Supporting Information

Evseenko et al. 10.1073/pnas.1002077107

SI Materials and Methods

Human Embryonic Stem Cell (hESC) Cultures. hESC lines H1, H9, and HES3 obtained from WiCell were maintained on irradiated primary mouse embryonic fibroblasts (MEFs) in media recommended by WiCell. hESCs were routinely characterized and found to have a normal karyotype and expression of pluripotency markers SSEA4, CD9, OCT4, and alkaline phosphatase.

Mesoderm Induction Conditions. To induce mesoderm differentiation, colonies of H1, H9, or HES3 were cut into uniform-sized pieces using the StemProEZPassage tool (Invitrogen), transferred into 6-well plates precoated for 1 h with Matrigel (growth factor reduced, no phenol red; BD Biosciences), and cultured initially in TESR medium (Stem Cell Technologies) until 50–60% confluent (typically 2 d). To induce differentiation, TESR medium was replaced with basal induction medium Stemline II (Sigma-Aldrich). Basal induction medium was supplemented with the following proteins either as single agents or in combination: human vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (bFGF), bone morphogenic protein 4 (BMP4), nodal, transforming growth factor-β1 (TGF-β1), and activin A (all at 10 ng/mL; R&D Systems) (Fig. S1*A*). On the basis of production of $CD326^{-}CD56^{+}$ cells (hEMP), the optimal induction medium was found to be basal medium supplemented with BMP4, VEGF, and bFGF, (all 10 ng/mL) with the inclusion of activin A (10ng/mL) on day 1 only (aka "A-BVF") (Fig. S1 *A* and *B*). Thus, for mesoderm induction in all subsequent experiments, hESCs were cultured in A-BVF medium, during which medium was refreshed every second day. After mesoderm induction, $CD326^{-}CD56^{+}$ cells were typically isolated by fluorescence activated cell sorting (FACS) at day 3.5 (unless otherwise described) and further differentiated into mesodermal lineages in hematoendothelial, cardiac, or mesenchymal stem cell conditions as described below.

Differentiation of $CD326^{-}CD56^{+}$ Cells to Hematoendothelial Lineages. For hematoendothelial differentiation, cell populations generated in the A-BVF mesodermal induction conditions, were seeded at 10–20,000/well into 48-well tissue culture plates, which had been prepared with the murine bone marrow cell line OP9 (American Type Culture Collection), 1 d before seeding as previously described (3). Hematoendothelial differentiation was first carried out in EGM-2 complete medium (Lonza) and after 7 d the EGM-2 medium was substituted with a new medium composed of Dulbecco's modified eagle medium (DMEM)/Hanks F-12 medium (Invitrogen) with 2 mM Glutamax (Invitrogen) in the presence of 10% fetal bovine serum (FBS) and the following cytokines: stem cell factor (SCF, 50 ng/mL), FMS-like tyrosine kinase-3 ligand (Flt-3, 10 ng/mL), thrombopoietin (TPO, 10 ng/mL), VEGF (10 ng/mL), interleukin-3 (IL-3, 5 ng/mL), erythropoietin (Epo, 2 U/mL) (R&D Systems), and with ALK 4/5/7 blocker SB-431542 (Sigma-Aldrich) added at a final concentration 5 μM. For endothelial differentiation complete EGM-2 media additionally supplemented with VEGF (50 ng/mL) was used during the entire period of differentiation (up to 14 d). Culture medium was replaced every 3 d. For clonal hematopoietic assays, 10,000 cells were directly plated into 6-well plates containing 2 mL semisolid MethoCult medium (Stem Cell Technologies) and cultured for up to 3 wk.

Differentiation of $CD326^{-}CD56^{+}$ Cells to Smooth Muscle Cells and Cardiomyocytes. $CD326^{-}CD56^{+}$ cells (hEMP) were isolated by FACS after 3.5 days of mesoderm induction in A-BVF medium, and reaggregated as previously described (4). In brief, FACS-isolated cells were resuspended in cold differentiation medium with 0.1 mg/mL Matrigel, transferred into low attachment 96-well plates (10,000 cells per well in 50 μL of media), and then centrifuged at 1,400 RPM for 5 min at 4 °C. After centrifugation, cultures were placed at 37 °C for 2 h and then reassembled cell colonies were carefully transferred onto Matrigel-coated chamber slides (smooth muscle cell differentiation) in SMGM-2 complete medium (Lonza). For cardiomyocyte differentiation cells were plated on gelatin-coated plates in the 1:1 mixture of SMGM-2 and EGM-2 medium supplemented with DKK-1 (150 ng/mL; R&D Systems).

Differentiation of $CD326^{-}CD56^{+}$ Cells to Mesenchymal Lineage. For mesenchymal cell differentiation, $CD326^{-}CD56^{+}$ cells were isolated by FACS after 3.5 days of induction in A-BVF conditions and plated on Matrigel-coated plates or OP9 stroma in EGM-2 complete medium (Lonza). After 7 additional days of culture, $CD73^{+}CD105^{+}CD34^{-}$ were isolated by FACS. These isolated $CD73^{+}CD105^{+}CD34^{-}$ cells were replated as large (10,000 cells) reaggregated colonies in culture plates or chamber slides in STEMPRO chondrogenic differentiation medium (Invitrogen) or STEMPRO osteogenic differentiation medium (Invitrogen) for cartilage and bone tissue differentiation, respectively. For adipocyte differentiation, isolated cells were cultured in DMEM with 12.5% FCS, 12.5% normal horse serum (both from Omega Scientific), 2 mM Glutamax (Invitrogen), and 100 nM hydrocortisone (Sigma-Aldrich) and cultured for up to 4 wk at 37 °C.

Mesenchymal cultures were fixed in 4% paraformaldehyde and then stained with Oil Red O for detection of neutral fat, von-Kossa reagent for detection of calcium deposits, and Alcian blue for cartilage-associated acid mucopolysaccharides and glycosaminoglycans (all reagents obtained from Sigma-Aldrich).

Differentiation to Ectodermal and Endodermal Lineages. For neuroectodermal cell differentiation, colonies of undifferentiated hESCs (H9 or HES3 lines) or $CD326^{-}CD56^{+}$ cells isolated at day 3.5 from A-BVF mesoderm induction culture were reaggregated and plated on Matrigel-coated plates or on MEF feeder layers in Neurobasal medium with B27 supplement (both obtained from Invitrogen) and noggin (250 ng/mL) as previously described (1). For endodermal differentiation, the same cell types were plated on Matrigel-coated plates or MEF feeders in previously reported endoderm-inducing conditions (2). In brief, cells were exposed to 25 ng/mL Wnt3a + 50 ng/mL activin A (both R&D Systems) in Advanced RPMI (Invitrogen) supplemented with 1× L −Glu and 0.2% FBS (Omega Scientific) for 1 d, then activin A in the same medium, and then cultured for 4–6 d to induce endoderm differentiation, and then the cells were transferred in DMEM supplemented with 1× L −Glu and 1× B27 (Invitrogen) (2). Both neuroectoderm and endoderm cultures were continued for up to 3 wk.

Antibody Staining and Flow Cytometry. For FACS analysis, cultured cells were dissociated into a single cell suspension with Tryple Select (Invitrogen) and then incubated with monoclonal antibodies against CD10, CD34, CD73, CD140a, CD143, CD144, CD235, CD326, SSEA4, CD34, CD45, CD117 (peridinin chlorophyll protein complex (PerCP)/Cy5.5 conjugated), CD9 CD31, CD34, CD9, CD56, CD105 [fluoresceinisothiocyanate (FITC)-conjugated] (all from BD Biosciences), KDR/VEGFR2 [allophycocyanin (APC)-conjugated, R&D Systems], SSEA4 (APC conjugated, BD Biosciences), and CD140a biotinylated, BioLegend).

Streptavidin-ECD (Immunotech) was used for the second step with CD140a biotinylated antibody. Note, the best separation of the $CD326^{+}CD56^{-}$ and $CD326^{-}CD56^{+}$ populations was achieved using PerCP/Cy5.5 conjugated anti-CD326 and FITC or PE labeled anti-CD56 antibodies.

For intracellular protein detection, cells were dissociated into single cell suspension using Tryple Select (Invitrogen) and processed with blocking and permeabilization buffer (BD Biosciences) and stained with PE-conjugated anti-CTNT (cardiac troponin T) according to the manufacturer's protocol. The conjugation of the CTNT antibody (clone 1C11, Abcam) with PE was carried using Zenon PE labeling kit (Invitrogen) according to the manufacturer's protocol. Control samples were processed without antibody against CTNT. After incubation, cells were washed in PBS containing 1% BSA and analyzed using a BD FACSAria or LSRII cytometer (BD Biosciences). FACS files were exported and analyzed using FACSDiva software (BD Biosciences).

Immunohistochemistry. For immunohistochemical analysis, colonies of undifferentiated hESC or differentiated cultures were fixed in 4% paraformaldehyde (Sigma-Aldrich) and further processed for immunostaining. Primary antibodies used were against SOX-1, FOXA2 (Abcam), CD31 (clone M20, Santa Cruz Biotechnology), cardiac troponin T (CTNT) (clone 1C11), h-caldesmon (clone hHCD; Sigma-Aldrich), α-actinin (clone EA53, Sigma-Aldrich), α-smooth muscle actin (clone 1A4; Sigma-Aldrich), sacromeric myosin (MF-20; Developmental Studies Hybridoma Bank), CD56, α-fetoprotein, HNF-4α (Epitomics), and Pax-6 (Millipore). For intracellular protein detection, cells were permeabilized with 0.5% saponin (Sigma-Aldrich) before the addition of primary antibody. For light microscopy, horse anti-rabbit HRP polymer antibody was used in combination with Vector-Vip substrate (Vector Labs). For florescent microscopy, alexa Fluor 488-conjugated goat anti-rabbit IgG (H+L) and anti-mouse IgG (1:250; all from (Invitrogen), and Cy.3 conjugated donkey anti-goat (1:500, Dako) were used. For counterstaining of cell nuclei, 4′-6-diamidino-2-phenylindole (Dapi) or TO-PRO-3 dye (both Sigma-Aldrich) were added to the final PBS washing. Staining without primary antibodies served as controls. Images were acquired using a confocal TCS SP2 AOBS laser-scanning microscope system (Leica Microsystems) with 40× [1.3 numerical aperture (NA)] and 63× (1.4 NA) oil-immersion objectives.

Teratoma Assay. Nonobese Diabetic/Severe Combined Immunodeficient/γc^{null} (NSG) mice, obtained from Jackson Laboratories, were used to assay the ability for cell populations to form in vivo teratomas, according to protocols approved by the Animal Research Committee of University of California Los Angeles. Either $CD326^{-}CD56^{+}$ cells isolated from A-BVF induction conditions or undifferentiated hESCs (H9 cell line) were resuspended in DMEM/F12 media and injected (500,000 cells/animal, $n = 3$ per group, 2 independent experiments) intramuscularly (IM, volume 50 μL). For IM injection, a sterile 1-mL syringe with a 23G needle was guided into the hind leg quadriceps along its long axis and toward the muscle center, following a well-established protocol (5).

Quantitative Real-Time PCR. SYBR Green RT-PCR amplification and detection was performed using an ABI Prism 7900 HT (Applied Biosystems) as previously described (6). The comparative C_t method for relative quantification ($2^{-\Delta\Delta Ct}$) was used to quantitate gene expression according to Applied Biosystems' recommendations (7900 HT Real-Time fast and SDS enterprise and database user guide). Expression of target genes was normalized to the level of RPL-7 and expressed relative to a calibrator (sample in each set with lowest expression). Primer sequences used for QPCR are available on request.

Semiquantitative RT-PCR. Total RNA was extracted from cells using the RNeasy mini kit, converted to cDNA using the Omniscript RT kit, and cDNA was subsequently subjected to multiplex PCR performed with Hot Start Master Mix kit (all kits from Qiagen) for the evaluation of the mRNA expression of genes of interest. PCR products were resolved by electrophoresis on 2% or 1.5% agarose gels containing ethidium bromide. Gels were scanned using the ImageStation 2000R (Kodak) and the bands were analyzed using Kodak 1D analysis software. RT-PCR primer sequences can be found in Table S1.

Microarray Hybridization and Data Analysis. Human U133plus2.0 array hybridizations were performed by the UCLA Clinical Microarray Core. Three independent differentiation experiments comparing cells from three different passages of undifferentiated hESC H9 (passages 36–41) to $CD326^{-}CD56^{+}$ cells isolated from A-BVF induction cultures initiated with the same hESCs, were analyzed separately and data processed following standard Affymetrix GeneChip Expression Analysis protocols. The acquisition of each array image was undertaken by using Affymetrix GeneChip Command Console 1.1 (AGCC). Subsequent raw data were analyzed using DNA-Chip Analyzer (D-Chip) with the .CEL files obtained from AGCC. A PM/MM difference model for estimating gene expression levels and a quantile approach for data normalization were used. Thresholds for selecting significant genes were set at a relative difference of twofold or more, absolute difference >100 signal intensity units and $P < 0.05$. Gene expression that met all three criteria simultaneously was considered as significantly changed. Global functional analyses, network analyses, and canonical pathway analyses were performed using Ingenuity Pathway Analysis.

Statistics. Descriptive statistics were performed for each dataset and the data combined for collective analysis. Data were converted to graphs with Microsoft Excel 2003. Statistical analysis was performed with SigmaStat software (Systat Software). Descriptive statistics, Student's *t*-test, and one-way ANOVA were applied followed by Student–Newman–Keul's test. $P < 0.05$ was considered to be significant.

1. Nasonkin IO, Koliatsos VE (2006) Nonhuman sialic acid Neu5Gc is very low in human embryonic stem cell-derived neural precursors differentiated with B27/N2 and noggin: implications for transplantation. *Exp Neurol* 201:525–529.
2. Borowiak M, et al. (2009) Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. *Cell Stem Cell* 4:348–358.
3. Hao QL, et al. (2008) Human intrathymic lineage commitment is marked by differential CD7 expression: identification of CD7- lympho-myeloid thymic progenitors. *Blood* 111: 1318–1326.
4. Evseenko D, et al. (2009) Identification of the critical extracellular matrix proteins that promote human embryonic stem cell assembly. *Stem Cells Dev* 18:919–928.
5. Hentze H, et al. Teratoma formation by human embryonic stem cells: Evaluation of essential parameters for future safety studies. *Stem Cell Res* February 12, epub ahead of print.
6. Evseenko DA, Paxton JW, Keelan JA (2006) ABC drug transporter expression and functional activity in trophoblast-like cell lines and differentiating primary trophoblast. *Am J Physiol Regul Integr Comp Physiol* 290:R1357–R1365.

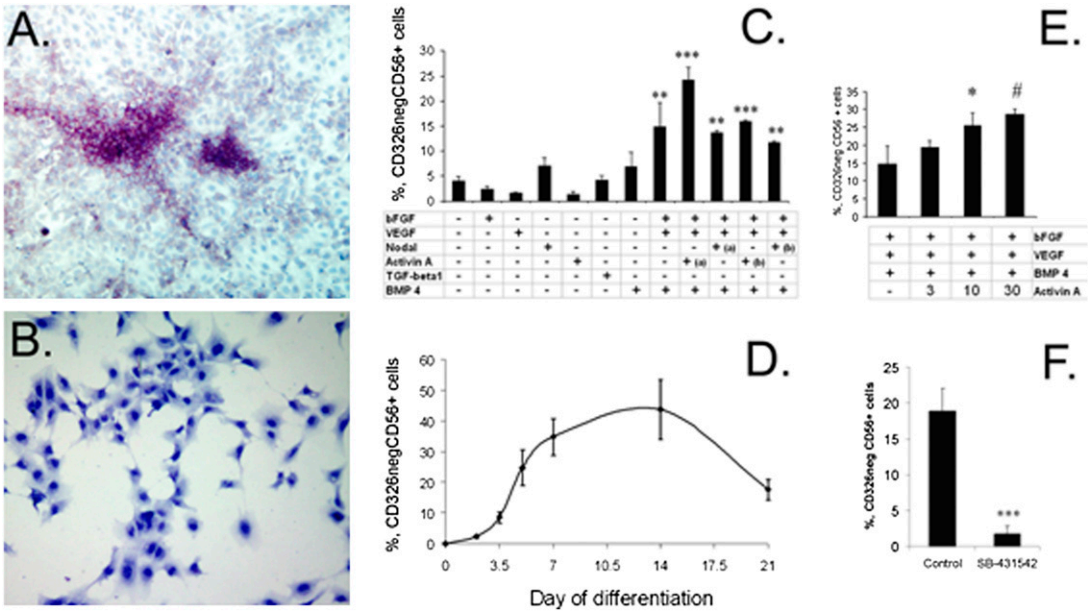

Fig. S1. Morphology, culture conditions, and time course of generation of $CD326^{-}CD56^{+}$ cells from hESCs. (*A*) Immunochistochemical detection of $CD56^{+}$ cells in differentiating hESC colonies on day 3.5 after mesoderm induction (magenta). Nuclei counterstained with hematoxylin (blue). (*B*) Morphological appearance of the $CD326^{-}CD56^{+}$ cells sorted on day 3.5 after mesoderm induction and plated on Matrigel-coated plates. (*C*) The effect of morphogens and cytokines on the generation of $CD326^{-}CD56^{+}$ cells following 5 d of mesoderm induction culture. All agents were used at a concentration of 10 ng/mL activin A and nodal were applied as single agents or in combination with BMP4, VEGF, and bFGF in two different formats, (*a*) transiently for the first 24 h of induction or (*b*) constantly for the entire duration of the culture. Shown is mean ± SD, $n = 3$ experiments. Significant increase in % $CD326^{-}CD56^{+}$ cells compared with control (no agents), $^{**}P < 0.01$; $^{***}P < 0.001$. (*D*) Time course of $CD326^{-}CD56^{+}$ cell generation in A-BVF induction conditions. (*E*) Effect on the generation of $CD326^{-}CD56^{+}$ cells of different concentrations of activin A (used transiently during the first 24 h of mesoderm induction) in combination with BMP4, VEGF, and bFGF. Shown are data from day 5 of mesoderm induction, mean ± SD, $n = 3$. Significant increase ($^{*}P = 0.011$; $^{\#}P = 0.007$) compared with no activin A. (*F*) The generation of $CD326^{-}CD56^{+}$ cells in A-BVF conditions was significantly inhibited in the presence of TGF-β inhibitor SB-431542 (5 μM, $P < 0.001$) (mean ± SD, $n = 3$. For statistical analysis, Student's *t*-test or one-way ANOVA was applied, followed by the Student–Newman–Keul's test. $P < 0.05$ was considered statistically significant.

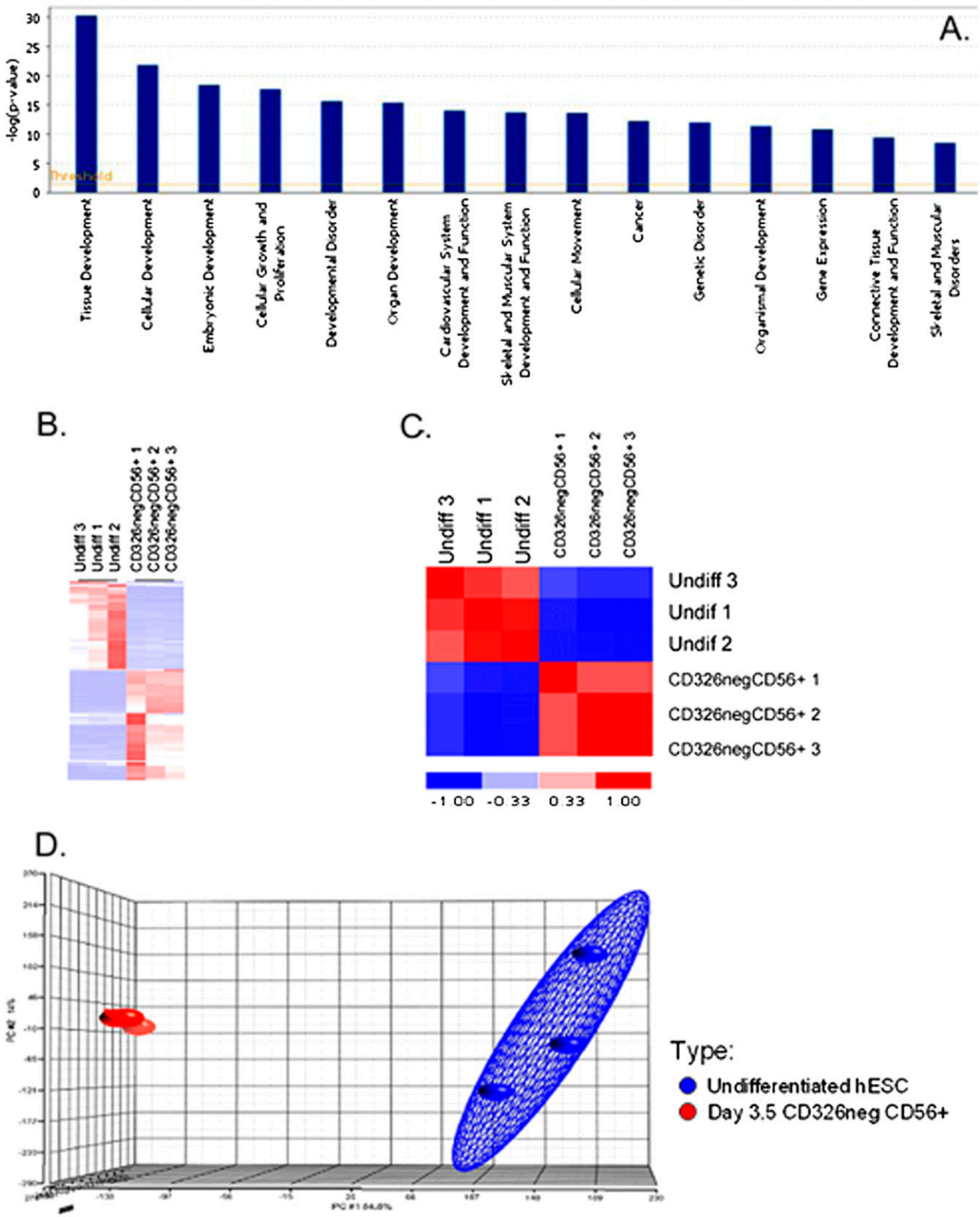

Fig. S2. Microarray analysis of day 3.5 CD326^{-}CD56^{+} cells compared with undifferentiated hESCs. (*A*) Significantly enriched biofunctional groups. Analysis of differentially expressed genes between day 3.5 CD326^{-}CD56^{+} cells ($n = 3$) and hESCs (H9) ($n = 3$) was performed using Ingenuity Pathway Analysis software. The *y* axis is log transformed *P* value. $P = 0.05$ is equivalent to *y* value of 1.3 (red line). (*B*) Unsupervised gene cluster, (*C*) correlation matrix, and (*D*) principal components analysis, demonstrate the reproducibility of data between biological replicates. The unsupervised cluster and correlation matrix were made by using 1,043 filtered genes (CV $\geq$ 1 across six samples for each gene), whereas the principal components analysis was made by using all genes.

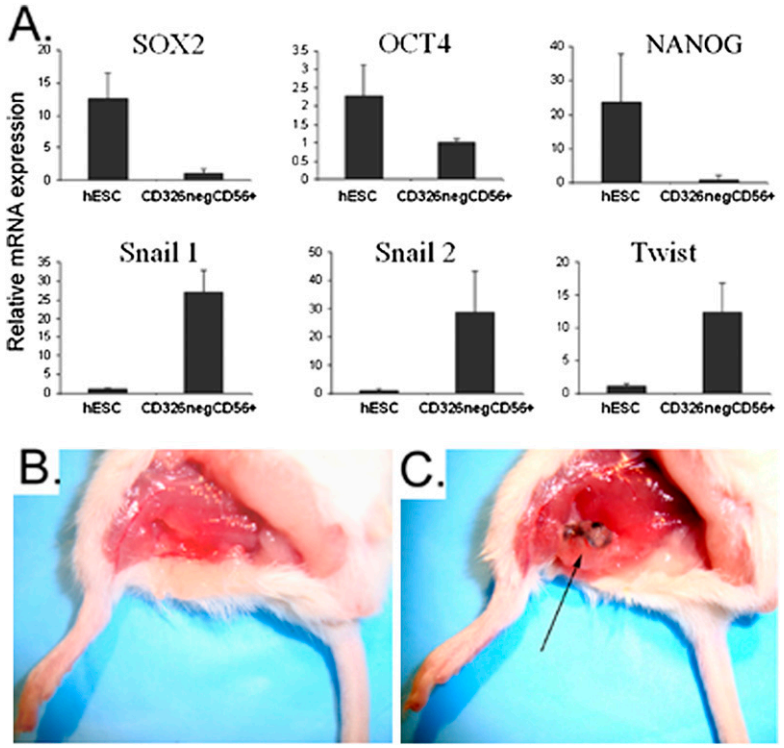

Fig. S3. Validation of the microarray data in Fig. 1*B* with real-time PCR analysis. (*A*) Relative expression of pluripotency and EMT genes in undifferentiated hESCs (H9 line) and CD326$^+$ cells isolated at day 3.5 after A-BVF induction. Teratoma formation assay. Day 3.5 CD326$^-$CD56$^+$ cells injected intramuscularly into the hind leg quadriceps along its long axis (500,000 cells per animal) of NSG mice (*B*) did not give rise to teratomas (one animal of total of six shown). (*C*) All control animals injected with equivalent numbers of undifferentiated hESCs developed teratomas (one animal of total of six shown).

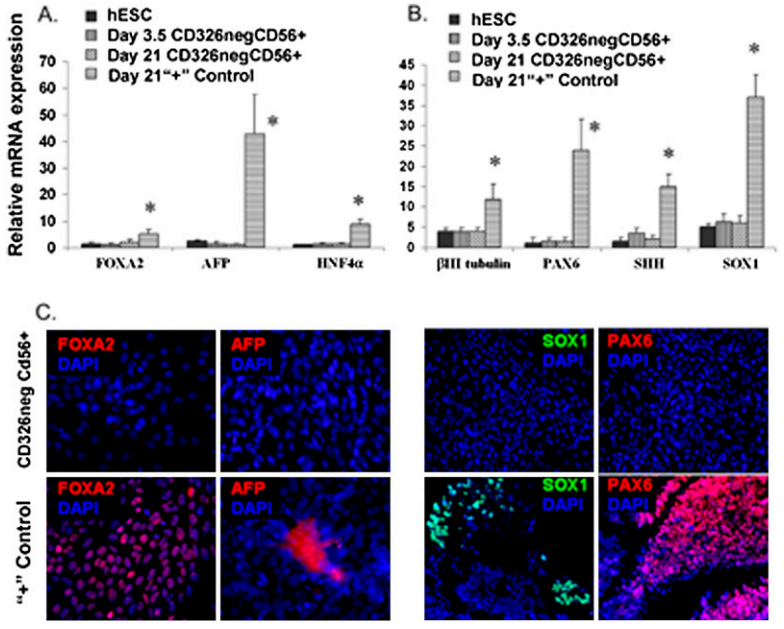

Fig. S4. Day 3.5 CD326$^-$CD56$^+$ cells fail to differentiate into endodermal or ectodermal lineages. CD326$^-$CD56$^+$ cells were isolated by FACS from the day 3.5 A-BVF induction conditions and plated into differentiation systems previously shown to be efficient for ectodermal or endodermal specification, respectively (*SI Materials and Methods*) (1, 2). No up-regulation of (*A*) endodermal genes [FOXA-2, α-Feto-Protein (AFP), or HNF-4α] or (*B*) ectodermal genes (βIII tubulin, Pax-6, sonic hedgehog (SHH), or Sox-1) was observed compared with either undifferentiated hESCs or day 3.5 CD326$^-$CD56$^+$ cells after up to 21 d in specific culture conditions. Day 21 "+" control cultures were generated in parallel from undifferentiated hESCs using identical endodermal or ectodermal conditions. For statistical analysis one-way ANOVA was performed, followed by Student–Newman–Keul's test. *$P < 0.05$. (*C*) Absence of FOXA-2 and AFP protein expressing cells in endoderm cultures or Sox-1 or Pax-6 expressing cells in ectoderm cultures were identified, whereas control cultures initiated from hESCs demonstrated robust generation of endodermal FOXA2 and AFP positive (red) or ectodermal lineages SOX1 (green) or Pax6 (red) expressing cells in the same conditions. Magnification ×200.

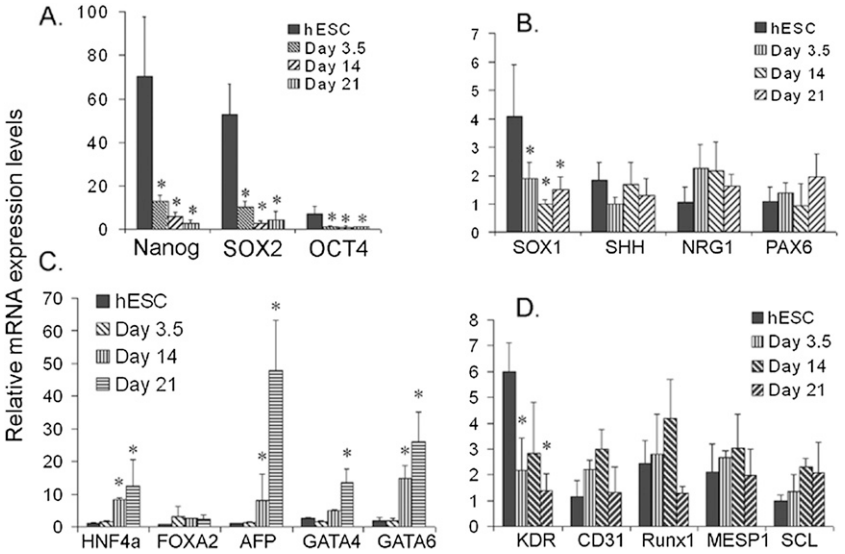

Fig. S5. CD326$^+$ cells remaining in A-BVF culture demonstrate a gene expression profile of primitive and visceral endoderm. Relative expression of (*A*) pluripotency, (*B*) ectoderm, (*C*) endoderm, and (*D*) mesoderm gene transcripts in undifferentiated hESCs (HES3 line) and CD326$^+$ cells isolated at days 3.5, 14, and 21 after A-BVF induction, determined by real-time PCR. For statistical analysis one-way ANOVA was performed, followed by Student–Newman–Keul's test. *$P <$ 0.05 significant difference between each population and hESCs.

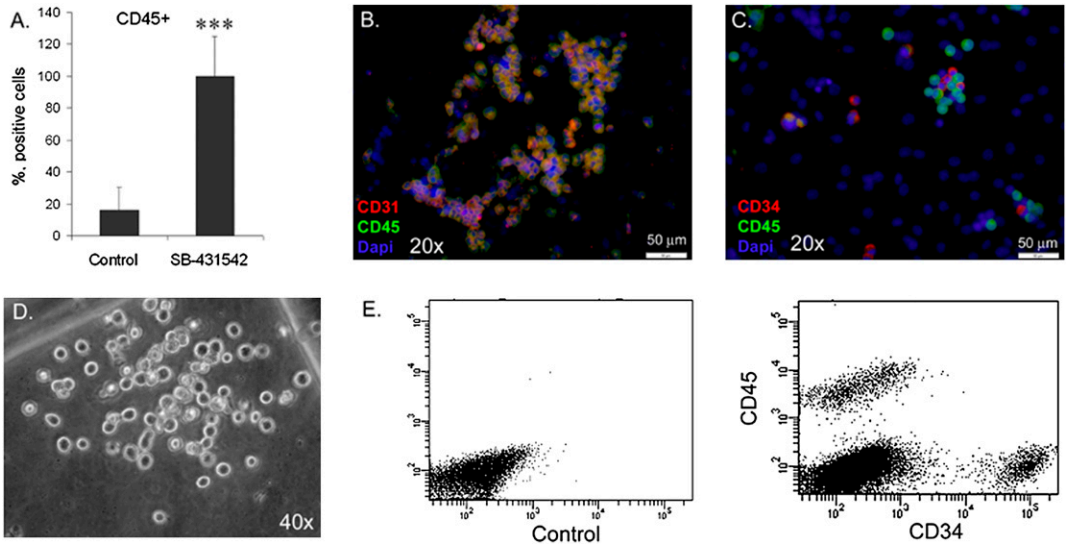

Fig. S6. Hematoendothelial differentiation from day 3.5 CD326$^-$CD56$^+$ population. (*A*) Inhibition of TGF-β signaling significantly increases the yield of hematopoietic cells generated from day 3.5 CD326$^-$CD56$^+$ cells. CD326$^-$CD56$^+$ cells were isolated by FACS 3.5 d after A-BVF induction and plated on OP9 murine stroma in EGM2 complete medium in the absence or presence of SB-431542 (5 μM). After 7 d of culture the EGM2 medium was replaced with DMEM/F12 medium supplemented with 10% FCS and hematopoietic cytokines in the absence (control) or presence of SB-431542 (5 μM). After an additional 7 d (total 14 d) total cell number and proportion of CD45$^+$ cells was measured by flow cytometry. Mean ± SD, $n = 3$ independent experiments, each in triplicate. ***$P < 0.001$. For statistical analysis, a Student's *t*-test was applied. $P < 0.05$ was considered as statistically significant. (*B* and *C*) Day 3.5 CD326$^-$CD56$^+$ cells were isolated by FACS from A-BVF induction conditions, and plated into chamber slides in hematoendothelial differentiation cultures. After 14 d, cells were fixed in methanol and stained with antibodies against CD45, CD31, and CD34. Nuclei were counterstained with DAPI. Representative images show coexpression of CD45 (green) and CD31 (red) in the membranes of hematopoietic cells, whereas the vast majority of CD45$^+$ (green) cells did not express CD34 (red). (*D*). Hematopoietic colonies generated from the day 3.5 CD326$^-$CD56$^+$ cells in semisolid methylcellulose culture. (*E*) Representative FACS plots showing CD45$^+$ and CD34$^+$ cells generated from the day 3.5 CD326$^-$CD56$^+$ cells on OP9 stromal cells in the presence of hematopoietic cytokines. Unstained sample was used as control.

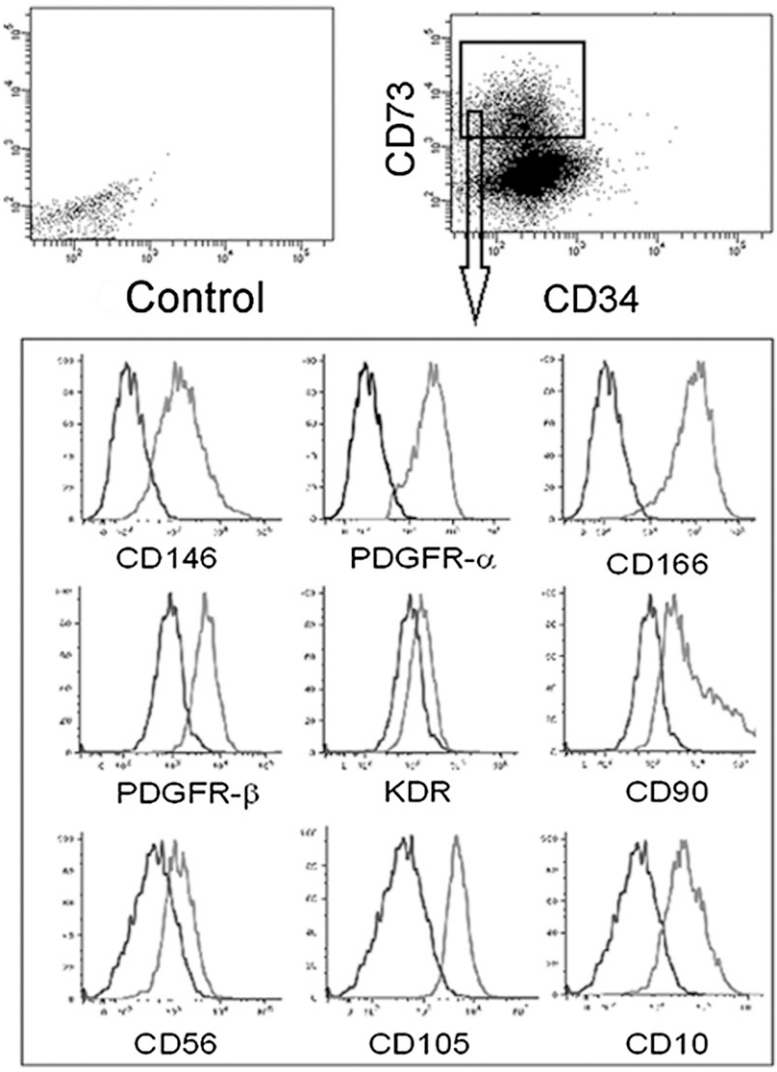

Fig. S7. Immunophenotype of mesenchymal cells generated from day 3.5 $CD326^{-}CD56^{+}$ cells. Flow cytometry of surface markers (gray line) of $CD73^{+}CD34^{-}$ gated cells generated after 2 wk of culture on Matrigel-coated plates in EGM2 complete media. Control (unstained) samples (dark line).

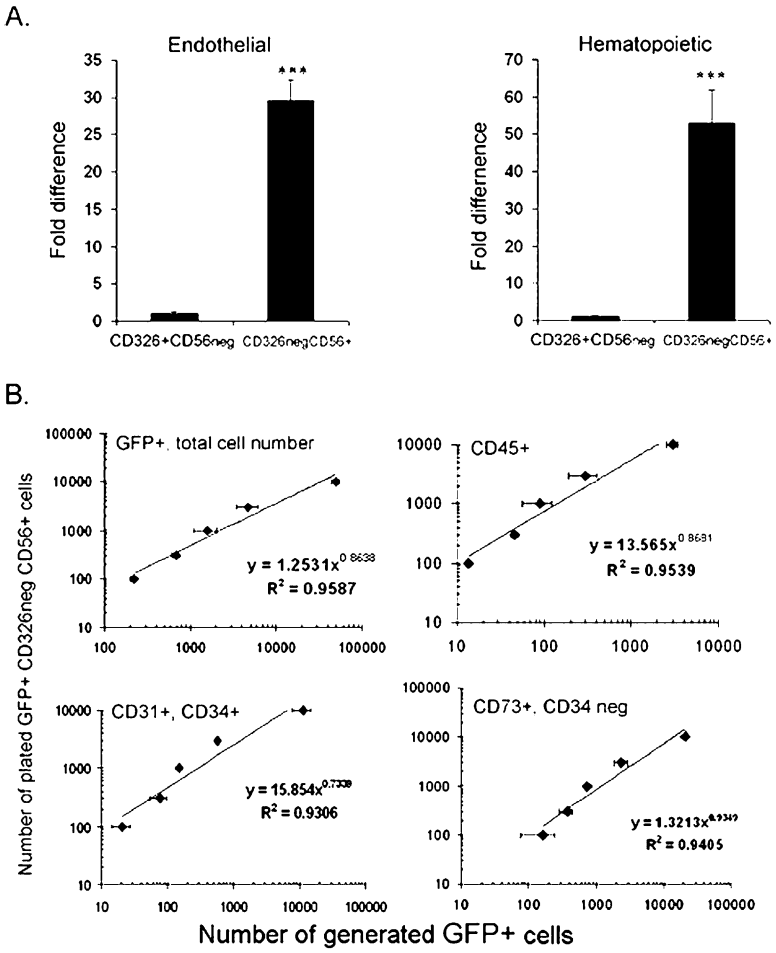

Fig. S8. Quantitative analysis of generation of mesodermal lineages from day 3.5 CD326$^-$CD56$^+$ cells. (*A*) Identical numbers of either CD326$^-$CD56$^+$ and CD326$^+$CD56$^-$ cells were isolated by FACS from the same day 3.5 A-BVF induction conditions, and plated into hematoendothelial differentiation cultures for 14 d. Numbers of CD31$^+$ and/or VE-cadherin$^+$ (endothelial lineage) and CD45$^+$ (hematopoietic lineage) cells generated from the CD326$^-$CD56$^+$ population were normalized to the cell numbers generated from the control CD326$^+$CD56$^-$ cells and are shown as a fold difference over control. Mean ± SD, $n = 3$ independent experiments. $P < 0.001$. (*B*) To quantitate the relative contribution of various mesodermal derivatives to total hEMP output, GFP expressing day 3.5 CD326$^-$CD56$^+$ cells (hEMP) were generated from a stable GFP$^+$ subclone of H9 hESCs created using lentiviral vector transduction. Day 3.5 GFP$^+$ CD326$^-$CD56$^+$ cells were seeded onto OP9 cells in serial dilution as 10,000, 3,000, 1,000, 300, and 100 cells per well. GFP$^-$ hEMP generated from untransduced parent H9 cells from identical A-BVF conditions were coseeded with the GFP$^+$ cells to keep equal cell numbers seeded into each well (total 10,000 CD326$^-$CD56$^+$ per well). After 14 d of culture, cells were counted and the absolute number of total GFP$^+$ cells, CD45$^+$ (hematopoietic), CD31$^+$CD34$^+$ (hematoendothelial), and CD73$^+$CD34$^-$ (mesenchymal) cells was measured by flow cytometry and plotted (on *y* axis) against initial numbers of the GFP$^+$ CD326$^-$CD56$^+$ cells seeded to the same wells (*x* axis). Correlation analysis was performed using Microsoft Excel software.

Table S1. Primers used for PCR analysis

Gene	GenBank accession	Forward primer	Reverse primer
CTNT	NM_000364	TGTTCTCCGAAACAGGATCAAC	CCGGTGACTTTAGCCTTCCC
TBX5	NM_000192	GACCATCCCTATAAGAAGCCCT	TGTGCCGACTCTGTCCTGTA
TBX20	NM_020417	AAGCCCCAACTCTCCTCTC	GGGTTTGATTGTGTTCTCCGT
Nkx2.5	NM_004387.2	AGAAGACAGAGGCGGACAAC	CGCCGCTCCAGTTCATAG
MLC2A	NM_021223	TGAGTGCCTTCCGCATGTTT	GTGATGATGTAGCACAGTGACTT
RPL7	NM_000971	ATGGCGAGGATGGCAAGAAAA	GCTCACTCCATTGATACCTCTGA

Movie S1. Cardiac potential of CD326^{-}CD56^{+} cells. Contracting aggregates of cells generated from CD326^{-}CD56^{+} cells (derived from HES3) isolated by FACS from A-BVF induction conditions at day 3.5 and cultured for an additional 11 d in cardiomyocyte conditions (*SI Materials and Methods*).

Movie S1