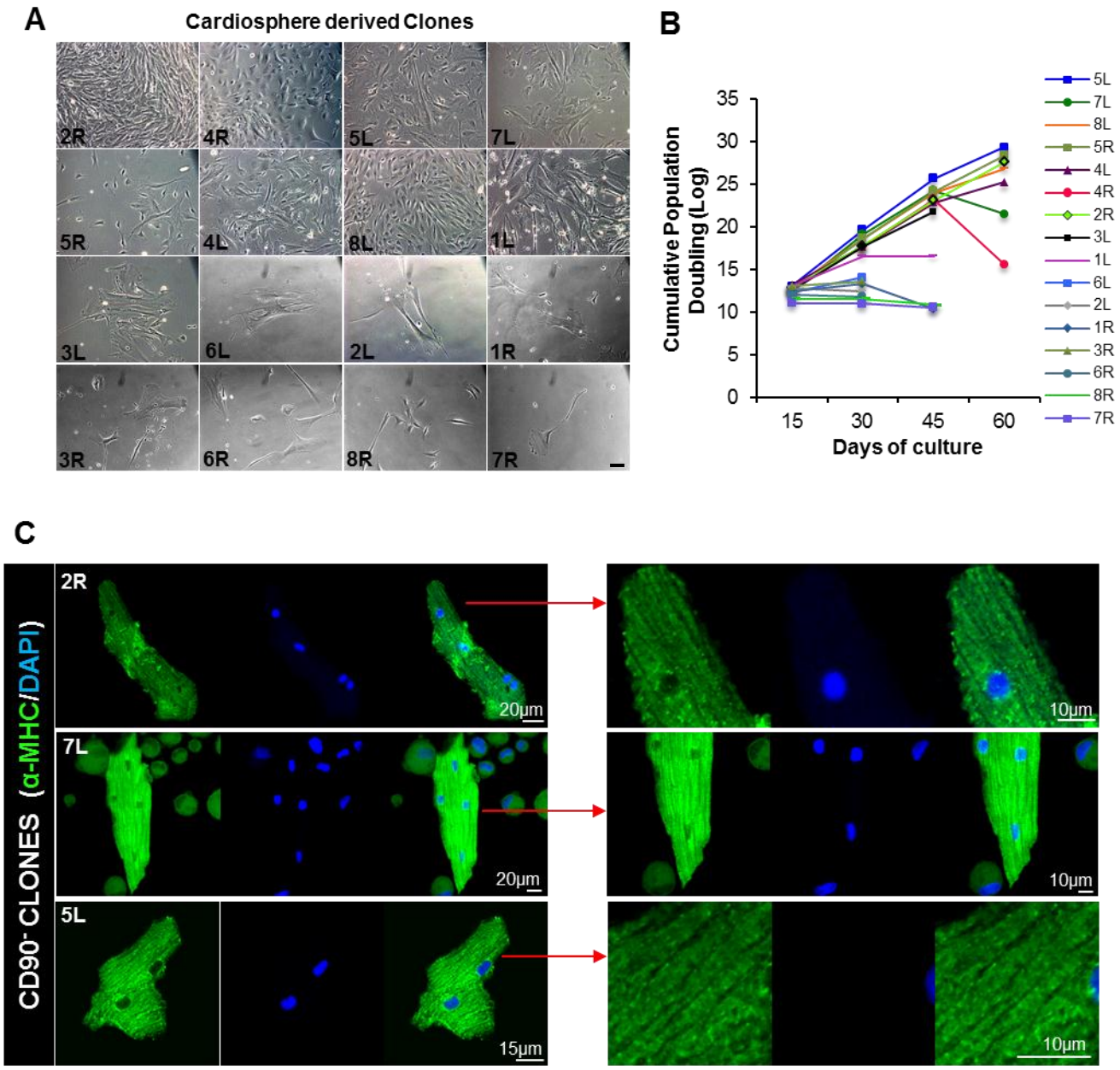

Figure S4. Isolation of clones from adult human CS-derived cells give rise mature cardiac myocytes. A, Imaging of CS-derived clones after 30 days in culture. Scale bar: 50 μm B, Cumulative

population doubling of CS-derived clones demonstrating clones with premature senescence and clones that demonstrated growth for at least 60 days in culture (clones# 2R, 5L, 7L, 5R, 4L and 8L). C, CS-derived CD90^{-} clones were differentiated and immunostained for alpha-myosin heavy chain (α-MHC,) imaged by confocal microscopy. Nuclei were stained with DAPI (blue).

FIGURE S5

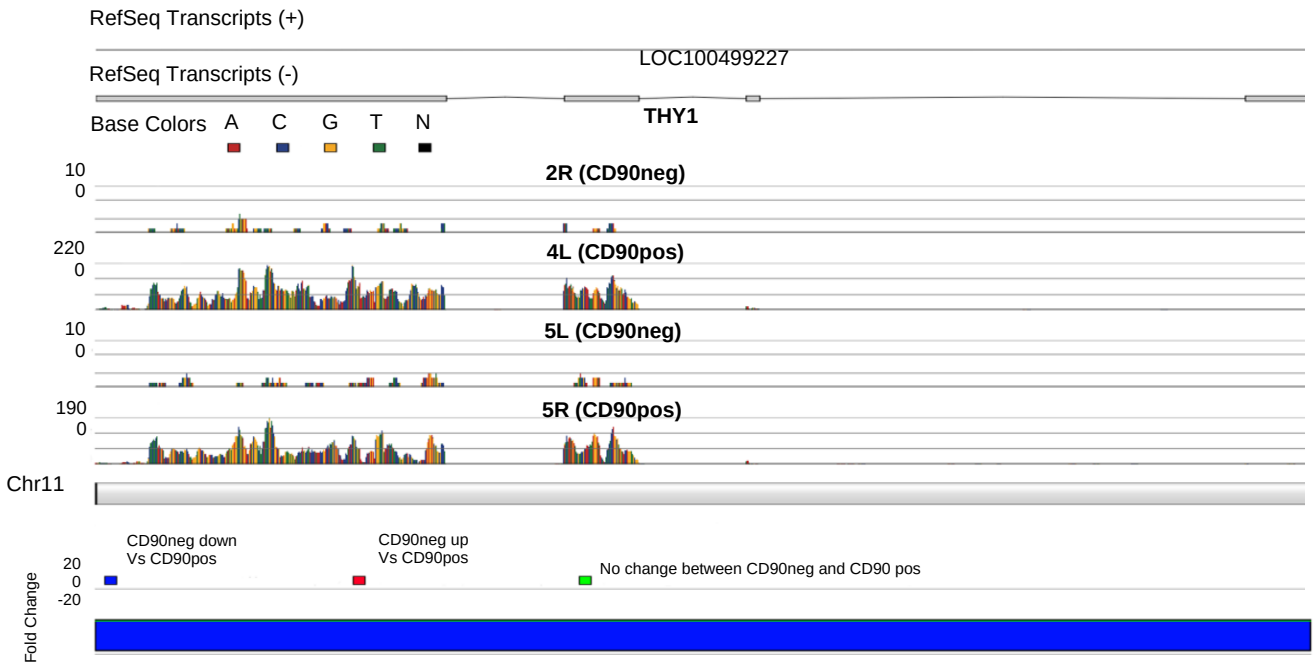

Figure S5. Chromosomal location and quantification of CD90 transcripts within adult human CDC clones in RNA-seq analysis. Quantification of CD90 transcripts for CD90^{+} (2R, 5L; 10 transcripts) versus CD90^{-} clones (4L, 5R; 190-220 transcripts) located on Chromosome 11. Blue line represents fold change in CD90^{-} versus CD90^{+} clones.

FIGURE S6

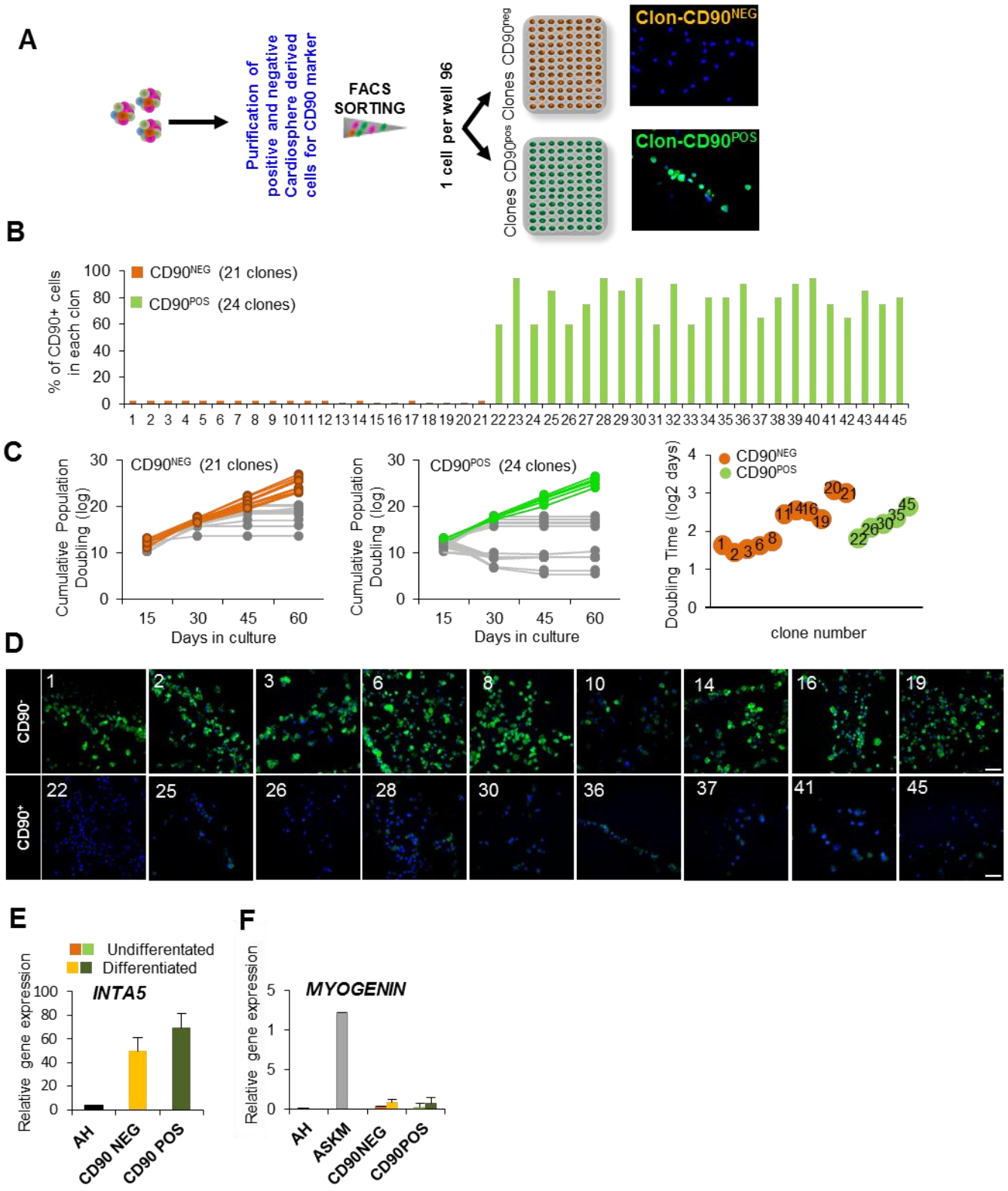
A
Purification of positive and negative Cardiosphere derived cells for CD90 marker
FACS SORTING
1 cell per well 96
Clones CD90neg
Clones CD90pos
Clon-CD90NEG
Clon-CD90POS
B
% of CD90+ cells in each clon
CD90NEG (21 clones)
CD90POS (24 clones)
C
Cumulative Population Doubling (log)
CD90NEG (21 clones)
CD90POS (24 clones)
Days in culture
Doubling Time (log2 days)
clone number
D
CD90-
CD90+
E
Undifferentated
Differentiated
INTA5
Relative gene expression
AH
CD90 NEG
CD90 POS
F
MYOGENIN
Relative gene expression
AH
ASKM
CD90NEG
CD90POS

Figure S6. Analysis of CD90$^-$ and CD90$^+$ CS-derived clones. A, Schematic diagram outlining strategy for purification, cloning and imaging of CD90$^-$ and CD90$^+$ CS-derived clones. B, Pure CD90$^-$ and pure CD90$^+$ CS-derived clones were choose by quantification of percentaje of cells positive or negative for CD90 marker. C, Cumulative population doubling of CS-derived clones demonstrating clones with premature senescence (left-CD90$^-$ and middle-CD90$^+$ graphs: grey lines) and clones that demonstrated growth for at least 60 days in culture (orange for CD90$^-$, green for CD90$^+$); Doubling cell time (days) for proliferative CD90$^-$ and CD90$^+$ CS-derived clones (right graph). D, After 15 days in differentiation of CD90$^-$ and CD90$^+$ CS-derived clones, cells were recollected, stained for α-actinin and cytospined for confocal imagen. Scale bar: 100 µm. E and F, Relative gene expression in undifferentiated and differentiated CD90$^-$ and CD90$^+$ CS-derived clones for *INTA5* (Integrin α5) and *MYOGENIN*. AH: Adult heart; ASKM: Adult skeletal muscle. N=26, **P*< 0.05

FIGURE S7

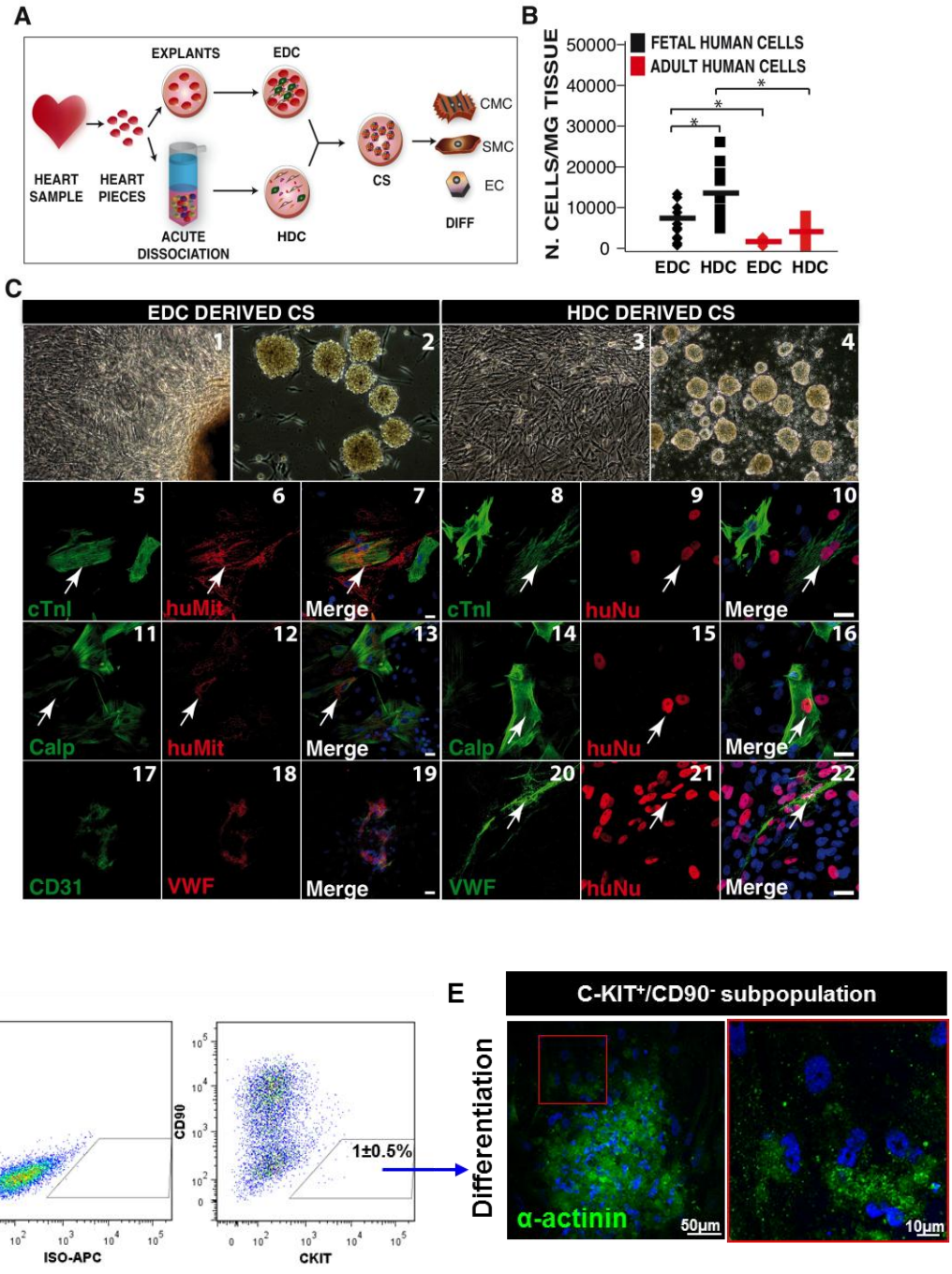

Figure S7. Yield and differentiation of CS derived cells from explant derived cells versus heart derived cells (A-C). Purification and cardiomyogenic differentiation of C-KIT^{+}/CD90^{-} cell fraction (D-E). A, Experimental design scheme: CS were created from cells obtained from acute dissociated heart tissue which we have called Heart derived cells (HDC) versus CS created from ourtgrowth cells

from heart explants called Explant derived cells (EDC). **B,** Comparison of yields of cells obtained of HDC versus EDC from fetal and adult heart biopsies. **C**, Comparison of CS formation (c1-4) and differentiation (c5-22) from HDC versus EDC cells. Cells from both sources readily formed cardiospheres that morphologically looked similar (Fig. C, c2, c4). Cells from both types of cardiospheres could differentiate into cardiomyocytes (cTroponinI, c5-c10), smooth muscle cells (Calponin, c11-c16) and endothelial cells (VWF or CD31, c17-c22) when placed in traditional differentiation condition coculture with neonatal rat ventricular cardiomyocytes. Human specific mitochondrial (HuMit) or/and Human specific nuclei (HuNu) were used to distinguish human versus rat cells. Nuclei were stained with DAPI (blue). Scale bar: 40 μm. **D-E,** Flow cytometry analysis and FACS sorting of the C-KIT^{+}/CD90^{-} cell fraction (**D**) and cardiomyogenic differentiation after 15 days in CMM medium (**E**). Nuclei were stained with DAPI (blue). All experiments were repeated with cells from three independent heart samples.

SUPPLEMENTARY TABLES

Supplementary Table 1. Human heart biopsies processed in this study.

ID # sample [(1)]	Age F-w A-y [(2)]	Sex [(3)]	Recovery cells / mg tissue	NO-VAD POST-VAD [(4)]	Remarks [(5)]
FH3	17 w	N/A*	11652	N/A	N/A
FH10	16 w	N/A	33333	N/A	N/A
FH13	15 w	N/A	21782	N/A	N/A
FH14	14 w	N/A	19444	N/A	N/A
FH21	15 w	N/A	4081	N/A	N/A
FH23	8 w	N/A	3333	N/A	N/A
FH24	16 w	N/A	7353	N/A	N/A
FH26	13 w	N/A	13676	N/A	N/A
FH27	14 w	N/A	30612	N/A	N/A
FH28	15 w	N/A	18656	N/A	N/A
FH34	16 w	N/A	12643	N/A	N/A

FH35	16 w	N/A	21839	N/A	N/A
FH36	14 w	N/A	8461	N/A	N/A
FH37	13 w	N/A	9803	N/A	N/A
FH40	10 w	N/A	8064	N/A	N/A
FH41	11 w	N/A	5833	N/A	N/A
AH9	17 y	M	3077	N/A	N/A
AH11	74 y	M	11206	NO-VAD	DCM
AH12	59 y	M	6450	NO-VAD	DCM
AH20	42 y	M	1500	NO-VAD	ICM
AH38	33 y	F	4742	NO-VAD	CHD
AH39	22 y	F	4545	POST-VAD	DCM
AH42	45 y	M	2857	N/A	N/A
AH43	10 y	F	3714	POST-VAD	DCM
AH44	49 y	M	4918	NO-VAD	DCM
AH45	42 y	M	6954	POST-VAD	ICM
AH48	57 y	F	2000	NO-VAD	ICM
AH49	53 y	M	2250	NO-VAD	ICM
AH50	56 y	F	1647	POST-VAD	DCM/ICM
AH51	17 y	M	1433	POST-VAD	DCM
AH52	59 y	M	5389	N/A	ICM
AH53	50 y	M	3540	N/A	DCM
AH54	30 y	M	3567	N/A	DCM

[1] FH = Fetal Heart; AH = Adult Heart
[2] F-w = Fetal age (weeks); A-y = Adult age (years)
[3] M = Male; F = Female
[4] VAD = Ventricular Assist Device
[5] DCM = Dilated Cardiomyopathy; ICM = Ischemic Cardiomyopathy; CHD = Coronary Heart Disease
* N/A = Not Applicable

Supplementary Table 2. RNA-seq differential gene expression CD90$^-$ vs CD90$^+$ clones.

(Posted as separate Excel File)

Supplementary Table 3. GO biological process involved in CD90$^-$ VS CD90$^+$ clones.

(Posted as separate Excel File)

Supplementary Table 4. Canonical Pathways and molecules enrichment into CD90$^-$ vs CD90$^+$ human cardiospheres derived clones.

Ingenuity Canonical Pathways	-log(p-value)	Ratio	Molecules
Role of Oct4 in Mammalian Embryonic Stem Cell Pluripotency	3	0.09	CCNF, PHC1, NR2F6, ASH2L
Methylglyoxal Degradation III	2	0.09	AKR1C1/AKR1C2, ADH4
GNRH Signaling	2	0.03	MAP3K10, PAK4, MAP2K7, KRAS, DNM2
FAK Signaling	2	0.04	DOCK1, PAK4, KRAS, GIT2
Sertoli Cell-Sertoli Cell Junction Signaling	2	0.03	MAP3K10, CDH1, MAP2K7, ZAK, KRAS, CLDN22
Glutamine Biosynthesis I	2	0.13	CCDC92
Glutamine Degradation I	2	0.20	GLS
SAPK/JNK Signaling	2	0.04	MAP3K10, MAP2K7, ZAK, KRAS
HGF Signaling	1	0.04	DOCK1, MAP3K10, MAP2K7, KRAS
Paxillin Signaling	1	0.04	DOCK1, PAK4, KRAS, GIT2
Glutathione Redox Reactions II	1	0.14	TXNDC12
Inosine-5'-phosphate Biosynthesis II	1	0.06	ATIC
Germ Cell-Sertoli Cell Junction Signaling	1	0.03	MAP3K10, PAK4, CDH1, MAP2K7, KRAS
Remodeling of Epithelial Adherens Junctions	1	0.04	CDH1, CLIP1, DNM2
Huntington's Disease Signaling	1	0.03	HSPA4, MAP3K10, MAP2K7, GLS, VTI1B, DNM2
Type II Diabetes Mellitus Signaling	1	0.02	MAP2K7, SLC27A1, CEBPB, SMPD2
NF-κB Signaling	1	0.03	AZI2, BMPR1B, MAP2K7, ZAP70, KRAS
Triacylglycerol Biosynthesis	1	0.04	DGAT1, ELOVL6
Stearate Biosynthesis I (Animals)	1	0.04	SLC27A1, ELOVL6
Signaling by Rho Family GTPases	1	0.02	MAP3K10, PAK4, CDH1, MAP2K7, SEPT1, CLIP1
VDR/RXR Activation	1	0.04	YY1, CEBPB, CALB1
NRF2-mediated Oxidative Stress Response	1	0.03	MAP2K7, VCP, DNAJC14, KRAS, JUNB
Regulation of IL-2 Expression in Activated and Anergic T Lymphocytes	1	0.03	MAP2K7, ZAP70, KRAS
EIF2 Signaling	1	0.03	RPL24, RPS4Y1, EIF3I, RPL35, KRAS

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Derivation of primary cardiac cell culture from human heart samples

To obtain acutely dissociated mononuclear heart cells (AD), the ventricular samples were washed with Hanks' balanced salt solution (HBSS), minced into small pieces (1-2 mm^3 pieces), and incubated in Type IV Collagenase (1mg/ml, Gibco), Dispase (0.15mg/ml, Gibco) and DNAse I (7.5µg/ml, Sigma) for 30-60

min at 37°C until partially digested. Partially digested tissues were manually dissociated by trituration until single cells are obtained. The cell suspension was centrifuged (5 min, 1200 rpm) and pellet resuspended and passed through a 40-µm cell strainer (BD Biosciences). 25,000 cells/cm^2 were cultured in T-75 culture flasks (BD Falcon) pre-coated with Fibronectin (20µg/mL DPBS) with growth media (GM) [IMDM with 20% FBS, 5 ng/ml bFGF (Peprotech), 1% penicillin-streptomycin, 1% L-glutamine, and 0.001% 2-mercaptoethanol]. HDC adopted fibroblast-like morphology after 3 days in culture (Fig1A).

Cardiovascular differentiation in vitro

Primary CS (5-10 units) or adherent cultures (10,000 cells /cm^2) were seeded on 12-mm diameter laminin-coated (Sigma-Aldrich) coverslips (Menzel-Gläser, Braunschweig, Germany, http://www.menzel.de). Four distinct differentiation medias were used: (a) *basal medium*: same as CS-GM without growth factors and supplemented with 10% FBS (Gibco); (b) *cardiomyogenic medium*: CS were incubated in basal medium for 3 days and then in DMEM-F12 3:1 supplemented with 1% N2 (Gibco, Grand Island, NY, http://www.invitrogen.com), and 100 ng/ml Heregulin-β1 (Peprotech, Rocky Hill, NJ, http://www.peprotech.com) for 12 days. For long differentiation (60-90 days), CS derived clones were incubated in GM without growth factors and supplemented with 2% B27; (c) *smooth-muscle medium*: same components as basal media with additional 10 ng/mL platelet-derived growth factor–beta (PDGF-β, Peprotech). (d) *Endothelial medium*: same components as basal media with additional 50 ng/mL vascular endothelial growth factor (VEGF, Peprotech). For endothelial cell tube formation assay we followed the BD protocol (BD Matrigel matrix cat. No. 354234). Briefly, 300 µl of BD Matrigel was added in 24-well culture plates on ice and incubates at 37ºC for 30-60 minutes. Then, 5-10 CS were seeded over polymerized matrigel and were grown in endothelial medium. Capillary tube formation was assessed after 24-48 h using an inverted microscope (Zeiss, model Axiovert 200) fitted with a AxioCam MRC digital camera (Photometrics, Tucson, AZ). Images were acquired and processed using AxioVision Release 4.6 software. All differentiation experiments were performed for 2-3 weeks, replacing differentiation media every 3 days.

Immunofluorescence in cover-slides and cytospin

Cells were rinsed with PBS and fixed with 4% paraformaldehyde for 15 minutes, PBS-washed three times, and processed for immunofluorescence. Cells were permeabilized with 0.5% Triton X-100 for 10 minutes, washed twice with PBS, and blocked with 10% normal donkey serum in PBS (Jackon InmunoResearch laboratories, Inc) for 20 minutes at RT. Primary and secondary antibodies were incubated with 10% normal donkey serum (in PBS) for 1 hour each at RT, with three 3 minutes PBS washes in between. Cells were washed again with PBS and nuclei were counterstained with Dapi (Molecular Probes Inc.) or Hoechst 33258 (Sigma-Aldrich) depending on the experiment. Primary antibodies used include human cTNT (R&D), cTNI (Santa Cruz), alpha-sarcomeric actin (Sigma), alpha-MHC (Abcam), Calponin (Abcam), VWF (Dako) and NKX2.5 (Santa Cruz clone A-16). Secondary antibodies conjugated to the appropriate fluorophores (Alexa Fluor 488, Alexa Fluor 555, Alexa Fluor 647; Molecular Probes Inc., Eugene, OR, http://probes.invitrogen.com). After staining, cells were then washed twice with PBS and mounted with Mowiol (Sigma) to be analyzed by confocal microscopy. For cytospin samples, 2.5-5x10^4 cells were suspended in 100ul of cold 1% FBS/ 1% BSA-PBS and spun down onto coated slides using a ThermoShandon Cytospin 4 apparatus (Thermo Shandon Inc., Pittsburgh,PA, http://www.thermo.com). The slides were then mounted with Mowiol and analyzed by confocal microscopy.

Calcium Fluorescence Imaging

Cell cultures were incubated with the fluorescent calcium indicator dye Fluo-4 AM (10 μM, Invitrogen) and nonionic surfactant Pluronic F-127 (0.02%, Invitrogen) in cardiomyogenic medium for 30 minutes. Following two gentle washes with PBS 1X, the cells were bathed in media without Fluo-4 for imaging. Spontaneous calcium fluorescence activity in cell cultures was imaged and recorded using an inverted Nikon Diaphot microscope (60X objective, Olympus) equipped with a charge-coupled device (CCD)-based Photometrics Cascade 128+ camera (105 frames per second, spatial resolution 128 x 128 pixels) operating under Imaging Workbench software (version 6.0, INDEC BioSystems). The acquired video image data were processed using ImageJ software (National Institutes of Health).

Flow Cytometry

Primary cultures, CS or differentiated cultures were dissociated with 0.1% Trypsin-EDTA (sigma) for 4 min at 37C, PBS-washed, counted, resuspended in PBS with 1% FBS, 1% BSA and 0.1% Sodium Azide (Staining buffer), and stained for 30-40 min at 4C with the following surface markers (BD Biosciences) depend on experiment: CD31 (ref 555445), CD144 (clon 55-7H1), CD34 (clon 8G12), CD90 (ref 555595), CD114 (ref 554538), C-KIT (ref 341096) CD184 (ref 555974), CD271 (Clon C40-1457) and from R&D: FLK1 (FAB357A), FLT1 (FAB321A), FLT4 (FAB3492P). To control for non-specifically bound fluorescence, isotype antibodies used (BD Biosciences) were FITC, PE and APC Mouse IgG1 k isotype controls. For intracellular proteins, staining was carried out on cells fixed with 2% paraformaldehyde (Electron Microscopy Sciences, Hatfield PA) in PBS for 10 min and washed with PBS. Staining with primary and secondary antibodies was done in staining buffer plus 0.25% Saponin (Sigma) for 45 min at room temperature washing with PBS three times. Matched control secondary IgG antibodies used were for FITC, PE and APC (BD Biosciences) experiments.

Cell Sorting

HDC and CS-derived cells from three independent fetal and/or adult human heart samples were resuspended in Ca++/Mg++-free Dulbecco's phosphate-buffered saline (DPBS, Cellgro) with 10% FBS and filtered throught a 40 µm cell strainer onto 5 ml polypropylene sterile tubes. Presorted samples were kept in agitation at 4ºC. Scatter parameters were acquired on linear mode and fluorescence parameter on log mode. PMT setting were established on live (AAD-7 negative) single cells by using unstained cells. Non-specific binding was evaluated with the corresponding isotype controls used at the same concentration to the antibody of interest. Sorting procedures were done excluding dead cells and doublets. Sorted cells were expanded in proliferation medium (with serum) and then used to create CS.

Clonal Assay

After 7 days in CS culture (suspension without serum), CS-derived cells were disaggregated in single cells to generated clones on 96 well plates treated with fibronectin (adhesion with serum). To obtain single cells, CS-derived cells were washed with DPBS, trypsinized with a 0.1% trypsin solution (Sigma) for 4 min at 37°C, neutralized with serum and centrifuged at 1,200 rpm for 5 min. Pellet was resuspended in Proliferating medium and cell number was determined using a hemocytometer. Cells were diluted with medium to a calculated concentration of 1 cell per 100 µl, and 100 µl of this suspension was then added to individual wells of a 96-well culture plate treated with fibronectin for adhesion culture. After several hours, each well was scored for the presence or absence of single cells. For each well containing a single cell, additional 100 µl of proliferating medium was added. Then, medium was changed two times in the week. Clones were monitored and photographed over a 4-6 week period, at the end of which the wells were confluent. Each clone was dissociated and transferred to a single well of a 24-well plate, and after 10-12 days in proliferation medium, cells were again dissociated and transferred to a flask 25 cm^2.

Direct cell cloning of CS-derived $CD90^-$ and $CD90^+$ cells was carried out using BD FACSAria III cell sorter. The cells were sorted directly into separate wells of a 96-well plate (one cell per well) containing 200 µl of medium using a CloneCyt automated cell deposition unit. Calibration of the clone sorting using fluorescent beads showed that <1% of the wells received more or less than one bead.

RNA isolation and quantitative real time-PCR

Total RNA was prepared with the RNeasy mini or micro kits (QIAGEN) and treated with RNase-free DNase (QIAGEN). 100 ng to 1 mg RNA was reverse transcribed into cDNA via random hexamers and Oligo (dT) with Superscript III Reverse Transcriptase (Roche). Real-time quantitative PCR was performed on a MasterCycler EP RealPlex (Eppendorf). All experiments were done in triplicate with SYBR Green JumpStart Taq ReadyMix (Sigma). The oligonucleotide sequences are *CD90* forward: TCAGGAAATGGCTTTTCCCA; *CD90* reverse: TCCTCAATGAGATGCCATAAGCT. *CD105* forward: CATCCTTGAAGTCCATGTCCTCTT; *CD105* reverse: GCCAGGTGCCATTTTGCTT. *NKX2-5* forward:

ACCTCAACAGCTCCCTGACTCT; *NKX2-5* reverse: ATAATCGCCGCCACAAACTCTCC. *ISL-1* forward: GAAGGTGGAGCTGCATTGGTTTGA; *ISL-1* reverse: TAAACCAGCTACAGGACAGGCCAA. *PERIOSTIN* forward: GGAGACAAAGTGGCTTCCGA; *PERIOSTIN* reverse: AATTGGGCCACAAGATCCGT. *ACTA2* forward: TCTCTATGCTAACAACGTCCTGTCA; *ACTA2* reverse: CCACCGATCCAGACAGAGTACTT. *TNNT2* forward: TTCACCAAAGATCTGCTCCTCGCT; *TNNT2* reverse: TTATTACTGGTGTGGAGTGGGTGTGG; *INTA7* forward: CTCCTGTGGAAGATGGGATTCT; *INTA7* reverse: GTCTTCTCCTCCTTGAACTGCT. *INTA5* forward: CCTATGAGGCTGAGCTTCGG; *INTA5* reverse: GGTGCAGTTGAGTCCCGTAA. *MYOGENIN* forward: AAACTACCTGCCTGTCCACC; *MYOGENIN* reverse: GAGCAGGGTGCTTCTCTTCA. Gene relative expression was calculated in relation to their *GAPDH* quantitative expression and normalized.

RNA-seq

Whole-genome transcriptomic analysis was performed by Next-Gen RNA-sequencing in two groups of CDC: CD90neg (clones 2R and 5L) and CD90pos (clones 5R and 4L). Next-Gen library construction and sequencing procedures were carried out by High Throughput Genomic Center facility in the Department of Genome Sciences at University of Washington. After confirmation of RNA quality by Agilent BioAnalyzer 2100, the stranded and indexed RNA-Seq libraries were constructed from total RNA using True Seq Stranded Total RNA LT with Ribo-Zero TM Human/Mouse/Rat Set A kit (Illumina, San Diego, CA) as per the manufacturer's recommendations. The libraries constructed underwent Pilot sequencing at 24-plex to assess library quality while providing a quantification for in-depth sequencing. The libraries were finally sequenced to provide single-end 1X36 reads to depths of 30-40 M tags per sample. Sequencing reads were filtered for polymers, primer adaptors, and ribosomal RNAs and then mapped against the human genome assembly (NCBI Build 37.1) using Bowtie pipeline. Data was imported to Partek Genomics Suite 6.12 for analysis of read alignment and counting, and evaluation of differential gene expression between CD90neg and CD90pos CDCs using ANOVA test on RPKM of genes. Gene reads between groups having a fold-change of 1.5 or more and $p<0.05$ were considered to be significant. These differentially expressed genes (DEGs) were further analyzed on canonical

Pathways and networks, as well as upstream regulators such as transcription factors with Ingenuity pathway Analysis (IPA, Ingenuity Systems, Inc, Redwood City, CA).

SUPPLEMENTARY VIDEO CAPTIONS

Video S1. Calcium fluorescence imaging of fetal human CS-derived cells demonstrating beating cardiomyocytes. Cells were incubated with the fluorescent calcium indicator dye Fluo-4 AM and nonionic surfactant Pluronic F-127 in cardiomyogenic medium for 30 minutes. Spontaneous calcium fluorescence activity in cell cultures was imaged and recorded. The acquired video image data were processed using ImageJ software.

(Attached as separate Movie File)

Video S2. Calcium fluorescence imaging from adult human CS-derived cardiac myocyte-like cells. Cell culture were incubated with the fluorescent calcium indicator dye Fluo-4 AM and nonionic surfactant Pluronic F-127 in cardiomyogenic medium for 30 minutes. Spontaneous calcium fluorescence activity in cell cultures was imaged and recorded but no calcium transients were observed. The acquired video image data were processed using ImageJ software.

(Attached as separate Movie File)